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Synthetic approaches to constructing proteolysis targeting chimeras (PROTACs)

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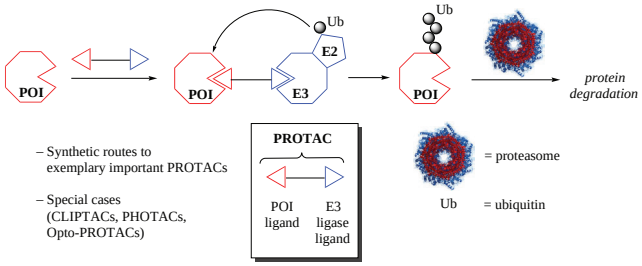
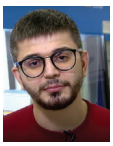
The development of various heterobifunctional constructs dubbed PRoteolysis-TArgeting Chimeras (PROTACs) has gained a significant impetus in the last few years. A viable alternative to the traditional occupancy-based inhibition of aberrantly hyperactive proteins, PROTACs operate by an event-based catalytic mechanism bringing together the protein of interest (POI, to be degraded) and E3 ubiquitin ligases. The formation of the ternary complex 'POI–PROTAC–E3 ubiquitin ligase' is the critical step which leads to the ubiquitination of the POI and its proteasomal degradation. The current Focused Review aims to highlight the syntheses of selected innovative PROTAC-type degraders of the therapeutically important protein targets as well as some notable chemical aspects of PROTAC construction. The overview is focusing on PROTACs aimed at recruiting Cereblon, the most exploited E3 ligase for targeted protein degradation.



Olga Bakulina was born in 1992 and raised near Saint Petersburg, Russia. She began her education in chemistry in 2007 at the Academic Gymnasium of Saint Petersburg State University followed by entering the university in 2009, from which she graduated in 2014 with Diploma in Chemistry (with Honors). In 2016 she obtained her PhD in organic chemistry at the same university. During 2017–2020 Olga developed her teaching career in SPbSU including positions of Assistant Professor, Senior Lecturer and Associate Professor, which she currently holds. Her research interests are in medicinal chemistry and the development of synthetic methodology based on multicomponent reactions.

Alexander Sapegin was born in 1983 in Yaroslavl, Russia. He graduated from Yaroslavl State Technical University in 2006 and obtained his PhD degree in organic chemistry from the Gubkin Russian State University of Oil and Gas. Currently, he is an Associate Professor at the Institute of Chemistry, Saint Petersburg State University. His research interests include developing new approaches to the synthesis of medium-sized rings, multicomponent reactions, chemistry of heterocyclic compounds.



Alexander S. Bunev was born in 1990 and graduated from Togliatti State University in 2018. Since 2019, he has been heading the Medicinal Chemistry Center of Togliatti State University. His current research interests include medicinal chemistry strategies for the development of kinase inhibitors and high throughput screening.

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Keywords: targeted protein degradation, proteolysis-targeting chimeras, E3 ubiquitin ligase, cereblon, recruiter ligands, linkers.

1. Introduction

Reduction of aberrantly overexpressed and/or hyperactive proteins in various disease states can provide a therapeutic benefit and can be achieved in several different ways. The use of small molecule inhibitors[1] or monoclonal antibodies[2] is a classical approach to lowering hyperactive enzyme activity. Protein overexpression can be reduced at the transcription level by employing such nucleic acid based methods as RNA interference[3] or CRISPR/Cas9 gene knockout.[4] The ubiquitin-proteasome system is an innate cellular mechanism for maintaining the protein homeostasis by proteolytic elimination of misfolded or damaged proteins.[5] The process of protein degradation involves several distinct steps one of which is the transfer of ubiquitin (Ub) from E2 to the protein of interest (POI) at its lysine residues mediated by E3 ligase enzymes – rendering the POI a client for the proteasome.[6] The idea of hijacking the ubiquitin–proteasome system to achieve the POI degradation was put forward over twenty years ago by Craig Crews of Yale University[7] and received its proof-of-concept in 2001 through the development of the first proteolysis-targeting chimeras (PROTACs).[8] These heterobifunctional molecular constructs consist of a POI ligand connected to an E3 ligase ligand with a linker and are aimed at bringing the two proteins in spatial proximity to each other as part of a ternary complex formation which results in the POI ubiquitination and ultimate proteosomal degradation.[9] This mechanism of PROTAC action (Figure 1) is currently well understood and supported by the recently obtained X-ray structure of a POI-PROTAC-E3 ternary complex.[10] The design principles for PROTAC molecules include the use of available POI ligands (typically, small-molecule inhibitors), various linkers (usually, of 5–15 atoms) and E3 ubiquitin ligase ligands.[11] Although there are over 600 E3 ubiquitin ligases encoded in the human genome,[6] only a handful with known small molecule ligands have been targeted by PROTACs, including Skp1-Cullin-F box complex containing Hrt11 (SCF),[12] Von Hippel–Lindau tumor suppressor (VHL),[13] inhibitor of apoptosis proteins (IAPs),[14] Cereblon (CRBN),[15] and mouse double minute 2 homolog (MDM2).[16] Despite the limited number of 'ligandable' E3 ubiquitin ligases known, the field has rapidly evolved and resulted in numerous small-molecule E3 ligands, which enabled the development of cell-permeable and biologically active PROTACs for over 50 proteins, many of which are clinically validated drug targets.[17] Two compounds developed by Arvinas, Inc. – androgen receptors (AR) degrader ARV-110 (**1**) and estrogen receptors (ER) degrader ARV-471 (**2**) – are currently in phase II clinical trials for the treatment of castration-resistant prostate cancer and locally advanced or metastatic ER positive/HER2 negative breast cancer, respectively.[18] Like the latter two most advanced compounds, PROTACs engaging Cereblon (CRBN) E3 ligase account for more that 30 different protein degraders reported to-date,[19] which makes CRBN the most exploited E3 ligase in the field. Most CRBN ligands are structurally distinct and belong to the class of immunomodulatory imide drugs (IMiDs), *i.e.*

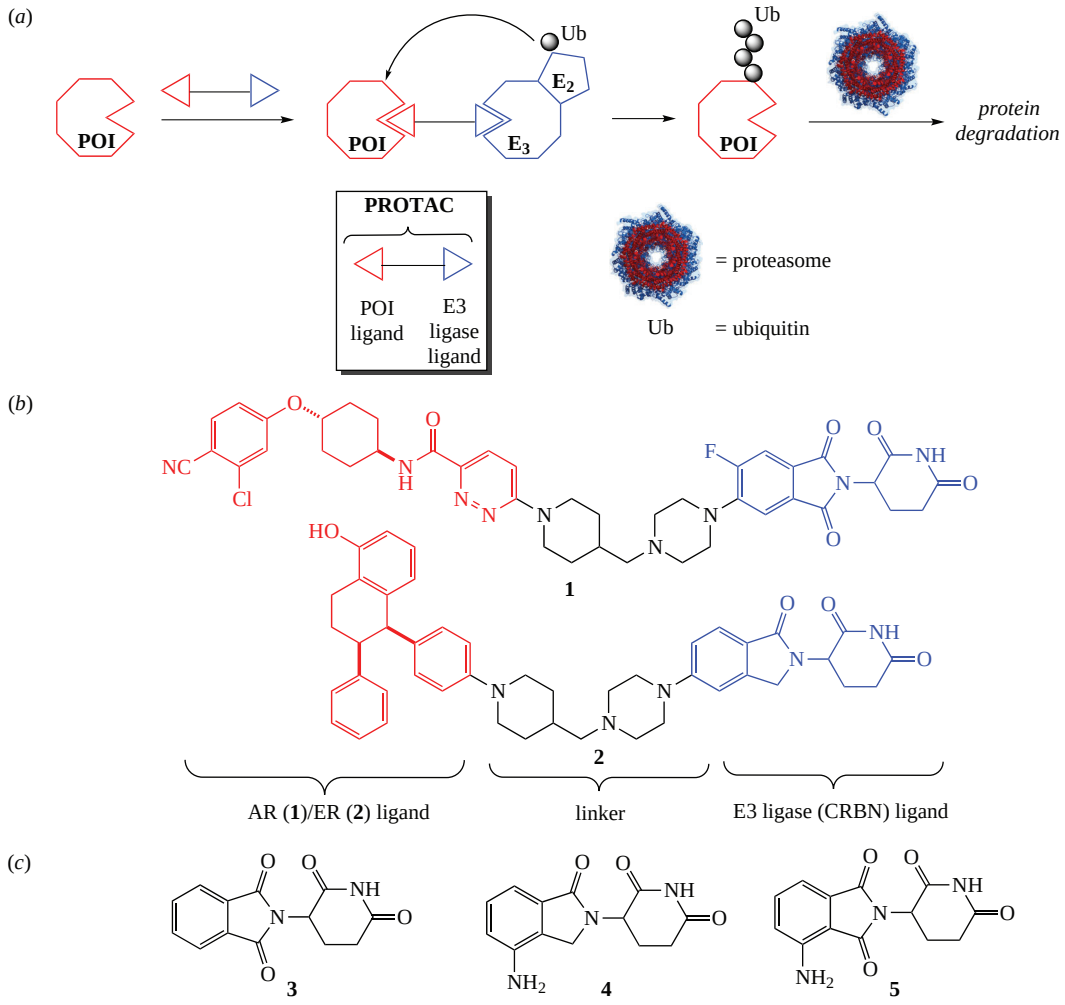


Figure 1 (*a*) Mechanism of PROTAC-mediated proteasomal degradation of protein of interest (POI); (*b*) Structures of advanced PROTACs (currently in phase II clinical trials); (*c*) Structures of CRBN IMiD ligands.

thalidomide (**3**), lenalidomide (**4**), and pomalidomide (**5**) (see Figure 1).[20,21] However, in the recent years, a plethora of new, non-IMiD CRBN ligands have been reported.[22]

The topic of PROTACs has been extensively reviewed in several recent publications.[23–27] The principal aim of the present Focused Review is to highlight the key synthetic approaches to constructing PROTAC degraders of therapeutically important proteins, with particular emphasis of the unique strategies for linking the POI and E3 ligase ligands. Additionally, special cases of PROTACs which are interesting from the chemical viewpoint (such as in-cell PROTAC assembly, photoactivated and photo-caged) will be discussed. Due to space limitations, the review will focus on PROTACs aimed at engaging CRBN E3 ligase. However, the approaches discussed herein have already been applied or are potentially applicable to PROTACs targeting other E3 ligases.[11]

