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Review

# Single Inhibitors versus Dual Inhibitors: Role of HDAC in Cancer

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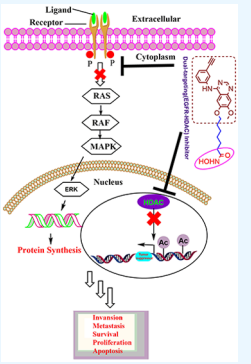
**ABSTRACT:** Due to the multimodal character of cancer, inhibition of two targets simultaneously by a single molecule is a beneficial and effective approach against cancer. Histone deacetylase (HDAC) was widely investigated as a novel category of anticancer drug targets due to its crucial role in various biological processes like cell-proliferation, metastasis, and apoptosis. Numerous HDAC inhibitors such as vorinostat and panobinostat are clinically approved but have limited usage due to their low efficacy, nonselectivity, drug resistance, and toxicity. Therefore, HDACs with a dual targeting ability have attracted great attention. The strategy of combining a HDAC inhibitor with other antitumor agents has been proved advantageous for combating the nonselectivity and drug resistivity problems associated with single-target drugs. Henceforth, we have highlighted dual-targeting inhibitors to target HDAC along with topoisomerase, receptor tyrosine kinase inhibitors, and the zeste homolog 2 enzyme. Our Review mainly focuses on the impact of the substituent effect along with the linker variation of well-known HDAC-inhibitor-conjugated anticancer drugs.




## 1. INTRODUCTION

Cancer, a life-threatening disease, is one of the foremost reasons for death across the world.[1] India ranks third among the nations in terms of the highest number of incidences of cancer. Every year, over 13 hundred thousand people in India are suffering from cancer, as per the National Cancer Registry Programme Report. According to a report by the Indian Council of Medical Research (ICMR), the expected cancer incidence will increase to 29.8 million in 2025. Chemotherapy, targeted therapy, radiation therapy, immunotherapy, and stem cell therapy are the most common treatments for cancer. Despite impressive progress in biotechnologies and diagnosis, the development of novel anticancer drugs is still necessary for the management of cancer patients, losing the great challenge for scientists to cure cancer successfully. For the betterment of cancer treatment and to overcome the toxicity and adverse effect of the implicated drug, scientists have already put their eyes on targeted drug therapy, for which we have seen how a single drug efficiently acts against a specific target with high selectivity.[2] Tamoxifen was the first reported drug used in targeted therapy against breast cancer by targeting estrogen receptors.[3] Though the "one drug, one target" concept is a highly potent and specific treatment against cancer due to the absence of off-target side-effects, the single-target drug is not having significant success due to drug resistance, low pharmacokinetics, and poor patient compliance.[4] Owing to the complex character of cancer, a new therapy needs to be developed for successful long-term outcomes; therefore, a new direction for cancer drug discovery is necessary. Thus, the combination therapy approach has achieved a remarkable place in the area of cancer drug discovery to maximize efficacy and minimize drug resistance. Initially, in drug combination therapy drug cocktails, physical mixtures of two or more compounds are used in combination.[5] Although combination therapy has had noteworthy success, many problems are associated with this therapy, such as drug solubility, drug resistance, and drug interactions. In addition, the possibility for drug−drug interactions and side effects are increasing, so dose adjustment is essential to avoid drug toxicity. For these boundaries of combination therapy, many research groups concentrate on molecular hybridization to develop a dual-targeting drug rather than a drug combination.[6] Thus, combination therapy has leaned toward the design of dual-target ligands in which a single molecule can target more than one site simultaneously, leading to synergistic effects and the potential to reduce the possible drug−drug interactions and drug-resistance and improve pharmacokinetics compared to physical mixtures of drugs molecules.[7]

## 2. COMPARISON OF SINGLE VERSUS DUAL INHIBITORS

A single inhibitor can specifically inhibit a target molecule. Therefore, a single inhibitor attacks only cancer cells, excluding normal cells; hence, it has high selectivity and effectivity and low toxicity. At first, although there is enthusiasm among clinicians, they soon have to face the problem that after giving

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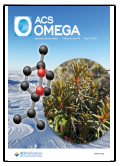

the drug repeatedly the cancer cells develop resistance against the drug. Therefore, patients cannot survive for long time. The resistance is mainly due to the epigenetic altering of the target, so a drug molecule is unable to interact with the target and therefore the targeted therapy is not anymore useful. Since cancer is a complex disease, it is very easy to manipulate the single target, resulting in treatment failure. Since then, combination drug treatment has replaced single targeted drug therapy using two or more drugs in together to enhance the efficacy. At the same time, multiple drugs may increase the risk of drug−drug interactions and dose-limiting toxicities. Although combination therapy has been successful in cancer treatment, it is not useful further due to these disadvantages. Therefore, a single drug with the ability to target two targets simultaneously could serve as an effective strategy in cancer treatments.[8] Overcoming drug resistance and the toxicity of single-targeted inhibitors is the basis for the development of dual-targeting inhibitors. A recent report indicated a more favorable outcome with dual-targeting inhibitors in cancer treatment. For an example, the single-targeted drug erlotinib is the first line drug treatment for non-small-cell lung carcinoma (NSCLC) but limited in its usage due to drug resistance. A synergistic antitumor effect is produced in NSCLC when vorinostat (SAHA) and erlotinib are combined. This observation led to the search for a new effective treatment for human lung cancer. Although the effectiveness increases in combination therapy, since the two individual drugs are given together, there are several limitations including drug−drug interactions, toxicity, and drug resistance. However, combining SAHA and erlotinib a single molecule leads to efficacious outcomes due to reduce toxicity and drug−drug interactions along with enhanced efficacy.

**2.1. Dual-Targeting Inhibitor and Its Importance.** The concepts of dual-targeting inhibitors have been used from the beginning of modern pharmacology and have also achieved success in clinics.[9]

A dual-targeting inhibitor acts on two molecular targets simultaneously. In the past decade, dual-target drugs (chimeric drugs) have attracted considerable attention, which is a sharply growing research area for medicinal chemists. Through the inhibition of two antitumor agents involved in disease progression simultaneously, drug resistance is improved. Additionally, dual-target therapies can overcome the limitations of single-targeted drugs including drug−drug interactions, poor safety, a low therapeutic index value, low efficacy, and other side effects.[4]

Here in this Review, we consider the bifunctional single drug, which is a product of two known inhibitors that acts on two specific targets and results in the advantages of enhanced antitumor activity and reduced toxic effects.[10] To enhance the affinity and efficacy compared to their individual parent compounds, two molecules are combined to design new hybrid compounds.[11] The advantages of dual inhibitors is that they are more active than their constituent parent molecules individually, are less toxic, and have improved therapeutic index values and bioavailability.[12] Few dual-targeting inhibitors are now in clinical trials against cancer. A dual-targeting inhibitor CUDC-101,[13] an inhibitor of epidermal growth factor receptor (EGFR)/histone deacetylase (HDAC), and another inhibitor CUDC-907, a dual phosphoinositide 3-kinase (PI3K)/HDAC inhibitor,[14] have completed phase I and phase II clinical trials against solid tumors and relapsed/refractory diffuse large B-cell and high-grade B-cell lymphoma, respectively. Therefore, the progress of dual-targeting inhibitors is gaining attention for discovering anticancer drugs that possess pharmacokinetic advantages compared to conventional cancer therapy.

