

PROTACs improve selectivity for targeted proteins

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Received: 10 March 2025; Revised: 26 March 2025; Accepted: 15 April 2025

Published online: 20 May 2025

DOI 10.15212/AMM-2025-0015

ABSTRACT

Proteolysis targeting chimera (PROTAC) technology has emerged as a powerful tool in drug discovery that enables targeted protein degradation through a unique bifunctional approach. This review provides a comprehensive overview of PROTACs with a focus on recent advances in enhancing selectivity and therapeutic potential. We begin with an in-depth discussion of the structure and mechanism of PROTACs, comparing PROTACs to traditional small molecule inhibitors and exploring how PROTACs overcome limitations, such as drug resistance and targeting previously undruggable proteins. Key to this discussion is the concept of selectivity in PROTAC design, including optimization of E3 ligases and linker structures to improve target engagement and minimize off-target effects. The review also highlights the potential of covalent PROTACs in enhancing specificity and efficacy. Furthermore, various target classes for PROTAC development are explored, including epigenetic regulators (e.g., BET proteins and histone deacetylases), cell cycle and signaling pathway proteins (e.g., CDKs, BCL-XL, and p38 MAPK), receptor and kinase targets (e.g., EGFR and BRAF), and immune regulators (e.g., CREBBP and IRAK3). We discuss the implications of targeting these proteins for cancer and other diseases, emphasizing the promise of PROTACs in transforming therapeutic strategies. Finally, the review highlights ongoing challenges, such as optimizing pharmacokinetics and clinical validation, and provides future perspectives on the evolution of PROTAC technology.

Keywords: PROTACs, targeting selectivity, ubiquitination, degradation, pan-inhibitors

1. INTRODUCTION

The concept of proteolysis targeting chimeras (PROTACs) was introduced by Crews and colleagues in 2001 [1], with the first all-small-molecule PROTAC developed in 2008 [2]. Over the past 2 decades, the field has had rapid advances, particularly through the discovery of small molecule ligands recruiting E3 ligases, such as small molecule ligands derived from von Hippel-Lindau (VHL) and cereblon (CRBN) [3, 4]. These discoveries have significantly expanded the toolbox for designing PROTACs, enabling the application of PROTACs in drug discovery and biological research [5-9] (Figure 1).

PROTACs are heterobifunctional molecules comprised of the following three key components: a ligand that binds to the target protein of interest (POI); a ligand that recruits an E3 ligase; and a linker that connects the POI and E3 ligase [10, 11] (Figure 2). By forming a ternary complex, PROTACs facilitate the ubiquitination and subsequent degradation of POIs via the ubiquitin-proteasome system (UPS), which distinguishes PROTACs from traditional small molecule inhibitors that work by blocking protein activity [12, 13]. This novel degradation mechanism holds great promise, particularly in overcoming the limitations of small molecule inhibitors, which often suffer from poor selectivity and off-target effects.

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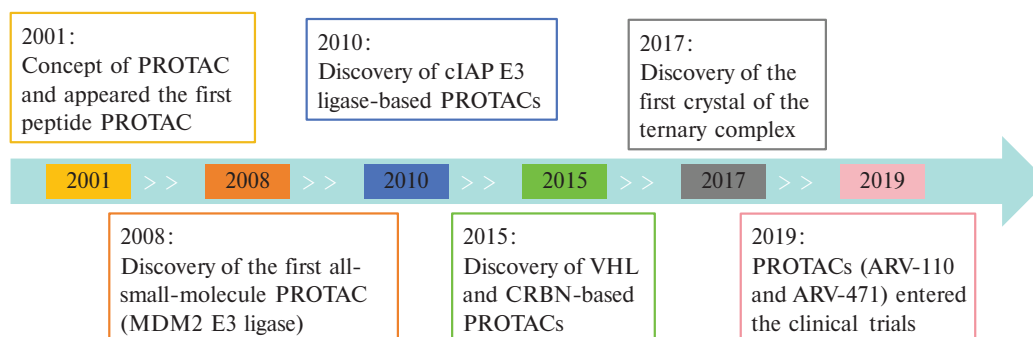


Figure 1 | Key milestones in the development of PROTAC technology.

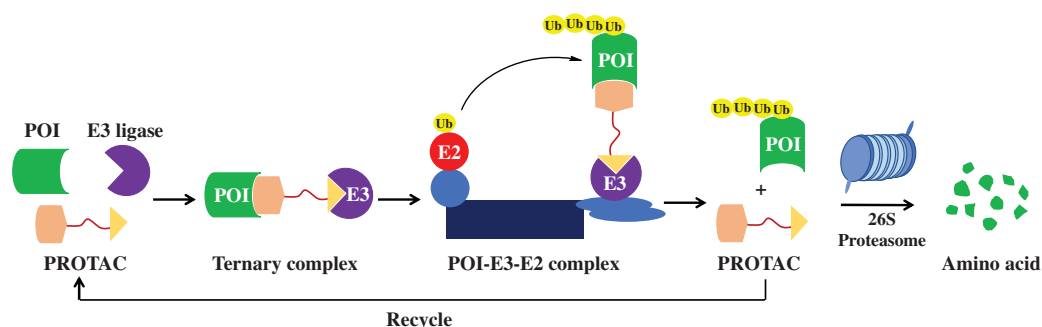


Figure 2 | Mechanism underlying PROTACs to degrade POI: PROTACs form a ternary complex with the POI and an E3 ligase, which mediates ubiquitination through the UPS, ultimately leading to proteasomal recognition and degradation of the target protein.

Despite the progress in PROTAC development, challenges remain in optimizing target selectivity, especially when targeting proteins with high-sequence homology. This review aims to address these challenges by focusing on the recent advances in enhancing target selectivity through PROTAC design. We will explore how the choice of linker and the recruitment of specific E3 ligases significantly influence selectivity, as well as how the ubiquitination process can be modulated to improve targeting precision. Furthermore, we will discuss the future directions for PROTAC development, including potential limitations and areas for further investigation.

This review provides a comprehensive overview of the current state of PROTAC technology, highlighting the importance of selectivity in drug discovery. By summarizing recent progress and offering insights into future developments, we aim to contribute to the ongoing effort to harness PROTACs as a powerful tool for precise and targeted therapeutic interventions. The logical flow of the review will first cover the fundamentals of PROTAC design, followed by recent advances in target selectivity and conclude with a discussion on future perspectives and potential challenges.

2. IMPROVEMENT IN PROTAC SELECTIVITY

2.1 Comparison between PROTACs and traditional small molecule inhibitors

Compared to traditional small molecule inhibitors, PROTACs offer significant advantages in terms of selectivity. Traditional small molecule drugs typically inhibit protein function by binding to the active site of the target protein. However, this mechanism encounters selectivity challenges when involving protein families because structurally similar proteins may share similar binding sites [14]. PROTACs induce degradation of the target protein, effectively reducing the expression, which provides a higher degree of selectivity, especially when targeting members of the same protein family [10, 15]. The selectivity advantage of PROTACs primarily arises from several factors. First, PROTACs simultaneously bind to the POI and E3 ubiquitin ligase, relying on specific interactions between the POI and E3 ligase. Second, PROTAC design can further enhance affinity and selectivity for specific targets by optimizing the linker and the choice of E3 ligands, thereby reducing non-specific interactions with other proteins. Finally, PROTACs function in different cellular environments,

ensuring more precise degradation of the POI, especially in distinguishing between members of the same protein family. This function enables PROTACs to selectively degrade specific members of a protein family and avoids the non-selective inhibition commonly observed with traditional small-molecule inhibitors [16].

2.2 Concept of selectivity and implementation in PROTACs

Selectivity in targeted therapy typically refers to the ability of a drug to precisely identify and act upon the target protein without affecting non-target proteins. PROTACs selectivity means that PROTACs can induce degradation of the POI with high precision by interacting specifically with the E3 ligase and target protein. E3 ligase selectivity is critical for the targeting capacity of PROTACs. Different E3 ligases exhibit varying substrate specificities, meaning that the appropriate selection of an E3 ligase can significantly enhance the selectivity of PROTACs. E3 ligases not only determine target protein degradation but also influence PROTAC affinity and selectivity through the interaction. For example, selecting a specific E3 ligase can help prevent the non-specific degradation of off-target proteins, ensuring that the degradation process is more precise [17].

Strategies to enhance PROTAC selectivity include optimizing the linker design [18–22], modulating the E3 ligase selectivity, and introducing covalent modifications [23]. Modifying the linker structure can improve PROTAC affinity for the target protein, thereby enhancing selectivity. Additionally, tuning the interaction between the E3 ligase and the POI or enhancing the stability of the ternary complex through linker modifications [24] can further increase selectivity. The introduction of covalent modifications, especially reversible covalent bonds, can further strengthen the binding between PROTACs and the target proteins and improve targeting efficiency [25–29].

2.3 Covalent PROTAC potential

Covalent PROTACs hold significant potential for enhancing selectivity and cellular uptake. Unlike traditional reversible PROTACs, covalent PROTACs form a stable covalent bond with the target protein, which may result in more durable and effective targeting in therapeutic applications. The introduction of covalent modifications not only increases PROTAC selectivity for the targets but also improves cellular uptake, thereby enhancing degradation efficiency. In contrast, reversible covalent PROTACs offer the advantage of maintaining selectivity while minimizing potential side effects, which provides a more controllable approach to targeted protein degradation [30].

Targeted therapy has become a crucial strategy in drug development for treating cancer and other diseases. Different types of targets have unique roles within cells and influence biological processes, such as

gene transcription, cell cycle regulation, signaling pathways, and immune responses, all of which affect tumor initiation, progression, and resistance to treatment. Therefore, understanding and precisely targeting these key molecules is fundamental to developing effective and specific therapeutic drugs. This review will categorize and describe these targets based on functions and biological characteristics and cover important areas, such as epigenetic regulation, cell cycle and signaling pathways, receptor kinases, tyrosine phosphatases, and immune regulation. Through in-depth research into these targets, the molecular mechanisms underlying diseases can be elucidated and theoretical foundations and practical guidance can be provided for developing the next generation of anti-cancer drugs.

