SUPPORTING INFORMATION

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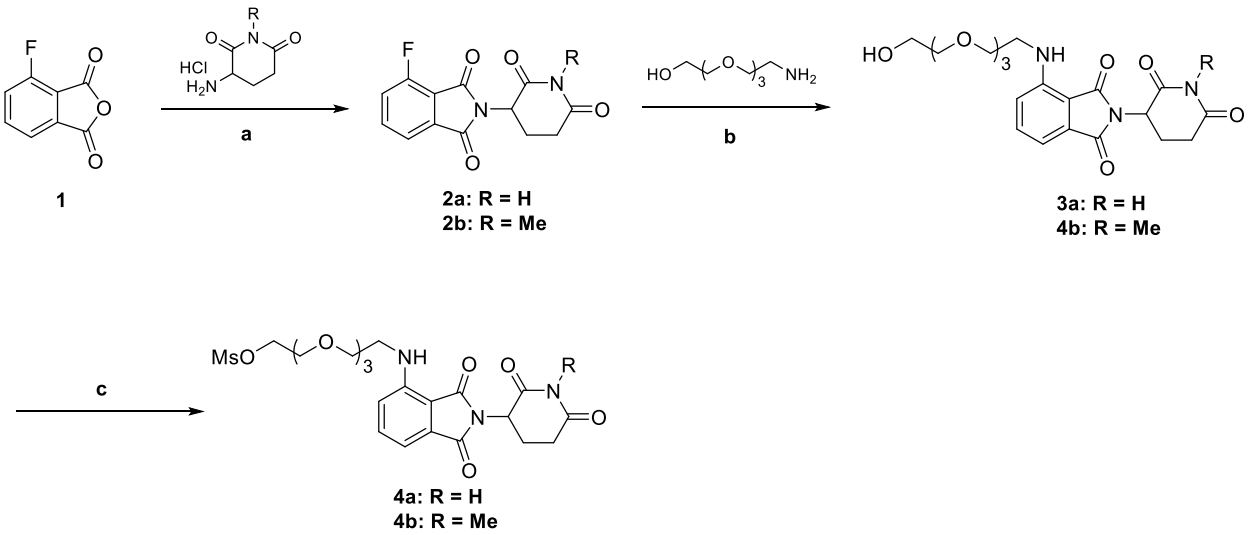
Experimental Procedures

Chemistry

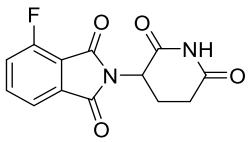
General Methods. Starting materials, reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise noted. All reactions were monitored using a Waters Acquity UPLC/MS system (Waters PDA eλ Detector, QDa Detector, Sample manager - FL, Binary Solvent Manager) using Acquity UPLC® BEH C18 column (2.1 x 50 mm, 1.7 µm particle size): solvent gradient = 85% A at 0 min, 1% A at 1.7 min; solvent A = 0.1% formic acid in Water; solvent B = 0.1% formic acid in Acetonitrile; flow rate : 0.6 mL/min. Reaction products were purified by flash column chromatography using CombiFlash®Rf with Teledyne Isco Redi*Sep*® normal-phase silica flash columns (4 g, 12 g, 24 g, 40 g or 80 g) and Waters HPLC system using SunFire™ Prep C18 column (19 x 100 mm, 5 µm particle size): solvent gradient = 80% A at 0 min, 10% A at 25 min; solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in MeOH; flow rate : 25 mL/min. ^{1}H NMR spectra were recorded on 500 MHz Bruker Avance III spectrometers and ^{13}C NMR spectra were recorded on 125 MHz Bruker Avance III spectrometer. 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 br (broad), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet).

Scheme S1. Synthesis of CRBN ligands linked to PEG3.[1]



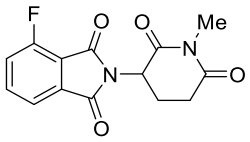
a) NaOAc, AcOH, 52%; b) DIEA, DMF, 53-62%; c) MsCl, Et_3N, CH_2Cl_2, 45 °C, 63-78%

1. 2a [2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione].



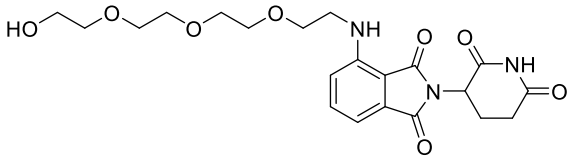
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.97 - 7.92 (m, 1H), 7.79 (d, *J* = 7.3 Hz, 1H), 7.73 (t, *J* = 8.9 Hz, 1H), 5.15 (dd, *J* = 12.8, 5.5 Hz, 1H), 2.94 - 2.83 (m, 1H), 2.65 - 2.45 (m, 2H), 2.10 - 2.03 (m, 1H); LC/MS (ESI) $C_{13}H_{10}FN_2O_4$ $[M+H]^+$ calcd. *m/z* 277.06; found 277.04.

2. 2b [4-fluoro-2-(1-methyl-2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione].



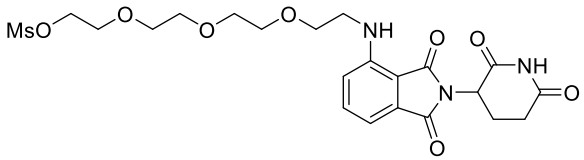
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 - 7.75 (m, 1H), 7.71 (d, *J* = 7.3 Hz, 1H), 7.43 (t, *J* = 8.5 Hz, 1H), 5.02 - 4.96 (m, 1H), 3.21 (s, 3H), 3.05 - 2.95 (m, 1H), 2.87 - 2.22 (m, 2H), 2.17 - 2.22 (m, 1H); LC/MS (ESI) $C_{14}H_{12}FN_2O_4$ $[M+H]^+$ calcd. *m/z* 291.08; found 291.25.

3. 3a [2-(2,6-dioxopiperidin-3-yl)-4-((2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)amino)isoindoline-1,3-dione].



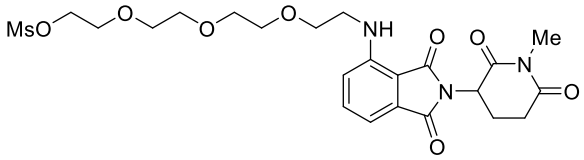
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 (br s, 1H), 7.50 (dd, *J* = 8.2, 7.3 Hz, 1H), 7.12 (d, *J* = 6.7 Hz, 1H), 6.94 (d, *J* = 8.5 Hz, 1H), 6.53 (t, *J* = 5.3 Hz, 1H), 4.95 - 4.90 (m, 1H), 3.76 - 3.67 (m, 12H), 3.64 - 3.61 (m, 2H), 3.49 (q, *J* = 5.4 Hz, 2H), 2.93 - 2.69 (m, 3H), 2.54 (t, *J* = 6.3 Hz, 1H), 2.18 - 2.10 (m, 1H); LC/MS (ESI) $C_{21}H_{28}N_3O_8$ $[M+H]^+$ calcd. *m/z* 450.19; found 450.46.