**2.2. HDAC as a Promising Dual-Targeting Inhibitor.** Mutations and aberrant expression of epigenetic regulators are important contributors that cause many types of cancer. HDAC plays an important role in tumor cell development and is an important target among epigenetic regulators. The HDAC enzyme catalyzes the deacetylation of both histone and nonhistone proteins.[15] SAHA, panobinostat, chidamide, belinostat, and romidepsin are known FDA-approved HDAC inhibitors.[16] In most of the HDAC inhibitors, three main pharmacophores are present: a Cap group (surface recognition group), a ZBG (zinc-binding group), and a linker required to connect the Cap group and the ZBG, which is represented in Figure 1. Hydroxamic acid, cyclic tetrapeptides, benzamides,

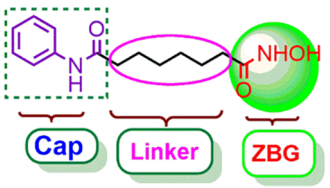


**Figure 1.** Schematic diagram of a HDAC inhibitor and its active functionality.

and fatty acids are the four types of HDAC inhibitors, which are categorized based on their structures.[17] HDAC inhibitors are efficient for the treatment of cancer, but there are several side effects. Due to the nonselective nature of the HDAC inhibitors, more efficient and selective HDAC inhibitors need to be developed. By developing isoform-selective HDAC inhibitors, toxicity is reduced but drug resistance is still a problem. HDAC inhibitors alone have been suffering some problems like efficacy, toxicity, and drug resistance, and these disadvantages are overcome by the strategy of designing HDAC inhibitors with dual-targeting capabilities.[18] Therefore, here in this Review we talk about HDAC-based dual-targeting inhibitors.[19] Other antitumor agents are easily linked with HDAC inhibitors due to their flexible structures. Other antitumor agents such as topoisomerase (Top) inhibitors and receptor tyrosine kinase (RTK) inhibitors, including vascular endothelial growth factor receptor (VEGFR), EGFR, zeste homolog 2 (EZH2), and c-Met inhibitors, exert a synergistic effect on the cellular process of cancer cells when combined with HDAC inhibitors. Therefore, the bifunctional single molecule (dual-targeted drug) has become a promising approach in the treatment of cancer and a rational strategy to solve the problems of single-targeted drugs.

## 3. STRATEGY TO DESIGN A DUAL-TARGETED INHIBITOR

The two known inhibitors are combined in a single molecule to act as a dual-targeting inhibitor. The inhibitors are combined through a linker or fused together to form a single bifunctional molecule that simultaneously inhibits two targets. Additionally, computation-based design has played an important role in drug development, which is expensive and time-intensive. The initial application of computational chemistry followed by high-throughput screening technologies has accelerated the drug discovery process. Therefore, this


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computational approach can be used also in the case of designing a dual-targeting inhibitor. Target identification, docking-based virtual screening, scoring functions, molecular similarity calculation, virtual library design, and sequence-based drug design are some methodologies involved in computer-aided drug design. Details of these two strategies are explained below.[20]

**3.1. Pharmacophore-Based Design of Dual-Target Kinase Drugs in Cancer.** In pharmacophore-based design, the distinct pharmacophores of two known inhibitors are combined in a single molecule. Pharmacophore-based design approaches are categorized into three types: (i) a suitable linker attached to an active moiety pharmacophore approach, (ii) the direct conjugation of the two active moieties pharmacophore approach, and (iii) the merged pharmacophore approach. In the linker pharmacophore approach, two different kinase drugs are combined via a linker and important structural features of two kinase inhibitors are retained in the new drug and may be effective against two targets (Figure 2). The growing amount of structural information is considered for linker-based drug design. This approach may be useful for designing a dual-target inhibitor. The fused pharmacophore approach is used to design a simple and small dual-targeting inhibitor. The fused pharmacophore approach allows for the overlap of the active space and ameliorates the pharmacokinetics as well as physicochemical properties of dual-target small molecules. The merged pharmacophore strategy requires lead compounds to have the same pharmacophore and share common fragments. Accordingly, the two small molecules are combined and synthesized into one molecule, but their respective pharmacophores remain, producing a smaller and simpler merged small molecule.[21]

**3.2. Computational Design of Dual-Target Kinase Drugs in Cancer.** Designing novel dual-targeted drugs based on a computational study is an alternative effective method, especially for novel kinase targets. From the knowledge of pharmacochemistry, proteomics, and computational chemistry, dual-targeted compounds are designed. For this design of dual-targeted drugs, the selection of target combinations and the virtual screening of dual-target small molecules are performed by applying techniques of data mining and structure analysis, respectively. The computational design strategies for dual-target kinase inhibitors are based on multiple docking, the use of pharmacophores, and fragment-based design.

Using multiple docking techniques of a small-molecule-based compound library with effective binding in the targeted site was evaluated.[22] Based on best hits, potential compounds were selected further for structural optimization for a molecular docking study. Due to the similar binding pocket present in the kinase, the result from the molecular docking study cannot offer appropriate predictions. In some cases, inverse docking approaches suffer from scoring algorithms and difficulty addressing target flexibility. Otherwise, improving the accuracy and false-positive rate for target selectivity prediction remains a challenge.

As an example, a hybrid molecule is formed by the combination of SAHA and the topoisomerase I (Top-I) inhibitor camptothecin.[23] It was reported experimentally that the newly formed hybrid compound is more effective than the parent molecules SAHA and camptothecin. To see if the new stuff actually works against HDAC and topoisomerase as confirmed by a computer-aided study, the binding modes of

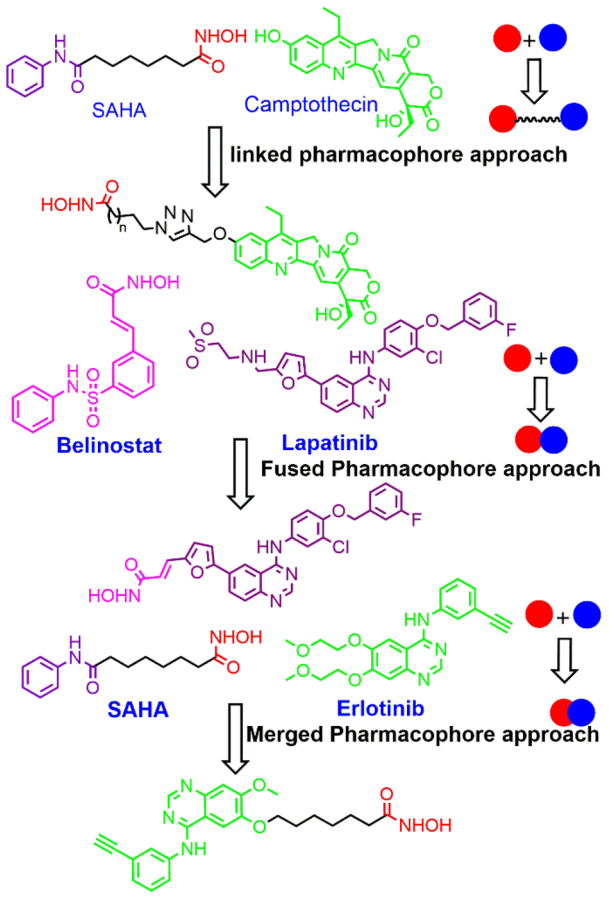


**Figure 2.** Schematic representation of the pharmacophore-based design of a dual-targeting inhibitor.

Top-I/HDAC hybrids have been predicted with their target receptors by a molecular docking study.

