

Review

# Proteolysis Targeting Chimeras (PROTACs): An Innovative Strategy for Targeted Protein Degradation and Disease Treatment

Jun Xia, James K.S. Norris, May-Li MacKinnon and Sam Butterworth \*

Division of Pharmacy and Optometry, Faculty of Biology Medicine and Health, The University of Manchester, Manchester M13 9PL, UK

\* Correspondence: sam.butterworth@manchester.ac.uk

Received: 18 May 2024; Revised: 9 July 2024; Accepted: 9 July 2024; Published: 26 August 2024

**Abstract:** Protein ubiquitination is a highly conserved post-translational modification through which cells initiate the proteasomal degradation of undesired, aberrant, or damaged proteins. Protein ubiquitination plays a crucial role in protein homeostasis and regulates a wide range of essential physiological processes including DNA repair, immunological response, cell survival and apoptosis. Dysregulation of ubiquitination is associated with various pathologies including cancers, neurodegenerative diseases, and immune disorders. The ubiquitin-proteasome system (UPS) machinery has been utilized in therapeutic research as it can be manipulated to induce the degradation of undruggable proteins in a superior manner to traditional drug modalities. One such a method of specific protein degradation is the use of heterobifunctional molecules such as proteolysis targeting chimeras (PROTACs). This literature review will focus on the composition, mechanism of action and developmental milestones of PROTACs, comparing these against traditional drug discovery and treatment approaches. In addition, the potential benefits of PROTAC usage will be highlighted by analyzing their practical applications in drug therapies.

**Keywords:** PROTAC; proteolysis; protein degradation; drug discovery

## 1. Introduction

The most extensively used paradigm in traditional drug discovery relies on small-molecule drugs that bind to and modulate the function of disease-associated macromolecules, typically proteins. There are, however, several drawbacks affecting the performance of small-molecule inhibitors, including poor selectivity, off-target toxicity, drug resistance in long-term therapy, and inability to target ‘undruggable’ proteins associated with diseases [1,2] Indeed, most disease-related proteins are still considered ‘undruggable’, because they lack active functional pockets and well-defined binding features that allow the discovery of high-affinity small molecule ligands [1,3] Additional mechanisms outside of traditional protein inhibition, based on existing human cell machinery, are now being harnessed for pharmaceutical innovation. One emerging technique disrupts protein homeostasis, the cellular process that regulates protein levels, conformation, and distribution [4,5]

The two main cellular organelles that cause the breakdown of proteins are lysosomes and proteasomes; protein complexes that cleave peptide bonds within proteins, generating polypeptides and/or amino acids through proteolysis [4]. These mechanisms act in synergy to breakdown misfolded proteins; in which lysosomes degrade long-lived proteins and insoluble protein aggregates whereas proteasomes target short-lived proteins [5]. The proteasome, which in mammalian cells accounts for 70% or more of protein degradation, shall be the focus of this review [6].



**Copyright:** © 2024 by the authors. This is an open access article under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Publisher’s Note:** Scilight stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

A cell uses the ubiquitin-proteasome system (UPS) process to access the 26S proteasome, the main proteolytic machine response in eukaryotic cells.[7]. The essential component of the UPS is ubiquitin (Ub), a 76-residue protein used to ‘tag’ proteins for degradation in a process called ubiquitination. This occurs after cellular free Ub is funneled through a cascade of enzymes, namely ubiquitin-activating enzymes (E1s) and ubiquitin-conjugating enzymes (E2s). The Ub transfer to a protein occurs after Ub is transferred from E1 to E2. The activated Ub-E2 complex interacts with ubiquitin E3 ligases and the specific protein. The E3 ligase acts as a catalyst in bringing together these proteins, and allows the transfer of ubiquitin from the E2 enzyme to the protein at lysine residues [5,8] Studies have shown that ubiquitin chains linked to proteins through the lysine 48 residue provide the most efficient degradation trigger, although ubiquitin linked through lysine 11 also tags proteins for degradation [9]. Once a protein has been tagged with K48/K11 polyubiquitination, the protein is recognized by the 26S proteasome and the substrate protein is degraded [1].

Deubiquitination also occurs in the UPS and this is driven by proteases called deubiquitinating enzymes (DUBs). DUBs reverse the effects of ubiquitination by cleaving the peptide or isopeptide bond between a protein and its Ub tag. In releasing Ub, DUBs in effect reverses the effect of the E3 ligases, which rescues a protein destined for degradation [10,11] The proteins involved in ubiquitination and de-ubiquitination act collaboratively, in opposing fashion to regulate the degradation of substrate proteins. To enhance proteostasis, they regulate biological processes in the cells [5,10]

Dysregulated ubiquitination and deubiquitination are implicated in multiple pathologies, including cancer, neurodegenerative diseases, and auto-inflammatory disorders [1]. Thus, the UPS machinery is suggested as a promising target for new drug discovery. Targeting the aberrant activity of the UPS may be an attractive strategy for developing novel therapeutic approaches. These approaches may provide deeper insights into disease pathology and lead to more effective clinical treatments. Traditional drug therapies against UPS disorders focus on developing small molecules which can bind to aberrant components of the UPS, such as E1, E2, E3 enzymes or deubiquitinases to modulate protein abundance and cellular functioning [12].