3. DISCUSSION OF VARIOUS TARGET TYPES

3.1 Epigenetic regulation targets

3.1.1 Bromo- and extra-terminal (BET) proteins. BET proteins harbor the bromodomain (BRD) domain. The four BRD family members (BRD2, BRD3, BRD4, and testis-specific BRDT) [31, 32] are epigenetic “readers” and key regulators of gene transcription [33, 34]. Moreover, BET proteins are regarded as potential therapeutic targets for the treatment of human cancers and other diseases.

The discovery of compound 1 [(+)-JQ-1] as the first potent and pan-BET inhibitor promoted the exploration of BET proteins as new therapeutic targets [35] (Figure 3). Several pan-BET inhibitors, such as compounds 2 (OTX015) [36–38] and 3 (I-BET762) [39], have advanced into clinical development [40, 41]. Compound 4 (QCA276) is used to treat conditions or disorders, such as cancer, which respond to BET BRD inhibition. The inhibitory effect of the compound on BRD2, BRD3, and BRD4 expression was assessed in the MV4:11 leukemia cell line with high expression of these proteins [42]. Compound 5 (WNY0824) is a dual BET and PLK1 (polo-like kinase 1) inhibitor with nanomolar and equipotent inhibition of BRD4 and PLK1 [43] (Figure 4A). However, adequate BET subtype selectivity is challenging to achieve due to the high homology among BET proteins. Based on the PROTACs concept, several degraders targeting BET proteins, including some selective BRD4 degraders, have been developed.

Ciulli and co-workers designed PROTACs that tether compound 1 to a ligand for the E3 ubiquitin ligase in 2015, resulting in the optimized compound 6 (MZ1), which was the first PROTAC reported to selectively degrade BRD4 and was evaluated in various human acute myeloid leukemia (AML) cell lines (NB4, K562, Kasumi-1, and MV4:11) as well as the mouse leukemia cell line, P388-D1. Compound 6 (MZ1) demonstrated potent and rapid degradation of BRD4 with minimal impact on BRD2 and BRD3 [44]. This finding was due to the more efficient formation of the VHL: PROTAC: BRD4 ternary complex (Figure 3). PROTAC

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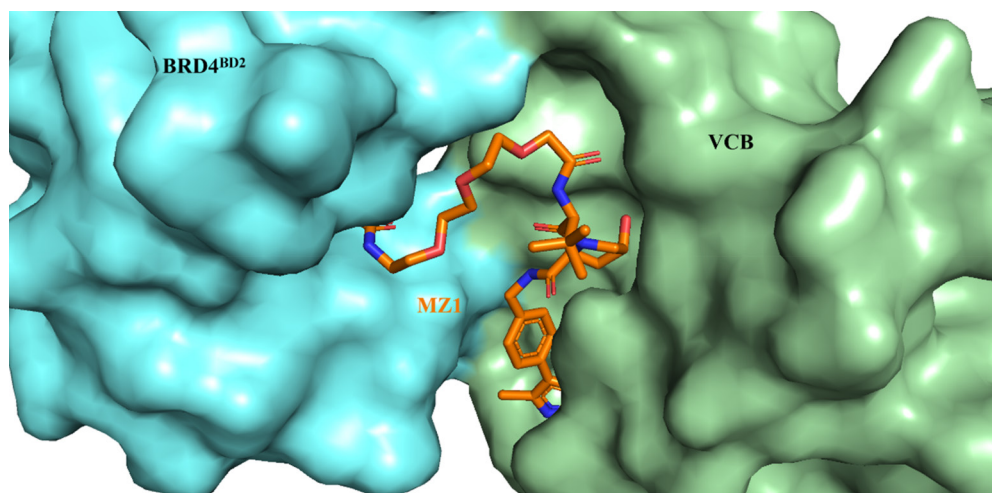


Figure 3 | The crystal structure of the ternary complex, BRD4^{BD2}: MZ1: VHL-elongin C-elongin B complex (PDB:5T35).

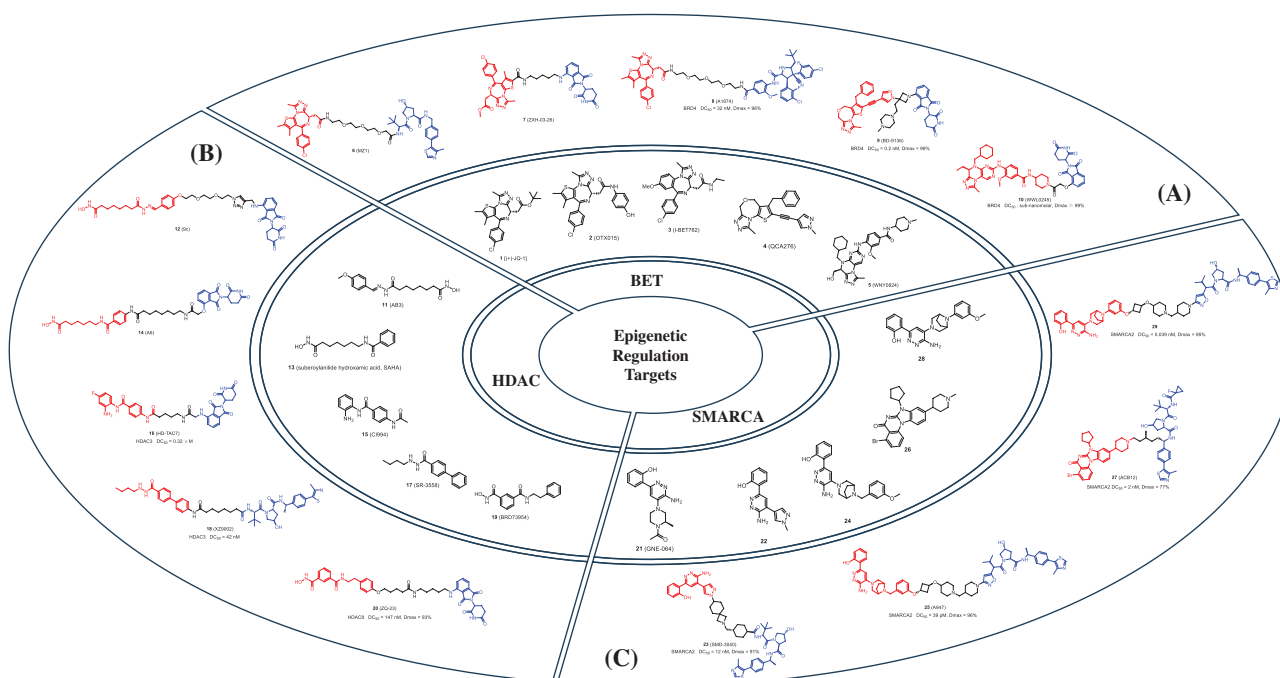


Figure 4 | (A) Selective BRD4 degraders based on pan-BET inhibitors and its analogues and a dual BET and PLK1 inhibitor. (B) Selective HDAC3/6/8 degraders based on pan-HDAC inhibitors. (C) Selective SMARCA2 degraders based on SMARCA2/4 ligands.

structure was analyzed in 2017, which revealed how PROTACs bind to the E3 ligase and target protein for the first time. The study revealed that MZ1 is positioned between the two proteins, facilitating the formation of novel protein-protein and protein-ligand interactions. These induced protein-protein interactions (PPIs) have a crucial role in determining the stability and abundance of the ternary complex. The PROTAC designed based on this mechanism exhibited remarkable selectivity toward BRD4.

Notably, this investigation into the ternary complex formation offered unique insights that had been infrequently explored in prior research [45].

Compound 7 (ZXH-03-26; Figure 4A) is a PROTAC molecule disclosed in a 2019 patent that was designed by combining a cereblon ligand with a BRD4-targeting ligand. Compound 7 was identified as the most potent degrader through systematic optimization of the linker. This PROTAC demonstrated a sub-nanomolar

half-maximal degrading concentration ($DC_{50/5h}$) of 5 nM and selectively degraded BRD4 without affecting BRD2/3. Notably, while compound **7** (10 μ M) was effective against the first bromine domain of BRD4, compound **7** (10 μ M) showed no degradation activity on BRD2/3. Western blot analysis revealed that **7** exhibited comparable efficacy to the pan-BET degrader, dBET6, in degrading endogenous BRD4 but was more selective in its action [46]. Another significant contribution to the development of BRD4-targeting PROTACs was reported by Crews and co-workers in 2019 with the identification of compound **8** (A1874; **Figure 4A**), a MDM2-based PROTAC. Compared to the PROTAC molecule, A743, which alters the E3 ligase MDM2 to VHL, A1874 showed superior efficacy in reducing cell viability in p53 wild-type cell lines. This finding also demonstrated that PROTAC activity can be enhanced by altering the E3 ligase. Unlike the earlier example, compound **8** led to robust and sustained BRD4 degradation through the ubiquitin-proteasome system, effectively inhibiting BRD4-dependent cancers. This finding demonstrated a new strategy for achieving selective protein degradation using PROTAC technology [47]. Wang and co-workers introduced compound **9** (BD-9136; **Figure 4A**), a highly potent and selective degrader targeting BRD4. At a low concentration of 1 nM, compound **9** induced rapid and selective degradation of BRD4 in 2023, exhibiting over 1000-fold greater selectivity compared to BRD2 and BRD3. Additionally, compound **9** was demonstrated to effectively suppress tumor growth *in vivo* with minimal toxicity. Compound **9** had a DC_{50} value of 0.2 nM for BRD4 in T47D cells, surpassing the potency of the pan-BET inhibitor, compound **4**. These findings underscore the promising potential of BRD4-specific PROTACs in cancer treatment [48]. The 2021 Wang study involving dual BET/PLK1 inhibitor-based PROTACs led to the identification of a BRD4-selective degrader, compound **10** (WWL0245; **Figure 4A**). This compound showed excellent selectivity for BRD4 with negligible degradation of other BET family members at concentrations as high as 10 μ M, further reinforcing the promise of isoform-selective BRD4 degraders in therapeutic applications [49].

