Dual inhibitors of histone deacetylases and other cancer-related targets: A pharmacological perspective

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ABSTRACT: Epigenetic enzymes histone deacetylases (HDACs) are clinically validated anticancer drug targets which have been studied intensively in the past few decades. Although several drugs have been approved in this field, they are still limited to a subset of hematological malignancies (in particular T-cell lymphomas), with therapeutic potential not fully realized and the drug-resistance occurred after a certain period of use. To maximize the therapeutic potential of these classes of anticancer drugs, and to extend their application to solid tumors, numerous combination therapies containing an HDACi and an anticancer agent from other mechanisms are currently ongoing in clinical trials. Recently, dual targeting strategy comprising the HDACs component has emerged as an alternative approach for combination therapies. In this perspective, we intend to gather all HDACs-containing dual inhibitors related to cancer therapy published in literature since 2015, classify them into five categories based on targets' biological functions, and discuss the rationale why dual acting agents should work better than combinatorial therapies using two separate drugs. The article discusses the pharmacological aspects of these dual inhibitors, including *in vitro* biological activities, pharmacokinetic studies, *in vivo* efficacy studies, as well as available clinical trials. The review of the current status and advances should provide better analysis for future opportunities and challenges of this field.

Key words: histone deacetylases (HDACs); dual inhibitor; anticancer drugs; kinases, epigenetics, enzymes, receptors, synergistic effects

1. Introduction

Mutations, deregulated expression, and aberrant recruitment of epigenetic regulators are recognized as important contributors to the onset and maintenance of many types of human

tumors. Among these regulators are epigenetic writers (such as DNA methyltransferases and histone acetylases or HATs), readers (such as bromodomain-containing proteins), and erasers (such as histone deacetylases or HDACs), all play crucial roles in the tumor pathological development. Particularly, chromatin remodeling regulators HDACs have received intensive studies in the past few decades, evidenced by the numbers from both scientific publications and clinical trials. Quick survey of literature reports on HDACs field using the SciFinder® search engine reveals that the publication (papers and patents) number dramatically increases from 200 in the year 1999 to 2200 in the year 2019. As of today, more than 700 clinical trials involving the use of HDACs inhibitors are listed in the clinicaltrials.gov website, of which 200 trials are in the active or recruiting stage [1].

HDACs are a family of enzymes that catalyze the deacetylation of lysine residues of both histone and non-histone protein substrates [2]. There are eighteen human HDACs enzymes, which can be divided into four different categories: class I (HDACs 1,2,3,8), class II (HDACs 4,5,6,7,9,10), class III (Sirtuins 1,2,3,4,5,6,7) and class IV (HDAC 11). The class III HDACs enzymes are NAD^+ dependent while the others belong to zinc dependent metal enzymes [3,4]. The expression levels of HDACs are usually higher in tumor cells than in normal cells. For instance, the following HDACs subtypes are found to be highly expressed in certain tumors: HDAC1 (gastric, lung, esophageal, colon, prostate, and breast cancers), HDAC2 (colorectal, cervical, and gastric cancers), HDAC3 (colon and breast tumors), HDAC6 (mammary tumors), HDAC8 (neuroblastoma), and HDAC11 (rhabdomyosarcoma).

Inhibition of HDACs can lead to pleiotropic effects on cellular signaling and transcription, including cell death, differentiation, autophagy, and enhancement of immunogenicity. The pharmacology of HDACs inhibitors (HDACi) has been clearly established and validated in clinic

for cancer therapy. To date, five drugs are approved for the treatment of various types of lymphoma: vorinostat (SAHA), romidepsin (FK-228), belinostat (PXD-101), tucidinostat, and panobinostat (LBH-589). All of them belong to pan-HDACs inhibitors (Fig. 1) [5-7].

<insert Figure 1>

Several excellent reviews have been published in recent years regarding to HDACi in cancer therapy [8-17]. In addition, HDACi as a potential treatment for other diseases such as fibrotic diseases [18], multiple sclerosis [19], fatty liver diseases [20], and aging [21] have also been reported.

The structure of a typical HDACi consists of three key components: the cap group (acts as surface binding, Cap), the linker, and the pharmacophore (zinc-binding group, ZBG) (**1**, Fig. 1). The Cap portion, which accounts for the affinity gain through hydrophobic interaction with protein, can be large or small in size, and can modulate the selectivity of the HDACs subtype. The linker is typically linear and hydrophobic as well. For example, straight carbon chain, trans-di-substituted olefin or di-substituted phenyl or heteroaryl are often used as the linker. Most of the ZBGs (pharmacophore or warhead) are hydroxamic acid or its corresponding prodrugs, and to less extent ZBGs can also be acyl aniline, cyclic tetrapeptides, thiol, and aliphatic carboxylic acids, or their isosteric replacements [22-24].

As mentioned above, five HDACi approved drugs are all for hematological malignancies. Despite many clinical trials using a single agent of HDACi have been conducted for solid tumors, none of them are effective. To overcome the drug resistance development as well as to expand HDACs inhibitors' therapeutic potential for solid tumors, the combination strategy [25,26] is currently being investigated in the clinic. Synergistic and enhancement effects using

the combination of an HDACi and a chemotherapy agent or targeted drug have been clearly demonstrated in both in vitro and in vivo studies [27,28]. Particularly encouraging, a recent phase III trial reveals that the combination of tucidinostat (chidamide) with exemestane improves progression-free survival in patients with advanced HR^+ and $HER2^-$ breast cancer compared to the exemestane treatment group [29,30].

2. Dual inhibitors of HDACs and one additional cancer-related target

Despite the success and promise in cancer treatment paradigms, an agent aiming at a single molecular mechanism (or pathway) faces limitations and obstacles, due to the compensation of defense mechanism by cancer cells, the complexity of tumor cell alterations, and the redundancy of survival pathways. The combination therapy has become a major trend in anti-cancer therapy, owing to its capability of both enhancing the efficacy and delaying or retarding the resistance. However, the combination therapy also faces several challenges yet to be addressed. Firstly, it has unpredictable drug-drug interactions between the two molecular entities. Secondly, the combination treatment might exacerbate the adverse effects of two agents. Thirdly, the pharmacokinetics complexity needs to be taken into consideration. Lastly, it could involve much higher developmental costs and significantly more clinical trial efforts. Thus, dual or multi-targeting drug strategy has emerged as an alternative approach in recent years [31]. Multi-targeting agents are capable of simultaneous intervention of two or more different biological targets (enzymes or signaling pathways) crucial for cancer progression. These agents were typically discovered through serendipity or screening in the early days. As the knowledge of molecular pathology of cancer deepens, scientists start the rational design of single agents, and use these agents on purposely to act on two (or more) different biological targets (or pathways) known to exhibit synergistic effects in the combination studies. Previous research studies in this

field have been reviewed by several research groups [12, 32-35]. Some of those reviews included dual targeting agents containing HDACi pharmacophore (being called as HDACs hybrid molecules), with the emphasis on other aspects such as medicinal chemistry. Due to the rapid development of this field and the increasing number of publications, summarizing the up-to-date advances not been covered in previous reviews is extremely necessary.

The accumulating knowledge of HDACi SAR in literature has revealed that the Cap can tolerate diverse structures while still maintaining a reasonably good in vitro activity. To design a dual inhibitor of HDACs and a target (kinases, epigenetic targets, other enzymes, receptors, and miscellaneous), one could simply swap the Cap with structure fragment from an inhibitor of the designated target (Fig. 2). The key is to identify a suitable attaching point where the HDACs pharmacophore side chain can be installed, and ensuring such chemical attachment with little to no influence on the biological activity against the original target. Additionally, an optimal attaching point should also allow the synthetic feasibility of the designed molecule. All these key factors should be taken into consideration while designing a dual targeting agent.

<insert Figure 2>

2.1 Dual inhibitors of HDACs and protein kinases

Drug discovery targeting protein kinases has been one of major focuses for pharmaceutical industries in the last few decades, and the efforts have produced fruitful success. By January 2020, the US FDA has approved 52 small molecule protein kinase inhibitors for various human diseases [36]. As many as 46 of them are used in the treatment of neoplastic diseases (41 against solid tumors, 8 against non-solid tumors, and some drugs are used against

both tumor types). Due to the tremendous efforts and fruitfulness in this field, protein kinases class has been considered by scientific community as one of the earliest target classes to be co-targeting with HDACs. In this session, we will discuss these dual targeting agents involving HDACs and protein kinases.

2.1.1 Dual inhibitors of HDACs and CDKs

Cyclin-dependent kinases (CDKs) are heterodimeric protein kinases that play a central role in the control of cell cycle progression. These kinases function through phosphorylation of well-defined enzymatic and structural targets, and are also involved in regulating transcription, mRNA processing, and the differentiation of nerve cells [37]. The US FDA approved the following three drugs targeting CDKs: palbociclib (2015), abemaciclib (2017), and ribociclib (2017). Conceptual design of a dual inhibitor targeting both HDACs and CDKs is supported by several scientific publications. In 2010, Keshelava and co-workers reported that the combination of vorinostat (SAHA) and flavopiridol (a pan-CDKs inhibitor) was significantly more cytotoxic to neuroblastomas with p53 LOF (loss of function) via TP53 mutations or p14ARF deletion [38]. Using a genomic approach, Bild and co-workers predicted that the combination of SAHA and palbociclib could have synergistic effects for the treatment of breast cancer. They then subsequently validated this prediction in an in vitro study [39]. Furthermore, Jochemsen and co-workers reported that the combination of quisinostat (a clinical pan-HDACs inhibitor developed by JNJ) and flavopiridol not only showed synergistic reduction effects of cell viability against several melanoma cell lines including $BRAF^{v600E}$ mutant melanoma [40], but also caused tumor regression in cutaneous melanoma PDX mice without increase of adverse effects.

The above scientific supports prompted several research groups to design and synthesize dual CDKs-HDACs inhibitors. In a 2017 patent application, Zhang and co-workers [41] disclosed a series of dual CDKs-HDACs inhibitors derived from palbociclib and SAHA. The representative compound **6** shows potent inhibitory activities against CDK4/6 and HDAC1 (IC_{50} for CDK4 and CDK6: <10 nM and <25 nM; IC_{50} for HDAC1: <50 nM). No further characterization for those dual inhibitors was given. In 2018, Xiang et al [42-44] reported two series of dual CDKs-HDACs inhibitors, one derived from abemaciclib and SAHA, and the other derived from ribociclib and SAHA. Compound **7**, a representative compound derived from abemaciclib series, shows potent inhibitory activities against both CDK4 and HDAC1 (IC_{50} for CDK4: 1.2 nM; HDAC1: 26 nM). In a cellular assay, compound **7** induces cell apoptosis and G1-phase arrest in various breast and ovarian cancer cells (IC_{50}: 15.8-44 nM in the following cell lines: 4T1, MDA-MB-468, MDA-MB-231, SK-OV-3, OVCAR-5, and H460). Importantly, compound **7** significantly inhibits tumor growth in breast cancer homograft and xenograft mice with low toxicity. Compound **8**, a representative compound derived from ribociclib series, not only shows potent inhibitory activities against both CDK4 and HDAC1 (IC_{50}: 8.8 nM and 2.2 nM respectively), but also exhibits greater anti-proliferative effects against 9 cancer cell lines (IC_{50} ranging from 1.11 to 3.78 μM) than ribociclib or SAHA separately. In in vivo studies, compound **8** is orally bioavailable (%F = 18.4%), and significantly inhibits tumor growth in 4T1 xenograft mice at 130 mg/kg dose without causing obvious toxicity (indicated by body weight decrease). In 2019, Gan et al [45] reported a series of dual CDKs-HDACs inhibitors derived from roscovitine (an investigational drug of CDKs inhibitor) and panobinostate. The representative compound **9** exhibits potent inhibitory activities against both CDK2 and HDAC1 and effectively induces apoptosis of three cancer cell lines (A549, HepG2, and CAL-148). Yuan

et al [46] reported a series of dual CDKs-HDACs inhibitors derived from a known CDK1/2 inhibitor AT7519 [47]. An HDACs pharmacophore (hydroxamic acid) side chain was attached to AT7519's phenyl portion, a region known to expose to the solvent region. Thus, the most active analog **10** from the series could arrest cell cycle in G2/M phase and promoted apoptosis in A75, HCT116, H460, and Hela cells. which was associated with increasing the intracellular reactive oxygen species (ROS) levels. In in vivo studies, compound **10** showed very good ip (intraperitoneal) bioavailability (%F = 63.6%) in ICR mice, and significantly inhibited tumor growth in HCT-116 xenograft mice upon 25 mg/kg once daily (qd) ip dosing for 21 days (Table 1).

<insert Table 1>

2.1.2 Dual inhibitors of HDACs and CK2

Casein kinase 2 (CK2) is a serine/threonine-selective protein kinase that has been implicated in cell cycle control, DNA repair, circadian rhythm regulation, and other cellular processes. High expression levels of CK2 have been associated with numerous solid tumors and hematologic malignancies [48]. The most advanced CK2 inhibitor is silmitasertib (CX-4945) [49], which is currently in several clinical trials for treatment of multiple myeloma, kidney cancer, medulloblastoma, and advanced solid tumors. In January 2017, it was granted orphan drug status by the US FDA for advanced cholangiocarcinoma. There is no approval drug for CK2 inhibitor.

Three different series of dual CK2-HDACs inhibitors reported to date are all from de Pascual-Teresa's group (Table 2) [50-52]. Compound **11** was derived from TBB [53] and HDAC pharmacophore hydroxamic acid using click chemistry. Despite the weaker CK2α activity

comparing to TBB (62% inhibition @50 μM vs IC_{50} value of 800 nM by TBB) and weaker HDAC1 activity comparing to SAHA (IC_{50}: 2.2 μM vs 33 nM), compound **11** exhibits significantly greater anti-proliferative effects against Jurkat cell line (human leukemia) than its parent TBB (LC_{50}: 2.5 μM vs 20 μM). Although the LC_{50} value of **11** is identical to that of SAHA, its proapoptotic activity is significantly higher, indicated by quantitative annexin V/PI apoptosis assay using flow cytometry. These results suggest synergistic and beneficial effects of inducing proapoptotic activities using a dual CK2-HDACs inhibitor. Compound **12**, a hybrid molecule derived from DMAT [54] and SAHA, shows moderate inhibitory activities against CK2 and HDAC1 and good anti-proliferative effects against 7 tumor cells (IC_{50} values ranging from 2 to 9 μM). Derived from clinical candidate silmitasertib and approved drug SAHA, compound **13** exhibits greater inhibitory activities against both CK2 and HDACs compared to its parents silmitasertib and SAHA (IC_{50} for CK2: 1.7 nM vs 1.8 nM of silmitasertib; IC_{50} for HDAC1: 3.3 nM vs 33 nM of SAHA; IC_{50} for HDAC6: 13 nM vs 33 nM of SAHA). Unfortunately, the excellent enzymatic activities of **13** did not transfer into good anti-proliferative effects against 4 different cancer cell lines. This might be due to the low permeability of compound **13** in cells.

<insert Table 2>

2.1.3 Dual inhibitors of HDACs and RTKs

Receptor tyrosine kinases (RTKs) are high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones. Inhibition of the kinase domain of RTKs has been validated as a strategy for cancer therapy [55]. The US FDA has approved several small

molecule drugs targeting the kinase domain of Rs, including fibroblast growth factor receptors (FGFRs), epidermal growth factor receptor (EGFR), and vascular endothelial growth factors (VEGFRs). For example, nintedanib (targeting FGFR1/2/3) was approved for treatment of idopathic pulmonary fibrosis in 2014, while erdafitinib (targeting FGFR1/2) was approved for urothelial bladder cancers in 2019. Approval drugs targeting EGFR are gefitinib (2003), erlotinib (2004), osimertinib (2015), lapatinib (2017), and dacomitinib (2018), for the treatment of NSCLC and breast cancers. Drugs targeting VEGFRs are sorafenib (2005), sunitinib (2006), pazopanib (2009), vandetanib (2011), axitinib (2012), cabozantinib (2012), regorafenib (2012), and lenvatinib (2015). Several research groups have reported dual inhibitors derived from some of the approved drugs listed above. Most of the dual inhibitors in this class reported before 2018 have been reviewed recently [56-58]. More recently, a rational designed dual EGFR and HDACs inhibitor compound **14** (derived from osimertinib and SAHA) shows interesting biological activities (Table 3) [59]. Despite having weak inhibitory activities against both $EGFR^{WT}$ and $EGFR^{T790M}$, compound **14** exhibits better anti-proliferative effects against several cancer cell lines (IC_{50} values ranging from 0.23 to 1.85 μM for the following cells: MDA-MB-231, MDA-MB-468, KG-1, HT-29, and HeLa) compared to its parent osimertinib. As reported by Hu et al [60], the dual FGFR1-HDACs inhibitor **15** exhibits moderate inhibitory activities against FGFR1 and HDAC1 (64% and 59% inhibition at 1 μM concentration, respectively) and potent inhibitory activity against HDAC6 (IC_{50}: 34 nM). No further pharmacological studies were reported. c-Met, also named tyrosine-protein kinase Met or hepatocyte growth factor receptor (HGFR), is known as a RTK. After binding to its specific ligand hepatocyte growth factor (HGF), c-Met activates a wide range of different cellular signaling pathways, including those involved in cancer cell proliferation, survival, invasion, metastasis, and angiogenesis etc. The c-Met pathway is

dysregulated in various cancers especially those associated with metastasis, and c-Met inhibition could effectively reduce c-Met signaling and metastatic phenotype in a range of preclinical models [61,62]. Several c-Met inhibitors were advanced to various stages of clinical trials [63]. In 2020, the first c-Met selective inhibitor capmatinib (INC280) was approved by the US FDA as a first-line treatment for non-small cell lung cancer (NSCLC) patients with the MET exon 14 skipping (METex14) mutation [64]. There are only a few series of dual c-Met-HDACs inhibitors reported to date. In 2017, Hu et al [65] reported first dual c-Met-HDACs inhibitors based on a known c-Met inhibitor and tucidinostat. Compound **16** shows potent inhibitory activities against c-Met and HDAC1 (IC_{50} values are 0.71 and 38 nM respectively) [65]. In cellular assays, compound **16** shows greater anti-proliferative effects against EBC-1 and HCT-116 cells than the parent tucidinostat. Compound **17** is a representative compound derived from crizotinib and SAHA [66]. It potently inhibits c-Met and HDAC1/2 enzymatically. Compound **18** [67] has the same quinoline scaffold as compound **16**, but with different side chains. It has a similar *in vitro* activity profile as compound **16**, not only in enzymatic levels, but also in cellular levels. However, comparing with an equimolar mixture of its parent compounds, SAHA and foretinib, compound **18** did not exhibit synergistic effect in inhibiting cancer cells proliferation.

