Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supplementary Information

Selective degradation-inducing probes for studying cereblon (CRBN) biology

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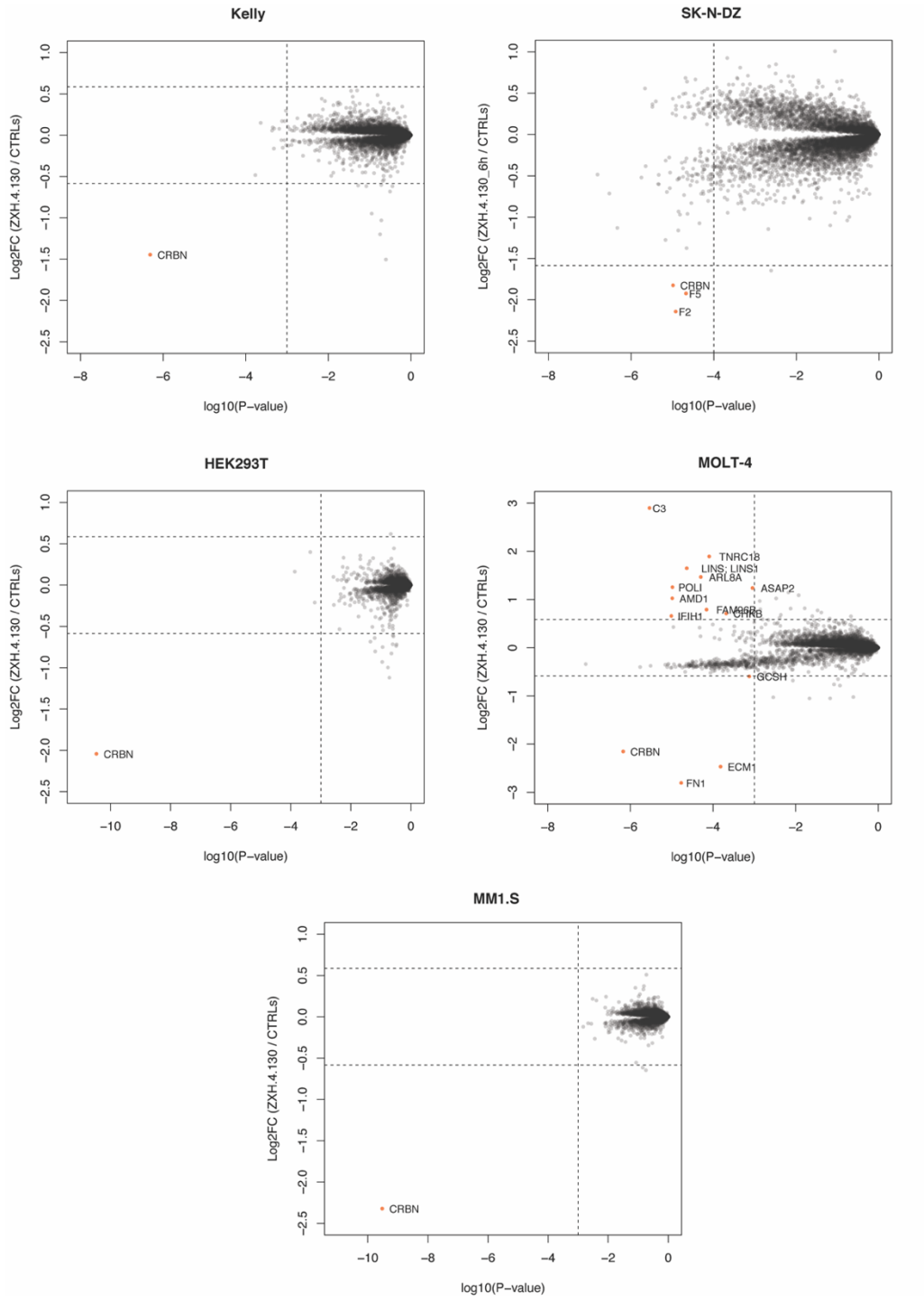


Figure S1. Expression proteomics for ZXH-4-130 versus DMSO control across multiple cell lines. 100 nM dose for Kelly and SK-N-DZ cells. 50 nM dose for HEK293T, MOLT-4, and MM1.S cells. 6 h treatment for all cell lines except Kelly, which was a 12 h treatment. Note that the MM1.S panel is from Figure 5, the SK-N-DZ panel is from Figure 6, and the MOLT-4 panel is from Figure 8 in the main text; they are provided here for direct comparison.

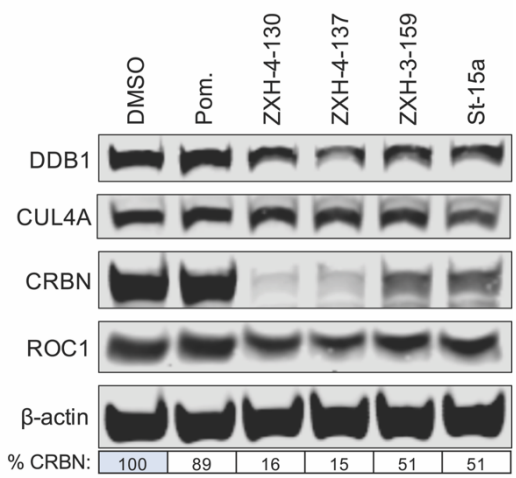


Figure S2. Degradative effects on members of the CRBN E3 ligase complex. Immunoblot after treatment with 50 nM of compound for 4 h. β-actin representative of 2 blots. Quantification shown as percentage of DMSO control normalized to β-actin.

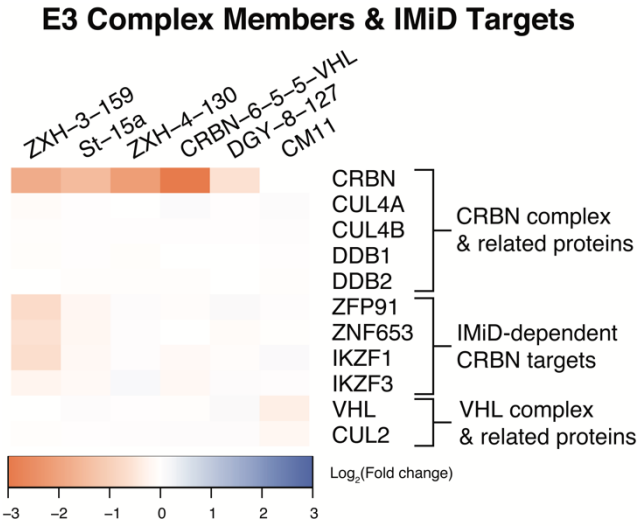
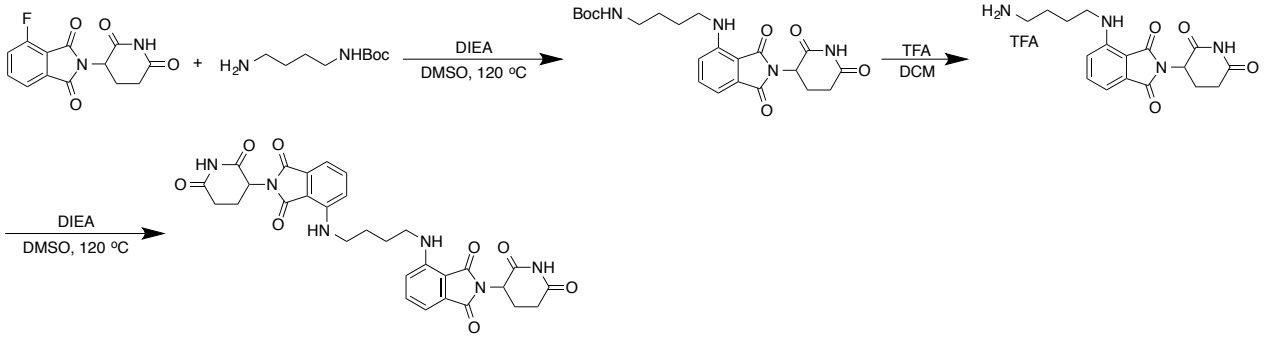


