SUPPORTING INFORMATION

The Discovery of I-BRD9, a Selective Cell Active Chemical Probe for Bromodomain Containing Protein 9 Inhibition

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Abbreviations

Å- Angstrom
Ala - Alanine
ApoA1 - Apolipoprotein A1
Arg - Arginine
Asn - Asparagine
ATAD2 - ATPase family, AAA domain containing 2
Aq. - Aqueous
BAF - BRG1/brm-associated factor
BAZ1B - Bromodomain adjacent to zinc finger domain, 1B
BCP - Bromodomain containing protein
BD - Bromodomain
BET - Bromodomain and Extra Terminal domain
BRD2 - Bromodomain containing protein 2
BRD3 - Bromodomain containing protein 3
BRD4 - Bromodomain containing protein 4
BRD7 - Bromodomain containing protein 7
BRD9 - Bromodomain containing protein 9
BRDT - Bromodomain containing protein, testis-specific
CECR2 - Cat eye syndrome chromosome region, candidate 2
CREBBP - cAMP response element-binding protein binding protein
DCM - Dichloromethane
DIPEA - *N*,*N*-Diisopropylethylamine
DME - 1,2-Dimethoxyethane
DMF - *N*,*N*-Dimethylformamide
DMSO - Dimethylsulfoxide
EDC - 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EP300 - E1A binding protein p300
ET – Extra terminal
FRET - Time-resolved Forster (fluorescence) resonance energy transfer
h – Hours
HAT - Histone acetyltransferases
HATU - O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate
HDAC - Histone deacetylase
HRMS- High resolution mass spectrometry
Ile – Isoleucine
IPA - Isopropyl alcohol
Kac - Acetyl-lysine
Leu - Leucine
LCMS - Liquid column mass spectrometry
LPS - Lipopolysaccharide
Lys - Lysine
M - Molar, moles/decimetre^{-3}
mmol - Millimol
m.p - Melting point

m/z - mass/charge ratio
NBS - *N*-Bromosuccinimide
nM - Nanomolar
NMC - NUT midline carcinoma
NMR - Nuclear magnetic resonance
PEPPSI – Palladium-enhanced Precatalyst Preparation Stabilisation and Initiation
rt - Room temperature
R_t - Retention time
sat. - Saturated
SAR - Structure activity relationship
SGC – Structural Genomics Consortium
SMARCA2 - SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 2
SMARCA4 - SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4
SWI/SNF - Switch/sucrose nonfermentable
THF – Tetrahydrofuran
TR-FRET - Time-resolved Forster (fluorescence) resonance energy transfer
w/v – weight per volume

General Experimental

All solvents were purchased from Sigma Aldrich (Hy-Dry anhydrous solvents) and commercially available reagents were used as received. All reactions were followed by TLC analysis (TLC plates GF254, Merck) or LCMS (liquid chromatography mass spectrometry) using a Waters ZQ instrument. IR spectra were obtained on a Perkin Elmer Spectrum 1 machine.

NMR spectra were recorded at ambient temperature using standard pulse methods on any of the following spectrometers and signal frequencies: Bruker AV-400 (^{1}H = 400 MHz, ^{13}C = 101 MHz), Bruker AV-500 (^{1}H = 500 MHz, ^{13}C = 125 MHz). Chemical shifts are reported in ppm and are referenced to tetramethylsilane (TMS) or the following solvent peaks: $CDCl_3$ (^{1}H = 7.27 ppm, ^{13}C = 77.00 ppm), DMSO-d_6 (^{1}H = 2.50 ppm, ^{13}C = 39.51 ppm) and MeOH-d_4 (^{1}H = 3.31 ppm, ^{13}C = 49.15 ppm). Coupling constants are quoted to the nearest 0.1 Hz and multiplicities are given by the following abbreviations and combinations thereof: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Column chromatography was performed on pre-packed silica gel columns (30-90 mesh, IST) using a biotage SP4.

High resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, with analytes separated on an Agilent 1100 Liquid Chromatograph equipped with a Phenomenex Luna C18(2) reversed phase column (100 mm x 2.1 mm, 3 µm packing diameter). LC conditions were 0.5 mL/min flow rate, 35 ºC, injection volume 2 - 5 µL. Gradient elution with (A) H_2O containing 0.1% (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. Gradient conditions were initially 5% B, increasing linearly to 100% B over 6 min, remaining at 100% B for 2.5 min then decreasing linearly to 5% B over 1 min followed by an equilibration period of 2.5 min prior to the next injection.

LCMS analysis was carried out on a H_2Os Acquity UPLC instrument equipped with a BEH column (50 mm x 2.1 mm, 1.7 µm packing diameter) and H_2Os micromass ZQ MS using alternate-scan positive and negative electrospray. Analytes were detected as a summed UV wavelength of 210 – 350 nm. Two liquid phase methods were used:

Formic – 40 °C, 1 mL/min flow rate. Gradient elution 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. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

High pH – 40 °C, 1 mL/min flow rate. Gradient elution 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. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

Mass-directed automatic purification (MDAP) was carried out using a H_2Os ZQ MS using alternate-scan positive and negative electrospray and a summed UV wavelength of 210 – 350 nm. Two liquid phase methods were used:

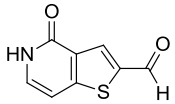
Formic – Sunfire C18 column (100 mm x 19 mm, 5 µm packing diameter, 20 mL/min flow rate) or Sunfire C18 column (150 mm x 30 mm, 5 µm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) H_2O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid.

High pH – Xbridge C18 column (100 mm x 19 mm, 5 µm packing diameter, 20 mL/min flow rate) or Xbridge C18 column (150 mm x 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.

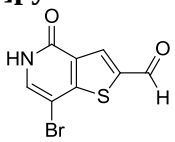
Synthetic Procedures

4-Oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbaldehyde (19)



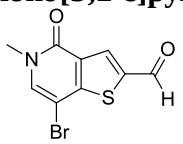
A suspension of 2-bromothieno[3,2-c]pyridin-4(5H)-one (**18**)[1] (33.40 g, 145.0 mmol) in anhydrous THF (250 mL) was cooled to –78 ºC, under an atmosphere of nitrogen. ^{n}BuLi (1.6 M in hexanes, 200 mL, 319.0 mmol) was added dropwise over 0.5 h (internal temperature < –60 ºC). After complete addition, the mixture was stirred at –60ºC for 1 h. A solution of DMF (23 mL, 290.0 mmol) in THF (20 mL) was added dropwise over 0.5 h, and the mixture was stirred for a further 1 h at –70 °C. H_2O (500 mL) was added dropwise and the mixture was allowed to warm to room temperature. Et_2O (500 mL) was added and the phases were separated. The aqueous layer was acidified to pH 4 with 2 M aq. HCl and the resulting suspension stirred for 0.5 h, then filtered under reduced pressure. The solid was washed with H_2O (50 mL) and Et_2O (50 mL), collected and dried under vacuum at 50 °C to give **19** (16.80 g, 65%) as an orange solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 11.71 (br.s, 1H), 10.02 (s, 1H), 8.42 (s, 1H), 7.49–7.44 (m, 1H), 6.93 (d, *J* = 6.8, 1H); LCMS (high pH) $(M + H)^+$ = 179.9, R_t = 0.57 min (94%).

7-Bromo-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbaldehyde (20)



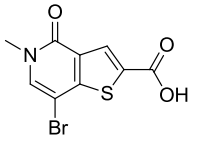
To a suspension of **19** (16.00 g, 89.0 mmol) in THF (200 mL), NBS (20.66 g, 116.0 mmol) was added. The reaction mixture was left to stir for 24 h at room temperature. The suspension was filtered under reduced pressure, washed with THF (50 mL) and Et_2O (50 mL), collected and dried under vacuum at 40 °C to give **20** (18.20 g, 79%) as a grey solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 12.07 (br.s, 1H), 10.03 (s, 1H), 8.52 (s, 1H), 7.80 (s, 1H); LCMS (formic acid) $(M + H)^+$ = 256.0, 258.0, R_t = 0.71 min (96%).

7-Bromo-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbaldehyde (21)



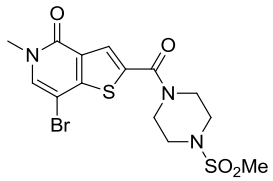
To a suspension of **20** (18.00 g, 69.7 mmol) in THF (200 mL), Cs_2CO_3 (68.20 g, 209.0 mmol) and MeI (8.7 mL, 139.0 mmol) were added. The reaction mixture was left to stir at room temperature for 18 h. The volatile components were removed *in vacuo* and the resulting residue was diluted with H_2O (200 mL) and stirred for 0.5 h. The suspension was filtered, washed with H_2O (50 mL), collected and dried under vacuum at 40 °C to give **21** (13.40 g, 71%) as a light brown solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 10.02 (s, 1H), 8.53 (s, 1H), 8.20 (s, 1H), 3.54 (s, 3H); LCMS (high pH) $(M + H)^+$ = 271.8, 273.6, R_t = 0.83 min (100%).

7-Bromo-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxylic acid (22)



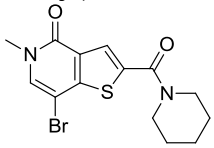
A suspension of **21** (6.80 g, 25.0 mmol) in DMSO (125 mL) and H_2O (125 mL) was cooled to 0 °C. Sodium chlorite (11.30 g, 125.0 mmol), sodium dihydrogen phosphate (14.99 g, 125.0 mmol) and hydrogen peroxide (35% weight in H_2O, 3.8 mL, 125.0 mmol) were added. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. Hydrogen peroxide (35% weight in H_2O, 1.0 mL, 32.9 mmol) was added in a single portion and the mixture was stirred for a further 20 h at room temperature. The reaction was acidified to pH 1 with glacial acetic acid and concentrated *in vacuo*. The residue was diluted with H_2O (500 mL) causing a solid to precipitate. The solid was filtered under reduced pressure, collected and dried under vaccum at 47 ºC to give **22** (3.19 g, 42%) as an orange solid ; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 13.00 (s, 1H), 8.12 (s, 1H), 8.02 (s, 1H), 3.51 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 287.0, 289.0, R_t = 0.67 min (95%).

7-Bromo-5-methyl-2-(4-(methylsulfonyl)piperazine-1-carbonyl)thieno[3,2-c]pyridin-4(5H)-one (23)



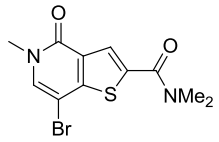
A mixture of **22** (183 mg, 0.64 mmol), 1-(methylsulfonyl)piperazine (128 mg, 0.78 mmol), HATU (290 mg, 0.76 mmol) and DIPEA (0.22 mL, 1.27 mmol) in DMF (5 mL) was left to stir at room temperature for 18 h. The volatile components were removed *in vacuo* and the resulting residue was dissolved in EtOAc (10 mL), washed with aq. citric acid (1 M, 3 × 10 mL), sat. aq. $NaHCO_3$ (3 × 10 mL), H_2O (10 mL) and brine (10 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give **23** (150 mg, 54%) as a brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.87 (s, 1H), 7.41 (s, 1H), 3.93 (t, *J* = 5.1, 4H), 3.65 (s, 3H), 3.35 (t, *J* = 5.1, 4H), 2.85 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 434.1, 436.1, R_t = 0.73 min (85%).

7-Bromo-5-methyl-2-(piperidine-1-carbonyl)thieno[3,2-c]pyridin-4(5H)-one (24)



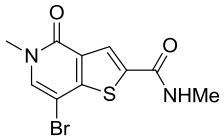
A mixture of **22** (250 mg, 0.87 mmol), piperidine (0.1 mL, 1.13 mmol), HATU (396 mg, 1.04 mmol), and DIPEA (0.3 mL, 1.74 mmol) in DMF (7.5 mL) was stirred at room temperature for 18 h. The volatile components were removed *in vacuo* and the resulting residue was dissolved in EtOAc (25 mL) and washed with aq. citric acid (10% w/v, 3 × 25 mL), sat. aq. $NaHCO_3$ (3 × 25 mL), H_2O (25 mL) and brine (25 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give **24** (244 mg, 79%) as a light brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.83 (s, 1H), 7.37 (s, 1H), 3.75–3.71 (m, 4H), 3.64 (s, 3H), 1.77–1.65 (m, 6H); LCMS (formic acid) $(M + H)^+$ = 355.2, 357.2, R_t = 0.89 min (100%).

7-Bromo-*N*,*N*,5-trimethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxamide (25)



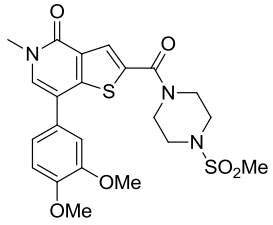
A mixture of **22** (150 mg, 0.52 mmol), dimethylamine hydrochloride (55 mg, 0.68 mmol), HATU (238 mg, 0.63 mmol), and DIPEA (0.18 mL, 1.04 mmol) in DMF (4.5 mL) was left to stir at room temperature for 20 h. The volatile components were removed *in vacuo* and the resulting residue was dissolved in EtOAc (15 mL), washed with aq. citric acid (10% w/v, 3 × 20 mL), sat. aq. $NaHCO_3$ (3 × 20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give **25** (70 mg, 43%) as an off white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.93 (s, 1H), 7.38 (s, 1H), 3.64 (s, 3H), 3.27 (br.s, 6H); LCMS (formic acid) $(M + H)^+$ = 315.1, 317.1, R_t = 0.70 min (100%).

7-Bromo-*N*,5-dimethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxamide (26)



A mixture of **22** (150 mg, 0.52 mmol), DIPEA (0.18 mL, 1.04 mmol), HATU (238 mg, 0.63 mmol) and methylamine hydrochloride (46 mg, 0.68 mmol) in DMF (3.5 mL) was left to stir at room temperature for 18 h. The volatile components were removed *in vacuo* and the resulting residue was dissolved in EtOAc (10 mL) and washed with aq. citric acid (10% w/v, 10 mL), causing precipitation of a solid. The solid was filtered under reduced pressure, rinsed with H_2O (15 mL), collected and dried under vacuum at 40 °C to give **26** (66 mg, 42%) as an off white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.81–8.76 (m, 1H), 8.30 (s, 1H), 8.07 (s, 1H), 3.52 (s, 3H), 2.79 (d, *J* = 4.7, 3H); LCMS (formic acid) $(M + H)^+$ = 301.1, 303.1, R_t = 0.67 min (82%).

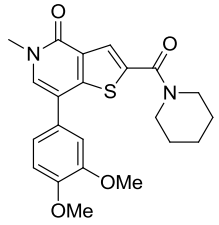
7-(3,4-Dimethoxyphenyl)-5-methyl-2-(4-(methylsulfonyl)piperazine-1-carbonyl)thieno[3,2-c]pyridin-4(5H)-one (17)



A mixture of **23** (140 mg, 0.32 mmol), (3,4-dimethoxyphenyl)boronic acid (72 mg, 0.40 mmol), K_2CO_3 (135 mg, 0.98 mmol) and bis(triphenylphosphine)palladium(II) chloride (21 mg, 0.03 mmol) in H_2O (1.2 mL) and DME (7 mL) was heated at 120 °C in a microwave reactor for 0.5 h. The mixture was allowed to cool to room temperature and partitioned between EtOAc (20 mL) and H_2O (20 mL). The organic layer was filtered through Celite®, passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (0–5% MeOH in EtOAc). The appropriate fractions were combined and solvent evaporated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and solvent evaporated *in vacuo* to give **17**

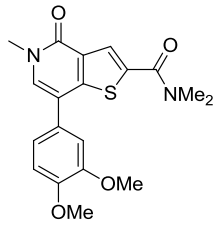
(70 mg, 44%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.87 (s, 1H), 7.26 (s, 1H), 7.12 (dd, *J* = 8.2, 2.1, 1H), 7.08 (d, *J* = 2.1, 1H), 6.99 (d, *J* = 8.2, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 3.95–3.92 (m, 4H), 3.72 (s, 3H), 3.36–3.33 (m, 4H), 2.85 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 492.3, R_t = 0.79 min (99%).

