Acta Pharmaceutica Sinica B 2024;14(2):533−578

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Overview of epigenetic degraders based on PROTAC, molecular glue, and hydrophobic tagging technologies

Xiaopeng Peng[a,†], Zhihao Hu[a,†], Limei Zeng[b], Meizhu Zhang[a], Congcong Xu[a], Benyan Lu[a], Chengpeng Tao[a], Weiming Chen[a], Wen Hou[a], Kui Cheng[c,*], Huichang Bi[c,*], Wanyi Pan[a,*], Jianjun Chen[c,*]

[a]*College of Pharmacy, Key Laboratory of Prevention and Treatment of Cardiovascular and Cerebrovascular Diseases of Ministry of Education, Gannan Medical University, Ganzhou 314000, China*
[b]*College of Basic Medicine, Gannan Medical University, Ganzhou 314000, China*
[c]*Guangdong Provincial Key Laboratory of New Drug Screening, NMPA Key Laboratory for Research and Evaluation of Drug Metabolism, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China*

Received 10 June 2023; received in revised form 21 July 2023; accepted 30 August 2023

KEY WORDS

Epigenetic;
Degrader;
PROTAC;
Molecular glue;
Hydrophobic tagging

Abstract Epigenetic pathways play a critical role in the initiation, progression, and metastasis of cancer. Over the past few decades, significant progress has been made in the development of targeted epigenetic modulators (*e.g.,* inhibitors). However, epigenetic inhibitors have faced multiple challenges, including limited clinical efficacy, toxicities, lack of subtype selectivity, and drug resistance. As a result, the design of new epigenetic modulators (*e.g.,* degraders) such as PROTACs, molecular glue, and hydrophobic tagging (HyT) degraders has garnered significant attention from both academia and pharmaceutical industry, and numerous epigenetic degraders have been discovered in the past decade. In this review, we aim to provide an in-depth illustration of new degrading strategies (2017−2023) targeting epigenetic proteins for cancer therapy, focusing on the rational design, pharmacodynamics, pharmacokinetics, clinical status, and crystal structure information of these degraders. Importantly, we also provide deep insights into the potential challenges and corresponding remedies of this approach to drug design and

*Corresponding authors.
E-mail addresses: chengk@smu.edu.cn (Kui Cheng), bihchang@smu.edu.cn (Huichang Bi), 270358051@qq.com (Wanyi Pan), jchen21@smu.edu.cn (Jianjun Chen).
[†]These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2023.09.003

development. Overall, we hope this review will offer a better mechanistic understanding and serve as a useful guide for the development of emerging epigenetic-targeting degraders.

1. Introduction

Epigenetics refer to changes in gene expression that can be inherited without altering the DNA sequence[1]. Epigenetic modifications, such as histone modifications, DNA methylation, and non-coding RNA modifications, are frequently encountered[2]. Enzymes regulating these modifications can be categorized as "writers," "erasers" or "readers." The "writers" are capable of catalyzing chemical modifications on histones and/or DNA substrates with examples including DNA methyltransferases (DNMTs), histone lysine methyltransferases (KMTs)[3]. Conversely, "erasers" can remove these modifications, for instance, histone lysine deacetylases (HDACs) and demethylases (KDMs). On the other hand, "readers" encompass proteins that can recognize specific epigenetic marks through protein−protein interaction (PPI) processes such as histone methylation-binding domains, like bromodomains (BRDs)[3]. Numerous studies have demonstrated the critical role of epigenetic modifications in the initiation, progression, and metastasis of cancer[4−6].

Currently, there are several inhibitors available on the market that target epigenetic processes. These include DNMT inhibitors (*e.g.,* azacitidine and decitabine)[7], HDAC inhibitors (*e.g.,* SAHA and belinostat)[8,9], and the first EZH2 inhibitor, tazemetostat[10], which is used exclusively for epithelioid sarcoma.

Targeted epigenetic inhibitors have demonstrated therapeutic efficacy in various diseases. However, they also faced several challenges, including toxicities due to the lack of subtype selectivity (*e.g.*, severe thrombocytopenia and neutropenia for the pan-HDAC inhibitor SAHA)[11] and the development of drug resistance[12].

Targeted protein degradation (TPD) technology such as proteolysis-targeting chimaeras (PROTACs), molecular glue, and hydrophobic tagging (HyT), represents an innovative drug discovery strategy[13,14]. Its primary mode of action involves the rapid degradation of disease-causing target proteins to produce therapeutic effects. TPD holds great promise for treating a broad range of diseases, including cancer, infection, inflammation, and neurodegenerative disorders.

Compared to conventional inhibitors, epigenetic degraders offer improved therapeutic effects and can serve as useful chemical tools for post-translational protein knockdown[15]. The sensitivity, specificity, and reversibility of epigenetic degraders provide a convenient and alternative gene knockout method for studying target proteins in physiological and pathophysiological processes. Additionally, the protein degradation mechanism of epigenetic degraders differs from that of inhibitors, resembling phenotypic genetic downregulation[16]. Ligand-directed protein degradation has the potential to improve target subtype selectivity beyond the binary target binding selectivity of constitutive inhibitors, enabling selective targeting of a single protein while retaining its homologue to better interpret its physiological role.

Moreover, the occupancy-driven paradigm prevails in small molecule drug development, aiming to inhibit abnormal protein function by occupying active or regulatory sites[17]. However, many pathogenic proteins are considered as "undruggable targets"[18], such as scaffold proteins, which play a crucial role in regulating cellular signaling pathways but often engage in simultaneous interactions with multiple signaling molecules, posing a challenge for their targeting with small molecules[19]. Additionally, small molecule inhibitors typically disrupt only one of the multi-domain in scaffold proteins, with the other domains and their respective interactions unaffected. These limitations hinder the widespread application of small molecules[20]. In contrast, TPD technology has a distinct advantage in eliminating disease-causing proteins rather than selectively inhibiting a fraction of their functionality. Consequently, TPD shows significant promise for cancer treatment.

Over the past 20 years, the field of PROTACs has witnessed rapid development primarily driven by the discovery of various small molecule E3 ligase ligands, particularly those based on VHL and CRBN. The discovery of epigenetic-targeting degraders has also been intensified in the last decade with many milestones achieved, as illustrated in Fig. 1. In 2015, Craig M. Crews[21] pioneered the development of BRD4 PROTAC ARV-825, comprising the BRD4 inhibitor OTX015 and CRBN ligand pomalidomide, for the selective degradation of BRD4 through a cereblon-mediated and proteasome-dependent pathway. Subsequently, Raina et al.[22] reported in 2016 the pan-BET PROTAC ARV-771, which exhibited significantly improved efficacy compared to BET inhibition in cellular models of castration-resistant prostate cancer (CRPC). In 2017, Gadd[23] disclosed the crystal structure of MZ1 (BRD4 PROTAC) within a ternary complex involving the second bromodomain (BD) of BRD4 ($BRD4^{BD2}$) and VHL. Later, Yang et al.[24] described the first HDAC PROTAC for cancer therapy. In 2019, Hsu et al.[25] developed the first EZH2 PROTAC, followed by the discovery of the first HDAC and EZH2 HyT degraders by the Schiedel group in 2020[26]. Notably, in 2021, the first epigenetic degrader FHD-609, which selectively targets BRD9, entered clinical trials for subjects with advanced synovial sarcoma[27]. Additionally, the second BRD9 selective degrader, CFT8634, is currently undergoing clinical trials for the treatment of synovial sarcoma and SMARCB1-Null tumors[28]. In terms of molecular glues, the Toriki[29] group developed the first HDAC and BRD4 molecular glue degraders in 2023 to improve the druggability.

In the past 5 years, there are several reviews focusing on epigenetic-targeting PROTACs. For example, Tomaselli et al.[30] summarized the progress in epigenetic PROTACs prior to 2020 by focusing on the pharmacological activities of these PROTACs. Similarly, Vogelmann et al.[31] provided a brief review in 2020, highlighting recent advances in PROTACs targeting epigenetic regulators (proteins).

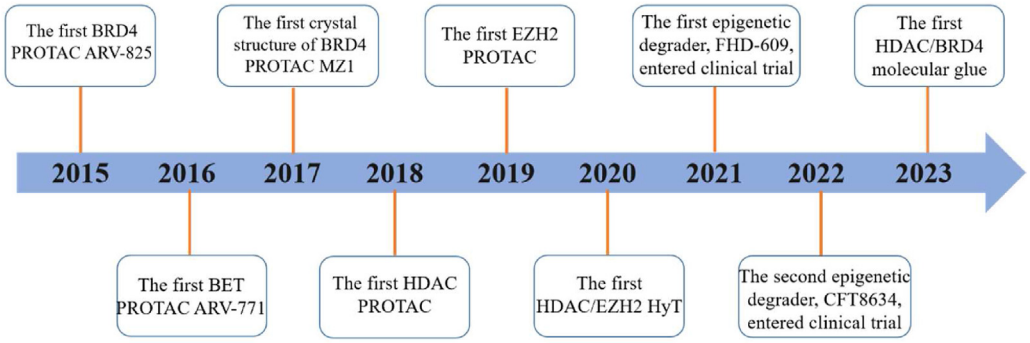

Figure 1 The development timeline of targeted epigenetic protein degradation.

The field of epigenetic degraders is rapidly evolving, with significant progress made over the past five years. In addition to PROTACs, breakthroughs have been made in epigenetic molecular glue and HyT degraders. Thus, it is of high importance to summarize the new progress in this fast-growing area from an overall perspective. Therefore, in this review, we aim to provide an in-depth illustration of the new degrading strategies targeting epigenetic proteins (2017−2023) for cancer therapy, focusing on the rational design, pharmacodynamics, pharmacokinetics, clinical status, and crystal structure of these degraders including epigenetic PROTACs, molecular glues and HyT degraders. Furthermore, this review also presents the potential challenges (such as uncontrollable properties and limited selectivity for specific tissues/cells, etc.) and their corresponding remedies associated with current targeted epigenetic degraders, including light-activated degraders, antibody/aptamer/folate-PROTAC conjugates, and covalent/trivalent degraders, etc.

2. TPD

Conventional modulators (*e.g.,* inhibitors/agonists) act by either inhibiting or enhancing target protein activity to treat diseases. They must effectively bind to the active site of their protein targets. However, analysis of known drug targets revealed that only ~20% of them have a targetable active site, while the remaining 80% are considered undruggable[32]. Excitingly, TPD technology has the potential to expand the druggable target landscape beyond previously undruggable targets and is expected to overcome drug resistance issues[32,33].

The two primary intracellular protein degradation pathways are the lysosomes and the ubiquitin−proteasome system (UPS), accounting for 80% of cellular protein degradation. Commonly used technologies for UPS-mediated protein degradation include PROTACs, molecular glues, hydrophobic tagging, chaperone-mediated protein degradation, and degrader−antibody-conjugates. This review focuses on epigenetic degraders (*e.g.,* PROTACs, molecular glue, hydrophobic tagging) that mediate targeted protein degradation through the UPS.

2.1. *PROTACs*

PROTACs are the most extensively studied and widely recognized protein degradation technology[34]. First proposed in 2001 by Craig M. Crews and Raymond J. Deshaies, PROTACs are bifunctional small molecules consisting of a ligand for targeting the protein of interest (POI), an E3 ubiquitin ligase ligand, and a linker connecting them. PROTACs induce the binding of the POI to the E3 ligase to form a ternary complex that activates ubiquitination and subsequent degradation of the POI by the 26S proteasome, and can be re-used after the catalytic cycle. Compared to traditional small molecule inhibitors, PROTACs are more effective and can work at low doses (catalytic), thus reducing systemic exposure and toxicity. In addition, PROTACs are not susceptible to target protein expression and mutation, enabling treatment of previously untargetable proteins[32]. The catalytic mode of action of PROTAC offers selectivity and efficacy, making it an attractive approach for drug development. PROTAC technology not only provides a new strategy for treating diseases but also advances our understanding of protein function and regulation. This innovative approach has shown therapeutic potential for various diseases, including cancer, inflammation, and neurodegeneration.

2.2. *Molecular glue*

In addition to PROTACs, molecular glue is another emerging TPD technology designed to bring two or more proteins into close proximity to promote their interaction and subsequent degradation through the UPS pathway[35]. Like PROTACs, molecular glues are capable of achieving TPD, but act *via* distinct mechanisms. Specifically, PROTACs induce the recruitment of an E3 ligase to a target protein, while molecular glues promote the direct engagement of a target protein with a component of the proteasome. Compared with PROTACs, molecular glues have lower molecular weights, higher cell permeability, and better pharmacokinetics (*e.g.,* oral absorption), rendering them more drug-like in nature[36].

Currently, there are three molecular glues approved for clinical use, including thalidomide and its derivatives (lenalidomide and pomalidomide). These drugs exhibit immunomodulatory, anti-inflammatory, and anticancer properties, and are used to treat various diseases such as cancer[35].

2.3. *HyT-based degraders*

HyT is a TPD technology developed by PROTAC pioneer Craig M. Crews in 2011[37]. HyT acts by appending hydrophobic moieties such as amantadane or Boc_3 arginine to the surface of a POI, thus mimicking the misfolded protein and recruiting companion proteins or proteasomes to degrade the POI. Another mode of action involves chaperones directly recognizing hydrophobic labels and mediating proteasomal degradation of labeled proteins. HyT technology offers advantages over PROTACs, including lower

molecular weight, better drug-like properties, and no teratogenic risk from thalidomide derivatives.

Fulvestrant, the only FDA-approved selective estrogen receptor degrader (SERD), is a representative degrader based on HyT technology[38]. The success of fulvestrant highlights the potential of HyT strategies for targeted protein degradation.

2.4. *Comparison of the merits and demerits for inhibitors and TPD technologies*

As mentioned above, the last decade has witnessed great advancements in TPD technology. Despite the many advantages of TPD technology (*i.e.*, the ability to overcome drug resistance, reduced toxicity, and targeting of undruggable proteins)[39] over conventional inhibitors, there are several limitations to be addressed, as outlined in Table 1.

Traditional occupation-driven small molecule inhibitors have several merits such as the ease of synthesis and favorable pharmacokinetic properties. In comparison, TPD technologies such as PROTACs possess unfavorable pharmacokinetics in general, including suboptimal oral bioavailability, and limited cell membrane permeability, likely due to their large molecular weight and poor physicochemical properties[34]. Additionally, the selection of E3 ligases is restricted despite the human genome encoding over 600 possibilities, with only a few extensively studied like CRBN, VHL, MDM2, and IAP and commonly utilized[17]. Moreover, PROTACs exhibit a "Hook effect" wherein they demonstrate weakened efficacy at high concentrations which hinders ubiquitination-mediated protein degradation due to the inability to form ternary complexes[40].

Compared to PROTACs, molecular glue molecules have a lower molecular weight with enhanced membrane permeability and improved absorption rates, thus are more likely to adhere to Lipinski's rule[41]. Additionally, molecular glues may have simpler SAR (structure−activity relationship) and are easier to synthesize. Notably, they can degrade target proteins that are otherwise inaccessible without requiring a binding pocket on the target protein. When molecular glue molecules are used alone, they primarily bind to the E3 ligase, with minimal affinity for binding to the target protein, minimizing the occurrence of a hook effect[35]. However, the major limitation of molecular glue is the lack of rational design principles to convert protein-targeting ligands into effective molecular glue degraders[42]. Consequently, most of the molecular glues rely on serendipitous discovery of different ligand combinations through randomization approaches.

The HyT technology is highly versatile and can be applied to a wide range of target proteins by incorporating a small hydrophobic moiety onto the protein[43]. Compared to PROTACs, HyT molecules typically have lower molecular weight, improved drug-like properties, and lower teratogenic risks associated with thalidomide moiety in PROTACs. In contrast to molecular glue technology, the design of HyT is simpler through selective combination of suitable ligands with hydrophobic tags tailored to the specific target. However, the availability of hydrophobic tag fragments for HyT is currently limited, presenting opportunities for optimization in terms of degradation activity and physicochemical properties. Furthermore, the precise mechanism underlying degradation of HyT remains unclear, necessitating further investigation and clarification[44].

3. Epigenetics

Epigenetic inheritance can produce heritable phenotypic changes without altering the DNA sequence, involving various mechanisms such as DNA methylation, histone modification, X-chromatin remodeling, non-coding RNA, nucleosome localization, and genomic imprinting[1]. The covalent modification of histones and nucleic acids is at the core of epigenetics, regulating chromatin structure and gene expression[2,3]. Epigenetic enzymes, known as "writers", control genome compression and gene expression, while effector proteins, or "readers", recognize these modifications (Fig. 2). Most epigenetic marks are reversible, and the enzymes that remove them are referred to as "erasers"[45].

Epigenetic modifications not only affect transcription factors but also are associated with other epigenetic mechanisms[46], such as chromatin remodeling and non-coding RNAs, to co-regulate neoplastic processes. DNA methylation, in particular, often affects gene expression, transcription, and activity by inducing hypermethylation of gene promoters and subsequent transcriptional inhibition, resulting in decreased gene expression.

Covalent histone modification is a crucial epigenetic mechanism that encompasses various modifications[47], including acetylation, phosphorylation, methylation, ADP ribosylation, ubiquitination, and citrullination. Many of these modifications (*e.g.,* acetylation, methylation, and phosphorylation) are involved in cancer occurrence and development, and the related enzymes include histone demethylases, histone methyltransferases, histone deacetylases, histone acetyltransferases (HATs), and ADP ribosyltransferases.

Table 1 The merits and demerits of conventional inhibitors and TPD technologies.

Inhibitors/TPD technology	Merits	Demerits
Conventional inhibitors	Ease of design and synthesis; high tissue penetration, oral bioavailability and druggability	Difficult to target undruggable proteins; drug resistance; potential toxicity
PROTAC degraders	Targeting of undruggable proteins; reversing drug resistance; rational design; minimizing potential toxicity	Poor PK properties; limited E3 ligases to select; hook effects
Molecular glue degraders	Lower molecular weights; higher cell permeability; better pharmacokinetics; simpler SAR; easier synthesis	Difficult to design rationally
HyT degraders	Broad range of target proteins; lower molecular weight, better drug-like properties; no teratogenic risk; ease of design and synthesis	Limited hydrophobic tag fragments; mechanism remains unclear

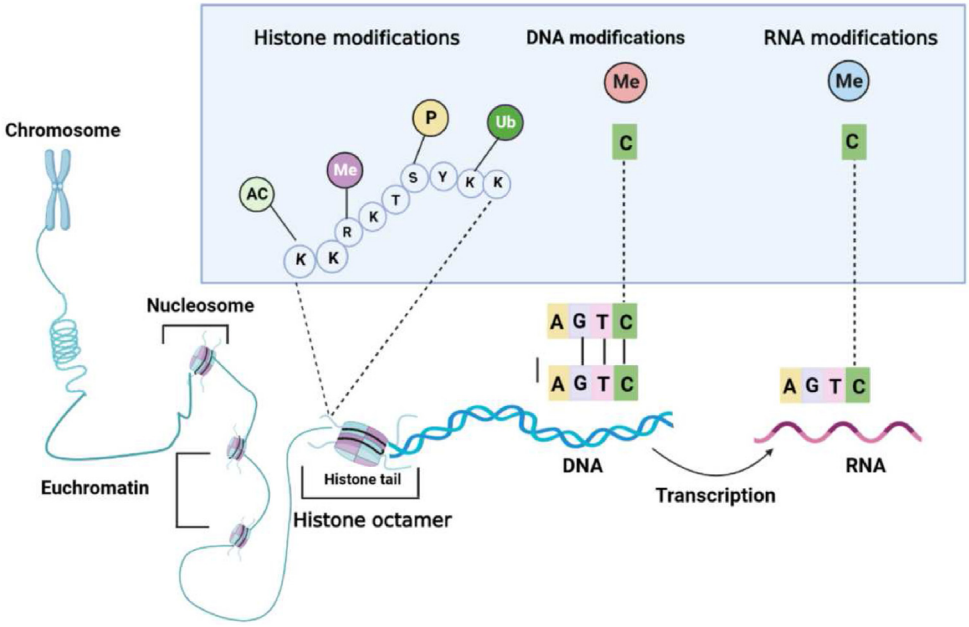

Figure 2 Therapeutic strategies to modulate the epigenome.

3.1. DNA modification (writer)

3.1.1. DNMTs

DNA methyltransferases (DNMTs) are a family of enzymes consisting of five members[48]: DNMT1, DNMT2, DNMT3A, DNMT3B, and DNMT3L. DNMT1 plays a vital role in maintaining DNA methylation patterns during cell division by copying them from the parent to the daughter strand. In contrast, DNMT2 modifies the 38th cytosine residue in the anticodon ring of some tRNAs by functioning as an RNA methyltransferase. DNMT3A has two subtypes, while DNMT3B has more than 30 subtypes. Both enzymes are involved in de novo DNA methylation, establishing new methylation patterns during development and differentiation. DNMT3L also belongs to the DNMT3 family but lacks methyltransferase activity. Instead, it promotes the de novo DNA methylation by interacting with DNMT3A/B (Fig. 3A).

DNA methylation is a biochemical process in which a methyl group is transferred to the 5′ carbon of cytosine within a CpG dinucleotide, producing 5-methylcytosine (5 mC)[49]. This modification is catalyzed by DNMTs, which recognize specific DNA sequences known as CpG islands (Fig. 3B). DNA methylation can regulate gene expression by modulating the ability of transcription factors and other regulatory proteins to access DNA. Mutations in DNMTs are important markers of malignant transformation, particularly in solid tumors and hematologic malignancies. For example, DNMT1 has been identified as an oncoprotein in breast cancer (BC) and lung cancer, where it promotes tumor growth and progression.

3.2. Histone modifications (eraser)

3.2.1. HATs

Histone modifications[50], such as phosphorylation, ubiquitination, ADP-ribosylation, deamination, and butyrylation, can alter the structure and function of chromosomes and have important biological roles. Acetylation and methylation of histones are among the most well-known examples of these processes. The regulation of histone acetylation is governed by two enzyme families: HATs and HDACs.

The histone tails of nucleosomes contain numerous lysine residues that can be post-translationally acetylated. Studies have demonstrated that abnormal histone acetylation is associated with the development of cancer by modulating cellular pH and gene transcriptional activity as well as chromatin architecture[51]. The opposing activities of HATs and HDACs govern the post-translational acetylation of proteins (Fig. 4). Imbalances between these enzymes have frequently been linked to tumorigenesis. HATs catalyze the transfer of acetyl groups from acetyl coenzyme A to the lysine side chains of histones, thereby neutralizing their positive charge and relaxing the adjacent chromatin structure[5]. These enzymatic complexes are generally composed of three main families located within the nucleus: the MYST family (Moz-Ybf2/Sas3-Sas2-Tip60), the p300/CREB-binding protein family (CBP/CREBBP), and the GCN5-related *N*-acetyltransferases family (GNAT).

3.2.2. HDACs

In contrast to HATs, HDACs can contribute to the development of cancer by disrupting the transcription of oncogenes and tumor suppressor genes through their ability to remove acetyl groups and reverse chromatin acetylation[52]. In addition, HDACs are capable of modifying a variety of non-histone substrates, including tubulin, ER-α, p53, and HSP90. Currently, 18 HDAC subtypes have been identified and can be grouped into four categories based on homology with yeast deacetylases: class I (HDAC1/2/3/8), class II (HDAC4/5/6/7/9/10), class III (Sirt1−7), and class IV (HDAC11). Class I, II, and IV HDACs are zinc-dependent enzymes that rely on zinc ions for catalysis. Conversely, class III HDACs are NAD^+-dependent enzymes that produce *O*-acetyl-ADP-ribose and nicotinamide *via* the transfer of acetyl groups[53].

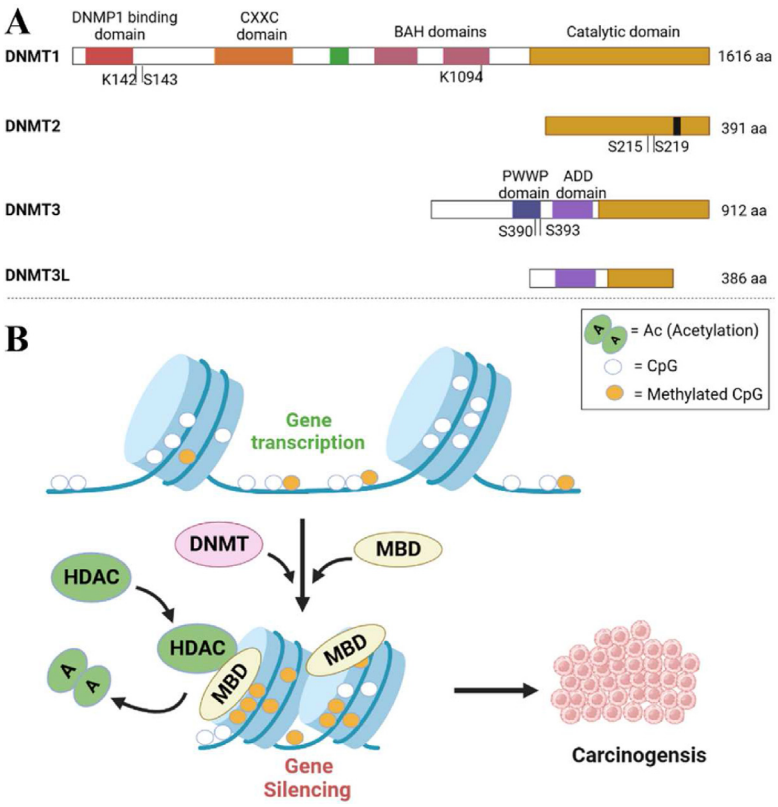

Figure 3 The bio-function of DNMTs.

HDACs are generally recognized as transcriptional inhibitors since they can stabilize and condense chromatin in cells, making it less accessible to transcription factors[52]. Moreover, HDACs can form complexes with other inhibitors that also contribute to the suppression of transcription. However, research has shown that HDACs can cooperate with transcriptional repressors and activate gene expression[54]. Apoptosis is the programmed cell death process initiated by internal or external stimuli and regulated by a complex interplay of proteins, including HDACs. As the central regulators of chromatin remodeling and acetylation levels affecting DNA damage-related proteins, HDACs play a crucial role in apoptosis (Fig. 5).

The Sirt family, a distinct group of HDACs, consists of seven members (Sirt1−7). Recent studies have revealed that Sirt proteins play a role in modulating these pathways[55]. Specifically, Sirt1 is significantly overexpressed in many types of cancers, including gliomas, breast cancer, and leukemia stem cells. Sirt2 also contributes to maintaining the stability of breast cancer stem cells (CSCs). Moreover, high expression of Notch has been shown to upregulate Sirt2 expression, which leads to activation of aldehyde dehydrogenase 1A1 (ALDH1A1) *via* Sirt2 deacetylation, ultimately promoting the proliferation of breast CSCs. Inhibition or knockdown of Sirt2 can potentially impede the progression of breast cancer (Fig. 6).

3.2.3. *Histone methyltransferases (HMTs)*

Histone methyltransferases (HMTs) comprise lysine methyltransferases (KMTs) and arginine methyltransferases (PRMTs)[3]. KMTs can be classified into SET domain-containing and non-SET domain-containing proteins based on their catalytic structure domain sequence[56]. The SET domain is a crucial structural domain for most methyltransferases, including the SUV39, SET1, SET2, EZH, RIZ, and other families, responsible for their enzymatic activity. However, proteins lacking a SET domain, such as DOT1L protein, are less common and are known to target histone H3K79 methylation.

Figure 4 HATs and HDACs regulate the histone acetylation process.

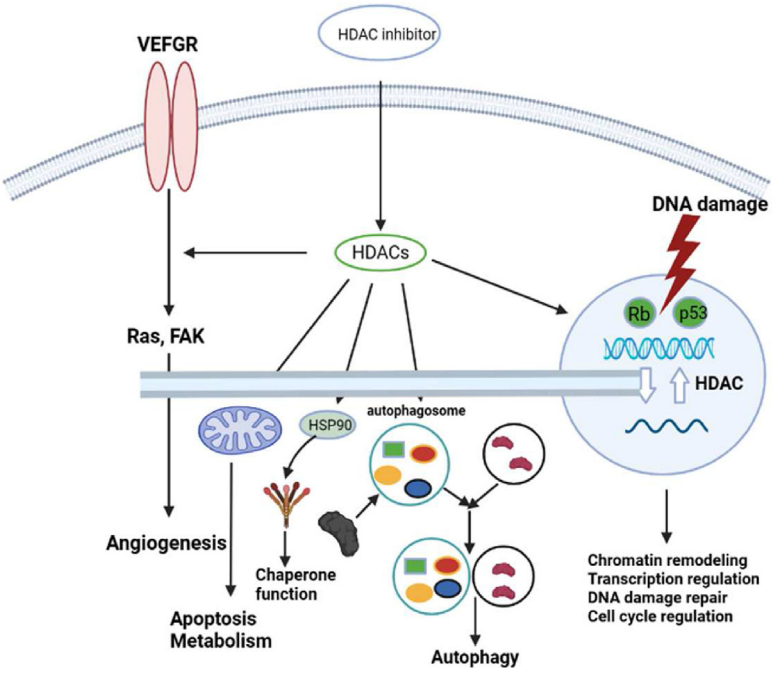

Figure 5 Biological functions of HDACs.

PRMTs can be categorized into three types based on their catalytic activity: monomethylarginine (MMA), asymmetric dimethylarginine (ADMA), or symmetric dimethylarginine (SDMA)[57]. Type I PRMTs (PRMT1, PRMT2, PRMT3, PRMT4, PRMT6, and PRMT8) produce mono- or asymmetrical dimethylarginine (ADMA), while type II PRMTs (PRMT5 and PRMT9) generate mono- or symmetrical dimethylarginine (SDMA). On the other hand, type III PRMT7 exclusively generates MMA.

3.2.3.1. EZH2. Polycomb repressive complex 2 (PRC2) is a highly conserved histone methyltransferase that targets lysine-27 of histone H3[58]. PRC2 comprises EZH2, EED, SUZ12, and RbAp46/48. Located on human chromosome 7q35, EZH2 is the core catalytic subunit of PRC2 and consists of 746 amino acid residues[59]. It induces histone H3 lysine 27 trimethylation (H3K27me3) and gains enzymatic activity by binding to other non-catalytic proteins such as EED, SUZ12, and RbAp46/48 (Fig. 7). EZH2 has four domains: SET and CXC domains mediate histone acetylation, and two SANT domains that enable EZH2 to bind to DNA, allowing for chromatin remodeling and transcription regulation. Studies have shown that overexpression of EZH2 suppresses the expression of tumor suppressor genes, triggers immune escape, and dysregulates the development of lymphatic system, leading to lymphoma and leukemia.

3.2.3.2. EED. Embryonic ectoderm development (EED) is one of the three core subunits of the PRC2 complex[60]. EED recognizes and binds to H3K27me3, thereby participating in the methylation

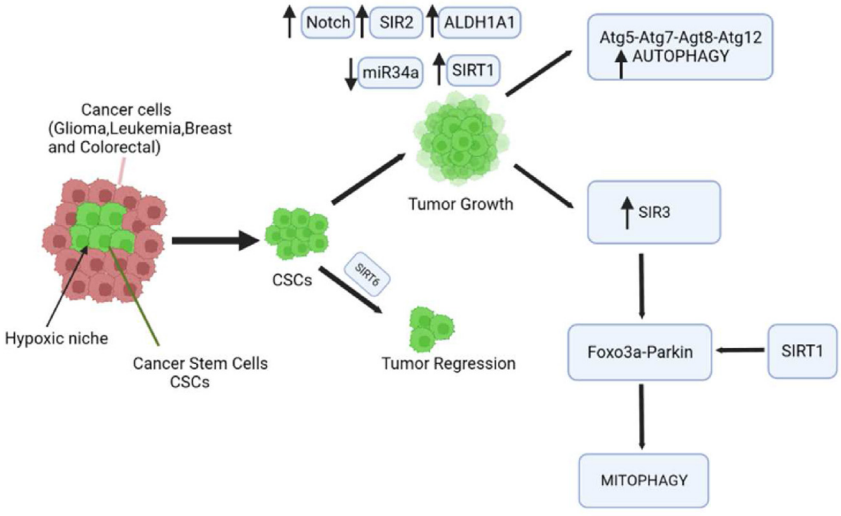

Figure 6 Biological functions of Sirt2.

of lysine 27 of histone H3 (H3K27). In addition, EED senses the methylation status of histones labeled with H3K27me3 and regulates PRC2 enzyme activity by interacting with EZH2 (Fig. 8). This interaction maintains PRC2 enzyme activity and jointly stimulates PRC2 activity. Research has shown that EED interacts with EZH2 protein to promote the proliferation and migration of triple-negative breast cancer cells (TNBC).

