

Protein Degradation by In-Cell Self-Assembly of Proteolysis Targeting Chimeras

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Supplementary Information

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Page 16 Synthesis of CLIPTAC Precursor Components.

Page 17 Supplementary Scheme 1.

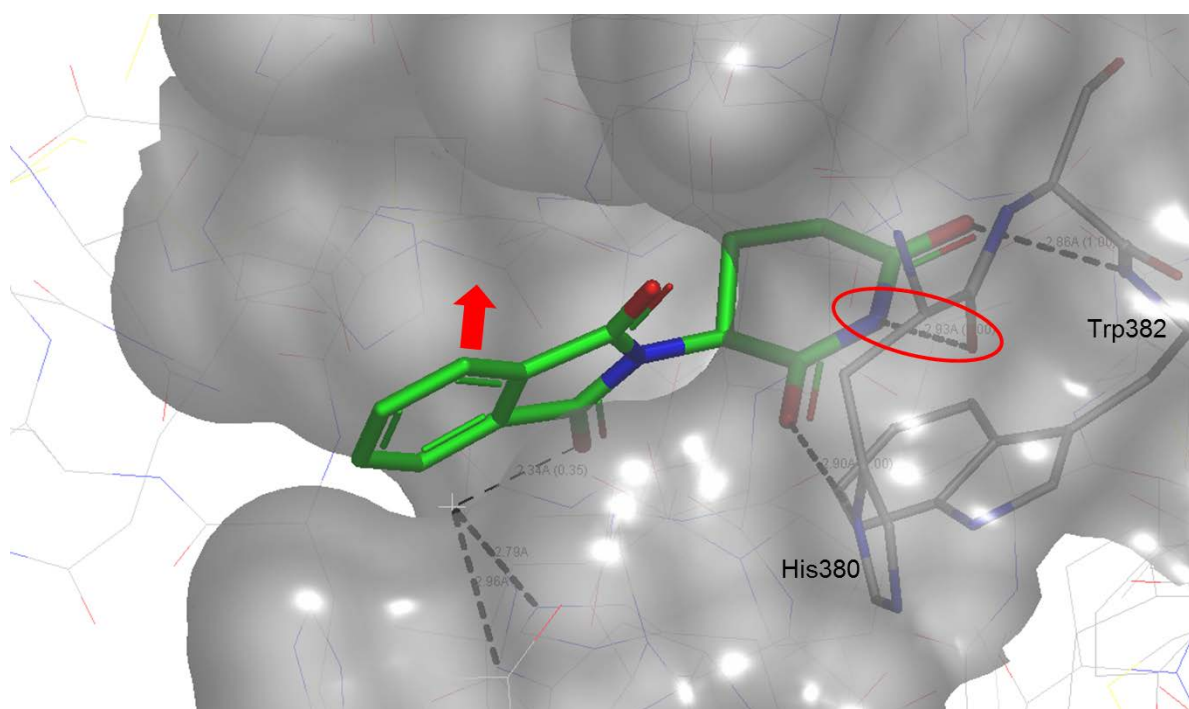
Supplementary Results

Supplementary Figures

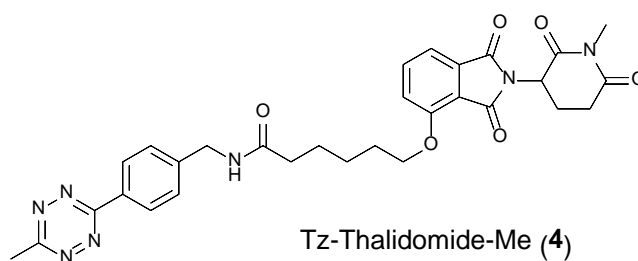
Supplementary Figure 1. Co-crystal structure of thalidomide in complex with CRBN (PDB: 4CI1).

a) The three key hydrogen bonds between the imide group of thalidomide and the His380 and Trp382 residues of CRBN are shown in dashed lines. The extension site is indicated towards the solvent (red arrow). The hydrogen bond perturbed by methylation of thalidomide is circled in red. b) Structure of Tz-Thalidomide-Me (**4**).

a)

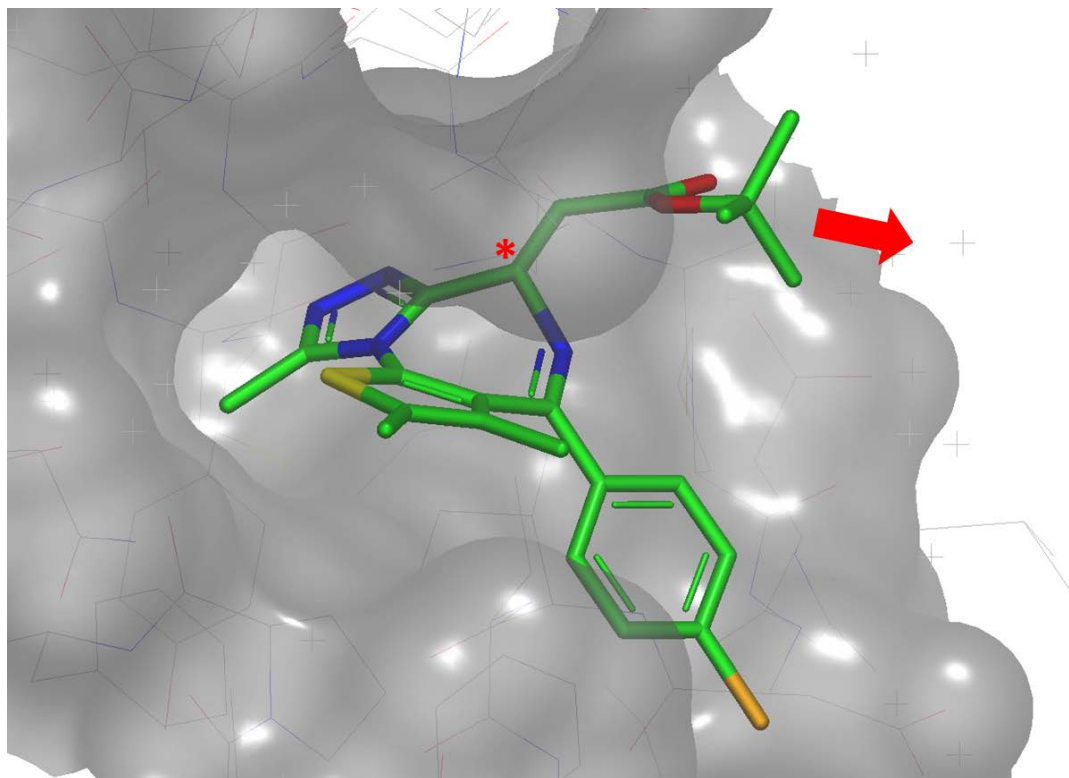


b)