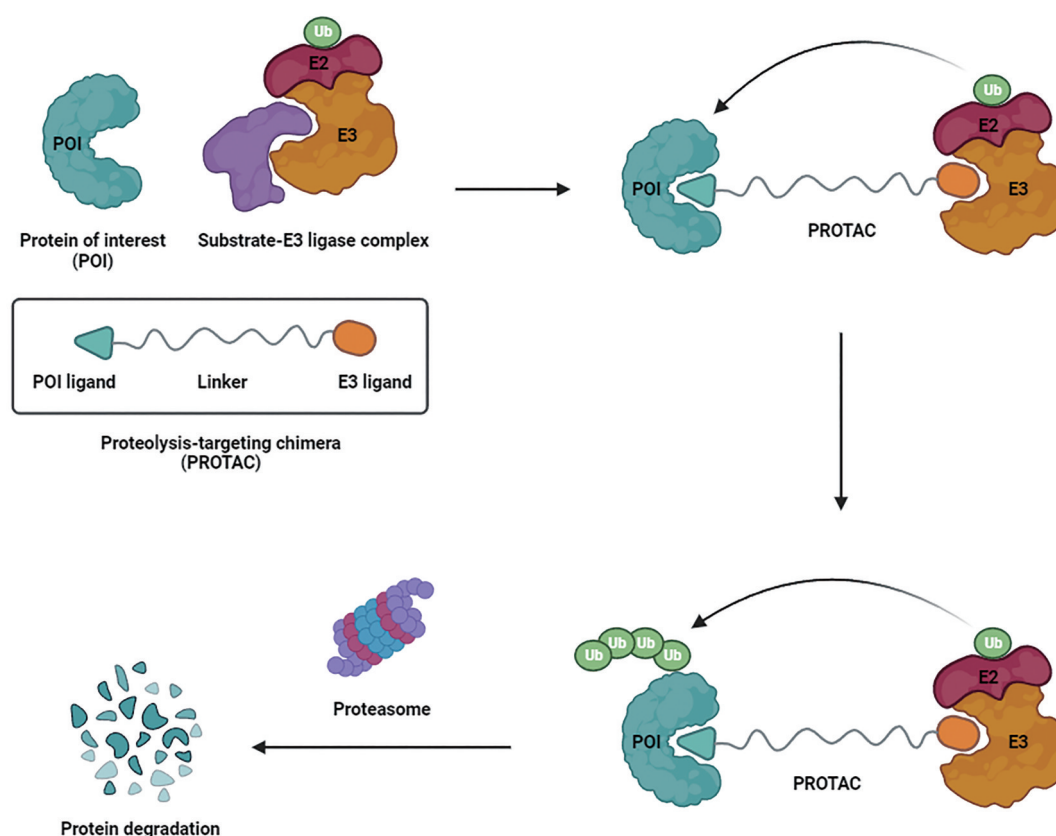
In this review, a therapeutic strategy that exploits the UPS machinery, is introduced. Proteolysis targeting chimeras (PROTACs) is an exciting area of discovery which has witnessed exponential growth in article publication, especially since 2016 [13]. In the following sections, their regulatory mechanisms, potential therapeutic values, and recent advances in drug development and clinical applications are summarized.

## **2. PROTAC: A Targeted Protein Degradation Technology Based on the UPS**

As a type of heterobifunctional small molecule, a PROTAC consists of three main components, including a targeting ligand (warhead) for a protein-of-interest (POI) to be degraded, a ligand for E3 ligase recruitment, and a chemical linker that connects the two different ligands and determines the proximity of the POI to the E3 ligase [2].

Regarding the mechanism of the PROTAC technology (Figure 1), targeted binding of PROTACs with POI and E3 ligases brings the two bound proteins in close proximity via the formation of a POI-PROTAC-E3 ligase ternary complex. By hijacking the UPS, the POI is polyubiquitinated, where ubiquitin molecules are transferred from a E3-conjugated E2 enzyme to a peripheral lysine residue on the POI in a catalytic manner as described in Section 1 above. The ubiquitin-tagged POI is then recognized by the proteasome which then undergoes proteasomal degradation, thereby eliminating the POI from eukaryotic cells [8,14].

During the design of PROTAC, POI and E3 ligase ligands are used as ‘small-molecule inhibitors’ and various linkers with appropriate length, chemical composition, and conformations are employed to connect the two selected ligands [15]. To avoid loss of binding affinity between the two bound ligands, amides, ethers, thioethers and C-C bonds are generally applied as linker attachments at points located at solvent-exposed regions [16,17] On this basis, structure-activity relationship (SAR) analysis of the linkers or entire molecules is conducted for PROTAC optimization to achieve desirable degradation potency, selectivity and physiochemical properties. [18]. Through variation of the three PROTAC components, one can construct heterobifunctional compound libraries to screen for selected targets.



**Figure 1.** Schematic of proteolysis-targeting chimera (PROTAC) mediated degradation of target protein. Created with BioRender.com (2024).