<insert Table 3>

2.1.4 Dual inhibitors of HDACs and JAKs

The Janus kinases (JAK1, JAK2, JAK3, and TYK2) are intracellular non-receptor tyrosine kinases. They play essential roles in the cell signaling pathway activated by a variety of cytokines and implicates in the pathogenesis of various cancer types [68]. Activation of JAKs by different cytokines results in phosphorylation and dimerization of the STAT (signal transducer

and activator of transcription) proteins, which further translocate to the nucleus and activate gene transcription [69]. The JAK-STAT signaling axis is associated with diverse biological functions including inflammation, immune function, and hematopoiesis [70,71]. Thus, JAKs are validated therapeutic targets for autoimmune diseases such as rheumatoid arthritis, myelofibrosis, and polycythemia vera. To date, there are four JAKs inhibitors approved by the US FDA, all for the treatment of diseases related to the immune system. The potential treatment for cancers using a JAKs inhibitor is still under clinical investigation. For example, itacitinib (INCB039110) is in various stages of clinical trials for advanced HCC (hepatocellular carcinoma), lymphoma, and other solid tumors [72]. Conceptual design of dual inhibitors targeting both JAKs and HDACs is supported by several scientific publications. In 2013, Radimerski and co-workers reported that the combination of ruxolitinib (a JAKs inhibitor approval drug) and panobinostat resulted in enhanced efficacy in mouse models of $JAK2^{V617F}$-driven disease compared to corresponding single agents [73]. In addition, the combination of HDACs inhibitor pracinostat (SB939) and JAK2 inhibitor pacritinib demonstrated synergistic effects on the reduction of tumor growth and the normalization of plasma cytokines/growth factors/chemokines [74]. Furthermore, Wang and co-workers reported that the combination of panobinostat and JAK2 inhibitor fedratinib (TG101209) could reduce $JAK2^{V617F}$ levels and could produce synergistic cytotoxic effects against human myeloproliferative neoplastic cells [75].

These synergy effects discussed above provide the scientific rationale for medicinal chemistry design of dual JAKs-HDACs inhibitors. Dymock's group published three articles and one patent describing three series of dual JAKs-HDACs inhibitors [76-79] derived from three different JAKs inhibitors namely pacritnib, ruxolitinib and XL019 [80]. Compound **19** (EY3238), a representative compound derived from pacritnib, shows potent dual inhibitory activities (IC_{50}

for JAK2, HDAC1, and HDAC6 are 1.4, 222, and 2.1 nM, respectively), and is highly selective against JAK2 over all other kinases tested. In cellular assays, compound **19** inhibits proliferation of various solid and hematological tumor cell lines. Its IC_{50} values range from 1.43 to 2.43 μM for solid tumor cells such as MDA-MB-231, HCT-116, PC-3, and MCF-7, and from 0.94 to 2.11 μM for hematological tumor cells such as HEL92.1.7, Jurkat, MOLM-14, NKYS, KG-1, OPM-2, KHYG, and KMS-12-BM. In the intracellular mechanism of action experiments, compound **19** clearly inhibits both JAK-STAT and HDACs pathways. When tested head-to-head with pacritnib in normal cells TAMH (transforming growth factor-α mouse hepatocytes), compound **19** has about 3-fold better safety window (the therapeutic windows are calculated by dividing normal cells IC_{50} value with cancer cells IC_{50} value). In the in vitro rat liver microsomes stability test, compound **19** has longer half-life than pacritnib, indicating better metabolic stability. Compound **20**, a dual JAKs-HDACs inhibitor based on ruxolitinib scaffold, shows potent JAKs and HDACs inhibitory activities in enzymatic assays (IC_{50} <10 nM for HDAC1/2/3/6; 17 nM and 75 nM for JAK1 and JAK2 respectively). In cellular assays, compound **20** shows potent anti-proliferative effects not only against various leukemia cells and hematological tumor cells (IC_{50}: 0.15-2.16 μM), but also against several solid tumor cells (MDA-MB-231, MCF-7, HCT-116, and PC-3). The anti-proliferative activities of **20** against solid tumor cells are greater or comparable to the activities of SAHA, and are significantly better than those of ruxolitinib (IC_{50} 0.79 to 2.41 μM vs >10 μM). Unfortunately, compound **20** has very poor oral bioavailability in Wistar rats (1.4%), and might be served as a tool molecule rather than a development candidate. Derived from JAK2-selective inhibitor XL019 (a terminated clinical candidate due to CNS penetration and related side effects), compound **21** exhibits potent inhibitory activities against JAK2, HDAC1, and HDAC6 (IC_{50} values are 3.1, 56, and 1.2 nM respectively). In cellular assays, compound **21**

exhibits better anti-proliferative effects against four solid tumor cells (HCT-116, MDA-MB-231, MCF-7, and PC-3) compared to XL019, and better or similar activities compared to SAHA. Mechanistic studies reveal that the apoptosis is responsible for tumor cell death, and the inhibition of both HDACs and JAK-STAT pathways contributes to the greater in vitro potency. It is worth mentioning that compound **21** shows less CNS penetration compared to XL019 and is expected to have better safety profile. In 2018, Yu et al [81] reported multi-targeting JAKs-FLT3-HDACs inhibitors derived from zotiraciclib (SB1317) and SAHA. The representative compound **22** shows potent inhibitory activities against JAK2, FLT3, and HDAC (IC_{50} values are 686, 87, and 87 nM respectively). In cellular assays, compound **22** exhibits potent antiproliferative effects against several leukemia and solid tumors cells (Table 4). In 2018, Huang et al [82] reported that the combination of momelotinib (CYT-387) and SAHA in 1:1 ratio exhibits greater antiproliferative activity against HL60 and K562 cells than that from either of the single agent alone. Based on the synergistic results, they designed and synthesized a series of dual JAKs-HDACs inhibitors derived from momelotinib and SAHA. The representative compound **23** is a potent JAK2/HDAC6-selective dual inhibitor (IC_{50} values for JAK2, HDAC1, HDAC3, and HDAC6 are 8, 1100, 234, and 46 nM respectively). In cellular assays, compound **23** induces cell apoptosis and G2/M-phase arrest in HEL cells (IC_{50}: 0.34 μM), more potent than the combination of momelotinib and SAHA (1:1). In addition, compound **23** also shows anti-proliferative effects against HL-60 and K562 cells (IC_{50}: 1.5 and 8.7 μM respectively). When administrated at a dose of 10 mg/kg to SD rats intraperitoneally, compound **23** shows good plasma exposure (AUC of 4470 h*ng/mL). In AML and HEL xenograft mice, compound **23** causes statistically significant inhibition of tumor growth upon ip dosing of 10 mg/kg for 21 days. Interestingly, the combination of compound **23** and fluconazole (FLC, a first-generation triazole

antifungal medication) exhibits excellent synergistic antifungal effects against the FLC-resistant C. albicans strain 0304103 in the *in vivo* studies. Specifically, ICR mice treated with the combination of compound **23** (5 mg/kg) and FLC (1 mg/kg) had significant improvement in survival rate without signs of toxicity. This is the first example of a small molecule possessing both anti-cancer (AML) and anti-fungal (against FLC-resistant infection) efficacy in vivo. In 2019, Zhang et al [83] reported a series of dual JAKs-HDACs inhibitors based on their previously reported N^2-(1*H*-pyrazol-4-yl)pyrimidine-2,4-diamine scaffold [84]. Among those analogs reported, compound **24** exhibits pan-JAKs and HDAC6-selective inhibitory activities (IC_{50} values for JAK1-3 and TYK2 ranging from 4 to 49 nM; IC_{50} values for HDAC6, HDAC2, and HDAC8 are 14, 120, and 2470 nM respectively). In cellular assays, compound **24** induces cell apoptosis and exhibits anti-proliferative effects against several tumor cells (IC_{50} values for HEL bearing the $JAK2^{V617F}$ mutation, K562, MOLT4, and Jurkat cells are 0.09, 0.49, 0.08, and 0.06 μM respectively). Dual inhibitors reported in this article exhibit more potent than the combination of ruxolitinib and SAHA in vitro. Unfortunately, the oral bioavailability of compound **24** is very poor (<1%), despite its ip bioavailability is good. Thus, the in vivo studies of compound **24** were conducted in HEL xenograft mice using ip dosing (100 mg/kg, qd, for 16 consecutive days), and moderate anti-tumor efficacy and good safety profiles (no significant body weight loss and no observable signs of toxicity) were obtained.

<insert Table 4>

2.1.5 Dual inhibitors of HDACs and PI3Ks (and/or mTOR)

The phosphatidylinositol-3-kinases (PI3Ks)/Akt and the mammalian target of rapamycin (mTOR) signaling pathways are crucial to many aspects of cell growth and survival, in both

physiological and pathological conditions [85-87]. Thus, targeting these pathways has been extensively studied from mechanism of action to preclinical experiment to clinical investigation. Inhibitors approved for cancer treatment by the US FDA are alpelisib (BYL-719, PI3Kα-selective inhibitor), copanlisib (BAY 80-6946, pan-PI3K inhibitor), and duvelisib (IPI-145, PI3Kδ-selective inhibitor). Among all kinases, PI3Ks class was probably the first kinase class investigated as dual inhibitor with HDACs. The synergistic anti-tumor effects have been reported using the combination of an HDACi and a PI3Ks inhibitor as well as using a dual PI3Ks-HDACs inhibitor [88-92]. Indeed, the first-in-class dual PI3Ks-HDACs inhibitor fimepinostat (CUDC-907) [93-102] developed by Curis, Inc. is the most advance compound in this field, and has entered into various stages of clinical trials. This review focuses dual PI3Ks-HDACs inhibitors reported in the literature after 2015. Compound **25** (Table 5) [103], a dual inhibitor derived from pyrimidine scaffold, exhibits balanced pan-inhibition across PI3Ks subtypes and selective inhibition against HDAC1, 2, 3, 6, and 10. In cellular assays, compound **25** shows greater anti-proliferative effects against several cancer cells (leukemia, lymphomas, and liver cancers) than SAHA or sorafenib alone. These cellular activities have been successfully transferred to in vivo efficacy in HCC xenograft mice. Importantly, compound **25** has good oral bioavailability (%F = 18%) in rats at 150 mg/kg dose to rats. Compound **26** [104], a close analog of fimepinostat, exhibits weaker inhibitory activity against PI3Ks (IC_{50} >1 μM) compared to fimepinostat, but remains potent and balanced inhibitory activities against all HDACs subtypes. Despite of the weaker activity against PI3Ks, compound **26** shows greater anti-proliferative effects against MV4-11 and HCT-116 cells than fimepinostat and SAHA alone. In the in vivo studies, compound **26** has good oral bioavailability in dog (%F = 41.8%), and shows good antitumor efficacy in various tumor cells xenograft mice models. Compound **27** [105], a dual PI3K-

HDACs inhibitor derived from copanlisib and SAHA, shows excellent inhibitory activities against HDACs and PI3Ks, and potent anti-proliferative effects against several tumor cells. Compound **28** [106], derived from the HDACs pharmacophore and a quinazoline scaffold of known PI3K inhibitors [107], possesses slightly different selectivity profiles of PI3Kδ and HDACs in enzymatic levels compared to clinical candidate fimepinostat. For instance, compound **28** is a potent PI3Kδ-selective inhibitor with 5 to 12-fold selectivity over other PI3K subtypes (IC_{50} for PI3Kδ: 8.1 nM), while fimepinostat exhibits PI3Kβ-selectivity (IC_{50} for PI3Kβ: 16 nM, 4 to 23-fold over other PI3K subtypes). Both compound **28** and fimepinostat have similar profiles of good selectivity against Class I (HDAC1, HDAC2, HDAC3, HDAC8) and Class IIB (HDAC6) HDACs. Interestingly, compound **28** has much weaker inhibitory activity against HDAC4 and HDAC11 than fimepinostat. Since the inhibition of HDAC4 and HDAC11 is known to cause potential side effects, compound **28** might have better safety profiles than fimepinostat [108,109]. In the cellular assays, compound **28** shows good anti-proliferative effects against several hematologic and solid tumor cells, although its anti-proliferative effects are relatively less potent than fimepinostat. In the pharmacokinetic study, compound **28** is orally bioavailable in mice (%F = 4.2%). Furthermore, compound **28** significantly inhibits tumor growth in HCT-116 and HGC-27 xenograft mice upon p.o. dosing (150 mg/kg, TGI: 45.8%) or ip dosing (30 mg/kg, TGI: 62.6%). PI-103 [110] is a potent PI3K and mTOR inhibitor derived from clinical candidate GDC-0941. Fraga et al [111] reported a series of PI3Kα and HDAC6/8-selective dual inhibitors based on the rational design from PI-103 and LASSBio-1911 [112]. Thus the representative compound **29** exhibits potent inhibitory activities against PI3Kα/β/δ and HDAC6/8 in enzymatic levels (IC_{50} values are 46, 73, 72, 15, and 68 nM, respectively). Recently Grewal et al [113] reported compound **30** as a PI3Kγ/δ– and HDAC6-selective dual inhibitor.

The target engagement of PI3Kδ and HDAC6 by **30** was validated in MV411 cells in a cellular thermal shift assay (CETSA). However, this compound has poor oral bioavailability (%F = 0.7%).

As early as 2012, Yu et al [114] observed the synergistic antitumor effects using the combination of an mTORi and an HDACi, both in vitro in HCC cells and in vivo in a HCC PDX (patient-derived xenograft) mice model. Despite of strong scientific evidence from both preclinical and clinical studies, the discovery of dual inhibitors targeting mTOR and HDACs progresses slowly. Up to date, there is only one report regarding to dual mTOR-HDACs inhibitors. Compound **31** [115] is a highly potent and selective inhibitor against mTOR and HDAC1/6 (IC_{50} values are 1.2, 0.19, and 1.8 nM respectively). The selectivity of mTOR over PI3Kα is greater than 416-fold. Molecular biology studies confirm that compound **31** upregulates acetylation of H3 and α-tubulin, and at the same time downregulates mTOR-related downstream signaling pathway. In MM1S xenograft mice, treatment using compound **31** exhibits comparable antitumor activity but fewer side effects compared to the treatment using combination SAHA and rapamycin.

<insert Table 5>

2.1.6 Dual inhibitors of HDACs and Syk

Spleen tyrosine kinase (Syk), a non-receptor cytoplasmic tyrosine kinase primarily expressed in hematopoietic tissues, mediates signal transduction downstream of a variety of transmembrane receptors including classical immune-receptors like the B-cell receptor (BCR).

Abnormal function of Syk has been implicated in several instances of hematopoietic malignancies. Fostamatinib is an FDA-approved Syk inhibitor for treatment of chronic immune thrombocytopenia. There are a number of clinical trials ongoing using fostamatinib alone or in combination for hematological malignancies and solid tumors such as ovarian cancer. To date, there is no report regarding the combination of an HDACi with a Syk inhibitor for cancer treatment in the literature. Compounds **32** and **33** (Table 6), the dual inhibitors in this class [116,117], can serve as tool molecules in elucidating whether a synergy effect exists for cancer treatment.

