

Fragment-based discovery of bromodomain inhibitors

part 2: optimization of phenylisoxazole sulfonamides

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Supplementary Methods

X-ray crystallography. E.coli expressed His6-tagged BRD2-BD1(67-200) was purified to homogeneity using a HisTrap column followed by gel filtration using a Sephadex S200. Typically the protein was concentrated to ~10mg/ml in 20mM HEPES pH7.5, 100mM NaCl. Apo crystals were grown in 100nl+100nl sitting drops using MRC or in 1ul+1ul hanging drops in Nextal plates at 20°C using 20-30% PEG3350, 0.1-0.3M (NH₄)₂SO₄, 100mM HEPES pH 7.0-8.5. Apo crystals were soaked with compound concentration ranging from 1mM to saturating concentrations, in 0-5% DMSO, between 1hr to overnight. Crystals were briefly transferred to a cryoprotectant buffer consisting of the well solution plus 20% ethylglycol prior to flash freezing. Data from single frozen crystals were collected at the European Synchrotron Radiation Facility, Grenoble or on an in house RIGAKU FRE+ SUPERBRIGHT/A200 system. Data processing was achieved using MOSFLM(14), HKL(15), or d*TREK(16) and scaled using SCALA(14) or SCALEPACK. The C2 cell has 3 molecules in the ASU and after rigid body refinement using a previously determined in house structure, model building was performed Coot(17) and refined using REFMAC(18). There was clear difference density for the ligands in at least one of the three acetyl-lysine binding sites and the binding modes could be unambiguously determined. The statistics for the data collection and refined co-ordinates are given in Supporting Information Fig. S1..

Peripheral blood mononuclear cell (PBMC) preparation. Blood containing 15% v/v ACD-A anticoagulant (Baxter Healthcare) was collected and 30ml poured into 50ml Leucosep tubes (Greiner Bio-one) pre-filled with Ficoll. The tubes were centrifuged for 15 minutes at 800 x g (r.t.). The enriched cell fractions from each Leucosep tube were pooled in a 500ml centrifuge tube. The pooled fractions were centrifuged at room temperature for 10 minutes at 400 x g, and the supernatant discarded. The cell pellet was resuspended in 40ml PBS and the cells counted on a haematology analyser (Sysmex). The cells were centrifuged at 300 x g for 10 minutes (r.t.), the supernatant discarded, and the cell pellet resuspended in freezing medium (90% heat-inactivated FBS, 10% DMSO) to make a final concentration of 4x10⁷ cells/ml. The cells were aliquoted and cryopreserved. Compound plates containing 0.5µl test sample in 1% DMSO were prepared (two replicates on account of PBMC donor variability). PBMCs were thawed from frozen stocks and resuspended in warm media (500ml DMEM, 50ml Heat Inactivated Australian Foetal Bovine Serum, 5ml Penicillin/Streptomycin, 5ml Glutamax) at a concentration of 2x10⁶ cells/ml. 1ng/ml lipopolysaccharide was added then 50µl added to each well of the compound plates. The seeded plates were then lidged and placed in the humidified primary cell incubator for 18-24 hours at 37°C, 5% CO₂.

Inhibition of IL-6 production. 10µl of cell supernatant was placed in a low-profile 384-well MSD plate pre-coated with human IL-6 capture antibody. The plates were sealed and placed on a shaker at 600rpm for 2 hours (r.t.). A 1% solution of MSD Blocker A (Mesoscale Discovery, USA) was prepared by dissolving solid Blocker A in Phosphate buffered saline (Invitrogen, UK). This was used to dilute the anti-human IL-6 antibody labelled with MSD SULFO-TAGTM reagent to 1µg/ml. 10µl of the antibody solution was then added to each well of the MSD plates. The plates were then re-sealed and shaken for another 2 hours before washing with PBS. 35µl of 2X MSD Read Buffer T (stock 4X MSD Read Buffer T was diluted 50:50 with de-ionised water) was then added to each well and the plates read on the MSD Sector Imager 6000.

Inhibition of TNFα production. 5µl of cell supernatant was placed in a white low volume Greiner 384-well plate. 2µl of Acceptor Mix Solution (0.7µg/ml biotinylated anti-human TNFα antibody (BAF210), 11.7µg/ml conjugated AlphaLISA acceptor beads, all in AlphaScreen buffer (30mM Tris pH7.4, 0.02% Tween 20, 0.02% Casein)) was added and the plates incubated for 90 minutes in the dark (r.t.). 2µl of Streptavidin donor bead solution (180µg/ml of Streptavidin donor beads in AlphaScreen buffer) was added followed by 90 min incubation in the dark (r.t.). Plates were then read on an EnVision spectrofluorometer (Perkin Elmer, UK) at an excitation wavelength of 680nm and an emission wavelength of 615nm.

Chemistry. All solvents were purchased from Fisher Scientific Ltd 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. The purity of all compounds was determined by LCMS and ¹H NMR. NMR spectra were recorded on a Bruker AVANCE 400 spectrometer or a Bruker Ultrashield 400 MHz instrument, both referenced as follows: ¹H (400 MHz), internal standard TMS at δ = 0.00; ¹³C (100.6 MHz), internal standard CDCl₃ at δ = 77.23 or DMSO-d₆ at δ = 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 a Waters ZQ (ESI-MS) spectrometer. All microwave reactions were carried out in a Biotage Initiator 60 focussed beam microwave (400 W). 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.

