Supporting Information

Induction of Apoptosis in MDA-MB-231 Breast Cancer Cells by a PARP1-Targeting PROTAC Small Molecule

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Cell lines and Materials: MDA-MB-231, HCC1937, MDA-MB-436 cells were grown in RPMI-1640 medium (Gibco) supplemented with 10% Fetal Bovine Serum (Gemini #900-108) and 1% penicillin-streptoycin (Gibco). MDA-MB-468 cells were grown in DMEM/F12 medium (Gibco) supplemented with 10% Fetal Bovine Serum and 1% penicillin-streptoycin. BEAS-2B, Hela and HepG2 cells were grown in DMEM medium (Gibco) supplemented with 10% Fetal Bovine Serum and 1% penicillin-streptoycin. MCF10A cells were cultured in DMEM/Ham's F-12 (Gibco) supplemented with 100 ng/ml cholera toxin, 20 ng/ml epidermal growth factor (EGF), 0.01 mg/ml insulin, 500 ng/ml hydrocortisone, and 5% chelex-treated horse serum (Gibco 16050122). All of the growth factors were purchased from MACGENE. Cells were incubated at 37°C and 5% CO2. Niraparib, Olaparib, Veliparib and Nutlin-3 were purchased from Selleck.

Western Blotting: Cells were lysed for 20 min on ice using RIPA buffer with added protease inhibitor cocktail (Selleck) and PMSF (Solarbio). The lysate was spun at 14,000 g for 15 min at 4 °C. Supernatant was collected and protein quantified using BCA Protein Assay (Solarbio). 40μg of protein were loaded into 8% SDS-PAGE gel. Proteins were transferred onto a PVDF methanol activated membrane (Millipore) using a wet transfer method (JUNYI, Beijing, China). Membranes were blocked in 5% non-fat milk diluted in 1xTBST for 1 hour at room temperature while gently rocking. Primary antibodies (listed below) were incubated in 5% BSA (MACGENE) in 1x TBST and gently rocked overnight at 4°C. HRP-conjugated secondary antibodies (CST, #7076s and #7074s) diluted at 1: 3,000 were incubated in 1xTBST and rocked for 1 hour at room temperature. ECL chemiluminescence kit (Beyotime) was used to detect protein expression. Western blot images were captured by Tanon 5200 chemiluminescent imaging system (Tanon, Shanghai, China). Quantification of band intensities has been performed using Tanon Gis software (Tanon, Shanghai, China).

Antibody Information:

ACTIN – ABclonal Biotechnology AC004 (1:10,000 dilution)

PARP1 – Santa Cruz SC-8007 (1:2000 dilution)
Cleaved Caspase-3 - Cell Signaling Technologies #9664T (1:1000 dilution)

Flow Cytometry of Apoptotic Cells. Annexin V-FITC/PI kit (MACGENE, CTK007-50) was used to assess the apoptosis of cells with different treatments. All of the steps were based on the instrument. Analysis was performed on a FACS LSRII (BD Biosciences) instrument and processed on FlowJo software.

Cell Viability Assay. Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8) assay. CCK-8 reagent was purchased from Medchem Express. $5x10^3$ cells/well were seeded in a 96-well plate. After growing for 24 or 48 hours, cells were treated with indicated reagents. Each group had triplicate wells. After incubation for the indicated hours, 10 μL CCK-8 reagent was added to each well. After incubation for 2-4 hour, the Optical density (OD) values at 490 nm were measured with a microplate reader. Data was analyzed using GraphPad Prism 7.0. Error bars represent ± SD for three independent experiments.