Figure S3. Degradative effects on E3 ligase complex members and IMiD targets. Heatmap of CRBN complex related proteins, IMiD-dependent CRBN targets, and VHL complex related proteins after 6 h treatments of compound or DMSO in MOLT-4 cells. All compounds were treated at 50 nM doses except DGY-8-127, which was treated at 100 nM. Singlicate analysis of compound; triplicate analysis of DMSO control. The VHL targeting homo-PROTAC CM11 is shown for comparison.[31]

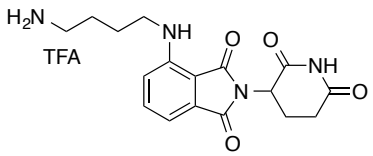
Experimental Methods

Chemical Synthesis

Unless otherwise noted, reagents and solvents were obtained from commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded on 500 MHz (Bruker A500), and chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane (TMS). Coupling constants (*J*) are reported in Hz. Spin multiplicities are described as s (singlet), br (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were obtained on a Waters Micromass ZQ instrument. Preparative HPLC was performed on a Waters Sunfire C18 column (19 x 50 mm, 5μM) using a gradient of 15-95% methanol in water containing 0.05% trifluoroacetic acid (TFA) over 22 min (28 min run time) at a flow rate of 20 mL/min. Purities of assayed compounds were in all cases greater than 95%, as determined by reverse-phase HPLC analysis.



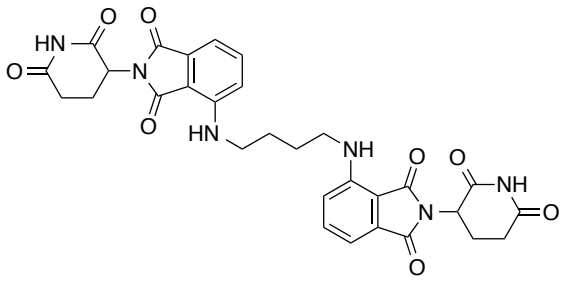
Scheme 1. Synthesis of **ZXH-3-152**.



4-((4-aminobutyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione.

To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (180 mg, 0.65 mmol) and *tert*-butyl (4-aminobutyl)carbamate (122 mg, 0.65 mmol) in DMSO (2 mL) was added DIEA (215 uL, 1.3 mmol), and then the mixture was stirred at 120 °C for 30 mins. After the starting material was consumed, the mixture was purified by prep-HPLC to obtain intermediate. The intermediate was then dissolved in TFA/DCM (v/v = 1/1), stirred at room temperature for 1 h, and then concentrated in vacuo to obtain desired product (190 mg, 64%) as TFA salt, which was used in next step without any purification.

LCMS: 345 $[M + H]^+$.

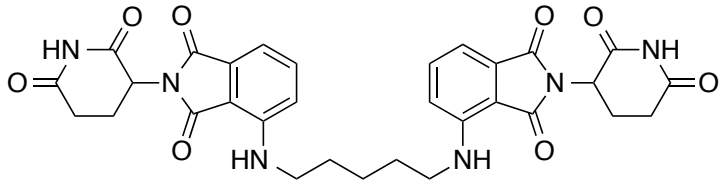


4,4'-(butane-1,4-diylbis(azanediyl))bis(2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione) (ZXH-3-152).

To a solution of 4-((4-aminobutyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (37 mg, 0.08 mmol) and 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (22 mg, 0.08 mmol) in DMSO (1 mL) was added DIEA (40 uL, 0.24 mmol), and then the mixture was stirred at 120 °C for 30 mins. After the starting material was consumed, the mixture was purified by prep-HPLC to obtain product (16 mg, 28%) as TFA salt.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 2H), 7.56 (dd, *J* = 8.6, 7.0 Hz, 2H), 7.12 – 7.06 (m, 2H), 7.00 (d, *J* = 7.0 Hz, 2H), 6.60 (t, *J* = 5.9 Hz, 2H), 5.04 (dd, *J* = 12.8, 5.4 Hz, 2H), 3.36 (d, *J* = 5.3 Hz, 4H), 2.88 (ddd, *J* = 17.0, 13.8, 5.4 Hz, 2H), 2.60 (d, *J* = 3.4 Hz, 1H), 2.56 (dt, *J* = 7.2, 4.3 Hz, 1H), 2.53 (d, *J* = 4.4 Hz, 1H), 2.03 (dtd, *J* = 13.2, 5.4, 2.4 Hz, 2H), 1.79 – 1.38 (m, 4H).

LCMS: 601 $[M + H]^+$.



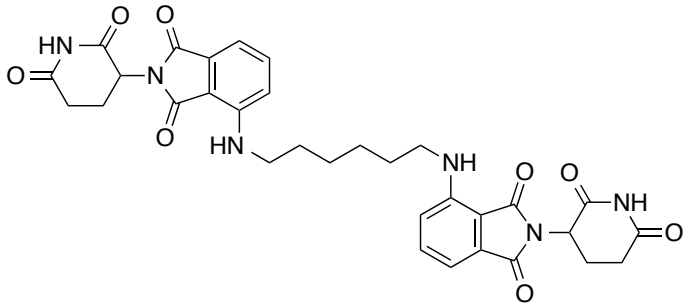
4,4'-(pentane-1,5-diylbis(azanediyl))bis(2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione) (ZXH-3-153.)

ZXH-3-153 (19 mg, 25%) was obtained according to the synthetic route of ZXH-3-152, changing from *tert-butyl (4-aminobutyl)carbamate* to *tert*-butyl (5-aminobutyl)carbamate.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 2H), 7.57 (dd, *J* = 8.6, 7.0 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H), 7.02 (d, *J* = 7.0 Hz, 2H), 6.56 (t, *J* = 6.0 Hz, 2H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 2H), 3.33 (s, 4H), 2.89 (ddd, *J* = 16.9, 13.9, 5.4 Hz, 2H), 2.63 – 2.53 (m, 3H), 2.03 (dddd, *J* = 15.6, 10.0, 5.9, 3.2 Hz, 2H), 1.63 (p, *J* = 7.4 Hz, 4H), 1.53 – 1.39 (m, 2H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.3, 170.6, 169.4, 167.8, 146.9, 136.7, 132.7, 117.7, 110.9, 109.5, 49.0, 42.3, 31.5, 28.9, 24.2, 22.6.

