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# E3 ligase ligand optimization of Clinical PROTACs

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Proteolysis targeting chimeras (PROTACs) technology can realize the development of drugs for non-druggable targets that are difficult to achieve with traditional small molecules, and therefore has attracted extensive attention from both academia and industry. Up to now, there are more than 600 known E3 ubiquitin ligases with different structures and functions, but only a few have developed corresponding E3 ubiquitin ligase ligands, and the ligands used to design PROTAC molecules are limited to a few types such as VHL (Von-Hippel-Lindau), CRBN (Cereblon), MDM2 (Mouse Doubleminute 2 homolog), IAP (Inhibitor of apoptosis proteins), etc. Most of the PROTAC molecules that have entered clinical trials were developed based on CRBN ligands, and only **DT2216** was based on VHL ligand. Obviously, the structural optimization of E3 ubiquitin ligase ligands plays an instrumental role in PROTAC technology from bench to bedside. In this review, we review the structure optimization process of E3 ubiquitin ligase ligands currently entering clinical trials on PROTAC molecules, summarize some characteristics of these ligands in terms of druggability, and provide some preliminary insights into their structural optimization. We hope that this review will help medicinal chemists to develop more druggable molecules into clinical studies and to realize the greater therapeutic potential of PROTAC technology.

## KEYWORDS

PROTACs, E3 ubiquitin ligase ligand, structure optimization, clinical trials, CRBN

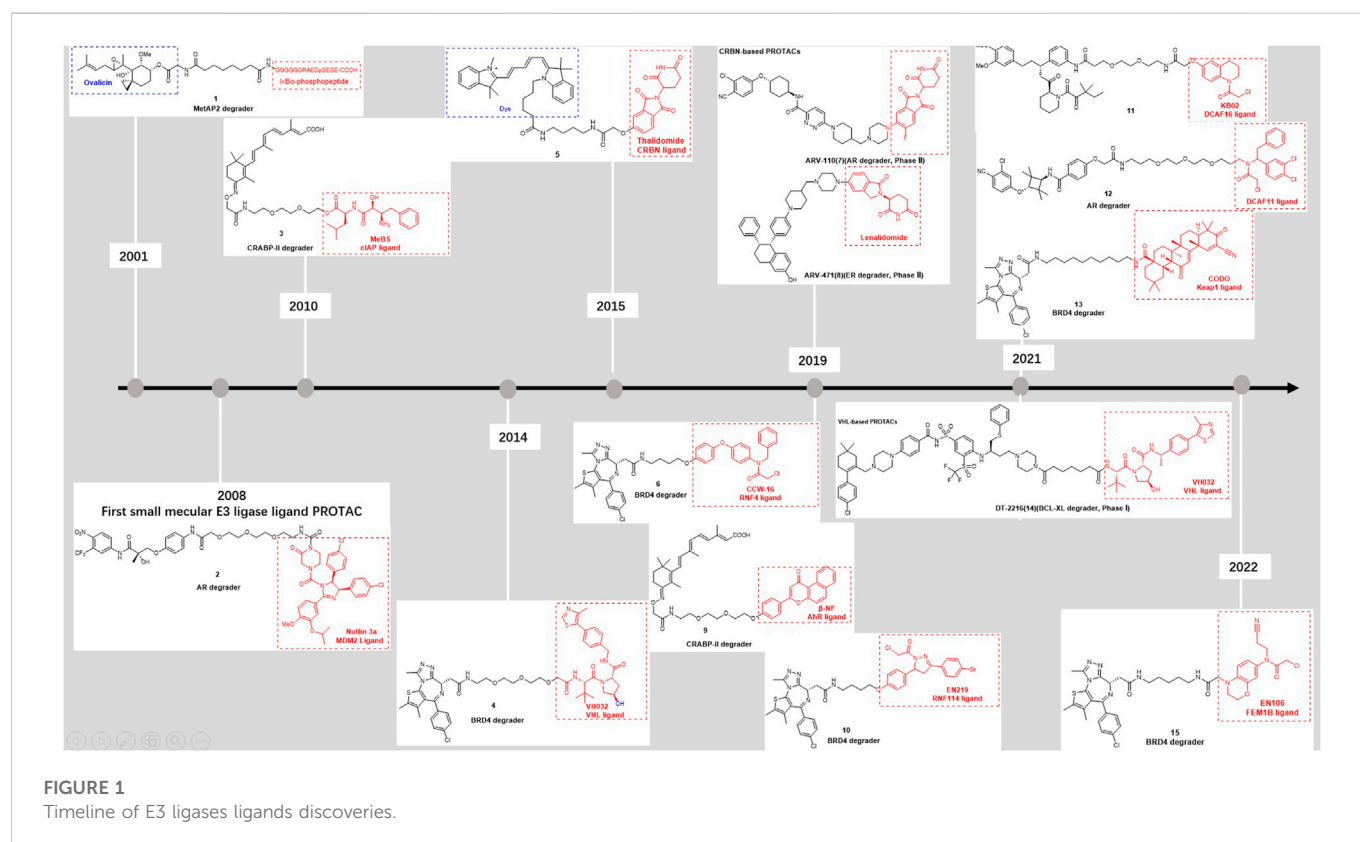
## 1 Introduction

The degradation of most damaged and soluble misfolded proteins is achieved by the 26S proteasome through ubiquitin-proteasome system (UPS)-mediated protein degradation (Paiva et al., 2019; Pohl et al., 2019; Kawahata et al., 2020). In the UPS, the proteasome conjugated to a protein substrate through enzymatic cascade (Hershko et al., 1983; Hershko et al., 1992; Komander et al., 2012). First, E1 (Ub activating enzyme) binds Ub (ubiquitin) via an ATP-dependent mechanism and then transfers Ub to E2 (Ubconjugating enzyme) by forming an E2 ubiquitin conjugate (Schulman et al., 2009; Ye et al., 2009). Next, the E3 (Ub ligase) mediates the transfer of the ubiquitin from E2 to the substrate protein, followed by 26S proteasome-induced degradation or post-translational modification of the substrate protein (Zheng et al., 2017). E3 ligase mediates the specificity of protein substrates through a non-covalent or covalent mechanism, and the type of E3 ligase determines the outcome of the substrate protein. For instance, TRAF6 (tumor necrosis factor receptor-associated factor 6) interacts with YAP (Yes-associated protein) and promotes its ubiquitination to enhance YAP stability (Liu et al., 2020). c-Cbl (Casitas B lymphoma) binds to the intracytoplasmic tail of PD-1 and targets it for ubiquitination-proteasomal degradation

TABLE 1 PROTAC degraders in clinical development.

Company	Degrader	Target	E3 ligase	ROA	Highest phase	Clinical trial no. (if applicable)
Arvinas	ARV-110	AR	CRBN	Oral	Phase II	NCT03888612
Arvinas	ARV-766	AR	Undisclosed	Oral	Phase I	NCT05067140
Arvinas/Pfizer	ARV-471	ER	CRBN	Oral	Phase II	NCT04072952
Accutar Biotech	AC682	ER	CRBN	Oral	Phase I	NCT05080842
Bristol Myers Squibb	CC-94676	AR	CRBN	Oral	Phase I	NCT04428788
Dialectic Therapeutics	DT2216	BCL-X <sub>L</sub>	VHL	I.v	Phase I	NCT04428788
Foghorn Therapeutics	FHD-609	BRD9	Undisclosed	Oral	Phase I	NCT04965753
Kymera	KT-413	IRAK4	CRBN	I.v	Phase I	NA
Kymera	KT-333	STAT3	Undisclosed	Undisclosed	Phase I	NA
Kymera/Sanofi	KT-474	IRAK4	Undisclosed	Oral	Phase I	NCT04772885
Nurix Therapeutics	NX-2127	BTK	CRBN	Oral	Phase I	NCT04830137
Nurix Therapeutics	NX-5948	BTK	CRBN	Oral	Phase I	NCT04830137
C4 Therapeutics	CFT8634	BRD9	CRBN	Oral	IND- e	NA
C4 Therapeutics	CFT8919	EGFR <sup>L858R</sup>	CRBN	Oral	IND- e	NA
Cullgen	CG001419	TRK	CRBN	Oral	IND- e	NA

NA, not applicable.



in macrophages, resulting in downregulation of PD-1 and reduced surface expression leading to increased tumor phagocytosis and tumor suppression (Lyle et al., 2019).

Proteolysis targeting chimeras (PROTACs) is based on proteasomes (Schapira et al., 2019). These bifunctional molecules consist of three parts: An E3-recruiting ligand, a POI (protein of

interest) targeting warhead, and a linker connecting the two ligands (Sun et al., 2019). PROTAC degraders mediate their own formation of POI-PROTAC-E3 complexes with substrate proteins and E3 ubiquitin ligases, which lead to the degradation of the substrate protein *via* UPS (Wang et al., 2020; Yang et al., 2021). There are many target-based POI warheads and linkers available for the design and optimization of PROTAC degraders for medicinal chemists, but only a few E3 ubiquitin ligases ligands have been developed (Sun et al., 2019).

