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Design, Synthesis and Biological Evaluation of Proteolysis Targeting Chimeras (PROTACs) as a BTK Degraders with Improved Pharmacokinetic Properties

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Abstract

A new series of Proteolysis Targeting Chimeras (PROTACs) targeting Bruton's Tyrosine Kinase (BTK) was synthesized, with the goal of improving the pharmacokinetic properties of our previously reported PROTAC, MT802. We recently described the ability of MT802 to induce degradation of both wild-type and C481S mutant BTK in immortalized cells and patient-derived B-lymphocytes. However, the pharmacokinetic properties of MT802 were not suitable for further *in vivo* development. Therefore, we undertook a systematic medicinal chemistry campaign to overcome this issue and made a series of PROTACs with structural modifications to the linker and E3-recruiting ligand; more specifically, the new PROTACs were synthesized with different von Hippel-Lindau (VHL) and cereblon (CRBN) ligands while keeping the BTK ligand and linker length constant. This approach resulted in an equally potent PROTAC, SJF620, with a significantly better pharmacokinetic profile than MT802. This compound may hold promise for further *in vivo* exploration of BTK degradation.

Graphical Abstract

Disclosures

C.M.C. is consultant and shareholder in Arvinas, Inc., which supports research in his lab.

Declaration of interests

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

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Keywords

PROTACs; Pharmacokinetics; Target protein degradation; BTK; CRBN; VHL

1. Introduction

Bruton's Tyrosine Kinase (BTK) plays a critical role in all aspects of B cell development, including cell growth, maturation, migration, and apoptosis^{1, 2}. Dysregulation of any of these pathways is associated with conditions such as cancer, autoimmunity and inflammation³. Therefore, development of reversible and irreversible inhibitors, has drawn the attention of many researchers and pharmaceutical companies in the last two decades^{3, 4}. As a result of these development efforts, two irreversible BTK inhibitors, ibrutinib and acalabrutinib, have garnered FDA approval (Fig. 1A) and many others are in clinical development^{3, 4}. These inhibitors exploit a cysteine (Cys481) within the active site of BTK to bind covalently to the kinase and inhibit its catalytic activity (Fig. 1B). Since this cysteine is conserved among several other kinases, such as EGFR and ITK, ibrutinib is a relatively promiscuous inhibitor with many side-effects. This realization prompted the development of acalabrutinib, a more potent and BTK selective inhibitor than ibrutinib⁵. However, in chronic lymphocytic leukemia (CLL) patients subjected to prolonged BTK inhibition, drug resistance often arises via mutation of the active site cysteine to serine (C481S)⁶. This renders covalent inhibitors capable of only reversibly binding to BTK. As a result, ibrutinib's IC₅₀ for BTK inhibition shifts from 2.2 nM to 1 μ M⁷. Most recently, several promising reversible inhibitors of BTK, most notably GDC-0853, have been applied to CLL patients with varying degrees of success (Fig. 1B)^{7, 8}. Despite such efforts, no effective second-line treatment options exist for relapsed CLL patients and outcome is poor. As a result, there is still a need for new approaches to address drug resistance in CLL.

Our group has pioneered the development of PROteolysis TARgeting Chimeraes (PROTACs), a technology for the targeted degradation of disease-relevant proteins⁹. Conceptually, PROTACs are hetero-bifunctional small molecules that simultaneously engage an E3 ubiquitin ligase and a target protein to be degraded. Bringing these two proteins into proximity results in a *de-novo* complex suitable for interfacing with the protein machinery required for ubiquitination. Once ubiquitinated, this 'neo-substrate' is then recognized by the proteasome and degraded (Fig. 2).

The PROTAC mechanism can be summarized as follows: **(1)** PROTACs are composed of three elements: an E3 ligand, a linker, and a ligand for the target protein. **(2)** An effective

PROTAC induces a complex between the target (e.g. BTK) and E3 ligase (e.g. CRBN or VHL) forming a ternary complex. (3) An E2 enzyme catalyzes transfer of ubiquitin onto the target protein, forming ubiquitin chains, which are eventually recognized by the proteasome. (4) Dissociation of the target from the E3 ligase allows recognition by the proteasome for degradation. (5) PROTACs can function catalytically whereby they can induce multiple rounds of target protein ubiquitination before clearance or metabolism. (6) The amino acids of the degraded protein and ubiquitin are recycled by the cell (Fig. 2). Because of their selectivity¹⁰, catalytic nature¹¹ and potency¹², PROTACs offer benefits not possible with regular inhibitors^{13–18}.

Our group recently reported the discovery of MT802¹⁹, a PROTAC that degrades BTK in cells at low nanomolar concentrations. PROTAC MT802 has a ligand specific for BTK, and a ligand specific to a E3 ligase cereblon (CRBN) connected by polyethylene glycol (PEG) linker (Fig. 3). Since a transient interaction with substrate is enough for ubiquitination, we reasoned that MT802 would be able to induce degradation of both wild-type and C481S BTK, despite the reduced affinity of the non-covalent BTK ligand.

Furthermore, because the BTK-targeting warhead in MT802 does not have the Michael acceptor scaffold does not bind BTK covalently (Fig. 4). As a result, MT802 should be comparatively more selective; and indeed, KinomeScan binding studies show MT802 binds fewer off-target kinases than ibrutinib¹⁹. Collectively, these findings make MT-802 and the PROTAC approach a promising avenue for overcoming ibrutinib resistance in CLL.

Because of its outstanding degradation profile, we selected MT802 as our initial lead to further advance into preclinical studies. However, the pharmacokinetic data on MT802 in mice showed the compound was not suitable for *in vivo* studies. Specifically, its clearance was too high (1662 mL/min/kg) and half-life was too short (0.119 h). To address these issues, we undertook a medicinal chemistry campaign to improve the pharmacokinetic properties of an MT802-derived PROTAC series. Herein we present two strategies to improve the pharmacokinetic profile on these PROTACs. Initially, we prepared three PROTACs where the CRBN ligand was replaced with a ligand targeting the von Hippel-Lindau E3 ligase (VHL). However, the VHL series suffered from poor potency and we did not pursue these compounds further. Thereafter, we explored structural modifications on the CRBN ligand of MT802. These modifications resulted in the discovery of SJF620, a new PROTAC that retains potent degradation of BTK in cellular assays and has a superior pharmacokinetic profile to MT802 in mice. Therefore, SJF620 is a promising compound for further exploration of BTK degradation *in vivo*.

2. Results and discussion

Based on our previous knowledge, we proposed that the replacement of the CRBN Ligand A warhead with a VHL ligand warhead could result in better pharmacokinetic properties. Therefore, our first efforts focused on the syntheses of analogs of MT802, containing VHL Ligands B²⁰ and C²¹ (Fig. 5a) as different E3-recruiting elements. These two ligands are structurally similar and bind at the same site on VHL, but they have different linker-attaching points (Fig. 5b). Given our recent work^{10,22} and other studies^{23, 24} about

characterization of PROTAC-induced ternary complexes, we explored both VHL ligands, which likely induce different *de-novo* interactions between the surfaces of BTK and VHL and therefore different orientations for the E3 ligase (Fig. 5b). Since we were recruiting a new E3 ligase, we could not rely on the optimized linker length identified in MT802. Thus, we screened many PROTACs with varying linker lengths (Table 1).

The synthesis of the first PROTAC containing VHL Ligand **B** as a warhead was achieved starting from commercially available 3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine, which was converted under Mitsunobu conditions to piperidine compound **1** following the procedure reported by J. Liu, et.al.²⁵. Alkylation of compound **1** with the ω -bromo *t*-butyl ester **2** provided the corresponding condensation product **3**. Cleavage of the *t*-butyl ester on **3**, followed by amide bond formation with amino VHL Ligand **B** afforded the final PROTAC SJF638 (Scheme 1).