LCMS: 615 $[M + H]^+$.



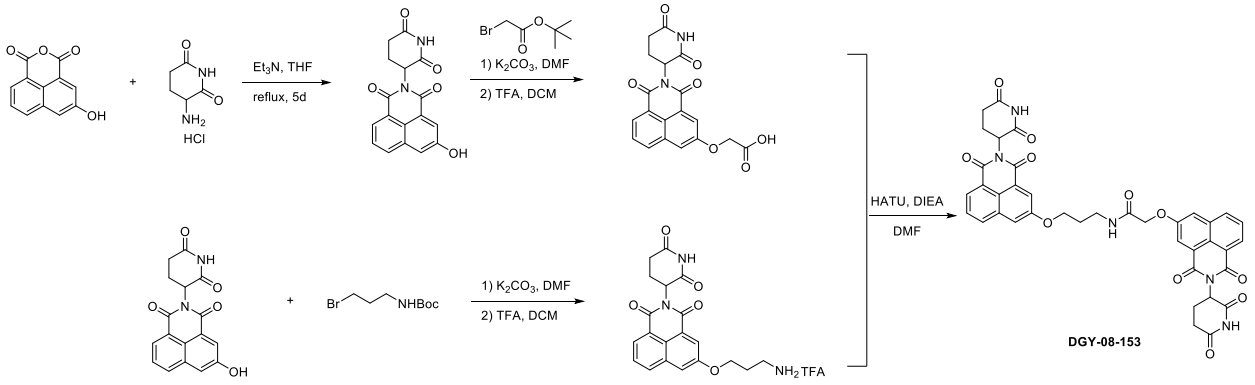
4,4'-(hexane-1,6-diylbis(azanediyl))bis(2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione) (ZXH-3-159).

ZXH-3-159 (19 mg, 15%) was obtained according to the synthetic route of ZXH-3-152, changing from *tert-butyl (4-aminobutyl)carbamate* to *tert*-butyl (6-aminobutyl)carbamate.

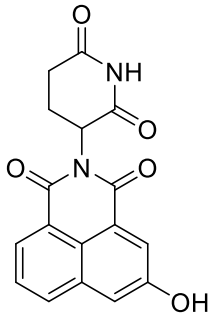
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 2H), 7.58 (dd, *J* = 8.5, 7.0 Hz, 2H), 7.09 (d, *J* = 8.6 Hz, 2H), 7.02 (d, *J* = 7.0 Hz, 2H), 6.54 (t, *J* = 6.0 Hz, 2H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 2H), 4.03 (q, *J* = 7.1 Hz, 1H), 3.33 (s, 4H), 2.89 (ddd, *J* = 16.9, 13.7, 5.4 Hz, 2H), 2.61 (dd, *J* = 4.5, 2.5 Hz, 1H), 2.58 (dt, *J* = 3.5, 1.6 Hz, 1H), 2.54 (d, *J* = 4.2 Hz, 1H), 2.08 – 1.93 (m, 3H), 1.59 (q, *J* = 6.7 Hz, 4H), 1.40 (q, *J* = 4.1, 3.6 Hz, 4H), 1.18 (t, *J* = 7.1 Hz, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.3, 170.6, 169.4, 167.8, 146.9, 136.8, 132.7, 117.7, 110.9, 109.5, 49.0, 42.3, 31.5, 29.1, 26.5, 22.6.

LCMS: 629 $[M + H]^+$.



Scheme 2. Synthesis of **DGY-8-153.**

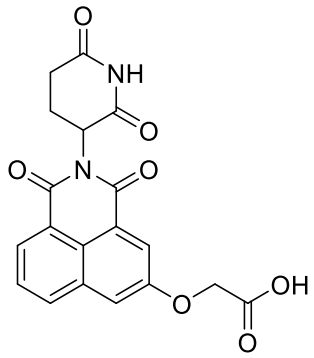


2-(2,6-dioxopiperidin-3-yl)-5-hydroxy-1H-benzo[de]isoquinoline-1,3(2H)-dione.

To the solution of 3-hydroxy-1,8-naphthalic anhydride (2.14 g, 10.0 mmol) and 3-aminopiperidine-2,6-dione (1.65 g, 10.0 mmol) in THF (40 mL) at room temperature, was added triethylamine (2.78 mL, 20.0 mmol). The suspension was then refluxed for 5 days, with a green precipitate forming in

the first 24 hours and eventually turning black. The solvent was evaporated andwater was added, and then mixture was acidified with HCl solution (1 N aq.), and stirred for another 1 hour. The suspension was then filtered to provide the titled compound (3.44g, 9.53 mmol, 95%) as a green solid.

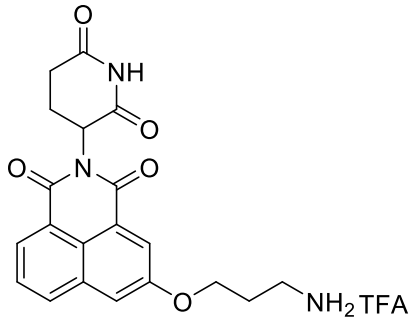
LC/MS *m/z* calculated for $[M+H]^+$ 325.1, found 325.1.



2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)acetic acid.

2-(2,6-dioxopiperidin-3-yl)-5-hydroxy-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (361 mg, 1.1 mmol) was suspended in 3 mL DMF, followed by addition of K_2CO_3 (276 mg, 2.0 mmol) and *tert*-butyl bromoacetate (234 mg, 1.2 mmol). The blue suspension was stirred at 35 ºC for 4 hours, at which point an additional 1.0 mmol of *tert*-butyl bromoacetate was added. After continuing to stir at 35 ºC overnight, water was added, and the suspension was filtered to provide the title compound (464 mg, 1.06 mmol, 95%) as a light gray solid. Then the solid was dissolved in DCM, TFA was added to the suspension at room temperature. After 2 hours, the solvent was removed to get crude product. (quant. yield for the second step.)

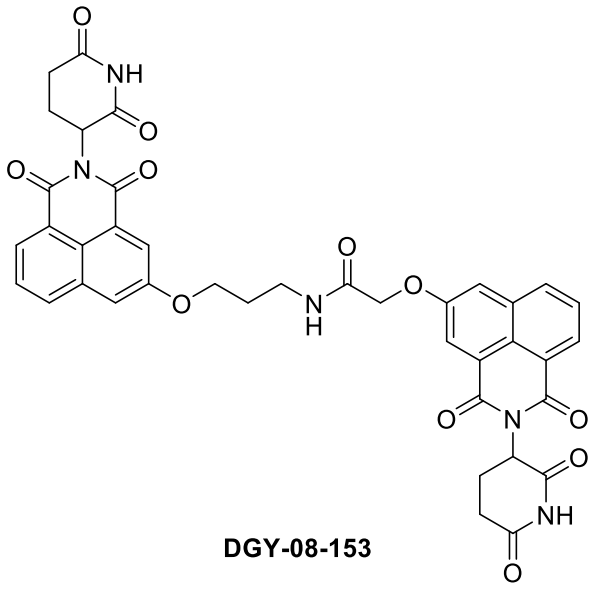
LC/MS *m/z* calculated for $[M+H]^+$ 383.1, found 383.2.



