Electronic Supplementary Material (ESI) for Chemical Communications.

Chemically induced degradation of CDK9 by a proteolysis targeting chimera (PROTAC)

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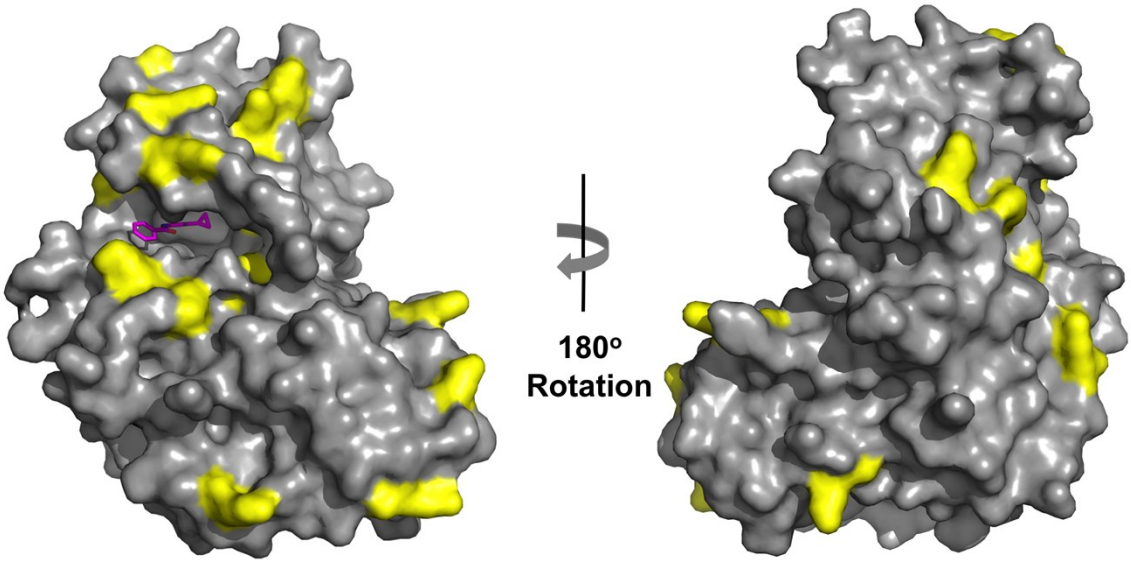
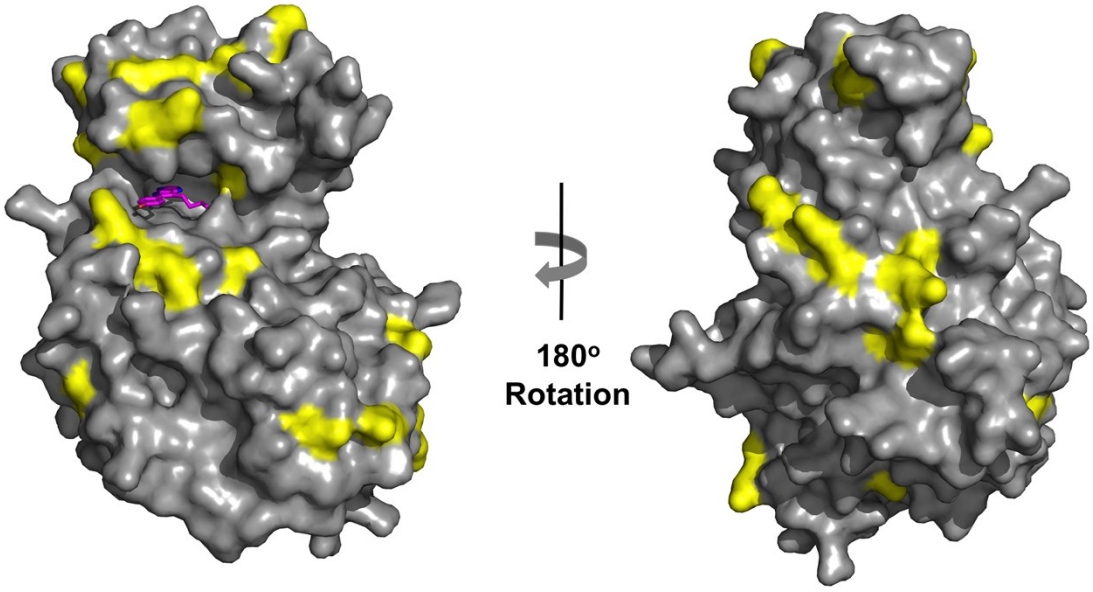
(# equal contribution)