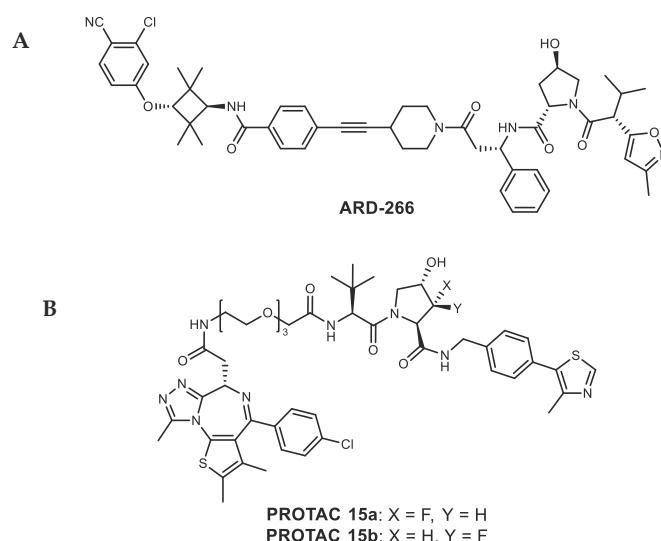
In 2001, Crews et al. designed and reported the first peptide-based PROTAC which could target protein methionine aminopeptidase-2 (MetAP-2) to E3 ubiquitin ligase SCF<sup>β-TrCP</sup> for selective MetAP-2 ubiquitination and degradation [19]. Following this first proof of concept of the PROTAC technology, rapid advancements in the development and application of PROTAC technology have been achieved as reflected in the several and quality of publications [13]. In the PROTAC field, one of the most commonly recruited E3 ligases is Von Hippel-Lindau (VHL), which was first recruited by a cell-penetrating peptide-based PROTAC designed by Schneekloth et al. in 2004 to induce targeted degradation of its substrate FKBP12 (FKBP12) [20]. Subsequently, the Crews group developed the first small molecule-based PROTAC in 2008, which recruited E3 ligase mouse double minute 2 (MDM2) to successfully degrade the androgen receptor (AR) [21]. About 600-700 E3 ligases encoded by the human genome have been reported [22]. However, besides MDM2, only a small number of small molecule E3 ligase ligands have been successfully recruited and utilized by PROTACs for targeted protein degradation (TPD). These include cellular inhibitor of apoptosis protein (cIAPs) [23], cereblon (CRBN) [24], VHL [25], DCAF15/16 [26,27], Kelch-like ECH-associated protein 1 (KEAP1) [28], RNF114 [29], and MDMX [30]. To date, CRBN and VHL are the two most frequently used E3 ligases using the current PROTAC technology, of which about 60% of reported PROTACs utilize CRBN while 30% utilize VHL [2].

### 3. Advantages of PROTACs over Other Approaches

The traditional drug treatment is based on the occupancy-driven strategy, where the pharmacological activities of small molecules mainly rely on occupation of functional binding pockets of target proteins to alter their functions. In contrast, PROTACs can completely eliminate all biological functions of targets by inducing the removal of the entire protein rather than altering specific protein functions [2]. Based on the event-driven mechanism of action, wherein even partial occupancy of the target protein can be sufficient to remove the protein from cells, this approach can create a cellular phenotype that closely replicates the genetic

knockdown of target proteins, as seen in the comparison of Aurora-A inhibition versus degradation [31,32] Although there are several challenges in the developmental of large PROTAC-type molecules, their intrinsic cell permeability and stability provide opportunities for utilizing structurally optimized examples with better pharmacokinetic properties, without the need for complicated and often tissue-restricted delivery systems required for RNA interference and microRNA therapeutics [33,34].

Researchers have shown that PROTACs can broaden the druggable space of the proteome. Disease-related proteins which are currently considered ‘undruggable’ with traditional small-molecule drugs, including transcription factors, scaffold proteins, and RAS superfamily proteins could be more accessible by PROTACs due to their ability to bypass the need for active functional pockets [35, 36] Given that the mechanism of PROTACs relies on the transient formation of POI-PROTAC-E3 ligase ternary complexes to induce ubiquitination and subsequent degradation of target proteins, ligands with lower affinity and more brief occupancy than small-molecule drugs can be utilized for PROTACs with appropriate linkers, as long as they can recruit an E3 ligase and POI to initiate effective ternary complex-mediated ubiquitination [36]. This can be demonstrated by the ARD-266 PROTAC (Figure 2A) and fluoro-hydroxyproline (F-Hyp)-containing PROTACs (e.g., PROTAC MZ1 derivatives: PROTAC 15a/b) (Figure 2B) [37,38].

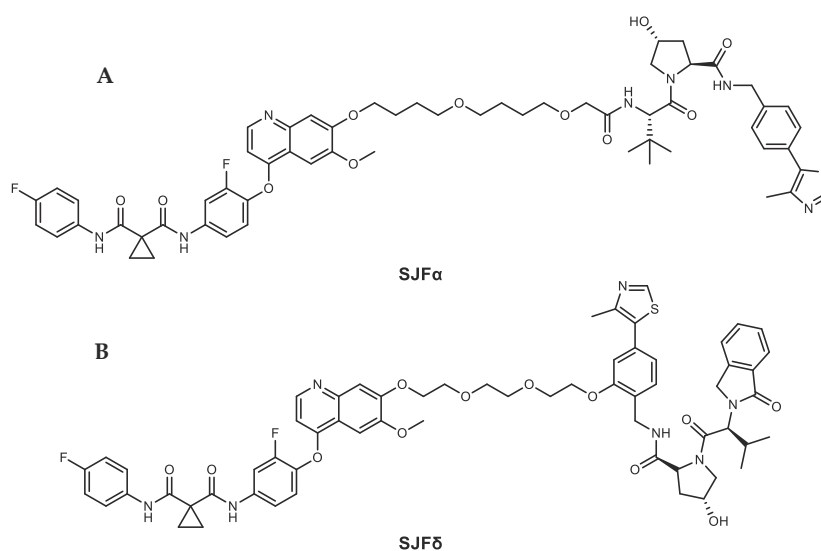


**Figure 2.** Chemical structures of (A) PROTAC ARD-266 and (B) PROTAC 15a/b.

Despite lower affinities of VHL ligands, PROTACs could still be recognized by POI and E3 ligases cooperatively to induce specific PPIs, as a result the enhanced PPI between POI and E3 ligases can promote ternary complex formation and lead to potent degradation [39]. Using VHL ligands with micromolar affinities, ARD-266 and the F-Hyp-containing PROTACs exerted even higher TPD activities than many analogues containing much stronger binding affinities to VHL. This study indicates that the design of PROTACs would no longer be limited to high-affinity E3 ligands, allowing expansion of druggable space for E3 ligase toward weak-affinity binding ligands.

