Discovery of PROTAC BCL-X_L degraders as potent anticancer agents with low on-target platelet toxicity

Xuan Zhang, Dinesh Thummuri, Xingui Liu, Wanyi Hu, Peiyi Zhang, Sajid Khan, Yaxia Yuan, Daohong Zhou, Guangrong Zheng

PII: S0223-5234(20)30153-7

DOI: https://doi.org/10.1016/j.ejmech.2020.112186

Reference: EJMECH 112186

To appear in: *European Journal of Medicinal Chemistry*

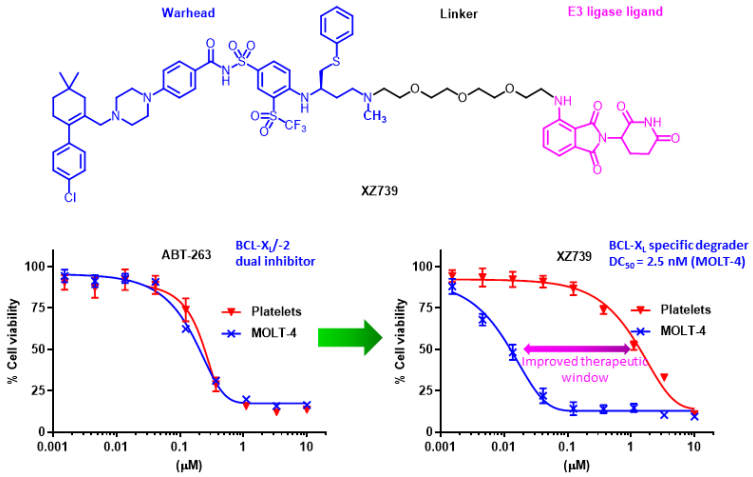
Received Date: 13 January 2020

Revised Date: 21 February 2020

Accepted Date: 23 February 2020

Please cite this article as: X. Zhang, D. Thummuri, X. Liu, W. Hu, P. Zhang, S. Khan, Y. Yuan, D. Zhou, G. Zheng, Discovery of PROTAC BCL-X_L degraders as potent anticancer agents with low on-target platelet toxicity, *European Journal of Medicinal Chemistry* (2020), doi: https://doi.org/10.1016/j.ejmech.2020.112186.

Graphical Abstract



Discovery of PROTAC BCL-X_L Degraders as Potent Anticancer Agents with Low On-target Platelet Toxicity

Xuan Zhang, [a, 1] Dinesh Thummuri, [b, 1] Xingui Liu, [b] Wanyi Hu, [a] Peiyi Zhang, [a] Sajid Khan, [b] Yaxia Yuan, [b] Daohong Zhou, [b, *] Guangrong Zheng [a, *]

[a] Department of Medicinal Chemistry and [b] Department of Pharmacodynamics, College of Pharmacy, University of Florida, 1333 Center Drive, Gainesville, FL, 32610, United States

*** Corresponding Authors.**

E-mail addresses: zhengg@cop.ufl.edu (G.Z.); zhoudaohong@cop.ufl.edu (D.Z.).

[1] Xuan Zhang and Dinesh Thummuri contributed equally.

Abstract: Anti-apoptotic protein BCL-X_L plays a key role in tumorigenesis and cancer chemotherapy resistance, rendering it an attractive target for cancer treatment. However, BCL-X_L inhibitors such as ABT-263 cannot be safely used in the clinic because platelets solely depend on BCL-X_L to maintain their viability. To reduce the on-target platelet toxicity associated with the inhibition of BCL-X_L, we designed and synthesized PROTAC BCL-X_L degraders that recruit CRBN or VHL E3 ligase because both of these enzymes are poorly expressed in human platelets compared to various cancer cell lines. We confirmed that platelet-toxic BCL-X_L/2 dual inhibitor ABT-263 can be converted into platelet-sparing CRBN/VHL-based BCL-X_L specific degraders. A number of BCL-X_L degraders are more potent in killing cancer cells than their parent compound ABT-263. Specifically, XZ739, a CRBN-dependent BCL-X_L degrader, is 20-fold more potent than ABT-263 against MOLT-4 T-ALL cells and has >100-fold selectivity for MOLT-4 cells over human platelets. Our findings further demonstrated the utility of PROTAC technology to achieve tissue selectivity through recruiting differentially expressed E3 ligases.

Keywords: BCL-X_L; PROTAC; apoptosis; platelet; degradation

1. Introduction

Apoptosis is a conserved and highly regulated biological process that plays a critical role in maintaining cellular homeostasis [1]. Resistance to apoptosis is a common feature in human malignancies and considered a key hallmark of cancer [2]. Therefore, targeting specific anti-apoptotic pathways is a promising cancer therapeutic strategy. Members of B-cell lymphoma 2 (BCL-2) protein family, consisting of both pro- and anti-apoptotic proteins, regulates the intrinsic apoptotic pathway. Several anti-apoptotic BCL-2 family proteins such as BCL-2, BCL-$X_{L,}$ and MCL-1 have been validated as anticancer targets [3-5]. Inhibition of these proteins with small-molecule inhibitors promotes Bax/Bak oligomerization and ultimately induces mitochondrial outer membrane permeabilization, followed by cytochrome *c* release and activation of caspases to execute apoptosis [1].

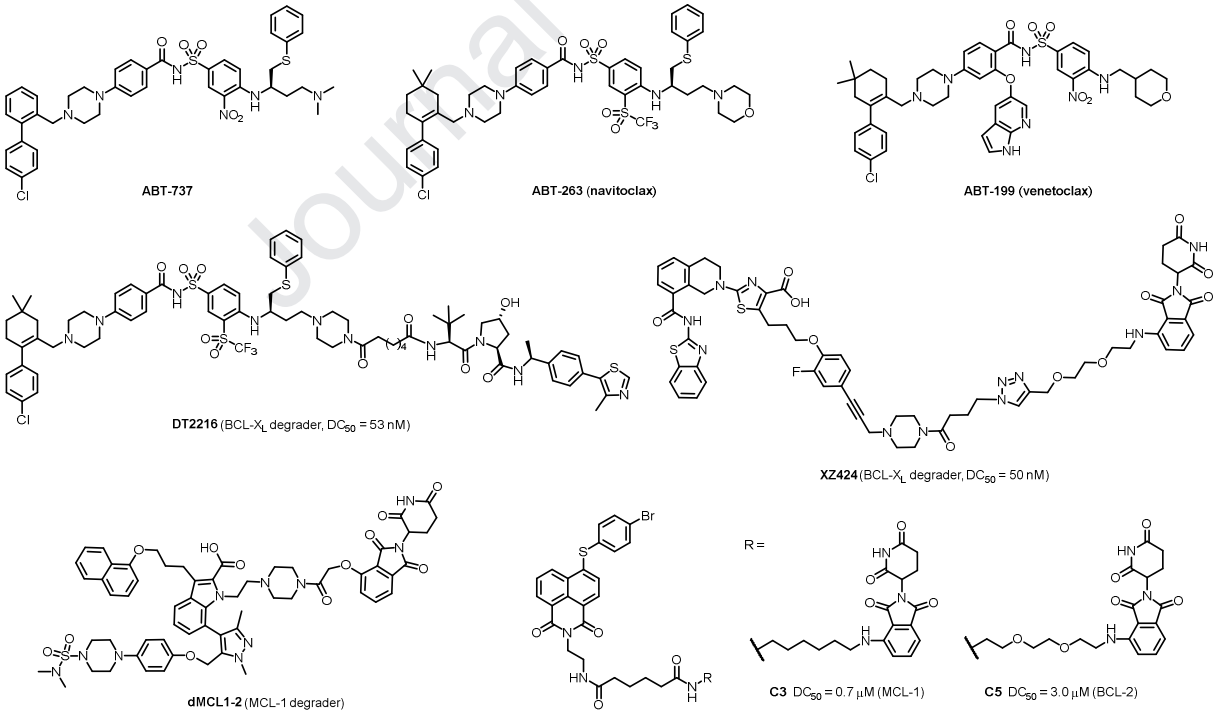


Figure 1. Chemical structures of representative BCl-2/BCL-X_L inhibitors and BCL-2 family protein degraders.

ABT-737 (Figure 1), the first potent BCL-2/BCL-X_L dual inhibitor, was developed *via* fragment-based drug discovery using NMR [6,7]. Optimization of ABT-737 afforded navitoclax (ABT-263) (Figure 1) [8,9], an orally bioavailable BCL-2/BCL-X_L dual inhibitor that has been in Phase II clinical trials for hematological malignancies and small cell lung cancer (SCLC). However, ABT-263 treatment leads to rapid and dose-dependent thrombocytopenia when dosed as a single agent [10], consistent with the studies showing the dependency of platelets on BCL-X_L to maintain their viability [11,12]. To overcome this on-target, dose-limiting toxicity, a selective BCL-2 inhibitor, venetoclax (ABT-199) (Figure 1), was developed. Venetoclax has shown antileukemic activity without the induction of thrombocytopenia [13]. Subsequently, it was approved by the FDA for the treatment of chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) as a single agent, and for acute myeloid leukemia (AML) in combination with low-intensity chemotherapy [14].

The overall response rate of CLL patients to venetoclax is 71-79% but the complete remission rate (20%) is relatively low [15]. Upregulation of BCL-X_L by microenvironmental survival signals has been identified as the major component accountable for the resistance, consistent with the high efficacy of BCL-2/BCL-X_L dual inhibitor ABT-263 in killing venetoclax resistant CLL cells [16]. In addition, venetoclax has limited utility for the treatment of solid tumors [17,18] because BCL-2 is mainly associated with the survival of hematological malignancies and BCL-X_L is the most common BCL-2 family member overexpressed in solid tumors, as well as in a subset of leukemia and lymphoma cells [19]. Bioinformatics analyses also reveal a strong correlation between the levels of BCL-X_L expression and resistance to chemotherapies [20]. Further, it has been well established that inhibition of BCL-X_L by ABT-263 is primarily

responsible for the observed synergy with chemotherapies in solid tumors [21,22]. Taken together, BCL-X_L is one of the most important validated cancer targets.

More recently, we and others discovered that ABT-263 and other BCL-X_L inhibitors, such as A-1331852 and A-1155463 are potent senolytics, referring to small-molecules that can selectively kill senescent cells [23-26]. This is because BCL-X_L is a key anti-apoptotic protein in many types of senescent cells. Subsequent studies on ABT-263 in mouse models have demonstrated that clearance of chemotherapy-induced senescent cells reduces several short- and long-term adverse effects of the chemotherapy, as well as cancer relapse and metastasis [27]. These findings suggest an added benefit of targeting BCL-X_L for cancer treatment. Therefore, it is highly desirable to develop a strategy that can retain the versatility and efficacy of BCL-X_L inhibitors, while reducing their on-target platelet toxicity.

Several strategies have been devised to minimize the on-target platelet toxicity associated with the inhibition of BCL-X_L. One strategy is to combine ABT-263 with chemotherapy or targeted therapies to reduce the dose regimen of ABT-263 so that thrombocytopenia can be manageable [22]. In another strategy, prodrugs of BCL-2/BCL-X_L dual inhibitors have been prepared to limit drug exposure to platelets [28], and the lead candidate, APG-1252, is currently in Phase I clinical trial [29]. Further, antibody-drug conjugates (ADCs) of BCL-X_L inhibitors have been designed to deliver the inhibitors to cancer cells more specifically. For example, ABBV-155, a BCL-X_L inhibitor ADC that targets B7H3 expressing cancer cells, is currently in Phase I clinical trial [30]. We have recently demonstrated that the proteolysis targeting chimera (PROTAC), an emerging therapeutic modality [31-33], is also a feasible solution to reduce platelet toxicity associated with BCL-X_L inhibition [34]. This was based on the hypothesis that because PROTACs engage E3 ligases to induce protein degradation, they can achieve cell/tissue

selectivity if the E3 ligase they recruit is differentially expressed in cells or tissues [34]. Von Hippel–Lindau (VHL) cullin-2 and cereblon (CRBN) cullin-4A RING E3 ligases, of which small-molecule ligands have been successfully employed in PROTAC design, were found to be minimally expressed in human platelets [35,36]. In a proof of concept study, we have shown that DT2216 (Figure 1), a VHL-based PROTAC derived from ABT-263, is more potent to a variety of BCL-X_L dependent cancer cells with significantly less platelet toxicity than its parent compound ABT-263 [34]. In another study, through XZ424 (Figure 1), which is constructed by tethering BCL-X_L specific inhibitor A-1155463 to CRBN ligand pomalidomide, we have demonstrated that CRBN could be an alternative E3 ligase to be recruited to build BCL-X_L degraders with low platelet toxicity [37].

The discovery of PROTAC MCL-1 and BCL-2 degraders (Figure 1) have been reported recently [38,39]. Herein, we describe our efforts in the discovery of potent PROTAC BCL-X_L degraders with low platelet toxicity. Emerging evidence suggests that it could be beneficial to parallelly develop two or more PROTAC series hijacking different E3 ligases [40-42]. Thus, we have evaluated both CRBN and VHL E3 ligases based BCL-X_L degraders. The critical role of linkerology in degradation potency and efficacy of PROTACs is well estabolished [43-45]. Therefore, we have also systematically varied the length and composition of the linker unit.

2. Results and discussion

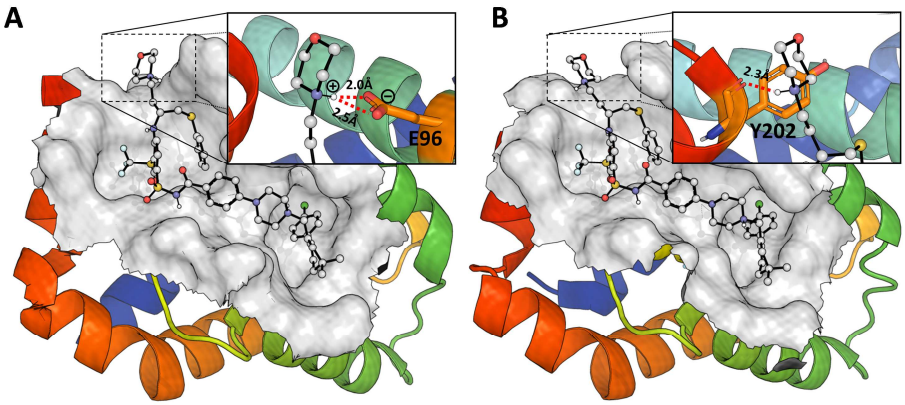
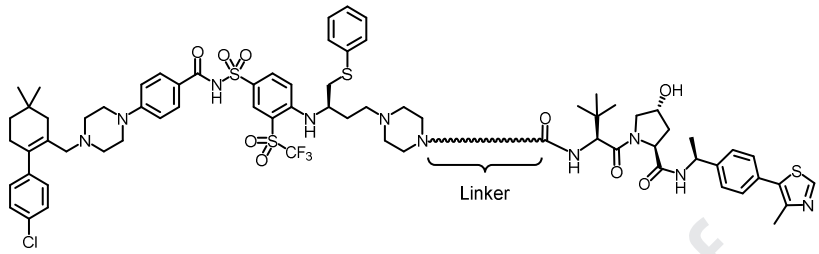
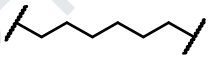
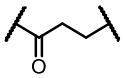
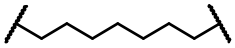
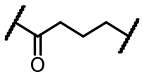
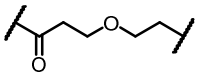
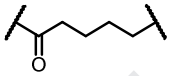
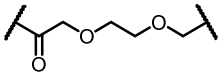
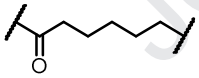
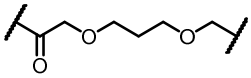
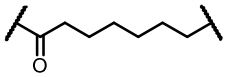
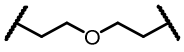
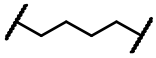
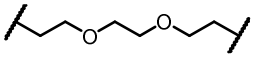


Figure 2. X-ray crystal structures of ABT-263 (black) bound to (A) BCL-X_L, available from PDB, code: 4QNQ, and (B) BCL-2, available from PDB, code: 4LVT. The local view of the key hydrogen bond between morpholine ring and protein is shown in the additional box.

Cytotoxicity of the designed PROTACs and their ability in inducing BCL-X_L degradation were primarily evaluated in MOLT-4, a T-cell acute lymphoblastic leukemia (T-ALL) cell line dependent on BCL-X_L for survival. We focused on ABT-263 as the 'warhead' in our PROTAC design. The co-crystal structures of ABT-263 in complex with BCL-2/BCL-X_L (PDB code 4LVT and 4QNQ, Figure 2) [13] indicate that the morpholine ring in ABT-263 is solvent-exposed, making it a suitable position for tethering to an E3 ligase binding unit through a linker. Accordingly, we initially converted ABT-263 to a 'PROTAC-ready' derivative by replacing the morpholine ring with a piperazine ring, followed by tethering it to a previously reported VHL ligand [46] through various linkers to yield compounds **1a-f**, **2a-d**, **3a-c**, and **4a-b** (Scheme 1, Table 1).

Table 1. BCL-X_L Degraders Designed with Various Linkers Tethering through a Piperazine Ring to a VHL Ligand



Compd	Linker	IC_{50} (nM)[a]	Compd	Linker	IC_{50} (nM)[a]
ABT-263	-	230	**2b**		82.0
1a		>2000	**2c**		94.5
1b		>2000	**2d**		181
1c		>2000	**3a**		324
1d		467	**3b**		512
1e (DT2216)		77.1	**3c**		232
1f		108	**4a**		151
2a		130	**4b**		914

[a] IC_{50} values are the means of at least three independent experiments; reduction of MOLT-4 cell viability after 48 h treatment.

We first evaluated the effects of these compounds on the viability of MOLT-4 T-ALL cells, with ABT-263 as the positive control (Table 1), followed by examining their ability to BCL-2/BCL-X_L degradation in the same cell line using Western blotting (Figure 3). In the series with

linkers containing an amide linkage and an alkane chain, we synthesized compounds **1a-f** with 1-6 methylene groups in the alkane chain (Table 1) and determined the optimal linker length by comparing their potencies in the cell viability assay. DT2216 (**1e**) with five methylene groups in the linker was the most potent in this series and 3 times more potent than ABT-263. Shortening the linker in **1e** afforded **1a-d**, which exhibited largely decreased cellular potencies in the same assay. However, the analog (**1f**) with one methylene unit longer than that in **1e** showed slightly reduced cytotoxicity against MOLT-4 cells. These results indicate that the optimal linker length is six carbon atoms. The cell viability assay data of **1a-f** were well correlated with the protein degradation data (Figure 3), indicating that the cytotoxicity is mainly derived from BCL-X_L protein degradation. Replacing the amide linkage in **1a-f** with a C-N linkage yielded analogs **2a-d**. The optimal linker length in this series is five carbon atoms (compound **2b**) and appears to be one carbon atom shorter than the amide linkage containing series, which could be attributed to the increased flexibility of the C-N bond in comparison to the amide bond. Replacement of one methylene group in the linker of **1e** with an oxygen atom afforded **3a**, which was ~4 times less potent than **1e**. In general, introduction of oxygen atoms into the linker unit is detrimental to cellular potency. None of the oxygen-containing PROTACs we synthesized showed better cytotoxicity to MOLT-4 cells in comparison to their corresponding alkane linker analogs (e.g. **3a** vs **1e**, **3b** vs **1f**, and **4a** vs **2b**). Interestingly, although **4b** was slightly more potent in inducing BCL-X_L degradation than **4a** at 100 nM (Figure 3B), it was ~6 times less potent in reducing MOLT-4 cell viability. The cytotoxicity of the PROTACs is derived from both direct inhibition of BCL-X_L and degradation of this protein. The relative high cellular potency of **4a** could be due to its higher cell permeability than **4b** despite its low efficiency in inducing BCL-X_L protein degradation.

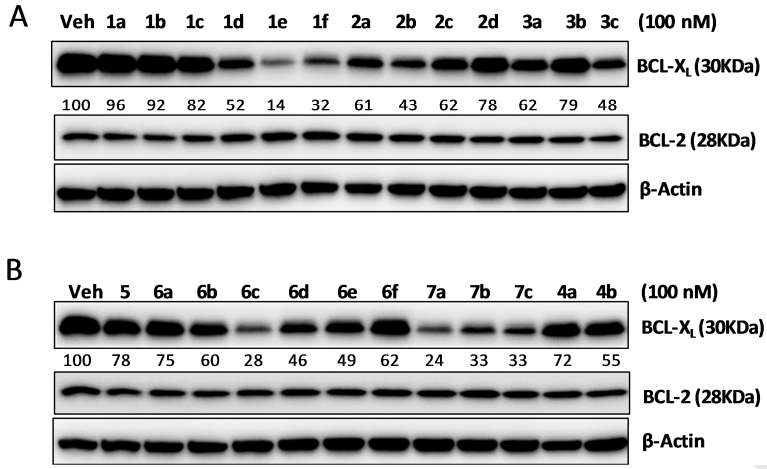
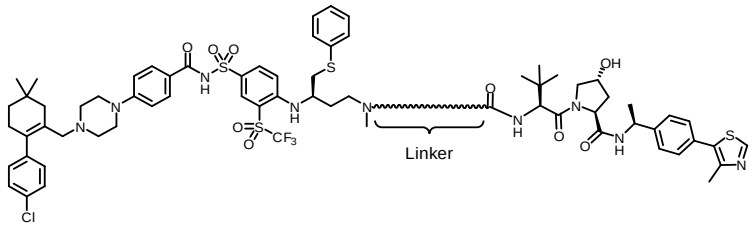
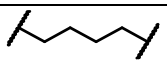
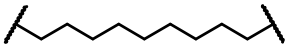
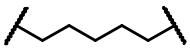
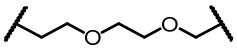
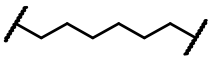
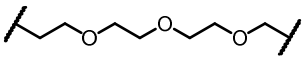
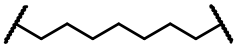
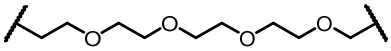


Figure 3. Western blot analysis of the effect of VHL-based BCL-X_L degraders on BCL-X_L and BCL-2 protein levels in MOLT-4 T-ALL cells. Cells were treated with 100 nM of each compound for 16 h before harvesting. Degradation activity is reported as % of total protein remaining after compound treatment relative to the vehicle after normalization with β-actin as quantified by Image J software.

Table 2. BCL-X_L Degraders Designed with Various Linkers Tethering through an *N*-Methylamino Group to a VHL Ligand



Compd	Linker	IC_{50} (nM) [a]	Compd	Linker	IC_{50} (nM) [a]
5		>2000	**6e**		139

6a		1243	**6f**		1058
6b		460	**7a**		97.0
6c		81.3	**7b**		200
6d		147	**7c**		206

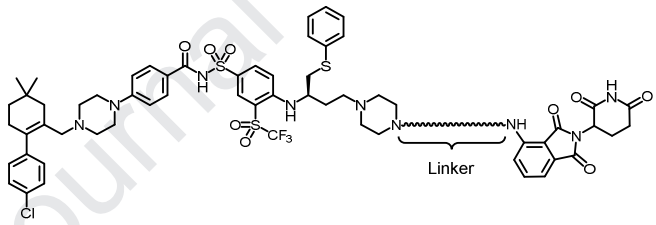
[a] IC_{50} values are the means of at least three independent experiments; reduction of MOLT-4 cell viability after 48 h treatment.