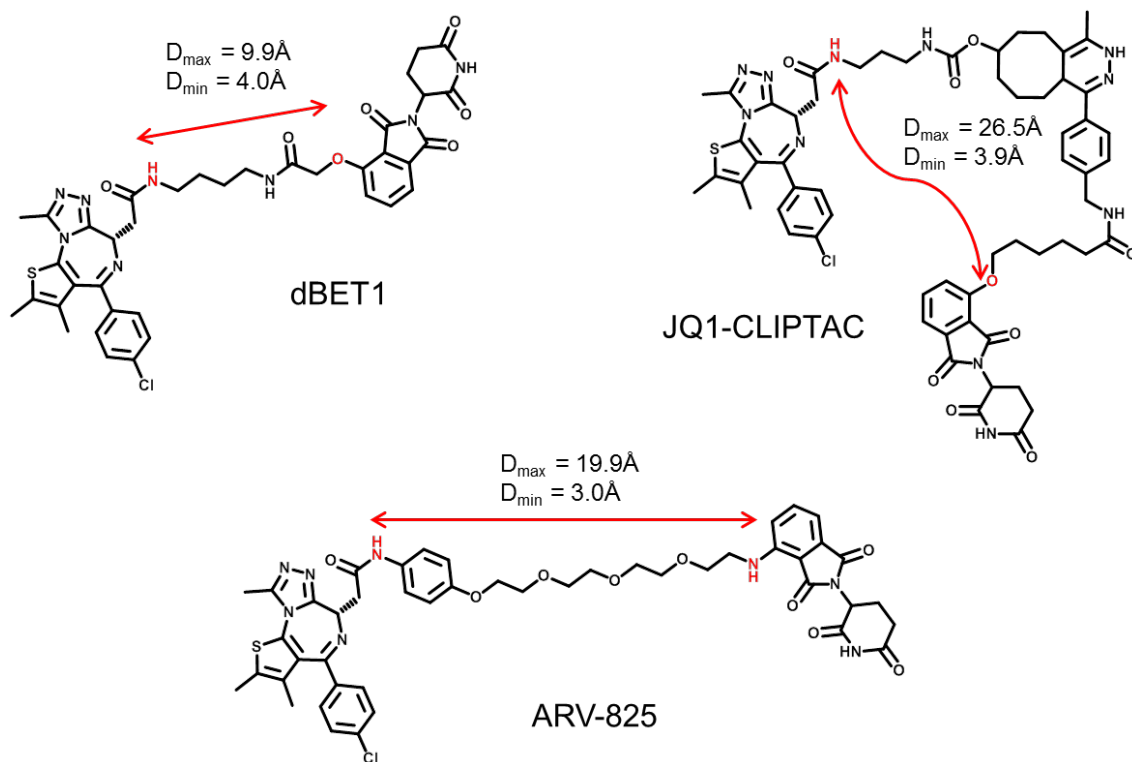
Supplementary Figure 2. Co-crystal structure of JQ1 with BRD4 (PDB: 3MXF).

The extension site is shown (red arrow). An asterisk marks the chiral carbon which is inverted in the negative control (-)JQ1.



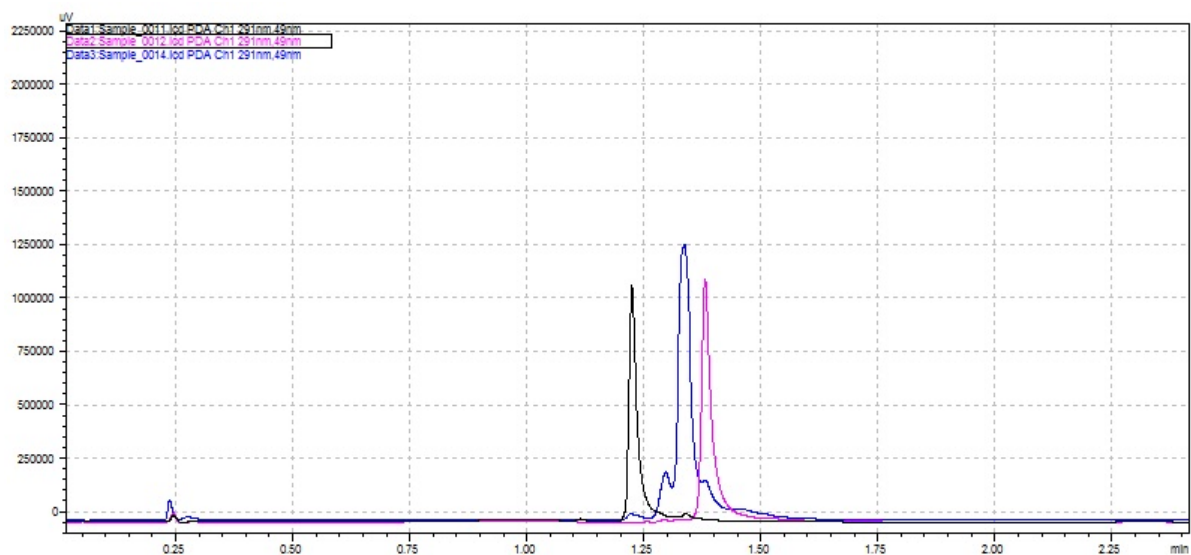
Supplementary Figure 3. Distances between the two recruiters within a PROTAC or CLIPTAC.

Maximum and minimum achievable distances between the linkage atoms in JQ-1 based PROTACs dBET1 and ARV-825 compared with JQ1-CLIPTAC. Calculations were performed using MOE 2015 in the absence of proteins. The results indicate that JQ1-CLIPTAC is able to elicit a similar range of proximities between the protein of interest (BRD4) and the E3 ligase (CRBN) as ARV-825.



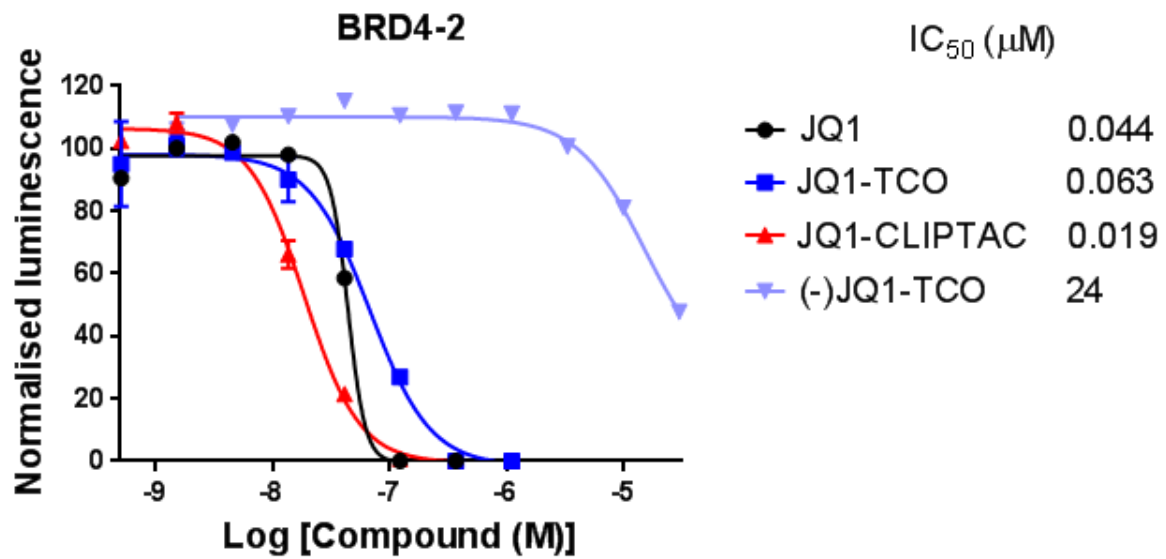
Supplementary Figure 4. LC-MS profile of the click reaction between JQ1-TCO and Tz-thalidomide.

LC-MS analysis of Tz-thalidomide (**1**, black), JQ1-TCO (**2**, pink) and the reaction mixture after 15 min (blue). Minor peaks are presumed to arise from diastereoisomers formed in the click reaction and which are not expected to show differences in biological activity.