<insert Table 6>

2.1.7 Dual inhibitors of HDACs and Bcr-Abl kinase

The fusion between the Abelson (Abl) tyrosine kinase gene at chromosome 9 and the break point cluster (Bcr) gene at chromosome 22 results in a chimeric oncogene (Bcr-Abl) and a constitutively active Bcr-Abl tyrosine kinase. Such abnormality is often called as Philadelphia chromosome, which is responsible for more than 90% of chronic myelogenous leukemia (CML). The US FDA has approved the following drugs for treatment of CML or AML: imatinib (2001), dasatinib (2006), nilotinib (2007), bosutinib (2012), and ponatinib (2012). Compound **34** (Table 7) [118], a dual Bcr-Abl and HDACs inhibitor derived from approved drug dasatinib and entinostat (MS-275), shows potent inhibitory activity against both Bcr-Abl and HDAC1 (IC_{50} values are 17.8 nM and 800 nM respectively). In the cellular assays, compound **34** exhibits potent anti-proliferative effects against K562 and DU145 cells (IC_{50} values are 2.62 μM and 0.6 μM respectively), better than dasatinib (IC_{50} are 7.4 μM and 2.43 μM respectively) and MS-275

(IC_{50} are 21.3 μM and 3.75 μM respectively) alone. These results clearly indicate the advantages of developing new anticancer drugs by targeting HDAC and Bcr-Abl simultaneously.

<insert Table 7>

2.1.8 Dual inhibitors of HDACs and Raf kinase

Raf (acronym for rapidly accelerated fibrosarcoma) kinases are a family of three serine/threonine-specific protein kinases namely A-Raf, B-Raf, and C-Raf. Raf kinases are core components of the RAS-RAF-MEK-ERK signal transduction cascade, and are also involved in the mitogen-activated protein kinase (MAPK) cascade. Vemurafenib, dabrafenib, and encorafenib are three drugs targeting B-Raf approved by the US FDA. Zhu et al [119,120] reported two closely related series of dual Raf-HDACs inhibitors based on sorafenib scaffold, with slightly different ZBG. The representative compounds **35** and **36** (Table 8) exhibit similar in vitro activities in both enzymatic assay and cellular assays.

<insert Table 8>

2.2 Dual inhibitors of HDACs and other epigenetic target

2.2.1 Dual inhibitors of HDACs and BET

Bromodomain-containing proteins (BRDs) are a family of 46 proteins consisting of 61 modules. These BRDs recognize acetylated lysine residues in histones and serve as readers responsible for post-translational modulation. Among these proteins, BRD2, BRD3, BRD4 and BRDT belong to bromodomain and extra-terminal (BET) family [121]. Like HATs (writers) and

HDACs (erasers), BET (readers) are important epigenetic modulators, which control the expression of genes related to regulation of various physiological functions, including inflammation, apoptosis, cell proliferation, cell cycle, pancreatic β cell function, and adipogenesis [122-125]. Thus, targeting BET has been validated in preclinical studies for treatment of various diseases including cancers [126-129]. To date, many BET inhibitors have been advanced to various stages of clinical trials: BMS-986158, birabresib (MK-8628), CPI-0610, mivebresib (ABBV-075), molibresib (I-BET762), and INCB-057643, etc [130]. Conceptual design of dual inhibitor targeting both BET and HDACs is supported by several scientific publications. In 2014, Nilsson and co-workers [131] reported that BETi and HDACi could induce similar genetic and biological effects and the combination of these two synergistically led to the death of murine lymphoma induced by Myc overexpression. When co-administrating BRD4 antagonist JQ1 and histone deacetylases inhibitor panobinostat to the NOD/SCID mice engrafted with OCI-AML3 or MOLM13 cells, the significant improvement of animals survival was observed indicating the synergistic effects [132]. In 2016, Geng and co-workers discovered that the feedback activation of leukemia inhibitory factor receptor (LIFR) signaling restrained the efficacy of HDACi in breast cancer while BRD4 inhibitor sensitized breast cancer to HDACi. This finding provides mechanistic foundation for breast cancer treatment using the combination of BRD4 inhibitor and HDACi [133].

The above discoveries strongly support the design of a single small molecule targeting both BET and HDACs. In 2014, Prinjha et al [134] reported compound **37** (Table 9), a potent dual BET-HDACs inhibitor structurally derived from I-BET295 and SAHA. This compound shows potent inhibitory activities against BRD4 and HDAC1 enzymatically (IC_{50} values are 50 and 250 nM respectively), and potent anti-proliferative effects against HL-60 and MV4-11 (IC_{50}

values for 764 and 334 nM respectively) in cellular assays. In 2016, Chen et al [135] reported a series of dual BRD4-HDACs inhibitors derived from dimethylisoxazole skeleton and SAHA. The representative compound **38** shows potent inhibitory activities against BRD4 and HDAC1 enzymatically and potent anti-proliferative effects against K562 and MV4-11 cells in cellular assays (IC_{50} values are 1860 and 910 nM respectively). Liu et al [136] published a patent about dual BRD4-HDACs inhibitors derived from JQ1 and entinostat. The representative compound **39** not only exhibits potent inhibitory activities against HDAC and BRD4 enzymatically (IC_{50} values are 31.2 and 29.6 nM, respectively), but also shows greater anti-proliferative effects than its parents JQ1 and entinostat alone. Compound **40** belongs to a series of dual BRD4-HDACs inhibitors derived from RVX-208 and panobinostat [137]. It shows potent inhibitory activities against BRD4/BD2 and HDAC1 enzymatically and greater anti-proliferative effects against various tumor cells than RVX-208 and panobinostat alone. In 2017, Noguchi-Yachide et al [138] reported a series of dual BRD4-HDACs inhibitors based on N^6-benzoyladenine scaffold [139]. Among those analogs reported, compound **41** is a dual BRD4-HDACs inhibitor (IC_{50} values are 2.7 and 0.26 μM for BRD4 and HDAC respectively). In cellular assays, compound **41** shows moderate anti-proliferative effects against HL-60, K562 and T-47D cells, less potent than SAHA alone. In 2019, Liu et al [140] reported a series of novel dual BRD4-HDACs inhibitors derived from P-0014 and SAHA. The representative compound **42** shows moderate inhibitory activities against BRD4 and potent inhibitory activities against HDAC1-3 (good selectivity over HDAC6), but relatively weak anti-proliferative effect against THP-1 cells in cellular assay. Molecular docking experiments reveal that the hydroxamic acid group of compound **42** might form hydrogen bonds with residues from both HDAC and BRD4. In 2020, Sheng et al [141] reported a series of novel dual BRD4-HDACs inhibitors derived from (+)-JQ1, tacedinaline (CI-994), and

SAHA. The representative compound **43** exhibits pan-inhibition against all BET subtypes (IC_{50} values ranging from 11 to 316 nM) and most of HDAC subtypes (HDAC1/2/3/6/8 IC_{50}: 21 to 192 nM). In cellular assays, compound **43** exhibits potent anti-proliferative effects against human pancreatic ductal adenocarcinoma Capan-1 cells (IC_{50} = 150 nM). Further studies indicate that compound **43** significantly induces the apoptosis in Capan-1 cells at a concentration of 0.5 μM and displays a much higher apoptosis rate (63.5%) than BET inhibitor JQ1 and HDAC inhibitor SAHA used alone or in the combination. These results validate the synergistic effects of dual inhibitor **43** at the cellular levels. Further in vivo studies were conducted in a Capan-1 human pancreatic cancer xenograft mice using ip bid dosing for 21 consecutive days, compound **43** significantly inhibited tumor growth in a dose-dependent manner, with greater efficacy than JQ1 and SAHA used alone or in combination. Notably, no significant body weight loss and no obvious adverse effects were observed during the study. These data support the development of a dual inhibitor as potential treatment for the pancreatic cancer, a cancer type lack of effective therapy to date. In 2020, He et al [142] reported a series of novel BRD4-HDACs dual inhibitors based on thieno[2,3-d]pyrimidine and hydroxamic acid. The representative compound **44** exhibits high selectivity against BRD4 and class I HDAC (BRD4 IC_{50}: 710 nM; HDAC1/2/3/6/8 IC_{50}: 46 - 167 nM). In cellular assays, compound **44** exhibits anti-proliferative effects in three different tumor cells (IC_{50} values for HCT-116, SW620 and DLD1 are 450, 1780 and 2110 nM respectively). Moreover, the anti-proliferative effects in HCT-116 cells were validated through its ability in inducing cell cycle arrest, apoptosis, autophagic cell death and suppressing IL6-JAK-STAT signaling pathways. In pharmacokinetic studies in rats, compound **44** shows good oral bioavailability (%F = 40.5%). Thus, the in vivo efficacy studies were carried out in HCT-116 xenograft mice using p.o. dosing for 17 consecutive days, compound **44** exerted excellent

anti-tumor effects with TGI of 42.7% (15 mg/kg) and 68.8% (30 mg/kg) respectively. No significant body weight loss and signs of toxicity were observed. These data provide opportunities for developing therapeutic drugs targeting BET-HDACs for the treatment of colorectal carcinoma.

<insert Table 9>

2.2.2 Dual inhibitors of HDACs-DNA and HDACs-DNMT

Targeting DNA of cancer cells using chemicals such as DNA alkylating agents results in DNA damage and the death of cancer cells. Such a strategy constitutes one of the earliest cancer treatment modalities. However, the DNA damage caused by genotoxic drugs such as chlormethine and bendamustine can be mitigated by cellular DNA repair machinery, which enables some cancer cells to evade and survive. Co-targeting an additional protein/pathway such as HDACs could prove to be a potential solution to the above issue. CY190602 is the first example targeting DNA and HDACs simultaneously [143]. It is approximately 20~100 fold more cytotoxic against human multiple myeloma cells compared to its parent bendamustine (a single acting agent targeting DNA alone). The synergistic effects provide the foundation for dual HDACs-DNA inhibitors as potential cancer therapy. Compounds **45**-**47** [144-146] are close analogs of CY190602, and are characterized in enzymatic level and/or in vivo studies. For example, the dual acting agent compound **45** shows pan-HDACs inhibitory activities. In the in vivo studies in Bcr-Abl cells transplanted mice, administrating compound **45** at 300 mg/kg dose resulted in significant improvement of the animal survival. Furthermore, administrating compound **45** to human lung cancer H460 xenograft mice resulted in potent antitumor effects.

DNA methyltransferases (DNMTs) are a family of enzymes catalyzing the transfer of a methyl group from *S*-adenosyl methionine (SAM) donor to DNA. As one of the important epigenetic modifications, DNA methylation regulates expression of genes in normal development of mammalian cells. Dysregulation in DNA methylation is connected to various cancers. Thus, inhibition of DNMTs has been validated in the clinic as cancer therapy. Azacitidine and decitabine are two DNMTi approved by the US FDA for treatment of hematologic malignancies. Several investigational drugs are currently in various stages of clinical trials [147]. To overcome the limitation of DNMTi in treating solid tumors, the combination of a DNMTi with other anticancer agents or the development of dual targeting agents is urgently needed. As early as 2010, Borden et al [148] discovered that the combination of decitabine and LHB589 synergistically reduced the proliferation of five small cell lung cancer (SCLC) cell lines. In 2017, Baylin et al [149] reported that the combination of a DNMTi and an HDACi induced a potent antitumor response in NSCLC xenograft mice, and these results became the scientific support for clinical studies. Russo et al [150] reported that the combination of SGI-110 (a DNMTi) and entinostat exerted greater antitumor effects both in vitro and in vivo, and such combination might lead to potential treatment for triple-negative breast cancer (TNBC), a type of breast cancers with greater aggressiveness and higher metastasis. Lübbert et al [151] also reported that the combination of decitabine (a DNMTi) and panobinostat produced synergistic anti-proliferative effects against U937 cells. The scientific evidence above prompted the discoveries of several series of dual DNMTs and HDACs targeting agents. Compound **48** (Table 10) [152] moderately inhibits DNMT1 and DNMT3B, but potently inhibits HDAC1/6 enzymatically. In cellular assays, compound **48** shows greater anti-proliferative effects against K562 and U937 cells than its parent NSC319745. Similarly, compound **49** shows inhibitory

activities against both DNMT1 and HDAC1/6, and strong anti-proliferative effects against histiocytic lymphoma U937 cells (IC_{50} = 1.3 μM) [153]. Compound **50** exhibits moderate inhibitory activities against both DNMTs and HDAC1, and greater anti-proliferative effects against MCF-7, A549, and MDA-MB-231 cells compared to SAHA and SGI-1027 alone [154].

<insert Table 10>

2.2.3 Dual inhibitors of HDACs and G9a

Euchromatic histone-lysine *N*-methyltransferase 2 (EHMT2), also known as G9a, is a histone methyltransferase catalyzing the mono- and di-methylated states of histone H3 at lysine residue 9 (i.e., H3K9me1 and H3K9me2) and lysine residue 27 (H3K27me1 and HeK27me2). G9a is overexpressed in various cancers including leukemia, HCC, and pulmonary carcinoma [155,156]. Thus targeting G9a might be potential strategy for cancer therapy, which requires further clinical validation. Since both G9a and HDACs belong to epigenetic modulators, development of dual G9a-HDACs inhibitors might produce synergistic and beneficial effects in cancer therapy. Wang and co-workers [157-159] reported a series of dual G9a-HDACs inhibitors based on quinazolin scaffold. The representative compound **51** (Table 11) exhibits moderate inhibitory activities against G9a and HDAC in enzymatic assays, and moderate anti-proliferative effects against MDA-MB-231, MCF-7, A549, and HEK293 cells in cellular assays.

<insert Table 11>

2.2.4 Dual inhibitors of HDACs and LSD1

Lysine-specific demethylase 1 (LSD1), also known as KDM1, is the first identified protein lysine demethylases. Through a FAD-dependent oxidative reaction, LSD1 specifically removes histone H3K4me2 to H3K4me1 or H3K4me0. LSD1 is highly expressed in both hematological and solid tumors [160]. Thus, drug discovery targeting LSD1 for cancer therapy has been intense both in molecular biology and preclinical stages. Despite several compounds are currently being investigated in clinical trials, there is no approved drug targeting LSD1. Since both LSD1 and HDACs belong to epigenetic modulators, design a small molecule targeting both enzymes might produce synergistic and beneficial effects in cancer therapy. The dual targeting agent compound **52** (Table 12) shows not only stronger LSD1 inhibitory activity than its parent 2-PCPA (IC_{50} 1.2 vs 29.3 μM), but also better selectivity against MAO-A and MAO-B [161]. When tested head-to-head with SAHA, compound **52** shows comparable inhibitory activities against HDAC1/2, but greater anti-proliferative effects against several cancer cells. Recently Cole and co-workers [162] reported a series of dual inhibitors targeting LSD1 and HDACs components of CoREST complex. The representative compound **53** potently inhibits LSD1 and HDACs enzymatically, and produces anti-proliferative effects against cancer cells in cellular assays. Particularly, compound **53** shows excellent anti-proliferative effects against IC1 and MET1 cells, in a level significantly more potent than its parent entinostat alone, or the combination of entinostat and a LSD1 inhibitor. In the in vivo studies in human melanoma SK-MEL-5 cells xenograft mice, administration of compound **53** at 30 mg/kg dose (ip qd) for 28 days led to significant tumor volume inhibition (TVI: 61%) without toxicity signs. In contract, when entinostat was administrated to animals at the same dose and schedule for just one week, 60% of animals died indicating intolerable toxicity. Domatinostat (4SC-202) was originally synthesized as an HDACs inhibitor [163], and is currently in several clinical trials. Golas and co-

workers reported that the incubation of domatinostat with CRC (C-terminal REST/NRSF-CoREST-LSD1-HDAC1 transcriptional repressor complex) resulted in a significant decrease of the demethylation activity. These results suggest that domatinostat is a dual LSD1-HDACs inhibitor [164]. However, Cole and co-workers did not observe obvious LSD1 inhibition with domatinostat in their experiment [162]. To the best of our knowledge, it is questionable whether domatinostat is a dual LSD1-HDACs inhibitor or just an HDACs inhibitor.

<insert Table 12>

2.2.5 Dual inhibitors of HDACs and EZH2

Enhancer of zeste homolog 2 (EZH2) is a histone-lysine *N*-methyltransferase, an enzyme capable of catalyzing the addition of methyl groups to histone H3 at lysine 27 position (H3K27). EZH2 is overexpressed in a wide range of cancers including breast, prostate, bladder, uterine, renal cancers, melanoma, and lymphoma than in healthy cells. Thus, targeting EZH2 for cancer therapy appears to be an attractive strategy for pharmaceutical industries and academic institutes [171]. In March 2020, the first-in-class EZH2 inhibitor tazemetostat (EPZ-6438) was approved by the US FDA for the treatment of adults and adolescents aged ≥ 16 years with locally advanced or metastatic epithelioid sarcoma not eligible for complete resection [172]. Huntsman et al [173] reported that the combination of tazemetostat and quisinostat (a pan-HDACs inhibitor experimental drug) led to rapid induction of apoptosis and growth suppression of SCCOHT (small cell carcinoma of the ovary hypercalcemic type, a rare but extremely lethal malignancy ovarian cancer) cells. Synergistic effects against lymphomas using the combination of EZH2 inhibitor GSK126 and HDACi romidepsin were reported by Amengual and co-worker [174] in

both cellular assays and in vivo study. Recently Valente et al [175] reported the first-in-class dual EZH2-HDACs inhibitor. The representative compound **55** (Table 13) is an HDAC6-selective EZH2-HDACs dual inhibitor. In cellular assays, compound **55** exhibits moderate anti-proliferative effects against cells of various cancer types including leukemia, rhabdomyosarcoma, and glioblastoma. However, there is no experimental data in the article to support the synergistic effects of compound **55** *vs* the combination of two individual drugs.

