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Rational Design and Synthesis of Novel Dual PROTACs for Simultaneous Degradation of EGFR and PARP

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of degrading two completely different types of targets simultaneously. A library of novel dual-targeting PROTAC molecules has been rationally designed and prepared. A convergent synthetic strategy has been utilized to achieve high synthetic efficiency. These dual PROTAC structures are characterized using trifunctional natural amino acids as star-type core linkers to connect two independent inhibitors and E3 ligands together. In this study,



gefitinib, olaparib, and CRBN or VHL E3 ligands were used as substrates to synthesize novel dual PROTACs. They successfully degraded both the epidermal growth factor receptor (EGFR) and poly(ADP-ribose) polymerase (PARP) simultaneously in cancer cells. Being the first successful example of dual PROTACs, this technique will greatly widen the range of application of the PROTAC method and open up a new field for drug discovery.

INTRODUCTION

In cancer, tumor cells often upregulate different growthpromoting factors, which can act independently or interfere with each other through a signal network.¹ It is easy for tumor cells to acquire drug resistance by resorting to the compensatory factors or switching the signal pathway that promotes proliferation.² Therefore, the treatment with drugs targeting only one single target inevitably exhibits limitations. In addition to drug resistance, the side effects and the tissue toxicity of single-target drugs often lead to reduced efficacy and decreased life quality of patients.³ To overcome these shortcomings of single-target drugs, drug combination targeting two or more different signal pathways of tumor has become a well-recognized effective method.⁴ Due to the synergistic effects of the drug combination, a smaller dose of each single drug is needed, thus reducing the side effects.

Another approach to improve therapeutic efficacy is to design a single hybrid molecule merged by two or more pharmacophores to target two or more antitumor epitopes or targets simultaneously.³ Regulating multiple targets or pathways at the same time, these hybrid molecules usually exhibit better efficacy while causing fewer side effects. In the past few decades, these hybrid molecules, including bispecific antibodies and other small molecule drugs with double or multiple targets,^{3,5} have attracted great interest and achieved considerable success due to their superiority in the treatment of

complex diseases. They have gradually become an alternative to combination therapy or the use of mixtures.^{6,7}

Proteolysis targeting chimera (PROTAC) is a kind of bifunctional small molecule, in which the target protein ligand and the E3 ubiquitin ligase ligand are linked together through a linker to form a triplet compound.⁸ Compared with traditional small molecule inhibitors, PROTAC has several advantages. It no more needs to bind to the active site of the target protein to exert effects, while it is capable of the degradation of "nondruggable" targets. There exists an event-driven mechanism rendering its catalytic properties, and it works at lower doses while providing great opportunities for the development of anticancer drugs.⁹ However, the majority of reported PROTAC molecules only connect one inhibitor with one E3 ligand, which often degrades only a single-target protein and not exceeding the limit of two or more similar proteins.

RESULTS AND DISCUSSION

Inspired by the great success of dual-targeting drugs, especially bispecific antibodies, we envisage that by combining the

Received: April 9, 2021 **Published:** May 26, 2021







Figure 1. Design of novel star-shaped dual PROTACs and the schematic diagram of dual-targeted protein degradation. Dual PROTACs consist of two ligands binding to two different target proteins and a ligand binding to the E3 ubiquitin ligase. Three ligands are linked through a linker to form a dual-targeting PROTAC, then recruit the E3 ligase to induce the ubiquitination of both two targets, and subsequent degradation.

concept of PROTAC and dual targeting, a dual PROTAC molecule with two independent inhibitor units and one E3 ligase ligand can be designed for degrading two targets simultaneously in completely different pathways (Figure 1). Dual PROTAC can not only degrade two targets at the same time, but also imbibe merits of both PROTACs and double targeting drugs, achieving similar efficacy as bispecific antibodies and still relatively conserving the advantages of small molecule drugs. To the best of our knowledge, there has been no report yet on this audacious and innovative idea. Therefore, it will be very interesting to design and synthesize such a novel dual PROTAC molecule for proof of concept.

To verify our hypothesis shown above, the rational design of the novel star-type linker should function as the most pivotal element for dual PROTACs. Taking biocompatibility into consideration, natural amino acids will be a good choice. More importantly, some amino acids, such as tyrosine and serine, bearing a third reactive site besides normal amine and acid functional groups, are ideal star-type linkers to connect three different small molecules (two inhibitors and one E3 ligase ligand) together (Scheme 1). In addition, the three potential reactive sites display different reactivities, which makes them very suitable for sequential organic synthesis manipulation according to our rational design.

Drug resistance in advanced cancers has been reported to be mediated by different factors, such as the epidermal growth factor receptor (EGFR) over-expression and DNA repair enzymes. Poly(ADP-ribose) polymerase (PARP) is one such protein that is known to be a key player in base excision repair (BER)¹⁰ and cellular signaling pathways.^{11,12} The inhibition of EGFR leads to the down-regulation of key players in BER and sensitizes cell response to alkylating agents and ionizing radiation.^{6,13} Receptor tyrosine kinase inhibitors (TKIs), just like EGFR inhibitors (e.g., gefitinib), are widely used in clinics and have shown excellent therapeutic efficacy.^{14,15} However, the emergence of drug resistance, such as that due to T790M mutation, greatly reduces their efficacy.¹⁶ EGFR mutant cancer cells have been shown to be sensitive to olaparib both in vivo and in vitro.¹⁷ Therefore, inhibition of both EGFR and PARP may produce a synergistic effect.³ To verify the concept of dual PROTAC shown above, we designed the first round of dual PROTAC molecules by merging EGFR inhibitors and PARP inhibitors with the E3 ligase ligand in one novel star-shaped molecule and evaluated their capability to degrade two independent targets at the cellular level.

As illustrated in Scheme 1, a convergent synthetic strategy was rationally designed based on our synthetic experience. The first etherification of the free hydroxyl group of tyrosine and serine with propargyl bromide provided the protected amino acid A. Subsequently, a sequential amide condensation reaction was utilized as a practical operation to induce Gefitinib and Olaparib to the star-type linker, generating the key intermediate B. Eventually, the classical copper-promoted click reaction of azide and alkene was used to connect E3 ligands, CRBN, and VHL, respectively. Based on this concise synthetic route, a small library of eight dual PROTACs, including four CRBD ligand-based PROTACs (DP-C 1-4, Figure S1), four VHL ligand-based PROTACs (DP-V 1-4, Figure S2), and the corresponding four mono PROTACs in which one single inhibitor was linked (MP-GC, MP-GV, MP-OC, MP-OV, Figure S3) were efficiently constructed. Scheme 2 illustrates the detailed synthetic routes for the four mono PROTACs and eight dual PROTACs.

Due to drug resistance shown in pancreatic cancer and nonsmall cell lung cancer, as well as the high expression of EGFR and PARP,^{18–23} SW1990 and H1299 cell lines were used in the experiments. Intracellularly, the degradation of EGFR in the SW1990 cell line was induced by the CRBN-based mono PROTAC of Gefitinib (**MP-GC**) (Figure 2A), and the CRBN-based mono PROTAC of Olaparib (**MP-OC**) also exhibited a PARP degradation effect in the SW1900 cell line (Figure 2B). However, neither Gefitinib nor Olaparib itself could induce the degradation of two targets in SW1990 (Figures S4 and S5).

Further experiments revealed that all four CRBN-based dual PROTACs (**DP-C 1-4**) had degradation effects on EGFR and PARP simultaneously in SW1990 cells. As shown in Figure 2C, compound **DP-C-1** showed the best degradation effect on

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Scheme 1. General Synthetic Route Design and the Structures of Dual PROTACs (DP-C 1-4, DP-V 1-4) and Mono PROTACs (MP-GC, MP-GV, MP-OC, MP-OV)^a



^aTyrosine and serine were used as star-type linkers to connect two inhibitors and one E3 ligase ligand together. Eight dual PROTACs and four mono PROTACs were constructed consequently.

both EGFR and PARP. The remaining compounds showed lower degradation ability on both targets compared to **DP-C-1**. These results also indicate that the degradation of EGFR and PARP by **DP-C 1-4** was enhanced by increasing concentrations. The simultaneous degradation of EGFR and PARP by a single dual PROTAC molecule successfully proves the concept of dual-targeting degradation.

To further demonstrate the degradation kinetics of DP-C-1, time-dependent experiments were carried out. The results showed that DP-C-1 began to degrade EGFR and PARP at 6 h. At first, the degradation rate increased with increasing time, but it started to decrease at 48 h (Figures 2D and S6). Therefore, DP-C-1 degrades EGFR and PARP in the SW1990 cell line in a time-dependent manner within 48 h and the optimal degradation rate was around 24 and 36 h.

PROTACs exert their degradation effect on target proteins through the ubiquitin-proteasome system. Therefore, MG132, a proteasome inhibitor, is widely used as a tool to verify the mechanism of such an action.²⁴⁻²⁶ To further verify that the degradation of proteins by **DP-C-1** functions via the

ubiquitination pathway, the effect of DP-C-1 was assayed by introducing the proteasome inhibitor MG132. It can be seen from Figure 2E that due to the inhibitory effect of MG132 itself on the SW1990 cells, the group with MG132 alone had a slightly lower expression level of both EGFR and PARP compared to that of the negative control (NC) group. However, the addition of DP-C-1 in various concentrations ceases to further decrease the expression levels of both targets, and they maintain approximately the same level. This shows that the degradation of DP-C-1 is inhibited by MG132. These results suggest that the degradation of the target protein induced by DP-C-1 functions via the proteasome pathway.

