Supplementary information

Systematic Exploration of Different E3 Ubiquitin Ligases: An Approach Towards Potent and Selective CDK6 Degraders

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Electronic Supplementary Material (ESI) for Chemical Science.
# Table of Content

Supplementary Schemes, Tables and Figures.........................................................3

Supplementary Information: Biology................................................................. 27

A. Cell Lines........................................................................................................... 27

B. CRISPR-mediated Knockout of CRBN and VHL........................................ 27

C. Immunoblotting............................................................................................... 28

D. Cell Viability Assay.......................................................................................... 29

E. Cell Migration Assay....................................................................................... 29

F. Statistical Analysis............................................................................................ 29

Supplementary Information: Chemistry............................................................ 30

G. General Remarks............................................................................................. 32

H. Synthesis of CRBN-based PROTACs............................................................. 33

J. Synthesis of VHL-based PROTACs................................................................. 45

K. Synthesis of IAP-based degraders................................................................. 77

L. Synthesis of MDM2-based PROTACs............................................................ 85

M. Selected Analytical Spectra........................................................................... 90

Supplementary Information: Physical Chemistry............................................. 93

N. Molecular Descriptor Calculation................................................................. 93

O. Physicochemical Measurements................................................................. 93

References............................................................................................................ 95
**Scheme S1**  Synthesis of the VHL-addressing CDK4/6 degrader 22. Reagents and conditions: (a) 8-Chloro-1-octanol, BAIB/TEMPO, MeCN/H$_2$O, rt, 16 h; (b) HATU, DIPEA, DMF, rt, 16 h; (c) NaI, acetone, 60 °C, 48 h; (d) DIPEA, DMSO/DMF, 80 °C, 24 h.
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**Table S1** Overview on synthesized CRBN-based CDK-PROTACs. Degrader score (Deg_S), topological polar surface area (TPSA), number of rotatable bonds (NRotB), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA)
<table>
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**Table S2** Overview on synthesized VHL-based CDK-PROTACs. Degrader score (Deg_S), topological polar surface area (TPSA), number of rotatable bonds (NRotB), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA)
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**Table S3** Overview on synthesized IAP-based CDK-degraders. Degradation score (Deg_S), topological polar surface area (TPSA), number of rotatable bonds (NRotB), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA)
### Table S4 Overview on synthesized MDM2-based PROTACs.

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*Defined value. Degradation score (Deg_S), topological polar surface area (TPSA), number of rotatable bonds (NRotB), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA)*
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Table S5  Activities of the MDM2-addressing CDK4/6 degrader. $^a$Experimental distribution coefficient at pH 7.4. $^b$CDK4 or CDK6 degradation indicated as remaining CDK4 or CDK6 levels after 16 h treatment of each compound at the indicated concentration. Percentage values are normalized to DMSO-treated MM.1S cells and the respective loading controls (100%). All of the data were the average of at least three independent experiments. $^c$Selectivity ratio for the degradation of CDK6 over CDK4. $^d$Not determined.
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**Table S6** Overview on different VHL ligands incorporated into final PROTACs (see also Table 2 and Table 3, respectively).
Amide-connected CRBN-addressing PROTACs degrade CDK4/6. MM.1S cells were treated with 0.1 µM pomalidomide (3, POM), the CDK6-selective degrader BSJ-03-123 (8),\(^2\) negative control 21, or PROTACs 11-20 for 16 h. In addition to the target proteins CDK4/6, the expression levels of CRBN as well as the neo-substrates IKZF1 and IKZF3 were blotted.

(A) CRBN-based PROTAC 11 induces CDK6-selective degradation in a dose-dependent manner. MM.1S cells were treated at indicated concentrations for 16 h. The CDK6-selective degrader BSJ-03-123 (8) was used as a standard; (B) Time course experiment with CDK4/6d 11 versus BSJ-03-123. MM.1S cells were treated at 0.1 µM for the indicated time.
Fig. S3  CDK4/6 degradation induced by PROTAC 11 is mediated via the E3 ubiquitin ligase CRL4<sup>CRBN</sup> and subsequent proteasomal degradation. (A) MM.1S cells were treated for 16 h with vehicle, pomalidomide (3, POM), the CDK6-selective degrader BSJ-03-123 (8), negative control 21, or PROTAC 11 at the indicated concentrations. Co-treatment of 11 and POM (100-fold excess) significantly reduced the effects on CDK4/6; (B) MM.1S cells were treated with 10 µM of the proteasome inhibitor MG132, 10 µM of the NEDD8-activating enzyme inhibitor MLN4924 or 0.1 µM PROTACs alone for 3 h or cells were pre-treated with inhibitors MG132 / MLN4924 for 1 h before addition of PROTACs for additional 3 h; (C) RPMI-8226 wild-type or RPMI-8226 <i>CRBN</i><sup>−/−</sup> cells were treated with compounds at 0.1 µM for 16 h.
CRISPR dependency scores for selected E3 ligases

**Fig. S4** CRISPR dependency scores for selected E3 ligases. CRISPR dependency scores from the DepMap database representing specific genetic vulnerabilities of cancer cells. Lower values indicate higher relative deleteriousness for cell growth.
Fig. S5  VHL-based PROTACs of the ‘amide’ subseries degrade CDK4/6. (A) MM.1S cells were treated with 0.1 µM of the VHL ligand VH298 (4), BSJ-03-123 (8), negative control 26, or PROTACs 22-25 and 27 for 16 h; (B) PROTAC 27 induces CDK6-selective degradation in a dose-dependent manner. MM.1S cells were treated with compounds for 16 h; (C) Quantification of (B) and calculation of the DC_{50}-value; (D) Time course experiment with the CDK6-selective, VHL-based PROTAC 27. MM.1S cells were treated at 0.1 µM for the indicated time.
CDK4/6 degradation induced by PROTAC 27 is mediated via the E3 ubiquitin ligase CRL2\textsuperscript{VHL} and subsequent proteasomal degradation. (A) MM.1S cells were treated for 16 h with vehicle, VH298 (4), the CDK6-selective degrader BSJ-03-123 (8), negative control 26, or PROTAC 27 at the indicated concentrations. Co-treatment of 27 and VH298 (4, 100-fold excess) significantly reduced the effects on CDK4/6; (B) MM.1S cells were treated with 10 µM of the proteasome inhibitor MG132, 10 µM of the NEDD8-activating enzyme inhibitor MLN4924 or 0.1 µM PROTACs alone for 3 h or cells were pre-treated with inhibitors MG132 / MLN4924 for 1 h before addition of PROTACs for additional 3 h; (C) RPMI-8226 wild-type or RPMI-8226 VHL\textsuperscript{−/−} cells were treated with compounds at 0.1 µM for 16 h.
**Fig. S7** CDK4/6 inhibition with palbociclib exhibits a stronger inhibitory effect of the kinase function of CDK4/6 compared to CDK4/6 degradation by CRBN- and VHL-based PROTACs. MM.1S cells were treated with BSJ-03-123 (B), 27, 34, and palbociclib for 16 h at the indicated concentrations. Target proteins CDK4 and CDK6, along with retinoblastoma (Rb) and phosphorylated retinoblastoma (p-Rb) (Ser807/811) were blotted.

**Fig. S8** VHL-based PROTAC 34 of the ‘phenoxy’ subseries induces CDK6-selective degradation in a dose-dependent manner. (A) MM.1S cells were treated with 1 µM of the VHL ligand VH298 (4), BSJ-03-123 (B), negative control 32, or PROTAC 34 at the indicated concentrations for 16 h; (B) Quantification of (A) and calculation of the DC50 value for CDK4. Parts of the blot (A) and the DC50 curve for CDK6 degradation by PROTAC 34 are presented in the main body of the manuscript.
**Fig. S9**  Long-term treatments with (A) BSJ-03-123 (8) versus VHL-based PROTAC 27 of the ‘amide’ subseries (B) and PROTAC 34 of the ‘phenoxy’ subseries (C). MM.1S cells were treated with PROTACs at 0.1 µM for the indicated time without washout.
**Fig. S10** Washout experiments with (A) BSJ-03-123 (8) versus VHL-based PROTAC 27 of the ‘amide’ subseries (B) and PROTAC 34 of the ‘phenoxy’ subseries (Fig. 3E). (C) Quantification of (A), (B), and Fig. 4E. MM.1S cells were treated with PROTACs at 0.1 µM for 16 h before washout with PBS (= 0 h), then kept in plain media until indicated time points.
**Fig. S11** VHL-based PROTACs are as potent as palbociclib (Palbo) on decreasing cell viability in various cell lines. (A) Human multiple myeloma cell lines MM.1S, LP-1, and AMO-1; (B) acute myeloid leukemia cell lines MOLM-13, HEL, KG-1; (C) chronic myelogenous leukemia cell line K562; and (D) acute lymphoblastic leukemia cell line Nalm-6 were treated with Palbo, 27, or 34 for 96 h at 0.1 µM or 1 µM. All results were normalized to non-treated conditions and data represent mean ±SD of biological triplicates.

**Fig. S12** CRBN- and VHL-based PROTACs are capable of inducing CDK6 degradation in human and murine cell lines. MM.1S cells, Ba/F3 or 32D cells were treated with the CRBN-based PROTAC 11 and VHL-addressing CDK4/6d 22 for 16 h at 1 µM.
Fig. S13  Sensitivity of human cancer cell lines to CDK4/6 PROTACs. (A) Palbociclib-sensitive MDA-MB-231 cells were treated with control compounds in a dose-dependent manner for 96 h. Viability data were normalized to non-treated conditions and data represent mean ±SD of biological triplicates; (B) BT549 were treated with palbociclib, VHL-based CDK4/6 PROTACs, and CRBN-based CDK4/6 PROTACs or control compounds in a dose-dependent manner for 96 h; (C) PROTAC-mediated CDK6-selective degradation in breast cancer. MDA-MB-231 cells were treated with the compounds at the concentrations indicated for 16 h. Whole cell extracts were collected and subjected to immunoblotting using primary antibodies against the proteins as indicated.
**Fig. S14** Cell migration assay in MDA-MB-231 cells. (A) Wound gap pictures; (B) Column diagram showing the open wound area. Cells were pre-treated with 0.1 µM of the corresponding compound, followed by the scratch and an additional treatment with 0.1 µM. Pictures were acquired 24 h post-scratch.
**Fig. S15** Putative MDM2-based degrader 37 does not induce CDK4/6 degradation. MM.1S cells were treated with idasanutlin (6), the CDK6-selective degrader BSJ-03-123 (8), or degrader 37 at the indicated concentrations for 16 h. In addition to the target proteins CDK4/6, the expression levels of the E3 ligase MDM2 and the tumour suppressor molecule p53 as well as its downstream effector p21 were blotted.

**Fig. S16** MDM2i and MDM2-based PROTACs induce lead to diminished CDK4 levels in a dose-and MDM2/p21-dependent manner. MM.1S cells were treated with idasanutlin (6), AMG232, or bifunctional probe 95 at the indicated concentrations for 16 h.
Off-target CDK4/6 protein decrease and p21 stabilization resulting from treatment with MDM2i or functionalized MDM2-ligands is dependent on the p53 status. The human chronic myelogenous leukemia (CML) cell line K562 and the human multiple myeloma cell line MM.1S were treated with 1 µM of MDM2i idasanutlin (6), CDK6-selective degrader BSJ-03-123 (8), MDM2i AMG232, putative MDM2-based CDK4/6 degrader 37, and MDM2-based bifunctional probe 95 for 16 h.

Fig. S17

<table>
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 OM90 | Idasanutlin | BSJ-03-123 | AMG232 | 37 | 95 | OM90 | Idasanutlin | BSJ-03-123 | AMG232 | 37 | 95 |
Supplementary Information: Biology

A. Cell Lines

Cell lines MM.1S, LP-1, AMO-1, MOLM13, HEL, KG-1, K562, Nalm6, RPMI-8226, 32D, Ba/F3, and HEK293T were obtained from the American Type Culture Collection (ATCC) or the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ). HEK293T cells were maintained in DMEM medium (Gibco) while all other cells were maintained in RPMI-1640 medium (Biochrom). All media contained 10% fetal bovine serum (FBS) (Merck Millipore) and was supplemented with 1% penicillin/streptomycin (Thermo Fisher) and 1% L-glutamine (Merck Millipore). Cell lines 32D and Ba/F3 were cultured in the presence of murine IL-3 (Preprotech). Cells were maintained at 37 °C with 5% CO₂ in humidified atmosphere.

Human breast cancer cell lines MDA-MB-231 and BT549 were cultured in RPMI-1640 medium (Lonza) supplemented with 10% fetal bovine serum (Gibco) and were cultured at 37 °C in a humidified 5 % CO₂ incubator.

B. CRISPR-mediated Knockout of CRBN and VHL

For the generation of knock-out cell lines utilizing CRISPR/Cas9, plasmids pLKO5d.SSF.SpCas9.P2a.BSD and pLKO5.hU6.sgRNA.dTom (gifts from D. Heckl, Hannover Medical School) were used. Single-guide RNAs targeting CRBN and VHL were cloned into the lentiviral vector pLKO5.hU6.sgRNA.dTom. Guide RNA sequences are as follows: CRBN: 5'-GTCCTGCTGATCTCCTTCGC-3'; VHL: 5'-GAGATGCAGGGACACACGAT-3'.

HEK293T cells were transfected with lentiviral vectors along with plasmids encoding for envelope proteins and packaging proteins. Viral supernatants were harvested 48 hours post-transfection and were used to transduce multiple myeloma cell line RPMI-8226. RPMI-8226 cells were first transduced with virus containing pLKO5d.SSF.SpCas9.P2a.BSD and were selected with blasticidin (Invivogen), followed by transduction with sgRNA-expressing lentiviral vectors. Transduction success was confirmed through FACS analysis 48 hours post-transduction with a minimum efficiency of 95% tomato fluorescence. CRBN or VHL knockout was confirmed through Western blot analysis.
C. Immunoblotting

Multiple myeloma, leukaemia, and murine cell lines:

After respective designated treatments, cells were washed with 1x PBS and lysed in IP lysis buffer (Pierce) containing HALT protease and phosphatase inhibitor cocktail (Thermo Scientific). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with 5–20 µg of protein loaded per sample. Gel electrophoresis was carried out at a constant voltage. Proteins were transferred onto Immobilon-P transfer membranes (Millipore). Membranes were blocked with 5% milk in Tris Buffered Saline with 0.05% Tween 20 (TBS-T) followed by overnight incubation at 4 °C with shaking in primary antibodies diluted in 5% BSA in TBS-T. After incubation with respective secondary HRP-conjugated antibodies diluted in 5% milk in TBST, detection of proteins was performed using either WesternBright ECL HRP substrate or WesternBright Sirius HRP substrate (Advansta). Chemiluminescence was detected with ChemiDoc™ XRS+ System (Bio-Rad). For further detection of other proteins on the same membrane, membranes were subjected to incubation in Restore Western Blot Stripping Buffer, followed by re-blocking, and further probing of proteins was carried out. Quantification was performed using ImageJ (National Institutes of Health). Antibodies used are as follows: CDK6 (Cell Signaling, clone DCS83, 3136), CDK4 (Cell signaling, clone D9G3E, 12790), Rb (Cell Signaling, clone 4H1, 9309), phospho-Rb (Ser807/811) (Cell Signaling, 9308), CRBN (Sigma, HPA045910), IKZF1 (Cell Signaling, clone D6N9Y, 14859), IKZF3 (Cell Signaling, clone D1C1E, 15103), VHL (Cell Signaling, 68547), p53 (Cell Signaling, clone 7F5, 2527), p21 Waf1/Cip1 (Cell Signaling, clone 12D1, 2947), MDM2 (Santa Cruz, clone SMP14, sc-965), BIRC2 (BioRad, clone AB01/3B4, VMA00532), c-IAP2 (Cell Signaling, clone 58C7, 3130), XIAP (Cell Signaling, clone D228W, 14334), and α-Tubulin (Sigma, clone B512, T5168).

Breast cancer cell lines:

To examine the effect of PROTAC-mediated target degradation, cells were treated with compounds at indicated concentrations for 16 hr. Whole cell extract was harvested in Laemmli sample buffer and subjected to SDS-PAGE followed by immunoblotting using primary antibodies raised against CDK4 (Cell Signaling Technology, #12790), CDK6 (Cell Signaling Technology, #3136), phospho-RB_Ser807/811 (Cell Signaling Technology, #8516), RB (Cell Signaling Technology, #9309), VHL (Cell Signaling Technology, #68547), CRBN (Sigma, #HPA045910), and GAPDH (Santa Cruz, #SC-47724). HRP-conjugated secondary antibodies used were from Jackson Immuno Research. Immunoblots were imaged using a ChemiDoc Touch Imaging System (Bio-Rad).
D. Cell Viability Assay

*Multiple myeloma, leukaemia, and murine cell lines:*

Cells were seeded in 96-well plates with media containing indicated concentrations of respective compounds and plates were incubated at 37 °C for 96 hours. Readout was performed with CellTiter-Glo® Luminescent Cell Viability Assay (Promega) according to the manufacturer’s protocol and luminescence was measured with POLARStar Omega plate reader (BMG LabTech). All results were normalized to non-treated conditions and data represent mean ±SD of biological triplicates.

*Breast cancer cell lines:*

To access the compound effect on cell viability, cells were plated in 384-well tissue culture-treated plates (Greiner Bio One). Twenty-four hours later, tested compounds were added into culture to the desired concentrations. Cell viability was measured by using CellTiter-Glo® Luminescent Cell Viability Assay (Promega) at day 4 or day 7 post-treatment and normalized by non-treated controls.

E. Cell Migration Assay

In total 800,000 MDA-MB-231 breast cancer cells were seeded on uncoated 24-well plates (Starlab). After 24 h DMEM medium (Pan Biotech) was removed, wells were washed twice with PBS and incubated with 0.1 µM palbociclib, 0.1 µM 34, 0.1 µM VH298 or DMSO in fresh FCS-free medium. After 24 h of incubation, a scratch was conducted with a 100 µL pipette tip (Starlab), medium was removed, wells were washed twice with PBS and again incubated with fresh FCS-free medium containing the compounds at the aforementioned concentrations. Wound healing was observed microscopically for 24 h with 10-fold magnification (Axiovert 100, Zeiss, Germany). Migration speed was quantified according to the reduced scratch wound area over time.

F. Statistical Analysis

Dose response curves were modeled and plotted by using GraphPad Prism software (GraphPad Software). Statistical analysis was performed using GraphPad Prism software (GraphPad Software). Variance of biological replicates is represented as the standard error of the mean (SEM) or as the standard deviation (SD). 

29
G. General Remarks

Preparative column chromatography was performed using Merck silica gel 60 (63 – 200 mesh) or using an automated flash chromatography system CombiFlash Rf 200. Indicated compounds were purified using a reversed-phase HPLC system from Knauer (C18 column, 250 × 20 mm, Eurospher 100-10). Petroleum ether used was a mixture of alkanes boiling between 40 – 60 °C. Melting points were determined on a Büchi 510 oil bath apparatus or on a Reichelt hot-stage apparatus and were uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer, Bruker Avance 500 MHz NMR spectrometer or on a Bruker Avance III 600 MHz NMR spectrometer, respectively. NMR spectra were processed and analyzed in MestReNova. Chemical shifts are given in parts per million (ppm), coupling constants $J$ are given in Hertz, and spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). All multiplets related with $J$(C,F) couplings in $^{13}$C NMR spectra are centred. In case of overlapping extraneous solvent peaks, multiplet analyses in $^1$H NMR spectra were performed using qGSD (quantitative Global Spectral Deconvolution). In case of rotamers only the peaks for the major rotamer are given, resonance assignments were made on the basis of one- and two-dimensional NMR techniques which include $^1$H, $^{13}$C, DEPT, HSQC, and HMBC experiments. HRMS was recorded on a micrOTOF-Q mass spectrometer (Bruker) with ESI-source coupled with an HPLC Dionex UltiMate 3000 (Thermo Scientific). The purity and identity of the compounds were determined by HPLC-UV obtained on an LC-MS instrument (Applied Biosystems API 2000 LC/MS/MS, HPLC Agilent 1100) or separately on an LC instrument (Acquity UPLC) and mass spectrometer (Thermo Scientific Q Exactive Plus). The purity of all the final compounds was confirmed to be ≥95% purity by LC.
H. Synthesis of CRBN-based PROTACs

General Procedure I: HATU-mediated coupling with palbociclib. The corresponding protected CRBN-linker conjugate (0.2 mmol) was dissolved in dry CH₂Cl₂ (5 mL) and TFA (5 mL) and stirred at 40 °C for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. Subsequently, the crude acid was dissolved in dry DMF (12 mL) and DIPEA (0.14 mL, 0.8 mmol) was added, followed by the addition of HATU (0.22 mmol). After stirring for 5 minutes, palbociclib (90 mg, 0.2 mmol) was added to the mixture. It was stirred at room temperature for 16 h, after which half-saturated brine (50 mL) was added, and the product was extracted with EtOAc (3 × 25 mL). The combined organic phases were washed with brine (50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo.

Palbociclib (1)

This compound was used as commercially supplied (Activate Scientific).

$^1$H NMR (600 MHz, 308 K, DMSO-d₆) δ 1.51 – 1.63 (m, 2H), 1.70 – 1.80 (m, 2H), 1.82 – 1.95 (m, 2H), 2.19 – 2.27 (m, 2H, 2''-H, 3'''-H), 2.30 (s, 3H, CH₃), 2.41 (s, 3H, 10-H), 2.81 – 2.88 (m, 4H), 3.02 – 3.08 (m, 4H, 2''-H, 3'''-H), 3.14 – 3.27 (m, 1H, 4''-H), 5.81 (p, J = 8.9 Hz, 1H, 1'''-H), 7.42 (dd, J = 3.1, 9.1 Hz, 1H, 4'-H), 7.82 (d, J = 9.0 Hz, 1H, 3'-H), 8.02 (d, J = 3.0 Hz, 1H, 6'-H), 8.93 (s, 1H, 4-H), 10.00 (s, 1H, NH); $^{13}$C NMR (151 MHz, 308 K, DMSO-d₆) δ 13.12 (CH₃), 25.17 (C-3'''), 27.65 (C-2''), 31.39 (C-10), 45.52, 49.55 (C-2'', C-3'''), 53.04 (C-1'''), 106.67 (C-4a), 115.34 (C-3'), 124.68 (C-4'), 129.32 (C-6), 135.45 (C-6'), 142.17 (C-5'), 144.22, 144.25 (C-8a, C-2''), 154.89 (C-5), 158.34 (C-4), 158.72 (C-2), 160.89 (C-7), 202.49 (C-9).
This compound was synthesized as reported previously. The crude material was purified by column chromatography (CH$_2$Cl$_2$/EtOH 19:1) to yield the title compound (free amine) as a yellow solid.

Yield (70%); mp 128 – 134 °C; $R_f = 0.29$ (CH$_2$Cl$_2$/MeOH 19:1); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.57 (t, $J$ = 5.8 Hz, 2H), 1.72 – 1.78 (m, 2H), 1.82 – 1.92 (m, 2H), 1.95 – 2.05 (m, 1H), 2.21 – 2.24 (m, 2H), 2.29 (s, 3H), 2.41 (s, 3H), 2.59 (t, $J$ = 5.0 Hz, 4H), 2.81 – 2.92 (m, 1H), 2.97 (s, 2H), 3.18 (t, $J$ = 5.1 Hz, 4H), 3.22 – 3.46 (m, 8H), 3.47 – 3.54 (m, 8H), 3.57 (t, $J$ = 5.4 Hz, 2H), 5.03 (dd, $J$ = 5.4, 12.8 Hz, 1H), 5.79 (q, $J$ = 8.7 Hz, 1H), 6.55 (t, $J$ = 5.8 Hz, 1H), 7.00 (dd, $J$ = 7.1 Hz, 2H), 7.08 (d, $J$ = 8.5 Hz, 1H), 7.44 (dd, $J$ = 3.1, 8.9 Hz, 1H), 7.54 (t, $J$ = 7.7 Hz, 1H), 7.70 (t, $J$ = 5.8 Hz, 1H), 7.83 (d, $J$ = 9.1 Hz, 1H), 8.04 (d, $J$ = 3.0 Hz, 1H), 8.92 (d, $J$ = 1.1 Hz, 1H), 10.00 (s, 1H), 11.05 (br s, 1H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 13.76, 22.31, 25.25, 27.71, 31.14, 31.45, 38.28, 41.87, 48.44, 48.74, 52.70, 53.10, 55.04, 61.27, 69.05, 69.14, 69.73, 69.95, 70.05, 106.76, 109.43, 110.82, 115.31, 117.55, 124.86, 129.41, 132.23, 135.52, 136.35, 142.23, 143.55, 144.49, 146.56, 154.93, 158.38, 158.72, 160.94, 167.44, 169.10, 169.27, 170.19, 172.94, 202.60; LC-MS (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-450 nm), $t_R = 11.30$ min, 99% purity, m/z [M + H]$^+$ calcd for C$_{47}$H$_{57}$N$_{11}$O$_{10}$, 936.43; found, 936.7; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{47}$H$_{57}$N$_{11}$O$_{10}$, 936.4363; found, 936.4364.

tert-Butyl 6-(benzyloxycarbonylamino)hexanoate (38)

This compound was synthesized as we described previously.
4-Fluoro-thalidomide (39)

This compound was synthesized as we described previously.\(^8\)

CRBN-6 building block (40)

This compound was synthesized as we described previously.\(^7\)

CRBN-based PROTAC 11

This compound was prepared using the General Procedure I and CRBN building block 40 (89 mg). The crude product was purified by column chromatography (gradient of CH\(_2\)Cl\(_2\)/EtOH 29:1 to 19:1) to obtain a yellow solid.

