Supplementary information

Targeting IRAK4 for Degradation with PROTACs

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Contents:

1. Synthetic details

NMR Spectroscopy

^{1}H NMR and ^{13}C NMR spectra were acquired and processed on a Bruker AVIII 400MHz instrument with a 5mm BBO probe. Chemical shifts (δ) are reported in parts per million (ppm) and referenced to the residual solvent peak. Coupling constants (J) are measured in hertz (Hz). Advanced experiments were acquired and processed on either a Bruker AVII+ 600MHz instrument or a Bruker AV4 700MHz instrument, each with a 5mm CPTCI cryo-probe. These were obtained by Sean Lynn of the Analytical Chemistry Department, GlaxoSmithKline, Stevenage.

Mass Spectrometry

High Resolution Mass Spectroscopy (HRMS)

Positive ion mass spectra were acquired using a Waters XEVO G2-XS Qtof mass spectrometer, equipped with an ESI interface, over a mass range of 100 – 1200 Da, with a scan time of 0.5 sec. The elemental composition was calculated using Masslynx software and processed for either the $[M+H]^{+}$, $[M+2H]^{2+}/2$, $[M+3H]^{3+}/3$ or $[M+Na]^{+}$ ion and the mass error quoted as ppm.

Low resolution mass spectra (LC-MS) were recorded using one of three methods:

1) System A: (Formic)

An Acquity UPLC CSH C18 column (50 mm × 2.1 mm i.d. 1.7 μm packing diameter) eluting with 0.1% formic acid in water (solvent A), and 0.1 % formic acid in acetonitrile (solvent B), using the following elution gradient 0.0 – 1.5 min 3 – 100 % B, 1.5 – 1.9 min 100% B, 1.9 – 2.0 min 100 – 3% B, at a flow rate of 1 $mLmin^{-1}$ at 40 °C.

2) System B: (HpH)

An Acquity UPLC CSH C18 column (50 mm × 2.1 mm i.d. 1.7 μm packing diameter) eluting with 10 mM NH_4HCO_3 in water adjusted to pH 10 with aqueous ammonia (solvent A), and acetonitrile (solvent B), using the following elution gradient: 0.0 – 1.5 min 3 – 95 % B, 1.5 – 1.9 min 95% B, 1.9 – 2.0 min 95 – 3% B, at a flow-rate of 1 $mLmin^{-1}$ at 40°C. The UV detection was an averaged signal from wavelength of 210 nm to 350 nm, and mass spectra were recorded on a mass spectrometer using alternate-scan electrospray positive and negative mode ionisation (ES+ve and ES-ve)

3) System C: (Formic)

A HALO C18 column (50 mm × 4.6 mm i.d. 2.7 μm packing diameter) eluting with 0.1% formic acid in water (solvent A), and 0.1 % formic acid in acetonitrile (solvent B), using the following elution gradient 0.0 – 1.0 min 5 – 95% B, 1.0 min – 2.0 min 95% B, 2.0 min – 2.5 min 95% – 5% B, at 40°C.

Chromatography

Normal phase

Column chromatography was performed on a Isolera purification system. The Isolera is an automated multi-user flash chromatography system, available from Biotage, which utilizes disposable, normal phase pre-packed silica, and C18 reverse phase cartridges (1 g to 340 g).

Reverse phase

Method A - Crude reaction mixtures were dissolved in the minimum amount of DMSO and loaded onto a pre-packed Biotage® C18 modified silica cartridge. Flash column chromatography was carried out using the Biotage Isolera apparatus.

Method B - Crude reaction mixtures were dissolved in the minimum amount of DMSO and purified on XBridge Shield reverse phase C18 column (150 mm × 30 mm, 5 μm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) methanol. Purification was carried out using Waters ZQ MS using positive electrospray and a summed UV wavelength of 210–350 nm.

Mass directed auto purification (MDAP). Mass-directed automatic purification was carried out using an H_2Os ZQ MS using alternate-scan positive and negative electrospray and a summed UV wavelength of 210–350 nm. Crude reaction mixtures were dissolved in 1:1 DMSO:MeOH (1 mL). Two liquid phase methods were used:

Method A - Formic – XSelect CSH Prep C18 column (150 mm × 30 mm, 5 µm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) H_2O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid.

Method B - High pH – XSelect CSH Prep C18 column (150 mm × 30 mm, 5 µm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile.

Phase Separators

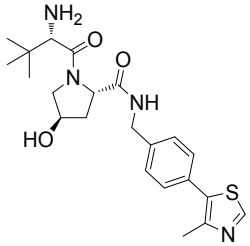
'Hydrophobic frits' refer to filtration tubes sold by Whatman.

All reagents were used as purchased from commercial suppliers. Solvents were purchased from Sigma Aldrich, anhydrous, sure-seal quality, and used with no further purification. Reactions were carried out in a fume hood, under air atmosphere, unless otherwise stated.

Synthetic procedures

(2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

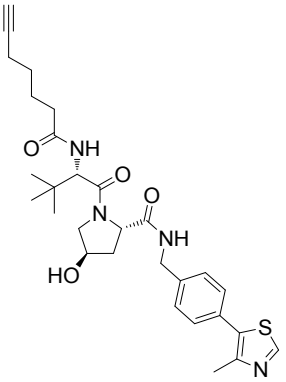
For prep. see ACS Chem. Biol. 2015, 10, 1831−1837



^{1}H NMR (MeOH, 500 MHz): δ = 8.87 (s, 1H), 7.43 (dd, *J* = 22.5, 8.3 Hz, 4H), 4.64 – 4.32 (m, 4H), 3.81 – 3.70 (m, 2H), 3.41 (s, 1H), 2.47 (s, 3H), 2.29 – 2.18 (m, 1H), 2.09 (ddd, *J* = 13.2, 8.9, 4.5 Hz, 1H), 1.01 (s, 9H). 4 exchangeable protons not seen.

^{13}C NMR (126 MHz, MeOH): δ = 175.28, 174.55, 152.80, 149.01, 140.24, 133.38, 131.48, 130.36, 128.94, 71.07, 61.21, 60.69, 57.69, 43.68, 38.91, 36.53, 26.77, 15.82.

(2*S*,4*R*)-1-((*S*)-2-(hept-6-ynamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

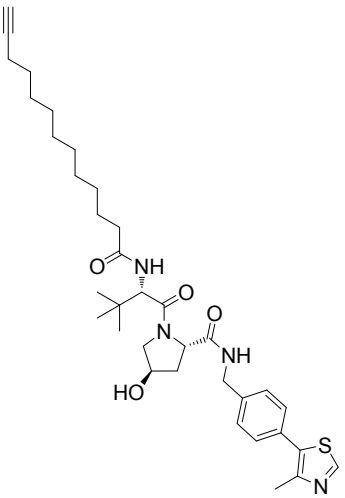


To a stirred solution of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (2.50 g, 5.81 mmol) in DMF (12 mL) was added hept-6-ynoic acid (0.732 g, 5.81 mmol), DIPEA (2.03 mL, 11.6 mmol) and HATU (2.43 g, 6.39 mmol). The resulting solution was stirred for 4 h at 25 °C. The reaction mixture was diluted with water (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, washed with brine (2 × 30 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica chromatography, eluting with DCM:MeOH (5% MeOH). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (1.00 g, 32%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.97 (s, 1H), 8.54 - 8.49 (m, 1H), 7.82 (d, *J*=9.3 Hz, 1H), 7.43 - 7.35 (m, 4H), 5.08 (d, *J*=3.5 Hz, 1H), 4.53 (d, *J*=9.3 Hz, 1H), 4.45 - 4.38 (m, 2H), 4.34 (br. s., 1H), 4.21 (dd, *J*=5.4, 15.7 Hz, 1H), 3.69 - 3.60 (m, 2H), 2.71 (t, *J*=2.6 Hz, 1H), 2.43 (s, 3H), 2.25 (dd, *J*=7.2, 14.4 Hz, 1H), 2.17 - 2.10 (m, 3H), 2.06 - 1.98 (m, 1H), 1.94 - 1.85 (m, 1H), 1.61 - 1.50 (m, 2H), 1.45 - 1.36 (m, 2H), 0.93 (s, 9H).

LC-MS (System B): calc for $C_{29}H_{38}N_4O_4S$ = 538.2, found [M+H$^+$] = 539.2, tR = 0.96 min.

(2*S*,4*R*)-1-((*S*)-3,3-dimethyl-2-(tridec-12-ynamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

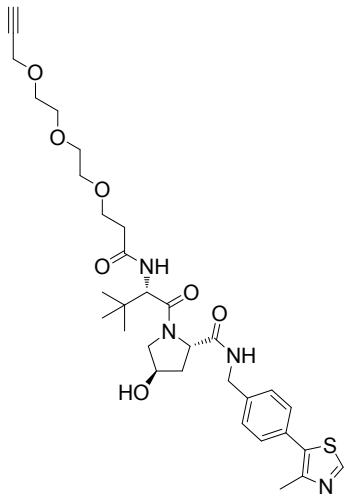


To a stirred solution of tridec-12-ynoic acid (901 mg, 4.28 mmol) and HATU (2.12 g, 5.57 mmol) in DMF (15 mL) at 25 °C was added DIPEA (1.87 mL, 10.7 mmol). This was stirred for 5 min, followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, hydrochloride (2.00 g, 4.28 mmol). The resulting solution was stirred for 16 h at 25 °C. The reaction mixture was diluted with water (30 mL), extracted with EtOAc (3 × 30 mL), the organic phases combined, washed with brine (30 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica chromatography, eluting with DCM:MeOH (0 – 2% MeOH). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (1.20 g, 45%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.98 (s, 1H), 8.52 (t, *J*=6.0 Hz, 1H), 7.79 (d, *J*=9.3 Hz, 1H), 7.45 - 7.35 (m, 4H), 5.09 (d, *J*=3.4 Hz, 1H), 4.54 (d, *J*=9.3 Hz, 1H), 4.48 - 4.39 (m, 2H), 4.35 (br. s., 1H), 4.22 (dd, *J*=5.5, 15.8 Hz, 1H), 3.70 - 3.60 (m, 2H), 2.70 (t, *J*=2.6 Hz, 1H), 2.44 (s, 3H), 2.30 - 2.20 (m, 1H), 2.17 - 1.98 (m, 5H), 1.91 (ddd, *J*=4.5, 8.3, 12.8 Hz, 1H), 1.43 (br. s., 5H), 1.37 - 1.18 (m, 10H), 0.96 - 0.91 (m, 9H).

LC-MS (System B): calc for $C_{35}H_{50}N_4O_4S$ = 622.4, found [M+H$^+$] = 623.2, tR = 1.30 min.

