



## REVIEW ARTICLE

# Recent advances of PROTACs technology in neurodegenerative diseases



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## KEYWORDS

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**Abstract** Neurodegenerative diseases, like Alzheimer's disease, Huntington's disease, Parkinson's disease, progressive supranuclear palsy, and frontotemporal dementia are among the refractory diseases that lack appropriate drugs and treatments. Numerous disease-causing proteins in neurodegenerative diseases are undruggable for traditional drugs. Many clinical studies of drugs for Alzheimer's disease have failed, and none of the substances that slowed the amyloid- $\beta$  (A $\beta$ ) accumulation process have been approved for use in clinical trials. A novel approach to addressing this issue is Proteolysis targeting chimeras (PROTACs) technology. PROTACs are heterogeneous functional molecules joined by a chemical linker and include binding ligands for the target protein and recruitment ligands for the E3 ligand. When a PROTAC binds to a target protein, the E3 ligand enzyme is brought into close contact and the target protein begins to be polyubiquitinated, followed by proteasome-mediated degradation. Numerous neurodegenerative disease-related targets, including  $\alpha$ -Synuclein, mHTT, GSK-3, LRRK2, Tau, TRKA, and TRKC have been successfully targeted by PROTACs to date. This article presents a comprehensive overview of the development of PROTACs in neurodegenerative diseases. These PROTACs' chemical structures, preparative routes, *in vitro* and *in vivo* activities, and pharmacodynamics are outlined. We also offer our viewpoint on PROTACs' probable challenges and future prospects.

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## 1. Introduction

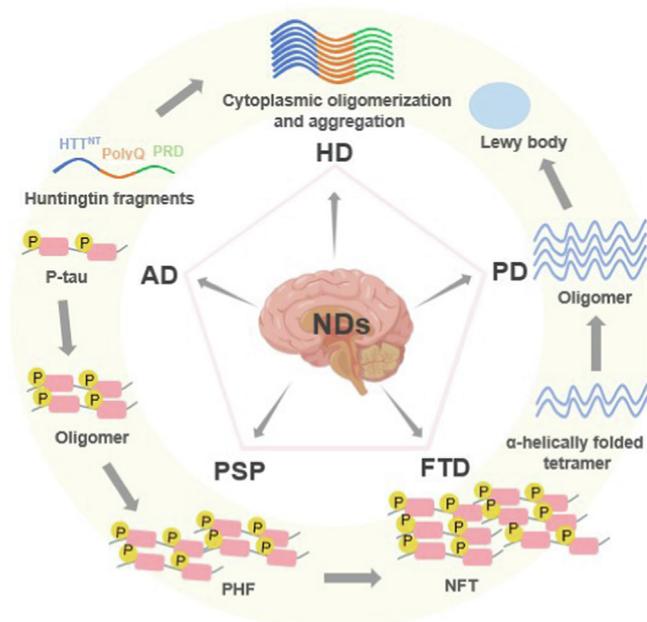
More than 10 million people worldwide are affected by neurodegenerative diseases (NDs) each year, including Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), progressive supranuclear palsy (PSP), and frontotemporal dementia (FTD) (Fig. 1). These diseases are the most common and difficult to treat affecting the central nervous system (CNS) because of their crippling effects (Alzheimer's Association, 2010; Deng et al., 2018; GBD 2016 Neurology Collaborators, 2019; Gribkoff et al., 2017; Scheltens

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**Fig. 1** Pathological sites and pathogenesis of pathogenic proteins of common neurodegenerative diseases.

et al., 2021). As life expectancy continues to increase and the number of patients grows each year, the need for novel drugs to treat chronic NDs is becoming more urgent. Unfortunately, much remains to be learned about the pathogenesis of these complex brain disorders, and the ability to treat them is often hampered by the difficulties associated with diagnosis, resulting in interventions that come too late. These diseases' heterogeneity adds to their complexity; frequently, it is this accumulation of toxic proteins that causes severe, gradual neuronal death (Breijyeh and Karaman, 2020; Maher, 2019; Inuzuka et al., 2022). Small-molecule inhibitors and immunotherapies that target disease-causing proteins are among the current treatments for NDs, however, they can only alleviate the symptoms of these diseases rather than reverse their progression. Many new NDs therapies are being explored, such as miRNA therapies, autophagy, and stem cell therapies, which also face problems such as low BBB permeability, target bias, and inability to act on intracellular proteins. The fact that the Food and Drug Administration (FDA) is about half as likely to approve CNS drugs as peripheral targets, and the approval process takes almost twice as long as for oncology drugs, further complicates the situation (Banks, 2016; Charvériat et al., 2021). Finally, many pharmaceutical companies have left the neuroscience research and development space as a result of the failure of numerous advanced clinical trials in the field of AD and other brain diseases, which has decreased the resources being invested to solve the issue. A portion of this failure can be related to a lack of knowledge about the illness itself, which makes it challenging to accurately target the main biological pathways that cause it (Farrell and Jarome, 2021; Ma et al., 2021). To date, a vast majority of NDs still lack effective therapeutic options.

Normal cells acquire the ability to regulate their self-controlled quality processes in order to maintain homeostasis by avoiding long-lasting damage as a result of external factors. Misfolded proteins that aggregate into  $\beta$ -sheet structures are a major cause of neurodegenerative diseases. Typically, the ubiquitin proteasome system (UPS) is the main defense mechanism that cells have against aggregated and misfolded proteins to maintain homeostasis (Pohl and Dikic, 2019). The UPS system, which is primarily made up of the protein ubiquitin (Ub), three catalytic enzymes, proteasomes, and certain substrates, is crucial to many biological processes. A series of enzymatic processes, including the cooperative action of Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub-ligase enzyme (E3), cause ubiquiti-

nation. Once the substrate protein has been polyubiquitinated, the proteasome will detect it and begin to break it down, and UPS can then break the protein down into minute peptides (Deol et al., 2019). E3 appears to have the ability to specifically recognize substrate proteins, and as a result, E3 is crucial in determining the specificity of Ub-mediated protein degradation.

Proteolysis targeting chimeras (PROTACs) are emerging as a novel technology to treat a variety of diseases and are considered the next "blockbuster" therapy for neurodegenerative diseases and other diseases with undruggable protein targets (An and Fu, 2018; Wang et al., 2021; Xiao et al., 2022). PROTACs are heterobifunctional compounds that use cellular degradation systems to degrade a target protein (Fig. 2). With the optimal linker, PROTACs recruit the target protein to the E3 ubiquitin ligase (e.g. CRBN, IAP, MDM2, VHL ligase, etc.). In eukaryotic cells, this complex formation between the protein of interest (POI) and the E3 ligases causes POI ubiquitination, which is then followed by POI degradation by the 26S proteasome. The use and advancement of PROTACs technology have significantly increased in both industry and academia following the verification of the proof of concept (Gadd et al., 2017; Martín-Acosta and Xiao, 2021; Zeng et al., 2021). This is primarily due to the following aspects of PROTACs: 1) ability to eliminate the target proteins that are "undruggable," 2) ability to decrease both the non-enzymatic and enzymatic functions of proteins, 3) reversible and rapid elimination of protein targets, 3) enhancing selectivity and specificity, and 5) promise in overcoming drug resistance. Drug resistance is a significant issue in treating diseases, therefore PROTACs technology will provide more potent medications to combat it. Because of its new mechanism of action for the reduction of target protein levels while using less dosage of the drug than traditional ones, the use of PROTACs should also be very advantageous (Gu et al., 2018; Sun et al., 2019; Toure and Crews, 2016; Wang et al., 2021). Currently, PROTACs have been successfully used to degrade different types of target proteins associated with various diseases, including neurodegenerative diseases, cancer, viral infections, and immune disorders (Fig. 3). Some of the reported cases include Bristol-Myers Squibb's PROTACs targeting the androgen receptor (AR), Dialectic's PROTACs targeting the B-cell lymphoma extra large (BCL-X<sub>L</sub>), Kymera's PROTACs targeting the interleukin-1 receptor-associated kinase 4 (IRAK4) and STAT3, Nurix' PROTACs targeting the BTK, C4 Therapeutic's PROTACs targeting the

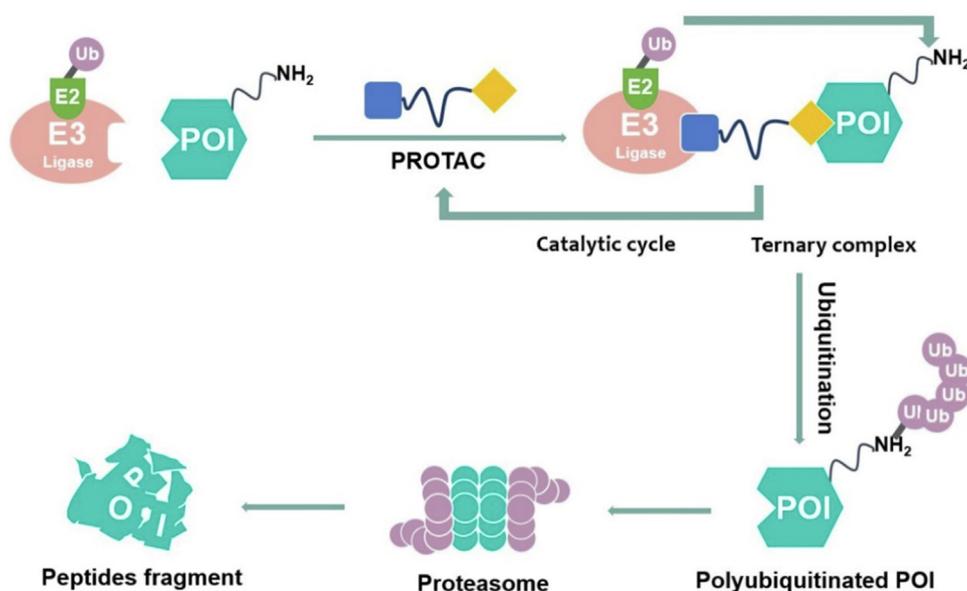


Fig. 2 The proposed mechanism of action of PROTACs.

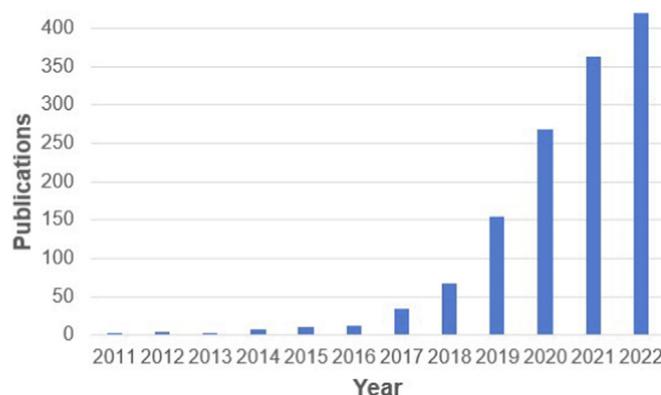


Fig. 3 The number of publications on PROTACs in PubMed (accessed on 27/11/2022).

epidermal growth factor receptor (EGFR), and Cullgen's PROTACs targeting the tropomyosin receptor kinases (TRKs) (Garber, 2022; Mullard, 2021). In short, PROTAC molecules have the potential to treat a variety of diseases, including neurodegenerative diseases (Table 1).

**Table 1** Comparisons of different targeted therapies for immune-related diseases.

	PROTAC	Small-Molecule Inhibitor
Target scaffolding functions	Yes	No
Potential to treat undruggable proteins	Yes	No
Broad tissue penetration	Yes	No
Iterative mechanism of action	Yes	No
Orally bioavailable	Yes	Yes
Ease of manufacturing	Yes	Yes
Preclinical validation, proof-of-concept established	Yes	Yes
Clinical validation	Yes	Yes

The concept of PROTACs was first introduced by Craig M. Crews and Raymond J. Deshaies in 2001. The first generation of PROTACs was a bifunctional molecule consisting of the 10 amino acid phosphopeptide DRHDSGLDSM and the angiogenesis inhibitor ovalicin. This peptide sequence could be recognized by the Fbox protein  $\beta$ -TRCP E3 ubiquitin protein ligase. The mechanism of the first generation PROTACs was the induction of MetAP2 degradation through the recruitment of  $\beta$ -TRCP E3 ligase. The problem with first generation PROTACs was that they have low cell permeability and stability in biological systems. In 2008, to address the aforementioned problem, Craig M. Crews and coworkers developed the first small-molecule PROTACs capable of successfully inducing intracellular degradation of the androgen receptor (AR) in human cervical cancer cells (Benowitz et al., 2021). This PROTAC associated the selective androgen receptor modulator SARM with nutlin, recruiting the E3 ubiquitin ligase human homolog of mouse double molecule 2 (MDM2). Recently, the study of new small-molecule PROTACs with cereblon (CRBN), inhibitors of apoptosis (IAPs), von Hippel-Lindau (VHL), DDB1 and CUL4-associated factors (DCAF15, DCAF16), and ring finger protein (RNF114) ligands had progressed rapidly, offering the potential for the development of new therapeutic drugs. Updated through November 2022, there were approximately a dozen PROTACs in clinical development globally (Table 2). Among them, Arvinas' ARV-471 has entered clinical phase III and is the most advanced PROTAC drug in clinical development. Some start-ups have

gained close attention from large global pharmaceutical companies, such as Gilead, Merck, Pfizer, Roche, Sanofi, etc. Additionally, Chinese companies are also actively involved in the development, including Hesco, Yaxin, Jinto, Novocain Jianhua, Hengrui, etc. It can be seen that the clinical application is currently in a state of explosive growth. Today, PROTAC technology has evolved into a new approach to the development of novel drugs and the treatment of diseases. A crucial time for the development of PROTACs will be in the upcoming years. To date, more than a dozen PROTACs molecules have entered preclinical and clinical studies. Future advances in PROTAC technology are anticipated to help the treatment of human disease and overall well-being (Chirnomas et al., 2023; Cao et al., 2022; Garber, 2022).

Target proteins associated with neurodegenerative diseases have been successfully degraded by PROTACs. We discovered a comprehensive list of these targets after searching the literature over the previous few years, mainly including  $\alpha$ -Synuclein, mutant huntingtin (mHTT), glycogen synthase kinase 3 $\beta$  (GSK-3 $\beta$ ), leucine-rich repeat kinase 2 (LRRK2), Tau, TRKA, and TRKC. Thus, PROTACs are today thought to be one of the most promising alternatives to chemical interventions in pharmacology and as potential therapeutic tools. The major turning points and events in the evolution of PROTACs associated with neurodegenerative diseases are shown in Fig. 4. According to drug target class criteria and alphabetical target order, we present PROTACs for drug targets for neurodegenerative diseases in this review. In addition, we focus on E3 ligase ligands utilized in successful PROTACs in NDs. We also discuss potential problems specific to this field. We hope that this article will serve as a complement to previous papers on protein degradation.