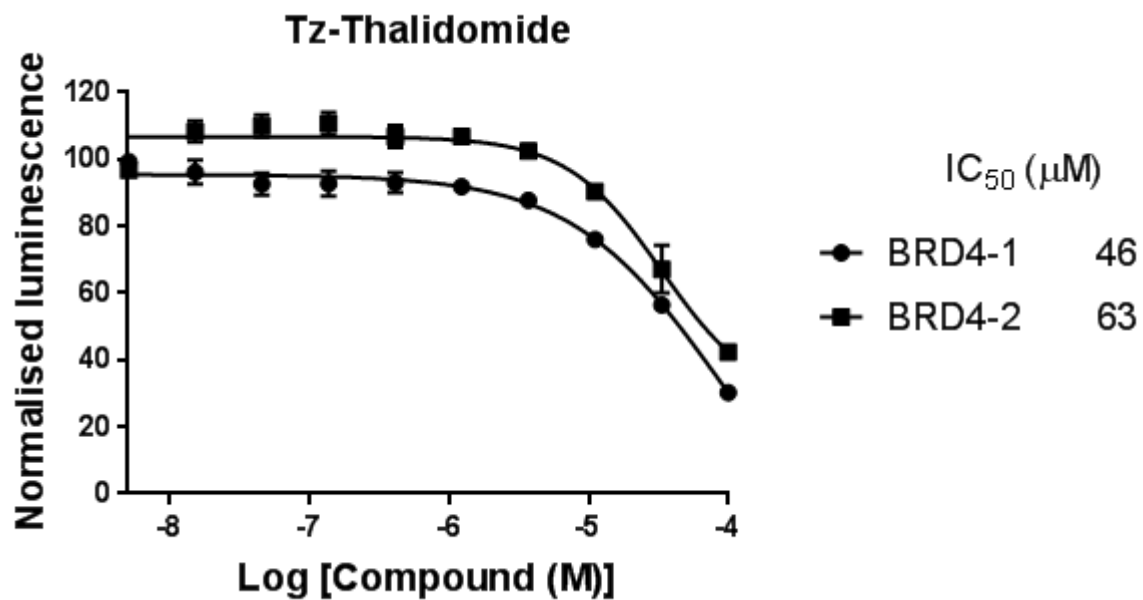
Supplementary Figure 5. Bioassay evaluation of JQ1 derivatives vs the BRD4 bromodomain BRD4-2.

Affinity of JQ1 (n=1), JQ1-TCO (2, n=2), JQ1-CLIPTAC (3, n=2) and (-)JQ1-TCO ((-)2, n=2) for BRD4-2 bromodomain as determined by AlphaScreen histone peptide displacement assays.



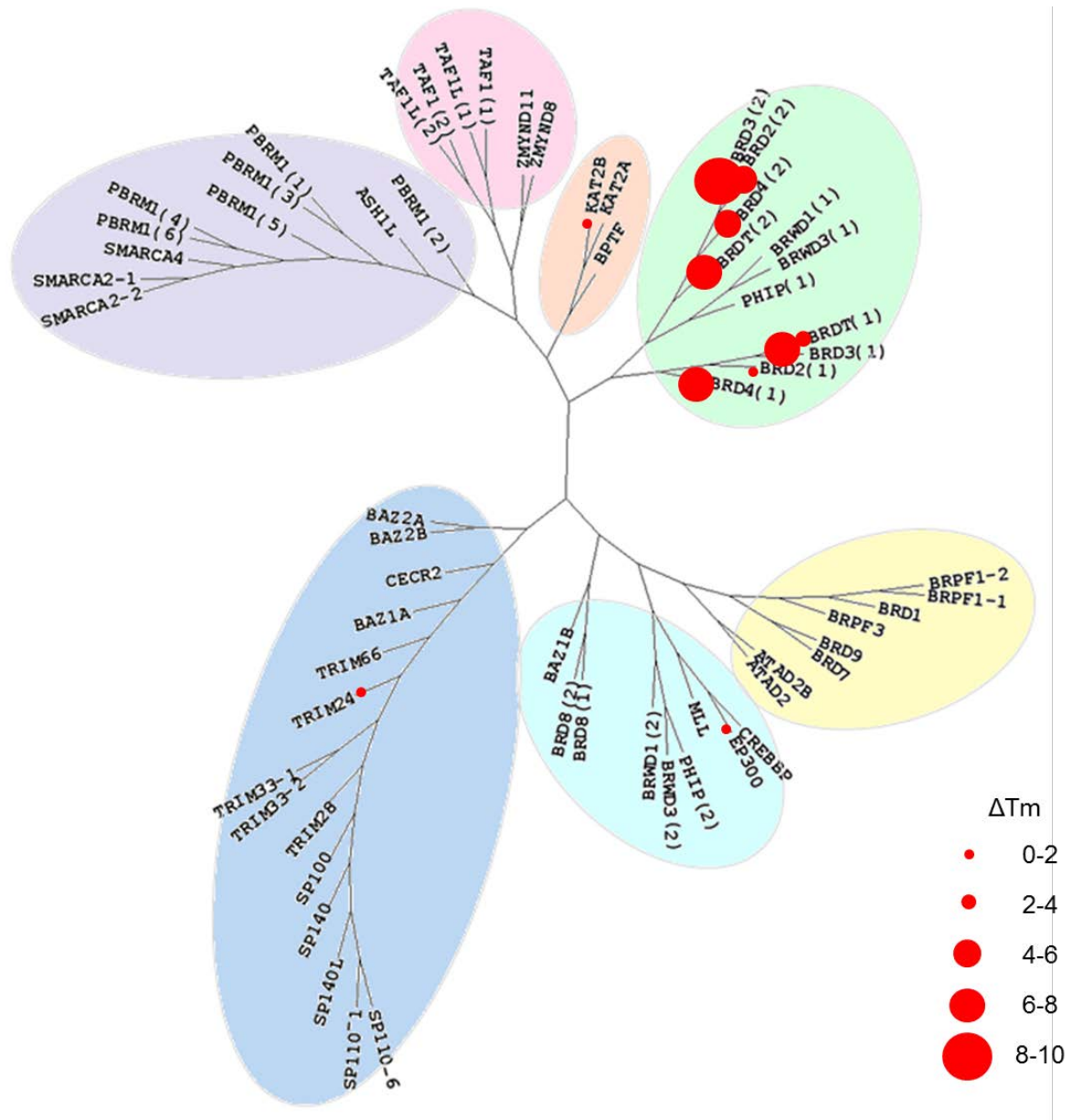
Supplementary Figure 6. Bioassay evaluation of Tz-thalidomide 1 against the BRD4 bromodomains BRD4-1 and BRD4-2.

Affinity of Tz-thalidomide 1 (n = 2) for BRD4-1 and BRD4-2 as determined by AlphaScreen histone peptide displacement assays.



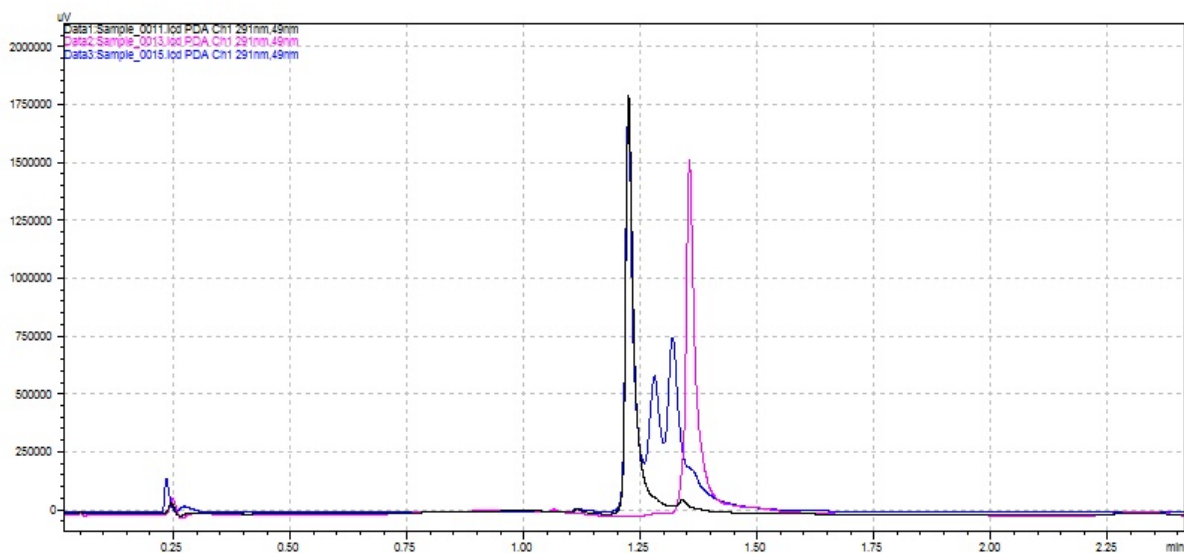
Supplementary Figure 7. Selectivity profile of JQ1-TCO against the BET family.