(2*S*,4*R*)-1-((*S*)-2-(tert-butyl)-4-oxo-7,10,13-trioxa-3-azahexadec-15-yn-1-oyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

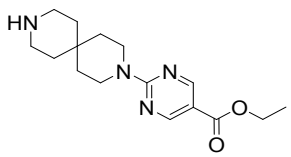


To a stirred solution of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, hydrochloride (750 mg, 1.61 mmol) in DMF (4 mL) was added 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoic acid (347 mg, 1.61 mmol), DIPEA (0.560 mL, 3.21 mmol) and HATU (611 mg, 1.61 mmol). The resulting solution was stirred for 1 h at 25 ^{o}C. The reaction mixture was diluted with DCM (100 mL) and washed with sat. aq. $NaHCO_3$ (100 mL) and sat. aq. LiCl (100 mL). The organic phase was separated, passed through a hydrophobic frit and concentrated *in vacuo* to give a brown oil. The residue was purified by silica chromatography, eluting with DCM:MeOH (0 – 7% MeOH). The appropriate fractions were combined and concentrated *in vacuo* to afford a brown gum. The residue was repurified by reverse phase chromatography (Method A), eluting with water + 0.1% formic acid:MeCN + 0.1% formic acid (5 – 55% MeCN). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound as an off yellow solid (324 mg, 32%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.98 (s, 1H), 8.53 (t, *J*=6.0 Hz, 1H), 7.88 (d, *J*=9.3 Hz, 1H), 7.44 - 7.36 (m, 4H), 4.55 (d, *J*=9.5 Hz, 1H), 4.47 - 4.39 (m, 2H), 4.35 (br. s., 1H), 4.22 (dd, *J*=5.4, 15.9 Hz, 1H), 4.13 (d, *J*=2.4 Hz, 2H), 3.71 - 3.44 (m, 14H), 3.39 (t, *J*=2.3 Hz, 1H), 2.45 - 2.43 (m, 3H), 2.40 - 2.31 (m, 1H), 2.07 - 1.99 (m, 1H), 1.95 - 1.86 (m, *J*=4.6, 8.4, 12.8 Hz, 1H), 0.96 - 0.90 (m, 9H).

LC-MS (System A): calc for $C_{32}H_{44}N_4O_7S$ =628.3, found [M+H$^+$] = 629.3, tR = 0.87 min.

ethyl 2-(3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylate

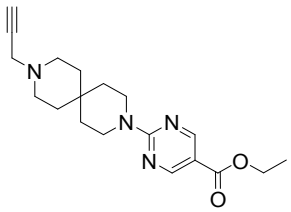


To a stirred solution of *tert*-butyl 3,9-diazaspiro[5.5]undecane-3-carboxylate (50.0 g, 197 mmol) and triethylamine (54.8 ml, 393 mmol) in DCM (700 mL) was added ethyl 2-chloropyrimidine-5-

carboxylate (36.7 g, 197 mmol). The resulting solution was stirred for 16 h at 25 °C. The reaction mixture was washed with water (3 × 500 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford crude *tert*-butyl 9-(5-(ethoxycarbonyl)pyrimidin-2-yl)-3,9-diazaspiro[5.5]undecane-3-carboxylate (96.0 g, 197 mmol). To a stirred solution of *tert*-butyl 9-(5-(ethoxycarbonyl)pyrimidin-2-yl)-3,9-diazaspiro[5.5]undecane-3-carboxylate (96.0 g, 197 mmol) in DCM (300 mL) was added HCl (4M in MeOH, 300 ml, 1200 mmol). The resulting solution was stirred for 16 h at 25 °C, then the reaction mixture was concentrated *in vacuo*. The residue was diluted with sat. aq. $NaHCO_3$ until pH = 8, extracted with DCM (10 × 500 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the title compound (57.0 g, 95%).

LC-MS (System C): calc for $C_{16}H_{24}N_4O_2$ = 304.2, found [M+H$^+$] = 305.2, tR = 1.15 min.

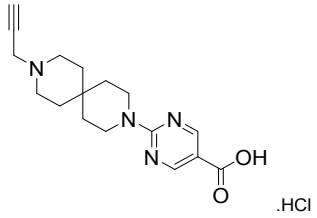
ethyl 2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylate



To a stirred solution of ethyl 2-(3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylate (57.0 g, 187 mmol) in THF (1000 mL) at 0 °C was added potassium carbonate (25.9 g, 187 mmol) followed by the slow addition of 3-bromoprop-1-yne (22.3 g, 187 mmol). The resulting solution was stirred for 16 h at 25 °C, then the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (1800 mL), washed with water (7 × 500 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford the title compound (42.0 g, 66%).

LC-MS (System C): calc for $C_{19}H_{26}N_4O_2$ = 342.2, found [M+H$^+$] = 343.1, tR = 1.17 min.

2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylic acid hydrochloride



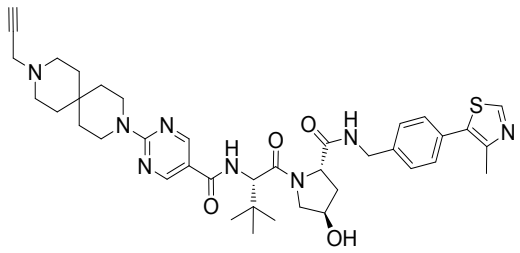
To a stirred solution of ethyl 2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylate (22.3 g, 65.1 mmol) in THF (150 mL) and MeOH (150 mL) was added aq. NaOH (1M, 150

mL, 150 mmol). The resulting solution was stirred for 3 h at 25 °C, before aq. HCl (1M, 160 mL) was added until pH = 5. The reaction mixture was concentrated *in vacuo*, washed with water (200 mL), dried over Na2SO4, filtered and concentrated in vacuo to afford the title compound (19.8 g, 97%).

^{1}H NMR (400 MHz, DMSO-d6) δ = 12.91 - 12.60 (m, 1H), 11.28 - 11.00 (m, 1H), 8.75 (s, 2H), 4.12 - 4.04 (m, 2H), 3.91 - 3.80 (m, 5H), 3.33 (br s, 2H), 3.22 - 3.03 (m, 2H), 1.91 (br d, J = 1.5 Hz, 2H), 1.81 - 1.58 (m, 4H), 1.42 (br s, 2H).

LC-MS (System B): calc for $C_{17}H_{22}N_4O_2$ = 314.2, found [M+H$^+$] = 315.1, tR = 0.56 min.

_N_-((_S_)-1-((2_S_,4_R_)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxamide

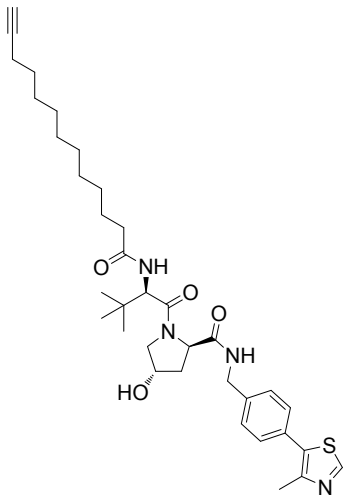


To a stirred solution of 2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylic acid (1.00 g, 3.18 mmol) in DMF (5 mL) at 25 °C was added DIPEA (1.67 mL, 9.54 mmol) and HATU (1.45 g, 3.82 mmol). This was stirred for 5 min, followed by addition of (2*S*,4*R*)-1-((*S*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, hydrochloride (1.56 g, 3.34 mmol). The resulting solution was stirred for 1 h at 25 °C. The reaction mixture was purified directly by reverse phase chromatography (Method A), eluting with water + 10mM ammonium bicarbonate:MeCN + 0.1% NH3 (10 – 65% MeCN). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (1.89 g, 82%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.97 (s, 1H), 8.77 (s, 2H), 8.53 (t, *J*=6.1 Hz, 1H), 8.02 (d, *J*=9.0 Hz, 1H), 7.43 - 7.36 (m, 4H), 5.11 (d, *J*=3.7 Hz, 1H), 4.72 (d, *J*=9.0 Hz, 1H), 4.47 - 4.34 (m, 3H), 4.27 - 4.19 (m, 1H), 3.83 - 3.78 (m, 4H), 3.71 (d, *J*=2.4 Hz, 2H), 3.24 (d, *J*=2.4 Hz, 2H), 3.10 (t, *J*=2.3 Hz, 1H), 2.45 - 2.40 (m, 7H), 2.02 (dd, *J*=2.4, 7.6 Hz, 1H), 1.91 (ddd, *J*=4.6, 8.4, 12.8 Hz, 1H), 1.54 - 1.47 (m, 4H), 1.45 - 1.38 (m, 4H), 1.00 (s, 9H).

LC-MS (System B): calc for $C_{39}H_{50}N_8O_4S$ = 726.4, found [M+H$^+$] = 727.3, tR = 1.06 min.

(2*S*,4*R*)-1-((*S*)-3,3-dimethyl-2-(tridec-12-ynamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide

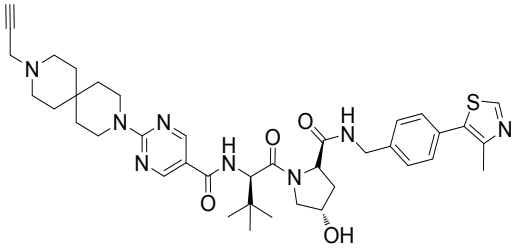


To a stirred solution of tridec-12-ynoic acid (108 mg, 0.514 mmol) in DMF (2 mL) at 25 °C was added DIPEA (0.299 mL, 1.71 mmol) and HATU (195 mg, 0.514 mmol). This was stirred for 5 min, followed by addition of (2*R*,4*S*)-1-((*R*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, hydrochloride (200 mg, 0.428 mmol). The resulting solution was stirred for 3 h at 25 °C, then the reaction mixture was blown down under a stream of nitrogen. The residue was diluted with 10% aq. LiCl (20 mL), extracted with EtOAc (3 × 20 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method B). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (120 mg, 45%)

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.97 (s, 1H), 8.52 (s, 1H), 7.79 (d, *J*=9.3 Hz, 1H), 7.46 - 7.30 (m, 4H), 5.09 (d, *J*=3.4 Hz, 1H), 4.54 (d, *J*=9.3 Hz, 1H), 4.47 - 4.38 (m, 2H), 4.34 (br. s., 1H), 4.21 (dd, *J*=5.6, 15.9 Hz, 1H), 3.72 - 3.59 (m, 2H), 2.69 (t, *J*=2.7 Hz, 1H), 2.46 - 2.41 (m, 3H), 2.31 - 2.20 (m, 1H), 2.17 - 2.08 (m, 3H), 2.06 (s, 3H), 1.96 - 1.85 (m, 1H), 1.53 - 1.37 (m, 4H), 1.27 - 1.20 (m, 10H), 0.98 - 0.88 (m, 9H).

LC-MS (System B): calc for $C_{35}H_{50}N_4O_4S$ = 622.4, found [M+H$^+$] = 623.9, tR = 1.28 min.

***N*-((*R*)-1-((2*S*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxamide**

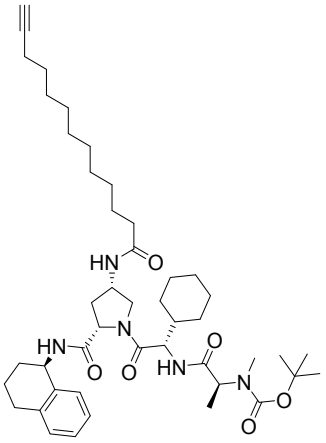


To a stirred solution of 2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxylic acid (241 mg, 0.766 mmol) in DMF (2 mL) at 25 °C was added DIPEA (0.487 mL, 2.79 mmol) and HATU (318 mg, 0.836 mmol). This was stirred for 5 min, followed by addition of (2*R*,4*S*)-1-((*R*)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (300 mg, 0.697 mmol). The resulting solution was stirred for 16 h at 25 °C. The reaction mixture was purified directly by reverse phase chromatography (Method A), eluting with water + 10mM ammonium bicarbonate:MeCN + 0.1% NH3 (10 – 65% MeCN). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (388 mg, 77%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.98 (s, 1H), 8.78 (s, 2H), 8.54 (t, *J*=6.0 Hz, 1H), 8.03 (d, *J*=9.0 Hz, 1H), 7.44 - 7.37 (m, 4H), 5.12 (d, *J*=3.4 Hz, 1H), 4.73 (d, *J*=9.0 Hz, 1H), 4.48 - 4.35 (m, 3H), 4.28 - 4.20 (m, 1H), 3.80 (d, *J*=5.6 Hz, 4H), 3.72 (d, *J*=2.4 Hz, 2H), 3.25 (d, *J*=2.2 Hz, 2H), 3.11 (t, *J*=2.4 Hz, 1H), 2.46 - 2.42 (m, 7H), 2.08 - 1.99 (m, 1H), 1.92 (ddd, *J*=4.6, 8.4, 12.8 Hz, 1H), 1.54 - 1.49 (m, 4H), 1.45 - 1.40 (m, 4H), 1.01 (s, 9H).

