Review

Inhibitors, PROTACs and Molecular Glues as Diverse Therapeutic Modalities to Target Cyclin-Dependent Kinase

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Simple Summary: Cyclin-dependent kinases (CDKs) are rich and viable therapeutic targets for various cancers. The emergence of event-driven pharmacology as an alternative to occupancy-driven pharmacology has begun to address the challenges associated with selectively targeting CDKs. In this review article, we summarize the CDK inhibitors that are currently in clinical trials. In addition, we provide an overview of PROTAC- and molecular glue-based strategies to modulate CDK function.

Abstract: The cyclin-dependent kinase (CDK) family of proteins play prominent roles in transcription, mRNA processing, and cell cycle regulation, making them attractive cancer targets. Palbociclib was the first FDA-approved CDK inhibitor that non-selectively targets the ATP binding sites of CDK4 and CDK6. In this review, we will briefly inventory CDK inhibitors that are either part of over 30 active clinical trials or recruiting patients. The lack of selectivity among CDKs and dose-limiting toxicities are major challenges associated with the development of CDK inhibitors. Proteolysis Targeting Chimeras (PROTACs) and Molecular Glues have emerged as alternative therapeutic modalities to target proteins. PROTACs and Molecular glues utilize the cellular protein degradation machinery to destroy the target protein. PROTACs are heterobifunctional molecules that form a ternary complex with the target protein and E3-ligase by making two distinct small molecule–protein interactions. On the other hand, Molecular glues function by converting the target protein into a "*neo-substrate*" for an E3 ligase. Unlike small molecule inhibitors, preclinical studies with CDK targeted PROTACs have exhibited improved CDK selectivity. Moreover, the efficacy of PROTACs and molecular glues are not tied to the dose of these molecular entities but to the formation of the ternary complex. Here, we provide an overview of PROTACs and molecular glues that modulate CDK function as emerging therapeutic modalities.

Keywords: kinase; CDK; cancer; PROTAC; molecular glue

1. Introduction

Protein kinases are a family of essential enzymes that make up ~2.6% of all human genes (based on estimated 20,000 human genes). They are clustered in different subgroups such as serine-threonine (S/T) kinases, tyrosine kinases, and tyrosine-like kinases, based on their sequence similarity and biochemical function [1]. Mechanistically, a kinase forms a ternary complex with ATP and its substrate to catalyze the transfer of the γ-phosphate group from an ATP to its substrate proteins. The resulting phosphorylation of the substrate serves as the on/off switch for downstream signal transduction pathways. Unlike

the classical kinases, histidine kinases are two-component signal transduction systems, i.e., the transfer of the γ-phosphate group from an ATP to the histidine residue of the kinase, followed by the transfer of phosphate to the aspartate residue of the downstream substrate [2,3].

Changes to the phosphoproteome regulate several aspects of cellular functions, such as growth, migration, and survival, etc. Therefore, dysregulation of the kinase function has been associated with the pathogenesis of several human diseases, including cancer and neurodegenerative diseases [4–6]. Therapeutically, a small, well-defined ATP binding pocket makes kinases an attractive target for drug discovery. Twenty years ago, Imatinib (Gleevec), a BCR-Abl kinase inhibitor, was the first Food and Drug Administration (FDA)-approved kinase inhibitor to treat Philadelphia chromosome-positive chronic myelogenous leukemia. As of 30 March 2021, 65 kinase inhibitors have been approved by the FDA, and 85% of them are prescribed to treat cancer [7].

A protein kinase core is composed of two lobes; a smaller N-terminal lobe that mainly consists of five β-sheets and a C_α-helix, and a larger C-terminal lobe that primarily consists of six α-helices. These two lobes are connected by a flexible hinge region that interacts with the ATP [8]. On the other side of the hinge region is a flexible activation loop that begins with the conserved DFG motif and extends up to the APE motif. In the active conformation, the Asp (DFG in) residue orients inwards towards the ATP binding site and binds to the magnesium ion that interacts with the ATP phosphate. In the inactive conformation, the Asp (DFG out) residue points away from the ATP binding site. Kinase inhibitors that bind to the active kinase conformation are called type I inhibitors while those that bind to the inactive conformation are called type II inhibitors. Representative examples of type I and type II inhibitors are Gefitinib, Baricitinib, Ribociclib and Imatinib, Ponatinib, Sorafenib, respectively. Type III kinase inhibitors, such as Cobimetinib, Trametinib, and Binimetinib bind to an allosteric site of the kinase. Covalent kinase inhibitors bind irreversibly to the kinase by forming a carbon–sulfur bond through the sulfhydryl group of the surface-exposed cysteine residue proximal to the ATP binding pocket site [4].

To date, ~89% of approved kinase inhibitors are reversible and follow an occupancy-driven pharmacology model wherein the efficacy of the inhibitor correlates with its off constant [9]. Since it is impossible to accurately model the amount of drug required to maximally inhibit kinase function in vivo, efficacy studies are conducted at concentrations as close to the maximum tolerated dose as possible. Irreversible or covalent inhibitors that follow a two-step binding model overcome the above issue. First, the reversible component of the covalent inhibitor binds to the kinase (K_i), followed by the attack of the proximal nucleophile with an appropriate trajectory to the electrophilic component of the covalent inhibitor (K_{inact}) [10,11].

Cyclin-dependent kinases (CDKs) are members of a S/T kinase subfamily that comprises 21 CDK enzymes. CDK1, 2, 3, 4, and 6 play a key role in cell cycle regulation, whereas CDK7, 8, 9, and 11 are involved in the regulation of transcription. The biological functions of CDK10, 11, 14–18, and 20 are yet to be fully elucidated [12,13]. Cyclins bind to CDKs to regulate their activity, except CDK5, which binds to and is regulated by p35/p39 and their cleaved products p25/p29 [14]. Multiple cyclins bind to the same CDK to regulate different phases of the cell cycle. For instance, the CDK2-cyclin E complex regulates cell cycle reentry, G1 progression, and S phase entry, whereas CDK2-cyclin A regulates the later part, S and G2/M progression, of the cell cycle [15]. CDKs bind to different cyclins, resulting in the phosphorylation of an array of substrates [16]. For example, CDK9 can complex with either cyclin T1, T2a, T2b, or K to form the positive transcription elongation factor b (P-TEFb), which phosphorylates the C-terminal domain of the large subunit of RNA polymerase II (RNA pol II) and facilitates mRNA synthesis [13,14]. However, the CDK9–cyclin K complex has additional functions in genome maintenance, [15] and induces the phosphorylation of Ser33 within the transactivation domain (TAD) of p53 [16].

CDKs play important roles in a variety of functions including cell proliferation, transcriptional activity, and neuronal activity, and dysregulation of these proteins is often

observed in cancer and neurological diseases [17]. Over the past two decades, several CDK inhibitors (**1–13**) entered clinical trials to treat cancer (Figure 1). In 2015, Palbociclib was the first CDK 4/6 inhibitor (**6**) to be approved by the FDA, [18] and shortly after, the CDK4/6 inhibitors ribociclib (**7**) [19] and abemaciclib (**8**) [20] also received FDA approval for the treatment of breast cancer. The above successes reinvigorated CDK inhibitor development, leading to several CDK inhibitors currently being evaluated alone or in combination with other drugs against several cancers (Table 1) [21].

Novel approaches to target dysregulated proteins are routinely explored. One such approach that has gained considerable attention is proteolysis targeting chimeras (PROTACs). PROTACs artificially induce the degradation of a targeted protein by hijacking the cellular quality-control machinery [22]. PROTACs are heterobifunctional molecules with two different ligands connected by a linker. One ligand attaches to the protein of interest (POI), whereas the other ligand binds to an E3 ligase, resulting in a ternary complex between POI, the PROTAC, and the E3 ligase. This enables proximity-driven polyubiquitination of the POI by the E3 ligase and the subsequent non-natural degradation of the target protein (Figure 2). A traditional small molecule inhibitor (SMI) primarily blocks the catalytic activity of the protein to inhibit the enzymatic function of the target protein via an occupancy-driven pharmacology. On the other hand, a PROTAC operates by degrading the target protein via an event-driven pharmacology model. Early studies showed superior effects via the PROTAC approach, likely due to the elimination of both catalytic and non-catalytic/scaffolding function of the target protein [23–30].
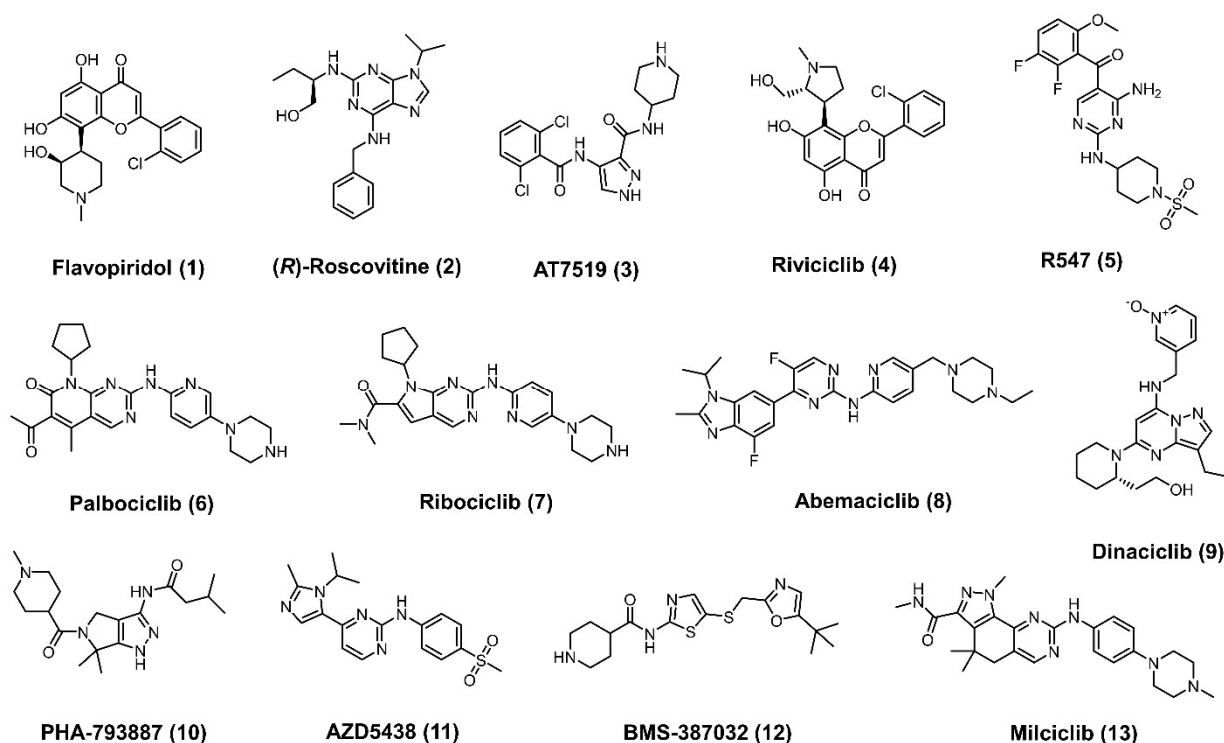

Figure 1. CDK inhibitors that are advanced to the clinic/clinical trials.

Table 1. CDK inhibitors in clinical trials.

NCT No.	Conditions	Interventions	Phase
04802759 [a]	BC	GDC-9545, Abemaciclib, Ipatasertib, GDC-0077, Ribociclib, Everolimus	I, II
04607668 [a]	MCC	Trilaciclib	III
04585724 [a]	BC	Abemaciclib, Palbociclib, Ribociclib	I
04553133 [a]	SCLC, NSCLC, TNBC, EGFR2, OC	PF-07104091 monotherapy, PF-07104091 + Palbociclib, PF-07104091 + Palbociclib + Letrozole	II
04469764 [a]	EC, OC	Abemaciclib, Anastrozole, Letrozole	II
04282031 [a]	ST, BC	BPI-1178 [c], Fulvestrant, Letrozole	I, II
04247126 [a]	ST, BC, SCLC	SY-5609 [d], Fulvestrant	I
04116541 [a]	ST	HDM201, Ribociclib, Cabozantinib, Alectinib	II
04049227 [a]	OC	Abemaciclib, Letrozole	I
04040205 [a]	STS	Abemaciclib	II
04010357 [a]	SCLC	Abemaciclib	II
03959891 [a]	BC	Ipatasertib, Fulvestrant, Palbociclib	I
03740334 [a]	ALL	Ribociclib, Dexamethasone, Everolimus	I
03675893 [a]	EC	Letrozole, Abemaciclib	II
03593915 [b]	MS	Alvocidib + Decitabine, or Azacitidine	I, II
03455270 [b]	BC	G1T48, Palbociclib	I
03439735 [a]	BC	AI and Palbociclib	II
03386929 [b]	NSCLC	Avelumab [e], Axitinib, Palbociclib	I, II
03363893 [a]	ST	CT7001, CT7001 + Fulvestrant	I, II
03310879 [a]	Cancer	Abemaciclib	II
03285412 [a]	BC	Ribociclib, ET	II
03280563 [a]	BC	Atezolizumab [f], Bevacizumab [g], Entinostat, Exemestane, Fulvestrant Ipatasertib, Tamoxifen, Abemaciclib	I, II
03227328 [a]	BC	CDK4/6 inhibitor + ET, chemotherapy + ET	II
03220178 [a]	BC	Palbociclib, Fulvestrant, Anastrozole, Letrozole, Exemestane	IV
03170206 [a]	LC	Binimetinib, Palbociclib	I, II
03155997 [a]	BC	Abemaciclib, ET	III
03110744 [a]	BC	Palbociclib	II
02712723 [a]	BC	Letrozole, Ribociclib	II
02644460 [a]	BT, ST, ES, BC	Abemaciclib	I
02632045 [a]	BC	LEE-011 (Ribociclib), Fulvestrant	II
02626507 [a]	BC	Gedatolisib, Faslodex, Palbociclib, Zoladex	I
02603679 [a]	BC	Paclitaxel, Tamoxifen + Palbociclib, AI + Palbociclib, Goserelin + AI + Palbociclib,	II
02599714 [b]	BC	AZD2014, Palbociclib, Fulvestrant	I
02592083 [b]	BC	Tamoxifen, Goserelin, Palbociclib	II
02586675 [b]	BC	Tamoxifen, Ribociclib, Goserelin	I
02503709 [b]	ST	AT7519, Onalespib	I
02499146 [b]	BC	Palbociclib, Letrozole	I
02095132 [b]	BT	Adavosertib, Irinotecan Hydrochloride	I, II
01864746 [b]	HR	Palbociclib	III
01723774 [a]	BC	PD0332991, Anastrozole, Goserelin	II
01676753 [b]	BC, TNBC	Dinaciclib, Pembrolizumab [h]	I
01522989 [b]	ST	PD-0332991, 5-FU, Oxaliplatin	I
01434316 [a]	ST	Dinaciclib, Veliparib	I