## 2. E3 ligase ligands

### 2.1. CRBN

CRBN is a 442-amino acid protein that associates with the adaptor molecule damaged DNA binding protein 1 (DDB1)

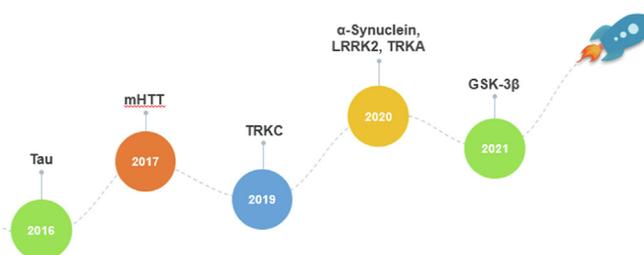
to generate the Cullin-4-RING E3 ubiquitin ligase (CRL4) complex (Chamberlain et al., 2014). CRBN functions as a substrate-specificity receptor inside the CRL4 complex. Thalidomide and other immunomodulatory imide drugs (IMiDs) are known CRBN ligands. The E3 ubiquitin ligase activity of CRBN is remodulated when IMiDs attach to it (Zhu et al., 2011). The transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) are consequently recruited more frequently, which results in their subsequent ubiquitination and proteasomal degradation. To date, PROTAC has been successful in using CRBN as the E3 ligase to target NDs-associated proteins. The CRBN targeting NDs PROTACs employ pomalidomide, 4-hydroxythalidomide, and 5-aminothalidomide (Fig. 5).

The synthetic routes to obtain CRBN E3 ligands are presented in Scheme 1a, Scheme 1b, Scheme 1c. Typically, 3-nitrophthalic anhydride was used as the starting compound in the synthesis of pomalidomide. 4-nitrothalidomide was created by condensing 3-nitrophthalic anhydride with the glutarimide ring. In comparison to employing iron-ammonium chloride or Pd/C and ammonium formate as reducing agents (Steinebach et al., 2018), the subsequent reduction to pomalidomide was significantly more efficient when using Pd/C-catalyzed hydrogenation with reports of nearly quantitative yield (Chen et al., 2018). A different approach was described by Huang et al. in which the 3-nitrophthalimide was first reacted with glutamine *via* amination to produce a carboxylic acid intermediate. Next, it was reduced to an amine intermediate and subsequently cyclized under CDI-mediated conditions (Huang et al., 2016). Low reduction conversion led to a low pomalidomide yield, which was the main cause of this.

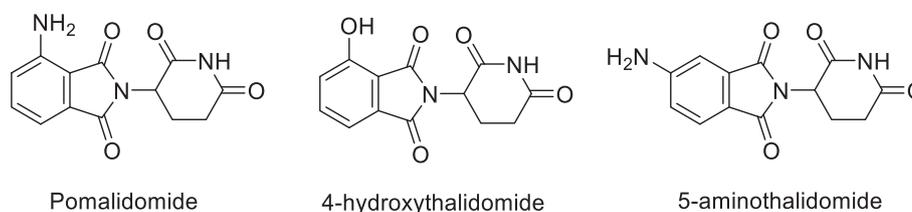
3-hydroxyphthalic anhydride was typically used as the starting compound in the preparation of 4-

**Table 2** Representative PROTACs in the clinic (<https://clinicaltrials.gov/>).

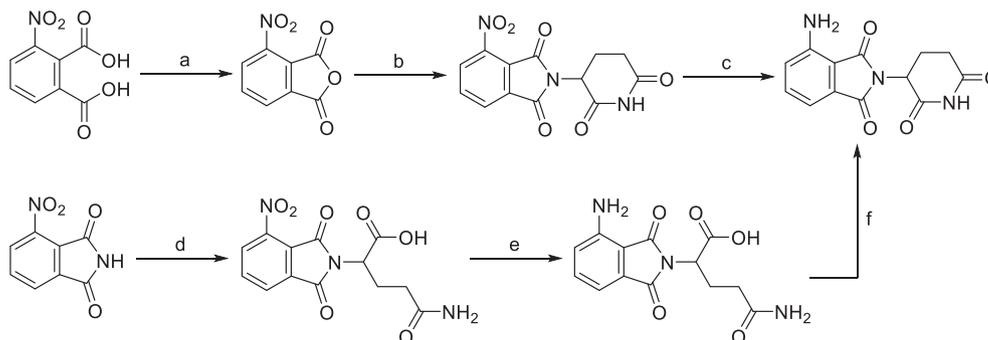
PROTAC	Target protein	Usage	Company	Condition
ARV-110	AR	Prostate cancer	Arvinas	Phase II
ARV-766	AR	Prostate cancer	Arvinas	Phase I
CC-94676	AR	Prostate cancer	Bristol Myers Squibb	Phase I
ARV-471	ER	Breast cancer	Arvinas	Phase II
DT2216	BCL-X <sub>L</sub>	Liquid and solid tumors	Dialectic	Phase I
FHD-609	BRD9	Synovial sarcoma	Foghorn	Phase I
NX-2127	BTK, Ikaros, Aiolos	B-cell malignancies	Nurix	Phase I
BGB-16673	BTK	B-cell malignancies	BeiGene	Phase I
NX-5948	BTK	B-cell malignancies	Nurix	Phase I
HK29116	BTK	B-cell malignancies	Haisco	Phase I
KT-474	IRAK4	Atopic Dermatitis	Kymera	Phase I
KT-413	IRAK4	B-cell NHL	Kymera	Phase I
LNK-01002	Ras GTPase	Myelofibrosis or Myeloid Leukemia	Lynk	Phase I



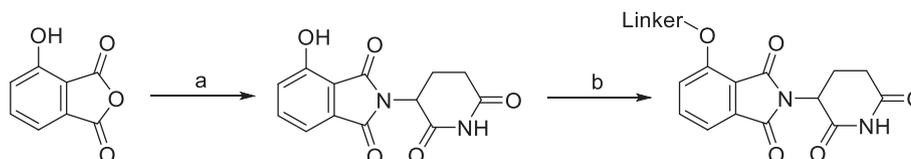
**Fig. 4** Timeline and major milestones for the development of neurodegenerative diseases.



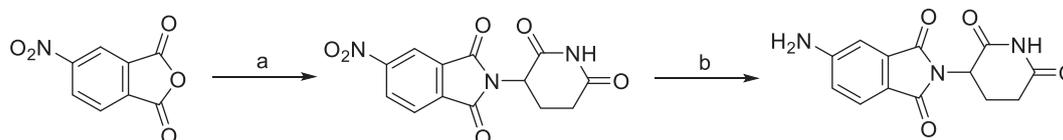
**Fig. 5** Chemical structures of CRBN ligase ligands used in NDs PROTACs.



**Scheme 1a** Synthesis of pomalidomide. Reagents and conditions: a)  $\text{Ac}_2\text{O}$ , reflux, 2 h; b) *tert*-butyl (2,6-dioxopiperidin-3-yl)carbamate, NaOAc, AcOH, reflux, 6 h; c) 3-aminopiperidine-2,6-dione hydrochloride, NaOAc, AcOH, 130 °C, 48 h; d) Pd/C,  $\text{H}_2$ , DMF, r.t., 24 h; e) glutamine, DMF, 80–87 °C, 8 h; f) Pd/C,  $\text{H}_2$ , 50 psi, MeOH, 2.5 h; g) CDI, MeCN, reflux, 4.5 h.



**Scheme 1b** Synthesis of 4-hydroxythalidomide. Reagents and conditions: a) 3-aminopiperidine-2,6-dione hydrochloride, KOAc, AcOH, reflux, 24 h; b) Linker-Br, KI,  $\text{NaHCO}_3$ , DMF, 60 °C, 12 h.



**Scheme 1c** Synthesis of 5-aminothalidomide. Reagents and conditions: a) 3-aminopiperidine-2,6-dione trifluoroacetate, AcOH, reflux, 2 h; b) Pd/C,  $\text{H}_2$ , r.t., 20 h.

hydroxythalidomide. With different reaction conditions, the condensation with the glutarimide ring was achievable using 3-hydroxyphthalic anhydride as the starting material. While yields for other reported procedures ranged between 83 and 90% (Burslem et al., 2018; Papatzimas et al., 2019), which were comparable to the reactions used to synthesize 4-fluoro- and 4-nitrothalidomide, the highest yield for the desired product 4-hydroxythalidomide was described to be 96% (KOAc, AcOH, reflux) (Robb et al., 2017). By adding a bromo group as a linker terminus, it was feasible to derivatize 4-hydroxythalidomide and create a derivative that had an ether bond. Through a Mitsunobu reaction, a linker with a terminal hydroxyl group could be joined to 4-hydroxythalidomide (Chessum et al., 2018).

Derivatives of 5-aminothalidomide were less frequently used in PROTACs, although this substitution pattern remained a valid option for targeting CRBN. 5-aminothalidomide was synthesized by condensation of 4-nitrophthalic anhydride with 3-aminopiperidine-2,6-dione trifluoroacetate to form 5-nitrothalidomide, which was then reduced to the desired product 5-aminothalidomide (Capitosti et al. 2003).

## 2.2. IAP

IAP are a group of apoptosis-inhibiting regulators that work by preventing caspase from activating. There are eight mem-

bers of the IAP family; the three most well researched members are c-IAP1, c-IAP2, and XIAP. IAP are members of the E3 ubiquitin ligase family and are important targets for cancer therapy. The term “specific and nongenetic IAP-dependent protein erasers (SNIPERs)” is also used to describe IAP-based PROTACs. IAP inhibitors and their derivatives are frequently employed as SNIPERs E3 ligase ligands. The cIAP1-binding ligands for the reported NDs PROTACs recruiting this E3 ligase were bestatin and MV1 (Fig. 6) (Ma et al., 2021). In comparison to early bestatin-based compounds, the effectiveness of SNIPERs was increased by the subsequent development of high-affinity IAP ligands and their incorporation into bifunctional molecules. The representative synthetic routes to obtain IAP E3 ligands are presented in Scheme 2a, Scheme 2b.

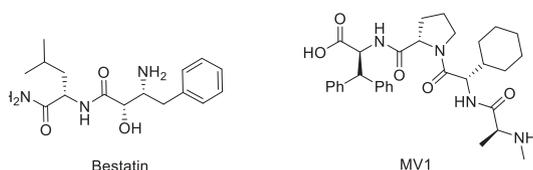
The representative synthetic route to bestatin involved the treatment of (2-nitroethyl)benzene and ethyl glyoxalate in Shibasaki's asymmetric Henry reaction catalyzed by optically active lanthanum-(*R*)-binaphthol complex. Then, ethyl (2*S*,3*R*)-2-hydroxy-3-nitro-4-phenylbutanoate was *O*-acetylated prior to the reduction of the nitro group to give (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid. After *N*-Boc protection, the phenylbutanoic acid derivative was coupled to *L*-leucine benzyl ester followed by immediate deprotection of the terminal carboxylic acid group. Both protecting groups of the precursor of bestatin were removed to give bestatin in an overall yield of 26% (Gogoi et al., 2005).

Scheme 2a, Scheme 2b outlines the solid-phase peptide synthesis of IAP ligand B derivative on a 2-chlorotrichloromethane resin. It was performed stepwise, using HCTU, HOBT, and DIPEA for coupling, followed by the addition of 20% piperidine in DMF and removal of the Fmoc moiety after each step. Finally, the Boc-protected IAP ligand was treated with 1% TFA in DCM to remove the resin to obtain MV1 (Ohoka et al., 2017).

### 2.3. VHL

The VHL protein is part of a multiprotein complex with elongin B and C, cullin 2, and Rbx-1, which have E3 ubiquitin ligase activity. In the complex, VHL folds into two structural domains, one of which is responsible for binding specific substrates (Van Molle et al., 2012), most notably hypoxia-inducible factor (HIF)-1 $\alpha$ , leading to its ubiquitination and proteasomal degradation. VHL, similar to CRBN, is a broad target for NPs PROTACs (Fig. 7). The synthetic routes to obtain VHL E3 ligands (VHL ligands 1, 2, and 3) are shown in Scheme 3a, Scheme 3b, Scheme 3c.

The most efficient procedure for the synthesis of VHL ligand 1 started with 4-bromobenzylamine, which was first



**Fig. 6** Chemical structures of IAP ligase ligands used in NDs PROTACs.

protected by Boc to *tert*-butyl (4-bromobenzyl)carbamate, and then underwent the Heck reaction and Boc deprotection to give the key intermediate (4-(4-methylthiazol-5-yl)phenyl)methanamine. The final VHL ligand 1 was prepared by coupling (4-(4-methylthiazol-5-yl)phenyl)methanamine with the dipeptide, which was prepared from *N*-Boc-*L*-*tert*-leucine and hydroxyproline methyl ester. Incorporating an element of convergent synthesis helped to increase the overall yield (Han et al., 2019).

In the design and optimization of VHL ligands, the introduction of an (*S*)-methyl group on the benzyl carbon atom improved the binding affinity for VHL. That was, the potency of the methyl-substituted ligand was three times better than that of the non-substituted VHL ligand 1 (Han et al., 2019). The synthesis of the important intermediate (*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethan-1-amine was similar to the synthetic route described in Scheme 3a, using (*S*)-(-)-4-bromo- $\alpha$ -methylbenzylamine as the starting material. The left-hand side dipeptide moiety could be assembled by linear synthesis (Hu et al., 2019) or convergent synthesis (Raina et al., 2016) to obtain VHL ligand 2.

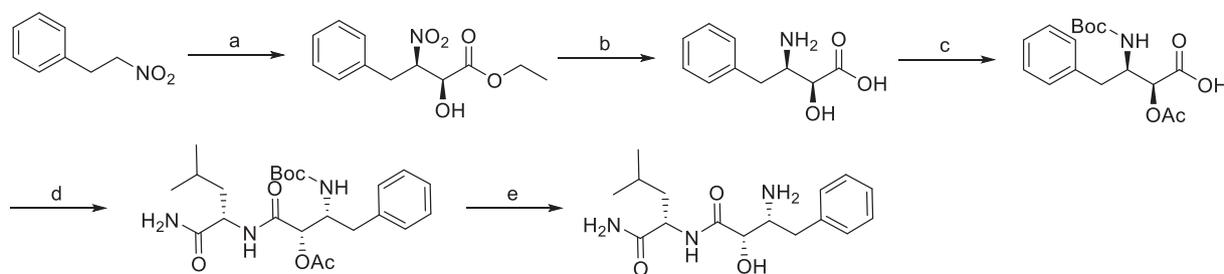
The synthesis of VHL ligand 3 started with *tert*-butyl (4-(4-methylthiazol-5-yl)benzyl)carbamate (Liu et al., 2022). The important intermediate, *tert*-butyl (2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carboxylate was generated from *tert*-butyl (4-(4-methylthiazol-5-yl)benzyl)carbamate and Boc-*L*-hydroxyproline, which was then Boc-protected and coupled with tributyl-protected penicillamine to give the sulfur-based intermediate which was derivatized into the precursor of VHL ligand 3. VHL ligand 3 was obtained from removing the trityl group.

