Supplemental information for:

Lessons in PROTAC design from selective degradation with a promiscuous warhead

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Supplemental Tables

Supplemental Table 1 – KinomeScan data for foretinib and foretinb-based PROTACs across 428 human kinases, related to Figure 1.

Supplemental Table 2 – Raw peptide quantification of MDA-MB-231 proteomes after 24 hour treatments with foretinib and foretinib-based PROTACs, related to Figure 2.

Supplemental Table 3 – Compilation of KinomeScan data and quantitative proteomics for the 54 kinases bound by foretinib and expressed in MDA-MB-231 cells, related to Figure 3.

Supplemental Figures

Figure S1. Kinase-binding abilities of compounds 1 and 2, related to Figure 1.
(A) Dendrogram for compound **1**. (B) Dendrogram for compound **2**. (C) Percent of control (KinomeScan) for compound 1 and 2 are plotted on the X- and Y-axes, respectively, for all protein kinases that bind to Foretinib with a percent of control less than 35. Cutoffs for binding at a percent of control of 35 are shown. (D) Western blot of samples submitted for whole cell proteomics.

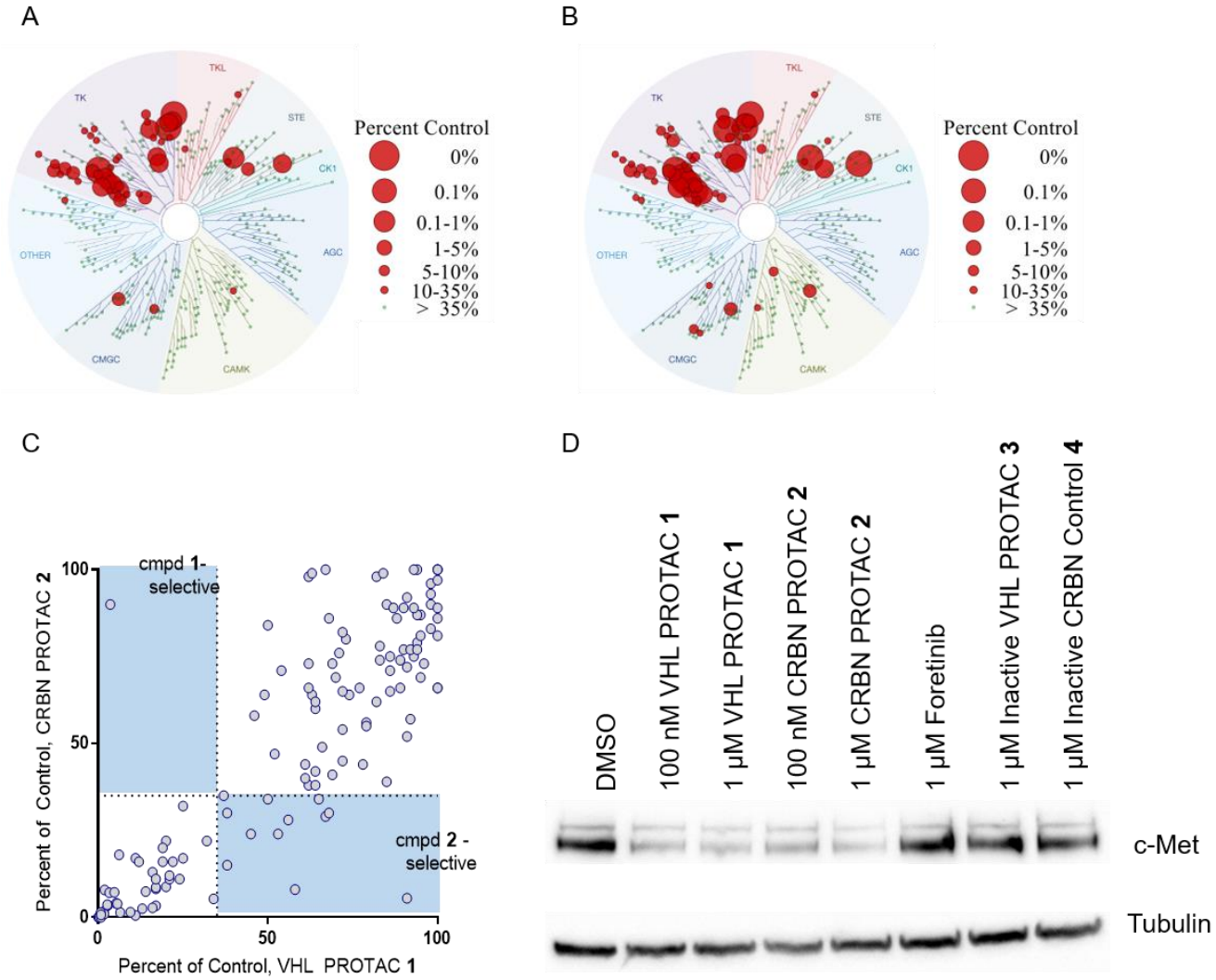


Figure S2. Confirmation of degradation and PROTAC affinity for kinase targets, related to Figure 3.

(A) MDA-MB-231 cells were treated for 24 hours with the compounds indicated and western blots were performed, probing for the indicated proteins. Representative blots for one of two experiments are shown here. (B) Determination of K_d for test kinases. For each of the 15 test kinases, the inhibition of phage-displayed kinase domains is shown for increasing concentrations of foretinib (gray), VHL PROTAC **1** (Teal), and CRBN PROTAC **2** (Blue). For p38α, the different compounds resulted in different absolute values and so compound **1** is plotted on the left Y-axis while compound **2** and foretinib are plotted on the right Y-axis. (C) Summary of the dissociation constants determined in (B). For each kinase, the K_d is shown for each compound as calculated using the Levenberg-Marquardt algorithm with a hill slope set to -1. (D) Comparison of degradation data from proteomics compared to western blot for VHL PROTAC **1**. The X-axis is the maximal percent (D_{max}) decrease in protein levels compared to DMSO after treatment with VHL PROTAC **1** as determined by western blotting. The Y-axis shows the percent of DMSO in the whole cell proteomics dataset for either the 100 nM (black dots) or 1 µM (gray dots) treatment. (E) Same as (D) but for the CRBN PROTAC **2.** (F) Comparison of binding 'affinity' by single point percent inhibition and multipoint K_d determination for the VHL PROTAC **1**. (G) Same as in (F), but for the CRBN PROTAC **2.**

Lane Legend for Test Kinase Western Blot (A)

Lanes	Compound and Dose
1	DMSO
2-3	Foretinib, 300 nM and 3 µM
4-9	VHL PROTAC **1**, 30 nM to 10 µM
10-11	Inactive VHL control **3,** 1 and 10 µM
12-17	CRBN PROTAC **2,** 30 nM to 10 µM
18-19	Inactive CRBN control **4,** 1 and 10 µM
20	DMSO