<insert Table 13>

2.3 Dual inhibitors of HDACs and other enzymes

2.3.1 Dual inhibitors of HDACs and IDO

Indoleamine 2,3-dioxygenase 1 (IDO1) is a heme-containing enzyme physiologically overexpressed in a wide variety of human tumors. IDO1 catalyzes the first and rate-limiting step in the kynurenine pathway, the O_2-dependent oxidation of L-tryptophan to *N*-formylkynurenine. Increased IDO1 expression is associated with a poor prognosis in cancer patients. Several IDO1 inhibitors such as linrodostat (BMS-986205) and epacadostat (INCB024360) are currently in various stages of clinical investigation in the combination with other therapies [176]. The mono-therapy using an IDO inhibitor is known to have limited antitumor effects in both preclinical and clinical studies. To explore the potential of IDO1 inhibition, Sheng and co-workers [177] designed and synthesized the first generation of dual IDO-HDACs inhibitors from epacadostat and mocetinostat (a pan-HDAC inhibitor experimental drug). The representative compound **56** (Table 14) is a dual IDO1-HDACs inhibitor (IDO1 and HDAC1 IC_{50} values are 69 and 66.5 nM respectively) with no selectivity across HDACs subtypes. Compound **56** is orally bioavailable

(F% = 18%) indicated by the pharmacokinetic studies in SD rats at 100 mg/kg oral dose and 2 mg/kg iv dose. Administration of compound **56** at 100 mg/kg bid dosing for 14 days in LLC mouse model resulted in -56% of tumor growth inhibition. Other dual inhibitors **57** and **58** are less characterized in term of in vitro activities and pharmacology [178,179].

<insert Table 14>

2.3.2 Dual inhibitors of HDACs and Topoisomerases

DNA topoisomerases (Topoisomerases, or Topos) are enzymes that modulate DNA topology such as overwinding or underwinding. These enzymes play essential roles in controlling topological DNA problems during cell proliferation, differentiation, and survival. Several drugs targeting Topos have been approved for cancer therapy [180,181]. However, drugs targeting Topos have limitations, such as dose-limiting toxicity, drug resistance, and development of secondary malignancies. Searching for dual inhibitors against Topos and an additional target could potentially overcome the above limitations. Dual Topos-HDACs inhibitors published prior to 2015 have been reviewed [182], and the dual inhibitors published after 2015 will be covered in this article. Dallavalle et al reported two series of dual Topos-HDACs inhibitors based on camptothecin (CPT, a known Topos inhibitor natural product currently in various stages of clinical trials) scaffold, but with the different side chain of HDAC pharmacophore. Compound **59** (Table 15) [183] is the representative compound from the first series bearing a unique HDAC pharmacophore of psammaplin A, while compound **60** [184] is from the second series bearing SAHA pharmacophore. Both compounds **59** and **60** are potent dual Topos-HDACs inhibitors, with excellent inhibitory activities against HDACs and potent

anti-proliferative effects against a wide range of cancer cells. In in vivo studies, compound **59** shows strong antitumor effects in human mesothelioma primary cell line MM487 xenograft CD-1 nude mice with a very high safety profile. On the other hand, compound **60** shows excellent antitumor effects in MM473 xenograft CD-1 nude mice after 8 days treatment. Compound **61** [185] was derived from amsacrine (a Topo II inhibitor medicine) and SAHA. It shows excellent inhibitory activities against HDACs and potent anti-proliferative effects against various cancer cells. Derived from a known Topos inhibitor [186], compound **62** [187] shows good inhibitory activities against HDACs in enzymatic assays, and good anti-proliferative effects against several cancer cells in cellular testing. Compound **63** [188] derived from pyrozoloquinazoline scaffold and SAHA, shows moderate inhibitory activity against Topo I and good inhibitory activity against HDAC1. In cellular assays, compound **63** exhibits strong anti-proliferative effects against several cancer cells.

<insert Table 15>

2.3.3 Dual inhibitors of HDACs and PARP

Poly (ADP-ribose) polymerase (PARP) family is a 17-membered family of enzymes comprising a DNA-binding domain, a catalytic domain, and an auto-modification domain. These enzymes participate in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death. PARP inhibitors' ability in causing cancer cells death through "synthetic lethality" mechanism has been validated in the clinic, resulting in several approved drugs in this field for cancer therapy [189-191]. Olaparib (2014), rucaparib (2016), niraparib (2017), and talazoparib (2018) are four drugs approved by the US FDA for treatment of ovarian cancer of all

settings and breast cancer with BRCA1/2 mutation. Despite the success of PARPi as a mono-therapy, two major limitations remain: efficacy largely restricted to BRCA1/2-mutated tumors and the resistance developed over time [192,193]. Besides the combination strategy currently being assessed in preclinical and clinical stages [194], development of a single molecule inhibiting PARP and other cancer-relevant targets might potentially overcome the above limitations. The synergistic effects of combining a PARPi and HDACi have been well documented in the literature [195-198]. Those results lay foundation for development of dual PARP-HDACs inhibitors. There are two series of dual PARP-HDACs inhibitors reported to date, both derived from olaparib scaffold. Compound **64** (Table 16) [199] incorporates part of the linker and hydroxamic acid from panobinostat into olaparib, while compound **65** [200] adopts the HDAC pharmacophore from chidamide. Both compounds show potent inhibitory activities against PARP and HDACs in enzymatic assays, and moderate anti-proliferative effects against various cancer cells in cellular assays.

<insert Table 16>

2.3.4 Dual inhibitors of HDACs and NAMPT

Nicotinamide phosphoribosyltransferase (NAMPT), a member of glycosyltransferases family, is a rate-limiting enzyme in the nicotinamide adenine dinucleotide (NAD^+) salvage pathway that converts nicotinamide to nicotinamide mononucleotide in mammals to enable NAD^+ biosynthesis. As various tumor cells exhibit an increased reliance on NAD^+ production pathways, targeting NAMPT might be a potential cancer therapy. Despite promising preclinical results in animal models, several NAMPT inhibitors showed limited anticancer efficacy in early

phase clinical trials [201]. Dual NAMPT-HDACs inhibitors might potentially enhance the efficacy compared to NAMPT inhibitors alone. Through pharmacophore analysis and molecular docking experiment, Sheng et al [202] inferred that the known HDACi drug compound **66** (chidamide) (Table 17) could potentially inhibit NAMPT as well. They then confirmed chidamide's inhibitory activity against NAMPT via biological assay (IC_{50}: 2.1 μM). Compound **66** appears to be the first known dual NAMPT-HDACs inhibitor. The characterization of compound **66** in various cancer cells was documented in literature.

<insert Table 17>

2.3.5 Dual inhibitors of HDACs and MMPs

Matrix metallopeptidases (MMPs) are calcium-dependent zinc-containing endopeptidases not only capable of degrading all kinds of extracellular matrix proteins, but also having ability to process a number of bioactive molecules. Besides cleaving cell surface receptors, releasing apoptotic ligands, and inactivating chemokine/cytokine, MMPs also play a major role in cell behaviors such as cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, apoptosis, and host defense. For at least half century, MMPs have been heralded as promising targets for cancer therapy based on their molecular biology and their compelling preclinical study results [203,204]. Unfortunately, the clinical results are disappointing. Co-targeting an additional cell signaling pathway or enzyme or protein could potentially enhance the efficacy of MMPs inhibitors. Dual MMPs-HDACs inhibitors reported prior to 2015 have been reviewed by Jha et al [205]. Compound **67** (Table 18) [206] shows moderate inhibitory activities against MMP-2, MMP-9, and HDAC8 with good selectivity over other NMPs and HDACs

subtypes. Zhang et al [207] utilized virtual screening to identify four natural products, which are potential dual MMP-2- HDAC6 inhibitors. However, no further follow-up work has been reported.

<insert Table 18>

2.4 Dual inhibitors of HDACs and nuclear receptors

2.4.1 Dual inhibitors of HDACs and androgen receptor (AR)

Androgen receptor (AR) is a type of nuclear receptor activated by androgenic hormones, including testosterone and dihydrotestosterone (DHT) in the cytoplasm and then translocating into the nucleus. AR increases the cell survival and proliferation by DNA dependent and in-dependent mechanism. Due to its important role in male reproductive systems and homeostasis of bone and muscle, targeting AR through antagonist, agonist, or selective modulator has been validated as potential treatment for cancers, especially for prostate cancer [208]. Apalutamide (ARN-509) is a nonsteroidal antiandrogen (NSAA) medication which is used in the treatment of prostate cancer. Derived from SAHA pharmacophore and apalutamide, compound **68** (Table 19) is a dual AR- HDACs inhibitor. In the anti-proliferation assay against LNCaP cells, compound **68** shows greater activity than apalutamide (ARN-509) [209]. The dual AR-HDACs inhibitor might have potential treatment benefit for anti-cachectic therapy [210] or castration-resistant prostate cancer [211].

<insert Table 19>

2.4.2 Dual estrogen receptor (ER)-HDACs targeting agent

Estrogen receptors (ERs) are a group of proteins found inside cells that are activated by the hormone estrogen (17β-estradiol). Abnormal ERs signaling leads to the development of a variety of diseases, especially breast cancer [212]. The approval drug ERs antagonist tamoxifen can be used for treatment of half of ERs^+ breast cancer, but not ERs^- or triple-negative breast cancer (TNBC). In addition, another half of ER^+ breast cancer cases often develop the resistance to tamoxifen due to a down-regulation of ERα corepressors. The link between ERα corepressors and HDACs in tamoxifen-resistance breast cancer reveals that an HDACi could effectively reverse tamoxifen resistance, and could be a potential therapy for this specific type of breast cancers [213]. Derived from known ER antagonist [214], compound **69** (Table 20) was reported as a dual ERs-HDACs targeting agent with potent inhibitory activity against ERα, ERβ, and HDAC1 [215]. In cellular assays, compound **69** shows greater potency than its parent OBHS against both breast cancer MCF-7 cells and prostate cancer DU-145 cells.

<insert Table 20>

2.5 Miscellaneous

2.5.1 Dual inhibitors of HDACs and HSP90

HSP90 (heat shock protein 90) is a chaperone protein that assists other proteins to fold properly, stabilizes proteins against heat stress, and aids in protein degradation. The ability of HSP90 to stabilize a number of proteins required for tumor growth makes it an attractive drug target for potential anti-cancer therapy. Several papers reported that the combination of HDACi and HSP90 inhibitor could produce synergistic anti-cancer effects. These results provide the

foundation for rational design of dual inhibitors targeting HDACs and HSP90 simultaneously [216-220]. Derived from a known HSP90 inhibitor and SAHA, compound **70** (Table 21) [221] is a potent HSP90α-HDAC6 dual inhibitor. When tested in several cancer cells, compound **70** shows anti-proliferative effects at 1 μM concentration, better than SAHA alone. Importantly, compound **70** can reduce programmed death-ligand 1 (PD-L1) expression in IFN-γ treated H1975 lung cancer cells in a dose dependent manner. The result suggests that dual HDACs-HSP90 inhibitors have capability to modulate immunosuppression of tumor microenvironment, and might provide direction for future cancer treatment. Recently Rastelli et al [222] predicted compound **71** as a dual HSP90-HDAC6 inhibitor using an integrated ligand-based and structure-based virtual screening approach. The prediction was further verified through chemical synthesis and biological testing, and compound **71** was reported to have moderate inhibitory activity against both HDAC6 and HSP90.

<insert Table 21>

2.5.2 Dual inhibitors of HDACs and Tubulin

Microtubules, a major component of the eukaryotic cytoskeleton, are formed from the polymerization of α- and β-tubulins and function in many essential cellular processes, including mitosis. Tubulin-binding drugs kill cancerous cells by inhibiting microtubule dynamics, which are required for DNA segregation and therefore cell division [223]. There are two classes of tubulin-targeting drugs: depolymerization inhibitors such as paclitaxel and epothilone and polymerization inhibitors such as colchicine and combretastatins. The synergistic effects of combining an anti-tubulin agent with an HDACi have been well documented in the literature

[224-226]. A couple of papers disclosed dual tubulin-HDACs inhibitors prior to 2015. This paper will focus on those dual inhibitors published since 2015. Derived from colchicine and entinostat, compound **72** (Table 22) [227] shows powerful tubulin inhibitory activity and moderate HDACs inhibitory activity, and also exhibits superior cytotoxicity comparing to the positive control (IC_{50} 2 to 105 nM). Compound **73** [228] shows potent inhibitory activities against tubulin polymerization and HDAC8, and impressive anti-proliferative effects against various cancer cells. Its close analog **74** [229] appears to be HDAC6-selective dual inhibitor with weaker anti-tubulin polymerization activity (IC_{50} 54.5 μM). In cellular assays, compound **74** shows potent anti-proliferative effects against several cancer cells including A549, HeLa, and SGC-7901. Compound **75** [230,231] is also an HDAC6-selective dual tubulin-HDACs targeting agent. In addition to HDACs inhibitory activities, it induces acetylation of α-tubulin and inhibits tubulin polymerization. In cellular assays, compound **75** exhibits greater anti-proliferation potency against a variety of cancer cells than ricolinostat (a pan-HDAC inhibitor). Compound **76** [232], an analog by incorporating a thiol ester ZBG into the same scaffold, is a dual tubulin-HDACs targeting agent as compound **75**, but with weaker biological activities comparing to **75**. BPR0L075 [233], an indole analog bearing colchicine binding site, shows antimitotic activity against several cancer cells. Compound **77** [234,235] was derived from installing belinostat's side chain to the indole nitrogen of BPR0L075. Cell cycle analysis indicated that mitotic arrest was induced by compound **77** with enhanced expression of G2/M transition proteins. Molecular docking experiment supports compound **77** as a dual tubulin-HDAC6 inhibitor. Furthermore, compound **77** exhibits tumor growth inhibition in HL-60 and PC-3 xenograft mice upon administrating compound to animals via both oral and ip dosing.

<insert Table 22>

2.5.3 Dual inhibitors of HDACs and MDM2

Mouse double minute 2 homolog, also known as E3 ubiquitin-protein ligase MDM2, is an important negative regulator of the p53 tumor suppressor gene. It functions as both an E3 ubiquitin ligase that recognizes the N-terminal trans-activation domain (TAD) of the p53 tumor suppressor and an inhibitor of p53 transcriptional activation. Blocking the p53-MDM2 protein-protein interaction with small-molecule inhibitors can reactivate the function of p53 and is emerging as a promising strategy in cancer therapy [236,237]. Sheng et al [238] reported a series of dual MDM2-HDACs inhibitors derived from nutlin-3 and SAHA. The representative compound **78** (Table 23) not only potently inhibits MDM2 and HDACs enzymatically but also exhibits strong anti-proliferation effects against several cancer cells in cellular level. Pharmacokinetic studies indicate that compound **78** is orally bioavailable in SD rats (%F = 18%). In A549 xenograft mice, compound **78** effectively inhibits tumor growth with no significant weight loss and no adverse effects. In a Chinese patent filed in 2018 [239], several series of dual MDM2-HDACs inhibitors based on multi-substituted or polycyclic pyrrolidine cores were claimed. The representative compound **79** shows potent inhibitory activity against both MDM2 and HDACs. No further characterization is available.

<insert Table 23>

2.5.4 Dual inhibitors of HDACs and CRBN

Cereblon (CRBN) functions as a substrate receptor for the CRL4 ubiquitin ligase complex to target protein, and promotes ubiquitination and proteasomal degradation of CRBN substrates, such as IKZF1 and IKZF3. Immunomodulatory drugs (IMiD) such as lenalidomide are used to treat hematologic malignances primarily through targeting CRBN [240]. Tang et al [241,242] designed a series dual CRBN-HDACs targeting agents by incorporating HDACs pharmacophore to pomalidomide scaffold via a triazole linker. The representative compound **80** (Table 24) not only potently inhibits HDAC6 ($IC_{50} \leq 10$ nM) but also exhibits a promising degradation activity (DC_{50} = 1.6 nM) against IKZF (Ikaros family zinc finger protein), 3 to 5-fold improved than the previous reported dual targeting agent [243]. Compound **80** exhibits greater anti-proliferative effects against MM1S multiple myeloma cells than the combination of pomalidomide and nexturastat A at an 1 to 1 ratio, and the result further testifies the advantage of dual acting agents over single acting agents alone or their combination.

<insert Table 24>

2.5.5 Dual inhibitors of HDACs and proteasomes

Proteasomes are protein complexes capable of performing proteolysis (a chemical reaction that breaks peptide bonds) to selectively degrade unneeded or damaged proteins labelled with ubtiquitins. Proteasomes play a crucial role in the fate of proteins that are involved in almost all of the major cellular processes. Small molecules targeting proteasomes through inhibition can induce apoptosis in tumor cells, and have been validated as effective cancer therapy in animal models and clinical trials. Three approval drugs for treatment of multiple myeloma are bortezomib, carfilzomib, and ixazomib. The synergistic effects of the combination

of a proteasome inhibitor with an HDACi for the treatment of multiple myeloma were well documented [244], and might provide scientific rationale for design dual proteasome-HDAC inhibitors. Compound **81** [245] is a dual proteasome-HDACs inhibitor supported by biochemical and cellular assays (Table 25). The X-ray co-crystal structures of the 20S proteasome complexed with **81** and HDAC6 complexed with **81** were solved to further verify its dual acting functions.