Yield (66 mg, 40%); mp 172 – 176 °C; \(R_f = 0.64\) (EtOAc/EtOH 9:1); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 1.32 – 1.44 (m, 2H), 1.50 – 1.65 (m, 6H), 1.69 – 1.81 (m, 2H), 1.83 – 1.94 (m, 2H), 1.97 – 2.07 (m, 1H), 2.18 – 2.27 (m, 2H), 2.30 (s, 3H), 2.36 (t, \(J = 7.4\) Hz, 2H), 2.41 (s, 3H), 2.44 – 2.62 (m, 2H), 2.80 – 2.92 (m, 1H), 3.06 – 3.18 (m, 4H), 3.26 – 3.33 (m, 2H), 3.52 – 3.68 (m, 4H), 5.03 (dd, \(J = 5.5, 12.7\) Hz, 1H), 5.81 (p, \(J = 9.0\) Hz, 1H), 6.51 (t, \(J = 6.0\) Hz, 1H), 7.00 (d, \(J = 7.0\) Hz, 1H), 7.08 (d, \(J = 8.6\) Hz, 1H), 7.47 (dd, \(J = 3.1, 9.1\) Hz, 1H), 7.56 (dd, \(J = 7.0, 8.7\) Hz, 1H), 7.86 (d, \(J = 9.0\) Hz, 1H), 8.06 (d, \(J = 3.0\) Hz, 1H), 8.94 (s, 1H), 10.06 (s, 1H), 11.05 (br s, 1H); \(^1\)C NMR (126 MHz, DMSO-\(d_6\)) \(\delta\) 13.75, 22.32, 24.66, 25.25, 26.23, 27.71, 28.70, 31.13, 31.44, 32.26, 40.85, 41.94, 44.80, 48.67, 48.72, 49.10, 53.11, 106.80, 109.19, 110.53, 115.22, 117.34, 125.47, 129.46, 132.35, 136.08, 136.43, 142.20, 143.36, 144.92,
146.61, 154.92, 158.37, 158.68, 160.91, 167.44, 169.11, 170.20, 170.79, 172.92, 202.56; **LC-MS** (ESI) (90% H$_2$O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), $t_R = 11.64$ min, 99% purity, $m/z$ [M + H]$^+$ calcd for $\text{C}_{43}\text{H}_{48}\text{N}_{10}\text{O}_7$, 817.37; found, 816.9; **HRMS** (ESI) $m/z$ [M + H]$^+$ calcd for $\text{C}_{43}\text{H}_{48}\text{N}_{10}\text{O}_7$, 817.3780; found, 817.3769.

**CRBN-2-2-2 building block (41)**

![Diagram of CRBN-2-2-2 building block](image)

This compound was synthesized as we described previously.  

**CRBN-based PROTAC 12**

![Diagram of CRBN-based PROTAC 1](image)

This compound was prepared using the General Procedure I and CRBN building block 41 (95 mg). The crude product was purified by column chromatography (gradient of EtOAc/EtOH 15:1 to 12:1) to obtain a yellow solid.

Yield (57 mg, 33%); mp 92 – 96 °C; $R_f = 0.31$ (CH$_2$Cl$_2$/EtOH 19:1); $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 1.50 – 1.62 (m, 2H), 1.70 – 1.81 (m, 2H), 1.84 – 1.92 (m, 2H), 1.96 – 2.04 (m, 1H), 2.18 – 2.28 (m, 2H), 2.30 (s, 3H), 2.41 (s, 3H), 2.46 – 2.63 (m, 2H), 2.81 – 2.91 (m, 1H), 3.03 – 3.23 (m, 4H), 3.46 (q, $J = 5.6$ Hz, 2H), 3.49 – 3.81 (m, 10H), 4.19 (s, 2H), 5.03 (dd, $J = 5.5$, 12.8 Hz, 1H), 5.81 (p, $J = 8.9$ Hz, 1H), 6.59 (t, $J = 5.8$ Hz, 1H), 7.00 (d, $J = 7.0$ Hz, 1H), 7.12 (d, $J = 8.6$ Hz, 1H), 7.44 (dd, $J = 3.1$, 9.1 Hz, 1H), 7.55 (dd, $J = 7.1$, 8.6 Hz, 1H), 7.85 (d, $J = 9.0$ Hz, 1H), 8.04 (d, $J = 3.0$ Hz, 1H), 8.94 (s, 1H), 10.06 (s, 1H), 11.07 (s, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 13.76, 22.29, 25.25, 27.70, 31.13, 31.44, 40.96, 41.88, 44.18, 48.55, 48.72, 48.92, 53.09, 62.94, 68.97, 69.70, 69.73, 69.98, 69.98, 106.78, 109.41, 110.82, 115.11, 117.55, 125.40, 129.45, 132.23, 136.03, 136.37, 142.20, 143.26, 144.91, 146.54, 154.91, 158.38, 158.64, 160.90, 167.41, 167.45, 169.10, 170.20, 172.91, 202.57; **LC-MS** (ESI) (90% H$_2$O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), $t_R = 8.39$ min, 98% purity, $m/z$ [M + H]$^+$
calcd for C_{43}H_{48}N_{10}O_{9}, 849.36; found, 849.5; **HRMS** (ESI) \( m/z \) [M + H]\(^+\) calcd for C_{43}H_{48}N_{10}O_{9}, 849.3678; found, 849.3619.

**CRBN-2-2-2-2 building block (42)**

![CRBN-2-2-2-2 building block (42)](image)

This compound was synthesized as we described previously.³

**CRBN-based PROTAC 13**

![CRBN-based PROTAC 13](image)

This compound was prepared using the General Procedure I and CRBN building block 42 (104 mg). The crude product was purified by column chromatography (gradient of CH\(_2\)Cl\(_2\)/EtOH 29:1 to 15:1) to obtain a yellow solid.

Yield (82 mg, 46%); mp 140 – 144 °C; \( R_f \) = 0.26 (CH\(_2\)Cl\(_2\)/EtOH 19:1); \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta \): 1.46 – 1.64 (m, 2H), 1.69 – 1.78 (m, 2H), 1.81 – 1.95 (m, 2H), 1.97 – 2.06 (m, 1H), 2.18 – 2.27 (m, 2H), 2.30 (s, 3H), 2.41 (s, 3H), 2.44 – 2.62 (m, 2H), 2.73 – 2.92 (m, 1H), 3.08 – 3.20 (m, 4H), 3.44 (q, \( J = 5.5 \) Hz, 2H), 3.50 – 3.67 (m, 14H), 4.17 (s, 2H), 5.03 (dd, \( J = 5.5, 12.7 \) Hz, 1H), 5.81 (p, \( J = 8.9 \) Hz, 1H), 6.57 (t, \( J = 5.8 \) Hz, 1H), 7.00 (d, \( J = 7.0 \) Hz, 1H), 7.10 (d, \( J = 8.7 \) Hz, 1H), 7.46 (dd, \( J = 3.1, 9.1 \) Hz, 1H), 7.54 (dd, \( J = 7.1, 8.6 \) Hz, 1H), 7.86 (d, \( J = 9.0 \) Hz, 1H), 8.05 (d, \( J = 3.0 \) Hz, 1H), 8.93 (s, 1H), 10.05 (s, 1H), 11.05 (br s, 1H); \(^{13}\)C NMR (126 MHz, DMSO-\( d_6 \)) \( \delta \): 13.75, 22.29, 25.24, 27.70, 31.13, 31.43, 40.96, 41.86, 44.23, 48.58, 48.73, 48.98, 53.10, 69.05, 69.69, 69.84, 69.91, 69.97, 106.79, 109.42, 110.79, 115.16, 117.54, 125.46, 129.46, 132.22, 136.08, 136.33, 142.19, 143.31, 144.93, 146.54, 154.91, 158.36, 158.66, 160.90, 167.41, 167.46, 169.08, 170.18, 172.91, 202.55; **LC-MS** (ESI) (90% H\(_2\)O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-450 nm), \( t_R \) = 8.56 min, 99% purity, \( m/z \) [M + H]\(^+\) calcd for C_{45}H_{52}N_{10}O_{10}, 893.39; found, 893.4; **HRMS** (ESI) \( m/z \) [M + H]\(^+\) calcd for C_{45}H_{52}N_{10}O_{10}, 893.3941; found, 893.3923.
CRBN-2-2-2-5 building block (43)

This compound was synthesized as we described previously.  

CRBN-based PROTAC 14

This compound was prepared using the General Procedure I and CRBN building block 43 (112 mg). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$/EtOH 29:1 to 19:1) to obtain a yellow solid.

Yield (126 mg, 67%); mp 112 – 114 °C; $R_f$ = 0.40 (CH$_2$Cl$_2$/EtOH 19:1); $^1$H NMR (600 MHz, DMSO-$d_6$) δ 1.43 – 1.65 (m, 6H), 1.70 – 1.81 (m, 2H), 1.82 – 1.94 (m, 2H), 1.97 – 2.07 (m, 1H), 2.17 – 2.27 (m, 2H), 2.29 (s, 3H), 2.34 (t, $J$ = 7.1 Hz, 2H), 2.41 (s, 3H), 2.45 – 2.63 (m, 2H), 2.74 – 2.94 (m, 1H), 3.03 – 3.22 (m, 6H), 3.33 – 3.64 (m, 16H), 5.03 (dd, $J$ = 5.4, 12.9 Hz, 1H), 5.80 (t, $J$ = 8.9 Hz, 1H), 6.57 (t, $J$ = 5.8 Hz, 1H), 7.01 (d, $J$ = 7.1 Hz, 1H), 7.11 (d, $J$ = 8.6 Hz, 1H), 7.47 (dd, $J$ = 3.0 Hz, 1H), 7.55 (dd, $J$ = 7.1, 8.6 Hz, 1H), 7.86 (d, $J$ = 9.0 Hz, 1H), 8.06 (d, $J$ = 3.0 Hz, 1H), 8.93 (s, 1H), 10.07 (s, 1H), 11.07 (br s, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 13.80, 21.80, 22.34, 25.30, 27.75, 28.95, 31.17, 31.48, 32.14, 40.23, 40.87, 41.91, 44.82, 48.70, 48.76, 49.13, 53.14, 69.08, 69.65, 69.99, 70.02, 70.06, 70.24, 106.83, 109.43, 110.86, 115.24, 117.62, 125.54, 129.49, 132.27, 136.12, 136.40, 142.26, 143.40, 144.96, 146.61, 154.95, 158.41, 158.71, 160.95, 167.48, 169.12, 170.23, 170.88, 172.98, 202.66; LC-MS (ESI) (90% H$_2$O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-450 nm), $t_R$ = 8.68 min, 96% purity, m/z [M + H]$^+$ calcd for C$_{48}$H$_{58}$N$_{10}$O$_{10}$, 935.44; found, 935.5; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{48}$H$_{58}$N$_{10}$O$_{10}$, 935.4410; found, 935.4373.
CRBN-3-4-3-4 building block (44)

This compound was synthesized as we described previously.  

CRBN-based PROTAC 15

This compound was prepared using the General Procedure I and CRBN building block 44 (123 mg). The crude product was purified by column chromatography (gradient of EtOAc to EtOAc/EtOH 9:1) to obtain a yellow solid.

Yield (162 mg, 82%); mp 176 – 180 °C; Rf = 0.17 (CH₂Cl₂/EtOH 19:1); ¹H NMR (600 MHz, DMSO-d₆) δ 1.46 – 1.64 (m, 8H), 1.71 – 1.82 (m, 4H), 1.83 – 1.94 (m, 2H), 1.97 – 2.05 (m, 1H), 2.14 – 2.28 (m, 2H), 2.30 (s, 3H), 2.32 (t, J = 7.0 Hz, 2H), 2.41 (s, 3H), 2.46 – 2.62 (m, 4H), 2.80 – 2.92 (m, 1H), 3.02 – 3.22 (m, 6H), 3.31 – 3.37 (m, 8H), 3.43 (t, J = 5.9 Hz, 2H), 3.47 – 3.77 (m, 4H), 5.03 (dd, J = 5.4, 12.8 Hz, 1H), 5.81 (p, J = 8.9 Hz, 1H), 6.63 (t, J = 5.9 Hz, 1H), 7.00 (d, J = 7.0 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 7.48 (dd, J = 3.0, 9.1 Hz, 1H), 7.56 (dd, J = 7.0, 8.6 Hz, 1H), 7.76 (t, J = 5.6 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 8.06 (d, J = 3.0 Hz, 1H), 8.94 (s, 1H), 10.08 (s, 1H), 11.06 (br s, 1H); ¹³C NMR (151 MHz, DMSO-d₆) δ 13.77, 22.32, 25.27, 26.14, 26.22, 27.71, 27.95, 29.05, 29.61, 30.59, 31.14, 31.45, 36.02, 40.98, 44.64, 48.60, 48.71, 48.98, 53.10, 67.83, 68.03, 70.00, 70.21, 106.79, 109.26, 110.54, 115.21, 117.19, 125.47, 129.45, 132.36, 136.07, 136.39, 142.22, 143.36, 144.93, 146.58, 154.92, 158.40, 158.68, 160.92, 167.47, 169.00, 170.16, 170.23, 171.38, 172.95, 202.59; LC-MS (ESI) (90% H₂O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), tᵣ = 8.63 min, 98% purity, m/z [M + H]⁺ calcd for C₅₁H₆₃N₁₁O₁₀, 990.48; found, 990.9; HRMS (ESI) m/z [M + H]⁺ calcd for C₅₁H₆₃N₁₁O₁₀, 990.4832; found, 990.4852.
CRBN-6-5-5 building block (45)

This compound was synthesized as we described previously.  

CRBN-based PROTAC 16

This compound was prepared using the General Procedure I and CRBN building block 45 (123 mg). The crude product was purified by column chromatography (gradient of EtOAc to EtOAc/EtOH 9:1) to obtain a yellow solid.

Yield (123 mg, 62%); mp 110 – 112 °C; \( R_f = 0.60 \) (EtOAc/EtOH 9:1); \(^1\)H NMR (600 MHz, DMSO-\( d_6 \)) \( \delta \) 1.23 – 1.38 (m, 6H), 1.41 – 1.62 (m, 14H), 1.71 – 1.81 (m, 2H), 1.82 – 1.94 (m, 2H), 1.97 – 2.05 (m, 1H), 2.17 – 2.27 (m, 2H), 2.29 (s, 3H), 2.35 (t, \( J = 7.2 \) Hz, 2H), 2.41 (s, 3H), 2.44 – 2.63 (m, 2H), 2.80 – 2.93 (m, 1H), 3.06 – 3.18 (m, 4H), 3.21 – 3.42 (m, 10H), 3.59 (q, \( J = 4.9, 5.5 \) Hz, 4H), 5.03 (dd, \( J = 5.5, 12.8 \) Hz, 1H), 5.81 (p, \( J = 8.9 \) Hz, 1H), 6.48 (t, \( J = 5.9 \) Hz, 1H), 6.99 (d, \( J = 7.0 \) Hz, 1H), 7.05 (d, \( J = 3.1 \) Hz, 1H), 7.48 (dd, \( J = 3.1, 9.1 \) Hz, 1H), 7.55 (dd, \( J = 7.1, 8.5 \) Hz, 1H), 7.87 (d, \( J = 9.0 \) Hz, 1H), 8.06 (d, \( J = 3.1 \) Hz, 1H), 8.93 (s, 1H), 10.07 (s, 1H), 11.06 (br s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\( d_6 \)) \( \delta \) 13.79, 21.88, 22.34, 22.73, 25.29, 25.62, 26.30, 27.73, 28.82, 28.99, 29.23, 29.26, 29.33, 31.15, 31.47, 32.18, 40.23, 40.86, 41.98, 44.82, 48.71, 48.73, 49.13, 53.13, 69.89, 70.01, 70.07, 106.81, 109.19, 110.55, 115.20, 117.32, 125.51, 129.48, 132.35, 136.11, 136.44, 142.24, 143.38, 144.97, 146.61, 154.94, 158.40, 158.69, 160.93, 167.48, 169.13, 170.24, 170.84, 172.97, 202.63; LC-MS (ESI) (90% H\(_2\)O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), \( t_R = 10.98 \) min, 99% purity, \( m/z \) [M + H]\(^+\) calcd for C\(_{53}\)H\(_{68}\)N\(_{10}\)O\(_9\), 989.52; found, 989.7; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for C\(_{53}\)H\(_{68}\)N\(_{10}\)O\(_9\), 989.5262; found, 989.5244.
CRBN-6-6-6 building block (46)

This compound was synthesized as we described previously.²

CRBN-based PROTAC 17

This compound was prepared using the General Procedure I and CRBN building block 46 (128 mg). The crude product was purified by column chromatography (gradient of EtOAc to EtOAc/EtOH 9:1) to obtain a yellow solid.

Yield (118 mg, 58%); mp 110 – 112 °C; Rf = 0.65 (CH₂Cl₂/EtOH 9:1); ¹H NMR (600 MHz, DMSO-d₆) δ 1.21 – 1.37 (m, 10H), 1.39 – 1.60 (m, 14H), 1.71 – 1.81 (m, 2H), 1.82 – 1.94 (m, 2H), 2.01 (ddd, J = 3.1, 5.5, 10.4 Hz, 1H), 2.19 – 2.27 (m, 2H), 2.29 (s, 3H), 2.33 (t, J = 7.4 Hz, 2H), 2.41 (s, 3H), 2.46 – 2.63 (m, 2H), 2.80 – 2.92 (m, 1H), 3.04 – 3.20 (m, 4H), 3.21 – 3.47 (m, 10H), 3.51 – 3.70 (m, 4H), 5.03 (dd, J = 5.5, 12.8 Hz, 1H), 5.81 (p, J = 9.0 Hz, 1H), 6.48 (t, J = 5.9 Hz, 1H), 7.00 (d, J = 7.0 Hz, 1H), 7.06 (d, J = 8.6 Hz, 1H), 7.48 (dd, J = 3.0, 9.1 Hz, 1H), 7.55 (dd, J = 7.0, 8.6 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 8.06 (d, J = 3.0 Hz, 1H), 8.94 (s, 1H), 10.07 (s, 1H), 11.06 (br s, 1H); ¹³C NMR (151 MHz, DMSO-d₆) δ 13.79, 22.34, 24.84, 25.29, 25.63, 25.74, 26.30, 27.73, 28.83, 29.27, 29.33, 29.38, 29.40, 31.15, 31.48, 32.37, 40.23, 40.87, 41.98, 44.84, 48.70, 48.73, 49.14, 53.13, 70.00, 70.05, 70.08, 106.82, 109.20, 110.56, 115.23, 117.33, 125.50, 129.48, 132.36, 136.11, 136.45, 142.24, 143.39, 144.96, 146.62, 154.94, 158.42, 158.71, 160.94, 167.49, 169.14, 170.24, 170.87, 172.98, 202.63; LC-MS (ESI) (90% H₂O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), tᵣ = 11.72 min, 99% purity, m/z [M + H]⁺ calcd for C₅₅H₇₂N₁₀O₉, 1017.55; found, 1017.4; HRMS (ESI) m/z [M + H]⁺ calcd for C₅₅H₇₂N₁₀O₉, 1017.5557; found, 1017.5563.
CRBN-4-4-4-6 building block (47)

This compound was synthesized as we described previously. 

CRBN-based PROTAC 18

This compound was prepared using the General Procedure I and CRBN building block 47 (132 mg). The crude product was purified by column chromatography (gradient of EtOAc to EtOAc/EtOH 19:1) to obtain a yellow solid.

Yield (151 mg, 75%); mp 96 – 100 °C; \( R_f = 0.30 \) (CH\(_2\)Cl\(_2\)/EtOH 19:1); \(^1\)H NMR (600 MHz, DMSO-d\(_6\)) \( \delta \) 1.26 – 1.38 (m, 2H), 1.41 – 1.64 (m, 18H), 1.69 – 1.79 (m, 2H), 1.82 – 1.94 (m, 2H), 1.97 – 2.05 (m, 1H), 2.19 – 2.27 (m, 2H), 2.30 (s, 3H), 2.33 (t, \( J = 7.5 \) Hz, 2H), 2.41 (s, 3H), 2.44 – 2.61 (m, 2H), 3.21 – 3.41 (m, 14H), 2.74 – 2.92 (m, 1H), 3.04 – 3.19 (m, 4H), 3.51 – 3.66 (m, 4H), 5.03 (dd, \( J = 5.5, 12.7 \) Hz, 1H), 5.81 (p, \( J = 8.8 \) Hz, 1H), 6.52 (t, \( J = 6.1 \) Hz, 1H), 6.99 (d, \( J = 7.0 \) Hz, 1H), 7.06 (d, \( J = 8.6 \) Hz, 1H), 7.47 (dd, \( J = 3.1, 9.1 \) Hz, 1H), 7.55 (dd, \( J = 7.0, 8.5 \) Hz, 1H), 7.87 (d, \( J = 9.0 \) Hz, 1H), 8.06 (d, \( J = 3.0 \) Hz, 1H), 8.94 (s, 1H), 10.06 (s, 1H), 11.05 (br s, 1H); \(^{13}\)C NMR (126 MHz, DMSO-d\(_6\)) \( \delta \) 13.74, 22.31, 24.80, 25.24, 25.71, 25.75, 26.23, 26.65, 27.69, 29.24, 31.12, 31.42, 32.33, 40.83, 41.78, 44.80, 48.70, 49.10, 53.11, 69.69, 69.86, 69.91, 69.94, 70.00, 106.78, 109.22, 110.50, 115.16, 117.29, 125.45, 129.45, 132.35, 136.08, 136.35, 142.19, 143.35, 144.92, 146.55, 154.91, 158.35, 158.66, 160.90, 167.43, 169.06, 170.17, 170.81, 172.90, 202.53; LC-MS (ESI) (90% H\(_2\)O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), \( t_R = 10.72 \) min, 99% purity, \( m/z \) [M + H]\(^+\) calcd for C\(_{55}\)H\(_{52}\)N\(_{10}\)O\(_{10}\), 1017.55; found, 1033.4; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for C\(_{55}\)H\(_{52}\)N\(_{10}\)O\(_{10}\), 1033.5513; found, 1033.5506.
CRBN-6-(4)$_2$-6 building block (48)

This compound was synthesized as we described previously.

CRBN-based PROTAC 19

This compound was prepared using the General Procedure I and CRBN building block 48 (152 mg). The crude product was purified by column chromatography (gradient of EtOAc to EtOAc/EtOH 9:1) to obtain a yellow solid.

Yield (172 mg, 76%); mp 68 – 72 °C; $R_f = 0.57$ (EtOAc/EtOH 9:1); $^1$H NMR (600 MHz, DMSO-$d_6$) δ 1.27 – 1.33 (m, 6H), 1.41 – 1.61 (m, 22H), 1.71 – 1.81 (m, 2H), 1.83 – 1.93 (m, 2H), 1.97 – 2.05 (m, 1H), 2.19 – 2.28 (m, 2H), 2.29 (s, 3H), 2.33 (t, $J = 7.4$ Hz, 2H), 2.41 (s, 3H), 2.50 – 2.61 (m, 2H), 2.82 – 2.90 (m, 1H), 3.07 – 3.18 (m, 4H), 3.22 – 3.40 (m, 18H), 3.60 (d, $J = 5.1$ Hz, 4H), 5.03 (dd, $J = 5.4$, 12.8 Hz, 1H), 5.82 (q, $J = 8.8$ Hz, 1H), 6.49 (t, $J = 5.9$ Hz, 1H), 7.00 (d, $J = 7.0$ Hz, 1H), 7.06 (d, $J = 8.6$ Hz, 1H), 7.48 (dd, $J = 3.1$, 9.0 Hz, 1H), 7.55 (dd, $J = 7.0$, 8.6 Hz, 1H), 7.87 (d, $J = 9.0$ Hz, 1H), 8.06 (d, $J = 3.1$ Hz, 1H), 8.94 (s, 1H), 10.08 (s, 1H), 11.06 (br s, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 13.77, 22.33, 24.83, 25.27, 25.63, 25.74, 26.24, 26.29, 27.72, 28.82, 29.27, 29.33, 31.14, 31.45, 32.36, 40.23, 40.85, 41.98, 44.82, 48.70, 48.72, 49.13, 53.11, 69.87, 69.90, 69.93, 69.98, 70.02, 106.80, 109.19, 110.53, 115.17, 117.30, 125.48, 129.47, 132.35, 136.10, 136.42, 142.22, 143.37, 144.96, 146.60, 148.55, 158.39, 158.68, 160.92, 167.46, 169.11, 170.30, 170.83, 172.94, 202.58; LC-MS (ESI) (90% H$_2$O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), $t_R = 11.94$ min, 99% purity, $m/z$ [M + H]$^+$ calcd for $C_{61}H_{84}N_{10}O_{11}$, 1017.55; found, 1133.9; HRMS (ESI) $m/z$ [M + H]$^+$ calcd for $C_{61}H_{84}N_{10}O_{11}$, 1133.6430; found, 1133.6394.
This compound was synthesized as we described previously.  

CRBN-based PROTAC 20

This compound was prepared using the General Procedure I and CRBN building block 49 (152 mg). The crude product was purified by column chromatography (gradient of EtOAc to EtOAc/EtOH 9:1) to obtain a yellow solid.

Yield (172 mg, 52%); mp 80 – 82 °C; \( R_f = 0.67 \) (EtOAc/EtOH 9:1); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \( \delta \) 1.26 – 1.40 (m, 6H), 1.40 – 1.63 (m, 10H), 1.71 – 1.81 (m, 2H), 1.81 – 1.94 (m, 2H), 1.96 – 2.06 (m, 1H), 2.18 – 2.27 (m, 2H), 2.29 (s, 3H), 2.33 (t, \( J = 7.5 \) Hz, 2H), 2.41 (s, 3H), 2.50 – 2.62 (m, 2H), 2.79 – 2.92 (m, 1H), 3.06 – 3.19 (m, 4H), 3.23 – 3.29 (m, 2H), 3.29 – 3.39 (m, 4H), 3.41 – 3.50 (m, 20H), 3.55 – 3.64 (m, 4H), 5.03 (dd, \( J = 5.5, 12.8 \) Hz, 1H), 5.81 (p, \( J = 8.9 \) Hz, 1H), 6.48 (t, \( J = 5.9 \) Hz, 1H), 6.99 (d, \( J = 7.0 \) Hz, 1H), 7.06 (dd, \( J = 8.6 \) Hz, 1H), 7.48 (dd, \( J = 3.1, 9.1 \) Hz, 1H), 7.55 (dd, \( J = 7.1, 8.6 \) Hz, 1H), 7.87 (d, \( J = 9.0 \) Hz, 1H), 8.06 (d, \( J = 3.1 \) Hz, 1H), 8.93 (s, 1H), 10.07 (s, 1H), 11.06 (br s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \( \delta \) 13.79, 22.35, 24.83, 25.29, 25.58, 25.67, 26.32, 27.74, 28.84, 29.25, 29.31, 31.16, 31.48, 32.38, 40.23, 40.88, 42.00, 44.84, 48.71, 48.74, 49.14, 53.14, 69.66, 69.68, 69.98, 70.00, 70.41, 70.45, 106.82, 109.20, 110.57, 115.22, 117.34, 122.53, 129.49, 132.37, 136.12, 136.46, 142.25, 143.40, 144.96, 146.62, 154.95, 158.42, 158.70, 160.94, 167.50, 169.14, 170.25, 170.88, 172.99, 202.64; LC-MS (ESI) (90% H2O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-400 nm), \( t_R = 9.82 \) min, 99% purity, \( m/z \) [M + H]\(^+\) calcd for \( C_{59}H_{80}N_{10}O_{13} \), 1017.55; found, 1137.3; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for \( C_{59}H_{80}N_{10}O_{13} \), 1137.5979; found, 1137.5838.
4-Fluoro-2-(1-methyl-2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (50)

This compound was synthesized as we described previously.8

(−)CRBN-6 building block (51)

Compound 38 (161 mg, 0.5 mmol) was dissolved in dry EtOAc (10 mL) and treated with 10% Pd/C (10% m/m). The reaction mixture was stirred under H2 (1 atm, balloon) overnight. The mixture was filtered through celite and the filtrate was concentrated. The oily residue was dissolved in dry DMSO (5 mL) and DIPEA (0.13 g, 0.17 mL, 1 mmol) as well as compound 50 (145 mg, 0.5 mmol) were added. The mixture was stirred at 90 °C for 24 h. After cooling, it was poured onto half-saturated brine (50 mL) and it was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with saturated NH4Cl solution (50 mL) 5% LiCl solution (50 mL) and brine (50 mL), dried over Na2SO4, filtered and concentrated. The crude product was purified by column chromatography (petroleum ether/EtOAc 2:1) to obtain a green oil.

