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Review Article

PROTACs targeting epigenetic proteins

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ABSTRACT

The field of epigenetics pertains to alterations in gene function that are inherited without changes in the DNA sequence, including histone modifications, post-translational modifications of amino acids, and covalent modifications of DNA bases. These alteration pathways modulate the transformation of genotypes into specific phenotypes. Epigenetics plays major roles in cell growth, development, and differentiation by dynamically regulating gene transcription and ensuring genomic stability. This regulation is performed by three key players: writers, readers, and erasers. In recent years, epigenetic proteins have been found to have crucial roles in epigenetic regulation, and have become important targets in drug research and development. Although targeted therapy is an essential treatment strategy, the effectiveness of targeted drugs is often limited by drug resistance, thus posing a major dilemma in clinical practice. Targeted protein degradation technologies, including proteolysis-targeting chimeras (PROTACs), have great potential in overcoming drug resistance and targeting undruggable targets. PROTACs are gaining increasing attention in the treatment of various epigenetic diseases. In this review, we summarize recently developed degraders targeting epigenetic readers, writers, and erasers. Additionally, we outline new applications for epigenetic protein degraders. Finally, we address several unresolved challenges in the PROTAC field, and suggest potential solutions from our perspective. As the field continues to advance, the integration of these innovative methods holds great promise in addressing the challenges associated with PROTAC development.

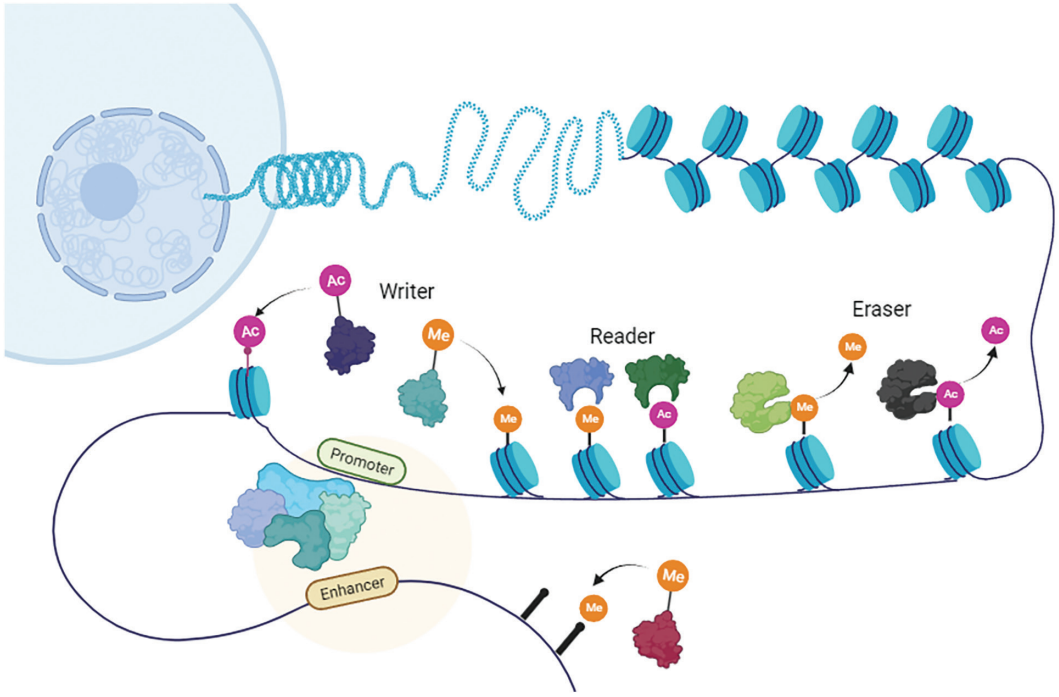
Keywords: PROTACs, Epigenetic protein degraders

1. INTRODUCTION

Epigenetics, which pertains to heritable changes in gene function that do not involve DNA sequence alterations, was first defined by Conrad Waddington in the early 1940s [1]. Originally, the epigenetic molecular pathways included histone modifications, post-translational modifications of amino acids, and covalent modifications of DNA bases, all of which modulate the translation of genotypes into specific phenotypes (**Scheme 1**) [2-6].

Epigenetics substantially contributes to cell growth, development, and differentiation through dynamic regulation of gene transcription and genomic stability [7-13], performed by writers (DNA methyltransferase [DNMT], histone acetyltransferase [HAT], ubiquitin E3 ligases, and histone methyltransferase [HMT]), readers (bromodomains), and erasers (histone deacetylases [HDACs], histone demethylases [KDMs], and deubiquitinating enzymes). Writers add epigenetic marks to DNA or histone tails; readers recognize epigenetic marks; and erasers remove epigenetic marks (**Scheme 2**) [14-18].

Because epigenetics is a key component of normal organism development, epigenetic dysregulation contributes to the origin and progression of human diseases such as cancer and metabolic diseases [19]. For example, HDACs are usually overexpressed in cancers [20]. DNMT3A (R882) mutation is associated with acute myeloid leukemia (AML) [21]. Vorinostat and romidepsin, derived from phenotypic screens, have been identified as HDAC inhibitors [22, 23]. Three other HDAC inhibitors—belinostat, panobinostat, and chidamide—have gained regulatory approval on the basis of lead-compound optimization [19]. Tazemetostat, an inhibitor of the Polycomb repressive subcomplex (PRC) protein enhancer of Zeste homolog 2 (EZH2), has been approved by the FDA for the treatment of relapsed or



Scheme 1 | Roles of epigenetics in human gene regulation.

refractory follicular lymphoma [24]. Progress has been made in using therapeutic epigenetic inhibitors in clinical settings to treat a wide range of tumoral and non-tumoral diseases, yet the applications have been limited primarily to hematological malignancies.

PROteolysis TArgeting Chimeras (PROTACs) are hetero-bifunctional molecules consisting of an E3 ligase ligand and a ligand of the target protein, connected by a linker. These bifunctional molecules are designed to bring the target protein and the E3 ligase into proximity, thus leading to ubiquitination and subsequent 26S proteasomal degradation of the target protein (**Figure 1**) [25-32]. In contrast to the traditional inhibitor approach to drug discovery, which is based on enzymatic activity that can be inhibited and consequently is limited by the target protein's "druggability," PROTACs eliminate target proteins through endogenous degradation pathways, thus achieving depletion of the whole protein rather than merely inhibiting protein function. Newly emerging PROTAC techniques provide major advantages. First, PROTACs require modest target protein binding affinity and have low susceptibility to mutation or overexpression. Second, PROTACs are event driven and initiate degradation in a repeatable manner, thus enabling low doses, administration frequencies, and toxicity. Therefore, PROTAC techniques have attracted wide research interest in academia as well as industry [33-36].

Because epigenetic proteins form complexes with multiple functions, concurrently inhibiting those functions by using inhibitors is difficult. However, elimination of epigenetic proteins can address this issue [19]. PROTACs have been successfully used to degrade epigenetic proteins (**Figure 2**). In this review, we provide a comprehensive summary of reported degraders of epigenetic proteins categorized as readers, erasers, and writers.

2. MAIN TEXT

2.1 Protein degraders of epigenetic readers

2.1.1 BET. The bromodomain is a highly conserved structure that recognizes acetylated lysine residues in histone tails, regulates transcription, and remodels chromatin [37]. The bromodomain-containing proteins Bromo and extra terminal domain family (BET) proteins, such as BRD2, BRD3, BRD4, BRD7, and BRD9, have attracted wide research interest. Recently, numerous degraders targeting BET proteins have been reported to demonstrate potent tumor growth suppression [38-40].

In recent years, PROTACs targeting BET proteins have attracted substantial interest, and numerous degraders have been reported [38, 41-44]. In 2016, the Crews group developed a pan-BET protein degrader named ARV-771 (**Figure 3**). This molecule, a von Hippel–Landau (VHL) E3 ligase-based PROTAC, has shown potent degradative activity in 22Rv1 enzalutamide-resistant prostate carcinoma cells (concentration achieving half-maximal degradation [DC_{50}] < 5 nM), thus resulting in downregulation of cMyc levels and cell apoptosis. Interestingly, ARV-766, a diastereomer of ARV-771, shows little c-MYC suppression. Thus,

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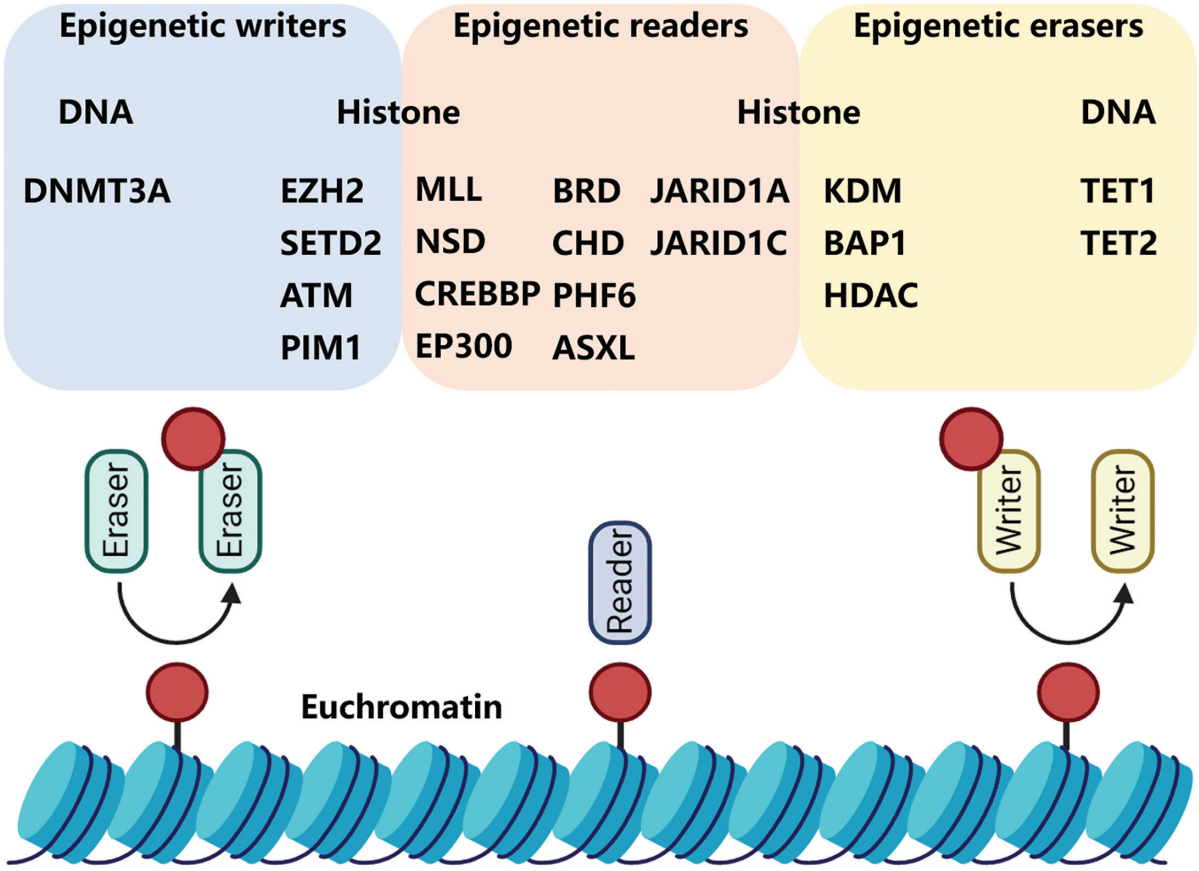