In summary, although BET proteins are essential mediators in the control of gene transcription, the high homology across family members has posed a challenge for selective inhibition in cancer treatment. The advent of PROTAC technology has provided a solution to this issue, enabling the development of selective BRD4 degraders which thereby enhance therapeutic targeting and tumor inhibition. By optimizing the linker and selecting different E3 ligase ligands, the selectivity of PROTACs can be further improved. Optimization of PROTAC selectivity is often achieved by adjusting the length, rigidity, or conformation of the linker, or by improving the binding characteristics of the E3 ligase with the target protein. These modifications can enhance PROTAC affinity and selectivity for specific targets, reducing off-target effects. The PROTAC molecules described in this paper have been optimized through

these two strategies to improve selectivity. As PROTAC technology continues to develop, the precise targeting of BET proteins is expected to offer more effective and selective therapeutic strategies for cancer and other diseases.

3.1.2 Histone deacetylases. Histone deacetylases (HDACs) are considered promising targets in cancer drug development. Mammalian genomes contain 11 proteins with a conserved deacetylase domain. HDACs are classified into four groups (I, IIa, IIb, and IV) and exhibit diversity in structure, enzymatic roles, intracellular localization, and expression dynamics. The class I HDAC family includes HDAC1, 2, 3, and 8, which are homologous to Rpd3, a key member from budding yeast [50, 51]. HDAC4, 5, 7, and 9 are members of the class IIa family of HDACs. HDAC6 and 10 comprise the class IIb family, while HDAC11 is the sole member of class IV. HDAC6 has a significant role as a major cytoplasmic deacetylase. HDAC6 functions as an α -tubulin deacetylase [52] and HSP90 deacetylase [53], regulating cell motility and adhesion and serving as a chaperone. HDAC6 carries out cellular roles independent of its deacetylase function. The acetylase, via its zinc finger domain, associates with ubiquitin and controls aggresome function, autophagy, heat shock factor-1 (HSF-1), and platelet-derived growth factor (PDGF) [54, 55]. Overexpression of HDAC6 has been associated with inflammation and various cancers [56-64].

Tang designed the first-in-class small molecule degrader, compound **12** (9c), in 2018 (**Figure 4B**) by conjugating compound **11** (AB3; **Figure 4B**) with thalidomide analogs. This degrader selectively degrades HDAC6, with minimal impact on other HDAC isoforms [65, 66]. Compound **13** (suberoylanilide hydroxamic acid [SAHA]; **Figure 4B**), an FDA-approved HDAC inhibitor for the treatment of advanced primary cutaneous T-cell lymphoma, demonstrated potent inhibition of HDACs 1, 2, 3, and 6 [67, 68]. In 2023 Weindl and co-workers developed an HDAC6-targeting PROTAC, compound **14** (A6; **Figure 4B**), based on the pan-HDAC inhibitor, compound **13**, and the cereblon E3 ligase ligand, thalidomide, which exhibits excellent selectivity for degrading HDAC6 [69]. In 2020 Dekker and co-workers reported the development of compound **16** (HD-TAC7; **Figure 4B**), a PROTAC that conjugates the class I HDAC inhibitor, compound **15** (CI994; **Figure 4B**), with the cereblon E3 ligase ligand, pomalidomide [70, 71], with the cereblon E3 ligase ligand, pomalidomide [72] (**Figure 4B**). This degrader demonstrated selective degradation of HDAC3 [72]. At the same time, Liao and co-workers developed a series of novel PROTACs based on class I HDAC inhibitor compound **17** (SR-3558; **Figure 4B**), which specifically degraded HDAC3 compared to other HDAC isoforms [73]. Compound **18** (XZ9002; **Figure 4B**) was the most potent PROTAC. HDAC3 is degraded in MDA-MB-468 cells in a dose-dependent fashion, reaching a DC_{50} value of 42 nM after 14 h. However, the levels of HDAC 1, 2, and 6 remain unchanged under the same treatment conditions [74]. In 2022 Chen and co-workers

Review Article

[76] synthesized HDAC8 degraders by attaching the dual inhibitor (Figure 4B) [75] to the cereblon E3 ligase ligand, pomalidomide (Figure 4B) [76]. Among these degraders, compound 20 (ZQ-23; Figure 4B) demonstrated selective degradation of HDAC8, achieving a DC_{50} of 147 nM and a D_{max} of 93% with no impact on HDAC1 or HDAC3 [76].

In summary, HDAC degraders based on PROTAC technology have demonstrated superior selectivity and efficacy compared to traditional inhibitors. As PROTAC technology continues to develop, it is expected that more selective degraders targeting the HDAC family will enter clinical use, providing more precise treatment options for cancer and other diseases.

3.1.3 BRD proteins. BRDs, which recognize acetyllysine, are found in multiple human proteins. Sixty-one human BRDs have been categorized into families based on sequence and structure. Family VIII consists of the single BRDs found in SMARCA2 and SMARCA4, which are catalytic subunits of cBAF, ncBAF, and PBAF (human SWI-SNF) chromatin remodeling complexes [77], along with the six BRDs contained in the hallmark PBAF subunit, PBRM1. Hence, designing BRD-targeting chemical probes [78] or SMARCA2 and SMARCA4 ATPase activity [79] has gained significant attention in elucidating the biological roles of these domains and the potential for therapeutic intervention in mutants. Achieving *in vivo* effectiveness with minimal toxicity in SMARCA4-deficient cancers requires selective SMARCA2 inhibition. Nevertheless, the close sequence homology of the ATPase domains in SMARCA2 and SMARCA4 has made it difficult to develop SMARCA2-specific inhibitors [80].

An optimized analogue, compound 21 (GNE-064; Figure 4C), serves as a chemical probe targeting the SMARCA2 and SMARCA4 BRDs and shows promise for *in vivo* application [81]. Experimental data demonstrated that targeting the BRM bromodomain alone with a high-affinity inhibitor fails to inhibit cell growth. SMARCA2 and SMARCA4 feature BRDs interacting with acetylated histone lysines and ATPase domains essential for SWI/SNF-mediated chromatin remodeling [82]. In 2023 Wang and co-workers created a series of highly effective and selective SMARCA2 degraders derived from the SMARCA BRD inhibitor compound 22, with compound 23 (SMD-3040; Figure 4C) serving as a key example. This PROTAC has a low nanomolar DC_{50} and a $D_{max} > 90\%$, highlighting its remarkable selectivity in degrading SMARCA2 over SMARCA4 [82]. Similarly, Yauch and co-workers synthesized the selective SMARCA2 PROTAC, compound 25 (A947; Figure 4C) in 2022, based on a SMARCA2/4 ligand (compound 24). Targeting SMARCA2 specifically and bypassing the need for SMARCA2/4 PROTAC engagement, the degrader achieves potent *in vitro* antiproliferative effects and superior *in vivo* activity in SMARCA4-mutant systems compared to wild-type counterparts [83]. In addition, Farnaby discovered compound 27 (ACBI2; Figure 4C), a PROTAC based on a SMARCA BRD binder, compound

26, which also demonstrated selective SMARCA2 degradation over SMARCA4 in *ex vivo* human whole blood assays and showed efficacy in SMARCA4-deficient cancer models [84]. More recently, Dragovic reported a series of VHL-binding PROTACs in 2024 based on potent BRM BRD ligands, including compound 28 (Figure 4C), which effectively degrades BRM (SMARCA2) in SW1573 cell-based experiments. These PROTACs exhibited 10- to 100-fold selectivity for BRM over the closely related paralog, SMARCA4, with representative PROTAC compound 29 (Figure 4C) showing significant degradation activity [85].

The development of these selective SMARCA2 degraders, such as compounds 23, 25, and 27, marks a significant advance in targeting the SWI/SNF complex. These compounds demonstrate potent *in vitro* and *in vivo* efficacy, especially in cancer models with SMARCA4 mutations. With the continued progress in PROTAC technology, it is anticipated that more precise and effective treatments will emerge for cancers driven by mutations in the SWI/SNF complex.

3.2 Cell cycle and signaling pathway targets

3.2.1 BCL-XL and BCL-2. BCL-XL and BCL-2 are part of the anti-apoptotic BCL protein family and are essential in supporting tumor initiation, progression, and development of drug resistance by preventing apoptosis in tumor cells [86-89].

Compound 30 (ABT-263) is an orally bioavailable inhibitor of the BCL-2 family, with a K_i (inhibition constant) < 1 nmol/L for BCL-2, BCL-XL, and BCL-W (Figure 5A). Compound 30 (ABT-263) exhibits strong binding affinity for BCL-XL and BCL-2 but a weaker affinity for other BCL-2 family members [90]. Conventional small molecule inhibitors, such as compound 30, target BCL-2 and BCL-XL with high affinity but bind weakly to other BCL-2 family members, potentially leading to significant toxicity. To overcome this effect, in 2019 Zhou and co-workers converted compound 30 into BCL-XL PROTAC (compound 31 [DT2216]; Figure 5A), which targets BCL-XL for degradation by the VHL E3 ligase. This modification greatly decreased toxicity, especially in platelets because VHL is minimally expressed in platelets. Unlike traditional small molecule inhibitors, PROTACs selectively degrade target proteins, like BCL-XL, without affecting BCL-2 levels, preventing non-specific inhibition of other BCL family members and reducing side effects. Moreover, compound 31 has enhanced efficacy against BCL-XL-dependent leukemia and cancer cells *in vitro* and *in vivo*, without inducing significant thrombocytopenia. As next-generation protein degraders, PROTACs offer promising potential for cancer therapy due to superior selectivity and reduced toxicity [91].