Yield (79 mg, 35%); Rf = 0.38 (petroleum ether/EtOAc 2:1); 1H NMR (600 MHz, DMSO-d6) δ 1.33 (dd, J = 6.5, 8.7 Hz, 2H, CH2), 1.36 (s, 9H, CH3), 1.48 – 1.61 (m, 4H, CH2), 1.99 – 2.07 (m, 1H, 4'-H), 2.18 (t, J = 7.3 Hz, 2H, CH2), 2.49 – 2.57 (m, 1H, 4'-H), 2.71 – 2.78 (m, 1H, 5'-H), 2.86 – 2.98 (m, 1H, 5'-H), 3.01 (s, 3H, CH3), 3.28 (d, J = 6.4 Hz, 2H, NHCH3), 5.10 (dd, J = 5.4, 13.0 Hz, 1H, 3'-H), 6.52 (t, J = 6.0 Hz, 1H, CH2), 7.01 (d, J = 7.0 Hz, 1H, NHCH3), 7.08 (d, J = 8.6 Hz, 1H, 5-H, 7-H), 7.57 (dd, J = 7.0, 8.5 Hz, 1H, 6-H); 13C NMR (151 MHz, DMSO-d6) δ 21.55 (C-4'), 24.51, 25.85 (CH2), 26.74 (CH3), 27.90 (C(CH3)3), 28.53 (CH3), 31.27 (C-5'), 34.84 (CH2), 41.85 (NHCH3), 49.26 (C-3'), 79.54 (C(CH3)3), 109.17 (C-3a), 110.56 (C-7), 117.38 (C-5), 132.35 (C-7a), 136.46 (C-6), 146.62 (C-4), 167.44 (C-1), 169.07 (C-3), 169.97 (C-2'), 171.96, 172.37 (C-6′, CO); MS (ESI) m/z [M + H]+ calcd for C24H31N3O6, 458.22; found, 458.3; HRMS (ESI) m/z [M + H]+ calcd for C24H31N3O6, 458.2286; found, 458.2285.
CRBN-based negative control 21

![Chemical structure](attachment:image.png)

This compound was prepared using the General Procedure I and CRBN building block 51 (92 mg). The crude product was purified by column chromatography (CH₂Cl₂/EtOH 29:1) to obtain a yellow solid.

Yield (140 mg, 84%); mp 102 – 106 °C; Rᵋ = 0.40 (CH₂Cl₂/EtOH 19:1); \(^1\)H NMR (600 MHz, DMSO-d₆) δ 1.31 – 1.46 (m, 2H), 1.46 – 1.66 (m, 6H), 1.66 – 1.83 (m, 2H), 1.84 – 1.94 (m, 2H), 1.99 – 2.08 (m, 1H), 2.17 – 2.28 (m, 2H), 2.30 (s, 3H), 2.36 (t, J = 7.4 Hz, 2H), 2.41 (s, 3H), 2.49 – 2.56 (m, 1H), 2.63 – 2.84 (m, 1H), 2.89 – 2.98 (m, 1H), 3.00 (s, 3H), 3.03 – 3.21 (m, 4H), 3.26 – 3.30 (m, 2H), 3.41 – 3.78 (m, 4H), 5.10 (dd, J = 5.4, 13.0 Hz, 1H), 5.81 (p, J = 8.9 Hz, 1H), 6.52 (t, J = 5.9 Hz, 1H), 7.00 (d, J = 7.0 Hz, 1H), 7.09 (d, J = 8.6 Hz, 1H), 7.47 (dd, J = 3.1, 9.1 Hz, 1H), 7.56 (dd, J = 7.0, 8.6 Hz, 1H), 7.87 (d, J = 9.1 Hz, 1H), 8.06 (d, J = 3.0 Hz, 1H), 8.94 (s, 1H), 10.08 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-d₆) δ 13.76, 21.53, 24.67, 25.26, 26.23, 26.74, 27.70, 28.70, 31.27, 31.44, 32.25, 40.84, 41.93, 44.79, 48.67, 49.09, 49.26, 53.09, 106.78, 109.13, 110.54, 115.19, 117.36, 125.47, 129.45, 132.34, 136.08, 136.46, 142.21, 143.35, 144.93, 146.62, 154.91, 158.39, 158.67, 160.90, 167.41, 169.07, 169.97, 170.76, 171.94, 202.56; LC-MS (ESI) (90% H₂O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-500 nm), tᵋ = 9.67 min, 99% purity, m/z [M + H]⁺ calcd for C₄₃H₄₈N₁₀O₇, 831.39; found, 831.5; HRMS (ESI) m/z [M + H]⁺ calcd for C₄₃H₄₈N₁₀O₇, 831.3937; found, 831.3928.
J Synthesis of VHL-based PROTACs

**General Procedure II: TEMPO/BAIB mediated oxidation of alcohols to carboxylic acids.** The corresponding alcohol (5 mmol) was dissolved in acetonitrile (12.5 mL) and H₂O (12.5 mL). TEMPO (0.17 g, 1.1 mmol) was added followed by the portionwise addition of (diacetoxyiodo)benzene (3.54 g, 11 mmol). The orange mixture was stirred at rt for 16 h. It was neutralized by the addition of saturated NaHCO₃ solution (100 mL) and the aqueous layer was washed with EtOAc (2 x 100 mL). The aqueous phase was then acidified by the careful addition of 2N HCl solution until pH = 1. The mixture was then extracted with EtOAc (2 x 100 mL) and the combined organic layers were dried over Na₂SO₄, filtered and concentrated.

**General Procedure III: HATU-mediated coupling to the VHL ligand.** The corresponding VHL ligand (1 mmol) was dissolved in dry CH₂Cl₂ (5 mL) and TFA (5 mL) was added. The mixture was stirred at room temperature for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. Subsequently, the appropriate acid (1 mmol) was dissolved in dry DMF (5 mL) and DIPEA (0.35 mL, 2 mmol) was added, followed by the addition of HATU (1.1 mmol). After stirring for 5 minutes, the deprotected amine was dissolved in dry DMF (5 mL) and DIPEA (0.70 mL, 4 mmol) and was added to the mixture. It was stirred at room temperature for 16 h, after which half-saturated brine (50 mL) was added, and the product was extracted with EtOAc (3 x 50 mL). The combined organic phases were washed with saturated NH₄Cl solution (50 mL), 5% LiCl solution (50 mL), brine (50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo.

**General Procedure IV: Finkelstein reaction and alkylation of palbociclib.** The corresponding VHL-linker conjugate (0.1 mmol) was dissolved in dry acetone (5 mL) and sodium iodide (150 mg, 1 mmol) was added. After stirring for 48 h, the yellow suspension was diluted with EtOAc (50 mL) and it was washed with 10% Na₂SO₄ solution, H₂O and brine (each 25 mL), dried over Na₂SO₄, filtered and concentrated. The intermediate was then dissolved in dry DMSO (4 mL) and dry DMF (4 mL), followed by the addition of DIPEA (39 mg, 52 µL, 0.3 mmol) and palbociclib (45 mg, 0.1 mmol). The yellow suspension was stirred at 80 °C for 24 h. After cooling, the yellow solution was diluted with EtOAc (100 mL) and it was washed with saturated NaHCO₃ solution (50 mL), 5% LiCl solution (50 mL), and brine (50 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated.
General Procedure V: Alkylation of phenolic VHL ligands. The corresponding VHL ligand (0.30 mmol) and the corresponding mesylate (0.36 mmol) were dissolved in dry DMF (6 mL) and Cs$_2$CO$_3$ (147 mg, 0.45 mmol) was added. After stirring for 18 h at rt, the beige suspension was heated at 60 °C for 3 h. After cooling, it was diluted with H$_2$O (50 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with 5% LiCl solution and brine (each 25 mL), dried over Na$_2$SO$_4$, filtered and concentrated.

8-Chlorooctanoic acid (52)

![8-Chlorooctanoic acid](image)

This compound was prepared using the General Procedure II and 8-chloro-1-octanol (0.82 g). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$ to CH$_2$Cl$_2$/MeOH 19:1) to obtain a colorless oil.

Yield (0.33 g, 37%); $R_f = 0.48$ (CH$_2$Cl$_2$/MeOH 9:1); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.20 – 1.35 (m, 4H), 1.36 (t, $J = 7.3$ Hz, 2H), 1.43 – 1.53 (m, 2H), 1.64 – 1.74 (m, 2H), 2.18 (t, $J = 7.4$ Hz, 2H), 3.61 (t, $J = 6.6$ Hz, 2H, CH$_2$), 11.86 (br s, 1H, COOH); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 24.55, 26.28, 28.08, 28.54, 32.16, 33.80 (CH$_3$), 45.54 (CH$_2$Cl), 174.65 (CO); HRMS (ESI) $m/z$ [M – H]$^-$ calcd for C$_8$H$_{15}$ClO$_2$, 177.0688; found, 177.0680.

VHL ligand a (53)

![VHL ligand a](image)

This compound was synthesized as we described previously. 

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8
This compound was prepared using the General Procedure III, VHL ligand a (53, 0.53 g) and acid 52 (0.18 g). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$/EtOH 29:1 to 19:1) to obtain a colorless solid.

Yield (0.46 g, 78%); mp 62 – 66 °C, $R_f$ = 0.92 (s, 9H, C(CH$_3$)$_3$), 1.18 – 1.30 (m, 4H), 1.30 – 1.38 (m, 2H), 1.39 – 1.55 (m, 2H), 1.62 – 1.72 (m, 2H, CH$_2$), 1.86 – 1.93 (m, 1H, 3-H), 1.98 – 2.05 (m, 1H, 3-H), 2.06 – 2.14 (m, 1H), 2.21 – 2.29 (m, 1H, CH$_2$), 2.43 (s, 3H, CH$_3$), 3.54 – 3.70 (m, 4H, S-H), 4.21 (dd, $J = 5.5$, 15.8 Hz, 1H), 4.32 – 4.36 (m, 1H), 4.39 – 4.45 (m, 2H), 4.53 (d, $J = 9.4$ Hz, 1H, NHCH$_2$), 5.09 (d, $J = 3.6$ Hz, 1H, OH), 7.35 – 7.43 (m, 4H, 2'-H, 3'-H), 7.80 (d, $J = 9.4$ Hz, 1H), 8.52 (t, $J = 6.1$ Hz, 1H, CONH), 8.97 (s, 1H, 2''-H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 16.09 (CH$_3$), 25.46, 26.32 (CH$_3$), 26.54 (C(CH$_3$)$_3$), 28.07, 28.63, 32.17, 34.97 (CH$_2$), 35.37 (C(CH$_3$)$_3$), 38.11 (C-3), 41.82 (NHCH$_2$), 45.54 (CH$_2$Cl), 56.45, 56.49, 58.85 (C-2, C-5, NHCH), 69.03 (C-4), 127.61, 128.81 (C-2'', C-3''), 129.82, 131.34 (C-1', C-5''), 139.68 (C-4''), 147.90 (C-4''), 151.61 (C-2'''), 169.90, 172.11, 172.26 (CO); MS (ESI) m/z [M + H]$^+$ calcd for C$_{30}$H$_{43}$ClN$_4$O$_4$S, 591.27; found, 591.2; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{30}$H$_{43}$ClN$_4$O$_4$S, 591.2766; found, 591.2762.

VHL(a)-based PROTAC 22

This compound was prepared using the General Procedure IV and precursor 54 (59 mg). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$/MeOH+7N NH$_3$ 29:1 to 19:1) to obtain the title compound as a yellow solid.
Yield (34 mg, 34%); mp 96 – 98 °C; \( R_f = 0.38 \) (CH\(_2\)Cl\(_2\)/MeOH+7N \( \text{NH}_3 \) 9:1); \(^1\)H NMR (600 MHz, DMSO-\( d_6 \)) \( \delta \); 0.92 (s, 9H), 1.16 – 1.34 (m, 7H), 1.35 – 1.65 (m, 7H), 1.72 – 1.78 (m, 2H), 1.84 – 1.92 (m, 3H), 1.98 – 2.37 (m, 12H), 2.41 (s, 3H), 2.43 (s, 3H), 3.10 – 3.16 (m, 4H), 3.58 – 3.71 (m, 2H), 4.20 (dd, \( J = 5.5, 15.9 \) Hz, 1H), 4.34 (p, \( J = 2.7, 3.2 \) Hz, 1H), 4.37 – 4.45 (m, 2H), 4.53 (d, \( J = 9.3 \) Hz, 1H), 5.10 (d, \( J = 3.6 \) Hz, 1H), 5.80 (p, \( J = 8.9 \) Hz, 1H), 7.35 – 7.45 (m, 5H), 7.81 (dd, \( J = 9.2, 13.1 \) Hz, 2H), 8.03 (d, \( J = 3.0 \) Hz, 1H), 8.52 (t, \( J = 6.1 \) Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.04 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\( d_6 \)) \( \delta \); 13.76, 16.08, 25.24, 25.55, 26.38, 26.54, 27.02, 27.70, 28.78, 28.82, 31.45, 35.03, 35.36, 38.10, 41.81, 48.50, 52.72, 53.06, 56.45, 56.48, 58.02, 58.85, 69.01, 106.71, 115.34, 124.77, 127.59, 128.79, 129.36, 129.80, 130.31, 135.44, 139.65, 142.24, 143.66, 144.37, 147.87, 151.58, 154.92, 158.40, 158.74, 160.92, 169.89, 172.09, 172.28, 202.60; LC-MS (ESI) (90% H\(_2\)O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220-420 nm), \( t_R = 9.34 \) min, 99% purity, \( m/z \) [M + H]\(^+\) calcd for \( C_{54}H_{71}N_{11}O_{6}S \), 1002.53; found, 1002.54; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for \( C_{54}H_{71}N_{11}O_{6}S \), 1002.5382; found, 1002.5428.

2-[2-(2-Chloroethoxy)ethoxy]acetic acid (55)

This compound was prepared using the General Procedure II and 2-[2-(2-chloroethoxy)ethoxy]ethanol (0.84 g). The crude product was purified by column chromatography (gradient of CH\(_2\)Cl\(_2\) to CH\(_2\)Cl\(_2\)/MeOH 9:1) to obtain a colorless oil.

Yield (0.74 g, 81%); \( R_f = 0.25 \) (CH\(_2\)Cl\(_2\)/MeOH 9:1); \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta \); 3.55 – 3.62 (m, 4H), 3.63 – 3.74 (m, 4H, CH\(_2\)Cl, OCH\(_2\)), 4.01 (s, 2H, OCH\(_2\)), 12.27 (br s, 1H, COOH); \(^{13}\)C NMR (126 MHz, DMSO-\( d_6 \)) \( \delta \); 43.68 (CH\(_2\)Cl), 67.84, 69.79, 69.95, 70.65 (OCH\(_2\)), 171.79 (CO); MS (ESI) \( m/z \) [M + H]\(^+\) calcd for \( C_{6}H_{12}ClO_{4} \), 183.04; found, 183.0.
This compound was prepared using the General Procedure III, VHL ligand a (53, 0.53 g) and acid 55 (0.18 g). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$/MeOH 29:1 to 19:1) to obtain a colorless solid.

Yield (0.35 g, 59%); mp 52 – 54 °C, $R_f = 0.29$ (CH$_2$Cl$_2$/MeOH 19:1); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 0.93 (s, 9H, C(CH$_3$)$_3$), 1.86 – 1.94 (m, 1H, 3-H), 2.01 – 2.09 (m, 1H, 3-H), 2.43 (s, 3H, CH$_3$), 3.56 – 3.75 (m, 10H, 5-H, CH$_2$Cl, OCH$_2$), 3.90 – 4.03 (m, 2H, OCH$_3$), 4.23 (dd, $J = 5.6$, 15.8 Hz, 1H), 4.34 (d, $J = 4.4$ Hz, 1H), 4.36 – 4.47 (m, 2H), 4.56 (d, $J = 9.6$ Hz, 1H, 2-H, 4-H, NHCH$_2$ NHCH$_2$), 5.13 (d, $J = 3.5$ Hz, 1H, OH), 7.35 – 7.46 (m, 5H, 2''-H, 3'-H, CONH), 8.55 (t, $J = 6.1$ Hz, 1H, CONH), 8.96 (s, 1H, 2''-H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 16.07 (CH$_3$), 26.36 (C(CH$_3$)$_3$), 35.88 (C(CH$_3$)$_3$), 38.07 (C-3), 41.88 (NHCH$_2$), 43.57 (CH$_2$Cl), 55.90 , 56.73, 58.92 (C-2, C-5, NHCH), 69.06 (C-4), 69.63, 69.80, 70.55, 70.80 (OCH$_2$), 127.64, 128.87 (C-2', C-3'), 129.89, 131.33 (C-1', C-5''), 139.61 (C-4'), 147.93 (C-4''), 151.61 (C-2''), 168.79, 169.35, 171.96 (CO); MS (ESI) m/z [M + H]$^+$ calcd for C$_{28}$H$_{39}$ClN$_4$O$_6$S, 595.23; found, 595.4; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{28}$H$_{39}$ClN$_4$O$_6$S, 595.2352; found, 595.2354.

This compound was prepared using the General Procedure IV and precursor 56 (60 mg). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$/MeOH+7N NH$_3$ 29:1 to 19:1) to obtain the title compound as a yellow solid.
Yield (67 mg, 67%); mp 122 – 124 °C; \( R_f = 0.12 \) (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\); 19:1); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \( \delta \) 0.95 (s, 9H), 1.49 – 1.62 (m, 2H), 1.70 – 1.80 (m, 2H), 1.81 – 1.95 (m, 3H), 2.02 – 2.11 (m, 1H), 2.12 – 2.26 (m, 2H), 2.29 (s, 3H), 2.41 (s, 3H), 2.42 (s, 3H), 2.51 – 2.61 (m, 6H), 3.06 – 3.15 (m, 4H), 3.53 – 3.72 (m, 8H), 3.97 (s, 2H), 4.15 – 4.31 (m, 1H), 4.31 – 4.41 (m, 2H), 4.41 – 4.51 (m, 1H), 4.57 (d, \( J = 9.5 \) Hz, 1H), 5.13 (d, \( J = 3.6 \) Hz, 1H), 5.75 – 5.86 (m, 1H), 7.31 – 7.47 (m, 6H), 7.83 (d, \( J = 9.0 \) Hz, 1H), 8.01 (d, \( J = 3.2 \) Hz, 1H), 8.54 (t, \( J = 6.1 \) Hz, 1H), 8.92 (s, 1H), 8.94 (s, 1H), 10.02 (s, 1H); \(^{13}\)C NMR (126 MHz, DMSO-\(d_6\)) \( \delta \) 13.73, 16.03, 25.22, 26.34, 27.68, 31.42, 35.86, 38.04, 41.84, 48.45, 53.01, 53.07, 55.83, 56.70, 57.28, 58.90, 68.60, 69.02, 69.62, 69.80, 70.64, 106.69, 115.26, 124.72, 127.58, 128.80, 129.35, 129.82, 131.26, 135.42, 139.55, 142.20, 143.60, 144.35, 147.86, 151.48, 154.90, 158.34, 158.69, 160.90, 168.72, 169.28, 171.86, 202.52; LC-MS (ESI) (90% H\(_2\)O to 100% MeCN in 10 min, then 100% MeCN to 20 min, DAD 220 – 420 nm), \( t_R = 8.61 \) min, 97% purity, \( m/z \) [M + H]\(^+\) calcd for C\(_{52}\)H\(_{67}\)N\(_{11}\)O\(_8\)S, 1006.49; found, 1006.8; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for C\(_{52}\)H\(_{67}\)N\(_{11}\)O\(_8\)S, 1006.4968; found, 1006.5152.

2-[2-[2-(Chloroethoxy)ethoxy]ethoxy]ethanol (57)

This compound was synthesized similar to a previously reported procedure.\(^9\) In brief, tetraethylene glycol (19.42 g, 100 mmol) was dissolved in CHCl\(_3\) (20 mL). Subsequently, pyridine (7.91 g, 8.07 mL, 100 mmol) was added followed by the dropwise addition of SOCl\(_2\) (11.90 g, 7.26 mL, 100 mmol) while cooling with a water bath. The mixture was then stirred at 77 °C for 2 h and stirring was continued at 60 °C overnight. All volatiles (including pyridine) were removed in vacuo and the residue was partitioned between H\(_2\)O (100 mL) and CH\(_2\)Cl\(_2\) (100 mL). The aqueous phase was extracted again with CH\(_2\)Cl\(_2\) (100 mL) and the combined organic layers were washed with brine (100 mL), dried over Na\(_2\)SO\(_4\), filtered and evaporated. The crude product was purified by column chromatography (gradient of petroleum ether/EtOAc 1:2 to EtOAc) to obtain a colourless oil.

Yield (4.45 g, 21%); \( R_f = 0.30 \) (EtOAc); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \( \delta \) 3.38 – 3.43 (m, 2H), 3.48 (q, \( J = 5.3 \) Hz, 2H), 3.48 – 3.66 (m, 8H), 3.63 – 3.73 (m, 4H, OCH\(_3\)), 4.53 (t, \( J = 5.5 \) Hz, 1H, OH); \(^{13}\)C NMR (126 MHz, DMSO-\(d_6\)) \( \delta \) 43.72 (CH\(_3\)Cl), 60.40 (CH\(_2\)OH), 69.86 (OCH\(_3\)), 69.94 (2 × OCH\(_3\)), 70.01, 70.72, 72.50 (OCH\(_3\)); MS (ESI) \( m/z \) [M + H]\(^+\) calcd for C\(_8\)H\(_{17}\)ClO\(_4\), 213.08; found, 213.1.
2-[2-[2-(Chloroethoxy)ethoxy]ethoxy]acetic acid (58)

This compound was prepared using the General Procedure II and alcohol 57 (1.06 g). The crude product was purified by column chromatography (gradient of CH₂Cl₂ to CH₂Cl₂/MeOH 9:1) to obtain a colorless oil.

Yield (0.52 g, 46%); Rf = 0.28 (CH₂Cl₂/MeOH 9:1); ¹H NMR (600 MHz, DMSO-d₆) δ 3.50 – 3.59 (m, 8H), 3.64 – 3.72 (m, 4H, OCH₂, CH₂Cl), 4.00 (s, 2H, OCH₂), 12.55 (br s, 1H, COOH); ¹³C NMR (151 MHz, DMSO-d₆) δ 43.74 (CH₂Cl), 67.81, 69.84, 69.89, 69.94, 70.00, 70.72 (OCH₂), 171.85 (CO); HRMS (ESI) m/z [M – H]⁻ calcd for C₈H₁₅ClO₅, 225.0535; found, 225.0531.

VHL(a)-2-2-2-2 building block (59)

This compound was prepared using the General Procedure III, VHL ligand a (53, 0.53 g) and acid 58 (0.23 g). The crude product was purified by column chromatography (gradient of CH₂Cl₂/MeOH 29:1 to 19:1) to obtain a colorless solid.

Yield (0.45 g, 70%); mp 52 – 54 °C, Rf = 0.42 (CH₂Cl₂/MeOH 9:1); ¹H NMR (500 MHz, DMSO-d₆) δ 0.94 (s, 9H, C(CH₃)₃), 1.86 – 1.94 (m, 1H, 3-H), 2.01 – 2.09 (m, 1H, 3-H), 2.43 (s, 3H, CH₃), 3.53 – 3.71 (m, 14H, 5-H, OCH₂, CH₂Cl), 3.96 (s, 2H, OCH₂), 4.25 (dd, J = 5.7, 15.8 Hz, 1H), 4.32 – 4.47 (m, 3H), 4.56 (d, J = 9.5 Hz, 1H, 2-H, 4-H, NHCH, NHCH₂), 5.12 (d, J = 3.7 Hz, 1H, OH), 7.35 – 7.46 (m, 5H, 2'-H, 3'-H, CONH), 8.55 (t, J = 6.1 Hz, 1H, CONH), 8.96 (s, 1H, 2''''-H); ¹³C NMR (126 MHz, DMSO-d₆) δ 16.05 (CH₃), 26.33 (C(CH₃)₃), 35.86 (C(CH₃)₃), 38.05 (C-3), 41.86 (NHCH₂), 43.69 (CH₂Cl), 55.86, 56.70, 58.89 (C-2, C-5, NHCH), 69.03 (C-4), 69.78 (2 × OCH₂), 69.84, 69.99, 70.62, 70.70 (OCH₂), 127.64, 128.85 (C-2', C-3'), 129.88, 131.29 (C-1', C-5''), 139.60 (C-4'), 147.92 (C-4''), 151.58 (C-2'''), 168.75, 169.32, 171.91 (CO); MS (ESI) m/z [M + H]⁺ calcd for C₃₀H₄₃ClN₄O₇S, 639.26; found, 639.3; HRMS (ESI) m/z [M + H]⁺ calcd for C₃₀H₄₃ClN₄O₇S, 639.2614; found, 639.2619.
VHL(a)-based PROTAC 24

This compound was prepared using the General Procedure IV and precursor 59 (64 mg). The crude product was purified by column chromatography (gradient of CH$_2$Cl$_2$/MeOH+7N NH$_3$ 29:1 to 19:1) to obtain the title compound as a yellow solid.

