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Small-Molecule PROTACS: New Approaches to Protein Degradation

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The current inhibitor-based approach to therapeutics has inherent limitations owing to its occupancy-based model: 1) there is a need to maintain high systemic exposure to ensure sufficient in vivo inhibition, 2) high in vivo concentrations bring potential for off-target side effects, and 3) there is a need to bind to an active site, thus limiting the drug target space. As an alternative, induced protein degradation lacks these limitations. Based on an event-driven model, this approach offers a novel catalytic mechanism to irreversibly inhibit protein function by targeting protein destruction through recruitment to the cellular quality control machinery. Prior protein degrading strategies have lacked therapeutic potential. However, recent reports of small-molecule-based proteolysis-targeting chimeras (PROTACs) have demonstrated that this technology can effectively decrease the cellular levels of several protein classes.

1. Introduction

Traditional small-molecule drugs have been limited to proteins with a tractable ligand binding site such as receptors and enzymes. Since only a small portion (ca. 25%) of the human proteome is pharmaceutically accessible based on contemporary drug strategies, the majority of proteins, for example, transcription factors, scaffolding proteins, and non-enzymatic proteins, remain "undruggable".[1] This is a product of the current occupancy-driven pharmacology model, which based on the idea that the longer a drug occupies and blocks an active site, the greater the clinical effect achieved. However, in addition to limiting drug targets to those proteins with an active site, occupancy-driven pharmacology requires high drug dosing in order to achieve adequate IC_{90-95} concentrations for therapeutic efficacy, which results in potential off-target binding and unwanted side effects.

In the past two decades, nucleic acid based strategies have been employed to knock down or decrease the expression of disease-causing proteins. However, while genetic knockdown techniques such as antisense oligonucleotides and RNAi can abolish or decrease protein levels, metabolic, biodistribution, and off-target issues have limited their therapeutic utility.[2] A novel approach to address these challenges, which combines the ADME properties of small molecules and the advantages of genetic knockdown techniques, is the small-molecule control of intracellular protein levels through recruiting targeted proteins to the ubiquitin/proteasome system (UPS) for selective degradation.[3] While E3 ubiquitin ligase recruitment by proteolysis-targeting chimeras (PROTACs) has been reported previously, these approaches primarily relied on peptidic ligands, which limited their therapeutic potential.[3b] In this Minireview, we discuss the recent progress in developing small-molecule-based PROTACs to degrade targeted proteins, as well as the therapeutic opportunities and challenges of this technology.

2. Early "Small Molecule" Inducers of Protein Instability

In an effort to develop new molecular tools and probes that could modulate intracellular protein stability, our group and others have reported different approaches to engage the UPS. These include the use of heat-shock protein 90 (HSP90) inhibitors (recently reviewed elsewhere),[4] and approaches that require genetic fusion to the target protein, for example, the use of a destabilizing domain (DD), ligand-induced degradation (LID), and hydrophobic tagging (HyT). All of these methods have served as useful chemical biology tools in studies of protein function and target validation.[3d,5]

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2.1. Destabilizing Domain (DD) and Ligand-Induced Degradation (LID) Technology: Shield-1

The fact that different proteins have different intracellular half-lives has been exploited to generate fusion proteins with tunable stability. Pioneering work from the Pickart and Deshaies groups employed this strategy to target or rescue Ub fusion protein from degradation.[5] Proteins can also be directly targeted for degradation when fused recombinantly with a destabilizing protein domain known as a degron. Since direct ligand binding affects protein stability through a number of mechanisms, one powerful technology to tune protein stability in cells involves the use of a small molecule that binds and stabilizes a degron, thereby rescuing the fusion protein from degradation (Figure 1).[6] For example, a constitutively unstable FKBP12–YFP fusion protein is stabilized in the presence of a high-affinity FKBP12 ligand termed Shield-1[7,8] In a follow-up study, a FKBP-based LID domain, the stability of which is inversely regulated by Shield-1, was reported. This alternative approach has the opposite effect of the DD technology: the addition of Shield-1 leads to degradation of the fusion protein. This strategy uses the tethering of FKBP to a degron sequence, along with a proline-rich sequence specifically designed to bind to the active site of FKBP. In the absence of Shield-1, the peptide appended to the C terminus of FKBP functions as a mask (cryptic degron). Shield-1 binding to FKBP exposes the degron, thereby leading to proteasome degradation of the fusion protein.[9] This system has proven quite general and has been utilized in numerous chemical genetics studies, for example, in the study of dendritic proteins[10] and transcription factors.[11] However, a distinct disadvantage of this approach for consideration as a therapeutic strategy is the need to genetically engineer and introduce the fusion protein into host cells/animals.

