Supplementary Information

Topical BET PROTACs for locally restricted protein degradation in the lung.

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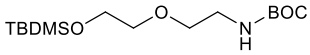
Contents

Synthesis of compounds 3-12 and 15-22

Compound Synthesis. All solvents and chemicals were used as purchased without further purification. Solvents for reactions were anhydrous (≤50 ppm of H2O) unless otherwise stated. Solvents for extraction and chromatographic purification were of HPLC grade. Compounds **1**, **dBET6, ZXH-3-26** are commercially available. The dihydrouracils **33** and **34** [1]and the 1H-benzo[d]imidazole-5-carbaldehyde **29** were synthesized according to the literature procedure. [2] All other reagents and intermediates were commercially available and used as arrived. Unless otherwise stated, operations discussed in the below examples were carried out at room/ambient temperature (18−25 °C). Oven-dried standard laboratory glassware was used, and routine manipulations were conducted at ambient temperature under a blanket of nitrogen. The reaction progress was monitored by LCMS.

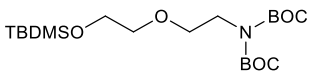
LCMS experiments were performed using either a Shimadzu LCMS-2010 or a Shimadzu LCMS-2020 system equipped with a photodiode array detector (190 to 400 nm) and electrospray ionization in positive ion detection mode (90.00 to 900.00 m/z), using either a Shim-pack XR-ODS column (2.2 µm, 3.0 × 50 mm) with a gradient elution (5−95%/2.0 min/flow rate 1.2 mL/min) of MeCN (+0.05% TFA) in water (+0.05% TFA) as mobile phase or a Kinetex EVO C18 column (2.6 µm, 2.1 × 50 mm) with a gradient elution (10− 95%/2.0 min/flow rate 1.0 mLmin−1) of MeCN in aqueous 46 mM ammonium carbonate/ammonia buffer (pH 10) as mobile phase. Evaporation was performed under reduced pressure using a rotary evaporator, and products were dried under reduced pressure at a suitable temperature. Test compounds were purified by reverse phase preparative HPLC, performed using either a Waters FractionLynx system with integrated MS detection or Gilson GX-281 with integrated UV detection (220/254 nm). All test compounds have purities >95%, as determined by reverse phase HPLC with a gradient MeCN/water in either acidic (TFA) or basic (ammonium carbonate/ammonia) conditions (for details for each compound, see Supporting Information). Purification conditions are described for each specific compound below. NMR spectra were recorded on Bruker Avance III spectrometers at a proton frequency of 300, 400, 500, or 600 MHz. The central peaks of chloroform δ (H 7.26 ppm), CD3OD (H 3.30 ppm), or DMSO-d6 (H 2.49 ppm) were used as internal references. HRMS experiments were run on a high-resolution (R = 9000 fwhm) LCMS system (Waters Acquity−Xevo Q-ToF) with an electrospray ionization(ESI) interface.

tert-butyl (2-(2-((tert-butyldimethylsilyl)oxy)ethoxy)ethyl)carbamate (40)



tert-butyl (2-(2-hydroxyethoxy)ethyl)carbamate (10.0 g, 48.7 mmol) was added to imidazole (8.29 g, 122 mmol) and TBSCl (8.81 g, 58.5 mmol) in DMF (100 mL) and the resulting solution was stirred at room temperature for 18 hours. The reaction mixture was concentrated, diluted with EtOAc (100 mL), and washed sequentially with water (3x100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The product was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.65 (t, *J* = 5.8 Hz, 1H), 3.67 (dd, *J* = 5.7, 4.6 Hz, 2H), 3.46 – 3.36 (m, 4H), 3.07 (q, *J* = 6.0 Hz, 2H), 1.37 (s, 9H), 0.86 (s, 9H). LCMS: m/z (ES+), TFA, found $[M+H]^+$ = 320.

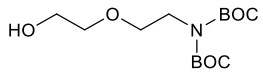
tert-butyl (tert-butoxycarbonyl)(2-(2-((tert-butyldimethylsilyl)oxy)ethoxy)ethyl)carbamate (41)



tert-butyl (2-(2-((tert-butyldimethylsilyl)oxy)ethoxy)ethyl)carbamate (**40**) (17.0 g, 53.2 mmol) was added to BOC_2O (23.3 g, 106 mmol) and DMAP (1.30 g, 10.6 mmol) in acetonitrile (200 mL). The

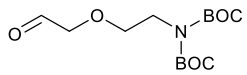
resulting solution was stirred at room temperature for 18 hours. The reaction mixture was concentrated, diluted with EtOAc (200 mL), and washed sequentially with water (2x200 mL), 0.1M HCl (150 mL), and brine (200 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The product tert-butyl (tert-butoxycarbonyl)(2-(2-((tert-butyldimethylsilyl)oxy)ethoxy)ethyl)-carbamate (20.3 g, 91%) was used in the next step without further purification. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.65 (q, *J* = 5.1 Hz, 4H), 3.54 – 3.48 (m, 2H), 3.43 (t, *J* = 5.2 Hz, 2H), 1.44 (s, 18H), 0.86 (s, 9H). LCMS: m/z (ES+), TFA, found $[M+H]^+$ = 420.

tert-butyl (tert-butoxycarbonyl)(2-(2-hydroxyethoxy)ethyl)carbamate (42)



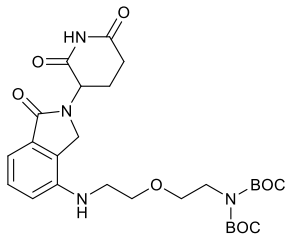
tert-butyl (tert-butoxycarbonyl)(2-(2-((tert-butyldimethylsilyl)oxy)-ethoxy)ethyl)carbamate (**41**) (18.0 g, 42.9 mmol) was added to TBAF (1 M in THF, 64 mL, 64.3 mmol) in THF (200 mL). The reaction was stirred at room temperature for 18 hours. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (200 mL) and washed sequentially with water (2x200 mL) and brine (2x200 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to afford crude product. The crude product was purified by silica column chromatography, elution gradient 0 to 20% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford tert-butyl (tert-butoxycarbonyl)(2-(2-hydroxyethoxy)ethyl)carbamate (11.5 g, 88%) as a colourless oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.49 (t, *J* = 5.3 Hz, 1H), 3.66 (t, *J* = 6.0 Hz, 2H), 3.49 (dt, *J* = 9.9, 5.3 Hz, 4H), 3.41 (dd, *J* = 6.7, 4.7 Hz, 2H), 1.45 (s, 18H). LCMS: m/z (ES+), TFA, found $[M+H]^+$ = 306.

tert-butyl (tert-butoxycarbonyl)(2-(2-oxoethoxy)ethyl)carbamate (43)



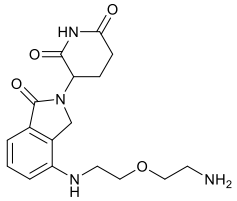
DMSO (4.3 mL, 60.6 mmol) in DCM (50 mL) was added dropwise over a period of 1 hour to a solution of oxalyl chloride (3.38 g, 26.7 mmol) in DCM (50 mL) at -78 °C under nitrogen atmosphere. The reaction mixture was stirred at the same temperature for 30 min and a solution of tert-butyl (tert-butoxycarbonyl)(2-(2-hydroxyethoxy)ethyl)carbamate (**42**) (3.70 g, 12.1 mmol) in DCM (50 mL) was added dropwise over 30 min. The mixture was stirred for 30 min at -78 °C. A solution of NEt_3 (15 mL, 109 mmol) in DCM (50 mL) was added dropwise over 30 min and the resulting solution was stirred for 6 hours at 78 °C. The reaction mixture was warmed to room temperature, concentrated and diluted with EtOAc (200 mL). The mixture was washed sequentially with water (2x200 mL) and brine (2x200 m-L). The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to afford tert-butyl (tert-butoxycarbonyl)(2-(2-oxoethoxy)ethyl)carbamate (3.30 g, 90%). The product was used in the next step without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (s, 1H), 4.15 (s, 1H), 3.65 - 3.70 (m, 2H), 3.55 - 3.59 (m, 2H), 1.43 (s, 18H).

tert-butyl (tert-butoxycarbonyl)(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)ethoxy)ethyl)carbamate (44)



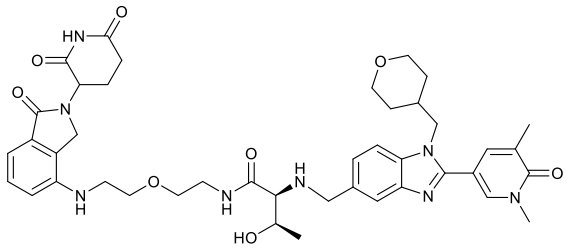
tert-butyl (tert-butoxycarbonyl)(2-(2-oxoethoxy)ethyl)carbamate (**43**) (2.34 g, 7.71 mmol) was added to 3-(4-amino-1-oxoisoindolin-2-yl)piperidine-2,6-dione (2.00 g, 7.71 mmol), AcOH (0.4 mL, 7.71 mmol) and sodium triacetoxyborohydride (3.27 g, 15.4 mmol) in DCE (22 mL). The resulting mixture was stirred at room temperature for 3 hours. The solvent was removed under reduced pressure and the crude product was purified by silica column chromatography, elution gradient 0 to 60% EtOAc in petroleum ether. Pure fractions were evaporated to dryness to afford tert-butyl (R)-(tert-butoxycarbonyl)(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4 yl)amino)ethoxy) -ethyl)carbamate (2.30 g, 55%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.98 (s, 1H), 7.27 (t, *J* = 7.8 Hz, 1H), 6.95 (d, *J* = 7.3 Hz, 1H), 6.78 (d, *J* = 8.1 Hz, 1H), 5.11 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.23 (d, *J* = 17.1 Hz, 1H), 4.12 (d, *J* = 17.1 Hz, 1H), 3.44 - 3.68 (m, 8H), 3.35 - 3.41 (m, 1H), 3.27 (t, J = 5.9 Hz, 1H), 2.86 - 2.97 (m, 1H), 2.61 (br d, *J* = 16.8 Hz, 1H), 2.23 - 2.36 (m, 1H), 1.98 - 2.06 (m, 1H), 1.43 (s, 9H), 1.42 (s, 9H). *Note: NH signal not visible.* LCMS m/z (ES+), TFA, found $[M+H]^+$ = 547.

3-(4-((2-(2-aminoethoxy)ethyl)amino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (23a)



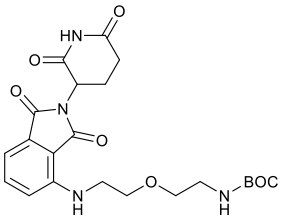
tert-butyl (tert-butoxycarbonyl)(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-ethoxy)ethyl)carbamate (**44**) (1.32 g, 2.41 mmol) was added to HCl (4 M in 1,4-dioxane, 15 mL, 49.4 mmol) . The resulting solution was stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the product (R)-3-(4-((2-(2-aminoethoxy)ethyl)amino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione was used in the next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.99 (s, 1H), 7.36 (t, *J* = 7.3 Hz, 1H), 7.12 (d, *J* = 7.5 Hz, 1H), 7.04 (br d, *J* = 7.9 Hz, 1H), 6.57 - 6.71 (m, 1H), 5.09 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.46 (d, *J* = 17.4 Hz, 1H), 4.29 (d, *J* = 17.4 Hz, 1H), 3.62 - 3.66 (m, 4H), 3.56 - 3.61 (m, 3H), 3.42 - 3.52 (m, 4H), 3.38 (br t, *J* = 5.3 Hz, 2H), 2.89 - 2.96 (m, 4H), 2.61 (br d, *J* = 16.6 Hz, 1H), 2.30 (qd, *J* = 13.3, 4.5 Hz, 1H), 1.98 - 2.07 (m, 1H). LCMS m/z (ES+), TFA, found $[M+H]^+$ = 347.

(2S,3R)-2-(((2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)methyl)amino)-N-(2-(2-((2-((R)-2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)ethoxy)ethyl)-3-hydroxybutanamide (3)



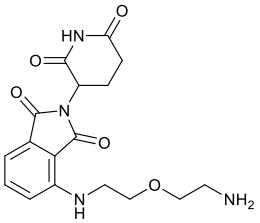
((2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)methyl)-L-threonine (**24**) (2.30 g, 4.91 mmol) was added to 3-(4-((2-(2-aminoethoxy)ethyl)amino)-1-oxoisoindolin-2-yl)piperidine-2,6-dione (**23a**) (1.70 g, 4.91 mmol), DIPEA (2.6 mL, 14.7 mmol) and HATU (2.43 g, 6.38 mmol) in DMF (20 mL). The resulting solution was stirred at room temperature for 2 hours. The crude product was purified by preparative HPLC Column: Xselect CSH C18 OBD Column 30*150mm 5μm, n; Mobile Phase A: Water(0.05%TFA), Mobile Phase B: MeOH--HPLC; Flow rate: 60 mL/min; Gradient: 18% B to 32% B in 8 min, 32% B. Fractions containing the desired compound were evaporated to dryness to afford (2S,3R)-2-(((2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)methyl)amino)-N-(2-(2-((2-((R)-2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)ethoxy)ethyl)-3-hydroxybutanamide (**3**) (0.43 g, 11%) as a white solid. Purity 94%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.02 (br s, 1 H), 8.05 - 8.14 (m, 1 H), 7.95 - 8.03 (m, 1 H), 7.72 (br d, *J*=0.7 Hz, 1 H), 7.59 (s, 1 H), 7.54 (dd, *J*=17.9, 8.3 Hz, 1 H), 7.20 - 7.28 (m, 2 H), 6.95 (d, *J*=7.3 Hz, 1 H), 6.77 (d, *J*=8.1 Hz, 1 H), 5.58 (q, *J*=5.7 Hz, 1 H), 5.06 - 5.13 (m, 1 H), 4.71 - 4.82 (m, 1 H), 4.07 - 4.28 (m, 4 H), 3.76 - 3.88 (m, 1 H), 3.58 - 3.74 (m, 6 H), 3.56 (s, 3 H), 3.46 - 3.52 (m, 2 H), 3.29 - 3.38 (m, 3 H), 3.21 - 3.28 (m, 1 H), 3.06 - 3.12 (m, 2 H), 2.86 - 2.95 (m, 2 H), 2.59 - 2.63 (m, 1 H), 2.49 - 2.52 (m, 1 H), 2.19 - 2.30 (m, 1 H), 2.08 (s, 3 H), 1.88 - 2.03 (m, 2 H), 1.03 - 1.24 (m, 7 H). ^{13}C NMR (151 MHz, DMSO) δ 172.9, 171.3, 168.8, 165.7, 161.8, 150.5, 143.5, 139.6, 136.1, 135.3, 132.1, 129.2, 128.2, 126.5, 126.2, 125.8, 119.7, 112.0, 111.9, 110.4, 105.5, 105.4, 68.7, 68.6, 66.3, 65.5, 64.6, 59.3, 51.5, 49.7, 49.2, 45.6, 42.5, 40.1, 38.7, 37.7, 35.0, 31.2, 29.7, 29.7, 22.8, 20.0, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 797.3986, found 797.3989.

tert-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-carbamate (45)



The reaction was carried out in 2 batches of the same size which were unified for workup and purification. 2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline-1,3-dione (811 mg, 2.94 mmol) was added to tert-butyl (2-(2-aminoethoxy)ethyl)carbamate (600 mg, 2.94 mmol), and DIPEA (1.5 mL, 8.81 mmol) in NMP (2 mL). The resulting solution was stirred at 90 °C for 6 hours. The solvent was removed under reduced pressure and the crude product was purified by C18 column chromatography, elution gradient 0 to 100% MeCN in water. Pure fractions were evaporated to dryness to afford tert-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)carbamate (1.70 g, 63%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.08 (s, 1 H), 7.57 (dd, *J*=8.5, 7.2 Hz, 1 H), 7.14 (d, *J*=8.6 Hz, 1 H), 7.03 (d, *J*=7.0 Hz, 1 H), 6.69 - 6.79 (m, 1 H), 6.59 (t, *J*=5.8 Hz, 1 H), 5.04 (dd, *J*=12.6, 5.4 Hz, 1 H), 3.55 - 3.70 (m, 3 H), 3.37 - 3.48 (m, 4 H), 2.97 - 3.16 (m, 3 H), 2.79 - 2.93 (m, 1 H), 2.55 - 2.63 (m, 1 H), 1.35 (s, 9 H). LCMS: m/z (ES+), found $[M+H]^+$ = 461.