## 3. PROTACs for neurodegenerative diseases

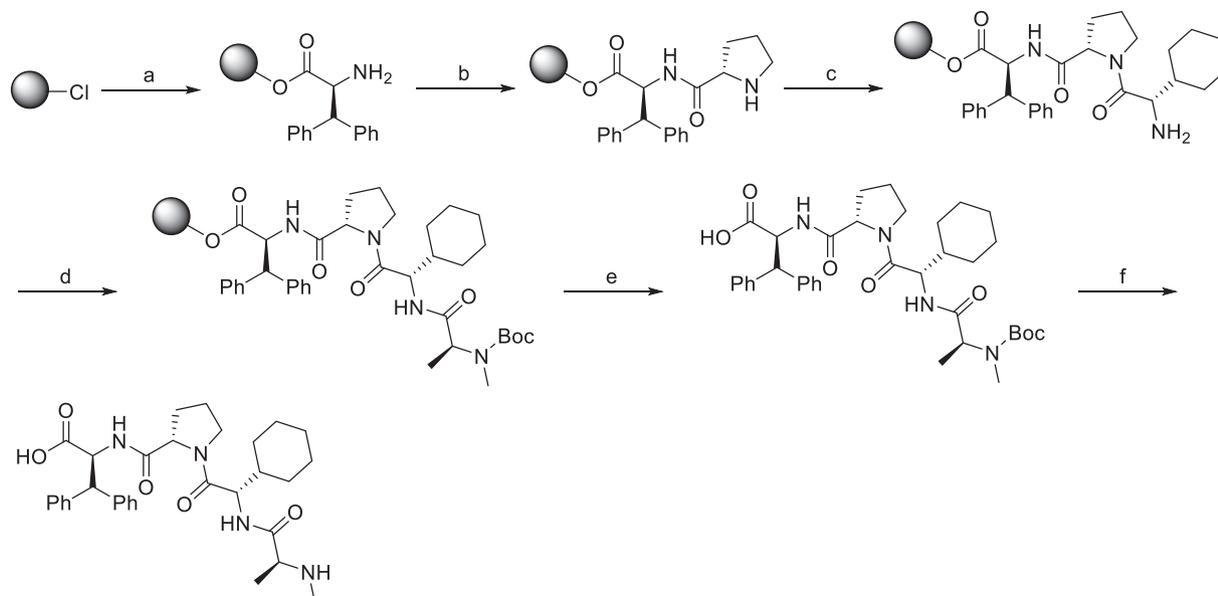
### 3.1. $\alpha$ -Synuclein

$\alpha$ -Synuclein is a protein that is found in abundance in neurons and is particularly enriched in presynaptic nerve terminals (Shahnawaz et al., 2020).  $\alpha$ -Synuclein has been linked to PD and other neurodegenerative diseases through neuropathology and genetic research (Schwab et al., 2020). Numerous neurodegenerative diseases known as synucleinopathies are characterized by the accumulation of misfolded oligomers and larger aggregates of  $\alpha$ -synuclein.

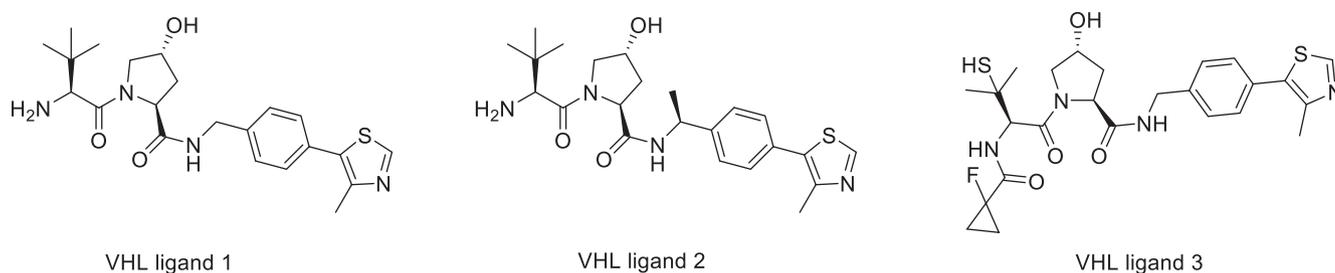
In 2020, a recent patent filing from Andrew P Crew and coworkers disclosed PROTAC molecules that are claimed to degrade  $\alpha$ -Synuclein. Exemplified compounds incorporated CRBN and VHL-binding ligands as well as chemically diverse binders to  $\alpha$ -Synuclein, and they were shown to significantly reduce  $\alpha$ -Synuclein in HEK293 TREX u-syn A53T cells which had been treated with pre-formed  $\alpha$ -Synuclein fibrils to induce insoluble  $\alpha$ -Synuclein aggregates (Kargbo, 2020). The authors found that representative compounds PROTAC1 (Fig. 8), PROTAC2 (Fig. 8), and PROTAC3 (Fig. 8) could induce to degrade  $\alpha$ -Synuclein, and the degradation activities were 30–65% at 1  $\mu$ M, demonstrating that neuronal diseases associated with  $\alpha$ -Synuclein aggregation and accumulation (dementia, PD, AD, etc.) could be treated by targeted degradation of  $\alpha$ -Synuclein. PROTAC1, PROTAC2, and PROTAC3 were synthesized *via* synthetic pathways shown in Scheme 4a, Scheme 4b, Scheme 4c.



**Scheme 2a** Synthesis of bestatin. Reagents and conditions: a) Ethyl glyoxalate, La-(*R*)-BINOL, THF,  $-50\text{ }^{\circ}\text{C}$ ; b) i) acetylation; ii) Pd/C,  $\text{NaBH}_4$ ,  $\text{H}_2$ , MeOH, r.t.; c) (Boc) $_2$ O,  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$ , EtOAc, r.t.; d) i) *L*-leucine benzyl ester, *N*-ethylmorpholine, isobutyl chloroformate, THF,  $-10\text{ }^{\circ}\text{C}$ ; ii) Pd/C,  $\text{H}_2$ , MeOH, r.t.; e) i)  $\text{K}_2\text{CO}_3$ , MeOH, r.t.; ii) TFA, MeOH, r.t..



**Scheme 2b** Synthesis of MV1. Reagents and conditions: a) i) Fmoc-*L*-3,3-diphenylalanine, DIPEA, DCM, r.t., 2 days; ii) 20% piperidine in DMF, r.t., 10 min; b) i) Fmoc-*L*-Pro-OH, HCTU, HOBT, DIPEA, DMF, r.t., 1.5 h; ii) 20% piperidine in DMF, r.t., 10 min; c) i) Fmoc-*L*-cyclohexylglycine, HCTU, HOBT, DIPEA, DMF, r.t., 1.5 h; ii) 20% piperidine in DMF, r.t., 10 min; d) i) Fmoc-*L*-Ala-OH, HCTU, HOBT, DIPEA, DMF, r.t., 1.5 h; ii) 20% piperidine in DMF, r.t., 10 min; e) 1% TFA in DCM, r.t., 5 min.

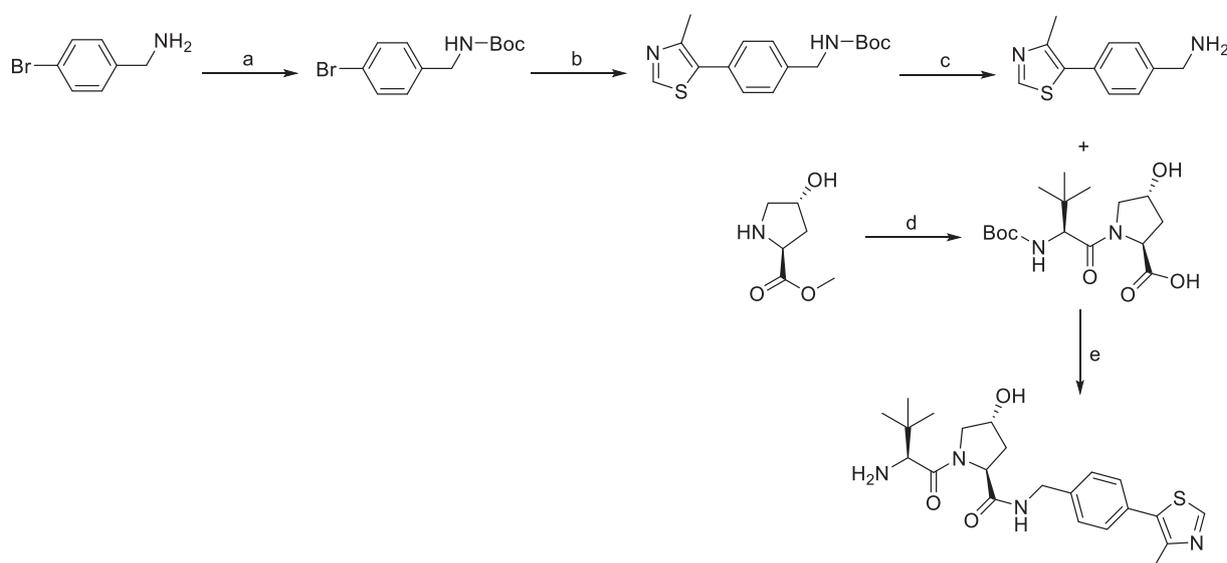


**Fig. 7** Chemical structures of VHL ligase ligands used in NDs PROTACs.

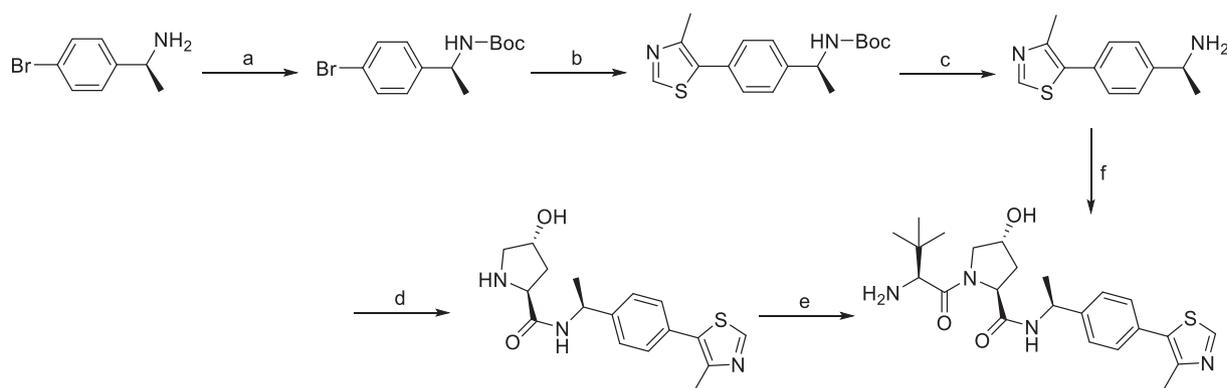
### 3.2. mHtt

The neurodegenerative disease HD is inherited autosomal dominant and characterized by a triad of psychiatric, cognitive, and motor features. All cases of HD are brought on by an abnormally expanded CAG repeat close to the huntingtin gene's *N* terminus, which results in the synthesis of mHTT

on translation (Reiner et al., 2011). Since the genetic mutation was discovered in 1993, 30 years have passed, and extensive studies have revealed numerous cellular pathogenic pathways driving disease onset. The mHTT protein, which is very universally expressed and is thought to cause disease through a predominate harmful gain-of-function mechanism, is mostly responsible for all of them (Sánchez et al., 2003). Reducing mHTT enhances disease phenotypes and reverses neuropathol-



**Scheme 3a** Synthesis of VHL ligand 1. Reagents and conditions: a)  $(\text{Boc})_2\text{O}$ ,  $\text{NaHCO}_3$ ,  $\text{EtOAc}/\text{H}_2\text{O}$ , r.t., 1 h; b) 4-methylthiazole,  $\text{Pd}(\text{OAc})_2$ ,  $\text{KOAc}$ ,  $\text{DMF}$ ,  $90^\circ\text{C}$ , 2 h; c)  $\text{TFA}$ ,  $\text{DCM}$ , r.t., 30 min; d) i)  $\text{Boc-Tle-OH}$ ,  $\text{HATU}$ ,  $\text{DIPEA}$ ,  $\text{DMF}$ , r.t., overnight; ii)  $\text{LiOH}$ ,  $\text{THF}/\text{H}_2\text{O}$ , r.t., 30 min; e) i)  $\text{HATU}$ ,  $\text{DIPEA}$ ,  $\text{DMF}$ , r.t., overnight; ii)  $\text{TFA}$ ,  $\text{DCM}$ , r.t., 30 min.



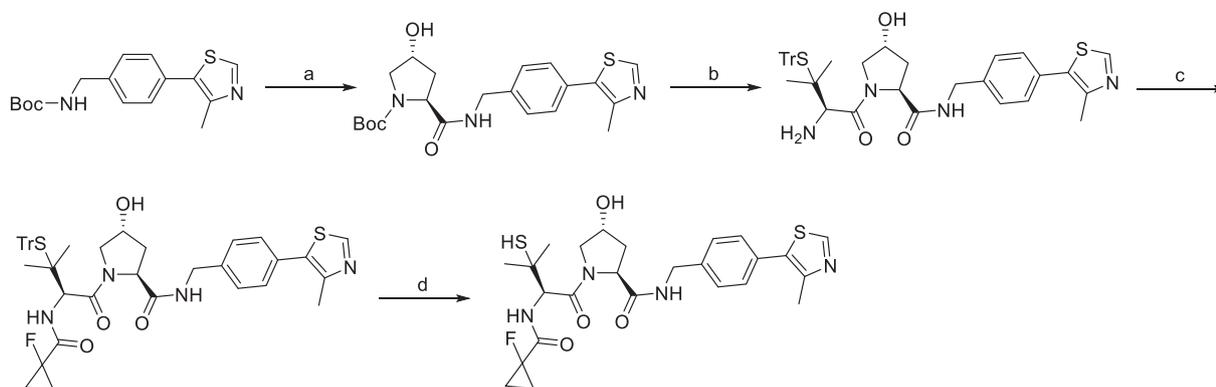
**Scheme 3b** Synthesis of VHL ligand 2. Reagents and conditions: a)  $(\text{Boc})_2\text{O}$ ,  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}/\text{EtOAc}$ , r.t., 2 h; b) 4-methylthiazole,  $\text{Pd}(\text{OAc})_2$ ,  $\text{KOAc}$ ,  $\text{DMA}$ ,  $90^\circ\text{C}$ , 18 h; c) 4 M  $\text{HCl}$  in  $\text{MeOH}$ , r.t., 3 h; c) 4 M  $\text{HCl}$  in dioxane,  $\text{MeOH}$ , r.t., 12 h; d) i)  $\text{Boc-Hyp-OH}$ ,  $\text{HATU}$ ,  $\text{DIPEA}$ ,  $\text{DMF}$ ,  $0^\circ\text{C}$ -r.t., 12 h; ii) 4 M  $\text{HCl}$  in dioxane,  $\text{MeOH}$ , r.t., 12 h; e) i)  $\text{Boc-Tle-OH}$ ,  $\text{HATU}$ ,  $\text{DIPEA}$ ,  $\text{DMF}$ ,  $0^\circ\text{C}$ -r.t., 12 h; ii) 4 M  $\text{HCl}$  in dioxane,  $\text{MeOH}$ , r.t., 12 h; f) i) dipeptide,  $\text{HATU}$ ,  $\text{DIPEA}$ ,  $\text{THF}$ , r.t., 2 h; ii) 4 M  $\text{HCl}$  in  $\text{MeOH}$ , r.t., 3 h.

ogy in HD animal models, demonstrating the significance of mHTT lowering as a treatment strategy (Bauer et al., 2010).