7-(3,4-Dimethoxyphenyl)-5-methyl-2-(piperidine-1-carbonyl)thieno[3,2-c]pyridin-4(5H)-one (27)



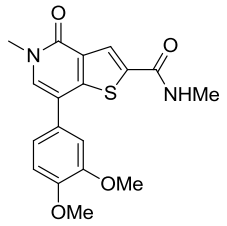
A mixture of **24** (230 mg, 0.65 mmol), (3,4-dimethoxyphenyl)boronic acid (147 mg, 0.81 mmol), K_2CO_3 (268 mg, 1.94 mmol) and bis(triphenylphosphine)palladium(II) chloride (45 mg, 0.07 mmol) in H_2O (1.25 mL) and DME (7.5 mL) was heated at 120 °C in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (20 mL), filtered through Celite®, passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (0–5%, MeOH in CH_2Cl_2). The appropriate fractions were combined and solvent evaporated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and the solvent was evaporated *in vacuo* to give **27** (121 mg, 45%) as an off white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.82 (s, 1H), 7.22 (s, 1H), 7.14–7.08 (m, 2H), 6.97 (d, *J* = 7.8, 1H), 3.95 (s, 6H), 3.75–3.71 (m, 4H), 3.70 (s, 3H), 1.76–1.61 (m, 6H); LCMS (formic acid) $(M + H)^+$ = 413.3, R_t = 0.93 min (100%).

7-(3,4-Dimethoxyphenyl)-*N*,*N*,5-trimethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxamide (28)



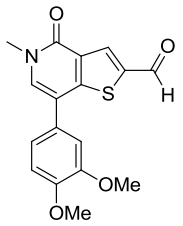
A mixture of **25** (70 mg, 0.22 mmol), (3,4-dimethoxyphenyl)boronic acid (49 mg, 0.27 mmol), K_2CO_3 (92 mg, 0.67 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in H_2O (0.4 mL) and DME (1.7 mL) was heated at 120 °C in a microwave reactor for 0.5 h. The mixture was allowed to cool to room temperature and diluted with EtOAc (20 mL). The solution was filtered through Celite®, passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and solvent evaporated *in vacuo* to give **28** (47 mg, 57%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.93 (s, 1H), 7.23 (s, 1H), 7.13 (dd, *J* = 8.2, 2.2, 1H), 7.10 (d, *J* = 2.2, 1H), 6.98 (d, *J* = 8.2, 1H), 3.96 (s, 3H), 3.95 (s, 1H), 3.71 (s, 3H), 3.26 (br.s, 6H); LCMS (formic acid) $(M + H)^+$ = 373.2, R_t = 0.78 min (100%).

7-(3,4-Dimethoxyphenyl)-*N*,5-dimethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxamide (29)



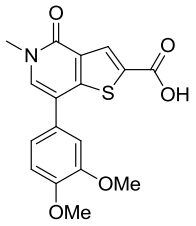
A mixture of **26** (66 mg, 0.22 mmol), (3,4-dimethoxyphenyl)boronic acid (44 mg, 0.24 mmol), K_2CO_3 (91 mg, 0.66 mmol) and bis(triphenylphosphine)palladium(II) chloride (14 mg, 0.02 mmol) in H_2O (0.25 mL) and DME (1.5 mL) was heated at 120 °C in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (15 mL), filtered through Celite®, passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and the solvent evaporated *in vacuo* to give **29** (19 mg, 24%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.12 (s, 1H), 7.24 (s, 1H), 7.14–7.11 (m, 1H), 7.10–7.08 (m, 1H), 6.98 (d, *J* = 8.1, 1H), 6.57–6.50 (m, 1H), 3.96 (s, 6H), 3.71 (s, 3H), 3.06 (d, *J* = 4.6, 3H); LCMS (formic acid) $(M + H)^+$ = 359.2, R_t = 0.74 min (98%).

7-(3,4-Dimethoxyphenyl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbaldehyde (30)



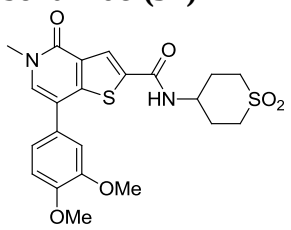
A mixture of **21** (1.00 g, 3.67 mmol), (3,4-dimethoxyphenyl)boronic acid (0.77 g, 4.23 mmol), K_2CO_3 (1.37 g, 9.92 mmol) and bis(triphenylphosphine)palladium(II) chloride (0.23 g, 0.33 mmol) in DME (12.0 mL) and H_2O (2.0 mL) was heated at 120 °C in a microwave reactor for 0.5 h. The mixture was allowed to cool to room temperature, diluted with EtOAc (25 mL) and H_2O (5 mL) and filtered through Celite®. The filtrate was concentrated *in vacuo*. The resulting solid was suspended in EtOAc (50 mL), filtered under reduced pressure, rinsed with EtOAc (50 mL), collected and dissolved in DMSO (10 mL). The resulting solution was blown down under a stream of nitrogen at 40 °C to give **30** (750 mg, 62%) as a yellow solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 10.04 (s, 1H), 8.51 (s, 1H), 7.96 (s, 1H), 7.21–7.17 (m, 2H), 7.11 (d, *J* = 8.8, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.62 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 320.2, R_t = 0.83 min (95%).

7-(3,4-Dimethoxyphenyl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxylic acid (31)



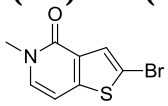
To a solution of **30** (750 mg, 2.27 mmol) in H_2O (10 mL) in DMSO (10 mL), sodium chlorite (1.44 g, 15.94 mmol), sodium dihydrogen phosphate (1.91 g, 15.94 mmol) and hydrogen peroxide (35% weight in H_2O, 0.5 mL, 15.94 mmol) were added. The mixture was left to stir at room temperature for 48 h. The mixture was acidified to pH 1 with glacial acetic acid (15 mL) and concentrated *in vacuo* (DMSO remained). Addition of H_2O (20 mL) caused precipitation of a solid, which was filtered under reduced pressure, rinsed with H_2O (10 mL), collected and dried under vacuum at 40 ˚C to give **31** (562 mg, 72%) as a light orange solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 13.42 (br.s, 1H), 8.02 (s, 1H), 7.87 (s, 1H), 7.22–7.16 (m, 2H), 7.10 (d, J = 8.8, 1H), 3.83 (s, 3H), 3.82 (s, 3H), 3.59 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 346.2, R_t = 0.75 min (100%).

7-(3,4-Dimethoxyphenyl)-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboxamide (32)



To a solution of **31** (52 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), and DIPEA (0.08 mL, 0.45 mmol) in DMF (0.4 mL), 4-aminotetrahydro-2H-thiopyran-1,1-dioxide hydrochloride (22 mg, 0.12 mmol) was added. The reaction mixture was left to stand for 18 h at room temperature. The mixture was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and the solvent evaporated under a stream of nitrogen at 40 °C to give **32** (10 mg, 13%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.71 (d, J = 7.9, 1H), 8.41 (s, 1H), 7.81 (s, 1H), 7.19 (dd, J = 8.1, 1.8, 1H), 7.17 (d, J = 1.9, 1H), 7.09 (d, J = 8.1, 1H), 4.21–4.14 (m, 1H), 3.83 (s, 3H), 3.81 (s, 3H), 3.60 (s, 3H), 3.30–3.25 (m, 2H), 3.18–3.12 (m, 2H), 2.17–2.03 (m, 4H); LCMS (formic acid) $(M + H)^+$ = 477.0, R_t = 0.78 min (97%).

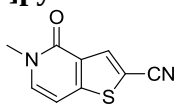
2-Bromo-5-methylthieno[3,2-c]pyridin-4(5H)-one (33)



To a stirred suspension of 2-bromothieno[3,2-c]pyridin-4(5H)-one (**18**)[1] (10.00 g, 43.5 mmol) in THF (500 mL), Cs_2CO_3 (42.50 g, 130.0 mmol) was added. The mixture was left to stir at room temperature for 1 h. MeI (5.4 mL, 87.0 mmol) was added and the reaction mixture was left to stir for a further 18 h at room temperature. The volatile components were removed *in vacuo* and the

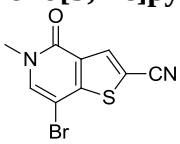
resulting solid was suspended in H_2O (200 mL), filtered under reduced pressure, washed with H_2O (100 mL), collected and dried under vacuum at 40 ˚C to give **28** (9.65 g, quant.) as a brown solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.64 (s, 1H), 7.16 (d, *J* = 7.1, 1H), 6.57 (d, *J* = 7.1, 1H), 3.62 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 243.9, 245.9, R_t = 0.81 min (100%).

5-Methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbonitrile (34)



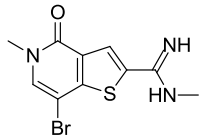
A mixture of **33** (1.50 g, 6.10 mmol), zinc cyanide (1.44 g, 12.30 mmol) and $Pd(PPh_3)_4$ (640 mg, 0.50 mmol) in DMF (10 mL) was heated at 115 ˚C for 4.5 h in a microwave reactor. This process was repeated to provide a further 4 identical batches. The 5 batches were allowed to cool to room temperature, combined, diluted with EtOAc (50 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (0–5%, MeOH in CH_2Cl_2). The appropriate fractions were combined and solvent evaporated *in vacuo* to give **34** (4.45 g, 68%) as a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.16 (s, 1H), 7.34 (d, *J* = 7.1, 1H), 6.66 (d, *J* = 7.1, 1H), 3.64 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 191.1, R_t = 0.63 min (95%).

7-Bromo-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbonitrile (35)



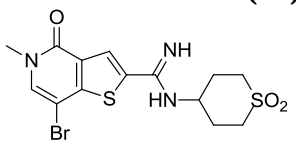
A solution of **34** (1.57 g, 7.80 mmol.) in THF (30 mL) was treated with NBS (2.09 g, 11.80 mmol) and the reaction mixture was stirred at room temperature for 72 h. The volatile components were removed *in vacuo* and the resulting solid was suspended in Et_2O (50 mL), filtered under reduced pressure, washed with Et_2O (50 mL), collected and dried under vacuum at 40 ˚C to give **35** (1.81 g, 86%) as an off white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.24 (s, 1H), 7.50 (s, 1H), 3.65 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 268.9, 270.9, R_t = 0.83 min (95%).

7-Bromo-*N*,5-dimethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (36)



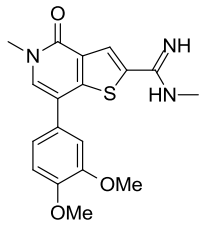
A solution of **35** (50 mg, 0.19 mmol) and NaOMe solution in MeOH (25% by weight, 0.05 mL, 0.20 mmol) in MeOH (4 mL) was heated at 75 ˚C for 16 h. Methylamine hydrochloride (38 mg, 0.56 mmol) was added and the solution was left to stir for a further 3 h. The reaction mixture was allowed to cool to room temperature. The volatile components were removed *in vacuo* to give **36** (107 mg, quant.) as a pale yellow solid; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.36 (s, 1H), 8.01 (s, 1H), 3.67 (s, 3H), 3.13 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 300.0, 302.0, R_t = 0.45 min (81%).

7-Bromo-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-4,5dihydrothieno[3,2-c]pyridine-2-carboximidamide (37)



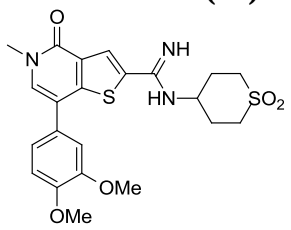
To a suspension of **35** (2.00 g, 7.43 mmol) in MeOH (30 mL), NaOMe solution in MeOH (25% by weight, 1.7 mL, 7.43 mmol) was added. The reaction mixture was heated to 75 °C for 3 h. 4-aminotetrahydro-2H-thiopyran-1,1-dioxide hydrochloride (1.66 g, 8.94 mmol) was added at 75 °C and the reaction mixture was stirred for a further 72 h. The volatile components were removed *in vacuo* and the resulting solid was suspended in MeOH (50 mL), filtered under reduced pressure, washed with MeOH (20 mL), collected and dried under vacuum at 40 ºC to give **37** (2.69 g, 87%) as a white solid ; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.17 (s, 1H), 7.99 (s, 1H), 6.79 (br.s, 2H), 3.67–3.59 (m, 1H), 3.51 (s, 3H), 3.27–3.17 (m, 2H), 3.10–3.01 (m, 2H), 2.08–1.99 (m, 2H), 1.98–1.88 (m, 2H); LCMS (formic acid) $(M + H)^+$ = 418.0, 420.0, R_t = 0.48 min (96%).

7-(3,4-Dimethoxyphenyl)-*N*,5-dimethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (38)



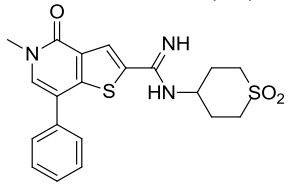
A mixture of **36** (107 mg, 0.21 mmol), (3,4-dimethoxyphenyl)boronic acid (47 mg, 0.26 mmol), K_2CO_3 (71 mg, 0.51 mmol) and PEPPSI-^{i}Pr (13 mg, 0.02 mmol) in IPA (1.5 mL) and H_2O (0.5 mL) was heated at 120 °C for 0.5 h in a microwave reactor. The reaction mixture was allowed to cool to room temperature and diluted with EtOAc (30 mL) and MeOH (20 mL). The solution was filtered through Celite®, passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue dissolved in MeOH (5 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo*. The resultant solid was purified by mass directed autopreparation (high pH). The appropriate fractions were combined and concentrated *in vacuo* to give **38** (5 mg, 7%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.04 (s, 1H), 7.64 (s, 1H), 7.23–7.17 (m, 2H), 7.08 (d, *J* = 8.1, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 3.72 (s, 3H), 2.96 (s, 3H); LCMS (formic acid) $(M + H)^+$ = 358.2, R_t = 0.58 min (100%).

7-(3,4-Dimethoxyphenyl)-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (39)



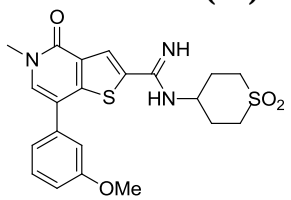
A mixture of **37** (128 mg, 0.18 mmol), (3,4-dimethoxyphenyl)boronic acid (43 mg, 0.24 mmol), K_2CO_3 (64 mg, 0.46 mmol) and PEPPSI-^{i}Pr (19 mg, 0.03 mmol) in H_2O (0.5 mL) and IPA (1.5 mL) was heated at 120 °C for 0.5 h in a microwave reactor. The reaction mixture was allowed to cool to room temperature and diluted with EtOAc (50 mL). The solution was filtered through Celite®, passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (10 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo* to give **39** (37 mg, 42%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.11 (s, 1H), 7.72 (s, 1H), 7.19 (dd, *J* = 8.1, 2.1, 1H), 7.16 (d, *J* = 2.1, 1H), 7.10 (d, *J* = 8.1, 1H), 6.74 (br.s, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.64–3.56 (m, 4H), 3.23–3.18 (m, 2H), 3.09–2.99 (m, 2H), 2.06–1.97 (m, 2H), 1.95–1.87 (m, 2H); LCMS (formic acid) $(M + H)^+$ = 476.2, R_t = 0.61 min (100%).

***N*-(1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-7-phenyl-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (40)**



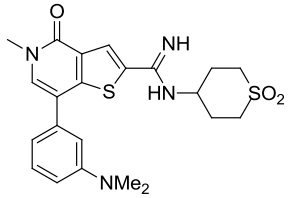
A mixture of **37** (100 mg, 0.24 mmol), phenylboronic acid (35 mg, 0.29 mmol), PEPPSI-^{i}Pr (15 mg, 0.02 mmol) and K_2CO_3 (79 mg, 0.57 mmol) in H_2O (1 mL) and IPA (3 mL) was heated at 120 ˚C in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (40 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (5 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo* to give **40** (42 mg, 42%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.16 (s, 1H), 7.77 (s, 1H), 7.65–7.61 (m, 2H), 7.56–7.50 (m, 2H), 7.46–7.41 (m, 1H), 6.78 (br.s, 2H), 3.67–3.56 (m, 4H), 3.21–3.12 (m, 2H), 3.11–3.01 (m, 2H), 2.08–1.97 (m, 2H), 1.97–1.86 (m, 2H); LCMS (formic acid) $(M + H)^+$ = 416.1, R_t = 0.63 min (100%).

***N*-(1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)-7-(3-methoxyphenyl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (41)**



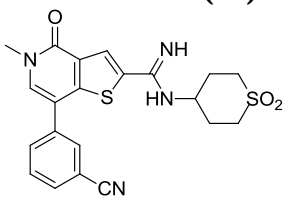
A mixture of **37** (63 mg, 0.15 mmol), (3-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), K_2CO_3 (50 mg, 0.36 mmol) and PEPPSI-^{i}Pr (9 mg, 0.01 mmol) in H_2O (0.3 mL) and IPA (0.9 mL) was heated at 120 ˚C in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (3 mL) and passed through a preconditioned (EtOAc, 5 mL) C-18 column (1 g). The column was rinsed with EtOAc (3 mL) and MeOH (2 mL). The filtrate was collected and blown down under a stream of nitrogen at 40 ˚C. The resulting residue was purified by mass directed autopreparation (formic acid). The solvent was evaporated under a stream of nitrogen at 40 ˚C. The resulting residue was dissolved in MeOH (5 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and solvent evaporated *in vacuo* to give **41** (5 mg, 7%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.13 (s, 1H), 7.79 (s, 1H), 7.47–7.41 (m, 1H), 7.24–7.20 (m, 1H), 7.17–7.15 (m, 1H), 7.00 (dd, *J* = 8.3, 2.3, 1H), 6.74 (br.s, 2H), 3.83 (s, 3H), 3.64–3.57 (m, 4H), 3.22–3.13 (m, 2H), 3.09–3.00 (m, 2H), 2.06–1.96 (m, 2H), 1.96–1.86 (m, 2H); LCMS (formic acid) $(M + H)^+$ = 446.3, R_t = 0.63 min (98%).