Arvinas is the first company to clinically implement two PROTAC degraders, the androgen receptor (AR) degrader **ARV-110** and the estrogen receptor (ER) degrader **ARV-471** (Mullard, 2019). The safety and effectiveness of **ARV-110** has been demonstrated in the treatment of metastatic castrated prostate cancer (mCRPC) (Neklesa et al., 2018; Neklesa et al., 2019; Gao et al., 2022), and **ARV-471** also has shown great potential in the treatment of breast cancer (Snyder et al., 2021). Since **ARV-110** and **ARV-471** entered clinic trials, an increasing number of protein targets have emerged to develop clinical degraders of PROTACs, such as BRD9 and IRAK4 (Table 1), while antitumor is currently the most concentrated field of research for PROTACs, except for KYMERA's degrader **KT-474**, which is the only clinical degrader for autoimmune-disease (Békés et al., 2022). These PROTACs degraders are based on different E3 ligands, but mainly on CRBN-based ligands.

The first PROTAC was discovered in 2001 by Craig Crews, founder of Arvinas (Sakamoto et al., 2001) (Figure 1). This compound consists of a covalent small molecule inhibitor of MetAP-2, and IκBα-phosphopeptide, enabling the ligase to ubiquitylate METAP2. As the first attempt to explore PROTACs, this compound exposed the poor cell permeability prevented it from being widely used, and the structure of phosphopeptides in this type of PROTAC is easily hydrolyzed by intracellular phosphatase, which reduces its stability. Therefore, the desired small molecule E3 ligase ligand, must have good membrane permeability, be stable *in vitro* environment and have strong affinity to E3 ubiquitin ligase, on the basis of which PROTACs will have stronger druggability.

In the past 2 decades, various E3 ligase ligands based on different functions have been reported. Such as MDM2 ligand (Honda et al., 1997; Schneekloth et al., 2008; Saadatzadeh et al., 2017), cIAP ligands (Sato et al., 2008; Itoh et al., 2011; Ohoka et al., 2017), VHL ligand (Schneekloth et al., 2004; Rodriguez-Gonzalez et al., 2008), CRBN ligand (Winter et al., 2015), AhR (Aryl hydrocarbon receptor) ligand (Ohtake et al., 2007; Ohoka et al., 2019), DCAF (DDB1- And CUL4-Associated Factor) 11 and 15 and 16 ligands (Han et al., 2017; Zhang et al., 2019; Zhang et al., 2021), RNF (RING finger protein) 4 and 114 ligands (Kumar et al., 2019; Ward et al., 2019; Luo et al., 2021), FEM1B (Fem-1 Homolog B) ligand (Henning et al., 2022), KEAP1 (Kelch Like ECH Associated Protein 1) ligand (Tong et al., 2020; Wei et al., 2021; Pei et al., 2022) (Figure 1). They were developed based on the function of different E3 ligases thus have different properties. For instance, VHL is the substrate receptor of CRL2VHL E3 ubiquitin ligase.

The Pro564 residue of HIF-1α (Hypoxia-Inducible Factor-1α) is hydroxylated by prolyl hydroxylase (also the hydroxyl group in the VHL ligands), bound to VHL proteins and subsequently ubiquitinated by CRL2VHL E3 (Ivan et al., 2001; Schneekloth et al., 2004). HIF-1α protects cells during hypoxia, and VHL-based PROTAC shows good degradation activity in most cases, and VHL ligands even reduce side effects in some cases (Jaakkola et al., 2001). However, in subsequent studies, the poor membrane permeability and low oral delivery rate of

VHL ligands limited their application. The E3 ubiquitin ligase ligands are like a toolbox for PROTACs, and the appropriate "tool" is selected based on the different properties of E3 ligands for the purpose of the researchers.

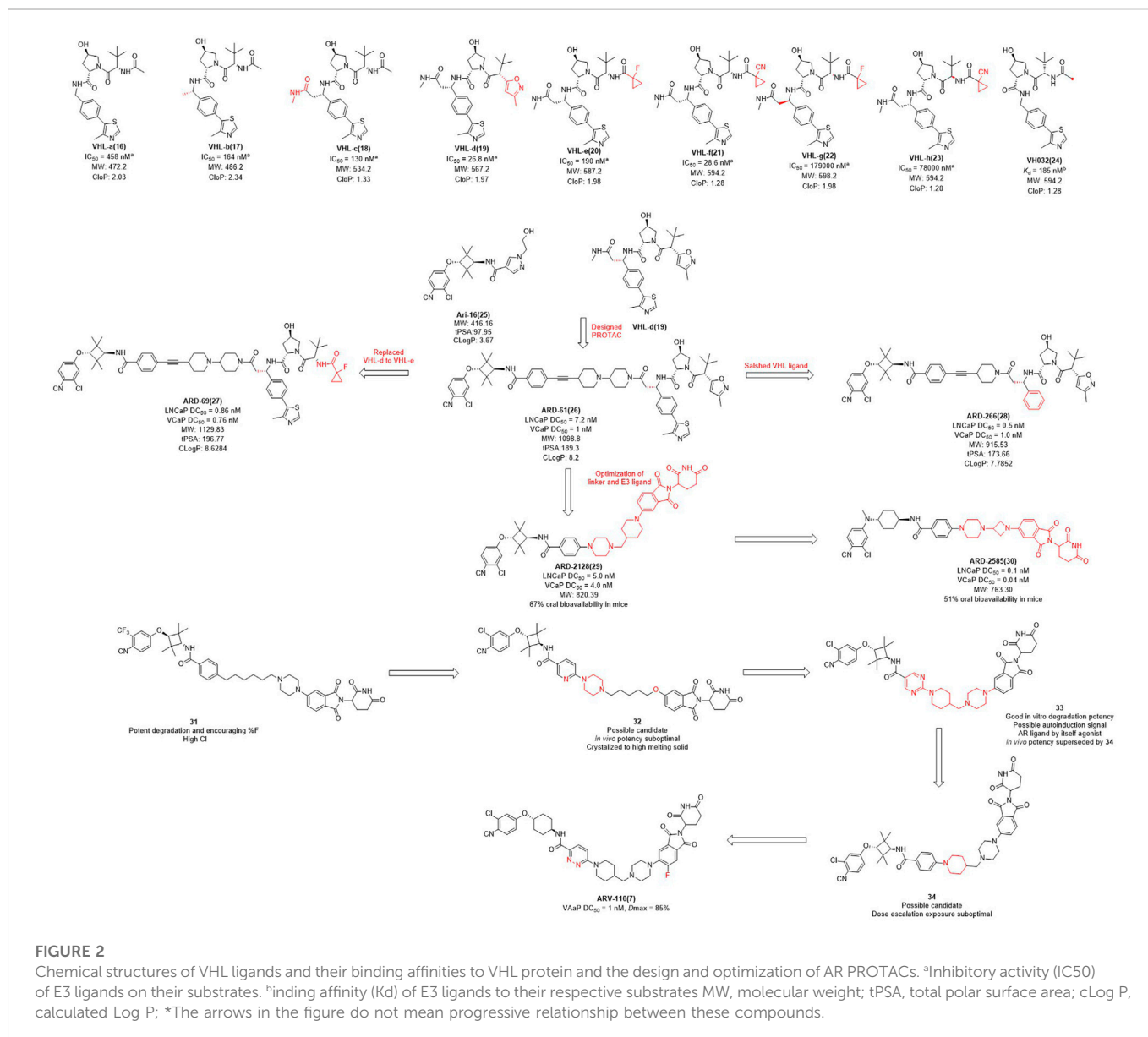
In this review, we will summarize the experience of small molecule PROTACs currently entering clinical trials in optimizing E3 ligase ligands for degraders from the perspective of chemical structure. It is expected to shed light on the optimization of E3 ligase ligands for degraders of PROTACs in the future.

## 2 E3 ligase ligands optimization of PROTACs for clinical application

### 2.1 E3 ligase ligands optimization of AR PROTACs

Annually, more than 350,000 deaths are associated with prostate cancer, making the disease one of the leading causes of cancer-related death in men, and the androgen receptor (AR) is believed to drive hormone dependency of prostate cancer (Rebello et al., 2021). Key AR gene alterations contribute to castration-resistant prostate cancer (CRPC) (de Bono et al., 2020). Both enzalutamide and abiraterone have shown good results as AR antagonists in the treatment of prostate cancer. However, the drug resistance is inevitable when mutations occur in the ligand-binding domain (Teo et al., 2019). Therefore, the development of AR degraders based on PROTACs technology has become a new strategy (Pan et al., 2007).