Scheme 2 | Epigenetic tools.
Brown circles represent methylation, acetylation, or phosphorylation marks. DNMT3A, DNA (cytosine-5)-methyltransferase 3A; EZH2, enhancer of Zeste homolog 2 protein; SETD2, SET domain containing 2 protein; ATM, ataxia-telangiectasia mutated protein; PIM1, proto-oncogene serine/threonine-protein kinase; MLL, mixed-lineage leukemia histone methyltransferase; NSD, nuclear receptor-binding SET domain protein; CREBBP, cAMP-responsive element-binding protein-binding protein; EP300, E1A binding protein P300; BRD, bromodomain-containing protein; CHD, congenital heart disease protein; PHF6, plant homeodomain factor 6 protein; ASXL, additional sex combs-like protein; JARID1A, JumonjiC and ARID domain-containing histone lysine demethylase 1A; JARID1C, JumonjiC and ARID domain-containing histone lysine demethylase 1C; KDM, histone lysine demethylase; BAP1, BRCA1-associated protein 1; HDAC, histone deacetylase; TET1, ten-eleven translocation protein 1; TET2, ten-eleven translocation protein 2.

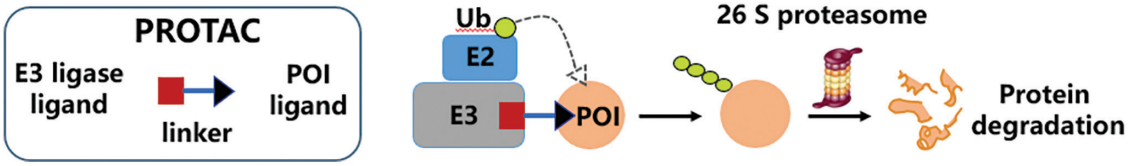

Figure 1 | Introduction to PROTAC technology.

in cellular degradative activity, ARV-771 functions through a catalytic event. In contrast to BET inhibitors, which may be affected by secondary resistance, ARV-771 shows potent efficacy in castration-resistant prostate cancer cells. Furthermore, similar effects have been observed in the VCaP tumor model, with 60% tumor growth inhibition and weight maintenance, whereas no apparent effects have been observed after treatment with enzalutamide [41].

dBET1, a conjugate of JQ1 and pomalidomide, was developed for BET protein degradation by the Bradner group in 2015 (Figure 3) [45]. Treatment of MV4-11 cells with dBET1, even at a low concentration (100 nM, 18 h), has been found to lead to a >85% decrease in BRD4.

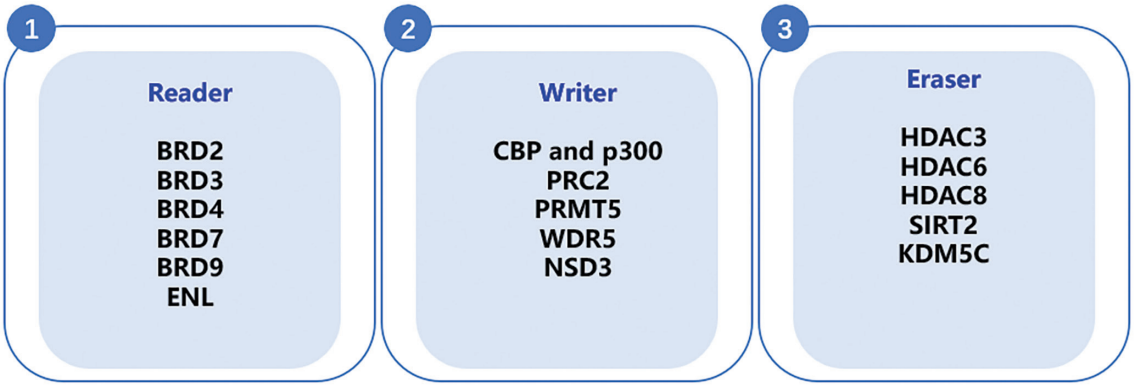

Figure 2 | Targeting epigenetic proteins with PROTAC degraders.

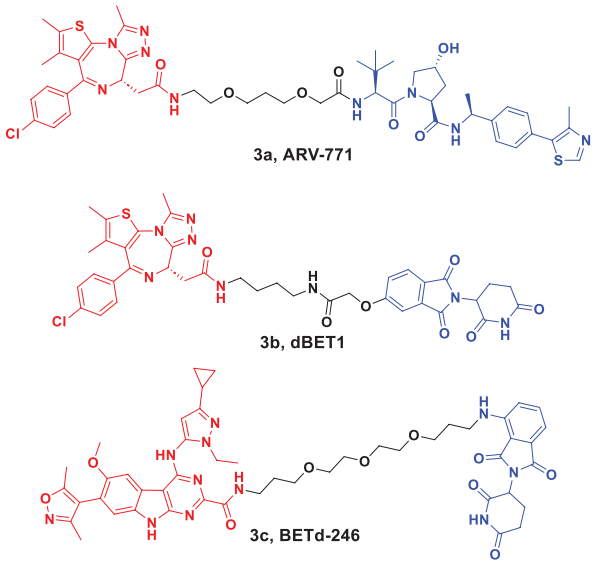

Figure 3 | Representative PROTACs targeting drug-resistant BET.

dBET1 is also more effective than JQ1 in inhibiting the proliferation of human MV4-11 leukemia cells. In vivo studies using a mouse hind-limb xenograft model with MV4-11 cells have demonstrated that dBET1 degrades BRD4 and inhibits tumor growth, without affecting animal weight or normal blood counts. Furthermore, the excised tumors showed a significant downregulation of MYC in these mice compared with vehicle-treated mice.

Triple-negative breast cancer (TNBC) typically responds well to chemotherapy [42]. However, high rates of metastatic disease often occur because of the amplification of MCL1 loci, as frequently observed in chemo-refractory tumors. In patients with TNBC, MCL1 has been found to be both an intrinsic and acquired resistance factor, thus limiting the application of numerous anticancer agents. In 2018, BETd-246, derived from BETi-211, was developed by the Wang group (**Figure 3**). As a degrader of BET proteins in TNBC, BETd-246 degrades BRD2, BRD3, and BRD4 in a dose-dependent manner (30–100 nmol/L, 1 or 3 hours), thus resulting in nearly complete depletion of the target proteins. A BETd-246 concentration of 10 nmol/L in TNBC cell lines has been found to inhibit cell growth and result in rapid downregulation of MCL1 protein. BETd-246 (5 mpk, i.v., triweekly, 3 weeks) has shown comparative anti-tumor activity to that of BETi-211 (50 mpk, p.o., daily, 3 weeks), thus providing an alternative approach to overcome drug resistance in TNBC [40].

Numerous BET protein degraders have been reported. BI2536, as a dual inhibitor, targets two important therapeutic targets for AML: Polo-like kinase 1 (PLK1) and BRD4. In 2020, the Lu group reported a dual degrader based on BI2536 connected to pomalidomide (HBL-4, **Figure 4a**), which targets BRD4 and PLK1 [46]. HBL-4 induces rapid protein degradation in human leukemia cells (e.g., MV4-11, MOLM-13, and KG1). Compared with BI2536, HBL-4 has been found to lead to more potent anti-proliferation and c-Myc suppression efficacy in an MV4-11 tumor xenograft model, and thus may provide a possible threptic option for acute myeloid leukemia. In 2019, the Ciulli group reported MacPROTAC-1, derived from the BET degrader MZ1 by introduction of a macrocycle into the molecule (**Figure 4a**) [47]. This conformationally constrained strategy enabled the molecule to maintain a bioactive configuration, thus decreasing the energetic penalty and providing an alternative approach to drug development. Compared with the BET degrader MZ1, MacPROTAC-1 has been found to result in a more pronounced difference in binding affinity between BD1 and BD2; furthermore, MacPROTAC-1 has been found to exhibit comparable degradation activity and cell proliferation inhibition in BET-sensitive 22RV1 cells.