LCMS methodology

a) 2-minute method – formate. 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 (HPLC grade), (B) 0.1% v/v solution of formic acid in acetonitrile (HPLC grade). 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 a 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

b) 25 minute method – TFA. The HPLC analysis was conducted on an X-Bridge C18 250 mm X 4.6 mm, 5 µm packing diameter). The solvents employed were (A) 0.1% v/v TFA in water (HPLC grade), (B) 0.1% v/v TFA in acetonitrile (HPLC grade). The gradient employed was:

Time (min)	Flow rate (mL/min)	%A	%B
0.01	1	90	10
9.00	1	10	90
11.00	1	0	100
20.00	1	0	100
20.01	1	90	10
25.00	1	90	10

UV detection was at a fixed wavelength of 267 nm.

Procedures: see Scheme 1.

a) For R₁=Me, a mixture of 4-iodo-3,5-dimethylisoxazole (1.0 g, 4.48 mmol), 4-tolylboronic acid (0.9 g, 6.73 mmol) and aqueous sodium carbonate solution (2M) (0.73 mL) in 1,2-dimethoxyethane (DME) was degassed and Pd(PPh₃)₄ (0.26 g, 0.22 mmol) was added. The reaction mixture was stirred at 85 °C for 2 h before being cooled to room temperature and concentrated. The residue was diluted with water and extracted with ethyl acetate (3 x 35 mL). The combined organic extracts were washed with brine (100 mL), dried (Na₂SO₄), filtered and concentrated. The residue was purified by flash column chromatography (silica gel, 0-10% ethyl acetate / hexanes) to yield 3,5-dimethyl-4-(4-methylphenyl)isoxazole **S1** (0.61 g, 3.26 mmol, 73%) as an orange solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.27 (m, 2H), 7.20 (m, 2H), 2.38 (s, 6H), 2.22 (s, 3H); LCMS (2 minute method): (ES⁺) 188, retention time 1.14 min., >99% pure.

For R₁=OMe, 4-methoxyphenylboronic acid (3.41 g, 22.42 mmol), 4-iodo-3,5-dimethylisoxazole (1.00 g, 4.48 mmol), Pd(PPh₃)₄ (104 mg, 0.09 mmol) and sodium carbonate (3.09 g, 29.10 mmol) as a suspension in 1,2-dimethoxyethane (DME) (100 mL) and water (15 mL) were stirred and heated at 85 °C for 3.5 days. After cooling, the reaction mixture was concentrated, water (100 mL) was added and the product extracted using ethyl acetate (3 x 100 mL). The combined organics were washed with brine (70 mL), dried and concentrated to yield a bright yellow residue. Purification by flash column chromatography (silica gel, 0-100% ethyl acetate/cyclohexane) afforded 3,5-dimethyl-4-[4-(methoxy) phenyl]isoxazole **S1** (810 mg, 3.99 mmol, 89%) as a white solid; ¹H NMR (400 MHz, DMSO-d₆) δ 7.31 (m, 2H), 6.03 (m, 2H), 3.79 (s, 3H), 2.37 (s, 3H), 2.20 (s, 3H); LCMS (2 minute method): (ES⁺) 204 [MH⁺], retention time 1.03 min., >96% pure.

b) For R₁=Me, to a solution of 3,5-dimethyl-4-(4-methylphenyl)isoxazole **S1** (0.61g, 3.26 mmol) in dichloromethane (DCM) was added chlorosulfonic acid (2.0 mL, 3.89 mmol) and PCl₅ (0.68 g, 3.26 mmol) at 0°C under N₂. After stirring at 0°C for 15 min., the reaction mixture was heated to 70°C for 4 h. The mixture was allowed to cool and then poured onto crushed ice/water with stirring. The resulting suspension was filtered through celite and the filtrate was extracted with DCM (2 x 75 mL). The combined organic extracts were washed with brine (70 mL), dried (Na₂SO₄), filtered and concentrated to afford 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (630mg, 68%) as a brown oil; ¹H NMR (400 MHz, DMSO-d₆) δ 7.67 (d, J = 2.0 Hz, 1H), 7.23 (m, 2H), 2.55 (s, 3H), 2.36 (s, 3H), 2.18 (s, 3H); LCMS (2 minute method): (ES⁺) No clear peak for product, retention time 1.23 min., >93% pure.

For R₁=OMe, 3,5-dimethyl-4-[4-(methoxy)phenyl]isoxazole **S1** (560 mg, 2.76 mmol) was dissolved in dichloromethane (DCM) (10 mL). Chlorosulfonic acid (183 µL, 2.76 mmol) was added dropwise to the reaction mixture at 0 °C. The reaction mixture was stirred under N₂ at room temperature overnight. Further chlorosulfonic acid (367 µL, 5.52 mmol) was added at 0 °C and the reaction mixture stirred for a further 4 h. The reaction mixture was poured onto ice (15 mL) before being cooled to 0 °C. The white solid which precipitated out was collected by filtration and dried under vacuum. The residue and phosphorus pentachloride (487 mg, 2.340 mmol) were dissolved in phosphoryl chloride (3 mL, 31.8 mmol) and the resulting solution

stirred under N₂ at 90 °C from for 4.5 h. Ice/water (6 eq, 190 g, 190 mmol) was added and a grey precipitate formed. More ice was added to the solution (~6 mL) and the precipitate was collected by filtration and dried to afford 5-(3,5-dimethylisoxazol-4-yl)-2-methoxybenzene-1-sulfonyl chloride **S2** (216 mg, 0.64 mmol, 41%) as an orange oil; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.86 (d, *J* = 2.5 Hz, 1H), 7.77 (dd, *J* = 8.5 and 2.5 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 4.10 (s, 3H), 2.41 (s, 3H), 2.25 (s, 3H); LCMS (2 minute method): (ES+) 302 [MH⁺], retention time 1.08 min., >90% pure.

c) *General procedure (A)* for formation of sulfonamides: to a solution of the amine (3 eq.) in ethyl acetate (100 mL/8 mmol) was added the sulfonyl chloride (1 eq.) under N₂. The reaction mixture was stirred overnight and then diluted with ethyl acetate. The mixture was washed with aqueous HCl (0.5 M) and brine before being dried (Na₂SO₄), filtered and concentrated to give a residue which was purified by flash column chromatography on silica gel to give the desired sulfonamide.