The selectivity profile of JQ1-TCO 2 (10 μ M, duplicate) was assessed using thermal shift assays (Reaction Biology) against the BET family. Red spots are representative of the ΔT_m shifts in degrees Celsius obtained from the thermal shift assay.



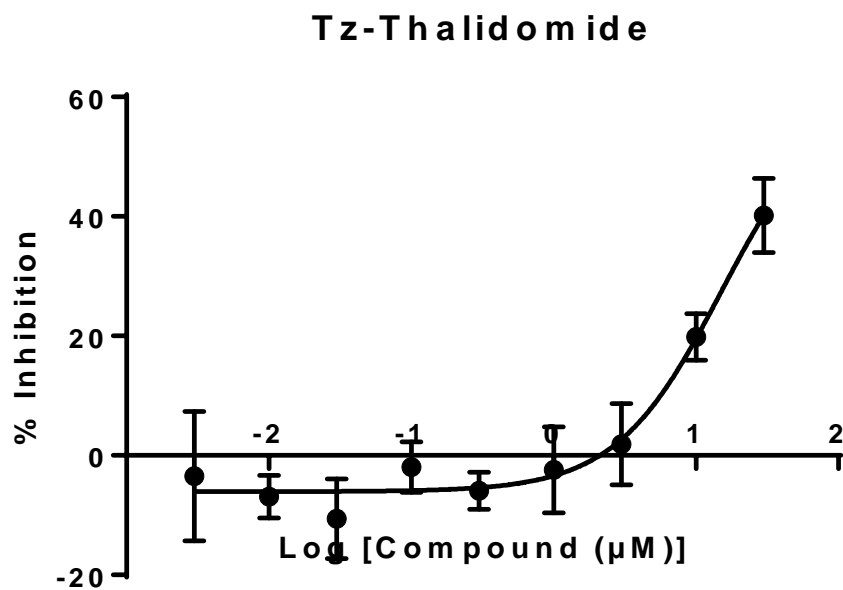
Supplementary Figure 8. LC-MS profile of the click reaction between Probe 1-TCO and Tz-thalidomide.

LC-MS analysis of Probe 1 (pink), Tz-thalidomide (black) and the reaction mixture after 15 min (blue). The double peaks are ascribed to diastereoisomers of ERK-CLIPTAC formed in the click reaction which are not expected to show differences in biological activity.



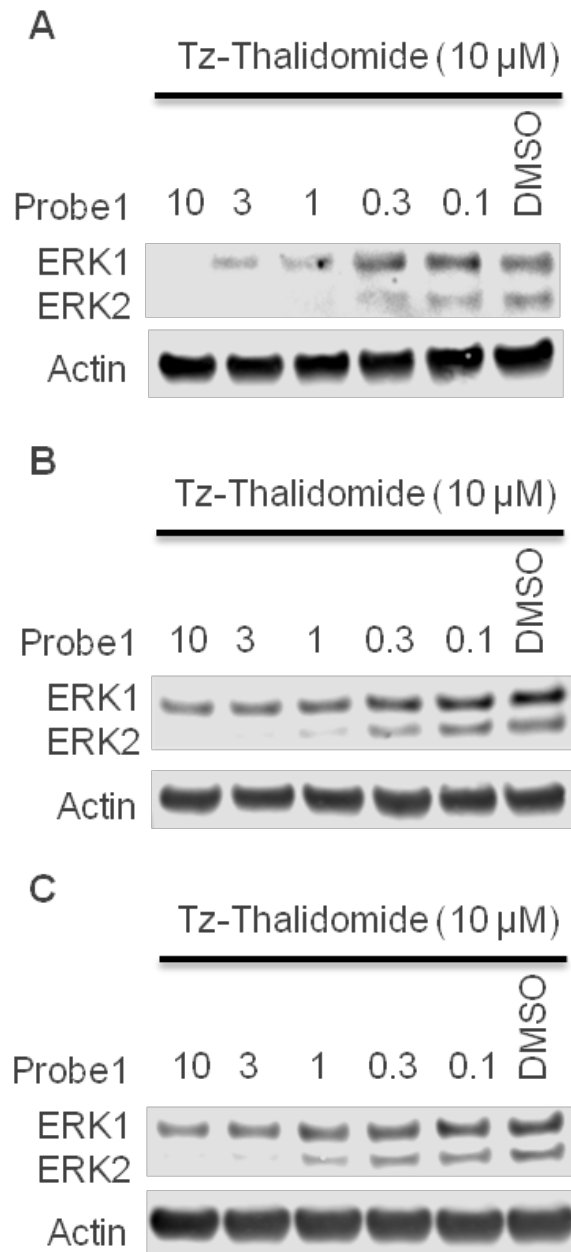
Supplementary Figure 9. Bioassay evaluation of Tz-thalidomide 1 against ERK2.

Table summarising the concentration-dependent inhibition of recombinant ERK2 by Tz-thalidomide 1 as determined by time-resolved fluorescence assay (n = 2, 46 ± 4% inhibition @ 30 μM). Full assay conditions are given in the Experimental section.



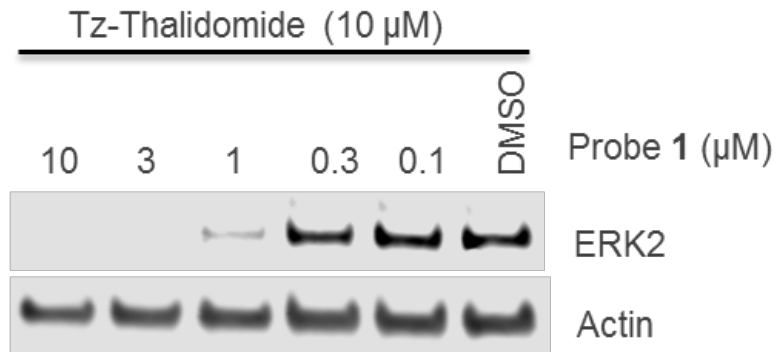
Supplementary Figure 10. Influence of Probe 1 incubation time on ERK1/2 degradation.

A375 cells were treated with Probe 1 for 24 h (A), 8 h (B) or 4 h (C) before the addition of Tz-thalidomide (10 μ M, 18 h). Following cell lysis, the levels of ERK1/2 were studied by Western Blots.



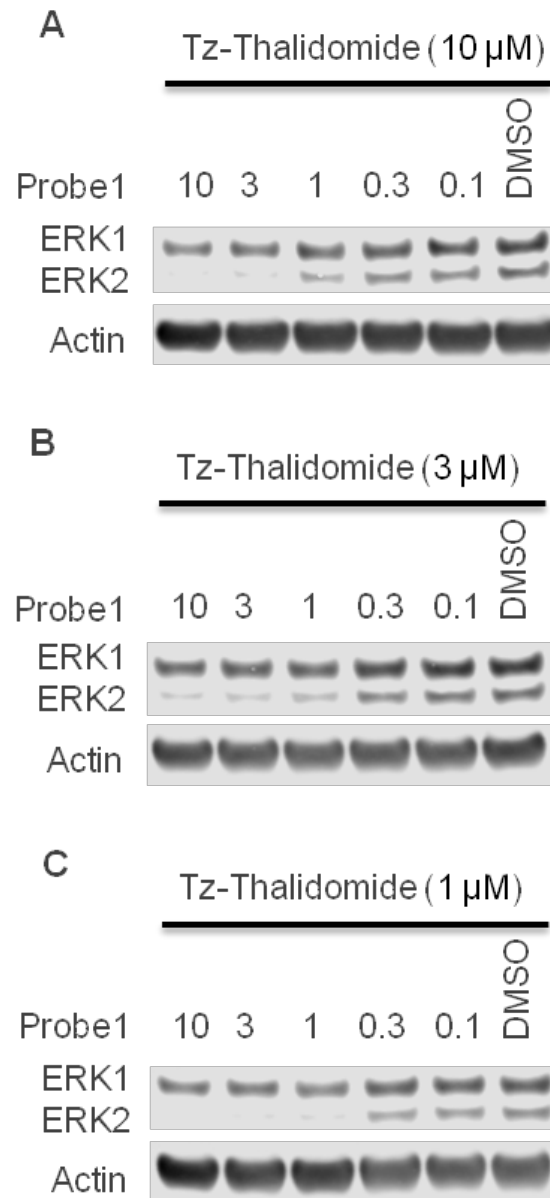
Supplementary Figure 11. Immunoblot for ERK2 and Actin showing Probe 1 dose-dependent downregulation of ERK2 protein levels in HCT116 cells.