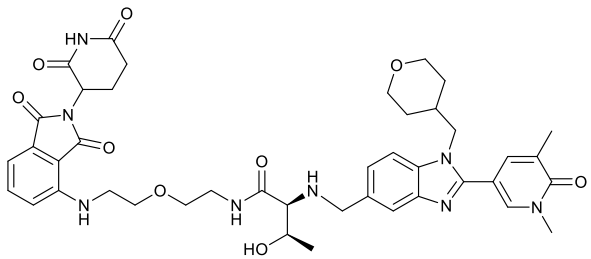
4-((2-(2-aminoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (23b)



tert-butyl (2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-carbamate (**45**) (0.55 g, 1.20 mmol) was dissolved in DCM (24 mL), 2,2,2-trifluoroacetic acid (1.9 mL, 24.0 mmol) was added at 0 °C, and the solution was stirred at room temperature for 2 hours. The solution was concentrated, and the crude material was purified by preparative HPLC on a Kromasil C8 column (10 µm 250x51 ID mm) using a gradient of 5-50% acetonitrile in H_2O/MeCN/TFA 95/5/0.2 buffer, over 20 min with a flow of 100 mL/min. Fractions containing the desired product were collected and evaporated to give 4-((2-(2-aminoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione as the TFA adduct (0.49 g, 86%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1H), 7.83 (s, 3H), 7.60 (dd, *J* = 8.6, 7.1 Hz, 1H), 7.17 (d, *J* = 8.6 Hz, 1H), 7.06 (d, *J* = 7.0 Hz, 1H), 6.64 (t, *J* = 5.9 Hz, 1H), 5.06 (dd, *J* = 12.8, 5.4 Hz, 1H), 3.58 – 3.71 (m, 4H), 3.51 (q, *J* = 5.5 Hz, 2H), 2.99 (h, *J* = 5.5

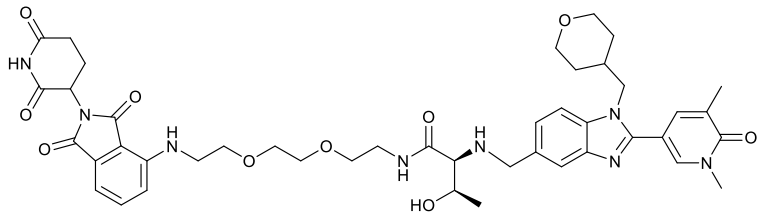
Hz, 2H), 2.89 (ddd, *J* = 17.0, 13.9, 5.5 Hz, 1H), 2.55 – 2.63 (m, 1H), 2.51 – 2.54 (m, 1H), 2.02 (dtd, *J* = 13.0, 5.4, 2.3 Hz, 1H).

(2S,3R)-2-(((2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)methyl)amino)-N-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethyl)-3-hydroxybutanamide (4)



Prepared following the same procedure as PROTAC **3** using 4-((2-(2-aminoethoxy)ethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**23b**). Purity 92%. ^{1}H NMR (300 MHz, METHANOL-d_4) δ 8.15 - 8.18 (m, 1 H), 7.90 (s, 1 H), 7.82 (d, *J*=8.3 Hz, 1 H), 7.69 - 7.73 (m, 1 H), 7.41 - 7.65 (m, 2 H), 6.94 - 7.07 (m, 2 H), 4.95 - 5.02 (m, 1 H), 4.26 - 4.46 (m, 4 H), 3.93 - 4.10 (m, 1 H), 3.74 - 3.90 (m, 5 H), 3.63 - 3.74 (m, 7 H), 3.48 - 3.54 (m, 1 H), 3.35 - 3.44 (m, 1 H), 3.18 - 3.31 (m, 2 H), 2.58 - 2.96 (m, 3 H), 2.25 (s, 3 H), 1.98 - 2.18 (m, 2 H), 1.29 - 1.39 (m, 3 H), 1.21 - 1.29 (m, 4 H). *Note: Exchanging protons not visible.* ^{13}C NMR (151 MHz, DMSO) δ 172.8, 170.1, 169.0, 167.2, 165.7, 161.8, 150.5, 146.3, 139.5, 136.2, 136.1, 135.3, 132.0, 128.2, 126.1, 125.8, 119.8, 117.3, 112.0, 110.7, 109.2, 105.5, 68.7, 68.6, 66.3, 65.5, 64.6, 64.6, 49.7, 49.2, 48.6, 41.7, 40.1, 38.7, 37.7, 35.0, 31.0, 29.7, 29.7, 22.2, 20.0, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 811.3779, found 811.3781.

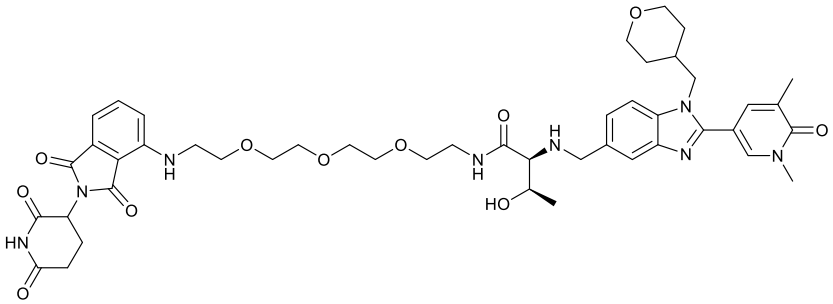
(2S,3R)-2-(((2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)methyl)amino)-N-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoiso-indolin-4-yl)amino)ethoxy)ethoxy)ethyl)-3-hydroxybutanamide (5)



Prepared following the same synthetic route as PROTAC **4**. Intermediate **23c** was prepared in analogy to **23b** using tert-butyl (2-(2-(2-hydroxyethoxy)ethoxy)-ethyl)carbamate. Purity 93%. ^{1}H NMR (300 MHz, METHANOL-d_4) δ 8.15 (d, *J*=2.2 Hz, 1 H), 7.88 - 7.92 (m, 1 H), 7.84 (d, *J*=8.4 Hz, 1 H), 7.71 - 7.79 (m, 1 H), 7.46 - 7.62 (m, 2 H), 6.97 - 7.08 (m, 2 H), 4.97 - 5.15 (m, 1 H), 4.90 - 4.95 (m, 1 H), 4.24 - 4.51 (m, 4 H), 3.91 - 4.08 (m, 1 H), 3.78 - 3.90 (m, 2 H), 3.53 - 3.74 (m, 12 H), 3.37 - 3.49 (m, 4 H), 3.19 -

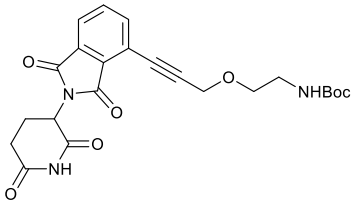
3.31 (m, 2 H), 2.64 - 2.94 (m, 3 H), 2.25 (s, 3 H), 2.08 - 2.18 (m, 2 H), 2.05 (s, 1 H), 1.20 - 1.43 (m, 8 H). *Note: Exchanging protons not visible.* HRMS m/z (ESI) calc. $[M+H]^+$ = 855.4041, found 855.4076.

(2S,3R)-2-(((2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)methyl)amino)-N-(2-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethoxy)ethoxy)ethoxy)ethyl)-3-hydroxybutanamide (6)



Prepared following the same synthetic route as PROTAC **4**. Intermediate **23d** was prepared in analogy to **23b** using tert-butyl (2-(2-(2-(2-hydroxyethoxy)ethoxy)-ethoxy)ethyl)carbamate. Purity 94%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.11 (s, 1 H), 9.35 - 9.46 (m, 1 H), 8.89 - 9.00 (m, 1 H), 8.63 - 8.69 (m, 1 H), 8.18 (d, *J*=2.2 Hz, 1 H), 7.77 - 7.82 (m, 2 H), 7.72 - 7.77 (m, 1 H), 7.58 (dd, *J*=8.5, 7.2 Hz, 1 H), 7.43 (d, *J*=8.2 Hz, 1 H), 7.13 (d, *J*=8.6 Hz, 1 H), 7.05 (d, *J*=7.1 Hz, 1 H), 6.56 - 6.62 (m, 1 H), 5.70 (br s, 1 H), 5.06 (dd, *J*=12.9, 5.4 Hz, 1 H), 4.33 (br d, *J*=7.2 Hz, 2 H), 4.18 - 4.29 (m, 1 H), 4.04 - 4.17 (m, 1 H), 3.84 - 3.91 (m, 1 H), 3.73 - 3.75 (m, 1 H), 3.58 - 3.60 (m, 2 H), 3.57 (s, 3 H), 3.53 - 3.55 (m, 1 H), 3.39 - 3.52 (m, 8 H), 3.24 - 3.35 (m, 2 H), 3.03 - 3.17 (m, 2 H), 2.89 (ddd, *J*=17.2, 13.9, 5.4 Hz, 1 H), 2.53 - 2.63 (m, 1 H), 2.45 - 2.49 (m, 1 H), 2.12 (s, 3 H), 1.91 - 2.10 (m, 2 H), 1.06 - 1.24 (m, 7 H). ^{13}C NMR (151 MHz, DMSO) δ 172.8, 170.1, 169.0, 167.3, 163.1, 161.8, 150.8, 146.4, 142.4, 138.3, 136.7, 136.2, 135.7, 132.1, 127.9, 123.6, 119.4, 119.4, 117.4, 110.9, 110.7, 109.2, 108.3, 69.8, 69.8, 69.8, 69.6, 68.9, 68.9, 66.7, 66.3, 51.0, 49.4, 48.6, 41.7, 40.1, 38.4, 37.5, 35.1, 31.0, 29.9, 22.2, 20.1, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 899.4303, found 899.4319.

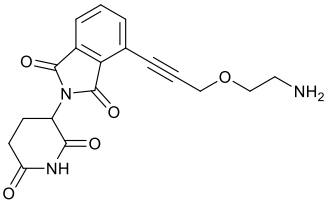
tert-butyl (2-((3-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)-ethyl)carbamate (28)



Cuprous iodide (85 mg, 0.44 mmol) was added to $PdCl_2(PPh_3)_2$ (208 mg, 0.30 mmol), 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (1.00 g, 2.97 mmol) and tert-butyl (2-(prop-2-yn-1-yloxy)ethyl)carbamate (887 mg, 4.45 mmol) and TEA (2.5 mL,17.8 mmol) in DMF (1 mL) at room

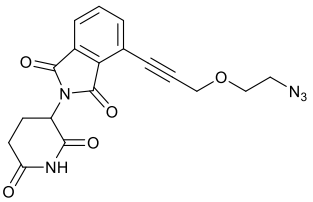
temperature. The resulting mixture was stirred at 80 °C for 2 hours. The solvent was removed under reduced pressure and the crude product was used in the next step without purification.

4-(3-(2-aminoethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (46)



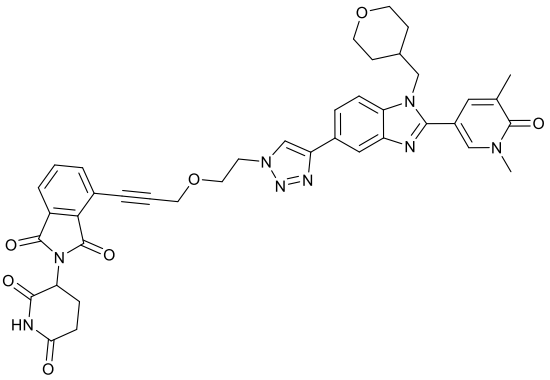
HCl (4 M in dioxane, 35 mL, 87.8 mmol) was added to tert-butyl (2-((3-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)prop-2-yn-1-yl)oxy)ethyl)carbamate (**28**) (2.00 g, 4.39 mmol) at room temperature. The resulting mixture was stirred for 1 hour. The solvent was removed under reduced pressure and the crude product was purified by C18 column chromatography, elution gradient 50 to 60% MeCN in water. Pure fractions were evaporated to dryness to afford 4-(3-(2-aminoethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (1.50 g, 96%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.14 (s, 1 H), 8.07 (br s, 2 H), 7.85 - 7.95 (m, 3 H), 5.14 (dd, *J*=12.7, 5.4 Hz, 1 H), 4.56 (s, 2 H), 3.80 (t, *J*=5.3 Hz, 2 H), 3.04 (br d, *J*=5.3 Hz, 2 H), 2.83 - 2.94 (m, 1 H), 2.52 - 2.65 (m, 2 H), 2.02 - 2.09 (m, 1 H). LCMS m/z (ES+), TFA, found $[M+H]^+$ = 356.

4-(3-(2-azidoethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (27a)



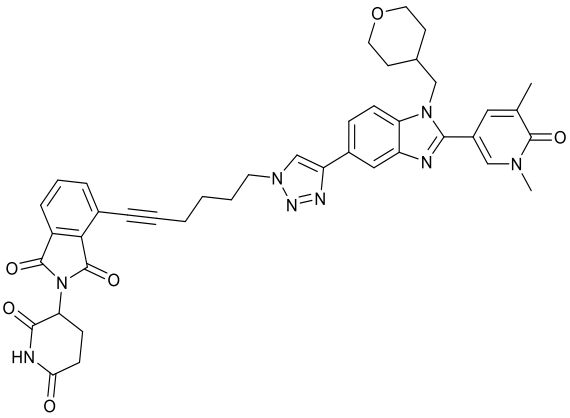
$CuSO_4$ (2.25 mg, 0.01 mmol) was added to potassium carbonate (38.9 mg, 0.28 mmol), 4-(3-(2-aminoethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**46**) (100 mg, 0.28 mmol) and sulfurazidic fluoride (70.4 mg, 0.56 mmol) in MeOH (3 mL) at room temperature. The resulting mixture was stirred for 2 hours. The solvent was evaporated and the product was used in the next step without purification.