Based on prior experience^{21, 22}, we also prepared PROTACs containing the VHL ligand **C**, which will induce a protein complex with a different E3 orientation relative to the target, BTK. We initiated the synthesis of one of these PROTACs starting from commercially available 1-bromo-4-chloro-butane, which was condensed with butane-1,4-diol to provide compound **5** (Scheme 2). This chloro-alkyl alcohol **5** was transformed to the corresponding mesylate, followed by *in-situ* conversion to the chloro-iodo alkyl linker **6**. Alkylation of VHL Ligand **C** with the iodo-linker **6** gave the chloro-compound **7**, which was converted under Finkelstein conditions to the iodo-compound **8**. Finally, condensation of **8** with the warhead **1** provided the PROTAC SJF678.

Following the approach shown on Scheme 3, we prepared a second PROTAC containing VHL Ligand **C**. Michael addition of 3-chloropropanol to *t*-butyl acrylate provided the chloro-alkyl linker **9**, which was transformed to the iodo-compound **10** under Finkelstein conditions. Condensation of VHL-Ligand **C** with the linker **10** gave *t*-butyl ester **11**. Cleavage of *t*-butyl ester **11** to the corresponding carboxylic acid, followed amide-bond formation with warhead **1** afforded the expected PROTAC SJF690 in good yields (Scheme 3).

Simultaneously, we explored a different strategy also aimed to improve on the pharmacokinetic properties of MT802. In this approach we made structural modifications on the linker and CRBN ligand. We hypothesized that replacing the amide connectivity with an ether moiety between the linker and CRBN would provide a more robust and metabolically stable compound than MT802 (Fig. 6). We initiated the synthesis of this PROTAC by preparing the N-protected-5-hydroxythalidomide **12** following a new methodology that we developed in-house²⁶. Alkylation of **12** with commercially available bis-iodo linker **13** provided the corresponding iodo-compound **14** in good yields. Alkylation of **14** with warhead **1** gave the protected PROTAC **15**. SEM deprotection of compounds **15** afforded the final PROTAC SJF608 in very high yields (Scheme 4).

We then decided to further modify the CRBN ligand in an effort to improve pharmacokinetic properties. Thus, we eliminated one of the carbonyls on the phthalimide moiety (lenalidomide analog) on MT802 (Fig. 6). This compound was synthesized starting from 5-

hydroxy-iso-indolinone **16**¹⁵, following the procedure described in the literature. Alkylation of compound **16** with commercially available 1,2-bis(2-iodoethoxy)ethane **17** provided the iodo-compound **18**, further condensation of this iodo-compound **18** with the warhead **1** gave the uncycled intermediate **19**. Finally, acidic catalyzed cyclization of **19** afforded to the final PROTAC SJF620 in overall good yields (Scheme 5).

With these synthetic routes fully developed, we evaluated their BTK degradation profile in NAMALWA cells a Burkitt's lymphoma cell line with robust BTK expression. Unfortunately, PROTACs harboring VHL Ligands **B** and **C** as the E3-targeting warhead showed significant reduction of target degradation ($D_{\max} \sim 50\%$) and less potency (Table 1). However, PROTAC SJF608, which contained the same CRBN ligand as MT802 but without the amide group in its linker, showed a similarly potent degradation profile as MT802 (Table 1 and Fig. 6). We observed similar conservation of potency and efficacy with SJF620, which contained a lenalidomide analog for recruiting CRBN (Table 1 and Fig. 7). Encouragingly, both PROTACs SJF608 and SJF620 proved to have superior pharmacokinetic profiles relative to MT-802, with SJF620 displaying a more robust clearance and exposure profile, and we believe that this compound can be progressed to further *in vivo* studies.

Experimental section

1. Chemistry—General comments. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification. Tetrahydrofuran (THF), dimethylformamide (DMF), and dichloromethane (DCM) were dried by a PureSolv™ solvent drying system. Flash column chromatography was performed using silica gel 60 (230–400 mesh). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV or KMnO₄. ¹H and ¹³C NMR spectra were recorded on an Agilent DD₂ 500 (500 MHz ¹H; 125 MHz ¹³C) or Agilent DD₂ 600 (600 MHz ¹H; 150 MHz ¹³C) or Agilent DD₂ 400 (400 MHz ¹H; 100 MHz ¹³C) spectrometer at room temperature. Chemical shifts were reported in ppm relative to the residual CDCl₃ (δ 7.26 ppm ¹H; δ 77.00 ppm ¹³C), CD₃OD (δ 3.31 ppm ¹H; δ 49.00 ppm ¹³C), or *d*⁶-DMSO (δ 2.50 ppm ¹H; δ 39.52 ppm ¹³C). NMR chemical shifts were expressed in ppm relative to internal solvent peaks, and coupling constants were measured in Hz. (bs = broad signal). Only peaks of the major rotamer are reported. Mass spectra were obtained using electrospray ionization (ESI) on a time of flight (TOF) mass spectrometer.

Preparation of 3-(4-phenoxyphenyl)-1-(piperidin-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine hydrochloride (Compound 1):

To a solution of triphenylphosphine (864.72 mg, 3.3 mmol) in THF (300 mL) was added DIAD (0.65 mL, 3.3 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 0.5 h under argon, then tert-butyl 4-hydroxypiperidine-1-carboxylate (663.53 mg, 3.3 mmol) was added. The reaction mixture was stirred at 0 °C for 0.5 h. After that, 3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (500 mg, 1.65 mmol) was added. The reaction mixture was allowed to warm to room temperature with stirring for 4 h. The resulting mixture was then concentrated to afford the crude product, which was purified by flash silica gel column chromatography (SiO₂-80g, Hex:EtOAc, gradient 3:7 to 100% in 15 min) to provide the desired Boc-protected compound as an off-white foam (504 mg). ¹H NMR (400 MHz, DMSO-d₆) δ 8.22 (s, 1H), 7.63 (d, J = 8.7 Hz, 2H), 7.49 – 7.33 (m, 2H), 7.27 – 6.98 (m, 5H), 4.97 – 4.80 (m, 1H), 4.08 (bs, 2H), 2.97 (bs, 2H), 2.12 – 1.75 (m, 4H), 1.40 (s, 9H). Then, this foam was dissolved in EtOAc (10 mL) and 4 N HCl in Dioxane (5 mL) was added. The reaction mixture was stirred at room temperature for 12 h (overnight). After complete conversion of the starting material, the solid was collected by filtration and washed with (EtOAc, 2mLx2) to give an off-white solid (**1**) (560 mg, 80% yield). ¹H NMR (500 MHz, DMSO-d₆) δ 9.35 (bs, 1H), 9.02 (bs, 1H), 8.54 (s, 1H), 7.66 (d, J = 8.2 Hz, 2H), 7.45 (t, J = 7.5 Hz, 2H), 7.17 (td, J = 19.2, 17.4, 7.6 Hz, 5H), 5.12 (t, J = 11.1 Hz, 1H), 3.43 (d, J = 12.5 Hz, 2H), 3.19 (q, J = 11.5 Hz, 2H), 2.39 (q, J = 10.9 Hz, 2H), 2.15 (d, J = 12.6 Hz, 2H). LC/MS: [M+H]⁺ for C₂₂H₂₃N₆O calculated. 387.1904. Found 387.1933.

tert-butyl 2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)acetate (Compound 3):