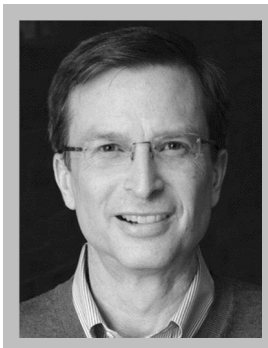
More recently, a new method that makes use of an auxin-inducible degron (AID) and similar to the LID system has been described. A photoactivatable auxin derivative is able to release auxin upon local illumination. Upon release, auxin binds to an artificial ubiquitin ligase and recruits the auxin-inducible degron fused with the target protein. This process is then followed by degradation of the protein of interest (POI) by the UPS.[12] In this manner, additional temporal and subcellular control of protein degradation permits finer control of intracellular protein function, although this system still requires the generation of fusion proteins.

2.2. Hydrophobic Tagging (HyT)

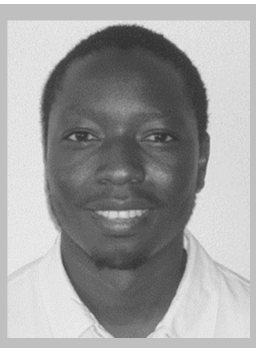
Based on the success of specific degrons, our group sought to develop "chemical degrons," or small-molecules that would directly mimic the unfolded state of a protein. This strategy, known as hydrophobic tagging (HyT), was first explored by using a proof-of-concept system consisting of chloroalkyl hydrophobic ligands that are capable of inducing the degradation of proteins fused to the dehalogenase HaloTag2.[13] This hydrophobic tag is believed to mimic a partially denatured protein folding state, recruiting chaperones to the protein of interest and thereby leading to chaperone-mediated degradation by the proteasome (Figure 2). HyTs with the hydrophobic adamantyl group were found to potently induce the degradation of numerous proteins (GFP, luciferase, $HRas1^{G12V}$, ROR2) fused to HaloTag2. Moreover, the HyT approach was effective in degrading a HRas–HaloTag2 fusion protein in vivo, which led to a reduction in Ras-driven tumor growth in mice.[14] Interestingly, we also identified a small molecule (HALTS) that is capable of stabilizing HaloTag2 fusion proteins.[14c] Thus, between HyT-induced degradation and HALTS-mediated stabilization, it is possible to modulate the intracellular stability of a single HaloTag fusion protein of interest over a 50-fold dynamic range.

Non-adamantyl moieties have also been used to confirm the concept of hydrophobic tags as "greasy tags" capable of inducing protein degradation.[15] Hedstrom and colleagues have shown that Boc-protected arginine (Boc_3-Arg) can also serve as a hydrophobic tag.[16] While significantly larger than adamantane, Boc_3-Arg can induce the degradation of glutathione-S-transferase (GST) and *E. coli* dihydrofolate reductase (DHFR) when coupled to corresponding ligands. This was the first demonstration that hydrophobic tags do not need to be covalently coupled to their cognate target protein in order to induce protein degradation.

More recently, hydrophobic tagging was employed to induce the degradation of the currently undruggable cancer target protein HER3. In this case, a potent ligand of HER3 (TX2-121-1) was coupled to an adamantane moiety via a short linker.[17] Biological data for these adamantane-tagged compounds revealed HER3 knockdown at 2 μM in PC9 GR4 cells, which resulted in decreased downstream signaling through phosphorylated Akt (p-Akt) and p-Erk upon neuregulin stimulation. Adamantyl-tagged TX2-121-1 shows antiproliferative activity, inhibiting cell growth seven-fold more than



Craig M. Crews studied chemistry at the University of Virginia and received his doctorate from Harvard University under the supervision of R. L. Erikson. Following a postdoctoral fellowship with S. L. Schreiber, he joined the Yale faculty in 1995. His research is focused on the small-molecule control of intracellular protein levels and led to the development of carfilzomib/Kyprolis, an FDA-approved next-generation proteasome inhibitor based on the lead compound YU101, which was synthesized in his laboratory.



Momar Toure studied chemistry at ENSCM, France (M.Sc. 2010) and completed his PhD in the research group of Dr. J.-L. Parrain and Dr. O. Chuzel at Aix-Marseille Université, France in 2013. He is currently a postdoctoral research associate in Prof. Crews' group at Yale University and works on the PROTAC technology.

