Supporting Information

A high affinity pan-PI3K binding module supports selective targeted protein degradation of PI3Kα

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Abstract: Class I phosphoinositide 3-kinases (PI3Ks) control cellular growth, but are also essential in insulin signaling and glucose homeostasis. Pan-PI3K inhibitors thus generate substantial adverse effects, a reality that has plagued drug development against this target class. We present here evidence that a high affinity binding module with the capacity to target all class I PI3K isoforms can facilitate selective degradation of the most frequently mutated class I isoform, PI3Kα, when incorporated into a cereblon-targeted (CRBN) degrader. A systematic proteomics study of linker variations guided the fine tuning of linker features to optimize degrader selectivity and potency. Our work resulted in the creation of WJ112-14, a PI3Kα-specific nanomolar degrader that should serve as an important research tool for studying PI3K biology. Given the toxicities observed in the clinic with unselective PI3Kα inhibitors, the results here offer a new approach toward selectively targeting this frequently mutated oncogenic driver.

DOI:

Experimental Procedures and Supplementary Figures/Tables.

Table of Contents

DataWarrior calculated properties of compound series

Name	Total Molweight	cLogP	cLogS	Druglikeness	Polar Surface Area
WJ111_11	847.882	1.7351	-6.663	-1.1412	251.17
WJ200_12	861.909	2.1895	-6.933	-1.1412	251.17
WJ201-13	875.936	2.6439	-7.203	-1.1412	251.17
WJ112_14	889.963	3.0983	-7.473	-1.1412	251.17
WJ202-15	903.99	3.5527	-7.743	-1.1412	251.17
WJ208-16	918.017	4.0071	-8.013	-1.1412	251.17
WJ209-17	932.043	4.4615	-8.283	-1.1412	251.17
WJ204-12	931.975	2.7842	-7.413	4.2325	233.59
WJ213-14	916.989	2.08	-5.87	2.2841	245.62
WJ214-14	884.943	4.0663	-8.408	-4.9998	239.14
WJ203-14	1047.29	5.3204	-8.757	-7.2311	278.39
WJ117-16	937.916	-0.7096	-5.328	-6.5193	295.93
Li_2018_D	884.899	3.1483	-5.561	-4.1235	234.26
WJ112-14-Me	903.99	3.3512	-7.111	-1.1039	242.38

Table S1 *Calculated properties for compound series (DataWarrior).*

Cell Culture – Procedure 1

MCF7 cells (RRID: CVCL_0031, human, female) were obtained from DSMZ (ACC 115) and grown in DMEM (Sigma Aldrich, D5796) supplemented with 10 % FCS (BioConcept, 2-01F00-I) and 1 % Penicillin-Streptomycin (BioConcept, 4-01F00-H). MCF7 cells were passaged by trypsination with 0.05 % trypsin (Gibco, 25300053). Cells were kept in a humidified incubator at 37 °C and 5 % CO_2.

Western Blotting – Procedure 1

Western blots presented or used for Figure 2E, 3B, 3C, 3D, 3F, 4B and all blots exhibited in this section refer to the western blotting – Procedure 1.
Cells were seeded at 800k in 2 ml complete growth medium in 6 well plates and grown for 24 h. Cells were treated from DMSO stocks at indicated concentrations. Final DMSO concentration was kept below 1 %. Cells were treated for the indicated time after which cells were washed twice with PBS (Sigma-Aldrich, D8537) and then harvested by scraping (Cell Scrapers, Sarstedt, 83.3951) and resuspended in PBS. Cells were collected by centrifugation (15 min, 8.8 krcf, 4 °C) and the supernatant was removed by suction. Cell pellets were stored at -80 °C until further processing.
Cell pellets were resuspended in RIPA lysis buffer (50 mM Tris at pH 8, 150 mM NaCl, 1 % Triton X-100, 0.25 % sodium deoxycholate, 0.1 % SDS, supplemented with cOmplete Mini Protease Inhibitor (Roche) and 1 mM sodium orthovanadate (Sigma Aldrich)), left on ice for 30 min and centrifuged at 16 krcf and 4 °C for 15 min. The supernatant was collected, and protein concentration was measured (DC Protein Assay, Bio-Rad). Aliquots of 50 µg of protein were combined with 3X loading dye (New England BioLabs, B7703S) and heated to 98 °C for 5 min shaking at 300 rpm. The samples were separated by 8 % SDS-PAGE applying 80 – 120 Volt. Analytes were transferred onto nitrocellulose membranes using a semi-dry blotting apparatus (Trans-Blot Turbo with corresponding transfer packs, BioRad). Membranes were blocked with Intercept® (TBS) Blocking Buffer (LI-COR) for 1 h at room temperature while shaking. Primary antibodies used for incubation at 4 °C overnight: anti-p110a (ref: Hu Q, Klippel A, Muslin A J, Fantl W J, Williams L T. Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase. Science. 1995; 268: 100–10), anti-p110b (#3011, CST), anti-mTOR (#2983, CST), anti-alpha Tubulin (ab7291, abcam). The blots incubated with secondary antibodies using anti-Mouse (Licor, IRDye® 680RD Goat anti-Mouse IgG, 1:10000 dilution) or anti-Rabbit secondary antibody (Licor, IRDye® 800CW Goat anti-Rabbit IgG, 1:10000 dilution) and the signals analyzed using LI-COR Odyssey CLx infrared scanner and processed using LI-COR image Studio software.

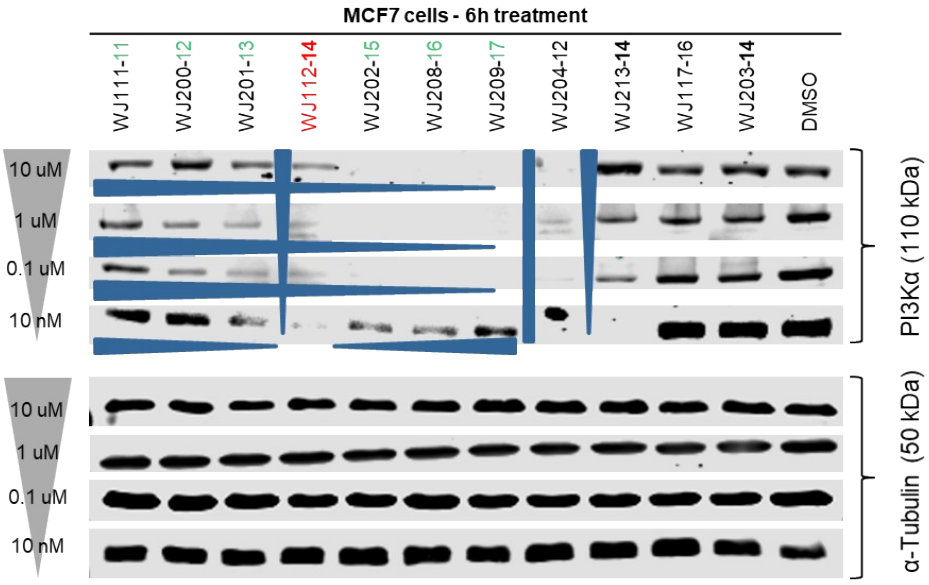


Figure S1 *Western blots used to create the bar plot in Figure 2E. All compounds were analyzed by WB in biological duplicates at their best concentration and at least in triplicates for the two lead compounds WJ112-14 and WJ213-14.*

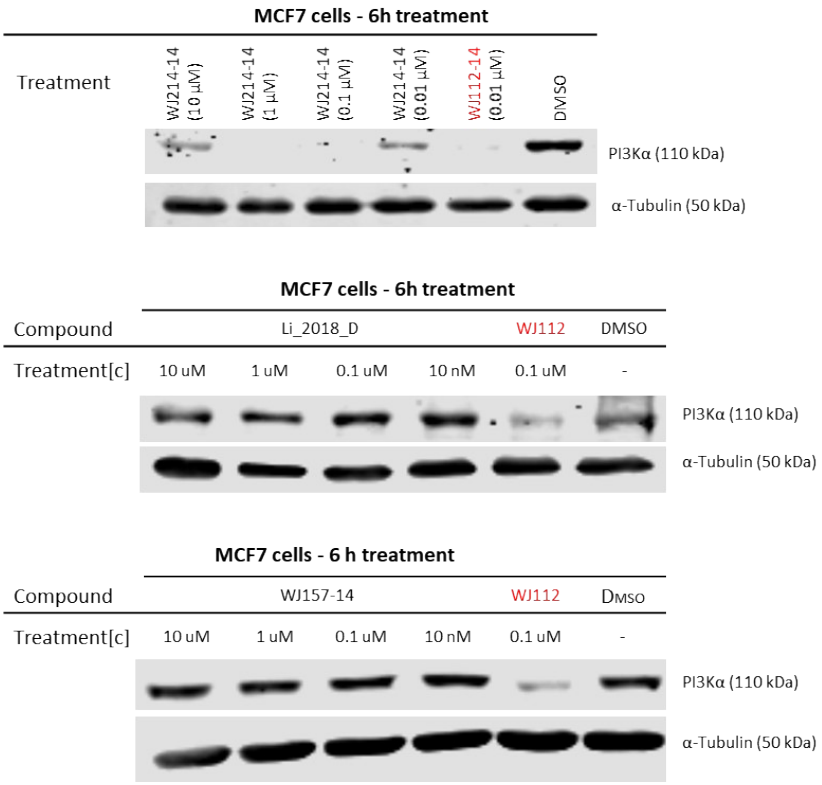


Figure S2 *Western blots used to create the bar plot in the Figure 2E. All compounds were analyzed by WB in biological duplicates at their best concentration and at least in triplicates for the two lead compounds WJ112-14 and WJ213-14.*

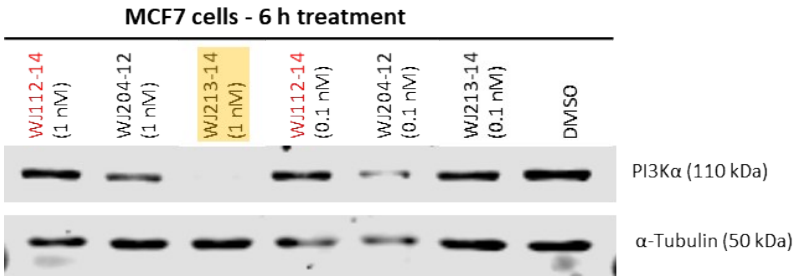


Figure S3 *Western blot to evaluate the degrader that is active at the lowest concentration.*

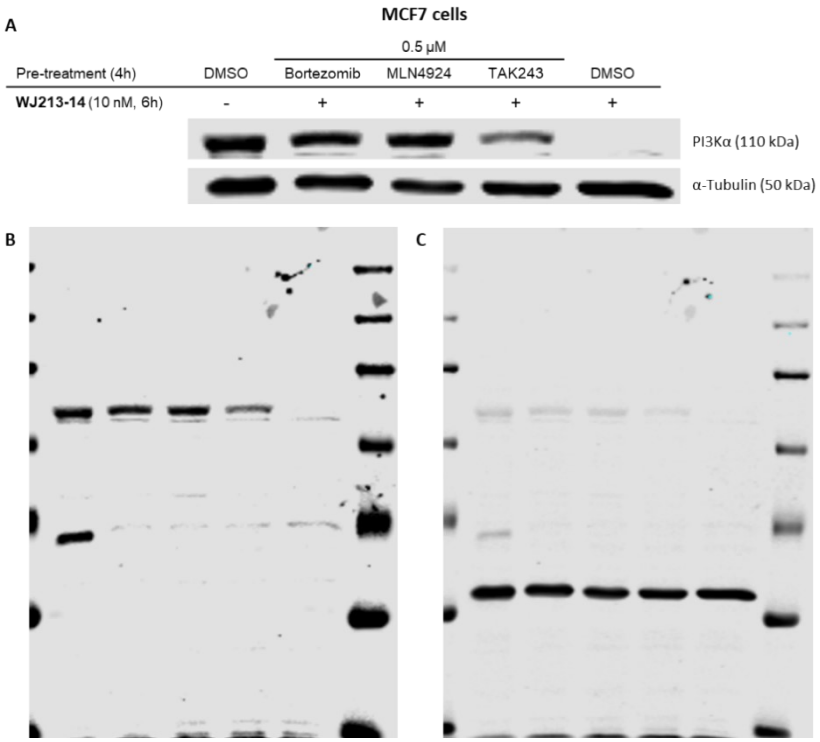


Figure S4 A) *Mechanistic study, treatment with WJ213-14 and UPP inhibitors in MCF7 cells. B) PI3Kα staining full membrane. C) αTubulin staining full membrane.*

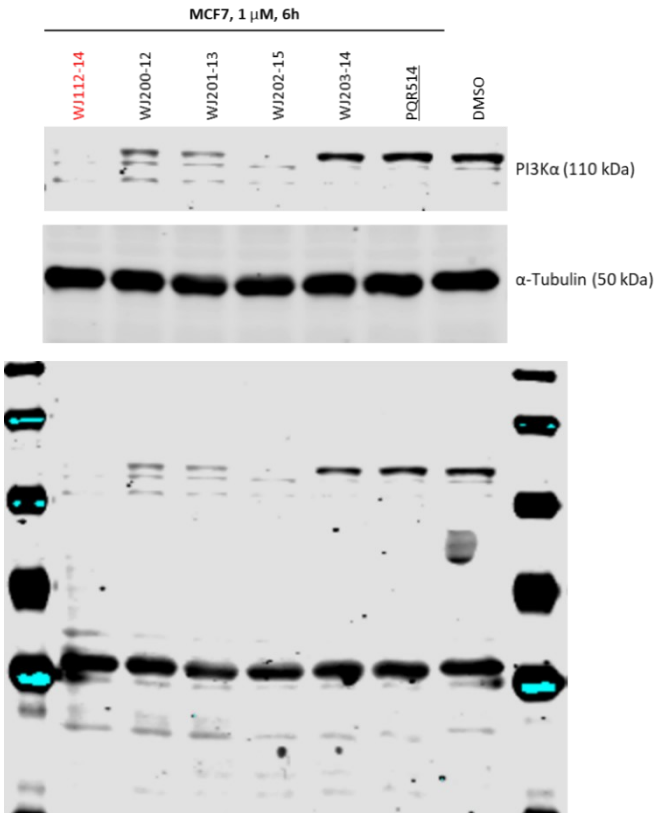


Figure S5 *Western blots, signals for PI3Kα and αTubulin, full membrane. Compound treatments in MCF7 cells for 6 h at 1 µM compared to PI3K inhibitor PQR514.*

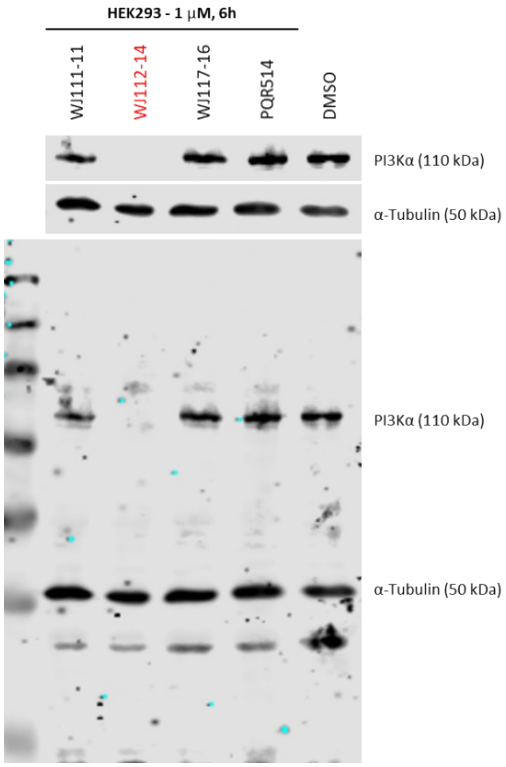


Figure S6 *Western blot stained for PI3Kα and αTubulin, full membrane. Compound treatments in HEK293 cells for 6 h at 1 µM compared to PI3K inhibitor PQR514.*

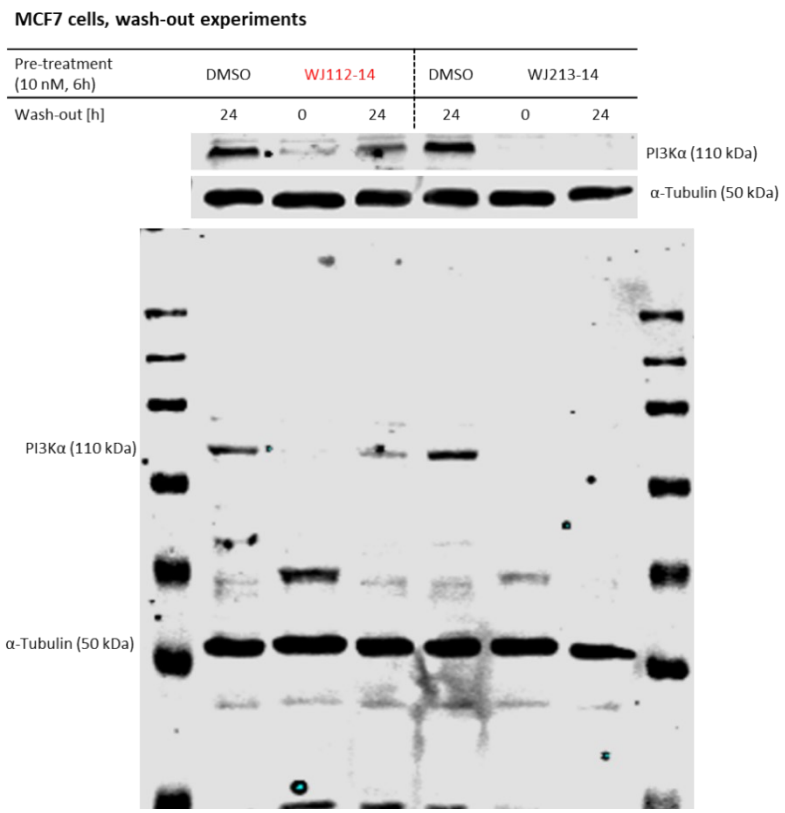


Figure S7 *Western blot stained for PI3Kα and αTubulin, full membrane. 24 h wash-out experiment upon WJ112-14 and WJ213-14 treatments.*

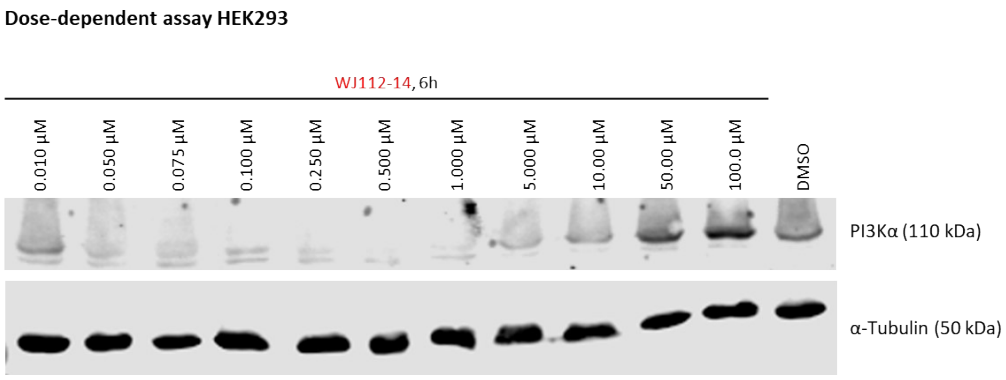


Figure S8 *Western blot stained for PI3Kα and αTubulin. Dose-dependent study with strong Hook-effect in HEK293 cells upon 6 hours treatment with WJ112-14.*

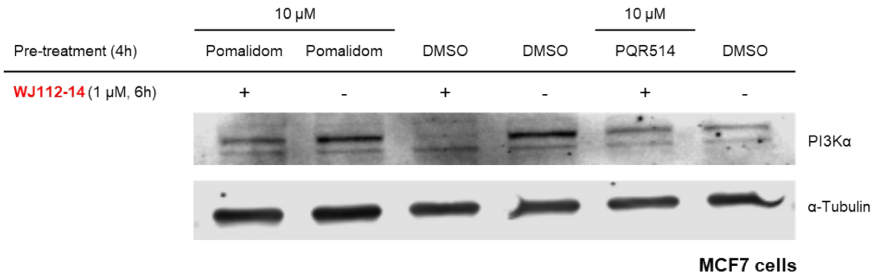


Figure S9 *In-cell analysis of target engagement (CRBN and PI3Kα). Blocking the enzymes with inhibitors rescues the PI3Kα signal in western blot assays.*

Western Blotting – Procedure 2

Western blots presented or used for Figure 4C, 4D, 5A and all blots exhibited in this section refer to the Western blotting – Procedure 2.

Unless indicated differently, inhibitor treatments were performed in 6-well dishes and on cell cultures that had reached a final density of no more but 80 - 90 % confluence at the time of harvest. For harvest, wells were first rinsed with 2x 2 mL of cold D-PBS buffer and cells detached from the growth support by scraping at cold. Cells were centrifuged (5 min, 100 rcf, 4 °C) and the resultant pellet flash-frozen and stored at minus 80 °C for later use. For western analyses, pellets were disrupted in 1x cold Lysis Buffer (#9803, Cell Signaling Technologies (CST), Danvers, MA, USA) supplemented with Protease/Phosphatase Inhibitor Cocktail (#78442, Thermo Fisher, Waltham, MA, USA) and cleared by centrifugation (15 min, 21 000 rcf, 4 °C). Protein content was determined by Bradford using a Coomassie Plus Protein Assay Reagent (Thermo Scientific, #1856210) and following the manufacturers' instructions. Cleared protein lysates were denatured with Laemmli buffer and an equivalent of 10 µg total protein per lane separated over 8 – 12 % (depending on the analyte's size) bis-acrylamide (#161-0148, BioRad, Hercules, CA, USA) gels by discontinuous SDS-PAGE. Analytes were transferred onto nitrocellulose membranes (#10600021, Amersham, GE Healthcare Life Sciences, Chalfont St. Giles, UK) using a semi-dry blotting apparatus (Trans-Blot Turbo with corresponding transfer packs, BioRad). Membranes were blocked for 1 h at RT with 10 % w/v nonfat dry milk (#9999S, Cell Signaling Technology (CST), Danvers, MA, US) diluted in TBS buffer supplemented with 0.1 % Tween-20 (p1379, Sigma), and proteins stained with the following primary antibodies (o/n at 4 °C): anti-p110a (ref: Hu Q, Klippel A, Muslin A J, Fantl W J, Williams L T. Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase. Science. 1995; 268: 100–10), anti-p110b (#3011, CST), anti-mTOR (#2983, CST), anti-p85a (ab133595, Abcam, Cambridge, UK), anti-p85b, (ab28356, Abcam), anti-pAKT_T308 (#13038, CST), anti-pAKT_S473 (#4058, CST), anti-pRPS6_S240/244 (#5364, CST), or anti-tubulin a (T9046, Sigma). Proteins were standardly detected by ECL reaction (immobilon HRP substrate solution, Millipore, Burlington, MA) involving HRP-linked anti-rabbit (#7074, CST) or anti-mouse (#7076, CST) secondary reagents. Where necessary, membranes were cleared of primary antibodies using the Restore Western Blot Stripping Buffer (#21059, Thermo Fisher) and re-cycled for iterative detection. Chemiluminescence was recorded on a Fusion FX image reader (Vilber, Collegien, France) set to autoexposure at high sensitivity.

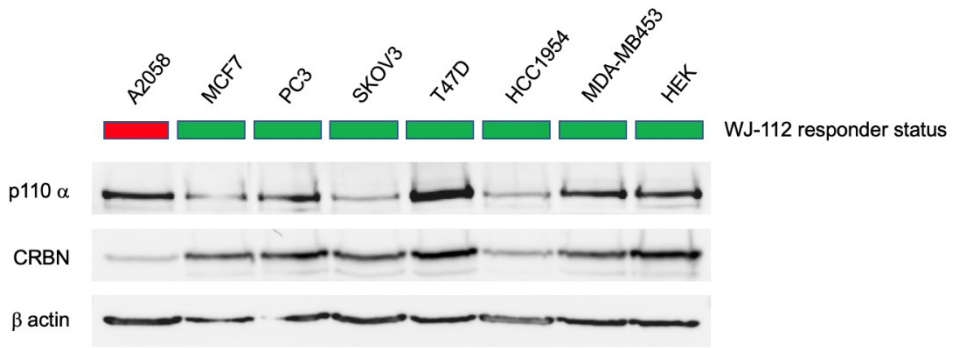


Figure S10 *CRBN and p110α levels in 8 cell lines. WJ112-14 responder cell lines are marked in green, non-responders in red. A2058 is very low in CRBN.*

Plasmid and Stable Cell Line Generation

Stable EGFP_PI3CA cell line was created by integration of the pcDNA5/FRT expression vector containing EGFP-PI3CA sequence into the genome of Flp-In-293 cells (Invitrogen) via FLP recombinase-mediated DNA recombination at the FRT site (Flp-In system, Invitrogen). Cells were transfected with the expression vector and selected in presence of hygromycin.
The pcDNA5/FRT/EGFP_PI3CA expression vector was generated by insertion of a PI3CA coding sequence fused to the C-terminus of EGFP (using GGGGSGGGGS linker) into pcDNA5/FRT/TO between AflII and XhoI restriction sites.
PI3CA coding sequence was obtained from the plasmid PIK3CA-WT, a kind gift from Bert Vogelstein (Addgene plasmid # 16643).[1]

qPCR

Total RNA was isolated from treated cells (as in treatments for western blots) by using TRI Reagent® (MRC, TR118) and RNA Clean & Concentrator-5 (Zymo Research, R1015) according to the manufacturer's protocol. The cDNA was synthesized using SuperScriptIV Reverse Transcriptase (Invitrogen) and assessed by qPCR (PowerUP SYBR Green Master Mix; Invitrogen) on an Applied Biosystems StepOnePlus Instrument.
Two different primer pairs were used for PIK3CA (1F 5'-GAA GCA CCT GAA TAG GCA AGT CG-3', 1R 5'-GAG CAT CCA TGA AAT CTG GTC GC-3' and 2F 5'-TGC TAA AGA GGA ACA CTG TCC A-3', 2R 5'-GGT ACT GGC CAA AGA TTC AAA G-3') and GAPDH was used as a housekeeping gene (GAPDH_F 5'-CCACTCCTCCACCTTTGAC-3'; GAPDH_R 5'-ACCCTGTTGCTGTAGCCA-3'). Each cDNA was analyzed in triplicate.

Flow Cytometry

800k cells were seeded in a 6 well-plate format in 1 ml growing medium. After 24 h the cells were treated. After 24 h, the growing medium was removed and 0.05 % Trypsin was added to detach the cells. The trypsin was neutralized by the addition of growing medium and the cells collected via centrifugation (8000 rcf at 4 °C for 5 min). The pellets were resuspended in 300 µl cold PBS supplemented with 2.5 % FCS. Centrifugation and resuspension were repeated two more times and then measured on a BD LSR Fortessa Analyzer. The data was analyzed with Cytoflow 1.2. Blue Laser: 488 nm, LP Mirror 505 nm, BP Filter 512 nm/25 nm.

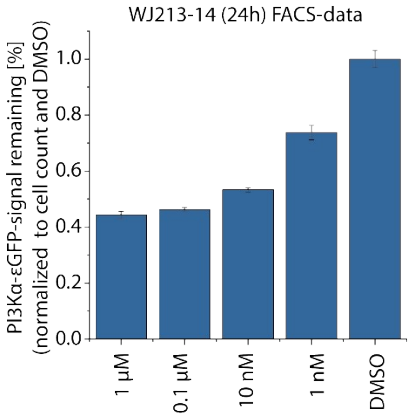
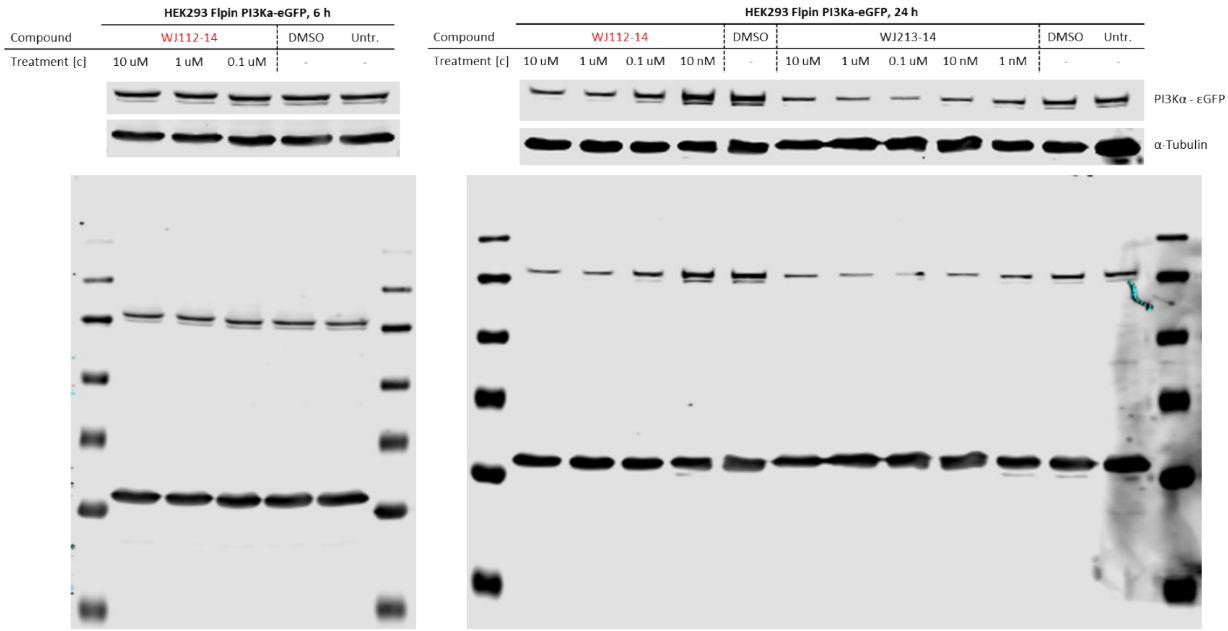


Figure S11 *WJ213-14 treatments for 24 h at four different concentrations. PI3Kα-εGFP signal normalized to cell count and DMSO.*



Technical replicates (n = 3).

Figure S12 *Western blots of 6 and 24 h treatments with WJ112-14 and WJ213-14 with the HEK293 Flpin PI3Kα-εGFP stable cell line.*

TR-FRET Assay

The kinetic constant (Ki) of compounds for p110α were determined by LanthaScreen Technology EU Kinase Binding Assay (Life Technologies). An N-terminally His6-tagged p110α recombinant protein was combined with a biotinylated anti-His6-tag antibody (2 nM, #6089), an Europium-labeled Streptavidin complex (2 nM, #5899) and an AlexaFluor647-labelled kinase Tracer (Tracer 314, #6087, Kd of 2.26 for p110α at 20 nM) in TR-FRET assay buffer (50 mM N-(2-hydroxyethyl)piperazine-N′-ethanesulfonic acid (HEPES) pH 7.5, 10 mM $MgCl_2$, 1 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), and 0.01 % (v/v) Brij-35). The prepared recombinant protein-antibody-tracer mix was dispensed in a 384-well plate (5 µl per well). The plate was then centrifuged (1700 rpm for 5 min) and incubated in dark at RT for 45 min. Subsequently, compounds were diluted in TR-FRET assay buffer and then dispensed into a 384-well plate (in triplicate) at a final concentration range between 10 µM and 0.2 nM using Dispendix I-DOT One and incubated in the dark at RT for 60 min. Time resolved FRET was measured with a Synergy Neo2 plate reader. (BioTek instruments; Emission filters: 665/8 nm and 620/10 nm; excitation with 337 nm TRF laser; 100 µs delay, 200 µs data collection; 37 °C; dichroic mirror 400 nm).[2] The emission ratio of 665/620 was normalized to a non-inhibitor control and subsequently, the data was fitted to the “log(inhibitor) vs. normalized response – variable slope” curve using Graphpad Prism software (Version 9.5.1).
The Ki values were calculated according to the following formula:

$$K_i = \frac{IC_{50}(inhibitor)}{(1 + \frac{[Tracer]}{K_d(Tracer)})}$$

Compound	Ki [nM]	SD	CI (95%, n=3)
WJ111-11	1.849	0.083	0.152
WJ200-12	1.837	0.018	0.033
WJ201-13	1.850	0.138	0.254
WJ112-14	1.662	0.034	0.062
WJ202-15	3.432	0.086	0.158
WJ208-16	3.985	0.413	0.759
WJ209-17	9.836	0.783	1.438
WJ204-12	1.163	0.110	0.202
WJ213-14	0.851	0.013	0.024
WJ203-14	2.809	0.021	0.039
WJ214-14	2.452	0.093	0.171
WJ112-14-Me	3.471	1.141	2.096
WJ117-16	4.990	0.109	0.200
Li_2018_D	19.448	0.007	0.013
PQR514	2.840	0.130	0.239

Table S2 *Mean K_i values with SD and confidence interval at 95 % for triplicates from TR-FRET.*

In-Cell Western

1800 MCF7 cells/well were seeded in a 96 well-plate format µClear® CELLSTAR® (Greiner Bio-One, Cat. #7.655 090) and grown for 24 h in 100 µl DMEM low glucose (Sigma, Cat. #D6046-1L) containing 10 % of heat inactivated FCS (Sigma, Cat. #F7524), 1 % of 200 mM L-glutamine (Sigma, Cat. #G7513) and 1 % of penicillin – streptomycin (Sigma, Cat. #P4333). The compounds for treatment were diluted from 2 mM stock solutions in DMSO into 150 µM, 15 µM, and 0.15 µM dilutions in 1X PBS (pH 7.40, 0.22 mm filtered, without Ca/Cl/Mg, Sigma, Cat. #D8537-500ML) in I-DOT PURE S Plates 100 (Art. Nr. D1610021800) and subsequently transferred via IDOT dispenser into the wells of the cell culture plate. The final treatment concentrations for each compound were 3000, 1024, 512, 256, 128, 64, 32, 16, 8, 4, and 0.8 nM. The cells were incubated with the compounds for 2 h. 10 % (w/v) PFA (Paraformaldehyde) in PBS (Sigma-Aldrich, Cat. #158127-500G) was filtered (0.22 µm filter), dispensed into the wells and incubated for fixation at room temperature for 30 min. The fixation-buffer was removed and the cells were washed with 150 µl 1 x PBS (3 times). For permeabilization 60 µl per well of 0.1 % (v/v) Triton X-100, 1 % (w/v) BSA (Bovine Serum Albumin), (Sigma, Cat. #T8787-250ML) in PBS (Sigma, Cat. #A3912-100G) were dispensed and incubated for 30 min at room temperature. The buffer was removed and 60 µl per well of a prepared buffer of 5 % Goat Serum (Sigma, Cat. #G6767), 1 % BSA, 0.1 % Triton in PBS was dispensed for blocking by incubating for 30 min. The buffer was removed and the plates were incubated overnight at 4 °C (sealed and shaking) with 50 µl per well of a mixture of rabbit anti-pPKB S473 antibodies (Cell Signaling Technology, Cat. #4058L) diluted 1:500 in PBS (containing 5 % Goat Serum, 1 % BSA, 0.1 % Triton) and Mouse anti-αTubulin antibodies (Sigma, Cat. #T9026) diluted 1:2000 in PBS (containing 5 % Goat Serum, 1 % BSA, 0.1 % Triton). The plates were washed with PBS three times. Secondary antibodies (Goat anti-mouse IRDye680 (LICOR, #D00115-03) and Goat anti-rabbit IRDye800 (LICOR, #D00304-15)) were diluted and dispensed the same way as the primary antibodies in the previous step, then incubated for 90 min at room temperature, protected from light and shaking. The buffer was removed and the plates were washed three times with PBS 1X. The plates were analyzed with the LICOR Infrared scanning system Odyssey CLx and the software image Studio. Images were acquired with the auto setting, scan control 84 µm, quality medium and focus distance 40 µm.

