Front. Med. 2023, 17(5): 823−854
https://doi.org/10.1007/s11684-023-1027-5

REVIEW

Discovery of small molecule degraders for modulating cell cycle

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Abstract **The cell cycle is a complex process that involves DNA replication, protein expression, and cell division. Dysregulation of the cell cycle is associated with various diseases. Cyclin-dependent kinases (CDKs) and their corresponding cyclins are major proteins that regulate the cell cycle. In contrast to inhibition, a new approach called proteolysis-targeting chimeras (PROTACs) and molecular glues can eliminate both enzymatic and scaffold functions of CDKs and cyclins, achieving targeted degradation. The field of PROTACs and molecular glues has developed rapidly in recent years. In this article, we aim to summarize the latest developments of CDKs and cyclin protein degraders. The selectivity, application, validation and the current state of each CDK degrader will be overviewed. Additionally, possible methods are discussed for the development of degraders for CDK members that still lack them. Overall, this article provides a comprehensive summary of the latest advancements in CDK and cyclin protein degraders, which will be helpful for researchers working on this topic.**

Keywords PROTAC; molecular glue; degrader; cell cycle; CDK; cyclin

Introduction

The cell cycle is a fundamental process in cells that involves DNA replication, protein expression, and cell division. Many proteins are known to modulate the cell cycle through various pathways. Among these proteins, cyclin-dependent kinases (CDKs) and cyclins are major members that play essential roles in cell cycle regulation. In this review, we focus solely on the degrader progress of CDKs and cyclins. CDKs are a type of serine/threonine kinases that form a complex with cyclin proteins, playing essential roles in cell cycle regulation [1]. As a kinase, CDK binds with ATP and its regulatory subunits cyclin to phosphorylate various substrates that regulate cell cycle processes and transcription through phosphate group transfer [2]. The activity of CDK/cyclin proteins changes periodically in order as the cell cycle progresses, playing essential roles in regulating the cell cycle and its essential components [3].

Cdc2, which is the homolog of mammalian CDK1, was the first CDK member identified in *Saccharomyces cerevisiae* [4,5]. Other CDKs were subsequently discovered with the guidance of CDK1 and new techniques [6–8]. Generally, the formation of a CDK-cyclin-ATP ternary complex is required to phosphorylate CDK substrates, as the binding of cyclin induces a conformational change in CDK from an inactive to active state, except for CDK5 [9]. There are 20 known subtypes of CDKs (numbered from CDK1 to CDK20) and 29 cyclins [1,10]. Generally, CDK family members play important roles in controlling cell cycle and transcription, as shown in Fig. 1. In recent years, some other CDK family members have been discovered, which have more complex functions, especially in some diseases represented by tumors and neurodevelopment (Table 1) [11]. CDKs could combine with temporal cyclin binding partners to form CDK/cyclin complex. A certain CDK usually binds with a specific type of cyclins, but not all cyclins. CDKs involved in cell cycle regulation can bind with multiple cyclins to exert multiple functions, while those involved in transcriptional regulation usually only bind with a single cyclin [1]. It should be noted that different cyclins binding to the same CDK can also regulate different cell cycle processes [12]. Fig. 1 and Table 1 below summarize the typical functions of CDKs.

Received May 17, 2023; accepted August 16, 2023

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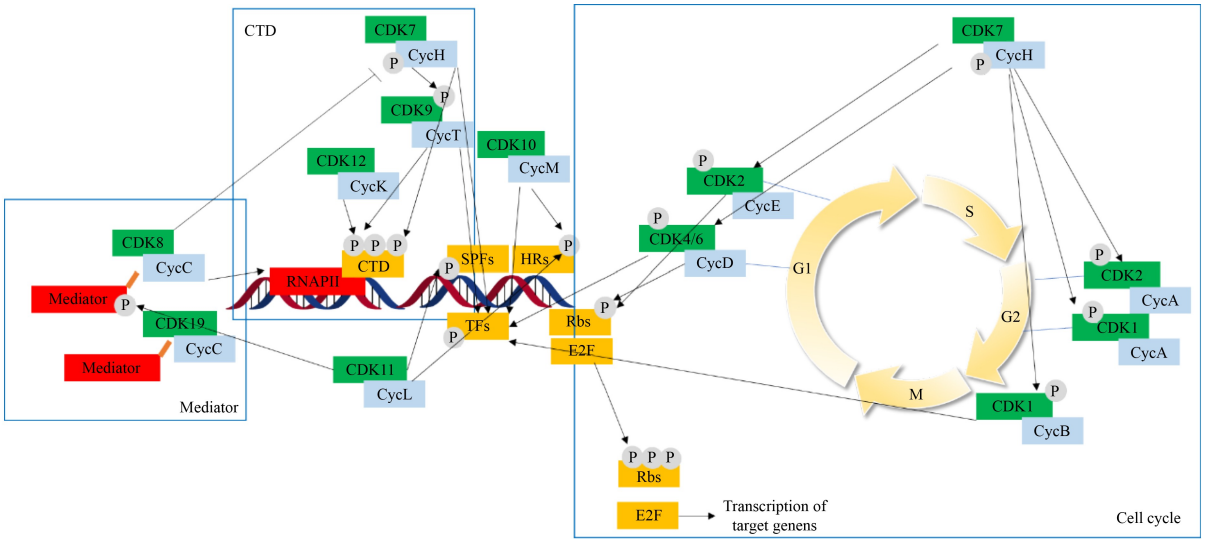


Fig. 1 Typical functions of CDKs. CDKs involved in cell cycle and transcription.

Table 1 Typical functions and corresponding cyclins of CDKs

Functions	CDKs	Cyclins	Main functions	Achieved degradation
Cell cycle	CDK1	A1, A2, B1, B2, D1, D3, E1, F, K	Trigger S–G2 and G2–M transitions and G2 progression	Y
	CDK2	A1, A2, B1, B2, B3, D1, D2, D3, E1, E2, G1, H, J, K	Trigger G1–S transition; control G1–S and G2–M transitions	Y
	CDK4/6	A2, D1, D2, D3, E1, T1, T2	Mediate the monophosphorylation of Rb in G1; promotes G1–S transition	Y
Transcription	CDK7	A2, B1, B2, E1, H	Mediate the activation of CDK1/2/4/6/11 by catalyzing the phosphorylation of a threonine residue within the T-loop or activation segment	Y
	CDK8/19	C, H	Regulation of RNA polymerase II transcription; phosphorylation of NOTCH leads to its degradation	Y
	CDK9	H, K, T1, T2A, T2B	Regulation of RNA polymerase II transcription; genome integrity maintenance	Y
	CDK12/13	K	Regulation of RNA polymerase II and transcription elongation	Y
Special roles	CDK3	A2, C, E1, E2	Function is poorly defined: may trigger reentry from G0–G1 and through phosphorylation of Rb; may trigger G1–S transitions by catalyzing the phosphorylation of E2F1/2/3	N
	CDK5	A2, B1, D2, G1, E1, I1, I2	Function is poorly defined: may produce neuronal cell cycle arrest and differentiation and participates in many aspects of neuronal function	Y
	CDK10	L2, M	Traversing start point and phosphorylation of transcription factor ETS2 leading to its degradation	Y
	CDK11	L1, L2	Regulation of apoptosis cytokinesis	Y
	CDK14	D3, Y	Regulate cell cycle and neuronal differentiation	Y
	CDK15	Y	Antiapoptotic	N
	CDK16	Y	Regulation of neuron differentiation and exocytosis	Y
	CDK17/18	K, Y	Play a role in terminally differentiated neurons	Y
	CDK20	H	Regulation of neural development	N

Due to the critical role of CDKs in the cell cycle, abnormalities in CDKs can lead to severe diseases, such as tumorigenesis [13], malformation [14], diabetes [15], and neurodegenerative diseases [16], particularly when CDKs and cyclins are overactivated. As a result, CDK inhibitors have attracted significant interest from both the clinical and research communities. Numerous CDK inhibitors have been reported with promising activity

in vitro and/or *in vivo* (animal models) [17]. Currently, over 50 CDK inhibitors have been investigated in clinical trials or are approved for clinical use [17]. However, only five CDK inhibitors targeting CDK4/6 have been approved for clinical use [17]. Hormone receptor positive (HR^+)/$HER2^-$ is the most common type of breast cancer in patients with breast cancer. In the original treatment regimen, HR^+ breast cancer patients can receive endocrine therapy with fluvestran, anastrozole, and other drugs, but they are prone to drug resistance. As a result, patients with advanced breast cancer often lack effective treatment options. CDK4/6 inhibitors effectively block the progression of tumor cells from G1 phase to S phase, thus blocking the proliferation of tumor cells and achieving the purpose of inhibiting the growth of breast cancer cells. This has changed the traditional model of endocrine therapy, and CDK4/6 inhibitor combined endocrine therapy has become the new standard of first- and second-line treatment for HR^+/$HER2^-$ breast cancer patients. For example, Palbociclib, Ribociclib, Abemaciclib, and Dalcilil, are approved for HR^+/$HER2^-$ advanced breast cancer. Currently, Pipercilil, Abesilil, and Dalcilil have been approved in China. A number of clinical data show that these inhibitors extended the overall survival of advanced breast cancer and achieved a good therapeutic effect [18,19].

Despite the development of high-throughput screening techniques, it remains challenging to develop CDK inhibitors with high potency, selectivity, and activity [20,21]. One major obstacle is the high conservation within the CDK family [17]. Most CDK inhibitors mimic the ATP binding pattern and occupy the ATP binding domain. Small molecules often fail to recognize the small differences between the primary target and other similar kinases. For example, CDK2 shares a highly identical sequence with other CDK members, with almost 70% similarity to CDK1 [22]. Although many inhibitors have successfully inhibited the enzymatic function of CDK2 with high potency, few have achieved selective CDK2 inhibition [22]. Thus, it is challenging to determine how much of the efficacy comes from CDK2 inhibition and how much from off-target inhibition. Moreover, selective inhibitors for other CDK members are also rare, not only for CDK2.

Proteolysis-targeting chimeras (PROTACs) and molecular glue are emerging techniques that use small molecules as carriers to eliminate proteins instead of inhibiting their enzymatic function [23]. PROTACs consist of three components: a binder with affinity for the protein of interest (POI), a binder with affinity for E3 ligase, and a linker that connects these two binders. Based on these components, a PROTAC molecule forms a dual affinity structure with both POI and E3 ligase, which attracts these two proteins to form a "POI-PROTAC-E3 ligase" ternary complex in space. Then, E3 ligase recruits other necessary components to facilitate POI ubiquitination and degradation (Fig. 2A). Generally, the commonly used E3 ligase ligands mainly include pomalidomide and its derivatives targeting cereblon, VH032 ligand targeting VHL, nutlin-3a ligand targeting MDM2, and MeBS ligand targeting cIAP. Among them, cereblon ligands are the most widely used in PROTAC development due to their small molecular weight and high activity. The representative molecular structures are shown in Fig. 2B. Although the concept of molecular glue has not been clearly defined, its mechanism appears to be much simpler in comparison (Fig. 2C) [24]. Molecular glue plays a key role in inducing protein–protein interactions (PPI). One component of the PPI is the POI, and the other may be an element in E3 ligase or a ubiquitin-associated protein. This means that the small molecule acts as a "glue" to bind the POI and the ubiquitination-related protein, which then recruits other ubiquitination proteins to facilitate POI ubiquitination and degradation. Once ubiquitination is completed, the molecule is released and can function as a catalyst to enter the catalytic cycle.

PROTACs and molecular glue offer a new way to address challenges that are difficult for conventional molecules. Although we do not fully understand the internal mechanisms of PROTAC and molecular glue, empirically guided drug design has successfully achieved selective degradation by a non-selective binder. It is worth noting that although we do not fully understand the degradation mechanism, it does not prevent PROTACs and molecular glues from playing an increasingly important role in the pharmaceutical field. Recently, with the improvement of ADME research, ARV-110 and ARV-471, as orally available PROTACs molecules, have been pushed to clinical trials. They target androgen receptor (AR) and estrogen receptor (ER) respectively for the treatment of metastatic castration-resistant cancer (NCT03888612) and metastatic ER^+/$HER2^-$ breast cancer (NCT04072952), and have entered phase III clinical studies respectively. In addition, many companies have also disclosed their preclinical and research projects of PROTACs [25]. In the field of molecular glues, traditional drugs such as Pomalidoimide, Thalidomide, Lenalidomide, etc., as immunomodulators, have been successfully applied in clinical settings for multiple myeloma, anti-inflammatory and other applications. Recently, Celgene's IMiD platform, CC-90009 (NCT04336982), CC-92480 (NCT05372354) and other molecules are also testing safety doses and therapeutic effects in clinical trials. This fully demonstrates the application prospects of PROTACs and molecular glues in the future disease treatment field.