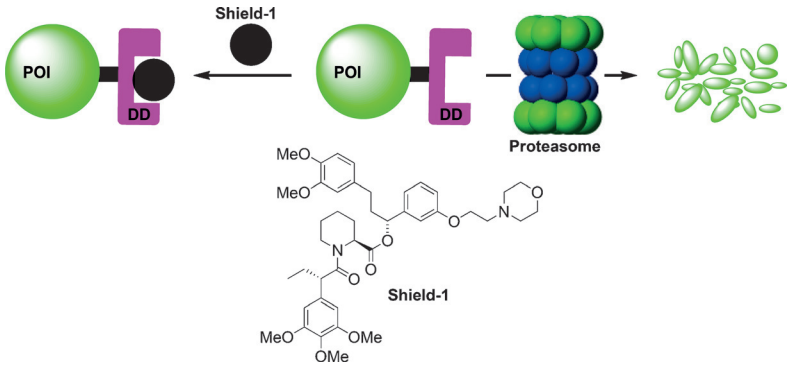


Figure 1. Shield-1 is used to stabilize mutant/fusion FKBP proteins. DD = destabilization domain, POI = protein of interest.

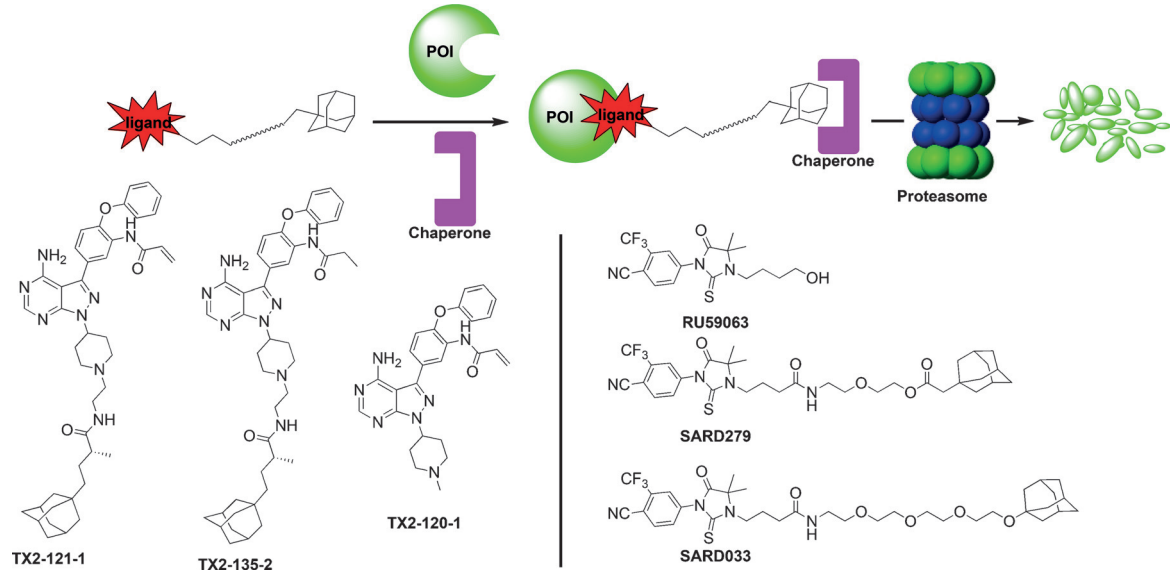


Figure 2. The hydrophobic tagging (HyT) technology and chemical structures of representative HyTs.

the negative control compound TX2-135-2.[17] Although these findings represent the first application of the HyT technology to the targeting of an important undruggable oncology pseudokinase protein (HER3) for degradation, the need for covalent binding and the low potency, which is in the single-digit μM range, represent key limitations for the future clinical advancement of a hydrophobic tagged TX2-121-1.