Compound	IC_{50}	SD	CI (95%, n=3)
PQR514	30.9	1.1	2.0
WJ111-11	67.3	4.9	9.0
WJ112-14	37.5	4.2	7.7
WJ117-16	410.1	39.0	71.6
WJ112-14-Me	124.6	5.2	9.6
Li_2018_D	2511.6	244.1	448.4
WJ200-12	44.1	2.5	4.6
WJ201-13	32.3	3.1	5.7
WJ202-15	42.0	3.4	6.2
WJ203-14	70.7	3.9	7.2
WJ204-12	8.9	0.4	0.7
WJ208-16	40.2	1.8	3.3
WJ209-17	29.0	1.2	2.2
WJ213-14	11.8	0.9	1.7
WJ214-14	31.2	1.7	3.1

Table S3 *Mean IC_{50} values with SD and CI for In-cell western blots in biological triplicates.*

Kinome Screen

These assays were performed by eurofins at their site in San Diego, California.
From the description from eurofins:
For most assays, kinase-tagged T7 phage strains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (6,000 x g) and filtered (0.2µm) to remove cell debris. The remaining kinases were produced in HEK-293 cells and subsequently tagged with DNA for qPCR detection. Streptavidin-coated magnetic beads were treated with biotinylated small molecule ligands for 30 minutes at room temperature to generate affinity resins for kinase assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining kinases, liganded affinity beads, and test compounds in 1x binding buffer (20 % SeaBlock, 0.17x PBS, 0.05 % Tween 20, 6 mM DTT). Test compounds were prepared as 40x stocks in 100% DMSO and directly diluted into the assay. All reactions were performed in polypropylene 384-well plates in a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05 % Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05 % Tween 20, 0.5 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The kinase concentration in the eluates was measured by qPCR.

KUW001-01-p-00001 Study Results

Table 1 - Matrix of Compound Screen for KUW001-01-p-00001

Target	WJ112-14	WJ213-14
Gene Symbol	%Ctrl @ 10000nM	%Ctrl @ 10000nM
PIK3C2B	47	1.9
PIK3C2G	20	0.75
PIK3CA	0	0
PIK3CA(C420R)	0	0
PIK3CA(E542K)	12	0.2
PIK3CA(E545A)	0.1	1.6
PIK3CA(E545K)	0	0
PIK3CA(H1047L)	0	0
PIK3CA(H1047Y)	0	0.15
PIK3CA(I800L)	0	0
PIK3CA(M1043I)	0	1.7
PIK3CA(Q546K)	2.2	0.2
PIK3CB	0.15	0
PIK3CD	0.05	0.55
PIK3CG	0.05	0
PIK4CB	88	76
PIKFYVE	50	4.8
PIP5K1A	90	93
PIP5K1C	98	96
PIP5K2B	89	85
PIP5K2C	74	67
VPS34	15	0.25

%Ctrl Legend

0≤x<.1	.1≤x<1	1≤x<10	10≤x<35	x≥35

KUW002-01-s-00001 Study Results

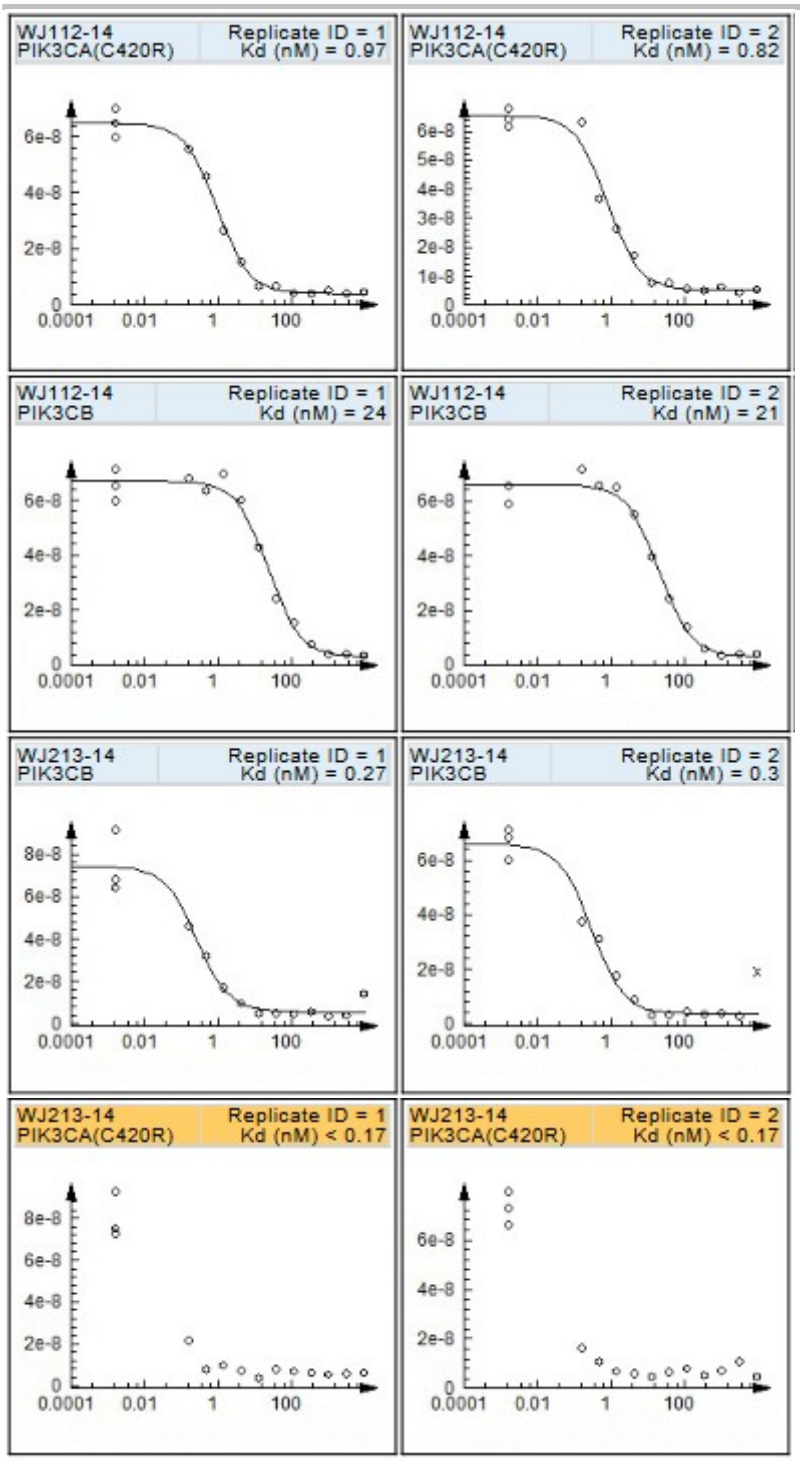
Table 1 - Matrix of Kds for KUW002-01-s-00001.

Target	WJ112-14	WJ213-14
Gene Symbol	Kd (nM)	Kd (nM)
MTOR	150	18

Kd Legend

x<100nM	100nM≤x<1uM	x≥1uM	No Binding	Not Requested

Table S4 *Results of the lipid kinome scan performed by eurofins.*



Target	WJ112-14	WJ213-14
Gene Symbol	Kd (nM)	Kd (nM)
PIK3CA (C420R)	0.89	<0.17
PIK3CB	22	0.29

Figure S13 *Binding curves and calculated/tabulated Kd values for* ***WJ112-14*** *and* ***WJ213-14*** *for PI3Kα/β.*

Targeted PRM-LC-MS Analysis

Parallel reaction-monitoring (PRM) assays[3] were generated from a mixture containing 50 fmol of each proteotypic heavy reference peptide of the target proteins PIK3CA (DDGQLFHIDFGHFLDHK, LINLTDILK, MDWIFHTIK, IMENIWQNQGLDLR, DLNSPHSR), PIK3CB (AAEIASSDSANVSSR, VFGEDSVGVIFK, VNELAIQK, EALELLDFNYPDQYVR, QVEALNK), PIK3CD (TGLIEVVLR, VNWLAHNVSK, NPGEALDR, HEVQEHFPEALAR, SDTIANIQLNK), PIK3CG (GIDIPVLPR, HQPTPDPEGDR, STTSQTIK, VPYDPGLK, EDIEYIR), MTOR (DLELAVPGTYDPNQPIIR, LFDAPEAPLPSR, TLDQSPELR, YHPQALIYPLTVASK, ETSFNQAYGR), PIK3R1 (TQSSSNLAELR, ISEIIDSR, LHEYNTQFQEK, GDFPGTYVEYIGR, DTADGTFLVR) and PIK3R2 (EAAGPVGPALEPPTLPLHR, INEWLGIK, APGPGPPPAAR, AALQALGVAEGGER, EYDQLYEEYTR), all JPT Peptide Technologies GmbH, Berlin, Germany. Peptides were subjected to LC–MS/MS analysis using an Orbitrap Fusion Lumos Mass Spectrometer fitted with an EASY-nLC 1200 (both Thermo Fisher Scientific) and a custom-made column heater set to 60 °C. Peptides were resolved using a RP-HPLC column (75 µm × 37 cm) packed in-house with C18 resin (ReproSil-Pur C18–AQ, 1.9 µm resin; Dr. Maisch GmbH) at a flow rate of 0.2 µl min-1. A linear gradient ranging from 5 % buffer B to 45 % buffer B over 60 min was used for peptide separation. Buffer A was 0.1 % formic acid in water and buffer B was 80 % acetonitrile, 0.1 % formic acid in water.
The mass spectrometer was operated in DDA mode with a cycle time of 3 s between master scans. Each master scan was acquired in the Orbitrap at a resolution of 120 000 FWHM (at 200 m/z) and a scan range from 250 – 1 600 m/z followed by MS2 scans of the most intense precursors in the Orbitrap with a resolution of 30 000 FWHM and with the isolation width of the quadrupole set to 1.4 m/z. Maximum ion injection time was set to 50 ms (MS1) and 54 ms (MS2) with an AGC target set to 106 and 105, respectively. Only peptides with charge state 2 – 5 were included in the analysis. Monoisotopic precursor selection (MIPS) was set to Peptide, and the Intensity Threshold was set to 2.5 x 104. Peptides were fragmented by HCD (Higher-energy collisional dissociation) with collision energy set to 35 %, and one microscan was acquired for each spectrum. The dynamic exclusion duration was set to 12 s.
The acquired raw-files were searched using the MaxQuant software (Version 1.6.2.3) against a human database (containing 20372 protein sequences downloaded from Uniprot on 2022-02-22) using default parameters except protein, peptide and site FDR were set to 1 and Lys8 and Arg10 were added as variable modifications. Search results were imported into SpectroDive (version 8, Biognosys, Schlieren) and a scheduled (window width 12 min) mass isolation list containing all peptides was exported and imported into the Orbitrap Fusion Lumos operating software for PRM analysis.
MCF7 cells were seeded at 500 k in 2 ml complete growth medium in 6-well plates and grown for 24 h. Cells were then treated either with control (DMSO) or compounds dissolved in DMSO according to the indicated concentrations. Final DMSO concentration in every well was 0.1 %. Treatments were done for 6 h after which cells were washed twice with PBS (Sigma-Aldrich, D8537) and then harvested by scraping (Cell Scrapers, Sarstedt, 83.3951) and resuspended in PBS. Cells were collected by centrifugation (15 min, 8.8 krcf, 4 °C) and the supernatant was removed by suction. Cell pellets were stored at -80 °C until further processing.
Cell pellets were resuspended in 100 µl lysis buffer (1 % sodium deoxycholate, 0.1 M Tris, 10 mM TCEP, pH = 8.5), transferred to 96-well round bottom plates (Corning, 3799) and subjected to ultrasonication using a PIXUL well plate sonicator (Active Motif, settings: Pulse N = 50, PRF = 1 kHz, Process Time = 10 min, Burst Rate = 20 Hz). Samples were transferred to 0.5 ml Eppendorf tubes and reduced for 10 min at 95 °C and 300 rpm, subsequently spun down at 21.3 krcf for 5 min and the supernatant was transferred to fresh Eppendorf tubes leaving any non-dissolved fractions. Protein concentration was determined by tryptophan fluorescence.[4]
Aliquots of 50 µg were normalized to equal volumes and alkylated with 15 mM chloroacetamide for 30 min at 37 °C and 500 rpm. Proteins were digested by incubation with sequencing-grade modified trypsin (1 µg per 50 µg of protein, Promega, V511C) for 12 h at 37 °C. Prior to trypsin addition the pH was checked to be around 8 – 9. Digests were then either stored at -20 °C until clean up or directly processed the next morning. Tryptic digests were acidified (pH < 3) by addition of 50 µl 5 % TFA in water. 100 µl 1 % TFA in isopropanol were added. The samples were thoroughly vortex and spun down at 21.3 krcf for 5 minutes to remove any precipitates before purification with iST spin columns. Samples were loaded onto columns, washed twice with 200 µl 1 % TFA in isopropanol, and twice with 0.2 % TFA in water. Peptides were then eluted into 2 ml Eppendorf tubes with two times 100 µl 1 % NH_3 in 19 % water and 80 % acetonitrile. Purified samples were dried in a SpeedVac vacuum concentrator and stored at -20 °C until further processing.
Peptide samples for PRM analysis were resuspended in 20 µl 0.1 % formic acid in water by sonication in a VialTweeter (settings: 10 pulses, amplitude = 100, cycle = 0.5, Hielscher) and subsequent shaking for 5 min at 1 400 rpm and 25 °C. Samples were spiked with the heavy reference peptide mix at a concentration of 8 fmol of heavy reference peptides per 1 µg of total endogenous peptide and subjected to LC–MS/MS analysis on the same LC-MS system described above using the following settings: The resolution of the Orbitrap was set to 60 000 FWHM (at 200 m/z), the fill time was set to 118 ms to reach an AGC target of 106, the normalized collision energy was set to 35 %, ion isolation window was set to 0.4 m/z and the scan range was set to 150 – 1500 m/z. A MS1 scan at 120 000 resolution (at 200 m/z), AGC target 106 and fill time of 100 ms was included in each MS cycle.
All raw files were imported into Skyline for protein/peptide quantification. A library was generated based on the MaxQuant analysis of the heavy peptides (see above) and the 6 highest ranking y ions were used for peptide quantification where applicable.
The data was exported from Skyline and further processed in R. Peptides with a library dot product ≥ 0.8 were considered for analysis. To account for technical variations in the experiment, the total ion chromatogram of each sample was determined and used for normalization. Further all sample signals were normalized to DMSO control.
Data for WJ111/WJ200, WJ201/WJ202, WJ208/WJ209 and WJ204/WJ214 were acquired in the same experiment and share the DMSO control.

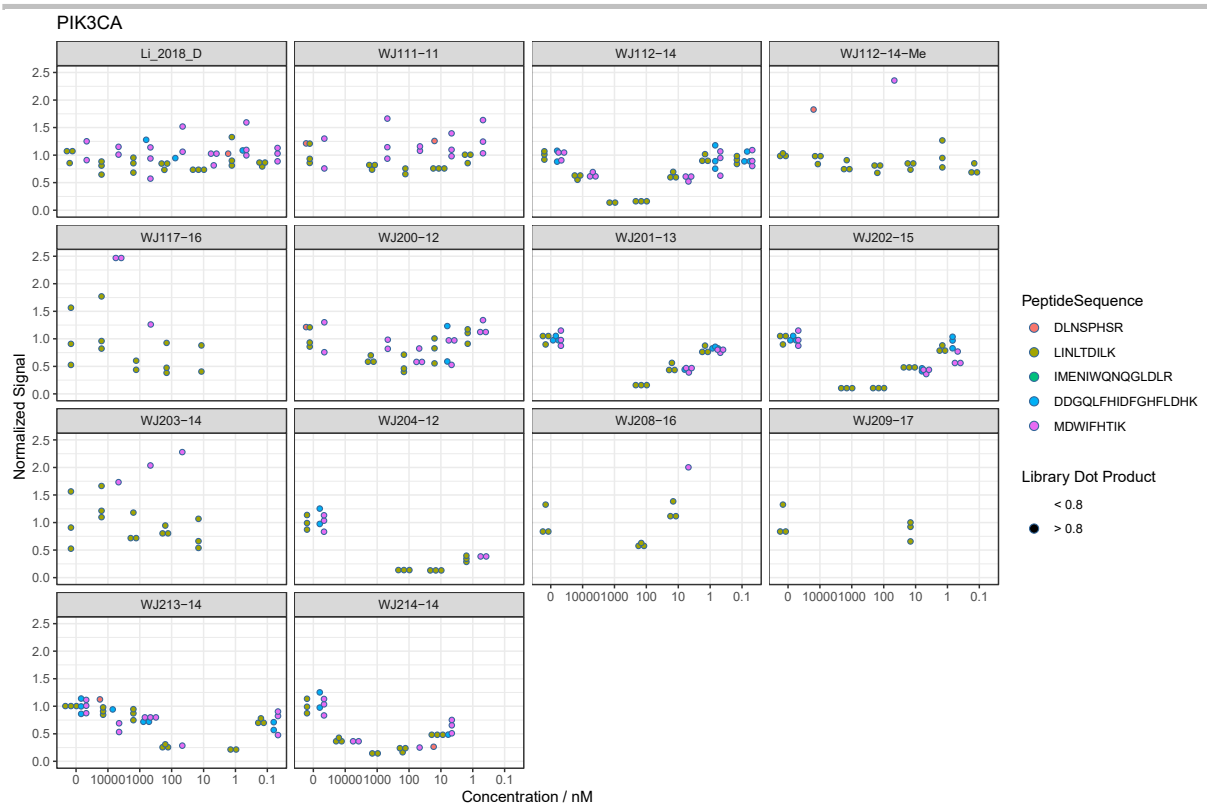


Figure S14 *Parallel Reaction Monitoring: full data set for PIK3CA. All experiments were done in MCF7 cells at indicated concentrations for 6 h in triplicates.*

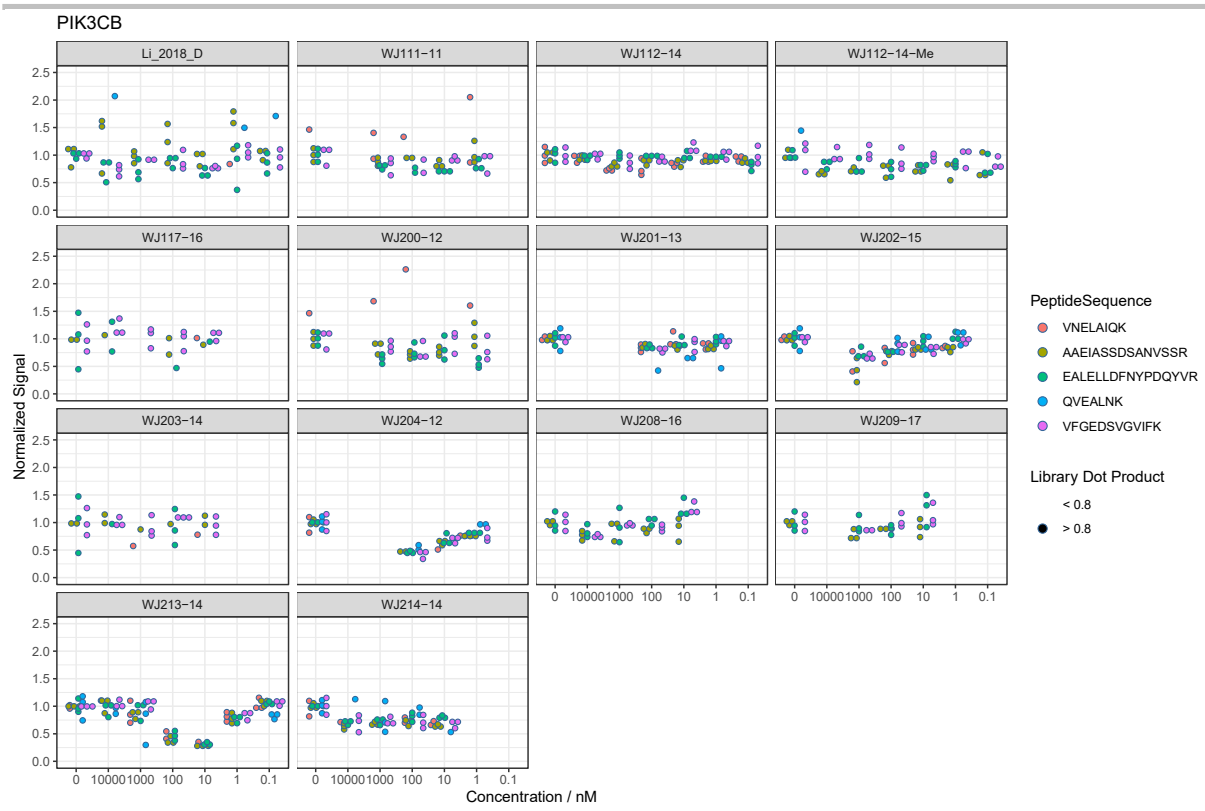


Figure S15 *Parallel Reaction Monitoring: full data set for PIK3CB. All experiments were done in MCF7 cells at indicated concentrations for 6 h in triplicates.*

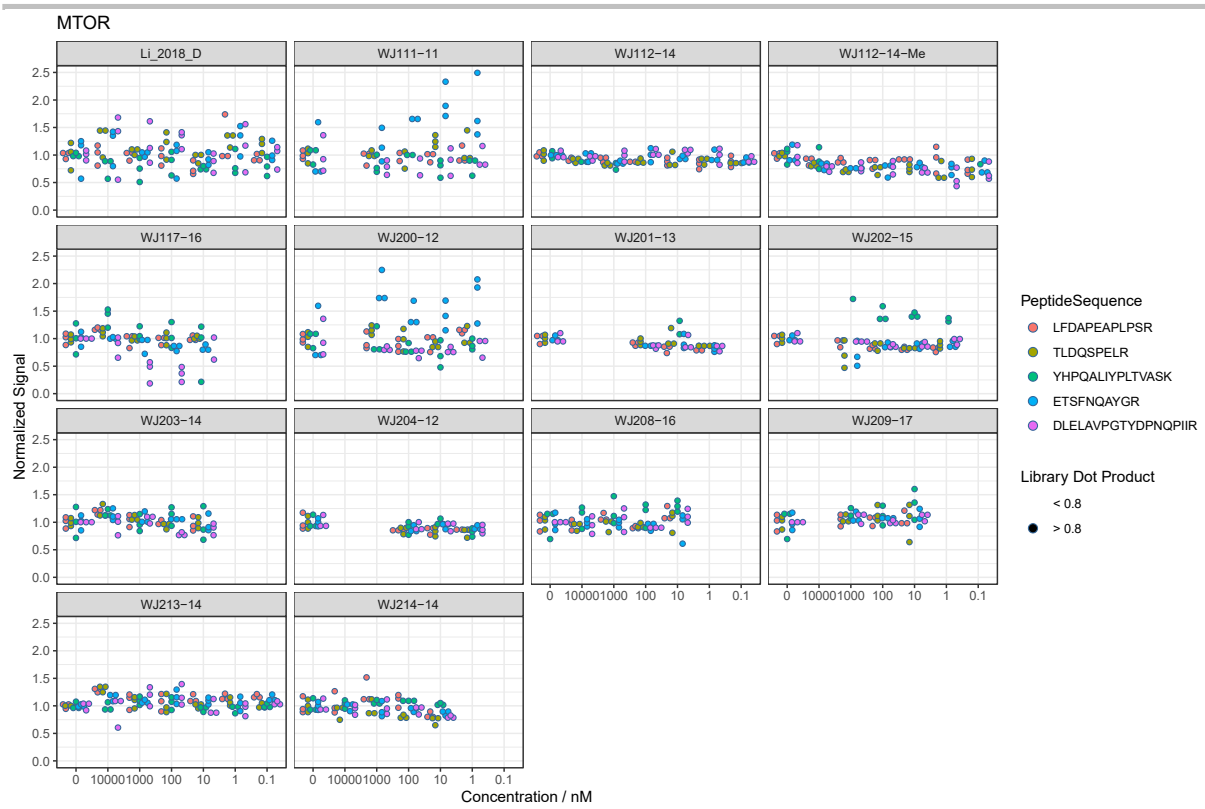


Figure S16 *Parallel Reaction Monitoring: full data set for MTOR. All experiments were done in MCF7 cells at indicated concentrations for 6 h in triplicates.*

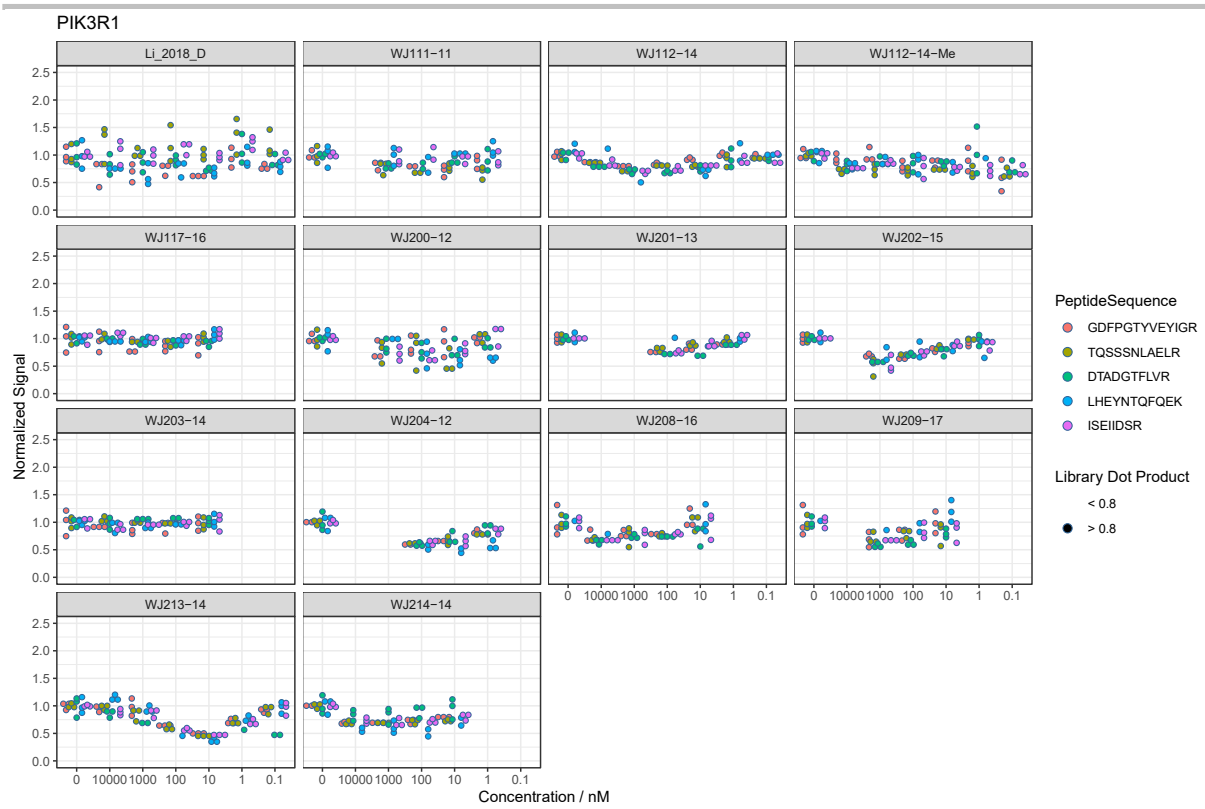


Figure S17 *Parallel Reaction Monitoring: full data set for PIK3R1. All experiments were done in MCF7 cells at indicated concentrations for 6 h in triplicates.*

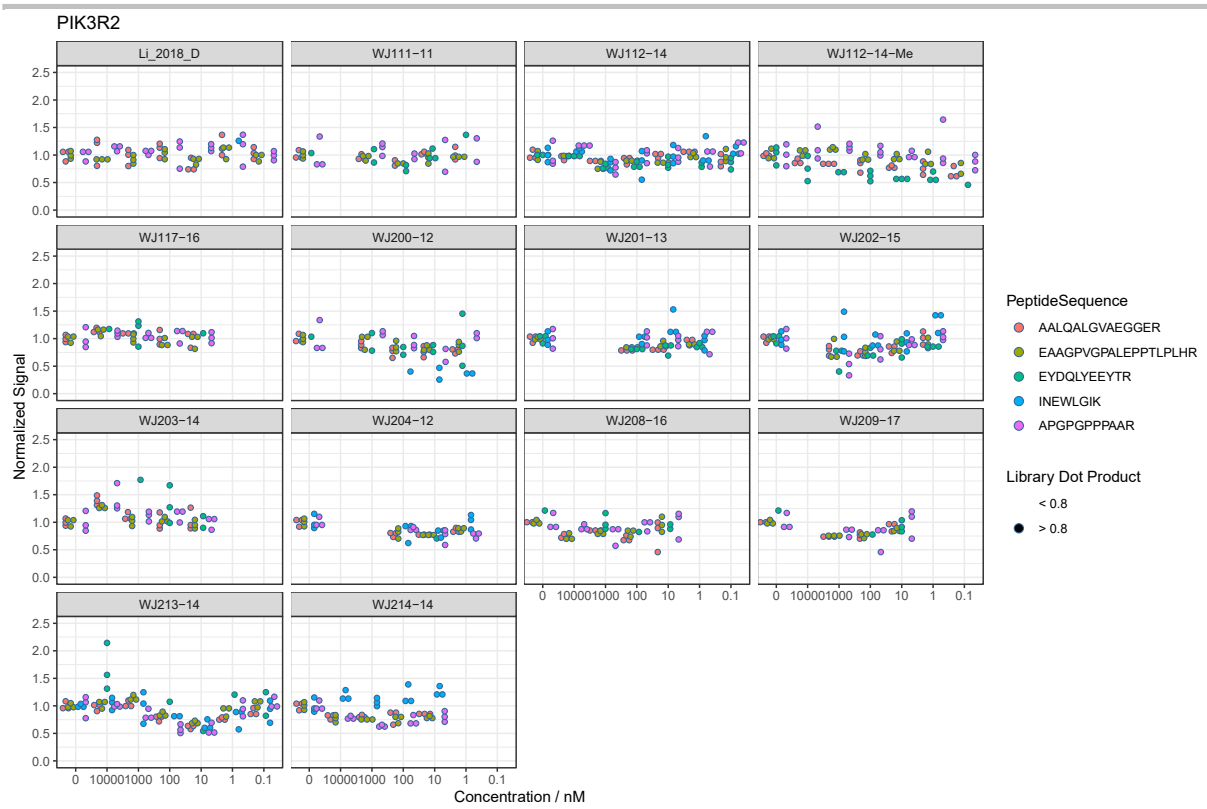


Figure S18 *Parallel Reaction Monitoring: full data set for PIK3R2. All experiments were done in MCF7 cells at indicated concentrations for 6 h in triplicates.*

TMT Labelling and LC-MS/MS Analysis of MCF7 Cells Treated with PROTACs

MCF7 cells were seeded at 500 k in 2 ml complete growth medium in 6-well plates and grown for 24 h. Cells were treated in quadruplicates with compounds dissolved in DMSO to a final concentration of 10 nM for WJ213-14, 100 nM for WJ112-14, and WJ204-12 for 100 nM, or DMSO as control. Final DMSO concentration in every well was 0.1 %. Treatments were done for 6 h after which cells were washed twice with PBS (Sigma-Aldrich, D8537) and then harvested by scraping (Cell Scrapers, Sarstedt, 83.3951) and resuspended in PBS. Cells were collected by centrifugation (15 min, 8.8 krcf, 4 °C) and the supernatant was removed by suction. Cell pellets were stored at -80 °C until further processing.

