Journal of Medicinal Chemistry

pubs.acs.org/jmc

Perspective

Advancing Strategies for Proteolysis-Targeting Chimera Design

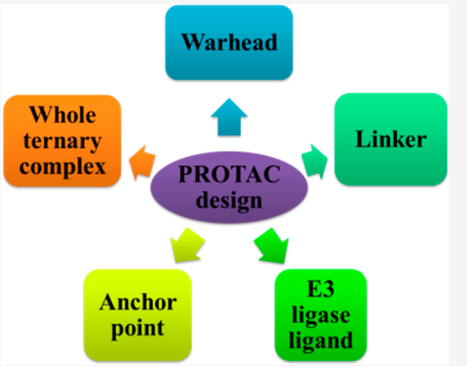
Minglei Li, Ying Zhi,* Bo Liu,* and Qingqiang Yao*

Cite This: J. Med. Chem. 2023, 66, 2308−2329

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Proteolysis-targeting chimeras (PROTACs) have shown great therapeutic potential by degrading various disease-causing proteins, particularly those related to tumors. Therefore, the introduction of PROTACs has ushered in a new chapter of antitumor drug development, marked by significant advances over recent years. Herein, we describe recent developments in PROTAC technology, focusing on design strategy, development workflow, and future outlooks. We also discuss potential opportunities and challenges for PROTAC research.



1. INTRODUCTION

Proteolysis-targeting chimeras (PROTACs), which induce the ubiquitination and degradation of targeted proteins through the ubiquitin−proteasome system, have gained considerable attention for the discovery and development of novel therapeutics.[1] PROTAC technology has been successfully applied to target cytoplasmic, nuclear, and membrane-associated proteins.[2−5] PROTACs have three building blocks: a ligand of the protein of interest (POI; warhead), an E3 ligase ligand, and a linker that tethers the two ligands (Figure 1).

Over the past 6 years, PROTACs have brought unprecedented opportunities to industry and academia (Figure 2). As a major milestone, androgen receptor (AR) degrader compound **1** (ARV-110) and estrogen receptor (ER) degrader compound **2** (ARV-471) have entered phase II clinical trials for the treatment of castration-resistant prostate cancer and ER$^+$/HER2$^-$ breast cancer, respectively (NCT03888612 and NCT04072952) (Figure 3). More than a dozen PROTACs have also entered clinical trials in the last 2 years (Table 1). Currently, two PROTAC databases exist: PROTACpedia (http://protacdb.weizmann.ac.il/ptcb/stats) and PROTAC-DB 2.0 (http://cadd.zju.edu.cn/protacdb/).[6,7]

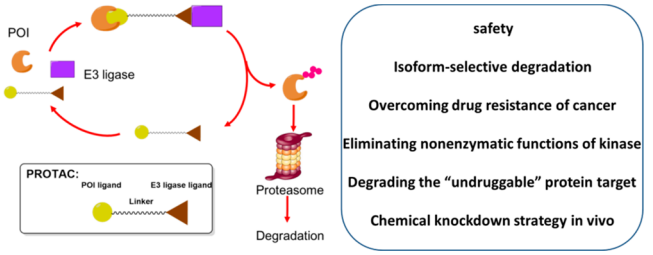


Figure 1. PROTAC-induced degradation mechanism and advantages. After a PROTAC molecule binds to the POI and E3 ligase and forms a ternary complex, ubiquitin is transferred to a lysine residue on the surface of the POI, resulting in the formation of polyubiquitinated POI that is subsequently degraded by the proteasome. At the same time, the PROTAC molecule dissociates from the complex and participates in the next degradation cycle. This degradation mechanism determines the therapeutic advantage of PROTAC technology.

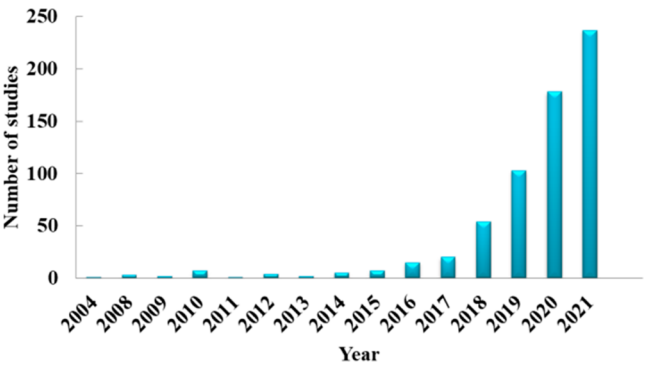


Figure 2. Search results for "PROTAC" as a topic on the "Web of Science" Web site. Field of PROTACs is in a period of explosively rapid development.

Received: September 22, 2022
Published: February 14, 2023

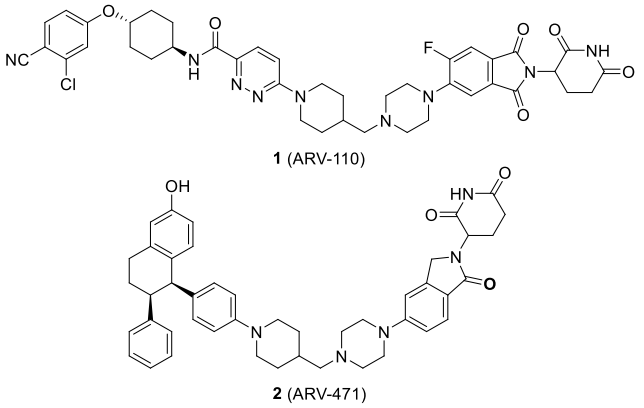


Figure 3. Chemical structures of compound **1** and **2**.

In the past decade, extensive efforts have been made to develop PROTAC-based therapeutics. Many reviews have clarified the advantages and disadvantages of PROTACs.[8−12] Despite all of the advantages and considerations of PROTAC technology, the design of PROTACs is still a matter of empirical trial and error. A review of recent design strategies is necessary, especially in a field with such rapid and explosive development. In this review, we discuss the recent advances focused on design strategy, development workflow, and future outlooks in this field.

2. PROTAC DESIGN STRATEGY

2.1. E3 Ligase Ligands. A variety of E3 ligases have been utilized in PROTAC therapeutics, including VHL, CRBN, MDM2,[13] IAP,[14] RNF4,[15] RNF114,[16,17] βTRCP,[18] parkin,[18] DCAF16,[2] KEAP,[19,20] and FEM1B.[21] Among these, VHL and CRBN are the two most widely applied. Compound **3** (VH032), a high-affinity ligand of VHL widely used in PROTAC formulations, was first reported in 2014 (Figure 4).[22] Recently, Li et al. reported a column chromatography-free process that enables the production of 42.5 g of compound **3** amine hydrochloride in 1 week with 65% overall yield and 97% purity, facilitating rapid construction of VHL-based PROTAC libraries.[23] In the course of optimization of VHL ligands, introduction of an (*S*)-methyl group on the benzylic carbon atom of compound **3** provided compound **4** (compound **11**, Figure 4) that has two times more affinity than the prototype.[24] Currently, approximately 51% of VHL ligands are compound **3**, and only 32% of VHL ligands are compound **4**. Therefore, the utilization of diverse VHL ligands, such as compound **4**, will enrich the VHL-based PROTAC library.

Diamine CRBN ligands are small-molecule drugs with molecular weights between 250 and 260 and are ideal for PROTAC design owing to their excellent physiochemical properties. However, hydrolysis of amide linkages in compound **5** (thalidomide, Figure 4) occurs in aqueous solutions at physiological pH, leading to chemical instability at pH > 7. The half-life of thalidomide at pH 7.4 is 5 h in vitro. The primary hydrolysis products are compounds **6**−**8**.[25,26] Several novel stable CRBN ligands have been discovered in recent years, including PG derivatives compound **9** (**2a**) and compound **10** (**2b**), which remain stable at pH 7.4 (Figure 4). The half-lives of compounds **9** and **10** are over 24 h at pH 7.4, longer than those of compounds **5**, **11** (lenalidomide), and **12** (pomalidomide) (3.3, 11.7, and 12.2 h, respectively).[27] Compound **13** (TD-106) is another novel CRBN modulator that induces the degradation of IKZF1/3 and inhibits the proliferation of multiple myeloma cells (Figure 4). Most importantly, PG derivatives and compound **13** have been used to design PROTACs with successful POI degradation.[28] Compound **14** (CC-122) is another new chemical entity that binds CRBN to degrade Aiolos and Ikaros (Figure 4).[29] Interestingly, hydrolysate compound **15** (Figure 4) was also shown to be a PROTAC CRBN recruiter.[30] Great effort has been devoted to discovering new CRBN ligands, which will contribute to improving chemical stability.

2.2. Anchor Point. In PROTACs, a linker tethers the ligand of the POI and an E3 ligase ligand. The linker can be attached at several exit vectors for both CRBN and VHL ligands. The binding site has an impact on not only PROTAC selectivity[31]

Table 1. PROTACs in clinical trials

compound	company	target	indication	phase	NCT no.
ARV-766	Arvinas	AR	prostate cancer	Phase 1	NCT05067140
AC0682	Accutar	ERα	breast cancer	Phase 1	NCT05080842
AC0176	Accutar	AR	prostate cancer	Phase 1	NCT05241613
CFT8634	C4	BRD9	synovial sarcoma, soft tissue sarcoma	Phase 1/2	NCT05355753
DT-2216	Dialectic	BCL-XL	liquid and solid tumors	Phase 1	NCT04886622
FHD609	Foghorn		synovial sarcoma	Phase 1	NCT04965753
NX-2127	Nurix	BTK	B-cell malignancies	Phase 1	NCT04830137
NX-5948	Nurix	BTK	lymphocytic leukemia	Phase 1	NCT05131022
KT-474	Kymera	IRAK4	autoimmune disease	Phase 1	NCT04772885
KT-413	Kymera	IRAK4	non-Hodgkin lymphoma; diffuse large B-cell lymphoma	Phase 1	NCT05233033
KT-333	Kymera	STAT3	lymphocytic leukemia; solid tumors	Phase 1	NCT05225584
BGB-16673	BeiGene	BTK	B-cell malignancies	Phase 1	NCT05294731
					NCT05006716
GT-20029	Kintor	AR	androgenic alopecia and acne	Phase 1	NCT05428449
HSK-29116	Haisco	BTK	B-cell malignancies	Phase 1	NCT04861779
HP518	Hinova	AR	Metastatic castration-resistant prostate cancer	Phase 1	NCT05252364
LNK-01002	Lynk		primary or secondary myelofibrosis; acute myeloid leukemia	Phase 1	NCT04896112
CC-94676	Bristol Myers Squibb	AR	prostate cancer	Phase 1	NCT04428788
RNK05047	Ranok	BRD4	advanced solid tumor	Phase 1/2	NCT05487170
ASP3082	Astellas	$KRAS^{G12D}$	advanced solid tumor	Phase 1	NCT05382559

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

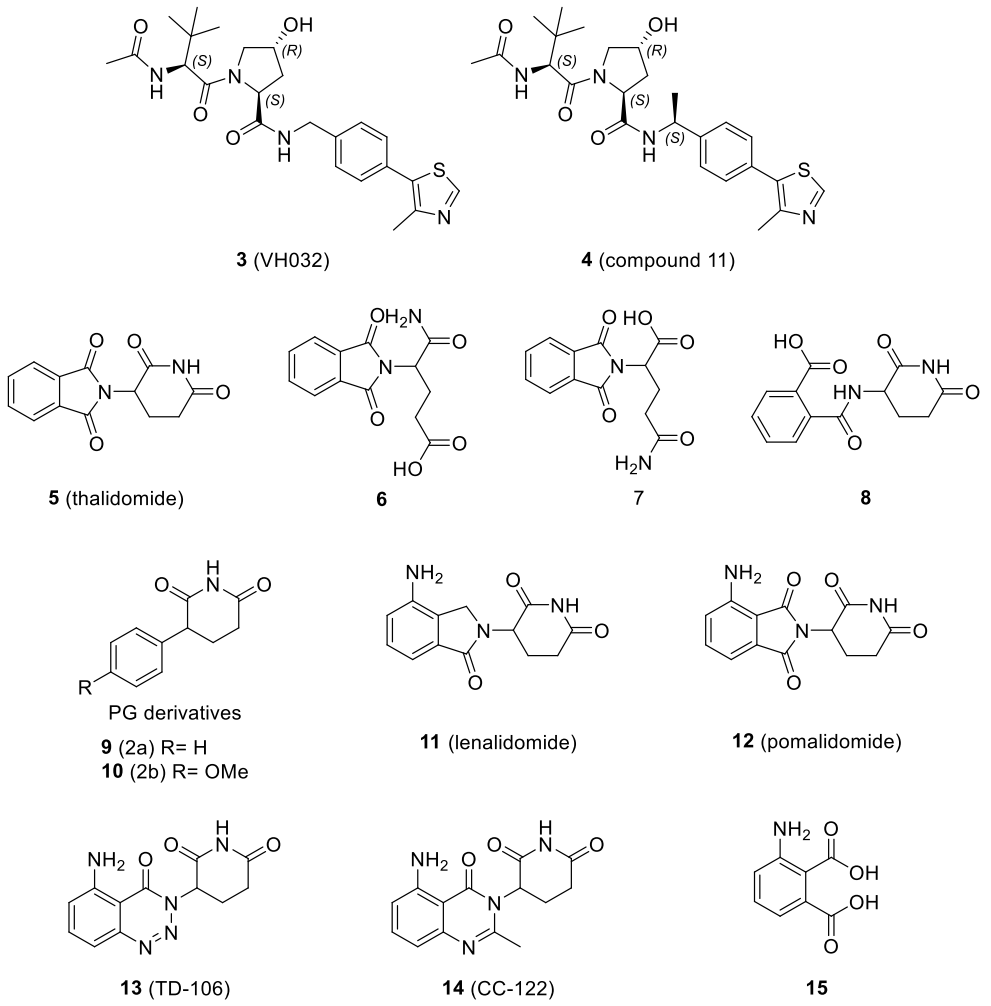


Figure 4. Representative ligands of E3 ligase used for PROTAC development.

but also DMPK parameters, such as microsomal stability.[32] W. Farnaby et al. introduced linkers at different locations of the VHL ligand, thereby forming "phenolic series" and "benzylic series" PROTACs for SMARCA2/4 degradation (Figure 5).[32] Pharmacokinetics studies revealed that, compared with the "phenolic series", "benzylic series", compound **16** (compound **6**) shows improved microsomal stability with a low clearance of 7 mL/min/kg in mice. Besides, compound **16** exhibited significant tumor growth inhibition in the NCI-H1568 tumor xenograft model with subcutaneous treatment. Furthermore, for CRBN-based PROTAC, replacing the amide connectivity at the 5′ position in Thalidomide with an ether moiety may provide a more robust and metabolically stable compound, such as compound **18** (MT802) ($t_{1/2}$ = 0.119 h) versus compound **19** (SJF608) ($t_{1/2}$ = 1.62 h) (Figure 5).[33] Interestingly, current CRBN-based PROTACs with oral bioavailability all exhibit linkers at the 5′ position (as mentioned in section 2.4.2). In conclusion, through the modification of the anchor point, PROTACs can acquire better microsomal stability, which provides a feasible way to optimize the pharmacokinetic properties of PROTACs.

The anchor point of the linker is important for degradation efficiency. In threonine tyrosine kinase degradation, for example, moving the anchor point in compound **20** (**8d**, Figure 5) from the 4′ site into the 7′ site generated compound **23** (**8p**), which has a DC_{50} value of 21.7 nM and is, therefore, 10 times less potent than compound **20** (DC_{50} = 2.2 nM).[34] Similarly, using 4′ site tethering in diamine compounds yielded a stronger inhibitory activity in RS4 11 cell line (compound **24**, IC_{50} = 1.5 nM) than tethering at the 5′ position (compound **25**, IC_{50} = 5 nM).[35] Similar phenomena are also observed with SHP2, BTK, p38α, HMGCR, and HDAC6 degraders.[36−40] Briefly, this suggests that the 4′ position in diamine compounds may be a better anchor point than the 5′ position in terms of obtaining high degradation efficiency.

It is well known that the binding of the ligand itself to the E3 ligase CRBN may result in a molecular glue effect or IMiD off-target effect, that is, CRBN neosubstrates would be degraded. This effect caused by the E3 ligase ligand in PROTAC is an important factor that has to be considered when designing PROTACs for drug safety. For example, when the anchor point of the linker is the 5′ position of compound **27** (C13, Figure 5), the substrates of IMiD, including IKZF1 and ZFP91, could not be degraded because the molecular glue function of the E3 ligase ligand was lost.[41] Lier et al. reported that linking the 5′ position of compound **28** (MDEG-541, Figure 5) can induce degradation of CRBN neosubstrates GSPT1(eRF3a) and GSPT2 (eRF3b).[42,43] Furthermore, neosubstrates ZNF653 and IKZF1 clearly favor 4′ position-linked degraders, whereas neosubstrate RNF166 favors 5′ position-linked degraders.[44,45] In addition to the linker exit vector, modification on the thalidomide aryl attachment point can also regulate IMiD

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

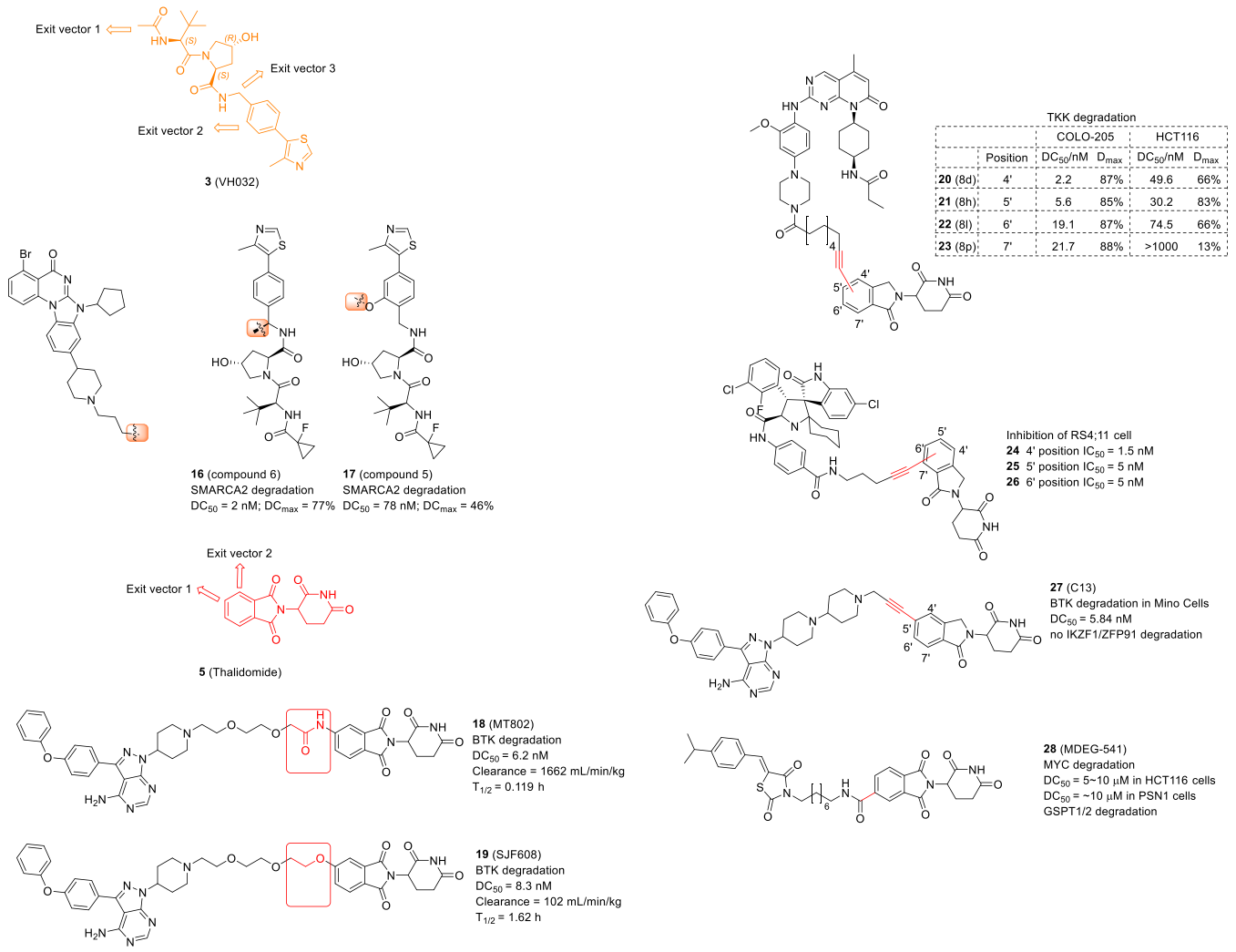


Figure 5. Anchor points of linkers in PROTACs.

substrate degradation. In a data set consisting of 68 CRBN-recruiting PROTACs targeting different targets, degraders with an aryl oxy acetamide conjugation to the linker tend to exhibit fewer IMiD off-target effects.[46] This suggests that the IMiD-like effect of the E3 ligase ligand in PROTACs might be avoided depending on the selection and modification of the anchor point.