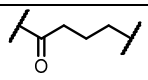
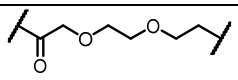
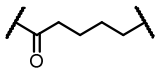
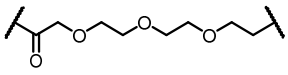
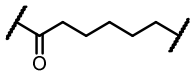
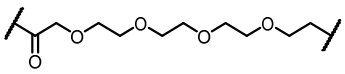
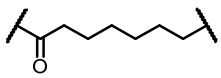
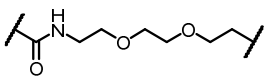
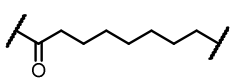
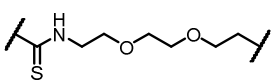
The salt bridge between Glu96 of BCL-X_L and the protonated nitrogen atom on the morpholine ring of ABT-263 is one of the crucial interactions for high BCL-X_L binding (Figure 2). However, the ring system is not required to maintain high binding affinity as the morpholine ring in ABT-263 can be replaced by a dimethylamino group as shown in ABT-737. Thus, we replaced the morpholine ring in ABT-263 with an *N*-methylamino group and tethered it to the same VHL binding moiety as compounds in Table 1 through an alkane chain to generate compounds **6a-f** (Table 2). Interestingly, despite the smaller linkage unit (a single *N* atom vs. a piperazine ring), the optimal linker length for this series of compounds was also six carbon atoms; compound **6c** was the most potent degrader among its analogs (Figure 3B) and exhibited a comparable cellular potency to **1e** in reducing MOLT-4 cell viability. Compound **5**, which was generated by replacing the C-N linkage in **6c** with an amide bond, had no effect on MOLT-4 cell viability (IC_{50} > 2 μM) and weak BCL-X_L degradation in MOLT-4 cells at 100 nM, confirming the importance of the salt bridge in maintaining high BCL-X_L protein binding and the subsequent protein degradation. Incorporation of oxygen atoms into the linker unit of **6d** caused small increases in both protein degradation and cellular potency (**7a** vs. **6d**). In addition, PEG-linker

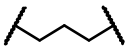
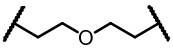
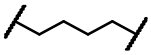
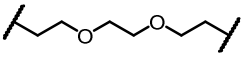
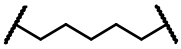
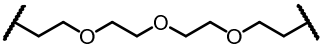
containing PROTACs, i.e. **7a-c**, are linker length independent in inducing BCL-X_L protein degradation and cell death.

Similar to DT2216 (**1e**) [34], none of these VHL-based PROTACs were able to degrade BCL-2 in MOLT-4 cells (Figure 3), further demonstrated the feasibility of achieving target specificity through conversion of non-selective inhibitors to PROTACs. Our studies with the most potent analog in this series, DT2216, have shown that BCL-X_L, but not BCL-2, can form stable ternary complexes with DT2216 and VHL in live cells as determined by nanoBRET assay [47], which could contribute to the specific BCL-X_L degradation [34].

Table 3. BCL-X_L Degraders Designed with Various Linkers Tethering through a Piperazine Ring to Pomalidomide



Compd	Linker	IC_{50} (nM) [a]	Compd	Linker	IC_{50} (nM) [a]
8a		82.0	**10a**		161
8b		346	**10b**		211
8c		518	**10c**		126
8d		>2000	**11a**		118
8e		>2000	**11b**		722

9a		28.3	**12a**		60.5
9b		43.0	**12b**		41.4
9c		100	**12c**		17.3

[a] IC_{50} values are the means of at least three independent experiments; reduction of MOLT-4 cell viability after 48 h treatment.

We next replaced the VHL binding moiety in compounds **1-4** with CRBN binding moiety pomalidomide. It is well established that different E3 ligases require different linker length and/or composition for optimal cellular potency. Thus, we also conducted a systematic investigation of linker length and composition in the CRBN-based PROTACs. As shown in Table 3, compounds with an amide linkage were generally less potent in reducing MOLT-4 cell viability when compared with their corresponding C-N linkage containing analogs with a similar linker length. Among the alkane-linker containing PROTACs (**8a-e** and **9a-c**), compounds with the shortest linkers, i.e. **8a** and **9a**, were the most potent. Compound **8a**, which contains an amide linkage and three methylene groups, was 3 times more potent than the parent compound ABT-263. Switching the amide linkage to a C-N linkage resulted in a significant improvement on cellular potency; compound **9a**, which contains three methylene groups in the linker, was 8 times more potent than ABT-263. Compounds with an amide linkage and a PEG-chain, **10a-c**, appeared to have little dependence on linker length for their cellular potency. Replacing the amide linkage in **10a-c** with a urea linkage, exemplified by **11a**, had little effect on the ability to inhibit MOLT-4 growth. However, the thiourea analog **11b** exhibited a ~6-fold decrease in potency compared to **11a**, in the same assay. In contrast to **9a-c** that contain a C-N linkage and an alkane-chain in their linkers, PEG-linker containing analogs **12a-c** preferred longer linker length for optimal cellular potency. Compound **12c**, which has the longest linker in this series

with 11 atoms, had the highest cellular potency that was ~12 times more potent than ABT-263 in reducing MOLT-4 cell viability. These results further demonstrate the empirical nature of PROTAC design, which is at least partially attributable to the fact that potencies of reducing cell viability for inhibitor-derived PROTACs are a combination of protein degradation and direct protein inhibition, both of which are dependent on cell permeability. As a result, analogs with similar intrinsic protein degradation efficiency may exhibit completely different ability to induce protein degradation in cells.

We evaluated all the compounds in Table 3 for their ability to induce BCL-X_L/BCL-2 protein degradation in MOLT-4 cells at 50 nM for 16 h. As shown in Figure 4, a good correlation between the ability to reduce MOLT-4 cell viability and inducing BCL-X_L degradation was observed within each analog series. However, there was no obvious correlation among compounds from different series. For example, compounds **9a-c** were more potent in the cell viability assay but much less effective in inducing protein degradation than **10a-c**, indicating the cellular activity of **9a-c** was more dependent on direct protein inhibitory effects and permeability. Similar to VHL-based PROTACs, no BCL-2 degradation was observed with these CRBN-based PROTACs.

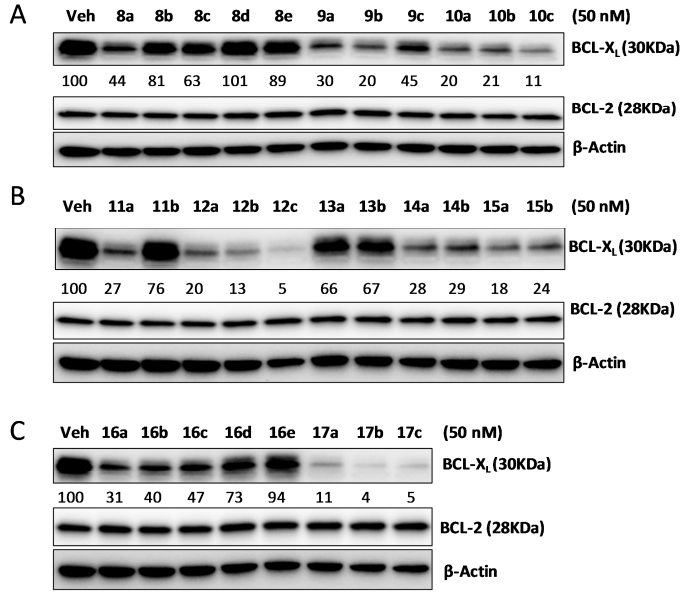
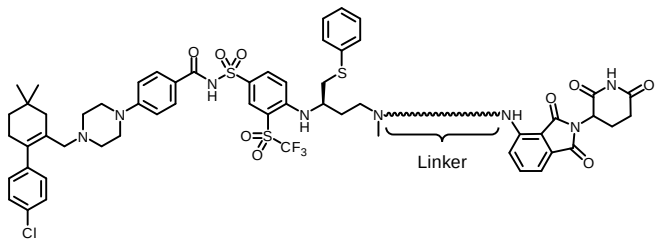
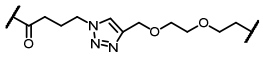
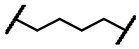
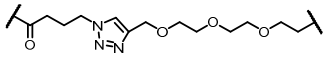
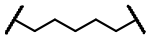
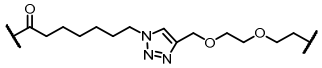
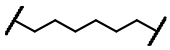
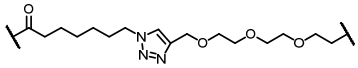
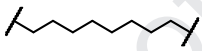
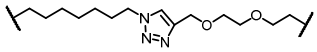
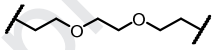
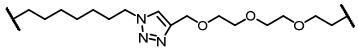
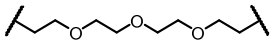
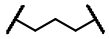
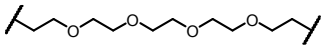


Figure 4. Western-blot analysis of BCL-X_L and BCL-2 protein levels in MOLT-4 T-ALL cells after treating with CRBN-based BCL-X_L degraders at 50 nM for 16 h. Degradation activity reported as % of total protein remaining after compound treatment relative to vehicle after normalization with β-actin as quantified by Image J software.

Table 4. BCL-X_L Degraders Designed with Various Linkers Tethering through an *N*-Methylamino Group to Pomalidomide



Compd	Linker	IC_{50} (nM)[a]	Compd	Linker	IC_{50} (nM)[a]

13a		770	**16b**		38.7
13b		769	**16c**		60.9
14a		177	**16d**		147
14b		179	**16e**		>2000
15a		29.2	**17a**		35.0
15b		35.0	**17b (XZ739)**		10.1
16a		32.5	**17c**		19.3

[a] IC_{50} values are the means of at least three independent experiments; reduction of MOLT-4 cell viability after 48 h treatment.

Subsequently, the *N*-methylamine precursor for analogs in Table 2 was employed for CRBN-based PROTACs and the cell viability data are shown in Table 4. Compounds **13a** and **13b** with an amide linkage to connect the 1,2,3-triazole containing linker and the precursor displayed moderate cytotoxicity against MOLT-4 cells. The extension of the alkane-chain portion of the linker resulted in an improvement in cellular activity (**13a** vs. **14a**, **13b** vs. **14b**). The PEG-chain portion was more tolerated for modification, supported by the similar activity for **13a** and **13b** (or **14a** and **14b**). Switching the amide linkage in compounds **14a-b** to C-N linkage, which resulted in **15a-b**, significantly increased cytotoxicity in MOLT-4 cells. Protein degradation data indicated that **15a** and **15b** are better BCL-X_L degraders than their amide analogs (Figure 4).

Similar to the compounds in Table 2, we also synthesized both alkane-chain and PEG-chain containing PROTACs with a C-N linkage. Compared to the corresponding VHL-based PROTACs with the same linker (Table 2, Figure 3), the CRBN-based PROTACs displayed much higher cytotoxicity and BCL-X_L degradation activity. Compound **17b** (XZ739), which contains a PEG linker with linker length of 11 atoms, was the most potent BCL-X_L degrader against MOLT-4 cells and was ~22 times more potent than ABT-263.

Table 5. Cellular Activity of Selected Analogs in Human Platelets and a Panel of Human Cancer Cell Lines

Compd	IC_{50} (nM)[a] 48 h treatment				DC_{50} (nM)[b]	IC_{50} ratio[c]
	MOLT-4	RS4;11	H146	Platelets		
ABT-263	227	49.0	43.8	242	ND	1.1
DT2216	77.1	213	278	>10,000	53	>130
2b	82.0	76	203	>10,000	93	>122
6c	81.3	189	265	>10,000	71	>123
12c	17.3	38.5	24.6	1,560	4.5	90
15a	29.2	62.2	61.7	6,250	6.3	214
16a	32.5	129	70.2	3,296	10.6	101
17b (XZ739)	10.1	41.8	25.3	1,217	2.5	120

[a] IC_{50} values are the means of at least three independent experiments; [b] in MOLT-4 cells, 16 h treatment; [c] IC_{50} ratio between human platelets and MOLT-4 cells.

We selected several potent BCL-X_L degraders for further investigation. The DC_{50} values (the concentrations at which 50% protein has been degraded) were determined in MOLT-4 cells after treatment with each individual degrader for 16 h. As shown in Table 5 and Figure S2, the CRBN-based PROTACs **12c**, **15a**, **16a**, and **17b** (XZ739) were more potent in inducing BCL-X_L degradation and reducing the viability of MOLT-4 cells than the VHL-based PROTACs DT2216,

2b, and **6c**. Among these PROTACs, XZ739 was the most potent BCL-X_L degrader with a DC_{50} value of 2.5 nM. Likely due to the reduced membrane permeability relative to their parent compound ABT-263, coupled with the lack of BCL-2 degradation, none of these ABT-263 derived BCL-X_L degraders were significantly more potent than ABT-263 in reducing the viability of RS4;11, a B-cell ALL cell line that mainly depends on BCL-2 for survival [13]. The CRBN-based BCL-X_L degraders were also more potent than the VHL-based degraders in H146 cells, a small cell lung cancer cell line that depends on both BCL-2 and BCL-X_L for survival and has low VHL expression (Figure S1). Subsequently, the BCL-X_L degraders in Table 5 were tested for their toxicity against human platelets and most displayed >100-fold selectivity for MOLT-4 cells over platelets. In contrast, ABT-263 showed no selectivity between these platelets and MOLT-4 cells.

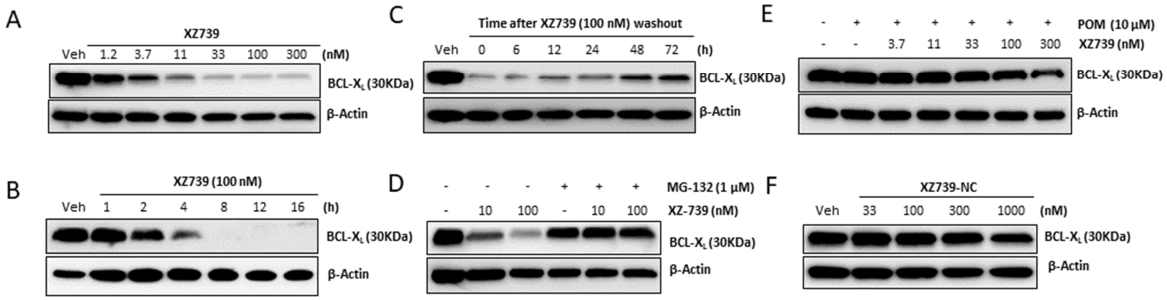


Figure 5. XZ739-induced BCL-X_L degradation. (A) Western blots showing the BCL-X_L protein levels in MOLT-4 cells treated with the indicated concentrations of XZ739 for 16 h. (B) BCL-X_L protein levels in MOLT-4 cells treated with 100 nM of XZ739 at the indicated time points. (C) BCL-X_L protein levels in MOLT-4 cells after incubation with 100 nM of XZ739 for 16 h followed by drug washout, resuspension and incubation of the cells for additional time as indicated in a drug-free medium. (D-E) Pretreatment with 10 μM pomalidomide (POM) or 1 μM MG-132 for 2 h blocked the degradation of BCL-X_L induced by XZ739. (F) Western blot

analysis of BCL-X_L in MOLT-4 cells treated with XZ739-NC at indicated concentrations for 16 h. Data are representative of two independent experiments.

Western blot analysis revealed that XZ739 dose-dependently induced BCL-X_L degradation in MOLT-4 cells (Figure 5A). In addition, the BCL-X_L degradation induced by XZ739 in MOLT-4 was rapid, starting within 2 h; and 8 h after drug treatment, more than 96% of the protein was degraded with 100 nM of XZ739 (Figure 5B). The effects of XZ739 on BCL-X_L protein levels in MOLT-4 were long-lasting and also reversible, as indicated in the 'washout' experiment (Figure 5C). Further, XZ739-induced BCL-X_L degradation can be abrogated by proteasome inhibitor MG-132 (Figure 5D) or excess competitive CRBN ligand pomalidomide (Figure 5E), indicating that the degradation depends on both proteasomes and the CRBN E3 ligase. To further confirm that the CRBN E3 ligase is involved in XZ739-induced BCL-X_L degradation. We synthesized XZ739-NC, a negative control compound of XZ739, in which a methyl group is installed on the amino group in the pomalidomide moiety of XZ739 to block CRBN binding. Not surprisingly, XZ739-NC did not induce BCL-X_L degradation in MOLT-4 cells (Figure 5F).

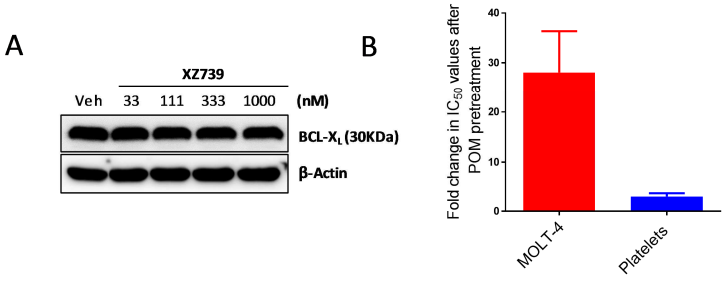


Figure 6. XZ739 shows improved selectivity because of its low activity in inducing BCL-X_L degradation in human platelets, and the remaining cytotoxicity to human platelets mainly depends on BCL-X_L inhibition. (A) Western blot analysis of BCL-X_L levels after treatment of

human platelets with indicated concentration of XZ739 for 16 h. (B) IC_{50} fold changes in MOLT-4 cells and human platelets after blocking with 10 μM pomalidomide (POM). IC_{50} values in MOLT-4 cells and platelets were normalized to 1.0.

In human platelets, no significant changes in BCL-X_L protein levels were observed after 16 h treatment with up to 1.0 μM of XZ739 (Figure 6A). The cytotoxicity of high concentrations of XZ739 to platelets most likely derived from BCL-X_L inhibition rather than degradation as pre-incubation of platelets with pomalidomide did not affect the cytotoxicity of XZ739 to platelets while a ~27-fold shift in IC_{50} values was observed in MOLT-4 cells with pomalidomide pretreatment (Figure 6B).

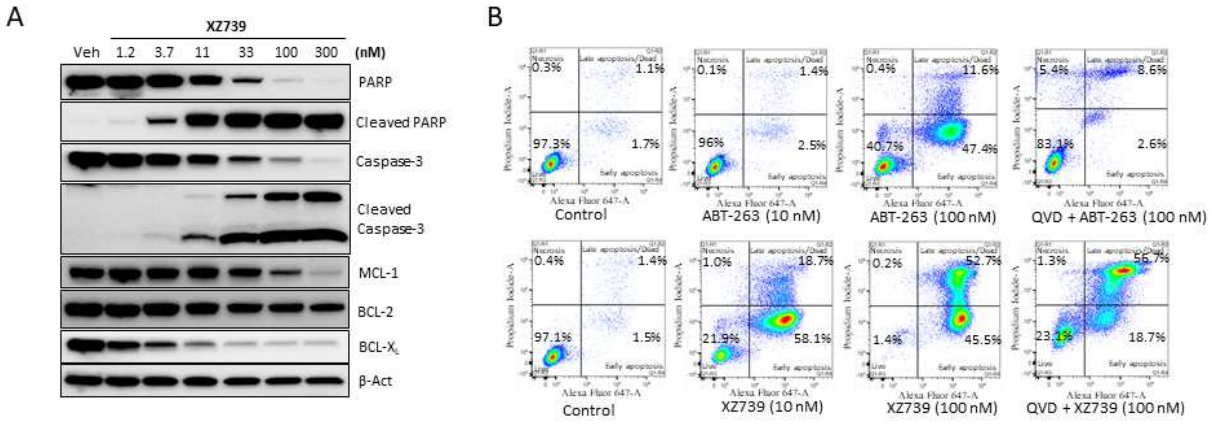


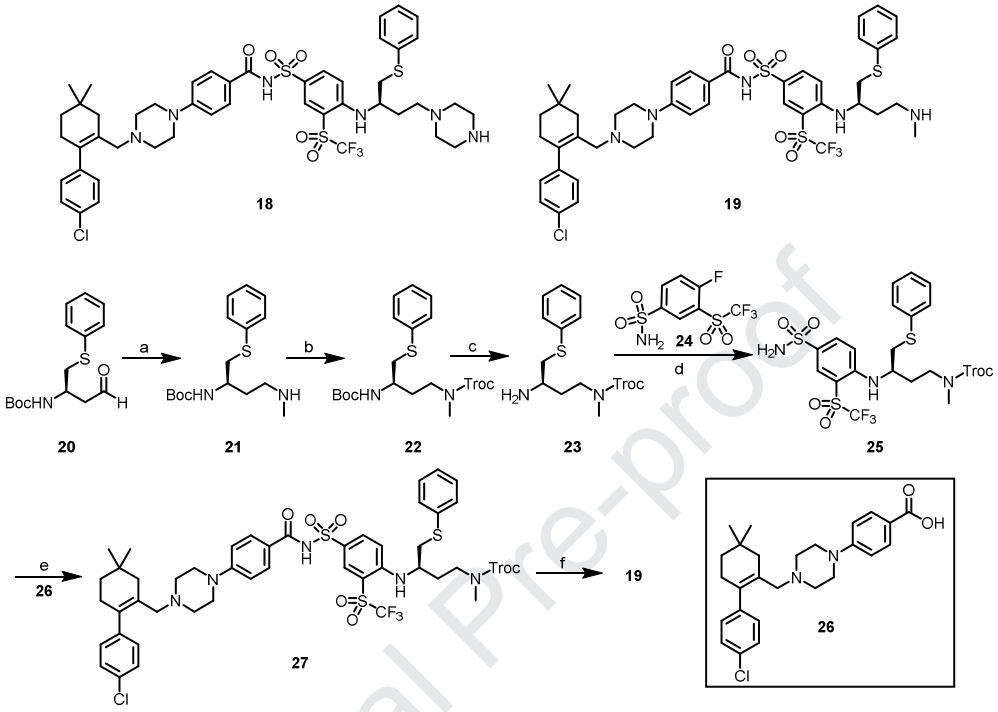
Figure 7. Characterization of XZ739-mediated apoptosis in MOLT-4 cells. (A) Western blot analysis of cleaved-PARP and cleaved-caspase-3 after 16 h treatment with indicated concentrations of XZ739. (B) Flow cytometry analysis of apoptosis using Annexin-V and PI staining. MOLT-4 cells were treated with XZ739 or ABT-263 at 10 and 100 nM for 48 h. XZ739 (100 nM) significantly increased the percentage of apoptotic cells, and QVD (10 μM) pre-

treatment for 2 h inhibited the apoptosis induced by XZ739. Data are representative of two independent experiments.

We next assessed the impact of XZ739 on apoptosis. Western blot analysis showed that XZ739 dose-dependently increased poly (ADP-ribose) polymerase (PARP) and caspase-3 cleavage in MOLT-4 cells (Figure 7A), suggesting the apoptotic cell-death mechanism. Further, to confirm that XZ739 induces cell death through caspase-mediated apoptosis, we did flow cytometry analysis of apoptosis using Annexin-V and propidium iodide (PI) staining (Figure 7B). We found that, 10 nM of XZ739 treatment for 48 h significantly increased the percentage of Annexin-V-positive cells in MOLT-4 cells compared to the vehicle group. In comparison, ABT-263 showed similar effect at much higher concentration (100 nM). In addition, pretreatment with 10 μM of pan-caspase inhibitor Q-VD-OPh (QVD) for 2 h partially inhibited the XZ739-induced apoptosis, which confirms that XZ739 induces cell death through a caspase-dependent mechanism.