3.2.2 Cyclin-dependent kinase (CDK) family (CDK2, CDK4, CDK6, and CDK9). The functional diversity among CDK family members makes CDK family members promising targets for designing potent therapeutic inhibitors [92-95]. CDK2, CDK4, and CDK6 directly regulate the cell

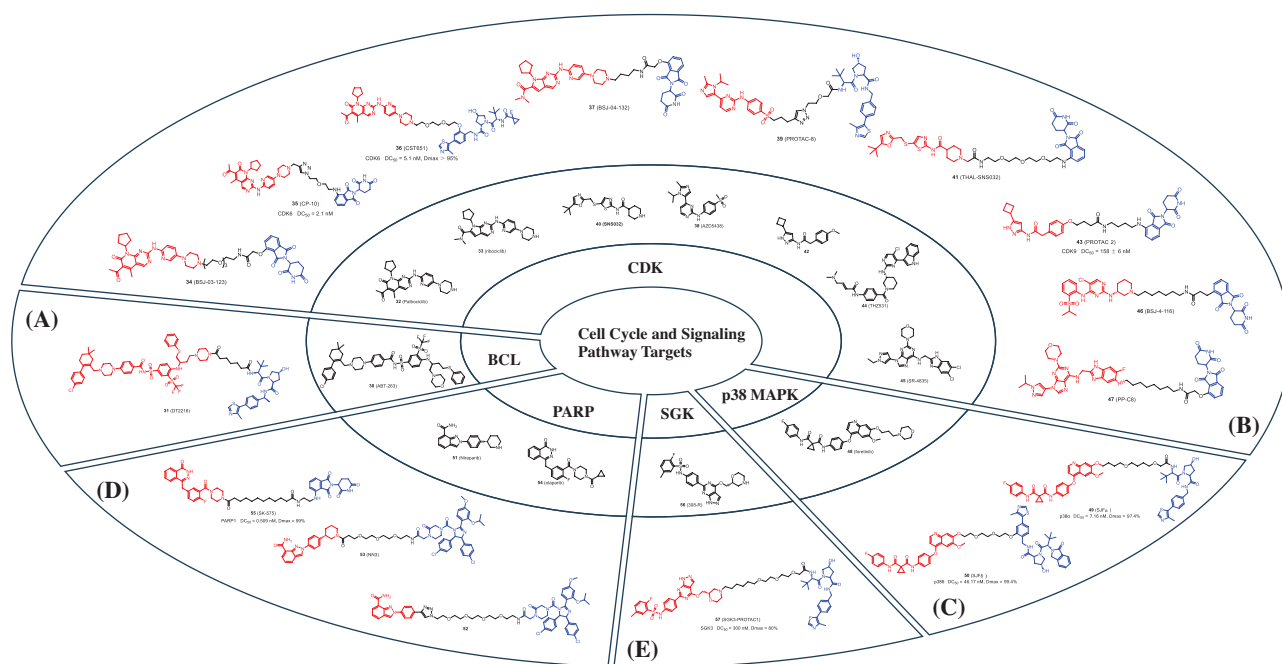


Figure 5 | (A) Selective BCL-XL degraders based on BCL-2/BCL-XL dual inhibitor. (B) Selective CDK2/4/6/9/12 degraders based on pan-CDK inhibitors. (C) Selective p38 α and p38 δ degraders based on p38 inhibitor. (D) Selective PARP1 degraders based on PARP1/2 inhibitors. (E) Selective SGK3 degraders based on pan-SGK inhibitor.

cycle [96, 97], whereas CDK9 primarily modulates transcription regulation [98–100]. Off-target interactions with other CDK family members lead to toxicity, posing a major challenge for the clinical development of these inhibitors [101–103]. The PROTACs approach enhances efficacy and reduces toxicity by selectively targeting specific CDKs, offering a more refined alternative to traditional small molecule inhibitors.

Dual CDK4/6 inhibitors, such as compounds **32** (palbociclib) [104] and **33** (ribociclib; **Figure 5B**) [105] have been FDA- and EMA-approved for combination therapy in advanced or metastatic breast cancer [106–109]. However, due to the high homology between CDK4 and CDK6 [110–113], existing inhibitors struggle to selectively target one of them, leading to the development of more selective compounds [114]. PROTACs have addressed this selectivity issue. Winter and co-workers introduced compound **34** (BSJ-03-123; **Figure 5B**) [115] in 2018, a phthalimide-based degrader that selectively targets and degrades CDK6 through protein-interface determinants. This degrader enables more precise targeting of genetic dependencies in acute myeloid leukemia (AML) cells, potentially minimizing toxicity and broadening therapeutic possibilities. Rao developed compound **35** (CP-10; **Figure 5B**) in 2019, a PROTAC that links palbociclib to pomalidomide. This compound effectively degrades CDK6 and CDK4 in breast cancer cell lines resistant to palbociclib and demonstrated activity against CDK6 mutant forms as well [116]. This finding demonstrates

the potential for treating various CDK6-related cancers. The 2020 Krönke study identified compound **36** (CST651; **Figure 5B**), a CDK6-selective degrader, which inhibits kinase-dependent and -independent functions of CDK6. This finding highlights the potential of PROTACs to uncover CDK6-specific roles and aid in cancer therapy development [117]. Gray and co-workers also developed selective PROTACs in 2019, like compound **37** (BSJ-04-132; **Figure 5B**), a ribociclib-based CDK4 degrader. The linker design determines the selectivity of these PROTACs, allowing for precise modulation of kinase activity and offering a solution to challenges, such as resistance and off-target effects [118].

Compound **38** (AZD5438; **Figure 5B**) is a potent pan-CDK inhibitor targeting CDK1, 2, and 9, with IC₅₀ values of 16, 6, and 20 nmol/L, respectively. Compound **38** (AZD5438) has been shown to markedly suppress tumor growth in human xenograft models and decrease the number of proliferating cells. Compound **38** (AZD5438) also inhibits key CDK substrates, such as pRb and nucleolin, in tumor cells, demonstrating potential as a broad-spectrum CDK-targeting agent for cancer therapy. Nevertheless, the clinical progression was halted due to poor tolerability and significant off-target effects identified in phase II trials [119–121]. PROTACs offer a solution by enhancing selectivity to overcome the limitations of pan-CDK inhibitors, like compound **38**. Zuo developed AZD5438-PROTAC compound **39** (PROTAC-8; **Figure 5B**) in 2021, which selectively induces partial CDK2

Review Article

degradation with reduced toxicity compared to the parent compound, demonstrating the potential of PROTACs to improve efficacy and selectivity in cancer treatment [122]. Subsequent advances in CDK inhibition led to the development of compound **40** (SNS032; **Figure 5B**), a non-covalent broad-spectrum CDK inhibitor targeting CDK2, CDK7, and CDK9. To further improve inhibitory efficacy, Marschall and co-workers designed compound **41** (THAL-SNS032; **Figure 5B**) in 2021, a CDK9-specific PROTAC based on SNS032. This new degrader demonstrated a four-fold increase in potency over SNS032 with an EC_{50} of 0.025 μ M. Additionally, compound **41** showed significant antiviral activity against both human and murine CMVs and effectively suppressed SARS-CoV-2 replication [123]. Natarajan and co-workers introduced compound **43** (PROTAC 2; **Figure 5B**) in 2021, a selective CDK9 degrader derived from the amino-pyrazole, compound **42**. With a DC_{50} near 150 nM, the compound specifically targets CDK9 for degradation, while leaving other CDKs largely unaffected. Compound **43** selectivity stems from variations in the topologic distribution of surface lysine residues within the CDK family, highlighting the potential as a CDK9-specific therapeutic in oncology [124].

The currently existing CDK12 inhibitors strongly inhibit the closest homolog, CDK13, which may lead to potential toxicity [125–128]. Therefore, developing CDK12 inhibitors with homolog-selectivity that effectively bypass CDK13 remains a challenge [129, 130]. To date, only a limited number of small-molecule inhibitors, such as the irreversible covalent inhibitor, compound **44** (THZ531; **Figure 5B**) [129], and the non-covalent inhibitor, compound **45** (SR-4835; **Figure 5B**) [131], have been identified to target CDK12. The high sequence homology between the kinase domains of CDK12 and CDK13 means that most inhibitors also strongly affect CDK13. Moreover, dual CDK12/13 inhibitors have encountered clinical setbacks because of severe toxicity. As a result, designing CDK12-selective inhibitors that avoid CDK13 inhibition has become essential for advancing cancer treatment. Gray and co-workers identified the first isoform-selective CDK12 degrader, compound **46** (BSJ-4-116; **Figure 5B**), based on compound **44** in 2021. This degrader effectively and durably degrades CDK12 without affecting the levels of CDK13 and cyclin K (CycK) proteins [132]. Zhu and co-workers reported a PROTAC degrader, compound **47** (PP-C8; **Figure 5B**), targeting the CDK12-CycK complex based on compound **45**. Inducing swift and selective degradation of CDK12-CycK in Bel-7402 hepatoma and MDA-MB-231 triple-negative breast cancer cells, compound **47** (PP-C8) did not cause degradation of CDK13 [133].

Transforming small molecule inhibitors into PROTACs offers significant advantages, especially in achieving selective degradation of CDK family members. While traditional small molecule inhibitors block the activity of target proteins through competitive binding, traditional small molecule inhibitors may also target unintended

proteins, leading to toxicity and adverse effects. In contrast, PROTACs, with a unique bifunctional structure, comprising a target-binding molecule and a degradation-inducing element, precisely direct the degradation of specific proteins, offering greater selectivity and specificity. Additionally, PROTACs exploit the proteasomal degradation pathway, which helps avoid the resistance issues often seen with traditional inhibitors. Overall, PROTACs represent a novel drug development strategy that provides more precise targeted therapies, enhancing both efficacy and safety, and showing immense potential, particularly in treating complex diseases, such as cancer and viral infections, in which multi-target engagement is often required.