The earlier AR PROTAC was designed using enzalutamide as the AR antagonist and VHL-b ligands as E3 ligase ligand. In 2019, Wang et al. selected **Ari-16** as the antagonist of the degrader by screening various VHL-based E3 ligase ligands (**compounds 16–24**) to build different AR degraders (Kregel et al., 2020; Zhao et al., 2020) (Figure 2). During E3 ligase ligand optimization process, they found that the (S)-methyl group in **VHL-b** exposed to the solvent environment could serve as a possible tethering point for the design of AR degraders (Han et al., 2019). Subsequently, they reported that **ARD-61** is an AR degrader with DC<sub>50</sub> (concentration that resulted in a 50% targeted protein degradation) values of 7.2 nM and 1.0 nM in LNCaP and VCaP cells, respectively. Apparently, the large molecular weight of the VHL ligand makes **ARD-61** too large (MW = 1095.8) for its degradation activity to be significant. In their following work, the E3 ligase ligand part of **ARD-61** was replaced with a VHL ligand and the optimized degrader **ARD-69** showed AR DC<sub>50</sub> values of 0.86 nM and 0.76 nM in LNCaP and VCaP cells, respectively. However, the molecular weight of **ARD-69** was still too large as an AR degrader. Subsequently, they shortened the linker length and modified the VHL ligand to decrease its molecular weight. Thus, led to degrader **ARD-266** with similar degradation activity, the AR DC<sub>50</sub> values were 0.5 nM and 1.0 nM in LNCaP and VCaP cells, respectively (Han et al., 2019). The poor membrane permeability and low oral availability of VHL ligands led to its eventual replacement by CRBN ligands which is also used in Bristol Myers Squibb's ER clinical degrader **CC-94676**. On the basis of the above three molecules, they discovered that **ARD-2128** showed the same degradation activity as **ARD-61** (MW = 820.4), but its molecular weight was significantly reduced and its bioavailability in mice reached 67% (Han et al., 2021). Finally, on the basis of CRBN ligand, they re-optimized the linker and the antagonist and disclosed **ARD-2585**, which has 51% oral bioavailability in mice and with AR



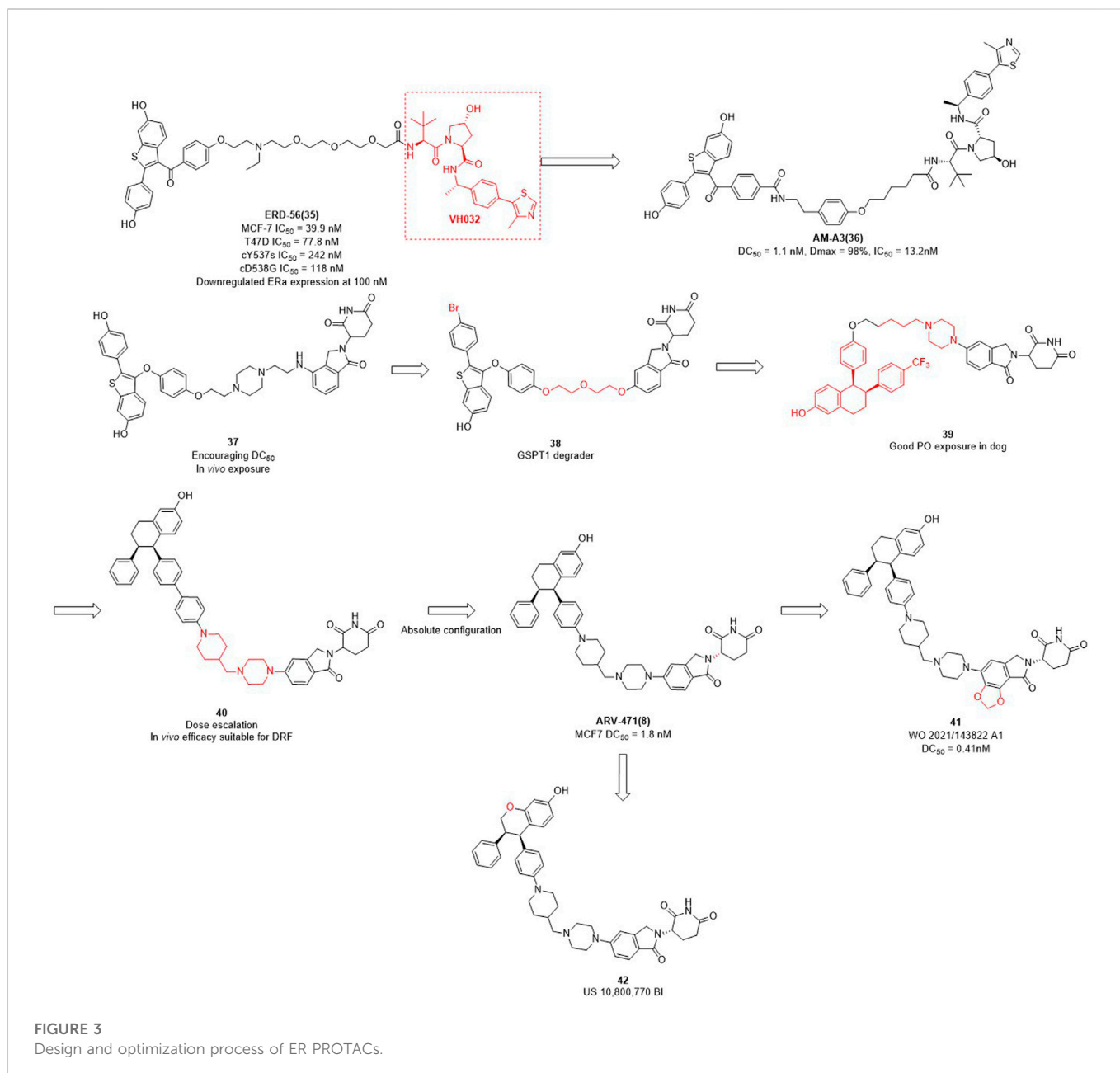
DC<sub>50</sub> of 0.10 nM and 0.04 nM in LNCaP and VCaP cells, respectively (Xiang et al., 2021).

In the pharmaceutical industry, AR degraders have also received the attention of Arvinas, whose CRBN-based AR degrader **ARV-110** (Figure 2) is currently in phase II clinical trials (Snyder et al., 2021). Arvinas has also performed many optimizations for AR degradation. First of all, compounds **31** and **32** showed good degradation abilities *in vitro*, but also with a high clearance rate *in vivo*. Subsequently, they disclosed that bi-functional compound **33** showed strong degradation activity *in vitro*, however, poor activity *in vivo*, perhaps due to the metabolism of **33**. The structure of compound **34** was optimized on the basis of compound **33** with improved activity *in vivo*, but it was dose dependent and needed further optimization. Finally, the H at position 3 of the benzene ring of the CRBN ligand of **34** was replaced with fluorine to obtain degrader **ARV-110** which improved the druggability. **ARV-110** was highly efficient in VCaP cells with a DC<sub>50</sub> value with 1 nM and D<sub>max</sub> (maximal levels of protein degradation) of 85% (Snyder et al., 2021). Above all, the

E3 ubiquitin ligand functions as the promoter of the degradation process, and optimizing the E3 ligand and modifying its molecular structure is expected to improve the activity and oral delivery rate of the degraders.

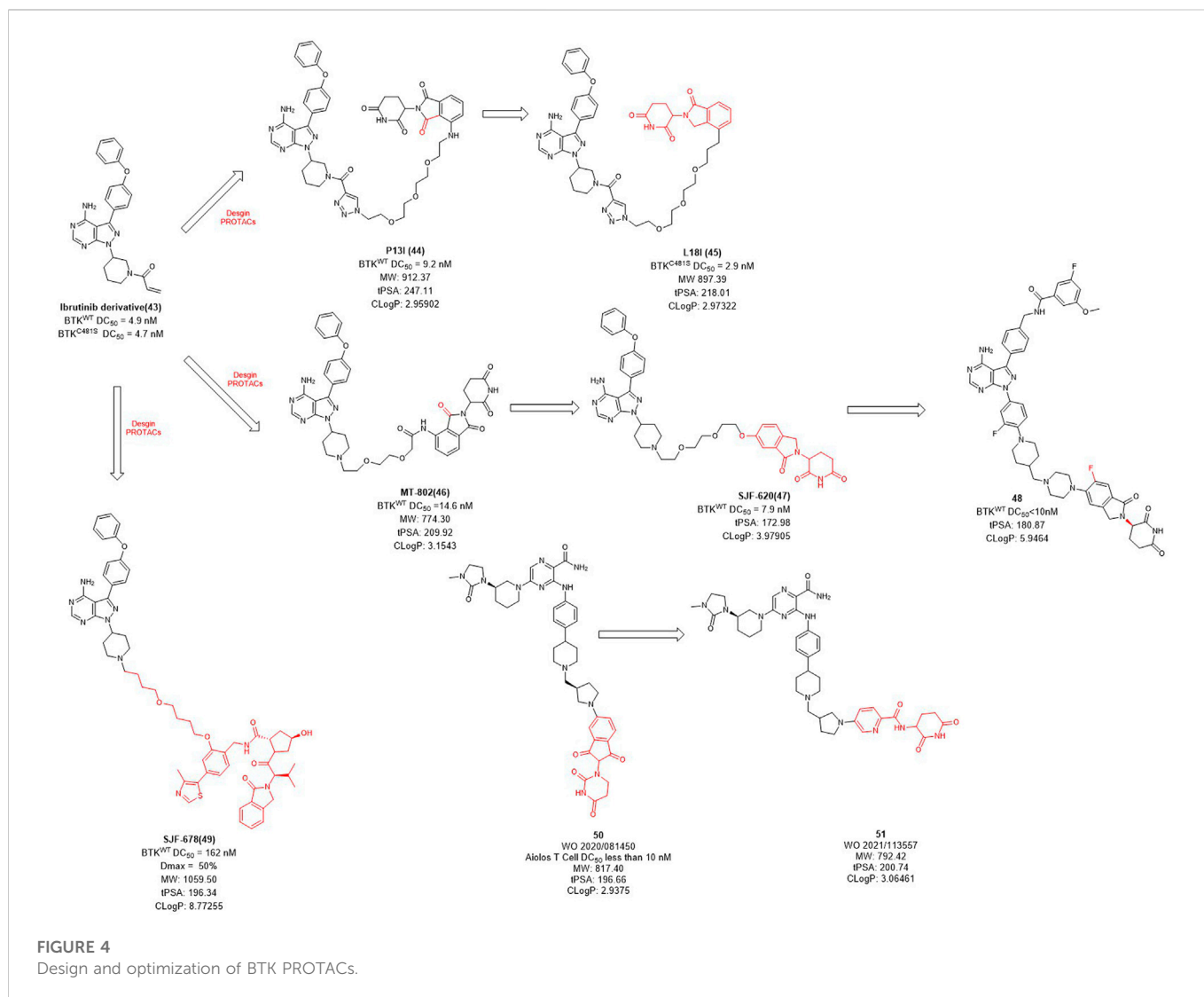
## 2.2 E3 ligase ligands optimization of ERα PROTACs