4. 4a [2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate]



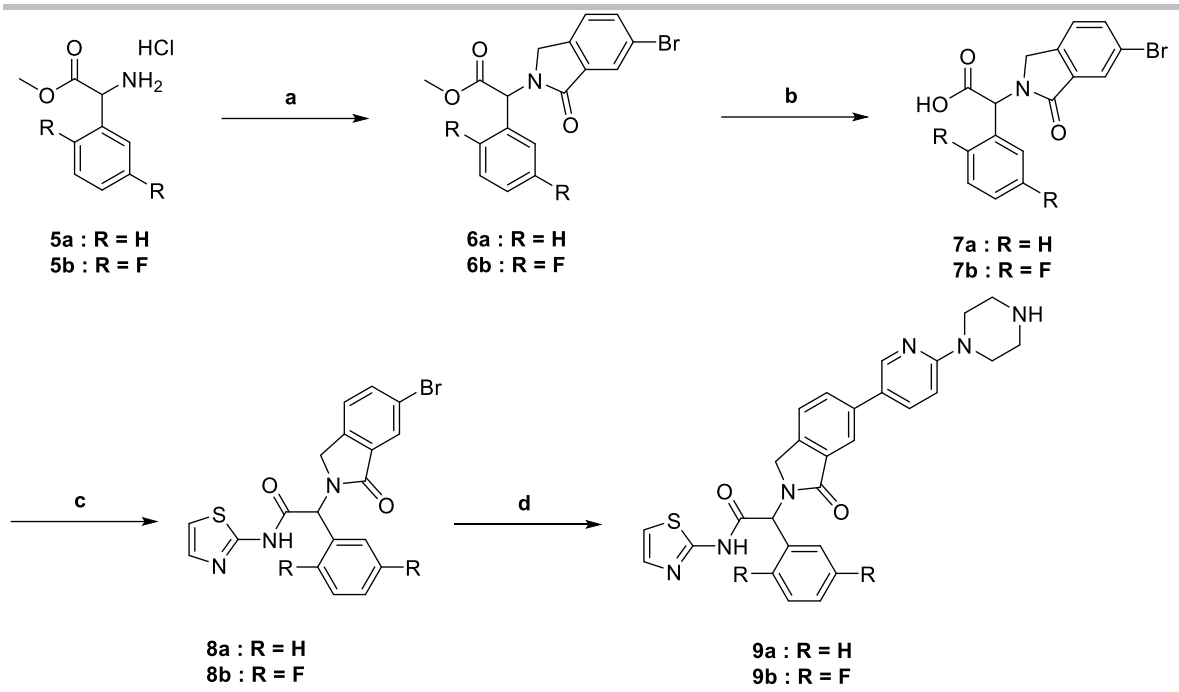
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.11 (br s, 1H), 7.51 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.12 (d, *J* = 7.0 Hz, 1H), 6.93 (d, *J* = 8.5 Hz, 1H), 6.50 (br s, 1H), 4.96 - 4.90 (m, 1H), 4.40 - 4.35 (m, 2H), 3.80 - 3.75 (m, 2H), 3.73 (t, *J* = 5.3 Hz, 2H), 3.70 - 3.64 (m, 8H), 3.48 (t, *J* = 5.3 Hz, 2H), 3.08 (s, 3H), 2.95 - 2.70 (m, 3H), 2.18 - 2.11 (m, 1H); LC/MS (ESI) $C_{22}H_{30}N_3O_{10}S$ $[M+H]^+$ calcd. *m/z* 528.16; found 528.47.

5. 4b [2-(2-(2-(2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl methanesulfonate].



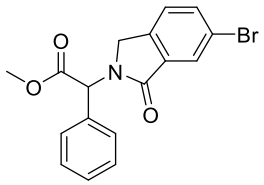
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.10 (d, *J* = 7.0 Hz, 1H), 6.92 (d, *J* = 8.5 Hz, 1H), 6.48 (br s, 1H), 4.96 - 4.87 (m, 1H), 4.38 - 4.34 (m, 2H), 3.78 - 3.74 (m, 2H), 3.72 (t, *J* = 5.5 Hz, 2H), 3.69 - 3.57 (m, 8H), 3.47 (t, *J* = 5.3 Hz, 2H), 3.20 (s, 3H), 3.06 (s, 3H), 3.01 - 2.91 (m, 1H), 2.83 - 2.70 (m, 2H), 2.13 - 2.01 (m, 1H); LC/MS (ESI) $C_{23}H_{32}N_3O_{10}S$ $[M+H]^+$ calcd. *m/z* 542.18; found 542.51.

Scheme S2. Synthesis of EGFR ligands.



a) methyl 5-bromo-2-(bromomethyl)benzoate, DIEA, dioxane/ *N*,*N*-dimethylformamide, 72-85%; b) $LiOH.H_2O$, THF/MeOH/H_2O (1:1:1), 87-90% c) 2-aminothiazole, HATU, DIEA, DMF, 45 °C, 63-78%; d) (6-(piperazin-1-yl)pyridin-3-yl)boronic acid, $PdCl_2(dppf)_2$, Xphos, 2 *N* Na_2CO_3, dioxane, 100 °C, 57-63%

Representative procedure

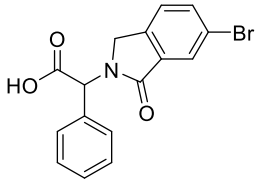


1. Synthesis of 6a [methyl 2-(6-bromo-1-oxoisoindolin-2-yl)-2-phenylacetate].

To a solution of **5a** (2.80 g, 13.92 mmol) and methyl 5-bromo-2-(bromomethyl)benzoate (3.90 g, 12.66 mmol) in *N*,*N*-dimethylformamide (120 mL) was added DIEA (6.60 mL, 37.98 mmol) at 0 °C. After stirring for 12 hours at 80 °C, the reaction mixture was cooled to room temperature and diluted with ethyl acetate. The resulting solution was washed with water five times and washed with brine. The organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by flash column chromatography (0 to 20% methanol in DCM) to give **6a** (3.28 g, 72%) as a brown solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (d, *J* = 1.8 Hz, 1H), 7.79 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.48 - 7.36 (m, 5H), 6.07 (s, 1H), 4.57 (d, *J* = 17.7 Hz, 1H), 3.94 (d, *J* = 18.0 Hz, 1H), 3.74 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.1, 166.1, 141.2, 134.6, 133.9, 133.4, 129.1, 128.8, 126.1, 125.6, 121.2, 58.0, 52.6, 47.7; LC/MS (ESI) *m/z* 359.98 $[M+H]^+$.