<insert Table 25>

2.5.6 Dual agents targeting HDACs and NO-donor

Nitric oxide (NO) is a well-known gaseous molecule that plays important roles in mammalian physiology and pathophysiology. Besides its roles in the cardiovascular system for vascular homeostasis [246], in the central nervous system for learning and memory formation [247], and in skeletal muscles for modulating contraction excitation coupling [248], nitric oxide also exhibits interesting inhibitory activity against tumor cells proliferation [249], angiogenesis, and metastasis [250]. Hence, nitric oxide could potentially accelerate tumor cell apoptosis [251] and retard the development of drug resistance [252]. The inducible nitric oxide synthase (iNOS) is an enzyme capable of producing a heavy dose of cellular NO biologically, while a chemical NO donor (or a small molecule bearing the such) is identified or devised by scientists to release cellular NO chemically. Among those chemical NO donors are oxadiazole [253], sodium nitroprusside (SNP) [254], and oleanolic acid derivatives [255], all exhibit interesting cytotoxicity and antitumor activity against various tumor cells. Fruttero et al [256] reported the first example of dual NO donor-HDACs targeting agent **82** (Table 26) by attaching a NO releasing group onto entinostat. In molecular biology studies, compound **82** affects a number of micro-RNAs not modulated by entinostat or NO-releasing group alone, indicating the synergy

effects produced from both HDAC inhibition and NO release. Derived from phenylsulfonylfuroxan and SAHA, compound **83** [257] displays strong NO-releasing ability and potent inhibitory activities against HDACs. Mechanistic studies reveal that **83** can induce a much stronger apoptotic effect and G1 phase arrest in HEL cells than SAHA, a result contributed by both HDACs inhibition and NO release. Compound **84** [258], a close analog of **82** but with different NO-releasing group, might be severed as a therapeutic tool molecule for cardiovascular, neuromuscular, and inflammatory diseases as it possesses muscle differentiation function. The thio ester prodrug **85** [259] is found to release largazole (an HDACs inhibitor) and NO upon ester hydrolysis. In cellular assay, **85** shows greater anti-proliferative effects against U-2OS and IMR-32 cells than largazole alone, indicating additive effects contributed from NO release.

<insert Table 26>

3. Clinical trials of dual targeting agents containing HDACs component

Dual targeting agents containing HDACs component have been an emerging area in cancer therapy, with the number of publications increasing significantly and several candidates advancing to various stages of clinical trials. Among all the dual acting agents, Curis, Inc.'s fimepinostat (CUDC-907) (Table 27) is the most advanced one, targeting PI3K and HDACs simultaneously. In 2013, fimepinostat entered into Phase I trial to determine the maximal tolerated dose, the recommended Phase II dose (RP2D), and the preliminary anti-cancer activity as monotherapy in patients with relapsed or refractory lymphomas or multiple myeloma. Within 25 patients with relapsed/refractory diffuse large B-cell lymphoma (DLBCL) enrolled in this study, 9 objective responses (3 complete responses and 6 partial responses) were reported in 21-

response-evaluable patients. Further analysis of the tumors from 9 patients who achieved objective responses confirmed 5 of them had MYC oncogene alterations. Thereby, MYC oncogene alteration could be an important biomarker for patient stratification in the future clinical trials. The recommended dose of fimepinostat for further Phase 2 development was determined to be once-daily oral administration of 60 mg dose using a 5 days "on" and 2 days "off" schedule, in a 21-day cycle. The most common drug-related adverse events (AEs) reported in the study were low grade (Grade 1 and 2) diarrhea, fatigue and nausea. Dose limiting toxicities (DLTs) consisted of diarrhea and hyperglycemia, however no DLTs occurred at the RP2D. Other drug-related Grade 3 or Grade 4 AEs reported in 3 or more patients included thrombocytopenia and neutrophil decrease (hematologic AEs) as well as diarrhea, hyperglycemia and fatigue (non-hematologic AEs). In 2015, the US FDA granted fimepinostat orphan drug designation for the treatment of patients with DLBCL. In 2018, the US FDA granted Fast Track designation for the development of fimepinostat in adult patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL) after two or more lines of systemic therapy [260]. Recent results reported by Ge et al [261] and Okabe et al [262] suggested that fimepinostat would be an effective treatment for ABL TKI-resistant cells (including cells harboring the T315I mutation) as well as resistant prostate cancer cells.

CUDC-101 [263,264], an investigational drug also developed by Curis, Inc., is a multi-target inhibitor of HDACs, EGFR, and HER2. There are 4 Phase I clinical trials on the US clinical trial website, 3 of them being listed as completed, and one being listed as terminated. The termination is likely due to the safety issues. As of today, CUDC-101 is no longer existing as developmental candidate in Curis, Inc. company website.

Domatinostat (4SC-202) was originally synthesized as a pan-HDACs inhibitor [163], and later was reported to also possess the inhibitory activity against LSD1. In a Phase I study with 24 heavily pretreated patients with several types of advanced hematologic cancers, domatinostat was found to be well tolerated, with positive signs of anti-tumor efficacy observed (one complete remission (28 months) and one partial responder (8 months)). It is currently in four active clinical trials either alone or in combination with a PD-1, PDL-1, or CTLA-4 antibodies. The clinical trials designs with antibodies are supported by preclinical studies that domatinostat favors the immunotherapy response by modulating the tumor immune microenvironment [265]. In January 2019, the Germany-based company 4SC AG announced that domatinostat began Phase II gastrointestinal cancer clinical trial.

Tinostamustine is a dual acting agent targeting both DNA and HDACs. It is being developed by Mundipharma EDO GmbH Therapeutics L.P., a subsidiary of Purdue Pharma L.P., for the treatment of hematological malignancies, solid tumors, multiple myeloma, and glioblastoma. The preclinical studies not only demonstrated that tinostamustine could improve access to cancer cells' DNA strands, break them, and suppress damage repair, but also exhibited the potential to overcome resistance toward some other cancer treatments utilizing its complementary and simultaneous actions on both DNA and HDACs. In April 2019, the US FDA has granted orphan drug status to tinostamustine for the treatment of T-cell prolymphocytic leukemia (T-PLL), an aggressive type of leukemia with very limited treatment options.

<insert Table 27>

4. Future directions

The number of publications describing dual inhibitors containing HDACs component increases significantly in the past 5 years, which indicates that this area of research excites great interests for the scientific community. From the analysis above, one can realize that HDACs inhibitory activity might be obtained in a relatively easy way, through attachment of the HDACs pharmacophore to lead molecules (or drugs) via a suitable linker. The design and construction of such dual acting agents are feasible and manageable using medicinal chemistry tactics so as not to compromise the biological activity of lead molecules (or drugs) on the parent targets. The said biological targets pairing with HDACs could be as broad as one can imagine, namely from kinases to epigenetic targets to receptors to other enzymes and so on, based on the target function category. In many cases, the synergistic anti-tumor effects are clearly established with these dual inhibitors in enzymatic levels, and/or in cellular levels, and in some cases in *in vivo* animal models as well. Besides those biological targets described in this article, more and more additional ones are being discovered to have synergistic effects with HDACs. For example, Ramakrishnan et al [266] reported that the combination of MEK1/2 inhibitor selumetinib (an investigational drug from AstraZeneca) with panobinostat (a pan-HDACs inhibitor approved drug) demonstrates synergistic apoptosis in Ras/RAF mutated multiple myeloma cell lines. Such results could further spark the discovery of dual MEK-HDACs inhibitors in the near future. It should be noted that this article only intended to include cancer-related targets as dual acting agents. Other non-cancer-related targets such as PDE are reported to have synergistic effects with HDACs as well [267-274], but they are out of the scope of this article.

One of the biggest challenges in this field is to improve oral bioavailability of the dual acting agents. Among those described above, several compounds show poor oral bioavailability despite of the good ip bioavailability (i.e. compound **24** oral bioavailability <1%, ip

bioavailability >100%). This could be in part due to the nature of HDACs pharmacophore (i.e. hydroxamic acid), which has been documented in literature as labile metabolic functional group. However, some compounds are reported to have good oral bioavailability and they also bear similar HDACs pharmacophore. For example, the following compounds have good oral bioavailability: compounds **7** (73%), **8** (18%), **25** (18%), **26** (41%), **44** (40.5%), **56** (18%), **75** (47%), and **78** (18%). It's convinced that the oral bioavailability might be governed by many factors including the overall physical chemical properties of the entire molecule, rather than just by the nature of HDACs pharmacophore hydroxamic acid functional group. Such hurdle might be addressed through medicinal chemistry design and iterative optimization.

When designing dual inhibitors targeting HDACs and additional cancer-related target, one might also need to consider the subtype-selectivity for HDACs. Recent advances suggest that selectivity of certain HDAC subtypes such as HDAC6 might produce better safety profiles comparing to pan-HDACs inhibition [275,276].

Either HDACs component or its counterpart, or both might contribute to potential side effects for HDACs-containing dual targeting agents. This should be taken into consideration when designing dual inhibitors. Unfornately, there is no rule or enough knowledge that can help us effectively predict such side effects. Testing these dual targeting agents in an in vivo experiment might be the only solution. Within the literature we discuss above, the body weights of tested animals were assessed as one of criteria for side effects. For example, CDKs-HDACs dual inhibitor **8**, JAK-HDACs dual inhibitor **24**, BRD-HDAcs dual inhibitors **43** and **44** all showed no significant weight loss at their efficacious doses.

Despite of large number of publications for dual targeting agents, there are only 9 papers describing in vivo pharmacological effects. The rest papers focus only on in vitro activity. Due to the limited data reported, it is difficult to conclude that single agents inhibiting both HDACs and other targets have definitely pharmacological superiority than the combination of the two individual drugs. We anticipate that more data for such direct comparison will appear in publications in the future.

The advantages of dual targeting agents over the combination strategy are being recognized gradually by scientific community, and the dual targeting agents might become the future trend for cancer therapy. Since this approach involves multiple biological targets (HDACs and other), in vivo studies for selected compounds require comprehensive design, for example taking consideration of the in vitro activities of all targets involved. In some cases, exploration through rational experimental studies might be the only solution. The dual PI3K-HDACs inhibitor fimepinostat (CUDC-907) is the most advanced compound in this field, which is currently in various stages of clinical trials. It's hopeful that more and more dual targeting agents will be identified and advanced to clinical stage, and will ultimately lead to approval drugs from this category to combat cancers.

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Notes

The authors declare no competing financial interest.

Abbreviations

AML, acute myeloid leukemia; AR, androgen receptor; ATC, anaplastic thyroid cancer; Bcr, break point cluster; AUC, area under curve; BET, bromodomain and extra-terminal; bid, bis in die; CDKs, Cyclin-dependent kinases; CETSA, cellular thermal shift assay; CK2, casein kinase 2; CML, chronic myelogenous leukemia; CNS, central nervous system; CRBN, Cereblon; DLBCL, diffuse large B-cell lymphoma; DMAT, 2-dimethylamino-4,5,6,7-tetrabromo-benzimidazole; DNMTs, DNA methyltransferases; EGFR, epidermal growth factor receptor; EHMT2, Euchromatic histone-lysine N-methyltransferase 2; ERs, Estrogen receptors; EZH2, Enhancer of zeste homolog 2; FGFRs, fibroblast growth factor receptors; FLC, fluconazole; GFR, growth factor receptor; HATs, histone acetylases; HCC, hepatocellular carcinoma; HDACi, histone deacetylase inhibitor; HDACs, histone deacetylases; HGFR, hepatocyte growth factor receptor; HSP90, heat shock protein 90; HEL, human erythroid leukemia; IDO1, indoleamine 2,3-dioxygenase 1; IMiD, immunomodulatory drugs; iNOS, inducible nitric oxide synthase; ip, intraperitoneal; isoCA-4, iso-Combretastatin A-4; ITK, interleukin-2-inducible T-cell kinase; ITP, Inhibition of Tubulin Polymerization; JAKs, Janus kinases; LIFR, leukemia inhibitory factor receptor; LOF, loss of function; LSD1, Lysine-specific demethylase 1; MAPK, mitogen-activated protein kinase; MDM2, Mouse double minute 2 homolog; MMPs, matrix metallopeptidases; NAMPT, nicotinamide phosphoribosyltransferase; NO, Nitric oxide; NOD/SCID, nonobese diabetic/severe combined immunodeficiency; NSCLC, non-small cell lung cancer; p.o., per os; PARP, Poly (ADP-ribose) polymerase; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft; PDE, phosphodiesterase; PI3K, phosphatidylinositol-3-kinase; qd, once daily; Raf, rapidly accelerated fibrosarcoma; SAM, S-adenosyl methionine;

SCCOHT, small cell carcinoma of the ovary, hypercalcemic type; SCLC, small cell lung cancer; SNP, sodium nitroprusside; STAT, signal transducers and activators of transcription; SyK, Spleen tyrosine kinase; TAD, trans-activation domain; TAMH, transforming growth factor-α mouse hepatocytes; TBB, tetrabromobenzotriazole; TGI, tumor growth inhibition; TNBC, triple negative breast cancer; TVI, tumor volume inhibition; VEGFRs, vascular endothelial growth factors; ZBG, zinc-binding group

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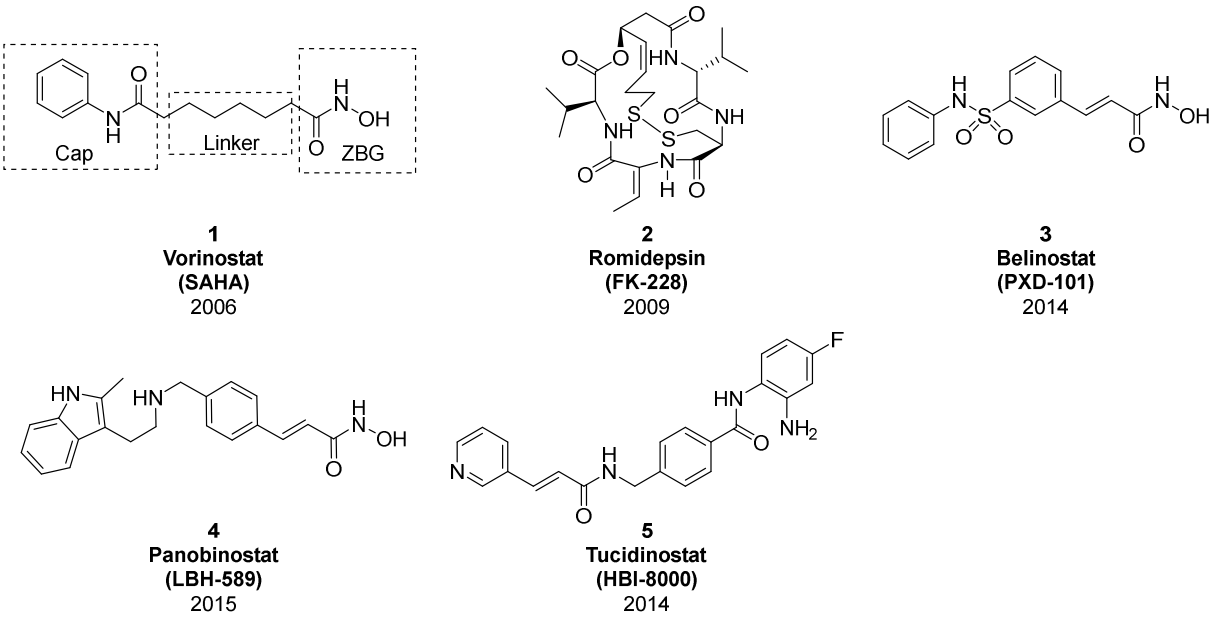


Figure 1. The approved anticancer drugs targeting HDACs.

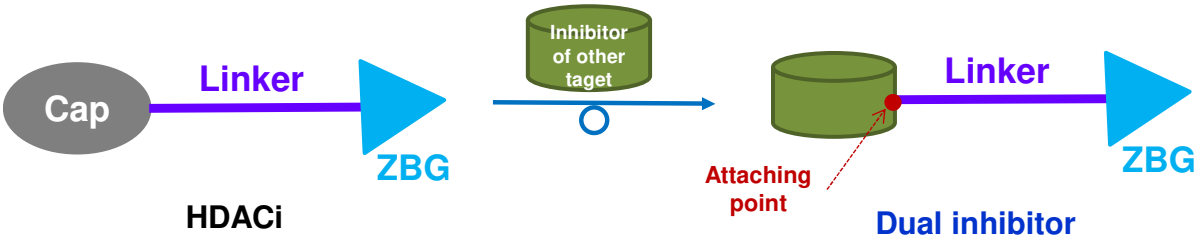
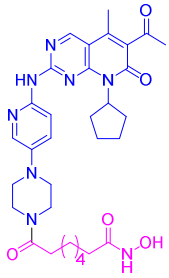
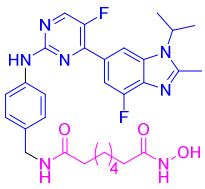
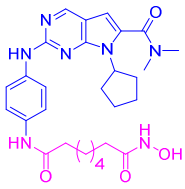
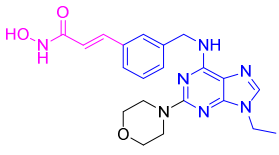
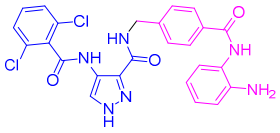


Figure 2. Conceptual formation of a dual inhibitor from a HDACi.