To a solution of 3-(4-phenoxyphenyl)-1-(4-piperidyl)pyrazolo[3,4-d]pyrimidin-4-amine (**1**) (7.51 mg, 0.02 mmol) and TEA (0.01 mL, 0.58 mmol) in DMF (1 mL) was added tert-butyl 2-(2-(2-bromoethoxy)ethoxy)acetate (**2**) (5.5 mg, 0.02 mmol) and the resulting solution

stirred for 36 h at room temperature. The reaction mixture was diluted with EtOAc (5 mL) and washed with water/brine (3×5 mL), organic phase was dried (Na₂SO₄, and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH₄OH, 91:8:1) to give 3 mg of product (**3**) (26%). ¹H NMR (500 MHz, DMSO-d₆) δ 8.24 (s, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.43 (t, J = 7.7 Hz, 2H), 7.19 (t, J = 7.3 Hz, 1H), 7.14 (dd, J = 12.3, 8.5 Hz, 5H), 4.72 (bs, 1H), 4.00 (s, 2H), 3.74 – 3.44 (m, 6H), 3.14 (bs, 2H), 2.85 – 2.55 (m, 3H), 2.38 – 2.14 (m, 3H), 2.02 – 1.80 (m, 2H), 1.40 (s, 9H). LC/MS (ESI); m/z [M+H]⁺: Calcd. for C₃₂H₄₁N₆O₅, 589.3138. Found 589.3347.

(2S,4R)-1-((S)-2-(2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (PROTAC SJF638):

SJF638

A solution of tert-butyl 2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]¹⁵pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)acetate (**3**) (3 mg, 0.01 mmol) in a mixture of TFA (1 mL, 13.46 mmol) and dichloromethane (3 mL) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 2 h. Crude product was used in the next step without any further purification (2.7 mg, quantitative yield). LC/MS (ESI); m/z: [M+H]⁺ Calcd. for C₂₈H₃₃N₆O₅, 533.2512. Found 533.2579. To a solution of 2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d] - pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)acetic acid (crude product from previous step) (2.7 mg, 0.01 mmol) and (2S,4R)-1-[(2S)-2-amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)-phenyl]methyl]pyrrolidine-2-carboxamide; hydro-chloride (**VHL ligand B**)²⁰ (2.6 mg, 0.006 mmol) in DMF (1 mL) was added TEA (0.1 mL, 0.72 mmol) and PyBOP (2.77 mg, 0.01 mmol) at room temperature. The reaction mixture was stirred for 4 h at the same temperature. TLC (DCM:MeOH:NH₄OH, 91:8:1) shows no starting materials. The DMF was removed under high vacuum. Crude product was filtered over a silica-carbonate cartridge using a mixture of DCM:MeOH:NH₄OH (91:8:1) as a eluent. Filtrate was evaporated under vacuum and crude product was purified by PTLC (DCM:MeOH:NH₄OH, 91:8:1) to give 2.5 mg of product **SJF638** (52% yield). ¹H NMR (600 MHz, DMSO-d₆) δ 8.96 (s, 1H), 8.60 (t, J = 6.0 Hz, 1H), 8.23 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.48 – 7.34 (m, 7H), 7.18 (t, J = 7.4 Hz, 1H), 7.16 – 7.07 (m, 4H), 5.16 (d, 1H), 4.63 (bs, 1H), 4.57 (d, J = 9.6 Hz, 1H), 4.44 (t, J = 8.2 Hz, 1H), 4.40 – 4.32 (m, 2H), 4.23 (dd, J = 15.8, 5.6 Hz, 1H), 3.98 (s, 2H), 3.72 – 3.50 (m, 8H), 3.01 (bs, 2H), 2.56 (bs, 2H), 2.43 (s, 3H), 2.26 – 2.11 (m, 4H), 2.10 – 1.98 (m, 1H), 1.94 – 1.78 (m, 3H), 0.95 (s, 9H).

^{13}C NMR (151 MHz, DMSO- d_6) δ 171.76, 169.10, 168.57, 158.15, 157.03, 156.29, 155.42, 153.60, 151.42, 147.72, 142.77, 139.42, 131.13, 130.13, 130.01, 129.67, 128.68, 128.11, 127.43, 123.79, 118.99, 118.94, 97.44, 70.51, 69.59, 69.46, 68.88, 58.74, 56.99, 56.58, 55.67, 54.93, 52.72, 52.66, 41.67, 37.90, 35.75, 30.99, 26.19, 15.93. LC/MS (ESI); m/z [$M+H$] $^+$: Calcd. for $\text{C}_{50}\text{H}_{61}\text{N}_{10}\text{O}_7\text{S}$, 945.4445. Found 945.4641.

4-(4-chlorobutoxy)butan-1-ol (Compound 5):

A solution of butane-1,4-diol (23.14 mL, 260.35 mmol) and KOH (2.43 g, 43.39 mmol) in DMSO (135 mL) and distilled water (15 mL) was stirred 15 min at rt. Then the mixture was cooled down to 0 °C and 1-bromo-4-chloro-butane (5 mL, 43.39 mmol) was added dropwise over 1 h. The resulting solution was stirred 1 h at 0 °C and then 12 h (overnight) at rt. After dilution with a mixture of ether/hexane (1:1, 250 mL) and water (250 mL), the organic phase was separated. Water layer was extracted with a mixture ether/hexane (2×150 mL). Organic extracts were combined and washed with water (4×150 mL), dried over Na_2SO_4 , and concentrated under vacuum. Crude product was subjected to flash column chromatography (SiO_2 -120 g, Gradient, Hexane 100% to hexane / EtOAc, 1/1 in 20 minutes) to give 3.18 g (41% yield) of product (**5**) as an oil. ^1H NMR (400 MHz, Chloroform- d) δ 3.64 (t, J = 5.7 Hz, 2H), 3.57 (t, J = 6.5 Hz, 2H), 3.51 – 3.43 (m, 4H), 2.15 (s, 1H), 1.91 – 1.81 (m, 2H), 1.79 – 1.61 (m, 6H). ^{13}C NMR (151 MHz, Chloroform- d) δ 71.09, 70.24, 62.92, 45.06, 30.46, 29.60, 27.13, 27.02. LC/MS (ESI); m/z : [$M+H$] $^+$ Calcd. for $\text{C}_8\text{H}_{18}\text{ClO}_2$, 181.0995. Found 181.0990

Chloro-4-(4-iodobutoxy)butane (Compound 6):

To a solution of 4-(4-chlorobutoxy)butan-1-ol (**5**) (271 mg, 1.5 mmol) in dichloromethane (5 mL) was added TEA (0.63 mL, 4.5 mmol), then reaction mixture was cooled to 0 °C (water ice/acetone bath) and Mesyl chloride (0.14 mL, 1.8 mmol) was added dropwise. The reaction mixture was stirred for 1 h at the same temperature. By TLC no starting material (Hex:EtOAc, 3:7), and a less polar compound was formed. Reaction mixture was poured into an aqueous solution of NaHCO_3 (20 mL) and product extracted with DCM (20 mL, 2x), the organic extracts were combined, dried (Na_2SO_4), and evaporated under vacuum. The crude product (mesylate) was used in the next step without any further purification (>95% pure by NMR): ^1H NMR (400 MHz, Chloroform- d) δ 4.26 (t, J = 6.5 Hz, 2H), 3.57 (t, J = 6.6 Hz, 2H), 3.44 (td, J = 6.2, 2.0 Hz, 4H), 3.01 (s, 3H), 1.92 – 1.78 (m, 4H), 1.76 – 1.62 (m, 4H). Crude mixture from previous step was diluted in Acetonitrile (5 mL) and NaI (247.32 mg, 1.65 mmol) was added, the reaction mixture was stirred at room temperature for 72 h (over the weekend). By TLC (Hex:EtOAc, 7:3) and NMR small amount of mesylate, the reaction was poured into an aqueous solution of $\text{Na}_2\text{S}_2\text{O}_3$ (10%, 20 mL) and product was

extracted with DCM (2×20 mL). Organic extracts were combined, dried (Na₂SO₄) and evaporated under vacuum. Crude product was purified by flash chromatography (SiO₂-40g, grad. Hex:EtOAc, 2 to 20% in 10 min), to give 310 mg of product (**6**) as an oil (71% yield): ¹H NMR (500 MHz, Chloroform-d) δ 3.57 (t, J = 6.6 Hz, 2H), 3.43 (td, J = 6.3, 2.4 Hz, 4H), 3.22 (t, J = 7.0 Hz, 2H), 2.01 – 1.80 (m, 4H), 1.77 – 1.61 (m, 4H). ¹³C NMR (151 MHz, Chloroform-d) δ 70.13, 69.77, 45.13, 30.74, 30.56, 29.68, 27.22, 7.02. LC/MS (ESI); m/z: [M+H]⁺ Calcd. for C₈H₁₇ClO, 291.001. Found 291.0060.