Due to the unique heterobifunctional structure of PROTACs, the selectivity and specificity of the whole drug molecules are highly dependent on the selective recognition of both E3 ligases and POIs. This implies that the rational design of E3 ligase and POI ligands with optimized binding affinity can cooperate and enhance the substrate selectivity and specificity of PROTACs [40]. On this basis, the additional protein-protein interaction (PPI) between E3 ligases and POIs also play a vital role in selective substrate recognition to form appropriate POI-PROTAC-E3 ternary complexes for TPD. These characteristics can collaboratively support desirable drug-target interactions and help filter out effects from other homologues proteins that can bind to the POI ligands.[2,8] A recent example of highly selective TPD is the SJF $\alpha$ /SJF $\delta$  PROTACs (Figure 3), which consist of one p38-Mitogen-activated protein kinase (MAPK) inhibitor foretinib, two different VHL-recruiting ligands and linkers to exert isoform-specific p38 $\alpha$ /p38 $\delta$  degradation [41]. The study demonstrates that the VHL-recruiting ligand and linker length differences between the two PROTACs significantly

contribute to their respective substrate specificity, which is the main driving force behind selective ternary complex formation and protein degradation. An additional example is the case of Class 1 histone deacetylase (HDAC) proteins. Different levels of degradation of HDACs 1, 2 and 3 can be achieved by altering the linker composition [42,43]



**Figure 3.** Chemical structures of (A) PROTAC SJF $\alpha$  and (B) PROTAC SJF $\delta$ .

In most cases, traditional small-molecule drugs that act in a dose-dependent manner need to be administered in relatively high dosages to maximize drug-receptor occupancy for sufficient and continuous clinical effects. Unfortunately, the efficacy of traditional drugs often comes with a high risk of undesirable toxic side effects and off-target effects, which are considered limitations inherent to these therapeutics.[8,40,44]. On the other hand, PROTACs can induce catalytic ubiquitination and degradation, exhibiting pharmacological activities without being constrained by long-lasting occupancy. The reversible POI-PROTAC-E3 ligase ternary complexes allow detachment of PROTACs in the proteasomal degradation process, preventing PROTACs from being destroyed and enabling their reuse in multiple further catalytic cycles of POI degradation. As a result, the catalytic nature of PROTACs would impart these molecules unique advantages of sub-stoichiometric dosage. While the catalytic function is theoretically a general feature of PROTACs, this has only been specifically demonstrated with a few examples to date, including the catalytic degradation of nuclear receptor ERR $\alpha$  and protein kinase RIPK2 [45,46]

Additionally, PROTACs hold an exciting potential to minimize drug resistance that frequently occurs with traditional drug therapy. This is because the unique mechanism of PROTAC-mediated catalytic ubiquitination can theoretically reduce the impact of site mutations that reduce target occupancy or overexpression of targets that are usually associated with resistance development. To date, PROTACs have been successfully applied to treat several drug-resistant diseases by degrading related target proteins, such as AR, BTK, and BCR-ABL [47].

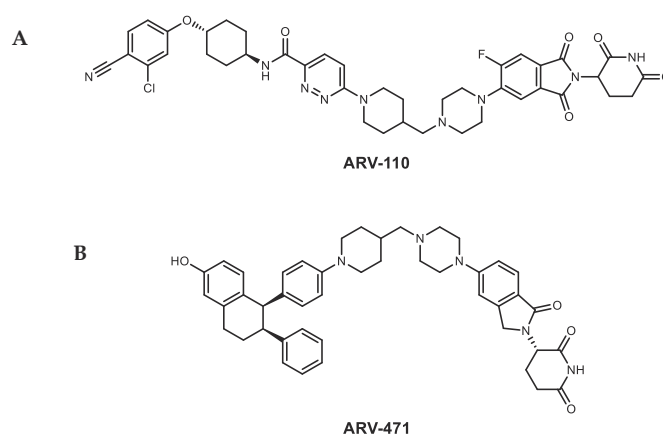
#### 4. Application of PROTACs in Drug Discovery for Disease Treatment

As highlighted above, PROTACs have demonstrated a new perspective on drug discovery as an emerging technology different from traditional small-molecule drugs, which has the potential to offer a range of clinical benefits. This has been evidenced recently by numerous PROTACs, which have entered clinical trials.