4-(3-(2-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)ethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (7)



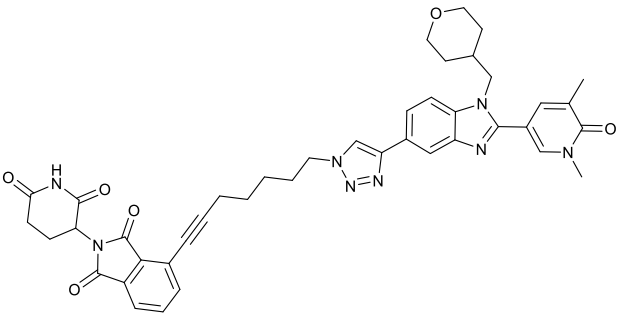
L-Ascorbic acid, sodium salt (104 mg, 0.52 mmol) was added to $CuSO_4$ (41.9 mg, 0.26 mmol), 4-(3-(2-azidoethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**27a**) (100 mg, 0.26 mmol) and 5-(5-ethynyl-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-2-yl)-1,3-dimethylpyridin-2(1H)-one (**30**) (123 mg, 0.34 mmol) in water/dioxane (3 mL) at room temperature. The resulting mixture was stirred for 1 hour. The solvents were removed under reduced pressure and the crude product was purified by C18 column chromatography, elution gradient 10 to 20% MeCN in water. Pure fractions were evaporated to dryness to afford 4-(3-(2-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)ethoxy)prop-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (**7**) (39.9 mg, 21%) as a white solid. Purity 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1 H), 8.58 (s, 1 H), 8.16 (d, *J*=2.3 Hz, 1 H), 8.02 - 8.04 (m, 1 H), 7.80 - 7.91 (m, 3 H), 7.71 - 7.78 (m, 3 H), 5.15 (dd, *J*=12.9, 5.2 Hz, 1 H), 4.66 - 4.71 (m, 2 H), 4.56 (s, 2 H), 4.30 (br d, *J*=7.1 Hz, 2 H), 4.08 - 4.15 (m, 2 H), 3.71 - 3.77 (m, 2 H), 3.58 (s, 3 H), 3.09 - 3.16 (m, 2 H), 2.83 - 2.94 (m, 1 H), 2.53 - 2.62 (m, 2 H), 2.13 (s, 3 H), 2.02 - 2.09 (m, 1 H), 1.94 - 2.02 (m, 1 H), 1.13 - 1.28 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 172.8, 169.8, 166.2, 165.7, 161.8, 151.2, 147.0, 142.7, 138.5, 138.2, 136.7, 135.9, 134.8, 132.0, 130.4, 127.9, 125.1, 123.4, 121.2, 120.1, 118.3, 115.2, 111.6, 108.2, 104.5, 92.9, 81.3, 67.7, 66.3, 58.0, 49.5, 49.5, 49.0, 40.1, 37.5, 35.1, 30.9, 29.9, 21.9, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 743.2941, found 743.2978.

4-(6-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)hex-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (8)



Prepared following the same synthetic route as PROTAC **13** using 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione and hex-5-yn-1-ol. Purity 92%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1 H), 8.64 (s, 1 H), 8.16 - 8.18 (m, 1 H), 8.06 (s, 1 H), 7.84 - 7.89 (m, 3 H), 7.75 - 7.80 (m, 3 H), 5.16 (dd, *J*=12.8, 5.4 Hz, 1 H), 4.49 (t, *J*=6.8 Hz, 2 H), 4.30 (br d, *J*=7.3 Hz, 2 H), 3.69 - 3.75 (m, 2 H), 3.57 (s, 3 H), 3.08 - 3.16 (m, 2 H), 2.84 - 2.94 (m, 1 H), 2.51 - 2.64 (m, 5 H), 2.12 (s, 3 H), 2.01 - 2.11 (m, 3 H), 1.57 - 1.65 (m, 2 H), 1.15 - 1.26 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 172.7, 169.8, 166.5, 166.5, 161.8, 151.2, 147.1, 142.7, 138.5, 137.5, 136.7, 135.9, 131.8, 129.9, 129.5, 127.9, 125.7, 125.1, 123.7, 120.8, 120.1, 115.2, 111.6, 108.2, 95.5, 79.8, 66.3, 49.4, 49.1, 49.1, 40.1, 38.4, 37.5, 35.1, 30.9, 29.9, 28.9, 24.9, 21.9, 18.3, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 741.3149, found 741.3159.

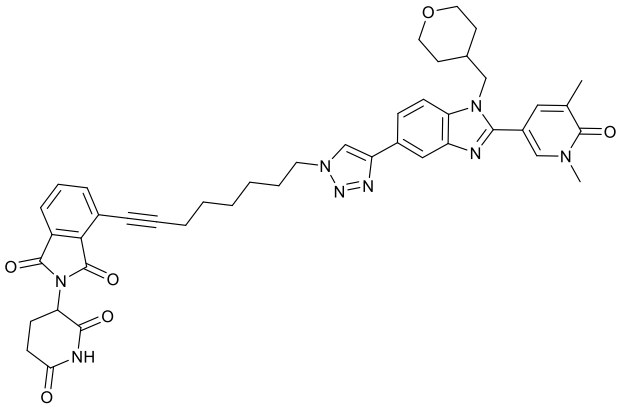
4-(7-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)hept-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (9)



Prepared following the same synthetic route as PROTAC **13** using 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione and hept-6-yn-1-ol. Purity 94%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 1 H), 8.62 (s, 1 H), 8.15 - 8.18 (m, 1 H), 8.06 (s, 1 H), 7.82 (s, 1 H), 7.71 - 7.79 (m, 5 H), 5.16 (dd, *J*=12.9,

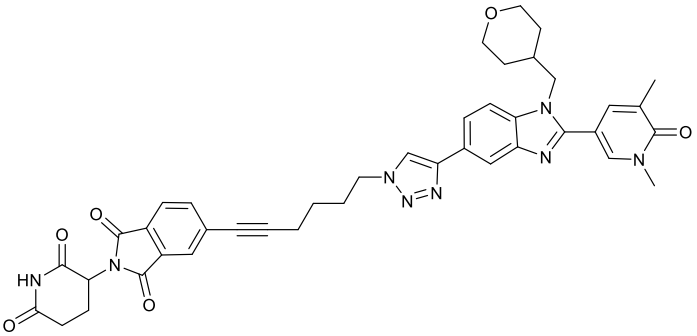
5.3 Hz, 1 H), 4.46 (t, *J*=6.6 Hz, 2 H), 4.29 (br d, *J*=7.2 Hz, 2 H), 3.69 - 3.75 (m, 2 H), 3.58 (s, 3 H), 3.08 - 3.16 (m, 2 H), 2.84 - 2.94 (m, 1 H), 2.53 - 2.64 (m, 2 H), 2.12 (s, 3 H), 2.04 - 2.10 (m, 1 H), 1.92 - 2.00 (m, 3 H), 1.59 - 1.68 (m, 2 H), 1.42 - 1.51 (m, 2 H), 1.12 - 1.27 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 172.7, 169.8, 166.5, 166.4, 161.8, 151.2, 147.1, 142.7, 138.5, 137.3, 136.7, 135.9, 131.7, 129.7, 129.6, 127.8, 125.5, 125.1, 123.5, 120.8, 120.0, 115.2, 111.6, 108.2, 95.8, 79.7, 66.3, 49.4, 49.3, 49.1, 40.1, 37.5, 35.1, 30.9, 29.9, 29.1, 27.1, 25.0, 21.9, 18.6, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 755.3306, found 755.3307.

4-(8-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)oct-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (10)



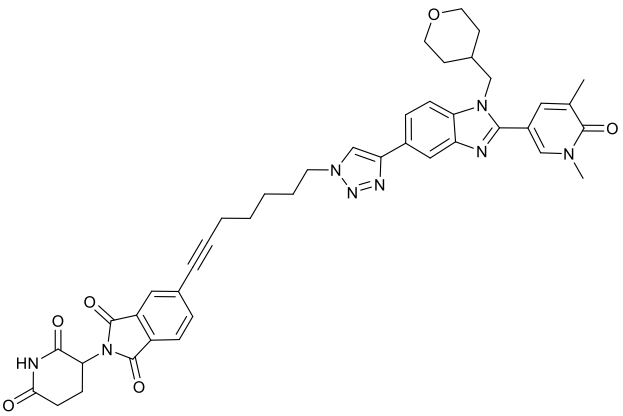
Prepared following the same synthetic route as PROTAC **13** using 4-bromo-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione. Purity 95%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.14 (s, 1 H), 8.60 (s, 1 H), 8.18 (d, *J*=2.2 Hz, 1 H), 8.07 (s, 1 H), 7.76 - 7.87 (m, 6 H), 5.15 (dd, *J*=12.9, 5.4 Hz, 1 H), 4.43 (t, *J*=7.1 Hz, 2 H), 4.31 (d, *J*=7.3 Hz, 2 H), 3.68 - 3.79 (m, 2 H), 3.58 (s, 3 H), 3.06 - 3.19 (m, 2 H), 2.89 (ddd, *J*=17.2, 13.8, 5.4 Hz, 1 H), 2.52 - 2.62 (m, 4 H), 2.12 (s, 3 H), 1.90 - 2.08 (m, 4 H), 1.52 - 1.64 (m, 4 H), 1.31 - 1.42 (m, 2 H), 1.14 - 1.26 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 172.8, 169.9, 166.3, 165.8, 163.0, 161.8, 151.1, 147.0, 142.3, 138.6, 138.2, 136.7, 135.7, 134.6, 132.0, 130.2, 127.9, 125.3, 122.6, 120.8, 120.2, 120.0, 114.9, 111.7, 107.8, 98.8, 76.2, 66.3, 49.5, 49.5, 48.9, 40.1, 37.5, 35.1, 30.9, 29.9, 29.5, 27.6, 27.5, 25.4, 21.9, 18.9, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 769.3462, found 769.3533.

5-(6-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)hex-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (11)



Prepared following the same synthetic route as PROTAC **13** using hex-5-yn-1-ol. Purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1 H), 8.64 (s, 1 H), 8.16 - 8.18 (m, 1 H), 8.06 (s, 1 H), 7.84 - 7.89 (m, 3 H), 7.75 - 7.80 (m, 3 H), 5.16 (dd, *J*=12.8, 5.4 Hz, 1 H), 4.49 (t, *J*=6.8 Hz, 2 H), 4.30 (br d, *J*=7.3 Hz, 2 H), 3.69 - 3.75 (m, 2 H), 3.57 (s, 3 H), 3.08 - 3.16 (m, 2 H), 2.84 - 2.94 (m, 1 H), 2.52 - 2.64 (m, 4 H), 2.12 (s, 3 H), 2.03 - 2.11 (m, 3 H), 1.93 - 2.02 (m, 1 H), 1.57 - 1.65 (m, 2 H), 1.15 - 1.26 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 172.8, 169.8, 166.5, 166.5, 161.8, 151.2, 147.1, 142.7, 138.5, 137.5, 136.7, 135.9, 131.8, 129.9, 129.5, 127.9, 125.7, 125.1, 123.7, 120.8, 120.1, 115.2, 111.6, 108.2, 95.5, 79.8, 66.3, 49.5, 49.1, 49.1, 40.1, 37.5, 35.1, 30.9, 29.9, 28.9, 24.9, 21.9, 18.3, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 741.3149, found 741.3162.

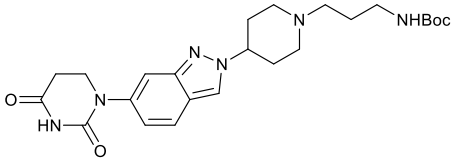
5-(7-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)hept-1-yn-1-yl)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (12)



Prepared following the same synthetic route as PROTAC **13** using hept-6-yn-1-ol. Purity 97%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.16 (s, 1 H), 8.62 (s, 1 H), 8.15 - 8.18 (m, 1 H), 8.06 (s, 1 H), 7.82 (s, 1 H), 7.71 - 7.79 (m, 5 H), 5.16 (dd, *J*=12.9, 5.3 Hz, 1 H), 4.46 (t, *J*=6.9 Hz, 2 H), 4.29 (br d, *J*=7.2 Hz, 2 H), 3.69 - 3.75 (m, 2 H), 3.58 (s, 3 H), 3.08 - 3.16 (m, 2 H), 2.84 - 2.94 (m, 1 H), 2.53 - 2.65 (m, 2 H), 2.12

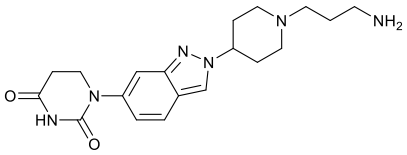
(s, 3 H), 2.04 - 2.10 (m, 1 H), 1.92 - 2.00 (m, 3 H), 1.59 - 1.68 (m, 2 H), 1.42 - 1.51 (m, 2 H), 1.12 - 1.27 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 172.8, 169.8, 166.5, 166.4, 161.8, 151.2, 147.1, 142.7, 138.5, 137.3, 136.7, 135.9, 131.7, 129.7, 129.6, 127.9, 125.5, 125.1, 123.5, 120.8, 120.1, 115.2, 111.6, 108.2, 95.8, 79.7, 66.3, 49.5, 49.3, 49.1, 40.1, 37.5, 35.1, 30.9, 29.9, 29.1, 27.1, 25.0, 21.9, 18.6, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 755.3306, found 755.3320.

tert-butyl (3-(4-(6-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-2H-indazol-2-yl)piperidin-1-yl)propyl)carbamate (47)



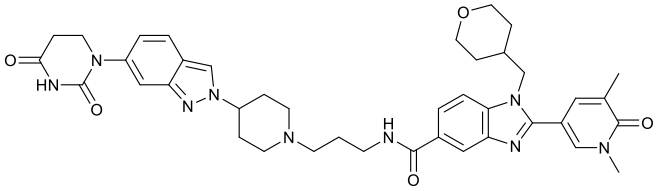
Sodium triacetoxyborohydride (812 mg, 3.83 mmol) was added to 1-(2-(piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**33**) (400 mg, 1.28 mmol) and tert-butyl (3-oxopropyl)carbamate (442 mg, 2.55 mmol) in DCM (10 mL) at room temperature under air. The resulting mixture was stirred for 3 hours. The solvent was removed under reduced pressure and the crude product was purified by silica column chromatography, elution gradient 0 to 20% MeOH in DCM. Pure fractions were evaporated to dryness to afford tert-butyl (3-(4-(6-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-2H-indazol-2-yl)piperidin-1-yl)propyl)carbamate (380 mg, 63%) as a yellow gum. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.38 (s, 1H), 8.44 (s, 1H), 7.67 (d, *J* = 8.9 Hz, 1H), 7.50 (s, 1H), 7.03 (d, *J* = 8.9 Hz, 1H), 6.87 (s, 1H), 4.52 (s, 1H), 3.83 (t, *J* = 6.7 Hz, 2H), 2.97 (d, *J* = 6.8 Hz, 3H), 2.74 (t, *J* = 6.7 Hz, 2H), 2.43 (d, *J* = 6.9 Hz, 2H), 2.14 (s, 4H), 1.91 (s, 3H), 1.61 (s, 2H), 1.39 (s, 9H). LCMS m/z (ES+), TFA, found $[M+H]^+$ = 471.

1-(2-(1-(3-aminopropyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (35)



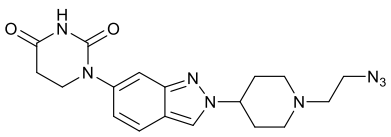
tert-butyl (3-(4-(6-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-2H-indazol-2-yl)piperidin-1-yl)propyl)carbamate (**50**) (360 mg, 0.77 mmol) was added to HCl in dioxane (4 M, 7.6 mL, 30.6 mmol) at RT under air. The resulting mixture was stirred for 2 hours and the solvent was removed under reduced pressure. The product was obtained as a yellow solid and used in the next step without purification. LCMS m/z (ES+), TFA, found $[M+H]^+$ = 371.