A

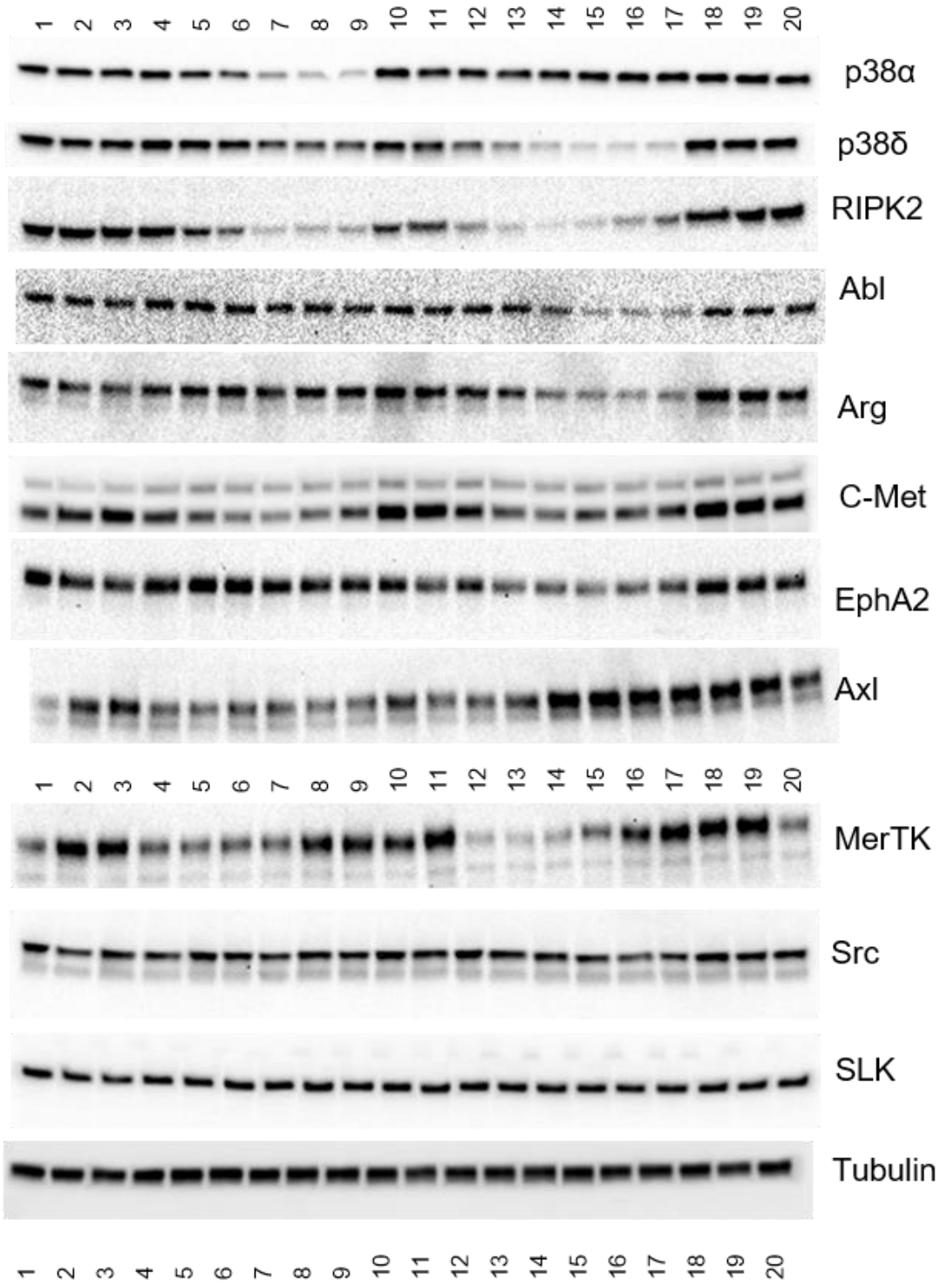

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
p38α
p38δ
RIPK2
Abl
Arg
C-Met
EphA2
Axl
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
MerTK
Src
SLK
Tubulin
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figure S2, continued

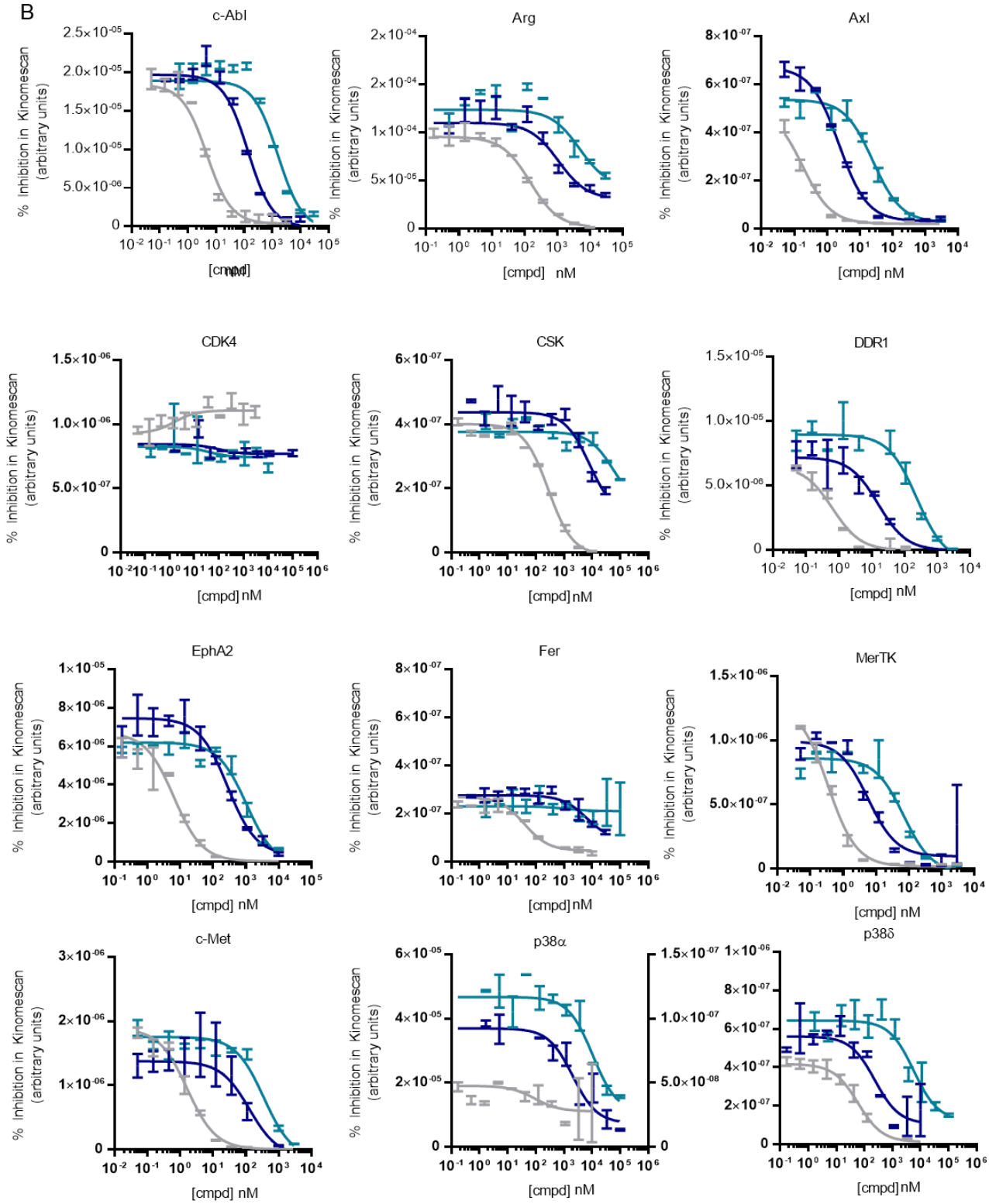
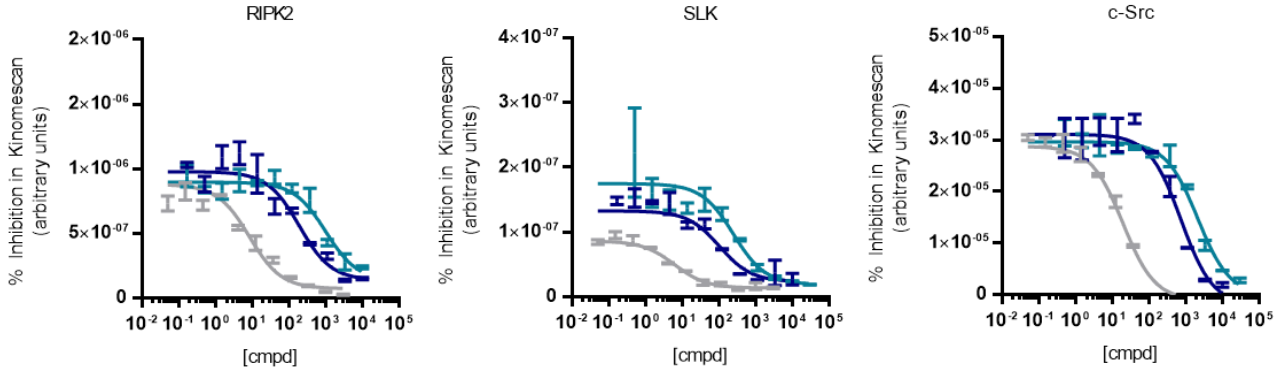


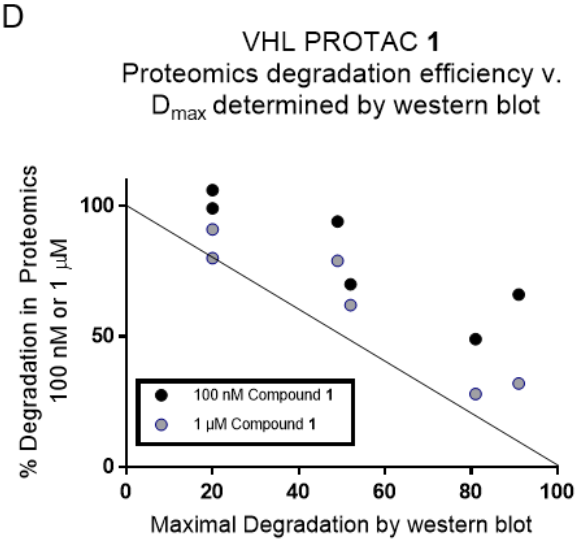
Figure S2, continued



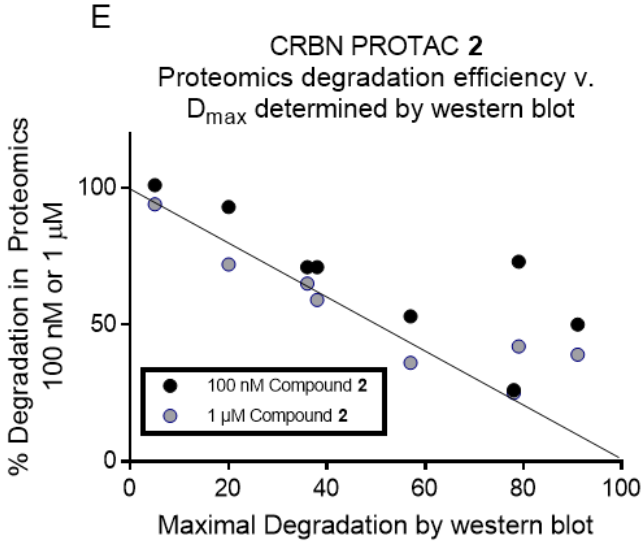
C Summary of Kinase K_d Values

Kinase	Foretinib K_d (nM)	VHL PROTAC 1 K_d (nM)	CRBN PROTAC 2 K_d (nM)
AXL	0.18	26	2.4
MERTK	0.36	64	7.8
DDR1	0.65	190	16
SLK	6.2	450	95
MET	1.7	310	100
ABL1	4.7	1,300	130
RIPK2	17	1,600	200
EPHA2	6.6	1,000	330
MAPK13	60	5,300	380
SRC	18	2,100	580
ABL2	160	15,000	1,100
MAPK14	910	11,000	2,000
FER	69	77,000	17,000
CSK	260	100,000	17,000
CDK4	3000	10,000	100,000