Although great progress has made in the development of BET protein degraders, selective subtype BET protein degraders avoiding off-target effects remain much needed. In 2020, the Wang group reported that one degrader, 4c, based on a selective BD1 inhibitor

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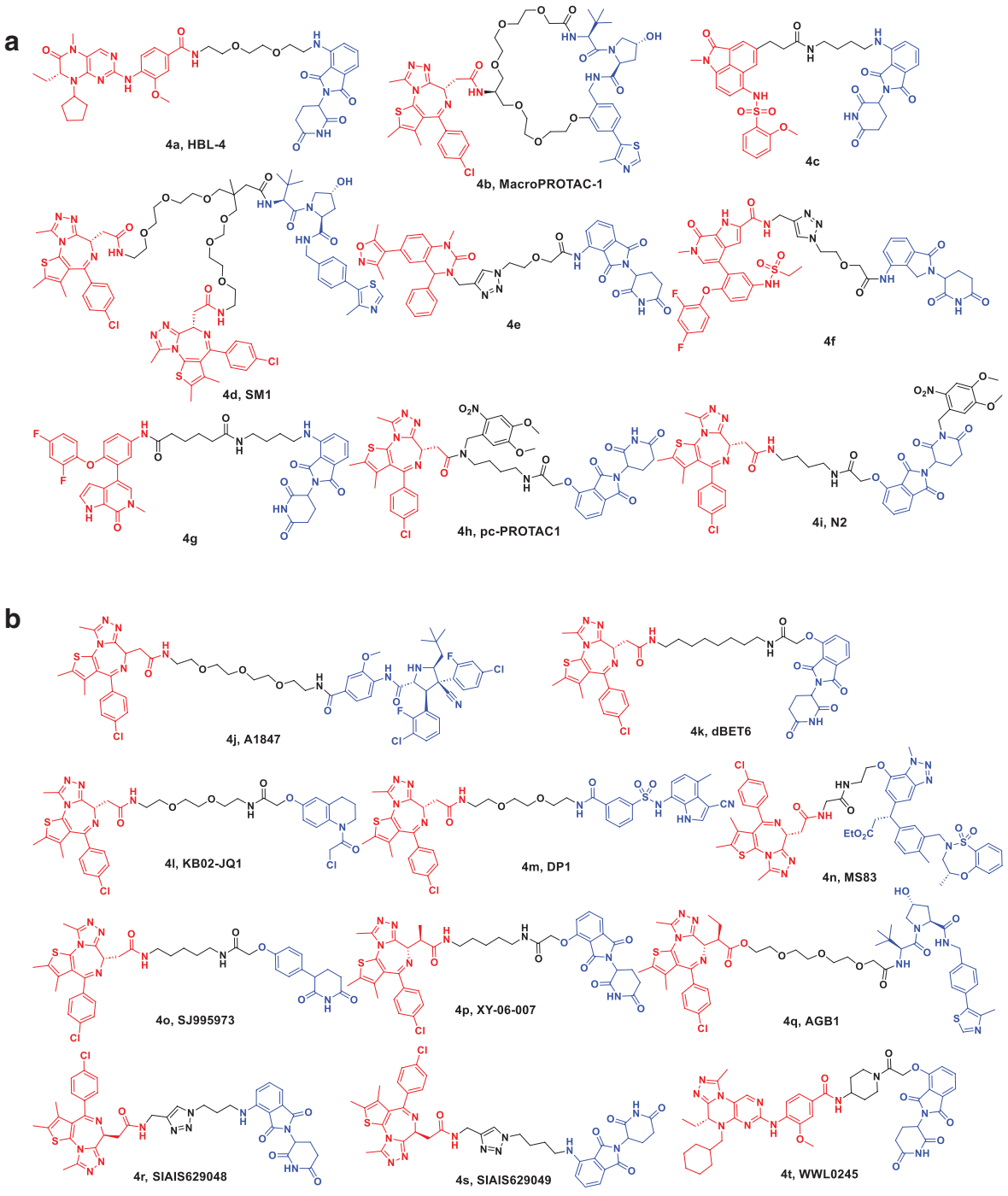

Figure 4 | (a) Representative PROTACs targeting BRD. (b) Representative PROTACs targeting BRD.

combined with thalidomide, demonstrates high selectivity toward BRD2 and BRD4 over other subtypes (**Figure 4a**) [48]. BRD4 has been found to be completely degraded after 8 h treatment with degrader 4c at a concentration at 1 μM in either hematoma or solid tumor cells, thus demonstrating potent cell growth suppression with no clear cytotoxicity.

In 2021, the Ciulli group developed trivalent PROTACs by connecting a bivalent BET inhibitor to an E3 ligand [49]. Compared with bivalent PROTACs, the trivalent degrader 4d with a VHL ligand moiety (SM1, **Figure 4a**) shows higher degradation potency and stronger anticancer activity. Mechanistically, the degrader 4d (SM1) forms a 1:1:1 ternary complex with VHL, BD1, and BD2,

thus prolonging the residence time. Despite its higher molecular weight, the trivalent degrader 4d (SM1) shows enhanced cell permeability and a highly favorable PK profile. In addition to common structures such as JQ1, an increasing number of BET inhibitors have been used in the development and production of PROTACs.

In 2019, Zhang's team created a novel degrader based on a highly effective dihydroquinazolinone-based BRD4 inhibitor. Degrader 4e (**Figure 4a**) at a concentration of 1 μM after a 3-hour treatment achieves complete BRD4 degradation. Additionally, the degrader has remarkable cell growth inhibition ability, with an IC_{50} of 0.81 μM in THP-1 human leukemia monocytic cells, surpassing the potency of dihydroquinazolinone-based BRD4 inhibitor by four-fold in antiproliferative assays. In subsequent studies, the Zhang group has used ABBV-075 derivatives to develop PROTACs [50]. In 2020, the Zhang group incorporated linkers at the pyrrole ring position, thus creating degrader 4f (**Figure 4a**). This degrader effectively induces degradation of BRD4, cell-cycle arrest, and apoptosis in the human pancreatic cancer cell line BxPC-3. The antiproliferative activity of degrader 4f against the BxPC-3 cell line (IC_{50} = 0.165 μM) is approximately seven-fold higher than that of ABBV-075. In 2021, the Yu group reported the development of another BRD4 degrader, 4g (**Figure 4a**), created by connecting an ABBV-075 derivative to an E3 ligand [51]. This molecule has been found to have a DC_{50} of 0.25 nM in MV4-11 cells and 3.15 nM in RS4-11 cells. Additionally, this degrader has been found to suppress proliferation of human leukemia cells (MV4-11 and RS4-11), with an IC_{50} of 0.5 nM and 4.8 nM, respectively.

Because of the crucial functions of BET proteins in cells, targeting these proteins with PROTACs may lead to harmful effects in healthy cells, thereby limiting their clinical use. To address this challenge, several strategies have been used to regulate PROTACs in space and time. One commonly used approach involves incorporating a photocaged group into the PROTACs. In 2019, the Pan research group modified dBET1 at the nitrogen site of JQ1 moiety with a bulky 4,5-dimethoxy-2-nitrobenzyl group, thus yielding the degrader 4h (pcPROTAC1, as shown in **Figure 4a**). Under light irradiation, this degrader induces degradation of BRD4 in live cells. In a zebrafish model, treatment with the degrader has been found to decrease BRD4 levels and lead to corresponding phenotypic changes [52]. The Li research group has developed a similar degrader (N2, **Figure 4a**) by incorporating the 4,5-dimethoxy-2-nitrobenzyl group onto the glutarimide nitrogen of dBET1. Under exposure to UV light, the degrader has been found to induce the degradation of BRD4 in HEK293T cells, whereas in a zebrafish xenograft model, it has been found to suppress the growth of tumors derived from HN-6 tongue squamous cell carcinoma cells [53].

In 2020, Lu et al. discovered that the BRD4-degrading PROTAC A1874 (**Figure 4b**) induces BRD4 protein degradation, and downregulates BRD-dependent genes, such as c-Myc, Bcl-2, and cyclin D1 in colon cancer. Furthermore, A1874 exhibits greater efficacy against colon cancer than BRD4 inhibitors including JQ1, CPI203, and I-BET151. Additionally, in BRD4-knockdown colon cancer, A1874 maintains its cytotoxicity, thus suggesting that an alternative BRD4-independent mechanism might potentially be mediated by A1874's ability to enhance p53 stability and ROS production in a dose-dependent manner. That study has confirmed the outstanding anticancer activity of A1874 against colon cancer cells, thus laying the groundwork for potential clinical translation [54].

In 2021, the Dashwood group investigated the antitumor activity of a BET degrader (dBET6) and HDAC3-specific inhibitor (BG45). In SW620 (metastatic colon cancer cell line) xenografts, the combination of BG45 and dBET6 (**Figure 4b**) demonstrates a greater antitumor efficacy compared to the individual inhibition observed with BG45 or dBET6 alone ($p < 0.05$), thus providing further support for the combination of HDAC3 with BRD4 [55].

Furthermore, novel E3 ligases appropriate for use in PROTACs have been evaluated for their potential to develop BRD4 degrading agents. The Cravatt research group reported DCAF16, a newly discovered E3 ligase, in 2019. As a result of this discovery, they designed a BRD4 degrader, called KB02-JQ1 (**Figure 4b**), using reversible ligands to target DCAF16 along with JQ1. However, to achieve degradation, a concentration of 20 μM is required [56]. In 2020, the Chen group developed the novel BRD4 degrader 4m by using E7820, a DCAF15 ligand, in combination with JQ1 (**Figure 4b**). This degrader has been found to have a DC_{50} of 10.84 μM in SUDHL-4 cells and an impressive 98% maximum degradation rate [57]. The Jin group has also developed the novel BET protein degrader 4n by conjugating JQ1 with KI696, a KEAP1 ligand (**Figure 4b**). Notably, in MDAMB-231 human breast cancer cells, this degrader decreases the protein levels of both BRD4 and BRD3, without affecting BRD2 protein levels. Additionally, the degrader selectively degrades the short isoform of BRD4 while leaving the long isoform unaffected [58]. The Rankovic group's recent discovery that phenyl glutarimide binds CRBN has led to the development of a new BRD4 degrader, SJ995973 (**Figure 4b**). This degrader has similar binding affinity to those of previously reported degraders that use CRBN as the E3 ligase, but has greater stability. In MV4-11 cells, SJ995973 at a concentration of only 0.87 nM induces 50% BRD4 degradation [59].

Through a bump-and-hole strategy, the Fischer group designed a CRBN-based degrader (XY-06-007) that specifically targets BRD4BD1L94V in 2021 (**Figure 4b**) [60]. According to proteomics analysis, this degrader has excellent BRD4BD1L94V selectivity over wild-type or other BET family bromodomains. Furthermore, XY-06-007 has shown good pharmacokinetics in vivo studies.

Recently, the Ciulli group developed the novel VHL-based degrader 4q (AGB1, **Figure 4b**) through a bump-and-hole approach [61]. In an inducible BromoTag degron system, the degrader 4q (AGB1) not only

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forms a strong, cooperative ternary complex between VHL and the BromoTag-BRD2, but also induces complete degradation of BromoTagged target proteins, with low-nanomolar potency. The degrader 4q (AGB1) exhibits excellent selectivity over the native wild-type BET, thereby avoiding cytotoxicity in several cancer relevant cell lines. In summary, these two different methods have provided useful tools to study the effects and implications of rapid, highly selective degradation of target proteins.