3.2.4. *Histone demethylases (HDMTs)*

Histone demethylases (HDMTs) have the opposite function to methyltransferases, as they remove methyl groups from both histone and non-histone substrates[61]. Currently, two evolutionarily conserved families of histone demethylases, namely the lysine-specific demethylase (LSD) family, and the Jumonji C (Jmc) protein family, have been identified as detailed below.

3.2.4.1. LSD1. LSD1 is an FAD-dependent amine oxidase (AO) that removes specifically monomethylated and dimethylated groups from histone H3K4 and H3K9 sites, activating or inhibiting gene transcription[62]. LSD1 mainly regulates gene transcription through two pathways: (1) interacting with the CoREST transcription repressor complex and the Mi-2/nucleosome remodeling and deacetylase complex (NuRD) to catalyze H3K4me1/me2 demethylation, leading to transcriptional inhibition; (2) binding to androgen/estrogen receptors and demethylating H3K9, resulting in transcriptional activation of hormone receptor-dependent genes (see Fig. 9). Furthermore, LSD1 demethylates non-histone proteins and participates in multiple cellular processes such as cell proliferation, differentiation, epithelial-mesenchymal transformation (EMT), cell viability, angiogenesis, and senescence.

3.3. *Reader*

3.3.1. *Bromodomain and extraterrestrial minal (BET)*

The BET domain protein family consists of BRD2, BRD3, BRD4, and BRDT[63]. BRD2 recognizes acetylated histones and regulates the repair of DNA and transcription of cell cycle genes. Although the biological function of BRD3 is largely unknown, it appears to

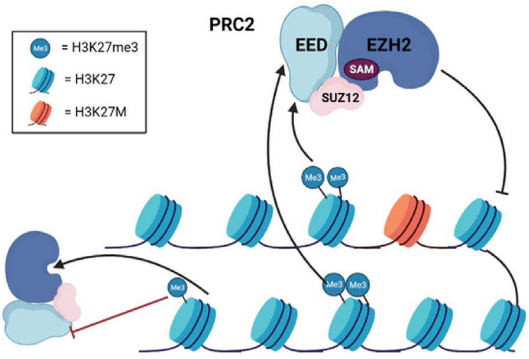

Figure 8 Biological functions of EED.

be involved in nucleosome remodeling and transcription of erythroid genes. BRD4 has three isoforms (BRD4A−C) that recognize acetylated histones and recruit the P-TEFb (positive transcription elongation factor b) complex, activating RNA polymerase II and stimulating oncogene expression such as C-myc. This stimulation can inhibit various tumor suppressor genes, including P21 and P53. Additionally, BRD4 modulates transcriptional regulation of cellular processes involved in cell cycle progression, proliferation, and immune response. Through interactions with P-TEFb, mediator complexes, and transcription factors, BRD4 promotes transcriptional elongation, ultimately resulting in phosphorylation of the CTD of RNA polymerase II at serine residues (Fig. 10)[64].

BRD9 is a member of the BET domain (IV) family and a subunit of a novel non-canonical barrier-autointegration factor (ncBAF) complex[65]. Its oncogenic biology functions through epigenetic modifications, mediated by its bromodomain, which stimulates tumor cell growth factors to promote tumorigenesis. Yuan et al. demonstrated that BRD9's bromodomain promotes the interaction between RAD51 and RAD54 by binding to the acetylation site of RAD54, thereby regulating DNA homologous recombination (HR) activity.

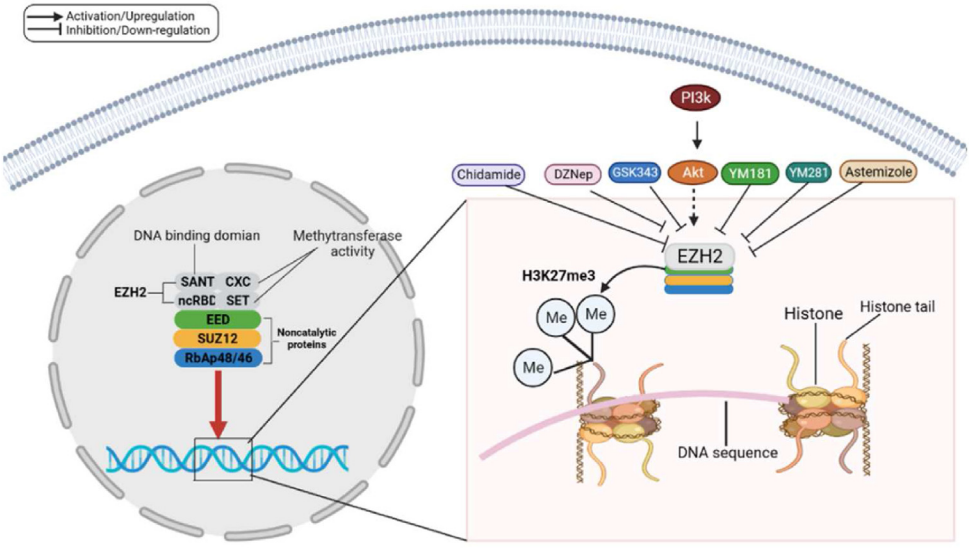

Figure 7 Biological functions of EZH2[59].

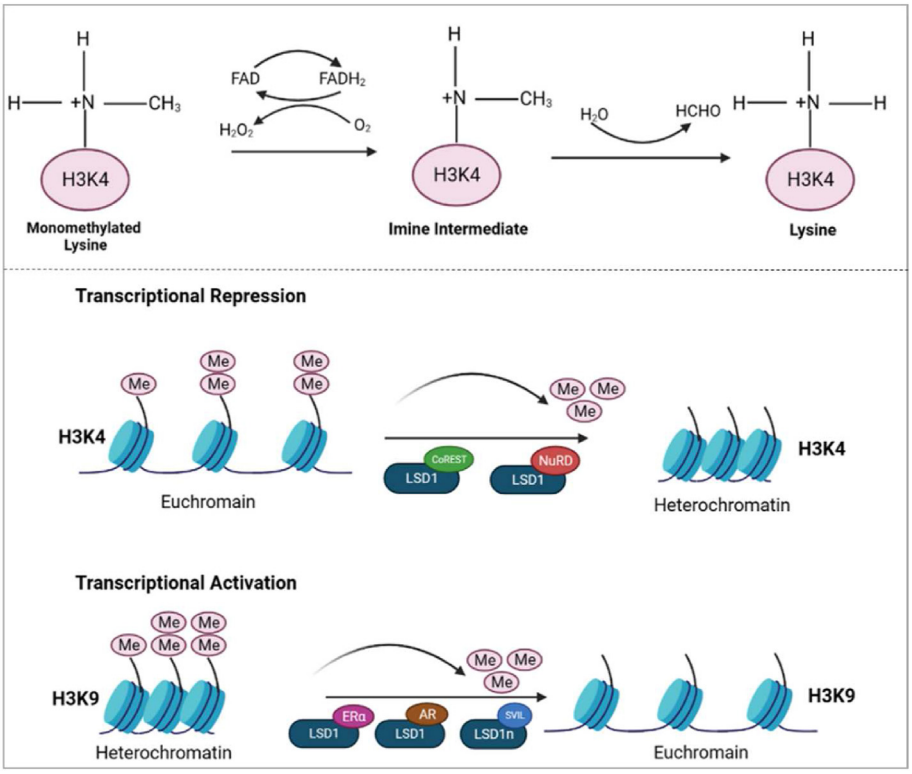

Figure 9 Biological functions of LSD1.

3.3.2. Eleven-nineteen leukemia (ENL)

The YEATS (Yaf9, ENL, AF9, Taf14, Sas5) domain is an emerging reader module that selectively recognizes histone lysine acylation, with a preference for crotonylation over acetylation[66,67]. Among these domains, ENL is a key component in the super elongation complex (SEC) and Dot1 Like (DOT1L) complexes, and functions as a reader of histone acetylation[68]. It is involved in regulating chromatin remodeling and gene expression of important proto-oncogenes such as myc and Hox genes by interacting with acetylated histones[69]. Moreover, ENL is closely related to AF9 and contribute to the regulation of promoter-proximal pause release and transcriptional elongation[70] (Fig. 11). Extensive research has demonstrated the indispensable role of ENL in disease maintenance, particularly in MLL-rearranged leukemia, which is a subtype of acute leukemias[66,71]. Depletion of ENL or disrupting the interaction between its YEATS domain and acetylated histones effectively suppresses leukemia progression[72]. Altogether, these findings underscore the potential therapeutic significance of targeting the YEATS domain of ENL.

4. Epigenetic degraders in clinical trials and the crystal structure of epigenetic degraders with their target proteins

4.1. PROTACs

With the deeper understanding of the mechanism of action of PROTACs and their immense potential in drug discovery, an increasing number of researchers are turning their attention towards this field[34,40,73]. As a result, the capacity of PROTACs to target more protein targets is continuously expanding.

Currently, a number of PROTACs are undergoing clinical trials, including ARV-110 (NCT03888612)[74] and ARV-471 (NCT04072952)[75], both developed by Arvinas, in Phase II clinical trials for prostate metastases and breast cancer, respectively[76,77]. DT2216 (NCT04886622), a potent B-cell CLL/lymphoma 2 (BCL-XL) degrader utilizing von Hippel-Lindau (VHL) E3 ligase recruitment, has entered Phase I clinical study for treating advanced liquid and solid tumors in 2021[78]. To date, more than 19 PROTACs are under evaluation in ongoing clinical trials, targeting POIs such as interleukin-1 receptor-associated kinase 4 (IRAK4) by degrader KT-413[79], signal transducer and activator of transcription 3 (STAT3) by degrader KT-333[80], and Bruton's tyrosine kinase (BTK) by degrader NX-5948[81].

While numerous PROTAC degraders have progressed to clinical trials, only a limited number of them fall under the category of epigenetic degraders. Notably, CFT8634, FHD-609, and RNK05047 (listed in Table 2) are currently undergoing clinical trials as potential treatments for late-stage synovial sarcoma patients (Fig. 12).

CFT8634 is an orally bioavailable PROTAC BRD9 degrader with a DC_{50} of 3 nmol/L. It has been granted orphan drug designation by the US FDA for treating synovial sarcoma[28]. BRD9 is an essential component of the BAF chromatin remodeling complex that contributes to synovial sarcoma cell formation. CFT8634 has demonstrated potent anti-tumor efficacy in two different synovial sarcoma patient-derived xenograft (PDX) models, with doses ranging from 1 to 50 mg/kg administered once

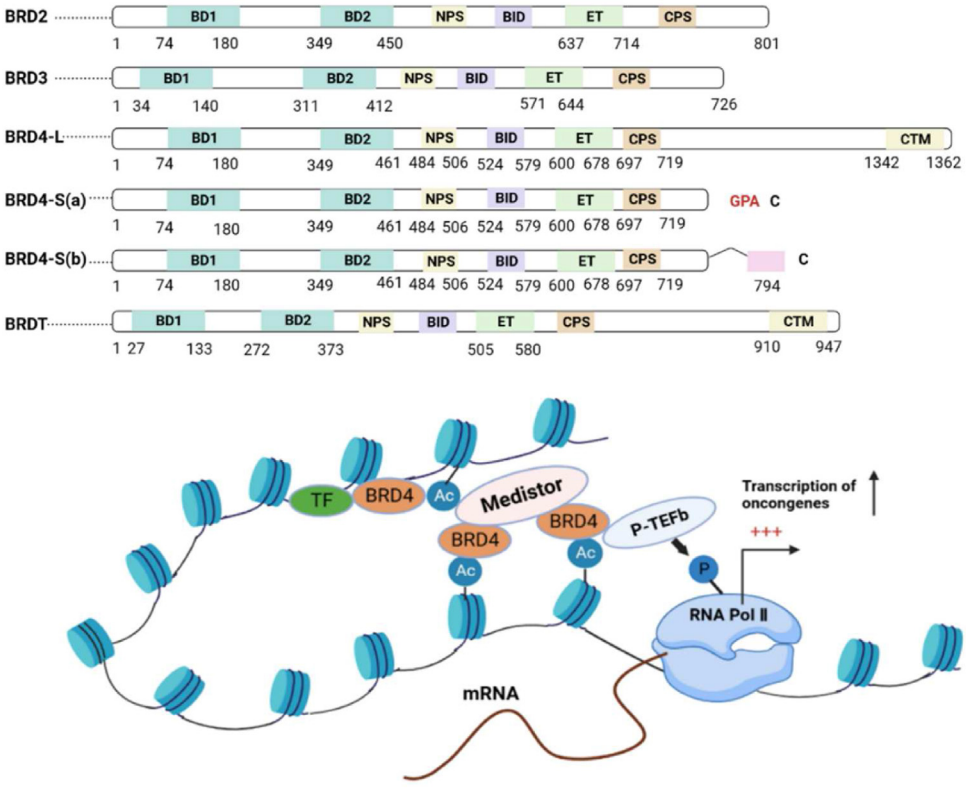

Figure 10 The BRD4 signaling pathway.

daily. Remarkably, the tumors continued to shrink even after CFT8634 treatment was discontinued.

FHD-609 is a PROTAC BRD9 degrader in clinical trials to treat synovial sarcoma and smarcb1-deficient tumors[27]. By degrading BRD9, FHD-609 prevents the formation of the BAF complex, thus treating synovial sarcoma. However, the clinical trial has been suspended by the FDA following severe cardiac-related adverse events (*e.g.,* grade 4 QTc prolongation and torsades de pointes ventricular tachycardia) observed in synovial sarcoma patients.

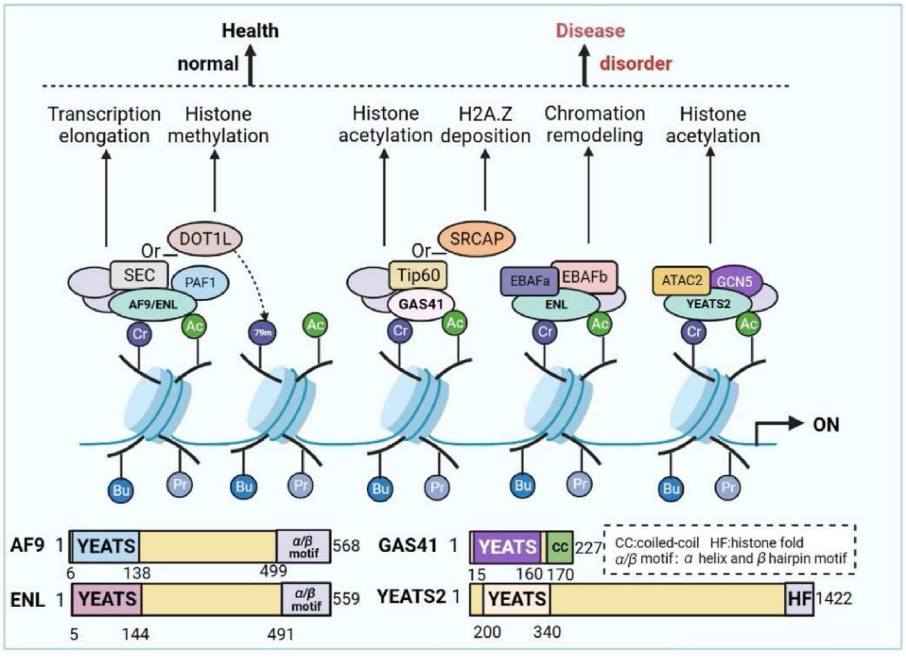

Figure 11 The involvement of ENL proteins in diverse cellular processes.

Table 2 Epigenetic degraders in clinical trials.

Drug name	Status	Trial number	Indications	Sponsor
CFT8634	Phase I/II	NCT05355753	Synovial sarcoma and smarcb1-deficient tumors	C4 Therapeutics, Inc.
FHD-609	Phase I	NCT04965753	Advanced synovial sarcoma and advanced smarcb1-loss tumors	Foghorn Therapeutics
RNK05047	Phase I/II	NCT05487170	Advanced solid tumors and lymphomas	Ranok Therapuetics Co., Ltd.

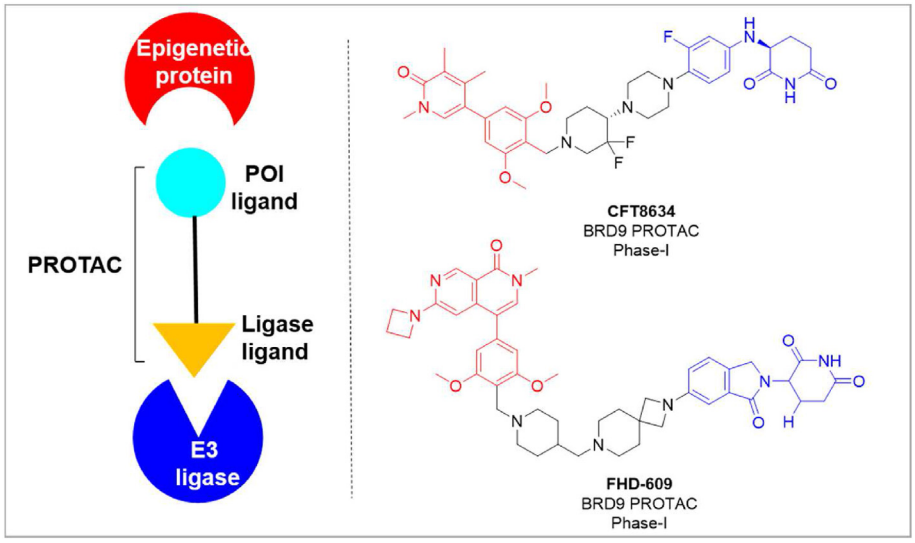

Figure 12 The BRD9 PROTACs in clinical trials.

RNK05047, a selective PROTAC degrader developed by Ranok Therapuetics, targets BRD4, a vital transcription factor that drives several types of cancer[82]. As of January 2022, RNK05047 has entered clinical trials to treat advanced solid tumors and lymphomas. Currently, the safety, tolerability, pharmacokinetics, anti-tumor activity, and pharmacodynamics of RNK05047 are being assessed in the ongoing Phase I/II clinical trial CHAMP-1.

4.2. *Molecular glues*

Since 2014, molecular glues have emerged as a new strategy for protein degradation[35]. As mentioned above, molecular glues exhibit desirable drug-like properties compared to PROTACs, including lower molecular weight, improved oral bioavailability, and enhanced PK/PD profiles. In recent years, important progress has been made in the rational discovery or design of molecular glue degraders *via* high-throughput screening (HTS) and/or chemogenomic screening. For instance, Li et al.[83] proposed a pioneering approach for identifying molecular glues that induce autophagic degradation by employing microarray-based HTS. Through this method, they identified several molecular glue molecules with the ability to target both the autophagosome protein LC3 (microtubule-associated protein 1A/1B light-chain 3) and the disease-causing mutant huntingtin protein (mHTT). However, at present, the identification of molecular glue degraders is largely serendipitous.

Currently, three molecular glue drugs, namely thalidomide and its derivatives (lenalidomide and pomalidomide, Fig. 13), have obtained approval for the treatment of multiple myeloma and other diseases. These drugs function by selectively ubiquitinating and degrading two lymphoid transcription factors, IKZF1 and IKZF3, using the CRBN−CRL4 ubiquitin ligases. Thalidomide exhibits an inhibitory effect on cereblon, with a K_d value of approximately 250 nmol/L. In addition, it also possesses notable immunomodulatory and anti-inflammatory properties. Furthermore, multiple molecular glue-based degraders have advanced to clinical trials, indicating the substantial market potential for these drugs in the future.

4.3. *HyT degraders*

HyT molecules consist of three essential components: a ligand specific for the POI, a substantial hydrophobic tag, and a linker that connects the ligand and hydrophobic tag, as illustrated in Fig. 14. Fulvestrant, a representative degrader based on HyT technology[84], stands as the sole FDA-approved selective estrogen receptor degrader (SERD). Upon binding to the estrogen receptor (ER), Fulvestrant augments the hydrophobicity of the ER protein surface, thereby initiating subsequent degradation. However, the poor oral bioavailability of Fulvestrant confine its administration exclusively to intramuscular injection, limiting its potential for effective binding and efficacy towards the intended target.

In a subsequent study, Wang et al. replaced the 3-OH group of fulvestrant with a boronic acid moiety and designed a modified form called ZB716[85]. This boronic acid modified fulvestrant binds competitively to ERα (IC_{50} = 4.1 nmol/L) and effectively downregulates ERα in both tamoxifen-sensitive and tamoxifen-resistant breast cancer cells. Additionally, ZB716 exhibits superior oral bioavailability in mice, suggesting its promising clinical utility as an orally administered SERD.

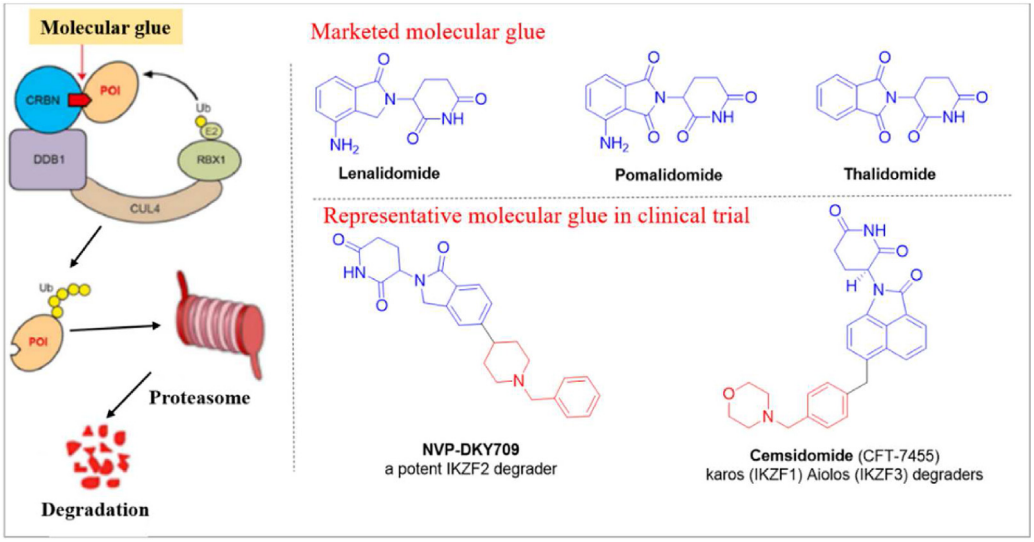

Figure 13 The marketed and representative molecular glues in clinical trial.

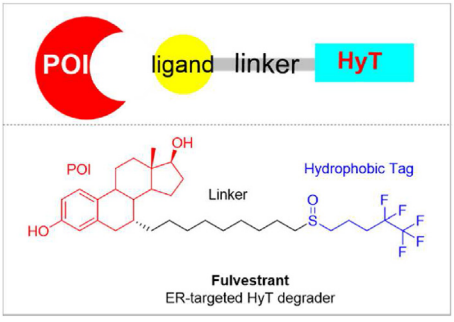

Figure 14 The marketed degrader (fulvestrant) based on HyT technology.

4.4. Crystal structures of epigenetic degraders in complex with their target proteins

With the disclosure of crystal structures of epigenetic PROTAC molecules in complex with their target proteins, there is greater promise for the rational design of more potent and selective epigenetic degraders. To date, several crystal structures of BRD4−PROTAC complexes have been determined.

In 2017, Gadd[23] disclosed the crystal structure of MZ1 in a ternary complex with the second bromodomain (BD) of BRD4 ($BRD4^{BD2}$) and VHL, resolving it to 2.7 Å, as shown in Fig. 15. MZ1 is bound within an extensive protein-protein interface shaped like a bowl, formed between $BRD4^{BD2}$ and VHL. The hydrophobic "base" of the bowl has two key points of contact. First, Trp374 from the characteristic hydrophobic "WPF shelf" (W374, P375, F376) of $BRD4^{BD2}$ interacts with residues Arg69, Pro71, and Tyr112 of VHL. Pro71 provides an additional stack to the WPF, forming an extended "PWPF" shelf. Second, Ala384 and Leu385 from the

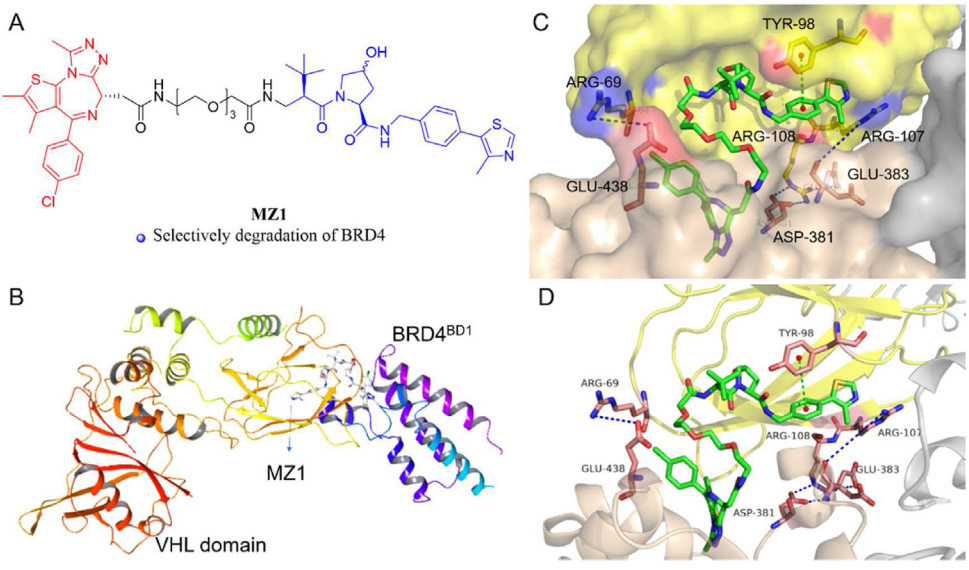

Figure 15 Overall structure of $BRD4^{BD2}$-MZ1-VHL in ribbon representation (PDB 5T35).

second helical turn of the ZA loop of $BRD4^{BD2}$ contact the hydrophobic side chains of Arg108, Ile109, and His110 in β4 of VHL. Two electrostatic"arms" complete the rim of the bowl. At one end, Asp381 and Glu383 in the ZA loop of $BRD4^{BD2}$ form a tight zipper structure of complementary charges with Arg107 and Arg108. At the opposite end, Glu438 residue of $BRD4^{BD2}$, located in the BC loop, makes contact with Arg69 from VHL.

In 2018, Nowak et al.[86] reported the crystal structures of ternary complexes of BRD4 PROTAC dBET6 with BRD4 and $CRL4^{CRBN}$, as illustrated in Fig. 16. In contrast, crystal structures of PROTACs/BRD4/CRBN complexes revealed the weak protein-protein interactions at the interface of both proteins. The observed interactions are predominantly hydrophobic, resulting in loose cooperative binding. Intriguingly, different binding conformations were observed between the BRD4 and CRBN complexes depending on the linker length and position of the bound PROTACs. This is believed to confer selectivity to the bound PROTACs.

5. HDAC-targeting degraders

In recent years, rapid progress has been made in the development of HDAC-targeting degraders, including PROTACs, molecular glue, and hydrophobic tag degraders. In this section, we will review the three types of HDAC degraders based on the specific subtypes of HDAC they target.

5.1. *HDACs-targeting PROTACs*

Achieving subtype specificity in PROTAC design for HDACs is highly challenging due to the structural similarities among different subtypes, especially the high homology shared between HDAC1 and HDAC2. Consequently, research on HDAC6 PROTACs has been more prevalent, likely because of an open hydrophobic pocket of approximately 14 Å width present in the HDAC6 protein that creates a notable structural difference from other HDAC subtypes.

5.1.1. *HDAC3-targeting PROTACs*

Numerous studies have reported the lethality associated with HDAC3 knockout, which may contribute to the significant cytotoxicity observed with HDAC3 selective PROTACs. In 2020, Xiao et al.[87] designed and synthesized a series of HDAC3 degraders by utilizing the lead compound SR-3558, a reported selective HDAC3 inhibitor. As shown in Fig. 17, the docking model of SR-3558 and HDAC3 protein suggested that the amide group of SR-3558 is exposed to solvent and can be conjugated *via* a linker to an E3 ligase ligand such as pomalidomide or VHL. Through structural optimization, the most promising PROTAC **1** was found to selectively and dose-dependently degrade HDAC3 (DC_{50} = 42 nmol/L) in MDA-MB-468 cells, with no effect on HDACs 1, 2, and 6. Moreover, PROTAC **1** exhibited excellent antiproliferative activity against four cancer cells with average IC_{50} values of 52 nmol/L. Utilizing partial subtype-selective HDAC3 inhibitors as ligands for the POI may lead to more efficient and selective degradation of HDAC3. In 2021, a novel HDAC3-targeting PROTAC degrader was developed by linking CI994 (a reported HDAC1/2/3 inhibitor) and pomalidomide (Fig. 17)[88]. Pleasurably, the representative compound **2** (HD-TAC7) exhibited excellent HDAC3 degradation potency with a DC_{50} of 320 nmol/L in RAW 264.7 cells.

5.1.2. *HDAC4-targeting PROTACs*

HDAC4 is implicated in controlling gene expression important for diverse cellular functions[89]. Besides, basic and clinical experimental evidence has identified HDAC4 as a potential therapeutic target in vascular senescence treatment. In 2019, Cristea reported that HDAC4 contributed to the progression of Huntington's disease (HD) and that HDAC4 knockdown significantly downregulated pathogenic gene expression and extended the lifespan[90].

Recently, Doherty et al.[90,91] identified two potent HDAC4 PROTACs, **3** and **4** as illustrated in Fig. 18. Degraders **3** and **4** exhibited significant and selective HDAC4 degradation activities with DC_{50} values of 11 and 2 nmol/L, respectively, in Jurkat

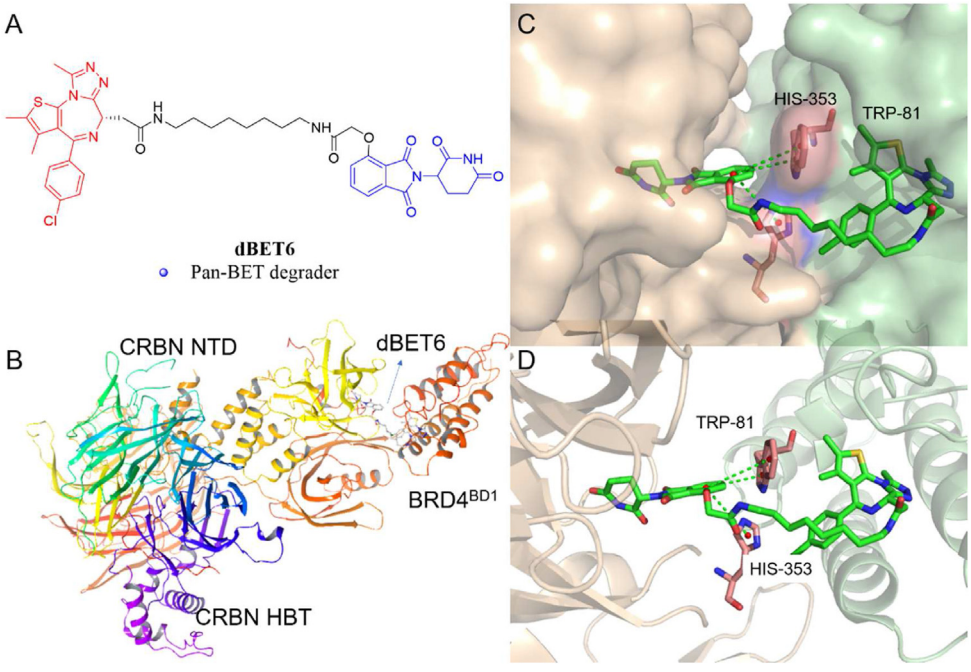

Figure 16 Crystal structure of dBET6/$BRD4^{BD1}$/CRBN (PDB: 6BOY).