Yield (62 mg, 59%); mp 96 – 98 °C; $R_f$ = 0.80 (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 9:1); $^1$H NMR (600 MHz, DMSO-$d_6$) δ 0.94 (s, 9H), 1.51 – 1.61 (m, 2H), 1.68 – 1.79 (m, 2H), 1.81 – 1.94 (m, 3H), 2.00 – 2.10 (m, 1H), 2.18 – 2.27 (m, 2H), 2.29 (s, 3H), 2.41 (s, 3H), 2.43 (s, 3H), 2.49 – 2.62 (m, 6H), 3.08 – 3.16 (m, 4H), 3.45 – 3.72 (m, 12H), 3.96 (s, 2H), 4.20 – 4.27 (m, 1H), 4.30 – 4.47 (m, 3H), 4.56 (d, $J$ = 9.6 Hz, 1H), 5.13 (d, $J$ = 3.6 Hz, 1H), 5.80 (p, $J$ = 8.9 Hz, 1H), 7.32 – 7.48 (m, 6H), 7.82 (d, $J$ = 9.0 Hz, 1H), 8.03 (d, $J$ = 3.0 Hz, 1H), 8.56 (t, $J$ = 6.0 Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.04 (s, 1H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) δ 13.75, 16.05, 25.22, 26.33, 27.68, 31.43, 35.86, 38.05, 41.82, 48.43, 53.02, 55.83, 56.70, 57.31, 58.88, 68.51, 69.01, 69.76, 69.84, 69.99, 70.66, 106.69, 115.31, 124.74, 127.59, 128.81, 129.35, 129.83, 131.27, 135.44, 139.58, 142.21, 143.60, 144.37, 147.88, 151.55, 154.90, 158.38, 158.72, 160.90, 168.73, 169.27, 171.89, 202.56; LC-MS (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $t_R$ = 11.83 min, 96% purity, $m/z$ [M + H]$^+$ calcd for C$_{54}$H$_{71}$N$_{11}$O$_9$S, 1050.52; found, 1051.0; HRMS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{54}$H$_{71}$N$_{11}$O$_9$S, 1050.5230; found, 1050.5260.

tert-Butyl 5-[5-(6-chlorohexoxy)pentoxy]pentanoate (60)

This compound was synthesized as we described previously.$^8$
VHL(a)-5-5-6 building block (61)

The linker building block 60 (1 mmol, 0.38 g) was dissolved in dry CH₂Cl₂ (5 mL) and TFA (5 mL) was added. The mixture was stirred at 40 °C for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. The so obtained free acid was then subjected to the General Procedure III with VHL ligand a (53, 0.53 g). The crude product was purified by column chromatography (gradient of CH₂Cl₂/MeOH 29:1 to 19:1) to obtain a colorless oil.

Yield (0.66 g, 90%); Rf = 0.35 (CH₂Cl₂/MeOH 19:1); ¹H NMR (600 MHz, DMSO-d₆) δ; 0.92 (s, 9H, C(CH₃)₃), 1.26 – 1.33 (m, 4H), 1.33 – 1.41 (m, 2H), 1.41 – 1.56 (m, 10H, 3-H, COCH₂), 1.65 – 1.73 (m, 2H, CH₂), 1.86 – 1.93 (m, 1H), 2.01 (d, J = 9.7 Hz, 1H), 2.07 – 2.15 (m, 1H, 3'-H, COCH₂), 2.22 – 2.30 (m, 1H, 3-H, COCH₂), 2.43 (s, 3H, CH₃), 3.57 – 3.64 (m, 3H), 3.66 (dd, J = 4.1, 10.6 Hz, 1H), 4.20 (dd, J = 5.6, 15.8 Hz, 1H), 4.34 (s, 1H), 4.39 – 4.45 (m, 2H, 2'-H, 4'-H, 5'-H, NHCH₂), 4.53 (d, J = 9.3 Hz, 1H, NHCH), 5.09 (d, J = 3.7 Hz, 1H, OH), 7.37 (d, J = 8.1 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H, 2'-H, 3'-H), 7.81 (d, J = 9.4 Hz, 1H ), 8.52 (t, J = 6.1 Hz, 1H, NH), 8.97 (s, 1H, 2''-H). The expected signal at 3.30 ppm for 4 × OCH₂ was unquantifiable (overlapping H₂O peak). ¹³C NMR (151 MHz, DMSO-d₆) δ 16.09 (CH₃), 22.42, 22.68, 25.14, 26.25 (CH₂), 26.54 (C(CH₃)₃), 28.98 (CH₂), 29.21 (2 × CH₃), 29.23, 32.18 (CH₂), 34.79, 35.37 (CH₂, C(CH₃)₃), 38.09 (C-3), 41.82 (NHCH₂), 45.51 (CH₂Cl), 56.46 , 56.49, 58.85 (C-2, C-5, NHCH), 69.02 (C-4), 69.80, 69.95 (OCH₂), 70.06 (2 × OCH₂), 127.60, 128.81 (C-2', C-3'), 129.82, 131.33 (C-5'', C-1''), 139.68 (C-4''), 147.89 (C-4''), 151.60 (C-2''), 169.88, 172.11, 172.16 (CO); MS (ESI) m/z [M + H]^+ calcd for C₃₈H₅₉ClN₄O₆S, 735.39; found, 735.3; HRMS (ESI) m/z [M + H]^+ calcd for C₃₈H₅₉ClN₄O₆S, 735.3917; found, 735.3887.

VHL(a)-based PROTAC 25

This compound was prepared using the General Procedure IV and precursor 61 (74 mg). The crude product was purified by column chromatography (CH₂Cl₂/MeOH+7N NH₃ 19:1) followed by HPLC
purification using a gradient of 70% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.

Yield (41 mg, 36%); mp 104 – 106 °C; $R_f = 0.25$ (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 19:1); $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 0.92 (s, 9H), 1.30 (dt, $J = 4.9, 7.4, 9.4$ Hz, 6H), 1.37 – 1.62 (m, 16H), 1.71 – 1.80 (m, 2H), 1.82 – 1.93 (m, 3H), 1.98 – 2.06 (m, 1H), 2.06 – 2.16 (m, 1H), 2.17 – 2.33 (m, 8H), 2.41 (s, 3H), 2.43 (s, 3H), 3.13 (t, $J = 5.0$ Hz, 4H), 3.27 – 3.34 (m, 10H), 3.51 – 3.77 (m, 2H), 4.20 (dd, $J = 5.5, 15.8$ Hz, 1H), 4.29 – 4.38 (m, 1H), 4.38 – 4.46 (m, 2H), 4.53 (d, $J = 9.4$ Hz, 1H), 5.09 (d, $J = 3.6$ Hz, 1H), 5.80 (p, $J = 8.9$ Hz, 1H), 7.34 – 7.46 (m, 5H), 7.82 (dd, $J = 9.2, 11.3$ Hz, 2H), 8.03 (d, $J = 3.0$ Hz, 1H), 8.52 (t, $J = 6.1$ Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.04 (s, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 13.76, 16.08, 22.43, 22.68, 25.24, 25.84, 26.03, 26.53, 26.97, 27.70, 28.98, 29.22, 29.39, 31.45, 34.79, 35.36, 38.09, 41.81, 48.49, 52.72, 53.06, 56.44, 56.49, 58.00, 58.84, 69.02, 69.80, 70.06, 106.71, 115.33, 124.76, 127.58, 128.78, 129.37, 129.80, 131.32, 135.44, 139.66, 142.24, 143.65, 144.38, 147.87, 151.57, 154.92, 158.40, 158.74, 160.92, 169.87, 172.12, 202.58; LC-MS (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $t_R = 12.92$ min, 99% purity, m/z [M + H]$^+$ calcd for C$_{62}$H$_{87}$N$_{11}$O$_8$S, 1146.65; found, 1146.65; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{62}$H$_{87}$N$_{11}$O$_8$S, 1146.6533; found, 1146.6573.

(-)VHL ligand a (62)

This compound was synthesized as described previously.$^{10}$

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 0.95 (s, 9H, C(CH$_3$)$_3$), 1.31 (s, 9H, COC(CH$_3$)$_3$), 1.92 – 2.00 (m, 1H, 3-H), 2.00 – 2.08 (m, 1H, 3-H), 2.44 (s, 3H, CH$_3$), 3.48 – 3.54 (m, 1H, 1H), 3.75 (dd, $J = 5.0, 10.6$ Hz, 1H), 4.10 (d, $J = 8.2$ Hz, 1H), 4.24 – 4.42 (m, 4H), 5.10 (d, $J = 3.8$ Hz, 1H, OH), 6.67 (d, $J = 8.0$ Hz, 1H, NH), 7.33 (d, $J = 7.9$ Hz, 2H, Ar-H), 7.41 (d, $J = 8.2$ Hz, 2H, Ar-H), 8.16 (t, $J = 8.9$ Hz, 1H, NH), 8.96 (s, 1H, 2"-H); $^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 16.07 (CH$_3$), 26.58 (C(CH$_3$)$_3$), 28.27 (C(CH$_3$)$_3$), 34.81(C(CH$_3$)$_3$), 38.01 (C-3), 41.71 (NHCH$_3$), 55.45, 58.70, 59.04 (C-2, C-5, NHCH), 68.70 (C-4), 78.45 (CO(C(CH$_3$)$_3$), 127.45, 128.97 (C-2', C-3'), 129.89, 131.28 (C-5", C-1"), 139.50 (C-4"), 147.93 (C-4"'), 151.55 (C-2"'), 155.97 (NCO$_2$), 170.04 (CO), 171.88 (CO); MS (ESI) m/z [M + H]$^+$ calcd for C$_{27}$H$_{38}$N$_4$O$_9$S, 531.26; found, 531.4.
This compound was prepared using the General Procedure III, (−)VHL ligand a (62, 0.53 g) and acid 52 (0.18 g). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in CH₂Cl₂) to yield the title compound as a colorless solid.

Yield (455 mg, 77%); mp 64 – 66 °C; R_f = 0.33 (CH₂Cl₂/MeOH 19:1); H NMR (600 MHz, DMSO-d₆) δ 0.96 (s, 9H, C(CH₃)₃), 1.09 – 1.21 (m, 4H), 1.21 – 1.43 (m, 4H), 1.56 – 1.66 (m, 2H, CH₂), 1.84 – 2.03 (m, 2H, 3-H), 2.01 – 2.11 (m, 1H), 2.12 – 2.23 (m, 1H, CH₂), 2.44 (s, 3H, CH₃), 3.51 (dd, J = 3.9, 10.4 Hz, 1H, 5-H'), 3.56 (t, J = 6.7 Hz, 2H, CH₂Cl), 3.81 (dd, J = 5.2, 10.5 Hz, 1H, 5-H''), 4.20 (dd, J = 5.6, 15.7 Hz, 1H), 4.30 – 4.50 (m, 4H, 2-H, 4-H, NHCH₂, NHCH₃), 5.11 (d, J = 4.0 Hz, 1H, OH), 7.31 (d, J = 8.0 Hz, 2H, 2'-H, 3'-H), 7.41 (d, J = 8.2 Hz, 2H, 2'-H, 3'-H), 7.89 (d, J = 7.8 Hz, 1H), 8.15 (t, J = 6.1 Hz, 1H, CONH), 8.97 (s, 1H, 2''-H); C NMR (151 MHz, DMSO-d₆) δ 16.13 (CH₃), 25.28, 26.26 (CH₂), 26.63 (CH(CH₃)), 28.06, 28.58, 32.13, 32.16, 34.33 (CH₃), 34.74 (CH(CH₃)₂), 38.03 (C-3), 41.71 (NHCH₂), 45.47 (CH₂Cl), 55.38, 57.32, 58.97 (C-2, C-5, NHCH), 68.60 (C-4), 127.45, 128.92 (C-2', C-3'), 129.86, 131.28 (C-1', C-5''), 139.51 (C-4'), 147.89 (C-4''), 151.57 (C-2''), 169.98, 171.73, 173.22 (CO); MS (ESI) m/z [M + H]^+ calcld for C₃₀H₄₃ClN₄O₄S, 591.28; found, 591.2; HRMS (ESI) m/z [M + H]^+ calcld for C₃₀H₄₃ClN₄O₄S, 591.2766; found, 591.2760.

VHL(a)-based negative control 26

This compound was prepared using the General Procedure IV and precursor 63 (59 mg). The crude product was purified by column chromatography (gradient of CH₂Cl₂/MeOH+7N NH₃ 29:1 to 19:1) followed by HPLC purification using a gradient of 70% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.
Yield (61 mg, 64%); mp 126 – 130 °C; \( R_f = 0.18 \) (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\), 19:1); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \( \delta \) 0.97 (s, 9H), 1.09 – 1.33 (m, 7H), 1.37 (s, 5H), 1.70 – 1.80 (m, 2H), 1.81 – 1.92 (m, 2H), 1.93 – 2.27 (m, 8H), 2.30 (s, 3H), 2.41 (s, 3H), 2.41 – 2.48 (m, 7H), 2.94 – 3.27 (m, 4H), 3.51 (dd, \( J = 4.0, 10.4 \) Hz, 1H), 3.81 (dd, \( J = 5.2, 10.5 \) Hz, 1H), 4.09 – 4.27 (m, 1H), 4.27 – 4.50 (m, 4H), 5.12 (d, \( J = 4.0 \) Hz, 1H), 5.81 (p, \( J = 8.9 \) Hz, 1H), 7.23 – 7.53 (m, 5H), 7.83 (d, \( J = 9.0 \) Hz, 1H), 7.89 (d, \( J = 7.7 \) Hz, 1H), 8.02 (d, \( J = 3.0 \) Hz, 1H), 8.15 (t, \( J = 6.1 \) Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.05 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \( \delta \) 13.76, 16.14, 25.24, 25.37, 26.37, 26.64, 26.97, 27.70, 28.76, 28.81, 31.44, 34.31, 34.81, 38.03, 41.73, 48.49, 52.69, 53.06, 55.37, 57.38, 58.00, 58.98, 68.60, 106.70, 115.30, 124.74, 127.45, 128.91, 129.36, 129.86, 131.29, 135.43, 139.50, 142.23, 143.64, 144.37, 147.89, 151.55, 154.92, 158.39, 158.72, 160.91, 170.01, 171.73, 173.30, 202.56; LC-MS (ESI) (90% H\(_2\)O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), \( t_R = 12.06 \) min, 99% purity, \( m/z \) [M + H]\(^+\) calcd for C\(_54\)H\(_51\)N\(_3\)O\(_6\)S, 1002.53; found, 1003.0; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for C\(_54\)H\(_51\)N\(_3\)O\(_6\)S, 1002.5382; found, 1002.5360.

VHL ligand b (64)

![VHL ligand b](image)

This compound was synthesized as described previously.\(^{11,12}\)

\(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 1.05 (s, 9H, C(CH\(_3\))\(_3\)), 1.41 (s, 9H, OC(CH\(_3\))\(_3\)), 1.46 (d, \( J = 7.0 \) Hz, 3H, CHCH\(_3\)), 2.03 – 2.10 (m, 1H, 3-H), 2.53 (s, 3H, CH\(_3\)), 2.58 – 2.66 (m, 1H, 3-H), 2.77 (d, \( J = 4.0 \) Hz, 1H), 3.55 (dd, \( J = 3.3, 11.5 \) Hz, 1H), 4.15 (d, \( J = 11.5 \) Hz, 1H), 4.19 (d, \( J = 9.0 \) Hz, 1H), 4.51 (br s, 1H), 4.80 (t, \( J = 7.9 \) Hz, 1H), 5.07 (symm m, 1H), 5.20 (d, \( J = 8.8 \) Hz, 1H), 7.34 – 7.44 (m, 4H, 2'-H, 3'-H), 7.70 (d, \( J = 7.7 \) Hz, 1H, NHCO), 8.67 (s, 1H, 2''-H); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \( \delta \) 0.93 (s, 9H, C(CH\(_3\))\(_3\)), 1.36 (d, \( J = 7.6 \) Hz, 3H, CHCH\(_3\)), 1.38 (s, 9H, OC(CH\(_3\))\(_3\)), 1.72 – 1.81 (m, 1H, 3-H), 1.98 – 2.06 (m, 1H, 3-H), 2.45 (s, 3H, CH\(_3\)), 3.53 – 3.62 (m, 2H), 4.13 (d, \( J = 9.2 \) Hz, 1H), 4.28 (br s, 1H), 4.44 (t, \( J = 8.1 \) Hz, 1H), 4.89 (symm m, 1H), 5.12 (d, \( J = 3.3 \) Hz, 1H, OH), 6.42 (d, \( J = 9.0 \) Hz, 1H, NHboc), 7.35 – 7.47 (m, 4H, 2'-H, 3'-H), 8.41 (d, \( J = 7.6 \) Hz, NHCO), 8.98 (s, 1H, 2''-H); \(^1\)H NMR (400 MHz, CD\(_2\)OD) \( \delta \) 1.02 (s, 9H, C(CH\(_3\))\(_3\)), 1.44 (s, 9H, OC(CH\(_3\))\(_3\)), 1.51 (d, \( J = 7.0 \) Hz, 3H, CHCH\(_3\)), 1.90 – 2.00 (m, 1H, 3-H), 2.16 – 2.24 (m, 1H, 3-H), 2.48 (s, 3H, CH\(_3\)), 3.74 (dd, \( J = 3.8, 10.9 \) Hz, 1H), 3.86 (d, \( J = 11.1 \) Hz, 1H), 4.29 (d, \( J = 9.3 \) Hz, 1H), 4.44 (br s, 1H), 4.59 (t, \( J = 8.3 \) Hz, 1H), 4.99 (symm m, 1H), 6.41 (d, \( J = 9.4 \) Hz, 1H), 7.39 – 7.47 (m, 4H, 2'-H, 3'-H), 8.88 (s, 1H, 2''-H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \) 16.03 (CH\(_3\)), 22.21 (CH(CH\(_3\))), 26.40 (C(CH\(_3\))\(_3\)), 28.29 (OC(CH\(_3\))\(_3\)), 34.97 (C(CH\(_3\))\(_3\)), 35.38 (C-3), 48.81 (CH(CH\(_3\))), 56.48, 58.32, 58.91 (C-2,
NH\textsubscript{2}, 70.00 (C-4), 80.44 (OC\textsubscript{3}(CH\textsubscript{3})\textsubscript{3}), 126.40 (C-2', C-6'), 129.53 (C-3', C-5'), 130.08 (C-1'), 131.59 (C-5''), 143.18 (C-4''), 148.43 (C-4''), 150.30 (C-2''), 156.41 (NHCOO), 169.67 (NHCO), 172.65 (NCO); HRMS (ESI) m/z [M + H]\textsuperscript{+} calcd for C\textsubscript{38}H\textsubscript{41}N\textsubscript{4}O\textsubscript{5}S, 545.2792; found, 545.2786.

**VHL(b)-2-2-2 building block (65)**

This compound was prepared using the General Procedure III, VHL ligand b (64, 0.54 g) and acid 58 (0.23 g). The crude product was purified by flash chromatography on silica gel (0% to 10% MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to yield the title compound as a colorless solid.

Yield (392 mg, 60%); mp 58 – 60 °C; R\textsubscript{f} = 0.34 (CH\textsubscript{2}Cl\textsubscript{2}/MeOH 9:1); \textsuperscript{1}H NMR (600 MHz, DMSO-\textsubscript{d}6) \(\delta\) 0.93 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}), 1.37 (d, \(\textit{J}\) = 7.0 Hz, 3H, CHCH\textsubscript{3}), 1.64 – 1.86 (m, 1H, 3-H'), 1.91 – 2.11 (m, 1H, 3-H''), 2.44 (s, 3H, CH\textsubscript{3}), 3.48 – 3.64 (m, 10H), 3.64 – 3.76 (m, 4H), 3.95 (d, \(\textit{J}\) = 1.3 Hz, 2H, OCH\textsubscript{2}, CH\textsubscript{2}Cl), 4.24 – 4.37 (m, 1H), 4.43 (t, \(\textit{J}\) = 8.3 Hz, 1H), 4.54 (d, \(\textit{J}\) = 9.5 Hz, 1H), 4.90 (p, \(\textit{J}\) = 7.3 Hz, 1H, 2-H, 4-H, 2 × NHCH), 5.10 (d, \(\textit{J}\) = 3.6 Hz, 1H, OH), 7.24 – 7.40 (m, 3H), 7.40 – 7.52 (m, 2H, CONH, 2'-H, 3'-H), 8.40 (d, \(\textit{J}\) = 7.8 Hz, 1H, NHCO), 8.97 (s, 1H, 2''-H); \textsuperscript{13}C NMR (151 MHz, DMSO-\textsubscript{d}6) \(\delta\) 16.12 (CH\textsubscript{3}), 22.58 (CH\textsubscript{3}CH), 26.37 (C(CH\textsubscript{3})\textsubscript{3}), 35.89 (C(CH\textsubscript{3})\textsubscript{3}), 37.86 (C-3), 40.22 (NHCH\textsubscript{3}), 43.73 (CH\textsubscript{2}Cl), 47.89 (CH\textsubscript{2}Cl), 55.83, 56.65, 58.70 (C-2, NHCHCO, C-5), 68.91 (OCH\textsubscript{2}), 69.77 and 69.81 (3 × OCH\textsubscript{2}), 70.00 (2 × C), 70.59, 70.71 (OCH\textsubscript{2}, C-4), 126.48 (C-2', C-6'), 128.97 (C-3', C-5'), 129.84 (C-1'), 131.25 (C-5''), 144.85 (C-4''), 147.90 (C-4'''), 151.60 (C-2''), 168.65, 169.16, 170.59 (CO); MS (ESI) m/z [M + H]\textsuperscript{+} calcd for C\textsubscript{31}H\textsubscript{43}ClN\textsubscript{4}O\textsubscript{5}S 653.28; found, 653.5; HRMS (ESI) m/z [M + H]\textsuperscript{+} calcd for C\textsubscript{31}H\textsubscript{45}ClN\textsubscript{4}O\textsubscript{5}S, 653.2770; found, 653.2759.

**VHL(b)-based PROTAC 27 (CST620)**
This compound was prepared using the General Procedure IV and precursor 65 (65 mg). The crude product was purified by column chromatography (gradient of CH₂Cl₂/MeOH+7N NH₃ 29:1 to 19:1) followed by HPLC purification using a gradient of 70% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.

Yield (61 mg, 57%); mp 116 – 120 °C; \( R_f = 0.13 \) (CH₂Cl₂/MeOH+7N NH₃; 19:1); \(^1\)H NMR (600 MHz, DMSO-d₆) \( \delta \) 0.94 (s, 9H), 1.36 (d, \( J = 7.0 \) Hz, 3H), 1.49 – 1.60 (m, 2H), 1.70 – 1.81 (m, 3H), 1.81 – 1.92 (m, 2H), 2.00 – 2.08 (m, 1H), 2.23 (q, \( J = 8.5, 9.2 \) Hz, 2H), 2.29 (s, 3H), 2.41 (s, 3H), 2.43 (s, 3H), 2.52 (t, \( J = 5.9 \) Hz, 2H), 2.57 (t, \( J = 5.0 \) Hz, 4H), 3.07 – 3.21 (m, 4H), 3.49 – 3.77 (m, 12H), 3.86 – 4.05 (m, 2H), 4.15 – 4.38 (m, 1H), 4.44 (t, \( J = 8.3 \) Hz, 1H), 4.54 (d, \( J = 9.5 \) Hz, 1H), 4.89 (p, \( J = 7.1 \) Hz, 1H), 5.11 (d, \( J = 3.6 \) Hz, 1H), 5.80 (p, \( J = 8.9 \) Hz, 1H), 7.26 – 7.57 (m, 6H), 7.83 (d, \( J = 8.9 \) Hz, 1H), 8.04 (d, \( J = 3.1 \) Hz, 1H), 8.39 (d, \( J = 7.7 \) Hz, 1H), 8.93 (s, 1H), 8.95 (s, 1H), 10.05 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-d₆) \( \delta \) 13.75, 16.11, 22.59, 25.24, 26.40, 27.69, 31.44, 35.88, 37.87, 47.89, 48.48, 53.06, 55.85, 56.66, 57.38, 58.72, 68.56, 68.93, 69.79, 69.81, 69.85, 70.02, 70.66, 106.70, 115.30, 124.75, 126.47, 128.95, 129.36, 129.84, 131.24, 135.45, 142.23, 143.63, 144.38, 144.82, 147.89, 151.56, 154.91, 158.38, 158.72, 160.91, 168.67, 169.18, 170.60, 202.56; LC-MS (ESI) (90% H₂O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), \( t_R = 11.92 \) min, 99% purity, \( m/z \) [M + H]⁺ calcd for C₅₅H₇₃N₁₁O₉S, 1064.53; found, 1064.5368; HRMS (ESI) \( m/z \) [M + H]⁺ calcd for C₅₅H₇₃N₁₁O₉S, 1064.5386; found, 1064.5368.

VHL ligand c (66)

This compound was synthesized as described previously.\(^{13}\)

\(^1\)H NMR (600 MHz, DMSO-d₆) \( \delta \) 0.73 (d, \( J = 6.6 \) Hz, 3H), 0.97 (d, \( J = 6.6 \) Hz, 3H, CH(CH₃)₂), 1.86 – 1.94 (m, 1H), 1.97 – 2.07 (m, 1H, 3-H), 2.23 – 2.40 (m, 1H, CH(CH₃)₂), 2.45 (s, 3H, CH₃), 3.64 – 3.80 (m, 2H, 5-H), 4.16 – 4.29 (m, 2H), 4.29 – 4.35 (m, 1H), 4.36 – 4.58 (m, 3H, 4-H), 4.70 (d, \( J = 10.8 \) Hz, 1H, 2-H, 4-H, NCH, NHCH₂NCH₂), 5.06 (d, \( J = 4.0 \) Hz, 1H, OH), 6.87 (dd, \( J = 1.8, 7.9 \) Hz, 1H, 5'-H), 6.92 (d, \( J = 1.8 \) Hz, 1H, 3'-H), 7.25 (d, \( J = 7.8 \) Hz, 1H, 6'-H), 7.49 (ddd, \( J = 2.3, 5.8, 7.8 \) Hz, 1H), 7.53 – 7.66 (m, 2H), 7.70 (d, \( J = 7.6 \) Hz, 1H, Ar”’-H), 8.37 (t, \( J = 5.9 \) Hz, 1H, CONH), 8.94 (s, 1H, 2”’-H), 9.79 (s, 1H, Ar-OH); \(^{13}\)C NMR (151 MHz, DMSO-d₆) \( \delta \) 16.26 (CH₃), 18.78, 19.06 (CH(CH₃)₂), 28.53 (CH(CH₃)₂), 37.34 (C-3), 38.27 (NHCH₂), 46.98 (C-2’’’), 55.54 (C-5), 57.96 (C-2), 58.81 (NCH), 68.76 (C-4), 115.27 (C-3’’), 119.59 (C-5’’), 58.
123.17, 123.77 (C-4′′', C-7′′'), 125.43 (C-1′), 128.06 (C-6′′'), 128.72 (C-6′), 130.93, 131.47, 131.55 (C-4′, C-5′', C-7a′′'), 131.74 (C-3a′′'), 142.37 (C-3a′′), 147.69 (C-4′), 151.42 (C-2′′), 155.04 (C-2'), 167.66, 168.25, 171.90 (CO); MS (ESI) m/z [M + H]⁺ calcd for C_{29}H_{32}N_{4}O_{5}S, 549.21; found, 549.4.

8-Chlorooctyl methanesulfonate (67)

To a solution of 8-chloro-1-octanol (2.20 g, 13.12 mmol) in dry CH₂Cl₂ (20 mL), Et₃N (1.99 g, 2.74 mL, 19.68 mmol) was added under argon atmosphere and the mixture was cooled to 0 °C. Subsequently, methanesulfonyl chloride (2.25 g, 1.52 mL, 19.68 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 3 h. After the reaction was complete (monitored by TLC), MeOH (20 mL) was added to the mixture carefully. The volatiles were then evaporated and the resultant residue was partitioned between EtOAc (100 mL) and H₂O (100 mL). The organic layer was further washed with brine (2 × 100 mL), dried over Na₂SO₄, filtered and evaporated. The crude product was purified by column chromatography (EtOAc/n-hexanes 2:1) to give a colorless oil.

