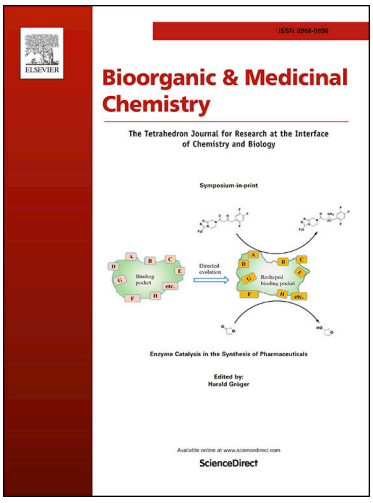
Journal Pre-proofs



Discovery of a new class of PROTAC BRD4 degraders based on a dihydroquinazolinone derivative and lenalidomide/pomalidomide

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PII: S0968-0896(19)31371-9
DOI: https://doi.org/10.1016/j.bmc.2019.115228
Reference: BMC 115228

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 14 August 2019
Revised Date: 4 November 2019
Accepted Date: 18 November 2019

Please cite this article as: F. Zhang, Z. Wu, P. Chen, J. Zhang, T. Wang, J. Zhou, H. Zhang, Discovery of a new class of PROTAC BRD4 degraders based on a dihydroquinazolinone derivative and lenalidomide/pomalidomide, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.115228

Discovery of a new class of PROTAC BRD4 degraders based on a dihydroquinazolinone derivative and lenalidomide/pomalidomide

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Abstract

BRD4 has emerged as an attractive target for anticancer therapy. However, BRD4 inhibitors treatment leads to BRD4 protein accumulation, together with the reversible nature of inhibitors binding to BRD4, which may limit the efficacy of BRD4 inhibitors. To address these problems, a protein degradation strategy based on the proteolysis targeting chimera (PROTAC) technology has been developed to target BRD4 recently. Herein, we present our design, synthesis and biological evaluation of a new class of PROTAC BRD4 degraders, which were based on a potent dihydroquinazolinone-based BRD4 inhibitor compound **6** and lenalidomide/pomalidomide as ligand for E3 ligase cereblon. Gratifyingly, several compounds showed excellent inhibitory activity against BRD4, and high anti-proliferative potency against human monocyte lymphoma cell line THP-1. Especially, compound **21** (BRD4 BD1, IC_{50} = 41.8 nM) achieved a submicromolar IC_{50} value of 0.81 μM in inhibiting the growth of THP-1 cell line, and was 4 times more potent than compound **6**. Moreover, the mechanism study established that **21** could effectively induce the degradation of BRD4 protein and suppression of c-Myc. All of these results suggested that **21** was an efficacious BRD4 degrader for further investigation.

Keywords: BRD4 degraders; PROTAC; protein degradation; dihydroquinazolinone

1. Introduction

As the epigenetic "readers", bromodomain and extra terminal (BET) proteins consist of BRD2, BRD3, BRD4 and testicle-specific BRDT, each of them contains two bromodomains (BD1 and BD2)[1]. Through recognition of specific ε-nacetyl modified lysine residues on the histone tails by two BDs, BET proteins play an important role in regulation of gene transcription[2-4]. Among BET proteins, BRD4 is the most widely investigated one, which has been emerged as an attractive therapeutic target for cancer therapy[5, 6]. By mediating the expression of oncogenes such as *c-myc*, *bcl-xL* and *bcl-6*[7, 8], BRD4 has been involved in a variety of cancers including midline carcinoma[9, 10], acute myeloid leukemia[11, 12], prostate cancer[13], multiple myeloma[14, 15] and breast cancer[16, 17]. As a result, several BRD4 inhibitors such as **JQ1**(**1**), **OTX015**(**2**) (**Fig. 1**) have been reported, and showed promising therapeutic potential in preclinical models of multiple cancers[18, 19]. However, limitations of these existed BRD4 inhibitors were also displayed. It was found that reversible binding and incomplete inhibition of BRD4 may reduce the activity of some BRD4 inhibitors in cancer cells[20, 21]. For example, high concentrations of **JQ1** and **OTX015** were required to suppress the expression of c-Myc in Burkitt's lymphoma cells[21]. Besides, recent researches also revealed that

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BRD4 inhibitors treatment results in obvious BRD4 protein accumulation, which may account for their limited suppression of c-Myc expression and modest antiproliferative activity[21, 22]. Thus, more studies are needed to address these problems.

Lately, a novel protein degradation strategy based on the proteolysis targeting chimera (PROTAC) technology has been developed to target BRD4[21, 23]. In this strategy, scientists designed a heterobifunctional molecule that contains a BRD4 inhibitor portion, a small-molecule ligand of E3 ubiquitin ligase complex, and a linker to connect these two ligands. Up to now, several PROTAC BRD4 degraders have been documented, including **ARV-825** (**3**)[21], **dBET1** (**4**)[23], and **ARV-771**[24] (**Fig. 1**). Significantly, these BRD4 degraders could effectively induce the degradation of BRD4, leading to overt and persistent downstream c-Myc suppression, more importantly, BRD4 degraders were more potent in proliferation inhibition and apoptosis induction of cancer cells than their corresponding BRD4 inhibitors[20, 21, 23-25]. For instance, **dBET1** was found to be more efficient than **JQ-1** in inhibition of tumor growth in a xenograft model of MV4;11 cell line[23]. Above all, these studies advocated that PROTAC BRD4 degraders may have a promising future for anticancer therapy. Therefore, in this paper, we report the discovery of a new class of PROTAC BRD4 degraders.

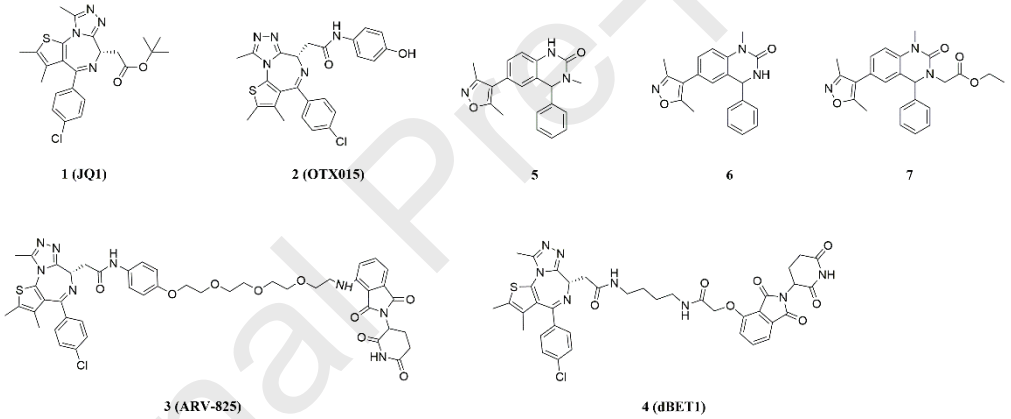


Fig. 1. Structures of representative BRD4 inhibitors and BRD4 degraders.

Compound **5**, a dihydroquinazolinone-based selective BRD4 inhibitor with the IC_{50} value of 44 nM for BRD4 BD1[26], was selected as the lead compound for BRD4 inhibitor portion of our BRD4 degraders (**Fig. 1**). Through modification of compound **5**, we designed two closely related and potent BRD4 inhibitors compound **6** and **7** (**Fig. 1**). Molecular docking study of compound **7** with BRD4 BD1 cocrystal structure (PDB code 3MXF) illustrated that the ethyl acetate side chain attached to the dihydroquinazolinone scaffold in **7** was exposed to solvent, making it an appropriate site to connect E3 ligase cereblon ligand lenalidomide/pomalidomide (**Fig. 2**). In other words, the ethyl acetate side chain in **7** may act as a linker to tether compound **6** and lenalidomide/pomalidomide for the design of potential PROTAC BRD4 degraders. According to these, we designed and synthesized a novel class of small-molecule BRD4 degraders using compound **6** for the BRD4 inhibitor portion, lenalidomide or pomalidomide as ligand for E3 ligase cereblon (**Fig. 3**). And several potent BRD4 degraders were acquired by optimizing the linker. Among these, compound **21** was the most potent one to inhibit the growth of human monocyte

lymphoma cell line THP-1, with the IC_{50} value of 0.81 μM. What's more, **21** could effectively induce the degradation of BRD4 and suppression of c-Myc expression. These results indicated that compound **21** was an efficacious BRD4 degrader for further investigation.

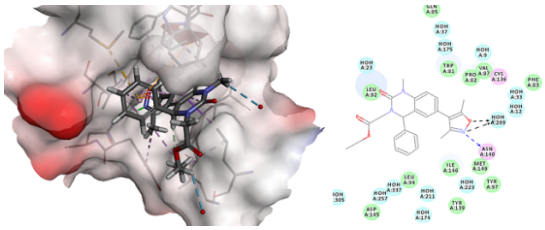


Fig. 2. Modeled structure of BRD4 BD1 complexed with **7** (PDB code 3MXF). The ethyl acetate side chain of **7** was exposed to solvent.

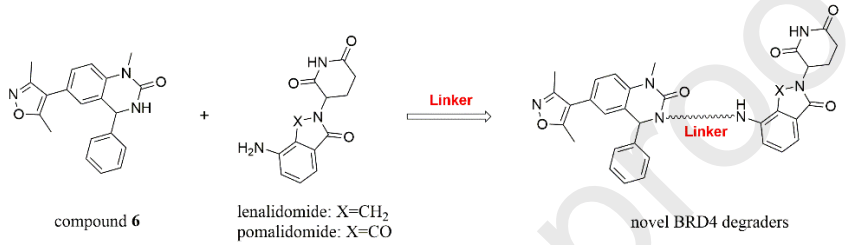
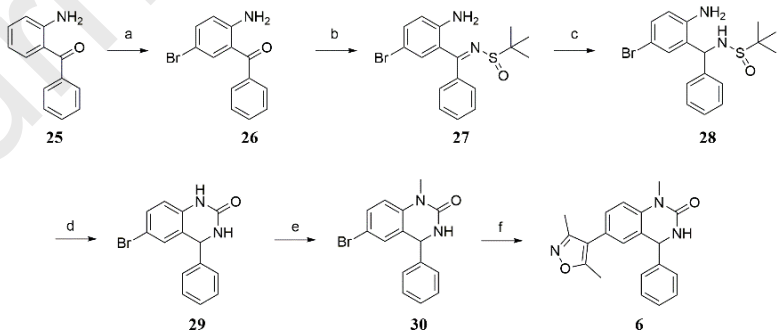


Fig. 3. The design of novel BRD4 degraders.