To further verify the concept of dual PROTAC, another E3 ligase ligand (VHL-L) was chosen to develop new dual PROTACs. First, the corresponding mono PROTACs, **MP-GV** and **MP-OV**, were synthesized based on Gefitinib, Olaparib, and VHL ligands. In the H1299 cell line, **MP-GV** and **MP-OV** could induce EGFR and PARP degradation, respectively, while exhibiting no effect on the other target proteins (Figure 3A,B). According to this result, four dual

Scheme 2. Synthetic Routes for Mono PROTACs (MP-GC, MP-GV, MP-OC, MP-OV) and Dual PROTACs (DP-C 1-4, DP-V 1-4)^a



"(a) Compound 1 was treated with 1 equiv of compound 2 and 2 equiv of N_i . A compound 1 was treated with 1 equiv of compound 2 and 2 equiv of N_i . A compound 1 was treated with 1 equiv of acid and amine was treated with 1.2 equiv of 1-ethyl-3(3-dimethylpropylamine) carbodiimide (EDCI), 1hydroxybenzotriazole (HOBt), and DIPEA in dry dichloromethane (DCM) at room temperature overnight; (c) compound 7 was treated with 1.1 equiv of compound 8 and 2 equiv of K_2CO_3 in acetone by refluxing overnight; (d) ester was treated with 5 equiv of NaOH in 1:1 mixture solvent of methanol and THF at room temperature overnight; (e) 1.0 equiv of alkyne and azide was treated with 1 equiv of $CuSO_4$ and 2 equiv of sodium ascorbate in 4:1 mixture solvent of THF and water at room temperature for a few hours; (f) phenol or alcohol was treated with 2 equiv of bromide

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Scheme 2. continued

17 and 2 equiv of K_2CO_3 or NaH in dry DMF for a few hours; and (g) the Boc-protecting group was removed by treatment with HCl ethyl acetate solution at room temperature overnight.



Figure 2. DP-C-1 degraded EGFR and PARP simultaneously in a dose- and time-dependent manner in SW1990 cells. (A) Changes in associated proteins 24 h after the addition of **MP-GC**. (B) Changes in associated proteins 24 h after the addition of **MP-OC**. (C) Effect of four dual PROTAC compounds (**DP-C 1-4**) on the related proteins after 24 h. (D) Effect of 5 μ M **DP-C-1** on related proteins at different time points. (E) Effect of **DP-C-1** on related proteins after the introduction of 700 nM MG132. EGFR and PARP degradation by **DP-C-1** in 24 h could be abrogated by pretreating with MG132 for 4 h in 700 nM.

PROTACs based on the VHL ligand were further developed and designated as **DP-V 1-4**, respectively. To evaluate their degradation ability of EGFR and PARP, H1299 cells were treated with these four compounds under six different concentrations for 36 h. Among the different compounds, **DP-V-4** exhibited the best degradation ability for both EGFR and PARP (Figures 3C,D and S7) and was selected for further investigation. Meanwhile, further experiments revealed that **DP-V-4** could also degrade EGFR and PARP in human epidermal carcinoma A431 cells simultaneously (Figure S8).

In H1299 cells, **DP-V-4** degraded EGFR and PARP simultaneously in a dose-dependent manner (Figure 3D). At a concentration of 0.47 μ M, **DP-V-4** could induce about 50% of PARP degradation, and as the concentration increased, it induced PARP degradation to a greater level. For EGFR, **DP-V-4** also induced degradation at higher levels when the compound concentration gradually increased. Moreover, **DP-V-4** could significantly induce EGFR and PARP degradation at 15 μ M. Apart from EGFR and PARP, p-EGFR concentrations decreased visibly when treated with **DP-V-4**, which could be the result of its degradation by **DP-V-4**, or reduced supply from EGFR. To further explore the mode of action, different time points were set in H1299 cells after being treated with 4 μ M of **DP-V-4**. At 6 h, **DP-V-4** showed the best degradation effect, and EGFR and PARP recovered as time went on (Figure 3E), which might be explained by the accumulation of newly synthesized proteins in cells.

To further verify that the degradation of proteins in cells by **DP-V-4** functions via the ubiquitination pathway, the effect of **DP-V-4** was assayed by introducing the proteasome inhibitor MG132. In H1299 cells, when pretreated with 1 μ M MG132 for 4 h, the degradation of EGFR and PARP by **DP-V-4** completely terminated (Figure 3F). These results proved that **DP-V-4** degrades EGFR and PARP through the ubiquitin–proteasome system.

Besides degradation ability, we attempted to identify the antiproliferative activity of dual PROTACs in H1299 tumor cells. The CCK-8 assay determined Gefitinib, Olaparib, and **DP-V-4** with IC₅₀s of 6.56 \pm 1.07, 35.93 \pm 1.05, and 19.92 \pm 1.08 μ M, respectively (Figure 3G). The relatively weaker antiproliferative activity of **DP-V-4** might be explained by its poorer solubility and cell penetrability, owing to its higher molecular weight.



Figure 3. DP-V-4 degraded EGFR and PARP simultaneously in a dose- and time-dependent manner in H1299 cells. (A) Changes in associated proteins 36 h after the addition of **MP-GV**. (B) Changes in associated proteins 36 h after the addition of **MP-OV**. (C) Effect of three dual PROTAC compounds (**DP-V 1-3**) on related proteins after 36 h. (D) Effect of **DP-V-4** on related proteins with different concentrations at 36 h. (E) Effect of 3.75 μ M **DP-V-4** on related proteins at different times. (F) Effect of **DP-V-4** on related proteins after the introduction of 1 μ M MG132. EGFR and PARP degradation by **DP-V-4** in 6 h could be abrogated by pretreating with MG132 for 4 h in 1 μ M. (G) The antiproliferative activity of Gefitinib, Olaparib, and **DP-V-4** at 72 h was identified by a cell counting kit-8 (CCK-8) assay. Half-maximal inhibitory concentration (IC₅₀) was expressed as mean \pm standard deviation (SD).

To further confirm the target engagement of these dual PROTACs, microscale thermophoresis (MST) was employed to assay the binding affinity between the two targets and two representative dual PROTACs, **DP-C-1** (CRBN based) and **DP-V-4** (VHL based). As shown in Figure 4, although a little weaker binding was observed for both **DP-C-1** and **DP-V-4** compared to Gefitinib with EGFR (K_d 0.47 ± 0.05 μ M) and Olaparib with PARP (K_d 5.31 ± 1.11 μ M), they still retained strong binding with EGFR and PARP, with the disassociation constants for **DP-C-1** at 2.74 ± 0.13 and 7.89 ± 0.96 μ M with EGFR and PARP, respectively, and for **DP-V-4** at 5.47 ± 0.67 and 12.80 ± 2.16 μ M with EGFR and PARP, respectively.

In this study, we proposed the new concept of dual-targeting PROTAC. Inspired by bispecific antibodies,⁵ we creatively proposed the idea of designing a dual-targeting PROTAC molecule with two different warheads, in which an E3 ligand was linked to two inhibitors with different types/pathways of targets, and was capable of simultaneously degrading two completely different target proteins in tumor cells. To verify this concept, we used Gefitinib (an existing EGFR inhibitor) and Olaparib (a PARP inhibitor)³ as substrates to synthesize dual-targeting degradation chimeric molecules (dual PRO-TAC) of EGFR and PARP proteins with different linker lengths and different E3 ligands (CRBN or VHL), which successfully degraded EGFR and PARP simultaneously in cancer cells. This is the first successful example of dual PROTACs.

In addition, we used biocompatible natural amino acids as a linker to achieve the rapid synthesis of star-type dual-targeting PROTACs. The technique and method established in this study can be easily adapted for the synthesis of more dualtargeting PROTAC molecules just like building blocks, and it will greatly expand the application of PROTAC technology while opening up a new avenue for drug discovery. In the present clinical practice, the combination therapy of two different inhibitors, or even the treatment of some bispecific antibodies, could be substituted by the strategy shown in this study through designing a corresponding single dual PROTAC molecule for double targeting degradation, with the same or even better therapeutic effects.

It is easy to recognize that in the field of combination therapy of cancer, dual PROTACs can be quickly applied to replace many current combinations. For example, we could design and synthesize the dual PROTACs targeting two kinases, which are synthetically lethal, or targeting a tumor immune target plus an adjuvant immune target, kinase, or energy metabolism target, or targeting an epigenetic target plus an antiapoptotic target, etc. Of course, in addition to the obvious advantages listed above, the increased molecular weight of dual PROTACs will give rise to some issues in the aspect of drug properties and pharmacokinetics. The approach to solving these problems lies in two directions. One is to employ a nanodrug delivery system to improve drug absorption, and the other is to simplify the inhibitor part while retaining the minimum pharmacophore. We are currently working on realizing these approaches.

CONCLUSIONS

In conclusion, we have designed and synthesized the first dualtargeting PROTAC molecules with two different warheads,



Figure 4. Binding affinity of **DP-C-1** and **DP-V-4** with EGFR and PARP. (A–C) Measurement of affinity of **DP-C-1**, **DP-V-4**, and Gefitinib to EGFR by MST with K_d values of 2.74 ± 0.13, 5.47 ± 0.67, and 0.47 ± 0.05 μ M, respectively. (D–F) Measurement of affinity of **DP-C-1**, **DP-V-4**, and Olaparib to PARP by MST with K_d values of 7.89 ± 0.96, 12.80 ± 2.16, and 5.31 ± 1.11 μ M, respectively.

and realized the simultaneous degradation of two completely different types of target proteins by one small molecule in tumor cells. We believe that this technique will greatly expand the application of PROTAC technology and open up a new field of drug discovery.