In the case of PROTACs, binding affinity is a necessary but not sufficient condition for degradation [26]. Although we cannot distinguish small differences within

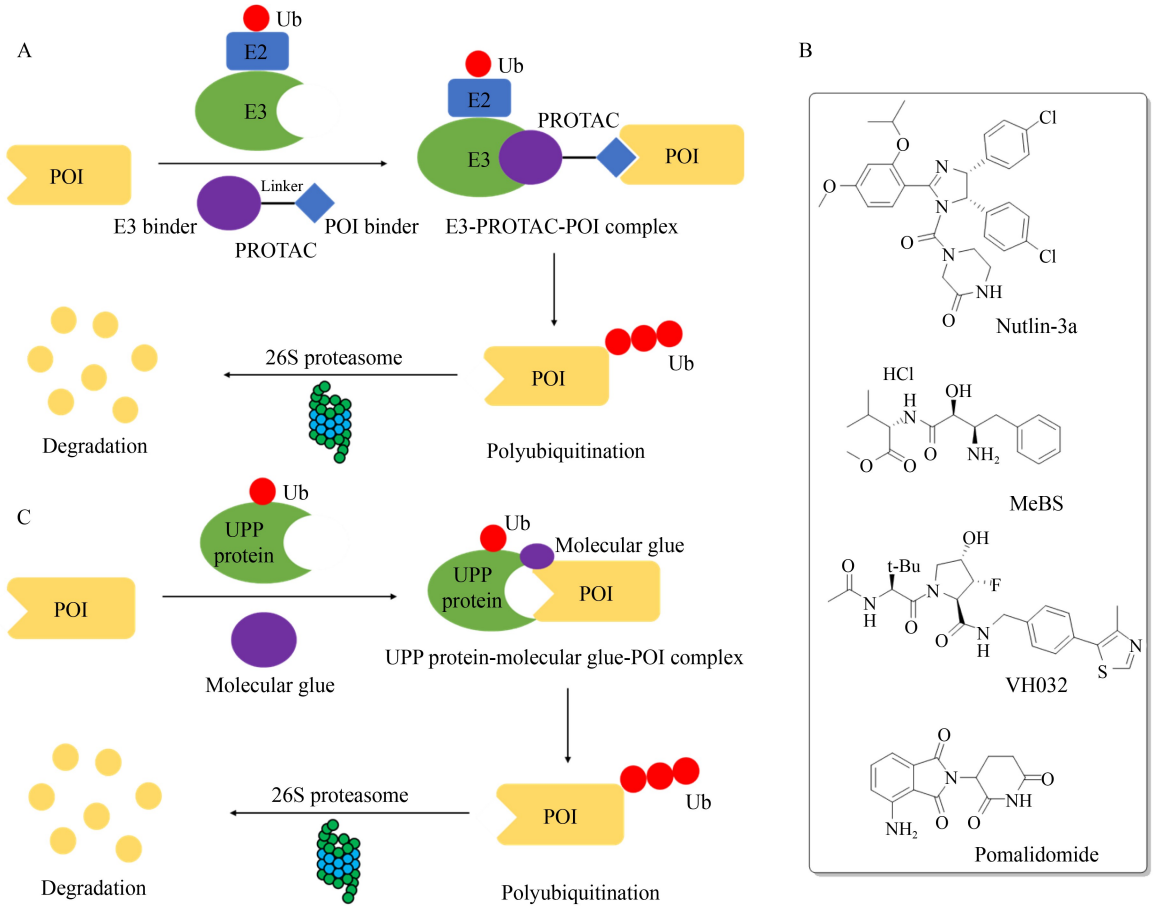


Fig. 2 The structure of PROTACs and molecule glue. The mechanism of PROTACs (A) and molecular glue (C). (B) Chemical structures of representative E3 ligands.

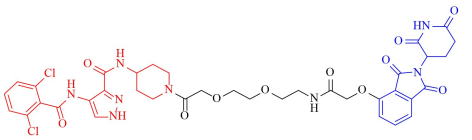
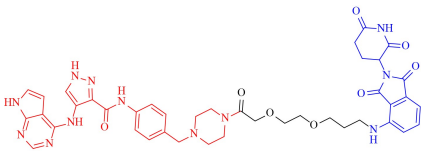
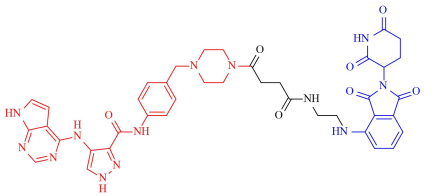
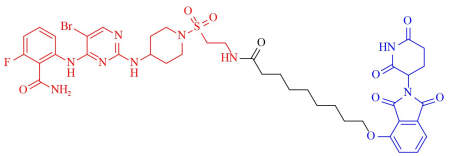
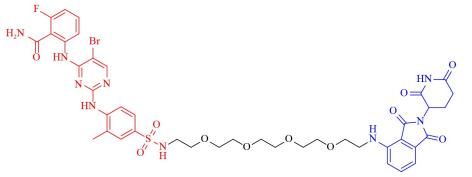
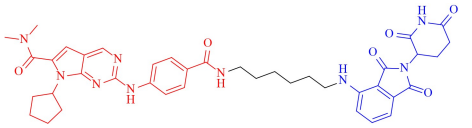
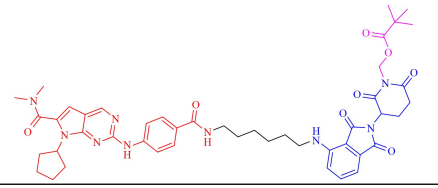
CDK members using small molecule chemical spaces alone, we may still have the opportunity to achieve selective degradation by differences across proteins. With improved selectivity, toxicity and efficacy can be greatly improved. Moreover, PROTACs and molecular glue are designed to eliminate the total structure of a protein rather than just inhibiting its enzymatic function. Thus, these new techniques could be a solution to eliminate the scaffold function of specific proteins, including CDKs. Additionally, PROTACs and molecular glue can be helpful for undruggable targets, as they only require general affinity and a catalytic cycle.

One feasible and simple approach with the help of PROTACs and molecular glue techniques is based on inhibitors and converting them into degraders. So far, numerous attempts have been successful, with over 2500 PROTACs based on this method [27]. Many reviews have systematically described how to design and the lessons learned in PROTACs and molecular glues [23,25,26,28,29]. However, few papers have focused specifically on the CDK field. Sandeep *et al*. previously wrote a review that systematically described the progress in PROTAC and molecular glue targeting CDK [30]. With the rapid development of technology, many aspects need to be updated. In this paper, we summarize all PROTACs and molecular glues targeting CDKs and their corresponding cyclins up to date. Additionally, based on recent developments in PROTAC and molecular glue techniques, we also indicate several possible future development tendencies for PROTAC and molecular glue targeting CDKs and cyclins.

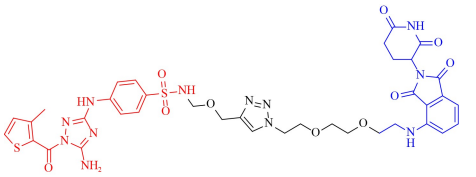
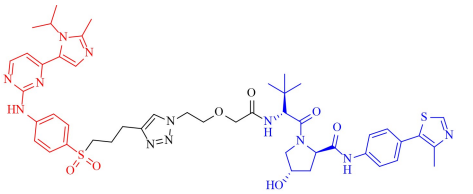
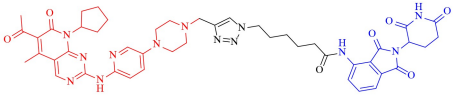
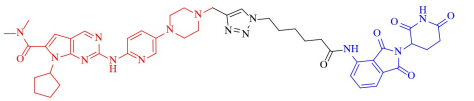
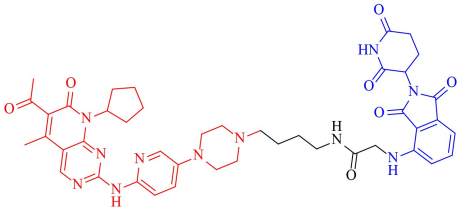
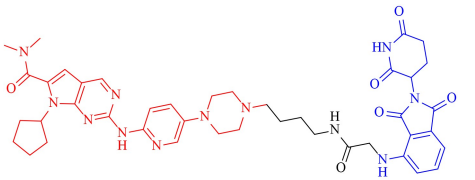
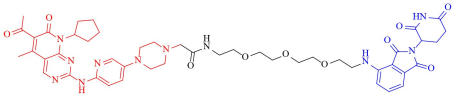
The latest research progress of CDK/cyclin degraders

In this section, we summarize recent research developments, with all molecules classified by their targets. There appears to be an imbalance in the degradable targets among CDK members. For targets such as CDK9 and CDK4/6, they can be easily degraded with many successful attempts. However, for CDK3, there seems to be no degrader reported. Since some PROTACs or molecular glues can degrade more than one CDK or cyclin, we will only discuss these molecules at the time of their first appearance and their main functions in this paper. For better displaying the representative compounds, we summarize the features of each compound into Table 2.

Table 2 Information of representative degraders

Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
1	A9	*In vitro* Rescue Concentration-dependent Cell inhibition	Tumor inhibition	CDK2		[31]
2	F9	*In vitro* Concentration-dependent Cell inhibition	Tumor inhibition	CDK9		[31]
3	F3	*In vitro* Rescue Concentration-dependent Cell inhibition Cell-cycle	Tumor inhibition	CDK2 CDK9		[31]
4	TMX-1160	*In vitro* Concentration-dependent	–	CDK2 CDK4 CDK5 CDK6		[32]
5	TMX-2172	*In vitro* Rescue Concentration-dependent Cell inhibition Proteomics	Tumor inhibition	CDK2 CDK5		[32]
6	Compound 3	*In vitro* and *in vivo* Concentration-dependent Rescue Cell inhibition Cell-cycle PK Xenograft IHC	Orally available Tumor inhibition	CDK2 CDK4 CDK6		[33]
7	Compound 11	Prodrug of 6	–	–		[33]

(*Continued*)

Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
8	CPS2	*In vitro* Time-course Concentration-dependent Rescue Cell inhibition Proteomics	AML differentiation	CDK2		[34]
9	PROTAC-8	*In vitro* and *in vivo* Concentration-dependent	Hearing loss	CDK2		[35]
10	pal-pom	*In vitro* Concentration-dependent Rescue Time-course	Tumor inhibition	CDK4 CDK6		[36]
11	rib-pom	*In vitro* Concentration-dependent	Tumor inhibition	CDK4 CDK6		[36]
12	BSJ-02-162	*In vitro* Rescue Concentration-dependent Cell-cycle Cell inhibition Proteomics	Tumor inhibition	CDK4 CDK6 IKZF1 IKZF3		[37]
13	BSJ-01-187	*In vitro* Rescue Concentration-dependent	–	CDK4 IKZF1 IKZF3		[37]
14	YKL-06-102	*In vitro* Rescue Concentration-dependent	–	CDK6 IKZF1 IKZF3		[37]

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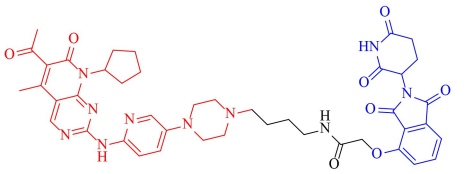
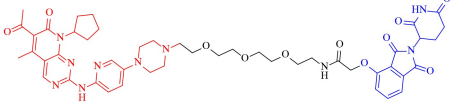
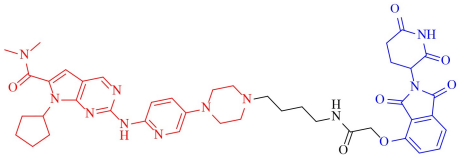
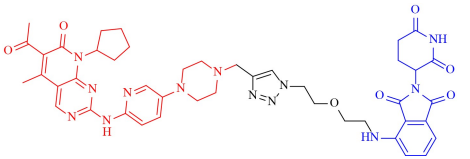
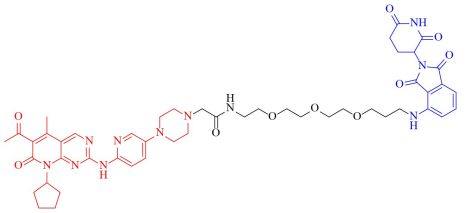
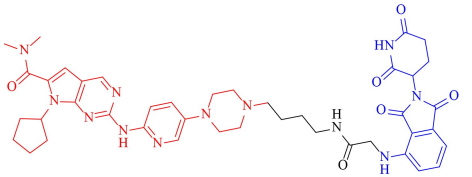
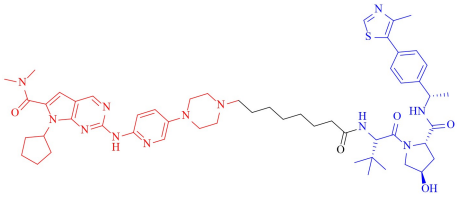
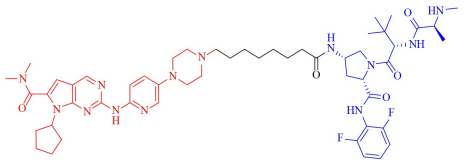
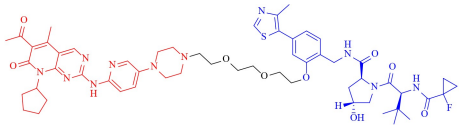
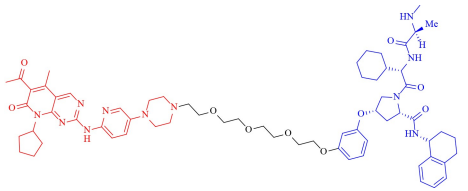
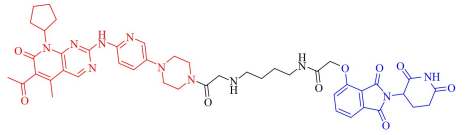
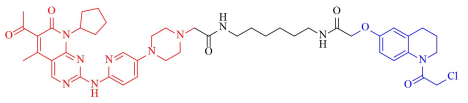
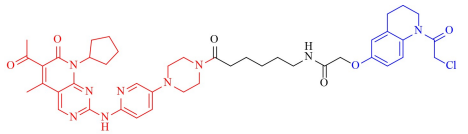
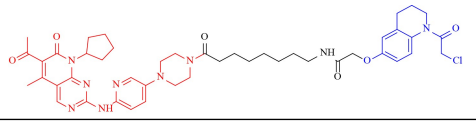
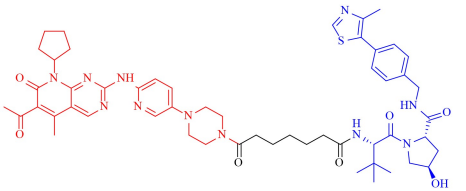
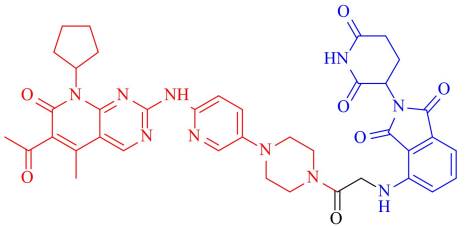
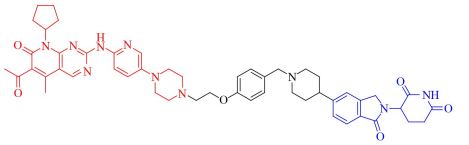
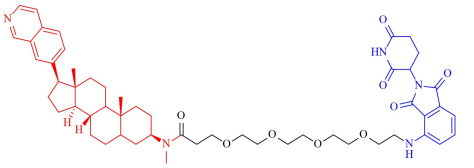
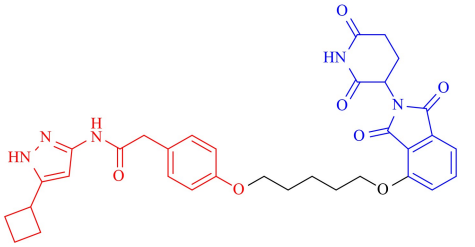
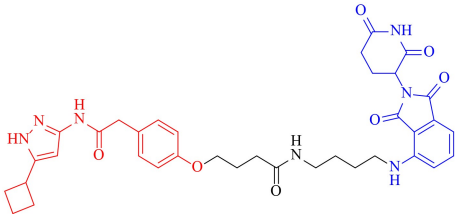
Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
15	BSJ-03-204	*In vitro* Rescue Concentration-dependent Cell-cycle Cell inhibition Proteomics	Tumor inhibition	CDK4 CDK6		[37]
16	BSJ-03-123	*In vitro* Rescue Concentration-dependent Proteomics	–	CDK6		[37]
17	BSJ-04-132	*In vitro* Rescue Concentration-dependent Proteomics	–	CDK4		[37]
18	CP-10	*In vitro* Rescue Concentration-dependent Proteomics	Tumor inhibition	CDK6		[38]
19	Degrader 6	*In vitro* Concentration-dependent Rescue Time-course	–	CDK6		[39]
20	PROTAC-10-CRBN	*In vitro* Concentration-dependent Rescue	–	CDK4 CDK6		[40]
21	PROTAC-4-VHL	*In vitro* Concentration-dependent Rescue	–	CDK4 CDK6		[40]