3.2.3 p38 MAPK family (p38 α , p38 β , p38 γ , and p38 δ). The p38 MAPK family, consisting of four isoforms (p38 α , p38 β , p38 γ , and p38 δ), is dominated by p38 α , the most abundant isoform in many cell types. Dysregulated p38 activity is linked to several diseases, including cancer and inflammatory disorders. However, the clinical success of p38 inhibitors has been challenged by restricted efficacy and safety concerns [134, 135].

Crews and co-workers developed p38 α and p38 δ selective PROTACs [134] based on compound **48** (foretinib) [136] and VHL ligands in 2019. Compound **49** (SJF α ; **Figure 5C**) was effective in degrading p38 α at low nanomolar concentrations but had poor degradation effects on p38 β , p38 γ , and p38 δ . In contrast, compound **50** (SJF δ ; **Figure 5C**) effectively degraded p38 δ but did not effectively degrade p38 α , p38 β , and p38 γ . The ternary complex pull-down assay indicated that this selectivity was related to PROTAC-induced ternary complex formation [134]. These findings highlight the potential of PROTACs to provide more targeted and effective therapies for p38-related diseases.

3.2.4 PARP [poly(ADP-ribose) polymerase]. PARP is a key protein involved in DNA damage repair and genome stability maintenance [137]. The PARP enzyme family includes 17 different protein members, which can exist in various subtypes, each possessing conserved domains but performing unique biological roles [138]. PARP1 is the most well-studied and predominant member of the PARP enzyme family. PARP1 functions as a DNA-binding protein, becoming activated in response to DNA damage caused by factors, such as inflammation, ischemia, neurodegenerative diseases, and cancer treatments [139–142].

Compound **51** (niraparib; **Figure 5D**), a dual PARP1/2 inhibitor (PARP-1: IC_{50} = 3.76 nM and PARP-2: IC_{50} = 2.15 nM), is the first PARP inhibitor approved for use in ovarian cancer patients without germ-line or somatic mutations in the *BRCA* gene [143–145]. While compound **51** demonstrates potent anti-tumor activity, compound **51** is associated with hematologic, gastrointestinal, and cardiovascular toxicities and is prone to resistance. To address these issues, PROTACs have been proposed to enhance

selectivity for PARP1/2, overcome resistance, and mitigate toxic effects [45, 146]. Rao and co-workers identified a series of degraders, such as compound **52** (Figure 5D) in 2018, which targeted PARP1 by connecting the dual PARP1/2 inhibitor, compound **51**, to E3 ligands through various linkers. These PROTACs effectively induce PARP1 cleavage and programmed cell death in MDA-MB-231 cells [147]. Wu and co-workers reported a PARP1-selective PROTAC, compound **53** (NN3; Figure 5D) in 2022, which showed superior anti-tumor proliferation effects and therapeutic tolerance compared to compound **51** in xenograft tumor models [148]. Olaparib (compound **54**; Figure 5D), a well-known oral PARP1/2 inhibitor, exerts its action not only by inhibiting PARP1 enzymatic activity but also through PARP1 trapping, which enhances tumor cell killing. However, this effect also induces toxicity in normal cells. The contribution of these two pathways to cytotoxicity is still unclear. Furthermore, olaparib faces challenges, such as resistance and a limited therapeutic scope. To overcome these limitations, Chen and co-workers designed selective PARP1 degraders in 2020 based on compound **54**. The resulting compound (compound **55** [SK-575]; Figure 5D), induced potent and selective degradation of PARP1 in various human PARP1-positive cancer cells, achieving a $D_{\max} > 95\%$, without affecting PARP2 [149].

These PROTACs, including compounds **52**, **53**, and **55**, have demonstrated improved specificity, reduced toxicity, and efficacy against resistant mutations, positioning PROTACs as a promising strategy to overcome the shortcomings of traditional PARP inhibitors and improve cancer therapy outcomes.

3.2.5 Serum/glucocorticoid-inducible protein kinase (SGK) family. SGK, a major downstream target of PI3K signaling, regulates cell proliferation, survival, invasion, and metastasis. SGK3, in particular, has a key role in cell growth and survival, especially in cancers, such as breast, liver, colorectal, and prostate. Recent studies have shown that inhibiting SGK3 overexpression significantly reduces breast cancer cell proliferation. However, existing SGK3 inhibitors are limited by poor IC_{50} values and low selectivity. Therefore, developing and optimizing a highly selective SGK3 targeting PROTAC is of great importance for advancing effective therapeutic strategies [150, 151].

Alessi and co-workers first designed compound **57** (SGK3-PROTAC1; Figure 5E) in 2019 by conjugating the pan-SGK inhibitor, compound **56** (308-R), with the VH032 VHL binding ligand to target SGK3 for degradation. Compound **57** induced 50% degradation of endogenous SGK3 at 300 nM within 2 h and maximal 80% degradation within 8 h of treatment in HEK293 cells. In contrast to the inhibitor (compound **56** [308-R]), compound **57** had good selectivity and did not degrade the closely related SGK1 and SGK2 isoforms. Compound **56** also suppressed the proliferation of ZR-75-1 and CAMA-1 cancer cell lines, outperforming a PI3K inhibitor (GDC0941) [152].

In conclusion, while SGK3 remains a critical target in cancer therapy, current inhibitors require optimization. PROTAC-based approaches offer a more precise and effective strategy for targeting SGK3 and future research should focus on enhancing potency, selectivity, and clinical applicability.

3.3 Receptor and kinase targets

3.3.1 BRAF. BRAF is a crucial regulator in the MAPK signaling pathway and is involved in controlling cell proliferation and survival. Mutations in BRAF can lead to the continuous activation of downstream kinases, resulting in uncontrolled cell growth and promoting cancer development [153-159]. The use of selective inhibitors to target BRAF represents one of the earliest achievements in precision cancer therapy. To date, multiple small-molecule inhibitors of BRAF kinase have received approval for treating melanomas with *BRAF* V600E mutations [160-162]. However, the benefits of BRAF kinase inhibitors are short-lived. Second-generation BRAF inhibitors also induce BRAF to form dimers with itself or with other RAF family proteins [163-165]. This unique mechanism might compromise the clinical efficacy of these drugs, promote acquired resistance [166], and induce adverse effects, such as secondary skin cancer [167, 168]. Although compound **58** (Figure 6A [vemurafenib]) and other approved BRAF kinase inhibitors have shown significant therapeutic responses in melanoma patients harboring *BRAF* V600E mutations [169-172]; the swift development of acquired resistance severely limits long-term effectiveness. Therefore, there is an urgent need for more potent, selective, and sustained strategies to target oncogenic *BRAF* mutations.

In 2023 Ayrapetov and co-workers designed a PROTAC, compound **60** (SJF-0628; Figure 6A), to degrade mutant BRAF across different cancer cell models and study degradation responses based on compound **58**. Treatment with compound **60** degrades *BRAF* V600E and reduces MEK activation. However, the effects of treatment on cell signaling and proliferation were cell-line-specific. BRAF inhibition by small molecule inhibitors, such as compounds **58** and **60**, exerted the same level of maximal inhibition of cell viability. However, compound **58** is a more potent inhibitor than compound **60** based on the IC_{50} [173]. Wang identified two degraders of *BRAF* V600E (compounds **61** [degrader 12] and **62** [degrader 23]; Figure 6A) in 2020. The IC_{50} value for compound **61** was approximately 10-fold higher than its binding ligand (compound **59** [BI882370]) and the IC_{50} values of compound **62** were similar. In *BRAF* V600E-dependent melanoma and colon cancer cells, compounds **61** and **62** triggered *in vitro* antineoplastic activities similar to the clinical stage BRAF kinase inhibitors, paving the way to target BRAF mutants for treatments [174].

In summary, although BRAF inhibitors have achieved clinical progress in treating *BRAF* V600E mutation-related cancers, like melanoma, the efficacy is limited by the rapid emergence of resistance and side effects. PROTAC

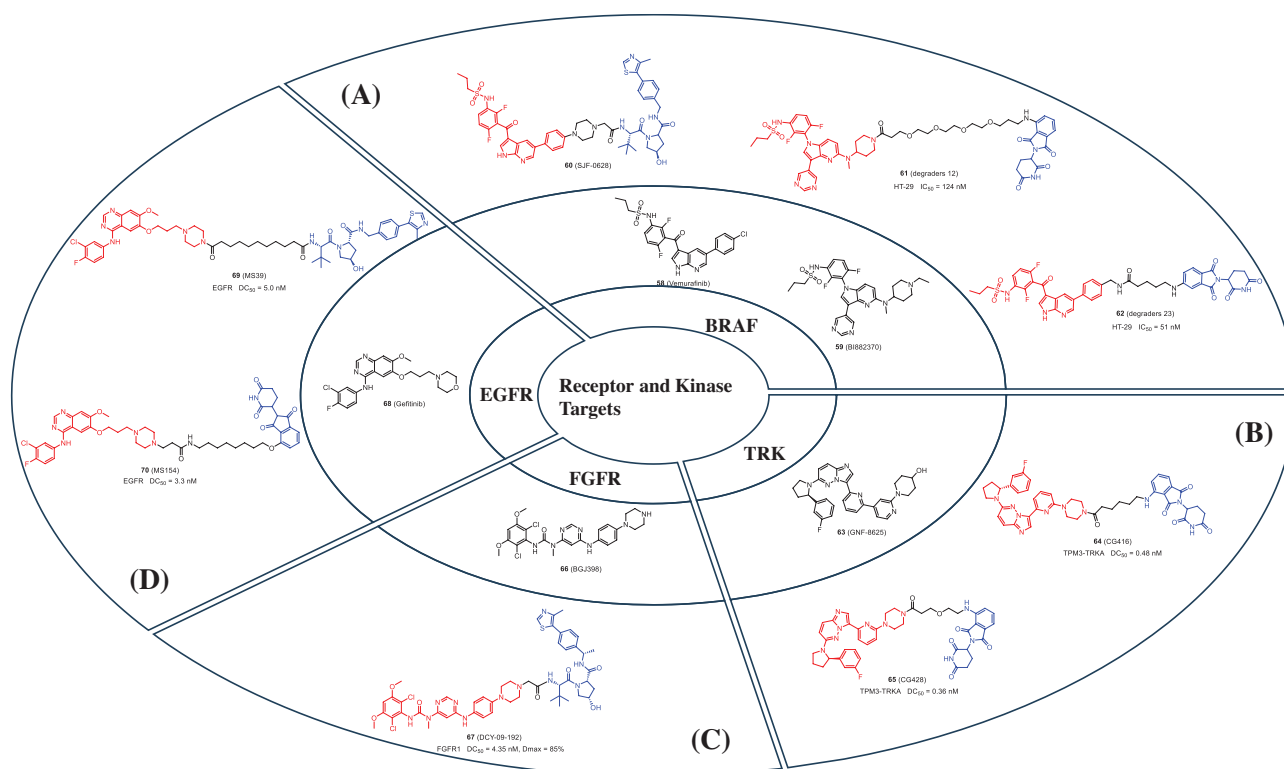


Figure 6 | (A) Selective mutant BRAF degraders based on BRAF inhibitors. (B) Selective TRKA degraders based on TRK A/B/C inhibitor. (C) Selective FGFR degrader based on pan-FGFR inhibitor. (D) Selective EGFR degraders based on pan-EGFR inhibitor.