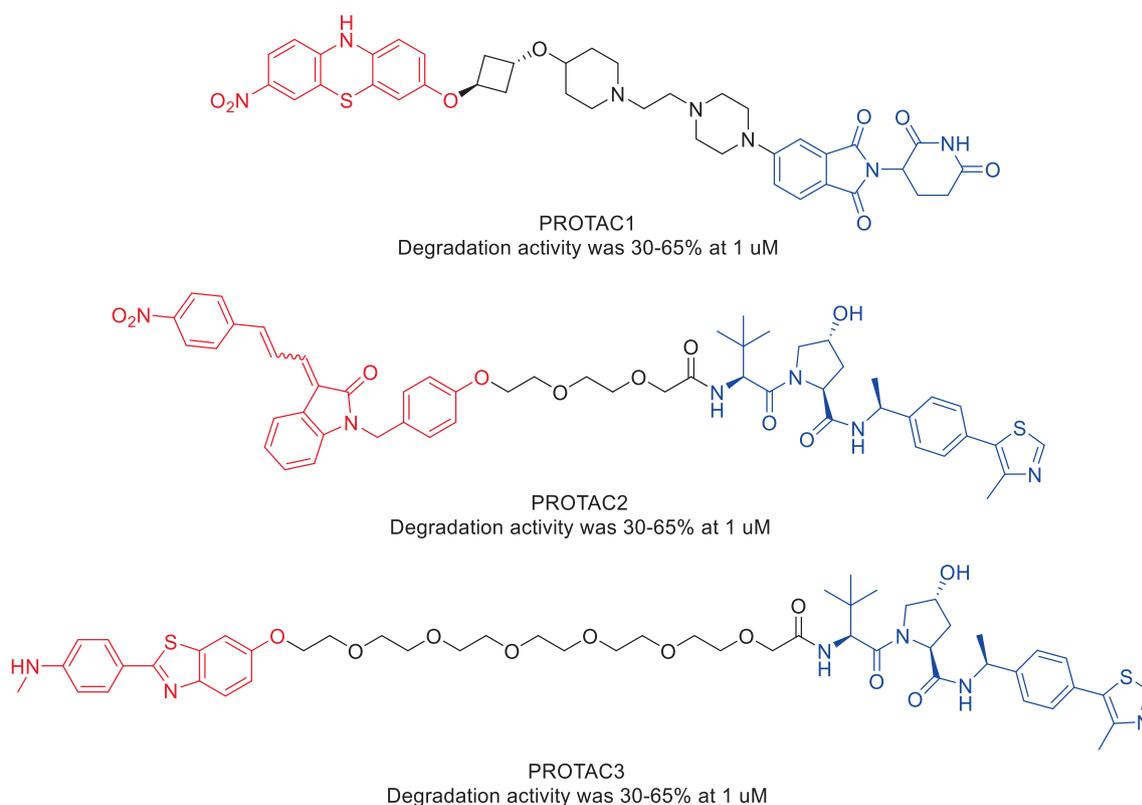
In 2017, Shusuke Tomoshige and coworkers developed two mHtt PROTACs (PROTAC4 and PROTAC5, Fig. 9), each of which was made up of the cIAP1 ligand BE04 coupled to either BTA or PDB (Tomoshige et al., 2017). They examined how PROTAC4 and PROTAC5 affected the levels of mHtt in fibroblasts taken from two HD patients (HD fb). It appeared that PROTAC4 and PROTAC5 function efficiently as mHtt down-regulators because they decreased the levels of mHtt in both sets of HD fb at a concentration of  $10\ \mu\text{M}$ . The mHtt level was decreased by PROTAC4 in a dose-dependent manner. There is mounting evidence that neurodegenerative diseases are affected by UPS. The design idea of posttranslational knockdown utilizing particular ligands to target proteins is different from the design concept of targeting the aggregated state of proteins. They thought that PROTACs

technology could be used to target other proteins that are prone to aggregation and are responsible for neurodegenerative illnesses like PD, AD, and other polyQ diseases.

To test the impact of changing the E3 ligand, Shusuke Tomoshige and colleagues developed a new Htt degrader called PROTAC6 in 2018 (Fig. 9). This version used PDB coupled to MV1 rather than BE04, which was used in their previously reported Htt degrader PROTAC5 (Tomoshige et al., 2018). PROTAC6 successfully decreased both wtHtt level and mHtt level. The efficacy of PROTAC6 was lower than that of PROTAC4 or PROTAC5, despite the fact that MV1 was thought to have a higher affinity than BE04 for IAPs, indicating that the linker length of PROTAC6 might not be optimal for protein knockdown of Htt. They confirmed that PROTAC6 induced an interaction between GST-BIR3 and insoluble model aggregates of 62Q peptides, supporting the idea that PROTAC6 worked by forming a non-physiological com-



**Scheme 3c** Synthesis of VHL ligand 3. Reagents and conditions: a) 2 M HCl in DCM/MeOH, r.t., overnight; (b) (2*S*,4*S*)-1-(*tert*-butoxycarbonyl)-4-hydroxypyrrolidine-2-carboxylic acid, HATU, Et<sub>3</sub>N, DCM, r.t., overnight; (c) Fmoc-*S*-trityl-l-penicillamine, HATU, TEA, DMF r.t., overnight; (d) 1-fluorocyclopropane-1-carboxylic acid, HATU, Et<sub>3</sub>N, DCM, r.t., overnight; (e) TFA, triisopropylsilane, DCM, 0 °C, 30 min.

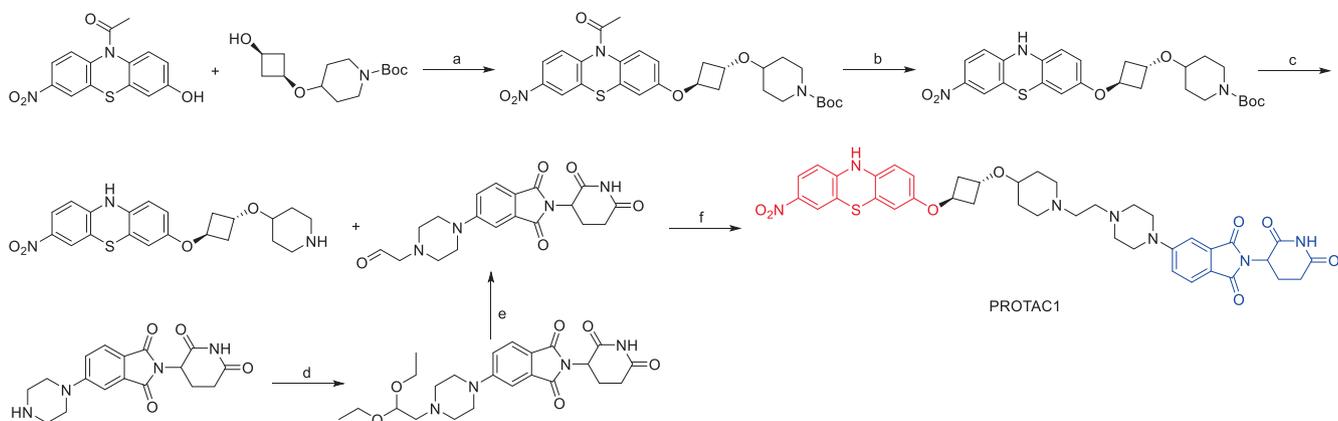


**Fig. 8** Representative PROTACs targeting  $\alpha$ -synuclein.

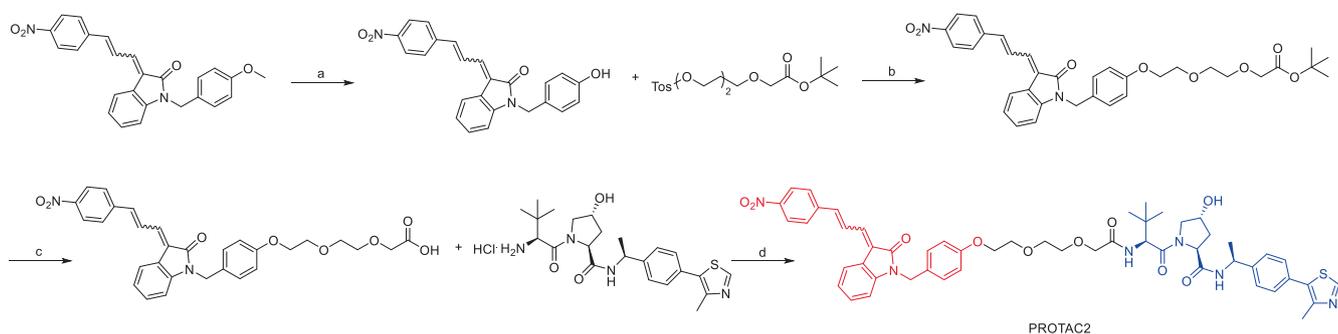
plex consisting of Htt aggregate, IAPs, and PROTAC6. Additionally, PROTAC6 induced an interaction between GST-BIR3 and soluble GST-62Q, which was the observation that it decreased the levels of both mHtt and wtHtt. Overall, their findings show that substituting the E3 ligase ligand for protein knockdown of Htt in hybrid molecules does not change the mode of action but may affect the efficacy. In these hybrids, it might be crucial to optimize the linker length for each unique E3 ligand. PROTAC4, PROTAC5, and PROTAC6 were synthesized *via* synthetic pathways shown in Scheme 5a, Scheme 5b.

### 3.3. GSK-3 $\beta$

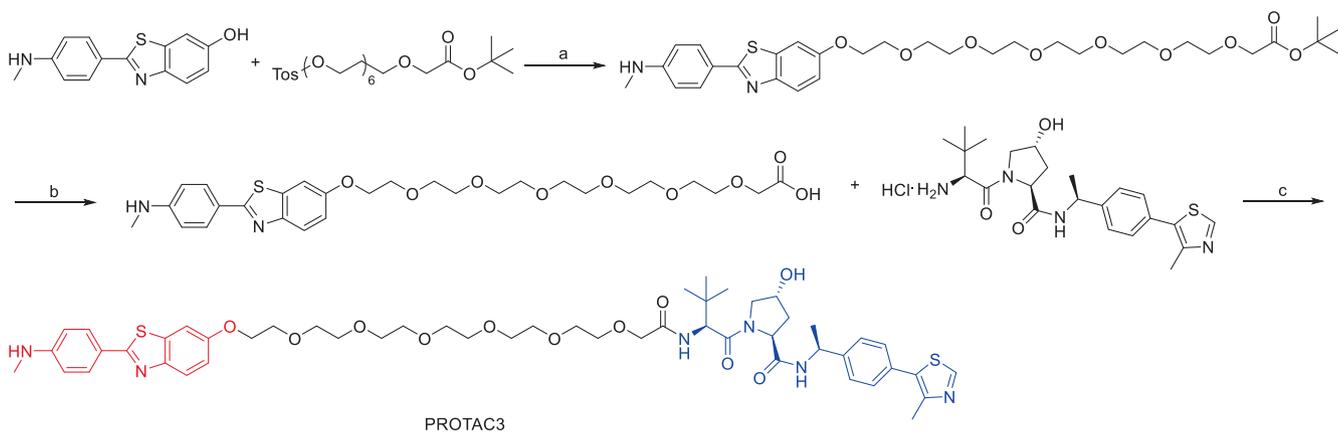
A highly conserved serine/threonine kinase is GSK-3 $\beta$ . Tau is made hyperphosphorylated by GSK-3 $\beta$ , which is a crucial step in the development of neurofibrillary tangles (NFTs). GSK-3 $\beta$  is the most abundant isoform in the CNS, and the expression level of GSK-3 $\beta$  increases with age (Embi et al., 1980). According to recent research, AD has higher levels of total and active GSK-3 $\beta$  than healthy controls. GSK-3 $\beta$  is active in pre-tangle neurons, preferentially co-localizes with NFTs, and aids in the development of paired helical filaments (PHF) in the AD



**Scheme 4a** Synthesis of PROTAC1. Reagents and conditions: a) ADDP,  $\text{Bu}_3\text{P}$ , Toluene, 15–110 °C, 16 h; b)  $\text{K}_2\text{CO}_3$ , MeOH, 15 °C, 10 h; c) 4 M HCl, Dioxane, 20 °C, 1 h; d) 2-bromo-1,1-dithoxyethane, DIEA, DMF, 100 °C, 2 h, m.w.; e) 2 M  $\text{H}_2\text{SO}_4$ , THF, 60 °C, 14 h; f)  $\text{NaBH}(\text{OAc})_3$ ,  $\text{Et}_3\text{N}$ , DCM, MeOH, 0–20 °C, 6 h.



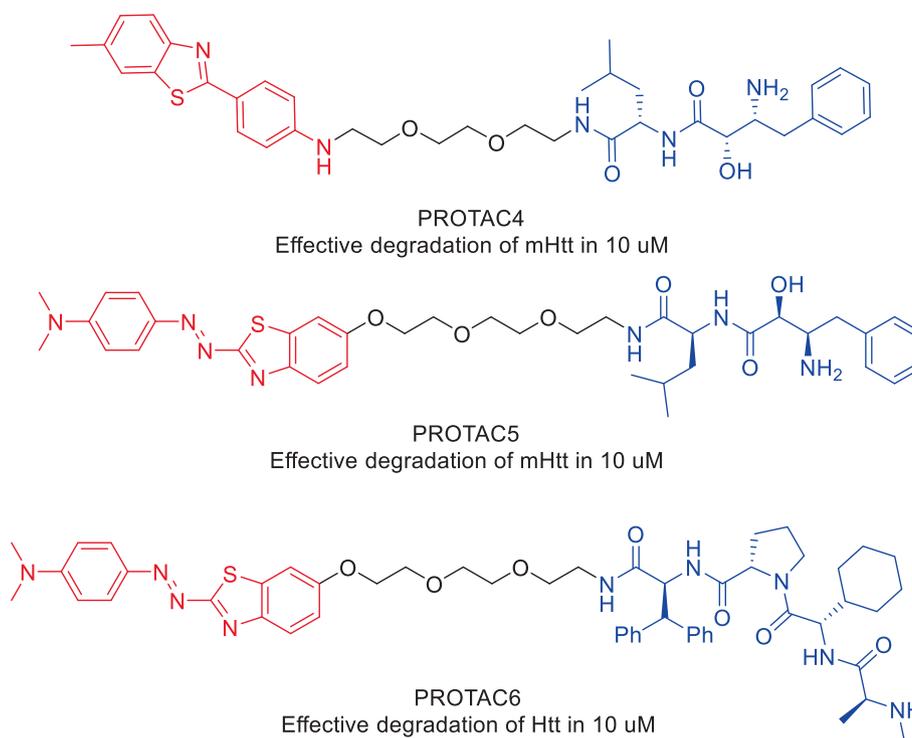
**Scheme 4b** Synthesis of PROTAC2. Reagents and conditions: a)  $\text{BBr}_3$ , DCM, –60–10 °C, 15 min; b)  $\text{Cs}_2\text{CO}_3$ , DMF, 85 °C, 1 h; c) TFA, DCM, 12 °C, 12 h; d) EDCI, HOBT, DMF, 12–30 °C, 12 h.