Given its role in prostate and breast cancer, the androgen receptor (AR) is the target of numerous antitumor chemotherapeutic agents, including the AR antagonist enzalutamide. With the success of HER3 degradation when using HyT technology, the AR was next targeted in order to develop a novel class of selective androgen receptor degraders (SARDs). Recently, our group reported the first small-molecule SARDs that mediate degradation of the androgen receptor through hydrophobic tagging.[18] The known AR agonist RU59063[19] was coupled via a short PEG linker to an adamantane moiety. The resulting compound SARD279 has a short linker length (8 atoms) and was able to induce 50% AR degradation at a concentration of 1 μM (the DC_{50} value), while SARD033, which possesses a longer ether linker (13 atoms) was half as potent. This finding highlights the fact that linker composition and length play important roles in optimal target protein recruitment and appropriate physicochemical properties in SARDS. SARD279 suppressed cell growth with similar efficacy to enzalutamide.[18] Despite the efficacy of enzalutamide against AR-driven castration-resistant prostate cancer (CRPC), patients usually relapse due to resistance attributed to the reactivation of AR signaling. Importantly, SARD-mediated degradation of AR could circumvent this resistance associated with enzalutamide.

3. PROTAC Technology

Like HyT molecules, proteolysis-targeting chimeras (PROTACs) are heterobifunctional compounds with two recruiting ligands connected via a linker. One ligand is specific to the protein of interest (POI) while the other moiety specifically recruits an E3 ligase. The PROTAC thus forms a ternary complex upon binding to both its E3 ligase target and the protein of interest. By hijacking the E3 ligase,

PROTACS position the POI in a spatially favorable presentation to facilitate substrate poly-ubiquitination, thereby selectively knocking down levels of the targeted protein (Figure 3).[20] An advantage of this approach is that unlike small-molecule inhibitors, PROTACs can potentially act catalytically by facilitating multiple rounds of POI degradation (Figure 3).

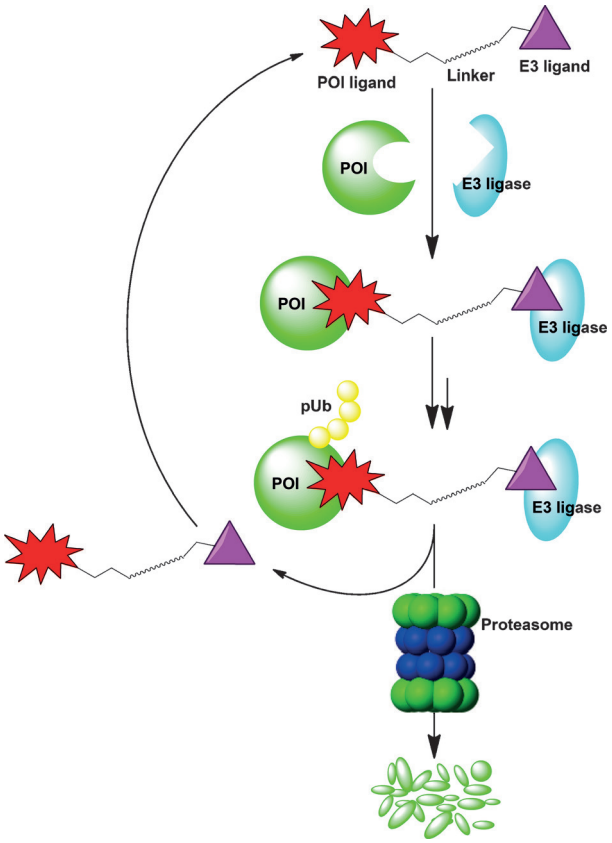


Figure 3. Direct recruitment of an E3 ligase by using the PROTACs.

3.1. Peptidic PROTACs

Given a dearth of small-molecule ligands for E3 ubiquitin ligases, first-generation PROTACs were based on large peptide motifs, for example, the degron for IkBα, a protein degraded by the SKP-Cullin-F-Box E3 ligase βTRCP.[21] While these provided the first proof of concept for the PROTAC technology, the peptidic E3 recruiting moiety of these early PROTACs lacked cell permeability, thus limiting their utility as a chemical probe.[22]

To address this issue, the first cell-permeable PROTACs were developed by coupling a poly-D-Arg sequence to a 7-amino acid sequence derived from HIF (ALAPYIP) that binds to the E3 ligase complex VHL (von Hippel Lindau); this peptide was then conjugated to a known "bump-hole" ligand (AP21998) of the FKBP12 F36V mutant.[23] The resulting PROTAC effectively degraded the fusion protein GFP-FKBP12 (F36V) at 25 μM in cells.[24] Interestingly, subsequent related peptidic PROTACs have been successfully used to target the estrogen receptor, as well as the aryl hydrocarbon receptor, without the need for a poly-D-Arg chain.[25]