2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-N-(3-(4-(6-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-2H-indazol-2-yl)piperidin-1-yl)propyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxamide (15)



HATU (267 mg, 0.70 mmol) was added to DIPEA (0.4 mL, 2.43 mmol), 1-(2-(1-(3-aminopropyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**35**) (200 mg, 0.54 mmol) and 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylic acid (**38**) (206 mg, 0.54 mmol) in DMF (3 mL) at room temperature under nitrogen. The resulting mixture was stirred for 2 hours, and the solvent was removed under reduced pressure. The crude product was purified by preparative HPLC (Column: XSelect CSH Fluoro Phenyl, 30*150 mm, 5µm; Mobile Phase A: Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 11% B to 17% B in 7 min, 17% B. Fractions containing the desired compound were evaporated to dryness to afford 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-N-(3-(4-(6-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-2H-indazol-2-yl)piperidin-1-yl)propyl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxamide (**15**) (77 mg, 18.62 %) as a yellow solid. Purity 97%. ^{1}H NMR (400 MHz, METHANOL-d_4) δ 8.41 (s, 1 H) 8.33 - 8.35 (m, 1 H) 8.22 - 8.24 (m, 1 H) 8.08 (d, *J*=2.2 Hz, 1 H) 7.90 - 7.94 (m, 1 H) 7.71 - 7.80 (m, 3 H) 7.57 - 7.60 (m, 1 H) 7.12 (dd, *J*=8.9, 1.7 Hz, 1 H) 4.72 - 4.82 (m, 1 H) 4.34 (d, *J*=7.6 Hz, 2 H) 3.95 (t, *J*=6.8 Hz, 2 H) 3.80 - 3.88 (m, 2 H) 3.71 (s, 3 H) 3.59 (br d, *J*=6.0 Hz, 4 H) 3.21 - 3.29 (m, 2 H) 3.04 - 3.13 (m, 2 H) 2.93 - 3.04 (m, 2 H) 2.86 (t, *J*=6.8 Hz, 2 H) 2.38 - 2.51 (m, 4 H) 2.24 (s, 3 H) 2.04 - 2.14 (m, 3 H) 1.23 - 1.36 (m, 4 H). *Note: Exchanging protons not visible.* ^{13}C NMR (151 MHz, DMSO) δ 170.7, 166.6, 163.2, 161.8, 152.4, 151.9, 147.4, 141.9, 139.8, 138.6, 138.0, 136.7, 128.8, 127.9, 122.1, 121.8, 120.9, 120.5, 119.3, 118.0, 112.6, 110.8, 108.0, 66.3, 59.4, 55.2, 51.8, 49.5, 45.0, 40.1, 37.8, 37.5, 35.1, 31.8, 31.1, 29.8, 26.2, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 734.3778, found 734.3807.

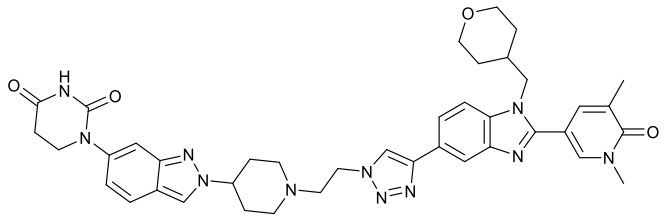
1-(2-(1-(2-azidoethyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (36a)



Diisopropylethylamine (1.4 mL, 7.98 mmol) was added to 2-azidoethyl 4-methylbenzenesulfonate (770 mg, 3.19 mmol) and 1-(2-(piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**33**) (500 mg, 1.60 mmol) in DMF (5 mL). The resulting mixture was stirred at 90 °C for 4 hours, then

cooled to room temperature and filtered through Celite. The solvent was removed under reduced pressure and the crude product was purified by C18 column chromatography, elution gradient 0 to 60% MeCN in water. Pure fractions were evaporated to dryness to afford 1-(2-(1-(2-azidoethyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (400 mg, 66%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.43 (d, *J* = 0.9 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 1H), 7.50 (d, *J* = 1.7 Hz, 1H), 7.02 (dd, *J* = 8.9, 1.8 Hz, 1H), 4.49 (p, *J* = 7.6 Hz, 1H), 3.83 (t, *J* = 6.6 Hz, 2H), 3.38 (t, *J* = 5.8 Hz, 2H), 3.06 (bd, *J* = 10.9 Hz, 1H), 2.73 (t, *J* = 6.7 Hz, 2H), 2.61 (t, *J* = 5.8 Hz, 1H), 2.15–2.29 (m, 2H), 2.10 (m, 3H), 1.06 (d, *J* = 6.5 Hz, 3H). LCMS: m/z (ES+), TFA, found $[M+H]^+$ = 383.

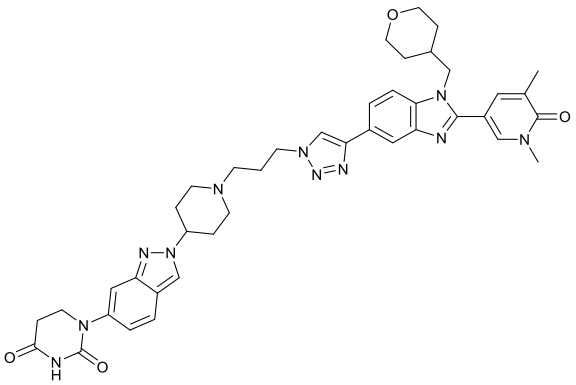
1-(2-(1-(2-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)ethyl)piperidin-4-yl)-2H-indazol-6-yl)dihydro-pyrimidine-2,4(1H,3H)-dione (16)



L-Ascorbic acid, sodium salt (121 mg, 0.61 mmol) was added to a mixture of $CuSO_4$ (48.6 mg, 0.30 mmol), 1-(2-(1-(2-azidoethyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**39a**) (151 mg, 0.40 mmol) and 5-(5-ethynyl-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-2-yl)-1,3-dimethylpyridin-2(1H)-one (**33**) (110 mg, 0.30 mmol) in water (2 mL) and 1,4-dioxane (2 mL) at room temperature under nitrogen. The resulting mixture was stirred for 2 hours. The solvents were removed under reduced pressure and the crude product was purified by preparative HPLC Column: XBridge Prep OBD C18 Column, 19*250 mm, 5µm; Mobile Phase A: Water(10 mmol/L NH4HCO3+0.1%NH3.H2O), Mobile Phase B: ACN; Flow rate: 25 mL/min; Gradient: 23% B to 33% B in 10 min. Fractions containing the desired compound were evaporated to dryness to afford 1-(2-(1-(2-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)ethyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**16**) (111 mg, 49%) as a white solid. Purity 98%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (s, 1 H), 8.61 (s, 1 H), 8.43 (s, 1 H), 8.16 - 8.19 (m, 1 H), 8.09 (s, 1 H), 7.75 - 7.83 (m, 3 H), 7.66 (d, *J*=8.9 Hz, 1 H), 7.50 (s, 1 H), 7.02 (dd, *J*=8.8, 1.5 Hz, 1 H), 4.55 - 4.63 (m, 2 H), 4.47 - 4.55 (m, 1 H), 4.31 (br d, *J*=7.2 Hz, 2 H), 3.82 (t, *J*=6.7 Hz, 2 H), 3.69 - 3.77 (m, 2 H), 3.58 (s, 3 H), 3.06 - 3.19 (m, 4 H), 2.86 - 3.01 (m, 2 H), 2.73 (t, *J*=6.6 Hz, 2 H), 2.23 - 2.41 (m, 2 H), 2.07 - 2.21 (m, 7 H), 1.91 - 2.06 (m, 1 H), 1.13 - 1.27 (m, 4 H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.1, 163.5, 162.3, 152.8, 151.7, 147.9, 147.4, 143.2, 140.2, 138.9, 137.2, 136.4, 128.3, 125.7, 122.5, 121.7, 121.3,

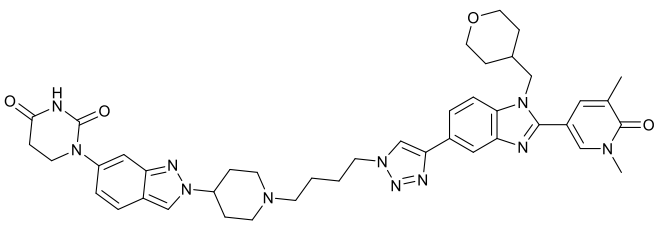
120.9, 120.6, 119.8, 115.7, 113.0, 112.1, 108.6, 66.8, 60.4, 57.2, 57.2, 52.3, 49.9, 47.7, 45.5, 37.9, 35.6, 32.8, 31.6, 30.4, 17.4. HRMS m/z (ESI) calc. $[M+H]^+$ = 744.3734, found 744.3771.

1-(2-(1-(3-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)propyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (17)



Prepared following the same synthetic route as PROTAC **16** using 1-(2-(piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**33**) and 3-azidopropyl 4-methylbenzenesulfonate. Purity 93%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (s, 1 H), 8.57 - 8.70 (m, 1 H), 8.37 - 8.42 (m, 1 H), 7.99 - 8.37 (m, 2 H), 7.74 - 7.92 (m, 2 H), 7.59 - 7.71 (m, 1 H), 7.44 - 7.56 (m, 1 H), 6.99 - 7.05 (m, 1 H), 4.41 - 4.57 (m, 3 H), 4.25 - 4.41 (m, 2 H), 3.83 (t, *J*=6.6 Hz, 3 H), 3.72 (br d, *J*=9.8 Hz, 2 H), 3.57 - 3.62 (m, 2 H), 3.06 - 3.17 (m, 3 H), 2.99 - 3.06 (m, 2 H), 2.74 (t, *J*=6.6 Hz, 2 H), 2.38 - 2.48 (m, 2 H), 2.05 - 2.19 (m, 10 H), 1.91 - 2.05 (m, 2 H), 1.10 - 1.30 (m, 4 H). ^{13}C NMR (126 MHz, DMSO) δ 170.7, 161.8, 160.9, 152.3, 147.4, 147.0, 139.9, 139.7, 138.2, 136.5, 127.9, 125.0, 122.0, 121.0, 120.8, 120.5, 120.4, 120.2, 119.4, 115.5, 112.6, 112.0, 66.3, 60.0, 59.6, 54.1, 51.9, 49.8, 47.8, 45.0, 43.7, 40.1, 37.6, 37.5, 35.0, 33.0, 32.4, 31.7, 31.1, 29.9, 27.2, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 758.3890, found 758.3907.

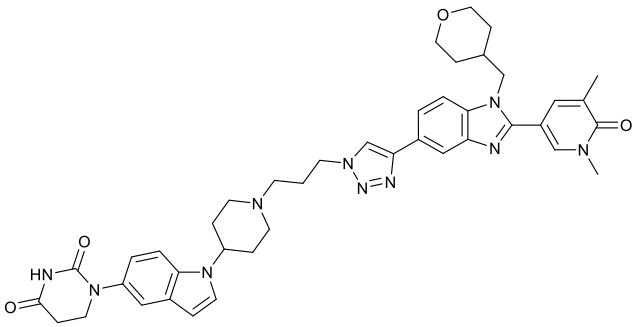
1-(2-(1-(4-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)butyl)piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (18)



Prepared following the same synthetic route as PROTAC **16** using 1-(2-(piperidin-4-yl)-2H-indazol-6-yl)dihydropyrimidine-2,4(1H,3H)-dione (**33**) and 4-azidobutyl 4-methylbenzenesulfonate. Purity 95%.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.36 (s, 1 H), 8.62 (s, 1 H), 8.41 (s, 1 H), 8.17 (s, 1 H), 8.06 - 8.10 (m, 1 H), 7.75 - 7.81 (m, 3 H), 7.66 (d, *J*=8.9 Hz, 1 H), 7.48 - 7.51 (m, 1 H), 7.02 (dd, *J*=8.8, 1.7 Hz, 1 H), 4.41 - 4.55 (m, 3 H), 4.30 (br d, *J*=7.3 Hz, 2 H), 3.83 (t, *J*=6.7 Hz, 2 H), 3.70 - 3.76 (m, 2 H), 3.57 (s, 3 H), 3.08 - 3.16 (m, 2 H), 2.96 - 3.05 (m, 2 H), 2.73 (t, *J*=6.7 Hz, 2 H), 2.40 - 2.45 (m, 2 H), 2.07 - 2.18 (m, 9 H), 1.88 - 2.03 (m, 3 H), 1.46 - 1.55 (m, 2 H), 1.13 - 1.26 (m, 4 H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 171.2, 163.7, 152.8, 151.7, 147.9, 147.6, 143.2, 140.2, 138.9, 137.2, 136.4, 128.3, 125.6, 122.5, 121.2, 120.9, 119.8, 115.7, 113.0, 112.1, 108.6, 66.8, 60.4, 57.1, 52.4, 49.9, 45.5, 40.6, 40.4, 40.2, 40.1, 37.9, 35.6, 32.7, 31.6, 30.4, 28.1, 23.8, 17.4. HRMS m/z (ESI) calc. $[M+H]^+$ = 772.4047, found 772.4072.

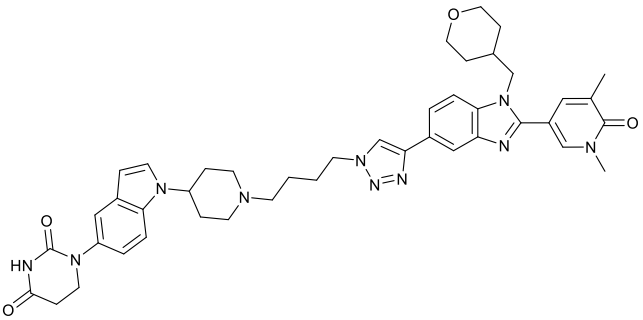
1-(1-(1-(3-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)propyl)piperidin-4-yl)-1H-indol-5-yl)dihydropyrimidine-2,4(1H,3H)-dione (19)



Prepared following the same synthetic route as PROTAC **16** using 1-(1-(piperidin-4-yl)-1H-indol-5-yl)dihydropyrimidine-2,4(1H,3H)-dione (**34**) and 3-azidopropyl 4-methylbenzenesulfonate.

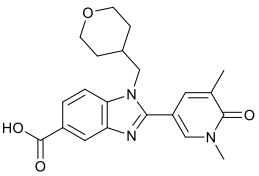
Purity 98%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.26 (s, 1 H) 8.64 (s, 1 H) 8.16 - 8.19 (m, 2 H) 8.08 - 8.09 (m, 1 H) 7.76 - 7.83 (m, 3 H) 7.44 - 7.56 (m, 3 H) 7.06 (dd, *J*=8.9, 2.00 Hz, 1 H) 6.42 (d, *J*=3.2 Hz, 1 H) 4.45 - 4.54 (m, 2 H) 4.28 - 4.40 (m, 3 H) 3.71 - 3.80 (m, 4 H) 3.08 - 3.18 (m, 2 H) 2.98 - 3.07 (m, 2 H) 2.72 (t, *J*=6.6 Hz, 2 H) 2.39 - 2.45 (m, 2 H) 2.06 - 2.24 (m, 8 H) 1.90 - 2.04 (m, 5 H) 1.13 - 1.29 (m, 5 H). ^{13}C NMR (151 MHz, DMSO) δ 170.8, 163.4, 161.8, 152.6, 151.2, 147.0, 142.8, 138.4, 136.7, 135.9, 134.2, 133.7, 127.9, 127.9, 126.0, 125.2, 121.0, 120.1, 119.7, 117.8, 115.2, 111.6, 109.9, 108.2, 101.1, 66.3, 54.4, 52.8, 52.4, 49.5, 47.9, 45.6, 40.1, 37.5, 35.1, 32.0, 31.3, 29.9, 27.2, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 757.3938, found 757.3950.