[a] recruiting; [b] active, not recruiting; [c] undisclosed CDK4/6 inhibitor; [d] undisclosed CDK7 inhibitor; [e] human IgG1 anti-PDL1 monoclonal antibody; [f] an anti-programmed death-ligand 1 antibody; [g] a humanized monoclonal antibody that binds to all VEGF-A isoforms; [h] a humanized antibody inhibiting PD-1 receptor; Non-small Cell Lung Cancer (NSCLC); Small Cell Lung Cancer (SCLC); Triple Negative Breast Cancer (TNBC); Breast Cancer (BC); Lung Cancer (LC); Myelodysplastic Syndromes (MS); Central Nervous System (CNS); Acute Lymphoblastic Leukemia (ALL); Solid Tumor (ST); Ewing Sarcoma (ES); Metastatic Colorectal Cancer (MCC); Brain Tumor (BT); Bone Cancer (BC); Endometrial Carcinoma (EC); Deficiency Disorder (DD); Soft Tissue Sarcoma (STS); Aromatase Inhibitor (AI); Endocrine therapy (ET).
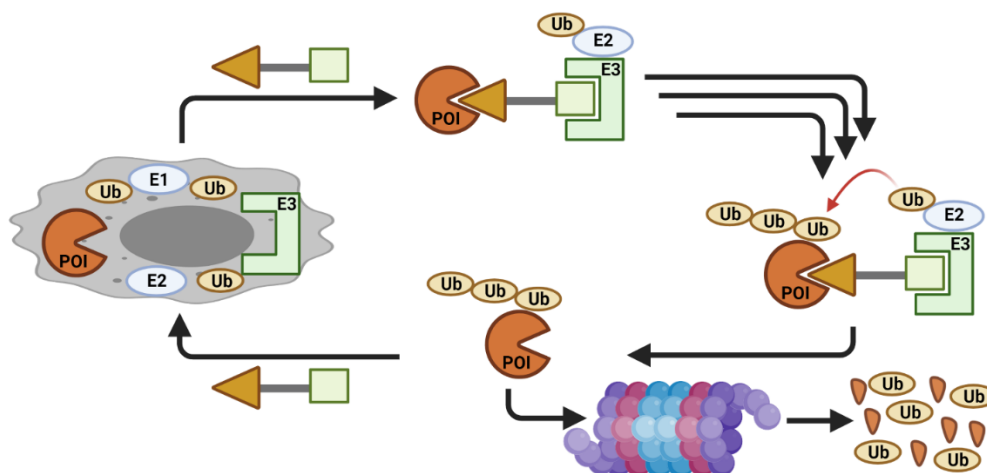
Figure 2. Schematic cartoon showing a PROTAC mechanism of action (created with BioRender.com).

The developmental arc of PROTACs went from a proof-of-concept study to clinical trials in ~20 years (Figure 3). A typical PROTAC consists of three parts: a POI binding ligand, an E3 ubiquitin-binding ligand, and a linker connecting two ligands. Despite the presence of >600 putative E3 ligases in the human proteome, only a handful of E3 ligase ligands (CRBN [31,32], VHL [33–35], MDM [36–38], IAP [39], RNF114 [40,41], DCAF15 [42], DCAF 16 [43], and FEM1B [44]) are readily available for the design of a PROTAC (Figure 4). The PROTAC approach has been successfully applied to degrade kinases, G protein-coupled receptors, nuclear receptors, membrane proteins, transcription factors, and neurodegenerative proteins aggregates [23,26–30,36,45–61]. Recent studies showed good potency and selectivity of PROTACs in animal models due to POI degradation [27–30]. Currently, two PROTAC candidates, ARV-471 (**14**, NCT04072952) and ARV-110 (**15**, NCT03888612), targeting the estrogen receptor and androgen receptor, respectively, are in Phase II clinical trials. Two additional PROTACs, viz. KT-474 (undisclosed structure) (NCT04772885) that degrades IRAK4 for the treatment of interleukin-1 receptor (IL-1R)/toll-like receptor (TLR)-driven immune-inflammatory diseases, and BTK degrader NX-2127 (NCT04830137) for the treatment of relapsed and refractory B-cell malignancies, are being evaluated in Phase I studies.
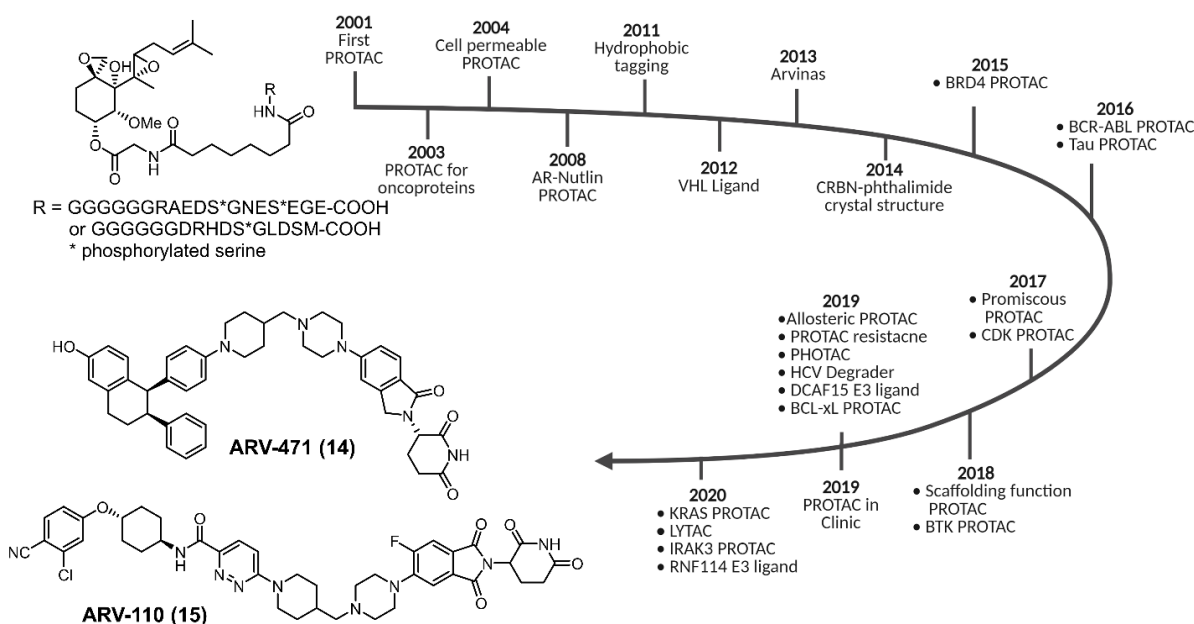

Figure 3. PROTACs evolution from bench to bedside (created with BioRender.com).
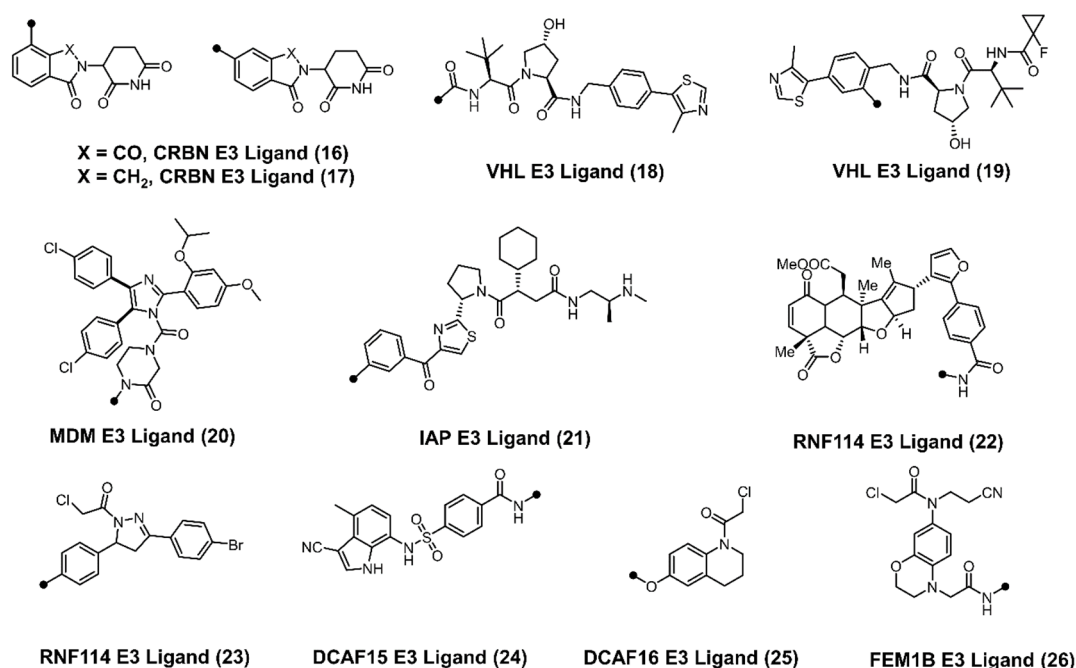
Figure 4. Chemical structures of different E3 ligands that have been used successfully to degrade a POI (• represent the site for linker conjugation).

A typical flowchart for the successful development of a PROTAC is summarized in Figure 5. The PROTAC strategy has been effective in converting promiscuous binders to selective degraders [62–64]. Due to the catalytic nature of PROTAC technology, a sub-stoichiometric amount of PROTAC is adequate to induce robust POI degradation, thereby mitigating the off-target inhibitory effects observed with promiscuous SMI. Since PROTACs degrade the POI, unlike SMI's, PROTAC treatment does not result in feedback induction of the POI. Future studies will focus on developing disease-specific PROTACs either by exploring novel disease-specific POI such as oncogenic KRAS^{G12C}, or by examining tissue-specific E3 ligases [65,66]. Alternative strategies to achieve tissue specificity could include the development of antibody PROTAC conjugates [67].
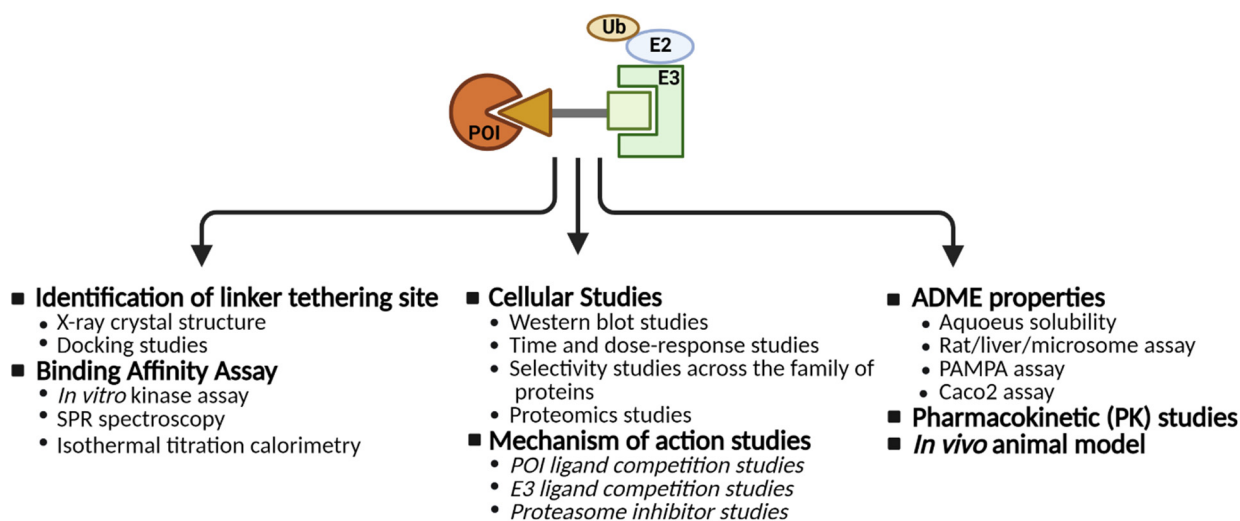
Figure 5. A flowchart highlighting the development of a PROTAC (created with BioRender.com).

2. CDK Degradation Using PROTAC

In the following sections, we summarize PROTACs developed against various CDKs and, where available, compare their effects with the corresponding inhibitors. In general, the identification of a successful CDK PROTAC requires a set of generic experiments that are common such as: (i) binding studies with the newly synthesized PROTACs via in vitro kinase assays; (ii) dose–response studies to determine their cellular DC_{50}; (iii) time-course studies to demonstrate the robust degradation of a CDK kinase at different time points; (iv) competition studies with an inhibitor, an E3 ligand, and a proteasome inhibitor to show the formation of a ternary complex and subsequent ubiquitin-mediated degradation of the CDK; and (v) whole cell proteomics studies to show selective degradation of the target CDK. In the subsequent sections, we provide the structures of selective CDK PROTACs along with an overview of experiments conducted to establish their MOA. Moreover, we highlight the CDK inhibitor component of a PROTAC with a yellow box and an E3 ligand component with a green box.

2.1. CDK2

Cyclin-dependent kinase 2 (CDK2) plays a crucial role in cell cycle regulation and other biological processes such as DNA damage, intracellular transport, and signal transduction [68]. CDK2 binds with cyclin E to phosphorylate numerous proteins required for the cell cycle progression, DNA replication, and centrosome duplication. During the S phase, cyclin E is degraded, and CDK2 then forms complexes with cyclin A [69–71]. Although CDK2 mutations are rarely observed in human cancers, the CDK2/cyclin E and CDK2/cyclin A pathways are often altered. For instance, in ovarian and breast cancers, cyclin E1 gene amplification results in increased activity of CDK2/cyclin E complexes [72,73]. Similarly, cyclin A is often overexpressed in colorectal [74], breast [75], and high-grade serous ovarian cancers (HGSOC) [76]. Recent studies also suggest that inhibiting CDK2 could be a promising therapeutic strategy to treat acute myeloid leukemia (AML) [77,78]. Despite extensive efforts put forth by both pharma and academic labs, the discovery of a highly selective CDK2 inhibitor remains elusive, primarily due to the high homology of the ATP binding sites among the CDKs [68,79].

