HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2023 February 24.

Published in final edited form as:

J Med Chem. 2022 February 24; 65(4): 3644–3666. doi:10.1021/acs.jmedchem.1c02165.

Discovery of Potent, Selective, and In Vivo Efficacious AKT Kinase Protein Degraders via Structure–Activity Relationship Studies

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Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c02165

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c02165.
Effects of compounds **19**–**23** on colony formation inhibition in BT474 cells; effects of compounds **35**–**41** on colony formation inhibition in BT474 cells; binding of benzylated VHL-1 (VHL-1-Bn) to the E3 ligase VHL assessed via ITC; effects of compound **20** on degradation of AKT1, AKT2, and AKT3 isoforms in PC3 cells; and ^{1}H NMR, ^{13}C NMR, and HPLC spectra of compounds **20**, **35**, **42**, and **43** (PDF)
Molecular formula strings for all compounds (CSV)

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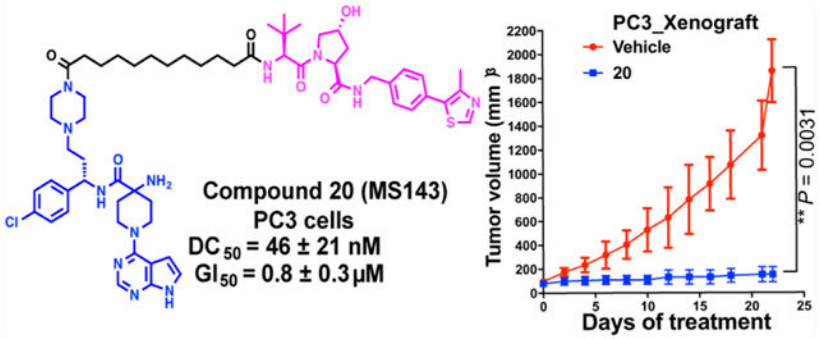
Abstract

We recently reported a potent, selective, and in vivo efficacious AKT degrader, MS21, which is a von Hippel–Lindau (VHL)-recruiting proteolysis targeting chimera (PROTAC) based on the AKT inhibitor AZD5363. However, no structure–activity relationship (SAR) studies that resulted in this discovery have been reported. Herein, we present our SAR studies that led to the discovery of MS21, another VHL-recruiting AKT degrader, MS143 (compound **20**) with similar potency as MS21, and a novel cereblon (CRBN)-recruiting PROTAC, MS5033 (compound **35**). Compounds **20** and **35** induced rapid and robust AKT degradation in a concentration- and time-dependent manner via hijacking the ubiquitin-proteasome system. Compound **20** suppressed cell growth more effectively than AZD5363 in multiple cancer cell lines. Furthermore, **20** and **35** displayed good plasma exposure levels in mice and are suitable for in vivo efficacy studies. Lastly, compound **20** effectively suppressed tumor growth in vivo in a xenograft model without apparent toxicity.

Graphical Abstract



INTRODUCTION

AKT [also known as protein kinase B (PKB)] has three isoforms (AKT1, AKT2, and AKT3) that share a high degree of sequence identity in their catalytic domains.[1–4] AKT functions as a central element of the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway, which is frequently hyperactivated in human

cancers and other human diseases.[5] The activation mechanisms include amplification and/or mutation of three *AKT* genes, somatic mutation of PI3K (encoded by the *PIK3CA* gene) at the p110α catalytic subunit, loss-of-function mutations or downregulation of tumor suppressor *PTEN*, and alterations of receptor tyrosine kinase (RTK) signaling.[4,6] Apart from the dysregulated PI3K/AKT/mTOR signaling pathway that plays a major role in tumor growth, survival, metabolism, and resistance to anticancer therapy,[7–10] the overexpression of AKT has also been implicated in cancer progression and development.[11] Therefore, AKT has been considered as an important therapeutic target for the prevention and treatment of human cancers.[12–14]

A number of AKT inhibitors including adenosine triphosphate (ATP)-competitive inhibitors (such as GSK690693,[15] GDC-0068,[16] and AZD5363[17]) and allosteric inhibitors (such as MK2206[18] and ARQ-092[19]) have entered clinical investigations. However, to date, none of these inhibitors have demonstrated robust clinical efficacy, under-scoring the urgent need for novel therapeutic strategies for the treatment of AKT-mediated cancers and other diseases.[13,20–23] Extensive studies have established that specific knockdown/knockout of AKT isoforms through genetic technologies significantly inhibited cancer cell proliferation and suppressed tumor progression in many AKT-driven human malignancies.[24–27] For example, depletion of AKT1 and AKT2 substantially inhibited the proliferation of prostate cancer cells,[28,29] and knockdown of AKT2 or AKT3 by RNA interference remarkably reduced the growth of glioma cells.[30] In addition, the downregulation of AKT3 attenuated the growth of triple-negative breast cancer (TNBC) cells.[31] Taken together, these studies highlighted that the downregulation or depletion of AKT protein levels can be a potential therapeutic strategy for treating AKT-dependent cancers.

Proteolysis targeting chimeras (PROTACs) are promising new therapeutic modalities with a unique mechanism of action (MOA), which induces the degradation of the target protein through hijacking the endogenous ubiquitin proteasome system (UPS).[32–38] PROTACs, which chemically down-regulate target protein levels, could recapitulate biological effects of genetic knockout/knockdown and provide a potentially more effective therapeutic strategy than pharmacological inhibition of AKT kinase activity.

A few AKT PROTAC degraders have been reported by us and others. You et al. reported INY-03–041 as the first GDC-0068-derived cereblon (CRBN)-recruiting AKT degrader.[39] We recently reported an extensive SAR study using GDC-0068 as an AKT binding moiety and the discovery of two additional AKT degraders that recruit CRBN (MS170) and von Hippel–Lindau (VHL) (MS98), respectively.[40] We also reported extensive in vitro and in vivo characterization of a potent, selective, and in vivo efficacious AKT degrader, MS21, which is VHL-recruiting and derived from the AKT inhibitor AZD5363.[41] However, SAR studies that resulted in the discovery of MS21 have not been reported. In this paper, we report the design, synthesis, and biological evaluation of a variety of AZD5363-derived AKT degraders. Through this extensive SAR campaign, we show how MS21[41] was discovered as a potent and selective AKT degrader. In addition, we report another potent VHL-recruiting AKT degrader **20** (MS143) and a novel CRBN-recruiting AKT degrader **35** (MS5033). Both **20** and **35** induced rapid and efficient AKT degradation in cancer cells and displayed good bioavailability in mice via intraperitoneal (IP) administration. Moreover,

20 effectively inhibited tumor growth in vivo in a PC-3 cell line xenograft mouse model. Compounds **20** and **35** could be useful chemical tools for further studying AKT functions in health and disease. Further optimization of these degraders could provide potential AKT degradation therapies for AKT-dependent cancers and other diseases.

RESULTS AND DISCUSSION

Design and Biological Evaluation of AZD5363-Based AKT PROTAC Degraders.

AZD5363 is a highly potent and selective ATP-competitive pan-AKT inhibitor, which has been advanced into clinical trials for the treatment of solid tumors.[17,21] The co-crystal structure of AKT1 in complex with AZD5363 revealed that the hydroxyl group of AZD5363 is solvent-exposed (Figure 1A, PDB: 4GV1).[17] Previous SAR studies indicated that the hydroxyl group can be replaced with a morpholinyl group without a significant decrease in potency against AKT isomers (Figure 1B).[17] To facilitate the linker installation, precursor **2** (AZD5363*), bearing a piperazine ring, was designed (Figure 1B).

VHL/cullin 2 and CRBN/cullin 4A E3 ligase complexes have been widely harnessed by PROTACs to induce the degradation of targeted proteins, mainly because well-characterized small-molecule ligands of VHL and CRBN are readily available.[42,43] VHL-1 and its analogues display good binding affinities to the VHL E3 ligase,[44–46] and immunomodulatory drugs (IMiDs), such as pomalidomide (POM), lenalidomide, and thalidomide, are well characterized CRBN ligands.[47,48] Therefore, we designed an initial set of AKT putative degraders by conjugating compound **2** to VHL-1 or POM through a variety of polyethylene glycol (PEG) or alkyl linkers (Figure 2).

These putative AKT degraders were evaluated using an immunoblotting assay through assessing the protein levels of total AKT (T-AKT), phosphorylated AKT (P-AKT), downstream signaling P-PRAS40, and biomarker P-S6 in BT474 cells, a breast cancer cell line (Figures 3 and 4). The cells were treated with the putative degraders at 1, 5, or 10 μM for 24 h using AZD5363 and the corresponding E3 ligase ligand (VHL-1 or POM) as controls. As shown in Figure 3, among the VHL-recruiting compounds with a PEG linker (**3**–**11**), compound **3**, with the shortest PEG linker, induced the most significant T-AKT degradation, and displayed the most effective inhibition of the downstream signaling. VHL-1 as a control had no effect on the T-AKT protein level and downstream signaling. Interestingly, while the AKT inhibitor AZD5363 effectively inhibited the phosphorylation of downstream PRAS40 and ribosomal protein S6 and paradoxically upregulated the phosphorylated AKT level as expected,[17,49] it also downregulated the T-AKT protein level. However, the effect of AZD5363 on the T-AKT protein level was less profound than that of **3**. Increasing the linker length by two methylene groups (**4**) decreased the AKT degradation effectiveness. Longer PEG linkers (**5**–**11**) further diminished the degradation effectiveness. This linker length related SAR trend for compounds with a PEG linker is opposite to that of compounds with an alkyl linker. Compounds with a short alkyl linker, such as **12** (ethylene) and **13** (propylene), were inactive at reducing the T-AKT protein level. Compounds with a medium length linker, such as **14** (butylene) and **15** (pentylene), showed improved AKT degradation and significant downstream signaling inhibition at 5 μM. Compounds with an

alkyl linker longer than pentylene (**16**–**19**) are the most potent AKT degraders and induced near-complete depletion of the T-AKT protein at concentrations as low as 1 μM. Among these compounds, compound **19** (MS21) exhibited the most profound AKT degradation and downstream signaling inhibition. Based on previous reports that a methylated VHL-1 analogue, (*S,R,S*)-AHPC-Me (VHL-2), can improve degradation potency,[50–52] we designed compound **23**, which incorporated this VHL ligand, as a direct analogue of **19**. However, **23** did not show significant difference from **19** in degrading AKT and inhibiting the downstream signaling and colony formation (Figures 3 and S1). We further extended the alkyl linker by 1–3 methylene units to obtain compounds **20**–**22**. Among these three analogues, compounds **20** and **21** displayed similar AKT protein degradation potency to **19** but slightly enhanced downstream signaling inhibition compared to **19** (Figure 3). Compounds **20** and **21** were also highly effective in inhibiting colony formation in BT474 cells, similar to compound **19** (Figure S1). Because compound **20** has a slightly shorter linker than compound **21**, we selected compound **20** for further studies. Taken together, our SAR results of VHL-recruiting AKT degraders demonstrated that compounds with an alkyl linker induced AKT degradation more effectively than the compounds with a PEG linker, and compounds with a relatively long alkyl linker are more potent than the ones with a short alkyl linker.

Compared with the VHL-recruiting degraders, the linker of the CRBN-recruiting compounds has different effects (Figure 4). Compounds with a medium length alkyl linker, such as **26**, **27**, and **28**, displayed moderate effectiveness in AKT degradation and downstream signaling inhibition. Compounds with a short (**24** and **25**) or long (**29** and **30**) alkyl linker induced negligible AKT degradation at 1–10 μM concentrations. Switching from alkyl linkers to PEG linkers resulted in much more potent CRBN-recruiting AKT degraders (**32**–**38**). While compound **31** with the shortest PEG linker induced only moderate AKT degradation at 10 μM, compounds with a longer PEG linker (**32**–**38**) significantly reduced the AKT protein level at 1 μM. Among them, compound **35** was most effective in inhibiting the phosphorylation of ribosomal protein S6. Next, we designed three analogues of **35** by modifying the CRBN binder moiety (Figure 2B). Removal of one carbonyl group of the phthalimide moiety (**39**) significantly decreased effectiveness in AKT degradation (Figure 4). Replacing the amino group in compound **35** with either oxygen (**40**) or methylene group (**41**) did not improve either the AKT degradation or inhibition of colony formation in BT474 cells (Figures 4 and S2). Based on these SAR results, we selected compound **35** for additional characterization. Overall, these studies revealed the following SAR trends for CRBN-recruiting AKT degraders: (1) compounds with a PEG linker showed better AKT degradation potency than compounds with an alkyl linker, and (2) compounds with a long PEG linker are more potent than compounds with a short PEG linker.

Through the extensive SAR studies of AZD5363-based AKT degraders, we identified compounds **20** and **35** as promising VHL- and CRBR-recruiting AKT degraders, respectively. We thus developed two structurally similar negative control compounds: compound **42** (MS143N) for degrader **20**, and compound **43** (MS5033N) for degrader **35** (Figure 5). Compound **42** incorporates a benzyl-protected hydroxyl proline group at the VHL binding region and preserves the same AKT binding motif and linker as compound

20 (Figure 5). The addition of the benzyl group to the hydroxyl proline moiety of VHL-1 (VHL-1-Bn) was designed to abrogate the key hydrogen bond interaction between the hydroxyl group and VHL.[53] Using isothermal titration calorimetry (ITC), we confirmed that benzyl-protected VHL-1 indeed did not bind VHL (Figure S3). Compound **43** contains a methylated glutarimide ring, which abrogates its binding to CRBN[54] and retains the identical AKT binding moiety and linker as compound **35** (Figure 5).

Binding of Compounds 20, 35, 42, and 43 to AKT.

We next assessed binding affinities of degraders **20** and **35**, and their negative compounds **42** and **43** to three AKT isoforms using a biochemical competition assay (Figure 6). The VHL-based degrader **20** showed good binding affinity to AKT1 (K_d = 26 ± 6.3 nM), AKT2 (K_d = 400 ± 200 nM), and AKT3 (K_d = 88 ± 140 nM), whereas the corresponding control compound **42** displayed weaker binding affinities to AKT1 (K_d = 150 ± 130 nM), AKT2 (K_d > 10 μM), and AKT3 (K_d = 2.9 ± 2.7 μM). The CRBN-based degrader **35** exhibited strong binding to AKT1 (K_d = 4.8 ± 5.6 nM), AKT2 (K_d = 160 ± 120 nM), and AKT3 (K_d = 22 ± 8.8 nM). Compared to **35**, the *N*-methyl glutarimide negative control **43** retained similar binding affinities to AKT1 and AKT3 and displayed two fold decreased affinity to AKT2. We previously reported binding affinities of compound **19** and AZD5363 to the AKT isoforms in the same assay.[41] The binding affinities of compound **20** to AKT1/2/3 are similar to those of compound **19**.[41] Compared to the parent inhibitor AZD5363,[41] these degraders and their corresponding controls displayed weaker binding affinities to all three AKT isoforms. While it was unexpected that the binding affinities of compound **42** to AKT isoforms were lower than that of **20**, compound **42** still retained good binding affinity to AKT1 and moderate binding affinity to AKT3. Because compounds **20** and **35** exhibited better affinity to AKT1 and AKT3 over AKT2, we sought to determine whether the observed binding selectivity for AKT1 and AKT3 over AKT2 could lead to selective degradation of AKT1 and AKT3 over AKT2. As illustrated in Figure S4, compound **20** at 100 nM reduced AKT1 and AKT3 protein levels by about 90% and the AKT2 protein level by about 50% in PC3 cells. To achieve approximately 90% of AKT2 degradation, a higher compound concentration (such as 300 nM–1 μM) was required. Therefore, degrader **20** indeed showed moderate degradation selectivity for AKT1 and AKT3 over AKT2.

Compounds 20 and 35 Potently Induce AKT Degradation in PC3 Cells.

We further assessed the AKT degradation potency of **20** and **35** in PC3 cells (Figure 7A–B). Both **20** and **35** effectively induced T-AKT degradation in a concentration-dependent manner. The VHL-recruiting degrader **20** (DC_{50} = 46 ± 21 nM) was significantly more potent than the CRBN-recruiting degrader **35** (DC_{50} = 430 ± 300 nM) at degrading T-AKT. The complete T-AKT depletion was achieved when PC3 cells were treated with **20** at 300 nM or higher concentrations. Importantly, the “hooker effect” was not observed for either compound at concentrations up to 10 μ**M**. To demonstrate that the observed AKT degradation is due to recruiting the corresponding E3 ligase, we evaluated the AKT degradation effect of negative controls **42** and **43**. As expected, both **42** and **43** did not reduce the T-AKT protein level in PC3 cells at concentrations up to 10 μM (Figure 7C,D),

suggesting that the VHL and CRBN E3 ligases were involved in the AKT degradation induced by degraders **20** and **35**, respectively.

We next assessed kinetics of the AKT degradation and downstream signaling inhibition in PC3 cells treated with **20** or **35** at 1 μM (Figure 8). As a control, the parent inhibitor AZD5363 did not significantly reduce the AKT protein level at 1 μM for 24 h, but effectively inhibited PRAS40 and S6 phosphorylation. On the other hand, compound **20** induced substantial AKT degradation and inhibited PRAS40 and S6 phosphorylation within 4 h (Figure 8A). Complete depletion of T-AKT was achieved within 8 h. Compared to the VHL-recruiting degrader **20**, the CRBN-recruiting degrader **35** displayed slower kinetics in degrading AKT. T-AKT was significantly downregulated at 8 h, and the maximum degradation was achieved at around 24 h (Figure 8B). We also performed washout studies for both **20** and **35**. Interestingly, treatment with degrader **20** led to profound and persistent degradation of both T-AKT and P-AKT. The T-AKT level and downstream signaling inhibition were partially recovered after 48 h post-treatment (Figure 8C). On the other hand, treatment with degrader **35**, the AKT protein level rebounded substantially at 24 h and was completely recovered at 48 h (Figure 8D). We also observed that downstream signaling inhibition was near fully recovered at 24 h after the treatment of **35** or the parent inhibitor AZD5363. Taken together, these results suggest that the VHL-recruiting degrader **20** mediates rapid and sustained AKT degradation and the CRBN-recruiting degrader **35** is less effective than the VHL-recruiting degrader **20**.

Compounds 20 and 35 Induce AKT Degradation through Hijacking the UPS.

To determine whether the AKT protein degradation induced by degraders **20** and **35** is mediated through the corresponding E3 ligase and UPS, we conducted a series of competition experiments in PC3 cells. As shown in Figure 9A, an excess amount of VHL ligand (*S,R,S*)-AHPC-Me (VHL-2), which competes with **20** at the VHL E3 ligase binding site, restored the AKT protein level effectively, suggesting that the AKT degradation induced by **20** is dependent on the VHL E3 ligase. Pretreatment with the NEDD8-activating enzyme (NAE) inhibitor MLN4924 or proteasome inhibitor MG-132 also significantly diminished the AKT degradation effect of **20**, suggesting that the AKT degradation mediated by **20** is dependent on the cullin E3 ligase complex and proteasome. Furthermore, saturating the AKT binding site with an excess amount of AZD5363 also rescued the AKT protein level, indicating that AKT binding is required for AKT degradation. Similar results were obtained for the CRBN-recruiting degrader **35**. Pretreatment with POM (1 μM), MLN4924 (1 μM), MG-132 (20 μM), or AZD5363 (1 μM) diminished **35**'s ability in reducing the AKT protein in PC3 cells (Figure 9B). Moreover, pretreatment with these compounds, except AZD5363, not only rescued the AKT protein level but also attenuated the downstream signaling inhibition. These MOA study results, together with our observation that negative controls **42** and **43** were unable to induce T-AKT degradation (Figure 7C,D), demonstrated that **20** and **35** were *bona fide* AKT degraders, promoting AKT degradation in an E3 ligase- and UPS-dependent manner.

Compounds 20 and 35 Potently Inhibit the Proliferation of Cancer Cells.