Similarly, another cell-permeable all-peptidic PROTAC that antagonizes and destroys the X-protein of the hepatitis B virus (HBV) was later reported. The oxygen-dependent degradation (ODD) domain of HIF-1α recognized by the VHL E3 ligase was conjugated to the X-protein oligomerization domain with an additional polyarginine cell-penetrating peptide. The corresponding peptidic PROTACS caused almost complete degradation of both the full-length and truncated forms of the X-protein.[26]

Inspired by early work on phosphopeptidic PROTACs, an all-peptide-based PhosphoPROTAC ($^{ErbB2}PP_{PI3K}$) was designed to induce conditional knockdown of PI3K upon growth-factor stimulation (Figure 4). Like earlier PROTACs,

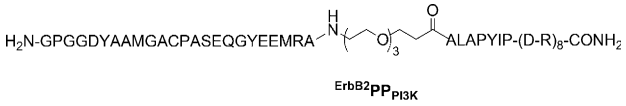


Figure 4. A VHL-based peptidic PhosphoPROTAC that binds VHL.

this degradation molecule consisted of the VHL peptidic binding fragment and a poly-D-Arg sequence to enhance cell permeability. However, instead of a small-molecule ligand to recruit a target protein, a phosphoPROTAC uses a "conditional" protein ligand, that is, one that binds its cognate partner only upon the addition of growth factor to the cells. This is accomplished through the incorporation of a peptide sequence from a receptor tyrosine kinase (e.g., ErbB3) that contains a phosphorylation site, which serves as a binding site for a downstream signaling component (e.g., PI3K). In a proof-of-concept experiment, the growth factor neuregulin induced the phosphorylation of both ErbB3 and the ErbB3 phosphorylation site on the phosphoPROTAC, thus resulting in the recruitment, ubiquitination, and proteasome-mediated degradation of PI3K. Moreover, in a mouse xenograft model, treatment with $^{ErbB2}PP_{PI3K}$ led to a 40% reduction in tumor size,[27] which represents the first demonstration of in vivo PROTAC activity. Despite the promising in vivo antitumor activity, phosphoPROTACs are unlikely to serve as good drug candidates owing to their high molecular weight, relatively poor cell permeability, and potential metabolic instability.

3.2. "Small-Molecule" PROTACs

Although the first-generation peptidic PROTACs lacked traditional drug-like properties, they proved that targeting E3 ligases to modulate protein half-life is a viable strategy for potential drug development. The next step in the development of PROTACs as a therapeutic strategy would require conversion into all-small-molecule, more drug-like drug candidates. These would have several advantages over previous peptidic PROTACs: better in vivo stability, better biodistribution, and potentially better potency. Fortunately, the development and incorporation of small-molecule ligands for several E3 ligases (MDM2, clAP1, CRBN (cereblon) and

VHL) have significantly advanced the PROTAC technology as a viable therapeutic strategy.

3.2.1. Recruiting the E3 Ubiquitin Ligase MDM2

The first entirely small-molecule PROTAC employed the known MDM2 ligand nutlin as an E3 ligase recruiting moiety.[28] (Figure 5). Coupling nutlin to an androgen receptor ligand yielded a PROTAC capable of inducing the degradation of the androgen receptor in prostate tumor cells. While promising as a critical first step away from peptide-based PROTACs, this initial small-molecule inducer of degradation proved to be less effective than its peptidic analogues.[29] Whether its potency could be improved through linker optimization remains to be determined.

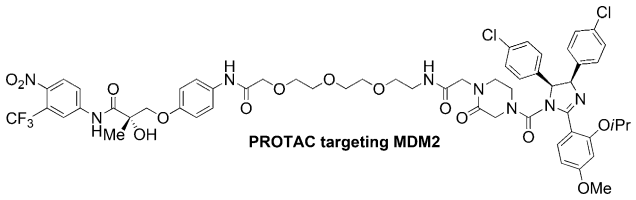


Figure 5. A small-molecule PROTAC that recruits the MDM2 E3 ligase.

3.2.2. Recruiting the E3 Ubiquitin Ligase cIAP1

More recently, the Hashimoto group used bestatin esters, which bind the cellular inhibitor of apoptosis protein 1 (cIAP1), to successfully induce the degradation of target proteins (Figure 6).[30] These bestatin-based PROTACs were found to effectively induce the degradation of nuclear receptors, such as the androgen receptor, the estrogen receptor, and the retinoic acid receptor.[31] However, this new class of small-molecule PROTACs has several key limitations. Notably, numerous off-target effects were observed, which were attributed to the bestatin moiety, a common aminopeptidase inhibitor.[32] In addition, it was shown

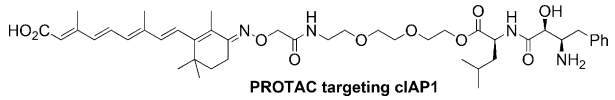


Figure 6. A small-molecule PROTAC that recruits the cIAP1 E3 ligase.

that high concentrations are needed to effectively degrade targeted proteins.[31] Finally, the IAP ligands used are known to often induce IAP autoubiquitination and degradation, which could be a concern for their use in therapeutic PROTACs.