1-(1-(1-(4-(4-(2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazol-5-yl)-1H-1,2,3-triazol-1-yl)butyl)piperidin-4-yl)-1H-indol-5-yl)dihydro-pyrimidine-2,4(1H,3H)-dione (20)



Prepared following the same synthetic route as PROTAC **16** using 1-(1-(piperidin-4-yl)-1H-indol-5-yl)dihydropyrimidine-2,4(1H,3H)-dione (**34**) and 4-azidobutyl 4-methylbenzenesulfonate. Purity 98%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.27 (s, 1 H) 8.63 (s, 1 H) 8.17 (d, *J*=2.5 Hz, 1 H) 8.06 - 8.08 (m, 1 H) 7.75 - 7.81 (m, 3 H) 7.50 - 7.56 (m, 2 H) 7.46 (d, *J*=2.0 Hz, 1 H) 7.07 (dd, *J*=8.8, 2.0 Hz, 1 H) 6.46 (d, *J*=2.9 Hz, 1 H) 4.35 - 4.54 (m, 3 H) 4.30 (br d, *J*=7.1 Hz, 2 H) 3.66 - 3.84 (m, 5 H) 3.52 - 3.63 (m, 4 H) 3.02 - 3.21 (m, 4 H) 2.72 (t, *J*=6.7 Hz, 2 H) 2.11 (s, 3 H) 1.87 - 2.09 (m, 8 H) 1.48 - 1.62 (m, 2 H) 1.10 - 1.31 (m, 5 H). ^{13}C NMR (151 MHz, DMSO) δ 170.8, 163.1, 161.8, 152.6, 151.2, 147.1, 142.8, 138.4, 136.7, 135.9, 134.3, 133.7, 127.9, 127.9, 126.0, 125.1, 120.8, 120.1, 119.8, 117.8, 115.2, 111.6, 109.9, 108.2, 101.2, 66.3, 56.3, 52.1, 49.5, 49.3, 45.6, 40.1, 37.5, 35.1, 31.3, 31.1, 29.9, 27.5, 22.9, 16.9. HRMS m/z (ESI) calc. $[M+H]^+$ = 771.4095, found 771.4098.

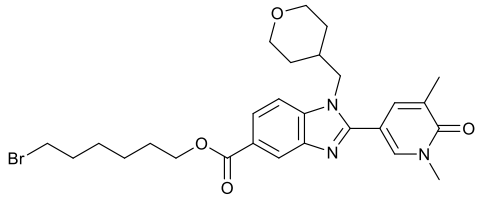
2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]-imidazole-5-carboxylic acid (38)



Sodium chlorite (2.450 g, 27.1 mmol) was added to hydrogen peroxide (0.55 g, 16.0 mmol), NaH_2PO_4 (0.59 g, 4.93 mmol) and 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carbaldehyde (**29**) (4.50 g, 12.3 mmol) in MeCN (25 mL) and water (25 mL) at 0 °C. The resulting mixture was stirred at room temperature for 1.5 hours. The crude product was purified by C18 column chromatography, elution gradient 0 to 70% MeCN in water. Pure fractions were evaporated to dryness to afford 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylic acid (4.60 g, 98%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.78 (s, 1H), 8.20 (dd, *J* = 6.8, 2.0 Hz, 2H), 7.89 (dd, *J* = 8.5, 1.6 Hz, 1H), 7.76–7.8 (m, 2H), 4.33 (d, *J* = 7.3 Hz, 2H), 3.66–3.79 (m, 2H), 3.57 (s, 3H), 3.11 (td, *J* =

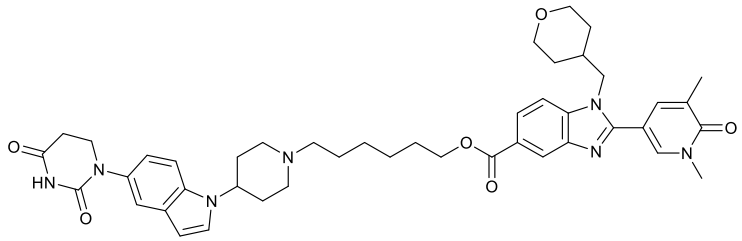
11.2, 3.2 Hz, 2H), 2.11 (s, 3H), 1.89–2.06 (m, 1H), 1.1–1.25 (m, 4H). LCMS m/z (ES+), TFA, found $[M+H]^+$ = 382.

6-bromohexyl 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylate (39b)



6-Bromohexan-1-ol (114 mg, 0.63 mmol) was added to DIPEA (0.2 mL, 0.94 mmol), DMAP (38.4 mg, 0.31 mmol), EDC (151 mg, 0.79 mmol) and 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylic acid (**38**) (120 mg, 0.31 mmol) in DCM (1 mL) over a period of 10 minutes. The resulting mixture was stirred at room temperature for 2 hours. The mixture was filtered through Celite and the solvent was removed under reduced pressure. The crude product was purified by C18 column chromatography, elution gradient 0 to 50% MeCN in water. Pure fractions were evaporated to dryness to afford 6-bromohexyl 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylate (85 mg, 50%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.21 (m, 2H), 7.90 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.84 (d, *J* = 8.6 Hz, 1H), 7.78 (dd, *J* = 2.6, 1.3 Hz, 1H), 4.28–4.37 (m, 4H), 3.72 (d, *J* = 11.0 Hz, 2H), 3.57 (s, 3H), 3.53 (t, *J* = 6.7 Hz, 2H), 3.08–3.15 (m, 2H), 2.11 (s, 3H), 1.96 (bs, 1H), 1.77 (dt, *J* = 22.5, 7.3 Hz, 4H), 1.34 (m, 4H), 1.20 (d, *J* = 6.5 Hz, 4H). LCMS m/z (ES+), TFA, found $[M+H]^+$ = 544.

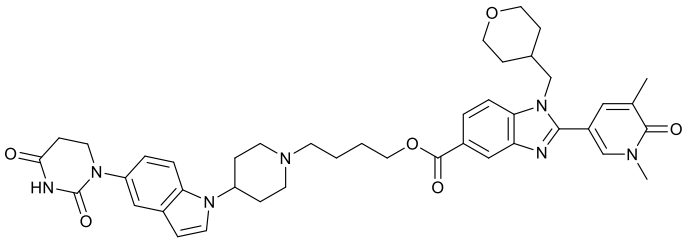
6-(4-(5-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-1H-indol-1-yl)piperidin-1-yl)hexyl 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylate (22)



DIPEA (0.401 mL, 2.30 mmol) was added to 6-bromohexyl 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylate (**39b**) (500 mg, 0.92 mmol) and 1-(1-(piperidin-4-yl)-1H-indol-5-yl)dihydropyrimidine-2,4(1H,3H)-dione (**37**) (239 mg, 0.77

mmol) in DMF (5 mL) at 25°C under nitrogen. The resulting solution was stirred at 80 °C for 12 h. The crude product was purified by preparative HPLC (Column: Xselect CSH C18 OBD Column 30*150mm 5µm, n; Mobile Phase A: Water(0.1%FA), Mobile Phase B: ACN; Flow rate: 60 mL/min; Gradient: 13% B to 32% B in 7 min, 32% B. Fractions containing the desired compound were evaporated to dryness to afford 6-(4-(5-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-1H-indol-1-yl)piperidin-1-yl)hexyl 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylate (272 mg, 45%) as a white solid. Purity 99%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.27 (s, 1 H), 8.21 - 8.26 (m, 1 H), 8.18 (d, *J*=2.3 Hz, 1 H), 7.88 - 7.95 (m, 1 H), 7.80 - 7.86 (m, 1 H), 7.74 - 7.78 (m, 1 H), 7.51 (dd, *J*=6.0, 2.8 Hz, 2 H), 7.46 (d, *J*=1.9 Hz, 1 H), 7.06 (dd, *J*=8.8, 2.0 Hz, 1 H), 6.44 (d, *J*=3.2 Hz, 1 H), 4.27 - 4.38 (m, 5 H), 3.63 - 3.80 (m, 4 H), 3.56 (s, 3 H), 2.97 - 3.16 (m, 4 H), 2.72 (t, *J*=6.7 Hz, 2 H), 2.32 - 2.41 (m, 2 H), 2.13 - 2.19 (m, 1 H), 2.08 - 2.12 (m, 4 H), 1.86 - 2.04 (m, 5 H), 1.71 - 1.81 (m, 2 H), 1.33 - 1.53 (m, 6 H), 1.10 - 1.25 (m, 4 H). ^{13}C NMR (126 MHz, DMSO) δ 170.8, 166.2, 163.1, 161.8, 152.6, 141.9, 139.5, 138.7, 136.5, 134.4, 133.6, 128.0, 128.0, 125.9, 123.8, 123.2, 120.3, 119.9, 117.9, 111.4, 109.9, 107.7, 101.4, 66.3, 64.4, 56.4, 51.8, 51.5, 49.6, 45.6, 40.1, 39.9, 39.8, 39.6, 37.5, 35.1, 31.3, 30.3, 29.8, 28.1, 26.2, 25.3, 25.0, 16.9. HRMS m/z (ES+) calc. $[M+H]^+$ = 776.4136, found 776.4154.

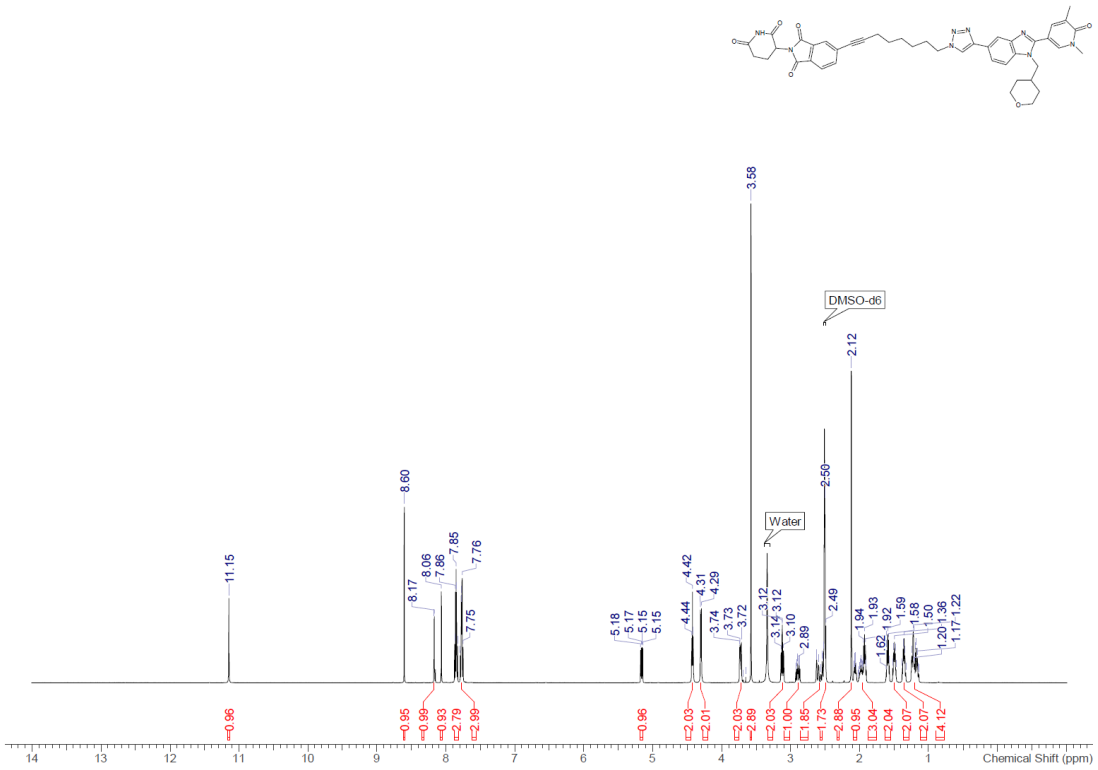
4-(4-(5-(2,4-dioxotetrahydropyrimidin-1(2H)-yl)-1H-indol-1-yl)piperidin-1-yl)butyl 2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1-((tetrahydro-2H-pyran-4-yl)methyl)-1H-benzo[d]imidazole-5-carboxylate (21)

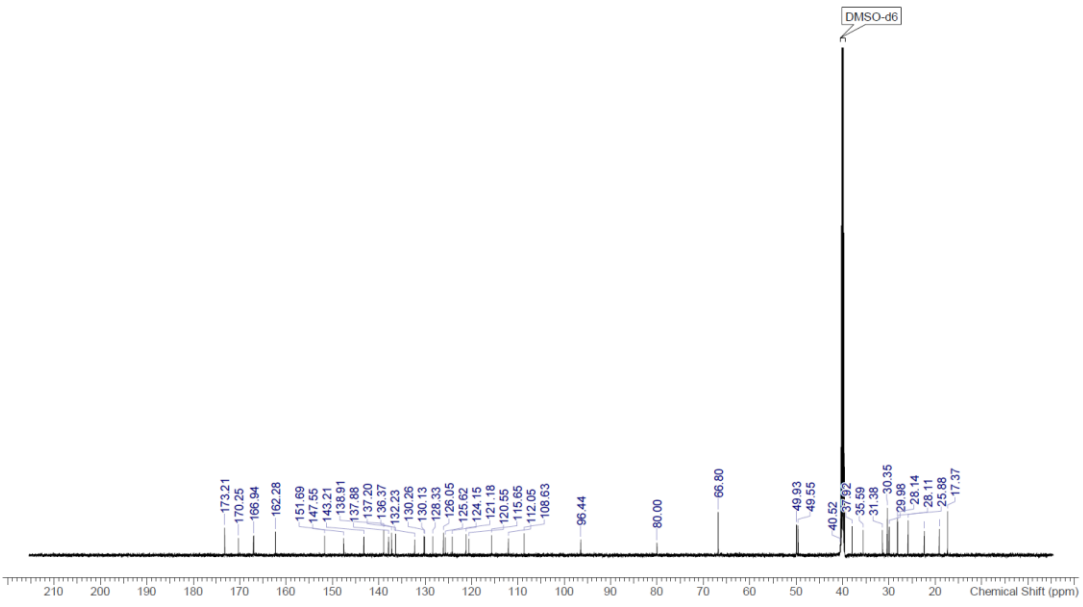


Prepared following the same synthetic route as PROTAC **22** using 1-(1-(piperidin-4-yl)-1H-indol-5-yl)dihydropyrimidine-2,4(1H,3H)-dione (**34**) and 3-bromopropan-1-ol. Purity 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.26 (s, 1 H) 8.23 - 8.25 (m, 1 H) 8.19 (d, *J*=2.5 Hz, 1 H) 7.90 - 7.94 (m, 1 H) 7.82 - 7.87 (m, 1 H) 7.75 - 7.79 (m, 1 H) 7.52 - 7.56 (m, 2 H) 7.46 (d, *J*=2.00 Hz, 1 H) 7.07 (dd, *J*=8.7, 1.8 Hz, 1 H) 6.45 (d, *J*=3.2 Hz, 1 H) 4.29 - 4.40 (m, 5 H) 3.68 - 3.81 (m, 4 H) 3.57 (s, 3 H) 2.99 - 3.17 (m, 4 H) 2.66 - 2.79 (m, 2 H) 2.41 - 2.48 (m, 2 H) 2.07 - 2.21 (m, 5 H) 1.90 - 2.04 (m, 5 H) 1.75 - 1.85 (m, 2 H) 1.60 - 1.70 (m, 2 H) 1.10 - 1.25 (m, 4 H). ^{13}C NMR (151 MHz, DMSO) δ 170.8, 166.1, 161.8, 152.6, 152.4, 140.8, 139.2, 139.0, 136.4, 134.6, 133.6, 128.1, 125.8, 124.0, 123.6, 120.1, 120.0, 118.0, 111.6, 110.0, 106.9, 101.8, 66.3, 63.9, 55.5, 51.3, 50.0, 49.7, 49.3, 48.9, 45.6, 40.1, 37.6, 35.1, 31.3, 29.8, 29.1, 25.5, 24.9, 21.2, 20.5, 16.9.HRMS m/z (ESI) calc. $[M+H]^+$ = 748.3823, found 748.3807.