The PROTAC strategy relies on the overall three-dimensional architecture of CDK and involves structural complementarity with the E3 ligase complex. This approach has been explored to develop a selective CDK2 degrader. Three different labs reported the development of CDK2 PROTACs using pomalidomide (**16**) and three different CDK targeting ligands (**27**, **29** and **31**, Figure 6). OVCAR8 cells treated with PROTAC **27** (TMX-2172) selectively degraded CDK2 and CDK5 over other CDKs [80]. The study also reported a control compound **28** (TMX-bump) in which the glutarimide nitrogen was methylated, thus blocking its ability to bind CRBN (E3 ligase). Ribociclib (**7**) does not inhibit CDK2; however, replacing the 1-(pyridyl-2-yl)piperazine with a methylbenzoate in ribociclib yielded an inhibitor that was selective of CDK2 (>50-fold) and bound CDK2 with improved affinity. PROTAC **29** derived from the modified ribociclib analog degraded CDK2 in addition to CDK4 and CDK6. PROTAC **29**, degraded CDK2/4/6 in melanoma cells (B16F10 and A375), and inhibited retinoblastoma (Rb) protein phosphorylation. Moreover, PROTAC **29** inhibited colony formation and induced apoptosis in A375 and B16F10 cells [81]. A prodrug **30** of PROTAC **29**, was synthesized by attaching a labile group on thalidomide which resulted in improvement of oral bioavailability from 1 to 68%. In a B16F10 xenograft model, oral administration (200 mg/kg) of **30** showed significant reduction in tumor growth. The third reported CDK2 PROTAC (**31**, Figure 6) was built using a CDK2 inhibitor with a diaminotriazole (J2) [82]. PROTAC **31** induced robust degradation of CDK2 in NB4 cells and Ramos cells at nanomolar concentrations. Accumulation of mutations within hematopoietic stem cells (HSCs) leads to AML [83]. In a cellular differentiation study, PROTAC **31** treatment induced the expression of CD11b, a marker for cell differentiation. Morphological changes indicated that PROTAC **31** could induce significant differentiation of myeloid cells [82].

Furthermore, PROTAC **31** induced granulocytic differentiation of HSCs, suggesting that PROTAC **31** is a promising lead for AML differentiation therapy.
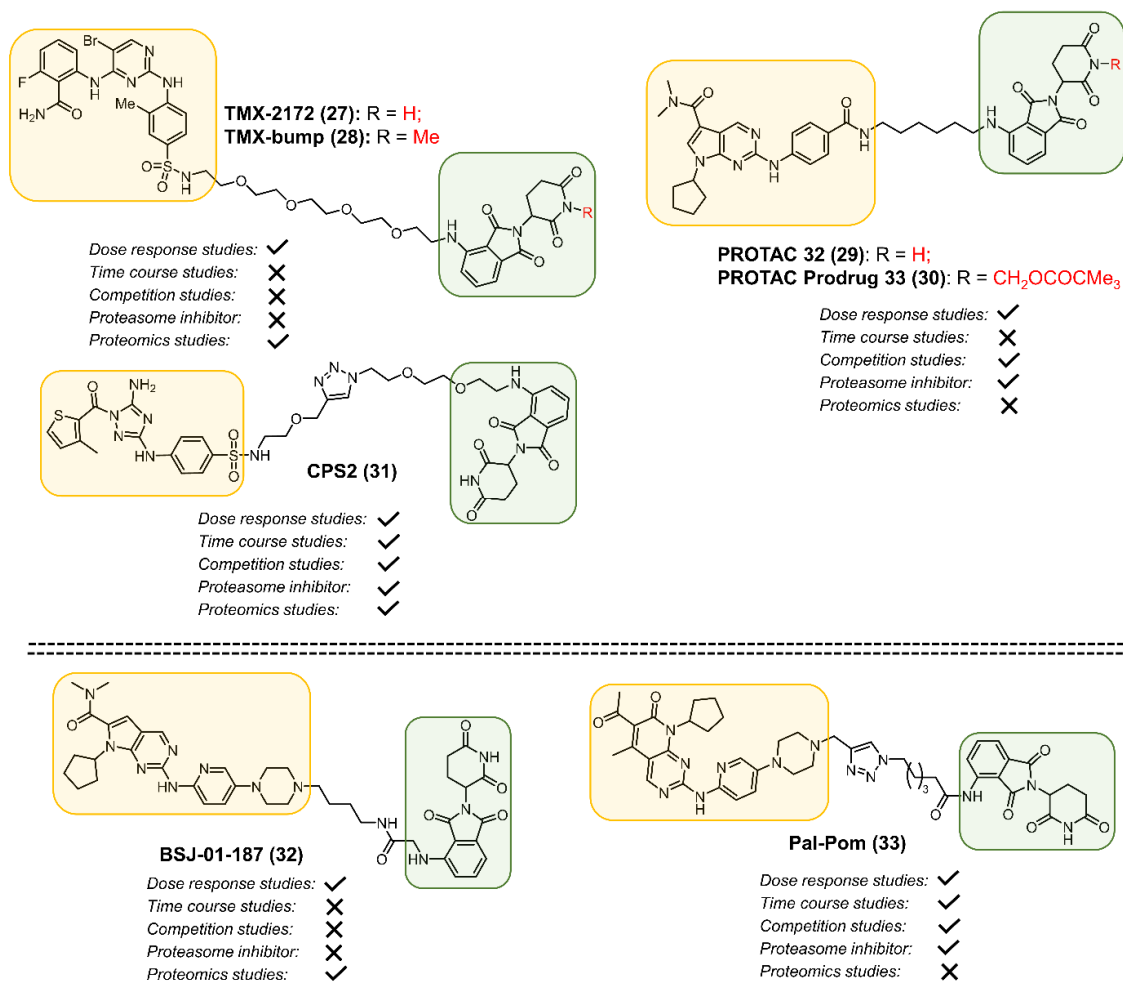
Figure 6. Structures of selective CDK2 (**27**, **29**, and **31**) and CDK4 (**32**, **33**) PROTACs.

2.2. CDK4

CDK4 and its close homolog CDK6 are a family of serine-threonine kinases that interact with D-type cyclins (D1, D2, and D3) to phosphorylate Rb to regulate the G1/S transition of the cell cycle [84]. Phosphorylation of Rb results in the release of transcription factors E2F to activate transcription of numerous genes that are responsible for cell cycle progression through the S phase as well as those involved in DNA replication and chromosome segregation [1]. Activation of the CDK4-Rb pathway is often observed in human cancers; for example, CDK4 amplification is observed in ~50% of glioblastomas [85]. Although CDK4 and CDK6 are highly homologous, they elicit different biological functions in cells. For instance, knockdown of CDK4 in the mouse model of NSCLC results in the CDK6-induced expression of mutant K-Ras [86] and CDK4/6 inhibitor treatment in breast cancer cells results in the amplification of CDK6 through a feedback mechanism [87]. These findings indicate the need to develop CDK4- and CDK6-selective inhibitors. The high homology (>90%) in the ATP binding pocket of CDK4 and CDK6 makes the development of CDK4- and CDK6-selective inhibitors a challenging task.

The synthesis and screening of a focused set of PROTACs derived from the CDK4/6 inhibitors palbociclib, ribociclib and abemaciclib identified selective and non-selective CDK4 and CDK6 degraders. The ribociclib-pomalidomide PROTAC **32** (Figure 6) with a 7-atom linker selectively degraded CDK4 in a CRBN-dependent manner in Jurkat cells [88]. Another study that evaluated a palbociclib and a ribociclib PROTAC linked through a triazole

showed that the palbociclib-based PROTAC pal-pom (**33**) degraded CDK4 with three-fold selectivity over CDK6 in MDA-MB-231 cells [60]. Despite available CDK4/6 inhibitors and E3-ligase binding ligands, the development of fully characterized CDK4-selective degraders are yet to be achieved.

2.3. CDK6

The Gray lab, our lab and Rao lab independently discovered CDK6-selective PROTACs (**34**, **36** and **37**, Figure 7) by conjugating the linker through the solvent exposed piperazine on Palbociclib and functionalizing the 4-position of phthalimide. The three PROTACs had different linker lengths and linker compositions [46]. PROTAC **34** exhibited potent and selective CDK6 degradation, while the designed *N*-methylated thalidomide containing compound BSJ-bump (**35**) did not degrade CDK4 or CDK6. Both PROTAC **34** and parent inhibitor **6** induced a similar transcriptional response in AML cells, suggesting that the transcriptional role of CDK6 in AML is mainly limited to its kinase-dependent function. PROTAC **36** exhibited similar in vitro potency for CDK4 and CDK6. In MiaPaCa2 cells, PROTAC **36** with a linker length of 17 atoms selectively degraded CDK6 while sparing its close homolog CDK4. We also observed Hook effects [89] at 5 and 10 µM concentrations in both HPNE and MiaPaCa2 cell lines [90,91]. PROTAC **37** (CP-10) showed robust CDK6 degradation in human glioblastoma U251 cells [92]. Since CDK6 overexpression could result in resistance to CDK6 inhibitors, additional copies of CDK6 were introduced in A-673, an Ewing's sarcoma cell line. In the above cell line, treatment with degrader **37** reduced CDK6 levels, suggesting that CDK6 PROTACs could be used to reverse CDK6 overexpression-induced drug resistance [92].

Multiple studies explored selectivity for CDK4 or CDK6 degradation by building focused sets of analogs with palbociclib, ribociclib and abemaciclib as the POI ligand, varying linker compositions, and E3-ligase ligands [60,93]. For the most part, PROTACs with shorter hydrophilic linker lengths and ribociclib as the POI ligand favored CDK4 degradation while those with longer hydrophobic linkers and palbociclib as the POI ligand resulted in selectivity for CDK6.

Steinebach and co-workers also explored the effect of linkers' lengths and the type of E3 ligand on the activity and selectivity of PROTACs derived from CDK4/6 dual inhibitors [94]. Several PROTACs with varied linker lengths and E3 ligands i.e., CRL4^{CRBN}, von Hippel Lindau (CRL2^{VHL}), a cellular inhibitor of apoptosis protein 1 (cIAP1) and mouse double minute 2 homolog (MDM2) were synthesized [94]. The pomalidomide-based PROTAC **38** (Figure 7) reduced CDK4 and CDK6 levels in multiple myeloma (MM) cell lines at 100 nM concentration, but also degraded the Ikaros (IKZF1) and Aiolos (IKZF3) transcription factors. Interestingly, the effect of the linker length was not significant but the lipophilicity of PROTACs was crucial in determining its selectivity. In general, PROTACs with log D < 4, showed superior CDK6 degradation. Additionally, the PROTACs derived from different VHL ligands also showed pronounced degradation of CDK4 and CDK6 with some selectivity for CDK6. PROTACs synthesized using VHL ligands linked through the phenyl ring showed excellent selectivity toward CDK6 compared to those derived from the terminal amine group (**39** vs. **40, Figure** 7). Both PROTACs **39** and **40** were effective in degrading CDK6 in multiple cell lines. The IAP-based PROTAC **41** (Analog **35**, Figure 7) non-selectively degraded CDK4 and CDK6. However, the MDM2-based PROTAC was unable to degrade CDK4 and CDK6, probably due to their poor cellular permeability.
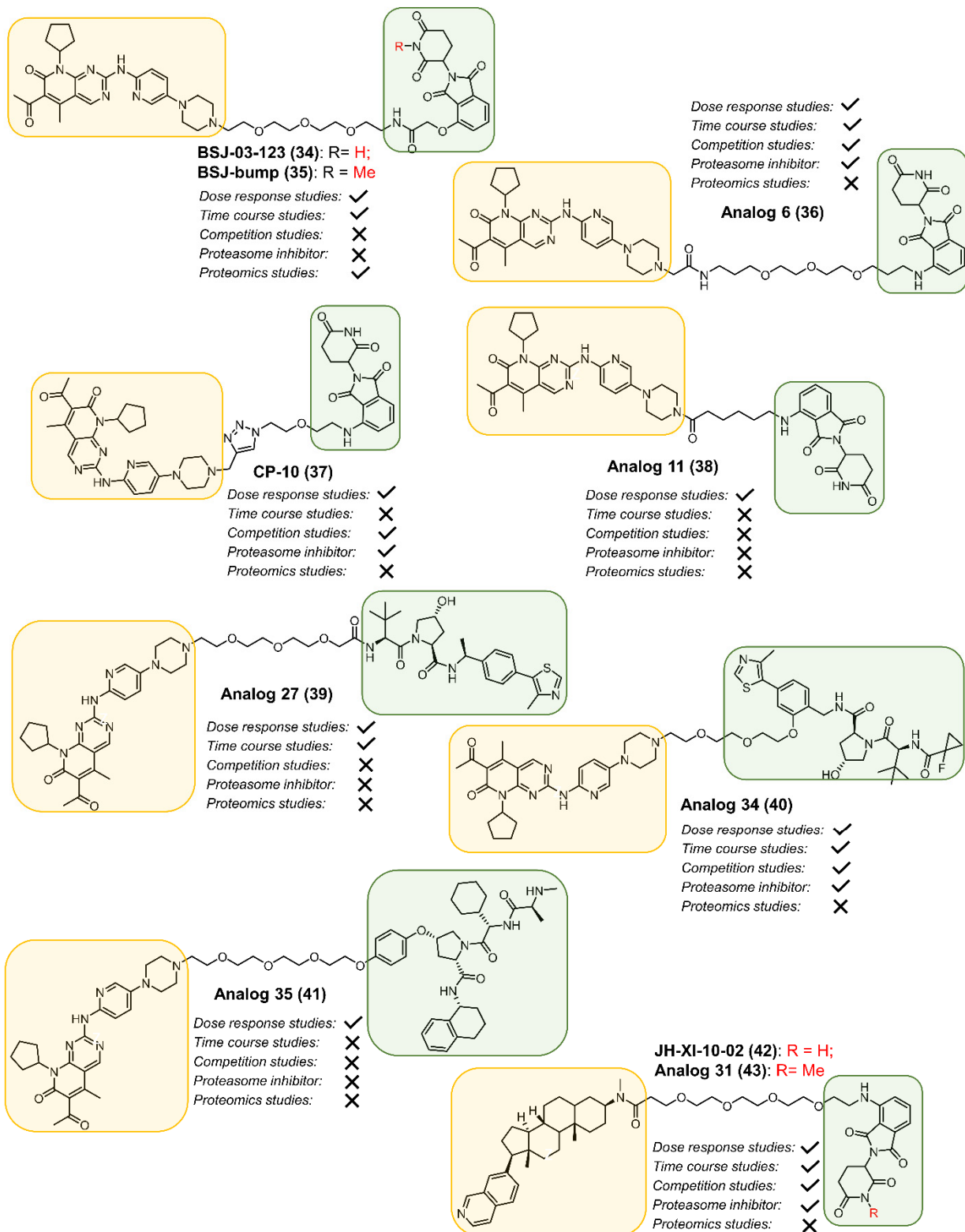
Figure 7. Structures of CDK6 (**34**, **36–41**) and CDK8 (**42**) PROTACs.