2. Synthesis of 7a [2-(6-bromo-1-oxoisoindolin-2-yl)-2-phenylacetic acid].

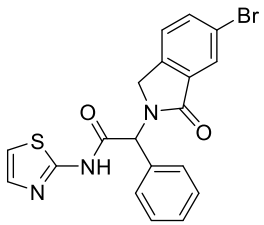


6a (3.20 g, 8.89 mmol) was dissolved in THF/MeOH/Water (1:1:1) mixture (30 mL) and lithium hydroxide monohydrate (2.65 g, 63.30 mmol) was added to the mixture. After stirring for 30 min, the reaction mixture was concentrated under reduced pressure. The residue

was diluted with ice water and acidified with conc. HCl. The precipitate was filtered and dried using blowing nitrogen gas to give **7a** (3.28 g, 87%) as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 13.47 (br s, 1H), 7.86 (d, *J* = 1.8 Hz, 1H), 7.77 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.47 - 7.37 (m, 5H), 5.97 (s, 1H), 4.61 (d, *J* = 17.7 Hz, 1H), 3.90 (d, *J* = 17.7 Hz, 1H), 3.72 (s, 3H), 3.33 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.1, 166.1, 141.2, 134.5, 133.7, 129.0, 128.9, 128.5, 126.0, 125.6, 121.1, 58.2, 47.6; LC/MS (ESI) *m/z* 345.97 $[M+H]^+$.

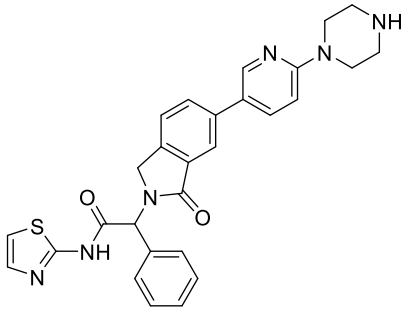
3. Synthesis of 8a [2-(6-bromo-1-oxoisoindolin-2-yl)-2-phenyl-*N*-(thiazol-2-yl)acetamide].



To a solution of **7a** (2.00 g, 5.78 mmol), 2-aminothiazole (1.10 g, 11.55 mmol) and HATU (4.40 g, 11.55 mmol) in DMF (30 mL) was added DIEA (4.00 mL, 23.12 mmol). After stirring at 45 °C for 8 hours, the reaction mixture was poured into excess water. The precipitate was filtered and dried using blowing nitrogen gas to give **8a** (1.93 g, 78%) as a pale yellowish solid which was used to next step without further purification.

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.71 (s, 1H), 7.87 (d, *J* = 1.8 Hz, 1H), 7.79 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.54 (d, *J* = 8.2 Hz, 1H), 7.49 (d, *J* = 3.4 Hz, 1H), 7.48 - 7.40 (m, 3H), 7.40 - 7.36 (m, 2H), 7.28 (d, *J* = 3.7 Hz, 1H), 6.29 (s, 1H), 4.73 (d, *J* = 18.0 Hz, 1H), 3.96 (d, *J* = 18.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.2, 166.3, 157.5, 141.5, 137.8, 134.5, 134.2, 133.6, 129.1, 128.8, 126.0, 125.6, 121.1, 114.1, 58.3, 48.5; LC/MS (ESI) *m/z* 428.04 $[M+H]^+$.

4. Synthesis of 9a (JBJ-07-149) [2-(6-(1*H*-indol-5-yl)-1-oxoisoindolin-2-yl)-2-phenyl-*N*-(thiazol-2-yl)acetamide].

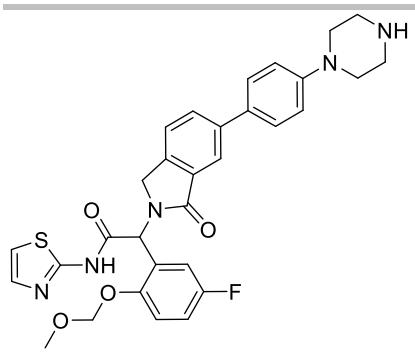


After degassing using sonication for 10 seconds, a mixture of **8a** (520 mg, 1.21 mmol), (1H-indol-5-yl)boronic acid (440 mg, 1.81 mmol) and 2 *N* sodium carbonate (3 mL, 6.00 mmol) in dioxane (12 mL) was preheated at 100 °C for 20 min. Then, $PdCl_2(dppf)_2$ (53 mg, 0.07 mmol) and Xphos (52 mg, 0.11 mmol) were added carefully to the reaction mixture. After stirring at 100 °C for 8 hours, the reaction mixture was cooled to room temperature, filtered through a pad of celite. The filtrate was diluted with DCM and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by preparative HPLC to obtain **9a** (292 mg, 57%) as an off-white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.72 (br s, 1H), 11.19 (br s, 1H), 7.95 - 7.87 (m, 3H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.52 - 7.38 (m, 9H), 7.27 (d, *J* = 3.7 Hz, 1H), 6.50 (t, *J* = 2.0 Hz, 1H), 6.34 (s, 1H), 4.81 (d, *J* = 17.7 Hz, 1H), 4.01 (d, *J* = 17.7 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.6, 167.8, 157.8, 142.1, 140.2, 137.8, 135.6, 134.6, 132.1, 130.5, 130.5, 129.1, 128.8, 128.7, 128.3, 126.2, 124.0, 120.5, 120.4, 118.5, 113.9, 112.0, 101.6, 58.3, 48.4; LC/MS (ESI) *m/z* 465.20 $[M+H]^+$.

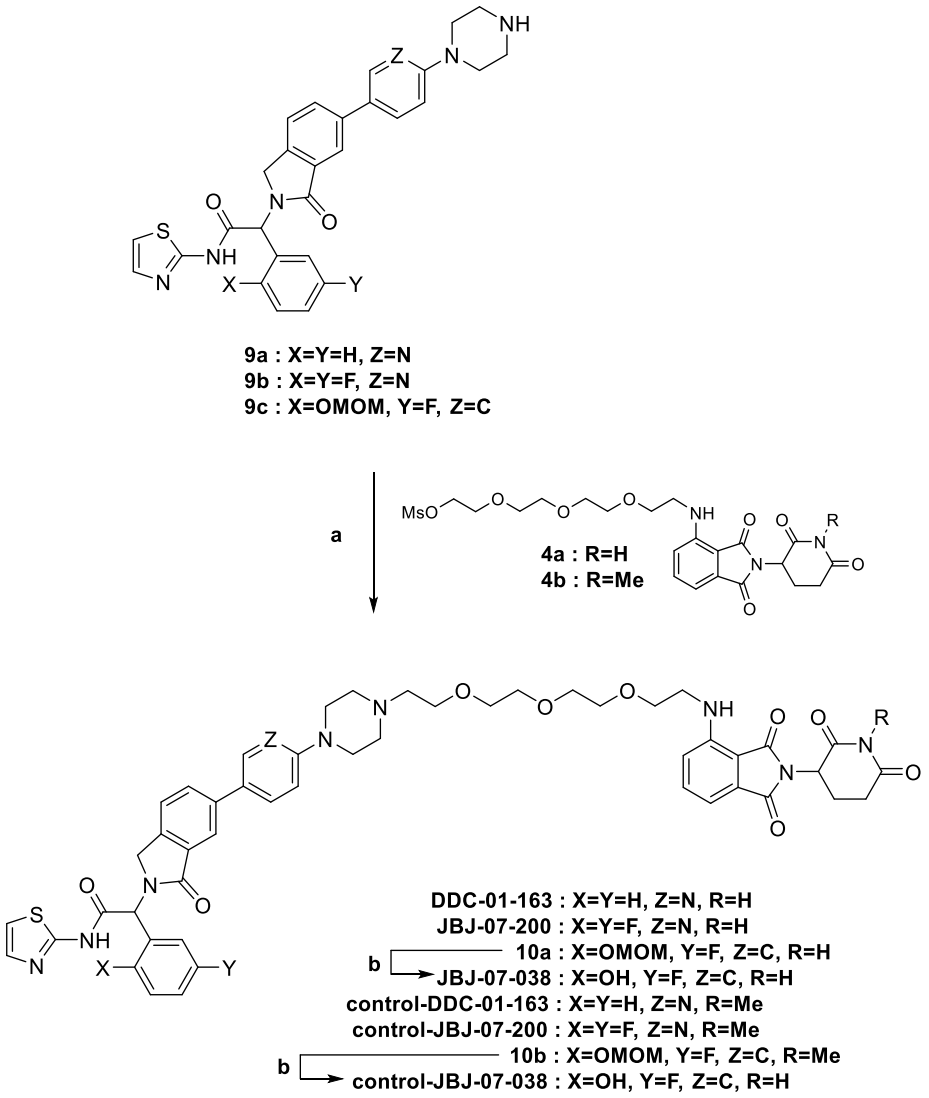
5. 9c [2-(5-fluoro-2-(methoxymethoxy)phenyl)-2-(1-oxo-6-(4-(piperazin-1-yl)phenyl)isoindolin-2-yl)-*N*-(thiazol-2-yl)acetamide].