General procedure (B) for formation of sulfonamides: the amine (2.5 eq.) and the sulfonyl chloride (1 eq.) in DCM (15 mL/mmole) were treated with DIPEA (1.5 eq.). The reaction mixture was then stirred for 2.5 h. The reaction mixture was washed with water (15 mL/mmole), dried and concentrated to yield the crude sulfonamide product. Purification by flash column chromatography on silica gel yielded the desired sulfonamide.

General procedure (C) for formation of sulfonamides: the amine (2.5 eq.) and the sulfonyl chloride (1 eq.) in DCM (15 mL/mmole) were treated with DIPEA (1.5 eq.). The reaction mixture was then stirred for 2.5 h. The reaction mixture was applied directly to a silica column. Purification by flash column chromatography yielded the desired sulfonamide.

General procedure (D) for Boc-deprotection: the Boc-protected amine (1 eq.) was dissolved in dichloromethane (DCM) (1 mL). Trifluoroacetic acid (3 mL/mmole) was added and the mixture stirred at room temperature for 1 h. The crude products were applied to SCX SPE columns and washed with MeOH (2CV) followed by 2M NH₃ in MeOH (4CV). The basic fractions were concentrated and dried. The residue was dissolved in methanol (15 mL/mmole) and HCl (1.25 M in methanol) (1 eq.) was added. The solution was concentrated by flow of nitrogen gas to leave the desired sulfonamide amine as the hydrochloride salt.

Following general procedure (A), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (200 mg, 0.70 mmol) and 2-methoxyethylamine (158 mg, 2.1 mmol) afforded 5-(3,5-dimethylisoxazol-4-yl)-N-(2-methoxyethyl)-2-methylbenzenesulfonamide **18a** (58 mg, 0.18 mmol, 26%); ¹H NMR (400 MHz, DMSO-d₆) δ 7.92 (m, 1H), 7.74 (s, 1H), 7.52 (m, 2H), 3.27 (t, *J* = 5.5 Hz, 2H), 3.10 (s, 3H), 3.00 (m, 2H), 2.61 (s, 3H), 2.41 (s, 3H), 2.23 (s, 3H); LCMS (25 minute method): (ES+) 325, retention time 9.70 min., >99% pure.

Combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (150 mg, 0.52 mmol) and 3-amino-1-propanol (119 mg, 1.57 mmol) by general procedure (A) afforded 5-(3,5-dimethylisoxazol-4-yl)-N-(3-hydroxypropyl)-2-methylbenzenesulfonamide **19a** (88 mg, 0.27 mmol, 51%); ¹H NMR (400 MHz, DMSO-d₆) δ 7.72 (m, 2H), 7.56 (dd, *J* = 8.0 and 2.0 Hz, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 4.42 (br, s, 1H), 2.88 (m, 2H), 2.60 (s, 3H), 2.52 (m, 2H), 2.41 (s, 3H), 2.23 (s, 3H), 1.51 (m, 2H); LCMS (25 minute method): (ES+) 325, retention time 9.18 min., >99% pure.

Following general procedure (A), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (150 mg, 0.52 mmol) and 0.88 S.G. ammonium hydroxide (27 mg, 1.57 mmol) afforded 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzenesulfonamide **21a** (44 mg, 0.16 mmol, 31%); ¹H NMR (400 MHz, DMSO-d₆) δ 7.78 (m, 1H), 7.50 (m, 4H), 2.62 (s, 3H), 2.42 (s, 3H), 2.23 (s, 3H); LCMS (25 minute method): (ES+) 266, retention time 7.76 min., >99% pure.

Following general procedure (C), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (39 mg, 0.14 mmol) and 1-Boc-piperazine (63 mg, 0.34 mmol) followed by flash column chromatography (silica gel, 7-35% ethyl acetate/cyclohexane) afforded *tert*-butyl 4-((5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)sulfonyl)piperazine-1-carboxylate. Deprotection following general procedure (D) afforded 3,5-dimethyl-4-(4-methyl-3-(piperazin-1-yl)sulfonyl)phenylisoxazole hydrochloride, **27a** (26 mg, 0.07 mmol, 66%) as a white solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.83 (s, 1H), 7.56 (m, 2H), 3.33 (m, 4H), 3.11 (m, 4H), 2.67 (s, 3H), 2.43 (s, 3H), 2.27 (s, 3H); LCMS (2 minute method): (ES+) 336 (MH⁺), retention time 0.65 min., >98% pure.

Following general procedure (C), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (39 mg, 0.14 mmol) and 4-amino-1-Boc-piperidine (68 mg, 0.34 mmol) followed by flash column chromatography (silica gel, 9-45% ethyl acetate/cyclohexane) afforded *tert*-butyl 4-(5-(3,5-dimethylisoxazol-4-yl)-2-methylphenyl)sulfonyl)piperidine-1-carboxylate. General procedure (D) deprotection gave 5-(3,5-dimethylisoxazol-4-yl)-2-methyl-N-(piperidin-4-yl)benzene sulfonamide hydrochloride **29a** (27 mg, 0.07 mmol, 66%) as a white solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.90 (s, 1H), 7.50 (s, 2H), 3.26 (m, 1H), 3.14 (m, 2H), 2.76 (m, 2H), 2.69 (s, 3H), 2.42 (s, 3H), 2.26 (s, 3H), 1.82 (m, 2H), 1.58 (m, 2H); LCMS (2 minute method): (ES+) 350 (MH⁺), retention time 0.61 min., >98% pure.