The importance of estrogen regulates (ER) for female breast cancer is similar to AR for male prostate cancer. ER targets are associated with 70%–80% of breast cancer profiles, and become the primary therapeutic target for this disease (Ohoka et al., 2018; Waks et al., 2019; Criscitiello et al., 2022). ERα is a member of the nuclear receptor family and plays a crucial role in mediating the estrogen signaling pathway within the mammary glands and female reproductive tract (Arnal et al., 2017). In contrast to the high expression of ERα in breast tumors, the ERα expression is low in



normal breast epithelium cells (Huang et al., 2014). ER $\alpha$  knockout experiments in rats demonstrated that ER $\alpha$  plays an important role in promoting the formation of breast cancer cells in the mammary gland (Zhang et al., 2011). In addition to ER $\alpha$  knockout, PROTAC can also reduce the expression levels of ER $\alpha$  in breast. Wang et al. focused not only on AR degraders but also on ER $\alpha$  degraders. In their initial studies of ER $\alpha$  degradation, they singled out raloxifene as ER $\alpha$ -binding ligand and CRBN and VHL as E3 ligase ligands, respectively. Interestingly, the VHL-based PROTACs were shown to induce significant degradation of the target protein, whereas no obvious protein degradation was observed for CRBN-based molecules. Therefore, they synthesized a series of PROTACs based on raloxifene and VH032 (Figure 3). Among them, ERD-56 showed significant degradation properties of ER $\alpha$  protein at 100 nM, and also showed good anti-proliferative activity in MCF-7 and T47D cells with  $IC_{50}$  (half maximal inhibitory concentration)

of 39.9 nM and 77.8 nM (Gonzalez et al., 2020). In order to improve the potency of ERD-56, they optimized the linker and ERD-308 showed the best efficacy with  $DC_{50}$  of 0.17 nM (Hu et al., 2019). Interestingly, the ER protein degraders reported above were all designed based on VH032 as an E3 ligase ligand. Due to the poor druggability of VH032 itself, the druggability of ER protein degraders developed based on it was also generally poor. Different from the above studies, Arvinas has also been working on the development of ER $\alpha$  degraders based on CRBN. First, they designed ER $\alpha$  degraders based on raloxifene and lenalidomide, and found that degrader 37 (Figure 3) showed good degradation activity. Based on 37, they optimized the linker and ER $\alpha$  ligands to obtain the degrader 38 targeting GSPT1. Then they modified the structure of raloxifene to obtain degrader 39, due to the poor druggability of the long-chain linker, they decided to use the same linker that existed in ARV-110 to obtain degrader 40. From the activity screening

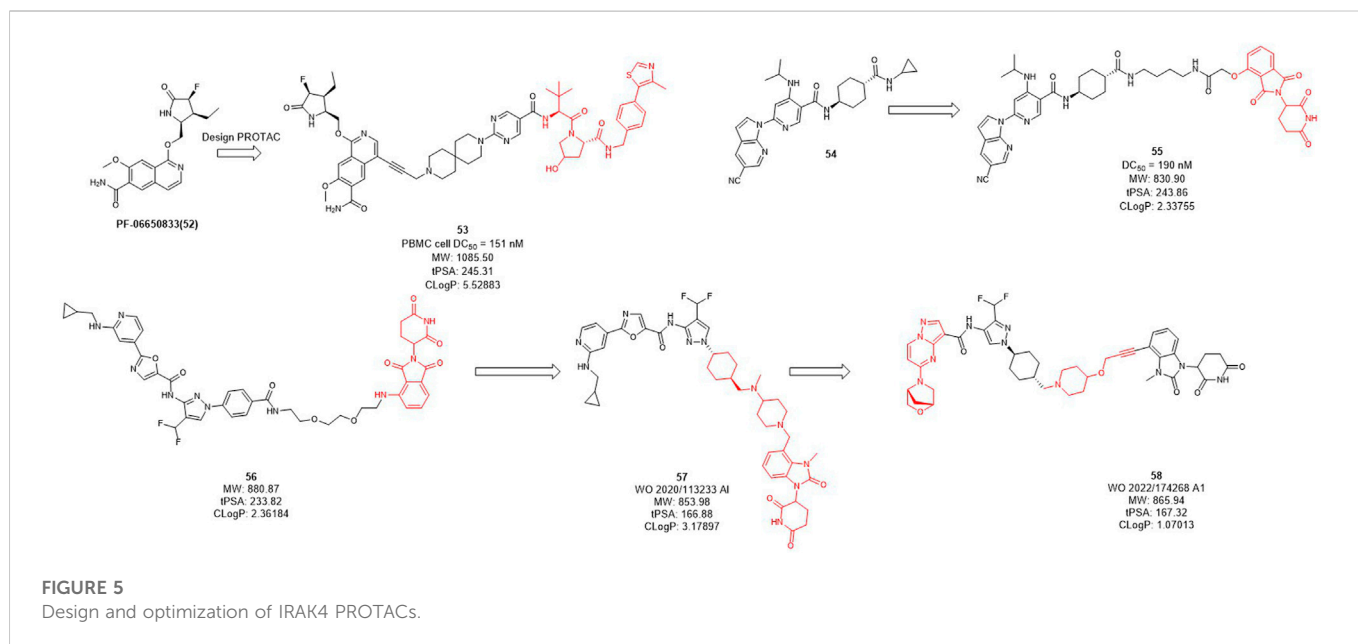


experiment, it was found that the chiral degrader **ARV-471** had better degradation activity than **40**. The ER $\alpha$  degradation activity DC<sub>50</sub> in MCF-7 cells was 1.8 nM and thus **ARV-471** became another clinical degrader of Arvinas (Snyder et al., 2021). Hengrui Medicine further optimized CRBN E3 ligase ligand and improve the degradation activity of ER degraders, and found compound **41** with DC<sub>50</sub> value of 0.41 nM in MCF-7 cells (Yang et al., 2021). Accutar Biotech undisclosed their structure of ER clinical degrader (AC682) (NCT05080842), in their research they found that the compound **42** with DC<sub>50</sub> value of 0.3 nM in MCF-7 cells (Fan et al., 2021). In summary, the optimization of E3 ubiquitin ligase ligand is the key to improve the oral availability and potency of PROTACs.

