Supplementary Information

Expanding the PROTAC Toolbox: Targeted Degradation of the Deubiquitinase USP7 in Cancer

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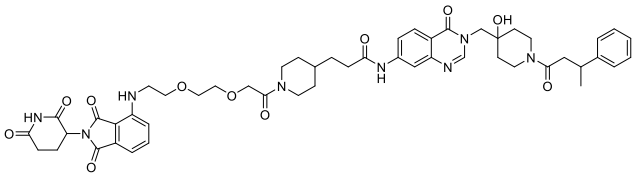
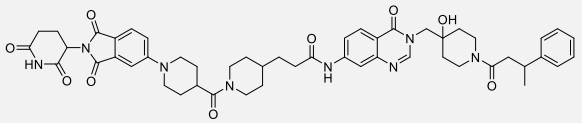
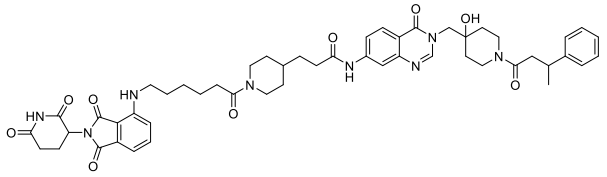
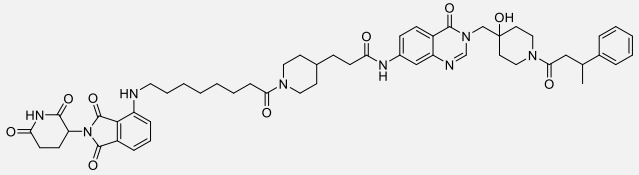
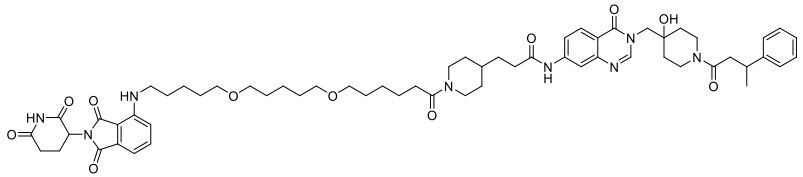
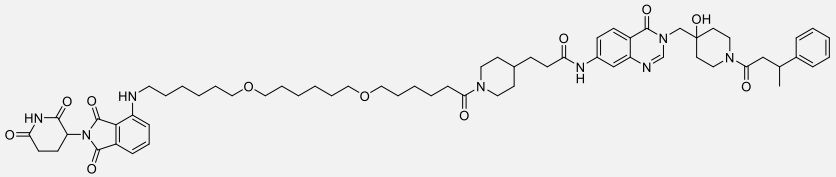
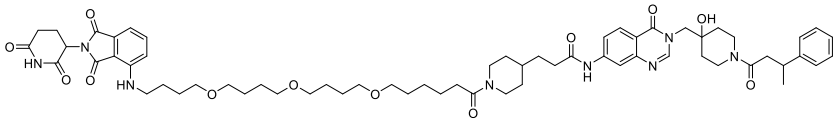
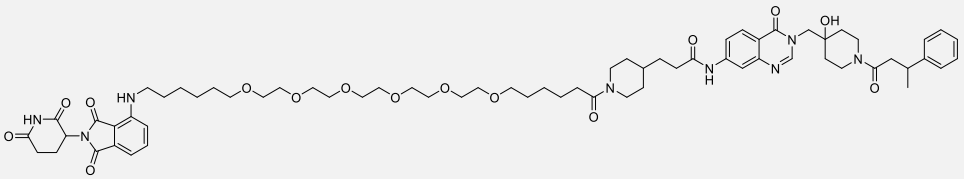
Supplementary Tables, Schemes and Figures

Cmpd	D_{USP7}[a] (%)	MW (g/mol)	logD[b]	TPSA[c] (A^2)	PPB[d] (%)	Structure
8	<5	961.09	2.3	237	n.d.[e]	
9	70	927.07	2.4	209	94	
10	<5	929.09	2.7	218	n.d.	

11	<5	957.14	3.2	218	n.d.	
12	<5	1101.36	4.1	237	n.d.	
13	<5	1129.41	4.6	237	96	
14	<5	1145.41	3.9	246	95	
15	<5	1249.41	3.3	274	94	

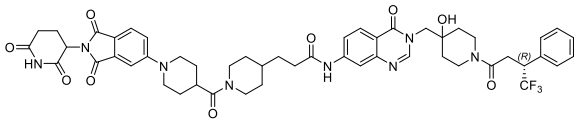
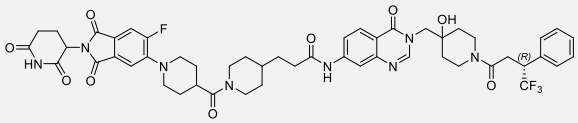
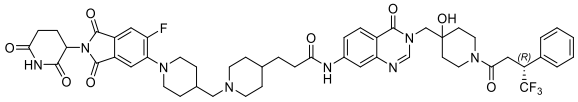
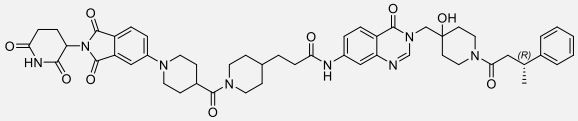
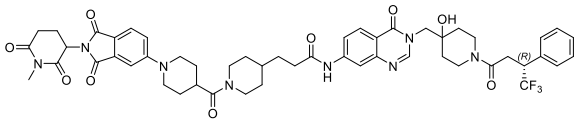
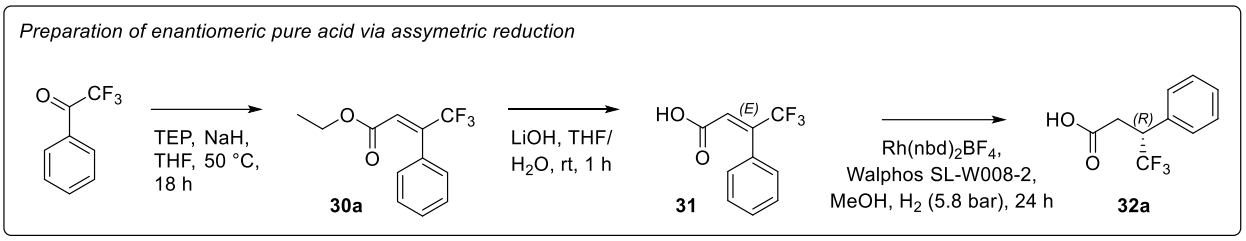
16	79	981.04	2.8	209	95	
17	<5	999.03	2.9	209	96	
18	<5	1021.51	2.6	192	95	
19	85	927.07	2.5	209	93	
20	n.d.	995.07	3.1	201	n.d.	

Table S1: Overview on synthesized USP7-targeting PROTACs. [a] USP7 depletion after 24 h treatment of MM.1S cells with 1 µM of each compound. Data are shown as mean (n=2). [b] Experimental partition coefficient at *p*H 7.4 (see below). [c] Topological polar surface area is given in Å². [d] Plasma protein binding (PPB) values were estimated by an HPLC-based method (see below). [e] not determined.



Scheme S1 Synthesis of the enantiopure building block **32a** by assymetric reduction.[1,2]

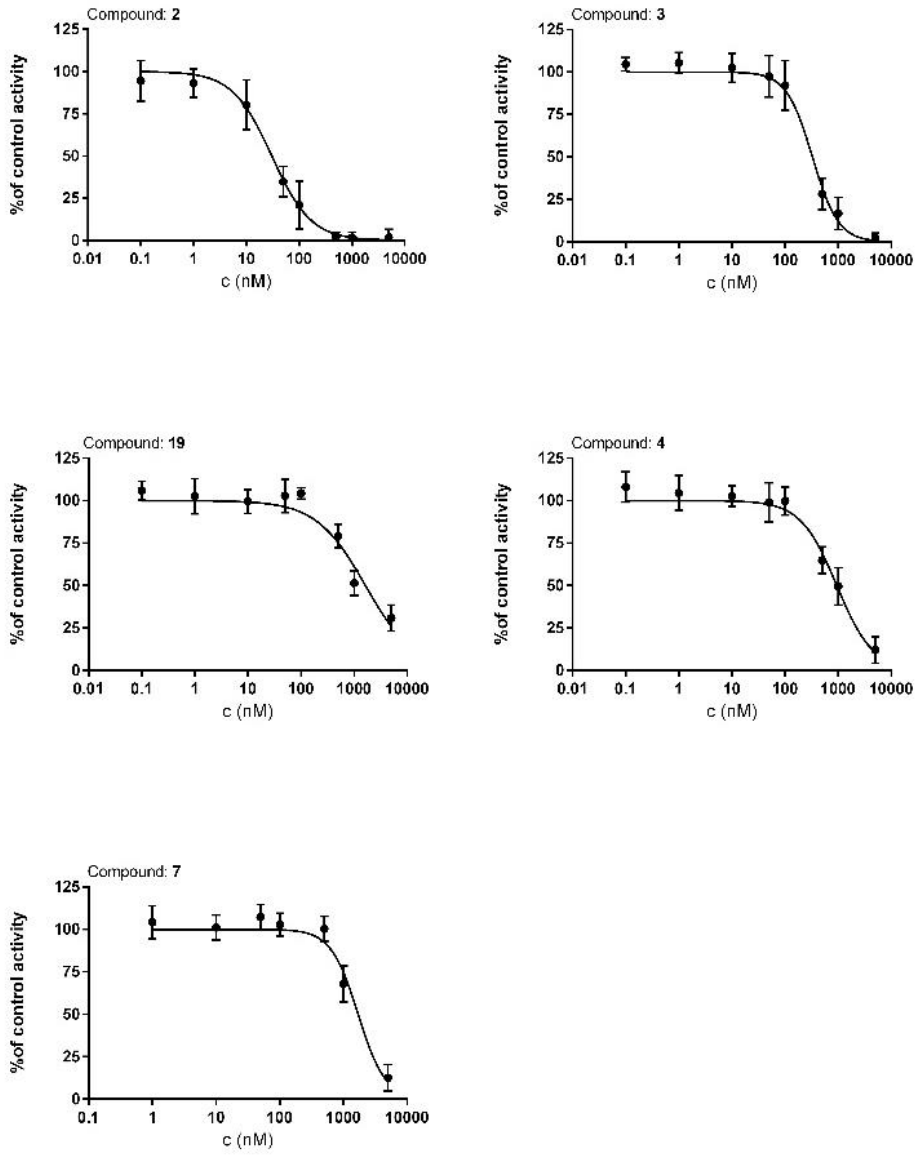


Figure S1 *In vitro* IC_{50} assays (dose-response curves) against USP7 activity at various concentrations of selected compounds, using a fluorogenic substrate (Ub-AMC) assay. Data for at least three independent determinations are presented.

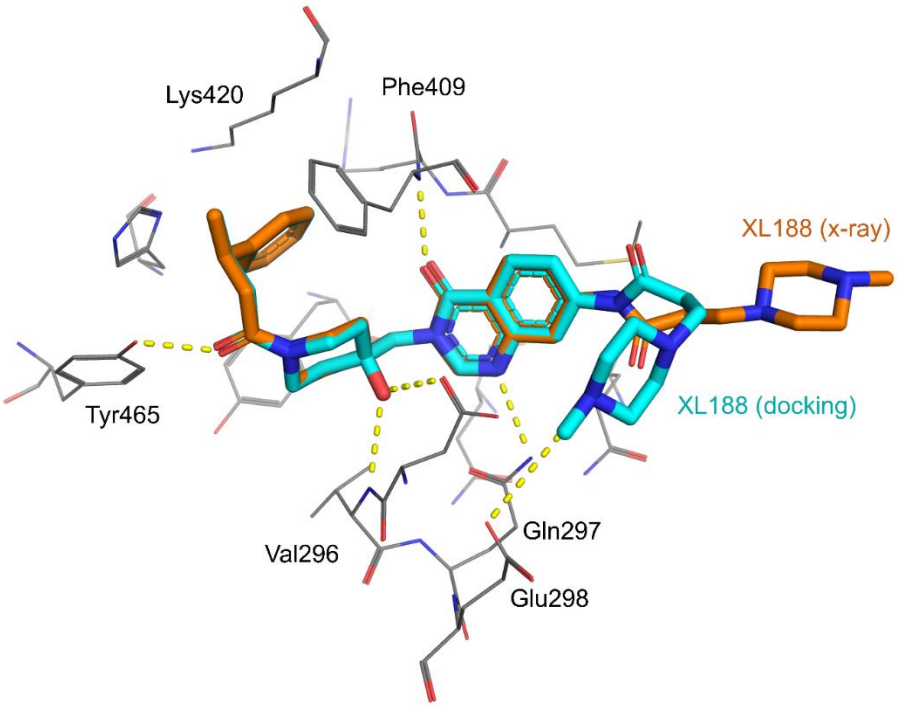


Figure S2 Redocking of the co-crystallized ligand **3**/XL188 into the crystal structure of USP7 (PDB: 5VS6) revealed that the binding pose allowed flexibility for the piperazinepropanamide portion of the ligand that points out of the binding site. Orange, co-crystallized pose; cyan, docked pose. Hydrogen bonds are represented with yellow dashed lines.

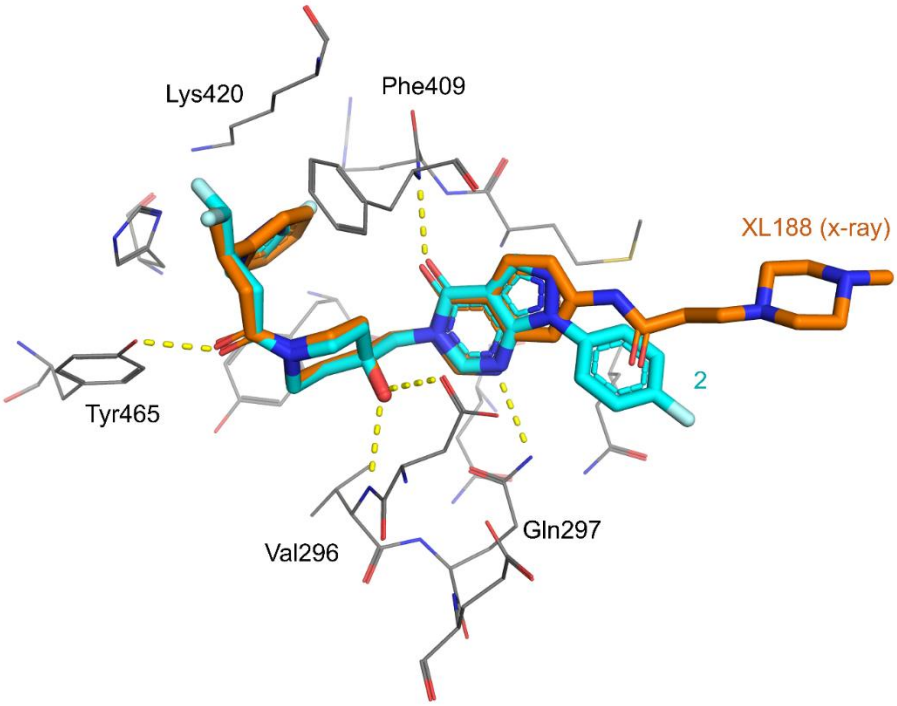


Figure S3 Docking of compound **2** (cyan) into the crystal structure of USP7 (PDB: 5VS6) co-crystallized with **3**/XL188 (orange). Hydrogen bonds are represented with yellow dashed lines.

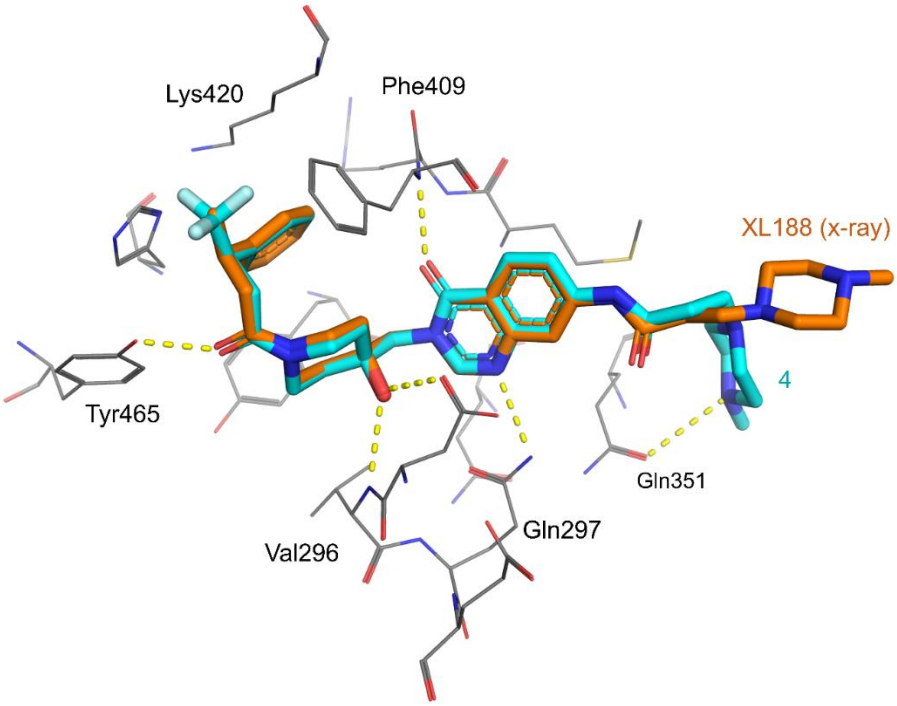


Figure S4 Docking of compound **4** (cyan) into the crystal structure of USP7 (PDB: 5VS6) co-crystallized with **3**/XL188 (orange). Hydrogen bonds are represented with yellow dashed lines.