2.3. Warhead. Binding between the warhead and the POI does not have to be strong, but POI ligands with low nanomolar affinity are ideal. Notably, de novo design of novel and potent POI ligands with improved physicochemical properties is more likely to arrive at orally available degraders.[32] Besides, for suitable conjugation and optimized physicochemical properties, the structure of the POI ligand can be slightly modified, for example, by replacing a piperidine with an *N,N*-diethylamino group,[24] a morpholine with piperazine,[47,48] or a quaternion ring with a topine ring and octahydropentalen,[49] or by omitting the noncritical *o*-fluoro substitution[50] (Figure 6).

Moreover, choosing the right protein target is key, as some proteins are highly expressed in normal tissues and their degradation may cause unpredictable toxicity. This can limit translation to clinical application. For example, Khan et al. found that ABT263-based VHL-recruiting PROTACs induce selective BCL-XL degradation in T-cell acute lymphoblastic leukemia (T-ALL) tumor models while sparing platelets due to low levels of VHL expression.[51] A similar phenomenon was observed for phthalimide-based PROTACs.[52] The best-case scenario is to identify targets and ligases highly expressed in tumor tissues but with low expression in normal tissues. Within this framework, Cristina et al. found that coupling the MDM2 ligase with inhibitors of targets BCL2L1, BRD4, CDK9, PLK1, and MCL1 in stomach cancer and with PIK3C3 inhibitors in breast cancer formed ideal combinations for PROTACs.[53] The tissue distribution studies of E3 ligase and the POI in different cancer types open up new opportunities for developing PROTACs with a wider therapeutic window. The narrow therapeutic window could also be addressed in many other ways. For example, due to the lack of small-molecule ligands, only less than 1% of the 600+ existing E3 ligases have been successfully utilized.[54] Therefore, the discovery of E3 ligase ligands is of great relevance. Moreover, consensus frameworks that integrate in silico methodology with in vivo and in vitro data to inform the potential clinical risk profile of a given compound are being devised.[55]

2.4. Linkers. *2.4.1. Linker Length.* The linker length is the most critical feature to be considered early during PROTAC development. Linker parameters such as the length can have profound effects on cell permeability, degradation, and specificity.[56−59] If the chain length is too short, the two ligands will not bind to the corresponding protein due to steric hindrance. Conversely, if the length is too long, proteins cannot

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

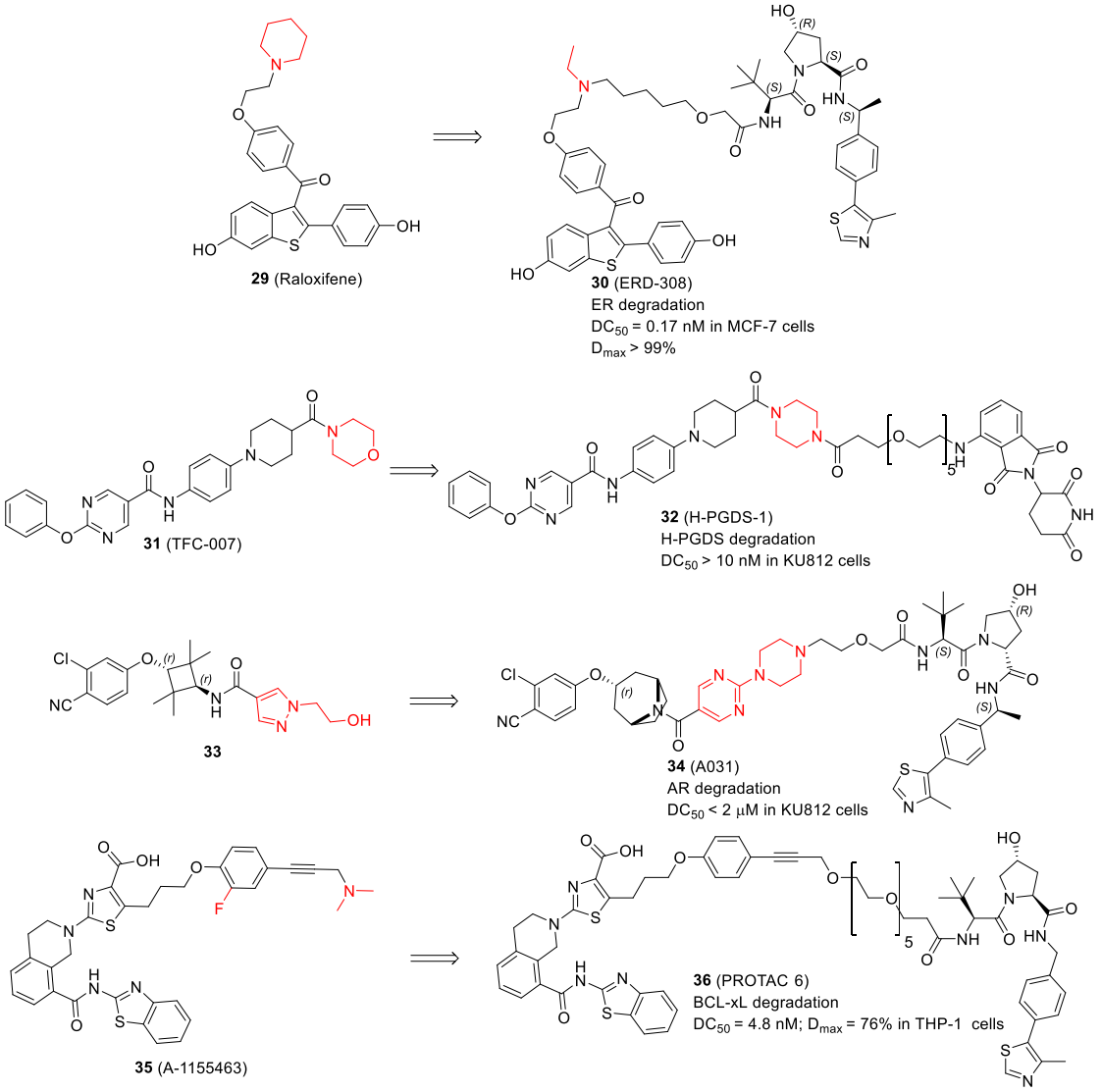


Figure 6. Representative warheads that were slightly modified.

be brought in close proximity for target ubiquitination. In addition, the linker length significantly affects the selectivity of PROTAC degradation. Nowak et al. observed that shorter linkers have better selectivity due to conformational restrictions.[56] Conversely, in a study conducted by Crews et al., a longer linker achieved selective degradation of EGFR.[57] Notably, for the same POI, the optimal linker length is different for degradation by different E3 ligases. As shown in Figure 7, for BRD4 degradation,[60,61] the linker length of FEM1B-based PROTAC is shortest[21] and that of CRBN-based PROTAC is longest.[28] The same phenomenon was found in ALK degrader[62,63] and BTK degrader.[64,65] Therefore, each target/E3 combination has its appropriate linker length, which reflects the degradation potency and selectivity.

Furthermore, Troy et al. showed correlations between the degrader efficacy and the linker length.[66] Their analysis suggested that to produce a lead degrader compound, a SAR study of the linker length is best started with longer lengths to test for compatibility between the E3 ligase and the POI. Systematically shortening the linker length will reveal a sharp cutoff point in potency, which may allow for the production of PROTACs with the greatest potency and selectivity.

2.4.2. Chemical Composition. The chemical composition of the linker is a major determinant of the rigidity, solubility, cell permeability, and pharmacokinetic/pharmacodynamic profile. A wide variety of linkers are based on polyethylene glycol (PEG), unsaturated alkane chains, and heterocyclic rings (Figure 8). Due to their polarity and flexible nature, PEG chains are essential scaffold elements for PROTAC design. However, PEG linkers with aliphatic and/or ether chains are often associated with an increased risk of oxidative metabolism,[11] which may be ameliorated via click chemistry to produce groups containing triazole.[67]

To modulate the PROTAC physicochemical properties, positively charged nitrogen heterocycles (such as piperidine and piperazine) can be introduced.[24] Rigid linkers can lead to more effective degradation, as seen for compounds **46** (GSK215, Figure 9) and **47** (DGY-09-192, Figure 9).[68,69] Furthermore, nitrogen heterocycles are beneficial for increasing solubility and improving oral bioavailability. Wang et al. reported two orally bioavailable AR degraders, compounds **48** (ARD-2128, Figure 10) and **49** (ARD-2585, Figure 10). The linkers of these molecules harbor nitrogen-containing heterocyclic rings piperazine and azetidine. In both molecules, rigid chains were shown

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

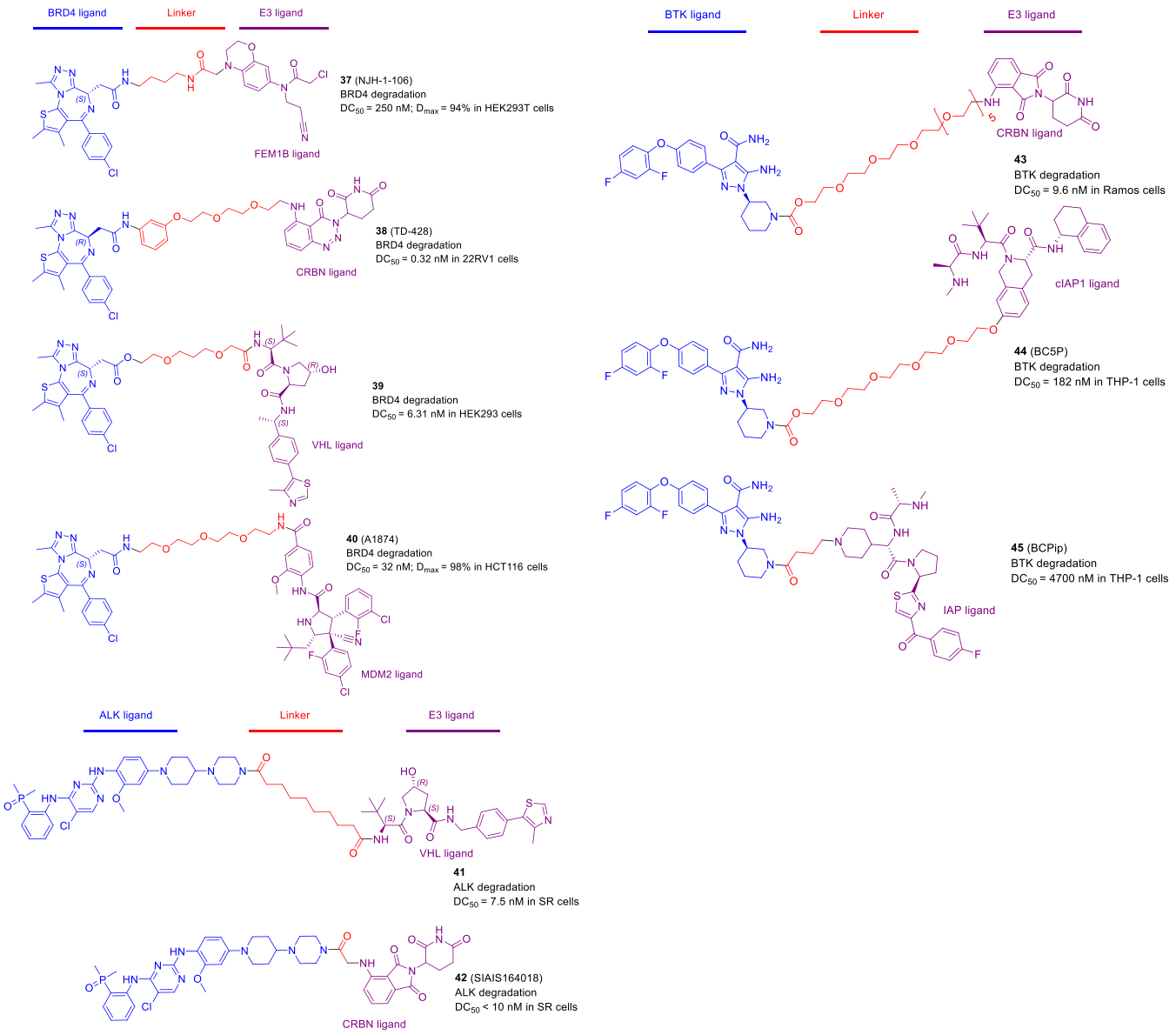


Figure 7. POI can be degraded by different E3 ligases through PROTACs with different optimal linker lengths.

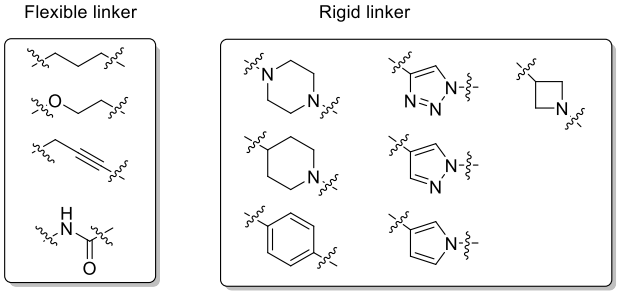


Figure 8. Common types of linkers used for PROTAC design.

to be essential for the development of AR degradants.[70,71] The oral bioavailable PROTACs, compounds **50** (HJM-561, Figure 10) and **51** (SIAIS001, Figure 10), both harbor piperidine and piperazine rings.[72,73] Recently, the orally bioavailable compound **27** (C13, Figure 10) was discovered, containing two piperidine rings.[41] Moreover, compounds **1** and **2** (Figure 10), which are in phase II clinical trials for prostate cancer and breast cancer, respectively, also contain the nitrogen heterocycles piperidine and piperazine.[74−76] This may indicate that linkers comprising nitrogen-containing heterocycles are more conducive to oral absorption. Researchers from AstraZeneca have recently suggested that linker modifications should be selected based on physical property optimization while taking full advantage of crystal structure and homologous modeling information. This will enable the identification of suitable attachment points or new interactions in the ternary complex without enduring the traditional "trial-and-error" approach.[33]

Due to the relatively large molecular weight of VHL ligands (compared with CRBN ligands), it is generally considered that VHL-based PROTACs are difficult to exhibit good bioavailability. However, W. Farnaby et al. recently reported the first oral bioavailable PROTAC based on VHL, compound **52** (ACBI2, Figure 10).[32] A slight linker modification in compound **53**, introducing a methyl group, increased oral bioavailability from 3% to 22%, which is the result of a more compact PROTAC conformation with a reduced 3D polar surface area and radius of gyration.[77] Compound **52** significantly inhibited tumor growth

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

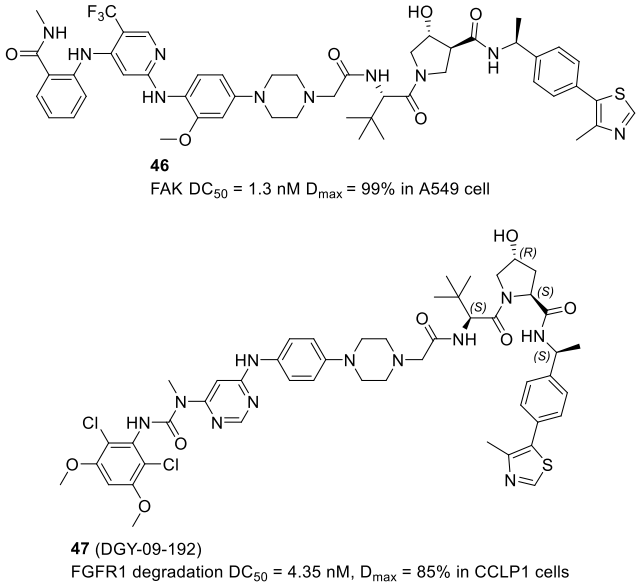


Figure 9. Chemical structures of compounds **46** and **47**.

in an A549 xenograft model with treatment at 80 mg/kg orally. Besides, compound **54** (XL01126, Figure 10), another VHL-based PROTAC for LRRK2 degradation, also exhibited modest oral bioavailability (F = 15%). More importantly, compound **54** is the first VHL-based PROTAC that is both orally bioavailable and blood−brain barrier permeable.[78] These are groundbreaking findings that have significant implications for the improvement of other VHL-based PROTAC oral bioavailability.

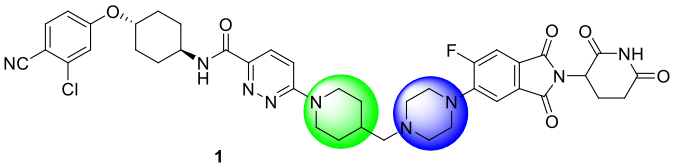
2.4.3. Molecular Glue Function. As mentioned in section 2.2, molecular glue function is an important factor that has to be considered for drug safety. The linker has a key impact on the molecular glue function. Recently, the structural mechanism for this function of IMiDs was elucidated.[79] The authors studied three molecules with different activities for ubiquitinylation of the neosubstrate Ikaros: compounds **12** (moderate), **55** (CC-220, potent), and **56** (CC-92480, most potent). Their data showed that pomalidomide induced 20% of CRBN to be closed, and compound **55** induced 50% of CRBN to be closed. Compound **56**, with the most potent activity, induced almost 100% of CRBN to be closed (Figure 11). Compared to compounds **12** and **55**, compound **56** makes extra interactions with the sensor loop and Lon domains of CRBN. Additional phenyl and morpholino moieties of compound **55** arch over the top of the sensor loop. The benzonitrile group in compound **56** is stabilized by aromatic or cation−π interactions with Phe102 and Phe150 in the CRBN Lon domain. This suggests that we should consider avoiding the interaction with the sensor loop and Lon domain of CRBN when designing the linker, so as to reduce this IMiD off-target effect.