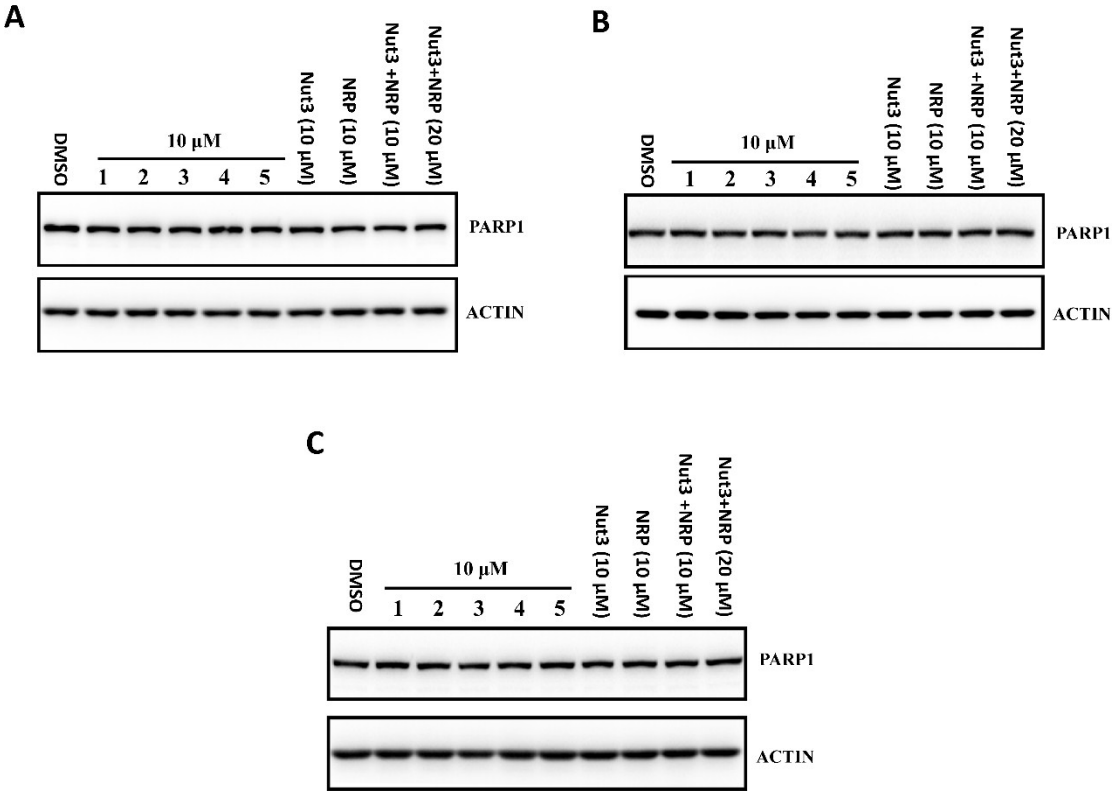


Fig. S1 Evaluation of PROTACs for PARP1 in HCC1937, MDA-MB-436 and MDA-MB-468 cell lines. (A) Immunoblot of PARP1 and actin following 24h of incubation with DMSO or the indicated small molecules in HCC1937 cells. (B) Immunoblot of PARP1 and actin following 24h of incubation with DMSO or the indicated small molecules in MDA-MB-436 cells. (C) Immunoblot of PARP1 and actin following 24h of incubation with DMSO or the indicated small molecules in MDA-MB-468 cells.

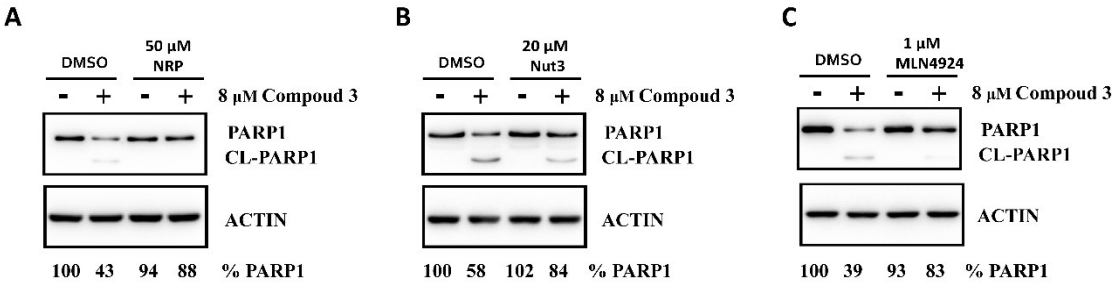


Fig. S2 Chemical rescue of compound **3** mediated PARP1 degradation (A) Immunoblot of PARP1 and actin after pre-treatment with DMSO and Niraparib (NRP) for 2h followed by compound **3** (8 μM) treatment for 20h. (B) Immunoblot of PARP1 and actin after a 4h pre-treatment with DMSO or Nutlin-3 (Nut3) followed by compound **3** (8 μM) treatment for 20h. (C) Immunoblot of PARP1 and actin after a 4h pre-treatment with DMSO and MLN4924 followed by compound **3** (8 μM) treatment for 20h.

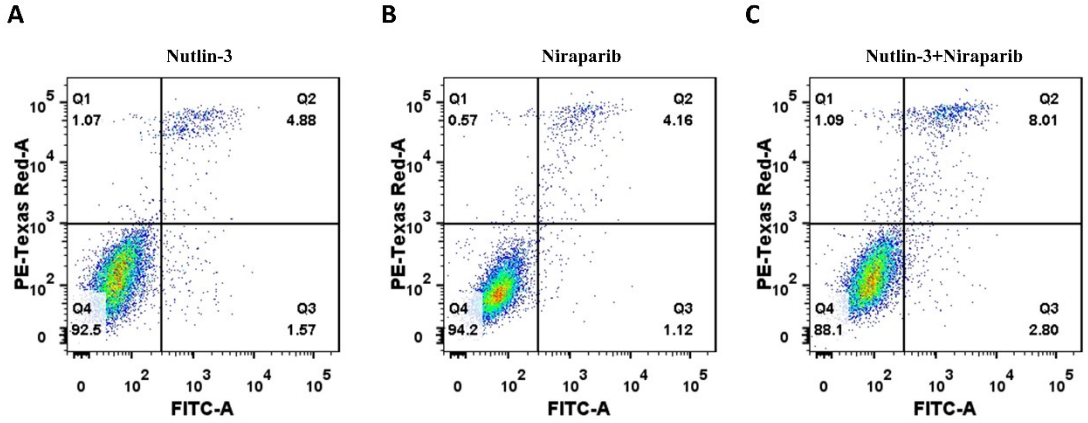


Fig. S3 Effects of treatment with Nutlin-3, Niraparib and the combination on apoptosis. From (A) to (C) MDA-MB-231 cells were treated with nutlin-3 (10 μM), niraparib (10 μM) or combination of nutlin-3 (10 μM) and niraparib (10 μM) for 24h, and apoptosis was assayed by flow cytometry after Annexin-V/PI co-staining.