3.2.3. Recruiting the E3 Ubiquitin Ligase CRBN

Although the first reported all-small-molecule PROTACS targeting cIAP1 and MDM2 E3 ligases were able to induce numerous proteins for degradation, these PROTACs were only active in the double-digit μM range. Therefore, there was a strong incentive to design better PROTACs in terms of potency and target selectivity. One such PROTAC series targeting the oncoprotein BRD4 uses the phthalimide family to hijack the E3 ubiquitin ligase cereblon (CRBN), a component of a cullin–RING ubiquitin ligase (CRL) complex (Figure 7). In recent years, interest in thalidomide and its derivatives lenalidomide and pomalidomide has intensified as a result of their utility as potent immunomodulatory drugs (IMiD). A recent study found that thalidomide binds to the E3 ligase cereblon, thereby inducing the degradation of IKZF1and IKZF3, which are essential transcription factors in multiple myeloma.[33] This suggests that thalidomide and its derivatives could be starting ligands for new PROTACs targeting the E3 ligase CRBN.

Starting with the potent BRD4-targeting drug candidate OTX015, a small-molecule PROTAC was developed to target the degradation of this protein, which is known to be critical for cancer cell growth and survival. This PROTAC (ARV-825) consists of OTX015 coupled to pomalidomide via a short alkyl linker.[34] ARV-825 caused almost complete BRD4 protein degradation at 10 nM within 6 h, and the effect was long lasting (greater than 24 h). It is therefore active at a substoichiometric concentration in mediating BRD4 degradation since the BRD4- and cereblon-binding motifs in ARV-825 have K_d values of 28–90 nM and approximately 3 μM with their respective targets. This finding represents some of the first evidence supporting the catalytic nature of PROTACs. Interestingly, the so-called "hook effect", which is characteristic of the mechanism by which degradation is dependent on PROTAC-mediated ternary-complex formation, was observed. A negative control PROTAC was obtained by methylation of the NH group in the piperidione fragment to afford the N-methylated ARV-825, which is

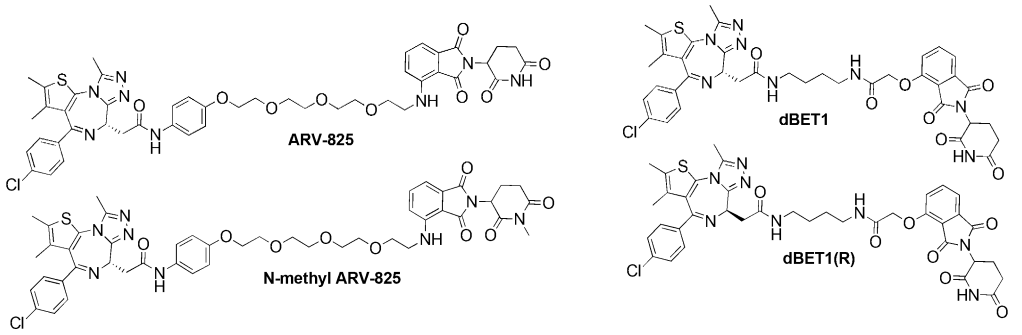


Figure 7. Small-molecule PROTACs that recruit the CRBN E3 Ligase.

incapable of either binding CRBN or promoting BRD4 degradation. Interestingly, further assays confirmed that ARV-825 has both antiproliferative and apoptosis-inducing advantages over the Brd4 inhibitors JQ1 and OTX015. These findings indicate that cereblon-based PROTACs provide a better and more efficient strategy for targeting BRD4 than current small-molecule inhibitors.