9c was synthesized using the same procedure reported previously.[2]



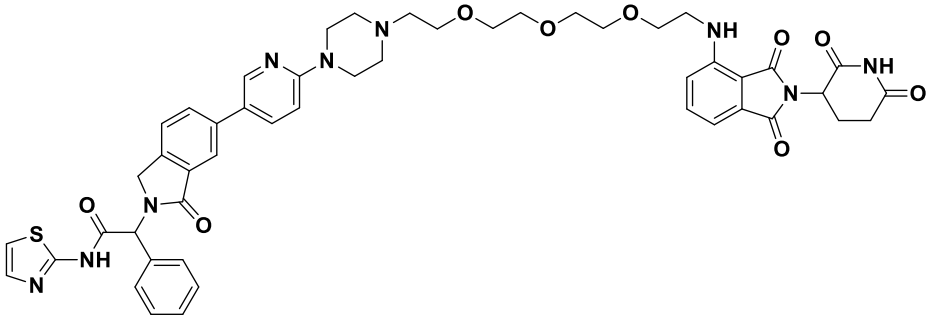
^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (br d, *J* = 1.2 Hz, 1H), 7.85 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 2H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 3.4 Hz, 1H), 7.29 - 7.19 (m, 3H), 7.02 (d, *J* = 8.9 Hz, 2H), 6.98 (dd, *J* = 9.2, 3.1 Hz, 1H), 6.42 (s, 1H), 5.20 (d, *J* = 6.7 Hz, 1H), 5.15 (d, *J* = 6.7 Hz, 1H), 4.64 (d, *J* = 17.4 Hz, 1H), 4.05 (d, *J* = 17.4 Hz, 1H), 3.25 (s, 3H), 3.16 - 3.11 (m, 2H), 2.91 - 2.85 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 168.1, 167.6, 158.0, 157.5, 155.6, 151.3, 151.1, 140.3, 140.2, 137.7, 132.2, 129.5, 129.3, 127.4, 125.4, 125.4, 124.1, 119.5, 116.4, 116.2, 116.0, 115.8, 115.6, 115.5, 113.9, 94.4, 55.7, 54.0, 48.6, 48.4, 45.2; LC/MS (ESI) *m/z* 588.28 $[M+H]^+$.

Scheme S3. Synthesis of bivalent allosteric EGFR PROTACs.



a) DIEA, DMF, 80 °C; b) TFA, CH_2Cl_2.

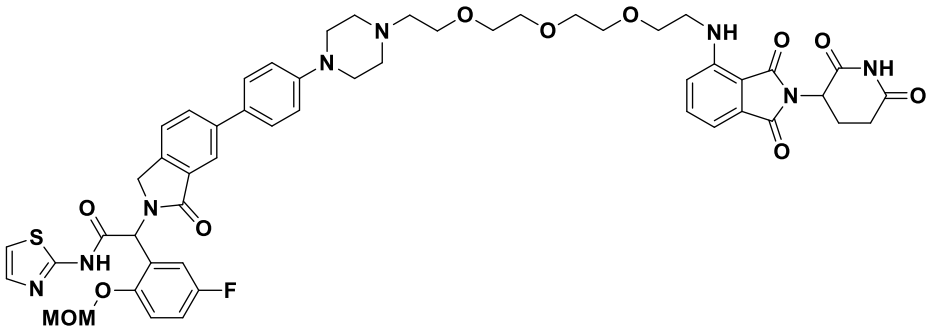
1. Synthesis of DDC-01-163 [2-(6-(6-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)pyridin-3-yl)-1-oxoisoindolin-2-yl)-2-phenyl-*N*-(thiazol-2-yl)acetamide].



To a solution of **9a** (66 mg, 0.13 mmol) and **4a** (70 mg, 0.13 mmol) in DMF (1 mL) was added DIEA (67 ml, 0.39 mmol). After stirring at 80 °C for 12 hours, the resulting mixture was diluted with DMSO and purified by preparative HPLC to obtain 2-(6-(6-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)pyridin-3-yl)-1-oxoisoindolin-2-yl)-2-phenyl-*N*-(thiazol-2-yl)acetamide (**DDC-01-163**, 29 mg, 24%) as a yellowish solid.

^{1}H NMR 500 MHz (DMSO-d_6) δ 12.64 (br s, 1H), 11.04 (s, 1H), 8.43 (d, *J* = 1.5 Hz, 1H), 7.91 - 7.83 (m, 2H), 7.82 - 7.77 (m, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.53 - 7.48 (m, 1H), 7.43 (d, *J* = 3.7 Hz, 1H), 7.41 (d, *J* = 7.6 Hz, 2H), 7.39 - 7.35 (m, 1H), 7.33 (d, *J* = 7.0 Hz, 2H), 7.22 (d, *J* = 3.4 Hz, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.96 (d, *J* = 7.0 Hz, 1H), 6.85 (d, *J* = 7.6 Hz, 1H), 6.54 (t, *J* = 5.6 Hz, 1H), 6.27 (s, 1H), 4.99 (dd, *J* = 12.7, 5.3 Hz, 1H), 4.72 (d, *J* = 17.4 Hz, 1H), 3.94 (d, *J* = 17.7 Hz, 1H), 3.57 (t, *J* = 5.3 Hz, 2H), 3.53 - 3.38 (m, 12H), 3.26 (br s, 4H), 2.87 - 2.77 (m, 1H), 2.59 - 2.36 (m, 8H), 2.03 - 1.92 (m, 1H); LC/MS (ESI) $C_{49}H_{52}N_9O_9S$ $[M+H]^+$ calcd. *m/z* 942.36; found 942.95.