Yield (2.74 g, 86%); Rf = 0.60 (EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 1.29 – 1.37 (m, 4H), 1.38 – 1.49 (m, 4H), 1.75 (symm m, 4H), 3.00 (s, 3H, CH₃), 3.53 (t, J = 6.7 Hz, 2H, CH₂Cl), 4.22 (t, J = 6.5 Hz, 2H, OCH₂); HRMS (ESI) m/z [M + H]⁺ calcd for C₉H₂₀O₃ClS, 243.0816; found, 243.0815.

VHL(c)-8 building block (68)

This compound was prepared using the General Procedure V, VHL ligand c (66, 165 mg) and mesylate 67 (87 mg). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in CH₂Cl₂) to yield the title compound as a colorless solid.

Yield (142 mg, 68%); mp 80 – 84 °C; Rf = 0.22 (CH₂Cl₂/MeOH 29:1); ¹H NMR (600 MHz, DMSO-d₆) δ 0.73 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H, CH(CH₃)₂), 1.27 – 1.42 (m, 6H), 1.42 – 1.49 (m, 2H), 1.66 – 1.79 (m, 4H, CH₃), 1.88 – 1.96 (m, 1H), 1.99 – 2.07 (m, 1H, 3'-H), 2.26 – 2.40 (m, 1H, CH(CH₃)₂), 2.46 (s, 3H, CH₃), 3.61 (t, J = 6.6 Hz, 2H, CH₂Cl), 3.64 – 3.72 (m, 1H), 3.77 (dd, J = 4.5, 10.6 Hz, 1H, 5-H), 4.04
(t, J = 6.3 Hz, 2H, OCH₃), 4.18 – 4.37 (m, 3H), 4.37 – 4.59 (m, 3H), 4.71 (d, J = 10.8 Hz, 1H, 2-H, 4-H, NCH₃, NHCH₂, NCH₂), 5.06 (d, J = 4.1 Hz, 1H, OH), 6.96 – 7.03 (m, 2H, 3'-H, 5'-H), 7.32 (d, J = 7.7 Hz, 1H, 6'-H), 7.46 – 7.55 (m, 1H), 7.55 – 7.64 (m, 2H), 7.68 – 7.76 (m, 1H, Ar'''-H), 8.33 (t, J = 6.0 Hz, 1H, CONH), 8.97 (s, 1H, 2''-H); ¹³C NMR (151 MHz, DMSO-d₆) δ 16.15 (CH₃), 18.76, 19.02 (CH(CH₃)₂), 25.63, 26.37, 28.34 (CH₃), 28.52 (CH(CH₃)₂), 28.77 (2 x CH₃), 32.19 (CH₂), 37.17 (C-3), 38.22 (NHCH₂), 45.54 (CH₂Cl), 46.95 (C-2'''), 55.53 (C-5), 57.93 (C-2), 58.84 (NCH), 67.88 (OCH₃), 68.76 (C-4), 111.87 (C-3'), 120.90 (C-5'), 123.15, 123.75 (C-4''', C-7''), 127.14, 127.83 (C-1', C-6'), 128.04 (C-6''), 131.13, 131.46, 131.53 (C-4', C-5', C-7'a'''), 131.71 (C-5'''), 142.34 (C-3'a'''), 148.03 (C-4'''), 151.56 (C-2''), 156.11 (C-2'), 167.62, 168.26, 171.66 (CO); MS (ESI) m/z [M + H]+ calcd for C₃₇H₄₇ClN₄O₅S, 695.30; found, 695.7; HRMS (ESI) m/z [M + H]+ calcd for C₃₇H₄₇ClN₄O₅S, 695.3006; found, 695.3029.

VHL(c)-based PROTAC 28

This compound was prepared using the General Procedure IV and precursor 68 (70 mg). The crude product was purified by column chromatography (CH₂Cl₂/MeOH+7N NH₃ 29:1) followed by HPLC purification using a gradient of 70% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.

Yield (55 mg, 50%); mp 134 – 138 °C; Rf = 0.10 (CH₂Cl₂/MeOH+7N NH₃ 29:1); ¹H NMR (600 MHz, DMSO-d₆) δ 0.73 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.4 Hz, 3H), 1.25 – 1.63 (m, 12H), 1.66 – 1.98 (m, 7H), 2.00 – 2.17 (m, 1H), 2.18 – 2.37 (m, 8H), 2.41 (s, 3H), 2.43 – 2.49 (m, 7H), 3.07 – 3.26 (m, 4H), 3.64 – 3.84 (m, 2H), 4.04 (t, J = 6.3 Hz, 2H), 4.17 – 4.60 (m, 6H), 4.71 (d, J = 10.8 Hz, 1H), 5.07 (s, 1H), 5.80 (p, J = 8.9 Hz, 1H), 6.90 – 7.04 (m, 2H), 7.33 (d, J = 7.7 Hz, 1H), 7.39 – 7.53 (m, 2H), 7.55 – 7.65 (m, 2H), 7.70 (d, J = 7.6 Hz, 1H), 7.83 (d, J = 9.0 Hz, 1H), 8.03 (d, J = 3.1 Hz, 1H), 8.33 (t, J = 6.0 Hz, 1H), 8.93 (s, 1H), 8.97 (s, 1H), 10.04 (s, 1H); ¹³C NMR (151 MHz, DMSO-d₆) δ 13.75, 16.15, 18.76, 19.03, 25.24, 25.68, 26.42, 27.06, 27.69, 28.52, 28.79, 28.89, 29.07, 31.44, 37.17, 38.22, 46.95, 48.50, 52.72, 53.05, 55.54, 57.93, 58.04, 58.84, 67.88, 68.76, 106.70, 111.87, 115.30, 120.89, 123.15, 123.73, 124.73, 127.13, 127.85, 128.03, 129.35, 131.13, 131.46, 131.53, 131.70, 135.44, 142.23, 142.33, 143.64, 144.37, 148.02, 151.55, 154.91, 156.12, 158.39, 158.72, 160.91, 167.62, 168.26, 171.66, 202.56; LC-MS (ESI) (90% H₂O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-420 nm), tᵣ = 60
12.84 min, 99% purity, m/z [M + H]^+ calcd for C_{61}H_{75}N_{11}O_{7}S, 1106.56; found, 1106.8; HRMS (ESI) m/z [M + H]^+ calcd for C_{61}H_{75}N_{11}O_{7}S, 1106.5644; found, 1106.5628.

2-[2-(2-Chloroethoxy)ethoxy]ethyl methanesulfonate (69)

To a solution of 2-[2-(2-chloroethoxy)ethoxy]ethanol (1.18 g, 7 mmol) in dry CH_{2}Cl_{2} (10 mL), DIPEA (1.36 g, 1.78 mL, 10.5 mmol) was added under argon atmosphere and the mixture was cooled to 0 °C. Subsequently, methanesulfonyl chloride (1.20 g, 0.81 mL, 10.5 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 3 h. After the reaction was complete (monitored by TLC), MeOH (20 mL) was added to the mixture carefully. The volatiles were then evaporated and the crude product was purified by column chromatography (EtOAc) to give a colorless oil.

Yield (1.62 g, 94%); R_{f} = 0.50 (EtOAc); ^1H NMR (400 MHz, CDCl_{3}) δ 3.07 (s, 3H, CH_{3}), 3.61 – 3.64 (m, 2H), 3.67 – 3.69 (m, 4H), 3.73 – 3.77 (m, 2H), 3.78 (symm m, 2H), 4.38 (symm m, 2H); ^13C NMR (101 MHz, CDCl_{3}) δ 37.67 (CH_{3}), 42.77 (CH_{2}Cl), 69.03, 69.17, 70.51, 70.53, 71.28 (OCH_{2}); HRMS (ESI) m/z [M + H]^+ calcd for C_{17}H_{16}O_{5}ClS, 247.0402; found, 247.0399.

VHL(c)-2-2-2 building block (70)

This compound was prepared using the General Procedure V, VHL ligand c (66, 165 mg) and mesylate 69 (89 mg). The crude material was purified by column chromatography (gradient of CH_{2}Cl_{2}/MeOH 29:1 to 19:1) to yield the title compound as a colorless solid.

Yield (117 mg, 56%); mp 74 – 76 °C; R_{f} = 0.28 (CH_{2}Cl_{2}/MeOH 15:1); ^1H NMR (600 MHz, DMSO-d_{6}) δ 0.73 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H, CH(CH_{3})_{3}), 1.81 – 1.97 (m, 1H), 1.99 – 2.15 (m, 1H, 3-H), 2.20 – 2.41 (m, 1H, CH(CH_{3})_{2}), 2.46 (s, 3H, CH_{3}), 3.57 – 3.62 (m, 2H, CH_{2}Cl), 3.60 – 3.71 (m, 7H), 3.74 – 3.82 (m, 3H, 5-H, OCH_{2}), 4.19 (t, J = 4.7 Hz, 2H, OCH_{2}), 4.21 – 4.37 (m, 3H), 4.37 – 4.59 (m, 3H), 4.71 (d, J = 10.8 Hz, 1H, 2-H, 4-H, NCH, NHCH_{2}, NHCH_{3}), 5.06 (d, J = 4.1 Hz, 1H, OH), 7.00 (dd, J = 1.6, 7.7 Hz, 1H, 5’-H), 7.04 (d, J = 1.8 Hz, 1H, 3’-H), 7.33 (d, J = 7.8 Hz, 1H, 6’-H), 7.41 – 7.56 (m, 1H), 7.56 – 7.66
(m, 2H), 7.70 (dd, J = 1.1, 7.5 Hz, 1H, Ar′′″-H), 8.33 (t, J = 6.0 Hz, 1H, CONH), 8.97 (s, 1H, 2′″-H);
\(^{13}\)C NMR (151 MHz, DMSO-\(d_6\))  δ 16.16 (CH\(_3\)), 18.77, 19.03 (CH(CH\(_3\))\(_2\)), 28.54 (CH(CH\(_3\))\(_2\)), 37.23 (C-3), 38.24 (NHCH\(_3\)), 43.71 (CH\(_2\)), 46.97 (C-2″), 55.56 (C-5), 57.95 (C-2), 58.86 (NCH), 68.08, 68.77, 69.17, 69.88, 70.22, 70.73 (C-4, OCH\(_2\)), 112.38 (C-3′), 121.25 (C-5′), 123.16, 123.76 (C-4″, C-7″), 123.38, 127.87 (C-1′, C-6′), 128.05 (C-6″), 131.15, 131.41, 131.54 (C-4′, C-5″, C-7a″), 131.72 (C-5″), 142.36 (C-3a″), 148.09 (C-4″), 151.60 (C-2″), 156.04 (C-2′), 167.64, 168.27, 171.70 (CO); MS (ESI) m/z [M + H]\(^{+}\) calcd for C\(_{35}\)H\(_{43}\)ClN\(_4\)O\(_7\)S, 699.26; found, 699.7; HRMS (ESI) m/z [M + H]\(^{+}\) calcd for C\(_{35}\)H\(_{43}\)ClN\(_4\)O\(_7\)S, 699.2614; found, 699.2589.

**VHL(c)-based PROTAC 29**

This compound was prepared using the General Procedure IV and precursor 70 (70 mg). The crude product was purified by column chromatography (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\) 29:1) followed by HPLC purification using a gradient of 70% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.

Yield (29 mg, 26%); mp 96 – 98 °C; \(R_f\) = 0.25 (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\) 19:1); \(^1\)H NMR (600 MHz, DMSO-\(d_6\))  δ 0.72 (d, J = 6.6 Hz, 3H), 0.95 (d, J = 6.5 Hz, 3H), 1.52 – 1.60 (m, 2H), 1.71 – 1.80 (m, 2H), 1.80 – 1.96 (m, 3H), 2.00 – 2.07 (m, 1H), 2.17 – 2.36 (m, 6H), 2.41 (s, 3H), 2.46 (s, 3H), 2.49 – 2.61 (m, 6H), 3.11 (t, J = 5.0 Hz, 4H), 3.45 – 3.59 (m, 4H), 3.60 – 3.86 (m, 6H), 4.19 (t, J = 4.7 Hz, 2H), 4.22 – 4.60 (m, 6H), 4.71 (d, J = 10.8 Hz, 1H), 5.06 (d, J = 4.1 Hz, 1H), 5.80 (p, J = 8.9 Hz, 1H), 7.00 (dd, J = 1.6, 7.7 Hz, 1H), 7.05 (d, J = 1.7 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.41 (dd, J = 3.1, 9.1 Hz, 1H), 7.44 – 7.54 (m, 1H), 7.54 – 7.63 (m, 2H), 7.69 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 8.9 Hz, 1H), 8.01 (d, J = 3.1 Hz, 1H), 8.33 (t, J = 6.0 Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.04 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) δ 13.74, 16.16, 18.75, 19.02, 25.23, 27.68, 28.52, 31.43, 37.22, 38.23, 46.94, 48.42, 53.02, 55.54, 57.34, 57.92, 58.84, 68.14, 68.53, 68.75, 69.14, 69.90, 70.23, 106.70, 112.34, 115.28, 121.22, 123.13, 123.72, 124.73, 127.36, 127.87, 128.02, 129.35, 131.14, 131.39, 131.51, 131.69, 135.43, 142.21, 142.32, 143.59, 144.36, 148.06, 151.57, 154.90, 156.05, 158.37, 158.71, 160.90, 167.61, 168.25, 171.67, 202.56; LC-MS (ESI) (90% H\(_2\)O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), \(t_R\) =
11.81 min, 99% purity, m/z [M + H]^+ calcd for C_{59}H_{71}N_{11}O_{9}S, 1110.52; found, 1111.2; HRMS (ESI) m/z [M + H]^+ calcd for C_{59}H_{71}N_{11}O_{9}S, 1110.5230; found, 1110.5179.

2-[2-[2-(Chloroethoxy)ethoxy]ethoxy]ethyl methanesulfonate (71)

To a solution of 2-[2-[2-(chloroethoxy)ethoxy]ethoxy]ethanol (0.88 g, 4.14 mmol) in dry CH_2Cl_2 (10 mL), Et_3N (0.63 g, 0.86 mL, 6.21 mmol) was added under argon atmosphere and the mixture was cooled to 0 °C. Subsequently, methanesulfonyl chloride (0.71 g, 0.45 mL, 6.21 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 3 h. After the reaction was complete (monitored by TLC), MeOH (20 mL) was added to the mixture carefully. The volatiles were then evaporated and the resultant residue was partitioned between EtOAc (100 mL) and H_2O (100 mL). The organic layer was further washed with brine (2 × 100 mL), dried over Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (EtOAc/n-hexanes 2:1) to give a colorless oil.

Yield (1.08 g, 90%); R_f = 0.20 (EtOAc/n-hexanes 2:1); ^1H NMR (400 MHz, CDCl_3) δ 3.08 (s, 3H, CH_3), 3.61 – 3.70 (m, 10H), 3.73 – 3.79 (m, 4H), 4.38 (symm m, 2H); HRMS (ESI) m/z [M + H]^+ calcd for C_{9}H_{20}O_{6}ClS, 291.0664; found, 291.0658.

VHL(c)-2-2-2-2 building block (72)

This compound was prepared using the General Procedure V, VHL ligand c (66, 165 mg) and mesylate 71 (105 mg). The crude material was purified by column chromatography (CH_2Cl_2/MeOH 29:1) to yield the title compound as a colorless solid.

Yield (120 mg, 54%); mp 56 – 58 °C; R_f = 0.39 (CH_2Cl_2/MeOH 15:1); ^1H NMR (600 MHz, DMSO-d_6) δ 0.73 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.5 Hz, 3H, CH(CH_3)_2), 1.87 – 1.96 (m, 1H), 1.99 – 2.07 (m, 1H, 3-H), 2.26 – 2.37 (m, 1H, CH(CH_3)_2), 2.46 (s, 3H, CH_3), 3.46 – 3.59 (m, 6H), 3.59 – 3.71 (m, 7H), 3.73 – 3.83 (m, 3H, 5-H, CH_2Cl, OCH_2), 4.15 – 4.20 (m, 2H, OCH_2), 4.21 – 4.36 (m, 3H), 4.36 – 4.58 (m, 3H), 4.71 (d,
\[ J = 10.8 \text{ Hz}, 1H, 2'-H, 4'-H, NCH, NHCH_2, NCH_3 \], \[ 5.06 \text{ (d, } J = 4.1 \text{ Hz, 1H, OH)} \], \[ 7.00 \text{ (dd, } J = 1.6, 7.7 \text{ Hz, 1H, 5'-H)} \], \[ 7.04 \text{ (d, } J = 1.8 \text{ Hz, 1H, 3'-H)} \], \[ 7.33 \text{ (d, } J = 7.8 \text{ Hz, 1H, 6'-H)} \], \[ 7.44 - 7.54 \text{ (m, 1H)} \], \[ 7.55 - 7.66 \text{ (m, 2H)} \], \[ 7.68 - 7.73 \text{ (m, 1H, Ar''''-H)} \], \[ 8.33 \text{ (t, } J = 6.0 \text{ Hz, 1H, CONH)} \], \[ 8.97 \text{ (s, 1H, 2''-H)} \]; \[ ^{13}C \text{ NMR (151 MHz, DMSO-}d_6) \] \( \delta \) 16.15 (CH_3), 18.76, 19.02 (CH(CH_3)_2), 28.53 (CH(CH_3)_2), 37.21 (C-3), 38.23 (NHCH_3), 43.68 (CH_2Cl), 46.96 (C-2'''), 55.54 (C-5), 57.93 (C-2), 58.85 (NCH), 68.08, 68.76, 69.14, 69.82, 69.94, 70.00, 70.22, 70.67 (C-4, OCH_3), 112.35 (C-3'), 121.23 (C-5'), 123.15, 123.75 (C-4'', C-7''), 127.36, 127.86 (C-1', C-6'), 128.04 (C-6'''), 131.13, 131.40, 131.53 (C-4', C-5'', C-7a'''), 131.72 (C-5'''), 142.34 (C-3a''), 148.08 (C-4''), 151.59 (C-2''), 156.03 (C-2'), 167.63, 168.26, 171.69 (CO); \[ MS \text{ (ESI)} \] \( m/z \) [M + H]^+ calcd for C_{37}H_{47}ClN_4O_8S, 743.28; found, 743.5; \[ HRMS \text{ (ESI)} \] \( m/z \) [M + H]^+ calcd for C_{37}H_{47}ClN_4O_8S, 743.2844; found, 743.2876.

**VHL(c)-based PROTAC 30**

This compound was prepared using the General Procedure IV and precursor 72 (74 mg). The crude product was purified by column chromatography (CH_2Cl_2/MeOH+7N NH_3 19:1) to obtain the title compound as a yellow solid.

Yield (80 mg, 69%); mp 118 – 120 °C; \( R_f \) = 0.25 (CH_2Cl_2/MeOH+7NH_3 19:1); \[ ^{1}H \text{ NMR (600 MHz, DMSO-}d_6) \] \( \delta \) 0.72 (d, \( J = 6.6 \) Hz, 3H), 0.96 (d, \( J = 6.5 \) Hz, 3H), 1.51 – 1.61 (m, 2H), 1.70 – 1.80 (m, 2H), 1.81 – 1.96 (m, 3H), 2.00 – 2.07 (m, 1H), 2.16 – 2.36 (m, 6H), 2.41 (s, 3H), 2.46 (s, 3H), 2.49 – 2.59 (m, 6H), 3.11 (t, \( J = 5.0 \) Hz, 4H), 3.44 – 3.60 (m, 8H), 3.60 – 3.86 (m, 6H), 4.14 – 4.21 (m, 2H), 4.21 – 4.60 (m, 6H), 4.71 (d, \( J = 10.8 \) Hz, 1H), 5.07 (d, \( J = 4.1 \) Hz, 1H), 5.74 – 5.85 (m, 1H), 7.00 (dd, \( J = 1.7, 7.8 \) Hz, 1H), 7.04 (d, \( J = 1.7 \) Hz, 1H), 7.33 (d, \( J = 7.8 \) Hz, 1H), 7.42 (t, \( J = 3.2, 9.1 \) Hz, 1H), 7.48 (dd, \( J = 2.2, 6.2, 8.1 \) Hz, 1H), 7.55 – 7.64 (m, 2H), 7.70 (d, \( J = 7.6 \) Hz, 1H), 7.78 – 7.86 (m, 1H), 8.02 (d, \( J = 3.0 \) Hz, 1H), 8.33 (t, \( J = 5.9 \) Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.04 (s, 1H); \[ ^{13}C \text{ NMR (151 MHz, DMSO-}d_6) \] \( \delta \) 13.75, 16.16, 18.76, 19.03, 25.23, 27.69, 28.53, 31.44, 37.23, 38.23, 46.95, 48.46, 53.03, 55.55, 57.36, 57.93, 58.85, 68.10, 68.51, 68.77, 69.16, 69.84, 69.97, 70.02, 70.27, 106.70, 112.35, 115.28, 121.23, 123.14, 123.73, 124.73, 127.37, 127.88, 128.03, 129.36, 131.14, 131.39, 131.53, 131.70, 135.43, 142.23, 142.33, 143.61, 144.37, 148.07, 151.55, 154.91, 156.04, 158.38, 158.71, 160.91, 167.62, 168.26, 171.68, 202.56; \[ LC-MS \text{ (ESI)} \] (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, 64
DAD 220-400 nm), $t_R = 12.13$ min, 97% purity, $m/z$ [M + H]$^+$ calcd for C$_{61}$H$_{75}$N$_{11}$O$_{10}$S, 1154.55; found, 1154.7; HRMS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{61}$H$_{75}$N$_{11}$O$_{10}$S, 1154.5492; found, 1154.5452.

**Linker Cl-6-5-5-OMs:**

![Diagram of the linker Cl-6-5-5-OMs]

Reagents and conditions: (a) 3,4-Dihydro-2H-pyran, CuSO$_4$·5H$_2$O, MeCN, rt, 4 h; (b) MsCl, DIPEA, CH$_2$Cl$_2$, rt, 3 h; (c) 50% NaOH (aq), DMSO, rt, 20 h; (d) Bu$_4$NHSO$_4$, 50% NaOH (aq), toluene, rt, 24 h; (e) p-TsOH·H$_2$O, MeOH, rt, 20 h; (f) MsCl, DIPEA, CH$_2$Cl$_2$, rt, 3 h

5-Tetrahydropyran-2-yloxypentan-1-ol (73)

To a solution of 1,5-pentanediol (5.00 g, 47.85 mmol) in dry MeCN (25 mL), 3,4-dihydro-2H-pyran (4.43 g, 4.81 mL, 52.64 mmol) was added under argon atmosphere. Subsequently, CuSO$_4$·5H$_2$O (2.39 g, 9.57 mmol) was added, followed by stirring of the mixture at rt for 4 h. After the reaction was complete, the mixture was filtered and the filtrate was concentrated. The crude product was purified by column chromatography (EtOAc/n-hexanes 1:1) to give a colorless oil.
Yield (4.41 g, 49%); R<sub>f</sub> = 0.20 (EtOAc/n-hexanes 1:1); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.39 – 1.75 (m, 12H), 1.76 – 1.87 (m, 1H), 3.39 (symm m, 1H), 3.45 – 3.53 (m, 1H), 3.64 (t, J = 6.6 Hz, 2H), 3.74 (symm m, 1H), 3.82 – 3.90 (m, 1H), 4.54 – 4.59 (m, 1H, CH); HRMS (ESI) m/z [M + H]<sup>+</sup> calcd for C<sub>10</sub>H<sub>21</sub>O<sub>3</sub>, 189.1485; found, 189.1482.

5-Tetrahydropyran-2-yloxypentyl methanesulfonate (74)

![Chemical structure of 5-Tetrahydropyran-2-yloxypentyl methanesulfonate (74)]

To a solution of 73 (4.94 g, 26.21 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (25 mL), DIPEA (10.16 g, 13.44 mL, 78.63 mmol) was added under argon atmosphere and the mixture was cooled to 0 °C. Subsequently, methanesulfonyl chloride (4.50 g, 3.04 mL, 39.32 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 3 h. After the reaction was complete (monitored by TLC), MeOH (20 mL) was added to the mixture carefully. The volatiles were then evaporated and the crude product was purified by column chromatography (EtOAc/n-hexanes 1:2) to give an orange oil.

Yield (4.159 g, 60%); R<sub>f</sub> = 0.24 (EtOAc/n-hexanes 1:2); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 1.47 – 1.58 (m, 6H), 1.60 – 1.72 (m, 3H), 1.75 – 1.85 (m, 3H), 3.00 (s, 3H, CH<sub>3</sub>), 3.39 (symm m, 1H), 3.46 – 3.53 (m, 1H), 3.75 (symm m, 1H), 3.81 – 3.89 (m, 1H), 4.23 (t, J = 6.5 Hz, 2H), 4.54 – 4.58 (m, 1H, CH); HRMS (ESI) m/z [M + H]<sup>+</sup> calcd for C<sub>11</sub>H<sub>23</sub>O<sub>5</sub>S, 267.1261; found, 267.1262.

5-(6-Chlorohexoxy)pentan-1-ol (75)

![Chemical structure of 5-(6-Chlorohexoxy)pentan-1-ol (75)]

This compound was synthesized as we described previously. 8

2-[5-[5-(6-Chlorohexoxy)pentoxy]pentoxy]tetrahydropyran (76)

![Chemical structure of 2-[5-[5-(6-Chlorohexoxy)pentoxy]pentoxy]tetrahydropyran (76)]

To a cooled (0 °C) solution of 75 (1.92 g, 8.637 mmol) and 74 (2.30 g, 8.637 mmol) in toluene (40 mL), Bu<sub>4</sub>NHSO<sub>4</sub> (1.354 g, 9.8 mmol) and 50% NaOH (aq) (4.5 mL) were added. The reaction mixture was stirred at rt for 24 h. H<sub>2</sub>O (70 mL) was then added and the mixture extracted with EtOAc (3 × 100 mL). The combined organic layers were further washed with H<sub>2</sub>O and brine (each 200 mL). The organic
layers were dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc/n-hexanes 1:2) to give a colorless oil.

Yield (1.81 g, 53%); $R_f$ = 0.48 (EtOAc/n-hexanes 1:2); $^1$H NMR (400 MHz, CDCl$_3$) δ 1.34 – 1.48 (m, 8H), 1.49 – 1.64 (m, 14H), 1.69 – 1.82 (m, 4H), 3.32 – 3.43 (m, 8H), 3.45 – 3.56 (m, 4H), 3.69 – 3.77 (m, 1H), 3.81 – 3.88 (m, 1H), 4.53 – 4.58 (m, 1H, CH); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 19.62, 22.44, 22.50, 25.43, 25.48, 26.68, 28.48 (2 × C), 29.30 (2 × C), 29.53, 30.69, 32.51, 45.03 (CH$_2$Cl), 62.30, 63.91, 67.22, 67.83, 70.55, 70.70 (OCH$_2$), 98.82 (CH); HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{21}$H$_{42}$O$_4$Cl, 393.2966; found, 393.2961.

