

SUPPORTING INFORMATION

1,3-Dimethyl benzimidazolones are potent, selective inhibitors of the BRPF1 bromodomain

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General Methods

General: 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. NMR spectra were recorded on a Bruker AVANCE 400 spectrometer and are referenced as follows: ^1H (400 MHz), internal standard TMS at $\delta = 0.00$; ^{13}C (100.6 MHz), internal standard CDCl_3 at $\delta = 77.23$ or $\text{DMSO-}D_6$ at $\delta = 39.70$. Column chromatography was performed on pre-packed silica gel columns (30-90 mesh, IST) using a Biotage SP4. Mass spectra were recorded on Waters ZQ (ESI-MS) and Q-Tof 2 (HRMS) spectrometers. Mass Directed Auto Prep was performed on a Waters 2767 with a MicroMass ZQ Mass Spectrometer using Supelco LCABZ++ column.

GLOBAL gradient for chromatography are as follows (solvent B polar component, CV = column volume): 10% GLOBAL: 3% B for 2 CV, 3 to 13% B over 10 CV then 13% B for 5 CV; 20% GLOBAL: 5% B for 2 CV, 5 to 20% B over 10 CV then 20% B for 5 CV; 30% GLOBAL: 8% B for 2 CV, 8 to 38% B over 10 CV then 38% B for 5 CV; 40% GLOBAL: 10% B for 2 CV, 10 to 50% B over 10 CV then 50% B for 5 CV; 50% GLOBAL: 13% B for 2 CV, 13 to 63% B over 10 CV then 63% B for 5 CV. 100% GLOBAL: 25% B for 2 CV, 25 to 100% B over 10 CV then 100% B for 10 CV.

Abbreviations for multiplicities observed in NMR spectra: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quadruplet; p, pentuplet; spt, septuplet; m, multiplet.

The purity of all compounds was determined by LCMS and ^1H NMR and was always > 95%.

LCMS methodology:

1) Method format

LC conditions

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm, i.d. 1.7 μm packing diameter) at 40°C. The solvents employed were:

A = 0.1% v/v solution of formic acid in water

B = 0.1% v/v solution of formic acid in acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS : Waters ZQ
Ionisation mode : Alternate-scan positive and negative electrospray
Scan range : 100 to 1000 AMU
Scan time : 0.27 sec
Inter scan delay : 0.10 sec

2) Method high pH

LC conditions

The UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm x 2.1 mm, i.d. 1.7 µm packing diameter) at 40°C. The solvents employed were:

A = 10 mM ammonium hydrogen carbonate in water adjusted to pH10 with ammonia solution

B = acetonitrile

The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS : Waters ZQ

Ionisation mode : Alternate-scan positive and negative electrospray

Scan range : 100 to 1000 AMU

Scan time : 0.27 sec

Inter scan delay : 0.10 sec

MDAP methodology:

1) Method Formate

LC conditions

The HPLC analysis was conducted on either a Sunfire C18 column (100 mm x 19 mm, i.d. 5 µm packing diameter) or a Sunfire C18 column (150 mm x 30 mm, i.d. 5 µm packing diameter) at ambient temperature.

The solvents employed were:

A = 0.1% v/v solution of formic acid in water

B = 0.1% v/v solution of formic acid in acetonitrile

Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm x 19 mm, i.d. 5 µm packing diameter) or 40 mL/min (150 mm x 30 mm, i.d. 5 µm packing diameter).

The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS : Waters ZQ

Ionisation mode : Alternate-scan positive and negative electrospray

Scan range : 100 to 1000 AMU

Scan time : 0.50 sec

Inter scan delay : 0.20 sec

2) Method high pH

LC conditions

The HPLC analysis was conducted on either an Xbridge C18 column (100 mm x 19 mm, i.d. 5 µm packing diameter) or a Xbridge C18 column (100 mm x 30 mm, i.d. 5 µm packing diameter) at ambient temperature.

The solvents employed were:

A = 10 mM ammonium bicarbonate in water, adjusted to pH10 with ammonia solution

B = acetonitrile

Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm x 19 mm, i.d. 5 µm packing diameter) or 40 mL/min (100 mm x 30 mm, i.d. 5 µm packing diameter).

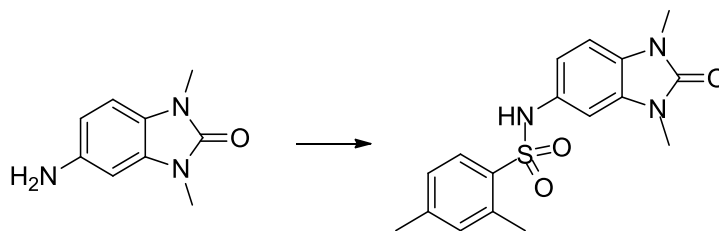
The UV detection was a summed signal from wavelength of 210 nm to 350 nm.

MS conditions

MS	:	Waters ZQ
Ionisation mode	:	Alternate-scan positive and negative electrospray
Scan range	:	100 to 1000 AMU
Scan time	:	0.50 sec
Inter scan delay	:	0.20 sec

Synthetic Methods

Compound 2



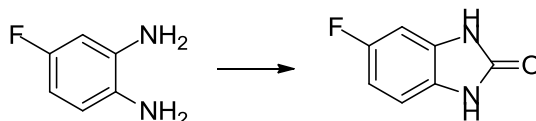
A solution of 5-amino-1,3-dimethyl-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (Fluorochem, 177 mg, 1.00 mmol) in CH_2Cl_2 (10 mL) at 0 °C under nitrogen was treated with triethylamine (0.42 mL, 3.0 mmol) then 2,4-dimethylbenzene-1-sulfonyl chloride (225 mg, 1.10 mmol) and the resulting mixture was stirred at this temperature for 1 h then was diluted with CH_2Cl_2 (10 mL). The organic phase was washed with a 2N HCl aqueous solution then with a saturated NaHCO_3 aqueous solution, and then was dried using a phase separator and concentrated *in vacuo*. Trituration of the residue with CH_2Cl_2 and small amounts of MeOH gave a solid which was filtered off and rinsed with CH_2Cl_2 to give N-(1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-2,4-dimethylbenzenesulfonamide (100 mg, 29%) as a pale brown solid.

LCMS (method high pH): Retention time 0.87 min; $[\text{M}+\text{H}]^+ = 346.2$

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.02 (s, 1H), 7.69 (d, $J = 8.1$ Hz, 1H), 7.16 (s, 1H), 7.10 (d, $J = 8.1$ Hz, 1H), 6.95 (d, $J = 8.3$ Hz, 1H), 6.83 (d, $J = 2.0$ Hz, 1H), 6.70 (dd, $J = 8.3, 2.0$ Hz, 1H), 3.23 (s, 3H), 3.22 (s, 3H), 2.54 (s, 3H), 2.27 (s, 3H).

Compound 3

Step 1

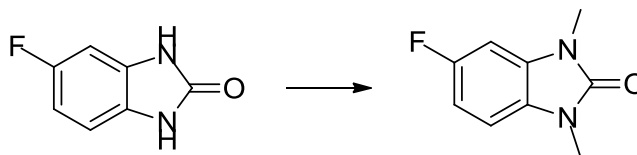


A solution of 4-fluorobenzene-1,2-diamine (15.15 g, 120 mmol) in THF (120 mL) under nitrogen was cooled using an ice-bath and then was treated with di(1H-imidazol-1-yl)methanone (23.37 g, 144 mmol) portion wise over 15 min. The resulting mixture was slowly warmed to room temperature then was concentrated *in vacuo* after 2.5 h. The residue was suspended in a mixture of water and CH_2Cl_2 (250 mL each) and filtered off. This residue was then washed with water (50 mL) and CH_2Cl_2 (50 mL), before being dried at 40°C under vacuum for 16 h to give 5-fluoro-1H-benzo[d]imidazol-2(3H)-one (16.0 g, 83 %) as a brown solid.

LCMS (method high pH): Retention time 0.57 min; $[\text{M}-\text{H}]^- = 151.15$

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.73 (br s, 1H), 10.61 (br s, 1H), 6.83-6.94 (m, 1H), 6.68-6.81 (m, 2H).

Step 2

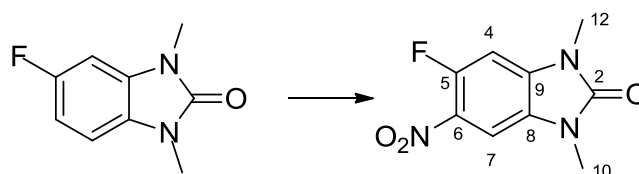


A solution of 5-fluoro-1H-benzo[d]imidazol-2(3H)-one (16.0 g, 105 mmol) in DMF (400 mL) under nitrogen was cooled with an ice-bath, using a mechanical stirrer for agitation. It was then treated over 10 min with sodium hydride (60% w/w in mineral oil, 13.1 g, 327 mmol) and the resulting mixture was stirred at this temperature for 30 min before being treated with iodomethane (26.3 mL, 422 mmol) over 30 min. The resulting mixture was then allowed to warm to room temperature and was carefully treated after 1 h with water (500 mL). The aqueous phase was extracted with EtOAc (3 x 800 mL) and the combined organics were washed with brine (1 L), dried over MgSO₄ and concentrated *in vacuo*. Purification of the brown residue by flash chromatography on silica gel (SP4, 1.5 kg column, gradient: 0 to 25% (3:1 EtOAc/EtOH) in cyclohexane) gave 5-fluoro-1,3-dimethyl-1H-benzo[d]imidazol-2(3H)-one (15.4 g, 77%) as pink solid.

LCMS (method high pH): Retention time 0.75 min; [M+H]⁺ = 181.14

¹H NMR (400 MHz, CDCl₃) δ 6.78-6.91 (m, 2H), 6.74 (dd, *J* = 8.3, 2.3 Hz, 1H), 3.42 (s, 3H), 3.41 (s, 3H).

Step 3



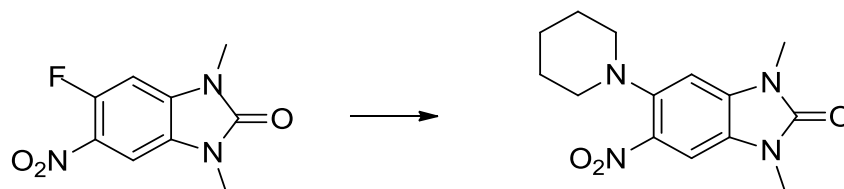
A solution of 5-fluoro-1,3-dimethyl-1H-benzo[d]imidazol-2(3H)-one (4.55 g, 25.3 mmol) in acetic anhydride (75 mL) under nitrogen was cooled to -30°C and then was slowly treated with fuming nitric acid (1.13 mL, 25.3 mmol) making sure that the temperature was kept below -25°C. The solution turned brown once the first drop of acid was added and a thick brown precipitate formed after the addition was complete. The mixture was allowed to slowly warm up to 0°C then was carefully treated after 1 h with ice-water (100 mL). EtOAc (15 mL) was then added and the resulting mixture was stirred for 20 min. The precipitate formed was filtered off, washed with water (10 mL) and EtOAc (10 mL), and then was dried under vacuum at 40°C for 16 h to give 5-fluoro-1,3-dimethyl-6-nitro-1H-benzo[d]imidazol-2(3H)-one (4.82 g, 81%) as a yellow solid.

LCMS (method high pH): Retention time 0.76 min; [M+H]⁺ not detected

¹H NMR (600 MHz, DMSO-d₆) δ 7.95 (d, *J* = 6.4 Hz, 1H, (H-7)), 7.48 (d, *J* = 11.7 Hz, 1H, (H-4)), 3.38 (s, 3H, (H-10)), 3.37 (s, 3H, (H-12)).

¹³C NMR (151 MHz, DMSO-d₆) δ 154.3 (s, 1C, (C-2)), 152.3 (d, *J* = 254.9 Hz, 1C, (C-5)), 135.5 (d, *J* = 13 Hz, 1C, (C-9)), 130.1 (d, *J* = 8.0 Hz, 1C, (C-6)), 125.7 (s, 1C, (C-8)), 104.4 (s, 1C, (C-7)), 97.5 (d, *J* = 28.5 Hz, 1C, (C-4)), 27.7 (s, 1C, (C-12)), 27.4 (s, 1C, (C-10)).