As described earlier, degrader **20** effectively inhibited colony formation in BT474 cells (Figure S1). We next investigated the antiproliferation activity of **20** and **35** in prostate cancer PC3 cells and TNBC MDA-MB-468 cells using AZD5363, **42**, and **43** as controls (Figure 10). The VHL-recruiting compound **20** (GI_{50} = 0.8 ± 0.3 μM) was approximately 8-fold more potent than AZD5363 (GI_{50} = 6.5 ± 2.9 μM) in inhibiting the cell growth of PC3 cells (Figure 10A). The CRBN-recruiting degrader **35** (GI_{50} = 10.8 ± 5.6 μM) was about 14-fold less potent than **20**, which is consistent to the weaker degradation potency of **35** (DC_{50} = 430 ± 300 nM) than that of **20** (DC_{50} = 46 ± 21 nM, Figure 7A,B). In MDA-MB-468 cells, compound **20** (GI_{50} = 1.0 ± 0.3 μM) was also more potent than AZD5363 (GI_{50} = 3.8 ± 1.6 μM) and **35** (GI_{50} = 4.8 ± 1.9 μM) in inhibiting cell growth. Negative controls **42** and **43** were inactive in these antiproliferation assays, suggesting that compound **20**'s improved potency in cell growth inhibition is at least partially due to its AKT degradation activity (Figure 10A,B). Furthermore, as described earlier, degrader **20** effectively inhibited colony formation in BT474 cells (Figure S1). Overall, these results indicate that AKT degraders **20** and **35** were more potent in suppressing cancer cell proliferation than their corresponding negative control compounds **42** and **43**. Importantly, the VHL-recruiting degrader **20** was more potent than AZD5363 and the CRBN-recruiting degrader **35** in inhibiting the growth in both PC3 and MDA-MB-468 cells.

Compounds 20 and 35 are Bioavailable in Mice.

We next assessed the pharmacokinetic (PK) properties of compounds **20** and **35** in mice (Figure 11). A single dose of compound **20** at 75 mg/kg by IP injection achieved the maximum plasma concentration (C_{max}) of 7 μM at 2 h (T_{max}). The exposure level of approximate 3.7 μM was maintained over the 12 h period post-injection with an area under the curve (AUC) value of 63 600 h·ng/mL. Furthermore, the CRBN-recruiting compound **35** showed comparable C_{max} (8 μM) at 1 h (T_{max}) to **20** at a higher dose (150 mg/kg) via IP injection with an AUC value of 32 500 h·ng/mL. Compared to their determined DC_{50} and GI_{50} values in cellular assays, these high plasma concentrations and exposure suggest that these degraders have the potential to degrade ATK in vivo. A limitation in these PK studies is that we did not determine free drug concentrations. Future studies to determine this parameter are warranted. It is worth noting that these degraders were well tolerated by the tested mice, suggesting that they could be suitable for in vivo efficacy studies.

Compound 20 Effectively Suppresses Tumor Growth In Vivo in a Xenograft Model.

Because compound **20** exhibited more potent cell antiproliferation activity and displayed higher exposure in mouse plasma than compound **35**, we selected compound **20** for testing its in vivo efficacy in a PC3 cell line xenograft model (Figure 12). We treated mice bearing PC3 xenografts with vehicle or compound **20** at a dose of 75 mg/kg once daily through IP injection. After the 22-day treatment, compound **20** drastically inhibited the tumor growth by 92% compared to the vehicle treatment (P = 0.0031, Figure 12A). In addition, compound **20** was well tolerated by the mice treated at this dose through the entire in vivo experiment, without significant body weight loss (Figure 12B) and other clinical signs. We previously reported that AZD5363 via the same dose regimen (75 mg/kg once daily IP injection)

achieved only 75% tumor growth inhibition in the same xenograft model.[41] Lastly, western blotting analysis of the tumor samples collected from mice treated with **20** or vehicle at the end of the study showed that compound **20** substantially degraded T-AKT and P-AKT and effectively inhibited the downstream signaling (PRAS40 phosphorylation) in vivo (Figure 12C).

Compound Synthesis.

The synthetic route for preparing key intermediate **2** is described in Scheme 1. The synthetic effort commenced with Boc-protection of commercially available (*S*)-3-amino-3-(4-chlorophenyl)propan-1-ol (**44**) and subsequent halogenation reaction, yielding iodide compound **45**. *N*-Alkylation of benzyl piperazine-1-carboxylate (**46**) with compound **45**, followed by the deprotection of the Boc group provided compound **47**. Finally, the amide coupling reaction between **47** and 4-[(*tert*-butoxycarbonyl)amino]-1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidine-4-carboxylic acid (**48**),[17] followed by the removal of the Cbz protecting group afforded the common intermediate **2**.

As illustrated in Scheme 2A, AZD5363-based VHL-recruiting AKT degraders **3**–**23** were synthesized from intermediate **2** and various linker attached VHL-1 derivatives (**49**–**69**) through amide coupling and subsequent Boc-deprotecting. Following the same reaction sequence, CRBN-recruiting compounds **24**–**41** were synthesized from intermediate **2** and various linker attached POM derivatives (**70**–**87**) (Scheme 2B). Compounds **49**–**65** and **70**–**81** were synthesized following the reported procedures.[55]

The synthetic routes for compounds **42** and **43** are described in Scheme 3. Intermediate **90** was synthesized using the amide coupling reaction between commercially available compounds **88** and **89**, followed by Boc-deprotection. Amide coupling between **90** and (*S*)-2-[(*tert*-butoxycarbonyl)-amino]-3,3-dimethylbutanoic acid (**91**) and subsequent Boc-deprotection led to intermediate **92**, which was then coupled with undecanedioic acid (**93**) to yield **94**. Negative control compound **42** was synthesized from intermediates **2** and **94** by amide coupling and Boc deprotection reactions (Scheme 3A). Intermediate **97** was obtained via a nucleophilic aromatic substitution reaction of the commercially available *tert*-butyl 1-amino-3,6,9,12,15-pentaoxaoctadecan-18-oate (**95**) and 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**96**),[56] and subsequent hydrolysis of the *t*-butyl ester. Amide coupling between **2** and **97**, followed by the removal of the Boc-protecting group provided compound **43** (Scheme 3B).

The syntheses of linker attached E3 ligase ligands **66**–**69** and **82**–**87** are outlined in Scheme 4. Compounds **66**–**68** were prepared using amide coupling between commercially available di-acids **93**, **98**–**99**, and VHL-1 (**101**). Compound **69** was synthesized using the same amide coupling reaction between undecanedioic acid (**100**) and VHL-2 (**102**) (Scheme 4A). Compounds **82**–**84** were synthesized through nucleophilic aromatic substitution of **106** and commercially available **103**–**105**, and subsequent Boc-protecting group removal (Scheme 4B). Compounds **85** and **86** were prepared through nucleophilic substitution of **108** and **109** with *tert*-butyl 1-bromo-3,6,9,12,15-pentaoxaoctadecan-18-oate (**107**) under mild basic conditions, followed by *t*-butyl ester hydrolysis (Scheme 4C).

Compound **87** was synthesized via a three-step reaction sequence: Sonogashira coupling of commercially available *tert*-butyl 4,7,10,13,16-pentaoxanonadec-18-ynoate (**110**) with 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**111**), reduction of the alkynyl moiety by hydrogenation, and hydrolysis of the *tert*-butyl ester group (Scheme 4D).

CONCLUSIONS

Starting from the ATP-competitive AKT inhibitor AZD5363, we designed and synthesized a series of putative AKT degraders by conjugating a solvent-exposed site of AZD5363 to two types of E3 ligase (VHL and CRBN) ligands via various linkers. Through extensive SAR studies on the composition and length of linkers and different VHL- and CRBN-recruiting ligands, we identified compounds **20** and **35** as potent VHL-and CRBN-recruiting AKT degraders, respectively. We also developed two structurally similar analogues, **42** and **43**, as the negative controls of degraders **20** and **35**, respectively. These two degraders, but not their negative controls, achieved rapid and effective AKT degradation and downstream signaling inhibition in PC3 cells in a concentration- and time-dependent manner. Washout experiments further demonstrated that the AKT degradation induced by compound **20** can be sustained for 1–2 days post treatment. Our MOA studies indicated that the AKT degradation induced by **20** and **35** is dependent on the corresponding E3 ligase (VHL/CRBN) and the UPS. In cell proliferation assays, degraders **20** and **35** were more potent than their corresponding negative controls in inhibiting the growth of cancer cells. Moreover, degrader **20** was more potent than AZD5363 and degrader **35** in suppressing cell proliferation in PC3 and MDA-MB-468 cells. In addition, both **20** and **35** displayed good plasma exposure levels in mouse PK studies, suggesting that they are potentially suitable for in vivo efficacy studies. Furthermore, degrader **20** effectively suppressed the tumor growth and reduced the AKT protein level in vivo in a PC3 cell line xenograft mouse model. Overall, we present a comprehensive SAR study to the targeted protein degradation research community, which resulted in the discovery of two in vivo efficacious VHL-recruiting AKT degraders (**19**[41] and **20**) and a novel CRBN-recruiting AKT degrader (**35**).

EXPERIMENTAL SECTION

Chemistry General Procedures.

All commercially available solvents and reagents were used as received without further purification. Microwave-heated reactions were carried out with a Discover SP microwave system with an Explorer 12 Hybrid Autosampler by CEM (Buckingham, UK). Normal and reverse-phase flash chromatographies were performed using a Teledyne ISCO *CombiFlash* instrument and HP C18 Redi*Sep* Rf reverse-phase silica columns, respectively. Final compounds for biological evaluation were purified with preparative high-performance liquid chromatography (HPLC) on an Agilent Prep 1200 series with the UV detector set to 254 or 220 nm with solvent A (0.1% of TFA in water) and solvent B (methanol or acetonitrile) as eluents with a flow rate of 40 mL/min at rt. Purities of the final compounds were determined by HPLC and were >95%. HPLC conditions to assess purity were as follows: Agilent 1200 series system, 2.1 mm × 150 mm Zorbax 300SB-C18 5 μm column 1–99% gradient of 0.1% trifluoroacetic acid in water, and 0.1% trifluoroacetic acid in acetonitrile; flow rate,

0.4 mL/min; acquisition time, 8 min; and high-resolution mass spectra (HRMS) data were acquired on an Agilent G1969A API-TOF with an electrospray ionization (ESI) source. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker DXI 600 MHz (or 800 MHz) or AVANCE NEO 600 MHz NMR spectrometer. Proton nuclear magnetic resonance spectra are reported in parts per million (ppm) on the δ scale and are referenced from the residual protium in the NMR solvent (CD_3OD, δ 3.31). C13 nuclear magnetic resonance spectra are reported in parts per million (ppm) on the δ scale and are referenced from the carbon resonances of the solvent (CD_3OD, δ 49.15). Data are reported in the following format: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet), coupling constant, and integration.

tert-Butyl(S)-(4-((1-(4-chlorophenyl)-3-(piperazin-1-yl)propyl)-carbamoyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidin-4-yl)-carbamate (2).—To the solution of (*S*)-3-amino-3-(4-chlorophenyl)-propan-1-ol (**44**, 1 g, 5.4 mmol) in DCM was added di-*tert*-butyl dicarbonate (1.42 g, 6.5 mmol). After the reaction was stirred for 2 h at rt, the solvent was removed. The resulting residue was purified by flash chromatography (MeOH/DCM = 1/9) to provide *tert*-butyl (*S*)-[1-(4-chlorophenyl)-3-hydroxypropyl]carbamate as a white solid (1.31 g, yield 85%). ESI-MS (*m/z*) $[M + H]^+$: 286.2; To a solution of triphenylphosphine (1.84 g, 7 mmol) and iodine (1.80 g, 7 mmol) in DCM (50 mL) was added imidazole (0.89 g, 14 mmol). The reaction was stirred at rt for 30 min before *tert*-butyl (*S*)-[1-(4-chlorophenyl)-3-hydroxypropyl]carbamate (1.0 g, 3.5 mmol) in 30 mL of DCM was added. After the resulting mixture was stirred for 3 h, saturated $NaHCO_3$ and 10% of $Na_2S_2O_3$ aqueous solution were added. The mixture was extracted with DCM (20 mL × 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated. The resulting residue was purified by silica gel flash chromatography (Hexane/EtOAc = 1/1) to provide compound **45** as a white solid (2.3 g, yield 65%). ^1H NMR (600 MHz, $CDCl_3$): δ 7.34−7.30 (m, 2H), 7.22 (d, J = 8.2 Hz, 2H), 4.85−4.61 (m, 1H), 3.11 (d, J = 8.5 Hz, 1H), 3.01 (ddd, J = 10.0, 8.0, 6.8 Hz, 1H), 2.37−2.14 (m, 2H), 1.41 (s, 9H); ESI-MS (*m/z*): $[M + H]^+$ 396.1.

To a solution of compound **45** (1.48 g, 3.7 mmol) in CH_3CN (30 mL) were added K_2CO_3 (2.1 g, 14.8 mmol) and benzyl piperazine-1-carboxylate (**46**, 1.65 g, 7.4 mmol). After stirring at 80 °C overnight, the mixture was purified by silica gel flash chromatography (Hexane/EtOAc = 1/1) to provide an intermediate. The obtained intermediate was dissolved in DCM (20 mL) and TFA (20 mL). After the mixture was stirred for 30 min, solvents were evaporated to afford (*S*)-4-[3-amino-3-(4-chlorophenyl)propyl]piperazine-1-carboxylate **47** as a white foam solid (1.35 g, yield 95%). ESI-MS (*m/z*): $[M + H]^+$ 388.3.

To a solution of benzyl (*S*)-4-[3-amino-3-(4-chlorophenyl)propyl]-piperazine-1-carboxylate (**47**, 1.3 g, 3.4 mmol) and 4-[(*tert*-butoxycarbonyl)amino]-1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)-piperidine-4-carboxylic acid[17] (**48**, 1.2 g, 3.4 mmol) in DMSO (15 mL) were added EDCI (0.98 g, 5.1 mmol), HOAt (0.69 g, 5.1 mmol), and NMM (1.0 g, 10.2 mmol). After stirring overnight at rt, the resulting mixture was purified by reverse-phase ISCO (10–100% methanol/0.1% TFA in H_2O) to afford a white solid (1.71 g, 69% yield). This product was dissolved in methanol (30 mL) and 10% palladium on carbon was added. After this reaction was stirred under H_2 for 4 h, it was filtered through celite. The filtrate was concentrated, and

the resulting mixture was purified by a reverse-phase column (0–100% methanol/0.1% TFA in H_2O) to afford the title compound **2** as a white solid (1.1 g, yield 78%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.43 (d, J = 8.3 Hz, 1H), 8.31 (s, 1H), 7.42−7.33 (m, 4H), 6.95 (d, J = 3.7 Hz, 1H), 5.09 (q, J = 7.2 Hz, 1H), 4.46−4.29 (m, 2H), 3.93−3.80 (m, 2H), 3.57 (t, J = 5.3 Hz, 4H), 3.52−3.38 (m, 4H), 3.24−3.09 (m, 2H), 2.42−2.14 (m, 6H), 1.45 (s, 9H); ^{13}C NMR (151 MHz, CD_3OD): δ 174.66, 155.30, 142.13, 139.81, 133.10, 128.62, 128.39 (2C), 128.19 (2C), 128.14, 123.58, 103.83, 101.96, 79.76, 56.68 (2C), 54.29 (2C), 50.34, 48.73 (2C), 43.01, 41.22 (2C), 31.55, 29.71, 27.34 (3C); HRMS (m/z): for $C_{30}H_{42}ClN_8O_3^+$ [M + H]$^+$, calcd 597.3063; found, 597.3079.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)acetyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (3).—To a solution of intermediate **2** (15.9 mg, 0.022 mmol) in DMSO (1 mL) were added intermediate **49** (12.2 mg, 0.022 mmol, 1.0 equiv), EDCI (6.5 mg, 0.033 mmol, 1.5 equiv), HOAt (4.5 mg, 0.033 mmol, 1.5 equiv), and NMM (6.7 mg, 0.066 mmol, 3.0 equiv). After stirring overnight at rt, the resulting mixture was purified by preparative HPLC (10%−100% methanol/0.1% TFA in H_2O) to afford a white solid (18.2 mg, yield 91%). The solid was dissolved in DCM (1 mL) and TFA (1 mL) and the reaction was stirred at rt for 30 min. Then, the solvent was evaporated, and the resulting mixture was purified by preparative HPLC (10%–100% methanol/0.1% TFA in H_2O) to afford compound **3** as a white solid (19.4 mg, yield 86%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.98 (s, 1H), 8.39 (s, 1H), 7.47−7.43 (m, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 3.7 Hz, 1H), 7.37−7.31 (m, 4H), 6.94 (d, J = 3.7 Hz, 1H), 5.00 (dd, J = 9.3, 5.8 Hz, 1H), 4.67 (s, 1H), 4.66−4.61 (m, 2H), 4.58−4.53 (m, 1H), 4.53−4.46 (m, 2H), 4.42−4.34 (m, 4H), 4.17−4.03 (m, 3H), 3.92−3.78 (m, 5H), 3.27 (dd, J = 12.2, 4.4 Hz, 2H), 3.14 (td, J = 12.1, 5.3 Hz, 2H), 2.72−2.58 (m, 3H), 2.47 (s, 3H), 2.39−2.14 (m, 5H), 2.12−2.00 (m, 3H), 1.04 (s, 9H). HRMS (m/z): for $C_{51}H_{66}ClN_{12}O_7S^+$ [M + H]$^+$, calcd 1025.4581; found, 1025.4590.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(3-(3-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)-propanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (4).—Compound **4** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **50** (12.7 mg, 0.022 mmol, 1.0 equiv). Compound **4** was obtained as a white solid (11.9 mg, yield 52%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.95 (s, 1H), 8.39 (s, 1H), 7.47−7.30 (m, 9H), 6.94 (d, J = 3.8 Hz, 1H), 5.01 (dd, J = 9.2, 5.9 Hz, 1H), 4.70−4.60 (m, 4H), 4.53 (dd, J = 9.4, 7.5 Hz, 1H), 4.49−4.36 (m, 4H), 3.90−3.76 (m, 5H), 3.75−3.66 (m, 5H), 3.26 (dd, J = 12.2, 4.5 Hz, 2H), 3.18−3.09 (m, 2H), 2.71−2.57 (m, 4H), 2.50 (t, J = 5.9 Hz, 2H), 2.47 (s, 3H), 2.39−2.13 (m, 5H), 2.11−1.98 (m, 3H), 1.02 (s, 9H). HRMS (m/z): for $C_{55}H_{70}ClN_{12}O_7S^+$ [M + H]$^+$, calcd 1053.4894; found, 1053.4895.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(2-(2-(2-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-

dimethyl-1-oxobutan-2-yl)amino)-2-oxoethoxy)ethoxy)acetyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (5).—Compound **5** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **51** (13 mg, 0.022 mmol, 1.0 equiv). Compound **5** was obtained as a white solid (21.1 mg, yield 89%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.00 (s, 1H), 8.40 (d, J = 2.8 Hz, 1H), 7.48−7.29 (m, 9H), 6.94 (d, J = 3.6 Hz, 1H), 5.01 (dd, J = 9.2, 5.8 Hz, 1H), 4.69 (d, J = 2.7 Hz, 1H), 4.66−4.61 (m, 2H), 4.56−4.51 (m, 1H), 4.47 (d, J = 17.9 Hz, 2H), 4.41−4.23 (m, 4H), 4.04 (t, J = 2.9 Hz, 2H), 3.90−3.78 (m, 5H), 3.75−3.68 (m, 5H), 3.26 (dd, J = 12.1, 4.3 Hz, 2H), 3.19−3.10 (m, 2H), 2.72−2.57 (m, 3H), 2.48 (s, 3H), 2.39−2.14 (m, 5H), 2.12−1.97 (m, 3H), 1.02 (s, 9H). HRMS (m/z): for $C_{53}H_{70}ClN_{12}O_8S^+$ $[M + H]^+$, calcd 1069.4843; found, 1069.4850.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(3-(2-(3-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)ethoxy)propanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (6).—Compound **6** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **52** (13.6 mg, 0.022 mmol, 1.0 equiv). Compound **6** was obtained as a white solid (18.3 mg, yield 76%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.00 (s, 1H), 8.39 (s, 1H), 7.49−7.31 (m, 9H), 6.94 (d, J = 3.7 Hz, 1H), 5.02 (dd, J = 9.2, 5.8 Hz, 1H), 4.64 (s, 4H), 4.59−4.45 (m, 4H), 4.36 (d, J = 15.5 Hz, 1H), 3.91−3.77 (m, 5H), 3.75−3.66 (m, 5H), 3.59−3.55 (m, 4H), 3.26 (dd, J = 12.3, 4.5 Hz, 2H), 3.13 (td, J = 12.1, 5.2 Hz, 2H), 2.74−2.58 (m, 5H), 2.54 (dt, J = 15.0, 5.9 Hz, 1H), 2.49−2.42 (m, 4H), 2.40−2.15 (m, 5H), 2.11−2.01 (m, 2H), 1.03 (s, 9H). HRMS (m/z): for $C_{55}H_{74}ClN_{12}O_8S^+$ $[M + H]^+$, calcd 1097.5156; found, 1097.5160.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-((S)-13-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbony l) - 14, 14 - dimethyl - 11 - oxo - 3, 6, 9 - trioxa - 12 - azapentadecanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (7).—Compound **7** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **53** (14 mg, 0.022 mmol, 1.0 equiv). Compound **7** was obtained as a white solid (19.2 mg, yield 78%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.01 (d, J = 3.0 Hz, 1H), 8.39 (d, J = 3.0 Hz, 1H), 7.48−7.30 (m, 9H), 6.95 (d, J = 3.7 Hz, 1H), 5.01 (dd, J = 9.2, 5.9 Hz, 1H), 4.66 (brs, 4H), 4.57−4.48 (m, 3H), 4.37 (d, J = 15.5 Hz, 1H), 4.30−4.17 (m, 2H), 4.04 (s, 2H), 3.93−3.77 (m, 5H), 3.72−3.62 (m, 9H), 3.26 (dd, J = 12.2, 4.5 Hz, 2H), 3.12 (td, J = 12.1, 5.2 Hz, 2H), 2.73−2.57 (m, 3H), 2.48 (d, J = 2.9 Hz, 3H), 2.39−2.15 (m, 5H), 2.13−1.98 (m, 3H), 1.03 (s, 9H). HRMS (m/z): for $C_{55}H_{74}ClN_{12}O_9S^+$ $[M + H]^+$, calcd 1113.5105; found, 1113.5120.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-((S)-15-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-16, 16-dimethyl −13-oxo - 4, 7, 10- trioxa - 14-azaheptadecanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (8).—Compound **8** was synthesized following the standard procedure for preparing compound **3** from