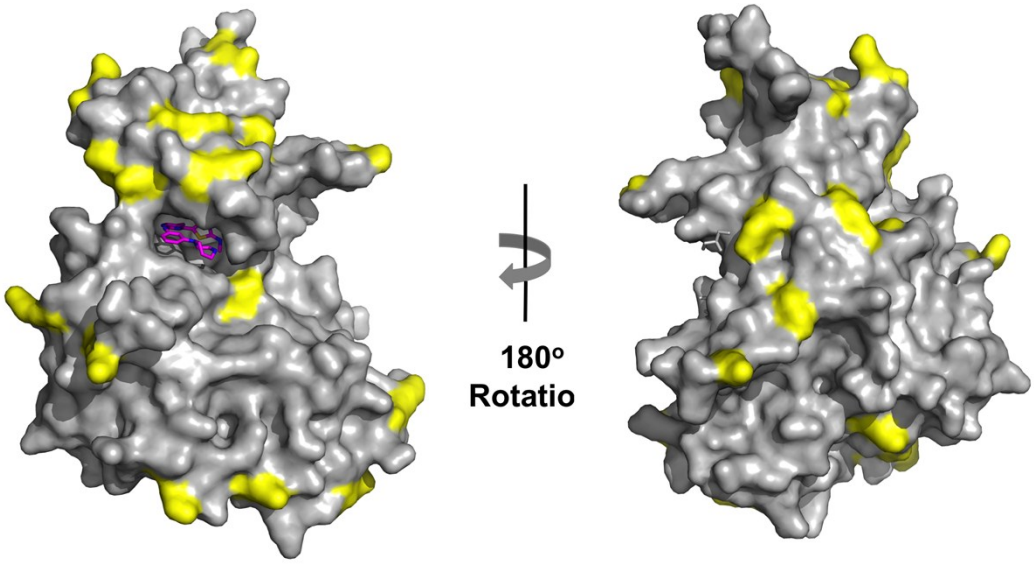
Figure S1: The distribution of lysine residues (yellow) for CDK2 (PDB id 1VYZ), CDK5 (PDB id 1UNG) and CDK9 (PDB id 4BCG) are shown below. Inhibitor is shown in magenta.



A. CDK2



B. CDK5



C. CDK9

Cell lines and Materials: HCT116 cells were grown in RPMI-1640 medium (HyClone #SH30027.01) supplemented with 10% Fetal Bovine Serum (Gibco by Life Technologies #26140-079) and 1x-penicillin-streptomycin (HyClone #SV30010). Cells were incubated at

37°C and 5% CO2. Cells were validated by STR profiling at the University of Nebraska Medical Center Human DNA Identification Laboratory.