Step 4



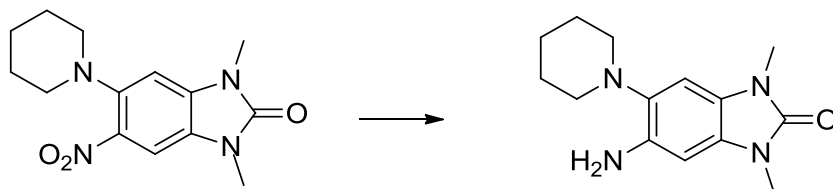
A mixture of 5-fluoro-1,3-dimethyl-6-nitro-1H-benzo[d]imidazol-2(3H)-one (450 mg, 2.0 mmol), piperidine (0.60 mL, 6.0 mmol) and DIPEA (1.75 mL, 10.0 mmol) in DMSO (2 mL) was stirred for 1 h under nitrogen at 120 °C under microwave

irradiation then was cooled to room temperature and diluted with water (20 mL). The aqueous phase was extracted three times with EtOAc and the combined organics were washed with water then brine, dried over MgSO_4 and concentrated *in vacuo* to give after trituration with Et_2O 1,3-dimethyl-5-nitro-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (460 mg, 79%) as an orange solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.11 min; $[\text{M}+\text{H}]^+ = 291.3$

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.73 (s, 1H), 7.12 (s, 1H), 3.36 (s, 3H), 3.33 (s, 3H), 2.93 (m, 4H), 1.65 (m, 4H), 1.54 (m, 2H).

Step 5

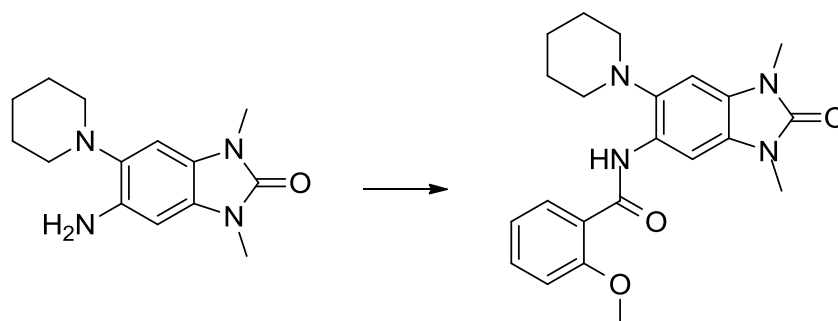


A solution of 1,3-dimethyl-5-nitro-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (440 mg, 1.52 mmol) in EtOH (30 mL) at room temperature was treated with palladium on carbon (10% w/w, 50% wet, 150 mg, 0.141 mmol) and the resulting mixture was stirred at this temperature under hydrogen (1 atm) for 16 h. The catalyst was filtered off using a pad of celite (2.5 g) and rinsed with EtOAc. The combined organics were concentrated *in vacuo* to give 5-amino-1,3-dimethyl-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (390 mg, 99%) as a white solid.

LCMS (method formate): Retention time 0.47 min; $[\text{M}+\text{H}]^+ = 261.3$

^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 6.79 (s, 1H), 6.47 (s, 1H), 4.55 (br s, 2H), 3.23 (s, 3H), 3.20 (s, 3H), 2.74 (br s, 4H), 1.66 (m, 4H), 1.52 (br s, 2H).

Step 6



A solution of 5-amino-1,3-dimethyl-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (200 mg, 0.768 mmol) in CH_2Cl_2 (8 mL) at room temperature was treated with pyridine (0.081 mL, 1.0 mmol) then 2-methoxybenzoyl chloride (0.11 mL, 0.84 mmol) and the resulting mixture was stirred at this temperature for 45 min before being diluted with CH_2Cl_2 . The organic phase was washed with a saturated NaHCO_3 aqueous solution and the aqueous phase was extracted with CH_2Cl_2 . The combined organics were washed with a 2N HCl aqueous solution and the aqueous phase was extracted with CH_2Cl_2 . The combined organics were washed with water, dried using a separator frit and concentrated *in vacuo*.

Purification of the residue by flash chromatography on silica gel (25 g column, 30% GLOBAL gradient using (3/1 EtOAc/EtOH) and cyclohexane) gave N-(1,3-dimethyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-2-

methoxybenzamide (68 mg, 22%) as a white solid. The remaining fractions containing the product were concentrated *in vacuo* and purified again by flash chromatography on silica gel (25 g column, 20% GLOBAL gradient using (3/1 EtOAc/EtOH) and cyclohexane) to give N-(1,3-dimethyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-2-methoxybenzamide (100 mg, 33%) as a white solid.

LCMS (method high pH): Retention time 1.23 min; $[M+H]^+ = 395.4$

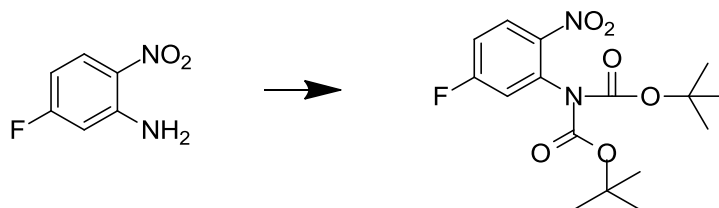
^1H NMR (600 MHz, CDCl_3) δ 10.61 (s, 1H), 8.43 (s, 1H), 8.29 (dd, $J = 7.9, 1.8$ Hz, 1H), 7.50-7.52 (m, 1H), 7.14-7.17 (m, 1H), 7.07 (d, $J = 8.1$ Hz, 1H), 6.83 (s, 1H), 4.10 (s, 3H), 3.44 (s, 3H), 3.42 (s, 3H), 2.80-2.93 (m, 4H), 1.75-1.78 (m, 4H), 1.63 (br s, 2H).

^{13}C NMR (151 MHz, CDCl_3) δ 163.24 (s, 1C), 157.36 (s, 1C), 155.05 (s, 1C), 138.43 (s, 1C), 132.89 (s, 1C), 132.36 (s, 1C), 128.75 (s, 1C), 126.45 (s, 1C), 125.96 (s, 1C), 122.84 (s, 1C), 121.49 (s, 1C), 111.68 (s, 1C), 101.11 (s, 1C), 99.93 (s, 1C), 56.32 (s, 1C), 54.49 (s, 2C), 27.28 (s, 1C), 27.13 (s, 1C), 26.71 (s, 2C), 24.00 (s, 1C).

Compound 4

Compound 4 was obtained according in part to: Chen, H.; McDaniel, K. F.; Green, B. E.; Shanley, J. P.; Kruger, A. W.; Gandarilla, J.; Welch, D. S.; Cink, R. D.; Gai, Y.; Wang, G. Or, Y. S. Preparation of macrocyclic proline-containing peptides as hepatitis C serine protease inhibitors. PCT Int. Appl.(2011), WO2011156337A2.

Step 1

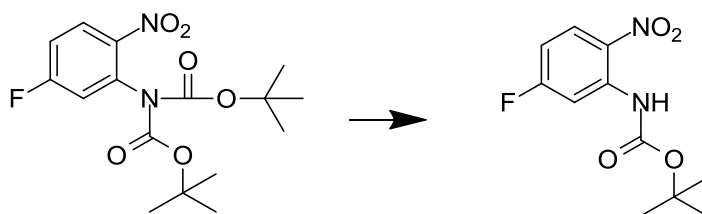


A solution of 5-fluoro-2-nitroaniline (10 g, 64 mmol) in 2-methyltetrahydrofuran (200 mL) at room temperature was treated with di-tert-butyl dicarbonate (29.7 mL, 128 mmol) and DMAP (160 mg, 1.31 mmol) and the resulting mixture was stirred at this temperature for 3 days. The mixture was washed with water and the layers were separated (an aliquot of brine was added to facilitate the separation of the phases). The aqueous phase was extracted with EtOAc and the combined organic phases were washed with water (again an aliquot of brine was added to facilitate the separation of the phases), dried over MgSO_4 and concentrated *in vacuo* to give di-tert-butyl (5-fluoro-2-nitrophenyl)carbamate (21.8 g, 96%) as a yellow solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.27 min; $[M+H]^+ = 357.3$

^1H NMR (400 MHz, CDCl_3) δ 8.12-8.21 (m, 1H), 7.17-7.26 (m, 1H), 7.04-7.11 (m, 1H), 1.43 (s, 18H).

Step 2

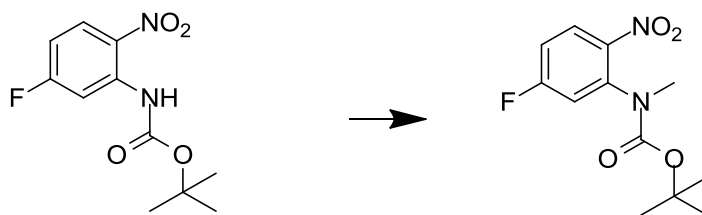


A solution of di-tert-butyl (5-fluoro-2-nitrophenyl)carbamate (11.3 g, 31.7 mmol) in CH_2Cl_2 (100 mL) at room temperature was treated with TFA (3.66 mL, 47.5 mmol) drop wise and the resulting mixture was stirred at this temperature for 80 min before being treated with a saturated NaHCO_3 aqueous solution. The layers were separated and the organic phase was washed with a saturated NaHCO_3 aqueous solution, dried using a phase separator and concentrated *in vacuo* to give tert-butyl (5-fluoro-2-nitrophenyl)carbamate (8.10 g, 100%) as a yellow solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.31 min; $[\text{M}-\text{H}]^- = 255.4$

^1H NMR (400 MHz, CDCl_3) δ 9.90 (br s, 1H), 8.42 (dd, $J = 11.7, 2.7$ Hz, 1H), 8.28 (dd, $J = 9.4, 5.7$ Hz, 1H), 6.79 (m, 1H), 1.57 (s, 9H).

Step 3

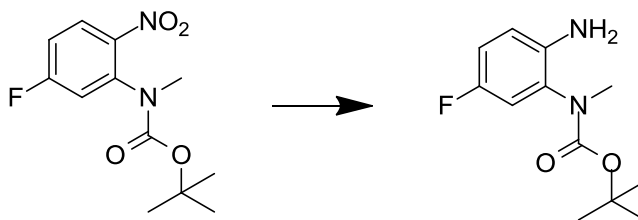


A solution of tert-butyl (5-fluoro-2-nitrophenyl)carbamate (13 g, 51 mmol) in THF (80 mL) and DMF (160 mL) at 0 °C under nitrogen was treated with sodium hydride (60% w/w in mineral oil, 2.43 g, 60.9 mmol) and the resulting mixture was stirred at this temperature for 15 min before being treated with methyl iodide (6.34 mL, 101 mmol). The resulting mixture was stirred at this temperature for 1 h. EtOH (2 mL) was added followed by a few drops of AcOH. The bright yellow solution was allowed to warm to room temperature and was concentrated *in vacuo*. The solid residue was partitioned between water and EtOAc and the layers were separated. The aqueous phase was extracted with EtOAc. The combined organics were washed twice with water then with brine, dried over MgSO_4 and concentrated *in vacuo* to give tert-butyl (5-fluoro-2-nitrophenyl)(methyl)carbamate (14.9 g, 109%) as a yellow oil which was used the next step without further purification.

LCMS (method high pH): Retention time 1.15 min; $[\text{M}+\text{H}]^+ = 271.2$

^1H NMR (400 MHz, CDCl_3) δ 7.94-8.03 (m, 1H), 7.02-7.13 (m, 2H), 3.32 (s, 3H), 1.33 (br s, 9H).

Step 4

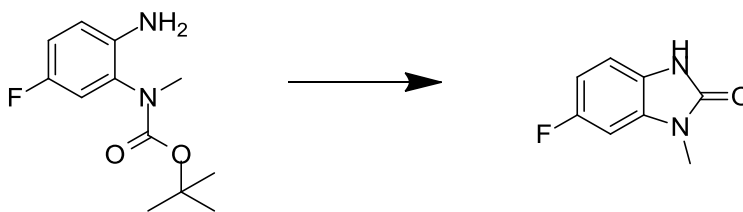


A solution of crude tert-butyl (5-fluoro-2-nitrophenyl)(methyl)carbamate (13.7 g, 50.7 mmol) in EtOH (300 mL) was treated with palladium on carbon (10% w/w, 50% wet, 1.5 g, 0.7 mmol) and the resulting mixture was stirred under hydrogen (1 bar) for 16 h. The catalyst was removed using a pad of celite (10 g) and rinsed with EtOH. The combined organics were concentrated *in vacuo* to give tert-butyl (2-amino-5-fluorophenyl)(methyl)carbamate (11.4 g, 94%) as a white solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 1.01 min; $[M+H]^+ = 241.2$

^1H NMR (400 MHz, DMSO- d_6 , T = 393K) δ 6.70-6.86 (m, 3H), 4.28-4.46 (br s, 2H), 3.05 (s, 3H), 1.40 (br s, 9H).