Following general procedure (C), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methylbenzene-1-sulfonyl chloride **S2** (39 mg, 0.14 mmol) and 3-amino-1-Boc-piperidine (68 mg, 0.34 mmol) followed by flash column chromatography (silica gel, 9-45% ethyl acetate/cyclohexane) afforded *tert*-butyl 3-(5-(3,5-dimethylisoxazol-4-

yl)-2-methylphenyl)sulfonyl)piperidine-1-carboxylate. Deprotection following general procedure (D) afforded 5-(3,5-dimethylisoxazol-4-yl)-2-methyl-N-(piperidin-3-yl)benzenesulfonamide hydrochloride **30a** (26 mg, 0.06 mmol, 63%) as a white solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.89 (s, 1H), 7.51 (s, 1H), 3.34 (s, 1H), 3.30 (m, 1H), 3.21 (m, 1H), 3.08 (m, 1H), 2.71 (m, 5H), 2.42 (s, 3H), 2.26 (s, 3H), 1.81 (m, 2H), 1.52 (m, 2H); LCMS (2 minute method): (ES+) 350 (MH⁺), retention time 0.63 min., >98% pure.

Following general procedure (B), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methoxybenzene-1-sulfonyl chloride **S2** (36 mg, 0.12 mmol) and 1-Boc-piperazine (56 mg, 0.30 mmol) followed by flash column chromatography (silica gel, 15-75% ethyl acetate/cyclohexane) afforded *tert*-butyl 4-((5-(3,5-dimethylisoxazol-4-yl)-2-methoxyphenyl)sulfonyl)piperazine-1-carboxylate. Deprotection following general procedure (D) afforded 4-(4-methoxy-3-(piperazin-1-yl)sulfonyl)phenyl-3,5-dimethylisoxazole hydrochloride **27b** (18 mg, 0.04 mmol, 64%) as a white solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.79 (d, *J* = 2.0 Hz, 1H), 7.63 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 4.02 (s, 3H), 3.48 (m, 4H), 3.21 (m, 4H), 2.41 (s, 3H), 2.24 (s, 3H); LCMS (2 minute method): (ES+) 352 (MH⁺), retention time 0.59 min., >98% pure.

Following general procedure (B), combination of 5-(3,5-dimethylisoxazol-4-yl)-2-methoxybenzene-1-sulfonyl chloride **S2** (36 mg, 0.12 mmol) and 4-amino-1-Boc-piperidine (60 mg, 0.30 mmol) followed by flash column chromatography (silica gel, 15-75% ethyl acetate/cyclohexane) afforded *tert*-butyl 4-(5-(3,5-dimethylisoxazol-4-yl)-2-methoxyphenyl)sulfonyl)piperidine-1-carboxylate. Deprotection following general procedure (D) afforded 5-(3,5-dimethylisoxazol-4-yl)-2-methoxy-N-(piperidin-4-yl)benzenesulfonamide hydrochloride **29b** (17 mg, 0.04 mmol, 59%) as a white solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.79 (d, *J* = 2.5 Hz, 1H), 7.59 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.32 (d, *J* = 8.5 Hz, 1H), 4.03 (s, 3H), 3.30 (m, 2H), 3.14 (m, 2H), 2.74 (m, 2H), 2.40 (s, 3H), 2.24 (s, 3H), 1.81 (dd, *J* = 3.5, 13.5 Hz, 2H), 1.57 (m, 2H); LCMS (2 minute method): (ES+) 366 (MH⁺), retention time 0.56 min., >99% pure.

Combining 5-(3,5-dimethylisoxazol-4-yl)-2-methoxy benzene-1-sulfonyl chloride **S2** (36 mg, 0.12 mmol) and 3-amino-1-Boc-piperidine (60 mg, 0.30 mmol) by general procedure (B) followed by flash column chromatography (silica gel, 11-55% ethyl acetate/cyclohexane) afforded *tert*-butyl 3-(5-(3,5-dimethylisoxazol-4-yl)-2-methoxyphenyl)sulfonyl)piperidine-1-carboxylate. Deprotection by general procedure (D) afforded (*rac*)-5-(3,5-dimethylisoxazol-4-yl)-2-methoxy-N-(piperidin-3-yl) benzene sulfonamide hydrochloride **30b** (21 mg, 0.05 mmol, 68%) as white solid; ¹H NMR (400 MHz, METHANOL-d₄) δ 7.80 (d, *J* = 2.5 Hz, 1H), 7.61 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 1H), 4.04 (s, 3H), 3.43 (m, 1H), 3.21 (m, 1H), 3.25 (m, 1H), 3.15 (m, 1H), 2.86 (m, 2H), 2.43 (m, 3H), 2.25 (s, 3H), 1.85 (m, 2H), 1.55 (m, 2H); LCMS (2 minute method): (ES+) 366 (MH⁺), retention time 0.56 min., >93% pure.