LC-MS (System B): calc for $C_{39}H_{50}N_8O_4S$ = 726.4, found [M+H$^+$] = 727.5, tR = 1.05 min.

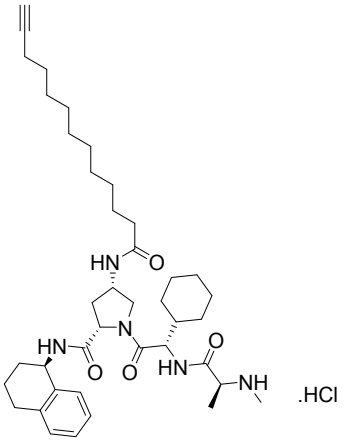
***tert*-butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-oxo-2-((2*S*,4*S*)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)-4-(tridec-12-ynamido)pyrrolidin-1-yl)ethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate**



To a stirred solution of tridec-12-ynoic acid (360 mg, 1.71 mmol) in DMF (8 mL) at 25 °C was added DIPEA (0.598 mL, 3.42 mmol) and HATU (846 mg, 2.23 mmol). This was stirred for 10 min, followed by addition of *tert*-butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-amino-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (999 mg, 1.71 mmol). The resulting solution was stirred for 16 h at 25 °C. The reaction mixture was diluted with water (50 mL), extracted with EtOAc (3 × 50 mL), the organic phases combined, washed with brine (50 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica chromatography, eluting with DCM:MeOH (0 – 2% MeOH). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (1.20 g, 90%).

LC-MS (System C): calc for $C_{45}H_{69}N_5O_6$ = 775.5, found [M+H$^+$] = 776.4, tR = 2.18 min.

tert-butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-oxo-2-((2*S*,4*S*)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)-4-(tridec-12-ynamido)pyrrolidin-1-yl)ethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate, hydrochloride

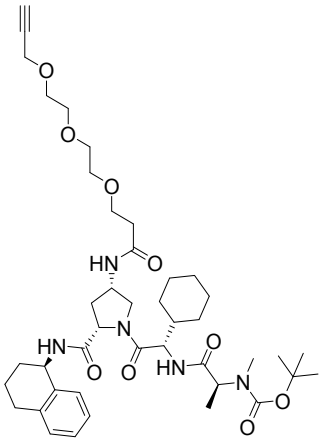


To a stirred solution of *tert*-butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-oxo-2-((2*S*,4*S*)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)-4-(tridec-12-ynamido)pyrrolidin-1-yl)ethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (1.20 g, 1.55 mmol) in Methanol (6 mL) at 0 °C was added HCl (4M in MeOH, 6 mL, 197 mmol) portion wise. The resulting solution was stirred for 2 h at 25 °C. The reaction mixture was concentrated *in vacuo* to afford the title compound (1.00 g, 91%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.80 - 8.70 (m, 2H), 8.40 (d, *J*=8.8 Hz, 1H), 8.13 (d, *J*=7.8 Hz, 1H), 7.30 (d, *J*=7.3 Hz, 1H), 7.15 (d, *J*=7.0 Hz, 1H), 7.10 (t, *J*=6.5 Hz, 2H), 4.95 (br. s., 1H), 4.40 (t, *J*=8.2 Hz, 1H), 4.34 - 4.25 (m, 2H), 4.13 - 4.06 (m, 1H), 3.84 (d, *J*=6.8 Hz, 1H), 2.77 - 2.70 (m, 3H), 2.69 - 2.66 (m, 1H), 2.34 (s, 1H), 2.14 (dt, *J*=2.8, 6.9 Hz, 2H), 2.06 (t, *J*=7.3 Hz, 2H), 1.95 - 1.83 (m, 3H), 1.80 - 1.59 (m, 10H), 1.54 - 1.40 (m, 6H), 1.33 (d, *J*=7.0 Hz, 6H), 1.26 (m, 14H).

LC-MS (System B): calc for $C_{40}H_{61}N_5O_4$ = 675.5, found [M+H$^+$] = 676.4, tR = 1.51 min.

***tert*-butyl((*S*)-1-(((*S*)-1-cyclohexyl-2-oxo-2-((2*S*,4*S*)-4-(3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanamido)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)ethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate**

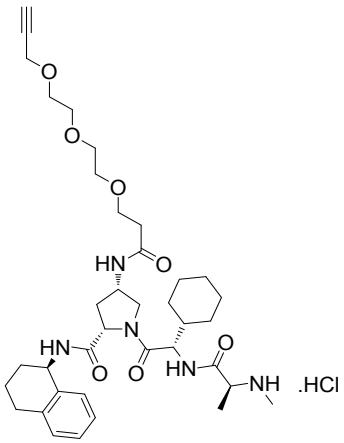


To a stirred solution of *tert*-butyl ((*S*)-1-(((*S*)-2-((2*S*,4*S*)-4-amino-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)-1-cyclohexyl-2-oxoethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate, hydrochloride (500 mg, 0.806 mmol) in DMF (3.5 mL) at 25 °C was added 3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanoic acid (209 mg, 0.967 mmol), DIPEA (0.422 mL, 2.42 mmol) and HATU (368 mg, 0.967 mmol). The resulting solution was stirred for 1 h at 25 °C. The reaction mixture was diluted with DCM (50 mL) and washed with sat. aq. $NaHCO_3$ (50 mL) and brine (50 mL). The organic phase was separated, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by reverse phase chromatography (Method A), eluting with water + 0.1% formic acid:MeCN + 0.1% formic acid (30 – 65% MeCN). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (500 mg, 79%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.35 (d, *J*=8.6 Hz, 1H), 8.15 (s, 1H), 7.31 (d, *J*=7.6 Hz, 1H), 7.16 - 7.06 (m, 3H), 4.93 (d, *J*=4.4 Hz, 1H), 4.66 - 4.42 (m, 1H), 4.40 - 4.21 (m, 3H), 4.14 (d, *J*=2.4 Hz, 2H), 4.08 (dd, *J*=7.2, 9.7 Hz, 1H), 3.61 (t, *J*=6.5 Hz, 2H), 3.56 - 3.52 (m, 4H), 3.52 - 3.46 (m, 4H), 3.39 (t, *J*=2.4 Hz, 1H), 3.34 - 3.24 (m, 1H), 2.76 - 2.72 (m, 4H), 2.38 (dd, *J*=4.9, 7.3 Hz, 1H), 2.31 (t, *J*=6.4 Hz, 2H), 2.07 (s, 2H), 1.91 - 1.82 (m, 3H), 1.80 - 1.56 (m, 8H), 1.44 - 1.31 (m, 9H), 1.28 - 1.09 (m, 6H), 1.06 - 0.86 (m, 2H).

LC-MS (System A): calc for $C_{42}H_{63}N_5O_9$ = 781.5, found [M+H$^+$] = 782.5, tR = 1.30 min.

(2*S*,4*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-4-(3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanamido)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)pyrrolidine-2-carboxamide, hydrochloride

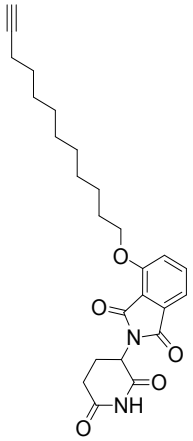


To a stirred solution of *tert*-butyl ((*S*)-1-(((*S*)-1-cyclohexyl-2-oxo-2-((2*S*,4*S*)-4-(3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanamido)-2-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-1-yl)ethyl)amino)-1-oxopropan-2-yl)(methyl)carbamate (500 mg, 0.639 mmol) in 1,4-Dioxane (1.314 mL) at 25 °C was added HCl (4 M in 1,4-dioxane, 1.60 mL, 6.39 mmol). The resulting solution was stirred for 2 h at 25 °C. The reaction mixture was blown down under a stream of nitrogen to give the title compound (440 mg, 96%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 9.41 (d, *J*=4.4 Hz, 1H), 8.86 (d, *J*=8.1 Hz, 1H), 8.78 (d, *J*=8.1 Hz, 1H), 8.42 - 8.35 (m, 1H), 8.26 (d, *J*=7.3 Hz, 1H), 7.34 - 7.28 (m, 1H), 7.20 - 7.07 (m, 4H), 5.00 - 4.88 (m, 1H), 4.39 (t, *J*=8.1 Hz, 1H), 4.33 - 4.22 (m, 2H), 4.19 - 4.09 (m, 3H), 3.90 - 3.79 (m, 1H), 3.65 (t, *J*=6.5 Hz, 2H), 3.60 - 3.55 (m, 3H), 3.42 (t, *J*=2.3 Hz, 1H), 3.33 - 3.26 (m, 3H), 2.74 (d, *J*=5.4 Hz, 2H), 2.49 - 2.44 (m, 3H), 2.34 (t, *J*=6.4 Hz, 2H), 1.96 - 1.54 (m, 13H), 1.43 - 1.32 (m, 3H), 1.26 - 0.96 (m, 6H).

LC-MS (System B): calc for $C_{37}H_{55}N_5O_7$ = 681.4, found [M+H$^+$] = 682.5, tR = 1.08 min.

2-(2,6-dioxopiperidin-3-yl)-4-(dodec-11-yn-1-yloxy)isoindoline-1,3-dione

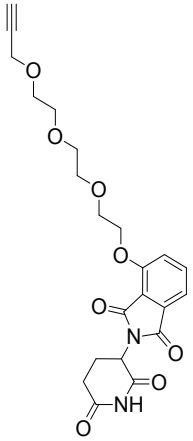


To a stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (1.96 g, 7.13 mmol) and triphenylphosphine (2.24 g, 8.56 mmol) in THF (20 mL) at -10 °C under an argon atmosphere was added DIAD (1.66 mL, 8.56 mmol) dropwise. The reaction mixture was stirred for 30 min at -10 °C, followed by the slow addition of dodec-11-yn-1-ol (1.30 g, 7.13 mmol) in THF (10 mL). The resulting solution was slowly warmed to 25 °C and stirred for 16 h. The reaction mixture was concentrated *in vacuo*. This was purified by silica chromatography, eluting with Pet. Ether:EtOAc (20 % EtOAc). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (1.30 g, 38%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 11.01 (br. s., 1H), 7.80 (dd, *J*=7.3, 8.6 Hz, 1H), 7.51 (d, *J*=8.6 Hz, 1H), 7.43 (d, *J*=7.1 Hz, 1H), 5.07 (dd, *J*=5.4, 13.0 Hz, 1H), 4.20 (t, *J*=6.5 Hz, 2H), 2.94 - 2.82 (m, 1H), 2.70 (t, *J*=2.6 Hz, 1H), 2.63 - 2.55 (m, 1H), 2.13 (dt, *J*=2.6, 6.9 Hz, 2H), 2.07 - 1.98 (m, 1H), 1.80 - 1.70 (m, 2H), 1.50 - 1.22 (m, 15H).

LC-MS (System B): calc for $C_{25}H_{30}N_2O_5$ = 438.2, found [M+H$^+$] = 439.2, tR = 1.38 min.