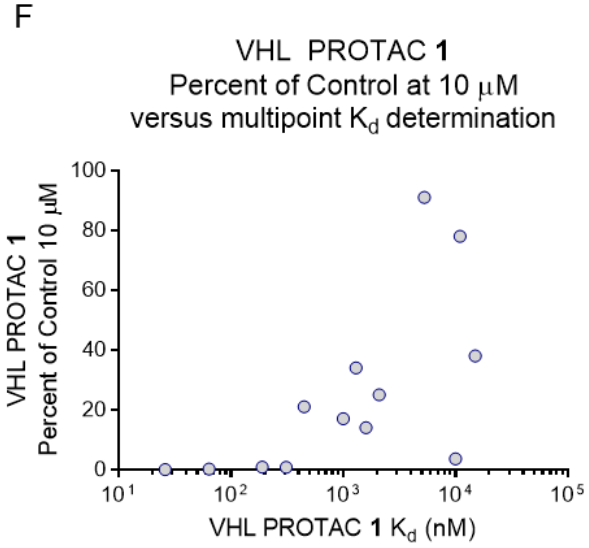
D


VHL PROTAC 1
Proteomics degradation efficiency v.
Dmax determined by western blot
% Degradation in Proteomics
100 nM or 1 μM
100 nM Compound 1
1 μM Compound 1
Maximal Degradation by western blot

E


CRBN PROTAC 2
Proteomics degradation efficiency v.
Dmax determined by western blot
% Degradation in Proteomics
100 nM or 1 μM
100 nM Compound 2
1 μM Compound 2
Maximal Degradation by western blot

F


VHL PROTAC 1
Percent of Control at 10 μM
versus multipoint Kd determination
VHL PROTAC 1
Percent of Control 10 μM
VHL PROTAC 1 Kd (nM)

G

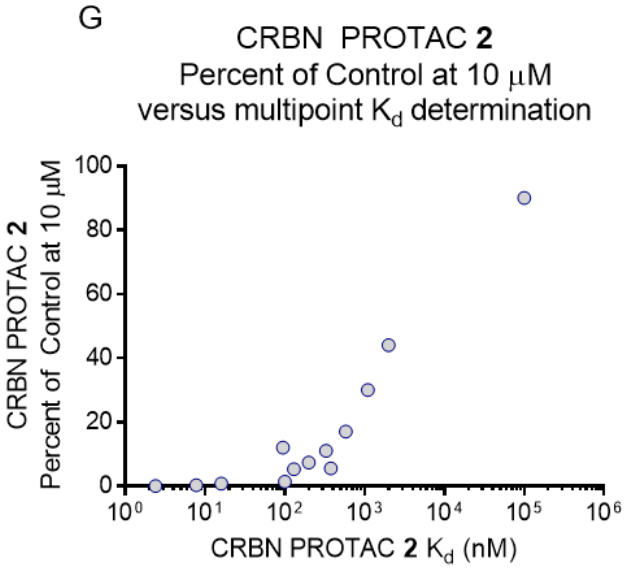
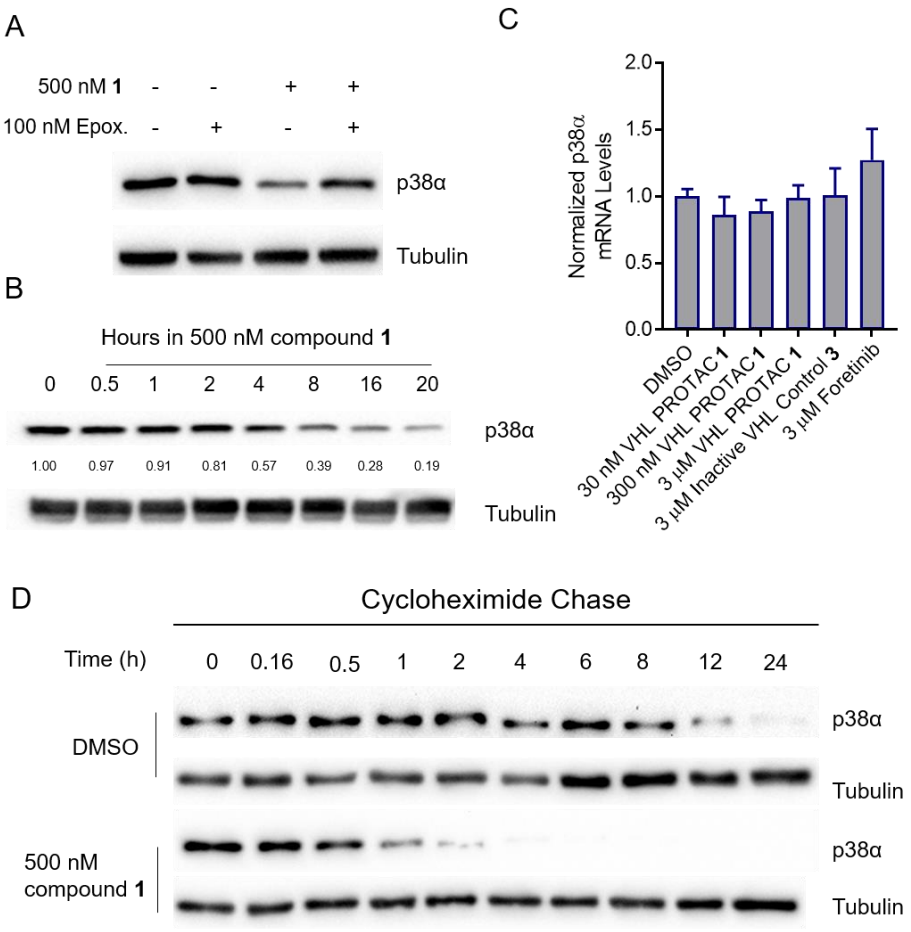

CRBN PROTAC 2
Percent of Control at 10 μM
versus multipoint Kd determination
CRBN PROTAC 2
Percent of Control at 10 μM
CRBN PROTAC 2 Kd (nM)

Figure S3. A low affinity target (p38α) is rapidly degraded in a PROTAC-consistent mechanism of action and high affinity targets are not degraded despite robust target engagement, related to Figure 3.

(A) Epoxomicin rescue of p38α degradation. MDA-MB-231 cells were pretreated with 100 nM Epoxomicin or vehicle for 30 minutes, followed by an 8 hour pulse of compound **1** or vehicle and total p38α levels were analyzed by western blot. (B) p38α is rapidly degraded by compound 1. MDA-MB-231 cells were treated for the indicated times with compound **1**, and total p38α levels analyzed by western blot. The values below the western data indicate quantitation of 3 parallel experiments. (C) MDA-MB-231 cells were pre-treated with cycloheximide for 1 hour at 100 μg mL^{-1}, followed by addition of DMSO or 500 nM PROTAC. At the indicated times, cells were lysed and p38α protein levels were analyzed by western blot. (E) PROTACs inhibit but do not degrade Axl. MDA-MB-231 cells were pretreated with the indicated compounds for 24 hours followed with a 10-minute pulse of Gas6 to simulate the Axl-AKT pathway. (F) Additional targets were analyzed by western blot from the experiment shown in Figure 3C.