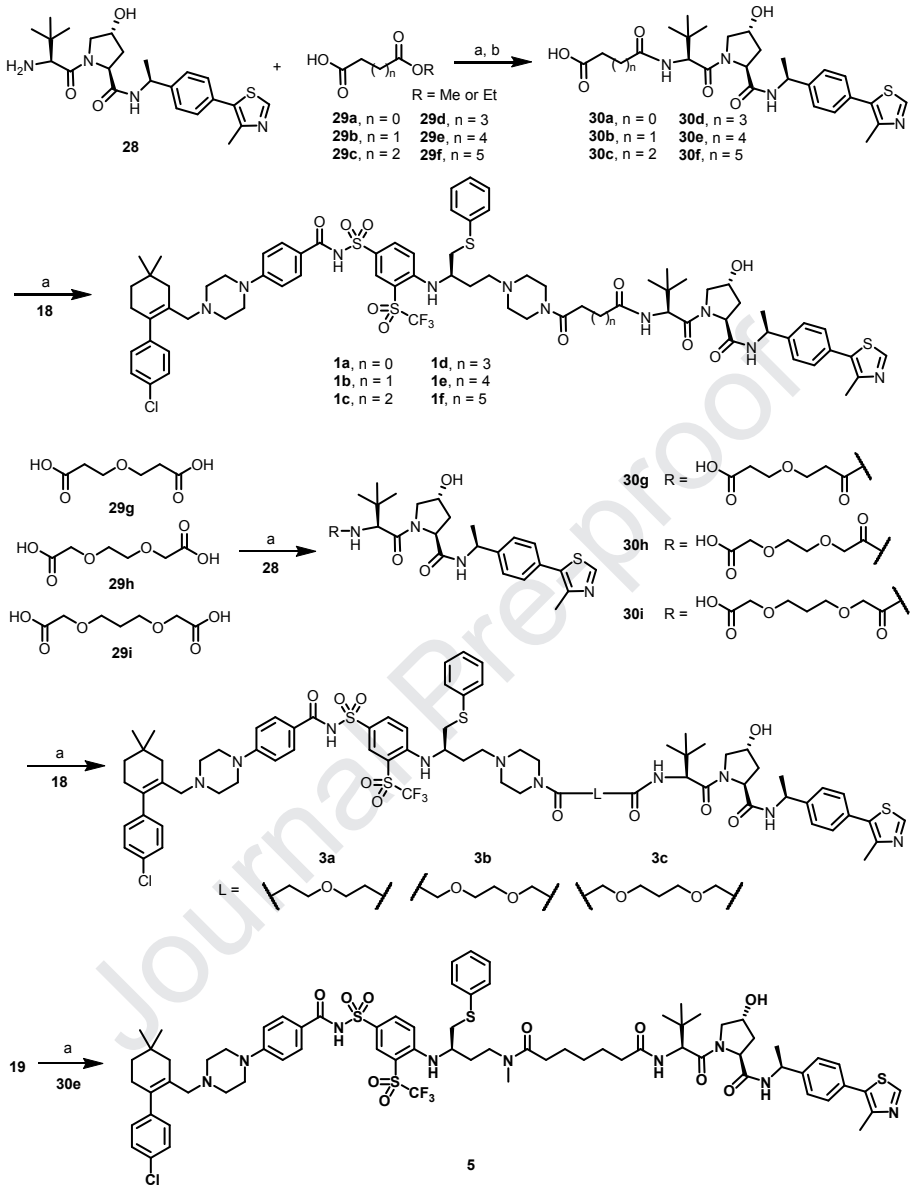
CRBN-targeting immunomodulatory drugs (IMiDs) such as thalidomide, pomalidomide and lenalidomide can induce degradation of a number of neo-substrate proteins through a C2H2 degron. PROTACs that are based on these IMiDs may have the potential to exert some off-target effects by degrading some of these C2H2 zinc finger proteins [48]. To test this possibility, we evaluated the effects of XZ739 on the expression of the Ikaros proteins that are well known targets of those CRBN E3 ligands, and found that IKZF1 and IKZF3 protein levels were not affected in MOLT-4 cells treated with 10 nM XZ739 but downregulated when the cells were treated with 100 nM XZ739 (Figure S6).

3. Chemistry



Scheme 1. Reagents and conditions: (a) i. $MeNH_2$, $MgSO_4$, THF, rt; ii. $NaBH_3CN$, THF, rt; (b) Troc-Cl, TEA, DCM, rt; (c) TFA, DCM, rt; (d) **24**, TEA, acetonitrile, reflux; (e) **26**, EDCI, DMAP, DCM, rt; (f) Zn, HOAc, THF, rt.

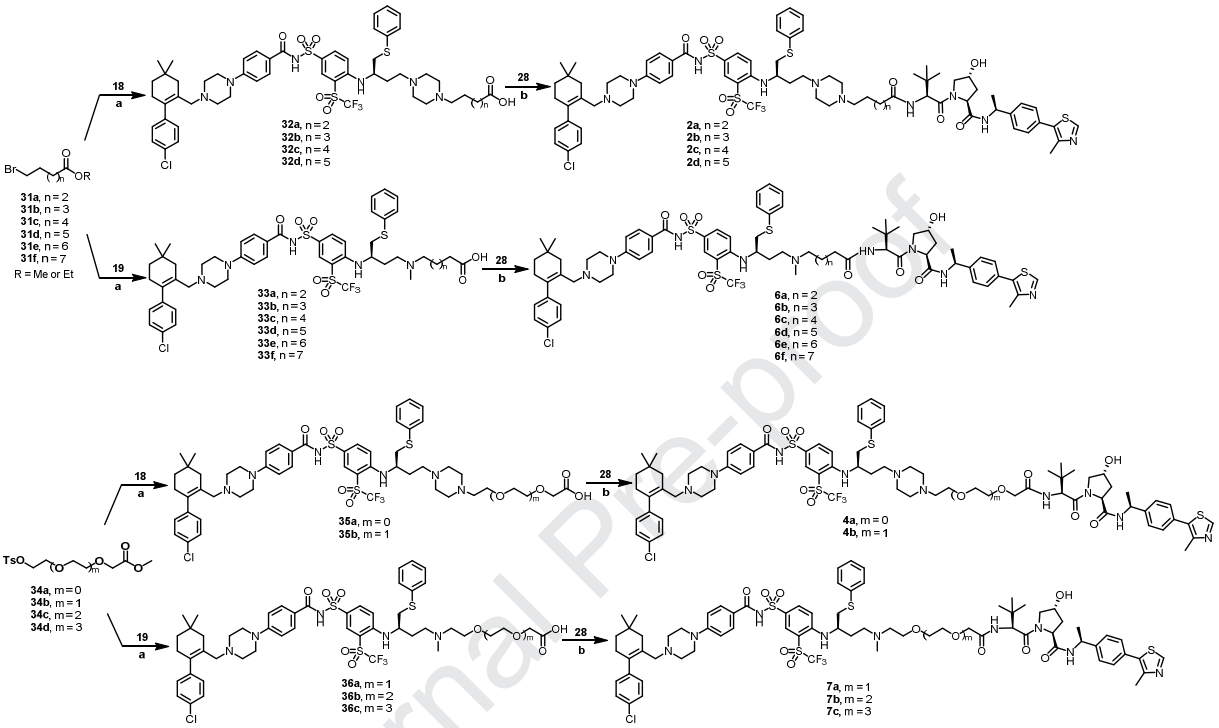
Precursor **18** that contains a piperazinyl moiety for linker attachment and aldehyde **20** were prepared as described in our recent publication [34]. The synthesis of methylamino group containing precursor **19** is outlined in Scheme 1. Briefly, aldehyde **20** was converted to amine **21** through a reductive amination reaction. The amino group in **21** was protected with 2,2,2-trichloroethoxycarbonyl (Troc) group by treating with trichloroethyl chloroformate (Troc-Cl) to give **22**. Removal of the Boc protection group in **22** afforded **23**, which was subjected to S_NAr substitution with **24** to yield *N*-acylsulfonamide **25**. Coupling of **25** with acid **26** in the presence of EDCI and DMAP afforded **27**. Removal of the Troc group in **27** with Zn/HOAc gave **19**.



Scheme 2. Reagents and conditions: (a) HATU, TEA, DCM, rt; (b) LiOH monohydrate, MeOH, H_2O, rt.

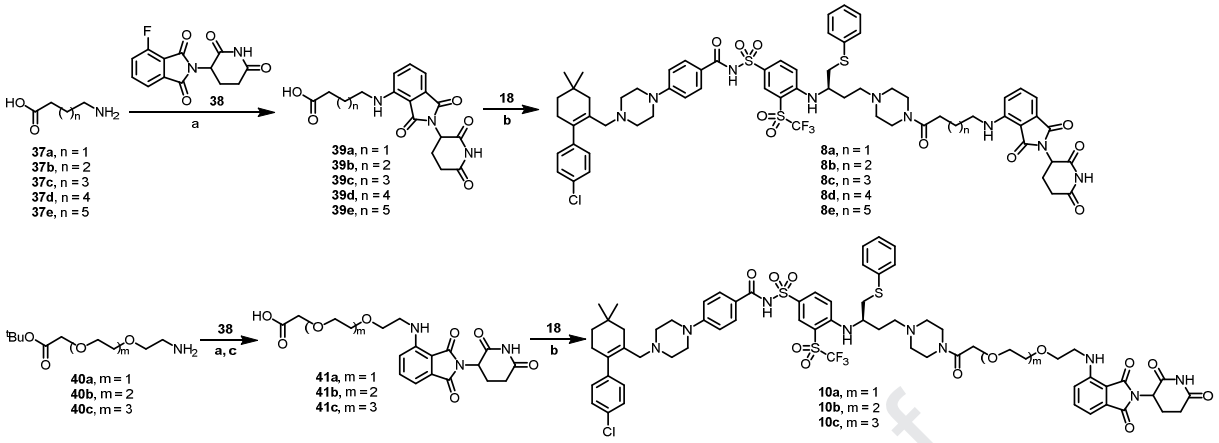
The syntheses of VHL-based PROTACs are illustrated in Schemes 2 and 3. The VHL ligand motif **28** was prepared by following reported methods [46]. Coupling of **28** with acid **29a-f** followed by hydrolysis with LiOH afforded **30a-f**. Direct coupling between dicarboxylic acid

29g-i and **28** also worked smoothly in good yields (62-89%). PROTACs **1a-f**, **3a-c**, and **5** were obtained *via* coupling of precursor **18/19** with **30a-i** (Scheme 2).



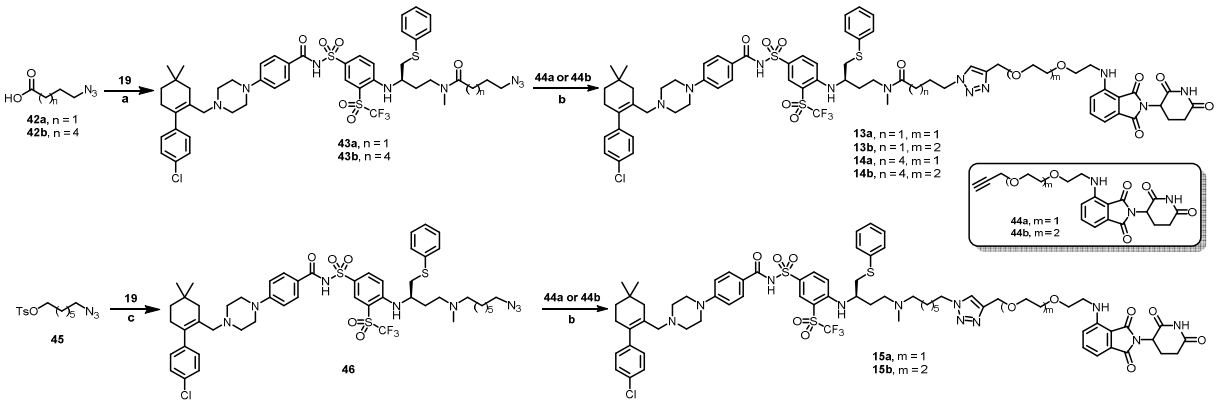
Scheme 3. Reagents and conditions: (a) i. K_2CO_3, NaI, DMSO, 80 °C; ii. LiOH monohydrate, MeOH, THF, H_2O, rt; (b) **28**, HATU, TEA, DCM, rt.

The synthesis of the series with C-N linkage started from bromides **31a-f** or tosylates **34a-d** (Scheme 3). Nucleophilic substitution with amine **18/19** in the presence of K_2CO_3 and NaI at 80 °C, followed by ester hydrolysis, afforded acid intermediates **32a-d**, **33a-f**, **35a-b**, and **36a-c**, respectively, which were coupled with VHL ligand **28** to generate the corresponding final products **2a-d**, **4a-b**, **6a-f**, and **7a-c**.



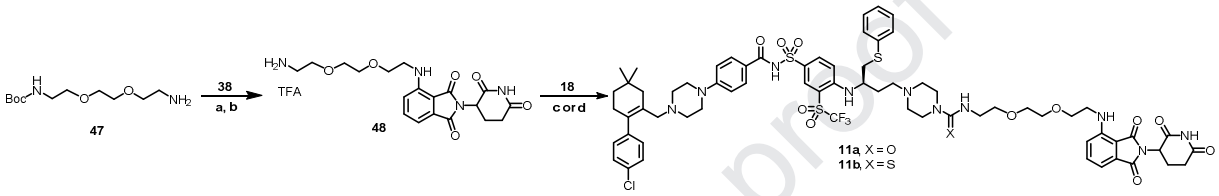
Scheme 4. Reagents and conditions. (a) DIPEA, DMF, 90 °C; (b) **18**, HATU, DIPEA, DCM, rt; (c) TFA, DCM, rt.

The syntheses of CRBN-based PROTAC BCL-X_L degraders are illustrated in Schemes 4-7. As shown in Scheme 4, amino acids **37a-e** were coupled with **38** in the presence of DIPEA to afford intermediates **39a-e**. Compound **8a-e** were synthesized by amide coupling of **39a-e** with **18**. PEG linker containing PROTACs **10a-c** were synthesized by initial coupling of amine **40a-c** with **38**, followed by removal of the *tert*-butyl on them to give intermediates **41a-c** and amide coupling with **18**.



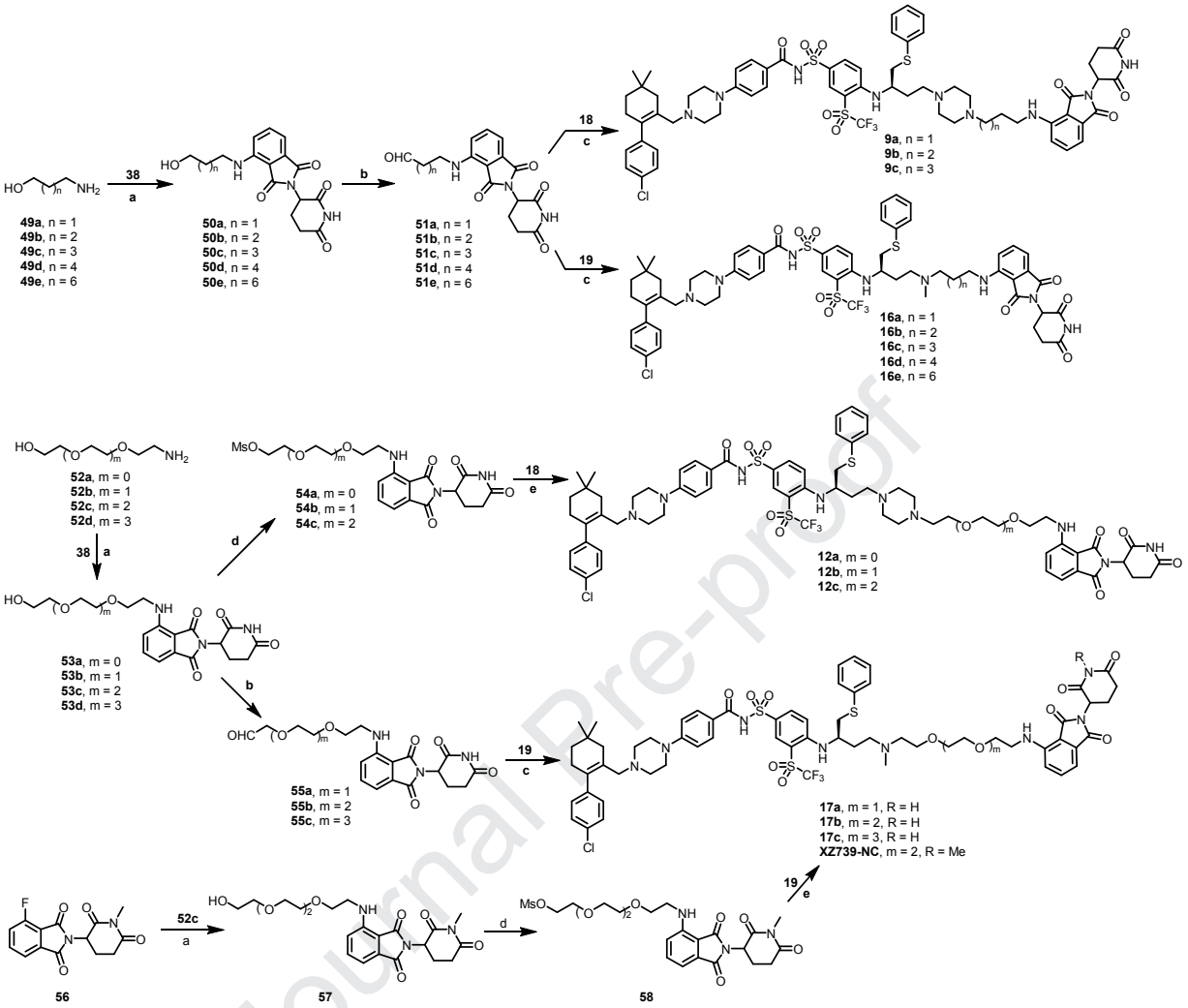
Scheme 5. Reagents and conditions: (a) HATU, DIPEA, DCM, rt; (b) $CuSO_4 \cdot 5H_2O$, sodium L-ascorbate, ^{t}BuOH, THF, H_2O, 50 °C; (c) K_2CO_3, NaI, DMSO, 80 °C.

As shown in Scheme 5, compounds **13a-b** and **14a-b** were produced through coupling of azido acid **42a-b** with precursor **19,** followed by a click reaction with alkynes **44a/44b** [37]. The synthesis of **15a-b** started from a nucleophilic substitution of tosylate **45** with **19**, followed by click reaction.



Scheme 6. Reagents and conditions. (a) DIPEA, DMF, 90 °C; (b) TFA, DCM, rt; (c) i. carbonyldiimidazole, TEA, DCM, rt; ii. **18**, DIPEA, rt; (d) i. 1,1'-thiocarbonyldiimidazole, TEA, DCM, rt; ii. **18**, DIPEA, rt.

The synthesis of compounds **11a** and **11b** is shown in Scheme 6. The key intermediate **48** was synthesized by coupling of compounds **47** and **38**, followed by removal of the Boc group by treating with TFA/DCM. Compound **48** was treated with carbonyldiimidazole or 1,1'-thiocarbonyldiimidazole and then with precursor **18** to afford compound **11a** and **11b**, respectively.



Scheme 7. Reagents and conditions. (a) DIPEA, DMF, 90 °C; (b) i. $COCl_2$, DMSO, DCM, -78 °C; ii. **50** or **53**, DCM, -78 °C; iii. TEA, DCM, -78 °C then rt; (c) $NaBH_3CN$, TEA, DCM, rt; (d) MsCl, TEA, DCM, rt; (e) DIPEA, NaI, 1,4-dioxane, 90 °C.

The syntheses of CRBN-based PROTACs with a C-N linkage are illustrated in Scheme 7. Since the pomalidomide moiety is unstable under the treatment of inorganic bases, they were synthesized either by nucleophilic substitution of mesylates with precursor **18**/**19** in the presence of DIPEA or by reductive amination of aldehydes with **18**/**19**. Briefly, coupling of **38** with **49a-e**/**52a-d** afforded **50a-e** and **53a-d**, respectively. Swern oxidation of **50a-e** produced aldehyde

51a-e. Other oxidation methods, including PCC, PDC, Dess-Martin periodinane, and IBX, afforded unsatisfactory results. Subsequent reductive amination of the aldehydes with the corresponding precursor **18** or **19** yielded compounds **9a-c** and **16a-e**, respectively. Compounds **17a-c** were obtained by using the same strategy. Compounds **12a-c** were synthesized by using a different method. Mesylates **54a-c**, which are more stable than the corresponding aldehyde intermediates, were prepared from **53a-c**. Nucleophilic substitution of **54a-c** with **18** in the presence of DIPEA and NaI afforded **12a-c**. Similarly, XZ739-NC, the negative control compound of XZ739, was synthesized starting from compound **56**.

4. Conclusions

In this study, we reported the design, synthesis, and evaluation of a series of PROTAC BCL-X_L degraders. Through tethering BCL-2/BCL-X_L dual inhibitor ABT-263 and a VHL ligand or a CRBN ligand, and varying the linker unit, we obtained a number of BCL-X_L degraders that showed improved cytotoxicity against BCL-X_L dependent MOLT-4 cells when compared with ABT-263. Protein degradation assay confirmed that these BCL-X_L PROTACs were able to degrade BCL-X_L but not BCL-2 despite that the warhead ABT-263 binds to both proteins. In addition, human platelets were more tolerant to the treatment of these degraders whereas the parent BCL-X_L inhibitor ABT-263 showed no selectivity for MOLT-4 cells over platelets. Both CRBN and VHL-based PROTACs were able to achieve >100-fold selectivity *in vitro* for MOLT-4 cells over human platelets. XZ739, a CRBN-based BCL-X_L PROTAC, was the most potent analog against various cancer cell lines. The mouse plasma stability assay and preliminary pharmacokinetic study revealed that XZ739 is bioavailable (Figure S4). Overall, we have

developed a novel strategy to reduce drug on-target toxicity by conversion of a conventional inhibitor into a protein degrader.

Despite the high molecular weight of PROTACs that are derived from PPI inhibitors, a number of PPI-targeting degraders have been successfully developed, including MCL-1 [38], BCL-2/MCL-1 [39], MDM2 [49], BCL-6 [50], and STAT3 [51,52]. Due to their catalytic mode of action, common 'rules' such as 'rule-of-five' may not be suitable to predict the drug-like properties of PROTACs. The size and molecular weight of our BCL-X_L specific degraders are among the largest PROTACs, which further highlighted the broad utility of this therapeutic modality.

5. Experimental

5.1. Chemistry

THF, DCM, toluene, and acetonitrile were obtained via a solvent purification system by filtering through two columns packed with activated alumina and 4 Å molecular sieve, respectively. All other chemicals obtained from commercial sources were used without further purification. Flash chromatography was performed using silica gel (230–400 mesh) as the stationary phase. Reaction progress was monitored by thin-layer chromatography (silica-coated glass plates) and visualized by UV light, and/or by LC-MS. NMR spectra were recorded in $CDCl_3$, CD_3OD, or acetone-*d6* at 400 or 600 MHz for ^1H NMR. Chemical shifts δ are given in using tetramethylsilane as an internal standard. Multiplicities of NMR signals are designated as singlet (s), broad singlet (*br* s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), and multiplet (m). All final compounds for biological testing were of ≥95.0% purity as analyzed by LC-MS, performed on an Advion AVANT LC system with the expression CMS using two

different columns: a Thermo Accucore™ Vanquish™ C18+ UHPLC Column (1.5 µm, 50 x 2.1 mm) at 40 °C and a Thermo Scientific™ BetaSil™ C18 Column (3.0 µm, 150 x 4.6 mm) at 25 °C. Gradient elution was used for UHPLC with a mobile phase of acetonitrile and water containing 0.1% formic acid.

5.1.1. General methods

5.1.1.1. General Method A. A mixture of acid (1.0 equiv.), amine (1.0 equiv.), HATU (1.05 equiv.) and TEA (5.0 equiv.) in DCM was stirred at room temperature for 1 h. The mixture was poured into water and extracted with DCM. The combined organic layers were washed with NH_4Cl (aq.) × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue crude product was purified by flash column chromatography to afford the desired compound.

5.1.1.2. General Method B. To a solution of ester (1.0 equiv.) in MeOH was added LiOH (5.0 equiv.). The mixture was stirred at room temperature for 2 h and the solvent was removed under reduced pressure. The residue crude product was purified by column chromatography to afford the corresponding carboxylic acid**.**

5.1.1.3. General Method C. A mixture of amine (1.0 equiv.), an appropriate bromide or tosylate (5.0 equiv.), K_2CO_3 (2.0 equiv.), and NaI (0.2 equiv.) in DMSO was stirred at 80 °C overnight. The mixture was poured into water and extracted with DCM. The combined organic layers were washed with NH_4Cl (aq.) × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The crude product was purified by column chromatography to afford the corresponding ester intermediate.