(2S,4R)-N-(2-(4-(4-chlorobutoxy)butoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (Compound 7):

To a mixture of (2S,4R)-4-hydroxy-N-[[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl]-1-[(2S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (**VHL ligand C**)²¹ (83 mg, 0.151 mmol) and 1-chloro-4-(4-iodobutoxy)butane (**6**) (44 mg, 0.151 mmol) in N,N-Dimethylformamide (1 mL) was added Cs₂CO₃ (49.34 mg, 0.151 mmol). After stirring at room temperature for 4 hrs, the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5×10 mL), organic phase was dried (Na₂SO₄, and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH₄OH, 92:7:1) to give 83 mg of product (**7**) (77% yield). ¹H NMR (500 MHz, DMSO-d₆) δ 8.99 (s, 1H), 8.36 (t, J = 5.7 Hz, 1H), 7.71 (d, J = 7.5 Hz, 1H), 7.65 – 7.57 (m, 2H), 7.50 (t, J = 7.7 Hz, 1H), 7.33 (d, J = 7.6 Hz, 1H), 7.02 – 6.96 (m, 2H), 5.08 (d, J = 3.9 Hz, 1H), 4.71 (d, J = 10.8 Hz, 1H), 4.59 – 4.18 (m, 6H), 4.07 (t, J = 5.0 Hz, 2H), 3.77 (dd, J = 10.5, 4.2 Hz, 1H), 3.69 (d, J = 10.4 Hz, 1H), 3.63 (t, J = 6.6 Hz, 2H), 3.49 – 3.36 (m, 4H), 2.47 (s, 3H), 2.37 – 2.29 (m, 1H), 2.07 – 2.01 (m, 1H), 1.96 – 1.89 (m, 1H), 1.85 – 1.56 (m, 8H), 0.96 (d, J = 6.4 Hz, 3H), 0.74 (d, J = 6.6 Hz, 3H). ¹³C NMR (151 MHz, DMSO-d₆) δ 171.54, 168.09, 167.48, 155.90, 151.48, 147.89, 142.21, 131.60, 131.37, 131.32, 130.98, 127.92, 127.69, 126.96, 123.63, 123.02, 120.77, 111.69, 69.68, 69.14, 68.62, 67.56, 58.70, 57.79, 55.42, 46.82, 45.36, 38.10, 37.6, 29.18, 28.40, 26.63, 25.90, 25.69, 18.89, 18.64, 16.03. LC/MS (ESI); m/z: [M+H]⁺ Calcd. for C₃₇H₄₈ClN₄O₆S, 711.2983. Found 711.3224.

(2S,4R)-N-(2-(4-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)butoxy)butoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (SJF678):

SJF678

To a solution of (2S,4R)-N-[[2-[4-(4-chlorobutoxy)butoxy]-4-(4-methylthiazol-5-yl)phenyl]methyl]-4-hydroxy-1-[(2S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (**7**) (75 mg, 0.11 mmol) in Acetone (10 mL) was added NaI (158.05 mg, 1.05 mmol). The reaction mixture was stirred at reflux temperature for 48 h, then the solvent was removed under vacuum and crude product was dissolved in EtOAc (15 mL) and an aqueous solution of Na₂SO₃ (10%, 10 mL), organic layer was separated, washed with water (10 mL), dried (Na₂SO₄) and evaporated under vacuum. Crude iodo-compound (**8**) was pure by NMR (quantitative yield) no further purification. ¹H (400 MHz, Chloroform-d) δ 8.68 (s, 1H), 7.78 (d, J = 7.6 Hz, 1H), 7.52 (t, J = 7.4 Hz, 1H), 7.47 – 7.37 (m, 2H), 7.34 – 7.23 (m, 2H), 6.96 (d, J = 8.7 Hz, 1H), 6.87 (s, 1H), 4.76 (s, 1H), 4.73 (d, J = 6.7 Hz, 1H), 4.64 (t, J = 7.8 Hz, 1H), 4.57 – 4.36 (m, 5H), 4.05 (t, J = 6.2 Hz, 2H), 3.65 (dd, J = 11.5, 3.5 Hz, 1H), 3.49 (t, J = 6.2 Hz, 2H), 3.44 (t, J = 6.2 Hz, 2H), 3.19 (t, J = 6.9 Hz, 2H), 2.58 – 2.45 (m, 1H), 2.53 (s, 3H), 2.47 – 2.27 (m, 2H), 2.12 – 2.00 (m, 2H), 1.98 – 1.84 (m, 3H), 1.79 (dt, J = 9.4, 6.5 Hz, 2H), 1.71 – 1.58 (m, 2H), 0.89 (dd, J = 6.0 Hz, 6H). ¹³C NMR (126 MHz, Chloroform-d) δ 170.54, 170.47, 169.71, 156.94, 150.41, 148.61, 142.23, 132.35, 131.99, 131.92, 131.73, 129.41, 128.15, 126.47, 123.97, 122.99, 121.65, 112.15, 70.62, 70.13, 69.83, 68.04, 58.81, 58.55, 56.7, 47.59, 39.05, 35.87, 30.75, 30.58, 29.85, 28.89, 26.55, 26.34, 19.23, 16.28, 6.98. LC/MS (ESI); m/z [M+H]⁺: Calcd. for C₃₇H₄₈IN₄O₆S, 803.2339. Found 803.2675.

To a solution of 3-(4-phenoxyphenyl)-1-(4-piperidyl)pyrazolo[3,4-d]pyrimidin-4-amine (**1**) (4.81 mg, 0.01 mmol) and (2S,4R)-4-hydroxy-N-(2-(4-(4-iodobutoxy)butoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (**8**) (10 mg, 0.01 mmol) in DMF (1 mL) was added TEA (0.01 mL, 0.04 mmol), the resulting solution stirred for 12 h (overnight) at room temperature. The DMF was evaporated under high vacuum and the crude product was filtered over a silica-carbonate cartridge using a mixture of DCM:MeOH:NH₄OH (91:8:1) as a eluent. Filtrate was evaporated and crude was purified by PTLC (DCM:MeOH:NH₄OH, 91:8:1) to give 4.8 mg of pure product **SJF678** (36% yield). ¹H NMR (500 MHz, Chloroform-d) δ 8.66 (s, 1H), 8.34 (s, 1H), 7.75 (d, J = 7.6 Hz, 1H), 7.64 (d, J = 8.5 Hz, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.44 – 7.33 (m, 4H), 7.33 – 7.26 (m, 2H), 7.15 (dd, J = 17.5, 8.0 Hz, 3H), 7.08 (d, J = 8.4 Hz,