2.4. CDK8

CDK8, a serine/threonine protein kinase, is overexpressed in colorectal, breast, and hematological malignancies, and in colon cancer, high CDK8 levels are associated with poor clinical outcomes [95]. CDK8 knockdown by short hairpin RNA (shRNA) suppressed proliferation in both in vitro and in vivo models [96]. CDK8 plays a key role in oncogenic

signaling pathways, including the Wnt/β-catenin pathway [96], the p53 pathway [97], the NOTCH signaling pathway [98], and the TGF-β signaling pathway [99]. CDK8 regulates transcription through both its kinase activity and its scaffolding function [100], therefore CDK8 PROTACs are an attractive therapeutic option [101].

CDK8 has a high sequence similarity (>90%) with CDK19 [102]. Cortistatin A is a potent ATP competitive CDK8/19 selective inhibitor [103]. The complex total synthesis of Cortistatin (16–30 steps), along with the poor overall yield (0.012 and 2%) [104–106], limited the development of Cortistatin A-based PROTACs. Based on the X-ray crystal structure and mutagenesis studies [107], Hatcher et al. designed an analog by replacing the complex core in Cortistatin A with a simple steroid scaffold such as dehydroepiandrosterone (DHEA). Further optimization of this core resulted in the development of a potent CDK8 inhibitor with IC_{50} of 17 nM. A PEG linker that conjugated CDK8 inhibitor to pomalidomide yielded PROTAC JH-XI-10-02 (**42**, Figure 7) that induced partial degradation of CDK8 in Jurkat cells at 1 μM [103]. Studies with a negative control PROTAC **43** (Analog **31**, Figure 7), that lacked the ability to bind CRBN, validated **42** as a CDK8-selective PROTAC.

2.5. CDK9

CDK9, a member of the positive transcription elongation factor b (P-TEFb) complex, associates with cyclin T and cyclin K to regulate transcriptional elongation by interacting with RNA polymerase II (RNAP II) [95]. Inhibition of CDK9 leads to reduced phosphorylation of RPB1, which results in a reduction in Mcl-1 levels. Mcl-1 is an anti-apoptotic protein that plays a crucial role in cancer cell survival [108–110]. Inhibition of CDK9 kinase activity resulted in the induction of apoptosis in several cancer cell lines [111–115]. These indicate that CDK9 is a promising cancer target, especially for cancers that are driven by transcriptional dysregulation [111,116].

We reported a PROTAC derived from a CDK2/5 aminopyrazole-based inhibitor that selectively degraded CDK9 without affecting the levels of CDK2, CDK5, AKT, FAK, and IKKβ [62,117]. In cellular studies, compound 3 reduced RPB1 phosphorylation, a direct substrate for CDK9, and reduced Mcl-1 levels. This was the first report that showed that the differential surface-exposed lysine residues among the CDKs could be exploited to convert a non-selective CDK inhibitor into a selective CDK9 degrader. In a follow-up study, we optimized linker length and linker composition to identify PROTAC 2 (**44**, Figure 8), which selectively degraded CDK9 with a DC_{50} value of 158 ± 6 nM [118]. Despite the remarkable selectivity for CDK9 degradation, as with compound 3, PROTAC **44** non-selectively inhibited CDK2, 5 and 9 in cell-free studies. Unlike the CDK9 inhibitor flavopiridol that reduced cyclin K levels, PROTAC **44** induced complete CDK9 degradation at 1 μM while sparing cyclin K. Recent studies demonstrated that concurrent inactivation of Mcl-1 and Bcl-xL resulted in robust induction of apoptosis [14,62,118–120] and CDK9 regulates the levels of pro-survival protein Mcl-1 [121,122]. Synergism studies showed PROTAC **44** sensitizes pancreatic cancer cells to Bcl2 inhibitors. PROTACs generated using two other aminopyrazole-based pan CDK inhibitors AT-7519, and FN-1501 resulted in CDK2/9 selective degraders. The FN-1501-based PROTAC induced G2/M arrest and inhibited the growth of PC3 cells with nanomolar potency [123].

Olson et al. developed a degrader, THAL-SNS-032 (**45, Figure 8**) by conjugating an N-acyl-2-aminothiazole CDK7/9 inhibitor **12** [124] to pomalidomide via a PEG linker. PROTAC **45** induced potent and selective degradation of CDK9 in a CRBN-dependent manner [111]. In a cell-free assay, PROTAC **45** non-selectivity inhibited CDK1/2/7/9. PROTAC **45** selectively induced CDK9 degradation and did not alter the levels of CDKs inhibited by **12** even at 5μM. At higher concentrations, PROTAC **45** exhibited a hook effect as well [125]. Consistent with CDK9's role in promoting transcriptional elongation, PROTAC **45** inhibited phosphorylation of Ser2 C-terminal domain (CTD) of RNA Polymerase II in dose- and time-dependent studies, without effecting Ser5 and Ser7 phosphorylation [111].
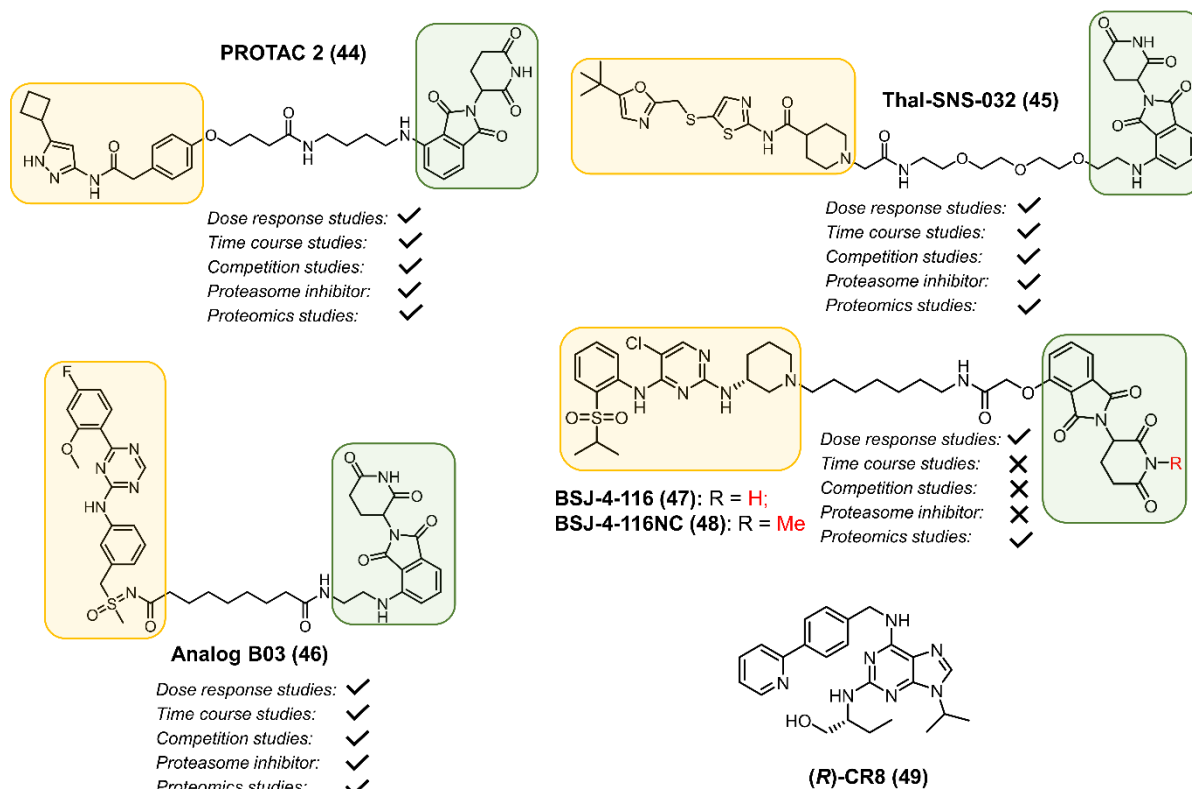
Figure 8. Structures of CDK9 PROTACs (**44**–**46**), CDK12 (**47**) PROTAC and a Cyclin K molecular glue (**49**).

Multiple independent PROTAC development studies with different potent and selective CDK9 ligands yielded mixed results. Replacing the N-acyl-2-aminothiazole kinase binding ligand in **45** with NVP-2, a highly selective aminopyrimidine-derived CDK9 inhibitor with sub-nM potency for CDK9, resulted in weaker CDK9 degradation [111,126]. Wogonin is a selective CDK9 inhibitor with an IC_{50} of 0.19 µM [127]. A docking guided PROTAC library design identified 11c that showed sub-micromolar biochemical inhibition of CDK9/CycT1 [128], but degraded CDK9 with a DC_{50} ~ 10 µM and reduced Mcl-1 levels in a dose-dependent study [129,130]. Conjugating the CDK9-selective inhibitor BAY-1143572 through the solvent-exposed group with pomalidomide led to the identification of PROTAC B03 (**46, Figure** 8) [131]. In cell-free studies, PROTAC **46** inhibited CDK9 with IC_{50} ~ 8 nM, which was comparable to the parent inhibitor (IC_{50} ~ 13 nM). PROTAC **46** reduced the levels of CDK9 (DC_{50} ~ 7 nM) and Mcl-1 levels in a time-dependent study. Moreover, PROTAC **46** exhibited improved anti-proliferative activity when compared to its parent inhibitor [131]. Together, these results suggest that the efficacy of CDK9 degradation by various PROTACs does not correlate with the potency and selectivity for CDK9 inhibition by the parent inhibitor.

2.6. CDK12

CDK12 phosphorylates RNA polymerase II to regulate transcription elongation [132]. In addition, CDK12 has also been reported to play a critical role in RNA splicing, DNA damage response (DDR), and the maintenance of genomic stability [133,134]. Loss of *CDK12* results in reduced transcription of *BRCA1* and increases sensitivity to PARP inhibitors and platinum chemotherapy. Thus, CDK12 inhibitors could be used in combination with DNA-damaging agents in HR-deficient cancers [12]. CDK12 is overexpressed in a variety of cancers, including breast cancer, ovarian cancer, prostate cancer, and gastric cancer, making it a viable cancer target [132]. The development of CDK12 inhibitors is particularly challenging due to high sequence similarity with its close homolog CDK13. The Gray lab developed a CDK12-selective degrader, BSJ-4-116 (**47**, Figure 8), by attaching

3-aminopiperidine moiety, which was important for the selectivity for CDK12 over its close homolog CDK13, to a promiscuous multi kinase degrader TL12-186 [64,135]. In cellular assays, PROTAC **47** selectively degraded CDK12 in a dose- and a time-dependent study, while sparing CDK13. A robust ternary complex was observed between CDK12 and CRBN in the presence of **47**, which was abolished with inactive PROTAC BSJ-4-116-NC (**48**). On the other hand, no such complex was induced between CDK13 and CRBN. The control **48** was ten-fold less potent than **47** in inhibiting cell growth, indicating CDK12 degradation as the primary mode of action of **47**. Since CDK12 is implicated in the regulation of DDR genes, degrader **47** treatment resulted in the downregulation of transcription genes involved in DDR pathways. In Jurkat and MOLT4 cells, PROTAC **47** and PARP inhibitors showed strong synergism. Interestingly, chronic exposure of MOLT-4 and Jurkat cells to degrader **47** resulted in the development of CDK12 degradation resistance via mutations of Ile733Val and Gly739Ser on the CDK12 G-loop, which resulted in reduced degrader **47** binding.

3. Perturbing CDK Function by a Molecular Glue

In contrast to heterobifunctional PROTAC degraders (Figure 2), molecular glues are low molecular weight inducers (novel de novo contacts) or stabilizers of protein–protein interactions [136,137]. Upon binding to a protein, the small molecule induces a conformational change and makes the small molecule–protein complex a "*neo-substrate*" for E3 ligase (Figure 9). Following the formation of a ternary complex, the "*neo-substrate*" is ubiquitinated, which leads to UPS-mediated protein degradation. Although these glues were identified serendipitously, this is an emerging and exciting therapeutic strategy for the inactivation of intractable targets.
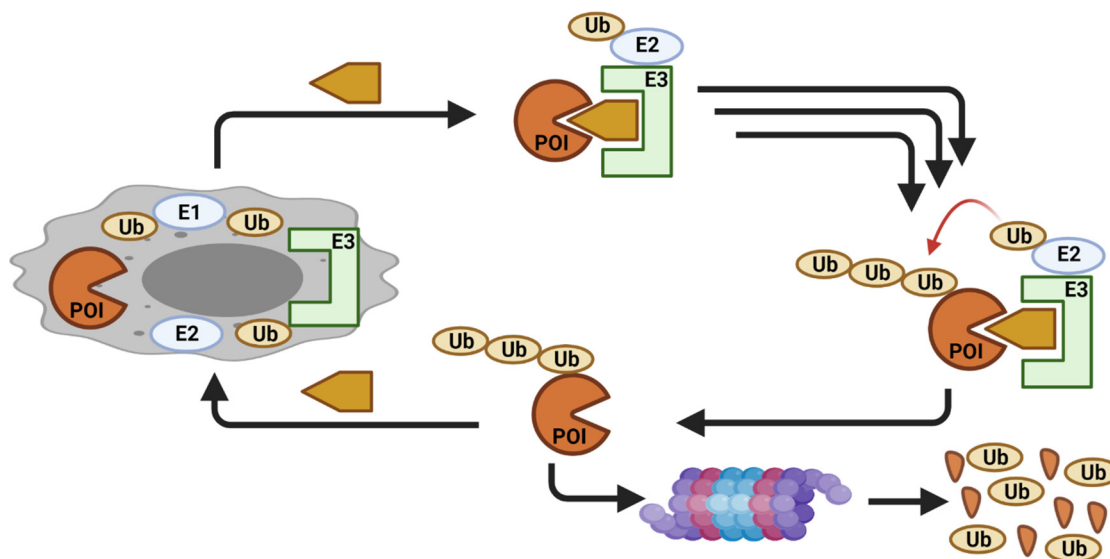

Figure 9. Mechanism of action of a prototypical molecular glue (created with BioRender.com).

