

Recent advances in IAP-based PROTACs (SNIPERs) as potential therapeutic agents

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ABSTRACT

Proteolytic targeting chimaeras (PROTACs) have been developed as an effective technology for targeted protein degradation. PROTACs are heterobifunctional molecules that can trigger the polyubiquitination of proteins of interest (POIs) by recruiting the ubiquitin-proteasome system, thereby inhibiting the intracellular level of POIs. To date, a variety of small-molecule PROTACs (CRBN, VHL, IAP, and MDM2-based PROTACs) have been developed. IAP-based PROTACs, also known as specific and nongenetic IAP-dependent protein erasers (SNIPERs), are used to degrade the target proteins closely related to diseases. Their structures consist of three parts, including target protein ligand, E3 ligase ligand, and the linker between them. So far, many SNIPERs have been extensively studied worldwide and have performed well in multiple diseases, especially cancer. In this review, we will present the most relevant advances in the field of SNIPERs and provide our perspective on the opportunities and challenges for SNIPERs to become therapeutic agents.

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1. Introduction

Traditional small-molecule drugs have dramatically changed the face of diseases treatment in the last few decades. A variety of inhibitors, agonists, and antagonists have entered clinical use^{1–3}. They are characterised by more acceptable pharmacokinetic properties and desirable oral bioavailabilities, as well as lower manufacturing costs. However, traditional small-molecule drugs still have many weaknesses that limit their application on non-pharmacological proteins such as transcription factors, scaffolding proteins, and non-enzymatic proteins. In terms of mode of action, traditional small-molecule drugs exert their effects by occupying active pocket sites, which requires high doses of administration to maintain activities. This increases the risk of off-targeting and leads to adverse effects. Moreover, refractory drug targets do not bind effectively to traditional small-molecule drugs. Furthermore, genetic mutations often lead to changes in protein conformation that can result in resistance to traditional small-molecule drugs. In addition, sustained inhibition of the target proteins may lead to compensatory overexpression of the proteins, which may greatly increase the risk of acquired drug resistances^{4–6}. Therefore, it is of great importance to develop new technologies to address these issues.

Alternatively, a new technology for targeting diseases-causing proteins is emerging. Proteolytic targeted chimaeras (PROTACs) are a new technique for targeted degradation of proteins of interest (POIs) that have attracted increasing research interest in recent years. PROTACs utilise ubiquitin-proteasome system (UPS) to

degrade POIs^{7–10}. During degradation, their E3 ligase ligands hijack the E3 ligase and label POIs with ubiquitin. PROTACs are fundamentally different from small-molecule drugs. Traditional small-molecule drugs bind specifically to the cavity of the target proteins in an occupation-driven manner. In contrast, PROTACs are event-driven and induce ubiquitination in a transient binding manner to eliminate the diseases-causing proteins. PROTACs themselves are not degraded, they are recycled to facilitate ubiquitination and degradation of other target proteins (Figure 1). Importantly, this mode of action avoids a high level of drug administrations and the corresponding adverse effects. Moreover, to some extent, PROTACs can solve the problem of acquired drug resistances caused by POIs mutations and conventional inhibitors. Thus, PROTACs can be used to overcome the common drawbacks of traditional occupation-driven small-molecule drugs^{11–13}.

The concept of PROTACs was proposed by Craig M. Crews and Raymond J. Deshaies in 2001¹⁴. The first-generation of PROTACs were heterofunctional molecules consisting of angiogenesis inhibitors and 10-amino acid phosphopeptides, which had the disadvantage of low cell permeability and stability in biological systems¹⁵. In order to solve the above issues, Craig M. Crews *et al.* developed the second-generation of PROTACs (small-molecule PROTACs) capable of successfully inducing androgen receptor (AR) degradation in 2008¹⁶. These PROTACs associated selective AR modulators with nutlin, recruiting the E3 ubiquitin ligase human homolog mouse double molecule 2 (MDM2). Recently, the third-generation of PROTACs with cereblon (CRBN), von Hippel-Lindau (VHL), and inhibitors of apoptosis (IAP) ligands have been

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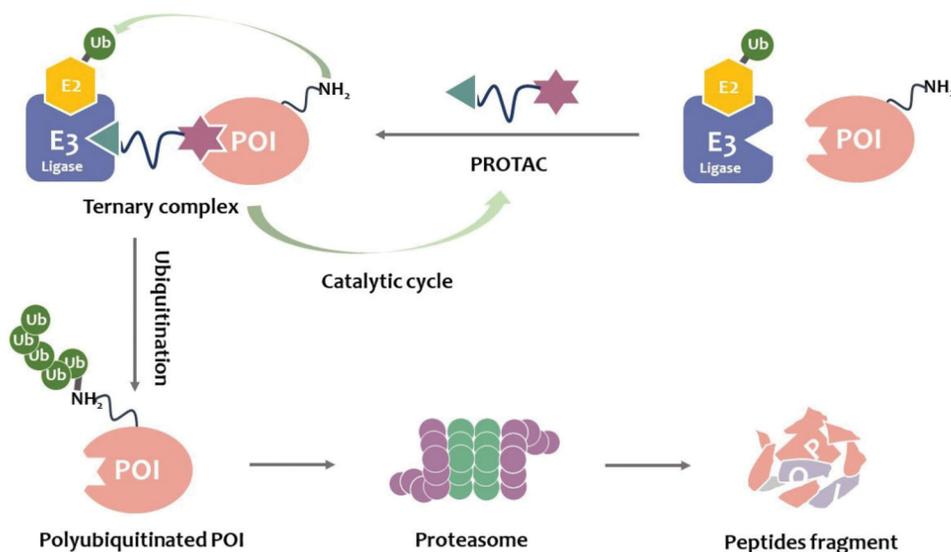


Figure 1. PROTACs-mediated degradation of target proteins through the UPS.

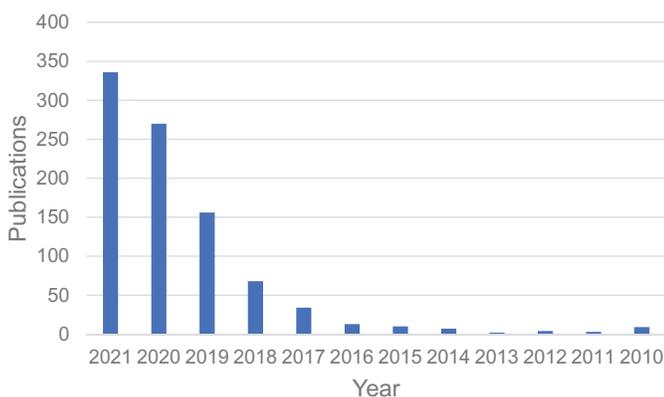


Figure 2. The number of publications on PROTACs/SNIPERs in PubMed (accessed on 12/13/2021).

developed rapidly, offering hope for the discovery and development of therapeutic agents with novel mechanisms and efficacy (Figure 2 and Table 1) ^{18–21}.

IAP are a class of negative regulators for apoptosis that can inhibit cell apoptosis by inhibiting caspase ^{22–24}. IAP family consists of eight members, of which c-IAP1, c-IAP2, and XIAP are the most widely studied members of IAP ^{25,26}. IAP are often overexpressed in cancer cells and are closely related to poor prognoses, thus they are shown to be important targets for cancer therapy ^{27,28}. Apart from being the key targets for cancer therapy, IAP belong to the E3 ubiquitin ligase family. IAP-based PROTACs are also called specific and nongenetic IAP-dependent protein erasers (SNIPERs) ^{29,30}. IAP inhibitors and their derivatives have been commonly used as E3 ligase ligands for SNIPERs. SNIPERs have been successfully employed in the degradation of different types of target proteins related to various diseases, including cancer, immune disorders, and neurodegenerative diseases. These target proteins mainly include AR, BCL-X_L, BCR-ABL, BTK, BRD4, CDK, CRABP1, EGFR, ER, HDACs, H-PGDS, IRAK4, JAK, mHtt, NOTCH1, P97, PDE4, RAR α , TACC3, etc. ^{29,31,32}. Therefore, SNIPERs are currently considered a promising approach for disease treatment. Figure 3 shows the milestones in the development of SNIPERs. In this review, we will summarise the most IAP E3 ligands and sum up the rapid

progress from 2010 to 2021 in SNIPERs. We will also provide our perspective on the opportunities and challenges for SNIPERs to become therapeutic agents.

2. IAP ligands

Because of IAP's involvement in multiple malignancies, inhibitors of IAP represent an attractive strategy for tumour therapy, and many potent peptidomimetic antagonists have been developed ^{33–35}. Interaction between IAP antagonists and their targets results in the autoubiquitylation and proteasomal degradation of cIAP1. The methyl bestatin derivative was used as the cIAP1-binding ligand for the early reported degraders recruiting this E3 ligase ³⁶. The subsequent development of high-affinity IAP ligands and their incorporation into bifunctional molecules improved the efficiency of SNIPERs in comparison with early bestatin-based compounds. Structures of IAP ligands utilised in chimeric molecules are presented in Figure 4.

Statistics were performed using data extracted from PubMed and PROTAC-DB to determine the frequency of various IAP ligands used in SNIPERs (Figure 5) ^{37,38}. The LCL-161 derivative was the most commonly used, with approximately 31% of SNIPERs employing its structure. It was closely followed by bestatin, with lower occurrence of MV1 derivatives and IAP ligand 4 at 10% and 9%, respectively. Other IAP ligands were less common, with less than 3% representation.