2. Results and discussion

2.1. Chemistry

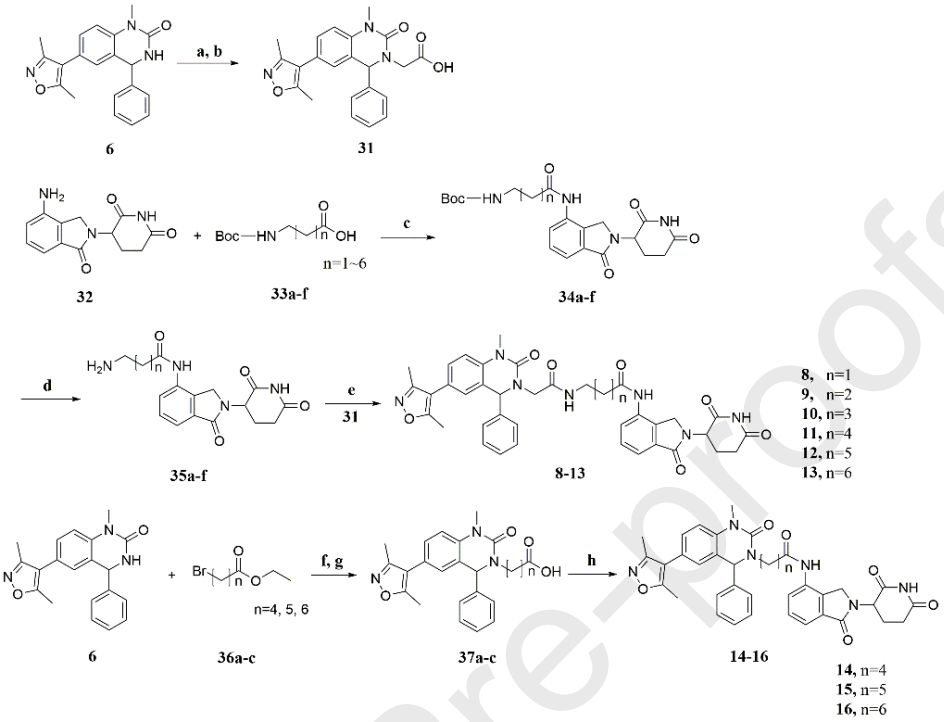
The synthesis of compound **6** is shown in **Scheme 1**. Briefly, bromination of 2-aminodibenzophenone (**25**) with N-bromosuccinimide (NBS) gave **26**, which was heated with 2-methylpropane-2-sulfinamide and tetraethyl titanate in tetrahydrofuran to generate **27**. Reduction of the alkene group of **27** by the sodium borohydride resulted in **28**. Compound **29** was obtained by treatment of **28** with triphosgene. Then methylation of **29** with dimethyl sulfate in MeCN afforded **30**, which was treated with the 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2yl)isoxazole produced compound **6** by the Suzuki coupling reaction.



Scheme 1. General synthesis of compound **6**. Reagents and conditions: (a) NBS, DMF, 0 ℃; (b) 2-methylpropane-2-sulfinamide, tetraethyl titanate, THF, 70 ℃; (c) $NaBH_4$, THF, H_2O, rt; (d) triphosgene, THF, rt; (e) dimethyl sulfate, Cs_2CO_3, MeCN, rt; (f) 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2yl)isoxazole, $Pd(PPh_3)_4$, Na_2CO_3, N_2, PhMe/EtOH/H_2O, 80 ℃.

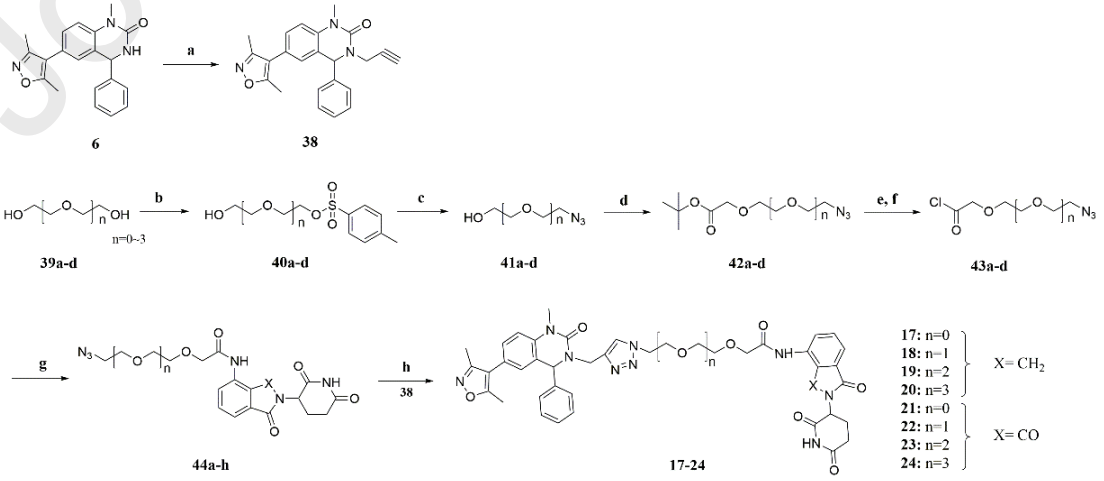
Target compounds **8-16** were synthesized according to the route shown in **Scheme 2.** Starting from compound **6**, the key intermediate **31** was obtained by two steps: alkylation and hydrolysis. Then lenalidomide (**32**) was condensed with **33a-f** respectively generated **34a-f**. Boc-deprotection

of the obtained **34a-f** afforded intermediates **35a-f**, which were subsequently condensed with **31** to generate target compounds **8-13**. Intermediates **37a-c** were synthesized from **6** and **36a-c** by the similar procedure that used for the synthesis of **31**. After that, **37a-c** were further converted to the target compounds **14-16** by condensation with lenalidomide.



Scheme 2. General synthesis of compounds **8-16**. Reagents and conditions: (a) ethyl bromoacetate, NaH, DMF, rt; (b) LiOH·H_2O, CH_3OH/H_2O, rt; (c) pyridine, $POCl_3$, acetonitrile, rt; (d) CF_3COOH, CH_2Cl_2, rt; (e) HATU, DIPEA, DMF, rt; (f) NaH, DMF, rt; (g) LiOH·H_2O, CH_3OH/H_2O, rt; (h) pyridine, $POCl_3$, acetonitrile, rt.

The synthetic route to compounds **17-24** is shown in **Scheme 3**. Alkylation of **6** with 3-bromopropyne gave the intermediate **38**. Afterwards, treatment of **39a-d** with tosyl chloride produced **40a-d**, which were reacted with the sodium azide to yield **41a-d**. The obtained **41a-d** were subsequently treated with tert-butyl bromoacetate respectively to afford **42a-d**, which were further converted to **43a-d** by hydrolysis and acylation reactions. Then condensation of **43a-d** with lenalidomide/pomalidomide resulted in key intermediates **44a-h**, which were transformed to the target compounds **17-24** by cyclization with **38** in the presence of sodium ascorbate and copper sulfate pentahydrate.



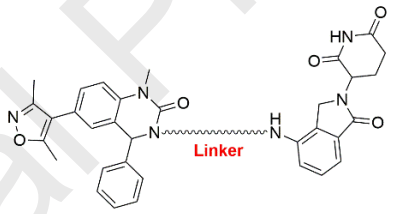
Scheme 3. General synthesis of compounds **17-24**. Reagents and conditions: (a) 3-bromopropyne, NaH, DMF, rt; (b) tosyl chloride, pyridine, rt; (c) NaN_3, acetone/H_2O, 60 ℃; (d) tert-butyl bromoacetate, NaH, THF, rt; (e) trifluoroacetic acid, CH_2Cl_2, rt; (f) sulfuryl dichloride, CH_2Cl_2, rt; (g) lenalidomide/pomalidomide, NMP, rt; (h) sodium ascorbate, $CuSO_4$•$5H_2O$, CH_2Cl_2/CH_3OH/H_2O, rt.

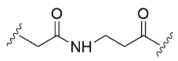
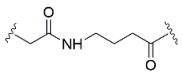
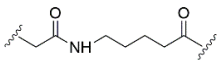
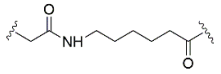
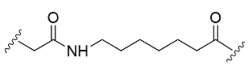
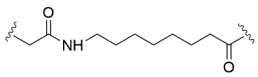
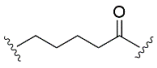
2.2. Biological evaluation

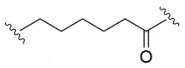
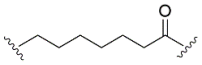
2.2.1. *In vitro* BRD4 inhibitory activity and SAR study

All synthesized compounds were evaluated for their ability to inhibit BRD4 BD1, and the results were listed in **Tables 1** and **2**. The binding affinity was investigated by the AlphaScreen assay to prove the rational structure-activity relationship. Compound **6** and **JQ1** were used for positive controls, and both of them exhibited high inhibitory activity against BRD4 BD1, with IC_{50} values of 46.4 nM and 27.9 nM respectively. First of all, we synthesized compounds **8-13** by using compound **6** as BRD4 inhibitor portion, lenalidomide as the cereblon ligand and similar linkers of **dBET1**. The linker of **8-13** was gradually extended by one methylene group progressively. As shown in **Table 1**, compounds **8-13** had high binding affinity for BRD4 BD1, although their IC_{50} values for BRD4 BD1 were all higher than compound **6** and **JQ1**. Interestingly, with the lengthening of the linker, the BRD4 inhibitory activity of **8-13** was gradually increased. Particularly, compound **13** possessed an IC_{50} value of 68.3 nM in inhibition of BRD4 BD1 (**Table 1**). To investigate the function of the amide group in the linker, we synthesized compounds **14-16**. As a consequence, IC_{50} values of **14-16** for BRD4 BD1 were only changed slightly compared with those of **8** and **9**, which denoted that the amide group in the linker may just make a little influence on the BRD4 binding potency of these BRD4 degraders.