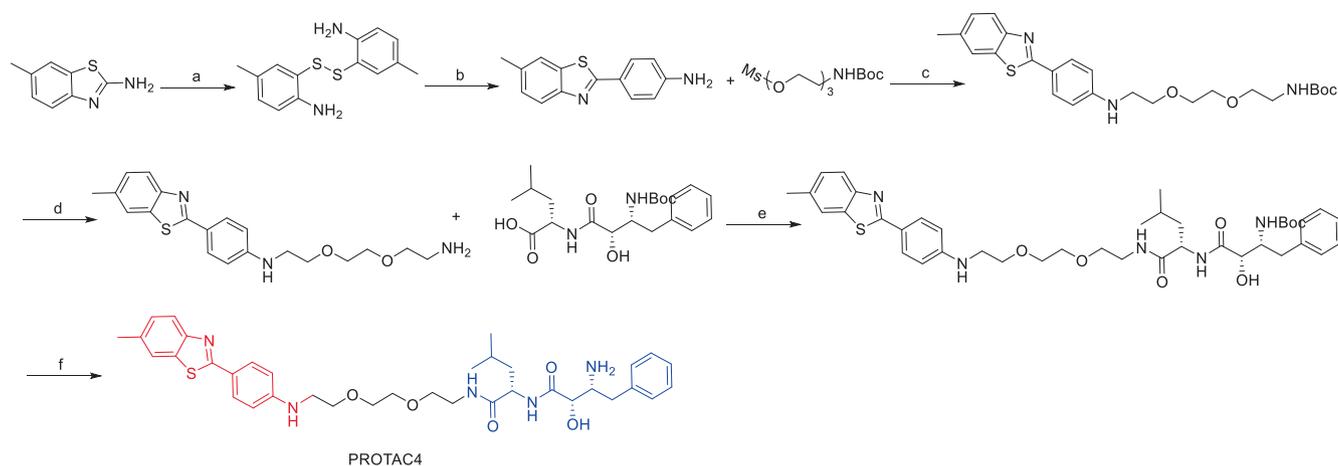
**Scheme 4c** Synthesis of PROTAC3. Reagents and conditions: a)  $\text{Cs}_2\text{CO}_3$ , DMF, 85 °C, 1 h; b) TFA, DCM, 12 °C, 12 h; c) EDCI, HOBT, DMF, 12–30 °C, 12 h.

brain. The efficacy of several GSK-3 $\beta$  inhibitors in the treatment of AD has been established. Clinical trial findings on the ATP noncompetitive GSK-3 $\beta$  inhibitor tideglusib showed that it could improve cognition and lower phospho-Tau levels in cerebrospinal fluid in AD patients (Eldar-Finkelman, 2002). Therefore, GSK-3 $\beta$  is considered a potential target for the treatment of AD.

Through the coupling of GSK-3 inhibitor G1 and CRBN ligand thalidomide, Xueyang Jiang and coworkers reported a family of GSK-3 $\beta$  PROTACs in 2021 (Jiang et al., 2021). They produced a potent small molecule GSK-3 $\beta$  degrader called PROTAC7 by extensively optimizing the length and composition of the linker. According to Western blot results, PROTAC7 (Fig. 10) could effectively and dose-dependently



**Fig. 9** Representative PROTACs targeting mHtt.



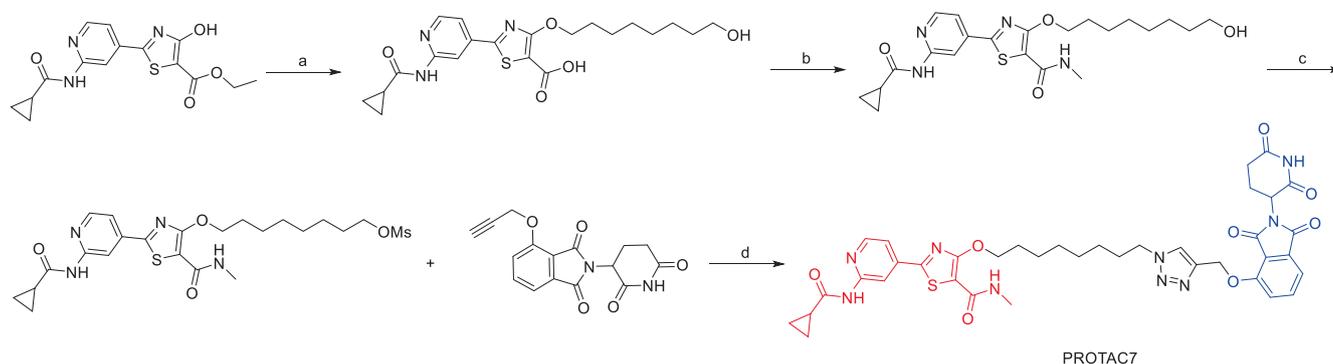
**Scheme 5a** Synthesis of PROTAC4. Reagents and conditions: a) 10 M NaOH, reflux, 18.5 h; b)  $\text{PPh}_3$ , 4-aminobenzoic acid, polyphosphoric acid, 220  $^\circ\text{C}$ , 4 h; c)  $\text{K}_2\text{CO}_3$ , KI, DMF, 90  $^\circ\text{C}$ , 3.5 h; d) 4 M HCl, 1,4-dioxane, DCM, r.t., 3 h; e) EDCI, HOBT, DIEA, DMF 0  $^\circ\text{C}$ -r.t., 24 h; f) 4 M HCl, 1,4-dioxane, DCM, r.t., 3 h.

degrade GSK-3 $\beta$ , leading to 44% protein degradation at a concentration of 2.8  $\mu\text{M}$ . Their mechanistic analysis revealed that to cause GSK-3 $\beta$  degradation, PROTAC7 must rely on the intracellular UPS. PROTAC7 displayed neuroprotective effects, according to preliminary *in vitro* cytotoxicity tests. This is the first instance of PROTAC targeting the GSK-3 $\beta$  protein. PROTAC7 is anticipated to be used as a cutting-edge tool to examine the function of GSK-3 $\beta$  in various pathological states because of its good kinase selectivity.

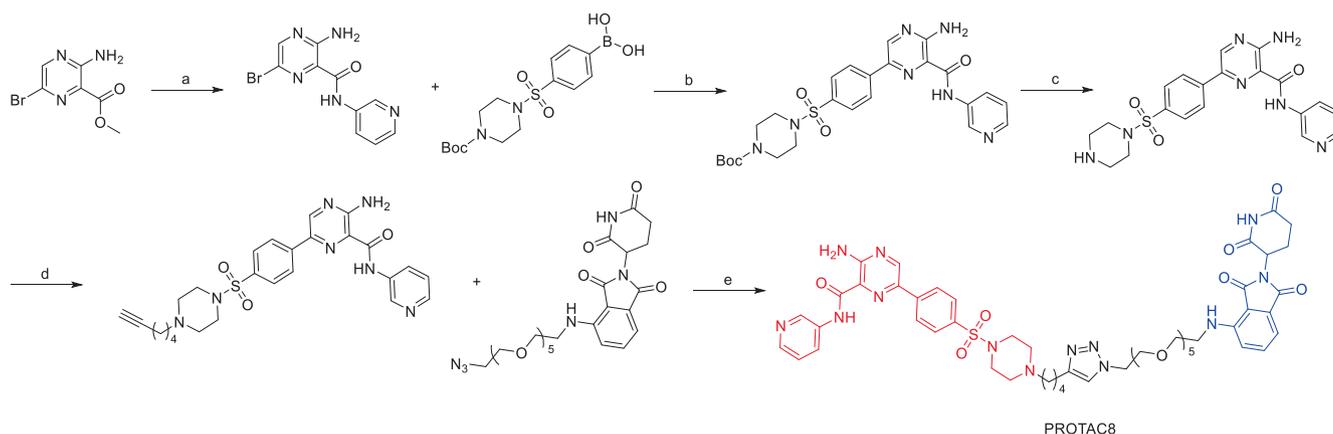
Through the optimization of GSK-3 $\beta$  ligands, linkers, and E3 ligase ligands based on a click chemistry platform, several GSK-3 $\beta$  PROTACs were developed (Qu et al., 2021). The

work of Lailiang Qu and coworkers produced numerous efficient GSK-3 $\beta$  PROTACs, with PROTAC8 serving as an example (Fig. 10). In SH-SY5Y cells, PROTAC8 was able to degrade GSK3 $\alpha$  and GSK3 $\beta$  with  $\text{DC}_{50}$  values of 28.3 and 34.2 nM, respectively. The SPR experiment demonstrated that PROTAC8 has a strong affinity for GSK-3 $\beta$  ( $K_D = 12.41$  nM), which underlies the strength of its GSK-3 $\beta$  degradation ability. And the proteomic investigation revealed that PROTAC8 was able to specifically promote the degradation of GSK-3 $\beta$ . Moreover, they confirmed that CRBN and the proteasome were responsible for the degradation of GSK-3 $\beta$ . In SH-SY5Y and HEK GSK3 293 T cells, PROTAC8 could efficiently





**Scheme 6a** Synthesis of PROTAC7. Reagents and conditions: a) 8-decamethylene glycol,  $\text{PPh}_3$  DIAD, THF, r.t., 24 h; b) i) 1.5 M LiOH, THF/MeOH, 0 °C-r.t., 6 h; ii) methylamine, DIPEA, HATU, DMF, r.t., 24 h; c) *p*-tosyl chloride,  $\text{Et}_3\text{N}$ , DCM, r.t., 24 h; d) i)  $\text{NaN}_3$ , DMF, 80 °C, 10 h; ii) sodium ascorbate,  $\text{Et}_3\text{N}$ ,  $\text{CuSO}_4$ , *n*-butanol, r.t., 8 h.



**Scheme 6b** Synthesis of PROTAC8. Reagents and conditions: a) 3-aminopyridine, DBU, 70 °C, 4 h; b)  $\text{Pd}(\text{dppf})\text{Cl}_2$ , DCM,  $\text{Na}_2\text{CO}_3$ , toluene/EtOH, 80 °C, overnight; c) TFA, DCM, r.t., 2 h; d) haloalkynyl, DIPEA, DMF, 80 °C, 2 h; e)  $\text{CuSO}_4$ , NaVic, *t*-BuOH/DCM/DMF/ $\text{H}_2\text{O}$ , r.t., 8 h.

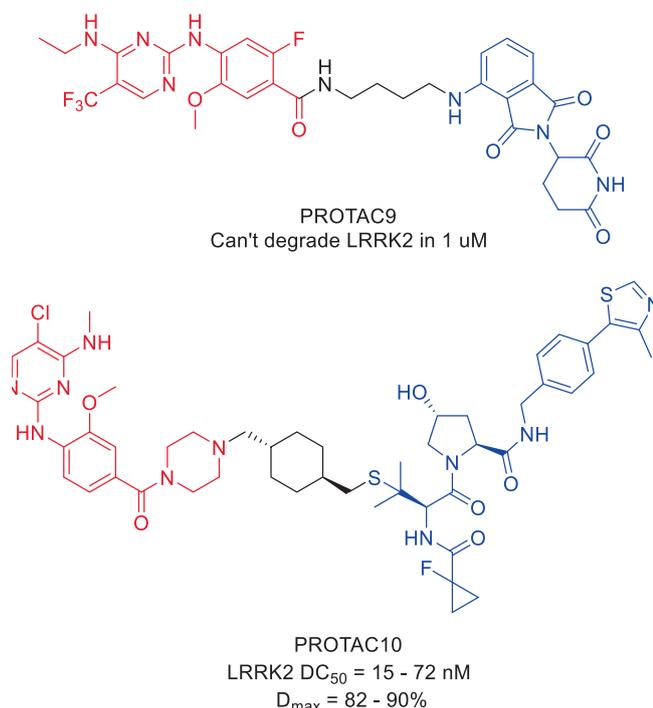
10  $\mu\text{M}$ ) for 24, 48, and 72 h during the initial screening (Fig. 11). Western blotting results showed no discernible differences in the amounts of LRRK2 protein between cells treated with PROTAC9 and cells treated with the original kinase inhibitors, proving that PROTACs were unable to degrade LRRK2. Although PROTACs have so far been successful in challenging targets, the development of degraders is not an easy task. To further explore the possibility of degrading LRRK2, which continues to be a difficult drug target, and to comprehend the reasons for the observed differences when highly effective LRRK2 inhibitors were modified into potential degraders, a better understanding of its structure and conformational changes, which have just recently been reported, is required. Overall, they wanted to highlight the difficulties of degrading a target whose complete structure is unknown in this work. They think that further research will make it possible to rationally create an effective LRRK2-targeting PROTAC after gaining a better understanding of the structure and dynamics of LRRK2.

As an alternate LRRK2-targeting strategy, Xingui Liu and colleagues reported the development of LRRK2 PROTACs in 2022, which culminated in the identification of degrader PROTAC10 (Fig. 11) (Liu et al., 2022). The most effective degrader contained thioether-conjugated VHL ligand VH101, as determined by initial designs and tests of PROTACs based on

ligands for E3 ligases VHL, CRBN, and cIAP. PROTAC10 was identified as a potent and powerful LRRK2 degrader in several cell lines during a second round of medicinal chemistry exploration, with  $\text{DC}_{50}$  values between 15 and 72 nM,  $\text{D}_{\text{max}}$  values between 82 and 90%, and degradation half-lives spanning from 0.6 to 2.4 h. PROTAC10 exhibited high cell permeability and formed a positively cooperative ternary complex with VHL and LRRK2 ( $\alpha = 5.7$ ), which compensated for a substantial loss of binary binding affinities to VHL and LRRK2, underscoring its strong degradation performance in cells. Importantly, PROTAC10 could penetrate the blood–brain barrier after being administered to mice either oral or parenteral dosing ( $F = 15\%$ ). When taken as a whole, these studies demonstrate the suitability of PROTAC10 as a degrader probe to investigate the noncatalytic and scaffolding roles of LRRK2 *in vitro* and *in vivo*, and they provide an alluring starting point for potential future therapeutic development. PROTAC9 and PROTAC10 were synthesized *via* synthetic pathways shown in Scheme 7a, Scheme 7b.

### 3.5. Tau

Tau is a protein that is associated with microtubules and is typically assumed to control microtubule stability and partici-



**Fig. 11** Representative PROTACs targeting LRRK2.

pate in axonal transport (Jeremic et al., 2021). As an essential pathogenic protein in AD, Tau has the ability to mediate the toxicity of A $\beta$ . It has been suggested that Tau is a particularly alluring target for future treatments of neurodegenerative disorders (Congdon and Sigurdsson, 2018). As a result, lowering Tau with chemical compounds might present a new approach to treating AD.

Finding small molecule compounds that will selectively cause Tau degradation is difficult. The first study on the precise knockdown of endogenous Tau protein *via* peptide-directed ubiquitin-proteasome degradation was published in 2016 by Tingting Chu and coworkers (Chu et al., 2016). They designed and produced several multifunctional compounds that contained moieties for interacting with E3 ligases, identifying Tau protein, and penetrating cell membranes. PROTAC11 (Fig. 12) was the synthetic compound that promoted intracellular Tau degradation the most. It managed to get through the cell membrane and enhance Tau's polyubiquitination in cells. Furthermore, the cytotoxicity brought on by A $\beta$  was lessened by Tau degradation mediated by PROTAC11. Additionally, it controlled the Tau level in the mouse model of AD. In a nutshell, their research has described a cutting-edge AD treatment approach. They believe that the use of their synthetic molecules will make it easier to create AD treatments that work.