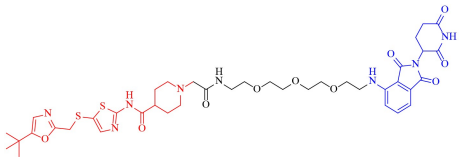
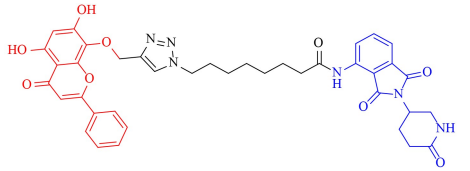
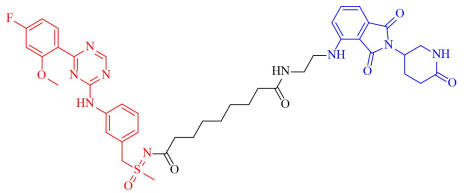
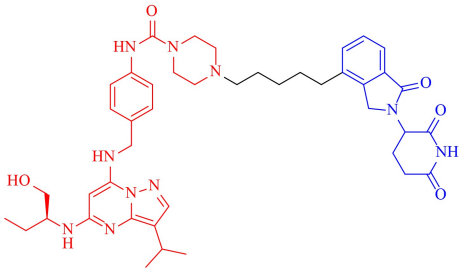
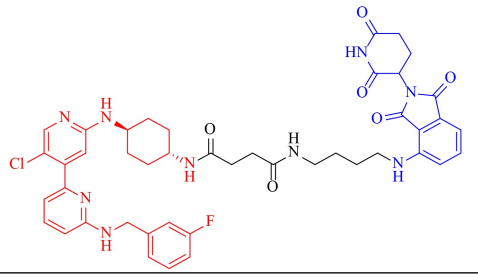
(*Continued*)

Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
22	PROTAC-7-IAP	*In vitro* Concentration-dependent Rescue	–	CDK4 CDK6		[40]
23	CST651	*In vitro* Concentration-dependent Time-course Cell inhibition Rescue Washout PK	Tumor inhibition	CDK4 CDK6		[41]
24	35	*In vitro* Concentration-dependent Cell inhibition	Tumor inhibition	CDK4 CDK6		[41]
25	YX-2-107	*In vitro* and *in vivo* Concentration-dependent Xenograft Proteomics Cell cycle PK	Tumor inhibition	CDK6		[42]
26	A4	*In vitro* Rescue Time-course Concentration-dependent Cell inhibition Cell cycle	Tumor inhibition	CDK4 CDK6		[43]
27	C6	*In vitro* Cell inhibition	Tumor inhibition	CDK4 CDK6		[43]
28	C7	*In vitro* Cell inhibition	Tumor inhibition	CDK4 CDK6		[43]

(*Continued*)

Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
29	MS28	*In vitro* Concentration-dependent Time-course Rescue Kinome Clonogenicity inhibition Proteomics	Tumor inhibition	Cyclin D1 CDK6		[44]
30	MS140	*In vitro* Concentration-dependent	–	CDK6		[44]
31	ALV-07-082-03	*In vitro* Rescue Concentration-dependent Proteomics Cell cycle	IL-2 derepression	CDK4/6, Helios		[45]
32	JH-XI-10-02	*In vitro* Time-course Concentration-dependent	–	CDK8		[46]
33	PROTAC-3-CDK9	*In vitro* Concentration-dependent	–	CDK9		[47]
34	PROTAC-2-CDK9	*In vitro* Concentration-dependent Proteomics Cell inhibition Rescue	Tumor inhibition	CDK9		[48]

(*Continued*)

Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
35	THAL-SNS-032	*In vitro* Time-course Concentration-dependent Rescue Cell inhibition Proteomics RNA-seq ChIP-seq Kinome	Tumor inhibition	CDK9		[49]
36	11c	*In vitro* Concentration-dependent Rescue Cell inhibition	Tumor inhibition	CDK9		[50]
37	B03	*In vitro* and *in vivo* Time-course Kinome Rescue Concentration-dependent Proteomics PK	Tumor inhibition	CDK9		[51]
38	Degrader 45	*In vitro* and *in vivo* Time-course Concentration-dependent Rescue Cell inhibition RNA-seq PK IHC Xenograft	Tumor inhibition	CDK9		[52]
39	CD-5	*In vitro* Proteomics Concentration-dependent Time-course Rescue	–	CDK9		[53]

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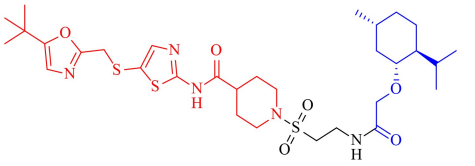
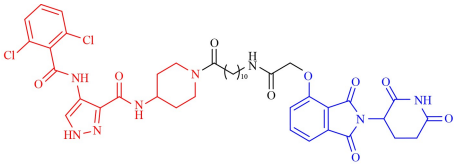
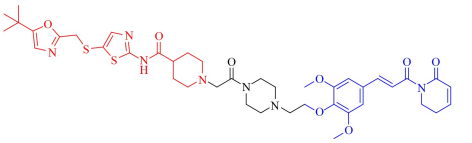
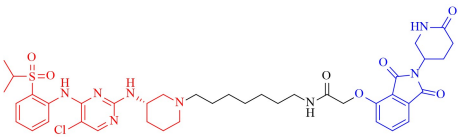
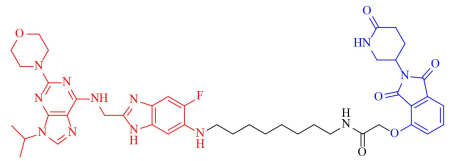
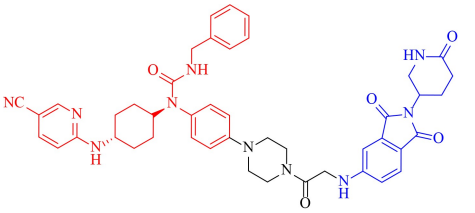
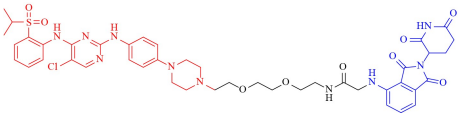
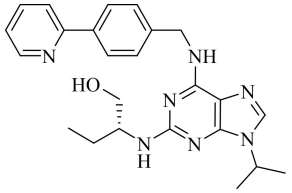
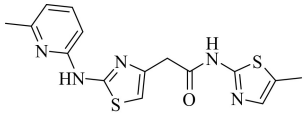
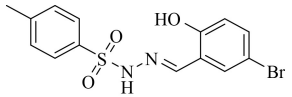
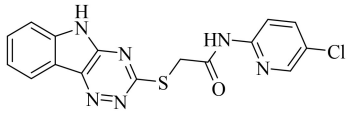
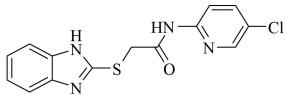
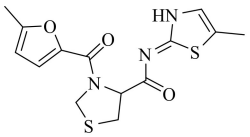
Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
40	LL-K9-3	*In vitro* Concentration-dependent Proteomics Time-course Rescue Cell inhibition RNA-seq	Tumor inhibition	CDK9 Cyclin T1		[54]
41	15e	*In vitro* Concentration-dependent Time-course Cell inhibition Proteomics	–	CDK9		[55]
42	955	*In vitro* Rescue Proteomics Concentration-dependent RNAseq	Tumor inhibition	CDK9 CDK10 And others		[56]
43	BSJ-4-116	*In vitro* Time-course Proteomics Rescue	Tumor inhibition	CDK12		[57]
44	PP-C8	*In vitro* Rescue Concentration-dependent Proteomics Cell inhibition RNA-seq	Tumor inhibition	CDK12 Cyclin K		[58]
45	7f	*In vitro* and *in vivo* Concentration-dependent Proteomics PK	Tumor inhibition	CDK12 CDK13		[59]
46	TL12-186	*In vitro* Proteomics	–	Multi-target		[60]

(*Continued*)

Compound No.	Original name	Validation	Claimed application	Claimed major target(s)	Structure	Reference
47	(R)-CR8	*In vitro* Rescue Time-course Concentration-dependent Proteomics Crystal structure Cell inhibition	–	Cyclin K		[61]
48	HQ461	*In vitro* Proteomics Time-course Concentration-dependent Rescue	Tumor inhibition	CDK12 Cyclin K		[62]
49	dCeMM1	*In vitro* Proteomics Rescue Cell inhibition Time-course RNA-seq	–	RPM39		[63]
50	dCeMM2	*In vitro* Proteomics Time-course Rescue Cell inhibition Cell-cycle RNA-seq	–	Cyclin K		[63]
51	dCeMM3	*In vitro* Proteomics Time-course Cell inhibition Cell-cycle RNA-seq	–	Cyclin K		[63]
52	dCeMM4	*In vitro* Proteomics Time-course Cell inhibition Cell-cycle RNA-seq	–	Cyclin K		[63]

CDK2 PROTACs

CDK2 plays a crucial role in cell cycle regulation. In most normal cells, CDK2 is expressed at relatively low levels [64]. During the G1 phase, the CDK2/cyclin E complex can phosphorylate retinoblastoma proteins (Rbs), which were previously phosphorylated by CDK4/6, leading to the release and activation of E2F transcription factors (E2F) [65]. E2F promotes the transition from G0 to G1 and protein synthesis. CDK2/cyclin A then maintains the phosphorylation of Rbs, which helps to propel the cell cycle from the S phase to the G2 phase [65]. However, there is also considerable evidence showing that CDK2 is dispensable for normal cells, except for genital system development [66], and its function can be partly replaced by CDK1 [67]. CDK2 knockout mice are viable but infertile [68]. Meanwhile, many diseases are highly related to the function of CDK2. For instance, some subtypes of ovarian cancer, neuroblastoma, and lung cancer are sensitive to CDK2 inhibition [69]. Recently, Ying *et al*. demonstrated that some subtypes of acute myeloid leukemia patients could benefit from CDK2 inhibition [70]. They found that AML could differentiate and exit the cell cycle. Therefore, CDK2 inhibition is a low-toxic but highly effective treatment for certain diseases. However, there are very few selective CDK2 inhibitors, and serious off-target effects can lead to severe toxicity.