intermediates **2** (15.9 mg, 0.022 mmol) and **54** (14 mg, 0.022 mmol, 1.0 equiv). Compound **8** was obtained as a white solid (13.2 mg, yield 53%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.99 (s, 1H), 8.40 (s, 1H), 7.51−7.28 (m, 9H), 6.94 (d, J = 3.6 Hz, 1H), 5.01 (dd, J = 8.9, 6.1 Hz, 1H), 4.74−4.61 (m, 4H), 4.60−4.46 (m, 4H), 4.37 (d, J = 15.5 Hz, 1H), 3.91−3.75 (m, 5H), 3.73−3.67 (m, 5H), 3.60−3.54 (m, 8H), 3.25 (dd, J = 12.2, 4.5 Hz, 2H), 3.13−3.08 (m, 2H), 2.73−2.52 (m, 6H), 2.48 (d, J = 7.7 Hz, 4H), 2.41−2.14 (m, 5H), 2.12−2.00 (m, 2H), 1.03 (s, 9H). HRMS (m/z): for $C_{57}H_{78}ClN_{12}O_9S^+$ $[M + H]^+$, calcd 1141.5418; found, 1141.5438.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-((S)-18-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-19,19-dimethyl-16-oxo-4,7,10,13-tetraoxa-17-azaicosanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (9).—Compound **9** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **55** (15.6 mg, 0.022 mmol, 1.0 equiv). Compound **9** was obtained as a white solid (14.0 mg, yield 54%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.95 (s, 1H), 8.39 (s, 1H), 7.51−7.26 (m, 9H), 6.93 (d, J = 3.7 Hz, 1H), 5.00 (dd, J = 8.8, 6.3 Hz, 1H), 4.70−4.60 (m, 4H), 4.60−4.45 (m, 4H), 4.36 (d, J = 15.5 Hz, 1H), 3.90−3.76 (m, 5H), 3.75−3.66 (m, 5H), 3.62−3.51 (m, 12H), 3.26 (td, J = 12.4, 4.7 Hz, 2H), 3.10 (td, J = 12.1, 5.3 Hz, 2H), 2.72−2.55 (m, 6H), 2.51−2.42 (m, 4H), 2.39−2.14 (m, 5H), 2.11−1.99 (m, 2H), 1.02 (s, 9H). HRMS (m/z): for $C_{59}H_{82}ClN_{12}O_{10}S^+$ $[M + H]^+$, calcd 1185.5681; found, 1185.5665.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-((S)-19-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-20,20-dimethyl-17-oxo-3,6,9,12,15-pentaoxa-18-azahenicosanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (10).—Compound **10** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **56** (15.9 mg, 0.022 mmol, 1.0 equiv). Compound **10** was obtained as a white solid (16.3 mg, yield 62%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.96 (s, 1H), 8.39 (s, 1H), 7.55−7.17 (m, 9H), 6.93 (d, J = 3.7 Hz, 1H), 5.01 (t, J = 7.7 Hz, 1H), 4.68−4.46 (m, 8H), 4.36 (d, J = 15.5 Hz, 2H), 4.16−4.02 (m, 3H), 3.93−3.82 (m, 4H), 3.78 (dd, J = 10.9, 3.8 Hz, 1H), 3.68−3.54 (m, 16H), 3.29−3.20 (m, 2H), 3.17−3.09 (m, 2H), 2.74−2.57 (m, 3H), 2.47 (s, 3H), 2.41−2.13 (m, 5H), 2.12−1.99 (m, 3H), 1.04 (s, 9H). HRMS (m/z): for $C_{59}H_{82}ClN_{12}O_{11}S^+$ $[M + H]^+$, calcd 1201.5630; found, 1201.5651.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-((S)-21-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-22,22-dimethyl-19-oxo-4,7,10,13,16-pentaoxa-20-azatricosanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (11).—Compound **11** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **57** (16.5 mg, 0.022 mmol, 1.0 equiv). Compound **11** was obtained as a white solid (17.3 mg, yield 64%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.00 (s, 1H), 8.40 (s, 1H), 7.53−7.28 (m, 9H), 6.94 (d, J = 3.7 Hz, 1H), 5.00 (dd, J = 8.9, 6.3 Hz, 1H), 4.70−4.60 (m, 4H), 4.60−4.46 (m, 4H), 4.36 (d, J = 15.5 Hz, 1H), 3.91−3.76 (m, 5H), 3.76−3.67

(m, 5H), 3.65−3.49 (m, 16H), 3.26 (dd, J = 12.3, 4.5 Hz, 2H), 3.11 (td, J = 12.2, 5.4 Hz, 2H), 2.72−2.54 (m, 6H), 2.52−2.43 (m, 4H), 2.40−2.13 (m, 5H), 2.12−1.99 (m, 2H), 1.02 (s, 9H). HRMS (m/z): for $C_{61}H_{86}ClN_{12}O_{11}S^+$ $[M + H]^+$, calcd 1229.5943; found, 1229.5950.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(4-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-oxobutanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (12).—Compound **12** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **58** (11.7 mg, 0.022 mmol, 1.0 equiv). Compound **12** was obtained as a white solid (19.9 mg, yield 90%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.99 (s, 1H), 8.40 (s, 1H), 7.52−7.25 (m, 9H), 6.95 (d, J = 3.8 Hz, 1H), 5.01 (dd, J = 9.2, 5.9 Hz, 1H), 4.71−4.60 (m, 3H), 4.60−4.45 (m, 5H), 4.37 (d, J = 15.6 Hz, 1H), 3.92−3.74 (m, 5H), 3.27 (dd, J = 12.3, 4.4 Hz, 2H), 3.13 (td, J = 12.2, 5.2 Hz, 2H), 2.74−2.52 (m, 7H), 2.48 (s, 3H), 2.41−2.13 (m, 5H), 2.13−1.98 (m, 3H), 1.02 (s, 9H). HRMS (m/z): for $C_{51}H_{66}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1009.4632; found, 1009.4638.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(5-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-5-oxopentanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (13).—Compound **13** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **59** (12 mg, 0.022 mmol, 1.0 equiv). Compound **13** was obtained as a white solid (13.9 mg, yield 62%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.00 (s, 1H), 8.40 (s, 1H), 7.51−7.26 (m, 9H), 6.95 (dd, J = 3.7, 1.7 Hz, 1H), 5.00 (dd, J = 9.2, 5.9 Hz, 1H), 4.70−4.61 (m, 3H), 4.60−4.45 (m, 5H), 4.37 (d, J = 15.4 Hz, 1H), 3.94−3.75 (m, 5H), 3.26 (dd, J = 12.2, 4.3 Hz, 2H), 3.12 (td, J = 12.1, 5.2 Hz, 2H), 2.72−2.57 (m, 3H), 2.48 (d, J = 1.5 Hz, 3H), 2.45−2.38 (m, 2H), 2.38−2.12 (m, 8H), 2.12−1.98 (m, 2H), 1.88 (p, J = 7.3 Hz, 2H), 1.03 (d, J = 1.7 Hz, 9H). HRMS (m/z): for $C_{52}H_{68}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1023.4789; found, 1023.4794.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(6-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (14).—Compound **14** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **60** (12.3 mg, 0.022 mmol, 1.0 equiv). Compound **14** was obtained as a white solid (14 mg, yield 61%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.00 (s, 1H), 8.39 (s, 1H), 7.53−7.22 (m, 9H), 6.95 (d, J = 3.7 Hz, 1H), 5.00 (dd, J = 9.2, 5.9 Hz, 1H), 4.69−4.59 (m, 4H), 4.59−4.44 (m, 4H), 4.36 (d, J = 15.5 Hz, 1H), 3.91−3.76 (m, 5H), 3.26 (dd, J = 12.3, 4.4 Hz, 2H), 3.13 (td, J = 12.1, 5.2 Hz, 2H), 2.73−2.57 (m, 3H), 2.48 (s, 3H), 2.43 (td, J = 7.1, 4.8 Hz, 2H), 2.40−2.13 (m, 8H), 2.11−2.01 (m, 2H), 1.68−1.57 (m, 4H), 1.03 (s, 9H). HRMS (m/z): for $C_{53}H_{70}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1037.4945; found, 1037.4923.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(7-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-7-oxoheptanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (15).—Compound **15** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **61** (12.6 mg, 0.022 mmol, 1.0 equiv). Compound **15** was obtained as a white solid (16.3 mg, yield 70%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.04 (s, 1H), 8.40 (s, 1H), 7.55−7.25 (m, 9H), 6.95 (d, J = 3.7 Hz, 1H), 5.01 (dd, J = 9.3, 5.8 Hz, 1H), 4.64 (d, J = 18.7 Hz, 4H), 4.58−4.45 (m, 4H), 4.37 (d, J = 15.5 Hz, 1H), 3.93−3.77 (m, 5H), 3.27 (dd, J = 12.3, 4.5 Hz, 2H), 3.14 (td, J = 12.2, 5.2 Hz, 2H), 2.74−2.55 (m, 3H), 2.48 (s, 3H), 2.41 (t, J = 7.5 Hz, 2H), 2.39−2.14 (m, 8H), 2.12−2.00 (m, 2H), 1.66−1.56 (m, 4H), 1.41−1.29 (m, 2H), 1.02 (s, 9H). HRMS (m/z): for $C_{54}H_{72}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1051.5102; found, 1051.5094.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(8-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-8-oxooctanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (16).—Compound **16** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **62** (12.9 mg, 0.022 mmol, 1.0 equiv). Compound **16** was obtained as a white solid (11.9 mg, yield 51%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.97 (s, 1H), 8.39 (s, 1H), 7.53−7.19 (m, 9H), 6.94 (d, J = 3.8 Hz, 1H), 5.01 (dd, J = 9.2, 5.9 Hz, 1H), 4.71−4.61 (m, 4H), 4.59−4.45 (m, 4H), 4.36 (d, J = 15.4 Hz, 1H), 3.92−3.76 (m, 5H), 3.26 (dd, J = 12.3, 4.5 Hz, 2H), 3.13 (td, J = 12.2, 5.3 Hz, 2H), 2.73−2.55 (m, 3H), 2.48 (s, 3H), 2.40 (t, J = 7.6 Hz, 3H), 2.37−2.14 (m, 7H), 2.11−2.00 (m, 2H), 1.64−1.55 (m, 4H), 1.35 (dd, J = 7.5, 4.0 Hz, 4H), 1.03 (s, 9H). HRMS (m/z): for $C_{55}H_{74}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1065.5258; found, 1065.5272.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(9-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-9-oxononanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (17).—Compound **17** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **63** (13.2 mg, 0.022 mmol, 1.0 equiv). Compound **17** was obtained as a white solid (10.4 mg, yield 44%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.98 (s, 1H), 8.40 (d, J = 1.9 Hz, 1H), 7.56−7.25 (m, 9H), 6.95 (dd, J = 3.7, 1.9 Hz, 1H), 5.01 (dd, J = 9.3, 5.8 Hz, 1H), 4.70−4.61 (m, 4H), 4.59−4.47 (m, 4H), 4.36 (d, J = 15.5 Hz, 1H), 3.92−3.78 (m, 5H), 3.26 (dd, J = 12.3, 4.3 Hz, 2H), 3.13 (td, J = 12.0, 5.0 Hz, 2H), 2.73−2.58 (m, 3H), 2.48 (s, 3H), 2.43−2.37 (m, 3H), 2.37−2.14 (m, 7H), 2.11−2.00 (m, 2H), 1.65−1.54 (m, 4H), 1.34 (s, 6H), 1.03 (d, J = 1.9 Hz, 9H). HRMS (m/z): for $C_{56}H_{76}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1079.5415; found, 1079.5414.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(10-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-10-oxodecanoyl)-piperazin-1-yl)propyl)-1-(7H-

pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (18).—Compound **18** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **64** (13.5 mg, 0.022 mmol, 1.0 equiv). Compound **18** was obtained as a white solid (13.6 mg, yield 60%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.00 (s, 1H), 8.40 (s, 1H), 7.53−7.22 (m, 9H), 6.95 (d, J = 3.8 Hz, 1H), 5.01 (dd, J = 9.3, 5.8 Hz, 1H), 4.71−4.61 (m, 4H), 4.59−4.46 (m, 4H), 4.36 (d, J = 15.5 Hz, 1H), 3.93−3.76 (m, 5H), 3.26 (dd, J = 12.3, 4.4 Hz, 2H), 3.13 (td, J = 12.2, 5.1 Hz, 2H), 2.73−2.57 (m, 3H), 2.48 (s, 3H), 2.40 (t, J = 7.6 Hz, 3H), 2.37−2.14 (m, 7H), 2.11–2.00 (m, 2H), 1.63−1.53 (m, 4H), 1.32 (s, 8H), 1.03 (s, 9H). HRMS (m/z): for $C_{57}H_{78}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1093.5571; found, 1093.5561.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(11-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-11-oxoundecanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (19).—Compound **19** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **65** (13.5 mg, 0.022 mmol, 1.0 equiv). Compound **19** was obtained as a white solid (16.0 mg, yield 66%). ^{1}H NMR (800 MHz, CD_3OD): δ 8.96 (s, 1H), 8.41 (s, 1H), 7.51−7.35 (m, 9H), 6.97 (d, J = 3.8 Hz, 1H), 5.05 (dd, J = 9.6, 5.7 Hz, 1H), 4.65 (d, J = 11.3 Hz, 3H), 4.62−4.50 (m, 3H), 4.39 (d, J = 15.4 Hz, 1H), 3.95−3.85 (m, 3H), 3.83 (dd, J = 10.9, 3.9 Hz, 1H), 3.31 (dt, J = 12.0, 6.3 Hz, 1H), 3.18 (td, J = 12.2, 5.1 Hz, 1H), 2.77−2.70 (m, 1H), 2.68 (s, 1H), 2.50 (s, 3H), 2.45−2.37 (m, 4H), 2.35−2.19 (m, 6H), 2.13−2.06 (m, 2H), 1.67−1.54 (m, 6H), 1.34 (d, J = 8.5 Hz, 14H), 1.06 (s, 9H). HRMS (m/z): for $C_{58}H_{80}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1107.5728; found, 1107.5751.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(12-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (20).—Compound **20** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **66** (12.8 mg, 0.02 mmol, 1.0 equiv). Compound **20** was obtained as a white solid (13.5 mg, yield 60%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.03 (s, 1H), 8.40 (s, 1H), 7.53−7.27 (m, 9H), 6.95 (d, J = 3.7 Hz, 1H), 5.03 (dd, J = 9.5, 5.7 Hz, 1H), 4.69−4.45 (m, 6H), 4.37 (d, J = 15.4 Hz, 1H), 3.99−3.85 (m, 3H), 3.81 (dd, J = 11.0, 3.9 Hz, 1H), 3.69−3.38 (m, 8H), 3.36−3.23 (m, 3H), 3.23−3.10 (m, 1H), 2.78−2.55 (m, 2H), 2.49 (s, 3H), 2.43−2.14 (m, 6H), 2.14−2.00 (m, 2H), 1.71−1.47 (m, 4H), 1.43−1.18 (m, 12H), 1.04 (s, 9H). ^{13}C NMR (151 MHz, CD_3OD): δ 174.66, 173.08, 172.75, 170.97, 169.25, 154.17, 151.87, 146.99, 142.67, 139.59, 139.10, 133.39, 129.73, 129.09, 128.95 (2C), 128.56 (2C), 128.06 (2C), 128.02, 127.61 (2C), 123.61, 103.43, 102.33, 69.68, 59.44, 58.13, 57.58, 56.60, 53.97, 51.61, 51.50, 51.38, 42.29, 42.17, 41.89, 38.20, 37.53, 35.27, 35.17, 32.17, 30.68, 30.60, 29.24, 29.18, 29.13 (2C), 29.06, 29.00, 28.94, 28.90, 25.64 (3C), 25.60, 24.74, 14.14. HRMS (m/z): for $C_{59}H_{82}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1121.5884; found, 1121.5868.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(13-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-13-oxotridecanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (21).—Compound **21** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **67** (13.1 mg, 0.02 mmol, 1.0 equiv). Compound **21** was obtained as a white solid (15 mg, yield 66%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.06−8.86 (m, 1H), 8.41−8.34 (m, 1H), 7.57−7.27 (m, 9H), 6.94 (q, J = 3.9 Hz, 1H), 5.00 (td, J = 8.9, 5.1 Hz, 1H), 4.71−4.64 (m, 3H), 4.63−4.46 (m, 3H), 4.35 (dd, J = 15.5, 7.0 Hz, 1H), 3.95−3.76 (m, 4H), 3.61−2.89 (m, 10H), 2.65−1.95 (m, 15H), 1.58 (dh, J = 15.0, 7.3 Hz, 4H), 1.30 (dd, J = 13.9, 6.9 Hz, 14H), 1.09−0.89 (m, 9H). HRMS (m/z): for $C_{60}H_{84}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1135.6041; found, 1135.6034.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(14-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-14-oxotetradecanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (22).—Compound **22** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **68** (13.4 mg, 0.02 mmol, 1.0 equiv). Compound **22** was obtained as a white solid (20.2 mg, yield 88%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.04 (s, 1H), 8.40 (s, 1H), 7.55−7.24 (m, 9H), 7.07−6.84 (m, 1H), 5.01 (dd, J = 9.3, 5.8 Hz, 1H), 4.71−4.63 (m, 3H), 4.63−4.43 (m, 3H), 4.36 (d, J = 15.5 Hz, 1H), 3.95−3.77 (m, 4H), 3.61−2.95 (m, 10H), 2.68−2.61 (m, 2H), 2.49 (s, 3H), 2.45−2.14 (m, 8H), 2.12−1.97 (m, 2H), 1.63−1.53 (m, 4H), 1.39−1.19 (m, 16H), 1.03 (s, 9H). HRMS (m/z): for $C_{61}H_{86}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1149.6197; found, 1149.6176.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(11-(((S)-1-((2S,4R)-4-hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-11-oxoundecanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (23).—Compound **23** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (23.5 mg, 0.022 mmol) and **69** (21.2 mg, 0.022 mmol, 1.0 equiv). Compound **23** was obtained as a white solid (22.4 mg, yield 61%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.95 (d, J = 6.3 Hz, 1H), 8.39 (s, 1H), 7.47−7.40 (m, 4H), 7.40−7.30 (m, 5H), 6.94 (d, J = 3.8 Hz, 1H), 5.08−4.95 (m, 1H), 4.69−4.63 (m, 3H), 4.61−4.52 (m, 1H), 4.46−4.35 (m, 1H), 3.98−3.81 (m, 4H), 3.74 (dd, J = 11.0, 4.0 Hz, 1H), 3.64−2.91 (m, 10H), 2.73−2.67 (m, 1H), 2.66−2.60 (m, 1H), 2.48 (s, 3H), 2.44−2.13 (m, 8H), 2.07−2.01 (m, 1H), 1.98−1.93 (m, 1H), 1.66−1.53 (m, 4H), 1.50 (d, J = 7.0 Hz, 3H), 1.41−1.23 (m, 10H), 1.04 (s, 9H). HRMS (m/z): for $C_{59}H_{82}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1121.5884; found, 1121.5869.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)glycyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (24).—Compound **24** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **70** (7.3 mg, 0.022 mmol, 1.0 equiv).