The hydroxyl group (ring E) and nitrogen atom (ring B) of camptothecin are formed hydrogen bonding with Asp533 and Arg364, respectively. The $\pi$−cation interaction is also observed with Lys425, and ring A and a hydroxamic acid group occupied the major groove cavity of the Top-I DNA complexes. Therefore, the interaction of camptothecin is not only preserved in the binding modes of the Top-I/HDAC hybrids but also enhanced due to the hydroxamic acid group of SAHA. Similarly, for the binding with the HDAC protein, we observe that camptothecin acts as a CAP group, oxygens of ring E form hydrogen bonds with Gly268, Asp269, Arg270, and Gly272, and hydroxamic acid coordinates with the $Zn^{2+}$ ion in a bidentate manner. A triazole ring is involved in the formation of additional hydrogen bonds with His178 and Phe205, and that is observed only in the case of linkers containing five and six methylene groups (Figure 3).[24]

From this observation, it is concluded that the hybrid HDAC/Top molecule interacts with both target proteins and thus inhibits the function of two target simultaneously.

## 4. DUAL TOPOISOMERASE/HISTONE DEACETYLASE INHIBITOR

DNA topoisomerase enzymes play crucial roles in many biological processes involving DNA cleavage and the relegation process.[25] To stop cell division and progression, topoisomerase enzymes that are responsible for breaking and reconnecting DNA strands are blocked by a topoisomerase inhibitor.[26]


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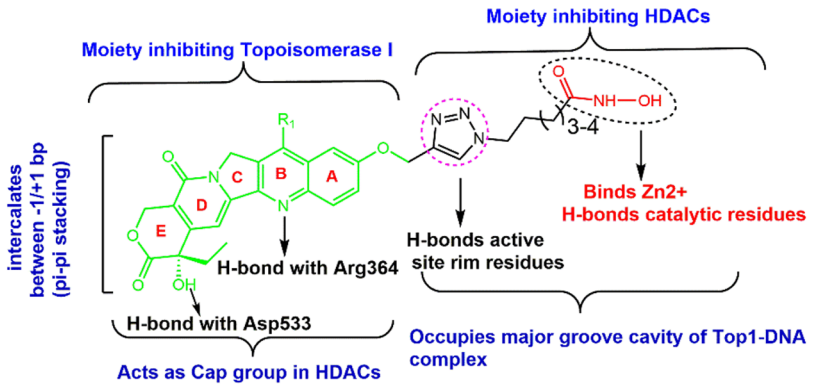


**Figure 3.** Binding of a dual HDAC/Top inhibitor with the HDAC protein and the Top-I DNA complex via a docking study.

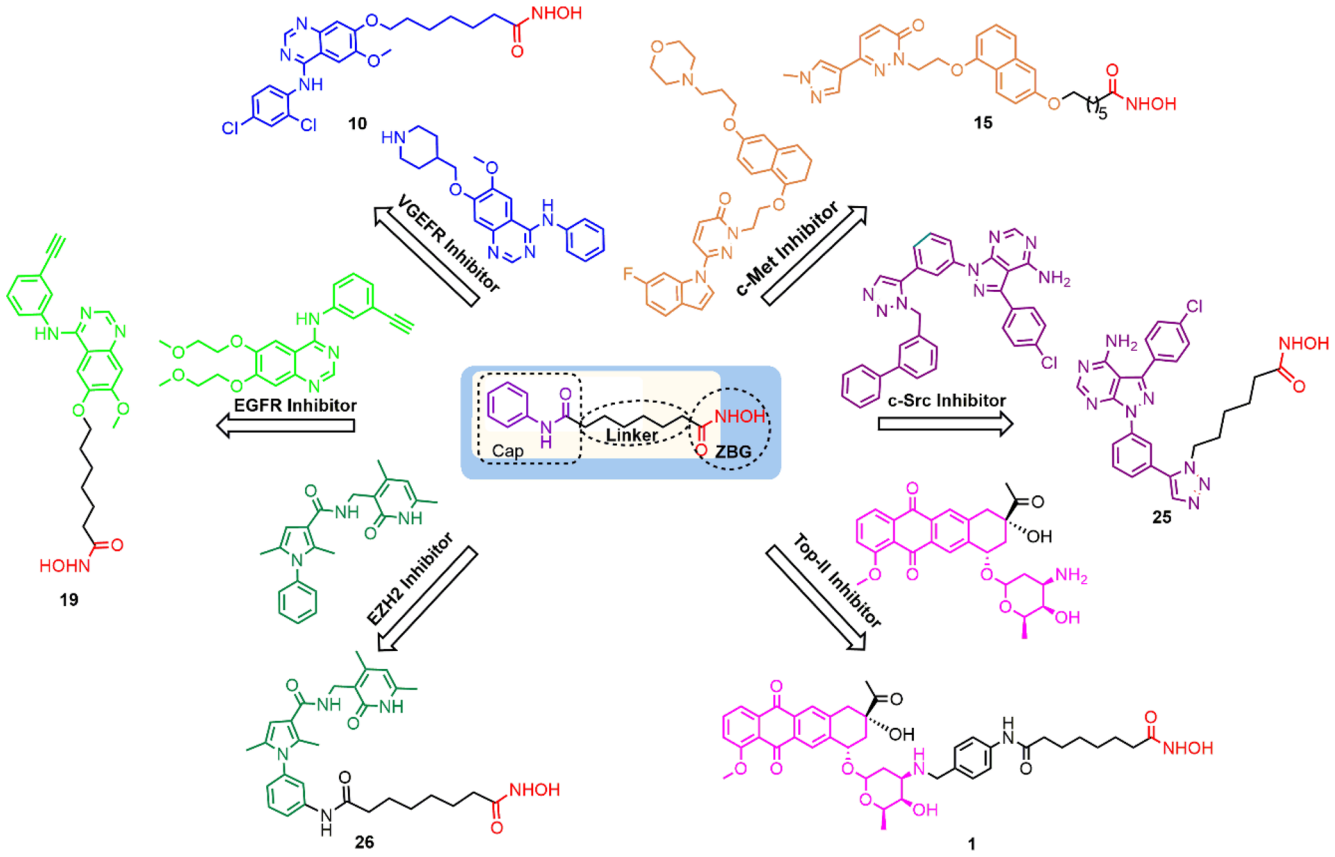


**Figure 4.** Respective dual-targeting inhibitors (**1**, **10**, **15**, **19**, **25**, and **26**) presented in a pictorial form from their precursor molecules.