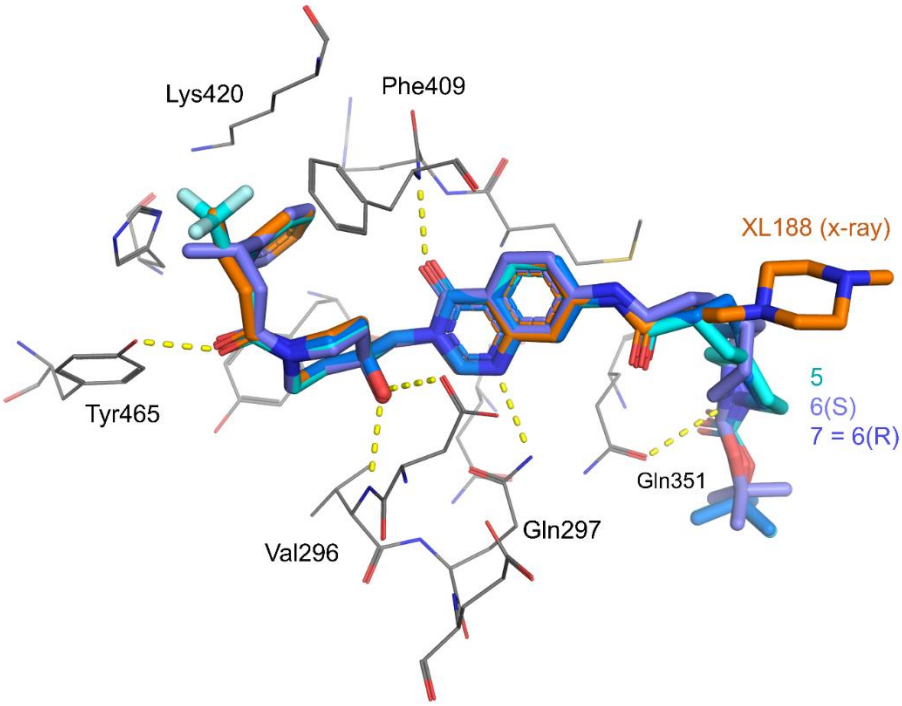


Figure S5 Docking of compounds **5** (cyan), **6** (isomer *S*, purple; isomer *R*, blue), and **7** (blue) into the crystal structure of USP7 (PDB: 5VS6) co-crystallized with **3**/XL188 (orange). Hydrogen bonds are represented with yellow dashed lines.

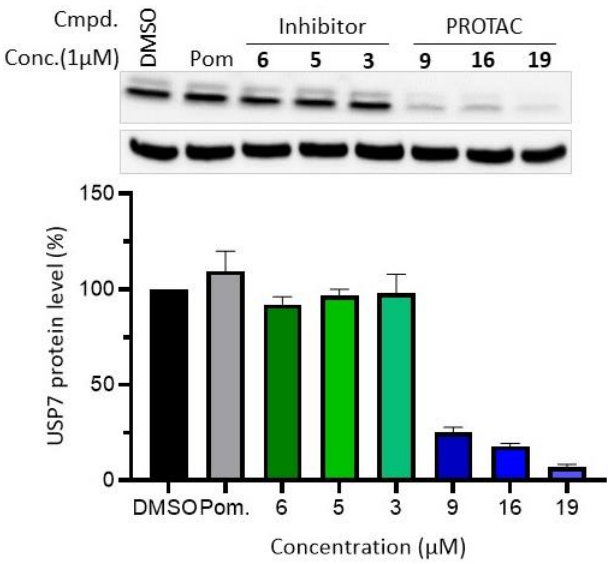


Figure S6 Comparative western blot analysis performed in MM.1S cells with different USP7 inhibitors and PROTACs treated for 24 h at a concentration of 1 μM (above). Bar graph depicting quantification of USP7 protein levels normalized to tubulin and DMSO (below). Data is represented as mean±s.d. (n=2).

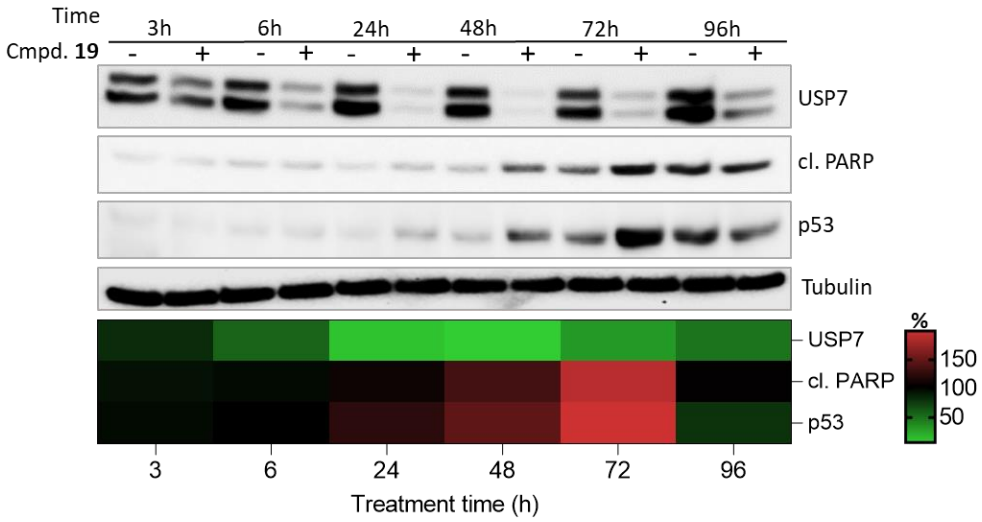


Figure S7 Western blot showing time course analysis with compound **19** at a concentration of 1 µM in MM.1S cells. Heatmap shows quantification of USP7 degradation levels, induction of apoptosis indicated by upregulation of cleaved PARP, upregulation of USP7 downstream target p53. Each protein level was normalized to tublin and to DMSO of the respective time point. Data is represented as mean±s.d. (n=2).

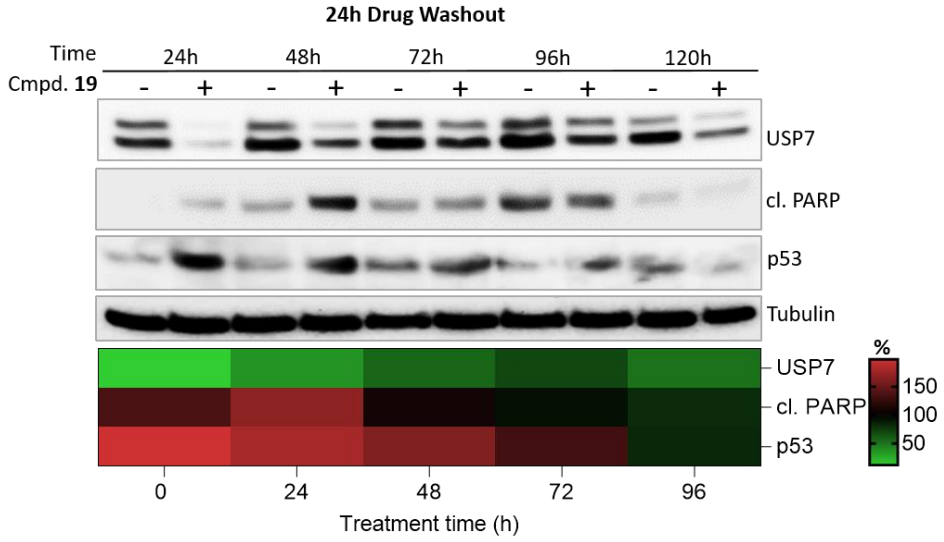


Figure S8 Western blot showing time course analysis with compound **19** at a concentration of 1 µM in MM.1S cells. After a 24 h-drug treatment the drug was washed out and samples were collected at the listed time points. Heatmap shows quantification of USP7 degradation levels, induction of apoptosis indicated by upregulation of cleaved PARP, upregulation of USP7 downstream target p53. Each protein level was normalized to tubulin and to DMSO of the respective time point. Data is represented as mean±s.d. (n=2).

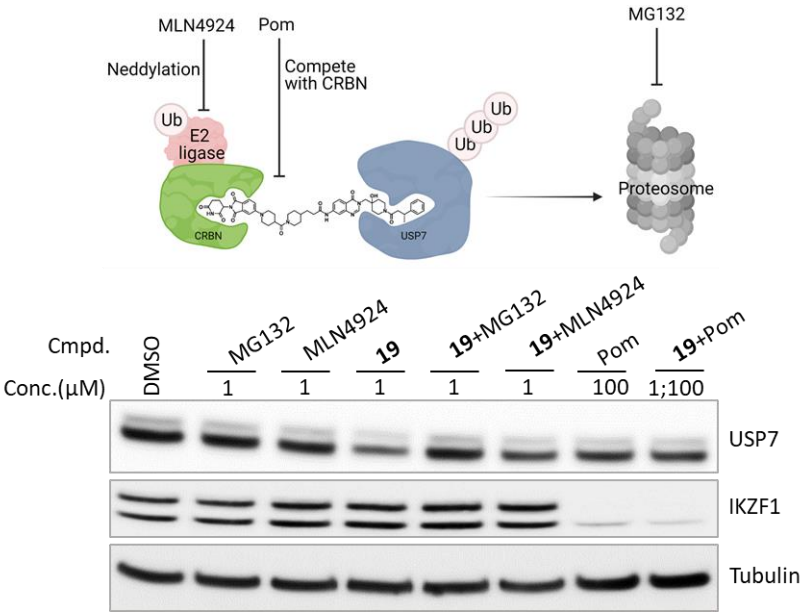


Figure S9 Schematic representation of mode of action of the listed compounds (above). Western blot in MM.1S cells pre-treated with MG132, MLN4924, 100-fold excess pomalidomide (Pom) for 1 h followed by treatment with compound **19** at a concentration of 1 µM for 3 h which successfully prevent degradation of USP7 (below).

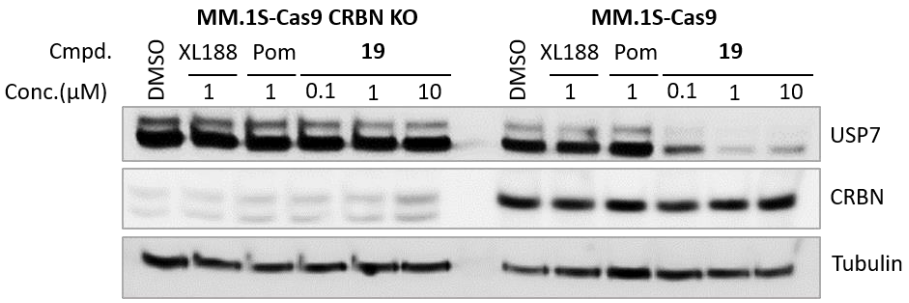


Figure S10 Western blot performed in CRISPR/Cas9 generated MM.1S-Cas9 CRBN KO or MM.1S-Cas9 cells. The cells were treated with the mentioned compounds for 24 h at the indicated concentrations. Western blot shows successful knockout of CRBN which prevents degradation of USP7 by compound **19**.

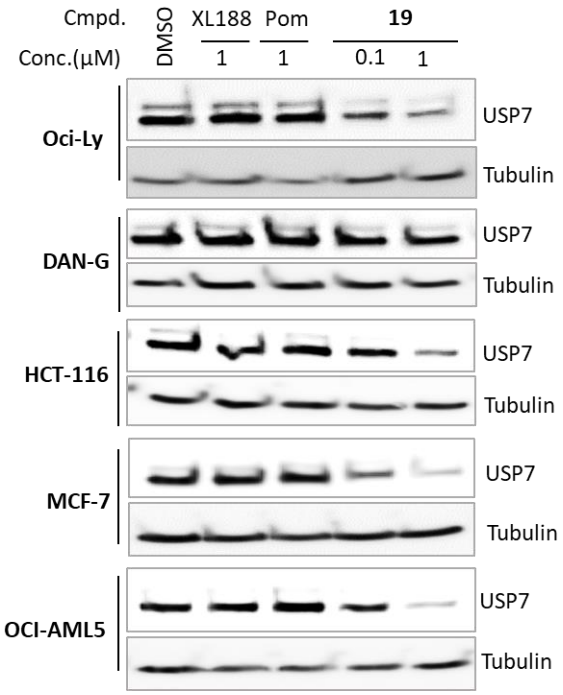


Figure S11 Western blot performed in various cancer cell lines showing degradation of USP7 by compound **19**.

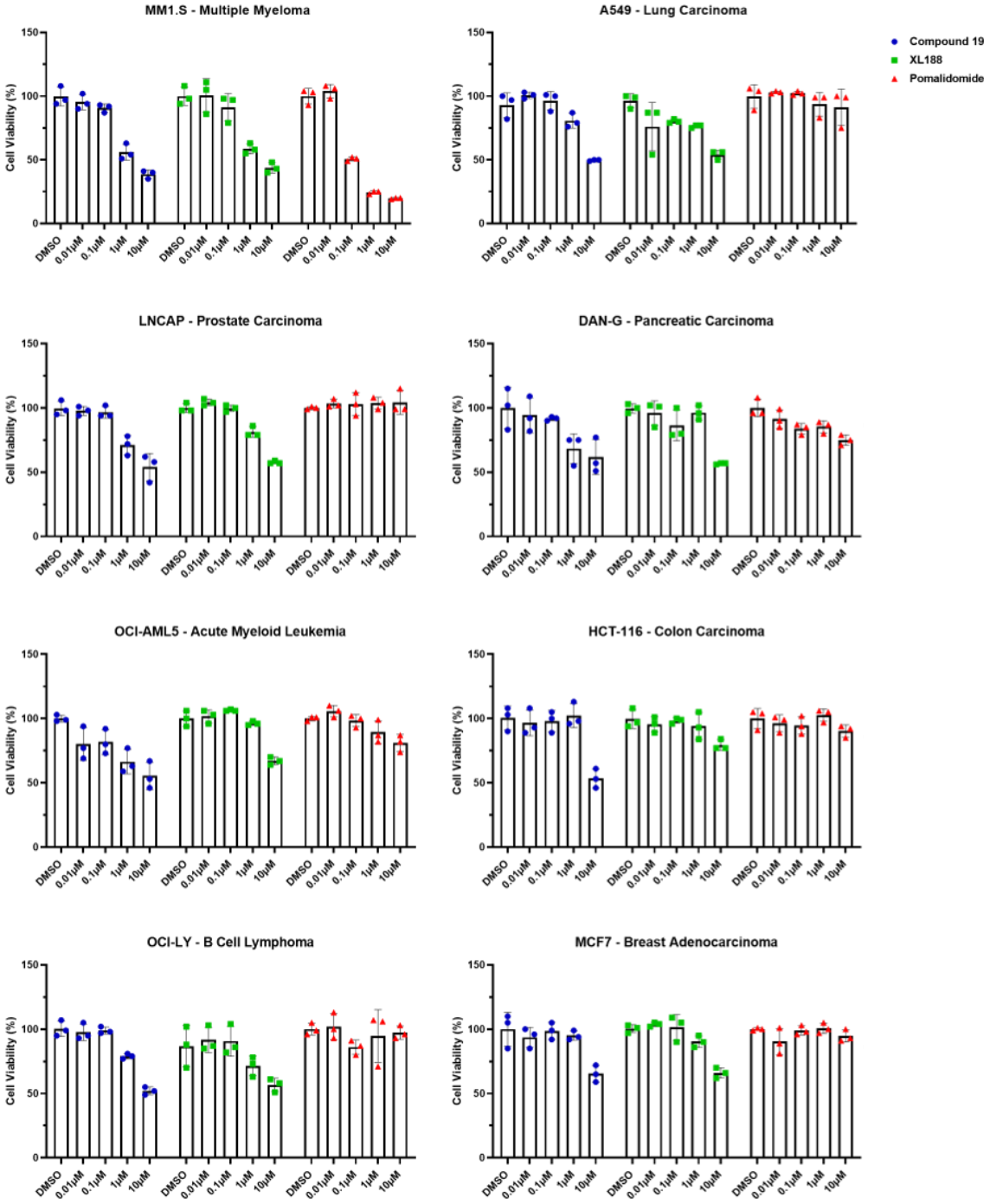


Figure S11 CellTiter-Glo luminescent cell viability assay performed in the mentioned cancer cell lines upon 96 h treatment with the listed compounds. Data is represented as mean±s.d. (n=3).

Supplementary Information: Biochemistry

A. USP7 inhibitor screening

The IC_{50} value determinations were carried out in 50 mM HEPES, 0.5 mM EDTA, 11 µM ovalbumin assay buffer (*p*H 7.6). USP7 (2.5 nM final concentration, #E-519, Boston Biochem) was pre-incubated with different concentrations of tested compounds or DMSO as control in black 384-well plates for 30 min at 25 °C. The enzymatic reactions were initiated with the addition of 250 nM ubiquitin-AMC (125 nM final concentration, #U-550, R&D systems). Fluorescence intensity was measured every minute over a 30-minute period on a Tecan Spark microplate reader (excitation at 340 nm, emission at 450 nm). The calculated initial rate values were plotted against inhibitor concentration to determine IC_{50} values in GraphPad Prism.

B. Computational docking

For compounds **2**–**7**, 3D structures were prepared using LigPrep (Schrödinger Suite 2020-2, Schrödinger, LLC, New York, NY, 2020) before docking. The crystal structure of USP7 in complex with XL188 (PDB: 5VS6)[3] was prepared using Protein Preparation Wizard.[4] Only chain A was used for the docking experiments. The co-crystallized ligand XL188 (**3**) was extracted, hydrogen atoms were added, residues were protonated at *p*H 7.0, the H-bonding network was refined, and restrained minimization was performed. The receptor's grid box was centered on the co-crystallized ligand XL188, and water molecules were removed. Docking was performed using Glide XP,[5] and the top-scoring ten poses for each compound were rescored with Prime MM-GBSA (molecular mechanics with generalised Born and surface area solvation) to estimate binding affinity. Validation of the docking protocol was performed by redocking the co-crystallized ligand. Structural visualizations were created in PyMOL 2.4.0.