In 2021, the Jiang group reported the synthesis of azide substrates based on IMiDs. These compounds were then associated with various proteins of interest (POIs) through a click reaction, thus enabling screening of degraders targeting BET family proteins. Among these compounds, the degraders SIAIS629048 and SIAIS629049 at 50 nM exhibited potent activity in degrading BET proteins and showed strong anti-proliferative activity against MV4-11 cells (**Figure 4b**) [62].

In 2022, the Wang group synthesized WWL0245, which selectively degrades BRD4 (**Figure 4b**). This degrader has shown potent antiproliferative effects in AR-positive prostate cancer cell lines. Additionally, WWL0245 has been found to induce the degradation of BRD4 with a sub-nanomolar DC_{50} and to achieve >99% maximum percentage degradation (D_{max}) in the aforementioned cell line. In BETi-sensitive cancer cells, such as AR-positive prostate cancer cells, WWL0245 has potent antiproliferative activity, with an IC_{50} of 3 nM in MV4-11 cells [63].

In 2017, the Bradner group first reported the degradation of BRD9, a subunit of the human BAF (SWI/SNF) nucleosome remodeling complex, by a molecule named dBRD9. This molecule at a concentration of 50 nM exhibits potent degradation ability toward BRD9. Additionally, in the MOLM13 human acute myeloid leukemia cell line, dBRD9 has shown superior antiproliferative effects to the non-degrading probe, with an excess ranging from 10 to 100 times (**Figure 5**) [64].

In 2019, the Ciulli group developed VZ185 by conjugating ligands of VHL and BRD9 (**Figure 5**). In addition to degrading BRD9 with a DC_{50} value of 1.8 nM, VZ185 degrades BRD7 with a DC_{50} value of 4.5 nM. In the EOL-1 acute myeloid eosinophilic leukemia and A-204 malignant rhabdoid tumor cell lines, VZ185 has been found to exhibit potent cytotoxic effects, with EC_{50} values of 3 and 40 nM, respectively [65].

The catalytic function of the BAF nucleosome complex is executed by an ATP-dependent helicase, either SMARCA2 or SMARCA4. These two proteins have a conserved bromodomain that interacts with acetylated chromatin. Both proteins share high protein sequence identity. Preclinical genetic studies have indicated that achieving selective inhibition of SMARCA2 is likely to be essential in developing successful therapeutics. In 2022, the Yauch group discovered A947, a potent and highly selective PROTAC molecule targeting SMARCA2 (**Figure 5**). In SW1573 cells, A947 has been found to degrade SMARCA2 with a DC_{50} of 39 pM, achieving 96% maximal degradation at 10 nM. In contrast, it requires a 28-fold higher concentration of A947 to achieve a DC_{50} (1.1 nM) toward SMARCA4, achieving 92% maximal degradation at approximately 100 nM. Global ubiquitin profiling and proteomic analysis have further confirmed

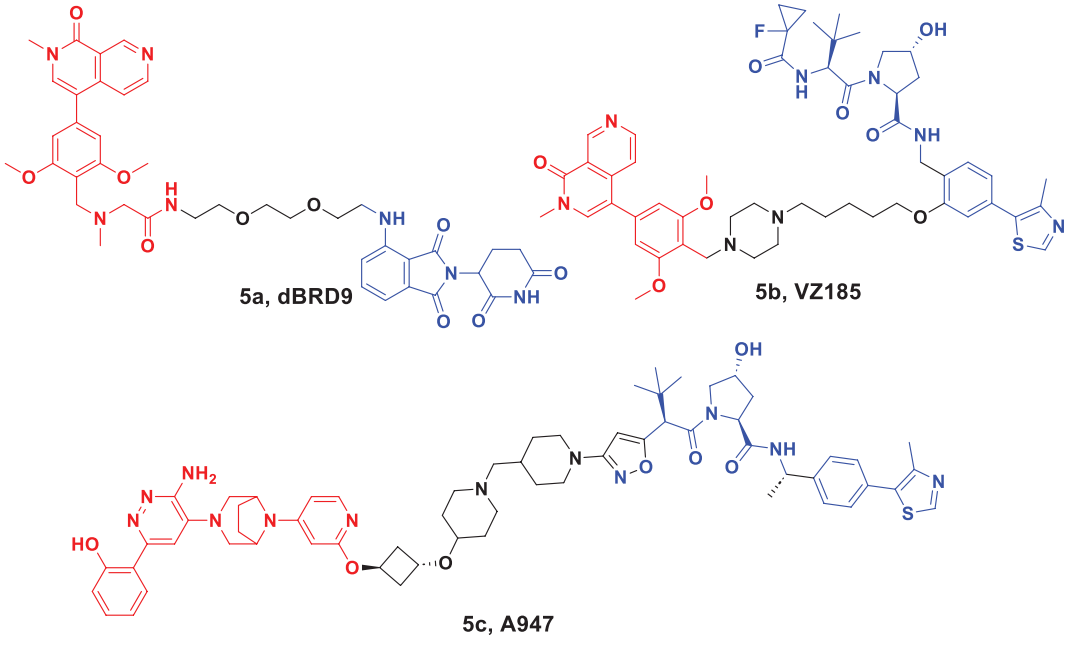


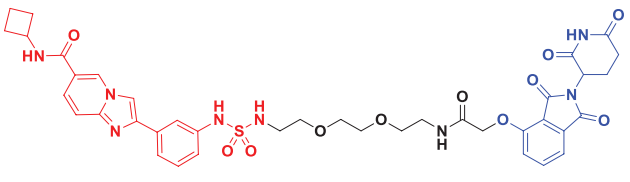
Figure 5 | Representative PROTACs targeting proteins of the BAF nucleosome complex.

the high specificity of A947 in degrading these target proteins at high concentrations [66].

2.1.2 ENL. The YEATS domain is classified as a histone acetylation "reader." ENL is one of the four human-genome-encoded proteins containing a YEATS domain. ENL protein is essential for survival in AML. Anti-leukemia effects and leukemia growth inhibition have been observed after ENL knockout. In 2021, the Erb group implemented a SuFEx-based strategy for high-throughput medicinal chemistry, thus leading to the discovery of a remarkably effective inhibitor, SR-0813, targeting the ENL YEATS domain. Building on the identification of SR-0813, the researchers developed SR-1114, a degrader that specifically targets ENL (**Figure 6**). In MV4-11 cells, treatment with SR-1114 has been found to achieve CRBN-dependent degradation of ENL with a DC_{50} value of 150 nM. Maximum degradation of ENL is observed within 4 hours of treatment with a concentration of 10 μM, but ENL can be resynthesized within 24 hours [67].

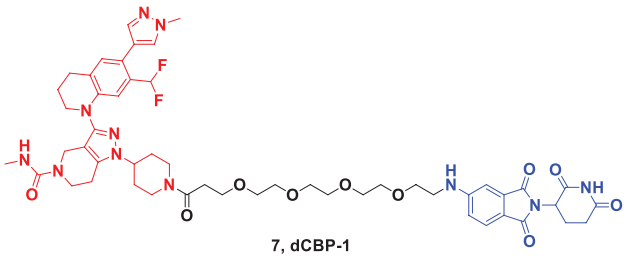
2.2 Protein degraders of epigenetic writers

2.2.1 CBP. The paralogous chromatin regulators CREB-binding protein (CBP) and p300 (also known as KAT3A and KAT3B) are key transcription factors that establish and activate enhancer mediations [68]. p300/CBP exert enzymatic function through a lysine acetyltransferase region, which can dynamically acetylate approximately 5000 lysines on more than 21000 proteins. These proteins also mediate protein-protein interactions on chromatin [69]. In cancer, p300/CBP is considered an oncogene and tumor suppressor, and selective inhibitors targeting its KAT domain have demonstrated to be a promising cancer treatment strategy [70]. However, inhibition of a single domain is not sufficient to completely eliminate p300/CBP activity in cells. Therefore, inhibitors that simultaneously inhibit multiple domains or even completely eliminate p300/CBP must be developed. In 2021, Ott et al. reported the first CRBN-based p300/CBP PROTAC, dCBP-1 (**Figure 7**) [71]. This degrader has high antiproliferative activity in multiple myeloma and significantly downregulates the oncogene MYC. Treatment with dCBP-1 also decreases the enhancer histone acetylation and chromatin accessibility, and is more effective than the KAT domain and bromine domain inhibitors alone or in combination. As a highly potent CBP/p300 degrader, dCBP-1 is a useful tool to investigate



6, SR-1114

Figure 6 | Representative PROTAC targeting ENL.



7, dCBP-1

Figure 7 | Representative PROTAC targeting CBP and p300.

the mechanisms through which these factors coordinate enhancer activity in cells.

2.2.2 PRC2. Polycomb repressive complex 2 (PRC2), an epigenetic regulator of transcription, consists of four subunits, EZH1/2, EED, SUZ12, and RbAp46/RbAp48. EZH2 is a key catalytic subunit in the PRC2 complex [72]. PRC2 has histone methyltransferase activity, and can methylate H3K27. Hyper-trimethylation of H3K27 is observed in various types of tumors. PRC2 is both an oncogene and a suppressor of tumorigenesis in multiple cancer types, such as colorectal, breast, and prostate cancers [73]. Existing inhibitors targeting EZH2 and EED subunits effectively inhibit the catalytic activity of the PRC2 complex and achieve anti-tumor activity. However, resistance mutations to this small-molecule inhibitor have been observed in clinical trials, and targeting PRC2 protein degradation may serve as an alternative strategy for this competitive inhibition [74].