Camptothecin (Top-I inhibitor), DACA (Top-II inhibitor), doxorubicin, and daunorubicin (DAU; Top-I and Top-II inhibitor) are known topoisomerase inhibitors.[27]

To design dual-targeted Top/HDAC inhibitors, the well-known SAHA has been incorporated or modified with other Top inhibitors. Herein, for the synthesis of a dual Top/HDAC inhibitor, the Top-II inhibitor DAU containing an amino group was conjugated via *N*-benzylation to HDAC. Additionally, a DAU-containing anthracycline moiety can act as a capping group that can conjugate with the hydroxamic acid of HDAC. Here, the hydroxamic acid of SAHA act as a zinc-binding pocket.[28]

Two types of dual HDAC and Top-II inhibitors were reported by Guerrant et al.,[29] where one is directly conjugated with DAU and SAHA and the other is DAU/triazolylaryl hydroxamate conjugates where SAHA and DAU are conjugated by a triazole ring. The hybrid compound made directly from SAHA and DAU (**1**) (in Table 1) has shown better HDAC inhibitory activity than SAHA and also has shown the best cytotoxicity compared to the other reported bifunctional (SAHA and DAU) compounds.

The triazole-linked conjugate compounds of different linker lengths effectively inhibit HeLa cell with $IC_{50}$ values in the nanomolar range. From the result, it was indicated that linker length also plays an important role in inhibiting HDAC. Among all the developed molecules, the compound containing six methylene linkers (**2**) (in Table 1) has expressed a potency 70-fold greater than that of the standard drug SAHA. However, it was also observed that, despite the similar length, the triazole-linked compounds are 40-fold more active in comparison to amide-linked compounds.



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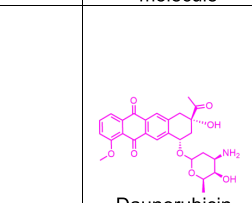
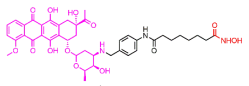
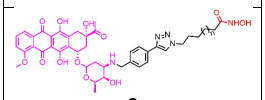
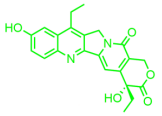
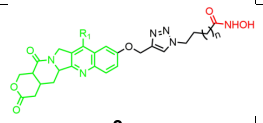
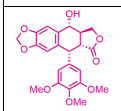
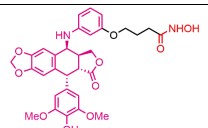
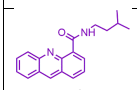
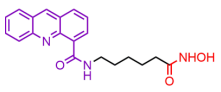
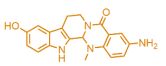
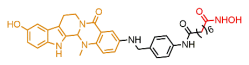
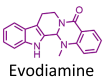
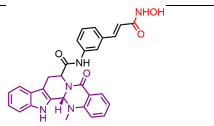
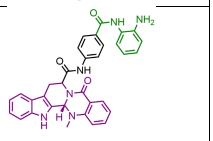
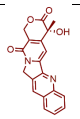
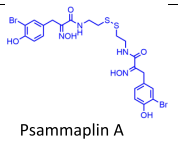
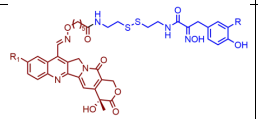
**Table 1. Novel Dual-Drug (HDAC/Top) Inhibitors and Their Anticancer Activities**

| Sl No | HDAC Inhibitor | Targeted therapeutic molecule | Hybrid Molecule (HDAC and Topo) | Activity as dual drug inhibitor |
|---|---|---|---|---|
| 1. |  SAHA |  Daunorubicin |  1  2 | Dual Topo II/HDAC inhibitor. Better HDAC inhibitory activity than SAHA and show best cytotoxicity against DU-145 prostate carcinoma cell line.[29] 70-fold more potent than SAHA and IC50 value in nano-molar range also 40-fold more active and amide linked compound.[29] |
| 2. | |  Camptothecin(SN38) |  3 | Dual Topo I/HDAC Inhibitor. Showed better cytotoxicity against DU-145 prostate cancer cell line.[23] |
| 3. | |  Podophyllotoxin |  4 | Dual Topo I/HDAC Inhibitor. 10- to 20 fold more potent than SAHA.[30] |
| 4. | |  DACA |  5 | Dual Topo I/HDAC inhibitor and potent anti-proliferative activity than SAHA and DACA. Induce cell-cycle arrest.[31] |
| 5. | |  3-amino-10-hydroxyevodiamine |  6 | Triple Top I/II/HDAC inhibitor. Good anti-proliferative activity against MDA-MB-231 (breast cancer), HCT116 (colon cancer) and HLF (liver cancer) cell lines.[32] |
| 6. | |  Evodiamine |  7  8 | Dual HDAC/Topo Inhibitor. Hydroxamic acid containing compounds (**7**) are potent than the ortho-aminoanilide(**8**).[33] |
| 7. |  CPT |  Psammaplin A |  9 | Dual Topo I/HDAC inhibitor. good antiproliferative activity and cytotoxic potency in nanomolar range in solid tumor cell lines.[34] |

Similarly, the same research group has designed molecules for getting dual-targeting Top/HDAC inhibitors by combining SN-38 (as the Top-I inhibitor) and SAHA via various linkers.[23] By further incorporating a triazole ring and modifying the linker length, plenty of compounds are synthesized. The HDAC inhibitory activity increases for the triazole ring present in the alkyl linker. The dual-acting inhibitors were screened against the DU-145 prostate cancer cell line, and the result indicated that the novel molecule (**3**) showed better cytotoxicity than standard SAHA molecules.

The Zhang et al.[30] research group designed and synthesized Top-I/HDAC molecules with podophyllotoxin derivatives. A



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Table 2. Novel Dual Drug Inhibitor HDAC as Common with Other Inhibitors (VGEFR/EGFR/PARP/c-Met)

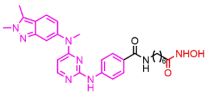
| Sl No | HDAC Inhibitor | Targeted molecule | Hybrid Molecule | Activity as dual drug inhibitor |
|---|---|---|---|---|
| 1. |  | Vandetinib | **10** | showed excellent inhibitor against HDAC and VEGFR-2, exert effectiveness towards MCF-7 cancer cell line.[37] |
| 2. | | Vandetinib | **11** | Most inhibition activity against HDAC and VEGFR-2. 7-fold higher in comparison to standard drug. Great anti-proliferation potency against MCF-7 solid tumor cell line.[38] |
| 3. | | Semaxanib | **12** | Good cytotoxicity on breast cancer cell line MDA-MB231, leukemia cell line, CCRF-CEM and melanoma cell line MALME-3 in compare to SAHA.[39] |
| 4. | | Pazopanib | **13**  **14** | Ortho-aminoanilide and hydroxamic acid showed comparable HDAC inhibitory activity to MS-275 and SAHA respectively.[40] |
| 5. | | Selective c-Met inhibitor | **15** | Four-fold more inhibitory activity than the standard drug Chidamide.[49] |
| 6. | | Foretinib | **16** | Good anti-proliferative activity against HCT-116, MCF-7 and A549 cell lines.[51] |
| 7. | Chidamide | Olaparib | **17** | Good anti-proliferative activity than olaparib and SAHA in various cancer cells lines and 4.1-fold less cytotoxicity compared with SAHA to normal cells MCF-10A.[55] |
| 8. | Panobinostat | Olaparib | **18** | Active against PARP-1 and HDAC-1, which is as potent as olaparib and chidamide respectively. potent inhibitory activities against BRCA1/2-proficient K562 and MDA-MB-231 cells.[56] |