7-(3-(Dimethylamino)phenyl)-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (42)



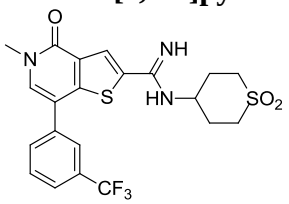
A mixture of **37** (36 mg, 0.15 mmol), (3-(dimethylamino)phenyl)boronic acid (30 mg, 0.18 mmol), K_2CO_3 (50 mg, 0.36 mmol,) and PEPPSI-^{i}Pr (9 mg, 0.01 mmol) in H_2O (0.3 mL) and IPA (0.9 mL) was heated at 120 ˚C in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (3 mL) and passed through a preconditioned (EtOAc, 5 mL) C-18 column (1 g). The column was rinsed with EtOAc (3 mL) and MeOH (2 mL). The filtrate was collected and blown down under a stream of nitrogen at 40 ˚C. The resulting residue was purified by mass directed autopreparation (high pH). The appropriate fractions were combined and solvent evaporated under a stream of nitrogen at 40 °C to give **42** (3 mg, 4%) as a white solid; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.98 (s, 1H), 7.62 (s, 1H), 7.36–7.30 (m, 1H), 7.01–6.98 (m, 1H), 6.94 (d, *J* = 8.2, 1H), 6.85 (dd, *J* = 8.2, 2.6, 1H), 3.78–3.71 (m, 4H), 3.31–3.25 (m, 2H), 3.16–3.08 (m, 2H), 3.02 (s, 6H), 2.28–2.13 (m, 4H); LCMS (high pH) $(M + H)^+$ = 459.4, R_t = 0.91 min (100%).

7-(3-Cyanophenyl)-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (43)



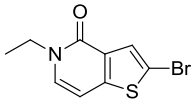
A mixture of **37** (100 mg, 0.24 mmol), (3-cyanophenyl)boronic acid (42 mg, 0.29 mmol), PEPPSI-^{i}Pr (15 mg, 0.02 mmol) and K_2CO_3 (79 mg, 0.57 mmol) in H_2O (1 mL) and IPA (3 mL) was heated at 120 ºC in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool room temperature, diluted with EtOAc (40 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (5 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo* to give **43** (34 mg, 32%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.16 (s, 1H), 8.08–8.07 (m, 1H), 8.05–8.01 (m, 1H), 7.92–7.87 (m, 2H), 7.77–7.73 (m, 1H), 6.77 (br.s, 2H), 3.63–3.55 (m, 4H), 3.21–3.11 (m, 2H), 3.10–3.01 (m, 2H), 2.07–1.97 (m, 2H), 1.95–1.85 (m, 2H); LCMS (formic acid) $(M + H)^+$ = 441.1, R_t = 0.59 min (100%).

***N*-(1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)-5-methyl-4-oxo-7-(3-(trifluoromethyl)phenyl)-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (44)**



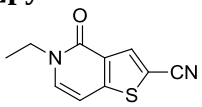
A mixture of **37** (100 mg, 0.24 mmol), (3-(trifluoromethyl)phenyl)boronic acid (54 mg, 0.29 mmol), PEPPSI-^{i}Pr (15 mg, 0.02 mmol) and K_2CO_3 (79 mg, 0.57 mmol) in H_2O (0.5 mL) and IPA (1.5 mL) was heated at 120 ºC in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (40 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (5 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo* to give **44** (60 mg, 52%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.16 (s, 1H), 8.02–7.98 (m, 1H), 7.94 (bs, 1H), 7.91 (s, 1H), 7.81–7.77 (m, 2H), 6.77 (br.s, 2H), 3.65–3.56 (m, 4H), 3.21–3.12 (m, 2H) 3.08–3.00 (m, 2H), 2.07–1.96 (m, 2H), 1.95–1.85 (m, 2H); LCMS (formic acid) $(M + H)^+$ = 484.1, R_t = 0.77 min (100%).

2-Bromo-5-ethylthieno[3,2-c]pyridin-4(5H)-one (47)



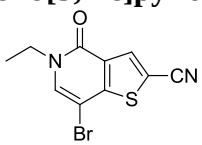
A single portion of ethyl iodide (1.40 mL, 17.39 mmol) was added to a solution of bromothieno[3,2-c]pyridin-4(5H)-one (**18**)[1] (2.00 g, 8.69 mmol) and Cs_2CO_3 (8.50 g, 26.10 mmol) in THF (35 mL) at room temperature. The reaction mixture was heated at 60 °C for 18 h, then allowed to cool to room temperature and diluted with EtOAc (50 mL) and H_2O (50 mL). The separated aqueous phase was extracted with EtOAc (3 × 50 mL) and the combined organic layers were passed through a hydrophobic frit and concentrated *in vacuo* to give **47** (2.30 g, quant.) as a light brown solid. 50 mg of **47** was taken forward for purification by mass directed autopreparation (formic acid), providing suitably pure material (44 mg, 0.17 mmol) for full characterisation; mp 124–125 °C; ν_{max} (solid)/cm^{-1}: 1632, 1577, 1082, 846, 761; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ ppm 7.62 (s, 1H), 7.56 (d, *J* = 7.2, 1H), 6.89 (d, *J* = 7.2, 1H), 3.99 (q, *J* = 7.2, 2H), 1.24 (t, *J* = 7.2, 3H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ ppm 156.9, 148.9, 134.3, 130.7, 127.5, 112.2, 101.3, 43.8, 15.1; HRMS $(M + H)^+$ calculated for C_9H_9BrNOS 257.9583; found 257.9585. LCMS (formic acid) $(M + H)^+$ = 258.0, 260.0, R_t = 0.89 min (98%).

5-Ethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbonitrile (48)



A mixture of **47** (1.10 g, 4.26 mmol), zinc cyanide (1.00 g, 8.52 mmol), and $Pd(PPh_3)_4$ (0.44 g, 0.3 mmol) in DMF (10 mL) was heated to 115 ˚C in a microwave reactor for 3.5 h. This process was repeated to provide a second identical batch. The two batches were allowed to cool to room temperature, diluted with EtOAc (50 mL each) and combined. The solution was filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (0–3% MeOH in CH_2Cl_2). The appropriate fractions were combined and concentrated *in vacuo* to give **48** (1.70 g, 67%) as a light brown solid. 50 mg of **48** was taken forward for purification by mass directed autopreparation (formic acid), providing suitably pure material (33 mg, 0.16 mmol) for full characterisation; mp 157–158 °C; ν_{max} (solid)/cm^{-1}: 2211, 1637, 1595, 1247, 1078, 879, 780, 769; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.32 (s, 1H), 7.82 (d, *J* = 7.2, 1H), 7.03 (d, *J* = 7.2, 1H), 4.00 (q, *J* = 7.2, 2H), 1.25 (t, *J* = 7.2, 3H); ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ ppm 157.6, 151.6, 137.6, 137.2, 129.4, 114.7, 106.9, 101.4, 43.9, 15.0; HRMS $(M + H)^+$ calculated for $C_{10}H_9N_2OS$ 205.0430; found 205.0429. LCMS (formic acid) $(M + H)^+$ = 205.0, R_t = 0.73 min (98%).

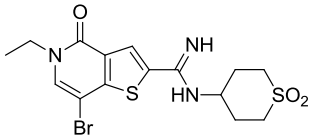
7-Bromo-5-ethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbonitrile (49)



To a solution of **48** (1.17 g, 5.73 mmol) in THF (20 mL), NBS (1.53 g, 8.59 mmol) was added portion-wise. The reaction mixture was left to stir at room temperature for 48 h. The volatile components were removed *in vacuo*. The resulting solid was suspended in Et_2O (50 mL), filtered under reduced pressure, washed with Et_2O (50 mL), collected and dried under vacuum at 40 ˚C

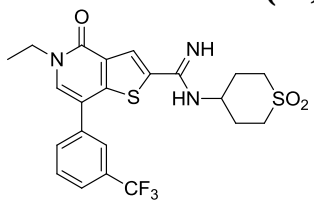
to give **49** (1.69 g, quant.) as an off white solid; mp 197–198 °C; ν_{max} (solid)/cm^{-1}: 3041, 2220, 1654, 1584, 1250, 1090, 766; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ ppm 8.50 (s, 1H), 8.26 (s, 1H), 4.02 (q, *J* = 7.2, 2H), 1.26 (t, *J* = 7.2, 3H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ ppm 156.7, 152.9, 138.7, 138.1, 128.8, 114.2, 107.5, 91.0, 44.4, 14.9; HRMS $(M + H)^+$ calculated for $C_{10}H_8BrN_2OS$ 282.9535; found 282.9534. LCMS (formic acid) $(M + H)^+$ = 283.0, 285.0, R_t = 0.93 min (100%).

7-Bromo-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-ethyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (50)



To a suspension of **49** (1.00 g, 3.53 mmol) in MeOH (30 mL) at room temperature, sodium methoxide (25% weight in MeOH, 0.81 mL, 3.53 mmol) was added in a single portion. The reaction mixture was heated at 75 ˚C for 5 h. 4-Aminotetrahydro-2H-thiopyran-1,1-dioxide hydrochloride (1.98 g, 10.60 mmol) was added and the solution was heated for a further 18 h. The volatile components were removed *in vacuo* and the resulting solid was suspended in Et_2O (50 mL), filtered under reduced pressure, washed with Et_2O (50 mL) collected and dried under vacuum at 40 ˚C to give **50** (1.55 g, quant.) as a white solid. 50 mg of **50** was taken forward for purification by mass directed autopreparation (high pH), providing suitably pure material (25 mg, 0.06 mmol) for full characterisation; mp 284–285 °C; ν_{max} (solid)/cm^{-1}: 1646, 1578, 1284, 1124, 853, 769; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ ppm 8.18 (s, 1H), 8.01 (s, 1H), 6.80 (br.s, 2H), 4.01 (q, *J* = 7.2, 2H), 3.68–3.61 (m, 1H), 3.27–3.20 (m, 2H), 3.10–3.03 (m, 2H), 2.09–2.00 (m, 2H), 1.99–1.90 (m, 2H), 1.26 (t, *J* = 7.2, 3H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ ppm 156.9, 150.4, 136.2, 130.2, 122.6, 109.0, 90.6, 48.6, 44.5, 30.0, 15.1; HRMS $(M + H)^+$ calculated for $C_{15}H_{19}BrN_3O_3S_2$ 432.0046; found 432.0046. LCMS (formic acid) $(M + H)^+$ = 432.1, 434.1, R_t = 0.53 min (100%).

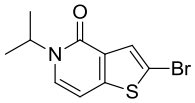
***N*-(1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)-5-ethyl-4-oxo-7-(3-(trifluoromethyl)phenyl)-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (45, I-BRD9)**



A mixture of **50** (150 mg, 0.35 mmol), (3-(trifluoromethyl)phenyl)boronic acid (79 mg, 0.42 mmol), PEPPSI-^{i}Pr (21 mg, 0.03 mmol) and K_2CO_3 (115 mg, 0.83 mmol) in H_2O (1 mL) and IPA (3 mL) was heated at 120 ºC in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (40 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (5 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo* to give **45** (50 mg, 29%) as a white solid; mp 228–229 °C; ν_{max} (solid)/cm^{-1}: 1640, 1587, 1340, 1303, 1117; ^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ ppm 8.15 (s, 1H), 8.03–7.98 (m, 1H), 7.98–7.94 (m, 1H), 7.91 (s, 1H), 7.82–7.76 (m, 2H), 6.76 (br.s, 2H), 4.10 (q, *J* = 7.0, 2H), 3.64–3.57 (m,

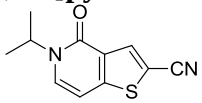
1H), 3.21–3.12 (m, 2H), 3.10–3.00 (m, 2H), 2.06–1.96 (m, 2H), 1.96–1.85 (m, 2H), 1.32 (t, J = 7.0, 3H); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ ppm 157.6, 149.7, 147.7, 142.2, 137.5, 134.1, 131.9, 130.7, 130.5, 130.3 (q, J_{C-F} = 31.7), 125.0 (q, J_{C-F} = 3.7), 124.60 (q, J_{C-F} = 3.7), 124.59 (q, J_{C-F} = 272.6), 123.1, 114.4, 49.9, 49.0, 44.0, 30.6, 15.2; ^{19}F NMR (376 MHz, $(CD_3)_2SO$) δ ppm -61.0; HRMS $(M + H)^+$ calculated for $C_{22}H_{23}F_3N_3O_3S_2$ 498.1128; found 498.1110. LCMS (formic acid) $(M + H)^+$ = 498.1, R_t = 0.81 min (100%).

2-Bromo-5-isopropylthieno[3,2-c]pyridin-4(5H)-one (51)



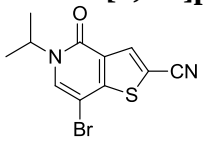
2-Iodopropane (1.3 mL, 13.04 mmol) was added in a single portion to a stirred suspension of 2-bromothieno[3,2-c]pyridin-4(5H)-one (**18**)[1] (1.5 g, 6.52 mmol) and K_2CO_3 (1.26 g, 9.13 mmol) in 1,4-dioxane (10 mL) at room temperature. The vial was sealed and heated at 150 ºC in a microwave reactor for 5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (30 mL) and H_2O (30 mL). The separated aqueous phase was extracted with EtOAc (3 × 30 mL) and the combined organic layers were passed through a hydrophobic frit and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (0–20% EtOAc in cyclohexane). The appropriate fractions were combined and solvent evaporated *in vacuo* to give **51** (1.37 g, 77%) as a pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.62 (s, 1H), 7.20 (d, J = 7.5, 1H), 6.61 (d, J = 7.5, 1H), 5.25–5.48 (m, 1H), 1.40 (d, J = 6.9, 6H); LCMS (formic acid) $(M + H)^+$ = 271.9, 273.9, R_t = 0.99 min (100%).

5-Isopropyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbonitrile (52)



A mixture of **51** (1.37 g, 5.02 mmol), zinc cyanide (1.18 g, 10.05 mmol) and $Pd(PPh_3)_4$ (0.52 g, 0.45 mmol) in DMF (12 mL) was heated at 115 °C in a microwave reactor for 5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (30 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (0–40% EtOAc in cyclohexane). The appropriate fractions were combined and solvent evaporated *in vacuo* to give **52** (998 mg, 77%) as an off white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.17 (s, 1H), 7.39 (d, J = 7.5, 1H), 6.71 (d, J = 7.5, 1H), 5.40–5.32 (m, 1H), 1.42 (d, J = 6.9, 6H); LCMS (formic acid) $(M + H)^+$ = 219.0, R_t = 0.82 min (85%).

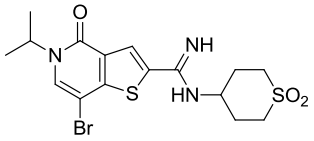
7-Bromo-5-isopropyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carbonitrile (53)



To a stirred solution of **52** (998 mg, 4.57 mmol) in THF (15 mL), NBS (1.22 g, 6.86 mmol) was added in a single portion. The reaction mixture was left to stir at room temperature for 3 h. The volatile components were removed *in vacuo* and the resulting solid was suspended in Et_2O (50 mL), filtered under reduced pressure, rinsed with Et_2O (50 mL), collected and dried under vacuum at 40 °C to give **53** (1.15 g, 85%) as a white solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm

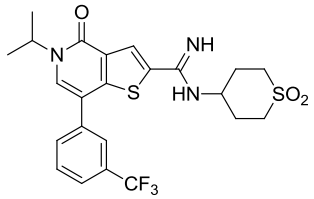
8.25 (s, 1H), 7.48 (s, 1H), 5.38–5.28 (m, 1H), 1.43 (d, J = 6.9, 6H); LCMS (formic acid) $(M + H)^+$ = 296.9, 298.9, R_t = 1.03 min (97%).