## 2.3 E3 ligase ligands optimization of BTK PROTACs

Bruton's tyrosine kinase (BTK) is highly expressed in various of lymphoma cells and plays an essential role in B-cell receptor (BCR) signal and B cell activation (Davis et al., 2010). Since the ATP binding

site of BTK is highly conserved, how to achieve kinase selectivity of BTK inhibitors becomes the key issue. Ibrutinib is the first approved BTK covalent inhibitor with high selectivity, strong activity and good oral bioavailability (Pan et al., 2007). The acrylamide warhead of ibrutinib forms a covalent bond with the sulfhydryl group of the cysteine residue 481 in BTK, which is irreversible and thus permanently inactivates BTK kinase with an IC<sub>50</sub> value of 0.5 nM after 2 h (Pan et al., 2007). However, the C481S BTK mutation (cysteine to serine mutation at position 481) prevents the formation of the critical covalent bond with ibrutinib, leading to drug resistance (Woyach et al., 2014). In order to overcome this challenge, in 2018, Rao et al. applied the PROTAC technology for ibrutinib-resistant BTK degradation and reported **P131** (Figure 4) which is an ibrutinib and pomalidomide-linked degrader with DC<sub>50</sub> value of 9.2 nM for wild-type and DC<sub>50</sub> value of 30 nM for ibrutinib-resistant C481S BTK in Mino cells (Sun et al., 2018). While ibrutinib has difficulty inhibiting the autophosphorylation of C481S mutant BTK, **P131** is effective at low concentrations. For HBL-1 cells expressing the C481S mutant BTK, the GI<sub>50</sub> (50% growth inhibitory concentration) of **P131** was about 28 nM compared to about 700 nM for ibrutinib, a 20-fold decrease in potency. In



addition, **P13I** showed no effect on ITK, EGFR and TEC family kinases that cause side effects. Subsequently, in order to improve the aqueous solubility of **P13I** for both *in vitro* and *in vivo* evaluations, Rao et al. further optimized the E3 ligase ligand of **P13I** with lenalidomide and to obtain a new degrader **L18I** (Sievers et al., 2018; Sun et al., 2019). **L18I** exhibited good solubility in phosphate buffered saline (PBS), and inhibit C481S BTK in DLBCL tumors growth *in vivo*. These efforts suggest that PROTACs may provide a new treatment strategy for ibrutinib-resistant tumors.

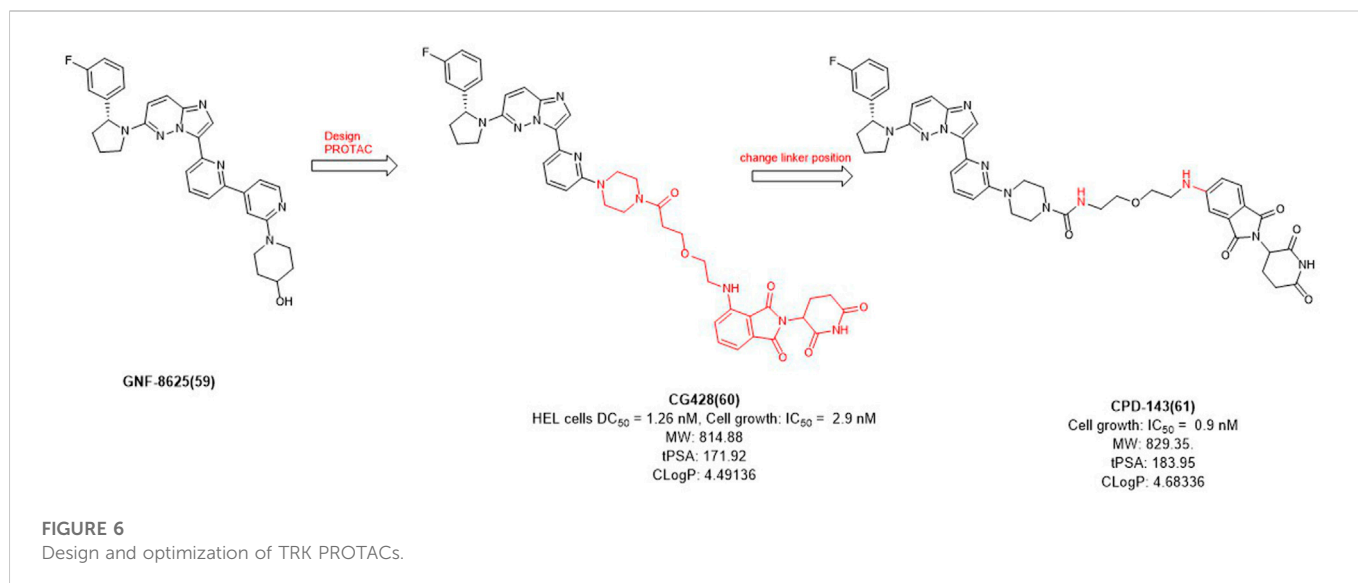
Unlike the usually used ibrutinib-based BTK PROTACs (In this section we only discuss the reversible non-covalent BTK PROTACs), Crews et al. reported another non-covalent analog of ibrutinib, and developed a novel CRBN-recruiting BTK PROTAC, **MT-802** (Figure 4), which induced the efficient degradation of both wild-type ( $DC_{50} = 14.6$  nM) and C481S mutation ( $DC_{50} = 14.9$  nM) BTK (Buhimschi et al., 2018). Meanwhile, they found that the degradation efficiency of VHL-recruited BTK PROTAC degrader **SJF678** was significantly weaker than that of CRBN-recruited BTK PROTACs. They then developed a BTK degrader **49** based on the E3 ligand of **ARV-110** with  $DC_{50}$  value less than 10 nM treated in RAMOS cell lines for 6 h. Nurix Therapeutics discovered two BTK degraders, NX-2127 and NX-5948, currently in Phase I clinical trials and their structures have not been disclosed. In recent years, they have disclosed oral PROTAC degraders **50** and **51** with good activities both *in vitro* and *in vivo*, as well as oral bioavailability (Robbins et al., 2020; Sands et al., 2020). The druggability of BTK degraders was significantly improved after modifying the linker to make it more rigid and reducing the molecular weight of the E3 ligand.

## 2.4 E3 ligase ligands optimization of IRAK4 PROTACs

Interleukin-1 receptor-associated kinase 4 (IRAK4) is a serine/threonine kinase that not only performs phosphorylation but also

functions as a scaffold role in Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling pathways (Brzezinska et al., 2009; Lim et al., 2013; Vollmer et al., 2017). As a promising therapeutic target for diffusing large B-cell lymphoma driven by the MYD88 L265P mutant, the IRAK4 target receives significant attention. While Previous inhibitors had only moderate effects on IRAK4 target because they inhibited kinase function but had no effect on scaffold function. Unlike traditional small molecule inhibitors, which only inhibit kinase activity, PROTACs for protein degradation may offer a solution to block both IRAK4 kinase activity and scaffold capabilities. In 2019, Anderson et al. selected **PF-06650833** as IRAK4 inhibitor and synthesized a series of compounds based on VHL, CRBN, and IAP ligands. Among them, only the degrader **53** (Figure 5) based on VHL showed degradation activity of IRAK, with the  $DC_{50}$  value of 151 nM in PBMC cell (Nunes et al., 2019). In 2020, Dai et al. designed and synthesized a series of CRBN-based IRAK4 degraders and compound **55** showed  $DC_{50}$  value of 190 nM *in vitro* (Zhang et al., 2020). However, its degradation activity may be weaker than that of inhibitors **54**. It may be due to the weak affinity between the inhibitor and the target protein, and replacing the E3 ligand will not obtain the desired potency.

Kymera has two IRAK4 degraders, KT-474 and KT-413 in phase I clinical trial while their structures are undisclosed (Kymera, 2022). From their published patent (WO 2020/113233 A1), they obtained a series of IRAK4 degraders based on CRBN in combination with different IRAK4 inhibitors, most of which such as compound **56** (Figure 5) showed more than 50% degradation of IRAK4 at 0.01 nM in PMBC cells (Kargbo, 2019) (e.g., compound **57**). Subsequently, the E3 ligase ligand part of **56** was replaced with the CRBN ligand, and the linker-optimized degrader **57** also showed  $DC_{50}$  less than 0.01 nM in PMBC cells (Mainolfi et al., 2020). Recently, they disclosed the structure of compound **58** which is also based on CRBN ligand and showed excellent *in vitro* and *in vivo* activity, as well as oral bioavailability (Gollob et al., 2022).



## 2.5 E3 ligase ligands optimization of TRK PROTACs

The tropomyosin receptor kinase (TRK) receptor family comprises three members: TRKA, TRKB, and TRKC that are encoded by the NTRK1, NTRK2, and NTRK3 genes, respectively, which plays an important role in regulating cell differentiation, proliferation, pain, and survival (Pulciani et al., 1982; Martin-Zanca et al., 1986; Klein et al., 1989). TRKs are tyrosine kinases receptors and their main implication is the development and function of neuronal tissues (Kargbo, 2020). Although the targeted treatment of TRK1 and TRK2 shows an overall good safety profile in the clinic trials, this strategy could also be improved because currently available pan-TRK kinase inhibitors may induce off-target adverse effects by modulating TRK family members present in the CNS. Currently, moderate off-target adverse effects have been observed, such as dizziness/ataxia, paresthesia, and weight gain (Drilon, 2019). Non-specific side effects and drug resistance to TRK kinase inhibitors remain great challenges for effective treatment (Kargbo, 2020). In contrast, PROTACs technology keep the target protein in the periphery without penetrating the blood-brain barrier, thus avoid the side effects of off-targeting to the CNS.