Thalidomide was prescribed to treat morning sickness in pregnant women in the late 1950s and was withdrawn in the early 1960s because of catastrophic teratogenicity. Studies conducted to understand the associated biology resulted in the characterization of Thalidomide as a molecular glue [138]. The mechanism of action of Thalidomide and its analogs, termed immune-modulatory imine drugs (IMiDs), involves the recruitment of the Ikaros zinc family transcription factor (IKZF1 and IKZF3), CK1α, GSPT1, and others to cullin-RING E3 ligase CUL4^{CRBN}, resulting in their proteasomal degradation [31,32,139–143]. IMiDs, thalidomide, lenalidomide, and pomalidomide are first line therapeutics for multiple myeloma [144,145]. This discovery of IMiDs binding to CRBN led to the explosion of heterobifunctional PROTACs that recruit novel proteins of interest for proteasomal degradation [62,90,118,125]. Another class of molecular glue degrader is Indisulam, an

aryl sulfonamides drug that recruits splicing protein RNF39 to DDB1-associated and CUL4-associated factor 15 (DCAF15 E3 ligase), which resulted in RNF39 ubiquitination, followed by its degradation [56,146–148]. As a result, Indisulam is being used as an E3 ligase recruiter for PROTAC development. Li et al. successfully demonstrated BRD4 degradation by recruiting DCAF15 in a PROTAC-dependent manner [42].

A rational design was used by Slabicki et al. to discover (*R*)-CR8 (**49, Figure** 8) as a cyclin K molecular glue degrader [149]. The glue **49** is an analog of roscovitine (**2**), which was previously reported as a CDK1/2/3/5/7/9 inhibitor [150]. A correlation of drug-sensitivity data of over 4500 drugs against 578 cancer cell lines and the expression of 499 E3 ligase components showed a positive correlation between the cytotoxicity of **49** and the mRNA levels of DDB1, a CUL4 adaptor protein. A quantitative proteome-wide mass spectrometry study showed a decrease in cyclin K in **49**-treated cells, which was rescued by the pretreatment of cells with MLN7243 (an E1 ubiquitin-activating enzyme inhibitor), MLN4924, and MG132. Biophysical, point mutation, and X-ray crystallographic studies showed that in the presence of **49**, CDK12 interacts with DDB1. Cyclin K is a binding partner of CDK12, and overlays showed that it did not make direct contact with DDB1. Interestingly, in the DDB1-CDK12-Cyclin K conformation, cyclin K was transformed into a "neo-substrate" for CUL4 and was degraded. Glue **49**-induced cyclin K degradation was blocked by co-treatment with either the inhibitor of E1 ubiquitin-activating enzyme (MLN7243) or MLN4924. CRISPR-Cas9 resistance screens demonstrated the involvement of the CUL4 complex components in mediating **49**-induced toxicity. In vitro ubiquitination assays showed that the CUL4 complex core that included RBX1-DDB1 was sufficient for **49** induced cyclin K degradation. Other CDKs (9 and 13) were also associated with DDB1 in the presence of **49**. However, these complexes did not degrade cyclin K to the same degree as the CDK12-DDB1 complex. X-ray crystallographic studies showed that the purine core of analog **49** occupied the ATP-binding site of CDK12, whereas the hydrophobic phenylpyridine ring interacted with the BPC domain of DDB1. Structure–activity relationship (SAR) studies showed that any alteration to the phenylpyridine ring resulted in diminished activity, suggesting the importance of the terminal groups that interact with DDB1 for robust cyclin K degradation. This study, along with another recent report, showed that the minor modification of a surface-exposed region of a small molecule converted it from an inhibitor to a glue [151,152], which suggests that exploiting the surface-exposed regions of CDK inhibitors could yield CDK-selective molecular glues.

4. Conclusions and Outlook

Novel small molecules that modulate the function or levels of specific proteins are beginning to emerge as the primary modality for cancer therapy over other strategies such as surgery, chemotherapy, and radiation therapy. In recent years, kinase inhibitors have emerged as a successful class of targeted therapeutics. Several kinase inhibitors are in clinical use or under clinical/preclinical studies. The CDK family of proteins are an important class of kinases that play prominent roles in regulating cellular transcription, mRNA processing, and the cell cycle, and thus, have become attractive therapeutic targets for cancer. The similar ATP binding sites among CDKs make the development of selective CDK inhibitors a challenging task. Even FDA-approved drugs such as palbociclib, ribociclib and abemaciclib are dual CDK4/6 inhibitors. Moreover, most of the CDKs inhibitors developed to date are non-covalent; thus, accurately estimating the dosage required to inhibit the kinase continues to vex translational scientists. PROTACs and molecular glues have emerged as an alternative approach that relies on artificially inducing degradation of the targeted protein by hijacking the cellular quality-control machinery. PROTACs and molecular glues rely on the topology of surface-exposed lysine residues for their activity and, therefore, provide a different modality to develop CDK-selective modulators. Moreover, the "catalytic nature" and "event-driven" pharmacology of PROTACs/molecular glues allows sub-stoichiometric dosing to eliminate the targeted CDK.

Here, we summarized the reported CDK selective PROTACs. Several factors such as the differential distribution of lysine residues, stable ternary complex formation, structure complementarity of CDK, and the components of the E-ligase complex dictate selective degradation. Unlike kinase inhibitors, whose efficacy is closely associated with its binding affinity, the linker length, linker composition, and the choice of the E3 ligand play a greater role in dictating the potency and selectivity of PROTACs. Nonetheless, the design of any PROTAC is an iterative process, and extensive optimization is needed to develop a potent and selective degrader. Among others, hook effects and unknown off-target effects are likely to be the growing pains as the PROTAC-based strategies mature. Most of the PROTACs developed thus far have not been evaluated for in vivo efficacy and, therefore, would require further optimization to achieve the desired pharmacokinetic (PK) profile. The successful development of prodrug **33** (Figure 7), which improved the oral bioavailability of parent PROTAC from 1 to 68%, suggests that PROTACs can be optimized for PK [81]. Presently, the PROTACs ARV-471 and ARV-110 are in Phase II clinical trials, and the PROTACs KT-474 and NX-2127 are in phase I clinical trials. These early clinical candidates provide the scientific community with the necessary drive to continue to pursue PROTACs as an alternative therapeutic strategy. Future studies will focus on developing disease-specific PROTACs either by exploring novel disease-specific POI and/or using ligands that target tissue-specific E3 ligases. Alternative strategies to achieve tissue specificity could include developing antibody PROTAC conjugates for targeted delivery to the tumors [67]. The recent discovery of molecular glues, which are small molecule inhibitors with minor modification of surface-exposed regions, for targeted protein degradation [151,152], also indicates a bright future for these therapeutic modalities that exploit the cellular protein degradation machinery.

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References

1. Duong-Ly, K.C.; Peterson, J.R. The human kinome and kinase inhibition. *Curr. Protoc. Pharmacol.* **2013**, *60*, 2–9. [CrossRef]
2. Gao, R.; Stock, A.M. Biological insights from structures of two-component proteins. *Annu. Rev. Microbiol.* **2009**, *63*, 133–154. [CrossRef]
3. Ferris, H.U.; Dunin-Horkawicz, S.; Hornig, N.; Hulko, M.; Martin, J.; Schultz, J.E.; Zeth, K.; Lupas, A.N.; Coles, M. Mechanism of regulation of receptor histidine kinases. *Structure* **2012**, *20*, 56–66. [CrossRef]
4. Bhullar, K.S.; Lagaron, N.O.; McGowan, E.M.; Parmar, I.; Jha, A.; Hubbard, B.P.; Rupasinghe, H.P.V. Kinase-targeted cancer therapies: Progress, challenges and future directions. *Mol. Cancer* **2018**, *17*, 48. [CrossRef]
5. Gross, S.; Rahal, R.; Stransky, N.; Lengauer, C.; Hoeflich, K.P. Targeting cancer with kinase inhibitors. *J. Clin. Investig.* **2015**, *125*, 1780–1789. [CrossRef]
6. Benn, C.L.; Dawson, L.A. Clinically Precedented Protein Kinases: Rationale for Their Use in Neurodegenerative Disease. *Front. Aging Neurosci.* **2020**, *12*, 242. [CrossRef]
7. Roskoski, R., Jr. Properties of FDA-approved small molecule protein kinase inhibitors: A 2021 update. *Pharmacol. Res.* **2021**, *165*, 105463. [CrossRef]
8. Modi, V.; Dunbrack, R.L., Jr. Defining a new nomenclature for the structures of active and inactive kinases. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 6818–6827. [CrossRef]
9. Bernetti, M.; Masetti, M.; Rocchia, W.; Cavalli, A. Kinetics of Drug Binding and Residence Time. *Annu. Rev. Phys. Chem.* **2019**, *70*, 143–171. [CrossRef]
10. Potashman, M.H.; Duggan, M.E. Covalent modifiers: An orthogonal approach to drug design. *J. Med. Chem.* **2009**, *52*, 1231–1246. [CrossRef]
11. Abdeldayem, A.; Raouf, Y.S.; Constantinescu, S.N.; Moriggl, R.; Gunning, P.T. Advances in covalent kinase inhibitors. *Chem. Soc. Rev.* **2020**, *49*, 2617–2687. [CrossRef]
12. Malumbres, M.; Barbacid, M. Cell cycle, CDKs and cancer: A changing paradigm. *Nat. Rev. Cancer* **2009**, *9*, 153–166. [CrossRef]

13. Sonawane, Y.A.; Taylor, M.A.; Napoleon, J.V.; Rana, S.; Contreras, J.I.; Natarajan, A. Cyclin Dependent Kinase 9 Inhibitors for Cancer Therapy. *J. Med. Chem.* **2016**, *59*, 8667–8684. [CrossRef]

14. Robb, C.M.; Kour, S.; Contreras, J.I.; Agarwal, E.; Barger, C.J.; Rana, S.; Sonawane, Y.; Neilsen, B.K.; Taylor, M.; Kizhake, S.; et al. Characterization of CDK(5) inhibitor, 20-223 (aka CP668863) for colorectal cancer therapy. *Oncotarget* **2018**, *9*, 5216–5232. [CrossRef]

15. Chi, Y.; Carter, J.H.; Swanger, J.; Mazin, A.V.; Moritz, R.L.; Clurman, B.E. A novel landscape of nuclear human CDK2 substrates revealed by in situ phosphorylation. *Sci. Adv.* **2020**, *6*, eaaz9899. [CrossRef] [PubMed]

16. Loyer, P.; Trembley, J.H.; Katona, R.; Kidd, V.J.; Lahti, J.M. Role of CDK/cyclin complexes in transcription and RNA splicing. *Cell. Signal.* **2005**, *17*, 1033–1051. [CrossRef]

17. Kour, S.; Rana, S.; Contreras, J.I.; King, H.M.; Robb, C.M.; Sonawane, Y.A.; Bendjennat, M.; Crawford, A.J.; Barger, C.J.; Kizhake, S.; et al. CDK5 Inhibitor Downregulates Mcl-1 and Sensitizes Pancreatic Cancer Cell Lines to Navitoclax. *Mol. Pharmacol.* **2019**, *96*, 419–429. [CrossRef] [PubMed]

18. Finn, R.S.; Dering, J.; Conklin, D.; Kalous, O.; Cohen, D.J.; Desai, A.J.; Ginther, C.; Atefi, M.; Chen, I.; Fowst, C.; et al. PD 0332991, a selective cyclin D kinase 4/6 inhibitor, preferentially inhibits proliferation of luminal estrogen receptor-positive human breast cancer cell lines in vitro. *Breast Cancer Res.* **2009**, *11*, R77. [CrossRef]

19. Rader, J.; Russell, M.R.; Hart, L.S.; Nakazawa, M.S.; Belcastro, L.T.; Martinez, D.; Li, Y.; Carpenter, E.L.; Attiyeh, E.F.; Diskin, S.J.; et al. Dual CDK4/CDK6 inhibition induces cell-cycle arrest and senescence in neuroblastoma. *Clin. Cancer Res.* **2013**, *19*, 6173–6182. [CrossRef]

20. Corona, S.P.; Generali, D. Abemaciclib: A CDK4/6 inhibitor for the treatment of HR+/HER2- advanced breast cancer. *Drug Des. Dev. Ther.* **2018**, *12*, 321–330. [CrossRef] [PubMed]

21. ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ (accessed on 31 March 2021).