Another peptide PROTAC was created and used to degrade intracellular Tau by recruiting Keap1-Cul3 ubiquitin E3 ligase (Lu et al., 2018). Keap1 and Tau exhibited robust *in vitro* interaction with PROTAC12 (Fig. 12). PROTAC12 was discovered to colocalize with cellular Keap1 with appropriate cell permeability, which led to the coimmunoprecipitation of Tau and Keap1. It was determined through the use of flow cytometry and western blotting assays that PRO-

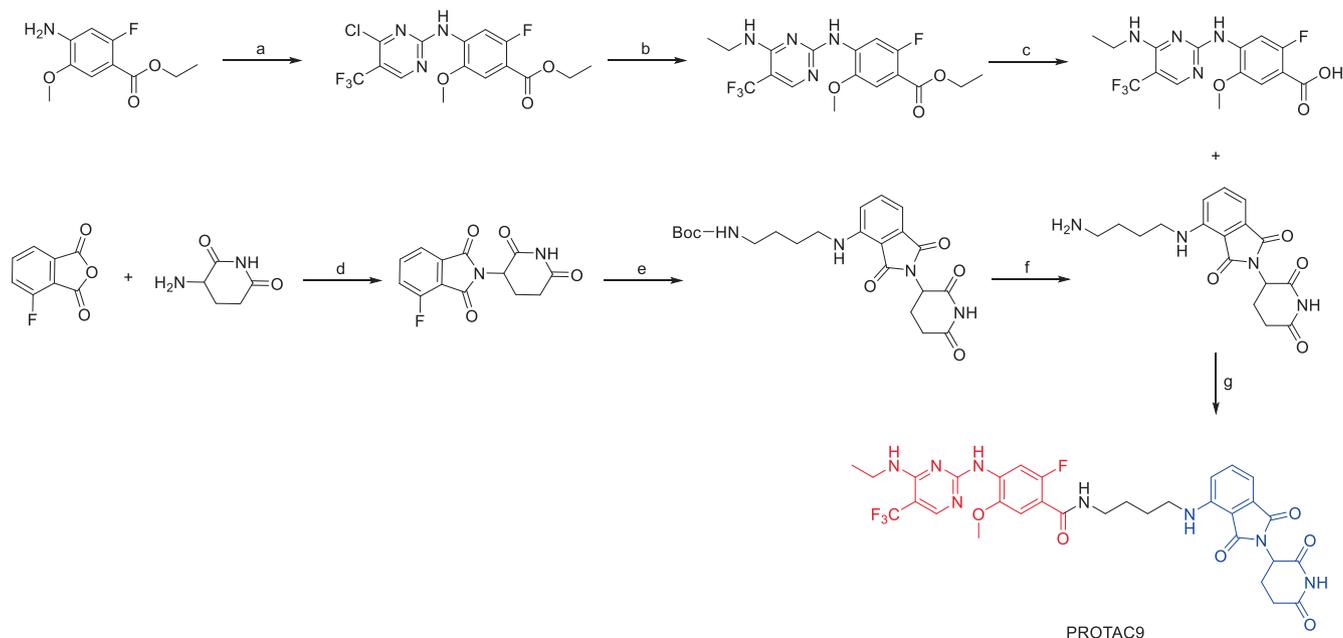
TAC12 has the ability to reduce intracellular Tau levels in a manner that is both concentration- and time-dependent. PROTAC12's ability to encourage Tau's poly-ubiquitination and proteasome-dependent degradation was validated by the use of Keap1 siRNA silencing and the proteasome inhibitor MG132. The findings suggested that employing PROTACs to enlist Keap1 and trigger Tau breakdown may have therapeutic potential for treating neurodegenerative diseases. Additionally, their research suggested that Keap1 could be a useful E3 ligase adaptor for the creation of new PROTACs. To test against therapeutically relevant protein phenotypes and species, their approach made use of already-existing PET probe binders in degrader design and well-characterized patient-derived neural models. PROTAC13 (Fig. 12) (Silva et al., 2019), a useful new research tool for examining Tau-mediated processes in human tauopathies, was the result of these efforts. Between 0.01  $\mu\text{M}$  and 10  $\mu\text{M}$ , PROTAC13 could effectively degrade both wild-type and mutant Tau in neurons. Moreover, when compared to healthy cells, PROTAC13 could preferentially degrade Tau in FTD neurons. In a mouse pharmacokinetic study to test PROTAC13's blood-brain barrier penetration, it was shown that a dose of 30 mg/kg produced compound brain levels that were equal to those used in *in vitro* experiments where Tau degradation was seen. According to their research, a promising method for improving our comprehension of human neurodegenerative disease and developing tailored treatments is small molecule-mediated protein degradation.

In 2021, Weijin Wang and coworkers designed a small molecule PROTAC, named PROTAC14 (Fig. 12) (Wang et al., 2021). Three components make up PROTAC14: a VHL ligand, a linker, and a Tau binder. They showed that PROTAC14 could effectively stimulate Tau protein clearance in both healthy and pathological conditions through *in vitro* and *in vivo* testing. Remarkably, even single-dose or seldom (once per 6 days) subcutaneous treatment of PROTAC14 achieved a sustained Tau decrease in the brain of 3xTg-AD and hTau transgenic mouse models. Low Tau levels can reduce the neurotoxicity caused by A $\beta$ , and Tau knockdown in mice does not cause obvious abnormalities. PROTAC14 is a promising therapeutic candidate for AD and the associated tauopathies as a result.

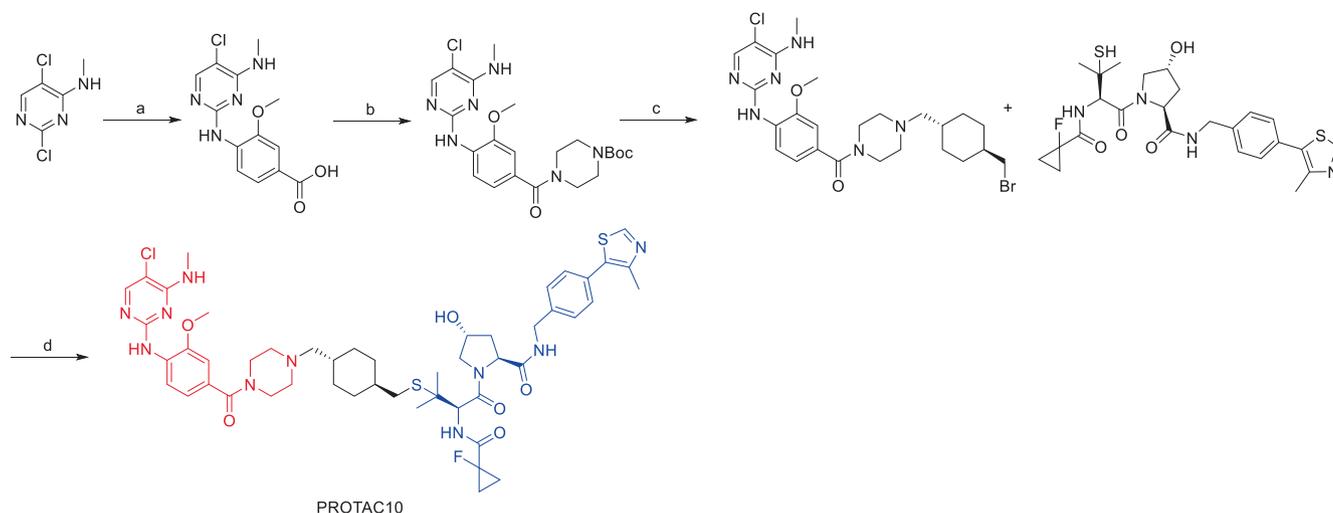
A small number of exemplified Tau-degrading PROTACs were disclosed by Mingliang Ma and colleagues in 2022 as part of a wider set of claims for CRBN-binders (Ma et al., 2022). In N2a/APP cells, the depicted Tau PROTAC (PROTAC15, Fig. 12) was found to be a moderately effective degrader. Similarly, a small number of exemplified Tau-degrading PROTACs in the context of claims describing VHL E3 ligase binders were also disclosed. The VHL-recruiting PROTAC (PROTAC16, Fig. 12) was likewise reported to be moderate to weak Tau-degrader in N2a/APP cells, with  $\text{D}_{\text{max}}$  values of 50% at 10  $\mu\text{M}$ . PROTAC13, PROTAC14, PROTAC15, and PROTAC16 were synthesized *via* synthetic pathways shown in Scheme 8a, Scheme 8b, Scheme 8c, Scheme 8d.

### 3.6. TRKs

The NTRK1, NTRK2, and NTRK3 genes, respectively, encode the three members of the TRKs receptor family: TRKA, TRKB, and TRKC. TRKs are receptor tyrosine



**Scheme 7a** Synthesis of PROTAC9. Reagents and conditions: a) 2,4-dichloro-5-(trifluoromethyl)pyrimidine, zinc chloride, diethyl ether, DCM, *tert*-butanol, triethylamine, 0 °C-r.t., 48 h; b) ethanamine, triethylamine, THF, 0 °C - r.t., 1h; c) LiOH, THF/H<sub>2</sub>O, 1 h; d) TFA, DCM, r.t., 2 h; e) sodium acetate, acetic acid, reflux, overnight; f) *N*-Boc-butane-1,4-diamine, DIPEA, DMF, reflux, overnight; g) 4 M HCl, 1,4-dioxane, DCM, r.t., 6 h; g) EDCI, HOBT, DIEA, DMF 0 °C-r.t., 24 h.



**Scheme 7b** Synthesis of PROTAC10. Reagents and conditions: a) 4 M HCl, 1,4-dioxane, H<sub>2</sub>O, 4-amino-3-methoxybenzoic acid, 100 °C, overnight; b) EDCI, HOBT, DIPEA, 1-Boc-piperazine, DMF, r.t., 16 h; c) i) 2 M HCl, 1,4-dioxane, DCM, r.t., overnight; ii) *trans*-1,4-bis(bromomethyl)cyclohexane, K<sub>2</sub>CO<sub>3</sub>, acetone, 50 °C, overnight; d) DBU, THF r.t., overnight.

kinases that are primarily involved in the development and function of neuronal tissue (Martin-Zanca et al., 1986). Nerve growth factor (NGF) for TRKA, brain-derived neurotrophic factor (BDNF) for TRKB, and neurotrophins for TRKC are the major ligands of TRKs. Induced dimerization and activation of the intracellular kinase domains by the binding of ligands to the extracellular domains of TRKs provides signals to downstream pathways, including RAF/MEK/ERK, PI3K/AKT, and phospholipase C gamma (PLC $\gamma$ ) (Rydén et al., 1996). There have been reports of chromosomal translocation, point mutations, amplification, and alternative splicing among

other genetic aberrations of NTRK genes. These modifications cause the TRK pathway to be constitutively activated, which encourages cellular growth, survival, and malignant transformation.

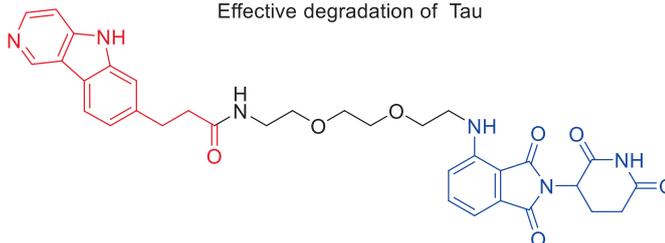
The first TRKC PROTACs were described by Bosheng Zhao and coworkers in 2019 and were based on the TRKC binder IY-IY ( $K_d = 112$  nM) and various E3 ligase ligands (Zhao et al., 2019). With an estimated DC<sub>50</sub> of 0.1–1.0  $\mu$ M, the pomalidomide-based degrader PROTAC17 (Fig. 13) demonstrated improved TRKC degradation activity. TRKC

**AQAYQAATADAQG -GSGS-ALAPYIP**

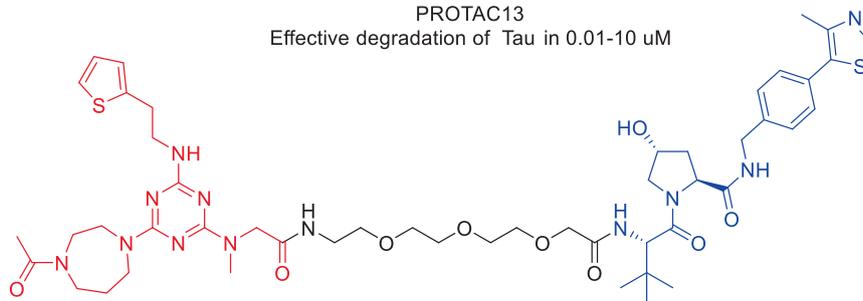
PROTAC11  
Effective degradation of Tau



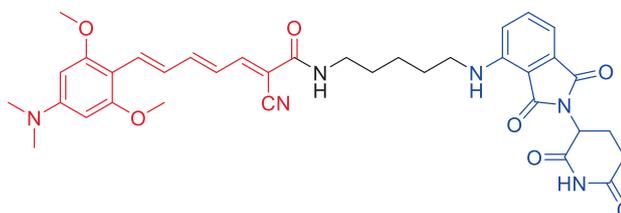
PROTAC12  
Effective degradation of Tau



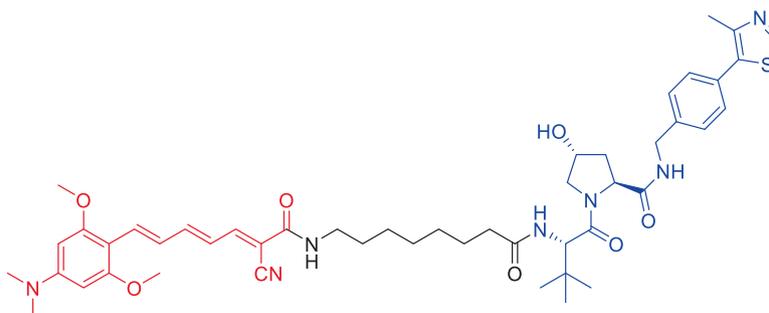
PROTAC13  
Effective degradation of Tau in 0.01-10 uM