d & e) For R₁=OMe, cyclopropylamine (1.21 mL, 17.5 mmol), 5-bromo-2-methoxy benzene sulfonyl chloride (2.00 g, 7.0 mmol) and DIPEA (1.84 mL, 10.5 mmol) were combined as a solution in DCM (50 mL) and stirred at room temperature under N₂ overnight. The reaction mixture was washed with water (50 mL), dried and concentrated *in vacuo* to yield impure desired product. Purification by flash column chromatography (silica gel, 7-100% ethyl acetate/cyclohexane) yielded pure 5-bromo-N-cyclopropyl-2-(methoxy)benzenesulfonamide **S3**, **S3** (1.94 g, 6.3 mmol), Pd(dppf)Cl₂ (463 mg, 0.63 mmol) and potassium carbonate (1.75 g, 12.7 mmol) were suspended in 1,4-dioxane (14 mL) and water (3.5 mL). The reaction mixture was heated at 100 °C for 30 min. in a microwave reactor. The reaction mixture was then heated for a further 15 min. at 100 °C in the microwave. The mixture was concentrated and ethyl acetate added (100 mL). The organics were washed with aqueous sodium carbonate solution (100 mL), dried and concentrated to yield a brown residue. Purification by flash column chromatography (silica gel, 12 – 60% ethyl acetate / cyclohexane) yielded N-cyclopropyl-5-(3,5-dimethyl-4-isoxazolyl)-2-(methoxy) benzenesulfonamide **5b** (1.38 g, 4.07 mmol, 64%) as a yellow solid; ¹H NMR (400 MHz, CHLOROFORM-d) δ 7.88 (d, *J* = 2.5 Hz, 1H), 7.45 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.14 (d, *J* = 8.5 Hz, 1H), 5.31 (s, 1H), 4.05 (s, 3H), 2.41 (s, 3H), 2.27 (s, 3H), 2.16 (m, 1H), 0.73 (m, 2H), 0.58 (m, 2H); LCMS (2 minute method): (ES+) 323, retention time 0.90 min., 97% pure.

d & e) For R₁=OMe, to a cold solution (ice bath) of 5-bromo-2-(methoxy)benzenesulfonyl chloride (2.9 g, 10.16 mmol) and DIPEA (4.43 mL, 25.4 mmol) in dichloromethane (DCM) (40 mL) stirred under nitrogen was added cyclopentyl amine (1.30 g, 15.23 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was filtered through a hydrophobic frit. The filtrate was washed with 1N HCl (50mL). The organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0-40% ethyl acetate/cyclohexane) to give 5-bromo-N-cyclopentyl-2-methoxybenzenesulfonamide **S3**. To a solution of **S3** (3.16 g, 9.45 mmol) in 1,4-dioxane (10 mL) was added 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (2.53 g, 11.35 mmol), Pd(dppf)Cl₂ (0.69 g, 0.95 mmol) and Cs₂CO₃ (9.45 g, 18.91 mmol). The mixture was degassed with nitrogen for 30 min. and then stirred at 100 °C for 1 h. The reaction mixture was filtered. The filtrate was concentrated under reduced pressure. The residue was partitioned between dichloromethane (50 mL) and water (50 mL). The layers were separated and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0-50% ethyl acetate/cyclohexane) to give N-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-methoxybenzenesulfonamide **6b** (2.85 g,

8.13 mmol, 86%) as a yellow solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.62 (m, 2H), 7.36 (d, *J* = 7.5 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 3.94 (s, 3H), 3.46 (m, 1H), 2.38 (s, 3H), 2.20 (s, 3H), 1.55 (br. s, 4H), 1.35 (br. s, 4H); LCMS (2 minute method): (ES+) 351, retention time 1.05 min., >90% pure.

d & e) For R₁=H, cyclopropylamine (136 μL, 1.96 mmol), 3-bromobenzenesulfonyl chloride (200 mg, 0.78 mmol) and DIPEA (205 μL, 1.17 mmol) as a solution in DCM (5 mL) were stirred at room temperature under N₂ overnight. The reaction mixture was washed with water (5 mL), dried and concentrated *in vacuo* to obtain 3-bromo-*N*-cyclopropylbenzenesulfonamide **S3** (200 mg, 0.72 mmol), Pd(dppf)Cl₂ (53 mg, 0.072 mmol) and potassium carbonate (200 mg, 1.45 mmol) were suspended in 1,4-dioxane (2.0 mL) and water (0.5 mL). The reaction mixture was heated in a microwave reactor at 100 °C for 30 min. The reaction mixture was concentrated and partitioned between ethyl acetate (12 mL) and aqueous sodium carbonate solution (12 mL). The aqueous layer was extracted with more ethyl acetate (12 mL). The organic extracts were combined, dried and concentrated to yield a brown residue. Purification by flash column chromatography (silica gel, 7-35% ethyl acetate/cyclohexane) yielded *N*-cyclopropyl-3-(3,5-dimethyl-4-isoxazolyl) benzenesulfonamide (147 mg, 0.48 mmol) as an orange oil. This was dissolved in methanol (1 mL) and HCl (1.25 M in methanol) (382 μL, 0.48 mmol) was added. The mixture was concentrated by flow of N₂ gas leaving the HCl salt of *N*-cyclopropyl-3-(3,5-dimethyl-4-isoxazolyl) benzenesulfonamide **31** (124 mg, 0.36 mmol, 50%) as an orange solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.03 (d, *J* = 2.8 Hz, 1H), 7.83 (m, 1H), 7.73 (m, 3H), 2.43 (s, 3H), 2.25 (s, 3H), 2.14 (m, 1H), 0.51 (m, 2H), 0.40 (m, 2H); LCMS (2 minute method): (ES+) 293, retention time 0.96 min., 98% pure.

f) To a stirred solution of *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-methoxy benzenesulfonamide **6b** (146 mg, 0.417 mmol) in dry dichloromethane (DCM) (8.3 mL) at 0 °C was added dropwise a solution of BBr₃ (1M in DCM) (4.17 mL, 4.17 mmol). The reaction mixture was allowed to stir at room temperature for 1 h. The reaction mixture was cooled to 0 °C and quenched with water (8.3 mL). The layers were separated and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 5-50% ethyl acetate/cyclohexane) to give *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-hydroxybenzenesulfonamide **32** (110 mg, 0.33 mmol, 78%) as a white solid; ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 8.83 (s, 1H), 7.53 (d, *J* = 2.0 Hz, 1H), 7.36 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 1H), 4.66 (d, *J* = 7.5 Hz, 1H), 3.67 (m, 1H), 2.40 (s, 3H), 2.26 (s, 3H), 1.84 (m, 2H), 1.60 (m, 4H), 1.39 (m, 2H); LCMS (2 minute method): (ES+) 339, retention time 0.99 min., >97% pure.