Western Blotting: HCT116 cells were treated with different compounds for 6 hours. Following treatment, cells were harvested and lysed using a buffer comprised of 50mM Tris, 100mM NaCl, 1% NP-40, 2mM EDTA, 20%SDS combined with 20xPPI (Na_3VO_4, NAF, β-glycerophosphate), and 1 mmol/L phenylmethane sulfonyl fluoride (PMSF). Samples were incubated on ice for 30 minutes and vortexed before being pelleted by centrifugation at 4°C. Supernatant was collected and protein quantified using BCA Protein Assay (Pierce # 23225). 40µg of protein were loaded into 4-15% gradient gels (BioRad). 1x TRIS-Glycine SDS running buffer (Research Products International Corporation #T32080) was used and gels were run at 120V for ~75 minutes and separated by SDS-PAGE electrophoresis. Proteins were transferred onto a PVDF methanol activated membrane using a semi-dry transfer method (ThermoScienctific, #35035) run at 18V for 35 minutes. Membranes were blocked in 5% milk diluted in 1X Tris Buffered Saline with 0.1%Tween (1xTBST) for 1 hour at room temperature while gently rocking at speed 2. Primary antibodies (listed below) were incubated in 5% milk in 1x TBST and rocked overnight at 4°C. Complimentary HRP-conjugated secondary antibodies diluted at 1:10,000 were incubated in 5% milk in 1xTBST and rocked on speed 2 for 1 hour at room temperature. ECL Prime (GE Healthcare #RPN2236) was used to detect protein expression. Quantification of protein expression after western blot analyses was performed using ImageJ.

Antibody Information:

α-tubulin - Cell Signaling Technologies # 3873 (1:10,000 dilution)
AKT - Cell Signaling Technologies # 9272 (1:2000 dilution)
FAK - Cell Signaling Technologies # 3285 (1:2000 dilution)
CDK2 - Cell Signaling Technologies # 2546 (1:2000 dilution)
CDK5 - Cell Signaling Technologies # 2506 (1:2000 dilution)
CDK9 - Cell Signaling Technologies # 2316 (1:2000 dilution)
Mcl-1 - Cell Signaling Technologies # 5453 (1:2000 dilution)
pRPB1 (S2) - Cell Signaling Technologies #13499 (1:20,000 dilution)
pFAK (S732) - Abcam ab4792 (1:2000 dilution)

pRB (S807/811) - CST #9308 (1:2000 dilution)

Figure S2: Effect of the aminopyrazole inhibitor **1** and **2** on phosphorylation status of CDK2, CDK5 and CDK9 and it substrates pRB, pFAK and pRPB1.

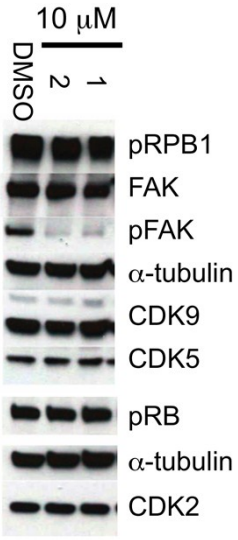
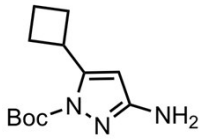


Figure S3: *In vitro* kinase assay for CDK5.

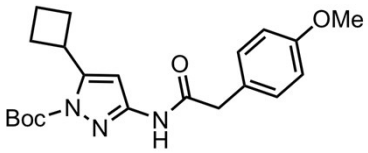
	IC_{50} (nM) ± SEM	
	1	**3**
CDK5/p35	0.54 ± 0.03	0.73 ± 0.05

General Methods: All reagents were purchased from commercial sources and were used without further purification. Flash chromatography was carried out on silica gel (200–400 mesh). Thin layer chromatography (TLC) were run on pre-coated EMD silica gel 60 F254 plates and observed under UV light at 254 nm and with basic potassium permanganate dip. Column chromatography was performed with silica gel (230-400 mesh, grade 60, Fisher scientific, USA). ^{1}H NMR (400 – 600 MHz) and ^{13}C NMR (100 – 150 MHz) spectra were recorded in chloroform-d, or DMSO-d6 on a Bruker instrument ($CDCl_3$ was 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C, DMSO-d_6 was 2.50 ppm for ^{1}H and 39.00 ppm for ^{13}C. Proton and carbon chemical shifts were reported in ppm relative to the signal from residual solvent proton and carbon. Preparative HPLC was carried out on 250 x 21.2 mm C-18 column using gradient conditions (10 – 100% B, flow

rate = 6.0 mL/min, 39 min). The eluents used were: solvent A (H_2O with 0.1% Formic acid) and solvent B (CH_3CN with 0.1% Formic acid). HRMS Mass spectra were obtained using Shimadzu LCMS IT-TOF with LCMS solution software version 3.70 (Shimadzu Scientific Instruments, Columbia, Maryland).