As mentioned above, the formation of the ternary complex is a necessary but not sufficient condition for degradation. By fine-tuning PROTACs, highly selective degradation may be achieved. The technique of PROTACs has been successfully applied to this target, albeit with many challenges. To date, CDK2 degradation has been achieved by several laboratories using different binders (Fig. 3). In 2019, Chen group reported the first series of CDK2 degraders [31]. They used AT-7519 or FN-1501 as the CDK binder and pomalidomide as the E3 ligase binder (pomalidomide targets CRBN protein). They experimented with different lengths and types of linkers to obtain the A1–10 and F1–10 two-series compounds. After further optimization, they obtained compounds that are mono-CDK2 degradable (compound 1), mono-CDK9 degradable (compound 2), and dual CDK2/9 degradable (compound 3). They also demonstrated a high proliferative inhibitory effect on PC-3 cells. However, they only tested CDK2/5/9 degradation in Western blot, and it is unclear if their compounds have any other possible off-target effects on the CDK family. In 2020, Gray group reported a series of CDK2 PROTACs [32]. In this case, they used TMX-2039, a pan-CDK inhibitor with inhibitory effects on CDK1, CDK2, CDK4, CDK6, CDK7, and CDK9, as the POI binder at first. CDK2,4,5,6 degradation was achieved with compound 4 (TMX-1160) treatment. Then, they made many attempts to synthesize PROTACs with

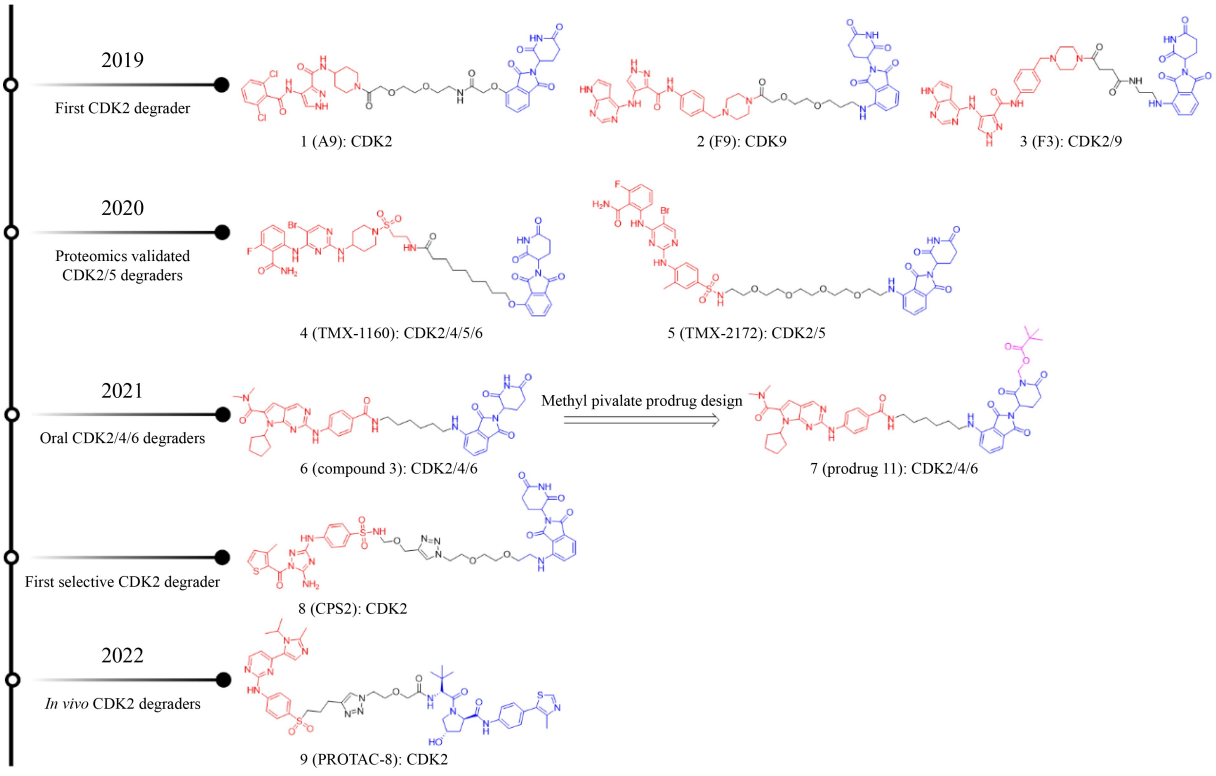


Fig. 3 Structure of CDK2 degraders.

various modified POI binders and linkers to improve efficacy and selectivity. Pomalidomide and its analogs were used as the E3 ligase ligands. Compound 5 (TMX-2172), a representative molecule, achieves CDK2 and CDK5 dual degradation over the CDK family in OVCAR8 cells. They also demonstrated that CDK2 degradation caused the proliferative inhibition of OVCAR8 cells. Later, Yang group reported the first orally bioavailable prodrug of PROTACs targeting CDK2/4/6 [33]. In this case, they used Ribociclib and its analog as the POI binder. Ribociclib has no inhibitory effect on CDK2. After many rounds of optimization, the analog of Ribociclib exhibited enhanced potency for CDK2, as well as CDK4 and CDK6. After conjugation with pomalidomide, they successfully obtained the CDK2/4/6 degrader (compound 6), which showed potent efficacy on B15F10 and A375 cells. They demonstrated that compound 6 significantly inhibited colony formation in malignant melanoma cells and induced apoptosis in cancer cells. However, after pharmacokinetic experiments, they found that compound 6 was nearly impossible to absorb orally. To improve the oral bioavailability, based on compound 6, they introduced a methyl pivalate on the pomalidomide arms to form a prodrug with much improved stability and oral bioavailability (compound 7). The prodrug also showed potent tumor suppression and CDK2/4/6 knockdown effects on mouse models. In 2021, our group reported the first CDK2 degrader with much improved selectivity over the CDK family [34]. We initially attempted to use JNJ-7706621 as the POI binder and pomalidomide as the E3 ligase binder to obtain CPF1. However, only weak CDK2 degradation was observed. We then refined the CDK2 binder design to obtain a family of molecules named CPS. After further optimization, we selected compound 8 (CPS2) as the lead compound for the next investigation. In this work, we evaluated CPS2 in more than 20 cell types, showing potent efficacy at nanomolar concentrations. CPS2 is capable of achieving fast CDK2 knockout even in 30 min. We also demonstrated the selectivity of CPS2 by Western blot and proteomics of CDK1/2/4/5/6/7/8/9, further confirming its good selectivity over the CDK family. We used engineered cells and epithelial cells 293T and Beas2b to test the toxicity of CPS2 in cells. Almost no suppression was observed, even at very high concentrations. In functional experiments, we treated AML cells with CPS2, which induced significant AML differentiation. Thus, CPS2, which exhibits high efficacy, high selectivity, wide application, and low toxicity, well reproduces the findings of RNAi and CRISPR-Cas9. In 2021, Zuo group successfully developed another selective CDK2 degrader [35]. In this study, they used AZD5438 or AZD7519-7 as the POI binder and pomalidomide or VH032 as E3 ligase ligand (VH032 targeted E3 ligase VHL). This was the first successful attempt to use the VHL ligand in their PROTACs design targeting CDK2. Their represented molecule compound 9 (PROTAC-8) achieved sub-micromolar degradation efficacy. They also attempted to use this molecule as a treatment to prevent acquired hearing loss *in vivo*.

In summary, several CDK2 degraders have been reported, but most of them were non-selective or lacked validation. Compound 8 is currently one of the validated and selective degraders targeting CDK2. Moving forward, more research should focus on improving selectivity and druggability of CDK2 degraders.

CDK4/6 PROTACs

Typically, CDK4 and its homolog CDK6 share a similar mode of action and have been shown to have redundant roles in many aspects [71]. CDK4/6 dual knockout probably results in embryonic lethality [72]. D-type cyclins (D1, D2, D3) activate CDK4/6, followed by an activating phosphorylation at $T172^{CDK4}$ and $T177^{CDK6}$ [73,74]. CDK4 and CDK6 play a critical role in mediating the cell G1/S phase transition [75]. They phosphorylate and inactivate the Rb protein to activate the function of E2F and activate relevant transcriptional elements for cell cycle and cell division. Thus, inhibiting the function of CDK4/6 may block cell proliferation. In addition, the inhibition of CDK4/6 could result in tumor cell senescence [76]. Several CDK4 and CDK6 inhibitors have been approved and applied for the treatment of many types of tumors in clinical treatment and trials [77]. CDK4 and CDK6 might share slight differences in phosphorylation pattern and their functions [78,79]. It is supposed that CDK4 regulates prometastatic inflammatory cytokine signaling while CDK6 takes part in DNA replication and repairing processes [79]. Meanwhile, CDK4 ablation could specifically result in the senescence of non-small-cell lung cancer but not CDK6 ablation in mice models [80]. As a result, there is a pressing need for compounds that specifically and selectively target CDK4 and/or CDK6. Although CDK4 and CDK6 are still the most successful and developed targets over all CDK members, due to the high similarity between CDK4 and CDK6, it is extremely difficult to develop traditional selective inhibitors at the ATP binding domain [81]. However, mono and dual CDK4/6 degradation can also be developed by the PROTACs technique.

Palbociclib, Ribociclib, and Abemaciclib are FDA-approved drugs for clinical treatment and are commonly used as POI binders in CDK4/6 degrader development (Fig. 4). In 2019, Burgess group reported the first CDK4/6 degraders using PROTACs [36]. They designed and synthesized two compounds based on Palbociclib and Ribociclib, respectively, and characterized the

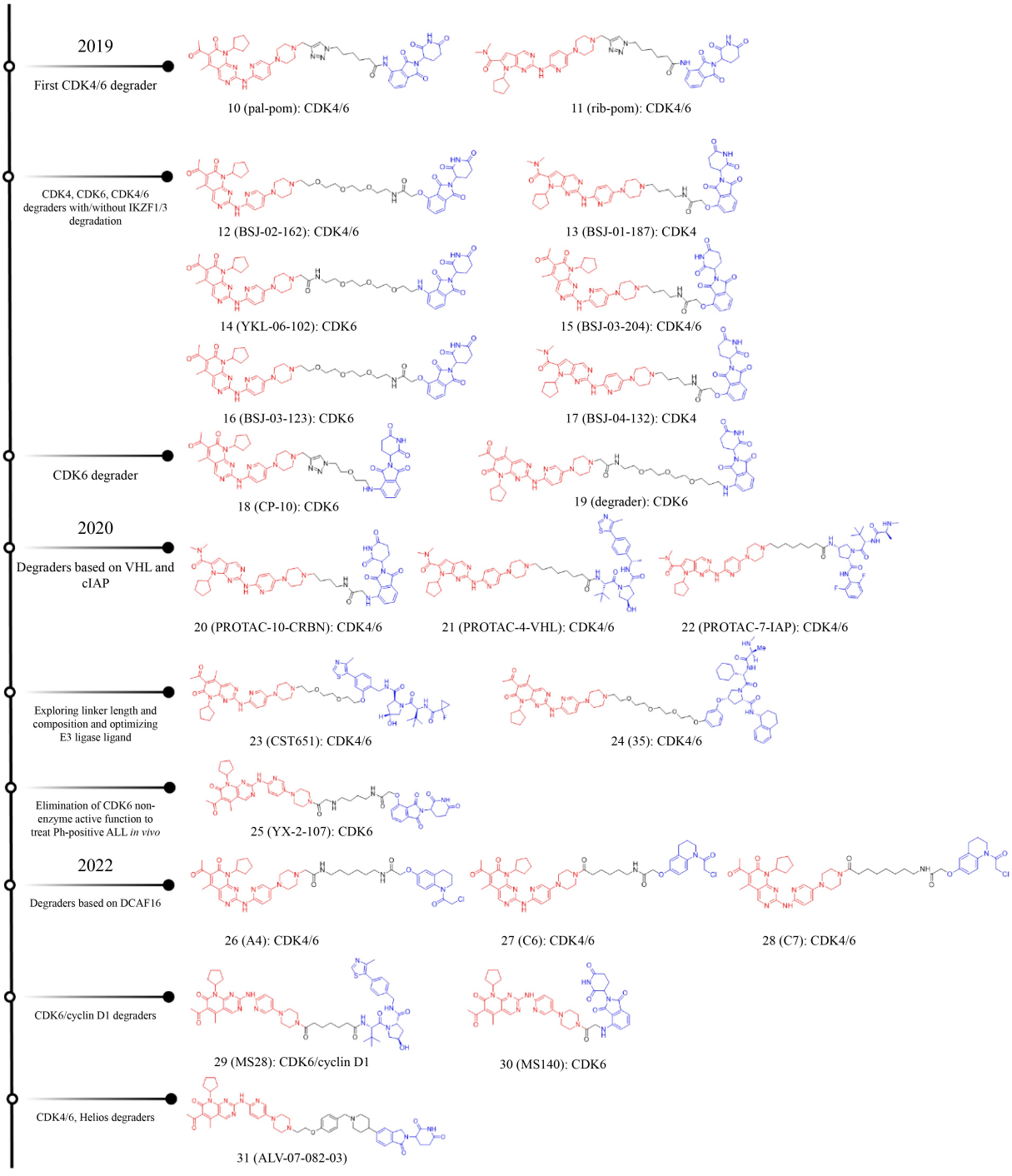


Fig. 4 Structure of CDK4/6 degraders.