Cell pellets were resuspended in 100 µl lysis buffer (1 % sodium deoxycholate, 0.1 M Tris, 10 mM TCEP, pH = 8.5) transferred to 96-well round bottom plates (Corning, 3799) and subjected to ultrasonication using a PIXUL well plate sonicator (Active Motif, settings: Pulse N = 50, PRF = 1 kHz, Process Time = 10 min, Burst Rate = 20 Hz). Samples were transferred to 0.5 ml Eppendorf tubes and heated at 95 °C for 10 min and 300 rpm, subsequently spun down at 21.3 krcf for 5 min and the supernatant was transferred to fresh Eppendorf tubes to remove any non-dissolved fractions. Protein concentration was determined by Tryptophan fluorescence[4].

Aliquots of 70 µg were normalized to equal volumes and alkylated with 15 mM chloroacetamide for 30 min at 37 °C. Proteins were digested by incubation with sequencing-grade modified trypsin (1 µg per 50 µg of protein, Promega) for 12 h at 37 °C. Prior to trypsin addition the pH was checked to be around 8-9. Digests were directly processed the next morning. Tryptic digests were acidified (pH < 3) by addition of 50 µl 5 % TFA in water. 100 µl 1 % TFA in isopropanol were added. The samples were thoroughly vortex and spun down at 21.3 krcf for 5 min to remove any precipitates before purification with iST spin columns. Samples were loaded onto the columns, washed twice with 200 µl 1 % TFA in isopropanol and twice with 0.2 % TFA in water. Peptides were then eluted with two times 100 µl 1 % NH_3 in 19 % water and 80 % acetonitrile. Purified samples were dried in a SpeedVac vacuum concentrator and stored at -20 °C until further processing.

Sample aliquots comprising 10 µg of peptides were labelled with isobaric tandem mass tags (TMTpro 16-plex, Thermo Fisher Scientific). Peptides were resuspended in 10 µl labelling buffer (2 M urea, 0.2 M HEPES, pH 8.3) by sonication and 2.5 µl of each TMT reagent were added to the individual peptide samples followed by a 1 h incubation at 25° C shaking at 500 rpm. To quench the labelling reaction, 0.75 µl aqueous 1.5 M hydroxylamine solution was added, and samples were incubated for 5 min at 25 °C shaking at 500 rpm followed by pooling of all samples. The pH of the sample pool was increased to 11.9 by adding 1 M phosphate buffer (pH 12) and incubated for 20 min at 25 °C and 500 rpm shaking to remove TMT labels linked to peptide hydroxyl groups. Subsequently, the reaction was stopped by adding 2 M hydrochloric acid until a pH < 2 was reached. Finally, peptide samples were further acidified using 5 % TFA, desalted using BioPureSPN MACRO™ SPE cartridges (Nest group) according to the manufacturer's instructions and dried under vacuum.

TMT-labeled peptides were fractionated by high-pH reversed phase separation using a XBridge Peptide BEH C18 column (3.5 µm, 130 Å, 1 mm x 150 mm, Waters) on an Ultimate 3000 system (Thermo Scientific). Peptides were loaded on column in buffer A and the system was run at a flow of 42 µl min^{-1}. The following gradient was used for peptide separation: from 2 % B to 15 % B over 3 min to 45 % B over 59 min to 80 % B over 3 min followed by 9 min at 80 % B then back to 2 % B over 1 min followed by 15 min at 2 % B. Buffer A was 20 mM ammonium formate in water, pH 10 and buffer B was 20 mM ammonium formate in 90 % acetonitrile, pH 10. Elution of peptides was monitored with a UV detector (205 nm, 214 nm) and a total of 36 fractions were collected, pooled into 12 fractions using a post-concatenation strategy as previously described[5] and dried under vacuum.

Dried peptides were resuspended in 0.1 % aqueous formic acid and subjected to LC–MS/MS analysis using an Orbitrap Eclipse Tribrid Mass Spectrometer fitted with Ultimate 3000 nano system and a FAIMS Pro interface (all Thermo Fisher Scientific) and a custom-made column heater set to 60 °C. Peptides were resolved using a RP-HPLC column (75 µm × 30 cm) packed in-house with C18 resin (ReproSil-Pur C18–AQ, 1.9 µm resin; Dr. Maisch GmbH) at a flow rate of 0.3 µl min^{-1}. The following gradient was used for peptide separation: from 2 % B to 12 % B over 5 min to 30 % B over 70 min to 50 % B over 15 min to 95 % B over 2 min followed by 18 min at 95 % B then back to 2 % B over 2 min followed by 18 min at 2 % B. Buffer A was 0.1 % formic acid in water and buffer B was 80 % acetonitrile, 0.1 % formic acid in water.

The mass spectrometer was operated in DDA mode with a cycle time of 3 s between master scans. Throughout each acquisition, the FAIMS Pro interface switched between CVs of −40 V and −70 V with cycle times of 1.5 s and 1.5 s, respectively. MS1 spectra were acquired in the Orbitrap at a resolution of 120 000 and a scan range of 400 to 1 600 m/z, AGC target set to «Standard» and maximum injection time set to «Auto». Precursors were filtered with precursor selection range set to 400 to 1 600 m/z, monoisotopic peak determination set to «Peptide», charge state set to 2 to 6, a dynamic exclusion of 45 s, a precursor fit of 50 % in a window of 0.7 m/z and an intensity threshold of 5x103.

Precursors selected for MS2 analysis were isolated in the quadrupole with a 0.7 m/z window and collected for a maximum injection time of 35 ms with AGC target set to «Standard». Fragmentation was performed with a CID collision energy of 30 % and MS2 spectra were acquired in the IT at scan rate «Turbo».

MS2 spectra were subjected to RTS using a human database containing 20362 entries downloaded from Uniprot on 20200417 using the following settings: enzyme was set to «Trypsin», TMTpro16plex (K and N-term) and Carbamidomethyl (C) were set as fixed modifications, Oxidation (M) was set as variable modifications, maximum missed cleavages were set to 1 and maximum variable modifications to 2. Maximum search time was set to 100 ms, the scoring threshold was set to 1.4 XCorr, 0.1 dCn, 10 ppm precursor tolerance, charge state 2 and «TMT SPS MS3 Mode» was enabled. Subsequently, spectra were filtered with a precursor selection range filter of 400–1600 m/z, precursor ion exclusion set to 25 ppm low and 25 ppm high and isobaric tag loss exclusion set to «TMTpro». MS/MS product ions of precursors identified via RTS were isolated for an MS3 scan using the quadrupole with a 2 m/z window and ions were collected for a maximum injection time of 200 ms with a normalized AGC target set to 200 %. SPS was activated and the number of SPS precursors was set to 10. Isolated fragments were fragmented with normalized HCD collision energy set to 55 % and MS3 spectra were acquired in the orbitrap with a resolution of 50 000 and a scan range of 100 to 500 m/z.

The acquired raw files were analyzed using the SpectroMine software (Biognosis AG, Schlieren, Switzerland). Spectra were searched against a human database consisting of 20372 protein sequences (downloaded from Uniprot on 20220222). Standard Pulsar search settings for TMT 16 pro («TMTpro_Quantification») were used and resulting identifications and corresponding quantitative values were exported on the PSM level using the «Export Report» function. Acquired reporter ion intensities were employed for automated quantification and statistical analysis using the in-house developed SafeQuant R script (v2.3).[6] This analysis included adjustment of reporter ion intensities, global data normalization by equalizing the total reporter ion intensity across all channels, data imputation using the knn algorithm, summation of reporter ion intensities per protein and channel and calculation of protein abundance ratios. To meet additional assumptions (normality and homoscedasticity) underlying the use of linear regression models and t-tests, MS-intensity signals were transformed from the linear to the log-scale. The summarized protein expression values were used for statistical testing of between condition differentially abundant proteins. Here, empirical Bayes moderated t-tests were applied, as implemented in the R/Bioconductor limma package (bioconductor.org/packages/release/bioc/html/limma.html). The resulting per protein and condition comparison p-values were adjusted for multiple testing using the Benjamini-Hochberg method.

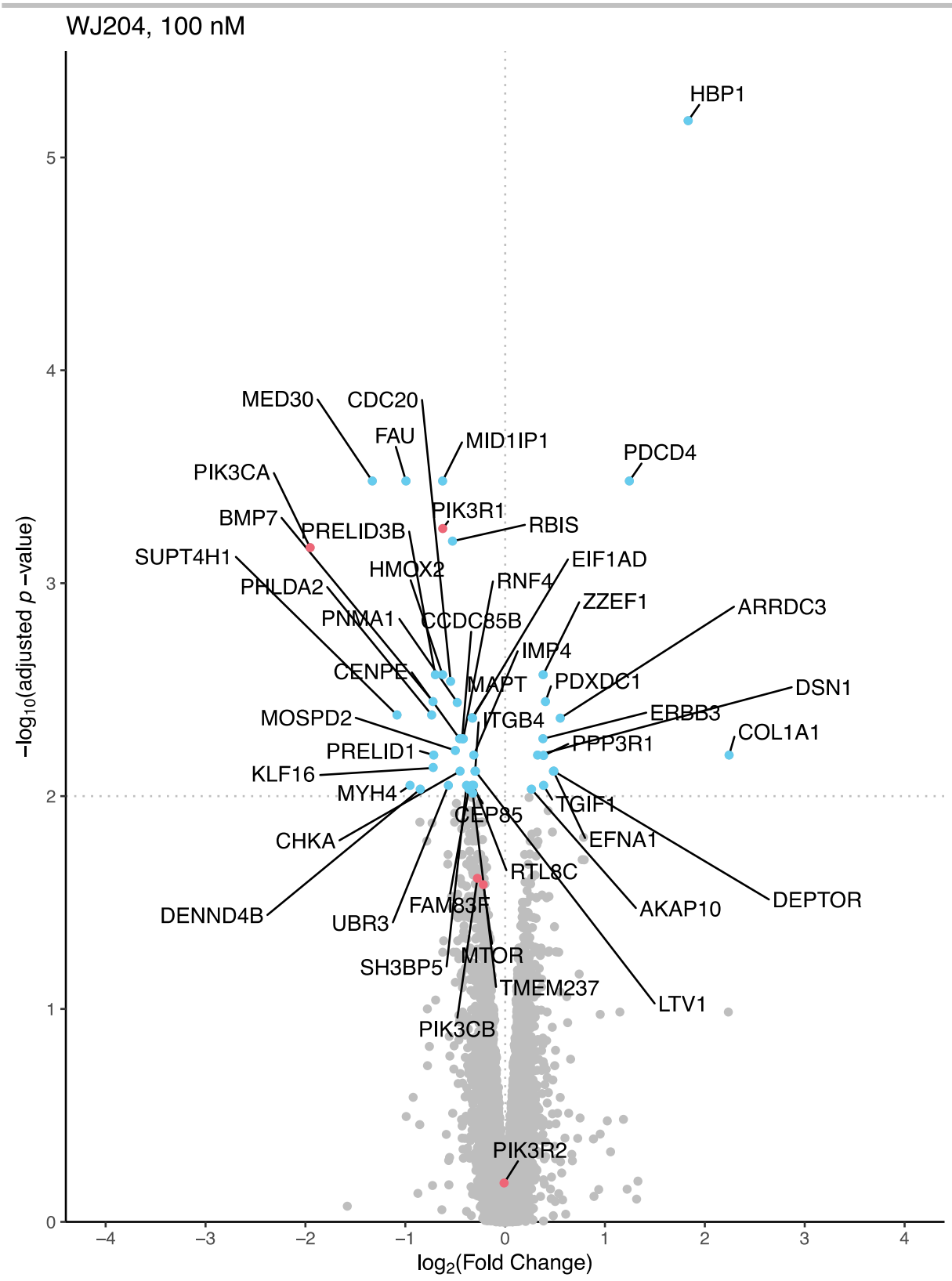
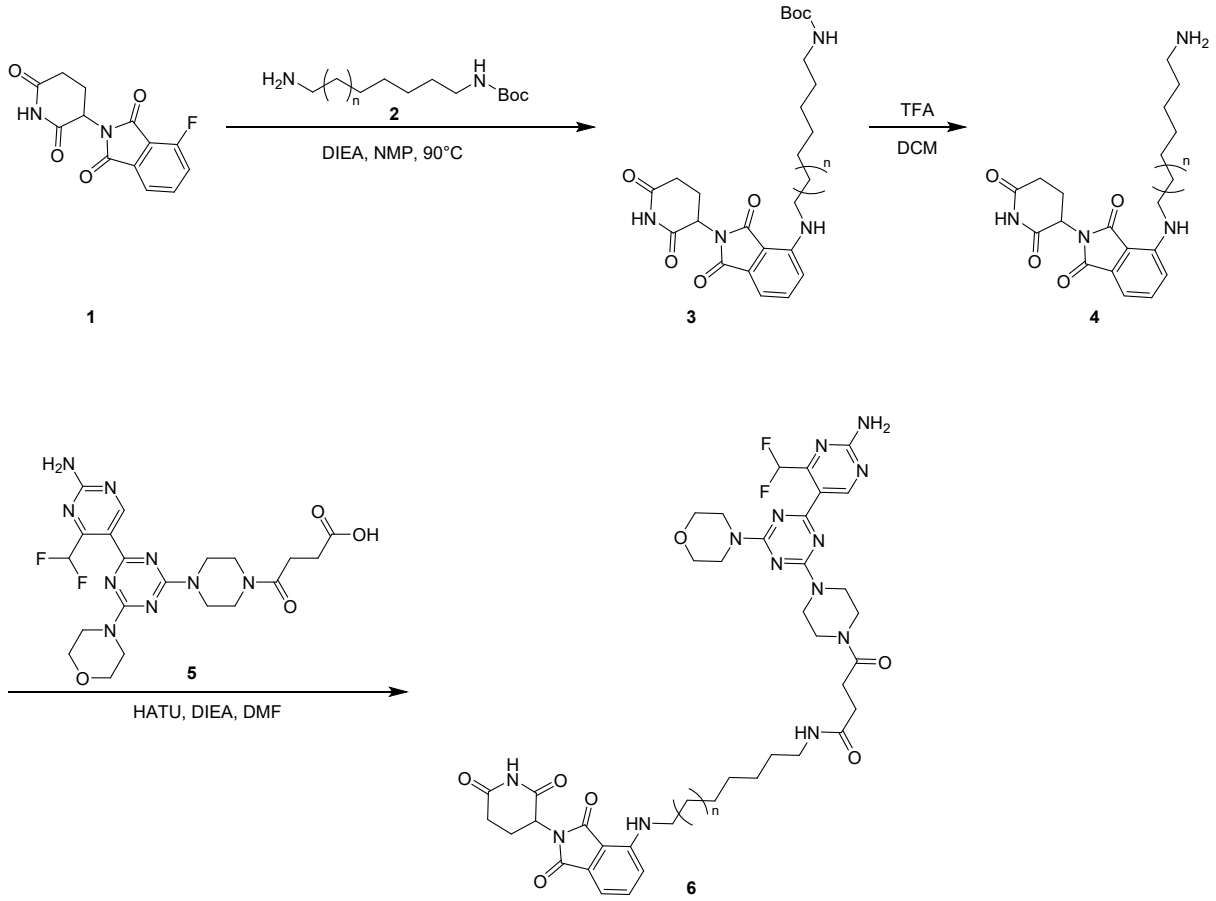


Figure S19 *TMT data for PROTAC WJ204-12.*

Synthesis

General procedure A



Synthesis of intermediate 3 (a,b,c,d,e,f)
To a mixture of 2-(2, 6-dioxo-3-piperidyl) -4-fluoro-isoindoline-1, 3-dione (**1**, 200 mg, 724 µmol, 1 eq), DIEA (187 mg, 1.45 mmol, 252 µl, 2 eq) in NMP (2 ml) was added **2** (1.1 eq), then the mixture was stirred at 90 °C for 12 h. LC-MS showed 2-(2, 6-dioxo-3-piperidyl) -4-fluoro-isoindoline-1, 3-dione consumed completely and one main peak with desired *m/z* was detected. TLC (Petroluem/EtOAc = 1:1) indicated the starting material was consumed completely and a major spot observed. The reaction mixture was diluted with EtOAc (30 ml) and the resulting mixture was washed with brine (3x 20 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0 to 50 % Ethyl acetate/Petroleum ethergradient @ 40 ml min^{-1}) to give compounds **3** (50 – 100 % yield) as yellow solids.

Synthesis of intermediate 4 (a,b,c,d,e,f)
To a solution of **3** (180 mg, 349 µmol, 1 eq) in DCM (0.5 ml) was added TFA (693 mg, 6.08 mmol, 450 µL, 17.4 eq), then the mixture was stirred at 10 °C for 0.5 h. LC-MS showed tert-butyl N-[9-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]nonyl]carbamate consumed completely and one main peak with desired mass was detected. The mixture was concentrated to give crude **4** as yellow solids which were used in the next step without further purification.

Synthesis of compound 6 (n = 1, 2, 3, 4, 5, 6)

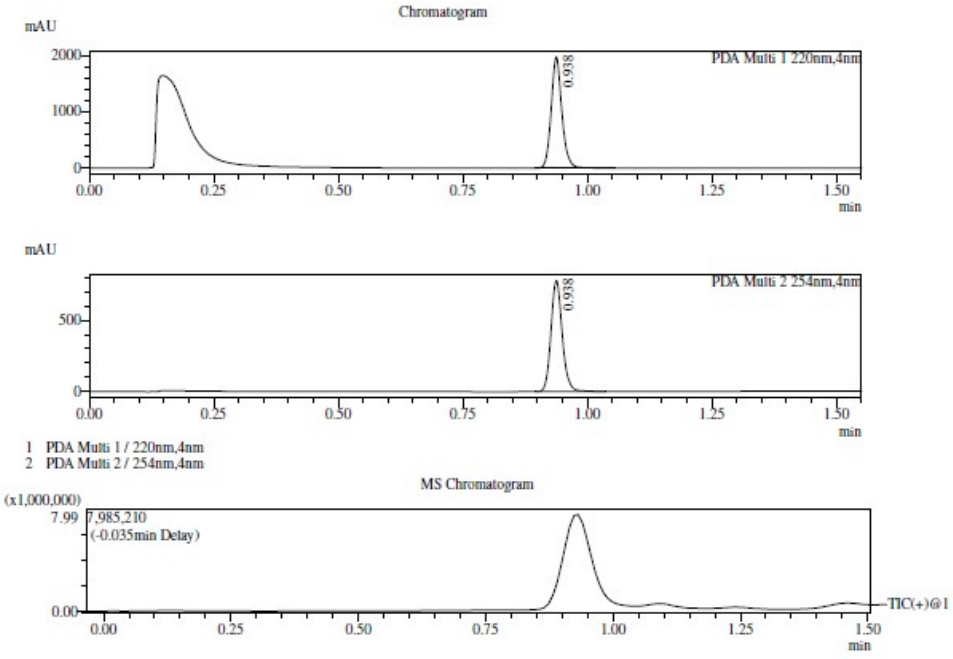
To a solution of **4** (170 mg, 410 µmol, 1 eq) and 4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6-morpholino-1,3,5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (255 mg, 517 µmol, 1.3 eq) in DMF (5 ml) was added HATU (340 mg, 894 µmol, 2.2 eq) and DIEA (255 mg, 1.97 mmol, 344 µl, 4.8 eq), then the resulting mixture was stirred at 20 °C for 2 h. LCMS showed full consumption of the starting material and the desired MS was detected. The reaction mixture was partitioned between ethyl acetate (60 ml) and brine (50 ml). The aqueous layer was extracted with ethyl acetate (100 ml) twice. The combined organic layers were dried over Na_2SO_4, filtrated and evaporated to give a crude material. The crude material was purified by Prep-HPLC (column: Unisil 3-100 C18 Ultra 150 x 50 mm x 3 µm; mobile phase: [water(FA)-ACN];B%: 48 %-78 %,7 min). The eluent solution was lyophilized to give final compounds **6** (20 – 60 % yield) as yellow solids.

The general procedure for the synthesis of the following final compounds:

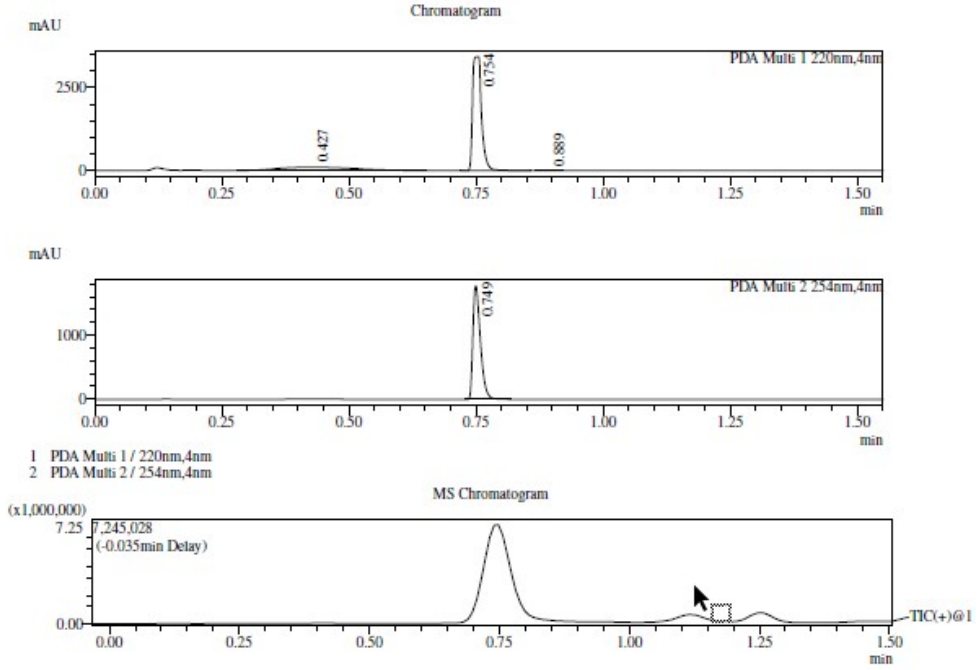
- WJ200-12 (linker 2a with n = 1, intermediate 3a and 4a)
- WJ201-13 (linker 2b with n = 2, intermediate 3b and 4b)
- WJ112-14 (linker 2c with n = 3, intermediate 3c and 4c)
- WJ202-15 (linker 2d with n = 4, intermediate 3d and 4d)
- WJ208-16 (linker 2e with n = 5, intermediate 3e and 4e)
- WJ209-17 (linker 2f with n = 6, intermediate 3f and 4f)
- WJ204-12 (linker 2g not linear, intermediate 3g and 4g, 1g starting material)
- WJ213-14 (linker 2h not linear, intermediate 3h and 4h)

WJ200-12 (linker 2a with n = 1, intermediate 3a and 4a)

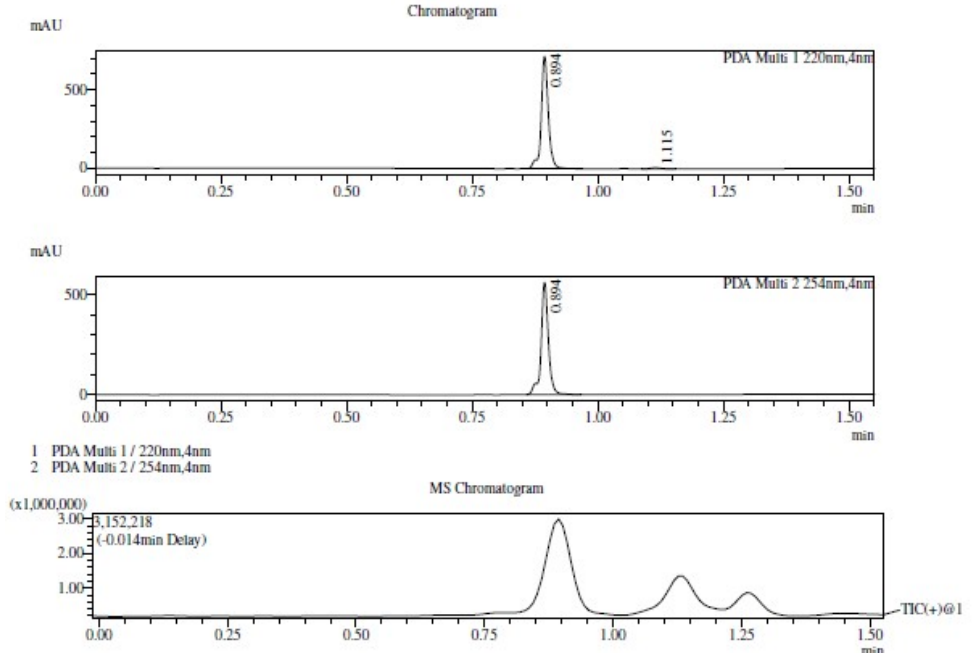
tert-butyl N-[7-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]heptyl]carbamate (3a)
UHPLC-ESIMS (m/z): 387.2 $[M\text{-}Boc+H]^+$, 509.2 $[M+Na]^+$



4-(7-aminoheptylamino) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (4a)
UHPLC-ESIMS (m/z): 387.1 $[M+H]^+$

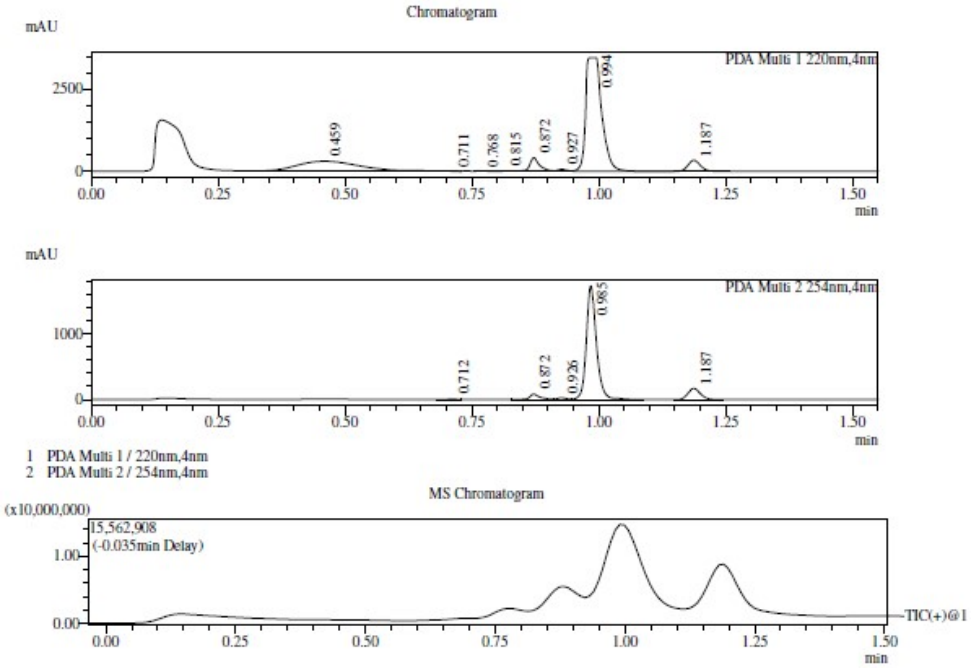


4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-N-[7-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]heptyl]-4-oxo-butanamide (WJ200-12)
^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 11.08 (s, 1H), 9.10 (s, 1H), 7.80 - 7.78 (m, 1H), 7.63 (t, J = 54.0 Hz, 1H), 7.62 - 7.50 (m, 3H), 7.08 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 7.0 Hz, 1H), 6.51 (br t, *J* = 5.8 Hz, 1H), 5.09 - 4.97 (m, 1H), 3.87 - 3.72 (m, 8H), 3.67 - 3.61 (m, 4H), 3.58 - 3.48 (m, 4H), 3.29 - 3.25 (m, 2H), 3.05 - 2.97 (m, 2H), 2.95 - 2.82 (m, 1H), 2.63-2.53 (m, 4H), 2.36 - 2.28 (m, 2H), 2.06 - 1.97 (m, 1H), 1.63 - 1.51 (m, 2H), 1.39 - 1.22 (m, 8H).
ESI-HRMS (m/z): $[M]+H^+$ calc. for $C_{40}H_{50}F_2N_{13}O_7$: 862.3916; found: 862.3919
UHPLC-ESIMS (m/z): 862.4 $[M+H]^+$

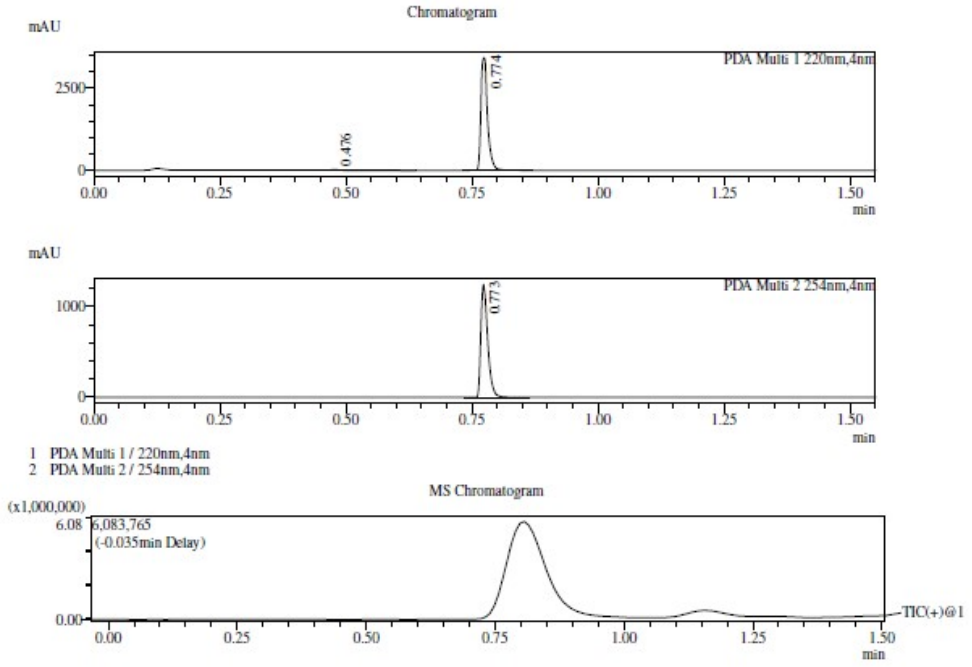


WJ201-13 (linker 2b with n = 2, intermediate 3b and 4b)

tert-butyl N-[8-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]octyl]carbamate (3b)
UHPLC-ESIMS (m/z): 401.2 $[M\text{-}Boc+H]^+$



4-(8-aminooctylamino) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (4b)
UHPLC-ESIMS (m/z): 401.2 $[M+H]^+$

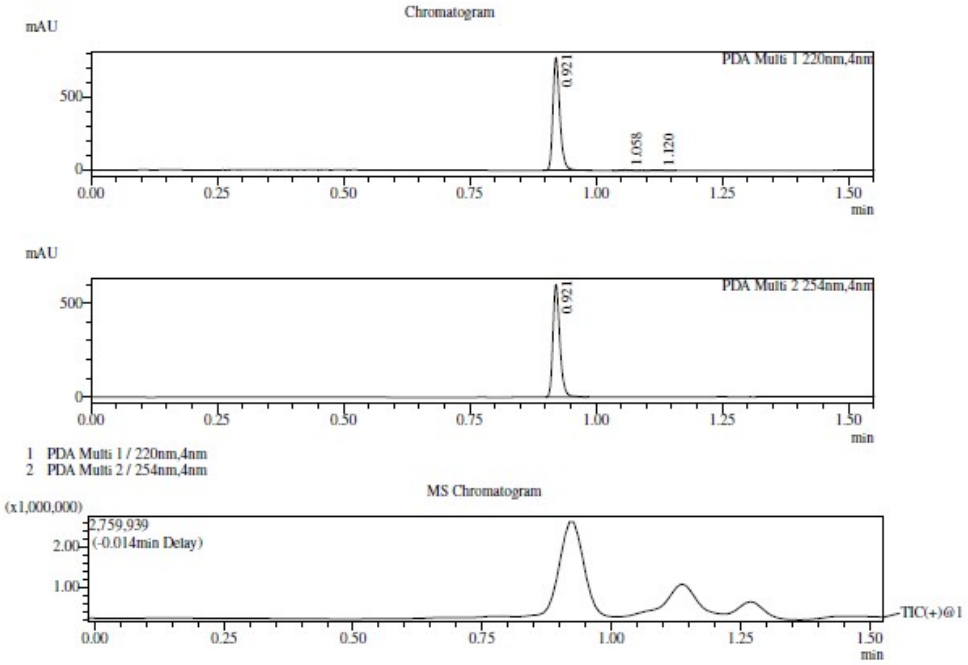


4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-N-[8-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]octyl]-4-oxo-butanamide (WJ201-13)

^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 11.08 (s, 1H), 9.11 (s, 1H), 7.77 (d, J = 5.6 Hz, 1H), 7.63 (t, J = 54.0 Hz, 1H), 7.62-7.51 (m, 3H), 7.08 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 7.0 Hz, 1H), 6.51 (t, J = 5.8 Hz, 1H), 5.11 - 4.96 (m, 1H), 3.86 - 3.71 (m, 8H), 3.68 - 3.62 (m, 4H), 3.58 - 3.48 (m, 4H), 3.30 - 3.25 (m, 2H), 3.06 - 2.95 (m, 2H), 2.93 - 2.82 (m, 1H), 2.63 - 2.53 (m, 4H), 2.37 - 2.29 (m, 2H), 2.08 - 1.96 (m, 1H), 1.63 - 1.51 (m, 2H), 1.39 - 1.23 (m, 10H).