EXPERIMENTAL SECTION

Cell Culture. Cells were purchased from the Chinese Typical Culture Collection Center. The cells were cultured in Dulbecco's modified Eagle's medium containing 25 mM glucose (high DMEM, GIBCO) supplemented with 10% (v/v) fetal bovine serum in a saturated humidified atmosphere of 5% CO₂ at 37 °C. All cell lines were authenticated by short tandem repeat (STR) analysis.

Immunoblotting. The cells were seeded in six-well plates at an individual density for 24 h and then treated with dimethyl sulfoxide (DMSO) or compounds at the indicated concentrations for various times. After being treated with the indicated concentrations of

compounds for the specific time, the cells were harvested and washed once with phosphate-buffered saline (PBS) and then lysed in radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, #P0013C) according to the directions. The suspension was centrifuged at 13 000 rpm for 15 min to remove insoluble material, and a bicinchoninic acid (BCA) assay was employed to determine the concentration of soluble proteins (Beyotime, #P0011). The lysate protein (20-40 μ g) was subjected to 10% sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred to poly(vinylidene difluoride) (PVDF) membranes (Millipore, #IPVH00010). The membranes were blocked with 5% nonfat milk or 5% bovine serum albumin (BSA) for 1 h and then incubated with the specific primary antibodies with a 1:1000 dilution, respectively, at 4 °C overnight. The primary antibodies and secondary antibodies were purchased from Abclonal and Biosharp, respectively. Membranes were imaged by the Tanon-5200 Chemiluminescent Imaging System with ECL luminescence reagents (Meilunbio, #MA0186).

Cell Proliferation Inhibition Assay. About 4000 cells per well were seeded in 96-well plates in 100 μ L of medium and incubated overnight. After that, the cells were treated with a series of concentrations of specific compounds for a further 72 h. Cell counting kit-8 (CCK-8) (Targetmol, #C0005) was used to identify the antiproliferative activity of compounds, and plates were read at 450 nm using a Bio-Tek Synergy 2 microplate reader. Data were analyzed using GraphPad Prism 7.0 Software.

Clone, Expression, and Purification of PARP. Human PARP-1 ART domain Asp788-Trp1014 (NCBI RefSeq: NP_001609.2) containing a GST tag followed by the HRV-3C cleavage site was cloned into a PGEX-6p-1 and heterologously expressed in *Escherichia* coli strain BL21 (DE3).²⁷ The cells were cultured at 37 °C for 3–4 h and supplemented with 0.4 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) and 0.1 mM ZnSO₄ to boost protein expression. The cells were collected by centrifugation at 3000g for 10 min after overnight growth at 16 °C. The cell pellet was resuspended in lysis buffer (50 mM N-(2-hydroxyethyl)piperazine-N'-ethanesulfonic acid (HEPES), pH 8.0, 200 mM NaCl, 5 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride (PMSF)) and homogenized using a homogenizer on ice, followed by centrifugation at 40 000g for 1 h. After centrifugation, the supernatant was collected and incubated with 3-4 mL of GST agarose (ABclonal) in a gravity column (Bio-Rad), washed with 30 mL of buffer A (50 mM HEPES, pH 8.0, 200 mM NaCl, 1 mM dithiothreitol (DTT)). The washed agarose was left oncolumn in buffer A and digested with HRV-3C protease overnight. After HRV-3C protease digestion, the flow-through containing an untagged PARP-1 ART domain was collected, concentrated, and purified by size-exclusion chromatography on a Superdex 200 10/300 GL column (GE Healthcare) pre-equilibrated with buffer C (20 mM HEPES, pH 8.0, 150 mM NaCl, 1 mM DTT). The protein peak was collected for further use.

Microscale Thermophoresis (MST) Assay. The MST assay was performed according to the supplied labeling protocol. The EFGR kinase domain (purchased from Sino Biological: Cat 10001-H20B2) and the PARP-1 ART domain were diluted with assay buffer (20 mM HEPES, pH 7.5, 150 mM NaCl) to 10 μ M and labeled with the Monolith NTTM Protein Labeling Kit RED (Cat # L001).28 After protein purification, the labeled EFGR and PARP concentration were adjusted to 200 nM. The high concentration of compounds was used as the initial concentration for subsequent gradient dilution. Mixed the different concentrations of compounds with EFGR or PARP and incubated for 10 min at room temperature. The samples were loaded into Monolith standard-treated capillaries, and the thermophoresis was measured at room temperature after 30 min incubation on a Monolith NT.115 instrument (Germany). Furthermore, the laser power was set to 40% using 15 s on-time. The light-emitting diode (LED) power was set to 100%. The dissociation constant $K_{\rm d}$ values were fitted using NT-Analysis software.²⁹

Synthesis of Compounds and Their Characterization. General Information. Compound 7 (CAS: 184475-71-6) and compound 13 (CAS: 763111-47-3) were both purchased from Bide Pharmatech Ltd. Unless otherwise noted, reagents and solvents were obtained from commercial suppliers and were used directly without further purification. Reactions were monitored by thin-layer chromatography (TLC) analysis, which was performed on aluminum plates precoated with silica gel (GF-254), and visualized by UV fluorescence ($\lambda_{max} = 254$ nm) and/or by staining with 1% w/v KMnO₄ in 0.5 M aqueous K₂CO₃. Products were purified by flash column chromatography, which was performed using silica gel (230-400 mesh). ¹H NMR spectra were recorded on a 400 MHz Bruker Avance III spectrometer. Chemical shifts for ¹H NMR spectra are reported in parts per million (ppm) from tetramethylsilane with the solvent resonance as the internal standard (CDCl₃, 7.26 ppm; DMSO- d_{6} , 2.50 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet, br = broad), coupling constants (Hz), and integration. A high-resolution mass spectrum (HRMS) was measured on a FLEX-PC (ultraflex TOF/TOF) instrument equipped with an atmospheric pressure chemical ionization (APCI) or electrospray

ionization (ESI) source in the positive-ion mode. The purity of compounds was determined by high-performance liquid chromatog-raphy (HPLC) analyses, and the purity was >95% for all tested compounds.

General Procedure for the Preparation of Compound **3**. A 25 mL reaction tube was charged with compound **1** (0.27 g, 1.0 mmol, 1.0 equiv) and 1 equiv of compound **2**. Then, 5 mL of dry DMF and 2 equiv of DIPEA were added to the mixture and the resulting mixture was stirred at 85 °C overnight. The reaction was monitored by TLC. When the reaction was completed, 50 mL of ethyl acetate was added and the mixture was washed with brine several times and then dried over Na_2SO_4 . The solvent was removed and the residue was purified with column chromatography on silica gel, eluting with petroleum ether and ethyl acetate (gradient elution from 2:1 to 1:1) to afford the corresponding product **3** as a yellow solid.

4-((2-(2-Azidoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**3a**). 0.11 g, 29% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.31 (s, 1H), 7.50 (t, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 1H), 6.50 (t, *J* = 5.6 Hz, 1H), 4.92 (dd, *J* = 12.0, 5.2 Hz, 1H), 3.73 (t, *J* = 5.2 Hz, 2H), 3.68 (t, *J* = 5.2 Hz, 2H), 3.50 (q, *J* = 5.6 Hz, 2H), 3.41 (t, *J* = 5.2 Hz, 2H), 2.87–2.72 (m, 3H), 2.14–2.10 (m, 1H).

4-((2-(2-(2-Azidoethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**3b**). 0.14 g, 33% yield. ¹H NMR (400 MHz, CDCl₃) δ 9.06 (brs, 1H), 7.49 (t, *J* = 7.8 Hz, 1H), 7.09 (dd, *J* = 7.2, 2.0 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.50 (t, *J* = 5.6 Hz, 1H), 4.96-4.92 (m, 1H), 3.74 (t, *J* = 5.2 Hz, 2H), 3.70-3.67 (m, 6H), 3.48 (q, *J* = 5.6 Hz, 2H), 3.38 (t, *J* = 4.4 Hz, 2H), 2.80-2.73 (m, 3H), 2.13-2.10 (m, 1H).

General Procedure for Amide Condensation. One equiv of acid and amine was added to 50 mL of dry dichloromethane at 0 °C, then 1.2 equiv of EDCI, HOBt, and DIPEA was added in a sequence. Then, the reaction mixture was warmed to room temperature and stirred overnight. The reaction was monitored by TLC. When the reaction was completed, 50 mL of dichloromethane and 50 mL of saturated aqueous NaHCO₃ were added. The organic phase was separated and washed with 50 mL of brine and then dried over Na₂SO₄. The solvent was removed with a rotary evaporator. The residue was purified with column chromatography on silica gel, eluting with dichloromethane and methanol to afford the corresponding amide product.

(25,4R)-1-((S)-2-(4-Azidobutanamido)-3,3-dimethylbutanoyl)-4hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**6**). **6** was prepared according to the general procedure for the amide condensation, starting from compound 4 (0.86 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound **5**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 50:1 to 20:1) to afford the corresponding product as a white solid, 0.71 g, 65% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.70 (s, 1H), 7.37–7.32 (m, 5H), 6.36 (d, *J* = 8.4 Hz, 1H), 4.70 (t, *J* = 8.0 Hz, 1H), 4.58–4.52 (m, 3H), 4.34 (dd, *J* = 15.2, 5.6 Hz, 1H), 4.06 (d, *J* = 11.6 Hz, 1H), 3.63 (dd, *J* = 11.2, 3.6 Hz, 1H), 3.32 (td, *J* = 6.4, 1.6 Hz, 2H), 2.50 (s, 3H), 2.48–2.45 (m, 1H), 2.36–2.34 (m, 2H), 2.17–2.11 (m, 1H), 1.87 (dtd, *J* = 14.2, 6.8, 3.2 Hz, 2H), 0.94 (s, 9H).