7-Bromo-*N*-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-isopropyl-4-oxo-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (54)



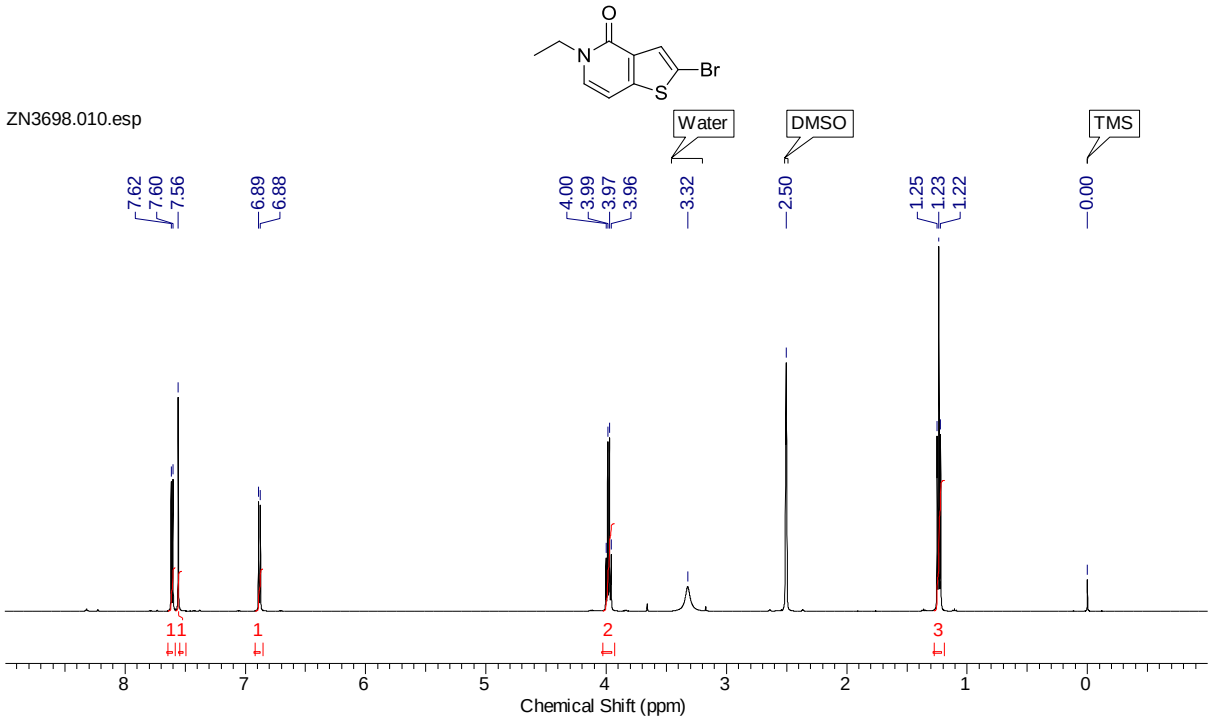
To a suspension of **53** (1.00 g, 3.37 mmol) in MeOH (30 mL), NaOMe (25% weight in MeOH, 0.8 mL, 3.37 mmol) was added in a single portion. The reaction mixture was heated at 75 ºC for 0.5 h. 4-Aminotetrahydro-2H-thiopyran-1,1-dioxide hydrochloride (1.87 g, 10.10 mmol) was added and the solution was heated at 75 ºC for a further 18 h. The volatile components were removed *in vacuo*. The resulting solid was suspended in MeOH (50 mL), filtered under reduced pressure, rinsed with MeOH (50 mL) collected and dried under vacuum at 40 °C to give **54** (1.55 g, 93%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.21 (s, 1H), 7.95 (s, 1H), 5.19–5.10 (m, 1H), 3.75–3.66 (m, 1H), 3.41–3.05 (m, 4H), 2.31–2.21 (m, 2H), 2.10–1.95 (m, 2H), 1.35 (d, J = 6.9, 6H); LCMS (formic acid) $(M + H)^+$ = 446.0, 448.0, R_t = 0.59 min (100%).

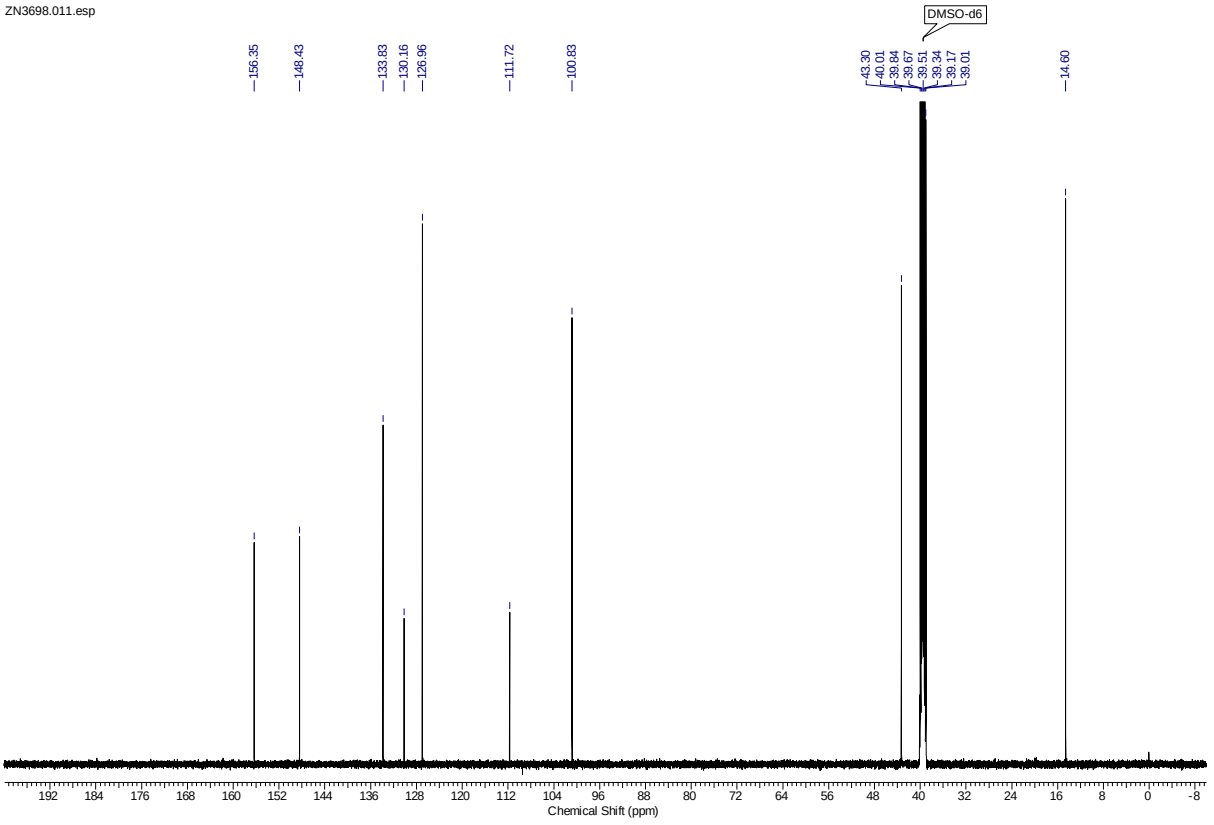
***N*-(1,1-Dioxidotetrahydro-2H-thiopyran-4-yl)-5-isopropyl-4-oxo-7-(3-(trifluoromethyl)phenyl)-4,5-dihydrothieno[3,2-c]pyridine-2-carboximidamide (46)**



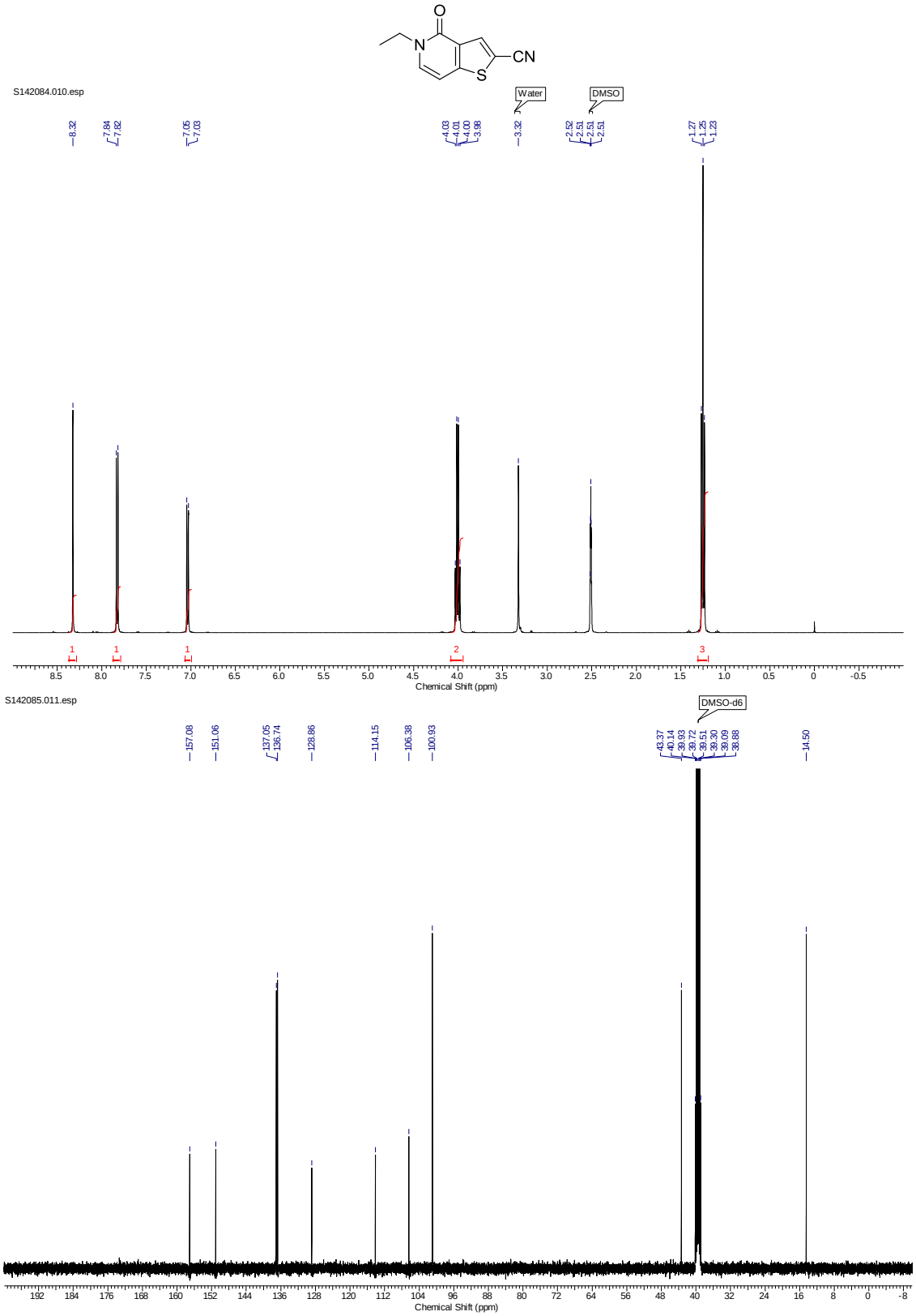
A mixture of **54** (100 mg, 0.22 mmol), 3-(trifluoromethyl)phenyl)boronic acid (51 mg, 0.27 mmol), PEPPSI-^{i}Pr (15 mg, 0.02 mmol) and K_2CO_3 (74 mg, 0.54 mmol) in H_2O (1 mL) and IPA (3 mL) was heated at 120 ºC in a microwave reactor for 0.5 h. The reaction mixture was allowed to cool to room temperature, diluted with EtOAc (40 mL), filtered through Celite® and concentrated *in vacuo*. The resulting residue was purified by mass directed autopreparation (formic acid). The appropriate fractions were combined and concentrated *in vacuo*. The resulting residue was dissolved in MeOH (20 mL) and passed through a preconditioned (MeOH, 20 mL) amino propyl column (10 g). The appropriate fractions were combined and concentrated *in vacuo* to give **46** (35 mg, 31%) as a white solid; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ ppm 8.15 (s, 1H), 8.02–7.93 (m, 2H), 7.82–7.74 (m, 3H), 6.76 (br.s, 2H), 5.27–5.18 (m, 1H), 3.65–3.56 (m, 1H), 3.21–3.10 (m, 2H), 3.10–2.98 (m, 2H), 2.06–1.95 (m, 2H), 1.95–1.86 (m, 2H), 1.42 (d, J = 6.8, 6H); LCMS (formic acid) $(M + H)^+$ = 512.1, R_t = 0.86 min (100%).

^{1}H NMR (500 MHz, $(CD_3)_2SO$) and ^{13}C NMR (125 MHz, $(CD_3)_2SO$) Spectra for compound 47

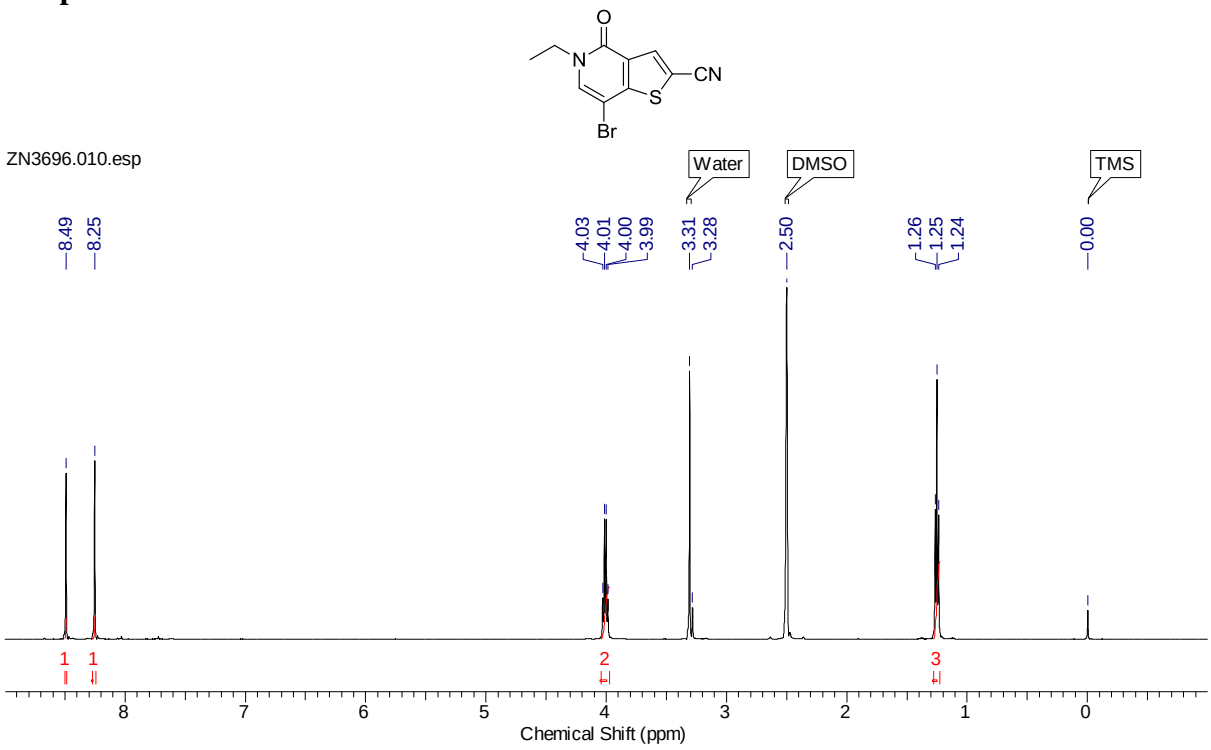


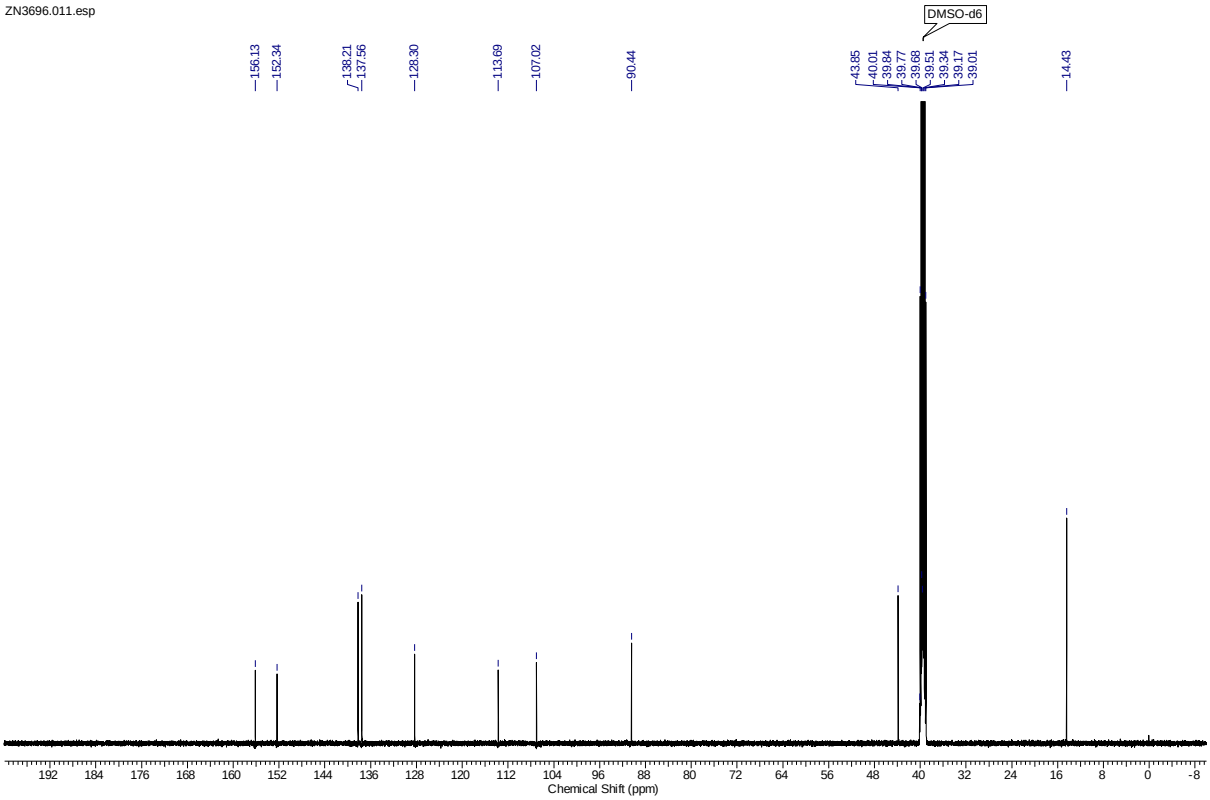


^{1}H NMR (400 MHz, $(CD_3)_2SO$) and ^{13}C NMR (100 MHz, $(CD_3)_2SO$) Spectra for compound 48