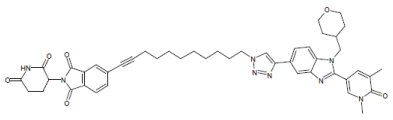
Spectra and analyses

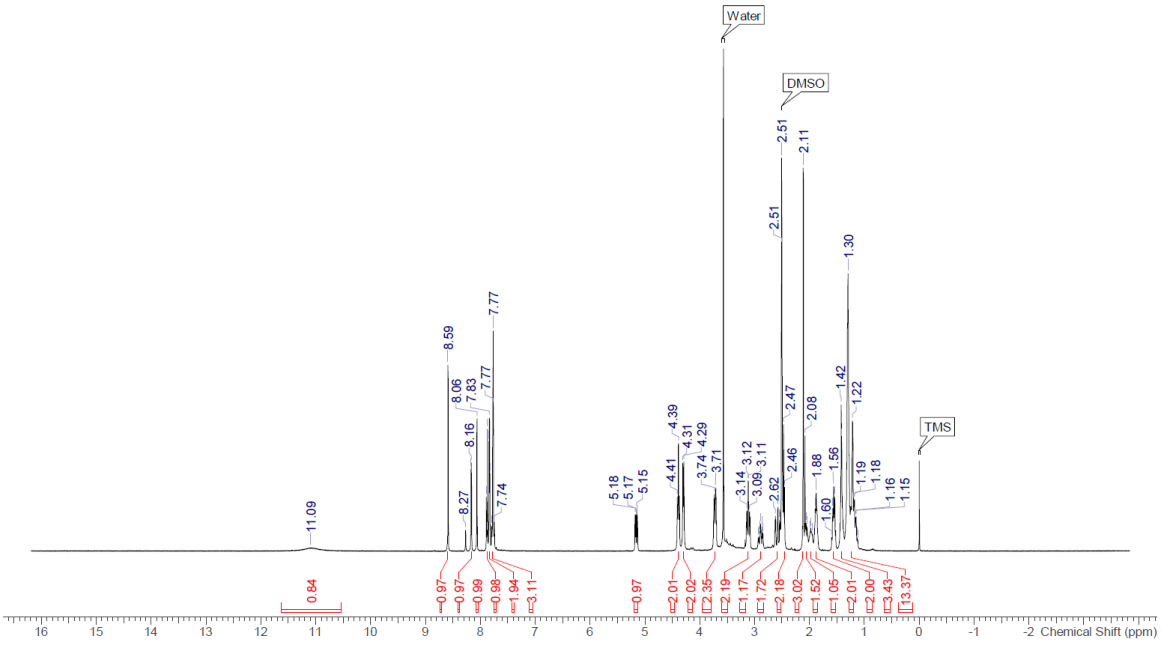
PROTAC **13**





PROTAC **14**

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8.59
8.27
8.06
8.16
7.83
7.77
7.77
7.74
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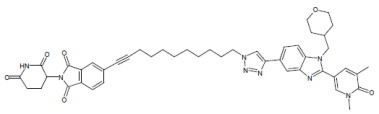
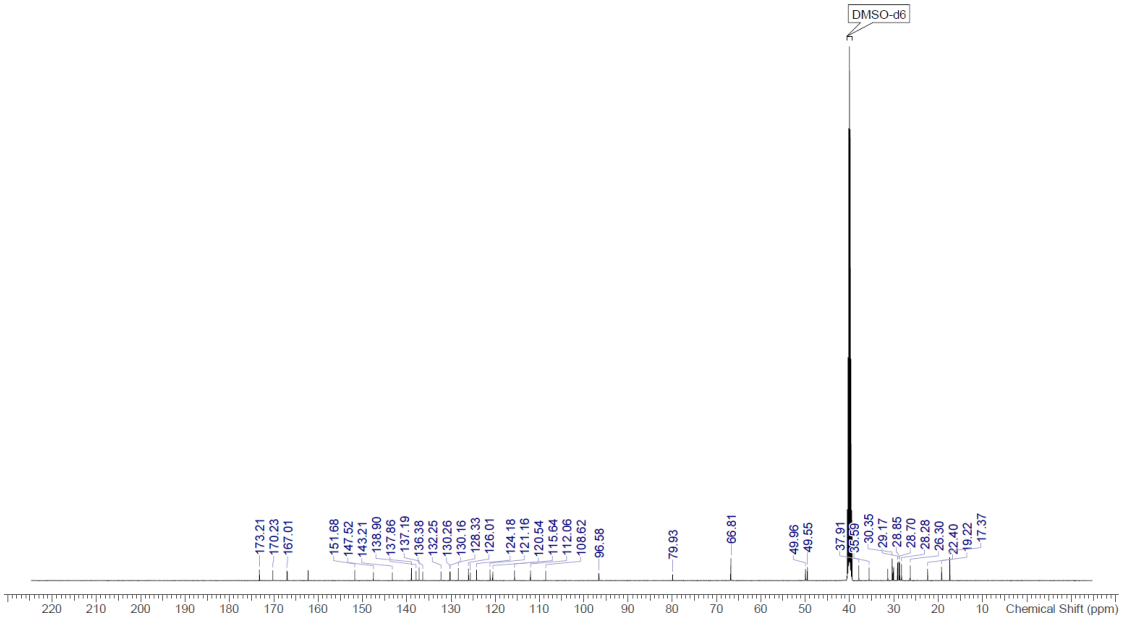
5.15
4.41
4.39
4.31
4.29
3.74
3.71
3.14
3.12
3.09
3.11
2.62
2.51
2.51
2.11
2.47
2.46
2.08
1.88
1.60
1.56
1.42
1.30
1.22
1.19
1.18
1.16
1.15
0.84
0.97
0.97
0.99
0.98
1.94
3.11
0.97
2.01
2.02
2.35
2.19
1.17
1.72
2.18
3.02
1.52
1.05
2.01
2.00
3.43
3.37
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 Chemical Shift (ppm)

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167.01
151.68
147.52
143.21
138.90
137.86
137.19
136.38
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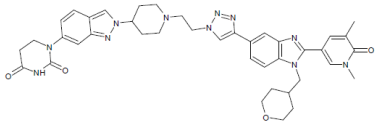
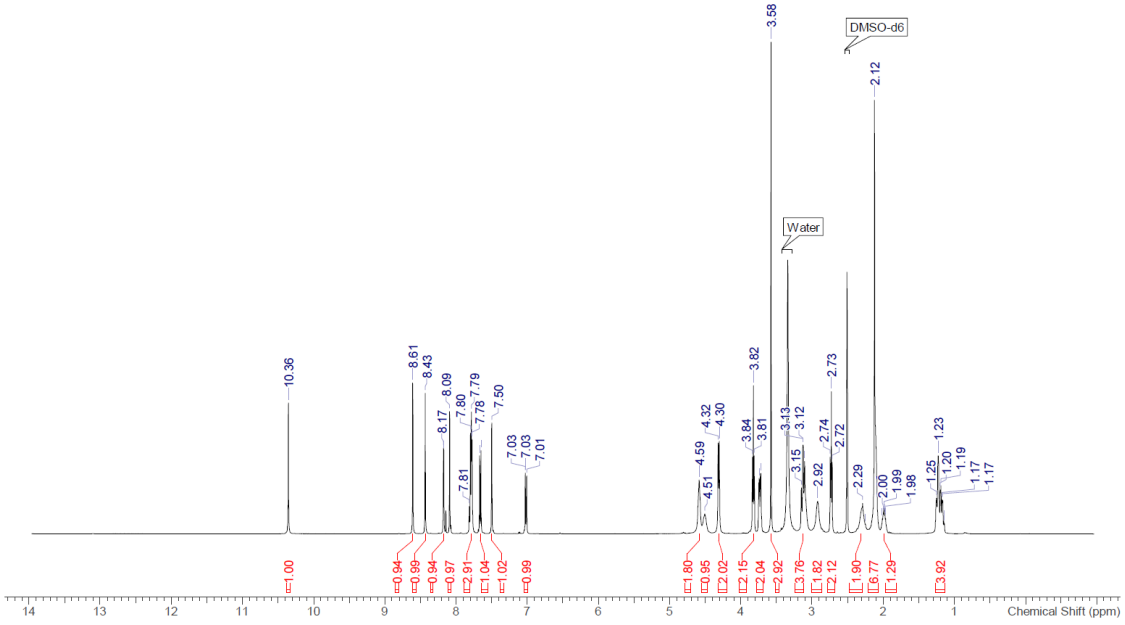
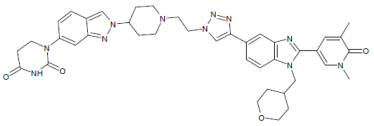
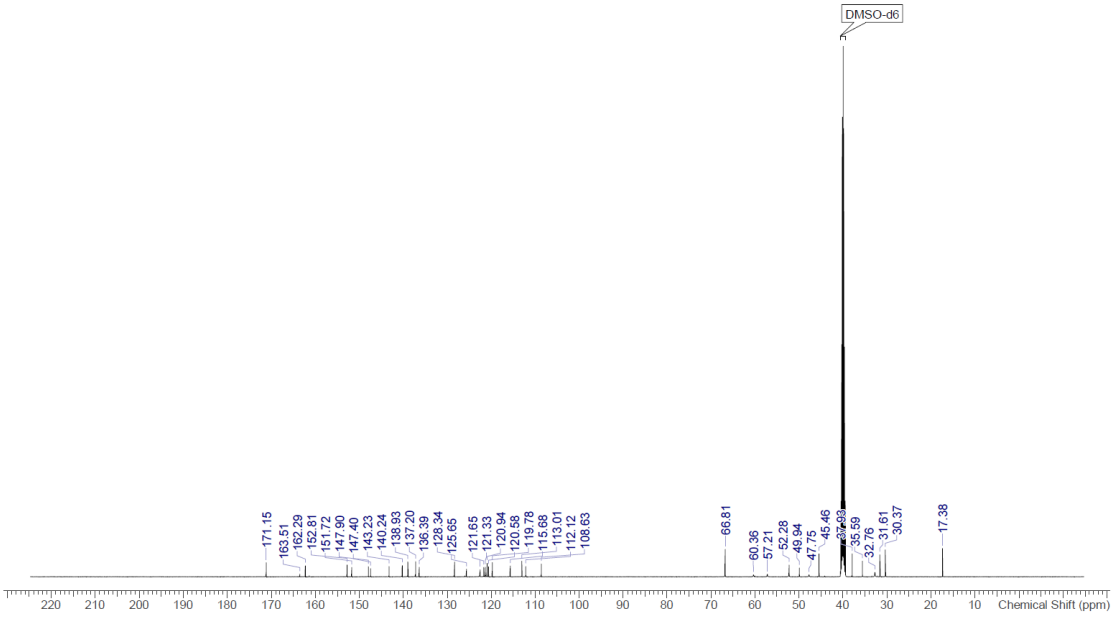
130.16
128.33
126.01
124.18
121.16
120.54
115.64
112.06
108.62
96.58
79.93
66.81
49.96
49.55
37.91
35.59
30.35
29.17
28.85
28.70
28.28
26.30
22.40
19.22
17.37
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 Chemical Shift (ppm)