2-(2,6-dioxopiperidin-3-yl)-4-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)isoindoline-1,3-dione



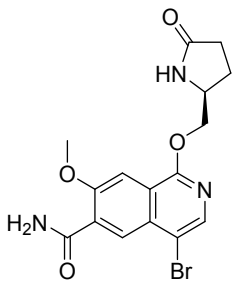
To a stirred solution of 2-(2,6-dioxopiperidin-3-yl)-4-hydroxyisoindoline-1,3-dione (500 mg, 1.82 mmol) and triphenylphosphine (956 mg, 3.65 mmol) in THF (18.2 mL) and DMF (0.912 mL) at 0 °C was added 2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethanol (343 mg, 1.82 mmol). The reaction was degassed and backfilled with nitrogen (× 3) followed by the addition of DIAD (0.709 mL, 3.65 mmol) dropwise. The resulting solution was slowly warmed to 25 °C and stirred for 16 h. The reaction mixture was concentrated *in vacuo*, then diluted with DCM (100 mL), washed with water (100 mL) and brine (100 mL) then concentrated *in vacuo*. The residue was purified by reverse phase chromatography (Method A), eluting with water + 0.1% formic acid:MeCN + 0.1% formic acid (5 – 40% MeCN). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (148 mg, 18%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 11.07 (br. s., 1H), 7.81 (dd, *J*=7.3, 8.6 Hz, 1H), 7.54 (d, *J*=8.6 Hz, 1H), 7.46 (d, *J*=7.3 Hz, 1H), 5.08 (dd, *J*=5.4, 12.7 Hz, 1H), 4.39 - 4.32 (m, 2H), 4.13 (d, *J*=2.2 Hz, 2H), 3.84 - 3.78 (m, 2H), 3.65 (dd, *J*=3.8, 5.7 Hz, 2H), 3.56 - 3.52 (m, 5H), 3.39 (t, *J*=2.3 Hz, 1H), 2.95 - 2.83 (m, 1H), 2.65 - 2.53 (m, 2H), 2.08 - 1.99 (m, 1H), 1.20 (dd, *J*=6.4, 13.0 Hz, 1H).

LC-MS (System A): calc for $C_{22}H_{24}N_2O_8$ = 444.2, found [M+H$^-$] = 443.2, tR = 0.78 min.

(*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 1

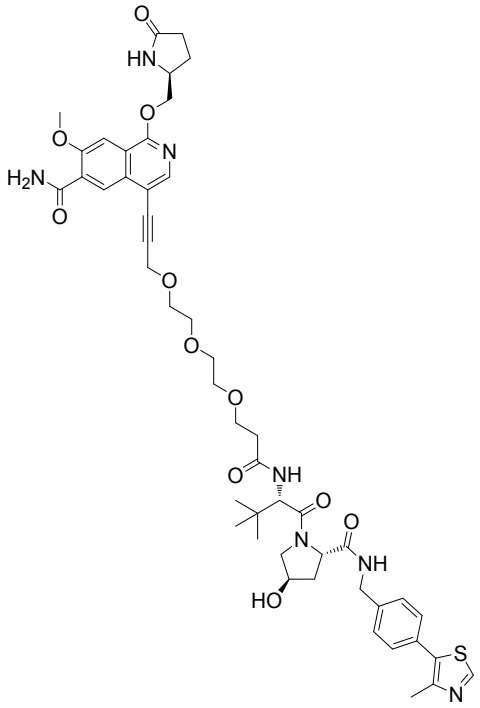
For prep. see WO 2015/150995 A1



^{1}H NMR (600 MHz, DMSO-d_6) δ = 8.26 (s, 1H), 8.15 - 8.11 (m, 2H), 7.92 (s, 1H), 7.82 (br s, 1H), 7.70 (s, 1H), 4.49 (dd, J = 3.9, 10.8 Hz, 1H), 4.29 (dd, J = 6.9, 10.8 Hz, 1H), 4.06 - 4.02 (m, 1H), 4.04 (s, 3H), 2.34 - 2.27 (m, 1H), 2.28 - 2.22 (m, 1H), 2.22 - 2.16 (m, 1H), 1.93 - 1.86 (m, 1H).

LC-MS (System A): calc for $C_{16}H_{16}BrN_3O_4$ = 393.0, found [M+H$^+$] = 394.0, tR = 0.75 min.

4-((*S*)-15-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-16,16-dimethyl-13-oxo-4,7,10-trioxa-14-azaheptadec-1-yn-1-yl)-7-methoxy-1-(((S)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 2

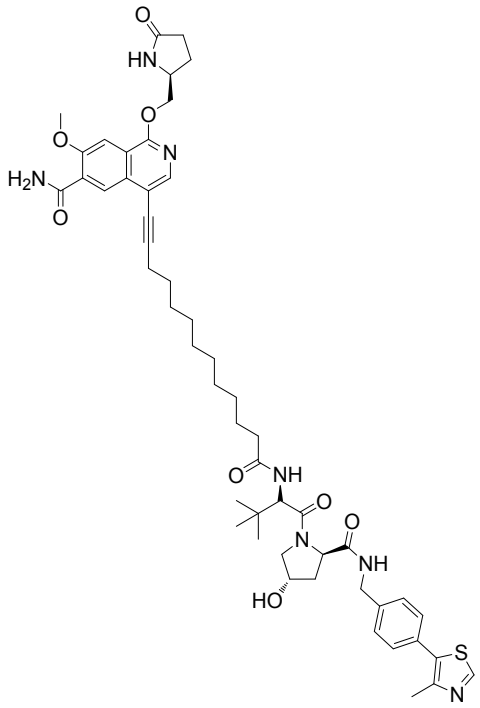


(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-4-oxo-7,10,13-trioxa-3-azahexadec-15-yn-1-oyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (28.7 mg, 0.0460 mmol), potassium carbonate (12.6 mg, 0.0910 mmol), (S)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (12.0 mg, 0.0300 mmol), XPhos (2.00 mg, 4.20 µmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 µmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 1 h. The reaction mixture was then cooled to 25 °C and further XPhos (4.00 mg, 8.39 µmol) and $Pd_2(dba)_3$ (4.00 mg, 4.37 µmol) were added and The reaction mixture was heated to 100 °C for a further 2 h. The reaction mixture was then cooled to 25 °C, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method A). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (10.3 mg, 36%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.97 (s, 1H), 8.57 - 8.47 (m, 1H), 8.32 (s, 1H), 8.13 (s, 2H), 7.94 - 7.84 (m, 2H), 7.77 (br. s., 1H), 7.69 (s, 1H), 7.44 - 7.34 (m, 4H), 4.57 - 4.49 (m, 4H), 4.46 - 4.38 (m, 2H), 4.37 - 4.29 (m, 2H), 4.22 (dd, *J*=5.3, 15.8 Hz, 1H), 4.08 - 3.97 (m, 4H), 3.72 - 3.48 (m, 14H), 2.44 (s, 3H), 2.38 - 2.31 (m, 1H), 2.22 (m, 2H), 2.08 - 2.00 (m, 2H), 1.95 - 1.85 (m, 2H), 0.92 (s, 9H).

LC-MS (System A): calc for $C_{48}H_{59}N_7O_{11}S$ = 941.4, found [M+H$^+$] = 942.4, tR = 0.87 min.

4-(13-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-13-oxotridec-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 3

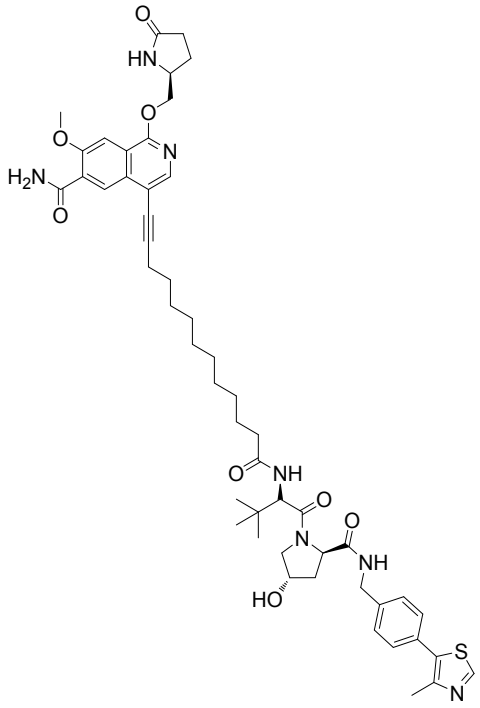


(2*S*,4*R*)-1-((*S*)-3,3-dimethyl-2-(tridec-12-ynamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (85.0 mg, 0.137 mmol), potassium carbonate (28.4 mg, 0.205 mmol), (*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (27.0 mg, 0.0680 mmol), XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 μmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 1.5 h. The reaction mixture was then cooled to 25 °C and further XPhos (4.00 mg, 8.39 μmol) and $Pd_2(dba)_3$ (4.00 mg, 4.37 μmol) were added. The reaction mixture was heated for a further 2 h. The reaction mixture was then cooled to 25 °C, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method A). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (7 mg, 11%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.97 (s, 1H), 8.52 (t, *J*=6.1 Hz, 1H), 8.33 (s, 1H), 8.12 (s, 1H), 8.03 (s, 1H), 7.87 (br. s., 1H), 7.79 (d, *J*=9.3 Hz, 1H), 7.73 (br. s., 1H), 7.67 (s, 1H), 7.43 - 7.36 (m, 4H), 5.10 (br. s., 1H), 4.56 - 4.47 (m, 2H), 4.46 - 4.38 (m, 2H), 4.37 - 4.27 (m, 2H), 4.23 (s, 1H), 4.08 - 3.96 (m, 5H), 3.70 - 3.60 (m, 3H), 2.59 - 2.53 (m, 3H), 2.44 (s, 3H), 2.35 - 1.98 (m, 4H), 1.90 (ddd, *J*=4.6, 8.2, 12.8 Hz, 2H), 1.67 - 1.56 (m, 3H), 1.46 (d, *J*=8.1 Hz, 4H), 1.37 - 1.18 (m, 8H), 0.92 (s, 9H).

LC-MS (System A): calc for $C_{51}H_{65}N_7O_8S$ = 935.5, found [M+H$^+$] = 936.5, tR = 1.18 min.

4-(13-(((*S*)-1-((2*R*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-13-oxotridec-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide

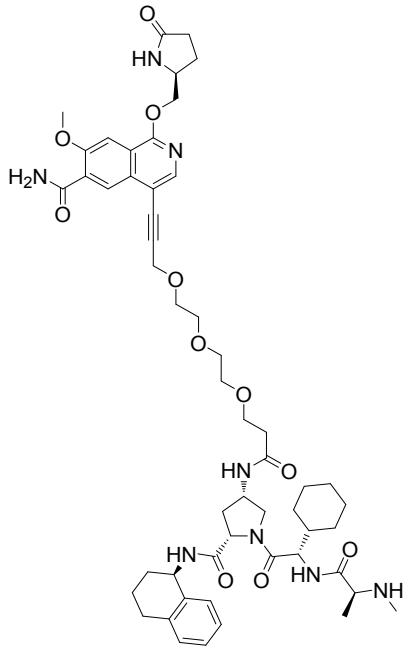


(2*R*,4*S*)-1-((*R*)-3,3-dimethyl-2-(tridec-12-ynamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (47.4 mg, 0.0760 mmol), (*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (25.0 mg, 0.0630 mmol), potassium carbonate (26.3 mg, 0.190 mmol), XPhos (3.93 mg, 8.24 μmol) and $Pd_2(dba)_3$ (4.07 mg, 4.44 μmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 2 h. The reaction mixture was concentrated under a stream of nitrogen. The residue was diluted with 10% aq. LiCl (20 mL), extracted with EtOAc (3 × 30 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method A). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (11.4 mg, 19%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.96 (s, 1H), 8.52 (t, *J*=6.0 Hz, 1H), 8.32 (s, 1H), 8.19 (s, 1H), 8.11 (s, 1H), 8.02 (s, 1H), 7.87 (br. s., 1H), 7.79 (d, *J*=9.3 Hz, 1H), 7.75 - 7.70 (m, 1H), 7.66 (s, 1H), 7.43 - 7.35 (m, 4H), 4.53 (d, *J*=9.5 Hz, 2H), 4.42 (s, 2H), 4.36 - 4.26 (m, 2H), 4.21 (dd, *J*=5.4, 15.9 Hz, 1H), 4.00 (s, 3H), 3.68 - 3.61 (m, 4H), 2.55 (t, *J*=7.0 Hz, 2H), 2.43 (s, 3H), 2.34 - 2.19 (m, 4H), 2.04 - 1.98 (m, 1H), 1.95 - 1.85 (m, 2H), 1.66 - 1.56 (m, 2H), 1.46 (d, *J*=6.4 Hz, 4H), 1.33 - 1.21 (m, 10H), 0.92 (s, 9H).