2.5. PROTAC Synthesis. Unlike traditional small-molecule inhibitors, the synthesis of a whole PROTAC molecule requires the linking of three parts (POI ligand, E3 ligase, and linker), which makes the synthesis of PROTACs time consuming and laborious. Many efforts have been made to improve the synthesis efficiency of PROTACs. Current strategies for increasing synthetic throughput have been introduced through Staudinger ligation chemistry,[80] solid-phase synthesis,[81] copper-catalyzed click chemistry,[82] activated esters,[83] the rapid synthesis of PROTACs (Rapid-TAC) platform,[84] and the modular synthetic platform.[85] Notably, a late-stage single-step reductive deoxygenation method allows immediate conversion from thalidomide-containing PROTACs to lenalidomide groups without a de novo synthesis.[86] A few studies have focused on the synthesis methodology of PROTAC fragments. For example, Duncan et al. reported a strategy for the reliable and succinct preparation of pomalidomide linkers.[87] Furthermore, Qiu et al. developed the organic base-promoted chemoselective alkylation of lenalidomide with different halides.[88] Some fragments have become commercially available. These advances will greatly benefit the establishment of a PROTAC molecular library and accelerate the discovery of PROTACs with clinical therapeutic applications.

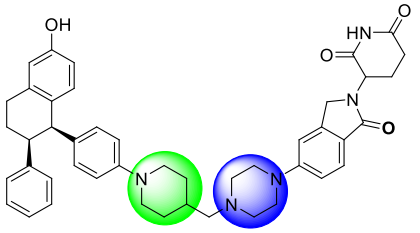
2.6. Whole Ternary Complex Formation. The ternary complex consists of the POI, PROTAC, and E3 ligase, which are the basis for the formation of the polyubiquitinated POI for degradation by the proteasome. Design strategies aimed at forming whole ternary complexes will be conducive to the discovery and development of PROTACs. The ternary complex crystal structure-based design and optimization is more attractive and efficient than the current multiturn, empirical trial-and-error approach. Crystallizing ternary complexes has been demonstrated to be challenging, but several examples at present have been reported (Table 2, Figure 12). For example, the first orally bioavailable VHL-based PROTAC (F = 22%), compound **52**, was discovered by taking advantage of the ternary complex crystal structure (see Figure 10). Besides, the first crystal structure of a ternary complex was published in 2017.[89] This PROTAC, compound **57** (MZ1), binds to the E3 ligase VHL, degrading BRD4. Compound **58** (AT1) was rationally designed with an unusual anchor replacing the *tert*-Leu group of compound **57**, improving its selectivity for BRD4 (Figure 13). Similarly, the macrocyclic PROTAC compound **59** (macro-PROTAC-1) was designed based on the crystal structure to lock the PROTAC conformation in the bound state by a macrocyclization strategy; cyclization is a feasible strategy to enhance potency and selectivity (Figure 13).[90] These studies show that the ternary complex cocrystal structure will guide us toward the design of a more optimal degrader, avoiding lengthy empirical exploration of structure−activity relationships based on PROTACs.

Compared to a single ligand contact with an individual protein, the ternary complex provides more interaction networks. For example, the POI can interact with E3 ligase, which is called de novo protein−protein interaction; the POI ligand in PROTAC can interact with E3 ligase or the E3 ligase ligand can interact with the POI, which is called "cross" protein−ligand interaction. These interactions are essential for the formation of ternary complexes, guiding us to improve the characteristics of the degrader. For example, to maintain de novo protein−protein interactions and "cross" protein−ligand interactions, simultaneously increase conformational restraint, and attempt to form π-stacking interactions to Y98, benzene rings were immediately introduced into the linker in compound **60** (PROTAC 1, Figure 14) to obtain compound **61** (PROTAC 2) with improved permeability. Indeed, the introduced benzene rings formed new π-stacking interactions with the Y98 in the cocrystal structure. Emboldened by crystallographic and biophysical data, for the same linker length of compound **60**, oxygen atoms were further introduced into the linker in compound **61**, yielding compound **62** (ACBI1), a more potent degrader with promising antiproliferative effects in acute myeloid leukemia cell lines.[91] Recently, X-ray crystallography of the FAK:compound **46**:VHL ternary complex was reported.

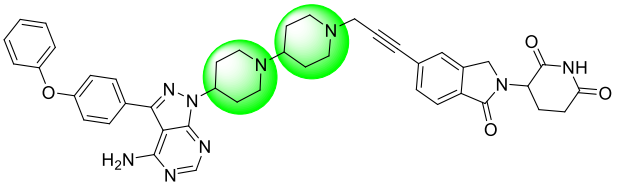
https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329



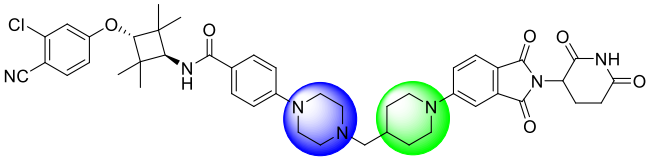
1
AR DC_{50} = 1 nM in LNCaP cell



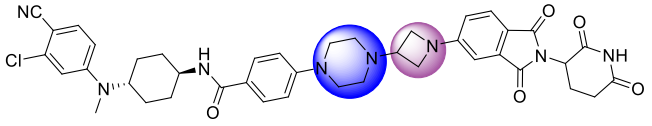
2
AR DC_{50} = 2 nM in in ER-positive breast cancer cell lines



27
BTK DC_{50} = 5.8 nM D_{max} = 96% in Mino cell
F = 12%

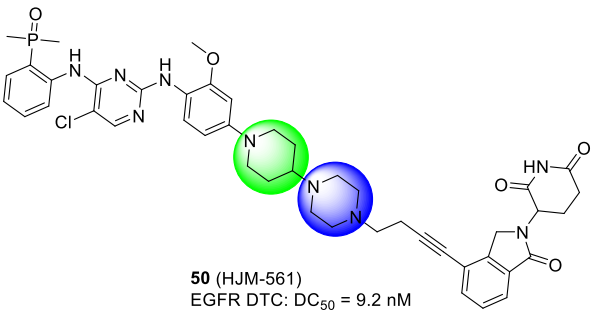


48 (ARD-2128)

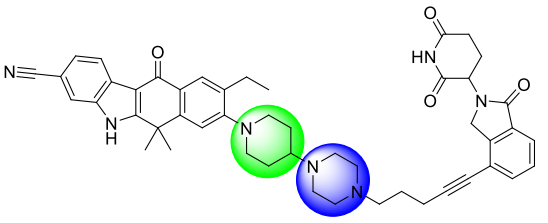


49 (ARD-2585)

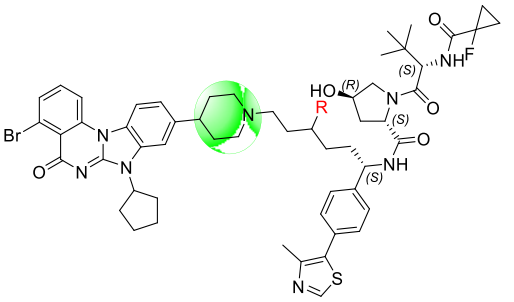
	LNCaP		VCaP		
	DC_{50}/nM	D_{max}	DC_{50}/nM	D_{max}	F
48	8.3	/	0.28	96%	67%
49	0.1	98%	0.04	99%	51%



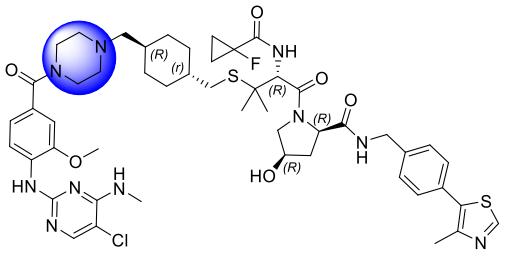
50 (HJM-561)
EGFR DTC: DC_{50} = 9.2 nM
EGFR LTC: DC_{50} = 5.8 nM
F = 62.8%



51 (SIAIS001)
ALK: DC_{50} = 3.9 nM; DC_{max} = 70.3 nM
G1202R ALK: DC_{50} = 200 nM
F = 16%



52 (ACBI2) R = *(S)*-Me
SMARCA 2 degradation
DC_{50} = 1 nM
F = 22%
53 R = H
SMARCA 2 degradation
DC_{50} = 32 nM
F = 3%



54 (XL01126)
LRRK2 degrader
WT LRRK2: DC_{50} = 32 nM; DC_{max} = 82%
G2019S LRRK2: DC_{50} = 14 nM; DC_{max} = 90%
F = 15%
Penetrate the blood brain barrier

Figure 10. Structures of orally bioavailable PROTACs. Piperidine (green) and piperazine (blue) were the most frequent nitrogen heterocycles present in PROTACs.

Compound **46** (see Figure 12) is a potent FAK degrader (DC_{50} = 1.3 nM, DC_{max} = 99%) and induced high ternary complex cooperativity as a result of various de novo protein−protein interactions in the cocrystal, including van der Waals

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

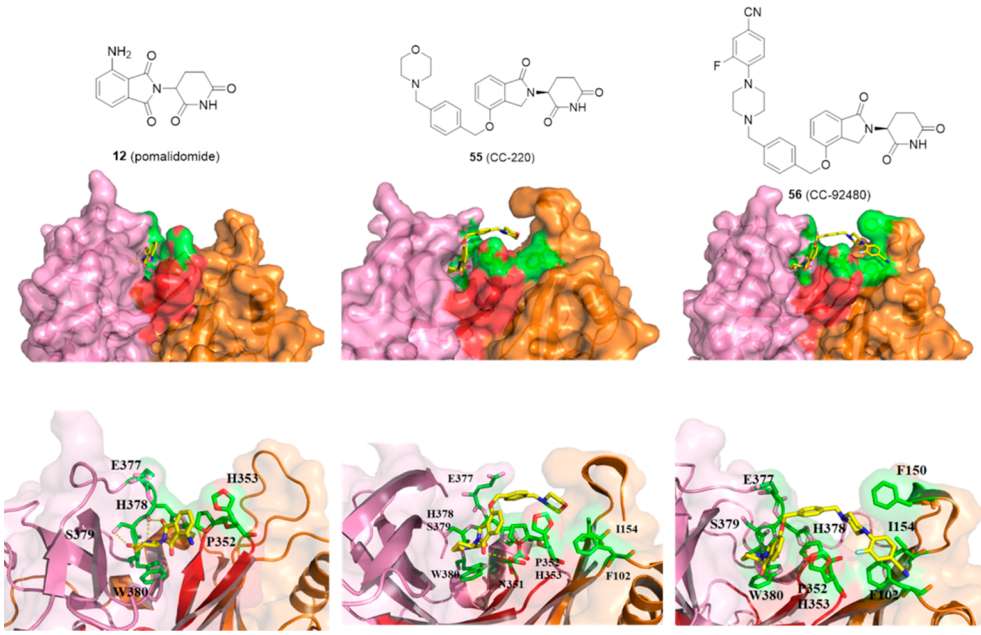


Figure 11. Cocrystal structure of compounds **12** (left row, PDB: 8D81), **55** (middle row, PDB: 5V3O), and **56** (right row, PDB: 8D7U) bound to CRBN. Compared to compound **12**, compound **55** additionally interacts with the sensor loop (red). Besides the sensor loop, compound **56** also interacts with the Lon domain (orange). Represented are the key residues (green) between each ligand and CRBN, highlighting the activity of molecular glue.

Table 2. Reported Ternary Cocrystal Structure

				neointeraction			
	PROTAC	E3	POI	E3 residues	POI residues	ref	PDB
1	**57** (MZ1)	VHL	$BRD4^{BD2}$	R69, P71, R107, R108, I109, H110, Y112	W374, P375, F376, D381, E383, A384, L385	89	5T35
2	**59** (Macro PROTAC-1)	VHL	$BRD4^{BD2}$			90	6SIS
3	**60** (PROTAC-1)	VHL	SMARCA2	R69, Y112	T1462, F1463, N1464	91	6HAY
4	**61** (PROTAC-2)	VHL	SMARCA2				6HAX
5	**61** (PROTAC-2)	VHL	SMARCA4				6HR2
6	**36** (PROTAC 6)	VHL	Bcl-xL	Arg60, Arg69, Gln90, Phe91, Gln96	Arg103, Ala104, Phe105, Asp133	50	6ZHC
7	**63** (BCPyr)	cIAP1	BTK	NR[a]	NR[a]	64	6W7O
8	**44** (BC5P)	cIAP1	BTK				6W8I
9	**46** (GSK215)	VHL	FAK	N67, R69	C427, E430	68	7PI4
10	**64** (dBET23)	CRBN	$BRD4^{BD1}$	residues 101−104 and 147−157	residues 76−104 and 145−161	56	6BN7
11	**65** (dBET6)	CRBN	$BRD4^{BD1}$	NR[a]	NR[a]		6BOY
12	**66** (dBET70)	CRBN	$BRD4^{BD1}$	NR[a]	NR[a]		6BN9
13	**67** (dBET55)	CRBN	$BRD4^{BD1}$	NR[a]	NR[a]		6BN8
14	**68** (dBET57)	CRBN	$BRD4^{BD1}$	NR[a]	NR[a]		6BNB
15	**69** (MS33)	VHL	WDR5	Arg69, Asp92	Asp172, Tyr191, Asp192, Asn214, Lys259	94	7JTO
16	**70** (MS67)	VHL	WDR5	Arg69, Asp92, Pro71, Arg107, Arg108, His110	Asp172, Tyr191, Asp192, Asn214, Leu234, Lys259		7JTP
17	**71**	VHL	$BRD4^{BD1}$	R107, Pro97	Asp145, Trp81	95	7KHH
18	**17**	VHL	SMARCA2	Asn67	Gln1469	32	7Z6L
19	**72** (compound **10**)	VHL	SMARCA2	NR[a]	NR[a]	32	7Z76
20	**16**	VHL	SMARCA2	NR[a]	NR[a]	32	7Z77

[a]NR: not reported.

interactions, direct H bonds, and salt bridges.[68] These suggest that de novo protein−protein interactions and "cross" protein−ligand interactions are crucial for more potent degrader discovery and should be considered seriously in PROTAC design.

To form productive ternary complexes, small E3 ligases seem to be most suitable for some membrane proteins that are barely exposed to the cytosol. For example, KRAS, a recognized antitumor target, is localized to the plasma membrane after post-translational modification. It can be degraded by a PROTAC recruiting a small CUL2-elongin B (ELOB)-ELOC-VHL-E2-Ub complex but not by those recruiting the large Cullin 4A (CUL4A)-DDB1-CRBN-E2-Ub complex, even when ternary complexes are formed.[92,93] It seems that those small E3 ligases can form ternary complexes more easily.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

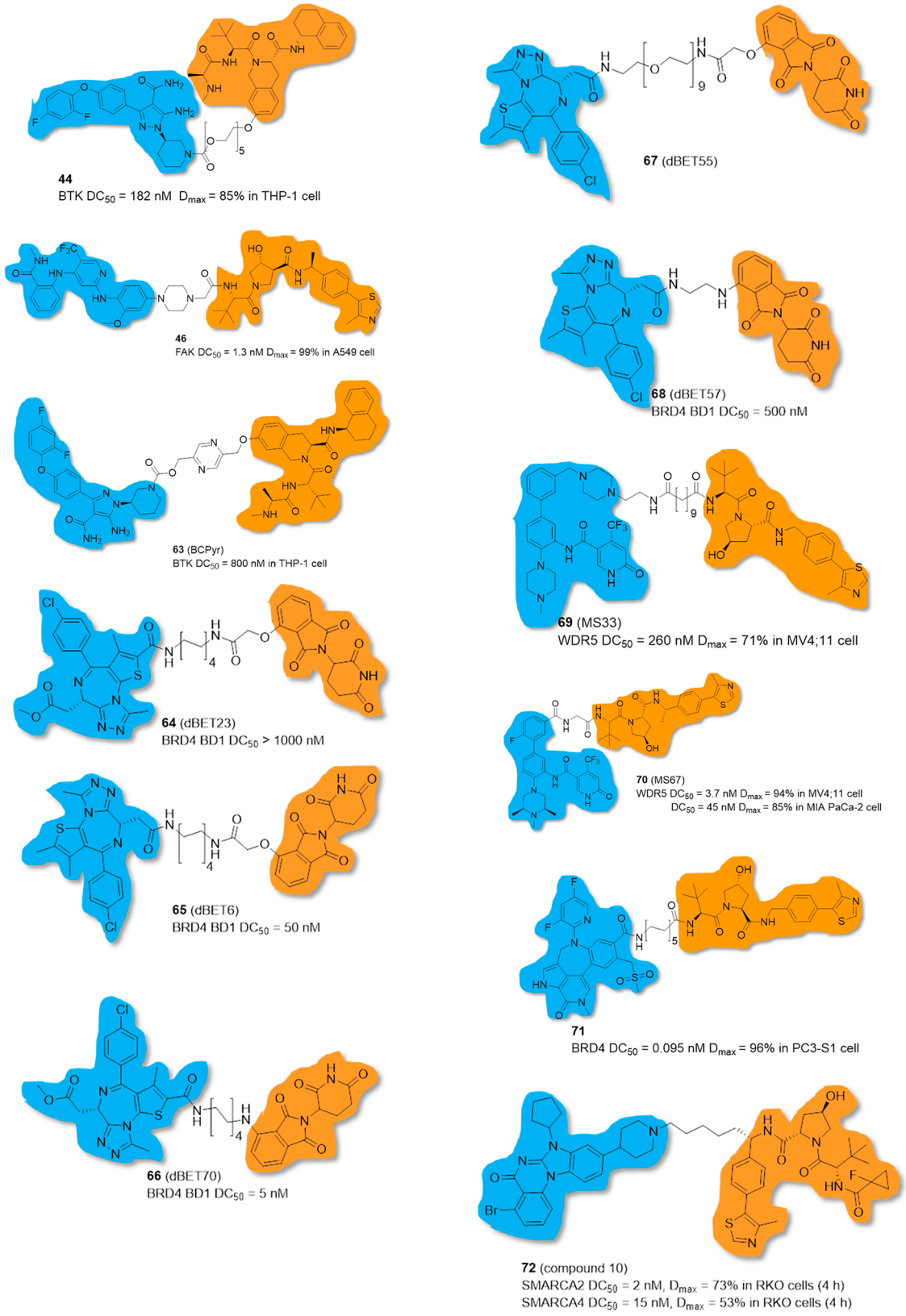


Figure 12. Representative PROTACs in ternary crystal structures.