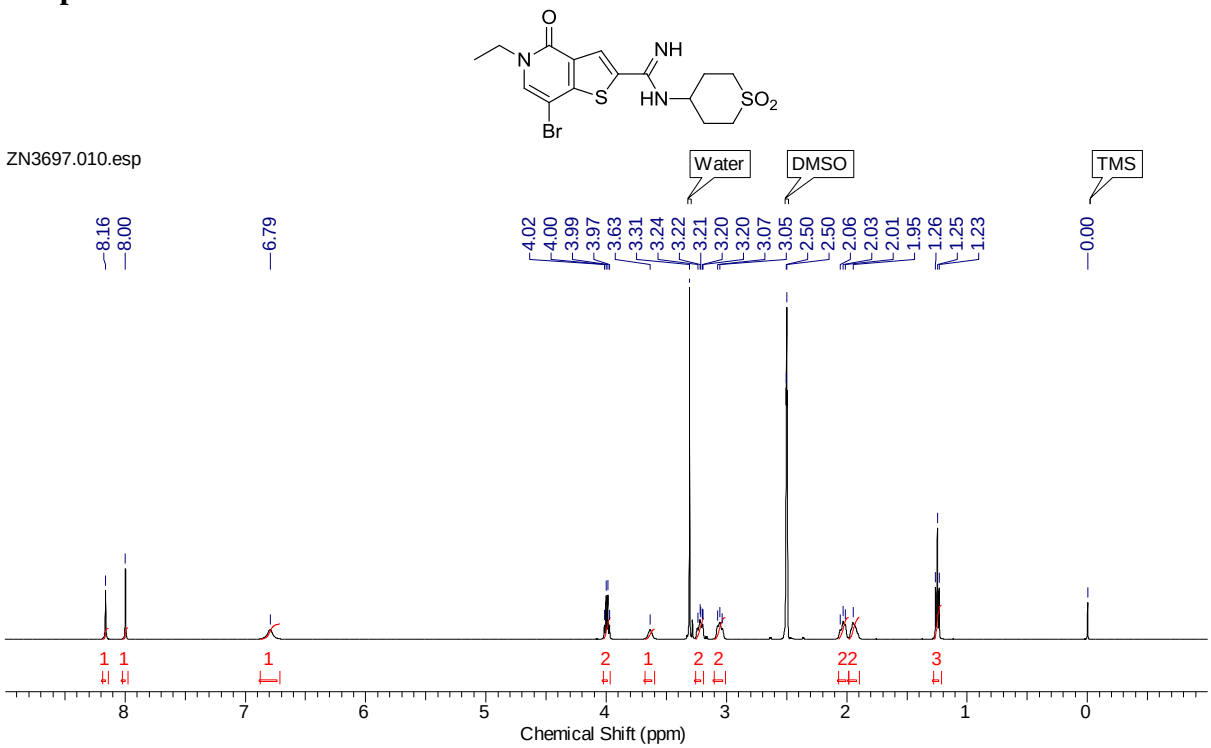


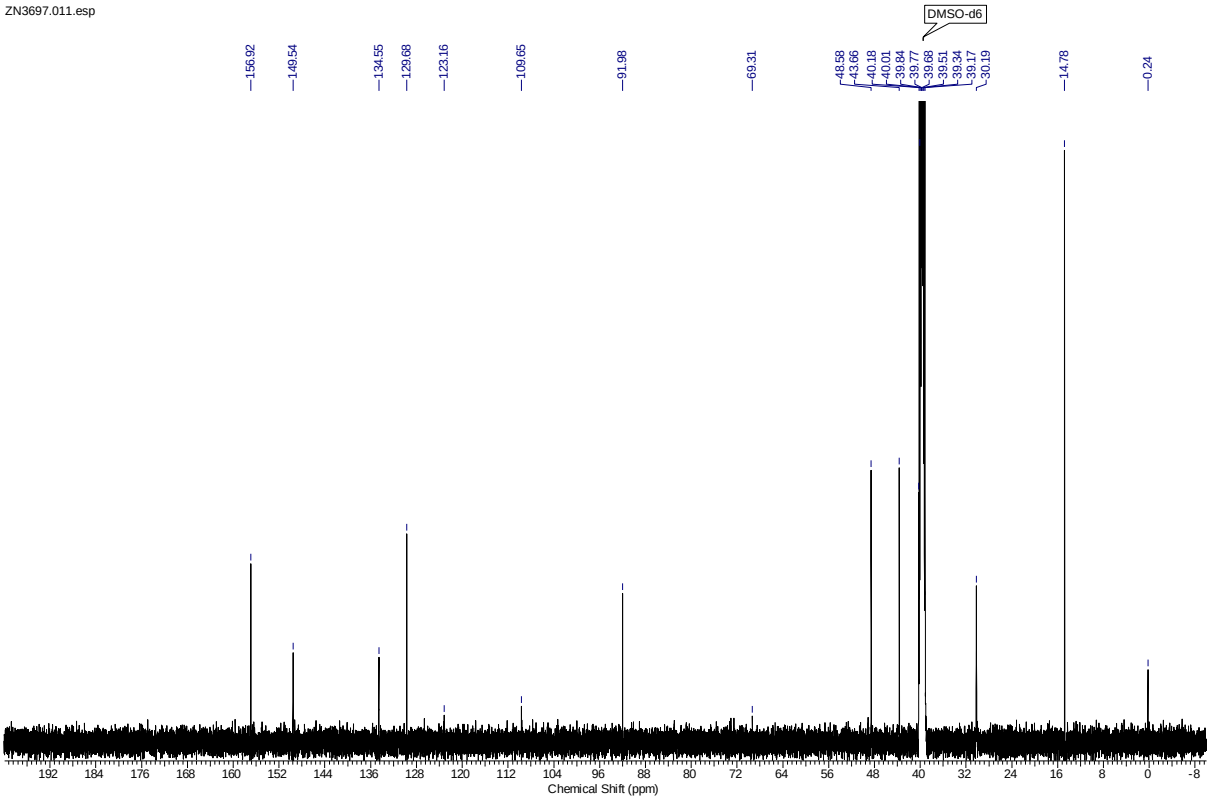
^{1}H NMR (500 MHz, $(CD_3)_2SO$) and ^{13}C NMR (125 MHz, $(CD_3)_2SO$) Spectra for compound 49



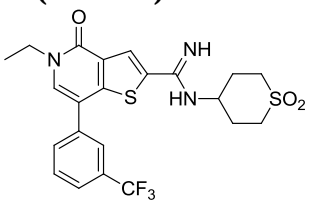


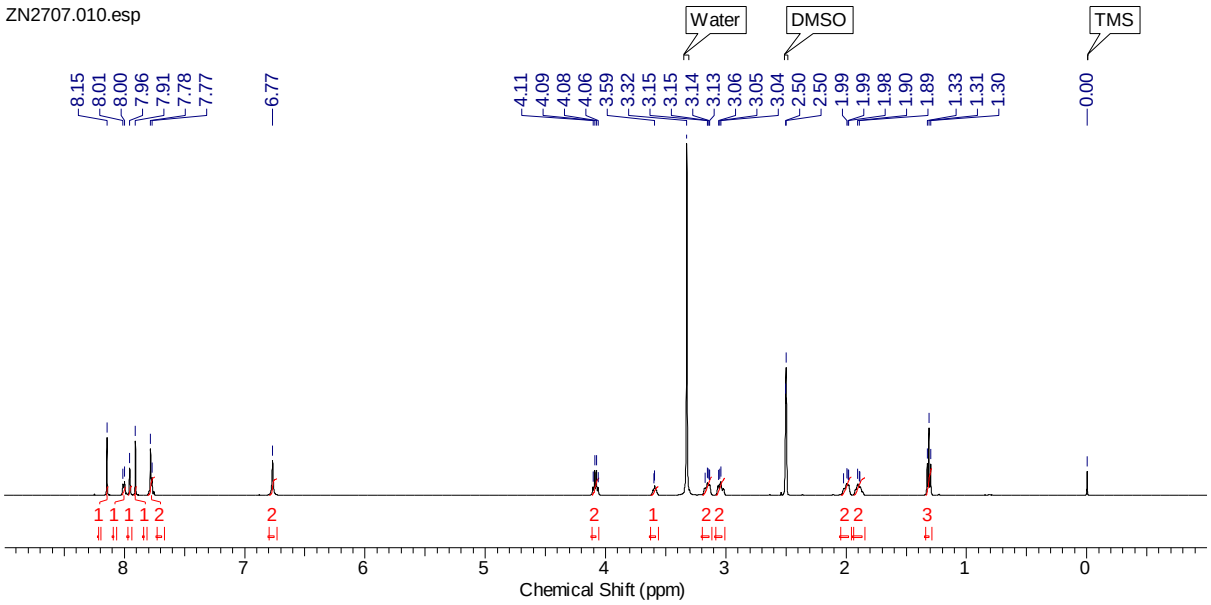
^{1}H NMR (500 MHz, $(CD_3)_2SO$) and ^{13}C NMR (125 MHz, $(CD_3)_2SO$) Spectra for compound 50

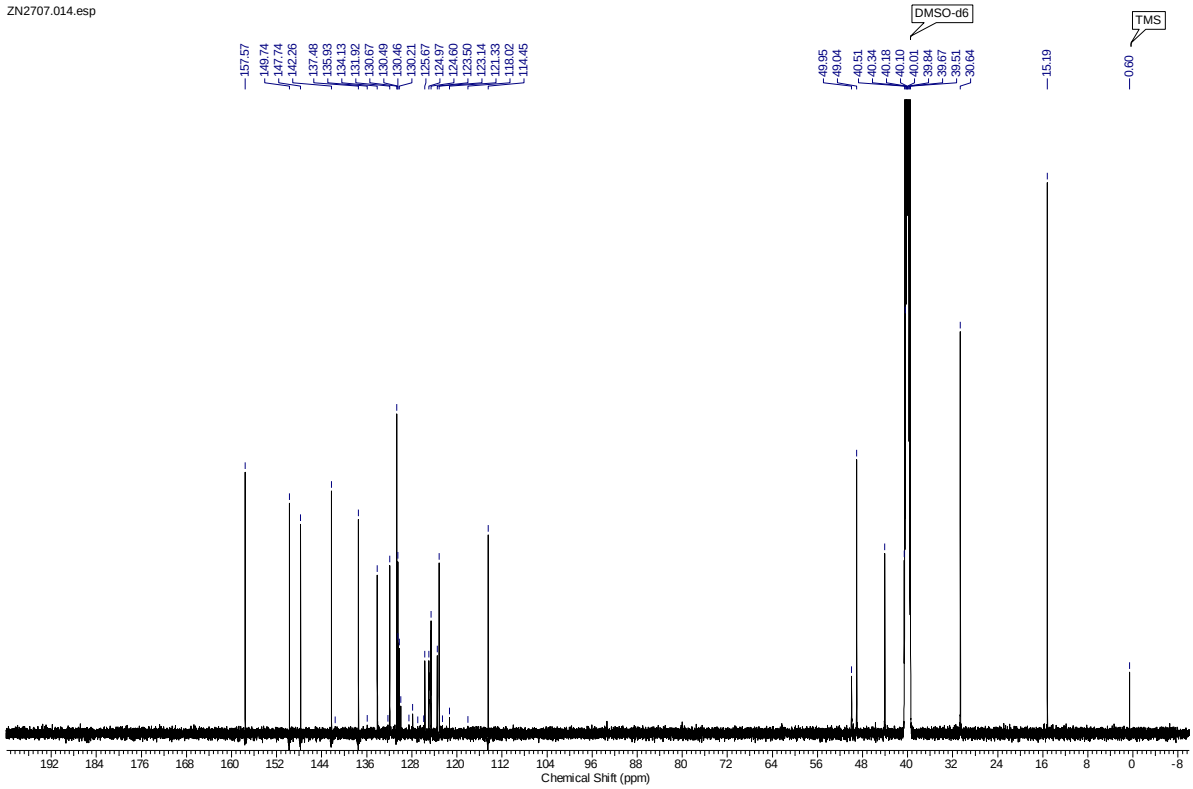


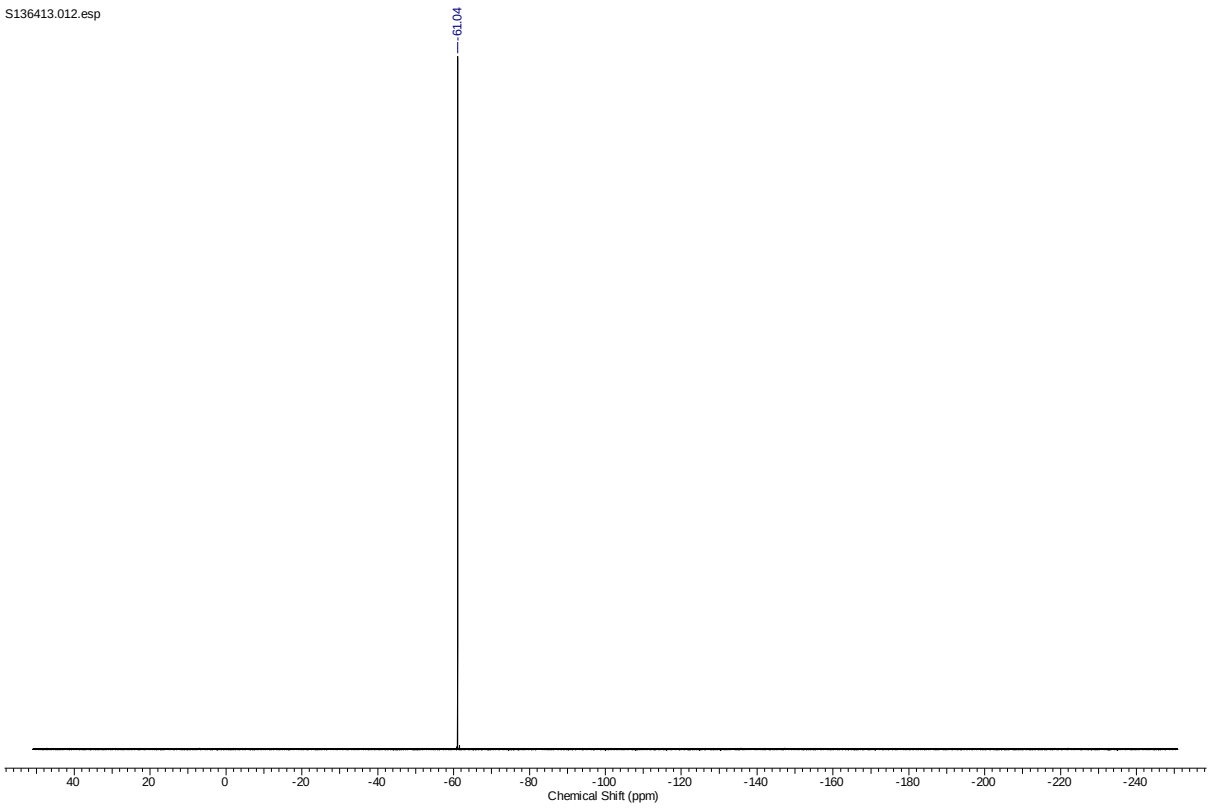


^{1}H NMR (500 MHz, $(CD_3)_2SO$), ^{13}C NMR (125 MHz, $(CD_3)_2SO$) and ^{19}F (376 MHz, $(CD_3)_2SO$) Spectra for compound 45 (I-BRD9)





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20
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-20
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-80
-100
-120
-140
-160
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-200
-220
-240
Chemical Shift (ppm)

TR-FRET Assays

BRD9 TR-FRET Assay

Ligand preparation

A solution of Alexa Fluor 647 hydroxysuccinimide ester in DMF was added to a 1.5 fold excess of a proprietary bromodomain binding small molecule containing a pendant primary amine, also in DMF, and when thoroughly mixed, the solution was basified by the addition of a 3 fold excess of diisopropylethylamine. Reaction progress was followed by electrospray LC/MS and when judged complete, the product was isolated and purified by reverse-phase C18 HPLC. The final compound was converted to the ammonium salt and characterised by mass spectroscopy and analytical reverse-phase HPLC.