LC-MS (System A): calc for $C_{51}H_{65}N_7O_8S$ = 935.5, found [M+H$^+$] = 936.7, tR = 1.20 min.

4-(3-(2-(2-(3-(((3*S*,5*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-5-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-3-yl)amino)-3-oxopropoxy)ethoxy)ethoxy)prop-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 6



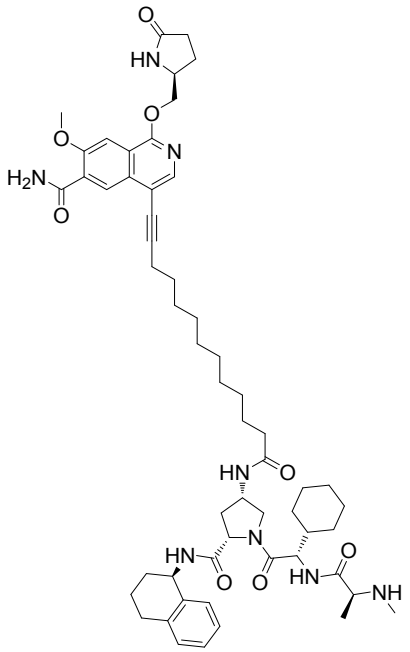
(2*S*,4*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-4-(3-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)propanamido)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)pyrrolidine-2-carboxamide (59.2 mg, 0.0820 mmol), potassium carbonate (12.6 mg, 0.0910 mmol), (*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (12.0 mg, 0.0300 mmol), XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 μmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 1 h. The reaction mixture was then cooled to 25 °C and further $Pd_2(dba)_3$ (4.00 mg, 4.37 μmol) was added. The reaction mixture was heated to

100 °C for a further 2 h. The reaction mixture was then cooled to 25 °C, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method B). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (4.8 mg, 8%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.38 - 8.34 (m, 1H), 8.32 (s, 1H), 8.19 - 8.15 (m, 1H), 8.14 (s, 2H), 7.90 (d, *J*=4.4 Hz, 2H), 7.77 (br. s., 1H), 7.70 (s, 1H), 7.31 (d, *J*=7.3 Hz, 1H), 7.18 - 7.04 (m, 3H), 4.98 - 4.89 (m, 1H), 4.55 (s, 2H), 4.52 (d, *J*=3.7 Hz, 1H), 4.43 - 4.21 (m, 4H), 4.15 - 3.99 (m, 5H), 3.75 - 3.68 (m, 2H), 3.65 - 3.58 (m, 4H), 3.57 - 3.49 (m, 4H), 3.02 - 2.93 (m, 1H), 2.76 - 2.66 (m, 2H), 2.43 - 2.15 (m, 9H), 1.94 - 1.80 (m, 4H), 1.78 - 1.55 (m, 9H), 1.26 - 0.89 (m, 9H).

LC-MS (System B): calc for $C_{53}H_{70}N_8O_{11}$ = 994.5, found [M+H$^+$] = 995.4, tR = 1.03 min.

4-(13-(((3*S*,5*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-5-(((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)carbamoyl)pyrrolidin-3-yl)amino)-13-oxotridec-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 7.



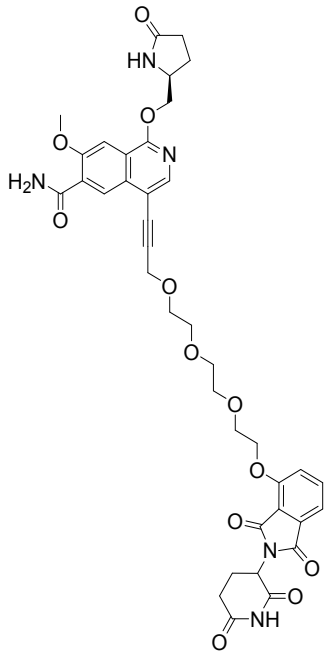
(2*S*,4*S*)-1-((*S*)-2-cyclohexyl-2-((*S*)-2-(methylamino)propanamido)acetyl)-*N*-((*R*)-1,2,3,4-tetrahydronaphthalen-1-yl)-4-(tridec-12-ynamido)pyrrolidine-2-carboxamide (55.7 mg, 0.0820 mmol), potassium carbonate (12.6 mg, 0.0910 mmol), (*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (12.0 mg, 0.0300 mmol), XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 μmol) were added to a microwave vial followed by DMA (1 mL). The vial

was sealed and heated to 100 °C for 1 h. The reaction mixture was then cooled to 25 °C and further $Pd_2(dba)_3$ (4.00 mg, 4.37 μmol) was added. The reaction mixture was heated to 100 °C for a further 2 h. The reaction mixture was then cooled to 25 °C, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method B). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (5.2 mg, 8%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.40 (d, *J*=8.6 Hz, 1H), 8.34 (s, 1H), 8.15 - 8.09 (m, 2H), 8.04 (s, 1H), 7.93 - 7.86 (m, 2H), 7.74 (br. s., 1H), 7.68 (s, 1H), 7.32 (d, *J*=7.1 Hz, 1H), 7.18 - 7.06 (m, 3H), 4.98 - 4.90 (m, 1H), 4.51 (dd, *J*=3.9, 11.0 Hz, 1H), 4.39 (t, *J*=8.1 Hz, 1H), 4.35 - 4.26 (m, 3H), 4.09 - 4.00 (m, 5H), 3.33 (br. s., 1H), 2.96 (q, *J*=6.9 Hz, 1H), 2.73 (d, *J*=5.4 Hz, 2H), 2.56 (t, *J*=7.0 Hz, 2H), 2.42 - 2.31 (m, 2H), 2.30 - 2.26 (m, 1H), 2.25 - 2.21 (m, 1H), 2.20 - 2.16 (m, 3H), 2.05 (t, *J*=7.2 Hz, 2H), 1.94 - 1.80 (m, 4H), 1.77 - 1.57 (m, 11H), 1.49 (br. s., 4H), 1.36 - 1.22 (m, 11H), 1.14 (d, *J*=12.0 Hz, 2H), 1.10 (d, *J*=6.8 Hz, 3H), 1.03 - 0.92 (m, 2H).

LC-MS (System B): calc for $C_{56}H_{76}N_8O_8$ = 988.6, found [M+H$^+$] = 989.5, tR = 1.37 min.

4-(3-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)ethoxy)ethoxy)ethoxy)prop-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 4.



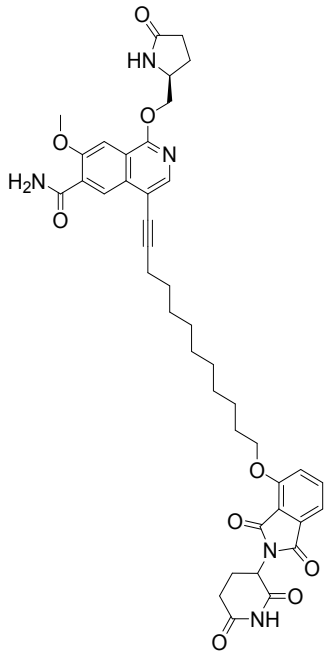
2-(2,6-dioxopiperidin-3-yl)-4-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethoxy)isoindoline-1,3-dione (36.6 mg, 0.0820 mmol), potassium carbonate (12.6 mg, 0.0910 mmol), (*S*)-4-bromo-7-methoxy-

1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (12.0 mg, 0.0300 mmol), XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 μmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 1 h. The reaction mixture was then cooled to 25 °C and further $Pd_2(dba)_3$ (4.00 mg, 4.37 μmol) was added. The reaction mixture was heated to 100 °C for a further 2 h. The reaction mixture was then cooled to 25 °C, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method A). The solvent was evaporated *in vacuo* and the resulting crude product was purified by MDAP (Method A). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (1.2 mg, 2%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 11.08 (br. s., 1H), 8.31 (s, 2H), 8.16 - 8.11 (m, 2H), 7.90 (br. s., 1H), 7.81 - 7.75 (m, 2H), 7.69 (s, 1H), 7.51 (d, *J*=8.6 Hz, 1H), 7.43 (d, *J*=7.3 Hz, 1H), 5.08 (dd, *J*=5.5, 12.8 Hz, 1H), 4.56 - 4.50 (m, 3H), 4.36 - 4.29 (m, 3H), 4.09 - 4.00 (m, 4H), 3.84 - 3.79 (m, 2H), 3.73 - 3.65 (m, 4H), 3.64 - 3.60 (m, 2H), 3.60 - 3.56 (m, 2H), 2.94 - 2.83 (m, 1H), 2.36 - 2.31 (m, 1H), 2.31 - 2.27 (m, 1H), 2.26 - 2.17 (m, 2H), 2.07 - 1.99 (m, 1H), 1.91 (dd, *J*=7.6, 12.7 Hz, 1H).

LC-MS (System A): calc for $C_{38}H_{39}N_5O_{12}$ = 757.3, found [M+H$^+$] = 758.5, tR = 0.83 min.

4-(12-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)dodec-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide, Compound 5.



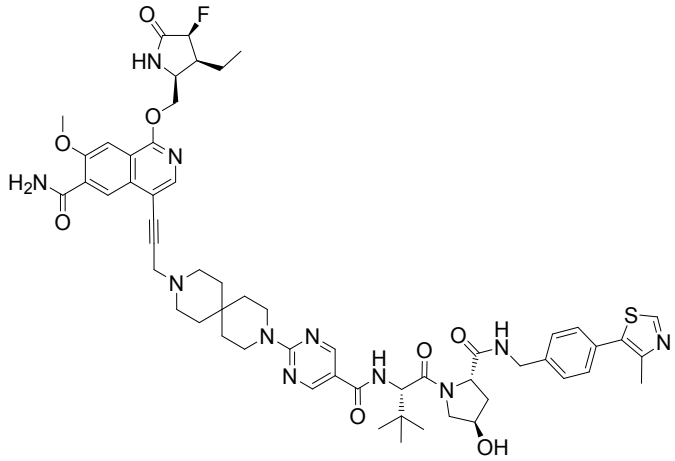
2-(2,6-dioxopiperidin-3-yl)-4-(dodec-11-yn-1-yloxy)isoindoline-1,3-dione (36.2 mg, 0.0820 mmol), potassium carbonate (12.6 mg, 0.0910 mmol), (*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-

yl)methoxy)isoquinoline-6-carboxamide (12.0 mg, 0.0300 mmol), XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.184 μmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 1 h. The reaction mixture was then cooled to 25 °C and further $Pd_2(dba)_3$ (4.00 mg, 4.37 μmol) was added. The reaction mixture was heated to 100 °C for a further 2 h. The reaction mixture was then cooled to 25 °C, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method A). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (4 mg, 8%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 11.08 (s, 1H), 8.33 (s, 1H), 8.12 (s, 1H), 8.03 (s, 1H), 7.88 (br. s., 1H), 7.80 (dd, *J*=7.3, 8.6 Hz, 1H), 7.73 (br. s., 1H), 7.67 (s, 1H), 7.49 (d, *J*=8.6 Hz, 1H), 7.43 (d, *J*=7.3 Hz, 1H), 5.07 (dd, *J*=5.4, 12.7 Hz, 1H), 4.50 (dd, *J*=3.9, 11.0 Hz, 1H), 4.30 (dd, *J*=6.8, 11.0 Hz, 1H), 4.18 (t, *J*=6.5 Hz, 2H), 4.07 - 3.98 (m, 4H), 2.94 - 2.82 (m, 1H), 2.61 (br. s., 1H), 2.59 - 2.53 (m, 1H), 2.36 - 2.15 (m, 4H), 2.08 - 1.98 (m, 1H), 1.93 - 1.85 (m, 1H), 1.74 (dd, *J*=7.0, 11.9 Hz, 2H), 1.66 - 1.57 (m, 2H), 1.53 - 1.25 (m, 13H).