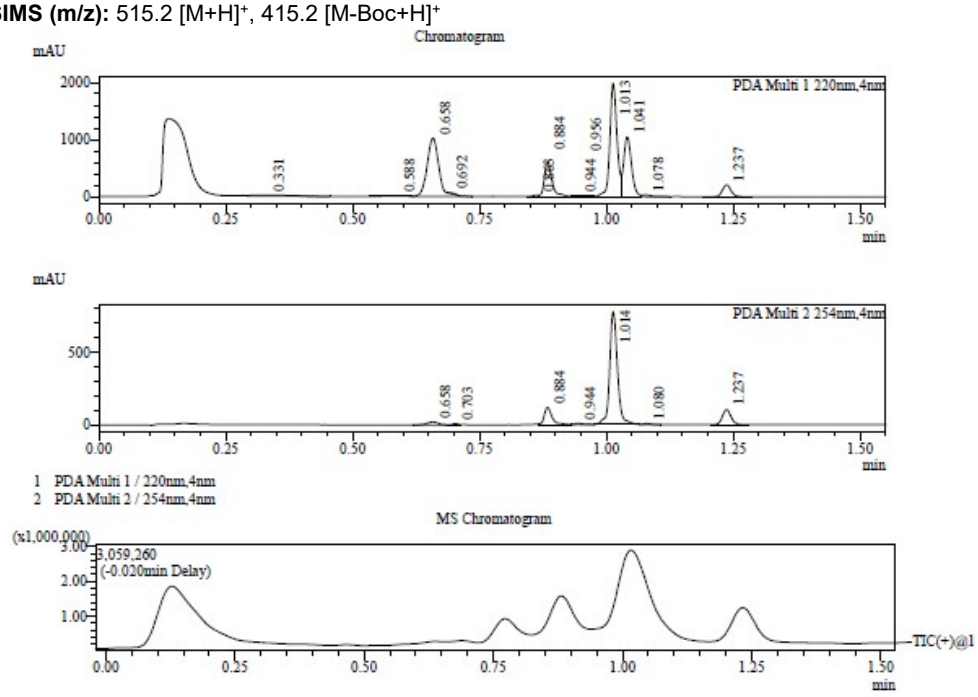
ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{41}H_{52}F_2N_{13}O_7$: 876.4070; found: 876.4075

UHPLC-ESIMS (m/z): 876.4 $[M+H]^+$

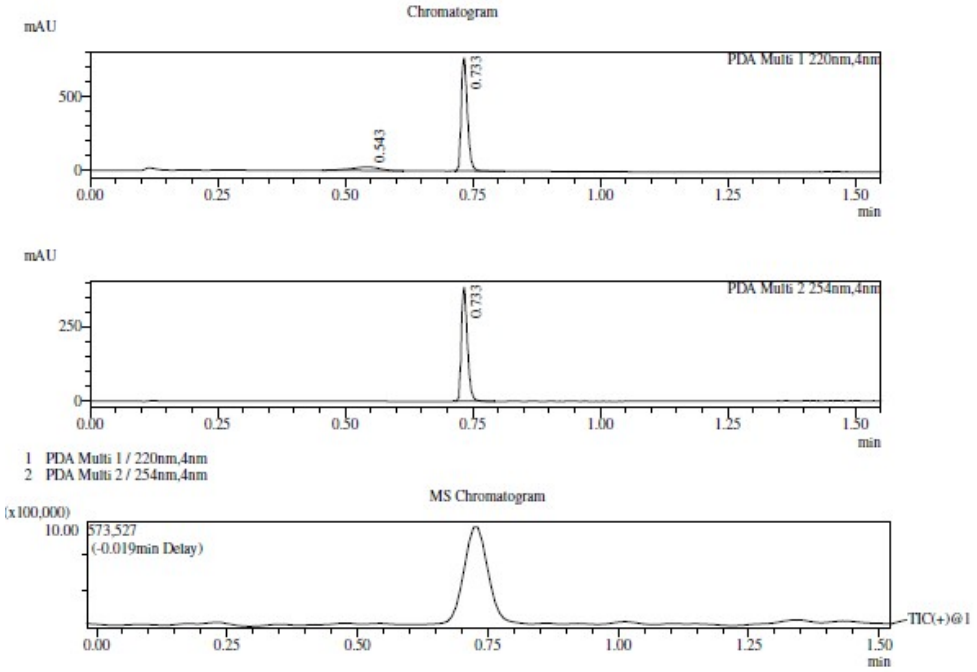


WJ112-14 (linker 2c with n = 3, intermediate 3c and 4c)

tert-butyl N-[9-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]nonyl]carbamate (3c)
UHPLC-ESIMS (m/z): 515.2 $[M+H]^+$, 415.2 $[M-Boc+H]^+$



4-(9-aminononylamino) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (4c)
UHPLC-ESIMS (m/z): 415.3 $[M+H]^+$

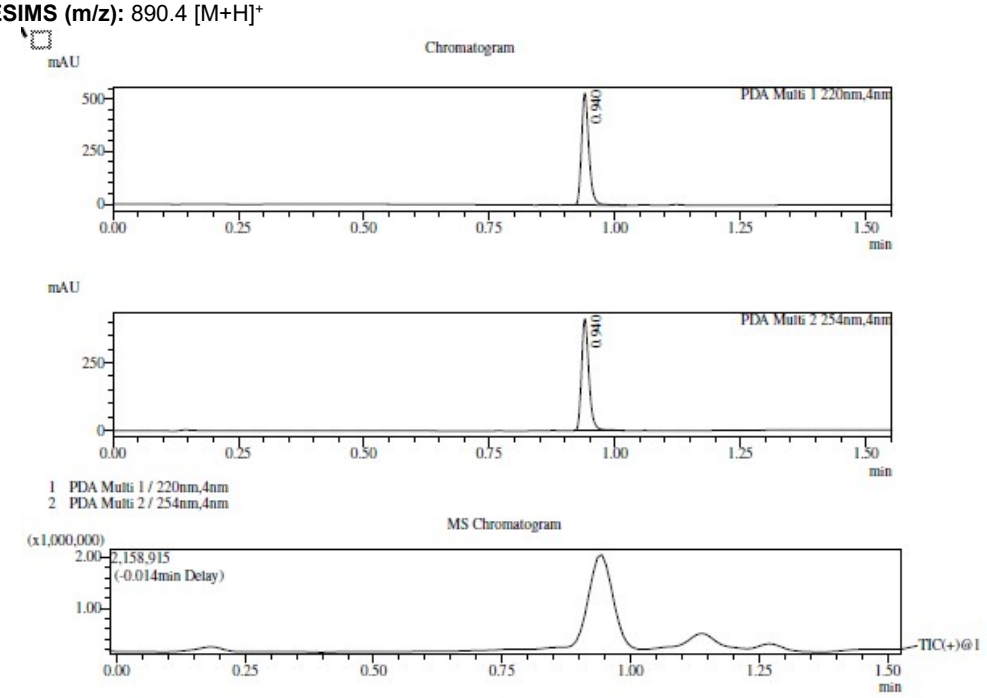


4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6-morpholino- 1,3,5-triazin-2-yl]piperazin-1-yl]-N-[9-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl] amino]nonyl]-4-oxo-butanamide (112-14)

^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 11.08 (s, 1H), 9.11 (s, 1H), 7.79-7.75 (m, 1H), 7.63 (t, J = 54.0 Hz, 1H), 7.61-7.52 (m, 3H), 7.08 (d, J = 8.6 Hz, 1H), 7.01 (d, J = 7.0 Hz, 1H), 6.57-6.46 (m, 1H), 5.09-4.97 (m, 1H), 3.87-3.71 (m, 8H), 3.67-3.61 (m, 4H), 3.57-3.48 (m, 4H), 3.29-3.23 (m, 2H), 3.05-2.96 (m, 2H), 2.93-2.81 (m, 1H), 2.65-2.54 (m, 4H), 2.38-2.26 (m, 2H), 2.08-1.97 (m, 1H), 1.61-1.51 (m, 2H), 1.37-1.21 (m, 12H).

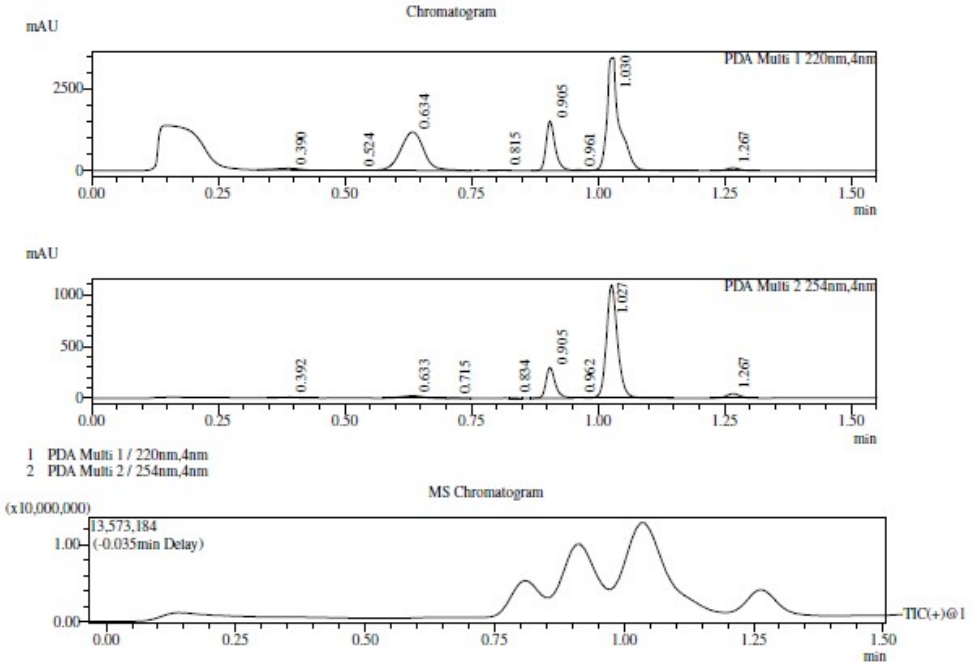
ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{42}H_{54}F_2N_{13}O_7$: 890.4228; found: 890.4232

UHPLC-ESIMS (m/z): 890.4 $[M+H]^+$

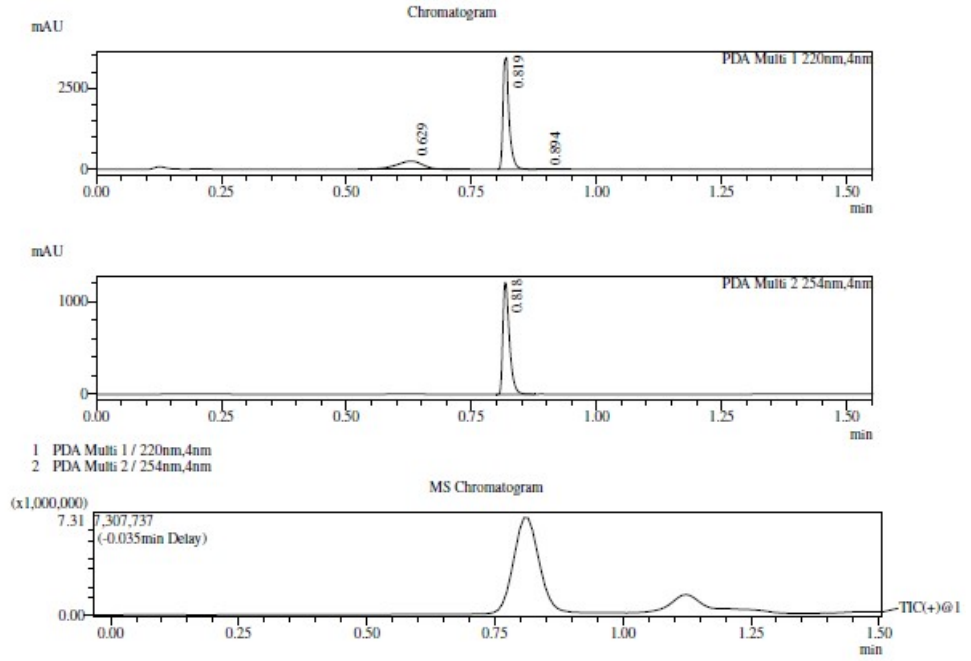


WJ202-15 (linker 2d with n = 4, intermediate 3d and 4d)

tert-butyl N-[10-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]decyl]carbamate (3d)
UHPLC-ESIMS (m/z): 429.2 $[M\text{-}Boc+H]^+$



4-(10-aminodecylamino) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (4d)
UHPLC-ESIMS (m/z): 429.2 $[M+H]^+$

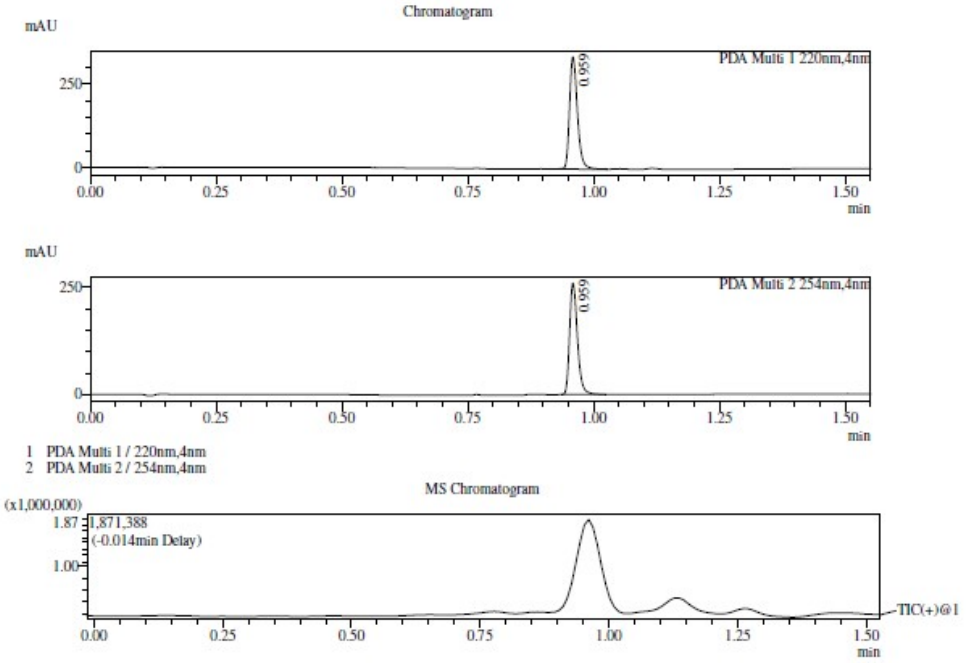


4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl] -6-morpholino-1,3,5-triazin-2-yl]piperazin-1-yl]-N-[10-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]decyl]-4-oxo-butanamide (WJ202-15)

^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 11.09 (s, 1H), 9.12 (s, 1H), 7.80 - 7.77 (m, 1H), 7.63 (t, J = 54.0 Hz, 1H), 7.61-7.51 (m, 3H), 7.09 (d, J = 8.6 Hz, 1H), 7.02 (d, J = 7.0 Hz, 1H), 6.52 (t, J = 5.9 Hz, 1H), 5.13 - 4.97 (m, 1H), 3.89 - 3.71 (m, 8H), 3.69 - 3.62 (m, 4H), 3.59 - 3.49 (m, 4H), 3.31 - 3.24 (m, 2H), 3.06 - 2.97 (m, 2H), 2.95 - 2.83 (m, 1H), 2.64 - 2.54 (m, 4H), 2.39 - 2.29 (m, 2H), 2.09 - 1.97 (m, 1H), 1.62 - 1.51 (m, 2H), 1.43 - 1.21 (m, 14H).

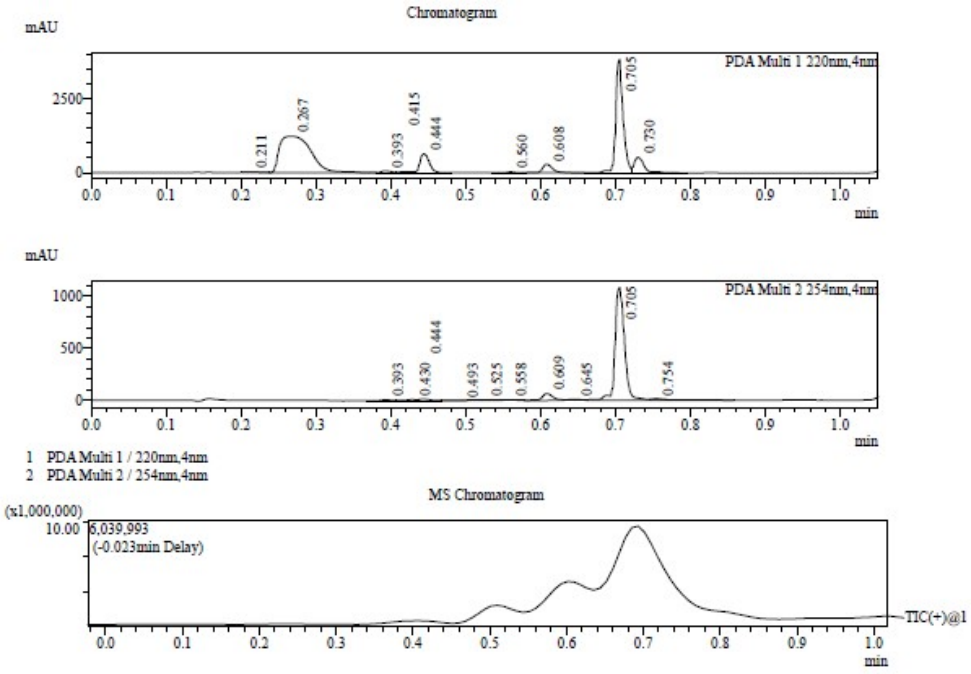
ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{43}H_{56}F_2N_{13}O_7$: 904.4382; found: 904.4388

UHPLC-ESIMS (m/z): 904.4 $[M+H]^+$

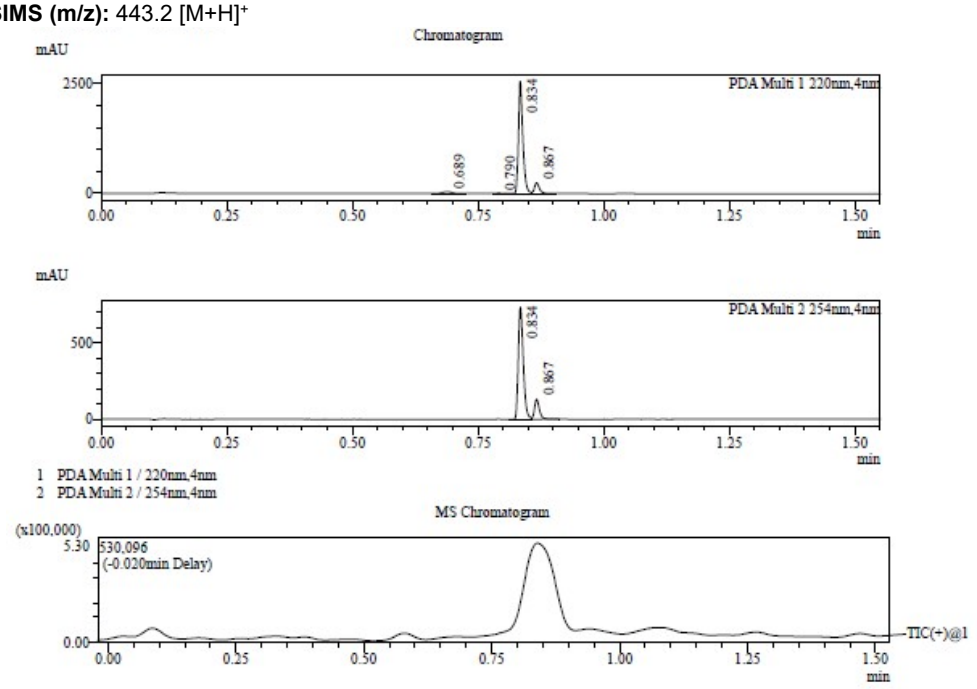


WJ208-16 (linker 2e with n = 5, intermediate 3e and 4e)

tert-butyl-N-[11-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]-amino]undecyl]carbamate (3e)
UHPLC-ESIMS (m/z): 443.2 $[M\text{-}Boc+H]^+$



4-(11-aminoundecylamino)-2-(2,6-dioxo-3-piperidyl)isoindoli-ne-1,3-dione (4e)
UHPLC-ESIMS (m/z): 443.2 $[M+H]^+$

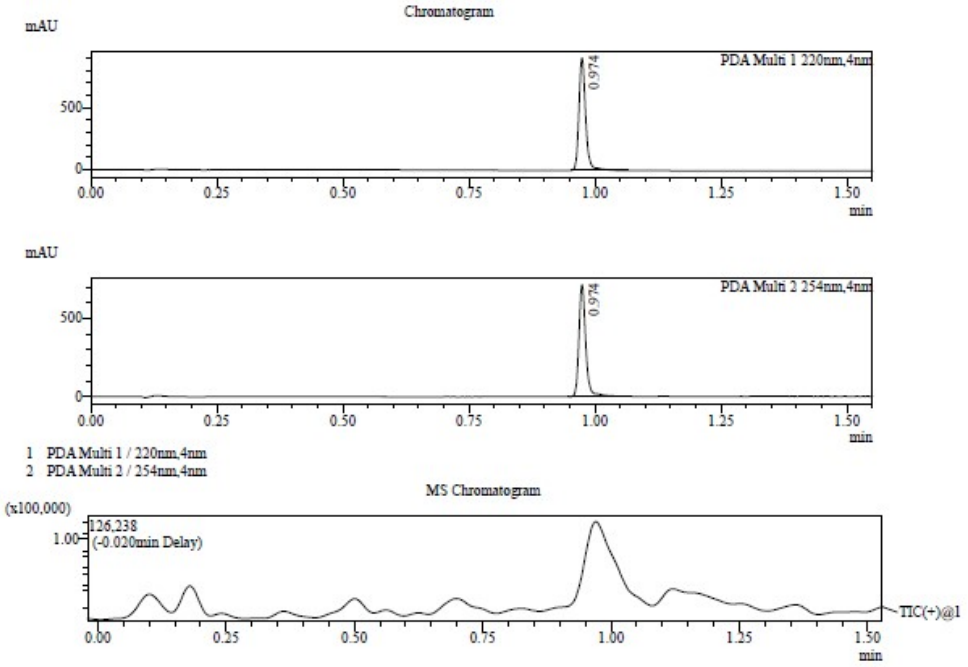


4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-N-[11-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]undecyl]-4-oxo-butanamide (WJ208-16)

^{1}H NMR (400 MHz, DMSO-d_6) *δ (ppm)*: 11.05 (br s, 1H), 9.11 (s, 1H), 7.80 - 7.76 (m, 1H), 7.63 (t, *J* = 54 Hz, 1H), 7.61 - 7.52 (m, 3H), 7.07 (d, *J* = 8.7 Hz, 1H), 7.01 (d, *J* = 7.0 Hz, 1H), 6.51 (br t, *J* = 5.8 Hz, 1H), 5.04 (dd, *J* = 5.4, 12.8 Hz, 1H), 3.85-3.70 (m, 8H), 3.67-3.62 (m, 4H), 3.56-3.49 (m, 4H), 3.29-3.24 (m, 2H), 3.04-2.96 (m, 2H), 2.93 - 2.82 (m, 1H), 2.63 - 2.54 (m, 4H), 2.36 - 2.28 (m, 2H), 2.07 - 1.97 (m, 1H), 1.60 - 1.51 (m, 2H), 1.36 - 1.22 (m, 16H).

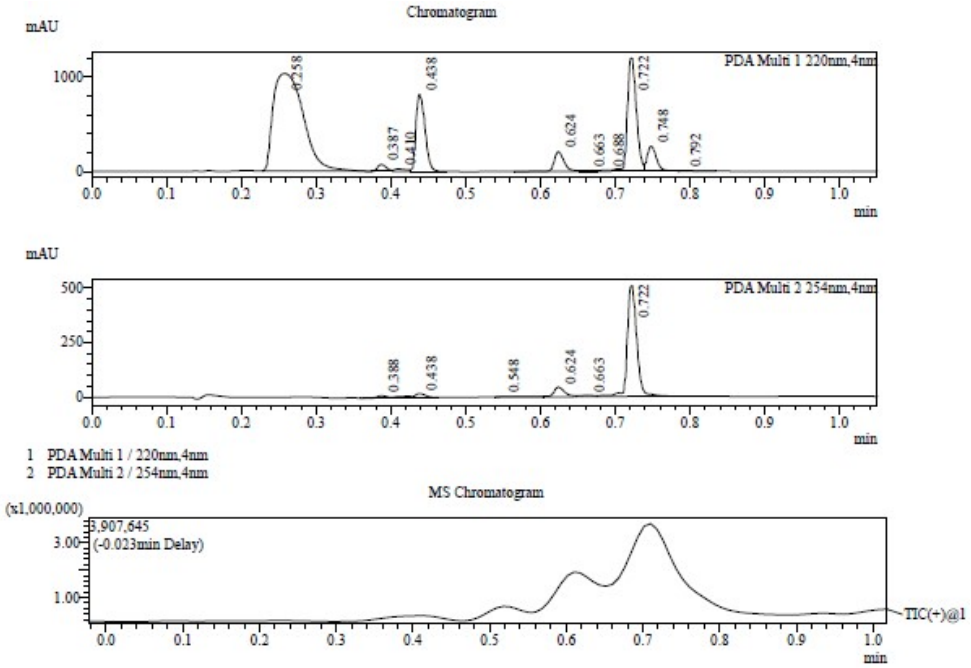
ESI-HRMS (m/z): $[M]+H^+$ calc. for $C_{44}H_{58}F_2N_{13}O_7$: 918.4539; found: 918.4545

UHPLC-ESIMS (m/z): 918.3 $[M+H]^+$

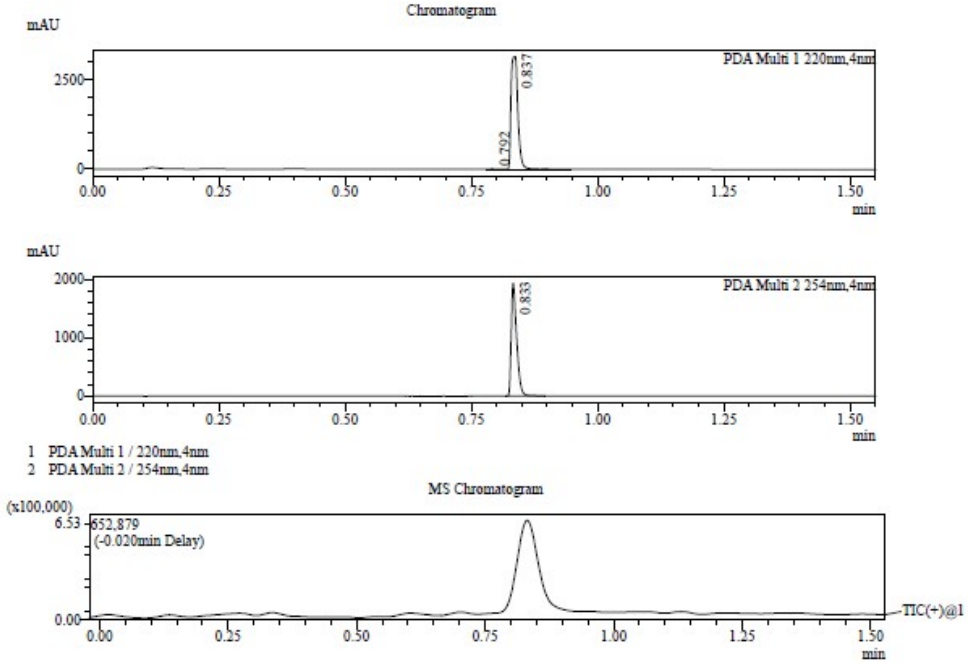


WJ209-17 (linker 2f with n = 6, intermediate 3f and 4f)

tert-butyl N-[12-[[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]amino]dodecyl]carbamate (3f)
UHPLC-ESIMS (m/z): 579.4 $[M+Na]^+$, 457.2 $[M-Boc+H]^+$



4-(12-aminododecylamino)-2-(2,6-dioxo-3-piperidyl)isoindo-line-1,3-dione (4f)
UHPLC-ESIMS (m/z): 457.3 $[M+H]^+$

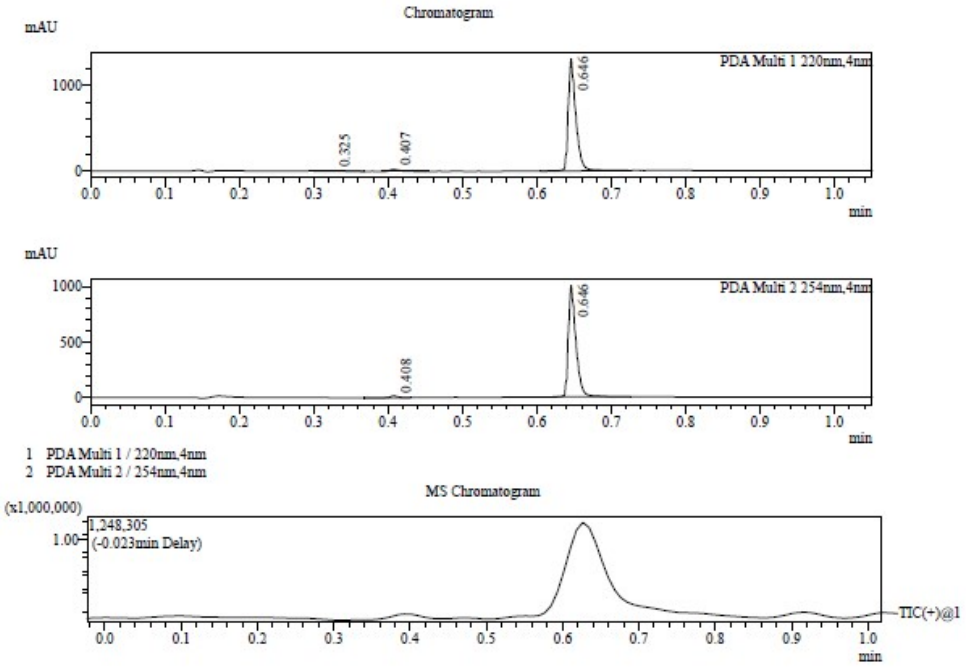


4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6-morpholino-1,3,5-triazin-2-yl]piperazin-1-yl]-N-[12-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]dodecyl]-4-oxo-butanamide (WJ209-17)

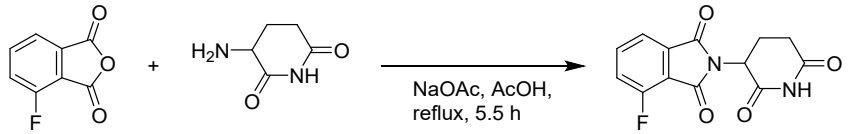
^{1}H NMR (400 MHz, DMSO-d_6) *δ (ppm)*: 11.07 (s, 1H), 9.10 (s, 1H), 7.80 - 7.73 (m, 1H), 7.63 (t, *J* = 54 Hz, 1H), 7.61 - 7.51 (m, 3H), 7.07 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 6.9 Hz, 1H), 6.50 (t, *J* = 5.2 Hz, 1H), 5.08-5.01 (m, 1H), 3.85 - 3.72 (m, 8H), 3.66-3.62 (m, 4H), 3.54-3.50 (m, 4H), 3.30 - 3.25 (m, 2H), 3.03 - 2.97 (m, 2H), 2.90 - 2.83 (m, 1H), 2.61 - 2.55 (m, 4H), 2.35 - 2.30 (m, 2H), 2.05 - 1.98 (m, 1H), 1.59-1.52 (m, 2H), 1.35 - 1.21 (m, 18H).

ESI-HRMS (m/z): $[M]+H^+$ calc. for $C_{45}H_{60}F_2N_{13}O_7$: 932.4697; found: 932.4701

UHPLC-ESIMS (m/z): 932.4 $[M+H]^+$



Synthesis of Compound 1



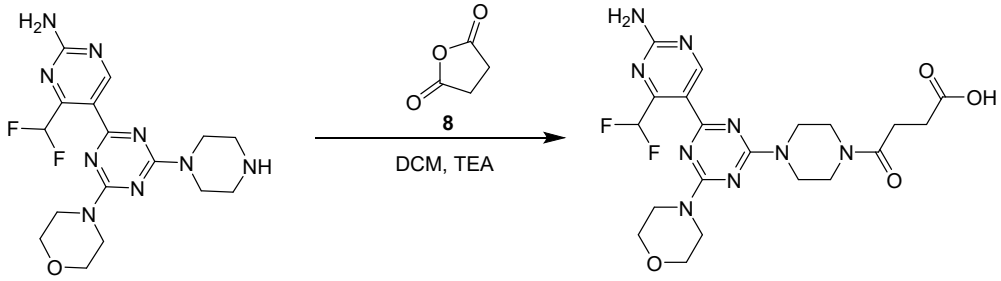
1

2-(2,6-Dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (7)

A round bottom flask was charged with 3-fluorophthalic anhydride (18.2 mmol, 3.02 g, 1 eq), 3-aminopiperidine-2,6-dione hydrochloride (3.00 g, 18.2 mmol, 1 eq) and sodium acetate (2.24 g, 27.3 mmol, 1.5 eq) in acetic acid (60 ml). The mixture was heated to reflux and stirred for 6 h. The reaction was allowed to cool to room temperature and the acetic acid was removed *in vacuo*. H_2O (80 ml) was added to the residue and subsequently extracted with EtOAc (4 x 80 ml). The combined organic layers were concentrated *in vacuo* and DCM was added to the crude and the insoluble solid was filtered off and washed with DCM to yield 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**1**, 4.32 g, 15.6 mmol, 86 %) as a grey powder.