Compound **24** was obtained as a yellow solid (2.9 mg, yield 16%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.36 (s, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.44 (dd, J = 8.5, 7.0 Hz, 1H), 7.36 (dd, J = 12.3, 2.7 Hz, 3H), 7.10 (d, J = 7.2 Hz, 1H), 7.04 (d, J = 7.0 Hz, 1H), 6.99 (dd, J = 8.4, 3.6 Hz, 1H), 6.89 (d, J = 3.7 Hz, 1H), 5.10−4.99 (m, 2H), 4.75−4.64 (m, 2H), 4.24 (s, 2H), 3.76 (q, J = 12.1 Hz, 4H), 3.44−3.32 (m, 2H), 3.28−3.22 (m, 2H), 3.12 (td, J = 12.2, 5.2 Hz, 2H), 2.90−2.80 (m, 2H), 2.78−2.51 (m, 5H), 2.40−2.24 (m, 2H), 2.17−2.06 (m, 2H), 2.04−1.96 (m, 1H). HRMS (*m*/*z*): for $C_{40}H_{45}ClN_{11}O_6$ $^+$[M + H]$^+$, calcd 810.3237; found, 810.3246.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)propanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (25).—Compound **25** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **71** (7.6 mg, 0.022 mmol, 1.0 equiv).
Compound **25** was obtained as a yellow solid (16.2 mg, yield 89%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.38 (s, 1H), 7.56 (dd, J = 8.5, 7.2 Hz, 1H), 7.39−7.31 (m, 5H), 7.11 (d, J = 8.6 Hz, 1H), 7.05 (d, J = 7.1 Hz, 1H), 6.93 (d, J = 3.7 Hz, 1H), 5.07−4.97 (m, 2H), 4.64 (s, 2H), 3.84 (q, J = 12.2 Hz, 4H), 3.68−3.63 (m, 4H), 3.26−3.15 (m, 2H), 3.08 (td, J = 11.9, 4.9 Hz, 2H), 2.90−2.79 (m, 2H), 2.77−2.71 (m, 3H), 2.71−2.58 (m, 3H), 2.37−1.98 (m, 6H). HRMS (*m*/*z*): for $C_{41}H_{47}ClN_{11}O_6^+$ [M + H]$^+$, calcd 824.3394; found, 824.3396.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoyl)piperazin-1-yl)-propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (26).—Compound **26** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **72** (7.9 mg, 0.022 mmol, 1.0 equiv).
Compound **26** was obtained as a yellow solid (12.5 mg, yield 68%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.54 (dd, J = 8.5, 7.2 Hz, 1H), 7.37 (dd, J = 17.5, 3.5 Hz, 5H), 7.10 (d, J = 8.6 Hz, 1H), 7.04 (d, J = 7.1 Hz, 1H), 6.94 (d, J = 3.7 Hz, 1H), 5.05 (dd, J = 12.8, 5.5 Hz, 1H), 5.00 (dd, J = 9.2, 5.9 Hz, 1H), 4.70−4.60 (m, 2H), 3.84 (dd, J = 13.8, 10.5 Hz, 4H), 3.39 (t, J = 6.7 Hz, 4H), 3.23 (t, J = 12.2 Hz, 2H), 3.11−3.05 (m, J2H), 2.93−2.79 (m, 2H), 2.78−2.57 (m, 5H), 2.53 (t, J = 6.9 Hz, 2H), 2.34 (d, J = 11.8 Hz, 1H), 2.26 (dd, J = 12.3, 6.2 Hz, 1H), 2.17 (d, J = 14.9 Hz, 1H), 2.12−2.08 (m, 1H), 2.04 (d, J = 14.7 Hz, 1H), 1.99−1.94 (m, 2H). HRMS (*m*/*z*): for $C_{42}H_{49}ClN_{11}O_6^+$[M + H]$^+$, calcd 838.3550; found, 838.3552.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)pentanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (27).—Compound **27** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **73** (8.2 mg, 0.022 mmol, 1.0 equiv).
Compound **27** was obtained as a yellow solid (9.6 mg, yield 51%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.38 (s, 1H), 7.54 (dd, J = 8.4, 7.2 Hz, 1H), 7.36 (dd, J = 6.5, 3.5 Hz, 5H), 7.07−7.00 (m, 2H), 6.91 (d, J = 3.7 Hz, 1H), 5.07−5.03 (m, 1H), 5.00 (dd, J = 9.2, 5.9 Hz, 1H), 4.71−4.61 (m, 2H), 3.82 (q, J = 12.0 Hz, 4H), 3.36 (d, J = 5.8 Hz, 4H), 3.28−3.20 (m, 2H), 3.14−3.09 (m, 2H), 2.88−2.82 (m, 2H), 2.78−2.55 (m, 5H), 2.48 (s, 2H), 2.38−2.29

(m, 1H), 2.28−2.23 (m, 1H), 2.19−2.06 (m, 2H), 2.02 (d, J = 14.6 Hz, 1H), 1.75−1.67 (m, 4H). HRMS (m/z): for $C_{43}H_{51}ClN_{11}O_6^+$ $[M + H]^+$, calcd 852.3707; found, 852.3710.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexanoyl)piperazin-1-yl)-propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (28).—Compound **28** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **74** (8.5 mg, 0.022 mmol, 1.0 equiv). Compound **28** was obtained as a yellow solid (13.9 mg, yield 73%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.59−7.48 (m, 1H), 7.43−7.24 (m, 5H), 7.03 (dd, J = 7.8, 5.8 Hz, 2H), 6.93 (d, J = 3.7 Hz, 1H), 5.09−4.96 (m, 2H), 4.70−4.57 (m, 2H), 3.85 (q, J = 12.1 Hz, 4H), 3.33 (t, J = 6.8 Hz, 4H), 3.29−3.22 (m, 2H), 3.14−3.08 (m, 2H), 2.92−2.79 (m, 2H), 2.78−2.57 (m, 5H), 2.43 (t, J = 7.4 Hz, 2H), 2.38−2.30 (m, 1H), 2.28−2.20 (m, 1H), 2.17 (d, J = 14.7 Hz, 1H), 2.13−2.07 (m, 1H), 2.07−1.99 (m, 1H), 1.73−1.61 (m, 4H), 1.48−1.44 (m, 2H). HRMS (m/z): for $C_{44}H_{53}ClN_{11}O_6^+$ $[M + H]^+$, calcd 866.3863; found, 866.3859.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)heptanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (29).—Compound **29** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **75** (8.9 mg, 0.022 mmol, 1.0 equiv). Compound **29** was obtained as a yellow solid (14.5 mg, yield 75%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.54 (dd, J = 8.6, 7.1 Hz, 1H), 7.40−7.30 (m, 5H), 7.03 (t, J = 7.3 Hz, 2H), 6.94 (d, J = 3.7 Hz, 1H), 5.08−4.96 (m, 2H), 4.65 (d, J = 13.8 Hz, 2H), 3.85 (q, J = 12.1 Hz, 4H), 3.36−3.30 (m, 4H), 3.29−3.25 (m, 2H), 3.15−3.10 (m, 2H), 2.91−2.78 (m, 2H), 2.77−2.59 (m, 5H), 2.41 (d, J = 1.6 Hz, 2H), 2.37−2.31 (m, 1H), 2.26 (d, 1H), 2.17 (d, J = 15.0 Hz, 1H), 2.14−2.07 (m, 1H), 2.07−1.98 (m, 1H), 1.68−1.63 (m, 4H), 1.49−1.35 (m, 4H). HRMS (m/z): for $C_{45}H_{55}ClN_{11}O_6^+$ $[M + H]^+$, calcd 880.4020; found, 880.4026.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)octanoyl)piperazin-1-yl)-propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (30).—Compound **30** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **76** (9.1 mg, 0.022 mmol, 1.0 equiv). Compound **30** was obtained as a yellow solid (15.5 mg, yield 78%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.54 (dd, J = 8.6, 7.1 Hz, 1H), 7.39−7.32 (m, 5H), 7.03 (dd, J = 7.8, 4.3 Hz, 2H), 6.94 (d, J = 3.7 Hz, 1H), 5.08−4.97 (m, 2H), 4.68−4.62 (m, 2H), 3.84 (q, J = 12.2 Hz, 4H), 3.35−3.31 (m, 4H), 3.28−3.24 (m, 2H), 3.16−3.11 (m, 2H), 2.89−2.81 (m, 2H), 2.78−2.56 (m, 5H), 2.39 (t, J = 7.5 Hz, 2H), 2.34 (t, J = 10.4 Hz, 1H), 2.29−2.22 (m, 1H), 2.17 (d, J = 14.8 Hz, 1H), 2.14−2.10 (m, 1H), 2.03 (d, J = 14.6 Hz, 1H), 1.69−1.64 (m, 2H), 1.62−1.56 (m, 2H), 1.47−1.33 (m, 6H). HRMS (m/z): for $C_{46}H_{57}ClN_{11}O_6^+$ $[M + H]^+$, calcd 894.4176; found, 894.4192.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(3-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)propanoyl)-piperazin-1-yl)propyl)-1-(7H-

pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (31).—Compound **31** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **77** (8.6 mg, 0.022 mmol, 1.0 equiv). Compound **31** was obtained as a yellow solid (16.7 mg, yield 87%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.59−7.52 (m, 1H), 7.37 (dd, J = 3.9, 1.1 Hz, 1H), 7.34−7.28 (m, 4H), 7.10−7.03 (m, 2H), 6.93 (dd, J = 3.7, 1.5 Hz, 1H), 5.06−5.03 (m, 1H), 4.68−4.59 (m, 3H), 3.91−3.74 (m, 6H), 3.72−3.67 (m, 3H), 3.51−3.45 (m, 3H), 3.19−3.11 (m, 2H), 2.98−2.93 (m, 2H), 2.88−2.78 (m, 2H), 2.77−2.54 (m, 6H), 2.32−2.06 (m, 5H), 2.02 (d, J = 14.6 Hz, 1H). HRMS (m/z): for $C_{43}H_{51}ClN_{11}O_7^+$ $[M + H]^+$, calcd 868.3656; found, 868.3661.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(3-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)-propanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (32).— Compound **32** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **78** (9.5 mg, 0.022 mmol, 1.0 equiv). Compound **32** was obtained as a yellow solid (15.8 mg, yield 79%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.38 (s, 1H), 7.54 (m, 1H), 7.37 (dd, J = 3.8, 1.3 Hz, 1H), 7.34−7.28 (m, 4H), 7.08 (d, J = 8.5 Hz, 1H), 7.04 (dd, J = 7.0, 2.7 Hz, 1H), 6.92 (dd, J = 3.8, 1.6 Hz, 1H), 5.08−5.03 (m, 1H), 4.99−4.90 (m, 1H), 4.68−4.54 (m, 3H), 3.89−3.80 (m, 3H), 3.78−3.72 (m, 2H), 3.69−3.66 (m, 2H), 3.65−3.54 (m, 5H), 3.46 (t, J = 5.1 Hz, 3H), 3.28−3.22 (m, 2H), 3.10−3.01 (m, 2H), 2.88−2.81 (m, 2H), 2.77−2.57 (m, 6H), 2.35−2.07 (m, 5H), 2.02 (d, J = 14.7 Hz, 1H). HRMS (m/z): for $C_{45}H_{55}ClN_{11}O_8^+$ $[M + H]^+$, calcd 912.3918; found, 912.3902.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(3-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)-ethoxy)propanoyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (33).—Compound **33** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **79** (10.5 mg, 0.022 mmol, 1.0 equiv). Compound **33** was obtained as a yellow solid (16.2 mg, yield 77%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.38 (s, 1H), 7.55−7.53 (m, 1H), 7.39−7.28 (m, 5H), 7.07 (d, J = 8.6 Hz, 1H), 7.04 (s, 1H), 6.92 (dd, J = 3.7, 1.6 Hz, 1H), 5.07−5.04 (m, 1H), 5.01−4.98 (m, 1H), 4.67−4.53 (m, 3H), 3.91−3.80 (m, 3H), 3.73−3.67 (m, 4H), 3.64−3.53 (m, 8H), 3.53−3.44 (m, 4H), 3.25 (t, J = 12.8 Hz, 2H), 3.11−3.07 (m, 2H), 2.91−2.80 (m, 2H), 2.77−2.57 (m, 7H), 2.37−2.23 (m, 2H), 2.20−2.07 (m, 2H), 2.03 (d, J = 14.7 Hz, 1H). HRMS (m/z): for $C_{47}H_{59}ClN_{11}O_9^+$ $[M + H]^+$, calcd 956.4180; found, 956.4183.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12-tetraoxapentadecan-15-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (34).— Compound **34** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **80** (11.5 mg, 0.022 mmol, 1.0 equiv). Compound **34** was obtained as a yellow solid (12.8 mg, yield 58%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.38 (s,

1H), 7.56−7.53 (m, 1H), 7.38−7.31 (m, 5H), 7.08 (d, J = 8.5 Hz, 1H), 7.04 (dd, J = 7.1, 2.1 Hz, 1H), 6.92 (dd, J = 3.8, 1.3 Hz, 1H), 5.05 (dd, J = 12.8, 5.5 Hz, 1H), 5.02−4.96 (m, 1H), 4.68−4.57 (m, 3H), 3.87−3.84 (m, 3H), 3.73−3.68 (m, 4H), 3.65−3.60 (m, 4H), 3.59−3.53 (m, 9H), 3.49 (t, J = 5.2 Hz, 3H), 3.28−3.24 (m, 2H), 3.12−3.07 (m, 2H), 2.89−2.80 (m, 2H), 2.77−2.56 (m, 7H), 2.38−2.24 (m, 2H), 2.19−2.08 (m, 2H), 2.03 (d, J = 14.7 Hz, 1H). HRMS (m/z): for $C_{49}H_{63}ClN_{11}O_{10}^{+}$ $[M + H]^{+}$, calcd 1000.4442; found, 1000.4462.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaoctadecan-18-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (35, MS5033).—Compound **35** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15.9 mg, 0.022 mmol) and **81** (9.5 mg, 0.022 mmol, 1.0 equiv). Compound **35** was obtained as a yellow solid (5.0 mg, yield 22%). ^{1}H NMR (600 MHz, CD_3OD) 8.41 (s, 1H), 7.56 (t, J = 8.0 Hz, 1H), 7.40 (d, J = 7.6 Hz, 3H), 7.36 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 8.5 Hz, 1H), 7.06 (d, J = 7.1 Hz, 1H), 6.95 (s, 1H), 5.07 (dd, J = 12.9, 5.5 Hz, 1H), 5.04 (t, J = 7.7 Hz, 1H), 4.66−4.53 (m, 2H), 3.99−3.90 (m, 2H), 3.74 (t, J = 5.8 Hz, 4H), 3.70−3.53 (m, 23H), 3.53−3.46 (m, 2H), 3.38 (s, 2H), 3.34 (s, 1H), 3.19−3.10 (m, 1H), 2.92−2.83 (m, 1H), 2.81−2.61 (m, 5H), 2.43−2.27 (m, 2H), 2.24−2.19 (m, 1H), 2.16−2.05 (m, 2H); ^{13}C NMR (201 MHz, CD_3OD): δ 173.27, 171.13, 170.29, 169.30, 169.25, 167.90, 154.31, 146.81, 143.35, 142.95, 139.52, 135.89, 133.43, 132.43, 128.61 (2C), 128.18 (2C), 123.56, 116.95, 110.71, 109.83, 103.36, 102.37, 70.22 (2C), 70.11, 70.07 (2C), 69.90 (2C), 69.85, 69.17, 67.14, 58.15, 53.94, 51.78, 51.67, 51.45, 48.84 (2C), 48.47, 42.75, 41.86, 38.36, 32.80, 30.83, 30.75, 30.62, 29.25, 22.42. HRMS (m/z): for $C_{51}H_{67}ClN_{11}O_{11}^{+}$ $[M + H]^{+}$, calcd 1044.4705; found, 1044.4708.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15,18-hexaoxahenicosan-21-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]-pyrimidin-4-yl)piperidine-4-carboxamide (36).—Compound **36** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **82**(12.2 mg, 0.02 mmol, 1.0 equiv). Compound **36** was obtained as a yellow solid (10.3 mg, yield 47%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.38 (s, 1H), 7.69 (d, J = 7.3 Hz, 1H), 7.61−7.51 (m, 1H), 7.44−7.28 (m, 5H), 7.07 (dd, J = 20.2, 7.8 Hz, 1H), 6.92 (d, J = 3.6 Hz, 1H), 5.09−4.98 (m, 2H), 4.65 (d, J = 12.4 Hz, 2H), 3.91−3.79 (m, 2H), 3.79−3.69 (m, 4H), 3.69−3.48 (m, 24H), 3.41−3.07 (m, 9H), 2.87 (t, J = 14.8 Hz, 1H), 2.80−2.53 (m, 5H), 2.41−2.24 (m, 2H), 2.18−2.11 (m, 2H), 2.04 (d, J = 14.7 Hz, 1H). HRMS (m/z): for $C_{53}H_{71}ClN_{11}O_{12}^{+}$ $[M + H]^{+}$, calcd 1088.4967; found, 1088.4974.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (37).—Compound **37** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **83** (14.1 mg, 0.02 mmol, 1.0 equiv). Compound **37** was obtained as a yellow solid (16.1 mg, yield 68%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.54 (t, J = 7.8 Hz,

1H), 7.41−7.26 (m, 5H), 7.06 (dd, *J* = 21.5, 7.8 Hz, 2H), 6.93 (d, *J* = 3.7 Hz, 1H), 5.13−5.01 (m, 2H), 4.61 (d, *J* = 14.1 Hz, 2H), 3.94−3.79 (m, 2H), 3.80−3.37 (m, 38H), 3.36−3.00 (m, 8H), 2.91−2.83 (m, 1H), 2.79−2.53 (m, 4H), 2.40−2.26 (m, 2H), 2.22−2.00 (m, 3H). HRMS (*m*/*z*): for $C_{57}H_{79}ClN_{11}O_{14}^{+}$ [M + H]$^{+}$, calcd 1176.5491; found, 1176.5475.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15,18,21,24,27-nonaoxatriacontan-30-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo-[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (38).—Compound **38** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **84** (14.8 mg, 0.02 mmol, 1.0 equiv). Compound **38** was obtained as a yellow solid (15.9 mg, yield 65%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.40 (s, 1H), 7.56 (t, *J* = 7.8 Hz, 1H), 7.46−7.30 (m, 5H), 7.10−7.07 (m, 2H), 6.94 (d, *J* = 3.6 Hz, 1H), 5.15−4.98 (m, 2H), 4.64 (d, *J* = 14.3 Hz, 2H), 3.87 (q, *J* = 10.9 Hz, 2H), 3.81−3.39 (m, 40H), 3.39−2.96 (m, 10H), 2.89−2.85 (m, 1H), 2.81−2.57 (m, 4H), 2.41−2.24 (m, 2H), 2.24−1.97 (m, 3H). HRMS (*m*/*z*): for $C_{59}H_{83}ClN_{11}O_{15}$ $^{+}$[M + H]$^{+}$, calcd 1220.5753; found, 1220.5767.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaoctadecan-18-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (39).—Compound **39** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **85** (11 mg, 0.02 mmol, 1.0 equiv). Compound **39** was obtained as a yellow solid (16.1 mg, yield 78%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.42 (s, 1H), 7.58−7.37 (m, 6H), 7.08 (d, *J* = 8.5 Hz, 1H), 7.05 (d, *J* = 7.1 Hz, 1H), 6.96 (s, 1H), 5.07 (dd, *J* = 12.9, 5.5 Hz, 1H), 5.04 (t, *J* = 7.7 Hz, 1H), 4.66−4.53 (m, 2H), 3.99−3.90 (m, 2H), 3.75 (d, *J* = 5.8 Hz, 4H), 3.69−3.54 (m, 23H), 3.53−3.47 (m, 2H), 3.38 (s, 2H), 3.35 (s, 1H), 3.19−3.10 (m, 1H), 2.92−2.83 (m, 1H), 2.83−2.62 (m, 7H), 2.42−2.24 (m, 2H), 2.23−2.18 (m, 1H), 2.15−2.05 (m, 2H); HRMS (*m*/*z*): for $C_{51}H_{69}ClN_{11}O_{10}^{+}$ [M + H]$^{+}$, calcd 1030.4912; found, 1030.4945.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)-3,6,9,12,15-pentaoxaoctadecan-18-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (40).—Compound **40** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **86** (11.2 mg, 0.02 mmol, 1.0 equiv). Compound **40** was obtained as a white solid (13.4 mg, 64%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.39 (s, 1H), 7.78 (t, *J* = 7.5 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.42−7.31 (m, 5H), 6.92 (d, *J* = 3.6 Hz, 1H), 5.11 (dd, *J* = 12.8, 5.4 Hz, 1H), 5.01 (d, *J* = 8.2 Hz, 1H), 4.79−4.60 (m, 3H), 4.38 (s, 1H), 3.92 (d, *J* = 4.5 Hz, 2H), 3.88−3.78 (m, 3H), 3.78−3.69 (m, 3H), 3.64−3.58 (m, 16H), 3.37−3.02 (m, 12H), 2.88 (t, *J* = 15.1 Hz, 1H), 2.81−2.54 (m, 3H), 2.43−2.25 (m, 2H), 2.22−2.10 (m, 1H), 2.04 (d, *J* = 14.6 Hz, 1H). HRMS (*m*/*z*): for $C_{51}H_{66}ClN_{10}O_{12}^{+}$ [M + H]$^{+}$, calcd 1045.4545; found, 1045.4534.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(19-(2-(2,6-dioxopiperidin-3-yl) - 1, 3-dioxoisoindolin-4 - yl) - 4, 7, 10, 13, 16-pentaoxanonadecanoyl)piperazin-1-

yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (41).— Compound **41** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (14.2 mg, 0.02 mmol) and **87** (11.4 mg, 0.02 mmol, 1.0 equiv). Compound **41** was obtained as a white solid (12.1 mg, yield 58%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.40 (s, 1H), 7.80−7.71 (m, 2H), 7.66 (dd, J = 6.1, 2.8 Hz, 1H), 7.45−7.26 (m, 5H), 6.95 (d, J = 3.6 Hz, 1H), 5.13 (dd, J = 12.8, 5.4 Hz, 1H), 5.02 (t, J = 7.7 Hz, 1H), 4.66 (s, 2H), 3.86 (q, J = 11.8 Hz, 2H), 3.73 (t, J = 6.1 Hz, 2H), 3.63−3.57 (m, 17H), 3.51 (t, J = 6.4 Hz, 2H), 3.36−3.24 (m, 9H), 3.23−3.06 (m, 3H), 2.94−2.84 (m, 1H), 2.81−2.55 (m, 5H), 2.42−2.25 (m, 2H), 2.16 (t, J = 17.3 Hz, 2H), 2.04 (d, J = 14.8 Hz, 1H), 1.99−1.91 (m, 2H). HRMS (m/z): for $C_{52}H_{68}ClN_{10}O_{11}^+$ $[M + H]^+$, calcd 1043.4752; found, 1043.4746.