General Procedure for the Preparation of Compound 9. A 50 mL reaction tube was charged with compound 7 (0.64 g, 2.0 mmol, 1.0 equiv), 1.1 equiv of compound 8, and 2 equiv of K_2CO_3 . Then, 10 mL of acetone was added. The reaction tube was sealed and the resulting mixture was heated to refluxing and stirred overnight. The reaction was monitored by TLC. When the reaction was completed, the mixture was cooled down to room temperature and filtered. The filtrate was removed using a rotary evaporator. The residue was purified with column chromatography on silica gel, eluting with petroleum ether and ethyl acetate (1:1) to afford the corresponding product 9 as a white solid.

Ethyl 2-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)acetate (**9a**). 0.34 g, 42% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 9.66 (brs, 1H), 8.48 (d, *J* = 4.4 Hz, 1H), 8.10 (d, *J* = 6.8 Hz, 1H), 7.87 (s, 1H), 7.74 (d, *J* = 5.0 Hz, 1H), 7.15 (td, *J* =

13.2, 2.8 Hz, 1H), 7.22 (d, *J* = 3.2 Hz, 1H), 4.94 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 3.96 (s, 3H), 1.24 (t, *J* = 7.2 Hz, 3H).

Methyl 4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)butanoate (**9b**). 0.43 g, 51% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.68 (s, 1H), 8.17 (brs, 1H), 8.05–8.02 (m, 2H), 7.87 (s, 1H), 7.26 (s, 1H), 7.16 (t, *J* = 8.8 Hz, 1H), 4.31 (t, *J* = 8.0 Hz, 2H), 4.02 (s, 3H), 3.86 (s, 3H), 2.59 (t, *J* = 6.0 Hz, 2H), 2.29–2.22 (m, 2H).

Methyl 6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)hexanoate (**9c**). 0.54 g, 60% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.64 (s, 1H), 7.90 (dd, J = 6.4, 2.4 Hz, 1H), 7.83 (brs, 1H), 7.63–7.58 (m, 1H), 7.24 (d, J = 5.2 Hz, 2H), 7.15 (t, J = 8.4 Hz, 1H), 4.11 (t, J = 8.4 Hz, 2H), 3.98 (s, 3H), 3.68 (s, 3H), 2.39 (t, J = 7.2 Hz, 2H), 1.91 (p, J = 7.2 Hz, 2H), 1.74 (p, J = 7.2 Hz, 2H), 1.55 (p, J = 7.2 Hz, 2H).

General Procedure for Saponification. One equiv of ester and 5 equiv of NaOH were added to the 1:1 mixture solvent of methanol and THF. The resulting mixture was stirred at room temperature overnight. The reaction was monitored by TLC. When the reaction was completed, 2 M HCl ethyl acetate solution was added to the mixture until the pH reached 2. Then, the solvent was removed to afford the corresponding acid product, which was used directly for the next step.

2-((*4*-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)acetic Acid (**10a**). **10a** was prepared according to the general procedure for saponification, starting from compound **9a** (0.41 g, 1.0 mmol, 1.0 equiv). 0.34 g, 90% yield, white solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.15 (brs, 1H), 12.06 (s, 1H), 8.85 (s, 1H), 8.78 (s, 1H), 8.12 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.86 (ddd, *J* = 9.0, 4.4, 2.6 Hz, 1H), 7.60 (s, 1H), 7.53 (t, *J* = 13.2 Hz, 1H), 5.09 (s, 2H), 4.00 (s, 3H).

4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)butanoic Acid (10b). 10b was prepared according to the general procedure for saponification, starting from compound 9b (0.42 g, 1.0 mmol, 1.0 equiv). 0.37 g, 92% yield, white solid. ¹H NMR (400 MHz, DMSO- d_6) δ 12.03 (s, 2H), 8.84 (s, 1H), 8.69 (s, 1H), 8.10 (dd, J = 6.8, 2.4 Hz, 1H), 7.87–7.84 (m, 1H), 7.56 (s, 1H), 7.53 (t, J = 9.2 Hz, 1H), 4.32 (t, J = 6.4 Hz, 2H), 3.99 (s, 3H), 2.46 (t, J =7.2 Hz, 2H), 2.03 (p, J = 6.8 Hz, 2H).

6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)hexanoic Acid (**10c**). **10c** was prepared according to the general procedure for saponification, starting from compound **9c** (0.45 g, 1.0 mmol, 1.0 equiv). 0.42 g, 96% yield, white solid. ¹H NMR (400 MHz, DMSO- d_6) δ 12.13 (s, 2H), 8.84 (s, 1H), 8.73 (s, 1H), 8.12 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.89–7.86 (m, 1H), 7.56 (s, 1H), 7.52 (t, *J* = 8.8 Hz, 1H), 4.29 (t, *J* = 6.4 Hz, 2H), 3.98 (s, 3H), 2.26 (t, *J* = 7.2 Hz, 2H), 1.81 (p, *J* = 7.2 Hz, 2H), 1.60 (p, *J* = 7.2 Hz, 2H), 1.48 (p, *J* = 7.2 Hz, 2H).

4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(prop-2-yn-1-yl)butanamide (12). 12 was prepared according to the general procedure for the amide condensation, starting from compound **10b** (81 mg, 0.2 mmol, 1.0 equiv) and 1 equiv of compound **11**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 80:1 to 30:1) to afford the corresponding product as a white solid, 55 mg, 63% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 9.54 (brs, 1H), 8.51 (s, 1H), 8.39 (t, J = 5.2 Hz, 1H), 8.13 (dd, J = 6.8, 2.4 Hz, 1H), 7.82– 7.79 (m, 2H), 7.45 (t, J = 8.8 Hz, 1H), 7.21 (s, 1H), 4.15 (t, J = 6.0 Hz, 2H), 3.95 (s, 3H), 3.88 (dd, J = 5.2, 2.4 Hz, 2H), 3.11 (t, J = 2.4 Hz, 1H), 2.36 (t, J = 7.2 Hz, 2H), 2.07 (p, J = 6.8 Hz, 2H).

4-(4-Fluoro-3-(4-(pent-4-ynoyl))piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one (15). 15 was prepared according to the general procedure for the amide condensation, starting from compound 13 (0.73 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound 14. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 100:1 to 50:1) to afford the corresponding product as a white solid, 0.69 g, 78% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.86 (brs, 1H), 8.49–8.47 (m, 1H), 7.79–7.72 (m, 3H), 7.38–7.27 (m, 2H), 7.05 (t, *J* = 9.2 Hz, 1H), 4.30 (s, 2H), 3.80 (brs, 3H), 3.59 (brs, 2H), 3.46 (brs, 1H), 3.30 (brs, 2H), 2.62-2.55 (m, 4H), 1.99-1.97 (m, 1H).

General Procedure for the Alkylation of the Hydroxyl Group. Phenol or alcohol was treated with 2 equiv of bromide 17 and 2 equiv of K_2CO_3 or NaH in dry DMF at room temperature or at 0 °C for a few hours. The reaction was monitored by TLC. When the reaction was completed, 200 mL of ethyl acetate and 200 mL of brine were added. The organic phase was separated and washed with 50 mL of brine, and then dried over Na₂SO₄. The solvent was removed using a rotary evaporator. The residue was purified with column chromatography on silica gel, eluting with petroleum ether and ethyl acetate to afford the corresponding product.

Methyl 2-((*tert-Butoxycarbonyl*)*amino*)-3-(4-(*prop-2-yn-1-yloxy*)*phenyl*)*propanoate* (**18**). **18** was prepared according to the general procedure for the alkylation of the hydroxyl group, starting from compound **16** (5.90 g, 20 mmol, 1.0 equiv), 2 equiv of compound **17**, and 2 equiv of K₂CO₃ at room temperature for 6 h. Chromatography purification used petroleum ether and ethyl acetate as elution solvents (8:1) to afford the corresponding product as a yellow oil, 6.50 g, 98% yield. ¹H NMR (400 MHz, CDCl₃) δ 7.06 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 4.98 (d, *J* = 8.0 Hz, 1H), 4.67 (d, *J* = 2.4 Hz, 2H), 4.55 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.72 (s, 3H), 3.03 (qd, *J* = 14.0, 6.0 Hz, 2H), 2.53 (t, *J* = 2.4 Hz, 1H), 1.42 (s, 9H).

2-((tert-Butoxycarbonyl)amino)-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic Acid (19). 19 was prepared according to the general procedure for saponification, starting from compound 18 (0.66 g, 2.0 mmol, 1.0 equiv). 0.54 g, 85% yield, yellow oil, which was used directly for the next step.

tert-Butyl(1-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-1-oxo-3-(4-(prop-2-yn-1-yloxy)phenyl)propan-2-yl)carbamate (**20**). **20** was prepared according to the general procedure for the amide condensation, starting from compound **13** (0.73 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound **19**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 80:1 to 30:1) to afford the corresponding product as a white solid, 0.60 g, 45% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.76 (brs, 1H), 8.49–8.46 (m, 1H), 7.80–7.69 (m, 3H), 7.30–7.28 (m, 2H), 7.13 (t, *J* = 8.8 Hz, 2H), 7.02 (dd, *J* = 15.2, 8.8 Hz, 1H), 6.99 (dd, *J* = 15.6, 8.4 Hz, 2H), 5.44 (t, *J* = 8.0 Hz, 1H), 4.84–4.71 (m, 1H), 4.67 (dd, *J* = 10.8, 6.0 Hz, 2H), 4.28 (s, 2H), 3.83 (brs, 1H), 3.60–3.35 (m, 3H), 3.27–3.21 (m, 1H), 3.08–3.01 (m, 2H), 2.97 (dd, *J* = 13.6, 5.6 Hz, 1H), 2.89 (dd, *J* = 12.8, 5.6 Hz, 1H), 2.53 (t, *J* = 2.4 Hz, 1H), 1.42 (s, 9H).