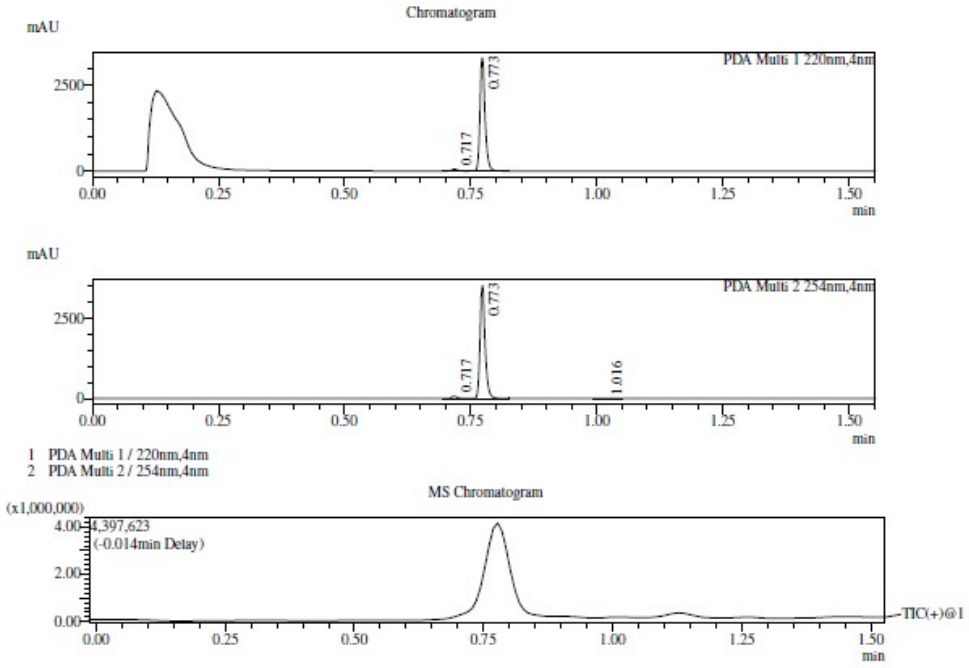
^{1}H-NMR (400 MHz, 298 K, DMSO-d_6, d/ppm): 11.14 (s, 1H), 7.97-7.92 (m, 1H), 7.80-7.71 (m, 2H), 5.18-5.14 (m, 1H), 2.94-2.85 (m, 1H), 2.64-2.53 (m, 2H), 2.10-2.03 (m, 1H).

Synthesis of Compound 5 (PQR514-derivative di-ketone exit vector)

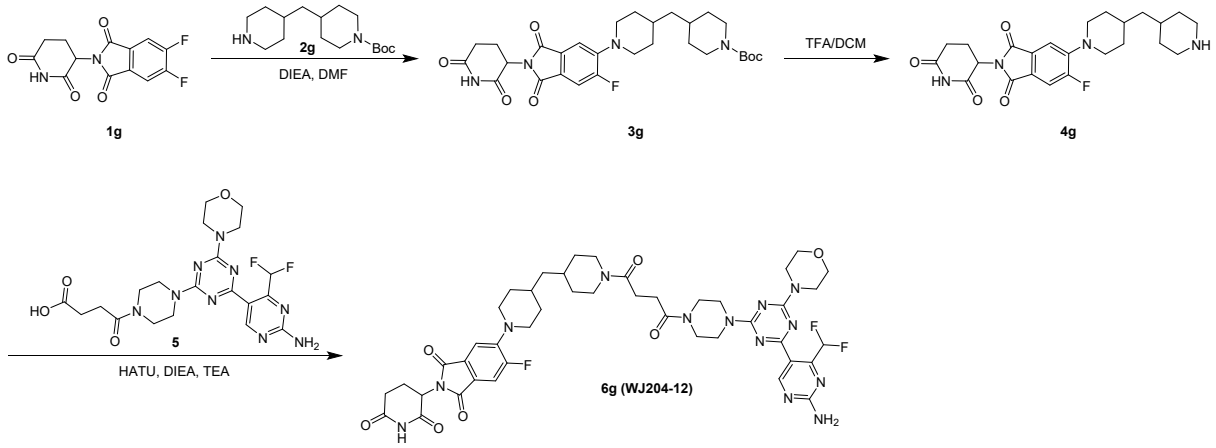


7 **5**

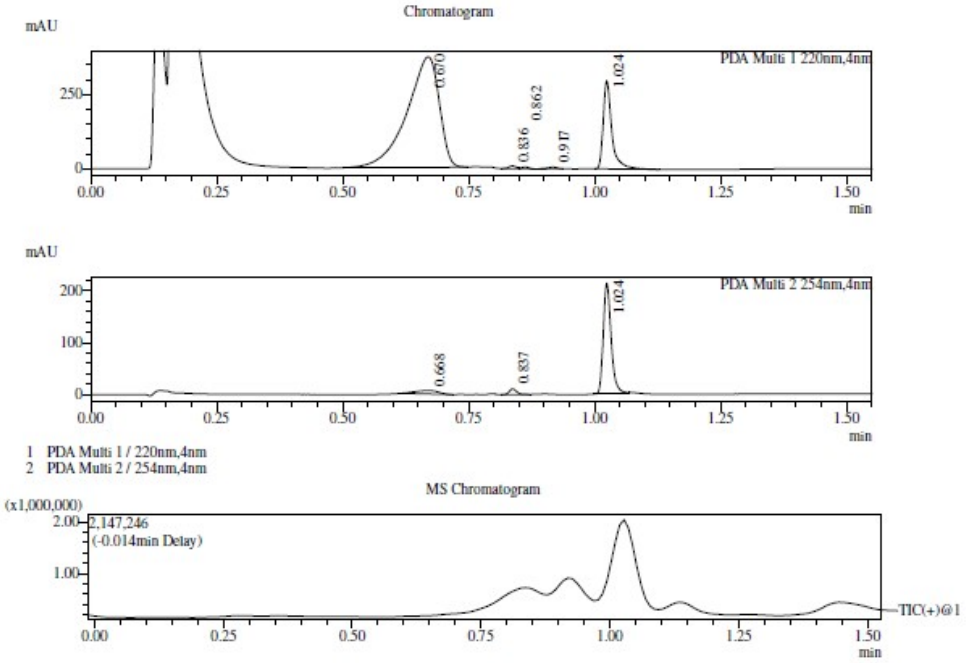
4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (5)
To a solution of 4-(difluoromethyl) -5-(4-morpholino-6-piperazin-1-yl-1, 3, 5-triazin-2-yl) pyrimidin-2-amine (**7**, 2.2 g, 4.34 mmol, 1 eq) and tetrahydrofuran-2, 5-dione (**8**, 500 mg, 5.00 mmol, 1.15 eq) in DCM (50 ml) was added TEA (1.45 g, 14.37 mmol, 2 ml, 3.31 eq) at 20 °C, then the reaction mixture was stirred at 20 °C for 12 h. LCMS showed some of 4-(difluoromethyl) -5-(4-morpholino-6-piperazin-1-yl-1, 3, 5-triazin-2-yl) pyrimidin-2-amine was consumed and desired MS was detected. The residue mixture was concentrated to give crude material. The crude product was triturated with 20 ml (PE/EtOAC=5/1), filtered to give 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (**5**, 3.00 g, crude) as a white solid.
^{1}H NMR (400 MHz, CDCl3) δ ppm: 8.93 (s, 1H), 7.63 (t, J = 54 Hz, 1H), 7.56 (s, 2H), 3.85 - 3.74 (m, 8H), 3.67-3.63 (m, 4H), 3.57-3.52 (m, 4H), 2.61 - 2.56 (m, 2H), 2.47 - 2.43 (m, 2H).
UHPLC-ESIMS (m/z): 494.2 $[M+H]^+$



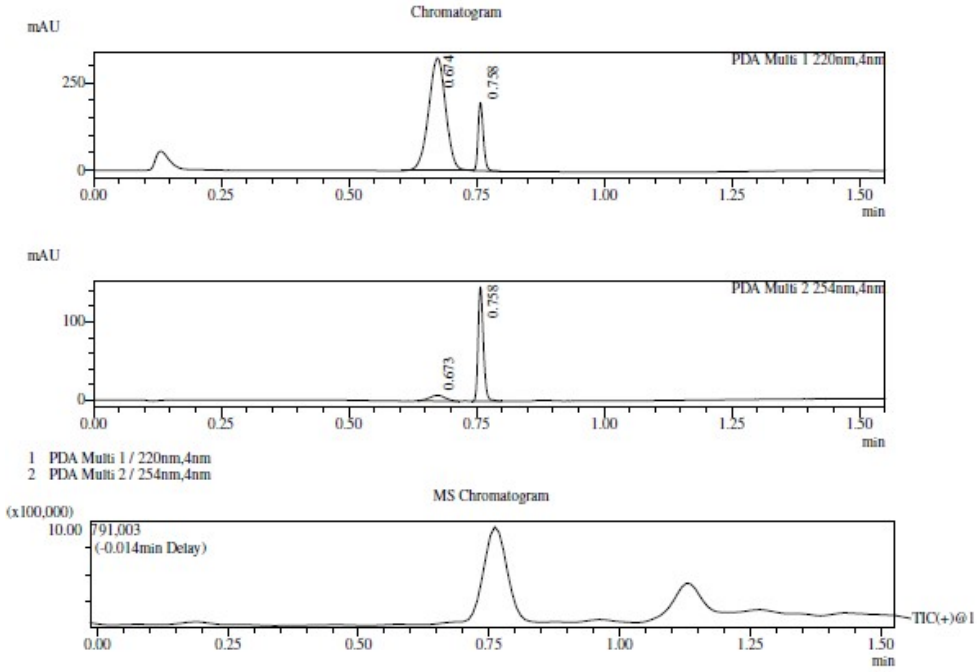
Synthesis of WJ204-12 (same General Procedure, different starting materials)



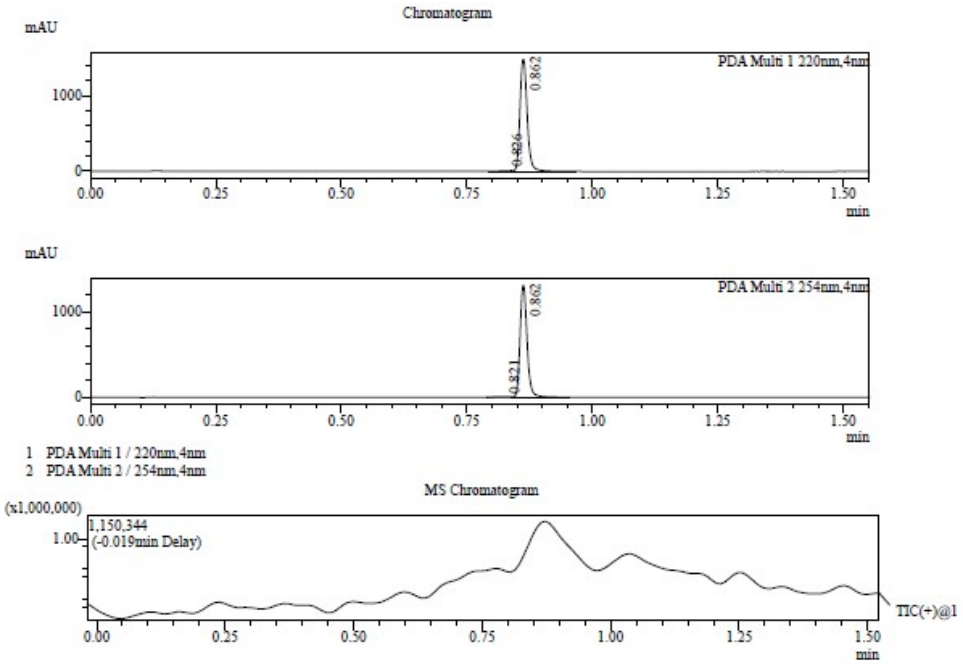
tert-butyl 4-[[1-[2-(2, 6-dioxo-3-piperidyl) -6-fluoro-1, 3-dioxo-isoindolin-5-yl]-4-piperidyl]methyl]piperidine-1-carboxylate (3g)
UHPLC-ESIMS (m/z): 579.3 $[M+Na]^+$, 457.2 $[M\text{-}Boc+H]^+$



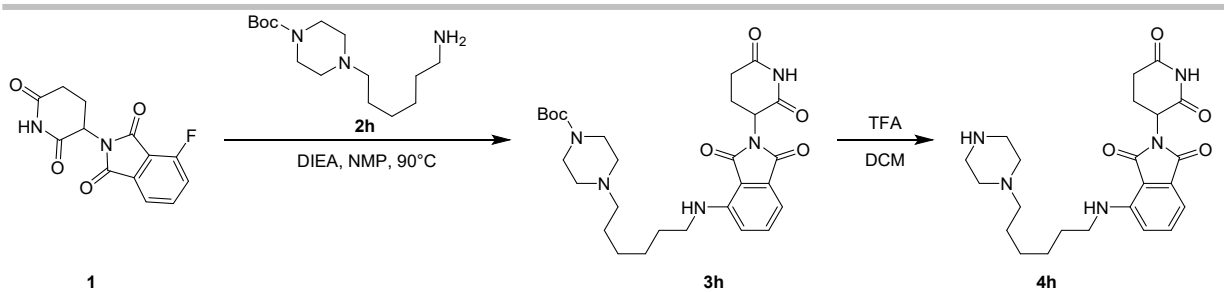
2-(2, 6-dioxo-3-piperidyl) -5-fluoro-6-[4-(4-piperidylmethyl) -1-piperidyl]isoindoline-1, 3-dione (4g)
UHPLC-ESIMS (m/z): 557.2 $[M+H]^+$

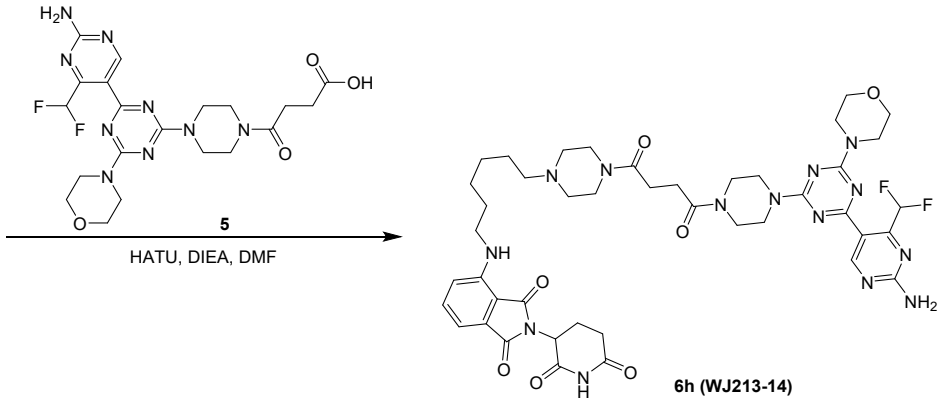


5-[4-[[1-[4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]- 6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]-4-piperidyl]methyl] -1-piperidyl]-2-(2, 6-dioxo-3-piperidyl) -6-fluoro-isoindoline-1, 3-dione (WJ204-12)
^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 11.09 (s, 1H), 9.11 (s, 1H), 7.69 (d, J = 11.4 Hz, 1H), 7.63 (t, J = 54.0 Hz, 1H), 7.57 (br s, 2H), 7.42 (d, J = 7.4 Hz, 1H), 5.13 - 5.05 (m, 1H), 4.40 - 4.30 (m, 1H), 3.95 - 3.73 (m, 8H), 3.71 - 3.62 (m, 4H), 3.62 - 3.54 (m, 4H), 3.54 - 3.48 (m, 2H), 3.05-2.89 (m, 2H), 2.88 - 2.80 (m, 2H), 2.68 - 2.52 (m, 6H), 2.08 - 1.97 (m, 1H), 1.82 - 1.72 (m, 2H), 1.72 - 1.49 (m, 4H), 1.32 - 1.21 (m, 2H), 1.20 - 1.12 (m, 2H), 1.10 - 0.98 (m, 1H), 0.97 - 0.82 (m, 1H).
ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{44}H_{53}F_3N_{13}O_7$: 932.4129; found: 932.4138
UHPLC-ESIMS (m/z): 932.4 $[M+H]^+$

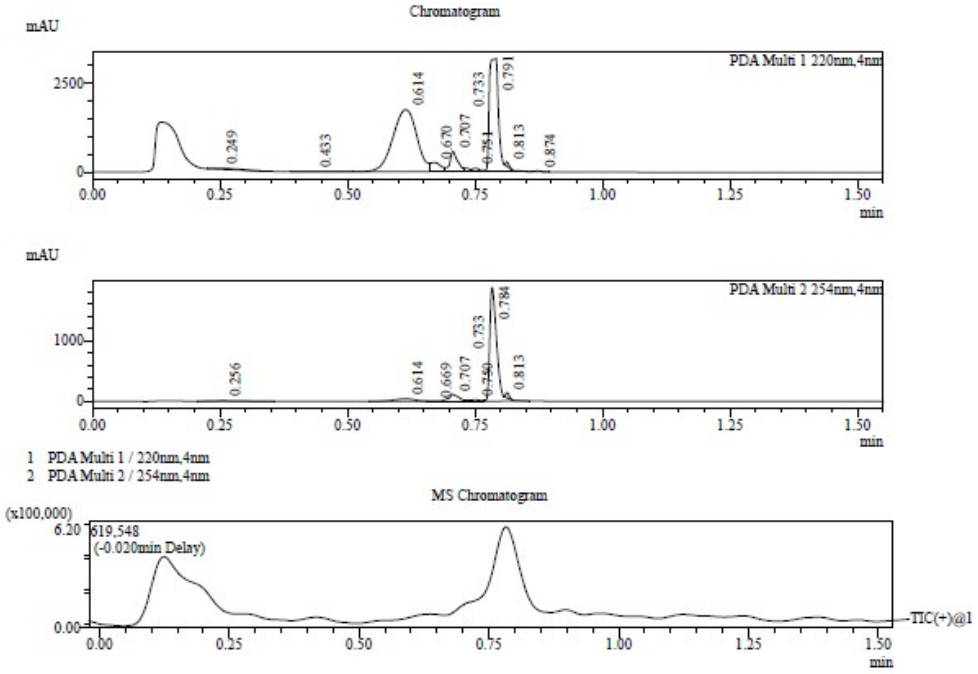


Synthesis of WJ213-14 (same General Procedure, not linear linker)

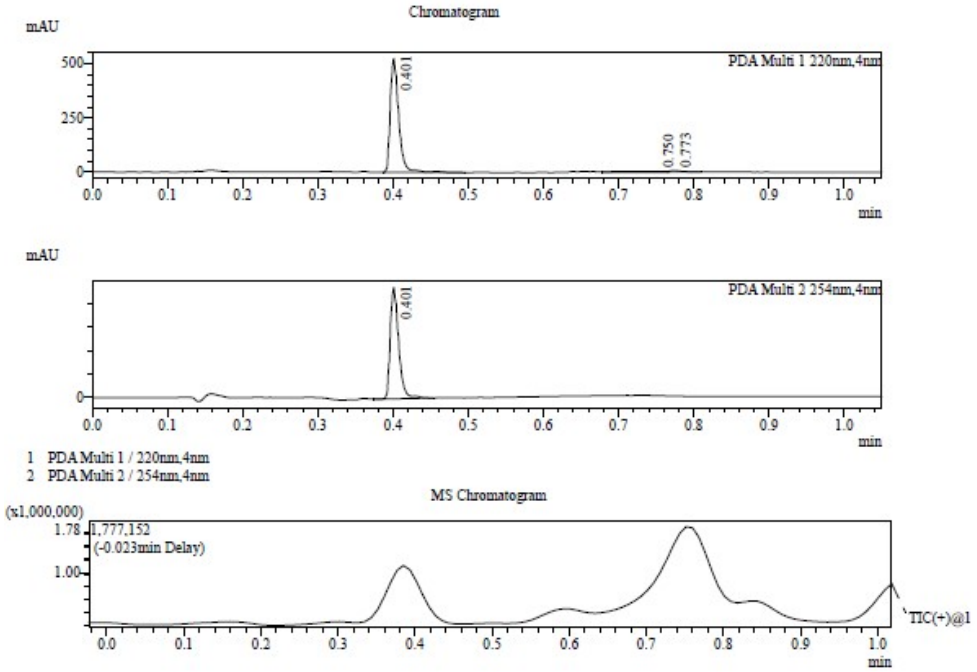




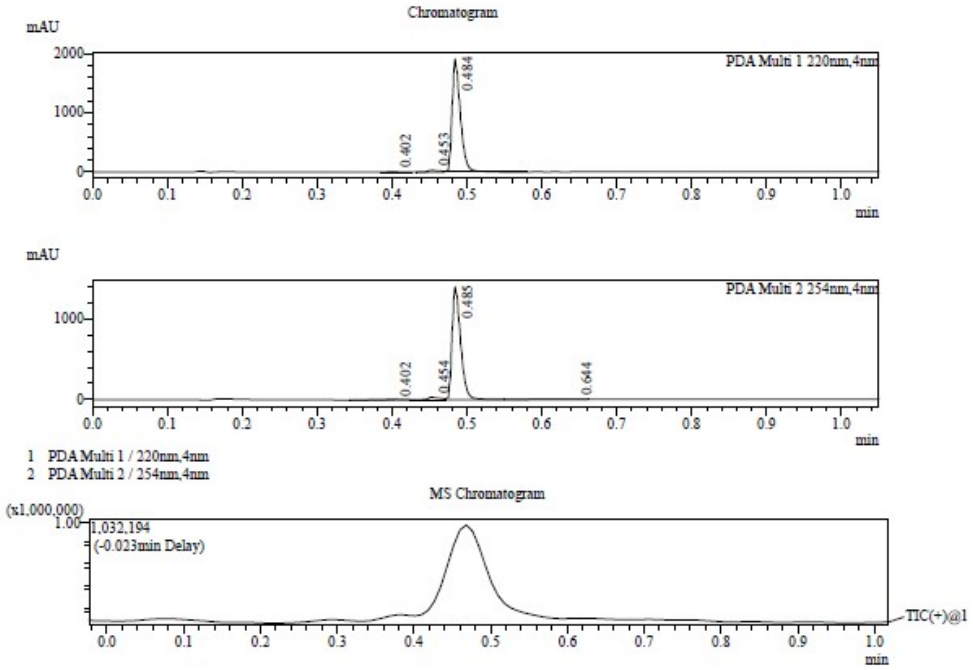
tert-butyl 4-[6-[[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]amino]hexyl]piperazine-1-carboxylate (3h)
UHPLC-ESIMS (m/z): 542.3 $[M+H]^+$



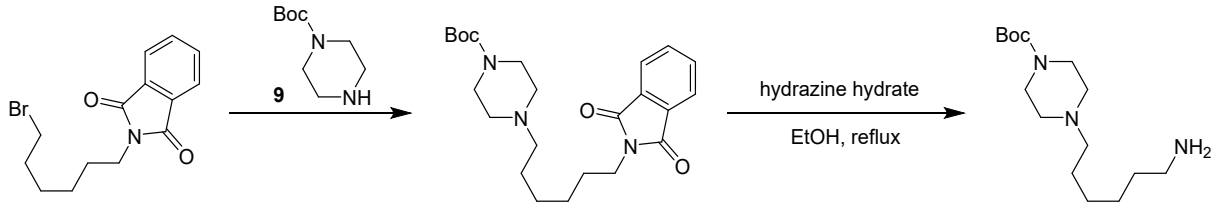
2-(2,6-dioxo-3-piperidyl)-4-(6-piperazin-1-ylhexylamino)isoindoline-1,3-dione (4h)
UHPLC-ESIMS (m/z): 442.2 $[M+H]^+$



4-[6-[4-[4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6-morpholino-1,3,5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]piperazin-1-yl]hexylamino]-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (WJ213-14)
^{1}H NMR (400 MHz, DMSO-d_6) δ = 11.09 (s, 1H), 9.10 (s, 1H), 7.50 (m, 5H), 7.17 - 6.91 (m, 2H), 6.51 (m, 1H), 5.04 (m, 1H), 3.75 (m, 12H), 3.64 (m, 12H), 3.29 (m, 2H), 2.97 - 2.78 (m, 2H), 2.71 - 2.59 (m, 3H), 2.38 - 2.22 (m, 6H), 2.08 - 1.97 (m, 1H), 1.57 (m, 2H), 1.49 - 1.26 (m, 6H).
ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{43}H_{55}F_2N_{14}O_7$: 917.4344; found: 917.4341
UHPLC-ESIMS (m/z): 917.4 $[M+H]^+$



Synthesis of linker 2h



tert-butyl 4-[6-(1,3-dioxoisoindolin-2-yl)hexyl]piperazine-1-carboxylate (10)

To a mixture of 2-(6-bromohexyl)isoindoline-1,3-dione (**8**, 1 g, 3.22 mmol, 1 eq), NaI (500 mg, 3.34 mmol, 1.03 eq) , TEA (650 mg, 6.42 mmol, 1 ml, 1.99 eq) in THF (10 ml) was added tert-butyl piperazine-1-carboxylate (**9**, 600 mg, 3.22 mmol, 1 eq), and then the mixture was stirred at 70 °C for 12 h. LC-MS indicated the mass of desired product was detected. TLC (Petroluem/ EtOAc=1:2) indicated a major spot observed. The reaction mixture was diluted with water (80 ml), extracted with EtOAc (3x 60 ml). The organic layer was washed with brine (2x 60 ml), dried over Na_2SO_4, filtered and concentrated to give the crude product. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~20% Ethyl acetate/Petroleum ethergradient @ 30 ml min^{-1}) to give tert-butyl 4-[6-(1,3-dioxoisoindolin-2-yl)hexyl]piperazine-1-carboxylate (**10**, 960 mg, 2.31 mmol, 72 % yield) as a white solid.

UHPLC-ESIMS (m/z): 416.2 $[M+H]^+$

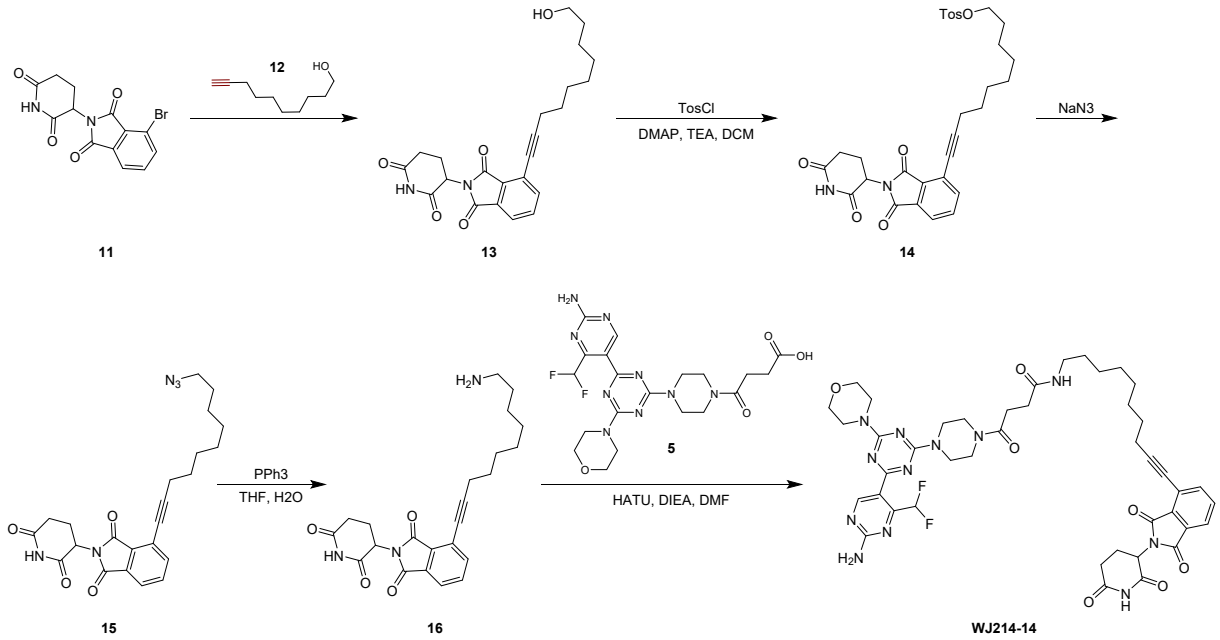
^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.90 - 7.80 (m, 4H), 3.55 (t, *J* = 7.1 Hz, 2H), 3.26 (d, *J* = 4.1 Hz, 4H), 2.27 - 2.21 (m, 6H), 1.59 (m, 2H), 1.43 - 1.34 (m, 11H), 1.32 - 1.24 (m, 4H).

tert-butyl 4-(6-aminohexyl) piperazine-1-carboxylate (2h)

To a mixture of tert-butyl 4-[6-(1, 3-dioxoisoindolin-2-yl) hexyl]piperazine-1-carboxylate (900 mg, 2.17 mmol) in EtOH (10 ml) was added $NH_2NH_2 \cdot H_2O$ (600 mg, 11.9 mmol), then the mixture was stirred at 90 °C for 1 h. The mixture was cooled to 20 °C and filtered. The filtrate was concentrated to give the crude product. The crude product was triturated with DCM (30 ml), filtered to give filtrate. The filtrate was concentrated to give tert-butyl 4-(6-aminohexyl) piperazine-1-carboxylate (**2h**, 400 mg, 1.40 mmol, 64.7 % yield) as yellow oil.

^{1}H NMR (400 MHz, $CHCl_3$-d) δ = 3.48-3.34 (m, 4 H), 2.68 (t, J=7.2 Hz, 2 H), 2.43-2.27 (m, 6 H), 1.56-1.39 (m, 17 H).

Synthesis of WJ214-14

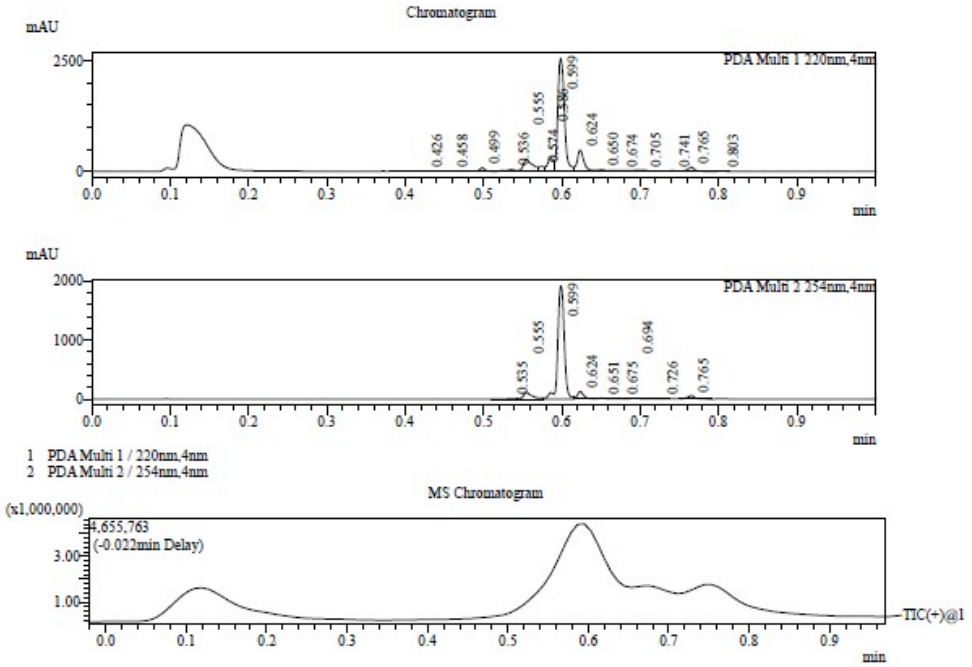


2-(2,6-dioxo-3-piperidyl)-4-(10-hydroxydec-1-ynyl)isoindoline-1,3-dione (13)

To a mixture of 4-bromo-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (**11**, 2 g, 5.9 mmol, 1 eq), dec-9-yn-1-ol (**12**, 1.00 g, 6.48 mmol, 1.1 eq), TEA (7.27 g, 71.9 mmol, 10 ml, 12 eq) in DMF (10 ml) was added $Pd(PPh_3)_2Cl_2$ (400 mg, 569 μmol,0.096 eq) and CuI (120 mg, 630 μmol, 0.1 eq), then the mixture was stirred at 80 °C for 12 h under N_2 atmosphere. LC-MS indicated desired MS was detected. TLC (Petroluem/ EtOAc=1:2) indicated a major spot observed. The mixture was poured into water (60 ml) and extracted with EtOAc (3x 60 ml). The organic layer was washed with NH_4Cl (2x 60 ml), brine (3x 60 ml), dried over Na_2SO_4, filtered and concentrated to give the crude product. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0~20% Ethyl acetate/Petroleum ethergradient @ 30 ml min^{-1}) to give 2-(2,6-dioxo-3-piperidyl)-4-(10-hydroxydec-1-ynyl)isoindoline-1,3-dione (**13**, 2.3 g, 5.60 mmol, 94.5 % yield) as a white solid.