Step 5

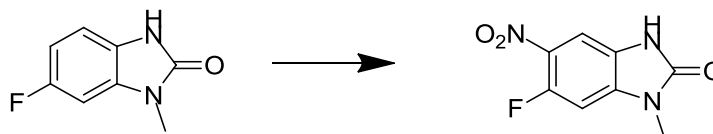


A solution of tert-butyl (2-amino-5-fluorophenyl)(methyl)carbamate (12.7 g, 53.0 mmol) in DMF (120 mL) and THF (80 mL) under nitrogen at room temperature was treated with sodium hydride (60% w/w in mineral oil, 2.76 g, 68.9 mmol) and the resulting mixture was stirred at 60°C for 2 h then was cooled to room temperature, treated with EtOH (2 mL, excess) and concentrated *in vacuo*. The residue was dissolved in water and treated with a few drops of acetic acid until a precipitate appeared. This precipitate was filtered off and rinsed with water. It was then suspended in Et₂O, filtered off and dried under vacuum at 40°C for 16 h to give 6-fluoro-1-methyl-1H-benzo[d]imidazol-2(3H)-one (7.6 g, 86%) as a grey solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.66 min; $[M+H]^+ = 167.0$

^1H NMR (400 MHz, DMSO- d_6) δ 10.74-11.09 (m, 1H), 7.05 (dd, $J = 9.0, 2.4$ Hz, 1H), 6.93 (dd, $J = 8.4, 4.8$ Hz, 1H), 6.78 (ddd, $J = 10.4, 8.4, 2.4$ Hz, 1H), 3.26 (s, 3H).

Step 6



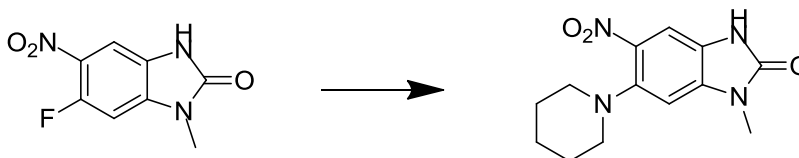
A suspension of 6-fluoro-1-methyl-1H-benzo[d]imidazol-2(3H)-one (5 g, 30 mmol) in acetic anhydride (75 mL) under nitrogen was cooled to -30°C and slowly treated with fuming nitric acid (1.34 mL, 30.1 mmol), keeping the temperature below -20°C. The resulting mixture was then allowed to warm slowly to 0°C. After 1 h, the mixture was poured into ice-water (100 mL) and EtOAc (10 mL) was added. The resulting mixture was stirred for 30 min. The precipitate formed was

collected by filtration and washed with water (20 mL) before being dried under vacuum at 40°C for 16 h to give 6-fluoro-1-methyl-5-nitro-1H-benzo[d]imidazol-2(3H)-one (4.75 g, 71%) as a light yellow solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.66 min; $[M-H]^- = 210.3$

1H NMR (400 MHz, DMSO- d_6) δ 11.39 (s, 1H), 7.63 (d, $J = 6.6$ Hz, 1H), 7.44 (d, $J = 11.7$ Hz, 1H), 3.33 (s, 3H).

Step 7

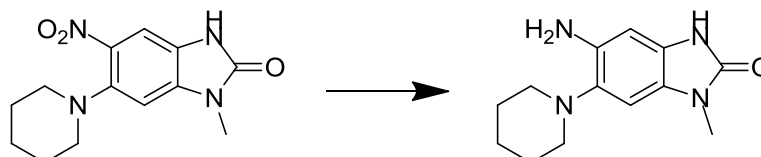


A mixture of 6-fluoro-1-methyl-5-nitro-1H-benzo[d]imidazol-2(3H)-one (715 mg, 3.39 mmol), piperidine (865 mg, 10.2 mmol) and DIPEA (2.19 g, 16.9 mmol) in DMSO (2 mL) was stirred at 120°C under microwave irradiation for 1 h then was cooled to room temperature and partitioned between EtOAc and water (50 mL each). The layers were separated and the aqueous phase was extracted with EtOAc (50 mL). The combined organics were washed with water then brine (100 mL each), dried over Na_2SO_4 , and concentrated *in vacuo* to give 1-methyl-5-nitro-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (540 mg, 52%) as an orange solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.96 min; $[M+H]^+ = 277.2$

1H NMR (400 MHz, $CDCl_3$) δ 8.55 (br s, 1H), 7.68 (s, 1H), 6.71 (s, 1H), 3.44 (s, 3H), 2.98-3.07 (m, 4H), 1.78 (m, 4H), 1.57-1.66 (m, 2H).

Step 8

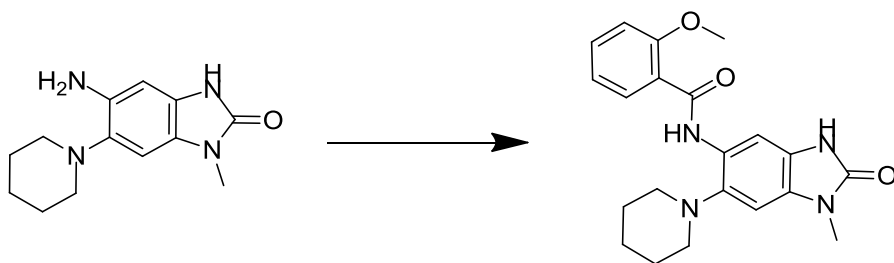


A mixture of 1-methyl-5-nitro-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (535 mg, 1.94 mmol), iron(0) (325 mesh, 541 mg, 9.68 mmol), and ammonium chloride (311 mg, 5.81 mmol) in EtOH (20 mL) and water (6.7 mL) was stirred at 90°C for 2.5 h then was cooled to room temperature and left to stand for 16 h. The mixture was filtered through celite and the filter cake was washed with EtOH (50 mL). The filtrate was concentrated *in vacuo* and the residue was partitioned between 1:1 CH_2Cl_2 : EtOAc (50 mL) and water (50 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (50 mL) and the combined organics were dried using an hydrophobic frit then were concentrated *in vacuo* to give 5-amino-1-methyl-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (110 mg, 21%) as an orange solid.

LCMS (method high pH): Retention time 0.83 min; $[M+H]^+ = 247.2$

1H NMR (400 MHz, DMSO- d_6) δ 10.28 (s, 1H), 6.71 (s, 1H), 6.39 (s, 1H), 4.49 (br s, 2H), 3.17 (s, 3H), 2.73 (br s, 4H), 1.59-1.71 (m, 4H), 1.44-1.57 (br s, 2H).

Step 9



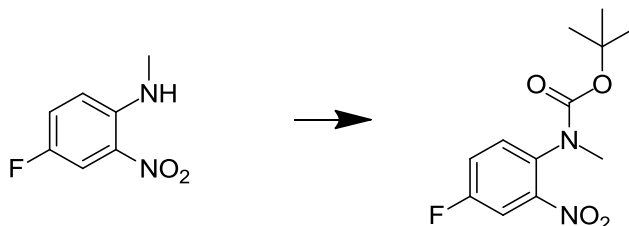
A solution of 5-amino-1-methyl-6-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (54.5 mg, 0.22 mmol) in DMF (1 mL) at room temperature was treated with pyridine (0.036 mL, 0.44 mmol) then 2-methoxybenzoyl chloride (0.036 mL, 0.27 mmol) and the resulting mixture was stirred at this temperature for 1 h before being purified by MDAP (method high pH) to give 2-methoxy-N-(1-methyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzamide (26 mg, 30%) as a cream solid.

LCMS (method high pH): Retention time 1.10 min; $[M+H]^+ = 381.4$

^1H NMR (400 MHz, CDCl_3) δ 10.59 (s, 1H), 8.50 (s, 1H), 8.33 (dd, $J = 7.8, 2.0$ Hz, 1H), 8.06 (s, 1H), 7.49-7.55 (m, 1H), 7.15-7.21 (m, 1H), 7.08 (d, $J = 8.3$ Hz, 1H), 6.82 (s, 1H), 4.10 (s, 3H), 3.41 (s, 3H), 2.87 (br s, 4H), 1.78 (m, 4H), 1.64 (br s, 2H).

Compound 5

Step 1

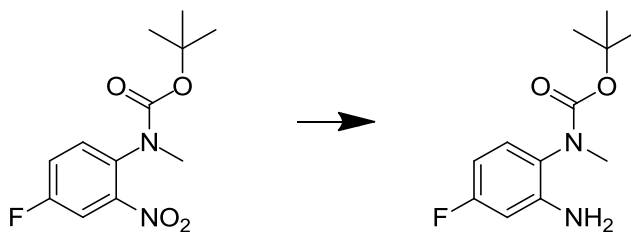


A solution of 4-fluoro-N-methyl-2-nitroaniline (5.00 g, 29.4 mmol) in 2-methyltetrahydrofuran (100 mL) at room temperature was treated with triethylamine (4.92 mL, 35.3 mmol), DMAP (0.18 g, 1.5 mmol) and then di-tert-butyl dicarbonate (7.16 mL, 30.9 mmol) and the resulting mixture was stirred at this temperature for 3 days, then at 80°C for 5 h, and then was cooled to room temperature. DMAP (300 mg, 2.46 mmol), triethylamine (2.46 mL, 17.6 mmol) and di-tert-butyl dicarbonate (3.58 mL, 15.4 mmol) were added and the mixture was stirred again at 80°C for 2.5 h before being cooled to room temperature and washed with a 2N HCl aqueous solution. The layers were separated and the aqueous phase was extracted with EtOAc. The combined organics were washed with a saturated NaHCO_3 aqueous solution then with brine, dried over MgSO_4 and concentrated *in vacuo*. Purification of the residue by flash chromatography on silica gel (100 g column, 0 to 50% EtOAc in hexanes) gave tert-butyl (4-fluoro-2-nitrophenyl)(methyl)carbamate (6.9 g, 87%).

LCMS (method high pH): Retention time 1.16 min; $[M+H]^+ = 271.1$

^1H NMR (400 MHz, CDCl_3) δ 7.62-7.71 (m, 1H), 7.34 (m, 2H), 3.29 (s, 3H), 1.50 (s, 9H).

Step 2

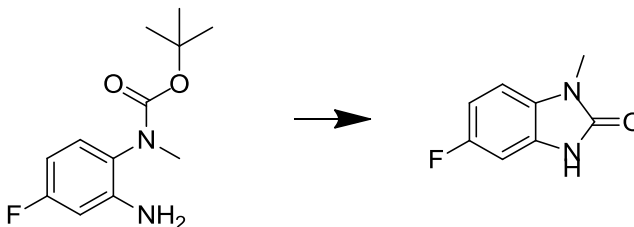


A solution of tert-butyl (4-fluoro-2-nitrophenyl)(methyl)carbamate (7.90 g, 29.2 mmol) in EtOH (200 mL) was treated with palladium on carbon (5% w/w, 50% wet, 3.1 g, 0.70 mmol) and the resulting mixture was stirred under hydrogen (1 bar) for 72 h. The catalyst was filtered off using a pad a celite and rinsed with EtOH. The combined organics were concentrated *in vacuo* to give tert-butyl (2-amino-4-fluorophenyl)(methyl)carbamate (6.85 g, 98%).

LCMS (method high pH): Retention time 1.04 min; $[M+H]^+ = 241.2$

^1H NMR (400 MHz, DMSO- d_6 , T = 393 K) δ 6.91 (dd, $J = 8.5, 6.3$ Hz, 1H), 6.52 (dd, $J = 11.2, 2.9$ Hz, 1H), 6.29 (ddd, $J = 8.5, 8.5, 2.9$ Hz, 1H), 4.74-4.85 (br s, 2H), 3.03 (s, 3H), 1.38 (s, 9H).

Step 3

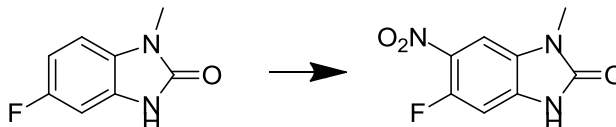


A solution of tert-butyl (2-amino-4-fluorophenyl)(methyl)carbamate (6.0 g, 25 mmol) in a mixture of THF (40 mL) and DMF (60 mL) at room temperature under nitrogen was treated portion wise with sodium hydride (60% w/w in mineral oil, 1.2 g, 30 mmol). The resulting mixture was stirred at this temperature for 1 h, then at 60°C for 1 h, before being cooled to room temperature and treated with MeOH (10 mL). The mixture was then concentrated *in vacuo* and the residue was dissolved in water (60 mL). The aqueous solution was treated with a saturated NH_4Cl aqueous solution (20 mL) and the precipitate formed was collected by filtration, rinsed with water and dried under vacuum at 40°C for 16 h to give 5-fluoro-1-methyl-1H-benzo[d]imidazol-2(3H)-one (4.1 g, 99%) as a beige solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.65 min; $[M+H]^+ = 167.1$

^1H NMR (400 MHz, DMSO- d_6) δ 10.90 (br s, 1H), 7.03-7.08 (m, 1H), 6.80-6.87 (m, 2H), 3.27 (s, 3H).