Since 2019, a series of PROTACs targeting PRC2 subunits including EED and EZH2 have been developed, which inhibit the activity of the PRC2 complex. Bloeche et al., by combining the existing EED inhibitor MAK683 with a VHL ligand, developed the first PRC2 degrader, 8a (**Figure 8**), which promotes ternary complex formation by VHL. EED-targeted PROTACs simultaneously induce efficient and selective degradation of EED, EZH2, and SUZ12; effectively inhibit PRC2 enzymatic activity (pIC_{50} ~8.1); and decrease EZH2-dependent cancer cell proliferation (GI_{50} = 49–58 nM) [75]. James et al. have also reported the PROTAC degrader UNC6852 (**Figure 8**), based on the EED ligand and VHL ligand. This degrader selectively degrades EED (DC_{50} = 0.79 μM), EZH2 (DC_{50} = 0.3 μM), and SUZ12, thus resulting in a loss of PRC2 catalytic activity and decreased H3K27me3 levels in HeLa cells. This degrader has antiproliferative effects in diffuse large B-cell lymphoma cell lines with EZH2 activating mutations [74]. In 2020, Jin et al. generated MS1943 (**Figure 8**), based on a selective non-covalent inhibitor of EZH2, by using a hydrophobic labeling method. This first-in-class EZH2 selective degrader has been found to effectively decrease levels of EZH2 in MDA-MB-468 cells at 5 μM. In addition, MS1943, the degrader targeting the EZH2 protein, compared with inhibitor treatment, significantly inhibits growth and induces apoptosis in TNBC cells [76]. In 2021, Yu et al. [77] developed the

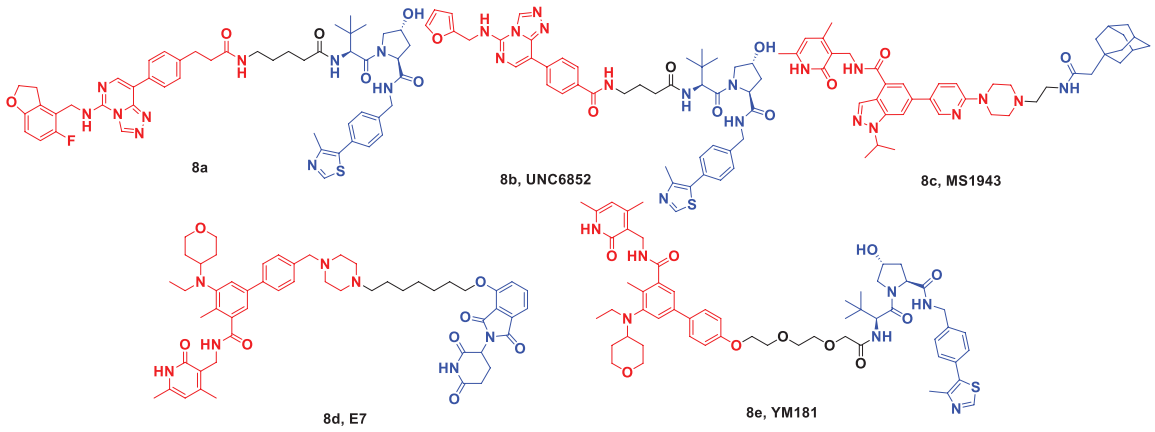


Figure 8 | Representative PROTACs targeting PRC2 (EZH2, EED).

degrader E7 (**Figure 8**) based on the CRBN ligand and the clinical EZH2 inhibitor EPZ6438; this degrader has been found to degrade all PRC2 subunits at 1 μM in WSU-DLCL-2 cells (EZH2 72%, SUZ12 81%, EED 75%, and RbAp48 74%). In the same year, Wen et al. [78] reported a similar VHL-based degrader that induces 50% degradation of EZH2 protein levels at 1 mM. The degrader YM181 (**Figure 8**) robustly inhibits cell viability in diffuse large B-cell lymphoma and other subtypes of lymphomas. Overall, degrading PRC2 in cancer therapy has been demonstrated to be an effective strategy, and some degraders have anticancer activity equal to or better than those of inhibitors.

2.2.3 PRMT5. Arginine methylation is a common post-translational modification, which is regulated primarily by protein arginine methyltransferase (PRMT). Nine PRMT members have been identified in mammals and found to catalyze the production of three forms of methylated arginine [79]. PRMTs are classified as type I, type II, or type III enzymes according to their methylation products. The function of type I PRMTs, including PRMT1, 2, 3, 4, 6, and 8, is to catalyze the formation of monomethylarginine, which is subsequently further catalyzed into asymmetric dimethylarginine [80]. Type II PRMTs, including PRMT5 and 7, catalyze the formation of monomethylarginine intermediates, which are further catalyzed into symmetric dimethyl intermediates. Type III PRMT enzymes, primarily PRMT7, catalyze the formation of only monomethylated products.

PRMT5 (also known as Hsl7, Jbp1, and Skb1) is often considered a strong transcriptional suppressor and was first identified as a JAK2-binding protein that methylates its H2A, H3, and H4. PRMT5 plays important roles in development and cancer. Thus, PRMT5 is an important drug target whose overexpression has been associated with heart disease; infectious diseases; and cancers, such as breast, lung, and liver cancer [81, 82]. In 2020, Jin et al. [83] reported the first PRMT5 selective degrader, MS4322, formed by linking the inhibitor EPZ01566619 and the VHL ligand through PEG chains (**Figure 9**). This degrader has been found to effectively decrease PRMT5 protein levels in the human breast cancer cell line MCF-7, with a DC_{50} of 1.1 μM. MS4322 also significantly decreases PRMT172 protein levels in other cancer lines, such as HeLa, A5, A549, and Jurkat cells, and inhibits the proliferation of these cells. This degrader is therefore a valuable chemical tool for exploring PRMT5's functions in health and disease.

2.2.4 WDR5. The chromatin associated WD50 repeat domain protein 5 (WDR5) is a functional subunit of the mixed lineage leukemia (MLL) histone methyltransferase complex (also known as the MLL complex), which catalyzes H3K4 methylation [84-86]. WDR5, a major component of this complex, is essential for chromatin methylation. WDR5 has a doughnut-shaped propeller structure with two main surface binding sites: WDR5-interaction site (WIN) and WDR5-binding motif (WBM) sites [87]. The WIN position is required for WDR5 chromatin recruitment and interaction with the KMT2 enzyme, and the function of MLL1 is particularly dependent on this interaction. WBM sites mediate protein-protein interactions with a variety

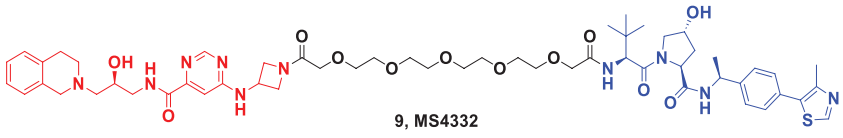


Figure 9 | Representative PROTAC targeting PRMT5.

of non-MLL partners, such as c-MYC. WDR5 is overexpressed in many solid tumors, including pancreatic ductal adenocarcinoma, and promotes tumorigenesis [88, 89]. Multiple inhibitors that block the binding of WDR5 to its partner have been successfully developed by targeting WIN and WBM binding sites; however, inhibitors that block protein-protein interactions between WDR5 and its binding partner generally have only relatively weak antitumor activity [90, 91]. On the one hand, the occupation-driven mode might prevent permanent and complete blocking of protein-protein interactions; on the other hand, the inhibitor might target only part of the WDR and might not affect all carcinogenic functions. Therefore, a new treatment strategy must be developed. PROTACs have been shown to have mechanisms unique from those of inhibitors, by pharmacologically enabling the degradation of POI, thereby temporarily eliminating all POI functions [92].

In May 2021, Knapp et al. [93] reported two families of WDR5 degraders with two different WIN site-binding stents: one based on the existing inhibitor OICR-9429 and one based on a modified pyrroimidazole scaffold. A variety of E3 ligands have been connected to the modified OICR-9429 scaffold with linkers of different lengths to obtain the degrader 10a (**Figure 10**). This degrader has been found to induce degradation of 58% of WDR5 with a DC_{50} value of 53 nM in MV4-11 cells. The 10b (**Figure 10**) molecule, developed on the basis of a modified pyrrole imidazole scaffold and VHL ligand, induces 53% degradation of WDR5 with a DC_{50} value of 1.24 μM. In September of the same year, Jin's research group also reported MS67 (**Figure 10**), based on the inhibitor OICR-9429 and the VHL ligand. In the first round of design, the MS33 degrader (**Figure 10**) (DC_{50} = 260 nM) was obtained, and the structure of the VHL-MS33-WDR5 ternary complex was successfully solved. On the basis of the crystal structure, the inhibitor ligand and linker were further optimized to obtain MS67 (DC_{50} = 3.7 nM) and the VHL-MS67-WDR5 ternary complex. MS67, compared with other WDR5 inhibitors, has been shown to significantly inhibit tumor growth in a mouse model of AML PDX [94].

2.2.5 NSD3. Nuclear receptor binding SET domain protein 3 (NSD3; also known as KMT3F or WHSC1L1), is a lysine methyltransferase at position 36 of histone H3 (H3K36) that catalyzes the dimethylation of H3K36 [95]. The NSD3 gene, localized within an amplicon locus of 8p11-p12 in breast and squamous lung cancers, encodes two splicing variants: the NSD3-short (NSD3S) and NSD3-long (NSD3L) isoforms [96]. Overactivity of the NSD3 protein is closely associated with development of many types of tumors, including human AML, breast tumors, and lung cancer [97-99]. Therefore, NSD3 is considered a potential target for novel anticancer drugs. Several inhibitors of NSD3 have been reported, among which BI-9321 blocks the NSD3 PWWP1 domain (a mode retained in both NSD3L and NSD3S), but this inhibitor does not have effective anti-cancer activity [100]. Using a PROTAC strategy to develop NSD3-targeting degraders is expected to yield more efficient antitumor compounds. In 2022, Wang et al. [101] reported the first selective degrader of NSD3, MS9715 (**Figure 11**), formed by linking the inhibitor BI-9321 to different E3 ligands via varying linker lengths. MS9715 showed the best NSD3 degradative activity in human acute myeloid leukemia cells (MOLM-13), with a DC50 of 4.9 μM and D_{max} greater than 80%. In addition, transcriptomic analysis indicated that MS9715 effectively inhibits the expression of NSD3 and cMyc-associated genes, and has better anti-tumor activity than inhibitors.