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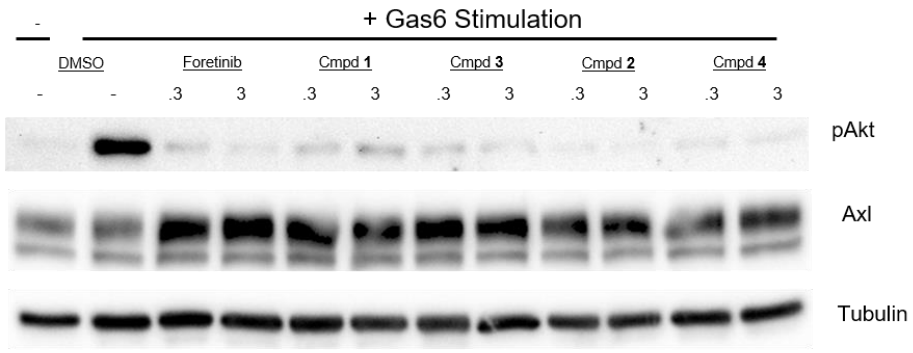
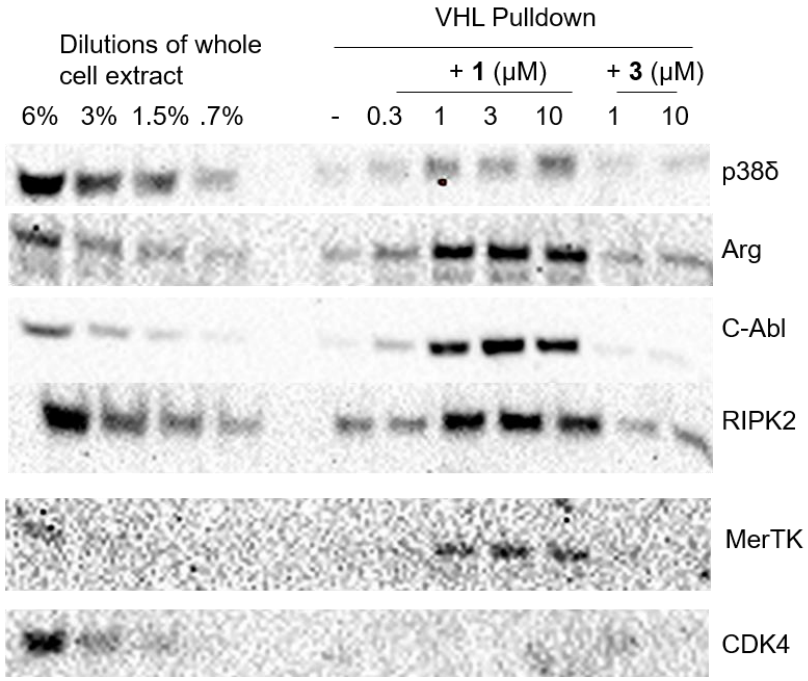
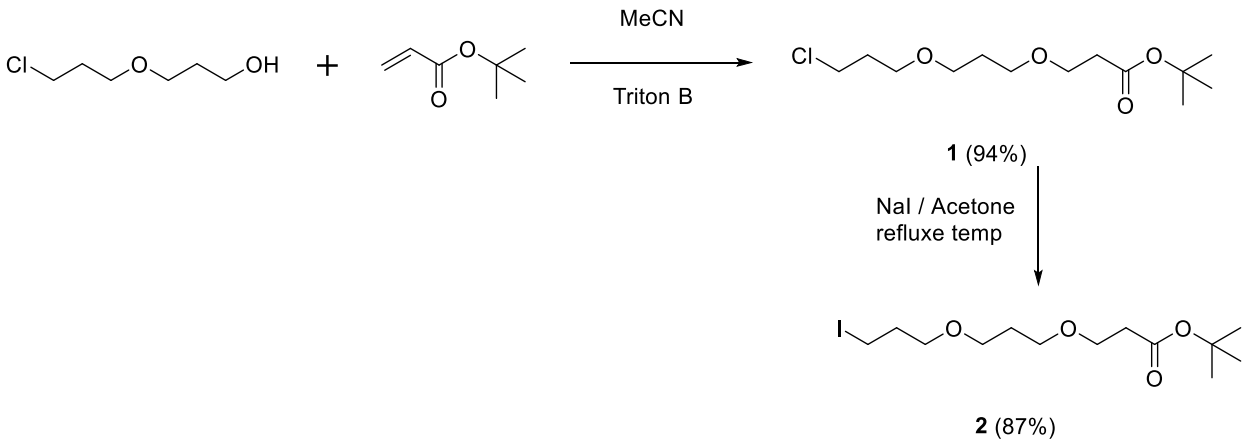

-
+ Gas6 Stimulation
DMSO
Foretinib
Cmpd 1
Cmpd 3
Cmpd 2
Cmpd 4
- - .3 3 .3 3 .3 3 .3 3 .3 3
pAkt
Axl
Tubulin

F

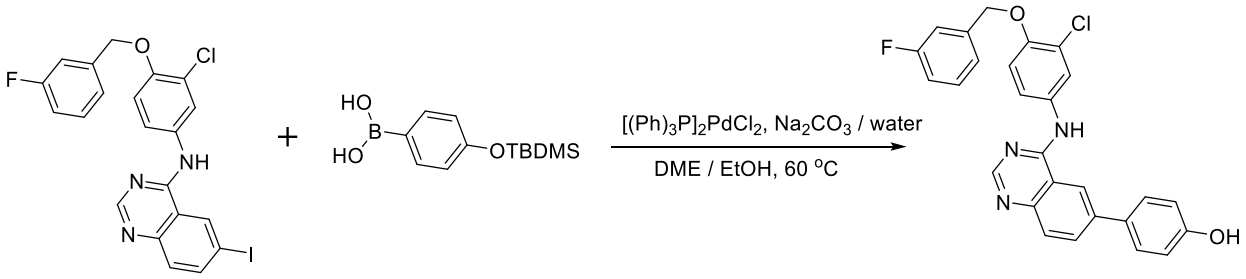

Dilutions of whole cell extract
6% 3% 1.5% .7%
VHL Pulldown
+ 1 (μM)
+ 3 (μM)
- 0.3 1 3 10 1 10
p38δ
Arg
C-Abl
RIPK2
MerTK
CDK4

Chemistry



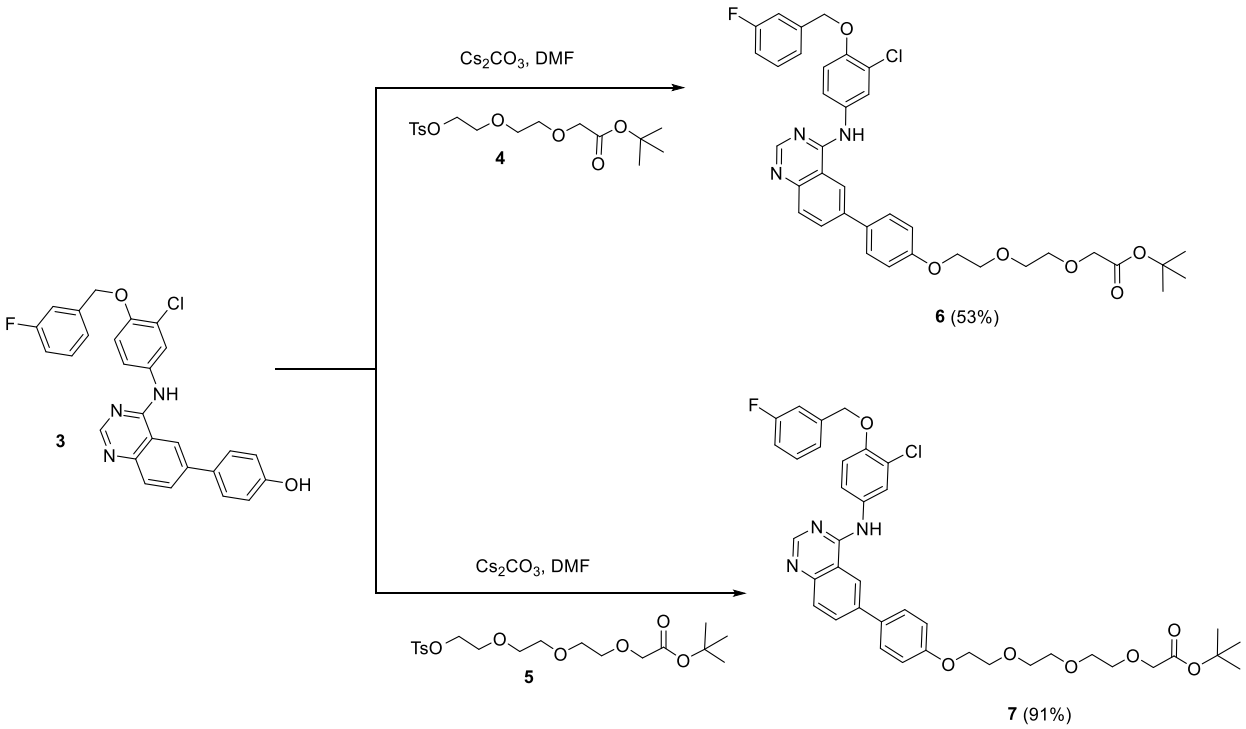
***tert*-Butyl 3-(3-(3-chloropropoxy)propoxy)propanoate (1).** 3-(3-chloropropoxy)propan-1-ol (66 mg, 0.43 mmol) in acetonitrile (3 mL) was added tert-butyl prop-2-enoate (0.31 ml, 2.16 mmol) followed by Triton B (54 mg, 0.1 mmol, 40% by weight in water). The mixture was stirred at room temperature for 72 hour. The mixture was concentrated under vacuum and crude product was purified by column chromatography (SiO_2, gradient Hex:EtOAc, 95:5 to 9:1) to give 115 mg of product (**1**) as an oil (94% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 3.70 – 3.59 (m, 4H), 3.59 – 3.42 (m, 6H), 2.47 (t, J = 6.5 Hz, 2H), 2.04 – 1.96 (m, 2H), 1.82 (p, J = 6.3 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-d) δ 171.13, 80.63, 68.02, 67.97, 67.27, 66.64, 42.17, 36.50, 32.88, 30.09, 28.25. LC-MS (ESI); m/z $[M+Na]^+$: Calcd. for $C_{13}H_{25}ClO_4Na$, 303.1339. Found 303.1381.