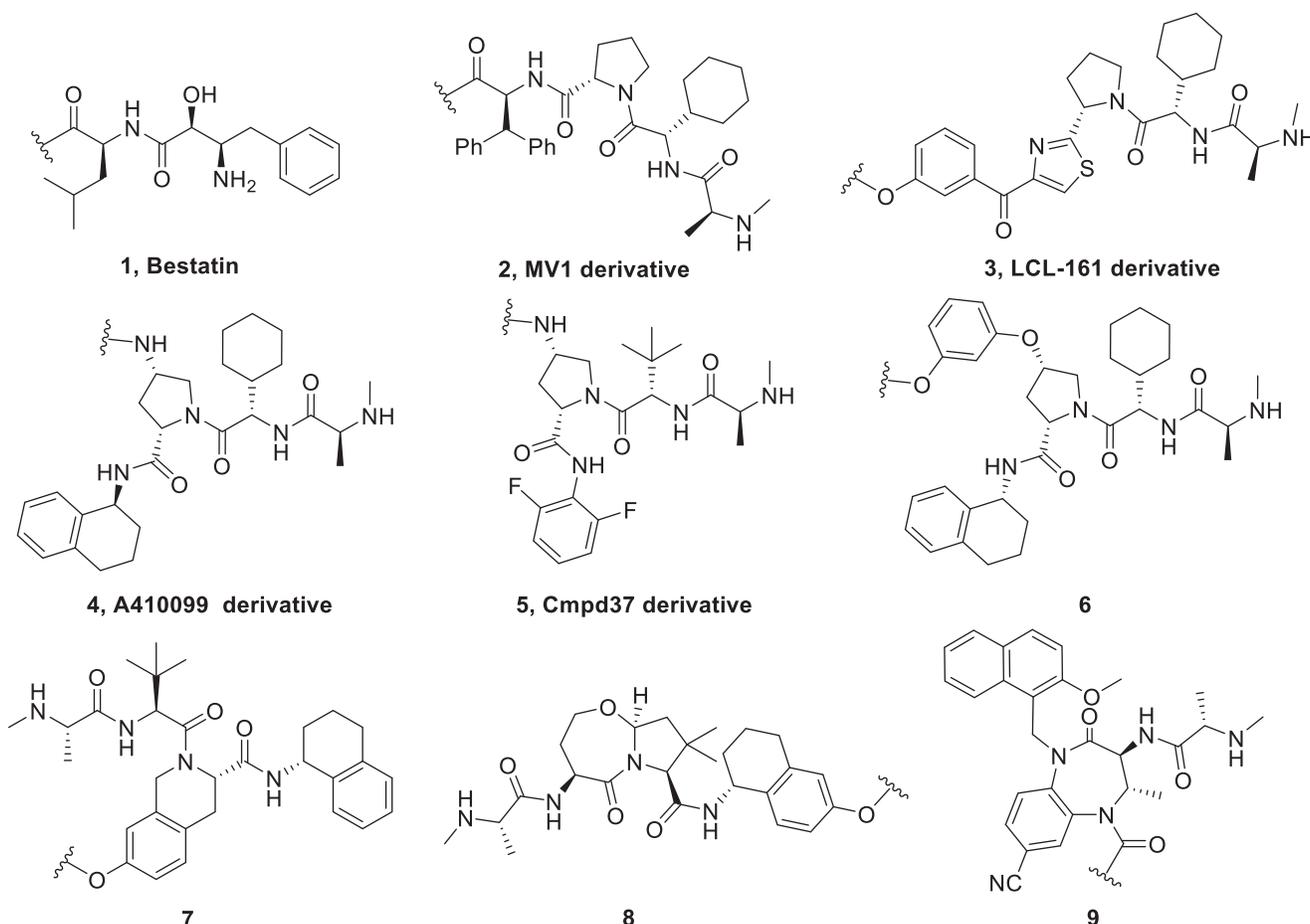
3. Snipers for cancers

3.1. Targeting AR

The AR, a member of the nuclear hormone receptor superfamily, plays a vital role in the maintenance of male secondary sexual characteristics and development of the prostate gland. Disorder of the androgen receptor is the main driving force for prostate cancer (PC). AR antagonists, such as enzalutamide, apalutamide, and darolutamide, are effective for the treatment of PC. Unfortunately, patients treated with these AR antagonists ultimately develop drug resistances. In most tumours that are resistant to AR antagonists, the AR signalling continues to be functional and drives tumour growth and progression. Some of the major resistance mechanisms to AR antagonists include AR gene amplification and

Table 1. Overview of current PROTACs¹⁷.

Degraders	Description	Advantages	Disadvantages
CRBN-based PROTACs	Bifunctionals recruiting E3 CRBN; by far most common degrading mechanism used.	Wide target scope; compact warhead can allow oral exposure.	Narrow warhead SAR; heterogeneous responses across cells; high resistance potential; toxicological issues.
VHL-based PROTACs	Bifunctionals recruiting E3 VHL; second only to CRBN in breadth of reported use.	Bifunctionals recruiting E3 VHL; second only to CRBN in breadth of reported use.	High complexity of warheads limits oral exposure, CNS penetration, etc.
IAP-based PROTACs (SNIPERs)	Bifunctionals recruiting multiple E3s including cAIP1, cIAP2, and XIAP.	Parallel degrading pathways with potential to increase degradation efficiency.	Target scope not as well defined due to fewer reports; high warhead complexity limits druglike properties.
PROTACs based on other E3 ligases	Bifunctionals recruiting other E3 ligases, such as MDM2, RNF114, and DCAF15, 16.	Can offer novel profiles and novel chemical warheads.	Few data yet to assess potential; dependency on single ligase can cause scope and resistance issues.

**Figure 3.** Timeline and major milestones for the development of SNIPERs.**Figure 4.** Chemical structures of small-molecular IAP ligands.

mutation and expression of AR variants^{39,40}. Therefore, new therapeutic strategies to effectively target the AR signalling in tumours resistant to AR antagonists are urgently needed.

In 2018, Mikihiro Naito et al. developed a series of AR SNIPERs by linking AR antagonists with IAP ligands⁴¹. SNIPER-1 (Table 2) showed the ability to effectively degrade AR at 3 μ M

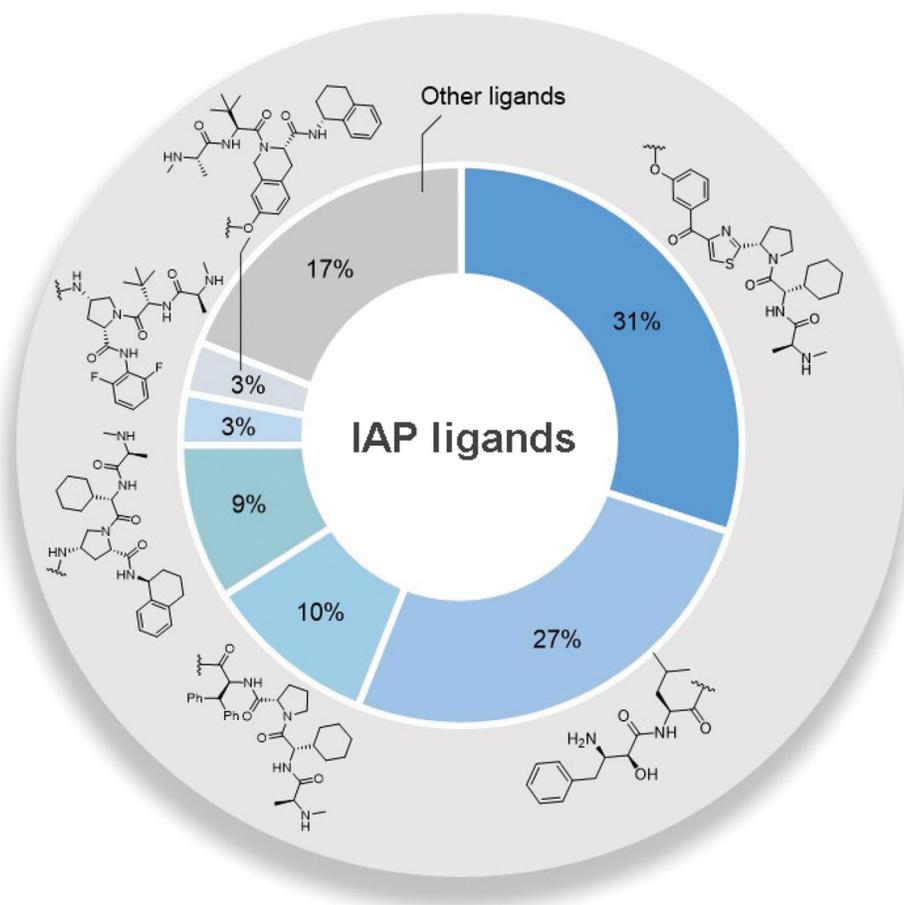


Figure 5. Frequency of IAP ligands used in SNIPERs.

Table 2. Representative SNIPER targeting AR.

Compound	Target protein	Structure	Reference
SNIPER-1	AR		41

concentration. Moreover, SNIPER-1 could efficiently inhibit AR-mediated gene expression and suppress the proliferation of androgen-dependent PC cells. In addition, SNIPER-1 was found to be able to induce caspase activation and apoptosis in PC cells efficiently, which didn't appear in the cells treated with AR antagonists. These results suggested that AR SNIPERs might serve as leads for an anticancer drug against PC that exhibit AR-dependent proliferation. AR SNIPERs are a promising strategy for the treatment of PC, further optimisation is necessary to develop more efficient AR degradation inducers for clinical use in the future.

3.2. Targeting BCL-X_L

B-cell lymphoma extra-large (BCL-X_L) is one of the important proteins in the B-cell lymphoma 2 (BCL-2) family, which plays a pivotal role in controlling the life-cycle of cell *via* regulating the intrinsic apoptotic pathway. BCL-X_L is a well-validated cancer target. Inhibition of these BCL-2 family proteins with small molecules has been extensively investigated as a therapeutic strategy for

cancers, resulting in the discovery of ABT263 (navitoclax, a BCL-2 and BCL-X_L dual inhibitor), ABT199 (venetoclax, a BCL-2 selective inhibitor), and several BCL-X_L and MCL-1 monoselective inhibitors as promising anticancer drug candidates. Currently, ABT199 is the only Food and Drug Administration (FDA)-approved antitumor agent targeting the BCL-2 family proteins. The use of BCL-X_L inhibitors in the clinic is largely limited by their on-target and dose-limiting platelet toxicity^{42,43}. Targeting BCL-X_L *via* PROTACs/SNIPERs is a promising strategy in reducing BCL-X_L inhibition associated platelet toxicity.

In 2019, Guangrong Zheng et al. reported the potent BCL-X_L degraders that recruit VHL or CRBN E3 ligase. However, low protein expression or mutation of the responsible E3 ligase has been known to result in decreased protein degradation efficiency of the corresponding PROTACs. To overcome these mechanisms of resistance, PROTACs based on recruiting alternative E3 ligases could be generated. In 2020, they designed and synthesised a series of PROTACs that recruit IAP E3 ligases for BCL-X_L degradation⁴⁴. Among those PROTACs, SNIPER-2 (Table 3) efficiently degraded

Table 4. Representative SNIPERs targeting BCR-ABL.