A similar PROTAC was generated using the BRD4 ligand JQ1 coupled to thalidomide.[35,36] The resulting PROTAC, called dBET1, induced 87% BRD4 degradation at 100 nM in the human AML cell line MV4-11 after 18 h, whereas the inactive dBET1(R) epimer did not. As expected, given the lack of binding specificity of JQ1, dBET1 also induced the degradation of other BET family members (e.g., BRD2 and BRD3). The pharmacological properties of dBET1 were further evaluated in proliferation assays; interestingly, BRD4 degradation suppressed growth in several lymphoma cells and was superior in efficacy to the parent inhibitor JQ1. Moreover, it was shown in a xenograft mouse model of human MV4-11 leukemia cells, that daily treatment with dBET1 led to tumor regression in 14 days. However, BRD4 protein levels partially recovered 24 h after treatment with dBET1, possibly as a result of PROTAC instability, a known liability of phthalimides. This finding could be a potential problem for the future development of dBET1 as a therapeutic agent in the clinic.

Interestingly, while ARV-825 is structurally similar to dBET1, it is 10-fold more potent than dBET1 in terms of inducing BRD4 degradation. Whether this is due to the phenyl ring in ARV-825 or other aspects of its linker warrants further investigation with an extensive linker optimization study.

3.2.4. Recruiting the E3 Ubiquitin Ligase von Hippel–Lindau Protein (VHL)

While initial VHL-based peptidic PROTACs proved successful in degrading targeted proteins, the move to small-molecule PROTACs that recruit VHL proved challenging given the lack of high-affinity small-molecule ligands for this E3 ligase. Fortunately, several recent studies report small molecules capable of competitively binding to the primary HIF-binding site on VHL.[37] With these ligands available, several PROTACs that engage VHL have now been reported. The first targeted ERRα, an orphan nuclear hormone receptor (Figure 8), by using a thiazolidinedione-based[38] selective ERRα ligand conjugated to a VHL ligand. This PROTAC was shown to induce 50% degradation of its target protein at 100 nM (the DC_{50} value). Importantly, this PROTAC proved to be active in vivo; treatment with the PROTAC reduced ERRα levels roughly 44%, 44%, and 39% in mouse heart, kidney, and MDA-MB-231 xenografted tumors, respectively.[39]

Based on these promising results, another PROTAC was developed to target the serine/threonine kinase RIPK2, an important mediator of innate immune signaling. A parent RIPK2 ligand was connected via a 12-atom linker to the VHL ligand to yield the PROTAC RIPK2, which effectively mediated ubiquitination and subsequent proteasome down-regulation of RIPK2 with a maximal degradation ability (D_{max}) of 95% at concentrations as low as 10 nM. The negative control epimer PROTAC_RIPK2_epi did not degrade RIPK2. Unlike CRBN-based PROTACs, VHL PROTACs have a perfect control construct (hydroproline epimer) that allows a more accurate comparison between degradation and inhibition. In addition, maximal RIPK2 knockdown with PROTAC_RIPK2 was achieved as early as 4 h, which is remarkably fast given that the natural half-life of the protein is approximately 60 h.

A key but previously unproven assumption of the PROTAC technology was its theoretical capacity to mediate multiple rounds of target protein ubiquitination, thus serving to catalytically induce protein degradation. While hints of this were provided by the PROTACS that potently degraded target proteins with DC_{50} values below their respective binding affinities for the target or E3 ligase, more definitive

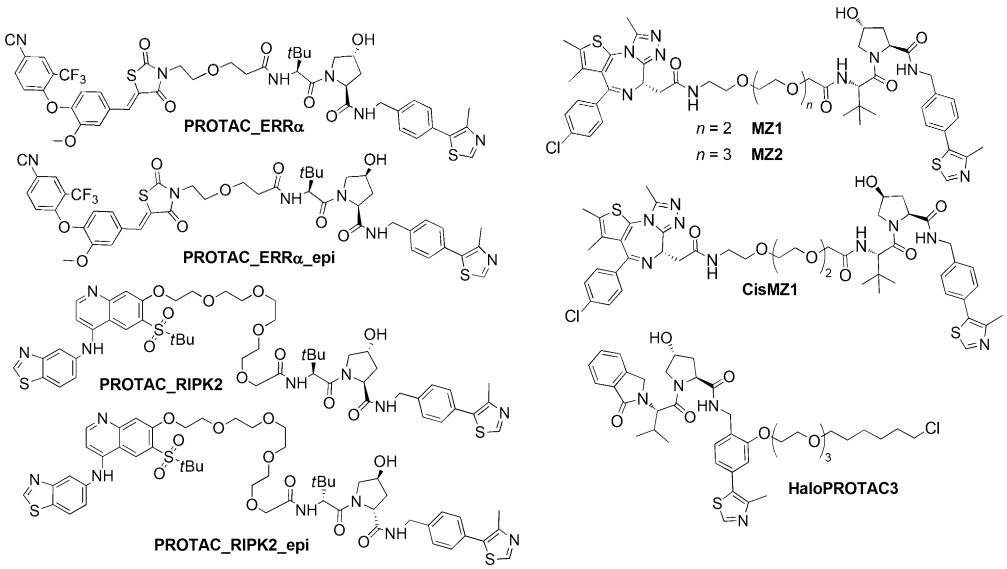


Figure 8. Small-molecule PROTACs that recruit the VHL E3 ligase.