compounds by performing rescue, time course, washout, and kinase affinity experiments. However, they did not provide further detailed information about these two compounds (compounds 10 and 11). Next, Zhang group and Gray group published their research on CDK4/6 degraders [37]. They used pomalidomide as the E3 ligase ligand and conjugated it respectively with Palbociclib, Ribociclib, and Abemaciclib at the piperazine NH to form three series of PROTAC compounds. They successfully achieved CDK4/6 dual degradation and selectively degraded either CDK4 or CDK6. However, the PROTACs based on Abemaciclib may induce potent CDK9 degradation in addition to CDK4 and CDK6 degradation. Compound 12 (BSJ-02-162), which used Palbociclib as the POI binder and a 4-carbon alkyl linker, degraded CDK4 and CDK6, while compound 13 (BSJ-01-187) used Ribociclib as the POI binder and the same linker and selectively degraded CDK4. Surprisingly,

compound 14 (YKL-06-102), which used Palbociclib as the POI binder and an extended PEG-3 linker, achieved CDK6 degradation without CDK4 degradation. They also evaluated the compound-induced degradation of IKZF1 and IKZF3, which are known targets of pomalidomide and its analogs. After further optimizations, they successfully removed the degradation of IKZF1 and IKZF3 and obtained compound 15 (BSJ-03-204, a dual CDK4 and CDK6 degrader), compound 16 (BSJ-03-123, a selective CDK6 degrader), and compound 17 (BSJ-04-132, a selective CDK4 degrader). They conducted further validation experiments, such as proteomics, cell cycle experiments, and proliferative inhibition, to better characterize their compounds. After a short while, our group independently reported on CDK4/6 degraders [38]. In this study, we used Abemaciclib, Palbociclib, and Ribociclib as the POI binders to form PROTACs based on CRBN, VHL, or cIAP, respectively. Following the analysis of the co-crystal structure of small molecules and CDK4/6, we hypothesized that the NH of piperazine might be a suitable site for PROTACs construction. In this work, we tested the effect of linker composition, linker length and rigidity, E3 ligase types, and POI binder on the degradation efficacy. Only PROTACs based on CRBN, which use pomalidomide as an E3 ligand, were able to degrade CDK6 with high efficiency. The PROTACs based on Palbociclib induced more potent degradation than the other two binders. Meanwhile, long and flexible linkers did not show advantages over short linkers. The degradation efficacy also decreased when the flexible imino group on the linkers was replaced by a rigid alkyne. Although Palbociclib has a similar affinity with CDK4 and CDK6, after our design, the representative compound 18 (CP-10) displayed a 50- to 80-fold degradation difference over CDK4 and CDK6. CP-10 was a potent and selective CDK6 degrader in this paper. Proteinomics experiments showed that the reduction in CDK6 was most significant with only a few target perturbations. We also observed comprehensive tumor cell proliferative inhibition with CP-10 treatment. In the same vein, Amarnath Natarajan's group also reported their CDK6 degraders based on Palbociclib, achieving selective degradation with compound 19 (Degrader 6), which was validated by rescue experiments [39]. In 2020, Benowitz group and Kronke group published their studies on CDK4/6 PROTACs involving different E3 ligase ligands based on Palbociclib and Ribociclib [40]. In their paper, they revisited the cereblon, VHL, MDM2, and IAP ligands previously used in our research. Interestingly, in their system, they found that both CDK4 and CDK6 could be degraded with VHL or IAP PROTACs. However, the Western blot images or methods used to measure protein levels were not published in Benowitz group's study. Compounds 20–22 were representative compounds based on cereblon, VHL, and cIAP1, respectively. The study by Kronke group was more detailed [41]. They explored the linker length and composition and optimized the E3 ligase ligand. For CRBN-based PROTACs, they found that the linker length had a less pronounced effect on selectivity than the lipophilicity of the PROTACs. PROTACs with log D below 4 tended to be potent CDK6 degraders. Although they further explored the CDK4/6 degraders based on CRBN, only one compound with a 28-atom linker based on cereblon ligand achieved more selective degradation than BSJ-03-123. They also noted that CRBN might be dispensable for several types of tumors, leading to drug resistance. This was an important reason why they tried other E3 ligase ligands. They developed highly active and selective CDK6 PROTACs based on VHL ligand (representative compound 23) and highly active CDK4/6 degraders based on IAP ligand (representative compound 24). The PROTACs based on VHL and cereblon ligands had the same preferred degradation of CDK6 over CDK4, while that based on cIAP1 ligand had no degradation bias of CDK4 and CDK6. The PROTACs based on MDM2 failed to achieve CDK4/6 degradation. Poor cell permeability might be responsible for the poor degradation capacity. Notably, the PROTACs based on IAP achieved IAP degradation, facilitating potent cell-killing function. Based on their PROTACs library, they summarized the characteristics of CRBN-, VHL-, IAP-, and MDM2-based PROTACs, which might guide the design of PROTACs. De Dominici *et al*. developed a new CDK6 selective degrader compound 25 (YX-2-107) in 2020, which could effectively inhibit tumor growth in a Philadelphia-positive acute lymphoblastic leukemia (ALL) xenograft mouse model [42]. In addition, the authors discussed the linker characteristics of the degrader in the paper. Although the pyrazole ring in the palbociclib molecular structure extends to the solvent region and has little effect on the binding to CDK6 from the crystal structure, the fragment molecules obtained by connecting different linkers from the pyrazole ring have a great impact on the inhibitory activity of CDK6, and the same phenomenon was observed in the assembled PROTAC molecules, which may directly affect the final PROTAC molecule's degradation activity of the target protein. At the same time, the authors suggested that YX-2-107 selectively degrades CDK6 by forming a ternary complex to generate new protein–protein interactions, which enables the degrader to ubiquitinate CDK6 selectively rather than CDK4. Recently, Rui Li group reported that CDK4/6 could be degraded using a DCAF16 ligand, which further expands the applicable E3 ligase ligands for CDK4/6 PROTACs design [43]. The representative compounds 26–28 (A4, C6 and C7) could degrade CDK4/6 and showed potent inhibitory activity in MDA-MB-231 cells. They also confirmed that compound A4 degraded CDK4/6 through the PROTACs mechanism.

In 2022, Jin group reported an interesting case of a PROTAC that targets CDK6 and cyclin D1, which they called a bridged PROTAC [44]. The representative compound 29 (MS28) was based on Palbociclib and VHL. Controlled experiments showed that BSJ-03-204, compound 30 (MS140), and CP-10, which are pomalidomide-based PROTACs, did not degrade cyclin D1. This phenomenon demonstrated that cyclin D1 degradation was not the result of CDK4/6 degradation. Next, they confirmed that cyclin D1 degradation was VHL, CDK6, and UPS dependent via the knockout or inhibition of each element. They also tested the protein abundance of major CDKs and cyclins via Western blot. The proteomics showed that cyclin D1 and CDK6 were the major degradation targets with a small number of off-targets. Verano *et al*. developed a CDK4/CDK6/Helios triple-targeted degrader in 2022, demonstrating the possibility of rationally redirecting PROTACs to new substrate specificities by using pomalidomide derivatives as E3 ligase ligands, and the authors found that targeting CDK4/6 and Helios together may have synergistic effects [45]. Ng *et al*. found in 2022 that overexpression of CDK6 reduced the sensitivity to IMiDs in multiple myeloma cell lines, and that inhibiting CDK6 with palbociclib or degrading CDK6 with YKL-06-102 had a high degree of synergy with IMiDs *in vitro* and *in vivo* [82]. They identified upregulation of CDK6 as a drug target for IMiD-resistant multiple myeloma. Du *et al*. explored the target range of PROTACs based on KEAP1 E3 ligase in 2022, and found that the molecule obtained by combining palbociclib and KI696 could not achieve efficient degradation of CDK6, indicating that PROTACs recruiting KEAP1 are not as universal as VHL or CRBN [83].

In summary, CRBN, VHL, cIAP, and DCAF16 have all been successfully applied in CDK4/6 degrader design. There are few relatively selective CDK4 degraders, such as BSJ-02-162 and BSJ-04-132, while there are relatively more selective CDK6 degraders without comparable CDK4 degradation, such as YKL-06-102, BSJ-03-123, CP-10, and compound 19. MS28, the VHL-based PROTAC, is the only known CDK6 and cyclin D1 degrader. Regarding drug efficacy improvement, there has been a case involving oral PROTAC targeting CDK2/4/6 discussed in the CDK2 session. Of note, all selectivity data should be validated under exactly same condition.

CDK5 PROTACs

CDK5 differs slightly from other common CDK members in that it is not typically activated by cyclin proteins, but mainly by p35 and p39 [84,85]. CDK5 is considered a crucial regulator of neuronal migration and post-mitotic neurons [86]. Interestingly, CDK5 is significantly expressed in terminally differentiated neuron cells, suggesting the necessity of CDK5 for neuronal differentiation [87]. Thus, many studies have investigated the function of CDK5 in the nervous system, and CDK5 dysfunction has been associated with many diseases such as neurotoxicity, neurodegeneration, Alzheimer's disease, APP processing and Aβ production, tauopathy, and DNA damage and cell cycle reentry [88]. Patients with these conditions may benefit greatly from drugs that target CDK5. On the one hand, in recent years, a large number of studies have shown that CDK5 mutation, overexpression and the occurrence of various cancers such as colon cancer, lung cancer, breast cancer, prostate cancer, pancreatic cancer, melanoma and hematological system tumors are closely related [89]. On the other hand, CDK5 has also been proven to maintain a close relationship with tumor cell resistance to chemotherapy, radiotherapy, targeted therapy, and proliferation activity [89]. Therefore, CDK5 is not only a driving force in tumors, directly or indirectly involved in tumor development; but also plays a role in maintaining proliferation and enhancing metastasis through other potential signaling pathways. Interestingly, CDK5 is not essential for mouse survival. Although CDK5 knockout mice show epilepsy and other problems, it is not a lethal target [90]. Its function can be partially compensated by CDK1, CDK2, CDK4, CDK6, and so on, which demonstrates the safety of CDK5 as a therapeutic target.

However, although many molecules have been shown to significantly inhibit the activity of CDK5, only a few achieve high selectivity [91]. Due to the importance of CDK5 in the neuronal system and cancer therapy, there is an urgent need for selective, low-toxicity molecules to perturb CDK5 function.

As far as we know, only two research groups have independently reported the development of CDK5 degraders [32,60]. However, the discovery of these degraders was by chance. In 2017, Gray group used proteomics to identify CDK5 as a potential degradable target [60]. We will discuss this paper in detail in the "Special degrader" section. In 2020, they reported the development of CDK2 and CDK5 degraders (represented by compound 4 and compound 5) [32], which was discussed in the CDK2 section. However, they did not provide any information on the phenotype of CDK5 knockdown. Selective CDK5 degraders are still urgently needed, and there may be new discoveries in the future.

CDK8 PROTACs

CDK8, originally known as K35, is involved in transcriptional regulation, with the C type cyclin serving as the kinase partner of CDK8 [92]. CDK8 is often involved in the composition of Mediator, a conserved transcriptional coactivator composed of CDK8, cyclin C,

MED12, and MED13 that facilitates signal transduction [93]. Initially, CDK8 and the Mediator complex were considered transcriptional repressors [94], but over the past few years, several reports have identified new positive roles of CDK8 in phosphorylation and gene-specific transcription [95]. CDK8 also acts as a coactivator in pathways such as JAK-STAT [96], Smad [97], NOTCH [98], and Wnt/β-catenin [99]. CDK8 is commonly overexpressed in specific tumor types such as colorectal cancer [100] and its knockdown does not affect the viability of 293FT and normal cells, suggesting CDK8 may be a suitable and safe target for the treatment of certain cancers [101]. CDK19 and CDK8 share 97% identical sequences in the catalytic site [102], making the development of selective CDK8 inhibitors challenging. While several CDK8 inhibitors exist, few have achieved selective inhibition [103,104], making PROTACs and molecular glues potentially useful applications for targeting CDK8.

In 2018, Gray group published their findings on CDK8 degrader [46] (Fig. 5). They utilized cortistatin A as a point of interest binder which is a natural product having an IC50 of 15 nmol/L for CDK8. However, cortistatin synthesis takes 16 to 30 steps and yields range from 0.012% to 2%. Thus, the authors aimed to modify cortistatin A's complex core to retain its binding affinity toward CDK8 and simplify its synthetic route. By replacing the core of cortistatin A with a simple steroid DHEA based on the 3D structure, they developed a simplified CDK8 inhibitor having a 16 nmol/L IC50 and 33% overall yield. Subsequently, they designed compound 32 with a long polyethylene glycol (PEG) linker, utilizing the new CDK8 inhibitor to achieve CDK8 degradation at 1 μmol/L concentration on Jurkat cells. Notably, PROTACs comprising short and alkyl linkers were found to be ineffective. The authors conducted rescue experiments to examine degradation by the PROTAC mechanism, confirming that compound 32 induced degradation via this pathway.

CDK9 PROTACs

CDK9 is a component of the P-TEFb complex [105], and it forms a complex with cyclin T and cyclin K as coactivators [106]. The active P-TEFb complex phosphorylates RNA polymerase II, which regulates transcriptional elongation [107]. Additionally, CDK9 and cyclin T play a role in the regulation of MCL-1 and MYC, both of which are important in tumorigenesis and tumor cell proliferation [108].