5-(3-aminopropoxy)-2-(2,6-dioxopiperidin-3-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione TFA salt.

2-(2,6-dioxopiperidin-3-yl)-5-hydroxy-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (100 mg, 0.31 mmol) was suspended in 2 mL DMF, followed by addition of K_2CO_3 (128 mg, 0.93 mmol) and tert-butyl (3-bromopropyl)carbamate (74 mg, 0.31 mmol). The suspension was stirred at room temperature overnight. After the reaction was finished, the solvent was removed under vacuum. The residue was purified with flash chromatography to yield the product. The product was dissolved in DCM, TFA was then added to the mixture. After 2 hours, the solvent was removed under vacuum to get the crude product TFA salt without any further purification (143.7 mg, 0.30 mmol, 97%).

LC/MS *m/z* calculated for $[M+H]^+$ 382.1, found 382.2.



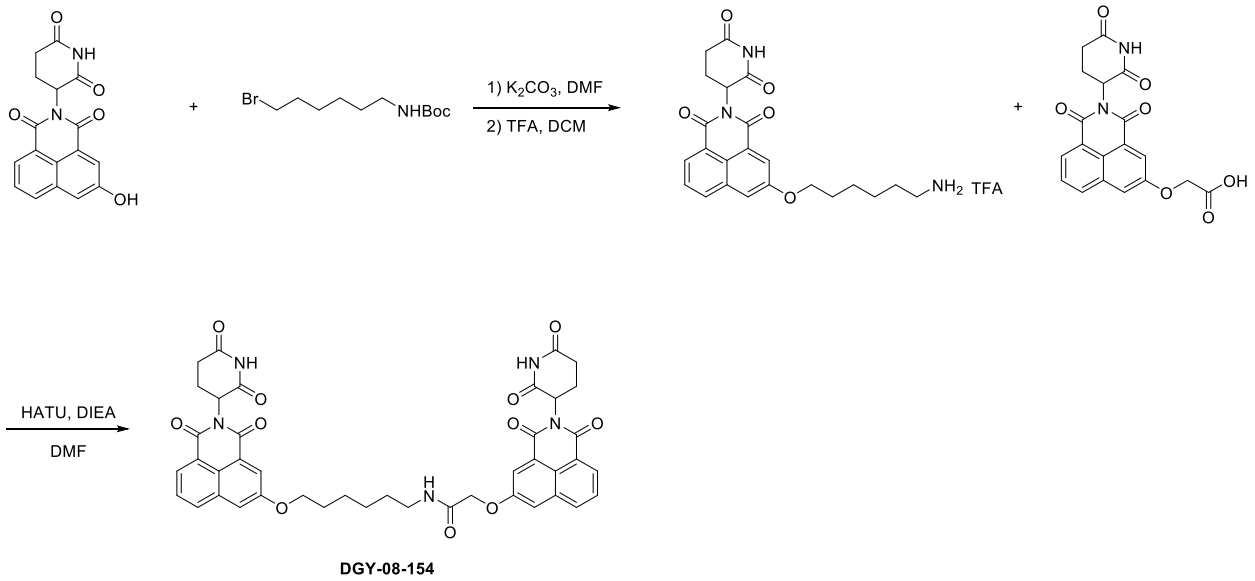
DGY-08-153

2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)-N-(3-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)propyl)acetamide (DGY-8-153).

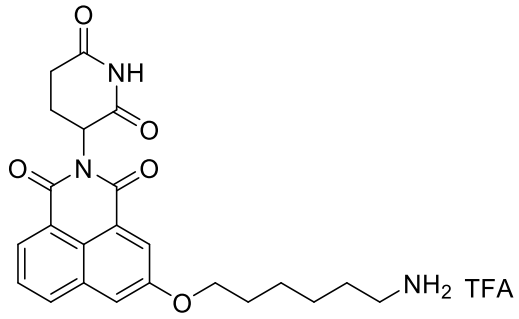
To the solution of 5-(3-aminopropoxy)-2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione TFA salt (10 mg, 0.02 mmol)and 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)acetic acid (7.7 mg, 0.02 mmol) in DMF (1 mL), HATU (20 mg, 0.052 mmol) and DIEA (17 mg, 0.13 mmol) was added at room temperature. After 10 mins, the reaction mixture was purified with reverse phase HPLC to yield the product (9.3 mg, 0.012 mmol, 60%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 2H), 8.37 (d, *J* = 25.2 Hz, 1H), 8.32 – 8.16 (m, 4H), 8.10 (d, *J* = 35.5 Hz, 1H), 8.01 – 7.81 (m, 2H), 7.80 – 7.63 (m, 3H), 5.74 (dd, *J* = 11.8, 5.9 Hz, 2H), 4.69 (d, *J* = 2.5 Hz, 2H), 4.17 – 4.02 (m, 2H), 3.40 – 3.30 (m, 2H), 2.87 (t, *J* = 15.9 Hz, 2H), 2.59 – 2.46 (m, 4H), 1.96 (s, 4H).

LC/MS *m/z* calculated for $[M+H]^+$ 746.2, found 746.2.



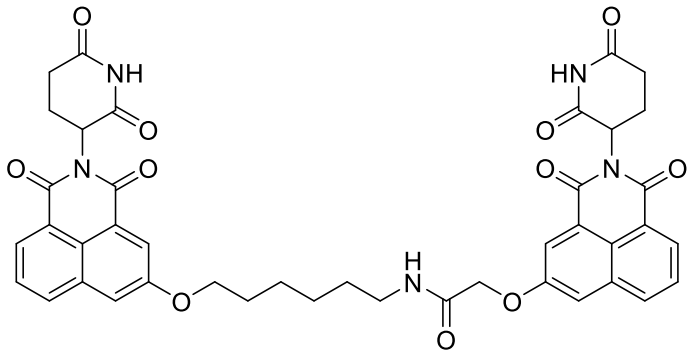
Scheme 3. Synthesis of **DGY-8-154**



5-((6-aminohexyl)oxy)-2-(2,6-dioxopiperidin-3-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione TFA salt.

This compound was synthesized via the same route with 5-(3-aminopropoxy)-2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione.

LC/MS *m/z* calculated for $[M+H]^+$ 424.2, found 424.2.