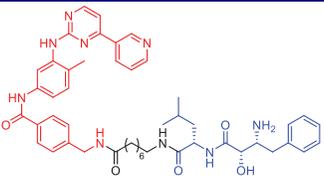
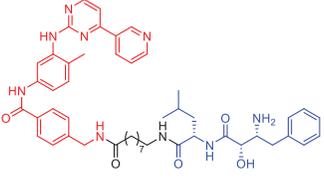
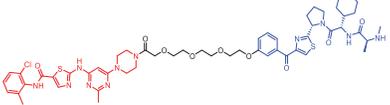
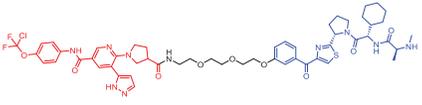
Compounds	Target protein	Structure	References
SNIPER-3	BCR-ABL		47
SNIPER-4	BCR-ABL		47
SNIPER-5	BCR-ABL		48
SNIPER-6	BCR-ABL		49

Table 5. Representative SNIPERs targeting BRD4.

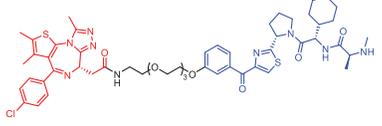
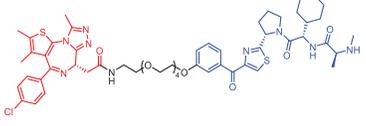
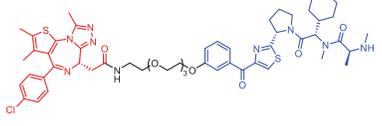
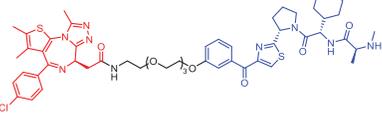
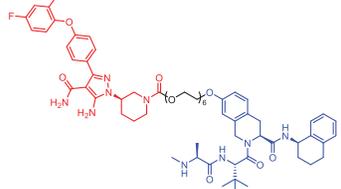
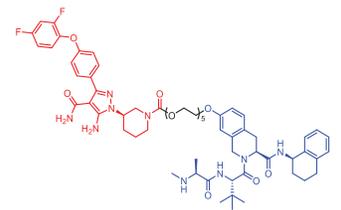
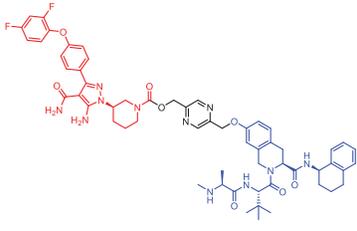
Compounds	Target protein	Structure	References
SNIPER-7	BRD4		52
SNIPER-8	BRD4		52
SNIPER-9	BRD4		52
SNIPER-10	BRD4		52

Table 6. Representative SNIPERs targeting BTK.

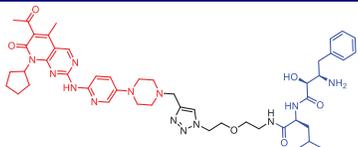
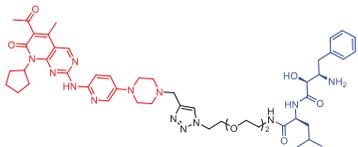
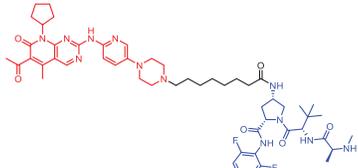
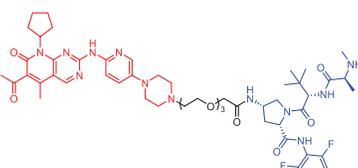
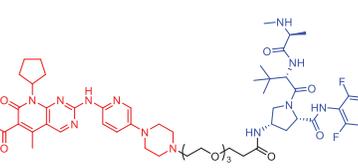
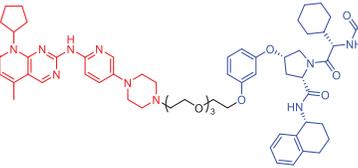
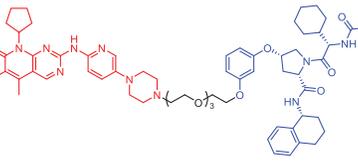
Compounds	Target protein	Structure	References
SNIPER-11	BTK		56
SNIPER-12	BTK		57
SNIPER-13	BTK		57

approved by the FDA for the treatment of several types of NHL. However, due to a missense mutation in BTK C481S, NHL patients have developed drug resistances after treatment with ibrutinib. Ibrutinib also lost the inhibitory effect on NHL tumour cell growth caused by the BTK C481S mutation⁵⁵.

In 2018, a series of new BTK PROTACs/SNIPERs were designed and synthesised by Matthew F. Calabrese et al.⁵⁶. The PROTACs/SNIPERs were developed through the conjugation of a BTK

inhibitor and CRBN/VHL/IAP ligands. Among all PROTACs/SNIPERs, CRBN-based PROTACs had their abilities to induce BTK degradation. The authors found that BTK degradation was inefficient

Table 7. Representative SNIPERs targeting CDK.

Compounds	Target protein	Structure	References
SNIPER-14	CDK		62
SNIPER-15	CDK		62
SNIPER-16	CDK		63
SNIPER-17	CDK		63
SNIPER-18	CDK		63
SNIPER-19	CDK		60
SNIPER-20	CDK		60

when either IAP or VHL was recruited instead of CRBN. Representative compound, SNIPER-11, was shown in Table 6.

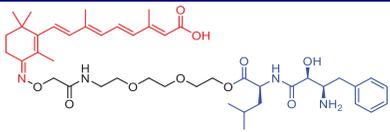
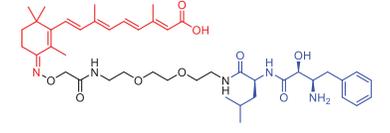
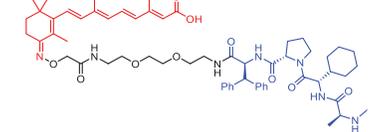
In 2020, Matthew F. Calabrese et al. reported a unique BTK degrader cIAP1 ternary complex crystal structure, providing a snapshot of three distinct binding poses between the target and E3 ligase⁵⁷. Using both HSQC NMR and computational modelling, they designed and synthesised two new BTK SNIPERs (SNIPER-12 and SNIPER-13, Table 6) by linking aminopyrazole derivatives to IAP ligands. Treatment of THP-1 cells with SNIPER-12 led to a dose-dependent loss of BTK, with a half-maximum degradation concentration (DC₅₀) of 182 ± 57 nM. This degradation was rescued by shortening the five-PEG linker to a non-permissive two-PEG

linker (SNIPER-13) consistent with the protein degrader mechanism of action.

3.6. Targeting CDK

Cell cycle regulation is one of the vital and housekeeping activities in most types of the cells. Cyclin-CDK dual complexes are the major driving machinery for cell cycle progression. Among the CDKs, CDK6 plays a significant role in cell cycle entrance and is frequently overexpressed or hyperactivated in cancer samples^{58,59}. Therefore, small molecule inhibitors of CDK6 have been officially

Table 8. Representative SNIPERs targeting CRABP-II.

Compounds	Target protein	Structure	References
SNIPER-21	CRABP-II		18
SNIPER-22	CRABP-II		65
SNIPER-23	CRABP-II		65

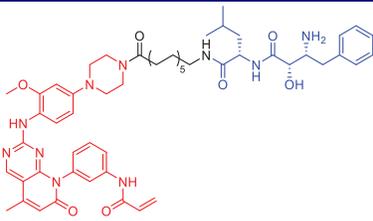
approved or clinically tested to act against cancers including breast cancer, lymphoma, and multiple myeloma. However, overexpression of CDK6 induced by gene amplification or loss of FAT1 gene functions has been reported to be correlated to CDK4/6 inhibitor resistance in breast cancer cell lines and patient samples. Moreover, point mutation of CDK6 could possibly result in attenuation of drug binding affinity or hyperactivation of CDK6, though not yet reported in clinical samples^{60,61}. Thus, there is an urgent need to develop a practical strategy against CDK6-centered malignancy.

In 2019, a focussed library of CDK6 degraders was developed and factors including linker length, spatial orientation, and binding affinity were systematically evaluated to help understand the match/mismatch between PROTACs/SNIPERs and targets and to deduce the best strategy for future design or optimisation of CDK6 degradation⁶². Yu Rao et al. found that CRBN-based PROTACs, instead of other tested E3 ligases, resulted in functional degraders. Representative IAP-based PROTACs, SNIPER-14 and SNIPER-15 (Table 7), were generated by linking E3 ligase IAP recruiter besatin to the FDA-approved CDK4/6 inhibitor palbociclib. Compared to CRBN-based PROTACs, SNIPER-14 and SNIPER-15 were not effective in degrading CDK6 protein and were not investigated more thoroughly by the authors.

In 2020, Andrew B. Benowitz et al. developed some novel palbociclib-based CDK4/6 SNIPERs that incorporate a novel IAP-binder⁶³. Following the preparation of SNIPER-16–SNIPER-18 (Table 7), they evaluated the degradation of CDK4 and CDK6 in Jurkat cells after 24 h compounds treatment in a dose–response manner using capillary electrophoresis combined with antibody labelling to measure protein levels. As expected, palbociclib did not cause any significant degradation of CDK4 or CDK6. However, they observed potent degradation of CDK4/6 for three SNIPERs.

In 2020, Jan Kronke et al. systematically explored the chemical space of CDK4/6 PROTACs by addressing E3 IAP ligase, and two novel multifunctional degraders (SNIPER-19 and SNIPER-20, Table 7), based on palbociclib and IAP ligands, were developed⁶⁰. SNIPER-19 and SNIPER-20 could simultaneously and effectively degrade CDK4/6 with D_{max} of > 77% at 0.1 μ M in MM.1S cells. Moreover, the new SNIPERs showed potent and long-lasting degrading activity in human and mouse cells and inhibited

Table 9. Representative SNIPER targeting EGFR.

Compound	Target protein	Structure	Reference
SNIPER-24	EGFR		69

proliferation of several leukaemia, myeloma, and breast cancer cell lines. IAP-based PROTACs are an attractive approach for targeted degradation of CDK4/6 in cancer.

3.7. Targeting CRABP-II

Cellular retinoic acid binding protein II (CRABP-II) resides in cytoplasm and specifically binds to all-trans retinoic acid (ATRA), an endogenous ligand of retinoic acid receptors (RARs). CRABP-II is expressed in several cancers, including neuroblastoma and Wilms tumour. In particular, it is thought to play a role in cancer development in MycN-amplified neuroblastoma IMR-32 cells. It is reported that decrease of CRABP-II induced down-regulation of MycN, activation of caspase and growth inhibition of MycN-amplified neuroblastoma IMR-32 cells^{18,64}.