^{1}H NMR (400 MHz, DMSO-d6) δ = 11.14 (s, 1H), 7.92 - 7.77 (m, 3H), 5.15 (dd, J = 5.3, 12.7 Hz, 1H), 4.33 (t, J = 5.1 Hz, 1H), 3.40 - 3.36 (m, 3H), 3.00 - 2.81 (m, 1H), 2.57 (br d, J = 11.9 Hz, 2H), 2.12 - 2.00 (m, 2H), 1.66 - 1.55 (m, 2H), 1.51 - 1.37 (m, 4H), 1.29 (br s, 6H).

UHPLC-ESIMS (m/z): 411.3 $[M+H]^+$



10-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]dec-9-ynyl 4-methylbenzenesulfonate (14)

To a mixture of 2-(2,6-dioxo-3-piperidyl)-4-(10-hydroxydec-1-ynyl)isoindoline-1,3-dione (**13**, 1 g, 2.44 mmol, 1 eq), DMAP (30 mg, 246 µmol, 0.1 eq), TEA (1.24 g, 12.2 mmol, 1.7 ml, 5 eq) in DCM (10 ml) was added 4-methylbenzenesulfonyl chloride (650 mg, 3.41 mmol, 1.4 eq), then the mixture was stirred at 0-20 °C for 12 h. TLC (Petroluem/ EtOAc=1:1) indicated the starting material was consumed completely and a major spot observed. The mixture was poured into water (60 ml) and extracted with EtOAc (3x 60 ml). The organic layer was washed with NH_4Cl (3x 60 ml), brine (3x 60 ml). The organic layer was dried over Na_2SO_4, filtered and concentrated to give 10-[2-(2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]dec-9-ynyl 4-methylbenzenesulfonate (**14**, 1.1 g, crude) as a colorless oil.

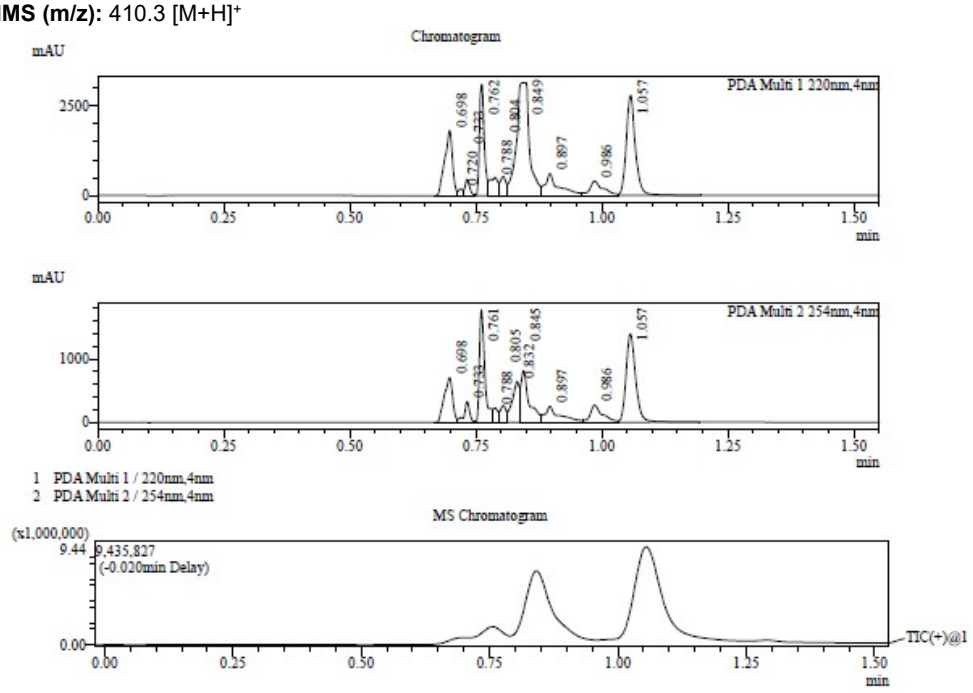
4-(10-azidodec-1-ynyl) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (15)

To a solution of 10-[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]dec-9-ynyl 4-methylbenzenesulfonate (**14**, 1.1 g, 1.95 mmol) in DMF (20 ml) was added NaN_3 (210 mg, 3.23 mmol), then the mixture was stirred at 60 °C for 2 h. TLC (Petroluem/ EtOAc=5:1) indicated a major spot observed. The mixture was poured into sat. $NaHCO_3$ (60 ml) and extracted with EtOAc (3x 60 ml). The organic layers were washed with brine (3x 100 ml), dried over Na_2SO_4, filtered and concentrated to give the crude product. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0 – 30 % Ethyl acetate/ Petroleum ethergradient @ 30 ml min^{-1}) to give 4-(10-azidodec-1-ynyl) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (**15**, 500 mg, 1.15 mmol, 58.9 % yield) as a white solid.

4-(10-aminodec-1-ynyl) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (16)

To a solution of 4-(10-azidodec-1-ynyl) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (**15**, 500 mg, 1.15 mmol, 1 eq) in THF (5 ml) and H_2O (2 ml) was added PPh_3 (364 mg, 1.39 mmol, 1.2 eq). The mixture was stirred at 60 °C for 2 h. TLC (Petroluem/ EtOAc=2:1) indicated a major spot observed. The mixture was poured into $NaHCO_3$ (60 ml) and extracted with EtOAc (3x 60 ml). The organic layer was washed with brine (3x 60 ml). The organic layer was dried over Na_2SO_4, filtered and concentrated to give the crude product. The residue was purified by flash silica gel chromatography (ISCO®; 20 g SepaFlash® Silica Flash Column, Eluent of 0 – 20 % Ethyl acetate/Petroleum ethergradient @ 40 ml min^{-1}) to give 4-(10-aminodec-1-ynyl) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (**16**, 560 mg, 172 µmol, 15 % yield, 12.6 % purity) as a white solid.

UHPLC-ESIMS (m/z): 410.3 $[M+H]^+$



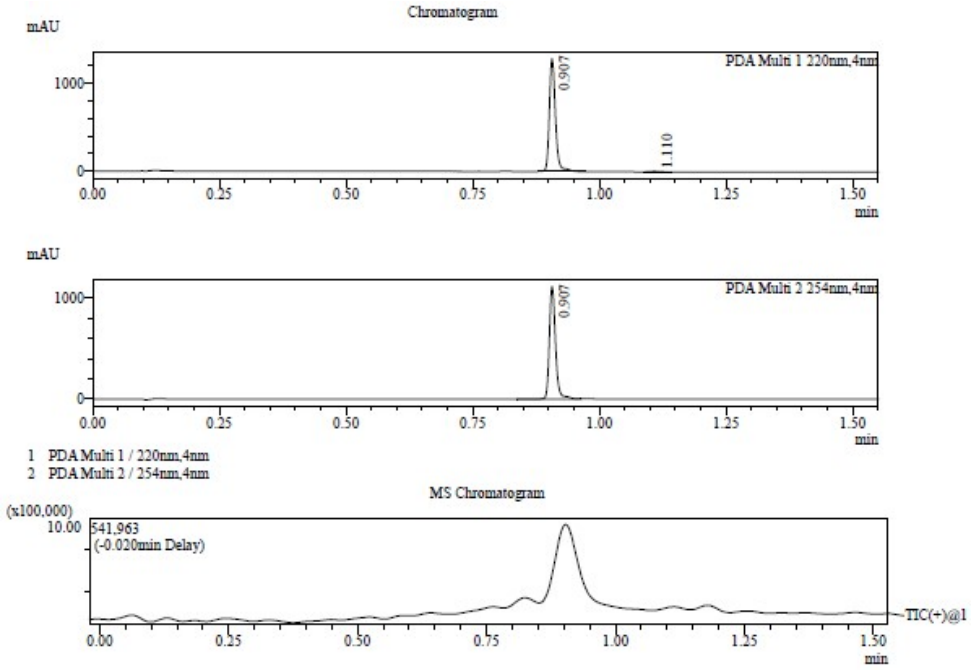
4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-N-[10-[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]dec-9-ynyl]-4-oxo-butanamide (WJ214-14)

To a solution of 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (200 mg, 405 μmol, 1.1 *eq*), HATU (190 mg, 500 μmol, 1.4 *eq*), DIEA (148.40 mg, 1.15 mmol, 200 μl, 3.1 *eq*) in DMF (5 ml) was added 4-(10-aminodec-1-ynyl) -2-(2, 6-dioxo-3-piperidyl) isoindoline-1, 3-dione (300 mg, 366 μmol, 50 % purity, 1 *eq*). The mixture was stirred at 20 °C for 2 h. LC-MS (EW35368-137-P1A) indicated the Ms of desired product was detected. The mixture was poured into water (60 mL) and extracted with EtOAc (60 mL*3). The organic layer was washed with $NaHCO_3$ (2x 60 ml), NH_4Cl (2x 60 ml), brine (3x 60 ml), dried over Na_2SO_4, filtered and concentrated to give the crude product. The crude product was purified by prep-HPLC (column: Phenomenex Luna C18 150 x 25 mm x 10μm;mobile phase: [water (FA) -ACN];B%: 39 % - 69 %, 10 min). After Prep-HPLC purification, the eluent was concentrated to remove organic solvents. The residual aqueous solution was lyophilized to give 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-N-[10-[2-(2, 6-dioxo-3-piperidyl) -1, 3-dioxo-isoindolin-4-yl]dec-9-ynyl]-4-oxo-butanamide (**WJ214-14**, 40 mg, 45 μmol, 12 % yield, 99 % purity) was obtained as a white solid.

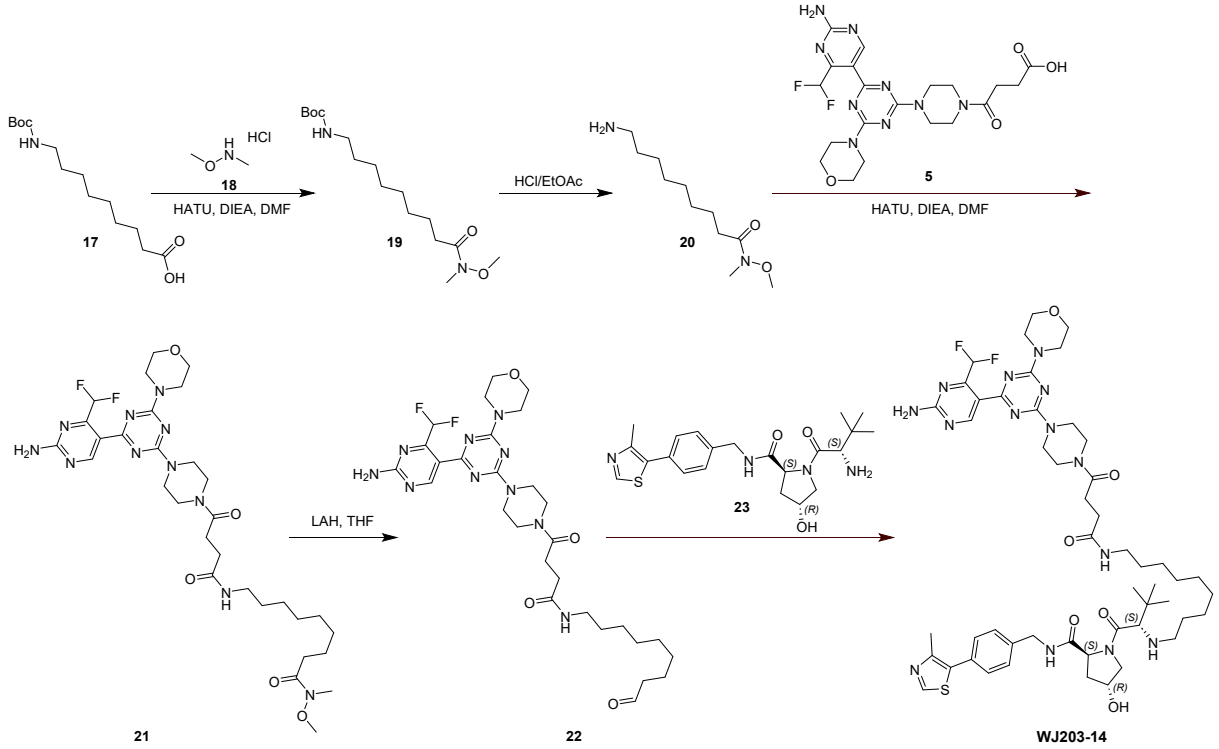
^{1}H NMR (400 MHz, DMSO) *δ(ppm)*: 11.31 (s, 1H), 9.11 (s, 1H), 7.87 - 7.47 (m, 7H), 5.13 (dd, *J* = 5.4, 12.7 Hz, 1H), 3.91 - 3.47 (m, 16H), 3.01 (m, 2H), 2.94 - 2.82 (m, 1H), 2.65 - 2.54 (m, 6H), 2.36 - 2.30 (m, 2H), 2.10 - 2.02 (m, 1H), 1.63 - 1.52 (m, 2H), 1.47 (br d, *J* = 5.1 Hz, 2H), 1.41 - 1.34 (m, 2H), 1.32 - 1.23 (m, 6H).

ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{43}H_{51}F_2N_{12}O_7$: 885.3952; found: 885.3966

UHPLC-ESIMS (m/z): 885.5 $[M+H]^+$



Synthesis of WJ203-14



tert-butyl N-[9-[methoxy (methyl) amino]-9-oxo-nonyl]carbamate (19)

To a mixture of 9-(tert-butoxycarbonylamino) nonanoic acid (**17**, 500 mg, 1.83 mmol, 1 *eq*), DIEA (742 mg, 5.74 mmol, 1 ml, 3.14 *eq*) in DMF (5 ml) was added HATU (800 mg, 2.1 mmol, 1.2 *eq*), then the mixture was stirred at 10 °C for 0.5 h. N-methoxymethanamine (**18**, 200 mg, 2 mmol, 1.1 *eq*, HCl) was added and the mixture was stirred at 10 °C for another 12 h. TLC (Petroluem/ EtOAc=1:1) indicated the starting material was consumed completely and a major spot observed. The reaction mixture was diluted with EtOAc (50 mL) and the resulting mixture was washed with brine (3x 30 ml). The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated to give the crude product. The residue was purified by flash silica gel chromatography (ISCO®; 24 g SepaFlash® Silica Flash Column, Eluent of 0-50 % Ethyl acetate/Petroleum ethergradient @ 50 mL/min) to give tert-butyl N-[9-[methoxy (methyl) amino]-9-oxo-nonyl]carbamate (**19**, 400 mg, 1.26 mmol, 69 % yield) as colorless oil.

9-amino-N-methoxy-N-methyl-nonanamide (20)

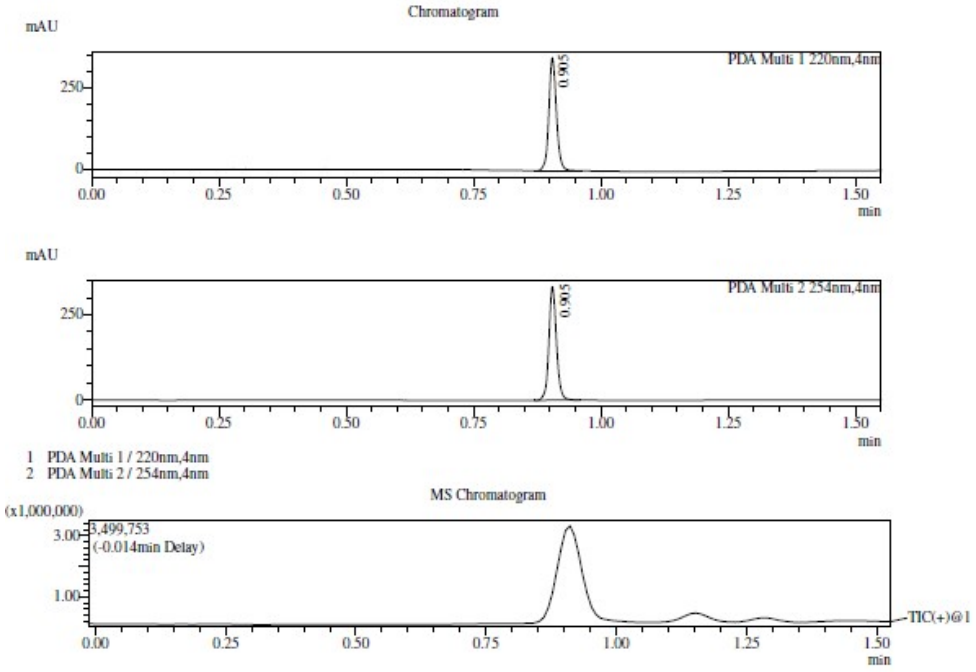
To a mixture of tert-butyl N-[9-[methoxy (methyl) amino]-9-oxo-nonyl]carbamate (**19**, 400 mg, 1.26 mmol, 1 *eq*) in EtOAc (1 ml) was added HCl/EtOAc (4 M, 800 µl, 2.53 *eq*), then the mixture was stirred at 10 °C for 2 h. TLC (Petroluem/ EtOAc=1:1) indicated the starting material was consumed completely and a major spot observed. The mixture was concentrated to give 9-amino-N-methoxy-N-methyl-nonanamide (**20**, 300 mg, crude, HCl) as white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 7.91 (s, 3H), 3.64 (s, 3H), 3.06 (s, 3H), 2.80 - 2.65 (m, 2H), 2.35 – 2.25 (m, 2H), 1.60 - 1.48 (m, 2H), 1.26 (s, 6H).

9-[[4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]amino]-N-methoxy-N-methyl-nonanamide (21)

To a solution of 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (**5**, 650 mg, 1.32 mmol, 1 *eq*) and 9-amino-N-methoxy-N-methyl-nonanamide (**20**, 280 mg, 1.29 mmol, 0.98 *eq*) in DMF (5 ml) was added HATU (800 mg, 2.1 mmol, 1.6 *eq*) and DIEA (371 mg, 2.87 mmol, 0.5 ml, 2.18 *eq*), then the resulting mixture was stirred at 20 °C for another 2 hr. LCMS showed all of 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid was consumed and desired MS was detected. The reaction mixture was partitioned between ethyl acetate (60 ml) and brine (3x 50 ml). The aqueous layer was extracted with ethyl acetate (100 ml) twice. The combined organic layers were dried over Na_2SO_4, filtrated and evaporated to give a crude material. The crude material was purified by Prep-HPLC (column: Unisil 3-100 C18 Ultra 150 x 50 mm x 3 μm;mobile phase: [water (FA) -ACN];B%: 33%-63%, 7min) and Prep-HPLC (column: Unisil 3-100 C_{18} Ultra 150 x 50 mm x 3 μm;mobile phase: [water (FA) -ACN];B%: 35%-65%, 7min). The residual aqueous solution was lyophilized to give 9-[[4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]amino]-N-methoxy-N-methyl-nonanamide (**21**, 400 mg, 578 μmol, 44 % yield, 100 % purity) as white solid.

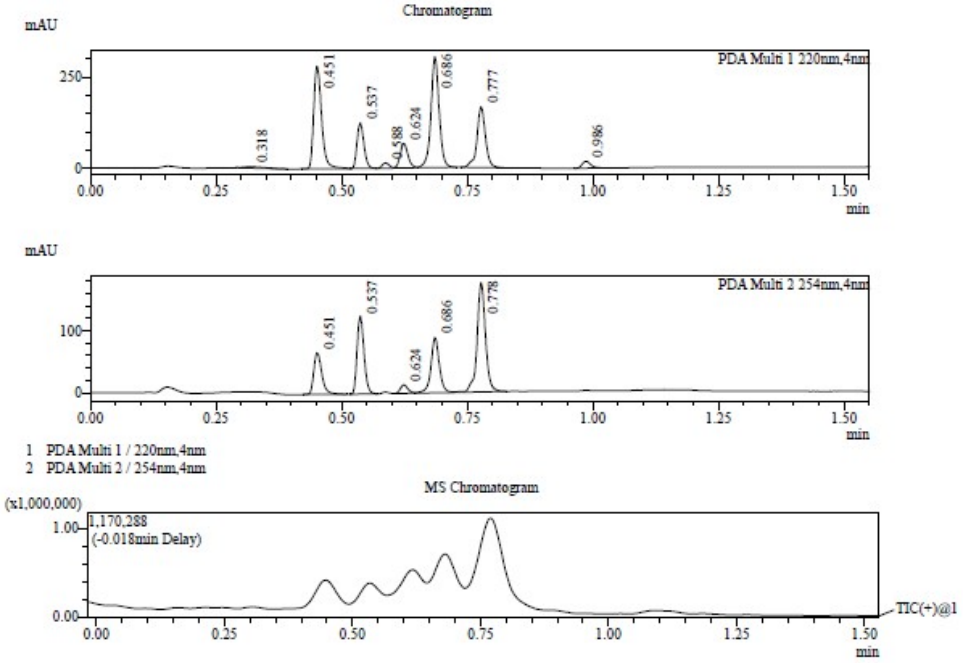
UHPLC-ESIMS (m/z): 692.3 $[M+H]^+$



4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-N-(9-oxononyl) butanamide (22)

To a solution of 9-[[4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]amino]-N-methoxy-N-methyl-nonanamide (**21**, 220 mg, 318 μmol, 1 *eq*) in THF (10 ml) was added $LiAlH_4$ (20 mg, 526 μmol, 1.7 *eq*), then the resulting mixture was stirred at 0 °C for another 2 h. LCMS showed some of 9-[[4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]amino]-N-methoxy-N-methyl-nonanamide was remained and desired MS was detected. The reaction mixture was added into Na_2SO_4•$10H_2O$ (0.05 g). The mixture was stirred for 0.5 h, filtered to give and evaporated to give a crude material. The residue mixture was concentrated to give the title compound 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-N-(9-oxononyl) butanamide (**22**, 200 mg, crude).

UHPLC-ESIMS (m/z): 633.3 $[M+H]^+$



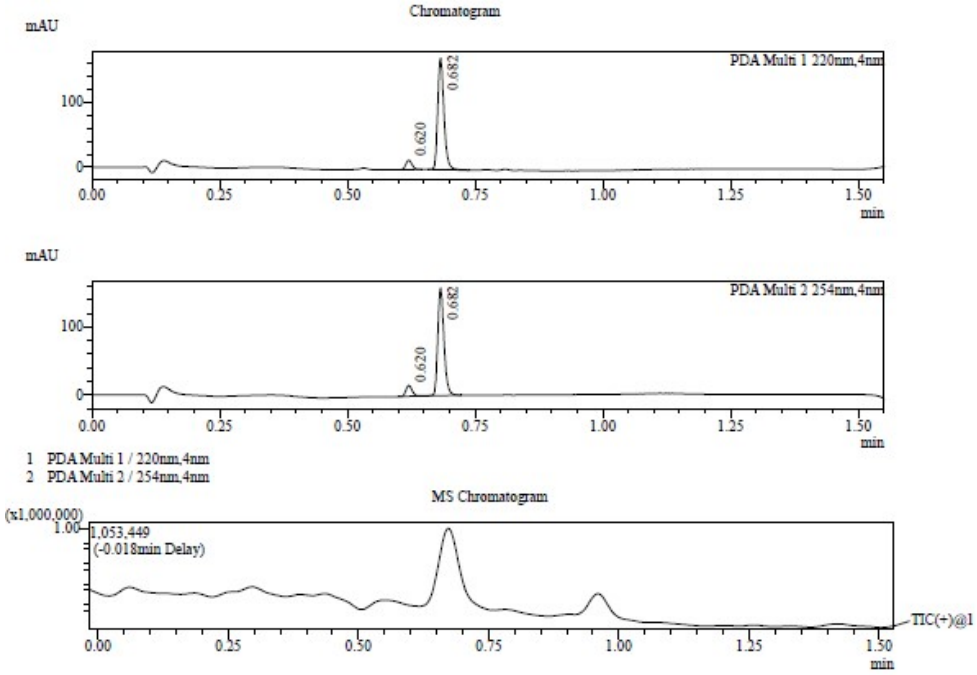
(2S, 4R)-1-[(2S)-2-[9-[[4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6- morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]amino] nonylamino]-3, 3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl) phenyl]methyl]pyrrolidine-2- carboxamide (WJ203-14)

To a solution of 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-N-(9-oxononyl) butanamide (**5**, 200 mg, 316 μmol, 1 *eq*) and (2S, 4R) -1-[(2S) -2-amino-3, 3-dimethyl-butanoyl]-4-hydroxy-N- [[4-(4-methylthiazol-5-yl) phenyl]methyl]pyrrolidine-2-carboxamide (**22**, 140 mg, 325 μmol, 1 *eq*) in DMF (3 ml) was added $NaBH(OAc)_3$ (160 mg, 754 μmol, 2.4 *eq*) and AcOH (60 mg, 999 μmol, 57 μL, 3.2 *eq*), then the resulting mixture was stirred at 20 °C for another 2 h. LCMS showed all of 4-[4-[4-[2-amino-4-(difluoromethyl) pyrimidin-5-yl]-6-morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-N-(9-oxononyl) butanamide was consumed and desired MS was detected. The reaction mixture was partitioned between ethyl acetate (30 ml) and brine (3x 20 ml). The aqueous layer was extracted with ethyl acetate (30 ml) twice. The combined organic layers were dried over Na_2SO_4, filtrated, and evaporated to give a crude material. The crude material was purified by Prep-HPLC (column: Unisil 3-100 C18 Ultra 150 x 50 mm x 3 μm;mobile phase: [water (FA) -ACN];B%: 21 % - 51 %, 7 min) three times. The residual aqueous solution was lyophilized to give (2S, 4R)-1-[(2S)-2-[9-[[4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6- morpholino-1, 3, 5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoyl]amino] nonylamino]-3, 3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl) phenyl]methyl]pyrrolidine-2- carboxamide (**WJ203-14**, 5 mg, 4.4 μmol, 1.4 % yield, 92% purity) as white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ *ppm*: 9.10 (s, 1H), 8.97 (s, 1H), 8.54 (t, J = 6.0 Hz, 1H), 7.80 - 7.76 (m, 1H), 7.63 (t, J = 54.0 Hz, 1H), 7.57 (s, 2H), 7.44 - 7.34 (m, 4H), 5.04 (s, 1H), 4.51 (t, J = 8.1 Hz, 1H), 4.43 - 4.23 (m, 3H), 3.87-3.71 (m, 10H), 3.69-3.62 (m, 6H), 3.57 - 3.52 (m, 4H), 3.03 - 2.97 (m, 3H), 2.43 (s, 3H), 2.41 - 2.38 (m, 1H), 2.36 - 2.24 (m, 4H), 2.07 - 2.00 (m, 1H), 1.94-1.87 (m, 1H), 1.41 - 1.10 (m, 14H), 0.90 (s, 9H).

ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{51}H_{73}F_2N_{14}O_6S$: 1047.5516; found: 1047.5521

UHPLC-ESIMS (m/z): 1047.6 $[M+H]^+$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1.50
min
mAU
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0
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0.25
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0.75
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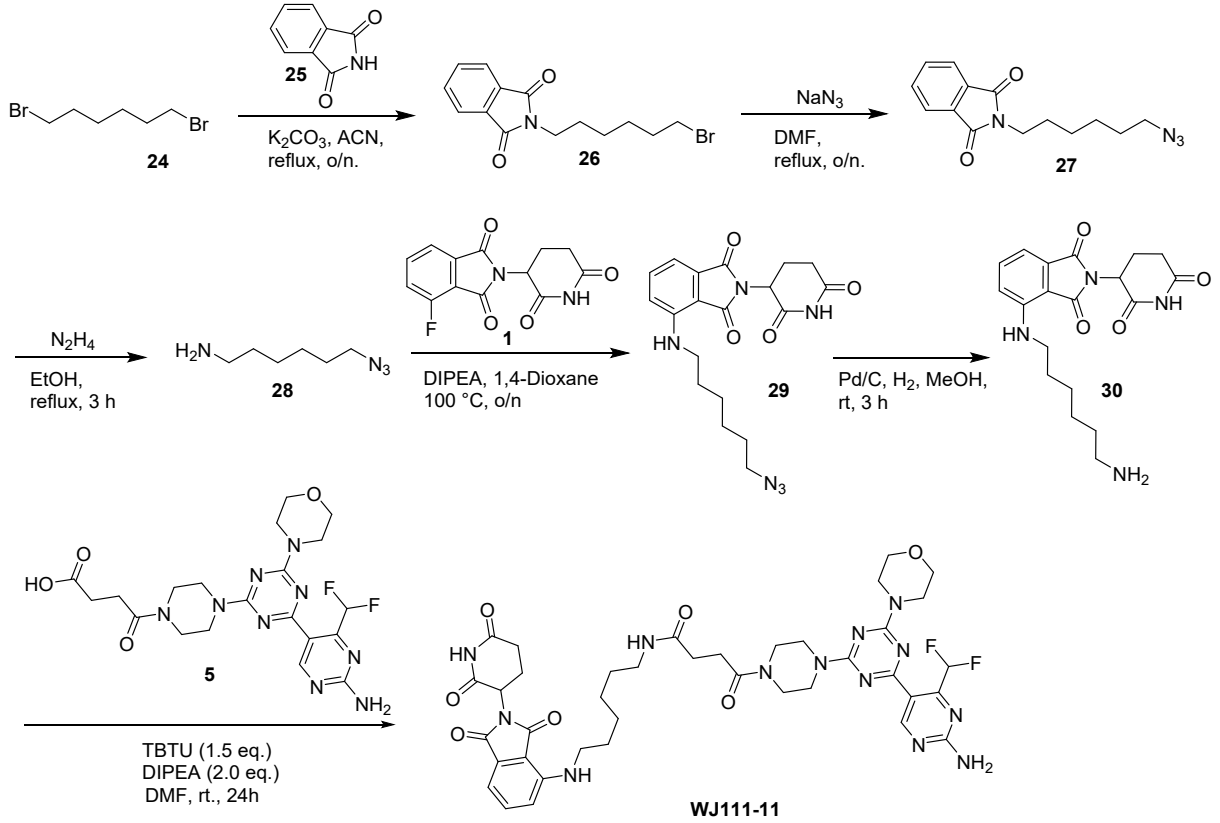
1.50
min
1 PDA Multi 1 / 220nm,4nm
2 PDA Multi 2 / 254nm,4nm
MS Chromatogram
(x1,000,000)
1.00
1,053,449
(-0.018min Delay)
TIC(+)@1
0.00
0.25
0.50
0.75
1.00
1.25
1.50
min

Synthesis of WJ111-11



2-(6-Bromohexyl)isoindoline-1,3-dione (26)

A round-bottom flask was charged with phthalimide (**25**, 2.65 g, 18 mmol, 1.0 eq.), 1,6-dibromohexane (**24**, 8.3 ml, 54 mmol, 3.0 eq.), potassium carbonate (9.95 g, 72 mmol, 4.0 eq.) and acetonitrile (60 ml). The mixture was then heated to reflux overnight. Precipitated solids were filtered off and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (SiO_2, cyclohexane+0.1% TFA/EtOAc+0.1% TFA) to yield 2-(6-bromohexyl)isoindoline-1,3-dione (**26**, 4.75 g, 15 mmol, 85%) as a colourless oil.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 7.86-7.82 (m, 2H), 7.73-7.69 (m, 2H), 3.70-3.67 (m, 2H), 3.41-3.37 (m, 2H), 1.89-1.82 (m, 2H), 1.73-1.65 (m, 2H), 1.52-1.45 (m, 2H), 1.40-1.33 (m, 2H).

2-(6-Azidohexyl)isoindoline-1,3-dione (27)

Sodium azide (2.30 g, 35.3 mmol, 4.0 eq.) was added to a solution of 2-(6-bromohexyl)isoindoline-1,3-dione (**26**, 2.74 g, 8.83 mmol, 1.0 eq.) in DMF (30 ml). The reaction was heated to 70 °C under reflux until no starting material was detected by TLC. The heating was removed and the reaction was quenched by the addition of water (30 ml). The aqueous layer was then extracted with DCM (3 x 30 mL) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to yield 2-(6-azidohexyl)isoindoline-1,3-dione (**27**, 2.35 g, 8.63 mmol, 98 %) as a pale yellow oil. The product was used without further purification.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 7.85-7.83 (m, 2H), 7.72-7.70 (m, 2H), 3.70-3.66 (m, 2H), 3.27-3.23 (m, 2H), 1.72-1.65 (m, 2H), 1.63-1.55 (m, 2H), 1.45-1.32 (m, 4H).

6-Azidohexan-1-amine (28)

2-(6-Azidohexyl)isoindoline-1,3-dione (**27**, 1.28 g, 4.7 mmol, 1.0 eq.) and hydrazine monohydrate (1.4 mL, 28.2 mmol, 6.0 eq,) were dissolved in EtOH (15 mL) and stirred under reflux for 3h. The reaction mixture was then filtered and concentrated *in vacuo*. The residue was redissolved in EtOAc and left at room temperature overnight whereupon a solid precipitated. The solid was filtered off and the filtrate was concentrated *in vacuo* to yield 6-azidohexan-1-amine (**28**, 611 mg, 4.30 mmol, 91 %) as a yellow oil. The product was used without further purification.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 3.28-3.24 (m, 2H), 2.72-2.68 (m, 2H), 1.97-1.96 (m, 2H), 1.64-1.57 (m, 2H), 1.48-1.43 (m, 2H), 1.40-1.34 (m, 4H).