In 2020, Cullgen et al. selected GNF-8625 (Figure 6) as TRK inhibitor and linked with CRBN to obtain a series of TRK degraders and CG428 proved to be the most promising degrader. It demonstrated that CG428 can induce the degradation of wild-type TRKA in HEL cells with  $DC_{50}$  value of 1.26 nM and also inhibit cell growth with  $IC_{50}$  value of 2.9 nM (Chen et al., 2020). They then changed the connect position of pomalidomide with linker and obtained compound CPD-143 with an increased activity which the cell growth  $IC_{50}$  was 0.9 nM (Kargbo, 2020). It can be seen that when optimizing PROTACs, once the POI and E3 ligands were identified, changing the linker position on the E3 ligase ligand could be considered to improve the potency of PROTACs.

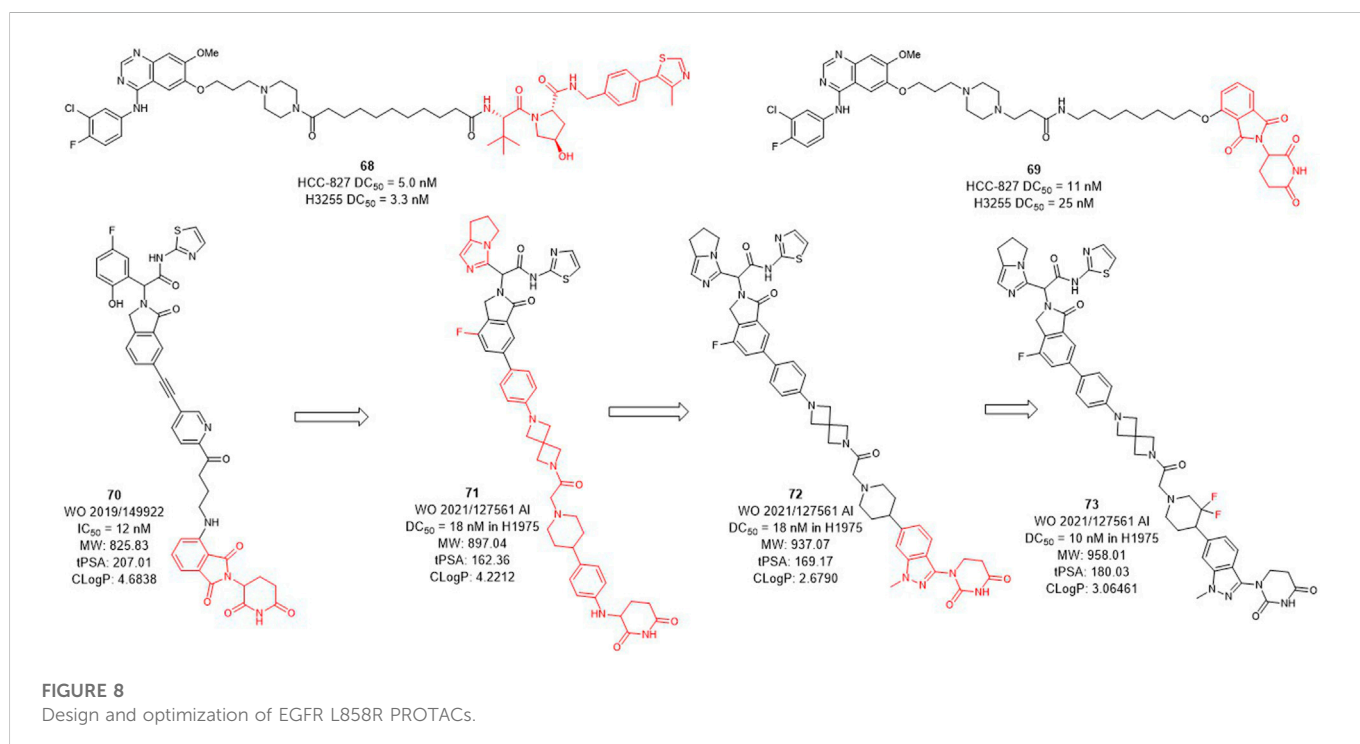
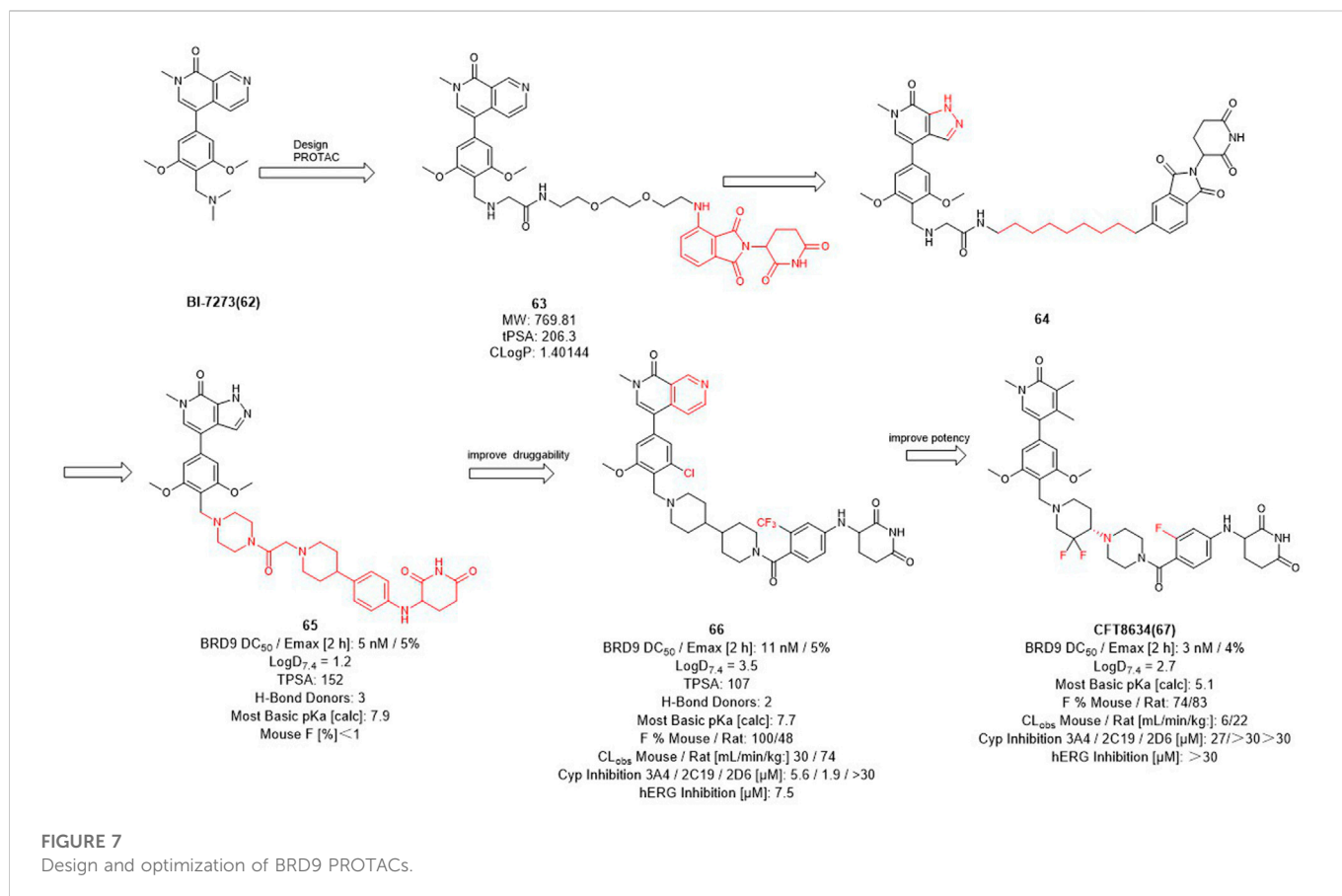
## 2.6 E3 ligase ligands optimization of BRD9 PROTACs

The BRD9 (bromodomain-containing protein 9) has gained special attention as a component of the human ATP-dependent chromatin remodeling BAF (BRG1/BRM-associated factor) complex (also known as mammalian SWI/SNF (SWItch Sucrose Non-Fermentable)) (Kadoch et al., 2013; Theodoulou et al., 2016). Studies have shown that BRD9 is preferentially used by cancers harboring SMARCB1 abnormalities, such as malignant rhabdoid tumors and several specific types of sarcomas (Kim et al., 2014). BRD9-containing complexes bind to active promoters and enhancers where they contribute to gene expression (Gatchalian et al., 2018). Loss of BRD9 leads to changes in gene expression related to apoptosis regulation, translation and development regulation. BRD9 is essential for the proliferation of SMARCB1-deficient cancer cell lines and is therefore a therapeutic target for these lethal cancers, and it is also a key target for causing acute myeloid leukemia (Hohmann et al., 2016; Michel et al., 2018). Despite the early discovery of BRD9 inhibitors, there is limited understanding of the function of BRD9 beyond acetyl lysine recognition based on early chemical probes.