2H), 6.95 (d, $J = 7.7$ Hz, 1H), 6.87 (s, 1H), 5.55 (s, 2H), 4.84 – 4.68 (m, 3H), 4.62 (t, $J = 7.8$ Hz, 1H), 4.58 – 4.28 (m, 5H), 4.05 (t, $J = 6.3$ Hz, 2H), 3.67 (dd, $J = 11.3, 3.5$ Hz, 1H), 3.50 (t, $J = 6.3$ Hz, 2H), 3.46 – 3.38 (m, 2H), 3.15 – 2.98 (m, 2H), 2.52 (s, 3H), 2.51 – 2.30 (m, 5H), 2.25 – 1.88 (m, 8H), 1.85 – 1.74 (m, 2H), 0.88 (dd, $J = 18.6, 6.5$ Hz, 6H). ^{13}C NMR (151 MHz, Chloroform- d) δ 170.62, 170.32, 169.48, 158.50, 157.89, 157.00, 156.50, 155.60, 153.97, 150.39, 148.58, 143.44, 142.19, 132.36, 132.36, 131.97, 131.82, 131.75, 130.08, 129.59, 128.16, 128.7, 126.39, 124.12, 123.89, 122.96, 121.60, 119.65, 119.20, 112.09, 98.71, 70.88, 70.54, 69.92, 68.02, 58.72, 58.71, 58.28, 56.07, 52.91, 47.53, 39.20, 36.21, 31.37, 29.83, 28.97, 27.85, 26.61, 26.31, 23.99, 19.19, 19.13, 16.27. LC/MS (ESI); m/z $[\text{M}+\text{H}]^+$: Calcd. for $\text{C}_{59}\text{H}_{69}\text{N}_{10}\text{O}_7\text{S}$, 1061.5071. Found 1061.5353.

tert-Butyl 3-(3-chloropropoxy)propanoate (Compound 9):

3-chloropropan-1-ol (8.84 mL, 105.78 mmol) in acetonitrile (50 mL) was added tert-butyl prop-2-enoate (15.35 mL, 105.78 mmol) followed by Triton B (1 mL, 2.54 mmol) in. The mixture was stirred at room temperature for 72 hour. The mixture was concentrated in vacuum and crude product was purified by CC (SiO_2 -250g, gradient Hex:EtOAc, 95:5 to 9:1) to give 14.2 g of product (**9**) as an oil (60% yield). ^1H NMR (500 MHz, Chloroform- d) δ 3.66 (t, $J = 6.4$ Hz, 2H), 3.62 (t, $J = 6.5$ Hz, 2H), 3.56 (t, $J = 5.9$ Hz, 2H), 2.51 – 2.42 (m, 2H), 1.99 (p, $J = 6.2$ Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (101 MHz, Chloroform- d) δ 171.03, 80.70, 67.32, 66.72, 42.02, 36.46, 32.79, 28.22. LC/MS (ESI); m/z $[\text{M}+\text{Na}]^+$: Calcd. for $\text{C}_{10}\text{H}_{19}\text{ClO}_3\text{Na}$, 245.0920. Found 245.0957.

tert-Butyl 3-(3-iodopropoxy)propanoate (Compound 10):

To a solution of tert-butyl 3-(3-chloropropoxy)propanoate (**9**) (1.36 g, 6.11 mmol) in acetone (100 mL) was added NaI (4.58 g, 30.53 mmol). The reaction mixture was stirred at reflux temperature for 72 h, then the solvent was removed under vacuum and crude product was re-dissolved in EtOAc (100 mL) and washed with water (100 mL), and an aqueous solution of Na_2SO_3 (10%, 50 mL), organic layer was separated, dried (Na_2SO_4) and evaporated under vacuum. Crude product (**10**) was pure by NMR (>98% purity). It was used in the next step without any further purification. ^1H NMR (500 MHz, Chloroform- d) δ 3.67 (t, $J = 6.4$ Hz, 2H), 3.49 (t, $J = 5.8$ Hz, 2H), 3.25 (t, $J = 6.8$ Hz, 2H), 2.47 (t, $J = 6.4$ Hz, 2H), 2.04 (p, $J =$

6.3 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, Chloroform- d) δ 171.01, 80.72, 70.26, 66.71, 36.46, 33.52, 28.25, 3.54. LC/MS (ESI); m/z $[\text{M}+\text{Na}]^+$: Calcd. for $\text{C}_{10}\text{H}_{19}\text{IO}_3\text{Na}$, 337.0276. Found 337.0351.

tert-Butyl 3-(3-(2-(((2S,4R)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl) - pyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propoxy)propanoate (Compound 11):

To a solution of (2S,4R)-4-hydroxy-N-[[2-hydroxy-4-(4-methylthiazol-5-yl)phenyl]methyl]-1-[(2S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl]pyrrolidine-2-carboxamide (**VHL-Ligand-C**)²¹ (40.0 mg, 0.0729 mmol) and tert-butyl 3-(3-iodopropoxy)propanoate (**10**) (34.4 mg, 0.109 mmol) in DMF (1 mL) was added Cs_2CO_3 (71 mg, 0.21 mmol) at room temperature, the reaction mixture was stirred for 12 h (overnight) at the same temperature. Reaction was diluted with EtOAc (10 mL) and washed with water (4×10 mL). Organic extract was dried (Na_2SO_4), and concentrated under vacuum. Crude product was purified by PTLC (DCM:MeOH: NH_4OH , 91:8:1) to give 38 mg of product (**11**) (71% yield). ^1H NMR (500 MHz, DMSO- d_6) δ 8.98 (s, 1H), 8.36 (t, J = 5.8 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.62 (q, J = 7.5, 6.5 Hz, 2H), 7.50 (t, J = 6.8 Hz, 1H), 7.35 (d, J = 7.7 Hz, 1H), 7.00 (d, J = 7.5 Hz, 2H), 5.09 (d, J = 4.1 Hz, 1H), 4.72 (d, J = 10.8 Hz, 1H), 4.61 – 4.19 (m, 6H), 4.09 (t, J = 6.1 Hz, 2H), 3.78 (dd, J = 10.5, 4.3 Hz, 1H), 3.70 (d, J = 10.6 Hz, 1H), 3.64 – 3.49 (m, 4H), 2.47 (s, 3H), 2.42 (t, J = 6.0 Hz, 2H), 2.39 – 2.27 (m, 1H), 2.12 – 1.87 (m, 4H), 1.34 (s, 9H), 0.97 (d, J = 6.5 Hz, 3H), 0.74 (d, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 171.49, 170.43, 168.09, 167.47, 155.86, 151.42, 147.86, 142.18, 131.56, 131.37, 131.26, 131.00, 127.88, 127.81, 126.99, 123.60, 123.00, 120.83, 111.63, 79.63, 68.61, 66.59, 65.95, 64.71, 58.69, 57.78, 55.40, 46.81, 38.08, 37.05, 35.85, 28.98, 28.38, 27.69, 18.88, 18.61, 15.99. LC/MS (ESI); m/z : $[\text{M}+\text{H}]^+$ Calcd. for $\text{C}_{39}\text{H}_{51}\text{N}_4\text{O}_8\text{S}$, 735.3427. Found 735.3720.

(2S,4R)-N-(2-(3-(3-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)-3-oxopropoxy)propoxy)-4-(4-methylthiazol-5-yl)benzyl)-4-hydroxy-1-((S)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)pyrrolidine-2-carboxamide (SJF690):

SJF690

A solution of tert-butyl 3-(3-(2-(((2*S*,4*R*)-4-hydroxy-1-((*S*)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)pyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propoxy)propanoate (19 mg, 0.03 mmol) in a mixture of TFA (0.7 mL, 9.42 mmol) and dichloromethane (2 mL) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 1 h. Crude carboxylic acid was used in the next step without any further purification (17.5 mg, quantitative yield). LC/MS (ESI); m/z : $[M+H]^+$ Calcd. for $C_{35}H_{43}N_4O_8S$, 679.2801. Found 679.3105.

