# PROTACs as a New Tactic to Treat Cancer

Siyi Zhang

Department of Chemistry, University of Nottingham, NG7 2RD, England

zhangsiyi0917@163.com

**Abstract.** Cancer's high mortality, medication resistance, and recurrence rates make it a significant worldwide health concern. Despite improvements in immunotherapy and targeted therapy, its effectiveness is hampered by issues including acquired resistance. Among the innovative treatment approaches being investigated to address these issues, proteolysis-targeting chimeras (PROTACs) have shown promise. By selectively degrading target proteins, PROTACs provide a catalytic, long-lasting and low side effect mechanism of action in contrast to conventional small molecule inhibitors that impede protein function. In this review, the design, mechanisms, and uses of PROTACs in cancer treatment are examined. The development of PROTAC based cancer treatments is given a wider vision and additional alternatives thanks to this review, which also examines current issues like pharmacokinetics, cell membrane permeability, E3 ligase selection, and off-target effects et al. The information in this evaluation is intended to be used as a guide for future clinical translation and optimization of medications based on PROTACs.

**Keywords:** PROTAC, Cancer Therapy, Drug Resistance, Clinical Trials.

## 1. Introduction

Despite significant advancements in cancer treatment in recent years, cancer remains a major problem due to its high recurrence rate, drug resistance, and mortality [1]. In recent years, PROTAC technology has emerged as an innovative anticancer approach [2]. PROTACs offer a novel strategy to address drug resistance in targeted therapies and significantly broaden the spectrum of druggable targets, including previously “undruggable” proteins. Compared with conventional small-molecule inhibitors, PROTACs function catalytically to induce selective protein degradation, offering advantages in potency, specificity, and durability of response [3]. This review provides a comprehensive overview of PROTAC technology, including its mechanisms of action, design strategies, and current progress in clinical and preclinical applications for cancer treatment.

## 2. The Mechanism of PROTAC

By grabbing the ubiquitin-proteasome system (UPS), PROTACs preferentially break down disease-related proteins. Protac is a bifunctional compound mechanistically made of a ligand binding a protein of interest (POI), a ligand recruiting an E3 ubiquitin ligase, and a chemical linker joining the two [2]. Once bound, the PROTAC helps a ternary complex to develop between the POI, the E3 ligase, and itself to bring the two proteins close together and enable the ubiquitin chains to lysine residues on the target protein transfer. The 26S proteasome then picks out and breaks down the polyubiquitinated target. PROTACs are particularly catalytic; hence, one PROTAC molecule can cause the degradation of several target proteins without being eaten (Fig. 1) [2-4].

Although the binary affinities of a PROTAC to its constituent components are significant, the mounting data point to ternary complex cooperativity as the main factor influencing degrading efficiency. Positive cooperativity arises from the binding of the PROTAC to one protein improving its binding to the second, hence producing a more stable complex and increased ubiquitination. Conversely, negative cooperativity generates poor degradation and less ternary complex stability [5].