2. Selected PROTACs aimed at Cereblon E3 recruitment and their chemical syntheses

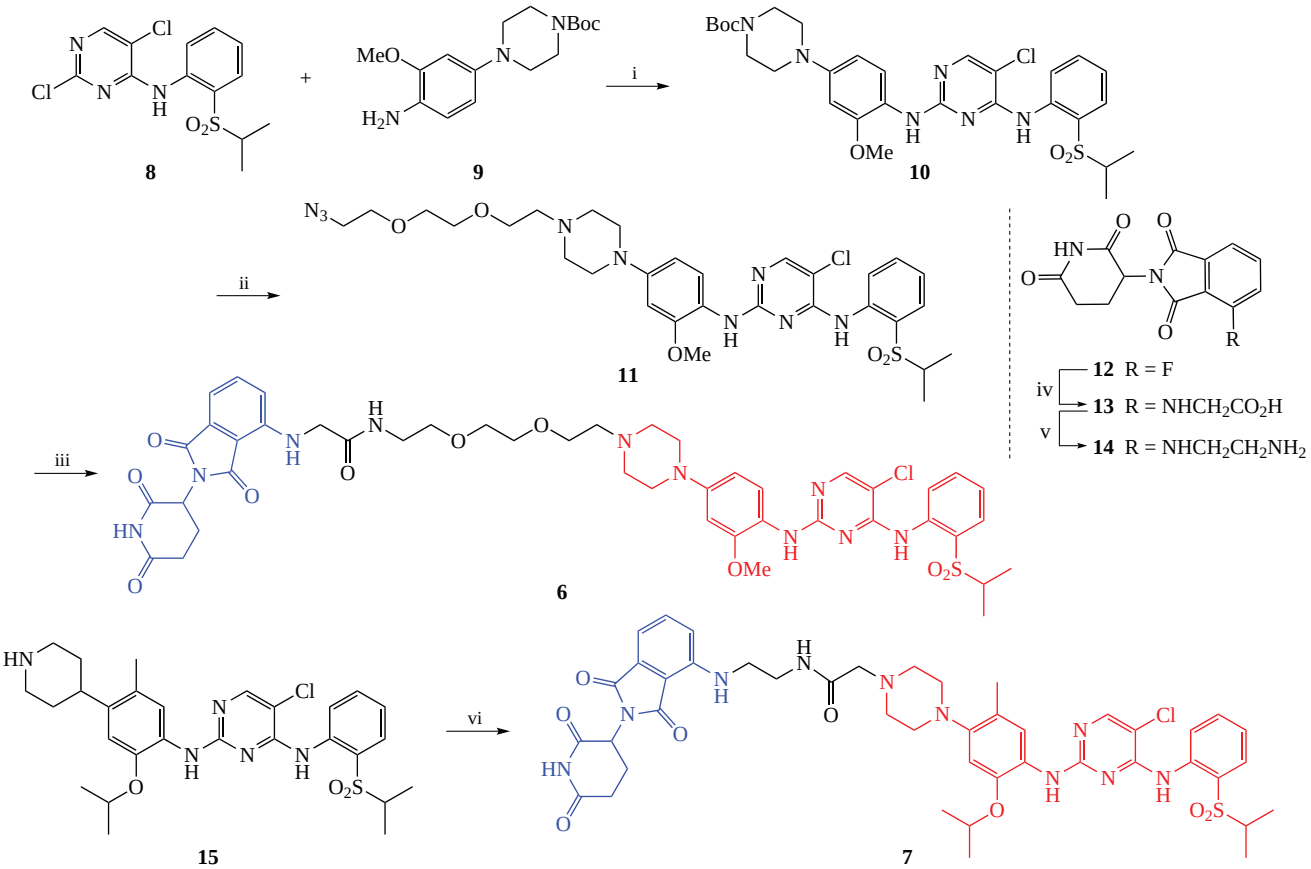
Designing new PROTACs is quite straightforward from the standpoint of the two ligands for the POI and E3 ligase (CRBN). The POI ligand is typically selected from the known small-molecule inhibitors of the POI while the CRBN ligand – from the pool of known IMiDs (**3**–**5**).[11] The design principles of the linker and its points of attachment to the POI and CRBN ligands are much less understood[28,29] and typically require that several candidate PROTACs are synthesized and tested for the POI degradation before the best compound is selected. The structure of the linker[30] and its topology within the PROTAC structure is crucial for the formation of the ternary complex (POI–PROTAC–CRBN) and determines the synthetic route by which a particular PROTAC will be accessed. In this section, we will review the syntheses of several efficacious PROTACs that showed notable degradation of their POIs based on CRBN E3 ligase recruitment.

2.1. Anaplastic lymphoma kinase (ALK)

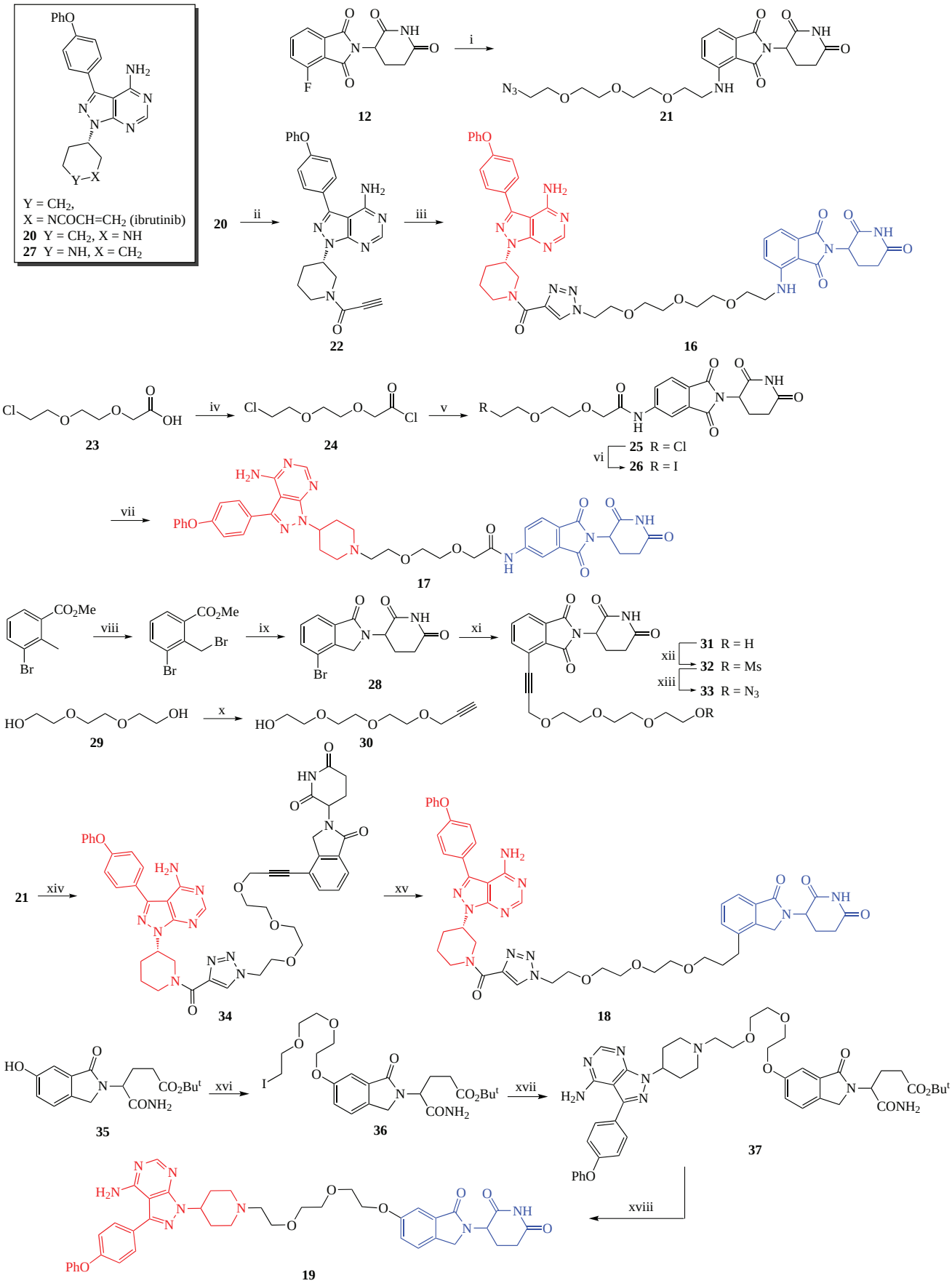
ALK is a tyrosine kinase whose oncogenic activation is related to the occurrence of many human cancers. In 2018, two research groups (Nathaniel Gray[31] and Jian Jin[32]) reported PROTACs [TL13-12 (**6**)[31] and MS4078 (**7**),[32] respectively] that are based on pomalidomide (**5**) as CRBN ligand and ALK inhibitors TAE684 (in **6**) and ceritinib (in **7**), highlighted red. While structurally similar, the two compounds required slightly different routes for their assembly. For the assembly of TL13-12 (**6**), Boc-protected compound TAE684 (**10**) was prepared *via* nucleophilic aromatic substitution reaction between commercially available building blocks **8** and **9**. Boc group in **10** was replaced with the azido-substituted linker (to afford compound **11**). The azide group was reduced and the resulting amine was used in the amide coupling with glycine-substituted phthalimide **13** to give final compound **6**. Notably, building block **13** was prepared from 4-fluorothalidomide (**12**) which is central to many syntheses of CRBN-targeting PROTACs. Replacement of the fluorine in **12** with ethylenediamine unit gave building block **14**. The latter was directly used in the assembly of compound MS4078 (**7**) from ceritinib (**15**) for which purpose an acetic acid side chain was installed and used in the amide coupling (Scheme 1).

2.2. Bruton's tyrosine kinase (BTK)

Bruton's tyrosine kinase (BTK) is indispensable for the activation and proliferation of B-cells.[33] BTK inhibition, particularly with the first-in-class inhibitor ibrutinib, has proven to be a viable treatment option for patients with chronic lymphocytic leukemia



Scheme 1 *Reagents and conditions*: i, TFA, Bu^{s}OH, 80 °C (75%); ii, a) TFA, CH_2Cl_2, room temperature; b) $N_3(CH_2CH_2O)_2CH_2CH_2Br$, K_2CO_3, MeCN, 80 °C (78%); iii, a) PPh_3, THF/H_2O, room temperature; b) **13**, HATU, DIEA, CH_2Cl_2, room temperature (71%); iv, a) NH_2–Gly–Bu^t, DIEA, DMSO, 90 °C; b) TFA, CH_2Cl_2, room temperature (68%); v, a) $BocNHCH_2CH_2NH_2$, NMP, DIPEA, microwave, 90 °C; b) TFA, CH_2Cl_2, room temperature (66%); vi, a) $BrCH_2CO_2Bu^t$, K_2CO_3, DMF, room temperature; b) TFA, CH_2Cl_2, room temperature (75%); b) EDCI, HOAt, NMM, DMSO, room temperature (80%).