5.1.1.4. General Method D. 2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (1.0 equiv.), amine (1.2 equiv.), and DIPEA (2.0 equiv.) in DMF were stirred at 90 °C overnight. The

reaction was cooled to room temperature and poured into water. The resulting mixture was extracted with ethyl acetate and the combined organic layers were washed with water × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under vacuum. The residue crude product was purified by column chromatography to afford the desired compound.

5.1.1.5. General Method E. To a solution of *tert*-butyl ester (1.0 equiv.) in DCM was added TFA (20-30 equiv.). The mixture was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. The residue crude product was washed with Et_2O to afford the desired product.

5.1.1.6. General Method F. To a mixture of azide (1.0 equiv.), alkyne (1.2 equiv.) in ^{t}BuOH-THF (1:1, v/v) under argon was added $CuSO_4 \cdot 5H_2O$ (0.2 equiv.) and sodium ascorbate (0.2 equiv.) in water. The mixture was stirred at 50 °C for 3 h and cooled to room temperature before poured into water. The resulting mixture was extracted with DCM. The combined organic layers were washed with brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crude product was purified via flash column chromatography using DCM and MeOH as eluents to afford the desired product.

5.1.1.7. General Method G. DMSO (3.0 equiv.) in DCM was cooled to -78 °C and $(COCl)_2$ (1.5 equiv.) was added dropwise. The mixture was stirred for 10 min and alcohol (1.0 equiv.) in DCM was added dropwise into the solution. TEA (6.0 equiv.) was added after 10 min and the resulting mixture was kept at -78 °C for 30 min and then warmed to room temperature. The mixture was poured into water and extracted with DCM. The combined organic layers were washed with water × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crude product was purified by flash column chromatography using DCM

and MeOH as eluents to afford the corresponding aldehyde, which was used directly in the next step.

5.1.1.8. General Method H. A mixture of amine (1.0 equiv.) and aldehyde (1.5 equiv.) in DCM was treated with triethylamine (4.0 equiv.) and $NaBH_3CN$ (2.0 equiv.). The mixture was stirred at room temperature overnight. The solution was poured into water and extracted with DCM. The combined organic layers were washed with water × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crude product was purified by flash column chromatography using DCM and MeOH as eluents to afford the desired product.

5.1.1.9. General Method I. To a mixture of alcohol (1.0 equiv.), TEA (4.0 equiv.) in DCM was added MsCl (1.2 equiv.). The mixture was stirred at room temperature for 3 h and poured into water. The resulting mixture was extracted with ethyl acetate and the combined organic layers were washed with water × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crude product was purified by flash column chromatography using DCM and MeOH as eluents.

5.1.1.10. General Method J. A mixture of amine (1.0 equiv.), an appropriate mesylate (3.0 equiv.), DIPEA (10 equiv.), and NaI (0.2 equiv.) in 1,4-dioxane was stirred at 90 °C overnight. The mixture was poured into water and extracted with ethyl acetate. The combined organic phases were washed with water × 1, NH_4Cl (aq.) × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crude product was purified by flash column chromatography using DCM and MeOH as eluents to afford the desired product.

(*R*)-*tert*-Butyl (4-(methylamino)-1-(phenylthio)butan-2-yl)carbamate (21). A mixture of compound **20** (450 mg, 1.53 mmol), $MeNH_2$ (2.0 M in MeOH; 7.5 mL, 15.0 mmol) and $MgSO_4$ (3.0 g, 24.9 mmol) in THF (50 mL) was stirred at room temperature overnight. $NaBH_3CN$ (143

mg, 2.27 mmol) was then added. The resulting mixture was stirred at room temperature for 30 min. The solution was diluted with water and extracted with DCM. The combined organic phases were washed with brine × 1, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified via column chromatography using DCM and MeOH as eluents to afford the title compound (280 mg, 56% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.28 (m, 4H), 7.27–7.23 (m, 1H), 5.09 (d, *J* = 8.1 Hz, 1H), 4.52–4.31 (m, 1H), 3.86–3.66 (m, 1H), 3.27–2.81 (m, 3H), 2.67 (s, 3H), 2.30–2.17 (m, 1H), 1.88–1.72 (m, 1H), 1.44 (s, 9H). LC-MS (ESI): m/z 311.1 $[M+H]^+$.

(*R*)-2,2,2-Trichloroethyl (3-((tert-butoxycarbonyl)amino)-4-(phenylthio)butyl)(methyl)carbamate (22). A mixture of compound **21** (280 mg, 0.90 mmol), Troc-Cl (137 μL, 1.00 mmol) and TEA (250 μL, 1.80 mmol) in DCM (10 mL) was stirred at room temperature for 3 h. The mixture was poured into water and extracted with DCM. The combined organic phases were washed with brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The residue crude product was purified via flash column chromatography using ethyl acetate and hexanes as eluents to afford the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.16 (m, 5H), 4.87–4.59 (m, 3H), 3.89–3.71 (m, 1H), 3.59–3.40 (m, 1H), 3.33–3.01 (m, 3H), 3.00–2.91 (m, 3H), 2.11–1.88 (m, 1H), 1.86–1.68 (m, 1H), 1.46–1.37 (m, 9H). LC-MS (ESI): m/z 484.9 $[M+H]^+$.

(*R*)-2,2,2-Trichloroethyl (3-amino-4-(phenylthio)butyl)(methyl)carbamate trifluoroacetate (23). To a solution of compound **22** (from above) in DCM (10 mL) was added TFA (700 μL, 9.15 mmol). The mixture was stirred at room temperature for 3 h and the solvent was removed under reduced pressure. The residue solid was washed with Et_2O to afford the title compound, which was used directly in the next step.

(*R*)-2,2,2-Trichloroethyl methyl(4-(phenylthio)-3-((4-sulfamoyl-2-((trifluoromethyl)sulfonyl)phenyl)amino)butyl)carbamate (25). A mixture of compound **23** (from above), 4-fluoro-3-((trifluoromethyl)sulfonyl)benzenesulfonamide **24** (280 mg, 0.91 mmol) and TEA (750 μL, 5.39 mmol) in acetonitrile (30 mL) was refluxed overnight. The reaction was cooled to room temperature and the solvent was evaporated under reduced pressure. The residue was purified via flash column chromatography using ethyl acetate and hexanes as eluents to afford the title compound (220 mg, 36% yield in 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.81 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.45–7.29 (m, 5H), 7.03–6.94 (m, 1H), 6.51–6.37 (m, 1H), 4.92 (s, 2H), 4.83–4.55 (m, 2H), 3.81–3.61 (m, 1H), 3.56–3.24 (m, 2H), 3.19–2.90 (m, 5H), 2.46–2.19 (m, 1H), 1.93–1.71 (m, 1H). LC-MS (ESI): m/z 672.0 $[M+H]^+$.

(*R*)-2,2,2-Trichloroethyl (3-((4-(*N*-(4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)(methyl)carbamate (27). A mixture of compound **25** (220 mg, 0.33 mmol), acid **26** (174 mg, 0.40 mmol), EDCI (151 mg, 0.79 mmol) and DMAP (96 mg, 0.79 mmol) in DCM (15 mL) was stirred at room temperature overnight. The mixture was poured into water and extracted with DCM. The combined organic layers were washed with 1N HCl (aq.) × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified via flash column chromatography using DCM and MeOH as eluents to afford the title compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (d, *J* = 2.2 Hz, 1H), 8.13 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.66 (d, *J* = 8.7 Hz, 2H), 7.45–7.28 (m, 6H), 7.08–6.95 (m, 3H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.48–6.38 (m, 1H), 4.83–4.50 (m, 2H), 3.82–3.61 (m, 1H), 3.48–3.26 (m, 6H), 3.22–2.88 (m, 7H), 2.60–2.19 (m, 7H), 2.12–2.06 (m, 2H), 1.93–1.74 (m, 1H), 1.54–1.41 (m, 2H), 0.99 (s, 6H). LC-MS (ESI): m/z 1091.9 $[M+H]^+$.

(*R*)-4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-((4-(methylamino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (19). Zinc powder (960 mg, 14.8 mmol) was added to a mixture of compound **27** and AcOH (600 μL, 10.5 mmol) in THF (20 mL). The reaction was stirred at room temperature overnight. Solid was removed by filtration and the filtrate was poured into water and extracted with ethyl acetate. The organic phases were washed brine × 1, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified via column chromatography using DCM, MeOH and TEA as eluents to afford the title compound (180 mg, 60% yield in 2 steps). ^{1}H NMR (600 MHz, CD_3OD) δ 8.29 (d, *J* = 2.3 Hz, 1H), 8.09 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.85–7.79 (m, 2H), 7.43–7.34 (m, 4H), 7.27–7.20 (m, 2H), 7.19–7.13 (m, 3H), 6.96–6.91 (m, 2H), 6.88 (d, *J* = 9.3 Hz, 1H), 4.14–4.01 (m, 1H), 3.53–3.34 (m, 7H), 3.24–3.21 (m, 1H), 3.13–3.02 (m, 2H), 3.02–2.82 (m, 3H), 2.70 (s, 3H), 2.42–2.35 (m, 2H), 2.29–2.19 (m, 1H), 2.12–1.97 (m, 4H), 1.57 (t, *J* = 6.5 Hz, 2H), 1.07 (s, 6H). LC-MS (ESI): m/z 918.1 $[M+H]^+$.

3-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropanoic acid (30a). Following general methods A and B, compound **30a** was obtained from **29a** and **28** (27 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.65 (s, 1H), 7.90 (s, 1H), 7.43–7.29 (m, 4H), 5.13–4.98 (m, 1H), 4.83–4.67 (m, 1H), 4.55–4.36 (m, 2H), 4.15 (d, *J* = 11.4 Hz, 1H), 3.64–3.49 (m, 1H), 3.27–3.09 (m, 2H), 2.50 (s, 3H), 2.43–2.23 (m, 1H), 2.21–2.06 (m, 1H), 1.45 (d, *J* = 7.0 Hz, 3H), 1.06 (s, 9H). LC-MS (ESI): m/z 531.1 $[M+H]^+$.

4-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-

oxobutanoic acid (30b). Following general methods A and B, compound **30b** was obtained from **29b** and **28** (84 mg, 99% yield) ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.94 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.44–7.34 (m, 4H), 5.13–5.03 (m, 1H), 4.81–4.73 (m, 1H), 4.51–4.38 (m, 2H), 4.15 (d, *J* = 11.4 Hz, 1H), 3.54 (dd, *J* = 11.4, 3.5 Hz, 1H), 2.64–2.37 (m, 8H), 2.16–2.06 (m, 1H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.05 (s, 9H). LC-MS (ESI): m/z 545.1 $[M+H]^+$.

5-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-5-oxopentanoic acid (30c). Following general methods A and B, compound **30c** was obtained from **29c** and **28**. (10 mg, 31% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 1H), 7.45–7.32 (m, 4H), 7.19 (s, 1H), 5.15–5.02 (m, 1H), 4.80–4.69 (m, 1H), 4.57 (d, *J* = 8.4 Hz, 1H), 4.46 (s, 1H), 4.16–4.03 (m, 1H), 3.60 (dd, *J* = 11.1, 3.8 Hz, 1H), 2.52 (s, 3H), 2.47–1.84 (m, 8H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.05 (s, 9H). LC-MS (ESI): m/z 559.2 $[M+H]^+$.

6-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoic acid (30d). Following general methods A and B, compound **30d** was obtained from **29d** and **28** (33 mg, 59% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.67 (d, *J* = 7.7 Hz, 1H), 7.43–7.32 (m, 4H), 6.85 (d, *J* = 8.9 Hz, 1H), 5.09 (t, *J* = 7.2 Hz, 1H), 4.72 (t, *J* = 8.1 Hz, 1H), 4.63 (d, *J* = 9.0 Hz, 1H), 4.46 (s, 1H), 4.06 (d, *J* = 11.3 Hz, 1H), 3.61 (dd, *J* = 11.2, 3.6 Hz, 1H), 2.52 (s, 3H), 2.41–2.05 (m, 6H), 1.73–1.52 (m, 4H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.03 (s, 9H). LC-MS (ESI): m/z 573.2 $[M+H]^+$.

7-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-7-oxoheptanoic acid (30e). Following general methods A and B, compound **30e** was obtained

from **29e** and **28** (100 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.72 (s, 1H), 8.05–7.89 (m, 1H), 7.43–7.33 (m, 4H), 7.24–7.08 (m, 1H), 5.14–4.95 (m, 1H), 4.73–4.40 (m, 3H), 4.00–3.93 (m, 1H), 3.76–3.59 (m, 1H), 2.52 (s, 3H), 2.38–2.05 (m, 6H), 1.71–1.49 (m, 9H), 1.04 (s, 9H). LC-MS (ESI): m/z 587.2 $[M+H]^+$.

8-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-8-oxooctanoic acid (30f). Following general methods A and B, compound **30f** was obtained from **29f** and **28** (90 mg, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.59 (d, *J* = 7.8 Hz, 1H), 7.40–7.33 (m, 4H), 6.92 (d, *J* = 8.7 Hz, 1H), 5.15–4.98 (m, 1H), 4.76–4.67 (m, 1H), 4.62 (d, *J* = 8.9 Hz, 1H), 4.52 (s, 1H), 4.04 (d, *J* = 11.2 Hz, 1H), 3.74–3.59 (m, 1H), 2.51 (s, 3H), 2.39–2.10 (m, 6H), 1.66–1.45 (m, 7H), 1.35–1.27 (m, 4H), 1.03 (s, 9H). LC-MS (ESI): m/z 601.2 $[M+H]^+$.

3-(3-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)propanoic acid (30g). Following general method A, compound **30g** was obtained from **29g** and **28** (21.2 mg, 62% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.48–7.32 (m, 4H), 7.10 (d, *J* = 8.3 Hz, 1H), 5.12–4.98 (m, 1H), 4.76 (t, *J* = 8.0 Hz, 1H), 4.55 (d, *J* = 8.4 Hz, 1H), 4.49–4.41 (m, 1H), 4.08 (d, *J* = 11.4 Hz, 1H), 3.79–3.63 (m, 4H), 3.57 (dd, *J* = 11.3, 3.7 Hz, 1H), 2.53–2.42 (m, 8H), 2.15–2.05 (m, 1H), 1.47 (d, *J* = 6.9 Hz, 3H), 1.03 (s, 9H). LC-MS (ESI): m/z 589.2 $[M+H]^+$.

2-(2-(2-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)acetic acid (30h). Following general method A, compound **30h** was obtained

from **29h** and **28** (22 mg, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.64–7.31 (m, 6H), 5.13–4.98 (m, 1H), 4.75 (t, *J* = 8.1 Hz, 1H), 4.57 (d, *J* = 8.8 Hz, 1H), 4.48–4.40 (m, 1H), 4.15–3.85 (m, 5H), 3.80–3.63 (m, 4H), 3.58 (dd, *J* = 11.3, 3.5 Hz, 1H), 2.55–2.37 (m, 4H), 2.16–2.02 (m, 1H), 1.47 (d, *J* = 6.8 Hz, 3H), 1.05 (s, 9H). Yield: 11.3 mg, 31%. LC-MS (ESI): m/z 605.3 $[M+H]^+$.

2-(3-(2-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)propoxy)acetic acid (30i). Following general method A, compound **30i** was obtained from **29i** and **28** (32 mg, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 7.69 (d, *J* = 7.7 Hz, 1H), 7.40–7.26 (m, 5H), 5.11–5.02 (m, 1H), 4.71 (t, *J* = 7.9 Hz, 1H), 4.57 (d, *J* = 9.0 Hz, 1H), 4.49–4.43 (m, 1H), 4.03–3.85 (m, 5H), 3.69–3.57 (m, 5H), 2.51 (s, 3H), 2.45–2.34 (m, 1H), 2.12–2.03 (m, 1H), 1.96–1.87 (m, 2H), 1.47 (d, *J* = 7.0 Hz, 3H), 1.03 (s, 9H). LC-MS (ESI): m/z 619.4 $[M+H]^+$.

(2*S*,4*R*)-1-((*S*)-2-(3-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-3-oxopropanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (1a). Following general method A, compound **1a** was obtained from **30a** and **18** (13.1 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74–8.54 (m, 2H), 8.35 (d, *J* = 2.2 Hz, 1H), 8.10 (dd, *J* = 9.3, 2.3 Hz, 1H), 7.79–7.58 (m, 3H), 7.41–7.27 (m, 10H), 7.26–7.19 (m, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 7.01–6.94 (m, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.64 (d, *J* = 9.4 Hz, 1H), 5.13–5.01 (m, 1H), 4.80–4.71 (m, 1H), 4.54–4.44 (m, 2H), 4.19–4.04 (m, 1H), 3.97–3.84 (m, 1H), 3.72–2.88 (m, 15H), 2.59–2.02 (m, 20H), 1.66 (d, *J* = 13.1 Hz,

1H), 1.45 (d, *J* = 6.9 Hz, 5H), 1.08 (s, 9H), 0.98 (s, 6H). LC-MS (ESI): m/z 1485.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(4-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-4-oxobutanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (1b). Following general method A, compound **1b** was obtained from **30b** and **18** (14.2 mg, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.37 (d, *J* = 2.2 Hz, 1H), 8.09 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.68 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 7.8 Hz, 1H), 7.43–7.28 (m, 10H), 7.26–7.21 (m, 1H), 7.11–6.89 (m, 4H), 6.76 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 9.4 Hz, 1H), 5.14–5.01 (m, 1H), 4.81–4.74 (m, 1H), 4.53 (d, *J* = 8.5 Hz, 1H), 4.47 (s, 1H), 4.06 (d, *J* = 11.5 Hz, 1H), 3.97–3.83 (m, 1H), 3.63–2.84 (m, 13H), 2.62–1.99 (m, 24H), 1.74–1.61 (m, 1H), 1.51–1.43 (m, 5H), 1.06 (s, 9H), 0.98 (s, 6H). LC-MS (ESI): m/z 1499.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(5-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-5-oxopentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (1c). Following general method A, compound **1c** was obtained from **30c** and **18**. (19.9 mg, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.34 (d, *J* = 2.2 Hz, 1H), 8.09 (dd, *J* = 9.3, 2.3 Hz, 1H), 7.71 (d, *J* = 8.7 Hz, 2H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.41–7.27 (m, 9H), 7.26–7.21 (m, 2H), 7.12–6.95 (m, 3H), 6.89 (d, *J* = 8.3 Hz, 1H), 6.76 (d, *J* = 8.8 Hz, 2H), 6.61 (d, *J* = 9.5 Hz, 1H), 5.14–5.02 (m, 1H), 4.79–4.68 (m, 1H),

4.58–4.44 (m, 2H), 4.12 (d, *J* = 11.3 Hz, 1H), 3.96–3.81 (m, 1H), 3.68–2.85 (m, 13H), 2.54–2.00 (m, 24H), 1.87 (m, *J* = 7.2 Hz, 2H), 1.78–1.60 (m, 1H), 1.46 (dd, *J* = 6.7, 3.6 Hz, 5H), 1.06 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1513.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(6-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-6-oxohexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (1d). Following general method A, compound **1d** was obtained from **30d** and **18** (19.4 mg, 88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.34 (d, *J* = 2.3 Hz, 1H), 8.09 (dd, *J* = 9.3, 2.3 Hz, 1H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.45–7.27 (m, 10H), 7.26–7.22 (m, 2H), 7.12–6.94 (m, 3H), 6.76 (d, *J* = 8.7 Hz, 2H), 6.62 (d, *J* = 9.4 Hz, 1H), 6.56 (d, *J* = 8.7 Hz, 1H), 5.14–5.01 (m, 1H), 4.80–4.69 (m, 1H), 4.61 (d, *J* = 8.8 Hz, 1H), 4.49 (s, 1H), 4.10 (d, *J* = 11.4 Hz, 1H), 3.98–3.84 (m, 1H), 3.70–2.85 (m, 13H), 2.54–2.00 (m, 24H), 1.45 (d, *J* = 6.7 Hz, 10H), 1.05 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1527.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(7-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-7-oxoheptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (1e). Following general method A, compound **1e** was obtained from **30e** and **18** (12.7 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.34 (d, *J* = 2.3 Hz, 1H), 8.08 (d, *J* = 9.1 Hz, 1H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.46–7.27 (m, 12H), 7.14–6.93 (m, 3H), 6.76 (d, *J* = 8.6 Hz, 2H), 6.61 (d, *J* = 9.4 Hz, 1H), 6.34 (d, *J* = 8.7 Hz, 1H),

5.13–5.00 (m, 1H), 4.79–4.69 (m, 1H), 4.60 (d, *J* = 8.7 Hz, 1H), 4.49 (s, 1H), 4.10 (d, *J* = 11.4 Hz, 1H), 3.96–3.83 (m, 1H), 3.72–2.81 (m, 13H), 2.55–2.00 (m, 24H), 1.77–1.30 (m, 12H), 1.03 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1541.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(8-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-8-oxooctanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (1f). Following general method A, compound **1f** was obtained from **30f** and **18** (21.7 mg, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.34 (d, *J* = 2.3 Hz, 1H), 8.14–8.05 (m, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.49–7.27 (m, 11H), 7.25–7.18 (m, 1H), 7.13–6.94 (m, 3H), 6.75 (d, *J* = 8.6 Hz, 2H), 6.61 (d, *J* = 9.4 Hz, 1H), 6.32 (d, *J* = 8.7 Hz, 1H), 5.14–5.02 (m, 1H), 4.77–4.67 (m, 1H), 4.60 (d, *J* = 8.8 Hz, 1H), 4.49 (s, 1H), 4.10 (d, *J* = 11.4 Hz, 1H), 3.97–3.84 (m, 1H), 3.74–2.85 (m, 13H), 2.54–1.99 (m, 24H), 1.76–1.23 (m, 14H), 1.04 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1555.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(3-(3-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-3-oxopropoxy)propanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (3a). Following general method A, compound **3a** was obtained from **30g** and **18** (25.0 mg, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.34 (d, *J* = 2.2 Hz, 1H), 8.00 (dd, *J* = 9.2, 2.2 Hz, 1H), 7.82 (d, *J* = 8.9 Hz, 2H), 7.44–7.32 (m, 7H), 7.31–7.26 (m, 3H), 7.26–7.21 (m, 2H), 7.01–6.91 (m, 4H), 6.76 (d, *J* = 9.0 Hz, 2H), 6.53 (d, *J* = 9.4 Hz, 1H), 5.12–4.99 (m, 1H), 4.73 (t, *J* = 8.0 Hz, 1H), 4.54 (d, *J* = 8.4 Hz, 1H),

4.50–4.42 (m, 1H), 4.09 (d, *J* = 11.4 Hz, 1H), 3.89–3.62 (m, 6H), 3.55 (dd, *J* = 11.4, 3.6 Hz, 1H), 3.48–3.30 (m, 3H), 3.27–3.20 (m, 4H), 3.09 (dd, *J* = 13.8, 4.8 Hz, 1H), 2.94 (dd, *J* = 13.8, 7.6 Hz, 1H), 2.82 (s, 2H), 2.59–2.00 (m, 24H), 1.69–1.60 (m, 1H), 1.48–1.41 (m, 5H), 1.03 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1543.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(2-(2-(2-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-2-oxoethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (3b). Following general method A, compound **3b** was obtained from **30h** and **18** (18.0 mg, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.35 (d, *J* = 2.3 Hz, 1H), 8.00 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.43–7.33 (m, 8H), 7.32–7.27 (m, 3H), 7.26–7.21 (m, 2H), 7.04–6.93 (m, 3H), 6.80–6.72 (m, 2H), 6.57 (d, *J* = 9.4 Hz, 1H), 5.11–5.00 (m, 1H), 4.71 (t, *J* = 8.0 Hz, 1H), 4.58 (d, *J* = 8.8 Hz, 1H), 4.51–4.42 (m, 1H), 4.25–3.94 (m, 5H), 3.90–3.81 (m, 1H), 3.72–3.37 (m, 9H), 3.28–3.22 (m, 4H), 3.11–3.06 (m, 1H), 2.96 (dd, *J* = 13.8, 7.4 Hz, 1H), 2.82 (s, 2H), 2.51–2.00 (m, 20H), 1.71–1.59 (m, 1H), 1.49–1.42 (m, 5H), 1.05 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1559.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(2-(3-(2-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)-2-oxoethoxy)propoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (3c). Following general method A, compound **3c** was obtained from **30i** and **18** (15.9 mg, 63% yield). ^{1}H NMR (400 MHz,

$CDCl_3$) δ 8.67 (s, 1H), 8.36 (d, *J* = 2.2 Hz, 1H), 8.03 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.78–7.71 (m, 2H), 7.46–7.33 (m, 7H), 7.32–7.27 (m, 3H), 7.26–7.22 (m, 3H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.02–6.95 (m, 2H), 6.80–6.68 (m, 2H), 6.60 (d, *J* = 9.4 Hz, 1H), 5.12–5.02 (m, 1H), 4.74 (t, *J* = 7.9 Hz, 1H), 4.58 (d, *J* = 8.8 Hz, 1H), 4.53–4.46 (m, 1H), 4.19–3.81 (m, 6H), 3.72–3.54 (m, 6H), 3.52–3.33 (m, 3H), 3.32–3.22 (m, 4H), 3.12–3.07 (m, 1H), 2.98 (dd, *J* = 13.8, 7.3 Hz, 1H), 2.84 (s, 2H), 2.50–1.88 (m, 22H), 1.69–1.62 (m, 1H), 1.50–1.42 (m, 5H), 1.05 (s, 9H), 0.97 (s, 6H). LC-MS (ESI): m/z 1573.6 $[M+H]^+$; HPLC: >95% purity.