General Procedure for the Deprotection of the Boc Group. Bocprotected amine was treated with HCl ethyl acetate solution at room temperature overnight to remove the Boc group. The reaction was monitored by TLC. When the reaction was completed, the solvent was removed to afford the corresponding product as a white solid, which was used directly for the next step.

4-(3-(4-(2-Amino-3-(4-(prop-2-yn-1-yloxy)phenyl)propanoyl)piperazine-1-carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (21). 21 was prepared according to the general procedure for the deprotection of the Boc group, starting from compound 20 (0.2 mmol, 1.0 equiv) to afford the corresponding product quantitatively as a white solid, which was used directly for the next step.

2-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(1-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-1-oxo-3-(4-(prop-2-yn-1-yloxy)phenyl)propan-2-yl)acetamide (22). 22 was prepared according to the general procedure for the amide condensation, starting from compound 10a (75 mg, 0.2 mmol, 1.0 equiv) and 1 equiv of compound 21. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 40:1 to 10:1) to afford the corresponding product as a white solid, 81 mg, 44% yield. ¹H NMR (400 MHz, CDCl₃) δ 12.58 (s, 1H), 9.50 (d, *J* = 2.4 Hz, 1H), 8.53 (s, 1H), 8.25 (d, *J* = 6.4 Hz, 1H), 8.22 (t, *J* = 6.8 Hz, 1H), 8.11 (dd, *J* = 5.2, 2.0 Hz, 1H), 7.96–7.93 (m, 1H), 7.90–7.86 (m, 1H), 7.84–7.77 (m, 3H), 7.47–7.42 (m, 2H), 7.33 (d, *J* = 4.0 Hz, 1H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.11 (dd, *J* = 11.2, 6.4 Hz, 2H), 6.81 (dd, *J* = 11.2, 6.4 Hz, 2H), 5.01 (ddd, *J* = 44.8, 13.8, 6.6 Hz, 1H),

4.71-4.69 (m, 4H), 4.32 (s, 2H), 3.94 (s, 3H), 3.56-3.44 (m, 7H), 3.11-3.08 (m, 2H), 2.97-2.84 (m, 3H).

2-((tert-Butoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoic Acid (24). 24 was prepared according to the general procedure for the alkylation of the hydroxyl group, starting from compound 23 (4.10 g, 20 mmol, 1.0 equiv), 2 equiv of compound 17, and 2 equiv of NaH at 0 °C, then the mixture was warmed to room temperature, and stirred overnight. Chromatography purification used petroleum ether and ethyl acetate as elution solvents (1:1) to afford the corresponding product as a yellow oil, 3.61 g, 74% yield. ¹H NMR (400 MHz, CDCl₃) δ 9.51 (brs, 1H), 5.42 (d, *J* = 8.4 Hz, 1H), 4.50 (dd, *J* = 5.2, 3.2 Hz, 1H), 4.19–4.18 (m, 2H), 4.00 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.81 (dd, *J* = 9.2, 3.2 Hz, 1H), 2.48 (t, *J* = 2.4 Hz, 1H), 1.46 (s, 9H).

tert-Butyl(1-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (25). 25 was prepared according to the general procedure for the amide condensation, starting from compound 13 (0.73 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound 24. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 100:1 to 50:1) to afford the corresponding product as a white solid, 0.59 g, 50% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.86 (brs, 1H), 8.49– 8.47 (m, 1H), 7.79–7.71 (m, 3H), 7.35–7.32 (m, 2H), 7.05 (t, J = 8.8 Hz, 2H), 5.51 (d, J = 8.0 Hz, 1H), 4.83 (d, J = 28.8 Hz, 1H), 4.30 (s, 2H), 4.15–4.11 (m, 2H), 3.98 (brs, 1H), 3.81–3.36 (m, 9H), 2.44 (d, J = 11.2 Hz, 1H), 1.43 (s, 9H).

4-(3-(4-(2-Amino-3-(prop-2-yn-1-yloxy)propanoyl)piperazine-1carbonyl)-4-fluorobenzyl)phthalazin-1(2H)-one (**26**). **26** was prepared according to the general procedure for the deprotection of the Boc group, starting from compound **25** (0.2 mmol, 1.0 equiv) to afford the corresponding product quantitatively as a white solid, which was used directly for the next step.

4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(1-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)butanamide (27). 27 was prepared according to the general procedure for the amide condensation, starting from compound 10b (81 mg, 0.2 mmol, 1.0 equiv) and 1 equiv of compound 26. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 40:1 to 20:1) to afford the corresponding product as a white solid, 110 mg, 64% yield. ¹H NMR (400 MHz, CDCl₃) δ 12.60 (s, 1H), 9.57 (brs, 1H), 8.51 (s, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.28-8.25 (m, 2H), 8.13 (dd, J = 6.8, 2.4 Hz, 1H), 7.98-7.91 (m, 3H), 7.87-7.78 (m, 3H), 7.40-7.36 (m, 1H), 7.24 (t, J = 9.2 Hz, 1H), 5.00–4.90 (m, 1H), 4.32 (s, 2H), 4.16 (s, 4H), 3.95 (s, 3H), 3.63-3.53 (m, 8H), 3.47 (s, 2H), 3.16-3.13 (m, 2H), 2.39 (q, J = 6.8 Hz, 2H), 2.05 (q, J = 6.0 Hz, 2H).

tert-Butyl(2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethyl)carbamate (**29a**). **29a** was prepared according to the general procedure for the amide condensation, starting from compound **13** (0.73 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound **28a**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 40:1 to 20:1) to afford the corresponding product as a white solid, 0.86 g, 82% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.91 (brs, 1H), 8.49–8.47 (m, 1H), 7.79–7.73 (m, 3H), 7.36–7.33 (m, 2H), 7.05 (t, *J* = 8.8 Hz, 1H), 5.51 (d, *J* = 16.0 Hz, 1H), 4.30 (s, 2H), 4.03–3.94 (m, 2H), 3.79 (brs, 3H), 3.60 (brs, 1H), 3.50 (brs, 1H), 3.37–3.33 (m, 3H), 1.45 (s, 9H).

tert-Butyl(4-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-4-oxobutyl)carbamate (**29b**). **29b** was prepared according to the general procedure for the amide condensation, starting from compound **13** (0.73 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound **28b**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 40:1 to 20:1) to afford the corresponding product as a white solid, 0.84 g, 76% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.94 (brs, 1H), 8.49–8.46 (m, 1H), 7.78–7.73 (m, 3H), 7.34–7.27 (m, 2H), 7.05 (t, J = 8.8 Hz, 1H), 4.81 (brs, 1H), 4.30 (s, 2H), 4.38 (brs, 3H), 3.57–3.56 (m, 2H), 3.43 (brs, 1H), 3.30 (brs, 2H), 3.20–3.17 pubs.acs.org/jmc

(m, 2H), 2.42 (t, *J* = 6.8 Hz, 1H), 2.35 (t, *J* = 6.8 Hz, 1H), 1.88–1.81 (m, 2H), 1.44 (s, 9H).

tert-Butyl(6-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-6-oxohexyl)carbamate (**29c**). **29c** was prepared according to the general procedure for the amide condensation, starting from compound **13** (0.73 g, 2.0 mmol, 1.0 equiv) and 1 equiv of compound **28c**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 40:1 to 20:1) to afford the corresponding product as a white solid, 0.98 g, 85% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.80 (brs, 1H), 8.49–8.47 (m, 1H), 7.78–7.72 (m, 3H), 7.33 (brs, 2H), 7.05 (t, *J* = 8.8 Hz, 1H), 4.60 (brs, 1H), 4.30 (s, 2H), 3.78 (brs, 3H), 3.57 (brs, 2H), 3.43 (brs, 1H), 3.29 (brs, 2H), 3.11 (brs, 2H), 2.37– 2.30 (m, 2H), 1.66–1.65 (m, 2H), 1.51–1.50 (m, 2H), 1.44 (s, 9H), 1.37–1.36 (m, 2H).

30 was prepared according to the general procedure for the deprotection of the Boc group, starting from compound **29** (0.2 mmol, 1.0 equiv) to afford the corresponding product quantitatively as a white solid, which was used directly for the next step.

tert-Butyl(1-((2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethyl)amino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (**31a**). **31a** was prepared according to the general procedure for amide condensation, starting from compound **24** (0.24 g, 1.0 mmol, 1.0 equiv) and 1 equiv of compound **30a**. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 30:1 to 15:1) to afford the corresponding product as a white solid, 0.27 g, 50% yield. ¹H NMR (400 MHz, CDCl₃) δ 11.27 (d, *J* = 12.0 Hz, 1H), 8.49–8.46 (m, 1H), 7.78–7.77 (m, 2H), 7.75–7.73 (m, 1H), 7.44 (d, *J* = 12.8 Hz, 1H), 7.36–7.28 (m, 2H), 7.05 (t, *J* = 9.2 Hz, 1H), 5.50 (d, *J* = 6.8 Hz, 1H), 4.39 (brs, 1H), 4.31 (s, 2H), 4.17–4.08 (m, 4H), 3.98 (brs, 1H), 3.79 (brs, 2H), 3.72–3.68 (m, 1H), 3.61 (brs, 1H), 3.53 (brs, 1H), 3.41 (brs, 1H), 3.33 (brs, 2H), 2.46 (q, *J* = 2.4 Hz, 1H), 1.46 (s, 9H).