5-[5-(6-Chlorohexoxy)pentoxy]pentan-1-ol (77)

To a solution of 76 (1.80 g, 4.58 mmol) in MeOH (20 mL), p-TsOH ×·H$_2$O (0.44 g, 2.29 mmol) was added. The reaction mixture was stirred at rt for 20 h. H$_2$O (70 mL) was added and it was extracted with CH$_2$Cl$_2$ (2 × 150 mL). The combined organic layers were washed with brine (200 mL). The organic layers were dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc/n-hexanes 1:2) to give a colorless oil.

Yield (1.045 g, 74%); $R_f$ = 0.14 (EtOAc/n-hexanes 1:2); $^1$H NMR (400 MHz, CDCl$_3$) δ 1.34 – 1.48 (m, 8H), 1.55 – 1.62 (m, 8H), 1.68 – 1.82 (m, 4H), 3.38 (td, $J$ = 2.2, 6.5 Hz, 8H), 3.52 (t, $J$ = 6.7 Hz, 2H), 3.63 (t, $J$ = 6.4 Hz, 2H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 21.97, 22.43, 25.46, 26.66, 28.41, 28.46, 29.28, 29.50, 32.19, 32.49, 45.03 (CH$_2$Cl), 62.55 (CH$_2$OH), 67.74, 67.86, 70.54, 70.69 (OCH$_2$); HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{16}$H$_{34}$O$_3$Cl, 309.2191; found, 309.2186.

5-[5-(6-Chlorohexoxy)pentoxy]penty methyl methanesulfonate (78)

To a solution of 77 (1.05 g, 3.38 mmol) in dry CH$_2$Cl$_2$ (10 mL), DIPEA (1.31 g, 1.73 mL, 10.14 mmol) was added under argon atmosphere and the mixture was cooled to 0 °C. Subsequently, methanesulfonyl chloride (0.58 g, 0.39 mL, 5.07 mmol) was added dropwise at 0 °C, followed by stirring of the mixture at rt for 3 h. After the reaction was complete (monitored by TLC), MeOH (20 mL) was added to the mixture carefully. The volatiles were then evaporated and the crude product was purified by column chromatography (EtOAc/n-hexanes 1:2) to give a colorless oil.
Yield (0.750 g, 57%); 

\[ R_f = 0.26 \text{ (EtOAc/n-hexanes 1:2)} \]

\[ \text{^1H NMR (400 MHz, CDCl}_3\text{)} \delta 1.32 – 1.48 (m, 8H), 1.52 – 1.62 (m, 8H), 1.72 – 1.80 (m, 4H), 2.98 (s, 3H, CH}_3\text{), 3.34 – 3.42 (m, 8H), 3.51 (t, } J = 6.8 \text{ Hz, 2H, CH}_2\text{Cl), 4.21 (t, } J = 6.5 \text{ Hz, 2H, OCH}_2\text{);} \]

\[ \text{^13C NMR (101 MHz, CDCl}_3\text{)} \delta 22.21, 22.77, 25.46, 26.65, 28.90, 29.08, 29.51 (3 \times C), 32.50, 37.28 (\text{CH}_3\text{), 45.01 (CH}_2\text{Cl), 69.95, 70.32, 70.63, 70.73, 70.82;} \]

\[ \text{HRMS (ESI) } m/z \text{ [M + H]}^+ \text{ calcd for C}_{17}H_{36}O_5ClS, 387.1967; \text{ found, 387.1960.} \]

**VHL(c)-5-5-6 building block (79)**

This compound was prepared using the General Procedure V, VHL ligand c (66, 165 mg) and mesylate 78 (139 mg). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to yield the title compound as a colorless resin.

Yield (123 mg, 49%); 

\[ R_f = 0.35 \text{ (CH}_2\text{Cl}_2/MeOH 19:1)} \]

\[ \text{^1H NMR (600 MHz, DMSO-d}_6\text{)} \delta 0.73 (d, } J = 6.7 \text{ Hz, 3H), 0.95 (d, } J = 6.5 \text{ Hz, 3H), CH(CH}_3\text{)_2), 1.24 – 1.40 (m, 6H), 1.41 – 1.52 (m, 8H), 1.52 – 1.64 (m, 2H), 1.65 – 1.72 (m, 2H), 1.73 – 1.83 (m, 2H, CH), 1.86 – 1.97 (m, 1H), 1.99 – 2.07 (m, 1H, 3-H), 2.27 – 2.39 (m, 1H, CH(CH}_3\text{)_2), 2.46 (s, 3H, CH}_3\text{), 3.31 – 3.39 (m, 8H), 3.59 (t, } J = 6.6 \text{ Hz, 2H), 3.62 – 3.73 (m, 1H), 3.73 – 3.82 (m, 1H), 4.04 (t, } J = 6.3 \text{ Hz, 2H, 5-H, OCH}_2\text{, CH}_2\text{Cl), 4.18 – 4.59 (m, 6H), 4.71 (d, } J = 10.8 \text{ Hz, 1H, 2-H, 4-H, NCH, NHCH}_3\text{, NCH}_2\text{), 5.06 (br s, 1H, OH), 6.96 – 7.02 (m, 2H, 3'-H, 5'-H), 7.32 (d, } J = 7.7 \text{ Hz, 1H, 6'-H), 7.45 – 7.55 (m, 1H), 7.57 – 7.64 (m, 2H), 7.70 (d, } J = 7.6 \text{ Hz, 1H, Ar''''-H), 8.33 (t, } J = 5.9 \text{ Hz, 1H, CONH), 8.97 (s, 1H, 2''-H);} \]

\[ \text{^13C NMR (151 MHz, DMSO-d}_6\text{)} \delta 16.15 (\text{CH}_3\text{), 18.76, 19.02 (CH(CH}_3\text{)_2), 22.55, 22.68, 25.13, 26.24 (CH}_3\text{), 28.53 (CH(CH}_3\text{)_2), 28.64, 29.10, 29.18, 29.19, 29.22, 32.17 (CH}_2\text{), 37.17 (C-3), 38.21 (NHCH}_3\text{), 45.48 (CH}_2\text{Cl), 46.96 (C-2'''), 55.53 (C-5), 57.94 (C-2), 58.84 (NCH), 67.86, 68.77, 69.92, 70.01, 70.04, 70.08 (C-4, OCH}_2\text{), 111.86 (C-3'), 120.90 (C-5'), 123.16, 123.75 (C-4'''', C-7'''), 127.14, 127.84 (C-1', C-6'), 128.04 (C-6'''), 131.13, 131.46, 131.53 (C-4', C-5'', C-7a'''), 131.72 (C-5'''), 142.35 (C-3a'''), 148.03 (C-4''), 151.56 (C-2'''), 156.10 (C-2'), 167.63, 168.26, 171.66 (CO); MS (ESI) m/z [M + H]^+ \text{ calcd for C}_{45}H_{63}ClN_4O_7S, 839.42; found, 839.8; HRMS (ESI) m/z [M + H]^+ \text{ calcd for C}_{45}H_{63}ClN_4O_7S, 839.4179; found, 839.4160.} \]
This compound was prepared using the General Procedure IV and precursor 79 (84 mg). The crude product was purified by column chromatography (CH₂Cl₂/MeOH+7N NH₃ 29:1) followed by HPLC purification using a gradient of 80% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.

Yield (64 mg, 51%); mp 104 – 108 °C; Rᵢ = 0.17 (CH₂Cl₂/MeOH+7N NH₃ 19:1); ¹H NMR (600 MHz, DMSO-d₆) δ 0.72 (d, J = 6.7 Hz, 3H), 0.95 (d, J = 6.4 Hz, 3H), 1.23 – 1.38 (m, 6H), 1.38 – 1.51 (m, 10H), 1.52 – 1.60 (m, 4H), 1.61 – 1.80 (m, 4H), 1.80 – 1.97 (m, 3H), 1.98 – 2.08 (m, 1H), 2.20 – 2.33 (m, 7H), 2.41 (s, 3H), 2.45 (s, 3H), 2.84 – 3.22 (m, 4H), 3.29 – 3.36 (m, 10H), 3.67 (d, J = 10.6 Hz, 2H), 3.76 (dd, J = 4.5, 10.6 Hz, 2H), 4.03 (t, J = 6.4 Hz, 2H), 4.18 – 4.57 (m, 6H), 4.70 (d, J = 10.8 Hz, 1H), 5.06 (d, J = 4.0 Hz, 1H), 5.80 (p, J = 8.9 Hz, 1H), 6.94 – 7.01 (m, 2H), 7.32 (d, J = 7.7 Hz, 1H), 7.40 – 7.45 (m, 1H), 7.46 – 7.52 (m, 1H), 7.57 – 7.62 (m, 2H), 7.70 (d, J = 7.5 Hz, 1H), 7.83 (d, J = 9.0 Hz, 1H), 8.02 (d, J = 2.9 Hz, 1H), 8.33 (t, J = 6.0 Hz, 1H), 8.93 (s, 1H), 8.93 (d, J = 1.6 Hz, 1H), 8.96 (s, 1H), 10.03 (s, 1H); ¹³C NMR (151 MHz, DMSO-d₆) δ 13.76, 16.15, 18.76, 19.03, 22.56, 22.69, 25.25, 25.83, 26.37, 26.94, 27.70, 28.53, 28.65, 29.11, 29.20, 29.38, 31.44, 37.18, 38.22, 46.96, 48.46, 52.69, 53.07, 55.54, 57.95, 58.85, 67.86, 68.78, 70.01, 70.05, 70.09, 106.72, 111.85, 115.31, 120.91, 123.17, 123.74, 124.77, 127.15, 127.85, 128.05, 129.37, 131.13, 131.47, 131.54, 131.73, 135.45, 142.24, 142.34, 143.63, 144.41, 148.03, 151.55, 154.92, 156.11, 158.39, 158.73, 160.92, 167.65, 168.28, 171.68, 202.59; LC-MS (ESI) (90% H₂O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), tᵣ = 13.24 min, 99% purity, m/z [M + H]⁺ calcd for C₆₁H₉₁N₁₁O₉S, 1250.68; found, 1251.0; HRMS (ESI) m/z [M + H]⁺ calcd for C₆₁H₉₁N₁₁O₉S, 1250.6795; found, 1250.6786.
This compound was synthesized by analogy with compound 66, but using Boc-D-Hyp-OH. The crude product was purified by column chromatography (gradient of CH₂Cl₂/MeOH 29:1 to 19:1) yielding 80 as a colorless solid.

Yield (71%); mp 106 – 108 °C; R_f = 0.30 (CH₂Cl₂/MeOH 9:1); ¹H NMR (600 MHz, DMSO-d₆) δ 0.70 (d, J = 6.6 Hz, 3H), 0.97 (d, J = 6.4 Hz, 3H, CH(CH₃)₂), 1.78 (ddd, J = 4.7, 7.8, 12.7 Hz, 1H), 2.04 (ddd, J = 3.0, 8.3, 11.2 Hz, 1H, 3-H), 2.29 – 2.40 (m, 1H, CH₂(CH₃)₂), 2.44 (s, 3H, CH₃), 3.53 – 3.65 (m, 2H, 5-H), 4.13 (dd, J = 5.7, 15.9 Hz, 1H), 4.19 – 4.27 (m, 2H), 4.28 – 4.35 (m, 1H), 4.43 – 4.53 (m, 2H), 4.67 (d, J = 10.5 Hz, 1H), 1H, 2-H, 4-H, NCH, NHCH₂, N(CH₃)), 5.09 (d, J = 3.4 Hz, 1H, OH), 6.84 (dd, J = 1.8 Hz, 7.8 Hz, 1H, 5'-H), 6.90 (d, J = 1.8 Hz, 1H, 3'-H), 7.19 (d, J = 7.8 Hz, 1H, 6'-H), 7.42 – 7.52 (m, 1H), 7.52 – 7.62 (m, 2H), 7.70 (d, J = 7.7 Hz, 1H, Ar''-H), 8.42 (t, J = 4.7, 5.7 Hz, 1H, CONH), 8.95 (s, 1H, 2''-H), 9.77 (s, 1H, Ar-OH); ¹³C NMR (151 MHz, DMSO-d₆) δ 16.25 (CH₃), 18.37, 19.36 (CH(CH₃)₂), 27.56 (CH(CH₃)₂), 37.24 (C-3), 37.91 (NHCH₃), 46.47 (C-2’’), 55.41 (C-5), 57.92 (C-2), 58.59 (NCH), 68.87 (C-4'), 115.21 (C-3’'), 119.55 (C-5’'), 123.26, 123.66 (C-4’, C-7’’), 125.30 (C-1’), 127.99 (C-6’’), 128.54 (C-6’), 130.88, 131.35, 131.42 (C-4’, C-5’, C-7’a’’), 131.71 (C-5’’), 142.30 (C-3a’’), 147.67 (C-4’’), 151.40 (C-2’’), 154.96 (C-2’), 167.44, 167.56, 172.17 (CO); MS (ESI) m/z [M + H]^+ calcd for C₂₉H₃₂N₄O₅S, 549.21; found, 549.3; HRMS (ESI) m/z [M + H]^+ calcd for C₂₉H₃₂N₄O₅S, 549.2166; found, 549.2155.

This compound was prepared using the General Procedure V, (–)VHL ligand c (80, 165 mg) and mesylate 69 (89 mg). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in CH₂Cl₂) to yield the title compound as a colorless solid.

Yield (111 mg, 53%); mp 72 – 76 °C; R_f = 0.33 (CH₂Cl₂/MeOH 9:1); ¹H NMR (600 MHz, DMSO-d₆) δ 0.71 (d, J = 6.7 Hz, 3H), 0.98 (d, J = 6.5 Hz, 3H, CH(CH₃)₂), 1.67 – 1.86 (m, 1H), 2.01 – 2.12 (m, 1H, 3-H), 2.26
- 2.43 (m, 1H, CH(CH$_3$)$_3$), 2.45 (s, 3H, CH$_3$), 3.25 – 3.71 (m, 10H), 3.78 (t, J = 4.7 Hz, 2H, CH$_2$Cl, OCH$_2$), 4.12 – 4.29 (m, 5H), 4.32 (d, J = 17.8 Hz, 1H), 4.44 – 4.54 (m, 2H), 4.68 (d, J = 10.5 Hz, 1H, OCH$_2$), 2-H, 4-H, NCH, NHCH$_3$, NCH$_2$), 5.09 (d, J = 3.5 Hz, 1H, OH), 6.94 – 7.00 (m, 1H, 5’-H), 7.02 (d, J = 1.6 Hz, 1H, 3’-H), 7.27 (d, J = 7.8 Hz, 1H, 6’-H), 7.44 – 7.58 (m, 3H), 7.70 (d, J = 7.5 Hz, 1H, Ar”’-H), 8.37 (t, J = 5.9 Hz, 1H, CONH), 8.97 (s, 1H, 2’’-H); $^{13}$C NMR (151 MHz, DMSO-d$_6$) δ 16.17 (CH$_3$), 18.39, 20.04 (CH(C$_3$H$_7$)$_2$), 27.59 (CH(C$_3$H$_7$)$_2$), 37.20 (C-3), 37.90 (NHCH$_3$), 43.70 (CH$_2$Cl), 46.47 (C-2’’), 55.44 (C-5), 57.93 (C-2), 58.66 (NCH), 68.06, 68.89, 69.15, 69.87, 70.19, 70.71 (C-4, OCH$_2$), 112.37 (C-3’), 121.26 (C-5’), 123.27, 123.62 (C-4’’, C-7’’), 127.26, 127.76 (C-1’, C-6’), 128.00 (C-6’’), 131.17, 131.35, 131.38 (C-4’, C-5’, C-7a’’), 131.70 (C-5’’), 142.27 (C-3a’’), 148.10 (C-4’’), 151.61 (C-2’’), 155.99 (C-2’), 167.46, 167.63, 172.06 (CO); MS (ESI) m/z [M + H]$^+$ calcd for C$_{35}$H$_{43}$ClN$_4$O$_7$S, 699.26; found, 699.8; HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{35}$H$_{43}$ClN$_4$O$_7$S, 699.2614; found, 699.2610.

VHL(c)-based negative control 32

This compound was prepared using the General Procedure IV and precursor 81 (70 mg). The crude product was purified by column chromatography (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 19:1) followed by HPLC purification using a gradient of 80% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation.

Yield (69 mg, 62%); mp 106 – 110 °C; R$_f$ = 0.13 (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 19:1); $^1$H NMR (600 MHz, DMSO-d$_6$) δ 0.70 (d, J = 6.7 Hz, 3H), 0.97 (d, J = 6.4 Hz, 3H), 1.50 – 1.65 (m, 2H), 1.68 – 1.83 (m, 3H), 1.83 – 1.89 (m, 2H), 2.03 – 2.09 (m, 1H), 2.19 – 2.26 (m, 2H), 2.30 (s, 3H), 2.32 – 2.39 (m, 1H), 2.41 (s, 3H), 2.45 (s, 3H), 2.49 – 2.59 (m, 6H), 3.04 – 3.12 (m, 4H), 3.38 – 3.67 (m, 8H), 3.70 – 3.83 (m, 2H), 4.13 – 4.35 (m, 6H), 4.41 – 4.54 (m, 2H), 4.67 (d, J = 10.5 Hz, 1H), 5.09 (d, J = 3.3 Hz, 1H), 5.80 (p, J = 8.9 Hz, 1H), 6.94 – 7.00 (m, 1H), 7.03 (d, J = 1.7 Hz, 1H), 7.28 (d, J = 7.8 Hz, 1H), 7.36 – 7.63 (m, 4H), 7.69 (d, J = 7.7 Hz, 1H), 7.81 (d, J = 9.1 Hz, 1H), 8.00 (d, J = 3.0 Hz, 1H), 8.38 (t, J = 5.9 Hz, 1H), 8.93 (s, 1H), 8.96 (s, 1H), 10.04 (s, 1H); $^{13}$C NMR (151 MHz, DMSO-d$_6$) δ 13.76, 16.18, 18.37, 20.04, 25.24, 27.58, 27.69, 31.45, 37.21, 37.90, 46.47, 48.45, 53.03, 55.44, 57.35, 57.92, 58.65, 68.13, 68.55, 68.90, 69.13, 69.89, 70.22, 106.70, 112.36, 115.29, 121.25, 123.26, 123.60, 124.72, 127.25, 127.78, 127.98,
This compound was synthesized as described previously.\textsuperscript{14}

\textbf{1}H NMR (600 MHz, DMSO-\textit{d}_6) δ 0.94 (s, 9H, C(CH\textsubscript{3})\textsubscript{3}), 1.45 – 1.52 (m, 2H), 1.57 – 1.65 (m, 2H, 2''''-H), 1.87 – 1.93 (m, 1H), 2.03 – 2.09 (m, 1H, 3-H), 2.43 (s, 3H, CH\textsubscript{3}), 3.55 (d, \textit{J} = 10.8, 1H), 3.60 – 3.65 (m, 1H, 5-H), 4.15 – 4.27 (m, 2H), 4.31 – 4.37 (m, 1H), 4.47 – 4.53 (m, 2H, 2-H, 4-H, NHCH, NHCH\textsubscript{2}), 5.13 (d, \textit{J} = 3.6 Hz, 1H, OH), 6.82 (d, \textit{J} = 7.8 Hz, 1H), 6.87 – 6.93 (m, 1H), 7.28 – 7.36 (m, 2H, Ar-H, CONH), 8.49 (t, \textit{J} = 6.0 Hz, 1H, CONH), 8.94 (s, 1H, 2''-H), 9.78 (s, 1H, Ar-OH); \textbf{13}C NMR (151 MHz, DMSO-\textit{d}_6) δ 13.90 (C-1''''), 16.22 (CH\textsubscript{3}), 16.77, 16.95 (C-2'''''), 26.25 (C(CH\textsubscript{3})\textsubscript{3}), 36.39 (C(CH\textsubscript{3})\textsubscript{3}), 37.48 (C-3), 38.02 (NHCH\textsubscript{2}), 56.78, 57.52, 58.93 (C-2, C-5, NHCH), 69.05 (C-4), 115.19 (C-3'), 119.49 (C-5'), 120.28 (CN), 125.39 (C-1'), 128.71 (C-6'), 130.83, 131.47 (C-4', C-5''), 147.63 (C-4'''), 151.40 (C-2'''), 154.97 (C-2'), 164.59, 168.87, 172.07 (CO); \textbf{MS} (ESI) \textit{m/z} [M + H]\textsuperscript{+} calcd for C\textsubscript{27}H\textsubscript{33}N\textsubscript{5}O\textsubscript{5}S, 540.22; found, 540.3.

\textbf{VHL(d)-2-2-2 building block (83)}

This compound was prepared using the General Procedure V, VHL ligand d (82, 162 mg) and mesylate 69 (89 mg). The crude product was purified by flash chromatography on silica gel (0% to 5% MeOH in CH\textsubscript{2}Cl\textsubscript{2}) to yield the title compound as a colorless solid.
Yield (108 mg, 52%); mp 67 – 70 °C; \( R_f = 0.30 \) (CH\(_3\)Cl/MeOH 19:1); \(^1\)H NMR (600 MHz, DMSO-\( d_6 \)) \( \delta \) 0.94 (s, 9H, (CH\(_3\))\(_3\)), 1.45 – 1.55 (m, 2H), 1.57 – 1.67 (m, 2H, 2""-H), 1.85 – 1.97 (m, 1H), 2.04 – 2.17 (m, 1H, 3-H), 2.45 (s, 3H, CH\(_3\)), 3.54 – 3.71 (m, 10H), 3.75 – 3.84 (m, 2H, 5-H, CH\(_3\)Cl, OCH\(_3\)), 4.15 – 4.39 (m, 5H), 4.48 – 4.53 (m, 2H, 2-H, 4-H, NHCH\(_3\), NHCH\(_2\), OCH\(_3\)), 5.14 (d, \( J = 3.7 \) Hz, 1H, OH), 6.95 (dd, \( J = 1.6, 7.7 \) Hz, 1H), 7.03 (d, \( J = 1.7 \) Hz, 1H), 7.37 (dd, \( J = 8.4, 21.6 \) Hz, 2H, Ar-H, CONH), 8.46 (t, \( J = 6.0 \) Hz, 1H, CONH), 8.97 (s, 1H, 2""-H); \(^{13}\)C NMR (151 MHz, DMSO-\( d_6 \)) \( \delta \) 13.88 (C-1""), 16.13 (CH\(_3\)), 16.75, 16.94 (C-2'''), 26.23 (C(CH\(_3\))\(_3\)), 36.37 (C(CH\(_3\))\(_3\)), 37.40 (C-3), 38.03 (NHCH\(_2\)), 43.68 (CH\(_3\)Cl), 56.79, 57.51, 58.98 (C-2, C-5, NHCH), 68.06, 69.06, 69.17, 69.88, 70.21, 70.72 (C-4, OCH\(_3\)), 112.30 (C-3'), 120.27 (CN), 121.13 (C-5'), 127.32, 127.87 (C-1', C-6'), 131.05, 131.42 (C-4', C-5''), 148.03 (C-4''), 151.57 (C-2""), 155.98 (C-2'), 164.58, 168.87, 171.93 (CO); MS (ESI) \( m/z \) [M + H]\(^+\) calcd for \( C_{33}H_{44}ClN_5O_7S \), 690.27; found, 699.6; HRMS (ESI) \( m/z \) [M + H]\(^+\) calcd for \( C_{33}H_{44}ClN_5O_7S \), 690.2723; found, 690.2707.

VHL(d)-based PROTAC 33

This compound was prepared using the General Procedure IV and precursor 83 (69 mg). The crude product was purified by column chromatography (CH\(_3\)Cl/MeOH+7N NH\(_3\) 19:1) to give the title product as a yellow solid.

Yield (65 mg, 59%); mp 118 – 122 °C; \( R_f = 0.23 \) (CH\(_3\)Cl/MeOH+7N NH\(_3\) 19:1); \(^1\)H NMR (600 MHz, DMSO-\( d_6 \)) \( \delta \) 0.93 (s, 9H), 1.42 – 1.65 (m, 6H), 1.66 – 1.82 (m, 2H), 1.82 – 1.97 (m, 3H), 2.03 – 2.12 (m, 1H), 2.23 (d, \( J = 3.3 \) Hz, 2H), 2.30 (s, 3H), 2.41 (s, 3H), 2.45 (s, 3H), 2.49 – 2.60 (m, 6H), 3.11 (t, \( J = 5.0 \) Hz, 4H), 3.48 – 3.68 (m, 8H), 3.70 – 3.86 (m, 2H), 4.15 – 4.39 (m, 5H), 4.46 – 4.57 (m, 2H), 5.14 (d, \( J = 3.7 \) Hz, 1H), 5.80 (p, \( J = 8.9 \) Hz, 1H), 6.95 (dd, \( J = 1.6, 7.7 \) Hz, 1H), 6.99 – 7.13 (m, 1H), 7.35 (d, \( J = 8.9 \) Hz, 1H), 7.37 – 7.51 (m, 2H), 7.82 (d, \( J = 9.0 \) Hz, 1H), 8.01 (d, \( J = 3.0 \) Hz, 1H), 8.46 (t, \( J = 6.1 \) Hz, 1H), 8.93 (s, 1H), 8.95 (s, 1H), 10.03 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\( d_6 \)) \( \delta \) 13.76, 13.88, 16.14, 16.75, 16.94, 25.24, 26.24, 27.69, 31.44, 36.38, 37.42, 38.04, 48.46, 53.05, 56.80, 57.37, 57.51, 58.98, 68.13, 68.57, 69.06, 69.15, 69.91, 70.24, 106.71, 112.29, 115.30, 120.27, 121.12, 124.73, 127.31, 127.91, 129.36, 131.07, 131.41, 135.44, 142.22, 143.62, 144.37, 148.02, 151.55, 154.92, 156.01, 158.38, 158.73, 160.91, 164.57, 168.87, 171.92, 202.57; LC-MS (ESI) (90% H\(_2\)O to 100% MeOH in 10 min, then
100% MeOH to 20 min, DAD 220-420 nm), $t_{R} = 11.77$ min, 99% purity, $m/z$ [M + H]$^+$ calcd for C$_{57}$H$_{72}$N$_{12}$O$_9$S, 1101.54; found, 1102.2; HRMS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{57}$H$_{72}$N$_{12}$O$_9$S, 1101.5339; found, 1101.5325.