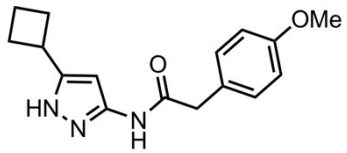


6. *tert*-butyl 5-amino-3-cyclobutyl-1*H*-pyrazole-1-carboxylate: In a round bottom flask, **5** (0.70g, 5.10 mmol) was dissolved in CH_2Cl_2 (20 mL) followed by addition of KOH (2.28g, 40.80 mmol) in 10 mL water. The reaction mixture was allowed to stir at room temperature for 10 min followed by addition of di-tert-butyl dicarbonate (1.17g, 5.35 mmol). The reaction mixture was allowed to stir for 3h and completion of reaction was monitored by thin layer chromatography. The reaction mixture was washed with brine and dried over $MgSO_4$. After filtration and evaporation, the crude residue was purified by flash column chromatography with hexane/ethyl acetate to give compound **6** (1.06g, 88% yield) as off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 5.39 (s, 1H), 5.31 (br, 2H), 3.49 – 3.42 (m, 1H), 2.29 – 1.82 (m, 6H), 1.62 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 160.92, 150.75, 150.61, 86.64, 84.99, 34.34, 28.49, 27.99, 18.66.

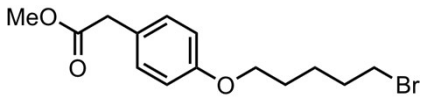


7. *tert*-butyl 3-cyclobutyl-5-(2-(4-methoxyphenyl)acetamido)-1H-pyrazole-1-carboxylate: To a stirred solution of **6** (25mg, 0.10 mmol) in DMF (1mL) were added *N*,*N*-Diisopropylethylamine (0.05 mL, 0.30 mmol) and T3P (0.2mL, 50% in DMF, 0.30 mmol) sequentially. A solution of 4-methoxyphenyl acetic acid (17mg, 0.10 mmol) in DMF (1 mL) was added and this was stirred at 50 °C for 3 h. Progress of the reaction was monitored by thin layer chromatography. Solvent was evaporated and purified by flash column chromatography with hexane/ethyl acetate to give compound **7** (38mg, 95% yield) as white solid. ^{1}H NMR (500 MHz,

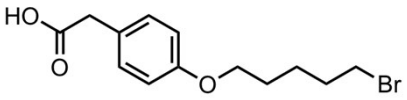
$CDCl_3$) δ (ppm) 10.19 (s, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 2H), 6.85 (s, 1H), 3.82 – 3.79 (m, 2H), 3.69 (s, 2H), 3.56 – 3.48 (m, 1H), 2.31 – 1.88 (m, 6H), 1.60 (s, 9H).



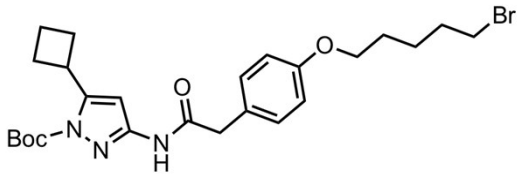
1. *N*-(3-cyclobutyl-1*H*-pyrazol-5-yl)-2-(4-methoxyphenyl)acetamide: To a stirred solution of **7** (16mg, 0.04 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added 1 mL of trifluoroacetic acid dropwise and reaction mixture was stirred for 3h. After completion, the reaction mixture was concentrated in vacuo to give 10.7 mg (92% yield) of **1** as a sticky solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 10.23 (s, 1H), 9.70 (br, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 6.79 (s, 1H), 3.79 (s, 2H), 3.71 (s, 2H), 3.62 – 3.55 (m, 1H), 2.46 – 1.95 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 170.43, 159.15, 153.54, 142.71, 130.37, 124.94, 114.42, 95.22, 55.27, 42.92, 31.21, 28.36, 18.67; HRMS (ESI-MS) calcd for $C_{16}H_{19}N_3O_2$ m/z 285.1477, found: 286.1664.



