Identification of a chemical probe for BET bromodomain inhibition through optimization of a fragment-derived hit

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SUPPORTING INFORMATION

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1. Supplementary Methods

X-ray crytallography

Crystallization: Aliquots of the purified proteins were set up for crystallization using a mosquito® crystallization robot (TTP Labtech, Royston UK). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nl, 75+75 nl and 50+100 nl). Initial hits were optimized further using Greiner 1-well plates and scaling up the drop sizes in steps. All crystallizations were carried out using the sitting drop vapor diffusion method at 4 °C. For example, BRD4(1) crystals with **8** (5 mM final concentration) were grown by mixing 150 nl of the protein (9 mg/ml) with an equal amount of reservoir solution containing 0.2 M $NaNO_3$, 0.1 M BTProp pH 6.5, 20 % PEG3350 and 10 % ethylene glycol.

Data Collection and Structure solution: Crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data were collected at a Rigaku FRE Superbright using an RAXIS-VI detector at 1.52 Å. Indexing and integration was carried out using MOSFLM[1] and scaling was performed with SCALA[2]. Initial phases were calculated by molecular replacement with PHASER[3] using an ensemble of known bromodomain models (PDB IDs 2OSS, 2OUO, 2GRC, 2OO1, 3DAI, 3D7C, 3DWY). Initial models were built by ARP/wARP[4] and building was completed manually with COOT[5]. Refinement was carried out in REFMAC5[6]. Thermal motions were analyzed using TLSMD[7] and hydrogen atoms were included in late

refinement cycles. Data collection and refinement statistics can be found in Supplemental Tables 1 and 2. The models and structure factors have been deposited with PDB accession codes: 4HBV, 4HBW, 4HBX, 4HBY and 4E96.

Biological Screening

AlphaScreen™

Cloning, protein expression and purification were performed as described previously by this group.[8] AlphaScreen™ assays were performed with minor modifications from the manufacturers protocol (PerkinElmer, USA). All reagents were diluted in the recommended buffer (50 mMHEPES, 100 mM NaCl, 0.1% BSA; pH = 7.4) supplemented with 0.05% CHAPS and allowed to equilibrate to room temperature prior to addition to plates. 4 ml of HIS-tagged protein was added to low-volume 384-well plates (ProxiPlatet-384 Plus, PerkinElmer, USA), followed by 4 ml of either buffer, non-biotinylated peptide, solvent or compound. Plates were sealed and incubated at room temperature for 30 minutes, before the addition of 4 ml biotinylated peptide, resealing and incubation for a further 30 minutes. 4 ml of streptavidin-coated donor beads (25 μg/ml) and 4 μl of nickel chelate acceptor beads (25 μg/ml) were then added under low light conditions. Plates were foil sealed to protect from light, incubated at room temperature for 60 minutes and read on a PHERAstar FS plate reader (BMG Labtech,Germany) using an AlphaScreen™ 680 excitation/570 emission filter set. IC50s were calculated in GraphPad Prism 5 (GraphPad Software, USA). Results for compounds dissolved in DMSO were normalised against corresponding DMSO controls prior to IC_{50} determination, which are given as the final concentration of compound in the 20 μl reaction volume.

Peripheral Blood Mononuclear Cell (PBMC) and inhibition of IL-6 production

Blood was collected from human volunteers into heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Mononuclear cells were prepared by density gradient separation of blood over

Histopaque 1077 (Sigma, St Louis, MO) for 20' at a 1000 x g. Mononuclear cells were washed one time by centrifugation in phosphate buffered saline without Ca and Mg at 300 x g and two times with RPMI culture medium + 5% fetal bovine serum. Mononuclear cells were plated at 2E5 c/well in 200 ul in RPMI culture medium + 5% fetal bovine serum in 96 well flat bottomed tissue culture plates. Test compounds were added to cells in a final concentration of 0.1% DMSO and incubated for 30 minutes. Ultrapure LPS O111:B4 (Invivogen, San Diego, CA) was dissolved in PBS and added at a final concentration of 0.5 ng/ml. Supernatants were collected after 20 hrs and cytokines were measured by multiplex elisa plates (Meso Scale Discovery, Gaithersburg, MD). Data was analyzed by determining the relative IC_{50} based on the effect of BRD inhibitors to inhibit 80 – 100% of IL6 and TNF in individual donors (n=2).

Bromdomain Thermal Shift[8] and Surface Plasmon Resonance[9]

Representative methods can be found within the indicated references in section 6 of these supplementary data.

Chemistry Methods and Compound Characterization

Proton (^{1}H NMR) and carbon (^{13}C NMR) magnetic resonance spectra where obtained in DMSO-d_6 at 400 MHz and 100 MHz, respectively unless otherwise noted. The following abbreviations were utilized to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, and m = multiplet. High-resolution mass measurements were obtained on an Agilent ToF mass spectrometer. Accurate Mass Spectrometry analyses were conducted on an Agilent 6220 TOF mass spectrometer (Agilent Technologies, Wilmington, DE) in positive or negative electrospray mode. The system was calibrated to greater than 1ppm accuracy across the mass range prior to analyses according to manufacturer's specifications. The samples were separated using UHPLC on an Agilent 1200 (Agilent Technologies, Wilmington, DE) system prior to mass spectrometric analysis. The resulting spectra were automatically lockmass corrected and the target mass ions and any confirming adducts (Na^+, NH_4^+) were extracted and