To a solution of 3-(3-(2-(((2*S*,4*R*)-4-hydroxy-1-((*S*)-3-methyl-2-(1-oxoisindolin-2-yl)butanoyl)-pyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)propoxy)propanoic acid (from previous step) (17.5 mg, 0.03 mmol) and 3-(4-phenoxyphenyl)-1-(piperidin-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (10.96 mg, 0.03 mmol) (**1**) in DMF (1 mL) was added TEA (0.1 mL, 0.72 mmol) and PyBOP (14.76 mg, 0.03 mmol) at room temperature. The reaction mixture was stirred for 4 h at the same temperature. TLC (DCM:MeOH:NH₄OH, 91:8:1) shows no starting materials. The reaction mixture was dissolved in EtOAc (10 mL) and washed with brine/water (3×5 mL). Organic extract was concentrated under vacuum and crude product was purified by PTLC (DCM:MeOH:NH₄OH, 91:8:1) to give 22 mg of **SJF690** (83% yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.91 (s, 1H), 8.33 (t, *J* = 5.5 Hz, 1H), 8.22 (s, 1H), 7.68 (d, *J* = 7.5 Hz, 1H), 7.63 – 7.52 (m, 4H), 7.50 – 7.43 (m, 1H), 7.43 – 7.34 (m, 2H), 7.31 (d, *J* = 7.6 Hz, 1H), 7.17 – 7.11 (m, 1H), 7.07 (dd, *J* = 11.4, 8.4 Hz, 4H), 6.98 – 6.90 (m, 2H), 5.05 (d, *J* = 3.9 Hz, 1H), 4.98 – 4.87 (m, 1H), 4.68 (d, *J* = 10.8 Hz, 1H), 4.59 – 4.16 (m, 7H), 4.06 (t, *J* = 5.6 Hz, 2H), 4.04 – 3.94 (m, 1H), 3.80 – 3.60 (m, 4H), 3.57 (t, *J* = 6.0 Hz, 2H), 3.20 (t, *J* = 12.5 Hz, 1H), 2.82 – 2.53 (m, 3H), 2.40 (s, 3H), 2.33 – 2.23 (m, 1H), 2.16 – 1.76 (m, 8H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.69 (d, *J* = 6.3 Hz, 3H). ¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.50, 168.79, 168.08, 167.46, 158.17, 157.01, 156.30, 155.88, 155.52, 153.62, 151.37, 147.85, 143.01, 142.19, 131.56, 131.37, 131.26, 130.99, 130.11, 130.00, 128.02, 127.89, 127.79, 127.00, 123.75, 123.60, 123.00, 120.82, 118.96, 118.91, 111.66, 97.42, 68.62, 66.75, 66.69, 64.81, 58.69, 57.78, 55.42, 53.33, 46.81, 44.11, 38.10, 37.06, 32.68, 31.52, 30.90, 29.05, 28.39, 18.87, 18.62, 15.98. LC/MS (ESI); m/z $[M+H]^+$: Calcd. for $C_{57}H_{63}N_{10}O_8S$, 1047.4551. Found 1047.5190.

2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)-5-(2-(2-(2-iodoethoxy)-ethoxy)ethoxy)isoindoline-1,3-dione (Compound 14):

To a mixture of 2-[2,6-dioxo-1-(2-trimethylsilylethoxymethyl)-3-piperidyl]-5-hydroxy - isoindoline-1,3-dione (**12**)²⁶ (64 mg, 0.16 mmol) and 1,2-bis(2-iodoethoxy)ethane (**13**) (702.45 mg, 1.9 mmol) in DMF (2 mL) was added Cs₂CO₃ (309.31 mg, 0.95 mmol). After stirring at room temperature for 2 hrs, the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5×10 mL), organic phase was dried (Na₂SO₄, and evaporated under vacuum. Crude product was filtered over a short column of SiO₂ (DCM 100%, then DCM:MeOH:NH₄OH, 92:7:1) to remove the excess of the bis-iodo reactant, then crude product was purified by PTLC (DCM:MeOH:NH₄OH, 92:7:1) to give 71 mg of product (**14**) (69% yield). ¹H NMR (500 MHz, DMSO-d₆) δ 7.83 (d, J = 8.3 Hz, 1H), 7.45 (d, J = 2.3 Hz, 1H), 7.38 (dd, J = 8.4, 2.3 Hz, 1H), 5.25 (dd, J = 13.1, 5.3 Hz, 1H), 5.08 (s, 2H), 4.41 – 4.23 (m, 2H), 3.87 – 3.76 (m, 2H), 3.66 (t, J = 6.4 Hz, 2H), 3.63 – 3.43 (m, 6H), 3.30 (t, J = 6.5 Hz, 2H), 3.03 (ddd, J = 18.8, 14.3, 5.3 Hz, 1H), 2.84 – 2.74 (m, 1H), 2.57 (qd, J = 13.4, 4.5 Hz, 1H), 2.16 – 2.03 (m, 1H), 0.84 (t, J = 7.7 Hz, 2H), –0.02 (s, 9H). ¹³C NMR (151 MHz, DMSO-d₆) δ 171.65, 169.90, 166.80, 166.73, 163.95, 133.90, 125.31, 123.04, 120.94, 108.94, 70.99, 69.95, 69.31, 68.68, 68.44, 68.30, 65.96, 49.54, 31.20, 21.07, 17.43, 5.43, –1.32. LC/MS (ESI); m/z: [M+Na]⁺ Calcd. for C₂₅H₃₅IN₂O₈SiNa, 669.1105. Found 669.1311.

5-(2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)ethoxy)-2-(2,6-dioxo-1-((2-(trimethylsilyl)ethoxy)methyl)piperidin-3-yl)isoindoline-1,3-dione (Compound 15):

To a solution of 3-(4-phenoxyphenyl)-1-(4-piperidyl)pyrazolo[3,4-d]pyrimidin-4-amine; - hydrochloride (**1**) (36.63 mg, 0.09 mmol) and TEA (0.1 mL, 0.57 mmol) in DMF (1 mL) was added 2-[2,6-dioxo-1-(2-trimethylsilylethoxymethyl)-3-piperidyl]-5-[2-[2-(2-iodoethoxy)ethoxy]ethoxy] - iso-indoline-1,3-dione (**14**) (56 mg, 0.09 mmol) and the resulting solution stirred for 36 h at rt. The reaction mixture was evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH₄OH, 92:7:1, and then again DCM:MeOH, 95:5) to give 36.5 mg of pure product (**15**) (47% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.22 (s, 1H), 7.80 (d, J = 8.3 Hz, 1H), 7.65 (d, J = 8.7 Hz, 2H), 7.50 – 7.33 (m, 4H), 7.15 (dt, J = 22.5, 7.4 Hz, 5H), 5.24 (dd, J = 13.1, 5.4 Hz, 1H), 5.06 (s, 2H), 4.68 – 4.56 (m, 1H), 4.39 – 4.24 (m, 2H), 3.84 – 3.76 (m, 2H), 3.65 – 3.42 (m, 8H), 3.09 – 2.94 (m, 3H), 2.77 (dd, J = 14.1, 3.3 Hz, 1H), 2.64 – 2.50 (m, 3H), 2.26 – 2.01 (m, 5H), 1.92 – 1.80 (m, 2H), 0.82 (t, J = 8.6 Hz, 2H), –0.04 (s, 9H). ¹³C NMR (151 MHz, DMSO-d₆) δ 171.62,

169.87, 166.78, 166.70, 163.96, 158.13, 157.03, 156.28, 155.41, 153.60, 142.75, 133.87, 130.11, 129.99, 128.11, 125.26, 123.78, 123.00, 120.93, 118.98, 118.92, 108.91, 97.43, 69.95, 69.71, 68.65, 68.58, 68.46, 68.29, 65.95, 57.06, 53.89, 52.70, 49.53, 31.19, 31.03, 21.05, 17.41, -1.35. LC/MS (ESI); m/z $[M+H]^+$: Calcd. for $C_{47}H_{57}N_8O_9Si$, 905.4017. Found 905.4290.