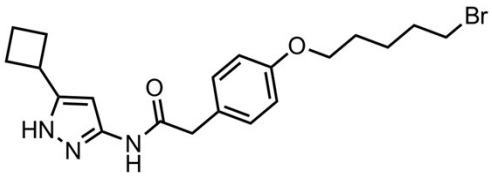
9. methyl 2-(4-((5-bromopentyl)oxy)phenyl)acetate: In a round-bottom flask, compound **8** (0.30g, 1.8 mmol) and K_2CO_3 (0.30g, 2.16 mmol) were dissolved in acetone (10 mL). Reaction mixture was stirred for 10 minutes followed by addition of 1,5-dibromopentane (0.82g, 3.6 mmol) and the resulting mixture was refluxed for 72 h. Acetone was evaporated ad the crude mixture was dissolved in ethyl acetate and washed with saturated ammonium chloride and brine, the combined organic layer was dried over $MgSO_4$. After filtration and evaporation, the residue was purified by flash column chromatography to give **9** as a colorless liquid (0.44g, 77% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.18 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 3.95 (t, *J* = 6 Hz, 2H), 3.68 (s, 3H), 3.56 (s, 2H), 3.43 (t, *J* = 7.0 Hz, 2H), 1.91 – 1.85 (m, 2H), 1.78 – 1.72 (m, 2H), 1.60 – 1.54 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 178.78, 158.26, 130.39, 125.28, 114.63, 67.56, 40.09, 33.54, 32.46, 28.40, 24.82.



10. 2-(4-((5-bromopentyl)oxy)phenyl)acetic acid: To a solution of **9** (0.44g, 1.4 mmol) in 10 mL of ethanol was added lithium hydroxide (0.10g, 4.19 mmol) in water (3 mL) and reaction mixture was stirred at room temperature for 16h. Solvent was evaporated and the crude mixture was dissolved in ethyl acetate (30 mL) and washed with 1N HCl and brine. The organic layers were combined and evaporated under reduced pressure to give **10** (0.42g, quantitative) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.18 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 3.95 (t, *J* = 6 Hz, 2H), 3.58 (s, 2H), 3.43 (t, *J* = 7.0 Hz, 2H), 1.96 – 1.90 (m, 2H), 1.83 – 1.77 (m, 2H), 1.67 – 1.59 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 172.35, 158.10, 130.25, 126.01, 114.58, 67.56, 51.97, 40.29, 33.56, 32.47, 28.42, 24.83.

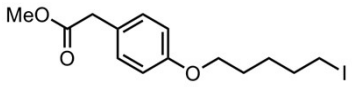


11. *tert*-butyl 5-(2-(4-((5-bromopentyl)oxy)phenyl)acetamido)-3-cyclobutyl-1*H*-pyrazole-1-carboxylate: Following the procedure for the synthesis of compound **7**, compound **11** was obtained in 71% yield as sticky oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 10.19 (s, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.90 (d, *J* = 8.5 Hz, 2H), 6.85 (s, 1H), 3.97 (t, *J* = 6.0 Hz, 2H), 3.68 (s, 2H), 3.56 – 3.49 (m, 1H), 3.44 (t, *J* = 7.0 Hz, 2H), 2.32 – 2.14 (m, 4H), 2.03 – 1.78 (m, 6H), 1.65 – 1.62 (m, 2H), 1.60 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 168.14, 161.08, 158.53, 150.89, 141.42, 130.51, 125.63, 115.06, 95.43, 86.26, 67.65, 43.64, 34.31, 33.53, 32.44, 28.39, 27.92, 24.83, 18.69.