C. Cell lines and treatments

Cell lines were obtained from the American Type Culture Collection (ATCC) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Cells were cultured in RPMI-1640 (Thermo Scientific) supplemented with 10% fetal bovine serum (FBS; Life Technologies) and 1% penicillin/streptomycin (Life Technologies) were maintained in a 37 °C humidified incubator with 5% CO_2. Cells were regularly tested for mycoplasma.

Cells were treated in 12-well cell culture dishes for western blots and 96-well microplates for cell viability with the designated compounds. The compounds used for treatments were synthesized where mentioned while the following compounds were obtained commercially: FT671 (MedChemExpress, #HY-107985), Pomalidomide (MedChemExpress, #HY-10984), Pevonedistat/MLN4924 (Selleckchem, #S7109-5), MG-132 (Selleckchem, #S2619-5).

D. Immunoblotting

Cells were treated with the designated compounds in 12-well cell culture dishes. Treated cells were harvested, washed with PBS and lysed in Pierce IP lysis buffer. Isolated protein was quantified using bicinchoninic acid (BCA) assay (Pierce). Equal amounts of protein were loaded and separated on a sodium dodecyl sulfate–polyacrylamide gel at a constant voltage of 100 V. Proteins were transferred onto a Immobilon-P transfer membrane (Millipore) under contstant current of 360 mA for 2 h. The membrane was washed in TBST (1 × Tris-Buffered Saline, 0.1% Tween) and blocked for 1 h in 5% milk in TBST. Primary antibodies were prepared in 5% Bovine serum albumin (BSA) and incubated overnight at 4 °C. This was followed by 3 × TBST washed and secondary HRP-antibody incubation for 1 h at room temperature. Chemiluminescence signal was detected using Immobilon Western Chemiluminescent HRP Substrate (Millipore) and imaged using LAS 4000 × (Fuji). Quantification of blots was performed using ImageJ software.

The following antibodies were used: HAUSP (D17C6) XP(R) rabbit mAb (Cell Signaling 4833S), cleaved PARP (Asp214) (D64E10) XP(R) rabbit mAb (Cell Signaling, #5625S), p53 (7F5) rabbit mAb (Cell Signaling, #2527S), anti-CRBN antibody produced in rabbit (Sigma, #HPA045910), Anti-

eRF3/GSPT1 (Abcam, #ab49878), monoclonal anti-α-Tubulin (Sigma, #T5168), anti-rabbit IgG, HRP-linked antibody (Cell Signaling, #7074S).

E. Cell viability assay

Cells were seeded in 96-well microplates and treated with the indicated compounds. Fresh media containing the compounds was added every 36 h. CellTiter-Glo Luminescent Cell Viability Assay (Promega) was performed after 96 h according to the manufacturer's instructions. Synergy LX Multi-Mode plate reader (BioTek) was used for luminescence readout. The different compound treatment conditions were normalized to DMSO.

F. Statistical analyses

Statistical analysis of western blots and cell viability were performed with Prism version 9.10 (GraphPad Software, San Diego, CA, USA). Variance of biological replicates is represented as standard deviation of mean.

Supplementary Information: Chemistry

G. Molecular descriptor calculations

Predicted values for the topological polar surface area (TPSA) were calculated using MarvinSketch 17.28.0 (ChemAxon).

H. logD measurements

The determination of the $logD_{7.4}$ values was performed by a chromatographic method as described previously.[6,7] The system was calibrated by plotting the retention times of six different drugs (atenolol, metoprolol, labetalol, diltiazem, triphenylene, permethrin) versus their literature known $logD_{7.4}$ in a calibration line (R^2 = 0.99). Subsequently, the mean retention times of the analytes were taken to calculate their $logD_{7.4}$ values with aid of the calibration line. At least two independent measurements of each analyte were performed.

I. Plasma protein binding studies

Plasma protein binding (%PPB) was estimated by correlating the logarithmic retention times of the analytes on a CHIRALPAK HSA 50 × 3 mm, 5 µm column with the literature known %PPB values (converted into logK values) of the following drugs: warfarin, ketoprofen, budesonide, nizatidine, indomethacin, acetylsalicylic acid, carbamazepine, piroxicam, nicardipine, and cimetidine (for details, see Valko *et al.*[8]). Samples were dissolved in MeCN/DMSO 9:1 to achieve a final concentration of 0.5 mg/mL. The mobile phase A was 50 mM ammonium acetate adjusted to *p*H 7.4 with ammonia solution, while mobile phase B was *i*PrOH. The flow rate was set to 1.0 mL/min, the UV detector was set to 254 nm, and the column temperature was kept at 30 °C. After injecting 2 µL of the sample, a linear gradient from 100% A to 30% *i*PrOH in 5.4 min was applied. From 5.4 to 18 min, 30% *i*PrOH was kept, followed by switching back to 100% A in 1.0 min and a re-equilibration time of 6 min. With the aid of the calibration line (R^2 = 0.94), the logK values of new

substances were calculated and converted to their %PPB values. At least two independent measurements of each analyte were performed. In case of racemic mixtures such as compound **6**, different retention times for each enantiomer were observed but the mean t_R was used to calculate %PPB.

J. Synthesis: General remarks

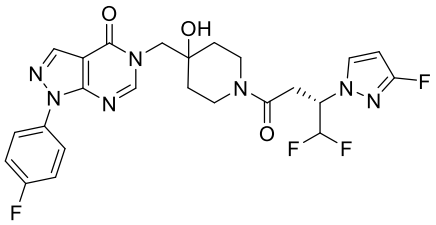
Preparative column chromatography was performed using Merck silica gel 60 (0.063 – 0.200 mm) or using an automated flash chromatography system puriFlash XS 520Plus. Melting points were determined on a Büchi 510 oil bath apparatus or on a Reichelt hot-stage apparatus and were uncorrected. ^{1}H NMR and ^{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). In case of overlapping extraneous solvent peaks, multiplet analyses in ^{1}H NMR spectra were performed using qGSD (quantitative Global Spectral Deconvolution). 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. *Important note*: The presence of amide rotamers significantly complicated the appearance and validation of the ^{1}H and ^{13}C NMR spectra associated with synthetic intermediates and final PROTACs. Thus, reported resonances and integrals may have limited accuracy. 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 compounds were determined on an Infinity Lab LC/MSD-system (Agilent) with ESI-source coupled with an HPLC 1260 Infinity II (Agilent) using a EC50/2 Nucleodur C18 Gravity 3 µm column (Macherey-Nagel). The column temperature was 40 °C. HPLC conditions started with 90% H_2O containing 2 mM NH_4Ac. The gradient ramped up to 100% MeCN in 10 min, followed by further flusing with 100% MeCN for 5 min. The flow rate was 0.5 mL/min. The samples were dissolved in H_2O, MeOH or MeCN (approx. 1 mg/mL), and 2 µL sample solution was injected. Positive total ion scans were observed from 100–1000 *m*/*z* (or more if necessary) and UV absorption was detected from 190–600 nm using a diode array detector (DAD). The purity was determinated at 220–600 nm. The enantiomeric purities of selected compounds were determined by polarimetric measurements performed with a Jasco-P2000 digital polarimeter

(600 mg of powder dissolved in 10.00 ml of solvent, λ = 589 nm, T = 20.00 °C, tube length equal to L = 10 cm).

K. Synthesis: USP7 inhibitors 4-7

FT671 (2)[9]

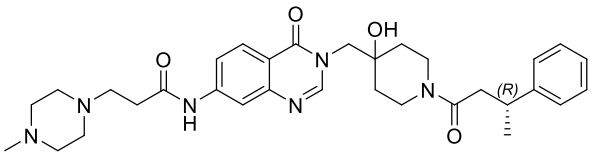
(CAS: 1959551-26-8)



This compound was used as commercially supplied (MedChemExpress).

XL188 (3)[3]

(CAS: 2305045-76-3)

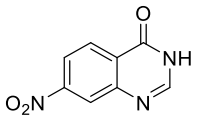


This compound was synthesised as described previously.[3]

R_f = 0.14 (40% MeOH/EtOAc +1% Et_3N); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.19 (d, *J* = 6.9 Hz, 3H), 1.22 – 1.54 (m, 4H), 2.13 (s, 3H), 2.26 – 2.47 (m, 8H), 2.51 – 2.66 (m, 6H), 2.88 (t, *J* = 12.6 Hz, 1H), 3.11 – 3.27 (m, 2H), 3.63 (t, *J* = 13.1 Hz, 1H), 3.87 – 4.04 (m, 3H), 4.90 (s, 1H), 7.10 – 7.18 (m, 1H), 7.20 – 7.29 (m, 4H), 7.61 (dd, *J* = 2.1, 8.8 Hz, 1H), 8.00 (d, *J* = 2.1 Hz, 1H), 8.07 (d, *J* = 8.7 Hz, 1H), 8.18 (d, *J* = 12.8 Hz, 1H), 10.48 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.00, 22.17, 34.38, 34.45, 35.07, 35.19, 36.15, 36.35, 37.08, 41.12, 41.22, 45.84, 52.48, 53.65, 54.88, 69.39, 114.88, 116.68, 118.45, 126.03, 126.08, 127.02, 127.39, 128.30, 128.33, 144.44, 146.70, 146.82, 149.14, 149.49, 160.28, 160.33, 169.24, 171.16; **LC-MS** (ESI) t_R = 4.78 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{32}H_{43}N_6O_4$, 575.33; found, 575.5; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{32}H_{43}N_6O_4$, 575.3340; found, 575.3322.

7-Nitro-3*H*-quinazolin-4-one (22)[10,11]

(CAS: 20872-93-9)

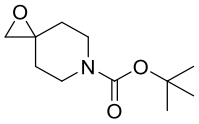


This compound was synthesized similar to a previously reported procedure.[3] In brief, 2-amino-4-nitrobenzoic acid (**21**, 7.29 g, 40 mmol) and formamide (7.21 g, 160 mmol) were heated (neat) at 150 °C for 16 h. The brown solid was suspended in H_2O (400 mL) and filtrated under vacuum. The residue was titruated with hot EtOAc (4 × 50 mL) and the solution/filtrate was evaporated to give the title compound as a light brown solid.

Yield (1.53 g, 20%); mp >250 °C, lit. mp 263–269 °C[11]; R_f = 0.48 (80% EtOAc/petroleum ether); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 8.20 – 8.27 (m, 2H), 8.30 – 8.37 (m, 2H), 12.63 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 120.52, 122.39, 127.16, 128.37, 147.87, 149.34, 151.24, 159.96; **MS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_8H_6N_3O_3$, 192.04; found, 192.1.

***tert*-Butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate (23)**[12]

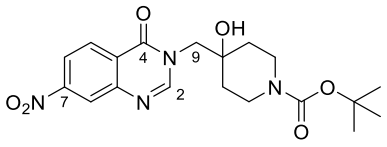
(CAS: 147804-30-6)



This compound was synthesized as reported previously.[3]

Yield (0.95 g, 22%); mp 82–84 °C, lit. mp 50–52 °C[12]; R_f = 0.70 (50% EtOAc/cyclohexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.40 (s, 11H), 1.59 – 1.67 (m, 2H), 2.64 (s, 2H), 3.30 – 3.41 (m, 2H), 3.45 – 3.53 (m, 2H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.18, 32.64, 42.39, 52.93, 56.96, 78.94, 153.98; **MS** (ESI) *m*/*z* $[M + H - C_4H_9]^+$ calcd for $C_7H_{12}NO_3$, 158.08; found, 158.2.

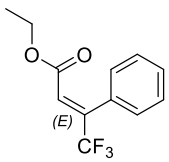
***tert*-Butyl 4-hydroxy-4-[(7-nitro-4-oxo-quinazolin-3-yl)methyl]piperidine-1-carboxylate (24)**[3]
(CAS: 2417090-44-7)



This compound was synthesized similar to a previously reported procedure.[3] In brief, compound **22** (1.91 g, 10 mmol), epoxid **23** (2.34 g, 11 mmol), and Cs_2CO_3 (9.77 g, 30 mmol) were suspended in dry DMF (50 mL) and stirred at 80 °C for 16 h. The mixture was diluted with EtOAc (200 mL) and washed with half-saturated NH_4Cl solution (100 mL). The aqueous layer was extracted with EtOAc (100 mL), and the combined organic layers were washed with 5% LiCl solution and brine (each 100 mL). It was dried over Na_2SO_4, filtered, and evaporated. The crude material was subjected to column chromatography (gradient from 50% to 80% EtOAc in cyclohexane), and the product enriched fractions were subjected to flash chromatography (80 g, 30 µm, 0 to 5% MeOH in CH_2Cl_2) to give the title compound as a colorless solid.

Yield (2.46 g, 61%); mp 184–186 °C; R_f = 0.50 (80% EtOAc/petroleum ether); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.38 (s, 11H), 1.45 – 1.53 (m, 2H), 3.04 (s, 2H), 3.65 (d, J = 13.0 Hz, 2H), 4.02 (s, 2H), 4.93 (s, 1H), 8.25 (dd, J = 2.3, 8.7 Hz, 1H), 8.36 – 8.40 (m, 3H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 28.23, 34.47, 40.23, 54.23, 69.37, 78.68, 120.68, 122.30, 125.96, 128.86, 148.41, 151.22, 151.30, 153.98, 160.03. The (^{1}H,^{13}C)-HMBC spectrum showed correlations between 9-H and C-2 as well as C-4. **MS** (ESI) *m/z* $[M + H - C_4H_9]^+$ calcd for $C_{19}H_{24}N_4O_6$, 349.11; found, 349.1.

Ethyl (*E*)-4,4,4-trifluoro-3-phenyl-but-2-enoate (30a)[13]
(CAS: 56210-74-3)



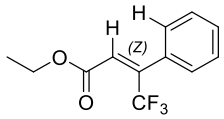
NaH (60% dispersion in mineral oil, 1.77 g, 44.4 mmol) was placed in an oven-dried Schlenk flask, which was then evacuated and backfilled with argon gas. Subsequently, dry THF (80 mL) was added, and the flask was cooled to 0 °C. Triethyl phosphonoacetate (8.9 mL, 44.4 mmol) was

slowly added, and the combined mixture was stirred until it became a clear solution (30 min). A solution of 2,2,2-trifluoro-acetophenone in dry THF (10 mL) was added over a period of 15 min, after which the reaction was heated to 50 °C for 16 h. After cooling, it was quenched by the careful addition of saturated NH_4Cl solution (200 mL), and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude material was divided in 5 portions, each of which were purified by flash chromatography on spherical silica gel (80 g, 30 µm, gradient from 0 to 5% EtOAc in cyclohexane) to give a major fraction of the (*E*)-isomer (**30a**) along with a minor fraction of the (*Z*)-isomer (**30b**, see below). Both isomers were colorless oils with a fruity smell that attracted Drosophila in the lab.

Yield (4.68 g, 65%); R_f = 0.45 (5% EtOAc/petroleum ether); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.95 (t, *J* = 7.1 Hz, 3H), 3.97 (q, *J* = 7.1 Hz, 2H), 6.86 (q, *J* = 1.4 Hz, 1H), 7.26 – 7.31 (m, 2H), 7.41 – 7.49 (m, 3H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 13.65, 60.92, 119.54 – 125.83 (m), 126.45 (q, *J* = 5.4 Hz), 128.60 (d, *J* = 11.0 Hz), 129.60, 130.64, 139.28 (q, *J* = 30.8 Hz), 163.82; **MS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{12}F_3O_2$, 245.08; mass not found.

Ethyl (*Z*)-4,4,4-trifluoro-3-phenyl-but-2-enoate (30b)[13]

(CAS: 56210-75-4)

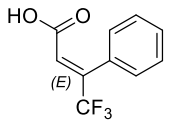


This isomer was isolated from the same batch as described above.

Yield (0.40 g, 5%); R_f = 0.39 (5% EtOAc/petroleum ether); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.25 (t, *J* = 7.1 Hz, 3H), 4.24 (q, *J* = 7.1 Hz, 2H), 6.92 (s, 1H), 7.42 – 7.51 (m, 5H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 13.96, 61.50, 122.54 (q, *J* = 275.9 Hz), 127.70, 129.05, 129.24 – 129.62 (m), 129.86, 132.56, 134.69 (q, *J* = 30.8 Hz), 164.65. The (^{1}H,^{1}H)-NOESY spectrum indicated a through space coupling between the aromatic and the olefinic hydrogen, which was not present in **30a**. **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{12}H_{12}F_3O_2$, 245.0784; found, 245.0782.

(*E*)-4,4,4-Trifluoro-3-phenyl-but-2-enoic acid (31)[1,14]

(CAS: 149819-78-3)

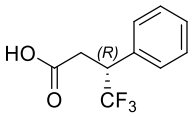


Compound **30a** (1.22 g, 5 mmol) was suspended in THF (10 mL) and H_2O (5 mL). LiOH × H_2O (232 mg, 5.5 mmol) was added, and the yellow suspension was stirred at RT for 2 h. The colorless solution was diluted with 1N HCl (50 mL), and it was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were washed with saturated NH_4Cl solution (50 mL), dried over Na_2SO_4, filtered, and evaporated to give the title compound as a colorless solid.

Yield (1.03 g, 95%); mp 94–96 °C, lit. mp 95–97 °C[14]; R_f = 0.48 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 6.84 (t, *J* = 1.5 Hz, 1H), 7.30 (dd, *J* = 2.8, 6.5 Hz, 2H), 7.44 (dd, *J* = 2.0, 4.8 Hz, 3H), 13.20 (br s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 123.07 (q, *J* = 274.4 Hz), 127.86 (q, *J* = 5.4 Hz), 128.71 (d, *J* = 20.0 Hz), 129.55, 130.90, 137.41 (q, *J* = 30.0 Hz), 165.56; **MS** (ESI) *m*/*z* $[M - H]^-$ calcd for $C_{10}H_6F_3O_2$, 215.03; found, 215.0.