Scheme 2 *Reagents and conditions*: i, $N_3(CH_2CH_2O)_3CH_2CH_2NH_2$, DIPEA, DMF, 90 °C (22%); ii, propiolic acid, DCC, $CHCl_3$, 0 °C (72%); iii, **21**, $CuSO_4$, Na ascorbate, H_2O, Bu^{t}OH, 70 °C, 50%; iv, $SOCl_2$, 60 °C (78%); v, 5-aminophthalimide, THF, reflux (65%); vi, NaI, acetone, 60 °C (92%); vii, **27**, TEA, DMF, room temperature (55%); viii, NBS, AIBN, $CHCl_3$, reflux; ix, 3-aminoglutarimide hydrochloride, TEA, MeCN, reflux (60%); x, propargyl bromide, Bu^{t}OK, THF, room temperature (70%); xi, **28**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C (41%); xii, MsCl, TEA, THF, room temperature (85%); xiii, NaN_3, DMF, 80 °C (84%); xiv, $CuSO_4$, Na ascorbate, H_2O, Bu^{t}OH, 70 °C, 52%; xv, 10% Pd/C, H_2 (1 atm.), DMF, MeOH, 38 °C (63%); xvi, $I(CH_2CH_2O)_2CH_2CH_2I$, Cs_2CO_3, DMF (59%); xvii, **26**, TEA, DMF, room temperature (69%); xviii, BSA, MeCN, reflux (51%).

(CLL) and other B-cell malignancies.[34] Ibrutinib is an irreversible covalent inhibitor which acts *via* binding in the ATP-binding pocket of BTK and forming a covalent bond with nearby Cys481 residue (through Michael addition at the inhibitor's acrylamide moiety).[35] Unfortunately, over 80% CLL patients develop resistance to ibrutinib as expression of C481S mutant BTK, unsusceptible to covalent inhibition mechanism, emerges.[36] Luckily, PROTACs employing ibrutinib moiety as the BTK recruiter were created in 2018–2019 and proved effective knocking down the drug-resistant kinase.

The four frontrunner BTK-degrading PROTACs – the pioneering P13I (**16**) containing a 1,2,3-triazole moiety in its linker,[37] next-in-class MT-802 (**17**) developed by the Crews team,[38] carbon-linked L18I (**18**)[39] and ether-linked SJF620 (**19**) also developed by Crews and co-workers[40] – are all based on the ibrutinib as the BTK-recruiting warhead (red) and thalidomide derivative moiety (blue) as the CRBN recruiter with significant differences in the linker type and its point of attachment to the CRBN ligand. This had a notable bearing on the synthetic strategies employed in the assembly of these PROTACs.

Assembly of P13I (**16**) started by installing a polyethylene glycol linker on the pomalidomide core *via* nucleophilic aromatic substitution reaction between 4-fluorothalidomide (**12**) and the suitable azido-functionalized linker amine. At the same time, des-acryloyl ibrutinib (**20**) was acylated with propiolic acid to install the site for the Huisgen cycloaddition at the next step. The latter reaction (**21** + **22**) produced P13I (**16**). BTK degrader MT-802 was based on a positional isomer **27** of des-acryloyl ibrutinib. Apparently, the exact topology of the BTK-recruiting portion of MT-802 did not matter while the modification eliminated the unwanted chirality. The linker was elaborated from commercially available acid **23** which was converted to acyl chloride **24** and the latter was used in the acylation of 5-aminothalidomide. The chloro substituent in **25** was converted to a better leaving group and the resulting iodide **26** was used to alkylate **27** and produce MT-802. L18I (**19**) is merely a carbon-linked, des-oxo version of P13I (**16**). Yet its synthesis was markedly more elaborate compared to that of **16**. The lenalidomide-like bromo synthon **28** was synthesized in two steps from methyl 3-bromo-2-methylbenzoate and coupled with alkyne **30** (synthesized, in turn, from symmetrical ethylene glycol diol **29**). The hydroxy group in coupling product **31** was activated by mesylation and, on reaction of mesylate **32** with sodium azide, produced **33**. The latter was ready to react with terminal alkyne **21** *via* Cu-promoted azide–alkyne cycloaddition to give alkyne-linked precursor to L18I (compound **34**). Target L18I (**18**) was obtained by catalytic hydrogenation of the triple bond in **34**. The synthesis of SJF620 (**19**) is different and interesting in the way that the glutarimide portion in this PROTAC is elaborate at the very last step. Readily available isoindolinone **35** was alkylated with symmetrical diiodide at its phenol function. The resulting iodide **36** was used to alkylate BTK-recruiting amine **27** and produce **37**, a very advanced precursor to the target molecule. Finally, exposure of **37** to benzenesulfonic acid in refluxing acetonitrile triggered removal of the *tert*-butyl group and the formation of glutarimide to give SJF620 (**19**) (Scheme 2).

2.3. Bromodomain and extraterminal (BET) proteins

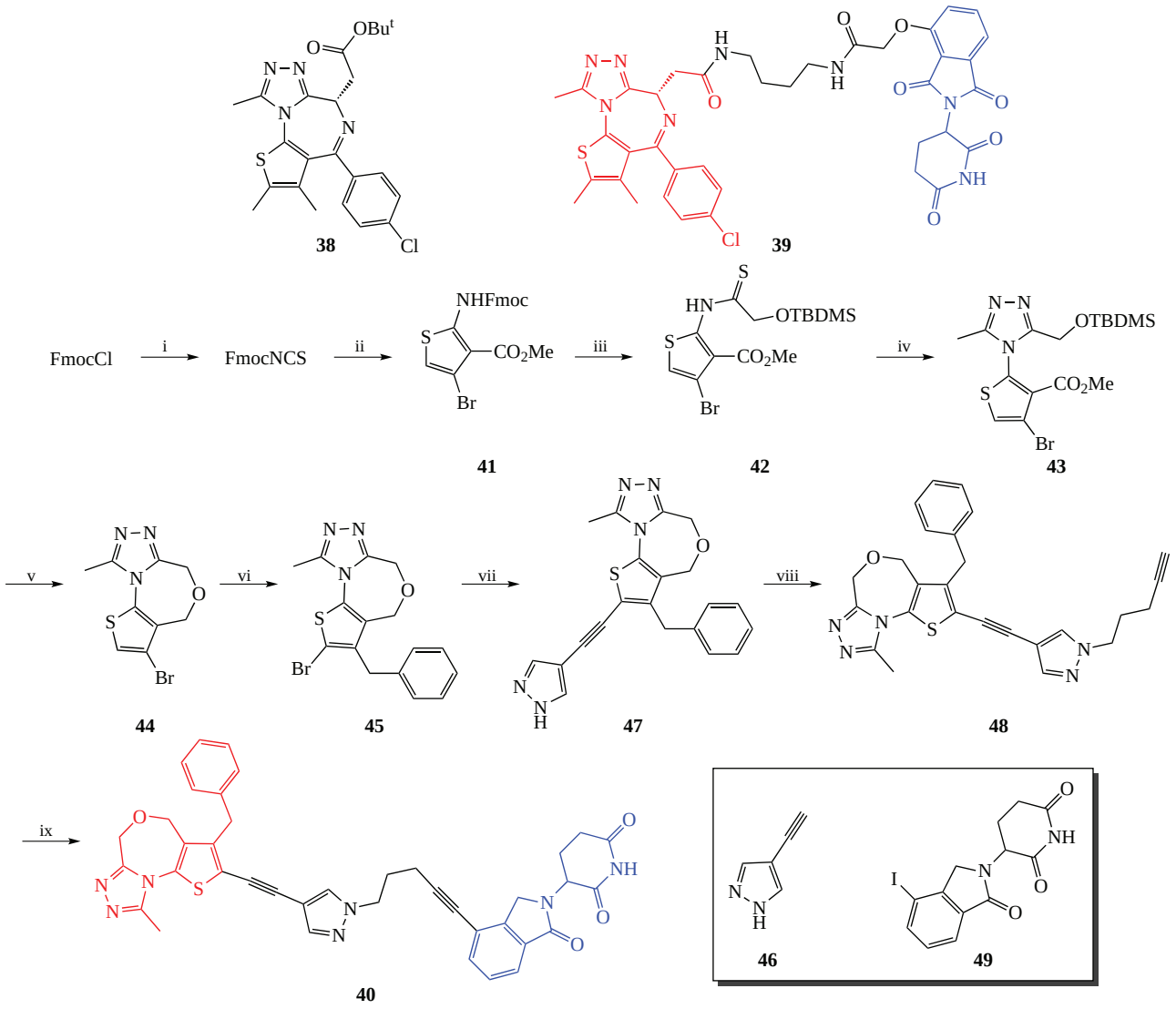
BET family and bromodomain proteins (and BRD4 protein in particular) are transcription factors which have emerged as drug targets for various cancers, especially castration-resistant prostate cancer.[41] Transcription factors are generally considered 'difficult-to-drug' targets. However, in 2010 James Bradner's team described the structure of JQ1 (**38**), a potent BET inhibitor.[42] Subsequently, it was hypothesized that PROTACs targeting BET proteins might have even better chance of lowering BET protein level and several BET protein degraders have been reported in the literature, including pioneering dBET1 (**39**) by Bradner[43] and the most potent and efficacious degrader of this class – QCAS570 (**40**) by Wang.[44] The design of dBET1 (**39**) was based on the general premise that the *tert*-butyl ester group of JQ1 can be used as the attachment point for the linker, without any detriment for the affinity to BRD4 (and other bromodomain proteins). The strategy to assemble **39** from readily available precursors is fairly straightforward and is discussed in detail in the recent publication.[45]

On the contrary, the structure of and the synthetic route to **40** are quite interesting and deserve special attention. Firstly, the structure of BRD4 ligand is quite distant from JQ1 and represents a significant bioisosteric alteration. Secondly, the lenalidomide-like CRBN recruiter part is connected to the POI recruiter *via* a rather intriguing bis-alkyne pyrazole linker which is fairly unique for the current PROTAC design. The assembly of 1,2,4-triazolyl thiophene core started with FmocCl which was sequentially condensed with potassium thiocyanide and methyl 4-chloro-3-oxobutanoate and then converted to bromo derivative **41**. The latter was deprotected, acylated with silyl-protected hydroxyacetyl chloride and the resulting amide was converted to thioamide **42** on reaction with the Lawesson's reagent. The thioamide moiety was elaborated into 1,2,4-triazole **43** by reaction with hydrazine hydrate and cyclocondensation with triethyl orthoacetate. Reduction of the ester group in **43**, its conversion to the respective alkyl chloride and silyl group deprotection set the scene for the 1,4-oxazepine ring closure to give compound **44**. The latter was coupled with potassium benzyltrufluoroborate *via* Suzuki reaction and ring-brominated with NBS to give **45**. The latter was introduced in the Sonogashira coupling with alkyne **46**. The pyrazolyl moiety in **47** was alkylated with homopropargyl bromide and intermediate **48** was again introduced in Sonogashira coupling with 4-iodo-isoindolinone **49** to afford QCAS570 (**40**) (Scheme 3).