g) To a cold solution of *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-hydroxy benzenesulfonamide **32** (45 mg, 0.13 mmol), triphenylphosphine (70 mg, 0.27 mmol) and diisopropylazodicarboxylate (53 μL, 0.27 mmol) in tetrahydrofuran (THF) (2 mL) stirred under N₂ at 0 °C was added 3-(4-morpholinyl)-1-propanol (39 mg, 0.27 mmol). The reaction mixture was stirred at room temperature for 2 h. The mixture was concentrated by flow of N₂ gas and the residue was purified by flash column chromatography (0-5% 2N ammonia in methanol/dichloromethane) to give the purified Boc-protected analogue of the desired product. Residue was dissolved in 1:1 methanol/dichloromethane and treated with 2N HCl in diethyl ether (1.1 eq). Mixtures were concentrated by flow of N₂ gas to give *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-(3-morpholinopropoxy) benzene sulfonamide hydrochloride **33** (62 mg, 0.12mmol, 93%) as a white solid; ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 12.92 (br. s, 1H), 7.86 (d, *J* = 2.5Hz, 1H), 7.39 (dd, *J* = 2.5, 8.5Hz, 1H), 7.03 (d, *J* = 8.5Hz, 1H), 4.31 (m, 4H), 4.01 (d, *J* = 11.5Hz, 2H), 3.55 (m, 4H), 2.88 (d, *J* = 11.5 Hz, 2H), 2.63 (br. s, 2H), 2.41 (s, 3H), 2.26 (s, 3H), 1.67 (m, 8H), 1.41 (d, *J* = 3.5Hz, 2H); LCMS (2 minute method): (ES+) 464 (MH+), retention time 0.82min., >83% pure.

To a cold solution of *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-hydroxy benzenesulfonamide **32** (45 mg, 0.13 mmol), triphenylphosphine (70 mg, 0.27 mmol) and diisopropylazodicarboxylate (53 μL, 0.27 mmol) in tetrahydrofuran (THF) (2 mL) stirred under N₂ at 0 °C was added 1,1-dimethylethyl 4-(2-hydroxyethyl)-1-piperazinecarboxylate (62 mg, 0.27 mmol). The reaction mixture was then stirred at room temperature for 2 h. The mixture was concentrated by flow of N₂ gas and the residue was purified by flash column chromatography (0-5% 2N ammonia in methanol/dichloromethane) to give the purified Boc-protected analogue of the desired product. The residue was dissolved in 1:1 methanol/dichloromethane and treated with 2N HCl in diethyl ether (1.1 eq). The mixtures were then concentrated by flow of N₂ gas to give *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-(2-(piperazin-1-yl)ethoxy)benzenesulfonamide hydrochloride **34** (45 mg, 0.09 mmol, 69%) as a white solid; ¹H NMR (250 MHz, DMSO-*d*₆) δ 7.70 (d, *J* = 2.5 Hz, 1H), 7.56 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 6.64 (br. s, 1H), 4.39 (t, *J* = 5.5 Hz, 2H), 3.57 (br. s, 2H), 3.04 (m, 11H), 2.38 (s, 3H), 2.20 (s, 3H), 1.57 (m, 8H); LCMS (2 minute method): (ES+) 449 (MH+), retention time 0.96 min., >99% pure.

h) For R₅=Me, to a cold solution (0 °C) of *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-methoxybenzenesulfonamide **6b** (42.6 mg, 0.12 mmol) in *N,N*-dimethylformamide (DMF) (2 mL) was added NaH (60 disp. In mineral oil, 5.35 mg, 0.13 mmol). The resulting mixture was stirred at 0 °C for 30 min. before MeI (8.36 μL, 0.13 mmol) was then added. The reaction mixture was stirred at room temperature for 3 h. The mixture was then re-cooled to 0 °C and additional NaH (60 disp. In mineral oil, 5.35 mg, 0.13 mmol) was added. After 15 min. at 0 °C, further MeI (8.36 μL, 0.13 mmol) was then added and the reaction was stirred at room temperature for 1 h. The reaction mixture was concentrated by flow of N₂ gas. The residue was

partitioned between water (5 mL) and dichloromethane (5 mL). The layers were separated and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0-50% ethyl acetate/cyclohexane) to give *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-2-methoxy-*N*-methylbenzenesulfonamide **23b** (35 mg, 0.10 mmol, 79%) as a off-white solid; ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 7.85 (d, *J* = 2.5 Hz, 1H), 7.38 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.07 (d, *J* = 8.50 Hz, 1H), 4.30 (m, 1H), 3.98 (s, 3H), 2.86 (s, 3H), 2.41 (s, 3H), 2.27 (s, 3H), 1.56 (m, 8H); LCMS (2 minute method): (ES+) 365 (MH+), retention time 1.14 min., >84% pure.