HCT116 cells were treated with Probe 1 for 18 hours followed by Tz-thalidomide (10 μ M) for 18 hours.



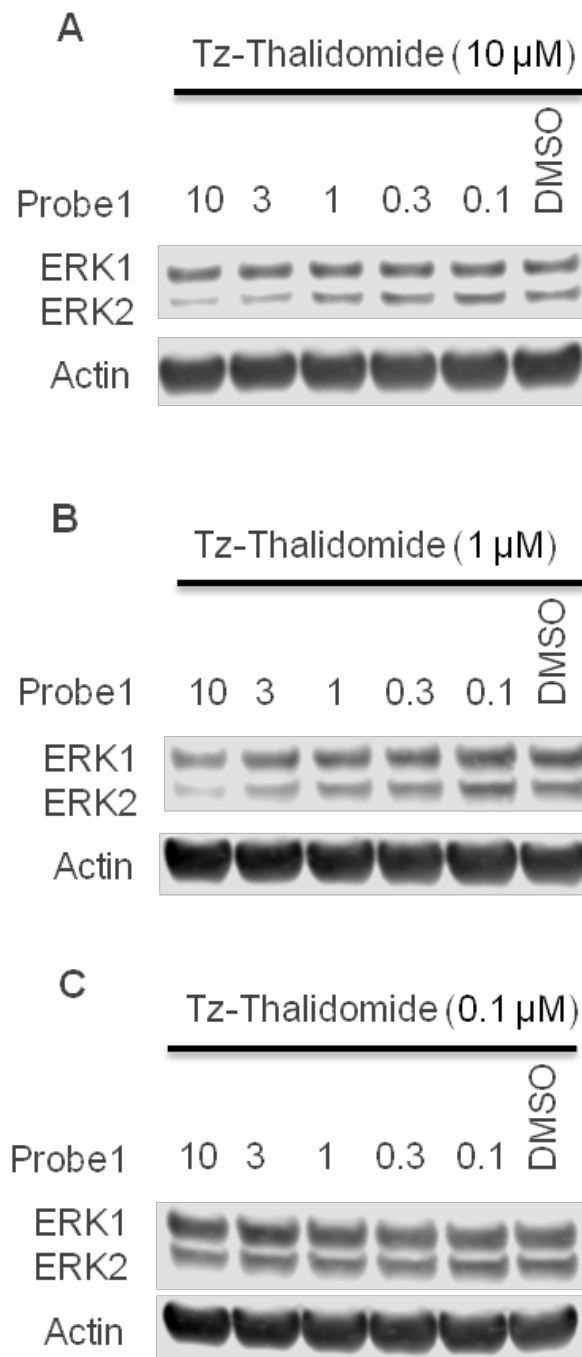
Supplementary Figure 12. Influence of Tz-thalidomide concentration on ERK1/2 degradation.

A375 cells were treated with Probe 1 for 4 h followed by Tz-thalidomide (10 μ M (A), 3 μ M (B), 1 μ M (C)) for 18 h. Following cell lysis, the levels of ERK1/2 were studied by Western Blots.



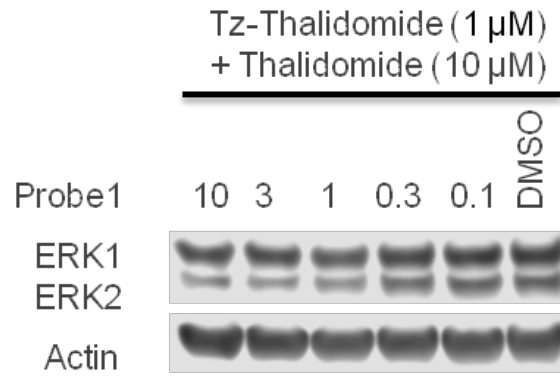
Supplementary Figure 13. Influence of a washout on ERK1/2 degradation.

A375 cells were treated with Probe 1 for 4 h. The cells were washed with fresh media to remove unreacted Probe 1 and treated with Tz-thalidomide (10 μ M (A), 1 μ M (B), 0.1 μ M (C)). Following cell lysis, the levels of ERK1/2 were studied by Western Blots.



Supplementary Figure 14. ERK1/2 degradation prevented by addition of thalidomide.

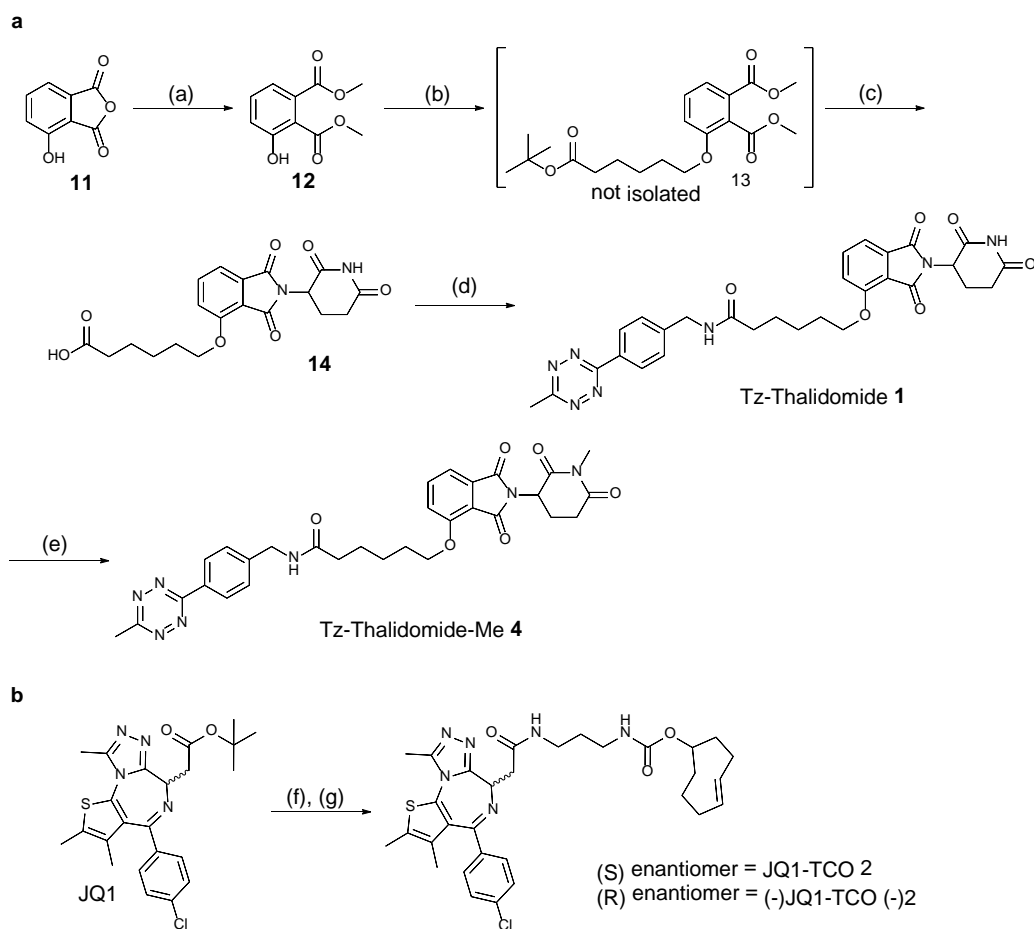
A375 cells were treated with Probe 1 for 4 h followed by thalidomide (10 μ M) and Tz-thalidomide (1 μ M) for 18 h. Following cell lysis, the levels of ERK1/2 were studied by Western Blots.