In 2021, Arvinas became the first company to bring a PROTAC to clinical trials, namely ARV-110 (Figure 4A). This is an AR degrader for treating castration-resistant metastatic prostate cancer caused by overexpression of AR signaling. While traditional AR inhibitor enzalutamide suffers from losing pharmacological activity in prolonged administration due to AR mutations and amplifications, PROTAC

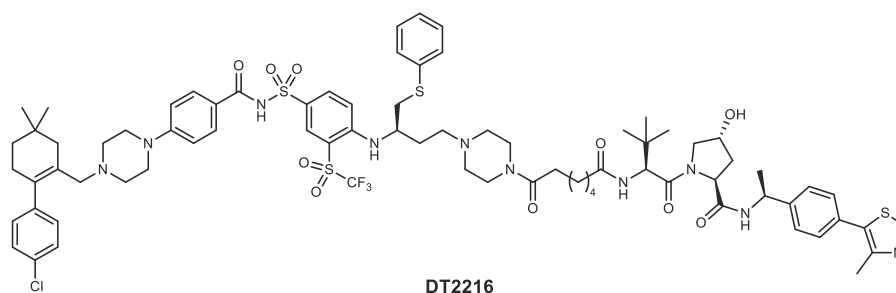
ARV-110 exhibits high potency of AR degradation and AR-associated gene suppression in both in-vivo and vitro studies, with half-maximal degradation concentration ( $DC_{50}$ ) below 1 nM [48]. It is currently under evaluation in phase II trial for further confirmation of its therapeutic potential (NCT03888612) [49].

ARV-471 (Figure 4B), also developed by Arvinas, underwent a clinical trial investigation as an estrogen receptor (ER) targeting degrader applied to ER<sup>+</sup>/HER2<sup>-</sup> breast cancer treatment. Preclinical studies have demonstrated that ARV-471 could lead to potent ER degradation in multiple ER-dependent breast cancer cell lines with  $DC_{50}$  of 0.9 nM [50]. According to the reported clinical data from the phase 1 trial, ARV-471 shows safety and tolerability and high clinical benefit rate (42%) through oral administration in late-line patients (NCT04072952). In combination with a CDK4/6 inhibitor palbociclib, ARV-471 exhibit 62% of mean ER degradation in metastatic tumors, suggesting promising anti-tumor activity and great potential to be a best-in-class ER-targeting therapy [49].



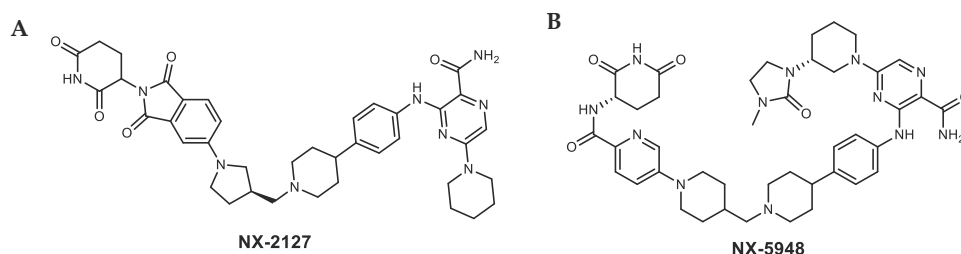
**Figure 4.** Chemical structures of (A) ARV-110 and (B) ARV-471.

In terms of targeting the anti-apoptotic proteins implicated in cancer progression, Dialectic Therapeutics reported the first PROTAC (DT2216) of selectively degrading B-cell lymphoma extra-large (BCL-XL) of the BCL-2 family in 2019 (Figure 5) [51]. ABT-263, a BCL-2/BCL-XL dual inhibitor previously limited by on-target toxicity and dose-limiting thrombocytopenia for traditional chemotherapy, was utilized in combination with a VHL ligand to construct DT2216. In-vitro studies have demonstrated that DT2216 can cause superior BCL-XL degradation and anti-tumor activity ( $EC_{50} < 10$  nM) compared to ABT-263 in multiple associated leukemia and tumor cells, while has less toxicity ( $EC_{50} > 3$   $\mu$ M) to human platelets than ABT-263. Platelets are spared from DT2216-mediated apoptosis due to the low expression of VHL E3 ligase. This allows DT2216 to selectively target cancer cells that express VHL E3 ligase. These in-vitro characteristics have been successfully translated into *in-vivo* cancer models, where DT2216 led to quick and potent tumor regression in the T-cell lymphoma xenografted mice without causing pronounced thrombocytopenia [51,52] With the significant efficacy in preclinical cell and tumor models, DT2216 was progressed to clinical trials and is currently under phase 1 evaluation for its safety, tolerability, and pharmacological activity in patients with relapsed/refractory malignancies (NCT04886622).[53].



**Figure 5.** Chemical structures of DT2216.

More recently, Nurix Therapeutics reported an oral CRBN-based PROTAC NX-2127 (Figure 6A) that targets Bruton's tyrosine kinase (BTK) degradation, suggesting a promising alternative approach for treatment of relapsed B-cell malignancies [54]. As a vital component of B-cell receptor signaling, BTK serves to regulate the proliferation, differentiation, and survival of B cells, while the overexpression of BTK is often found in malignant B-cell neoplasms associated with mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) [54]. In contrast with the previously mentioned PROTACs that can only target a single POI, NX-2127 is a triple-degrader that possesses degradation activity on not only BTK but also two additional therapeutic validated targets including transcription factors Ikaros family zinc finger protein (IKZF) 1 and 3. Preclinical *in vitro* and *in vivo* studies have demonstrated the potent and selective degradation of POIs by NX-2127. This triple degradation, combined with its immunomodulatory activity, results in superior anti-tumor efficacy against resistant B-cell malignancies compared to traditional small-molecule BTK inhibitors. [55]. Furthermore, another BTK degrader NX-5948 (Figure 6B) was designed by Nurix Therapeutics about one year after the announcement of NX-2127. First-in-human phase I clinical trials are currently underway in patients with relapsed/refractory CLL and primary central nervous system lymphoma to respectively evaluate the safety, tolerability and activity of NX-2127 (NCT04830137) and NX-5987 (NCT05131022). [56].