The structures of CDK9 degraders are listed in Fig. 6. CDK9 was the first CDK member to be successfully degraded by PROTAC. In 2017, Natarajan and Rana group reported the first CDK9 degrader [47], using aminopyrazole analogs evaluated as CDK9 inhibitors as the POI binder and pomalidomide analogs as the E3 ligase ligand. The representative CDK9 degrader 29 achieved selective CDK9 degradation in micromolar concentrations without affecting AKT, FAK, IKKβ, CDK2, and CDK5. Compound 33 reduced levels of p-RPB1 and Mcl-1, revealing its potential for use in anti-cancer therapy. In 2021, they updated their research on the PROTACs based on aminopyrazole targeting CDK9 [48]. In this paper, they further explored the linker length and composition. Representative compound 34 degraded CDK9 at sub-micromolar concentrations, a significant improvement over compound 33. The selectivity of compound 34 was measured by Western blot and proteome. Among the 13 tested CDK members and 3433 quantified proteins, CDK9 stood out as the target of the largest reduction. They then applied a combination treatment of compound 34 and a BCL inhibitor to achieve enhanced proliferative inhibition. Notably, compound 33 and compound 34 were both based on a multi-target POI binder, and selective degradation was achieved through PROTAC modification. In 2018, Gray group published their research [49] using SNS-032 and NVP-2 as POI binders, which are considered multi-target inhibitors and relatively selective CDK9 inhibitors, respectively. Although NVP-2 is more selective than SNS-032 and has sub-nanomolar potency against CDK9, PROTACs based on SNS-032 are much more potent than those based on NVP-2. The representative compound 35 (THAL-SNS-032) could degrade CDK9 at sub-nanomolar concentration even within 1 h. Meanwhile, the selectivity of THAL-SNS-032 based on SNS-032 was further validated by proteomics, demonstrating excellent and most significant CDK9 degradation over other targets. They also showed that THAL-SNS-032 induced more similar transcriptional changes to NVP-2 than SNS-032 did. After washout, THAL-SNS-032 had a longer-lasting effect on cell apoptosis than NVP-2, demonstrating a more profound cytotoxic effect than the inhibitor. In this case, they converted a non-selective inhibitor into a

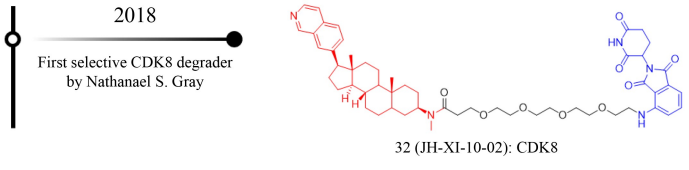


Fig. 5 Structure of CDK8 degraders.

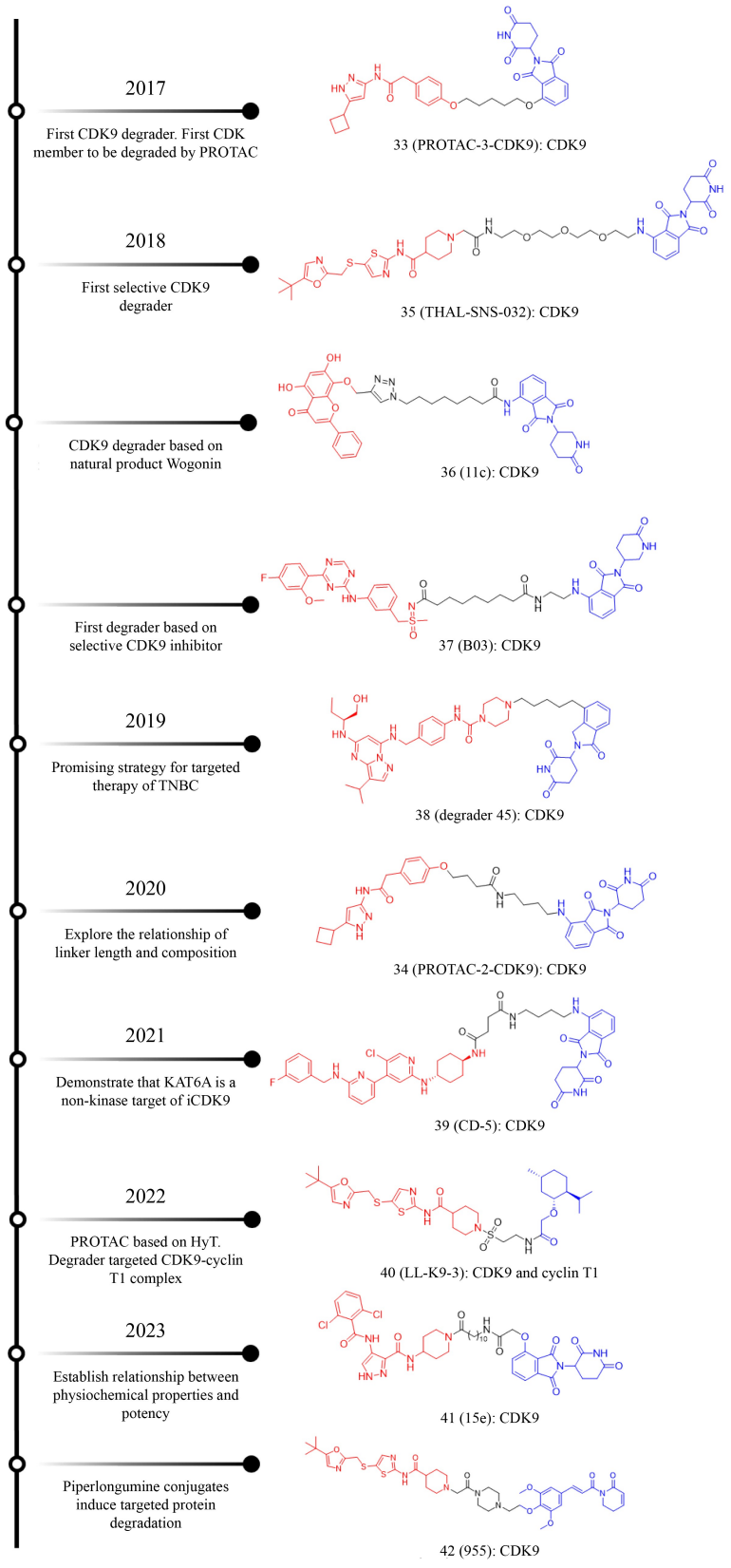


Fig. 6 Structure of CDK9 degraders.

selective degrader. In 2018, Zhiyu Li group reported their CDK9 degrader based on Wogonin and Pomalidomide [50]. Wogonin is a natural product with an IC50 of 0.19 μmol/L against CDK9 and 12.3 μmol/L against CDK7. The potency of their compounds was relatively weak. Representative compound 36 achieved selective CDK9 degradation only at sub-micromolar concentrations. Compound 36 might be able to inhibit the proliferation of CDK9-overexpressing cancer cells. In 2019, Yuanwei Chen group reported the CDK9 degrader (compound 2), which was discussed in the CDK2 section. In 2020, Jinlei Bian and colleagues developed their PROTACs based on BAY-1143572, a selective CDK9 inhibitor [51]. Previously, PROTACs targeting CDK9 employed non-selective inhibitors. In this paper, they synthesized two series of compounds with attached linkers at different sites. Several compounds were shown to induce CDK9 degradation in acute myeloid leukemia cells at nanomolar concentrations. The representative compound 37 had a more potent inhibitory effect on cells than the warhead alone. The half-life of compound 37 was 1.3 h, which was acceptable in the PROTAC field. Later, Xiao-Hua Chen group published their CDK9 degraders and their use in triple-negative breast cancer treatment [52]. Notably, previous reports mainly focused on the inhibitory effect *in vitro*, while this study claimed that their compounds were effective in TNBC *in vivo*. They analyzed the structure of NVP-2, which was proven to have poor degradation ability in Nathanael S. Gray's research, and proposed that the different geometry of certain inhibitors could aid in CDK9 PROTAC design. Therefore, they employed a heterocycle scaffold as the POI binder and thalidomide as the E3 ligase binder. The inhibitory effects were initially unsatisfactory but were significantly improved after optimization of the binding ligand and linker. Compound 38 was able to inhibit TNBC cells with an IC50 of 4 nmol/L. Several mechanism researches showed that compound 38 effectively induced the downregulation of downstream targets of CDK9 (Mcl-1 and MYC). Compound 38 was subsequently tested on MDA-MB-231 xenograft mouse models to assess *in vivo* efficacy, demonstrating a clear knock-down effect on several CDK9 and tumor suppressor effects. In 2022, Wu group used iCDK9 as the POI binder and synthesized CD-5, which could degrade CDK9 in nanomolar concentration [53]. Interestingly, in this work, they adopted proteomics to evaluate the selectivity of compound 39 (CD-5) and found an unexpected degradation of KAT6A. Further experiments demonstrated that KAT6A was a non-kinase target of iCDK9. Moreover, Luo group reported their unusual PROTACs [54]. In this paper, they induced synchronous degradation of CDK9 and cyclin T1. They did not employ the common cereblon, VHL, MDM2, and cIAP ligands. Instead, they used the HyT Tag, which contained a hydrophobic core to mimic the misfolded protein. SNS-032 was used as the POI binder. Then, they focused on the optimization of linkers and HyT Tag and obtained compound 40 (LL-K9-3), which has DC50 (the half-maximal degradation concentration) values of 0.662 μmol/L and 0.589 μmol/L for CDK9 and cyclin T1, respectively, making it the most potent degrader. With the treatment of compound 40, CDK9 stood out as the most decreased target, while the abundances of other CDK members were hardly affected. They then compared the differences in proteomics, cell proliferation, apoptosis, AR signaling, and RNAseq between SNS032, THAL-SNS-032, and compound 40, showing that compound 40 has a unique mode of action. Compound 40 exhibited a more profound inhibitory effect than SNS032 and the selective CDK9 degrader THAL-SNS-032. In 2023, Fuchs's group developed two classes of compounds with distinct types of linkers [55]. Based AT7519 as POI binder, they obtained the compounds with different physiochemical properties and established the relationship between potency and physiochemical property. Herein, we show compound 41 as an example of their representative selective degraders with sub-micromolar DC_{50}. In addition, Pei *et al*. used SNS032 as a binder for CDK9 and piperlongumine as a binder for E3 ligase, and developed a novel CDK9 degrader compound 42 [56], which proved that the E3 ligase recruited by 955 was KEAP1, and that 955 bound to KEAP1 covalently. 955 had better anti-tumor activity than the inhibitor SNS032.

In summary, CDK9 is a relatively well-developed target with many molecules validated by proteomics that have achieved CDK9 degradation. Notably, compound 3 achieved CDK2/9 dual degradation, and compound 40 with the HyT technique achieved CDK9 and cyclin T1 dual degradation. The selectivity for CDK9 is closely related to the POI binders and E3 ligase ligand. Readers may choose suitable molecules for personalized applications. However, only a few cases have reported *in vivo* stability and use, and this warrants further exploration in the future.

CDK12 PROTACs

CDK12 plays an important role in transcriptional and post-transcriptional regulation, essential for embryonic development and DNA-damage response (DDR) [109]. Knockout of CDK12 *in vivo* is embryonic lethal [110]. CDK12 normally forms an activation co-complex with cyclin K, which phosphorylates Pol II and is involved in transcriptional elongation [109]. Dysregulated CDK12 may lead to various types of tumors, such as esophageal cancer, bladder cancer, colorectal cancer, and ovarian cancer [109]. It should be noted that CDK12 and CDK13 have high homology [111]. Selective CDK12 inhibition may benefit specific tumors. Representative known

CDK12 inhibitors include THZ1 [112], SR-4835 [113], Dinaciclib [112], and SY-5609 [114], but none of them are selective inhibitors. CDK7 and CDK13 are common off-targets of these inhibitors.

In 2021, Gray group reported the first degrader that induced selective CDK12 degradation [57] (Fig. 7). They first analyzed the protein plasticity of CDK12 and CDK13 kinase domains and found that CDK12 should be more flexible in its conformations, thus having a large tolerance for the exit vector of the linker installment. After optimizing the binders and linkers, they obtained compound 43 (BSJ-4-116) as a selective CDK12 degrader with sub-nanomolar degradation ability, which employed a pan-kinase inhibitor as the POI binder and pomalidomide as the E3 binder. The selectivity was demonstrated by Western blot and proteomics, with the abundance of CDK13 remaining almost unaffected. Compound 43 showed a potent inhibitory effect on tumor cells, even those resistant to covalent CDK12 inhibitors. They also investigated the potential mechanisms of acquired resistance to compound 43, which may guide future PROTAC design. In 2022, Zhu group reported on degraders that achieved CDK12-cyclin K dual degradation [58]. They suggested that the dual degradation of cyclin K and CDK12 could induce a more potent inhibitory effect on tumors than the single CDK12 degradation. The representative compound 44 (PP-C8) could induce remarkable degradation of CDK12 and cyclin K, with DC50 values of 416 nmol/L and 412 nmol/L, respectively, without CDK7, CDK9, CDK13, cyclin H, and cyclin T1 degradation. Further selectivity experiments were performed via proteomics to demonstrate selectivity among all CDK members. Compound 44 was then applied to the treatment of TNBC, where it was shown to be synergistic with PARP inhibition, potentially for use in PARP-resistant cancers.

In summary, two research groups have reported their respective degraders targeting CDK12. Compound 43 selectively degrades CDK12, while compound 44 degrades both CDK12 and cyclin K.

CDK13 PROTACs

CDK12 and CDK13 share similar structures and functions. Cyclin K is the partner of CDK13 and forms the CDK13-cyclin K complex, which phosphorylates Pol II and is involved in DDR regulation together with the CDK12-cyclin K complex [115,116]. Studies on the differences between CDK12 and CDK13 are still lacking. Few papers have demonstrated clear differences between CDK12 and CDK13. However, several disorders, including developmental delay, craniofacial features, intellectual disability, feeding difficulties, structural brain abnormality, and structural heart defects, have been associated with CDK13 abnormalities and mutations [14].