LC-MS (System A): calc for $C_{41}H_{45}N_5O_9$ = 751.3, found [M+H$^+$] = 752.5, tR = 1.25 min.

1-(((2S,3S,4S)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl)methoxy)-4-(3-(9-(5-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)pyrimidin-2-yl)-3,9-diazaspiro[5.5]undecan-3-yl)prop-1-yn-1-yl)-7-methoxyisoquinoline-6-carboxamide, Compound 9.



N-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxamide (125 mg, 0.172 mmol), 4-bromo-1-(((2S,3S,4S)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl)methoxy)-7-methoxyisoquinoline-6-carboxamide (72.0 mg, 0.164 mmol), potassium carbonate (67.8 mg, 0.491 mmol), XPhos (4.00 mg, 8.39 μmol) and $Pd_2(dba)_3$ (4.00 mg, 4.37 μmol) were added to a

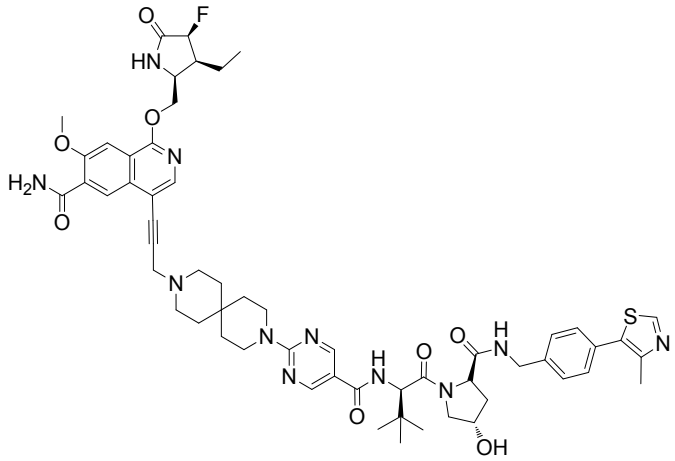
microwave vial followed by DMA (2 mL). The vial was sealed and heated to 100 °C for 3 h. The reaction mixture was then cooled to 25 °C and further XPhos (4.00 mg, 8.39 µmol) and $Pd_2(dba)_3$ (4.00 mg, 4.37 µmol) was added. The reaction mixture was heated to 100 °C for a further 3 h. The reaction mixture was then cooled to 25 °C and further XPhos (4.00 mg, 8.39 µmol), $Pd_2(dba)_3$ (4.00 mg, 4.37 µmol) and *N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxamide (10.0 mg, 0.0138 mmol) was added. The reaction mixture was heated to 100 °C for a further 2 h. The reaction mixture was passed through a plug of cotton wool and blown down under a stream of nitrogen. The resulting crude product was purified by MDAP (Method B). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (15 mg, 8% yield).

HRMS: calc for $C_{57}H_{68}FN_{11}O_8S$ [$M+H^+$] = 1086.5035, found 1086.5050

^{1}H NMR (700 MHz, DMSO-d6) δ = 8.98 (s, 1H), 8.85 (s, 1H), 8.77 (s, 2H), 8.55 (br t, J = 5.8 Hz, 1H), 8.38 (s, 1H), 8.11 (s, 1H), 8.04 (br d, J = 8.9 Hz, 1H), 7.89 (br s, 1H), 7.78 (s, 1H), 7.73 (br s, 1H), 7.43 - 7.41 (m, 2H), 7.40 - 7.38 (m, 2H), 5.13 (br d, J = 3.0 Hz, 1H), 4.97 - 4.84 (m, 1H), 4.73 (br d, J = 9.1 Hz, 1H), 4.57 (dd, J = 3.3, 11.1 Hz, 1H), 4.45 - 4.40 (m, 1H), 4.48 - 4.39 (m, 1H), 4.39 - 4.35 (m, 1H), 4.29 (br dd, J = 6.4, 11.0 Hz, 1H), 4.24 (br dd, J = 5.5, 15.7 Hz, 1H), 4.15 - 4.06 (m, 1H), 3.99 (s, 3H), 3.86 - 3.77 (m, 4H), 3.72 (br s, 2H), 3.67 - 3.65 (m, 2H), 2.64 - 2.60 (m, 1H), 2.67 - 2.57 (m, 4H), 2.45 (s, 3H), 2.06 - 2.01 (m, 1H), 1.92 (ddd, J = 4.7, 8.3, 12.7 Hz, 1H), 1.65 - 1.55 (m, 2H), 1.59 - 1.54 (m, 4H), 1.46 (br s, 4H), 1.01 (s, 9H), 1.05 - 0.98 (m, 3H).

^{13}C NMR (176 MHz, DMSO-d6) δ = 171.9 (s, 1C) 171.0 (d, J=19 Hz, 1C) 169.4 (s, 1C) 166.0 (s, 1C) 164.0 (s, 1C) 161.4 (s, 1C) 158.1 (s, 1C) 158.0 (s, 2C) 155.7 (s, 1C) 151.4 (s, 1C) 147.7 (s, 1C) 141.6 (s, 1C) 139.4 (s, 1C) 131.1 (s, 1C) 131.0 (s, 1C) 130.9 (s, 1C) 129.6 (s, 1C) 128.6 (s, 2C) 127.4 (s, 2C) 126.5 (s, 1C) 119.7 (s, 1C) 115.2 (s, 1C) 109.7 (s, 1C) 103.8 (s, 1C) 91.4 (s, 1C) 89.9 (d, J=180 Hz, 1C) 79.6 (s, 1C) 68.9 (s, 1C) 66.7 (s, 1C) 58.8 (s, 1C) 57.0 (s, 1C) 56.3 (s, 1C) 56.1 (s, 1C) 53.9 (s, 1C) 47.3 - 47.4 (m, 1C) 47.3 (s, 2C) 42.1 (d, J=19 Hz, 1C) 41.6 (s, 1C) 39.3 (s, 2C) 37.9 (s, 1C) 35.4 (s, 1C) 35.0 (s, 4C) 29.2 (s, 1C) 26.5 (s, 3C) 16.3 (d, J=8 Hz, 1C) 15.9 (s, 1C) 12.1 (s, 1C).

1-(((2*S*,3*S*,4*S*)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl)methoxy)-4-(3-(9-(5-(((*R*)-1-((2*S*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)carbamoyl)pyrimidin-2-yl)-3,9-diazaspiro[5.5]undecan-3-yl)prop-1-yn-1-yl)-7-methoxyisoquinoline-6-carboxamide.

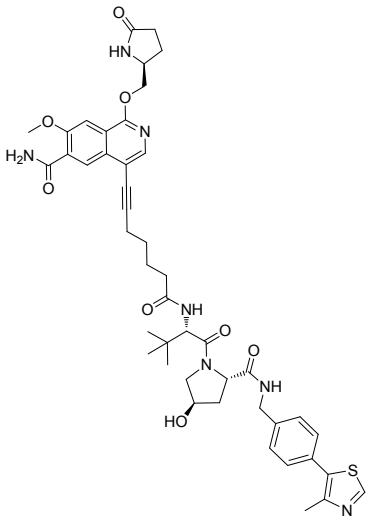


N-((*R*)-1-((2*R*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)-2-(9-(prop-2-yn-1-yl)-3,9-diazaspiro[5.5]undecan-3-yl)pyrimidine-5-carboxamide (41.3 mg, 0.0570 mmol), 4-bromo-1-(((2*S*,3*S*,4*S*)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl)methoxy)-7-methoxyisoquinoline-6-carboxamide (25.0 mg, 0.0570 mmol), potassium carbonate (23.5 mg, 0.170 mmol), XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 μmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 2 h. The reaction mixture was then cooled to 25 °C and further XPhos (2.00 mg, 4.20 μmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 μmol) were added. The reaction mixture was heated to 100 °C for a further 2 h. The reaction mixture was passed through a plug of cotton wool and blown down under a stream of nitrogen. The residue was purified by MDAP (Method B). Collection failed, the waste was concentrated *in vacuo*. The residue was purified by MDAP (Method B). Collection failed, the waste was concentrated *in vacuo*. The residue was purified by MDAP (Method B). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (5.3 mg, 9%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.98 (s, 1H), 8.85 (s, 1H), 8.77 (s, 2H), 8.54 (t, *J*=6.1 Hz, 1H), 8.38 (s, 1H), 8.11 (s, 1H), 8.03 (d, *J*=9.0 Hz, 1H), 7.88 (br. s., 1H), 7.79 (s, 1H), 7.72 (br. s., 1H), 7.44 - 7.37 (m, 4H), 5.12 (d, *J*=3.4 Hz, 1H), 5.00 - 4.82 (m, 1H), 4.73 (d, *J*=9.3 Hz, 1H), 4.57 (dd, *J*=3.5, 11.1 Hz, 1H), 4.48 - 4.34 (m, 3H), 4.33 - 4.19 (m, 2H), 4.10 (br. s., 1H), 3.99 (s, 3H), 3.81 (br. s., 4H), 3.72 (br. s., 2H), 3.66 (s, 2H), 2.69 - 2.58 (m, 5H), 2.45 (s, 3H), 2.09 - 2.00 (m, 1H), 1.92 (dt, *J*=4.3, 8.5 Hz, 1H), 1.58 (br. s., 6H), 1.46 (br. s., 4H), 1.05 - 0.96 (m, 12H).

LC-MS (System B): calc for $C_{57}H_{68}FN_{11}O_8S$ = 1085.5, found $[M+2H]^{2+}/2$ = 543.9, tR = 1.11 min.

4-(7-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-7-oxohept-1-yn-1-yl)-7-methoxy-1-(((*S*)-5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide.