PROTAC **16**

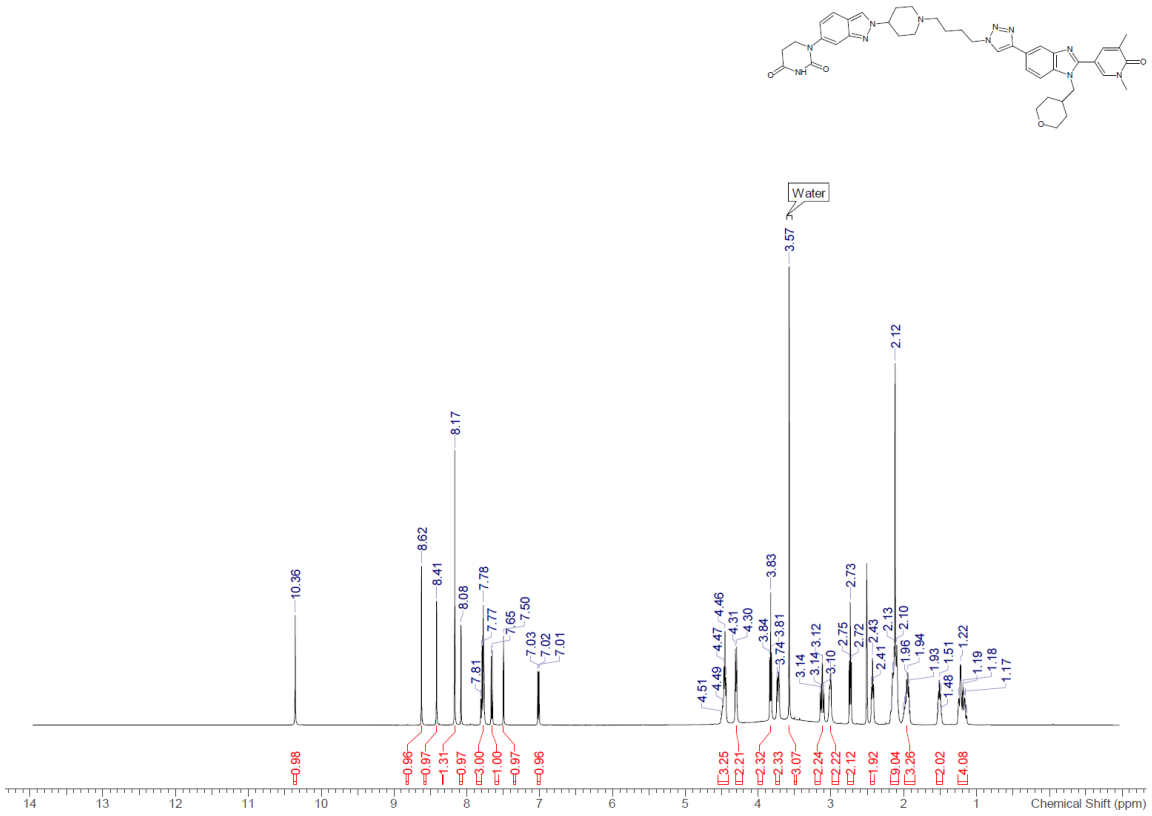








PROTAC **18**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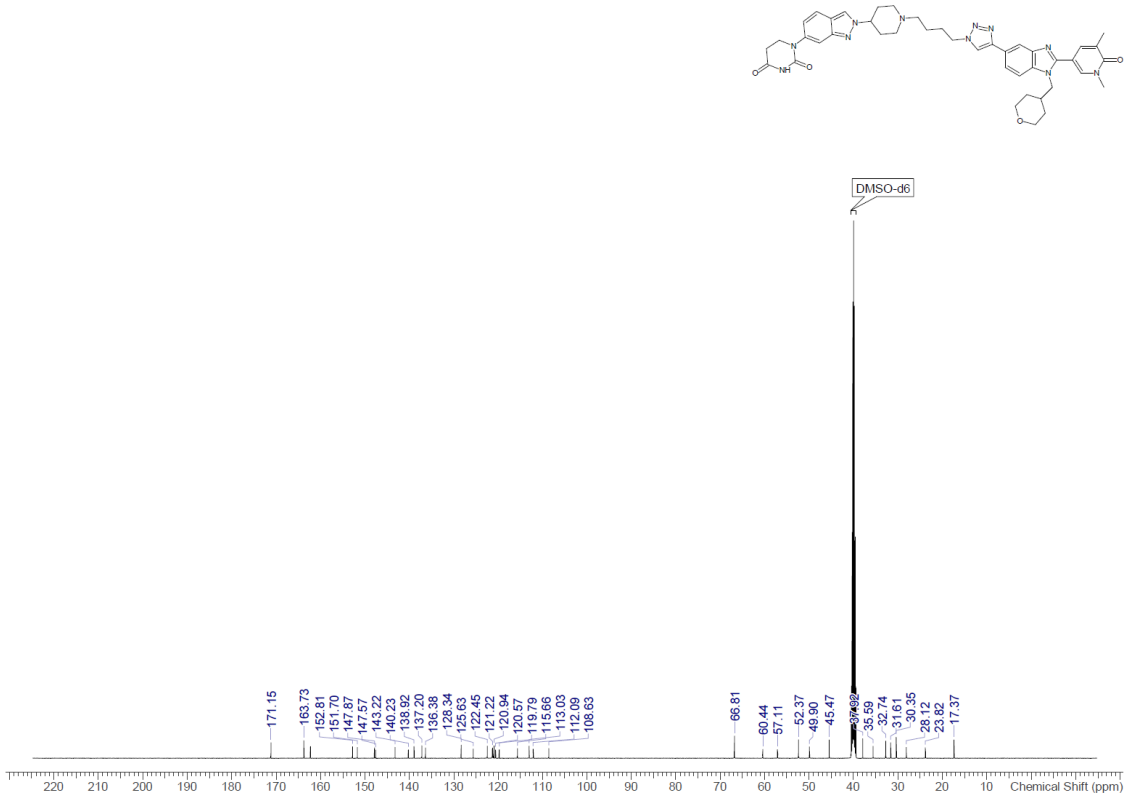
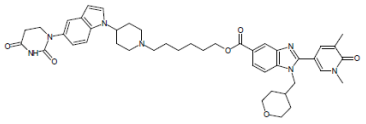
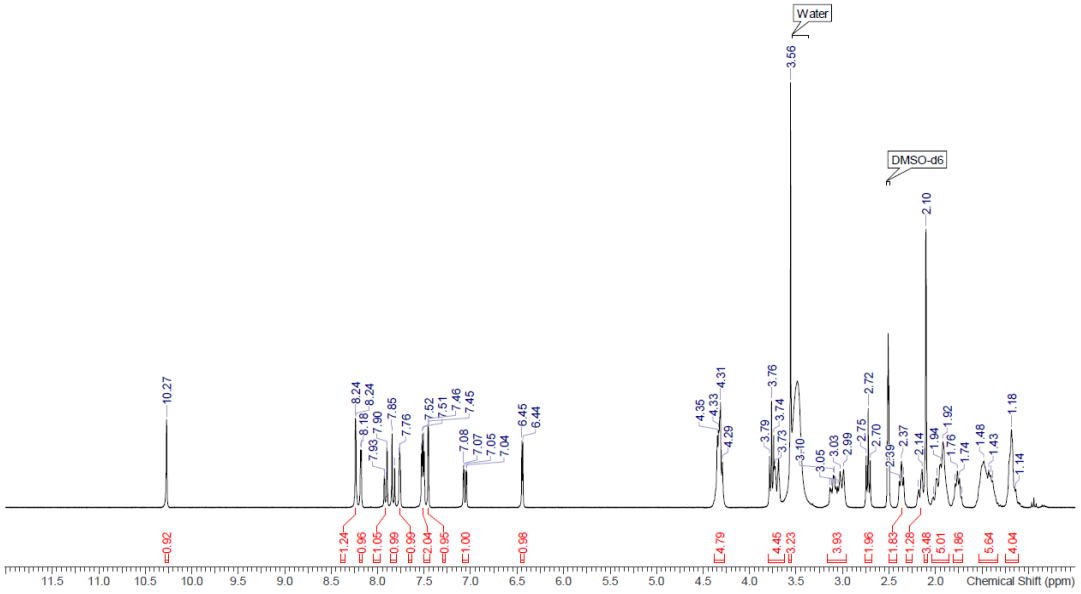
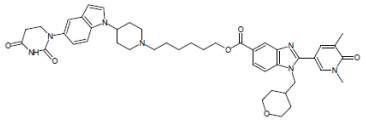
8.17
8.62
8.41
8.08
7.78
7.77
7.65
7.50
7.03
7.02
7.01
7.81
10.36
3.83
4.46
4.47
4.31
4.30
3.84
3.81
3.74
3.14
3.12
3.10
2.75
2.73
2.72
2.43
2.41
2.13
2.10
1.96
1.94
1.93
1.51
1.48
1.22
1.19
1.18
1.17
4.51
4.49
=0.98
=0.96
=0.97
=1.31
=0.97
=3.00
=1.00
=0.97
=0.96
=3.25
=2.21
=2.32
=2.33
=3.07
=2.24
=2.22
=2.12
=1.92
=9.04
=3.26
=2.02
=4.08
Chemical Shift (ppm)

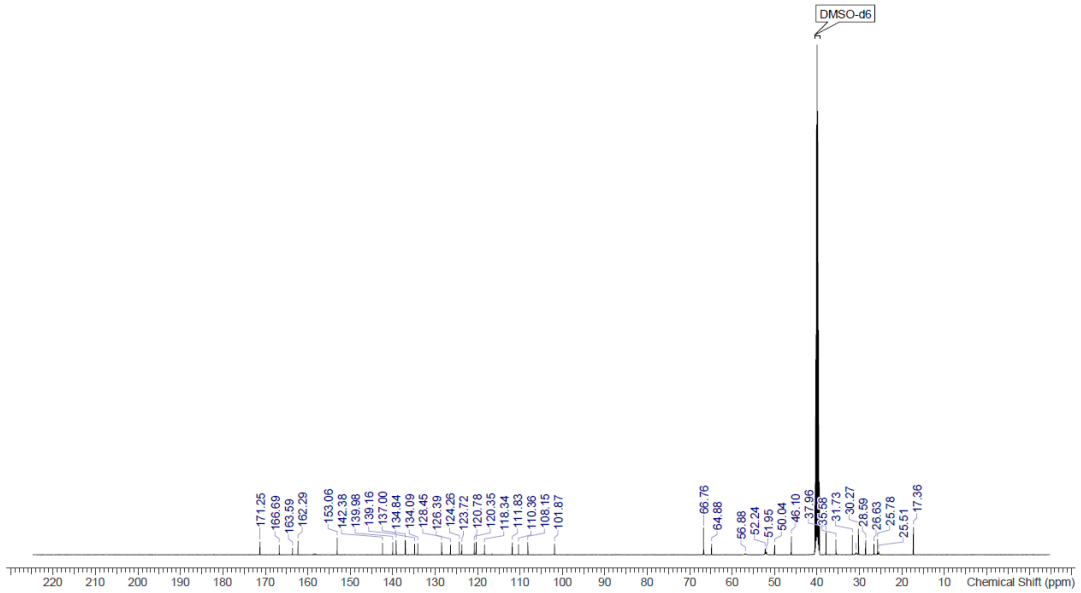

DMSO-d6
171.15
163.73
152.81
151.70
147.87
147.57
143.22
140.23
138.92
137.20
136.38
128.34
125.63
122.45
121.22
120.94
120.57
119.79
115.66
113.03
112.09
106.63
66.81
60.44
57.11
52.37
49.90
45.47
39.99
35.56
32.74
31.61
30.35
28.12
23.82
17.37
Chemical Shift (ppm)

PROTAC **22**









Molecular Docking

All calculations were performed within Maestro software. [3] For docking of the POI warhead (benzimidazole **1**) we used the ternary complex structure of dBET23 with BRD4-BD1 and CRBN (PDB code: 6BN7). The E3 warheads were docked into human CRBN (PDB code: 4TZ4) and then mouse CRBN (PDB code:5YIZ) was overlaid on it to see the residue differences. Molecular docking was performed using Glide program. [4] Before docking, the PDB structures were prepared with Protein Preparation Workflow. [5] The protonation state of each warhead was determined at pH=7.4 using Epik program. [6] For docking into CRBN we applied H-bond constraints in the active site to account for key interactions between DHU/glutarimide warhead and the protein.

In vitro biology

BD1 and BD2 fluorescence polarization binding assays. PROTAC binding potencies to BRD2(BD1) and BRD2(BD2) were measured in fluorescence polarization (FP) binding assays by following similar procedure as described previously. [7] In brief, both BRD2(BD1) and BRD2(BD2 FP assays were performed in buffer containing 50 mM Hepes, 1 mM TCEP, 0.01% Brij-35. The assays were run in 384-well black low volume plates (Greiner Bio-One, #784076) containing 20 nL compound or DMSO (predispensed with Echo 665 acoustic dispenser (Labcyte)). To the assay ready plates, 90 nM recombinant human BRD2(BD1) or 30 nM recombinant human BRD2(BD2) was added followed by 10 nM fluorescent probe (Alexafluor-647-JQ1). The inhibitor control in the assays was JQ-1 at a concentration of 25 µM. Final concentration of DMSO in the 8 µL assay volume was 0.25% (v/v). Plates were briefly centrifuged (30 sec at 1000 RPM), incubated for 1 hour at room temperature prior to reading on a PHERAstar FSX plate reader (BMG LABTECH, Germany) using a FP optical module (Excitation 590-50, Emission 675-50).

HEK293 HiBiT-BRD2-4 degradation assay. PROTAC degradation potency was measured in HEK293 cells endogenously expressing HiBiT-tagged BRD2, BRD3 or BRD4 according to the protocol described previously. [8] In brief, cryopreserved HEK293/HiBiT-BRD2-4 cells were31 ff thawed in a 37°C water bath with gentle shaking and reconstituted at 2 x 10^5 cells/mL in Opti-MEM™ media (Gibco) containing 2% FBS (Gibco cat#10270). 25 µL/well of cell suspension were dispensed to a 384-well assay plate (Greiner, 781080). The assay plates were incubated at room temperature (RT) for 20 minutes. Thereafter, 75 nl of PROTAC DMSO solutions (diluted at appropriate concentrations) were dispensed on top of cells from a 384-well LDV compound source plate with an Echo 655 acoustic dispenser system (Beckman). DMSO (v/v 0.3%) and dBET6 PROTAC (fc 1 µM) served as a neutral and 100 % degradation control, respectively. After addition of PROTACs, assay plates were incubated at

37°C in a cell culturing incubator for 5 hours and HiBiT-BRD2-4 levels were measured via nanoluciferase activity using a NanoGlo HiBiT lytic assay kit (Promega, N3040) following manufacturer instructions. Plates were measured in a Pherastar FSX plate reader (BMG Labtech). Data normalization to neutral and 100% inhibition controls, curve fitting, DC_{50} and D_{max} calculations were carried out using Genedata screener software using the bell-shape fit condensing method.

Table S1. BRD1 BD1 and BD2 binding and BRD2-4 degradation with standard error.

table	#	BRD2-BD1 Hu Bind FP[a,b] pIC_{50}± Std Err	BRD2-BD2 Hu Bind FP[a,b] pIC_{50}± Std Err	Hu BRD4 HiBit degr[a,c] pDC_{50}± Std Err	Hu BRD2 HiBit degr[a,c] pDC_{50}± Std Err	Hu BRD3 HiBit degr[a,c] pDC_{50}± Std Err
1	**dBET6**	7.36±0.17	7.27±0.11	8.32±0.03	8.76±0.48	9.13±0.48
1	**ZXH-3-26**	7.63[d]	7.51[d]	8.15±0.04	8.11±0.07	8.33±0.11
1	**2**	7.26±0.05	6.89±0.04	<5[d]	<5.02[d]	<5.02[d]
1	**3**	7.18±0.06	6.82±0.09	6.53[d]	nt[e]	nt[e]
1	**4**	7.19±0.06	6.74±0.05	6.42±0.01	nt[e]	nt[e]
1	**5**	7.26[d]	6.93[d]	6.76±0.04	nt[e]	nt[e]
1	**6**	7.19[d]	6.82[d]	7.29±0.18	nt[e]	nt[e]
2	**7**	7.21±0.02	6.81±0.02	7.82±0.18	7.44[e]	8.04[e]
2	**8**	nt[e]	nt[e]	9.83±0.13	nt[e]	nt[e]
2	**9**	7.34±0.01	6.87±0.12	9.23±0.09	nt[e]	nt[e]
2	**10**	7.35±0.01	7.14±0.03	9.59±0.09	>9.57[d]	>9.57[d]
2	**11**	7.64±0.08	7.30±0.06	9.60±0.04	9.55[d]	>9.57[d]
2	**12**	7.51±0.07	6.94±0.10	9.34±0.02	9.38±0.14	>9.60[d]
2	**13**	7.44±0.06	7.15±0.07	9.72±0.04	9.69±0.05	>9.61±0.28
2	**14**	7.04±0.02	6.62±0.10	9.05±0.04	9.10±0.07	>9.60±0.23
3	**15**	7.37±0.12	7.34±0.08	7.17±0.15	nt[e]	nt[e]
3	**16**	7.83[d]	7.54[d]	9.02±0.02	8.86±0.04	9.61±0.03
3	**17**	7.46±0.08	7.27±0.06	8.79±0.04	8.80[d]	>9.02[d]
3	**18**	7.62[d]	7.42[d]	9.15±0.08	9.14±0.15	>9.59±0.20
3	**19**	7.64[d]	7.34[d]	9.61±0.03	>9.43±0.20	>9.43±0.20
3	**20**	7.60[d]	7.36[d]	>9.46±0.07	9.33[d]	>9.57[d]
3	**21**	7.38±0.05	7.00±0.04	10.1±0.18	>9.57[d]	>9.57[d]
3	**22**	7.56±0.05	7.13±0.01	10.1±0.18	>9.57[d]	>9.57[d]

[a]Values are means of at least two independent experiments with two replicates each, unless otherwise indicated; [b]compound binding to BRD2 BD1 or BD2 by displacement of a fluorescent Alexafluor 647-JQ-1-like probe in −log10(M). The 95% confidence interval of $pIC5_0$ values was ±0.24 for BD1 ±0.23 for BD2; [c]NanoGlo degradation assay in a HEK293 cell line expressing human BRD2-Hibit, BRD3-HiBit or BRD4-HiBit fusion at endogenous expression levels in −log10(M). The 95% confidence interval of pDC_{50} values were ±0.08 (BRD2), ±0.16 (BRD3), ±0.14 (BRD4) respectively. [d]one test occasion; [e]not tested

BRD4 degradation assay in lung fibroblasts. Previously expanded primary normal human lung fibroblasts (NHLF) (Lonza #CC-2512) from three donors (passage 6) were thawed and seeded in collagen I-coated 96-well plates (Revvity #6055308) at 13 000 cells/well, or PDL-coated 384-well plates (Revvity #6057500) at 3 000 cells/well, in growth medium composed of DMEM (Thermo Fisher Scientific #31966-029) with 10% (v/v) fetal bovine serum (FBS) (Thermo Fisher Scientific #12070-106, lot #42F6280K), 100 units/ml penicillin and 100 µg/ml streptomycin (Thermo Fisher Scientific #15140122). Primary mouse lung fibroblasts were also thawed and seeded with the same medium and seeding density. Three days after seeding, serum starvation was induced by replacing the growth medium with a starvation medium (growth medium without FBS or growth medium with 3% FBS for human and mouse fibroblasts, respectively). DMSO solutions of the compounds were added using a D300e digital dispenser (Tecan), and the cells were incubated with the compounds for 4 hours at 37°C and 5% CO_2 in starvation medium (growth medium without FBS or growth medium with 1% FBS for human and mouse fibroblasts, respectively), followed by fixation with 4% formaldehyde (VWR #9713.1000) at room temperature for 20 minutes. The final concentration of DMSO in all wells during the assay was 0.1% (v/v).