For R₅=Et, to a cold solution (0 °C) of *N*-cyclopentyl-5-(3,5-dimethyl-4-isoxazolyl)-2-(methoxy)benzenesulfonamide **6b** (105 mg, 0.30 mmol) in *N,N*-dimethylformamide (DMF) (2 mL) was added NaH (13.2 mg, 0.33 mmol). The resulting mixture was stirred at 0 °C for 30 min. and iodoethane (26 μL, 0.33 mmol) was then added. The reaction mixture was stirred at room temperature for 3 h. The mixture was cooled to 0 °C and NaH (5.35 mg, 0.13 mmol) was added. After 15 min. at 0 °C, MeI (8.36 μL, 0.13 mmol) was then added and the reaction was stirred at room temperature for 1 h. The reaction mixture was concentrated by flow of N₂ gas. The residue was partitioned between water (5 mL) and dichloromethane (5 mL). The layers were separated and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, 0-50% ethyl acetate/cyclohexane) to give *N*-cyclopentyl-5-(3,5-dimethylisoxazol-4-yl)-*N*-ethyl-2-methoxybenzenesulfonamide **24b** (88 mg, 0.23 mmol, 78%) as a yellow solid; ¹H NMR (400 MHz, CHLOROFORM-*d*) δ 7.87 (d, *J* = 2.5 Hz, 1H), 7.37 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 4.15 (m, 1H), 3.98 (s, 3H), 3.37 (q, *J* = 7.0 Hz, 2H), 2.40 (s, 3H), 2.26 (s, 3H), 1.60 (m, 8H), 1.24 (t, *J* = 7.0 Hz, 3H); LCMS (2 minute method): (ES+) 379 (MH+), retention time 1.19 min., 77% pure.

pdbcode	Compound 3 4a9p	Compound 4 4a9o	Compound 5a 4a9n	Compound 6a 4a9m
Data collection^a				
Space Group	C2	C2	C2	C2
Cell Dimensions				
a,b,c (Å)	114.282,55.697,68.713	113.628,55.692,68.077	113.967, 55.725,68.126	115.577,55.682,67.986
α, β, γ (°)	90.00,94.92,90.00	90.00,94.00,90.00	90.00,94.96,90.00	90.00,94.61,90.00
Resolution (Å)	1.97 (2.01)	1.78 (1.81)	1.86 (1.89)	2.06 (2.17)
R _{merge} ^b	0.086 (0.449)	0.054 (0.267)	0.048 (0.380)	0.082 (0.374)
Average I/ σ I	17.6/2.0	22.9/4.0	22.2/2.1	12.3 (3.2)
Completeness (%)	99.4 (100)	99.0 (99.7)	97.7 (86.0)	99.3 (99.3)
Redundancy	3.5 (3.4)	3.6 (3.5)	3.6 (2.9)	3.5 (3.4)
No. Reflections	105258	146198	126924	92087 (13164)
No. Unique Reflections	30411	40262	35351 (1524)	26642 (3830)
Refinement				
Resolution (Å)	68.5-1.97	67.88-1.78	67.88-1.86	33.88 -2.06
R _{work} /R _{free}	0.173/0.203	0.172/0.198	0.174/0.195	0.180/0.225
No. Reflections	29172	38647	33945	25297
No. atoms	3180	3377	3204	3242
Protein	2809	2893	2833	2846
Ligand/ion	39/28	42/23	42/48	69/15
Water	304	419	281	312
B-factors				
Protein	25.8	23.1	27.0	37.5
Ligand/ion	39.2/63.5	27.8/48.3	40.0/66.6	58.1/85.1
Water	40.2	38.7	41.2	49.1
R.m.s deviations				
Bond lengths (Å)	0.006	0.008	0.006	0.008
Bond angles (°)	0.933	0.935	0.904	1.139

^a Data for the highest resolution shell are given in parentheses.

^b $R_{\text{merge}} = \Sigma |I_j - \langle I_j \rangle| / \Sigma \langle I_j \rangle$.