Synthesis of CLIPTAC Precursor Components

Tz-thalidomide **1** was synthesised in 7 steps as illustrated in **Supplementary Scheme S1a**, starting with the opening of the anhydride moiety of compound **11** with methanol and methylation of the carboxylic acid generated to give the dimethyl ester **12**. Alkylation of the phenol group was performed under Mitsunobu conditions to afford compound **13** which underwent a selective hydrolysis under basic conditions followed by amination with the hydrochloride salt of 3-amino piperidine-2,6-dione, and hydrolysis of the *tert*-butyl ester under acidic conditions to form the diacid **14**. The tetrazine moiety was inserted by amide coupling to give Tz-thalidomide **1**. Finally, the imide group was methylated to form Tz-thalidomide-Me **4**, an inactive CLIPTAC partner to be used in control experiments. JQ1-TCO **2** was synthesised in two steps as illustrated in **Supplementary Scheme 1b**, with first, the cleavage of the *tert*-butyl ester under acidic conditions. The amide coupling was then carried out with the commercially available TCO-amine reagent to afford JQ1-TCO **2**. The same synthesis was performed on the inactive enantiomer of JQ1, to give (-)JQ1-TCO (-)**2** for use in control experiments.

Supplementary Scheme 1. Syntheses of CLIPTAC Precursor Components.^a



^a**Reagents and conditions:** (a) 1. MeOH, reflux, 3 h. 2. NaHCO₃, MeI, DMF, 55 °C, 3 h, 94%; (b) *tert*-butyl 6-hydroxyhexanoate, PPh₃, DIAD, THF, r.t., 18 h; (c) 1. NaOH, THF, MeOH, r.t., 2 h. 2. 3-amino piperidine-2,6-dione HCl, pyridine, 110 °C, 17 h. 3. TFA, r.t., 3 h, 10% over 4 steps; (d) Tz-amine, DIPEA, HATU, DMF, r.t., 2 h, 57%; (e) NaH, MeI, THF, r.t., 18 h, 69%; (f) DCM, TFA, r.t., 3 h, quant.; (g) DIPEA, HATU, TCO-amine, DMF, r.t., 16 h, 70% (JQ1-TCO) and 48% ((-)-JQ1-TCO).

General Notes for Syntheses

Anhydrous solvents were purchased either from VWR or SeccoSolv and were stored under nitrogen. Other solvents were purchased from Fisher Chemicals. Commercially available reagents were used as received. TCO-Amine and TCO-NHS ester were purchased from Jena Bioscience. Petrol refers to the fraction with a boiling range between 40 and 60 °C. All reactions were followed by TLC analysis (pre-coated TLC sheets ALUGRAM[®] SIL G/UV₂₅₄, Macherey-Nagel) or LC-MS (liquid chromatography mass spectrometry) on Agilent 1200 HPLC and 6140 MS using a YMC-Triart C18 column (50 x 2.0 mm, 1.9 µm). ¹H NMR spectra were recorded on a Bruker 400 UltraShield[™] spectrometer. Chemical shifts are reported in parts per million (δ) referenced to the appropriate deuterated solvent employed and relative to TMS. Multiplicities are indicated by s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet). 'Flash' column chromatography was performed on pre-packed silica cartridges (Biotage SNAP cartridges, KP-Sil) on Biotage Isolera Four. All reactions were carried out under nitrogen.

The purity of the final probes was determined by LC-MS and ¹H NMR and was always >95%.

LC-MS methodology

Eluent A: 10 mmol ammonium bicarbonate pH 9.4

Eluent B: acetonitrile

Gradient: 3 – 99% B over 0.7 min

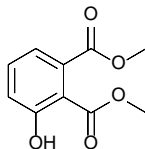
Flow: 0.7 mL/min

Column T: 45 °C

Procedures for the preparation of CLIPTAC components

Synthesis of Tz-thalidomide and Tz-thalidomide-Me

Procedure for Compound **12**: 1,2-Dimethyl 3-hydroxybenzene-1,2-dicarboxylate.



12

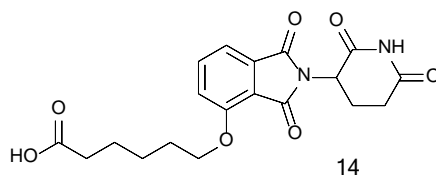
A mixture of 3-hydroxyphthalic anhydride **11** (1.6 g, 9.8 mmol) in MeOH (25 mL) was refluxed for 3 hours. The mixture was cooled to room temperature and concentrated. The residue and NaHCO₃ (2.3 g, 27.3 mmol) were stirred in DMF (20 mL). Methyl iodide (1.46 mL, 23.4 mmol) was added and the reaction mixture was heated at 55 °C for 3 hours. The reaction was cooled to room temperature and diluted with EtOAc (80 mL) and water (40 mL). The mixture was acidified with an aqueous solution of HCl (4N) and the aqueous layer was extracted with EtOAc (2 x 40 mL). The combined organic layers were washed with water (2 x 100), brine (2 x 100) and dried over MgSO₄. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography with 3:7 EtOAc: Petrol to give 1,2-dimethyl 3-hydroxybenzene-1,2-dicarboxylate **12** (1.9 g, 9.1 mmol, 94%) as a pale pink oil.

LCMS: Retention time 1.14 min, [M+H]⁺ = 211

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.28 (s, 1H), 7.44-7.30 (m, 2H), 7.17 (dd, *J* = 7.1, 2.2 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ ppm 167.61, 166.10, 155.04, 130.96, 129.04, 122.99, 120.92, 120.42, 52.90, 52.53.

Procedure for Compound 14: 6-[[2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl]oxy] hexanoic acid.



To a 0 °C mixture of *tert*-butyl 6-hydroxyhexanoate **12** (0.90 g, 4.76 mmol), triphenylphosphine (0.75 g, 2.86 mmol) and 1,2-dimethyl 3-hydroxybenzene-1,2-dicarboxylate (0.5 g, 2.38 mmol) in THF (4 mL), was added DIAD (0.56 mL, 2.86 mmol) dropwise. The mixture was stirred at room temperature for 18 hours. The solvent was removed *in vacuo* and the crude product was partitioned between EtOAc (20 mL) and water (20 mL). The organic phase was washed with brine (2 x 20 mL), dried over MgSO₄ and the solvent was removed *in vacuo*.

LCMS: Retention time 1.51 min, [M+H]⁺ = 381

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 7.58-7.45 (m, 2H), 7.40 (dd, *J* = 7.0, 2.3 Hz, 1H), 4.04 (t, *J* = 6.2 Hz, 2H), 3.82 (s, 3H), 3.79 (s, 3H), 2.19 (m, 2H), 1.71-1.62 (m, 2H), 1.58-1.50 (m, 2H), 1.44-1.37 (m, 11H).

The crude product was solubilised in THF/MeOH (1:1, 15 mL) and NaOH (1M aqueous, 3.03 mL, 3.03 mmol, 3eq) was added. The resulting solution was stirred at room temperature for 2 hours. The mixture was then acidified to pH 4-5 with a 1M HCl aqueous solution. The organic layer was extracted with DCM. The organic layers were combined, dried over MgSO₄ and the solvent was removed *in vacuo*. The crude product was carried forward without further purification.

LCMS: Retention time 1.16 min, [M-H]⁻ = 365

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 13.23 (br s, 1H), 7.53-7.44 (m, 2H), 7.35 (dd, *J* = 5.2, 4.1 Hz, 1H), 4.03 (t, *J* = 6.1 Hz, 2H), 3.75 (s, 3H), 2.20 (t, *J* = 7.2 Hz, 2H), 1.73-1.61 (m, 2H), 1.61-1.50 (m, 2H), 1.44-1.33 (m, 11H).