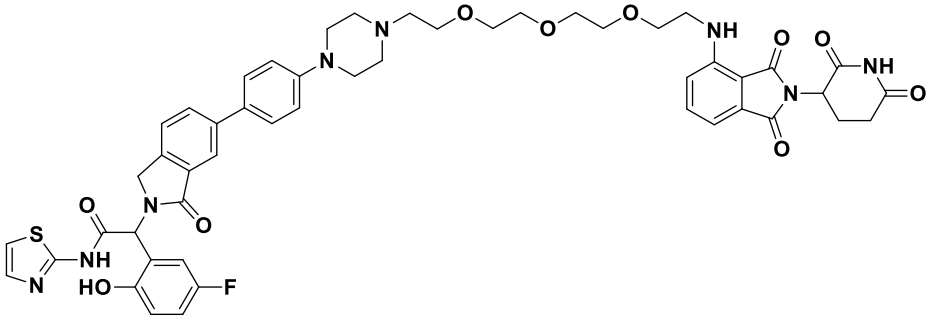
2. Synthesis of 6a [2-(6-(4-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)phenyl)-1-oxoisoindolin-2-yl)-2-(5-fluoro-2-(methoxymethoxy)phenyl)-N-(thiazol-2-yl)acetamide].



To a solution of **4a** (54 mg, 0.102 mmol) and **9c** (60 mg, 0.102 mmol) in DMF (2 mL) was added DIEA (53 µL, 0.307 mmol). After stirring at 80 °C for 4 hr, the reaction mixture was diluted with DMSO and purified by prep HPLC to obtain **10a** (88 mg, 85%).

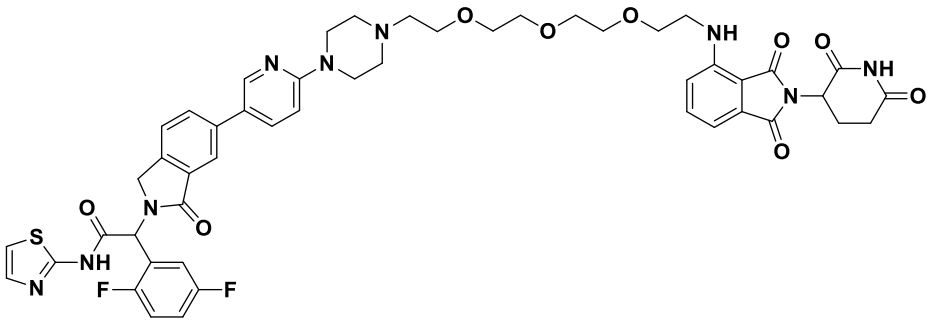
^{1}H NMR 500 MHz ($CDCl_3$) δ 7.96 (s, 1H) 7.59 (d, *J* = 7.9 Hz, 1H), 7.52 (d, *J* = 3.4 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 2H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.04 (dd, *J* = 8.5, 2.7 Hz, 1H), 6.99 - 6.94 (m, 2H), 6.90 - 6.80 (m, 4H), 6.76 (d, *J* = 8.5 Hz, 1H), 6.66 (s, 1H), 6.39 (t, *J* = 5.2 Hz, 1H), 6.33 (s, 1H), 4.90 (dd, *J* = 24.5, 6.9 Hz, 2H), 4.76 (d, *J* = 16.5 Hz, 1H), 4.77 - 4.70 (m, 1H), 4.06 (d, *J* = 17.1 Hz, 1H), 3.62 - 3.48 (m, 12H), 3.37 - 3.28 (m, 2H), 3.16 - 3.09 (m, 7H), 2.69 - 2.47 (m, 10H), 1.99 - 1.91 (m, 1H); LC/MS (ESI) $C_{52}H_{55}FN_8O_{11}S$ $[M+H]^+$ calcd. *m/z* 1019.38; found 1019.93.

3. Synthesis of JBJ-07-038 [2-(6-(4-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)phenyl)-1-oxoisoindolin-2-yl)-2-(5-fluoro-2-hydroxyphenyl)-*N*-(thiazol-2-yl)acetamide].



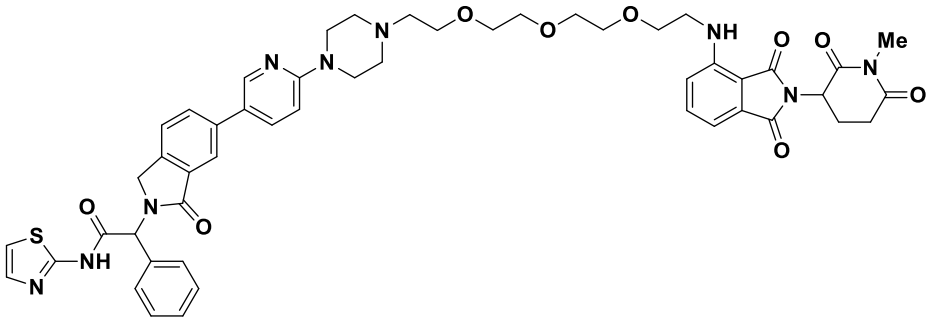
To a solution of **10a** (88 mg, 0.086 mmol) in dry DCM (4 mL) was added TFA (1 mL) at 0 °C. After stirring for 5 hr, the reaction mixture was concentrated under reduced pressure and purified by flash column chromatography (DCM : 1 *N* NH_3 in MeOH = 100 : 0 to 80 : 20) to afford 2-(6-(4-(4-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)phenyl)-1-oxoisoindolin-2-yl)-2-(5-fluoro-2-hydroxyphenyl)-*N*-(thiazol-2-yl)acetamide (**JBJ-07-038**, 27 mg, 32%) as a yellowish solid. ^{1}H NMR 500 MHz (DMSO-d_6) δ 12.61 (br s, 1H), 11.10 (s, 1H), 9.96 (br s, 1H), 7.86 (s, 1H) 7.84 (d, *J* = 8.2 Hz, 1H), 7.64 - 7.54 (m, 4H), 7.49 (d, *J* = 3.7 Hz, 1H), 7.27 (d, *J* = 3.7 Hz, 1H), 7.18 - 7.08 (m, 2H), 7.04 (d, *J* = 7.0 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 2H), 6.91 (dd, *J* = 8.9, 4.9 Hz, 1H), 6.86 (dd, *J* = 9.2, 3.1 Hz, 1H), 6.61 (t, *J* = 5.5 Hz, 1H), 6.33 (s, 1H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.62 (d, *J* = 17.7 Hz, 1H), 4.00 (d, *J* = 17.4 Hz, 1H), 3.63 (t, *J* = 5.3 Hz, 2H), 3.60 - 3.42 (m, 12H), 3.17 (br s, 4H), 2.96 - 2.80 (m, 1H), 2.64 - 2.46 (m, 8H), 2.07 - 1.97 (m, 1H); LC/MS (ESI) $C_{50}H_{52}FN_8O_{10}S$ $[M+H]^+$ calcd. *m/z* 975.35; found 975.41.