Lung fibroblast-to-myofibroblast transition (FMT) assay. Previously expanded primary NHLF (Lonza #CC-2512) from three donors (passage 6) were thawed, seeded and starved as described for the BRD4 degradation assay. DMSO solutions of the compounds were added using a digital dispenser (Tecan D300e) and the cells were incubated with the compounds for 2 hours at 37°C and 5% CO_2, followed by stimulation with 0.12 ng/ml (96-well plates) or 1 ng/ml (384-well plates) human recombinant human TGF-β1 (Bio-techne #240-B-010) for another 24 hours in the same environment. The TGF-β1 concentration had previously been titrated and optimized for each plate format. The cells were fixed with 4% formaldehyde (VWR #9713.1000) at room temperature for 20 minutes. The final concentration of DMSO in all wells during the assay was 0.1% (v/v).

Immunofluorescence. The fixed cells were permeabilized with 0.2% Tween-20 (Thermo Fisher Scientific #851113) for 20 minutes on an orbital shaker, followed by incubation with blocking buffer (5% bovine serum albumin [w/v] in PBS) for 1 hour. Primary antibodies against alpha smooth muscle actin (α-SMA) (Agilent/Dako #M0851, mouse, 1/250 dilution) or BRD4 (Bethyl Laboratories #A700-004, rabbit, 1/1000 dilution) were added to the FMT and BRD4 degradation plates, respectively, followed by incubation for 1 hour and washing in PBS. Alexa Fluor 647-conjugated goat anti-mouse IgG (Thermo Fisher Scientific #A-21235) or Alexa Fluor 594-conjugated goat anti-rabbit IgG (Thermo

Fisher Scientific #A-11037) were added to the FMT and BRD4 degradation plates, respectively, followed by incubation for 1 hour and washing in PBS. The nuclear dye Hoechst 33342 (Thermo Fisher Scientific #H3570, 1/10 000 dilution) was added to the secondary antibody solutions. Finally, the cells were incubated with the cytoplasmic dye CellMask Blue (Thermo Fisher Scientific #H32720), 1/5000 dilution) in PBS for 30 minutes followed by washing with PBS. All antibodies were diluted in blocking buffer, and incubations were performed at room temperature. The fluorescence was quantified using a CellVoyager CV8000 high-throughput confocal microscope (Yokogawa).

Image analysis and calculation of potencies. The Columbus image analysis software (Perkin Elmer) was used to quantify the mean α-SMA staining intensity per cell in cells from the FMT assay, while mean BRD4 staining intensity per cell was quantified in the nuclei of cells from the BRD4 degradation assays. The cytoplasm and nuclei were detected using the CellMask Blue and Hoechst 33342 dyes, respectively. The α-SMA intensities were normalized to % inhibition of α-SMA, using the intensities from compound-free control wells with TGF-β1 (minimum inhibition) or without TGF-β1 (maximum inhibition). Curve fitting was performed, and potencies calculated in Prism 10.1.2 (GraphPad Software Inc.) using a log[compound] vs. response – variable slope (four parameters) model, based on % inhibition of α-SMA for FMT data and mean BRD4 staining intensity for BRD4 degradation data.

Drug metabolism and pharmacokinetics (DMPK).

In vitro assays were performed as routine screening assays and have been described in detail elsewhere as indicated below.

Lipophilicity: Lipophilicity was measured as the distribution coefficient (logD7.4) between octanol and 10 mM sodium phosphate buffer with the solution adjusted to pH 7.4, in a high-throughput assay based on the traditional shake flask method, [9] using 96-well plates.
HLM: Metabolic stability was determined by incubating 1 µM compound solution in pooled human liver microsomes (1 mg/mL) at 37 °C and pH 7.4. Compound concentration at different time points (typically 0.5, 1, 10, 15, 20 and 30 minutes) was quantified by LC−MS/MS and Clint calculated from compound disappearance. [10]
Hepatocyte CLint (Mouse): Metabolic stability was determined by incubating 1 µM compound solution in cryopreserved hepatocytes (1 million cells/mL) at 37 °C and pH 7.4. Compound concentration at different time points (typically 2, 15,30, 45, and 60 min) was quantified by

LC−MS/MS and Clint calculated from compound disappearance. [10] Pooled human, rat (Wistar Han), and mice (C57/Bl) hepatocyte batches were used as available for screening at the time of assay.

Plasma protein Binding (Human): Plasma protein binding experiments were performed as reported previously [11] using blood plasma samples from humans.

Plasma stability: Stability of compound in plasma was determined after 18h incubation as % remaining in the plasma protein binding assay above.

Caco2 Papp: The assay has been described in detail elsewhere. [12] An automated assay to study Caco-2 monolayer intrinsic permeability in the apical to basolateral direction including a pH gradient (pH 6.5 in apical donor compartment and pH 7.4 in basolateral receiver compartment) was used.

In vivo

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of AstraZeneca and approved by the local Ethical Committee for Laboratory Animals in Gothenburg.

Intravenous administration in rats: Male Sprague Dawley rats were dosed with **13** intravenously at 1 mg/kg (5% DMSO and 95% SBE-B-CD (30% w/v) in water pH 4 adjusted by HCl).

Intratracheal administration in rats. Male Wistar Han rats were obtained from Charles River Laboratories (Sulzfeld, Germany). **13** was given intratracheally (195 nmol/kg) as a solution in 5% w/v Captisol® (sulfobutylether-beta-cyclodextrin, Cydex, USA) in 10 mM citrate buffer saline pH 4, or as a suspension in 0.2 mg/mL Tween 80 (polysorbate 80, Seppic, France) in saline (9 mg/mL NaCl, B. Braun, Germany). Homogenisation of the suspension was done by 7 hours of magnetic stirring and 10 minutes in an ultrasonication bath type Elmasonic S (Elma, Germany). The particle size of the suspension, Dv50= 4.3 µm and Dv90=7.1 µm, was measured by laser diffraction (Horiba Partica LA960V2, Refractive Index =1.71). 10 mM citrate buffer saline pH 4 was made from citric acid anhydrous (Sigma-Aldrich, Austria), sodium citrate tribasic dihydrate (Sigma-Aldrich, Belgium), saline (B. Braun, Germany) and adjusted to pH 4 using HCl or NaOH (Labservice AB, Sweden).

Table S2. Rat pharmacokinetics in after intravenous and intratracheally administration

Comp	iv[a] Sprague Dawley rats			it[b], Wistar Han rats	
	CL (mL/min/kg)	Vss (L/kg)	MRT (h)	Lung $T_{1/2}$ (h) suspension	Lung $T_{1/2}$ (h) solution
13	80.3±7.1 (n=2)	1.8±0.1 (n=2)	0.4±0.0 (n=2)	>12	< 0.2

[a] iv, intravenous; [b]it, intratracheally; [c]CL, clearance; [d]Vss, volume of distribution at steady state; [e]MRT, mean residence time; [f]Lung $T_{1/2}$, half-life in lung after it administration of a suspension of amorphous material or as a solution as indicated.

Pharmacokinetic experiments and assessment of BRD4 in mice. Female C57BL/6 mice were obtained from Charles River Laboratories (Sulzfeld, Germany). Following intravenous dosing, blood sampling was done at 5, 15 and 30 minutes as well as 2, 5 and 24 hours after dosing. Following intratracheal dosing (*method see below*) blood and lung sampling was done at 1, 7 and 24 hours after dosing (**13** and **14**) or at the same time-points as for intravenous dosing (**22**).
Doses and formulations for compound **22:** iv 2.5 mg/kg (5 % kleptose in citrate buffer saline, pH 5-6) and it 1 mg/kg (5 % captisol in saline, pH 5); for compound **13** iv 1 mg/kg (1.6% captisol in saline pH 7 for **13** and it 0.8 mg/kg (citrate buffer pH 5-6 and 0.2 mg/ml polysorbate 80); for compound **14** iv 1 mg/kg (11 % captisol in pH 5) and it 0.8 mg/kg (citrate buffer pH 5-6 and 0.2 mg/ml polysorbate 80). Suspensions were homogenised by 45 minutes of magnetic stirring and 15 minutes in ultrasonication bath. The particle size of **13** suspension, Dv50= 2.4 µm and Dv90=4.5 µm, was measured by laser diffraction (Horiba Partica LA960V2, Refractive Index =1.71). The particle size value for **14** suspension was Dv50= 5.9 µm and Dv90= 9.8 µm (Refractive Index =1.69).
Compound concentrations in blood, plasma or lung homogenates were measured using LC-MS/MS. BRD4 levels were assessed with Immunohistochemical analysis, see below.

Dose response study in mice. Female C57BL/6 mice at an average weight of 18-20 g were obtained from Charles River Laboratories (Sulzfeld, Germany). The study design and group size (n=6) was designed based on good statsitical practice methods. Animals were randomized upon arrival and housed in temperature-controlled, individually ventilated macrolon cages in groups of six, with food and water provided *ad libitum*. Animals underwent acclimatization for at least 1 week before experimentation. For intratracheal administration of the compound, mice were anaesthetised with Isoflurane mixture (air/oxygen and 3.5% isoflurane), put in a supine position with 30-40˚angle and instilled with vehicle or compound (0.1, 0.3, 1 and 3 mg/kg in suspension). Mice were then placed in cages in a supine position with the head up until consciousness was regained. Seven hours after administration, mice were euthanised with an intraperitoneal injection of 0.2 mL pentobarbitol (Allfatal®) diluted in saline. The left lung lobe was inflated with 4% formaldehyde and tied off. Spleens were collected and divided longitudinally in two parts. All tissue samples were then transferred into tubes containing 4% formaldehyde for fixation for 48 hours and further processed and embedded for histological evaluation. For dosing, a 10 mg/mL stock suspension of **13** in 0.2 mg/mL Tween 80 + 3.55 mM citrate buffered saline pH 5.5 was prepared. The suspension was homogenised by overnight magnetic stirring and 2 × 10 minutes in ultrasonication bath. The particle

size of the suspension, Dv50= 3.1 µm and Dv90= 5.3 µm, was measured with laser diffraction (Horiba Partica LA960V2, Refractive Index = 1.71). The suspension was diluted with vehicle, 0.2 mg/mL Tween 80 + 3.55 mM citrate buffered saline pH 5.5, to the desired doses.

Immunohistochemical analysis. Formalin-fixed pulmonary tissues were embedded in paraffin. Section of 4 µm thickness were cut and subjected to immunohistochemical (IHC) staining for BRD4 (Bethyl Laboratories, A700-004) using the Ventana Discovery Ultra autostainer (Roche). Serial sections were utilized as negative control (without primary antibodies). The slides were digitized using an Aperio ScanScope XT (Leica Biosystems, Wetzlar, Germany), and the resulting digital files were analysed with Visiopharm® (VP) software (Version 2023-09.5.15777 x64). BRD4-positive and -negative nuclei were counted in the peripheral lung (alveoli).

Statistics. Statistical analysis of in vivo study data was performed using log-transformed data and GraphPad Prism (version 10.1.2, GraphPad, San Diego, CA, USA). Analysis of variance (ANOVA) multiple comparisons with Dunnett's *post hoc* test were performed to detect significant effects of treatment with **13** and **14**. Asterisks denote significant differences between vehicle- and compound treated groups (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

References:

[1] U. Börjesson et al., **2022** WO2022069520

[2] R. A. Bit et al., **2016** WO2016146738.

[3] Schrödinger Release 2023-1: Maestro, Schrödinger, LLC, New York, NY, 2023.

[4] R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, D. E. Shaw, P. Francis, P. S. Shenkin, J *Med Chem 2***004,** 4*7,* 1739-1749.

[5] G. M. Sastry, M. Adzhigirey, T. Day, R. Annabhimoju, W. Sherman, J *Comput Aided Mol Des 2***013,** 2*7,* 221-234.

[6] J. C. Shelley, A. Cholleti, L. L. Frye, J. R. Greenwood, M. R. Timlin, M. Uchimaya, J *Comput Aided Mol Des 2***007,** 2*1,* 681-691.

[7] P. Hansson, H. Boyd, I. L. Dale, G. Dahl, F. Nicolaus, W. Bowen, K. Doering, C. Dunsmore, G. Cotton, H. Lindmark, 2**018,** 1*6,* 372-383.

[8] M. Plesniak, E. K. Taylor, F. Eisele, C. M. B. K. Kourra, I. Michaelides, A. Oram, J. Wernevik, Z. Santisteban-Valencia, H. Rowbottom, N. Mann, L. Fredlund, V. Pivnytska, A. Noven, M. Pirmoradian, T. Lundback, R. I. Storer, M. Pettersson, G. M. De Donatis, M. Rehnstrom, 2**023.**

[9] M. C. Wenlock, T. Potter, P. Barton, R. P. Austin, J *Biomol Screen 2***011,** 1*6,* 348-355.

[10] A. K. Sohlenius-Sternbeck, C. Jones, D. Ferguson, B. J. Middleton, D. Projean, E. Floby, J. Bylund, L. Afzelius, X*enobiotica 2***012,** 4*2,* 841-853.

[11] H. Wan, M. Rehngren, J *Chromatogr A 2***006,** 1*102,* 125-134.

[12] L. Fredlund, S. Winiwarter, C. Hilgendorf, M*ol Pharm 2***017,** 1*4,* 1601-1609.