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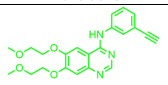
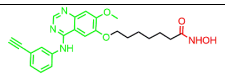
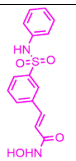
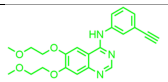
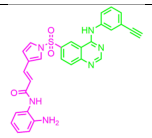
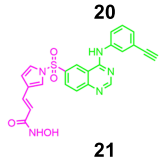
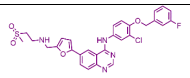
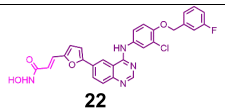
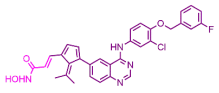
**Table 2. continued**

| Sl No | HDAC Inhibitor | Targeted molecule | Hybrid Molecule | Activity as dual drug inhibitor |
|---|---|---|---|---|
| 9. |  SAHA |  Erlotinib |  **19** | CUDC-101 showed potent anti-proliferative activity than vorinostat (SAHA), erlotinib, lapatinib, and combinations of vorinostat/erlotinib and vorinostat/lapatinib.[79] |
| 10. |  Belinostat |  Erlotinib |  **20**  **21** | improved HDAC inhibition than SAHA and exhibited cytotoxic activity on all tested tumor cell lines. hydroxamic acid moiety containing hybrid molecule inhibit both HDAC class I and class II isoforms.[64] |
| 11. | |  Lapatinib |  **22**  **23** | displayed the most potential activity in inhibiting HDAC. potent and selective inhibition against EGFR/HER2 kinase.[65] |

more flexible linker was introduced to increase the affinity of the hybrid molecules for the surfaces of HDAC enzymes. Therefore, a new series of hybrid molecules attached with an ether linkage were designed. Noticeable is that as the chain length increases the HDAC inhibitory activity increases according to the SAR study. The larger capping group PPT is associated with the HDAC inhibitor through a flexible ether linker, promoting the affinity between the compounds and the HDAC enzymes. The substitutions on the aromatic ring have shown further activity variability, indicating *meta*-substituted compounds have shown 10−20-fold higher potency than SAHA. *meta*-Substituted ether-linked podophyllotoxin derivatives (**4**) exhibited the best inhibitory activity against HDAC.

In 2014, the Yu et al. group reported a synthetic molecule WJ35435, a new class of hybrid molecule, by merging the pharmacophoric features of SAHA and DACA.[31] WJ35435, a dual targeting Top/HDAC inhibitor, showed better anti-proliferative activity than individual known HDAC and Top inhibitors. The hybrid molecule WJ35435 (**5**) showed improved antiproliferative activity greater than that of DACA and vorinostat. The dual-targeted hybrid molecule inhibits the activity of both HDAC and topoisomerase I; as a result, DNA damage and cell cycle arrest happen at both G1 and G2 phases.

He et al. designed and synthesized a new series of hybrid compounds as triple-targeting antitumor agents by combining 3-amino-10-hydroxylevodiamine, a Top-I/Top-II inhibitor, and SAHA.[32] SAHA was conjugated to the evodiamine at the amino portion of 3-amino-10-hydroxylevodiamine to synthesize new dual Top/HDAC inhibitors. The evodiamine scaffold is connected with the zinc-binding group of SAHA via a linker oxadiazole ring. To improve the lipophilicity and pharmacokinetic profiles, the incorporation of 1,3,4-oxadiazole rings was an effective strategy. All the hybrid compounds were reported HDAC1 inhibitors and have shown comparable activity to SAHA. Additionally, the hybrid 1,2,4-oxadiazole-containing compound (**6**) showed good antiproliferative activity against MDA-MB-231 (breast cancer), HCT116 (colon cancer), and HLF (liver cancer) cell lines compared to the individual parent drug.

Inspired by the above-mentioned work, the same research group has given more effort to design antitumor evodiamine derivatives. This group previously reported a triple Top-I/Top-II/HDAC inhibitor based on the synergistic effect of Top and HDAC inhibitors. The hybrid compound has shown good antiproliferative activity and a pro-apoptotic effect. However, in vivo antitumor effects led to in poor outcomes against antitumor activity, and further development is necessary. New HDAC/Top dual inhibitors were further developed and reported with admirable antitumor efficacy in both in vitro and in vivo.[33] In addition, hydroxamic acid (ZBG) containing compounds (**7**) are more potent than *ortho*-amino aniline (**8**). The *ortho*-amino aniline compound effectively induced apoptosis with G2/M cell cycle arrest. Further structural modifications were performed to increase efficacy.

Cincinelli et al. designed a new Top-I and HDAC hybrid inhibitor.[34] A series of dual-targeting inhibitors were synthesized by conjugating substituted (*E*)-7-oxyminomethyl CPTs with the active fragment of psammaplin A via an amide bond. The choice of the spacer length is a crucial factor for HDAC inhibition. The newly developed hybrid compounds are potent inhibitors of Top-I/HDAC dual inhibitory activity. The novel hybrid compound 6-(10-hydroxycamptothecin-7-



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ylmethyleneaminooxy)hexanoic acid hydroxyamide (**9**) has good antiproliferative activity and cytotoxic potency in the nanomolar range in solid tumor cell lines compared to SAHA and irinotecan.

## 5. DUAL VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (VEGFR) AND HDAC INHIBITORS

Vascular endothelial growth factor (VEGF) and VEGFR-2, the receptor tyrosine kinase of VEGF, are the key mediators for angiogenesis. When VEGFR-2 binds to VEGF, dimerization occurs between the ligand and the receptor and phosphorylation happens, thereby initiating downstream signaling; as a result, angiogenesis, tumor survival, and proliferations occur.[35] Therefore, the VEGFR/VGFR-2 pathway is an important target for antiangiogenic therapy in cancer treatment. Bevacizumab is an FDA-approved monoclonal antibody, and the other small molecules sorafenib, sunitinib, vandetanib, and pazopanib are FDA-approved VEGFR-2 tyrosine kinase inhibitors used in various types of cancers.[36] However, VEGF/VEGFR-2 targeted therapy is not successful for a large number of patients. To overcome this problem, Shi's group, i.e., Peng et al., have developed novel VEGFR-2/HDAC dual inhibitors by combining 4-anilinoquinazoline with hydroxamic acid motifs of the HDAC inhibitor SAHA.[37] A series of compounds were designed and synthesized that simultaneously target both the VEGFR-2 and HDAC. All the compounds have modest VEGFR-2 inhibition in comparison to standard drugs. However, among them, the compound containing a 2,4-Cl substitution on the phenyl ring (**10**) showed an excellent inhibition against HDAC and VEGFR-2, exerting effectiveness toward the MCF-7 cancer cell line.