tert-Butyl(1-((4-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1yl)methyl)benzoyl)piperazin-1-yl)-4-oxobutyl)amino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (31b). 31b was prepared according to the general procedure for the amide condensation, starting from compound 24 (0.24 g, 1.0 mmol, 1.0 equiv) and 1 equiv of compound 30b. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 30:1 to 15:1) to afford the corresponding product as a white solid, 0.35 g, 61% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.60 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.90 (t, J = 7.6 Hz, 2H), 7.84 (t, J = 7.6 Hz, 1H), 7.44 (brs, 1H), 7.37 (t, J = 6.4 Hz, 1H), 7.24 (t, J = 9.2 Hz, 1H), 6.81 (t, J = 6.4 Hz, 1H), 4.33 (s, 2H), 4.12 (d, J = 12.4 Hz, 2H), 4.06-4.02 (m, 1H), 3.64 (brs, 1H), 3.58-3.46 (m, 5H), 3.41–3.38 (m, 1H), 3.19 (brs, 1H), 3.14 (brs, 1H), 3.08 (brs, 2H), 2.33 (t, J = 6.8 Hz, 1H), 2.26 (t, J = 6.8 Hz, 1H), 1.63 (q, J = 5.6 Hz, 1H), 1.35 (d, J = 24.8 Hz, 9H), 1.24 (s, 2H).

tert-Butyl(1-((6-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1yl)methyl)benzoyl)piperazin-1-yl)-6-oxohexyl)amino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)carbamate (31c). 31c was prepared according to the general procedure for the amide condensation, starting from compound 24 (0.24 g, 1.0 mmol, 1.0 equiv) and 1 equiv of compound 30c. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 30:1 to 15:1) to afford the corresponding product as a white solid, 0.46 g, 76% yield. ¹H NMR (400 MHz, CDCl₃) δ 11.06 (d, J = 46.8 Hz, 1H), 8.49-8.46 (m, 1H), 7.79-7.70 (m, 3H), 7.34-7.32 (m, 2H), 7.05 (t, J = 9.2 Hz, 1H), 6.57 (brs, 1H), 5.51-5.46 (m, 1H), 4.30 (s, 2H), 4.28-4.23 (m, 1H), 4.19-4.11 (m, 2H), 3.93-3.89 (m, 1H), 3.70-3.66 (m, 4H), 3.55 (brs, 2H), 3.43 (brs, 1H), 3.28 (brs, 4H), 2.48 (brs, 1H), 2.37 (t, J = 7.2 Hz, 1H), 2.29 (t, J = 7.2 Hz, 1H), 1.66 (p, J = 7.2 Hz, 2H), 1.55 (p, J = 7.2 Hz, 2H), 1.45 (s, 9H), 1.38 (p, J = 7.2 Hz, 2H).

32 was prepared according to the general procedure for the deprotection of the Boc group, starting from compound 31 (0.2 mmol, 1.0 equiv) to afford the corresponding product quantitatively as a white solid, which was used directly for the next step.

4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(1-((4-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-4-oxobutyl)amino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)butanamide (33a). 33a was prepared according to the general procedure for the amide condensation, starting from compound 10b (40 mg, 0.1 mmol, 1.0 equiv) and 1 equiv of compound 32b. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 30:1 to 10:1) to afford the corresponding product as a white solid, 37 mg, 38% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.60 (s, 1H), 9.56 (s, 1H), 8.51 (s, 1H), 8.26 (dd, J = 7.6, 1.2 Hz, 1H), 8.15 (t, J = 7.6 Hz, 1H), 8.13 (dd, J = 7.2, 2.4 Hz, 1H), 8.04-7.99 (m, 1H), 7.95 (t, J = 8.4 Hz, 1H), 7.88 (t, J = 7.6 Hz, 1H), 7.85-7.77 (m, 3H), 7.46-7.42 (m, 2H), 7.36 (brs, 1H), 7.25–7.21 (m, 2H), 4.43 (p, J = 6.8 Hz, 1H), 4.32 (s, 2H), 4.15-4.11 (m, 4H), 3.94 (s, 3H), 3.60-3.57 (m, 4H), 3.50-3.99 (m, 4H), 3.30 (brs, 1H), 3.19 (brs, 1H), 3.13 (brs, 1H), 3.09-3.03 (m, 2H), 2.43-2.41 (m, 2H), 2.31 (t, J = 7.2 Hz, 1H), 2.24 (t, J = 7.2 Hz, 1H), 2.05 (p, J = 7.2 Hz, 2H), 1.61 (brs, 2H).

6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(1-((2-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethyl)amino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)hexanamide (33b). 33b was prepared according to the general procedure for the amide condensation, starting from compound 10c (43 mg, 0.1 mmol, 1.0 equiv) and 1 equiv of compound 32a. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 30:1 to 10:1) to afford the corresponding product as a white solid, 43 mg, 45% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.60 (s, 1H), 9.77 (s, 1H), 8.54 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 8.14-8.10 (m, 1H), 8.02 (t, J = 4.8 Hz, 1H), 7.97–7.95 (m, 1H), 7.90–7.78 (m, 3H), 7.45 (t, J = 8.8 Hz, 2H), 7.36 (brs, 1H), 7.24 (brs, 1H), 7.21 (s, 1H), 4.56 (brs, 1H), 4.33 (s, 2H), 4.16-4.13 (m, 3H), 3.99 (brs, 1H), 3.95 (s, 3H), 3.63-3.44 (m, 6H), 3.19 (brs, 1H), 3.13 (brs, 1H), 3.05-2.99 (m, 1H), 2.75 (d, J = 4.4 Hz, 1H), 2.22 (t, J = 6.0 Hz, 2H), 1.86–1.82 (m, 2H), 1.61 (t, J = 6.4 Hz, 2H), 1.49 (p, J = 7.6 Hz, 2H).

6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(1-((6-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-6-oxohexyl)amino)-1-oxo-3-(prop-2-yn-1-yloxy)propan-2-yl)hexanamide (33c). 33c was prepared according to the general procedure for the amide condensation, starting from compound 10c (43 mg, 0.1 mmol, 1.0 equiv) and 1 equiv of compound 32c. Chromatography purification used dichloromethane and methanol as elution solvents (gradient elution from 30:1 to 10:1) to afford the corresponding product as a white solid, 53 mg, 52% yield. ¹H NMR (400 MHz, CDCl₃) δ 11.91 (d, J = 53.2 Hz, 1H), 9.15 (s, 1H), 8.58 (s, 1H), 8.40 (d, J = 7.2, 1H), 7.88-7.86 (m, 1H), 7.74-7.67 (m, 4H), 7.64-7.62 (m, 2H), 7.30-7.28 (m, 2H), 7.23-7.19 (m, 2H), 7.09–6.99 (m, 2H), 4.70–4.64 (m, 1H), 4.25 (s, 2H), 4.15-4.00 (m, 2H), 3.99 (brs, 2H), 3.94 (s, 3H), 3.82-3.65 (m, 6H), 3.51 (brs, 3H), 3.38-3.21 (m, 6H), 2.45 (s, 1H), 2.35-2.23 (m, 4H), 1.81 (t, J = 6.8 Hz, 2H), 1.66 (t, J = 6.8 Hz, 2H), 1.57–1.43 (m, 5H).

General Procedure for Copper-Promoted Click Reaction to Access the Corresponding Mono and Dual PROTACs. A 5 mL reaction tube was charged with 1.0 equiv of alkyne and 1 equiv of azide. Then, 2 mL of THF was added. The reaction mixture was stirred at room temperature. One equiv of CuSO4 and 2 equiv of sodium ascorbate were dissolved in 0.5 mL of water and the solution color became brown. The brown aqueous solution was added dropwise to the THF solution. Then, the resulting mixture was stirred at room temperature for 0.5-3 h. The reaction was monitored by TLC. When the reaction was completed, 10 mL of ethyl acetate and 10 mL of brine were added. The organic phase was separated and dried over Na2SO4. The solvent was removed using a rotary evaporator. The residue was purified with column chromatography on silica gel, eluting with dichloromethane and methanol (gradient elution from 30:1 to 8:1) to afford the corresponding PROTAC products as a yellow or white solid. In the case of Mono PROTACs MP-GC, MP-GV, MP-PC, MP-PV, the click reaction was performed on a 0.05 mmol scale, while the reaction for dual PROTACs DP-C 1-4 and DP-V 1-4 was performed on a 0.02 mmol scale.

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4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-((1-(2-(2-(2-(2-(6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methyl)butanamide (**MP-GC**). Yellow solid, 13 mg, 32% yield. ¹H NMR (400 MHz, DMSO-d₆) δ 11.10 (s, 1H), 9.59 (s, 1H), 8.51 (s, 1H), 8.42 (t, *J* = 5.6 Hz, 1H), 8.13 (dd, *J* = 6.8, 2.4 Hz, 1H), 7.89 (s, 1H), 7.82 (s, 1H), 7.80–7.77 (m, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 7.44 (t, *J* = 9.2 Hz, 1H), 7.20 (s, 1H), 7.07 (d, *J* = 8.8 Hz, 1H), 7.02 (d, *J* = 7.2 Hz, 1H), 6.56 (t, *J* = 6.0 Hz, 1H), 5.06 (dd, *J* = 12.8, 5.6 Hz, 1H), 4.49 (t, *J* = 4.8 Hz, 2H), 4.31 (d, *J* = 5.6 Hz, 2H), 4.15 (t, *J* = 6.0 Hz, 2H), 3.94 (s, 3H), 3.80 (t, *J* = 5.2 Hz, 2H), 3.58 (t, *J* = 5.2 Hz, 2H), 3.41 (dd, *J* = 10.8, 5.2 Hz, 2H), 2.94–2.84 (m, 1H), 2.61–2.54 (m, 2H), 2.36 (t, *J* = 7.2 Hz, 2H), 2.10–2.02 (m, 3H). HRMS (pos. ESI): m/z [M + H]⁺ for C₃₉H₃₈CIFN₁₀O₈ calcd: 829.2619, found: 829.2631.