4-Amino-N-((S)-1-(4-chlorophenyl)-3-(4-(11-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-11-oxoundecanoyl)-piperazin-1-yl)propyl)-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-piperidine-4-carboxamide (42, MS143N).— Compound **42** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (15 mg, 0.025 mmol) and 12-(((*S*)-1-((2*S*,4*R*)-4-(benzyloxy)-2-((4-(4-methylthiazol-5-yl)benzyl)-carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecanoic acid (**94**, 18.4 mg, 0.025 mmol, 1.0 equiv). Compound **42** was obtained as a white solid (15.8 mg, yield 52%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.80 (s, 1H), 8.26 (s, 1H), 7.41−7.07 (m, 14H), 6.78 (d, J = 3.7 Hz, 1H), 4.92 (dd, J = 9.2, 5.9 Hz, 1H), 4.61 (s, 1H), 4.58−4.47 (m, 3H), 4.47−4.35 (m, 3H), 4.26 (d, J = 15.4 Hz, 1H), 4.22−4.14 (m, 2H), 3.76−3.60 (m, 3H), 3.28−3.19 (m, 8H), 3.18−3.07 (m, 1H), 3.07−2.97 (m, 1H), 2.58−2.43 (m, 2H), 2.37 (s, 3H), 2.33−2.22 (m, 4H), 2.22−1.95 (m, 5H), 1.92 (dd, J = 14.9, 4.1 Hz, 1H), 1.55−1.41 (m, 4H), 1.28−1.12 (m, 12H), 0.94 (s, 9H). ^{13}C NMR (151 MHz, CD_3OD): δ 174.69, 172.96, 172.73, 171.07, 169.44, 154.95, 151.52, 147.60, 144.60, 139.53, 138.88, 138.02, 133.46, 130.09, 129.10, 128.97 (2C), 128.60 (2C), 128.09 (2C), 127.98, 127.96 (2C), 127.60 (2C), 127.58 (2C), 127.43, 127.30, 123.12, 102.71, 77.13, 75.29, 70.26, 59.44, 58.34, 57.42, 54.00, 53.11, 51.59, 51.43, 42.30, 42.22, 41.52, 38.25, 36.08, 35.34, 35.27, 35.02, 32.17, 30.77, 30.65, 29.22, 29.15 (2C), 29.09, 29.01, 28.96, 28.90, 25.65, 25.60 (3C), 24.75, 14.38. HRMS (m/z): for $C_{66}H_{88}ClN_{12}O_6S^+$ $[M + H]^+$, calcd 1211.6354; found, 1211.6365.

4-Amino-N-((1S)-1-(4-chlorophenyl)-3-(4-(1-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-3,6,9,12,15-pentaoxaoctadecan-18-oyl)piperazin-1-yl)propyl)-1-(7H-pyrrolo-[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamide (43).— Compound **43** was synthesized following the standard procedure for preparing compound **3** from intermediates **2** (57.3 mg, 0.096 mmol) and 1-{[2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}−3,6,9,12,15-pentaoxaoctadecan-18-oic acid (**97**, 55.4 mg, 0.096 mmol, 1.0 equiv). Compound **43** was obtained as a yellow solid (63.7 mg, yield 63%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.42 (s, 1H), 7.57 (t, J = 7.8 Hz, 1H), 7.44−7.34 (m, 5H), 7.09 (dd, J = 28.6, 7.8 Hz, 2H), 6.97 (d, J = 3.5 Hz, 1H), 5.10 (dd, J = 13.0, 5.4 Hz, 1H), 5.05 (t, J = 7.6 Hz, 1H), 4.61 (d, J = 14.4 Hz, 2H), 4.00−3.90 (m, 2H), 3.79−3.70 (m, 4H), 3.70−3.66 (m, 4H), 3.66−3.46 (m, 20H), 3.39 (s, 2H),

3.36−3.33 (m, 2H), 3.16 (s, 3H), 2.94−2.85 (m, 2H), 2.77−2.54 (m, 5H), 2.44−2.28 (m, 2H), 2.23 (d, J = 14.7 Hz, 1H), 2.16−2.05 (m, 2H); ^{13}C NMR (201 MHz, CD_3OD): δ 172.30, 171.10, 170.07, 169.33, 169.24, 167.92, 154.31, 146.84, 143.33, 142.91, 139.56, 135.90, 133.44, 132.44, 128.62 (2C), 128.19 (2C), 123.59, 116.96, 110.72, 109.85, 103.39, 102.38, 70.22 (2C), 70.14 (2C), 70.09 (2C), 69.93 (2C), 69.86, 69.22, 67.16, 58.15, 53.95, 51.79, 51.68, 51.46, 49.50, 48.51, 42.76, 41.88, 38.38, 32.81, 31.14, 30.77, 30.64, 29.28, 26.05, 21.68; HRMS (m/z): for $C_{52}H_{69}ClN_{11}O_{11}^+$ [M + H]$^+$, calcd 1058.4861; found, 1058.4864.

12-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)-benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-amino)-12-oxododecanoic Acid (66).—To the solution of commercially available (2*S*,4*R*)-1-[(*S*)-2-amino-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide (**101**, 215 mg, 0.5 mmol) in DMSO (1.5 mL) were added dodecanedioic acid (**93**, 138 mg, 0.6 mmol, 1.5 equiv), EDCI (215 mg, 0.75 mmol, 1.5 equiv), HOAt (147 mg, 0.75 mmol, 1.5 equiv), and NMM (102 mg, 1.5 mmol, 3.0 equiv). After stirring overnight at rt, the resulting mixture was purified by reverse-phase ISCO (methanol/0.1% TFA in H_2O) to afford compound **66** as a white solid (154 mg, yield 48%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.91 (s, 1H), 7.49 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 4.69−4.63 (m, 1H), 4.61−4.49 (m, 3H), 4.38 (dd, J = 15.4, 5.3 Hz, 1H), 3.92 (d, J = 11.0 Hz, 1H), 3.82 (dd, J = 11.0, 3.9 Hz, 1H), 2.50 (s, 3H), 2.36−2.19 (m, 5H), 2.14−2.06 (m, 1H), 1.65−1.58 (m, 4H), 1.34 (t, J = 4.9 Hz, 12H), 1.06 (s, 9H). HRMS (m/z): for $C_{34}H_{51}N_4O_6S^+$ [M + H]$^+$, calcd 643.3524; found, 643.3523.

13-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)-benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-amino)-13-oxotridecanoic Acid (67).—Compound **67** was synthesized following the standard procedure for preparing compound **66** from (2*S*,4*R*)-1-[(*S*)-2-amino-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide (**101**, 215 mg, 0.5 mmol) and tridecanedioic acid (**98**, 147 mg, 0.6 mmol, 1.0 equiv). Compound **67** was obtained as a white solid (158 mg, yield 48%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.91 (s, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 4.68−4.64 (m, 1H), 4.61−4.49 (m, 3H), 4.38 (dd, J = 15.4, 5.1 Hz, 1H), 3.92 (d, J = 11.0 Hz, 1H), 3.82 (dd, J = 11.0, 3.9 Hz, 1H), 2.50 (s, 3H), 2.38−2.20 (m, 5H), 2.15−2.05 (m, 1H), 1.65−1.58 (m, 4H), 1.42−1.29 (m, 14H), 1.06 (s, 9H). HRMS (m/z): for $C_{35}H_{53}N_4O_6S^+$ [M + H]$^+$, calcd 657.3680; found, 657.3683.

14-(((S)-1-((2S,4R)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)-benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-amino)-14-oxotetradecanoic Acid (68).—Compound **68** was synthesized following the standard procedure for preparing compound **66** from (2*S*,4*R*)-1-[(*S*)-2-amino-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide (**101**, 215 mg, 0.5 mmol) and tetradecanedioic acid (**99**, 155 mg, 0.6 mmol, 1.0 equiv). Compound **68** was obtained as a white solid (145 mg, yield 43%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.92 (s, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 4.68−4.63 (m, 1H), 4.62−4.50 (m, 3H), 4.39

(dd, J = 15.4, 5.1 Hz, 1H), 3.92 (d, J = 11.0 Hz, 1H), 3.83 (dd, J = 11.0, 3.9 Hz, 1H), 2.50 (s, 3H), 2.36−2.20 (m, 5H), 2.17−2.03 (m, 1H), 1.67−1.59 (m, 4H), 1.43−1.29 (m, 16H), 1.06 (s, 9H). HRMS (m/z): for $C_{36}H_{55}N_4O_6S^+$ $[M + H]^+$, calcd 671.3837; found, 671.3875.

11-(((S)-1-((2S,4R)-4-Hydroxy-2-(((S)-1-(4-(4-methylthiazol-5-yl)-phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-11-oxoundecanoic Acid (69).—To a solution of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide[51] (**102**, 150 mg, 0.34 mmol) in DMSO (1.5 mL) were added undecanedioic acid (**100**, 88 mg, 0.41 mmol, 1.5 equiv), EDCI (98 mg, 0.51 mmol, 1.5 equiv), HOAt (70 mg, 0.51 mmol, 1.5 equiv), and NMM (103 mg, 1.02 mmol, 3.0 equiv). After stirring overnight at rt, the resulting mixture was purified by preparative HPLC (10%−100% methanol/0.1% TFA in H_2O) to afford compound **69** as a white solid (134.8 mg, yield 62%). ^{1}H NMR (600 MHz, CD_3OD): δ 9.01 (s, 1H), 7.47−7.40 (m, 4H), 5.00 (q, J = 7.0 Hz, 1H), 4.62 (s, 1H), 4.59−4.54 (m, 1H), 4.46−4.42 (m, 1H), 3.89−3.87 (m, 1H), 3.74 (dd, J = 11.0, 4.0 Hz, 1H), 2.49 (s, 3H), 2.34−2.15 (m, 5H), 1.96−1.92 (m, 1H), 1.63−1.55 (m, 4H), 1.50 (d, J = 7.0 Hz, 3H), 1.32 (t, J = 4.4 Hz, 10H), 1.04 (s, 9H). HRMS (m/z): for $C_{34}H_{51}N_4O_6S^+$ $[M + H]^+$, calcd 643.3524; found, 643.3527.

1-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}−3,6,9,12,15,18-hexaoxahenicosan-21-oic Acid (82).—To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**106**, 169 mg, 0.61 mmol) in NMP (2 mL) were added commercially available *tert*-butyl 1-amino-3,6,9,12,15,18-hexaoxahenicosan-21-oate (**103**, 250 mg, 0.61 mmol, 1.0 equiv) and DIPEA (266 μL, 1.53 mmol, 2.5 equiv). The reaction was heated at 100 °C for 1 h under microwave. The reaction mixture was purified by reverse-phase ISCO (acetonitrile/0.1% TFA in H_2O) to afford the intermediate as a yellow solid. This intermediate was dissolved in DCM (3 mL) and TFA (3 mL). After the reaction was stirred for 30 min, solvents were removed. The resulting residue was purified by reverse-phase ISCO (acetonitrile/0.1% TFA in H_2O) to afford the title compound **82** as a yellow solid (124 mg, yield 33%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.83 (s, 1H), 7.57−7.52 (m, 2H), 5.05 (dd, J = 12.6, 5.5 Hz, 1H), 3.85 (t, J = 5.8 Hz, 1H), 3.78−3.63 (m, 20H), 3.57−3.46 (m, 4H), 2.95−2.81 (m, 1H), 2.73 (t, J = 16.1 Hz, 1H), 2.63−2.45 (m, 4H), 2.11 (s, 1H). HRMS (m/z): for $C_{28}H_{40}N_3O_{12}^+$ $[M + H]^+$, calcd 610.2607; found, 610.2612.

1-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}−3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic Acid (83).—Compound **83** was synthesized following the standard procedure for preparing compound **82** from 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**106**, 139 mg, 0.5 mmol) and *tert*-butyl 1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oate (**104**, 250 mg, 0.5 mmol, 1.0 equiv). Compound **83** was obtained as a yellow solid (188 mg, yield 54%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.56 (t, J = 7.8 Hz, 1H), 7.10−7.08 (m, 2H), 5.05 (dd, J = 12.9, 5.4 Hz, 1H), 3.84−3.47 (m, 34H), 2.94−2.81 (m, 1H), 2.73 (t, J = 15.5 Hz, 2H), 2.54 (t, J = 6.3 Hz, 2H), 2.11 (d, J = 12.4 Hz, 1H). HRMS (m/z): for $C_{32}H_{48}N_3O_{14}^+$ $[M + H]^+$, calcd 698.3131; found, 698.3138.

1-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}−3,6,9,12,15,18,21,24,27-nonaoxatriacontan-30-oic Acid (84).— Compound **84** was synthesized following the standard procedure for preparing compound **82** from 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**106**, 51 mg, 0.18 mmol) and *tert*-butyl 1-amino-3,6,9,12,15,18,21,24,27-nonaoxatriacontan-30-oate (**105**, 100 mg, 0.18 mmol, 1.0 equiv). Compound **84** was obtained as a yellow solid (107 mg, yield 81%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.56 (t, J = 7.9 Hz, 1H), 7.09−7.07 (dd, 2H), 5.05 (dd, J = 12.9, 5.4 Hz, 1H), 3.81−3.48 (m, 38H), 2.85 (d, J = 15.4 Hz, 1H), 2.73 (t, J = 15.6 Hz, 2H), 2.54 (t, J = 6.2 Hz, 2H), 2.16−2.05 (m, 1H). HRMS (m/z): for $C_{34}H_{52}N_3O_{15}^+$ $[M + H]^+$, calcd 742.3393; found, 742.3397.

1-{[2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]amino}−3,6,9,12,15-pentaoxaoctadecan-18-oic Acid (85).—To a solution of 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**108**, 50 mg, 0.19 mmol) in DMF (2 mL) were added *tert*-butyl 1-bromo-3,6,9,12,15-pentaoxaoctadecan-18-oate (**107**, 100 mg, 1.2 mmol, 1.2 equiv), $NaHCO_3$ (37 mg, 0.44 mmol, 2.3 equiv), and NaI (6.3 mg, 0.038 mmol, 0.2 equiv). After the reaction was stirred at 60 °C overnight, it was quenched by water. The reaction was extracted with EtOAc (3 × 10 mL), dried over Na_2SO_4, filtered, and concentrated. The resulting residue was dissolved in DCM (2 mL) and TFA (2 mL). After the reaction was stirred for 30 min, solvents were removed. The residue was purified by preparative HPLC (10%–100% methanol/0.1% TFA in H_2O) to afford compound **85** as a yellow solid (84.8 mg, yield 81%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.44−7.33 (m, 2H), 7.15 (d, J = 7.8 Hz, 1H), 5.22 (d, J = 12.4 Hz, 1H), 4.02 (s, 2H), 3.78−3.56 (m, 22H), 3.05−2.84 (m, 2H), 2.61−2.39 (m, 3H), 2.25−2.14 (m, 1H). HRMS (m/z): for $C_{26}H_{38}N_3O_{10}^+$ $[M + H]^+$, calcd 552.2552; found, 552.2556.

1-{[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]oxy}−3,6,9,12,15-pentaoxaoctadecan-18-oic Acid (86).—Compound **86** was synthesized following the standard procedure for preparing compound **85** from 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (**109**, 79 mg, 0.29 mmol) and **107** (150 mg, 0.35 mmol, 1.2 equiv). Compound **86** was obtained as a white solid (113 mg, yield 69%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.77 (t, J = 8.0 Hz, 1H), 7.47 (dd, J = 11.5, 7.8 Hz, 2H), 5.09 (dd, J = 12.9, 5.4 Hz, 1H), 4.38 (t, J = 4.6 Hz, 2H), 3.92 (t, J = 4.6 Hz, 2H), 3.83−3.55 (m, 18H), 2.95−2.83 (m, 1H), 2.79−2.66 (m, 2H), 2.60−2.50 (m, 2H), 2.19−2.03 (m, 1H). HRMS (m/z): for $C_{26}H_{35}N_2O_{12}^+$ $[M + H]^+$, calcd 567.2185; found, 567.2197.

19-[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]-4,7,10,13,16-pentaoxanonadecanoic Acid (87).—To a solution of 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**111**, 33.7 mg, 0.1 mmol) and *tert*-butyl 4,7,10,13,16-pentaoxanonadec-18-ynoate (**110**, 72 mg, 0.2 mmol, 2 equiv) in DMA (1.5 mL) were added tetrakis(triphenylphosphine)palladium (0) (2.3 mg, 2 mol %), copper iodide (1 mg, 4 mol %), and triethylamine (22 mg, 0.3 mmol, 3.0 equiv). After the reaction was stirred at 100 °C for 3 h, the mixture was purified by preparative HPLC (10%−100% acetonitrile/0.1% TFA in H_2O) to afford the desired product (49.1 mg, yield 80%). This intermediate was dissolved in MeOH, and palladium on carbon (5 mg, 10%)

was added. The reaction was stirred at rt under hydrogen for 1 h, before it was filtered. The filtrate was concentrated. The resulting residue was dissolved in DCM (1 mL) and TFA (1 mL). After the reaction was stirred for 30 min, solvents were removed. The resulting residue was purified by preparative HPLC (10%−100% acetonitrile/0.1% TFA in H_2O) to afford the title compound **87** as a white solid (24 mg, yield 57%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.74−7.63 (m, 2H), 7.58−7.47 (m, 1H), 5.12 (dd, J = 12.7, 5.2 Hz, 1H), 3.71 (t, J = 6.1 Hz, 2H), 3.67−3.55 (m, 16H), 3.50 (t, J = 6.1 Hz, 2H), 3.17 (t, J = 7.8 Hz, 2H), 2.95−2.81 (m, 1H), 2.74 (t, J = 14.8 Hz, 2H), 2.53 (t, J = 6.2 Hz, 2H), 2.13 (t, J = 8.9 Hz, 1H), 1.94 (t, J = 7.5 Hz, 2H). HRMS (*m*/*z*): for $C_{27}H_{37}N_2O_{11}^+$ [M + H]$^+$, calcd 565.2392; found, 565.2388.