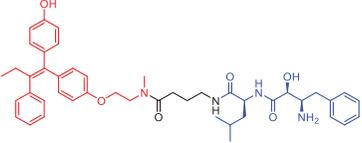
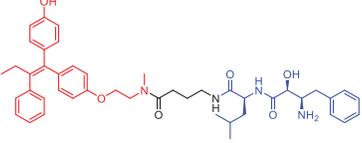
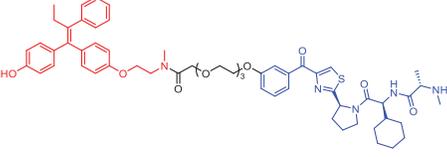
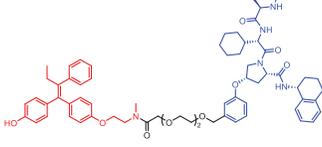
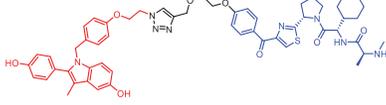
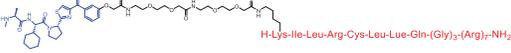
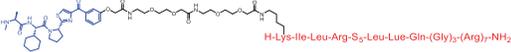
In 2010, Yuichi Hashimoto et al. first reported a series of CRABP-II SNIPERs through connecting ATRA and bestatin *via* a linker, which could recruit cIAP1 and induce selective poly-ubiquitylation of CRABP-II to cause proteasomal degradation¹⁸. Among them, SNIPER-21 (Table 8) displayed the best potency, which could degrade CRABP-II in HT1080 cells at 1 μ M, and showed a concentration-dependent trend. However, SNIPER-21 often induced cIAP1 autoubiquitination and degradation, which could be a concern for their use in therapy.

In 2012, Yuichi Hashimoto et al. designed and synthesised SNIPER-22 (Table 8), which contains bestatin as the E3 ligase ligand and induces selective degradation of CRABP-II but not of IAP⁶⁵. They found that SNIPER-22 exerted an almost equivalent effect with SNIPER-21 in the aspect of degrading CRABP-II. Importantly, SNIPER-22 produced a more long-lasting degradation effect. However, SNIPER-22 was less effective than SNIPER-21 in inhibiting the proliferation of neuroblastoma IMR32 cells. Subsequently, taking IAP inhibitor MV-1 as E3 ligase ligand, they developed SNIPER-23 (Table 8), which was nearly 10 times more potent than SNIPER-21 in the area of degrading CRABP-II and cIAP1, and also could effectively inhibit the proliferation of IMR32 cells.

3.8. Targeting EGFR

Epidermal growth factor receptor (EGFR) is a glycoprotein with tyrosine kinase activity that is a major member of the erythroblastosis oncogene B (ErbB) family. EGFR is involved in tumour cell proliferation, angiogenesis, tumour invasion, metastasis, and inhibition of apoptosis. The overexpression of EGFR plays an important role in the progression of malignant tumours, such as glioblastoma, head and neck cancer, NSCLC, pancreatic cancer, breast cancer, and so on. Through decades of development, three generations of EGFR inhibitors have emerged. Despite great

Table 10. Representative SNIPERs targeting ER.

Compounds	Target protein	Structure	References
SNIPER-25	ER		72
SNIPER-26	ER		73
SNIPER-27	ER		74
SNIPER-28	ER		74
SNIPER-29	ER		75
SNIPER-30	ER		76
SNIPER-31	ER		77
SNIPER-32	ER		78
SNIPER-33	ER		78

therapeutic successes, the clinical application of three generations of EGFR inhibitors inevitably leads to acquired drug resistances, which has presented a new challenge of treating cancers^{66–68}.

In 2020, a series of selective EGFR^{L858R/T790M} mutant PROTACs/SNIPERs were designed and synthesised based on pyrido [2,3-d]pyrimidin-7-one selective EGFR^{L858R/T790M} inhibitor XTF-262⁶⁹. The VHL-based PROTACs effectively and selectively degraded EGFR^{L858R/T790M} with a DC₅₀ value of 5.9 nM. In contrast to VHL-based PROTACs, IAP-based PROTAC (SNIPER-24, Table 9) was unable to degrade EGFR^{L858R/T790M} protein, which was not overly described by the authors.

3.9. Targeting ER

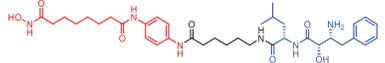
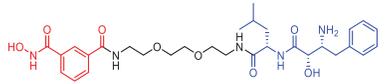
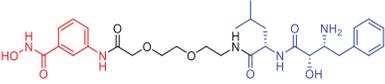
Breast cancer (BC) is one of the most common malignancies in women worldwide. Oestrogen receptors+ (ER+) breast cancer occurs in approximately 80% of newly diagnosed breast cancer

cases. As members of the nuclear receptor family, oestrogen receptors ER α and ER β are transcription factors regulating gene expression and mediating the biological effects of the oestrogen. Both ER α and ER β are widely expressed in different tissues, and ER α is considered to be the major mediator which transduces the oestrogen signalling in the female reproductive tract and mammary glands. ER α has therefore been pursued as a promising therapeutic target in multiple pathological settings, particularly in cancer and osteoporosis^{70,71}.

In 2011, Yuichi Hashimoto et al. reported SNIPER-25 (Table 10) targeting ER by linking oestrone ligand and IAP ligand (bestatin) within a single molecule⁷². They found that SNIPER-25 at 1 μ M significantly decreased ER α level in human breast cancer cell MCF-7.

In 2012, Masaaki Kurihara et al. also synthesised three ER α SNIPERs by means of connecting 4-hydroxytamoxifen (4-OHT) with bestatin⁷³. Among them, SNIPER-26 (Table 10) was effective in reducing ER α levels even at low concentrations of 10 μ M. Moreover, SNIPER-26 was found to be able to induce the

Table 11. Representative SNIPERs targeting HDAC.

Compounds	Target protein	Structure	References
SNIPER-34	HDACs		81
SNIPER-35	HDACs		81
SNIPER-36	HDACs		81

production of reactive oxygen species (ROS) in MCF-7 cells and then lead to cell death. In addition, the down-regulation of ER α by SNIPER-26 was also observed in breast cancer cell T47D⁷³.

PERM3 is a peptide analogue of Steroid Receptor Coactivator 1 (SRC-1) that reacts with the ER surface R7, a fragment of heptarginine, is capable of improving the permeability of PERM3. In 2016, Masaaki Kurihara *et al.* discovered SNIPER-27 (Table 10) and SNIPER-28 (Table 10) based on PERM3-R7⁷⁴. SNIPER-27 could decrease the levels of ER α and clAP1 in a concentration-dependent manner, but toxic effects began to appear when the concentration was more than 6 μ M in MCF-7 cells. The ability of SNIPER-28 for degrading ER α was slightly weaker than that of SNIPER-27, but toxic effects appeared at concentration of 20 μ M.

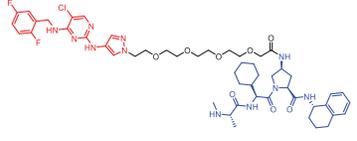
In 2017, Mikihiro Naito *et al.* disclosed some SNIPERs. These compounds were developed by ligating 4-OHT with different IAP-binding compounds (bestatin, MV1, and LCL161)⁷⁵. However, bestatin-based SNIPER could only induce ER α degradation at 10 μ M or higher. Then they developed MV1-based SNIPER, in which the IAP antagonist MV1 was substituted for bestatin. It successfully improved ER α degradation activity. They further developed SNIPER-29 (Table 10), with higher degradation activity, by using a derivative of LCL161. Unlike SNIPERs targeting ER α mentioned above, SNIPER-29 recruited XIAP instead of clAP1 to ubiquitinate ER α for degradation. SNIPER-29 at 3 nM began to show degradation activity, and the best effect appeared at concentration of 100 nM. In MCF-7 tumour xenotransplantation mouse model, SNIPER-29 could significantly inhibit tumour growth, and displayed no obvious toxic side effects.

In 2018, they continued to develop a series of ER α SNIPERs using other reported IAP inhibitors⁷⁶. Representative compound, SNIPER-30 (Table 10), was more effective than SNIPER-29 in inducing ER α degradation and apoptosis of breast cancer cell MCF-7. In addition, its ability of degrading ER α was better than that of SNIPER-29 in MCF-7 xenotransplantation mouse model.

In 2020, utilising DECL platform, Shilpi Arora *et al.* described some PROTACs/SNIPERs targeting ER α ⁷⁷. The VHL-based PROTACs effectively degraded ER α in ER $+$ cells. They also tested VHL-based PROTACs in normal immortalised breast cells and observed no off-target effects. These PROTACs also demonstrated suitable properties for *in vivo* applications and were efficacious in an ER α -dependent xenograft model. Compared with VHL-based PROTACs, IAP-based PROTAC (SNIPER-31, Table 10) degraded ER α proteins with low efficiency, which had not been studied much by the authors.

In 2021, Yosuke Demizu *et al.* succeeded in developing the stapled peptide stPERML-R7, which was based on the ER α -binding

Table 12. Representative SNIPER targeting JAK.

Compounds	Target protein	Structure	Reference
SNIPER-37	JAK		84

peptide PERML and composed of natural amino acids⁷⁸. stPERML-R7, which included a hepta-arginine motif and a hydrocarbon stapling moiety, showed increased α -helicity and similar binding affinity towards ER α when compared with those of the parent peptide PERML. On this basis, they used stPERML-R7 to develop a peptide-based degrader LCL-stPERML-R7 (SNIPER-32, Table 10) targeting ER α . The chimeric peptide SNIPER-32 induced sustained degradation of ER α and potently inhibited ER α -mediated transcription more effectively than the unstapled chimaera LCL-PERML-R7 (SNIPER-33, Table 10). These results suggested that a stapled structure was effective in maintaining the intracellular activity of peptide-based degraders.

3.10. Targeting HDACs

Histone deacetylases (HDACs) can catalyse the removal of acetyl group from lysine residues of histones or some non-histone proteins, therefore playing an essential role in the regulation of gene expression and protein function. Studies have shown that HDACs are closely associated with tumorigenesis and progression. To date, only five HDAC inhibitors have been approved as anticancer agents^{79,80}. Therefore, more drugs targeting HDACs are eager to be discovered.