2-(2,6-Dioxopiperidin-3-yl)-4-((2-(2-(4-(3-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)ethoxy)ethyl)amino)isoindoline-1,3-dione (**MP-OC**). Yellow solid, 16 mg, 39% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.61 (s, 1H), 11.11 (s, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.89 (td, *J* = 8.0, 1.6 Hz, 1H), 7.85–7.80 (m, 2H), 7.56 (dd, *J* = 12.8, 7.6 Hz, 1H), 7.46–7.43 (m, 1H), 7.37 (d, *J* = 6.4 Hz, 1H), 7.24 (t, *J* = 8.8 Hz, 1H), 7.11 (dd, *J* = 8.4, 5.6 Hz, 1H), 7.03 (dd, *J* = 6.8, 4.4 Hz, 1H), 6.58 (d, *J* = 4.4 Hz, 1H), 5.07 (dd, *J* = 12.8, 5.6 Hz, 1H), 3.45 (brs, 2H), 3.37 (brs, 1H), 3.30 (brs, 1H), 3.16 (brs, 2H), 2.90–2.82 (m, 3H), 2.72–2.54 (m, 4H), 2.06–2.03 (m, 1H). HRMS (pos. ESI): m/z [M + H]⁺ for C₄₂H₄₂FN₁₀O₈ calcd: 833.3166, found: 833.3161.

(25,4R)-1-((S)-2-(4-(4-((4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)butanamido)methyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**MP-GV**). White solid, 17 mg, 35% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.95 (brs, 1H), 8.67 (s, 1H), 8.61 (s, 1H), 8.04 (d, *J* = 4.4 Hz, 1H), 7.96 (brs, 1H), 7.88 (s, 1H), 7.46 (s, 1H), 7.36–7.29 (m, 5H), 7.20 (s, 1H), 7.14 (t, *J* = 8.8 Hz, 1H), 7.07 (d, *J* = 5.6 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 4.74 (t, *J* = 4.8 Hz, 1H), 4.59–4.47 (m, 4H), 4.44 (d, *J* = 8.8 Hz, 1H), 4.38–4.34 (m, 2H), 4.25–4.22 (m, 1H), 4.19–4.15 (m, 2H), 4.07 (d, *J* = 12.0 Hz, 1H), 3.97 (s, 3H), 3.62 (dd, *J* = 11.2, 3.2 Hz, 1H), 2.50 (s, 3H), 2.45 (t, *J* = 4.8 Hz, 3H), 2.22–2.01 (m, 7H), 0.93 (s, 9H). HRMS (pos. ESI): m/z [M + H]⁺ for C₄₈H₅₆ClFN₁₁O₇S calcd: 984.3752, found: 984.3759.

(25,4*R*)-1-((5)-2-(4-(4-(3-(4-(2-*F*luoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl))methyl)benzoyl)piperazin-1-yl)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**MP-OV**). White solid, 14 mg, 36% yield. ¹H NMR (400 MHz, CDCl₃) δ 11.33 (brs, 1H), 8.69 (s, 1H), 8.43 (s, 1H), 7.78–7.71 (m, 4H), 7.44–7.29 (m, 6H), 7.24–7.19 (m, 2H), 7.07–7.00 (m, 1H), 4.78–4.72 (m, 1H), 4.64–4.55 (m, 2H), 4.34–4.22 (m, 5H), 4.15 (t, *J* = 11.2 Hz, 1H), 3.70–3.67 (m, 2H), 3.48 (brs, 2H), 3.31 (brs, 1H), 3.17 (brs, 1H), 3.02 (brs, 2H), 2.76 (brs, 2H), 2.48 (s, 3H), 2.43–2.07 (m, 10H), 0.99 (s, 9H). HRMS (pos. ESI): m/z [M + Na]⁺ for C₅₁H₅₈FN₁₁NaO₇S calcd: 1010.4118, found: 1010.4128.

2-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(3-(4-((1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-1-oxopropan-2-yl)acetamide (DP-C-1). Yellow solid, 11 mg, 42% yield. ¹H NMR (400 MHz, DMSOd₆) δ 12.58 (s, 1H), 11.08 (s, 1H), 9.49 (s, 1H), 8.53 (s, 1H), 8.25 (d, J = 5.2 Hz, 1H), 8.21 (t, J = 6.8 Hz, 1H), 8.11–8.10 (m, 2H), 7.94 (t, J = 7.2 Hz, 1H), 7.87 (t, J = 7.2 Hz, 1H), 7.84–7.82 (m, 1H), 7.80– 7.76 (m, 1H), 7.54 (t, J = 6.0 Hz, 1H), 7.44–7.42 (m, 2H), 7.35 (brs, 1H), 7.22 (t, J = 6.8 Hz, 1H), 7.13–7.07 (m, 3H), 7.01 (d, J = 6.0 Hz, 1H), 6.84 (t, J = 7.6 Hz, 2H), 6.57 (t, J = 4.8 Hz, 1H), 5.04-4.99 (m, 4H), 4.70 (brs, 2H), 4.54 (brs, 2H), 4.32 (brs, 2H), 4.06-4.02 (m, 1H), 3.96 (s, 3H), 3.84 (t, J = 4.0 Hz, 2H), 3.60 (t, J = 4.0 Hz, 2H), 3.51 (brs, 2H), 3.43 (q, J = 4.0 Hz, 4H), 3.10 (brs, 2H), 2.94-2.81 (m, 4H), 1.99–1.97 (m, 2H), 1.76–1.71 (m, 1H). HRMS (pos. ESI):

 $m/z [M + H]^+$ for $C_{66}H_{60}ClF_2N_{14}O_{12}$ calcd: 1313.4166, found:1313.4152.

4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6yl)oxy)-N-(3-((1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-1-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-1-oxopropan-2-yl)butanamide (DP-C-2). Yellow solid, 12 mg, 48% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.59 (s, 1H), 11.09 (s, 1H), 9.52 (s, 1H), 8.50 (s, 1H), 8.32 (d, J = 8.0 Hz, 1H), 8.26-8.24 (m, 1H), 8.11 (dd, J = 6.8, 2.4 Hz, 1H), 8.01-7.99 (m, 1H), 7.94–7.92 (m, 1H), 7.88 (td, J = 7.2, 1.2 Hz, 1H), 7.82– 7.76 (m, 2H), 7.53 (t, J = 8.0 Hz, 1H), 7.45-7.41 (m, 2H), 7.35 (brs, 1H), 7.20 (brs, 2H), 7.08–7.02 (m, 2H), 7.01 (d, J = 7.2 Hz, 1H), 6.56 (brs, 1H), 5.05 (dd, J = 12.8, 5.2 Hz, 1H), 4.97-4.92 (m, 1H), 4.50 (brs, 4H), 4.32 (brs, 2H), 4.14-4.13 (m, 2H), 3.94 (s, 3H), 3.83 (brs, 2H), 3.58-3.42 (m, 12H), 3.12 (brs, 2H), 2.93-2.85 (m, 1H), 2.51-2.50 (m, 2H), 2.49-2.36 (m, 2H), 2.04 (brs, 2H), 1.83-1.81 (m, 1H). HRMS (pos. ESI): $m/z [M + H]^+$ for $C_{62}H_{60}ClF_2N_{14}O_{12}$ calcd: 1265.4166, found: 1265.4172.

4-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyguinazolin-6yl)oxy)-N-(3-((1-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-1-((4-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzovl)piperazin-1-vl)-4-oxobutvl)amino)-1-oxopropan-2-vl)butanamide (DP-C-3). Yellow solid, 12 mg, 45% yield. ¹H NMR (400 MHz, DMSO-d₆) δ 12.59 (s, 1H), 11.10 (s, 1H), 9.56 (s, 1H), 8.50 (s, 1H), 8.25 (d, J = 7.6 Hz, 1H), 8.12 (dd, J = 6.8, 2.4 Hz, 2H), 8.00-7.76 (m, 6H), 7.53 (t, J = 8.0 Hz, 1H), 7.43 (t, J = 8.8 Hz, 2H), 7.35 (brs, 1H), 7.24–7.19 (m, 2H), 7.07 (d, J = 8.8 Hz, 1H), 7.01 (d, J = 7.2 Hz, 1H), 6.56 (t, J = 5.6 Hz, 1H), 5.05 (dd, J = 12.8, 5.2 Hz, 1H), 4.50–4.42 (m, 4H), 4.31 (d, J = 7.6 Hz, 2H), 4.12 (t, J = 5.2 Hz, 2H), 3.94 (s, 3H), 3.83 (brs, 2H), 3.58-3.57 (m, 5H), 3.49-3.42 (m, 5H), 3.29 (brs, 1H), 3.17 (brs, 1H), 3.12 (brs, 1H), 3.05-3.04 (m, 2H), 2.93-2.85 (m, 1H), 2.51-2.50 (m, 2H), 2.42 (t, J = 8.0 Hz, 2H), 2.28 (t, J = 7.2 Hz, 1H), 2.21 (t, J = 7.2 Hz, 1H), 2.03 (brs, 3H), 1.59 (brs, 2H), 1.23 (brs, 2H). HRMS (pos. ESI): m/z [M + H]⁺ for C₆₆H₆₇ClF₂N₁₅O₁₃ calcd: 1350.4694, found: 1350.4681.