(3*R*)-4,4,4-Trifluoro-3-phenyl-butanoic acid (32a)[1,2]

(CAS: 374782-31-7)



Asymmetric hydrogenation was performed as described previously.[2] In brief, *bis*(norbornadiene) rhodium(I) tetrafluoroborate (15.6 mg, 41.6 µmol) and Walphos SL-W008-2 (39.2 mg, 41.6 µmol) were weighed into a 50 mL glass reactor and purged with argon. MeOH (40 mL) was added and the resulting orange solution thoroughly degassed with argon for 20 min. Then, compound **31** (450 mg, 2.08 mmol) was added and the reaction mixture hydrogenated under 7 bar H_2 at RT for 24 h. The volatiles were then evaporated, saturated aqueous solution of $NaHCO_3$ (70 mL) was added to the crude residue, and extracted with EtOAc (2 × 50 mL). The aqueous layer was acidified with 8M HCl to *p*H = 1 and extracted with EtOAc (4 × 50 mL). The combined organic

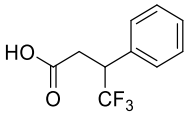
layers were dried over Na_2SO_4, filtered, and evaporated to give the title compound as a white solid.

Yield (432 mg, 95%); mp 55–56 °C; $[\alpha]_D^{20}$ = –19.2 (*c* 0.06, $CHCl_3$); R_f = 0.45 (10% MeOH/CH_2Cl_2 +1% AcOH); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.94 (dd, A of ABX, J_{AB} = 16.8 Hz, J_{AX} = 9.7 Hz, 1H), 3.08 (dd, A of ABX, J_{AB}= 16.8, J_{BX} = 4.9 Hz, 1H), 3.87 (pd, *J* = 4.9, 9.3 Hz, 1H), 7.28 – 7.40 (m, 5H), 10.46 (br s, 1H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 34.14 (q, *J* = 2.2 Hz), 45.70 (q, *J* = 27.9 Hz), 126.84 (q, *J* = 279.5 Hz), 128.67, 128.79, 128.85, 133.33 (q, *J* = 1.5 Hz), 176.05; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{10}H_8F_3O_2$, 217.0482; found, 217.0475.

The enantiomeric excess (ee) of the methyl ester of this compound was determined by HPLC (see below).

4,4,4-Trifluoro-3-phenyl-butanoic acid (32b)[15]

(CAS: 149680-95-5)



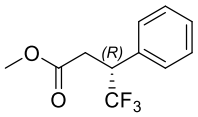
For comparison, hydrogenation was performed in the absence of the chiral catalyst. To a flame-dried round bottom flask, compound **31** (77 mg, 0.36 mmol) was added and dissolved in MeOH (10 mL). The solution was degassed and purged with argon before the addition of 10% Pd/C (10 mg). The mixture was stirred under a hydrogen atmosphere at RT for 24 h. The suspension was then filtered through Celite, and the filtrate concentrated *in vacuo* to give the title compound as a white solid.

Yield (58 mg, 74%); mp 55–56 °C; R_f = 0.47 (10% MeOH/CH_2Cl_2 +1% AcOH); **^{1}H NMR** (400 MHz, $CDCl_3$) δ 2.93 (dd, A of ABX, J_{AB} = 16.8 Hz, J_{AX} = 9.7 Hz, 1H), 3.07 (dd, A of ABX, J_{AB}= 16.8, J_{BX} = 4.9 Hz, 1H), 3.87 (pd, *J* = 4.9, 9.3 Hz, 1H), 7.28 – 7.41 (m, 5H); **^{13}C NMR** (101 MHz, $CDCl_3$) δ 34.14 (q, *J* = 2.2 Hz), 45.73 (q, *J* = 27.9 Hz), 126.16 (q, *J* = 279.5 Hz), 128.67, 128.79, 128.85, 133.32 (q, *J* = 1.5 Hz), 176.06; **HRMS** (ESI) *m/z* $[M - H]^-$ calcd for $C_{10}H_8F_3O_2$, 217.0482; found, 217.0475.

The ee of the methyl ester of this compound was determined by HPLC (see below).

Methyl (3*R*)-4,4,4-trifluoro-3-phenyl-butanoate (33a)[1]

(CAS: 2202738-44-9)

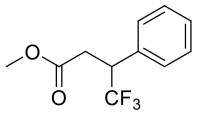


Compound **32a** (65 mg, 0.3 mmol) was dissolved in dry CH_2Cl_2 (5 mL). DMAP (4 mg, 0.03 mmol) and MeOH (60 µL) were added, followed by addition of DCC (68 mg, 0.33 mmol). The mixture was stirred at RT for 16 h, after which it was filtered and evaporated *in vacuo*. The crude product was subjected to purifaction by flash chromatography on spherical silica gel (4 g, 15 µm, gradient from 0 to 10% EtOAc in cyclohexane) to give the title compound as colorless oil.

Yield (56 mg, 80%); R_f = 0.42 (10% EtOAc/cyclohexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.97 – 3.13 (m, 2H), 3.52 (s, 3H), 4.00 – 4.12 (m, 1H), 7.30 – 7.47 (m, 5H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 33.30, 45.04 (q, *J* = 26.9 Hz), 51.88, 126.73 (q, *J* = 279.9 Hz), 128.56, 128.72, 129.13, 133.81, 170.25; **MS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{12}F_3O_2$, 233.08; mass not found.

Methyl 4,4,4-trifluoro-3-phenyl-butanoate (33b)[16]

(CAS: 1104029-00-6)



This compound was synthesized by analogy with **33a** but using acid **32b** (65 mg, 0.3 mmol). The crude product was subjected to purifaction by flash chromatography on spherical silica gel (4 g, 15 µm, gradient from 0 to 10% EtOAc in cyclohexane) to give the title compound as colorless oil.

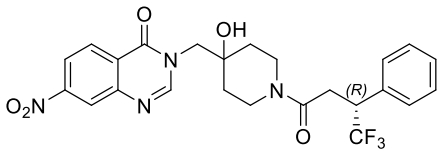
Yield (30 mg, 43%); R_f = 0.42 (10% EtOAc/cyclohexane); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.98 – 3.13 (m, 2H), 3.52 (s, 3H), 3.99 – 4.12 (m, 1H), 7.31 – 7.44 (m, 5H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 33.30, 45.03 (q, *J* = 26.8 Hz), 51.89, 126.73 (q, *J* = 280.0 Hz), 128.57, 128.72, 129.14, 133.80, 170.25; **MS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{12}F_3O_2$, 233.08; mass not found.

The ee of **33a** and **33b** was measured using a Chiralcel OJ-RH column (150 × 4.6 mm, 5 µm) and 60% H_2O and 40% MeCN as eluent at a flow rate of 0.5 mL/min. After injection of 20 µL of a 1 mg/mL solution in MeCN, UV-absorption was monitored at 254 nm.

Cmpd	t_{R1} (min)	area (%)	t_{R2} (min)	area (%)	ee
33a	32.067	1.08	34.042	98.93	>97%
33b	31.875	49.93	34.283	50.08	<1%

As reported previously,[1] for the enantioenriched **33a**, the later peak represented the major constituent and was assigned to be the (*R*)-configurated enantiomer based on the elution order and the negative specific rotation of its synthetic precursor **32a**.[2]

3-[[4-Hydroxy-1-[(3*R*)-4,4,4-trifluoro-3-phenyl-butanoyl]-4-piperidyl]methyl]-7-nitro-quinazolin-4-one (34)

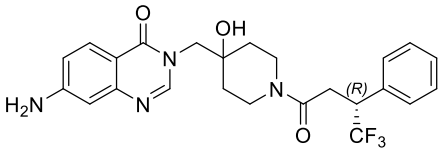


Compound **24** (1.46 g, 3.6 mmol) was suspended in dry CH_2Cl_2 (36 mL) and TFA (9 mL) was added. The yellow solution was stirred at RT for 2 h. The volatiles were removed under vacuum, and the residue was dried. Acid **32a** (0.79 g, 3.6 mmol) was dissolved in dry DMF (8 mL) and DIPEA (1.25 mL, 7.2 mmol) as well as HATU (1.51 g, 3.96 g) were added. After stirring for 10 min at RT, a second solution consisting of the deprotected **24**, dry DMF (8 mL) and DIPEA (2.5 mL, 14.4 mmol) was added. The combined mixture was stirred at RT for 16 h. The mixture was diluted with half-saturated NH_4Cl solution (200 mL) and it was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with 5% LiCl solution and brine (each 100 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was subjected to purifaction by flash

chromatography on spherical silica gel (80 g, 30 µm, gradient from 60 to 100% EtOAc in cyclohexane) to give the title compound as a light yellow solid.

Yield (1.62 g, 89%); mp 90–92 °C; R_f = 0.55 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.19 – 1.66 (m, 4H), 2.78 – 3.01 (m, 2H), 3.10 – 3.30 (m, 2H), 3.72 – 3.80 (m, 1H), 3.93 – 4.15 (m, 4H), 4.96 (s, 1H), 7.28 – 7.44 (m, 5H), 8.25 (dd, *J* = 2.3, 8.8 Hz, 1H), 8.34 – 8.41 (m, 3H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 31.64, 31.69, 34.27, 34.32, 35.01, 35.06, 37.43, 37.51, 40.95, 45.44 (dd, *J* = 16.0, 25.9 Hz), 54.20, 54.28, 69.35, 69.46, 120.70, 122.32, 119.89 – 129.23 (m), 125.95, 125.98, 128.17, 128.20, 128.52, 128.55, 128.84, 129.35, 134.91, 148.42, 151.23, 151.27, 159.97, 160.02, 166.76 (d, *J* = 16.6 Hz); **LC-MS** (ESI) t_R = 6.71 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{24}F_3N_4O_5$, 505.17; found, 505.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{24}F_3N_4O_5$, 505.1693; found, 505.1674.

7-Amino-3-[[4-hydroxy-1-[(3*R*)-4,4,4-trifluoro-3-phenyl-butanoyl]-4-piperidyl]methyl]quinazolin-4-one (35)

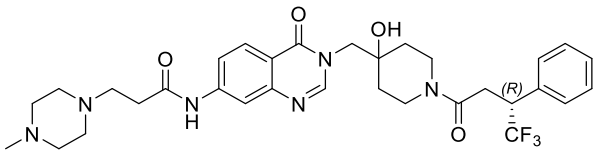


Compound **34** (1.57 g, 3.11 mmol) was suspended in EtOH (30 mL) and AcOH (7.5 mL). Iron powder (1.39 g, 24.88 mmol) was suspended in H_2O (25 mL) and AcOH (25 mL) and stirred for 5 min. Subsequently, the compound solution was added and it was stirred at 110 °C for 30 min. After cooling and filtration, saturated $NaHCO_3$ solution was added (caution: CO_2 release!) until a neutral *p*H was reached. The product was extracted from the aqueous solution with $CHCl_3$ (3 × 100 mL), and the combined organic layers were washed with H_2O and brine (each 100 mL). It was dried over Na_2SO_4, filtered, concentrated and subjected to purifaction by flash chromatography on spherical silica gel (80 g, 30 µm, dryload on Celite, gradient from 0 to 10% MeOH in $CHCl_3$) to give the title compound as a colorless solid.

Yield (1.00 g, 72%); mp 120–124 °C; R_f = 0.29 (10% MeOH/$CHCl_3$); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.11 – 1.64 (m, 4H), 2.80 – 2.99 (m, 2H), 3.09 – 3.30 (m, 2H), 3.69 – 3.77 (m, 1H), 3.75 – 3.95 (m, 2H), 3.89 – 3.97 (m, 1H), 4.03 – 4.13 (m, 1H), 4.89 (s, 1H), 6.07 (d, *J* = 1.9 Hz, 2H), 6.61 (t, *J* =

2.2 Hz, 1H), 6.72 (dd, *J* = 2.2, 8.8 Hz, 1H), 7.25 – 7.34 (m, 2H), 7.32 – 7.36 (m, 1H), 7.36 – 7.41 (m, 2H), 7.78 (dd, *J* = 3.6, 8.7 Hz, 1H), 8.01 (d, *J* = 15.0 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 31.60, 31.64, 34.34, 34.38, 35.00, 35.08, 37.49, 37.58, 41.04, 45.45 (dd, *J* = 15.3, 26.3 Hz), 53.33, 53.40, 69.34, 69.43, 79.33, 106.96, 110.43, 115.14, 124.19 – 130.57 (m), 127.76, 128.18, 128.53, 129.32, 129.35, 134.84, 134.93, 148.84, 148.87, 150.17, 154.50, 160.45, 160.39, 166.72 (d, *J* = 10.5 Hz); **LC-MS** (ESI) t_R = 5.23 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{26}F_3N_4O_3$, 475.20; found, 475.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{26}F_3N_4O_3$, 475.1952; found, 475.1937.

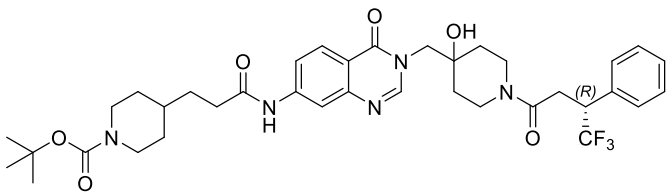
_N_-[3-[[4-hydroxy-1-[(3_R_)-4,4,4-trifluoro-3-phenyl-butanoyl]-4-piperidyl]methyl]-4-oxo-quinazolin-7-yl]-3-(4-methylpiperazin-1-yl)propanamide (4)



This compound was synthesized by analogy with **3**. In brief, compound **35** (0.24 g, 0.5 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled to –10 °C. After the addition of Et_3N (70 µL, 0.5 mmol), 3-bromopropionyl chloride (86 mg, 0.5 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise. The mixture was stirred at 0 °C for 3 h. The colorless suspension was diluted with CH_2Cl_2 (50 mL), washed with half-saturated NH_4Cl solution (50 mL), and the aqueous layer was extracted again with CH_2Cl_2 (50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, concentrated and subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in $CHCl_3$). The first two major peaks were collected and evaporated. The crude bromo alkyl intermediate (122 mg, 0.2 mmol) was dissolved in dry DMF (2 mL), and Et_3N (55 µL, 0.4 mmol) and *N*-methylpiperazine (24 mg, 0.24 mmol) were added. The mixture was heated at 80 °C for 3 h. The organic solvent was removed under high vacuum and the crude product was directly subjected to flash chromatography on spherical silica gel (25 g, 15 µm, dryload on Celite, gradient from 10 to 50% MeOH in EtOAc +1% Et_3N) to give the title compound as a colorless solid.

Yield (97 mg, 77%); mp 196–198 °C; R_f = 0.19 (40% MeOH/EtOAc +1% Et_3N); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.13 – 1.49 (m, 4H), 1.52 – 1.66 (m, 1H), 2.13 (s, 3H), 2.22 – 2.38 (m, 4H), 2.39 – 2.45 (m, 2H), 2.52 (t, *J* = 7.0 Hz, 2H), 2.63 (t, *J* = 7.0 Hz, 2H), 2.79 – 3.00 (m, 2H), 3.09 – 3.29 (m, 2H), 3.71 – 3.79 (m, 1H), 3.80 – 4.03 (m, 4H), 4.04 – 4.13 (m, 1H), 4.93 (s, 1H), 7.25 – 7.36 (m, 3H), 7.36 – 7.42 (m, 2H), 7.58 – 7.63 (m, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 8.06 (dd, *J* = 3.5, 8.7 Hz, 1H), 8.17 (d, *J* = 13.6 Hz, 1H), 10.50 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 31.64, 34.32, 34.37, 34.47, 35.01, 35.08, 37.47, 37.56, 41.02, 45.46 (dd, *J* = 16.0, 26.3 Hz), 45.88, 52.51, 53.68, 54.91, 69.35, 69.44, 114.91, 116.71, 118.49, 124.38 – 130.28 (m), 127.43, 128.19, 128.54, 129.35, 134.86, 134.93, 144.48, 149.17, 149.51, 149.55, 160.31, 160.37, 166.76 (d, *J* = 12.2 Hz), 171.22; **LC-MS** (ESI) t_R = 5.37 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{32}H_{40}F_3N_6O_4$, 629.31; found, 629.5; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{32}H_{40}F_3N_6O_4$, 629.3058; found, 629.3028.

tert-Butyl 4-[3-[[3-[[4-hydroxy-1-[(3*R*)-4,4,4-trifluoro-3-phenyl-butanoyl]-4-piperidyl]methyl]-4-oxo-quinazolin-7-yl]amino]-3-oxo-propyl]piperidine-1-carboxylate (5)



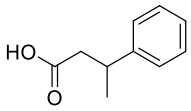
3-(1-(*tert*-Butoxycarbonyl)piperidin-4-yl)propanoic acid (0.56 g, 2.18 mmol) was dissolved in dry CH_2Cl_2 (10 mL) and cooled to 0 °C. Oxalyl chloride solution (2M in CH_2Cl_2, 1.6 mL, 3.27 mmol) and 1 drop dry DMF were added. The mixture was stirred at 0 °C for 3 h, after which it was evaporated at ambient temperature and further dried under high vacuum. Aniline **35** (0.52 g, 1.09 mmol) was suspended in a mixture of dry THF (20 mL) and dry CH_2Cl_2 (20 mL), Et_3N (0.3 mL, 2.18 mmol) was added, and finally a solution of the *in situ* generated ROCl in dry CH_2Cl_2 (10 mL) was introduced by syringe. After stirring for 1 h at 0 °C, it was quenched with half-saturated NH_4Cl solution (100 mL), and it was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on spherical silica gel (50 g, 30 µm, dryload on Celite, gradient from 0 to 10% MeOH in $CHCl_3$) to give the title compound as a colorless solid.