Protocol for BRD9 ligand FRET asssay

All assay components were dissolved in buffer composition of 50 mM HEPES pH7.4, 50 mM NaCl, 5% Glycerol, 1mM DTT and 1mM CHAPS. The final concentration of BRD9 protein was at 10 nM and the Alexa Fluor647 ligand (GSK2833930A) was at *Kd* (~100nM for BRD9). These components were premixed and 5 µl of this reaction mixture was added to all wells containing 50nl of various concentrations of test compound or DMSO vehicle (0.5% DMSO final) in Greiner 384 well black low volume microtitre plates and incubated in dark for 30 min at room temperature. Detection reagents were prepared in assay buffer by diluting Eu-W1024 Anti-6xHis Antibody (AD0111 PerkinElmer) to 1.5 nM FAC. 5µl of this solution was then added to all wells. The plates were read on the Envision reader and the donor and acceptor counts were determined. From this, the ratio of acceptor/donor was calculated (λex = 337 nm, λem donor = 615 nm, em acceptor = 665 nm) and used for data analysis. All data was normalized to the robust mean of 16 high and 16 low control wells on each plate. A four parameter curve fit of the following form was then applied.

$$y = \frac{a - d}{1 + \left(x/c\right)^{b}} + d$$

Where '*a*' is the minimum, '*b*' is the Hill slope, '*c*' is the pIC_{50} and'd' is the maximum.

BRD4 TR-FRET ASSAY

The binding of the compounds to BRD4 was assessed using a mutated protein to detect differential binding to Binding Domain 1 (BD1) on the bromodomain containing protein. The single residue mutation in the Binding Domain 2 (BD2) acetyl lysine binding pocket (Y390A) greatly lowers the affinity of the fluoroligand for the mutated BD2 domain (>1000 fold selective for the non-mutated domain). Therefore in the final assay conditions, binding of the fluoroligand to the mutated BD2 domain cannot be detected and subsequently the assay is suitable to determine the binding of compounds to the single non-mutated BD1 bromodomain.

Ligand Preparation

A solution of Alexa Fluor 647 hydroxysuccinimide ester in DMF was added to a 1.8 fold excess of N-(5-aminopentyl)-2-((4S)-6-(4-chlorophenyl)-8-methoxy-1-methyl-4H-benzo[f][1,2,4]triazolo[4,3-a][1,4]diazepin-4-yl)acetamide, also in DMF, and when thoroughly mixed, the solution was basified by the addition of a 3 fold excess of diisopropylethylamine. Reaction progress was followed by electrospray LC/MS and when judged complete, the product was isolated and purified by reverse-phase C18 HPLC. The final compound was characterised by mass spectroscopy and analytical reverse-phase HPLC.

Protocol for BRD4 BD1 ligand FRET assay

All assay components were dissolved in buffer composition of 50 mM HEPES pH7.4, 50 mM NaCl, 5% Glycerol, 1mM DTT and 1mM CHAPS. The final concentration of BRD4 protein (1-477, Y390A) was at 10 nM and the Alexa Fluor647 ligand was at *Kd* (~50 nM for BRD4). These components were premixed and 5 μL of this reaction mixture was added to all wells containing 50 nL of various concentrations of test compound or DMSO vehicle (0.5% DMSO final) in Greiner 384 well black low volume microtitre plates and incubated in dark for 30 min at room temperature. Detection reagents were prepared in assay buffer by diluting Eu-W1024 Anti-6xHis Antibody (AD0111 PerkinElmer) to 1.5nM FAC. 5 μl of this solution was then added to all wells. The plates were read on the Envision reader and the donor and acceptor counts were determined. From this, the ratio of acceptor/donor was calculated (λex = 337 nm, λem donor = 615 nm, em acceptor = 665 nm) and used for data analysis. All data was normalized to the robust mean of 16 high and 16 low control wells on each plate. A four parameter curve fit of the following form was then applied.

$$y = \frac{a-d}{1+\left(\frac{x}{c}\right)^b} + d$$

Where '*a*' is the minimum, '*b*' is the Hill slope, '*c*' is the pIC_{50} and'd' is the maximum.

NanoBRET Assay

HEK293 cell (8×10^5) were plated in each well of a 6-well plate and co-transfected with Histone H3.3-HaloTag (NM_002107) and NanoLuc-BRD9 (Q9H8M2) BD domain amino acids 120-240. Twenty hours post-transfection cells were collected, washed with PBS, and exchanged into media containing phenol red-free DMEM and 4% FBS in the absence (control sample) or the presence (experimental sample) of 100nM NanoBRET 618 fluorescent ligand (Promega). Cell density was adjusted to 2×10^5 cells/ml and then re-plated in a 96-well assay white plate (Corning Costar #3917). Inhibitor was then added directly to media at final concentrations between 0-33 μM and the plates were incubated for 18hrs at 37°C in the presence of 5% CO_2. NanoBRET furimazine substrate (Promega) was added to both control and experimental samples at a final concentration of 10 μM. Readings were performed within 5 minutes using the CLARIOstar (BMG) equipped with 450/80 nm bandpass and 610 nm longpass filters with a 0.5sec reading setting. A corrected BRET ratio was calculated and is defined as the ratio of the emission at 610 nm/450 nm for experimental samples (i.e. those treated with NanoBRET fluorescent ligand) subtracted by and the emission at 610 nm/450 nm for control samples (not treated with NanoBRET fluorescent ligand). BRET ratios are expressed as milliBRET units (mBU), where 1 mBU corresponds to the corrected BRET ratio multiplied by 1000.

BROMO*scan*® Bromodomain Profiling

Bromodomain profiling was provided by DiscoveRx Corp. (Fremont, CA, USA) on the basis of BROMO*scan*®. This screen accounted for the determination of the K_d between compound **45** (I-BRD9) and each of the 34 DNA tagged bromodomains included in the assay, by binding competition against a reference immobilized ligand (http://www.discoverx.com).

Full BROMO*scan*® data for compound 45 (I-BRD9)

Gene Symbol	K_d (nM)	Gene Symbol	K_d (nM)
ATAD2A	> 30000	BRPF1	= 2100
ATAD2B	> 30000	BRPF3	> 30000
BAZ2A	> 30000	CECR2	= 140
BAZ2B	> 30000	CREBBP	= 740
BRD1	= 17000	EP300	= 770
BRD2 BD1	= 3200	FALZ	= 22000
BRD2 BD2	> 30000	GCN5L2	> 30000
BRD3 BD1	= 3000	PBRM1 BD2	> 30000
BRD3 BD2	= 16000	PBRM1 BD5	> 30000
BRD4 BD1	= 1400	PCAF	> 30000
BRD4 BD2	= 17000	SMARCA2	> 30000
BRD7	= 380	SMARCA4	> 30000
BRD8 BD1	= 13000	TAF1 BD2	= 7500
BRD8 BD2	> 30000	TAF1L BD2	= 12000
BRD9	= 1.9	TRIM24(PHD,Bromo.)	> 30000
BRDT BD1	= 1500	TRIM33(PHD,Bromo.)	> 30000
BRDT BD2	= 15000	WDR9 BD2	> 30000

Chemoproteomic Profiling

Preparation of cell fractions

Nuclear extract was produced from fresh HuT78 cells grown at $5 \times 10^6 - 1 \times 10^6$ cells/mL in spinner flasks. Cells were collected by centrifugation, washed with PBS and resuspended in 3 volumes with hypotonic buffer A (10 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 10 mM KCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, and 1 Roche protease inhibitor tablet per 25 ml). After approximately 15 min cells were homogenized with a Dounce homogenizer. Nuclei were collected by centrifugation (2500xg), washed with hypotonic buffer A and homogenized in one volume of extraction buffer B (50 mM Tris-Cl, pH 7.4, 1.5 mM $MgCl_2$, 20 % glycerol, 420 mM NaCl, 25 mM NaF, 1 mM Na_3VO_4, 1 mM DTT, 400 Units/ml DNase I, and 1 Roche protease inhibitor tablet per 25 ml). Extraction was allowed to proceed under agitation for 30 min at 4°C before the extract was clarified by centrifugation at 13000xg. The extract was diluted in buffer D (50 mM Tris-Cl, pH 7.4 (RT), 1.5 mM $MgCl_2$, 25 mM NaF, 1 mM Na_3VO_4, 0.6 % NP40, 1 mM

DTT, and Roche protease inhibitors), and aliquots were snap frozen in liquid nitrogen and stored at -80°C.

Tight chromatin-associated proteins enriched fractions were prepared by resuspending the remaining pellet in 10 volumes of high salt extraction buffer (20 mM Hepes pH7.4, 1.5mM $MgCl_2$, 1000mM KCl, 10% glycerol, 0.1% NP40, 0.5mM DTT, and Roche protease inhibitors) with 6 cycles of 1minute sonication with an ultrasound homogenizer (Bandelin Sonoplus). After sonication and incubation for 45 min at 4 °C, the homogenate was clarified at 8000xg. The salt concentration was adjusted to 150 mM KCl through stepwise dialysis before ultracentrifugation at 100,000xg for 20 min. Aliquots were snap frozen in liquid nitrogen and stored at −80°C.

Chemoproteomic assay with dose-dependent competition

Affinity profiling assays were performed as described previously.[2] Briefly, sepahrose beads were derivatised with 2.0 mM of a proprietary bromodomain binding small molecule containing a pendant primary amine (GSK2893910A) as described previously.[3]

Compound **45** (I-BRD9) was spiked into HuT78 mixed nuclear and chromatin extracts and incubated for 45min at 4 °C. Derivatized sepharose beads (35 µl beads per sample) were equilibrated in lysis buffer and incubated with cell extract pre-incubated with compound. Beads were washed with lysis buffer containing 0.2 % NP-40 and eluted with 2x SDS sample buffer supplemented with DTT.

Western Blot

Aliquots of the eluates from chemoproteomic assays were separated on 4–12 % gel (NuPAGE, Invitrogen) and this was used for Western Blot analysis with anti-BRD9 (abcam, ab-66443) and anti-BRD3 (santa cruz, sc-81202) antibodies.

Microarray Analysis

Kasumi-1 cells were treated with I-BRD9 (10 µM), I-BET151 (1 µM) and DMSO for 6 hours. Total RNA was extracted using Qiagen RNeasy mini kit according to the manufacturer's protocol. Quality analysis of total RNA, hybridisation and data captured were performed at the Cambridge Genomic Services. HumanHT-12 v4 beadchips were used for gene expression screening. Results were analysed using the Lumi Bioconductor package in R.[4] Differentially expressed genes were identified in each cell line using limma,[5] and all resulting p-values were corrected for multiple testing by using the Benjamini and Hochberg False Discovery Rate correction. Genes were defined as being significantly altered following I-BRD9 treatment in each cell line if they showed a fold change greater than 1.5 (up or down compared to non-treated) with an adjusted p- value lower than 0.01.

Quantitative PCR

Kasumi-1 cells were treated with I-BRD9 (10 µM), I-BET151 (1 µM) and DMSO for 6 hours. Total RNA was extracted as described above. cDNA synthesis was carried out using the SuperScript III reverse transcriptase (Life technologies). Gene expression was performed by taqman quantitative real-time PCR assay on an ABI StepOne plus system. Results were normalized to GAPDH expression. The catalog number of each taqman probes (Life technologies) are compiled in the following table:

CLECL1	Hs00416849_m1
DUSP1	Hs04329643_s1
FES	Hs01120751_m1

GAPDH	Hs02758991_g1
SNHG15	Hs03301061_g1

Artificial Membrane Permeability Measurement
Permeability across a lipid membrane was measured using the published protocol.[6]

CLND Solubility Measurement
Solubility was determined by precipitation of 10 mM DMSO stock concentration to 5% DMSO pH7.4 phosphate buffered saline, with quantification by ChemiLuminescent Nitrogen Detection.

Selectivity Data for I-BRD9 Versus 49 Unrelated Proteins
I-BRD9 was screened against a range of protein types using the specified assay. n is the number of replicates.

TARGET CLASS	TARGET	ASSAY	I-BRD9 XC_{50} (µM) (n)
Ion Channel	Human hERG Barracuda	Electrophysiology	16 (3)
Ion Channel	Human CaV1.2 Barracuda	Electrophysiology	40 (3)
Ion Channel	Human NaV1.5	Electrophysiology	> 100 (3)
Ion Channel	Human Kv1.5	Electrophysiology	> 50 (2)
Ion Channel	Human KCNQ1/minK	Electrophysiology	> 25 (2)
Ion Channel	Human NMDA/NR2B receptor antagonism	Intracellular Ca (Fluoresence)	> 50 (2)
Ion Channel	Human GABA-A receptor agonism	Electrophysiology	> 100 (4)
Ion Channel	Human GABA-A receptor antagonism	Electrophysiology	> 100 (2)
Ion Channel	Human GABA-A receptor positive modulator	Electrophysiology	> 100 (2)
Ion Channel	Human Alpha 1 nicotinic AChR opening	Intracellular Ca (Fluoresence)	> 50 (2)
Ion Channel	Human KCNQ1/minK	Electrophysiology	> 25 (2)
GPCR	Human Alpha 1b adrenergic receptor antagonism	Intracellular Ca (Fluoresence)	> 25 (2)
GPCR	Human Adenosine 2a receptor agonism	TR-FRET	> 100 (2)

GPCR	Human Alpha 2c adrenergic receptor agonism	TR-FRET	> 100 (2)
GPCR	Human Beta 2 adrenergic receptor agonism	TR-FRET	> 100 (2)
GPCR	Human Beta 2 adrenergic receptor antagonism	TR-FRET	> 100 (2)
GPCR	Human Dopamine (D1) receptor antagonism	TR-FRET	> 100 (2)
GPCR	Human Dopamine (D2) receptor agonism	LEADseeker SPA	> 100 (2)
GPCR	Human Dopamine (D2) receptor antagonism	LEADseeker SPA	10 (4)
GPCR	Human Histamine 1 (H1) receptor antagonism	Intracellular Ca (Fluoresence)	> 25 (3)
GPCR	Human Muscarinic 1 receptor agonism	Intracellular Ca (Fluoresence)	> 50 (2)
GPCR	Human Muscarinic 2 receptor agonism	Intracellular Ca (Fluoresence)	> 50 (2)
GPCR	Human Muscarinic 2 receptor antagonism	Intracellular Ca (Fluoresence)	> 50 (2)
GPCR	Human Mu Opioid receptor agonism	LEADseeker SPA	> 100 (2)
GPCR	Human Vasopressin 1a antagonism	Intracellular Ca (Fluoresence)	> 50 (2)
GPCR	Human CB2 agonism	Yeast FDLu	20 (2)
GPCR	Human Serotonin (5HT1B) receptor agonism	LEADseeker SPA	> 100 (2)
GPCR	Human Serotonin (5HT1B) receptor antagonism	LEADseeker SPA	> 100 (2)
GPCR	Human Serotonin (5HT2A) receptor agonism	Intracellular Ca (Fluoresence)	> 25 (2)
GPCR	Human Serotonin (5HT2A) receptor antagonism	Intracellular Ca (Fluoresence)	> 25(2)

GPCR	Human Serotonin (5HT2C) receptor agonism	Intracellular Ca (Fluoresence)	> 25 (2)
GPCR	Human Serotonin (5HT2C) receptor antagonism	Intracellular Ca (Fluoresence)	13 (2)
Ion Channel	Human Serotonin (5-HT3) Receptor opening	Intracellular Ca (Fluoresence)	> 50 (2)
Ion Channel	Human Serotonin (5-HT3) Receptor blocking	Intracellular Ca (Fluoresence)	6 (2)
GPCR	Human Kappa opioid agonism	LEADseeker SPA	10 (2)
GPCR	Human Neurokinin Type 1 (NK1) receptor antagonism	Intracellular Ca (Fluoresence)	> 25 (2)
Nuclear Receptor	Human PXR (NR1l2) Agonist	Luciferase Reporter	< 50 (1)
Transporter	Human Norepinephrine (NE) transporter antagonism	LEADseeker SPA	8 (2)
Transporter	Human Serotonin (SERT) transporter antagonism	LEADseeker SPA	40 (2)
Enzyme	Human Monoamine oxidase (MAO-A) inhibition	FLINT	> 100 (2)
Enzyme	Human Monoamine oxidase (MAO-B) inhibition	FLINT	80 (2)
Enzyme	Human Phosphodiesterase type 3 (PDE3A) inhibition	Luminescence	> 100 (2)
Enzyme	Human Phosphodiesterase type 4 (PDE4B) inhibition	Luminescence	100 (2)
Enzyme	Human Cyclooxygenase 2 (COX-2) inhibition	FLINT	> 100 (2)
Enzyme	Human Acetylcholinesterase inhibition	Mass Spectrometry	10 (2)
Enzyme	CYP3A4 (substrate preincubation inhibition)	Mass Spectrometry	> 40 (2)
Kinase	Human AuroraB antagonism	IMAP	> 32 (2)
Kinase	Human PI3Kγ antagonism	TR-FRET	> 32 (1)

Kinase	Human LCK antagonism	IMAP	> 32 (2)

Crystallization and Crystallography Materials

BRD4-BD1 Crystallography Methods

Crystal structures of BRD4 complexes:

Methods have been previously described.[7] Specific successful co-crystallization conditions, data collection details and refinement procedures for each complex are given below and in supplementary table 1. In all instances *E. coli* expressed tag cleaved His_6-TEV-tagged BRD4-BD1(44-168) was used.