technology offers a new approach to overcome these challenges. By specifically degrading mutant BRAF, PROTACs not only reduce kinase activity but may also help avoid the resistance issues associated with traditional inhibitors. However, current degraders have not yet surpassed traditional inhibitors in terms of potency and further optimization of the selectivity and degradation efficiency is needed. Future research should focus on enhancing the clinical efficacy of PROTACs to provide more durable and effective targeted treatments for BRAF-mutant cancers.

3.3.2 Tropomyosin receptor kinase (TRK) family (TRKA, TRKB, and TRKC). The TRK family includes three receptors (TRKA, TRKB, and TRKC) encoded by the *NTRK1*, *NTRK2*, and *NTRK3* genes, respectively [175]. Previous research has demonstrated the abnormal activation of TRK pathways in different human cancers, highlighting the need for TRK inhibitors (TKIs). Several TKIs targeting TRKA, TRKB, and/or TRKC with varying levels of effectiveness are currently available. First-generation pan-TRK inhibitors (larotrectinib and entrectinib [176]) have limitations, including CNS-related on-target side effects and the development of drug resistance due to mutations in TRK proteins. To overcome mutation-induced resistance and reduce CNS toxicity, second-generation

pan-TRK inhibitors, like selitrectinib and repotrectinib, have been engineered [177].

Liu and co-workers designed a series of TRK degraders in 2020 by tethering a pan-TRK inhibitor, compound **63** (GNF-8625; Figure 6B) [178] to a cereblon E3 ligase ligand (pomalidomide). Consequently, compounds **64** (CG416) and **65** (CG428; Figure 6B), two potent small-molecule degraders selective for TRKA over TRKB and TRKC, were identified [179].

While current TRK inhibitors have made progress in treating TRK-driven cancers, the efficacy is often limited by resistance and side effects. The TRK degraders developed by Liu [179] represent an innovative breakthrough in this field. By linking a pan-TRK inhibitor with a cereblon E3 ligase ligand, these degraders specifically degrade TRKA, avoiding the non-specific effects and resistance issues associated with traditional small molecule inhibitors. This novel approach not only offers strong targeting potential but also holds promise in overcoming the limitations of existing treatments through precise protein degradation mechanisms. Therefore, TRK degraders provide new hope for the treatment of TRK-driven cancers. Future research should focus on optimizing the degradation efficiency and selectivity to facilitate the clinical translation of this new therapeutic strategy.

3.3.3 FGFR family (FGFR1, FGFR2, FGFR3, and FGFR4).

FGFR1, FGFR2, FGFR3, and FGFR4 make up the fibroblast growth factor receptor (FGFR) family of RTKs, which share a high degree of sequence homology. Structurally, each receptor includes an extracellular portion with three Ig-like domains (a membrane-spanning segment, an intracellular section containing a split kinase domain, and a tyrosine autophosphorylation site). These RTKs are high-affinity receptors for FGFs that regulate cell proliferation, migration, apoptosis, and differentiation, and contribute to both developmental and adult tissue homeostasis [180]. The oncogenic impact of abnormal FGFR signaling has driven significant research into developing targeted therapies for this pathway. Abnormal FGFR activation is found in various cancers. Although ATP-competitive FGFR inhibitors show clinical effectiveness, ATP-competitive FGFR inhibitors cause dose-dependent toxicity. This issue may stem from the lack of selectivity among FGFR family members and poor tolerance to these inhibitors [181].

In 2021 Gray and co-workers identified compound **67** (DGY-09-192; **Figure 6C**) [182], a bivalent degrader that connects with the pan-FGFR inhibitor, compound **66** (BGJ398) [183] to a CRL2^{VHL} E3 ligase recruiting ligand. This ligand preferentially induces FGFR1/2 degradation, while largely sparing FGFR3/4. Moreover, compound **67** induces degradation of a clinically relevant FGFR2 fusion protein in a xenograft mouse model [182].

In conclusion, although FGFR inhibitors are effective in targeting aberrant FGFR signaling, the lack of selectivity and dose-dependent toxicity highlight the need for more refined therapeutic approaches. PROTAC degraders, such as compound **67**, offer a promising solution by selectively degrading FGFR1/2, while sparing FGFR3/4, potentially overcoming the limitations of traditional inhibitors. This strategy could provide a more precise, targeted, and sustainable treatment option for cancers driven by FGFR dysregulation.

3.3.4 EGFR. Epidermal growth factor receptor (EGFR), also known as HER1 or ErbB1, is a member of the ErbB receptor family and is a transmembrane protein with tyrosine kinase activity. Abnormal expression and activation of EGFR contribute to tumorigenesis, particularly in non-small cell lung cancer (NSCLC). The ErbB family consists of four transmembrane receptors in humans (EGFR, ErbB2, ErbB3, and ErbB4). EGFR is the product of the proto-oncogene, c-ErbB1. Although the FDA has approved three generations of small molecule EGFR inhibitors for NSCLC, resistance due to heterogeneous mutations, like *EGFR*C797S, remains an unresolved challenge [184, 185].

Jin and co-workers designed a series of EGFR PTOACs based on compound **68** (gefitinib) and different E3 ligands in 2020 [186]. The degraders, compounds **69** (MS39) and **70** (MS154; **Figure 6D**), which are based on VHL and CRBN E3 ligase ligands, induce selective degradation of EGFR mutants but not EGFR^{WT}, and inhibit proliferation of

lung cancer cells (**Figure 15**). Compounds **69** and **70** effectively trigger mutant EGFR degradation in EGFR-mutant cell lines (HCC827 [EGFR^{E19D}] and H3255 [EGFR^{L858R}]), with DC₅₀ values of 5.0 nM and 3.3 nM for HCC827 and 11 nM and 25 nM for H3255, respectively. In contrast, no degradation was noted in wild-type EGFR cell lines (OVCAR8 and H1299), highlighting the mutant-specific degradation selectivity of these compounds.

The development of PROTACs targeting EGFR mutants marks a significant advance in cancer therapy. While small molecule inhibitors have been effective for NSCLC, limitations in overcoming resistance emphasize the need for alternative strategies. PROTACs offer a promising solution by selectively degrading mutant EGFR proteins and addressing these challenges. This approach aligns with the growing interest in targeted protein degradation as a therapeutic modality. If validated in clinical trials, PROTACs could become a mainstream treatment for patients with EGFR-mutant NSCLC and provide more durable responses and reduced side effects compared to traditional inhibitors. However, further clinical testing is necessary to assess the long-term effectiveness and safety.

3.4 Tyrosine phosphatase targets

3.4.1 Protein tyrosine phosphatases (PTPs). PTPs catalyze the removal of phosphate groups from tyrosine-phosphorylated proteins and counteracting the action of tyrosine kinases (TKs). Despite the significance as therapeutic targets in cancer, metabolic disorders, and autoimmune diseases, PTPs have not been extensively investigated in drug discovery. Compared to TKs, the high conservation of catalytic sites poses challenges for achieving subtype selectivity [187]. PTPN2, also known as T cell protein tyrosine phosphatase (TCPTP), belongs to the PTP family. PTPN2 was identified through screening a T cell cDNA library with a similar subtype (PTPN1 [PTP1B]) [188, 189]. PTPN2 dephosphorylates and inactivates various proteins involved in hematopoiesis and T cell activation, including Src family kinases, Janus kinases, and signal transducers and activators of transcription (STATs). These actions make PTPN2 a crucial negative regulator of the T cell receptor (TCR) signaling pathway and other cytokine-driven signaling pathways [190–193]. Therefore, PTPN2 is regarded as an attractive target for cancer immunotherapy.

Wang and co-workers [195] reported a series of small molecule PTPN2 degraders to address the PTPN2 subtype-selectivity issue based on compound **71** (A650; **Figure 7B**) [194], a dual PTPN1/2 inhibitor, and a VHL E3 ligase ligand to obtain the first subtype-selective PTPN2 degrader, compound **72** (PVD-06; **Figure 7B**) [195]. Interestingly, compound **72** demonstrated remarkable selectivity for the PTP1B subtype, exhibiting a selectivity index exceeding 60-fold. In contrast, several other PTPs triggered concentration-, time-, VHL-, and proteasome-dependent degradation of PTPN2 across multiple cell lines. Additionally, compound **72** has strong selectivity at the proteome level.