12-(((S)-1-((2S,4R)-4-(Benzyloxy)-2-((4-(4-methylthiazol-5-yl)-benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-amino)-12-oxododecanoic Acid (94).—To a solution of [4-(4-methylthiazol-5-yl)phenyl]methanamine (**88**, 1 mmol, 204 mg) in DMSO (5 mL) were added commercially available (2*S*,4*R*)-4-(benzyloxy)-1-(*tert*-butoxycarbonyl)pyrrolidine-2-carboxylic acid (**89**, 1 mmol, 321 mg), EDCI (288 mg, 1.5 mmol), HOAt (204 mg, 1.5 mmol), and NMM (303 mg, 3 mmol). After stirring for 2 h at rt, the resulting mixture was purified by reverse-phase ISCO (methanol/0.1% TFA in H_2O) to afford an intermediate, which was dissolved in TFA (5 mL) and DCM (5 mL). After the resulting mixture was stirred for 30 min, solvents were removed. The resulting residue was purified by reverse-phase ISCO (methanol/0.1% TFA in H_2O) to afford (2*S*,4*R*)-4-(benzyloxy)-*N*-[4-(4-methylthiazol-5-yl)-benzyl]pyrrolidine-2-carboxamide (**90**, 276 mg, yield 68%). To a solution of **90** (84 mg, 0.2 mmol) in DMSO (1 mL) was added commercially available (*S*)-2-[(*tert*-butoxycarbonyl)amino]-3,3-dimethylbutanoic acid (**91**, 48.5 mg, 0.2 mmol), EDCI (11.6 mg, 0.06 mmol), HOAt (8.2 mg, 0.06 mmol), and NMM (12.7 mg, 0.12 mmol), the reaction was stirred at rt for 3 h, and the mixture was purified by preparative HPLC (10%−100% acetonitrile/0.1% TFA in H_2O) to afford the intermediate. This intermediate was dissolved in DCM (1 mL) and TFA (1 mL) and the resulting mixture was stirred at rt for 30 min. The solvent was evaporated and the resulting mixture was purified by preparative HPLC (10%−100% acetonitrile/0.1% TFA in H_2O) to afford (2*S*,4*R*)-1-[(*S*)-2-amino-3,3-dimethylbutano-yl]-4-(benzyloxy)-*N*-[4-(4-methylthiazol-5-yl)benzyl]pyrrolidine-2-carboxamide (**92**, 39.5 mg, yield 38% for two steps) as a white solid. ESI (*m*/*z*): [M + H$^+$]: 521.3; To a solution of **92** (21 mg, 0.04 mmol) in DMSO (1 mL) were added dodecanedioic acid (**93**, 18.5 mg, 0.08 mmol), EDCI (11.6 mg, 0.06 mmol), HOAt (8.2 mg, 0.06 mmol), and NMM (12.7 mg, 0.12 mmol). After stirring for 2 h at rt, the resulting mixture was purified by preparative HPLC (10%−100% acetonitrile/0.1% TFA in H_2O) to afford the title compound **94** as a white solid (18.4 mg, yield 63%). ^{1}H NMR (600 MHz, CD_3OD): δ 8.94 (s, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.2 Hz, 2H), 7.38−7.31 (m, 4H), 7.31−7.25 (m, 1H), 4.73 (s, 1H), 4.62 (d, J = 11.6 Hz, 1H), 4.58−4.49 (m, 3H), 4.38 (d, J = 15.4 Hz, 1H), 4.35−4.28 (m, 2H), 3.76 (dd, J = 11.3, 3.6 Hz, 1H), 2.50 (s, 3H), 2.46–2.38 (m, 1H), 2.32−2.20 (m, 4H), 2.15−2.08 (m, 1H), 1.66−1.54 (m, 4H), 1.38−1.25 (m, 12H), 1.06 (s, 9H). HRMS (*m*/*z*): for $C_{41}H_{57}N_4O_6S^+$ [M + H]$^+$, calcd 733.3993; found, 733.3987.

1-{[2-(1-Methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl]amino}−3,6,9,12,15-pentaoxaoctadecan-18-oic Acid (97).—Compound **97** was synthesized

following the standard procedure for preparing compound **82** from commercially available *tert*-butyl 1-amino-3,6,9,12,15-pentaoxaoctadecan-18-oate (**95**, 74 mg, 0.2 mmol) and 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione[56] (**96**, 58 mg, 0.2 mmol). Compound **97** was obtained as a yellow solid (56.4 mg, yield 49%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.54 (dd, J = 8.5, 7.1 Hz, 1H), 7.09 (d, J = 8.5 Hz, 1H), 7.04 (d, J = 7.0 Hz, 1H), 5.07 (dd, J = 12.9, 5.4 Hz, 1H), 3.74−3.68 (m, 4H), 3.67−3.55 (m, 16H), 3.50 (t, J = 5.3 Hz, 2H), 3.14 (s, 3H), 2.90−2.85 (m, 2H), 2.74−2.62 (m, 1H), 2.53 (t, J = 6.2 Hz, 2H), 2.12−2.05 (m, 1H); HRMS (m/z): for $C_{27}H_{38}N_3O_{11}^+$ $[M + H]^+$, calcd 580.2501; found, 580.2513.

Cell Culture.

All cell lines were purchased from ATCC and authenticated. Cell lines were regularly tested in the lab for mycoplasma. All cells were cultured at 37 °C under 5% CO_2. Cell lines were cultured in 1× DMEM (Corning, 10-013-CV) with 10% fetal bovine serum (Atlanta Biologicals S11150) and 1X Penicillin/Streptomycin. Cells were split using 0.05 or 0.25% trypsin (Corning 25-051-Cl or 25-053-Cl, respectively) before they reached full confluence and media were changed every 3–4 days.

Western Blotting Assay.

Cells were lysed in a 2× sample buffer (125 mM Tris–HCl at pH 6.8, 10% βME, 2% sodium dodecyl sulfate, 20% glycerol, 0.05% Bromophenol Blue, 8 M urea). Protein lysates were loaded into 4–12% Bis-Tris gels and resolved by electrophoresis. Samples were then blotted on a poly(vinylidene difluoride) membrane (Millipore IPVH00010) using the wet transfer technique (Invitrogen). Membranes were blocked in 5% milk-TBST for 30 min, and incubated in primary antibody in 5% milk-TBST or 5% bovine serum albumin-TBST at 4 °C for 16 h. Membranes were rinsed (3 × 10 min) in TBST and incubated in horseradish peroxidase-conjugated secondary antibodies in 5% milk-TBST for at least 2 h and rinsed again in TBST (3 × 6 min). Membranes were visualized using the chemiluminescence system (Thermo 34080, 37075) on an auto-radiography film (Denville E3018). Primary antibodies: β-actin (Sigma A5316), p-AKT (Ser473 CST-9721), total AKT (CST-9272), AKT1 (2H10, CST-2967), AKT2 (CST-3063), AKT3 (CST-8018), p-S6 (Ser240/244, CST-5364), and p-PRAS40 (Thr246, CST-2997). Secondary antibodies: Mouse (Thermo 31432) and Rabbit (Thermo 31460).

AKT Binding Assay.

Binding affinities of **20**, **35**, **42**, and **43** to three AKT isoforms (AKT1, AKT2, and AKT3) were determined by DiscoverX using a competition binding assay (KINOME*scan* assay) to quantitatively measure the ability of a compound to compete with an immobilized, active-site directed ligand. The assay comprises three components: DNA-tagged AKT, immobilized ligand, and a test compound. The ability of the test compound to compete with the immobilized ligand was measured by quantitative polymerase chain reaction of the DNA tag. The K_d values were determined by using an 11-point threefold compound dilution (the top concentration of 30 μM) with three DMSO control points in duplicates.

ITC Assay.

VHL (Elongin C, Elongin B, and Von Hippel–Lindau) protein was prepared for ITC by desalting and equilibrating using ITC buffer (25 mM Tris pH 7.5 RT, 500 mM NaCl) in a GE healthcare PD-20 Sephadex column by gravity. Protein was then concentrated to stock 650 μM and final sample concentrations prepared (40 μM) with DMSO added from 1% (v/v) to minimize buffer mismatch between the cell and syringe. Compounds were diluted to a final concentration of 400 μM in a buffer with additional 1% DMSO (v/v). ITC was performed on a MicroCal ITC 200 device (Malvern, Worcestershire, UK) at 25 °C with an initial injection of 0.4 μL and 19 injections of 2.0 μL (180 s spacing, reference power 11) at a stirring rate of 750 rpm. Data were analyzed with Origin MicroCal Analysis software using a chi-squared fit model.

Cell Proliferation Assay.

Experiments were carried out in 96-well plates in triplicates (Corning 720089). A total of 1–3 × 10^3 cells per well were grown in the presence of indicated compounds, and cells were then monitored for 3–5 days using the IncuCyte live cell imaging system (Essen BioScience, Ann Arbor, MI, USA), which was placed in a cell culture incubator operated at 37 °C under 5% CO_2. Cell confluence was determined using calculations derived from phase-contrast image readings on an IncuCyte ZOOM (Essen Biosciences) on live cells over time.

Cell Colony Formation Assay.

Cells were cultured for 14 days in the presence of different compounds. Media with compound were replenished every 2 days. At the end of the experiment, media were aspirated and viable cells were stained with 0.5% crystal violet dye.

Mouse Pharmacokinetic Study.

Compounds **20** and **35** in HCl salt form were dissolved in a formulation of 5% DMA, 5% Solutol HS-15, and 90% normal saline. Six male Swiss Albino mice were administered with a solution formulation of each compound at an indicated dose via IP injection. All samples were maintained below −70 ± 10 °C until performing bioanalysis. Approximately 60 μL of plasma samples were collected from three mice at 0.5, 1, 2, 4, 8, and 12 h. Pharmacokinetic analysis was conducted using the NCA module of Phoenix WinNonlin (Version 8.0) and plasma concentrations were quantified by fit-for-purpose LC–MS/MS method (LLOQ: 10.18 ng/mL). Compound concentrations in plasma at each time point are average values from three tested mice. Error bars represent ±SEM. Experiments involving mice were performed according to the Institutional Animal Care and Use Committee (IACUC)-approved protocol.

Mice Xenograft Studies.

Male immunocompromised NU/J mice (6 weeks old) (The Jackson Laboratory) were engrafted with PC-3 human prostate cancer cells. After tumor volumes reach ~100 mm^3, mice were randomized into different treatment arms and administrated with vehicle control or 75 mg/kg compound **20** daily for 22 days via IP injections. The experimenters were not blinded. Tumor volume was calculated as follows: tumor size (mm^3) = (longer measurement

× shorter measurement^2) × 0.5. Tumor sizes were recorded every other day over the course of the studies. All procedures involving mice and experimental protocols (LA13–00024) were approved by the Institutional Animal Care and Use Committee (IACUC) of Icahn School of Medicine at Mount Sinai (ISMMS).

Statistical Analysis.

No statistical methods were used to determine the sample size. Student *t*-tests (parametric) were used to compare two data sets. GraphPad Prism was used to make these simple predetermined statistical comparisons.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

J.J. acknowledges the support by an endowed professorship by the Icahn School of Medicine at Mount Sinai. This research was supported by the grant R35CA220491 (to R.P.) from the National Cancer Institute (NCI) at the National Institutes of Health (NIH). R.P. and J.J. were also supported by P30 CA196521 grant from the NCI at the NIH. J.X. is supported by T32 postdoc fellow training grant (T32CA078207) and Leo and Julia Forchheimer Foundation Postdoc Fellowship. This work utilized the NMR Spectrometer Systems at Mount Sinai acquired with funding from National Institutes of Health SIG grants 1S10OD025132 and 1S10OD028504.

The authors declare the following competing financial interest(s): J.J., R.P., J.L. J.X., and X.Y. are inventors of a patent application filed by the Icahn School of Medicine at Mount Sinai. J.J. is a cofounder, scientific advisory board member, and equity shareholder in Cullgen, Inc. and a consultant for Cullgen, Inc., EpiCypher, Inc., and Accent Therapeutics, Inc. R.P. is a shareholder and advisor of Therapten Bioscience, Inc. The Jin laboratory received research funds from Celgene Corporation, Levo Therapeutics, Inc., Cullgen, Inc., and Cullinan Oncology, Inc.

ABBREVIATIONS USED

ATP	adenosine triphosphate
AUC	area under the curve
DIPEA	diisopropylethylamine
DMSO	dimethyl sulfoxide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOAt	1-hydroxy-7-azabenzo-triazole
IMiDs	immunomodulatory drugs
IP	intraperitoneal
ITC	isothermal titration calorimetry
MOA	mechanism of action
m-TOR	mammalian target of rapamycin
NAE	NEDD8-activating enzyme

NMM	N-methylmorpholine
NMP	N-methyl-2-pyrrolidone
P-AKT	phosphorylated AKT
PEG	polyethylene glycol
PI3K	phosphatidylinositol 3-kinase
PK	pharmacokinetic
POI	protein of interest
POM	pomalidomide
PROTACs	proteolysis targeting chimeras
RTKs	receptor tyrosine kinases
SAR	structure–activity relationship
T-AKT	total AKT
TNBC	triple-negative breast cancer
UPS	ubiquitin proteasome system
VHL	von Hippel–Lindau

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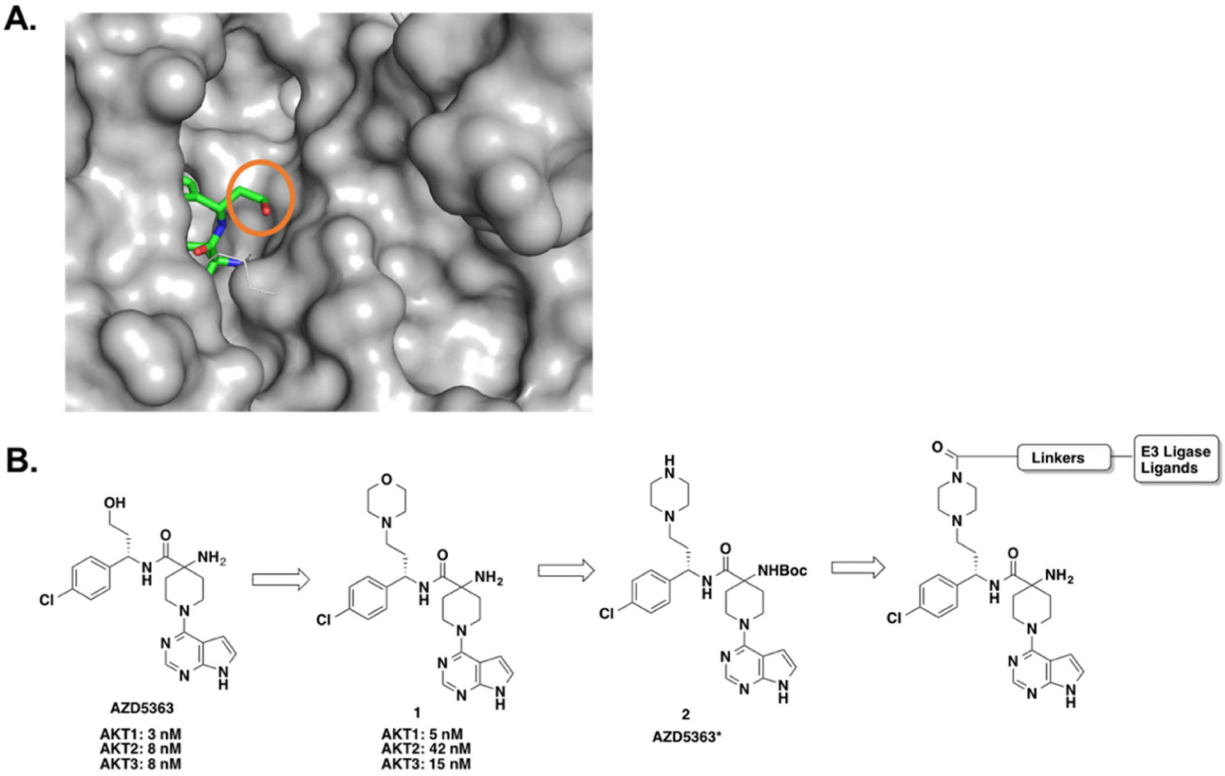


Figure 1.

Design of putative AKT degraders. (A) Co-crystal structure of AKT1 (in gray) in complex with AZD5363 (in green, blue, and red) (PDB: 4GV1). The hydroxyl group of AZD5363 (marked by the orange cycle) is solvent-exposed. (B) Schematic design of AKT putative degraders based upon the AKT inhibitor AZD5363.

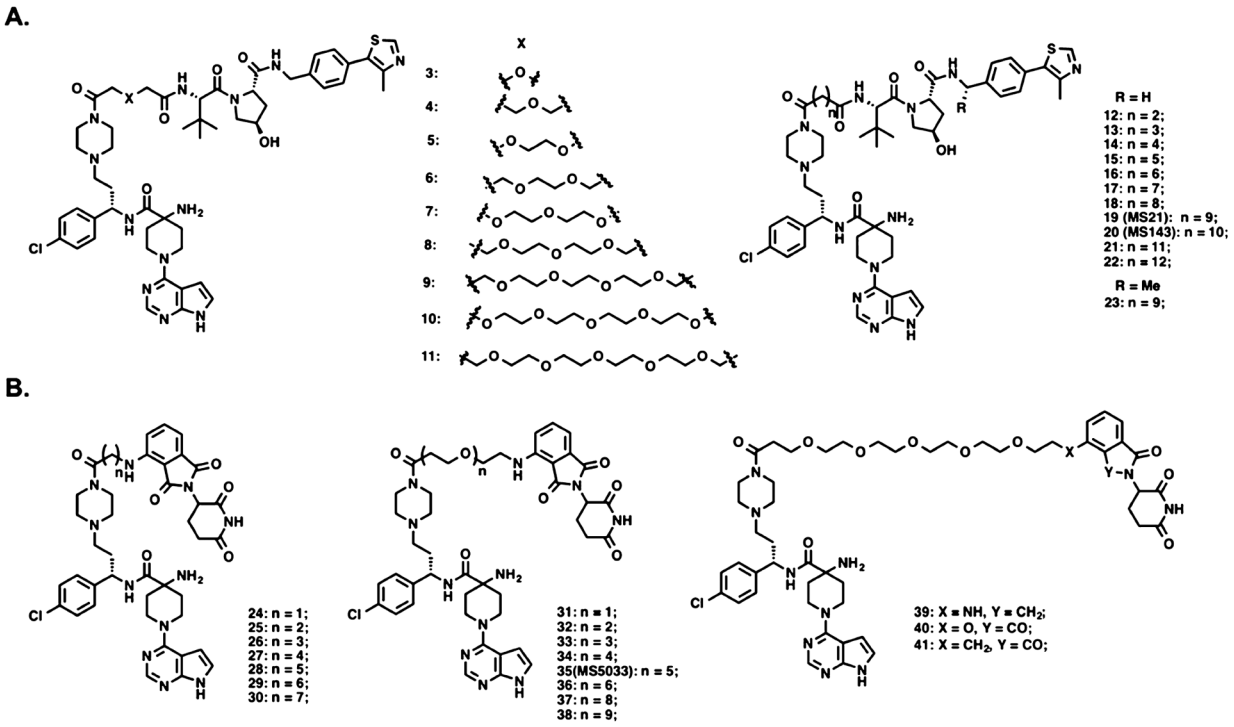


Figure 2.

Chemical structures of designed AKT degraders based on AZD5363. (A) VHL-recruiting AKT degraders. (B) CRBN-recruiting AKT degraders.