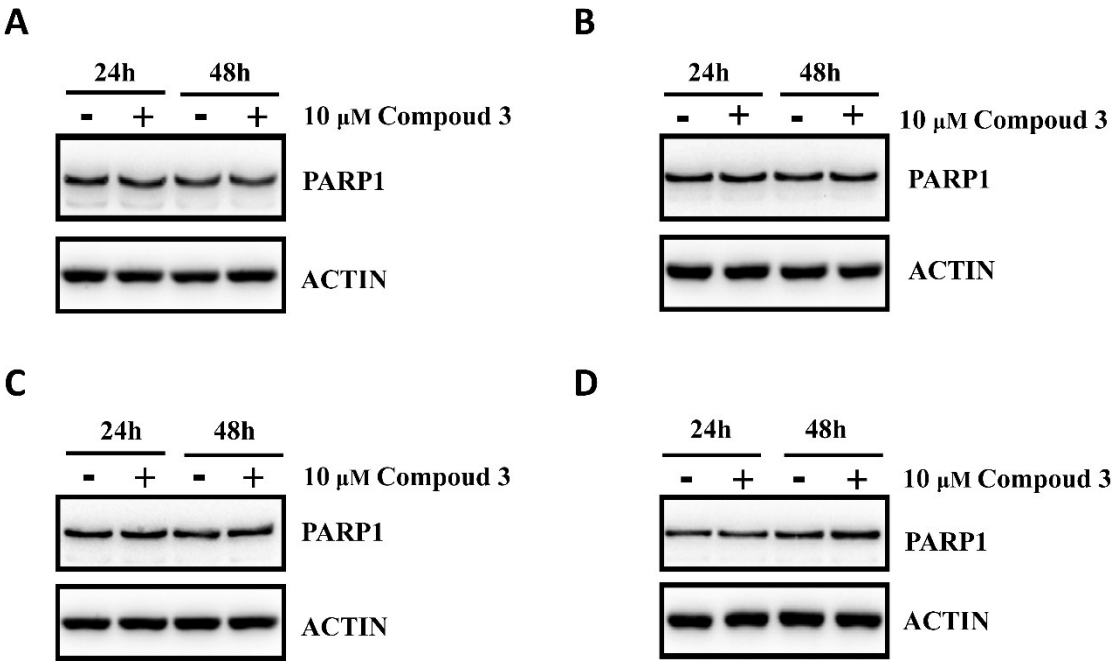
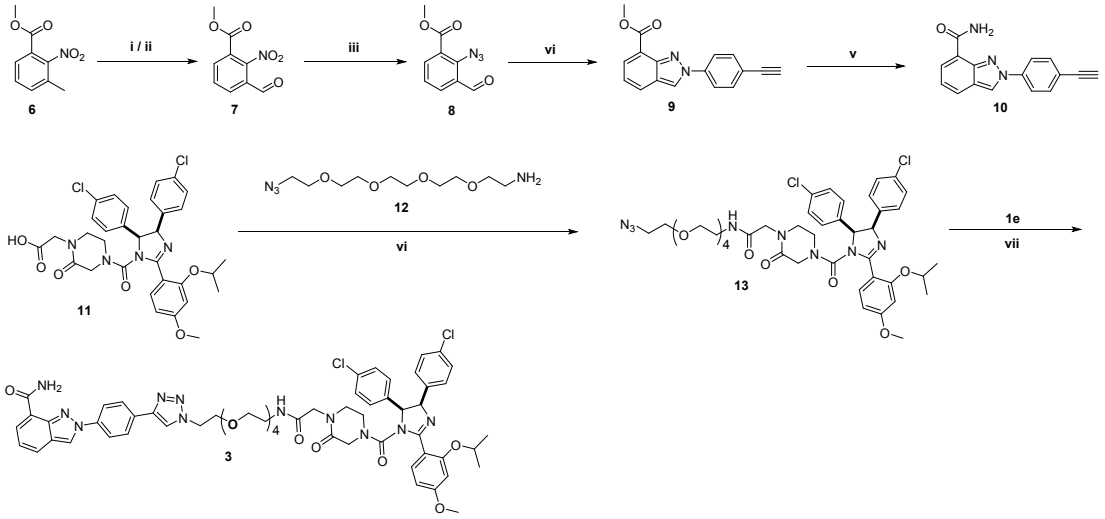


Fig. S4 Effect of compound **3** on PARP1 in MCF10A, BEAS-2B, HepG2 and Hela cells, no obvious PARP1 level change was detected by immunoblot in these cell lines after compound 3 incubation. (A) to (D) Immunoblot of PARP1 and actin following 24h and 48h of incubation with DMSO or 10 μM compound **3** in MCF10A, BEAS-2B, HepG2 and Hela cells.