Table 1. Structures of compounds **8-16**, and their inhibitory activity for BRD4 BD1.



Compound	Linker	BRD4 (BD1) Inh% (at 500 nM) [a]	BRD4 (BD1) IC_{50} (nM) [b]
8		94	132.2 ± 13.8
9		89	138.4 ± 15.4
10		91	133.6 ± 14.2
11		98	99.1 ± 8.7
12		100	80.6 ± 6.5
13		96	68.3 ± 7.1
14		90	143.7 ± 13.6

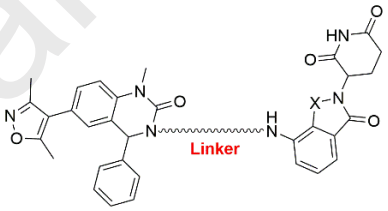
15		93	135.1 ± 14.0
16		96	118.2 ± 12.6
6		100	46.4 ± 6.7
JQ1		100	27.9 ± 4.5

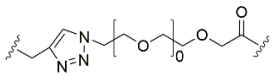
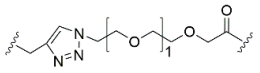
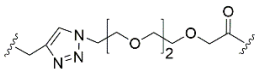
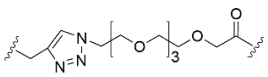
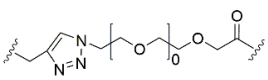
[a] Inhibition rate mean values at a screening concentration of 500 nM were obtained from three independent experiments.

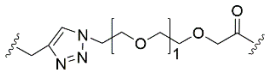
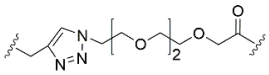
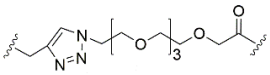
[b] IC_{50} values are the mean of at least three independent assays, presented as mean ± SD.

To further optimize the linker, we considered to enhance the polarity of the linker to improve the binding affinity with BRD4, by forming additional interactions between the linker and BRD4 protein. So, based upon compound **10**, one triazole and one oxygen atom were introduced to replace the amide group and carbon atom respectively in the linker to generate compound **17**. As expected, compound **17** achieved an IC_{50} value of 14.2 nM against BRD4 BD1 (**Table 2**), which was 9.4 times more potent than **10**, and 3 times more potent than **6**. Then, compounds **18-20** were obtained through farther extension of the linker of **10**. They all possessed strong potency to inhibit BRD4 BD1, which was comparable to compound **17** (**Table 2**). Additionally, to explore the effect of different cereblon ligands on BRD4 binding affinity of these degraders, we replaced the lenalidomide of **17-20** with pomalidomide to afford compounds **21-24**. Unfortunately, the binding affinity of **21-24** was lower than **17-20** correspondingly, even if compound **21** was more potent than compound **6** with an IC_{50} value of 41.8 nM against BRD4 BD1. These data indicated that comparing with lenalidomide, pomalidomide may reduce the BRD4 binding affinity of these degraders. Furthermore, consistent with compounds **17-20**, the BRD4 binding affinity of **21-24** was gradually decreased with the extension of the linker. These proposed that compound **17** or **21** may have an optimal linker length.

Table 2. Structures of compounds **17-24**, and their inhibitory activity for BRD4 BD1.



Compound	Linker	X	BRD4 (BD1) Inh% (at 500 nM) [a]	BRD4 (BD1) IC_{50} (nM) [b]
17		CH_2	99	14.2 ± 1.6
18		CH_2	100	15.4 ± 2.3
19		CH_2	100	22.1 ± 2.9
20		CH_2	100	22.6 ± 3.7
21		CO	99	41.8 ± 7.8

22		CO	93	67.7 ± 9.0
23		CO	86	68.5 ± 8.5
24		CO	60	173.4 ± 23.2
6			100	46.4 ± 6.7
JQ1			100	27.9 ± 4.5

[a] Inhibition rate mean values at a screening concentration of 500 nM were obtained from three independent experiments.

[b] IC_{50} values are the mean of at least three independent assays, presented as mean ± SD.

2.2.2. Anti-proliferative effects against cancer cell lines

In this section, firstly, we conducted a primary screening to assess the *in vitro* antiproliferative effect of the potent compounds **17-23** against cancer cell lines, including HL-60, Raji and THP-1 at the concentration of 2.5 μM. As seen from **Fig. 4**, most of the selected compounds showed high inhibitory activity against THP-1 cell line, and moderate activity against Raji and HL-60 cell lines. It's worth noting that compounds **17-23** were all more effective than compound **6** and **JQ1** to inhibit the above three cancer cell lines. In addition, compounds **21-23** possessed stronger anti-proliferative effect than the simple combination of compound **6** and pomalidomide. Thus, we deeply detected the IC_{50} values of compound **17** and **21-23** in inhibition of THP-1 cell growth. As shown in **Table 3**, the most potent compound **21** achieved an IC_{50} value of 0.81 μM against THP-1 cell line, which was 4 times more potent than compound **6** (IC_{50} = 3.26 μM). Intriguingly, although with the highest binding affinity of BRD4 BD1, compound **17** was less potent than **21** in inhibiting the growth of THP-1 cell line.

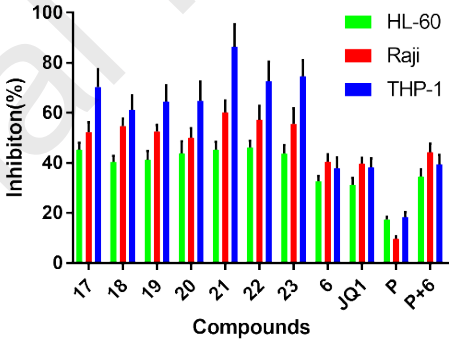


Fig. 4. Preliminary screening of *in vitro* antiproliferative activity of selected compounds against HL-60, Raji, and THP-1 cancer cell lines at the concentration of 2.5 μM. Inhibition values were obtained from three independent experiments. Compound **6**, **JQ1** and pomalidomide (P indicates pomalidomide) were used as the positive controls.

Table 3. The IC_{50} values of selected compounds against THP-1 cancer cell line

Compound	IC_{50} (THP-1 cell lines) [a] (μM)
17	1.83 ± 0.016
21	0.81 ± 0.0073
22	1.66 ± 0.012
23	1.75 ± 0.0091
6	3.26 ± 0.27

[a] IC_{50} is the average value of three independent assays, presented as mean ± SD.

2.2.3. Western blot assay

In order to examine the action mechanism of compounds **8-24**, the best compound **21** was selected to evaluate the ability to degrade BRD4 protein and suppress the expression of c-Myc by Western blot assay in THP-1 cell line. As displayed in **Fig. 5**, at the concentration of 1 μM, compound **21** effectively induced the degradation of BRD4 protein and suppression of c-Myc, while compound **6** and pomalidomide couldn't degrade BRD4. These results distinctly proposed that **21** was a real and highly potent BRD4 degrader. What's more, compound **6** could lead to BRD4 protein accumulation, which was consistent with the previous studies[21, 22].

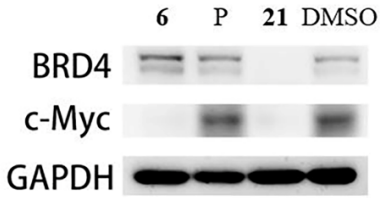


Fig. 5. Western blotting analysis of BRD4, c-Myc proteins in THP-1 cells treated with compound **21** at 1 μM. Compound **6** (3 μM) and pomalidomide (1μM, P indicates pomalidomide) were used as positive controls, THP-1 cells were treated for 3 h with individual compounds, and proteins were probed by specific antibodies. GAPDH was used as the loading control.

3. Conclusion

In summary, we designed and synthesized a new class of PROTAC BRD4 degraders which were based on a novel dihydroquinazolinone-based BRD4 inhibitor compound **6** and cereblon ligand lenalidomide/pomalidomide. All the obtained target compounds were evaluated for their binding affinity with BRD4, and the results showed that most of these compounds displayed excellent BRD4 inhibitory activity *in vitro*. In particular, compound **17** had an IC_{50} value of 14.2 nM against BRD4 BD1. Further anti-proliferative evaluation showed that most of the selected compounds possessed high inhibitory activity against human monocyte lymphoma cell line THP-1. And unlike the compound **17**, which owned the highest inhibitory activity of BRD4, compound **21** was the most potent one in inhibiting the growth of THP-1 cell line, with a submicromolar IC_{50} value of 0.81 μM. Moreover, mechanism study established that **21** could effectively induce the degradation of BRD4 protein and suppression of c-Myc expression at the concentration of 1 μM with a 3 h treatment. Overall, these results demonstrated that compound **21** was a highly potent BRD4 degrader and worthy for further investigation.

4. Experimental section

4.1. Chemistry

All chemical reagents were commercially available and treated with standard methods before use. Column chromatography were performed using silica gel (200-300 mesh). Melting points were measured on capillary tube and were uncorrected. IR spectra (in KBr pellets) were taken using Shimadzu FTIR-8400S spectrophotometer. ^{1}H and ^{13}C NMR spectra (DMSO-d6) were measured on Bruker AV-300 or AV-400 spectrometers at 25 ℃ and referenced to TMS. Chemical shifts were reported in ppm (d) using the residual solvent line as internal standard. Analytical thin-layer chromatography (TLC) was performed on the glass-backed silica gel sheets (silica gel 60 Å GF 254). High-resolution mass spectra were recorded using an Agilent QTOF 6520 (Beijing, China).