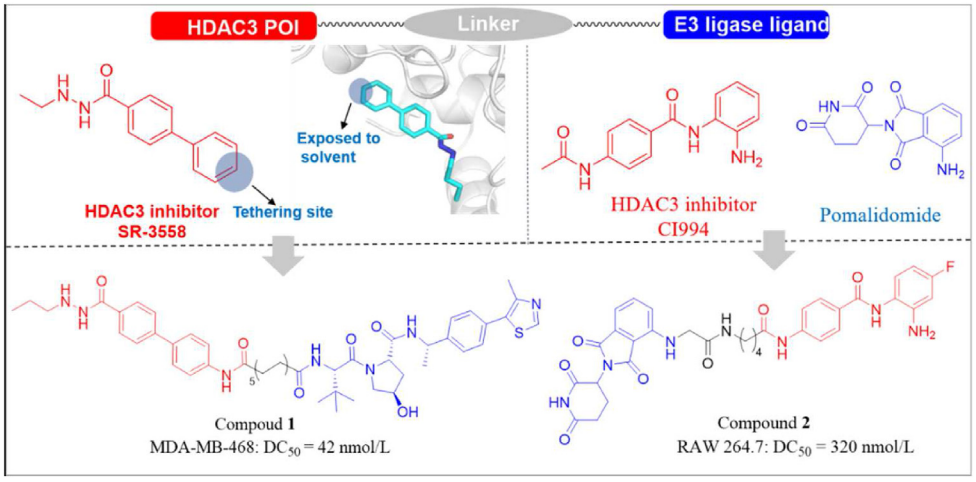

Figure 17 The representative HDAC3-targeted PROTACs **1**, **2**.

E6-1 cells, without affecting other HDAC isoforms. Furthermore, *in vitro* enzyme inhibition assays demonstrated that degraders **3** and **4** have moderate HDAC4 inhibitory activity, with IC_{50} values of 0.12 and 0.092 μmol/L, respectively. Notably, degrader **4** showed effectiveness in multiple cell lines, including HD mouse model-derived cortical neurons. This is the first reported degrader targeting HDAC for central nervous system diseases.

5.1.3. HDAC6-targeting PROTACs

The deregulation of HDAC6 is associated with numerous diseases such as cancer, neurodegenerative diseases[92], and pathological autoimmune responses[93–95]. Therefore, it is essential to directly control cellular HDAC6 protein levels for therapeutic purposes.

In 2018, the Yang group[24] pioneered the development of the first-in-class PROTAC-based HDAC6 degraders by conjugating WT161 (a highly selective HDAC6 inhibitor) to different E3 ligase ligands using alkyl or heterocyclic linkers (Fig. 19). Among them, degrader **5** significantly and selectively degraded HDAC6 in MCF-7 cells in a concentration-dependent manner at 12 h (DC_{50} = 34 nmol/L, D_{max} = 75%) without depleting other HDAC proteins. Furthermore, compound **5** exhibited HDAC enzyme inhibitory activity by increasing acetylated histone levels. It is highly likely that the excessively long linker of the POI ligand does not affect the enzyme inhibitory activity. However, the hydrazone linker of these HDAC6 degraders is not hydrolytically stable, which limits the further application.

In 2022, by employing solid-phase synthesis protocols, Sinatra and co-workers[96] developed two series of SAHA-like HDAC6 PROTACs. Among them, compounds **6** and **7** exhibited efficient and selective HDAC6-degradation activities in HL-60 cell lines at low nanomolar concentrations (DC_{50} of **6**: 3.4 nmol/L and **7**: 19.3 nmol/L) with comparable D_{max} values exceeding 80%, achieved *via* ternary complex formation and the UPS pathway.

Nexturastat A is a highly selective and potent inhibitor of HDAC6[97]. It demonstrated anti-inflammatory and anticancer activities in preclinical studies. Because of its high subtype selectivity, numerous research groups have chosen Nexturastat A as the warhead of PROTACs targeting HDAC6, as illustrated in Fig. 20.

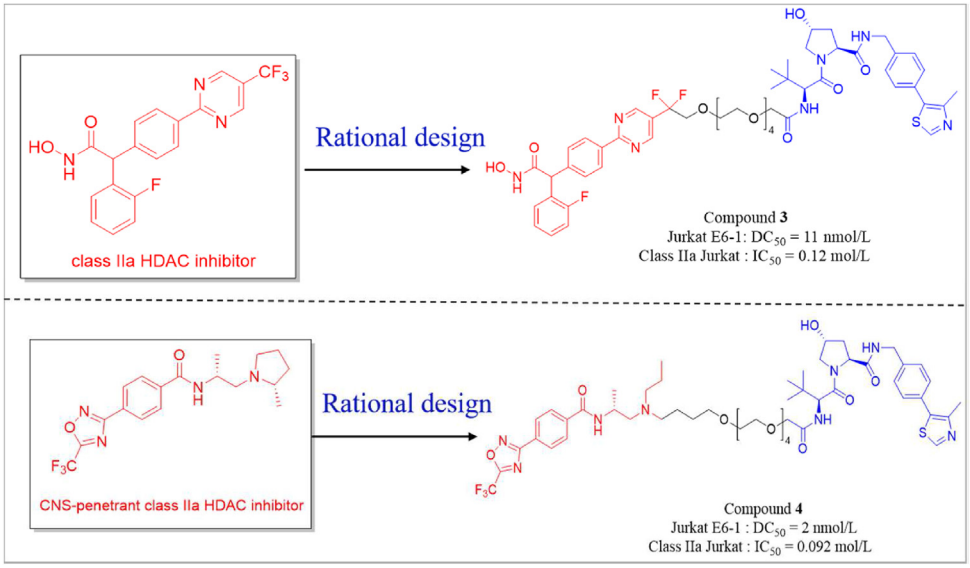

Figure 18 The design of HDAC4-targeting PROTACs **3**, **4**.

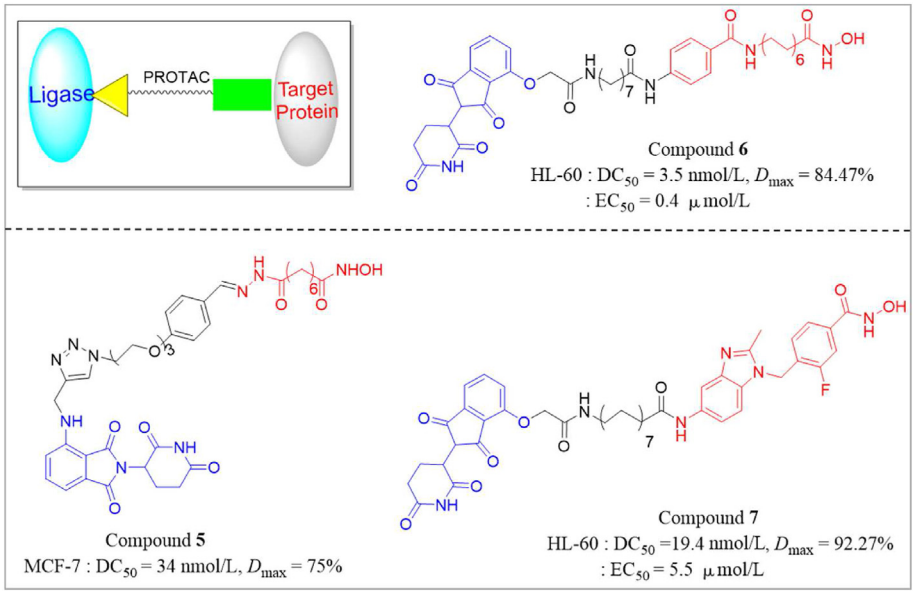

Figure 19 The representative HDAC6 PROTACs **5**–**7**.

Most of the research on Nexturastat A-based HDAC6 PROTACs is based on the co-crystal structure of the *h*HDAC6-Nexturastat A complex. This structure revealed that the propyl group and phenyl moiety of Nexturastat A are solvent-exposed fragments, and are suitable tethering sites (sites A and B) for the ligand. Yang and colleagues[98] improved the selectivity and potency of HDAC6 degraders by attaching pomalidomide to site A of Nexturastat A (Fig. 20). The most potent compound **8** degraded HDAC6 with a DC_{50} of 1.6 nmol/L and a D_{max} of 86% in MM.1S cell lines. Compared to the poor antiproliferative activity of Nexturastat A, compound **8** also demonstrated better cytotoxicity by degrading HDAC6.

Based on Nexturastat A, Yang and colleagues[99] reported a new HDAC6 degrader **9**, which exhibited excellent and comparable HDAC6-degradative activity in different cell lines (average DC_{50} of 3.2 nmol/L), including Mino, Jeko-1, HUVEC, and MDA-MB-231 cells. Most recently, using the same POI ligand, Wu and colleagues[97] developed another HDAC6 PROTAC degrader **10**, which exhibited significant HDAC6-degradation activity in the in-cell ELISA assay, with a DC_{50} of 1.9 nmol/L. At a concentration of 300 nmol/L, the "hook effect" was observed. Importantly, compound **10** had no effect on other targets, including HDAC4, IKZFs, and GSPT1.

In addition, An et al.[100] reported a class of HDAC6 PROTACs by appending a CRBN ligand to the site B of Nexturastat A. The representative degrader **11** induced HDAC6 degradation (DC_{50} = 3.8 nmol/L) specifically, without affecting other HDAC isoforms. Moreover, compound **11** exerted significant and comparable antiproliferative activity to Nexturastat A, with IC_{50} values of 1.21 and 2.25 μmol/L, respectively.

The multi-functionality of CRBN-based PROTACs could restrict their use as specific chemical probes to further study isoform-related cellular pathways. In 2020, Yang et al.[101] designed the first cell-permeable HDAC6 degraders by using VHL compound as an E3 ligase ligand. PROTAC **12** exhibited the best HDAC6 degradation activity in human MM1S and mouse 4935 cell lines, with DC_{50} values of 7.1 and 4.3 nmol/L, respectively. Importantly, compound **12** did not affect the levels of IKZF1/3, which are usually targets of CRBN-recruiting PROTAC degraders.

5.1.4. HDAC8-targeting PROTACs

In 2022, Chotitumnavee et al.[102] developed a series of novel HDAC8-targeting PROTAC degraders by linking pomalidomide and NCC149, a highly selective HDAC8 inhibitor. As shown in Fig. 21, the X-ray crystal structure of HDAC8 in complex with NCC149 revealed that the phenyl group of NCC149 was exposed on the surface of the protein, indicating that the phenyl moiety could serve as a suitable site for introducing a linker. By varying the linker position and length, compound **13** was identified as the most potent HDAC8 degrader, which selectively induced HDAC8 degradation (DC_{50} = 0.702 μmol/L) without affecting the levels of other representative HDAC enzymes, such as HDAC1, HDAC2, and HDAC6. Furthermore, even in the presence of a longer linker, degrader **13** maintained potent and selective HDAC8-inhibitory activity with an IC_{50} of 0.372 μmol/L and increased the acetylated SMC3 in Jurkat-T cells.

Based on the co-crystal structure, Darwish et al.[103] reported another pomalidomide-containing HDAC8 PROTAC **14** (Fig. 22). Degrader **14** exhibited strong HDAC8 degradation activity in SK-N-BE(2)-C neuroblastoma cells, with a DC_{50} value of 0.25 μmol/L. Moreover, compound **14** also had excellent anti-neuroblastoma activity and enhanced the differentiation phenotype.

In 2022, our group[104] designed and synthesized a series of novel HDAC8-selective PROTAC degraders based on BRD73954 (a reported dual HDAC6/8 inhibitor) and pomalidomide (Fig. 23A). By varying the linker length, flexibility/rigidity, and substitution position at pomalidomide, compound **15** was identified to be the most effective HDAC8 degrader with DC_{50} value of 147 nmol/L and a D_{max} of 93% in HCT-116 cells. Moreover, compound **15** exhibited the ability to degrade HDAC6 with a DC_{50} value of 4.95 μmol/L. This finding suggests that at higher concentrations, compound **15** can induce the degradation of HDAC6, albeit with a ~30-fold weaker effect compared to HDAC8 (DC_{50} = 147 nmol/L). It is worth noting that this phenomenon

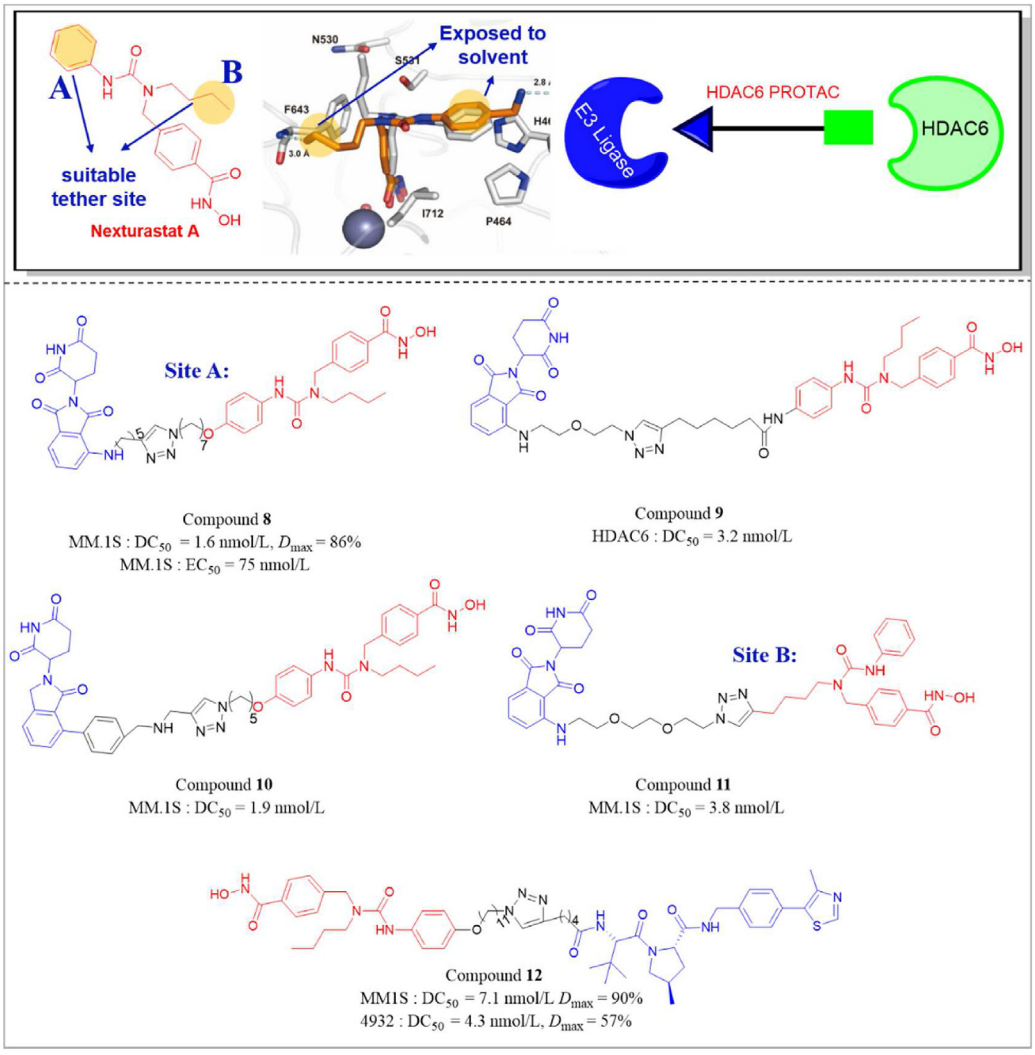

Figure 20 The Nexturastat A-based HDAC6 degraders **8**−**12**.

might be attributed to the dual inhibition of HDAC6 and HDAC8 by BRD73954.

Huang et al.[105] discovered a new class of HDAC8 PROTAC degraders based on the HDAC8 inhibitor PCI-34051 (Fig. 23B). The most potent compound **16** (SZUH280) induced ~80% HDAC8 protein degradation at a concentration of 2 μmol/L and had a DC_{50} of 0.58 μmol/L in A549 cells. Additionally, compound **16** exhibited potent antiproliferative activity (IC_{50} = 9.55 μmol/L) as well as significant *in vivo* antitumor efficacy in an A549 tumor model.

5.1.5. *Class I HDAC-targeting PROTACs*

The development of subtype-selective class I HDAC PROTACs is challenging due to the high homology in protein structures within the class I HDAC family. Therefore, a significant amount of research has been directed towards the development of partially selective PROTACs that target class I HDACs.

In 2021, Smalley et al.[106] reported a series of class I HDACs PROTAC degraders by using CI-994 as the POI ligand and VHL as the E3 ligase ligand. As shown in Fig. 24, the co-crystal structure of CI-994-HDAC complex revealed that the amide of CI-994 is exposed to solvent and can be conjugated *via* a linker to an E3 ligase ligand. By optimizing the linker component, compound **17** was identified as the best PROTAC degrader, selectively degrading HDAC1−3 with submicromolar DC_{50} values in HCT116 cells. Further mechanistic studies revealed that the degradation activity of these degraders were correlated with enhanced global gene expression and apoptosis.

Another degrader **18** exhibited the highest class I HDAC degradation activity, with approximately 50% degradation observed for HDAC1−3 at 1 μmol/L.

Recently, utilizing the Click chemistry strategy, Cross et al.[107] prepared a library of class I HDAC PROTACs based on Entinostat (a class I HDAC inhibitor currently in clinical trials) (Fig. 25). Among these, degrader JMC-137 (compound **19**) was identified as the most potent compound to deplete class I HDAC protein in a time- and dose-dependent manner through the proteasomal pathway (DC_{50} value of 2.84 μmol/L against HDAC1). Importantly, incorporating click reaction synthons into PROTACs generates a Ligation to Scavenging effect, which enables the controlled activation or inactivation of the degradation process of target proteins. This effect facilitates precise regulation of the levels of the target protein.

Due to the significant binding affinity of peptide macrocycles, Roatsch et al.[108] designed the first macrocyclic tetrapeptide-

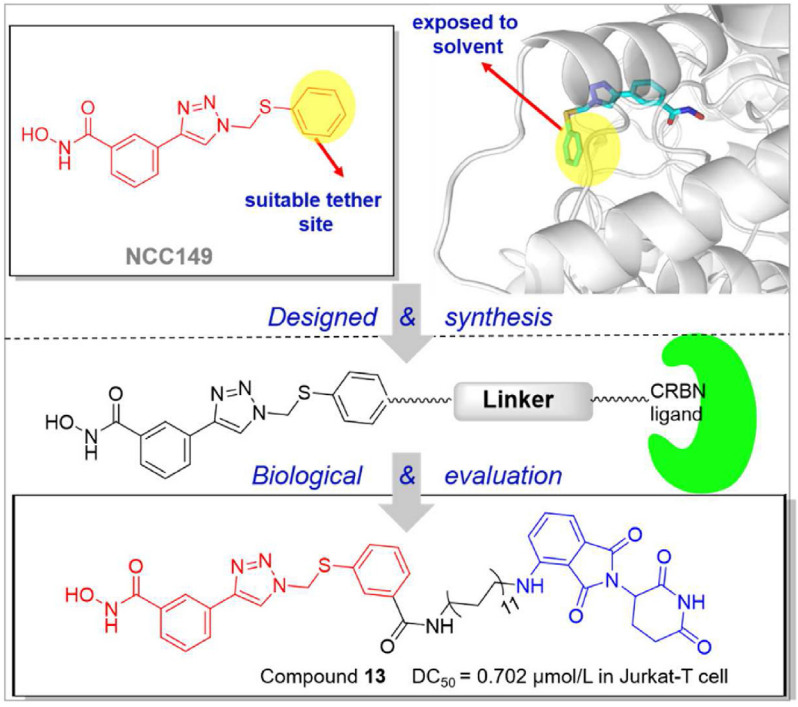

Figure 21 The representative HDAC8-targeted PROTAC **13**.

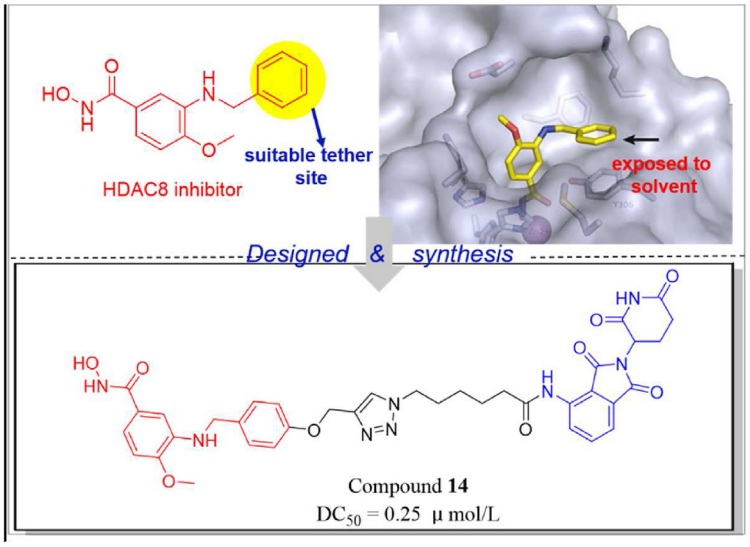

Figure 22 The structure of representative HDAC8-targeting PROTAC **14**.

containing class I HDAC PROTAC degraders based on the macrocyclic peptide HDAC inhibitors (TpxBAoda and TpxBAsuha) (Fig. 25). These conjugates caused the degradation of HDAC1-3 in HEK293T cells in a time- and concentration-dependent manner. Additionally, the hydroxamate-containing degraders exhibited moderate to excellent HDAC enzyme inhibitory activities, and the most potent compound **20** selectively inhibited HDAC1, HDAC2, and HDAC3 with IC_{50} values of 77.1, 92.3, and 4.43 nmol/L, respectively.

It is worth mentioning that, in comparison to class I HDAC inhibitors which exhibit significant toxicity towards both cancer cells and normal cells[109], class I HDAC degraders offer certain advantages in terms of safety and drug resistance. Class I HDAC inhibitors rely on strong binding to the target protein (class I HDAC), usually at the active site, to achieve an "occupancy-driven" effect. This often requires reaching effective drug concentrations. In contrast, PROTAC molecules employ an "event-driven" binding mode, allowing for weak interactions with the target protein to facilitate protein degradation[73]. As a consequence, they can exert their functions effectively at remarkably low doses, providing a safer alternative to class I HDAC inhibitors that require higher drug concentrations. Furthermore, in cases where target proteins undergo mutations that result in reduced affinity for traditional small molecule inhibitors, PROTAC

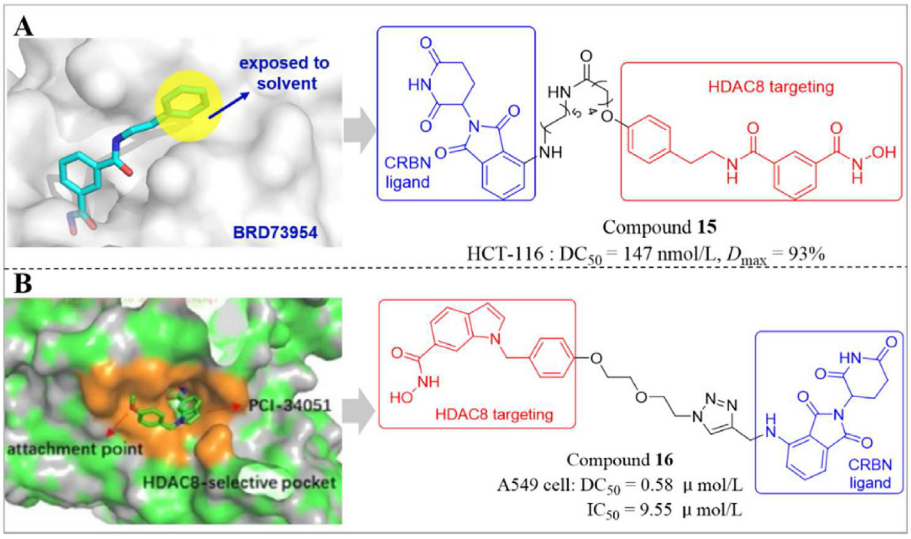

Figure 23 The HDAC8 PROTACs **15** and **16**.

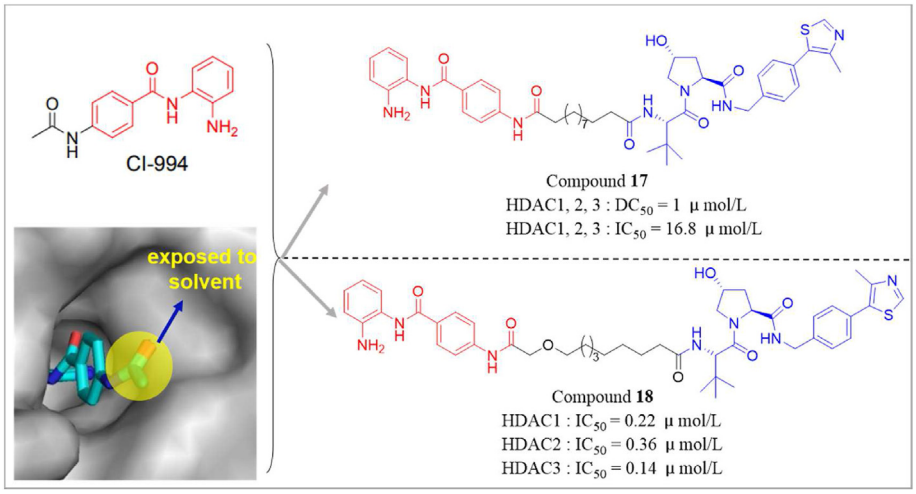

Figure 24 CI-994-based class I HDAC PROTACs **17**, **18**.

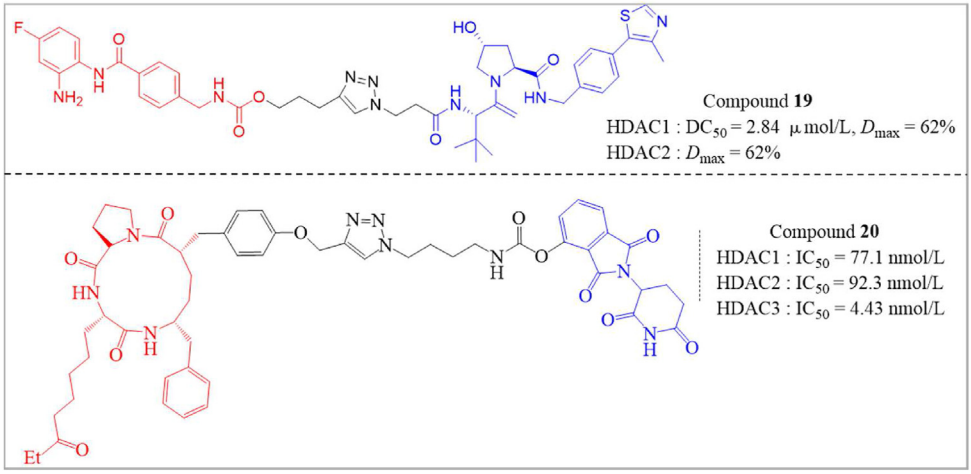

Figure 25 Class I HDAC PROTAC degraders **19**, **20**.

molecules leverage the ubiquitin-proteasome system to degrade the target protein[110]. This confers significant advantages in overcoming drug resistance.

5.1.6. *Class III HDAC (Sirtuins)-targeting PROTACs*

Sirtuins are a family of NAD^+-dependent deacetylases (class III HDACs) composed of seven mammalian enzymes (Sirt1−7), each with vastly different functions and locations. Modulators that target sirtuins have shown the potential for treating various human diseases, including cancer, type II diabetes, and other age-related diseases. Therefore, Sirtuin-targeting modulators, such as inhibitors or degraders, have attracted significant attention from the medicinal chemistry community.

In 2017, Schiedel et al.[111] developed a series of novel triazole-derived Sirt2 PROTACs by conjugating the sirtuin rearranging ligand **21** (SirReals, a potent Sirt2 inhibitor) with thalidomide, as shown in Fig. 26. Most of the newly synthesized SirReal-based PROTACs induced selective degradation of Sirt2, with concomitantly increased efficacy of tubulin hyperacetylation and enhanced process elongation. Additionally, the representative compound **22** displayed selective Sirt2 inhibitory activity with an IC_{50} of 0.25 μmol/L. This is the first example of a PROTAC targeting the epigenetic eraser protein.

5.1.7. *Other HDAC-targeting PROTACs*

In 2020, Sinatra et al.[112] developed an efficient solid-phase supported approach using hydroxamic acids immobilized on resins (HAIRs) as versatile building blocks for the preparation of a library of DNA-damaging and HDAC dual inhibitors. Compound **23** exhibited the highest antiproliferative and anti-HDAC6 activity with IC_{50} values of 2.68 and 0.62 μmol/L, respectively. To explore the utility of the preloaded resins strategy, the HAIR protocol was extended to the synthesis of proof-of-concept HDAC PROTACs, as shown in Fig. 27. Among the new synthesized PROTACs, degrader **24** effectively degraded HDAC6 and HDAC1 protein in a concentration-dependent manner.

5.2. *HDAC-targeting molecular glue degraders*

PROTACs can achieve targeted degradation of some proteins that are deemed "undruggable" previously. However, there is currently a dearth of rational design principles for converting protein-targeting ligands into molecular glue degraders. Therefore, most studies on molecular glues rely on fortuitous discovery of different ligand combinations through randomization.

Recently, Toriki et al.[29] developed a modular chemical handle (**25**) capable of converting various types of protein-targeting

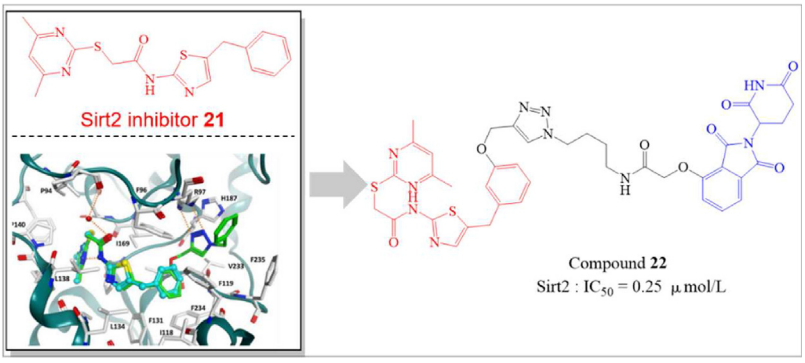

Figure 26 The Sirt2-targeting PROTAC **22**.

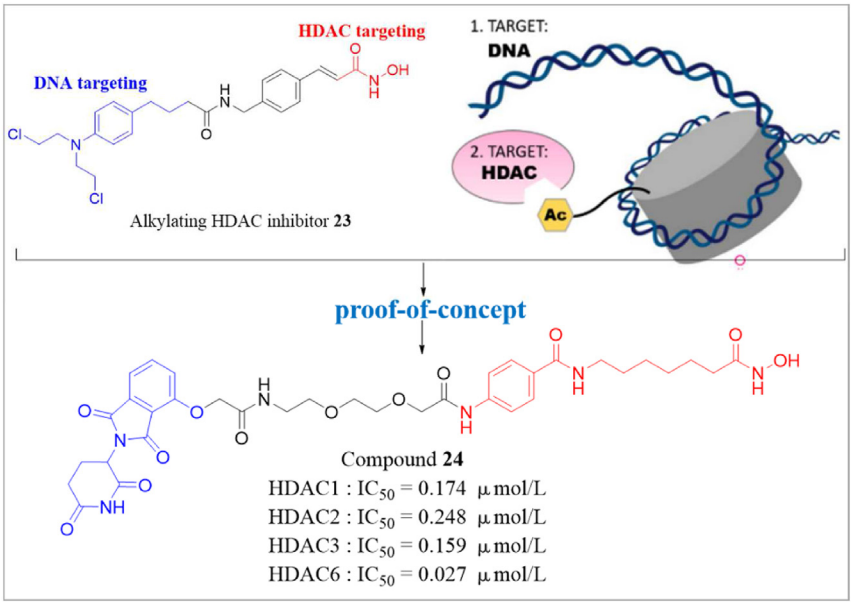

Figure 27 The design strategy of PROTAC **24**.