22. Sakamoto, K.M.; Kim, K.B.; Kumagai, A.; Mercurio, F.; Crews, C.M.; Deshaies, R.J. Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 8554–8559. [CrossRef] [PubMed]

23. Cromm, P.M.; Samarasinghe, K.T.G.; Hines, J.; Crews, C.M. Addressing Kinase-Independent Functions of Fak via PROTAC-Mediated Degradation. *J. Am. Chem. Soc.* **2018**, *140*, 17019–17026. [CrossRef]

24. Burslem, G.M.; Smith, B.E.; Lai, A.C.; Jaime-Figueroa, S.; McQuaid, D.C.; Bondeson, D.P.; Toure, M.; Dong, H.; Qian, Y.; Wang, J.; et al. The Advantages of Targeted Protein Degradation Over Inhibition: An RTK Case Study. *Cell Chem. Biol.* **2018**, *25*, 67–77. [CrossRef]

25. Wang, S.; Han, L.; Han, J.; Li, P.; Ding, Q.; Zhang, Q.-J.; Liu, Z.-P.; Chen, C.; Yu, Y. Uncoupling of PARP1 trapping and inhibition using selective PARP1 degradation. *Nat. Chem. Biol.* **2019**, *15*, 1223–1231. [CrossRef]

26. Burslem, G.M.; Song, J.; Chen, X.; Hines, J.; Crews, C.M. Enhancing Antiproliferative Activity and Selectivity of a FLT-3 Inhibitor by Proteolysis Targeting Chimera Conversion. *J. Am. Chem. Soc.* **2018**, *140*, 16428–16432. [CrossRef]

27. Bai, L.; Zhou, H.; Xu, R.; Zhao, Y.; Chinnaswamy, K.; McEachern, D.; Chen, J.; Yang, C.-Y.; Liu, Z.; Wang, M.; et al. A Potent and Selective Small-Molecule Degrader of STAT3 Achieves Complete Tumor Regression In Vivo. *Cancer Cell* **2019**, *36*, 498–511. [CrossRef] [PubMed]

28. Han, X.; Wang, C.; Qin, C.; Xiang, W.; Fernandez-Salas, E.; Yang, C.-Y.; Wang, M.; Zhao, L.; Xu, T.; Chinnaswamy, K.; et al. Discovery of ARD-69 as a Highly Potent Proteolysis Targeting Chimera (PROTAC) Degrader of Androgen Receptor (AR) for the Treatment of Prostate Cancer. *J. Med. Chem.* **2019**, *62*, 941–964. [CrossRef] [PubMed]

29. He, Y.; Zhang, X.; Chang, J.; Kim, H.-N.; Zhang, P.; Wang, Y.; Khan, S.; Liu, X.; Zhang, X.; Lv, D.; et al. Using proteolysis-targeting chimera technology to reduce navitoclax platelet toxicity and improve its senolytic activity. *Nat. Commun.* **2020**, *11*, 1996. [CrossRef]

30. Hu, J.; Hu, B.; Wang, M.; Xu, F.; Miao, B.; Yang, C.-Y.; Wang, M.; Liu, Z.; Hayes, D.F.; Chinnaswamy, K.; et al. Discovery of ERD-308 as a Highly Potent Proteolysis Targeting Chimera (PROTAC) Degrader of Estrogen Receptor (ER). *J. Med. Chem.* **2019**, *62*, 1420–1442. [CrossRef]

31. Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H. Identification of a Primary Target of Thalidomide Teratogenicity. *Science* **2010**, *327*, 1345–1350. [CrossRef]

32. Fischer, E.S.; Boehm, K.; Lydeard, J.R.; Yang, H.; Stadler, M.B.; Cavadini, S.; Nagel, J.; Serluca, F.; Acker, V.; Lingaraju, G.M.; et al. Structure of the DDBI-CRBN E3 ubiquitin ligase in complex with thalidomide. *Nature* **2014**, *512*, 49–53. [CrossRef]

33. Buckley, D.L.; Gustafson, J.L.; Van Molle, I.; Roth, A.G.; Tae, H.S.; Gareiss, P.C.; Jorgensen, W.L.; Ciulli, A.; Crews, C.M. Small-Molecule Inhibitors of the Interaction between the E3 Ligase VHL and HIF1a. *Angew. Chem. Int. Ed.* **2012**, *51*, 11463–11467. [CrossRef]

34. Buckley, D.L.; Van Molle, I.; Gareiss, P.C.; Tae, H.S.; Michel, J.; Noblin, D.J.; Jorgensen, W.L.; Ciulli, A.; Crews, C.M. Targeting the von Hippel-Lindau E3 Ubiquitin Ligase Using Small Molecules To Disrupt the VHL/HIF-1 alpha Interaction. *J. Am. Chem. Soc.* **2012**, *134*, 4465–4468. [CrossRef] [PubMed]

35. Galdeano, C.; Gadd, M.S.; Soares, P.; Scaffidi, S.; Van Molle, I.; Birced, I.; Hewitt, S.; Dias, D.M.; Ciulli, A. Structure-guided design and optimization of small molecules targeting the protein-protein interaction between the von Hippel-Lindau (VHL) E3 ubiquitin ligase and the hypoxia inducible factor (HIF) alpha subunit with in vitro nanomolar affinities. *J. Med. Chem.* **2014**, *57*, 8657–8663. [CrossRef]

36. Hines, J.; Lartigue, S.; Dong, H.; Qian, Y.; Crews, C.M. MDM2-Recruiting PROTAC Offers Superior, Synergistic Antiproliferative Activity via Simultaneous Degradation of BRD4 and Stabilization of p53. *Cancer Res.* **2019**, *79*, 251–262. [CrossRef]

37. Tovar, C.; Rosinski, J.; Filipovic, Z.; Higgins, B.; Kolinsky, K.; Hilton, H.; Zhao, X.L.; Vu, B.T.; Qing, W.G.; Packman, K.; et al. Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: Implications for therapy. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 1888–1893. [CrossRef]

38. Wang, S.; Zhao, Y.; Aguilar, A.; Bernard, D.; Yang, C.-Y. Targeting the MDM2-p53 Protein-Protein Interaction for New Cancer Therapy: Progress and Challenges. *Cold Spring Harb. Perspect. Med.* **2017**, *7*, a026245. [CrossRef] [PubMed]

39. Shibata, N.; Nagai, K.; Morita, Y.; Ujikawa, O.; Ohoka, N.; Hattori, T.; Koyama, R.; Sano, O.; Imaeda, Y.; Nara, H.; et al. Development of Protein Degradation Inducers of Androgen Receptor by Conjugation of Androgen Receptor Ligands and Inhibitor of Apoptosis Protein Ligands. *J. Med. Chem.* **2018**, *61*, 543–575. [CrossRef]

40. Tong, B.; Spradlin, J.N.; Novaes, L.F.T.; Zhang, E.; Hu, X.; Moeller, M.; Brittain, S.M.; McGregor, L.M.; McKenna, J.M.; Tallarico, J.A.; et al. A Nimbolide-Based Kinase Degrader Preferentially Degrades Oncogenic BCR-ABL. *ACS Chem. Biol.* **2020**, *15*, 1788–1794. [CrossRef] [PubMed]

41. Luo, M.; Spradlin, J.N.; Boike, L.; Tong, B.; Brittain, S.M.; McKenna, J.M.; Tallarico, J.A.; Schirle, M.; Maimone, T.J.; Nomura, D.K. Chemoproteomics-enabled discovery of covalent RNF114-based degraders that mimic natural product function. *Cell Chem. Biol.* **2021**, *28*, 559–566. [CrossRef]

42. Li, L.; Mi, D.; Pei, H.; Duan, Q.; Wang, X.; Zhou, W.; Jin, J.; Li, D.; Liu, M.; Chen, Y. In vivo target protein degradation induced by PROTACs based on E3 ligase DCAF15. *Signal Transduct. Target. Ther.* **2020**, *5*, 129. [CrossRef]

43. Zhang, X.; Crowley, V.M.; Wucherpfennig, T.G.; Dix, M.M.; Cravatt, B.F. Electrophilic PROTACs that degrade nuclear proteins by engaging DCAF16. *Nat. Chem. Biol.* **2019**, *15*, 737–746. [CrossRef] [PubMed]

44. Henning, N.J.; Manford, A.G.; Spradlin, J.N.; Brittain, S.M.; McKenna, J.M.; Tallarico, J.A.; Schirle, M.; Rape, M.; Nomura, D.K. Discovery of a Covalent FEM1B Recruiter for Targeted Protein Degradation Applications. *bioRxiv* **2021**. [CrossRef]

45. An, Z.; Lv, W.; Su, S.; Wu, W.; Rao, Y. Developing potent PROTACs tools for selective degradation of HDAC6 protein. *Protein Cell* **2019**, *10*, 606–609. [CrossRef] [PubMed]

46. Brand, M.; Jiang, B.; Bauer, S.; Donovan, K.A.; Liang, Y.; Wang, E.S.; Nowak, R.P.; Yuan, J.C.; Zhang, T.; Kwiatkowski, N.; et al. Homolog-Selective Degradation as a Strategy to Probe the Function of CDK6 in AML. *Cell Chem. Biol.* **2019**, *26*, 300–306. [CrossRef]

47. Burslem, G.M.; Schultz, A.R.; Bondeson, D.P.; Eide, C.A.; Stevens, S.L.S.; Druker, B.J.; Crews, C.M. Targeting BCR-ABL1 in Chronic Myeloid Leukemia by PROTAC-Mediated Targeted Protein Degradation. *Cancer Res.* **2019**, *79*, 4744–4753. [CrossRef]

48. Cance, W.G.; Kurenova, E.; Marlowe, T.; Golubovskaya, V. Disrupting the Scaffold to Improve Focal Adhesion Kinase-Targeted Cancer Therapeutics. *Sci. Signal.* **2013**, *6*, pe10. [CrossRef]

49. Crew, A.P.; Raina, K.; Dong, H.; Qian, Y.; Wang, J.; Vigil, D.; Serebrenik, Y.V.; Hamman, B.D.; Morgan, A.; Ferraro, C.; et al. Identification and Characterization of Von Hippel-Lindau-Recruiting Proteolysis Targeting Chimeras (PROTACs) of TANK-Binding Kinase 1. *J. Med. Chem.* **2018**, *61*, 583–598. [CrossRef]

50. Demizu, Y.; Okuhira, K.; Motoi, H.; Ohno, A.; Shoda, T.; Fukuhara, K.; Okuda, H.; Naito, M.; Kurihara, M. Design and synthesis of estrogen receptor degradation inducer based on a protein knockdown strategy. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1793–1796. [CrossRef]

51. Demizu, Y.; Shibata, N.; Hattori, T.; Ohoka, N.; Motoi, H.; Misawa, T.; Shoda, T.; Naito, M.; Kurihara, M. Development of BCR-ABL degradation inducers via the conjugation of an imatinib derivative and a cIAP1 ligand. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4865–4869. [CrossRef] [PubMed]

52. Farnaby, W.; Koegl, M.; Roy, M.J.; Whitworth, C.; Diers, E.; Trainor, N.; Zollman, D.; Steurer, S.; Karolyi-Oezguer, J.; Riedmueller, C.; et al. BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat. Chem. Biol.* **2019**, *15*, 672–680. [CrossRef]

53. Fry, D.W.; Harvey, P.J.; Keller, P.R.; Elliott, W.L.; Meade, M.A.; Trachet, E.; Albassam, M.; Zheng, X.X.; Leopold, W.R.; Pryer, N.K.; et al. Specific inhibition of cyclin-dependent kinase 4/6 by PD 0332991 and associated antitumor activity in human tumor xenografts. *Mol. Cancer Ther.* **2004**, *3*, 1427–1437. [PubMed]

54. Gabizon, R.; Shraga, A.; Gehrtz, P.; Livnah, E.; Shorer, Y.; Gurwicz, N.; Avram, L.; Unger, T.; Aharoni, H.; Albeck, S.; et al. Efficient Targeted Degradation via Reversible and Irreversible Covalent PROTACs. *J. Am. Chem. Soc.* **2020**, *142*, 11734–11742. [CrossRef]

55. Gechijian, L.N.; Buckley, D.L.; Lawlor, M.A.; Reyes, J.M.; Paulk, J.; Ott, C.J.; Winter, G.E.; Erb, M.A.; Scott, T.G.; Xu, M.; et al. Functional TRIM24 degrader via conjugation of ineffectual bromodomain and VHL ligands. *Nat. Chem. Biol.* **2018**, *14*, 405–412. [CrossRef]

56. Han, T.; Goralski, M.; Gaskill, N.; Capota, E.; Kim, J.; Ting, T.C.; Xie, Y.; Williams, N.S.; Nijhawan, D. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science* **2017**, *356*, eaal3755. [CrossRef] [PubMed]

57. Han, X.; Zhao, L.; Xiang, W.; Qin, C.; Miao, B.; Xu, T.; Wang, M.; Yang, C.-Y.; Chinnaswamy, K.; Stuckey, J.; et al. Discovery of Highly Potent and Efficient PROTAC Degraders of Androgen Receptor (AR) by Employing Weak Binding Affinity VHL E3 Ligase Ligands. *J. Med. Chem.* **2019**, *62*, 11218–11231. [CrossRef] [PubMed]

58. Hsu, J.H.-R.; Rasmusson, T.; Robinson, J.; Pachl, F.; Read, J.; Kawatkar, S.; O'Donovan, D.H.; Bagal, S.; Code, E.; Rawlins, P.; et al. EED-Targeted PROTACs Degrade EED, EZH2, and SUZ12 in the PRC2 Complex. *Cell Chem. Biol.* **2020**, *27*, 41–46. [CrossRef]

59. Humphreys, P.G.; Bamborough, P.; Chung, C.-w.; Crags, P.D.; Gordon, L.; Grandi, P.; Hayhow, T.G.; Hussain, J.; Jones, K.L.; Lindon, M.; et al. Discovery of a Potent, Cell Penetrant, and Selective p300/CBP-Associated Factor (PCAF)/General Control Nonderepressible 5 (GCN5) Bromodomain Chemical Probe. *J. Med. Chem.* **2017**, *60*, 695–709. [CrossRef] [PubMed]

60. Jiang, B.; Wang, E.S.; Donovan, K.A.; Liang, Y.; Fischer, E.S.; Zhang, T.; Gray, N.S. Development of Dual and Selective Degraders of Cyclin-Dependent Kinases 4 and 6. *Angew. Chem. Int. Ed.* **2019**, *58*, 6321–6326. [CrossRef]

61. Khan, S.; Zhang, X.; Lv, D.; Zhang, Q.; He, Y.; Zhang, P.; Liu, X.; Thummuri, D.; Yuan, Y.; Wiegand, J.S.; et al. A selective BCL-X-L PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* **2019**, *25*, 1938–1947. [CrossRef]

62. Robb, C.M.; Contreras, J.I.; Kour, S.; Taylor, M.A.; Abid, M.; Sonawane, Y.A.; Zahid, M.; Murry, D.J.; Natarajan, A.; Rana, S. Chemically induced degradation of CDK9 by a proteolysis targeting chimera (PROTAC). *Chem. Commun.* **2017**, *53*, 7577–7580. [CrossRef] [PubMed]

63. Bondeson, D.P.; Smith, B.E.; Burslem, G.M.; Buhimschi, A.D.; Hines, J.; Jaime-Figueroa, S.; Wang, J.; Hamman, B.D.; Ishchenko, A.; Crews, C.M. Lessons in PROTAC Design from Selective Degradation with a Promiscuous Warhead. *Cell Chem. Biol.* **2018**, *25*, 78–87. [CrossRef] [PubMed]

64. Huang, H.-T.; Dobrovolsky, D.; Paulk, J.; Yang, G.; Weisberg, E.L.; Doctor, Z.M.; Buckley, D.L.; Cho, J.-H.; Ko, E.; Jang, J.; et al. A Chemoproteomic Approach to Query the Degradable Kinome Using a Multi-kinase Degrader. *Cell Chem. Biol.* **2018**, *25*, 88–99. [CrossRef] [PubMed]