**Figure 6.** Chemical structures of (A) NX-2127 and (B) NX-5948.

## 5. Conclusion

Following the first demonstration of PROTACs ability to selectively degrade proteins in 2001, this novel technology has rapidly developed in academia, industry and the clinic. These techniques present a wealth of opportunities to discover previously unexploited or inaccessible target proteins and elucidate their functions. PROTACs can demonstrate significant degradation activity and, therefore, superior therapeutic efficacy to traditional drug treatment against multiple types of diseases in both preclinical investigation and clinical trials. Compared to small-molecule drugs functioning through the occupancy-driven mechanism, the distinct event-driven mechanism of action of PROTACs provides a range of advantages in their clinical applications, including substantial potential to target ‘undruggable’ proteins excluded in traditional drug discovery, increased level of selectivity and specificity, sub-stoichiometric dosage, and limited off-target effects.

Despite the encouraging advantages and performances of PROTACs in disease treatment, there are still several disadvantages and challenges that should be taken into account for the design and application of PROTACs as valid therapeutic agents. First, as heterobifunctional molecules with high molecular weights, the majority of PROTACs break many of Lipinski's rule of five which researchers use to explain their problematic pharmacodynamic and pharmacokinetic properties such as limited cell permeability, solubility, and oral bioavailability, therefore the optimization of PROTACs to clinical candidates and drugs remains a challenging, if achievable, goal.

Secondly, although the event-driven based PROTACs allow moderate-affinity ligands to be used for degrading ‘undruggable’ proteins without active functional pockets, it still remains challenging to identify appropriate structural elements on the POI surface for ligand recognition and simultaneous PPIs with E3 ligases. The limited number of reported PROTACs targeting ‘undruggable’ proteins highlights the need for further structural analysis of both protein-of-interest (POI) and E3 ligase molecules. This analysis can guide the exploration of new chemical space and optimize PROTAC design.

Thirdly, as discussed earlier PROTACs can reduce the risk of off-target effects due to their enhanced

selectivity and specificity. However, this point has not been fully investigated, so the potential of PROTACs to cause off-target effects by forming undesirable ternary complexes should not be underestimated. Furthermore, proteolysis products arising from PROTAC-induced target degradation could also engage proteins that are capable of inducing or attenuating additional physiological signaling, resulting in unexpected biological effects. For example, a study on BET-targeting PROTACs reveals that neo-amino-terminal peptides generated from induced BRD4 degradation, neutralize inhibitor of Apoptosis (IAP) proteins to drive caspase activation and cell apoptosis, which may explain the enhanced cytotoxicity of BET-targeting PROTACs over traditional inhibitors [57].

Nonetheless, having reached the clinic within just two decades of their discovery, PROTACs demonstrate remarkable progress. It is likely that some will soon be approved for clinical use. Indeed, this is an exciting time to be involved in this rapidly evolving field; it is expected that additional optimization and refinement of PROTACs will be achieved through the discovery of additional E3 ligase ligands for recruitment and by improving predictive in silico PROTAC technology. Finally, PROTAC research could benefit from the use of modern high-throughput-screening technology, such as the use of DNA encoded libraries, which would allow a large number of PROTACs to be synthesized with a variety of different E3 ligands and linkers. Indeed, early publications in this area demonstrate the feasibility of applying large encoded libraries to rapidly identify PROTACs [58].

**Author Contributions:** Writing—original draft preparation, J.X.; writing—review and editing, M.-L.M., J.K.S.N., S.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Acknowledgments:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Burslem, G.M.; Crews, C.M. Proteolysis-Targeting Chimeras as Therapeutics and Tools for Biological Discovery. *Cell* **2020**, *181*, 102–114.
- Liu, Z.; Hu, M.; Yang, Y.; et al. An overview of PROTACs: A promising drug discovery paradigm. *Mol. Biomed.* **2022**, *3*, 46.
- Békés, M.; Langley, D.R.; Crews, C.M. PROTAC targeted protein degraders: THE past is prologue. *Nat. Rev. Drug Discov.* **2022**, *21*, 181–200.
- Ciechanover, A. Proteolysis: FROM the lysosome to ubiquitin and the proteasome. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 79–87.
- Zhao, L.; Zhao, J.; Zhong, K.; et al. Targeted protein degradation: Mechanisms, strategies and application. *Signal Transduct. Target. Ther.* **2022**, *7*, 113.
- Sha, Z.; Zhao, J.; Goldberg, A.L. Measuring the Overall Rate of Protein Breakdown in Cells and the Contributions of the Ubiquitin-Proteasome and Autophagy-Lysosomal Pathways. *Methods Mol. Biol.* **2018**, *1844*, 261–276.
- Bard, J.A.M.; Goodall, E.A.; Greene, E.R.; et al. Structure and Function of the 26S Proteasome. *Annu. Rev. Biochem.* **2018**, *87*, 697–724.
- Bhole, R.P.; Kute, P.R.; Chikhale, R.V.; et al. Unlocking the potential of PROTACs: A comprehensive review of protein degradation strategies in disease therapy. *Bioorg. Chem.* **2023**, *139*, 106720.
- Middleton, A.J.; Day, C.L. *The molecular basis of lysine 48* ubiquitin chain synthesis by Ube2K. *Sci. Rep.* **2015**, *5*, 16793.
- Snyder, N. A.; Silva, G. M. Deubiquitinating enzymes (DUBs): Regulation, homeostasis, and oxidative stress response. *J. Biol. Chem.* **2021**, *297*, 101077.
- Liu, X.; Ciulli, A. DUB be good to me. *Nat. Chem. Biol.* **2022**, *18*, 358–359.
- Zhang, X.; Linder, S.; Bazzaro, M. Drug Development Targeting the Ubiquitin–Proteasome System (UPS) for the Treatment of Human Cancers. *Cancers* **2020**, *12*, 902.
- Li, D.; Yu, D.; Li, Y.; et al. A bibliometric analysis of PROTAC from 2001 to 2021. *Eur. J. Med. Chem.* **2022**, *244*, 114838.
- Martin-Acosta, P.; Xiao, X. PROTACs to address the challenges facing small molecule inhibitors. *Eur. J. Med. Chem.* **2021**, *210*, 112993.
- Bondeson, D.P.; Smith, B.E.; Burslem, G.M.; et al. Lessons in PROTAC Design from Selective Degradation with a Promiscuous Warhead. *Cell Chem. Biol.* **2018**, *25*, 78–87.