3. PROTAC DEVELOPMENT WORKFLOW

This section describes the general process of PROTAC development at the molecular level (Figure 15). The time and concentration dependence of POI degradation in tumor cells are first determined universally[1] and then by DC_{50} and D_{max}. In addition, detection of POI mRNA levels is necessary to verify whether the decrease in POI content is caused by protein expression.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

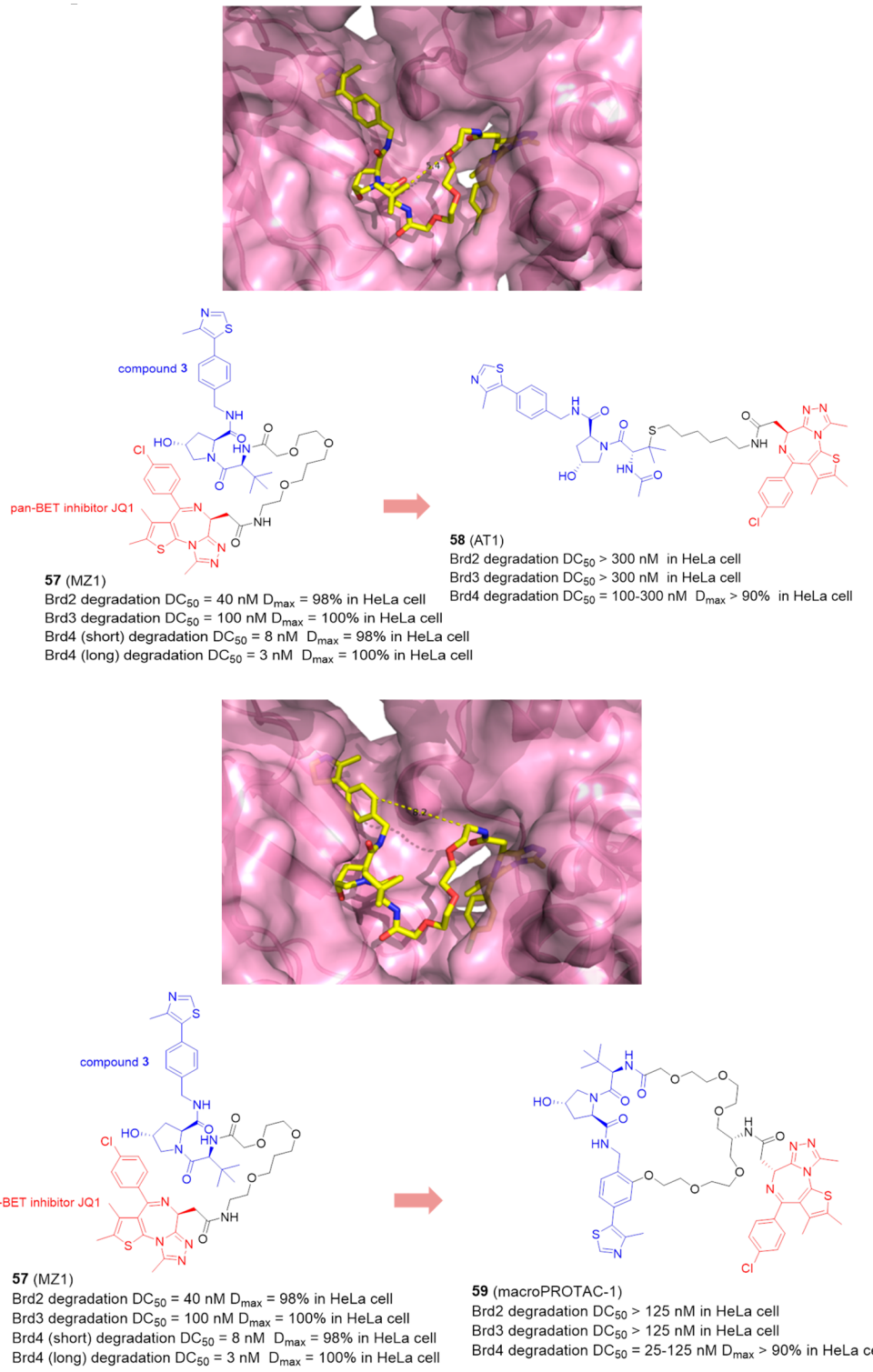


Figure 13. Design of compounds **58** and **59**. Cocrystal structure (PDB: 5T35) is shown to allow the amide groups to form new linkers with *tert*-Leu and the benzene ring of compound **3**, respectively.

Caution should be paid to the degradation kinetics. For example, using luminescence and bioluminescence resonance energy transfer (NanoBRET), Riching et al. found that BRD4 levels rebounded after 10 h of compound **73** (dBET1, Figure 16) treatment.[96] This phenomenon is called "rapid recovery". In addition, the establishment of a PROTAC-mediated ternary complex is dependent on PROTAC concentration. At high concentrations, ineffective binary complexes (PROTAC-POI or PROTAC-E3 ligase) are often observed in the range of 1−10 μM, representing the "hook effect". This effect is not always observed[1,47] and might be avoided or reduced by improving cooperative interactions in the ternary complex that favor stability. For example, with significant positive cooperativity, the BTK-targeting PROTAC compound **18** did not exhibit an observable hook effect at concentrations below 2.5 μM.[14] Briefly, as heterobifunctional molecules, PROTACs exhibit

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

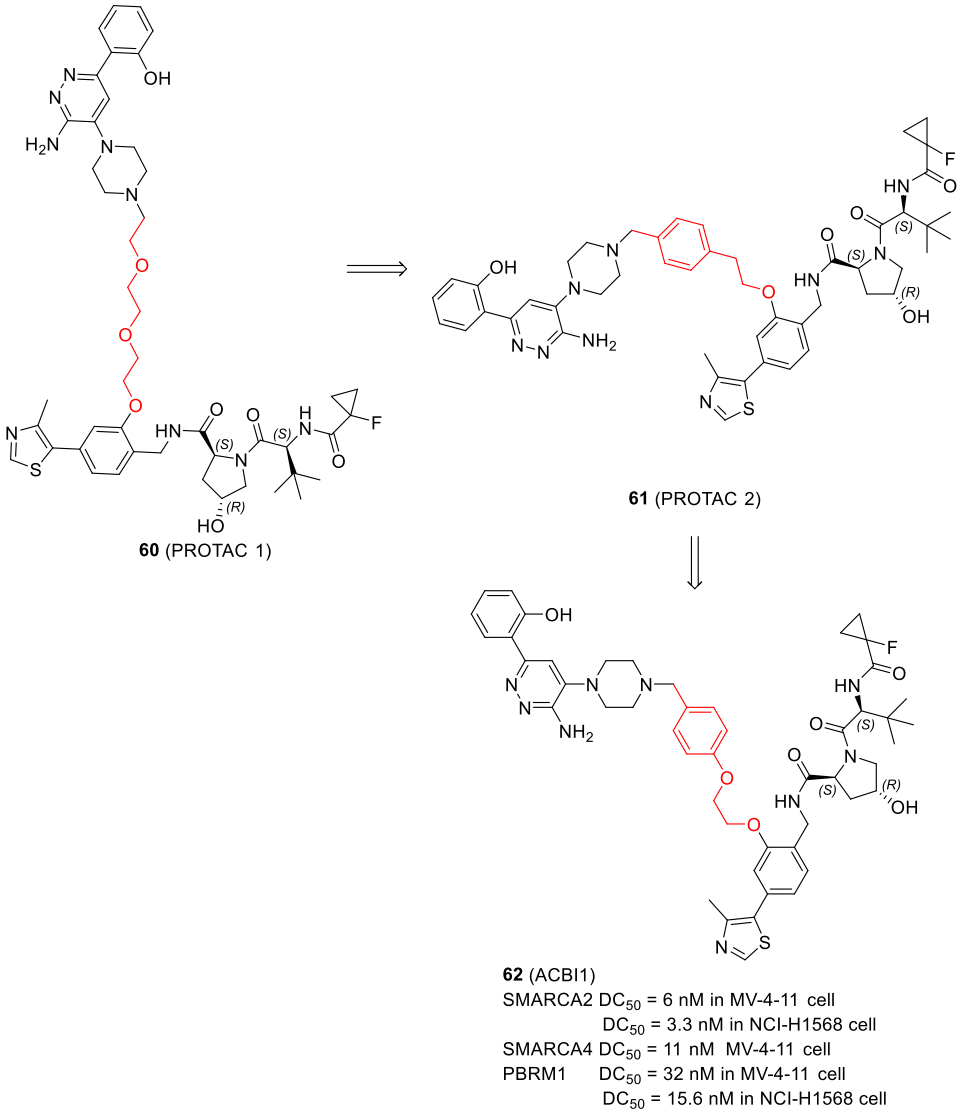


Figure 14. Design strategy of compound **62**.

unique degradation kinetic profiles, which require particular attention.

To confirm that the POI is degraded according to the expected mechanism of action, pretreatments with excessive target protein inhibitors, E3 ligase ligands (such as compounds **11**[1] and **12**,[48,92] see Figure 4), proteasome inhibitors (such as compounds **74** and **75**[92]), and NEDD8-activating enzyme inhibitors (such as compound **76**[1,92]) are required, as these demonstrate whether the degradation is dependent on the ubiquitin−proteasome system (Figure 17). Interestingly, some PROTAC-inducing EGFR degradation is not only dependent on ubiquitination/proteasome but also on autophagy/lysosome pathways.[97−99] Additionally, evaluation of negative controls is always needed for on-target validation. For example, methyl-modified glutarimide compounds and (*S*)-OH compound **3** (Figure 17) have been used to block binding to CRBN and VHL, respectively.[94,100] In conclusion, it is necessary to prove the degradation mechanism of PROTACs.

Affinity for the target protein is also usually measured, for example, through homogeneous time-resolved fluorescence (HTRF) assays.[50] It is worth noting that the affinity was demonstrated to rarely correlate with the selectivity observed in cellular assays. For example, Jin et al. reported that PROTACs compounds **77** (MS9449, Figure 17) and **78** (MS9427, Figure 17) have high binding affinity to both WT EGFR and mutant EGFR but effectively degraded only mutant EGFR without the degradation of WT EGFR.[97] This lack of correlation between binding affinity and degradation selectivity was also found in a selective $BRAF^{V600E}$ degrader.[101] To uncover any potential off-target effects, it is necessary to elucidate the proteome-wide selectivity of PROTACs through proteomic analysis.[1] Furthermore, the primary determinant of effective degradation is the ternary complex formation rather than the binding ability of the PROTACs to the target protein.[102,103] For example, BRD4 inhibitor **79** (JQ1)-based PROTACs showed more potent degradation than compound **80** (I-BET726)-based PROTACs, even though compound **80** has a higher binding affinity (K_d = 4 nM vs K_d = 100 nM for BRD4)[104] (Figure 17). As shown in Figure 18, the α value is a ratio of binary to ternary affinities, thus indicating the degree of cooperativity. Generally, $\alpha > 1$ describes a system that favors stable ternary complex formation, facilitating ubiquitin transfer and subsequent target degradation.[89] However, Zorba et al. found that the highest α value was not associated with the most efficient degradation of BTK.[65] In

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

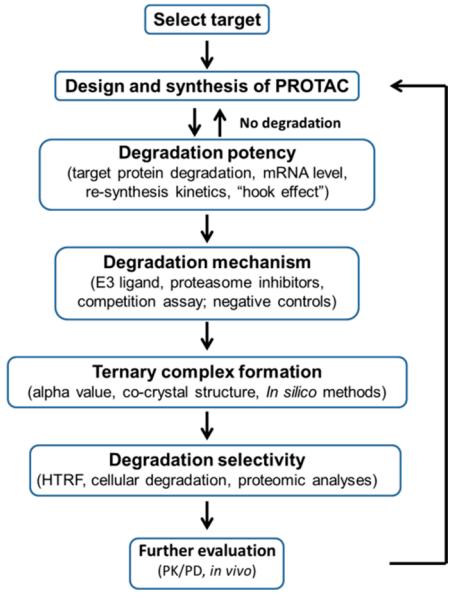


Figure 15. PROTAC development workflow.

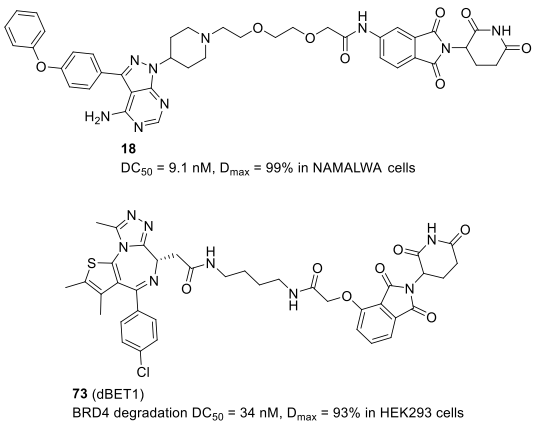


Figure 16. Chemical structures of compounds **18** and **73**.

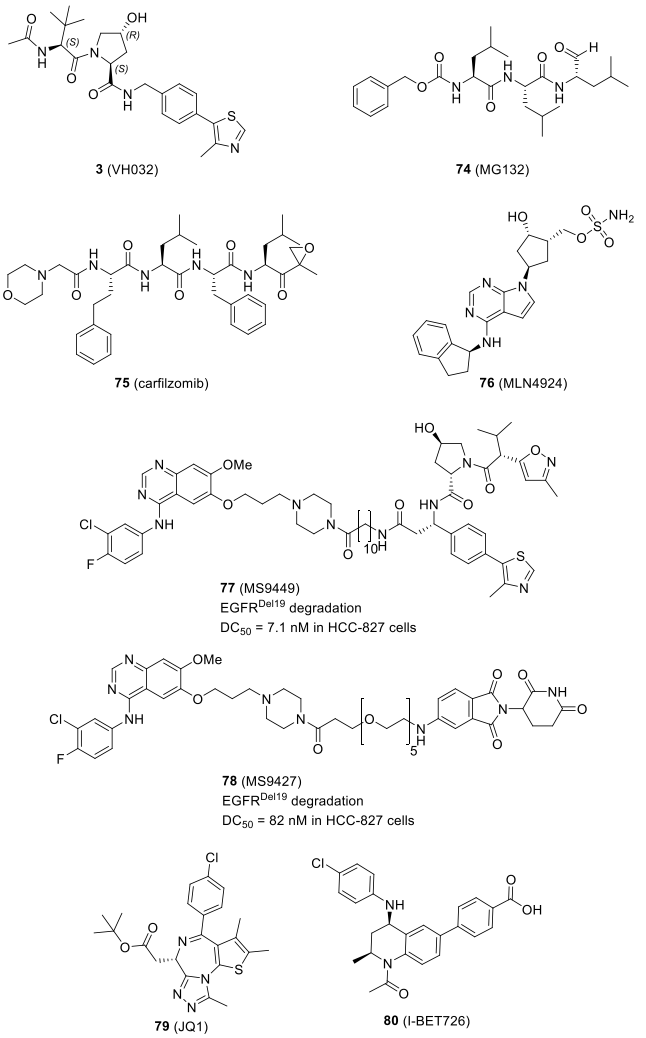


Figure 17. Chemical structures of compounds **3** and **74**−**80**.

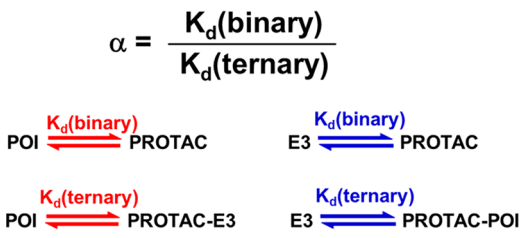


Figure 18. α value calculation diagram: binary, binary complex; ternary, ternary complex; E3, E3 ligase.

conclusion, α is often determined as a priority to indicate whether an effective ternary complex is formed.

To speed up PROTAC optimization, C4 Therapeutics employs a three-pronged, enzymology-based approach. At the early stage of hit identification, the kinetic constants, or the rate of degradation, are derived. Simulated or measured pharmacokinetic profiles are then layered in to predict how degradation would affect the body.[12] Together, these data provide directions for further optimization by chemists.

To further understand and recognize the workflow, a typical example, compound **54** recently reported for LRRK2 degradation, is given (Figure 19). At the beginning of the project, an LRRK2 inhibitor with favorable physicochemical properties was selected as the warhead, followed by conversion of the morpholine ring to piperazine for linker attachment facilitation. Then, the degradation activity was evaluated by Western blotting, which revealed the first-generation PROTACs with 30−70% degradation at 1 μM/4 h. Among the first-generation PROTACs, compound **81** (SD13, Figure 19) exhibited a "hook effect" with 60% of G2019S LRRK2 degraded at 33 nM/4 h but less degraded at 1 μM. For PROTAC improvement, further structure−activity relationship studies were carried out, focusing on the LRRK2 ligand, the linker, and the VHL ligand. This round of optimization led to the development of second-generation PROTACs, including the most potent PROTAC compound **54** that degraded 20−30% of WT LRRK2 and 50−60% of G2019S LRRK2 with a 33 nM/4 h treatment. Subsequent degradation kinetics studies showed that compound **54** was the most efficient and fastest degrader for G2019S LRRK2 ($DC_{50,4h}$ = 14 nM; $D_{max,4h}$ = 90%) and WT LRRK2 ($DC_{50,\ 4\ h}$ = 32 nM; $D_{max,\ 4\ h}$ = 82%). Moreover, in line with its "most potent" activity, compound **54** could induce the most cooperative ternary complex, as indicated by an α value of 5.7 in the NanoBRET-based assay, despite the weak binary binding affinity of the PROTAC to E3 or LRRK2.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

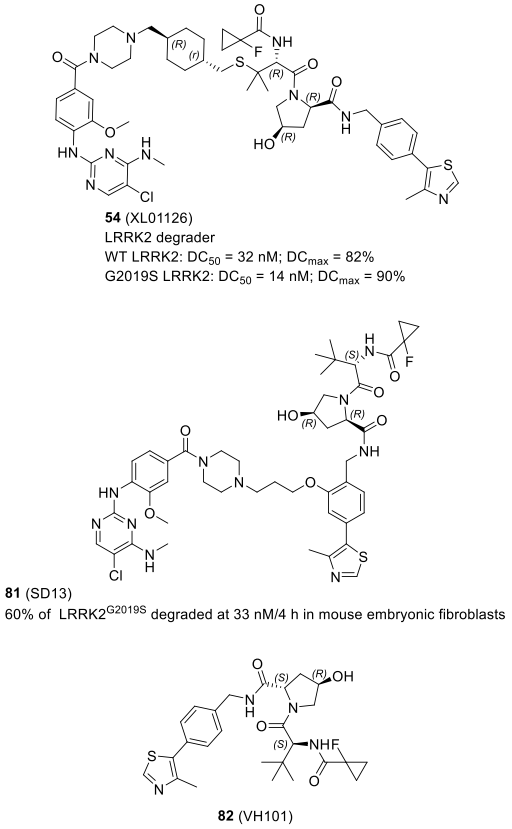


Figure 19. Chemical structures of compounds **54**, **81**, and **82**.

Besides, both WT and G2019S LRRK2 degradation could be blocked by the VHL ligand (compound **82**, Figure 19), neddylation inhibitor (compound **76**, see Figure 17), proteasome inhibitor (compound **74**, see Figure 17), and a PROTAC with an inverted hydroxyl group of hydroxyproline, and the degradation was mediated by the ubiquitin−proteasome system. Further, to assess its drug development potential, the in vitro physicochemical and ADME properties of compound **54** were detected including $t_{1/2}$ in mouse plasma, liver microsome, and hepatocytes. Finally, an in vivo pharmacokinetic profile study of compound **54** showed fast absorption in treatment with both IP (30 mg/kg, C_{max} = 7700 ng/mL, t_{max} = 0.25 min) and PO (30 mg/kg, C_{max} = 3620 ng/mL, t_{max} = 2 h) and was orally bioavailable (F = 15%). Notably, compound **54** is capable of penetrating the blood−brain barrier. Overall, this example can reflect the current commonly used process of developing PROTACs.