N^1-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)-N^7-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-N^1-methylheptanediamide (5). Following general method A, compound **5** was obtained from **30e** and **19** (6.6 mg, 29% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.69 (s, 1H), 8.44–8.30 (m, 1H), 8.10–7.96 (m, 1H), 7.92–7.57 (m, 3H), 7.43–7.34 (m, 6H), 7.31–7.18 (m, 4H), 7.08–6.73 (m, 6H), 6.56–6.26 (m, 1H), 5.12–4.94 (m, 1H), 4.72–4.40 (m, 3H), 4.12–3.88 (m, 1H), 3.81–2.77 (m, 15H), 2.51 (s, 3H), 2.45–1.93 (m, 15H), 1.83–1.16 (m, 12H), 1.08–0.94 (m, 15H). LC-MS (ESI): m/z 1486.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(5-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)pentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (2a). Following general methods C, B and A, compound **2a** was obtained from **31a**, **18** and **28** (7.3 mg, 27% yield). ^{1}H NMR (400 MHz,

$CDCl_3$ and CD_3OD) δ 8.64 (s, 1H), 8.26 (d, *J* = 2.2 Hz, 1H), 8.05–7.94 (m, 1H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.56 (d, *J* = 7.7 Hz, 1H), 7.38–7.29 (m, 6H), 7.25–7.15 (m, 5H), 7.00–6.83 (m, 4H), 6.73 (d, *J* = 8.6 Hz, 2H), 6.54 (d, *J* = 9.3 Hz, 1H), 5.08–4.95 (m, 1H), 4.59 (t, *J* = 8.2 Hz, 1H), 4.52 (d, *J* = 9.0 Hz, 1H), 4.42 (s, 1H), 3.95 (d, *J* = 11.3 Hz, 1H), 3.84–3.69 (m, 1H), 3.56 (dd, *J* = 11.3, 3.4 Hz, 1H), 3.23–3.15 (m, 4H), 3.05 (dd, *J* = 13.7, 5.0 Hz, 1H), 2.95 (dd, *J* = 13.8, 7.0 Hz, 1H), 2.78 (s, 2H), 2.67–1.92 (m, 28H), 1.69–1.40 (m, 10H), 0.99 (s, 9H), 0.94 (s, 6H). LC-MS (ESI): m/z 1499.8 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(6-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (2b). Following general methods C, B and A, compound **2b** was obtained from **31b**, **18** and **28** (8.4 mg, 30% yield). ^1H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.67 (s, 1H), 8.30 (d, *J* = 2.2 Hz, 1H), 8.01 (dd, *J* = 9.1, 2.2 Hz, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.57 (d, *J* = 7.7 Hz, 1H), 7.42–7.32 (m, 6H), 7.29–7.19 (m, 5H), 7.03–6.90 (m, 3H), 6.77 (t, *J* = 9.1 Hz, 3H), 6.54 (d, *J* = 9.3 Hz, 1H), 5.06 (p, *J* = 7.0 Hz, 1H), 4.66 (t, *J* = 8.2 Hz, 1H), 4.57 (d, *J* = 9.1 Hz, 1H), 4.45 (s, 1H), 4.01 (d, *J* = 11.4 Hz, 1H), 3.79 (s, 1H), 3.58 (dd, *J* = 11.2, 3.3 Hz, 1H), 3.22 (t, *J* = 4.9 Hz, 4H), 3.08 (dd, *J* = 13.7, 4.8 Hz, 1H), 3.01–2.92 (m, 1H), 2.82 (s, 2H), 2.79–1.94 (m, 28H), 1.75–1.18 (m, 12H), 1.02 (s, 9H), 0.97 (d, *J* = 1.8 Hz, 6H). LC-MS (ESI): m/z 1513.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(7-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-

yl)heptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (2c). Following general methods C, B and A, compound **2c** was obtained from **31c**, **18** and **28** (22.6 mg, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.75 (s, 1H), 8.33 (d, *J* = 2.2 Hz, 1H), 8.25 (d, *J* = 7.6 Hz, 1H), 8.07 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.81 (d, *J* = 8.7 Hz, 2H), 7.56 (s, 1H), 7.41–7.17 (m, 11H), 7.13–7.00 (m, 3H), 6.84 (d, *J* = 9.0 Hz, 2H), 6.69 (d, *J* = 9.4 Hz, 1H), 5.09–4.98 (m, 1H), 4.65–4.46 (m, 3H), 4.00–3.82 (m, 2H), 3.77–3.65 (m, 1H), 3.39–2.92 (m, 12H), 2.75–1.95 (m, 24H), 1.86–1.21 (m, 14H), 1.04 (s, 9H), 1.02 (s, 6H). LC-MS (ESI): m/z 1527.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(8-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (2d). Following general methods C, B and A, compound **2d** was obtained from **31d**, **18** and **28** (18.3 mg, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.75 (s, 1H), 8.34 (d, *J* = 2.2 Hz, 1H), 8.20 (d, *J* = 7.6 Hz, 1H), 8.08 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.78 (d, *J* = 8.7 Hz, 2H), 7.56 (s, 1H), 7.46–7.19 (m, 11H), 7.13 (d, *J* = 8.4 Hz, 1H), 7.07–6.99 (m, 2H), 6.88–6.80 (m, 2H), 6.68 (d, *J* = 9.4 Hz, 1H), 5.11–4.98 (m, 1H), 4.61–4.41 (m, 3H), 3.99–3.82 (m, 2H), 3.78–3.67 (m, 1H), 3.37–2.91 (m, 12H), 2.65–1.99 (m, 24H), 1.85–1.30 (m, 16H), 1.04 (s, 9H), 1.01 (s, 6H). LC-MS (ESI): m/z 1541.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(5-(((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-

(phenylthio)butyl)(methyl)amino)pentanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (6a). Following general methods C, B and A, compound **6a** was obtained from **31a**, **19** and **28** (11.1 mg, 47% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.43–8.34 (m, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.48–7.29 (m, 8H), 7.28–7.18 (m, 4H), 7.05–6.92 (m, 3H), 6.84–6.74 (m, 3H), 6.34 (*br* s, 1H), 5.15–5.07 (m, 1H), 4.87–4.77 (m, 1H), 4.60 (d, *J* = 8.8 Hz, 1H), 4.53–4.46 (m, 1H), 4.19–4.02 (m, 2H), 3.66–3.58 (m, 1H), 3.30–3.20 (m, 4H), 3.13–3.03 (m, 2H), 2.86–2.81 (m, 2H), 2.74–2.49 (m, 7H), 2.40–2.02 (m, 16H), 1.85–1.73 (m, 1H), 1.49–1.36 (m, 9H), 1.05 (s, 9H), 1.00 (s, 6H). LC-MS (ESI): m/z 1444.5 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(6-(((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)(methyl)amino)hexanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (6b). Following general methods C, B and A, compound **6b** was obtained from **31b**, **19** and **28** (5.9 mg, 19% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.32 (d, *J* = 2.2 Hz, 1H), 8.00–7.91 (m, 1H), 7.85 (d, *J* = 8.5 Hz, 2H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.42–7.31 (m, 6H), 7.30–7.29 (m, 1H), 7.28–7.19 (m, 4H), 7.05–6.89 (m, 3H), 6.82–6.65 (m, 3H), 6.43 (d, *J* = 8.6 Hz, 1H), 5.12–5.03 (m, 1H), 4.78 (t, *J* = 8.2 Hz, 1H), 4.60 (d, *J* = 8.7 Hz, 1H), 4.50–4.42 (m, 1H), 4.09 (d, *J* = 11.3 Hz, 1H), 4.02–3.92 (m, 1H), 3.63–3.55 (m, 1H), 3.29–3.19 (m, 4H), 3.10 (dd, *J* = 13.9, 4.7 Hz, 1H), 3.03 (dd, *J* = 13.8, 6.4 Hz, 1H), 2.87–2.71 (m, 3H), 2.70–2.41 (m, 9H), 2.40–2.23 (m, 7H), 2.19–1.99 (m, 6H), 1.89–1.80 (m, 1H), 1.48–1.38 (m, 7H), 1.35 (t, *J* = 7.3 Hz, 2H), 1.20–1.13 (m, 2H), 1.05 (s, 9H), 1.00 (s, 6H). LC-MS (ESI): m/z 1458.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(7-(((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)(methyl)amino)heptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (6c). Following general methods C, B and A, compound **6c** was obtained from **31c**, **19** and **28** (7.3 mg, 23% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.34 (d, *J* = 2.2 Hz, 1H), 7.93 (d, *J* = 9.0 Hz, 1H), 7.88 (d, *J* = 8.6 Hz, 2H), 7.45–7.29 (m, 8H), 7.28–7.21 (m, 4H), 7.03–6.94 (m, 3H), 6.78 (d, *J* = 9.0 Hz, 2H), 6.70–6.62 (m, 1H), 6.46–6.38 (m, 1H), 5.11–5.02 (m, 1H), 4.77 (t, *J* = 8.2 Hz, 1H), 4.62 (d, *J* = 8.8 Hz, 1H), 4.51–4.45 (m, 1H), 4.16–4.10 (m, 1H), 4.03–3.94 (m, 1H), 3.62–3.56 (m, 1H), 3.27–3.19 (m, 4H), 3.10 (dd, *J* = 13.8, 4.8 Hz, 1H), 3.01 (dd, *J* = 13.8, 6.7 Hz, 1H), 2.82–2.49 (m, 9H), 2.44–2.25 (m, 10H), 2.19–2.01 (m, 6H), 1.85–1.75 (m, 1H), 1.46–1.33 (m, 9H), 1.19–1.10 (m, 4H), 1.07 (s, 9H), 1.00 (s, 6H). LC-MS (ESI): m/z 1472.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(8-(((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)(methyl)amino)octanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (6d). Following general methods C, B and A, compound **6d** was obtained from **31d**, **19** and **28** (5.1 mg, 21% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.51–8.39 (m, 1H), 8.10–8.02 (m, 1H), 7.91–7.79 (m, 2H), 7.44–7.29 (m, 8H), 7.28–7.19 (m, 4H), 7.06–6.97 (m, 3H), 6.85–6.76 (m, 3H), 6.46–6.36 (m, 1H), 5.06–4.93 (m, 1H), 4.80 (t, *J* = 8.5 Hz, 1H), 4.71–4.62 (m, 1H), 4.56–4.49 (m, 1H), 4.22–4.16 (m, 1H),

4.16–4.07 (m, 1H), 3.71–3.58 (m, 1H), 3.35–3.23 (m, 4H), 3.22–3.14 (m, 2H), 2.89–2.51 (m, 9H), 2.47–2.01 (m, 16H), 1.95–1.85 (m, 1H), 1.48 (t, *J* = 6.5 Hz, 9H), 1.27–1.16 (m, 6H), 1.06 (s, 9H), 1.01 (s, 6H). LC-MS (ESI): m/z 1486.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(9-(((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)(methyl)amino)nonanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (6e). Following general methods C, B and A, compound **6e** was obtained from **31e**, **19** and **28** (9.1 mg, 40% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.36 (d, *J* = 2.1 Hz, 1H), 7.99 (d, *J* = 9.1 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.41–7.29 (m, 9H), 7.28–7.21 (m, 3H), 7.07–6.96 (m, 3H), 6.83–6.67 (m, 3H), 6.44 (d, *J* = 8.9 Hz, 1H), 5.10–5.01 (m, 1H), 4.76 (t, *J* = 8.1 Hz, 1H), 4.65 (d, *J* = 8.9 Hz, 1H), 4.54–4.49 (m, 1H), 4.18–4.00 (m, 2H), 3.65–3.58 (m, 1H), 3.32–3.24 (m, 4H), 3.13–3.03 (m, 2H), 2.88–2.46 (m, 11H), 2.44–2.01 (m, 14H), 1.95–1.85 (m, 1H), 1.54–1.43 (m, 9H), 1.22–1.10 (m, 8H), 1.06 (s, 9H), 1.00 (s, 6H). LC-MS (ESI): m/z 1500.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(10-(((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)(methyl)amino)decanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (6f). Following general methods C, B and A, compound **6f** was obtained from **31f**, **19** and **28** (7.8 mg, 43% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.33 (s, 1H), 8.04–7.94 (m, 1H), 7.90–7.76 (m, 2H), 7.46–7.32 (m, 7H),

7.30–7.29 (m, 1H), 7.28–7.19 (m, 4H), 7.07–6.95 (m, 3H), 6.84–6.57 (m, 3H), 6.46–6.29 (m, 1H), 5.15–4.99 (m, 1H), 4.76 (t, *J* = 8.0 Hz, 1H), 4.63 (d, *J* = 8.6 Hz, 1H), 4.57–4.47 (m, 1H), 4.26–3.97 (m, 2H), 3.65–3.54 (m, 1H), 3.32–3.19 (m, 4H), 3.15–3.03 (m, 2H), 2.89–2.52 (m, 8H), 2.51–2.02 (m, 17H), 1.87–1.84 (m, 1H), 1.53–1.45 (m, 9H), 1.22–1.09 (m, 10H), 1.04 (s, 9H), 1.00 (s, 6H). LC-MS (ESI): m/z 1514.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(2-(2-(4-((*R*)-3-((4-(*N*-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (4a). Following general methods C, B and A, compound **4a** was obtained from **34a**, **18** and **28** (7.4 mg, 49% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.37 (d, *J* = 2.1 Hz, 1H), 8.09 (d, *J* = 8.9 Hz, 1H), 7.91–7.79 (m, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.46–7.28 (m, 11H), 7.25–7.16 (m, 1H), 6.99 (d, *J* = 8.1 Hz, 2H), 6.77 (d, *J* = 8.6 Hz, 2H), 6.70–6.57 (m, 1H), 5.20–5.00 (m, 1H), 4.91–4.77 (m, 1H), 4.67 (d, *J* = 9.3 Hz, 1H), 4.47 (s, 1H), 4.04–2.65 (m, 21H), 2.60–1.93 (m, 20H), 1.85–1.43 (m, 6H), 1.05 (s, 9H), 1.00 (s, 6H). LC-MS (ESI): m/z 1501.6 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((*S*)-2-(2-(2-(2-(4-((*R*)-3-((4-(N-(4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-4-(phenylthio)butyl)piperazin-1-yl)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (4b). Following general methods C, B and A, compound **4b** was obtained from **34b**, **18** and **28** (5.4 mg, 54% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.40 (d, *J* = 2.2 Hz, 1H), 8.01 (d, *J* = 9.3 Hz, 1H), 7.70 (d, *J* = 8.6

Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 1H), 7.43–7.27 (m, 12H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 6.70–6.61 (m, 1H), 5.15–5.03 (m, 1H), 4.83–4.73 (m, 1H), 4.67 (d, *J* = 9.3 Hz, 1H), 4.45 (s, 1H), 4.18–2.65 (m, 25H), 2.61–1.93 (m, 20H), 1.85–1.44 (m, 6H), 1.05 (s, 9H), 0.99 (s, 6H). LC-MS (ESI): m/z 1545.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((2*S*,15*R*)-2-(tert-Butyl)-15-((4-(*N*-(4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-12-methyl-4-oxo-16-(phenylthio)-6,9-dioxa-3,12-diazahexadecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (7a). Following general methods C, B and A, compound **7a** was obtained from **34b**, **19** and **28** (7.5 mg, 48% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (s, 1H), 8.37 (d, *J* = 2.2 Hz, 1H), 8.07–7.99 (m, 1H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.39–7.27 (m, 8H), 7.25–7.10 (m, 4H), 7.01–6.95 (m, 2H), 6.79–6.70 (m, 3H), 5.13–5.03 (m, 1H), 4.84 (t, *J* = 8.1 Hz, 1H), 4.60 (d, *J* = 8.9 Hz, 1H), 4.53–4.46 (m, 1H), 4.11–3.98 (m, 2H), 3.87 (d, *J* = 15.4 Hz, 1H), 3.66–3.58 (m, 2H), 3.52–3.33 (m, 6H), 3.27–3.20 (m, 4H), 3.13–2.99 (m, 2H), 2.83–2.78 (m, 2H), 2.75–2.49 (m, 6H), 2.47–1.98 (m, 15H), 1.79–1.68 (m, 1H), 1.51–1.43 (m, 5H), 1.04 (s, 9H), 0.98 (s, 6H). LC-MS (ESI): m/z 1490.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((2*S*,18*R*)-2-(*tert*-Butyl)-18-((4-(*N*-(4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-15-methyl-4-oxo-19-(phenylthio)-6,9,12-trioxa-3,15-diazanonadecanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (7b). Following general methods C, B and A, compound **7b** was obtained from **34c**, **19** and **28** (7.8 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.35 (d, *J* = 2.0 Hz, 1H), 8.01 (d, *J* = 8.6 Hz, 1H), 7.76 (d, *J* = 8.5 Hz,

2H), 7.49 (d, *J* = 7.8 Hz, 1H), 7.39–7.32 (m, 6H), 7.29–7.27 (m, 1H), 7.26–7.10 (m, 5H), 7.03–6.95 (m, 2H), 6.82–6.68 (m, 3H), 5.13–5.01 (m, 1H), 4.82 (t, *J* = 8.1 Hz, 1H), 4.61 (d, *J* = 8.9 Hz, 1H), 4.48 (s, 1H), 4.12–4.00 (m, 2H), 3.91–3.48 (m, 13H), 3.30–3.19 (m, 4H), 3.13–3.06 (m, 2H), 2.84–2.45 (m, 10H), 2.39–1.98 (m, 13H), 1.93–1.81 (m, 1H), 1.52–1.43 (m, 5H), 1.04 (s, 9H), 0.98 (s, 6H). LC-MS (ESI): m/z 1534.7 $[M+H]^+$; HPLC: >95% purity.

(2*S*,4*R*)-1-((2*S*,21*R*)-2-(*tert*-Butyl)-21-((4-(*N*-(4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-((trifluoromethyl)sulfonyl)phenyl)amino)-18-methyl-4-oxo-22-(phenylthio)-6,9,12,15-tetraoxa-3,18-diazadocosanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (7c). Following general methods C, B and A, compound **7c** was obtained from **34d**, **19** and **28** (8.5 mg, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.40 (s, 1H), 8.03 (d, *J* = 9.1 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 2H), 7.48–7.32 (m, 8H), 7.31–7.28 (m, 2H), 7.25–7.12 (m, 3H), 7.02–6.96 (m, 2H), 6.92–6.67 (m, 3H), 5.17–4.99 (m, 1H), 4.83 (t, *J* = 8.1 Hz, 1H), 4.61 (d, *J* = 8.9 Hz, 1H), 4.55–4.44 (m, 1H), 4.24–4.03 (m, 2H), 3.92–3.43 (m, 17H), 3.36–3.07 (m, 6H), 2.91–2.49 (m, 8H), 2.46–1.99 (m, 15H), 1.95–1.85 (m, 1H), 1.52–1.44 (m, 5H), 1.05–0.96 (m, 15H). LC-MS (ESI): m/z 1578.7 $[M+H]^+$; HPLC: >95% purity.

4-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoic acid (39a). Following general method D, compound **39a** was obtained from **37a** and **38** (34 mg, 26% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 7.50 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.11 (dd, *J* = 7.1, 0.6 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.36–6.25 (m, 1H), 4.92 (dd, *J* = 12.1, 5.3 Hz, 1H), 3.43–3.29 (m, 2H), 2.97–2.67 (m, 3H), 2.49 (t, *J* = 7.0 Hz, 2H), 2.16–2.08 (m, 1H), 2.03–1.96 (m, 2H). LC-MS (ESI): m/z 360.1 $[M+H]^+$.

5-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentanoic acid (39b). Following general method D, compound **39b** was obtained from **37b** and **38** (30 mg, 21% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.49 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.10 (dd, *J* = 7.1, 0.6 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.36–6.25 (m, 1H), 4.92 (dd, *J* = 12.1, 5.3 Hz, 1H), 3.30–3.20 (m, 2H), 2.97–2.67 (m, 3H), 2.45–2.35 (m, 2H), 2.16–2.05 (m, 1H), 1.80–1.65 (m, 4H). LC-MS (ESI): m/z 374.1 $[M+H]^+$.

6-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexanoic acid (39c) Following general method D, compound **39c** was obtained from **37c** and **38** (45 mg, 16% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.54–7.44 (m, 1H), 7.09 (dd, *J* = 7.2, 0.6 Hz, 1H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.27–6.10 (m, 1H), 4.91 (dd, *J* = 12.2, 5.3 Hz, 1H), 3.36–3.16 (m, 2H), 2.99–2.63 (m, 3H), 2.39 (t, *J* = 7.4 Hz, 2H), 2.18–2.06 (m, 1H), 1.82–1.62 (m, 4H), 1.52–1.39 (m, 2H). LC-MS (ESI): m/z 388.2 $[M+H]^+$.

7-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptanoic acid (39d). Following general method D, compound **39d** was obtained from **37d** and **38** (26 mg, 36% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.48 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.07 (dd, *J* = 7.1, 0.6 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.31–6.10 (m, 1H), 4.91 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.32–3.18 (m, 2H), 2.97–2.66 (m, 3H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.17–2.09 (m, 1H), 1.73–1.59 (m, 4H), 1.51–1.34 (m, 4H). LC-MS (ESI): m/z 402.1 $[M+H]^+$.