To the best of our knowledge, there is no selective CDK13 degrader available. However, in 2022, Ding group reported a dual PROTAC degrader targeting both CDK12 and CDK13 [59] (Fig. 7). In this work, they utilized CDK12/13 inhibitors discovered by Yasuhiro Imaeda in 2018 [117] and pomalidomide as the POI binder and E3 ligase ligand, respectively. The linker was attached at the 1-methylpyridin-2(1H)-one group based on computational simulations to avoid possible clashes with CDK12/13. After two rounds of optimization of linkers and E3 ligase ligands, they obtained compound 45 (7f) as the most potent degrader, which could degrade CDK12 and CDK13 with respective DC50 values of 2.2 nmol/L and 2.1 nmol/L. The selectivity was further explored via proteomics, and their results showed a

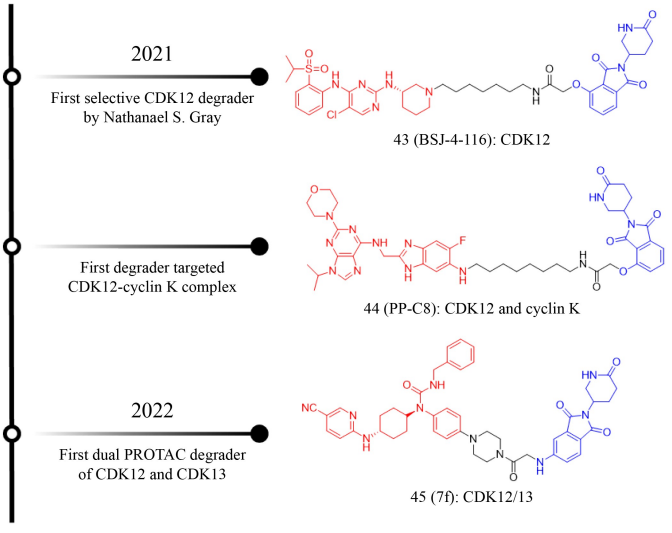


Fig. 7 Structure of CDK12/13 degraders.

decrease in the levels of PIK3R3, USP6NL, PSMB1, and PCBD1, which could be attributed to off-target effects due to CDK12/13 inhibition and degradation. Further characterization experiments are still required. The researchers applied compound 45 to treat TNBC, inducing a more potent inhibitory effect than single knockout of either CDK12 or CDK13, and suppressing the expression of DDR genes. They also performed a preliminary *in-vivo* pharmacodynamic experiment, which was conducted on a xenograft mouse model. After treatment, levels of CDK12 and CDK13 were significantly reduced compared to the vehicle.

Special PROTACs for CDK1/7/10/11/14/15/16/17/18/19

In this section, we have summarized the CDK members that have been partially shown to be degradable by PROTACs. While none of these degraders have been validated yet, there is a significant demand for the development of selective PROTACs targeting these CDK members.

In 2020, Fischer group reported on their method for rapidly developing degraders by mapping the degradable kinome [60] (Fig. 8). They created a large library of various multi-target PROTACs, such as compound 46, and treated cells with this library to identify and quantify changes in protein abundance via proteomics. Although they did not specifically discuss the degradation of CDK members in their paper, the abundance of CDK was significantly decreased with pan-degrader treatment. However, it is unclear if this reduction is specific or a second-order effect from other target perturbations. Their study suggests that CDK targets may be degradable, and selective and specific CDK degraders may be developed in the future using this approach. Nevertheless, further work, including rescue experiments and time course experiments, will need to be done to confirm the specificity of such degraders.

Molecular glues in CDK and cyclin degrader design

Most of the molecules discussed in this paper are constructed using classical PROTACs, which are designed to function by forming ternary complexes. However, another technique for inducing protein degradation by small molecules has also been developed, known as molecular glue. Using cyclin K as an example, Winter group, Ebert group, and Han group have successfully achieved the degradation of this target through molecular glue mechanisms, respectively [61–63]. As noted above, cyclin K is the activated partner of CDK9, CDK12, and CDK13. Therefore, degradation of cyclin K would hinder the phosphorylation of Pol II, which is an essential step in transcriptional regulation.

In 2020, Ebert group identified the first molecular glue, compound 47 ((R)-CR8), with nanomolar DC50 targeting cyclin K by constructing libraries [61] (Fig. 9).

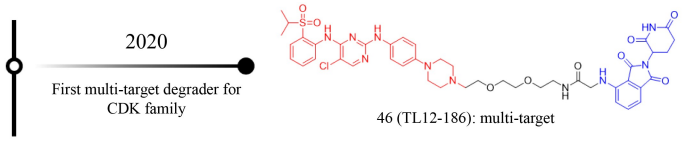


Fig. 8 Structure of special degraders.

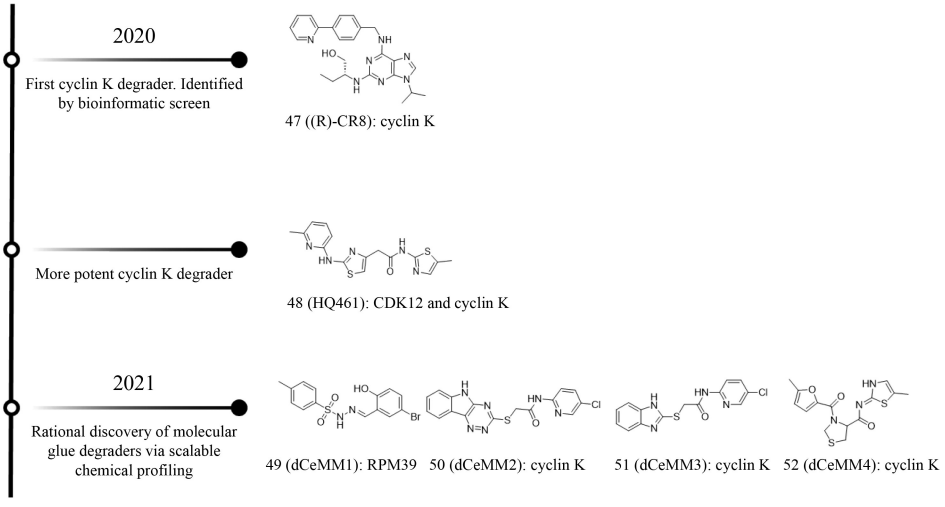


Fig. 9 Structure of cyclin K degraders.

Previously, (R)-CR8 was regarded as an inhibitor targeting CDK1, CDK2, CDK5, CDK7, CDK9, and CDK12. The discovery of such compounds was based on screening. First, they constructed a library of compounds to verify the correlation between the toxicity of these compounds and mRNA expression. The toxicity of (R)-CR8 is correlated with mRNA levels of DDB1, the CUL4 adaptor protein. Proteomics analysis confirmed that, with the treatment of (R)-CR8, cyclin K was the most decreased protein among over 8000 proteins. An sgRNA library based on CRISPR-Cas9 technique was also constructed to dissect the essential proteins in (R)-CR8-induced degradation, which confirmed that the degradation was associated with DDB1, CUL4B, RBX1, and NEDD8. Further co-crystal structures showed that CDK12 is the platform and mediates protein–protein interactions with DDB1 and cyclin K, forming the DDB1-CDK12-cyclin K complex (Fig. 10). Typically, cyclin K is bound to CDK9, CDK12, and CDK13. Therefore, they tested if (R)-CR8 could recruit these CDKs. Although the major difference between these CDKs is in the C-terminal extension, the results showed that the C-terminal extension was not essential for (R)-CR8-mediated ubiquitination. The PPI could be induced by (R)-CR8 via CDK12 or CDK13 and DDB1. They further analyzed the binding pattern of (R)-CR8, which occupies the ATP binding pocket of CDK12 and contacts the BPC domain of DDB1 with its phenylpyridine. The analogs of (R)-CR8, which have the same core structure but are different at the phenylpyridine, as well as the mutation of the DDB1 residues that are involved in the binding pocket, significantly reduced the binding affinity. Although the analogs of (R)-CR8 have reduced affinity to drive complex formation, they could not induce the degradation of cyclin K, suggesting that the subtle difference in the orientation of phenylpyridine might have a profound impact on degradation. At the same time, the binding surface and modes are plastic and differ in their ability to stabilize the DDB1-CDK12 complex when different ligands are applied.

Later, Ting Han group reported another molecular glue for cyclin K degradation [62] (Fig. 9). Compound 48 (HQ461) was previously designed for NRF2 inhibition, exhibiting a potent inhibitory effect on A549 cells. The group designed a CRISPR-Cas9-based sgRNA library to identify genes enriched in cells resistant to compound 48 treatment. By analyzing the enriched genes, they inferred that the toxicity of compound 48 was highly correlated with proteasomal degradation. After identifying CDK12 as a key factor in resistance, Han group found that compound 48 induced an interaction between CDK12 and DDB1, leading to the ubiquitination of cyclin K. They then optimized the structure of compound 48 and obtained a more potent degrader, compound 48 (HQ463) with a DC50 of 4.6 nmol/L, which exhibited potent anti-tumor activity *in vitro* and *in vivo*.

Winter group also published their research on molecular glue targeting cyclin K [63] (Fig. 9). In contrast to the above two papers, which aimed to identify target binders, they attempted to formulate a method for finding molecular glues. They first mutated UBE2M, a member of the E2 enzyme, in cells and then screened a library of 2000 cytosolic/cytotoxic compounds for viability on cells with or without the UBE2M mutation. Four compounds (compounds 49–52) that showed significant differences in viability between wild type and

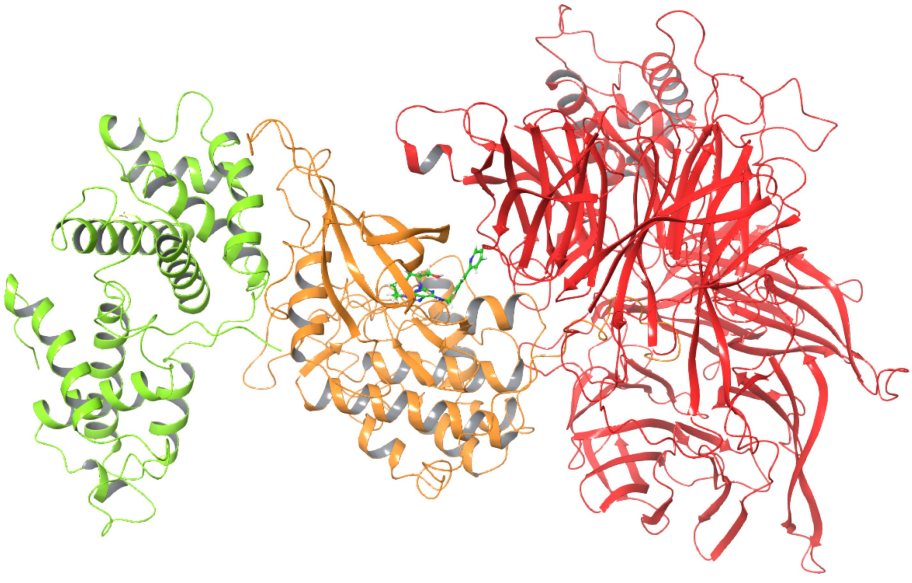


Fig. 10 Structure of DDB1-CR8-CDK12-cyclin K complex. Cyclin K (green, left), CDK12 (orange, middle) and DDB1 (red, right). PDB code: 6TD3.

mutated cells were selected for further validation. Proteomics showed potent cyclin K degradation and mild CDK12 and CDK13 degradation after treatment with compound 49, but Western blot analysis confirmed that cyclin K was the most decreased target after short-term treatment with micromolar concentrations. The degradation of CDK12/13 may be an indirect effect of these degraders. Moreover, they confirmed that cyclin K degradation is mediated via the formation of the CRL4B complex, using a library of sgRNA and cells harboring mutations that confer resistance to the drugs. They demonstrated that these compounds induced an interaction between DDB1 and CDK12-cyclin K.

It is worth noting that the discovery of molecular glues has been achieved through constructing libraries and high-throughput screening, and their rational design remains a challenge. The compounds in all three cases were discovered either by accident or through screening. Interestingly, although they started from different libraries, the common finding was that small molecules could act as molecular glues to induce interactions between DDB1 and the CDK12-cyclin K complex, which showed strong plasticity at the surface between DDB1 and CDK12. The degradation of cyclin K led to the inactivation of CDK9, CDK12, and CDK13. However, there is still a lack of molecular glue for other CDKs and cyclins. It is not clear if other CDKs could bind with specific elements of the ubiquitination pathway. It may still be a feasible approach to obtain CDK target degradation that is difficult to achieve with traditional PROTAC technology through high-throughput screening without relying on binder pre-discovery. The number of sgRNA libraries, compound libraries, and specialized cell lines needs to be expanded urgently.