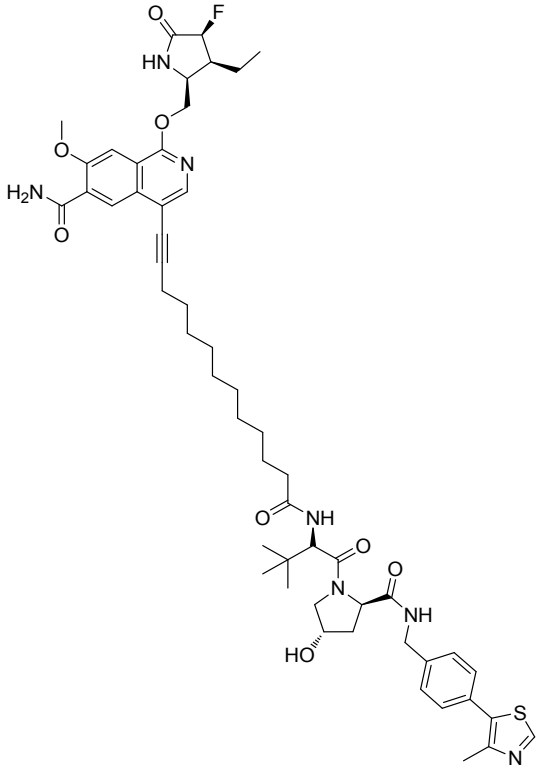


(2*S*,4*R*)-1-((*S*)-2-(hept-6-ynamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (44.4 mg, 0.0820 mmol), potassium carbonate (26.3 mg, 0.190 mmol), (*S*)-4-bromo-7-methoxy-1-((5-oxopyrrolidin-2-yl)methoxy)isoquinoline-6-carboxamide (25.0 mg, 0.0630 mmol), XPhos (2.00 mg, 4.20 µmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 µmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 1.5 h. The reaction mixture was then cooled to 25 °C and further XPhos (2.00 mg, 4.20 µmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 µmol) were added. The reaction mixture was heated to 100 °C for a further 1 h. The reaction mixture was then cooled to 25 °C and further XPhos (2.00 mg, 4.20 µmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 µmol) were added. The reaction mixture was heated to 100 °C for a further 1 h. The reaction mixture was then cooled to 25 °C and further XPhos (2.00 mg, 4.20 µmol) and $Pd_2(dba)_3$ (2.00 mg, 2.18 µmol) were added. The reaction mixture was heated to 100 °C for a further 0.5 h. The reaction mixture was then cooled to 25 °C, concentrated *in vacuo*, diluted with 10% aq. LiCl (50 mL), extracted with EtOAc (2 × 50 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by MDAP (Method A). The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (3.9 mg, 7%).

^{1}H NMR (400MHz, DMSO-d_6) δ = 8.98 (s, 1H), 8.53 (t, *J*=6.1 Hz, 1H), 8.32 (s, 1H), 8.13 (s, 1H), 8.04 (s, 1H), 7.91 - 7.85 (m, 2H), 7.73 (br. s., 1H), 7.67 (s, 1H), 7.45 - 7.36 (m, 4H), 5.10 (br. s., 1H), 4.56 (d, *J*=9.3 Hz, 1H), 4.51 (dd, *J*=3.9, 11.0 Hz, 1H), 4.47 - 4.39 (m, 2H), 4.38 - 4.27 (m, 2H), 4.22 (dd, *J*=5.5, 15.8 Hz, 1H), 4.01 (s, 4H), 3.71 - 3.63 (m, 2H), 2.58 (t, *J*=7.0 Hz, 2H), 2.44 (s, 3H), 2.39 - 2.17 (m, 5H), 2.07 - 1.99 (m, 1H), 1.95 - 1.86 (m, 2H), 1.75 - 1.58 (m, 4H), 0.94 (s, 9H).

LC-MS (System A): calc for $C_{45}H_{53}N_7O_8S$ = 851.4, found [M+H$^+$] = 852.6, tR = 0.94 min.

1-(((2*S*,3*S*,4*S*)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl)methoxy)-4-(13-(((*S*)-1-((2*R*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-13-oxotridec-1-yn-1-yl)-7-methoxyisoquinoline-6-carboxamide, Compound 8.



(2*S*,4*R*)-1-((*S*)-3,3-dimethyl-2-(tridec-12-ynamido)butanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (46.7 mg, 0.0750 mmol), 4-bromo-1-(((2*S*,3*S*,4*S*)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl)methoxy)-7-methoxyisoquinoline-6-carboxamide (22.0 mg, 0.0500 mmol), potassium carbonate (20.7 mg, 0.150 mmol), XPhos (1.00 mg, 2.10 µmol) and $Pd_2(dba)_3$ (1.00 mg, 1.09 µmol) were added to a vial followed by DMA (1 mL). The vial was sealed and heated to 100 °C for 2 h. The reaction mixture was then cooled to 25 °C, concentrated *in vacuo*, diluted with 10% aq. LiCl (20 mL), extracted with EtOAc (3 × 30 mL), the organic phases combined, passed through a hydrophobic frit and concentrated *in vacuo*. The residue was purified by reverse phase chromatography (Method B)**.** The appropriate fractions were combined and concentrated *in vacuo* to afford the title compound (14 mg, 29%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.97 (s, 1H), 8.83 (s, 1H), 8.52 (t, *J* = 6.1 Hz, 1H), 8.31 (s, 1H), 8.03 (s, 1H), 7.86 (br s, 1H), 7.82 - 7.74 (m, 2H), 7.72 (br s, 1H), 7.43 - 7.35 (m, 4H), 5.09 (br s, 1H), 4.98 - 4.80 (m, 1H), 4.58 - 4.50 (m, 2H), 4.46 - 4.38 (m, 2H), 4.34 (br s, 1H), 4.30 - 4.18 (m, 2H), 4.08 (br d, *J* = 3.4 Hz, 1H), 3.97 (s, 3H), 3.70 - 3.60 (m, 2H), 2.71 - 2.60 (m, 1H), 2.55 (t, *J* = 6.8 Hz, 3H), 2.44 (s, 3H), 2.29 - 2.19 (m, 1H), 2.14 - 1.97 (m, 3H), 1.90 (ddd, *J* = 4.6, 8.4, 12.8 Hz, 1H), 1.66 - 1.55 (m, 4H), 1.52 - 1.41 (m, 4H), 1.37 - 1.19 (m, 8H), 1.01 (t, *J* = 7.3 Hz, 3H), 0.92 (s, 9H).

LC-MS (System A): calc for $C_{53}H_{68}FN_7O_8S$ = 981.5, found [M+H$^+$] = 982.7, tR = 1.28 min.

2. Biology details

The human biological samples were sourced ethically, and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol.

Cell culture

THP-1 cells (purchased authenticated ATCC cell line) were cultured in complete growth medium RPMI 1640 medium (Life Technologies), supplemented with 2 mM L-glutamine (Gibco), 10% heat inactivated foetal bovine serum (FBS, Gibco), 100 units/mL penicillin and 100 µg/mL streptomycin (Gibco). PBMCs were cultured in RPMI 1640 + Glutamax + Hepes (Gibco) supplemented with 10% heat inactivated FBS (Gibco), 1X MEM non-essential amino acids (Gibco) and 50U/mL penicillin + 50µg/mL streptomycin (Gibco). Dermal Fibroblast were cultured in DMEM medium containing 4.5g/l D-glucose, L-glutamine and Pyruvate (Gibco), supplemented with 1X MEM non-essential amino acids (Gibco), 10% heat inactivated FBS (Gibco) and 50U/mL penicillin + 50µg/mL streptomycin (Gibco). Incubation for all cell types was performed at 37°C with 5% CO_2.

PBMCs isolation from blood

Human whole blood was collected in tubes containing 1 unit/mL Sodium Heparin (Wockhardt) and diluted 1:1 in PBS without Ca2+/ Mg2+ (Gibco). Blood was slowly layered onto a 50ml falcon tube containing 15ml of Histopaque, centrifuged at 800rcf without break for 20min at room temperature. The PBMC layer was collected with a Pasteur pipette into a separate tube and washed twice in PBS. Cells were then resuspended in growth media and total number of cells were counted using nucleocounter NC2000 (Chemometec).

Degradation assay

THP-1 or PBMCs were seeded at a density of 1x10^6 cells per well in 1ml of culture media in a 24-well plate, increasing concentration of each compounds was then added and cells were incubated for a period of 24 hours. For PBMCs stimulation assay, cells were stimulated with 2.5µg/ml of Resiquimod (R848, Invivogen) for further 8 hours. Cells were collected into separate tubes and centrifuged at 300rcf for

5min, growth media was collected and stored at -80C for cytokine analyses. Cells were washed with PBS, lysed with Ice cold RIPA buffer (Thermo Scientific) containing protease-inhibitors (Roche) and incubated on ice for 30min followed by 15min centrifugation at 13000 rcf. Cell lysate was collected and stored at -80°C.

Dermal Fibroblasts were seeded at a density of $5x10^5$ cells per well in 6-well plate in a final volume of 2mls. Compounds were added with the mentioned concentrations and cells were left incubated for a period of 24 hours at 37°C and 5% CO_2. For dermal fibroblast stimulation assay, cells were stimulated with 10ng/ml (R&D systems) and left incubating for further 24hours. Growth media was collected and stored at -80C for cytokine analysis. Fibroblasts were washed with PBS followed by the addition of ice-cold ripa buffer containing protease inhibitor cocktail. Cells lysate was obtain as described above for PBMCs.

Western Blotting

Total protein concentrations were determined by Pierce BCA Protein Assay kit (Thermo Scientific). 25 µg of total protein containing NuPAGE LDS sample buffer 4x (Invitrogen) and NuPAGE sample reducing agent 10x (Invitrogen) were boiled at 75°C and loaded on 4-12% NuPAGE gels (Life Technologies) and transferred onto PVDF immobilon membranes (Millipore). Membranes were blocked with Odyssey Blocking Buffer (LI-COR) for 1 hour followed by overnight incubation with the IRAK4 primary antibodies (1:1000, R&D systems), IRAK-1 (1:1000, SantaCruz) and LRKK2 (1:1000, abcam). The following day anti-β-tubulin (1:10,000, Sigma) was added for 2 hours at room temperature on a rocker. Membranes were washed using PBS with 0.1% Tween and were incubated with anti-goat IRdye 800CW (LI-COR) and anti-mouse IRdye 680RD (LI-COR) for 1 hour. The bands were visualised using an Odyssey scanner (LI-COR Biosciences) and the intensity of the bands was quantified using Image Studio Lite v5.2 software (LI-COR Biosciences). Data were analysed and plotted using Microsoft Office Excel and GraphPad Prism software.

Cytokine analysis

Supernatant from PBMCs or Fibroblasts were analysed on MSD plates (Meso Scale Discovery), which were run according to the manufacturer's protocols. Plates were read on an MSD Sector Imager S 600 Reader (Meso Scale Discovery) and data were analysed using Discovery Workbench 4.0.12.1 software. Data were analysed and plotted using Microsoft Office Excel and GraphPad Prism software.