8-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octanoic acid (39e). Following general method D, compound **39e** was obtained from **37e** and **38** (28 mg, 19% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 (s, 1H), 7.49 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.32–6.16 (m, 1H), 4.92 (dd, *J* = 12.1, 5.4 Hz, 1H), 3.31–3.20 (m,

2H), 3.02–2.65 (m, 3H), 2.35 (t, *J* = 7.4 Hz, 2H), 2.19–2.08 (m, 1H), 1.73–1.57 (m, 4H), 1.50–1.32 (m, 6H). LC-MS (ESI): m/z 416.0 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (8a). Following general method A, compound **8a** was obtained from **39a** and **18** (3.1 mg, 14% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60–8.30 (m, 2H), 8.10–8.00 (m, 1H), 7.74–7.64 (m, 2H), 7.53–7.40 (m, 1H), 7.39–7.27 (m, 5H), 7.26–7.22 (m, 1H), 7.12–7.02 (m, 2H), 7.01–6.91 (m, 3H), 6.78–6.67 (m, 2H), 6.65–6.58 (m, 1H), 6.33–6.25 (m, 1H), 5.00–4.82 (m, 1H), 3.98–3.84 (m, 1H), 3.82–3.62 (m, 1H), 3.48–3.21 (m, 9H), 3.13–2.96 (m, 2H), 2.91–2.66 (m, 5H), 2.47–1.93 (m, 20H), 1.73–1.58 (m, 1H), 1.46 (t, *J* = 6.5 Hz, 2H), 0.97 (s, 6H). LC-MS (ESI): m/z 1314.4 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (8b). Following general method A, compound **8b** was obtained from **39b** and **18** (11.9 mg, 63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57–8.26 (m, 2H), 8.13–8.04 (m, 1H), 7.68 (d, *J* = 8.9 Hz, 2H), 7.50–7.44 (m, 1H), 7.39–7.26 (m, 5H), 7.26–7.23 (m, 1H), 7.11–6.95 (m, 4H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.80–6.71 (m, 2H), 6.59 (dd, *J* = 9.6, 2.6 Hz, 1H), 6.24 (t, *J* = 5.6 Hz, 1H), 4.97–4.80 (m, 1H), 3.94–3.84 (m, 1H), 3.73–3.68 (m, 1H), 3.50–3.22 (m, 9H), 3.10–2.96 (m, 2H), 2.92–2.66 (m, 5H), 2.44–2.00 (m, 18H), 1.74–1.49 (m, 7H), 0.98 (s, 6H). LC-MS (ESI): m/z 1328.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (8c). Following general method A, compound **8c** was obtained from **39c** and **18** (15.4 mg, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60–8.26 (m, 2H), 8.09 (t, *J* = 8.6 Hz, 1H), 7.71–7.59 (m, 2H), 7.51–7.45 (m, 1H), 7.42–7.27 (m, 5H), 7.26–7.25 (m, 1H), 7.16–6.94 (m, 4H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.80–6.54 (m, 3H), 6.31–6.15 (m, 1H), 4.97–4.82 (m, 1H), 4.01–3.61 (m, 2H), 3.51–3.19 (m, 9H), 3.14–2.96 (m, 2H), 2.92–2.69 (m, 5H), 2.45–1.98 (m, 18H), 1.67–1.46 (m, 9H), 0.99 (s, 6H). LC-MS (ESI): m/z 1342.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (8d). Following general method A, compound **8d** was obtained from **39d** and **18** (17.6 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 13.2 Hz, 1H), 8.36 (d, *J* = 2.1 Hz, 1H), 8.17–8.04 (m, 1H), 7.72–7.64 (m, 2H), 7.51–7.43 (m, 1H), 7.40–7.27 (m, 5H), 7.26–7.23 (m, 1H), 7.14–6.95 (m, 4H), 6.86 (d, *J* = 8.5 Hz, 1H), 6.74 (d, *J* = 8.7 Hz, 2H), 6.59 (dd, *J* = 9.5, 4.1 Hz, 1H), 6.21 (t, *J* = 5.6 Hz, 1H), 4.96–4.85 (m, 1H), 3.94–3.62 (m, 2H), 3.50–3.21 (m, 9H), 3.13–2.97 (m, 2H), 2.92–2.65 (m, 5H), 2.52–1.98 (m, 18H), 1.72–1.39 (m, 11H), 0.98 (s, 6H). LC-MS (ESI): m/z 1356.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-

yl)amino)octanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (8e). Following general method A, compound **8e** was obtained from **39e** and **18** (2.3 mg, 13% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.37 (s, 1H), 8.10 (d, *J* = 9.2 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.48 (t, *J* = 7.8 Hz, 1H), 7.42–7.27 (m, 6H), 7.13–6.95 (m, 4H), 6.87 (d, *J* = 8.6 Hz, 1H), 6.74 (d, *J* = 8.6 Hz, 2H), 6.61 (d, *J* = 9.3 Hz, 1H), 6.21 (t, *J* = 5.4 Hz, 1H), 4.98–4.84 (m, 1H), 3.95–3.57 (m, 2H), 3.55–3.20 (m, 9H), 3.14–2.63 (m, 7H), 2.61–1.97 (m, 18H), 1.71–1.33 (m, 13H), 0.98 (s, 6H). LC-MS (ESI): m/z 1370.5 $[M+H]^+$; HPLC: >95% purity.

2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)acetic acid (41a). Following general methods D and E, compound **41a** was obtained from **40a** and **38** (67 mg, 19% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.05 (s, 1H), 7.51–7.43 (m, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.90 (d, *J* = 8.6 Hz, 1H), 5.01–4.87 (m, 1H), 4.18 (s, 2H), 3.80–3.65 (m, 6H), 3.53–3.41 (m, 2H), 2.91–2.82 (m, 1H), 2.81–2.69 (m, 2H), 2.16–2.07 (m, 1H). LC-MS (ESI): m/z 420.2 $[M+H]^+$.

2-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)acetic acid (41b). Following general methods D and E, compound **41b** was obtained from **40b** and **38** (19 mg, 37% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.47 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.08 (d, *J* = 7.0 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 4.97–4.89 (m, 1H), 4.14 (s, 2H), 3.77–3.65 (m, 10H), 3.49–3.43 (m, 2H), 2.91–2.81 (m, 1H), 2.80–2.69 (m, 2H), 2.15–2.06 (m, 1H). LC-MS (ESI): m/z 464.2 $[M+H]^+$.

14-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecanoic acid (41c). Following general methods D and E, compound **41c** was obtained from **40c** and **38** (14 mg, 15% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.84 (s, 1H), 7.48

(dd, *J* = 8.4, 7.2 Hz, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 4.97–4.89 (m, 1H), 4.13 (s, 2H), 3.74–3.69 (m, 4H), 3.69–3.62 (m, 10H), 3.48–3.44 (m, 2H), 2.91–2.81 (m, 1H), 2.81–2.68 (m, 2H), 2.15–2.04 (m, 1H). LC-MS (ESI): m/z 508.3 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)acetyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (10a). Following general method A, compound **10a** was obtained from **41a** and **18** (6.6 mg, 47% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 9.11 (*br* s, 1H), 8.36 (s, 1H), 8.08 (d, *J* = 9.1 Hz, 1H), 7.66 (d, *J* = 8.2 Hz, 2H), 7.46 (t, *J* = 7.8 Hz, 1H), 7.39–7.27 (m, 6H), 7.14–7.02 (m, 2H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 8.6 Hz, 2H), 6.58 (d, *J* = 5.8 Hz, 1H), 6.52–6.43 (*br* s, 1H), 4.91–4.81 (m, 1H), 4.26–4.15 (m, 2H), 3.94–3.81 (m, 1H), 3.70–3.33 (m, 12H), 3.31–3.22 (m, 4H), 3.12–2.93 (m, 2H), 2.89–2.55 (m, 5H), 2.49–2.00 (m, 16H), 1.74–1.70 (m, 1H), 1.51–1.45 (m, 2H), 0.98 (s, 6H). LC-MS (ESI): m/z 1374.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)acetyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (10b). Following general method A, compound **10b** was obtained from **41b** and **18** (6.2 mg, 44% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.33–8.28 (m, 1H), 8.07 (d, *J* = 9.0 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.54–7.45 (m, 1H), 7.41–7.34 (m, 2H), 7.33–7.20 (m, 5H), 7.09 (d, *J* = 7.1 Hz, 1H), 7.06–6.98 (m, 3H), 6.94 (d, *J* = 8.6 Hz, 1H), 6.79 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 9.4 Hz, 1H), 5.00–4.86 (m, 1H), 4.21 (s, 2H), 3.93–3.84 (m, 1H), 3.70–3.39 (m, 16H), 3.32–3.25 (m, 4H), 3.12–3.00 (m, 2H),

2.93–2.71 (m, 5H), 2.46–2.24 (m, 12H), 2.09–2.00 (m, 4H), 1.75–1.63 (m, 1H), 1.47 (t, *J* = 6.3 Hz, 2H), 0.99 (s, 6H). LC-MS (ESI): m/z 1418.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(14-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxatetradecanoyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (10c). Following general method A, compound **10c** was obtained from **41c** and **18** (13.6 mg, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.81 (*br* s, 1H), 8.33 (s, 1H), 8.13–7.99 (m, 1H), 7.79–7.60 (m, 2H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.38–7.26 (m, 5H), 7.24–7.17 (m, 1H), 7.12–6.94 (m, 4H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.73 (d, *J* = 8.8 Hz, 2H), 6.61–6.50 (m, 1H), 6.46 (*br* s, 1H), 4.95–4.84 (m, 1H), 4.16 (s, 2H), 3.88–3.80 (m, 1H), 3.72–3.39 (m, 20H), 3.32–3.25 (m, 4H), 3.12–3.01 (m, 2H), 2.93–2.71 (m, 5H), 2.46–2.24 (m, 12H), 2.09–2.00 (m, 4H), 1.75–1.63 (m, 1H), 1.47 (t, *J* = 6.3 Hz, 2H), 0.96 (s, 6H). LC-MS (ESI): m/z 1462.5 $[M+H]^+$; HPLC: >95% purity.

(*R*)-*N*-((4-((4-(4-Azido-*N*-methylbutanamido)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (43a). Following general method A, compound **43a** was obtained from **42a** and **19** (24 mg, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.49–8.35 (m, 1H), 8.23–8.12 (m, 1H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.48–7.33 (m, 5H), 7.31–7.25 (m, 1H), 7.08–6.75 (m, 5H), 6.59–6.33 (m, 1H), 3.85–2.82 (m, 16H), 2.56–1.74 (m, 14H), 1.57–1.46 (m, 2H), 1.03 (s, 6H). LC-MS (ESI): m/z 1029.3 $[M+H]^+$.

(*R*)-*N*-((4-((4-(7-Azido-N-methylheptanamido)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (43b). Following general

method A, compound **43b** was obtained from **42b** and **19** (30 mg, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$ and CD_3OD) δ 8.40–8.29 (m, 1H), 8.11 (dd, *J* = 9.2, 2.3 Hz, 1H), 7.68 (d, *J* = 8.7 Hz, 2H), 7.49–7.27 (m, 5H), 7.24–7.18 (m, 1H), 7.09–6.92 (m, 3H), 6.81–6.71 (m, 2H), 6.56–6.25 (m, 1H), 3.77–2.85 (m, 16H), 2.52–1.15 (m, 22H), 0.96 (s, 6H). LC-MS (ESI): m/z 1071.1 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(4-((2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-methylbutanamido)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (13a). Following general method F, compound **13a** was obtained from **43a** and **44a** (14.1 mg, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.26 (s, 1H), 8.35 (s, 1H), 8.08 (d, *J* = 9.0 Hz, 1H), 7.82–7.27 (m, 7H), 7.26–7.15 (m, 3H), 7.11–6.81 (m, 5H), 6.78–6.36 (m, 4H), 5.00–4.86 (m, 1H), 4.73–4.56 (m, 2H), 4.39–4.17 (m, 2H), 3.85–2.64 (m, 25H), 2.47–1.95 (m, 14H), 1.83–1.66 (m, 1H), 1.51–1.40 (m, 2H), 0.97 (s, 6H). LC-MS (ESI): m/z 1428.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(4-((2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-methylbutanamido)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (13b). Following general method F, compound **13b** was obtained from **43a** and **44b** (14.3 mg, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 8.47–8.29 (m, 1H), 8.16–7.99 (m, 1H), 7.82–7.27 (m, 8H), 7.26–7.17 (m, 2H), 7.12–6.36 (m, 9H), 5.00–4.81 (m, 1H), 4.70–4.52 (m,

2H), 4.35–4.13 (m, 2H), 3.88–2.67 (m, 29H), 2.46–1.94 (m, 14H), 1.81–1.66 (m, 1H), 1.50–1.41 (m, 2H), 0.97 (s, 6H). LC-MS (ESI): m/z 1472.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(7-(4-((2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-methylheptanamido)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (14a). Following general method F, compound **14a** was obtained from **43b** and **44a** (16.8 mg, 82% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.40–8.32 (m, 1H), 8.15–8.00 (m, 1H), 7.71 (d, *J* = 8.5 Hz, 2H), 7.60–7.52 (m, 1H), 7.50–7.42 (m, 1H), 7.39–7.27 (m, 4H), 7.26–7.18 (m, 2H), 7.10–7.04 (m, 1H), 7.03–6.86 (m, 4H), 6.80–6.65 (m, 2H), 6.56–6.40 (m, 2H), 4.97–4.85 (m, 1H), 4.76–4.63 (m, 2H), 4.33–4.14 (m, 2H), 3.78–2.68 (m, 25H), 2.41–1.98 (m, 12H), 1.85–1.67 (m, 3H), 1.53–1.42 (m, 4H), 1.33–1.21 (m, 4H), 0.98 (s, 6H). LC-MS (ESI): m/z 1470.5 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(7-(4-((2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)-*N*-methylheptanamido)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (14b). Following general method F, compound **14b** was obtained from **43b** and **44b** (16.4 mg, 77% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00–8.85 (m, 1H), 8.41–8.35 (m, 1H), 8.16–8.02 (m, 1H), 7.71 (d, *J* = 8.6 Hz, 2H), 7.57–7.27 (m, 6H), 7.26–7.19 (m, 2H), 7.11–6.85 (m, 5H), 6.78–6.68 (m, 2H), 6.54–6.28 (m, 2H), 4.99–4.86 (m, 1H), 4.73–4.59 (m, 2H), 4.34–4.10 (m, 2H), 3.81–2.67 (m, 29H), 2.42–1.97 (m, 12H), 1.87–1.68 (m, 3H), 1.55–1.41 (m, 4H), 1.34–1.10 (m, 4H), 0.98 (s, 6H). LC-MS (ESI): m/z 1514.6 $[M+H]^+$; HPLC: >95% purity.

(*R*)-*N*-((4-((4-((7-Azidoheptyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)-4-(4-((4'-chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)benzamide (46). Following general method C, compound **46** was obtained from **45** and **19** (11 mg, 47% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 2.2 Hz, 1H), 7.93–7.78 (m, 3H), 7.37–7.27 (m, 3H), 7.26–7.18 (m, 3H), 7.05–6.88 (m, 3H), 6.75 (d, *J* = 8.6 Hz, 2H), 6.56 (d, *J* = 9.3 Hz, 1H), 3.96–3.79 (m, 1H), 3.29–3.15 (m, 6H), 3.11–2.95 (m, 2H), 2.87–2.67 (m, 6H), 2.55 (s, 3H), 2.45–2.31 (m, 4H), 2.30–2.08 (m, 3H), 2.05–1.84 (m, 3H), 1.64–1.39 (m, 6H), 1.36–1.18 (m, 6H), 0.97 (s, 6H). LC-MS (ESI): m/z 1057.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((7-(4-((2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)heptyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (15a). Following general method F, compound **15a** was obtained from **46** and **44a** (12.4 mg, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 7.84 (d, *J* = 8.4 Hz, 2H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.38–7.30 (m, 2H), 7.28–7.26 (m, 1H), 7.25–7.16 (m, 3H), 7.05 (d, *J* = 7.1 Hz, 1H), 7.03–6.96 (m, 3H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.71 (d, *J* = 8.5 Hz, 2H), 6.58 (d, *J* = 9.3 Hz, 1H), 6.47 (t, *J* = 5.7 Hz, 1H), 4.96–4.81 (m, 1H), 4.66 (s, 2H), 4.24 (t, *J* = 7.2 Hz, 2H), 4.02–3.84 (m, 1H), 3.79–3.60 (m, 6H), 3.46–3.38 (m, 2H), 3.27–3.14 (m, 4H), 3.11–2.96 (m, 2H), 2.81–1.99 (m, 22H), 1.88–1.70 (m, 3H), 1.51–1.38 (m, 4H), 1.24–1.08 (m, 6H), 0.97 (s, 6H). LC-MS (ESI): m/z 1456.6 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((7-(4-((2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-

yl)amino)ethoxy)ethoxy)ethoxy)methyl)-1*H*-1,2,3-triazol-1-yl)heptyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (15b). Following general method F, compound **15b** was obtained from **46** and **44b** (17.4 mg, 62% yield). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.00 (s, 1H), 8.31 (s, 1H), 8.12–7.97 (m, 2H), 7.93–7.80 (m, 2H), 7.61–7.52 (m, 1H), 7.46–7.31 (m, 4H), 7.31–7.23 (m, 2H), 7.22–6.99 (m, 6H), 6.92–6.81 (m, 2H), 6.66–6.58 (m, 1H), 5.16–5.00 (m, 1H), 4.75–4.51 (m, 2H), 4.46–4.19 (m, 3H), 3.82–3.48 (m, 12H), 3.45–3.22 (m, 6H), 3.11–2.65 (m, 13H), 2.55–2.13 (m, 9H), 1.94–1.55 (m, 5H), 1.52–1.44 (m, 2H), 1.39–1.15 (m, 6H), 1.00 (s, 6H). LC-MS (ESI): m/z 1500.6 $[M+H]^+$; HPLC: >95% purity.

4-((2-(2-(2-Aminoethoxy)ethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione trifluoroacetate (48). Following general methods D and E, compound **48** was obtained from **47** and **38** (164 mg, 44% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (*br* s, 1H), 7.82 (*br* s, 2H), 7.48 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.04 (d, *J* = 7.0 Hz, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 5.13–4.82 (m, 1H), 3.85–3.60 (m, 8H), 3.55–3.37 (m, 2H), 3.28–3.12 (m, 2H), 2.81–2.58 (m, 3H), 2.09–1.88 (m, 1H). LC-MS (ESI): m/z 405.1 $[M+H]^+$.

4-((*R*)-3-(4-(*N*-(4-(4-((2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)benzoyl)sulfamoyl)-2-(trifluoromethylsulfonyl)phenylamino)-4-(phenylthio)butyl)-*N*-(2-(2-(2-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)ethoxy)ethoxy)ethyl)piperazine-1-carboxamide (11a). A mixture of compound **48** (20 mg, 0.039 mmol), CDI (10 mg, 0.062 mmol) and TEA (7.0 μL, 0.050 mmol) in 3 mL DCM was stirred at room temperature for 2 h. Then compound **18** (15 mg, 0.015 mmol) and DIPEA (50 μL, 0.303 mmol) were added into the above solution. The reaction was stirred overnight and quenched by the addition of NH_4Cl (aq.). Subsequently, it was poured into water and extracted

with DCM. The organic phase was washed with water × 1, brine × 1, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography using DCM and MeOH as eluents to afford the title compound (6.7 mg, 32% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (*br* s, 1H), 8.35 (s, 1H), 8.14–7.98 (m, 1H), 7.81–7.62 (m, 2H), 7.52–7.40 (m, 1H), 7.39–7.27 (m, 4H), 7.24–7.15 (m, 1H), 7.12–6.94 (m, 4H), 6.87 (d, *J* = 8.6 Hz, 1H), 6.73 (d, *J* = 7.2 Hz, 2H), 6.66–6.57 (m, 1H), 6.56–6.46 (m, 1H), 5.20–5.02 (*br* s, 1H), 5.00–4.83 (m, 1H), 3.95–3.81 (m, 1H), 3.75–3.69 (m, 2H), 3.67–3.61 (m, 4H), 3.61–3.53 (m, 2H), 3.49–3.38 (m, 4H), 3.38–3.18 (m, 8H), 3.12–2.95 (m, 2H), 2.88–2.66 (m, 5H), 2.47–2.18 (m, 12H), 2.17–1.98 (m, 4H), 1.69–1.57 (m, 1H), 1.46 (t, *J* = 6.3 Hz, 2H), 0.97 (s, 6H). LC-MS (ESI): m/z 1403.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((2-(4-Chlorophenyl)-5,5-dimethylcyclohex-1-enyl)methyl)piperazin-1-yl)-*N*-(4-((2*R*)-4-(4-(2-(2-(2-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-ylamino)ethoxy)ethoxy)ethylcarbamothioyl)piperazin-1-yl)-1-(phenylthio)butan-2-ylamino)-3-(trifluoromethylsulfonyl)phenylsulfonyl)benzamide (11b). A mixture of compound **48** (12 mg, 0.023 mmol), 1,1'-thiocarbonyldiimidazole (6.0 mg, 0.034 mmol) and TEA (4.2 μL, 0.030 mmol) in 2 mL DCM was stirred at room temperature for 1 h. Then compound **18** (6.5 mg, 0.0067 mmol) and DIPEA (0.05 mL) were added into the above solution. The reaction was stirred overnight and quenched by the addition of NH_4Cl (aq.). Subsequently, it was poured into water and extracted with DCM. The organic phase was washed with water x1, brine x1, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was purified by column chromatography using DCM and MeOH as eluents to afford the title compound (6.4 mg, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (*br* s, 1H), 8.37 (d, *J* = 2.1 Hz, 1H), 8.15–8.00 (m, 1H), 7.65 (d, *J* = 7.6 Hz, 2H), 7.51–7.42 (m, 1H), 7.38–7.27 (m, 5H),

7.12–7.05 (m, 2H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.75 (dd, *J* = 9.0, 3.8 Hz, 2H), 6.62 (dd, *J* = 12.9, 9.5 Hz, 1H), 6.51 (t, *J* = 5.2 Hz, 1H), 6.22 (*br* s, 1H), 4.95–4.80 (m, 1H), 3.90–3.61 (m, 15H), 3.48–3.41 (m, 2H), 3.30–3.20 (m, 4H), 3.13–2.96 (m, 2H), 2.88–2.71 (m, 5H), 2.47–2.22 (m, 12H), 2.13–2.00 (m, 4H), 1.75–1.70 (m, 1H), 1.46 (t, *J* = 6.4 Hz, 2H), 0.99 (s, 6H). LC-MS (ESI): m/z 1419.4 $[M+H]^+$; HPLC: >95% purity.