General Methods: All reagents were purchased from commercial sources and were used without further purification. The ^{1}H-NMR spectra were recorded on a Bruker AVANCEⅢ400 MHz spectrometer in $CDCl_3$. Low-resolution mass spectral analyses were performed with a Waters AQUITY UPLCTM/MS. Analytical TLC was performed on Yantai Chemical Industry Research Institute silica gel 60 F254 plates and flash column chromatography was performed on Qingdao Haiyang Chemical Co. Ltd silica gel 60 (200-300mesh). The rotavapor was BUCHI's Rotavapor R-3.

Synthesis of compound 3



methyl 3-formyl-2-nitrobenzoate (7). In a 250ml round bottom flask, compound **6** (4.7g, 24.1 mmol), AIBN (1.6g, 9.5 mmol) and N-bromosuccinimide (5.1g, 28.8 mmol) in acetonitrile (150 ml) was heated at reflux for 12 hr. The mixture was cooled to ambient temperature, and then the solvent was removed under reduced pressure. To the solid residue, 100ml water was added and sonicated subsequently. The mixture was filtered to collect the solid. After drying, a mixture of compound **6** and **7** (6.7g in total) was obtained. Then, in a 100ml round bottom flask, the aforementioned mixture (6.7g) and 4-methylmorpholine N-oxide (2.82g, 24.1 mmol) in 70ml acetonitrile were stirred at room temperature for 3 hr. The mixture was diluted in water and extracted with ethyl acetate for 3 times. The combined organic layer was dried by Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (PE:EA=3:1) to yield the title compound (2.0g, 40% over 2 steps).

methyl 2-azido-3-formylbenzoate (8). In a 50ml round bottom flask, **7** (0.49g, 2.34mmol), NaN_3 (0.46g, 7.03mmol), and 10ml DMF were added. The mixture was stirred at 85°C for 2 hr. After cooled to room temperature, the mixture was poured into 200ml water and extracted with EA (20ml*3 times). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (PE:EA=1:1) to yield the title compound (0.4g, 83%).

methyl 2-(4-ethynylphenyl)-2H-indazole-7-carboxylate (9). In a 10ml round bottom flask, **8** (100mg, 0.49 mmol), 4-ethynylaniline (60mg, 0.51 mmol), acetic acid (30ul, 0.5 mmol) and 3ml DMF were added, the mixture was stirred at 105°C for 24 hr. After cooled to ambient temperature, the mixture was diluted in 100ml water and extracted with EA for 3 times (20ml*3), the combined organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to silica gel chromatography (PE:EA=3:1) to yield 22mg title compound (yield 16%);

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 8.54 (s, 1H), 8.14 (d, *J*=6.92Hz, 1H), 7.99-7.94 (m, 3H), 7.66 (d, *J*=8.48Hz, 2H), 7.20 (t, *J*=7.48Hz, 1H), 4.05 (s, 3H), 3.19 (s, 1H);

LC-MS (ESI+): m / z calculated for $C_{17}H_{12}N_2O_2$ (M+H)+: 277.09 found 277.18.

2-(4-ethynylphenyl)-2H-indazole-7-carboxamide (10). In a 10ml sealed tube, **9** (22mg, 0.08 mmol), 2ml ethanol and 5ml ammonium hydroxide were added, the tube was sealed with a screw cap (PTFE). The reaction mixture was stirred at 70°C for 24 hr. After cooled to ambient temperature, the mixture was diluted in water and extracted with DCM (20ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was

subjected to silica gel chromatography (PE:EA=1:1) to give the title compound (16mg, 76%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 8.98 (s, 1H), 8.55 (s, 1H), 8.32 (dd, *J*=7.00Hz, *J*=0.84Hz, 1H), 7.92-7.88 (m, 3H), 7.68 (d, *J*=8.72Hz, 2H), 7.28 (dd, *J*=8.32Hz, *J*=7.12Hz, 1H), 5.98 (s, 1H), 3.21 (s, 1H);

LC-MS (ESI+): m / z calculated for $C_{16}H_{12}N_3O$ (M+H)+: 262.09 found 262.55.

intermediate 13. To a mixture of **11** (15mg. The synthesis of **11**, a mixture of two enantiomers, can be found in the literature[1]), HATU (2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate, 7mg, 1.2equiv.) in 2ml DMF:DCM=1:1 co-solvent, Et_3N (7ul, 3equiv.) was added and the resulting mixture was stirred at ambient temperature for 5 minutes till amine **12** (9mg, 2equiv. The synthesis of **12** can be found in the literature[2]) in 1ml DCM was added. Then the mixture was stirred at room temperature for overnight. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH=20:1) to give the title compound (13mg, 63%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 7.59 (d, *J*=8.48Hz, 1H), 7.08 (d, *J*=8.40 Hz, 2H), 7.03 (d, *J*=8.48Hz, 2H), 6.93 (d, *J*=8.36Hz, 2H), 6.88 (d, *J*=8.28Hz, 2H), 6.54 (dd, *J*=8.48Hz, *J*=2.12Hz, 1H), 6.47 (d, *J*=2.04Hz, 1H), 5.55 (d, *J*=9.68Hz, 1H), 5.48 (d, *J*=9.60Hz, 1H), 4.64-4.57 (m, 1H), 3.94 (d, *J*=15.60Hz, 1H), 3.85 (s, 3H), 3.84 (d, *J*=18.20Hz, 1H), 3.78-3.72 (m, 2H), 3.68-3.59 (m, 15H), 3.53-3.47 (m, 3H), 3.42-3.37 (m, 3H), 3.30-3.24 (m, 1H), 3.17-3.08 (m, 2H), 1.38 (t, *J*=6.04Hz, 3H), 1.34 (t, *J*=6.00Hz, 3H);