2.4. Histone deacetylase 6 (HDAC6)

Histone deacetylase 6 (HDAC6) catalyzes the deacetylation of histones and non-histone substrates, and plays important roles in cell migration, protein degradation and other cellular processes. Aberrantly high protein level of HDAC6 has been linked to various pathological conditions, such as cancer, neuro-degenerative diseases, and inflammatory disorders.[46] Therefore, the expression level of HDAC6 should be precisely controlled. Numerous HDAC6 inhibitors have been reported; however, achieving the desired level of selectivity against other HDAC isoforms remains a pressing issue. The development of PROTACs, even based on non-selective HDAC ligands, aimed at HDAC6 degradation offered new opportunities both in terms of efficacy and selectivity.[47]

Historically, the first selective HDAC6 degrader **50** was reported in 2018 based on a completely non-selective zinc-binding hydroxamic acid motif.[48] Alkyne **51** was prepared from 4-fluorothalidomide (**12**) and introduced in copper-promoted azide–alkyne cycloaddition reaction with azide **52**. Resulting 1,2,3-triazole-linked aldehyde **53** was condensed with hydroxamic acid-containing acyl hydrazine **54** to give the target PROTAC molecule **50**. The following two next-generation HDAC6 degraders are based on linking selective HDAC6 inhibitor Nexturastat A (**55**)[49] to pomalidomide. In one case (compound **56**), the linking was undertaken from the phenyl ring of the urea portion of Nexturastat A. Boc-Protected *p*-aminophenol was alkylated with 1,4-dibromobutane, the second bromine was replaced with azido group and the Boc group was removed to give aniline **57**. The latter was coupled with secondary amine **58** through a urea linkage and the resulting



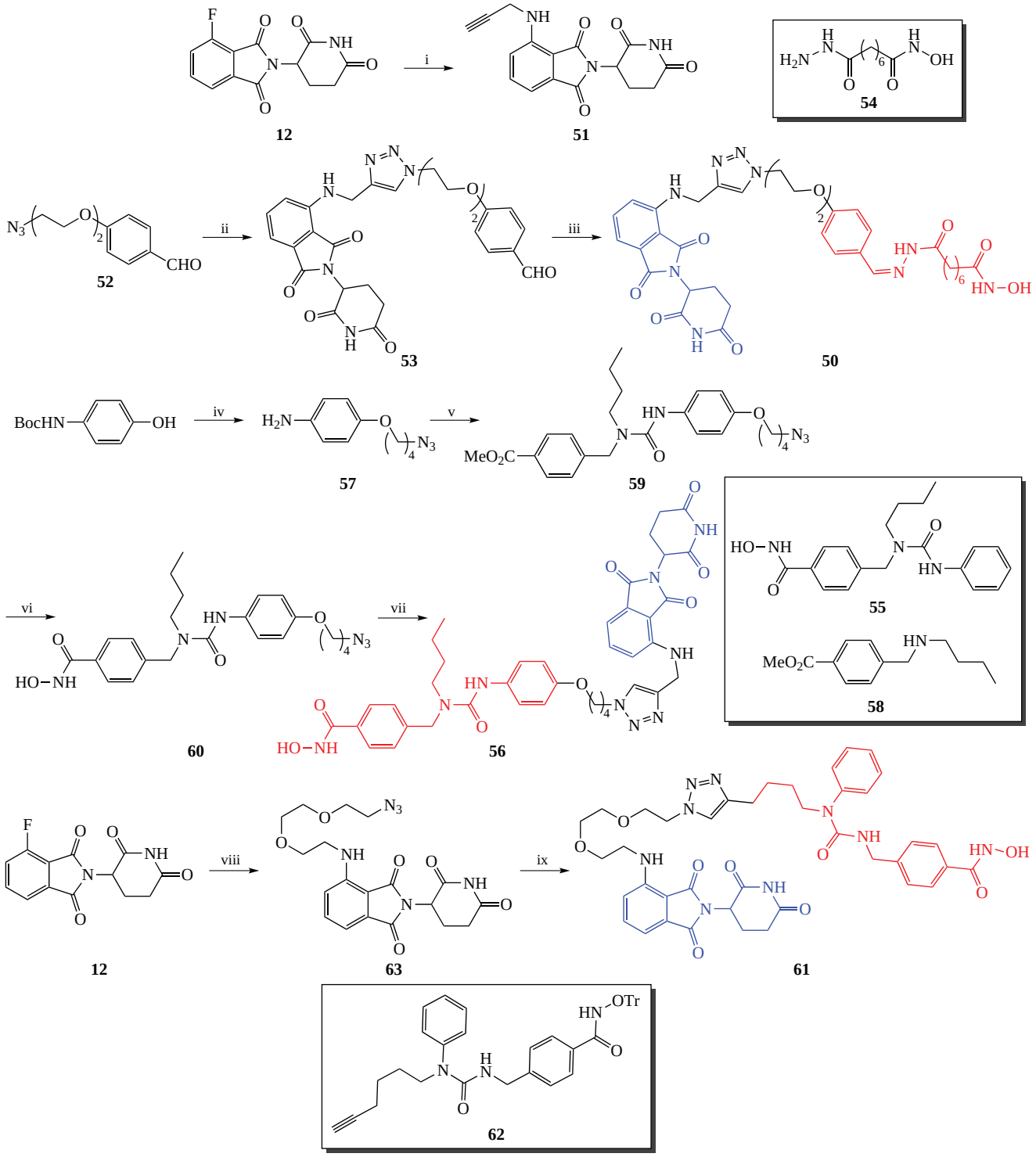
Scheme 3 *Reagents and conditions*: i, KNCS, EtOAc, 0 °C → room temperature (92%); ii, a) NaH, $ClCH_2COCH_2CO_2Me$, THF, 0 °C; b) $POBr_3$ (62%); iii, a) morpholine (52%); b) $TBDMSOCH_2COCl$, DIPEA, DCM (99%); c) Lawesson's reagent, 1,4-dioxane, room temperature (57%); iv, a) hydrazine hydrate; b) $MeCH(OEt)_3$; c) AcOH (58%); v, a) $LiBH_4$; b) $SOCl_2$ (77%); c) TBAF; d) $NaOBu^t$ (66%); vi, a) $BnBF_3K$, $Pd(allyl)Cl_2$, ^{s}SPhos, Na_2CO_3, H_2O, toluene; b) NBS, AcOH (84%); vii, **46**, $PdCl_2(PPh_3)_2$, CuI, THF, TEA (70%); viii, homopropargyl iodide, K_2CO_3, DMF (80%); ix, **49**, $PdCl_2(PPh_3)_2$, CuI, THF, TEA (54%).

ester **59** was converted to hydroxamic acid **60**. The latter was introduced in copper-promoted azide–akyne cycloaddition reaction with **51** to give target PROTAC molecule **56**.[50] In the other case (compound **61**), the structure of Nexturastat A (**55**) was modified so that CRBN ligand (pomalidomide) was attached *via* the butyl portion of the urea portion.[51] To this end, trityl-protected hydroxamic acid urea **62** was employed. The synthesis of **62** was not described in detail; however, one may assume that it was prepared in a similar fashion to compound **60** followed by *O*-tritylation of the hydroxamide acid portion.[52] 4-Fluorothalidomide (**12**) was converted to azide **63** *via* sequential aromatic nucleophilic substitution reaction with 2-[2-(2-bromoethoxy)ethoxy]ethan-1-amine followed by displacement of the bromine with an azido group. Azide **63** and alkyne **62** were brought together in a copper-promoted azide–alkyne cycloaddition reaction to furnish PROTAC **61** (Scheme 4).

2.5. Poly (ADP-ribose) polymerase 1 (PARP1)

Poly (ADP-ribose) polymerase 1 (PARP1) is an important DNA repair enzyme which has been pursued as an attractive cancer therapeutic target.[53] Inhibition of PARP1 itself does not lead to cancer cell death; however, it is lethal to cancer cells carrying BRCA1/2 mutations. Several PARP1 inhibitors are currently approved for medical use for the treatment of breast, ovarian and prostatic cancer. However, these treatments are not fully devoid of unwanted side effects and the development of new, 'cleaner' PARP1 inhibitors is hampered by the difficulties to achieve selectivity to the target.[54] Thus, PROTAC-type degrader have recently emerged to circumvent this problem and also potentially reduce the drug dosage required to achieve therapeutic effect.