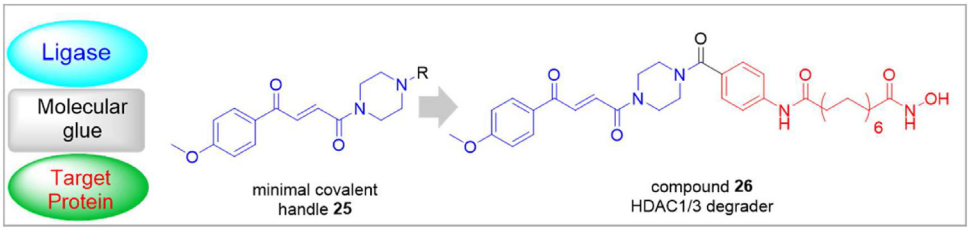

Figure 28 The HDAC-targeting molecular glue degrader **26**.

ligands into molecular glue degraders. Based on the structure of SAHA (Fig. 28), they synthesized HDAC1/3 degrader **26**, which demonstrated greater degradation efficacy than HDAC1/3 PROTACs. This study presents the first HDAC-targeting molecular glue degrader, which provides several advantages such as low weight and high druggability, and may offer a promising pathway for developing HDAC-targeted degraders.

5.3. *HDAC-targeting HyT degraders*

As previously mentioned, HyT degraders have several advantages over PROTAC molecules, including their lower molecular weight and the absence of teratogenic risks associated with thalidomide derivatives. Consequently, medicinal chemistry researchers have shown a keen interest in HyT degraders targeting HDACs.

In 2020, Schiedel et al.[26] utilized Halo-tagged parkin as E3 ubiquitin ligases for targeted protein degradation and discovered a novel class of chloroalkylated sirtuin rearranging ligand (SirReal)-based HyTs that enabled small-molecule-induced selective degradation of Sirt2 (Fig. 29). Among of them, compound **27** efficiently induced Sirt2 degradation at a concentration of 20 nmol/L in HeLa cells while also inhibiting the activity of Sirt1, Sirt2, and Sirt3 with IC_{50} values of 103, 0.74, and 165 nmol/L, respectively. Furthermore, they also demonstrated the degradation of Sirt2 induced by compound **27** was accompanied by acetylation of the microtubule network. Importantly, this study validates and highlights the effectiveness of HyT degraders for target protein degradation.

In addition, Huang et al.[113] utilized 18b-glycyrrhetinic acid (GA) as the HyT warhead to synthesize a novel series of HDAC-targeting HyT degraders that tethered SAHA and GA *via* different linkers. Compound **28**, bearing a piperazine fragment, exhibited the most potent HDAC3/6 degradation activity. In addition, **28** displayed enhanced or comparable antiproliferative activities (IC_{50} = 0.47 μmol/L, IC_{50} = 0.37 μmol/L) in PC-3 and HL-60 cells compared to SAHA (IC_{50} = 1.81 μmol/L, IC_{50} = 0.42 μmol/L). Furthermore, **28** inhibited HDAC3 and HDAC6 enzyme activities with IC_{50} values of 0.635 and 0.368 μmol/L, respectively. Moreover, **28** also possessed favorable pharmacokinetic properties with a long half-life of 16.75 h.

Compared to the HDAC-targeting PROTAC degraders, the research on the mechanism and clinical application of HyT technology is relatively scarce. Even though multiple hydrophobic tags with unique structural types have been reported, a majority of them exhibit unfavorable physicochemical or pharmacokinetic properties. Furthermore, incomplete degradation of target proteins owing to low bioavailability poses a significant challenge for the development and clinical application of hydrophobic tag molecules.

6. Bromodomain and extra-terminal (BET) domain-targeting degraders

To date, many potent BET inhibitors (BETis) have been developed[114], and a large number of them are currently undergoing

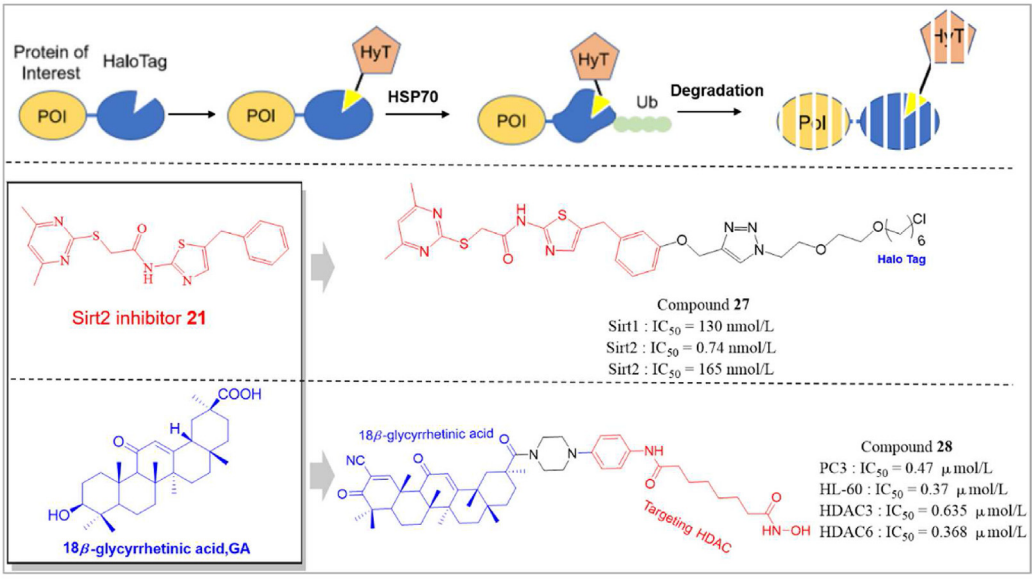

Figure 29 The HyT-based degraders **27**, **28**.

various clinical trials for cancer therapy. However, the lack of selectivity of BET inhibitors can lead to toxicity, which limits their use as anticancer agents. Additionally, BETis exhibit only modest clinical efficacy as single agents in clinical trials, and combination therapy with other drugs is more common. Therefore, in recent years, an increasing number of BET PROTAC degraders have emerged to address the limitations of BETis.

6.1. *BET PROTAC degraders*

6.1.1. *Pan degrader of BET*

BET inhibitors have demonstrated growth-inhibitory activity in preclinical models of castration-resistant prostate cancer (CRPC). Raina et al.[22] identified a novel PROTAC degrader, ARV-771 (Fig. 30, compound **29**), which displayed pan-BET degradation activity with $DC_{50} < 1$ nmol/L. Interestingly, compared to the moderate BET inhibitory activity of JQ-1 and OTX015, ARV-771 was approximately 10- and 100-fold more potent. Importantly, ARV-771 induced significantly stronger apoptotic effects and demonstrated dramatically higher efficacy in CRPC models than BET inhibitors.

Additionally, by using a long alkyl chain as the linker, Winter et al.[115] prepared a novel BET degrader **30**, which demonstrated remarkable degradation activity against BET proteins at 100 nmol/L. In addition, compound **30** induced subsequent downregulation of C-myc and apoptosis of cancer cells. Moreover, mice treated with **30** (7.5 mg/kg) exhibited significant survival benefits compared to JQ1 (7.5 mg/kg).

Most of the studies mentioned above used JQ1 as the POI ligand to target BET proteins. However, Ciulli and colleagues examined the impact of derivatizing two different BET inhibitors, JQ1 and the more potent tetrahydroquinoline I-BET726, on the activity and intra-BET selectivity profile of BET-targeting PROTACs. The authors demonstrated that JQ1-based PROTACs exhibited positive cooperativity of ternary complex formation and were more potent degraders than I-BET726-based degraders. Furthermore, the length of the linker significantly impacted the BET-degrading effect and C-myc-driven antiproliferative activities.

In 2017, Zhou et al.[116] designed a series of BET PROTACs based on the BET inhibitor RX-37 (Fig. 31). Using the co-crystal complex structure of RX-37 and $BRD4^{BD2}$, the 2-carboxamide moiety in RX-37 was identified as an appropriate site for tethering thalidomide/lenalidomide. The most potent compound **31** could degrade BRD2/3/4 in the RS4; 11 leukemia cell line with DC_{50} of 0.1, 0.1, and 0.03 nmol/L, respectively. Moreover, compound **31** exhibited potent anti-proliferative activity in the RS4; 11 leukemia cell line ($IC_{50} = 51$ nmol/L), comparable to that of RX-37 ($IC_{50} = 24$ nmol/L). Compound **31** also exerted potent antitumor activity in RS4; 11 leukemia tumor xenografts by reducing the level of BRD2/3/4 proteins in the tumor tissues. Furthermore, compound **31** displayed favorable PK properties with both intravenous and oral route of administration in mice and achieved extensive tissue distribution.

Further, Bai and colleagues[117] discovered the second-generation BET inhibitor BETi-211, based on RX-37 and further prepared the small-molecule hetero-bifunctional molecule **32** (BETd-246) as a BET PROTAC (Fig. 31). At low nanomolar concentrations (10, 30 nmol/L), **32** induced the degradation of BET proteins (BRD2/3/4) in a dose-dependent manner in TNBC cells, resulting in robust antiproliferative activity with IC_{50} of <10 nmol/L against 9 TNBC cell lines. Compared to BET inhibitor BETi-211, **32** was much more potent in inducing cancer cell apoptosis by downregulating the MCL1 gene, a critical downstream effector of these BET degraders. Moreover, BET degrader **32** effectively suppressed breast tumor growth in xenograft mouse tumor models at a dose of 10 mg/kg. Although the molecular weight and tissue penetration of PROTACs present certain challenges, both aforementioned studies successfully demonstrate the *in vivo* activity of PROTAC degraders.

In 2019, Qiu et al.[118] disclosed a DIPEA-promoted chemoselective alkylation of lenalidomide with different halides to prepare various functionalized lenalidomide-based PROTACs (Fig. 31). The practical strategy was further applied to synthesize BET-targeting PROTACs, and the representative compound **33** degraded BET proteins and effectively inhibited cancer cell proliferation.

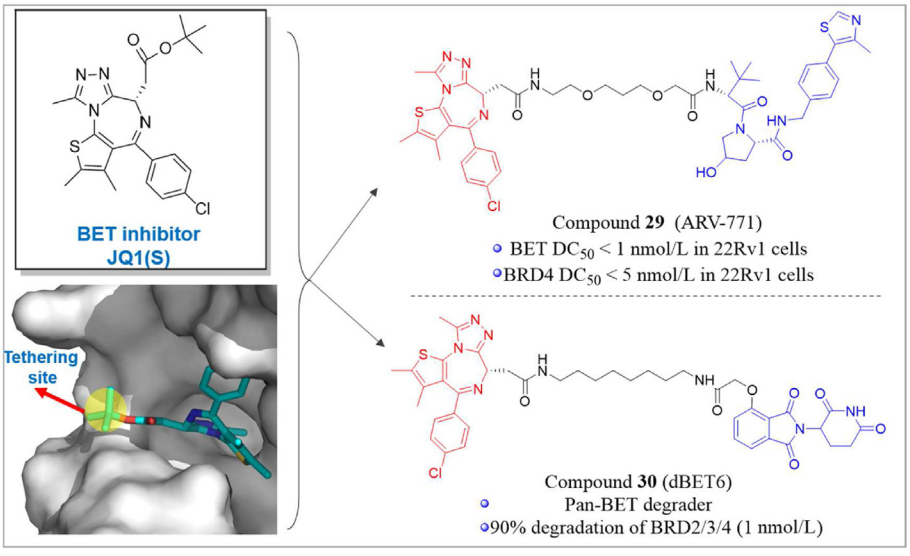

Figure 30 BRD4-targeting degraders **29**, **30**.

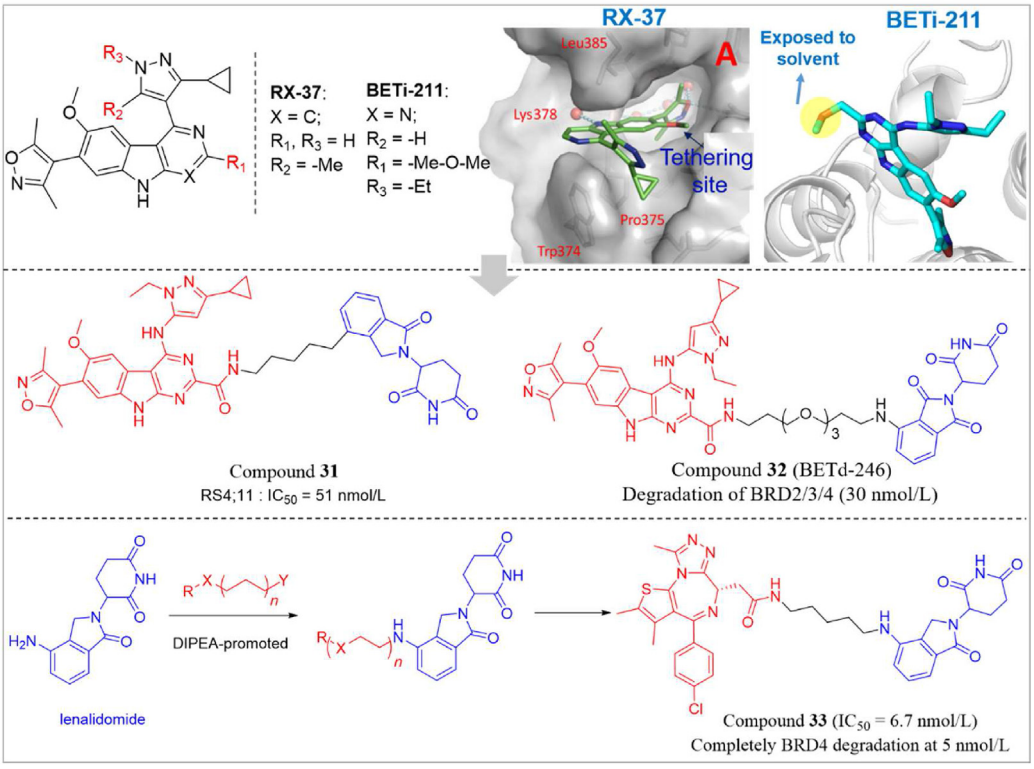

Figure 31 Crystal structure guided design of BET-targeting degraders **31**−**33**.

6.1.2. BRD4 selective degraders

In 2015, Crews et al.[21] designed and synthesized a series of BET degraders by conjugating OTX015 (a reported BRD4 inhibitor) with pomalidomide *via* a flexible PEG linker (Fig. 32). The representative compound **34** (ARV-825) effectively induced the degradation of BRD4 in different Burkitt's Lymphoma (BL) cell lines, with DC_{50} below 1 nmol/L and almost complete degradation of BRD4 protein at a concentration of 10 nmol/L within 6 h. Moreover, compared to JQ1 and OTX015, ARV-825 has advantages in terms of its anti-proliferation and apoptotic effects, likely due to the suppression of C-myc expression in cancer cells. Further mechanism studies showed that ARV-825 led to strong BRD4 degradation through a cereblon-mediated and proteasome-dependent pathway.

Crystallographic studies have indicated that the carboxyl moiety of JQ1 and the aryl ring of thalidomide can tolerate chemical substitutions. Based on this information, Zengerle and Winter designed a number of BET-targeting PROTACs using different linkers and E3 ligase ligands (Fig. 33)[119,120]. Zengerle et al. reported that compound **35** (MZ1, Fig. 33A), which bears a VHL-based ligand, had the highest activity with a BRD4-degradation rate of 90% at 1 μmol/L, without effecting BRD2/3 protein, although the selectivity toward BRD4 still needs to be improved. In addition, the protein degradation induced by **35** is dependent on binding to VHL, can be reversed by blocking the proteasome activity, and does not interfere with the endogenous, physiological levels of VHL and its natural substrate HIF-1α. In contrast, Winter et al. employed pomalidomide as an E3 ligase

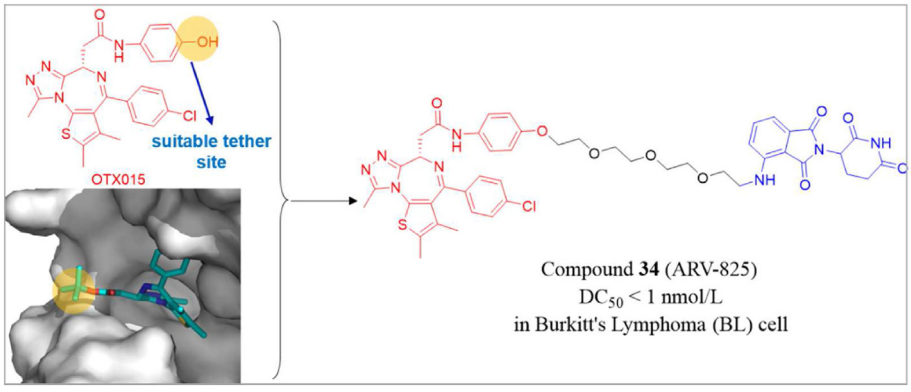

Figure 32 BRD4-targeting degrader **34**.

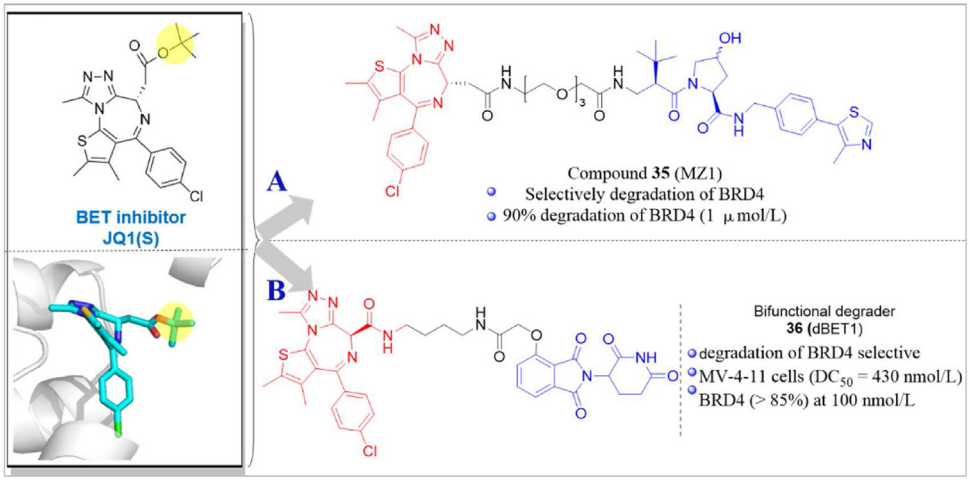

Figure 33 The JQ1-based BRD4 PROTAC degraders **35**, **36**.

ligand to synthesize compound **36** (dBET1, Fig. 33B) as a PROTAC BRD4 degrader that depleted BRD4 with a DC_{50} of 430 nmol/L in MV4; 11 AML cells[120]. Furthermore, the tolerability and antitumor efficacy of **36** also evaluated in a human leukemia xenograft model and **36** had favorable drug exposure in tumor-bearing mice, probably due to the pharmacologic destabilization of BRD4 *in vivo*.

To enhance the cell selectivity of degrader **35** (MZ1), Gadd et al.[23] modified its linker based on the crystal structure of MZ1 in complex with a BET bromodomain and VHL. The structure revealed that the two ligands of MZ1 were in close spatial proximity within the ternary complexes, which hindered the interactions with BRD4 (Fig. 34). Consequently, Gadd developed a novel BRD4-specific degrader named AT1 (degrader **37**) by using different linkers. AT1 bears an alkyl linkage and exerted highly cell selective depletion of BRD4 at 1 μmol/L after 24 h of treatment, with negligible activity against BRD2/3, and depletion in normal cells.

Testa et al.[121] further provided the first proof-of-concept validation of a macrocyclic PROTAC as a novel strategy to lock the PROTAC conformation in the bound state, thereby improving the selective degradation efficacy of BRD4 (Fig. 34). By appending another PEG linker to "close a circle" between the two ligand moieties of MZ1, they obtained a first macrocyclic BRD4 PROTAC named macroPROTAC **38**. Comparable to MZ1 (DC_{50} ≈ 500 nmol/L), macroPROTAC **38** induced potent and rapid degradation of BRD4 in 22RV1 cells with a DC_{50} of 25−125 nmol/L, without affecting BRD2/3. Furthermore, **38** displayed notable cytotoxicity with IC_{50} values of 640 and 300 nmol/L in 22RV1 and MV4; 11 cells, respectively.

Based on the binding conformation of a selective-BD1 BET inhibitor **39** to the BRD4 protein, Jiang et al.[122] designed and

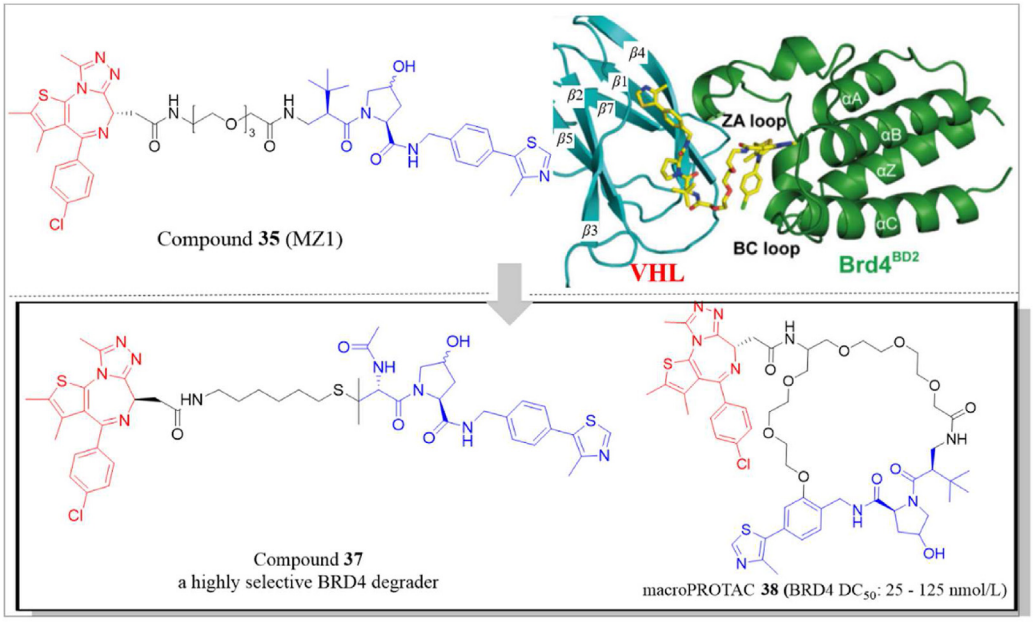

Figure 34 MZ1-based BRD4-targeting degraders **37**, **38**.

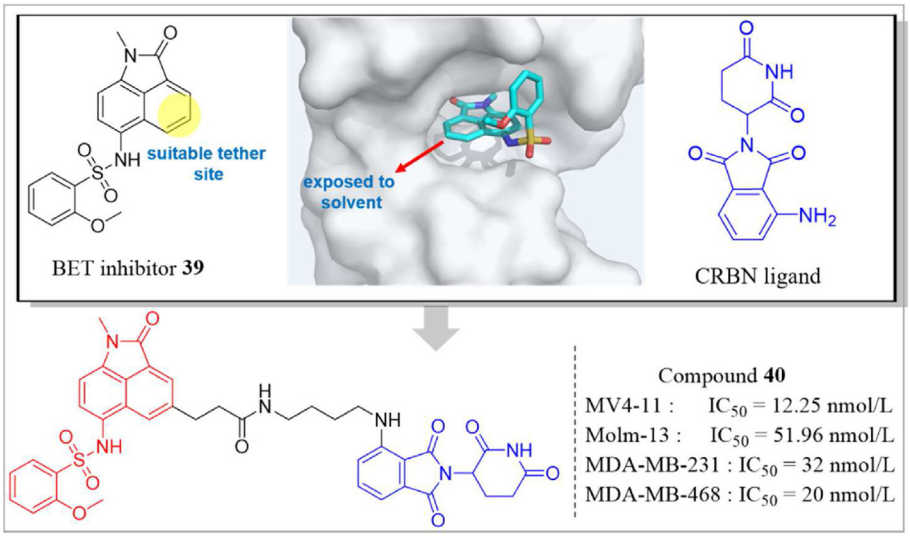

Figure 35 The representative BRD2/4-targeting PROTAC degrader **40**.

synthesized a series of BRD2/4 dual-targeting PROTACs (Fig. 35). Most of them enabled the degradation of both BRD2 and BRD4 simultaneously. In particular, compound **40** completely degraded BRD4 in MV4-11 cells at 1 μmol/L for 8 h and remained effective at concentrations as low as 10 nmol/L. Moreover, degrader **40** exhibited high anti-proliferative activity in leukemia cells, such as MV4-11 (IC_{50} = 12.25 nmol/L) and Molm-13 cells (IC_{50} = 51.96 nmol/L). Furthermore, **40** was effective against solid tumor cells, such as MDA-MB-231 and MDA-MB-468, with IC_{50} values of 32 and 20 nmol/L, respectively.

Although several E3 ligases have been successfully utilized in the design of PROTAC BET degraders, this has been limited to those for which small molecule ligands (*e.g.,* pomalidomide and VHL ligand) have been developed. In 2019, Hines et al.[123] discovered a novel class of MDM2-recruiting BRD4 degraders by linking the MDM2 inhibitor idasanutlin with JQ1 (Fig. 36). Among them, compound **41** with a polyethylene glycol (PEG) linker dose-dependently degraded BRD4 in HCT116 cells with a DC_{50} of 32 nmol/L. Moreover, compared with CRBN or VHL-based BRD4 PROTACs (*e.g.,* ARV-825), MDM2-recruiting BRD4 degrader **41** only partially degraded BRD2/3 and exhibited stronger antiproliferative activity in cancer cell lines with wild-type p53. These results suggest that the length of the linker and different E3 ligase ligands may achieve isoform-selective BET degradation.

BRD4 contains two isoforms, BRD4 short (BRD4-S) and BRD4 long (BRD4-L). Recent research has shown that BRD4-S

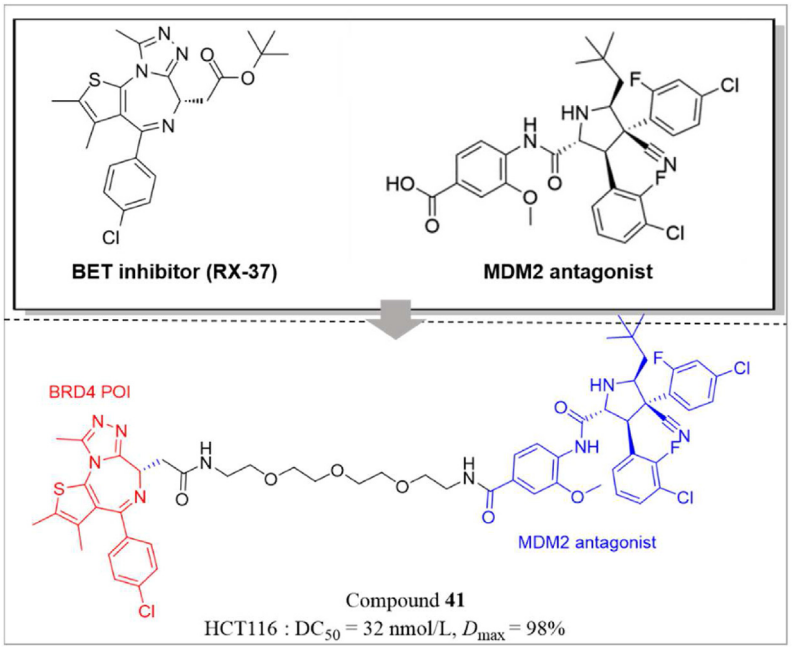

Figure 36 The structure of MDM2-recruiting BRD4-degrading PROTAC **41**.

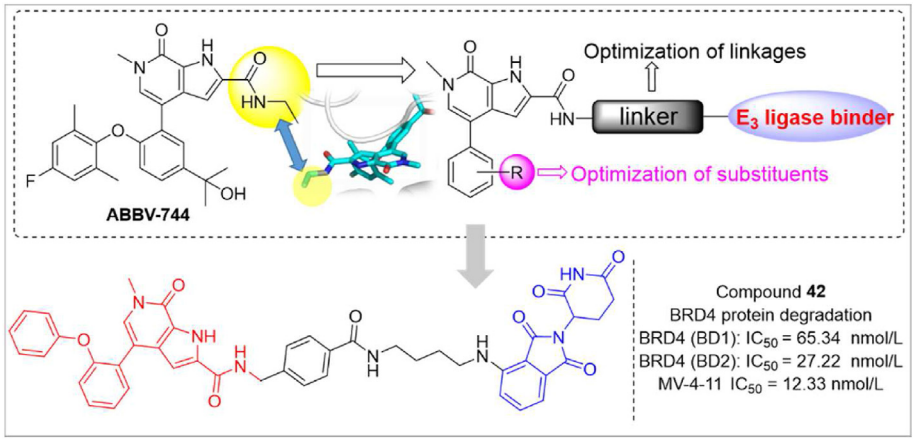

Figure 37 The structure of CRBN-based BRD4-degrading PROTAC **42**.

and BRD4-L are close related to the development of breast cancer and current BRD4 PROTACs are non-selective against BRD4-S and BRD4-L.

In 2022, Chen et al.[124] reported a pyrrolopyridone-based BRD4 degrader for the first time by linking ABBV-744 (a known BET inhibitor) as the warhead (Fig. 37). Those degraders effectively induced the degradation of both BRD4-S and BRD4-L isoforms in breast cancer cells. Particularly, degrader **42** displayed the highest degradation potency for BRD4-L/S in 4 and 24 h, respectively, and downregulated C-myc protein, which is essential for the cytotoxicity of a BET inhibitor or degrader. In addition, **42** demonstrated significant BRD4 inhibitory activity ($BRD4^{BD1}$, IC_{50} = 65.34 nmol/L; $BRD4^{BD2}$, IC_{50} = 27.22 nmol/L) and excellent antiproliferative activity (IC_{50} = 12.33 nmol/L), better than that of ABBV-744 (IC_{50} = 279 nmol/L).

Recently, Yan et al.[125] reported a novel dual PROTAC molecule that selectively degraded cellular BRD3 and BRD4-L without influencing BRD2 or BRD4-S in a panel of six cancer cell lines (Fig. 38). Fluorescence polarization (FP) protein binding assays demonstrated that the most potent compound **43** bound to BRD3 BD1 and BD2 with K_i values of 16.9 and 2.8 nmol/L, respectively. Degrader **43** efficiently promoted selective degradation of BRD3 and BRD4-L *in vitro* and *in vivo*.

Although a family of BET PROTAC degraders has been identified, the selectivity issue of BET PROTACs has not been fully addressed. Ding and Nowak designed and synthesized novel small-molecule BRD4 degraders **44**[126] and **45**[86] by using different linkages to conjugate JQ1 and E3 ligase ligands, respectively (Fig. 39). Degrader **44** exhibited excellent cytotoxic activity against MM.t1S and MV-4-11 cell lines with IC_{50} of 0.201 and 0.042 nmol/L, respectively. Moreover, **44** induced the degradation of BRD4 in MV-4-11 cell in a time- and concentration-dependent manner. Compared to **44**, small molecule **45** bearing a longer alkyl linkage showed significantly better BRD4 degradation activity with a DC_{50} of 5 nmol/L. This study indicated that linker length and linkage position influence binding conformations of CRBN−BRD4 complex and ultimately result in different BRD4 degradation activity.

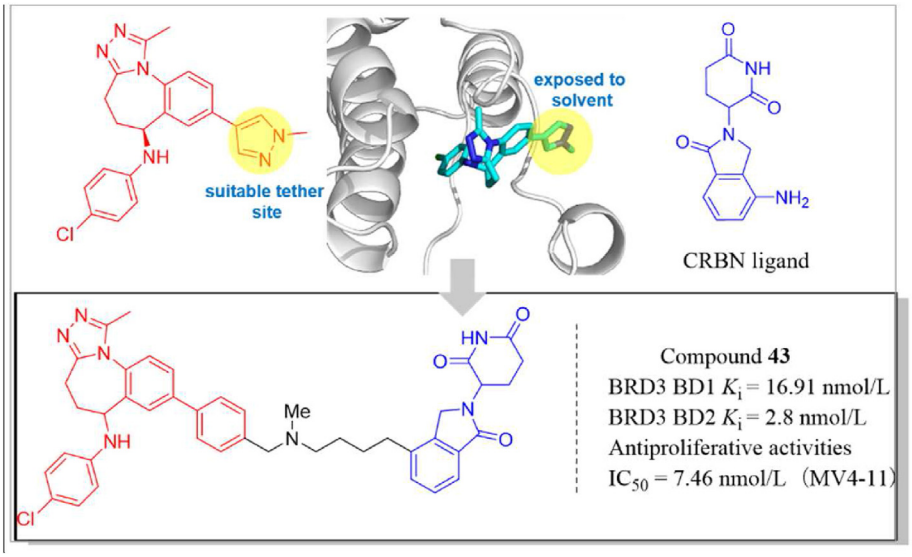

Figure 38 Chemical structure of BRD4-degrading PROTAC **43**.