5-(2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)ethoxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (PROTAC SJF608):

A solution of 5-[2-[2-[2-[4-[4-amino-3-(4-phenoxyphenyl)pyrazolo[3,4-d]pyrimidin-1-yl]-1-piperidyl]ethoxy]ethoxy]ethoxy]-2-[2,6-dioxo-1-(2-trimethylsilylethoxymethyl)-3-piperidyl]isoindoline-1,3-dione (**15**) (30 mg, 0.03 mmol) in a mixture of TFA (1 mL, 13.46 mmol) and dichloromethane (2 mL) was stirred for 1 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 1 h. Crude product was purified by PTLT (DCM:MeOH:NH₄OH, 92:7:1) to give 24.2 mg of PROTAC **SJF608** (94% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 11.11 (bs, 1H), 8.22 (s, 1H), 7.80 (d, J = 8.3 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.50 – 7.30 (m, 4H), 7.15 (dt, J = 23.0, 7.7 Hz, 5H), 5.10 (dd, J = 12.9, 5.3 Hz, 1H), 4.72 – 4.62 (m, 1H), 4.39 – 4.23 (m, 2H), 3.86 – 3.74 (m, 2H), 3.70 – 3.45 (m, 6H), 3.14 – 3.00 (m, 1H), 2.93 – 2.81 (m, 1H), 2.73 – 2.50 (m, 4H), 2.37 – 2.14 (m, 4H), 2.07 – 1.98 (m, 1H), 1.96 – 1.82 (m, 2H). ¹³C NMR (151 MHz, DMSO-d₆) δ 172.76, 169.92, 166.84, 166.77, 163.94, 158.15, 157.05, 156.27, 155.43, 153.61, 142.79, 133.89, 130.12, 130.00, 128.10, 125.24, 123.79, 123.03, 120.90, 119.00, 118.93, 108.87, 97.44, 73.80, 69.94, 69.71, 68.66, 68.48, 68.46, 56.99, 53.79, 52.65, 48.96, 30.96, 22.08. LC/MS (ESI); m/z : $[M+H]^+$ Calcd. for $C_{41}H_{43}N_8O_8$, 775.3203. Found 775.3290.

tert-Butyl 5-amino-4-(5-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)-1-oxoisoindolin-2-yl)-5-oxopentanoate (Compound 18):

To a mixture of tert-butyl 5-amino-4-(5-hydroxy-1-oxo-isoindolin-2-yl)-5-oxo-pentanoate (**16**)²⁷ (50 mg, 0.15 mmol) and 1,2-bis(2-iodoethoxy)ethane (663.88 mg, 1.79 mmol) in DMF (2 mL) was added Cs₂CO₃ (243.61 mg, 0.75 mmol). After stirring at room temperature for 2 hrs, the reaction mixture was diluted with EtOAc (10 mL) and washed with water (5×10 mL), organic phase was dried (Na₂SO₄), and evaporated under vacuum.

Crude product was filtered over a short column of SiO₂ (DCM 100%, then DCM:MeOH:NH₄OH, 91:8:1) to remove the excess of the bis-iodo reactant. Product was purified again by PTLC (DCM:MeOH:NH₄OH, 92:7:1) to give 51 mg of product (**18**) (59% yield). ¹H NMR (500 MHz, Chloroform-d) δ 7.71 (d, J = 8.4 Hz, 1H), 7.00 (dd, J = 8.4, 2.0 Hz, 1H), 6.95 (d, J = 2.1 Hz, 1H), 6.58 (bs, 1H), 5.54 (bs, 1H), 4.86 (dd, J = 8.9, 6.2 Hz, 1H), 4.42 (dd, 2H), 4.18 (t, 2H), 3.89 (t, 2H), 3.81 – 3.61 (m, 6H), 3.25 (t, J = 6.8 Hz, 2H), 2.40 – 2.07 (m, 4H), 1.40 (s, 9H). ¹³C NMR (126 MHz, Chloroform-d) δ 172.01, 171.93, 169.27, 162.42, 144.08, 125.25, 124.58, 115.72, 108.51, 80.97, 72.12, 70.99, 70.39, 69.75, 68.04, 53.95, 47.16, 32.01, 28.16, 24.31, 2.99. LC/MS (ESI); m/z: [M+H]⁺ Calcd. for C₂₃H₃₄N₂O₇, 577.1410. Found 577.1578.

tert-butyl 5-amino-4-(5-(2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)ethoxy)-1-oxoisindolin-2-yl)-5-oxopentanoate (Compound 19):

To a solution of 3-(4-phenoxyphenyl)-1-(4-piperidyl)pyrazolo[3,4-d]pyrimidin-4-amine; hydrochloride (36.68 mg, 0.09 mmol) (**1**) and TEA (0.1 mL, 0.57 mmol) in DMF (1 mL) was added tert-butyl 5-amino-4-[5-[2-[2-(2-iodoethoxy)ethoxy]ethoxy]-1-oxo-isoindolin-2-yl]-5-oxo-pentanoate (**18**) (50 mg, 0.09 mmol) and the resulting mixture was stirred for 72 h at rt. The reaction mixture was evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH₄OH, 91:8:1) to give 50 mg of pure product (**19**) (69% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 8.22 (s, 1H), 7.65 (d, J = 7.6 Hz, 2H), 7.60 – 7.51 (m, 2H), 7.43 (t, J = 7.4 Hz, 2H), 7.25 – 7.07 (m, 7H), 7.02 (d, J = 8.4 Hz, 1H), 4.75 – 4.66 (m, 1H), 4.66 – 4.60 (m, 1H), 4.54 (d, J = 17.5 Hz, 1H), 4.36 (d, J = 17.5 Hz, 1H), 4.24 – 4.12 (m, 2H), 3.83 – 3.74 (m, 2H), 3.69 – 3.46 (m, 6H), 3.05 – 2.96 (m, 2H), 2.57 – 2.43 (m, 2H), 2.29 – 2.04 (m, 7H), 2.02 – 1.80 (m, 3H), 1.31 (s, 9H). ¹³C NMR (151 MHz, DMSO-d₆) δ 171.96, 171.36, 167.69, 161.49, 158.15, 157.04, 156.28, 155.42, 153.61, 144.66, 142.77, 130.12, 130.00, 128.11, 124.41, 124.16, 123.78, 118.99, 118.93, 115.16, 108.58, 97.44, 79.73, 69.93, 69.71, 68.79, 68.60, 67.66, 57.06, 53.91, 53.29, 52.71, 46.68, 31.77, 31.04, 27.65, 24.88. LC/MS (ESI); m/z [M+H]⁺: Calcd. for C₄₅H₅₅N₈O₈.