4. Synthesis of JBJ-07-200 [**2-(2,5-difluorophenyl)-2-(6-(4-(4-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)phenyl)-1-oxoisoindolin-2-yl)-*N*-(thiazol-2-yl)acetamide].**



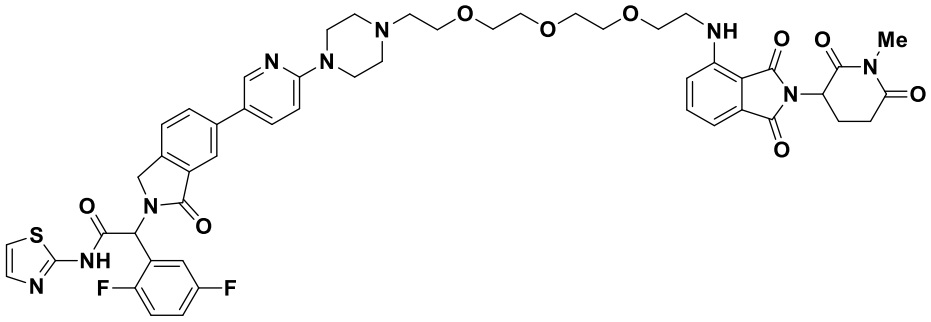
^{1}H NMR 500 MHz (DMSO-d_6) δ 12.75 (br s, 1H), 11.10 (s, 1H), 8.49 (d, *J* = 2.4 Hz, 1H), 7.95 - 7.86 (m, 2H), 7.88 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.57 (dd, *J* = 8.4, 7.2Hz, 1H), 7.50 (d, *J* = 3.7 Hz, 1H), 7.44 - 7.35 (m, 2H), 7.29 (d, *J* = 3.7 Hz, 1H), 7.21 - 7.15 (m, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 1H), 6.60 (t, *J* = 5.6 Hz, 1H), 6.46 (s, 1H), 5.05 (dd, *J* = 12.8, 5.5 Hz, 1H), 4.75 (d, *J* = 17.4 Hz, 1H), 4.16 (d, *J* = 17.4 Hz, 1H), 3.63 (t, *J* = 5.5 Hz, 2H), 3.60 - 3.44 (m, 12H), 3.32 (br s, 4H), 2.93 - 2.83 (m, 1H), 2.66 - 2.47 (m, 8H), 2.07 - 1.98 (m, 1H); LC/MS (ESI) $C_{49}H_{49}F_2N_9O_9S$ $[M+H]^+$ calcd. *m/z* 978.34; found 978.88.

5. Synthesis of control-DDC-01-163 [2-(6-(6-(4-(2-(2-(2-(2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)pyridin-3-yl)-1-oxoisoindolin-2-yl)-2-phenyl-*N*-(thiazol-2-yl)acetamide].



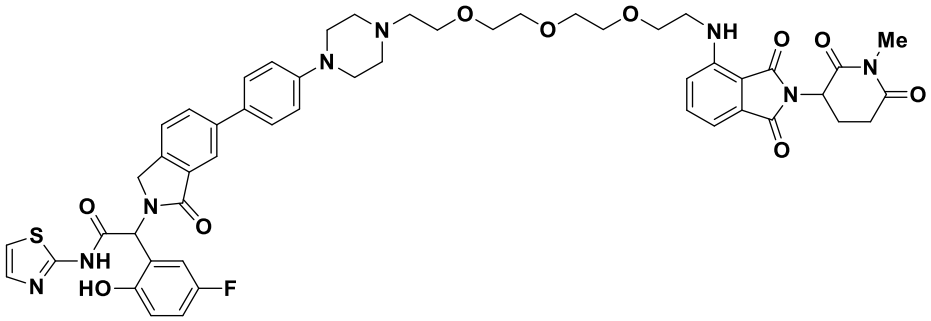
^{1}H NMR 500 MHz (DMSO-d_6) δ 12.70 (br s, 1H), 8.48 (d, *J* = 2.4 Hz, 1H), 7.94 - 7.88 (m, 2H), 7.86 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.58 (dd, *J* = 8.4, 7.2 Hz, 1H), 7.49 (d, *J* = 3.7 Hz, 1H), 7.48 - 7.41 (m, 3H), 7.41 - 7.36 (m, 2H), 7.28 (d, *J* = 3.4 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 7.0 Hz, 1H), 6.90 (d, *J* = 9.2 Hz, 1H), 6.60 (t, *J* = 5.8 Hz, 1H), 6.33 (s, 1H), 5.12 (dd, *J* = 13.1, 5.5 Hz, 1H), 4.78 (d, *J* = 17.4 Hz, 1H), 4.00 (d, *J* = 17.7 Hz, 1H), 3.63 (t, *J* = 5.5 Hz, 2H), 3.59 - 3.43 (m, 16H), 3.01 (s, 3H), 2.99 - 2.89 (m, 1H), 2.79 - 2.71 (m, 1H), 2.60 - 2.44 (m, 7H), 2.07 - 1.99 (m, 1H); LC/MS (ESI) $C_{50}H_{54}N_9O_9S$ $[M+H]^+$ calcd. *m/z* 956.38; found 956.47.

6. Synthesis of control-JBJ-07-200 [2-(2,5-difluorophenyl)-2-(6-(6-(4-(2-(2-(2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)pyridin-3-yl)-1-oxoisoindolin-2-yl)-*N*-(thiazol-2-yl)acetamide].



^{1}H NMR 500 MHz (DMSO-d_6) δ 12.75 (br s, 1H), 8.49 (d, *J* = 2.7 Hz, 1H), 7.95 - 7.90 (m, 2H), 7.88 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.58 (dd, *J* = 8.5, 7.0 Hz, 1H), 7.50 (d, *J* = 3.7 Hz, 1H), 7.45 - 7.35 (m, 2H), 7.30 (d, *J* = 3.7 Hz, 1H), 7.20 - 7.14 (m, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 7.03 (d, *J* = 6.7 Hz, 1H), 6.90 (d, *J* = 8.9 Hz, 1H), 6.60 (t, *J* = 5.8 Hz, 1H), 6.46 (s, 1H), 5.12 (dd, *J* = 13.1, 5.5 Hz, 1H), 4.75 (d, *J* = 17.4 Hz, 1H), 4.16 (d, *J* = 17.4 Hz, 1H), 3.63 (t, *J* = 5.5 Hz, 2H), 3.59 - 3.44 (m, 12H), 3.32 (br s, 4H), 3.01 (s, 3H), 2.99 - 2.88 (m, 1H), 2.79 - 2.71 (m, 1H), 2.65 - 2.47 (m, 7H), 2.08 - 1.99 (m, 1H); LC/MS (ESI) $C_{50}H_{52}F_2N_9O_9S$ $[M+H]^+$ calcd. *m/z* 992.36; found 992.51.