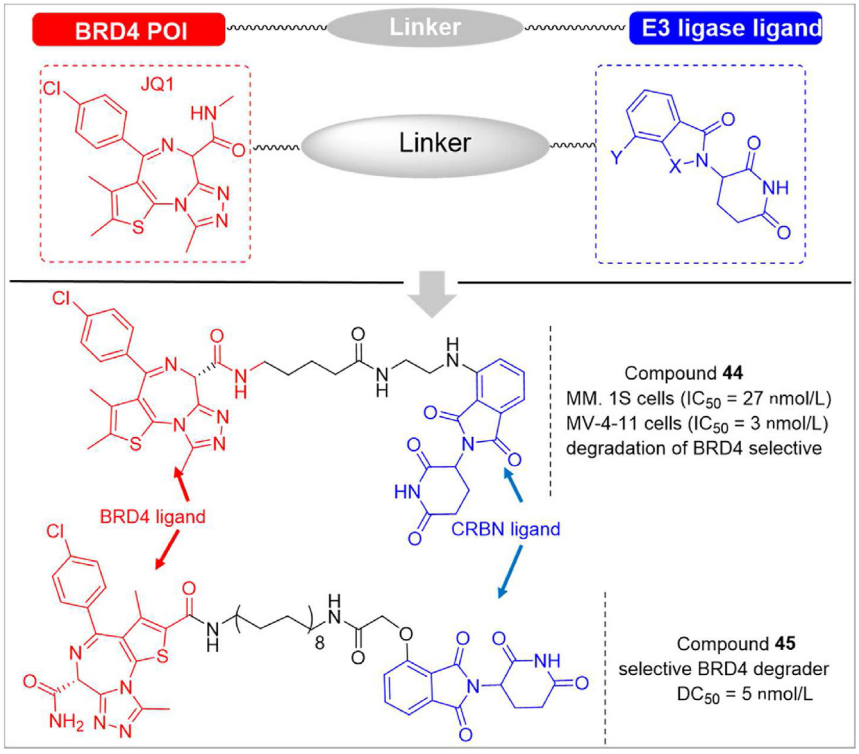

Figure 39 Chemical structure of BRD4-degrading PROTACs **44**, **45**.

6.1.3. BRD9 selective degraders

BRD9 represents an important therapeutic target for treating hematological diseases[127]. In recent years, several BRD9 degraders have been reported and are detailed below.

In 2017, Zoppi et al.[128] firstly identified the BRD9 PROTAC **46**, by linking BI-7273 (a selective BRD9 inhibitor) with pomalidomide as a CRBN ligand, as depicted in Fig. 40. The co-crystal structure of the BI-7273 with BRD9 complex revealed that the *N*,*N*-dimethyl group of BI-7273 was exposed to solvent and could serve as a suitable tethering site for linking the CRBN ligand. Among the synthesized compounds, degrader **46** induced selective BRD9 degradation in MOLM-13 cells at 100 nmol/L after 2 h. As expected, **46** exhibited markedly enhanced antiproliferative potency compared to BI-7273 (10- to 100-fold).

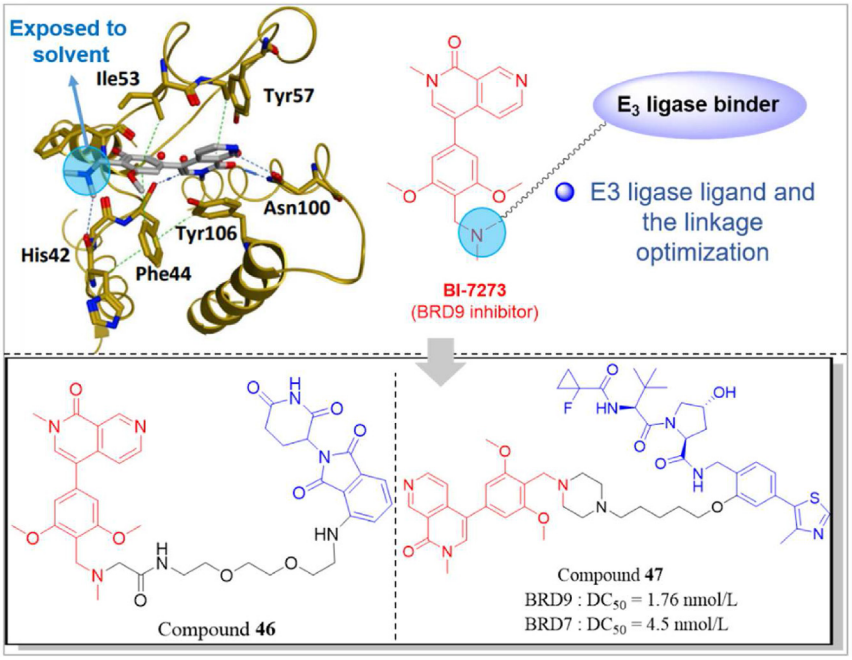

Figure 40 The structure of BRD9 PROTACs **46**, **47**.

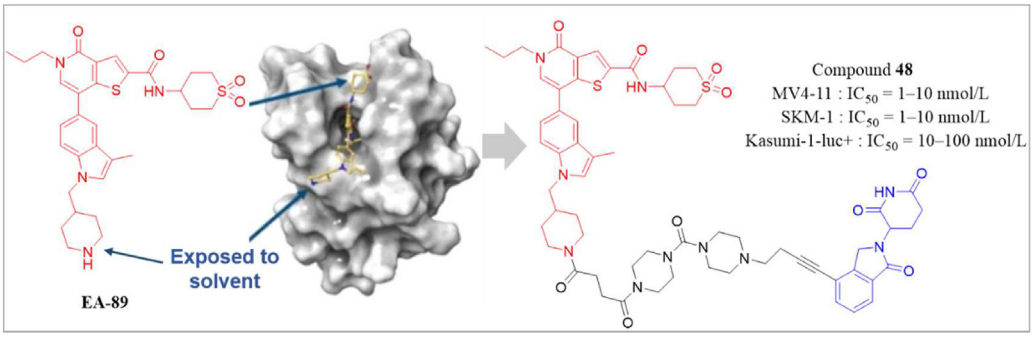

Figure 41 Structure-based design of BRD9 PROTAC **48**.

In addition, based on BI-7273, Remillard et al.[129] designed a series of novel PROTAC degraders targeting BRD9 and BRD7, as shown in Fig. 40. The conjugation patterns and linker composition were systematically optimized, resulting in the identification of **47** that dose-dependently depleted BRD9 and BRD7 in RI-1 cells (DC_{50} = 1.76, 4.5 nmol/L). In RI-1 cells, **47** degraded 50% or more of BRD9 and BRD7 within 30 min at 100 nmol/L and achieved 90% degradation efficacy after 4 h of treatment.

In 2022, Weisberg et al.[130] described a novel BRD9 PROTAC **48** (QA-68), which incorporates the EA-89 (a BRD9 inhibitor previously discovered by the authors) warhead into a CRBN ligand (Fig. 41). Degrader **48** displayed excellent antiproliferative activity against MV4-11, SKM-1, and Kasumi-1-luc + cells, with IC_{50} values of 1−10, 1−10, and 10−100 nmol/L, respectively. Compared to EA-89, **48** exhibited over a 100-fold increase in antiproliferative potency and robustly degraded BRD9 in AML cells. Moreover, **48** potentiated the antitumor effects of chemotherapeutic agents and presented a therapeutic strategy of combining BRD9 degraders with other agents for hematological malignancy.

Furthermore, researchers from C4 Therapeutics Inc. and Novartis AG in Switzerland have attempted to design BRD9 degraders **49**−**54** (Fig. 42)[131,132]. These compounds possess selective and remarkable BRD9 degradation activity, as detailed in Table 3.

6.2. *BRD4 molecular glue degrader*

Due to the significant advantages of molecular glues in terms of drug-likeness, Toriki et al.[29] developed the molecular glue JP-2-197 (**55**) by connecting a chemical handle to JQ1, as depicted in Fig. 43. JP-2-197 induced dose- and time-dependent degradation of the BRD4 protein in HEK293T cells. Additionally, moderate attenuation of BRD4 degradation at higher concentrations was observed, suggesting a potential "hook effect". Further mechanistic studies confirmed that JP-2-197 degraded BRD4 through the proteasome pathway.

Table 3 The BRD9 degradation activity of BRD9 degraders **49**−**54**.

Compd.	BRD9 DC_{50} (nmol/L)	Compd.	BRD9 DC_{50} (nmol/L)
49	1.0	**52**	<100 nmol/L
50	1.0	**53**	<100 nmol/L
51	1.0	**54**	<100 nmol/L

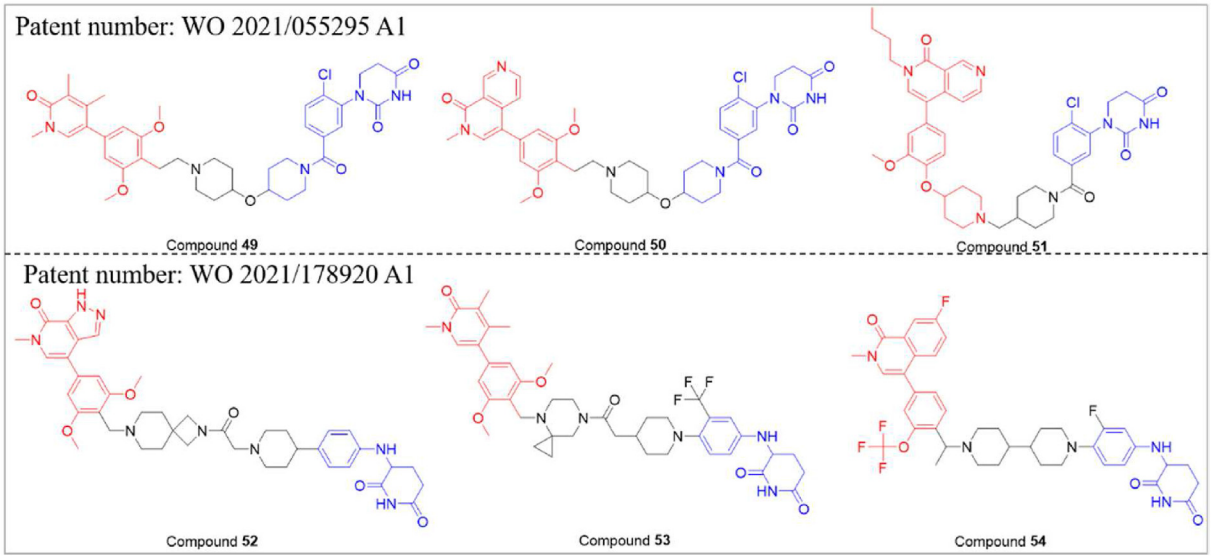

Figure 42 The representative BRD9 degraders **49**−**54**.

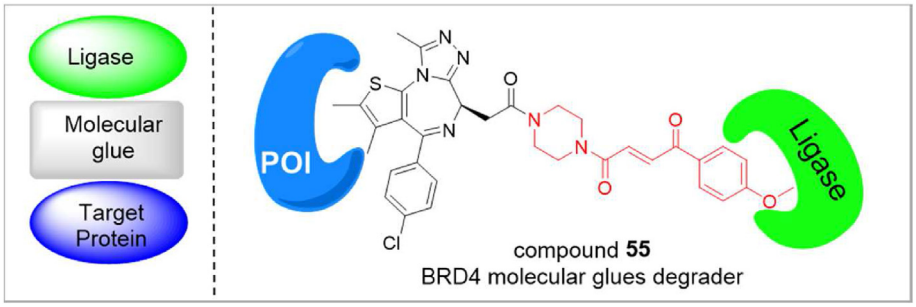

Figure 43 The BRD4 targeting molecular glue compound **55**.

Collectively, PROTACs can be rationally designed based on the binding mode of ligands with target proteins. In contrast, the discovery of molecular glues is often more serendipitous and lacks systematic discovery approaches and rational design strategies. Molecular glues cannot be obtained through large-scale screening of various components like PROTACs, which highlights the importance and challenges of rational designing molecular glues for further drug development.

7. EZH2-targeting degraders

EZH2 induces the agglutination of chromosomes and represses the transcription of target genes. Numerous reports have confirmed that EZH2 is a promising target for cancer treatment[59,133]. New technologies have been employed to enhance the anticancer activity of EZH2 modulators, such as dual-target inhibitors[134] and targeted protein degradation technology[44]. This section will review EZH2-targeting TPD technology, including PROTACs (Sections 7.1, 7.2), and hydrophobic tagging (HyT) approach (Section 7.3).

7.1. *EZH2-targeting PROTACs*

In 2021, Liu et al.[135] reported EZH2 PROTAC **56** by linking GSK126 (an EZH2 inhibitor) to pomalidomide (Fig. 44). Degrader **56** displayed excellent EZH2 inhibitory activity in an Alpha-Screen assay (IC_{50} = 2.7 nmol/L) and selectively degraded EZH2 in WSU-DLCL-2 cells. Furthermore, compound **56** exhibited significant degradation activity for all PRC2 subunits (EZH2: 72%, SUZ12: 81%, EED: 75%, RbAp48: 74%) and achieved maximal degradation of H3K27me3 at 1 μmol/L after 48 h treatment (D_{max} = 83%). In addition, **56** dose-dependently degraded the PRC2 subunits and H3K27me3 at 1 μmol/L.

In 2021, based on EPZ6438 (an EZH2 inhibitor), Tu and coworkers[136] designed two series of EZH2 degraders *via* hijacking E3 ligase systems containing either von Hippel−Lindau (VHL) or

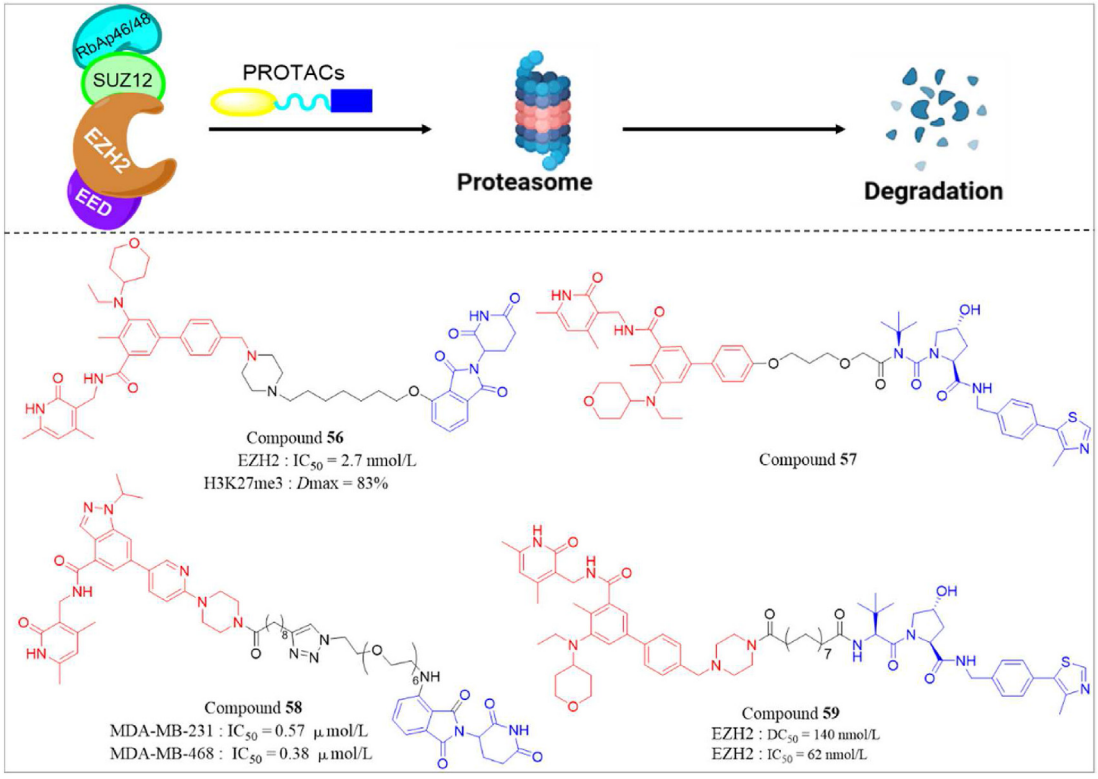

Figure 44 The representative PROTAC EZH2 degraders **56**−**59**.

cereblon (CRBN), and identified the VHL-based degrader **57** that could efficiently induce EZH2 degradation (Fig. 44). Within 24 h, degrader **57** concentration-dependently downregulated EZH2 protein levels and H3K27me3 levels in tumor cells, but had little effect on EZH1, demonstrating the high selectivity. Furthermore, **57** exhibited stronger anti-proliferative activity than EPZ6438 in lymphoma cells and potent *in vivo* anti-tumor efficacy in lymphoma xenografts, suggesting that **57** represents a promising new type of EZH2 degrader.

Wang et al.[137] synthesized a series of novel PROTAC EZH2 degraders. Among them, compound **58** inhibited EZH2 in MDA-MB-231 and MDA-MB-468 cells with IC_{50} of 0.57 and 0.38 μmol/L, respectively, while also exhibiting high affinity for the PRC2 complex (K_D = 16.19 nmol/L) (Fig. 44). Importantly, **58** induced the degradation of EZH2 in TNBC cells, leading to apoptosis of TNBC cells while causing little damage to normal cells.

In 2022, Dale et al.[138] reported EZH2 PROTAC **59**, which efficiently degraded EZH2 in a time- and concentration-dependent manner in MDA-MB-453 cells with a DC_{50} of 140 nmol/L (Fig. 44). Furthermore, **59** displayed excellent inhibitory activity against EZH1/2 with IC_{50} values of 8.6 and 62 nmol/L, respectively. Additionally, **59** exerted moderate antiproliferative activity against MDA-MB-453 cells (IC_{50} = 2.3 μmol/L).

7.2. *EED-targeting PROTACs*

EED could activate the methyltransferase activity of EZH2 by recognizing the basal levels of H3K27me3 in cells. Therefore, EED inhibitors theoretically could produce the same anti-tumor effects as EZH2 inhibitors and may even have the potential to generate stronger activity by overcoming resistance to EZH2 inhibitors. As a result, EED-targeting PROTACs have gained extensive attention.

In 2020, Potjewyd et al.[139] developed a novel VHL-based EED PROTAC degrader **60** (Fig. 45) based on EED226 (an EED inhibitor). Degrader **60** elicited significant degradation of EZH2, EED, and SUZ12 in HeLa cells with DC_{50} of 0.3, 0.79, and 0.59 μmol/L, respectively. In contrast, EED226 only inhibited H3K27 trimethylation with no effect on EED, EZH2, and SUZ12. Excitingly, **60** induced degradation of EED (DC_{50} = 0.61 μmol/L, D_{max} = 94%), EZH2 (DC_{50} = 0.67 μmol/L, D_{max} = 96%), and SUZ12 (DC_{50} = 0.59 μmol/L, D_{max} = 82%) in an EZH2 gain-of-function mutation DB cells upon testing.

Additionally, Hsu et al.[25] developed two new EED degraders (**61**, **62**) by conjugating the EED inhibitor MAK683 and the E3 ligand VH032 with different linkage motifs (Fig. 45). Degraders **61** and **62** exhibited good binding to EED with pK_D values of 9.27 and 9.02, respectively. **61** and **62** significantly depleted EED, as well as reduced EZH2 and SUZ12 at 1.0 μmol/L within 24 h in the KARPAS422 cell line, achieving remarkable antiproliferative activities with IC_{50} values of 57 and 45 nmol/L, respectively. Further mechanistic studies demonstrated that the degradation of PRC2 components induced by **61** and **62** was dependent on the ubiquitin-proteasome pathway.

7.3. *HyT degraders*

In 2020, Ma et al.[44] firstly reported a selective EZH2 HyT degrader MS1943 (**63**) by connecting the piperazine group of C24 (a reported EZH2 inhibitor) to the hydrophobic adamantine through a linker (Fig. 46). MS1943 could degrade EZH2 concentration- and time-dependently in various cancer cells. Moreover, compared to the low antiproliferative activity of EZH2 inhibitors against TNBC cells, MS1943 exerted stronger cytotoxicity in multiple TNBC cells, without affecting normal cells. In addition, MS1943 also possessed decent oral bioavailability (F = 46.3%). This is the first report of an EZH2 degrader for cancer therapy utilizing HyT strategy.

Hydrophobic tags including adamantane, pyrene, fluorene, and carborane have been successfully used for degradation of various proteins. However, existing hydrophobic tags still have limitations such as poor physicochemical properties, low bioavailability, and low degradation efficiency, which hinder their further applications. To address these issues, Xie et al.[140] employed dehydroabietic acid as a new hydrophobic tag to tethering the Tazemetostat (a marketed EZH2 inhibitor) and synthesized the novel hydrophobic tagging EZH2 degrader Hyt-13 (**64**, Fig. 46). Hyt-13 exhibited

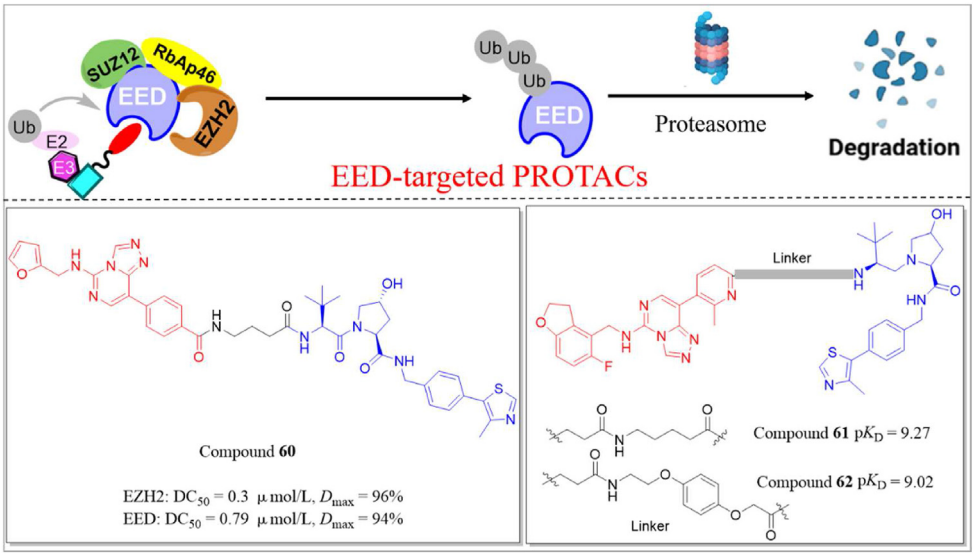

Figure 45 The EED-targeting PROTACs **60**–**62**.

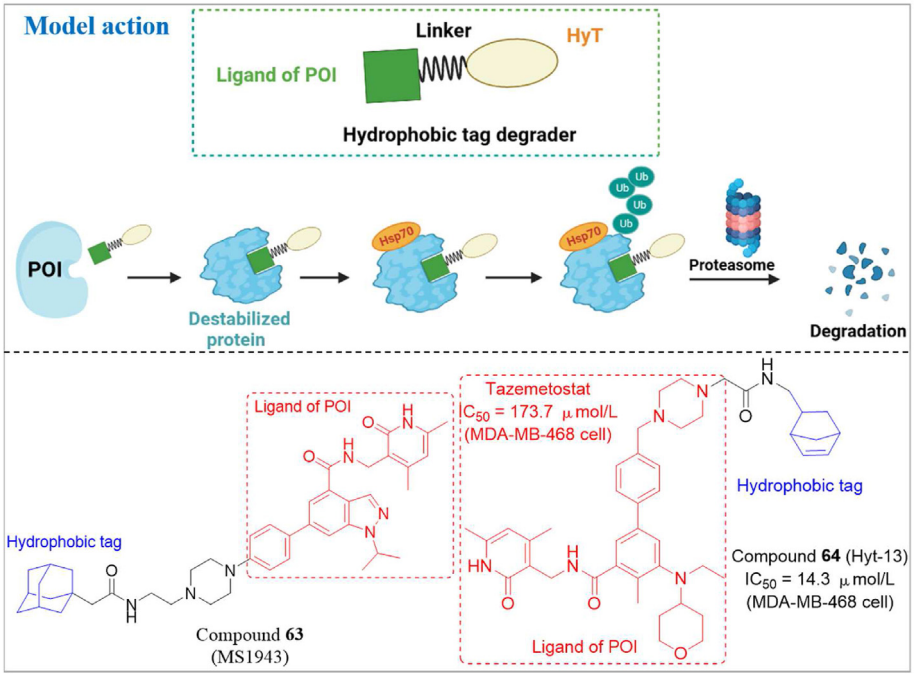

Figure 46 The mode of action of hydrophobic tagging degraders and the representative compounds **63**, **64**.

significantly better *in-vitro* antiproliferative activity (IC_{50} = 14.3 μmol/L) than EZH2 inhibitor Tazemetostat (IC_{50} = 173.7 μmol/L, 5.2%), and decent bioavailability (8.6%).

8. ENL-targeting degraders

Recent genetic loss-of-function studies have identified ENL as a crucial transcriptional coactivator necessary for the survival of acute leukemia. Its YEATS domain, involved in chromatin reading, plays an essential role in this process.

In 2020, Garnar et al.[141] conducted a screening of nearly 300,000 small molecules and discovered an amido-imidazopyridine inhibitor (**65**) targeting the ENL YEATS domain with an IC_{50} value of 7 μmol/L (Fig. 47). Through improvements using a SuFEx-based approach, they further identified SR-0813 (**66**), a potent and selective inhibitor of the ENL/AF9 YEATS domain (IC_{50} = 25 nmol/L). Building upon this finding, they developed SR-1114 (**67**), an ENL PROTAC that induced rapid degradation of ENL in MV4; 11, MOLM-13, and OCI/AML-2 cells with DC_{50} values of 150, 311 nmol/L, and 1.65 μmol/L, respectively. Notably, SR-1114 treatment selectively suppressed several ENL target genes, such as HOXA10, MYC, MYB, FLT3, ZEB2, and SATB1, resembling the transcriptional effects of dTAG-mediated ENL degradation. Additionally, SR-1114 treatment promoted myeloid differentiation marker CD11b and supported the role of ENL in preventing terminal differentiation of AML cells. While providing confidence in ENL as a target for leukemia treatment, proteomics studies revealed weak off-target degradation of IKZF1, potentially due to the ability of thalidomide to recruit IKZF1 to CRBN for degradation.

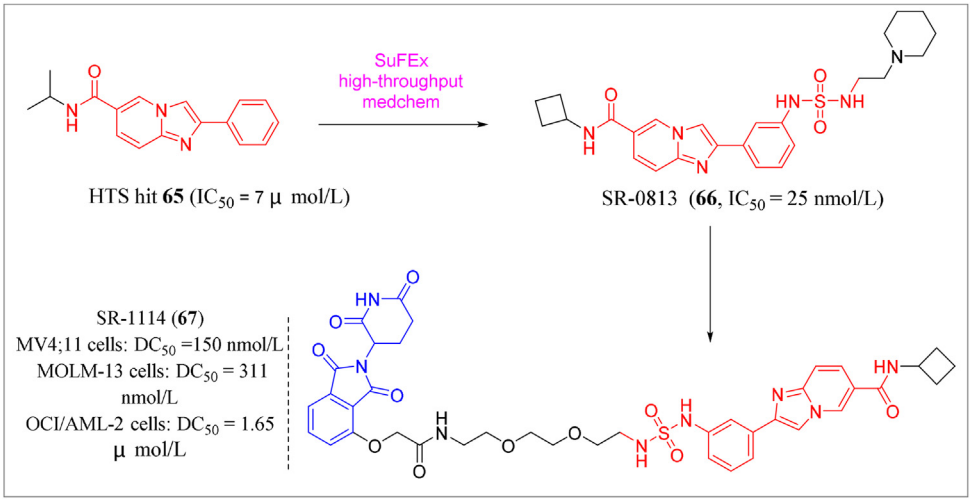

Figure 47 The ENL-targeting PROTAC **67**.

ENL plays a crucial role in MLL1-rearranged leukemia, and recurrent ENL mutations are associated with Wilms tumor and oncogenesis[142]. Thus, depleting ENL shows promise for Wilms tumor treatment. In 2022, Li et al.[143] developed novel PROTAC **69** and HyT degrader **70** targeting ENL by linking the YEATS inhibitor SGC-iMLLT **68** with thalidomide or hydrophobic tagging. PROTAC **69** efficiently degraded ENL in MV4; 11 leukemia cells, exhibiting a DC_{50} of 37 nmol/L and almost complete depletion at ~500 nmol/L (D_{max} ~95%) (Fig. 48). Notably, compound **69** selectively degraded ENL without affecting AF9 levels. However, HyT degrader **70** did not reduce ENL even at 5 μmol/L. Mechanistic studies revealed that compound **69**-mediated reduction of ENL significantly suppressed malignant gene signatures, and selectively inhibited cell proliferation in MLL1-rearranged leukemia and Myc-driven cancer cells, with EC_{50}s as low as 320 nmol/L. Additionally, compound **69** demonstrated excellent antitumor activity against MLL1-rearranged leukemia and mutant ENL in Wilms tumor using a mouse model, thereby showing potent anti-resistance effects against Wilms tumor.

Interestingly, degrader **69** demonstrated potent antiproliferative activity against MLL1-r leukemia cells Molm-13 and MV4; 11, with EC_{50} values of 320 and 570 nmol/L, respectively. In contrast, the parent inhibitor SGC-iMLLT and thalidomide showed no activity (EC_{50} > 50 μmol/L) against these cancer cells. This highlights the potential of PROTACs to convert non-active or minimally active inhibitors into potent degraders, with the rationales listed below:

Firstly, mechanistically, PROTACs possess a remarkable feature of recruiting E3 ligase even when the target protein-binding affinity of the small molecule inhibitor is low or absent. Consequently, PROTACs effectively utilize the existing binding interactions between the small molecule inhibitor and the target protein, along with the newly introduced interaction between the E3 ligase and the PROTAC molecule, to induce target protein degradation[39]. This strategy enhances the potency of weak or ineffective inhibitors, transforming them into highly effective degraders.

Secondly, compared to inhibitors that do not affect the target protein at genetic levels, PROTACs deplete the target protein, leading to global gene expression changes or aberrant gene alterations associated with cancer[135,143]. These changes result in improved bioactivity through enhanced cell proliferation, cell differentiation, or apoptosis. For instance, ENL depletion by degrader **69** significantly suppressed aberrant gene signatures in MLL1-r leukemia, including the reduced expression of characteristic genes like HoxA9 and Myc. Furthermore, the global gene expression changes mediated by degrader **69** resulted in inhibited cell proliferation, differentiation, and apoptosis[143]. Similarly, EZH2 PROTAC **56** exhibited significantly stronger antiproliferative activity compared to GSK126 (the parent EZH2 inhibitor) due to its selective depletion of EZH2 and degradation activity towards all PRC2 subunits, including SUZ12, EED, and RbAp48[135].

Lastly, PROTAC molecules enhance the selectivity of target protein degradation compared to parent inhibitors. For example, degrader **69** was found to be a highly efficient and ENL-specific PROTAC molecule that selectively degraded ENL without affecting AF9 levels, while the parent compound SGC-iMLLT exhibited non-selective inhibition against ENL and AF9[143].

9. Challenges and potential directions of epigenetic targeting degraders

Despite the high efficiency and "always-on" properties of epigenetic-targeting degraders such as PROTACs, molecular glue, and HyT in degrading POI, the field of epigenetic-targeting TPD has experienced explosive growth over the past decade. However, several challenges and limitations, including low subtype selectivity, poor drug-likeness and uncontrollable PK properties, must be addressed to increase the likelihood of successful clinical translation of epigenetic-targeting TPD drugs. Therefore, this section aims to highlight the challenges, opportunities and representative case studies involved in developing effective epigenetic-targeting TPD drugs for further application.