4.1.1. Synthesis of compound **6**.

2-Aminobenzophenone (**25**, 8 g, 40.56 mmol) in dichloromethane (80 mL) was stirred for 15 min in an ice bath. Then, N-bromosuccinimide (7.58 g, 42.59 mmol) was added slowly and the resulting solution was continually stirred at 0 ℃ for 2 h. After the reaction was completed, the mixture was diluted with water (40 mL) and extracted with dichloromethane (100 mL × 3). The combined organic layer was washed twice with saturated brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 6:1) to afford (2-amino-5-bromophenyl)(phenyl)methanone (**26**) as a yellow solid (9.83 g, 88% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.63 – 7.43(m, 5H), 7.40 (dd, J = 8.7, 2.4 Hz, 1H), 7.31 (d, J = 2.4 Hz, 1H), 7.24 (s, 2H), 6.86 (d, J = 8.9 Hz, 1H).

Tetraethyl titanate (24.36 g, 106.80 mmol) was added to a solution of **26** (9.83 g, 35.60 mmol) and tert-butylsulfinamide (12.94 g, 106.80 mmol) in THF (60 mL). The reaction was heated to 70 ℃ and stirred for 48 h. After cooling to room temperature, the solution was diluted with water (55 mL) and ethyl acetate (70 mL), then filtered through Celite and the filtrate was extracted with ethyl acetate (50 mL × 3). The organic layer was washed with saturated brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 6:1) to afford (E)-*N*-((2-amino-5-bromophenyl)(phenyl)methylene)-2-methylpropane-2-sulfinamide (**27**) as a yellow solid (12.33 g, 91% yield).

In a round-bottom flask, the intermediate **27** (12.33 g, 32.51 mmol) was dissolved in a mixture of tetrahydrofuran (49 mL) and water (1 mL), after that, the sodium borohydride (4.92 g, 130.02 mmol) was added portionwise to the solution. The reaction mixture was stirred at room temperature for 3 h. After the reaction was completed, 25 mL of water was added, then extraction with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate. After filtration, the filtrate was evaporated to give *N*-((2-amino-5-bromophenyl)(phenyl)methyl)-2-methylpropane-2-sulfinamide (**28**) as a white solid (12.14 g, 98% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.42 – 7.20 (m, 5H), 7.12 – 7.00 (m, 2H), 6.61 (d, J = 8.6 Hz, 1H), 5.96 (d, J = 5.9 Hz, 1H), 5.48(d, J = 6.1 Hz, 1H), 5.24 (s, 2H), 1.14 (s, 9H).

In a round-bottom flask, the intermediate **28** (12.14 g, 31.84 mmol) was dissolved in tetrahydrofuran (50 mL), thereafter, trichloromethyl carbonate (14.17 g, 47.75 mmol) was added slowly. The resulting reaction mixture was stirred at room temperature for 3 h. Then the reaction mixture was concentrated. Next, 20 mL of water was added and pH value was adjusted to 7 with saturated sodium carbonate solution. Subsequently the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine and dried over anhydrous sodium sulfate. After filtration and concentration, the crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give 6-bromo-4-phenyl-3,4-dihydroquinazolin-2(1*H*)-one (**29**) as a yellow solid (9.28 g, 96% yield).

Dimethyl sulfate (0.28 g, 2.22 mmol) in acetonitrile(3 mL) was dripped into a solution of **29** (0.56 g, 1.85 mmol) and cesium carbonate (1.81 g, 5.54 mmol) in acetonitrile (8 mL) at 0 ℃. Then the solution was stirred at room temperature for 3 h. After the reaction was completed, the reaction mixture was concentrated. Next, dilution with water (10 mL) and extraction with ethyl acetate (20 mL × 3), the organic layer was dried over anhydrous sodium sulfate. After filtration and evaporation, the crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give 6-bromo-1-methyl-4-phenyl-3,4-dihydroquinazolin-2(1*H*)-one (**30**) as a white solid (0.41 g,

70% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ7.47 – 7.43 (m, 4H), 7.29 – 7.25 (m, 2H), 7.15 (dd, J = 11.6, 3.4 Hz, 2H), 6.95 (d, J = 8.1 Hz, 1H), 5.77 (s, 1H), 2.97 (s, 3H).

In a round-bottom flask, the intermediate **30** (0.50 g, 1.58 mmol), 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2yl)isoxazole (0.51 g, 1.73 mmol), sodium carbonate (0.50 g, 4.73 mmol) and tetraphenylphosphine palladium (0.18 g, 0.16 mmol) were dissolved in a mixture of toluene (3 mL), ethanol (1 mL) and water (3 mL). Then the reaction solution was heated to 80 ℃ and stirred for 18 h under nitrogen. After that, the reaction mixture was filtered through Celite and the filtrate was extracted with ethyl acetate. The organic layer was washed with saturated sodium bicarbonate and saturated brine, then dried over anhydrous sodium sulfate. After evaporation, the crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate) to give 6-(3,5-dimethylisoxazol-4-yl)-1-methyl-4-phenyl-3,4-dihydroquinazolin-2(1*H*)-one (**6**) as a white solid (0.36 g, 70% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ7.53 – 7.48 (m, 4H), 7.31 – 7.26 (m, 2H), 7.17 (dd, J = 11.6, 3.4 Hz, 2H), 6.92 (d, J = 8.1 Hz, 1H), 5.73 (s, 1H), 2.92 (s, 3H), 2.47 (s, 3H), 2.27 (s, 3H).

4.1.2. 2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid (**31**)**.** In a round-bottom flask, sodium hydride (0.64 g, 2.7 mmol) was added portionwise to a solution of compound **6** (0.6 g, 1.8 mmol) in DMF (2 mL) which was stirred in an ice bath. After 10 minutes, ethyl bromoacetate (0.60 g, 3.6 mmol) was added dropwise, then the reaction solution was stirred at room temperature for 2 h. Next, saturated ammonium chloride solution (10 mL) was added, and the mixture was extracted with ethyl acetate. After evaporation, the crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give 2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetate (0.71 g, 1.69 mmol). Then the intermediate and lithium hydroxide hydrate (0.08 g, 3.39 mmol) were dissolved in a mixture of methanol (4 mL) and water (2 mL), the resulting reaction mixture was stirred at room temperature for 2 h. The reaction solution was concentrated and then diluted with water (5 mL), pH value was adjusted to 3 with 1 M HCl solution. The mixture was filtered and the precipitate was collected to afford compound **31** as a white solid (0.63 g, 96% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.59 (s, 1H), 7.47 – 7.14 (m, 7H), 7.09 (d, J = 8.5 Hz, 1H), 5.74 (s, 1H), 3.48 (d, J = 17.4 Hz, 5H), 2.32 (d, J = 3.4 Hz, 3H), 2.15 (d, J = 3.4 Hz, 3H).

4.1.3. General procedure for the preparation of **35a-f.**

A mixture of **33a-f** (0.85 mmol), lenalidomide (0.77 mmol), pyridine (2.31 mmol) and phosphorus oxychloride (0.93 mmol) in acetonitrile (10 mL) was stirred at room temperature for 3 h. After the reaction was completed, the mixture was concentrated and then diluted with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (dichloromethane/methanol = 35:1) to give **34a-f** (about 40% yield). Next, the intermediates **34a-f** (0.3 mmol) were dissolved in dichloromethane (2 mL) and TFA (1 mL), the reaction mixture was stirred at room temperature for 2 h. After evaporation of the solvent, 5 mL of ethyl ether was added. The mixture was filtered and the precipitate was collected to afford compound **35a-f**.

4.1.3.1. 3-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (**35a**). Yellow solid, 97% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 10.08 (s, 1H), 7.83 (d, *J* = 6.6 Hz, 4H), 7.57 – 7.46 (m, 2H), 5.16 (dd, *J* = 13.1, 4.9 Hz, 1H), 4.38 (q, *J* = 17.6 Hz, 2H), 3.10 (d, *J* = 6.0 Hz, 2H), 3.00 – 2.85 (m, 1H), 2.75 (t, *J* = 6.6 Hz, 2H), 2.61 (d, *J* = 17.3 Hz, 1H), 2.31 (d, *J* = 9.1 Hz, 1H), 2.05 (s, 1H).

4.1.3.2. 4-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)butanamide (**35b**). Yellow solid, 95% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.93 (s, 1H), 7.82 (d, *J* = 6.3 Hz, 4H), 7.51 (d, *J* = 6.8 Hz, 2H), 5.16 (dd, *J* = 13.2, 4.8 Hz, 1H), 4.37 (q, *J* = 17.6 Hz, 2H), 2.92 (d, *J* = 31.8 Hz, 3H), 2.61 (d, *J* = 16.8 Hz, 2H), 2.47 – 2.21 (m, 2H), 2.05 (s, 1H), 1.94 – 1.79 (m, 2H).

4.1.3.3. 5-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pentanamide (**35c**). Yellow solid, 95% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.84 (s, 1H), 7.91 – 7.60 (m, 4H), 7.50 (dd, *J* = 9.5, 4.8 Hz, 2H), 5.16 (dd, *J* = 13.3, 4.9 Hz, 1H), 4.37 (q, *J* = 17.5 Hz, 2H), 2.82 (s, 2H), 2.53 – 2.49 (m, 4H), 2.40 (d, *J* = 6.8 Hz, 2H), 1.63 (s, 4H).

4.1.3.4. 6-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hexanamide (**35d**). Yellow solid, 96% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.83 (s, 1H), 7.82 (s, 3H), 7.51 (s, 2H), 5.15 (d, *J* = 9.0 Hz, 1H), 4.36 (s, 2H), 2.79 (t, *J* = 43.8 Hz, 4H), 2.21 (d, *J* = 97.6 Hz, 5H), 1.48 (d, *J* = 72.3 Hz, 7H).

4.1.3.5. 7-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)heptanamide (**35e**). Yellow solid, 97% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.79 (s, 1H), 7.88 – 7.57 (m, 4H), 7.49 (d, *J* = 6.8 Hz, 2H), 5.15 (d, *J* = 8.4 Hz, 1H), 4.36 (q, *J* = 17.7 Hz, 2H), 3.08 – 2.66 (m, 4H), 2.42 – 2.12 (m, 4H), 1.54 (s, 4H), 1.33 (s, 4H).