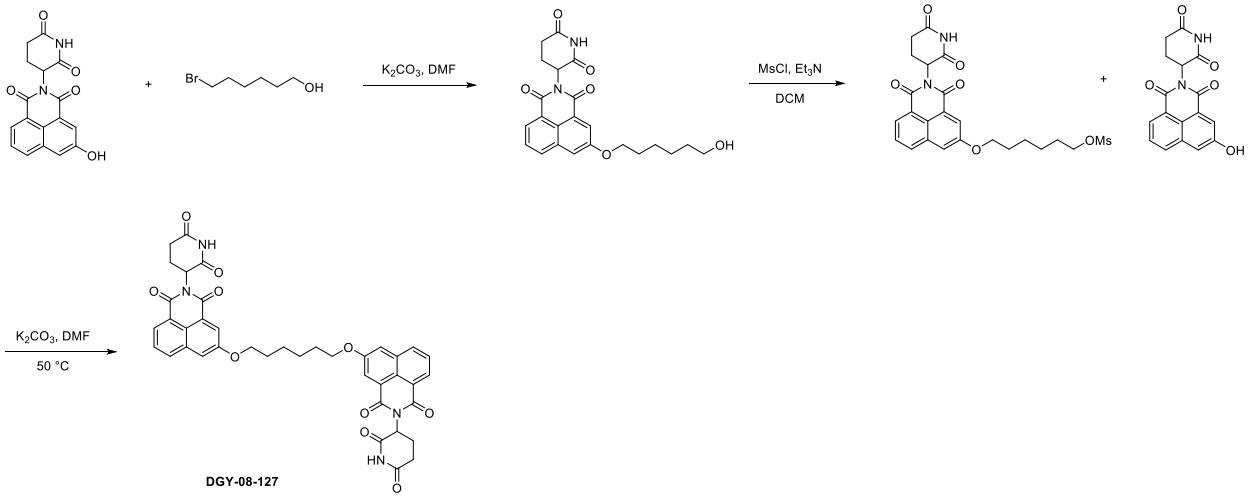
DGY-08-154

2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)-N-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)hexyl)acetamide (DGY-8-154).

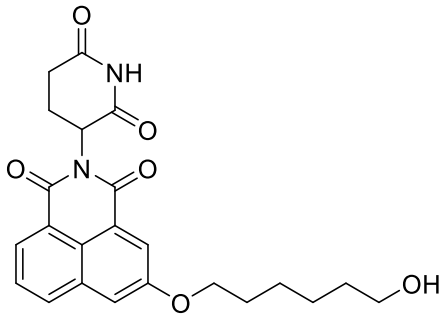
This compound was synthesized via the same route with **DGY-8-153**.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.95 (s, 2H), 8.32 – 8.03 (m, 6H), 8.01 – 7.68 (m, 5H), 5.75 (dd, *J* = 11.9, 5.8 Hz, 2H), 4.65 (d, *J* = 8.1 Hz, 2H), 4.08 (s, 2H), 3.12 (s, 2H), 2.93 – 2.82 (m, 2H), 2.53 (d, *J* = 16.1 Hz, 4H), 1.99 (d, *J* = 13.0 Hz, 2H), 1.68 (s, 2H), 1.49 – 1.34 (m, 4H), 1.27 (d, *J* = 7.4 Hz, 2H).

LC/MS *m/z* calculated for $[M+H]^+$ 788.2, found 788.2.



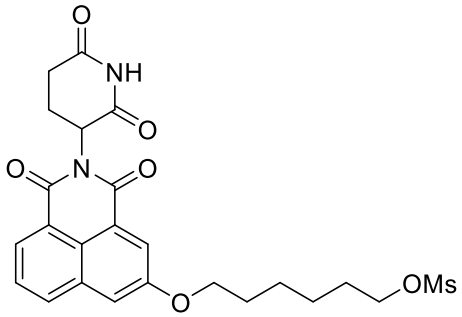
Scheme 4. Synthesis **DGY-8-127**



2-(2,6-dioxopiperidin-3-yl)-5-((6-hydroxyhexyl)oxy)-1H-benzo[de]isoquinoline-1,3(2H)-dione.

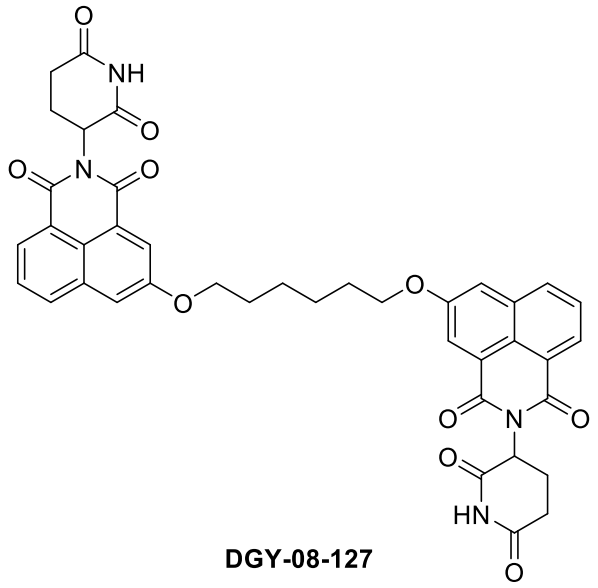
This compound was synthesized via the same route with 5-(3-aminopropoxy)-2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione.

LC/MS *m/z* calculated for $[M+H]^+$ 425.2, found 425.2.



6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-benzo[de]isoquinolin-5-yl)oxy)hexyl methanesulfonate.

To the solution of 2-(2,6-dioxopiperidin-3-yl)-5-((6-hydroxyhexyl)oxy)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (17 mg, 0.04 mmol) and Et_3N (16 mg, 0.16 mmol) in DCM (1 mL), MsCl (4.6 μL, 0.06 mmol) was added at 0 ℃. After 2 hours, the solvent was removed under vacuum. The residue was purified with flash chromatography to obtain the product (7 mg, 0.014 mmol, 35%).



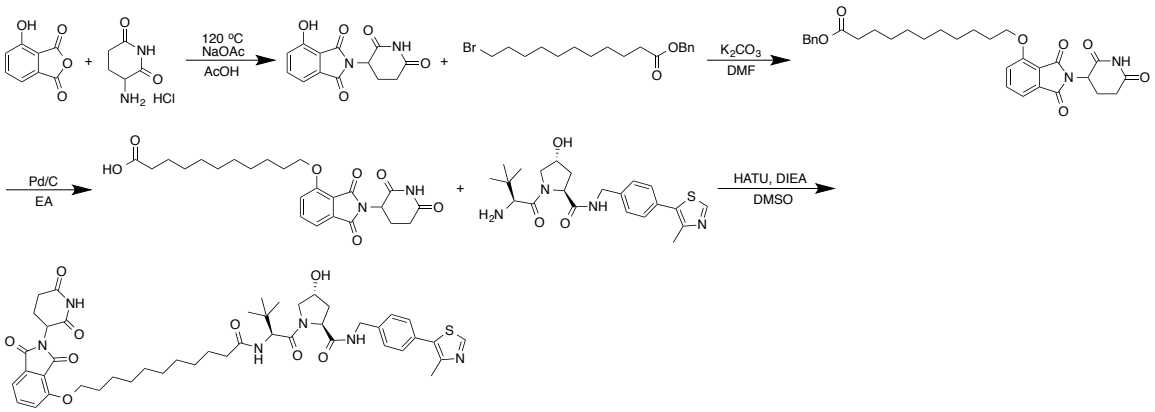
DGY-08-127

5,5'-(hexane-1,6-diylbis(oxy))bis(2-(2,6-dioxopiperidin-3-yl)-1H-benzo[de]isoquinoline-1,3(2H)-dione) (DGY-8-127).