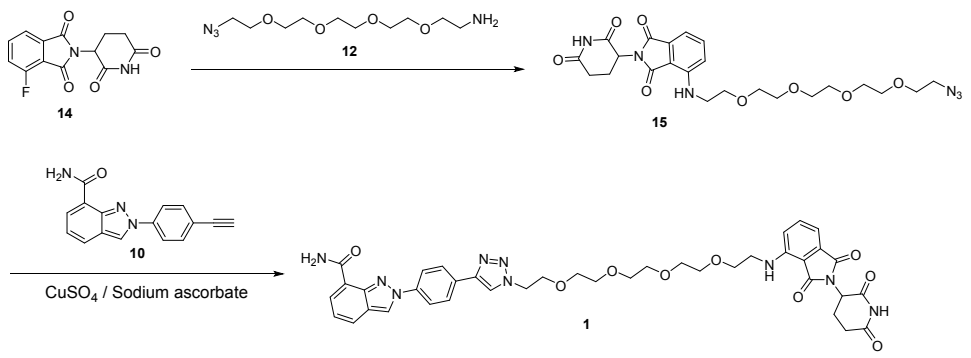
LC-MS (ESI+): m / z calculated for $C_{42}H_{53}C_{l2}N_8O_9$ (M+H)+: 883.32 found 883.88.

compound 3. To the mixture of **10** (5mg) and **13** (13mg, 0.77eq) dissolved in 2ml DMF:H_2O=5:1 co-solvent in a 5ml round bottom flask, sodium ascorbate (50mg, ~11eq) and $CuSO_4$ (3mg, ~1eq) were sequentially added. The resulting mixture was stirred at room temperature for 6 hr. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH=20:1to 10:1) to give the title compound (10mg, 45%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 9.06 (s, 1H), 8.59 (s, 1H), 8.29 (d, *J*=6.88Hz, 1H), 8.16 (s, 1H), 8.03 (d, *J*=8.56Hz, 2H), 7.97 (d, *J*=8.60Hz, 2H), 7.91 (d, *J*=8.32Hz, 1H), 7.57 (d, *J*=8.48Hz, 1H), 7.27 (t, *J*=7.64Hz, 1H), 7.10 (m, 1H), 7.07 (d, *J*=8.28Hz, 2H), 7.01 (d, *J*=8.24Hz, 2H), 6.92 (d, *J*=8.24Hz, 2H), 6.86 (d, *J*=8.12Hz, 2H), 6.52 (d, *J*=8.36Hz, 1H), 6.46 (s, 1H), 6.25 (s, 1H), 5.57 (d, *J*=9.64Hz, 1H), 5.47 (d, *J*=9.64Hz, 1H), 4.63-4.58 (m, 3H), 3.96-3.92 (m, 3H), 3.86-3.72 (m, 5H), 3.66-3.52 (m, 13H), 3.50-3.47 (m, 3H), 3.39-3.36 (m, 2H), 3.27-2.24 (m, 1H), 3.12-3.08 (m, 2H), 1.37 (t, *J*=5.96Hz, 3H), 1.32 (t, *J*=5.96Hz, 3H);

LC-MS (ESI+): m / z calculated for $C_{58}H_{64}Cl_2N_{11}O_{10}$ (M+H)+: 1144.41 found 1144.86.

Synthesis of compound 1



4-((14-azido-3,6,9,12-tetraoxatetradecyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (15). The mixture of **14** (0.3g, 1.08 mmol. The synthesis of **14** can be found in the literature[3]), **12** (0.28g, 1.08 mmol) and DIEA (0.36ml, 2.16 mmol) in 2ml DMF was heated at 90°C for 14hr. After cooled to ambient temperature, the mixture was diluted in 200ml water and extracted with ethyl acetate for 5 times (20ml*5), the combined organic layer was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to silica gel chromatography (PE:EA=6:1 to1:1) to yield 0.16g title compound (yield 29%);