2-(2,6-Dioxopiperidin-3-yl)-4-((3-hydroxypropyl)amino)isoindoline-1,3-dione (50a). Following general method D, compound **50a** was obtained from **49a** and **38** (19 mg, 11% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18 (s, 1H), 7.52 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 6.52–6.45 (m, 1H), 4.94 (dd, *J* = 12.4, 5.3 Hz, 1H), 3.84 (t, *J* = 5.8 Hz, 2H), 3.46 (q, *J* = 6.4 Hz, 2H), 2.95–2.69 (m, 3H), 2.18–2.12 (m, 1H), 1.94 (p, *J* = 6.3 Hz, 2H). LC-MS (ESI): m/z 332.1 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((4-hydroxybutyl)amino)isoindoline-1,3-dione (50b). Following general method D, compound **50b** was obtained from **49b** and **38** (59 mg, 24% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.97 (s, 1H), 7.52 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.36–6.29 (m, 1H), 4.94 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.75 (q, *J* = 5.8 Hz, 2H), 3.35 (q, *J* = 6.8 Hz, 2H), 2.97–2.72 (m, 3H), 2.19–2.13 (m, 1H), 1.84–1.78 (m, 2H), 1.75–1.69 (m, 2H), 1.37 (t, *J* = 5.1 Hz, 1H). LC-MS (ESI): m/z 346.1 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((5-hydroxypentyl)amino)isoindoline-1,3-dione (50c). Following general method D, compound **50c** was obtained from **49c** and **38** (34 mg, 17% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.52 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.31–6.22 (m, 1H), 4.94 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.76–3.67 (m, 2H), 3.37–3.28 (m, 2H), 2.96–2.71 (m, 3H), 2.20–2.12 (m, 1H), 1.78–1.70 (m, 2H), 1.69–1.62 (m, 2H), 1.56–1.51 (m, 2H). LC-MS (ESI): m/z 360.1 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((6-hydroxyhexyl)amino)isoindoline-1,3-dione (50d). Following general method D, compound **50d** was obtained from **49d** and **38** (56 mg, 21% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.52 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.11 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.29–6.22 (m, 1H), 4.94 (dd, *J* = 12.4, 5.3 Hz, 1H), 3.72–3.65 (m, 2H), 3.33–3.27 (m, 2H), 2.92–2.72 (m, 3H), 2.18–2.12 (m, 1H), 1.76–1.68 (m, 2H), 1.65–1.60 (m, 2H), 1.52–1.42 (m, 4H). LC-MS (ESI): m/z 374.1 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((8-hydroxyoctyl)amino)isoindoline-1,3-dione (50e). Following general method D, compound **50e** was obtained from **49e** and **38** (79 mg, 27% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.57–7.48 (m, 1H), 7.14–7.09 (m, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 6.30–6.21 (m, 1H), 4.93 (dd, *J* = 12.4, 5.3 Hz, 1H), 3.67 (t, *J* = 6.6 Hz, 2H), 3.32–3.24 (m, 2H), 2.94–2.71 (m, 3H), 2.19–2.13 (m, 1H), 1.72–1.66 (m, 2H), 1.59–1.36 (m, 10H). LC-MS (ESI): m/z 402.1 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (9a). Following general methods G and H, compound **9a** was obtained from **50a** and **18** (17.2 mg, 65% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.72 (*br* s, 1H), 8.35 (s, 1H), 8.09–7.97 (m, 1H), 7.80 (d, *J* = 8.4 Hz, 2H), 7.51–7.43 (m, 1H), 7.40–7.34 (m, 2H), 7.33–7.29 (m, 2H), 7.28–7.19 (m, 2H), 7.09 (d, *J* = 7.1 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.96–6.85 (m, 2H), 6.78–6.68 (m, 2H), 6.60 (d, *J* = 9.2 Hz, 1H), 6.48–6.35 (m, 1H), 4.98–4.90 (m, 1H), 3.88 (s, 1H), 3.34–3.18 (m, 6H), 3.13–3.06 (m, 1H), 3.00 (dd, *J* = 14.0, 6.9 Hz, 1H), 2.93–2.23 (m, 23H), 2.16–2.08 (m, 2H), 2.04–1.99 (m, 2H), 1.92–1.81 (m,

2H), 1.77–1.68 (m, 1H), 1.47 (t, *J* = 6.5 Hz, 2H), 0.98 (d, *J* = 4.1 Hz, 6H). LC-MS (ESI): m/z 1286.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (9b). Following general methods G and H, compound **9b** was obtained from **50b** and **18** (3.5 mg, 19% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.55 (*br* s, 1H), 8.37 (dd, *J* = 7.6, 2.2 Hz, 1H), 8.04 (t, *J* = 7.7 Hz, 1H), 7.77 (d, *J* = 8.4 Hz, 2H), 7.54–7.46 (m, 1H), 7.43–7.36 (m, 2H), 7.34–7.29 (m, 3H), 7.28–7.24 (m, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 7.05–6.97 (m, 3H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.82–6.72 (m, 2H), 6.59 (t, *J* = 7.8 Hz, 1H), 6.29–6.20 (m, 1H), 4.97–4.90 (m, 1H), 3.94–3.83 (m, 1H), 3.34–3.21 (m, 6H), 3.10 (dd, *J* = 13.8, 5.0 Hz, 1H), 3.02–2.96 (m, 1H), 2.91–2.25 (m, 21H), 2.19–2.00 (m, 6H), 1.79–1.66 (m, 5H), 1.47 (t, *J* = 6.5 Hz, 2H), 0.99 (d, *J* = 5.0 Hz, 6H). LC-MS (ESI): m/z 1300.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (9c). Following general methods G and H, compound **9c** was obtained from **50c** and **18** (2.9 mg, 11% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (*br* s, 1H), 8.37–8.33 (m, 1H), 8.02 (d, *J* = 9.2 Hz, 1H), 7.83 (d, *J* = 8.6 Hz, 2H), 7.51–7.45 (m, 1H), 7.40–7.36 (m, 2H), 7.34–7.29 (m, 3H), 7.28–7.24 (m, 1H), 7.09 (d, *J* = 7.0 Hz, 1H), 7.03–6.94 (m, 3H), 6.86 (d, *J* = 8.6 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.60–6.45 (m, 1H), 6.20 (t, *J* = 5.6 Hz, 1H), 4.97–4.87 (m, 1H), 3.84–3.76 (m, 1H), 3.30–3.20 (m, 6H), 3.09

(dd, *J* = 13.8, 4.7 Hz, 1H), 2.99–2.93 (m, 1H), 2.91–2.66 (m, 9H), 2.58–2.00 (m, 18H), 1.74–1.59 (m, 5H), 1.49–1.36 (m, 4H), 0.98 (d, *J* = 5.9 Hz, 6H). LC-MS (ESI): m/z 1314.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (16a). Following general methods G and H, compound **16a** was obtained from **50a** and **19** (9.0 mg, 33% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.73–8.33 (m, 2H), 8.28–8.04 (m, 1H), 7.70–7.56 (m, 2H), 7.43–7.29 (m, 6H), 7.26–7.20 (m, 1H), 7.15–6.85 (m, 4H), 6.77–5.55 (m, 5H), 5.02–4.84 (m, 1H), 4.09–3.91 (m, 1H), 3.30–3.07 (m, 6H), 3.06–2.69 (m, 7H), 2.60–2.10 (m, 15H), 2.05–2.00 (m, 2H), 1.77–1.54 (m, 3H), 1.50–1.47 (m, 2H), 1.01 (d, *J* = 4.9 Hz, 6H). LC-MS (ESI): m/z 1231.4 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (16b). Following general methods G and H, compound **16b** was obtained from **50b** and **19** (3.0 mg, 16% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.53–8.35 (m, 1H), 8.11 (s, 1H), 7.72–7.60 (m, 2H), 7.44–7.29 (m, 7H), 7.27–7.12 (m, 2H), 7.06–6.98 (m, 3H), 6.81–6.52 (m, 4H), 5.92–5.73 (m, 1H), 5.03–4.95 (m, 1H), 4.06–3.87 (m, 1H), 3.24–2.71 (m, 13H), 2.61–2.12 (m, 15H), 2.05–2.01 (m, 2H), 1.73–1.43 (m, 7H), 1.01 (d, *J* = 2.3 Hz, 6H). LC-MS (ESI): m/z 1245.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (16c). Following general methods G and H, compound **16c** was obtained from **50c** and **19** (3.0 mg, 11% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.38 (s, 1H), 8.18–8.01 (m, 1H), 7.71–7.62 (m, 2H), 7.47–7.29 (m, 7H), 7.27–7.19 (m, 2H), 7.07–6.96 (m, 3H), 6.82–5.60 (m, 5H), 4.98–4.90 (m, 1H), 4.06–3.90 (m, 1H), 3.25–2.69 (m, 13H), 2.59–2.11 (m, 15H), 2.03 (s, 2H), 1.61–1.47 (m, 9H), 1.01 (d, *J* = 3.7 Hz, 6H). LC-MS (ESI): m/z 1259.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (16d). Following general methods G and H, compound **16d** was obtained from **50d** and **19** (5.9 mg, 21% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58–8.39 (m, 1H), 8.36–8.33 (m, 1H), 8.02–7.91 (m, 1H), 7.81–7.70 (m, 2H), 7.48–7.36 (m, 3H), 7.33–7.29 (m, 3H), 7.27–7.21 (m, 1H), 7.18–6.97 (m, 4H), 6.88–6.51 (m, 4H), 6.21–5.94 (m, 1H), 4.98–4.88 (m, 1H), 4.00–3.86 (m, 1H), 3.27–2.98 (m, 8H), 2.90–2.70 (m, 5H), 2.57–2.11 (m, 15H), 2.06–2.01 (m, 2H), 1.88–1.78 (m, 1H), 1.52–1.26 (m, 10H), 1.00 (s, 6H). LC-MS (ESI): m/z 1273.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (16e). Following general methods G

and H, compound **16e** was obtained from **50e** and **19** (8.5 mg, 32% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.01–7.88 (m, 1H), 7.79 (d, *J* = 8.3 Hz, 2H), 7.52–7.43 (m, 1H), 7.42–7.34 (m, 2H), 7.33–7.29 (m, 2H), 7.28–7.23 (m, 3H), 7.08 (d, *J* = 7.1 Hz, 2H), 7.04–6.98 (m, 2H), 6.90–6.82 (m, 1H), 6.78–6.70 (m, 2H), 6.58 (d, *J* = 9.2 Hz, 1H), 6.25–6.14 (m, 1H), 4.97–4.88 (m, 1H), 3.98–3.85 (m, 1H), 3.23–2.68 (m, 13H), 2.62–2.13 (m, 15H), 2.03 (s, 2H), 1.89–1.86 (m, 1H), 1.62–1.57 (m, 2H), 1.48 (t, *J* = 6.5 Hz, 2H), 1.37–1.24 (m, 10H), 1.00 (s, 6H). LC-MS (ESI): m/z 1301.5 $[M+H]^+$; HPLC: >95% purity.

2-(2,6-Dioxopiperidin-3-yl)-4-((2-(2-hydroxyethoxy)ethyl)amino)isoindoline-1,3-dione (53a). Following general methods D, compound **53a** was obtained from **52a** and **38** (83 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (*br* s, 1H), 7.58–7.46 (m, 1H), 7.11 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.60–6.52 (m, 1H), 4.92 (dd, *J* = 12.2, 5.3 Hz, 1H), 3.81–3.71 (m, 4H), 3.66–3.61 (m, 2H), 3.48 (dd, *J* = 10.7, 5.3 Hz, 2H), 2.92–2.67 (m, 3H), 2.32 (*br* s, 1H), 2.18–2.07 (m, 1H). LC-MS (ESI): m/z 362.1 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((2-(2-(2-hydroxyethoxy)ethoxy)ethyl)amino)isoindoline-1,3-dione (53b). Following general methods D, compound **53b** was obtained from **52b** and **38** (230 mg, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (*br* s, 1H), 7.55–7.44 (m, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.62–6.54 (m, 1H), 4.91 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.85–3.65 (m, 8H), 3.64–3.59 (m, 2H), 3.51–3.43 (m, 2H), 2.92–2.68 (m, 3H), 2.57 (*br* s, 1H), 2.18–2.07 (m, 1H). LC-MS (ESI): m/z 406.0 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)amino)isoindoline-1,3-dione (53c). Following general methods D, compound **53c** was obtained from **52c** and **38** (84 mg, 40% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (*br* s, 1H), 7.58–7.40 (m, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.92 (d, *J* = 8.6 Hz,

1H), 6.56–6.48 (m, 1H), 4.92 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.77–3.65 (m, 12H), 3.63–3.58 (m, 2H), 3.52–3.44 (m, 2H), 3.00–2.59 (m, 4H), 2.24–2.04 (m, 1H). LC-MS (ESI): m/z 450.1 $[M+H]^+$.

2-(2,6-Dioxopiperidin-3-yl)-4-((14-hydroxy-3,6,9,12-tetraoxatetradecyl)amino)isoindoline-1,3-dione (53d). Following general methods D, compound **53d** was obtained from **52d** and **38** (92 mg, 19% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (*br* s, 1H), 7.49 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.11 (d, *J* = 6.8 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.55–6.47 (m, 1H), 4.90 (dd, *J* = 12.0, 5.4 Hz, 1H), 3.76–3.58 (m, 18H), 3.50–3.43 (m, 2H), 2.92–2.74 (m, 3H), 2.18–2.07 (m, 1H). LC-MS (ESI): m/z 494.3 $[M+H]^+$.

2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl methanesulfonate (54a). Following general methods I, compound **54a** was obtained from **53a** (49 mg, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (*br* s, 1H), 7.63–7.44 (m, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.54–6.45 (m, 1H), 4.91 (dd, *J* = 12.1, 5.3 Hz, 1H), 4.48–4.35 (m, 2H), 3.86–3.66 (m, 4H), 3.58–3.41 (m, 2H), 3.13–2.69 (m, 6H), 2.23–2.05 (m, 1H). LC-MS (ESI): m/z 440.2 $[M+H]^+$.

2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl methanesulfonate (54b). Following general methods I, compound **54b** was obtained from **53b** (20 mg, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (*br* s, 1H), 7.65–7.45 (m, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.56–6.48 (m, 1H), 4.94 (dd, *J* = 12.0, 5.3 Hz, 1H), 4.39 (dd, *J* = 5.3, 3.7 Hz, 2H), 4.00–3.66 (m, 8H), 3.52–3.44 (m, 2H), 3.05 (s, 3H), 2.93–2.62 (m, 3H), 2.28–2.06 (m, 1H). LC-MS (ESI): m/z 484.2 $[M+H]^+$.

2-(2-(2-(2-((2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate (54c). Following general methods I, compound **54c** was obtained from **53c** (64 mg, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20

(*br* s, 1H), 7.60–7.41 (m, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.52–6.44 (m, 1H), 4.92 (dd, *J* = 11.8, 5.4 Hz, 1H), 4.36 (dd, *J* = 5.3, 3.7 Hz, 2H), 3.82–3.60 (m, 12H), 3.54–3.40 (m, 2H), 3.07 (s, 3H), 2.98–2.65 (m, 3H), 2.24–2.06 (m, 1H). LC-MS (ESI): m/z 528.2 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (12a). Following general method J, compound **12a** was obtained from **54a** and **18** (8.8 mg, 26% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.09–7.98 (m, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.40–7.29 (m, 5H), 7.25–7.20 (m, 1H), 7.10 (d, *J* = 7.1 Hz, 1H), 7.06–6.95 (m, 3H), 6.89 (d, *J* = 8.4 Hz, 1H), 6.73 (d, *J* = 9.1 Hz, 2H), 6.66–6.55 (m, 1H), 6.53–6.42 (m, 1H), 4.98–4.82 (m, 1H), 3.93–3.80 (m, 1H), 3.76–3.40 (m, 6H), 3.32–2.64 (m, 17H), 2.43–1.97 (m, 16H), 1.70–1.66 (m, 1H), 1.52–1.41 (m, 2H), 1.01–0.95 (m, 6H). LC-MS (ESI): m/z 1316.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (12b). Following general method J, compound **12b** was obtained from **54b** and **18** (5.8 mg, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.02 (t, *J* = 8.9 Hz, 1H), 7.76 (d, *J* = 7.0 Hz, 2H), 7.52–7.46 (m, 1H), 7.41–7.33 (m, 2H), 7.32–7.27 (m, 3H), 7.25–7.21 (m, 1H), 7.10 (dd, *J* = 7.1, 2.3 Hz, 1H), 7.04–6.92 (m, 3H), 6.88 (d, *J* = 8.6 Hz, 1H), 6.75 (d, *J* = 8.4 Hz, 2H), 6.56–6.40 (m, 2H), 4.96–4.73 (m, 1H),

3.86–3.40 (m, 11H), 3.33–2.51 (m, 17H), 2.50–1.79 (m, 16H), 1.74–1.60 (m, 1H), 1.48–1.37 (m, 2H), 0.95 (d, *J* = 5.4 Hz, 6H). LC-MS (ESI): m/z 1360.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (12c). Following general method J, compound **12c** was obtained from **54c** and **18** (16.4 mg, 25% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.01 (d, *J* = 8.9 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 2H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.38–7.28 (m, 5H), 7.25–7.21 (m, 1H), 7.08 (d, *J* = 7.1 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 2H), 6.96–6.85 (m, 2H), 6.75 (d, *J* = 8.7 Hz, 2H), 6.54–6.43 (m, 2H), 4.96–4.83 (m, 1H), 3.90–3.39 (m, 15H), 3.28–2.68 (m, 17H), 2.51–1.95 (m, 16H), 1.61–1.57 (m, 1H), 1.47–1.41 (m, 2H), 0.97–0.93 (m, 6H). LC-MS (ESI): m/z 1404.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((2*R*)-4-((2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethyl)(methyl)amino)-1-(phenylthio)butan-2-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (17a). Following general methods G and H, compound **17a** was obtained from **53b** and **19** (16.2 mg, 60% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.07–7.93 (m, 1H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.48–7.39 (m, 1H), 7.33–7.27 (m, 3H), 7.25–7.14 (m, 3H), 7.09–6.95 (m, 4H), 6.84 (dd, *J* = 8.6, 3.2 Hz, 1H), 6.74–6.56 (m, 3H), 6.46–6.36 (m, 1H), 4.98–4.87 (m, 1H), 3.99–3.88 (m, 1H), 3.73–3.52 (m, 8H), 3.41–3.33 (m, 2H), 3.29–3.18 (m, 4H), 3.10–2.69 (m, 11H), 2.60–1.99 (m, 13H), 1.95–1.85 (m, 1H), 1.45 (t, *J* = 6.5 Hz, 2H), 0.97 (s, 6H). LC-MS (ESI): m/z 1305.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((15*R*)-1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-12-methyl-16-(phenylthio)-3,6,9-trioxa-12-azahexadecan-15-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (17b). Following general methods G and H, compound **17b** was obtained from **53c** and **19** (14.6 mg, 53% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37–8.26 (m, 1H), 8.06–7.95 (m, 1H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.50–7.39 (m, 1H), 7.38–7.31 (m, 2H), 7.29–7.27 (m, 1H), 7.26–7.17 (m, 3H), 7.16–7.04 (m, 2H), 7.02–6.95 (m, 2H), 6.87 (d, *J* = 8.5 Hz, 1H), 6.72 (d, *J* = 8.9 Hz, 2H), 6.61 (d, *J* = 9.4 Hz, 1H), 6.48–6.40 (m, 1H), 4.94–4.82 (m, 1H), 3.94–3.82 (m, 1H), 3.75–3.37 (m, 14H), 3.29–3.18 (m, 4H), 3.10–2.97 (m, 2H), 2.92–2.60 (m, 9H), 2.44–2.20 (m, 9H), 2.11–1.97 (m, 4H), 1.88–1.78 (m, 1H), 1.45 (t, *J* = 6.5 Hz, 2H), 0.97 (s, 6H). LC-MS (ESI): m/z 1349.5 $[M+H]^+$; HPLC: >95% purity.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((18*R*)-1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-15-methyl-19-(phenylthio)-3,6,9,12-tetraoxa-15-azanonadecan-18-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (17c). Following general methods G and H, compound **17c** was obtained from **53d** and **19** (12.1 mg, 42% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40–8.33 (m, 1H), 8.12–8.04 (m, 1H), 7.76–7.68 (m, 2H), 7.51–7.42 (m, 1H), 7.39–7.33 (m, 2H), 7.31–7.27 (m, 2H), 7.26–7.20 (m, 3H), 7.12–7.06 (m, 1H), 7.03–6.94 (m, 2H), 6.87 (d, *J* = 8.6 Hz, 1H), 6.78–6.67 (m, 3H), 6.45 (t, *J* = 5.6 Hz, 1H), 4.96–4.80 (m, 1H), 4.02–3.92 (m, 1H), 3.72–3.40 (m, 18H), 3.31–3.19 (m, 4H), 3.14–3.02 (m, 2H), 2.91–2.17 (m, 18H), 2.13–1.99 (m, 4H), 1.80–1.69 (m, 1H), 1.46 (t, *J* = 6.5 Hz, 2H), 0.98 (s, 6H). LC-MS (ESI): m/z 1393.5 $[M+H]^+$; HPLC: >95% purity.

4-((2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (57). Following general method D, compound **57** was obtained from **56** and **52c** (80 mg, 34% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.51 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.55–6.49 (m, 1H), 4.97–4.89 (m, 1H), 3.76–3.69 (m, 12H), 3.64–3.62 (m, 2H), 3.51 (q, *J* = 5.6 Hz, 2H), 3.23 (s, 3H), 3.05–2.95 (m, 1H), 2.84–2.72 (m, 2H), 2.17–2.05 (m, 1H). LC-MS (ESI): m/z 464.2 $[M+H]^+$.

2-(2-(2-(2-((2-(1-Methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate (58). Following general method I, compound **58** was obtained from **57** (75.3 mg, 81% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52 (dd, *J* = 8.5, 7.1 Hz, 1H), 7.13 (d, *J* = 7.1 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 6.55–6.45 (m, 1H), 4.98–4.89 (m, 1H), 4.42–4.34 (m, 2H), 3.81–3.77 (m, 2H), 3.74 (t, *J* = 5.4 Hz, 2H), 3.72–3.66 (m, 8H), 3.50 (q, *J* = 5.4 Hz, 2H), 3.23 (s, 3H), 3.08 (s, 3H), 3.03–2.95 (m, 1H), 2.85–2.72 (m, 2H), 2.17–2.09 (m, 1H). LC-MS (ESI): m/z 542.1 $[M+H]^+$.

4-(4-((4'-Chloro-4,4-dimethyl-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)methyl)piperazin-1-yl)-*N*-((4-(((15*R*)-12-methyl-1-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-16-(phenylthio)-3,6,9-trioxa-12-azahexadecan-15-yl)amino)-3-((trifluoromethyl)sulfonyl)phenyl)sulfonyl)benzamide (XZ739-NC). Following general method J, compound XZ739-NC was obtained from **58** and **19** (4.9 mg, 11% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, *J* = 2.3 Hz, 1H), 8.12–8.05 (m, 1H), 7.70 (d, *J* = 8.7 Hz, 2H), 7.51–7.45 (m, 1H), 7.42–7.29 (m, 6H), 7.27–7.22 (m, 1H), 7.09 (d, *J* = 7.0 Hz, 1H), 7.03–6.97 (m, 2H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 8.8 Hz, 2H), 6.68 (dd, *J* = 9.4, 3.9 Hz, 1H), 6.49–6.42 (m, 1H), 4.97–4.88 (m, 1H), 4.02–3.92 (m, 1H), 3.74–3.43 (m, 14H), 3.31–3.24 (m, 4H), 3.21 (s, 3H), 3.13–3.05 (m, 2H), 3.00–2.47 (m, 9H), 2.39–2.26 (m, 9H), 2.12–2.02 (m, 4H), 1.81–1.76

(m, 1H), 1.48 (t, *J* = 6.5 Hz, 2H), 1.00 (s, 6H). LC-MS (ESI): m/z 1363.5 $[M+H]^+$; HPLC: >95% purity.

5.2 Biology

5.2.1. Cell lines and culture. T-ALL MOLT-4 (Cat# CRL-1582), B-ALL RS4;11 (RS4, Cat# CRL-1873), and SCLC NCI-H146 (H146, Cat# HTB-173) cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). MOLT-4, RS4;11 and H146 cells were cultured in RPMI 1640 media (Life Technologies, Carlsbad, CA, USA) supplemented with 10% FBS (Atlanta Biologicals, Flowery Branch, GA, USA) and 1% penicillin-streptomycin solution (Thermo Fisher Scientific, Waltham, MA, USA).

5.2.2. Cell viability assay. Cell viability was measured by Tetrazolium-based MTS assay (Promega, Madison, WI, USA). 5×10^4 to 1×10^5 suspension cells were seeded and treated in 96-well plates for 48 h or 72 h. The EC_{50} values of individual agents were calculated with GraphPad Prism 7 software (GraphPad Software, La Jolla, CA, USA).

5.2.3. Immunoblotting. Cells were collected and lysed in Lysis buffer (Boston Bio Products, Ashland, MA, USA) supplied with protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO, USA). The equal amount of protein lysates was separated on pre-casted 4-20% Tris-glycine gels (Bio-Rad, Hercules, CA, USA). Thereafter, the proteins were transferred to PVDF membranes (MilliporeSigma, Billerica, MA, USA). The membranes were blocked with 5% w/v nonfat dry milk in TBS + Tween-20 (0.1% v/v), and then probed with primary antibodies overnight at 4° C. Next day, the membranes were washed and incubated with appropriate HRP-conjugated secondary antibodies. The signal was detected using ECL substrate (MilliporeSigma) and captured on X-ray films or ChemiDoc MP Imaging System (Bio-Rad).

The band intensities were calculated on ImageJ software and normalized to equal loading control *β*-actin.

5.2.4. Human platelet isolation and viability assays. Platelet-rich plasma (PRP) was purchased from Life South Community Blood Center (Gainesville, FL, USA). Platelets were separated from PRP as previously described [34]. Briefly, PRP was transferred into a 50 mL tube containing 5 mL acid citrate buffer (Cat. No. sc-214744, Santa Cruz Biotechnology). To prevent clotting, prostaglandin E1 (PGE1, Cat. No. sc-201223A, Santa Cruz Biotechnology) and apyrase (Cat. No. A6237, Sigma-Aldrich) were added to final concentrations of 1 μM and 0.2 units/mL, respectively. After centrifugation, platelet number was adjusted to 2×10^8/mL in HEPES Tyrode's buffer containing 10% FBS, 1 μM PGE1 and 0.2 units/mL apyrase, and treated with various compounds. After 48 h of treatment, platelet viability was measured using the MTS reagent (Cat. No. G1111, Promega, Madison, WI, USA). The data were analyzed by GraphPad Prism 7 software for the calculation of IC_{50} values.