Yield (0.30 g, 42%); mp 108–110 °C; R_f = 0.37 (10% MeOH/$CHCl_3$); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.93 – 1.05 (m, 2H), 1.18 – 1.35 (m, 2H), 1.38 (s, 9H), 1.40 – 1.68 (m, 7H), 2.40 (t, *J* = 7.6 Hz, 2H), 2.56 – 2.76 (m, 3H), 2.78 – 3.02 (m, 2H), 3.06 – 3.27 (m, 1H), 3.71 – 3.78 (m, 1H), 3.79 – 3.98 (m,

5H), 4.08 (d, *J* = 10.0 Hz, 1H), 4.91 (s, 1H), 7.24 – 7.36 (m, 3H), 7.36 – 7.42 (m, 2H), 7.62 (dd, *J* = 2.1, 8.5 Hz, 1H), 8.01 (d, *J* = 2.0 Hz, 1H), 8.05 (dd, *J* = 2.7, 8.6 Hz, 1H), 8.17 (d, *J* = 11.5 Hz, 1H), 10.30 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 28.26, 31.53, 31.65, 33.97, 34.35, 35.00, 35.07, 37.46, 37.55 , 41.01, 43.83, 45.10 – 45.78 (m), 53.66, 53.73, 69.33, 69.42, 78.56, 79.31, 114.91, 116.63, 118.50, 123.75 – 130.39 (m), 127.33, 128.17, 128.52, 129.32, 134.90, 144.57, 149.14, 149.46, 154.03, 160.30, 160.36, 166.74 (d, *J* = 9.8 Hz), 172.25; **LC-MS** (ESI) t_R = 7.24 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{37}H_{47}F_3N_5O_6$, 714.35; found, 714.4; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{37}H_{47}F_3N_5O_6$, 714.3473; found, 714.3447.

3-Phenylbutyric acid (36)

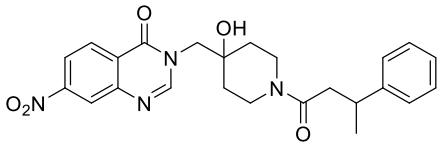
(CAS: 4593-90-2)



This compound was used as commercially supplied (Sigma Aldrich).

3-[[4-Hydroxy-1-(3-phenylbutanoyl)-4-piperidyl]methyl]-7-nitro-quinazolin-4-one (37)

(CAS: 1978361-18-0)



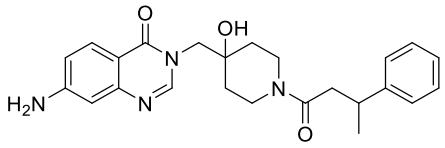
This compound was synthesized by analogy with **34** but using acid **36** (0.59 g, 3.6 mmol). The crude product was subjected to purifaction by flash chromatography on spherical silica gel (80 g, 30 µm, dryload on Celite, gradient from 70 to 100% EtOAc in cyclohexane) to give the title compound as colorless solid.

Yield (1.23 g, 76%); mp 138–140 °C; R_f = 0.30 (5% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.20 (d, *J* = 6.9 Hz, 3H), 1.28 – 1.59 (m, 3H), 1.98 (s, 1H), 2.49 – 2.65 (m, 2H), 2.81 – 2.91 (m, 1H), 3.09 – 3.27 (m, 2H), 3.64 (t, *J* = 12.8 Hz, 1H), 3.92 – 4.12 (m, 3H), 4.94 (d, *J* = 4.8 Hz, 1H), 7.11 – 7.19 (m, 1H), 7.21 – 7.30 (m, 4H), 8.25 (dd, *J* = 2.3, 8.7 Hz, 1H), 8.34 – 8.41 (m, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 14.21, 20.87, 21.99, 22.18, 34.35, 34.47, 35.06, 35.19, 36.13, 36.36, 37.04,

41.07, 41.18, 54.22, 59.86, 69.42, 69.47, 120.66, 122.29, 125.95, 126.04, 126.10, 127.00, 127.04, 128.31, 128.34, 128.83, 146.71, 146.82, 148.40, 151.23, 159.95, 160.00, 169.26; **LC-MS** (ESI) t_R = 6.03min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{24}H_{27}N4O_5$, 451.20; found, 451.3.

7-Amino-3-[[4-hydroxy-1-(3-phenylbutanoyl)-4-piperidyl]methyl]quinazolin-4-one (38)

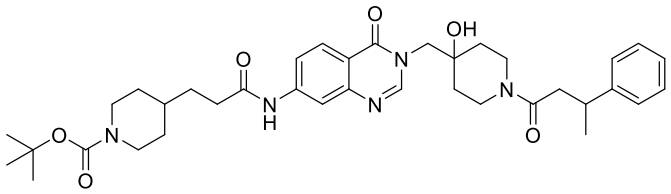
(CAS: 1978361-34-0)



This compound was synthesized by analogy with **35** but using aniline derivative **37** (0.59 g, 3.6 mmol). The crude product was subjected to purifaction by flash chromatography on spherical silica gel (80 g, 30 µm, dryload on Celite, gradient from 0 to 10% MeOH in $CHCl_3$) to give the title compound as colorless solid.

Yield (0.81 g, 62%); mp 204–206 °C; R_f = 0.19 (10% MeOH/EtOAc); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.09 – 1.55 (m, 7H), 2.43 – 2.64 (m, 2H), 2.88 (t, *J* = 12.0 Hz, 1H), 3.11 – 3.41 (m, 2H), 3.62 (s, 1H), 3.77 – 4.10 (m, 3H), 4.86 (d, *J* = 6.3 Hz, 1H), 6.06 (s, 2H), 6.61 (d, *J* = 2.2 Hz, 1H), 6.72 (dd, *J* = 2.2, 8.7 Hz, 1H), 7.10 – 7.16 (m, 1H), 7.24 (dd, *J* = 3.5, 5.8 Hz, 4H), 7.78 (d, *J* = 8.7 Hz, 1H), 8.02 (d, *J* = 13.5 Hz, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.03, 22.20, 34.41, 34.56, 35.09, 35.21, 36.18, 36.37, 37.14, 40.39, 40.43, 41.18, 41.28, 53.38, 69.42, 69.47, 106.96, 110.42, 110.45, 115.14, 126.06, 126.11, 127.02, 127.75, 128.33, 128.36, 146.72, 146.83, 148.85, 150.16, 154.48, 160.41, 160.46, 169.25, 169.27; **LC-MS** (ESI) t_R = 4.71min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{24}H_{29}N_4O_3$, 421.22; found, 421.3; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{24}H_{29}N_4O_3$, 421.2234; found, 421.2233.

***tert*-Butyl 4-[3-[[3-[[4-hydroxy-1-(3-phenylbutanoyl)-4-piperidyl]methyl]-4-oxo-quinazolin-7-yl]amino]-3-oxo-propyl]piperidine-1-carboxylate (6)**

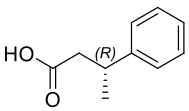


This compound was synthesized by analogy with **5** but using aniline derivative **38** (0.46 g, 1.09 mmol). The crude product was purified by flash chromatography on spherical silica gel (50 g, 30 µm, dryload on Celite, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as a colorless solid.

Yield (0.31 g, 43%); mp 128–130 °C; R_f = 0.33 (10% MeOH/$CHCl_3$); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.93 – 1.05 (m, 2H), 1.12 – 1.48 (m, 16H), 1.45 – 1.60 (m, 2H), 1.65 (dd, *J* = 3.6, 13.6 Hz, 2H), 2.40 (t, *J* = 7.7 Hz, 2H), 2.54 – 2.77 (m, 4H), 2.83 – 2.92 (m, 1H), 3.09 – 3.27 (m, 2H), 3.57 – 3.70 (m, 1H), 3.87 – 4.08 (m, 6H), 4.89 (d, *J* = 5.9 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.22 – 7.26 (m, 4H), 7.62 (dd, *J* = 2.0, 8.7 Hz, 1H), 8.01 (d, *J* = 2.1 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 8.17 (d, *J* = 12.8 Hz, 1H), 10.29 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.00, 22.17, 28.24, 31.50, 31.66, 33.94, 34.37, 34.51, 35.05, 35.19, 36.14, 36.34, 37.08, 40.36, 40.39, 41.12, 41.22, 43.51, 53.68, 59.85, 69.39, 69.44, 78.52, 114.88, 116.62, 118.46, 126.02, 126.07, 126.99, 127.01, 127.31, 128.30, 128.32, 144.54, 146.70, 146.82, 149.12, 149.44, 153.99, 160.28, 160.33, 169.23, 172.20; **LC-MS** (ESI) t_R = 7.13min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{37}H_{50}N_5O_6$, 660.38; found, 660.6; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{37}H_{50}N_5O_6$, 660.3756; found, 660.3753.

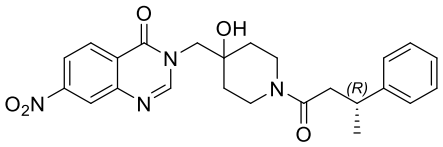
(*R*)-3-Phenylbutyric acid (25)

(CAS: 772-14-5)



This compound was used as commercially supplied and possessed >97% ee (Activate Scientific).

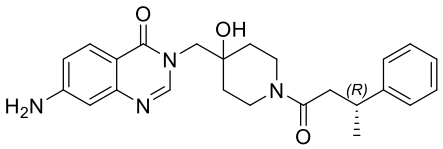
3-[[4-Hydroxy-1-[(3*R*)-3-phenylbutanoyl]-4-piperidyl]methyl]-7-nitro-quinazolin-4-one (26)[3]
(CAS: 1978361-18-0)



This compound was synthesized by analogy with **34** but using **24** (0.62 g, 1.52 mmol) and acid **25** (0.25 g, 1.52 mmol). The crude product was subjected to purifaction by flash chromatography on spherical silica gel (40 g, 30 µm, dryload on Celite, gradient from 70 to 100% EtOAc in cyclohexane) to give the title compound as colorless solid.

Yield (0.51 g, 75%); mp 96–98 °C; R_f = 0.54 (5% MeOH/EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.08 – 1.60 (m, 7H), 2.51 – 2.64 (m, 2H), 2.81 – 2.90 (m, 1H), 3.11 – 3.26 (m, 2H), 3.60 – 3.69 (m, 1H), 3.94 (s, 1H), 3.95 – 4.09 (m, 2H), 4.94 (d, *J* = 6.2 Hz, 1H), 7.11 – 7.18 (m, 1H), 7.21 – 7.33 (m, 4H), 8.25 (dd, *J* = 2.3, 8.8 Hz, 1H), 8.34 – 8.41 (m, 3H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.01, 22.21, 34.36, 34.48, 35.07, 35.20, 36.15, 36.38, 37.06, 40.24, 40.40, 41.08, 41.20, 54.24, 69.44, 69.49, 120.69, 122.32, 125.96, 126.06, 126.12, 127.03, 127.07, 128.33, 128.37, 128.85, 146.73, 146.84, 148.41, 151.22, 151.26, 151.28, 159.96, 160.02, 169.27; **LC-MS** (ESI) t_R = 6.00 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{27}N_4O_5$, 451.20; found, 451.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{27}N_4O_5$, 451.1976; found, 451.1964.

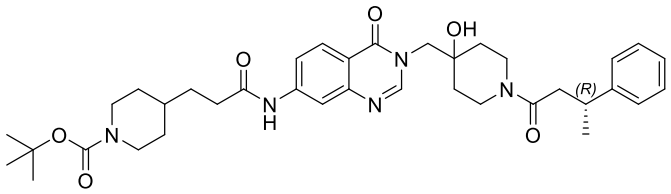
7-Amino-3-[[4-hydroxy-1-[(3*R*)-3-phenylbutanoyl]-4-piperidyl]methyl]quinazolin-4-one (27)[3]
(CAS: 2305046-93-7)



This compound was synthesized by analogy with **35** on a smaller scale and by using **26** (0.50 g, 1.1 mmol) instead. The crude product was subjected to purifaction by flash chromatography on spherical silica gel (40 g, 30 µm, dryload on Celite, gradient from 5 to 10% MeOH in $CHCl_3$) to give the title compound as colorless solid.

Yield (0.36 g, 78%); mp 208–210 °C; R_f = 0.17 (10% MeOH/$CHCl_3$); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.08 – 1.59 (m, 7H), 2.51 – 2.63 (m, 2H), 2.84 – 2.92 (m, 1H), 3.10 – 3.26 (m, 2H), 3.58 – 3.67 (m, 1H), 3.78 – 4.11 (m, 3H), 4.86 (d, *J* = 7.9 Hz, 1H), 6.07 (s, 2H), 6.61 (d, *J* = 2.2 Hz, 1H), 6.72 (dd, *J* = 2.2, 8.7 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.21 – 7.28 (m, 4H), 7.78 (d, *J* = 8.7 Hz, 1H), 8.02 (d, *J* = 16.1 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.04, 22.21, 34.41, 34.55, 35.09, 35.20, 36.17, 36.36, 37.12, 40.24, 40.37, 40.41, 41.17, 41.26, 53.37, 69.41, 69.46, 106.94, 110.41, 110.44, 115.12, 126.05, 126.10, 127.03, 127.75, 128.33, 128.36, 146.72, 146.84, 148.85, 150.16, 154.48, 160.39, 160.44, 169.24; **LC-MS** (ESI) t_R = 4.71 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{24}H_{29}N_4O_3$, 421.22; found, 421.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{24}H_{29}N_4O_3$, 421.2234; found, 421.2222.

***tert*-Butyl 4-[3-[[3-[[4-hydroxy-1-[(3*R*)-3-phenylbutanoyl]-4-piperidyl]methyl]-4-oxo-quinazolin-7-yl]amino]-3-oxo-propyl]piperidine-1-carboxylate (7)**



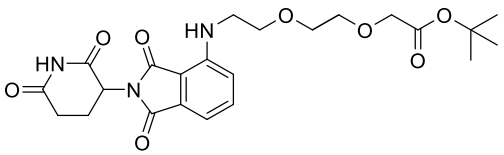
This compound was synthesized by analogy with **5** on a smaller sclae and by using aniline derivative **27** (185 mg, 0.44 mmol). The crude product was purified by flash chromatography on spherical silica gel (40 g, 30 µm, dryload on Celite, gradient from 2 to 10% MeOH in $CHCl_3$) to give the title compound as a colorless solid.

Yield (91 mg, 31%); mp 128–130 °C; R_f = 0.33 (10% MeOH/$CHCl_3$); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.93 – 1.05 (m, 2H), 1.38 (s, 16H), 1.56 (q, *J* = 7.3 Hz, 2H), 1.65 (d, *J* = 13.2 Hz, 2H), 2.40 (t, *J* = 7.7 Hz, 2H), 2.51 – 2.72 (m, 4H), 2.87 (d, *J* = 15.4 Hz, 1H), 3.07 – 3.25 (m, 2H), 3.63 (t, *J* = 12.9 Hz, 1H), 3.86 – 3.95 (m, 4H), 3.97 – 4.06 (m, 2H), 4.89 (d, *J* = 5.9 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.24 (t, *J* = 2.8 Hz, 4H), 7.62 (dd, *J* = 2.1, 8.8 Hz, 1H), 8.01 (d, *J* = 2.1 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 8.17 (d, *J* = 12.8 Hz, 1H), 10.29 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.00, 22.18, 28.24, 31.50, 31.66, 33.94, 34.37, 34.51, 35.05, 35.19, 36.14, 36.35, 37.08, 41.12, 41.22, 43.58, 53.68, 69.39, 69.44, 78.53, 114.88, 116.62, 118.46, 126.03, 126.08, 127.00, 127.02, 127.32, 128.30, 128.33, 144.54, 146.70, 146.82, 149.13, 149.45, 154.00, 160.29, 160.33, 169.23, 172.21; **LC-MS** (ESI) t_R = 7.13 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{37}H_{50}N_5O_6$, 660.38; found, 660.6; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{37}H_{50}N_5O_6$, 660.3756; found, 660.3737.