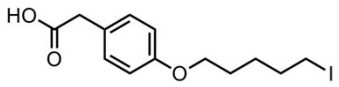


2. 2-(4-((5-bromopentyl)oxy)phenyl)-N-(3-cyclobutyl-1H-pyrazol-5-yl)acetamide: Following the procedure for the synthesis of compound **1**, compound **2** was obtained in quantitative yield as sticky solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 10.49 (s, 1H), 7.20 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.28 (s, 1H), 4.67 (br, 1H), 3.93 (t, *J* = 6.0 Hz, 2H), 3.55 (t, *J* = 7.0 Hz, 2H), 3.49 (s, 2H), 3.46 – 3.42 (m, 1H), 2.27 – 2.21 (m, 2H), 2.09 – 2.05 (m, 2H), 1.96 – 1.80 (m, 4H), 1.75 – 1.69 (m, 2H), 1.55 – 1.49 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 168.02,

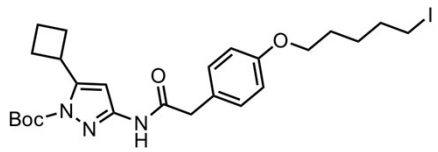
157.92, 157.61, 156.84, 129.51, 127.45, 113.73, 92.76, 66.71, 41.11, 34.55, 31.44, 30.54, 28.35, 27.29, 23.79, 17.57; HRMS (ESI-MS) calcd for $C_{20}H_{26}BrN_3O_2$ m/z 419.1208, found: 420.1462.



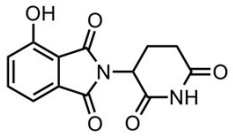
12. Methyl 2-(4-((5-iodopentyl)oxy)phenyl)acetate: To a solution of **9** (0.55g, 1.75 mmol) in acetone (15 ml) was added NaI (1.31g, 8.75 mmol). The reaction mixture was stirred at reflux temperature for 16h, then the solvent was removed under vacuum and crude product was dissolved in EtOAc (15 mL) and washed with an aqueous solution of $NaHSO_3$ (10%, 30 mL), dried using $MgSO_4$ and evaporated under vacuum. Crude product (0.58g, 90% yield) was used as such without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.18 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 3.95 (t, *J* = 6.0 Hz, 2H), 3.68 (s, 3H), 3.56 (s, 2H), 3.22 (t, *J* = 7.0 Hz, 2H), 1.94 – 1.86 (m, 2H), 1.82 – 1.76 (m, 2H), 1.62 – 1.54 (m, 2H); $CDCl_3$) δ (ppm) 172.31, 158.06, 130.22, 125.97, 114.45, 67.51, 51.94, 40.25, 33.16, 28.17, 27.12, 6.59.



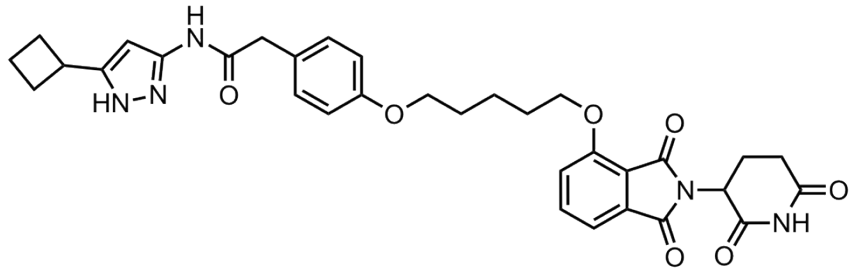
13. 2-(4-((5-iodopentyl)oxy)phenyl)acetic acid: Following the procedure for the synthesis of compound **10**, compound **13** was obtained in 87% yield as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 7.18 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 3.95 (t, *J* = 6 Hz, 2H), 3.58 (s, 2H), 3.21 (t, *J* = 7 Hz, 2H), 1.94 – 1.76 (m, 4H), 1.62 – 1.54 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm) 177.36, 158.26, 130.38, 125.28, 114.64, 67.55, 40.03, 33.18, 28.18, 27.14.