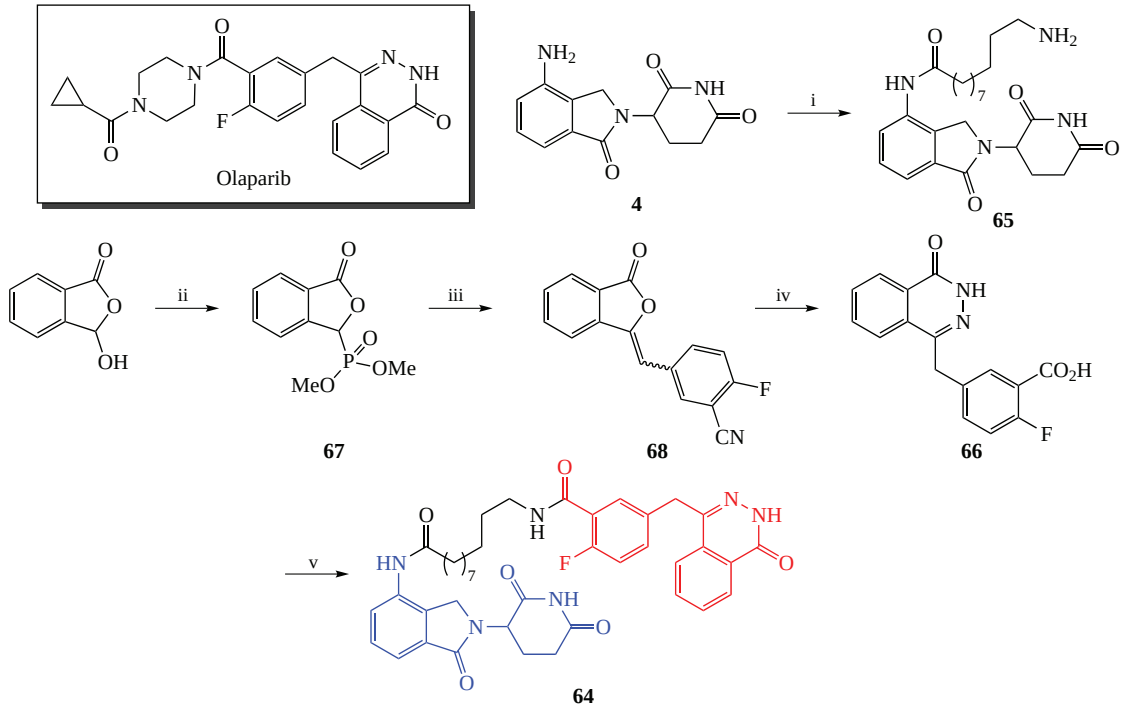
The first PROTAC to degrade PARP1 (compound **64**) was reported in 2020.[55] It was based on the structure of clinically used drug Olaparib which was linked to CRBN ligand lenalidomide (**4**) *via* an optimized alkanoic acid linker. An interesting feature of PROTAC **64** is the fact that the structure of Olaparib was significantly reduced by eliminating the 4-(cyclopropylcarbonyl)piperazin-1-yl moiety altogether and replacing it with the linker. Although such truncated Olaparib most likely has a reduced affinity to PARP1, this modification was tolerated for PARP1-recruting moiety. Installation of the linker was fairly straightforward: lenalidomide (**4**) was acylated with Boc-protected 12-aminododecanoic acid and the Boc group was removed to give amine **65**. Olaparib carboxylic acid **66** was synthesized *de novo* from *o*-phthalaldehydic acid which was reacted with dimethyl phosphite to generate phosphonate **67**. The Horner–Wadsworth–Emmons olefination of 3-cyano-4-



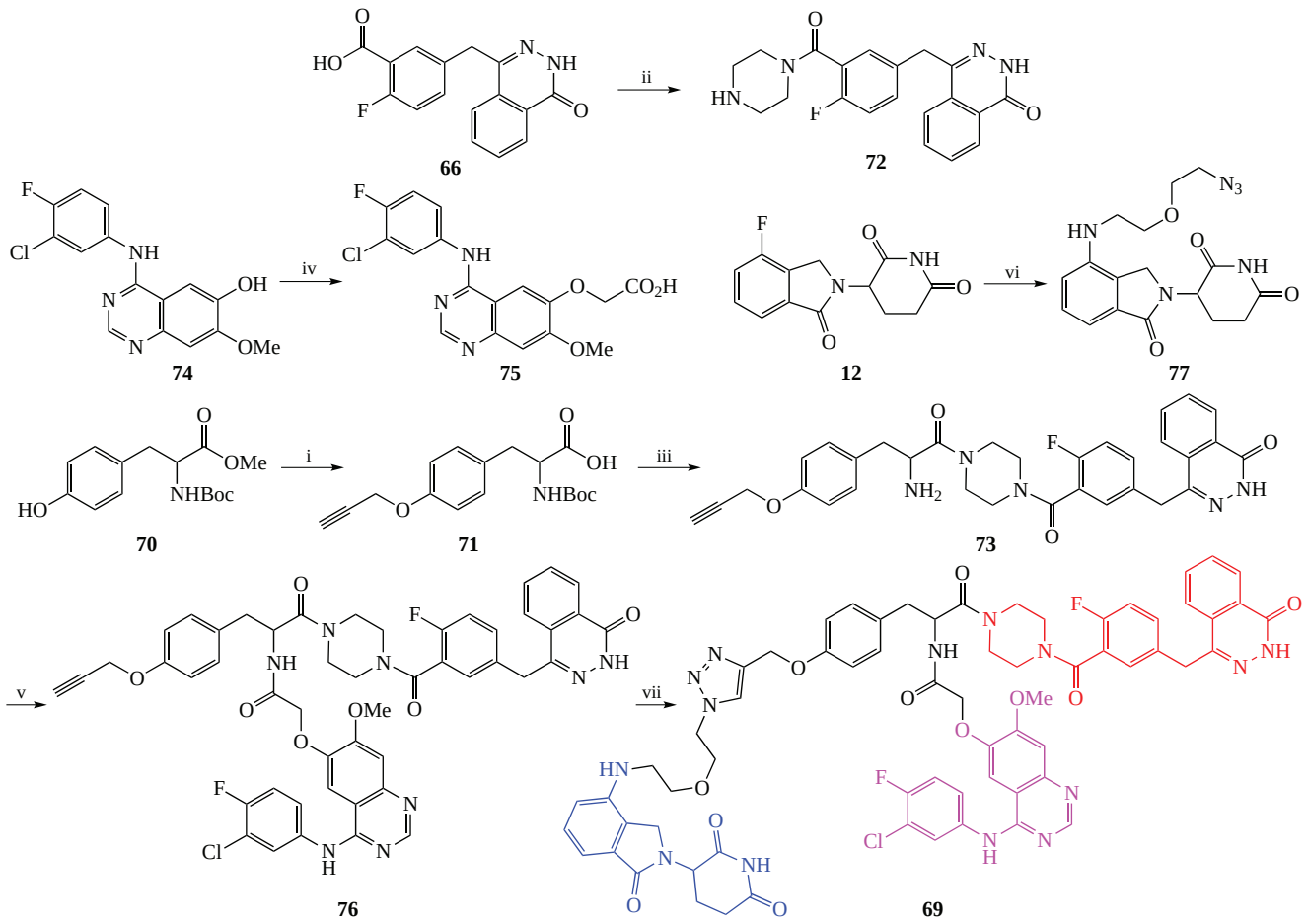
Scheme 4 *Reagents and conditions*: i, propargylamine hydrochloride, DIPEA, DMF, 90 °C (30%); ii, **51**, $CuSO_4$, tris(benzyltriazolylmethyl)amine (TBTA), Na ascorbate, H_2O/Bu^{t}OH (1 : 5), room temperature (60%); iii, HOAc, DMSO, 65 °C; iv, a) $Br(CH_2)_4Br$, K_2CO_3, MeCN, reflux (45%); b) NaN_3, DMF, 50 °C (66%); c) TFA, DCM, 0 °C to room temperature (94%); v, **58**, CDI, THF, 0 °C to room temperature (55%); vi, NaOH, NH_2OH/H_2O, THF, MeOH, 0 °C to room temperature (87%); vii, **51**, $CuSO_4$, TBTA, Na ascorbate, H_2O/Bu^{t}OH (1 : 1.5), room temperature (70%); viii, a) $H_2N(CH_2CH_2O)_2CH_2CH_2Br$, DIPEA, DMF, 90 °C; b) NaN_3, DMF, 50 °C (52%); ix, **62**, $CuSO_4$, Na ascorbate, H_2O/Bu^{t}OH, DCM, room temperature (85%).

fluorobenzaldehyde with the latter gave compound **68** as mixture of geometric isomers. The mixture of *E* and *Z* isomers was treated with hydrazine hydrate to produce the phthalazinone core. Base hydrolysis of the pendant nitrile provided the key carboxylic acid intermediate **66**. Finally, amidation of **66** with **65** promoted by HATU gave PROTAC **64** (Scheme 5).

Particularly interesting is PARP1 degrader **69** that also contains a recruiter ligand moiety for epithelial growth factor receptor (EGFR), another protein linked to oncogenesis (the respective recruiter moiety is highlighted pink).[56] While PARP1-recruiting motif of **69** was still based on Olaparib core, the EGFR-recruiting portion of this trifunctional PROTAC was built based on the structure of anticancer kinase inhibitor Gefitinib. The tripod linker for **69** was designed based on tyrosine in which the phenolic hydroxyl group, the amino and the carboxylic functions served as the points of attachment of PARP1-, EGFR- and CRBN-recruiting moieties. Boc-Protected tyrosine methyl ester **70** was alkylated by propargyl bromide at its phenolic hydroxy group and the ester was hydrolyzed to give carboxylic acid **71**. Olaparib acid **66** was amidated with *N*-Boc-piperazine



Scheme 5 *Reagents and conditions*: i, a) $BocNH(CH_2)_{10}CO_2H$, HATU, TEA, DMF, room temperature; b) TFA, DCM, room temperature (77%); ii, $(MeO)_2PHO$, MeONa, MeOH, 0–25 °C (89%); iii, 2-fluoro-5-formylbenzonitrile, TEA, THF, 10–20 °C (84%); iv, a) $N_2H_4 \cdot H_2O$, 70 °C; b) NaOH, H_2O, 90 °C; c) 2 M HCl, H_2O, room temperature (73%); v, **65**, HATU, TEA, DMF, room temperature (51%).