4.1.3.6. 8-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)octanamide (**35f**). Yellow solid, 97% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.79 (s, 1H), 7.79 (dd, *J* = 25.5, 18.9 Hz, 4H), 7.55 – 7.44 (m, 2H), 5.15 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.36 (q, *J* = 17.6 Hz, 2H), 2.91 (d, *J* = 12.0 Hz, 1H), 2.77 (d, *J* = 6.7 Hz, 2H), 2.61 (d, *J* = 17.6 Hz, 1H), 2.36 (t, *J* = 7.4 Hz, 3H), 2.05 (s, 1H), 1.56 (d, *J* = 28.1 Hz, 4H), 1.31 (s, 6H).

4.1.4. General procedure for the preparation of compounds **8-13.**

In a round-bottom flask, HATU (0.26 mmol) was added to a mixture solution of **31** (0.26 mmol), **35a-f** (0.26 mmol) and DIPEA (1.02 mmol) in DMF (2 mL). The reaction solution was stirred at room temperature for 4 h. After the reaction was completed, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (10 mL × 2). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (dichloromethane/methanol = 30:1) to give compounds **8-13.**

4.1.4.1. 3-(2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propanamide (**8**). White solid, 46% yield; m.p.:204-206 ℃; MS (ESI, m/z):702.00 $[M-H]^-$; IR (KBr, cm^{-1}) 3407.19, 2916.37, 1670.66, 1611.55, 1522.26, 1479.79, 1458.15, 1432.96, 1339.47, 1271.25, 1236.89, 1192.71,

847.91, 752.20, 577.80; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.02 (s, 1H), 9.85 (s, 1H), 8.11 (s, 1H), 7.80 (d, *J* = 7.0 Hz, 1H), 7.53 – 7.43 (m, 2H), 7.36 – 7.23 (m, 6H), 7.18 (d, *J* = 11.0 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 5.65 (d, *J* = 3.4 Hz, 1H), 5.14 (d, *J* = 8.4 Hz, 1H), 4.38 (d, *J* = 8.4 Hz, 2H), 3.33 (s, 5H), 3.17 (s, 2H), 2.56 (s, 2H), 2.31 (d, *J* = 2.8 Hz, 3H), 2.14 (d, *J* = 1.9 Hz, 3H), 1.99 (s, 2H), 1.24 (s, 2H).

4.1.4.2. 4-(2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)butanamide (**9**). White solid, 41% yield; m.p.:192-194 ℃; MS (ESI, m/z):716.05 [M-H]$^-$; IR (KBr, cm^{-1}) 3411.60, 1663.36, 1611.38, 1521.96, 1479.62, 1452.12, 1432.17, 1340.02, 1272.12, 1236.66, 1193.67, 847.03, 751.78, 577.65; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.03 (s, 1H), 9.82 (s, 1H), 8.03 (s, 1H), 7.81 (d, *J* = 6.8 Hz, 1H), 7.50 (d, *J* = 7.4 Hz, 2H), 7.36 (s, 4H), 7.29 (d, *J* = 8.1 Hz, 3H), 7.10 (d, *J* = 8.5 Hz, 1H), 5.69 (s, 1H), 5.15 (dd, *J* = 13.3, 4.8 Hz, 1H), 4.38 (d, *J* = 5.6 Hz, 2H), 3.35 (s, 5H), 3.16 (s, 2H), 2.36 (d, J = 13.0 Hz, 5H), 2.17 (d, *J* = 1.4 Hz, 3H), 2.03 (s, 2H), 1.81 – 1.68 (m, 2H), 1.26 (s, 2H).

4.1.4.3. 5-(2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pentanamide (**10**). White solid, 35% yield; m.p.:186-188 ℃; MS (ESI, m/z):730.05 [M-H]$^-$; IR (KBr, cm^{-1}) 3407.78, 3088.45, 2925.87, 2854.39, 1670.34, 1611.65, 1522.90, 1479.57, 1458.34, 1432.02, 1339.65, 1271.98, 1236.62, 1194.41, 1109.51, 847.76, 751.83, 577.67; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.77 (s, 1H), 7.96 (s, 1H), 7.80 (d, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 4.3 Hz, 4H), 7.26 (d, *J* = 7.2 Hz, 3H), 7.07 (d, *J* = 9.0 Hz, 1H), 5.66 (s, 1H), 5.18 – 5.08 (m, 1H), 4.35 (d, *J* = 5.2 Hz, 2H), 3.30 (s, 3H), 3.08 (d, *J* = 6.6 Hz, 2H), 2.36 (s, 2H), 2.30 (s, 3H), 2.14 (s, 3H), 1.98 (s, 2H), 1.51 (d, *J* = 47.6 Hz, 6H), 1.23 (s, 2H).

4.1.4.4. 6-(2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hexanamide (**11**). White solid, 35% yield; m.p.:167-169 ℃; MS (ESI, m/z):744.00 [M-H]$^-$; IR (KBr, cm^{-1}) 3303.68, 3084.59, 2928.27, 2856.84, 1670.00, 1611.82, 1522.05, 1477.72, 1458.11, 1431.25, 1338.56, 1270.76, 1235.65, 1193.01, 1108.09, 751.60, 701.88; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.91 (d, *J* = 17.4 Hz, 1H), 8.42 (s, 1H), 7.76 – 7.46 (m, 3H), 7.29 (d, *J* = 32.1 Hz, 5H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.80 (s, 1H), 6.48 (s, 1H), 5.58 (s, 1H), 5.01 (s, 1H), 4.30 (s, 2H), 3.33 (s, 3H), 3.03 (s, 2H), 2.67 (s, 3H), 2.22 (s, 6H), 2.08 (s, 3H), 1.26 (d, *J* = 39.9 Hz, 8H).

4.1.4.5. 7-(2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)heptanamide (**12**). White solid, 38% yield; m.p.:157-159 ℃; MS (ESI, m/z):758.10 [M-H]$^-$; IR (KBr, cm^{-1}) 3306.34, 2927.60, 2855.29, 1660.63, 1611.72, 1521.79, 1477.91, 1456.75, 1431.10, 1338.20, 1235.31, 1190.78, 1146.96, 1107.67, 829.99, 751.15, 700.94; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.75 (s, 1H), 7.82 (dd, *J* = 15.5, 10.5 Hz, 2H), 7.49 (t, *J* = 6.7 Hz, 2H), 7.35 (d, *J* = 4.0 Hz, 4H), 7.27 (d, *J* = 9.1 Hz, 3H), 7.08 (d, *J* = 8.2 Hz, 1H), 5.66 (s, 1H), 5.14 (dd, *J* = 13.1, 5.1 Hz, 1H), 4.36 (d, *J* = 4.1 Hz, 2H), 3.31 (s, 5H), 3.05 (d, *J* = 6.3 Hz, 2H), 2.36 (d, *J* = 7.3 Hz, 2H), 2.32 (s, 3H), 2.15 (s, 3H), 2.01 (s, 2H), 1.60 (s, 2H), 1.33 (dd, *J* = 33.6, 12.8 Hz, 8H).

4.1.4.6. 8-(2-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)acetamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)octanamide (**13**). White solid, 43% yield; m.p.:146-148 ℃; MS (ESI, m/z):772.05 $[M-H]^-$; IR (KBr, cm^{-1}) 3306.00, 2927.83, 2854.60, 1666.50, 1611.86, 1521.91, 1477.52, 1457.10, 1431.08, 1338.48, 1270.74, 1235.44, 1191.79, 1107.96, 829.99, 751.48; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.74 (s, 1H), 7.87 (s, 1H), 7.82 (d, *J* = 6.8 Hz, 1H), 7.49 (d, *J* = 7.1 Hz, 2H), 7.35 (d, *J* = 3.9 Hz, 4H), 7.27 (d, *J* = 10.7 Hz, 3H), 7.08 (d, *J* = 8.3 Hz, 1H), 5.66 (s, 1H), 5.14 (dd, *J* = 13.1, 4.9 Hz, 1H), 4.34 (t, *J* = 10.8 Hz, 2H), 3.28 (d, *J* = 20.6 Hz, 5H), 3.04 (d, *J* = 5.7 Hz, 2H), 2.40 – 2.34 (m, 2H), 2.32 (s, 3H), 2.16 (s, 3H), 2.03 (d, *J* = 9.4 Hz, 2H), 1.60 (s, 2H), 1.37 (d, *J* = 6.2 Hz, 2H), 1.26 (d, *J* = 12.7 Hz, 8H).

4.1.5. General procedure for the preparation of **37a-c.**

In a round-bottom flask, compound **6** (0.15 g, 0.45 mmol), sodium hydride (0.016 g, 0.67 mmol) and **36a-c** (0.9 mmol) were dissolved in DMF (2 mL). The reaction mixture was stirred at room temperature for 2 h. After that, the mixture was diluted with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL × 3). After concentration, the crude residue was purified by flash column chromatography (dichloromethane/methanol = 40:1) to give the ester intermediates (about 85% yield). Next, the ester intermediates (0.39 mmol) and lithium hydroxide monohydrate (0.78 mmol) were dissolved in a mixed solution of methanol (2 mL) and water (1 mL) and stirred at room temperature for 2 h. Then the reaction mixture was concentrated and diluted with water (5 mL), afterwards the pH value was adjusted to 3 with 1 M HCl solution. The mixture was extracted with ethyl acetate (10 mL × 3), and the combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford compounds **37a-c**.

4.1.5.1. 5-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)pentanoic acid (**37a**). Pale yellow viscous liquid, 88% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.97 (s, 1H), 7.37 (d, *J* = 1.7 Hz, 1H), 7.33 (d, *J* = 4.3 Hz, 4H), 7.26 (dt, *J* = 6.7, 2.8 Hz, 2H), 7.01 (dd, *J* = 13.4, 5.2 Hz, 1H), 5.72 (s, 1H), 3.79 (dd, *J* = 13.8, 7.6 Hz, 1H), 3.29 (s, 3H), 2.79 (dd, *J* = 13.4, 7.7 Hz, 1H), 2.35 (s, 3H), 2.22 – 2.14 (m, 5H), 1.45 (dd, *J* = 6.2, 3.1 Hz, 2H), 1.24 (d, *J* = 7.2 Hz, 2H).