3-(5-(2-(2-(2-(4-(4-amino-3-(4-phenoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)piperidin-1-yl)ethoxy)ethoxy)ethoxy)-1-oxoisindolin-2-yl)piperidine-2,6-dione (PROTAC SJF620):

To a solution of tert-butyl 5-amino-4-[5-[2-[2-[2-[4-[4-amino-3-(4-phenoxyphenyl)pyrazolo[3,4-d]pyrimidin-1-yl]-1-piperidyl]ethoxy]ethoxy]ethoxy]-1-oxo-isoindolin-2-yl]-5-oxo-pentanoate (**19**) (27.0 mg, 0.0323 mmol) in MeCN (15 mL) was added benzenesulfonic acid (10.2 mg, 0.0647 mmol) and the reaction mixture was heated at reflux temperature for 24 h (Dean-Stark distilling trap, with molecular sieves). The reaction progress was monitored by LC-MS. The reaction mixture was evaporated to dryness under reduced pressure. The crude was purified by PTLC (DCM:MeOH:NH₄OH, 91:8:1) to give 13 mg of **PROTAC SJF620** (51% yield). ¹H NMR (400 MHz, DMSO-d₆) δ 10.93 (s, 1H), 8.19 (s, 1H), 7.61 (d, J = 8.6 Hz, 2H), 7.56 (d, J = 8.4 Hz, 1H), 7.39 (t, J = 8.0 Hz, 2H), 7.21 – 7.05 (m, 6H), 7.04 – 6.97 (m, 1H), 5.02 (dd, J = 13.3, 4.9 Hz, 1H), 4.69 – 4.52 (m, 1H), 4.40 – 4.07 (m, 4H), 3.84 – 3.68 (m, 2H), 3.63 – 3.43 (m, 6H), 3.06 – 2.92 (m, 2H), 2.92 – 2.77 (m, 1H), 2.67 – 2.46 (m, 3H), 2.39 – 2.23 (m, 1H), 2.25 – 2.04 (m, 4H), 2.00 – 1.74 (m, 3H). ¹³C NMR (151 MHz, DMSO-d₆) δ 172.89, 171.15, 167.89, 161.67, 158.15, 157.05, 156.28, 155.44, 153.62, 144.40, 142.79, 130.13, 130.01, 128.10, 124.31, 124.18, 123.80, 119.00, 118.94, 115.40, 108.63, 97.45, 69.93, 69.72, 68.79, 68.52, 67.72, 57.02, 53.88, 52.67, 51.50, 46.96, 31.25, 30.97, 22.52. LC/MS (ESI); m/z [M+H]⁺: Calcd. For C₄₁H₄₅N₈O₇, 761.3411. Found 761.3866

2. Biology—Culturing of Cell Lines. NAMALWA cells line were purchased from ATCC and cultured according to supplier guidelines at 37 °C and 5% CO₂ in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum, 100 pg/mL streptomycin, and 100 units/mL penicillin-G (Gibco).

Cell Treatment and Immunoblotting. For each treatment concentration, 2 × 10⁶ NAMALWA cells were plated and then incubated with PROTAC for 24 hours. The cells were then collected and rinsed once with ice-cold PBS, followed by lysis in buffer containing 20 mM Tris (pH 8.0), 0.25% sodium deoxycholate, and 1% Triton X-100, supplemented with protease inhibitors (Roche) and phosphatase inhibitors (10 mM NaF, 2 mM Na₃VO₄, 10 mM β-glycerophosphate, and 10 mM sodium pyrophosphate). Lysates were centrifuged at 15000g for 10 min at 4 °C and the supernatant was quantified for total protein content using the BCA protein assay (Thermo Fisher Scientific). Thirty micrograms of protein were loaded onto sodium dodecyl sulfate–polyacrylamide gels (Bio-Rad) for electrophoretic separation, followed by transfer of the resolved proteins onto nitrocellulose membranes, which probed with the specified primary antibodies overnight while being rocked at 4°C in 1×TBS-T (TBS + 0.1% Tween 20) containing 5% nonfat milk. HRP-conjugated secondary antibodies (Pierce) were incubated with the membranes for 1 h at room temperature at 1:10,000 dilutions in 5% nonfat milk in 1× TBS-T. Imaging was performed using the ECL Prime chemiluminescent western blot detection reagents (GE Healthcare) followed by visualization with the Bio-Rad ChemiDoc imaging instrument. All western blots were subsequently processed and quantified using the accompanying Bio-Rad Image Lab software. Anti-BTK (catalog no. 8547) anti-GAPDH (catalog no. 2118) antibodies were purchased from Cell Signaling Technology and used at 1:1000 dilutions in 5% nonfat milk in 1× TBS-T.

Pharmacokinetic Data: Pharmacokinetic properties were estimated externally at Pharmaron Inc.; samples and protocols were prepared following the guidelines of this company.

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Figure 1.
(**A**) Structures of currently FDA-approved BTK inhibitors. (**B**) Aligned structures of ibrutinib (Magenta, PDBID: 5P9J) and GDC-0853 (Blue, PDBID: 5VFI) bound to BTK.

Figure 2.
General schematic for how PROTACs work.

Figure 3.
Chemical structure of MT802

Figure 4.
Ligand interactions diagrams; a) Crystal structure of BTK bound to ibrutinib (PDB, 5PNI).
b) MT802 warhead dock into BTK.

Figure 5.

a) CRBN and VHL ligands. b) Crystal structures of VHL in complex with VHL ligand (PDB code 4W9H)²⁰.

MT802-warhead

SJF608-warhead

SJF620-warhead

Figure 6.
Structural modifications on linker and CRBN ligand in MT802

Figure 7.

BTK degradation profile in NAMALWA cells for PROTACs SJF608 and SJF620. **(A)** Structures of PROTAC derivatives screened for cellular degradation of BTK. **(B)** Representative immunoblots showing degradation of BTK relative to GAPDH loading control. All compounds were screened at indicated concentrations in biological duplicate in NAMALWA. **(C)** Quantitation of DC₅₀ and D_{Max} values of these compounds by non-linear regression.

Scheme 1.

Synthesis of PROTAC **SJF638**. Reagents and conditions: (a) Et₃N, DMF; (b) TFA, DCM; (c) VHL Ligand, PyBOP, Et₃N, DMF.

Scheme 2.

Synthesis of PROTAC **SJF678**. Reagents and conditions: (a) 1,4-butanediol, KOH, DMSO; (b) MsCl, Et₃N, DCM; (c) NaI, MeCN; (d) VHL Ligand C, Cs₂CO₃, DMF; (e), NaI, acetone; (f) **1**, Et₃N, DMF.

Scheme 3.

Synthesis of PROTAC **SJF690**. Reagents and conditions: (a) Triton B, MeCN; (b) NaI, acetone; (c) VHL ligand C, Cs₂CO₃, DMF; (d) TFA, DCM; (e) PyBOP, Et₃N, DMF.

Scheme 4.

Synthesis of PROTAC **SJF608**. Reagents and conditions: (a) Cs_2CO_3 , DMF; (b) Et_3N , DMF; (c) TFA, DCM.

Scheme 5.

Synthesis of PROTAC **SJF620**. Reagents and conditions: (a) Cs_2CO_3 , DMF; (b) Et_3N , DMF; (c) BSA, MeCN, reflux temperature.

Table 1. -

Degradation profile, and pharmacokinetic data after IV compound administration in mice.

PROTAC		In NAMALWA cells line		IV mice (1 mg/Kg)			
Compound	E3 ligase recruited	DC ₅₀ (nM)	D _{max}	Clearance (Cl) mL/min/kg	Half Life (t _{1/2}) h	C _{max} ^{**} (ug/mL)	Exposure (AUC _{last}) min*ng/mL
MT802	CRBN	6.2	99%	1662	0.119	0.073	10.2
SJF638	VHL	374	49%	---	---	---	---
SJF678	VHL	162	50%	---	---	---	---
SJF690	VHL	>1000	---	---	---	---	---
SJF608	CRBN	8.3	91%	102	1.62	0.831	166
SJF620	CRBN	7.9	95%	40.8	1.64	2.1	405

^{**}
T_{max} (h) = 0.033