4. OUTLOOK FOR PROTAC DEVELOPMENT

4.1. Improving Physicochemical Properties. The Ro5, introduced in 1997, aids in the identification of drug candidates that are likely to have high oral bioavailability (MW ≤ 500, logP ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10).[105] Besides the traditional Ro5, the extended Ro5 (eRo5) and the beyond Ro5 (bRo5) have been introduced, with the former extending just outside Ro5 thresholds and the latter not conforming to Ro5 rules.[106,107] With advances in PROTAC research, the physicochemical properties associated with a favorable pharmacokinetics profile are becoming predictable. By searching the Roche compound library, Cantrill et al. found that PROTAC molecules fit into the bRo5 space in a 3D plot of PSA, MW, and AlogP, with a tendency for all three to decrease over time.[108] Moreover, accurate pK_a values of PROTACs can be measured through intact PROTAC molecules instead of fragments, even though PROTACs harbor multiple ionizable centers. The distribution coefficient logD is best measured via chromatographic methods.

Permeability is best defined via cellular permeability assays, for which incubation conditions need to be optimized. The rate of invalid PAMPA data for compounds in the bRo5 space is higher than that in the Ro5 space (14% versus 8%),[109] even though Cantrill et al. investigated several mechanistic refinements (i.e., incubation concentration and absolute quantification of compounds) for improving the permeability prediction by PAMPA.[108] Therefore, PAMPA may not be suitable for the permeability assessment of degraders. Thermodynamic solubility experiments should be used to obtain reliable values in aqueous media. Solubility is the important optimization parameter to focus on for improving the oral absorption of new degrader molecules. Two studies have shown that traditional in silico lipophilicity prediction methods may fail in the case of degraders.[110,111] Most recently, the PROTAC random tree model, a solubility decision-making tool, was developed, which can be implemented in pharma companies' drug discovery pipelines.[112] This model is based on one polarity (computed) and one lipophilicity (chromatographic) descriptors, TPSA and BRlogD,[113] respectively. One PROTAC with a TPSA value equal to or higher than 289.31 Å^2 is classified as highly soluble; otherwise, one PROTAC with BRlogD values equal to or higher than 2.58 are classified as low solubility. Moreover, flexibility should be considered with great attention, especially when molecules with cyclic moieties are investigated.[114] A strategy of amide-to-ester substitutions can be employed to improve the physicochemical properties of PROTAC-like model compounds.[60] Moreover, the design philosophy behind the orally dosed PROTACs compounds **1** and **2** (see Figure 3) includes a low hydrogen-bond donor count, high lipophilicity, and the incorporation of semirigid linkers. In the process of developing PROTACs, permeability may be the priority test item over solubility. Medium-chain fatty acid permeation enhancers and solid dispersion formulations can improve oral exposure to account for limited permeability and low solubility, respectively.[115]

Metrics, such as the multiparametric scoring function AB-MPS, have been developed to provide guidelines for bRo5 compounds (a lower AB-MPS score indicates a higher likelihood of absorption),[109] and recent analyses of these compounds have provided new descriptors for predicting absorption.[116,117] Edmondson et al. used the AB-MPS score alongside various in silico metrics associated with permeability/absorption [HBDs and HBAs, PSA, nRotB, $N_{rule\text{-}of\text{-}5}$, nAr, and cLogP/D] to analyze a representative set of 38 PROTACs across the 4 most commonly recruited E3 ligases (VHL, CRBN, cIAP, and MDM2).[118]

Most efforts focused on CRBN-targeting molecules because the lower molecular weight, reduced number of hydrogen-bond donors and receptors, and greater liposolubility of IMiDs offer a more oral drug-like starting point.[118,119] The Yang group developed an orally bioavailable prodrug for a CRBN-based PROTAC by increasing F from 1% to 68%.[120] In the PROTAC molecule library of AstraZeneca, thalidomide/pomalidomide/lenalidomide-derived CRBN E3 ligase ligands were used to show that high oral bioavailability (>30%) can be regularly achieved with PROTACs from multiple projects, which was rather unexpected.[121] Researchers concluded the following: (1) CRBN-based PROTACs show better oral bioavailability than

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

VHL-based PROTACs, (2) a value of chromatographic $LogD_{7.4}$ between 4.5 and 6.5 may indicate ideal oral bioavailability, and (3) PROTACs with high oral bioavailability violate 2−3 items of Ro5. Furthermore, the maximum achievable oral bioavailability tails off as lipophilicity is reduced. Due to high lipophilicity and chemical instability at a specific pH, especially for the glutarimide moiety of thalidomide/pomalidomide/lenalidomide-derived E3 ligase ligands, in most cases, conventional water-soluble and CACO-2 screening assay data indicate that orally bioavailable compounds would have restricted oral absorption. Simple kinetic aqueous solubility is an inadequate measure for predicting PROTAC absorption. However, solubility in biorelevant systems, such as FaSSIF/FeSSIF, showed marked improvements (>50-fold in some instances) and may be suitable for routine use.

Overall, compared to small-molecule drugs, prediction of the physical and chemical properties of PROTAC is still in the scattered exploration stage, and no obvious rules have been formed. We believe that "5 Rules of PROTAC" will definitely appear in the near future.

4.2. Computer-Aided Drug Design. In this rapidly growing field, the synthesis of numerous analogs to explore structural variations is required for the identification of potent degraders. Rational and efficient design PROTACs without extensive trial and error remains a challenge. In silico methods have been increasingly utilized for the efficient design of PROTACs and for predicting the structure of formed ternary complexes.

In 2019, Drummond et al. reported four modeling methods using the molecular operating environment modeling package to generate ensembles of PROTAC-mediated ternary complexes. In particular, the protein−protein docking-based Method 4 accurately complemented structural campaigns, representing a potential starting point for testing hypotheses and refining principles of PROTAC design.[122] In 2020, Method 4 was further improved by the same group to produce Method 4B, which yields noticeably superior predictions. Method 4B could be applied to a more realistic scenario in which the cocrystal structure of protein−ligand complexes is unavailable.[123] In addition, the PRosettaC model developed by Zaidman et al. in 2020 alternates between sampling of the protein−protein interaction space and the PROTAC molecule conformational space. The ternary complexes were successfully recapitulated in 6 out of 10 cases using PRosettaC, even though the global docking step was not optimized.[124] Furthermore, a structure-based modeling approach was developed by Bai et al. for predicting the efficacy and selectivity of a given PROTAC through the Rosetta software suite[125] and is freely available for research use.[126] More recently, the Hou group developed an integrative computational method named PROTAC-Model to predict ternary complex structures. This method can predict 8 cases out of 14 with medium or high quality, thus performing better than PRosettaC developed by Drummond et al.[127] Moreover, docking simulations and molecular dynamics have been used to discover optimal linker lengths and ideal sites for linker connection.[128,129] Recently, the HAPOD scoring method was developed based on an MD scoring approach for native configuration identification. This method accounts for both enthalpic and entropic contributions to ternary complex stability. It also allows the candidate pose to be generated through other sources, such as the online server PRosettaC. Such compatibility is very practical for medicinal chemists with limited MD experience.[130] Most recently, the Wang group reported that the MD-based MM/GBSA method is a useful tool for compound ranking, pose rescoring, and cooperativity prediction to further relax the ternary complex structure and visualize PROTAC-induced cooperativity.[131] The Yang group proposed a novel deep generative model for the rational design of PROTACs with optimal pharmacokinetics. Importantly, as a proof of concept, they experimentally tested 6 PROTACs, and 1 candidate demonstrated favorable pharmacokinetics in mice.[132] Therefore, greater expediency and lower cost are achieved for the structure-based iterative design of novel PROTACs.

With continuous advances in the field, an increasing number of potential PROTACs can be efficiently designed and synthesized through in silico methods.[124,125,133−135] For example, a global docking experiment using Rosetta was performed with the crystal structures of compound **11**-bound CRBN (PDB: 4TZ4) and compound **79**-bound $BRD4^{BD1}$ (PDB: 3MXF)[56] (Figure 20). The top 200 low-energy binding

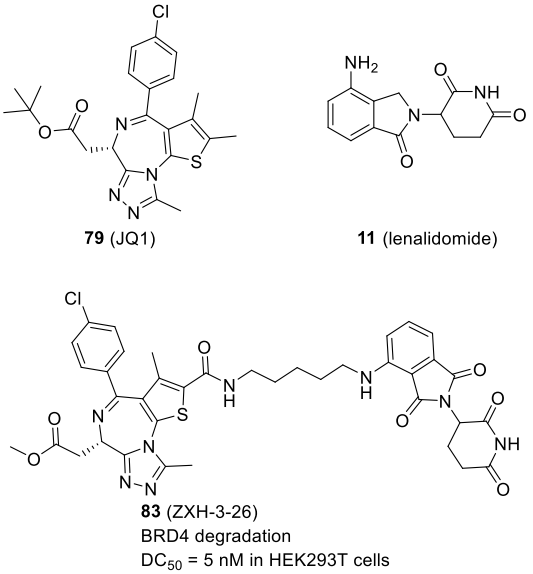


Figure 20. Chemical structures of compounds **11**, **79**, and **83**.

conformations were clustered to provide a rationale for the design of required minimal linker lengths. The distance between the selected solvent-exposed atoms of compounds **79** and **11** was 3−4 Å for all top 200 conformations, indicating that 2−3 atoms were sufficient to bridge the two moieties. Compound **83** (ZXH-3-26, Figure 20) was then synthesized, and immunoblot analysis confirmed that it was a highly selective degrader of BRD4 (DC_{50} = 5 nM) while inactive on BRD2 and BRD3. These novel in silico methods hold great promise for the future of PROTAC development.

5. PERSPECTIVES

PROTACs are bifunctional molecules that bind to an E3 ligase and target a POI, forming a ternary complex that leads to target ubiquitination and subsequent proteasomal degradation. In this paper, we provide advance strategies about PROTAC design from different aspects. Importantly, linker length, chemical composition of linker, and anchor points are critical for the physicochemical properties, degradation selectivity, and potential. In addition, we introduce the latest research progress on computational approaches to PROTACs design. We believe that the design will become more efficient, such as by utilizing CADD

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

or ternary complex crystal structure. Furthermore, due to the large molecular weight of PROTACs, the improvement of the PK/PD is challenging. Now the research of physicochemical properties is getting out of a trial-and-error situation, for example, rigid linker seems to be more conducive to achieving oral bioavailability. As more and more PROTACs are reported, the five rules belonging to PROTACs will definitely appear.

Notably, the development of E3 ligases in the PROTAC field is far from enough, and only a small part of E3 ligases in the human body has been utilized. Making full use of E3 ligases that have not been used in PROTACs will be conducive to the development of PROTACs with more therapeutic advantages. In addition, employment of E3 ligases with differential expression patterns is important to mitigate the limitations and increase efficiency of PROTACs. Currently, the most widely applied E3 ligases are CRBN and VHL. For development of CRBN-based PROTACs, efforts should be focused on the chemical stability of CRBN ligands and controllability of IMiD off-target effect. As the first oral bioavailable VHL-based PROTAC is reported, many other VHL-based PROTACs with good oral bioavailability will appear more and more quickly, which has important implications for the further cognition of good PK/PD properties in the PROTAC field.

However, one limitation needs to be further improved: Chronic PROTAC exposure can induce acquired drug resistance in tumor cells.[136,137] Drug resistance arises through point mutations in the target protein, which cause failure to form a ternary complex and enhanced target protein activity.[134] Zhang et al. found that genomic alteration of the E3 complex also led to resistance against PROTACs.[138] For the emergence of resistance, the following three aspects could be considered.

(1) Application of a new POI ligand or new E3 ligase. PROTACs containing different POI ligands that bind a new pocket of POI could be employed to slow down the resistance, such as the case of BCR-ABL inhibition by both a competitive and an allosteric inhibitor.[139] Moreover, expanding the E3 ligase toolbox is an attractive way. Mitsiades et al. found that the cancer cell that has been resistant to CRBN-based PROTAC is still sensitive to VHL-based PROTAC targeting the same oncoprotein or vice versa.[140]

(2) Dual-targeting treatment. On one hand, combined application of PROTAC and a small-molecule inhibitor may potentially prevent resistance. For example, Duncan et al. found that inhibition of multidrug-resistance receptor 1 resensitizes degrader-resistant cells to PROTACs targeting BET or CDK9.[141] On the other hand, the development of dual-targeting PROTACs should also be considered for overcoming the resistance. Such PROTACs contain either a dual-targeting warhead or two warheads that bind different targets separately. The former is like compound **84** (753b, Figure 21), a potent dual-BCL-2/BCL-xL degrader that was developed based on a dual-targeting POI ligand, compound **85** (ABT-263, Figure 21).[142] As an example of the latter, dual-targeting PROTACs, compound **86** (DP-C-1, Figure 21) or **87** (DP-V-4, Figure 21), encompass two warheads, compounds **88** (gefitinib, Figure 21) and **89** (olaparib, Figure 21), for simultaneous degradation of EGFR and PARP.[143]

(3) Inhibition of deubiquitinase. Deubiquitinase removed ubiquitin from target proteins, playing an important role in protein homeostasis mediated by the ubiquitin−proteasome system.[144−146] Much research has shown that deubiquitinase plays an important role in drug resistance.[147] Notably, deubiquitinase USP15 prevents CRBN-mediated substrate degradation by antagonizing ubiquitylation. Depletion of USP15 resensitizes IMiD-resistant multiple myeloma cells by promoting IMiD-induced degradation.[148] Therefore, identification and inhibition deubiquitinase that mediate resistance to

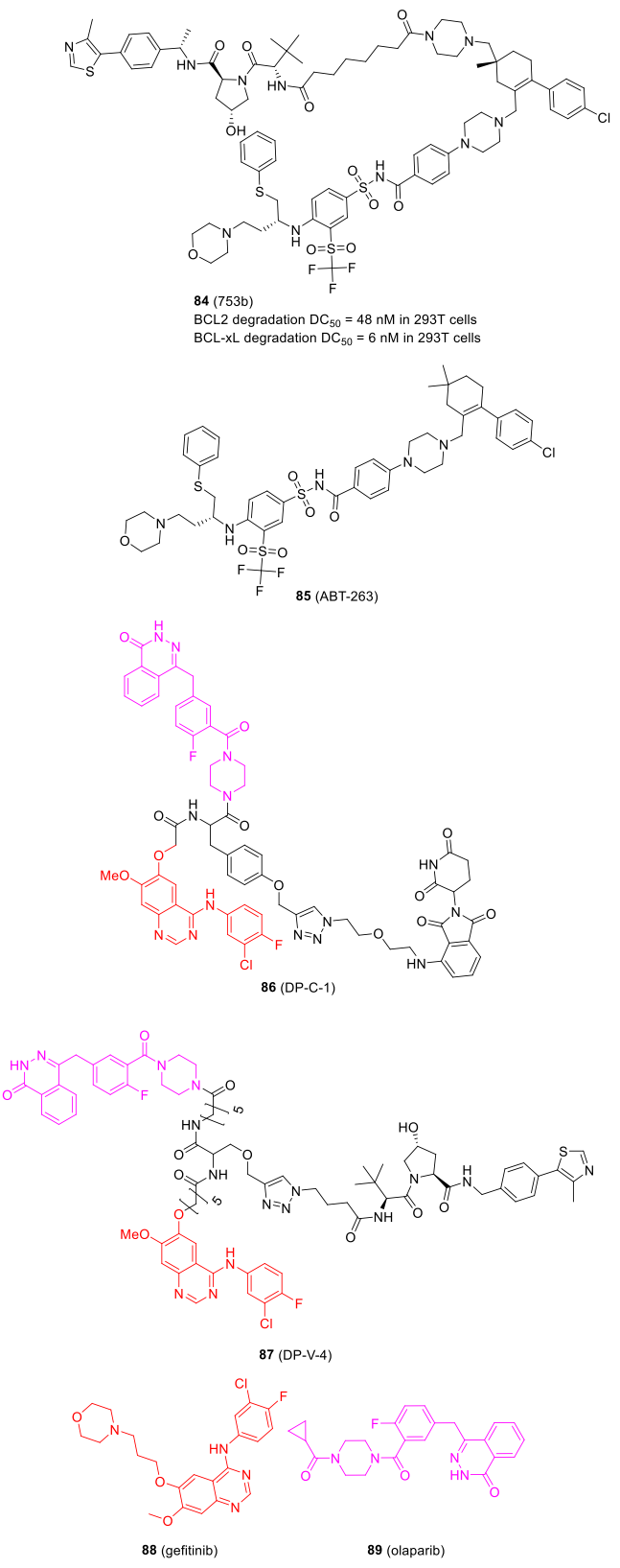


Figure 21. Chemical structures of compounds **84**−**89**.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

PROTACs may also an important method to achieve overcoming resistance and therapeutic response in cancer.

In conclusion, PROTAC technology has shown bright therapeutic prospects. We believe that PROTACs have the potential to give rise to novel targeted therapy drugs.

AUTHOR INFORMATION

Corresponding Authors

Ying Zhi − *School of Pharmacy and Pharmaceutical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117 Shandong, P. R. China*; Email: zhiying@sdfmu.edu.cn

Bo Liu − *School of Pharmacy and Pharmaceutical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117 Shandong, P. R. China*; Email: liubo@sdfmu.edu.cn

Qingqiang Yao − *School of Pharmacy and Pharmaceutical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117 Shandong, P. R. China*; Email: qqyao@sdfmu.edu.cn

Author

Minglei Li − *School of Pharmacy and Pharmaceutical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117 Shandong, P. R. China*; orcid.org/0000-0002-8381-2455

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.2c01555

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Minglei Li received his Ph.D. degree from China Pharmaceutical University in 2020 and is currently a postdoctoral student at the School of Pharmacy and Pharmaceutical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences. His research interests focus on the discovery, optimization, and molecular mechanism of candidate small-molecule compounds with antitumor activity.

Ying Zhi received her Ph.D. degree from Shanghai Institute of Materia Medica under the supervision of Ya-qiu Long in 2012. After performing postdoctoral studies at the RWTH Aachen with Dieter Enders in the area of organocatalysis, she returned to China. Since 2020, she has been an associate professor at Shandong First Medical University. Her research interests include synthesis of drug-like skeletons based on asymmetric catalysis and innovative drug discovery.

Bo Liu received his Ph.D. degree in Organic Chemistry from the University of Toyama in 2011. He is currently a assistant professor at the Shandong First Medical University & Shandong Academy of Medical Sciences. His current research interests focus on the design, synthesis, and biological evaluation of candidate small-molecule compounds (e.g., sphingosine kinase inhibitors) with potential anticancer activity.

Qingqiang Yao was born in 1965 in Shandong Province, People's Republic of China. He obtained his Ph.D. degree from Peking Union Medical College. His research is mainly focused on the design and development of (i) small-molecule drugs treating nonalcoholic steatohepatitis (NASH) and (ii) innovative antitumor drugs based on PROTACs.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No. 81903473), the Academic Promotion Programme of Shandong First Medical University (No. 2019LJ003), and the Postdoctoral Innovation Project of Shandong Province (No. 202103081).