6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-soindolin-4-yl)amino)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)-1-((6-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-6-oxohexyl)amino)-1-oxopropan-2-yl)hexanamide (DP-C-4). Yellow solid, 12 mg, 41% yield. ¹H NMR $(400 \text{ MHz}, \text{CDCl}_3) \delta 11.06 \text{ (d, } J = 16.08 \text{ Hz}, 1\text{H}), 10.01 \text{ (s, 1H)}, 8.99$ (s, 1H), 8.59 (s, 1H), 8.41 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 6.4 Hz, 1H), 7.74-7.70 (m, 4H), 7.59-7.57 (m, 2H), 7.46 (t, I = 8.0 Hz, 1H), 7.33–7.28 (m, 3H), 7.08–7.04 (m, 4H), 6.86 (dd, J = 8.8, 4.4 Hz, 1H), 6.50 (d, J = 4.4 Hz, 1H), 4.94-4.89 (m, 1H), 4.54-4.49 (m, 5H), 4.25 (s, 2H), 4.02 (t, J = 7.2 Hz, 2H), 3.98 (s, 3H), 3.84 (brs, 3H), 3.68-3.64 (m, 5H), 3.60 (s, 4H), 3.52 (brs, 2H), 3.36 (brs, 2H), 3.28 (brs, 2H), 3.21 (brs, 3H), 2.80-2.70 (m, 2H), 2.32-2.29 (m, 3H), 2.22-2.21 (m, 1H), 1.84 (brs, 2H), 1.69 (brs, 2H), 1.57-1.43 (m, 7H). HRMS (pos. ESI): $m/z [M + H]^+$ for $C_{72}H_{79}ClF_2N_{15}O_{14}$ calcd: 1450.5582, found: 1450.5536.

(2S,4R)-1-((2S)-2-(4-(4-((4-(2-(2-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)acetamido)-3-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1yl)-3-oxopropyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DP-V-1). White solid, 14 mg, 48% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.60 (s, 1H), 9.54 (s, 1H), 8.98 (s, 1H), 8.57 (t, J = 6.0 Hz, 1H), 8.54 (s, 1H), 8.25 (d, J = 7.6 Hz, 2H), 8.18 (d, J = 7.6 Hz, 1H), 8.11 (dd, J = 6.8, 2.4 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.94 (t, J = 7.2 Hz, 1H), 7.89-7.78 (m, 4H), 7.47-7.37 (m, 6H), 7.26 (brs, 1H), 7.23 (t, J = 8.8 Hz, 1H), 7.11 (t, J = 8.8 Hz, 2H), 6.86 (t, J = 8.4 Hz, 2H), 5.14 (d, J = 3.2 Hz, 1H), 5.06-4.98 (m, 3H), 4.71 (brs, 2H), 4.54 (d, J = 9.2 Hz, 1H), 4.46-4.41 (m, 2H), 4.35-4.33 (m, 5H), 4.21 (dd, J = 16.0, 5.2 Hz, 1H), 3.96 (s, 3H), 3.66-3.43 (m, 7H), 3.11 (brs, 2H), 2.93-2.85 (m, 3H), 2.44 (s, 3H), 2.30-2.15 (m, 2H), 2.04-2.02 (m, 3H), 0.94 (s, 9H). HRMS (pos. ESI): m/z [M + H]⁺ for C₇₅H₇₇ClF₂N₁₅O₁₁S calcd: 1468.5299, found: 1468.5287.

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(2S,4R)-1-((2S)-2-(4-(4-((2-(4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)butanamido)-3-(4-(2-fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-3-oxopropoxy)methyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DP-V-2). White solid, 10 mg, 36% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.60 (s, 1H), 9.57 (s, 1H), 8.98 (s, 1H), 8.57 (t, J = 6.0 Hz, 1H), 8.51 (s, 1H), 8.34 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 7.6 Hz, 1H), 8.11 (dd, J = 6.8, 2.4 Hz, 1H), 8.05 (d, *J* = 13.6 Hz, 1H), 7.99 (d, *J* = 9.2 Hz, 1H), 7.97–7.93 (m, 1H), 7.88 (td, I = 8.0, 1.2 Hz, 1H), 7.84-7.76 (m, 3H), 7.46-7.35 (m, 7H),7.21 (s, 2H), 5.13 (d, J = 3.6 Hz, 1H), 4.97-4.92 (m, 1H), 4.55-4.50 (m, 3H), 4.46-4.41 (m, 2H), 4.32 (brs, 5H), 4.22 (dd, J = 16.0, 5.6 Hz, 1H), 4.14 (d, J = 5.6 Hz, 2H), 3.94 (s, 3H), 3.65–3.41 (m, 10H), 3.13 (brs, 2H), 2.44 (s, 3H), 2.38 (d, J = 6.4 Hz, 2H), 2.28-2.15 (m, 2H), 2.03-2.01 (m, 5H), 0.92 (s, 9H). HRMS (pos. ESI): m/z [M + H^{+} for $C_{71}H_{77}ClF_{2}N_{15}O_{11}S$ calcd: 1420.5299, found: 1420.5281.

(2S,4R)-1-((2S)-2-(4-(4-((2-(6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxvauinazolin-6-vl)oxv)hexanamido)-3-((2-(4-(2fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-2-oxoethyl)amino)-3-oxopropoxy)methyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DP-**V-3**). White solid, 12 mg, 41% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.60 (s, 1H), 10.05 (brs, 1H), 8.98 (s, 1H), 8.59 (brs, 2H), 8.25 (d, I = 7.6 Hz, 1H), 8.13 (d, I = 7.6 Hz, 1H), 8.11-8.08 (m, 2H),8.03-7.99 (m, 2H), 7.97-7.93 (m, 2H), 7.89 (brs, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.79-7.76 (m, 1H), 7.45-7.36 (m, 6H), 7.22 (s, 2H), 5.15 (brs, 1H), 4.55-4.52 (m, 4H), 4.45-4.50 (m, 2H), 4.34-4.30 (m, 5H), 4.21 (dd, J = 16.0, 5.6 Hz, 1H), 4.14 (t, J = 6.4 Hz, 2H), 3.99 (brs, 2H), 3.95 (s, 3H), 3.64-3.57 (m, 6H), 3.51-3.46 (m, 2H), 3.19 (brs, 1H), 3.13 (brs, 1H), 2.43 (s, 3H), 2.28-2.15 (m, 5H), 2.06-2.01 (m, 3H), 1.83-1.81 (m, 3H), 1.60 (brs, 2H), 1.46 (brs, 2H), 0.92 (s, 9H). HRMS (pos. ESI): m/z [M + H]⁺ for C₇₅H₈₄ClF₂N₁₆O₁₂S calcd: 1505.5826, found: 1505.5829.

(2S,4R)-1-((2S)-2-(4-(4-((2-(6-((4-((3-Chloro-4-fluorophenyl)amino)-7-methoxyquinazolin-6-yl)oxy)hexanamido)-3-((6-(4-(2fluoro-5-((4-oxo-3,4-dihydrophthalazin-1-yl)methyl)benzoyl)piperazin-1-yl)-6-oxohexyl)amino)-3-oxopropoxy)methyl)-1H-1,2,3-triazol-1-yl)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DP-V-4). White solid, 11 mg, 35% yield. ¹H NMR (400 MHz, DMSO- d_6) δ 12.60 (s, 1H), 10.29 (brs, 2H), 8.98 (s, 1H), 8.65 (s, 1H), 8.59 (t, J = 6.0 Hz, 1H), 8.25 (d, J = 7.6 Hz, 1H), 8.08 (dd, J = 6.8, 2.0 Hz, 1H), 8.05 (brs, 1H), 8.02-7.94 (m, 4H), 7.88 (t, J = 7.6 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 7.78–7.76 (m, 1H), 7.49 (t, J = 8,8 Hz, 1H), 7.42-7.36 (m, 5H), 7.21-7.21 (m, 2H), 5.15 (brs, 1H), 4.55-4.40 (m, 5H), 4.32 (brs, 5H), 4.21 (dd, J = 15.6, 5.6 Hz, 1H), 4.14 (t, J = 5.2 Hz, 2H), 3.96 (s, 3H), 3.65–3.62 (m, 3H), 3.55–3.49 (m, 5H), 3.15 (d, J = 16.4 Hz, 3H), 3.02 (brs, 3H), 2.43 (s, 3H), 2.28-2.19 (m, 7H), 2.06-2.00 (m, 3H), 1.89-1.83 (m, 1H), 1.82 (brs, 2H), 1.58 (brs, 2H), 1.44-1.34 (m, 7H), 0.92 (s, 9H). HRMS (pos. ESI): m/z $[M + H]^+$ for $C_{79}H_{92}ClF_2N_{16}O_{12}S$ calcd: 1561.6452, found: 1561.6462.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00649.

¹H NMR spectra of compounds in the text, HRMS spectra of MP-GC, MP-OC, MP-GV, MP-OV, DP-C 1-4 and DP-V 1-4 and HPLC for purity determination of DP-C 1-4, DP-V 1-4, MP-GC, MP-OC, MP-GV, and MP-OV (PDF)

Molecular formula strings (CSV)

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The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (NSFC) (grant numbers 81773637, U1803122, and 81903863), the National Mega-project for Innovative Drugs (grant number 2019ZX09721001-004-007, China), the Chunhui Program-Cooperative Research Project of the Ministry of Education, the Natural Science Foundation of Hubei Province (no. 2020CFB642), the 100 Talents Program of the Hubei Provincial Government, the Liaoning Revitalization Talents Program (no. XLYC1807182), and the Liaoning Province Natural Science Foundation (no. 2020-MZLH-31). The Center of Analysis and Testing of Huazhong University of Science and Technology is gratefully acknowledged for the characterization of the new compounds. We thank Kexing Li from the School of Life Sciences, Tsinghua University, for English editing help.

ABBREVIATIONS

EDCI, 1-ethyl-3(3-dimethylpropylamine)carbodiimide; HOBt, 1-hydroxybenzotriazole; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMSO, dimethyl sulfoxide

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