4.1.5.2. 6-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)hexanoic acid (**37b**). Pale yellow viscous liquid, 89% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.00 (s, 1H), 7.38 – 7.29 (m, 5H), 7.25 (d, *J* = 5.8 Hz, 2H), 7.02 (d, *J* = 8.5 Hz, 1H), 5.74 (d, *J* = 7.7 Hz, 1H), 3.85 – 3.69 (m, 1H), 3.20 (s, 3H), 2.86 – 2.73 (m, 1H), 2.35 (s, 3H), 2.23 – 2.11 (m, 5H), 1.24 (m, 6H).

4.1.5.3. 7-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)heptanoic acid (**37c**). Pale yellow viscous liquid, 89% yield; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.97 (s, 1H), 7.29 (d, *J* = 25.7 Hz, 7H), 7.04 (s, 1H), 5.72 (s, 1H), 3.76 (s, 1H), 3.29 (s, 3H), 2.81 (s, 1H), 2.35 (d, *J* = 1.4 Hz, 3H), 2.16 (d, *J* = 10.3 Hz, 5H), 1.43 (s, 4H), 1.22 (s, 4H).

4.1.6. General procedure for the preparation of compounds **14-16.**

In a round-bottom flask, lenalidomide (0.27 mmol), **37a-c** (0.27 mmol), pyridine (0.81 mmol) were dissolved in acetonitrile (4 mL), then phosphorus oxychloride (0.32 mmol) in acetonitrile (2 mL) was added dropwise. After stirring at room temperature for 3 h, the mixture was diluted with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined organic layer was washed with saturated brine and dried over anhydrous Na_2SO_4. After evaporation, the crude residue was purified by flash column chromatography (dichloromethane/methanol = 35:1) to give **14-16.**

4.1.6.1. 5-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pentanamide (**14**). White solid, 46% yield; m.p.:148-150℃; MS (ESI, m/z): 673.00 $[M-H]^-$; IR (KBr, cm^{-1}) 3433.89, 2918.46, 2360.62, 1674.81, 1610.46, 1491.58, 1465.12, 1431.42, 1339.37, 1237.42, 1201.53, 750.89; ^1H NMR (300 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.75 (s, 1H), 7.80 (s, 1H), 7.49 (s, 2H), 7.34 (s, 3H), 7.21 (d, *J* = 15.6 Hz, 3H), 6.91 (d, *J* = 7.2 Hz, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 5.74 (s, 1H), 5.12 (s, 1H), 4.15 (dd, *J* = 32.1, 16.8 Hz, 2H), 3.29 (s, 2H), 2.92 (s, 2H), 2.34 (s, 5H), 2.17 (s, 2H), 1.34 (s, 2H), 1.20 (d, *J* = 21.4 Hz, 8H).

4.1.6.2. 6-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hexanamide (**15**). White solid, 41% yield; m.p.:140-142 ℃; MS (ESI, m/z): 687.00 $[M-H]^-$; IR (KBr, cm^{-1}) 3431.69, 2922.20, 2851.33, 1673.94, 1610.58, 1522.68, 1488.01, 1455.58, 1431.35, 1337.57, 1267.46, 1234.79, 1200.87, 751.04; ^1H NMR (300 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.74 (t, *J* = 17.6 Hz, 1H), 7.87 (d, *J* = 44.1 Hz, 1H), 7.51 (dd, *J* = 27.1, 21.2 Hz, 3H), 7.23 (ddd, *J* = 24.5, 20.6, 6.5 Hz, 5H), 7.07 – 6.65 (m, 2H), 5.41 (s, 1H), 5.12 (t, *J* = 10.3 Hz, 1H), 4.53 – 4.25 (m, 2H), 3.24 (d, *J* = 21.7 Hz, 2H), 3.04 – 2.79 (m, 2H), 2.42 – 2.22 (m, 5H), 2.17 (s, 2H), 2.01 (d, *J* = 17.5 Hz, 2H), 1.39 – 1.08 (m, 10H).

4.1.6.3. 7-(6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)heptanamide (**16**). White solid, 41% yield; m.p.:136-138 ℃; MS (ESI, m/z): 701.00 $[M-H]^-$; IR (KBr, cm^{-1}) 3433.24, 2927.27, 1686.02, 1612.24, 1522.26, 1477.33, 1458.76, 1431.06, 1338.07, 1269.45, 1235.73, 1200.00, 1147.64, 1105.37, 751.21; ^1H NMR (300 MHz, DMSO-d_6) δ 11.01 (s, 1H), 9.74 (s, 1H), 7.80 (d, *J* = 6.3 Hz, 1H), 7.61 – 7.41 (m, 2H), 7.40 – 7.18 (m, 7H), 7.03 (d, *J* = 8.4 Hz, 1H), 5.73 (s, 1H), 5.24 – 5.05 (m, 1H), 4.35 (s, 2H), 3.28 (s, 2H), 2.99 – 2.74 (m, 2H), 2.42 – 2.25 (m, 6H), 2.18 (s, 3H), 1.56 (s, 3H), 1.29 (dd, *J* = 30.3, 5.6 Hz, 9H).

4.1.7. 6-(3,5-dimethylisoxazol-4-yl)-1-methyl-4-phenyl-3-(prop-2-yn-1-yl)-3,4-dihydroquinazolin-2(1*H*)-one (**38**). In a round-bottom flask, compound **6** (1 g, 3 mmol) and 3-bromopropyne (0.53g, 4.5 mmol) were dissolved in DMF (4 mL), then sodium hydride (0.22 g, 9 mmol) was added slowly. After stirring at room temperature for 2 h, the reaction was quenched with saturated ammonium chloride solution (10 mL). Then the mixture was extracted with ethyl acetate (30 mL × 3) and the combined organic layer was washed three times with saturated brine and dried over anhydrous Na_2SO_4. After evaporation, the crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 5:1) to give **38** as a yellow solid (1.01g, 92% yield). ^1H NMR (300 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.40 – 7.25 (m, 6H), 7.09 (d, *J* = 8.2 Hz, 1H), 5.86 (s, 1H), 4.68

(d, *J* = 17.8 Hz, 1H), 3.61 (d, *J* = 17.4 Hz, 1H), 3.24 (s, 1H), 2.88 (d, *J* = 2.7 Hz, 3H), 2.33 (d, *J* = 2.8 Hz, 3H), 2.16 (d, *J* = 2.7 Hz, 3H).

4.1.8. General procedure for the preparation of **40a-d.**

39a-d (78.68 mmol) and p-toluenesulfonic acid (39.34 mmol) were dissolved in pyridine (10 mL). The mixture was stirred at room temperature for 6 h, then 1 M HCl (20 mL) was added dropwise, and a large amount of solid salt was precipitated. After filtration, the filtrate was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give **40a-d.**

4.1.8.1. 2-hydroxyethyl 4-methylbenzenesulfonate (**40a**). Yellow liquid, 34% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H), 7.36 (d, *J* = 8.0 Hz, 2H), 4.16 – 4.12 (m, 2H), 3.85 – 3.78 (m, 2H), 2.45 (s, 3H).

4.1.8.2. 2-(2-hydroxyethoxy)ethyl 4-methylbenzenesulfonate (**40b**). Yellow liquid, 29% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 4.25 – 4.16 (m, 2H), 3.73 – 3.63 (m, 4H), 3.53 (dd, *J* = 5.1, 3.7 Hz, 2H), 2.45 (s, 3H).

4.1.8.3. 2-(2-(2-hydroxyethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**40c**). Yellow liquid, 26% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, *J* = 6.2 Hz, 2H), 7.36 (d, *J* = 7.0 Hz, 2H), 4.25 – 4.15 (m, 2H), 3.67 (dt, *J* = 29.3, 4.4 Hz, 10H), 2.46 (s, 3H).

4.1.8.4. 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**40d**). Yellow liquid, 30% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.2 Hz, 2H), 7.34 (d, *J* = 8.0 Hz, 2H), 4.19 – 4.12 (m, 2H), 3.78 – 3.54 (m, 14H), 2.45 (s, 3H).

4.1.9. General procedure for the preparation of **41a-d.**

In a round-bottom flask, compounds **40a-d** (13.27 mmol) and sodium azide (26.54 mmol) were dissolved in a mixed solution of acetone (5 mL) and water (5 mL). The mixture solution was heated to 60 ℃ and stirred for 12 h. Then saturated sodium carbonate solution (5 mL) was added to quench the reaction, subsequently, the mixture was extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and concentrated. The crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 30:1) to give **41a-d**.

4.1.9.1. 2-azidoethan-1-ol (**41a**). Light yellow liquid, 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 – 3.74 (m, 2H), 3.49 – 3.39 (m, 2H), 2.01 (s, 1H).

4.1.9.2. 2-(2-azidoethoxy)ethan-1-ol (**41b**). Light yellow liquid, 96% yield; ^{1}H NMR (300 MHz, $CDCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 – 3.65 (m, 4H), 3.62 (dd, *J* = 5.1, 3.8 Hz, 2H), 3.48 – 3.35 (m, 2H), 2.12 (dd, *J* = 12.1, 6.1 Hz, 1H).

4.1.9.3. 2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol (**41c**). Light yellow liquid, 95% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.53 (d, *J* = 82.8 Hz, 12H), 2.49 (s, 1H).

4.1.9.4. 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethan-1-ol (**41d**). Light yellow liquid, 97% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.72 (dd, *J* = 5.3, 3.6 Hz, 2H), 3.70 – 3.64 (m, 10H), 3.63 – 3.59 (m, 2H), 3.42 – 3.36 (m, 2H), 2.39 (d, *J* = 29.6 Hz, 1H).