***tert*-Butyl 3-(3-(3-iodopropoxy)propoxy)propanoate (2)**. To a solution of tert-butyl 3-[3-(3-chloropropoxy)propoxy]propanoate (161 mg, 0.57 mmol) in Acetone (5 ml) was added NaI (429 mg, 2.87 mmol). The reaction mixture was stirred at reflux temperature for 24 h, then the solvent was removed under vacuum and crude product was dissolved in EtOAc (15 mL), washed with water (10 mL), and with an aqueous solution of Na_2SO_3 (10%, 10 mL). Organic layer was separated, washed with water (10 mL), dried (Na_2SO_4) and evaporated under vacuum. Crude product was pure by NMR (>98% purity, 186 mg, 87% yield), product (**2**) was used in the next step without any further purification. ^{1}H NMR (400 MHz, Chloroform-d) δ 3.66 (t, J = 6.5 Hz, 2H), 3.57 – 3.40 (m, 6H), 3.27 (t, J = 6.8 Hz, 2H), 2.48 (t, J = 6.5 Hz, 2H), 2.08 – 1.99 (m, 2H), 1.82 (p, J = 6.4 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-d) δ 171.13, 80.64, 70.18, 68.01, 67.98, 66.65, 36.50, 33.57, 30.10, 28.26, 3.72. LC-MS (ESI): m/z $[M+Na]^+$ Calcd. for $C_{13}H_{25}IO_4Na$: 395.0695, Found: 395.0719.

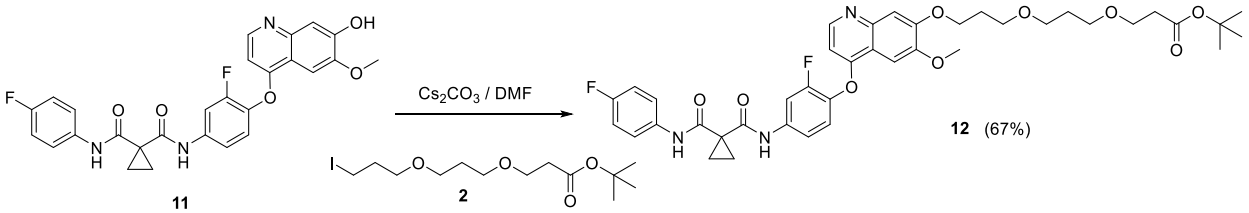


3 (75%)

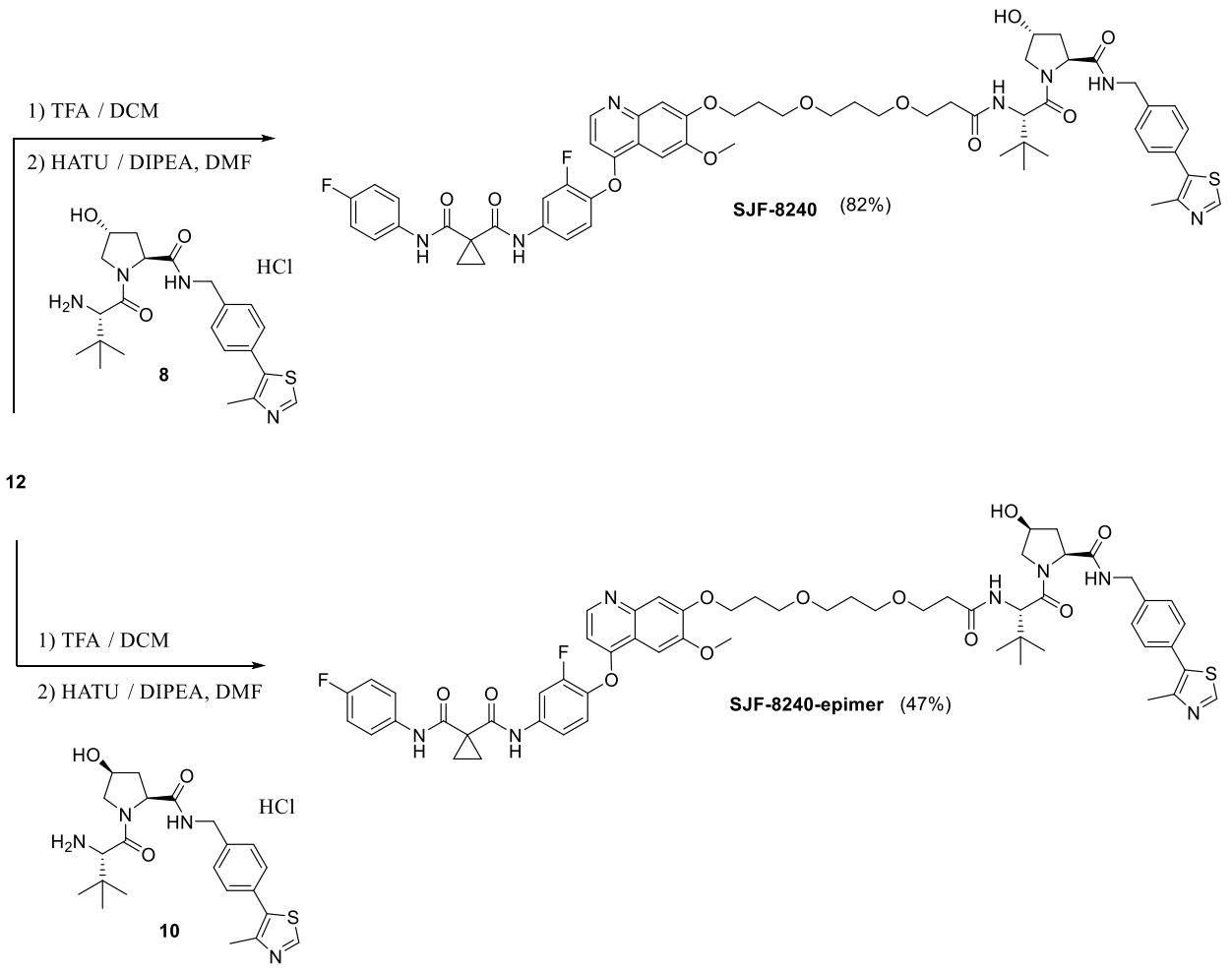
4-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenol (3). A suspension of N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-iodo-quinazolin-4-amine (300 mg, 0.59 mmol) in a mixture of 1,2-Dimethoxyethane (12 ml) and Ethanol (8 ml) was evacuated in vacuum and purged with argon (5x), then 2M Na_2CO_3 in water (6.5 ml) was added and the reaction mixture was again evacuated in vacuum and purged with argon (5x), then [4-[tert-butyl(dimethyl)silyl]oxyphenyl]boronic acid (209 mg, 0.831 mmol) was added into, and $[(Ph)_3P]_2PdCl_2$ (70 mg, 0.08 mmol). The reaction mixture was heated to 60 °C for 3h. The reaction mixture was cooled to room temperature and the reaction mixture was poured into an aqueous saturated solution of $NaHCO_3$ (30 mL) and product was extracted with AcOEt (2x30 mL). Organic extracts were combined, dried (Na_2SO_4), filtered over a celite pad, and evaporated under vacuum. Crude product was purified by flash chromatography (SiO_2-25g. dry silica-dispersion loading, gradient Hex:AcOEt, 9:1 to 100% AcOEt in 15 min) to give 210 mg of product (75% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.85 (s, 1H), 9.68 (s, 1H), 8.69 (s, 1H), 8.56 (s, 1H), 8.12 (d, J = 8.7 Hz, 1H), 8.03 (s, 1H), 7.87 – 7.67 (m, 4H), 7.48 (td, J = 8.0, 6.0 Hz, 1H), 7.39 – 7.25 (m, 3H), 7.19 (tt, J = 7.8, 1.4 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H), 5.27 (s, 2H). ^{13}C NMR (151 MHz, dmso) δ 163.06, 161.44, 157.66, 157.59, 154.07, 149.69, 148.44, 139.73, 139.69, 138.23, 133.18, 131.45, 130.69, 130.64, 129.86, 128.36, 128.32, 124.20, 123.45, 123.43, 122.38, 121.01, 118.95, 115.88, 115.33, 114.87, 114.73, 114.28, 114.21, 114.07, 69.37. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{27}H_{20}ClFN_3O_2$, 472.1228. Found 472.1283.