proof was missing. Bondeson et al. directly addressed the question of catalysis by using a reconstituted in vitro ubiquitination assay. One pmole of PROTAC_RIPK2 was shown to result in 3.4 pmoles of ubiquitinated RIPK2, which corresponds to a 3.4 stoichiometry.[39] This result clearly confirms the substoichiometric catalytic nature of PROTACs, which is a key advantage over traditional small-molecule inhibitors.

Small-molecule VHL ligands have also been used in PROTACs targeting BRD4. The BRD4 inhibitor JQ1 was linked to a VHL ligand to afford PROTACs MZ1 and MZ2.[40] While both PROTACS induce degradation of BRD4 within 24 h, MZ1, which possesses a shorter PEG linker, exhibited higher efficacy. This finding demonstrates once again that linker composition and length are crucial variables in determining the potency of PROTACs.[41] Interestingly, only partial degradation of BRD2 and BRD3 was observed after 24 h with these compounds,[40] in contrast with the cereblon-based BRD4-targeting PROTACs ARV-825[34] and dBET1.[36] These conflicting results raise the question of whether recruiting different E3 ligases can lead to different selectivity profiles. However, even though MZ1 (90% BRD4 reduction at 100 nM) is less potent than ARV-825 in decreasing BRD4 levels, it is clearly as potent as dBET1. These observations indicate that at least with regards to targeting BRD4, either VHL or CRBN E3 ligases can be recruited for efficient PROTAC-mediated degradation. Nevertheless, a head-to-head comparison of the two E3 ligases could help to better inform which E3 ligase to choose for next-generation PROTACs in order to gain increased potency and selectivity.

To begin to address the role that the linker plays in optimal PROTAC-mediated protein degradation, a simplified chemical biology assay was recently reported whereby various VHL-based PROTACs were tested for their ability to induce the degradation of intracellular GFP fused to HaloTag7.[13a] Various linker lengths as well as linker attachment points were explored and the best molecule, HaloPROTAC3, was found to induce more than 90% GFP–Halotag7 degradation, with 50% degradation by 6 h. While this exercise generated a very potent PROTAC, optimal linker lengths of future PROTACs are expected to be highly target dependent and will therefore need to be determined on a case-by-case basis. Impressively, HaloPROTAC3 was found to induce complete knockdown of important kinases fused to HaloTag7 (HaloTag7–ERK1 and HaloTag7–MEK1) at 500 nM.[42] Together, these findings suggest that the HaloPROTAC3 system could serve as an alternative to the widely used Shield-1 technology as a new molecular tool and chemical probe of the biological roles of target proteins.

4. Conclusions and Outlook

In the last decade, "hijacking" E3 ligases with PROTACs to induce targeted protein degradation has emerged as a viable therapeutic strategy. Numerous oncology target proteins, including the androgen receptor, estrogen receptor, BRD4, ERRα, and RIPK2, have been successfully targeted and degraded by using this novel technology, which has proven to be more robust than other induced-degradation strategies such as hydrophobic tagging. In addition, a recent study highlighted the catalytic nature of PROTACs, suggesting that PROTAC concentrations lower than those required for a simple inhibitor could be sufficient to achieve the equivalent therapeutic effects. The PROTAC strategy holds much promise for targeting those proteins that are currently "undruggable" in that they either lack an active site for an inhibitor to bind or have a scaffolding function that is not addressable by an inhibitor, for example, pseudokinases. Nevertheless, much work remains to be done in order to advance PROTACs into the clinic. Challenges such as optimal linker-length determination, manipulation of linker composition to achieve optimal physicochemical properties, and removal of metabolic "hotspots" will need to be determined for each target protein. However, these issues fall well within the realm of modern medicinal chemistry and while not trivial, they are not insurmountable, which means that advancing the PROTAC technology closer to the clinic should be achievable.

Acknowledgments

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