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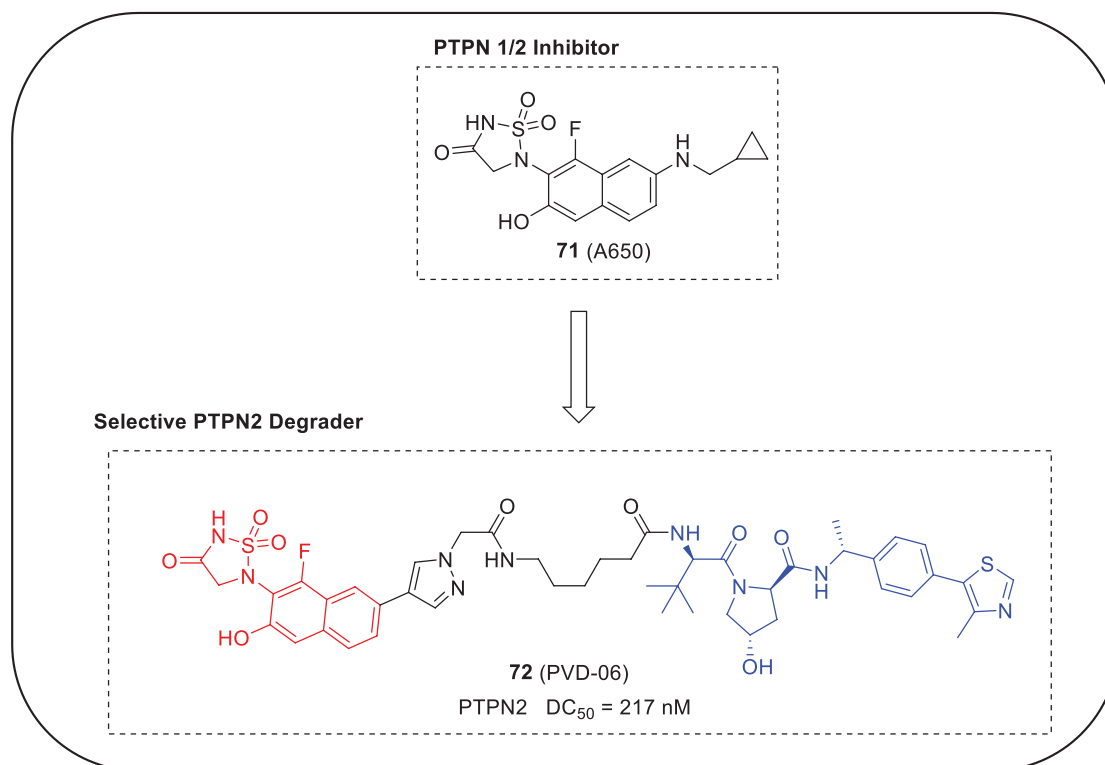


Figure 7 | Selective PTPN2 degraders based on PTPN1/2 inhibitor.

Immunologic evaluations revealed that targeted degradation of PTPN2 by compound **72** enhances T cell activation and proliferation, while also intensifying the inhibitory effects of IFN- γ on B16F10 melanoma cells. These findings highlight the promise of selectively degrading PTPN2 as a strategy for advancing cancer immunotherapy.

In summary, PTPs are key regulators in various diseases but the conserved catalytic sites hinder selective targeting. PTPN2, a negative regulator of T cell activation, is an attractive target for cancer immunotherapy. Compound **72**, the first subtype-selective PTPN2 degrader, has excellent selectivity and induces PTPN2 degradation in a proteasome-dependent manner. This selective degradation enhances T cell activation and amplifies anti-tumor effects, demonstrating the potential of PTPN2 degraders in cancer immunotherapy.

3.5 Immune regulation targets

3.5.1 CREBBP/EP300. Disordered histone acetylation has emerged as a key mechanism in promoting hematologic malignancies [196]. CREB-binding protein (CREBBP) and E1A-binding protein P300 (EP300) are key acetyltransferases and transcriptional coactivators that regulate gene expression through acetylation of histone and non-histone proteins [197]. CREBBP and its paralog, EP300, are evolutionarily conserved enzymes with similar functional roles. CREBBP and EP300 share a comparable domain structure

that includes various protein- and DNA-binding modules [198]. Dysregulation of CREBBP/EP300 and the associated complexes has a crucial role in the initiation, progression, and chemoresistance of hematologic cancers. These proteins also influence tumor immune responses by modulating the differentiation and function of various immune cells [199].

Compound **73** (A485; **Figure 8A**) is a dual EP300 and CREBBP inhibitor. Compound **73** inhibited proliferation of > 120 different cancer cell lines with high dose [200]. Qi used compound **73** in 2022 as a binding ligand and developed compound **74** (JQAD1; **Figure 8A**), a selective degrader to target EP300. Although the mechanism underlying the selective degradation of compound **74** was not investigated, selectivity was suggested to stem from the three-dimension interactions between the POI and the E3 ligase complex mediated by different PROTACs components. This finding underscores the potential of PROTACs to overcome the limitations of small molecule inhibitors, such as off-target effects and incomplete inhibition, by enabling more precise and tunable protein degradation [201].

CREBBP and EP300 are key regulators in hematologic malignancies and dysregulation contributes to tumor growth and chemoresistance. The dual inhibitor, compound **73**, shows significant anticancer activity, while compound **74** targets EP300 with greater precision.

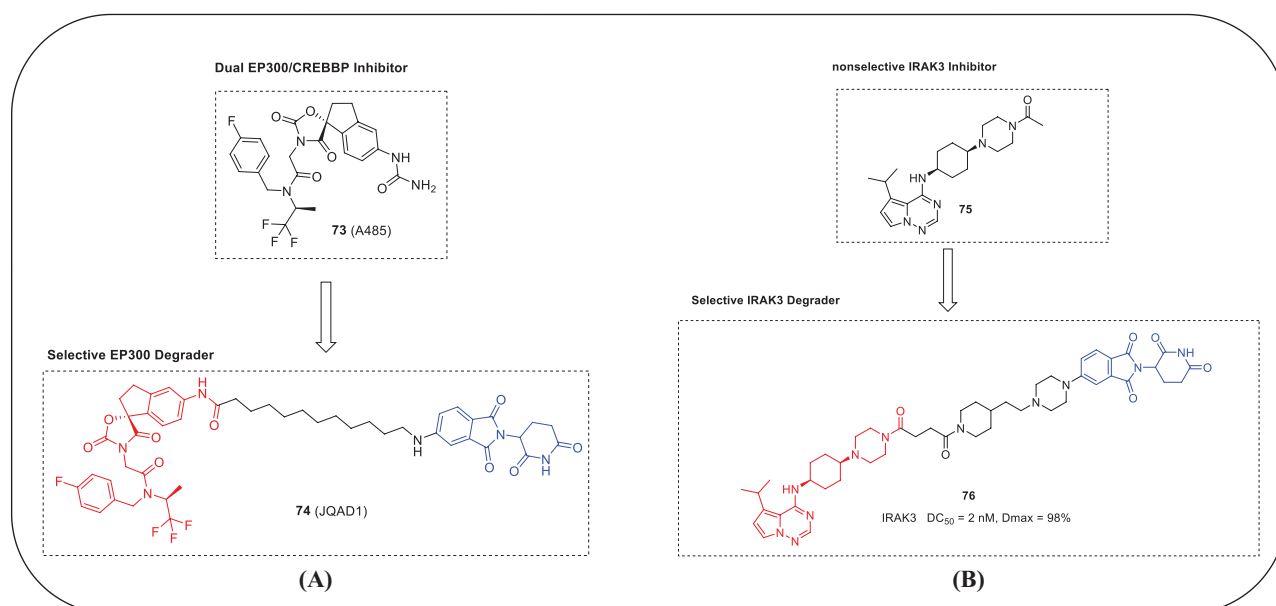


Figure 8 | (A) Selective EP300 degrader based on EP300/CREBBP inhibitor. (B) Selective IRAK3 degrader based on a non-selective IRAK3 inhibitor.

PROTACs offer a promising strategy to overcome the limitations of traditional inhibitors by enabling more targeted and tunable protein degradation. In our view, this approach holds great potential for improving cancer treatment specificity and minimizing off-target effects with further optimization needed for clinical success.

3.5.2 IRAK3 (IRAK-M). IRAK3 or IRAK-M is a class-1 pseudokinase within the IRAK family that also includes IRAK1, IRAK2, and IRAK4. Unlike other IRAKs, IRAK3 operates through a non-catalytic mechanism, likely functioning as a scaffold. IRAK3 is predominantly expressed in leukocytes, where IRAK3 suppresses proinflammatory signaling in innate immune cells, such as monocytes, macrophages, and neutrophils. Notably, IRAK3 knockout in mice reprograms myeloid cells to promote immune activation and enhances effector T-cell proliferation, thereby reversing immunosuppression and improving the response to checkpoint inhibitors [202, 203].

Edmondson and co-workers designed the first IRAK3 PROTACs in 2020 by conjugating a non-selective IRAK3 inhibitor (compound **75** [Figure 8B]) with CRBN ligands. After a 16-h treatment, PROTAC compound **76** (Figure 8B) was shown to mediate IRAK degradation with a DC_{50} of 2 nM and D_{max} of 98% in THP1 cells and primary macrophages [204].

Targeting IRAK3 with PROTACs represents a promising approach to modulating immune responses, particularly in cancer immunotherapy. By selectively degrading IRAK3, which suppresses immune activation, this strategy potentially enhances the efficacy of checkpoint inhibitors and improves immune surveillance in tumors.

To fully understand the potency and selectivity of the different degraders, all the PROTACs included in the current review have been summarized, including the basic structure, DC_{50} , D_{max} , IC_{50} , selectivity enhancement mechanisms, and the clinical status (Table 1).

4. CONCLUSION AND FUTURE PERSPECTIVES

PROTACs are bifunctional small molecules composed of three parts (target protein ligands, E3 ubiquitin ligase ligands, and linkers) that join these ligands together. When first proposed over 2 decades ago, PROTACs were anticipated to address some of the limitations associated with small molecule inhibitors, such as overcoming drug resistance, targeting “undruggable” proteins, and enhancing target selectivity. Since then, PROTACs have been known to induce the degradation of > 130 target proteins. Due to a deeper understanding of the underlying mechanisms and the significant potential in biological research and disease treatment, many researchers have turned their attention to this field, identifying potential targets suitable for PROTAC development. Approximately 20 protein degraders are currently in phase I or I/II clinical trials involving patients with various types of tumors; one phase III trial was launched in 2022.