tert-Butyl 2-(2-(2-(2-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)-ethoxy)ethoxy)ethoxy)acetate (7). To a mixture of 4-[4-[3-chloro-4-[(3-fluorophenyl)methoxy]anilino]-quinazolin-6-yl]phenol (7.2 mg, 0.015 mmol) and tert-butyl 2-[2-[2-[2-(p-tolylsulfonyloxy)ethoxy]ethoxy] -ethoxy]-acetate (8.3 mg, 0.02 mmol) in N,N-Dimethylformamide (2 mL) was added Cs_2CO_3 (14.91 mg, 0.05 mmol). Reaction mixture was heated at 50 ^oC for 2 h. Reaction mixture was diluted with AcOEt (20 mL), washed with water (4x15 mL), dried Na_2SO_4 and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1) to give 10 mg of product (**7**) (91% yield). ^1H NMR (500 MHz, DMSO-d6) δ 9.87 (s, 1H), 8.74 (d, J = 2.1 Hz, 1H), 8.57 (s, 1H), 8.16 (dd, J = 8.8, 1.9 Hz, 1H), 8.03 (s, 1H), 7.83 (dd, J = 8.6, 5.7 Hz, 3H), 7.76 (dd, J = 9.0, 2.6 Hz, 1H), 7.47 (td, J = 8.0, 6.1 Hz, 1H), 7.38 – 7.25 (m, 3H), 7.22 – 7.14 (m, 1H), 7.13 (d, J = 8.8 Hz, 2H), 5.27 (s, 2H), 4.23 – 4.13 (m, 2H), 3.98 (s, 2H), 3.84 – 3.74 (m, 2H), 3.68 – 3.46 (m, 8H), 1.41 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d6) δ 169.36, 163.01, 161.39, 158.54, 157.60, 154.15, 149.70, 148.57, 139.69, 139.64, 137.72, 133.14, 131.44, 130.61, 130.55, 128.32, 128.25, 124.19, 123.36, 123.34, 122.37, 121.03, 119.29, 115.28, 115.02, 114.78, 114.64, 114.30, 114.13, 113.98, 80.64, 69.95, 69.86, 69.78, 69.72, 69.38, 68.95, 68.09, 67.29, 27.76. LC-MS (ESI): m/z $[M+H]^+$ Calcd. For $C_{39}H_{42}ClFN_3O_7$, 718.2695. Found 718.3026.



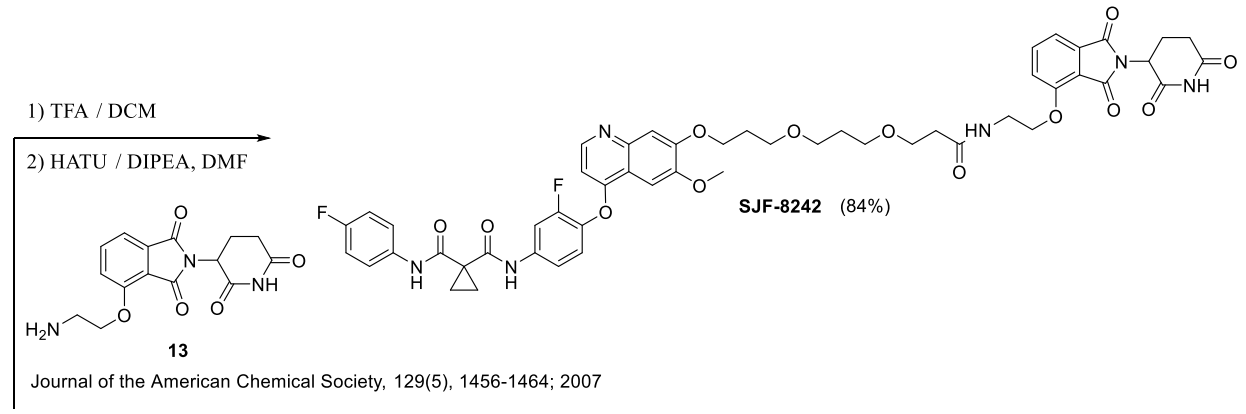
tert-Butyl 3-(3-(3-((4-(2-fluoro-4-(1-((4-fluorophenyl)carbamoyl)cyclopropane-1-carboxamido)-phenoxy)-6-methoxyquinolin-7-yl)oxy)propoxy)propoxy)propanoate (**12**). To a mixture of N1'-[3-fluoro-4-[(7-hydroxy-6-methoxy-4-quinolyl)oxy]phenyl]-N1-(4-fluorophenyl)cyclopropane -1,1-dicarboxamide (**11**) (15 mg, 0.03 mmol) and tert-butyl 3-[3-(3-iodopropoxy)propoxy]propanoate (**2**) (16.57 mg, 0.04 mmol) in N,N-Dimethylformamide (1 mL) was added Cs_2CO_3 (29.01 mg, 0.09 mmol). After stirring at room temperature for 12 hrs (overnight), the reaction mixture was diluted with AcOEt (20 mL) and washed with water (5x10 mL), organic phase was evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1) to give 15 mg of product (**12**) (67% yield). ^1H NMR (400 MHz, DMSO-d6) δ 10.39 (s, 1H), 10.01 (s, 1H), 8.46 (d, J = 5.2 Hz, 1H), 7.90 (d, J = 13.2 Hz, 1H), 7.71 – 7.58 (m, 2H), 7.51 (d, J = 7.4 Hz, 2H), 7.46 – 7.35 (m, 2H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.1 Hz, 1H), 4.21 (t, J = 6.2 Hz, 2H), 3.95 (s, 3H), 3.60 – 3.37 (m, 8H), 2.37 (d, J = 12.2 Hz, 2H), 2.04 (p, J = 6.4 Hz, 2H), 1.71 (p, J = 6.4 Hz, 2H), 1.47 (s, 4H), 1.37 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d6) δ 170.45, 168.27, 167.87, 159.29, 159.07, 157.48, 154.07, 152.44, 151.89, 149.56, 148.82, 146.37, 138.05, 137.98, 135.70, 135.61, 135.20, 135.19, 123.82, 122.46, 122.41, 116.90, 115.11, 115.09, 114.96, 114.47, 109.04, 108.88, 108.50, 101.95, 99.01, 79.64, 67.07, 66.55, 65.92, 65.45, 55.79, 35.87, 31.93, 29.53, 28.90, 27.76, 27.73, 15.31. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{40}H_{46}F_2N_3O_9$, 750.3202 . Found 750.3509.