3. Supplementary Information

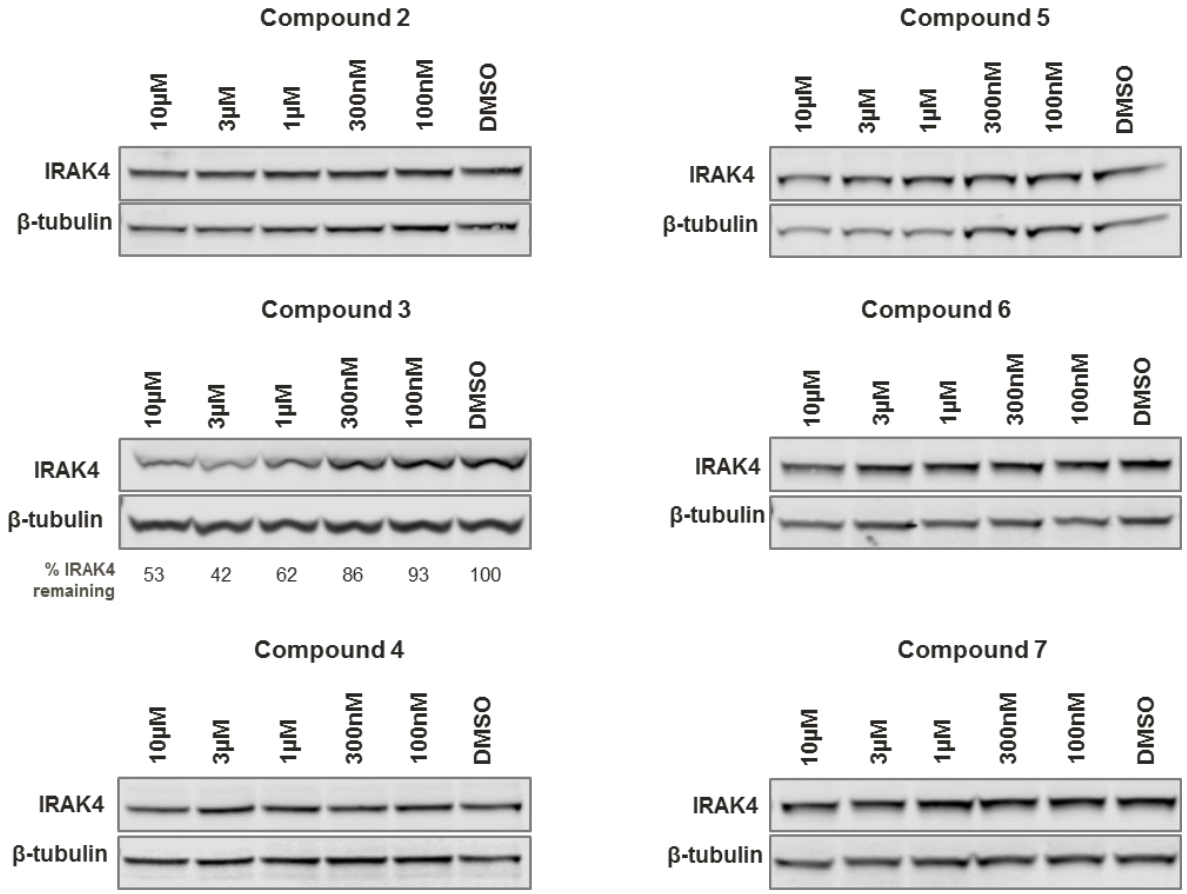


Figure S1: IRAK4 degradation in THP-1 cells. THP-1 cells were treated with increasing amounts of each compound for 24 hours. IRAK4 levels were determined by western blotting. % values represent the amount of IRAK4 remaining relative to DMSO treated cells.

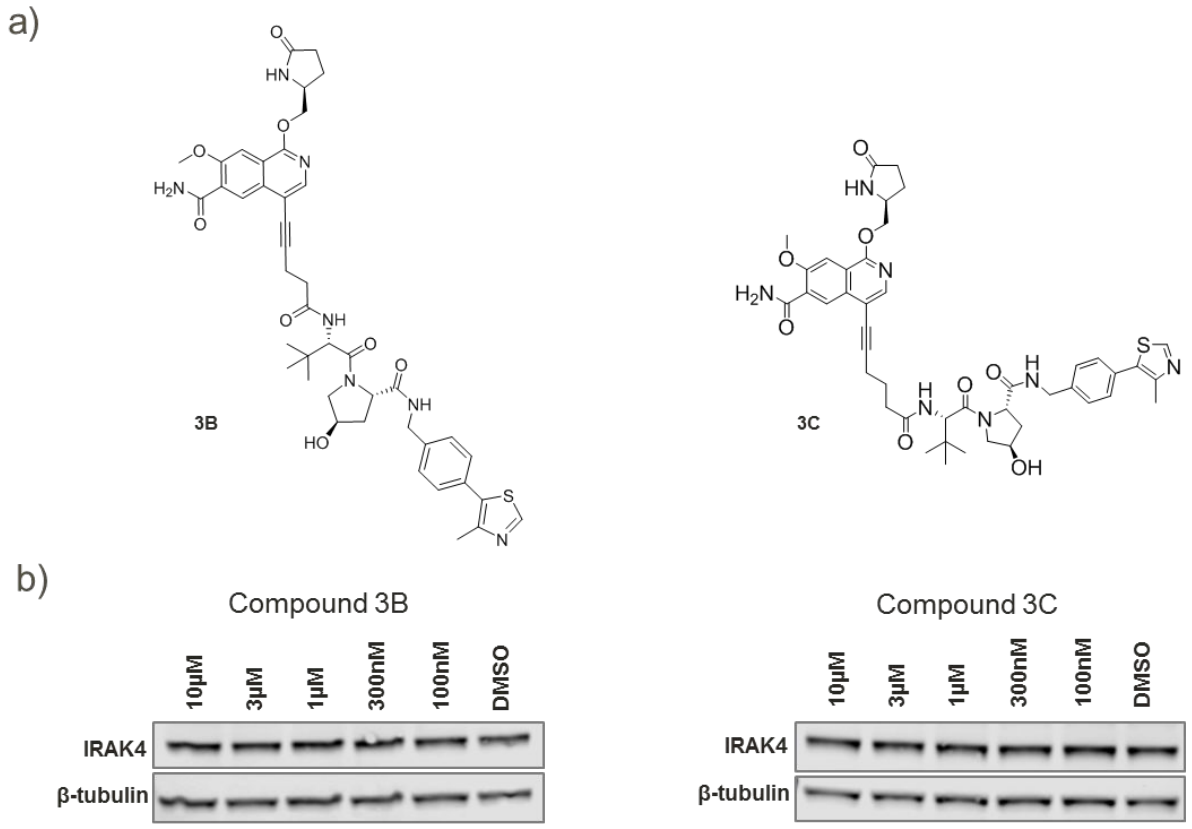


Figure S2: IRAK4 degradation by compounds 3B and 3C in THP-1 cells. a) Chemical structure of compound 3B and 3C b) THP-1 cells were treated with increasing amounts of each compound for 24 hours. IRAK4 levels were determined by western blotting.

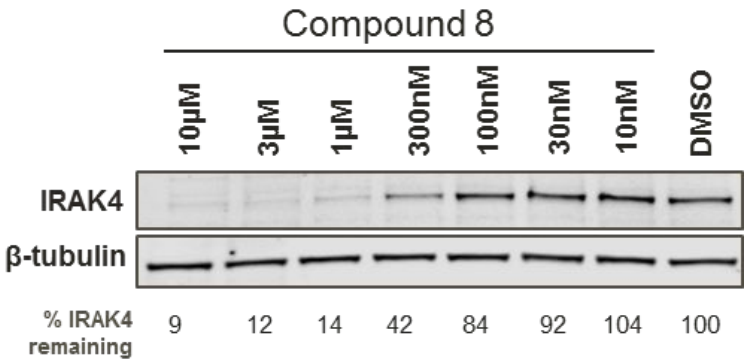


Figure S3: IRAK4 degradation by compound 8. PBMC cells were treated with increasing amounts of compound **8** for 24 hours. IRAK4 levels were determined by western blotting. % values represent the amount of remaining IRAK4 relative to DMSO treated cells.

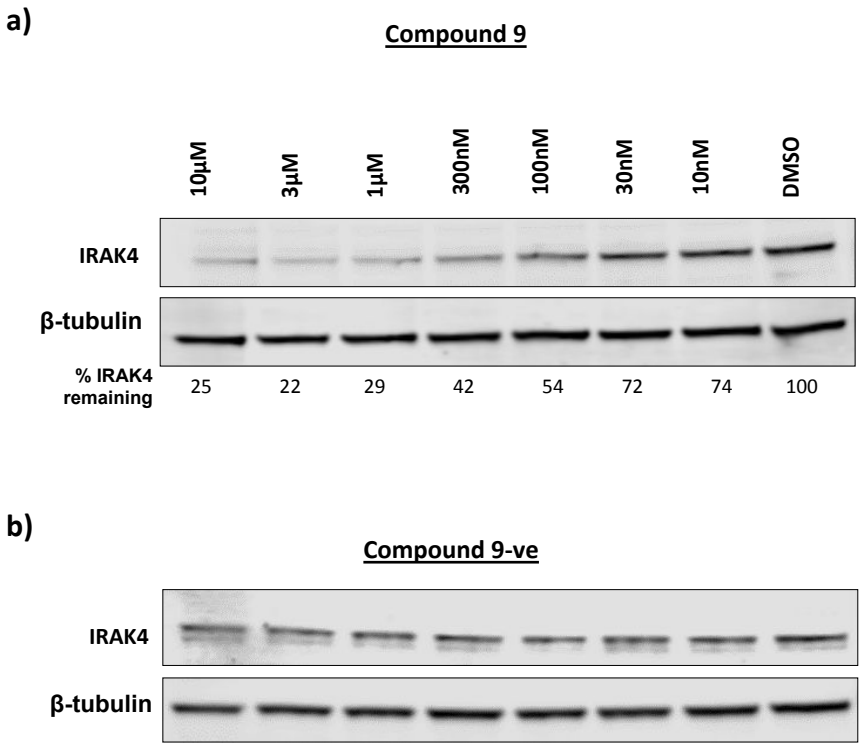


Figure S4: IRAK4 degradation by compound 9. a-b) PBMC cells were treated with increasing amounts of compound **9** or with VHL-non-binding enantiomeric control (Compound 9-ve) for a period of 24 hours. IRAK4 levels were determined by western blotting. % values represent the amount of remaining IRAK4 relative to DMSO treated cells.

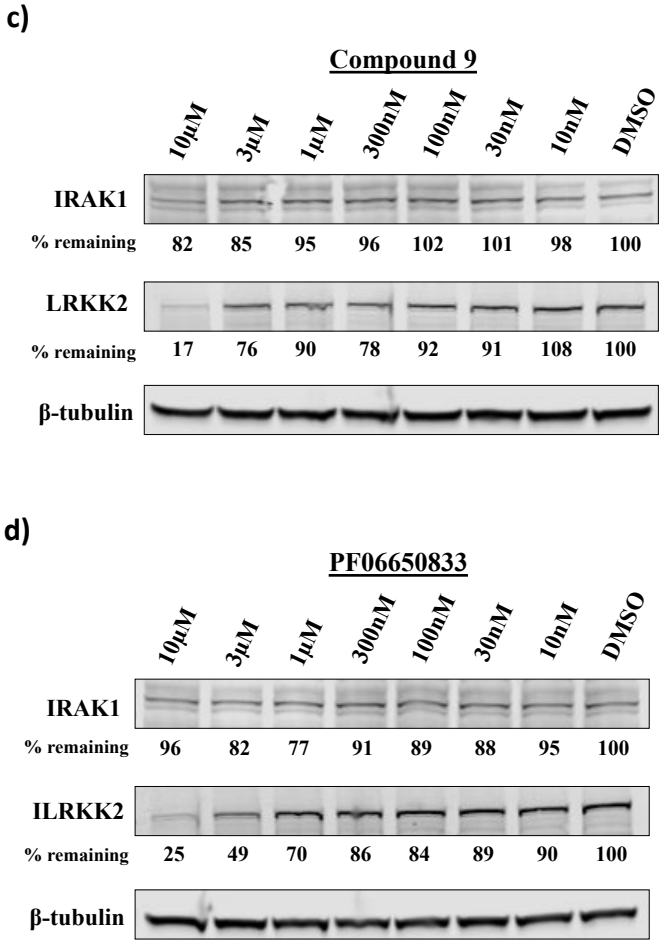


Figure S4c-d: Selectivity profile of compound 9. THP-1 cells were treated with increasing amounts of compound **9** for 24 hours. IRAK-1 and LRKK2 levels were determined by western blotting. % values represent the amount of remaining IRAK4 relative to DMSO treated cells