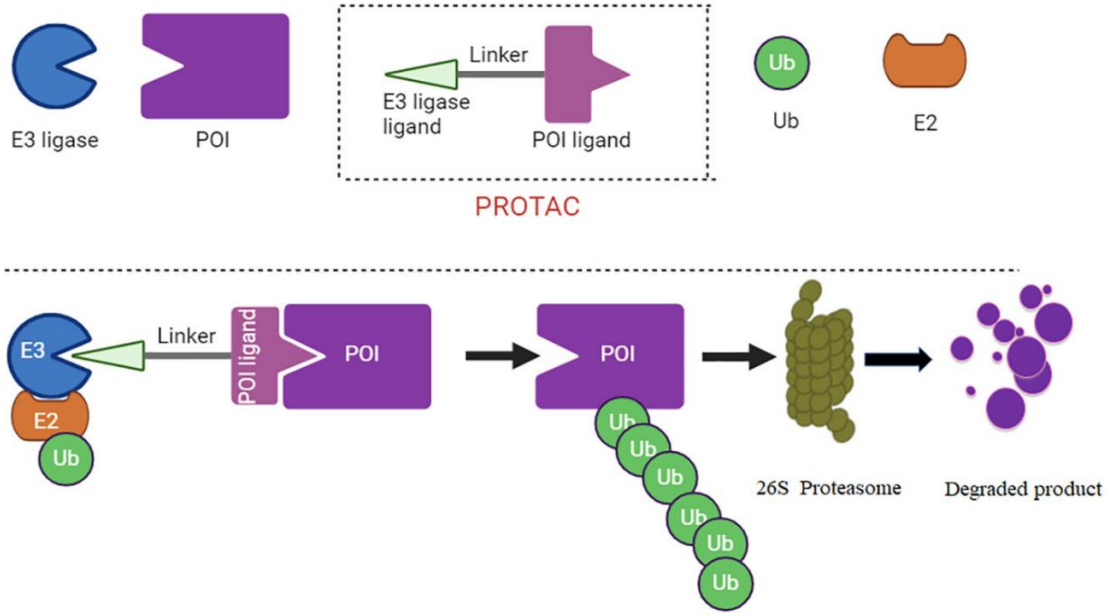


**Figure 1.** The mechanism of PROTAC

## 3. Design and Optimization

### 3.1. Target Ligand Specificity

Although PROTACs have shown great promise, one of their key problems is still off-target consequences [6]. Non-specific protein breakdown brought on by these off-target effects may impair treatment efficacy, cause toxicity, and interfere with cellular processes. The off-target effects of PROTACs are mostly dependent on the target protein ligand's selectivity. A PROTAC may attach to unwanted proteins and cause unintentional protein breakdown if its target protein ligand has poor selectivity. Certain protein families share highly similar domains, such as the BET (Bromodomain and Extra-Terminal domain) protein family, including BRD2, BRD3, and BRD4 (Bromodomain-containing protein 4), or multiple members of the kinase family that share ATP-binding pockets. Consequently, a PROTAC designed to degrade a specific target protein may also affect other proteins with similar binding sites. This non-specific binding increases the risk of off-target effects, causing the degradation of multiple related proteins within the cell rather than a single intended target [7, 8].

Moreover, the expression levels of E3 ligases vary significantly across different tissues and cancer types. The structural design of PROTAC molecules is another important factor influencing off-target effects. The length and chemical properties of the linker directly affect the stability of PROTAC binding to both the target protein and the E3 ligase. If the linker is excessively long or overly flexible, the PROTAC may freely switch between different E3 ligases, thereby increasing its impact on non-target proteins. Additionally, PROTAC molecules often exhibit high lipophilicity, which enhances their ability to diffuse across cell membranes [9]. This can lead to their accumulation in non-target tissues, further exacerbating non-specific degradation. Furthermore, many proteins share similar ubiquitination recognition signals, enabling E3 ligases to degrade multiple proteins rather than being limited to the intended target protein designed for the PROTAC, further increasing the risk of non-specific degradation.

### 3.2. E3 Ligase Selection

The choice of E3 ligase is very important, as different cancers exhibit varying expression levels of these ligases, which directly affects the efficiency of protein degradation [8]. Currently, the most used E3 ligases in PROTAC development are CRBN and VHL, as they are broadly expressed across various cancer types and have well-characterized small-molecule binders.