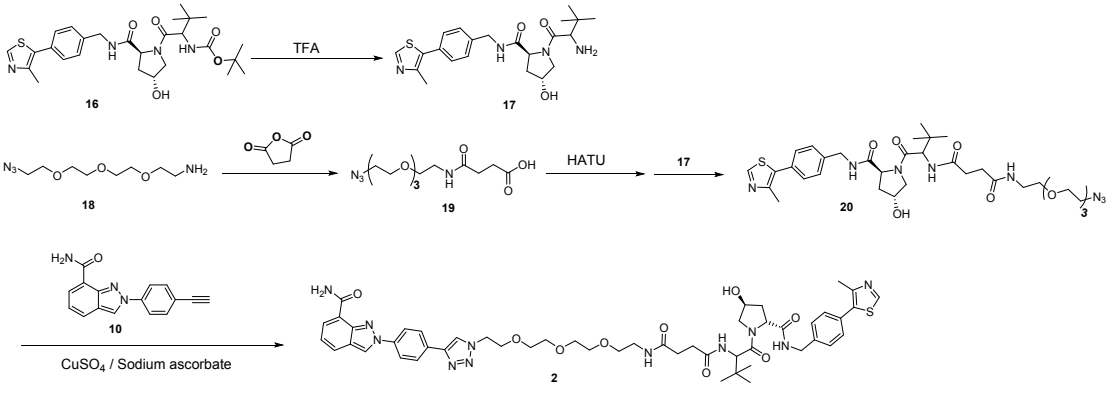
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 8.11 (s, 1H), 7.49 (t, *J*=7.36Hz, 1H), 7.10 (d, *J*=7.08Hz, 1H), 6.92 (d, *J*=8.40Hz, 1H), 6.49 (t, *J*=5.64Hz, 1H), 4.93-4.89 (m, 1H), 3.72 (t, *J*=5.44Hz, 2H), 3.68-3.66 (m, 14H), 3.49-3.45 (m, 2H), 3.39 (t, *J*=5.16Hz, 2H), 2.91-2.68 (m, 3H), 2.15-2.10 (m, 1H);

compound 1. To the mixture of **10** (15mg) and **15** (20mg, 0.67eq) dissolved in 2ml DMF:H_2O=5:1 co-solvent in a 5ml round bottom flask, sodium ascorbate (100mg, ~8.8eq) and $CuSO_4$ (3mmg, ~0.3eq) were sequentially added. The resulting mixture was stirred at room temperature for 6 hr. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH:Et_3N=90:10:1) to give the title compound (9mg, 20%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 9.07 (s, 1H), 8.77 (s, 1H), 8.59 (s, 1H), 8.32 (d, *J*=6.80Hz, 1H), 8.17 (s, 1H), 8.05 (d, *J*=8.32Hz, 2H), 7.97 (d, *J*=8.32Hz, 2H), 7.92 (d, *J*=8.40Hz, 1H), 7.44 (t, *J*=8.00Hz, 1H), 7.06 (d, *J*=7.04Hz, 1H), 6.84 (d, *J*=8.40Hz, 1H), 6.44 (s, 1H), 6.33 (s, 1H), 4.94-4.90 (m, 1H), 4.64 (t, *J*=4.32Hz, 2H), 3.95 (t, *J*=4.40Hz, 2H), 3.66 (m, 14H), 3.40 (d, *J*=5.08Hz, 2H), 3.49-3.45 (m, 2H), 3.39 (t, *J*=5.16Hz, 2H), 2.90-2.69 (m, 3H), 2.13-2.11 (m, 1H);

LC-MS (ESI+): m / z calculated for $C_{39}H_{42}N_9O_9$ (M+H)+: 780.30 found 780.99.

Synthesis of compound 2



(2S,4R)-1-(2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (17). To a solution of **16** (70mg. The synthesis of 16 can be found in the literature[4]) in 5ml DCM, 0.5ml TFA was added. The resulting mixture was stirred at room temperature till the disappearance of 2a monitored by TLC. The solvent was removed under reduced pressure. The residue was alkalified by adding 0.5ml Et_3N and used for the next step without purification.

1-azido-13-oxo-3,6,9-trioxa-12-azahexadecan-16-oic acid (19). The mixture of **18** (340mg, 2mmol, the synthesis of **18** can be found in the literature[5]) and succinic anhydride (200mg, 2mmol) in 5ml DCM was refluxed at 45°C for 1.5 hr. The mixture was concentrated under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH=20:1) to give the title compound (450mg, 83%).

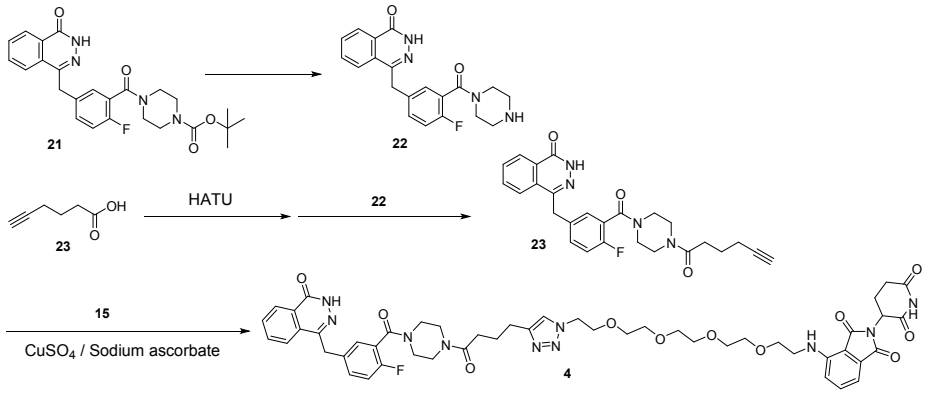
N1-(2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-N4-(1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)succinamide (20). To a mixture of **19** (36mg, 0.13mmol) and HATU (60mg, 0.16mmol) in 4ml DMF:DCM=1:1 co-solvent, Et_3N (0.055ml, 0.4mmol) was added and the resulting mixture was stirred at room temperature for 5 minutes. Then the above-mentioned mixture of **17** was added. The mixture was stirred at room temperature for overnight. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH=20:1) to give the title compound (26mg, 27%).