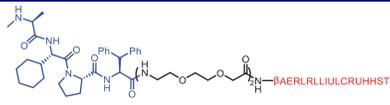
In 2021, three bestatin-based hydroxamic acids (SNIPER-34, SNIPER-35, and SNIPER-36, Table 11) were designed and synthesised by Yingjie Zhang *et al.*⁸¹. Those SNIPERs could work as HDACs degraders by recruiting clAP1 E3 ubiquitin ligase. Among them, SNIPER-34 exhibited comparable even more potent inhibitory activity against HDAC1, HDAC6, and HDAC8 relative to the approved HDACs inhibitor SAHA. It is worth noting that although SNIPER-34 could not lead to intracellular HDACs degradation after 6 h of treatment, it could dramatically decrease the intracellular levels of HDAC1, HDAC6, and HDAC8 after 24 h of treatment. Moreover, all three SNIPERs exhibited more potent aminopeptidase N (APN, CD13) inhibitory activities than the approved APN inhibitor bestatin, which translated to their superior anti-angiogenic activities.

3.11. Targeting JAK

Janus kinases (JAKs) are a family of non-receptor tyrosine kinases, composed of four members, JAK1, JAK2, JAK3, and TYK2. JAKs are involved in different inflammatory and autoimmune diseases, as well as in malignancies, through the activation of the JAK/STAT signalling pathway. Furthermore, the V617F mutation in JAK2 was identified in patients affected by myeloproliferative neoplasms. This knowledge prompted researchers from academia and pharmaceutical companies to investigate this field in order to discover small molecule JAK inhibitors and degraders^{82,83}.

In 2020, Rishi R. Shah *et al.* successfully described a set of novel SNIPERs targeting the JAK proteins by conjugating JAK inhibitor Quinoxaline with an IAP ligand⁸⁴. SNIPER-37 was capable

Table 13. Representative SNIPER targeting NOTCH1.

Compounds	Target protein	Structure	Reference
SNIPER-38	NOTCH1		85

of inducing JAK1 and JAK2 degradation. At the highest concentration (5 μ M) tested, where SNIPER-37 (Table 12) could induce JAK1 degradation by 48% and JAK2 degradation by 65%. The authors found that PROTACs bearing an IAP ligand induced significantly more JAK degradation over VHL- and CRBN-based PROTACs. In addition, the mechanism of action of the JAK SNIPERs was elucidated, and it was confirmed that JAK degradation was both IAP- and proteasome-dependent.

3.12. Targeting NOTCH1

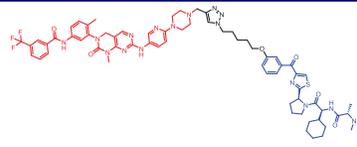
Oncogenic transcriptional factor NOTCH1 plays a critical role in several biological functions such as cellular differentiation, proliferation, and death. Activated NOTCH1 is closely related to the initiation and development of some cancers. Ligands that bind to the extracellular domain of NOTCH receptors induce their proteolytic cleavage catalysed by ADAM family metalloproteinase and γ -secretase, leading to the cytoplasmic release of the intracellular domain of NOTCH1 (ICN1). ICN1 translocates to the nucleus and regulates the activity of CSL, a DNA-binding transcription factor, together with MAML. ICN1 interacts with CSL, which forms a shallow groove along the surface of the two proteins that acts as a binding surface for the co-activator MAML1^{85,86}.

In 2017, Mikihiro Naito et al. reported peptide-based SNIPERs for NOTCH1 degradation by conjugating MAML1 (21–26) or SAHM1 to MV-1⁸⁵. Western blot showed that representative compound, SNIPER-38 (Table 13), effectively reduced NOTCH1 protein level in a dose-dependent manner, which was more effective than other SNIPERs. Moreover, after incubation with MOLT-4 human T-ALL cells within 2 h, SNIPER-38 also rapidly degraded the protein levels of c-MYC (a downstream target of NOTCH1) in a time-dependent manner. The above results indicate that the conjugation of MV1 and stapled peptides *via* an appropriate linker makes it feasible to develop potent inducers for NOTCH1 protein degradation.

3.13. Targeting P97

The AAA + ATPase, P97, also referred to as VCP, plays an essential role in cellular homeostasis by regulating endoplasmic reticulum-associated degradation (ERAD), mitochondrial-associated degradation (MAD), chromatin-associated degradation, autophagy, and endosomal trafficking. Mutations in P97 have been linked to several neuro-degenerative diseases, and overexpression of wild type P97 is observed in numerous cancers. Furthermore, P97 activity has been shown to be essential for the replication of certain viruses, including poliovirus, herpes simplex virus (HSV), cytomegalovirus (CMV), and influenza. Taken together, these observations highlight the potential for targeting P97 as a therapeutic approach in neurodegeneration, cancer, and certain infectious diseases^{87,88}. It is unclear what factors determine whether degradation of a ubiquitinated protein occurs in a P97-dependent or P97-independent manner. The ability to induce rapid

Table 14. Representative SNIPER targeting P97.

Compounds	Target protein	Structure	Reference
SNIPER-39	P97		89

polyubiquitination of large numbers of kinases with multitargeted degraders provided an opportunity to examine whether degradation of protein kinases is P97-dependent and if this dependency changes with differences in recruited E3 ligase.

To assess P97 dependence across the kinome, Eric S. Fischer et al. reported multiple P97 PROTACs/SNIPERs (e.g. SNIPER-39, Table 14) by tethering different E3 ligase ligands (CRBN, VHL, or IAP) with the P97 inhibitor GNF-7 in 2020⁸⁹. Analysis of protein expression of the top kinase hits from each experiment revealed that the proposed P97 dependence was independent of the ligase responsible for mediating ubiquitination.

3.14. Targeting TACC3

Transforming acidic coiled-coil-3 (TACC3) belongs to spindle regulatory protein family. During mitosis, TACC3 localises to the mitotic spindle and plays a key role in spindle assembly, chromosome function, and mitotic progression. TACC3 was found to be overexpressed in a variety of human cancers, including ovarian cancer, breast cancer, squamous cell carcinoma, and lymphoma. Therefore, TACC3 is a target that suitable for anti-cancer drug discovery, which has attracted increasing attention from researchers, recently^{90,91}.

In 2014, Mikihiro Naito et al. developed SNIPER-40 (Table 15) targeting TACC3 protein by connecting KHS101 to bestatin, which induced proteasomal degradation of the TACC3 proteins in HT080 cells after treated for 6 h at 30 μ M and 24 h at 10 μ M⁹². SNIPER-40 at $\geq 10 \mu$ M efficiently killed HT1080 and MCF7 cells after treatment for 48 h. SNIPER-40 selectively killed cancer cells that aberrantly express large amount of TACC3 proteins, but exerted no effect on normal cells. Therefore, targeting TACC3 for degradation by SNIPERs offers a new option for cancer therapy.

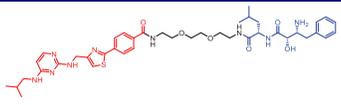
4. Snipers for immune diseases

4.1. Targeting H-PGDS

Overproduction of PGD2 is related to a variety of diseases, including allergic diseases, physiological sleep, and Duchenne muscular dystrophy. H-PGDS is one of the potential therapeutic targets for such diseases. *In vivo* studies have demonstrated that H-PGDS inhibition is effective in the treatment of allergic inflammation. To date, several types of H-PGDS inhibitors have been developed as therapies for allergic and inflammatory responses. However, the advancement of these inhibitors into clinical studies has not been satisfactory. Thus, the development of novel agents for clinical investigation with modes of action other than H-PGDS inhibition is required^{93,94}.

In 2021, Yosuke Demizu et al. successfully developed some PROTACs/SNIPERs of the H-PGDS protein by conjugating H-PGDS ligand (TFC-007) to CRBN ligand (pomalidomide) or IAP ligand (LCL161 derivative)⁹⁵. CRBN-based PROTACs effectively induced

Table 15. Representative SNIPER targeting TACC3.

Compounds	Target protein	Structure	Reference
SNIPER-40	TACC3		92

the selective degradation of H-PGDS protein *via* the UPS and showed sustained suppression of PGD2 production. CRBN-based PROTACs with a new mechanism of action were expected to be as effective or more effective than conventional inhibitors and might allow a reduction in the number of doses for the treatment of chronic inflammation. Compared to CRBN-based PROTACs, protein reduction activity for IAP-based PROTAC (SNIPER-41, Table 16) was not observed in MEG-01s cells expressing H-PGDS protein.

4.2. Targeting IRAK4

Interleukin-1 receptor-associated kinase 4 (IRAK4) belongs to a family of four kinases (IRAK4, IRAK1, interleukin-1 receptor-associated kinase 4 (IRAK4), IRAK2, and IRAK-M). IRAK4 is a serine/threonine kinase that is involved in transduction pathways stimulated by the Toll-like receptors (TLRs) and the interleukin-1 (IL-1) family of receptors. Recognition of foreign pathogens and inflammatory signals by these receptors promotes IRAK4 binding to the adapter protein myeloid differentiation primary response gene 88 (MyD88) resulting in IRAK4 activation that in turn leads to the production of pro-inflammatory cytokines *via* the NF- κ B pathway. IRAK4 deficiency or loss of function has been reported to increase susceptibility to several pathogens, while kinase activation has been linked with various autoimmune diseases such as systemic lupus erythematosus, psoriasis, rheumatoid arthritis, and cancer^{96,97}.

In 2019, Niall A. Anderson et al. reported many PROTACs/SNIPERs targeting IRAK4 protein by using different E3 ligase ligands⁹⁸. IAP-based PROTACs (SNIPER-42 and SNIPER-43, Table 17) were not able to degrade IRAK4 protein. The authors believed that there were many potential reasons why SNIPERs were not found to degrade IRAK4. For example, the linker may be the wrong length to facilitate efficient ternary complex formation. The orientation of the protein-IAP E3 ligase ternary complex may also not be able to promote efficient ubiquitin transfer onto an IRAK4 surface lysine residue. Finally, even though these compounds may bind to the protein, this does not always translate into degradation.