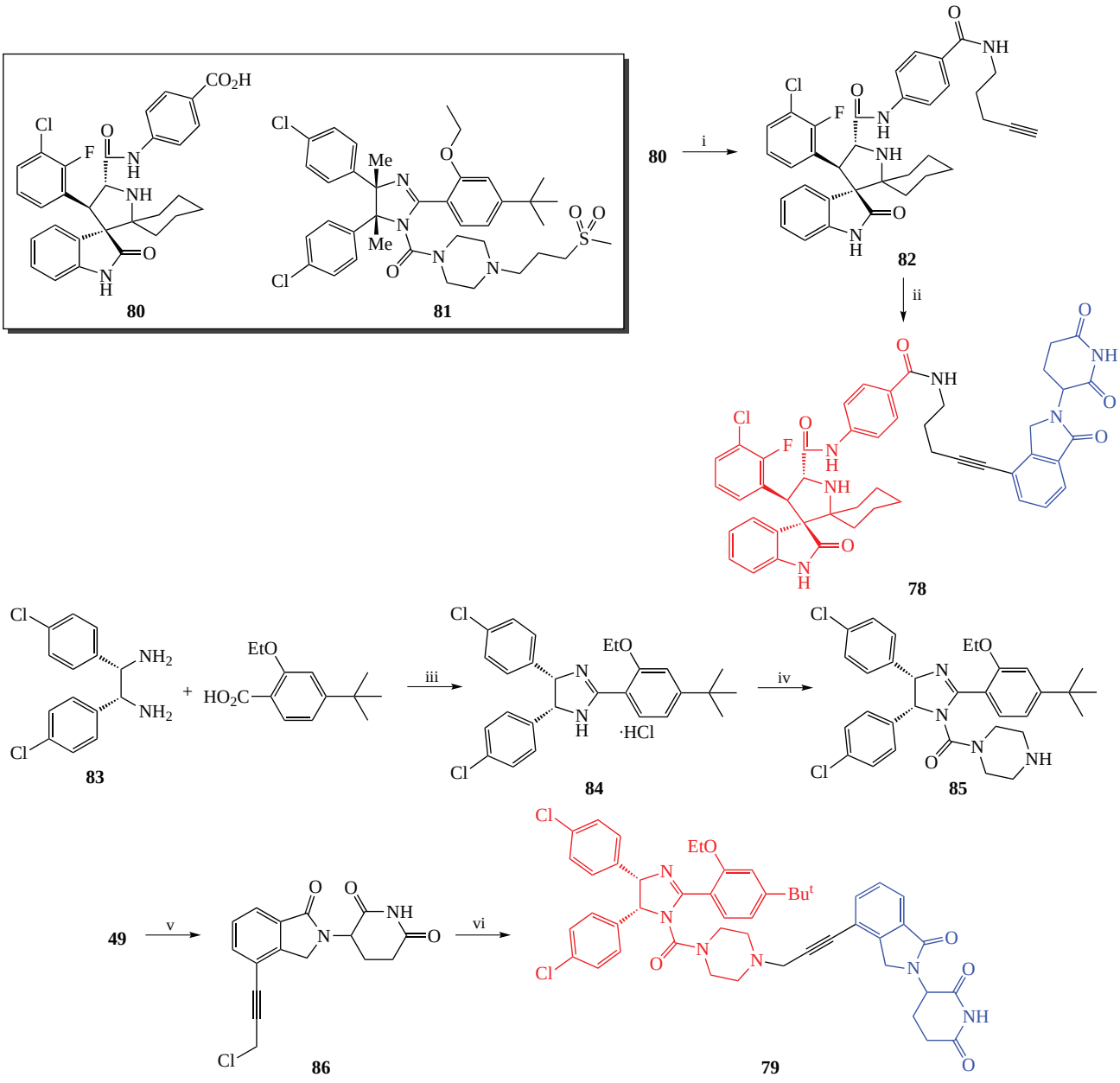
Scheme 6 *Reagents and conditions*: i, a) propargyl bromide, K_2CO_3, DMF, room temperature; b) NaOH, 1 : 1 MeOH/THF, room temperature (98%); ii, *N*-Boc-piperazine, HATU, TEA, DMF, room temperature; b) TFA, DCM, room temperature (87%); iii, a) **72**, EDCI, HOBt, DIPEA, DCM, room temperature (45%); b) TFA, DCM, room temperature (quant.); iv, a) $BrCH_2CO_2Et$, K_2CO_3, acetone, reflux (42%); b) NaOH, 1 : 1 MeOH/THF, room temperature (90%); v, **75**, EDCI, HOBt, DIPEA, DCM, room temperature (44%); vi, $H_2NCH_2CH_2OCH_2CH_2N_3$, DIPEA, DMF, 85 °C (29%); vii, **77**, $CuSO_4$, Na ascorbate, 4 : 1 THF/H_2O, room temperature (42%).

and the Boc group was removed to yield compound **72**. The latter was used in the amidation of carboxylic acid **71** thereby linking the PARP1-recruiting moiety to the tyrosine-based tripod. After removal of the Boc group intermediate **73** was obtained. Desmethyl Gefitinib **74** was alkylated with ethyl bromoacetate and the ethyl ester group was hydrolyzed to give carboxylic acid **75**. The latter was amidated with **73** in EDCI/HOBt-promoted reaction to give alkyne-tagged Gefitinib/Olaparib hybrid **76**. The alkyne moiety of the latter was involved in copper-promoted azide–alkyne cycloaddition reaction with azide **77** which was synthesized from 4-fluorothalidomide (**12**) *via* aromatic nucleophilic substitution reaction. Thus, trifunctional PARP1/EGFR degrader **69** was obtained in 42% yield (Scheme 6).

2.6. Murine double minute 2 protein (MDM2)

Tumor suppressor p53 is crucial for regulating many cellular processes and for preventing cancer.[57] In roughly 50% of human cancers, p53 is mutated and this results in the inactivation of its tumor suppressor ability.[58] Murine double minute 2 (MDM2) protein is an endogenous regulator of p53 activity. MDM2 is a ubiquitin E3 ligase and when it binds to and ubiquitinates p53, the latter is ultimately degraded by the proteasome.[59] In order to restore the activity of p53 in cancer, its inactivation by MDM2 could be antagonized by small-molecule inhibitors. To this end, many novel p53–MDM2 protein–protein interaction disruptors have been developed although none of them found its way to the clinic.[60] Unfortunately, the strategy based on p53 inhibition leads to MDM2 being overexpressed and accumulated, which results in toxicity issues. This setback is exacerbated by the rapidly developing drug resistance. In this context, an alternative approach offered by PROTAC-based MDM2 degradation (*i.e.* its complete removal from possible interaction with p53 and deactivation of the latter) is particularly attractive.

The selection of optimized ligands for MDM2 to be chosen from the literature is quite ample. In 2019, two MDM2 degraders were reported – **78**[61] and **79**[62] – that were designed based on MDM2 ligands MI-1061 (**80**)[63] and RG-7112 (**81**)[64] belonging to well-studied classes of spiro-oxindoles and *cis*-imidazolines (Nutlins), respectively.

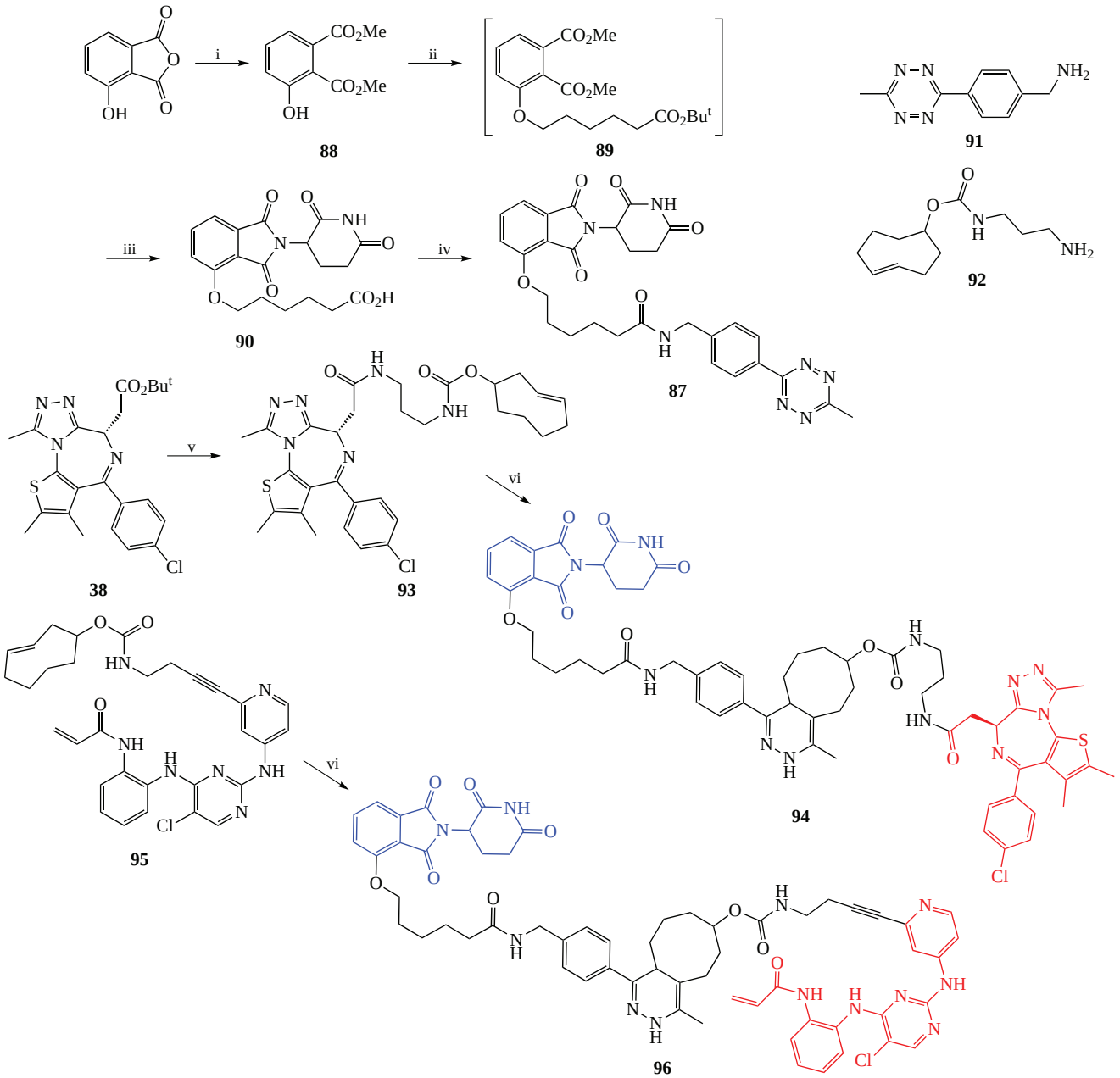


Scheme 7 *Reagents and conditions*: i, 5-aminopent-1-yne, EDCI, HOBt, DIPEA, DCM, room temperature (75%); ii, **49**, $PdCl_2(PPh_3)_2$, CuI, THF, TEA (60%); iii, a) $B(OH)_3$ (cat.), xylenes, reflux; b) HCl (conc.) (81%); iv, a) triphosgene, DIPEA, DCM, *N*-Boc-piperazine (74%); b) TFA, DCM (90%); v, a) propargyl alcohol, $PdCl_2(PPh_3)_2$, CuI, THF, TEA (81%); b) SO_2Cl_2, TEA, DCM (64%); vi, **85**, K_2CO_3, acetone, reflux (68%).