Unlike small molecules, PROTACs drive the formation of ternary complexes between target protein ligands and E3 ligase ligands. Linkers regulate the interaction between these complexes. Hitherto, only a limited number of crystal structures of ternary complexes have been elucidated. Thus, enhancing target selectivity may be dependent on

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Table 1 | Data of PROTACs mentioned in this review (^aNot available).

Compound	POI	DC ₅₀ (nM)	D _{max} (%)	IC ₅₀ (nM)	Clinical status
6 (MZ1)	BRD4	— ^a	—	—	preclinical
7 (ZXH-03-26)	BRD4	5	—	—	preclinical
8 (A1874)	BRD4	32	98	—	preclinical
9 (BD-9136)	BRD4	0.2	99	—	preclinical
10 (WWL0245)	BRD4	sub-nanomolar	>99	8 (MV4-11)	preclinical
12 (9c)	HDAC6	—	—	—	preclinical
14 (A6)	HDAC6	—	—	—	preclinical
16 (HD-TAC7)	HDAC3	320	—	—	preclinical
18 (XZ9002)	HDAC3	42	—	—	preclinical
20 (ZQ-23)	HDAC8	147	93	—	preclinical
23 (SMD-3040)	SMARCA2	12	91	—	preclinical
25 (A947)	SMARCA2	0.039	96	—	preclinical
27 (ACB12)	SMARCA2	2	77	—	preclinical
29	SMARCA2	0.039	95	—	preclinical
31 (DT2216)	BCL-XL	63	90.8	—	preclinical
34 (BSJ-03-123)	CDK6	—	—	—	preclinical
35 (CP-10)	CDK6	2.1	—	—	preclinical
36 (CST651)	CDK6	5.1	95	—	preclinical
37 (BSJ-04-132)	CDK4	—	—	—	preclinical
39 (PROTAC-8)	CDK2	—	—	—	preclinical
41 (THAL-SNS032)	CDK9	—	—	—	preclinical
43 (PROTAC 2)	CDK9	158 ± 6	—	—	preclinical
46 (BSJ-4-116)	CDK12	—	—	—	preclinical
47 (PP-C8)	CDK12	416	—	—	preclinical
49 (SJFα)	p38α	7.16	97.4	—	preclinical
50 (SJFδ)	p38δ	46.17	99.4	—	preclinical
52	PARP1	—	—	8450 (MDA-MB-231)	preclinical
53 (NN3)	PARP1	—	—	11840 (MDA-MB-468)	preclinical
55 (SK-575)	PARP	0.509	99	19 (MDA-MB-436)	preclinical
57 (SGK3-PROTAC 1)	SGK3	300	80	—	preclinical
60 (SJF-0628)	BRAF	—	—	—	preclinical
61 (degraders 12)	BRAF	—	—	124 (HT-29)	preclinical
62 (degraders 23)	BRAF	—	—	51 (HT-29)	preclinical
64 (CG416)	TPM3-TRKA	0.48	—	—	preclinical
65 (CG428)	TPM3-TRKA	0.36	—	—	preclinical
67 (DCY-09-192)	FGFR1	4.35	85	—	preclinical
69 (MS39)	EGFR	5.0	—	—	preclinical
70 (MS154)	EGFR	3.3	—	—	preclinical

Table 1 | Continued

Compound	POI	DC ₅₀ (nM)	D _{max} (%)	IC ₅₀ (nM)	Clinical status
72 (SHP2-D26)	SHP2	2.6	>95	–	preclinical
73 (SP4)	SHP2	–	–	4.3 (Hela cell line)	preclinical
75 (ZB-S-29)	SHP2	6.02	–	–	preclinical
77 (PVD-06)	PTPN2	217	–	–	preclinical
79 (JQAD1)	EP300	–	–	–	preclinical
81	IRAK3	2	98	–	preclinical

the crystallographic data of the structure of these ternary complexes. Intuitively, combination and recognition in PROTACs is crucial for enhancing targeting selectivity. Numerous studies have demonstrated that recruiting different E3 ligases significantly influence targeting selectivity. Various E3 ligases possess distinct ubiquitin recruitment capabilities and differing quantities of lysine residues on the target protein surface, which affect selectivity. A higher number of surface lysine residues typically leads to increased targeting selectivity because the surface lysine residues are essential for binding ubiquitin units. This selectivity can also be attributed to the enhanced stability of the ternary complex formed by the target protein, which improves the effective binding of ubiquitin to lysine. As a result, the selectivity of targeted degradation is further increased. The linker has a critical role in stabilizing these ternary complexes. The composition, length, and rigidity can significantly influence the interaction between the two proteins. Thus, the processes of combination and recognition not only complement but reinforce the formation of ternary complexes, ultimately improving targeting selectivity.

Furthermore, with the identification of > 600 E3 ligases to date, the development of small molecule ligands for E3 ligases, which is essential for PROTACs design, is limited. E3 ubiquitin ligases can be classified into three families based on the structural characteristics and mechanisms of action (RING E3s, HECT E3s, and RBR E3s). After E3 ligases bind to the E2 ubiquitin complex and the substrate, RING E3s facilitate the direct transfer of ubiquitin from E2 to the lysine residues on the substrate. In contrast, HECT and RBR E3 ligases catalyze the transfer of ubiquitin from E2 to a cysteine residue, which is located at the active site of the E3 ligase, then subsequently transfer ubiquitin to the substrate protein. Ligands for VHL and CRBN are currently predominantly utilized. The discovery of new E3 ligase ligands has been lagging due to advances in PROTAC technology. Because PROTACs have emerged as powerful tools for novel drug discovery and development, we anticipate that additional small molecule ligands for E3 ligases will be identified, offering further versatility in PROTACs design. Thus, further exploration is needed to understand the relationship between the degree of ubiquitination at the

seven lysine sites and the selective degradation of target proteins.

Although PROTAC technology offers significant advantages in targeted protein degradation, PROTAC technology still faces several challenges and limitations. First, PROTAC molecules typically have large molecular weights that can affect cell permeability and bioavailability. To address this limitation, researchers can optimize molecular design by simplifying the structure, using smaller ligands or small molecule linkers to reduce molecular size. Second, the poor cell permeability of PROTACs may hinder degradation activity inside cells. This issue can be mitigated by introducing lipophilic groups or utilizing delivery systems, such as nanoparticles or liposomes to enhance cellular uptake. Additionally, off-target toxicity is a potential concern because the PROTAC degradation mechanism may impact non-target proteins, reducing the safety profile. To minimize off-target effects, enhancing targeting selectivity, optimizing E3 ligase specificity, and conducting multi-target screening can be effective solutions. Finally, although E3 ubiquitin ligases have a crucial role in the PROTAC degradation mechanism, clinical validation remains lacking. Therefore, developing highly efficient, specific E3 ligases and their ligands, along with strengthening clinical research on E3 ligases, will be essential for advancing PROTAC technology. Overall, despite these challenges, with the introduction of new technologies and solutions PROTAC has the potential to overcome these bottlenecks and offer promising prospects for targeted drug development.

PROTAC technology has achieved relatively mature progress. Through target selection, linker optimization, and the rational choice of E3 ubiquitin ligases, PROTAC molecules can be effectively designed. However, several challenges remain, including high molecular weight, poor cellular permeability, suboptimal drug formulations, and off-target toxicity. Future research should focus on the following key areas: developing novel, efficient, and highly specific E3 ubiquitin ligases and their ligands to enhance the selectivity and specificity of PROTACs; optimizing the pharmacokinetic properties of PROTACs, including improving stability and cellular permeability

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in vivo; and exploring the application of PROTACs in non-cancer diseases, such as the potential in neurodegenerative diseases and immune disorders. Although no PROTAC drugs have been approved for clinical use, the lack of biological and clinical validation remains a significant barrier. However, as more compounds enter clinical trials and potentially receive market approval, the future of PROTAC technology remains promising. Through multifaceted exploration and innovation, PROTAC technology is expected to provide more effective and selective therapeutic strategies, leading to revolutionary breakthroughs in the treatment of cancer and other diseases.

ABBREVIATIONS

PROTAC, proteolysis-targeting chimeras; VHL, von Hippel-Lindau; CRBN, cereblon; POI, protein of interest; Ubs, ubiquitin; UPS, ubiquitin-proteasome system; E1, ubiquitin-activating enzyme; E2, ubiquitin-conjugating enzyme; E3, ubiquitin ligase; BET, bromo- and extra-terminal; PPI: protein-protein interaction; MDM2, mouse double minute 2 homolog; HDACs, histone deacetylases; HSF-1, heat shock factor-1; PDGF, platelet-derived growth factor; SAHA, suberoylanilide hydroxamic acid; CDK, cyclin-dependent kinase; AML, acute myeloid leukemia; PARP, poly(ADP-ribose) polymerase; SGK, serum/glucocorticoid-inducible protein kinase; MAPK, mitogen-activated protein kinase; TRKs, tropomyosin receptor family kinases; TKIs, TRK inhibitors; FGFR, fibroblast growth factor receptor; RTKs, receptor tyrosine kinases; EGFR, epidermal growth factor receptor; SHP2, Src homology region 2-containing phosphatase 2; PTP, protein tyrosine phosphatase; NCSCl, non-small cell lung cancer; PTPs, protein tyrosine phosphatases; PTPN2, protein tyrosine phosphatase non-receptor type 2; TCPTP, T cell protein tyrosine phosphatase; STATs, signal transducers and activators of transcription; TCR, T cell receptor; CREBBP, CREB-binding protein; EP300, E1A-binding protein P300; IRAK3, interleukin-1 receptor-associated kinase 3.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (82272637 and 82204429) and State Key Laboratory for Chemistry and Molecular Engineering of Medicinal Resources (Guangxi Normal University, CMEMR2023-B03).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in this work.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

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