65. Bond, M.J.; Chu, L.; Nalawansha, D.A.; Li, K.; Crews, C.M. Targeted Degradation of Oncogenic KRAS(G12C) by VHL-Recruiting PROTACs. *ACS Cent. Sci.* **2020**, *6*, 1367–1375. [CrossRef]

66. Zeng, M.; Xiong, Y.; Safaee, N.; Nowak, R.P.; Donovan, K.A.; Yuan, C.J.; Nabet, B.; Gero, T.W.; Feru, F.; Li, L.; et al. Exploring Targeted Degradation Strategy for Oncogenic KRAS(G12C). *Cell Chem. Biol.* **2020**, *27*, 19–31 e16. [CrossRef]

67. Maneiro, M.; Forte, N.; Shchepinova, M.M.; Kounde, C.S.; Chudasama, V.; Baker, J.R.; Tate, E.W. Antibody-PROTAC Conjugates Enable HER2-Dependent Targeted Protein Degradation of BRD4. *ACS Chem. Biol.* **2020**, *15*, 1306–1312. [CrossRef]

68. Tadesse, S.; Anshabo, A.T.; Portman, N.; Lim, E.; Tilley, W.; Caldon, C.E.; Wang, S. Targeting CDK2 in cancer: Challenges and opportunities for therapy. *Drug Discov. Today* **2020**, *25*, 406–413. [CrossRef]

69. Otto, T.; Sicinski, P. Cell cycle proteins as promising targets in cancer therapy. *Nat. Rev. Cancer* **2017**, *17*, 93–115. [CrossRef]

70. Ma, T.; Van Tine, B.A.; Wei, Y.; Garrett, M.D.; Nelson, D.; Adams, P.D.; Wang, J.; Qin, J.; Chow, L.T.; Harper, J.W. Cell cycle-regulated phosphorylation of p220(NPAT) by cyclin E/Cdk2 in Cajal bodies promotes histone gene transcription. *Genes Dev.* **2000**, *14*, 2298–2313. [CrossRef]

71. Okuda, M.; Horn, H.F.; Tarapore, P.; Tokuyama, Y.; Smulian, A.G.; Chan, P.K.; Knudsen, E.S.; Hofmann, I.A.; Snyder, J.D.; Bove, K.E.; et al. Nucleophosmin/B23 is a target of CDK2/cyclin E in centrosome duplication. *Cell* **2000**, *103*, 127–140. [CrossRef]

72. Scaltriti, M.; Eichhorn, P.J.; Cortes, J.; Prudkin, L.; Aura, C.; Jimenez, J.; Chandarlapaty, S.; Serra, V.; Prat, A.; Ibrahim, Y.H.; et al. Cyclin E amplification/overexpression is a mechanism of trastuzumab resistance in HER2+ breast cancer patients. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 3761–3766. [CrossRef]

73. Etemadmoghadam, D.; Weir, B.A.; Au-Yeung, G.; Alsop, K.; Mitchell, G.; George, J.; Australian Ovarian Cancer Study Group; Davis, S.; D'Andrea, A.D.; Simpson, K.; et al. Synthetic lethality between CCNE1 amplification and loss of BRCA1. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 19489–19494. [CrossRef] [PubMed]

74. Handa, K.; Yamakawa, M.; Takeda, H.; Kimura, S.; Takahashi, T. Expression of cell cycle markers in colorectal carcinoma: Superiority of cyclin A as an indicator of poor prognosis. *Int. J. Cancer* **1999**, *84*, 225–233. [CrossRef]

75. Michalides, R.; van Tinteren, H.; Balkenende, A.; Vermorken, J.B.; Benraadt, J.; Huldij, J.; van Diest, P. Cyclin A is a prognostic indicator in early stage breast cancer with and without tamoxifen treatment. *Br. J. Cancer* **2002**, *86*, 402–408. [CrossRef]

76. Han, Y.; Wei, Y.; Yao, J.; Chu, Y.Y.; Li, C.W.; Hsu, J.L.; Nie, L.; Hung, M.C. Inhibition of CDK2 reduces EZH2 phosphorylation and reactivates ERα expression in high-grade serous ovarian carcinoma. *Am. J. Cancer Res.* **2020**, *10*, 1194–1206. [PubMed]

77. Ying, M.; Shao, X.; Jing, H.; Liu, Y.; Qi, X.; Cao, J.; Chen, Y.; Xiang, S.; Song, H.; Hu, R.; et al. Ubiquitin-dependent degradation of CDK2 drives the therapeutic differentiation of AML by targeting PRDX2. *Blood* **2018**, *131*, 2698–2711. [CrossRef]

78. Berthet, C.; Aleem, E.; Coppola, V.; Tessarollo, L.; Kaldis, P. Cdk2 knockout mice are viable. *Curr. Biol.* **2003**, *13*, 1775–1785. [CrossRef] [PubMed]

79. Tadesse, S.; Caldon, E.C.; Tilley, W.; Wang, S. Cyclin-Dependent Kinase 2 Inhibitors in Cancer Therapy: An Update. *J. Med. Chem.* **2019**, *62*, 4233–4251. [CrossRef] [PubMed]

80. Teng, M.; Jiang, J.; He, Z.; Kwiatkowski, N.P.; Donovan, K.A.; Mills, C.E.; Victor, C.; Hatcher, J.M.; Fischer, E.S.; Sorger, P.K.; et al. Development of CDK2 and CDK5 Dual Degrader TMX-2172. *Angew. Chem. Int. Ed. Engl.* **2020**, *59*, 13865–13870. [CrossRef] [PubMed]

81. Wei, M.; Zhao, R.; Cao, Y.; Wei, Y.; Li, M.; Dong, Z.; Liu, Y.; Ruan, H.; Li, Y.; Cao, S.; et al. First orally bioavailable prodrug of proteolysis targeting chimera (PROTAC) degrades cyclin-dependent kinases 2/4/6 in vivo. *Eur. J. Med. Chem.* **2021**, *209*, 112903. [CrossRef]

82. Wang, L.; Shao, X.; Zhong, T.; Wu, Y.; Xu, A.; Sun, X.; Gao, H.; Liu, Y.; Lan, T.; Tong, Y.; et al. Discovery of a first-in-class CDK2 selective degrader for AML differentiation therapy. *Nat. Chem. Biol.* **2021**, *17*, 567–575. [CrossRef]

83. Corces, M.R.; Chang, H.Y.; Majeti, R. Preleukemic Hematopoietic Stem Cells in Human Acute Myeloid Leukemia. *Front. Oncol.* **2017**, *7*, 263. [CrossRef]

84. O'Leary, B.; Finn, R.S.; Turner, N.C. Treating cancer with selective CDK4/6 inhibitors. *Nat. Rev. Clin. Oncol.* **2016**, *13*, 417–430. [CrossRef]

85. Schmidt, E.E.; Ichimura, K.; Reifenberger, G.; Collins, V.P. CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. *Cancer Res.* **1994**, *54*, 6321–6324.

86. Puyol, M.; Martín, A.; Dubus, P.; Mulero, F.; Pizcueta, P.; Khan, G.; Guerra, C.; Santamaría, D.; Barbacid, M. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell* **2010**, *18*, 63–73. [CrossRef] [PubMed]

87. Yang, C.; Li, Z.; Bhatt, T.; Dickler, M.; Giri, D.; Scaltriti, M.; Baselga, J.; Rosen, N.; Chandarlapaty, S. Acquired CDK6 amplification promotes breast cancer resistance to CDK4/6 inhibitors and loss of ER signaling and dependence. *Oncogene* **2017**, *36*, 2255–2264. [CrossRef]

88. Zhao, B.; Burgess, K. PROTACs suppression of CDK4/6, crucial kinases for cell cycle regulation in cancer. *Chem. Commun.* **2019**, *55*, 2704–2707. [CrossRef] [PubMed]

89. An, S.; Fu, L. Small-molecule PROTACs: An emerging and promising approach for the development of targeted therapy drugs. *EBioMedicine* **2018**, *36*, 553–562. [CrossRef] [PubMed]

90. Rana, S.; Bendjennat, M.; Kour, S.; King, H.M.; Kizhake, S.; Zahid, M.; Natarajan, A. Selective degradation of CDK6 by a palbociclib based PROTAC. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1375–1379. [CrossRef]

91. Lu, H.; Schulze-Gahmen, U. Toward understanding the structural basis of cyclin-dependent kinase 6 specific inhibition. *J. Med. Chem.* **2006**, *49*, 3826–3831. [CrossRef]

92. Su, S.; Yang, Z.; Gao, H.; Yang, H.; Zhu, S.; An, Z.; Wang, J.; Li, Q.; Chandarlapaty, S.; Deng, H.; et al. Potent and Preferential Degradation of CDK6 via Proteolysis Targeting Chimera Degraders. *J. Med. Chem.* **2019**, *62*, 7575–7582. [CrossRef] [PubMed]

93. Anderson, N.A.; Cryan, J.; Ahmed, A.; Dai, H.; McGonagle, G.A.; Rozier, C.; Benowitz, A.B. Selective CDK6 degradation mediated by cereblon, VHL, and novel IAP-recruiting PROTACs. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127106. [CrossRef]

94. Steinebach, C.; Ng, Y.L.D.; Sosic, I.; Lee, C.S.; Chen, S.; Lindner, S.; Vu, L.P.; Bricelj, A.; Haschemi, R.; Monschke, M.; et al. Systematic exploration of different E3 ubiquitin ligases: An approach towards potent and selective CDK6 degraders. *Chem. Sci.* **2020**, *11*, 3474–3486. [CrossRef]

95. Firestein, R.; Shima, K.; Nosho, K.; Irahara, N.; Baba, Y.; Bojarski, E.; Giovannucci, E.L.; Hahn, W.C.; Fuchs, C.S.; Ogino, S. CDK8 expression in 470 colorectal cancers in relation to beta-catenin activation, other molecular alterations and patient survival. *Int. J. Cancer* **2010**, *126*, 2863–2873. [CrossRef] [PubMed]

96. Firestein, R.; Bass, A.J.; Kim, S.Y.; Dunn, I.F.; Silver, S.J.; Guney, I.; Freed, E.; Ligon, A.H.; Vena, N.; Ogino, S.; et al. CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. *Nature* **2008**, *455*, 547–551. [CrossRef] [PubMed]

97. Donner, A.J.; Szostek, S.; Hoover, J.M.; Espinosa, J.M. CDK8 is a stimulus-specific positive coregulator of p53 target genes. *Mol. Cell* **2007**, *27*, 121–133. [CrossRef]

98. Fryer, C.J.; White, J.B.; Jones, K.A. Mastermind recruits CycC:CDK8 to phosphorylate the Notch ICD and coordinate activation with turnover. *Mol. Cell* **2004**, *16*, 509–520. [CrossRef]

99. Alarcón, C.; Zaromytidou, A.I.; Xi, Q.; Gao, S.; Yu, J.; Fujisawa, S.; Barlas, A.; Miller, A.N.; Manova-Todorova, K.; Macias, M.J.; et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways. *Cell* **2009**, *139*, 757–769. [CrossRef] [PubMed]

100. Philip, S.; Kumarasiri, M.; Teo, T.; Yu, M.; Wang, S. Cyclin-Dependent Kinase 8: A New Hope in Targeted Cancer Therapy? *J. Med. Chem.* **2018**, *61*, 5073–5092. [CrossRef]

101. Menzl, I.; Witalisz-Siepracka, A.; Sexl, V. CDK8-Novel Therapeutic Opportunities. *Pharmaceuticals* **2019**, *12*, 92. [CrossRef]

102. Dannappel, M.V.; Sooraj, D.; Loh, J.J.; Firestein, R. Molecular and in vivo Functions of the CDK8 and CDK19 Kinase Modules. *Front. Cell Dev. Biol.* **2018**, *6*, 171. [CrossRef]

103. Hatcher, J.M.; Wang, E.S.; Johannessen, L.; Kwiatkowski, N.; Sim, T.; Gray, N.S. Development of Highly Potent and Selective Steroidal Inhibitors and Degraders of CDK8. *ACS Med. Chem. Lett.* **2018**, *9*, 540–545. [CrossRef]

104. Nicolaou, K.C.; Sun, Y.P.; Peng, X.S.; Polet, D.; Chen, D.Y. Total synthesis of (+)-cortistatin A. *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 7310–7313. [CrossRef]

105. Shenvi, R.A.; Guerrero, C.A.; Shi, J.; Li, C.C.; Baran, P.S. Synthesis of (+)-cortistatin A. *J. Am. Chem. Soc.* **2008**, *130*, 7241–7243. [CrossRef]

106. Simmons, E.M.; Hardin-Narayan, A.R.; Guo, X.; Sarpong, R. Formal total synthesis of (+/−)-cortistatin A. *Tetrahedron* **2010**, *66*, 4696–4700. [CrossRef]

107. Pelish, H.E.; Liau, B.B.; Nitulescu, I.I.; Tangpeerachaikul, A.; Poss, Z.C.; Da Silva, D.H.; Caruso, B.T.; Arefolov, A.; Fadeyi, O.; Christie, A.L.; et al. Mediator kinase inhibition further activates super-enhancer-associated genes in AML. *Nature* **2015**, *526*, 273–276. [CrossRef]

108. Albert, T.K.; Rigault, C.; Eickhoff, J.; Baumgart, K.; Antrecht, C.; Klebl, B.; Mittler, G.; Meisterernst, M. Characterization of molecular and cellular functions of the cyclin-dependent kinase CDK9 using a novel specific inhibitor. *Br. J. Pharmacol.* **2014**, *171*, 55–68. [CrossRef]

109. Yin, T.; Lallena, M.J.; Kreklau, E.L.; Fales, K.R.; Carballares, S.; Torrres, R.; Wishart, G.N.; Ajamie, R.T.; Cronier, D.M.; Iversen, P.W.; et al. A novel CDK9 inhibitor shows potent antitumor efficacy in preclinical hematologic tumor models. *Mol. Cancer Ther.* **2014**, *13*, 1442–1456. [CrossRef]

110. Contreras, J.I.; Robb, C.M.; King, H.M.; Baxter, J.; Crawford, A.J.; Kour, S.; Kizhake, S.; Sonawane, Y.A.; Rana, S.; Hollingsworth, M.A.; et al. Chemical Genetic Screens Identify Kinase Inhibitor Combinations that Target Anti-Apoptotic Proteins for Cancer Therapy. *ACS Chem. Biol.* **2018**, *13*, 1148–1152. [CrossRef]