Step 4



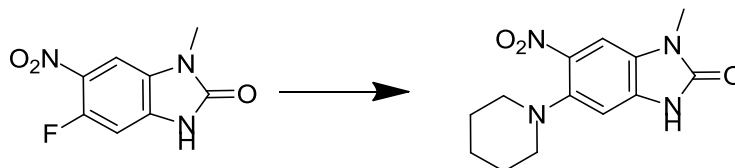
A solution of 5-fluoro-1-methyl-1H-benzo[d]imidazol-2(3H)-one (4.19 g, 25.2 mmol) in acetic anhydride (60 mL) under nitrogen was cooled to -30°C and then was slowly treated with fuming nitric acid (1.13 mL, 25.2 mmol), keeping the temperature below -20°C. The resulting mixture was then allowed to warm to 0°C. After 1 h, the reaction was treated with ice-water (100 mL) and then diluted with EtOAc (10 mL). The resulting mixture was stirred for 30 min. The precipitate

formed was filtered off, washed with water (10 mL) and dried at 40°C under vacuum for 72 h to give 5-fluoro-1-methyl-6-nitro-1H-benzo[d]imidazol-2(3H)-one (4.28 g, 76%) as a light yellow solid which was used in the next step without further purification.

LCMS (method high pH): Retention time 0.64 min; $[M-H]^- = 210.2$

1H NMR (400 MHz, DMSO- d_6) δ 11.75 (br s, 1H), 7.93 (d, $J = 6.6$ Hz, 1H), 7.15 (d, $J = 11.5$ Hz, 1H), 3.35 (s, 3H).

Step 5

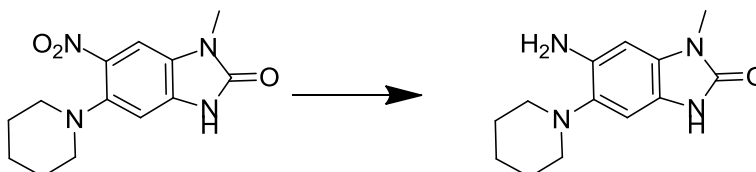


A solution of 5-fluoro-1-methyl-6-nitro-1H-benzo[d]imidazol-2(3H)-one (383 mg, 1.82 mmol), piperidine (0.54 mL, 5.4 mmol) and DIPEA (1.59 mL, 9.08 mmol) in DMSO (2 mL) was stirred at 120°C under microwave irradiation for 1 h then was cooled to room temperature and diluted with water (20 mL). The aqueous phase was extracted with EtOAc (3 x 20 mL) and the combined organics were washed with water (2 x 50 mL) and brine (50 mL), and then were dried over Na_2SO_4 and concentrated *in vacuo* to give 1-methyl-6-nitro-5-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (413 mg, 74%) as an orange solid.

LCMS (method high pH): Retention time 0.93 min; $[M+H]^+ = 277.2$

1H NMR (400 MHz, $CDCl_3$) δ 9.27 (br s, 1H), 7.61 (s, 1H), 6.89 (s, 1H), 3.44 (s, 3H), 2.95-3.04 (m, 4H), 1.77 (m, 4H), 1.61 (m, 2H).

Step 6

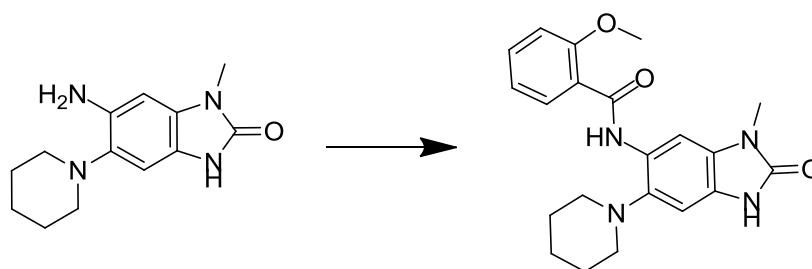


A mixture of 1-methyl-6-nitro-5-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (405 mg, 1.47 mmol), iron(0) (325 mesh, 409 mg, 7.33 mmol) and ammonium chloride (235 mg, 4.40 mmol) in EtOH (15 mL) and water (5 mL) was stirred at 90°C for 3 h then was cooled to room temperature and filtered through celite. The insoluble material was washed with EtOH (20 mL) and the combined filtrates were concentrated *in vacuo*. The residue was partitioned between EtOAc and water (25 mL each) and the layers were separated. The aqueous layer was extracted with EtOAc (25 mL) and the combined organics were dried over $MgSO_4$ and concentrated *in vacuo* to give 6-amino-1-methyl-5-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (252 mg, 66%) as a light yellow solid.

LCMS (method high pH): Retention time 0.79 min; $[M+H]^+ = 247.2$

1H NMR (400 MHz, DMSO- d_6) δ 10.22 (s, 1H), 6.58 (s, 1H), 6.41 (s, 1H), 4.50 (s, 2H), 3.15 (s, 3H), 2.68 (br s, 4H), 1.64 (m, 4H), 1.51 (br s, 2H).

Step 7

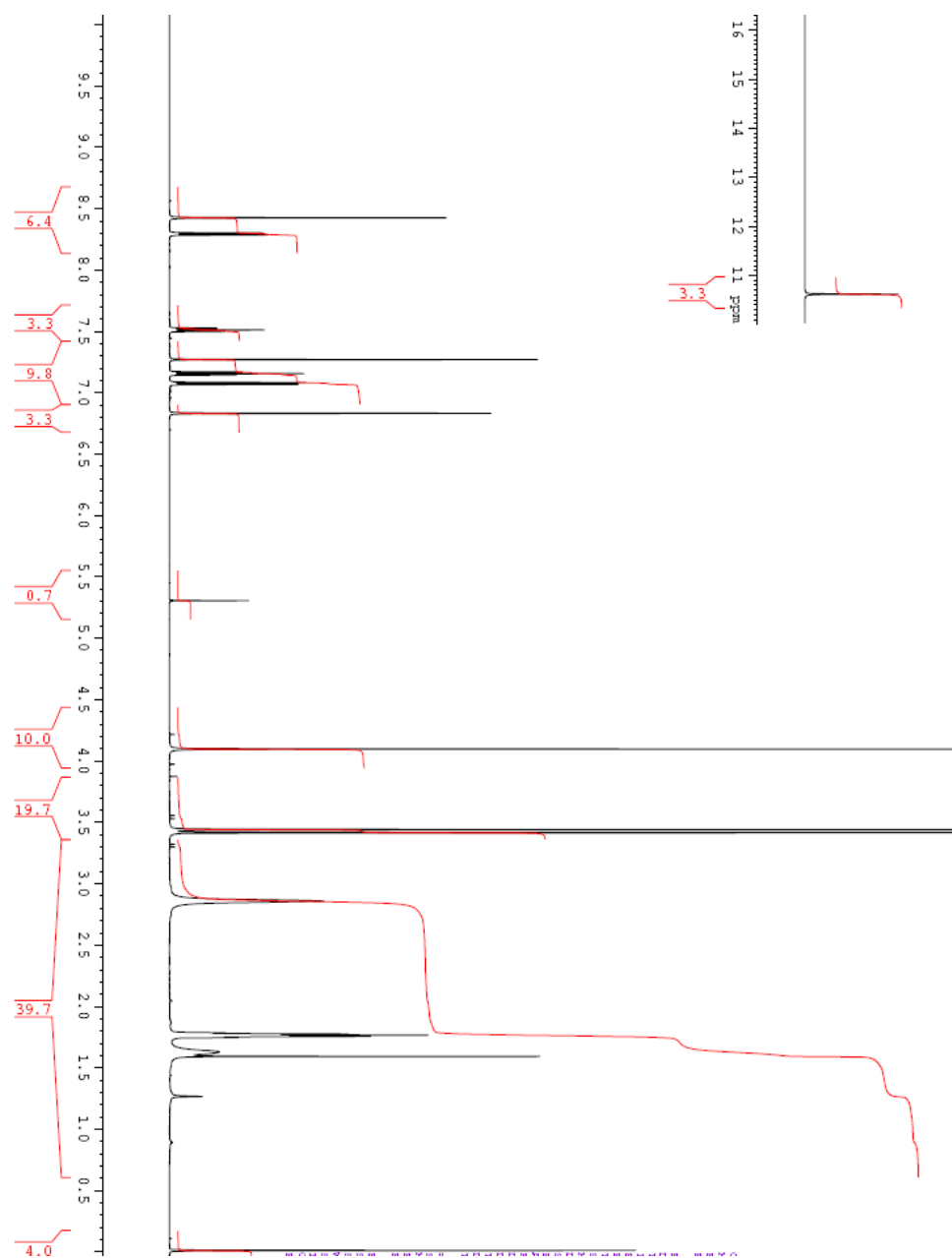


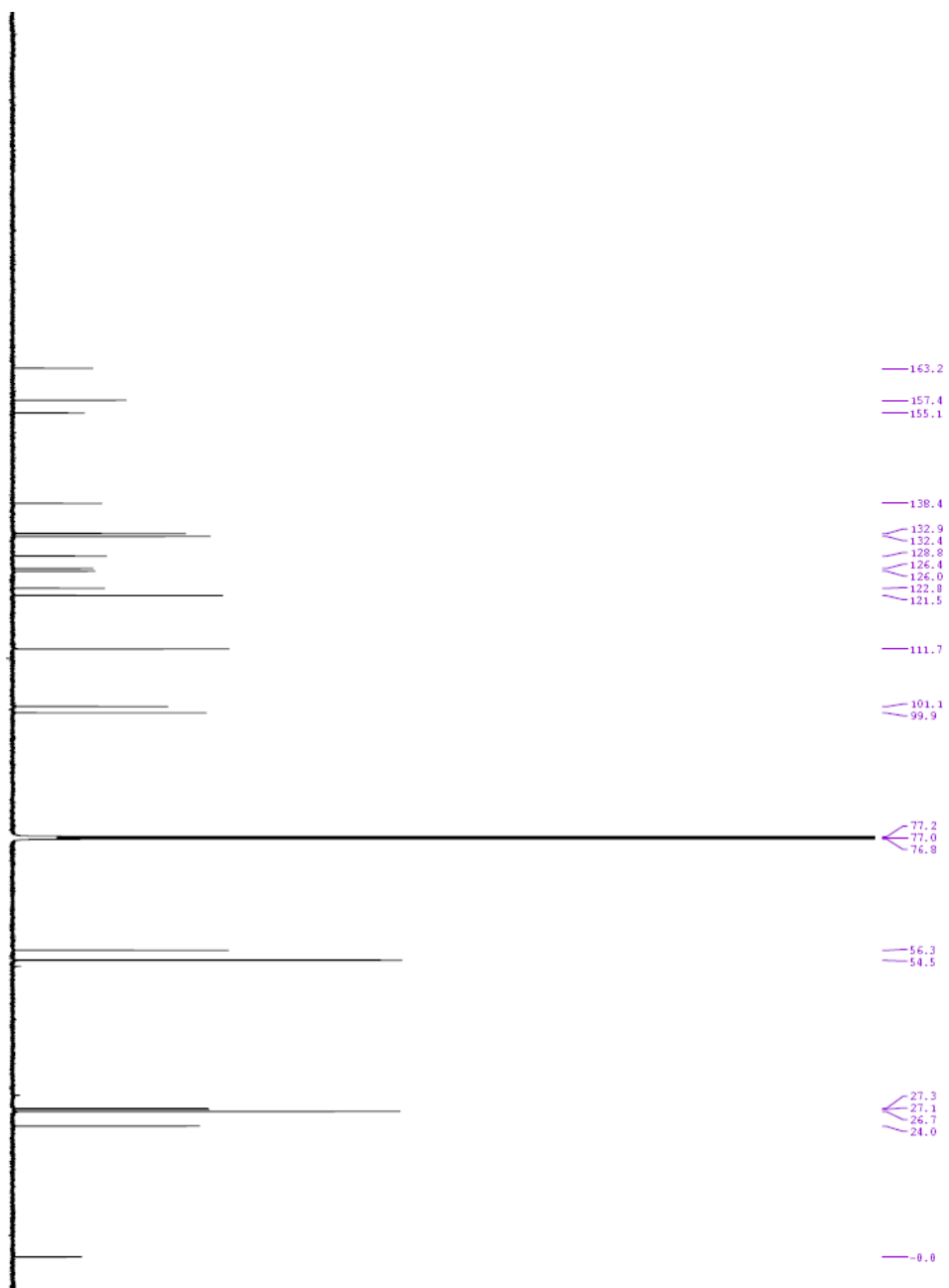
A solution of 6-amino-1-methyl-5-(piperidin-1-yl)-1H-benzo[d]imidazol-2(3H)-one (50 mg, 0.20 mmol) in DMF (1 mL) at room temperature was treated with pyridine (0.033 mL, 0.41 mmol) then 2-methoxybenzoyl chloride (0.033 mL, 0.24 mmol) and the resulting mixture was stirred at this temperature for 1 h before being purified by MDAP (high pH method) to give 2-methoxy-N-(3-methyl-2-oxo-6-(piperidin-1-yl)-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzamide (26 mg, 32%) as a cream solid.