BRD4-BD1/compound **32** was co-crystallized with 3:1 excess of compound @~11 mg/mL in 120nl+120nl sitting drops using MRC 2 sq with a well solution of 0.2 M $CaCl_2$, 0.1 M Hepes, PEG 6K at 20 °C. Crystals were cryoprotected using well with 20% ethylene glycol added prior to flash freezing in liquid nitrogen. Data from a single crystal was collected at the European Synchrotron Radiation Facility, Genoble and processed[8] to 1.58 Å using XDS[9] and SCALA.[10] Molecular replacement solution was determined with a previously collected in house structure. The $P2_1$ cell ($\alpha=\beta=90^\circ$, $\gamma=90.09^\circ$, a=63.640, Å b=44.670 Å, c=77.550 Å) has 3 molecules in the ASU. Manual model building was performed using Coot[11] and refined using refmac.[12] There was clear difference density for the ligands at all 3 AcK binding sites (OMIT maps show in Supplementary Fig. 1), allowing the ligand molecules to be uniquely placed. The statistics for the data collection and refined co-ordinates are given in (Supplementary Table 1). The final model has been deposited to the protein data bank under the accession code XXX.

BRD4-BD1/compound **44** was co-crystallized with 3:1 excess of compound @~11 mg/mL in 120nl+120nl sitting drops using MRC 2 sq with a well solution of 20% PEG3350, 0.2 M KSCN, 0.1M BTP pH 7.5 at 20 °C. Crystals were cryoprotected using well with 20% ethylene glycol added prior to flash freezing in liquid nitrogen. Data from a single crystal was collected on ID29 at the European Synchrotron Radiation Facility, Genoble and processed[8] to 1.30 Å using XDS[9] and aimless.[13] Molecular replacement solution was determined with a previously collected in house structure. The $P2_12_12_1$ cell ($\alpha=\beta=\gamma=90^\circ$, a=37.350, Å b=44.240 Å, c=78.640 Å) has a single molecule in the ASU. Manual model building was performed using Coot[11] and refined using refmac.[12] There was clear difference density for the ligands at KAc binding site (OMIT maps show in Supplementary Fig. 2), allowing the ligand to be unambiguously modeled. The statistics for the data collection and refined co-ordinates are given in (Supplementary Table 1). The final model has been deposited to the protein data bank under the accession code XXX.

BRD4-BD1/compound **17** was co-crystallized with 3:1 excess of compound @~11 mg/mL in 120nl+120nl sitting drops using MRC 2 sq with a well solution of 0.1 M BTP pH 6.5, 20% PEG3350, 0.2 M Naformate at 20 °C. Crystals were cryoprotected using well with 20% ethylene glycol added prior to flash freezing in liquid nitrogen. Data from a single crystal was collected on I04 at the Diamond Synchrotron Radiation Facility, Didcot, and processed[8] to 1.19 Å using XDS[9] and aimless.[13] Molecular replacement solution was determined with a previously collected in house structure. The $P2_12_12_1$ cell ($\alpha=\beta=\gamma=90^\circ$, a=37.373, Å b=44.555 Å, c=78.698 Å) has a single molecule in the ASU. Manual model building was performed using Coot[11] and refined using refmac.[12] There was clear difference density for the ligand at KAc binding site (OMIT maps show in Supplementary Fig. 2), allowing the ligand to be easily modelled. The statistics for

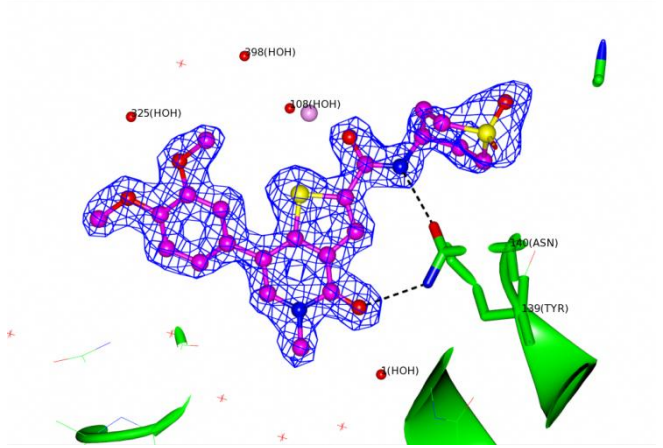
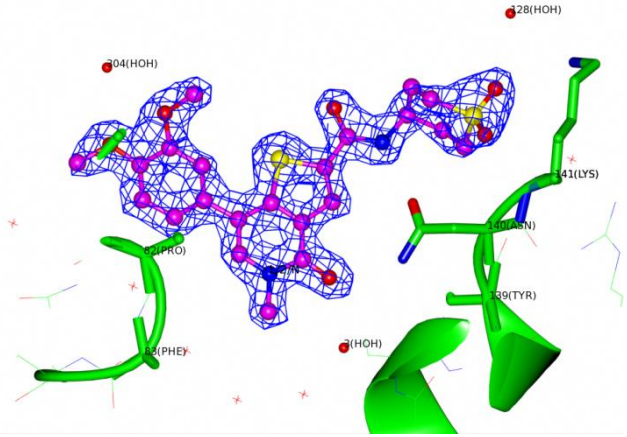
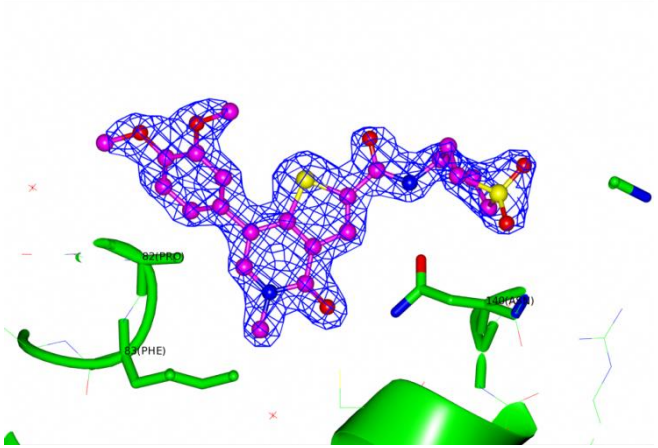
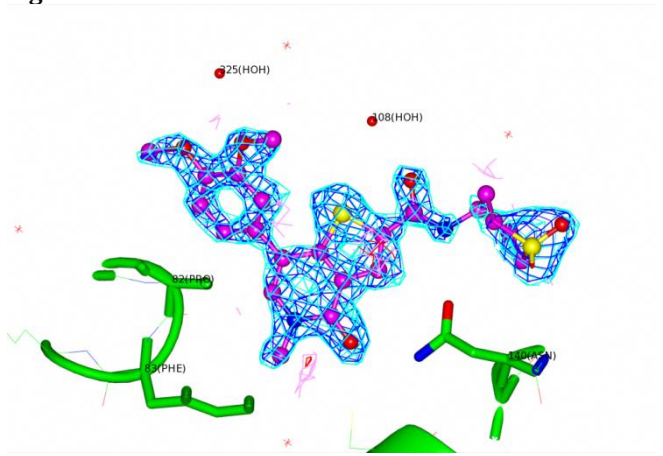
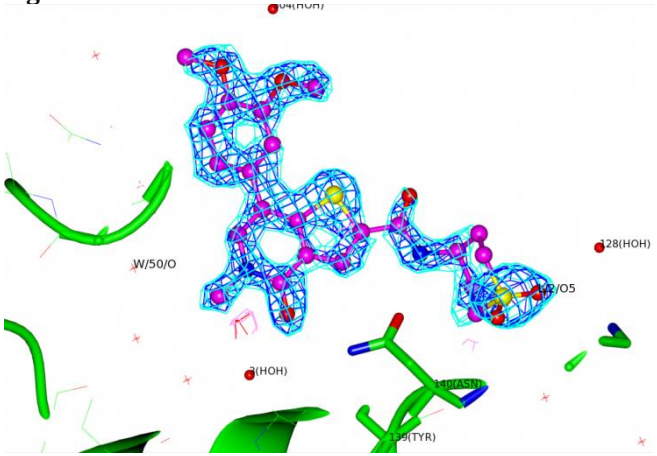
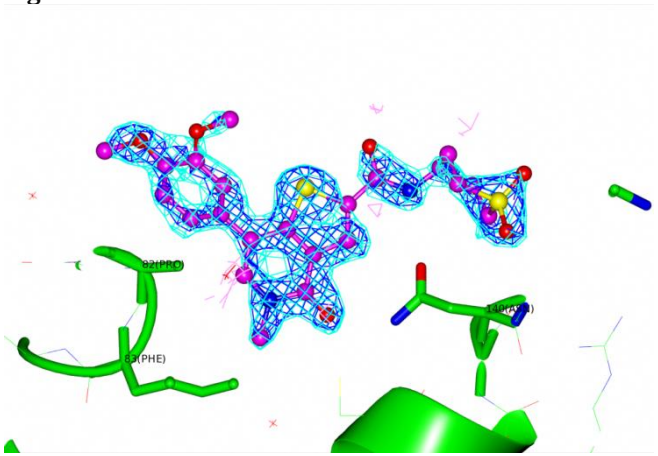
the data collection and refined co-ordinates are given in (Supplementary Table 1). The final model has been deposited to the protein data bank under the accession code XXX.

Supplementary Table 1 Data collection and refinement statistics for BRD4-BD1 (Molecular replacement)

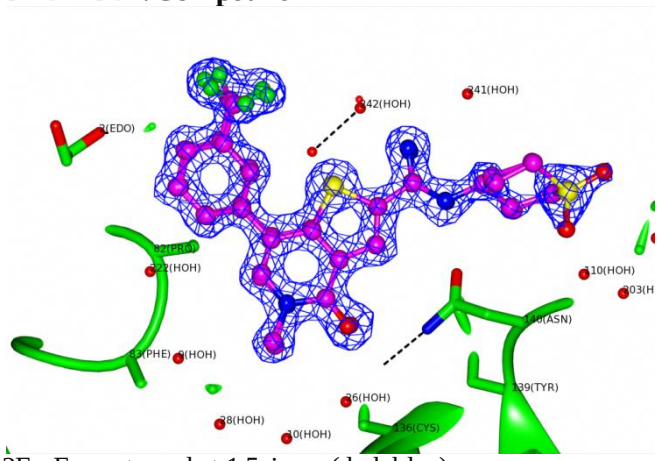
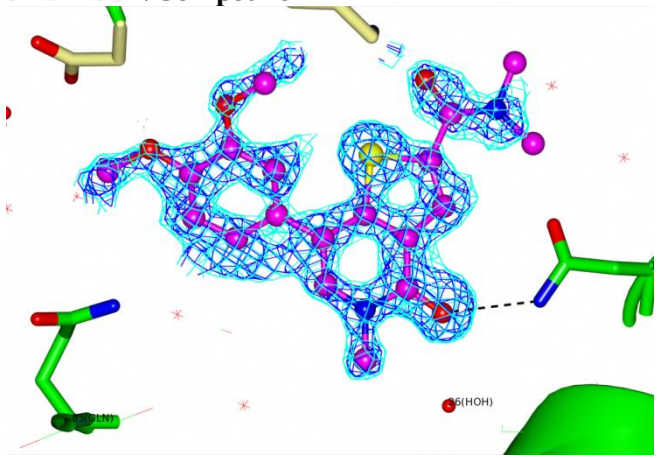
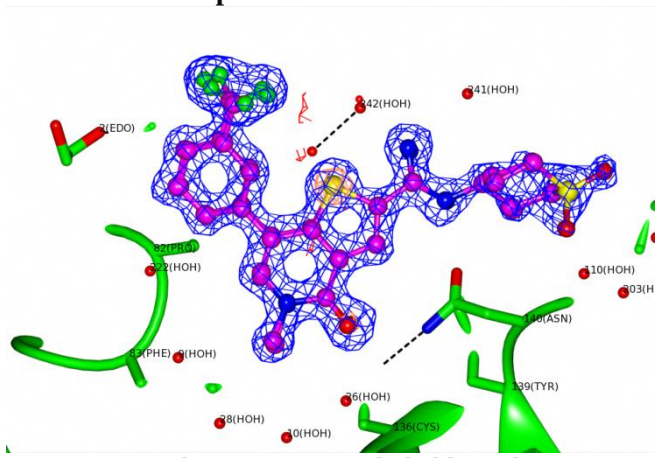
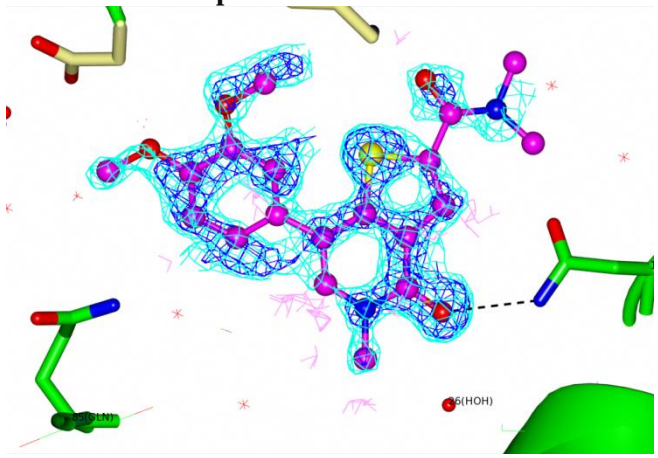
(collection on a single crystal)	BRD4-BD1/ Compound **32**	BRD4-BD1/ Compound **44**	BRD4-BD1/ Compound **17**
Data collection			
Space group	P 1 21 1	P 21 21 21	P 21 21 21
Cell dimensions			
a, *b*, *c* (Å)	63.640,44.670,77.550	37.350,44.240,78.640	37.373,44.555,78.698
α, β, γ (°)	90.00, 90.09, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	1.58 (1.64)	1.30 (1.37)	1.19 (1.25)
R_{merge}	0.106 (0.392)	0.051 (0.240)	0.036 (0.156)
$I/\sigma I$	6.4 (2.5)	16.5 (5.1)	28.3 (8.1)
Completeness (%)	97.7 (97.1)	96.6 (86.5)	96.7 (81.4)
Redundancy	3.3(3.3)	5.0 (4.1)	5.8 (3.5)
No. reflections	192523 (18467)	156253 (16544)	240569 (17662)
No. uniq reflections	58489 (5652)	31523 (4031)	41778 (5043)
Refinement			
Resolution (Å)	49.23-1.58	39.32-1.30	38.77-1.19
R_{work}/ R_{free}	0.20/0.24	0.158/0.185	0.167/0.175
No. Reflections	55517	29935	39614
No. atoms	3633	1414	1449
Protein	3095	1074	1124
Ligand/ion	96/4	64/12	33/8
Water	428	264	284
B-factors			
Protein	24.569	15.692	12.236
Ligand/ion	22.475/27.803	14.285/26.161	28.732/21.183
Water	34.548	32.759	28.340
R.m.s deviations			
Bond lengths (Å)	0.0070	0.0138	0.0091
Bond angles (°)	1.127	1.619	1.5574

*Highest resolution shell is shown in parenthesis.