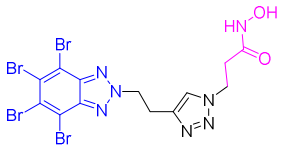
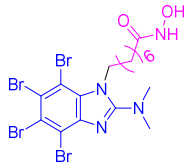
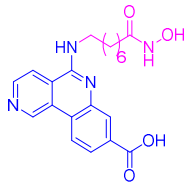
Table1. Dual inhibitors of HDACs and CDKs.

Structure	Derived from	IC_{50} (nM): enzymatic and cellular activities	PK	In vivo	Ref.
 **6**	palbociclib and SAHA	CDK4 (<10); CDK6 (<25); HDAC1 (<50); HDAC6 (<10)	NA	NA	[41]
 **7**	abemaciclib and SAHA	CDK4 (1.2); HDAC1 (26); Anti-proliferation: 4T1 (18.3); MDA-MB-468 (32.3); MDA-MB-231 (23.9); SK-OV-3 (44); OVCAR-5 (15.8); H460 (32.8)	SD rats (10 mg/kg), p.o. %F = 73%;	In breast cancer homograft and xenograft mice efficacious	[42]
 **8**	ribociclib and SAHA	CDK4 (8.8); HDAC1 (2.2); Anti-proliferation: 4T1 (1110); MDA-MB-468 (1820); MDA-MB-231 (1860); T47D (2590); A549 (1330); H460 (3780); H1299 (1870); Hep G2 (3370); Hep 3B (1240)	rats (20 mg/kg), p.o. %F = 18.4%; ip %F = 34.7%	In 4T1 xenograft mice, 90 mg/kg qd ip for 25 days efficacious	[43,44]
 **9**	roscovitine and panobinostat	CDK2 (56); HDAC1 (5.8); Anti-proliferation: Hep G2 (770); CAL-148 (1140); A549 (1490)	NA	NA	[45]

 **10**	AT7519 [47] and tucidinostat	CDK1 (8.63); CDK2 (0.3); CDK4,6,7 (>1000); HDAC1 (6.4); HDAC2 (0.25); HDAC3 (45); HDAC6,8 (>1000); Anti-proliferation: HCT-116 (710); A375 (1200); Hela (1830); H460 (4190); SMMC7721 (7760)	ICR mice (20 mg/kg), ip %F = 63.6%	In HCT-116 xenograft mice, 25 mg/kg qd ip for 21 days efficacious, TGI: 51%	[46]

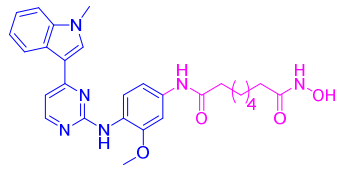
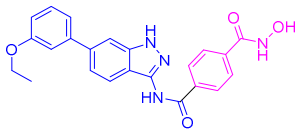
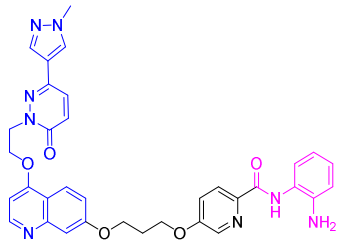
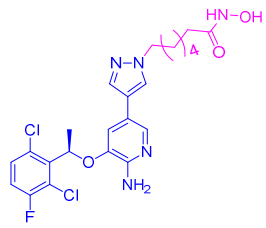
NA: not available.

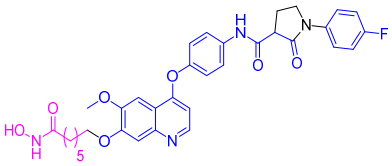
Table 2. Dual inhibitors of HDACs and CK2.

Structure	Derived from	IC_{50} (nM): enzymatic and cellular activities	PK	In vivo	Ref.
 **11**	TBB and hydroxamic acid	CK2□ (62% @50000); HDAC1 (2200); Anti-proliferation: Jurkat (2870); MCF-7 (4260); L1210 (13090); 293T (14550); HaCat (13660)	NA	NA	[50]
 **12**	DMAT and SAHA	CK2 (5890); HDAC1 (13700); HDAC6 (8980); Anti-proliferation: Jurkat (5300); MCF-7 (9020); HCT-116 (3100); HEK293 (8410); HL-60 (4690); HL-60/adr (8400); HL-60/vinc (2320)	NA	NA	[51]
 **13**	silmitasertib and SAHA	CK2 (1.7); HDAC1 (3.3); HDAC6 (13); Anti-proliferation: LNCaP (16310); PC-3 (40420); MCF-7 (52480); A549 (104730)	NA	NA	[52]

NA: not available.

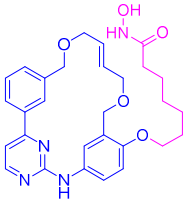
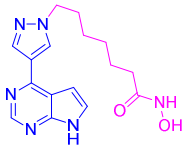
Table 3. Dual inhibitors of HDACs and RTKs.

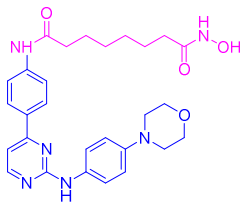
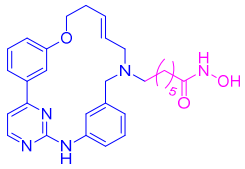
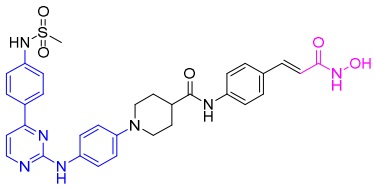
Structure	Derived from	IC_{50} (nM): enzymatic and cellular activities	PK	In vivo	Ref.
 **14**	osimertinib and SAHA	$EGFR^{WT}$ (5700); $EGFR^{T790M}$ (5000); HDAC (85); Anti-proliferation: A549 (2190); HeLa (1850); MDA-MB-231 (600); MDA-MB-468 (230); HT-29 (790); KG-1 (240); PC-3 (3390)	NA	NA	[59]
 **15**	Indazole scaffold and nexturastat A	FGFR1 (64% @1000); HDAC1 (59% @1000); HDAC6 (34); HDAC8 (20% @1000); Anti-proliferation: MCF-7 (9000)	NA	NA	[60]
 **16**	c-Met inhibitor and tucidinostat	c-Met (0.71); HDAC1 (38); Anti-proliferation: EBC-1 (58); HCT-116 (1300)	NA	NA	[65]
 **17**	crizotinib and SAHA	c-Met (31.6); HDAC1 (82); HDAC2 (181.7)	NA	NA	[66]

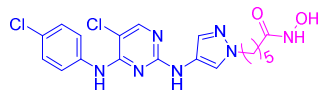
 **18**	foretinib and tucidinostat	c-Met (12.5); HDAC1 (27); Anti-proliferation: HCT-116 (540); MCF-7 (280); A549 (1080)	NA	NA	[67]

NA: not available.

Table 4. Dual inhibitors of HDACs and JAKs.

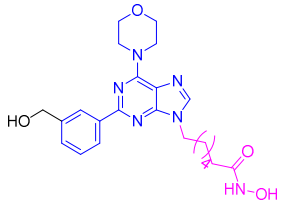
Structure	Derived from	IC_{50} (nM): enzymatic and cellular activities	PK	In vivo	Ref.
 **19**	pacritnib and SAHA	JAK2 (1.4); HDAC1 (222); HDAC2 (49); HDAC6 (2.1); HDAC8 (740); HDAC10 (80); HDAC11 (930); Anti-proliferation: MDA-MB-231 (1430); HCT-116 (2230); PC-3 (1700); MCF-7 (1470); HEL92.1.7 (940); Jurkat (1190); KMS-12-BM (2110); OPM-2 (2050); KG-1 (1630); MOLM-14 (1140); NKYS (1080); KHYG (1090)	NA	NA	[76,77]
 **20**	ruxolitinib and SAHA	JAK1 (17); JAK2 (75); JAK3 (569); TYK2 (188); HDAC1 (6.9); HDAC2 (5.8); HDAC3 (3.9); HDAC6 (1.4); HDAC10 (19); HDAC11 (31); Anti-proliferation: MDA-MB-231 (790); MCF-7 (840); HCT-116 (2320); PC-3 (2410); KMS-12-BM (2020); XG-6 (2010); MOLM-14 (740); MV4-11	Wistar rats (5 mg/kg) p.o. %F = 1.4%	NA	[78]

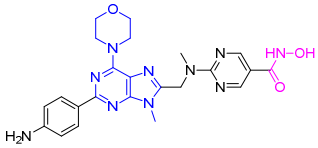
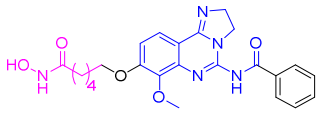
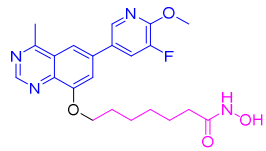
		(460); NKYS (450); HL-60 (7360); HEL92.1.7 (1330); Jurkat (470); OPM-2 (2160); KG-1 (150); KHYG (570)			
 **21**	XL019 and SAHA	JAK1 (52.1); JAK2 (3.1); JAK3 (80.1); TYK2 (79.4); HDAC1 (56); HDAC6 (1.2); Anti-proliferation: HCT-116 (1050); MDA-MB-231 (990); MCF-7 (700); PC-3 (640)	NA	NA	[79]
 **22**	zotiraciclib (SB1317) and SAHA	JAK2 (686); FLT3 (87); HDAC (87); Anti-proliferation: HL-60 (1030); MV4-11 (270); K562 (1020); HEL (340); HCT-116 (820); MCF-7 (950); MDA-MB-435 (1770); NCI-H460 (1070); Ovcar-5 (1220)	NA	NA	[81]
 **23**	momelotinib (CYT-387) and SAHA	JAK1 (359); JAK2 (8.4); JAK3 (121); TYK2 (46); HDAC1 (1100); HDAC2 (7472); HDAC3 (234); HDAC6 (46); Anti-proliferation: HEL (340); HL-60 (1500); K562 (8700)	SD rats (10 mg/kg), ip: $T_{1/2}$ = 5.14 h; C_{max} = 1603 ng/mL; AUC = 4470 h*ng/mL	In HEL AML xenograft mice model, 10 mg/kg qd ip for 21 days efficacious	[82]

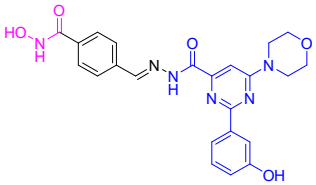
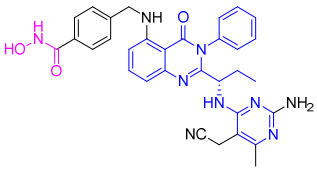
 **24**	known JAK inhibitor [77] and SAHA	JAK1 (4.8); JAK2 (4); JAK3 (7.4); TYK2 (49); HDAC2 (120); HDAC6 (14); HDAC8 (2470); Anti-proliferation: HEL (90); K562 (490); MOLT4 (80); Jurkat (60)	SD rats p.o. (10 mg/kg) %F <1%; ip (1 mg/kg) %F = 116%	In HEL AML xenograft mice model, 100 mg/kg ip for 16 days moderate efficacious, TGI: 36%	[83]

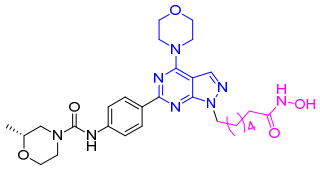
NA: not available.

Table 5. Dual inhibitors of HDACs and PI3Ks (and/or mTOR).

Structure	Derived from	IC_{50} (nM): enzymatic and cellular activities	PK	In vivo	Ref.
 **25**	pyrimidine scaffold and SAHA	PI3Kα (28); PI3Kβ (212); PI3Kγ (177); PI3Kδ (37); mTOR (1946); PI3K2β (103); HDAC1 (1.1); HDAC2 (6.0); HADC3 (1.1); HADC4 (4591); HDAC5 (4800); HADC6 (4.2); HDAC7 (2305); HDAC8 (320); HDAC9 (1282); HDAC10 (2.5); HDAC11 (9700); Anti-proliferation: K562 (350); MOLT4 (140); MV4-11 (47); PC-3 (1080); Raji (240); Ramos (400); SU-DHL-6 (190); Hep 3B (1250); Hep G2 (590); HuH-7 (480)	Rats: p.o. (50 mg/kg) % F = 5.8%, $T_{1/2}$ = 2.01 h; p.o. (150 mg/kg) % F = 18.1%, $T_{1/2}$ = 4.41 h;	In Hep G2 xenograft mice: 150 mg/kg, TGI: 91% (14 days); TGI: 95% (18 days), survivors: 6/6 (32 days). In Hep 3B xenograft mice: 100 mg/kg, TGI: 82% (21 days); TGI: 86% (23 days), survivors: 7/7 (23 days). In HuH-7 xenograft mice: 150 mg/kg, TGI: 78% (12 days), survivors: 5/5 (22 days).	[103]

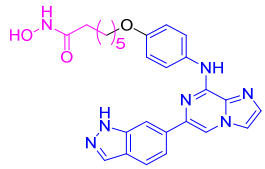
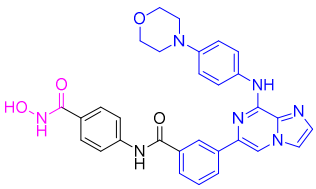
 **26**	pyrimidine scaffold and hydroxamic acid	PI3Kα (>1000); HDAC1 (0.7); HDAC2 (2.9); HDAC3 (3.4); HDAC6 (4.7); HDAC8 (24); HDAC10 (2.8); Anti-proliferation: MV4-11 (0.15); A2780s (7); HCT-116 (0.7)	doge (6 mg/kg) PK: %F = 41.8%	In MV4-11 xenograft mice, 10 mg/kg q2dx11 iv, TGI: 65%. In HCT-116 xenograft mice, 10 mg/kg q2dx8 iv, TGI: 68%. In Ramos xenograft mice, 10 mg/kg q2dx6 iv, TGI: 83%. In MM1S xenograft mice, 10 mg/kg q2dx6 iv, TGI: 75%.	[104]
 **27**	copanlisib and SAHA	PI3Kα (3); PI3Kβ (14); PI3Kγ (30); PI3Kδ (4.5); HDAC1 (50); HDAC6 (62); HDAC8 (153); Anti-proliferation: HCT-116 (330); K562 (95); Hut98 (62)	NA	NA	[105]
 **28**	quinazoline scaffold [107] and SAHA	PI3Kα (42); PI3Kβ (101); PI3Kγ (67); PI3Kδ (8.1); mTOR (2861); HDAC1 (1.4); HDAC2 (3.0); HDAC4 (>1000); HDAC6 (6.6); HDAC8 (18); HDAC11 (>1000); Anti-proliferation:	Mice: p.o. (30 mg/kg), % F = 4.2%, $T_{1/2}$ = 2.07 h;	In HCT-116 xenograft mice: p.o. (100 mpk), TGI: 18.3%; (150 mpk), TGI: 45.8%. In HGC-27 xenograft mice: p.o. (100 mpk), TGI: 15.2%; p.o. (200 mpk), TGI: 45.9%; ip	[106]

		THP-1 (5400); K562 (6800); MCF-7 (2000); MDA-MB-453 (300); HCT-8 (320); HCT-116 (150); Capan 2 (430); SW1990 (900); DU145 (540); HGC-27 (110); Hep G2 (1100)		(30 mpk), TGI: 62.6%	
 **29**	PI-103 and LASSBio-1911	PI3Kα (46.3); PI3Kβ (72.8); PI3Kγ (1300); PI3Kδ (72.4); mTOR (464); HDAC1,4,11 (>3000); HDAC6 (15.3); HDAC8 (67.6); CYP3A4 (3.6)	NA	NA	[111]
 **30**	idelalisib and hydroxamic acid	PI3Kα (47); PI3Kγ (9); PI3Kδ □□□;□ HDAC6 (12); Anti-proliferation: CCRF-CEM (700); SR (700); HOP-92 (900); SNB-75 (700); IGROV1 (1500); A498 (1000); CAKI-1 (1300); RXF 393 (1200); U266 (1900); MV4-11 (2500); U937 (2600); K562 (4600)	Female Balb/c mice: p.o. (10 mg/kg), %F = 0.7%, $T_{1/2}$ =1.4 h; ip (50 mg/kg and 150 mg/kg), $T_{1/2}$ =2.7 h and 1.7 h (the 24-hour data point was excluded for $T_{1/2}$ calculated), %F not calculated	NA	[113]

 **31**	mTOR inhibitor and SAHA	PI3Kα (57.3% @1000, 28.3% @100); mTOR (1.2); HDAC1 (0.19); HDAC2 (0.61); HDAC3 (1.47); HDAC6 (1.8); HDAC8 (1.28); HDAC10 (0.58); HDAC4,5,7,9,11 (>1000); Anti-proliferation: HCT-116 (17.2); Raji (1.9); MM1S (7.3); OCI-AML2 (9.01); OCI-AML3 (9.98); MV4-11 (4050)	NA	In MV4-11 xenograft NOD/SCID mouse model, 10 mg/kg q2d×6 iv, TGI: 53.1%, survivors: 6/6. In MM1S xenograft model, 10 mg/kg q2d×5 iv, TGI: 48.1%, survivors: 6/6; 20 mg/kg q2d×5 iv, TGI: 72.5%, survivors: 6/6.	[115]

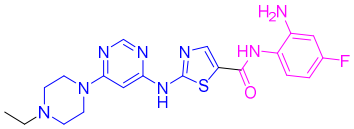
NA: not available.