9.1. *Poor subtype selectivity*

In the past few decades, significant strides have been made in the discovery of small molecules targeting the epigenetic processes. However, due to the structural similarities among epigenetic-related proteins and/or cofactor binding sites, developing highly selective epigenetic-targeting inhibitors remains a long-term challenge in drug discovery. Similarly, subtype selectivity is crucial for epigenetic PROTAC discovery, though it provides opportunities when compared to traditional small molecules.

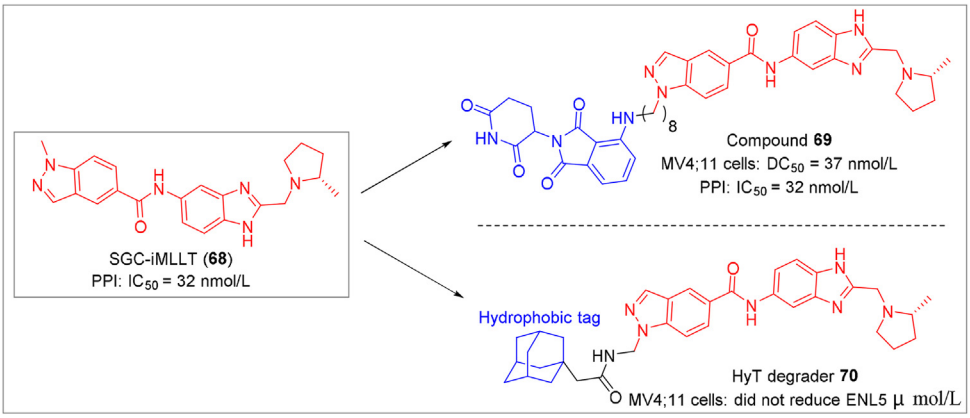

Figure 48 The ENL-targeting PROTAC **69** and HyT degrader **70**.

To enhance the subtype selectivity of PROTACs, the Crews lab investigated the correlation between ligand binding selectivity and degradation selectivity[144]. They found that selective degradation of target POIs does not necessarily correlate with either ligand binding selectivity or affinity. Notably, extensive datasets confirm prior observations that high-affinity binding to a specific target is not a prerequisite for selective target degradation[145]. Moreover, since PROTACs induce new protein−protein interactions (PPIs) between E3s and target POIs, unlike single binding events, these PPIs can vary significantly based on the length and composition of the linker and the ligands used. Thus, utilizing partial or pan inhibitors as POI warheads may yield highly subtype-selective PROTACs. Moreover, to achieve this selectivity, it is necessary to systematically investigate the tethering site and length of the linker and the structure of the POI ligand.

Furthermore, multiple studies indicate that pairing of E3 ligases with target proteins is a critical factor in generating selective PROTACs[146,147]. Recently, Xiong et al.[146] examined this factor by studying the degradation of several HDAC protein family members using degraders that combined a pan-HDAC inhibitor (dacinostat) with three different E3 ligase ligands (CRBN, VHL, and IAP) (Fig. 49). Their results revealed that selective degradation was frequently associated with the specific E3 ligase used. For example, CRBN-based dacinostat PROTACs preferentially degraded HDAC6 and HDAC8, while IAP-recruiting degraders exhibited weak but selective degradation of HDAC6.

The above studies suggest that the pairing of the E3 ligase with the target protein is one of the most critical factors involved in the generation of potent and selective PROTACs[144,148]. Moreover, the choice of specific E3 ligase has a substantial impact on PROTAC activity and selectivity, likely attributed to the varying tissue expression of different E3 ligases[146,149]. Nonetheless, the current focus in PROTAC design primarily revolves around VHL, CRBN, and IAP ligands. Thus, prioritizing the development of ligands for tissue-restricted ligases holds significant potential for disease treatment. These strategies offer the opportunity to mitigate both on- and off-target toxicities associated with undesired target depletion. For instance, in the case of bromodomain PROTACs targeting BRD4 degradation, which is known to cause gut toxicity[150], restricting PROTAC activity to cancer or immune cells may establish a therapeutic window.

Furthermore, pairing of the E3 ligase from the tissue expression with the target protein is an important strategy for designing PROTACs to enhance therapeutic effectiveness and reduce toxicity. For instance, FBXO41, FBXL16, RNF167, TRIM9, TRAF3, and TRIM2 exhibit predominant expression in the nervous system[151,152], rendering them attractive targets for treating neurological disorders. Considering the widespread distribution of HDAC6 in neuronal cells[153], employing FBXO41, FBXL16, RNF167, TRIM9, TRAF3, or TRIM2 as E3 ligases when designing HDAC6-PROTAC can significantly improve target specificity and selectivity.

9.2. *Undisclosed target degraders*

As of now, only a limited proportion of protein targets, including those related to epigenetic regulation (approximately 20%−25%)[154], have been utilized for degrader discovery. However, certain critical factors involved in epigenetic regulation, such as LSD1, DOT1L, and G9a, have not yet been targeted by degraders. The development of degrader technology for new drug targets is gaining momentum, as epigenetic degraders offer several advantages over the conventional small molecule inhibitors, such as improved therapeutic efficacy and the potential to serve as valuable chemical tools for post-translational protein knockdown[154].

In terms of existing targets, such as LSD1 and DOT1L, adjusting the linker and E3 ligase ligand may yield a desired degrader. For POI lacking known ligands, such as JmjC, structural modifications to a substrate can generate a compound that competes with the substrate for binding pockets, thereby serving as ligands for PROTAC design[155]. It is important to recognize that designing a PROTAC without a known ligand is an intricate undertaking, necessitating multiple iterations of optimization and validation. Furthermore, the physiological functions and structural features of the target protein should be taken into account to avoid unacceptable side effects.

9.3. *Poor pharmacokinetics and oral bioavailability*

The development of novel TPD drugs poses several challenges, among which poor pharmacokinetics is particularly problematic[156]. Achieving adequate oral bioavailability and cell permeation presents a challenge for TPD agents, especially for PROTACs, due to their

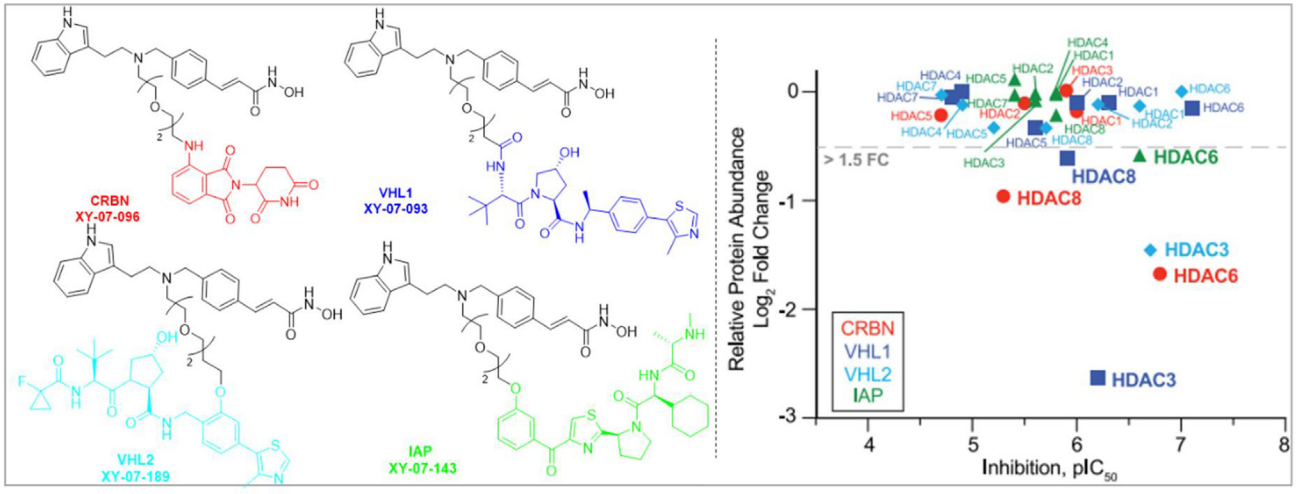

Figure 49 E3 ligase ligands correlated with subtype selectivity.

complex structures that often fall outside the traditional small-molecule property space and struggle to meet Lipinski's rule of five[157]. As such, intravenous TPDs could be a potential option; however, frequent infusions can reduce patient compliance and hands-on practice, while a single high-dose infusion with reduced frequency may increase the likelihood of potential toxicities.

Modifying the linker moiety of PROTACs is an established strategy for enhancing their pharmacokinetic properties, with polar groups introduced into linkers proving particularly useful in adjusting the PK/PD profiles of these bifunctional molecules. Despite the flexible and straightforward chemical synthesis of alkyl linkers, they present a challenge to the development of PROTACs due to their high hydrophobicity, which may restrict cell membrane penetration. To overcome this limitation, heteroatom-containing linkers, incorporating heteroatoms such as oxygen or nitrogen atoms, are often introduced to the alkyl chain to reduce its hydrophobicity and improve cell membrane permeability. For example, PEG chains are frequently employed as linkers to increase the solubility and cellular uptake of PROTACs.

Compared to heteroatom-containing alkyl linkers, the inclusion of heterocycles such as triazole motifs has been shown to enhance the solubility and rigidity of PROTACs[158]. This results in an improvement in their pharmacokinetic properties and degradation efficiency. Moreover, the incorporation of triazole moieties, known for their resistance to *in vivo* metabolism relative to alkyl linkers, may further increase the metabolic stability and catalytic cycling of PROTACs.

Enhancing the drug-like properties of PROTACs can be achieved by identifying new E3 ligase ligands possessing favorable molecular properties[159]. Among the four most common E3 ligands used in PROTAC design (CRBN, VHL, MDM2, and IAP), only CRBN ligands currently exhibit suitable prospects as orally bioavailable drugs, as demonstrated by their application in the orally-active clinical candidates ARV-110 and ARV-471. Conversely, E3 ligands such as VHL, MDM2, and IAP display unfavorable drug-like properties such as greater molecular weights, TPSAs, and flexibility. It is worth noting that the utility of CRBN ligands is limited by their poor chemical and metabolic stability, attributed to the racemization of the glutarimide group and hydrolysis of the imide groups. Consequently, the identification of novel E3 ligands possessing acceptable physicochemical attributes offers a promising strategy for improving the drug-like nature of PROTACs.

9.4. *Uncontrolled action of PROTACs*

Despite exhibiting superior catalytic behavior for protein degradation and demonstrating potential in clinical trials, PROTACs are constrained by significant limitations, such as poor controllability[14]. These drawbacks result in an inability to regulate drug release and the emergence of noticeable side effects. Thus, it is critical to modulate the activity of PROTACs, enabling their spatiotemporal activation within favorable tissues and cells to minimize off-target effects while improving therapeutic efficacy. Notably, recent years have seen considerable efforts devoted to the development of light-controlled, radiation-activatable, and ligation to scavenging strategy, which have made significant progress.

9.4.1. *Light-controlled epigenetic-targeting PROTACs*

The caging strategy of PROTACs leverages the state of inactivity assumed by these molecules when a photocleavable group hinders their binding with either the POI or the E3 ligase. The blockade can be reversed through exposure to light at an optimal wavelength, thereby enabling the production of active PROTACs that facilitate POI degradation (Fig. 50A).

Based on above strategy, Xue et al.[160] introduced the pioneering POI-caging BRD4 degrader **71** by incorporating a substantial 4,5-dimethoxy-2-nitrobenzyl (DMNB) group onto the POI motif of the original PROTAC **72**, as displayed in Fig. 50B. The compound remained inactive as the DMNB group hindered inhibitor binding to the POI. However, upon exposure to ultraviolet (UV) light, the DMNB group was removed, thereby initiating the degradation of POI induced by PROTACs. It is noteworthy, however, that even following caging using this approach, CRBN-based PROTACs could still act as molecular glues and attract additional substrates to the E3 ligase.

Caging E3 ligase might mitigate this problem. Liu and coworkers[161] developed E3 ligase ligand-caging PROTAC **73** (Fig. 51). They added a photolabile caging group (4,5-dimethoxy-2-nitrobenzyl) to CRBN-ligand of degrader **36** (dBET1) to block its interaction with the E3 ligase CRBN. As expected, *opto*-PROTAC **73** induced BRD4 depletion at a concentration of 50 nmol/L, with controllable degradation.

Furthermore, multiple studies have verified the efficacy of light-controllable PROTACs (**74**–**77**) in promoting the degradation of epigenetically targeting proteins, as illustrated in Fig. 52[161–164]. These photo-caged PROTACs manifest stability

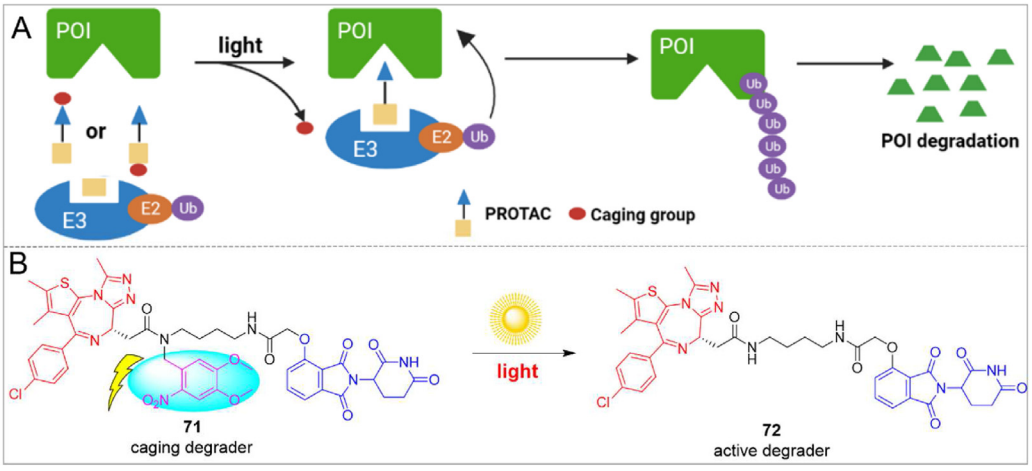

Figure 50 (A) Action mode of caging PROTACs. (B) Uncaging reaction of caging degrader **71**.

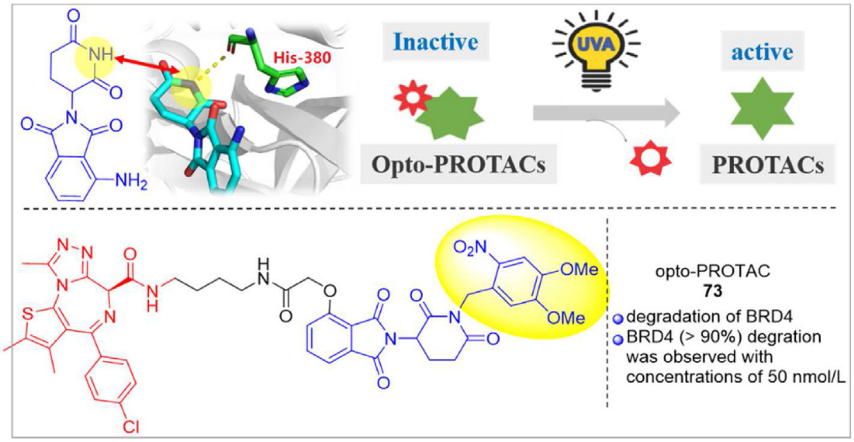

Figure 51 The degrader **36**-based light-inducible PROTAC **73**.

and dormancy in the absence of light but exhibit protein degradation capabilities exclusively upon photo-stimulation in cells. This fact serves to demonstrate the controllability of this approach and accentuate the potential of light-inducible PROTACs as promising contenders for accurate medical therapies for cancer and convenient tools for biological research.

9.4.2. Radiation-activatable epigenetic-targeting PROTACs

As opposed to light activation, X-ray radiation has been widely utilized clinically in cancer therapy owing to its exceptional precision and deep tissue penetration[165]. Consequently, it is a preferred exogenous stimulus employed for the development of stimuli-activatable (RT) PROTACs. Yang et al.[166] conducted a proof-of-concept study in which they designed a derivative of ARV-771 as a model radiotherapy-triggered PROTAC **78** by introducing a masking group of (4-azido-2,3,5,6-tetrafluorophenyl) methanol to the VHL ligand, effectively blocking the interaction between the E3 ligase and the RT-PROTAC, as shown in Fig. 53. Upon exposure to X-ray radiation, the masking moiety was reduced to 4-(hydroxymethyl)-2,3,5,6-tetrafluoroaniline and subsequently removed through a 1,6-elimination procedure. The restoration of the PROTAC prodrug to ARV-771 enabled the degradation of the BRD4 protein. Further Western blot assays confirmed that RT-PROTAC did not affect BRD4 expression without radiation, thus demonstrating its controllability.

9.4.3. Ligation to scavenging strategy

PROTACs are molecules that act *via* an event-driven mechanism, and are not consumed in the process of protein degradation. Instead, PROTACs facilitate the continuous and catalytic initiation of target protein degradation. Apart from the strategies mentioned above, chemical methods may present a plausible option.

Most recently, Oleinik et al.[167] introduced a flexible chemical knockdown approach, termed the "ligation to scavenging" strategy, which aims to terminate event-driven protein degradation (Fig. 54). This method specifically targets epigenetic regulation and involves a ligation to scavenging system consisting of a TCO-modified dendrimer (PAMAM-G5-TCO) and tetrazine-modified BRD4 PROTAC **79**. PAMAM-G5-TCO acts as an efficient intracellular scavenger, rapidly eliminating free PROTACs through an inverse electron demand Diels−Alder (IEDDA) reaction, effectively halting the degradation of BRD4 protein, thereby achieving termination control of targeted protein degradation. This innovative chemical strategy represents a promising avenue for achieving controlled target protein degradation.

9.5. Poor tissues/cell selectivity

Despite their ability to function at catalytic doses and exhibiting fewer side effects than traditional small-molecule inhibitors, PROTACs still have the potential to induce toxicity due to nonselective effects in normal cells and tissues[35]. To address this

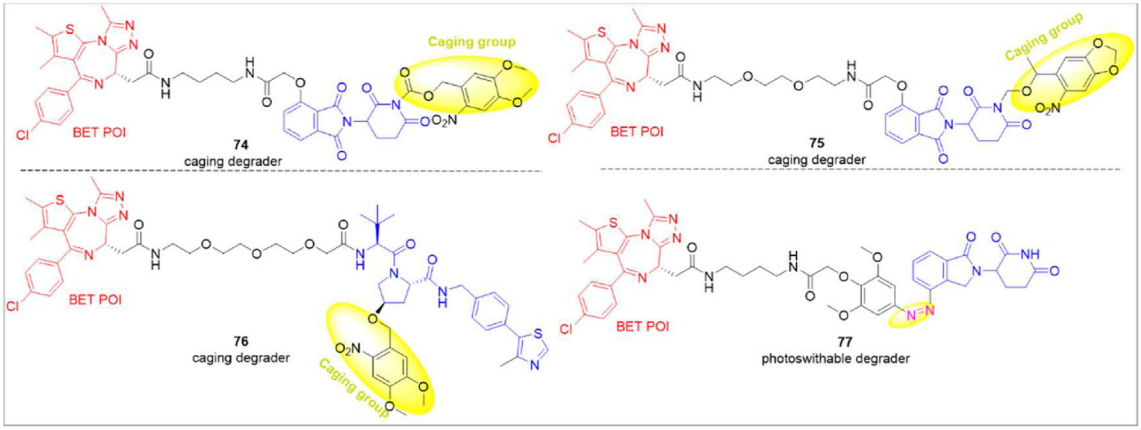

Figure 52 The representative of photo-controlled PROTACs **74**−**77**.

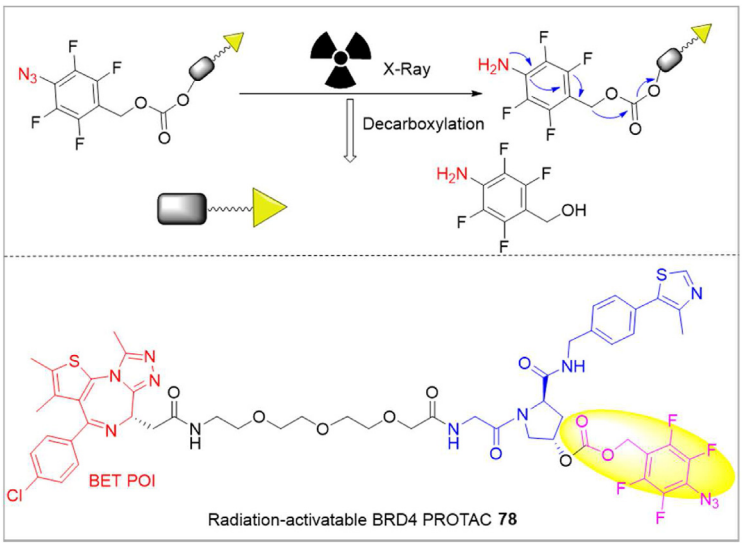

Figure 53 (A) Reaction mechanism of the X-ray radiation-activatable PROTAC. (B) Chemical structure of the X-ray-responsive BRD4 PROTAC **78**.

problem, the development of tissue- or cell-selective degraders, such as antibody−PAROTAC conjugates, aptamer−PROTAC conjugates, or folate−PROTAC conjugates, may enable precise release of PROTACs and minimize off-target effects.

9.5.1. Antibody−PROTAC conjugates

In order to enhance the tissue/cell selectivity of epigenetic-targeting PROTACs, Maneiro et al.[168] designed and synthesized curcumin−antibody−PROTAC conjugates (Ab−PROTAC) based on the BET PROTAC **80**. The conjugates hydrolyze and release active PROTAC after antibody−PROTAC internalization, resulting in catalytic degradation of target proteins (Fig. 55). Conjugate **81** exhibited excellent selectivity and complete BRD4 degradation in HER2-positive breast cancer cell lines at 100 nmol/L for 4 h. Using live cell confocal microscopy, the authors validated the mechanism of degradation *via* endocytosis and release of PROTAC molecules in

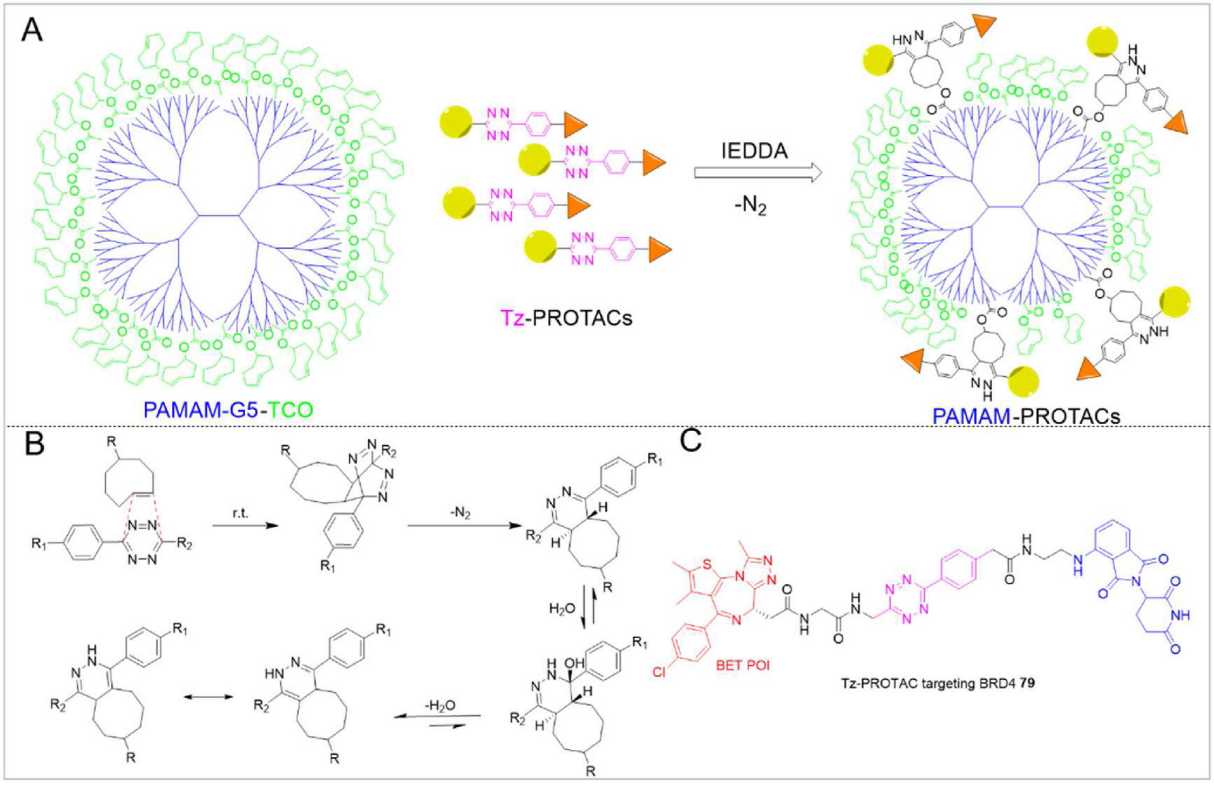

Figure 54 (A) Schematic diagram of the ligation to scavenging system. (B) Mechanism of inverse electron demand Diels−Alder (IEDDA) reaction between 1,2,4,5-tetrazines (Tz) and *trans*-cyclooctene (TCO). (C) Design of Tz-PROTAC **79** (TCB-series) targeting BRD4.

HER2-positive cells. Importantly, this study demonstrates proof-of-concept of the antibody−PROTAC with the potential to overcome the limitations (*e.g.,* selectivity) of conventional PROTACs.

Subsequently, a multitude of studies have been conducted on antibody-based BRD4 PROTACs[169−172], as illustrated in Fig. 56. In 2020, Pillow et al.[171] successfully attached a highly potent VHL-based chimeric BRD4 degrader, GNE-987, to a C-type lectin-like molecule-1 (CLL1)-targeting antibody using an innovative disulfide-containing cleavable linker. The resulting conjugate **82** demonstrated strong dose-dependent efficacy *in vivo* in HL-60 and EOL-1 AML xenograft models following intravenous administration, while GNE-987 exhibited negligible activity in these models. However, the carbonate moiety (yellow oval) posed a potential risk of *in vivo* metabolic instability. Therefore, another group employed an ester moiety (yellow oval) to connect the VHL-based PROTAC **83** and the HER2 antibody to improve metabolic stability[172]. Importantly, conjugate **84** enabled dose-dependent BRD4 degradation in two HER2-positive cell lines but not in the HER2-negative control lines. These findings emphasize the effectiveness of antibody−PROTAC conjugates for enhancing tissue or cell selectivity.

9.5.2. *Aptamer−PROTAC conjugates*

Nucleic acid aptamers are a type of single-stranded nucleic acid that exhibits high specificity and affinity for binding to target proteins[173]. Aptamers can serve as carriers, offering significant advantages in delivering therapeutic agents to specific cells or tissues[173]. Recently, He et al.[174] designed a novel aptamer-PROTAC conjugate **85** (APCs) by linking MZ1 to an aptamer targeting nucleolin (AS1411) using a glutathione (GSH)-sensitive linker, as depicted in Fig. 57. The aim was to improve tumor tissue specificity of conventional BRD4 PROTACs. Conjugate **85** demonstrated remarkable BRD4 degradation with a DC_{50} of 22 nmol/L, similar to MZ1 (DC_{50} = 13 nmol/L). Notably, the authors confirmed that the aptamer component selectively recognized the cell membrane receptor nucleolin in MCF-7 cancer cells, which highly express nucleolin, promoting endocytosis.

The newly developed aptamer-PROTAC conjugate exhibited selectivity towards tumor cells. However, the negatively charged nature of aptamers still presents a challenge for the efficient intracellular penetration of the aptamer-PROTACs. Specific recognition of cell membrane receptors, such as nucleolin, for inducing endocytosis could be a promising strategy for delivering PROTACs and merits further investigation.

9.5.3. *Folate−PROTAC conjugates*

The substantial variation in the expression levels of folate receptor alpha (FOLR1) between normal tissues or cells and those in several human cancers, presents a promising opportunity for targeted cancer therapeutics[175,176]. Therefore, specific recognition of FOLR1 using a chemical ligand, such as folic acid, represents a viable strategy for delivering degraders into cancer cells with high FOLR1 expression. This approach facilitates controlled degradation of POIs and enables selective targeting of specific tissues or cells.

Liu et al.[177] applied the aforementioned principle to conjugate a folate group to the VHL ligand section of ARV-771 using click chemistry, as illustrated in Fig. 58. Subsequent investigations demonstrated that the resulting folate-caged PROTAC **86** exhibited efficient degradation of target proteins in tumor cells with minimal activity in normal cells. The remarkable anti-proliferative activity observed confirmed stable activation of the folate-caged PROTAC and successful release of

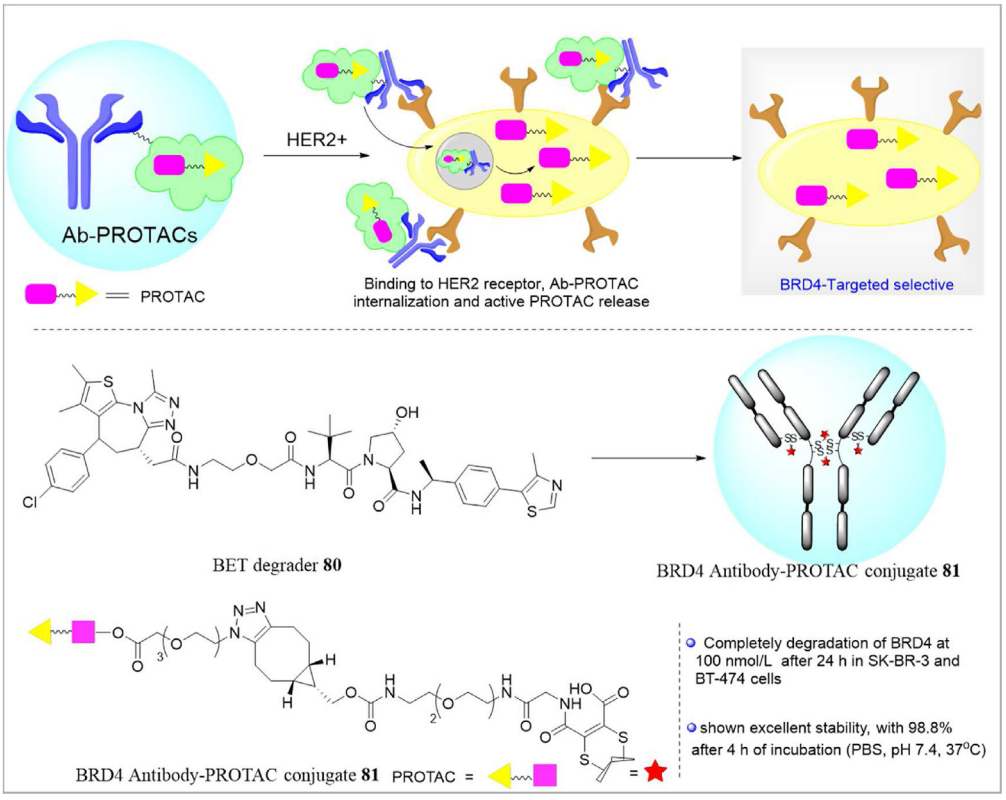

Figure 55 The mode of action of an antibody−PROTAC conjugate and the structure of antibody−PROTAC **81**.