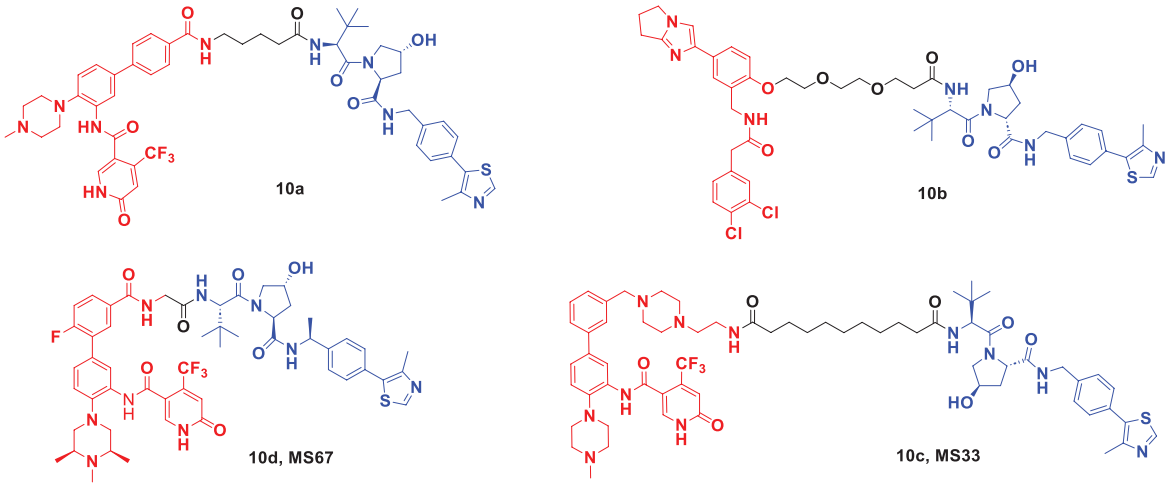


Figure 10 | Representative PROTACs targeting WDR5.

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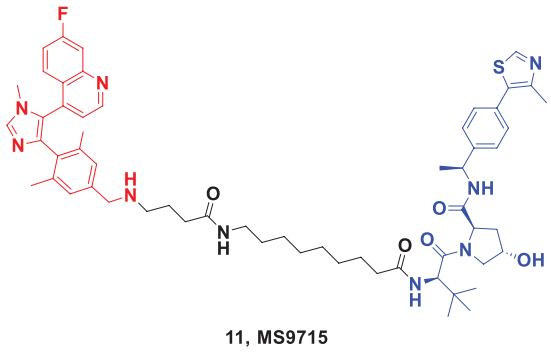

11, MS9715

Figure 11 | Representative PROTAC targeting NSD3.

2.3 Protein degraders of epigenetic erasers

2.3.1 HDACs. HDACs are a class of important epigenetic regulatory factors, which are responsible for catalyzing histone deacetylation, as "erasers." Histones tightly bind negatively charged DNA and inhibit gene transcription. HDACs play key roles in regulating both gene expression and cell signal transduction pathways, and are considered attractive therapeutic targets [102]. Currently, 18 HDAC enzymes are known in humans, and can be divided into four classes. Class 1 consists of HDACs 1, 2, 3, and 8. Class 2 is divided into class 2a (HDACs 4, 5, 7, and 9) and class 2b (HDACs 6 and 10). Class 3, also known as SIRT, consists of SIRTs 1, 2, 3, 4, 5, 6, and 7. Class 4 is represented by HDAC11 [103, 104]. Class I HDACs are expressed in a variety of tissues and are located primarily in the nucleus. HDACs 1, 2, and 3 isoenzymes exist in large multiprotein complexes, and HDAC8 acts independently of the multiprotein complex [105-107]. Class II enzymes show tissue specificity, responding to different cell signaling responses, and shuttling between the nucleus and cytoplasm. HDAC6 is the only protein in the HDACs family with two functionally independent catalytic domains and a ubiquitin-bound zinc finger domain [108, 109]. Class III is completely different from the atypical histone deacetylase family of other HDACs. HDAC11, type IV, is expressed in the brain, heart, kidneys, testes, and skeletal muscle, and has nuclear localization characteristics. HDACs have also been shown to have varying catalytic reactivity toward acetylated lysine, and only HDACs 1, 2, 3, and 6 have shown lysine deacetylase activity in vitro [110].

Dozens of drugs with HDAC-inhibiting properties have been developed, mainly for the treatment of hematoma, and promising advances have been made in inflammatory diseases and neurodegenerative diseases. However, some HDACs, such as HDAC3, have scaffold functions beyond their catalytic functions, thereby limiting the efficacy of inhibitors [111]. In addition, many currently approved HDAC inhibitors non-selectively target various HDACs and exhibit significant toxicity. PROTACs, as a new protein degradation technology, are expected to overcome various drawbacks of inhibitors.

In 2018, Tang et al. developed dHDAC6 (**Figure 12a**), the first degrader of the HDAC family; this degrader, based on a non-selective HDAC inhibitor and the E3 ligand pomalidomide [112], has a DC_{50} and maximum percentage degradation (D_{max}) of 34 nM and 70.5%, respectively. In 2019, Rao et al. obtained a novel HDAC6 degrader, NP8 (**Figure 12a**), based on the selective HDAC6 inhibitor Nexturastat A (Nex A) and the CRBN ligand pomalidomide. Subsequently, the researchers connected pomalidomide to the benzene ring of NexA from an alternative site and obtained degrader NH2 for HDAC6 (**Figure 12a**), with significantly improved activity with respect to that of NP8, and a DC_{50} of 3.2 nM in MM.1S human multiple myeloma cells. These findings indicated that the good flexibility of the ternary compound [113, 114]. In 2020, the Tang group obtained the first selective HDAC6 degrader, 12d (**Figure 12a**), based on the NexA and VHL ligand. The DC_{50} values of the most potent degrader, 12d, are 7.1 nM and 4.3 nM in human MM1S and mouse 4935 cell lines, respectively. The researchers then developed a competitive assay to evaluate the binding affinity of different E3 ligands in cells and screened for libraries of thalidomide analogs, including those with partial linkers. By combining the most active E3 ligand with the pan inhibitor SAHA, the researchers identified a selective HDAC6 degradation product, YZ167 (**Figure 12a**), with a DC_{50} of 1.94 nM in MM.1S cells. In addition, the degradation compound YZ268 (**Figure 12a**), based on the selective HDAC6 inhibitor Next-A, also has selective degradation activity toward HDAC6, without affecting the new substrates IKZFs and GSPT1 [115-117]. In 2021, He et al. coupled a selective HDAC6 inhibitor derived from the natural product indirubin with pomalidomide to obtain the new degrader 12g (**Figure 12b**), with a DC_{50} of 108.9 nM and a D_{max} of 88%. The application of this HDAC6 reducer in LPS-induced mice attenuated NLRP3 inflammasome activation, thus providing the first demonstration that an HDAC6 PROTAC may be a novel strategy for the treatment of NLRP3 inflammasome-associated diseases [118].

In addition to the above-mentioned degradation agents that selectively target HDAC6, many degradation agents targeting other HDAC subtypes, including HDAC1, HDAC2, HDAC3, and HDAC8, have been reported in the past 2 years. Hodgkinson's group has reported the class I HDAC (HDAC1/2/3) degrader 12 h (**Figure 12b**), based on the VHL and HDAC inhibitor CI-994, which induces degradation of HDAC1/2/3 in HCT116 cells. Subsequently, optimization of the linker of 12 h and VHL ligands on the basis of the developed class I histone deacetylase PROTACs yielded a selective degrader targeting HDAC1/2, and further demonstrated that HDAC1/2 is essential for inducing apoptosis and cell arrest in cancer cells [119, 120].

Hansen et al. have discovered a series of alkylated HDACIs by using pharmacophore linking strategies and applied HAIR technology to synthesize the

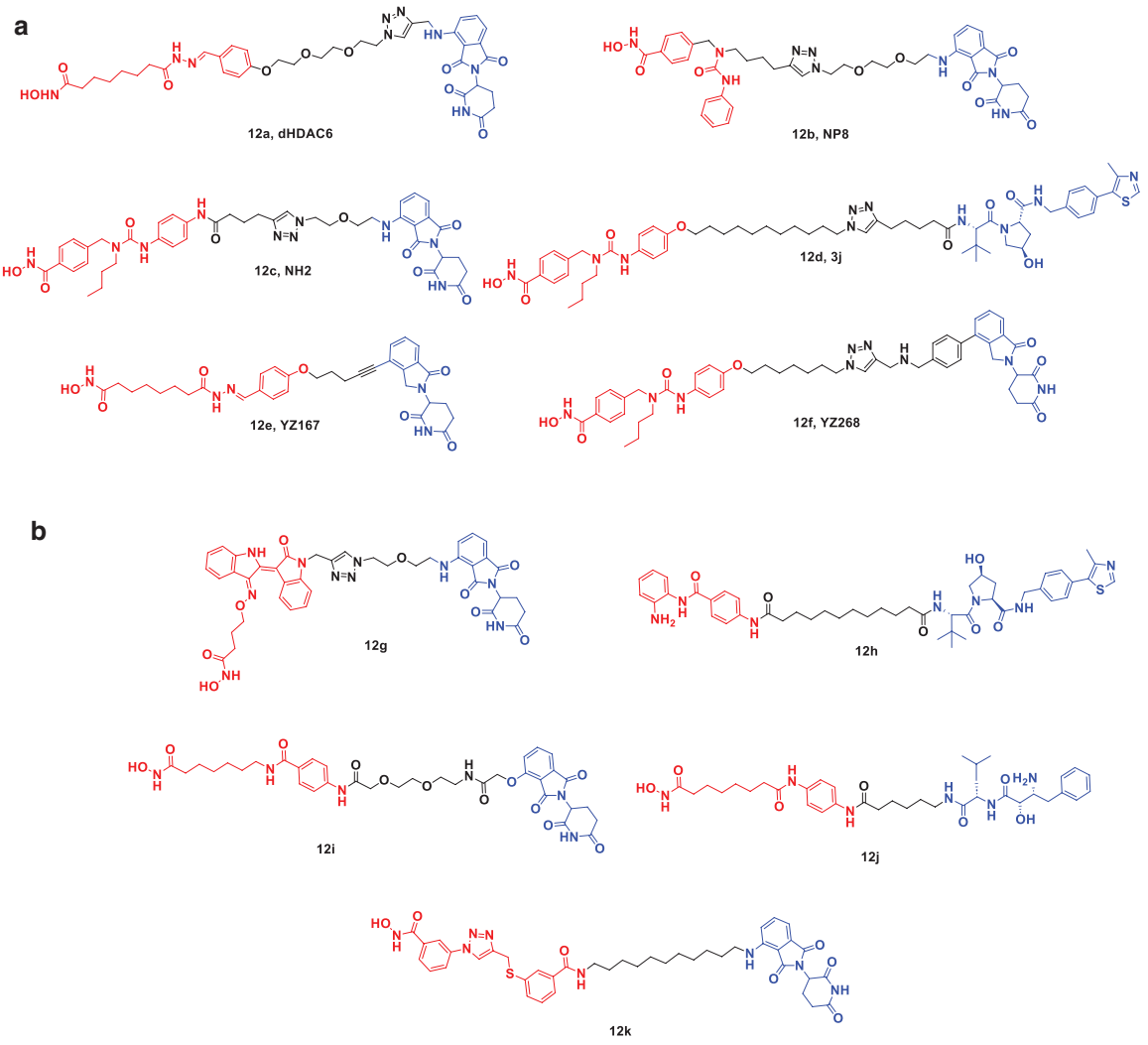

Figure 12 | (a) Representative PROTACs targeting HDAC. (b) Representative PROTACs targeting HDAC.

proof-of-concept HDAC degrader 12i (**Figure 12b**), based on pomalidomide and SAHA. Degrader 12i has strong inhibitory activity toward a variety of HDAC subtypes but induces the degradation of only HDAC1 and HDAC6; therefore, the selectivity of this degrader differs from its affinity, thereby providing a reference for the subsequent development of selective degraders [121].