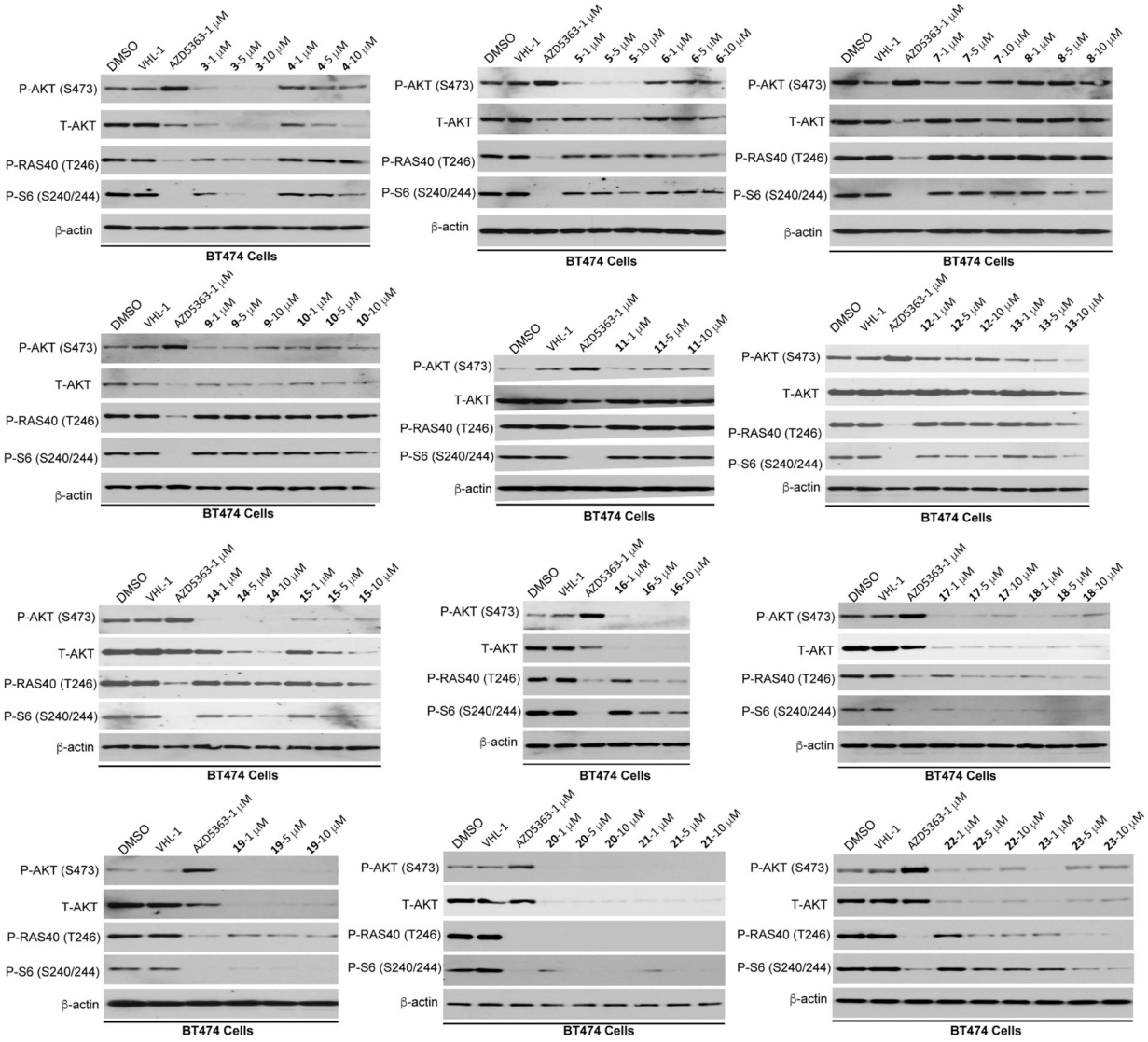


Figure 3.

Effects of compounds **3**–**23** on reducing the AKT protein level and inhibiting the downstream signaling. BT474 cells were treated with AZD5363 (1 μM), VHL-1 (1 μM), or the indicated compound at 1, 5, and 10 μM for 24 h. The cell lysates were analyzed by western blotting to examine the protein levels of T-AKT, P-AKT (S473), P-PRAS40 (T246), and P-S6 (S240/244). β-Actin was used as the loading control.

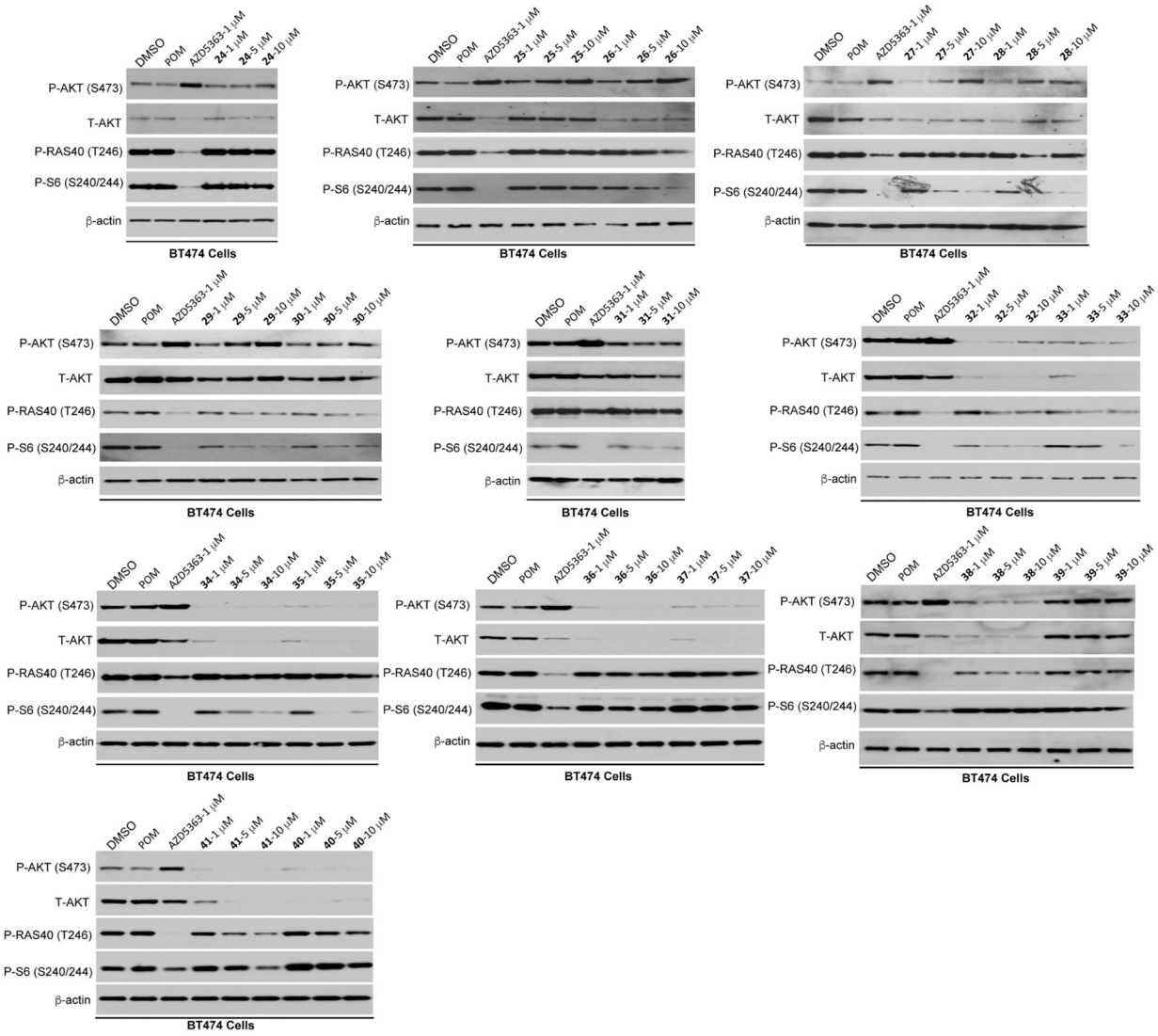


Figure 4.

Effects of compounds **24**–**41** on reducing the AKT protein level and inhibiting the downstream signaling. BT474 cells were treated with AZD5363 (1 μM), POM (1 μM), or indicated compound at 1, 5, and 10 μM for 24 h. The cell lysates were analyzed by western blotting to examine the protein levels of T-AKT, P-AKT (S473), P-PRAS40 (T246), and P-S6 (S240/244). β-Actin was used as the loading control.

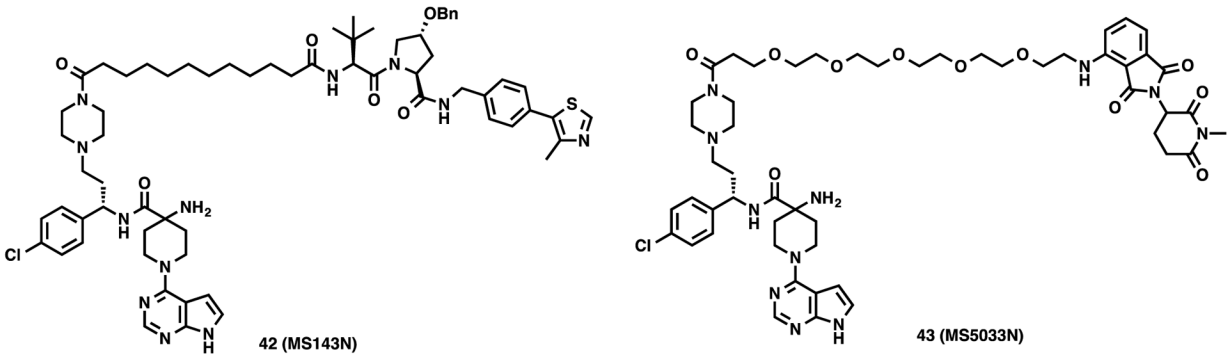


Figure 5.
Chemical structures of negative control compounds **42** and **43**.

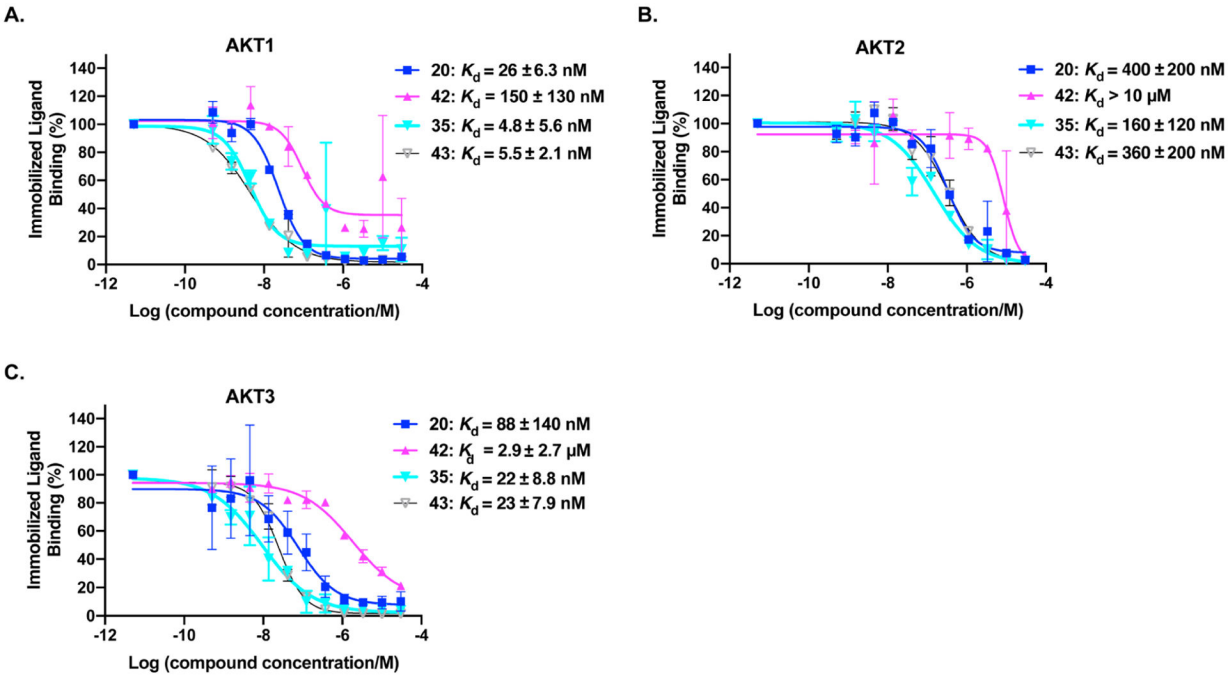


Figure 6.

Binding affinities of degraders **20** and **35**, and negative controls **42** and **43** to AKT1 (A), AKT2 (B), and AKT3 (C). The K_d determination experiments were performed in a competitive binding assay (KINOMEscan assay by Eurofins DiscoverX) in duplicate. The lowest concentration points represent data from the DMSO samples. Error bars represent ± SEM in duplicate independent experiments.

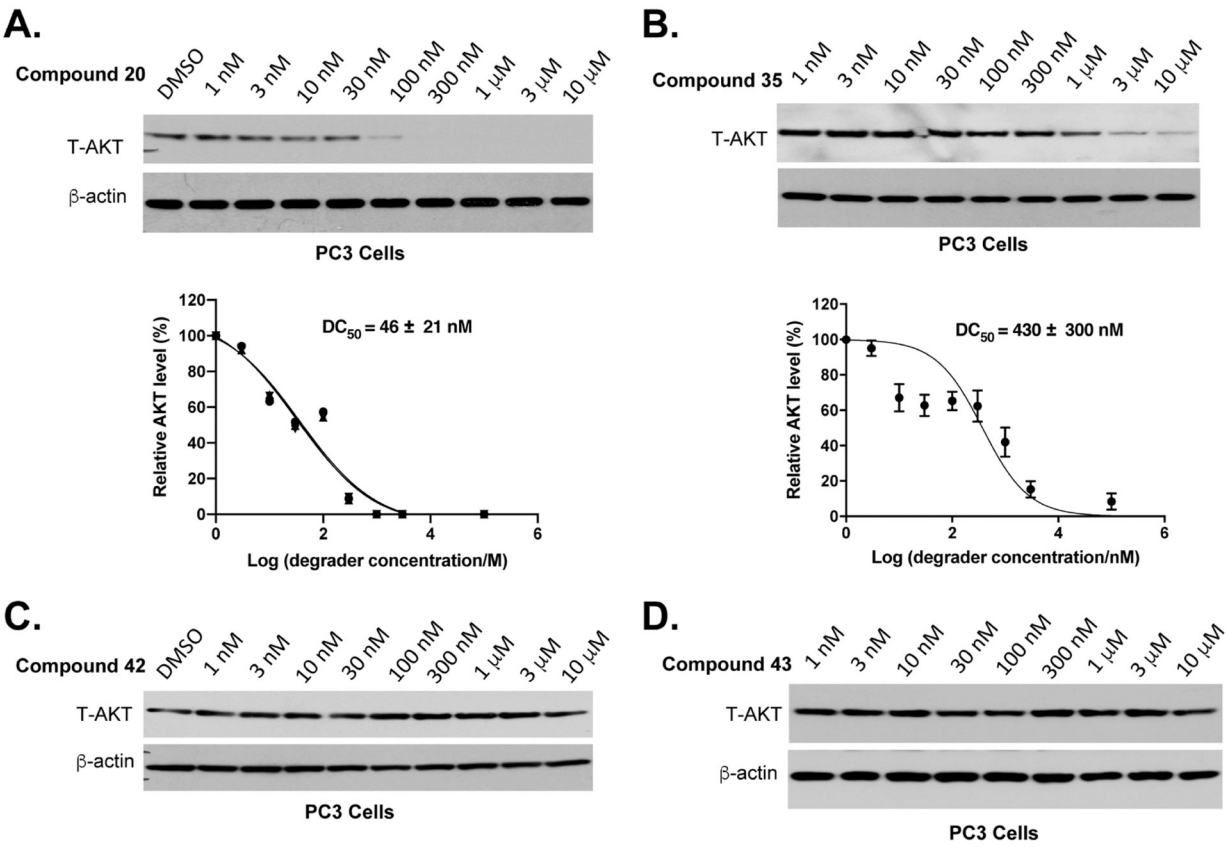


Figure 7.

Effects of compounds **20**, **35**, **42**, and **43** on reducing the AKT protein level in PC3 cells. PC3 cells were treated with DMSO or compound **20** (A), **35** (B), **42** (C), or **43** (D) at the indicated concentrations for 24 h. The T-AKT protein level was determined using western blots. The DC_{50} values of **20** (A) and **35** (B) were quantified from three independent biological experiments.

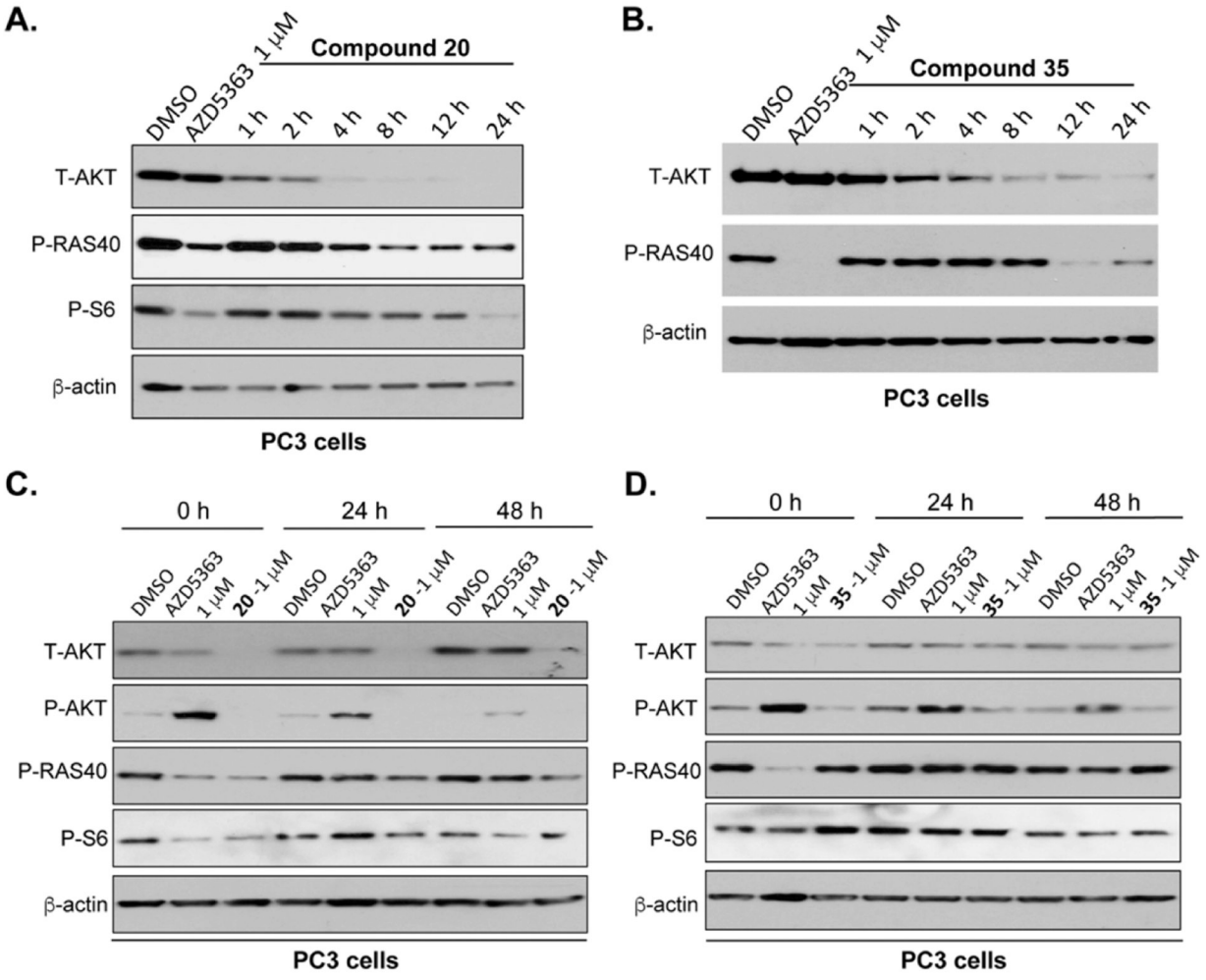


Figure 8.

Compounds **20** and **35** induced AKT degradation and inhibited the downstream signaling in a time-dependent and reversible manner. (A,B) PC3 cells treated with DMSO (24 h), AZD5363 (24 h), **20**, or **35** at 1 μM were collected at the indicated time points for western blotting analysis. (C,D) Western blots of T-AKT, P-AKT, P-PRAS40, and P-S6 and β-actin post-treatment of PC3 cells with DMSO, AZD5363, **20**, or **35** at 1 μM for 24 h at the indicated time. The compounds were washed out with fresh medium after the 24 h treatment.

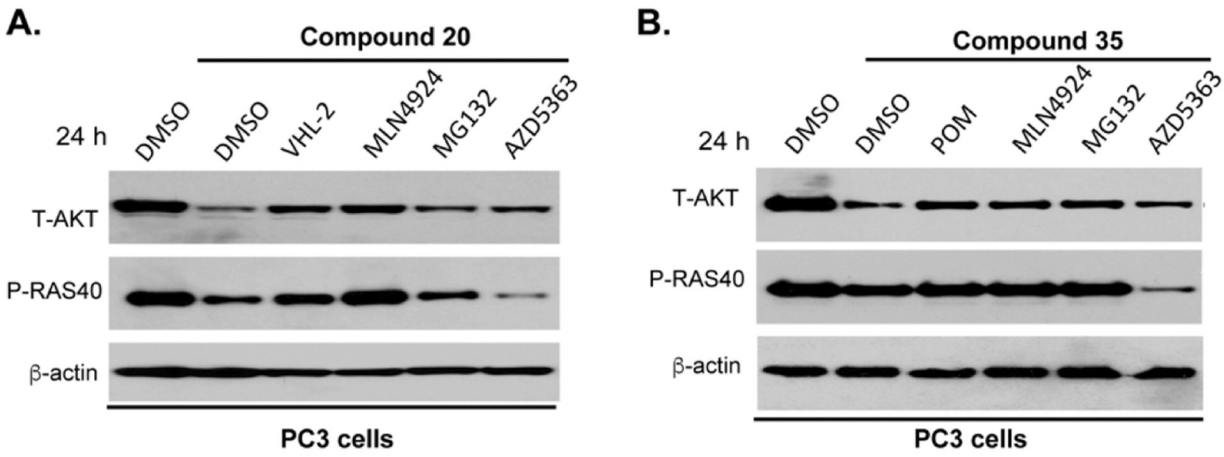


Figure 9.