LCMS (method high pH): Retention time 1.07 min; $[M+H]^+ = 381.2$

^1H NMR (400 MHz, CDCl_3) δ 10.63 (s, 1H), 8.93 (br s, 1H), 8.44 (s, 1H), 8.31 (dd, $J = 7.8, 2.0$ Hz, 1H), 7.53 (ddd, $J = 8.3, 7.3, 2.0$ Hz, 1H), 7.13-7.21 (m, 1H), 7.09 (d, $J = 8.3$ Hz, 1H), 6.98 (s, 1H), 4.11 (s, 3H), 3.46 (s, 3H), 2.84 (br s, 4H), 1.76 (m, 4H), 1.62 (br s, 2H).

NMR spectra of Compound 3 (^1H 600MHz, ^{13}C 151 MHz)





Supplementary Methods

Expression & purification of BET and BRPF1 proteins. BET proteins were produced using protocols given in the literature.¹ 6H-Flag-TEV-BRPF1 (622-738) was expressed in *E.coli* BL21 (DE3) cells. The pellet from the *E.coli* culture was resuspended in buffer A (50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM imidazole, and 0.5 mM TCEP) plus 0.1 mg/ml lysozyme (Sigma 92971-50G-F) and 1 μ l/ml Protease Inhibitor Cocktail (Sigma P8340). Cells were lysed by sonication, on ice, and centrifuged at 100,000 x g for 90 minutes at 4 °C. The supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02) equilibrated with buffer A, washed with ten column volumes buffer A, and the bound protein eluted using a linear gradient of 10-500 mM imidazole over twenty column volumes. Eluted BRPF1 protein was cleaved with rTEV protease at a ratio of 1:200, and dialysed overnight at 4°C against buffer B (50 mM HEPES pH 7.5, 150 mM NaCl and 0.5 mM TCEP) using SnakeSkin Dialysis Tubing 3.5K MWCO (Thermo Scientific #68035). Cleaved protein was applied to a HiLoad 26/60 Superdex 75 prep grade size exclusion column (GE Healthcare 45-002-489), equilibrated with buffer B. Fractions containing BRPF1 were pooled, concentrated to 5.9 mg/ml using an Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-3 membrane (Millipore UFC900308), aliquoted and stored at -80°C. Protein identity was confirmed by peptide mass fingerprinting and predicted molecular weight confirmed by Liquid Chromatography/Mass Spectrometry.

Purification of ¹⁵N 6H-FLAG-TEV-BRPF1 (622-738). The pellet from the *E.coli* culture was resuspended in buffer A (50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM imidazole and 0.5 mM TCEP) plus 0.1 mg/ml lysozyme (Sigma 92971-50G-F) and 1 μ l/ml Protease Inhibitor Cocktail (Sigma P8340). Cells were lysed by sonication, on ice, and centrifuged at 100,000 x g for 90 minutes at 4 °C. The supernatant was applied to a HisTRAP HP Column (GE Healthcare 17-5248-02) equilibrated with buffer A. The column was washed with ten column volumes of buffer A and bound protein was eluted using a linear gradient of buffer B (50 mM HEPES pH 7.5, 150 mM NaCl, 500 mM imidazole and 0.5 mM TCEP) over twenty column volumes. The BRPF1 protein eluted between 100 mM and 230 mM imidazole. Eluted BRPF1 protein was cleaved with rTEV protease at a ratio of 1:200 and dialysed overnight at 4 °C against buffer C (50 mM HEPES pH 7.5, 150 mM NaCl and 0.5 mM TCEP). The cleaved protein was concentrated 2.5-fold (Amicon Ultrafree-15 3 kDa, Millipore UFC900308) and loaded onto a HiLoad 26/60 Superdex 75 prep grade size exclusion column (GE Healthcare 17-1070-01), equilibrated with buffer C. Fractions containing ¹⁵N BRPF1 protein were pooled, concentrated to 5.5mg/ml, aliquoted and stored at -80°C. Analysis by mass spectrometry showed an increase in expected molecular weight of +157.03 Da which was correlated to be consistent with 94.0% ¹⁵N incorporation.

STD-NMR of BRD4. STD experiments were run on a Bruker Avance III spectrometer equipped with a 5 mm z-gradient TCI cryoprobe with 32k data points, transmitter 4.7 ppm, 16 ppm sweepwidth, 2s interscan delay acquiring a total of 128 scans. The samples contained 5 μ M of His-tagged-BRD4(1-477) with 1 mM of fragment 1, in the presence and absence of 1 mM of a potent acetyl-lysine site inhibitor GW841819.¹ The experiments were run at 278K.

BRPF1 ¹⁵N-¹H HSQC NMR. SOFAST HSQC experiments were run at 300K on a Bruker Avance III spectrometer equipped with a 5 mm z-gradient TCI cryoprobe. The NMR data were collected with ¹H and ¹⁵N radiofrequency pulses applied at 4.7 ppm and 120 ppm, respectively; 2048 \times 128 complex data points with spectral widths of 16 ppm and 35 ppm, respectively, were collected along the ¹H and ¹⁵N dimensions, with 64 scans per free induction decay and an inter-scan delay of 0.1 s, resulting in a total experimental time of about 30 min for each HSQC spectrum. BRPF1 (TEV-cleaved 622-738) was present at 80 μ M and compound at a nominal concentration of 300 μ M for compounds 2 and 3, and 1 mM for fragment 1.

BRD4-BD1 Crystallography. Crystallography was performed using *E.coli* expressed de-His-tagged BRD4-BD1(44-168). The protein was at typically ~11 mg/ml in 10mM HEPES pH7.5, 100 mM NaCl and purified to homogeneity using a HisTrap column followed by gel filtration, Tev protease cleavage and gel filtration using a Sephadex 75 column. Compounds were added to the protein at 3:1 excess and spun prior to co-crystallisation in 120 nl +120 nl sitting drops @ 20°C using MRC plates. The successful co-crystallisation conditions are given in Fig. S4. Crystals were briefly transferred into cryo buffer of the well solution with 10-20% ethylene glycol added before flash freezing in liquid nitrogen. Data from a single crystal was collected at the ESRF (European Synchrotron Radiation Facility, Grenoble, ID23-1). Molecular replacement solution was performed using an in-house structure and PHASER (CCP4)². Rounds of manual model building were accomplished using Coot³ and refinement used REFMAC⁴. Clear difference density for the ligands in the acetyl-lysine binding site could be unambiguously modelled. Data collection and refinement statistics are given in Fig. S4 alongside 2Fc-Fc maps in Fig. S3. The final model has been deposited in the protein databank under the accession code xxx (structures will be deposited on acceptance and released immediately on publication).

BRPF1 Bromodomain Crystallography. *E.coli* expressed 6H-Flag-Tev-BRPF1 (622-738) was purified to homogeneity as described above. Compounds were added to the protein at 3:1 excess and spun prior to co-crystallisation in 120 nl +120 nl

sitting drops @ 4°C and 20°C using MRC plates. The successful co-crystallisation conditions are given in Fig. S4. Crystals were briefly transferred to a cryo buffer consisting of the well solution +20% glycerol prior to flash freezing. Data from a single frozen crystal was collected at the Diamond Light Source. Data were processed using XDS and AIMLESS. The C2 cell contains 2 molecules in the ASU. Molecular replacement used a previously determined in-house structure as model, with PHASER (CCP4)². Model building was performed using Coot³ and refinement used REFMAC⁴. Clear difference density for the ligands in the acetyl-lysine binding sites of both chains A and B could be unambiguously modelled. Data collection and refinement statistics are given in Fig. S4 alongside 2Fc-Fc maps in Fig. S3. The final model has been deposited in the protein data bank under the accession code xxx (structures will be deposited on acceptance and released immediately on publication).

BRD4 TR-FRET assays. Compounds were screened against either 6H-Thr BRD4 (1-477) (Y390A) (BRD4 BD2 mutation to monitor compound binding to BD1) or 6H-Thr BRD4 (1-477) (Y97A) (BRD4 BD1 mutation to monitor compound binding to BD2) in a dose-response format in a TR-FRET assay measuring competition between test compound and an Alexa Fluor 647 derivative of I-BET762.¹ Compounds were titrated from 10 mM in 100% DMSO and 50 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Combi was used to dispense 5 µL of 20 nM protein in an assay buffer of 50 mM HEPES, 150 mM NaCl, 5% glycerol, 1 mM DTT and 1 mM CHAPS, pH 7.4, and in the presence of 100 nM fluorescent ligand (~*K_d* concentration for the interaction between BRD4 BD1 and ligand). After equilibrating for 30 mins in the dark at rt, the bromodomain protein:fluorescent ligand interaction was detected using TR-FRET following a 5 µL addition of 3 nM europium chelate labelled anti-6His antibody (Perkin Elmer, W1024, AD0111) in assay buffer. Time resolved fluorescence (TRF) was then detected on a TRF laser equipped Perkin Elmer Envision multimode plate reader (excitation = 337 nm; emission 1 = 615 nm; emission 2 = 665 nm; dual wavelength bias dichroic = 400 nm, 630 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 665 nm) / (Donor fluorescence at 615 nm)) * 1000. TR-FRET ratio data was normalised to high (DMSO) and low (compound control derivative of I-BET762) controls and IC₅₀ values determined for each of the compounds tested by fitting the fluorescence ratio data to a four parameter model: $y = a + ((b - a) / (1 + (10^x / 10^c)^d))$ where 'a' is the minimum, 'b' is the Hill slope, 'c' is the IC₅₀ and 'd' is the maximum.

BRPF1, 2 and 3 TR-FRET assays. Compounds were screened against 6H-Flag-Tev-BRPF1 (622-738), 6HisFlag-Tev-BRPF2 (also known as BRD1) (551-673) or 6His-Flag-Tev-BRPF3 (579-706) protein in dose-response format in a TR-FRET assay measuring competition between test compound and a synthetic fluorescent ligand. Compounds were titrated from 10 mM in 100% DMSO and 100 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Combi was used to dispense 5 µL of 4 nM BRPF1, 20 nM BRPF2 or 40 nM BRPF3 protein respectively in an assay buffer of 50 mM HEPES, 150 mM NaCl, 5% glycerol, 1mM DTT and 1 mM CHAPS, pH 7.4, and in the presence of the appropriate fluorescent ligand concentration (~*K_d* concentration for the interaction between protein and ligand). After equilibrating for 30 mins in the dark at rt, the bromodomain protein:fluorescent ligand interaction was detected using TR-FRET following a 5 µL addition of either 3 nM Lanthascreen Elite Tb-anti His antibody (Invitrogen PV5863) for the Alexa 488 ligands, or 3 nM europium chelate labelled anti-6His antibody (Perkin Elmer, W1024, AD0111) for the Alexa 647 ligand, in assay buffer. Time resolved fluorescence energy transfer (TR-FRET) was then detected on a time-resolved fluorescence laser equipped Perkin Elmer Envision multimode plate reader using the appropriate protocol (excitation = 337 nm; emission 1 Alexa 488 = 495 nm; emission 2 Alexa 488 = 520 nm, emission 1 Alexa 647 = 615 nm; emission 2 Alexa 647 = 665 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 520 or 665 nm) / (Donor fluorescence at 495 or 615 nm)) * 1000. Data were analysed as for the BRD4 assay.

BRPF1
Alexa Fluor 488 labelled ligand
(GSK2941799A) 100nM

BRPF2
Alexa Fluor 488 labelled ligand
(GSK2997657A) 200nM

BRPF3
Alexa Fluor 647 labelled ligand
(GSK3103956A) 400nM

An alternative peptide-based TR-FRET BRPF1 assay was used for hit identification, in which compounds were screened at 10µM (n=2). pIC₅₀ results from the peptide and fluoroligand assays correlate well (*r*²=0.74 for 135 compounds from a variety of inhibitor classes). The peptide assay buffer was 30mM HEPES, 50mM NaCl, plus 1mM CHAPS and 1mM DTT added prior to use. 5 µL of assay buffer containing 100nM 6H-Flag-Tev-BRPF1 (622-738) and 1000nM peptide (C-terminally biotinylated tri-acetylated histone H4(1-21) K5ac/K8ac/K12ac, AltaBioScience), incubated for 30 minutes rt, and 5 µL detection solution (assay buffer with 2.5 nM LANCE Eu-W1024 anti-6His and 80nM LANCE APC SureLight-streptavidin), were added to test compounds in low-volume black 384-well micro titre plates. After 30 minutes incubation, time resolved fluorescence energy transfer (TR-FRET) was detected on a time-resolved fluorescence laser equipped Perkin Elmer Envision multimode plate reader (excitation = 337 nm; emission 1 = 665 nm; emission 2 = 615 nm).