By using bestatin and SAHA, Zhang et al. have recruited apoptosis protein 1 (cIAP1) E3 ubiquitin ligase to achieve targeted degradation of HDAC6. Treatment with the degrader 12j (**Figure 12b**) in human multiple myeloma cells (RPMI-8226) for 24 h has been found to achieve effective degradation of HDAC1/6/8. In addition, the degrader exhibits more potent aminopeptidyl N (APN, CD13) inhibitory activity and antiangiogenic activity than the approved APN inhibitor bestatin. Therefore, the compound is both an HDAC1/6/8 degrader and a dual inhibitor of APN and HDAC [122]. In 2022, Suzuki et al. successfully developed the selective HDAC8 degrader 12k (**Figure 12b**), based on the HDAC8 selective inhibitor and CRBN ligand reported previously by the same researchers. Compared with HDAC8 inhibitors, the deactivator more effectively inhibits the growth of T-cell leukemia Jurkat cells [123].

2.3.2 SIRT2. Mammalian sirtuin protein is a niacinamide adenine (NAD^+)-dependent histone deacetylase that uses NAD^+ as a co-substrate to regulate the acetylation and ribosylation of a variety of proteins [124]. The human sirtuin family consists of seven subtypes, SIRT1 to SIRT7, all of which have highly conserved NAD-binding domains and catalytic functional domains. Of the seven sirtuins, SIRT2 is the only member that resides primarily in the cytoplasm, and can remove acetyl and other acyl groups from protein lysine residues [125, 126]. SIRT2 promotes tumor growth and regulates various biological

pathways through lysine deacetylation and degreasing acylation, and thus may serve as an attractive target for cancer therapy.

In 2018, Manfred et al. reported 13a, the first PROTAC (**Figure 13**) degrading SIRT2 [127]. They combined the structural characteristics of sirt2 selectivity and high-efficiency triazolyl SirReals with the ligand thalidomide to achieve chemically induced degradation of Sirt2. This is the first reported PROTAC targeting an epigenetic erasure protein. Compound 12 has shown significant SIRT2 protein selective degradation in human cervical cancer cells (HeLa) at 10 μM for 2 hours. In 2020, Lin et al. reported the PROTAC molecule TM-P4-Thal (**Figure 13**), combined with the thiomyristoyl lysine-based SIRT2 selective inhibitor TM and CRBN ligand through a PEG linker, which simultaneously inhibits the activity of SIRT2 protein deacetylase and ester acylase [128]. The degrader TM-P4-Thal at a concentration of 0.5 μM for 48 hours has been found to effectively and selectively degrade SIRT2 in MCF7 cells; moreover, the deacetylation of α-tubulin, a downstream target of SIRT2, is significantly inhibited by exposure to 10 μM for 12 hours. TM-P4-Thal at 1 μM for 48 hours also effectively inhibits the defatting acylation of the SIRT2 downstream target K-Ras4a in HEK 293T cells.

2.3.3 KDM5C. KDMs play important roles in the epigenetic modification of histone methylation and demethylation. KDM family proteins can be divided into two types according to their demethylation mechanisms. One type is composed of FAD-dependent enzymes, including KDM1A and KDM1B, and the other is composed of Fe(II)/α-ketoglutarate-dependent enzymes, including KDM2-7 [129]. The KDM5 family (KDMA-D) interacts with the chromatin remodeling NuRD complex and histone deacetylase complex, which catalyzes the demethylation of lysine 4 histone H3 (H3K4me3/2) that is dimethylated or trimethylated [130, 131]. KDM5 enzymes are associated with the occurrence of cancer and neurodegenerative diseases regulated by epigenetic mechanisms [132]. Several KDM5 inhibitors have been successfully developed and have shown good inhibitory activity in vitro but have not demonstrated effective anticancer effects. KDM proteins regulate genetic gene expression through both enzymatic and scaffold functions: the catalytic function oxidizes methyl groups in lysine residues in histones and removes them, and the scaffold function interacts with transcription factors and forms protein complexes [133]. Traditional KDM5 inhibitors inhibit only the enzymatic function without interfering with the scaffold function, thus potentially explaining the poor clinical efficacy of inhibitors. Developing KDM5 degraders based on protein degradation technology is expected to overcome the limitations of inhibitors. In 2021 [134], Suzuki et al. reported the first histone demethylase KDM degrader, 14 (**Figure 14**), which is based on a KDM5C inhibitor and has been found to achieve significant protein degradation at a concentration of 5 μM in prostate cancer PC-3 cells. Degrader 14 also has been found to have significantly better anti-proliferative activity than that of conventional KMD5 inhibitors. This first report of a histone demethylase KDM degrader lays a foundation for the development of related target degraders.

2.4 Representative ternary complex crystal structures

In 2017, the Ciulli group successfully solved the first ternary crystal structure of the VHL-MZ1-BRD4 complex (**Figure 15a**, PDB: 5T35). This groundbreaking structure revealed the presence of a novel protein-protein interaction between VHL and the second bromodomain of BRD4 (BD2 domain). This structural insight may provide valuable clues as to why MZ1 selectively binds BRD4 and not other isoforms [135]. For another commonly used E3 ligase, cereblon (CRBN), the Fischer group has successfully solved the ternary complex structure of CRBN-dBET23-BRD4 (**Figure 15b**, PDB: 6BN7). In this complex, dBET23 induces the second bromodomain of BRD4 (BD1 domain) to form de novo protein-protein interactions with both the thalidomide-binding domain and the LON N domain of CRBN [136]. These ternary-complex crystal structures provide a framework for the development of selective PROTACs for epigenetic proteins.

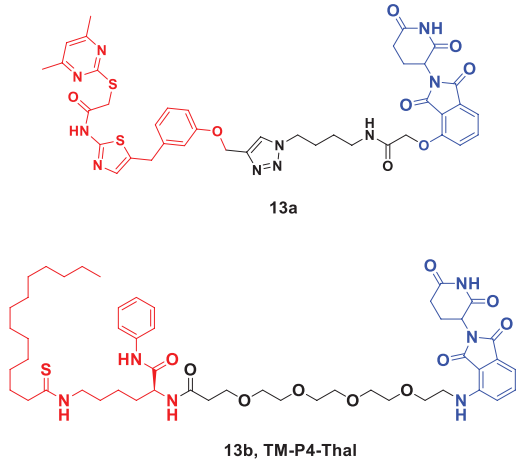

Figure 13 | Representative PROTAC targeting SIRT2.

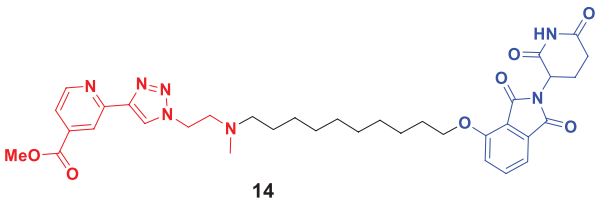

Figure 14 | Representative PROTAC targeting KDM5C.

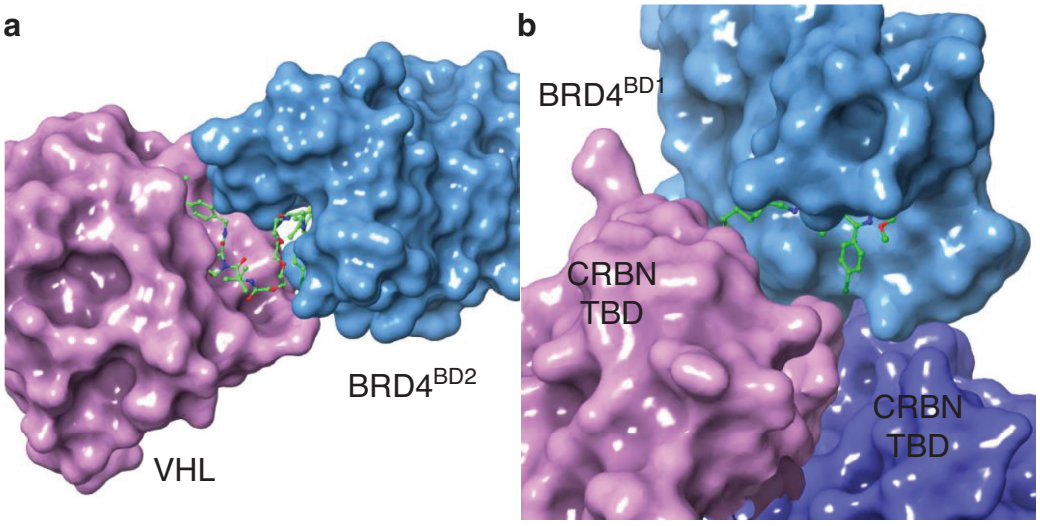

Figure 15 | Representative ternary complex of E3 ligase, PROTAC, and epigenetic protein.
(a) VHL-MZ1-BRD4 complex (PDB: 5T35); (b) CRBN-dBET23-BRD4 complex (PDB: 6BN7).

2.5 New applications of epigenetic protein degraders

2.5.1 Antibody-BRD 4 degrader conjugate. Although PROTAC technology has demonstrated substantial advantages, most PROTAC molecules exhibit poor tissue selectivity and cannot distinguish among different cell types. These drawbacks have hindered further application of this technology. In 2020, the Tate research group developed AbPROTAC technology and conjugated trastuzumab to the BRD4 degrader molecule 15a (**Figure 16**) [137], thus enabling selective targeting of HER2-positive cells. Confocal microscopy indicated that internalization and lysosomal transport occurred specifically in HER2-positive cells. The release of the PROTAC molecule inside the cells led to the degradation of BRD4.