111. Olson, C.M.; Jiang, B.; Erb, M.A.; Liang, Y.; Doctor, Z.M.; Zhang, Z.; Zhang, T.; Kwiatkowski, N.; Boukhali, M.; Green, J.L.; et al. Pharmacological perturbation of CDK9 using selective CDK9 inhibition or degradation. *Nat. Chem. Biol.* **2018**, *14*, 163–170. [CrossRef]

112. Bajrami, I.; Frankum, J.R.; Konde, A.; Miller, R.E.; Rehman, F.L.; Brough, R.; Campbell, J.; Sims, D.; Rafiq, R.; Hooper, S.; et al. Genome-wide profiling of genetic synthetic lethality identifies CDK12 as a novel determinant of PARP1/2 inhibitor sensitivity. *Cancer Res.* **2014**, *74*, 287–297. [CrossRef]

113. Franco, L.C.; Morales, F.; Boffo, S.; Giordano, A. CDK9: A key player in cancer and other diseases. *J. Cell Biochem.* **2018**, *119*, 1273–1284. [CrossRef] [PubMed]

114. Rahaman, M.H.; Lam, F.; Zhong, L.; Teo, T.; Adams, J.; Yu, M.; Milne, R.W.; Pepper, C.; Lokman, N.A.; Ricciardelli, C.; et al. Targeting CDK9 for treatment of colorectal cancer. *Mol. Oncol.* **2019**, *13*, 2178–2193. [CrossRef] [PubMed]

115. Wang, J.; Dean, D.C.; Hornicek, F.J.; Shi, H.; Duan, Z. Cyclin-dependent kinase 9 (CDK9) is a novel prognostic marker and therapeutic target in ovarian cancer. *FASEB J.* **2019**, *33*, 5990–6000. [CrossRef]

116. Krystof, V.; Uldrijan, S. Cyclin-dependent kinase inhibitors as anticancer drugs. *Curr. Drug Targets* **2010**, *11*, 291–302. [CrossRef] [PubMed]

117. Rana, S.; Sonawane, Y.A.; Taylor, M.A.; Kizhake, S.; Zahid, M.; Natarajan, A. Synthesis of aminopyrazole analogs and their evaluation as CDK inhibitors for cancer therapy. *Bioorg. Med. Chem. Lett.* **2018**, *28*, 3736–3740. [CrossRef]

118. King, H.M.; Rana, S.; Kubica, S.P.; Mallareddy, J.R.; Kizhake, S.; Ezell, E.L.; Zahid, M.; Naldrett, M.J.; Alvarez, S.; Law, H.C.; et al. Aminopyrazole based CDK9 PROTAC sensitizes pancreatic cancer cells to venetoclax. *Bioorg. Med. Chem. Lett.* **2021**, *43*, 128061. [CrossRef]

119. Lopez, H.; Zhang, L.; George, N.M.; Liu, X.; Pang, X.; Evans, J.J.D.; Targy, N.M.; Luo, X. Perturbation of the Bcl-2 network and an induced Noxa/Bcl-xL interaction trigger mitochondrial dysfunction after DNA damage. *J. Biol. Chem.* **2010**, *285*, 15016–15026. [CrossRef]

120. Rana, S.; Kour, S.; Sonawane, Y.A.; Robb, C.M.; Contreras, J.I.; Kizhake, S.; Zahid, M.; Karpf, A.R.; Natarajan, A. Symbiotic prodrugs (SymProDs) dual targeting of NFkappaB and CDK. *Chem. Biol. Drug Des.* **2020**, *96*, 773–784. [CrossRef]

121. MacCallum, D.E.; Melville, J.; Frame, S.; Watt, K.; Anderson, S.; Gianella-Borradori, A.; Lane, D.P.; Green, S.R. Seliciclib (CYC202, R-Roscovitine) induces cell death in multiple myeloma cells by inhibition of RNA polymerase II-dependent transcription and down-regulation of Mcl-1. *Cancer Res.* **2005**, *65*, 5399–5407. [CrossRef]

122. Abid, M.; Sonawane, Y.A.; Contreras, J.I.; Rana, S.; Natarajan, A. Recent Advances in Cancer Drug Development: Targeting Induced Myeloid Cell Leukemia-1 (Mcl-1) Differentiation Protein. *Curr. Med. Chem.* **2017**, *24*, 4488–4514. [CrossRef] [PubMed]

123. Zhou, F.; Chen, L.; Cao, C.; Yu, J.; Luo, X.; Zhou, P.; Zhao, L.; Du, W.; Cheng, J.; Xie, Y.; et al. Development of selective mono or dual PROTAC degrader probe of CDK isoforms. *Eur. J. Med. Chem.* **2020**, *187*, 111952. [CrossRef] [PubMed]

124. Misra, R.N.; Xiao, H.Y.; Kim, K.S.; Lu, S.; Han, W.C.; Barbosa, S.A.; Hunt, J.T.; Rawlins, D.B.; Shan, W.; Ahmed, S.Z.; et al. N-(cycloalkylamino)acyl-2-aminothiazole inhibitors of cyclin-dependent kinase 2. N-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4- piperidinecarboxamide (BMS-387032), a highly efficacious and selective antitumor agent. *J. Med. Chem.* **2004**, *47*, 1719–1728. [CrossRef]

125. Winter, G.E.; Buckley, D.L.; Paulk, J.; Roberts, J.M.; Souza, A.; Dhe-Paganon, S.; Bradner, J.E. Phthalimide conjugation as a strategy for in vivo target protein degradation. *Science* **2015**, *348*, 1376–1381. [CrossRef]

126. Barsanti, P.A.; Hu, C.; Jeff, J.; Keyes, R.; Kucejko, R.; Xiaodong, L.; Yue, P.; Pfister Kb, S.M.; Sutton, J.; Lifeng, W. Pyridine and Pyrzaine Derivatives as Protein Kinase Modulators. International Patent PCT/JP2008/073864 (WO/2011/012661), 3 February 2011.

127. Polier, G.; Ding, J.; Konkimalla, B.V.; Eick, D.; Ribeiro, N.; Kohler, R.; Giaisi, M.; Efferth, T.; Desaubry, L.; Krammer, P.H.; et al. Wogonin and related natural flavones are inhibitors of CDK9 that induce apoptosis in cancer cells by transcriptional suppression of Mcl-1. *Cell Death Dis.* **2011**, *2*, e182. [CrossRef]

128. Bian, J.; Ren, J.; Li, Y.; Wang, J.; Xu, X.; Feng, Y.; Tang, H.; Wang, Y.; Li, Z. Discovery of Wogonin-based PROTACs against CDK9 and capable of achieving antitumor activity. *Bioorg. Chem.* **2018**, *81*, 373–381. [CrossRef]

129. Chen, R.; Keating, M.J.; Gandhi, V.; Plunkett, W. Transcription inhibition by flavopiridol: Mechanism of chronic lymphocytic leukemia cell death. *Blood* **2005**, *106*, 2513–2519. [CrossRef]

130. Chen, R.; Guo, L.; Chen, Y.; Jiang, Y.; Wierda, W.G.; Plunkett, W. Homoharringtonine reduced Mcl-1 expression and induced apoptosis in chronic lymphocytic leukemia. *Blood* **2011**, *117*, 156–164. [CrossRef]

131. Qiu, X.; Li, Y.; Yu, B.; Ren, J.; Huang, H.; Wang, M.; Ding, H.; Li, Z.; Wang, J.; Bian, J. Discovery of selective CDK9 degraders with enhancing antiproliferative activity through PROTAC conversion. *Eur. J. Med. Chem.* **2021**, *211*, 113091. [CrossRef] [PubMed]

132. Liang, S.; Hu, L.; Wu, Z.; Chen, Z.; Liu, S.; Xu, X.; Qian, A. CDK12: A Potent Target and Biomarker for Human Cancer Therapy. *Cells* **2020**, *9*, 1483. [CrossRef]

133. Chen, H.H.; Wang, Y.C.; Fann, M.J. Identification and characterization of the CDK12/cyclin L1 complex involved in alternative splicing regulation. *Mol. Cell. Biol.* **2006**, *26*, 2736–2745. [CrossRef]

134. Liang, K.; Gao, X.; Gilmore, J.M.; Florens, L.; Washburn, M.P.; Smith, E.; Shilatifard, A. Characterization of human cyclin-dependent kinase 12 (CDK12) and CDK13 complexes in C-terminal domain phosphorylation, gene transcription, and RNA processing. *Mol. Cell. Biol.* **2015**, *35*, 928–938. [CrossRef]

135. Jiang, B.; Gao, Y.; Che, J.; Lu, W.; Kaltheuner, I.H.; Dries, R.; Kalocsay, M.; Berberich, M.J.; Jiang, J.; You, I.; et al. Discovery and resistance mechanism of a selective CDK12 degrader. *Nat. Chem. Biol.* **2021**, *17*, 1057–1064. [CrossRef]

136. den Besten, W.; Lipford, J.R. Prospecting for molecular glues. *Nat. Chem. Biol.* **2020**, *16*, 1157–1158. [CrossRef]

137. Schreiber, S.L. The Rise of Molecular Glues. *Cell* **2021**, *184*, 3–9. [CrossRef]

138. Munn, J.D. Thalidomide and Congenital Malformations. *Can. Med. Assoc. J.* **1962**, *86*, 665. [PubMed]

139. Kronke, J.; Udeshi, N.D.; Narla, A.; Grauman, P.; Hurst, S.N.; McConkey, M.; Svinkina, T.; Heckl, D.; Comer, E.; Li, X.; et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. *Science* **2014**, *343*, 301–305. [CrossRef] [PubMed]

140. Asatsuma-Okumura, T.; Ando, H.; De Simone, M.; Yamamoto, J.; Sato, T.; Shimizu, N.; Asakawa, K.; Yamaguchi, Y.; Ito, T.; Guerrini, L.; et al. p63 is a cereblon substrate involved in thalidomide teratogenicity. *Nat. Chem. Biol.* **2019**, *15*, 1077–1084. [CrossRef]

141. Donovan, K.A.; An, J.; Nowak, R.P.; Yuan, J.C.; Fink, E.C.; Berry, B.C.; Ebert, B.L.; Fischer, E.S. Thalidomide promotes degradation of SALL4, a transcription factor implicated in Duane Radial Ray syndrome. *eLife* **2018**, *7*, e38430. [CrossRef] [PubMed]

142. Matyskiela, M.E.; Lu, G.; Ito, T.; Pagarigan, B.; Lu, C.-C.; Miller, K.; Fang, W.; Wang, N.-Y.; Nguyen, D.; Houston, J.; et al. A novel cereblon modulator recruits GSPT1 to the CRL4(CRBN) ubiquitin ligase. *Nature* **2016**, *535*, 252–257. [CrossRef] [PubMed]

143. Sievers, Q.L.; Petzold, G.; Bunker, R.D.; Renneville, A.; Slabicki, M.; Liddicoat, B.J.; Abdulrahman, W.; Mikkelsen, T.; Ebert, B.L.; Thoma, N.H. Defining the human C2H2 zinc finger degrome targeted by thalidomide analogs through CRBN. *Science* **2018**, *362*, eaat0572. [CrossRef] [PubMed]

144. Zangari, M.; Elice, F.; Tricot, G. Immunomodulatory drugs in multiple myeloma. *Expert Opin. Investig. Drugs* **2005**, *14*, 1411–1418. [CrossRef]

145. Quach, H.; Ritchie, D.; Stewart, A.K.; Neeson, P.; Harrison, S.; Smyth, M.J.; Prince, H.M. Mechanism of action of immunomodulatory drugs (IMiDS) in multiple myeloma. *Leukemia* **2010**, *24*, 22–32. [CrossRef]

146. Bussiere, D.E.; Xie, L.; Srinivas, H.; Shu, W.; Burke, A.; Be, C.; Zhao, J.; Godbole, A.; King, D.; Karki, R.G.; et al. Structural basis of indisulam-mediated RBM39 recruitment to DCAF15 E3 ligase complex. *Nat. Chem. Biol.* **2020**, *16*, 15–23. [CrossRef]

147. Du, X.; Volkov, O.A.; Czerwinski, R.M.; Tan, H.; Huerta, C.; Morton, E.R.; Rizzi, J.P.; Wehn, P.M.; Xu, R.; Nijhawan, D.; et al. Structural Basis and Kinetic Pathway of RBM39 Recruitment to DCAF15 by a Sulfonamide Molecular Glue E7820. *Structure* **2019**, *27*, 1625–1633. [CrossRef]

148. Faust, T.B.; Yoon, H.; Nowak, R.P.; Donovan, K.A.; Li, Z.; Cai, Q.; Eleuteri, N.A.; Zhang, T.; Gray, N.S.; Fischer, E.S. Structural complementarity facilitates E7820-mediated degradation of RBM39 by DCAF15. *Nat. Chem. Biol.* **2020**, *16*, 7–14. [CrossRef]

149. Slabicki, M.; Kozicka, Z.; Petzold, G.; Li, Y.D.; Manojkumar, M.; Bunker, R.D.; Donovan, K.A.; Sievers, Q.L.; Koeppel, J.; Suchyta, D.; et al. The CDK inhibitor CR8 acts as a molecular glue degrader that depletes cyclin K. *Nature* **2020**, *585*, 293–297. [CrossRef] [PubMed]

150. Bettayeb, K.; Oumata, N.; Echalier, A.; Ferandin, Y.; Endicott, J.A.; Galons, H.; Meijer, L. CR8, a potent and selective, roscovitine-derived inhibitor of cyclin-dependent kinases. *Oncogene* **2008**, *27*, 5797–5807. [CrossRef] [PubMed]

151. Slabicki, M.; Yoon, H.; Koeppel, J.; Nitsch, L.; Roy Burman, S.S.; Di Genua, C.; Donovan, K.A.; Sperling, A.S.; Hunkeler, M.; Tsai, J.M.; et al. Small-molecule-induced polymerization triggers degradation of BCL6. *Nature* **2020**, *588*, 164–168. [CrossRef]

152. Rana, S.; Natarajan, A. Small molecule induced polymerization of BCL6 facilitates SIAH1 mediated degradation. *Signal Transduct. Target. Ther.* **2021**, *6*, 142. [CrossRef]