The crude material and 3-aminopiperidine-2,6-dione hydrochloride (0.12 g, 0.75 mmol, 1.1 eq) were dissolved in pyridine (2.7 mL) and heated to 110 °C for 17 hours. The mixture was cooled to room temperature. DCM (20 mL) and a 0.5 M HCl aqueous solution (10 mL) were added. The organic phase was extracted with DCM (3 x 20 mL). The organic phases were combined, dried over MgSO₄ and the solvent was removed *in vacuo*. The crude product was dissolved in TFA (4 mL) and the mixture was stirred at room temperature for 3 hours. The solvent was removed *in vacuo* and the crude material was dissolved in DCM (10 mL). The organic phase was extracted with a saturated solution of NaHCO₃ (4 x 20 mL). The aqueous phases were combined and acidified to pH 3 with a 1M HCl aqueous solution. The aqueous phase was then extracted with DCM (4 x 50 mL). The organic phases were combined, dried over MgSO₄ and the solvent was removed *in vacuo* to give 6-[[2-(2,6-dioxopiperidin-3-yl)-

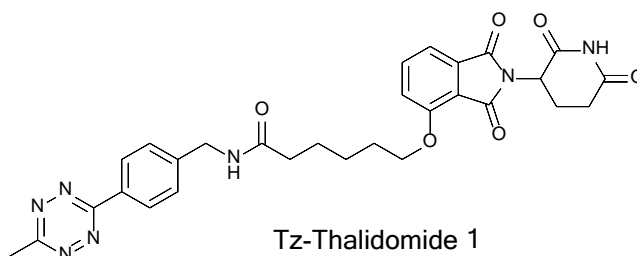
1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}hexanoic acid **14** (0.16 g, 0.40 mmol, 58%) as a pale yellow foam.

LCMS: Retention time 1.06 min, $[M-H]^- = 387$

1H NMR (400 MHz, DMSO- d_6) δ ppm 11.96 (br s, 1H), 11.08 (s, 1H), 7.81 (dd, $J = 8.5, 7.2$ Hz, 1H), 7.52 (d, $J = 8.5$ Hz, 1H), 7.45 (d, $J = 7.2$ Hz, 1H), 5.08 (dd, $J = 12.8, 5.4$ Hz, 1H), 4.21 (t, $J = 6.4$ Hz, 2H), 2.95-2.82 (m, 1H), 2.64-2.52 (m, 2H), 2.24 (t, $J = 7.2$ Hz, 2H), 2.11-1.96 (m, 1H), 1.83-1.72 (m, 2H), 1.62-1.54 (m, 2H), 1.52-1.42 (m, 2H).

^{13}C NMR (101 MHz, DMSO- d_6) δ ppm 174.84, 173.23, 170.39, 167.30, 165.76, 156.49, 137.49, 133.76, 120.31, 116.74, 115.63, 69.22, 49.24, 34.12, 31.45, 28.66, 25.42, 24.65, 22.49.

Procedure for Tz-thalidomide 1: 6-{{2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-{[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]methyl}hexanamide.



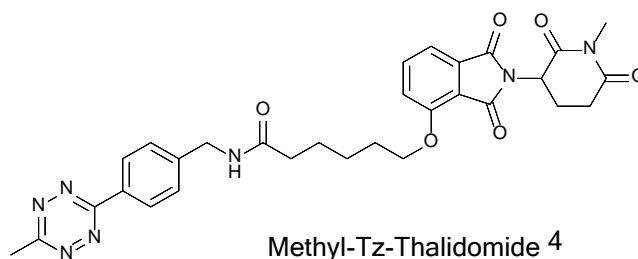
6-{{2-(2,6-Dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}hexanoic acid **14** (0.16 g, 0.40 mmol), tetrazine amine (0.09 g, 0.4 mmol), DIPEA (0.2 mL, 1.2 mmol) and HATU (0.15 g, 0.40 mmol) were mixed in DMF (4 mL) and the reaction was stirred at room temperature for 2 hours. The reaction was diluted with DCM (20 mL) and washed with brine (3 x 20 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed in *vacuo*. The crude product was purified by flash column chromatography with 9:1 EtOAc: Petrol. The compound was then solubilised in MeCN (20 mL) and water (15 mL) and was dried on the freeze dryer to give 6-{{2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-{[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]methyl}hexanamide (0.13 g, 0.23 mmol, 57%) as a pink solid.

LCMS: Retention time 1.3 min, $[M-H]^- = 570$

1H NMR (400 MHz, DMSO- d_6) δ ppm 11.07 (s, 1H), 8.46-8.38 (m, 3H), 7.80 (dd, $J = 8.6, 7.2$ Hz, 1H), 7.55-7.49 (m, 3H), 7.44 (d, $J = 7.2$ Hz, 1H), 5.07 (dd, $J = 12.7, 5.4$ Hz, 1H), 4.40 (d, $J = 5.8$ Hz, 2H), 4.21 (t, $J = 6.3$ Hz, 2H), 3.00 (s, 3H), 2.89-2.82 (m, 1H), 2.63-2.53 (m, 2H), 2.23 (t, $J = 7.3$ Hz, 2H), 2.09-1.98 (m, 1H), 1.84-1.74 (m, 2H), 1.70-1.59 (m, 2H), 1.54-1.43 (m, 2H).

^{13}C NMR (101 MHz, DMSO- d_6) δ ppm 173.20, 172.69, 170.38, 167.52, 167.31, 165.78, 163.67, 156.50, 145.04, 137.48, 133.73, 130.80, 128.52, 127.90, 120.30, 116.73, 115.63, 69.25, 49.23, 42.32, 35.79, 31.44, 28.69, 25.50, 25.46, 22.49, 21.29.

Procedure for Tz-thalidomide-Me 4: *N*-{[4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenyl]methyl}-6-{[2-(1-methyl-2,6-dioxo piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}hexanamide.



NaH (60% dispersion, 9 mg, 0.23 mmol) was added to a stirred solution of 6-{[2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}-*N*-{[4-(6-methyl-1,2,4,5-tetrazin-3-yl)phenyl]methyl}hexanamide (50 mg, 0.19 mmol) in dry THF (2 mL) at 0 °C. The suspension was stirred at room temperature for 30 min, then methyl iodide (14 μ L, 0.23 mmol) was added. The reaction was stirred at room temperature for 18 hours. The reaction was quenched and diluted with water (10 mL). The aqueous phase was extracted with DCM. The combined organic phases were dried over MgSO₄ and the solvent was removed *in vacuo*. The product was purified by flash column chromatography with 100 EtOAc to give *N*-{[4-(6-Methyl-1,2,4,5-tetrazin-3-yl)phenyl]methyl}-6-{[2-(1-methyl-2,6-dioxo piperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-isoindol-4-yl]oxy}hexanamide (45 mg, 0.13 mmol, 69%) as a pink solid.

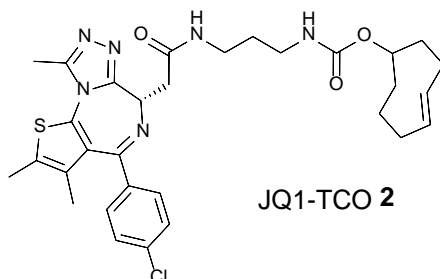
LCMS: Retention time 1.33 min, [M-H]⁻ = 584.2

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.48-8.34 (m, 3H), 7.81 (dd, *J* = 8.8, 7.0 Hz, 1H), 7.59-7.47 (m, 3H), 7.44 (d, *J* = 7.0 Hz, 1H), 5.14 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.39 (d, *J* = 5.9 Hz, 2H), 4.21 (t, *J* = 6.5 Hz, 2H), 3.01 (s, 3H), 3.00 (s, 3H), 2.97-2.87 (m, 1H), 2.81-2.71 (m, 1H), 2.61-2.54 (m, 1H), 2.22 (t, *J* = 7.5 Hz, 2H), 2.09-2.00 (m, 1H), 1.85-1.73 (m, 2H), 1.70-1.60 (m, 2H), 1.54-1.43 (m, 2H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ ppm 172.69, 172.21, 170.13, 167.51, 167.28, 165.77, 163.66, 156.53, 145.04, 137.51, 133.71, 130.79, 128.52, 127.89, 120.31, 116.70, 115.63, 69.24, 49.79, 42.31, 35.7, 31.57, 28.68, 27.05, 25.49, 25.44, 21.68, 21.27.