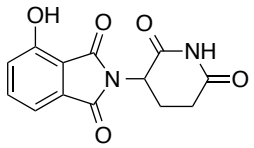
To the solution of 6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)hexyl methanesulfonate (7 mg, 0.014 mmol) and 2-(2,6-dioxopiperidin-3-yl)-5-hydroxy-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (4.5 mg, 0.014 mmol) in DMF (1 mL), K_2CO_3 (5.8 mg, 0.042 mmol) was added at room temperature. Then the reaction was heated to 50 ℃ overnight. The reaction mixture was purified with reverse phase HPLC to obtain the titled product (1.3 mg, 0.0018 mmol, 13%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.94 (s, 2H), 8.37 – 8.17 (m, 4H), 8.03 (d, *J* = 6.2 Hz, 1H), 7.96 – 7.84 (m, 3H), 7.75 (d, *J* = 6.4 Hz, 2H), 5.75 (dt, *J* = 11.7, 5.9 Hz, 2H), 4.23 – 4.10 (m, 4H), 2.95 – 2.81 (m, 2H), 2.52 (dd, *J* = 31.9, 15.8 Hz, 4H), 2.15 – 2.04 (m, 2H), 1.99 (d, *J* = 16.5 Hz, 2H), 1.82 (s, 2H), 1.54 (s, 2H), 1.40 (t, *J* = 7.2 Hz, 2H).

LC/MS *m/z* calculated for $[M+H]^+$ 731.2, found 731.2.

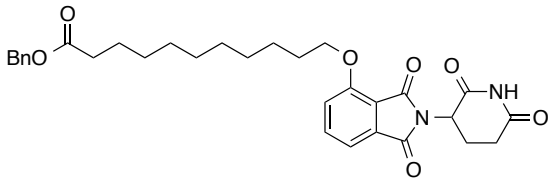


Scheme 5. Synthesis of **ZXH-4-130**.



2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione.

To a solution of 4-hydroxyisobenzofuran-1,3-dione (1.64 g, 10 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (1.65 g, 10 mmol) in acetic acid (30 mL) was added NaOAc (984 mg, 12 mmol), then the mixture solution was stirred at 120 °C for 12 hrs. After completed, the mixture was the cooled down to room temperature, filtered, and the solid was washed with some water and hexane, and then air dried for overnight to obtain crude product (2.23 g, 82%) as gray powder. LCMS: 275 $[M + H]^+$.

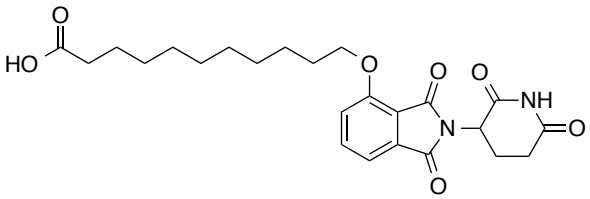


benzyl 11-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)undecanoate.

To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (200 mg, 0.73 mmol) and benzyl 11-bromoundecanoate (310 mg, 0.87 mmol) in DMF (4 mL) was added K_2CO_3 (152 mg, 1.1 mmol), and then the mixture was stirred at room temperature until reaction completed.

The mixture was then filtered, and the filtrate was collected, purified by HPLC (MeOH/water, 0.035% TFA) to obtain product (180 mg, 37%) as TFA salt.

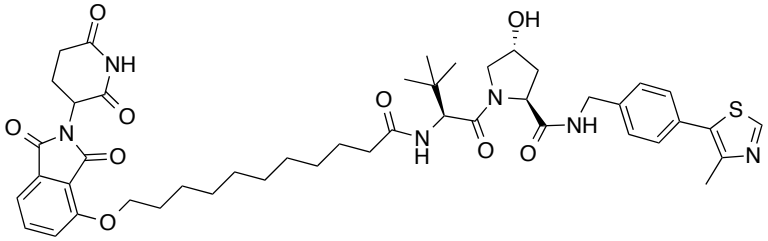
LCMS: 549 $[M + H]^+$.



11-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)undecanoic acid.

To a solution of benzyl 11-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)undecanoate (180 mg, 0.27 mmol) in EA (20 mL) was added Pd/C (20 mg, 60% w.t. in mineral oil), and then the mixture was stirred at N_2 atmosphere until reaction completed. The mixture was then filtered, and the filtrate was collected, concentrated in vacuo to obtain product (105 mg, 82%) which was used in the next step without any purification.

LCMS: 459 $[M + H]^+$.



(2S,4R)-1-((2S)-2-(11-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)undecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (ZXH-4-130).

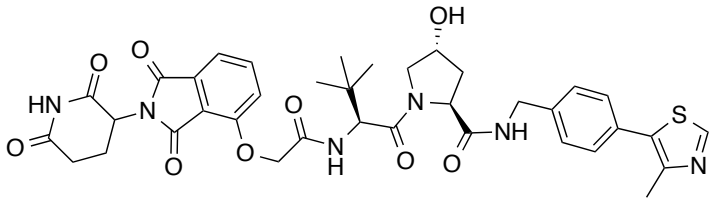
To a solution of 11-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)undecanoic acid (7 mg, 0.016 mmol) and (4*R*)-3-methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-*L*-prolinamide (7 mg, 0.016 mmol) in DMSO (1 mL) were added HATU (7 mg, 0.019 mmol) and

DIEA (8 uL, 0.048 mmol). The mixture was stirred at room temperature for 1 h, and then purified by HPLC (MeOH/water, 0.035% TFA) to obtain product (1 mg, 6%) as TFA salt.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.98 (s, 1H), 8.55 (t, *J* = 6.1 Hz, 1H), 7.86 – 7.76 (m, 2H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.46 – 7.40 (m, 3H), 7.38 (d, *J* = 8.4 Hz, 2H), 5.08 (dt, *J* = 12.7, 7.2 Hz, 2H), 4.54 (d, *J* = 9.3 Hz, 1H), 4.48 – 4.40 (m, 2H), 4.37 – 4.30 (m, 1H), 4.27 – 4.11 (m, 3H), 3.72 – 3.58 (m, 2H), 2.88 (ddd, *J* = 16.9, 13.9, 5.5 Hz, 1H), 2.59 (dt, *J* = 17.0, 2.7 Hz, 1H), 2.54 (s, 1H), 2.44 (s, 3H), 2.30 – 2.21 (m, 1H), 2.10 (ddd, *J* = 14.2, 8.1, 6.2 Hz, 1H), 2.02 (dtt, *J* = 11.8, 6.4, 3.4 Hz, 2H), 1.90 (ddd, *J* = 12.9, 8.5, 4.6 Hz, 1H), 1.82 – 1.68 (m, 2H), 1.54 – 1.39 (m, 4H), 1.36 – 1.18 (m, 9H), 0.93 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.3, 172.6, 172.4, 170.4, 170.2, 167.3, 165.8, 156.5, 151.9, 148.2, 140.0, 137.5, 133.7, 131.6, 130.1, 129.3, 129.1, 128.5, 127.9, 120.3, 116.7, 115.6, 69.3, 59.2, 56.8, 49.2, 42.1, 40.9, 38.4, 35.7, 35.3, 31.4, 29.4, 29.2, 29.1, 28.9, 26.8, 25.9, 25.8, 22.5, 16.4.