After careful analysis of the structure–activity relationships (SAR) information available for analogs of compound **80**, the carboxylic acid functionality was deemed the most suitable point for the attachment of the linker and, ultimately, the CRBN ligand. Indeed, amidation of **80** with 5-aminopent-1-yne gave compound **82** which was introduced directly in Sonogashira coupling with 4-iodoisondolinone **49** to afford PROTAC **78**. Transformation of Nutlin derivative **81** into MDM2 PROTAC-type degrader **79** was based on the two assumptions (both driven by the available SAR information): (i) that piperazine urea moiety is the most suitable attachment point for the CRBN ligand's linker; (ii) that angular methyl groups in **81** can be omitted as long as the *cis* relationship between the 4-chlorophenyl groups is preserved. Both assumptions proved correct as compound **79** showed a potent MDM2 degradation.[62] The synthesis of **79** commenced with *meso*-diamine **83** which gave imidazoline **84** on boric acid-catalyzed cyclocondensation with suitable carboxylic acid.[65] Treatment of **84** with triphosgene followed by *N*-Boc-piperazine installed the urea linkage and, after the Boc group removal, MDM2 ligand **85** was obtained. The lenalidomide moiety was prepared for linking to MDM2 ligand *via* the Sonogashira coupling of 4-iodoisondolinone **49** with propargyl alcohol and conversion of the alcohol moiety into propargyl chloride (**86**). Alkylation of piperazine **85** with propargyl chloride **86** in the presence of DIPEA as the base afforded the target PROTAC **79** (Scheme 7).

3. Special cases of Cereblon-targeting PROTACs

Besides the significant achievements in the construction of PROTAC degraders of therapeutically relevant proteins some of which are reviewed in the previous section of this Focused Review, the PROTAC technology has been extended to several notable cases which are of interest from the chemical point of view and can potentially give valuable clues to novel applications of these pilot examples in the development of future target protein degrader.



Scheme 8 *Reagents and conditions*: i, a) MeOH, reflux; b) MeI, $NaHCO_3$, DMF, 55 °C (94%); ii, *tert*-butyl 6-hydroxyhexanoate, PPh_3, DIAD, THF, room temperature; iii, a) NaOH, THF, MeOH, room temperature; b) 3-amino piperidine-2,6-dione hydrochloride, pyridine, 110 °C; c) TFA, room temperature (10% over 4 steps); iv, **91**, DIPEA, HATU, DMF, room temperature (57%); v, a) TFA, DCM, room temperature (quant.); b) **92**, DIPEA, HATU, DMF, room temperature (70%); vi, **87**, DMSO, room temperature, 15 min (quant.).

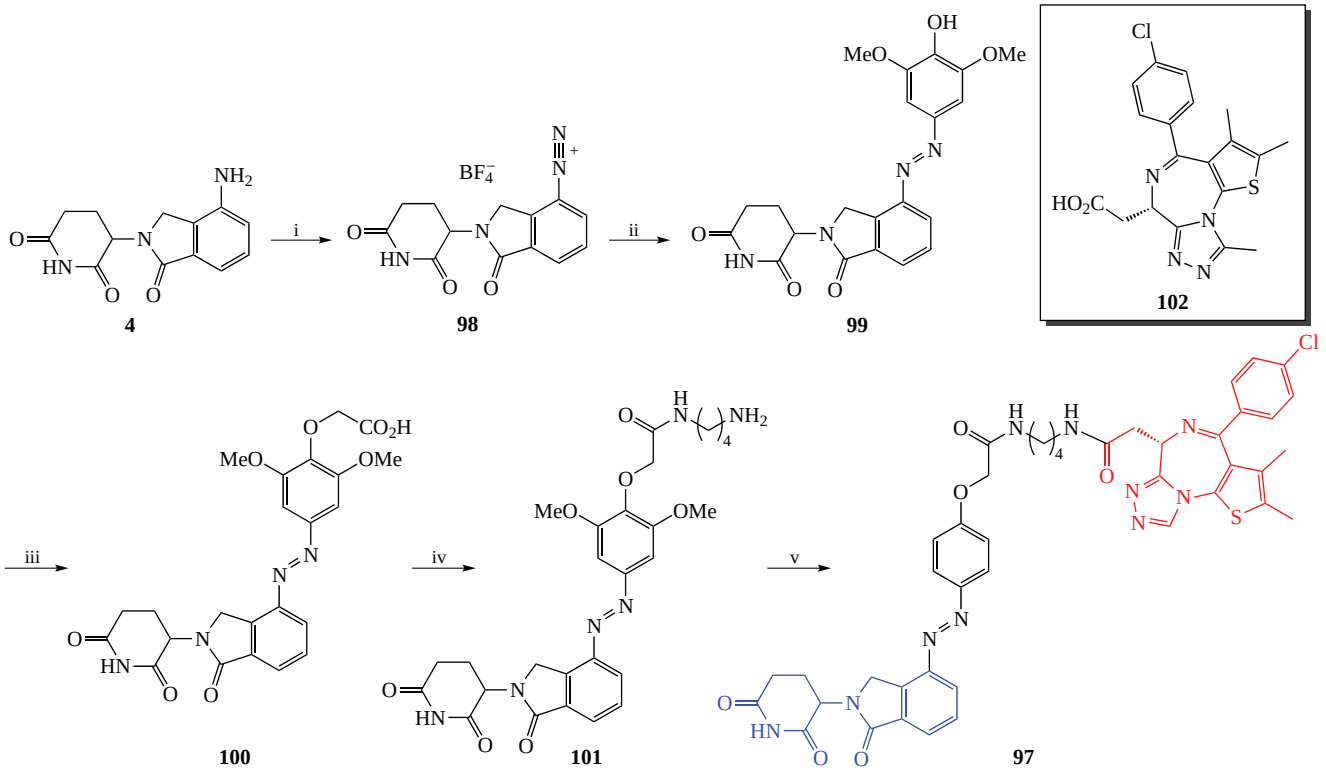
3.1. CLIPTACs

The principal drawback of PROTAC molecules which hampers their development as pharmaceutical agents is their molecular characteristics, mostly the overly molecular weight and total polar surface area, which fall outside the range of the Lipinski rules for druglikeness[66] and the generally acceptable range which ensures good cell membrane permeability.[67] Tom Heightman and co-workers from Astex Pharmaceuticals proposed to design suitable tagged building blocks for a PROTAC molecule that would be sufficiently small to permeate the cell membrane and which would rapidly form a full-size PROTAC molecule once within the cell.[68] As the chemical event for joining the two building blocks together they envisioned an inverse electron demand Diels–Alder (IEDDA) cycloaddition between a *trans*-cyclooctene (TCO) and a tetrazine (Tz) fragments, a versatile reaction employed in biorthogonal conjugation.[69] To realize this strategy, Tz-tagged thalidomide **87** was synthesized from 4-hydroxyphthalic anhydride. The latter was converted to phthalic dimethyl ester **88** and the phenolic hydroxy group was alkylated with *tert*-butyl 6-hydroxyhexanoate *via* the Mitsunobu reaction. Without isolation, resulting compound **89** was hydrolyzed and converted to phthalimide **90** *via* the reaction with 3-amino piperidine-2,6-dione. Amidation with 'Tz-amine' ([4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]methanamine, **91**) promoted by HATU gave Tz-thalidomide **87**. BRD4 ligand JQ1 (**38**) was hydrolyzed at its ester group and the resulting acid was amidated with 'TCO-amine' [(*E*)-cyclooct-4-en-1-yl (3-amino-propyl)carbamate, **92**] to give JQ1-TCO (**93**). Incubation of HeLa cells with **87** and **93** led to a notable lowering of intracellular levels of BRD4. At the same time, treatment of HeLa cell with full-size PROTAC **94** synthesized by IEDDA cycloaddition led to no BRD4 degradation, thus demonstrating that **94** itself is not cell membrane permeable and should be synthesized within the cell. Similar concept was successfully realized for TCO-tagged covalent inhibitor of kinases ERK **95**.[70] Treatment of A375 cells with **87** and **95** led to degradation of ERK1 and, to a lesser extent, ERK2 kinase while full-size PROTAC **96** synthesized *via* the click reaction did not degrade neither of these proteins (Scheme 8).

3.2. PHOTACs and opto-PROTACs

The need to control the ability of PROTAC molecules to induce protein degradation arises from the so-called off-tissue problems associated with the PROTAC use, *i.e.* PROTACs affecting untargeted tissue, similarly to the use of protein inhibitors. This led to an idea of creating controllable PROTACs whose activity could be switched on and off by external, tissue penetrating stimuli such as UV light.[71] This idea was realized by using azobenzene linkers between the POI ligand and the E3 ligase (CRBN) ligand which could be switched between active (*trans*) and inactive (*cis*) isomer simply by applying UV light of appropriate wavelength. The rationale behind such an approach is in the critical distance between the two protein-recruiting moieties necessary for PROTAC's functioning. If this difference is too short (as in the *cis*-isomer of the photoswitchable azobenzene compound), the ternary complex 'POI–PROTAC–E3 ligase' cannot form and the POI degradation does not occur. Turning such a PROTAC on with UV light would require light-induced isomerization of the N=N double bond to the *trans*-configuration. Such new-generation azobenzene-based PROTACs controllable by light were dubbed PHOTACs (PHOtochemically TArgeting Chimeras).[72]

In 2020, Dirk Trauner and co-workers reported novel azobenzene-based PROTAC molecules that show little or no proteolytic activity in the dark but can be activated with blue-violet light (380 to 440 nm).[73] Particularly notable are PHOTAC degraders of BET protein BRD4 (based on ligand JQ1, **38**) that afford optical control of protein levels and cell proliferation, survival, and viability. A highly stable, inactive *cis*-PHOTAC is isomerized by means of a light stimulus to a catalytically active *trans*-PHOTAC that induces polyubiquitination of BRD4 by complexation with an E3 ligase. The polyubiquitinated BRD4 then proceeds to degradation by the proteasome while the *trans*-PHOTAC remains



Scheme 9 *Reagents and conditions*: i, $NaNO_2$, HCl, HBF_4, MeOH (aq.); ii, 2,6-dimethoxyphenol, $NaHCO_3$, Na_2CO_3, 0 °C (69%); iii, a) *tert*-butyl bromoacetate, K_2CO_3, DMF, room temperature; b) TFA, DCM, room temperature (78%); iv, a) *N*-Boc-1,4-diaminopentane, HATU, DIPEA, DMF, room temperature; b) TFA, DCM, room temperature (75%); v, **102**, HATU, DIPEA, DMF, room temperature (91%).