16. Zagidullin, A.; Milyukov, V.; Rizvanov, A.; et al. Novel approaches for the rational design of PROTAC linkers, *Explor. Target. Anti-tumor Ther.* **2020**, *1*, 381–390.
17. Bricelj, A.; Steinebach, C.; Kuchta, R.; et al. E3 Ligase Ligands in Successful PROTACs: An Overview of Syntheses and Linker Attachment Points. *Front. Chem.* **2021**, *9*, 707317.
18. Hendrick, C. E.; Jorgensen, J. R.; Chaudhry, C.; et al. Direct-to-Biology Accelerates PROTAC Synthesis and the Evaluation of Linker Effects on Permeability and Degradation. *ACS Med. Chem. Lett.* **2022**, *13*, 1182–1190.
19. Sakamoto, K. M.; Kim, K. B.; Kumagai, A.; et al. Deshaies, Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8554–8559.
20. Schneekloth, J. S.; Fonseca, F. N.; Koldobskiy, M.; et al. Chemical Genetic Control of Protein Levels: Selective in Vivo Targeted Degradation. *J. Am. Chem. Soc.*, **2004**, *126*, 3748–3754.
21. Schneekloth, A. R.; Pucheault, M.; Tae, H. S.; et al. Targeted intracellular protein degradation induced by a small molecule: En route to chemical proteomics. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5904–5908.
22. Zheng, N.; Shabek, N. Ubiquitin Ligases: Structure, Function, and Regulation. *Annu. Rev. Biochem.* **2017**, *86*, 129–157.
23. Itoh, Y.; Ishikawa, M.; Naito, M.; et al. Protein knockdown using methyl bestatin-ligand hybrid molecules: Design and synthesis of inducers of ubiquitination-mediated degradation of cellular retinoic acid-binding proteins. *J. Am. Chem. Soc.* **2010**, *132*, 5820–5826.
24. Lu, J.; Qian, Y.; Altieri, M.; et al. Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4. *Chem. Biol.* **2015**, *22*, 755–763.
25. Setia, N.; Almuqadi, H. T. A.; Abid, M. Journey of Von Hippel-Lindau (VHL) E3 ligase in PROTACs design: From VHL ligands to VHL-based degraders. *Eur. J. Med. Chem.* **2024**, *265*, 116041.
26. Zhang, X.; Crowley, V. M.; Wucherpfennig, T. G.; et al. Electrophilic PROTACs that degrade nuclear proteins by engaging DCAF16. *Nat. Chem. Biol.* **2019**, *15*, 737–746.
27. Han, T.; Goralski, M.; Gaskill, N.; et al. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science* **2017**, *356*, eaal3755.
28. Lu, M.; Liu, T.; Jiao, Q.; et al. Discovery of a Keap1-dependent peptide PROTAC to knockdown Tau by ubiquitination-proteasome degradation pathway. *Eur. J. Med. Chem.* **2018**, *146*, 251–259.
29. Luo, M.; Spradlin, J. N.; Boike, L.; et al. Chemoproteomics-enabled discovery of covalent RNF114-based degraders that mimic natural product function. *Cell Chem. Biol.* **2021**, *28*, 559–566.e15.
30. Chen, S.; Li, X.; Li, Y.; et al. Design of stapled peptide-based PROTACs for MDM2/MDMX atypical degradation and tumor suppression. *Theranostics* **2022**, *12*, 6665–6681.
31. Adhikari, B.; Bozilovic, J.; Diebold, M.; et al. PROTAC-mediated degradation reveals a non-catalytic function of AURORA-A kinase. *Nat. Chem. Biol.* **2020**, *16*, 1179–1188.
32. Chirnomas, D.; Hornberger, K. R.; Crews, C. M. Protein degraders enter the clinic—A new approach to cancer therapy. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 265–278.
33. Yang, W.; Saboo, S.; Zhou, L.; et al. Early evaluation of opportunities in oral delivery of PROTACs to overcome their molecular challenges. *Drug Discov. Today* **2024**, *29*, 103865.
34. Conde, J.; Artzi, N. Are RNAi and miRNA therapeutics truly dead? *Trends Biotechnol.* **2015**, *33*, 141–144.
35. Lazo, J. S.; Sharlow, E. R. Drugging Undruggable Molecular Cancer Targets. *Annu. Rev. Pharmacol. Toxicol.* **2016**, *56*, 23–40.
36. Li, X.; Song, Y. Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *J. Hematol. Oncol.* **2020**, *13*, 50.
37. Han, X.; Zhao, L.; Xiang, W.; et al. Discovery of Highly Potent and Efficient PROTAC Degraders of Androgen Receptor (AR) by Employing Weak Binding Affinity VHL E3 Ligase Ligands. *J. Med. Chem.* **2019**, *62*, 11218–11231.
38. Testa, A.; Lucas, X.; Castro, G. V.; et al. Fletcher and A. Ciulli, 3-Fluoro-4-hydroxyprolines: Synthesis, Conformational Analysis, and Stereoselective Recognition by the VHL E3 Ubiquitin Ligase for Targeted Protein Degradation, *J. Am. Chem. Soc.* **2018**, *140*, 9299–9313.
39. Lee, J.; Lee, Y.; Jung, Y. M.; et al. Discovery of E3 Ligase Ligands for Target Protein Degradation. *Molecules* **2022**, *27*, 6515.
40. Zeng, S.; Huang, W.; Zheng, X.; et al. Proteolysis targeting chimera (PROTAC) in drug discovery paradigm: Recent progress and future challenges. *Eur. J. Med. Chem.* **2021**, *210*, 112981.
41. Smith, B. E.; Wang, S. L.; Jaime-Figueroa, S.; et al. Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase. *Nat. Commun.* **2019**, *10*, 131.
42. Smalley, J. P.; Adams, G. E.; Millard, C. J.; et al. PROTAC-mediated degradation of class I histone deacetylase enzymes in corepressor complexes. *Chem. Commun.* **2020**, *56*, 4476–4479.
43. Smalley, J. P.; Baker, I. M.; Pytel, W. A.; et al. Optimization of Class I Histone Deacetylase PROTACs Reveals that HDAC1/2 Degradation is Critical to Induce Apoptosis and Cell Arrest in Cancer Cells, *J. Med. Chem.* **2022**, *65*, 5642–5659.
44. Koravovic, M.; Markovic, B.; Kovacevic, M.; et al. Protein degradation induced by PROTAC molecules as an emerging drug discovery strategy. *J. Serbian Chem. Soc.* **2022**, *87*, 785–811.
45. Bondeson, D. P.; Mares, A.; Smith, I. E. D.; et al. Catalytic in vivo protein knockdown by small-molecule PROTACs. *Nat Chem Biol.* **2015**, *11*, 611–617.
46. Graham, H. The mechanism of action and clinical value of PROTACs: A graphical review. *Cell. Signal.* **2022**, *99*,