5.2.5. Flow cytometry. MOLT-4 cells were plated in 12 well plates at 4×10^5 cells/well and treated with Veh, ABT-263, or XZ739 for 48 h. Caspase activity was blocked using Q-VD-OPh (QVD, Cat. No. S7311, Selleckchem, Houston, TX, USA) pretreatment for 2 h. After treatment, cells were stained with Alexa Fluor 647-Annexin V (1: 50, Cat. No. 640912, BioLegend, San Diego, CA, USA) and PI (10 μg/mL, Cat. No. 421301, BioLegend, San Diego, CA, USA) at room temperature for 30 min. For apoptosis analysis, the stained cells were analyzed on an Aurora flow cytometer (Cytek Aurora, Fremont, CA, USA).

Notes

The authors declare the following competing financial interest(s): X. Zhang, P. Zhang, S. Khan, D. Zhou, and G. Zheng are co-inventors for BCL-X_L PROTACs disclosed in this study. D. Zhou

and G. Zheng are co-founders and shareholders of Dialectic Therapeutics, a company that is developing BCL-X_L PROTACs to treat cancers.

Acknowledgements

This study was supported in part by NIH grants R01CA211963, R01CA219836, and R21CA223371.

Appendix A. Supplementary data

Supplementary data related to this article can be found at xxxxxx. CRBN and VHL expression in human platelets and cancer cell lines; DC_{50} of representative PROTACs; BCL-X_L and BCL-2 binding affinities of ABT-263, DT2216, and XZ739; pharmacokinetics (PK) profile of XZ739; ^{1}H-NMR spectrum of XZ739; and protein levels of IZFK1 and IZFK3 in MOLT-4 cells treated with XZ739 or pomalidomide are available in SI (PDF).

References

[1] Singh, R.; Letai, A.; Sarosiek, K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 175–193.

[2] Hanahan, D.; Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell*. **2011**, *144*, 646–674.

[3] Delbridge, ARD, Grabow, S, Strasser, A, Vaux, DL. (2016). Thirty years of BCL-2: Translating cell death discoveries into novel cancer therapies. *Nat*. *Rev*. *Cancer*. **2016**, *16*, 99–109.

[4] Yap, J. L.; Chen, L.; Lanning, M. E.; Fletcher, S. Expanding the cancer arsenal with targeted therapies: Disarmament of the antiapoptotic Bcl□2 proteins by small molecules. *J*. *Med*. *Chem*. **2017**, *60*, 821–838.

[5] Garner, T. P.; Lopez, A.; Reyna, D. E.; Spitz, A. Z.; Gavathiotis, E. Progress in targeting the BCL-2 family of proteins. *Curr*. *Opin*. *Chem*. *Biol*. **2017**, *39*, 133–142.

[6] Oltersdorf, T.; Elmore, S. W.; Shoemaker, A. R.; Armstrong, R. C.; Augeri, D. J.; Belli, B. A.; Bruncko, M.; Deckwerth, T. L.; Dinges, J.; Hajduk, P. J.; Joseph, M. K.; Kitada, S.; Korsmeyer, S. J.; Kunzer, A. R.; Letai, A.; Li C.; Mitten, M. J.; Nettesheim, D. G.; Ng S.; Nimmer, P. M.; O'Connor, J. M.; Oleksijew, A.; Petros, A. M.; Reed, J. C.; Shen, W.; Tahir, S. K.; Thompson, C. B.; Tomaselli, K. J.; Wang, B.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature*. **2005**, *435*, 677–681.

[7] Bruncko, M.; Oost, T. K.; Belli, B. A.; Ding, H.; Joseph, M. K.; Kunzer, A.; Martineau, D.; McClellan, W. J.; Mitten, M.; Ng, S.-C.; Nimmer, P. M.; Oltersdorf, T.; Park, C.-M.; Petros, A. M.; Shoemaker, A. R.; Song, X.; Wang, B.; Wendt, M. D.; Zhang, H.; Fesik, S. W.; Rosenberg, S. H.; Elmore, S. W. Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL. *J*. *Med*. *Chem*. **2007**, *50*, 641–662.

[8] Tse, C.; Shoemaker, A. R.; Adickes, J.; Anderson, M. G.; Chen, J.; Jin, S.; Johnson, E. F.; Marsh, K. C.; Mitten, M. J.; Nimmer, P.; Roberts, L.; Tahir, S. K.; Xiao, Y.; Yang, X.;

Zhang, H.; Fesik, S.; Rosenberg, S. H.; Elmore, S. W. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Research*. **2008**, *68*, 3421–3428.

[9] Park, C.-M.; Bruncko, M.; Adickes, J.; Bauch, J.; Ding, H.; Kunzer, A.; Marsh, K. C.; Nimmer P.; Shoemaker, A. R.; Song, X.; Tahir, S. K.; Tse, C.; Wang, X.; Wendt, M. D.; Yang, X.; Zhang, H.; Fesik, S.; Rosenberg, S. H.; Elmore, S. W. Discovery of an orally bioavailable small molecule inhibitor of prosurvival B-cell lymphoma 2 proteins. *J. Med. Chem*. **2008**, *51*, 6902–6915.

[10] Wilson, W. H.; O'Connor, O. A.; Czuczman, M. S.; LaCasce, A. S.; Gerecitano, J. F.; Leonard, J. P.; Tulpule, A.; Dunleavy, K.; Xiong, H.; Chiu, Y. L.; Cui, Y.; Busman, T.; Elmore, S. W.; Rosenberg, S. H.; Krivoshik, S. P.; Enschede, S. H.; Humerickhouse, R. A. Navitoclax, a targeted high-affi nity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *Lancet Oncol*. **2010**, *11*, 1149–1159.

[11] Mason, K. D.; Carpinelli, M. R.; Fletcher, J. I.; Collinge, J. E.; Hilton, A. A.; Ellis, S.; Kelly, P. N.; Ekert, P. G.; Metcalf, D.; Roberts, A. W.; Huang, D. C. S.; Kile, B. T. Programmed anuclear cell death delimits platelet life span. *Cell*. **2007**, *128*, 1173–1186.

[12] Zhang, H.; Nimmer, P. M.; Tahir, S. K.; Chen, J.; Fryer, R. M.; Hahn, K. R.; Iciek, L. A.; Morgan, S. J.; Nasarre, M. C.; Nelson, R.; Preusser, L. C.; Reinhart, G. A.; Smith, M. L.; Rosenberg, S. H.; Elmore, S. W.; Tse, C. Bcl-2 family proteins are essential for platelet survival. *Cell Death Differ*. **2007**, *14*, 943–951.

[13] Souers, A. J.; Leverson, J. D.; Boghaert, E. R.; Ackler, S. L.; Catron, N. D.; Chen, J.; Dayton, B. D.; Ding, H.; Enschede, S. H.; Fairbrother, W. J.; Huang, D. C.; Hymowitz, S.

G.; Jin, S.; Khaw, S. L.; Kovar, P. J.; Lam, L. T.; Lee, J.; Maecker, H. L.; Marsh, K. C.; Mason, K. D.; Mitten, M. J.; Nimmer, P. M.; Oleksijew, A.; Park, C. H.; Park, C. M.; Phillips, D. C.; Roberts, A. W.; Sampath, D.; Seymour, J. F.; Smith, M. L.; Sullivan, G. M.; Tahir, S. K.; Tse, C.; Wendt, M. D.; Xiao, Y.; Xue, J. C.; Zhang, H.; Humerickhouse, R. A.; Rosenberg, S. H.; Elmore, S. W. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat*. *Med*. **2013**, *19*, 202–208.

[14] DiNardo, C. D.; Pratz, K.; Pullarkat, V.; Jonas, B. A.; Arellano, M.; Becker, P. S.; Frankfurt, O.; Konopleva, M.; Wei, A. H.; Kantarjian, H. M.; Xu, T.; Hong, W.-J.; Chyla, B.; Potluri, J.; Pollyea, D. A.; Letai, A. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood*. **2018**, *133*, 7–17.

[15] Roberts, A. W.; Davids, M. S.; Pagel, J. M.; Kahl, B. S.; Puvvada, S. D.; Gerecitano, J. F.; Kipps, T. J.; Anderson, M. A.; Brown, J. R.; Gressick, L.; Wong, S.; Dunbar, M.; Zhu, M.; Desai, M. B.; Cerri, E.; Enschede, S. H.; Humerickhouse, R. A.; Wierda, W. G.; Seymour, J. F. Targeting BCL2 with venetoclax in relapsed chronic lymphocytic leukemia. *N*. *Engl*. *J*. *Med*. **2016**, *374*, 311–322.

[16] Oppermann, S.; Ylanko, J.; Shi, Y.; Hariharan, S.; Oakes, C. C.; Brauer, P. M.; Zúñiga-Pflücker, J. C.; Leber, B.; Spaner, D. E.; Andrews, D. W. (2016). From high-content screening identifies kinase inhibitors that overcome venetoclax resistance in activated CLL cells. *Blood*. **2016**, *128*, 934–947.

[17] Mihalyova, J.; Jelinek, T.; Growkova, K.; Hrdinka, M.; Simicek, M.; Hajek, R. Venetoclax: A new wave in hematooncology. *Exp*. *Hematol*. **2018**, *61*, 10–25.

[18] Perini, G. F.; Ribeiro, G. N.; Neto, J. V. P.; Campos, L. T.; Hamerschlak, N. BCL-2 as therapeutic target for hematological malignancies. *J. Hematol. Oncol*. **2018**, *11*, 65.

[19] Vogler, M. Targeting BCL2-proteins for the treatment of solid tumours. *Adv. Med*. **2014**, Article ID 943648.

[20] Amundson, S. A.; Myers, T. G.; Scudiero, D.; Kitada, S.; Reed, J. C.; Fornace, A. J. An informatics approach identifying markers of chemosensitivity in human cancer cell lines. *Cancer Res*. **2000**, *60*, 6101–6110.

[21] Leverson, J. D.; Phillips, D. C.; Mitten, M. J.; Boghaert, E. R.; Diaz, D.; Tahir, S. K.; Belmont, L. D.; Nimmer, P.; Xiao, Y.; Ma, X. M.; Lowes, K. N.; Kovar, P.; Chen, J.; Jin, S.; Smith, M.; Xue, J.; Zhang, H.; Oleksijew, A.; Magoc, T. J.; Vaidya, K. S.; Albert, D. H.; Tarrant, J. M.; La, N.; Wang, L.; Tao, Z. F.; Wendt, M. D.; Sampath, D.; Rosenberg, S. H.; Tse, C.; Huang, D. C. S.; Fairbrother, W. J.; Elmore, S. W.; Souers, A. J. Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. *Sci. Transl. Med*. **2015**, *7*, 279ra40.

[22] Mullard, A. Pioneering apoptosis-targeted cancer drug poised for FDA approval. *Nat. Rev. Drug Discov*. **2016**, *15*, 147–149.

[23] Chang, J.; Wang, Y.; Shao, L.; Laberge, R. M.; Demaria, M.; Campisi, J.; Janakiraman, K.; Sharpless, N. E.; Ding, S.; Feng, W.; Luo, Y.; Wang, X.; Aykin-Burns, N.; Krager, K.; Ponnappan, U.; Hauer-Jensen, M.; Meng, A.; Zhou, D. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat. Med*. **2016**, *22*, 78–83.

[24] Zhu, Y.; Tchkonia, T.; Fuhrmann-Stroissnigg, H.; Dai, H. M.; Ling, Y. Y.; Stout, M. B.; Pirtskhalava, T.; Giorgadze, N.; Johnson, K. O.; Giles, C. B.; Wren, J. D.; Niedernhofer, L. J.; Robbins, P. D.; Kirkland, J. L. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell*. **2016**, *15*, 428–435.

[25] Yosef, R.; Pilpel, N.; Tokarsky-Amiel, R.; Biran, A.; Ovadya, Y.; Cohen, S.; Vadai, E.; Dassa, L.; Shahar, E.; Condiotti, R.; Ben-Porath, I.; Krizhanovsky, V. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat. Comm*. **2016**, *7*, 11190.

[26] Zhu, Y.; Doornebal, E. J.; Pirtskhalava, T.; Giorgadze, N.; Wentworth, M.; Fuhrmann-Stroissnigg, H.; Niedernhofer, L. J.; Robbins, P. D.; Tchkonia, T.; Kirkland, J. L. New agents that target senescent cells: the flavone, fisetin, and the BCL-XL inhibitors, A1331852 and A1155463. *Aging*. **2017**, *9*, 955−963.

[27] Demaria, M.; O'Leary, M. N.; Chang, J.; Shao, L.; Liu, S.; Alimirah, F.; Koenig, K.; Le, C.; Mitin, N.; Deal, A. M.; Alston, S.; Academia, E. C.; Kilmarx, S.; Valdovinos, A.; Wang, B.; de Bruin, A.; Kennedy, B. K.; Melov, S.; Zhou, D.; Sharpless, N. E.; Muss, H.; Campisi, J. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. *Cancer Discov*. **2017**, *7*, 165–176.

[28] Bai, L.; Chen, J.; Liu, L.; McEachern, D.; Aguilar, A.; Zhou, H.; Yang, C. Y.; Wang, H.; Wen, J.; Wang, G.; Zhai, Y.; Guo, M.; Yang, D.; Wang, S. BM-1252 (APG-1252): a potent dual specific Bcl-2/Bcl-xL inhibitor that achieves complete tumor regression with minimal platelet toxicity. *Eur. J. Cancer*. **2014**, *50*, 109–110.

[29] Lakhani, N. J.; Rasco, D. W.; Tolcher, A. W.; Huang, Y.; Ji, J.; Wang, H.; Dong, Q.; Men, L.; O'Rourke, T. J.; Chandana, S. R.; Amaya, A.; Cole, Y.; Kaiser, B.; Mays, T. A.;

Patnaik, A.; Papadopoulos, K. P.; Yang, D.; Zhai, Y. A phase I study of novel dual Bcl-2/Bcl-xL inhibitor APG-1252 in patients with advanced small cell lung cancer (SCLC) or other solid tumor. *J. Clin. Oncol.* **2018**, *36*, suppl. abstr. 2594.

[30] https://clinicaltrials.gov/ct2/show/NCT03595059

[31] Lai, A. C.; Crews, C. M. Induced protein degradation: an emerging drug discovery paradigm. Nature Reviews Drug Discovery. *Nat. Rev. Drug Discov.* **2017**, *16*, 101–114.

[32] Churcher, I. Protac-induced protein degradation in drug discovery: breaking the rules or just making new ones? *J. Med. Chem.* **2018**, *61*, 444–452.

[33] Schapira, M.; Calabrese M. F.; Bullock, A. N.; Crews, C. M. Targeted protein degradation: expanding the toolbox. *Nat. Rev. Drug Discov.* **2019**, 19, DOI:10.1038/s41573-019-0047-y

[34] Khan, S.; Zhang, X.; Lv, D.; Zhang, Q.; He, Y.; Zhang, P.; Liu, X.; Thummuri, D.; Yuan, Y.; Wiegand, J. S.; Pei, J.; Zhang, W.; Sharma, A.; McCurdy, C. R.; Kuruvilla, V. M.; Baran, N.; Ferrando, A. A.; Kim, Y.; Rogojina, A.; Houghton, P. J.; Huang, G.; Hromas, R.; Konopleva, M.; Zheng, G.; Zhou, D. A selective BCL-XL PROTAC degrader achieves safe and potent antitumor activity. *Nat. Med.* **2019**, DOI: 10.1038/s41591-019-0668-z

[35] Bray, P. F.; McKenzie, S. E.; Edelstein, L. C.; Nagalla, S.; Delgrosso, K.; Ertel, A.; Kupper, J.; Jing, Y.; Londin, E.; Loher, P.; Chen, H. W.; Fortina, P.; Rigoutsos, I. The complex transcriptional landscape of the anucleate human platelet. *BMC Genomics*. **2013**, *14*, 1.

[36] Kissopoulou, A.; Jonasson, J.; Lindahl, T. L.; Osman, A. Next generation sequencing analysis of human platelet PolyA$^+$ mRNAs and rRNA-depleted total RNA. *PLoS One*. **2013**, *8*, e81809.

[37] Zhang, X.; Thummuri, D.; He, Y.; Liu, X.; Zhang, P.; Zhou, D.; Zheng, G. Utilizing PROTAC technology to address the on-target platelet toxicity associated with inhibition of BCL-XL. *Chem*. *Commun*. **2019**, *55*, 14765–14768.

[38] Papatzimas, J. W.; Gorobets, E.; Maity, R.; Muniyat, M. I.; MacCallum, J. L.; Neri, P.; Bahlis, N. J.; Derksen, D. J. From inhibition to degradation: targeting the antiapoptotic protein myeloid cell leukemia 1 (MCL1). *J. Med. Chem*. **2019**, *62*, 5522–5540;

[39] Wang, Z.; He, N.; Guo, Z.; Niu, C.; Song, T.; Guo, Y.; Cao, K.; Wang, A.; Zhu, J.; Zhang X.; Zhang, Z. Proteolysis targeting chimeras for the selective degradation of Mcl-1/Bcl-2 derived from nonselective target binding ligands. *J. Med. Chem*. **2019**, *62*, 8152–8163.

[40] Lai, A. C.; Toure, M.; Hellerschmied, D.; Salami, J.; JaimeFigueroa, S.; Ko, E.; Hines, J.; Crews, C. M. Modular PROTAC Design for the Degradation of Oncogenic BCR-ABL. *Angew*. *Chem*. *Int*. *Ed*. **2016**, *55*, 807−810.

[41] Han, X.; Wang, C.; Qin, C.; Xiang, W.; Fernandez-Salas, E.; Yang, C.-Y.; Wang, M.; Zhao, L.; Xu, T.; Chinnaswamy, K.; Delproposto, J.; Stuckey, J.; Wang, S. Discovery of ARD-69 as a highly potent proteolysis targeting chimera (PROTAC) degrader of androgen receptor (AR) for the treatment of prostate cancer. *J. Med. Chem*. **2019**, *62*, 941–964.

[42] Hu, J.; Hu, B.; Wang, M.; Xu, F.; Miao, B.; Yang, C.-Y.; Wang, M.; Liu, Z.; Hayes, D. F.; Chinnaswamy, K.; Delproposto, J.; Stuckey, J.; Wang, S. Discovery of ERD-308 as a

highly potent proteolysis targeting chimera (PROTAC) degrader of estrogen receptor (ER). *J. Med. Chem.* **2019**, *62*, 1420–1442.

[43] Crew, A. P.; Raina, K.; Dong, H.; Qian, Y.; Wang, J.; Vigil, D.; Serebrenik, Y. V.; Hamman, B. D.; Morgan, A.; Ferraro, C.; Siu, K. Identification and characterization of Von Hippel-Lindau-recruiting proteolysis targeting chimeras (PROTACs) of TANK-binding kinase 1. *J. Med. Chem.* **2018**, *61*, 583–598.

[44] Zoppi, V.; Hughes, S. J.; Maniaci, C.; Testa, A.; Gmaschitz, T.; Wieshofer, C.; Koegl, M.; Riching, K. M.; Daniels, D. L.; Spallarossa, A.; Ciulli, A. Iterative design and optimization of initially inactive proteolysis targeting chimeras (PROTACs) identify VZ185 as a potent, fast, and selective von Hippel–Lindau (VHL) based dual degrader probe of BRD9 and BRD7. *J. Med. Chem.* **2018**, *62*, 699–726.

[45] Wurz, R. P.; Dellamaggiore, K.; Dou, H.; Javier, N.; Lo, M. C.; McCarter, J. D.; Mohl, D.; Sastri, C.; Lipford, J. R.; Cee, V. J. A "click chemistry platform" for the rapid synthesis of bispecific molecules for inducing protein degradation. *J. Med. Chem.* **2018**, *61*, 453–461.

[46] Raina, K.; Lu, J.; Qian, Y.; Altieri, M.; Gordon, D.; Rossi, A. M.; Wang, J.; Chen, X.; Dong, H.; Siu, K.; Winkler, J. D.; Crew, A. P.; Crews, C. M.; Coleman, K. G. PROTAC-induced BET protein degradation as a therapy for castration-resistant prostate cancer. *Proc. Natl. Acad. Sci. USA.* **2016**, *113*, 7124–7129.

[47] Riching, K.M.; Mahan, S.; Corona, C. R.; McDougall, M.; Vasta, J. D.; Robers, M. B.; Urh, M.; Daniels, D. L. Quantitative live-cell kinetic degradation and mechanistic profiling of PROTAC mode of action. *ACS Chem. Biol*. **2018**, *13*, 2758–2770.

[48] Moreau, K.; Coen, M.; Zhang, A. X.; Pachl, F.; Castaldi, M. P.; Dahl, G.; Boyd, H.; Scott, C.; Newham, P. PROTAC (PROteolysis TArgeting Chimeras) in drug development: a safety perspective. *Br. J. Pharmacol*. **2020**, doi.org/10.1111/bph.15014.

[49] Li, Y.; Yang, J.; Aguilar, A.; et al. Discovery of MD-224 as a first-in-class, highly potent, and efficacious proteolysis targeting chimera murine double minute 2 degrader capable of achieving complete and durable tumor regression. *J. Med. Chem*. **2018**, *62*, 448−467.

[50] McCoull, W.; Cheung, T.; Anderson, E.; Barton, P.; Burgess, J.;Byth, K.; Cao, Q.; Castaldi, M. P.; Chen, H.; Chiarparin, E.; Carbajo, R. J.; Code, E.; Cowan, S.; Davey, P. R.; Ferguson, A. D.; Fillery, S.; Fuller, N. O.; Gao, N.; Hargreaves, D.; Howard, M. R.; Hu, J.; Kawatkar, A.; Kemmitt, P. D.; Leo, E.; Molina, D. M.; O'Connell, N.; Petteruti, P.; Rasmusson, T.; Raubo, P.; Rawlins, P. B.; Ricchiuto, P.; Robb, G. R.; Schenone, M.; Waring, M. J.; Zinda, M.; Fawell, S.; Wilson, D. M. Development of a novel B-cell lymphoma 6 (BCL6) PROTAC to provide insight into small molecule targeting of BCL6. *ACS Chem. Biol*. **2018**, *13*, 3131−3141.

[51] Bai, L.; Zhou, H.; Xu, R.; Zhao, Y.; Chinnaswamy, K.; McEachern, D.; Chen, J.; Yang, C-Y.; Liu, Z.; Wang, M.; Liu, L.; Jiang, H.; Wen, B.; Kumar, P.; Meagher, J. L.; Sun, D.; Stuckey, J. A.; Wang, S. A potent and selective small-molecule degrader of STAT3 achieves complete tumor regression in vivo. Cancer Cell, **2019**, *36*, 498–511.

[52] Zhou, H.; Bai, L.; Xu, R.; Zhao, Y.; Chen, J.; McEachern, D.; Chinnaswamy, K.; Wen, B.; Dai, L.; Kumar, P.; Yang, C-Y.; Liu, Z.; Wang, M.; Liu, L.; Meagher, J. L.; Yi, H.; Sun, D.; Stuckey, J. A.; Wang, S. Structure-based discovery of SD-36 as a potent, selective and efficacious PROTAC degrader of STAT3 protein. *J. Med. Chem.* DOI: 10.1021/acs.jmedchem.9b01530.

- BCL-X_L PROTACs have significantly improved cytotoxicity in MOLT-4 cells
- XZ739 treatment leads to remarkable BCL-X_L degradation with a DC_{50} at 2.5 nM
- XZ739 exhibits >100-fold selectivity for MOLT-4 cells over human platelets, whereas ABT-263 has no selectivity

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:

X. Zhang, P. Zhang, S. Khan, D. Zhou, and G. Zheng are co-inventors for BCL-X_L PROTACs disclosed in this study. D. Zhou and G. Zheng are co-founders and shareholders of Dialectic Therapeutics, a company that is developing BCL-X_L PROTACs to treat cancers.