7. Synthesis of control-JBJ-07-038 [2-(5-fluoro-2-hydroxyphenyl)-2-(6-(4-(4-(2-(2-(2-((2-(1-methyl-2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)phenyl)-1-oxoisoindolin-2-yl)-*N*-(thiazol-2-yl)acetamide].



^{1}H NMR 500 MHz (DMSO-d_6) δ 12.61 (br s, 1H), 9.96 (br s, 1H), 7.86 (s, 1H), 7.84 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.62 - 7.55 (m, 3H), 7.49 (d, *J* = 3.7 Hz, 1H), 7.26 (d, *J* = 3.7 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 7.11 (td, *J* = 8.5, 3.1 Hz, 1H), 7.04 (d, *J* = 7.0 Hz, 1H), 7.00 (d, *J* = 9.2 Hz, 2H), 6.91 (dd, *J* = 9.0, 4.7 Hz, 1H), 6.86 (dd, *J* = 9.2, 3.1 Hz, 1H), 6.61 (t, *J* = 5.6 Hz, 1H), 6.33 (s, 1H), 5.12 (dd, *J* = 13.1, 5.5 Hz, 1H), 4.62 (d, *J* = 17.4 Hz, 1H), 4.01 (d, *J* = 17.7 Hz, 1H), 3.63 (t, *J* = 5.5 Hz, 2H), 3.59 - 3.44 (m, 12H), 3.21- 3.12 (m, 4H), 3.01 (s, 3H), 3.00 - 2.89 (m, 1H), 2.79 - 2.71 (m, 1H), 2.61 - 2.44 (m, 7H), 2.07 - 1.98 (m, 1H); LC/MS (ESI) $C_{51}H_{54}FN_8O_{10}S$ $[M+H]^+$ calcd. *m/z* 989.37; found 989.47.

SUPPORTING INFORMATION

Cell lines and drug compounds

BaF/3 cells were a generous gift from the laboratory of Dr. David Weinstock (in 2014). Wildtype EGFR, L858R/T790M and L858R/T790M/C797S mutant EGFR Ba/F3 cells were previously generated and characterized as described (Zhou et al, Nature 2009, Jia et al, Nature 2016). L858R/T790M/L718Q is generated using the QuikChange II XL Site Mutagenesis Kit from Agilent (Cat#200521) and cells were infected as previously described (Zhou et al, Nature 2009). H1975 cells were purchased from ATCC (Cat#CRL-5908). All cells were cultured in RPMI1640 media with 10% fetal bovine serum (FBS) and 1% penicilllin and streptomycin. Wildtype EGFR Ba/F3 cells were supplemented with 10 ng/ml of EGF purchased from Life Technologies (Cat#PHG0311L). All cell lines were tested negative for *Mycoplasma* using Mycoplasma Plus PCR Primer Set (Agilent) and were passaged and/or used for no longer than 4 weeks for all experiments. Pomalidomide, gefitinib, afatinib and osimertinib were purchased from Selleckchem (Houston, TX, Cat#S1567, S1025, S7810, S7297 respectively) and MLN4924 was purchased from Active Biochem (Hong Kong, Cat#A1139). MTS and Cell Titer Glo reagents were purchased from Promega (Cat#G111, G7570 respectively) and Sigma (Cat#P9625). Cetuximab was purchased from Eli Lilly and Company (Cat#66733-948-23).

Cell viability assays

Ba/F3 cells were treated with increasing concentrations of inhibitors for 72 hours and growth or the inhibition of growth were assessed by MTS assay or Cell Titer Glo Assays per manufacturer's protocol. For experiments that investigate the effect of compounds in wildtype EGFR Ba/F3 cells and/or in the presence of cetuximab, 10ng/ml EGF and/or 1ug/ml of cetuximab were added at the time of drug treatment. For combination studies with osimertinib and DDC-01-163, drugs were treated at the same time unless otherwise stated in the figure legend.

Antibodies and Western Blotting

Ba/F3 cells and H1975 cells were treated for the time and inhibitors indicated in the figure legends and were lysed and processed for Western Blotting analyses. Phospho-EGFR and total EGFR antibodies were purchased from Cell Signaling Technologies (Danvers, MA, Cat#3777 and 4267 respectively). Tubulin was purchased from Sigma Aldrich (T5168).

Densitometry Quantitation Analyses

Analyses were performed using ImageJ per the method outlined in ImageJ documentation for *Gel Analysis*. To assess protein degradation, total EGFR is normalized to its loading control (Tubulin) and then compared to DMSO control. To assess EGFR activity, phospho-EGFR is normalized to total EGFR and compared to DMSO control.

Kinase inhibition assay[2]

The preparation of purified EGFR kinase proteins (696-1022) with L858R/T790M mutation and the kinase inhibition assay using a HTRF(homogeneous time-resolved fluorescence) technique were carried out as described. Assays were performed under 100 μM ATP concentration. Compound IC_{50} values were determined by 12-point of inhibitor concentration (from 20 to 0.0000191 μM and control) in triplicate.

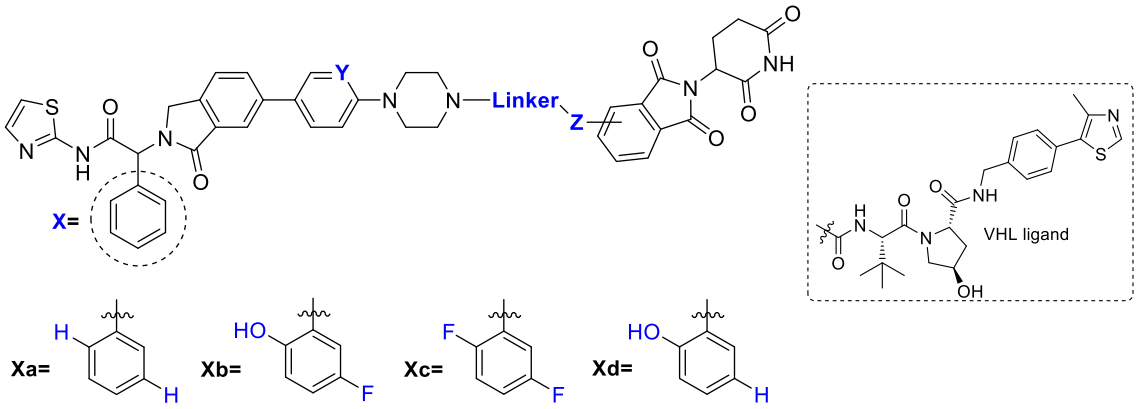
CRBN cellular engagement assay[3]