ABBREVIATIONS USED

AB-MPS, AbbVie multiparametric score; bRo5, beyond Ro5; CRBN, cereblon; eRo5, extended Ro5; HAPOD, heating-accelerated pose departure; HBAs, hydrogen-bond acceptors; HBDs, hydrogen-bond donors; IMiDs, immunomodulatory imide drugs; MetAP-2, metaphydes-2; NAE, NEDD8-activating enzyme; nAr, number of aromatic rings; nRotB, number of rotatable bonds; $N_{rule\text{-}of\text{-}5}$, number of violations of Lipinski's rule of 5; PAMPA, parallel artificial membrane permeation assay; PDB, Protein Data Bank; POI, protein of interest; PROTAC, proteolysis-targeting chimera; PSA, polar surface area; Ro5, Lipinski's rule of 5; SCF, SKP1-Cullin-F-box; TPSA, topological polar surface area; UPS, ubiquitin-proteasome system; VHL, von Hippel−Lindau

REFERENCES

(1) Bai, L.; Zhou, H.; Xu, R.; Zhao, Y.; Chinnaswamy, K.; McEachern, D.; Chen, J.; Yang, C.-Y.; Liu, Z.; Wang, M.; Liu, L.; Jiang, H.; Wen, B.; Kumar, P.; Meagher, J. L.; Sun, D.; Stuckey, J. A.; Wang, S. A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression in vivo. *Cancer Cell.* **2019**, *36*, 498−511.

(2) 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.

(3) Burslem, G. M.; Schultz, A. R.; Bondeson, D. P.; Eide, C. A.; Savage Stevens, S. L.; 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.

(4) 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.

(5) Burslem, G. M.; Crews, C. M. Proteolysis-targeting chimeras as therapeutics and tools for biological discovery. *Cell.* **2020**, *181*, 102−114.

(6) Ermondi, G.; Garcia-Jimenez, D.; Caron, G. PROTACs and building blocks: The 2D chemical space in very early drug discovery. *Molecules.* **2021**, *26*, 672.

(7) Weng, G.; Cai, X.; Cao, D.; Du, H.; Shen, C.; Deng, Y.; He, Q.; Yang, B.; Li, D.; Hou, T. PROTAC-DB 2.0: an updated database of PROTACs. *Nucleic Acids Res.* **2023**, *51*, D1367.

(8) Sun, X.; Rao, Y. PROTACs as potential therapeutic agents for cancer drug resistance. *Biochemistry.* **2020**, *59*, 240−249.

(9) Gao, H.; Sun, X.; Rao, Y. PROTAC technology: opportunities and challenges. *ACS Med. Chem. Lett.* **2020**, *11*, 237−240.

(10) Nalawansha, D. A.; Crews, C. M. PROTACs: An emerging therapeutic modality in precision medicine. *Cell Chem. Biol.* **2020**, *27*, 998−1014.

(11) Zeng, S.; Huang, W.; Zheng, X.; Cheng, L.; Zhang, Z.; Wang, J.; Shen, Z. Proteolysis targeting chimera (PROTAC) in drug discovery paradigm: Recent progress and future challenges. *Eur. J. Med. Chem.* **2021**, *210*, 112981.

(12) Garber, K. The PROTAC gold rush. *Nat. Biotechnol.* **2022**, *40*, 12−16.

(13) Zhao, Q.; Lan, T.; Su, S.; Rao, Y. Induction of apoptosis in MDA-MB-231 breast cancer cells by a PARP1-targeting PROTAC small molecule. *Chem. Commun.* **2019**, *55*, 369−372.
(14) Buhimschi, A. D.; Armstrong, H. A.; Toure, M.; Jaime-Figueroa, S.; Chen, T. L.; Lehman, A. M.; Woyach, J. A.; Johnson, A. J.; Byrd, J. C.; Crews, C. M. Targeting the C481S ibrutinib-resistance mutation in Bruton's tyrosine kinase using PROTAC-mediated degradation. *Biochemistry.* **2018**, *57*, 3564−3575.
(15) Ward, C. C.; Kleinman, J. I.; Brittain, S. M.; Lee, P. S.; Chung, C. Y. S.; Kim, K.; Petri, Y.; Thomas, J. R.; Tallarico, J. A.; McKenna, J. M.; Schirle, M.; Nomura, D. K. Covalent ligand screening uncovers a RNF4 E3 ligase recruiter for targeted protein degradation applications. *ACS Chem. Biol.* **2019**, *14*, 2430−2440.
(16) 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.; Schirle, M.; Maimone, T. J.; Nomura, D. K. A nimbolide-based kinase degrader preferentially degrades oncogenic BCR-ABL. *ACS Chem. Biol.* **2020**, *15*, 1788−1794.
(17) Spradlin, J. N.; Hu, X.; Ward, C. C.; Brittain, S. M.; Jones, M. D.; Ou, L.; To, M.; Proudfoot, A.; Ornelas, E.; Woldegiorgis, M.; Olzmann, J. A.; Bussiere, D. E.; Thomas, J. R.; Tallarico, J. A.; McKenna, J. M.; Schirle, M.; Maimone, T. J.; Nomura, D. K. Harnessing the anti-cancer natural product nimbolide for targeted protein degradation. *Nat. Chem. Biol.* **2019**, *15*, 747−755.
(18) Ottis, P.; Toure, M.; Cromm, P. M.; Ko, E.; Gustafson, J. L.; Crews, C. M. Assessing different E3 ligases for small molecule induced protein ubiquitination and degradation. *ACS Chem. Biol.* **2017**, *12*, 2570−2578.
(19) Tong, B.; Luo, M.; Xie, Y.; Spradlin, J. N.; Tallarico, J. A.; McKenna, J. M.; Schirle, M.; Maimone, T. J.; Nomura, D. K. Bardoxolone conjugation enables targeted protein degradation of BRD4. *Sci. Rep.* **2020**, *10*, 15543.
(20) Lu, M.; Liu, T.; Jiao, Q.; Ji, J.; Tao, M.; Liu, Y.; You, Q.; Jiang, Z. Discovery of a Keap1-dependent peptide PROTAC to knockdown Tau by ubiquitination-proteasome degradation pathway. *Eur. J. Med. Chem.* **2018**, *146*, 251−259.
(21) Henning, N. J.; Manford, A. G.; Spradlin, J. N.; Brittain, S. M.; Zhang, E.; McKenna, J. M.; Tallarico, J. A.; Schirle, M.; Rape, M.; Nomura, D. K. Discovery of a covalent FEM1B recruiter for targeted protein degradation applications. *J. Am. Chem. Soc.* **2022**, *144*, 701−708.
(22) 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.
(23) Yan, W.; Pan, B.; Shao, J.; Lin, H.; Li, H. Feasible column chromatography-free, multi-gram scale synthetic process of VH032 amine, which could enable rapid PROTAC library construction. *ACS Omega.* **2022**, *7*, 26015−26021.
(24) Hu, J.; Hu, B.; Wang, M.; Xu, F.; Miao, B.; Yang, C.-Y.; Wang, M.; Liu, Z.; Hayes, D. F.; Chinnaswamy, K.; Delproposto, J.; Stuckey, J.; Wang, S. Discovery of ERD-308 as a highly potent proteolysis targeting chimera (PROTAC) degrader of estrogen receptor (ER). *J. Med. Chem.* **2019**, *62*, 1420−1442.
(25) Schumacher, H.; Smith, R. L.; Williams, R. T. The metabolism of thalidomide: The spontaneous hydrolysis of thalidomide in solution. *Br. J. Pharmacol. Chemother.* **1965**, *25*, 324−337.
(26) Schumacher, H.; Smith, R. L.; Williams, R. T. The metabolism of thalidomide: the fate of thalidomide and some of its hydrolysis products in various species. *Br. J. Pharmacol. Chemother.* **1965**, *25*, 338−351.
(27) Min, J.; Mayasundari, A.; Keramatnia, F.; Jonchere, B.; Yang, S. W.; Jarusiewicz, J.; Actis, M.; Das, S.; Young, B.; Slavish, J.; Yang, L.; Li, Y.; Fu, X.; Garrett, S. H.; Yun, M.; Li, Z.; Nithianantham, S.; Chai, S.; Chen, T.; Shelat, A.; Lee, R. E.; Nishiguchi, G.; White, S. W.; Roussel, M. F.; Potts, P. R.; Fischer, M.; Rankovic, Z. Phenyl-Glutarimides: Alternative cereblon binders for the design of PROTACs. *Angew. Chemie Int. Ed.* **2021**, *60*, 26663−26670.
(28) Kim, S. A.; Go, A.; Jo, S.-H.; Park, S. J.; Jeon, Y. U.; Kim, J. E.; Lee, H. K.; Park, C. H.; Lee, C.-O.; Park, S. G.; Kim, P.; Park, B. C.; Cho, S. Y.; Kim, S.; Ha, J. D.; Kim, J.-H.; Hwang, J. Y. A novel cereblon modulator for targeted protein degradation. *Eur. J. Med. Chem.* **2019**, *166*, 65−74.
(29) Hagner, P. R.; Man, H.-W.; Fontanillo, C.; Wang, M.; Couto, S.; Breider, M.; Bjorklund, C.; Havens, C. G.; Lu, G.; Rychak, E.; Raymon, H.; Narla, R. K.; Barnes, L.; Khambatta, G.; Chiu, H.; Kosek, J.; Kang, J.; Amantangelo, M. D.; Waldman, M.; Lopez-Girona, A.; Cai, T.; Pourdehnad, M.; Trotter, M.; Daniel, T. O.; Schafer, P. H.; Klippel, A.; Thakurta, A.; Chopra, R.; Gandhi, A. K. CC-122, a pleiotropic pathway modifier, mimics an interferon response and has antitumor activity in DLBCL. *Blood.* **2015**, *126*, 779−789.
(30) Yan, Y.; Shao, J.; Ding, D.; Pan, Y.; Tran, P.; Yan, W.; Wang, Z.; Li, H.; Huang, H. 3-Aminophthalic acid, a new cereblon ligand for targeted protein degradation by O'PROTAC. *Chem. Commun.* **2022**, *58*, 2383−2386.
(31) Smith, B. E.; Wang, S. L.; Jaime-Figueroa, S.; Harbin, A.; Wang, J.; Hamman, B. D.; Crews, C. M. Differential PROTAC substrate specificity dictated by orientation of recruited E3 ligase. *Nat. Commun.* **2019**, *10*, 131.
(32) Kofink, C.; Trainor, N.; Mair, B.; Wohrle, S.; Wurm, M.; Mischerikow, N.; Roy, M. J.; Bader, G.; Greb, P.; Garavel, G.; Diers, E.; McLennan, R.; Whitworth, C.; Vetma, V.; Rumpel, K.; Scharnweber, M.; Fuchs, J. E.; Gerstberger, T.; Cui, Y.; Gremel, G.; Chetta, P.; Hopf, S.; Budano, N.; Rinnenthal, J.; Gmaschitz, G.; Mayer, M.; Koegl, M.; Ciulli, A.; Weinstabl, H.; Farnaby, W. A selective and orally bioavailable VHL-recruiting PROTAC achieves SMARCA2 degradation in vivo. *Nat. Commun.* **2022**, *13*, 5969.
(33) Jaime-Figueroa, S.; Buhimschi, A. D.; Toure, M.; Hines, J.; Crews, C. M. Design, synthesis and biological evaluation of proteolysis targeting chimeras (PROTACs) as a BTK degraders with improved pharmacokinetic properties. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 126877.
(34) Lu, J.; Huang, Y.; Huang, J.; He, R.; Huang, M.; Lu, X.; Xu, Y.; Zhou, F.; Zhang, Z.; Ding, K. Discovery of the first examples of threonine tyrosine kinase PROTAC degraders. *J. Med. Chem.* **2022**, *65*, 2313−2328.
(35) Li, Y.; Yang, J.; Aguilar, A.; McEachern, D.; Przybranowski, S.; Liu, L.; Yang, C.; Wang, M.; Han, X.; Wang, S. Discovery of MD-224 as a first-in-class, highly potent, and efficacious proteolysis targeting chimera murine double minute 2 degrader capable of achieving complete and durable tumor regression. *J. Med. Chem.* **2019**, *62*, 448−466.
(36) Yang, X.; Wang, Z.; Pei, Y.; Song, N.; Xu, L.; Feng, B.; Wang, H.; Luo, X.; Hu, X.; Qiu, X.; Feng, H.; Yang, Y.; Zhou, Y.; Li, J.; Zhou, B. Discovery of thalidomide-based PROTAC small molecules as the highly efficient SHP2 degraders. *Eur. J. Med. Chem.* **2021**, *218*, 113341.
(37) Guo, W.; Qi, X.; Yu, X.; Liu, Y.; Chung, C.; Bai, F.; Lin, X.; Lu, D.; Wang, L.; Chen, J.; Su, L.; Nomie, K. J.; Li, F.; Wang, M.; Shu, X.; Onuchic, J.; Woyach, J. A.; Wang, M.; Wang, J. Enhancing intracellular accumulation and target engagement of PROTACs with reversible covalent chemistry. *Nat. Commun.* **2020**, *11*, 4268.
(38) Donoghue, C.; Cubillos-Rojas, M.; Gutierrez-Prat, N.; Sanchez-Zarzalejo, C.; Verdaguer, X.; Riera, A.; Nebreda, A. R. Optimal Linker length for small molecule PROTACs that selectively target p38α and p38β for degradation. *Eur. J. Med. Chem.* **2020**, *201*, 112451.
(39) Li, M.; Yang, Y.; Zhao, Q.; Wu, Y.; Song, L.; Yang, H.; He, M.; Gao, H.; Song, B.; Luo, J.; Rao, Y. Degradation versus inhibition: development of proteolysis-targeting chimeras for overcoming statin-induced compensatory upregulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. *J. Med. Chem.* **2020**, *63*, 4908−4928.
(40) Wu, H.; Yang, K.; Zhang, Z.; Leisten, E. D.; Li, Z.; Xie, H.; Liu, J.; Smith, K. A.; Novakova, Z.; Barinka, C.; Tang, W. Development of multifunctional histone deacetylase 6 degraders with potent anti-myeloma activity. *J. Med. Chem.* **2019**, *62*, 7042−7057.
(41) Zhang, J.; Che, J.; Luo, X.; Wu, M.; Kan, W.; Jin, Y.; Wang, H.; Pang, A.; Li, C.; Huang, W.; Zeng, S.; Zhuang, W.; Wu, Y.; Xu, Y.; Zhou, Y.; Li, J.; Dong, X. Structural feature analyzation strategies toward