4-((6-Azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (29)

DIPEA (0.8 mL, 4.64 mmol, 5.5 eq.) was added to a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (**1**, 233 mg, 0.84 mmol, 1.0 eq.) and 6-azidohexan-1-amine (**28**, 300 mg, 2.11 mmol, 2.5 eq.) in dioxane (3.0 ml). The reaction was then stirred at 100 °C overnight. The solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, cyclohexane + 0.1 % TFA/EtOAc+0.1 % TFA) to yield 4-((6-azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**29**, 57.4 mg, 0.14 mmol, 17 %) as a green solid.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 7.93 (s, 1H), 7.52-7.48 (m, 1H), 7.11-7.09 (m, 1H), 6.89-6.87 (m, 1H), 6.23 (s, 1H), 4.94-4.89 (m, 1H), 3.30-3.27 (m, 3H), 2.92-2.72 (m, 4H), 2.16-2.11 (m, 1H), 1.71-1.61 (m, 4H), 1.46-1.44 (m, 4H).

4-((6-Aminohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (30)

4-((6-Azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**29**, 57.4 mg, 144 µmol, 1.0 eq.) was dissolved in methanol (2.0 mL) and treated with Pd/C (10 wt % on activated carbon, 5.80 mg). The mixture was stirred under a hydrogen atmosphere for 3 h and subsequently filtered over celite. The filtrate was concentrated *in vacuo* to yield 4-((6-aminohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**30**, 9.4 mg, 25 µmol, 18 %) as a yellow solid. The product was used without further purification.

^{1}H-NMR (400 MHz, 298 K, DMSO-d_6, d/ppm): 7.61-7.53 (m, 1H), 7.11-6.98 (m, 1H), 6.55-6.50 (m, 1H), 5.07-5.03 (m, 1H), 3.31 (s, 4H), 2.78-2.61 (m, 3H), 2.34-2.26 (m, 2H), 2.04-2.01 (m, 1H), 1.57-1.52 (m, 2H), 1.40-1.14 (m, 4H).

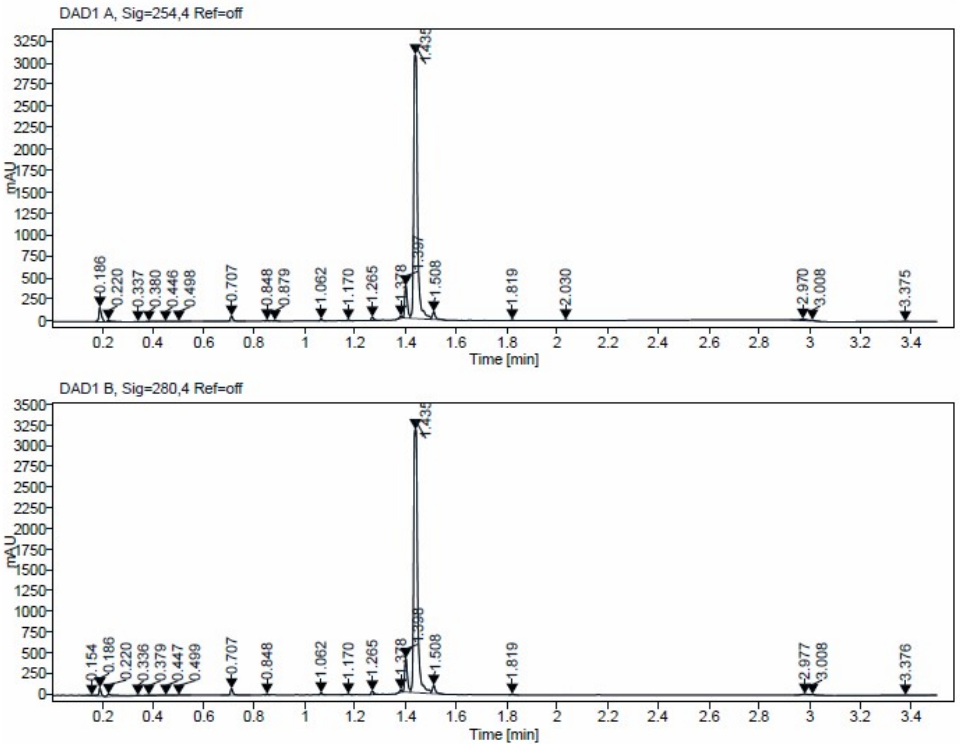
4-(4-(4-(2-amino-4-(difluoromethyl)pyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)-4-oxobutanamide (WJ111-11)

To a solution of 4-[4-[4-[2-amino-4-(difluoromethyl)pyrimidin-5-yl]-6-morpholino-1,3,5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (13.3 mg, 26.9 µmol, 1.0 eq.) and DIEA (9.19 µL, 53.7 µmol, 2.0 eq.) in DMF (1.0 mL) was added TBTU (12.9 mg, 40 µmol, 1.5 eq.). The solution was stirred at rt for 30 min. 4-((6-aminohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**30**, 10 mg, 27 µmol, 1 *eq*) was added to the reaction mixture and stirred for 24 h. LC-MS showed full consumption of the starting material and the desired MS was detected. The solvent was removed *in vacuo* and the precipitate was dissolved in water/MeCN (1:1). The crude material was purified by Prep-HPLC [water(FA)-ACN];B %: 0 – 99 %). The eluent solution was lyophilized to give final compounds 4-(4-(4-(2-amino-4-(difluoromethyl)pyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)hexyl)-4-oxobutanamide (**WJ111-11**, 2.30 mg, 10.1 % yield) as yellow solid.

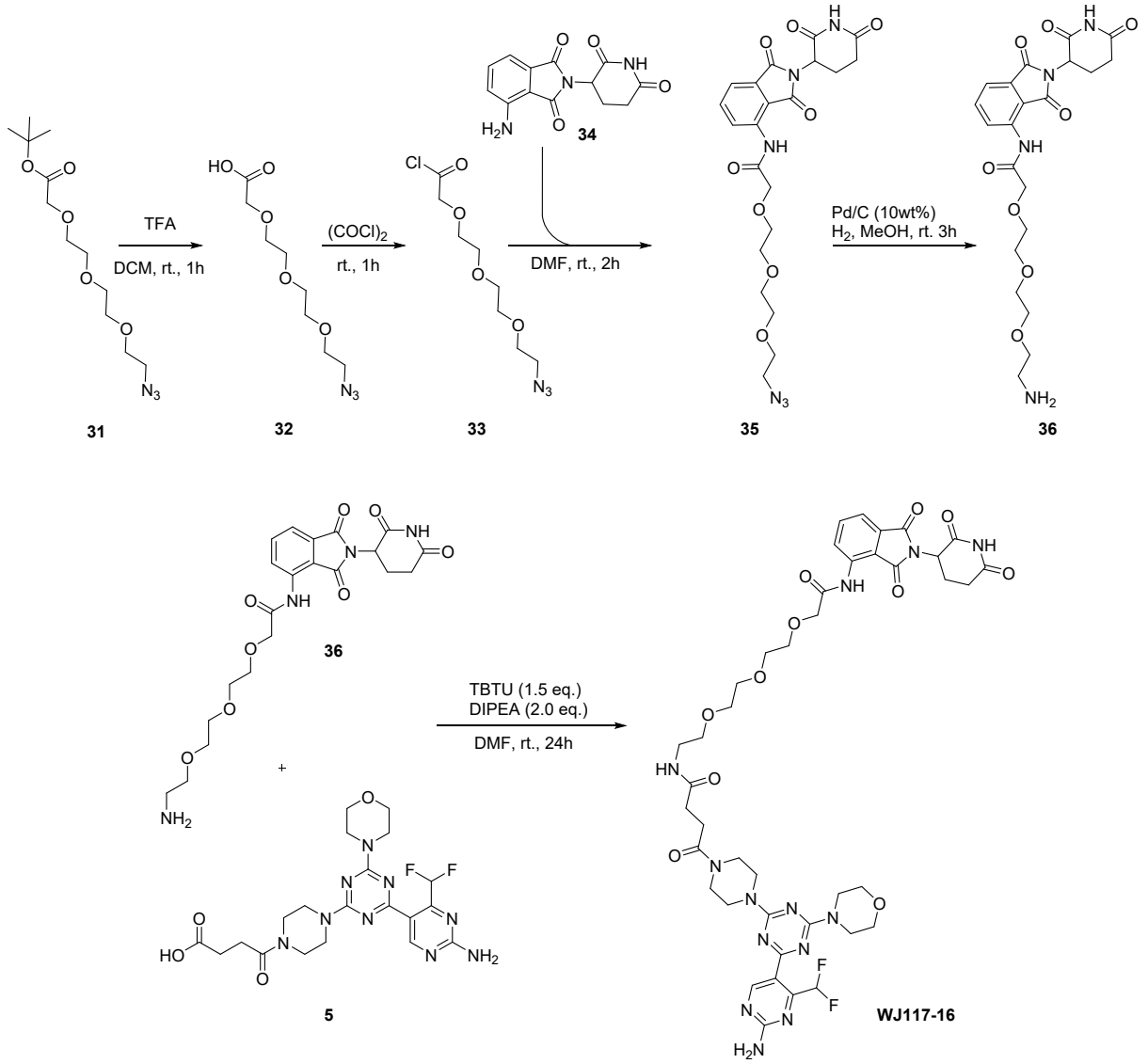
^{1}H {19F} NMR (*cryo*-600 MHz, DMSO-d_6) δ *ppm*: 11.11 (s, 1H), 9.11 (s, 1H), 7.80 (t, *J* = 5.7 Hz, 1H), 7.67 – 7.55 (m, 3H), 7.09 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 7.0 Hz, 1H), 6.55 (br s, 1H), 5.07 – 5.04 (m, 1H), 3.79 – 3.71 (m, 8H), 3.67 – 3.63 (m, 4H), 3.54 – 3.52 (m, 4H), 3.29 (t, *J* = 6.3 Hz, 2H), 3.02 (q, *J* = 6.5 Hz, 2H), 2.90 – 2.84 (m, 1H), 2.63 – 2.55 (m, 4H), 2.33 (t, *J* = 7.0 Hz, 2H), 2.03 – 2.01 (m, 1H), 1.57 - 1.54 (m, 2H), 1.40 – 1.38 (m, 2H), 1.34 – 1.29 (m, 8H).

ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{39}H_{47}F_2N_{13}NaO_7$: 870.3581; found: 870.3582

UHPLC-ESIMS (m/z): 848.4 $[M+H]^+$, 870.4 $[M+Na]^+$



Synthesis of WJ117-16



2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid (32)

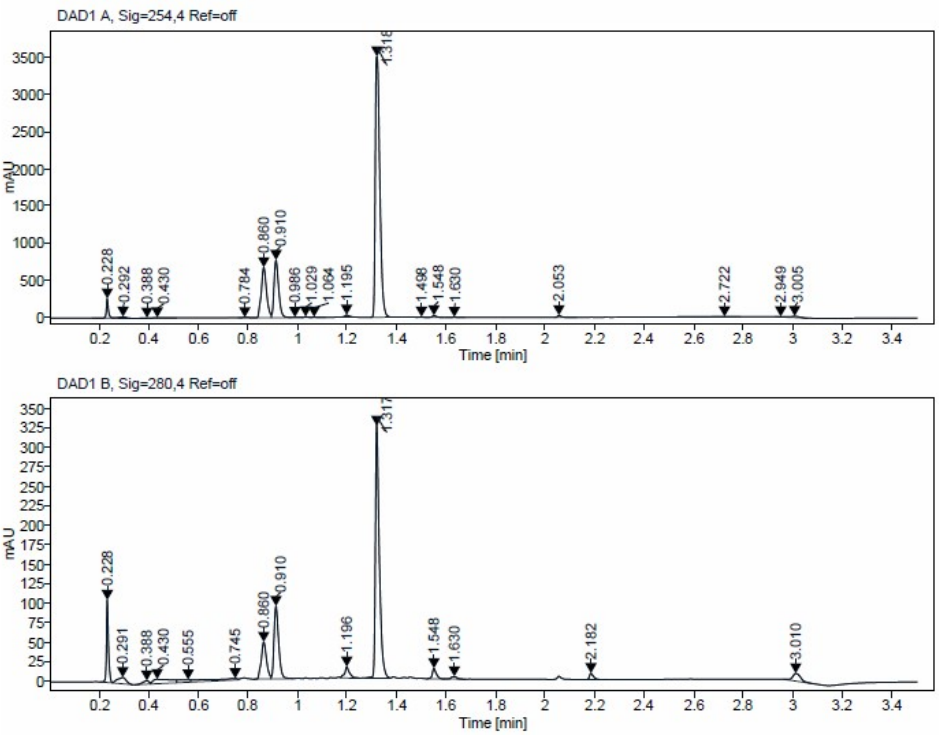
tert-butyl 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetate (**31**, 300 mg, 1 mmol, 1 eq.) was dissolved in DCM/TFA (1:1, 20 ml) and stirred at rt for 15 h. The solvent was removed *in vacuo* to yield the product 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid (**32**, 251 mg, 104 %) as yellow oil.

^{1}H-NMR (400 MHz, 298 K, DMSO-d_6, d/ppm): 4.17 (s, 2H), 3.79 – 3.75 (m, 2H), 3.73 – 3.69 (m, 4H), 3.69 – 3.65 (m, 4H), 3.42 – 3.37 (m, 2H).

2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (35)

To a solution of 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)acetic acid (**32**, 50.0 mg, 173 µmol, 1 eq.) in DCM (3 mL) oxalyl chloride (73 µL, 864 µmol, 5 eq.) was added dropwise under inert conditions. After full conversion of **32** to the respective acyl chloride **33** (monitored by UHPLC-ESIMS) the solvent was evaporated. The precipitate was dissolved in DMF (1.5 ml), 4-amino-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (**34**, 47 mg, 173 µmol, 1 eq) was added and the solution stirred for 1 h at room temperature. The solvent was removed *in vacuo*, the mixture was purified by preparative HPLC (SiO_2-C18, ACN + 0.1 % TFA/H_2O + 0.1 % TFA) and lyophilized to yield 2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (**35**, 80 mg, 164 µmol, 95 %) as a white solid.

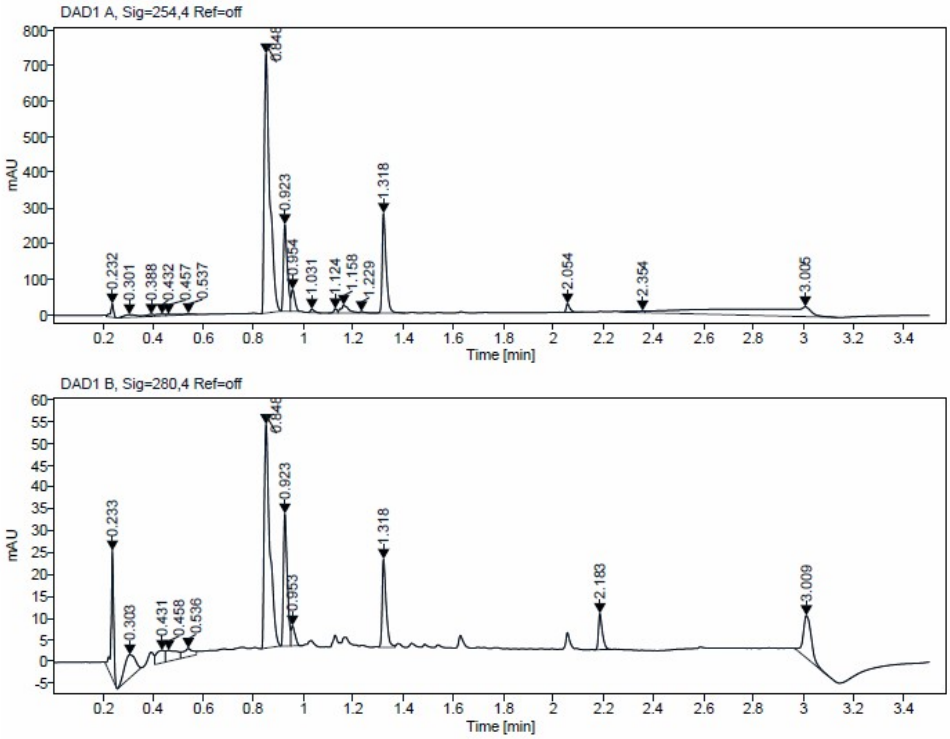
UHPLC-ESIMS (m/z): 489.2 $[M+H]^+$



2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (36)

2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (**35**, 80 mg, 164 µmol, 1 eq) was dissolved in methanol (5 ml) and treated with Pd/C (10 wt % on activated carbon, 8 mg). The mixture was stirred under a hydrogen atmosphere for 3 h and subsequently filtered over celite. The filtrate was concentrated *in vacuo* to yield 2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (**36**, quantitative) as a white solid. The product was used without further purification.

UHPLC-ESIMS (m/z): 463.3 $[M+H]^+$



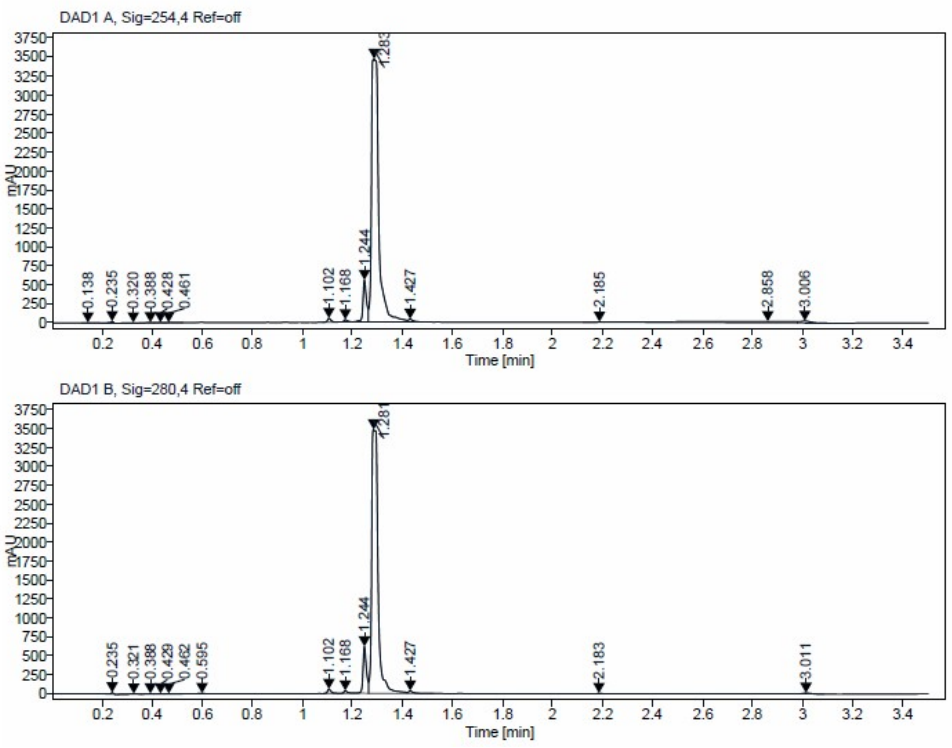
4-(4-(4-(2-amino-4-(difluoromethyl)pyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-2-oxoethoxy)ethoxy)ethoxy)ethyl)-4-oxobutanamide (WJ117-16)

4-(4-(4-(2-amino-4-(difluoromethyl)pyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanoic acid (**5**, 85 mg, 173 µmol, 1 eq), TBTU (83 mg, 259 µmol, 1.5 eq) and DIPEA (59 µl, 346 µmol, 2 eq) were dissolved in DMF (2 ml) and stirred at room temperature for 30 min. 2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)acetamide (**36**, 80 mg, 173 µmol, 1 eq) was dissolved in DMF (2 ml) and the solution was added to the reaction mixture and stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, cyclohexane + 0.1 % TFA/EtOAc + 0.1 % TFA) to yield *tert*-butyl 6-(4-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)hexanoate (**WJ117-16**, 20 mg, 21 µmol, 12 %) as white solid.

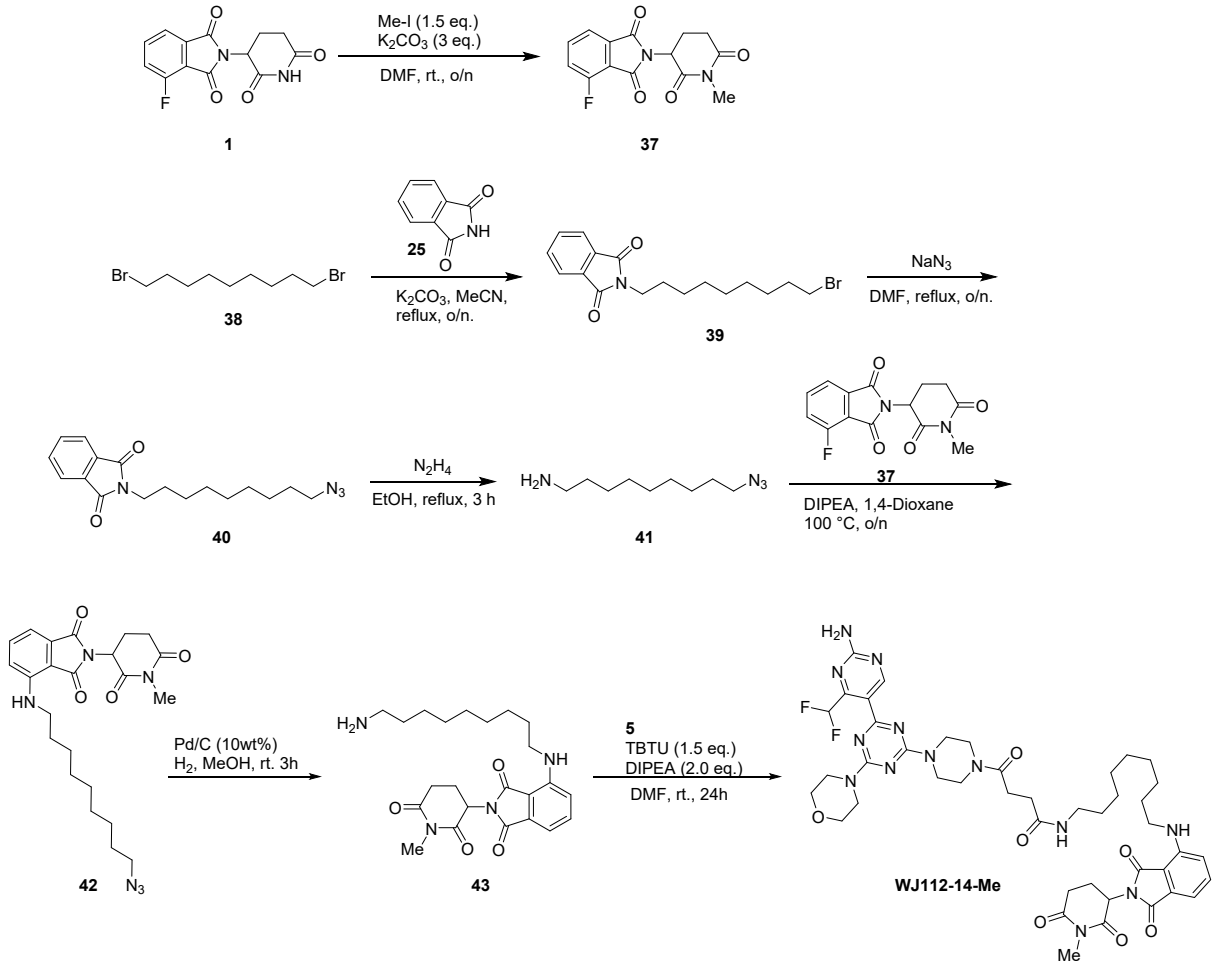
^{1}H-NMR (500 MHz, 298 K, DMSO-d_6, d/ppm): 11.15 (s, 1H), 10.36 (s, 1H), 9.11 (s, 1H), 8.73 (dd, *J* = 8.4, 0.7 Hz, 1H), 7.93 – 7.82 (m, 2H), 7.66 – 7.60 (m, 1H), 7.55 (d, *J* = 26.0 Hz, 2H), 5.17 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.21 (s, 2H), 3.80 – 3.72 (m, 4H), 3.72 – 3.61 (m, 4H), 3.60 – 3.48 (m, 8H), 3.37 (dd, *J* = 12.4, 5.5 Hz, 8H), 3.17 (q, *J* = 5.9 Hz, 2H), 2.90 (ddd, *J* = 17.1, 13.9, 5.4 Hz, 1H), 2.64 (dt, *J* = 4.0, 1.9 Hz, 1H), 2.57 – 2.54 (m, 2H), 2.53 – 2.52 (m, 2H), 2.48 – 2.46 (m, 1H), 2.38 – 2.32 (m, 2H).

ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{41}H_{50}F_2N_{13}O_{11}$: 938.3719; found: 938.3715

UHPLC-ESIMS (m/z): 938.3 $[M+H]^+$



Synthesis of WJ112-14-Me

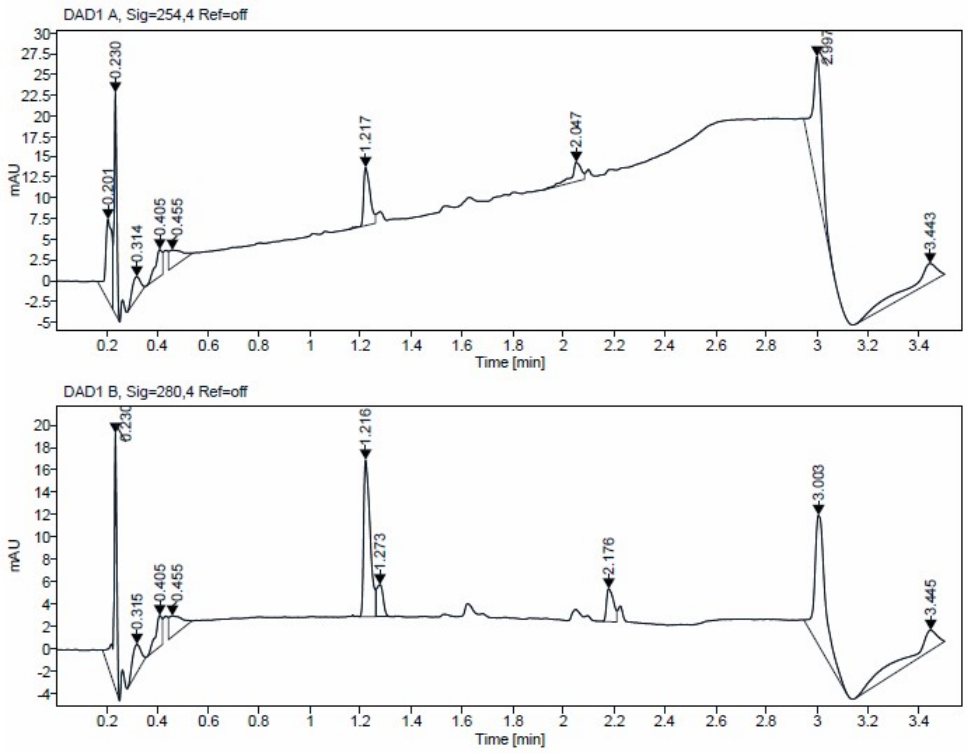


4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)-2,3-dihydro-1H-isoindole-1,3-dione (37)

Toa solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoro-2,3-dihydro-1H-isoindole-1,3-dione (500 mg, 1.8 mmol, 1 eq) in DMF (10 ml) was added CH_3I (385 mg, 2.7 mmol, 1.5 eq) and K_2CO_3 (750 mg, 5.4 mmol, 3 eq). The resulting solution was stirred overnight at 25 °C. The solids were filtered out. The resulting mixture was concentrated. The residue was applied onto a silica gel column with ethyl acetate/petroleum ether (1:2). This resulted in 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)-2,3-dihydro-1H-isoindole-1,3-dione (480 mg, 91 %) as a white solid. LCMS (ESI) m/z: [M-H]+ = 291.

UHPLC-ESIMS (m/z): 291.1 $[M+H]^+$

^{1}H NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 7.77 (td, *J* = 7.7, 4.3 Hz, 1H), 7.71 (d, *J* = 7.3 Hz, 1H), 7.43 (td, *J* = 8.5, 1.0 Hz, 1H), 5.05 – 4.92 (m, 1H), 3.04 – 2.97 (m, 1H), 2.83 – 2.74 (m, 2H), 2.12 (ddd, *J* = 8.0, 4.4, 1.9 Hz, 1H).



2-(9-Bromononyl)isoindoline-1,3-dione (39)
A round-bottom flask was charged with phthalimide (821 mg, 5.6 mmol, 1 eq), 1,9-dibromononane (**17**, 2.5 ml, 17 mmol, 3 eq), potassium carbonate (3.08 g, 22 mmol, 4 eq) and acetonitrile (20 ml). The mixture was then heated to reflux overnight. Precipitated solids were filtered off and the filtrate was concentrated *in vacuo*. The crude was purified by column chromatography (SiO_2, cyclohexane + 0.1 % TFA/EtOAc + 0.1 % TFA) to yield 2-(9-bromononyl)isoindoline-1,3-dione (**18**, 1.77 g, 5 mmol, 90 %) as a colourless oil.
^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 7.85-7.83 (m, 2H), 7.72-7.70 (m, 2H), 3.69-3.65 (m, 2H), 3.41-3.38 (m, 2H), 1.87-1.80 (m, 2H), 1.68-1.65 (m, 2H), 1.42-1.39 (m, 2H), 1.33-1.27 (m, 8H).

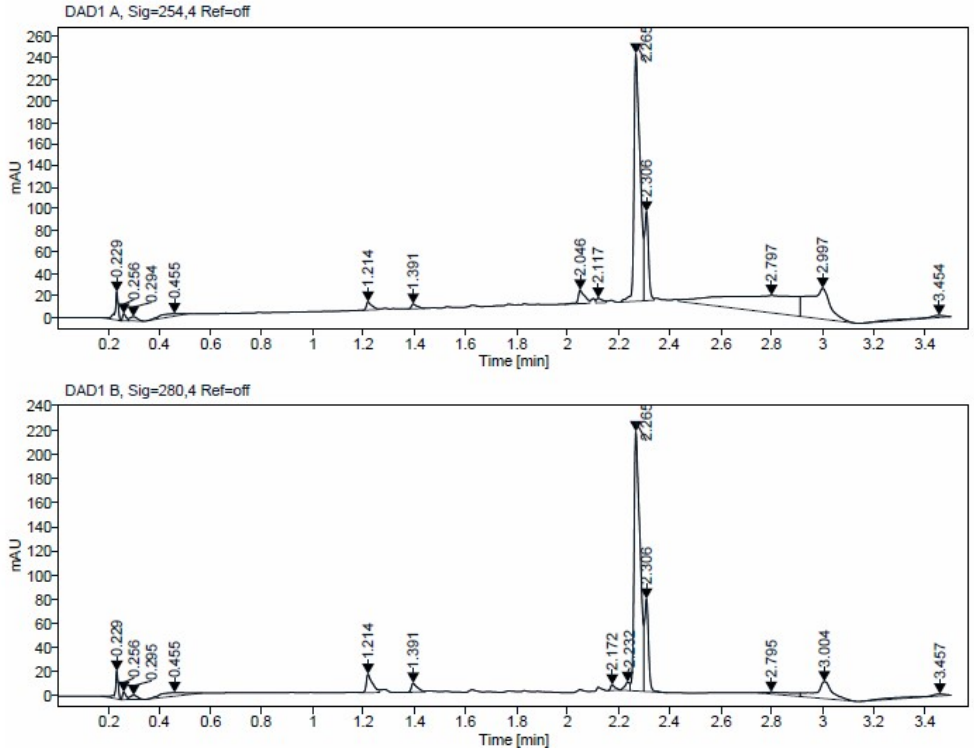
2-(9-Azidononyl)isoindoline-1,3-dione (40)
Sodium azide (726 mg, 11 mmol, 4 eq) was added to a solution of 2-(9-bromononyl)isoindoline-1,3-dione (**18**, 983 mg, 2.8 mmol, 1 eq) in DMF (10 ml). The reaction was heated to 70 °C under reflux until no starting material was detected by TLC. The heating was removed and the reaction was quenched by the addition of water (10 ml). The aqueous layer was then extracted with DCM (3 x 10 ml) and the combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to yield 2-(9-azidononyl)isoindoline-1,3-dione (**19**, 877 mg, 2.8 mmol, quantitative) as a colourless oil. The product was used without further purification.
^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 7.81-7.79 (m, 2H), 7.68-7.66 (m, 2H), 3.65-3.19 (m, 2H), 1.64-1.61 (m, 2H), 1.56-1.50 (m, 2H), 1.31-1.25 (m, 10H).

9-Azidononan-1-amine (41)
2-(9-Azidononyl)isoindoline-1,3-dione (**19**, 440 mg, 1.4 mmol, 1 eq) and hydrazine monohydrate (0.4 ml, 8.4 mmol, 6 eq) were dissolved in EtOH (5 ml) and stirred under reflux for 3 h. The reaction mixture was then diluted with EtOAc (5 ml), whereupon a solid precipitated. The solid was filtered off and washed with EtOAc (3 x 5 ml). Additional EtOAc (10 ml) was added to the filtrate and the solution was placed in a fridge for 30 min, whereupon additional solids precipitated. The filtration step was repeated, and the filtrate was concentrated *in vacuo* to yield 9-azidononan-1-amine (**20**, 258 mg, 1.4 mmol, quantitative) as a pale-yellow oil.
^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 3.27-3.23 (m, 2H), 2.70-2.66 (m, 2H), 1.61-1.56 (m, 4H), 1.45-1.42 (m, 2H), 1.37-1.34 (m, 2H), 1.31-1.29 (m, 6H).