AKT degradation induced by compound **20** (A) or compound **35** (B) is mediated through the UPS. PC3 cells were treated with DMSO, AZD5363 (1 μM), MLN4924 (1 μM), MG132 (10 μM), VHL-2 (1 μM), or POM (1 μM) for 2 h, before the cells were treated with **20** or **35** for additional 24 h. The T-AKT and P-PRAS40 protein levels were determined by western blots.

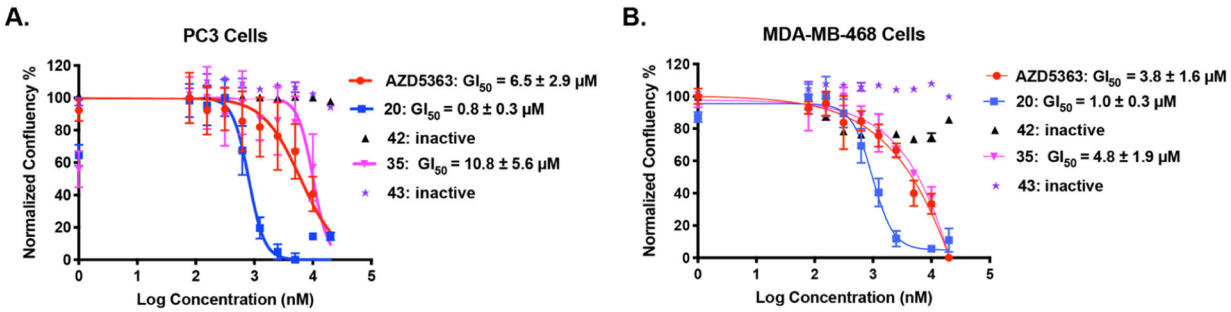


Figure 10.

Compounds **20** and **35** inhibit cancer cell proliferation. PC3 (A) and MDA-MB-468 (B) cells were treated with serial dilutions of AZD5363, **20**, **42**, **35**, or **43** for 5 days. Cell growth was determined using calculations derived from phase-contrast images in IncuCyte. Error bars indicate the standard errors in three independent experiments.

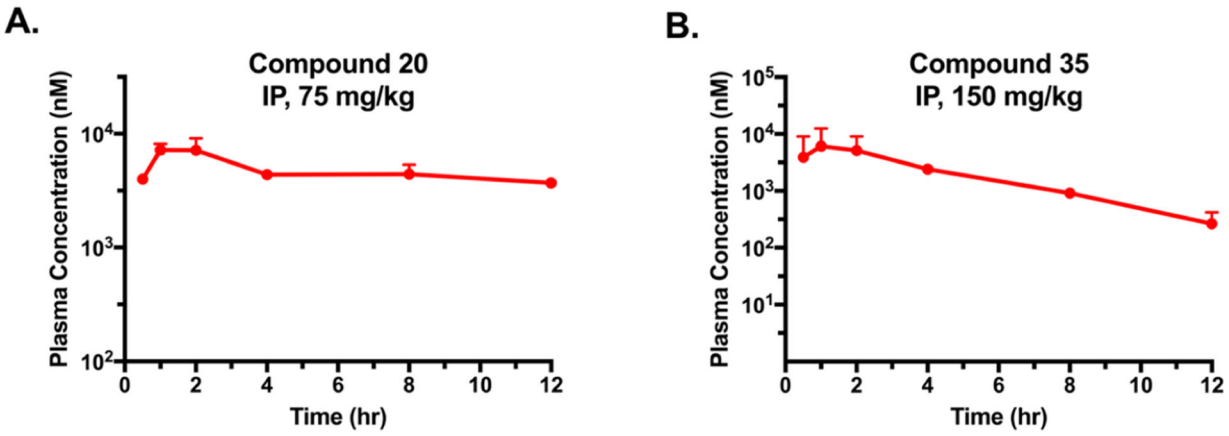


Figure 11.

AKT degraders **20** and **35** are bioavailable in mice. Plasma concentrations of **20** (A) following a single 75 mg/kg IP injection over 12 h and **35** (B) following a single 150 mg/kg IP injection over 12 h in male Swiss Albino mice. Experiments were carried out in biological triplicate per time point, with the values representing mean concentrations ±SEM.

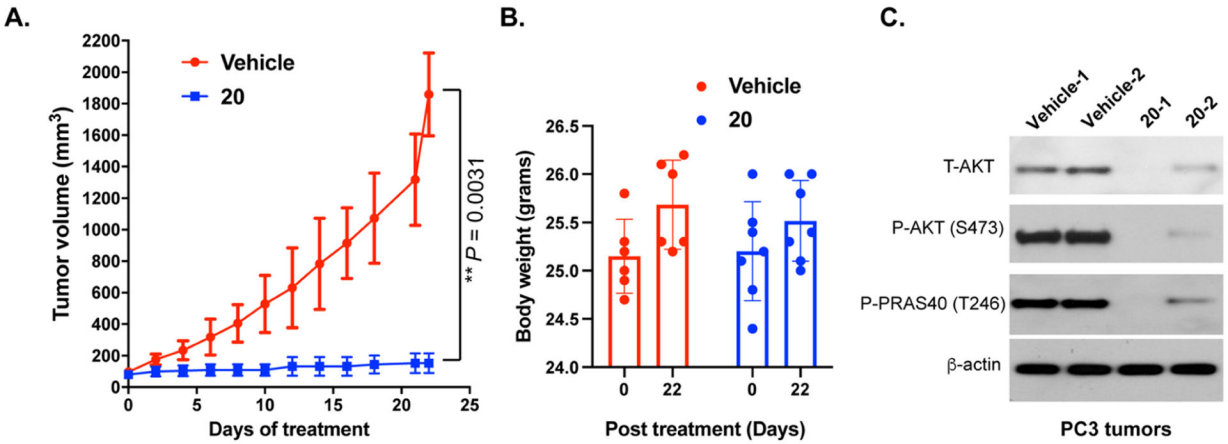
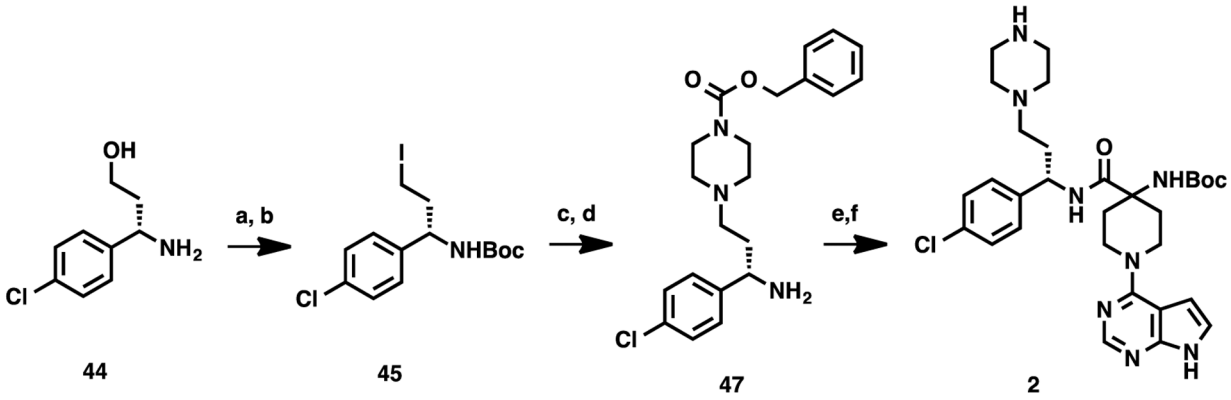


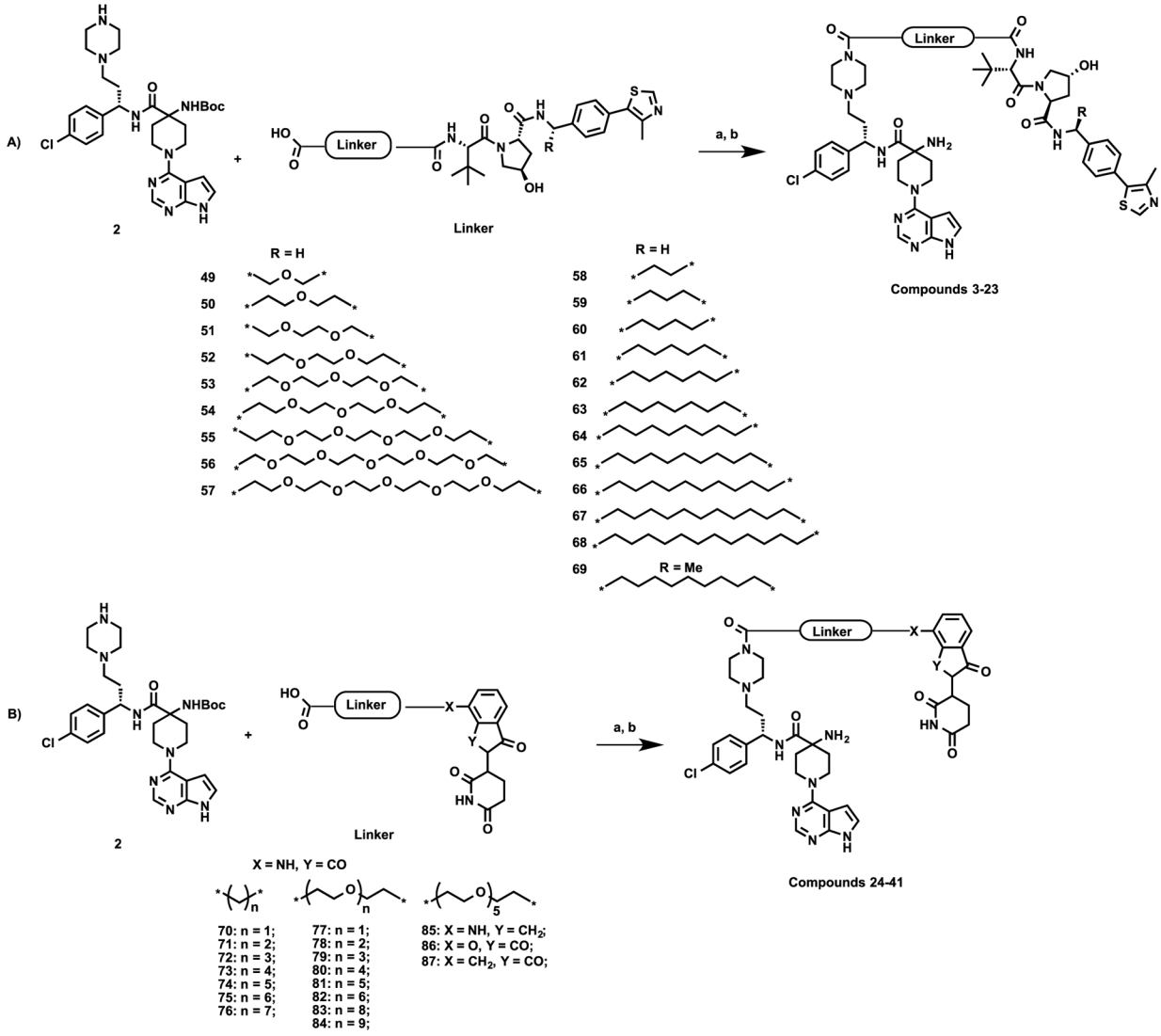
Figure 12.

AKT degrader **20** suppresses the tumor growth in vivo in a PC3 cell line xenograft mouse model. (A) Nude mice (*Foxn1*^{nu}, athymic nude) bearing established PC-3 xenografts were treated with vehicle (n = 6) or **20** (n = 7, 75 mg/kg, i.p., once daily) for 22 days. Data points represent mean tumor volume ±SE. (B) Body weights of mice at the beginning and the end of the in vivo studies. (C) Western blotting analysis of P-AKT and T-AKT protein levels, and downstream signaling inhibition (P-PRAS40) in the tumor samples isolated from the nude mice treated with vehicle or **20**.



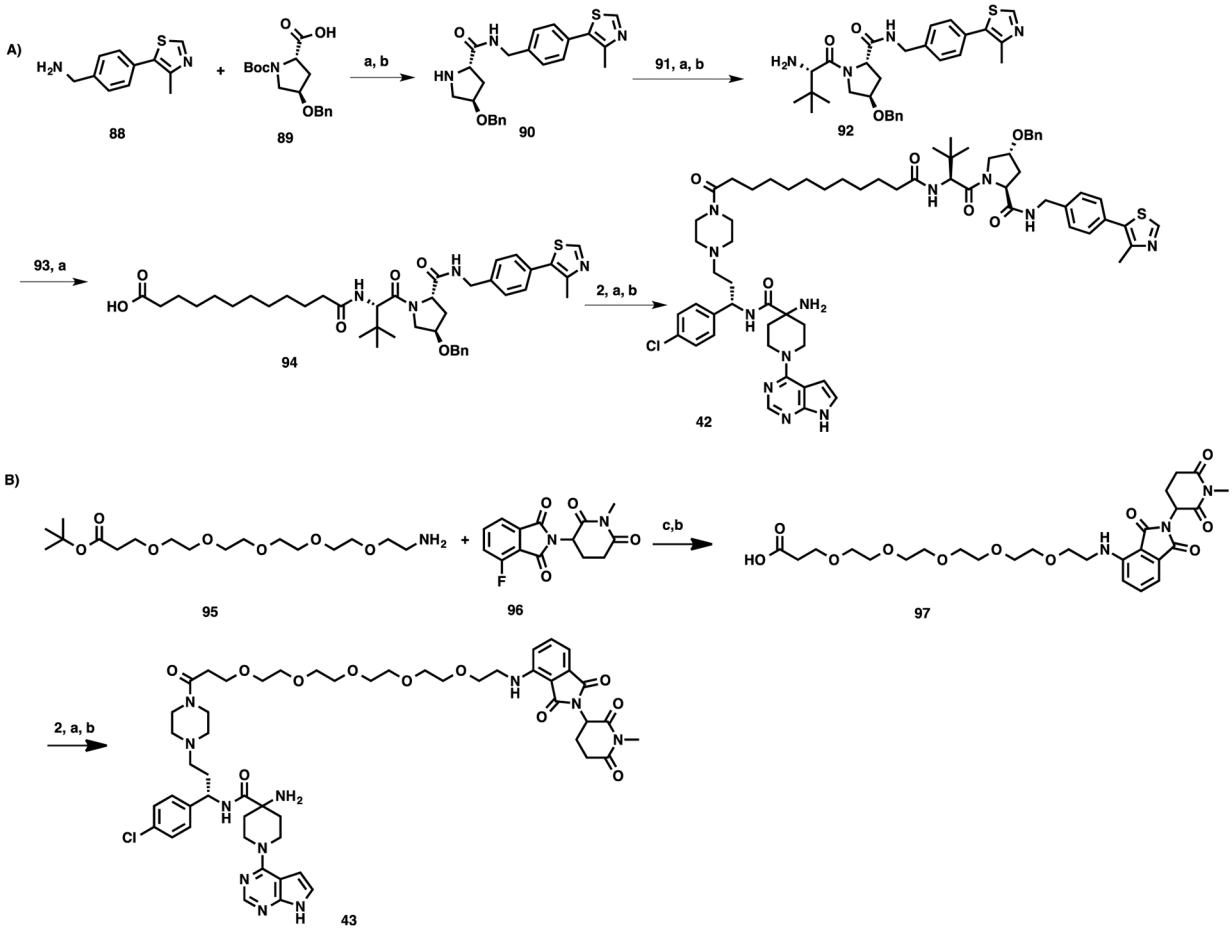
Scheme 1. Synthesis of Key Intermediate 2[a]

[a]Reaction conditions: (a) Boc_2O, NEt_3, rt, 2 h; (b) PPh_3, I_2, imidazole, DCM, 4 h; (c) Benzyl piperazine-1-carboxylate (**46**), K_2CO_3, CH_3CN, 80 °C, overnight; (d) TFA/DCM, rt, 30 min; (e) 4-[(*tert*-butoxycarbonyl)amino]-1-(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidine-4-carboxylic acid (**48**), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxy-7-azabenzo-triazole (HOAt), *N*-methylmorpholine (NMM), dimethyl sulfoxide (DMSO), rt; and (f) Pd/C, H_2, MeOH, rt.



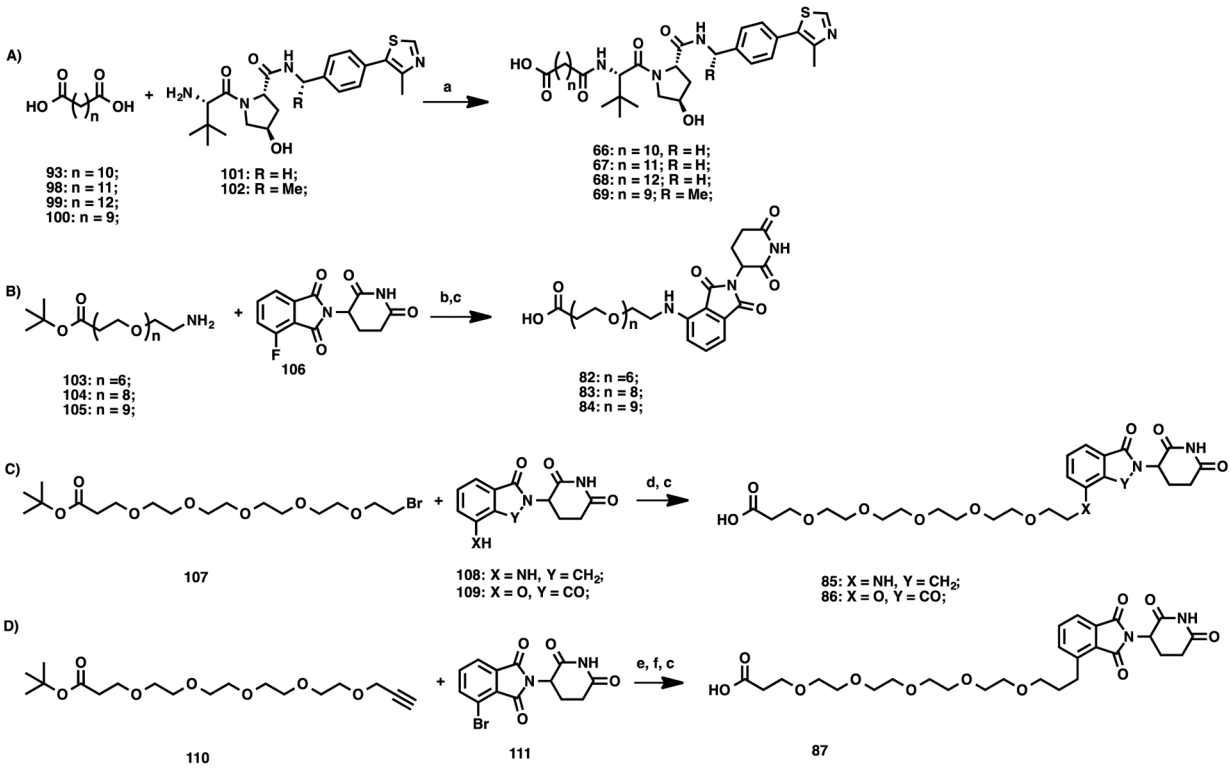
Scheme 2. Syntheses of Compounds 3–41[a]

[a]Reaction conditions: (a) EDCI, HOAt, NMM, DMSO, rt, 12 h; (b) TFA, DCM, rt, 30 min.



Scheme 3. Syntheses of Negative Control Compounds 42–43[a]

[a]Reaction conditions: (a) EDCI, HOAt, NMM, DMSO, rt, 12 h; (b) TFA, DCM, rt, 30 min; and (c) *N*-methyl-2-pyrrolidone (NMP), diisopropylethylamine (DIPEA), MW, 100 °C, 1 h.



Scheme 4. Syntheses of Linkers 66–69 and 82–87[a]

[a]Reaction conditions: (a) EDCI, HOAt, NMM, DMSO, rt, 12 h; (b) NMP, DIPEA, MW, 100 °C, 1 h; (c) TFA, DCM, rt, 30 min; (d) $NaHCO_3$, KI, DMF, 60 °C, overnight; (e) $Pd(PPh_3)_2Cl_2$, CuI, NEt_3, DMF, 70 °C, 2 h; and (f) Pd/C, H_2, MeOH, rt, 1 h.