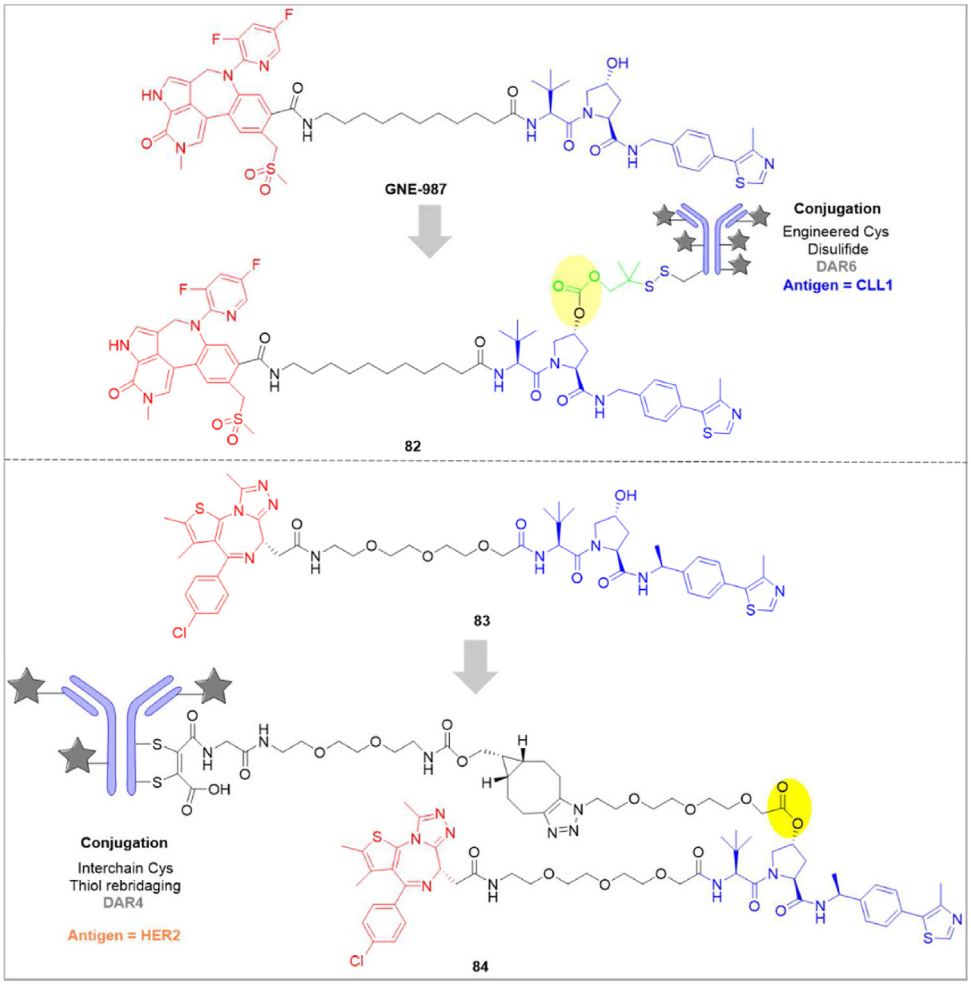

Figure 56 Representative antibody–PROTAC degraders.

active PROTAC molecules in cell lines with high FOLR1 expression. Importantly, this strategy enables selective targeting of cancer cells while sparing normal cells, making it a promising approach for degrader development.

9.6. Low-affinity binding of degraders

Effective degradation by degraders typically requires achieving high affinity with the target protein. However, the majority of

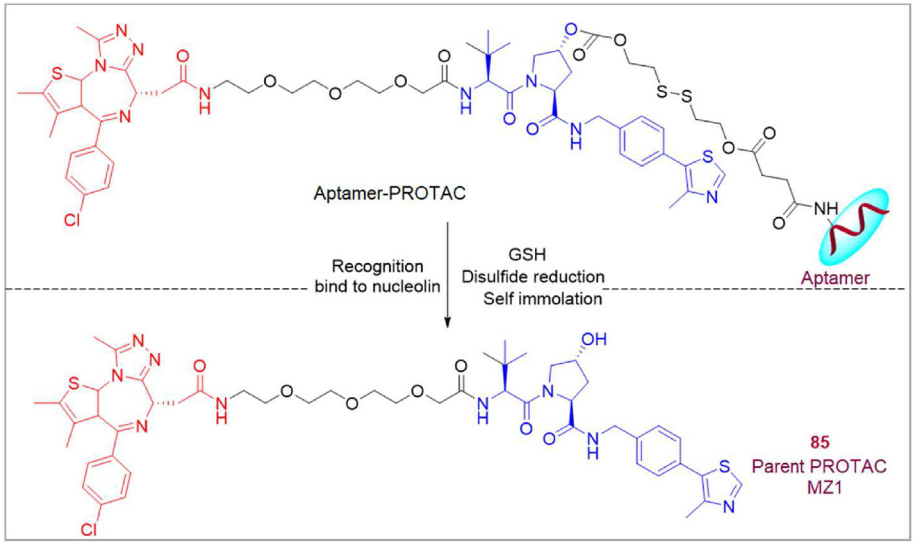

Figure 57 Chemical structures of aptamer–PROTACs and the anticipated intracellular release mechanism to generate the parent degrader.

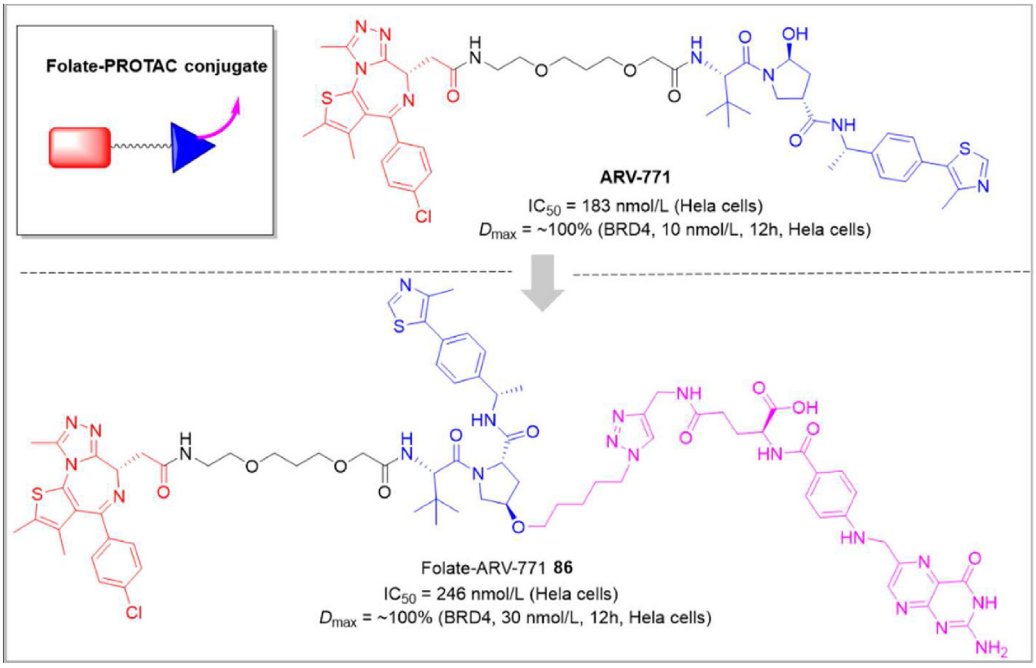

Figure 58 Schematic illustration of folate−PROTAC.

existing degraders (*e.g.,* PROTACs, molecular glues) bind to target proteins and E3 enzymes through reversible interactions[178]. This can result in limitations such as low activity and short degradation maintenance time stemming from their low affinity. Covalent degraders incorporate an electrophilic moiety that enables covalent binding with the target, resulting in high affinity binding and efficient degradation (Fig. 59A)[179]. Recent years have seen a rapid emergence of epigenetic-targeting covalent degraders (*e.g.,* PROTACs, molecular glues), which offer advantages in improving efficiency, ternary complex formation kinetics, and selectivity.

9.6.1. *Covalent PROTACs*

In 2019, Spradlin et al.[180] utilized Nimbolide, a terpenoid natural compound, to construct the BRD4 covalent degrader by acting as the ligand of the E3 ligase RNF114 and covalently reacting with Cys8 (Fig. 59B). They performed an *in vitro* activity-based protein profiling (ABPP)-based covalent ligand screen. The representative compound XH-2 (**87**) selectively degraded BRD4 after a 12-h treatment at 0.1 μmol/L in 231MFP cells without impacting BRD2 and BRD3.

The chloroacetamide scaffold is a crucial recruiter of RNF114. Luo et al.[181] integrated the chloroacetamide motif into a degrader linked to the BET ligand JQ1, building upon their discovery of nimbolide as a covalent ligand of RNF114. The resulting compound, CCW 28-3 (**88**), could proteasomally and RNF4-dependently degrade BRD4. Furthermore, Luo et al.[181] identified synthetic chloroacetamide EN219 as a covalent E3 ligase ligand. Drawing inspiration from nimbolide-based PROTACs, they designed the RNF114 PROTAC ML 2-14 (**89**) based on EN219, utilizing JQ1 as the POI ligand. In 231MFP breast cancer cells, ML 2-14 demonstrated the most effective degradation of BRD4, with DC_{50}s of 36 and 14 nmol/L for the long and short isoforms of BRD4, respectively.

Zhang et al.[182] developed covalent PROTACs that utilize synthetic electrophilic ligands to covalently react with specific cysteine, leading to the degradation of target proteins and expanding the E3 ligase toolbox. Using a similar chemoproteomic strategy, they identified the chloroacetamide cysteine-reactive electrophilic fragment, which was coupled with the synthetic ligand of BRD4-binding protein to evaluate BRD4 degradation. Among these compounds, KB02-JQ1 (**90**) triggered proteasome-dependent degradation of nuclear BRD4. Moreover, by screening a library of 566 covalent ligands, the Henning et al.[183] identified a cysteine-reactive ligand (EN106) that effectively bound to the CUL2 E3 ligase FEM1B and specifically reacted with Cys186 of FEM1B. Subsequently, covalent PROTACs were developed by linking EN106 with a BET ligand. NJH-1-106 (**91**) demonstrated effective degradation of BRD4 at low nanomolar activity levels across a range of cell lines. These results highlight the importance of reactive covalent fragment screening as a critical starting point for expanding the E3 ligase toolbox.

9.6.2. *Covalent molecular glue*

Molecular glue degraders have emerged as a potent therapeutic approach for eliminating traditionally undruggable disease-causing proteins *via* proteasome-mediated degradation. However, there is currently a lack of rational chemical design principles for transforming protein-targeting ligands into molecular glue degraders. In principle, covalent strategies could expedite the discovery of molecular glues by stabilizing neo-protein interfaces.

In 2023, Li et al.[184] developed a new covalent molecular glue degrader MMH2 (**92**) based on JQ1 by utilizing a *trans*-labeling mechanism (Fig. 60A). This degrader is capable of recruiting the CUL4 DCAF16 ligase to $BRD4^{BD2}$, leading to efficient degradation of BRD4. This study established for the first time the mechanism of "template-assisted covalent modification" for covalent molecular glues, thereby paving the way for proximity-driven pharmacology.

King et al.[185] recently employed a combination of phenotypic screening of covalent ligand libraries and chemoproteomic approaches to discover a covalent molecular glue degrader EN450 (**93**), as illustrated in Fig. 60B. The study also identified the

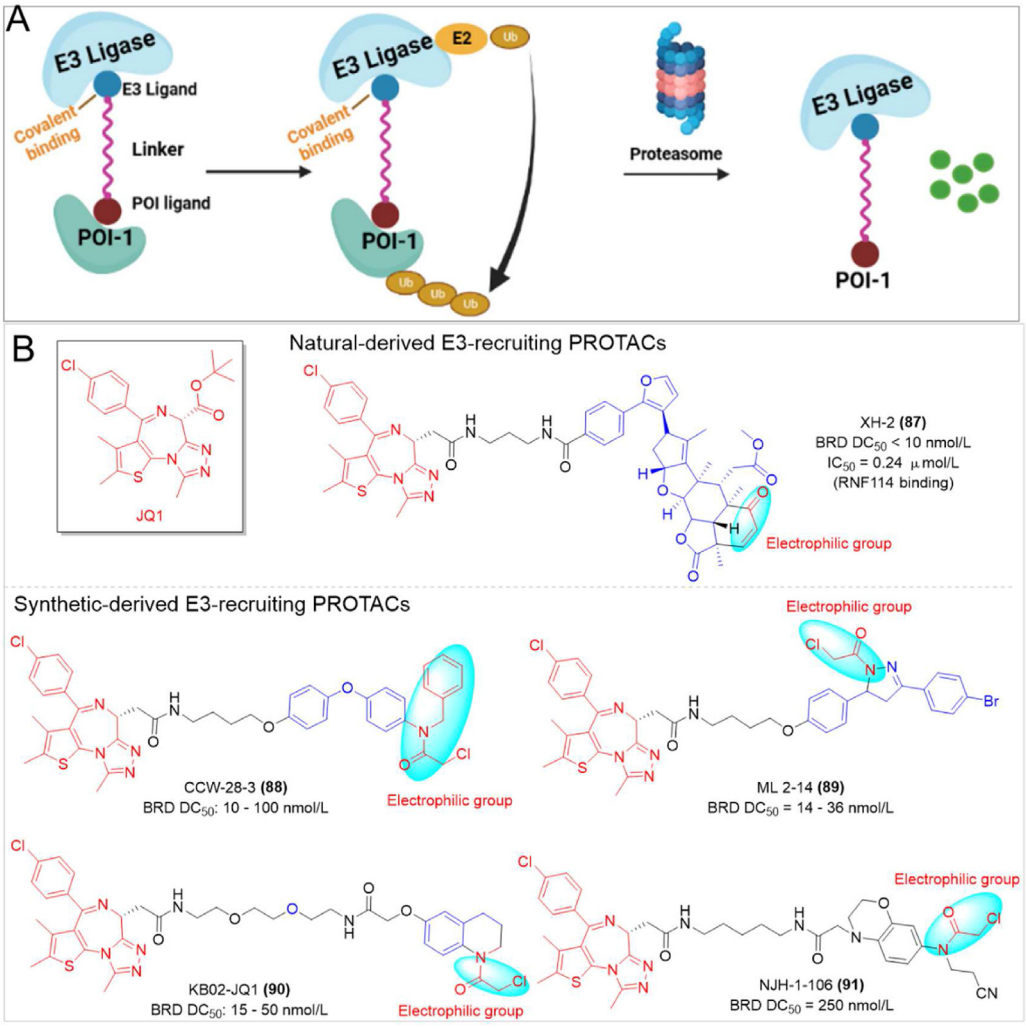

Figure 59 Examples of reported covalent PROTACs binding to BRD4.

oncogenic transcription factor NF-κB1 as a putative degradation target of EN450. Significantly, this research introduced a novel covalent molecular glue degrader that induced the proximity of an E2 and a transcription factor, leading to its degradation in cancer cells.

9.6.3. Dual-activity (trivalent) PROTACs

Currently, the majority of PROTACs are developed to degrade individual targets or a subset of targets within a protein family. However, considering the advantages and accomplishments of

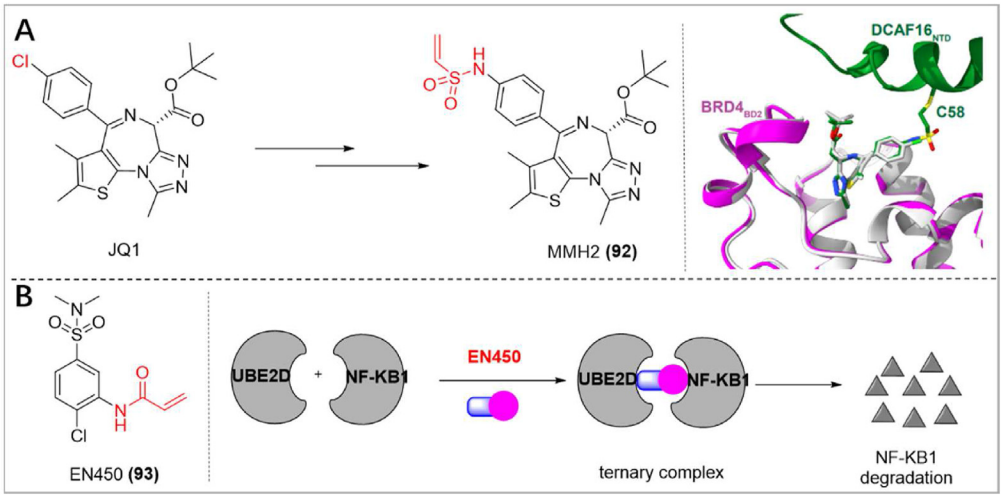

Figure 60 The representative covalent molecular glues.

multi-targeting agents and bispecific antibodies, it may be beneficial to design PROTACs capable of degrading two or more distinct target proteins[186]. Such an approach could potentially result in higher affinity and improved therapeutic outcomes.

Upon the MZ1 and BRD4^{BD2}-VHL co-crystal complex, Gadd et al.[23] design trivalent PROTACs that contained two POI ligands tethered to VHL or CRBN ligands *via* PEG0 or PEG1 linkers, with the aim of enhancing targeted protein degradation (Fig. 61). SIM1 (**94**), a VHL ligand-based degrader, showed higher efficacy in depleting BRD2/3/4 in human HEK293 cells over 4 h at 1 μmol/L compared to MZ1. Conversely, slower and partial loss of BRDs was observed for the CRBN-trivalent PROTAC SIM4 (**96**) under the same conditions[187]. Importantly, they also demonstrated positive cooperativity and high stability in a ternary complex with VHL at a molar ratio of 1:1:1, leading to an extended residence time in cells.

In addition, Huang et al.[188] replaced the 1,2-disubstituted ethyl group of MZ1 with a planar benzene, resulting in the novel trivalent PROTAC 1, 2D-EG2-MZ1 (**95**). This compound exhibited superior activity and the smallest "hook effect" in degrading BRD4, with an activity profile similar to that of MZ1. Additionally, they developed PROTAC 1, 2, 5T-EG2-MZ1 (**97**) by utilizing a *tert*-butyl ester moiety as a functionalization handle, which maintained BRD4 degradation activity comparable to that of 1, 2D-EG2-MZ1 (**95**). Notably, this study provided a platform for introducing functional

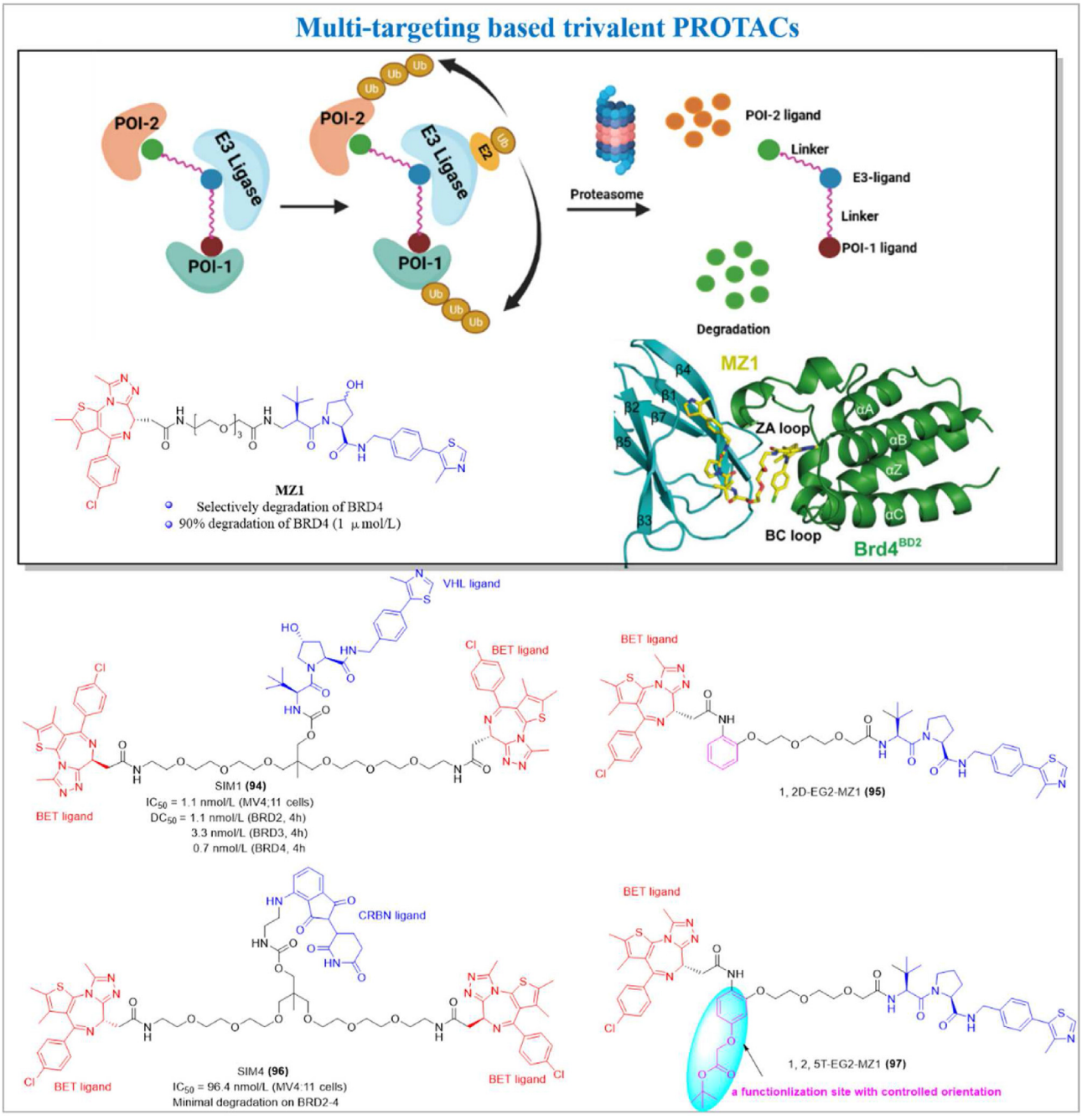

Figure 61 The representative dual-activity PROTACs.

fragments into parent compounds to analyze the mode of action during and after formation of the ternary complex.

9.7. *Further directions for novel degraders*

Although molecular glue possesses low weight and potential favorable druggability. Compared to PROTACs, the discovery of molecular glues is often more serendipitous and lacks systematic discovery approaches and rational design strategies. In addition, the mechanism of action for molecular glue remains relatively poorly understood. Therefore, a comprehensive understanding of the mechanism of action for molecular glue, along with their structural biology and medicinal chemistry characteristics, is crucial for the successful clinical application of this drug type.

Furthermore, the development of degradation agents based on HyT technology is still in its exploratory stage. On one hand, there are only a few reported hydrophobic tag fragments, leaving significant room for optimization in terms of their degradation activity and physicochemical properties. On the other hand, the degradation mechanism remains unclear. Unlike PROTACs, which rely on the ubiquitin−proteasome system of E3 ligases, the degradation mechanism of HyT involves the participation of molecular chaperones such as HSP70, as observed in the reported mechanisms to date[140]. Therefore, exploring more hydrophobic fragments with high activity and excellent physicochemical properties, as well as elucidating their corresponding degradation mechanisms, is crucial for advancing the development of hydrophobic tag-based drugs towards clinical application.

10. Conclusions

In recent years, significant progress has been made in the field of epigenetic-targeting degraders, including PROTACs, molecular glue, and HyT. Compared to small molecules, degraders offer a number of advantages, including enhanced selectivity, the ability to overcome drug resistance, targeting of undruggable proteins, and elimination of entire proteins (rather than just inhibiting enzyme function). However, these degraders also faced several problems. Therefore, in this review, we have overviewed the recent progress of the epigenetic-targeting degrader with regard to the rational design, pharmacodynamics, pharmacokinetics, clinical status, and crystal structure information of these degraders. Importantly, as for the challenges of these degraders, we also provide the further directions and corresponding remedies of this approach to drug design and development.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 82173668, 82260676); Jiangxi Provincial Natural Science Foundation (20232BAB216131, China); the Scientific and Technological Key Projects of Guangdong Province (Nos. 2021B1111110003, 2019B020202002, China); the Science and Technology Projects of Ganzhou (202101094462, China), the Start-Up Foundation of Gannan Medical University (No. QD202144-2067, China).

Author contributions

In this article, each author participated in article preparation. Jianjun Chen and Wanyi Pan designed the review. Xiaopeng Peng and Zhihao Hu searched the literatures and wrote the manuscript. Limei Zeng, Meizhu Zhang, and Congcong Xu wrote part of the introduction. Benyan Lu, Chengpeng Tao, Weiming Chen, and Wen Hou wrote part of the epigenetic PROTACs. Kui Cheng and Huichang Bi provided the new idea and reviewed this manuscript. Jianjun Chen edited the language, conceived the study and provided the guidance of the whole study. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Harvey ZH, Chen Y, Jarosz DF. Protein-based inheritance: epigenetics beyond the chromosome. *Mol Cell* 2018;**69**:195−202.
2. Ling C, Ronn T. Epigenetics in human obesity and type 2 diabetes. *Cell Metabol* 2019;**29**:1028−44.
3. Bhat KP, Umit Kaniskan H, Jin J, Gozani O. Epigenetics and beyond: targeting writers of protein lysine methylation to treat disease. *Nat Rev Drug Discov* 2021;**20**:265−86.
4. Toh TB, Lim JJ, Chow EK. Epigenetics in cancer stem cells. *Mol Cancer* 2017;**16**:29.
5. Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov* 2022;**12**:31−46.
6. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell* 2012;**150**:12−27.
7. Zhang J, Gao K, Xie H, Wang D, Zhang P, Wei T, et al. SPOP mutation induces DNA methylation *via* stabilizing GLP/G9a. *Nat Commun* 2021;**12**:5716.
8. Sun X, Shu Y, Ye G, Wu C, Xu M, Gao R, et al. Histone deacetylase inhibitors inhibit cervical cancer growth through Parkin acetylation-mediated mitophagy. *Acta Pharm Sin B* 2022;**12**:838−52.
9. Peng X, Sun Z, Kuang P, Chen J. Recent progress on HDAC inhibitors with dual targeting capabilities for cancer treatment. *Eur J Med Chem* 2020;**208**:112831.
10. Morschhauser F, Tilly H, Chaidos A, McKay P, Phillips T, Assouline S, et al. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. *Lancet Oncol* 2020;**21**:1433−42.
11. Yin L, Liu Y, Peng Y, Peng Y, Yu X, Gao Y, et al. PARP inhibitor veliparib and HDAC inhibitor SAHA synergistically co-target the UHRF1/BRCA1 DNA damage repair complex in prostate cancer cells. *J Exp Clin Cancer Res* 2018;**37**:153.
12. Chen CP, Chen K, Feng Z, Wen X, Sun H. Synergistic antitumor activity of artesunate and HDAC inhibitors through elevating heme synthesis *via* synergistic upregulation of ALAS1 expression. *Acta Pharm Sin B* 2019;**9**:937−51.
13. Zhao L, Zhao J, Zhong K, Tong A, Jia D. Targeted protein degradation: mechanisms, strategies and application. *Signal Transduct Targeted Ther* 2022;**7**:113.
14. Wang Y, Jiang X, Feng F, Liu W, Sun H. Degradation of proteins by PROTACs and other strategies. *Acta Pharm Sin B* 2020;**10**:207−38.
15. Dale B, Cheng M, Park KS, Kaniskan HU, Xiong Y, Jin J. Advancing targeted protein degradation for cancer therapy. *Nat Rev Cancer* 2021;**21**:638−54.
16. Lv L, Chen P, Cao L, Li Y, Zeng Z, Cui Y, et al. Discovery of a molecular glue promoting CDK12−DDB1 interaction to trigger cyclin K degradation. *Elife* 2020;**9**:e59994.
17. Cao C, He M, Wang L, He Y, Rao Y. Chemistries of bifunctional PROTAC degraders. *Chem Soc Rev* 2022;**51**:7066−114.
18. Lazo JS, Sharlow ER. Drugging undruggable molecular cancer targets. *Annu Rev Pharmacol Toxicol* 2016;**56**:23−40.
19. Su Z, Dhusia K, Wu Y. Understand the functions of scaffold proteins in cell signaling by a mesoscopic simulation method. *Biophys J* 2020;**119**:2116−26.

20. Jin L, Wang W, Fang G. Targeting protein−protein interaction by small molecules. *Annu Rev Pharmacol Toxicol* 2014;**54**:435−56.
21. Lu J, Qian Y, Altieri M, Dong H, Wang J, Raina K, et al. Hijacking the E3 ubiquitin ligase cereblon to efficiently target BRD4. *Chem Biol* 2015;**22**:755−63.
22. Raina K, Lu J, Qian Y, Altieri M, Gordon D, Rossi AM, et al. PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. *Proc Natl Acad Sci U S A* 2016;**113**:7124−9.
23. Gadd MS, Testa A, Lucas X, Chan KH, Chen W, Lamont DJ, et al. Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat Chem Biol* 2017;**13**:514−21.
24. Yang K, Song Y, Xie H, Wu H, Wu YT, Leisten ED, et al. Development of the first small molecule histone deacetylase 6 (HDAC6) degraders. *Bioorg Med Chem Lett* 2018;**28**:2493−7.
25. Hsu JH, Rasmusson T, Robinson J, Pachl F, Read J, Kawatkar S, et al. EED-targeted PROTACs degrade EED, EZH2, and SUZ12 in the PRC2 complex. *Cell Chem Biol* 2020;**27**:41−46 e17.
26. Schiedel M, Lehotzky A, Szunyogh S, Olah J, Hammelmann S, Wossner N, et al. HaloTag-targeted sirtuin-rearranging ligand (SirReal) for the development of proteolysis-targeting chimeras (PROTACs) against the lysine deacetylase sirtuin 2 (Sirt2). *Chembiochem* 2020;**21**:3371−6.
27. Hescheler DA, Hartmann MJM, Riemann B, Michel M, Bruns CJ, Alakus H, et al. Targeted therapy for adrenocortical carcinoma: a genomic-based search for available and emerging options. *Cancers* 2022;**14**:2721.
28. Cg N. Compounds for targeted degradation of BRD9. WO20211789320A1.
29. Toriki ES, Papatzimas JW, Nishikawa K, Dovala D, Frank AO, Hesse MJ, et al. Rational chemical design of molecular glue degraders. *ACS Cent Sci* 2023;**9**:915−26.
30. Tomaselli D, Mautone N, Mai A, Rotili D. Recent advances in epigenetic proteolysis targeting chimeras (Epi-PROTACs). *Eur J Med Chem* 2020;**207**:112750.
31. Vogelmann A, Robaa D, Sippl W, Jung M. Proteolysis targeting chimeras (PROTACs) for epigenetics research. *Curr Opin Chem Biol* 2020;**57**:8−16.
32. Zhang G, Zhang J, Gao Y, Li Y, Li Y. Strategies for targeting undruggable targets. *Expet Opin Drug Discov* 2022;**17**:55−69.
33. Lu B, Ye J. Commentary: PROTACs make undruggable targets druggable: challenge and opportunity. *Acta Pharm Sin B* 2021;**11**:3335−6.
34. Bekes M, Langley DR, Crews CM. PROTAC targeted protein degraders: the past is prologue. *Nat Rev Drug Discov* 2022;**21**:181−200.
35. Domostegui A, Nieto-Barrado L, Perez-Lopez C, Mayor-Ruiz C. Chasing molecular glue degraders: screening approaches. *Chem Soc Rev* 2022;**51**:5498−517.
36. Wu H, Yao H, He C, Jia Y, Zhu Z, Xu S, et al. Molecular glues modulate protein functions by inducing protein aggregation: a promising therapeutic strategy of small molecules for disease treatment. *Acta Pharm Sin B* 2022;**12**:3548−66.
37. Burslem GM, Crews CM. Proteolysis-targeting chimeras as therapeutics and tools for biological discovery. *Cell* 2020;**181**:102−14.
38. Slamon DJ, Neven P, Chia S, Jerusalem G, De Laurentiis M, Im S, et al. Ribociclib plus fulvestrant for postmenopausal women with hormone receptor-positive, human epidermal growth factor receptor 2-negative advanced breast cancer in the phase III randomized MONALEESA-3 trial: updated overall survival. *Ann Oncol* 2021;**32**:1015−24.
39. Paiva SL, Crews CM. Targeted protein degradation: elements of PROTAC design. *Curr Opin Chem Biol* 2019;**50**:111−9.
40. Li X, Song Y. Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *J Hematol Oncol* 2020;**13**:50.
41. Dong G, Ding Y, He S, Sheng C. Molecular glues for targeted protein degradation: from serendipity to rational discovery. *J Med Chem* 2021;**64**:10606−20.
42. Mayor-Ruiz C, Bauer S, Brand M, Kozicka Z, Siklos M, Imrichova H, et al. Rational discovery of molecular glue degraders *via* scalable chemical profiling. *Nat Chem Biol* 2020;**16**:1199−207.
43. Neklesa TK, Tae HS, Schneekloth AR, Stulberg MJ, Corson TW, Sundberg TB, et al. Small-molecule hydrophobic tagging-induced degradation of HaloTag fusion proteins. *Nat Chem Biol* 2011;**7**:538−43.
44. Ma A, Stratikopoulos E, Park KS, Wei J, Martin TC, Yang X, et al. Discovery of a first-in-class EZH2 selective degrader. *Nat Chem Biol* 2020;**16**:214−22.
45. Zaccara S, Ries RJ, Jaffrey SR. Reading, writing and erasing mRNA methylation. *Nat Rev Mol Cell Biol* 2019;**20**:608−24.
46. Zhao LY, Song J, Liu Y, Song CX, Yi C. Mapping the epigenetic modifications of DNA and RNA. *Protein Cell* 2020;**11**:792−808.
47. Strahl BD, Allis CD. The language of covalent histone modifications. *Nature* 2000;**403**:41−5.
48. Roberts RJ, Belfort M, Bestor T, Bhagwat AS, Bickle TA, Bitinaite J, et al. A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes. *Nucleic Acids Res* 2003;**31**:1805−12.
49. Jeltsch A, Adam S, Dukatz M, Emperle M, Bashtrykov P. Deep enzymology studies on DNA methyltransferases reveal novel connections between flanking sequences and enzyme activity. *J Mol Biol* 2021;**433**:167186.
50. Morgan MAJ, Shilatifard A. Reevaluating the roles of histone-modifying enzymes and their associated chromatin modifications in transcriptional regulation. *Nat Genet* 2020;**52**:1271−81.
51. Sharda A, Rashid M, Shah SG, Sharma AK, Singh SR, Gera P, et al. Elevated HDAC activity and altered histone phospho-acetylation confer acquired radio-resistant phenotype to breast cancer cells. *Clin Epigenet* 2020;**12**:4.
52. Witt O, Deubzer HE, Milde T, Oehme I. HDAC family: what are the cancer relevant targets? *Cancer Lett* 2009;**277**:8−21.
53. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J* 2003;**370**:737−49.
54. Lapierre LR, Kumsta C, Sandri M, Ballabio A, Hansen M. Transcriptional and epigenetic regulation of autophagy in aging. *Autophagy* 2015;**11**:867−80.
55. Wu QJ, Zhang TN, Chen HH, Yu XF, Lv JL, Liu YY, et al. The sirtuin family in health and disease. *Signal Transduct Targeted Ther* 2022;**7**:402.
56. Husmann D, Gozani O. Histone lysine methyltransferases in biology and disease. *Nat Struct Mol Biol* 2019;**26**:880−9.
57. Jarrold J, Davies CC. PRMTs and arginine methylation: cancer's best-kept secret? *Trends Mol Med* 2019;**25**:993−1009.
58. Margueron R, Reinberg D. The polycomb complex PRC2 and its mark in life. *Nature* 2011;**469**:343−9.
59. Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nat Med* 2016;**22**:128−34.
60. Tomassi S, Romanelli A, Zwergel C, Valente S, Mai A. Polycomb repressive complex 2 modulation through the development of EZH2−EED interaction inhibitors and EED binders. *J Med Chem* 2021;**64**:11774−97.
61. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 2011;**21**:381−95.
62. Li Z, Ding L, Li Z, Wang Z, Suo F, Shen D, et al. Development of the triazole-fused pyrimidine derivatives as highly potent and reversible inhibitors of histone lysine specific demethylase 1 (LSD1/KDM1A). *Acta Pharm Sin B* 2019;**9**:794−808.
63. Stathis A, Bertoni F. BET proteins as targets for anticancer treatment. *Cancer Discov* 2018;**8**:24−36.
64. Eagen KP, French CA. Supercharging BRD4 with NUT in carcinoma. *Oncogene* 2021;**40**:1396−408.
65. Hu J, Pan D, Li G, Chen K, Hu X. Regulation of programmed cell death by Brd4. *Cell Death Dis* 2022;**13**:1059.