4-((9-azidononyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (42)

DIPEA (0.372 ml, 2.2 mmol, 4 eq) was added to a solution of 4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**37**, 158 mg, 0.54 mmol, 1 eq) and 9-azidononan-1-amine (**41**, 100 mg, 0.54 mmol, 1 eq) in dioxane (5 ml). The reaction was then stirred at 100 °C overnight. The solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, cyclohexane + 0.1 % TFA/EtOAc + 0.1 % TFA) to yield 4-((9-azidononyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**42**, 66 mg, 0.15 mmol, 27 %) as a green solid.

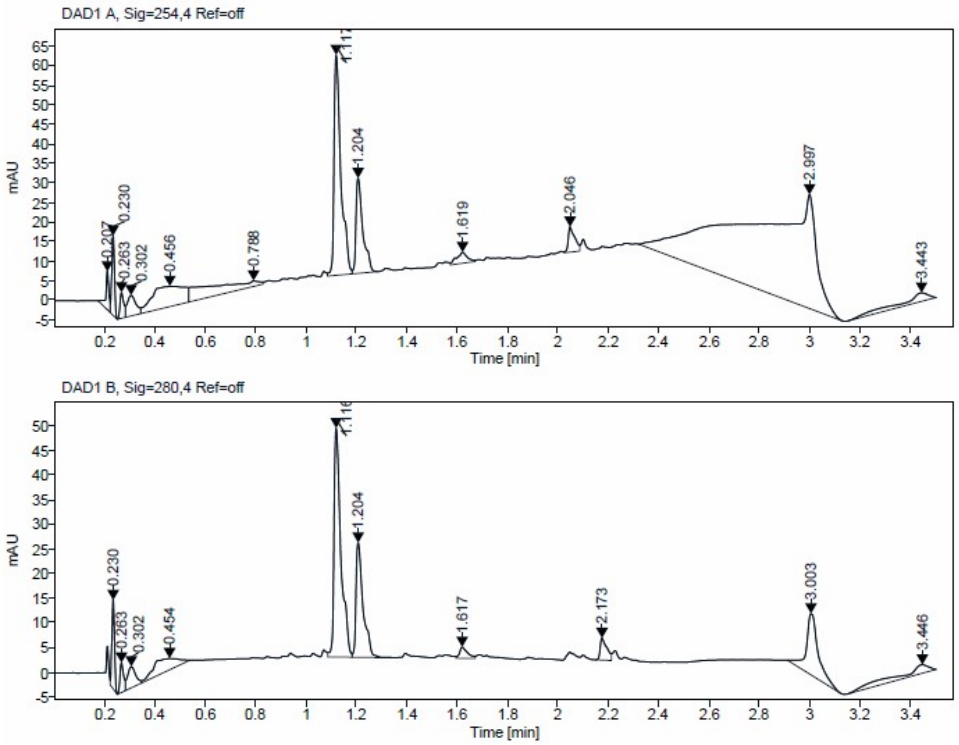
UHPLC-ESIMS (m/z): 455.4 $[M+H]^+$



4-((9-aminononyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (43)

4-((9-azidononyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**42**, 60.0 mg, 132 µmol, 1 eq) was dissolved in methanol (10 ml) and treated with Pd/C (10 wt % on activated carbon, 12.5 mg). The mixture was stirred under a hydrogen atmosphere for 3 h and subsequently filtered over celite. The filtrate was concentrated *in vacuo* to yield 4-((9-aminononyl)amino)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**43**, 60 mg, 140 µmol, 104 %) as a yellow solid. The product was used without further purification. The product was identified by UHPLC-ESIMS.

UHPLC-ESIMS (m/z): 429.3 $[M+H]^+$



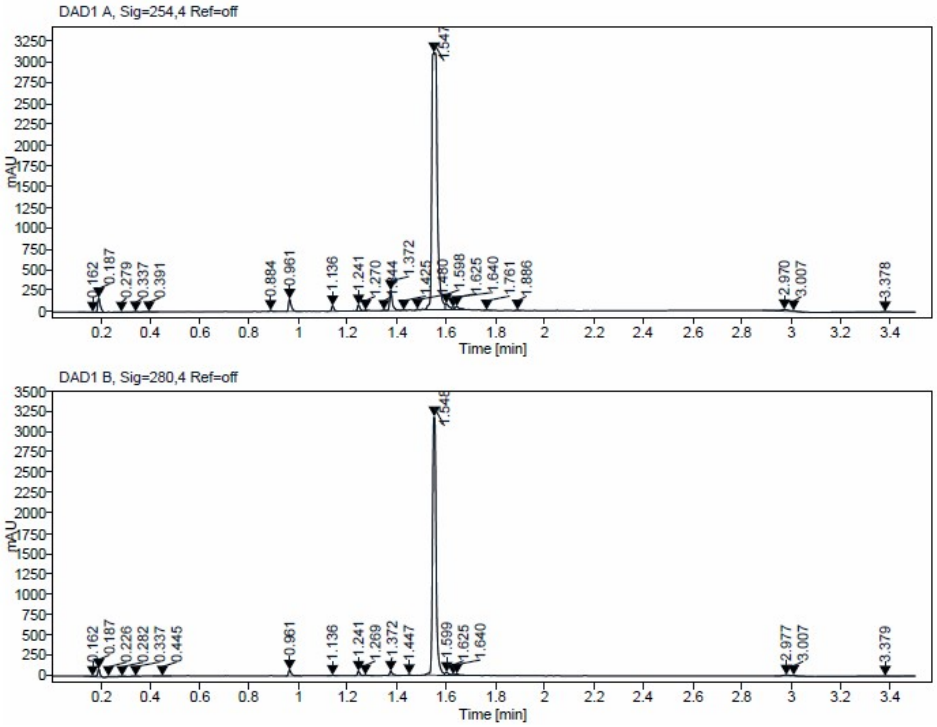
4-(4-(4-(2-amino-4-(difluoromethyl)pyrimidin-5-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-*N*-(9-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)nonyl)-4-oxobutanamide (WJ112-14-Me)

4-[4-[4-[2-Amino-4-(difluoromethyl)pyrimidin-5-yl]-6-morpholino-1,3,5-triazin-2-yl]piperazin-1-yl]-4-oxo-butanoic acid (**5**, 32.5 mg, 65 µmol, 1 eq), TBTU (56 mg, 175 µmol, 2.7 eq.) and DIPEA (40 µL, 233 µmol, 3.6 eq.) were dissolved in DMF (2.5 ml) and stirred at room temperature for 30 min. 4-(9-Aminononylamino)-2-(1-methyl-2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (**43**, 50 mg, 117 µmol, 1.8 eq.) was dissolved in DMF (2.5 ml) and the solution was added to the reaction mixture and stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, cyclohexane + 0.1 % TFA/EtOAc + 0.1 % TFA) to yield 4-((6-azidohexyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**WJ112-14-Me**, 4.6 mg, 5 µmol, 8 %) as a yellow solid.

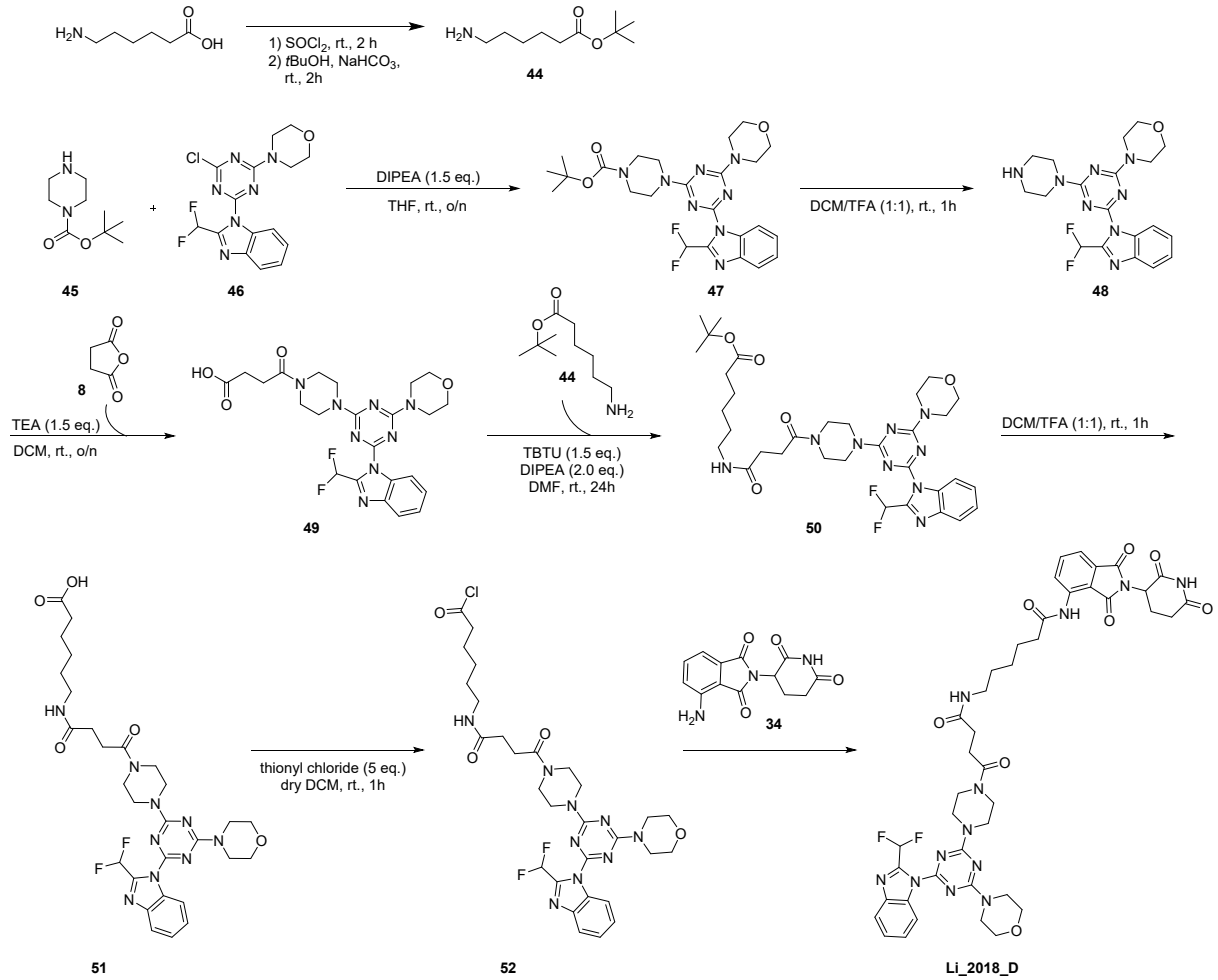
^{1}H-NMR (*cryo*-600 MHz, 298 K, DMSO-d_6, d/ppm): 9.10 (s, 1H), 7.78 (t, *J* = 5.5 Hz, 1H), 7.74 – 7.51 (m, 3H), 7.08 (d, *J* = 8.6 Hz, 1H), 7.01 (d, *J* = 6.9 Hz, 1H), 6.53 (s, 1H), 5.11 (dd, *J* = 13.0, 5.4 Hz, 1H), 3.90 – 3.78 (m, 10H), 3.53 (d, *J* = 16.2 Hz, 6H), 3.27 (d, *J* = 7.4 Hz, 2H), 3.01 (d, *J* = 3.3 Hz, 3H), 2.99 (s, 1H), 2.94 (ddd, *J* = 17.4, 14.0, 5.5 Hz, 1H), 2.80 – 2.72 (m, 1H), 2.64 – 2.53 (m, 3H), 2.43 – 2.37 (m, 1H), 2.31 (t, *J* = 7.0 Hz, 1H), 2.08 – 1.99 (m, 1H), 1.55 (q, *J* = 7.2 Hz, 2H), 1.33 (dt, *J* = 27.7, 6.5 Hz, 4H), 1.24 (d, *J* = 4.5 Hz, 8H).

ESI-HRMS (m/z): $[M]+H^+$ calcd. for $C_{43}H_{56}F_2N_{13}O_7$: 904.4384; found: 904.4388

UHPLC-ESIMS (m/z): 904.4 $[M+H]^+$



Synthesis of Li_2018_D



***tert*-Butyl-6-aminohexanoate (44)**

6-Aminocaproic acid (3.94 g, 30 mmol, 1 eq) was added to thionyl chloride (9.8 ml, 125 mmol, 4.5 eq) under a nitrogen atmosphere and stirred at room temperature for 2 h. The mixture was then concentrated *in vacuo* and subsequently $NaHCO_3$ (5.04 g, 60 mmol, 2 eq) and *tert*-butanol (15.6 ml, 171 mmol, 5.7 eq) were added. The mixture was stirred at room temperature overnight, before removing the solvent *in vacuo*. The residue was then diluted with EtOAc (50 ml) and washed with NaOH (4 x 50 ml), H_2O (3 x 50 ml) and brine (50 ml). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to yield *tert*-butyl-6-aminohexanoate (**44**, 2.82 g, 15 mmol, 50 %) as a yellow oil. The product was used without further purification.

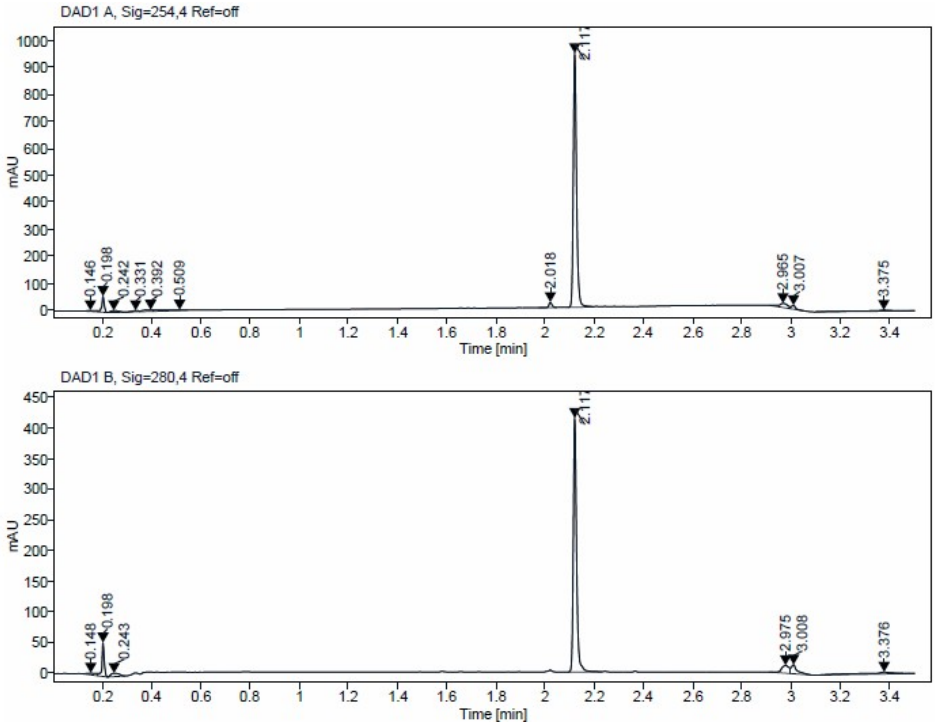
^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 2.69-2.66 (m, 2H), 2.22-2.18 (m, 2H), 1.62-1.54 (m, 2H), 1.48-1.41 (m, 11H), 1.36-1.31 (m, 2H).

***tert*-butyl 4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carboxylate (47)**

tert-Butyl piperazine-1-carboxylate (**45**, 55.9 mg, 0.3 mmol, 1.1 eq), 4-(4-chloro-6-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-1,3,5-triazin-2-yl)morpholine (**46**, 100 mg, 0.27 mmol, 1 eq) and DIPEA (70 µL, 0.4 mmol, 1.5 eq) were dissolved in THF (5 ml) and stirred for 1 h at RT. The solvent was removed *in vacuo* to give *tert*-butyl 4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carboxylate (**47**, 151 mg, quantitative) as a white solid. The product was used without further purification.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 8.38 – 8.29 (m, 1H), 7.95 – 7.85 (m, 1H), 7.63 (d, *J* = 53.6 Hz, 1H), 7.50 – 7.37 (m, 2H), 4.12 (q, *J* = 7.2 Hz, 1H), 3.88 (d, *J* = 5.5 Hz, 10H), 3.54 – 3.52 (m, 3H), 3.43 – 3.33 (m, 1H), 2.82 – 2.79 (m, 1H), 1.50 (s, 9H).

UHPLC-ESIMS (m/z): 516.2 $[M+H]^+$

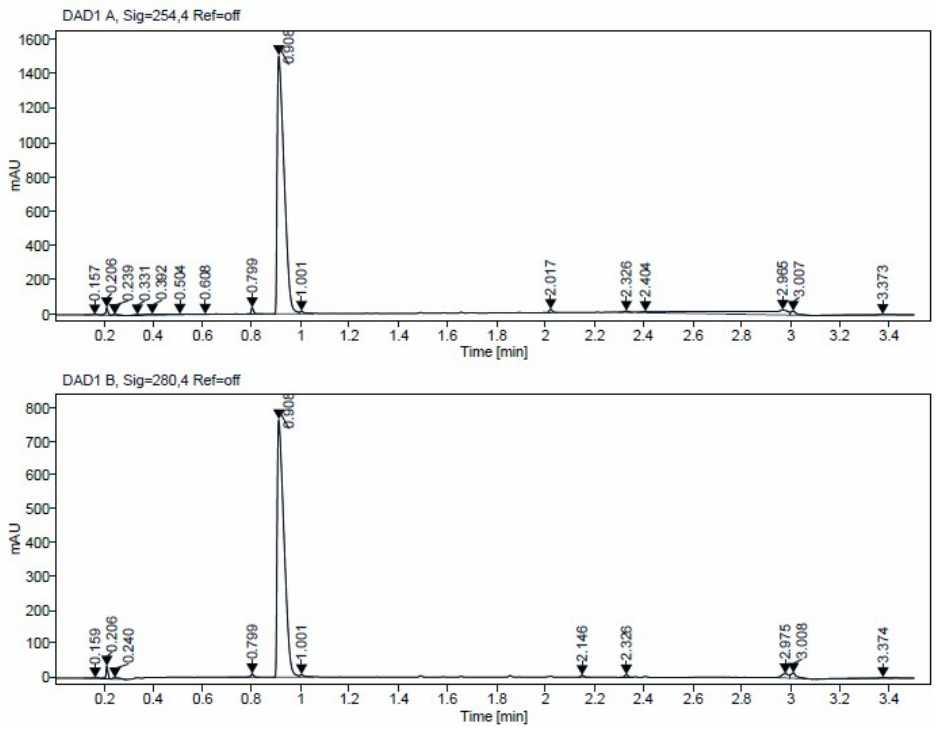


4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (48)

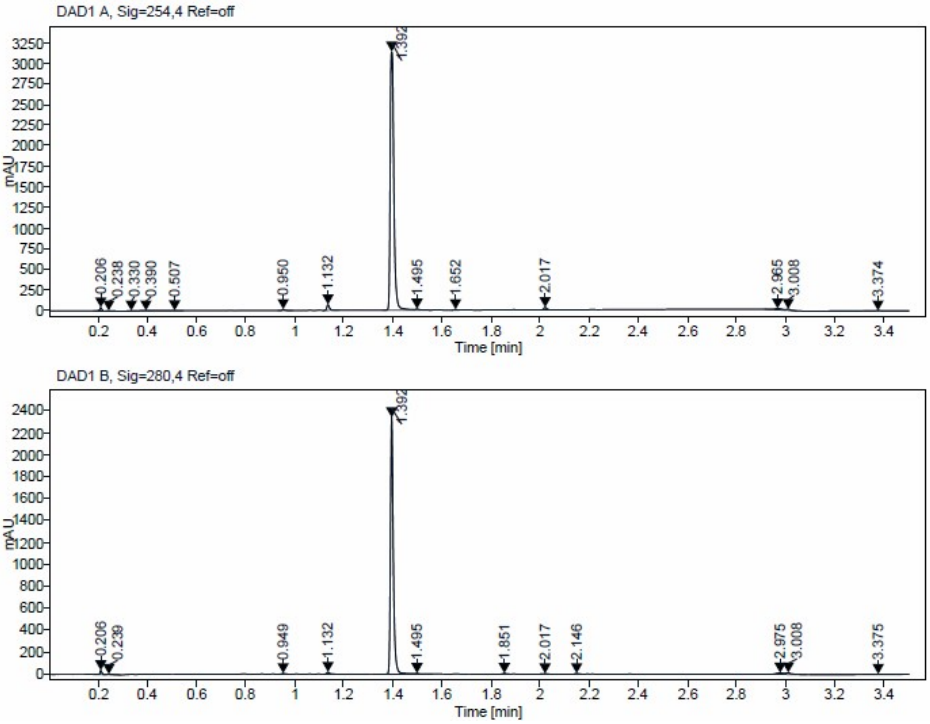
tert-Butyl-4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazine-1-carboxylate (**47**, 141 mg, 0.27 mmol, 1 eq.) was dissolved in DCM/TFA (1:1, 5 ml) and stirred at RT for 1 h. The solvent was removed *in vacuo* to yield the product 4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**48**, 120 mg, quantitative) as white solid.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 9.50 (s, 1H), 8.28 (d, *J* = 8.1 Hz, 1H), 7.95 (d, *J* = 7.2 Hz, 1H), 7.67 – 7.32 (m, 3H), 4.22 (s, 2H), 3.97 – 3.75 (m, 4H), 3.36 (s, 2H), 3.05 (s, 8H).

UHPLC-ESIMS (m/z): 416.4 $[M+H]^+$



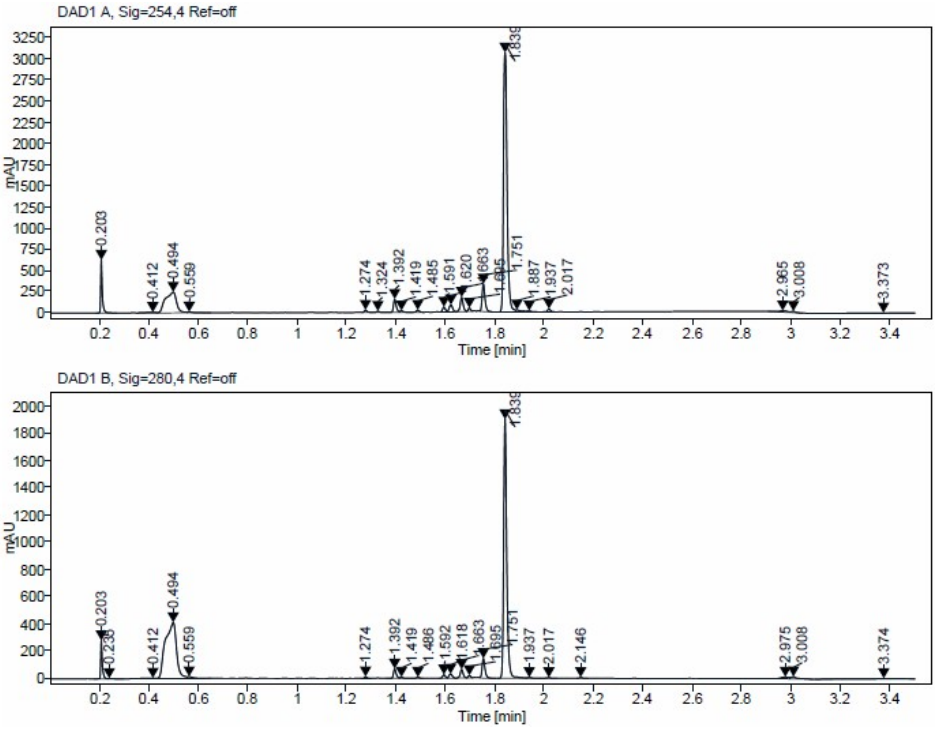
4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanoic acid (49)
To a solution of 4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-(piperazin-1-yl)-1,3,5-triazin-2-yl)morpholine (**48**, 114 mg, 0.27 mmol, 1 eq.) and tetrahydrofuran-2, 5-dione (**8**, 27.4 mg, 0.27 mmol, 1 eq) in DCM (2 ml) was added TEA (57 µL, 0.41 mmol, 1.5 eq) and stirred at room temperature for 12 h. UHPLC-ESIMS showed some of 4-(difluoromethyl) -5-(4-morpholino-6-piperazin-1-yl-1, 3, 5-triazin-2-yl) pyrimidin-2-amine was consumed and the desired mass was detected. The reaction mixture was concentrated *in vacuo* to give crude product 4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanoic acid (**49**, 135 mg, 0.26 mmol, 96 %) as white solid.
^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 8.38 – 8.30 (m, 1H), 7.90 (d, *J* = 7.4 Hz, 1H), 7.68 – 7.55 (m, 1H), 7.49 – 7.36 (m, 2H), 4.00 – 3.71 (m, 14H), 3.64 (s, 2H), 2.75 (s, 4H).
UHPLC-ESIMS (m/z): 516.5 $[M+H]^+$



***tert*-butyl 6-(4-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)hexanoate (50)**

4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanoic acid (**49**, 50 mg, 97 µmol, 1 eq), TBTU (47 mg, 145 µmol, 1.5 eq) and DIPEA (33 µl, 194 µmol, 2 eq) were dissolved in DMF (0.5 ml) and stirred at room temperature for 30 min. *tert*-Butyl 6-aminohexanoate (**44**, 22 mg, 116 µmol, 1.2 eq) was dissolved in DMF (0.25 ml) and the solution was added to the reaction mixture and stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude was purified by column chromatography (SiO_2, cyclohexane + 0.1 % TFA/EtOAc + 0.1 % TFA) to yield *tert*-butyl 6-(4-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)hexanoate (**50**, 41 mg, 60 µmol, 62 %) as white solid.

UHPLC-ESIMS (m/z): 685.4 $[M+H]^+$

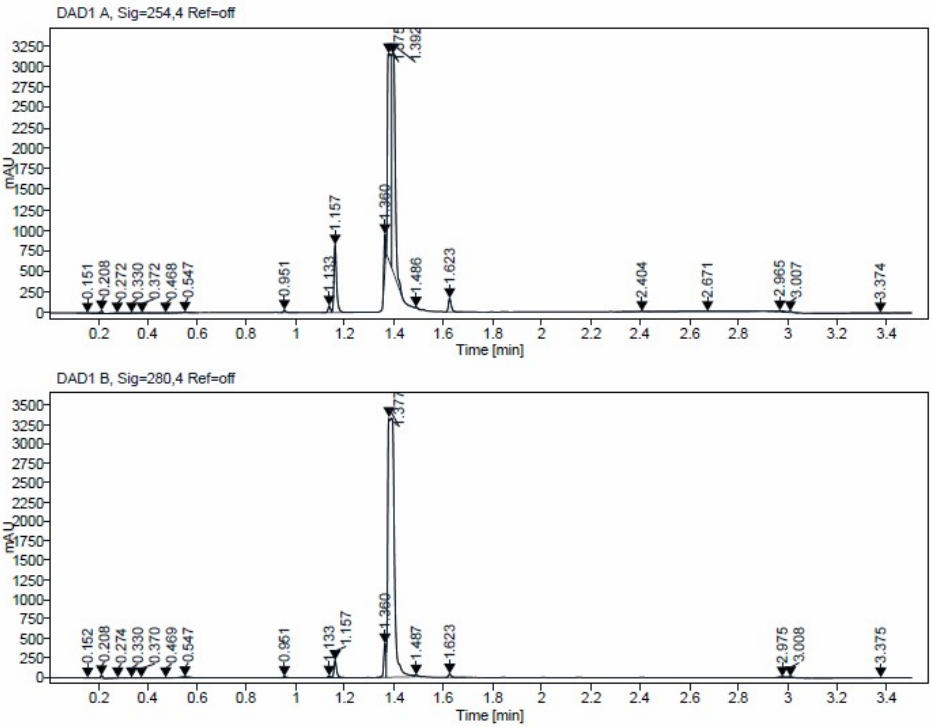


6-(4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)hexanoic acid (51)

tert-butyl-6-(4-(4-(4-(2-(difluoromethyl)-1H-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)hexanoate (**50**, 50 mg, 0.28 mmol, 1 eq) was dissolved in DCM/TFA (1:1, 2 ml) and stirred at rt for 2 h. The solvent was removed *in vacuo* and the precipitate was dissolved in water, quenched with NaOH (1 M) ant extracted with DCM to yield the product 6-(4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)hexanoic acid (**51**, 40 mg, 64 µmol, 88 %) as white solid.

^{1}H-NMR (400 MHz, 298 K, $CDCl_3$, d/ppm): 8.35 (d, *J* = 8.2 Hz, 1H), 7.91 – 7.58 (m, 3H), 7.51 (t, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.6 Hz, 1H), 3.93 – 3.50 (m, 16H), 3.01 (q, *J* = 6.5 Hz, 2H), 2.59 (t, *J* = 7.1 Hz, 2H), 2.33 (t, *J* = 7.1 Hz, 2H), 2.19 (t, *J* = 7.4 Hz, 2H), 1.48 (p, *J* = 7.4 Hz, 2H), 1.37 (q, *J* = 7.1 Hz, 2H), 1.25 (tt, *J* = 10.5, 6.0 Hz, 2H).

UHPLC-ESIMS (m/z): 629.3 $[M+H]^+$



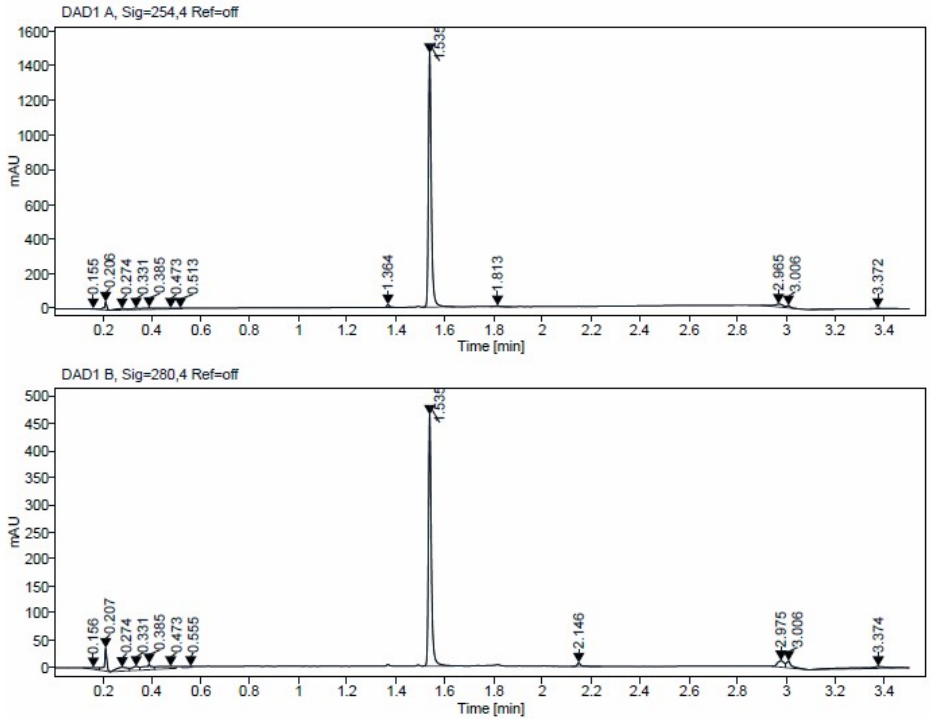
6-(4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hexanamide (Li_2018_D)

To a solution of 2-(2-(2-(2-azidoethoxy)ethoxy)acetic acid (**51**, 11 mg, 17 µmol, 1 eq) in DCM (3 ml) thionyl chloride (6 µl, 84 µmol, 5 eq) was added dropwise under inert conditions. After full conversion of **51** to the respective acyl chloride **52** (monitored by UHPLC-ESIMS) the solvent was evaporated. The precipitate was dissolved in DMF (3 ml), 4-amino-2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (**34**, 9 mg, 34 µmol, 2 eq) was added and the solution stirred for 1 h at room temperature. The solvent was removed *in vacuo*, the mixture was purified by preparative HPLC (SiO_2-C18, ACN + 0.1 % TFA/H_2O + 0.1% TFA) and lyophilized to yield 6-(4-(4-(4-(2-(difluoromethyl)-1*H*-benzo[d]imidazol-1-yl)-6-morpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-4-oxobutanamido)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hexanamide (**Li_2018_D**, 1.3 mg, 15 µmol, 9 %) as a white solid.

^{1}H-NMR (*cryo*-600 MHz, 298 K, $CDCl_3$, d/ppm): 11.16 (s, 1H), 9.70 (s, 1H), 8.45 (d, *J* = 8.4 Hz, 1H), 8.35 (d, *J* = 8.3 Hz, 1H), 7.83 (dt, *J* = 16.2, 8.1 Hz, 3H), 7.60 (d, *J* = 7.3 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.43 (t, *J* = 7.7 Hz, 1H), 5.14 (dd, *J* = 12.9, 5.4 Hz, 1H), 3.92 – 3.66 (m, 11H), 3.65 – 3.53 (m, 5H), 3.03 (q, *J* = 6.6 Hz, 2H), 2.89 (ddd, *J* = 17.5, 13.9, 5.5 Hz, 1H), 2.63 – 2.57 (m, 3H), 2.45 (t, *J* = 7.5 Hz, 2H), 2.33 (t, *J* = 7.1 Hz, 2H), 2.09 – 2.02 (m, 1H), 1.62 (p, *J* = 7.5 Hz, 2H), 1.41 (q, *J* = 7.5 Hz, 2H), 1.33 (q, *J* = 8.0 Hz, 2H).

ESI-HRMS (m/z): $[M]+H^+$ calc. for $C_{42}H_{47}F_2N_{12}O_8$: 885.3602; found: 885.3602

UHPLC-ESIMS (m/z): 885.4 $[M+H]^+$



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