L. Synthesis: Phthalimide-linker conjugates

***tert*-Butyl 2-[2-[2-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]ethoxy]ethoxy]acetate (39)**[17]

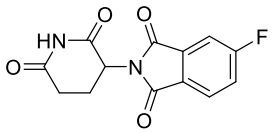
(CAS: 2143097-01-0)



This compound was synthesized as we described previously.[17]

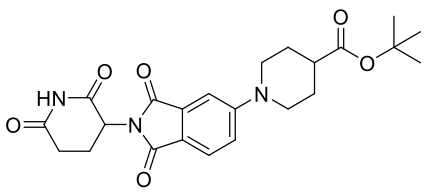
5-Fluorothalidomide (28)[18]

(CAS: 835616-61-0)



This compound was synthesized as we described previously.[18]

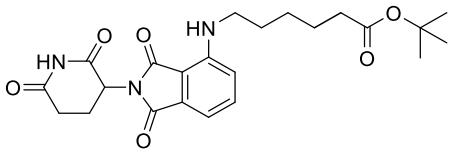
***tert*-Butyl 1-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]piperidine-4-carboxylate (29)**[18]



This compound was synthesized as we described previously.[18]

***tert*-Butyl 6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexanoate (40)**[17]

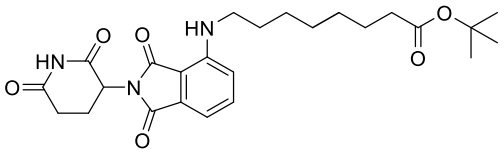
(CAS: 2226139-38-2)



This compound was synthesized as we described previously.[17]

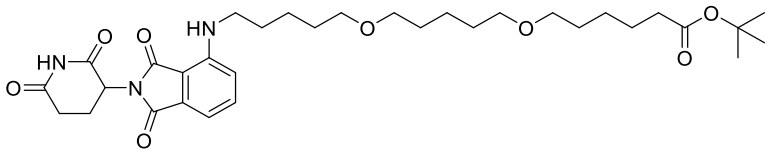
***tert*-Butyl 8-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]octanoate (41)**[18]

(CAS: 2226139-39-3)



This compound was synthesized as we described previously.[18]

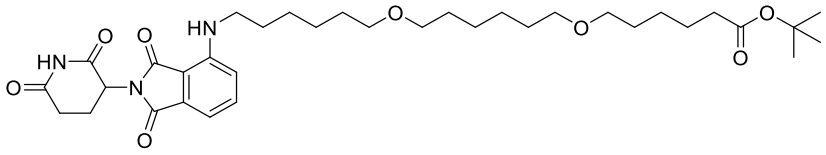
***tert*-Butyl 6-[5-[5-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]pentoxy]pentoxy] hexanoate (42)**[19]



This compound was synthesized as we described previously.[19]

***tert*-Butyl 6-[6-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy]hexoxy] hexanoate (43)**[19]

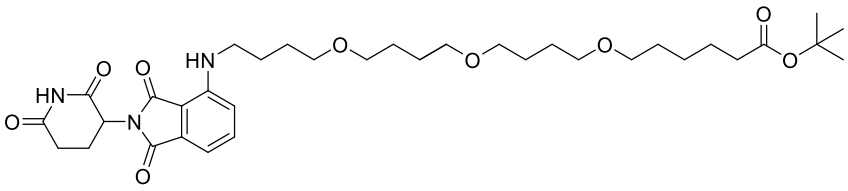
(CAS: 2362575-54-8)



This compound was synthesized as we described previously.[19]

***tert*-Butyl 6-[4-[4-[4-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]butoxy]butoxy] butoxy]hexanoate (44)**[19]

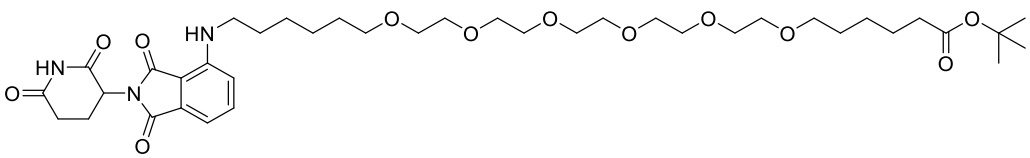
(CAS: 2362575-55-9)



This compound was synthesized as we described previously.[19]

***tert*-Butyl 6-[2-[2-[2-[2-[2-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexoxy] ethoxy]ethoxy]ethoxy]ethoxy]ethoxy]hexanoate (45)**[19]

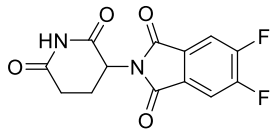
(CAS: 2362575-56-0)



This compound was synthesized as we described previously.[19]

5,6-Difluorothalidomide (46)

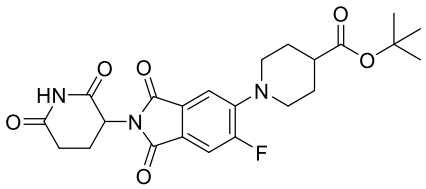
(CAS: 1496997-41-1)



This compound was synthesized using our previously published general procedure to produce aryl-substituted thalidomide derivatives.[20] In brief, 5,6-difluoroisobenzofuran-1,3-dione (5.08 g, 27.6 mmol), 3-aminopiperidine-2,6-dione hydrochloride (3.03 g, 18.4 mmol), and sodium acetate (1.81 g, 22.9 mmol) were stirred in AcOH (75 mL) at 120 °C for 4 h. After cooling, the mixture was poured onto icewater (200 mL) and stirred for 5 min. A precipitate was formed, which was collected by suction filtration, and it was washed with H_2O and *n*-hexanes (each 3 × 20 mL). The gray solid was further dried *in vacuo*.

Yield (4.69 g, 87%); mp 226–228 °C; R_f = 0.67 (60% EtOAc/petroleum ether); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 2.02 – 2.11 (m, 1H), 2.43 – 2.64 (m, 4H), 2.83 – 2.94 (m, 1H), 5.16 (dd, *J* = 5.4, 12.9 Hz, 1H), 8.13 (t, *J* = 7.6 Hz, 2H), 11.11 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.04, 31.04, 49.53, 114.02 (dd, *J* = 7.0, 15.0 Hz), 128.71 (t, *J* = 5.6 Hz), 152.92 (d, *J* = 15.2 Hz), 154.97 (d, *J* = 15.5 Hz), 165.49, 169.73, 172.79; **LC-MS** (ESI) t_R = 4.05 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{13}H_9F_2N_2O_4$, 295.05; found, 295.1.

tert-Butyl 1-[2-(2,6-dioxo-3-piperidyl)-6-fluoro-1,3-dioxo-isoindolin-5-yl]piperidine-4-carboxylate (47)

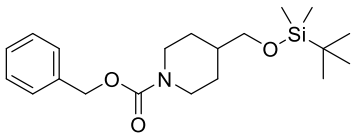


This compound was synthesized by analogy with **28** but using thalidomide derivative **46**. In brief, **46** (0.88 g, 3.0 mmol), *tert*-butyl piperidine-4-carboxylate hydrochloride (0.665 g, 3.0 mmol), and DIPEA (1.60 mL, 9.0 mmol) were dissolved in dry DMSO (30 mL) and heated under argon at 90 °C for 18 h. After cooling, it was diluted with half-saturated NH_4Cl solution (100 mL), and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with 5% LiCl solution and brine (each 50 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was by

subjected to purifaction by flash chromatography on spherical silica gel (40 g, 30 µm, dryload on Celite, gradient from 20 to 50% EtOAc in cyclohexane) to give the title compound as yellow solid.

Yield (1.01 g, 73%); mp 100–104 °C; R_f = 0.50 (50% EtOAc/cyclohexane); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.40 (s, 9H), 1.61 – 1.71 (m, 2H), 1.87 – 1.94 (m, 2H), 1.98 – 2.06 (m, 1H), 2.40 – 2.49 (m, 1H), 2.50 – 2.62 (m, 2H), 2.82 – 2.91 (m, 1H), 2.91 – 2.98 (m, 2H), 3.50 – 3.57 (m, 2H), 5.09 (dd, *J* = 5.4, 12.9 Hz, 1H), 7.43 (d, *J* = 7.5 Hz, 1H), 7.70 (d, *J* = 11.3 Hz, 1H), 11.10 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.25, 27.89, 27.98, 31.13, 49.23, 49.30, 49.33, 79.93, 112.12 (d, *J* = 25.2 Hz), 114.02 (d, *J* = 4.5 Hz), 123.39 (d, *J* = 9.8 Hz), 128.94, 145.80 (d, *J* = 8.8 Hz), 157.51 (d, *J* = 253.1 Hz), 166.38, 166.84, 170.09, 172.94, 173.53; **LC-MS** (ESI) t_R = 7.21 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{23}H_{27}FN_3O_6$, 460.19; found, 460.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{23}H_{27}FN_3O_6$, 460.1878; found, 460.1873.

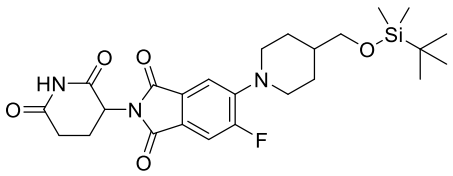
Benzyl 4-[[*tert*-butyl(dimethyl)silyl]oxymethyl]piperidine-1-carboxylate (48)



1-*N*-Cbz-hydroxymethyl-piperidine (3.17 g, 12.7 mmol) was dissolved in MeCN (80 mL), and imidazole (2.59 g, 38.1 mmol) and TBDMSCl (2.87 g, 19.05 mmol) were added. The mixture was stirred at RT for 16 h, after which it was filtrated, and the filtrate was evaporated. It was subjected to purifaction by flash chromatography on spherical silica gel (80 g, 30 µm, dryload on Celite, gradient from 0 to 10% EtOAc in cyclohexane) to give the title compound as a colorless oil.

Yield (4.12 g, 89%); R_f = 0.42 (10% EtOAc/petroleum ether); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.01 (s, 6H), 0.85 (s, 9H), 0.97 – 1.09 (m, 2H), 1.55 – 1.66 (m, 3H), 2.77 (s, 2H), 3.41 (d, *J* = 5.7 Hz, 2H), 3.97 – 4.05 (m, 2H), 5.05 (s, 2H), 7.26 – 7.39 (m, 5H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ –5.28, 18.08, 25.93, 28.29, 38.10, 43.55, 66.14, 67.15, 127.56, 127.87, 128.50, 137.22, 154.51; **LC-MS** (ESI) t_R = 10.49 min, 97% purity, *m/z* $[M + H]^+$ calcd for $C_{20}H_{34}NO_3Si$, 364.23; found, 364.3; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{20}H_{34}NO_3Si$, 364.2303; found, 364.2295.

5-[4-[[*tert*-Butyl(dimethyl)silyl]oxymethyl]-1-piperidyl]-2-(2,6-dioxo-3-piperidyl)-6-fluoro-isoindoline-1,3-dione (49a)

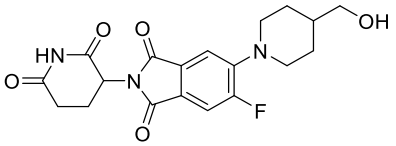


Carbamate **48** (0.91 g, 2.5 mmol) and Pd/C 10% w/w (91mg, 10% w/w) were added to a Schlenk tube. The vessel was closed, evacuated and refilled with nitrogen gas (3 ×), followed by hydrogen gas. Dry EtOAc (25 mL) was added, and the black mixture was stirred for 24 h at RT. The hydrogen gas was removed, and the flask was refilled with nitrogen gas, the vessel was opened, and it was filtrated through a pad of celite, and washed with EtOAc (3 × 20 mL). After evaporation of the solvent, the crude residue was further dried *in vacuo* to give a colorless oil. The crude amine was dissolved in dry DMSO (25 mL) and **46** (0.74 g, 2.5 mmol) and DIPEA (0.87 mL, 5.0 mmol) were added. The mixture was heated at 90 °C for 18 h. After cooling, it was diluted with half-saturated NH_4Cl solution (100 mL), and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with 5% LiCl solution and brine (each 50 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (50 g, 30 µm, dryload on Celite, gradient from 30 to 100% EtOAc in cyclohexane) to give the title compound as yellow solid.

Yield (306 mg, 24%, 2 steps); mp 102–104 °C; R_f = 0.60 (50% EtOAc/petroleum ether); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.03 (s, 6H), 0.86 (s, 9H), 0.97 – 1.05 (m, 1H), 1.31 (qd, *J* = 4.0, 12.4 Hz, 2H), 1.76 (dd, *J* = 3.6, 13.6 Hz, 2H), 1.98 – 2.06 (m, 1H), 2.46 – 2.62 (m, 2H), 2.83 – 2.92 (m, 3H), 3.48 (d, *J* = 6.2 Hz, 2H), 3.60 (dd, *J* = 6.2, 9.5 Hz, 2H), 5.08 (dd, *J* = 5.4, 12.9 Hz, 1H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.67 (d, *J* = 11.4 Hz, 1H), 11.07 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 18.13, 22.24, 25.97, 26.49, 28.34, 31.11, 37.86, 49.20, 49.96, 67.23, 112.00 (d, *J* = 25.3 Hz), 113.89 (d, *J* = 5.1 Hz), 123.01 (d, *J* = 9.1 Hz), 128.94, 146.01 (d, *J* = 8.7 Hz), 157.42 (d, *J* = 253.0 Hz), 166.36, 166.83, 170.03, 172.87. The signal for Si-CH_3 was beyond the recorded range; **LC-MS** (ESI) t_R = 10.46 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{25}H_{35}FN_3O_5Si$, 504.23; found, 504.4; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{25}H_{35}FN_3O_5Si$, 504.2325; found, 504.2309.

2-(2,6-Dioxo-3-piperidyl)-5-fluoro-6-[4-(hydroxymethyl)-1-piperidyl]isoindoline-1,3-dione (49b)

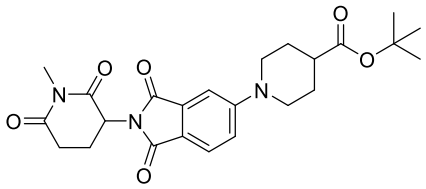
(CAS: 2505501-99-3)



During the reaction between the deprotected amine **48** and **46**, a side product consisting of the cleaved silyl ether was formed, presuambly from the fluoride release during the nucleophilic aromatic substitution reaction. This desired product was collected during the flash purification as stated above.

Yield (262 mg, 27%, 2 steps); mp 114–116 °C; R_f = 0.50 (EtOAc); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 1.24 – 1.33 (m, 2H), 1.77 (dd, *J* = 3.6, 13.7 Hz, 2H), 1.97 – 2.06 (m, 1H), 2.46 – 2.62 (m, 5H), 2.83 – 2.92 (m, 3H), 3.57 – 3.63 (m, 2H), 4.48 (t, *J* = 5.3 Hz, 1H), 5.08 (dd, *J* = 5.4, 13.0 Hz, 1H), 7.42 (d, *J* = 7.4 Hz, 1H), 7.67 (d, *J* = 11.5 Hz, 1H), 11.07 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.24, 28.62, 31.11, 38.07, 49.21, 50.08, 65.78, 112.01 (d, *J* = 25.2 Hz), 113.86, 122.93 (d, *J* = 9.7 Hz), 128.96, 146.06 (d, *J* = 8.8 Hz), 157.41 (d, *J* = 253.1 Hz), 166.37, 166.86, 170.05, 172.88; **LC-MS** (ESI) t_R = 4.75 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{25}H_{35}FN_3O_5Si$, 390.15; found, 390.2; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{25}H_{35}FN_3O_5Si$, 390.1460; found, 390.1446.

***tert*-Butyl 1-[2-(1-methyl-2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-5-yl]piperidine-4-carboxylate (50)**[18]

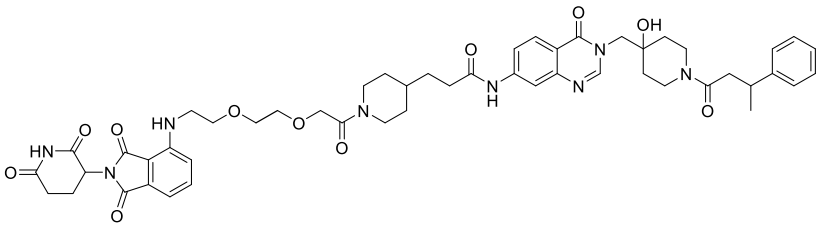


This compound was synthesized as we described previously.[18]

M. Synthesis: USP7-targeting PROTACs 8-20

General Procedure I: Assembly of final USP7 PROTACs. The corresponding modified USP7 ligang (0.1 mmol) was dissolved in dry CH_2Cl_2 (8 mL) and TFA (2 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. In a separate flask, the protected CRBN ligand-linker conjugate (0.1 mmol) was dissolved in dry CH_2Cl_2 (4 mL) and TFA (4 mL) was added. This mixture was heated at 40 °C for 2 h. After removal of the volatiles, the oily residue was further dried under high vacuum. Both deprotected precursors were dissolved in dry DMF (each 4 mL) and DIPEA (each 70 µL, 0.4 mmol) was added. HATU (42 mg, 0.11 mmol) was added to the acid, and after stirring for 5 minutes, the deprotected amine was added to the mixture. It was stirred at RT for 16 h, after which half-saturated NH_4Cl solution (50 mL) was added, and the product was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with 5% LiCl solution and brine (each 50 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*.

USP7-targeting PROTAC 8

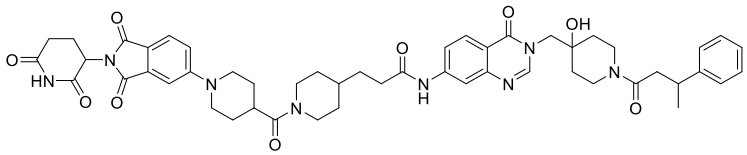


This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **39** (48 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 1 to 12% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (70 mg, 73%); mp >180 °C; R_f = 0.37 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.91 – 1.72 (m, 14H), 1.98 – 2.06 (m, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.46 – 2.63 (m, 4H), 2.81 – 2.95 (m, 3H), 3.11 – 3.28 (m, 2H), 3.46 (q, *J* = 5.6 Hz, 2H), 3.52 – 3.63 (m, 5H), 3.60 – 3.68 (m, 2H), 3.75

(d, *J* = 13.2 Hz, 1H), 3.86 – 3.95 (m, 2H), 3.97 – 4.04 (m, 2H), 4.11 (q, *J* = 13.5 Hz, 2H), 4.29 (d, *J* = 12.9 Hz, 1H), 4.89 (d, *J* = 5.9 Hz, 1H), 5.04 (dd, *J* = 5.5, 12.7 Hz, 1H), 6.58 (t, *J* = 5.9 Hz, 1H), 7.02 (d, *J* = 7.1 Hz, 1H), 7.10 – 7.17 (m, 2H), 7.20 – 7.28 (m, 4H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.62 (dd, *J* = 2.1, 8.7 Hz, 1H), 8.01 (d, *J* = 2.0 Hz, 1H), 8.06 (d, *J* = 8.8 Hz, 1H), 8.17 (d, *J* = 12.9 Hz, 1H), 10.28 (s, 1H), 11.05 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.02, 22.19, 22.31, 31.14, 31.45, 32.26, 33.97, 34.39, 34.53, 35.11, 35.21, 36.17, 36.37, 37.11, 40.40, 41.15, 41.25, 41.41, 41.92, 44.54, 48.74, 53.70, 69.00, 69.42, 69.46, 69.74, 69.94, 109.44, 110.83, 114.91, 116.64, 117.57, 118.49, 126.06, 126.10, 127.02, 127.03, 127.34, 128.33, 128.35, 132.25, 136.36, 144.55, 146.57, 146.71, 146.83, 149.14, 149.47, 160.31, 160.36, 167.00, 167.42, 169.09, 169.27, 170.17, 172.20, 172.90; **LC-MS** (ESI) t_R = 6.26 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{61}N_8O_{11}$, 961.45; found, 961.7; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{61}N_8O_{11}$, 961.4454; found, 961.4448.