Peng et al. demonstrated a set of hybrid molecules containing *N*-phenylquinazolin-4-amine and hydroxamic acid side chain moieties of SAHA.[38] The hybrid molecule displayed great antiproliferation potency against MCF-7 solid tumors. All the synthesized compounds exhibited moderate inhibition against VEGFR-2 compared to vandetanib. The compound containing *N*-phenylquinazolin-4-amine moiety acts as a capping group for HDAC inhibitors. The length of the carbon chain linker highly influences HDAC inhibition, and for VEGFR-2 inhibition the position and type of halogen substitution on the phenyl ring are also important. Among the hybrids, the compound containing a 2-Br-substituted phenyl ring (**11**) exhibited the most inhibition activity against HDAC and VEGFR-2, and the inhibition was seven-fold higher in comparison to the standard drug. From further investigation, it was observed that compound **11** (in Table 2) has shown significant inhibitory activity against HDAC1, HDAC2, HDAC6, and HDAC8.

Additionally, Patel et al. reported dual-targeted inhibitors by combining semaxanib (SU5416), a known VEGFR-2 inhibitor, with SAHA.[39] From SAR, docking studies and in vitro cancer cell-based assays enzyme inhibitory activity has indicated that (*Z*)-*N*1-(3-((1*H*-pyrrol-2-yl)methylene)-2-oxoindolin-5-yl)-*N*8-hydroxyoctanediamide (**12**) is the lead molecule. The compound also showed cytotoxic effects against breast cancer cell line MDA-MB231, leukemia cell line CCRF-CEM, and melanoma cell line MALME-3 in comparison to SAHA. These results indicate for the further improvement of the hybrid compounds.

The FDA-approved drug pazopanib is effective against VEGFR inhibitors and used for the treatment of advanced renal cell carcinoma.[40] Drug resistance and tumor relapsing have been reported in most patients who have received pazopanib.[41] A report indicates that combination therapy using pazopanib and HDAC enhances antitumor efficacy and minimizes drug resistance.[42] Based on this result, Zang et al. reported a novel set of molecules based on combining HDAC and pazopanib inhibitors.[43] Pazopanib is incorporated into the HDAC inhibitor pharmacophore to form pazopanib-based dual HDAC and VEGFR inhibitors. The two-hybrid compounds containing *ortho*-aminoanilide (**13**) and hydroxamic acid (**14**) have shown similar HDAC inhibitory activity to MS-275 and SAHA, respectively. Moreover, a phase I clinical study indicates that the combination of pazopanib and a HDAC inhibitor has shown an amazing result on pazopanib refractory diseases, so further assessment of *ortho*-aminoanilide-derived compounds is required.

## 6. DUAL C-MET AND HDAC INHIBITORS

Mesenchymal epithelial transition factor, a receptor of hepatocyte growth factor (HGF), is a prototypic member of the receptor tyrosine kinase (RTK) family. When HGF binds to c-Met, phosphorylation occurs and thereby activates c-Met signaling through the RAS/MAPK and PI3K/AKT pathways, which is involved in several biological activities.[44] Cell proliferation, progression, and metastasis happens due to the overexpression of HGF/c-Met signaling pathways.[45] Increased levels of both the c-Met and HGF are associated with poor clinical results in cancer patients.[46] Therefore, c-Met is a potential and attractive target for cancer therapy and drug development.[47] To stop tumor progression, the inhibition of c-Met alone is not sufficient and thus exhibits low efficacy or acquired resistance in clinical trials. The report indicates that HDACs influence c-Met and its downstream signaling pathway.[48] Therefore, the development of single hybrid molecules is needed that concurrently block both c-Met and HDAC, a promising strategy for cancer treatment.

The first c-Met/HDAC dual inhibitor was reported by Lu et al. by hybridizing the c-Met inhibitor and HDAC inhibitors together.[49] Modifications of the side chain of a known c-Met inhibitor do not show any significant effect.[50] To design a new hybrid compound, ZBG was incorporated at the quinoline moiety of the c-Met inhibitor, which conjugated with a pyridazinone-quinazoline moiety as a Cap group via a proper linker. All the designed compounds are effective toward c-Met kinase inhibition similar to c-Met inhibitors exhibit four fold greater inhibitory activity than the standard drug chidamide. The compound containing six carbon linkers (**15**) is most potent as a dual HDAC/c-Met inhibitor. For c-Met inhibition, linker length does not influence any significant role. The results confirmed that blockage of the c-Met and an HDAC pathway simultaneously by a single molecule was a novel approach for anticancer drug development.

A dual HDAC/c-Met inhibitor was discovered by Gong et al., via merging vorinostat (SAHA) and foretinib (a known c-Met inhibitor).[51] The hybrid molecules were synthesized using the pharmacophores of HDAC and c-Met inhibitor. All the synthesized compounds displayed antiproliferative activity against HCT-116, MCF-7, and A549 cell lines. The results showed that among all the synthesized hybrids, the hybrid compound (**16**) can promote the induction of apoptosis and block the G2/M phase.


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## 7. DUAL POLY(ADP-RIBOSE) POLYMERASE (PARP) AND HDAC INHIBITORS

The PARP enzyme is involved in DNA repairing and various cellular processes including transcription. PARP 1 and PARP 2 are involved in the function of base excision pair (BER) and BER repairs single-strand DNA breaks. Inhibition of BER resulted as cell death. PARP inhibitors interfere with BER and as a result DNA repair occurs.[52] Therefore, PARP proteins are ideal targets for anticancer therapy.[53]

The first FDA-approved drug olaparib (PARP inhibitor) is used for the treatment of BRCA-mutated ovarian cancer. Rucaparib and niraparib were also approved by FDA to be used for BRCA-mutated ovarian cancer. However, these drugs have some disadvantages such as poor prognosis rate, low efficacy, and poor clinical outcomes in cancer patients.[54] Therefore, improvement of these drugs are necessary. It is reported that combinations of olaparib and HDAC inhibitors have enhanced anticancer effects. Therefore, to develop dual PARP and HDAC inhibitors, olaparib is conjugated with hydroxamic acid of SAHA.[55] All the synthesized compounds displayed PARP1/2 and HDAC1/6 inhibition activity compared to olaparib and vorinostat (SAHA). The piperazine unit plays an indispensable role in PARP-1 inhibition, not in PARP-2 inhibition. In particular, (*E*)-2-fluoro-*N*-(4-(3-(hydroxyamino)-3-oxoprop-1-en-1-yl)phenethyl)-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzamide (**17**) showed antiproliferative activity in compared with olaparib and SAHA in various cancer cell lines and was 4.1-fold less cytotoxic than SAHA to normal cells MCF-10A. Further study revealed that the compound **17** could induce the cleavage of PARP and regulate tumor cell growth and apoptosis.