LCMS: 871 $[M + H]^+$.

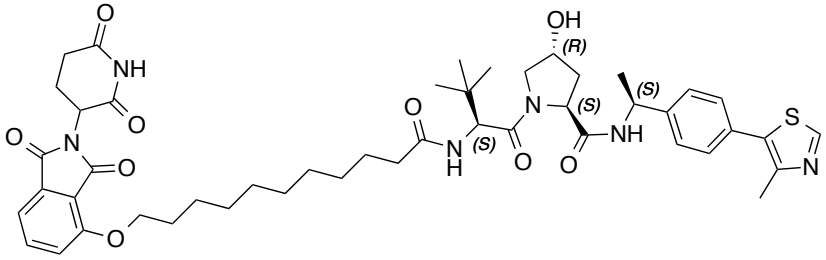


(2S,4R)-1-((2S)-2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (ZXH-4-132).

ZXH-4-132 (2.7 mg, 19%) was obtained according the synthetic route of **ZXH-4-130**, changing from benzyl 11-bromoundecanoate to benzyl 2-bromoacetate.

LCMS: 745 $[M + H]^+$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 8.99 (s, 1H), 8.59 (d, *J* = 5.1 Hz, 1H), 8.01 (dd, *J* = 9.3, 3.6 Hz, 1H), 7.82 (t, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 7.2 Hz, 1H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.41 (d, *J* = 2.9 Hz, 3H), 5.11 (dd, *J* = 13.4, 5.3 Hz, 2H), 4.95 (dd, *J* = 14.9, 2.8 Hz, 1H), 4.87 (dd, *J* = 14.8, 2.2 Hz, 1H), 4.60 (dd, *J* = 9.5, 3.5 Hz, 1H), 4.45 (dt, *J* = 8.2, 4.2 Hz, 1H), 4.36 (d, *J* = 5.5 Hz, 2H), 4.29 (ddd, *J* = 15.7, 5.9, 2.9 Hz, 1H), 3.74 – 3.58 (m, 2H), 2.97 – 2.79 (m, 1H), 2.45 (s, 3H), 2.05 (dd, *J* = 12.1, 5.6 Hz, 2H), 1.91 (ddd, *J* = 12.9, 9.3, 4.5 Hz, 1H), 1.00 (s, 7H).

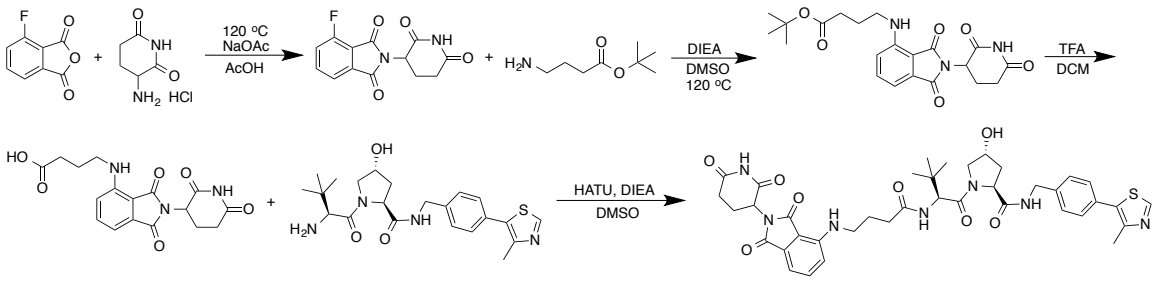


(2S,4R)-1-((2S)-2-(11-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)undecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (ZXH-4-137).

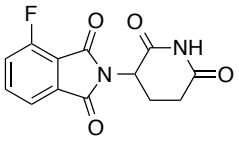
ZXH-4-137 (14.2 mg, 65%) was obtained according to the synthetic route of **ZXH-4-130**, changing from (4*R*)-3-methyl-L-valyl-4-hydroxy-*N*-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-*L*-prolinamide to (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl) phenyl)ethyl)pyrrolidine-2-carboxamide dihydrochloride (E3 ligase ligand 1).

LCMS: 885 $[M + H]^+$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.00 (s, 1H), 8.37 (d, *J* = 7.8 Hz, 1H), 7.86 – 7.76 (m, 2H), 7.52 (d, *J* = 8.6 Hz, 1H), 7.46 – 7.41 (m, 3H), 7.39 (d, *J* = 8.1 Hz, 2H), 5.09 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.92 (p, *J* = 7.2 Hz, 1H), 4.52 (d, *J* = 9.3 Hz, 1H), 4.43 (t, *J* = 8.0 Hz, 1H), 4.28 (t, *J* = 3.6 Hz, 1H), 4.21 (t, *J* = 6.4 Hz, 2H), 3.67 – 3.55 (m, 2H), 2.89 (ddd, *J* = 16.9, 13.8, 5.4 Hz, 1H), 2.61 (d, *J* = 3.4 Hz, 2H), 2.46 (s, 3H), 2.26 (dd, *J* = 14.4, 7.5 Hz, 1H), 2.17 – 2.09 (m, 1H), 2.08 – 1.98 (m, 2H), 1.76 (p, *J* = 6.8, 6.1 Hz, 2H), 1.47 – 1.23 (m, 15H), 0.94 (s, 9H).

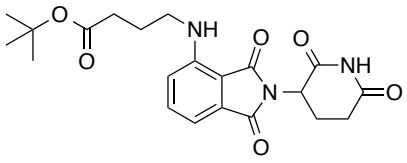


Scheme 6. Synthesis of (2*S*,4*R*)-1-((2*S*)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**ZXH-4-133**)



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

To a solution of 4-fluoroisobenzofuran-1,3-dione (1660 mg, 10 mmol) and 3-aminopiperidine-2,6-dione hydrochloride (1650 mg, 10 mmol) in acetic acid (30 mL) was added NaOAc (984 mg, 12 mmol), then the mixture solution was stirred at 120 °C for 12 hrs. After completed, the mixture was the cooled down to room temperature, filtered, and the solid was washed with some water and hexane, and then air dried for overnight to obtain crude product (1.68 g, 50%) as gray powder. LCMS: 277 $[M + H]^+$.

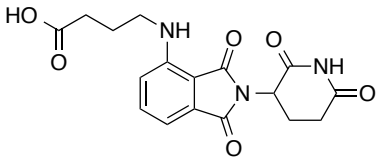


tert-butyl 4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoate

To a solution of 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (55 mg, 0.2 mmol) and *tert*-butyl 4-aminobutanoate (32 mg, 0.2 mmol) in DMSO (2 mL) was added DIEA (66 uL, 0.4

mmol). The mixture was stirred at 120 °C for 1 h, and then purified by HPLC (MeOH/water, 0.035% TFA) to obtain product as TFA salt, which was used in the next step directly.