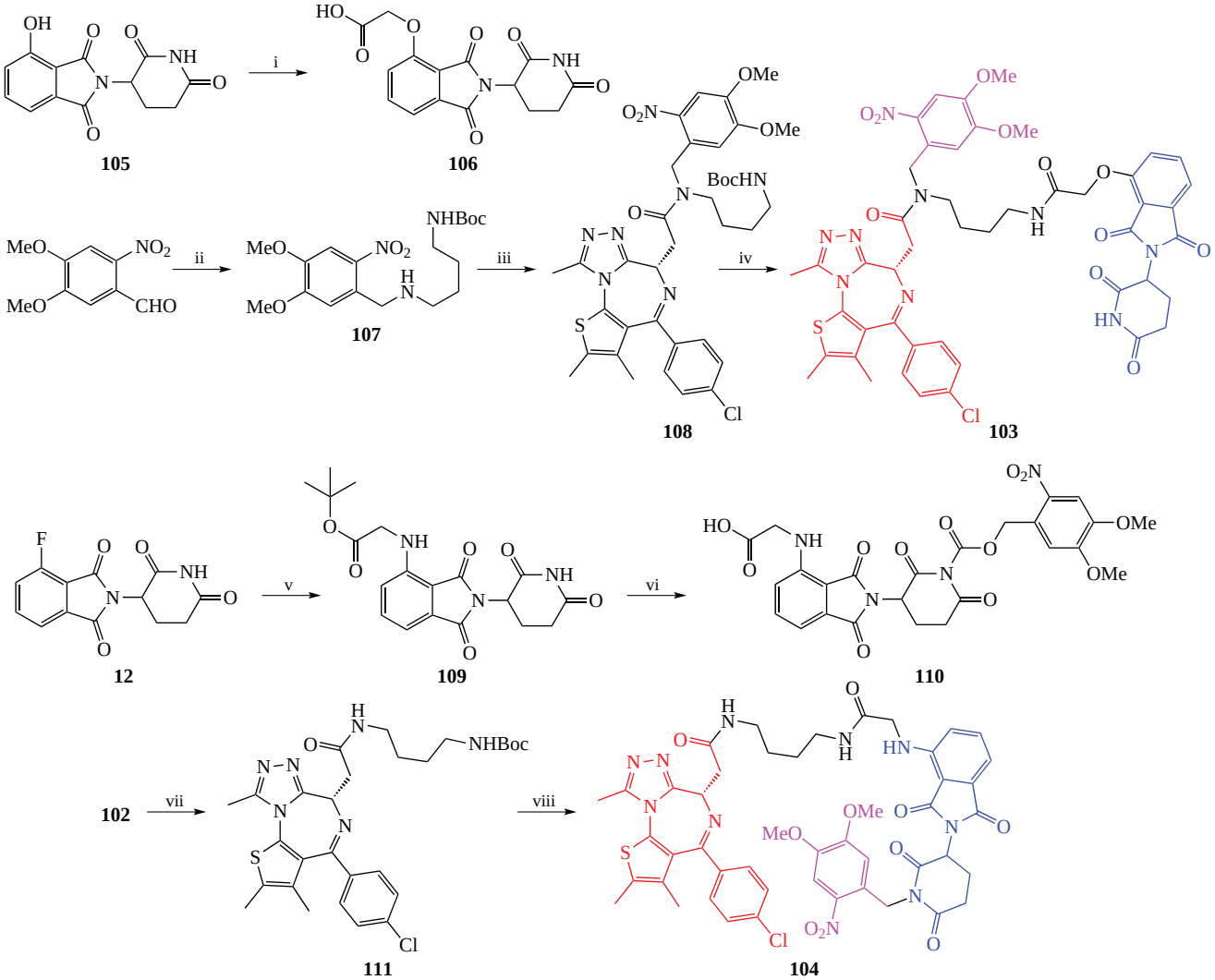
active until isomerization by a second light stimulus of a different wavelength regenerates the inactive *cis*-PHOTAC.

The synthesis of a representative PHOTAC **97** commenced with the diazotization of lenalidomide (**4**) and coupling of the resulting diazonium ion **98** to 2,6-dimethoxyphenol, which yielded azobenzene **99**. Alkylation with *tert*-butyl bromoacetate and subsequent deprotection then afforded the key intermediate **100**. Amide coupling of this carboxylic acid with *N*-Boc-butane-1,4-diamine and deprotection then gave **101**, which underwent peptide coupling with the free acid of JQ1 (**102**) to afford PHOTAC **97** (Scheme 9).

Another approach to controlling the ability of PROTACs to degrade proteins was in blocking the binding of PROTACs to E3 uqibuitin ligase by putting a photocleavable group (such as 4,5-dimethoxy-2-nitrobenzyl, DMNB) on the sites of the PROTAC molecule that are crucial for such binding. DMNB group can be efficiently cleaved upon irradiation at 365 nm and has been used in numerous cellular studies.[74] Such PROTACs capped with DMNB (and other photocleavable groups[75]) were dubbed 'opto-PROTACs' or 'photocaged PROTACS'.[76] In 2019, Pan and co-workers reported two photocaged versions of BET protein degrader dBET1 which contained a DMNB group placed either at the amide nitrogen atom in the linker (**103**) or at the glutarimide nitrogen atom in the pomalidomide portion of the molecule (**104**).[77] 4-Hydroxythalidomide (**105**) was alkylated with *tert*-butyl bromoacetate and the ester group was hydrolyzed to give carboxylic acid **106**. 4,5-Dimethoxy-2-nitrobenzaldehyde was reductively aminated with mono-Boc-protected butane-1,4-diamine and the resulting amine (**107**) was acylated with JQ1 carboxylic acid (**102**) to afford compound **108**. Removal of the Boc group and acylation with carboxylic acid 106 gave target photo-caged PROTAC **103** (the photolabile 4,5-dimethoxy-2-nitrobenzyl group is highlighted pink). 4-Fluorothalidomide (**12**) was involved in aromatic nucleophilic substitution reaction with glycine *tert*-butyl ester to yield compound **109**. Installment of the photolabile 4,5-dimethoxy-2-nitrobenzyl group was achieved *via* a urethane linkage and the *tert*-butyl ester group was hydrolyzed to give carboxylic acid **110**. The latter and JQ1 carboxylic acid (**102**) were joined *via* a butan-1,4-diamine linkage by sequential amidation of **102** with mono-Boc-protected butane-1,4-diamine, Boc group removal and acylation with carboxylic acid **110**. Thus, target photo-caged PROTAC **104** was obtained (Scheme 10).

3.3. Other special cases

In the recent few years, novel approaches to designing controllable-activity PROTACs have emerged. These are all aimed at minimizing the tissue-nonspecific side effects of



Scheme 10 *Reagents and conditions*: i, a) $Bu^tO_2CCH_2Br$, K_2CO_3, acetone, 50 °C; b) TFA, DCM, room temperature (67%); ii, *tert*-butyl (4-aminobutyl)-carbamate, HOAc, $NaBH(OAc)_3$, DCM, room temperature (32%); iii, **102**, HATU, DIPEA, DMF, room temperature (52%); iv, a) TFA, DCM, room temperature; b) **106**, HATU, DIPEA, DMF, room temperature (36%); v, NH_2-Gly-Bu^t, DIPEA, DMSO, 90 °C (72%); vi, a) NaHMDS, DCM, from –80 °C to –30 °C; b) 4,5-dimethoxy-2-nitrobenzyl carbonochloridate, from –30 °C to room temperature (27%); c) TFA, CH_2Cl_2, room temperature; vii, *tert*-butyl (4-aminobutyl)carbamate, HATU, DIPEA, DMF, room temperature (30%); viii, a) TFA, CH_2Cl_2, room temperature; b) **110**, HATU, DIPEA, DMF, room temperature (36%).

PROTACs whose protein degradation activity could be switched on only at the intended site of action.

Noteworthy examples of such switchable PROTACs include semiconducting polymer nano-PROTACs (SPNpro) for activatable photo-immunometabolic cancer therapy invented by the Pu team at Nanyang Technological University[78] and folate-caged PROTACs (folate-PROTACs) for targeted degradation of proteins of interest (POIs) in cancer cells *versus* noncancerous normal cells introduced by the Jin and Wei team at Mount Sinai and Harvard Medical School.[79]

Also quite remarkable is the targeted protein degradation platform invented by the same team which is aimed at initiating proteasomal degradation of transcription factors. In these constructs termed 'TF-PROTACs', an E3 ubiquitin ligase ligand is conjugated to an oligonucleotide whose sequence is specific to a particular transcription factor. Such a TF-PROTAC brings together the ubiquitin ligase and the oligonucleotide-bound transcription factor. This leads to the ubiquitination and ultimate proteasomal degradation of the latter.[80]

Similarly intriguing is the recently introduced novel technology for tumor-targeted protein degradation termed chaperone-mediated protein degradation (CHAMP) which is somewhat distinct from PROTACs.[81] These chimeric constructs chemically induce the proximity of the BET protein BRD4 and heat shock protein 90 (HSP90) chaperone in a ternary complex, which leads to the ubiquitination and proteasomal degradation of BRD4.

All these innovative approaches to targeted protein degradation were reported in 2021 and have not yet involved the recruitment of CRBN E3 ubiquitin ligase though they likely will in the future.

4. Conclusions and future prospects

Put forward 20 years ago, the idea of utilizing cell's own protein degradation machinery, the ubiquitin–proteasome system, for lowering intracellular levels of aberrantly hyperactive proteins fruited to become a well-understood technology. While still awaiting ultimate validation through the approval of PROTAC drug candidates currently in clinical trials, the field has matured to the stage where virtually any protein could be eliminated through judicious design of small-molecule heterobifunctional constructs. While the choice of ligands for the degraded 'protein of interest' and the E3 ubiquitin ligase is rather straightforward at this point, the less understood aspect of PROTAC design is the choice of linker to connect the two moieties. Essentially, any new combination of a POI and an E3 ligase ligand currently requires that an optimum linker is found through trial-and-error synthesis and elaborate, low-throughput screening. Eliminating this shortcoming is likely to become the focal point of future research into PROTAC technology and so is the search for new ligands for E3 ubiquitin ligases beyond the currently used handful of enzymes (Cereblon, VHL and other few). The current review clearly demonstrates that on synthetic level, any developments in this field are solidly supported by the recently developed synthetic routes to assemble PROTACs and turn them, if needed, into controllable protein degradation tools such as PHOTACs or photocaged PROTACs. Likewise, the novel protein degradation approaches reported last year (SPNpro, folate-PROTACs, TF-PROTACs and CHAMP) are likely to evolve into reliable technology platforms.

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