4.1.10. General procedure for the preparation of **42a-d.**

41a-d (12.63 mmol) were dissolved in THF (3 mL), then sodium hydride (25.26 mmol) was added portionwise. After stirring in an ice bath for half an hour, tert-butyl bromoacetate (12.63 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h. Then saturated ammonium chloride solution (10 mL) was added, and the mixture was extracted with ethyl acetate, the organic layer was dried over anhydrous sodium sulfate and concentrated. The crude residue was purified by flash column chromatography (petroleum ether/ethyl acetate = 25:1) to give **42a-d.**

4.1.10.1. tert-butyl 2-(2-azidoethoxy)acetate (**42a**). Light yellow liquid, 33% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.06 – 4.00 (m, 2H), 3.76 – 3.70 (m, 2H), 3.48 – 3.41 (m, 2H), 1.49 (d, *J* = 5.8 Hz, 9H).

4.1.10.2. tert-butyl 2-(2-(2-azidoethoxy)ethoxy)acetate (**42b**). Light yellow liquid, 36% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.17 – 3.94 (m, 2H), 3.77 – 3.56 (m, 6H), 3.45 – 3.30 (m, 2H), 1.55 – 1.34 (m, 9H).

4.1.10.3. tert-butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (**42c**). Light yellow liquid, 37% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.02 (s, 2H), 3.72 – 3.65 (m, 10H), 3.41 – 3.36 (m, 2H), 1.47 (s, 9H).

4.1.10.4. tert-butyl 17-azido-3,6,9,12,15-pentaoxaheptadecanoate (**42d**). Light yellow liquid, 39% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.02 (s, 2H), 3.71 – 3.64 (m, 14H), 3.38 (t, *J* = 5.1 Hz, 2H), 1.47 (s, 9H).

4.1.11. General procedure for the preparation of **43a-d.**

In a round-bottom flask, **42a-d** (4.17 mmol) were dissolved in a mixed solution of dichloromethane (2 mL) and trifluoroacetic acid (1 mL). The mixture was stirred at room temperature for 2 h. Then, the solution was concentrated and diluted with dichloromethane (5 mL). Next, thionyl chloride (1 mL) was added dropwise to the solution, after stirring for 2 h, the solvent was evaporated to afford **43a-d**, which were used in the following steps without further purification.

4.1.12. General procedure for the preparation of **44a-h.**

Lenalidomide (0.73 mmol) and **43a-d** (1.46 mmol) were dissolved in NMP (3 mL) and stirred at room temperature for 3 h. Then the reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (15 mL). The organic layer was washed twice with saturated brine and dried over anhydrous sodium sulfate. After concentration, the crude residue was purified by flash column chromatography (dichloromethane/methanol = 30:1) to give **44a-d**. Intermediates **44e-h** were obtained by the same procedure starting from pomalidomide and **43a-d**.

4.1.13. General procedure for the preparation of compounds **17-24.**

Compound **38** (0.27 mmol) and **44a-h** (0.27 mol) were dissolved in a mixed solution of dichloromethane (2 mL), methanol (2 mL) and water (1 mL). Copper sulfate pentahydrate (0.027 mmol) in 5 mL of water then was added, after stirring for 5 min at room temperature, sodium ascorbate (0.11 mmol) in 5 mL of water was added. Next, the reaction solution was stirred at room temperature for 12 h, then diluted with water (10 mL) and extracted with ethyl acetate (10 mL × 3). The organic layer was dried over anhydrous sodium sulfate and concentrated, the crude residue was purified by flash column chromatography (dichloromethane/methanol = 20:1) to give **17-24.**

4.1.13.1. 2-(2-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (**17**). White solid, 40% yield; m.p.:172-174 ℃; MS (ESI, m/z):780.10 $[M+Na]^+$; IR (KBr, cm^{-1}) 3465.75, 3085.33, 2923.46, 1704.72, 1611.00, 1521.12, 1475.81, 1455.16, 1432.80, 1337.97, 1190.92, 1144.95, 1104.33, 824.01, 751.24; ^1H NMR (300 MHz, $CDCl_3$) δ 8.81 (d, *J* = 34.0 Hz, 1H), 8.40 (s, 1H), 8.17 (s, 1H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 7.1 Hz, 1H), 7.31 (s, 2H), 7.24 (d, *J* = 4.9 Hz, 4H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 7.9 Hz, 2H), 5.70 (s, 1H), 5.26 (s, 1H), 5.02 (s, 1H), 4.53 (s, 6H), 4.11 (s, 2H), 3.98 (s, 2H), 3.43 (s, 3H), 2.82 (s, 2H), 2.27 (s, 3H), 2.14 (s, 4H).

4.1.13.2. 2-(2-(2-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (**18**). White solid, 43% yield; m.p.:165-167℃; MS (ESI, m/z):824.15 $[M+Na]^+$; IR (KBr, cm^{-1}) 3467.42, 2919.90, 1697.04, 1611.62, 1520.83, 1475.59, 1457.81, 1432.10, 1336.93, 1268.12, 1235.49, 1193.97, 1144.82, 1103.95, 827.84, 751.29, 736.80, 702.17; ^1H NMR (300 MHz, $CDCl_3$) δ 9.18 (d, *J* = 78.9 Hz, 1H), 8.63 (d, *J* = 14.2 Hz, 1H), 7.81 (dd, *J* = 13.0, 7.8 Hz, 2H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.45 (dd, *J* = 13.2, 7.5 Hz, 1H), 7.38 – 7.27 (m, 5H), 7.06 (d, *J* = 8.5 Hz, 1H), 6.88 (dd, *J* = 8.2, 4.1 Hz, 2H), 5.75 (d, *J* = 10.6 Hz, 1H), 5.22 (d, *J* = 9.3 Hz, 1H), 5.13 – 4.99 (m, 1H), 4.51 – 4.31 (m, 4H), 4.07 (dt, *J* = 15.2, 8.7 Hz, 3H), 3.87 (s, 2H), 3.76 – 3.57 (m, 4H), 3.38 (d, *J* = 2.0 Hz, 3H), 2.82 (dd, *J* = 11.9, 4.6 Hz, 2H), 2.26 (d, *J* = 1.5 Hz, 4H), 2.15 (t, *J* = 12.0 Hz, 4H).

4.1.13.3. 2-(2-(2-(2-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (**19**). White solid, 43% yield; m.p.:154-156 ℃; MS (ESI, m/z):868.15 $[M+Na]^+$; IR (KBr, cm^{-1}) 3466.23, 2912.82, 1696.91, 1611.73, 1521.09, 1475.88, 1458.88, 1432.22, 1338.32, 1268.31, 1237.09, 1194.97, 1144.98, 1104.35, 1050.66, 828.82, 751.78, 737.07, 703.06; ^1H NMR (300 MHz, $CDCl_3$) δ 9.17 (d, *J* = 49.8 Hz, 2H), 7.75 (d, *J* = 7.0 Hz, 1H), 7.63 (s, 1H), 7.58 – 7.27 (m, 7H), 7.08 (d, *J* = 7.9 Hz, 1H), 6.90 (s, 2H), 5.82 (s, 1H), 5.23 (s, 1H), 4.29 (d, *J* = 81.8 Hz, 8H), 3.77 (s, 2H), 3.61 (d, *J* = 20.4 Hz, 6H), 3.43 (s, 6H), 2.87 (s, 2H), 2.27 (s, 4H), 2.13 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 173.34, 171.50, 168.89, 168.25, 165.28, 158.56, 142.16, 137.26, 135.29, 133.36, 133.21, 129.42, 129.12, 128.30, 127.22, 126.79, 126.43, 124.52, 123.80, 120.22, 115.61, 114.17, 70.83, 70.42, 70.09, 70.06, 69.97, 69.09, 61.00, 52.00, 49.77, 46.89, 41.36, 31.67, 30.61, 23.04, 11.73, 10.86.

4.1.13.4. 14-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-3,6,9,12-tetraoxatetradecanamide (**20**). White solid, 50% yield; m.p.:143-145 ℃; MS (ESI, m/z):912.15 $[M+Na]^+$; IR (KBr, cm^{-1}) 3447.07, 2915.75, 1708.00, 1612.00, 1521.28, 1459.49, 1432.60, 1338.76, 1234.78, 1195.98, 1104.21, 751.46; ^1H NMR (300 MHz, $CDCl_3$) δ 9.36 (d, *J* = 24.8 Hz, 1H), 9.17 (s, 1H), 7.82 (d, *J* = 15.7 Hz, 1H), 7.74 (d, *J* = 7.2 Hz, 1H), 7.66 (s, 1H), 7.48 (d, *J* = 7.6 Hz, 1H), 7.34 (s, 5H), 7.07 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 12.3 Hz, 2H), 5.79 (s, 1H), 5.18 (s, 1H), 4.45 (s, 4H), 4.13 (s, 2H), 3.70 (t, *J* = 21.2 Hz, 9H), 3.44 (d, *J* = 15.2 Hz, 5H), 3.22 (d, *J* = 26.0 Hz, 4H), 2.87 (s, 2H), 2.20 (d, *J* = 41.3 Hz, 9H). ^{13}C NMR (101 MHz, DMSO) δ 173.34, 171.49, 168.91, 168.25, 165.27, 158.55, 153.88, 143.49, 142.15, 137.26, 135.34, 133.36, 133.20, 129.42, 129.13, 128.30, 127.22, 126.83, 126.43, 124.51, 124.43, 123.79, 120.24, 115.60, 114.17, 70.85, 70.41, 70.22, 70.14, 70.06, 69.94, 69.09, 60.96, 52.00, 49.77, 46.90, 41.29, 31.67, 30.61, 23.11, 23.03, 11.73, 10.85.