compound 2. To the mixture of **10** (11mg) and **20** (26mg, 0.85eq) dissolved in 1.2ml DMF:H_2O=5:1 co-solvent in a 5ml round bottom flask, sodium ascorbate (40mg, ~5eq) and $CuSO_4$ (3mg, 0.44eq) were sequentially added. The resulting mixture was stirred at room temperature for 6 hr. The mixture was diluted in water and extracted with EA (10ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH:Et_3N=90:10:1) to give the title compound (10mg, 24%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 9.00 (d, *J*=2.96 Hz, 1H), 8.63 (s, 1H), 8.55 (s, 1H), 8.23 (dd, *J*=7.04 Hz, *J*=0.96 Hz, 1H), 8.12 (s, 1H), 7.96 (d, *J*=8.80 Hz, 2H), 7.91 (d, *J*=8.80 Hz, 2H), 7.86 (dd, *J*=8.40 Hz, *J*=0.92 Hz, 1H), 7.67 (t, *J*=5.80 Hz, 1H), 7.31 (s, 4H), 7.20 (dd, *J*=8.32 Hz, *J*=7.08 Hz, 1H), 7.12 (d, *J*=8.76 Hz, 1H), 6.73 (d, *J*=2.52 Hz, 1H), 6.61 (t, *J*=5.44 Hz, 1H), 4.74 (t, *J*=8.04 Hz, 1H), 4.60 (t, *J*=4.64 Hz, 2H), 4.56-4.50 (m, 3H), 4.32 (dd, *J*=15.08 Hz, *J*=5.36 Hz, 1H), 4.02 (d, *J*=11.24 Hz, 1H), 3.91 (t, *J*=4.80 Hz, 2H), 3.64-3.59 (m, 5H), 3.55-3.51 (m, 4H), 3.44 (t, *J*=5.28 Hz, 2H), 3.34-3.31 (m, 2H), 2.57-2.37 (m, 8H), 2.21-2.15 (m, 1H);

LC-MS (ESI+): m / z calculated for $C_{50}H_{62}N_{11}O_9S$ (M+H)+: 992.44 found 992.76.

Synthesis of compound 4



4-(4-fluoro-3-(piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one (22). To a solution of **21** (70mg. The synthesis of 4a can be found in the literature[6]) in 2ml DCM, 0.5ml TFA was added. The resulting mixture was stirred at room temperature for 5 hr. The solvent was removed under reduced pressure. The residue was alkalified by adding 0.5ml Et_3N and used for the next step without purification.

4-(4-fluoro-3-(4-(hex-5-ynoyl)piperazine-1-carbonyl)benzyl)phthalazin-1(2H)-one (23). To a mixture of **23** (12mg, 0.1mmol) and HATU (42mg, 0.11mmol) in 2ml DMF, Et_3N (0.042ml, 0.3mmol) was added and the mixture was stirred at room temperature for 5 minutes. Then, the mixture of **22** was added and the resulting mixture was stirred at room temperature for overnight. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (100% EA) to give the title compound (25mg, 51%).

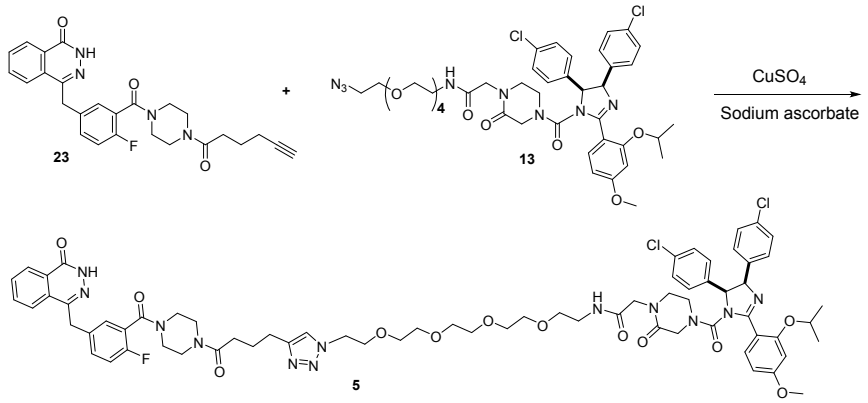
^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 10.17, 10.15 (s, 1H), 8.48-8.45 (m, 1H), 7.78-7.77 (m, 2H), 7.73-7.71 (m, 1H), 7.37-7.31 (m, 2H), 7.07-7.03 (m, 1H), 4.28 (s, 2H), 3.79-3.59 (m, 5H), 3.47 (s, 1H), 3.33-3.28 (m, 2H), 2.51, 2.44 (t, *J*=7.12 Hz, 2H), 2.32-2.28 (m, 2H), 1.94, 1.91 (brs, 1H), 1.90-1.85 (m, 2H);