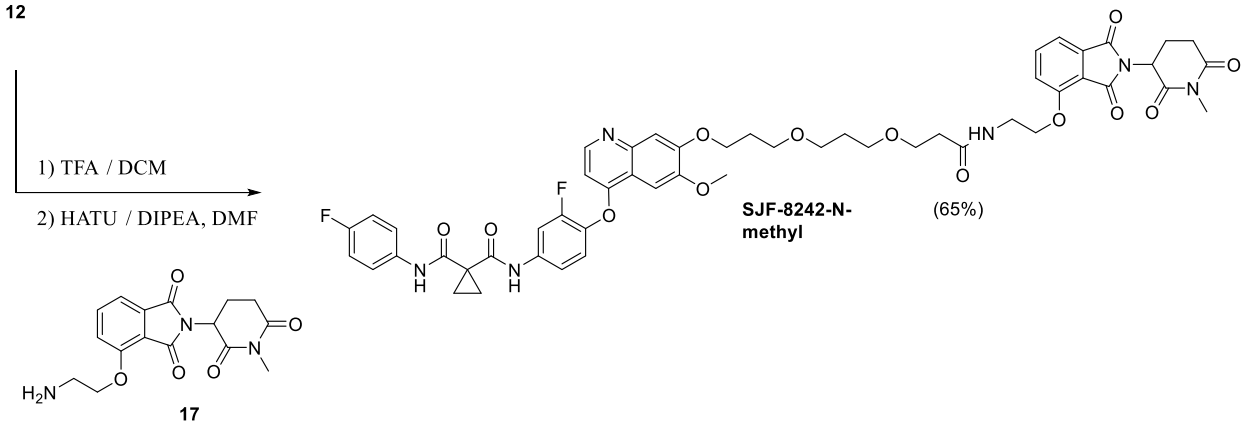


N-(3-Fluoro-4-((7-(3-(3-(3-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)propoxy)propoxy)-6-methoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (**Compound 1 in Main Text**). A solution of tert-butyl 3-[3-[3-[[4-[2-fluoro-4-[[1-[(4-fluorophenyl)carbamoyl]cyclopropanecarbonyl] amino]phenoxy]-6-methoxy-7-quinolyl]oxy]propoxy]propoxy]propanoate (**12**) (15 mg, 0.02 mmol) in a mixture of TFA (1 ml, 13.46 mmol) and Dichloromethane (3 ml) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 2 h. Crude product was used in the next step without any further purification (13.8 mg, quantitative yield). LC-MS (ESI): m/z [M+H]+ Calcd. for $C_{36}H_{38}F_2N_3O_9$, 694.2576. Found 694.2324. To a solution of crude product from above (13.8 mg, 0.02 mmol) and (2S,4R)-1-[(2S)-2-amino -3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]-pyrrolidine-2-carboxamide;hydrochloride (**8**) (11.15 mg, 0.02 mmol) in N,N-Dimethylformamide (2 ml) was added DIPEA (0.17 ml, 0.99 mmol) and HATU (11.35 mg, 0.03 mmol) at room temperature. The reaction mixture was stirred for 12 h (overnight) at the same temperature. Reaction mixture was diluted with ACOEt (20 mL), washed with water (4x15 mL), dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1), to give 18 mg of product (82 % yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.38 (s, 1H), 10.00 (s, 1H), 8.97 (s, 1H), 8.56 (t, J = 6.1 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 7.96 – 7.85 (m, 2H), 7.69 – 7.59 (m, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.45 – 7.33 (m, 5H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.1 Hz, 1H), 5.12 (d, J = 3.3 Hz, 1H), 4.55 (d, J = 9.4 Hz, 1H), 4.43 (ddd, J = 10.9, 6.7, 3.3 Hz, 2H), 4.27 – 4.16 (m, 3H), 3.94 (s, 3H), 3.76 – 3.33 (m, 10H), 2.58 – 2.51 (m, 1H), 2.43 (s, 3H),

2.35 – 2.25 (m, 1H), 2.03 (p, J = 5.7 Hz, 3H), 1.95 – 1.83 (m, 1H), 1.72 (p, J = 6.4 Hz, 2H), 1.48 (d, J = 3.9 Hz, 4H), 0.92 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d6) δ 171.89, 169.97, 169.51, 168.26, 167.88, 159.31, 159.22, 157.31, 154.21, 152.26, 151.90, 151.39, 149.56, 148.75, 147.69, 146.29, 139.47, 138.01, 137.94, 135.70, 135.60, 135.17, 135.15, 131.13, 129.61, 128.81, 128.61, 127.40, 123.77, 122.46, 122.40, 116.90, 115.09, 114.92, 114.47, 109.53, 109.05, 108.87, 108.45, 101.94, 99.03, 68.85, 67.16, 67.09, 66.62, 66.54, 65.47, 58.69, 56.35, 56.24, 55.77, 41.64, 37.92, 35.69, 35.36, 31.87, 29.60, 28.89, 26.28, 15.91, 15.31. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{58}H_{66}F_2N_7O_{11}S$, 1106.4509. Found 1106.4510.

N-(3-Fluoro-4-((7-(3-(3-(3-(((S)-1-((2S,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)propoxy)propoxy)-6-methoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (**Compound 3 in Main Text**). It was prepared from (2S,4R)-1-[(2S)-2-amino -3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide;hydrochloride (**10**) (10.42 mg, 0.022 mmol) and following the same procedure than above. Crude product was purified by PTLC (DCM:MEOH:NH_4OH, 90:9:1), to give 9.7 mg of the expected product (47 % yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.38 (s, 1H), 10.00 (s, 1H), 8.97 (s, 1H), 8.63 (t, J = 6.0 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 7.90 (d, J = 9.7 Hz, 2H), 7.71 – 7.57 (m, 2H), 7.51 (d, J = 8.7 Hz, 2H), 7.44 – 7.28 (m, 5H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.1 Hz, 1H), 5.43 (d, J = 7.2 Hz, 1H), 4.56 – 4.38 (m, 2H), 4.36 (dd, J = 8.6, 6.1 Hz, 1H), 4.32 – 4.13 (m, 4H), 3.94 (s, 3H), 3.97 – 3.82 (m, 1H), 3.64 – 3.46 (m, 4H), 3.48 – 3.35 (m, 4H), 2.57 – 2.45 (m, 2H), 2.43 (s, 3H), 2.36 – 2.26 (m, 2H), 2.03 (p, J = 6.3 Hz, 2H), 1.73 (dp, J = 13.0, 6.2 Hz, 3H), 1.55 – 1.38 (m, 4H), 0.93 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d6) δ 171.89, 169.97, 169.51, 168.26, 167.88, 159.31, 159.22, 157.31, 154.21, 152.26, 151.90, 151.39, 149.56, 148.75, 147.69, 146.29, 139.47, 138.01, 137.94, 135.70, 135.60, 135.17, 135.15, 131.13, 129.61, 128.81, 128.61, 127.40, 123.77, 122.46, 122.40, 116.90, 115.09, 114.92, 114.47, 109.53, 109.05, 108.87, 108.45, 101.94, 99.03, 68.85, 67.16, 67.09, 66.62, 66.54, 65.47, 58.69, 56.35, 56.24, 55.77, 41.64, 37.92, 35.69, 35.36, 31.87, 29.60, 28.89, 26.28, 15.91, 15.31. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{58}H_{66}F_2N_7O_{11}S$, 1106.4509. Found 1106.5096.