Fig. S1. X-ray data collection and refinement statistics

	BRD2 TR-FRET			BRD3 TR-FRET			BRD4 TR-FRET			BRD2 FA			BRD3 FA			BRD2 FA			IL-6 PBMC		
	pIC ₅₀	SD	n	pIC ₅₀	SD	n	pIC ₅₀	SD	n	pIC ₅₀	SD	n	pIC ₅₀	SD	n	pIC ₅₀	SD	n	pIC ₅₀	SD	n
5a	5.8	0.01	2	6.0	0.03	2	6.0	0.2	6	5.3	0.09	6	5.9	0.09	6	5.7	0.1	6	5.5	0.13	2
5b	5.7	0.01	2	5.9	0.02	2	5.5	0.05	6	5.4	0.14	5	5.8	0.13	6	5.5	0.04	5	5.5	0.15	2
6a	6.3	0.01	2	6.3	0.01	2	6.3	0.11	10	5.4	0.05	2	6.1	0.1	2	5.8	0.03	2	5.5	0.08	2
6b	5.9	0	2	6.0	0.01	2	5.7	0.05	4	5.4	0.21	8	5.9	0.1	9	5.6	0.12	8	5.9	0.06	2
7a	6.0	0.01	2	6.1	0.02	2	6.0	0.07	2	5.6	0.24	5	6.0	0.05	5	5.7	0.05	5	5.4	0.1	2
8a	6.0	0.11	2	6.0	0.04	2	5.7	0.02	2	5.5	0.3	5	5.8	0.21	5	5.6	0.16	5	4.9	0	2
8b	5.6	0.01	2	5.7	0.15	2	5.3	0.02	2	5.0	0.13	5	5.4	0.23	5	5.2	0.12	5	5.1	0.06	2
9a	5.7	0.03	4	5.7	0.03	4	5.5	0.05	4	5.3	0.28	7	5.5	0.36	7	5.3	0.11	6			
9b	5.4	0.03	4	5.3	0.05	4	5.1	0.06	4	5.0	0.22	7	5.2	0.15	7	5.0	0.13	7			
10a	5.6	0.1	4	5.7	0.04	4	5.5	0.08	4	5.3	0.23	7	5.4	0.08	7	5.3	0.12	7			
11a	5.4	0.01	2 ⁺	5.4	0.01	2 ⁺	5.4	0.11	2 ⁺	5.3	0.18	6	5.1	0.15	6	4.9	0.13	6			
12a	5.8	0.03	4	5.7	0.03	4	5.6	0.04	4	5.3	0.25	7	5.6	0.18	7	5.3	0.13	7			
12b	5.3	0.04	4	5.3	0.05	4	5.1	0.05	4	5.0	0.43	4 [†]	5.4	0.12	6	4.8	0.21	6			
13a	5.7	0.06	4	5.7	0.03	4	5.6	0.01	4	5.2	0.11	3	5.5	0.12	7	5.2	0.17	7			
13b	5.3	0.03	4	5.3	0.03	4	5.2	0.04	4	4.8	0.16	3 [*]	5.1	0.2	7	4.8	0.2	7			
14a	5.2	0.04	4	5.2	0.02	4	5.3	0.02	4	4.8	0.11	5 [†]	5.0	0.07	6	4.9	0.11	6			
14b	4.9	0.02	4	4.9	0.05	4	4.8	0.12	4	4.5	0.31	4 [†]	4.6	0.13	6	4.4	0.11	3 ⁺			
15a	5.3	0.01	4	5.4	0.04	4	5.2	0.03	4	4.9	0.3	6	5.3	0.14	6	4.9	0.16	5			
15b	4.9	0.04	4	4.9	0.04	4	4.7	0.05	4	4.9	0.17	3 ⁺	4.9	0.15	5	4.5	0.2	4 [†]			
16a	5.4	0.05	4	5.5	0.03	4	5.4	0.05	4	4.9	0.14	5	5.3	0.2	6	5.0	0.08	6			
17a	5.4	0.02	4	5.5	0.02	4	5.5	0.04	4	5.0	0.32	4 [*]	5.3	0.06	8	5.1	0.16	7			
18a	4.9	0.11	4	5.1	0.01	4	4.9	0.07	5	4.7	0.17	5	5.0	0.08	5	4.7	0.14	5			
19a	5.2	0.04	5	5.4	0.06	5	5.2	0.05	5	4.9	0.21	4	5.4	0.09	4	5.1	0.06	4			
20a	5.6	0.01	2	5.7	0.01	2	5.7	0.2	6	5.2	0.38	4	5.8	0.14	4	5.5	0.02	4	5.2	0.06	2
21a	5.1	0.06	5	5.1	0.1	5	5.1	0.08	5	4.6	0.1	3 [†]	5.0	0.03	4	4.7	0.1	4			
22a	5.7	0	2	5.8	0.04	2	5.6	0.04	2	5.1	0.21	5	5.7	0.14	5	5.4	0.07	5	5.1	0.02	2
23b	6.1	0.05	2	6.2	0.01	2	6.0	0.2	4	5.7	0	2	6.1	0.12	2	5.7	0.08	3	5.8	0.11	2
24b	6.1	0.02	2	6.2		1	5.9	0.08	6	5.8	0.15	4	6.2	0.08	4	5.8	0.13	4			
25a	5.8	0.03	2	6.0	0	2	5.8	0	2	5.2	0.33	5	5.8	0.16	5	5.5	0.07	5	5.6	0.02	2
26a	6.0	0.01	2	6.1	0.01	2	5.9	0.01	2	5.4	0.3	4	6.0	0.18	4	5.8	0.13	4	5.5	0.08	2
27a	4.9	0.02	2	5.1	0.02	2	4.9	0.08	2	4.4	0.09	4 [†]	4.9	0.1	6	4.6	0.07	6	4.7	0.24	2
27b	4.8	0.08	2	4.9	0.01	2	4.8	0.06	2	4.4	0.25	3 [†]	4.5	0.34	4	4.3	0.27	4 [†]			
28a	5.4	0.01	2	5.5	0.06	2	5.4	0.02	2	4.7	0.29	4	5.3	0.19	4	5.0	0.12	4	5.4	0.06	2
28b	5.0	0.03	2	5.2	0.1	2	5.0	0	2	4.5	0.13	4	5.0	0.2	4	4.7	0.17	4	4.8	0.04	2
29a	4.7	0	2	5.0	0.06	2	4.8	0.01	2	4.4	0.1	4 [†]	4.6	0.12	6	4.3	0.05	6	4.7	0.1	2
29b	4.5	0.01	2	4.7	0.06	2	4.7	0.12	4	4.2	0.15	3 [†]	4.3	0.18	5	< 4.0		5	4.3	0.19	3
30a	5.0	0.04	4	5.1	0.03	4	5.0	0.03	4	4.5	0.06	6 [†]	4.7	0.16	8	4.5	0.09	8			
30b	5.0	0.08	4	4.9	0.07	4	4.8	0.05	4	4.4	0.22	5	4.6	0.15	6	4.3	0.15	7			
31	5.4	0.03	4	5.5	0.03	4	5.5	0.03	4	5.1	0.27	6	5.5	0.13	6	5.2	0.27	6			
32	5.8	0.03	4	5.9	0.02	4	5.6	0.04	4	5.5	0.17	7	5.8	0.03	8	5.5	0.05	7			
33	5.4	0.05	2	5.7	0.01	2	5.3	0.08	4	5.1	0.19	4	5.6	0.09	4	5.2	0.06	4	5.5	0.09	2
34	5.8	0.06	2	5.9	0	2	5.6	0.07	4	5.5	0.13	4	6.0	0.06	4	5.5	0.05	4	5.5	0.11	2

Fig. S2. TR-FRET and FA assay data for BRD2, BRD3 and BRD4, and IL-6 inhibition in LPS-stimulated PBMC cells. Data shown: mean pIC₅₀ (-log₁₀ IC₅₀) and standard deviation, with the number of replicates used to calculate the average. For marked values, the mean excludes experiments for which IC₅₀ values were <4.0 on one[†], two⁺, or three^{*} occasions. These generally occur for weaker compounds where the maximum %I does not quite reach 50%. In some cases this may reflect compound insolubility at higher concentration.