66. Erb MA, Scott TG, Li BE, Xie H, Paulk J, Seo HS, et al. Transcription control by the ENL YEATS domain in acute leukaemia. *Nature* 2017;**543**:270−4.
67. Andrews FH, Shinsky SA, Shanle EK, Bridgers JB, Gest A, Tsun IK, et al. The Taf14 YEATS domain is a reader of histone crotonylation. *Nat Chem Biol* 2016;**12**:396−8.
68. Klein BJ, Ahmad S, Vann KR, Andrews FH, Mayo ZA, Bourriquen G, et al. Yaf9 subunit of the NuA4 and SWR1 complexes targets histone H3K27ac through its YEATS domain. *Nucleic Acids Res* 2018;**46**:421−30.
69. Mi W, Guan H, Lyu J, Zhao D, Xi Y, Jiang S, et al. YEATS2 links histone acetylation to tumorigenesis of non-small cell lung cancer. *Nat Commun* 2017;**8**:1088.
70. He N, Chan CK, Sobhian B, Chou S, Xue Y, Liu M, et al. Human polymerase-associated factor complex (PAFc) connects the super elongation complex (SEC) to RNA polymerase II on chromatin. *Proc Natl Acad Sci U S A* 2011;**108**:E636−45.
71. Zhao D, Guan H, Zhao S, Mi W, Wen H, Li Y, et al. YEATS2 is a selective histone crotonylation reader. *Cell Res* 2016;**26**:629−32.
72. Wan L, Chong S, Xuan F, Liang A, Cui X, Gates L, et al. Impaired cell fate through gain-of-function mutations in a chromatin reader. *Nature* 2020;**577**:121−6.
73. Guenette RG, Yang SW, Min J, Pei B, Potts PR. Target and tissue selectivity of PROTAC degraders. *Chem Soc Rev* 2022;**51**:5740−56.
74. Trial of ARV-110 in patients with metastatic castration resistant prostate cancer (mCRPC). Available online: https://classicclinicaltrialsgov/ct2/show/NCT03888612?term=NCT03888612&draw=2&rank=1..
75. A Phase 1/2 trial of ARV-471 alone and in combination with Palbociclib (IBRANCE®) in patients with ER+/HER2− locally advanced or metastatic breast cancer (mBC). Available online: https://classicclinicaltrialsgov/ct2/show/NCT04072952?term=NCT04072952&draw=2&rank=1..
76. Qi SM, Dong J, Xu ZY, Cheng XD, Zhang WD, Qin JJ. PROTAC: an effective targeted protein degradation strategy for cancer therapy. *Front Pharmacol* 2021;**12**:692574.
77. Hemkens M, Stamp K, Loberg LI, Moreau K, Hart T. Industry perspective on the nonclinical safety assessment of heterobifunctional degraders. *Drug Discov Today* 2023;**28**:103643.
78. Thummuri D, Khan S, Underwood PW, Zhang P, Wiegand J, Zhang X, et al. Overcoming gemcitabine resistance in pancreatic cancer using the BCL-X(L)-specific degrader DT2216. *Mol Cancer Therapeut* 2022;**21**:184−92.
79. Smith MA, Choudhary GS, Pellagatti A, Choi K, Bolanos LC, Bhagat TD, et al. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. *Nat Cell Biol* 2019;**21**:640−50.
80. Eyre TA, Riches JC. The evolution of therapies targeting Bruton tyrosine kinase for the treatment of chronic lymphocytic leukaemia: future perspectives. *Cancers* 2023;**15**:2596.
81. Bai L, Zhou H, Xu R, Zhao Y, Chinnaswamy K, McEachern D, et al. A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression *in vivo*. *Cancer Cell* 2019;**36**:498−511 e17.
82. Yang T, Hu Y, Miao J, Chen J, Liu J, Cheng Y, et al. A BRD4 PROTAC nanodrug for glioma therapy *via* the intervention of tumor cells proliferation, apoptosis and M2 macrophages polarization. *Acta Pharm Sin B* 2022;**12**:2658−71.
83. Li Z, Wang C, Wang Z, Zhu C, Li J, Sha T, et al. Allele-selective lowering of mutant HTT protein by HTT-LC3 linker compounds. *Nature* 2019;**575**:203−9.
84. Guo M, He S, Cheng J, Li Y, Dong G, Sheng C. Hydrophobic tagging-induced degradation of PDEdelta in colon cancer cells. *ACS Med Chem Lett* 2022;**13**:298−303.
85. Liu J, Zheng S, Akerstrom VL, Yuan C, Ma Y, Zhong Q, et al. Fulvestrant-3 boronic acid (ZB716): an orally bioavailable selective estrogen receptor downregulator (SERD). *J Med Chem* 2016;**59**:8134−40.
86. Nowak RP, DeAngelo SL, Buckley D, He Z, Donovan KA, An J, et al. Plasticity in binding confers selectivity in ligand-induced protein degradation. *Nat Chem Biol* 2018;**14**:706−14.
87. Xiao Y, Wang J, Zhao LY, Chen X, Zheng G, Zhang X, et al. Discovery of histone deacetylase 3 (HDAC3)-specific PROTACs. *Chem Commun* 2020;**56**:9866−9.
88. Cao F, de Weerd S, Chen D, Zwinderman MRH, van der Wouden PE, Dekker FJ. Induced protein degradation of histone deacetylases 3 (HDAC3) by proteolysis targeting chimera (PROTAC). *Eur J Med Chem* 2020;**208**:112800.
89. Kong Q, Hao Y, Li X, Wang X, Ji B, Wu Y. HDAC4 in ischemic stroke: mechanisms and therapeutic potential. *Clin Epigenet* 2018;**10**:117.
90. Federspiel JD, Greco TM, Lum KK, Cristea IM. HDAC4 interactions in Huntington's disease viewed through the prism of multiomics. *Mol Cell Proteomics* 2019;**18**:S92−113.
91. Macabuag N, Esmieu W, Breccia P, Jarvis R, Blackaby W, Lazari O, et al. Developing HDAC4-selective protein degraders to investigate the role of HDAC4 in Huntington's disease pathology. *J Med Chem* 2022;**65**:12445−59.
92. Pulya S, Amin SA, Adhikari N, Biswas S, Jha T, Ghosh B. HDAC6 as privileged target in drug discovery: a perspective. *Pharmacol Res* 2021;**163**:105274.
93. Wu Y, Yang Z, Cheng K, Bi H, Chen J. Small molecule-based immunomodulators for cancer therapy. *Acta Pharm Sin B* 2022;**12**:4287−308.
94. Peng X, Yu Z, Surineni G, Deng B, Zhang M, Li C, et al. Discovery of novel benzohydroxamate-based histone deacetylase 6 (HDAC6) inhibitors with the ability to potentiate anti-PD-L1 immunotherapy in melanoma. *J Enzym Inhib Med Chem* 2023;**38**:2201408.
95. Peng X, Li L, Chen J, Ren Y, Liu J, Yu Z, et al. Discovery of novel histone deacetylase 6 (HDAC6) inhibitors with enhanced antitumor immunity of anti-PD-L1 immunotherapy in melanoma. *J Med Chem* 2022;**65**:2434−57.
96. Sinatra L, Yang J, Schliehe-Diecks J, Dienstbier N, Vogt M, Gebing P, et al. Solid-phase synthesis of cereblon-recruiting selective histone deacetylase 6 degraders (HDAC6 PROTACs) with antileukemic activity. *J Med Chem* 2022;**65**:16860−78.
97. Wu H, Yang K, Zhang Z, Leisten ED, Li Z, Xie H, et al. Development of multifunctional histone deacetylase 6 degraders with potent antimyeloma activity. *J Med Chem* 2019;**62**:7042−57.
98. Yang K, Zhao Y, Nie X, Wu H, Wang B, Almodovar-Rivera CM, et al. A cell-based target engagement assay for the identification of cereblon E3 ubiquitin ligase ligands and their application in HDAC6 degraders. *Cell Chem Biol* 2020;**27**:866−876.e8.
99. Yang H, Lv W, He M, Deng H, Li H, Wu W, et al. Plasticity in designing PROTACs for selective and potent degradation of HDAC6. *Chem Commun* 2019;**55**:14848−51.
100. An Z, Lv W, Su S, Wu W, Rao Y. Developing potent PROTACs tools for selective degradation of HDAC6 protein. *Protein Cell* 2019;**10**:606−9.
101. Yang K, Wu H, Zhang Z, Leisten ED, Nie X, Liu B, et al. Development of selective histone deacetylase 6 (HDAC6) degraders recruiting Von Hippel-Lindau (VHL) E3 ubiquitin ligase. *ACS Med Chem Lett* 2020;**11**:575−81.
102. Chotitumnavee J, Yamashita Y, Takahashi Y, Takada Y, Iida T, Oba M, et al. Selective degradation of histone deacetylase 8 mediated by a proteolysis targeting chimera (PROTAC). *Chem Commun* 2022;**58**:4635−8.
103. Darwish S, Ghazy E, Heimburg T, Herp D, Zeyen P, Salem-Altintas R, et al. Design, synthesis and biological characterization of histone deacetylase 8 (HDAC8) Proteolysis Targeting Chimeras (PROTACs) with anti-neuroblastoma activity. *Int J Mol Sci* 2022;**23**:7535.
104. Sun Z, Deng B, Yang Z, Mai R, Huang J, Ma Z, et al. Discovery of pomalidomide-based PROTACs for selective degradation of histone deacetylase 8. *Eur J Med Chem* 2022;**239**:114544.

105. Huang J, Zhang J, Xu W, Wu Q, Zeng R, Liu Z, et al. Structure-based discovery of selective histone deacetylase 8 degraders with potent anticancer activity. *J Med Chem* 2023;**66**:1186−209.
106. Smalley JP, Adams GE, Millard CJ, Song Y, Norris JKS, Schwabe JWR, et al. PROTAC-mediated degradation of class I histone deacetylase enzymes in corepressor complexes. *Chem Commun* 2020;**56**:4476−9.
107. Cross JM, Coulson ME, Smalley JP, Pytel WA, Ismail O, Trory JS, et al. A 'click' chemistry approach to novel entinostat (MS-275) based class I histone deacetylase proteolysis targeting chimeras. *RSC Med Chem* 2022;**13**:1634−9.
108. Roatsch M, Vogelmann A, Herp D, Jung M, Olsen CA. Proteolysis-targeting chimeras (PROTACs) based on macrocyclic tetrapeptides selectively degrade class I histone deacetylases 1−3. *Biomo Med Chem* 2020;**40**:245.
109. Petrich A, Nabhan C. Use of class I histone deacetylase inhibitor romidepsin in combination regimens. *Leuk Lymphoma* 2016;**57**:1755−65.
110. Burke MR, Smith AR, Zheng G. Overcoming cancer drug resistance utilizing PROTAC technology. *Front Cell Dev Biol* 2022;**10**:872729.
111. Schiedel M, Herp D, Hammelmann S, Swyter S, Lehotzky A, Robaa D, et al. Chemically induced degradation of sirtuin 2 (Sirt2) by a proteolysis targeting chimera (PROTAC) based on sirtuin rearranging ligands (SirReals). *J Med Chem* 2018;**61**:482−91.
112. Sinatra L, Bandolik JJ, Roatsch M, Sonnichsen M, Schoeder CT, Hamacher A, et al. Hydroxamic acids immobilized on resins (HAIRs): synthesis of dual-targeting HDAC inhibitors and HDAC degraders (PROTACs). *Angew Chem Int Ed Engl* 2020;**59**:22494−9.
113. Huang M, Xie X, Gong P, Wei Y, Du H, Xu Y, et al. A 18β-Glycyrrhetinic acid conjugate with Vorinostat degrades HDAC3 and HDAC6 with improved antitumor effects. *Eur J Med Chem* 2020;**188**:111991.
114. Ghoshal A, Yugandhar D, Srivastava AK. BET inhibitors in cancer therapeutics: a patent review. *Expert Opin Ther Pat* 2016;**26**:505−22.
115. Winter GE, Mayer A, Buckley DL, Erb MA, Roderick JE, Vittori S, et al. BET bromodomain proteins function as master transcription elongation factors independent of CDK9 recruitment. *Mol Cell* 2017;**67**:5−18.e19.
116. Zhou B, Hu J, Xu F, Chen Z, Bai L, Fernandez-Salas E, et al. Discovery of a small-molecule degrader of bromodomain and extra-terminal (BET) proteins with picomolar cellular potencies and capable of achieving tumor regression. *J Med Chem* 2018;**61**:462−81.
117. Bai L, Zhou B, Yang CY, Ji J, McEachern D, Przybranowski S, et al. Targeted degradation of BET proteins in triple-negative breast cancer. *Cancer Res* 2017;**77**:2476−87.
118. Qiu X, Sun N, Kong Y, Li Y, Yang X, Jiang B. Chemoselective synthesis of lenalidomide-based PROTAC library using alkylation reaction. *Org Lett* 2019;**21**:3838−41.
119. Zengerle M, Chan KH, Ciulli A. Selective small molecule induced degradation of the BET bromodomain protein BRD4. *ACS Chem Biol* 2015;**10**:1770−7.
120. DRUG DEVELOPMENT Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, et al. Phthalimide conjugation as a strategy for *in vivo* target protein degradation. *Science* 2015;**348**:1376−81.
121. Testa A, Hughes SJ, Lucas X, Wright JE, Ciulli A. Structure-based design of a macrocyclic PROTAC. *Angew Chem Int Ed Engl* 2020;**59**:1727−34.
122. Jiang F, Wei Q, Li H, Li H, Cui Y, Ma Y, et al. Discovery of novel small molecule induced selective degradation of the bromodomain and extra-terminal (BET) bromodomain protein BRD4 and BRD2 with cellular potencies. *Bioorg Med Chem* 2020;**28**:115181.
123. Hines J, Lartigue S, Dong H, Qian Y, Crews CM. MDM2-recruiting PROTAC offers superior, synergistic antiproliferative activity *via* simultaneous degradation of BRD4 and stabilization of p53. *Cancer Res* 2019;**79**:251−62.
124. Chen D, Lu T, Yan Z, Lu W, Zhou F, Lyu X, et al. Discovery, structural insight, and bioactivities of BY27 as a selective inhibitor of the second bromodomains of BET proteins. *Eur J Med Chem* 2019;**182**:111633.
125. Yan Z, Lyu X, Lin D, Wu G, Gong Y, Ren X, et al. Selective degradation of cellular BRD3 and BRD4-L promoted by PROTAC molecules in six cancer cell lines. *Eur J Med Chem* 2023;**254**:115381.
126. Ding M, Shao Y, Sun D, Meng S, Zang Y, Zhou Y, et al. Design, synthesis, and biological evaluation of BRD4 degraders. *Bioorg Med Chem* 2023;**78**:117134.
127. Zhou Q, Huang J, Zhang C, Zhao F, Kim W, Tu X, et al. The bromodomain containing protein BRD-9 orchestrates RAD51−RAD54 complex formation and regulates homologous recombination-mediated repair. *Nat Commun* 2020;**11**:2639.
128. Zoppi V, Hughes SJ, Maniaci C, Testa A, Gmaschitz T, Wieshofer C, et al. Iterative design and optimization of initially inactive Proteolysis Targeting Chimeras (PROTACs) identify VZ185 as a potent, fast, and selective von Hippel-Lindau (VHL) based dual degrader probe of BRD9 and BRD7. *J Med Chem* 2019;**62**:699−726.
129. Remillard D, Buckley DL, Paulk J, Brien GL, Sonnett M, Seo HS, et al. Degradation of the BAF complex factor BRD9 by heterobifunctional ligands. *Angew Chem Int Ed Engl* 2017;**56**:5738−43.
130. Weisberg E, Chowdhury B, Meng C, Case AE, Ni W, Garg S, et al. BRD9 degraders as chemosensitizers in acute leukemia and multiple myeloma. *Blood Cancer J* 2022;**12**:110.
131. Sabnis RW. Novel compounds for targeted degradation of BRD9 and their use for treating cancer. *ACS Med Chem Lett* 2022;**13**:17−8.
132. Sabnis RW. BRD9 bifunctional degraders for treating cancer. *ACS Med Chem Lett* 2021;**12**:1879−80.
133. Duan R, Du W, Guo W. EZH2: a novel target for cancer treatment. *J Hematol Oncol* 2020;**13**:104.
134. Zeng J, Zhang J, Sun Y, Wang J, Ren C, Banerjee S, et al. Targeting EZH2 for cancer therapy: from current progress to novel strategies. *Eur J Med Chem* 2022;**238**:114419.
135. Liu Z, Hu X, Wang Q, Wu X, Zhang Q, Wei W, et al. Design and synthesis of EZH2-based PROTACs to degrade the PRC2 complex for targeting the noncatalytic activity of EZH2. *J Med Chem* 2021;**64**:2829−48.
136. Tu Y, Sun Y, Qiao S, Luo Y, Liu P, Jiang ZX, et al. Design, synthesis, and evaluation of VHL-based EZH2 degraders to enhance therapeutic activity against lymphoma. *J Med Chem* 2021;**64**:10167−84.
137. Wang C, Chen X, Liu X, Lu D, Li S, Qu L, et al. Discovery of precision targeting EZH2 degraders for triple-negative breast cancer. *Eur J Med Chem* 2022;**238**:114462.
138. Dale B, Anderson C, Park KS, Kaniskan HU, Ma A, Shen Y, et al. Targeting triple-negative breast cancer by a novel proteolysis targeting chimera degrader of enhancer of Zeste Homolog 2. *ACS Pharmacol Transl Sci* 2022;**5**:491−507.
139. Potjewyd F, Turner AW, Beri J, Rectenwald JM, Norris-Drouin JL, Cholensky SH, et al. Degradation of polycomb repressive complex 2 with an EED-targeted bivalent chemical degrader. *Cell Chem Biol* 2020;**27**:47−56.e15.
140. Xie S, Zhan F, Zhu J, Sun Y, Zhu H, Liu J, et al. Discovery of norbornene as a novel hydrophobic tag applied in protein degradation. *Angew Chem Int Ed Engl* 2023;**62**:e202217246.
141. Garnar-Wortzel L, Bishop TR, Kitamura S, Milosevich N, Asiaban JN, Zhang X, et al. Chemical inhibition of ENL/AF9 YEATS domains in acute leukemia. *ACS Cent Sci* 2021;**7**:815−30.
142. Perlman EJ, Gadd S, Arold ST, Radhakrishnan A, Gerhard DS, Jennings L, et al. MLLT1 YEATS domain mutations in clinically

distinctive Favourable Histology Wilms tumours. *Nat Commun* 2015;**6**:10013.
143. Li X, Yao Y, Wu F, Song Y. A proteolysis-targeting chimera molecule selectively degrades ENL and inhibits malignant gene expression and tumor growth. *J Hematol Oncol* 2022;**15**:41.
144. Bondeson DP, Smith BE, Burslem GM, Buhimschi AD, Hines J, Jaime-Figueroa S, et al. Lessons in PROTAC design from selective degradation with a promiscuous warhead. *Cell Chem Biol* 2018;**25**:78−87.e5.
145. Hughes SJ, Testa A, Thompson N, Churcher I. The rise and rise of protein degradation: opportunities and challenges ahead. *Drug Discov Today* 2021;**26**:2889−97.
146. Xiong Y, Donovan KA, Eleuteri NA, Kirmani N, Yue H, Razov A, et al. Chemo-proteomics exploration of HDAC degradability by small molecule degraders. *Cell Chem Biol* 2021;**28**:1514−1527.e4.
147. Huang HT, Dobrovolsky D, Paulk J, Yang G, Weisberg EL, Doctor ZM, et al. A chemoproteomic approach to 1uery the degradable kinome using a multi-kinase degrader. *Cell Chem Biol* 2018;**25**:88−99.e6.
148. Donovan KA, Ferguson FM, Bushman JW, Eleuteri NA, Bhunia D, Ryu S, et al. Mapping the degradable kinome provides a resource for expedited degrader development. *Cell* 2020;**183**:1714−1731.e10.
149. Steinebach C, Ng YLD, Sosic I, Lee CS, Chen S, Lindner S, et al. Systematic exploration of different E3 ubiquitin ligases: an approach towards potent and selective CDK6 degraders. *Chem Sci* 2020;**11**:3474−86.
150. Bolden JE, Tasdemir N, Dow LE, van Es JH, Wilkinson JE, Zhao Z, et al. Inducible *in vivo* silencing of BRD4 identifies potential toxicities of sustained BET protein inhibition. *Cell Rep* 2014;**8**:1919−29.
151. Mukherjee C, Holubowska A, Schwedhelm-Domeyer N, Mitkovski M, Lee SJ, Kannan M, et al. Loss of the neuron-specific F-box protein FBXO41 models an ataxia-like phenotype in mice with neuronal migration defects and degeneration in the cerebellum. *J Neurosci* 2015;**35**:8701−17.
152. Menon S, Boyer NP, Winkle CC, McClain LM, Hanlin CC, Pandey D, et al. The E3 ubiquitin ligase TRIM9 is a filopodia off switch required for netrin-dependent axon guidance. *Dev Cell* 2015;**35**:698−712.
153. LoPresti P. HDAC6 in diseases of cognition and of neurons. *Cells* 2020;**10**:12.
154. Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Adv Exp Med Biol* 2020;**1253**:3−55.
155. Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, et al. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 2006;**439**:811−6.
156. Hu Z, Crews CM. Recent developments in PROTAC-mediated protein degradation: from bench to clinic. *Chembiochem* 2022;**23**:e202100270.
157. Pike A, Williamson B, Harlfinger S, Martin S, McGinnity DF. Optimising proteolysis-targeting chimeras (PROTACs) for oral drug delivery: a drug metabolism and pharmacokinetics perspective. *Drug Discov Today* 2020;**25**:1793−800.
158. Wang L, Shao X, Zhong T, Wu Y, Xu A, Sun X, et al. Discovery of a first-in-class CDK2 selective degrader for AML differentiation therapy. *Nat Chem Biol* 2021;**17**:567−75.
159. Simpson LM, Glennie L, Brewer A, Zhao JF, Crooks J, Shpiro N, et al. Target protein localization and its impact on PROTAC-mediated degradation. *Cell Chem Biol* 2022;**29**:1482−1504.e7.
160. Xue G, Wang K, Zhou D, Zhong H, Pan Z. Light-induced protein degradation with photocaged PROTACs. *J Am Chem Soc* 2019;**141**:18370−4.
161. Liu J, Chen H, Ma L, He Z, Wang D, Liu Y, et al. Light-induced control of protein destruction by opto-PROTAC. *Sci Adv* 2020;**6**:eaay5154.
162. Reynders M, Matsuura BS, Berouti M, Simoneschi D, Marzio A, Pagano M, et al. PHOTACs enable optical control of protein degradation. *Sci Adv* 2020;**6**:eaay5064.
163. Pfaff P, Samarasinghe KTG, Crews CM, Carreira EM. Reversible spatiotemporal control of induced protein degradation by bistable photoPROTACs. *ACS Cent Sci* 2019;**5**:1682−90.
164. Naro Y, Darrah K, Deiters A. Optical control of small molecule-induced protein degradation. *J Am Chem Soc* 2020;**142**:2193−7.
165. Petroni G, Cantley LC, Santambrogio L, Formenti SC, Galluzzi L. Radiotherapy as a tool to elicit clinically actionable signalling pathways in cancer. *Nat Rev Clin Oncol* 2022;**19**:114−31.
166. Yang C, Yang Y, Li Y, Ni Q, Li J. Radiotherapy-triggered proteolysis targeting chimera prodrug activation in tumors. *J Am Chem Soc* 2023;**145**:385−91.
167. Oleinik II , Orlov VN, Ponomareva AG, Koroteeva GP, Tsarev VN. Cellular immunity indices in myocardial infarct. *Voenno-Med Zh* 1981:30−3.
168. Maneiro MA, Forte N, Shchepinova MM, Kounde CS, Chudasama V, Baker JR, et al. Antibody−PROTAC conjugates enable HER2-dependent targeted protein degradation of BRD4. *ACS Chem Biol* 2020;**15**:1306−12.
169. Dragovich PS, Pillow TH, Blake RA, Sadowsky JD, Adaligil E, Adhikari P, et al. Antibody-mediated delivery of chimeric BRD4 degraders. Part 2: improvement of *in vitro* antiproliferation activity and *in vivo* antitumor efficacy. *J Med Chem* 2021;**64**:2576−607.
170. Dragovich PS, Pillow TH, Blake RA, Sadowsky JD, Adaligil E, Adhikari P, et al. Antibody-mediated delivery of chimeric BRD4 degraders. Part 1: exploration of antibody linker, payload loading, and payload molecular properties. *J Med Chem* 2021;**64**:2534−75.
171. Pillow TH, Adhikari P, Blake RA, Chen J, Del Rosario G, Deshmukh G, et al. Antibody conjugation of a chimeric BET degrader enables *in vivo* activity. *ChemMedChem* 2020;**15**:17−25.
172. Kolakowski RV, Haelsig KT, Emmerton KK, Leiske CI, Miyamoto JB, Cochran JH, et al. The methylene alkoxy carbamate self-immolative unit: utilization for the targeted delivery of alcohol-containing payloads with antibody−drug conjugates. *Angew Chem Int Ed Engl* 2016;**55**:7948−51.
173. Zhou J, Rossi J. Aptamers as targeted therapeutics: current potential and challenges. *Nat Rev Drug Discov* 2017;**16**:181−202.
174. He S, Gao F, Ma J, Ma H, Dong G, Sheng C. Aptamer-PROTAC Conjugates (APCs) for tumor-specific targeting in breast cancer. *Angew Chem Int Ed Engl* 2021;**60**:23299−305.
175. Scaranti M, Cojocaru E, Banerjee S, Banerji U. Exploiting the folate receptor alpha in oncology. *Nat Rev Clin Oncol* 2020;**17**:349−59.
176. Numasawa K, Hanaoka K, Saito N, Yamaguchi Y, Ikeno T, Echizen H, et al. A fluorescent probe for rapid, high-contrast visualization of folate-receptor-expressing tumors *in vivo*. *Angew Chem Int Ed Engl* 2020;**59**:6015−20.
177. Liu J, Chen H, Liu Y, Shen Y, Meng F, Kaniskan HU, et al. Cancer selective target degradation by folate-caged PROTACs. *J Am Chem Soc* 2021;**143**:7380−7.
178. Kiely-Collins H, Winter GE, Bernardes GJL. The role of reversible and irreversible covalent chemistry in targeted protein degradation. *Cell Chem Biol* 2021;**28**:952−68.
179. Nemec V, Schwalm MP, Muller S, Knapp S. PROTAC degraders as chemical probes for studying target biology and target validation. *Chem Soc Rev* 2022;**51**:7971−93.
180. Spradlin JN, Hu X, Ward CC, Brittain SM, Jones MD, Ou L, et al. Harnessing the anti-cancer natural product nimbolide for targeted protein degradation. *Nat Chem Biol* 2019;**15**:747−55.
181. Luo M, Spradlin JN, Boike L, Tong B, Brittain SM, McKenna JM, et al. Chemoproteomics-enabled discovery of covalent RNF114-based degraders that mimic natural product function. *Cell Chem Biol* 2021;**28**:559−566.e15.
182. Zhang X, Luukkonen LM, Eissler CL, Crowley VM, Yamashita Y, Schafroth MA, et al. DCAF11 supports targeted protein degradation by electrophilic Proteolysis-Targeting Chimeras. *J Am Chem Soc* 2021;**143**:5141−9.

183. Henning NJ, Manford AG, Spradlin JN, Brittain SM, Zhang E, McKenna JM, et al. Discovery of a covalent FEM1B recruiter for targeted protein degradation applications. *J Am Chem Soc* 2022;**144**:701−8.
184. Li YD, Ma MW, Hassan MM, Hunkeler M, Teng M, Puvar K, et al. Template-assisted covalent modification of DCAF16 underlies activity of BRD4 molecular glue degraders. *bioRxiv* 2023;**14**:528208.
185. King EA, Cho Y, Hsu NS, Dovala D, McKenna JM, Tallarico JA, et al. Chemoproteomics-enabled discovery of a covalent molecular glue degrader targeting NF-kappaB. *Cell Chem Biol* 2023;**30**:394−402.e9.
186. Cyrus K, Wehenkel M, Choi EY, Swanson H, Kim KB. Two-headed PROTAC: an effective new tool for targeted protein degradation. *Chembiochem* 2010;**11**:1531−4.
187. Imaide S, Riching KM, Makukhin N, Vetma V, Whitworth C, Hughes SJ, et al. Trivalent PROTACs enhance protein degradation *via* combined avidity and cooperativity. *Nat Chem Biol* 2021;**17**:1157−67.
188. Huang Y, Yokoe H, Kaiho-Soma A, Takahashi K, Hirasawa Y, Morita H, et al. Design, synthesis, and evaluation of trivalent PROTACs having a functionalization site with controlled orientation. *Bioconjugate Chem* 2022;**33**:142−51.