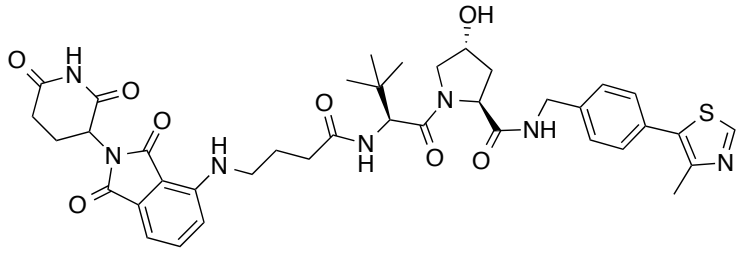
LCMS: 416 $[M + H]^+$.



4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoic acid

To a solution of *tert*-butyl 4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoate (0.2 mmol) in DCM (3 mL) was added TFA (1 mL), and then stirred at room temperature for 1 h, then concentrated in *vacuo* to obtain product (34 mg, 36%) as TFA salt, which was used in the next step without any purification.

LCMS: 360 $[M + H]^+$.



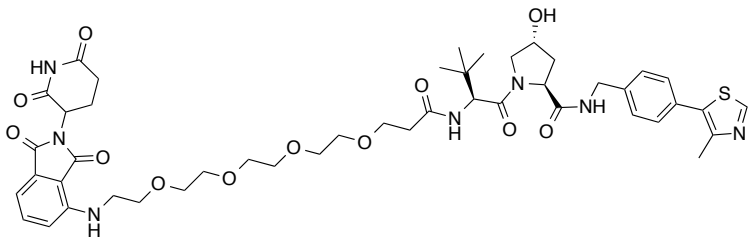
(2S,4R)-1-((2S)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (ZXH-4-133)

To a solution of 4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanoic acid (8 mg, 0.016 mmol) and (4*R*)-3-methyl-L-valyl-4-hydroxy-*N*-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-*L*-prolinamide (7 mg, 0.016 mmol) in DMSO (1 mL) were added HATU (7 mg, 0.019 mmol) and

DIEA (8 uL, 0.048 mmol). The mixture was stirred at room temperature for 1 h, and then purified by HPLC (MeOH/water, 0.035% TFA) to obtain product (3 mg, 21%) as TFA salt.

LCMS: 772 $[M + H]^+$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H), 9.00 (s, 1H), 8.56 (t, *J* = 6.1 Hz, 1H), 7.99 (d, *J* = 9.3 Hz, 1H), 7.59 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.43 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.3 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 1H), 7.02 (d, *J* = 7.0 Hz, 1H), 6.64 (s, 1H), 5.06 (dd, *J* = 12.7, 5.5 Hz, 1H), 4.57 (d, *J* = 9.4 Hz, 1H), 4.47 – 4.34 (m, 4H), 4.22 (dd, *J* = 15.9, 5.5 Hz, 1H), 3.68 (d, *J* = 3.5 Hz, 2H), 2.89 (ddd, *J* = 16.9, 13.7, 5.4 Hz, 1H), 2.66 – 2.57 (m, 1H), 2.45 (s, 3H), 2.36 (dd, *J* = 14.6, 7.2 Hz, 1H), 2.26 (dt, *J* = 14.4, 7.1 Hz, 1H), 2.08 – 1.99 (m, 2H), 1.91 (ddd, *J* = 12.9, 8.6, 4.6 Hz, 1H), 1.79 (dq, *J* = 17.9, 6.8 Hz, 2H), 0.95 (s, 9H).

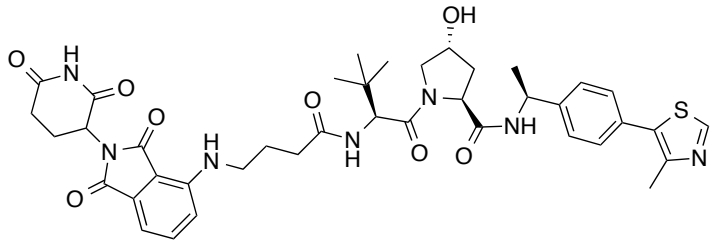


(2S,4R)-1-((17S)-17-(tert-butyl)-1-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-15-oxo-3,6,9,12-tetraoxa-16-azaoctadecan-18-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (ZXH-4-135)

ZXH-4-135 (1 mg, 6%) was obtained according to the synthetic route of **ZXH-4-133**, changing from *tert*-butyl 4-aminobutanoate to 3,6,9,12-Tetraoxapentadecan-15-oic acid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.99 (s, 1H), 8.56 (t, *J* = 6.1 Hz, 1H), 7.90 (d, *J* = 9.4 Hz, 1H), 7.58 (dd, *J* = 8.6, 7.0 Hz, 1H), 7.49 – 7.32 (m, 4H), 7.14 (d, *J* = 8.6 Hz, 1H), 7.04 (d, *J* = 7.1 Hz, 1H), 6.60 (s, 1H), 5.05 (dd, *J* = 12.7, 5.5 Hz, 1H), 4.55 (d, *J* = 9.4 Hz, 1H), 4.47 – 4.39 (m, 2H), 4.35 (dp, *J* = 4.5, 2.4 Hz, 1H), 4.22 (dd, *J* = 15.9, 5.5 Hz, 1H), 3.75 – 3.38 (m, 21H), 2.88 (ddd, *J* = 16.8, 13.7, 5.4 Hz, 1H), 2.63 – 2.55 (m, 1H), 2.55 – 2.53 (m, 1H), 2.44 (s, 3H), 2.34 (dt, *J* = 14.7, 6.2 Hz, 1H), 2.08 – 1.98 (m, 2H), 1.90 (ddd, *J* = 12.9, 8.6, 4.6 Hz, 1H), 0.93 (s, 9H).

LCMS: 934 $[M + H]^+$.



(2S,4R)-1-((2S)-2-(4-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)butanamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-((S)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide (ZXH-4-136)

ZXH-4-136 (20.2 mg, 61%) was obtained according to the synthetic route of **ZXH-4-133**, changing from (4*R*)-3-methyl-*L*-valyl-4-hydroxy-*N*-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-*L*-prolinamide to (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl) phenyl)ethyl)pyrrolidine-2-carboxamide dihydrochloride (E3 ligase ligand 1).

LCMS: 786 $[M + H]^+$.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.10 (s, 1H), 9.00 (s, 1H), 8.37 (d, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.59 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.39 (d, *J* = 8.0 Hz, 2H), 7.11 (d, *J* = 8.7 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.65 (s, 1H), 5.06 (dd, *J* = 12.7, 5.4 Hz, 1H), 4.92 (p, *J* = 7.1 Hz, 1H), 4.55 (d, *J* = 9.3 Hz, 1H), 4.43 (t, *J* = 8.0 Hz, 1H), 4.29 (t, *J* = 3.8 Hz, 1H), 3.63 (d, *J* = 3.2 Hz, 2H), 2.89 (ddd, *J* = 16.7, 13.7, 5.4 Hz, 1H), 2.65 – 2.57 (m, 2H), 2.46 (s, 3H), 2.34 (dt, *J* = 14.7, 7.4 Hz, 1H), 2.25 (dt, *J* = 14.6, 7.2 Hz, 1H), 2.03 (dtd, *J* = 12.9, 7.9, 7.1, 3.5 Hz, 2H), 1.85 – 1.72 (m, 3H), 0.95 (s, 9H).