NanoBRET assays. HEK293 cells (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-BRPF1 isoform 1 (P55201-1) bromodomain amino acids 625-735 or isoform 2 (P55201-2) bromodomain amino acids 625-741. Isoform 2 has an insertion S660 -> SEVTELD in the bromodomain. 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 100 nM 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). Inhibitors were then added directly to media at final concentrations between 0-33 μ M and the plates were incubated for 18hrs at 37°C in the presence of 5% CO₂. NanoBRET furimazine substrate (Promega) was added to both control and experimental samples at a final concentration of 10 μ M. 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 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.

Artificial membrane permeability measurement. Permeability across a lipid membrane was measured using the published protocol.⁵

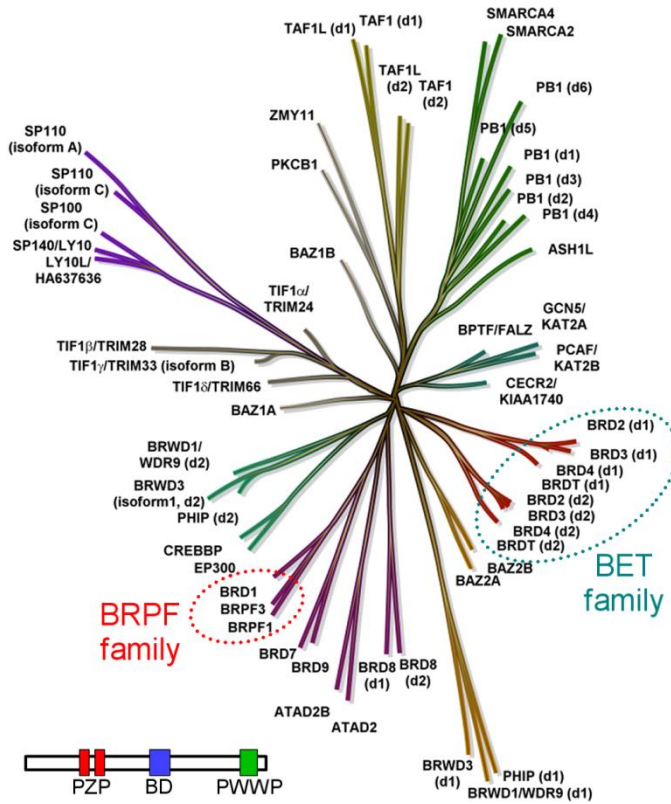
CLND solubility measurement. Solubility was determined by precipitation of 10 mM DMSO stock concentration to 5% DMSO pH7.4 phosphate buffered saline, with quantification by ChemiLuminescent Nitrogen Detection.

Supplementary References.

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Figure S1. A) Bromodomain phylogenetic tree, with BRPF1 domain structure inset. B) Aligned acetyl-lysine site residues of the BET family BD1 and BRPF family bromodomains. C) Sequence alignment of the BET and BRPF family bromodomains. The BET WPF motif is coloured blue, the water-binding tyrosine red, the conserved asparagine green, and the gatekeeper magenta.

A)



B)

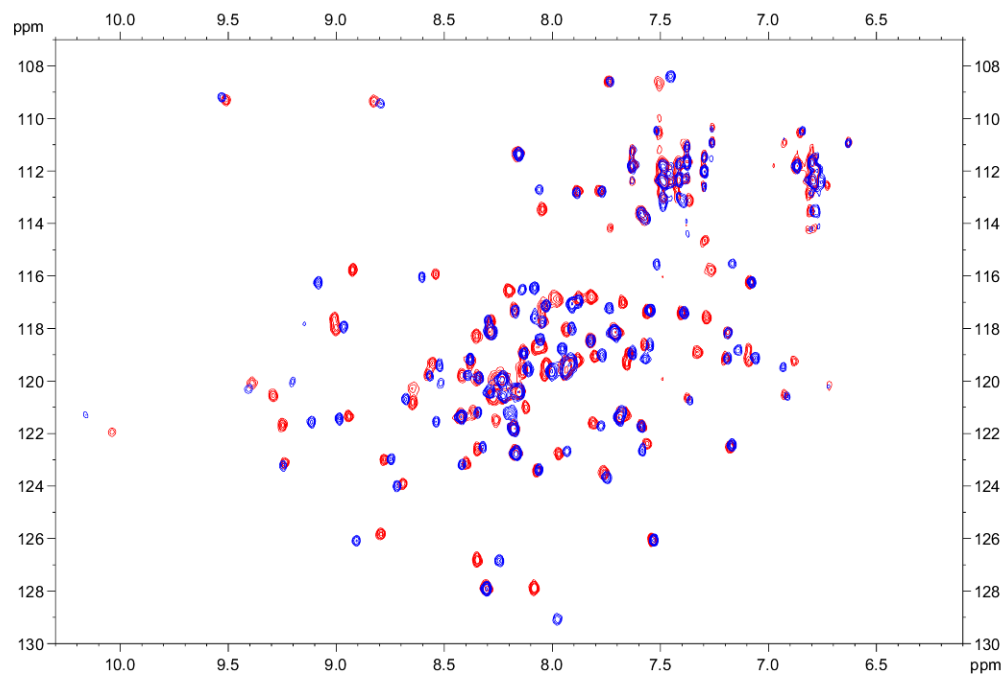
BRD2 BD1	BRD3 BD1	BRD4 BD1	BRPF1 / BRD1	BRPF2 / BRD1	BRPF3	Comment
W97	W57	W81	N651	R585	H612	WPF motif
P98	P58	P82	I652	I586	I613	WPF motif
F99	F59	F83	F653	F587	F614	WPF motif
R100	Y60	Q84	S654	A588	A615	ZA channel
Q101	Q61	Q85	E655	Q589	E616	ZA channel
P102	P62	P86	P656	P590	P617	ZA channel
V103	V63	V87	V657	V591	V618	ZA channel
D104	D64	D88	P658	S592	N619	ZA loop
A105	A65	A89	L659	L593	L620	ZA loop
K107	K67	K91				ZA loop
L108	L68	L92				ZA loop
G109	N69	N93	E661	E595	E622	ZA loop
L110	L70	L94	V662	V596	V623	ZA loop
Y113	Y73	Y97	Y665	Y599	Y626	Water-binding Tyr
H114	H74	Y98	L666	L600	L627	
I117	I77	I101	I669	I603	I630	
M148	M108	M132	I700	I634	I661	
N151	N111	N135	N703	N637	N664	
C152	C112	C136	C704	C638	C665	Conserved Cys
Y155	Y115	Y139	Y707	Y641	Y668	Conserved Tyr
N156	N116	N140	N708	N642	N669	Conserved Asn
D160	D120	D144	T712	T646	T673	
D161	D121	D145	I713	V647	I674	
I162	I122	I146	F714	F648	F675	Gatekeeper
V163	V123	V147	Y715	Y649	H676	
M165	M125	M149	A717	A651	A678	
A166	A126	A150	A718	A652	A679	

C)

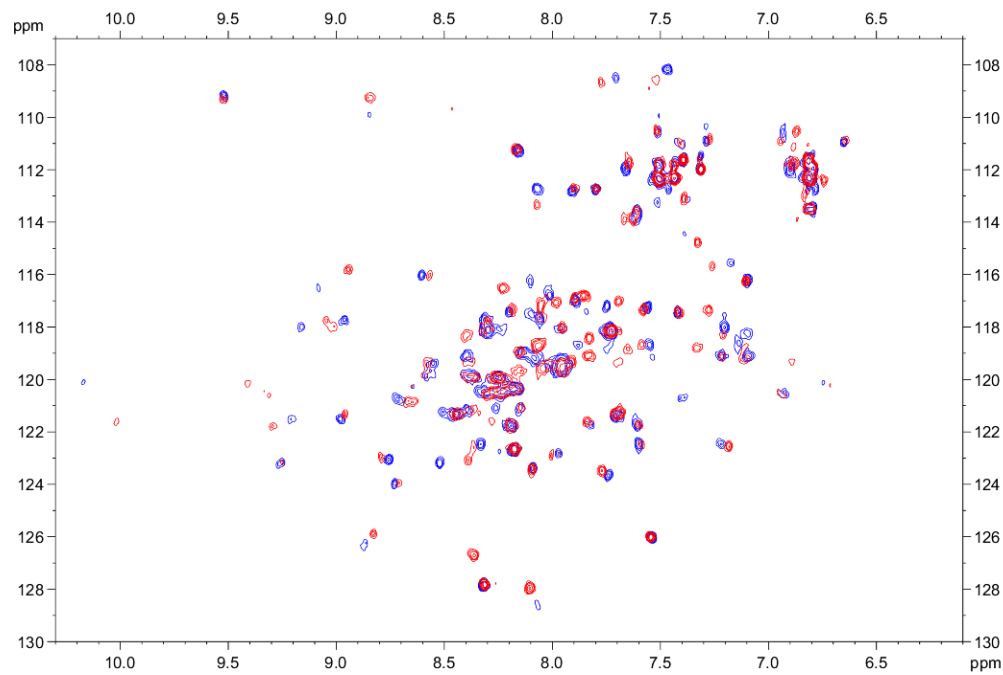
BRPF1	631	TPFLILLRKTLEQLQE...	KDTGNIFSEPVPLS...	EVPDYLDHIKKPMDFFTMKQNL	E
BRD1	565	TPLTVLLRSVLDQLQD...	KDPARIFAQPVSLK...	EVPDYLDHIKKPMDFATMRKRLE	E
BRPF3	592	MPFNVLRLRTLDLLQE...	KDPAHIFAEPVNL...	EVPDYLEFISKPMDFSTMRKRLE	E
BRD2_1	77	NQLQYLHKVVMKALWK...	HQFAWPFQRPVDAVKLGLPDYHKI	IKQPMDMGTIKRRLE	E
BRD3_1	37	NQLQYMQNVVVKTLWK...	HQFAWPFYQPVDAIKLNLPDYHKI	IKNPMDMGTIKRRLE	E
BRD4_1	61	NQLQYLLRVVLKTLWK...	HQFAWPFQQPVDAVKLNLPDYK	IKTPMDMGTIKRRLE	E
BRD2_2	347	SEQLKHCNGILKELLSSKHAAYAWPFYK	PVDASALGLHDYHDI	IKHPMDLSTVVRKME	E
BRD3_2	309	SEHLRYCDSILREMLSSKHAAYAWPFYK	PVDAEALGLHDYHDI	IKHPMDLSTVVRKMD	E
BRD4_2	351	SEQLKCCSGILKEMFAKKHAAYAWPFYK	PVDVEALGLHDYCDI	IKHPMDMSTIKSKLE	E
		<-	αZ	->	
				ZA loop	
				<-	αA ->
BRPF1	684	AYRYLNFDFFEDFNLI	VSNCILKYNKADTIF	YRAAVRLREQGAVLRQA	E
BRD1	618	AQGYKNLHEFEEDFDLI	IDNCMKYNARDTV	FYRAAVRLRDQGGVLRQA	E
BRPF3	645	SHLYRTLLEFEEDFNLI	VTNCMKYNKADTIF	HRAAVRLRDLGGAILRHA	E
BRD2_1	132	NNYYWAASECMQDFNT	MFTNCYIYNKPTDDI	VLMAQTLKIFLQKVASMPQEEQ	E
BRD3_1	92	NNYYWSASECMQDFNT	MFTNCYIYNKPTDDI	VLMAQALEKIFLQKVAQMPQEEV	E
BRD4_1	116	NNYYWNAQECIQDFNT	MFTNCYIYNKPGDDI	VLMAEALKFLQKINELPTEET	E
BRD2_2	405	NRDYRDAQEFADVRL	MFSNCKYNPPDHDV	VAMARKLQDVFEFRYAKMPDEPL	E
BRD3_2	367	GREYPDAQEGFADVRL	MFSNCKYNPPDHEV	VAMARKLQDVFEFRYAKMPDEPV	E
BRD4_2	409	AREYRDAQEFGADVRL	MFSNCKYNPPDHEV	VAMARKLQDVFEFRYAKMPDEPE	E
		<-	αB	->	
				αC	

Figure S2. ^{15}N - ^1H HSQC NMR Spectra of 80 μM BRPF1 bromodomain without ligand (red), and with A) fragment **1**, B) compound **2**, C) compound **3** shown in blue.