Supplementary Figure 1 : OMIT maps for BRD4-BD1/Compound 32

BRD4-BD1/Compound 32 **Ligand in chain A**  2Fo-Fc contoured at 1.5sigma (dark blue)	**BRD4-BD1/Compound 32** **Ligand in chain B**  2Fo-Fc contoured at 1.5sigma (dark blue)	**BRD4-BD1/Compound 32** **Ligand in chain C**  2Fo-Fc contoured at 1.5sigma (dark blue)
BRD4-BD1/Compound 32 **Ligand in chain A**  Fo-Fc contoured at +/-3sigma (dark blue,red), +/-2.5sigma (cyan,pink)	**BRD4-BD1/Compound 32** **Ligand in chain B**  Fo-Fc contoured at +/-3sigma (dark blue,red), +/-2.5sigma (cyan,pink)	**BRD4-BD1/Compound 32** **Ligand in chain C**  Fo-Fc contoured at +/-3sigma (dark blue,red), +/-2.5sigma (cyan,pink)

Supplementary Figure 2: OMIT maps for BRD4-BD1/Compound 39 and BRD4-BD1/Compound 17

BRD4-BD1/Compound 44  2Fo-Fc contoured at 1.5sigma (dark blue)	**BRD4-BD1/Compound 17**  2Fo-Fc contoured at 1sigma (dark blue), 0.75sigma (cyan)	
BRD4-BD1/Compound 44  Fo-Fc contoured at +/-3sigma (dark blue,red), +12sigma (orange)	**BRD4-BD1/Compound 17**  Fo-Fc contoured at +/-3sigma (dark blue,red), =/-2sigma (cyan,pink)	

BRD9 Crystallography Methods

BRD9 protein production and crystallisation: Human BRD9 bromodomain (His6-FLAG-TEV-BRD9(134-239)) was cloned into Kanamycin resistant pET28a with an N-terminal TEV cleavage site. The protein was expressed in Escherichia coli. Purification used an affinity 10 mL HisTrap column followed by overnight TEV cleavage at 4C with Supertev (1/100) and then SEC using Superdex 75 26/60. The purified protein was stored in buffer (25 mM HEPES pH 7.5, 300 mM NaCl, 0.5 mM TCEP) and concentrated to at least 20 mg/mL for crystallography. Crystals of BRD9 were grown by sitting drop vapour diffusion at 4 °C in MRC plates using a variety of conditions (as specified for each complex) with a well:protein drop volume of 120 nl:120 nl.

BRD9/Compound **17**. Apo crystals grown in 0.2 M ZnAc, 20% PEG3350 at 4 °C were transferred to soaking buffer comprising well solution supplemented with the compound (from a stock solution dissolved in DMSO) at nominal soaking concentration of 2 mM overnight. Crystals were cryoprotected using well with 20% glycerol added prior to flash freezing in liquid nitrogen. Data from a single crystal was collected at the Diamond Light Source (Harwell) and processed[8] to 1.3 Å using XDS[9] and SCALA.[10] Structures were solved by Fourier synthesis using REFMAC[12] starting from a previously determined in house structure, model-building was performed using COOT[11] and refined using REFMAC[12] via CCP4. The P1 cell (α=70.26˚,β= 73.80˚,γ=74.24˚, a=24.700 Å, b=34.110Å, c=39.680 Å) has 1 molecule in the ASU. There was clear difference density for the ligand at the KAc binding site (OMIT maps show in Supplementary Fig. 3), allowing the ligand to be uniquely placed. The statistics for the data collection and refined co-ordinates are given in (Supplementary Table 2). The final model has been deposited to the protein data bank under the accession code XXX.

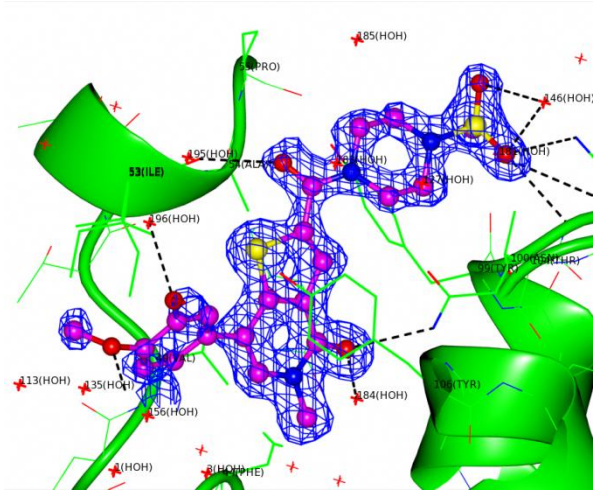
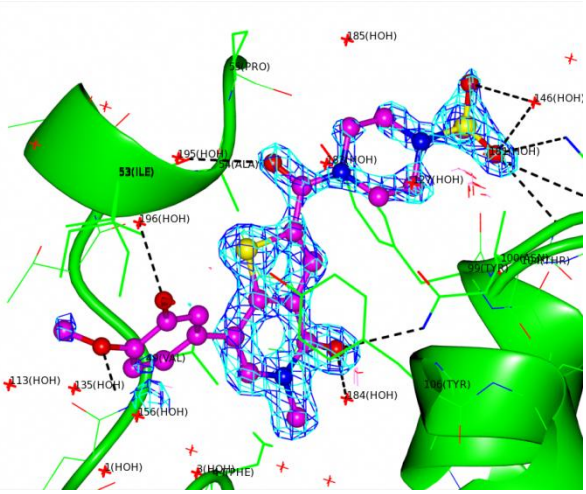
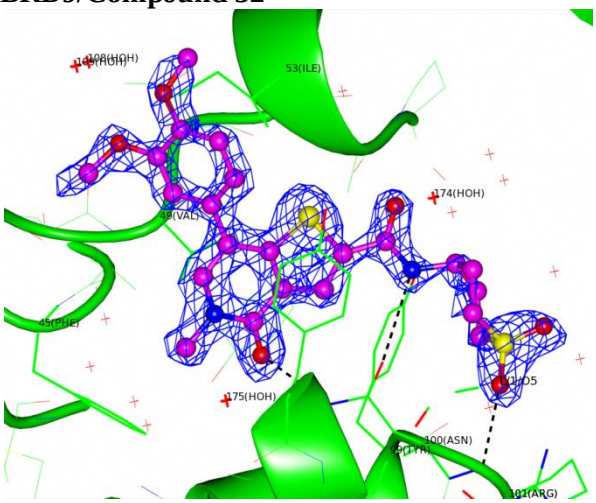
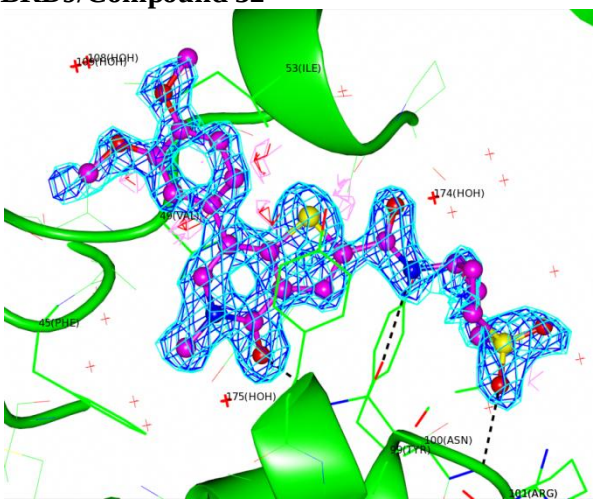
BRD9 X-ray complexes with Compound **32**, Compound **44** and Compound **45**: Apo crystals grown in Morpheus condition 86 (0.1 M morpheus buffer pH 6.5, 30% morpheus_EDO_P8K) were transferred to soaking buffer comprising well solution supplemented with the compound (from a stock solution dissolved in DMSO) at nominal soaking concentration of 2 mM. Crystals were soaked overnight then were briefly washed free from compound precipitate with well solution before being plunge-frozen into liquid nitrogen before loading in a puck for mounting with a sample collector. Data from single crystals were collected on an in-house RIGAKU FR-E+ SUPERBRIGHT/Saturn A200 detector/ACTOR robotic system. Data processing was achieved using XDS[9] (within AUTOPROC [Global Phasing Limited]) and scaled using SCALA[10] within the CCP4 programming suite. Structures were solved by Fourier synthesis using REFMAC[12] (via CCP4) starting from a previously determined in house structure, model-building was performed using COOT[11] and refined using REFMAC[12] via CCP4. The P1 cell has 1 molecule in the ASU. There was clear difference density for all ligands at the KAc binding site (OMIT maps show in Supplementary Fig. 3), allowing the ligand to be unambiguously placed in every case. The statistics for the data collection and refined co-ordinates for each complex are given in (Supplementary Table 2). The final models have been deposited to the protein data bank under the accession codes XXX, XXX and XXX.

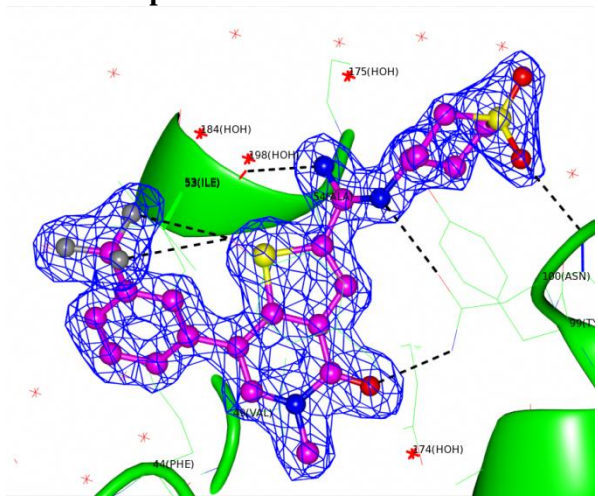
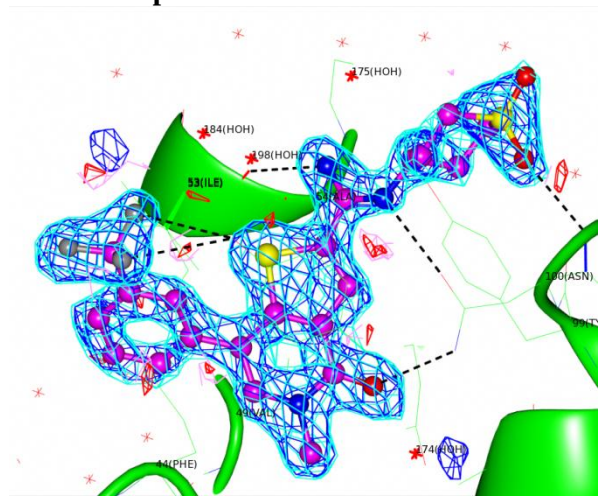
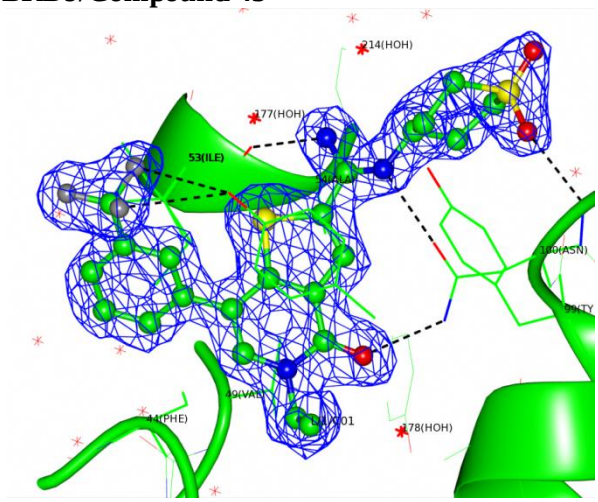
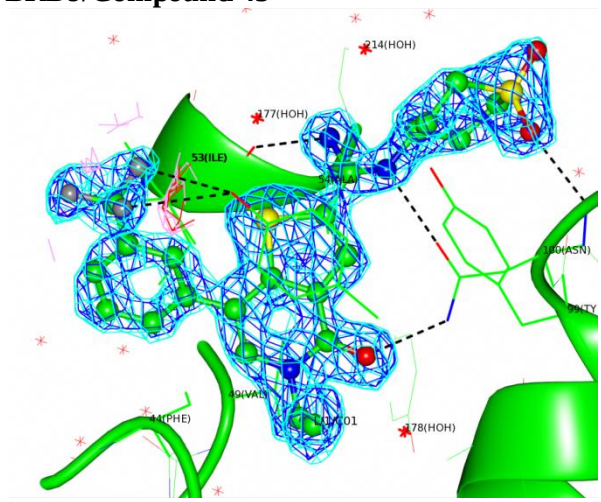
Supplementary Table 2 Data collection and refinement statistics for BRD9 (Molecular replacement)

(collection on a single crystal)	BRD9/ **Compound 17**	BRD9/ **Compound 32**	BRD9/ **Compound 44**	BRD9/ **Compound 45**
Data collection				
Space group	P 1	P 1	P 1	P 1
Cell dimensions				
a, *b*, *c* (Å)	24.700, 34.110, 39.680	24.548, 33.942, 39.465	24.682, 33.836, 39.542	24.689, 33.918, 39.578
α, β, γ (°)	70.26, 73.80, 74.24	70.39, 73.02, 74.10	68.09, 73.70, 73.35	68.84, 73.76, 73.83
Resolution (Å)	1.30 (1.37)	1.64 (1.72)	1.72 (1.82)	1.73 (1.83)
R_{merge}	0.032 (0.050)	0.016 (0.026)	0.021 (0.060)	0.021 (0.074)
$I/\sigma I$	19.4 (12.7)	41.4 (24.8)	28.1 (12.0)	26.1 (7.6)
Completeness (%)	81.9 (51.8)	91.0 (84.3)	91.5 (83.0)	92.1 (85.3)
Redundancy	1.9 (1.9)	2.1 (2.1)	2.1 (1.8)	2.2 (1.9)
No. reflections	44972 (4145)	26838 (3162)	22831 (2568)	24049 (2778)
No. uniq reflections	23127 (2129)	12650 (1704)	10790 (1435)	10779 (1457)
Refinement				
Resolution (Å)	36.54-1.30	36.26-1.64	28.22-1.72	31.00-1.73
R_{work}/ R_{free}	0.152/0.173	0.164 /0.187	0.139/0.173	0.135/0.180
No. Reflections	21959	11887	10171	10155
No. atoms	1086	1106	1089	1112
Protein	846	836	845	851
Ligand/ion	33	32	32/4	33/4
Water	207	238	208	224
B-factors				
Protein	13.689	11.889	17.517	17.529
Ligand/ion	24.538	17.141	20.265/18.347	22.941/17.830
Water	28.154	26.448	34.475	33.711
R.m.s deviations				
Bond lengths (Å)	0.0073	0.0043	0.0159	0.0156
Bond angles (°)	1.122	0.8226	1.641	1.606

*Highest resolution shell is shown in parenthesis.

Supplementary Figure 3: OMIT maps for BRD9 complexes

2Fo-Fc Maps	Fo-Fc maps
BRD9/**Compound 17**  2Fo-Fc contoured at 1.5sigma (dark blue)	BRD9/**Compound 17**  Fo-Fc contoured at +/-3sigma (dark blue,red), +/-2.5sigma (cyan,pink)
BRD9/Compound 32  2Fo-Fc contoured at 1.5sigma (dark blue)	**BRD9/Compound 32**  Fo-Fc contoured at +/-3sigma (dark blue,red), =/-2sigma (cyan,pink)

BRD9/Compound 44  2Fo-Fc contoured at 1.5sigma (dark blue)	**BRD9/Compound 44**  Fo-Fc contoured at +/-3sigma (dark blue,red), =/-2sigma (cyan,pink)
BRD9/Compound 45  2Fo-Fc contoured at 1.5sigma (dark blue)	**BRD9/Compound 45**  Fo-Fc contoured at +/-3sigma (dark blue,red), =/-2sigma (cyan,pink)

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