USP7-targeting PROTAC 9

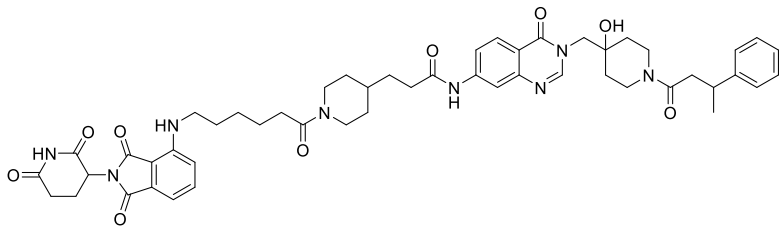


This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **29** (44 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 1 to 20% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (65 mg, 70%); mp 198–202 °C; R_f = 0.37 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.89 – 1.13 (m, 2H), 1.16 – 1.24 (m, 4H), 1.26 – 1.79 (m, 12H), 1.93 – 2.08 (m, 1H), 2.41 (t, *J* = 7.5 Hz, 2H), 2.49 – 2.61 (m, 4H), 2.80 – 3.25 (m, 8H), 3.57 – 3.70 (m, 1H), 3.86 – 4.07 (m, 6H), 4.37 (d, *J* = 12.7 Hz, 1H), 4.89 (d, *J* = 5.5 Hz, 1H), 5.05 (ddd, *J* = 1.7, 5.4, 12.8 Hz, 1H), 5.73 (d, *J* = 1.7 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.18 – 7.27 (m, 5H), 7.30 (t, *J* = 2.0 Hz, 1H), 7.60 – 7.67 (m, 2H), 8.02 (d, *J* = 2.0 Hz, 1H), 8.06 (dd, *J* = 1.6, 8.7 Hz, 1H), 8.17 (dd, *J* = 1.6, 12.9 Hz, 1H), 10.30 (s, 1H), 11.03 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.00, 22.18, 22.34, 27.56, 27.74, 31.12, 31.42, 31.59, 32.82, 33.98, 34.38, 34.52, 35.08, 35.20, 35.33, 36.15, 36.36, 37.00, 37.09, 41.13, 41.23, 41.50, 45.05,

46.86, 48.90, 53.69, 55.02, 69.40, 69.45, 107.90, 114.90, 116.63, 117.73, 118.49, 125.13, 126.04, 126.09, 127.00, 127.02, 127.34, 128.31, 128.34, 134.19, 144.55, 146.70, 146.82, 149.14, 149.47, 154.97, 160.30, 160.35, 167.09, 167.73, 169.26, 170.19, 172.03, 172.23, 172.90; **LC-MS** (ESI) t_R = 6.33 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{59}N_8O_9$, 927.44; found, 927.7; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{59}N_8O_9$, 927.4400; found, 927.4395.

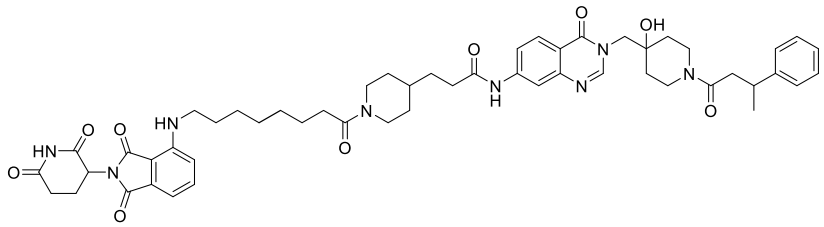
USP7-targeting PROTAC 10



This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **40** (44 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 1 to 12% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (71 mg, 76%); mp >180 °C; R_f = 0.35 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.90 – 1.08 (m, 2H), 1.11 – 1.62 (m, 16H), 1.68 (t, *J* = 14.2 Hz, 2H), 1.97 – 2.06 (m, 1H), 2.28 (t, *J* = 7.4 Hz, 2H), 2.36 – 2.65 (m, 6H), 2.82 – 2.97 (m, 3H), 3.11 – 3.38 (m, 5H), 3.63 (t, *J* = 12.8 Hz, 1H), 3.79 – 4.06 (m, 4H), 4.36 (d, *J* = 13.0 Hz, 1H), 4.89 (s, 1H), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 7.01 (d, *J* = 7.1 Hz, 1H), 7.08 (d, *J* = 8.5 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.20 – 7.28 (m, 4H), 7.56 (dd, *J* = 7.0, 8.6 Hz, 1H), 7.63 (dd, *J* = 2.1, 8.8 Hz, 1H), 8.00 – 8.09 (m, 2H), 8.17 (d, *J* = 12.9 Hz, 1H), 10.30 (s, 1H), 11.05 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.01, 22.19, 22.32, 24.79, 26.26, 28.71, 31.13, 31.47, 31.59, 32.43, 32.47, 33.99, 34.38, 34.52, 35.08, 35.24, 36.16, 36.37, 37.10, 40.40, 41.14, 41.28, 41.95, 45.24, 48.71, 53.70, 69.41, 69.46, 109.18, 110.52, 114.89, 116.63, 117.32, 118.50, 126.05, 126.10, 127.02, 127.34, 128.33, 132.35, 136.42, 144.56, 146.60, 146.70, 146.82, 149.12, 149.47, 160.31, 160.35, 167.44, 169.09, 169.27, 170.19, 170.31, 172.23, 172.91; **LC-MS** (ESI) t_R = 6.79 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{61}N_8O_9$, 929.46; found, 929.7; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{61}N_8O_9$, 929.4556; found, 929.4548.

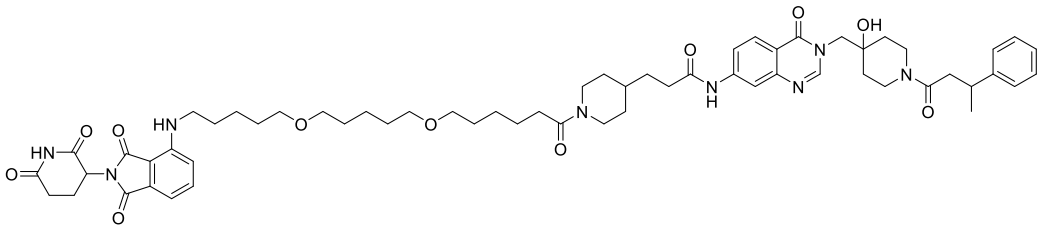
USP7-targeting PROTAC 11



This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **40** (47 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 1 to 20% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (83 mg, 87%); mp 150–154 °C; R_f = 0.40 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.88 – 1.10 (m, 2H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.22 – 1.62 (m, 17H), 1.68 (t, *J* = 14.8 Hz, 2H), 1.98 – 2.09 (m, 1H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.36 – 2.49 (m, 3H), 2.51 – 2.65 (m, 3H), 2.82 – 2.97 (m, 3H), 3.09 – 3.41 (m, 5H), 3.63 (t, *J* = 13.2 Hz, 1H), 3.82 (d, *J* = 13.5 Hz, 1H), 3.91 (d, *J* = 24.1 Hz, 2H), 4.00 (d, *J* = 13.9 Hz, 1H), 4.36 (d, *J* = 12.9 Hz, 1H), 4.90 (d, *J* = 5.9 Hz, 1H), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H), 6.49 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.1 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.20 – 7.28 (m, 4H), 7.56 (dd, *J* = 7.1, 8.5 Hz, 1H), 7.62 (dd, *J* = 2.1, 8.8 Hz, 1H), 8.02 (d, *J* = 1.9 Hz, 1H), 8.06 (d, *J* = 8.7 Hz, 1H), 8.17 (d, *J* = 12.8 Hz, 1H), 10.29 (s, 1H), 11.05 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.01, 22.19, 22.31, 25.00, 26.38, 28.72, 28.80, 28.89, 31.13, 31.48, 31.60, 32.48, 32.50, 33.99, 34.38, 34.52, 35.08, 35.24, 36.17, 36.37, 37.10, 40.40, 41.15, 41.26, 41.99, 45.25, 48.72, 53.70, 55.03, 69.41, 69.46, 109.19, 110.52, 114.91, 116.64, 117.32, 118.49, 126.05, 126.10, 127.02, 127.34, 128.33, 132.34, 136.41, 144.56, 146.61, 146.70, 146.82, 149.14, 149.46, 160.31, 160.36, 167.44, 169.12, 169.27, 170.20, 170.40, 172.23, 172.91; **LC-MS** (ESI) t_R = 7.32 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{53}H_{65}N_8O_9$, 957.49; found, 957.8; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{53}H_{65}N_8O_9$, 957.4869; found, 957.4848.

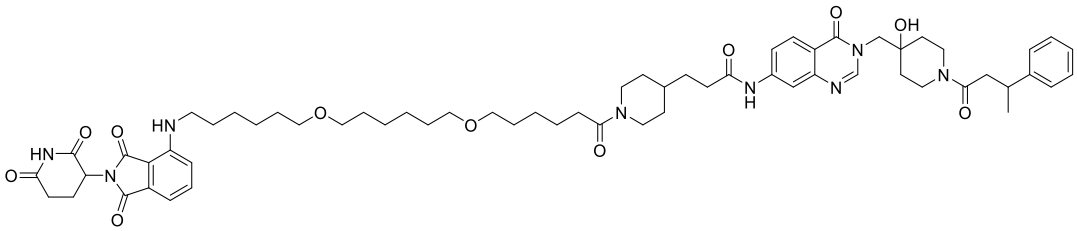
USP7-targeting PROTAC 12



This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **42** (62 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 1 to 20% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (95 mg, 86%); mp 114–118 °C; R_f = 0.37 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.86 – 1.11 (m, 2H), 1.12 – 1.61 (m, 30H), 1.68 (t, *J* = 14.5 Hz, 2H), 1.97 – 2.05 (m, 1H), 2.23 – 2.30 (m, 2H), 2.40 (t, *J* = 7.5 Hz, 2H), 2.43 – 2.66 (m, 5H), 2.80 – 2.99 (m, 3H), 3.10 – 3.22 (m, 2H), 3.31 (s, 8H), 3.63 (t, *J* = 13.3 Hz, 1H), 3.73 – 4.08 (m, 4H), 4.36 (d, *J* = 13.0 Hz, 1H), 4.89 (d, *J* = 6.0 Hz, 1H), 5.03 (dd, *J* = 5.4, 12.7 Hz, 1H), 6.48 (t, *J* = 6.0 Hz, 1H), 7.00 (d, *J* = 6.9 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.09 – 7.17 (m, 1H), 7.20 – 7.28 (m, 4H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.62 (dd, *J* = 2.1, 8.8 Hz, 1H), 7.99 – 8.08 (m, 2H), 8.17 (d, *J* = 12.8 Hz, 1H), 10.29 (s, 1H), 11.05 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 21.94, 22.00, 22.17, 22.30, 22.68, 25.59, 26.26, 28.80, 28.99, 29.19, 29.21, 29.29, 31.12, 31.47, 31.58, 32.30, 32.48, 33.98, 34.37, 34.51, 35.07, 35.23, 36.15, 36.36, 37.09, 40.39, 41.13, 41.25, 41.96, 45.21, 48.70, 53.68, 69.40, 69.45, 69.86, 69.97, 70.02, 109.19, 110.50, 114.89, 116.62, 117.27, 118.47, 126.04, 126.08, 127.01, 127.32, 128.32, 132.33, 136.38, 144.55, 146.58, 146.70, 146.81, 149.13, 149.44, 160.29, 160.34, 167.42, 169.09, 169.25, 170.16, 170.31, 172.19, 172.88; **LC-MS** (ESI) t_R = 8.21 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{61}H_{81}N_8O_{11}$, 1101.60; found, 1101.9; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{61}H_{81}N_8O_{11}$, 1101.6019; found, 1101.6015.

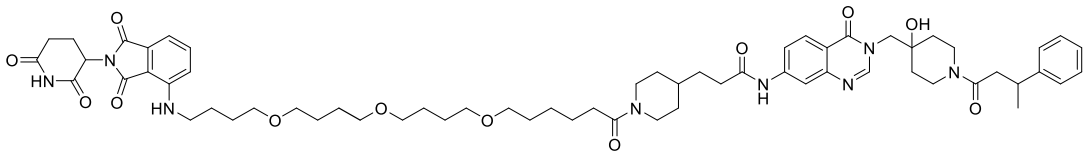
USP7-targeting PROTAC 13



This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **43** (64 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (49 mg, 43%); mp 94–98 °C; R_f = 0.50 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.87 – 1.09 (m, 2H), 1.11 – 1.63 (m, 34H), 1.64 – 1.73 (m, 2H), 1.98 – 2.05 (m, 1H), 2.20 – 2.29 (m, 2H), 2.40 (t, *J* = 7.5 Hz, 2H), 2.42 – 2.65 (m, 5H), 2.81 – 2.99 (m, 3H), 3.12 – 3.19 (m, 2H), 3.20 – 3.33 (m, 8H), 3.59 – 3.68 (m, 1H), 3.78 – 4.08 (m, 4H), 4.36 (d, *J* = 12.8 Hz, 1H), 4.89 (d, *J* = 7.5 Hz, 1H), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H), 6.49 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.1 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 1H), 7.10 – 7.16 (m, 1H), 7.24 (t, *J* = 5.8 Hz, 4H), 7.56 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.62 (dd, *J* = 2.1, 8.7 Hz, 1H), 8.00 – 8.08 (m, 2H), 8.17 (d, *J* = 15.5 Hz, 1H), 10.30 (s, 1H), 11.06 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.02, 22.21, 22.32, 24.94, 25.62, 25.72, 25.75, 26.28, 28.82, 29.26, 29.32, 29.37, 31.13, 31.50, 31.61, 32.49, 32.52, 33.99, 34.38, 34.53, 35.08, 35.20, 35.26, 36.16, 36.37, 37.10, 40.24, 40.39, 41.13, 41.23, 41.27, 41.97, 45.25, 48.70, 53.70, 69.41, 69.46, 69.97, 70.03, 70.06, 109.19, 110.51, 114.89, 116.63, 117.29, 118.47, 126.05, 126.10, 127.04, 127.34, 128.33, 128.35, 132.34, 136.40, 144.56, 146.59, 146.72, 146.84, 149.15, 149.47, 160.30, 160.35, 167.44, 169.10, 169.25, 170.19, 170.33, 172.21, 172.91; **LC-MS** (ESI) t_R = 8.39 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{63}H_{85}N_8O_{11}$, 1129.63; found, 1130.0; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{63}H_{85}N_8O_{11}$, 1129.6332; found, 1129.6320.

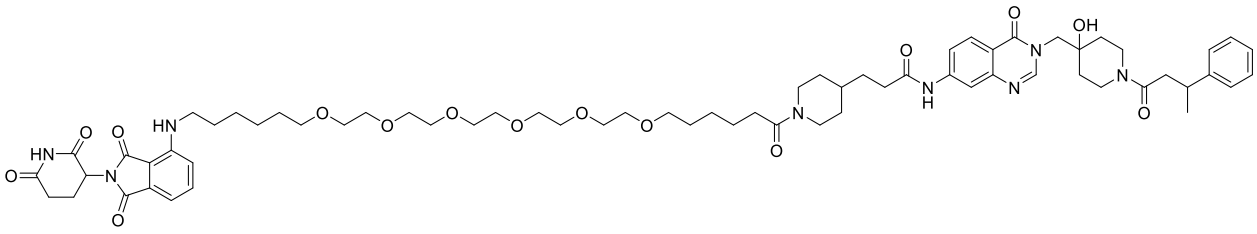
USP7-targeting PROTAC 14



This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **44** (66 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (61 mg, 53%); mp 104–108 °C; R_f = 0.20 (7% MeOH/CH_2Cl_2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.87 – 1.08 (m, 2H), 1.10 – 1.65 (m, 30H), 1.64 – 1.73 (m, 2H), 1.98 – 2.05 (m, 1H), 2.21 – 2.29 (m, 2H), 2.40 (t, *J* = 7.5 Hz, 2H), 2.43 – 2.63 (m, 5H), 2.81 – 2.98 (m, 3H), 3.10 – 3.25 (m, 2H), 3.26 – 3.40 (m, 12H), 3.59 – 3.68 (m, 1H), 3.78 – 4.05 (m, 4H), 4.36 (d, *J* = 12.9 Hz, 1H), 4.89 (d, *J* = 7.5 Hz, 1H), 5.03 (dd, *J* = 5.5, 12.8 Hz, 1H), 6.54 (t, *J* = 6.0 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.10 – 7.16 (m, 1H), 7.24 (t, *J* = 5.8 Hz, 4H), 7.56 (dd, *J* = 7.0, 8.6 Hz, 1H), 7.62 (dd, *J* = 2.1, 8.7 Hz, 1H), 8.00 – 8.08 (m, 2H), 8.17 (d, *J* = 15.4 Hz, 1H), 10.30 (s, 1H), 11.06 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.03, 22.21, 22.32, 24.94, 25.75, 26.24, 26.66, 29.26, 31.13, 31.50, 31.61, 32.49, 32.52, 33.99, 34.38, 34.53, 35.08, 35.20, 35.25, 36.16, 36.37, 37.10, 40.24, 40.39, 41.13, 41.24, 41.27, 41.78, 45.25, 48.70, 53.70, 69.41, 69.46, 69.70, 69.86, 69.91, 69.95, 70.01, 109.22, 110.51, 114.89, 116.63, 117.31, 118.47, 126.05, 126.10, 127.03, 127.34, 128.33, 128.35, 132.37, 136.36, 144.56, 146.56, 146.72, 146.84, 149.15, 149.47, 160.30, 160.35, 167.44, 169.07, 169.25, 170.19, 170.33, 172.21, 172.91; **LC-MS** (ESI) t_R = 7.72 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{63}H_{85}N_8O_{12}$, 1145.63; found, 1145.9; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{63}H_{85}N_8O_{12}$, 1145.6282; found, 1145.6268.