4.3. Targeting RIPK2

Receptor-interacting serine/threonine protein kinase 2 (RIPK2) is activated following intracellular recognition of the bacterial peptidoglycan fragments D-glutamyl-meso-diaminopimelic acid (iE-DAP) and muramyl dipeptide (MDP) by the pattern recognition receptors NOD1 and NOD2, respectively. Activation of RIPK2 results in its autophosphorylation and K-63 linked polyubiquitination which promotes recruitment of MAPK and NF- κ B signalling scaffolds and leads to transcriptional activation of multiple inflammatory cytokine genes. Dysregulation of this pathway has been implicated in several diseases such as inflammatory bowel disease, severe pulmonary sarcoidosis, and multiple sclerosis^{99,100}.

Table 16. Representative SNIPER targeting H-PGDS.

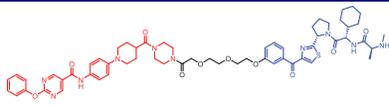
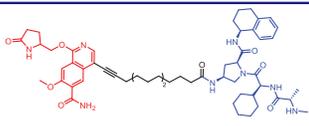
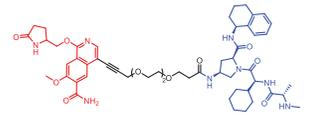
Compounds	Target protein	Structure	Reference
SNIPER-41	H-PGDS		95

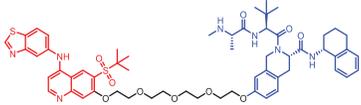
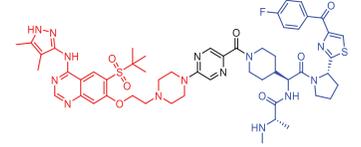
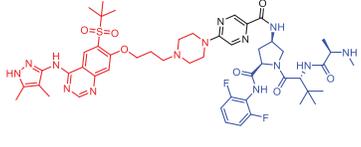
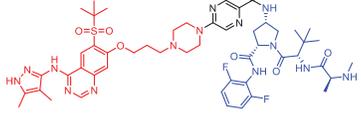
Table 17. Representative SNIPERs targeting IRAK4.

Compounds	Target protein	Structure	References
SNIPER-42	IRAK4		98
SNIPER-43	IRAK4		98

In 2020, John D. Harling et al. reported some IAP-based RIPK2 SNIPERs utilising an RIPK2 binder (aminobenzothiazole-quinoline)¹⁰¹. All three PROTACs potently degraded RIPK2 in a concentration-dependent manner. SNIPER-44 (Table 18) was found to have a log half-maximal degradation concentration (pDC₅₀) value of 9.4 ± 0.1 and was more potent than either the equivalent VHL-based PROTAC (pDC₅₀ = 8.7 ± 0.1) or the CRBN-based PROTAC (pDC₅₀ = 8.6 ± 0.4). To obtain SNIPERs with better physicochemical properties and *in vivo* efficacies, the authors designed and synthesised new SNIPER-45 (Table 18) and SNIPER-46 (Table 18). SNIPER-46 contained an additional methylene spacer at the junction of the RIPK2 binding moiety and the linker, as well as a modified IAP binder. SNIPER-46 produced a concentration- and time-dependent decrease in RIPK2 protein levels in human PBMCs such that the pDC₅₀ and D_{max} increased with longer incubation times, and a pDC₅₀ of 9.4 ± 0.2 and D_{max} of 94.3 ± 3.2% was determined for 6 following 24 h incubation. SNIPER-46 completely inhibited TNF α , IL1 β , IL-6, and IL-10 release following L18-MDP-stimulation with pIC₅₀ values >8.8, but only partially inhibited IL-8 release (pIC₅₀ = 8.5 and I_{max} ~50%). SNIPER-46 was equally effective at inhibiting spontaneous cytokine release across CD and UC patient biopsy samples with IC₅₀ values of ~1–3 nM. RIPK2 protein levels were also assessed by western blot upon treatment of the CD and UC donor colon tissue with SNIPER-46.

In 2021, John D. Harling et al. described the optimisation of a series of IAP-based PROTACs targeting RIPK2¹⁰². They focussed on reducing the lipophilicity of the early lead which resulted in the identification of analogues with improved solubility and increased human and rat microsomal stability. Representative compound, SNIPER-47 (Table 18), represented a highly potent and selective RIPK2 PROTAC. SNIPER-47, with attractive pharmacokinetic profiles, possessed the best overall profile with good solubility, potent degradation of RIPK2, and associated inhibition of TNF α release. It utilised a novel IAP binder which has reduced cIAP1 binding potency and increased XIAP BIR2 binding potency compared to most published SMAC mimetics. This allowed degradation of RIPK2 in the absence of cIAP1 autoubiquitination over a range of concentrations.

Table 18. Representative SNIPERs targeting RIPK2.

Compounds	Target protein	Structure	References
SNIPER-44	RIPK2		101
SNIPER-45	RIPK2		101
SNIPER-46	RIPK2		101
SNIPER-47	RIPK2		102

5. Snipers for neurodegenerative diseases

5.1. Targeting mHtt

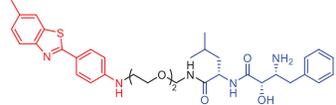
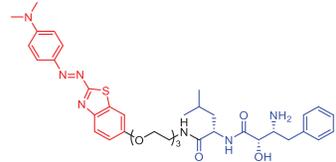
Huntington's disease (HD), an autosomal dominant neurodegenerative disorder, is caused by aggregation of mutant huntingtin (mHtt). Thus, removal of toxic mHtt may be an effective therapeutic approach for HD. Various approaches targeting the toxic mHtt aggregates have been developed and reported, including small molecules. Although many efforts have been made, effective clinical treatment is yet not available, owing to the modes of action of the chemical modulators for aggregation still remaining unclear. Additionally, biological approaches show several disadvantages, such as poor metabolic stability and selectivity, and low permeability¹⁰³.

In 2017, Minoru Ishikawa et al. successfully achieved the targeting of mHtt protein aggregation with small-molecule SNIPERs. They designed and synthesised SNIPER-48 (Table 19) and SNIPER-49 (Table 19) by connecting BTA and PDB to bestatin *via* proper linkers, respectively¹⁰⁴. Western blot results disclosed that SNIPER-48 and SNIPER-49 at 10 μ M could effectively reduce mHtt protein in two fibroblasts in a dose-dependent manner. Results of biological activity suggested that SNIPER-48 and SNIPER-49 worked effectively as down-regulators of mHtt in HD patients' fibroblasts and were expected to be promising candidates for treatment of patients with HD. In conclusion, the authors described SNIPERs that can degrade mHtt *in vitro*. The authors didn't describe much about the druglike properties of SNIPERs molecules, such as membrane permeability and metabolic stability.

5.2. Targeting PDE4

Phosphodiesterases (PDE) are a diverse family of enzymes responsible for the degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) which are involved in several cellular and biochemical functions.

Table 19. Representative SNIPERs targeting mHtt.

Compounds	Target protein	Structure	References
SNIPER-48	mHtt		104
SNIPER-49	mHtt		104

Phosphodiesterase 4 (PDE4) is the major isoform within this group and is highly expressed in the mammalian brain. An inverse association between PDE4 and cAMP levels is the key mechanism in various pathophysiological conditions like airway inflammatory diseases-chronic obstruction pulmonary disease (COPD), asthma, psoriasis, rheumatoid arthritis, and neurological disorders. Due to the adverse effects like unbearable nausea and vomiting, dose intolerance and diarrhoea, PDE4 inhibitors have very less clinical compliance. Efforts are being made to develop PDE4 PROTACs having better efficacy and lesser adverse effects^{105,106}.

In 2021, Mikihiro Naito et al. developed the discovery of SNIPER-50 (Table 20) against therapeutic target protein, PDE4, by conjugating the LCL161 derivative to a PDE4 inhibitor¹⁰⁷. SNIPER-50 showed efficient protein knockdown activity against PDE4 protein at nanomolar concentration. The authors did not describe the blood-brain barrier permeability of SNIPER-50.

6. Snipers for others

6.1. Targeting RAR α

Nuclear receptors (NRs), which are activated by small-molecular ligands, are transcription factors that regulate gene expression in the nucleus and play key roles in biological functions such as embryonic development and homeostasis. In addition, NRs are targets for the treatment of various diseases. Among NRs, retinoic acid receptors (RAR α , RAR β , and RAR γ) are receptors of all-trans retinoic acid (ATRA). Retinoids modulate the growth and differentiation of a wide variety of normal and transformed cells, and ATRA is involved in the control of embryonic development and cell differentiation^{108,109}.

To achieve selective degradation for RARs, Yuichi Hashimoto et al. designed and synthesised SNIPER-51 (Table 21) by using Ch55 as ligand for RAR and bestatin as ligand for E3 ligase⁷². Western blot analysis showed that SNIPER-51 could downregulate RAR α levels in a concentration-dependent manner in HT1080 cells expressing FLAG-tagged cIAP1, but exhibited no effect on the amount of CRABP-II, which suggested that SNIPER-51 showed selectivity for RAR knockdown activity against target protein at nanomolar concentration.

7. Conclusion

In this review, we provide a comprehensive update on the latest research progress in protein degraders that induce IAP-mediated ubiquitination. SNIPERs have emerged as a novel small molecule-

Table 20. Representative SNIPER targeting PDE4.

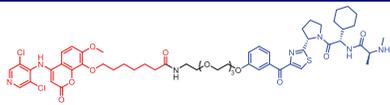
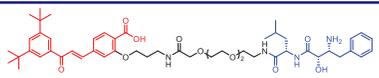
Compounds	Target protein	Structure	Reference
SNIPER-50	PDE4		107

Table 21. Representative SNIPER targeting RAR.

Compounds	Target protein	Structure	Reference
SNIPER-51	RAR α		72

based therapeutic approach in the treatment of many diseases, employing UPS to induce selective degradation of target proteins by hijacking E3 ligases, representing a very promising new paradigm for drug discovery. Compared to small-molecule drugs, SNIPERs display many features: (1) improving selectivities and specificities; (2) overcoming drug resistances; (3) eliminating the enzymatic and non-enzymatic functions of kinases; (4) degrading "non-pharmacological" protein targets; (5) rapid and reversible knockdown of POIs. Mikihiro Naito et al. reported the first small molecule that induces IAP E3-mediated ubiquitination and degradation of a target protein. Both selective knockdown of the target protein, and double knockdown of the target protein/cIAP1 were achieved by changing the chemical structure of the cIAP1 ligand. Indeed, double-proteins knockdown of IAP and cancer-related proteins is a promising approach for cancer treatment. This is a major advantage over other PROTACs that utilise the ubiquitin ligases CRBN and VHL. SNIPERs have performed well not only in cancer diseases but also in immune diseases, and neurodegenerative diseases. In recent years, more than 20 proteins^{9,10}, including undruggable targets, can be degraded by SNIPERs.