**VHL ligand e (84)**

![VHL ligand e (84)](image)

This compound was synthesized as described previously.$^{14}$

$^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 0.95 (s, 9H, C(CH$_3$)$_3$), 1.18 – 1.23 (m, 2H), 1.27 – 1.39 (m, 2H, 2”‘-H), 1.87 – 1.95 (m, 1H), 2.02 – 2.14 (m, 1H, 3-H), 2.44 (s, 3H, CH$_3$), 3.56 – 3.68 (m, 2H, 5-H), 4.12 – 4.20 (m, 1H), 4.20 – 4.30 (m, 1H), 4.31 – 4.36 (m, 1H), 4.50 (t, $J = 8.2$ Hz, 1H), 4.55 – 4.62 (m, 1H, 2-H, 4-H, NHCH$_2$, NHCH$_3$), 5.14 (d, $J = 3.7$ Hz, 1H, OH), 6.83 (dd, $J = 1.8$, 7.8 Hz, 1H), 6.90 (d, $J = 1.8$ Hz, 1H), 7.25 (dd, $J = 2.8$, 9.3 Hz, 1H), 7.31 (d, $J = 7.8$ Hz, 1H, Ar-H, CONH), 8.48 (t, $J = 6.0$ Hz, 1H, CONH), 8.94 (s, 1H, 2”‘-H), 9.77 (s, 1H, Ar-OH); $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 12.95 (d, $^2$(C,F) = 40.1 Hz), 13.02 (d, $^2$(C,F) = 39.8 Hz, C-2”‘), 16.21 (CH$_3$), 26.32 (C(CH$_3$)$_3$), 36.20 (C(CH$_3$)$_3$), 37.46 (C-3), 38.02 (NHCH$_2$), 56.70, 56.79, 58.90 (C-2, C-5, NHCH), 69.05 (C-4), 78.27 (d, $^3$(C,F) = 232.2 Hz, C-1”‘), 115.18 (C-3’), 119.51 (C-5’), 125.40 (C-1’), 128.69 (C-6’), 130.81, 131.47 (C-4’, C-5’), 147.63 (C-4’‘), 151.37 (C-2’), 154.95 (C-2’), 168.21 (d, $^2$(C,F) = 19.9 Hz), 169.04, 172.09 (CO); MS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{108}$H$_{133}$FN$_4$O$_5$S, 533.22; found, 533.4.

**VHL(e)-2-2-2 building block (85)**

![VHL(e)-2-2-2 building block (85)](image)

This compound was prepared using the General Procedure V, VHL ligand e (84, 160 mg) and mesylate 69 (89 mg). The crude product was purified by flash chromatography on silica gel (0% to 4% MeOH in CH$_2$Cl$_2$) to yield the title compound as a colorless solid.
Yield (107 mg, 52%); mp 58 – 60 °C; \(R_f = 0.21 \) (CH\(_2\)Cl\(_2\)/MeOH 19:1); \(^1\text{H} \text{NMR} \) (600 MHz, DMSO-\(d_6\)) \(\delta\) 0.95 (s, 9H, C(CH\(_3\))\(_3\)), 1.17 – 1.25 (m, 2H), 1.30 – 1.42 (m, 2H, 2"",-H), 1.86 – 1.97 (m, 1H), 2.04 – 2.12 (m, 1H, 3-H), 2.45 (s, 3H, CH\(_3\)), 3.55 – 3.71 (m, 10H), 3.76 – 3.83 (m, 2H, 5-H, CH\(_2\)Cl, OCH\(_3\)), 4.15 – 4.24 (m, 3H), 4.26 – 4.38 (m, 2H), 4.51 (t, \(J = 8.2 \) Hz, 1H), 4.59 (dd, \(J = 1.1, 9.1 \) Hz, 1H, 2-H, 4-H, NHCH, NHCH\(_3\), OCH\(_3\)), 5.14 (d, \(J = 3.6 \) Hz, 1H, OH), 6.96 (dd, \(J = 1.6, 7.7 \) Hz, 1H), 7.03 (d, \(J = 1.6 \) Hz, 1H), 7.27 (dd, \(J = 2.8, 9.3 \) Hz, 1H), 7.39 (d, \(J = 7.8 \) Hz, 1H, Ar-H, CONH), 8.45 (t, \(J = 6.0 \) Hz, 1H, CONH), 8.97 (s, 1H, 2"",-H); \(^{13}\text{C} \text{NMR} \) (151 MHz, DMSO-\(d_6\)) \(\delta\) 12.95 (d, \(^2J(C,F) = 39.6\) Hz), 13.02 (d, \(^2J(C,F) = 39.7\) Hz, C-2")", 16.13 (CH\(_3\)), 26.31 (C(CH\(_3\))\(_3\)), 36.19 (C(C(CH\(_3\))\(_3\)), 37.39 (C-3), 38.04 (NHCH\(_2\)), 43.68 (CH\(_2\)Cl), 56.70, 56.81, 58.97 (C-2, C-5, NHCH), 68.05, 69.06, 69.16, 69.87, 70.21, 70.72 (C-4, OCH\(_3\)), 78.27 (d, \(^1J(C,F) = 232.5\) Hz, C-1""), 112.28 (C-3'), 121.17 (C-5'), 127.33, 127.86 (C-1', C-6'), 131.03, 131.42 (C-4', C-5''), 148.03 (C-4"), 151.56 (C-2"'), 155.96 (C-2'), 168.21 (d, \(^2J(C,F) = 20.7\) Hz), 169.06, 171.97 (CO); \textbf{MS} (ESI) \(m/z\) [M + H]\(^+\) calcd for C\(_{32}\)H\(_{44}\)ClF\(_4\)N\(_2\)O\(_5\)S, 683.26; found, 683.6; \textbf{HRMS} (ESI) \(m/z\) [M + H]\(^+\) calcd for C\(_{32}\)H\(_{44}\)ClF\(_4\)N\(_2\)O\(_5\)S, 683.2676; found, 683.2662.

VHL(e)-based PROTAC 34 (CST651)

This compound was prepared using the General Procedure IV and precursor 85 (68 mg). The crude product was purified by column chromatography (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\) 29:1) followed by HPLC purification (gradient of 80% to 100% v/v MeOH) to give the title compound as a yellow solid.

Yield (38 mg, 34%); mp 120 – 124 °C; \(R_f = 0.25 \) (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\) 19:1); \(^1\text{H} \text{NMR} \) (600 MHz, DMSO-\(d_6\)) \(\delta\) 0.95 (s, 9H), 1.18 – 1.25 (m, 2H), 1.29 – 1.40 (m, 2H), 1.51 – 1.62 (m, 2H), 1.71 – 1.79 (m, 2H), 1.83 – 1.95 (m, 3H), 2.04 – 2.11 (m, 1H), 2.18 – 2.27 (m, 2H), 2.30 (s, 3H), 2.41 (s, 3H), 2.45 (s, 3H), 2.50 – 2.59 (m, 6H), 3.07 – 3.13 (m, 4H), 3.49 – 3.70 (m, 8H), 3.74 – 3.85 (m, 2H), 4.15 – 4.37 (m, 5H), 4.51 (t, \(J = 8.2 \) Hz, 1H), 4.58 (d, \(J = 9.0 \) Hz, 1H), 5.14 (d, \(J = 3.7 \) Hz, 1H), 5.80 (p, \(J = 8.9 \) Hz, 1H), 6.96 (dd, \(J = 1.7, 7.8 \) Hz, 1H), 7.01 – 7.07 (m, 1H), 7.26 (dd, \(J = 2.8, 9.2 \) Hz, 1H), 7.40 (dd, \(J = 3.0, 8.7 \) Hz, 2H), 7.82 (d, \(J = 8.9 \) Hz, 1H), 8.01 (d, \(J = 3.0 \) Hz, 1H), 8.46 (s, 1H), 8.93 (s, 1H), 8.95 (s, 1H), 10.03 (s, 1H); \(^{13}\text{C} \text{NMR} \) (151 MHz, DMSO-\(d_6\)) \(\delta\) 12.95 (d, \(^2J(C,F) = 39.8\) Hz), 13.02 (d, \(^2J(C,F) = 40.4\) Hz), 13.76, 16.14, 16.18, 25.24, 26.31, 27.69, 31.45, 36.19, 37.41, 38.04, 48.46, 53.03, 56.69, 56.82, 57.37, 58.96,
68.12, 68.57, 69.06, 69.15, 69.91, 70.24, 78.27 (d, $^1J(C,F) = 232.3$ Hz), 106.70, 112.27, 115.29, 121.16, 124.72, 127.33, 127.89, 129.36, 131.04, 131.36, 131.42, 135.44, 142.22, 143.62, 144.37, 148.03, 151.55, 154.92, 155.99, 158.39, 158.72, 160.91, 168.20 (d, $^2J(C,F) = 19.9$ Hz), 169.05, 171.96, 202.57;

**LC-MS** (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-420 nm), $t_R = 11.95$ min, 99% purity, $m/z$ [M + H]$^+$ calcd for C$_{56}$H$_{72}$FN$_{11}$O$_9$S, 1094.53; found, 1095.2; **HRMS** (ESI) $m/z$ [M + H]$^+$ calcd for C$_{56}$H$_{72}$FN$_{11}$O$_9$S, 1094.5292; found, 1094.5271.
K. Synthesis of IAP-based degraders

Monomeric IAP ligand (5)

This compound was synthesized as described previously.\textsuperscript{15}

\textit{\textsuperscript{1}H NMR} (500 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) 0.88 – 1.18 (m, 9H, CH\textsubscript{2}, CH\textsubscript{3}), 1.52 – 1.85 (m, 10H, CH\textsubscript{2}), 2.16 (s, 3H, NHCH\textsubscript{3}), 2.04 – 2.13 (m, 1H), 2.50 – 2.59 (m, 1H), 2.62 – 2.77 (m, 2H), 2.87 – 3.00 (m, 1H), 3.64 (dd, \(J = 4.5, 10.8\) Hz, 1H, \(2'-\)H, NHCH\textsubscript{3}, \(3'-\)H), 4.45 (dd, \(J = 5.2, 9.0\) Hz, 1H, CH\textsubscript{2}), 4.87 – 4.95 (m, 1H), 5.04 (p, \(J = 5.4\) Hz, 1H, \(1'\)-H, \(4'\)-H), 6.88 – 6.92 (m, 2H, Ar-H), 6.95 (t, \(J = 7.5\) Hz, 1H, Ar-H), 7.02 – 7.19 (m, 3H, Ar-H), 7.21 – 7.33 (m, 3H, Ar-H), 7.84 (d, \(J = 8.6\) Hz, 1H), 7.89 (d, \(J = 8.7\) Hz, 1H, CONH); \textit{\textsuperscript{13}C NMR} (126 MHz, DMSO-\textit{d}\textsubscript{6}) \(\delta\) 19.19 (CH\textsubscript{3}), 19.97 (C-3), 25.55, 25.75, 25.92 (CH\textsubscript{2}), 27.91, 28.86, 29.18, 29.77 (CH\textsubscript{2}, C-2, C-4), 34.39, 34.54 (C-3', NHCH\textsubscript{3}), 46.73 (C-1), 52.15 (NHCH\textsubscript{3}), 54.45 (C-5'), 58.63 (CH\textsubscript{2}), 59.28 (NCH), 74.96 (C-4'), 115.69 (C-3''), 121.20 (C-4''), 125.86, 126.84, 128.54, 128.76 (C-5, C-6, C-7, C-8), 129.69 (C-2''), 137.14, 137.35 (C-4a, C-8a), 157.06 (C-1''), 169.98, 170.57, 174.56 (CO). The signal for NHCH\textsubscript{3} is missing (overlapping solvent peaks); \textbf{LC-MS} (ESI) (90% H\textsubscript{2}O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 205-220 nm), \(t_R = 11.98\) min, 97% purity, \(m/z\) [M + H]\textsuperscript{+} calcd for C\textsubscript{33}H\textsubscript{44}N\textsubscript{4}O\textsubscript{4}, 561.34; found, 561.3.

IAP ligand (86)

This compound was synthesized as described previously.\textsuperscript{16}
\(^1\text{H NMR}\) (600 MHz, DMSO-\(d_6\)) \(\delta\) 0.71 – 1.24 (m, 9H, CH\(_2\), CH\(_3\)), 1.38 (s, 9H, C(CH\(_3\))\(_3\)), 1.52 – 1.84 (m, 10H), 2.01 – 2.14 (m, 1H), 2.43 – 2.53 (m, 1H), 2.64 – 2.76 (m, 5H), 3.60 (dd, \(J = 4.4, 10.8\) Hz, 1H, 2-\(H\), 3-\(H\), 4-\(H\), CH, CH\(_2\), NCH\(_3\)), 4.18 – 4.27 (m, 1H), 4.32 (\(t, J = 7.8\) Hz, 1H, 2′-\(H\), NHCH\(_2\)), 4.44 (dd, \(J = 5.1, 9.0\) Hz, 1H, CHCH\(_3\)), 4.85 – 5.00 (m, 2H, 1-\(H\), 4′-\(H\)), 6.23 – 6.40 (m, 3H, 2′′-\(H\), 4′′-\(H\), 6′′-\(H\)), 7.00 – 7.16 (m, 4H, 5-\(H\), 6-\(H\), 7-\(H\), 5′′-\(H\)), 7.24 (d, \(J = 7.5\) Hz, 1H, 8-\(H\)), 7.84 (d, \(J = 8.5\) Hz, 1H), 8.31 (d, \(J = 8.4\) Hz, 1H, CONH), 9.40 (s, 1H, OH); \(^{13}\text{C NMR}\) (151 MHz, DMSO-\(d_6\)) \(\delta\) 15.11 (br s, suppressed CH\(_3\)), 20.04 (C-3), 25.61, 25.78, 25.92 (CH\(_2\)), 28.17 (C(CH\(_3\))\(_3\)), 28.90, 29.07, 29.65, 29.81 (C-2, C-4, CH\(_2\)), 30.16 (NCH\(_3\)), 34.64 (C-3′), 39.52 (CH), 46.78 (C-1), 52.25 (NHCH), 53.27 (br s, suppressed CHCH\(_3\)), 55.23 (C-5′), 58.65 (NCH), 74.96 (OCH), 79.17 (C(CH\(_3\))\(_3\)), 103.14 (C-2′′), 106.14 (C-4′′), 108.55 (C-6′′), 125.89, 126.86, 128.51 (C-6, C-7, C-8), 128.79 (C-5), 130.06 (C-5′′), 137.17, 137.40 (C-4a, C-8a), 155.27 (br s, suppressed CO), 158.29, 158.76 (C-1′′, C-3′′), 169.40, 169.97, 170.38 (CO); \(\text{MS (ESI) } m/z\) [M + H]\(^+\) calcd for C\(_{38}\)H\(_{52}\)O\(_7\)Na\(_2\), 677.39; found, 677.6; \(\text{HRMS (ESI) } m/z\) [M + H]\(^+\) calcd for C\(_{38}\)H\(_{52}\)O\(_7\)Na\(_2\), 677.3909; found, 677.3899.

\(^1\text{H NMR}\) (400 MHz, CDCl\(_3\)) \(\delta\) 0.75 – 1.00 (m, 5H, CH\(_2\)), 1.30 (d, \(J = 7.3\) Hz, 3H, CHCH\(_3\)), 1.37 – 1.43 (m, 1H, CH\(_3\)), 1.47 (s, 9H, C(CH\(_3\))\(_3\)), 1.48 – 1.59 (m, 4H, CH\(_2\)), 1.70 – 1.85 (m, 4H, 2-\(H\), 3-\(H\)), 1.94 – 2.05 (m, 1H, CH), 2.28 (symm m, 1H, 4′-\(H\)), 2.70 – 2.76 (m, 2H, 4-\(H\), 3′-\(H\)), 2.77 (s, 3H, NCH\(_3\)), 2.84 (d, \(J = 13.8\) Hz, 1H, 4-\(H\)), 3.75 (d, \(J = 11.4\) Hz, 1H, 5′-\(H\)), 4.17 (dd, \(J = 4.6, 11.4\) Hz, 1H, 5′-\(H\)), 4.41 (t, \(J = 8.1\) Hz, 1H, 1-\(H\)), 4.52 – 4.70 (m, 1H, 4′-\(H\)), 4.75 (dd, \(J = 2.0, 9.2\) Hz, 1H, NHCH), 4.86 (t, \(J = 4.3\) Hz, 1H, NCH), 5.10 (q, \(J = 7.3\) Hz, 1H, CHCH\(_3\)), 6.27 (t, \(J = 1.8\) Hz, 1H, 2′′-\(H\)), 6.33 (dd, \(J = 1.8, 8.3\) Hz, 1H, 6′′-\(H\)), 6.47 (dd, \(J = 1.8, 8.1\) Hz, 1H, 4′′-\(H\)), 6.62 (d, \(J = 7.3\) Hz, 1H, CONH), 6.70 (br s, 1H, CONH), 7.03 – 7.15 (m, 4H, 5-\(H\), 6-\(H\), 7-\(H\), 8-\(H\)), 7.30 (d, \(J = 7.6\) Hz, 1H, 5′′-\(H\)). The resonance for OH is missing; \(^{13}\text{C NMR}\) (101 MHz, CDCl\(_3\)) \(\delta\) 14.45 (br s, suppressed CH\(_2\)), 19.83 (C-3), 25.44, 25.55, 25.79 (CH\(_2\)), 28.35 (C(CH\(_3\))\(_3\)), 28.45, 29.21, 29.74, 29.81 (C-2, C-4, CH\(_2\)), 30.16 (NCH\(_3\)), 33.40 (C-3′), 40.52 (CH), 47.69 (C-1), 53.40 (NHCH), 53.74 (br s, suppressed CHCH\(_3\)), 55.39 (C-5′), 60.01 (NCH), 75.95 (OCH), 80.85 (C(CH\(_3\))\(_3\)), 103.41 (C-2′′), 107.52 (C-4′′), 109.06 (C-6′′), 126.38 (C-6), 127.22 (C-7), 128.91 (C-8), 129.10 (C-5), 130.26 (C-5′′), 136.39 (C-4a), 137.45 (C-8a), 156.45 (br s, suppressed CO), 157.75 (C-3′′), 157.82 (C-1′′), 169.67, 171.89, 172.22 (CO).
To a solution of IAP ligand 86 (0.20 g, 0.30 mmol) in dry DMF (3 mL), K$_2$CO$_3$ (62 mg, 0.45 mmol) was added under argon atmosphere. Subsequently, a solution of 71 (105 mg, 0.36 mmol) in dry DMF (5 mL) was added, followed by stirring of the mixture at 60 °C for 24 h. After the reaction was complete, the volatiles were evaporated and the crude product was purified by column chromatography (CH$_2$Cl$_2$/MeOH 50:1) to give a colorless oil.

Yield (105 mg, 40%); $R_f$ = 0.12 (CH$_2$Cl$_2$/MeOH 20:1); $^1$H NMR (400 MHz, CDCl$_3$) δ 0.75 – 0.98 (m, 5H, CH$_2$), 1.30 (d, $J$ = 7.2 Hz, 3H, CH$_3$), 1.38 – 1.42 (m, 1H, CH$_2$), 1.47 (s, 9H, C(CH$_3$)$_3$), 1.50 – 1.63 (m, 4H, CH$_2$), 1.75 – 1.90 (m, 4H, 2-H, 3-H), 1.98 – 2.10 (m, 1H, CH), 2.32 (symm m, 1H, 4’-H), 2.68 – 2.82 (m, 5H, 4-H, 3’-H, NCH$_3$), 2.89 (d, $J$ = 13.7 Hz, 1H, 4-H), 3.62 (t, $J$ = 5.6 Hz, 2H, CH$_2$), 3.66 – 3.78 (m, 11H, CH$_2$, 5’-H), 3.84 (t, $J$ = 4.8 Hz, 2H, CH$_2$Cl), 4.06 (t, $J$ = 5.1 Hz, 2H, OCH$_2$), 4.19 (dd, $J$ = 4.9, 11.5 Hz, 1H, 1-H), 4.42 (t, $J$ = 7.9 Hz, 1H, 1-H), 4.50 – 4.68 (m, 1H, 4’-H), 4.76 (dd, $J$ = 1.7, 9.5 Hz, 1H, NHCH), 4.93 (t, $J$ = 4.6 Hz, 1H, NCH), 5.12 (q, $J$ = 7.2 Hz, 1H, CHCH$_3$), 6.38 – 6.44 (m, 2H, 2’’-H, 6’’-H), 6.55 (dd, $J$ = 1.7, 8.1 Hz, 1H, 4’’-H), 6.59 (d, $J$ = 8.1 Hz, 1H, CONH), 6.56 (br s, 1H, CONH), 7.02 – 7.20 (m, 4H, 5-H, 6-H, 7-H, 8-H), 7.29 (d, $J$ = 7.2 Hz, 1H, 5’’-H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 13.41 (br s, CH$_3$), 19.94 (C-3), 25.40, 25.51, 25.75 (CH$_3$), 28.29 (C(CH$_3$)$_3$), 28.38, 29.20, 29.70, 29.89 (C-2, C-4, CH$_2$), 30.00 (NCH$_3$), 33.35 (C-3’’), 40.52 (CH), 42.68 (CH$_2$Cl), 47.57 (C-1), 53.54 (NHCH), 53.68 (br s, suppressed CHCH$_3$), 55.20 (C-5’’), 60.05 (NCH), 67.39, 69.63, 70.57, 70.60, 70.64, 70.76, 71.29 (OCH$_3$), 76.13 (OCH), 80.67 (C(CH$_3$)$_3$), 102.97 (C-2’’), 107.63 (C-4’’), 108.35 (C-6’’), 126.16 (C-6), 127.09 (C-7), 128.60 (C-8), 129.04 (C-5), 130.03 (C-5’’), 130.62 (C-4a), 137.33 (C-8a), 156.24 (br s, CO), 157.78 (C-3’’), 160.03 (C-1’’), 169.32 (CO), 171.54 (CO), 172.22 (CO); HRMS (ESI) m/z [M + H]$^+$ calcd for C$_{46}$H$_{67}$O$_{10}$N$_4$Cl, 871.4619; found, 871.4586.
Boc-protected intermediate 88

This compound was prepared using the General Procedure IV and precursor 87 (87 mg). The crude product was purified by column chromatography (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 29:1) followed by HPLC purification using a gradient of 80% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation and was directly used in the next step.

Yield (82 mg, 62%); mp 120 – 122 °C; $R_f = 0.15$ (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 29:1); **LC-MS** (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $t_R = 13.21$ min, 99% purity, $m/z$ [M + H]$^+$ calcd for C$_{70}$H$_{95}$N$_{11}$O$_{12}$, 1220.50; found, 1282.9; **HRMS** (ESI) $m/z$ [M + H]$^+$ calcd for C$_{70}$H$_{95}$N$_{11}$O$_{12}$, 1282.7234; found, 1282.7221.

IAP-based degrader 35

PROTAC precursor 88 (47 µmol, 61 mg) was dissolved in 1M HCl in EtOAc (3 mL) and stirred at rt for 4 h. The yellow precipitate was filtered off, washed with dry Et$_2$O (3 × 5 mL) and dried in vacuo. The title compound (HCl salt) was then neutralized by eluting it with CH$_2$Cl$_2$/MeOH+7N NH$_3$ 19:1 on a small silica gel column. After evaporation of the solvent, a yellow solid was obtained.

Yield (44 mg, 79%); mp 94 – 96 °C; $R_f = 0.30$ (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 19:1); $^1$H NMR (600 MHz, DMSO-$d_6$) δ 0.86 – 1.13 (m, 8H), 1.50 – 1.83 (m, 15H), 1.83 – 1.92 (m, 2H), 2.02 – 2.12 (m, 1H), 2.17 (s, 3H), 2.19 – 2.27 (m, 3H), 2.29 (s, 3H), 2.41 (s, 3H), 2.48 – 2.55 (m, 2H), 2.56 (t, $J = 5.0$ Hz, 4H), 2.62 – 2.85 (m, 2H), 2.97 (q, $J = 6.9$ Hz, 1H), 3.12 (t, $J = 5.0$ Hz, 4H), 3.48 – 3.65 (m, 11H), 3.69 – 3.76 (m, 2H), 3.95
- 4.15 (m, 2H), 4.28 (dd, J = 6.1, 10.9 Hz, 1H), 4.38 (t, J = 7.9 Hz, 1H), 4.43 (dd, J = 5.4, 9.0 Hz, 1H), 4.91 (q, J = 7.5, 8.0 Hz, 1H), 5.03 (p, J = 5.3 Hz, 1H), 5.80 (p, J = 8.9 Hz, 1H), 6.45 – 6.59 (m, 3H), 6.93 – 7.19 (m, 4H), 7.23 (d, J = 7.5 Hz, 1H), 7.43 (dd, J = 3.1, 9.1 Hz, 1H), 7.80 – 7.88 (m, 2H), 7.93 (d, J = 8.6 Hz, 1H), 8.02 (d, J = 3.0 Hz, 1H), 8.93 (s, 1H), 10.04 (s, 1H); 13C NMR (151 MHz, DMSO-d6) δ 13.76, 19.09, 20.04, 25.24, 25.58, 25.76, 25.94, 27.69, 27.94, 28.89, 29.17, 29.82, 31.44, 34.28, 34.56, 40.23, 46.75, 48.47, 52.13, 53.04, 54.51, 57.36, 58.61, 59.16, 67.32, 68.53, 69.07, 69.84, 69.96, 69.99, 70.11, 74.99, 102.42, 106.71, 107.45, 108.05, 115.32, 124.74, 125.86, 126.86, 128.52, 128.78, 129.36, 130.21, 135.44, 137.15, 137.38, 142.23, 143.63, 144.38, 154.92, 158.27, 158.40, 158.73, 159.88, 160.92, 170.02, 170.55, 174.37, 202.58; LC-MS (ESI) (90% H2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), tR = 13.00 min, 99% purity, m/z [M + H]+ calcd for C70H95N11O12, 1182.67; found, 1183.3; HRMS (ESI) m/z [M + H]+ calcd for C65H87N11O10, 1182.6710; found, 1182.6700.