A)



B)



C)

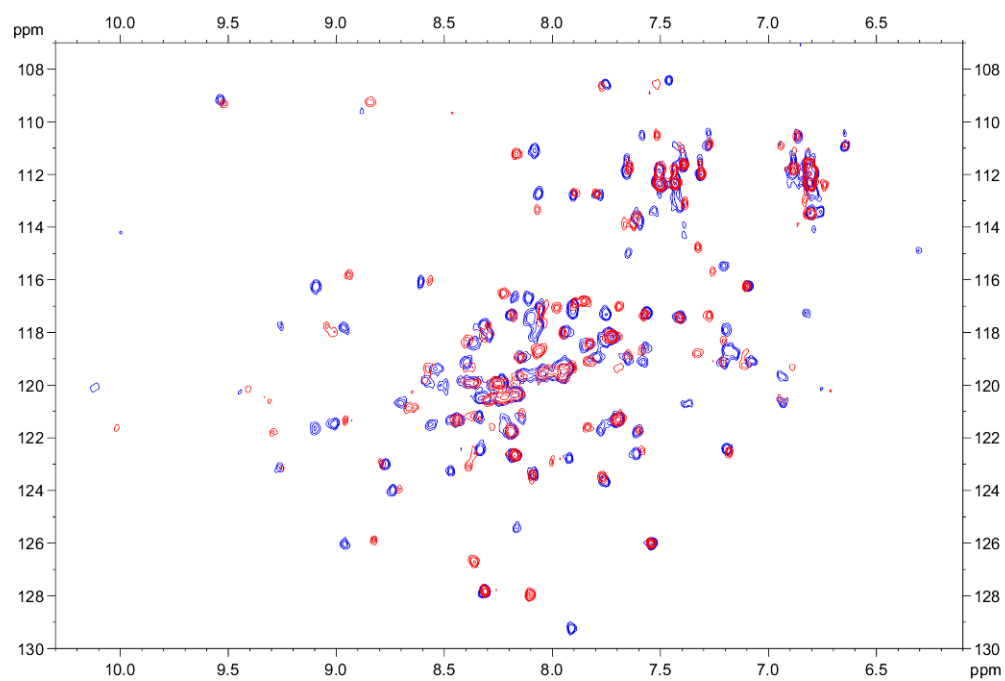


Figure S3. 2Fo-Fc and Fo-Fc omit maps for the ligands contoured at sigma levels indicated.

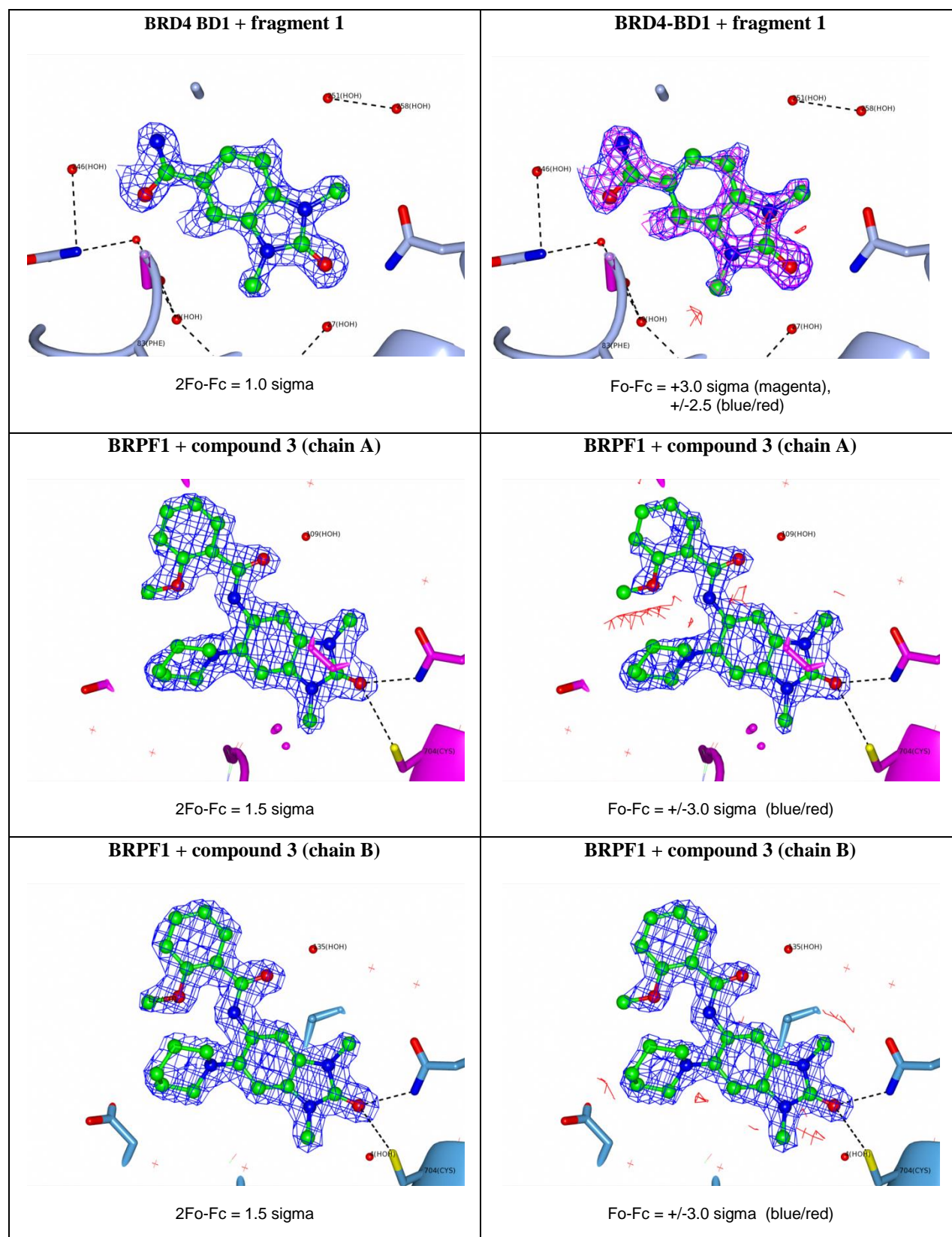


Figure S4. Data collection and refinement statistics for BRD4-BD1 and BRPF1 crystal structures. Each dataset was collected from a single crystal. Values in parentheses are for the highest resolution shell.

Databank deposition code	BRD4 BD1 with 1 4uyd	BRPF1 with 3 4uye
Crystallisation	20% PEG3350, 0.2M NaAc at 20°C	0.1 M MES/imidazole pH 6.5, 10% morpheus divalents MPD_P1K_P3350 at 4°C
Data collection		
Space Group	P 21 21 21	C2
Cell Dimensions		
a,b,c (Å)	37.420, 44.200, 78.260	89.060, 68.120, 58.750
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 126.13, 90.00
Resolution (Å)	1.37 (1.44)	1.65 (1.74)
R _{merge}	0.072 (0.281)	0.060 (0.349)
Average I/ σ I	15.7 (5.3)	10.4 (3.0)
Completeness (%)	98.7 (92.1)	95.7 (94.6)
Redundancy	6.1 (4.4)	3.3 (3.2)
No. Reflections	170004 (16431)	107705 (14826)
No. Unique Reflections	27651 (3695)	32628 (4687)
Wilson Bfactor	10.64	24.76
Refinement		
Resolution (Å)	39.13-1.37	49.47-1.65
R _{work} /R _{free}	0.174 / 0.188	0.176 / 0.201
No. Reflections	26265	30953
No. atoms	1377	2157
Protein	1079	1855
Ligand/ion	15/8	58/4
Water	275	240
B-factors		
Protein	11.51	26.02
Ligand/ion	15.72 / 24.96	22.14 / 47.04
Water	25.61	41.17
R.M.S. deviations		
Bond lengths (Å)	0.006	0.008
Bond angles (°)	1.144	1.183
Coordinate Errors		
ESR based on Free R	0.060	0.091
ESR based on Max Likelihood	0.035	0.057

Figure S5. Comparison of BRPF1 and BRPF2/BRD1 crystal structures. The BRPF1 X-ray complex with **3** is shown in blue. Contact between the C5-carbon of the benzimidazolone of **3** and the C δ atom of Pro658 is marked by a magenta dashed line. Chain A of the apo BRPF2 structure (PDB code 3rcw; Filippakopoulos et al. *Cell*, **2012**, 149, 214-231) is shown in orange. The equivalent of BRPF1 Pro658 in BRPF2 is Ser592, whose sidechain points away from the KAc site. Ser592 hydrogen-bonds to a water molecule through its backbone NH group (grey dashed line). The potentially unfavourable interaction between this water molecule and the benzimidazolone is shown as a yellow dashed line.

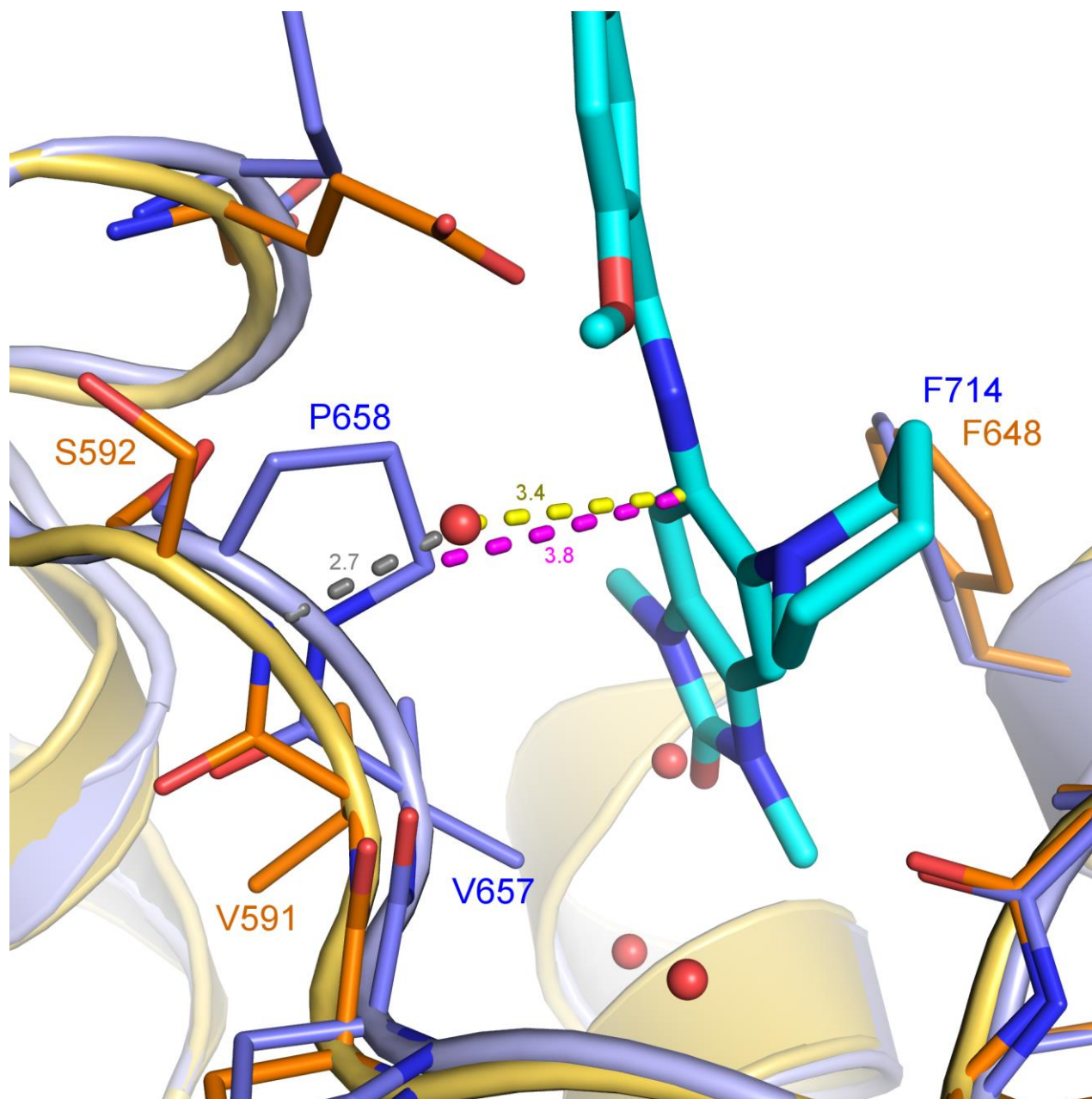


Figure S6. (A,B) Dose response curve NanoBRET experiments, showing inhibition of the NanoLuc-BRPF1 bromodomain interaction with Histone H3.3-HaloTag in HEK293 cells with A) compound **3**, as compared to B) the less active control, analogue **5**. A cellular IC_{50} value of $0.98\ \mu\text{M}$ was calculated for **3**. (C,D) NanoBRET experiments in HEK293 between the BRPF1 bromodomain isoform 2, which contains a 6 amino acid insert close to the acetyl-lysine site, and Histone H3.3. Dose response curves with either compound **3** (C) or **5** (D) showed no change in BRET signal, indicating that neither compound inhibits the interaction between BRPF1 bromodomain isoform 2 and Histone H3.3. See Supplementary Methods, p19.