PROTAC14  
Effective degradation of Tau

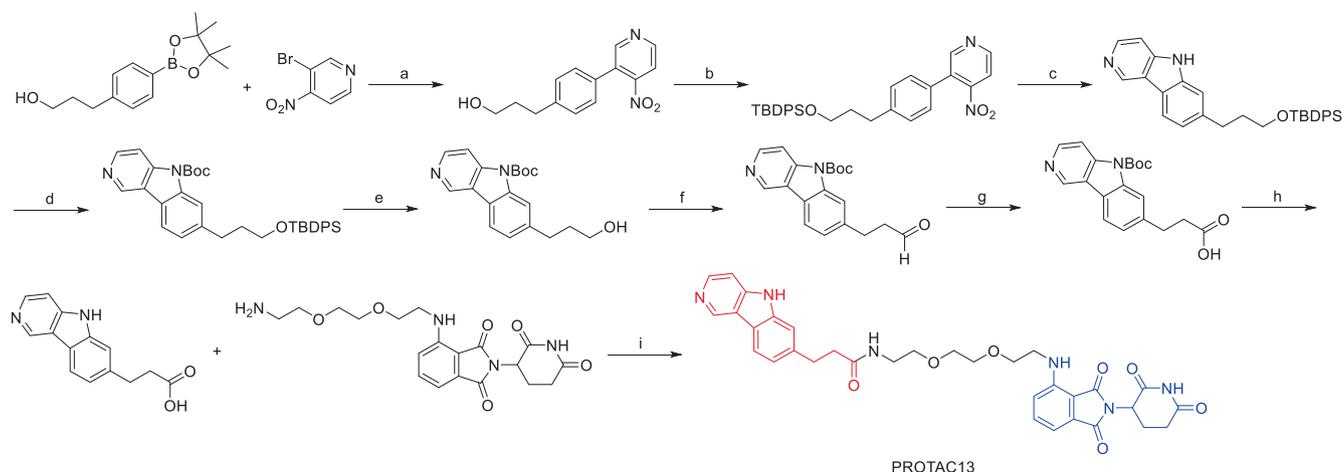


PROTAC15  
Effective degradation of Tau

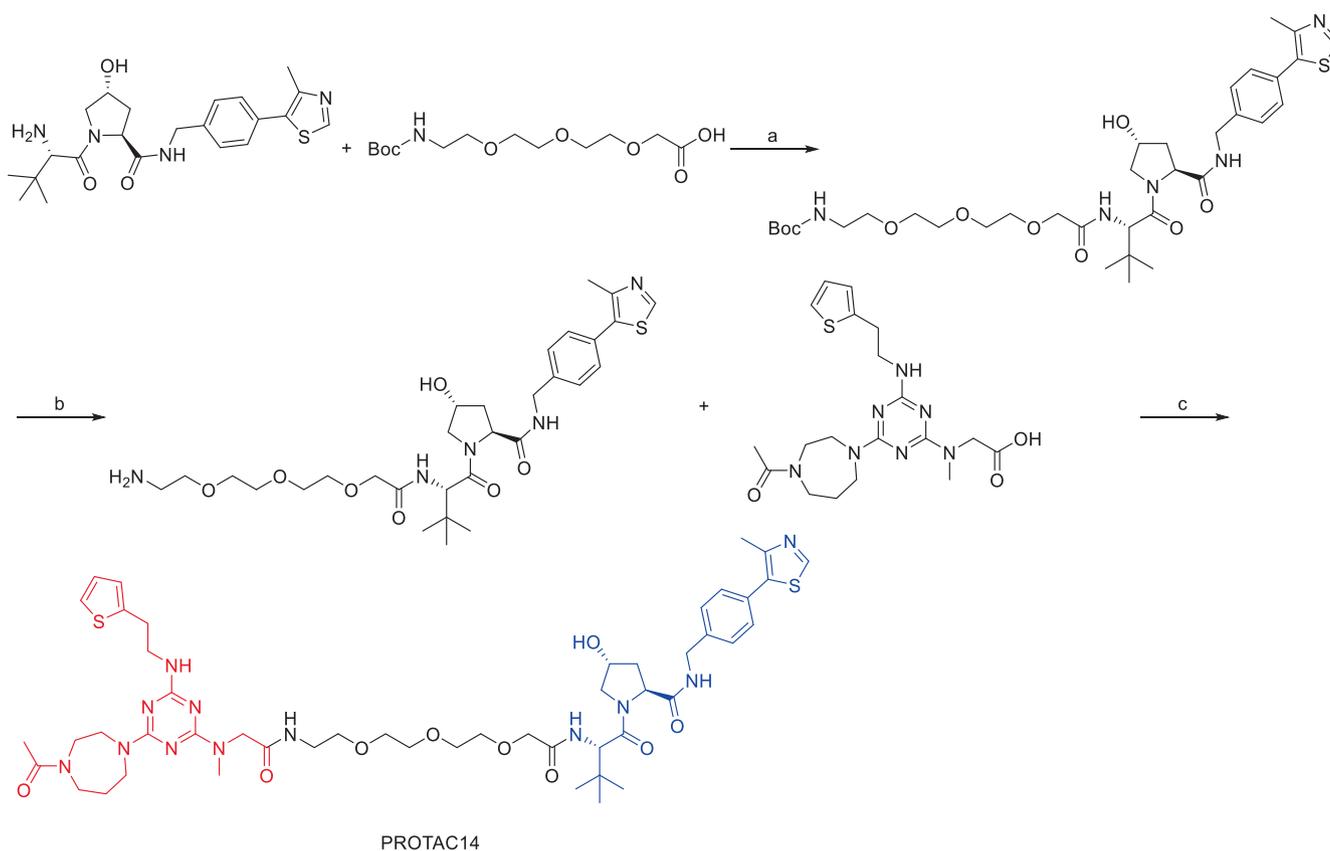


PROTAC16  
Effective degradation of Tau

**Fig. 12** Representative PROTACs targeting Tau.



**Scheme 8a** Synthesis of PROTAC13. Reagents and conditions: a) Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, 1,4-dioxane, H<sub>2</sub>O, 110 °C, 16 h; b) TBDPSCl, imidazole, DCM, r.t., 3 h; c) P(OEt)<sub>3</sub>, 110 °C, 3 h; d) (Boc)<sub>2</sub>O, DMAP, Et<sub>3</sub>N, DCM, r.t., 2 h; e) TBAF, THF, 0 °C, 2 h; f) (COCl)<sub>2</sub>, DMSO, Et<sub>3</sub>N, DCM, -78 °C-0 °C, 1.5 h; g) NaClO<sub>2</sub>, NaH<sub>2</sub>PO<sub>4</sub>, 2-methyl butene, THF/*t*-BuOH/H<sub>2</sub>O, r.t., 2 h; h) TFA, DCM, r.t., 12 h; i) EDCI, DMAP, DIPEA, DCM, r.t., 4 h.

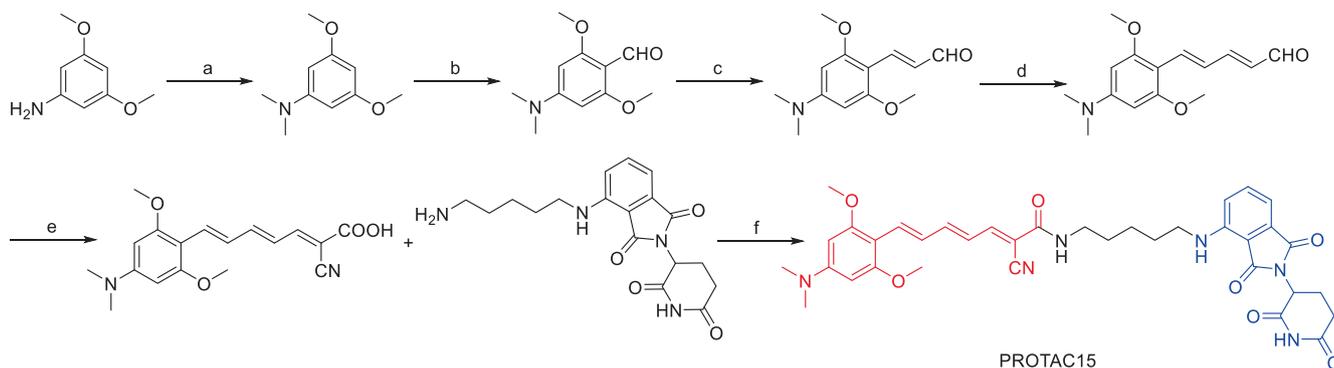


**Scheme 8b** Synthesis of PROTAC14. Reagents and conditions: a) DIPEA, HATU, DMF, r.t., overnight; b) 2 M HCl/EA, DCM, r.t., 1 h; c) DIPEA, HATU, DMF, r.t., overnight.

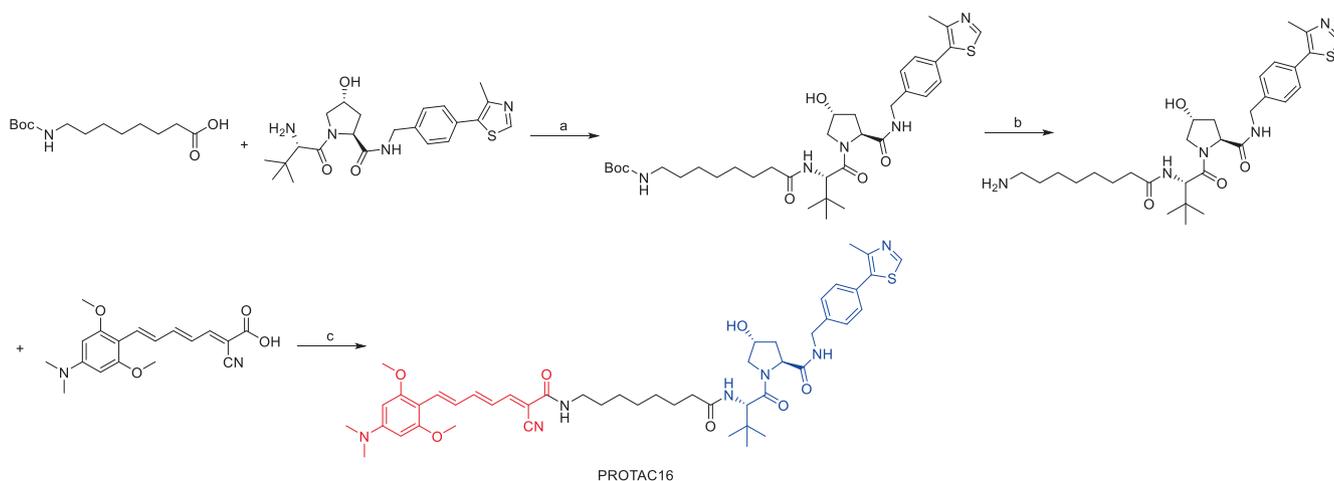
degraders may present prospects for alternate therapeutic approaches for TRKC-related diseases.

Jing Liu and coworkers published a patent filing disclosing TRKA degraders, comprising more than 200 exemplars of TRK PROTACs (Liu et al., 2020). Exemplified compounds in this filing all employ binders to CRBN and VHL as the

E3 ligases recruiting moiety. Representative compounds PROTAC18 and PROTAC19 (Fig. 13) could effectively and selectively inhibit the proliferation of KM12 cells (PROTAC18 IC<sub>50</sub> = 0.9 nM, PROTAC19 IC<sub>50</sub> = 0.9 nM). PROTAC18 and PROTAC19 could degrade TRKA protein in a dose-dependent manner by the ubiquitin–proteasome pathway. This



**Scheme 8c** Synthesis of PROTAC15. Reagents and conditions: a) Dimethyl sulfate,  $K_2CO_3$ , DMF,  $-4^\circ C$ , 4–6 h; b)  $POCl_3$ , DMF,  $0^\circ C$ , 12–14 h; c) ((1,3-Dioxolan-2-yl)methyl) triphenylphosphonium bromide,  $CH_3ONa/MeOH$  (25%), THF,  $78^\circ C$ , 12–16 h; d) ((1,3-Dioxolan-2-yl)methyl) triphenylphosphonium bromide,  $CH_3ONa/MeOH$  (25%), THF,  $78^\circ C$ , 12–16 h; e) cyanoacetic acid,  $K_2CO_3$ , MeOH,  $70^\circ C$ , 12 h; f) DIPEA, DMAP, HATU, r.t., 2 h.



**Scheme 8d** Synthesis of PROTAC16. Reagents and conditions: a) DIPEA, HATU, DMAP, THF,  $25^\circ C$ , 2–3 h; b) 2 M HCl/EtOH,  $CH_3OH$ ,  $25^\circ C$ , 6–8 h; c) DIPEA, DMAP, HATU, r.t., 2 h.

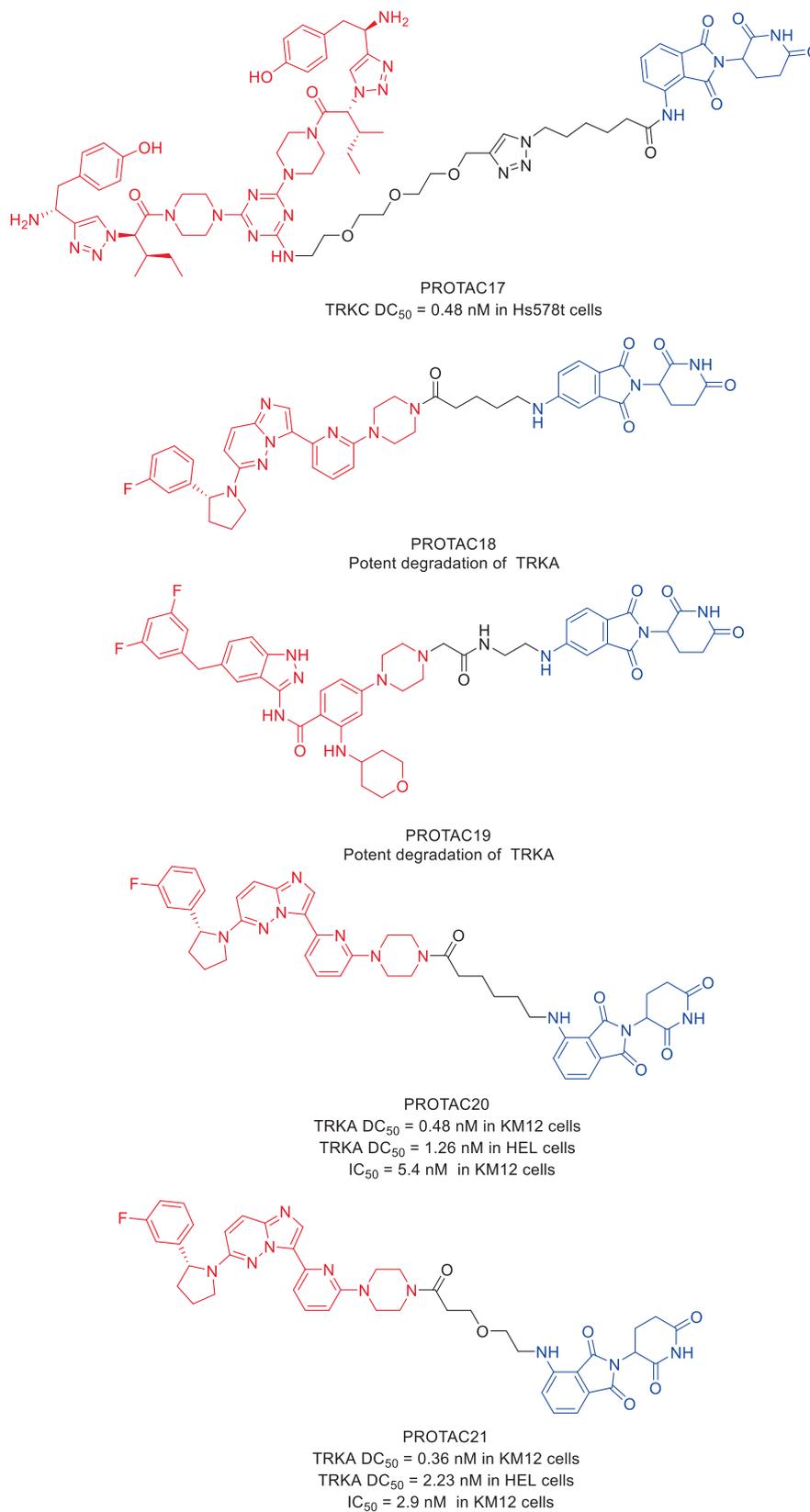
filling also contains voluminous biological data to support the potential utility of these TRK degraders to treat NDs and oncology.