Table 6. Dual inhibitors of HDACs and Syk.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **32**	entospletinib and SAHA	Syk (<20); HDAC1 (<10); HDAC6 (<10)	NA	NA	[116]
 **33**	entospletinib and hydroxamic acid	Syk (<100); HDAC1 (<200)	NA	NA	[117]

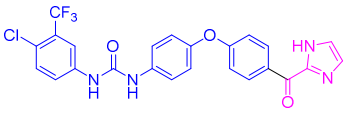
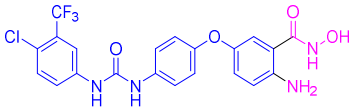
NA: not available.

Table 7. Dual inhibitors of HDACs and Bcr-Abl.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **34**	dasatinib and MS-275	Bcr-Abl (17.8); HDAC1 (810); Anti-proliferation: K562 (2620); Hep G2 (10440); DU145 (600)	NA	NA	[118]

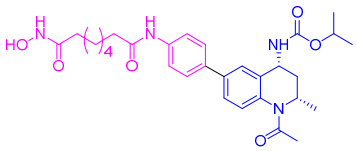
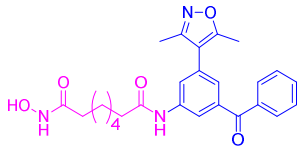
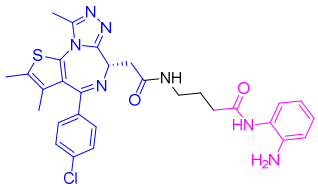
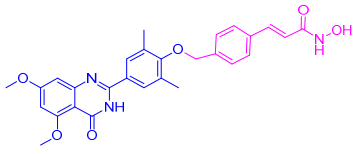
NA: not available.

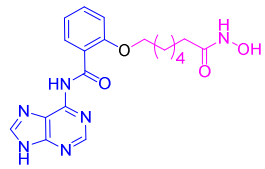
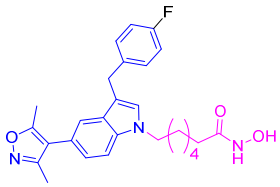
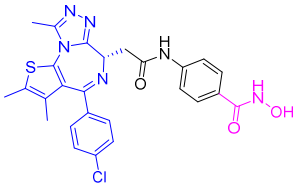
Table 8. Dual inhibitors of HDACs and Raf.

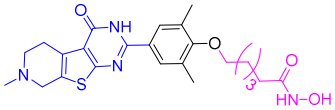
Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **35**	sorafenib and immidazole	ARaf (45); $BRaf^{V600E}$ (86); CRaf (102); HDAC1 (1710); Anti-proliferation: A549 (9110); SK-Mel-2 (5400); MV4-11 (380); K562 (9130)	NA	NA	[119]
 **36**	sorafenib and amide	ARaf (183); $BRaf^{V600E}$ (73); CRaf (92); HDAC1 (1170); Anti-proliferation: K562 (2730); MV4-11 (1120); Hep G2 (1330); MDA-MB-468 (570)	NA	NA	[120]

NA: not available.

Table 9. Dual inhibitors of HDACs and BET.

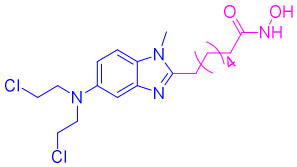
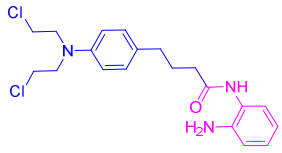
Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **37**	I-BET295 and SAHA	BRD4 (50); HDAC1 (250); HDAC6 (420); HDAC7 (14130); HDAC9 (34890); Anti-proliferation: HL-60 (764); MV4-11 (334)	NA	NA	[134]
 **38**	dimethylisoxazole skeleton and SAHA	BRD4 (670); HDAC1 (150); Anti-proliferation: K562 (1860); MV4-11 (910)	NA	NA	[135]
 **39**	(+)-JQ1 and entinostat	BRD4 (29.6); HDAC (31.2); Anti-proliferation: U937 (84% @3000)	NA	NA	[136]
 **40**	RVX-208 and panobinostat	BRD4/BD1 (>5000); BRD4/BD2 (401); HDAC1 (204); Anti-proliferation: MV4-11 (560); OCI-AML2 (380); OCI-AML3 (430)	NA	NA	[137]

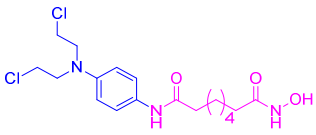
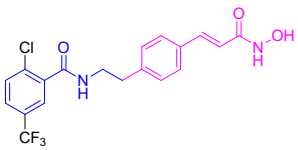
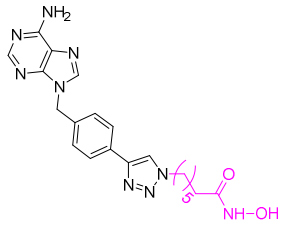
 **41**	known BET inhibitor [139] and SAHA	BRD4 (2700); HDAC (260); Anti-proliferation: HL-60 (4400); K562 (7100); MCF-7 (34000); MDA-MB-231 (10000); T-47D (2300); A2780 (14000); OVCAR-5 (18000)	NA	NA	[138]
 **42**	P-0014 and SAHA	BRD4 (28% @1000, 88% @10000); HDACs (291); HDAC1 (181); HDAC2 (298); HDAC3 (5); HDAC6 (>1000); Anti-proliferation: THP-1 (15500)[a]	NA	NA	[140]
 **43**	(+)-JQ1, tacedinaline and SAHA	BRD2/BD1 (316); BRD2/BD2 (78); BRD3/BD1 (133); BRD3/BD2 (38); BRD4/BD1 (11); BRD4/BD2 (69); BRDT/BD1 (254); BRDT/BD2 (302); BRD1 (32200); HDAC1 (21); HDAC2 (52); HDAC3 (39); HDAC6 (34); HDAC8 (192); Capan-1 (150)	NA	In capan-1 xenograft mice, 15 mg/kg bid×21 ip, TGI: 69%; 20 mg/kg, bid×21 ip, TGI: 87.7%.	[141]

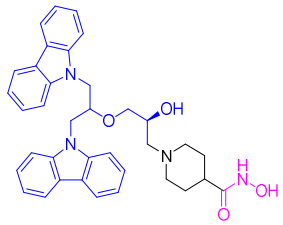
 **44**	known BRAD4 inhibitor and SAHA	BRD2/BD1 (16400); BRD2/BD2, BRD3/BD1, BRD3/BD2, BRDT/BD1 (>20000); BRD4/BD1 (1100); BRD4/BD2 (2050); BRD4 (710); HDAC1 (46); HDAC2 (58); HDAC3 (75); HDAC6 (73); HDAC8 (167); HDAC10 (923); HDAC4,5,7,9 (>10000); Anti-proliferation: HCT-116 (450); SW620 (1780); DLD1 (2110)	SD rats, 30 mg/kg, p.o., %F = 40.5%, $T_{1/2}$ = 4.8 h	In HCT-116 xenograft mice model, 15 mg/kg and 30 mg/kg, p.o., 17 days, TGI: 42.7% and 68.8%	[142]

[a]GI_{50} the concentration that results in inhibiting cell growth by 50%. NA: not available.

Table 10. Dual inhibitors of HDACs and DNA and DNMT.

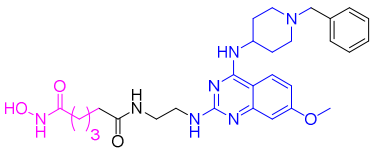
Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **45** (tinostamustine)	bendamustine and SAHA	HDAC1 (17); HDAC2 (10); HDAC3 (25); HDAC4 (6.4); HDAC5 (107); HDAC6 (72); Anti-proliferation: NCI60 (2200)[a]; MEFS (5700)[a]; CLL cell (≈100% @5000)	NA	In Bcr-Abl cells transplanted mice, 300 mg/kg, average survival extension of 14 days. In human lung cancer H460 xenograft mice. 20, 40 and 60 mg/kg, showed potent anticancer activity	[144]
 **46**	chlorambucil and tacedinaline	DNA damage (159% @8000, 213.3% @16000); HDAC1,6 (>160000); HDAC2 (32900); HDAC3 (9520); HDAC8 (75200); Anti-proliferation: A549 (6200); A375 (6100); SMMC7721 (4000); Hep G2 (14200); H1299 (5500); H460 (3100)	NA	NA	[145]

 **47**	chlorambucil and SAHA	DNA damage (224.1% @16000); HDAC1 (270); HDAC2 (240); HDAC6 (620); Anti-proliferation: Hep G2 (6100); A549 (4400); HCT-116 (3200); A375 (6200)	NA	NA	[146]
 **48**	NSC319745 and panobinostat	DNMT1 (70% @100 □□□); DNMT3B (48.5% @100000); HDAC1 (56.8); HDAC6 (17.4); Anti-proliferation: K562 (2850); U937 (1060)	NA	NA	[152]
 **49**	5-azacytidine /decitabine and SAHA	DNMT1 (44.48% @50000); HDAC1 (2.4); HDAC6 (4.8); Anti-proliferation: U937 (1300)	NA	NA	[153]

 **50**	DC-517 and SAHA	DNMT1 (2020); DNMT3A/3L (930); DNMT3B/3L (1320); HDAC1 (4160); HDAC6 (>100000); Anti-proliferation: MCF-7 (1880); A549 (3920); MDA-MB-231 (4650)	NA	In 4T1 breast tumor xenograft mice: 5 and 15 mg/kg qd administration significantly reduced tumor volume and mass models	[154]

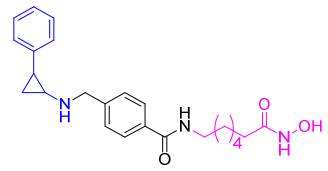
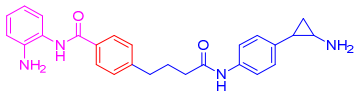
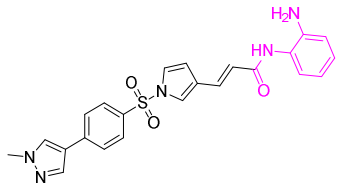
[a]GI_{50} the concentration that results in inhibiting cell growth by 50%. NA: not available.

Table 11. Dual inhibitors of HDACs and G9a.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **51**	BIX01294 and SAHA	G9a (7136); HDAC (Hela: 15330); HDAC (K562: 5735); Anti-proliferation: MDA-MB-231 (10020); MCF-7 (37360); A549 (36240); HEK293 (19950)	NA	NA	[157-159]

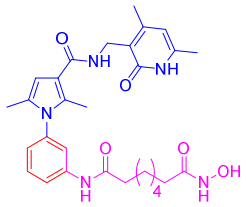
NA: not available.

Table 12. Dual inhibitors of HDACs and LSD1.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **52**	2-PCPA and SAHA	LSD1 (1200); MAO-A (21110); MAO-B (87090); HDAC1 (15); HDAC2 (23); HDAC5 (16840); Anti-proliferation: MGC-803 (810); MCF-7 (4280); SW-620 (2350); A549 (1340); PC-3 (5480)	NA	NA	[161]
 **53**	tranylcypromine analog and entinostat	HDAC1 (147); CoREST complex: LSD1 (330); CoREST complex: HDAC1 (206); Anti-proliferation: IC1 (41); MET1 (6); WM983B (~200); SK-MEL-5 (~130)	NA	In SK-MEL-5 melanoma xenograft mice, 30 mg/kg qd ip, 28 days, 61% reduction in tumor volume relative to vehicle	[162]
 **54** (domatinostat)	know LSD1 inhibitor and HDAC pharmacophore	Anti-proliferation: various urothelial cancer cells (150 -510); active in the following cells: HCC cells; lymphoma cells; central nervous system atypical teratoid/rhabdoid tumor cells	NA	In HT-29 xenograft nude mice, active. Antitumor effects when combined with metformin in 4NQO-induced mice OSCC model.	[165-170]

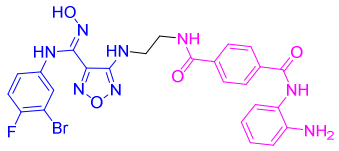
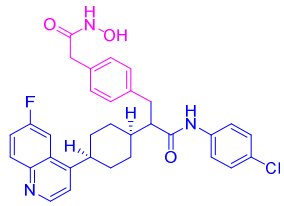
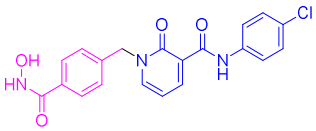
NA: not available.

Table 13. Dual inhibitors of HDACs and EZH2.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **55**	known EZH2 inhibitor and SAHA	EZH2/PRC2 (7370); HDAC1 (430); HDAC2 (1330); HDAC3 (450); HDAC6 (5); HDAC8 (110); Anti-proliferation: U937 (9000); RH4 (13000); THP1 (12000); SH-N-SK (25000); U87 (20000)	NA	NA	[175]

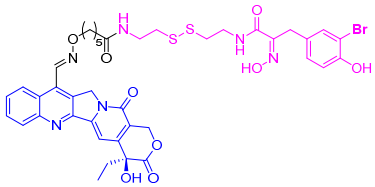
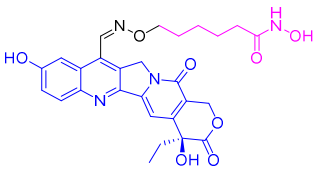
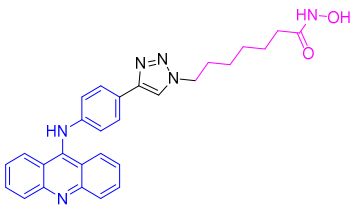
NA: not available.

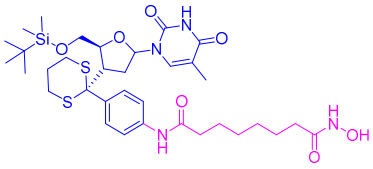
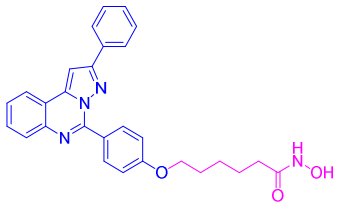
Table 14. Dual inhibitors of HDACs and IDO1.

Structure	Derived from	IC_{50} (nM) Enzymatic cellular	PK	In vivo	Ref.
 **56**	epacadostat and mocetinostat	IDO1 (69); HDAC1 (66.5); HDAC2 (179); HDAC3 (45); HDAC6 (70). Anti-proliferation: LLC (17620); CT26 (59840); A549 (16730); HCT-116 (5120); HT-29 (11710)	SD rats p.o. (100 mg/kg) %F = 18%	In LLC xenograft mice, 100 mg/kg bid×14, TGI: 56%	[177]
 **57**	linrodostat and HDAC pharmacophore	IDO1 in HEK293T (>100); Anti-proliferation: Hela (>100)	NA	NA	[178]
 **58**	-- and HDAC pharmacophore	IDO1 (1000~5000); HDAC1 (<1000); HDAC6 (<1000); Anti-proliferation: Hela (<1000)	NA	NA	[179]

NA: not available.