4.1.13.5. 2-(2-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)acetamide (**21**). White solid, 37% yield; m.p.:156-158 ℃; MS (ESI, m/z):794.05 $[M+Na]^+$; IR (KBr, cm-1) 3089.51, 2918.85, 1769.34, 1708.09, 1638.27, 1619.13, 1533.81, 1478.29, 1430.93, 1399.78, 1351.11, 1303.52, 1263.55, 1194.78, 1122.13, 1043.03, 1029.04, 823.38, 767.73, 748.27, 592.77, 533.82, 468.38; ^1H NMR (300 MHz, $CDCl_3$) δ 10.89 (s, 1H), 9.49 – 8.95 (m, 1H), 8.81 (s, 1H), 7.66 (d, *J* = 36.2 Hz, 3H), 7.36 (d, *J* = 46.0 Hz, 5H), 6.99 (d, *J* = 40.8 Hz, 3H), 6.23 (s, 1H), 5.03 (s, 2H), 4.72 (s, 2H), 4.11 (s, 6H), 3.43 (s, 3H), 2.90 (s, 4H), 2.28 (s, 5H). ^{13}C NMR (101 MHz, DMSO) δ 173.36, 171.50, 168.49, 168.25, 165.27, 158.55, 153.95, 142.23, 142.20, 137.24, 135.12, 135.07, 133.28, 133.22, 129.40, 129.38, 129.14, 128.28, 127.23, 126.62, 126.43, 124.54, 123.80, 120.21, 115.60, 114.18, 70.07, 69.75, 61.08, 52.02, 49.80, 46.87, 41.49, 31.69, 30.61, 23.03, 11.73, 10.86.

4.1.13.6. 2-(2-(2-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (**22**). White solid, 38% yield; m.p.:147-149 ℃; MS (ESI, m/z):838.05 $[M+Na]^+$; IR (KBr, cm^{-1}) 3458.03, 3314.01, 2922.18, 1771.43, 1708.11, 1654.18, 1615.70, 1524.75, 1478.42, 1397.84, 1348.60, 1261.90, 1197.81, 1105.01, 823.74, 747.68; ^1H NMR (300 MHz, $CDCl_3$) δ 10.44 (d, *J* = 5.9 Hz, 1H), 9.20 (s, 1H), 8.85 (d, *J* = 8.2 Hz, 1H), 7.78 – 7.66 (m, 2H), 7.57 (d, *J* = 7.3 Hz, 1H), 7.33 (d, *J* = 13.0 Hz, 5H), 7.07 (d, *J* = 8.4 Hz, 1H), 6.90 (d, *J* = 8.7 Hz, 2H), 5.79 (d, *J* = 11.5 Hz, 1H), 5.10 (s, 1H), 4.98 (s, 1H), 4.50 (s, 2H), 4.15 (s, 2H), 3.93 (d, *J* = 5.4 Hz, 2H), 3.75 (s, 4H), 3.41 (s, 3H), 2.86 (d, *J* = 13.4 Hz, 4H), 2.27 (s, 3H), 2.13 (s, 4H).

4.1.13.7. 2-(2-(2-(2-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (**23**). White solid, 40% yield; m.p.:142-144 ℃; MS (ESI, m/z):882.30 $[M+Na]^+$; IR (KBr, cm^{-1}) 3461.92, 3317.59, 2916.72, 1771.32, 1708.26, 1654.65, 1615.73, 1524.75, 1478.29, 1397.88, 1348.82, 1262.18, 1197.53, 1105.40, 824.15, 747.90; ^1H NMR (300 MHz, $CDCl_3$) δ 10.47 (s, 1H), 9.08 (s, 1H), 8.86 (d, *J* = 8.3 Hz, 1H),

7.88 – 7.53 (m, 3H), 7.34 (s, 5H), 7.09 (d, *J* = 7.7 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 2H), 5.84 (s, 1H), 5.31 (s, 1H), 4.98 (s, 1H), 4.51 (s, 2H), 4.20 (s, 2H), 3.78 (d, *J* = 8.5 Hz, 4H), 3.54 (dd, *J* = 36.9, 14.2 Hz, 9H), 2.82 (s, 4H), 2.29 (s, 3H), 2.15 (s, 4H).

4.1.13.8. 14-(4-((6-(3,5-dimethylisoxazol-4-yl)-1-methyl-2-oxo-4-phenyl-1,4-dihydroquinazolin-3(2*H*)-yl)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)-3,6,9,12-tetraoxatetradecanamide (**24**). White solid, 45% yield; m.p.:135-137 ℃; MS (ESI, m/z):926.05 $[M+Na]^+$; IR (KBr, cm^{-1}) 3458.36, 3313.68, 2871.07, 1770.88, 1708.15, 1654.52, 1615.68, 1524.77, 1478.12, 1348.95, 1261.11, 1198.01, 1104.39, 824.01, 747.83; [1] 1H NMR (300 MHz, $CDCl_3$) δ 10.50 (s, 1H), 9.14 (s, 1H), 8.84 (d, *J* = 8.4 Hz, 1H), 8.04 – 7.65 (m, 2H), 7.57 (d, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 25.2 Hz, 5H), 7.07 (d, *J* = 8.6 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 2H), 5.80 (s, 1H), 5.18(s, 1H), 4.98 (s, 1H), 4.49 (s, 2H), 4.18 (s, 3H), 3.95 – 3.15 (m, 16H), 2.78 (s, 4H), 2.21 (d, *J* = 41.5 Hz, 7H). ^{13}C NMR (101 MHz, DMSO) δ 173.21, 170.21, 169.86, 168.71, 167.16, 165.26, 158.54, 153.87, 142.16, 137.26, 136.98, 136.43, 131.78, 129.41, 129.12, 128.29, 127.22, 126.43, 124.85, 124.50, 123.78, 118.79, 116.53, 115.59, 114.15, 71.22, 70.68, 70.28, 70.17, 70.07, 69.95, 69.10, 60.95, 49.78, 49.45, 41.29, 31.40, 30.59, 22.42, 11.73, 10.85.

4.2. Docking study.

The crystal structure (PDB code 3MXF) obtained from RCSB Protein Data Bank was used as the docking template. The docking study was processed with the Glide docking protocol in Maestro 10.6. Hydrogen atoms were added to the structure and the water was removed. The ligand molecule was drawn in ChemDraw 14.0, and saved as sdf style. Then ligand was processed at a simulated pH of 7.4 ± 1.0 to generate all possible tautomers, stereoisomers, and protonation states and were finally minimized at the OPLS force field with Ligand preparation protocol of Maestro 10.6. After docking finished, only one docking conformation was saved. The graphical image was produced using Pymol.

4.3. Determination of biochemical binding affinity to BRD4 BD1.

All the synthetic compounds were evaluated against BRD4 BD1 *in vitro* with compound **6** and **JQ1** as the positive controls. BRD4 (44-168 aa) was purchased from Active Motif. After preparing 1 x assay buffer (modified HEPES Buffer), target compounds were transferred to assay plate by Echo, and the final concentration of DMSO solution was 0.1%. Then the preparation of protein solution and substrate solution in 1 x assay buffer was performed. After that, 5 μL of protein solution was transferred to assay plate and incubated at room temperature for 15 min. The protein concentration was 5 nM. Then 5 μL of substrate solution was added to each well to start reaction with an incubation period for 60 min at room temperature. Finally, 15 μL acceptor and donor solution were added, followed by another incubation period for 60 min at room temperature under subdued light. The IC_{50} values were calculated from the endpoints of EnSpire with Alpha mode.

4.4. Anti-proliferative assay and Western blot assay.

HL-60, Raji and THP-1 cells were seeded in 96-well tissue culture plates and incubated for 24 h at 37 ℃ in an atmosphere of 5% CO_2. Each compound tested was serially diluted in the appropriate medium, and 100 μL of the diluted solution containing the tested compound was added to the appropriate wells of the cell plate After that, cells were further incubated for 72 h at 37 ℃ in an

atmosphere of 5% CO_2. Cell proliferation was then determined using CellTiter-Glo agents that were added into every well and shaken for 10 min. Then optical signal was stabilized at room temperature for two minutes and tested by Envision. The data were processed in XL fit. The anti-proliferative assay was processed to obtain the IC_{50} for the selected compounds reported in **Table 3**.

For Western blot analysis, 2×10^6 cells/well were treated with compounds at the indicated concentrations for various times. Cells were collected and lysed in RIPA buffer containing protease inhibitors. An amount of 30 μg of lysate was run in each lane of a PAGE-SDS and blotted into PVDF membranes. Antibodies for immunoblotting were all bought from Active Motif.

Acknowledgement

This study was supported by the Natural Science Foundation of Jiangsu Province (No. BK 20141349) and the China National Key HiTech Innovation Project for the R&D of Novel Drugs (No. 2013ZX09301303-002).

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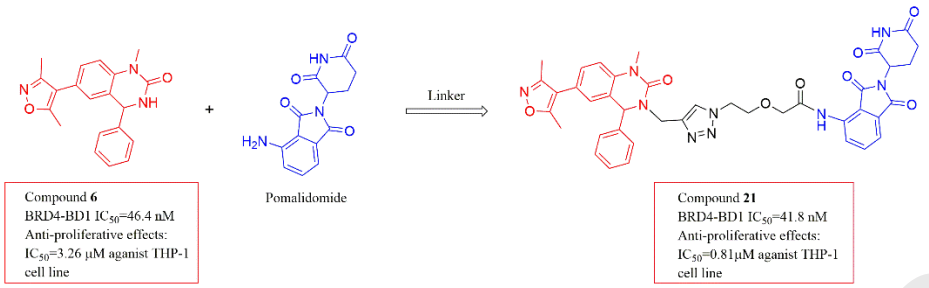
Graphical abstract



Compound **6**
BRD4-BD1 IC_{50}=46.4 nM
Anti-proliferative effects:
IC_{50}=3.26 μM aganist THP-1 cell line

Pomalidomide

Compound **21**
BRD4-BD1 IC_{50}=41.8 nM
Anti-proliferative effects:
IC_{50}=0.81μM aganist THP-1 cell line

Highlights

1. A new class of BRD4 degraders were designed and synthesized.

2. All synthesized compounds were evaluated for their ability to inhibit BRD4.

3. Cell proliferation inhibition was assessed in the HL-60, Raji and THP-1 cell lines.

4. Compound **21** effectively induced the degradation of BRD4 protein and suppression of c-Myc expression.

Conflict of Interest

Declarations of interest: the authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.