In 2020, Liqun Chen and coworkers developed a variety of putative protein PROTACs molecules with various types of linkers based on the X-ray cocrystal structure of TRKA in complex with an analog of GNF-8625 (Chen et al., 2020). The most promising degraders that target the intracellular kinase domain of TRKs are PROTAC20 and PROTAC21 (Fig. 13). In KM12 colorectal cancer cells, PROTAC20 ( $DC_{50} = 0.48$  nM) and PROTAC21 ( $DC_{50} = 0.36$  nM) decreased tropomyosin 3 (TPM3)-TRKA fusion protein levels and blocked downstream PLC1 signaling at sub-nanomolar concentrations. Both degraders also degraded human wild-type TRKA with  $DC_{50}$  values of 1.26 nM and 2.23 nM in HEL cells. It is interesting to note that in KM12 cells, both degraders, particularly PROTAC21, showed selectivity for the degradation of endogenous TPM3-TRKA over ectopically produced ATP/GTP binding protein-like 4 (AGBL4)-TRKB or ETS variant transcription factor 6 (ETV6)-TRKC fusion proteins. Assays using a global proteome profiler showed that PROTAC20 is very selective for the intended target. It was fur-

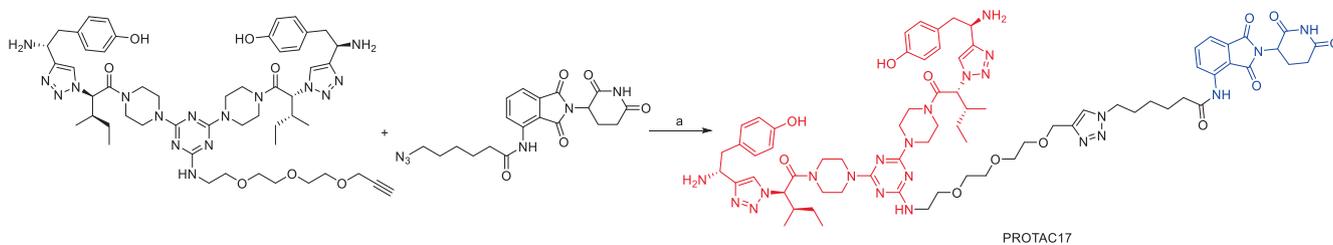
ther established that CRBN and the ubiquitin–proteasome system are responsible for the TPM3-TRKA protein degradation caused by PROTAC20 and PROTAC21. Both degraders showed more potency for preventing the proliferation of KM12 cells as compared to the parental TRKs kinase inhibitor. Additionally, both PROTAC20 and PROTAC21 showed good plasma exposure levels in mice. Therefore, PROTAC20 and PROTAC21 are potential chemical tool compounds to study the *in vivo* function of TRKs fusions during tumorigenesis. This study also points the way for the pharmacological degradation of TRKs. PROTAC17, PROTAC18, PROTAC19, PROTAC20, and PROTAC21 were synthesized *via* synthetic pathways shown in Scheme 9a, Scheme 9b, Scheme 9c, Scheme 9d.

#### 4. Conclusion and perspectives

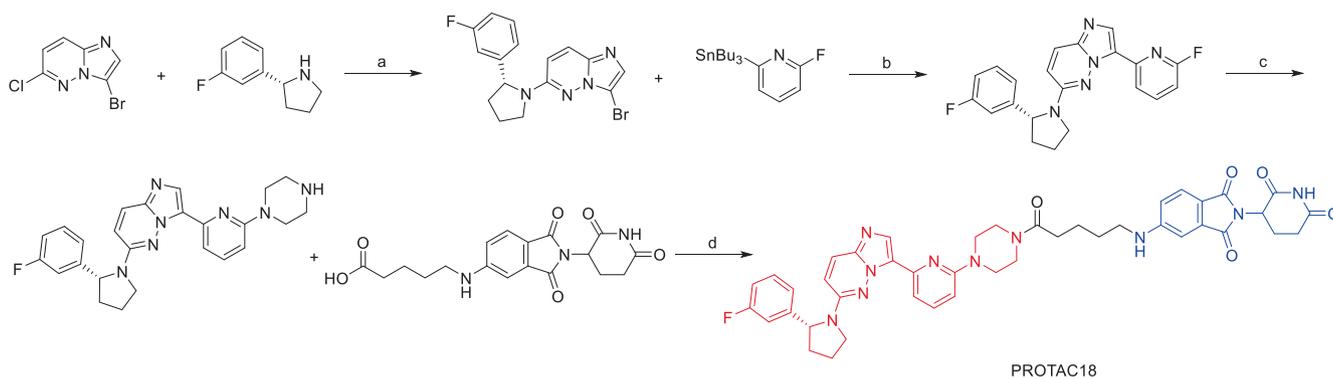
NDs are heterogeneous, currently incurable diseases that cause nerve cells to gradually deteriorate. There is presently no truly effective treatment for NDs, despite the fact that the World Health Organization predicts they will overtake heart disease as the second most common cause of mortality in the next 20 years. Treatments for NDs, such as



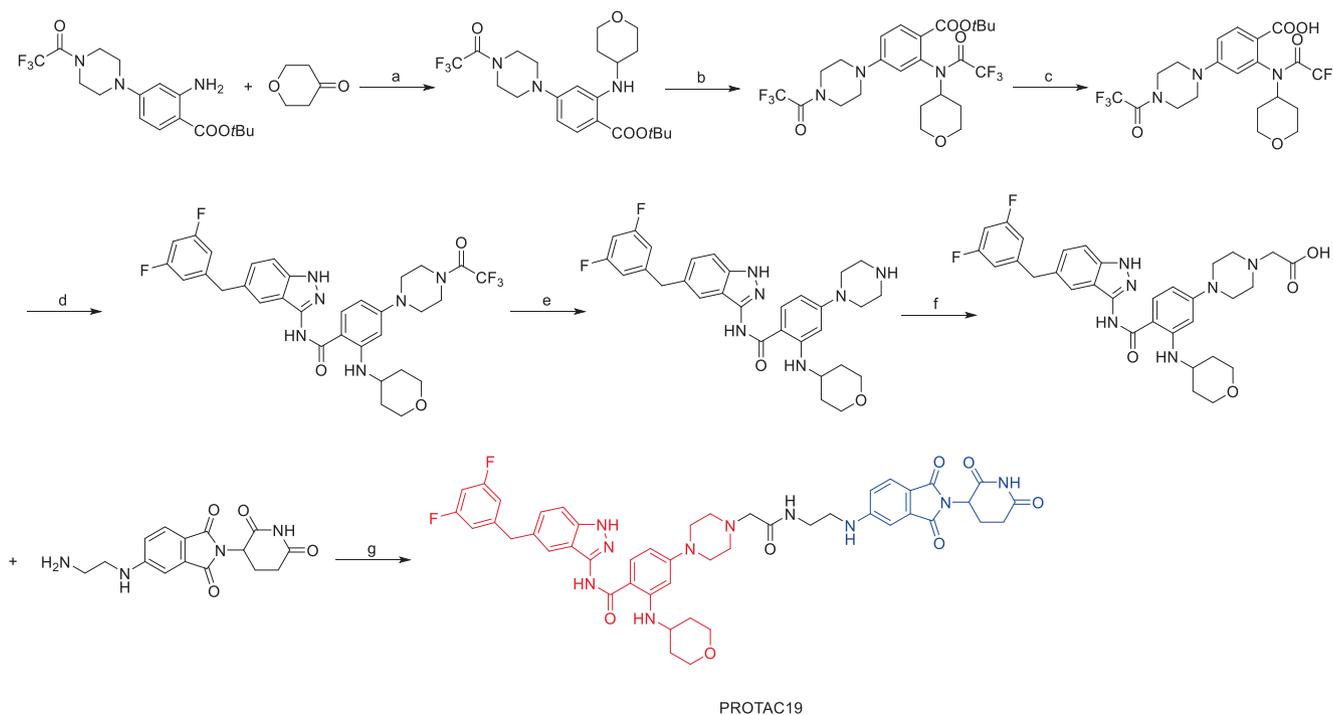
**Fig. 13** Representative PROTACs targeting TRKs.



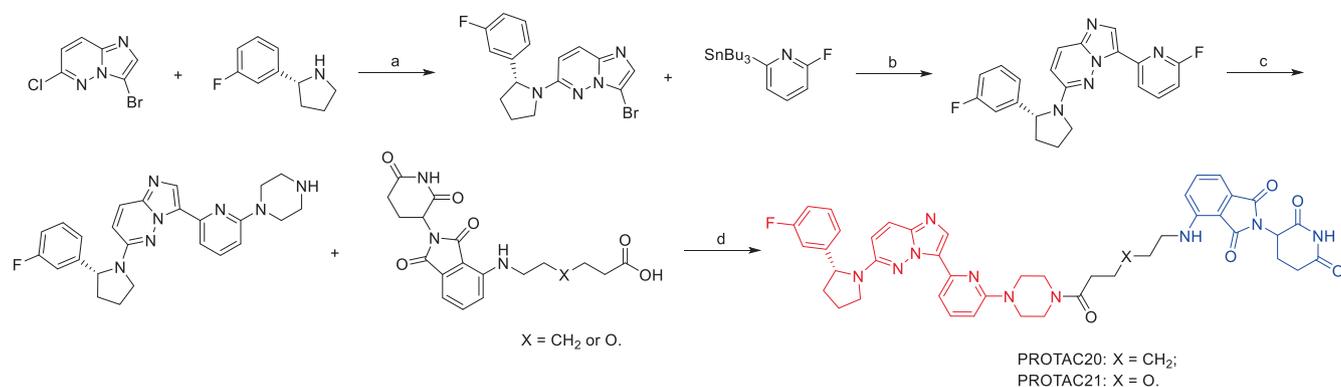
**Scheme 9a** Synthesis of PROTAC17. Reagents and conditions: a)  $\text{CuSO}_4$ ,  $\text{DMSO}/\text{H}_2\text{O} = 2/1$ , r.t., 24 h.



**Scheme 9b** Synthesis of PROTAC18. Reagents and conditions: a)  $\text{KF}$ ,  $\text{DMSO}$ ,  $100^\circ\text{C}$ , 12 h; b)  $\text{Pd}(\text{PPh}_3)_4$ , toluene,  $110^\circ\text{C}$ , 12 h; c) piperazine,  $\text{KF}$ ,  $\text{DMSO}$ ,  $130^\circ\text{C}$ , 12 h; d)  $\text{DIPEA}$ ,  $\text{DMAP}$ ,  $\text{HATU}$ , r.t., 2 h.



**Scheme 9c** Synthesis of PROTAC19. Reagents and conditions: a)  $\text{TFA}$ ,  $\text{Me}_4\text{N}^+\text{BH}(\text{OAc})_3$ ,  $\text{DCM}$ , r.t., 16 h; b)  $\text{TFAA}$ ,  $\text{Et}_3\text{N}$ ,  $\text{DCM}$ , r.t., 1 h; c)  $\text{TFA}$ ,  $\text{DCM}$ , r.t., 16 h; d) i)  $(\text{COCl})_2$ ,  $\text{DCM}$ ,  $0^\circ\text{C}$ , 30 min; ii) 5-(3,5-difluorobenzyl)-1H-indazol-3-amine,  $\text{Et}_3\text{N}$ ,  $\text{DCM}$ , r.t., 16 h; e)  $\text{K}_2\text{CO}_3$ ,  $\text{MeOH}$ , r.t., 2 h; f) i) *tert*-butyl-2-bromoacetate,  $\text{K}_2\text{CO}_3$ ,  $\text{DMF}$ , r.t., 3 h; ii)  $\text{TFA}$ ,  $\text{DCM}$ , 3 h; g)  $\text{DIPEA}$ ,  $\text{DMAP}$ ,  $\text{HATU}$ , r.t., 2 h.



**Scheme 9d** Synthesis of PROTAC20 and PROTAC21. Reagents and conditions: a) KF, DMSO, 100 °C, 12 h; b) Pd(PPh<sub>3</sub>)<sub>4</sub>, toluene, 110 °C, 12 h; c) piperazine, KF, DMSO, 130 °C, 12 h; d) EDCl, HOAt, NMM, DMSO, r.t., overnight.

reliance on small molecule drug therapy and immunotherapy targeting disease-causing proteins, can only alleviate the symptoms of the disease and cannot achieve reversal of disease progression. Even while innovative NDs treatment modalities like autophagy therapy, miRNA therapy, and stem cell therapy have been established, they still have limitations like poor BBB penetration, off-target effects, and the inability to affect intracellular proteins. PROTACs are a potent and alluring technique that has been researched and developed in both academia and industry. A critical property of PROTACs is the ability to degrade proteins that cannot be typically targeted by small-molecule inhibitors due to the lack of active sites. Due to this characteristic, the PROTACs method may be used to treat a number of neurodegenerative illnesses caused by the toxic accumulation of proteins including  $\alpha$ -synuclein, GSK-3 $\beta$ , LRRK2, mHTT, Tau, TRKA, and TRAC. There have been more in-depth studies done on the treatments for NDs, and the advancement of PROTACs technology has demonstrated significant promise in addressing the issue of undruggable protein targeted degradation. As shown by the aforementioned instances, PROTAC technology has been flourishing in recent years, displaying successful outcomes in the induction of the degradation of a variety of NDs proteins and overcoming some of the issues related to traditional small-molecule inhibitors. Unlike traditional small molecule inhibitors, PROTACs function through a unique event-driven model in which they induce the formation of ternary complexes and cycle through many rounds of target protein recruitment. In addition to being able to degrade undruggable targets, PROTACs have a number of distinct benefits over traditional small-molecule inhibitors due to their degradation mechanism. First off, PROTACs' catalytic properties make it possible to remove proteins even at sub-stoichiometric concentrations. Because of their catalytic character, PROTACs can be used without the need for high medication doses, which are frequently linked to toxicity and side effects. Moreover, PROTACs offer a higher level of target selectivity than POI ligands: for instance, the degree to which E3 and POI cooperate can affect the PROTACs' target selectivity. As a result, by carefully choosing linkers and E3 ligands, it is possible to design selective PROTACs for specific proteins over related homologues. Furthermore, PROTAC technology can overcome the resistance mechanisms common to small-molecule inhibitors. Ibrutinib (Timofeeva, et al., 2021), for instance, is frequently ineffective in treating AML when it causes the BTK C481S mutation; yet, PROTACs based on ibrutinib can still effectively degrade BTK<sup>C481S</sup> (Wang, et al., 2021). Additionally, protein overexpression is a common form of resistance to treatment with small-molecule inhibitors; however, degradation induced by PROTACs can offer an effective and prolonged effect due to their event-driven model, as shown by the fact that the KRAS<sup>G12C</sup>-targeting PROTAC demonstrated longer KRAS<sup>G12C</sup> downregulation than the parent inhibitor (MRTX849) did (Farrell,

et al., 2021). Finally, in contrast to inhibitors, PROTACs can block both the scaffolding and enzymatic functions of POIs. PROTACs may significantly improve the limited clinical efficacy of traditional small-molecule inhibitors, offering novel therapeutic possibilities for the treatment of neurodegenerative diseases.

Despite the fact that neurodegenerative diseases PROTACs present a promising strategy for a variety of areas, such as drug discovery and solutions to biological issues, difficulties still lie ahead for these PROTACs in the future. Only a select few E3 ligases have thus far been exploited in the development of useful PROTACs, despite the fact that different E3 ligases have demonstrated varying activity and selectivity for protein degradation. To achieve selectivity in the subcellular or cellular degradation of particular proteins, it is possible to take advantage of the different localization and expression variations among the E3 ligases. The varied protein-protein interactions in various situations may further enlist distinctive selectivity of protein degradation at particular contexts, a trait not conceivable with genetic ablation of proteins, although this has not yet been confirmed. The linker's chemical makeup and physical attributes appear to have a significant impact on PROTAC activity. Linker design essentials like composition and length have not yet been fully understood. Work must be completed fast in order to obtain the greatest linkers. Traditional guidelines like Lipinski's rule might not be appropriate to make a go/no go judgment in a drug discovery project due to their unique mechanisms of action. Even though we noted above that many endogenous targets were efficiently degraded, other proteins have been found to be resistant to PROTAC-mediated degradation. In addition, more investigation is needed to evaluate the absorption, distribution, metabolism, excretion, and toxicity of PROTACs for neurodegenerative diseases. This is because the mechanism underlying PROTACs is not well understood. PROTACs for neurodegenerative diseases have the potential to be developed as therapeutic agents for many deadly diseases, but they confront significant challenges that must be solved.

#### Author contributions

Concept design: CW, YZ, and DX; CW, YZ, and SY wrote the manuscript; CW and DX directed the study.

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### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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