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

discovery of orally bioavailable PROTACs of Bruton's tyrosine kinase for the treatment of lymphoma. *J. Med. Chem.* **2022**, *65*, 9096−9125.
(42) Lier, S.; Sellmer, A.; Orben, F.; Heinzlmeir, S.; Krauß, L.; Schneeweis, C.; Hassan, Z.; Schneider, C.; Schafer, A. P. G.; Pongratz, H.; Engleitner, T.; Ollinger, R.; Kuisl, A.; Bassermann, F.; Schlag, C.; Kong, B.; Dove, S.; Kuster, B.; Rad, R.; Reichert, M.; Wirth, M.; Saur, D.; Mahboobi, S.; Schneider, G. A novel cereblon E3 ligase modulator with antitumor activity in gastrointestinal cancer. *Bioorg. Chem.* **2022**, *119*, 105505.
(43) Matyskiela, M. E.; Lu, G.; Ito, T.; Pagarigan, B.; Lu, C.; Miller, K.; Fang, W.; Wang, N.; Nguyen, D.; Houston, J.; Carmel, G.; Tran, T.; Riley, M.; Nosaka, L.; Lander, G. C.; Gaidarova, S.; Xu, S.; Ruchelman, A. L.; Handa, H.; Carmichael, J.; Daniel, T. O.; Cathers, B. E.; Lopez-Girona, A.; Chamberlain, P. P. A novel cereblon modulator recruits GSPT1 to the CRL4CRBN ubiquitin ligase. *Nature* **2016**, *535*, 252−257.
(44) Donovan, K. A.; Ferguson, F. M.; Bushman, J. W.; Eleuteri, N. A.; Bhunia, D.; Ryu, S.; Tan, L.; Shi, K.; Yue, H.; Liu, X.; Dobrovolsky, D.; Jiang, B.; Wang, J.; Hao, M.; You, I.; Teng, M.; Liang, Y.; Hatcher, J.; Li, Z.; Manz, T. D.; Groendyke, B.; Hu, W.; Nam, Y.; Sengupta, S.; Cho, H.; Shin, I.; Agius, M. P.; Ghobrial, I. M.; Ma, M. W.; Che, J.; Buhrlage, S. J.; Sim, T.; Gray, N. S.; Fischer, E. S. Mapping the degradable kinome provides a resource for expedited degrader development. *Cell* **2020**, *183*, 1714−1731.
(45) Wu, H.; Yang, K.; Zhang, Z.; Leisten, E. D.; Li, Z.; Xie, H.; Liu, J.; Smith, K. A.; Novakova, Z.; Barinka, C.; Tang, W. Development of multifunctional histone deacetylase 6 degraders with potent anti-myeloma activity. *J. Med. Chem.* **2019**, *62*, 7042−7057.
(46) Donovan, K. A.; Ferguson, F. M.; Bushman, J. W.; Eleuteri, N. A.; Bhunia, D.; Ryu, S.; Tan, L.; Shi, K.; Yue, H.; Liu, X.; Dobrovolsky, D.; Jiang, B.; Wang, J.; Hao, M.; You, I.; Teng, M.; Liang, Y.; Hatcher, J.; Li, Z.; Manz, T. D.; Groendyke, B.; Hu, W.; Nam, Y.; Sengupta, S.; Cho, H.; Shin, I.; Agius, M. P.; Ghobrial, I. M.; Ma, M. W.; Che, J.; Buhrlage, S. J.; Sim, T.; Gray, N. S.; Fischer, E. S. Mapping the degradable kinome provides a resource for expedited degrader development. *Cell* **2020**, *183*, 1714−1731.
(47) Yu, X.; Xu, J.; Shen, Y.; Cahuzac, K. M.; Park, K.; Dale, B.; Liu, J.; Parsons, R. E.; Jin, J. Discovery of potent, selective, and in vivo efficacious AKT kinase protein degraders via structure−activity relationship studies. *J. Med. Chem.* **2022**, *65*, 3644−3666.
(48) Yokoo, H.; Shibata, N.; Naganuma, M.; Murakami, Y.; Fujii, K.; Ito, T.; Aritake, K.; Naito, M.; Demizu, Y. Development of a hematopoietic prostaglandin D synthase-degradation inducer. *ACS Med. Chem. Lett.* **2021**, *12*, 236−241.
(49) Chen, L.; Han, L.; Mao, S.; Xu, P.; Xu, X.; Zhao, R.; Wu, Z.; Zhong, K.; Yu, G.; Wang, X. Discovery of A031 as effective proteolysis targeting chimera (PROTAC) androgen receptor (AR) degrader for the treatment of prostate cancer. *Eur. J. Med. Chem.* **2021**, *216*, 113307.
(50) Chung, C.; Dai, H.; Fernandez, E.; Tinworth, C. P.; Churcher, I.; Cryan, J.; Denyer, J.; Harling, J. D.; Konopacka, A.; Queisser, M. A.; Tame, C. J.; Watt, G.; Jiang, F.; Qian, D.; Benowitz, A. B. Structural insights into PROTAC-mediated degradation of Bcl-xL. *ACS Chem. Biol.* **2020**, *15*, 2316−2323.
(51) Khan, S.; Zhang, X.; Lv, D.; Zhang, Q.; He, Y.; Zhang, P.; Liu, X.; Thummuri, D.; Yuan, Y.; Wiegand, J. S.; Pei, J.; Zhang, W.; Sharma, A.; McCurdy, C. R.; Kuruvilla, V. M.; Baran, N.; Ferrando, A. A.; Kim, Y.; Rogojina, A.; Houghton, P. J.; Huang, G.; Hromas, R.; Konopleva, M.; Zheng, G.; Zhou, D. A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* **2019**, *25*, 1938−1947.
(52) He, Y.; Zhang, X.; Chang, J.; Kim, H.-N.; Zhang, P.; Wang, Y.; Khan, S.; Liu, X.; Zhang, X.; Lv, D.; Song, L.; Li, W.; Thummuri, D.; Yuan, Y.; Wiegand, J. S.; Ortiz, Y. T.; Budamagunta, V.; Elisseeff, J. H.; Campisi, J.; Almeida, M.; Zheng, G.; Zhou, D. Using proteolysis-targeting chimera technology to reduce navitoclax platelet toxicity and improve its senolytic activity. *Nat. Commun.* **2020**, *11*, 1996.
(53) Nieto-Jiménez, C.; Morafraile, E. C.; Alonso-Moreno, C.; Ocaña, A. Clinical considerations for the design of PROTACs in cancer. *Mol. Cancer.* **2022**, *21*, 67.
(54) Ishida, T.; Ciulli, A. E3 ligase ligands for PROTACs: How they were found and How to discover new ones. *SLAS Discovery* **2021**, *26*, 484−502.
(55) Jones, L. H.; Mitchell, C. A.; Loberg, L.; Pavkovic, M.; Rao, M.; Roberts, R.; Stamp, K.; Volak, L.; Wittwer, M. B.; Pettit, S. Targeted protein degraders: a call for collective action to advance safety assessment. *Nat. Rev. Drug Discovery* **2022**, *21*, 401−402.
(56) Nowak, R. P.; DeAngelo, S. L.; Buckley, D.; He, Z.; Donovan, K. A.; An, J.; Safaee, N.; Jedrychowski, M. P.; Ponthier, C. M.; Ishoey, M.; Zhang, T.; Mancias, J. D.; Gray, N. S.; Bradner, J. E.; Fischer, E. S. Plasticity in binding confers selectivity in ligand-induced protein degradation. *Nat. Chem. Biol.* **2018**, *14*, 706−714.
(57) 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.; Crew, A. P.; Hines, J.; Crews, C. M. The advantages of targeted protein degradation over inhibition: An RTK case study. *Cell Chem. Biol.* **2018**, *25*, 67−77.
(58) Zoppi, V.; Hughes, S. J.; Maniaci, C.; Testa, A.; Gmaschitz, T.; Wieshofer, C.; Koegl, M.; Riching, K. M.; Daniels, D. L.; Spallarossa, A.; Ciulli, A. Iterative design and optimization of initially inactive proteolysis targeting chimeras (PROTACs) identify VZ185 as a potent, fast, and selective von Hippel−Lindau (VHL) based dual degrader probe of BRD9 and BRD7. *J. Med. Chem.* **2019**, *62*, 699−726.
(59) Ohoka, N.; Okuhira, K.; Ito, M.; Nagai, K.; Shibata, N.; Hattori, T.; Ujikawa, O.; Shimokawa, K.; Sano, O.; Koyama, R.; Fujita, H.; Teratani, M.; Matsumoto, H.; Imaeda, Y.; Nara, H.; Cho, N.; Naito, M. In vivo knockdown of pathogenic proteins via specific and nongenetic inhibitor of apoptosis protein (IAP)-dependent protein erasers (SNIPERs). *J. Biol. Chem.* **2017**, *292*, 4556−4570.
(60) Klein, V. G.; Bond, A. G.; Craigon, C.; Lokey, R. S.; Ciulli, A. Amide-to-ester substitution as a strategy for optimizing PROTAC permeability and cellular activity. *J. Med. Chem.* **2021**, *64*, 18082−18101.
(61) 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.
(62) Ren, C.; Sun, N.; Liu, H.; Kong, Y.; Sun, R.; Qiu, X.; Chen, J.; Li, Y.; Zhang, J.; Zhou, Y.; Zhong, H.; Yin, Q.; Song, X.; Yang, X.; Jiang, B. Discovery of a brigatinib degrader SIAIS164018 with destroying metastasis-related oncoproteins and a reshuffling kinome profile. *J. Med. Chem.* **2021**, *64*, 9152−9165.
(63) Sun, N.; Ren, C.; Kong, Y.; Zhong, H.; Chen, J.; Li, Y.; Zhang, J.; Zhou, Y.; Qiu, X.; Lin, H.; Song, X.; Yang, X.; Jiang, B. Development of a Brigatinib degrader (SIAIS117) as a potential treatment for ALK positive cancer resistance. *Eur. J. Med. Chem.* **2020**, *193*, 112190.
(64) Schiemer, J.; Horst, R.; Meng, Y.; Montgomery, J. I.; Xu, Y.; Feng, X.; Borzilleri, K.; Uccello, D. P.; Leverett, C.; Brown, S.; Che, Y.; Brown, M. F.; Hayward, M. M.; Gilbert, A. M.; Noe, M. C.; Calabrese, M. F. Snapshots and ensembles of BTK and cIAP1 protein degrader ternary complexes. *Nat. Chem. Biol.* **2021**, *17*, 152−160.
(65) Zorba, A.; Nguyen, C.; Xu, Y.; Starr, J.; Borzilleri, K.; Smith, J.; Zhu, H.; Farley, K. A.; Ding, W.; Schiemer, J.; Feng, X.; Chang, J. S.; Uccello, D. P.; Young, J. A.; Garcia-Irrizary, C. N.; Czabaniuk, L.; Schuff, B.; Oliver, R.; Montgomery, J.; Hayward, M. M.; Coe, J.; Chen, J.; Niosi, M.; Luthra, S.; Shah, J. C.; El-Kattan, A.; Qiu, X.; West, G. M.; Noe, M. C.; Shanmugasundaram, V.; Gilbert, A. M.; Brown, M. F.; Calabrese, M. F. Delineating the role of cooperativity in the design of potent PROTACs for BTK. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, E7285−E7292.
(66) Bemis, T. A.; La Clair, J. J.; Burkart, M. D. Unraveling the role of linker design in proteolysis targeting chimeras. *J. Med. Chem.* **2021**, *64*, 8042−8052.
(67) Xia, L.; Ba, M.; Liu, W.; Cheng, W.; Hu, C.; Zhao, Q.; Yao, Y.; Sun, M.; Duan, Y. Triazol: a privileged scaffold for proteolysis targeting chimeras. *Future Med. Chem.* **2019**, *11*, 2919−2973.
(68) Law, R. P.; Nunes, J.; Chung, C.; Bantscheff, M.; Buda, K.; Dai, H.; Evans, J. P.; Flinders, A.; Klimaszewska, D.; Lewis, A. J.; Muelbaier, M.; Scott-Stevens, P.; Stacey, P.; Tame, C. J.; Watt, G. F.; Zinn, N.;

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

Queisser, M. A.; Harling, J. D.; Benowitz, A. B. Discovery and characterisation of highly cooperative FAK-Degrading PROTACs. *Angew. Chemie Int. Ed.* **2021**, *60*, 23327−23334.
(69) Du, G.; Jiang, J.; Wu, Q.; Henning, N. J.; Donovan, K. A.; Yue, H.; Che, J.; Lu, W.; Fischer, E. S.; Bardeesy, N.; Zhang, T.; Gray, N. S. Discovery of a potent degrader for fibroblast growth factor receptor 1/2. *Angew. Chemie Int. Ed.* **2021**, *60*, 15905−15911.
(70) Xiang, W.; Zhao, L.; Han, X.; Qin, C.; Miao, B.; McEachern, D.; Wang, Y.; Metwally, H.; Kirchhoff, P. D.; Wang, L.; Matvekas, A.; He, M.; Wen, B.; Sun, D.; Wang, S. Discovery of ARD-2585 as an exceptionally potent and orally active PROTAC degrader of androgen receptor for the treatment of advanced prostate cancer. *J. Med. Chem.* **2021**, *64*, 13487−13509.
(71) Han, X.; Zhao, L.; Xiang, W.; Qin, C.; Miao, B.; McEachern, D.; Wang, Y.; Metwally, H.; Wang, L.; Matvekas, A.; Wen, B.; Sun, D.; Wang, S. Strategies toward discovery of potent and orally bioavailable proteolysis targeting chimera degraders of androgen receptor for the treatment of prostate cancer. *J. Med. Chem.* **2021**, *64*, 12831−12854.
(72) Du, Y.; Chen, Y.; Wang, Y.; Chen, J.; Lu, X.; Zhang, L.; Li, Y.; Wang, Z.; Ye, G.; Zhang, G. HJM-561, a potent, selective, and orally bioavailable EGFR PROTAC that overcomes Osimertinib-resistant EGFR triple mutations. *Mol. Cancer Ther.* **2022**, *21*, 1060−1066.
(73) Ren, C. W.; Sun, N.; Kong, Y.; Qu, X. J.; Liu, H. X.; Zhong, H.; Song, X. L.; Yang, X. B.; Jiang, B. Structure-based discovery of SIAIS001 as an oral bioavailability ALK degrader constructed from Alectinib. *Eur. J. Med. Chem.* **2021**, *217*, 113335.
(74) Neklesa, T.; Snyder, L. B.; Willard, R. R.; Vitale, N.; Pizzano, J.; Gordon, D. A.; Bookbinder, M.; Macaluso, J.; Dong, H.; Ferraro, C.; Wang, G.; Wang, J.; Crews, C. M.; Houston, J.; Crew, A. P.; Taylor, I. ARV-110: an oral androgen receptor PROTAC degrader for prostate cancer. *J. Clin. Oncol.* **2019**, *37*, 259.
(75) Flanagan, J.; Qian, Y; Gough, S.; Andreoli, M; Bookbinder, M; Cadelina, G; Bradley, J; Rousseau, E; Willard, R; Pizzano, J; Crews, C.; Crew, A.; Taylor, I; Houston, J Abstract P5-04-18: ARV-471, an oral estrogen receptor PROTAC degrader for Breast Cancer. *Cancer Res.* **2019**, *79*, P5-04-18.
(76) Snyder, L. B.; Flanagan, J. J.; Qian, Y.; Gough, S. M.; Andreoli, M.; Bookbinder, M.; Cadelina, G.; Bradley, J.; Rousseau, E.; Chandler, J.; Willard, R.; Pizzano, J.; Crews, C. M.; Crew, A. P.; Houston, J.; Moore, M. D.; Peck, R.; Taylor, I. The discovery of ARV-471, an orally bioavailable estrogen receptor degrading PROTAC for the treatment of patients with breast cancer. *Cancer Res.* **2021**, *81*, 44.
(77) Atilaw, Y.; Poongavanam, V.; Svensson Nilsson, C.; Nguyen, D.; Giese, A.; Meibom, D.; Erdelyi, M.; Kihlberg, J. Solution conformations shed light on PROTAC cell permeability. *ACS Med. Chem. Lett.* **2021**, *12*, 107−114.
(78) Liu, X.; Kalogeropulou, A. F.; Domingos, S.; Makukhin, N.; Nirujogi, R. S.; Singh, F.; Shpiro, N.; Saalfrank, A.; Sammler, E.; Ganley, I. G.; Moreira, R.; Alessi, D. R.; Ciulli, A. Discovery of XL01126: a potent, fast, cooperative, selective, orally bioavailable and blood brain barrier penetrant proteolysis targeting chimera degrader of leucine rich repeat kinase 2. *J. Am. Chem. Soc.* **2022**, *144*, 16930−16952.
(79) Watson, E. R.; Novick, S.; Matyskiela, M. E.; Chamberlain, P. P.; H. de la Pena, A.; Zhu, J.; Tran, E.; Griffin, P. R.; Wertz, I. E.; Lander, G. C. Molecular glue CELMoD compounds are regulators of cereblon conformation. *Science* **2022**, *378*, 549−553.
(80) Bemis, T. A.; La Clair, J. J.; Burkart, M. D. Traceless Staudinger ligation enabled parallel synthesis of proteolysis targeting chimera linker variants. *Chem. Commun.* **2021**, *57*, 1026−1029.
(81) Krajcovicova, S.; Jorda, R.; Hendrychova, D.; Krystof, V.; Soural, M. Solid-phase synthesis for thalidomide-based proteolysis-targeting chimeras (PROTAC). *Chem. Commun.* **2019**, *55*, 929−932.
(82) Wurz, R. P.; Dellamaggiore, K.; Dou, H.; Javier, N.; Lo, M.-C.; McCarter, J. D.; Mohl, D.; Sastri, C.; Lipford, J. R.; Cee, V. J. A "Click Chemistry Platform" for the rapid synthesis of bispecific molecules for inducing protein degradation. *J. Med. Chem.* **2018**, *61*, 453−461.
(83) Papatzimas, J.; Gorobets, E.; Brownsey, D.; Maity, R.; Bahlis, N.; Derksen, D. A general strategy for the preparation of thalidomide-conjugate linkers. *Synlett.* **2017**, *28*, 2881−2885.
(84) Guo, L.; Zhou, Y.; Nie, X.; Zhang, Z.; Zhang, Z.; Li, C.; Wang, T.; Tang, W. A platform for the rapid synthesis of proteolysis targeting chimeras (Rapid-TAC) under miniaturized conditions. *Eur. J. Med. Chem.* **2022**, *236*, 114317.
(85) Bhela, I. P.; Ranza, A.; Balestrero, F. C.; Serafini, M.; Aprile, S.; Di Martino, R. M. C.; Condorelli, F.; Pirali, T. A versatile and sustainable multicomponent platform for the synthesis of protein degraders: Proof-of-Concept application to BRD4-degrading PROTACs. *J. Med. Chem.* **2022**, *65*, 15282−15299.
(86) Kawamata, Y.; Hayashi, K.; Carlson, E.; Shaji, S.; Waldmann, D.; Simmons, B. J.; Edwards, J. T.; Zapf, C. W.; Saito, M.; Baran, P. S. Chemoselective electrosynthesis using rapid alternating polarity. *J. Am. Chem. Soc.* **2021**, *143*, 16580−16588.
(87) Brownsey, D. K.; Rowley, B. C.; Gorobets, E.; Gelfand, B. S.; Derksen, D. J. Rapid synthesis of pomalidomide-conjugates for the development of protein degrader libraries. *Chem. Sci.* **2021**, *12*, 4519−4525.
(88) Qiu, X.; Sun, N.; Kong, Y.; Li, Y.; Yang, X.; Jiang, B. Chemoselective synthesis of lenalidomide-based PROTAC library using alkylation reaction. *Org. Lett.* **2019**, *21*, 3838−3841.
(89) Gadd, M. S.; Testa, A.; Lucas, X.; Chan, K.-H.; Chen, W.; Lamont, D. J.; Zengerle, M.; Ciulli, A. Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat. Chem. Biol.* **2017**, *13*, 514−521.
(90) Testa, A.; Hughes, S. J.; Lucas, X.; Wright, J. E.; Ciulli, A. Structure-based design of a macrocyclic PROTAC. *Angew. Chemie Int. Ed.* **2020**, *59*, 1727−1734.
(91) Farnaby, W.; Koegl, M.; Roy, M. J.; Whitworth, C.; Diers, E.; Trainor, N.; Zollman, D.; Steurer, S.; Karolyi-Oezguer, J.; Riedmueller, C.; Gmaschitz, T.; Wachter, J.; Dank, C.; Galant, M.; Sharps, B.; Rumpel, K.; Traxler, E.; Gerstberger, T.; Schnitzer, R.; Petermann, O.; Greb, P.; Weinstabl, H.; Bader, G.; Zoephel, A.; Weiss-Puxbaum, A.; Ehrenhöfer-Wölfer, K.; Wöhrle, S.; Boehmelt, G.; Rinnenthal, J.; Arnhof, H.; Wiechens, N.; Wu, M.-Y.; Owen-Hughes, T.; Ettmayer, P.; Pearson, M.; McConnell, D. B.; Ciulli, A. BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat. Chem. Biol.* **2019**, *15*, 672−680.
(92) Zeng, M.; Xiong, Y.; Safaee, N.; Nowak, R. P.; Donovan, K. A.; Yuan, C. J.; Nabet, B.; Gero, T. W.; Feru, F.; Li, L.; Gondi, S.; Ombelets, L. J.; Quan, C.; Jänne, P. A.; Kostic, M.; Scott, D. A.; Westover, K. D.; Fischer, E. S.; Gray, N. S. Exploring targeted degradation strategy for oncogenic KRASG12C. *Cell Chem. Biol.* **2020**, *27*, 19−31.
(93) 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.
(94) Yu, X.; Li, D.; Kottur, J.; Shen, Y.; Kim, H. S.; Park, K.-S.; Tsai, Y.-H.; Gong, W.; Wang, J.; Suzuki, K.; Parker, J.; Herring, L.; Kaniskan, H.Ü.; Cai, L.; Jain, R.; Liu, J.; Aggarwal, A. K.; Wang, G. G.; Jin, J. A selective WDR5 degrader inhibits acute myeloid leukemia in patient-derived mouse models. *Sci. Transl. Med.* **2021**, *13*, eabj1578.
(95) Dragovich, P. S.; Pillow, T. H.; Blake, R. A.; Sadowsky, J. D.; Adaligil, E.; Adhikari, P.; Chen, J.; Corr, N.; dela Cruz-Chuh, J.; Del Rosario, G.; Fullerton, A.; Hartman, S. J.; Jiang, F.; Kaufman, S.; Kleinheinz, T.; Kozak, K. R.; Liu, L.; Lu, Y.; Mulvihill, M. M.; Murray, J. M.; O'Donohue, A.; Rowntree, R. K.; Sawyer, W. S.; Staben, L. R.; Wai, J.; Wang, J.; Wei, B.; Wei, W.; Xu, Z.; Yao, H.; Yu, S.-F.; Zhang, D.; Zhang, H.; Zhang, S.; Zhao, Y.; Zhou, H.; Zhu, X. Antibody-mediated delivery of chimeric BRD4 degraders. Part 2: Improvement of in vitro antiproliferation activity and in vivo antitumor efficacy. *J. Med. Chem.* **2021**, *64*, 2576−2607.
(96) Riching, K. M.; Mahan, S.; Corona, C. R.; McDougall, M.; Vasta, J. D.; Robers, M. B.; Urh, M.; Daniels, D. L. Quantitative live-cell kinetic degradation and mechanistic profiling of PROTAC mode of action. *ACS Chem. Biol.* **2018**, *13*, 2758−2770.
(97) Yu, X.; Cheng, M.; Lu, K.; Shen, Y.; Zhong, Y.; Liu, J.; Xiong, Y.; Jin, J. Exploring degradation of mutant and wild-type epidermal growth factor receptors induced by proteolysis-targeting chimeras. *J. Med. Chem.* **2022**, *65*, 8416−8443.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