Although SNIPERs depict a promising technology for a variety of aspects, including drug discovery and answers to biological issues, challenges exist for SNIPERs in the future. First, most of them have a high molecular weight and do not meet the requirements of the classical Lipinski's "Rule of Five." Second, linkers are also critical for SNIPERs, including membrane permeability and metabolic stability. To date, the principles guiding linker design, including composition and length, have not been rigorously mastered. There is an urgent need to do a lot of work to obtain the best linkers. Third, the mechanism of SNIPERs is not well studied, so more practices are needed to evaluate the absorption, distribution, metabolism, excretion, and toxicity of SNIPERs. Although SNIPERs face many challenges that need to be solved, they are potential to be developed as therapeutic agents for many incurable diseases.

Disclosure statement

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References

1. Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature* 2007; 450:1437–9.
2. Gu S, Cui D, Chen X, et al. PROTACs: an emerging targeting technique for protein degradation in drug discovery. *Bioessays* 2018;40:e1700247.
3. Yang J, Li Y, Aguilar A, et al. Simple structural modifications converting a bona fide MDM2 PROTAC degrader into a molecular glue molecule: a cautionary tale in the design of PROTAC degraders. *J Med Chem* 2019;62:9471–87.
4. Toure M, Crews CM. Small-molecule PROTACs: new approaches to protein degradation. *Angew Chem Int Ed Engl* 2016;55:1966–73.
5. Kathleen MS, Kyung BK, Akiko K, et al. Protacs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *P Nant Acad Sci USA* 2001;98:8554–9.
6. Liang D, Yu Y, Ma Z. Novel strategies targeting bromodomain-containing protein 4 (BRD4) for cancer drug discovery. *Eur J Med Chem* 2020;200:112426.
7. Drummond ML, Williams CI. In silico modeling of PROTAC-mediated ternary complexes: validation and application. *J Chem Inf Model* 2019;59:1634–44.
8. An S, Fu L. Small-molecule PROTACs: an emerging and promising approach for the development of targeted therapy drugs. *EBioMedicine* 2018;36:553–62.
9. Wang C, Zhang Y, Wang J, et al. VHL-based PROTACs as potential therapeutic agents: recent progress and perspectives. *Eur J Med Chem* 2022;227:113906.
10. Wang C, Zhang Y, Wu Y, et al. Developments of CRBN-based PROTACs as potential therapeutic agents. *Eur J Med Chem* 2021;225:113749.
11. Farnaby W, Koegl M, Roy MJ, et al. BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat Chem Biol* 2019;15:672–80.
12. Martin-Acosta P, Xiao X. PROTACs to address the challenges facing small molecule inhibitors. *Eur J Med Chem* 2021;210:112993.
13. 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.
14. Sakamoto KM, Kim KB, Kumagai A, et al. PROTACs: chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc Natl Acad Sci U S A* 2001;98:8554–9.
15. Schneekloth JS Jr, Fonseca FN, Koldobskiy M, et al. Chemical genetic control of protein levels: selective in vivo targeted degradation. *J Am Chem Soc* 2004;126:3748–54.
16. Schneekloth AR, Puchault M, Tae HS, et al. Targeted intracellular protein degradation induced by a small molecule: en route to chemical proteomics. *Bioorg Med Chem Lett* 2008;18:5904–8.

17. Hughes SJ, Testa A, Thompson N, et al. The rise and rise of protein degradation: opportunities and challenges ahead. *Drug Discov Today* 2021;21:1359–6446.
18. 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;132:5820–6.
19. Okuhira K, Ohoka N, Sai K, et al. Specific degradation of CRABP-II via cIAP1-mediated ubiquitylation induced by hybrid molecules that crosslink cIAP1 and the target protein. *FEBS Lett* 2011;585:1147–52.
20. Okuhira K, Demizu Y, Hattori T, et al. Development of hybrid small molecules that induce degradation of estrogen receptor-alpha and necrotic cell death in breast cancer cells. *Cancer Sci* 2013;104:1492–8.
21. Buckley DL, Van Molle I, Gareiss PC, et al. Targeting the von Hippel-Lindau E3 ubiquitin ligase using small molecules to disrupt the VHL/HIF-1 α interaction. *J Am Chem Soc* 2012;134:4465–8.
22. Salvesen GS, Duckett CS. IAP proteins: blocking the road to death's door. *Nat Rev Mol Cell Biol* 2002;3:401–10.
23. Deveraux QL, Reed JC. IAP family proteins-suppressors of apoptosis. *Genes Dev* 1999;13:239–52.
24. Birnbaum MJ, Clem RJ, Miller LK. An apoptosis-inhibiting gene from a nuclear polyhedrosis virus encoding a polypeptide with Cys/His sequence motifs. *J Virol* 1994;68:2521–8.
25. Crook NE, Clem RJ, Miller LK. An apoptosis-inhibiting baculovirus gene with a zinc finger-like motif. *J Virol* 1993;67:2168–74.
26. Ndubaku C, Cohen F, Varfolomeev E, et al. Targeting inhibitor of apoptosis proteins for therapeutic intervention. *Future Med Chem* 2009;1:1509–25.
27. Yang L, Cao Z, Yan H, et al. Coexistence of high levels of apoptotic signaling and inhibitor of apoptosis proteins in human tumor cells: implication for cancer specific therapy. *Canc Res* 2003;63:6815–24.
28. Tamm I, Kornblau SM, Segall H, et al. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. *Clin Canc Res* 2000;6:1796–803.
29. Ma Z, Ji Y, Yu Y, et al. Specific non-genetic IAP-based protein erasers (SNIPERs) as a potential therapeutic strategy. *Eur J Med Chem* 2021;216:113247.
30. Naito M, Ohoka N, Shibata N. SNIPERs-Hijacking IAP activity to induce protein degradation. *Drug Discov Today Technol* 2019;31:35–42.
31. Ishikawa M, Tomoshige S, Demizu Y, et al. Selective degradation of target proteins by chimeric small-molecular drugs, PROTACs and SNIPERs. *Pharmaceuticals (Basel)* 2020;13:74.
32. Naito M, Ohoka N, Shibata N, et al. Targeted protein degradation by chimeric small molecules, PROTACs and SNIPERs. *Front Chem* 2019;7:849.
33. Scott DE, Bayly AR, Abell C, et al. Small molecules, big targets: drug discovery faces the protein-protein interaction challenge. *Nat Rev Drug Discov* 2016;15:533–50.
34. Cong H, Xu L, Wu Y, et al. Inhibitor of apoptosis protein (IAP) antagonists in anticancer agent discovery: current status and perspectives. *J Med Chem* 2019;62:5750–72.
35. Li L, Thomas RM, Suzuki H, et al. A small molecule Smac mimic potentiates TRAIL- and TNF α -mediated cell death. *Science* 2004;305:1471–4.
36. Sekine K, Takubo K, Kikuchi R, et al. Small molecules destabilize cIAP1 by activating auto-ubiquitylation. *J Biol Chem* 2008;283:8961–8.
37. Weng G, Shen C, Cao D, et al. PROTAC-DB: an online database of PROTACs. *Nucleic Acids Res* 2021;49:1381–7.
38. 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.
39. Campos C, Sotomayor P, Jerez D, et al. Exercise and prostate cancer: from basic science to clinical applications. *Prostate* 2018;78:639–45.
40. Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev* 2004;25:276–308.
41. Shibata N, Nagai K, Morita Y, et al. Development of protein degradation inducers of androgen receptor by conjugation of androgen receptor ligands and inhibitor of apoptosis protein ligands. *J Med Chem* 2018;61:543–75.
42. Czabotar PE, Lessene G, Strasser A, et al. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. *Nat Rev Mol Cell Biol* 2014;15:49–63.
43. Tse C, Shoemaker AR, Adickes J, et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res* 2008;68:3421–8.
44. Zhang X, He Y, Zhang P, et al. Discovery of IAP-recruiting BCL-X_L PROTACs as potent degraders across multiple cancer cell lines. *Eur J Med Chem* 2020;199:112397.
45. Ren R. Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer* 2005;5:172–83.
46. Lai AC, Toure M, Hellerschmied D, et al. Modular PROTAC design for the degradation of oncogenic BCR-ABL. *Angew Chem Int Ed Engl* 2016;55:807–10.
47. Demizu Y, Shibata N, Hattori T, et al. Development of BCR-ABL degradation inducers via the conjugation of an imatinib derivative and a cIAP1 ligand. *Bioorg Med Chem Lett* 2016;26:4865–9.
48. Shibata N, Miyamoto N, Nagai K, et al. Development of protein degradation inducers of oncogenic BCR-ABL protein by conjugation of ABL kinase inhibitors and IAP ligands. *Cancer Sci* 2017;108:1657–66.
49. Shimokawa K, Shibata N, Sameshima T, et al. Targeting the allosteric site of oncoprotein bcr-abl as an alternative strategy for effective target protein degradation. *ACS Med Chem Lett* 2017;8:1042–7.
50. Zengerle M, Chan KH, Ciulli A. Selective small molecule induced degradation of the BET bromodomain protein BRD4. *ACS Chem Biol* 2015;10:1770–7.
51. Kounde CS, Shchepinova MM, Saunders CN, et al. A caged E3 ligase ligand for PROTAC-mediated protein degradation with light. *Chem Commun (Camb)* 2020;56:5532–5.
52. Ohoka N, Ujikawa O, Shimokawa K, et al. Different degradation mechanisms of inhibitor of apoptosis proteins (IAPs) by the specific and non-genetic IAP-dependent protein eraser (SNIPER). *Chem Pharm Bull (Tokyo)* 2019;67:203–9.
53. Pan ZY, Scheerens H, Li SJ, et al. Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem* 2007;2:58–61.
54. Sun Y, Zhao X, Ding N, et al. PROTAC-induced BTK degradation as a novel therapy for mutated BTK C481S induced ibrutinib-resistant B-cell malignancies. *Cell Res* 2018;28:779–81.
55. Sun Y, Ding N, Song Y, et al. Degradation of Bruton's tyrosine kinase mutants by PROTACs for potential treatment of