To address the issues of oral bioavailability and solubility of PROTAC molecules, in 2021, Dragovich and colleagues connected MZ1 to an antibody specifically recognizing the cell surface antigen STEAP1. In prostate cancer cells, degrader-antibody conjugate 15b shows

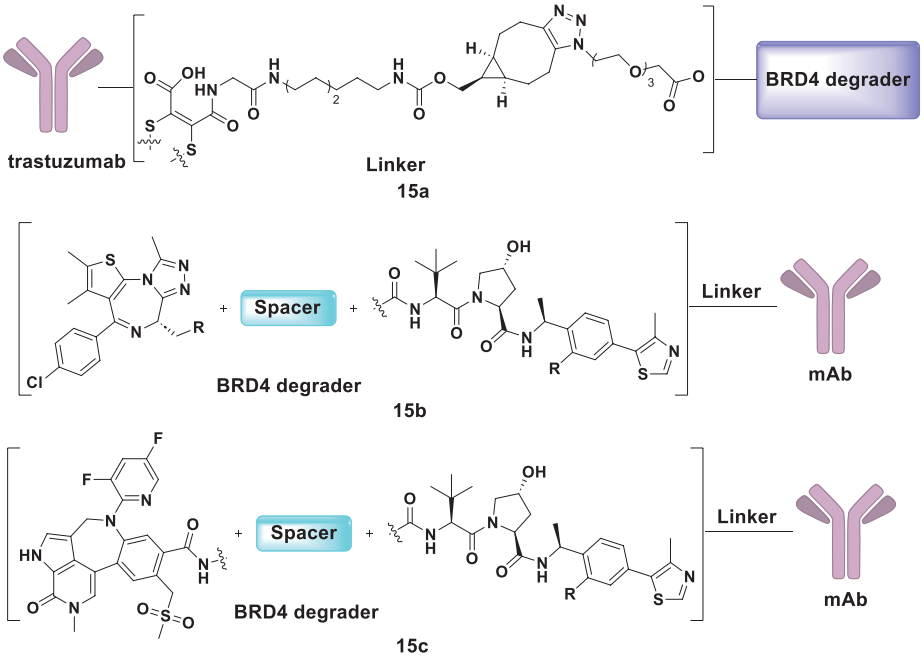

Figure 16 | Representative PROTAC-antibody conjugate.

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potent antigen-dependent anti-proliferative activity (**Figure 16**) [138]. Subsequently, the same research team used novel BRD4 degrader molecule to synthesize degrader-antibody conjugate 15c, which displayed even more potent antigen-dependent anti-tumor activity in the HL-60 human prostate cancer cell line mouse xenograft (**Figure 16**) [139].

2.5.2 BRD4 degrader in phase separation. Biomolecular condensates play crucial roles in various biological processes. However, specific regulators of these condensates are currently lacking. PROTACs can dynamically modulate biomolecular condensates by degrading key molecules within them. Recently, the Rao group has discovered that the BRD4 degrader ZXH-3-26 significantly

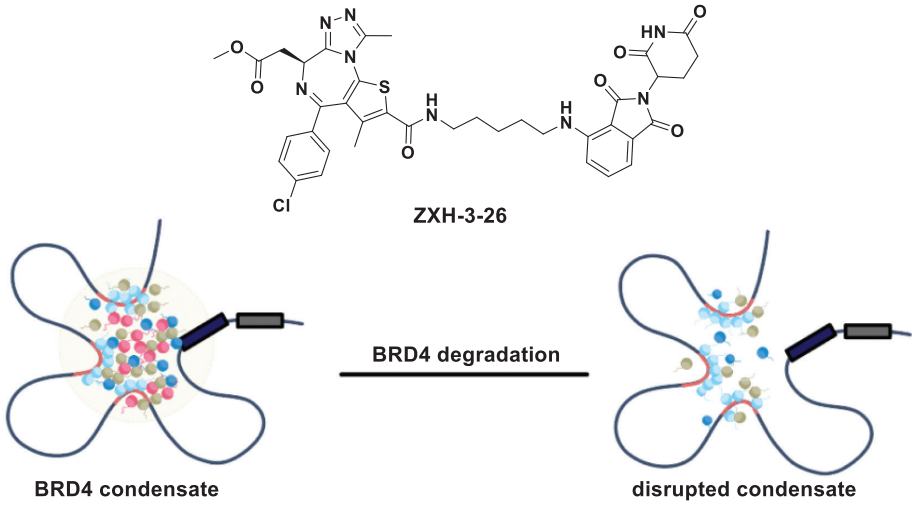

Figure 17 | Modulation of BRD4 condensate through PROTACs.

Table 1 | Summary of currently available PROTACs targeting epigenetic proteins.

NO.	Name	Target	NO.	Name	Target
1	3a, ARV-771	BET	**19**	8b, UNC6852	PRC2
2	3b, dBET1	BET	**20**	8c, MS1943	EZH2
3	3c, BETd-246	BET	**21**	8d, E7	PRC2
4	4a, HBL-4	BRD4	**22**	9, MS4332	PRMT5
5	4b, MacroPROTAC-1	BET	**23**	10a	WDR5
6	4d, SM1	BET	**24**	10d, MS67	WDR5
7	4f	BRD4	**25**	11, MS9715	NSD3
8	4j, A1847	BRD4	**26**	12a, dHDAC6	HDAC6
9	4l, KB02-JQ1	BRD4	**27**	12b, NP8	HDAC6
10	4n	BRD3/4	**28**	12c, NH2	HDAC6
11	4p, XY-06-007	BRD4	**29**	12e, YZ167	HDAC6
12	4q, AGB	BRD2	**30**	12h	HDAC1/2/3
13	5a, dBRD9	BRD9	**31**	12i	HDAC1/6
14	5b, VZ185	BRD7/9	**32**	12j	HDAC1/6/8
15	5c, A547	SMARCA2	**33**	12k	HDAC8
16	6, SR1114	ENL	**34**	13a	SIRT2
17	7, dCBP-1	CBP	**35**	13b, TM-P4-Thal	SIRT2
18	8a	PRC2	**36**	14	KDM5C

diminishes BRD4 condensates. The researchers also identified that BRD4 condensates form preferentially, serving specific functions in the regulation of biological processes. PROTAC technology offers an effective targeted approach for studying biomolecular condensates (**Figure 17**) [140].

2.5.3 Delivery systems for BRD4 degraders. In 2023, the Gao team developed a tumor-targeting copolymer designed for the co-delivery of doxorubicin (DOX) and the BRD4 degrader ARV-825, referred to as ARV-DOX/cRGD-P, as a potential treatment for colorectal cancer (CRC). The researchers identified BRD4 as a promising therapeutic target for adriamycin-resistant CRC and indicated that the use of ARV-825 as a PROTAC degrader enhanced the sensitivity of CRC to adriamycin. The ARV-DOX/cRGD-P copolymer has been reported to have significant anti-tumor effects and thus may have potential applications in clinical CRC treatment [141].

2.6 Available PROTACs targeting epigenetic proteins

As illustrated in **Table 1**, this review comprehensively outlines the existing PROTACs designed to target epigenetic proteins. This burgeoning class of therapeutic agents has immense promise in modulating the activity of proteins associated with epigenetic regulation. In this context, the field of epigenetic-targeted PROTACs is dynamic, and continuing advancements are propelling therapeutic breakthroughs.

3. CONCLUSION AND OUTLOOK

In this review, we summarized the degraders reported for epigenetic targets, including writes, readers, and erasers. These epigenetic regulators have been implicated in various diseases and therefore are attractive therapeutic targets. Notably, researchers have developed innovative strategies to target epigenetic targets, which play crucial roles in modulating chromatin structure and gene expression. As it continues to evolve, the field of epigenetic degradation holds immense promise for the development of novel therapeutics across diseases as diverse as cancer and neurodegenerative disorders. The successful clinical investigation of BRD9 degraders, such as FHD-609 and CFT8634, underscores the translational potential of this approach and paves the way to further advancements in epigenetic target degradation strategies [26]. With ongoing research and innovative techniques, the future of epigenetics-based therapies appears bright, and may offer new avenues for precision medicine and improved patient outcomes.

Despite the promise of the PROTAC field, several challenges remain unresolved, including the following: 1) discovery of PROTAC molecules is currently confined to known ligands and established binding pockets, and prolonged treatment can lead to drug resistance; 2) some undruggable epigenetic targets lack suitable ligands for degrader development; and 3) the availability of E3 ligases is limited, and only CRBN and VHL have been used in degrader development.

Addressing the challenges in the PROTAC field has spurred the development of innovative methods aimed at overcoming existing limitations and broadening the scope of targetable proteins. These emerging strategies have the potential to revolutionize the field of targeted protein degradation, as follows:

1. Expanding ligand discovery and binding pockets: To overcome the limitation of relying solely on known ligands and binding pockets, researchers are actively exploring new approaches, including using computational methods to predict potential binding sites on target proteins, thus enabling the design of PROTACs that can engage previously unexplored regions. Additionally, advancements in chemical synthesis and high-throughput screening techniques have facilitated the identification of novel ligands, thus supporting the development of PROTACs with a wider range of protein targets. These efforts have not only enhanced target diversity but also decreased the risk of drug resistance due to prolonged treatment.
2. Exploration of new E3 ligases: Although CRBN and VHL have been the primary E3 ligases used in PROTAC development, efforts are underway to identify new E3 ligases. This diversification may allow for targeting different cellular compartments, optimizing degradation kinetics, and minimizing off-target effects. By harnessing a wider array of E3 ligases, the precision and versatility of PROTAC-based therapies can be enhanced.
3. Advanced delivery systems: Developing effective delivery systems is critical for the clinical translation of PROTACs. Researchers are actively exploring nanoparticle-based delivery platforms, cell-penetrating peptides, and other innovative techniques to enhance the intracellular uptake and stability of PROTAC molecules. These advancements are aimed at improving target engagement and tissue specificity while minimizing off-target effects.

As the field continues to evolve, the combination of these novel methods holds great promise in addressing the challenges posed by PROTAC development. With concerted efforts geared toward innovation and collaboration, researchers may harness the full therapeutic potential of targeted protein degradation across a wide range of diseases and previously challenging target proteins.

ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The authors declare no competing financial interests.

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