(98) Qu, X.; Liu, H.; Song, X.; Sun, N.; Zhong, H.; Qiu, X.; Yang, X.; Jiang, B. Effective degradation of EGFRL858R+T790M mutant proteins by CRBN-based PROTACs through both proteosome and autophagy/lysosome degradation systems. *Eur. J. Med. Chem.* **2021**, *218*, 113328.
(99) Zhao, H.; Yang, X.; Lei, H.; Xi, X.; Lu, S.; Zhang, J.; Xin, M.; Zhang, S. Discovery of potent small molecule PROTACs targeting mutant EGFR. *Eur. J. Med. Chem.* **2020**, *208*, 112781.
(100) Fischer, E. S.; Böhm, K.; Lydeard, J. R.; Yang, H.; Stadler, M. B.; Cavadini, S.; Nagel, J.; Serluca, F.; Acker, V.; Lingaraju, G. M.; Tichkule, R. B.; Schebesta, M.; Forrester, W. C.; Schirle, M.; Hassiepen, U.; Ottl, J.; Hild, M.; Beckwith, R. E. J.; Harper, J. W.; Jenkins, J. L.; Thomä, N. H. Structure of the DDB1−CRBN E3 ubiquitin ligase in complex with thalidomide. *Nature* **2014**, *512*, 49−53.
(101) Han, X. R.; Chen, L.; Wei, Y.; Yu, W.; Chen, Y.; Zhang, C.; Jiao, B.; Shi, T.; Sun, L.; Zhang, C.; Xu, Y.; Lee, M. R.; Luo, Y.; Plewe, M. B.; Wang, J. Discovery of selective small molecule degraders of BRAF-V600E. *J. Med. Chem.* **2020**, *63*, 4069−4080.
(102) 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.
(103) Crew, A. P.; Raina, K.; Dong, H.; Qian, Y.; Wang, J.; Vigil, D.; Serebrenik, Y. V.; Hamman, B. D.; Morgan, A.; Ferraro, C.; Siu, K.; Neklesa, T. K.; Winkler, J. D.; Coleman, K. G.; Crews, C. M. Identification and characterization of von Hippel-Lindau-recruiting proteolysis targeting chimeras (PROTACs) of TANK-binding kinase 1. *J. Med. Chem.* **2018**, *61*, 583−598.
(104) Chan, K.; Zengerle, M.; Testa, A.; Ciulli, A. Impact of target warhead and linkage vector on inducing protein degradation: comparison of bromodomain and extra-terminal (BET) degraders derived from triazolodiazepine (JQ1) and tetrahydroquinoline (I-BET726) BET inhibitor scaffolds. *J. Med. Chem.* **2018**, *61*, 504−513.
(105) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **2012**, *64*, 4−17.
(106) Doak, B. C.; Over, B.; Giordanetto, F.; Kihlberg, J. Oral druggable space beyond the rule of 5: insights from drugs and clinical candidates. *Chem. Biol.* **2014**, *21*, 1115−1142.
(107) Matsson, P.; Doak, B. C.; Over, B.; Kihlberg, J. Cell permeability beyond the rule of 5. *Adv. Drug Delivery Rev.* **2016**, *101*, 42−61.
(108) Cantrill, C.; Chaturvedi, P.; Rynn, C.; Petrig Schaffland, J.; Walter, I.; Wittwer, M. B. Fundamental aspects of DMPK optimization of targeted protein degraders. *Drug Discovery Today.* **2020**, *25*, 969−982.
(109) DeGoey, D. A.; Chen, H.-J.; Cox, P. B.; Wendt, M. D. Beyond the rule of 5: lessons learned from AbbVie's drugs and compound collection. *J. Med. Chem.* **2018**, *61*, 2636−2651.
(110) Steinebach, C.; Sosič, I.; Lindner, S.; Bricelj, A.; Kohl, F.; Ng, Y. L. D.; Monschke, M.; Wagner, K. G.; Krönke, J.; Gütschow, M. A MedChem toolbox for cereblon-directed PROTACs. *Medchemcomm.* **2019**, *10*, 1037−1041.
(111) Ermondi, G.; Vallaro, M.; Caron, G. Degraders early developability assessment: face-to-face with molecular properties. *Drug Discovery Today.* **2020**, *25*, 1585−1591.
(112) García Jiménez, D.; Rossi Sebastiano, M.; Vallaro, M.; Mileo, V.; Pizzirani, D.; Moretti, E.; Ermondi, G.; Caron, G. Designing soluble PROTACs: strategies and preliminary guidelines. *J. Med. Chem.* **2022**, *65*, 12639−12649.
(113) Ermondi, G.; Vallaro, M.; Goetz, G.; Shalaeva, M.; Caron, G. Experimental lipophilicity for beyond Rule of 5 compounds. *Future Drug. Discovery* **2019**, *1*, 2631−3316.
(114) Caron, G.; Digiesi, V.; Solaro, S.; Ermondi, G. Flexibility in early drug discovery: focus on the beyond-Rule-of-5 chemical space. *Drug Discovery Today.* **2020**, *25*, 621−627.
(115) Benowitz, A. B.; Scott-Stevens, P. T.; Harling, J. D. Challenges and opportunities for in vivo PROTAC delivery. *Future Med. Chem.* **2022**, *14*, 119−121.
(116) Doak, B. C.; Zheng, J.; Dobritzsch, D.; Kihlberg, J. How beyond rule of 5 drugs and clinical candidates bind to their targets. *J. Med. Chem.* **2016**, *59*, 2312−2327.
(117) Poongavanam, V.; Doak, B. C.; Kihlberg, J. Opportunities and guidelines for discovery of orally absorbed drugs in beyond rule of 5 space. *Curr. Opin. Chem. Biol.* **2018**, *44*, 23−29.
(118) Edmondson, S. D.; Yang, B.; Fallan, C. Proteolysis targeting chimeras (PROTACs) in 'beyond rule-of-five' chemical space: Recent progress and future challenges. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1555−1564.
(119) Maple, H. J.; Clayden, N.; Baron, A.; Stacey, C.; Felix, R. Developing degraders: principles and perspectives on design and chemical space. *Medchemcomm.* **2019**, *10*, 1755−1764.
(120) Wei, M.; Zhao, R.; Cao, Y.; Wei, Y.; Li, M.; Dong, Z.; Liu, Y.; Ruan, H.; Li, Y.; Cao, S.; Tang, Z.; Zhou, Y.; Song, W.; Wang, Y.; Wang, J.; Yang, G.; Yang, C. 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.
(121) Pike, A.; Williamson, B.; Harlfinger, S.; Martin, S.; McGinnity, D. F. Optimising proteolysis-targeting chimeras (PROTACs) for oral drug delivery: A drug metabolism and pharmacokinetics perspective. *Drug Discovery Today.* **2020**, *25*, 1793−1800.
(122) Drummond, M. L.; Williams, C. I. In silico modeling of PROTAC-mediated ternary complexes: validation and application. *J. Chem. Inf. Model.* **2019**, *59*, 1634−1644.
(123) Drummond, M. L.; Henry, A.; Li, H.; Williams, C. I. Improved accuracy for modeling PROTAC-mediated ternary complex formation and targeted protein degradation via new in silico methodologies. *J. Chem. Inf. Model.* **2020**, *60*, 5234−5254.
(124) Zaidman, D.; Prilusky, J.; London, N. PRosettaC: Rosetta based modeling of protac mediated ternary complexes. *J. Chem. Inf. Model.* **2020**, *60*, 4894−4903.
(125) Bai, N.; Miller, S. A.; Andrianov, G. V.; Yates, M.; Kirubakaran, P.; Karanicolas, J. Rationalizing PROTAC-mediated ternary complex formation using Rosetta. *J. Chem. Inf. Model.* **2021**, *61*, 1368−1382.
(126) Leman, J. K.; Weitzner, B. D.; Lewis, S. M.; Adolf-Bryfogle, J.; Alam, N.; Alford, R. F.; Aprahamian, M.; Baker, D.; Barlow, K. A.; Barth, P.; Basanta, B.; Bender, B. J.; Blacklock, K.; Bonet, J.; Boyken, S. E.; Bradley, P.; Bystroff, C.; Conway, P.; Cooper, S.; Correia, B. E.; Coventry, B.; Das, R.; De Jong, R. M.; DiMaio, F.; Dsilva, L.; Dunbrack, R.; Ford, A. S.; Frenz, B.; Fu, D. Y.; Geniesse, C.; Goldschmidt, L.; Gowthaman, R.; Gray, J. J.; Gront, D.; Guffy, S.; Horowitz, S.; Huang, P.-S.; Huber, T.; Jacobs, T. M.; Jeliazkov, J. R.; Johnson, D. K.; Kappel, K.; Karanicolas, J.; Khakzad, H.; Khar, K. R.; Khare, S. D.; Khatib, F.; Khramushin, A.; King, I. C.; Kleffner, R.; Koepnick, B.; Kortemme, T.; Kuenze, G.; Kuhlman, B.; Kuroda, D.; Labonte, J. W.; Lai, J. K.; Lapidoth, G.; Leaver-Fay, A.; Lindert, S.; Linsky, T.; London, N.; Lubin, J. H.; Lyskov, S.; Maguire, J.; Malmström, L.; Marcos, E.; Marcu, O.; Marze, N. A.; Meiler, J.; Moretti, R.; Mulligan, V. K.; Nerli, S.; Norn, C.; Ó'Conchúir, S.; Ollikainen, N.; Ovchinnikov, S.; Pacella, M. S.; Pan, X.; Park, H.; Pavlovicz, R. E.; Pethe, M.; Pierce, B. G.; Pilla, K. B.; Raveh, B.; Renfrew, P. D.; Burman, S. S. R.; Rubenstein, A.; Sauer, M. F.; Scheck, A.; Schief, W.; Schueler-Furman, O.; Sedan, Y.; Sevy, A. M.; Sgourakis, N. G.; Shi, L.; Siegel, J. B.; Silva, D. A.; Smith, S.; Song, Y.; Stein, A.; Szegedy, M.; Teets, F. D.; Thyme, S. B.; Wang, R.Y.-R.; Watkins, A.; Zimmerman, L.; Bonneau, R. Macromolecular modeling and design in Rosetta: recent methods and frameworks. *Nat. Methods.* **2020**, *17*, 665−680.
(127) Weng, G.; Li, D.; Kang, Y.; Hou, T. Integrative modeling of PROTAC-mediated ternary complexes. *J. Med. Chem.* **2021**, *64*, 16271−16281.
(128) Yokoo, H.; Shibata, N.; Endo, A.; Ito, T.; Yanase, Y.; Murakami, Y.; Fujii, K.; Hamamura, K.; Saeki, Y.; Naito, M.; Aritake, K.; Demizu, Y. Discovery of a highly potent and selective degrader targeting hematopoietic prostaglandin D Synthase via in silico design. *J. Med. Chem.* **2021**, *64*, 15868−15882.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329

(129) 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.
(130) Liao, J.; Nie, X.; Unarta, I. C.; Ericksen, S. S.; Tang, W. In silico modeling and scoring of PROTAC-mediated ternary complex poses. *J. Med. Chem.* **2022**, *65*, 6116−6132.
(131) Li, W.; Zhang, J.; Guo, L.; Wang, Q. Importance of three-body problems and protein−protein interactions in proteolysis-targeting chimera modeling: Insights from molecular dynamics simulations. *J. Chem. Inf. Model.* **2022**, *62*, 523−532.
(132) Zheng, S.; Tan, Y.; Wang, Z.; Li, C.; Zhang, Z.; Sang, X.; Chen, H.; Yang, Y. Accelerated rational PROTAC design via deep learning and molecular simulations. *Nat. Mach. Intell.* **2022**, *4*, 739−748.
(133) Luo, G.; Li, Z.; Lin, X.; Li, X.; Chen, Y.; Xi, K.; Xiao, M.; Wei, H.; Zhu, L.; Xiang, H. Discovery of an orally active VHL-recruiting PROTAC that achieves robust HMGCR degradation and potent hypolipidemic activity in vivo. *Acta Pharm. Sin. B* **2021**, *11*, 1300−1314.
(134) Jiang, B.; Gao, Y.; Che, J.; Lu, W.; Kaltheuner, I. H.; Dries, R.; Kalocsay, M.; Berberich, M. J.; Jiang, J.; You, I.; Kwiatkowski, N.; Riching, K. M.; Daniels, D. L.; Sorger, P. K.; Geyer, M.; Zhang, T.; Gray, N. S. Discovery and resistance mechanism of a selective CDK12 degrader. *Nat. Chem. Biol.* **2021**, *17*, 675−683.
(135) Vannam, R.; Sayilgan, J.; Ojeda, S.; Karakyriakou, B.; Hu, E.; Kreuzer, J.; Morris, R.; Herrera Lopez, X. I.; Rai, S.; Haas, W.; Lawrence, M.; Ott, C. J. Targeted degradation of the enhancer lysine acetyltransferases CBP and p300. *Cell Chem. Biol.* **2021**, *28*, 503−514.
(136) Mayor-Ruiz, C.; Jaeger, M. G.; Bauer, S.; Brand, M.; Sin, C.; Hanzl, A.; Mueller, A. C.; Menche, J.; Winter, G. E. Plasticity of the cullin-RING ligase repertoire shapes sensitivity to ligand-induced protein degradation. *Mol. Cell* **2019**, *75*, 849−858.
(137) Ottis, P.; Palladino, C.; Thienger, P.; Britschgi, A.; Heichinger, C.; Berrera, M.; Julien-Laferriere, A.; Roudnicky, F.; Kam-Thong, T.; Bischoff, J. R.; Martoglio, B.; Pettazzoni, P. Cellular resistance mechanisms to targeted protein degradation converge toward impairment of the engaged ubiquitin transfer pathway. *ACS Chem. Biol.* **2019**, *14*, 2215−2223.
(138) Zhang, L.; Riley-Gillis, B.; Vijay, P.; Shen, Y. Acquired resistance to BET-PROTACs (proteolysis-targeting chimeras) caused by genomic alterations in core components of E3 ligase complexes. *Mol. Cancer Ther.* **2019**, *18*, 1302−1311.
(139) Schoepfer, J.; Jahnke, W.; Berellini, G.; Buonamici, S.; Cotesta, S.; Cowan-Jacob, S. W.; Dodd, S.; Drueckes, P.; Fabbro, D.; Gabriel, T.; Groell, J. M.; Grotzfeld, R. M.; Hassan, A. Q.; Henry, C.; Iyer, V.; Jones, D.; Lombardo, F.; Loo, A.; Manley, P. W.; Pelle, X.; Rummel, G.; Salem, B.; Warmuth, M.; Wylie, A. A.; Zoller, T.; Marzinzik, A. L.; Furet, P. Discovery of Asciminib (ABL001), an allosteric inhibitor of the tyrosine kinase activity of BCR-ABL1. *J. Med. Chem.* **2018**, *61*, 8120−8135.
(140) Shirasaki, R.; Matthews, G. M.; Gandolfi, S.; de Matos Simoes, R.; Buckley, D. L.; Raja Vora, J.; Sievers, Q. L.; Bruggenthies, J. B.; Dashevsky, O.; Poarch, H.; Tang, H.; Bariteau, M. A.; Sheffer, M.; Hu, Y.; Downey-Kopyscinski, S. L.; Hengeveld, P. J.; Glassner, B. J.; Dhimolea, E.; Ott, C. J.; Zhang, T.; Kwiatkowski, N. P.; Laubach, J. P.; Schlossman, R. L.; Richardson, P. G.; Culhane, A. C.; Groen, R. W.J.; Fischer, E. S.; Vazquez, F.; Tsherniak, A.; Hahn, W. C.; Levy, J.; Auclair, D.; Licht, J. D.; Keats, J. J.; Boise, L. H.; Ebert, B. L.; Bradner, J. E.; Gray, N. S.; Mitsiades, C. S. Functional genomics identify distinct and overlapping genes mediating resistance to different classes of heterobifunctional degraders of oncoproteins. *Cell Rep.* **2021**, *34*, 108532.
(141) Kurimchak, A. M.; Herrera-Montavez, C.; Montserrat-Sangra, S.; Araiza-Olivera, D.; Hu, J. P.; Neumann-Domer, R.; Kuruvilla, M.; Bellacosa, A.; Testa, J. R.; Jin, J.; Duncan, J. S. The drug efflux pump MDR1 promotes intrinsic and acquired resistance to PROTACs in cancer cells. *Sci. Signal* **2022**, *15*, eabn2707.
(142) Lv, D.; Pal, P.; Liu, X. G.; Jia, Y. N.; Thummuri, D.; Zhang, P. Y.; Hu, W. Y.; Pei, J.; Zhang, Q.; Zhou, S.; Khan, S.; Zhang, X.; Hua, N.; Yang, Q. P.; Arango, S.; Zhang, W. Z.; Nayak, D.; Olsen, S. K.; Weintraub, S. T.; Hromas, R.; Konopleva, M.; Yuan, Y. X.; Zheng, G. R.; Zhou, D. H. Development of a BCL-xL and BCL-2 dual degrader with improved anti-leukemic activity. *Nat. Commun.* **2021**, *12*, 6896.
(143) Zheng, M. Z.; Huo, J. F.; Gu, X. X.; Wang, Y. L.; Wu, C. R.; Zhang, Q. Z.; Wang, W.; Liu, Y.; Liu, Y.; Zhou, X. C.; Chen, L. X.; Zhou, Y. R.; Li, H. Rational design and synthesis of novel dual PROTACs for simultaneous degradation of EGFR and PARP. *J. Med. Chem.* **2021**, *64*, 7839−7852.
(144) Harrigan, J. A.; Jacq, X.; Martin, N. M.; Jackson, S. P. Deubiquitylating enzymes and drug discovery: emerging opportunities. *Nat. Rev. Drug Discovery* **2018**, *17*, 57−77.
(145) Li, M. L.; Liu, S. J.; Chen, H.; Zhou, X. Y.; Zhou, J.; Zhou, S. X.; Yuan, H. L.; Xu, Q. L.; Liu, J.; Cheng, K. G.; Sun, H. B.; Wang, Y.; Chen, C. P.; Wen, X. A. N-benzylpiperidinol derivatives as novel USP7 inhibitors: Structure-activity relationships and X-ray crystallographic studies. *Eur. J. Med. Chem.* **2020**, *199*, 112279.
(146) Li, X.; Yang, S. L.; Zhang, H. H.; Liu, X. P.; Gao, Y. C.; Chen, Y. Q.; Liu, L.; Wang, D. L.; Liang, Z. J.; Liu, S. J.; Dai, L.; Xu, Q. L.; Yuan, H. L.; Chen, C. P.; Sun, H. B.; Wen, X. A. Discovery of orally bioavailable N-benzylpiperidinol derivatives as potent and selective USP7 inhibitors with in vivo antitumor immunity activity against colon cancer. *J. Med. Chem.* **2022**, *65*, 16622−16639.
(147) Ge, F. J.; Li, Y. K.; Yuan, T.; Wu, Y. L.; He, Q. J.; Yang, B.; Zhu, H. Deubiquitinating enzymes: Promising targets for drug resistance. *Drug Discov Today* **2022**, *27*, 2603−2613.
(148) Nguyen, T. V. USP15 antagonizes CRL4(CRBN)-mediated ubiquitylation of glutamine synthetase and neosubstrates. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118*, e2111391118.

https://doi.org/10.1021/acs.jmedchem.2c01555
J. Med. Chem. 2023, 66, 2308−2329