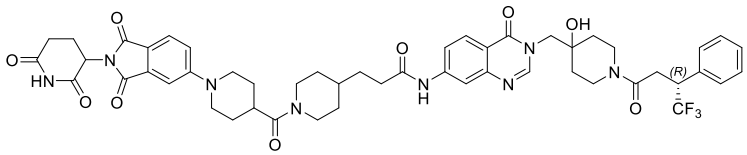
USP7-targeting PROTAC 15



This compound was prepared using the General Procedure **I**, USP7 ligand **6** (66 mg) and CRBN building block **45** (76 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (62 mg, 50%); mp 86–90 °C; R_f = 0.42 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.88 – 1.09 (m, 2H), 1.17 – 1.61 (m, 26H), 1.64 – 1.73 (m, 2H), 1.99 – 2.05 (m, 1H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.40 (t, *J* = 7.5 Hz, 2H), 2.42 – 2.64 (m, 4H), 2.82 – 2.97 (m, 3H), 3.15 (q, *J* = 7.6 Hz, 1H), 3.27 (q, *J* = 6.7 Hz, 2H), 3.35 (q, *J* = 6.6 Hz, 4H), 3.42 – 3.46 (m, 4H), 3.49 (s, 16H), 3.58 – 3.67 (m, 1H), 3.79 – 4.06 (m, 4H), 4.36 (d, *J* = 12.8 Hz, 1H), 4.89 (d, *J* = 7.5 Hz, 1H), 5.03 (dd, *J* = 5.4, 12.8 Hz, 1H), 6.50 (t, *J* = 5.9 Hz, 1H), 7.00 (d, *J* = 7.0 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.10 – 7.17 (m, 1H), 7.21 – 7.28 (m, 4H), 7.56 (dd, *J* = 7.0, 8.6 Hz, 1H), 7.60 – 7.65 (m, 1H), 8.00 – 8.08 (m, 2H), 8.17 (d, *J* = 15.6 Hz, 1H), 10.30 (s, 1H), 11.06 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.02, 22.20, 22.31, 24.92, 25.55, 25.67, 26.29, 28.82, 29.23, 29.28, 31.13, 31.49, 31.60, 32.48, 32.52, 33.98, 34.38, 34.52, 35.08, 35.20, 35.25, 36.16, 36.37, 37.09, 40.23, 40.39, 41.13, 41.25, 41.96, 45.23, 48.70, 69.41, 69.46, 69.63, 69.95, 70.37, 70.42, 109.18, 110.52, 114.88, 116.63, 117.30, 118.47, 126.05, 126.10, 127.03, 127.34, 128.32, 128.35, 132.34, 136.41, 144.56, 146.59, 146.72, 146.84, 149.15, 149.47, 160.30, 160.35, 167.44, 169.10, 169.25, 170.20, 170.31, 172.21, 172.92; **LC-MS** (ESI) t_R = 7.22 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{67}H_{93}N_8O_{15}$, 1249.68; found, 1250.1; **HRMS** (ESI) *m/2* $[M + H]^+$ calcd for $C_{67}H_{94}N_8O_{15}$, 625.3414; found, 625.3403.

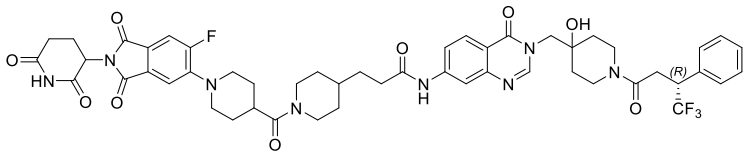
USP7-targeting PROTAC 16



This compound was prepared using the General Procedure **I**, USP7 ligand **5** (71 mg) and CRBN building block **29** (44 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as colorless solid.

Yield (69 mg, 62%); mp 208–210 °C; R_f = 0.39 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.88 – 1.84 (m, 16H), 1.97 – 2.04 (m, 1H), 2.39 – 2.44 (m, 2H), 2.46 – 2.61 (m, 2H), 2.80 – 3.29 (m, 8H), 3.72 – 3.87 (m, 2H), 3.88 – 4.13 (m, 7H), 4.37 (d, *J* = 12.8 Hz, 1H), 4.92 (s, 1H), 5.05 (dd, *J* = 5.4, 12.8 Hz, 1H), 7.22 (dd, *J* = 2.3, 8.8 Hz, 1H), 7.25 – 7.37 (m, 4H), 7.37 – 7.41 (m, 2H), 7.60 – 7.67 (m, 2H), 8.02 (d, *J* = 2.1 Hz, 1H), 8.06 (dd, *J* = 3.5, 8.7 Hz, 1H), 8.17 (d, *J* = 13.6 Hz, 1H), 10.32 (d, *J* = 1.7 Hz, 1H), 11.05 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.35, 27.58, 27.76, 31.14, 31.44, 31.63, 32.85, 33.99, 34.31, 34.36, 35.01, 35.08, 35.35, 37.03, 37.47, 37.55, 40.23, 41.01, 41.51, 45.07, 45.22 – 45.79 (m), 46.89, 48.91, 53.66, 53.74, 69.34, 69.43, 107.93, 114.91, 116.65, 117.75, 118.50, 125.17, 124.26 – 130.53 (m), 127.36, 128.18, 128.54, 129.34, 134.21, 134.86, 134.92, 144.58, 149.16, 149.49, 154.99, 160.30, 160.37, 166.75 (d, *J* = 12.6 Hz), 167.11, 167.76, 170.23, 172.04, 172.26, 172.95; **LC-MS** (ESI) t_R = 6.36 min, 98% purity, *m*/*z* $[M + H]^+$ calcd for $C_{51}H_{56}F_3N_8O_9$, 981.41; found, 981.7; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{51}H_{56}F_3N_8O_9$, 981.4117; found, 981.4079.

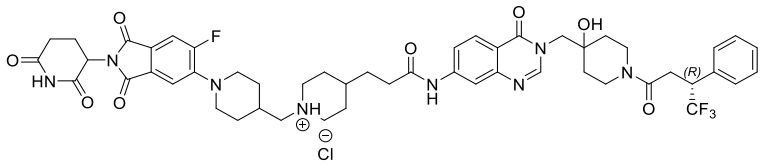
USP7-targeting PROTAC 17



This compound was prepared using the General Procedure **I**, USP7 ligand **5** (71 mg) and CRBN building block **47** (46 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (68 mg, 68%); mp 252–254 °C; R_f = 0.41 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.91 – 1.80 (m, 16H), 1.99 – 2.06 (m, 1H), 2.39 – 2.44 (m, 2H), 2.50 – 2.62 (m, 2H), 2.82 – 2.92 (m, 3H), 2.94 – 3.02 (m, 3H), 3.07 – 3.28 (m, 3H), 3.62 (d, *J* = 12.0 Hz, 2H), 3.70 – 4.13 (m, 6H), 4.40 (d, *J* = 12.8 Hz, 1H), 4.92 (s, 1H), 5.09 (dd, *J* = 5.4, 12.9 Hz, 1H), 7.25 – 7.36 (m, 3H), 7.36 – 7.44 (m, 3H), 7.60 – 7.65 (m, 1H), 7.69 (d, *J* = 11.4 Hz, 1H), 8.02 (d, *J* = 2.1 Hz, 1H), 8.06 (dd, *J* = 3.5, 8.7 Hz, 1H), 8.17 (d, *J* = 13.5 Hz, 1H), 10.32 (s, 1H), 11.08 (s, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 22.24, 28.30, 28.49, 31.11, 31.44, 31.62, 32.84, 33.99, 34.31, 34.36, 35.01, 35.08, 35.34, 36.87, 37.46, 37.55, 40.24, 41.00, 41.52, 45.07, 45.22 – 45.72 (m), 49.21, 49.49, 53.66, 53.73, 69.34, 69.43, 112.02, 112.18, 113.74, 113.77, 114.90, 116.64, 118.49, 123.03, 123.10, 124.35 – 130.30 (m), 127.36, 128.18, 128.53, 128.97, 129.34, 134.86, 134.93, 144.58, 145.74, 145.80, 149.16, 149.49, 156.55, 158.22, 160.30, 160.36, 166.35, 166.74 (d, *J* = 12.1 Hz), 166.84, 170.04, 172.06, 172.25, 172.89; **LC-MS** (ESI) t_R = 6.50 min, 99% purity, *m*/*z* $[M + H]^+$ calcd for $C_{51}H_{55}F_4N_8O_9$, 999.40; found, 999.7; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{51}H_{55}F_4N_8O_9$, 999.4023; found, 999.3992.

USP7-targeting PROTAC 18

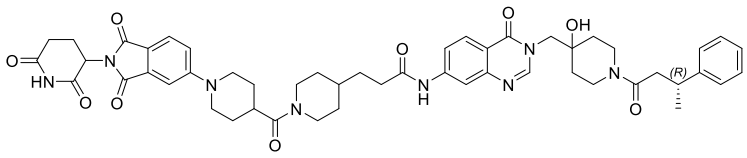


USP7 ligand **5** (143 mg, 0.2 mmol) was dissolved in dry CH_2Cl_2 (8 mL) and TFA (2 mL) and stirred at RT for 2 h. After evaporation of all volatiles and coevaporation with dry CH_2Cl_2 (2 × 5 mL), it was further dried under high vacuum. The TFA salt was suspended in dry CH_2Cl_2 (5 mL) and subjected to a short silica gel column (2 × 10 cm), and eluated with a mixture of 7N NH_3 in MeOH/CH_2Cl_2 (3:7). The collected fraction was evaporated *in vacuo*. Alcohol-functionalized CRBN ligand **49b** (78 mg, 0.2 mmol) was dissolved in dry CH_2Cl_2 and TEMPO (6 mg, 0.04 mmol) and BAIB (71 mg, 0.22 mmol) were added. This mixture was stirred at RT for 16 h, after which the crude deprotected amine, dissolved in dry CH_2Cl_2 (10 mL) was added. The combined mixture was stirred at RT for 30 min, followed by the addition of sodium triacetoxyborohydride (85 mg, 0.4 mmol). Subsequently, it was stirred at RT for 3 h. The mixture was directly subjected to a silica gel column and the compound was eluated with 7N NH_3 in MeOH/CH_2Cl_2 (1:9). Product-enriched fractions were pooled and subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 4 to 15% MeOH in EtOAc +1% Et_3N) to give a yellow solid after evaporation. The solid material was dissolved in dry dioxane (4 mL) and 4N HCl in dioxane (4 mL) was added. After stirring at RT for 1 h, the solvent was evaporated and the material was suspended in dry Et_2O (5 mL). The suspension was filtered, and the yellow solid was dried *in vacuo* to give the title HCl salt of PROTAC **18**.

Yield (127 mg, 64%); mp >252 °C; R_f = 0.33 (10% 7N NH_3 in MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 1.15 – 2.07 (m, 16H), 2.43 – 2.63 (m, 4H), 2.78 – 3.00 (m, 8H), 3.07 – 3.28 (m, 2H), 3.49 (d, *J* = 11.5 Hz, 2H), 3.61 (d, *J* = 12.2 Hz, 2H), 3.75 (s, 1H), 3.86 – 4.06 (m, 6H), 5.09 (dd, *J* = 5.4, 12.9 Hz, 1H), 7.26 – 7.47 (m, 7H), 7.69 (d, *J* = 11.4 Hz, 1H), 7.75 (dd, *J* = 2.0, 8.9 Hz, 1H), 8.09 (dd, *J* = 3.5, 8.7 Hz, 1H), 8.14 (d, *J* = 2.1 Hz, 1H), 8.52 (d, *J* = 15.1 Hz, 1H), 9.93 (s, 1H), 10.72 (s, 1H), 11.07 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.22, 28.56, 29.93, 30.29, 31.00, 31.10, 31.65, 32.75, 33.74, 34.22, 34.27, 34.95, 35.01, 37.43, 37.52, 40.97, 45.03 – 45.84 (m), 49.22, 49.49, 52.65, 53.88, 53.96, 61.44, 65.03, 66.50, 69.22, 69.32, 111.97, 112.17, 112.97, 113.95, 115.98, 119.00, 123.15, 123.22, 127.64, 128.17, 128.46 (dd, *J* = 279.9, 289.5 Hz), 128.54, 128.93, 129.32, 134.88, 145.06, 145.65, 145.72, 146.42, 150.12, 156.41, 158.42, 159.75, 159.80, 166.32, 166.75

(d, *J* = 11.7 Hz), 166.82; **LC-MS** (ESI) t_R = 6.50 min, 99% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{57}F_4N_8O_8$, 985.42; found, 985.7; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{57}F_4N_8O_8$, 985.4230; found, 985.4186.

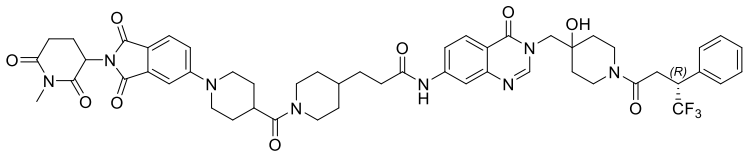
USP7-targeting PROTAC 19 (CST967)



This compound was prepared using the General Procedure **I**, USP7 ligand **7** (66 mg) and CRBN building block **29** (44 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 1 to 20% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (62 mg, 67%); mp 216–220 °C; R_f = 0.41 (10% MeOH/CH_2Cl_2); **^{1}H NMR** (500 MHz, DMSO-d_6) δ 0.91 – 1.10 (m, 2H), 1.13 – 1.24 (m, 4H), 1.25 – 1.44 (m, 3H), 1.47 – 1.79 (m, 10H), 2.00 (ddd, *J* = 3.0, 5.6, 10.5 Hz, 1H), 2.39 – 2.47 (m, 2H), 2.49 – 2.64 (m, 4H), 2.82 – 3.25 (m, 8H), 3.63 (t, *J* = 13.1 Hz, 1H), 3.86 – 4.07 (m, 6H), 4.37 (d, *J* = 12.8 Hz, 1H), 4.91 (s, 1H), 5.05 (dd, *J* = 5.4, 12.9 Hz, 1H), 7.02 – 7.17 (m, 2H), 7.22 – 7.25 (m, 4H), 7.30 (d, *J* = 2.2 Hz, 1H), 7.61 – 7.67 (m, 2H), 8.01 – 8.09 (m, 2H), 8.19 (d, *J* = 12.8 Hz, 1H), 10.36 (s, 1H), 11.03 (s, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 22.00, 22.18, 22.33, 27.56, 27.74, 31.12, 31.42, 31.60, 32.82, 33.97, 34.37, 34.51, 35.07, 35.20, 35.32, 36.15, 36.35, 37.00, 37.09, 41.13, 41.23, 41.49, 45.04, 46.86, 48.89, 53.66, 69.40, 69.45, 107.90, 114.88, 116.62, 117.73, 118.48, 125.13, 126.04, 126.08, 127.00, 127.02, 127.32, 128.31, 128.33, 134.19, 144.58, 146.70, 146.82, 149.12, 149.47, 154.97, 160.29, 160.34, 167.08, 167.73, 169.24, 170.18, 172.02, 172.24, 172.89; **LC-MS** (ESI) t_R = 6.12 min, 98% purity, *m/z* $[M + H]^+$ calcd for $C_{51}H_{59}N_8O_9$, 927.44; found, 927.7; **HRMS** (ESI) *m/z* $[M + H]^+$ calcd for $C_{51}H_{59}N_8O_9$, 927.4400; found, 927.4376.

USP7-targeting PROTAC 20 (*negative control*)



This compound was prepared using the General Procedure **I**, USP7 ligand **5** (71 mg) and CRBN building block **50** (46 mg). The crude product was by subjected to purifaction by flash chromatography on spherical silica gel (25 g, 15 µm, gradient from 0 to 10% MeOH in CH_2Cl_2) to give the title compound as yellow solid.

Yield (47 mg, 47%); mp 208–210 °C; R_f = 0.39 (7% MeOH/CH_2Cl_2); **^{1}H NMR** (600 MHz, DMSO-d_6) δ 0.87 – 1.83 (m, 16H), 1.96 – 2.11 (m, 2H), 2.39 – 2.44 (m, 2H), 2.46 – 2.59 (m, 1H), 2.69 – 2.99 (m, 5H), 3.00 (s, 3H), 3.02 – 3.28 (m, 4H), 3.56 – 3.89 (m, 2H), 3.89 – 4.13 (m, 6H), 4.37 (d, *J* = 12.7 Hz, 1H), 4.92 (s, 1H), 5.12 (dd, *J* = 5.4, 13.0 Hz, 1H), 7.23 (dd, *J* = 2.4, 8.6 Hz, 1H), 7.25 – 7.36 (m, 4H), 7.36 – 7.42 (m, 2H), 7.60 – 7.67 (m, 2H), 8.03 (d, *J* = 2.0 Hz, 1H), 8.06 (dd, *J* = 3.5, 8.7 Hz, 1H), 8.17 (d, *J* = 13.5 Hz, 1H), 10.32 (d, *J* = 1.7 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-d_6) δ 21.54, 26.74, 27.58, 27.76, 30.83, 31.29, 31.44, 31.62, 32.85, 33.98, 34.30, 34.36, 35.00, 35.08, 35.34, 37.02, 37.45, 37.54, 40.24, 41.00, 41.51, 45.06, 45.21 – 45.75 (m), 46.87, 49.48, 53.65, 53.73, 55.05, 69.33, 69.43, 107.93, 114.90, 116.64, 117.70, 117.78, 118.49, 125.18, 124.31 – 129.97 (m), 127.36, 128.17, 128.53, 129.34, 134.20, 134.89, 144.57, 149.16, 149.49, 155.00, 160.29, 160.36, 166.74 (d, *J* = 12.1 Hz), 167.09, 167.75, 169.99, 171.94, 172.03, 172.25; **LC-MS** (ESI) t_R = 6.77 min, 98% purity, *m*/*z* $[M + H]^+$ calcd for $C_{52}H_{58}F_3N_8O_9$, 995.43; found, 995.7; **HRMS** (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{52}H_{58}F_3N_8O_9$, 995.4273; found, 995.4243.

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