For the synergistic inhibition of PARP-1 and histone deacetylases (HDACs), a hybrid molecule was developed by combining olaparib and chidamide, and it is an effective strategy for cancer treatment.[56] Chidamide, a HDAC inhibitor containing a benzamide group, is used in the treatment of refractory peripheral T-cell lymphoma.[57] Tian et al. designed a series of novel 2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoic acid derivatives.[56] Different ZBGs such as benzamide or fluorine-substituted benzamide are introduced to replace hydroxamic acid, and various linkers that connect 3,4-(dihydrophthalazin-1-yl)methyl)benzoic acid to the ZBG moiety of chidamide were investigated to get most potent inhibitor. A benzamide-substituted olaparib hybrid compound (**18**) is active against PARP-1 and HDAC-1 and is as strong as olaparib and chidamide, respectively. Compound **18** has superior inhibitory activity toward BRCA1/2-proficient K562 and MDA-MB-231 cells. Results indicate that compound **18** can be a promising dual PARP-1/HDAC-1 inhibitor for further studies.

## 8. EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)/HDAC DUAL INHIBITORS

EGFR is the key mediator for cancer cell progression and an important target among protein tyrosine kinase (PKT) inhibitors.[58] Erlotinib, gefitinib, and lapatinib, FDA-approved EGFR inhibitors, are effective against multiple solid tumors.[59] However, these drugs are only effective for a small portion of patients because of molecular heterogeneity and drug resistance.[60] To overcome drug resistance and poor prognosis, dual-targeted therapy is one of the recently developed strategies.[61] The pathways are inhibited by the inhibition of histone deacetylases, encouraging the development of EGFR/HDAC inhibitors.[62]

The dual-targeted EGFR/HDAC inhibitor combining the main pharmacophores of erlotinib and SAHA was reported by Cai et al..[63] Quinazoline and phenyl-amino moieties of erlotinib and the hydroxamic acid of SAHA are important for EGFR and HDAC inhibition, respectively. The methoxyethoxy functional group of erlotinib was suitable for incorporation in the hydroxamic acid functionality without affecting EGFR/HER2 binding affinity. The modification of chain length, the repositioning of hydroxamic acid, and substitution in the quinazoline ring have given a series of novel molecules that were mentioned. Among them, the hybrid compound 7-(4-(3-ethynylphenylamino)-7-methoxyquinazolin-6-yloxy)-*N*-hydroxyheptanamide (CUDC-101) has displayed excellent inhibitory activity against HDAC, EGFR, and HER2. CUDC-101 (**19**) has shown more potent antiproliferative activity than vorinostat (SAHA), erlotinib, lapatinib, and combinations of vorinostat/erlotinib and vorinostat/lapatinib. CUDC-101 has completed phase I clinical trials against solid tumors.

A novel dual-targeting inhibitor against EGFR and HDAC was developed by Baer et al. by the combining 4-anilinoquinazoline scaffold of erlotinib with ZBG hydroxamate or 2-aminoanilide moieties of HDAC inhibitors.[64] The combination of erlotinib with an *N*-(2-aminoaryl)benzamide motif linked by methylene ether can selectively inhibit class I HDAC isoforms in the submicromolar range. When the benzamide motif (**20**) is changed to a hydroxamic acid moiety, the hybrid molecule (**21**) inhibits both class I and class II HDAC isoforms. The resultant compound showed improve HDAC inhibition compared to SAHA and exhibited cytotoxic activity.

A series of another set of dual-targeting inhibitors against HDAC and EGFR were synthesized by Mahboobi et al. by combining lapatinib with a HDAC inhibitor.[65] The hydroxamic acid and benzamide were chosen as HDAC inhibitory headgroups and combined with the [3-chloro-4-(3-fluorobenzyloxy)phenyl]quinazolin-4-yl-amine to obtain a chimerically designed compound. The designed hybrid molecules were structurally similar to belinostat, containing (*E*)-3-(aryl)-*N*-hydroxycarbamide as the ZBG, and displayed the most potent activity in inhibiting HDAC. The HDAC inhibitory activity is drastically reduced by positional changes in the *N*-hydroxyacrylamide moiety. The hybrid compounds 3-{5-[4-(3-chloro-4-(3-fluorobenzyloxy)phenylamino]-quinazolin-6-yl}furan-2-yl)-*N*-hydroxyacrylamide hydrochloride monohydrate (**22**) and (*E*)- 3-{3-[4-(3-chloro-4-(3-fluorobenzyloxy)phenylamino)quinazolin-6-yl)phenyl)-*N*-hydroxyacrylamide (**23**) have also shown potent and selective inhibition against the EGFR/HDAC kinase.

## 9. DUAL-TARGETED AGENTS TARGETING HDAC AND C-SRC KINASE

A nonreceptor tyrosine kinase, c-Src, plays a crucial role in various cellular processes. Overexpression of c-Src is correlated with malignant potential.[66] Therefore, c-Src is an important therapeutic target in cancer therapy.[67] Compound **24** is the first selective inhibitor for c-Src.[68] A library of targeted inhibitors that may show a synergistic effect with the c-Src inhibitor was evaluated by Soellner et al.[69] It was reported that panobinostat was combined with c-Src and showed a synergistic effect.[70] HDAC inhibitors can downregulate c-Src


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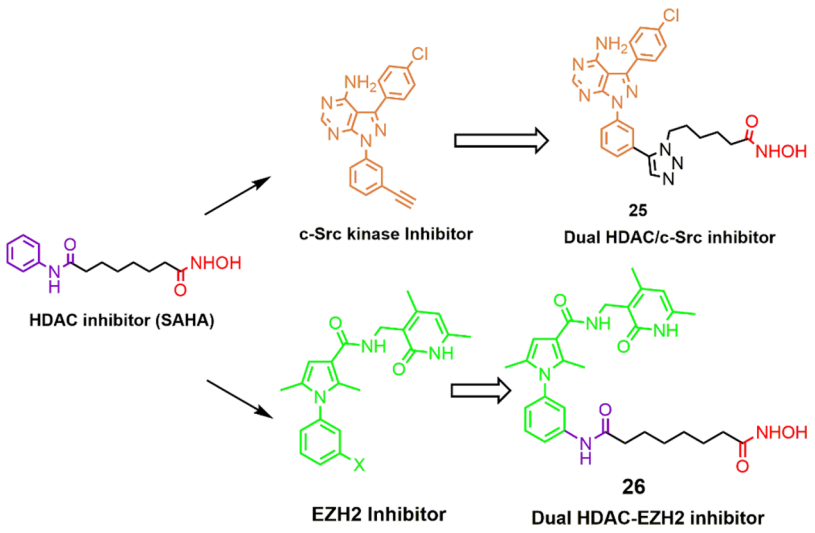


**Figure 5.** Synthetic approach of dual HDAC/c-Src and HDAC/EZH2 inhibitors.

levels through SRC transcription.[71] Taking into account the synergistic efficacy of c-Src and HDAC inhibitors, a set of dual-targeting inhibitors were designed and synthesized. In Figure 5 is shown that the PP2−alkyne scaffold of the c-Src inhibitor[68] attached to HDAC inhibitors via a triazole ring. The evolved hybrid compound has shown optimal efficiency to inhibit both the c-Src kinase and HDAC1. The excessive enhancement efficacy suggests that the chimera (**25**) acts more significantly in comparison to a HDAC inhibitor alone.