Synthesis of JQ1-TCO (2)

Procedure for JQ1-TCO 2: (4E)-Cyclooct-4-en-1-yl N-(3-{2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido}propyl)carbamate.



(+)-JQ-1 (0.10 g, 0.22 mmol) was dissolved in DCM (1.1 mL) and TFA (1.1 mL). The solution was stirred at room temperature for 3 hours. The solvent was removed *in vacuo* to give 2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetic acid, TFA salt (0.11 g, 0.22 mmol, quant.) as a yellow foam which was used without further purification.

LCMS: Retention time 1.14 min, $[M+H]^+ = 401$

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.12-8.20 (br s, 2H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.3 Hz, 2H), 4.46 (dd, *J* = 7.0, 7.0 Hz, 1H), 3.43 (dd, *J* = 16.7, 7.0 Hz, 1H), 3.34 (dd, *J* = 16.7, 7.0 Hz, 1H), 2.62 (s, 3H), 2.42 (s, 3H), 1.64 (s, 3H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ ppm 172.41, 163.69, 158.74 (q, *J* = 39 Hz), 155.27, 150.46, 137.08, 135.81, 132.63, 131.39, 130.66, 130.39, 130.09, 128.98, 115.76 (q, *J* = 288 Hz), 54.03, 36.98, 14.52, 13.16, 11.74.

2-[(9S)-7-(4-Chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetic acid. TFA salt (40 mg, 0.08 mmol), *N,N*-diisopropylethylamine (41 μ L, 0.23 mmol), HATU (30 mg, 0.08 mmol) and TCO-amine (18 mg, 0.08 mmol) were mixed in DMF (1 mL) and the reaction was stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and the crude product was solubilised in diethyl ether (10 mL) and washed with a cold saturated solution of NaHCO₃ (3 x 10 mL). The organic phase was dried over MgSO₄ and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 1: 9 MeOH: DCM to give (4E)-cyclooct-4-en-1-yl N-(3-{2-[(9S)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido}propyl)carbamate (33 mg, 0.05 mmol, 70%) as a white solid.

LCMS: Retention time 1.47 min, $[M+H]^+ = 609$

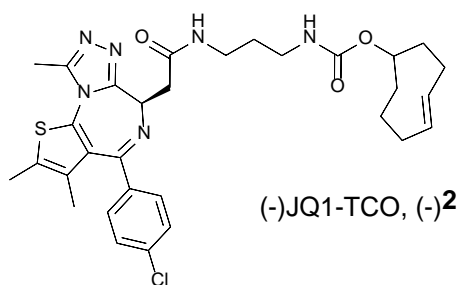
¹H NMR (400 MHz, CDCl₃) δ ppm 7.41 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 6.98 (t, *J* = 6.9 Hz, 1H), 5.66-5.46 (m, 2H), 5.40-5.32 (m, 1H), 4.66 (dd, *J* = 6.8 Hz, 1H), 4.41-4.29 (m, 1H), 3.56 (ddd, *J* = 14.4 Hz, 7.5 Hz, 2.1 Hz, 1H), 3.46-3.32 (m, 3H), 3.24-3.12 (m, 2H),

2.70 (s, 3H), 2.43 (s, 3H), 2.41-2.31 (m, 3H), 2.06-2.00 (m, 1H), 2.00-1.88 (m, 3H), 1.80-1.66 (m, 7H), 1.59-1.51 (m, 1H).

^{13}C NMR (101 MHz, CDCl_3) δ ppm 170.97, 163.87, 156.46, 155.91, 149.82, 136.80, 136.58, 134.90, 132.93, 130.88, 129.82, 128.70, 80.49, 54.42, 41.40, 39.24, 38.96, 37.36, 36.23, 34.21, 32.52, 30.92, 29.94, 14.29, 13.02, 11.56. (2 quaternary C not visible)

Synthesis of (-)JQ1-TCO, (-)2

Procedure for (-)JQ1-TCO, (-)2: (4E)-Cyclooct-4-en-1-yl N-(3-{2-[(9R)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido}propyl)carbamate.



(-)-JQ-1 (0.05 g, 0.11 mmol) was dissolved in DCM (0.6 mL) and TFA (0.6 mL). The solution was stirred at room temperature for 3 hours. The solvent was removed *in vacuo* to give 2-[(9R)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetic acid, TFA salt (0.06 g, 0.11 mmol, quant.) as a yellow foam which was used without further purification.

LCMS: Retention time 1.13 min, $[\text{M}+\text{H}]^+ = 401$

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ ppm 7.80-6.85 (br s, 2H), 7.50 (d, $J = 8.4$ Hz, 2H), 7.45 (d, $J = 8.4$ Hz, 2H), 4.46 (dd, $J = 6.9, 6.9$ Hz, 1H), 3.43 (dd, $J = 16.7, 6.9$ Hz, 1H), 3.33 (dd, $J = 16.7, 6.9$ Hz, 1H), 2.62 (s, 3H), 2.42 (s, 3H), 1.64 (s, 3H).

^{13}C NMR (101 MHz, $\text{DMSO}-d_6$) δ ppm 172.40, 163.73, 158.76 (q, $J = 38$ Hz), 155.28, 150.53, 137.06, 135.83, 132.59, 131.44, 130.67, 130.40, 130.12, 128.98, 115.74 (q, $J = 289$ Hz), 54.00, 36.97, 14.50, 13.18, 11.72.

2-[(9R)-7-(4-Chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetic acid. TFA salt (40 mg, 0.08 mmol), *N,N*-diisopropylethylamine (41 μL , 0.23 mmol), HATU (30 mg, 0.08 mmol) and TCO-amine (18 mg, 0.08 mmol) were mixed in DMF (1 mL) and the reaction was stirred at room temperature for 16 hours. The solvent was removed *in vacuo* and the crude product was solubilised in diethyl ether (10 mL) and washed with a cold saturated solution of NaHCO_3 (3 x 10 mL). The organic phase was dried over MgSO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography with 1: 9 MeOH: DCM to give (4E)-cyclooct-4-en-1-yl N-(3-{2-[(9R)-7-(4-chlorophenyl)-4,5,13-trimethyl-3-thia-1,8,11,12-

tetraazatricyclo[8.3.0.0^{2,6}]trideca-2(6),4,7,10,12-pentaen-9-yl]acetamido}propyl)carbamate (23 mg, 0.04 mmol, 48%) as a pale yellow solid.

¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.16 (t, *J* = 5.3 Hz, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.42 (d, *J* = 8.5 Hz, 2H), 6.92-6.89 (m, 1H), 5.62-5.54 (m, 1H), 5.47-5.40 (m, 1H), 4.50 (dd, *J* = 8.5, 5.7 Hz, 1H), 4.24-4.19 (m, 1H), 3.27-3.24 (m, 1H), 3.20-3.13 (m, 2H), 3.08-2.98 (m, 3H), 2.60 (s, 3H), 2.42 (s, 3H), 2.29-2.21 (m, 3H), 1.94-1.80 (m, 4H), 1.67-1.60 (m, 4H), 1.59-1.52 (m, 4H).

¹³C NMR (101 MHz, DMSO-*d*₆) δ ppm 169.94, 163.43, 155.54, 151.90, 150.51, 137.22, 135.70, 135.31, 132.91, 130.92, 130.69, 130.46, 128.99, 79.42, 54.41, 41.20, 38.68, 38.46, 38.12, 36.72, 34.22, 32.70, 31.11, 30.04, 14.54, 13.17, 11.80.