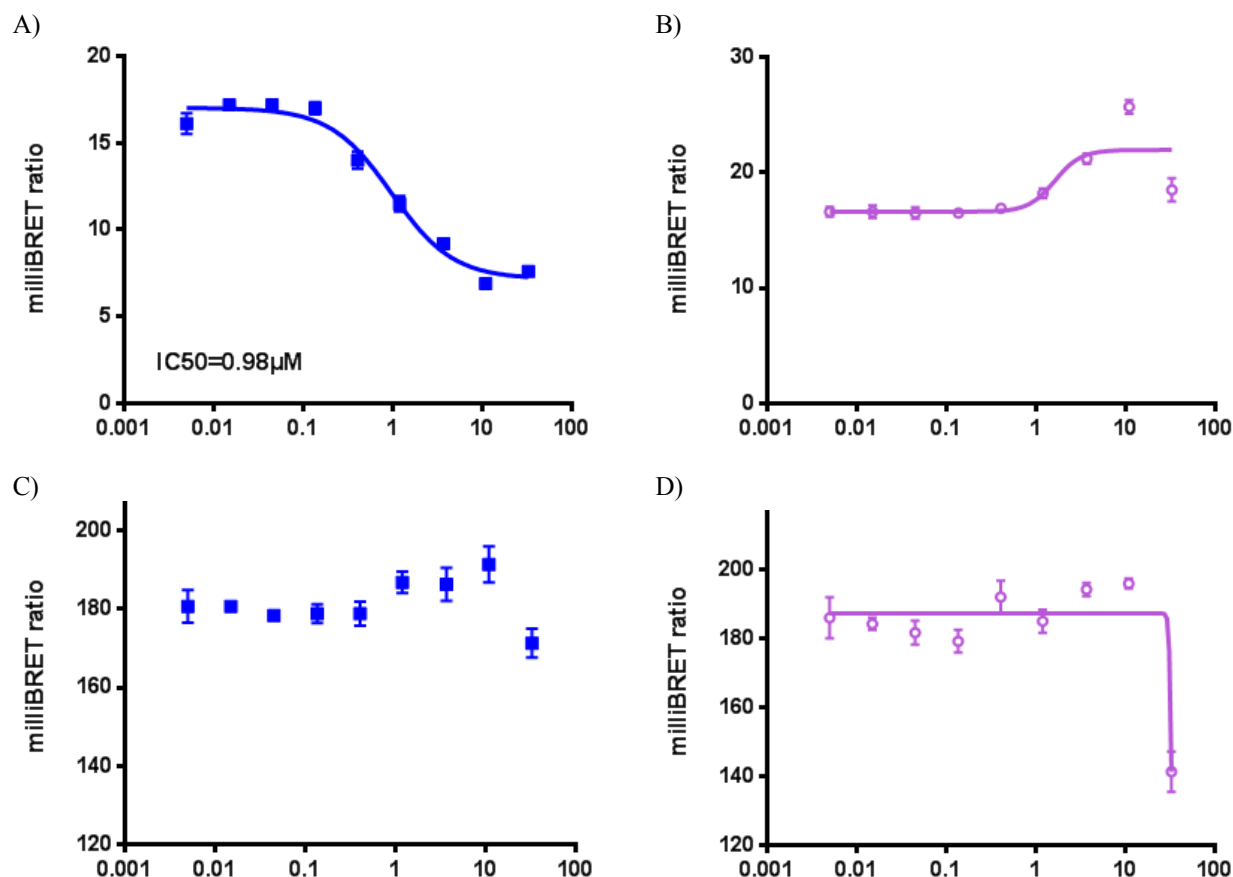
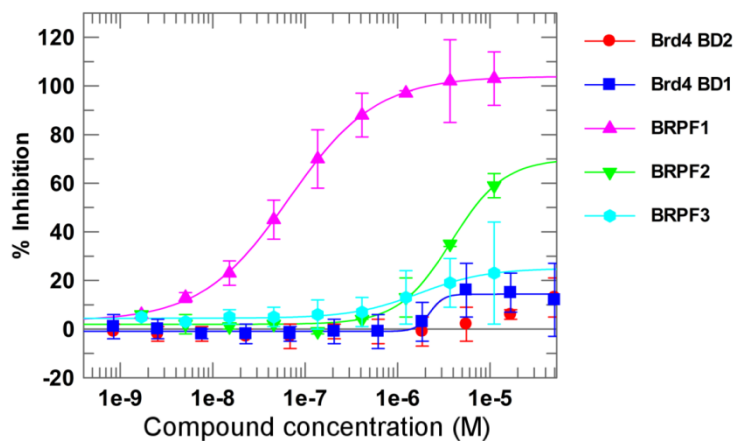


Table S1. A) TR-FRET pIC_{50} data for BRPF1-3 and BRD4 BD1 (N-terminal) and BD2 (C-terminal) bromodomains, including mean, standard deviation and number of test occasions. B) Dose-response replicates for compound **3** against BRPF1-3 and BRD4 BD1 and BD2. C) BRD4 BD1 dose-response curves for compounds **3**, **14**, **15** and **17**.

A)

ID	BRPF1 pIC_{50}	BRPF1 IC_{50} (μ M)	BRPF2 pIC_{50}	BRPF3 pIC_{50}	BRD4 BD1 pIC_{50}	BRD4 BD2 pIC_{50}
1	5.0 \pm 0.13 (6)	9.4	4.2 \pm 0.08 (2)	3.3 \pm 0.11 (2)	4.1 (2)	3.6 (2)
2	6.7 \pm 0.15 (6)	0.22	5.4 \pm 0.11 (2)	4.8 \pm 0.01 (2)	5.6 \pm 0.21 (6)	4.6 (2) and < 4.3 (4)
3	7.1 \pm 0.2 (5)	0.08	5.1 \pm 0.25 (2)	< 4.0 (2)	< 4.3 (9)	< 4.3 (9)
4	6.2 \pm 0.21 (3)	0.64	< 4.0 (2)	< 4.0 (2)	< 4.3 (3)	< 4.3 (3)
5	4.7 \pm 0.18 (3)	18	< 4.0 (2)	< 4.0 (2)	5.8 \pm 0.02 (3)	5.0 \pm 0.05 (3)
6	7.0 \pm 0.26 (4)	0.10	< 4.0 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
7	7.0 \pm 0.21 (5)	0.10	< 4.0 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
8	6.5 \pm 0.08 (4)	0.34	5.3 (2)	4.7 \pm 0.1 (2)	< 4.3 (1)	4.5 (1)
9	6.2 \pm 0.15 (4)	0.58	4.6 \pm 0.13 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
10	6.4 \pm 0.3 (3)	0.36	4.8 \pm 0.02 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
11	7.3 \pm 0.13 (5)	0.045	5.6 \pm 0.01 (2)	4.6 \pm 0.01 (2)	4.7 (1) and < 4.3 (1)	< 4.3 (2)
12	6.5 \pm 0.12 (5)	0.32	5.5 \pm 0.02 (2)	4.6 \pm 0.01 (2)	< 4.3 (1)	< 4.3 (1)
13	6.1 \pm 0.07 (3)	0.72	4.8 \pm 0.08 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
14	6.9 \pm 0.26 (4)	0.11	5.3 \pm 0.02 (2)	< 4.0 (2)	< 4.3 (3)	< 4.3 (3)
15	6.9 \pm 0.2 (7)	0.12	5.1 (1) and < 5.0 (1)	< 4.0 (2)	4.9 (1) and < 4.3 (1)	< 4.3 (2)
16	5.8 \pm 0.16 (6)	1.50	4.8 \pm 0.01 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
17	5.6 \pm 0.11 (4)	2.70	4.6 \pm 0.15 (2)	< 4.0 (2)	< 4.3 (1)	4.3 (1)
18	6.2 \pm 0.08 (4)	0.65	< 4.0 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
19	5.5 \pm 0.08 (3)	3.40	4.8 \pm 0.07 (2)	< 4.0 (2)	< 4.3 (1)	< 4.3 (1)
20	5.5 \pm 0.09 (4)	3.30	4.4 \pm 0.04 (2)	< 4.0 (2)	4.8 (1)	4.4 (1)

B)



C)

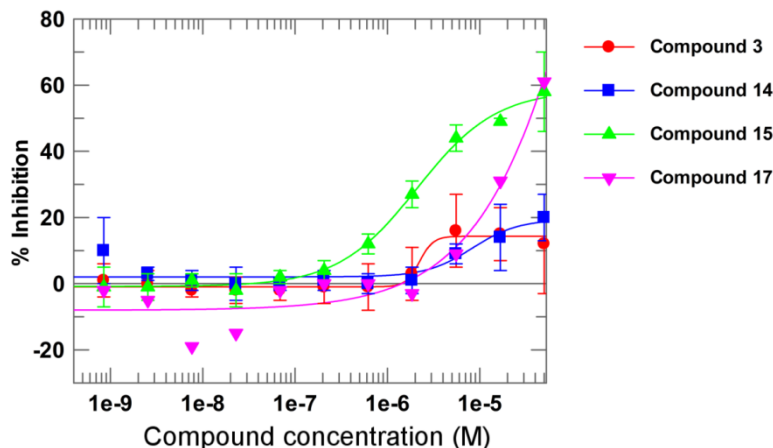
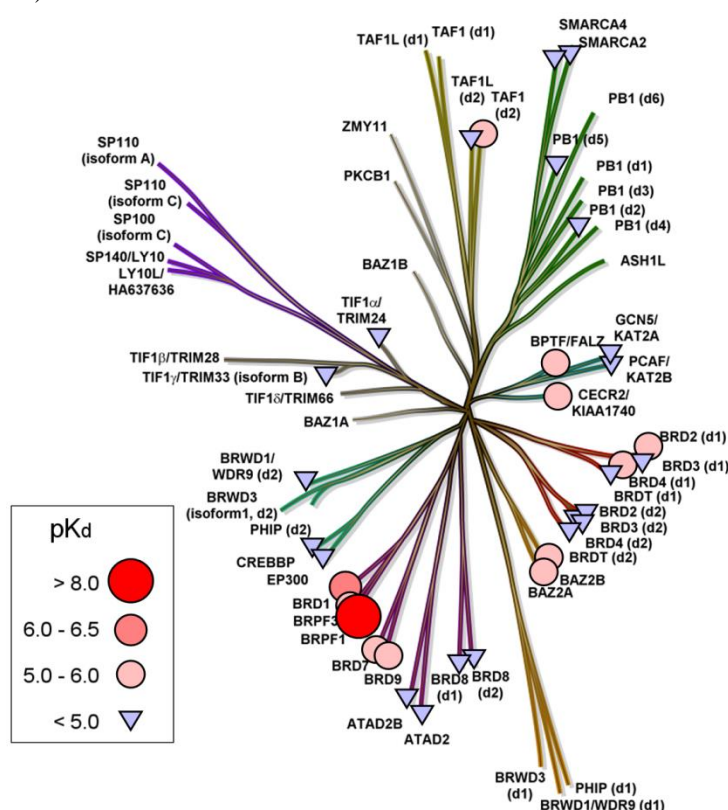


Table S2. A) Bromodomain selectivity of **3** in the BROMOscan™ panel (DiscoverRx Corp., Fremont, CA, USA, <http://www.discoverrx.com>). This screen measures competition against reference immobilized ligands for 35 DNA-tagged bromodomains. Results are mean, n=2. B) Graphical representation of data in A. C) Duplicate BRPF1 dose-response curves for compound **3**.

A)

Bromodomain	pK _d
ATAD2	< 5.00
ATAD2B	< 5.00
BAZ2A	= 5.07
BAZ2B	= 5.75
BRD2 (BD1)	= 5.10
BRD2 (BD2)	< 5.00
BRD3 (BD1)	< 5.00
BRD3 (BD2)	< 5.00
BRD4 (BD1)	= 5.25
BRD4 (BD2)	< 5.00
BRD7	= 5.51
BRD8 (BD1)	< 5.00
BRD8 (BD2)	< 5.00
BRD9	= 5.80
BRDT (BD1)	< 5.00
BRDT (BD2)	< 5.00
BRPF1	= 8.02
BRPF2 / BRD1	= 6.06
BRPF3	= 5.51
CECR2	= 5.62
CREBBP	< 5.00
EP300	< 5.00
FALZ / BPTF	= 5.18
GCN5L2	< 5.00
PBRM1 (BD2)	< 5.00
PBRM1 (BD5)	< 5.00
PCAF	< 5.00
SMARCA2	< 5.00
SMARCA4	< 5.00
TAF1 (BD2)	= 5.70
TAF1L (BD2)	< 5.00
TIF1α / TRIM24 (bromodomain)	< 5.00
TIF1α / TRIM24 (PHD/bromodomain)	< 5.00
TIF1γ / TRIM33 (PHD/bromodomain)	< 5.00
WDR9 (2)	< 5.00

B)



C)