- 110446.
47. Sun, X.; Rao, Y. PROTACs as Potential Therapeutic Agents for Cancer Drug Resistance. *Biochemistry* **2020**, *59*, 240–249.
  48. Snyder, L.B.; Neklesa, T.K.; Chen, X.; et al. Abstract 43: Discovery of ARV-110, a first in class androgen receptor degrading PROTAC for the treatment of men with metastatic castration resistant prostate cancer. *Cancer Res.* **2021**, *81*, 43–43.
  49. Xie, H.; Liu, J.; Alem Glison, D.M.; et al. The clinical advances of proteolysis targeting chimeras in oncology. *Explor. Target. Anti-Tumor Ther.* **2021**, *2*, 511–521.
  50. Flanagan, J.; Qian, Y.; Gough, S.; et al. Abstract P5-04-18: ARV-471, an oral estrogen receptor PROTAC degrader for breast cancer. *Cancer Res.* **2019**, *79*, P5-04.
  51. Khan, S.; Zhang, X.; Lv, D.; et al. A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* **2019**, *25*, 1938–1947.
  52. He, Y.; Koch, R.; Budamagunta, V.; et al. DT2216—A Bcl-xL-specific degrader is highly active against Bcl-xL-dependent T cell lymphomas. *J. Hematol. Oncol.* **2020**, *13*, 95.
  53. He, Y.; Koch, R.; Budamagunta, V.; et al. Weinstock and D. Zhou, DT2216, a BCL-XL Proteolysis Targeting Chimera (PROTAC), Is a Potent Anti T-Cell Lymphoma Agent That Does Not Induce Significant Thrombocytopenia. *Blood* **2019**, *134*, 303.
  54. Robbins, D.W.; Kelly, A.; Tan, M.; et al. Nx-2127, a Degradator of BTK and IMiD Neosubstrates, for the Treatment of B-Cell Malignancies. *Blood* **2020**, *136*, 34.
  55. Noviski, M.A.; Ma, J.; Lee, E.; et al. Abstract 1126: Concurrent degradation of BTK and IMiD neosubstrates by NX-2127 enhances multiple mechanisms of tumor killing. *Cancer Res.* **2022**, *82*, 1126.
  56. Robbins, D.W.; Noviski, M.; Rountree, R.; et al. Nx-5948, a Selective Degradator of BTK with Activity in Preclinical Models of Hematologic and Brain Malignancies. *Blood* **2021**, *138*, 2251.
  57. Chen S.-H.; Prakash, S.; Helgason, E.; et al. Constitutive protein degradation induces acute cell death via proteolysis products. *bioRxiv* 2023. <https://doi.org/10.1101/2023.02.06.527237>.
  58. Chen, Q.; Liu, C.; Wang, W.; et al. Optimization of PROTAC ternary complex using DNA encoded library approach. *ACS Chem. Biol.* **2023**, *18*, 25–33.