Therefore, Bradner et al. designed the first BRD9 degrader 63 in 2017 to provide a tool compound (Figure 7) (Remillard et al., 2017). Compound 63 was designed by using BI-7273 as inhibitor of BRD9 and CRBN ligand pomalidomide as E3 ligase ligand, it turned out to be valuable for exploring the biological and therapeutic potential of degrading BRD9. C4 Therapeutics (C4T) started with 64 and optimized both BRD9 inhibitors and E3 ligands and finally obtained the tool degrader 65 with  $DC_{50}$  value of 5 nM.

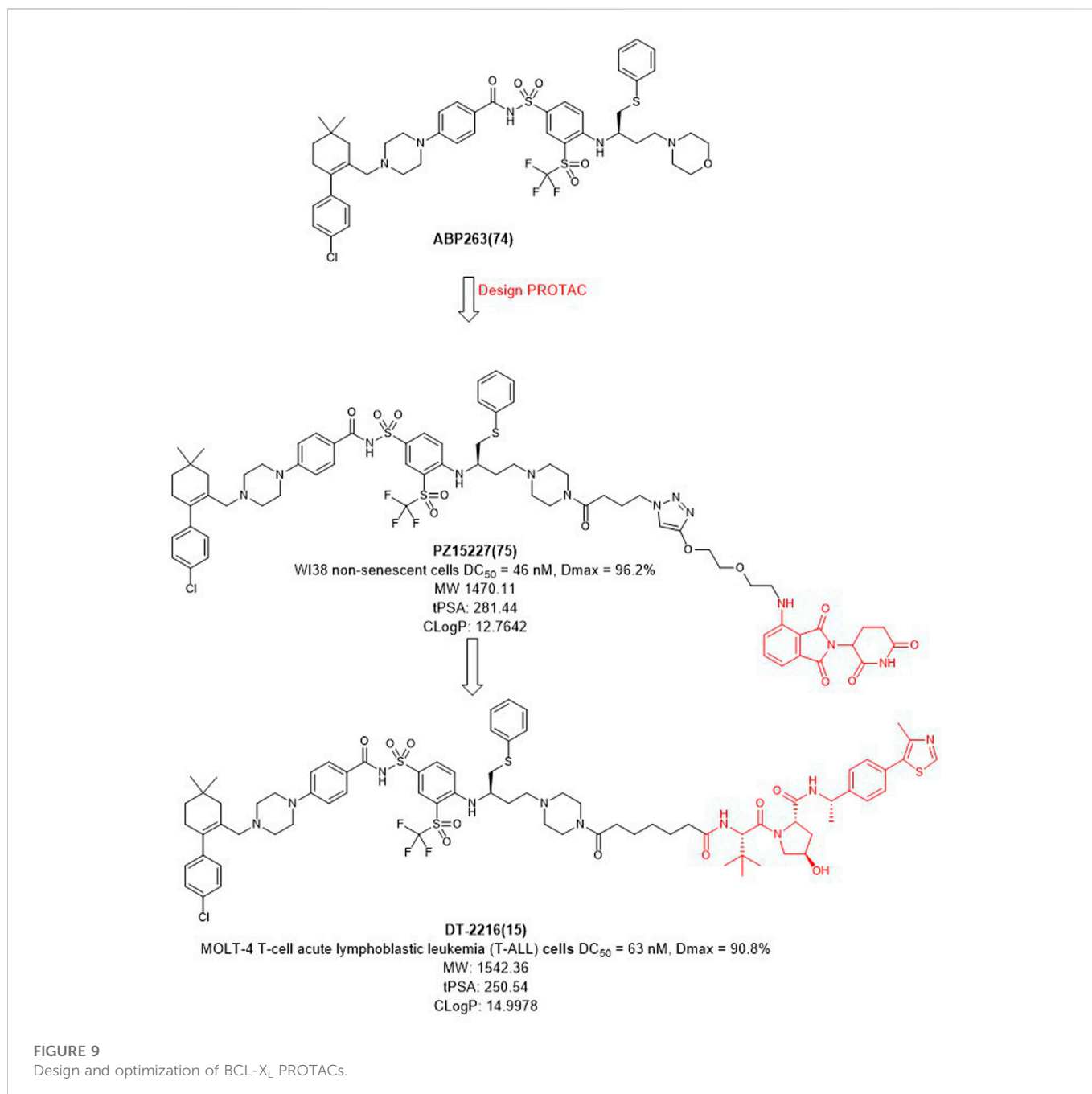
Compound 66 was optimized based on the structure of compound 65 with fewer hydrogen bond donors and its bioavailability (F %) in mice was increased to 100%, thus improving the druggability of the degrader. Subsequently, they made minor modifications of the POI and linker of compound 65, more importantly, for the E3 ligase ligand part, they replaced the F to trifluoromethyl group of the pomalidomide to obtain the degrader CFT8634 with high oral bioavailability (Jackson et al., 2022). Finally, Food and drug





Administration (FDA) has granted orphan drug designation (ODD) to CFT8634 (Figure 7) for the treatment of soft tissue sarcoma which is an orally bioavailable, selective degrader of BRD9 (DC<sub>50</sub> = 3 nM)

(Sabnis, 2021). In summary, the optimization process from 64 to CFT8634 indicates that the dramatic change in their E3 ligands improves their treatment potential.

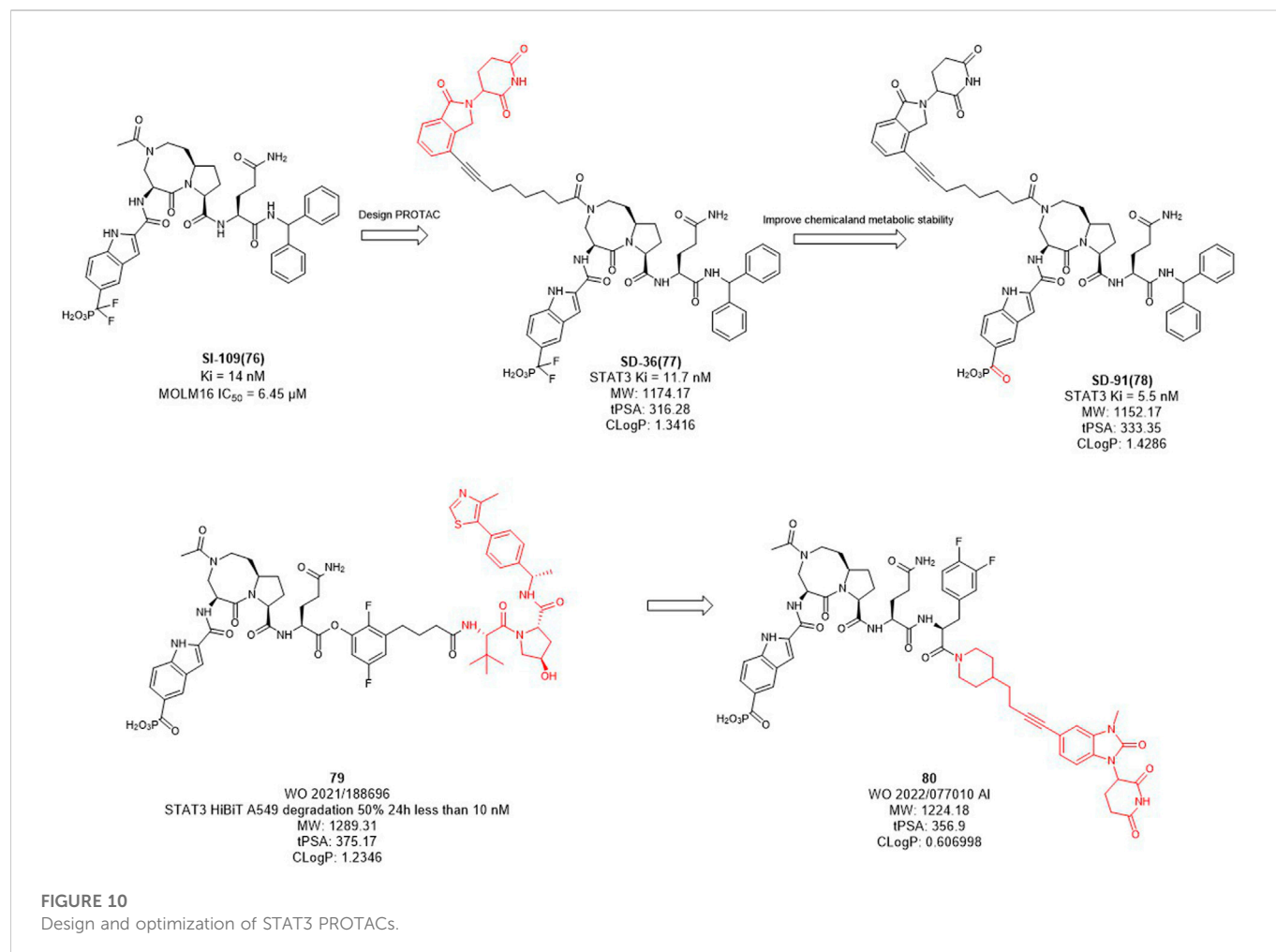


## 2.7 E3 ligase ligands optimization of EGFR L858R PROTACs

Several epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors have been developed and approved by the FDA for the treatment of non-small-cell lung cancer, but their efficacy may be compromised by drug resistance in EGFR-mutant variants (Sharma et al., 2007; Hirsch et al., 2017). Activating mutations, mainly in-frame deletions in exon 19 and L858R mutations, the former occurring in the  $\alpha$ C-helix domain and the latter in the adenosine triphosphate (ATP) binding domain of the EGFR kinase. The EGFR L858R variant leads to poor prognosis and high incidence of malignant pleural effusion in non-small cell lung cancer, and the current small molecule drugs are only

moderately effective against this variant (Kohno et al., 2021; Matsui et al., 2021). The development of EGFR L858R degraders based on PROTACs technology has become a new strategy. In 2020, Jin et al. designed and synthesized EGFR degraders with gefitinib and VHL or CRBN-recruited E3 ligands. The  $DC_{50}$  values of VHL-based degrader **68** (Figure 8) were 5.0 nM in HCC-827 cells and 3.3 nM in H3255 cells. The  $DC_{50}$  values of compound **69** which was based on CRBN ligand were 11 nM and 25 nM, respectively. In addition, they also showed good plasma exposure in mice.