14. tert-butyl 3-cyclobutyl-5-(2-(4-((5-iodopentyl)oxy)phenyl)acetamido)-1H-pyrazole-1-carboxylate Following the procedure for the synthesis of compound **11**, compound **14** was obtained in 34% yield as sticky white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) 10.19 (s, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.84 (s, 1H), 3.96 (t, *J* = 6.0 Hz, 2H), 3.68 (s, 2H), 3.56 – 3.48 (m, 1H), 3.22 (t, *J* = 7.0 Hz, 2H), 2.32 – 1.78 (m, 12H), 1.60 (s, 9H).



4. 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione: In a round-bottom flask, 3-hydroxyphthalic anhydride **15** (0.5g, 3.05 mmol), KOAc (0.93g, 9.45 mmol) and 3-aminoperidine-2,6-dione hydrochloride (0.55g, 3.35 mmol) were mixed in acetic acid (10 mL). The resulting reaction mixture was heated to reflux for 24h. After cooling to ambient temperature, solvent was evaporated and washed with water to yield brown solid which was purified by flash column chromatography with to obtain the desired product **4** as a pale yellow solid (0.81g, 96% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 11.17 (s, 1H), 11.09 (s, 1H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.32 (d, *J* =7.0 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 5.07 (dd, *J* = 13.0 Hz, *J* = 5.5 Hz, 1H), 2.93−2.85 (m, 1H), 2.60−2.47 (m, 2H), 2.04−1.91 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 172.28, 169.49, 166.50, 165.29, 154.94, 135.86, 132.63, 123.04, 113.85, 113.77, 48.12, 30.44, 21.51.



3. *N*-(3-cyclobutyl-1H-pyrazol-5-yl)-2-(4-((5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoiso indo lin-4-yl)oxy)pentyl)oxy)phenyl)acetamide: In a round-bottom flask, compound **4** (8.21mg, 0.03 mmol) was dissolved in DMF (1 mL) followed by addition of $NaHCO_3$ (6.20mg, 0.074 mmol). A solution of **14** (10mg, 0.04 mmol) in DMF (1mL) was added and reaction mixture was stirred at 70 °C for 6 h. Reaction solvent was evaporated and crude mixture was partially purified by flash column chromatography with DCM/MeOH to get the intermediate (14mg). The intermediate was dissolved in CH_2Cl_2 (3 mL) at 0 °C was added 1 mL of trifluoroacetic acid. After stirring for 3h, the reaction mixture was concentrated in vacuo. The crude product was dissolved in MeOH/CH_3CN (5 mL) and subjected to HPLC purification using 5-95% acetonitrile to give **3** (6.0mg, 32% yield over two steps) as off-white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 12.04 (br, 1H), 11.09 (s, 1H), 10.40 (s, 1H), 8.52 (s, 1H), 7.79 (dd, *J* = 8.4 Hz, *J* = 1.2

1H), 7.51 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 7.2 Hz, 1H), 7.19 (d, *J* = 9.0 Hz, 2H), 6.86 – 6.84 (m, 2H), 6.28 (br, 1H), 5.06 (dd, *J* = 12.6 Hz, J = 7.2 Hz, 1H), 4.22 (t, *J* = 7.2 Hz, 2H), 3.94 (t, *J* = 6.0 Hz, 2H), 3.47 (s, 2H), 3.43 – 3.40 (m, 1H), 2.89 – 2.83 (m, 1H), 2.60−2.47 (m, 2H), 2.25 – 2.20 (m, 2H), 2.07 – 1.75 (m, 6H), 1.62 – 1.58 (m, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.82, 169.98, 168.39, 166.85, 165.70, 165.31, 157.34, 155.98, 147.31, 147.03, 137.02, 133.23, 129.99, 128.04, 119.83, 116.22, 115.14, 114.23, 93.32, 68.73, 67.32, 48.72, 41.62, 30.94, 28.90, 28.30, 28.12, 22.03, 21.99, 18.07; HRMS (ESI-MS) calcd for $C_{33}H_{35}N_5O_7$ m/z 613.2536, found: 614.2698.