## 10. DUAL EZH2/HDAC INHIBITOR

An epigenetic enzyme, polycomb repressive complex 2 (PRC2), is the histone 3 lysine 27 (H3K27) methyltransferase that enhances zeste homologue 2 (EZH2), an epigenetic target, as it helps in the cell cycle, proliferation, and differentiation.[72] A high level of EZH2 is related to a variety of cancers. Some 2-pyridone-based EZH2 inhibitors are under clinical trial, and tazemetostat is approved for the treatment of cancer.[73]

Romanelli et al.[74] reported a series of EZH2 inhibitors that showed significant results by inducing cell growth arrest and apoptosis in different cell lines in breast MDA-MB231, leukemia K562, and neuroblastoma SK-N-BE cells. A branch of compounds were synthesized where pyrazole was replaced by a pyrrole ring to evaluate potent activity against EZH2. The compound 2-pyridone pyrrole decreased the H3K27me3 level and increased the level of p21 and p27 expression, weakening primary glioblastoma. Additionally, when the compound was combined with temozolomide and tazemetostat it showed a remarkable effect on cell viability. To improve the poor efficacy of HDAC inhibitors in solid tumors, the combination of EZH2 with HDAC inhibitors has been developed to boost apoptosis and DNA damage in patient-derived brain-tumor-initiating cell lines. Encouraged by these findings, dual EZH2/HDAC inhibitors have been designed and synthesized by combining EZH2 and SAHA. The new hybrid compound (**26**) in Figure 5 has displayed a noticeable inhibition in several cancers like leukemia U937 and THP1 along with solid cancers such as rhabdomyosarcoma RH4, glioblastoma U87, and neuroblastoma SH-N-SK; specifically, in U937 and RH4 cells the hybrid can control HDAC- and EZH2-dependent histone markers to induce cell cycle arrest in the G1 phase, which results in apoptosis with an increase in the expression of cell differentiation markers. Lastly, it could be concluded that these findings must open a new era with the foremost application of EZH2/HDAC synchronized inhibition in cancer therapy in the near future.

## 11. SIGNIFICANCE OF THE REVIEW

Dual-target therapies can overcome the limitations over single-targeted drugs therapy or a physical mixture combination, including drug−drug interaction, poor safety, low therapeutic index value and other side effects. Histone deacetylase was widely investigated as an anticancer drug target due to its crucial role in various biological processes like cell-proliferation, metastasis, and apoptosis. In this Review, we mainly focus on dual-targeting inhibitors based on HDAC with other antitumor agents and their effectivity in cancer treatments compared to single-target HDAC inhibitors. Numerous known HDAC inhibitors, including vorinostat and panobinostat, are clinically approved but limited in their usage due to their low efficacy, drug resistance, and toxicities; additionally they are nonselective in nature. Therefore, combining HDAC inhibitors with other antitumor agents, topoisomerase inhibitors, RTK pathway inhibitors, and EZH2 in a single molecule exert a synergistic effect on cellular processes in cancer cells.[75] The rationale for the development of dual-targeting inhibitors is to enhance efficacy over the single-target drugs against cancer.

The reported literature indicates that CUDC-101 acted as a dual-targeting EGFR/HDAC inhibitor by combining SAHA and erlotinib. The compound CUDC-101 inhibited the breast cancer cell line MCF-7, and the result shows that the hybrid molecule was more effective than the parent compounds SAHA, erlotinib, and lapatinib. Additionally, CUDC-101 was reported to be more effective than the different combinations of SAHA + erlotinib and SAHA + lapatinib against the MCF-7 cell line. In addition, an in vivo experiment indicates the effectiveness of CUDC-101 against non-small-cell lung cancer (NSCLC), liver, breast, head and neck, colon, and pancreatic cancers.[6] From these results, we can say that a single bifunctional molecule that simultaneously inhibits HDAC and EGFR will be more beneficial than single targeting inhibitor in cancer resistance.


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## 12. CONCLUSION

A new avenue will be opened in cancer treatment through the discovery of dual-targeting drugs. The FDA approved 124 small molecules of single targeted drugs until 2022, and thousands of small molecules are also in clinical trial. However, the single-target small molecules have some disadvantages like drug resistance and low efficiency. In order to solve these problems, a single molecule with a dual targeting ability has evolved for cancer treatment and has entered a rapid development stage. The limitations of small molecules of single-targeted drugs or drug combinations will be overcome with the discovery of dual-targeted drugs. The dual-targeting inhibitors target two oncogenic targeted sites simultaneously and thus have low adverse effects and high therapeutic value, as well as show synergistic effects. In this Review we focus on HDAC-based dual-targeted inhibitors. HDAC has a key role in regulating gene expression and cellular function. Over-expression of HDAC is responsible for many types of cancer. Therefore, it is an important chemotherapeutic target for the treatment of multiple cancers. All the dual-targeting inhibitors mentioned above are laboratory-based synthesized molecules, and they have great opportunities to enter into clinic. For an example, CUDC-101, a dual-targeting inhibitor against EGFR and HDAC, and CUDC-907, a dual-targeting inhibitor against PI3K and HDAC, have entered clinics. The phase I clinical trial of CUDC-101 was done against head and neck squamous cell carcinoma and showed good preclinical activity. The phase I and phase II studies of femepinostat (CUDC-907) were reported with relapsed/refractory diffuse large B cell and high-grade B-cell lymphoma.[14,76] The phase II study of the compound CUCD-907 gives encouraging results, and it has been approved by FDA for the treatment of relapsed or refractory DLBCL. A new kind of HDAC dual drug inhibitor EDO-S101, a combination of SAHA and bendamustine, inhibits both DNA and HDAC. A phase I study of this drug was done against chronic lymphoblastic leukemia (CLL) to investigate its safety and pharmacokinetics. The FDA has approved the dual drug EDO-S101 against cutaneous T-cell lymphoma.[77] In spite of these advantages, there are some disadvantages. In dual-targeted drugs, two main pharmacophores of the known inhibitor are combined and converted into a larger volume molecule than the parent compounds. Therefore, the problems of dual-targeting drugs are poor selectivity, membrane permeability, binding affinity, and pharmacological properties. It is a challenging task to select multiple targets in combination with HDAC with high specificity. Therefore, the knowledge of target−disease relationships, and pathway−target−drug−disease correlations are needed for in-depth understanding. Thus, the challenge for dual-target agents is to balance the two activities when combining two different pharmacophores with the desired pharmacological properties and safety profiles. Therefore, it is very much emergent and necessary to explore further, and structural optimization is also needed due to the improvement of cytotoxicity and antiproliferative activity. Despite the challenges, it is expected that in the near future the development of HDAC-based dual-targeted inhibitors will play a prominent role in cancer drug discovery.

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### Notes

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