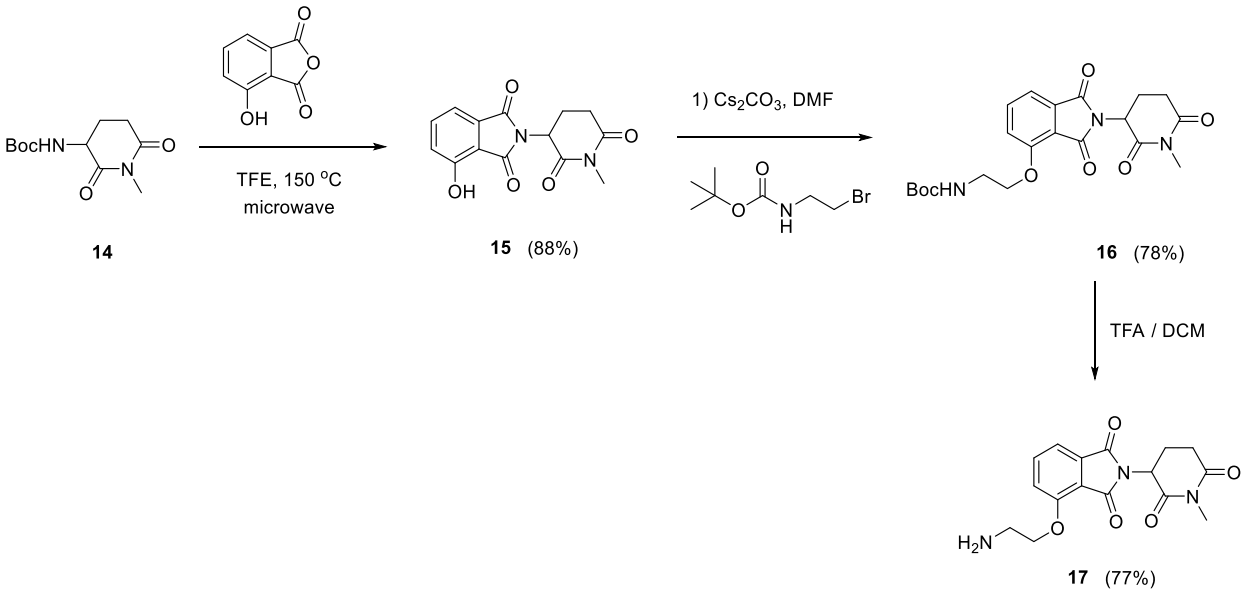




N-(4-((7-(3-(3-(3-((2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethyl)amino)-3-oxopropoxy)propoxy)propoxy)-6-methoxyquinolin-4-yl)oxy)-3-fluorophenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (Compound 2 in main text). A solution of the *tert*-butyl ester **12** (17 mg, 0.023 mmol) in a mixture of TFA (1 ml) and DCM (3 ml) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 2 h, to give 15.7 mg of 3-(3-(3-((4-(2-fluoro-4-(1-((4-fluorophenyl)carbamoyl)cyclopropane-1-carboxamido)phenoxy)-6-methoxyquinolin-7-yl)oxy)propoxy)propoxy)propanoic acid (quantitative yield). Crude product was used in the next step without any further purification. LC-MS (ESI): m/z $[M+H]^+$ Calcd. For $C_{36}H_{38}F_2N_3O_9$, 694.2576 . Found 694.2605. To a solution of the crude product from above (15 mg, 0.02 mmol) and 4-(2-aminoethoxy) -2-(2,6-dioxo-3-piperidyl)isoindoline-1,3-dione as a TFA salt (**13**) (11 mg, 0.03 mmol) in DMF (2 ml) was added DIPEA (0.2 ml, 1.15 mmol) and HATU (12.91 mg, 0.03 mmol) at room temperature. The reaction mixture was stirred for 12 h (overnight) at the same temperature. Reaction mixture was diluted with ACOEt (20 mL), washed with water (4x15 mL), dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1), to give 19 mg of product (84 % yield). ^1H NMR (400 MHz, DMSO-d6) δ 11.12 (s, 1H), 10.39 (s, 1H), 10.01 (s, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.10 (t, J = 5.5 Hz, 1H), 7.90 (dd, J = 13.2, 2.4 Hz, 1H), 7.79 (dd, J = 8.5, 7.3 Hz, 1H), 7.71 – 7.55 (m, 2H), 7.58 – 7.46 (m, 2H), 7.48 – 7.32 (m, 3H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.0 Hz, 1H), 5.08 (dd, J = 12.8, 5.4 Hz, 1H), 4.21 (dt, J = 15.8, 6.1 Hz, 4H), 3.95 (s, 3H), 3.63 – 3.34 (m, 10H), 2.88 (ddd, J = 17.1, 14.0, 5.4 Hz, 1H), 2.63 – 2.43 (m, 2H), 2.31 (t, J = 6.3 Hz, 2H), 2.09 – 1.93 (m, 3H), 1.68 (p, J = 6.4 Hz, 2H), 1.53 – 1.39 (m, 4H). ^{13}C NMR (151 MHz, dmso) δ 172.78, 170.65, 169.92, 168.26, 167.88, 166.77, 165.22, 159.32, 159.07, 157.48, 155.69, 154.06, 152.44, 151.89, 149.54, 148.79, 146.29, 138.05, 137.99, 137.00, 135.68, 135.60, 135.20, 135.18, 133.25, 123.82, 122.46, 122.40, 120.04, 116.90, 116.48, 115.51, 115.11, 114.96, 114.46, 109.03, 108.88, 108.44, 101.95,

99.02, 67.49, 67.08, 67.05, 66.55, 66.38, 65.47, 55.79, 48.74, 37.98, 36.03, 31.93, 30.95, 29.50, 28.88, 22.02, 15.32. LC-MS (ESI): m/z $[M+H]^+$ Calcd. For $C_{51}H_{51}F_2N_6O_{13}$, 993.3482. Found 993.3468.

N-(3-fluoro-4-((6-methoxy-7-(3-(3-(3-((2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethyl)amino)-3-oxopropoxy)propoxy)propoxy)quinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (**Compound 4 in main text**). It was prepared from 4-(2-aminoethoxy)-2-(1-methyl-2,6-dioxo-3-piperidyl)isoindoline-1,3-dione;2,2,2-trifluoroacetic acid (**17**) and following the same procedure than above. Crude product was purified by PTLC (DCM:MEOH:NH_4OH, 90:9:1), to give 24 mg of product (65 % yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.39 (s, 1H), 10.01 (s, 1H), 8.46 (d, J = 5.2 Hz, 1H), 8.08 (t, J = 5.1 Hz, 2H), 7.90 (d, J = 13.2 Hz, 1H), 7.84 – 7.74 (m, 1H), 7.69 – 7.58 (m, 2H), 7.57 – 7.48 (m, 3H), 7.48 – 7.31 (m, 3H), 7.15 (t, J = 8.8 Hz, 2H), 6.41 (d, J = 5.2 Hz, 1H), 5.15 (dd, J = 13.0, 5.4 Hz, 1H), 4.21 (dt, J = 16.4, 6.3 Hz, 4H), 3.95 (s, 3H), 3.63 – 3.36 (m, 10H), 3.01 (d, J = 1.2 Hz, 3H), 2.97 – 2.86 (m, 1H), 2.82 – 2.67 (m, 1H), 2.50 (dt, J = 3.5, 1.9 Hz, 1H), 2.32 (t, J = 6.4 Hz, 2H), 2.13 – 1.96 (m, 3H), 1.77 – 1.61 (m, 2H), 1.48 (bs, 4H). 13C NMR (151 MHz, DMSO-d6) δ 171.76 , 170.65 , 169.65 , 168.27 , 167.89 , 166.74 , 165.22 , 159.29 , 158.28 (d, J = 239.9 Hz), 155.72 , 153.26 (d, J = 245.1 Hz), 151.87 , 149.53 , 148.82 , 146.35 , 138.01 (d, J = 10.1 Hz), 137.03 , 135.66 (d, J = 12.3 Hz), 135.19 (d, J = 2.6 Hz), 133.23 , 123.81 , 122.43 (d, J = 7.8 Hz), 120.06 , 116.90 , 116.45 , 115.54 , 115.03 (d, J = 22.2 Hz), 114.46 , 108.96 (d, J = 23.0 Hz), 108.49 , 101.94 , 99.01 , 67.50 , 67.09 , 67.05 , 66.56 , 66.39 , 65.46 , 55.78 , 49.31 , 37.98 , 36.04 , 31.91 , 31.09 , 29.50 , 28.89 , 26.60 , 21.23 , 15.33 . LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{52}H_{53}F_2N_6O_{13}$, 1007.3638. Found 1007.4191.



14 **15** (88%) **16** (78%) **17** (77%)

4-hydroxy-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**15**). A suspension of tert-butyl N-(1-methyl-2,6-dioxo-3-piperidyl)carbamate (97 mg, 0.4 mmol) and 4-hydroxyisobenzofuran-1,3-dione (65.71 mg, 0.4 mmol) in Trifluoroethanol (2 mL) was heated at 150 oC for 2 h under microwave assisted conditions. After cooling the reaction mixture to room temperature and by addition of AcOEt (2 mL), a solid crystallise out from the reaction mixture. The solid was collected by filtration to give 102 mg of pure product (**15**) (88% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 11.17 (s, 1H), 7.66 (dd, J = 8.4, 7.2 Hz, 1H), 7.32 (d, J = 7.2 Hz, 1H), 7.25 (d, J = 8.4 Hz, 1H), 5.14 (dd, J = 13.0, 5.4 Hz, 1H), 3.01 (s, 3H), 3.00 – 2.87 (m, 1H), 2.82 – 2.69 (m, 1H), 2.61 – 2.49 (m, 1H), 2.09 – 1.96

(m, 1H). ^{13}C NMR (151 MHz, dmso) δ 171.79, 169.76, 167.00, 165.79, 155.49, 136.42, 133.13, 123.58, 114.34, 114.30, 49.20, 31.11, 26.60, 21.23. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{14}H_{13}N_2O_5$, 289.0824. Found 289.0789.

tert-Butyl (2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethyl)carbamate (**16**). To a solution of 4-hydroxy-2-(1-methyl-2,6-dioxo-3-piperidyl)isoindoline-1,3-dione (**15**) (18 mg, 0.06 mmol), tert-butyl N-(2-bromoethyl)carbamate (15.39 mg, 0.07 mmol), in DMF (1 mL) was added Cs_2CO_3 (20.35 mg, 0.06 mmol) and the reaction mixture was heated at 60°C for 12h. Reaction mixture was poured into an aqueous solution of HCL (1M, 5 mL) and AcOEt (10 mL), organic layer was separated, washed (water, 4x), dried (Na_2SO_4), and evaporated under vacuum. Crude product was purified by PTLC (DCM:MEOH:NH_4OH, 90:9:1) to give 22 mg (81%) of (**16**). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.81 (t, J = 7.9 Hz, 1H), 7.53 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 7.2 Hz, 1H), 6.95 (s, 1H), 5.15 (dd, J = 12.9, 5.4 Hz, 1H), 4.23 (t, J = 5.7 Hz, 3H), 3.40 – 3.29 (m, 2H), 3.02 (s, 3H), 3.00 – 2.87 (m, 1H), 2.76 (dt, J = 17.5, 3.6 Hz, 1H), 2.61 – 2.50 (m, 1H), 2.08 – 1.97 (m, 1H), 1.37 (s, 9H).^{13}C NMR (151 MHz, dmso) δ 171.78, 169.65, 166.76, 165.21, 155.75, 155.66, 137.04, 137.03, 133.25, 120.05, 116.44, 115.48, 77.92, 67.49, 49.30, 31.08, 28.20, 27.60, 26.60, 21.25. LC-MS (ESI): m/z $[M+Na]^+$ Calcd. for $C_{21}H_{25}N_3O_7Na$, 454.1590. Found 454.1507.

4-(2-Aminoethoxy)-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**17**). A solution of tert-butyl N-[2-[2-(1-methyl-2,6-dioxo-3-piperidyl)-1,3-dioxo-isoindolin-4-yl]oxyethyl]carbamate (23 mg, 0.05 mmol) in a mixture of DCM:TFA (3:1 mL) was stirred for 1 h at room temperature. Solvent was removed under vacuum. By TLC and LC-MS crude product was pure, crude product was used in the next step without any further purification (23 mg, quantitative yield). LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{16}H_{18}N_3O_5$, 332.1246. Found 332.1283.