- ibrutinib-resistant non-Hodgkin lymphomas. *Leukemia* 2019;33:2105–10.
56. Zorba A, Nguyen C, Xu Y, et al. Delineating the role of cooperativity in the design of potent PROTACs for BTK. *Proc Natl Acad Sci U S A* 2018;115:7285–92.
 57. Schiemer J, Horst R, Meng Y, et al. Snapshots and ensembles of BTK and cIAP1 protein degrader ternary complexes. *Nat Chem Biol* 2021;17: 152–60.
 58. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: from discovery to therapy. *Cancer Discov* 2016;6:353–67.
 59. Walker AJ, Wedam S, Amiri-Kordestani L, et al. FDA approval of palbociclib in combination with fulvestrant for the treatment of hormone receptor-positive, HER2-negative metastatic breast cancer. *Clin Cancer Res* 2016;22:4968–72.
 60. Steinebach C, Ng YLD, Susic I, et al. Systematic exploration of different E3 ubiquitin ligases: an approach towards potent and selective CDK6 degraders. *Chem Sci* 2020;11: 3474–86.
 61. Dominici MD, Porazzi P, Xiao Y, et al. Selective inhibition of Ph-positive ALL cell growth through kinase-dependent and-independent effects by CDK6-specific PROTACs. *Blood* 2020;135:1560–73.
 62. Su S, Yang Z, Gao H, et al. Potent and preferential degradation of cdk6 via proteolysis targeting chimera degraders. *J Med Chem* 2019;62:7575–82.
 63. Anderson NA, Cryan J, Ahmed A, et al. Selective CDK6 degradation mediated by cereblon, VHL, and novel IAP-recruiting PROTACs. *Bioorg Med Chem Lett* 2020;30:127106.
 64. Itoh Y, Ishikawa M, Kitaguchi R, et al. Development of target protein-selective degradation inducer for protein knockdown. *Bioorg Med Chem* 2011;19:3229–41.
 65. Itoh Y, Ishikawa M, Kitaguchi R, et al. Double protein knockdown of cIAP1 and CRABP-II using a hybrid molecule consisting of ATRA and IAPs antagonist. *Bioorg Med Chem Lett* 2012;22:4453–577.
 66. Sharma SV, Bell DW, Settleman J, et al. Epidermal growth factor receptor mutations in lung cancer. *Nat Rev Cancer* 2007;7:169–81.
 67. Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. *Nat Rev Cancer* 2005;5: 341–54.
 68. Burslem GM, Smith BE, Lai AC, et al. The advantages of targeted protein degradation over inhibition: an RTK case study. *Cell Chem Biol* 2018;25:67–77.
 69. Zhang X, Xu F, Tong L, et al. Design and synthesis of selective degraders of EGFR L858R/T790M mutant. *Eur J Med Chem* 2020;192:112199.
 70. Georgiadis D, Mpakali A, Koumantou D, et al. Inhibitors of ER aminopeptidase 1 and 2: from design to clinical application. *Curr Med Chem* 2019;26:2715–29.
 71. Demizu Y, Misawa T, Nagakubo T, et al. Structural development of stabilized helical peptides as inhibitors of estrogen receptor (ER)-mediated transcription. *Bioorg Med Chem* 2015;23:4132–8.
 72. Itoh Y, Kitaguchi R, Ishikawa M, et al. Design, synthesis and biological evaluation of nuclear receptor-degradation inducers. *Bioorg Med Chem* 2011;19:6768–78.
 73. Demizu Y, Okuhira K, Motoi H, et al. Design and synthesis of estrogen receptor degradation inducer based on a protein knockdown strategy. *Bioorg Med Chem Lett* 2012;22: 1793–6.
 74. Demizu Y, Ohoka N, Nagakubo T, et al. Development of a peptide-based inducer of nuclear receptors degradation. *Bioorg Med Chem Lett* 2016;26:2655–8.
 75. Ohoka N, Okuhira K, Ito M, et al. In vivo knockdown of pathogenic proteins via specific and nongenetic inhibitor of apoptosis protein (IAP)-dependent protein erasers (SNIPERs). *J Biol Chem* 2017;292:4556–70.
 76. Ohoka N, Morita Y, Nagai K, et al. Derivatization of inhibitor of apoptosis protein (IAP) ligands yields improved inducers of estrogen receptor α degradation. *J Biol Chem* 2018;293: 6776–90.
 77. Disch JS, Duffy JM, Lee ECY, et al. Bispecific estrogen receptor α degraders incorporating novel binders identified using DNA-encoded chemical library screening. *J Med Chem* 2021;64:5049–66.
 78. Yokoo H, Ohoka N, Takyo M, et al. Peptide stapling improves the sustainability of a peptide-based chimeric molecule that induces targeted protein degradation. *Int J Mol Sci* 2021;22:8772.
 79. Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007;26: 5541–52.
 80. Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. *Nat Rev Drug Discov* 2014;13:673–91.
 81. Cao J, Zhao W, Liu Q, et al. Development of a Bestatin-SAHA hybrid with dual inhibitory activity against APN and HDAC. *Molecules* 2020;25:4991.
 82. O'Shea JJ, Schwartz DM, Villarino AV, et al. The JAK-STAT pathway: impact on human disease and therapeutic intervention. *Annu Rev Med* 2015;66:311–28.
 83. Field SD, Arkin J, Li J, et al. Selective downregulation of JAK2 and JAK3 by an ATP-competitive pan-JAK inhibitor. *ACS Chem Biol* 2017;12:1183–7.
 84. Shah RR, Redmond JM, Mihut A, et al. Hi-JAK-ing the ubiquitin system: the design and physicochemical optimization of JAK PROTACs. *Bioorg Med Chem* 2020;28:115326.
 85. Ohoka N, Misawa T, Kurihara M, et al. Development of a peptide-based inducer of protein degradation targeting NOTCH1. *Bioorg Med Chem Lett* 2017;27:4985–8.
 86. Moellering RE, Cornejo M, Davis TN, et al. Direct inhibition of the NOTCH transcription factor complex. *Nature* 2009; 462:182–8.
 87. Huryn DM, Kornfilt DJP, Wipf P. p97: an emerging target for cancer, neurodegenerative diseases, and viral infections. *J Med Chem* 2020;63:1892–907.
 88. Barthelme D, Sauer RT. Origin and functional evolution of the Cdc48/p97/VCP AAA + protein unfolding and remodeling machine. *J Mol Biol* 2016;428:1861–9.
 89. Donovan KA, Ferguson FM, Bushman JW, et al. Mapping the degradable kinome provides a resource for expedited degrader development. *Cell* 2020;183:1714–31.
 90. Gergely F, Karlsson C, Still I, et al. The TACC domain identifies a family of centrosomal proteins that can interact with microtubules. *Proc Natl Acad Sci U S A* 2000;97:14352–7.
 91. LeRoy PJ, Hunter JJ, Hoar KM, et al. Localization of human TACC3 to mitotic spindles is mediated by phosphorylation on Ser558 by Aurora A: a novel pharmacodynamic method for measuring Aurora A activity. *Cancer Res* 2007;67: 5362–70.
 92. Ohoka N, Nagai K, Hattori T, et al. Cancer cell death induced by novel small molecules degrading the TACC3

- protein via the ubiquitin-proteasome pathway. *Cell Death Dis* 2014;5:e1513.
93. Rittchen S, Heinemann A. Therapeutic potential of hematopoietic prostaglandin D2 synthase in allergic inflammation. *Cells* 2019;8:619.
 94. Aritake K, Kado Y, Inoue T, et al. Structural and functional characterization of HQL-79, an orally selective inhibitor of human hematopoietic prostaglandin D synthase. *J Biol Chem* 2006;281:15277–86.
 95. Yokoo H, Shibata N, Naganuma M, et al. Development of a hematopoietic prostaglandin D synthase-degradation inducer. *ACS Med Chem Lett* 2021;12:236–41.
 96. Chaudhary D, Robinson S, Romero DL. Recent advances in the discovery of small molecule inhibitors of interleukin-1 receptor-associated kinase 4 (IRAK4) as a therapeutic target for inflammation and oncology disorders. *J Med Chem* 2015;58:96–110.
 97. Zhang J, Fu L, Shen B, et al. Assessing IRAK4 functions in ABC DLBCL by IRAK4 kinase inhibition and protein degradation. *Cell Chem Biol* 2020;27:1500–9.
 98. Nunes J, McGonagle GA, Eden J, et al. Targeting IRAK4 for degradation with PROTACs. *ACS Med Chem Lett* 2019;10:1081–5.
 99. Jensen S, Seidelin JB, LaCasse EC, et al. SMAC mimetics and RIPK inhibitors as therapeutics for chronic inflammatory diseases. *Sci Signal* 2020;13:8295.
 100. Hyun S, Shin D. Chemical-mediated targeted protein degradation in neurodegenerative diseases. *Life(Basel)* 2021;11:607.
 101. Mares A, Miah AH, Smith IED, et al. Extended pharmacodynamic responses observed upon PROTAC-mediated degradation of RIPK2. *Commun Biol* 2020;3:140.
 102. Miah AH, Smith IED, Rackham M, et al. Optimization of a series of RIPK2 PROTACs. *J Med Chem* 2021;64:12978–3003.
 103. Reiner A, Dragatsis I, Dietrich P. Genetics and neuropathology of Huntington's disease. *Int Rev Neurobiol* 2011;98:325–72.
 104. Tomoshige S, Nomura S, Ohgane K, et al. Discovery of small molecules that induce the degradation of Huntingtin. *Angew Chem Int Ed Engl* 2017;56:11530–3.
 105. Schafer PH, Parton A, Capone L, et al. Apremilast is a selective PDE4 inhibitor with regulatory effects on innate immunity. *Cell Signal* 2014;26:2016–29.
 106. Bhat A, Ray B, Mahalakshmi AM, et al. Phosphodiesterase-4 enzyme as a therapeutic target in neurological disorders. *Pharmacol Res* 2020;160:105078.
 107. Tsukumo Y, Tsuji G, Yokoo H, et al. Protocols for synthesis of SNIPERs and the methods to evaluate the anticancer effects. *Methods Mol Biol* 2021;2365:331–47.
 108. Wang KLIF, Cao WB, Lv XX, et al. TRIB3 promotes APL progression through stabilization of the oncoprotein PML-RAR α and inhibition of p53-mediated senescence. *Cancer Cell* 2017;31:697–710.
 109. Lo-Coco F, Hasan SK. Understanding the molecular pathogenesis of acute promyelocytic leukemia. *Best Pract Res Clin Haematol* 2014;27:3–9.