Brief summary of CDK/cyclin degraders

As previously mentioned, there are numerous PROTACs that target CDKs and cyclins. To better classify these compounds, we have summarized their characteristics in Tables 3 and 4 below, considering only their known targets and selectivity in CDKs and cyclins. Proteomics

Table 3 Features of the PROTACs or molecular glue targeting CDKs/cyclins

Target(s)	Proteomics?	Type	Compound No. and references
CDK2	Yes	PROTACs	8 [34]
	No	PROTACs	1 [31], 9 [35]
CDK4	Yes	PROTACs	17 [37]
	No	PROTACs	13 [37]
CDK6	Yes	PROTACs	16 [37], 18 [38], 25 [42]
	No	PROTACs	14 [37], 19 [39], 30 [44]
CDK8	No	PROTACs	32 [46]
CDK9	Yes	PROTACs	34 [48], 35 [49], 37 [51], 39 [53], 41 [55]
	No	PROTACs	2 [31], 33 [47], 36 [50], 38 [52]
CDK12	Yes	PROTACs	43 [57]
Cyclin K	Yes	Molecular glue	47 [61], 50 [63], 51 [63], 52 [63]
CDK2/9	No	PROTACs	3 [31]
CDK2/4/5/6	No	PROTACs	4 [32]
CDK2/5	Yes	PROTACs	5 [32]
CDK2/4/6	No	PROTACs	6 [32]
CDK4/6	Yes	PROTACs	12 [37], 15 [37]
	No	PROTACs	10 [36], 11 [36], 20 [40], 21 [40], 22 [40], 23 [41], 24 [41], 26 [43], 27 [43], 28 [43]
CDK4/6, Helios	Yes	PROTACs (Helios for MGs)	31 [45]
CDK6, cyclin D1	Yes	PROTACs	29 [44]
CDK9, cyclin T1	Yes	PROTACs	40 [54]
CDK12/13	Yes	PROTACs	45 [59]
CDK12, cyclin K	Yes	PROTACs	44 [58]
		Molecular glue	48 [62]
CDK1/7/10/11/14/15/16/17/18/19	Yes	PROTACs	46 [60]
CDK9/10	Yes	PROTACs	42 [56]

Table 4 The development of corresponding CDK degraders

Characters	CDKs
Exist degrader[a]	CDK2/4/5/6/8/9/10/12/13
Suggested to be degradable[b]	CDK1/7/11/14/15/16/17/18/19
No data	CDK3/20

[a]Western blot data confirmed. [b]Only proteomics data confirmed.

was used to indicate whether there is a corresponding compound with proteomic validation. While PROTACs and molecular glues have greatly improved selectivity, achieving high selectivity remains a challenging problem. Most compounds are still non-selective degraders. However, there have been selective CDK2/4/6/9/12 degraders with proteomic validation, as well as selective CDK8 degraders without proteomic validation. Notably, there is no available data on degradation for CDK3 and CDK20. Further research is needed to determine whether CDK1/7/10/11/14/15/16/17/18/19 and CDK3/20 are degradable in the future.

In 2018 [118] and 2021 [119], Daniels group reported on the kinetic responses observed with the treatment of PROTACs. Using the CRISPR-Cas9 technique, they constructed HiBiT tags for the N- or C-terminals of target genes alongside HaloTag-CRBN and HaloTag-Ubiquitin techniques to measure ternary complex formation and remaining protein through luminescence energy transfer in real time. Different degradation patterns were observed, with some behaving as classical single-exponential models with a plateau while others exhibited periodic changes or no plateau at all. In their 2021 work, they treated cells with TL12-186, a multi-CDK degrader. Interestingly, CDKs involved in cell cycle (CDK1, 2, 4, and 6) clustered together with poor degradation efficacy. Furthermore, ternary complex formation and degradation were found to be largely influenced by the cell cycle, which occurs during G1 but not S or G2/M phases. The group also provided evidence that exogenous CDK1/7/10/11/14/15/16/17/18 with HiBiT tags are degradable, thus highlighting the underlying difficulties in developing PROTACs for these targets.

Future development direction of CDK/cyclin degraders

Design degraders by non-ATP-based binders

CDKs belong to the family of protein kinases, which utilize ATP to phosphorylate downstream targets. Kinase inhibitors have been classified and labeled as various types, such as Type I to Type VI, based on their binding patterns and conformational changes [120–123]. By the definition, Type I inhibitors bind to the active conformation (DFG-in) and occupy the ATP binding pocket. Type II inhibitors usually bind with the inactive conformation (DFG-out) and may occupy the adjacent allosteric area of ATP binding domain. Type III inhibitors normally have an allosteric binding pattern with non-ATP competitive binding. They typically bind at adjacent allosteric regions of the ATP binding domain that do not overlap with the ATP binding pocket. Type IV inhibitor is allosteric inhibitor not bound next to the ATP-site. Type V inhibitor is bivalent inhibitor spanning two regions. Type VI inhibitor is covalent inhibitors. There are also sub-types in each type [124]. Currently, most kinase binders and CDK binders in PROTAC designs are sourced from Type I inhibitors, which are designed based on ATP binding patterns. However, this has resulted in poor selectivity. Developing allosteric inhibitors is also a promising idea. Allosteric inhibitors normally do not rely on mimicking ATP to blocking function. In 2014, Rauh group developed a high-throughput screening technology for screening allosteric inhibitors that stabilize inactive kinase conformations by binding within allosteric pockets in the kinase domain [125]. And in 2017, Morris group developed a conformation-sensitive fluorescent biosensor called CDKCONF that specifically reports on conformational changes of the T-loop of CDK2 and this biosensor was successfully applied to screen CDK2 allosteric inhibitors [126–128]. Designing PROTACs with allosteric binders presents several challenges, including the limited availability of non-Type I inhibitors and the allosteric mechanism itself, which can impede PROTACs design. So far, only a few cases have demonstrated that PROTACs can be constructed using allosteric binders [129]. Thus, it is necessary to fully evaluate the advantages and cost among several types of inhibitors and PROTACs, especially developing PROTACs based on allosteric binder of POI. It should be noted that in designing PROTACs, it is critical to consider POI binders with high binding affinity rather than inhibitory effects. Focusing on inhibitory effects alone can result in unwanted side effects. As mentioned earlier, Wu group identified KAT6A as a non-kinase target of iCDK9 by monitoring its degradation [53]. Most studies on PROTACs employ known inhibitors that have a definite affinity for the POI; however, this approach has limitations. As such, there is a pressing need to develop new techniques that can screen compounds based on their binding affinity beyond the ATP binding pockets. While Fischer group previously demonstrated the degradability of CDK1/7/10/11/14/15/16/17/18/19 using Type I pan binder [60], our study revealed that degradation efficiency can be significantly affected by the binding position. Thus, exploring other binding patterns may lead to more potent degradation on both degradable and non-degradable CDK targets.

Computer-aided degrader design

The introduction of computer-aided drug design has led to the development of multiple methods for designing proteolysis-targeting chimeras (PROTACs). In 2022, we published a review on the design of PROTACs based on experience and force field analysis [23]. However, some medicinal chemistry experts may find these methods overly intricate and challenging. As a result, several laboratories have proposed AI-based or integrated methods for PROTACs and molecular glue design. For instance, Gurram group recently described an *in-silico* approach for designing molecular glue (Fig. 11) [130]. Their methodology involved protein–protein docking, pocket analysis to identify small molecule binding sites, use of generative deep AI to generate suitable ligands, selection of the best model using deep learning scores, and molecular dynamics simulations to evaluate interactions and stability. Although they did not disclose further details about their algorithm, it is noteworthy that the crystal structures of DDB1 and CDK12-cyclin K complexes can be recovered. However, it is important to note that MD simulations may be too brief for evaluating such large complexes. Other researchers have also used AI-based methods for PROTACs design. Yang group applied a reinforcement learning method followed by filtering and traditional energy calculation (Fig. 12) [131]. They trained a model named PROTAC-RL with Quasi-PROTACs, which generated linkers with desired properties such as pharmacokinetics, length, and solubility. Further filtration was conducted by ML- or CADD-based methods to reduce the number of generated molecules. Notably, the entire design process only took 15 days, and the synthesized PROTACs were subsequently bio-evaluated. Another example is the work of Bai group who developed a deep-learning neural network named deepPROTACs, which embedded POI pockets, E3 pockets, PROTAC warheads, PROTAC linkers, and E3 ligands [132]. Their model had good predictive ability, as indicated by an AUROC of 0.8531.

Although various methods claim to have successfully assisted in the design of PROTACs and molecular glues, practical applications may still be far off. While some methods have produced near-to-actual bioactivities for limited types of proteins, different scoring functions can result in significant performance divergences on other proteins. As such, there is a need for CDK-specific methods for designing PROTACs and molecular glues. Moreover, additional and diverse examples should be evaluated to determine the real-world performance of these models. While AI methods have been used to successfully find and identify degraders for highly homologous CDK proteins, force field-based methods for CDK proteins remain largely unexplored. Further research in this area could lead to new insights into the design of effective degraders for CDK proteins.

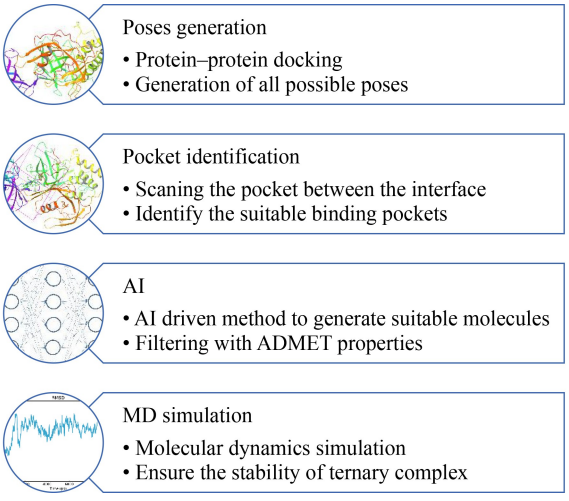


Fig. 11 The workflow of the method of AI-aided molecular glue design by Kishan Gurram and colleagues.

CDK inactivation by cyclin degradation

Typically, a CDK requires a corresponding cyclin to perform its function, and one effective method for inhibiting CDK function is through cyclin inactivation. Several cases of cyclin degradation have been achieved, including degradation of cyclin D1, cyclin K, and cyclin T1 [44,54]. In the field of PROTACs, pomalidomide and its derivatives are commonly used as E3 ligase ligands, with cereblon being a popular choice due to its potent degradation capacity and the stability of its ligand. However, despite success in inducing complex degradation for other targets such as EZH2/EED [133], cereblon-based PROTACs have failed to achieve cyclin

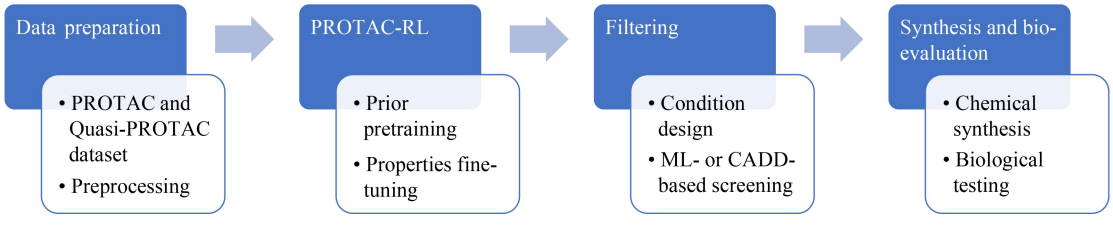


Fig. 12 The workflow of the method of AI-aided molecular glue design by Yang group.

degradation in all known CDK PROTACs. It is unclear why this is the case, as other E3 ligands like HyT tags for cyclin T1 degradation and VHL ligands for cyclin D1 degradation have shown promise. In the molecular glue field, three groups have independently reported successfully targeting cyclin K by recruiting the interaction between ubiquitination-proteasome associated protein and CDK/cyclin, but there is currently no available protocol for designing such molecular glues. High-throughput screening remains critical in this area. Although few cyclin binders exist, it is theoretically possible to achieve cyclin degradation via the CDK-cyclin complex.

Library construction and screening

The rational design of CDK degraders has proved challenging, leading to a greater reliance on compound library construction and high-throughput screening in their discovery. With the development of high-throughput screening techniques and the involvement of artificial intelligence in molecule design, it is possible that AI-guided molecule design and high-throughput screening may become the mainstream approach for discovering CDK degraders. However, this shift will require the creation of more diverse compound libraries, which will present a new major challenge in the field.

The rational design of CDK degraders has proved challenging, leading to a greater reliance on compound library construction and high-throughput screening in their discovery. Diversity-oriented organic synthesis (DOS) was first proposed in 2000 [134], and since then, related methodologies have seen significant developments over the past two decades [135]. Unlike traditional target-oriented organic synthesis (TOS), which follows retrosynthetic analysis to obtain a specific target product, DOS synthetic strategy employs forward-synthetic analysis to diversify functional groups as much as possible at each step to construct different molecular skeletons. With this approach, compound libraries with high diversity and complexity can be easily constructed using available starting materials, resulting in unique structural features such as medium-sized rings [136] and bridged bicyclic rings [137]. While DOS strategies make it possible to construct large compound libraries in a relatively short period of time, high-throughput screening techniques allow for quick and automated screening of these libraries. Generally, high-throughput screening experiments can parallelly screen 10^3 to 10^6 small molecules [138,139]. We speculate that diversity-oriented synthesis and high-throughput screening may become the mainstream approach for discovering CDK degraders in the future. However, the main challenge will be gaining a compound library with higher diversity and structural complexity, as well as developing more sensitive and precise screening assays.

Summary

CDK protein is an important regulatory factor in the cell cycle, and plays critical roles in various physiologic processes as well as in the development of diseases. While existing small molecule inhibitors have achieved some success, they are limited in their ability to fully meet certain needs. The advent of CDK degraders has potential to address these limitations, with PROTAC and molecular glue technology being used effectively to degrade different CDK members. In this review, we provide a summary of reported CDK degraders and the underlying techniques used to implement them, and propose possible future directions for the development of CDK degraders. With the development of target protein degradation technologies including new design methods, screening and evaluation systems, we believe that in the future, more CDK-targeting PROTAC and molecular glue molecules will not only be developed as tools for fundamental biological research, but will also enter the clinical study to solve the medical needs of patients.

Acknowledgements

This work was supported by National Key R&D Program of China (Nos. 2021YFA1302100, 2020YFE0202200, and 2021YFA1300200), National Natural Science Foundation of China (No. 82125034), Fellowship of China Postdoctoral Science Foundation (No. 2021M701953), and the Foundation of Shuimu Tsinghua Scholar Program (No. 2021SM110).

Compliance with ethics guidelines

Conflicts of interest Liguo Wang, Zhouli Yang, Guangchen Li, Yongbo Liu, Chao Ai, and Yu Rao declare no conflict interests.

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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