(−)IAP ligand (89)

This compound was synthesized as described previously.16

1H NMR (600 MHz, DMSO-d6) δ 0.82 – 1.28 (m, 9H, CH2, CH3), 1.35 (s, 9H, C(CH3)3), 1.53 – 1.82 (m, 10H), 2.01 – 2.21 (m, 1H), 2.27 – 2.49 (m, 1H), 2.62 – 2.71 (m, 2H), 2.74 (s, 3H), 3.62 (br s, 1H, 2- H, 3-H, 4-H, CH, CH2, NCH3), 4.31 (p, J = 13.5, 15.6 Hz, 2H, 2’-H, NCH), 4.44 (dd, J = 4.8, 9.0 Hz, 1H, CHCH3), 4.88 – 4.96 (m, 2H, 4’-H), 5.05 – 5.15 (m, 3H, 2’’-H, 4’’-H, 6’’-H), 6.93 – 7.19 (m, 4H, 5-H, 6-H, 7-H, 5’-H), 7.25 (d, J = 7.5 Hz, 1H, 8-H), 7.86 (br s, 1H), 7.91 (br s, 1H, CONH), 9.39 (s, 1H, OH); 13C NMR (151 MHz, DMSO-d6) δ 15.73 (br s, suppressed CH3), 19.94 (C-3), 25.59, 25.73, 25.98 (CH2), 28.15 (C(CH3)3), 28.88, 29.04, 29.65, 29.75 (C-2, C-4, CH2), 30.19 (NCH3), 34.77 (C-3’), 46.79 (C-1), 52.27 (NHCH), 52.84 (br s, suppressed CHCH3), 54.93 (C-5’), 58.57 (NCH), 74.96 (OCH2), 79.23 (C(CH3)3), 103.14 (C-2’’), 106.09 (C-4’’), 108.55 (C-6’’), 125.88, 126.87, 128.59 (C-6, C-7, C-8), 128.79 (C-5), 130.06 (C-5’’), 137.17, 137.36 (C-4a, C-8a), 155.19 (br s, CO), 158.24, 158.75, (C-1’’, C-3’’), 169.41, 170.47, 170.99 (CO); MS (ESI) m/z [M + H]+ calcd for C38H39N2O2, 677.39; found, 677.7; HRMS (ESI) m/z [M + H]+ calcd for C38H39N2O2, 677.3909; found, 677.3896.
To a solution of (−)IAP ligand 89 (0.20 g, 0.30 mmol) in dry DMF (3 mL), K$_2$CO$_3$ (62 mg, 0.45 mmol) was added under argon atmosphere. Subsequently, a solution of 71 (105 mg, 0.36 mmol) in dry DMF (5 mL) was added, followed by stirring of the mixture at 60 °C for 24 h. After the reaction was complete, the volatiles were evaporated and the crude product was purified by column chromatography (CH$_2$Cl$_2$/MeOH 20:1) to give a colorless oil.

Yield (120 mg, 46%); $R_f$ = 0.12 (CH$_2$Cl$_2$/MeOH 20:1); $^1$H NMR (400 MHz, CDCl$_3$) δ 0.80 – 1.00 (m, 5H, CH$_2$), 1.32 (d, $J$ = 6.8 Hz, 3H, CH$_3$), 1.40 – 1.44 (m, 1H, CH$_2$), 1.47 (s, 9H, C(CH$_3$)$_3$), 1.50 – 1.62 (m, 4H, CH$_2$), 1.75 – 1.91 (m, 4H, 2-H, 3-H), 2.00 – 2.08 (m, 1H, CH), 2.33 (symm m, 1H, 4'-H), 2.64 – 2.82 (m, 5H, 4-H, 3'-H, NCH$_3$), 2.87 (d, $J$ = 13.9 Hz, 1H, 4-H), 3.62 (t, $J$ = 5.9 Hz, 2H, CH$_2$), 3.65 – 3.78 (m, 11H, CH$_2$, 5'-H), 3.84 (t, $J$ = 4.5 Hz, 2H, CH$_2$Cl), 4.06 (t, $J$ = 5.0 Hz, 2H OCH$_2$), 4.16 (dd, $J$ = 4.8, 11.4 Hz, 1H, 5'-H), 4.42 (t, $J$ = 6.5 Hz, 1H, 1-H), 4.55 – 4.80 (m, 2H, 4'-H, NHCH$_3$), 4.94 (t, $J$ = 4.5 Hz, 1H, NCH), 5.12 (q, $J$ = 6.8 Hz, 1H, CH$_3$), 6.36 – 6.44 (m, 2H, 2''-H, 6''-H), 6.51 – 6.57 (m, 2H, 4''-H, CONH), 6.60 (br s, 1H, CONH), 7.02 – 7.19 (m, 4H, 5-H, 6-H, 7-H, 8-H), 7.29 (d, $J$ = 7.6 Hz, 1H, 5''-H); $^{13}$C NMR (101 MHz, CDCl$_3$) δ 13.47 (br s, CH$_3$), 19.97 (C-3), 25.40, 25.58, 25.71 (CH$_2$), 28.27 (C(CH$_3$)$_3$), 28.27, 29.20, 29.74, 29.92 (C-2, C-4), 30.10 (NCH$_3$), 33.41 (C-3'), 40.52 (CH), 42.68 (CH$_2$Cl), 47.56 (C-1), 53.49 (NHCH$_3$), 53.75 (br s, suppressed CH$_3$), 55.25 (C-5'), 60.03 (NCH), 67.39, 69.62, 70.57, 70.60, 70.64, 70.76, 71.29 (OCH$_2$), 76.24 (OCH), 80.66 (C(CH$_3$)$_3$), 102.97 (C-2'''), 107.63 (C-4'''), 108.35 (C-6''), 126.16 (C-6), 127.09 (C-7), 128.60 (C-8), 129.04 (C-5), 130.03 (C-5''), 136.52 (C-4a), 137.33 (C-8a), 156.24 (br s, CO), 157.78 (C-3'''), 160.03 (C-1''), 169.32 (CO), 171.54 (CO), 172.22 (CO); HRMS (ESI) m/z calcd for C$_{46}$H$_{67}$O$_{20}$N$_4$Cl, 871.4619; found, 871.5594.
Boc-protected intermediate 91

This compound was prepared using the General Procedure IV and precursor 90 (87 mg). The crude product was purified by column chromatography (CH₂Cl₂/MeOH+7N NH₃ 29:1) followed by HPLC purification using a gradient of 80% to 100% v/v MeOH. The title compound was obtained as a yellow solid after lyophilisation and was directly used in the next step.

Yield (87 mg, 68%); mp 110 – 114 °C; Rₛ = 0.30 (CH₂Cl₂/MeOH+7N NH₃ 19:1); LC-MS (ESI) (90% H₂O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-420 nm), tᵣᵣ = 13.19 min, 99% purity, m/z [M + H]+ calcd for C₇₀H₉₅N₁₁O₁₂, 1220.50; found, 1282.9; HRMS (ESI) m/z [M + H]+ calcd for C₇₀H₉₅N₁₁O₁₂, 1282.7234; found, 1282.7221.

IAP-based negative control 36

PROTAC precursor 91 (51 µmol, 65 mg) was dissolved in 1M HCl in EtOAc (5 mL) and stirred at rt for 4 h. The yellow precipitate was filtered off, washed with dry Et₂O (3 × 5 mL) and dried in vacuo. The title compound (HCl salt) was then neutralized by eluting it with CH₂Cl₂/MeOH+7N NH₃ 19:1 on a small silica gel column. After evaporation of the solvent, a yellow solid was obtained.
Yield (52 mg, 86%); mp 100 – 104 °C; \( R_f = 0.28 \) (CH\(_2\)Cl\(_2\)/MeOH+7N NH\(_3\); 19:1); \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \( \delta \) 0.70 – 1.29 (m, 9H), 1.41 – 1.82 (m, 14H), 1.82 – 1.90 (m, 2H), 1.99 – 2.14 (m, 1H), 2.18 (s, 3H), 2.19 – 2.27 (m, 3H), 2.29 (s, 3H), 2.41 (s, 3H), 2.48 – 2.54 (m, 2H), 2.54 – 2.60 (m, 4H), 2.60 – 2.76 (m, 2H), 2.87 – 3.04 (m, 1H), 3.12 (t, \( J = 5.0 \) Hz, 4H), 3.48 – 3.65 (m, 11H), 3.67 – 3.78 (m, 2H), 4.04 (dd, \( J = 3.7, 6.0 \) Hz, 2H), 4.30 (dd, \( J = 6.0, 10.7 \) Hz, 1H), 4.33 – 4.40 (m, 1H), 4.43 (dd, \( J = 5.4, 8.9 \) Hz, 1H), 4.74 – 4.94 (m, 1H), 5.02 (p, \( J = 5.3 \) Hz, 1H), 5.80 (p, \( J = 8.9 \) Hz, 1H), 6.45 – 6.58 (m, 3H), 6.94 – 7.20 (m, 4H), 7.20 – 7.32 (m, 1H), 7.43 (dd, \( J = 3.1, 9.1 \) Hz, 1H), 7.83 (dd, \( J = 8.7, 10.6 \) Hz, 2H), 7.95 (d, \( J = 8.5 \) Hz, 1H), 8.02 (d, \( J = 3.0 \) Hz, 1H), 8.93 (s, 1H), 10.04 (s, 1H); \(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \( \delta \) 13.75, 19.16, 20.05, 25.23, 25.58, 25.76, 25.96, 27.69, 27.98, 28.88, 29.24, 29.82, 31.44, 34.51, 34.57, 40.23, 46.75, 48.47, 52.11, 53.04, 54.65, 57.36, 58.61, 59.01, 67.32, 68.53, 69.07, 69.84, 69.96, 69.98, 70.10, 75.00, 102.41, 106.70, 107.44, 108.04, 115.31, 124.73, 125.87, 126.85, 128.49, 128.78, 129.35, 130.20, 135.43, 137.14, 137.39, 142.22, 143.62, 144.38, 154.91, 158.28, 158.39, 158.73, 159.88, 160.91, 170.01, 170.49, 174.53, 202.56; HPLC (95% H\(_2\)O (with 0.1% TFA) to 95% MeCN in 10 min, then 95% MeCN for 4 min), \( t_R = 5.60 \) min, 98.48% purity, detection at 254 nm; MS (ESI) m/z [M + H]\(^+\) calcd for C\(_{65}\)H\(_{87}\)N\(_{11}\)O\(_{10}\), 1182.67; found, 1183.4; HRMS (ESI) m/z [M + H]\(^+\) calcd for C\(_{65}\)H\(_{87}\)N\(_{11}\)O\(_{10}\), 1182.6710; found, 1182.6694.
L. Synthesis of MDM2-based PROTACs

Idasanutlin (6)

This compound was used as commercially supplied (MedChemExpress).

\(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 0.96 (s, 9H (C(CH\(_3\))\(_3\)), 1.26 (d, \(J = 14.1\) Hz, 1H), 1.63 (dd, \(J = 9.8, 14.2\) Hz, 1H, CH\(_2\)), 3.90 – 3.98 (m, 4H, OCH\(_3\), 5'-H), 4.33 – 4.41 (m, 1H, NH), 4.56 – 4.62 (m, 2H, 2'-H, 3'-H), 7.31 – 7.41 (m, 3H), 7.49 – 7.59 (m, 4H), 7.72 (t, \(J = 7.1\) Hz, 1H, Ar-H), 8.36 (d, \(J = 8.3\) Hz, 1H, 2'-H), 10.46 (s, 1H, CONH), 12.84 (s, 1H, COOH).

\(^13\)C NMR (151 MHz, DMSO-\(d_6\)) \(\delta\) 29.67 (C(C(C(H\(_3\))\(_3\))), 30.26 (C(C(H\(_3\))\(_3\))), 44.10 (CH\(_2\)), 50.35 (C-3'), 55.97 (OCH\(_3\)), 63.43, 63.56 (C-4', C-5'), 64.82 (C-2'), 111.22 (C-2), 117.40, 117.48 (C-1, C-3''), 117.65, 117.83, 119.35 (d, \(^2\)J(C,F) = 9.6 Hz, C-3''), 119.65 (d, \(^2\)J(C,F) = 18.7 Hz, C-1''), 122.92, 125.47 (d, \(^3\)J(C,F) = 4.5 Hz), 125.80 (d, \(^3\)J(C,F) = 2.7 Hz, Ar-CH), 126.07 (Ar-CH), 126.15 (d, \(^3\)J(C,F) = 13.3 Hz, C-1''), 128.78, 130.22, 131.01, 131.13 (d, \(^3\)J(C,F) = 4.4 Hz, Ar-CH), 134.94 (d, \(^3\)J(C,F) = 11.1 Hz, C-4''), 147.72 (C-3), 155.76 (d, \(^3\)J(C,F) = 246.6 Hz, C-2''), 159.70 (d, \(^3\)J(C,F) = 251.0 Hz, C-2''), 167.03 (COOH), 171.41 (CONH).


This compound was synthesized as we described previously.\(^7\)
A mixture of palbociclib (0.18 mmol, 81 mg) and methanesulfonate 92 (0.36 mmol, 134 mg) in dry DMSO (5 mL) and DIPEA (0.54 mmol, 94 µL) was stirred at 80 °C for 24 h. After cooling, it was partitioned between saturated NaHCO$_3$ solution (50 mL) and EtOAc (100 mL), and the organic layer was washed with 5% LiCl solution and brine (each 50 mL), dried over Na$_2$SO$_4$, filtered and evaporated. The crude product was purified by column chromatography ($\text{CH}_2\text{Cl}_2$/MeOH+7N NH$_3$ 49:1) to obtain a yellow solid.

Yield (99 mg, 76%); mp 78 – 82 °C; $R_f$ = 0.54 ($\text{CH}_2\text{Cl}_2$/MeOH+7N NH$_3$ 19:1); $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 1.36 (s, 9H, (C(CH$_3$)$_3$), 1.50 – 1.63 (m, 2H), 1.63 – 1.80 (m, 2H), 1.80 – 1.91 (m, 2H), 2.19 – 2.27 (m, 2H, 2''-H, 3''-H), 2.30 (s, 3H, CH$_3$), 2.41 (s, 3H, 10-H), 2.52 (t, $J$ = 5.9 Hz, 2H), 2.53 – 2.62 (m, 4H), 3.05 (d, $J$ = 6.0 Hz, 2H), 3.14 (t, $J$ = 5.0 Hz, 4H), 3.37 (t, $J$ = 6.1 Hz, 2H), 3.43 – 3.60 (m, 10H, 2''-H, 3''-H, NCH$_2$, OCH$_2$, NHCH$_2$), 5.80 (p, $J$ = 8.9 Hz, 1H, 1'''-H), 6.72 (t, $J$ = 5.9 Hz, 1H, NHCH$_2$), 7.44 (dd, $J$ = 3.1, 9.1 Hz, 1H, 4'-H), 7.83 (d, $J$ = 9.0 Hz, 1H, 3'-H), 8.03 (d, $J$ = 3.0 Hz, 1H, 6'-H), 8.93 (s, 1H, 4-H), 10.05 (s, 1H, NH); $^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 13.74 (CH$_3$), 25.23 (C-3'''), 27.68 (C-2'''), 28.37 (C(CH$_3$)$_3$), 31.43 (C-10), 48.46 (2C, C-2''), 53.04 (3C, C-3'', C-1'''), 57.35 (NCH$_2$), 68.53, 69.32, 69.68, 69.83, 69.90, 69.94 (OCH$_3$), 77.71 (C(CH$_3$)$_3$), 106.70 (C-4a), 115.29 (C-3'), 124.75 (C-4'), 129.35 (C-6), 135.44 (C-6'), 142.22 (C-5'), 143.61, 144.38 (C-8a, C-2'), 154.91 (C-5), 155.72 (CO), 158.38 (C-4'), 158.71 (C-2), 160.90 (C-7), 202.55 (C-9). The signal for NHCH$_2$ is missing (overlapping solvent peaks); MS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{37}$H$_{54}$N$_8$O$_7$, 723.41; found, 723.7; HRMS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{37}$H$_{54}$N$_8$O$_7$, 723.4149; found, 723.4179.
Palbociclib-2-2-2 conjugate 93 (82 µmol, 66 mg) was dissolved in dry CH$_2$Cl$_2$ (4 mL) and TFA (4 mL) and stirred at rt for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. Subsequently, it was dissolved in dry DMF (4 mL) and DIPEA (60 µL, 0.34 mmol) and idasanutlin (68 µmol, 42 mg) were added. After stirring for 5 minutes, HATU (75 µmol, 28 mg) was added to the mixture and it was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated NaHCO$_3$ solution (100 mL) and the product was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with 5% LiCl solution and brine (each 50 mL), dried over Na$_2$SO$_4$, filtered and concentrated in vacuo. The crude product was purified by column chromatography (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 35:1) followed by HPLC purification using a gradient of 60% to 90% v/v MeCN + 0.05% TFA. The title compound (TFA salt) was obtained as a light yellow solid after lyophilisation. The pure product was then neutralized by eluting it with CH$_2$Cl$_2$/MeOH+7N NH$_3$ 19:1 on a small silica gel column. After evaporation of the solvent, a light yellow solid was obtained.

Yield (44 mg, 53%); mp 142 – 146 °C; $R_f$ = 0.16 (CH$_2$Cl$_2$/MeOH+7N NH$_3$ 29:1); $^1$H NMR (600 MHz, DMSO-$d_6$) δ 0.95 (s, 9H), 1.25 (d, $J$ = 13.8 Hz, 1H), 1.51 – 1.67 (m, 3H), 1.69 – 1.79 (m, 2H), 1.81 – 1.92 (m, 2H), 2.17 – 2.27 (m, 2H), 2.29 (s, 3H), 2.41 (s, 3H), 2.49 – 2.65 (m, 6H), 2.93 – 3.23 (m, 4H), 3.41 (q, $J$ = 5.9 Hz, 2H), 3.44 – 3.64 (m, 12H), 3.90 (s, 3H), 3.91 – 4.01 (m, 1H), 4.16 – 4.48 (m, 1H), 4.52 – 4.64 (m, 2H), 5.80 (p, $J$ = 8.9 Hz, 1H), 7.30 – 7.40 (m, 3H), 7.43 (dd, $J$ = 3.1, 9.2 Hz, 1H), 7.45 – 7.59 (m, 4H), 7.63 – 7.75 (m, 1H), 7.82 (d, $J$ = 9.0 Hz, 1H), 8.03 (d, $J$ = 3.0 Hz, 1H), 8.30 (d, $J$ = 8.4 Hz, 1H), 8.45 (t, $J$ = 5.7 Hz, 1H), 8.93 (s, 1H), 10.04 (s, 1H), 10.38 (s, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 13.75, 25.23, 27.69, 29.63, 30.21, 31.44, 44.04, 48.45, 50.32, 53.00, 53.05, 55.95, 57.33, 63.38, 63.58, 64.78, 68.51, 69.14, 69.83, 69.91, 69.93, 106.71, 109.76, 115.30, 117.17, 117.45, 117.61, 117.79, 119.32, 119.61, 120.18, 124.75, 125.41, 125.77, 126.17, 128.74, 129.40, 129.77, 130.15, 131.11, 134.90, 135.45, 142.21, 143.60, 144.40, 147.65, 154.91, 155.72, 158.38, 158.72, 159.65, 160.91, 165.68, 171.16, 202.56; LC-MS (ESI) (90% H$_2$O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-420 nm), $t_R$ = 13.33 min, 99% purity, $m/z$ [M + H]$^+$ calcd for C$_{63}$H$_{73}$Cl$_2$F$_2$N$_{11}$O$_8$, 1220.50; found, 1220.9; HRMS (ESI) $m/z$ [M + H]$^+$ calcd for C$_{63}$H$_{73}$Cl$_2$F$_2$N$_{11}$O$_8$, 1220.5062; found, 1220.5050.
This compound was synthesized as reported previously.\textsuperscript{17}

\textbf{\textsuperscript{1}H NMR} (600 MHz, DMSO-\textit{d}_6) \( \delta \) 1.35 (s, 9H, CH\textsubscript{3}), 1.92 – 2.15 (m, 1H, 4'-H), 2.50 – 2.71 (m, 2H, 4'-H, 5'-H), 2.76 – 2.95 (m, 1H, 5'-H), 3.04 (q, \( J = 6.0 \) Hz, 2H), 3.26 – 3.33 (m, 2H), 3.36 (t, \( J = 6.1 \) Hz, 2H), 3.45 (t, \( J = 5.7 \) Hz, 2H), 3.49 (h, \( J = 2.2 \) Hz, 4H, NHCH\textsubscript{2}, OCH\textsubscript{2}), 4.78 (s, 2H, OCH\textsubscript{2}), 5.10 (dd, \( J = 5.5, 12.9 \) Hz, 1H, 3'-H), 6.71 (t, \( J = 5.8 \) Hz, 1H, NHCH\textsubscript{2}), 7.39 (d, \( J = 8.5 \) Hz, 1H, 5'-H), 7.49 (d, \( J = 7.2 \) Hz, 1H, 7-H), 7.80 (dd, \( J = 7.3, 8.5 \) Hz, 1H, 6-H), 7.98 (t, \( J = 5.7 \) Hz, 1H, NHCH\textsubscript{2}), 11.09 (br s, 1H, NH); \textbf{\textsuperscript{13}C NMR} (151 MHz, DMSO-\textit{d}_6) \( \delta \) 22.14 (C-4'), 28.36 (C(CH\textsubscript{3})\textsubscript{3}), 31.08 (C-5'), 38.55 (NHCH\textsubscript{2}), 48.95 (C-3'), 67.68, 68.96, 69.31, 69.60, 69.71 (OCH\textsubscript{2}), 77.73 (C(CH\textsubscript{3})\textsubscript{3}), 116.18 , 116.93 (C-3a, C-5), 120.52 (C-7), 133.19 (C-6), 137.06 (C-7a), 155.14 (C-4), 155.72, 165.58 (CO), 166.87, 167.03 (C-1, C-3), 169.98 (C-2'), 172.88 (C-6'); one signal for NHCH\textsubscript{2} is missing (overlapping residual solvent peaks). \textbf{MS} (ESI) \textit{m/z} [M + H]\textsuperscript{+} calcd for C\textsubscript{26}H\textsubscript{34}N\textsubscript{4}O\textsubscript{10}, 563.23; found, 563.5.
Pomalidomide conjugate 94 (63 µmol, 36 mg) was dissolved in dry CH₂Cl₂ (4 mL) and TFA (4 mL) and stirred at rt for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. Subsequently, it was dissolved in dry DMF (4 mL) and DIPEA (55 µL, 0.32 mmol) and idasanutlin (63 µmol, 39 mg) were added. After stirring for 5 minutes, HATU (70 µmol, 27 mg) was added to the mixture and it was stirred at room temperature for 16 h. The reaction mixture was quenched with saturated NaHCO₃ solution (100 mL) and the product was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with 5% LiCl solution and brine (each 50 mL), dried over Na₂SO₄, filtered and concentrated in vacuo. The crude product was purified by column chromatography (CH₂Cl₂/MeOH+7N NH₃ 29:1) followed by HPLC purification using 80% v/v MeCN + 0.05% TFA. The title compound (TFA salt) was obtained as a colorless solid after lyophilisation. The pure product was then neutralized by eluting it with CH₂Cl₂/MeOH+7N NH₃ 19:1 on a small silica gel column. After evaporation of the solvent, a colorless solid was obtained.

Yield (53 mg, 72%); mp 116 – 120 °C; Rf = 0.32 (CH₂Cl₂/MeOH+7N NH₃ 19:1); ¹H NMR (600 MHz, DMSO-d₆) δ 0.98 (s, 9H), 1.28 (d, J = 13.9 Hz, 1H), 1.65 (dd, J = 9.8, 14.2 Hz, 1H), 2.00 – 2.09 (m, 1H), 2.51 – 2.73 (m, 2H), 2.77 – 3.02 (m, 1H), 3.27 – 3.36 (m, 2H), 3.41 (q, J = 6.0 Hz, 2H), 3.47 (t, J = 5.7 Hz, 2H), 3.51 – 3.67 (m, 6H), 3.92 (s, 3H), 3.93 – 4.01 (m, 1H), 4.07 – 4.56 (m, 1H), 4.56 – 4.72 (m, 2H), 4.78 (s, 2H), 5.12 (dd, J = 5.5, 12.9 Hz, 1H), 7.26 – 7.44 (m, 4H), 7.44 – 7.51 (m, 2H), 7.51 – 7.57 (m, 2H), 7.58 (dd, J = 2.1, 12.6 Hz, 1H), 7.65 – 7.78 (m, 1H), 7.80 (dd, J = 7.2, 8.5 Hz, 1H), 7.99 (t, J = 5.7 Hz, 1H), 8.31 (d, J = 8.3 Hz, 1H), 8.46 (t, J = 5.7 Hz, 1H), 10.40 (s, 1H), 11.10 (br s, 1H); ¹³C NMR (151 MHz, DMSO) δ 22.14, 29.65, 30.23, 31.09, 38.55, 40.23, 44.05, 48.97, 50.33, 55.95, 63.38, 63.55, 64.77, 67.68, 68.97, 69.16, 69.74, 109.75, 116.18, 116.93, 117.17, 117.46, 117.62, 117.80, 119.33, 119.62, 120.18, 120.50, 125.43, 125.77, 126.17, 128.75, 129.44, 129.74, 130.16, 131.11, 133.18, 134.89, 137.04, 147.64, 155.11, 155.73, 159.67, 165.58, 165.71, 166.86, 167.04, 169.99, 171.17, 172.89; LC-MS (ESI) (90% H₂O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), tᵣ = 12.67 min, 99% purity, m/z [M + H]⁺ calcd for C₅₂H₃₃Cl₂F₂N₇O₁₁, 1060.32; found, 1060.6; HRMS (ESI) m/z [M + Na]⁺ calcd for C₅₂H₃₃Cl₂F₂N₇O₁₁, 1082.3040; found, 1082.2934.
M. Selected Analytical Spectra

Liquid chromatography analysis of 34 (CST651).
High resolution mass spectrometry analysis of 34 (CST651).

Elemental composition search on mass 1094.5271

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$^1$H and $^{13}$C NMR spectrum of compound 34 (CST651).
Supplementary Information: Physical Chemistry

N. Molecular Descriptor Calculation

Predicted values for the topological polar surface area (TPSA), the number of rotatable bonds (NRotB), as well as hydrogen bond donors (HBD) and acceptors (HBA) were calculated using MarvinSketch 17.28.0 (ChemAxon). The degrader score (Deg_S) as an overall measure of PROTAC efficacy was calculated based on our western blotting data (see Table 1–5).

O. Physicochemical Measurements

The determination of the elog $D_{7.4}$ values was performed by a chromatographic method according to the literature. HPLC analysis was conducted on a Waters 2696 Separation Module coupled with a Waters 996 Photodiode Array Detector. The column was a Polaris C18-A column (2 mm I.D., 50 mm length, 3 μm particle size) from Agilent Technologies. A linear mobile phase gradient was applied with 20 μM TRIS buffer adjusted to pH 7.4 and MeCN. Therefore, the gradient was run from 5% MeCN to 95% within 5 min, maintained for 1.5 min to be followed by an equilibration time of 5.5 min between the runs with a constant flow-rate of 0.8 ml/min and a temperature of 25 °C. The output wavelength of the detector was set to 269.5 nm. In order to calibrate the log $D_{7.4}$ to the retention time, 6 different drugs with known log $D_{7.4}$ were dissolved in DMSO and 10 μl were injected (Table S7). By plotting the log $D_{7.4}$ versus the retention times a linear calibration line ($R^2 = 0.996$) could be obtained (Figure S11). Approximately 1 mg of the samples was dissolved in DMSO and 5 μl were injected. Analyses were conducted in duplicates. Subsequently, the mean retention times of the analytes were taken to calculate their log $D_{7.4}$ values with aid of the calibration line.
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**Table S7**: Determined retention times of the calibration compounds and log $D_{7.4}$ values.

**Fig. S18**: Calibration line – log $D_{7.4}$ versus retention time.