C4T discovered the CRBN-based EGFR L858R degrader **70** (Figure 8) with an  $IC_{50}$  (BaF3 EGFR T790M/L858R/C797S degradation) value of 12 nM (Duplessis et al., 2019). After the optimization of the linker and the CRBN ligand, compounds **71** and **72** were subsequently synthesized, both showed a  $DC_{50}$  value



**TABLE 2** Summary of the chemical properties of E3 ligase ligands in clinical trials.

E3 ligase ligand	E3 ligase	MW	ROA	tPSA	cLog P
	CRBN	244.08	Oral	66.48	0.305
	CRBN	276.05	Oral	83.55	0.747
	CRBN	222.08	Oral	58.2	1.184
	VHL	486.23	Iv	111.1	2.343

Red spot: Linking site.

of 18 nM in H1975 cells. Based on the structure of 72, compound 73 was further identified, which improved the DC<sub>50</sub> value to 10 nM in H1975 cells. C4T replaced the lenalidomide ligand with a new CRBN-

based derivative ligand, resulted in a better activity of compound 73 than compound 70 on the basis of structural optimization (Nasveschuk et al., 2021).

## 2.8 E3 ligase ligands optimization of BCL-X<sub>L</sub> PROTACs

BCL-X<sub>L</sub> belongs to the anti-apoptotic BCL-2 protein family and plays a key role in determining cell life and death by regulating the intrinsic apoptotic pathway (Czabotar et al., 2014). The anti-apoptotic function of BCL-X<sub>L</sub> protects cancer cells and induces drug resistance, which also promotes tumor progression (Igney et al., 2002). Inhibition of BCL-X<sub>L</sub> has been of great interest as a potential cancer therapeutic strategy. However, traditional BCL-X<sub>L</sub> inhibitors, such as **ABT263** (Figure 9), exhibit targeted and dose-limited platelet toxicity (Zhang et al., 2019). Since the tissue distribution studies of VHL and CRBN have shown that its expression in platelets is minimal, degraders of BCL-X<sub>L</sub> could be developed through PROTAC technology.

In 2019, Zheng et al. selected **ABT263** (Figure 9) as BCL-X<sub>L</sub> inhibitor connected with CRBN to obtain the degrader **PZ15227** with DC<sub>50</sub> value of 46 nM and Dmax of 96.2% in WI38 non-senescent cells (NCs) (He et al., 2020). Compared with **ABT263**, **PZ15227** showed reduced toxicity to platelets but remained toxicity against senescent cells because CRBN was less expressed in platelets. Subsequently, they found that CRBN-based PROTACs were highly potent against other cancer cell lines, but less potent in MOLT-4 cells, possibly due to the low expression of CRBN (Zhang et al., 2020).

In 2021, Zheng et al. discovered **DT2216** (NCT04886622) as an effective BCL-X<sub>L</sub> degrader based on VHL E3 ligase with a DC<sub>50</sub> value of 63 nM and Dmax of 90.8% in MOLT-4 cells. Compared with **ABT263** (EC<sub>50</sub> = 0.191 μM, half max effective concentration) and **DT2216** (EC<sub>50</sub> = 0.052 μM), the latter showed increased cytotoxicity to MOLT-4 cells. More importantly, **DT2216** exerted almost no effect on the viability of platelets up to a concentration of 3 μM which showed better effect than **PZ15227**. **DT2216** was found to have enhanced efficacy against a variety of BCL-X<sub>L</sub>-dependent leukemia cell lines and exhibited much less toxic to platelets than **ABT263** (Khan et al., 2019). Therefore, **DT2216** was approved by FDA to enter phase I clinical trials for the treatment of advanced liquid and solid tumors. These findings demonstrated the potential of using PROTAC to reduce the toxicity of targeted drugs.

## 2.9 E3 ligase ligands optimization of STAT3 PROTACs

In mammalian cells, the signal transducer and activator of transcription 3 (STAT3) is an essential component of the seven members of the STAT family (STAT1, 2, 3, 4, 5a, 5b, 6). STAT3 is widely expressed in a variety of cells and tissues and activates the expression of downstream genes in response to various cytokines, growth factors and other signals (Yu et al., 2009). Under normal physiological conditions, STAT3 activation is rapid and transient, mainly due to the presence of negative regulators in cells. However, STAT3 is continuously activated and expressed at high levels in tumor cells. Overexpression of STAT3 is strongly associated with cancer cell survival, proliferation, invasion, metastasis, drug resistance, and immune evasion, among other related genes. Since STAT3 dysregulation contributes to many human cancers and other human diseases (Wang et al., 2018). Inhibition or downregulation of STAT3 expression has become a main strategy for cancer therapy. However, to date, no drugs based on STAT3 targets have been approved in the market. Wang et al. designed several

STAT3 degraders based on **SI-109** (Figure 10) and lenalidomide, and discovered that degrader **SD-36** exhibited good degradation activity (Zhou et al., 2019). In the subsequent studies, they converted the difluoro methylene group of **SD-36** to a ketone group to obtain degrader **SD-91** with improved potency both *in vitro* and *in vivo* (Zhou et al., 2021). Kymera Therapeutics chose the similar inhibitor, **SI-109** (Figure 10), but changed the linker attachment site and synthesized a series of degraders based on VHL ligand or CRBN ligand derivatives (Yang et al., 2022). From their disclosed data, the degradation activity of VHL-based degraders was generally better than that of CRBN-based derivatives. However, their recent patent selected CRBN-based ligands for further optimization may due to the large molecular of VHL ligand. It is hypothesized that reducing the molecular weight and the hydrogen bond receptors of E3 ligands may be the trend for PROTACs to be more druggable.

## 3 Conclusions and perspectives

Since PROTAC technology was first identified as a clinical therapeutic strategy, both academia and industry have shown great interest in PROTACs technology. However, PROTACs degraders have relatively more complex chemical structures and biological mechanisms than traditional small molecule drugs, which require efforts in the fields of organic synthetic chemistry and medicinal chemistry.

Although PROTACs technology has shown many advantages over traditional small molecule drugs for antitumor therapy, unlike traditional small molecule drugs, they are mostly regarded as “beyond rule-of-five”. PROTACs molecules have more hydrogen bond donors and acceptors and larger molecular weights, mostly around 1,000 Da. Therefore, the poor membrane permeability and low bioavailability of PROTACs molecules limit its clinical application. In the process of optimizing E3 ligase ligands by medicinal chemists, it is important to ensure a high affinity between the E3 ligase ligands and E3 ubiquitin ligases. The excessive molecular weight of E3 ligands leads to poor membrane permeability. Based on the suitable molecular weight size of CRBN ligand, **ARV-110** and **CFT8634** were rationally designed. In addition, they both introduced F in the aromatic ring of CRBN ligands, probably to improve their druggability. Most of the other CRBN ligands modifications are designed to improve the affinity with E3 ligases or to improve the druggability properties of these degraders in oral PROTACs.

Typically, the POIs based on agonists or antagonists of target proteins, as well as the traditional pharmacological optimization of these small molecules, have been studied in the design and optimization of PROTACs. The optimization of linkers tends to have more rigid structures. However, although more than 600 E3 ligases have been identified, the number of small molecule ligands available to design PROTAC molecules for E3 ligands is rather limited, and only CRBN-based PROTACs have been clinically achieved for oral application (Table 2).

It is believed that advances in artificial intelligence techniques (protein structure prediction), virtual drug screening, and other technologies will facilitate the discovery of E3 ligands and provide more tools for PROTAC design. These advances will greatly facilitate the transition of PROTACs degraders from being considered as tool molecules to small molecule clinical drug candidates.

## Author contributions

Concept, writing, review, and revision of the manuscript: MW, S-XG, HJ, and HX; all authors have approved the final version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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