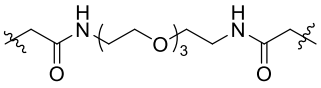
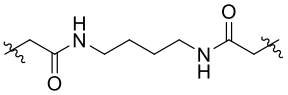
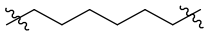
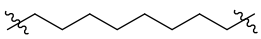
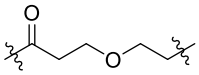
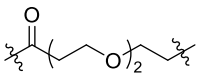
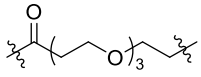
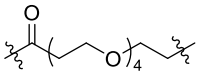
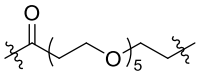
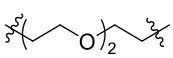
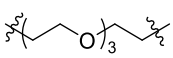
$BRD4_{BD2}$ cells were seeded at 30-50% confluency in either 96 well plates (3596, Costar) a day before compound treatment. Titrated compounds (see Figure legends) and 100 nM of dBET6 were incubated with cells for 5h following trypsinization and resuspension in DMEM media, transferred into 96-well plates (353910, Falcon) and analyzed by flow cytometer (guava easyCyte HT, Millipore). Signal from minimal 3000 events per well was acquired and the eGFP and mCherry florescence monitored. Data was analyzed using FlowJo (FlowJo, LCC). Forward and side scatter outliers, frequently associated with cell debris, were removed leaving >90% of total cells, followed by removal of eGFP and mCherry signal outliers, leaving 88-90% of total cells creating the set used for quantification. The eGFP protein abundance relative to mCherry was then quantified as a ten-fold amplified ratio for each individual cell using the formula: 10 × eGFP/mCherry. The median of the ratio was then calculated per set, normalized to the median of the DMSO ratio.

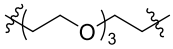
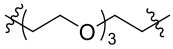
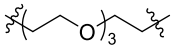
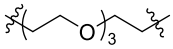
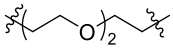
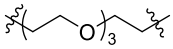
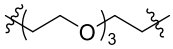
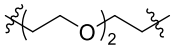
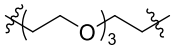
SUPPORTING INFORMATION

Supplementary table and figures

Table S1. The sturucture-acitivity relationship (SAR) of allosteric EGFR degraders assessed by anti-proliferative activities against L858R/T790M mutant EGFR Ba/F3 cells.



Compds.	X	Y	Linker	Z	Position	IC_{50} (μM)[a] (L858R/T790M)
1	Xa	C		O	*ortho*	>10
2	Xa	C		O	*ortho*	>10
3	Xa	C		NH	*ortho*	>10
4	Xa	C		NH	*ortho*	>10
5	Xa	C		NH	*ortho*	>10
6	Xa	C		NH	*ortho*	>10
7	Xa	C		NH	*ortho*	0.126
8	Xa	C		NH	*ortho*	>10
9	Xa	C		NH	*ortho*	>10
10	Xa	C		NH	*ortho*	>10
11	Xa	C		NH	*ortho*	0.161

12	Xa	N		NH	*ortho*	0.096
13	Xb	C		NH	*ortho*	0.477
14	Xc	N		NH	*ortho*	0.153
15	Xd	N		NH	*ortho*	>10
16	Xa	N		NH	*meta*	>10
17	Xa	N		NH	*meta*	>10
18	Xa	N		O	*ortho*	2.07
19[(b)]	Xa	N		None	None	>10
20[(b)]	Xa	N		None	None	>10

(a) Antiproliferative activity against L858R/T790M EGFR-Ba/F3 cells. IC_{50} values were obtained by single experiment with six replicates; (b) VHL-based PROTACs containing a VHL ligand (dotted box in the structures)

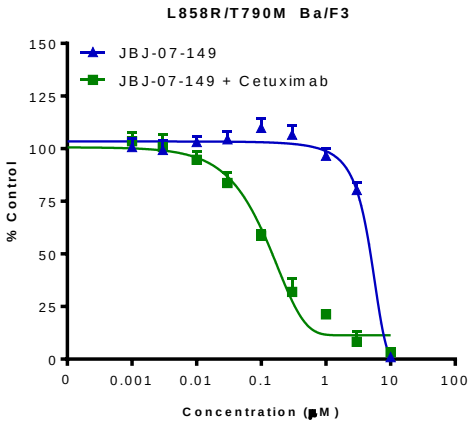


Figure S1. Anti-proliferative assay examining the growth inhibitory effect of dose escalated JBJ-07-149 in the presence or absence of 1 µg/ml of cetuximab in L858R/T790M mutant EGFR Ba/F3 cells.

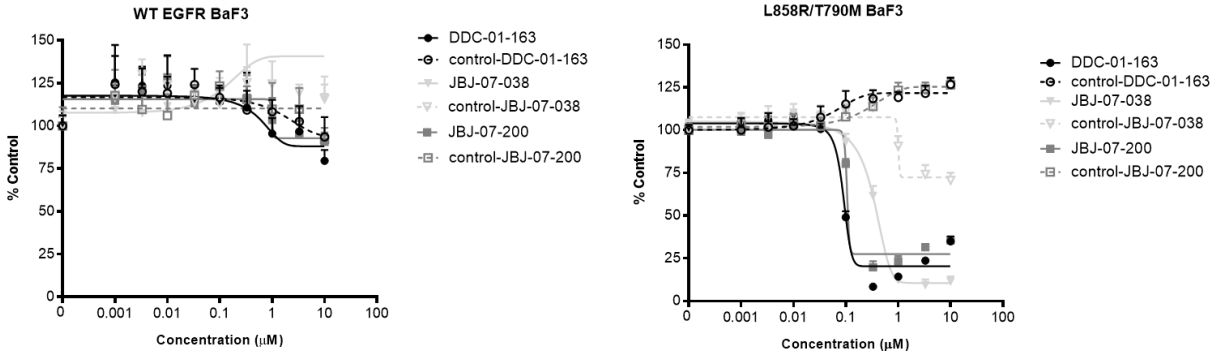


Figure S2. Anti-proliferative assays assessing the growth inhibitory effect of increasing concentrations of DDC-01-163, control-DDC-01-163, JBJ-07-038, control-JBJ-07-038, JBJ-07-200, control-JBJ-07-200 in wildtype EGFR and L858R/T790M mutant EGFR Ba/F3 cells.

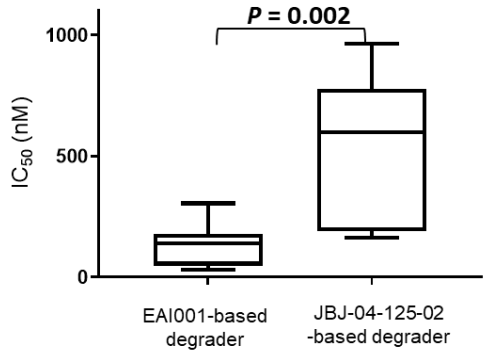


Figure S3. IC_{50} values of EAI001-based degraders and JBJ-04-125-02-based degraders in protection of CRBN-mediated degradation of $BRD4^{BD2}$ measured by cellular CRBN engagement assay. The graph was generated using GraphPad Prism.

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