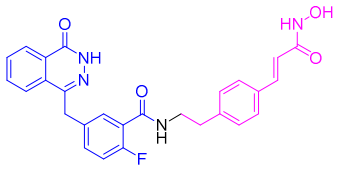
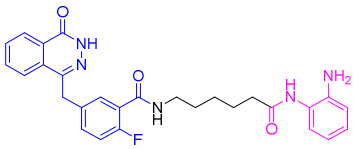
Table 15. Dual inhibitors of HDACs and Topoisomerases.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **59**	camptothecin (CPT) and psammaplin A	HDAC1 (150); HDAC2 (327); HDAC6 (709); HDAC10 (7.55); Anti-proliferation: MINO (35); MAVER-2 (300); JECO-1 (60); NB4 (47); CAPAN1 (50); MM487 (130); U-2932 (110); OCI-LY3 (100); DU145 (110)	NA	In MM473 xenograft CD-1 nude mice model, 90 mg/kg iv, 4 days, TVI: 78%.	[183]
 **60**	CPT and SAHA	HDAC1 (52.6); HDAC2 (93.3); HDAC6 (1.56); HDAC8 (224); Anti-proliferation: Capan1 (35); DU145 (70); MINO (31); MAVER-2 (12); JECO-1 (23); U-2932 (44); RAJI (77); Z-138 (6); MM472 (48)	NA	In human mesothelioma primary cell line MM473-Luc orthotopically xenografted in CD-1 nude mice, 45 mg/kg iv, 8 days, the tumor has largely disappeared	[184]
 **61**	amsacrine (m-AMSA) and SAHA	HDAC1 (3.9); HDAC6 (2.9); Anti-proliferation: U937 (900); MDA-MB-231 (7090); HCT-116 (2110)	NA	NA	[185]

 **62**	known Topo inhibitor [186] and SAHA	HDAC1 (730); HDAC6 (51); Anti-proliferation: MCF-7 (3240); HCT-116 (3390); DU-145 (3980)	NA	NA	[187]
 **63**	pyrozoloquinazoline scaffold and SAHA	Topo I (16590); HDAC1 (39.6); Anti-proliferation: A549 (720); H1299(810); MCF-7 (1390); MDA-MB-231 (1220); Colon cancer (1680)	NA	NA	[188]

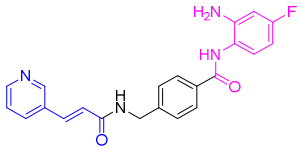
NA: not available.

Table 16. Dual inhibitors of HDACs and PARP.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **64**	olaparib and panobinostat	PARP1 (68.15); PARP2 (5.02); HDAC1 (27.26); HDAC6 (8.21); Anti-proliferation: T47D (2140); MCF-7 (3550); MDA-MB-231 (72 h) (1540); MDA-MB-231 (5 days) (220); HCC827 (1790); Hela (5100); K562 (5550); U937 (4500); MCF-10A (8650); Raji (1290); HCC1937 (2020)	NA	NA	[199]
 **65**	olaparib and chidamide	PARP1 (4.2); HDAC1 (340); Anti-proliferation: K562 (5620); MDA-MB-231 (4350); MCF-7 (no active)	Not tested	Not tested	[200]

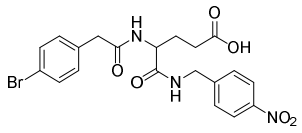
NA: not available.

Table 17. Dual inhibitors of HDACs and NAMPT.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **66**	similarity of pharmacophore between NAMPT and HDAC inhibitors	NAMPT (2100); HDAC1 (130); HDAC2 (110); HDAC3 (330); Anti-proliferation: HCT-116 (340); K562 (320); HL-60 (2.2); HEL (13); HCT-116-siRNA (>20000); K562-siRNA (2400); HL-60-siRNA (3200); HEL-siRNA (1800)	NA	NA	[202]

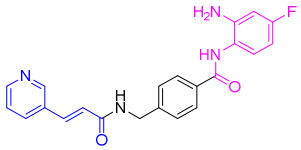
NA: not available.

Table 18. Dual inhibitors of HDACs and MMP.

Structure	Derived from	IC_{50} (nM) Enzymatic cellular	PK	In vivo	Ref.
 **67**		MMP-2 (6400); MMP-9 (4830); HDAC8 (2890)	NA	NA	[206]

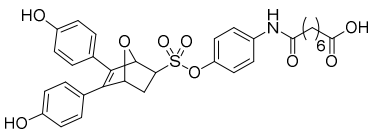
NA: not available.

Table 19. Dual inhibitors of HDACs and AR.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **66**	similarity of pharmacophore between NAMPT and HDAC inhibitors	NAMPT (2100); HDAC1 (130); HDAC2 (110); HDAC3 (330); Anti-proliferation: HCT-116 (340); K562 (320); HL-60 (2.2); HEL (13); HCT-116-siRNA (>20000); K562-siRNA (2400); HL-60-siRNA (3200); HEL-siRNA (1800)	NA	NA	[202]

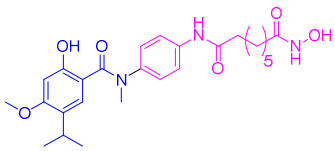
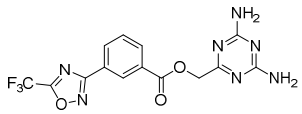
NA: not available.

Table 20. Dual inhibitors of HDACs and ER.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **69**	Known ER antagonist [214]	ERα (50); ERβ (160); HDAC1 (107); HDAC6 (8340); Anti-prolieration: MCF-7 (19100); DU-145 (34800)	NA	NA	[215]

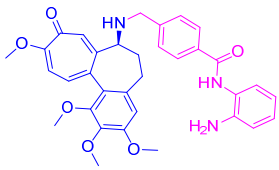
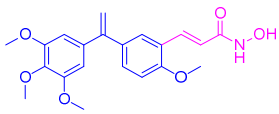
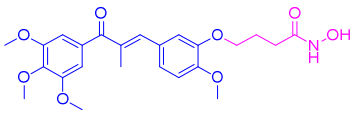
NA: not available.

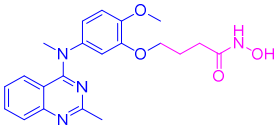
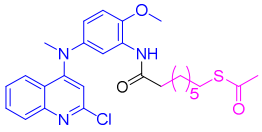
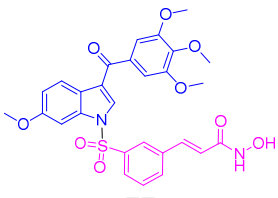
Table 21. Dual inhibitors of HDACs and HSP90.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **70**	HSP90 inhibitor and SAHA	HSP90α (153); HDAC1 (1310); HDAC2 (1190); HDAC3 (2060); HDAC6 (40); HDAC8 (422); anti-proliferation: A549 (770); HCT-116 (830); H1975 (690)	NA	NA	[221]
 **71**	ligand-based and structure-based virtual screening	HDAC6 (53% at 5000); HSP90 (32% at 50000)	NA	NA	[222]

NA: not available.

Table 22. Dual inhibitors of HDACs and tubulin.

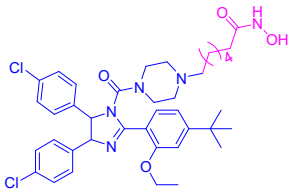
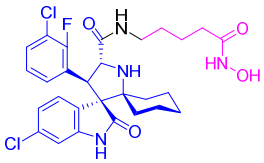
Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **72**	colchicine and entinostat	HDAC1 (12500); HDAC2 (6730); HDAC3 (11230); Anti-proliferation: A549 (106); HCT-116, SW620, Hep 3B, Hep G2, MHCC97H, SNU-5, SNU-16, MKN-45, PANC-1, SJSA-1 (ranging from 2~43)	NA	NA	[227]
 **73**	*iso*CA-4 and belinostat	HDAC8 (340); HDAC6 (15000); HDAC11 (10000); ITP (1600); Anti-proliferation: U87 (1.6)[a]; PC-3 (0.4)[a]; HCT-116 (1.5)[a]; A549 (1.7)[a]; K562 (1.6)[a]; K562R (1.4)[a]; MCF-7 (2)[a]; BXPC3 (2.9)[a]; MiaPacaz (5.1)[a]; HT-29 (2)[a]	NA	NA	[228]
 **74**	CA-4 like chalcone [229] and SAHA	HDAC1 (996); HDAC6 (470); HDAC8 (1700); tubulin polymerization (54500); Anti-proliferation: A549 (550); HeLa (470); SGC-7901 (1060); other additional cell lines (ranging from 2400~7540)	NA	NA	[229]

 **75**	tubulin inhibitor and hydroxamic acid	HDAC1 (422); HDAC6 (17); HDAC8 (3398); induction of acetylation of α-tubulin, inhibition of tubulin polymerization; Anti-proliferation: HCT-116 (39); HT29 (72); H460 (78); A549 (75); A2780s (37); SKOV3 (40); MCF-7 (83); MDA-MB-231 (66); A375 (48); Hep G2 (31); MM1S (67); RPMI-8226 (50); ARD (93); U266 (87); Romos (74); HBL-1 (121); Jeko-1 (116); LAMA-84s (84); K562 (117); MV4-11 (68)	rats, p.o. (12 mg/kg), iv (12 mg/kg), %F = 47%	Xenograft mice models, p.o., thrice a week: HCT116: 50 mg/kg, TGI: 66%; A2780s: 25 mg/kg, TGI: 77%; MCF-7: 50 mg/kg, TGI: 66%	[230,231]
 **76**	tubulin inhibitor and thio ester ZBG	HDAC6 (5800); Anti-proliferation: MCF-7, A549, SGC-7901, Hep G2, HCT-116, PC-3 (ranging 110~1600)	NA	NA	[232]
 **77**	BPR0L075 [233] and belinostat	HeLa nuclear HDACs (135.5); HDAC1 (467.8); HDAC2 (3216); HDAC6 (275.4); Anti-proliferation: PC-3 (33.1); HL-60 (42); A549 (72.5); HCT-116 (43.9)	NA	In HL-60 xenograft mice, 100 mpk p.o., TGI: 40.9%; in PC-3 xenograft mice, 100	[234,235]

		mg/kg qd ip, TGI: 31.1%

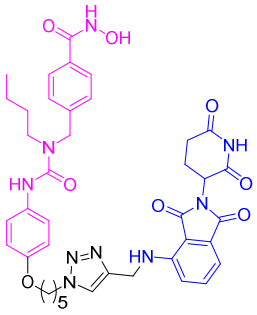
[a]GI_{50} the concentration that results in inhibiting cell growth by 50%. NA: not available.

Table 23. Dual inhibitors of HDACs and MDM2.

Structure	Derived from	IC_{50} (nM) Enzymatic cellular	PK	In vivo	Ref.
 **78**	nutlin-3 and SAHA	MDM2 (110); HDAC1 (820); HDAC2 (420); HDAC3 (178); HDAC6 (17.5); HDAC8 (1224); Anti-proliferation: A549 (910); HCT-116 (1080); MCF-7 (4340); NCI-H1299 (4160)	SD rats p.o. (20 mg/kg), iv (2 mg/kg), %F = 18%; $T_{1/2}$ = 5.87 h	In A549 xenograft mice, qd, p.o., 21 days, TGI: 74.5% (150 mg/kg); TGI: 65.4% (100 mg/kg)	[238]
 **79**	MDM2 inhibitor and SAHA	MDM2 (48); HDAC (>500)	NA	NA	[239]

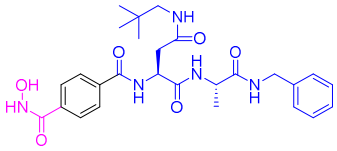
NA: not available.

Table 24. Dual inhibitors of HDACs and CRBN.

Structure	Derived from	IC_{50} (nM) Enzymatic cellular	PK	In vivo	Ref.
 **80**	pomalidomide and nexturastat A	HDAC6 (74.9% @10); IKZF (1.6)[a]; Anti-proliferation: MM1S (74.9)[b]	NA	NA	[241,242]

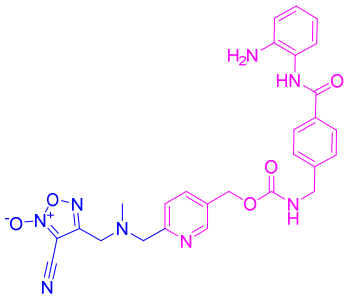
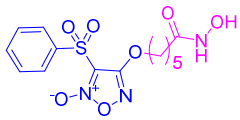
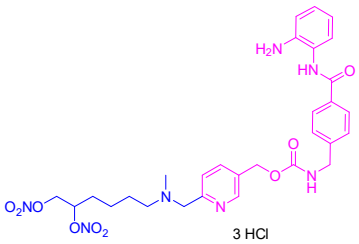
[a]DC_{50} refers to the concentration at which half-maximal degradation was achieved; [b]EC_{50} refers to the concentration at which half-maximal growth inhibition was achieved. NA: not available.

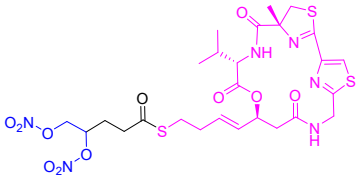
Table 25. Dual inhibitors of HDACs and proteasome.

Structure	Derived from	IC_{50} (nM) enzymatic cellular	PK	In vivo	Ref.
 **81**	ML16 and hydroxamic acid	HDAC1 (6900); HDAC6 (270); HDAC8 (530); Anti-proliferation: HL-60 (260.7); SEM (394.2); SUP-B15r (820.5)	NA	NA	[245]

NA: not available.

Table 26. Dual NO donor-HDACs targeting agents.

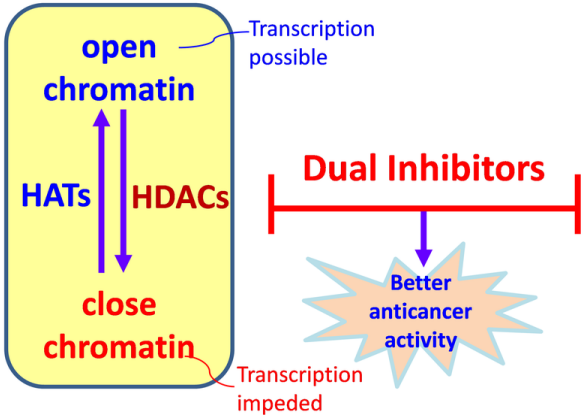
Structure	Derived from	IC_{50} (nM) Enzymatic cellular	PK	In vivo	Ref.
 **82**	NO donor motif and entinostat	No specific value obtained	NA	NA	[256]
 **83**	Phenylsulfonylfuroxan and SAHA	Nitrate increase in HEL cell (≈11% @100000, 3 h; ≈19% @100000, 5 h); HDAC1 (241); HDAC2 (380.5); HDAC3 (532); HDAC4 (30); HDAC6 (7.4); HDAC8 (343); HDAC11 (608); Anti-proliferation: Hela extract (38); HEL (380); HCT-116 (1510); Hela (1550); U937 (3100); 3-AO (5840); MDA (2250); ES-2 (1390); KG1 (1750)	NA	In HEL xenograft mice, 100 and 120 mg/kg, qd p.o., 21 days, TGI: 38% and 48%.	[257]
 **84**	NO donor motif and entinostat	HDAC1 (1040); HDAC2 (390); HDAC3 (1220); Vasodilator activity (290)[a]	NA	NA	[258]

 **85**	NO donor and largazole	Anti-proliferation (72 h): U-2OS (6000); IMR-32 (7300); CaCo-2 (8350)	NA	NA	[259]

[a]EC_{50}. NA: not available.

Table 27. Dual inhibitors in clinical trials.

Compound	Mechanism	Cancer types	Phase	Status	Clinical trial ID
Fimepinostat (CUDC-907)	PI3K-HDACs	relapsed or refractory solid tumors, CNS tumors, or lymphoma, brain tumor; prostate cancer; large B-cell lymphoma; TNBC; thyroid cancer (terminated, Phase II); lymphoma	I/II	recruiting unless otherwise indicated	NCT03893487; NCT02909777; NCT02913131; NCT02674750 (completed); NCT02307240 (completed); NCT03002623 (terminated); NCT01742988 (active, not recruiting).
CUDC-101	EGFR-HER2-HDAC-	advanced and refractory solid tumors, advanced or locally advanced head and neck, gastric, breast, liver and non-small cell lung cancer tumors	I	completed or terminated	NCT01702285 (terminated); NCT00728793 (completed); NCT01171924 (completed); NCT01384799 (completed).
Domatinostat (4SC-202)	KDM1A-LSD-HDACs	cutaneous melanoma primary refractory, advanced hematologic malignancies, gastrointestinal cancers, malignant melanoma; merkel cell carcinoma	I/II	recruiting unless otherwise indicated	NCT03278665; NCT01344707 (completed); NCT03812796; NCT04393753; NCT04133948.
Tinostamustine (EDO-S101)	DNA-HDACs	refractory, locally advanced or metastatic melanoma; relapsed / refractory multiple myeloma, diffuse large B-cell lymphoma, and hematologic malignancies; MGMT-promoter unmethylated glioblastoma; solid tumors; SCLC; leukemia; ovarian cancer; endometrial cancer; soft tissue sarcoma	I/II	recruiting unless otherwise indicated	NCT03903458; NCT03687125 (terminated); NCT02576496; NCT03452930; NCT03345485 (active, not recruiting); NCT04279938 (withdrawn due to safety issues)



1. Kinases: CDK, CK2, RTK (EGFR, FGFR, VEGFR, c-MET), JAKs, PI3Ks, mTOR, Syk, Bcr-Abl, Raf
2. Epigenetic: BET, DNA, DNMT, G9a, LSD1, EZH2
3. Other enzymes: IDO, Topo, PARP, NAMPT, MMPs
4. Receptors: AR, ER
5. Miscellaneous: HSP90, Tubulin, MDM2, CRBN, proteasomes , NO-donor