In renal cell carcinoma, VHL is highly expressed and serves as a natural target for PROTAC design, whereas, in multiple myeloma, CRBN is significantly overexpressed, making it a preferred ligase for targeted protein degradation [10]. Other E3 ligases, such as MDM2, are frequently overexpressed in breast cancer and glioblastoma, allowing for MDM2-recruiting PROTACs to restore p53 activity in tumors with excessive MDM2-mediated degradation. Similarly, IAP family ligases, which are upregulated in ovarian and pancreatic cancers, are being explored for their role in modulating apoptotic pathways [6]. To investigate the broad applicability of PROTACs based on VHL and CRBN in different tumor types, after screening with 56 cell lines representing different cancer types, each cell was treated with MZ1 and dBET1 for four hours, its degradation effect on BRD4 was evaluated, and its protein quantification was performed using the MSD (Mesoscale Discovery) platform. IC50 and DC50 values were calculated to evaluate PROTAC activity. The study found that CRBN-dependent dBET1 activity is often inhibited in cancer cell lines, while VHL expression and mutation can predict its activity [11]. Novel E3 ligases, such as DCAF15 in acute myeloid leukemia, are also gaining interest for their potential applications in PROTAC design [12].

**Table 1.** E3 ligases and their relevance in cancer types

| E3 Ligase | Highly Expressed In | Representative Targets |
|---|---|---|
| CRBN | Multiple Myeloma | AR, ERα |
| VHL | Renal Cell Carcinoma | BRD4 |
| MDM2 | Breast Cancer, Glioblastoma | p53 |
| IAP | Ovarian, Pancreatic Cancer | XIAP, cIAP1 |
| DCAF15 | Acute Myeloid Leukemia | RBM39 |

### 3.3. Linker Optimization

The linker plays a crucial role in PROTAC design by influencing ternary complex formation, cellular permeability, pharmacokinetics, and degradation efficiency. Optimizing linkers involves adjusting their length, flexibility, and chemical properties. An appropriate linker length ensures proper spatial positioning of the target protein and E3 ligase for efficient ubiquitination. Overly short linkers can cause steric hindrance, while excessively long or flexible ones may destabilize the ternary complex and reduce degradation efficiency. [9] Beyond length, chemical features such as polarity and rigidity also affect PROTAC performance. Flexible linkers like PEG or alkyl chains provide adaptability but may reduce complex stability due to entropy costs. In contrast, rigid linkers help maintain defined conformations, enhancing complex formation [13].

Hydrophobicity or hydrophilicity also impacts solubility and membrane permeability. Hydrophobic linkers may improve cell entry but risk nonspecific interactions and aggregation, whereas hydrophilic linkers enhance solubility but can limit intracellular transport. Given the typically large size of PROTACs, linker properties are central to maintaining bioavailability. Hydrophilic groups like PEG improve solubility, though excessive hydrophilicity can impair permeability [14]. Conversely, highly hydrophobic linkers may promote membrane diffusion but reduce free drug concentration by increasing plasma protein binding. Additionally, linker stability affects the compound’s half-life, as unstable linkers may undergo enzymatic degradation before reaching the target [9].

### 3.4. Clinical Progress of PROTACs

Several PROTAC compounds have entered clinical trials to show oncology's translational potential of focused protein breakdown as a treatment approach. Two prominent ones are ARV-471 and ARV-110. Designed to break down the androgen receptor, a main driver of metastatic castration-resistant prostate cancer, ARV-110 is a PROTAC. ARV-110 exhibited encouraging anti-tumor effectiveness in patients harboring particular AR mutations, which are known to give resistance to standard AR antagonists, in a Phase I clinical trial (NCT03888). Currently under study for estrogen receptor-positive/-negative breast cancer, ARV-471 targets the estrogen receptor alpha. ARV-471

showed stable disease in mostly pretreated patients and partial responses in Phase I/II trial (NCT0407). These findings lead ARV-471 to be under evaluation in randomized clinical studies together with CDK4/6 inhibitors. These early clinical investigations confirm the feasibility, selectivity, and safety of PROTACs in human cancer patients, therefore supporting the idea of induced protein degradation as a new modality.