compound 4. To the mixture of **23** (15mg) and **15** (17mg, 1eq) dissolved in 1.2ml DMF:H_2O=5:1 co-solvent in a 5ml round bottom flask, sodium ascorbate (50mg, ~8eq) and $CuSO_4$ (5mg, ~1eq) were sequentially added. The resulting mixture was stirred at room temperature for 4 hr. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH =10:1) to give the title compound (14mg, 44%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 10.73, 10.57 (s, 1H), 9.02 (s, 1H), 8.45 (d, *J*=6.60 Hz, 1H), 7.80-7.70 (m, 3H), 7.53 (s, 1H), 7.48 (t, *J*=7.84 Hz, 1H), 7.33-7.29 (m, 2H), 7.09 (d, *J*=7.08 Hz, 1H), 7.04 (d, *J*=8.96 Hz, 1H), 6.91 (d, *J*=8.56 Hz, 1H), 6.49 (s, 1H), 4.94-4.90 (m, 1H), 4.49 (t, *J*=4.92 Hz, 2H), 4.28 (s, 2H), 3.84 (s, 2H), 3.76-3.53 (m, 19H), 3.47-3.42 (m, 3H), 3.39-3.26 (m, 2H), 2.89-2.74 (m, 5H), 2.46-2.32 (m, 2H), 2.15-2.10 (m, 1H), 2.02-2.00 (m, 2H);

LC-MS (ESI+): m / z calculated for $C_{49}H_{56}FN_{10}O_{11}$ (M+H)+: 979.40 found 979.83.

Synthesis of compound 5



compound 5. To the mixture of **23** (7mg) and **13** (13mg, ~1eq) dissolved in 2ml DMF:H_2O=5:1 co-solvent, sodium ascorbate (30mg, ~10eq) and $CuSO_4$ (3mg, 1.2eq) were sequentially added. The resulting mixture was stirred at room temperature for overnight. The mixture was diluted in water and extracted with EA (15ml*3). The combined organic layer was dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was subjected to silica gel chromatography (DCM:MeOH:Et_3N =95:5:1) to give the title compound (10mg, 49%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 8.47-8.45 (m, 1H), 7.80-7.74 (m, 4H), 7.61 (d, *J*=8.32 Hz, 1H), 7.53 (s, 1H), 7.34-7.32 (m, 3H), 7.11-7.07 (m, 3H), 7.04 (d, *J*=8.36 Hz, 2H), 6.96 (d, *J*=8.28 Hz, 2H), 6.91 (d, *J*=8.24 Hz, 2H), 6.55 (dd, *J*=8.60 Hz, *J*=1.92 Hz, 1H), 6.49 (s, 1H), 5.58 (d, *J*=9.68Hz, 1H), 5.51 (d, *J*=9.64Hz, 1H), 4.63 (m,1H), 4.52 (t, *J*=4.72Hz, 2H), 4.30 (s, 2H), 3.86 (m, 5H), 3.66-3.52 (m, 22H), 3.46-3.39 (m, 4H), 3.34-3.27 (m, 3H), 3.15-3.14 (m, 2H), 2.82-2.78 (m, 2H), 2.47-2.41 (m, 2H), 2.06-2.02 (m, 2H), 1.40 (d, *J*=6.00Hz, 3H), 1.35 (d, *J*=6.00Hz, 3H);

LC-MS (ESI+): m / z calculated for $C_{68}H_{78}Cl_2FN_{12}O_{12}$ (M+H)+: 1343.51 found 1343.71.

References

1 A. R. Schneekloth, M. Pucheault, H. S. Tae and C. M. Crews, *Bioorg Med Chem Lett*.,2008, **18**, 5904-5908.

2 W.-H. Wen, M. Lin, C.-Y. Su, S.-Y. Wang, Y.-S. E. Cheng, J.-M. Fang and C.-H. Wong, *J. Med. Chem.*, 2009, **52**, 4903-4910.

3 B. Zhou, J. Hu, F. Xu, Z. Chen, L. Bai, E. Fernandez-Salas, M. Lin, L. Liu, C.-Y. Yang, Y. Zhao, D. McEachern, S. Przybranowski, B. Wen, D. Sun and S. Wang, *J. Med. Chem.*, 2018, **61**, 462-481.

4 J. Frost, C. Galdeano, P. Soares, M. S. Gadd, K. M. Grzes, L. Ellis, O. Epemolu, S. Shimamura, M. Bantscheff, P. Grandi, K. D. Read, D. A. Cantrell, S. Rocha and A. Ciulli, *Nat Commun.*, 2016, **7**, 13312.

5 D. Lahav, B. Liu, R. J. B. H. N. van den Berg, A. M. C. H. van den Nieuwendijk, T. Wennekes, A. T. Ghisaidoobe, I. Breen, M. J. Ferraz, C.-L. Kuo, L. Wu, P. P. Geurink, H. Ovaa, G. A. van der Marel, M. van der Stelt, R. G. Boot, G. J. Davies, J. M. F. G. Aerts and H. S. Overkleeft, *J. Am. Chem. Soc.*, 2017, **139**, 14192–14197.

6 K. A. Menear, C. Adcock, R. Boulter, X. Cockcroft, L. Copsey, A. Cranston, K. J. Dillon, J. Drzewiecki, S. Garman, S. Gomez, H. Javaid, F. Kerrigan, C. Knights, A. Lau, V. M. Loh, I. T. W. Matthews, S. Moore, M. J. O'Connor, G. C. M. Smith and N. M. B. Martin, *J. Med. Chem.*, 2008, **51**, 6581-6591.