## 4. PROTACs in Cancer Therapy

### 4.1. Overcoming Drug Resistance

Drug resistance remains a major challenge in cancer therapy, often driven by phenotypic changes, genetic mutations, or activation of bypass signaling pathways. Traditional small-molecule inhibitors, which act by blocking protein activity or binding sites, can be evaded through structural alterations or overexpression of the target protein. In contrast, PROTACs (proteolysis-targeting chimeras) eliminate the entire target protein by recruiting E3 ubiquitin ligases, thereby overcoming resistance mechanisms linked to mutations or compensatory signaling [1]. BCL-2, an anti-apoptotic protein that inhibits BAX/BAK-mediated mitochondrial apoptosis, is frequently overexpressed in AML and CLL, contributing to treatment resistance. In small cell lung cancer (SCLC), a highly aggressive malignancy with a five-year survival rate below 7%, BCL-2 and BCL-xL are recognized targets. However, clinical use of these inhibitors, like navitoclax, is limited by platelet toxicity. PROTACs offer a safer alternative by selectively degrading BCL-xL in tumor cells without harming platelets [15].

### 4.2. Immunotherapy Application (PD-L1)

#### 4.2.1 Small-Molecule-Based PD-L1 PROTACs

The basis for the creation of the first PD-L1-targeting PROTACs was the small-molecule inhibitor BMS-202, which binds to PD-L1, causes it to dimerize, and thus reduces the PD-1 interaction to boost T-cell-mediated antitumor effectiveness. At present, it has been demonstrated to induce CD8+ T cell infiltration, hence enhancing antitumor immune responses [16].

#### 4.2.2 Peptide-Based PD-L1 PROTACs

In addition to small-molecule PROTACs, some studies have utilized peptides as PD-L1 ligands conjugated with E3 ligase ligands to construct peptide-based PROTACs. These can efficiently degrade PD-L1 while minimizing off-target effects.

Peptide-PROTACs are designed with CPPs (cell-penetrating peptides), TPRs (target protein recognition peptides), and E3 ligase recruitment peptides. Some target PD-1, while others target PD-L1 for direct degradation. Suitable for PD-L1-overexpressing tumors due to cell membrane permeability. Low toxicity and can enhance anticancer effects when combined with cisplatin.

SP-PROTACs (stapled peptide PROTACs) are based on stapled peptides; these specifically degrade ZDHHC3 (palmitoyl transferase), leading to a reduction in PD-L1 expression. Experimental data show that SP-PROTACs can significantly reduce PD-L1 levels in both in vitro studies and mouse models, enhancing antitumor immune responses [17].

#### 4.2.3 CDTACs (Carbon Dot-Based PROTACs)

CDTACs are carbon dot-based PROTACs designed to induce proteasomal degradation of membrane proteins via the UPS (ubiquitin-proteasome system).

These CD-based PROTACs bind PD-L1, recruit the CRBN E3 ligase, and induce PD-L1 ubiquitination and degradation. They have been shown to significantly enhance T-cell immune responses by activating the STING pathway. When combined with fasting-mimicking diets (FMDs), CDTACs can inhibit tumor growth effectively [18].

## 5. Conclusion

PROTACs have shown a large step in targeted therapy, offering a new way to degrade target proteins rather than merely inhibiting them. It can also target resistant and mutant target proteins and overcome the limitations of TKIs and hormone therapy. PD-L1 overexpression is a significant mechanism of immune evasion, limiting the efficacy of immune checkpoint inhibitors (ICIs). While PD-L1 PROTACs can degrade PD-L1, restoring T-cell activity and immune surveillance. Preclinical studies demonstrate promising results: Enhanced anti-tumor immune response, potential synergy with anti-PD-1 antibodies, and lower toxicity compared to PD-L1 monoclonal antibodies.

However, there are still some drawbacks, such as limited cell permeability, bioavailability, and E3 ligase selectivity and specificity. Potential off-target effects and toxicity. Clinical translation is still in the early stages, so it needs more clinical trials to confirm efficacy and safety.

Development of next-generation PROTACs, such as improved linker designs for better stability and cell permeability and finding new E3 ligases to increase the range of druggable targets. Since PROTACs are a targeted protein degradation tool, they can be used in combination with other therapeutic approaches to increase their effectiveness.

## References

[1] Liao M, Qiang X, Sheng Z, Peng P. Mechanisms of drug resistance and coping strategies for small-molecule targeted anticancer drugs [J]. Progress in Pharmaceutical Sciences, 2023, 47 (9): 693–705.

[2] Zattoni, J., et al., A comprehensive primer and review of PROTACs and their in silico design. Comput Methods Programs Biomed, 2025. 264: p. 108687.

[3] Kim, H., J. Park, and J.M. Kim, Targeted Protein Degradation to Overcome Resistance in Cancer Therapies: PROTAC and N-Degron Pathway. Biomedicines, 2022. 10 (9).

[4] Ren, X., et al., PTMs of PD-1/PD-L1 and PROTACs application for improving cancer immunotherapy. Front Immunol, 2024. 15: p. 1392546.

[5] Wurz, R.P., et al., Affinity and cooperativity modulate ternary complex formation to drive targeted protein degradation. Nat Commun, 2023. 14 (1): p. 4177.

[6] Ocana, A. and A. Pandiella, Proteolysis targeting chimeras (PROTACs) in cancer therapy. J Exp Clin Cancer Res, 2020. 39 (1): p. 189.

[7] Danishuddin, et al., Revolutionizing Drug Targeting Strategies: Integrating Artificial Intelligence and Structure-Based Methods in PROTAC Development. Pharmaceuticals (Basel), 2023. 16 (12).

[8] Sobierajski, T., et al., The impact of E3 ligase choice on PROTAC effectiveness in protein kinase degradation. Drug Discov Today, 2024. 29 (7): p. 104032.

[9] Cecchini, C., S. Tardy, and L. Scapozza, Linkers as Game-changers in PROTAC Technology: Emphasizing General Trends in PROTAC Pharmacokinetics for their Rational Design. Chimia (Aarau), 2022. 76 (4): p. 341-345.

[10] Lee, J., et al., Discovery of E3 Ligase Ligands for Target Protein Degradation. Molecules, 2022. 27 (19).

[11] Luo, X., et al., Profiling of diverse tumor types establishes the broad utility of VHL-based ProTaCs and triages candidate ubiquitin ligases. iScience, 2022. 25 (3): p. 103985.

[12] Grothusen, G.P., et al., DCAF15 control of cohesin dynamics sustain acute myeloid leukemia. Nat Commun, 2024. 15 (1): p. 5604.

[13] Dong, Y., et al., Characteristic roadmap of linker governs the rational design of PROTACs. Acta Pharm Sin B, 2024. 14 (10): p. 4266-4295.

[14] Poongavanam, V., et al., Linker-Dependent Folding Rationalizes PROTAC Cell Permeability. J Med Chem, 2022. 65 (19): p. 13029-13040.

[15] Khan, S., et al., PROTAC-Mediated Dual Degradation of BCL-xL and BCL-2 Is a Highly Effective Therapeutic Strategy in Small-Cell Lung Cancer. Cells, 2024. 13 (6).

[16] Wang, Y., et al., In vitro and in vivo degradation of programmed cell death ligand 1 (PD-L1) by a proteolysis targeting chimera (PROTAC). Bioorg Chem, 2021. 111: p. 104833.

[17] Shi, Y.Y., et al., Stapled peptide PROTAC induced significantly greater anti-PD-L1 effects than inhibitor in human cervical cancer cells. Front Immunol, 2023. 14: p. 1193222.

[18] Su, W., et al., Targeted Degradation of PD-L1 and Activation of the STING Pathway by Carbon-Dot-Based PROTACs for Cancer Immunotherapy. Angew Chem Int Ed Engl, 2023. 62 (11): p. e202218128.