combined as a chromatogram. The mass accuracy was calculated for all observed isotopes against the theoretical mass ions derived from the chemical formula using MassHunter software (Agilent Technologies, Wilmington, DE). All air and moisture sensitive reactions were carried out under an atmosphere of dry nitrogen using heat-dried glassware and standard syringe techniques. Tetrahydrofuran (THF) and acetonitrile were purchased from EMD anhydrous and were used without further drying. Flash chromatography was performed using an Analogix Intelliflash 280 with Sepra Si 50 silica gel using ethyl acetate/heptane mixtures as solvent unless otherwise indicated. Certain compounds from the examples described were purified on reversed-phase using an automated preparative High Performance Liquid Chromatography (HPLC) on a Gilson GX281, Shimadzu CL-2010C, or Agilent 1200 system. Samples were submitted dissolved in 1mL of DMSO. Depending on the nature of the compounds and the results of a pre-analysis, the purification was performed under a variety conditions at ambient temperature. HPLC was carried out on an Agella Venusil ASB C18 column (21.2 x 150 mm, 5 μm),. A flow rate of 0.5-150 mL/min was used with mobile phase A: water + 0.1% modifier (v/v) and B: acetonitrile + 0.1% modifier (v/v). The modifier was formic acid, trifluoroacetate, ammonia acetate, or hydrochloric acid. A Shimadzu MS2010EV MS2525 binary LC or Waters 2545 BGM pump supplied a mobile phase with a composition of 5% B for 1 min. then ran from 5% to 98% B over 6 min. followed by a 2 min. hold at 98% B. Detection was achieved using a Shimadzu SPD-20AV or Waters 2998 PDA wavelength absorbance detector set at 220 or 200 nm followed in series by a Shimadzu MS2010EV or Waters 3100 mass spectrometer. The Shimadzu MS2010EV MS was tuned with the following parameters:

ES+ Cone voltage: 30 v Capillary: 1.5 kv

Desolvation gas: Nitrogen

Source Temp: 250°C.

Scan range 100-1200 Da

The fraction collection was triggered by both MS.

The Waters 3100 MS was tuned with the following parameters:

ES+ Cone voltage: 35 v Capillary: 3.0 kv

Desolvation gas: 800 (L/H)

Source Temp: 130 °C.

Scan range 100-800 Da

The fraction collection was triggered by MS or by UV.

Quality control (QC) analysis was performed using a LCMS method. Acidic runs were carried out on a Shimadzu XB-C18 (2.1 x 30 mm, 5 μm), X-Bridge (50 x 4.6 mm, 5 μm), Gemini NX C18 (50 x 4.6, 3 μm), or Gemini NX C18 (50 x 4.6, 5 μm). A flow rate of 1.0-1.2 mL/min was used with mobile phase A: water + 0.1% modifier (v/v) and B: acetonitrile + 0.1% modifier (v/v). For acidic runs the modifier was trifluoroacetic acid. A Shimadzu 20AB pump ran a gradient elution from 0% to 98% B over 2 min followed by a 1 min hold at 95% B. Detection was achieved using a Shimadzu 10A detector set at 220 or 260 nm followed in series by a Shimadzu MS2010EV or Applied Biosystem API 2000 mass spectrometer in parallel. The Shimadzu MS2010EV MS was tuned with the following parameters:

ES+ Cone voltage: 25 v Capillary: 1.50 kv

ES- Cone voltage:-30 v Capillary:-1.50 kv

Desolvation gas: Nitrogen

Source Temp: 250°C.

Scan range 100-1000 Da

The Applied Biosystem API 2000 MS was tuned with the following parameters:

ES+ Cone voltage: 50 v Capillary: 1.50 kv

ES- Cone voltage:-50 v Capillary:-1.50 kv

Desolvation gas: 40-50 psi

Source Temp: 200°C.

Scan range 100-800 Da

Compound purity was >95% other than intermediate **9** (91%) which, as a reactive sulfonyl chloride, was used satisfactorily with no further chromatographic purification.

3-methyl-2-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonyl chloride (9): 3-methyl-3,4-dihydroquinazolin-2(1H)-one (5.0 g, 30.828 mmol, 1.0 equiv) was dissolved in chlorosulfonic acid (15 mL) at 0 °C. The pale orange solution was stirred at room temperature for 2 h and monitored by TLC. When the starting material was judged to be consumed the reaction mixture was quenched with ice. The reaction mixture was filtered to provide a white solid that was azeotroped with dichloromethane to provided the desired material as a white solid (1.2 g, 15%). ^{1}H NMR (400 MHz, Methanol-d_4): δ = 3.00 (s, 3 H), 4.58 (s, 2 H), 6.98 (d, *J*=9.18 Hz, 1 H), 7.77 - 7.90 (m, 2 H); LCMS [M+H] = 261, 91% (t=2.57 min).

3-methyl-6-nitro-3,4-dihydroquinazolin-2(1H)-one (10): [10]

6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (11): To a solution of 3-methyl-6-nitro-3,4-dihydroquinazolin-2(1H)-one (5.00g, 24.13 mmol, 1.0 equiv) in acetic acid (50 mL) was added Raney Nickel (500 mg) and the reaction mixture was stirred at room temperature under H_2 balloon (1 atm) for 16 h. After completion of the reaction (via TLC 10% MeOH in DCM) the reaction mixture was filtered through celite pad, evaporated to provided the crude product. The crude material was purified by titration with ethyl acetate to provide the desired material as a brown solid which was an acetic acid salt (4.1 g, 72%). ^{1}H NMR (400 MHz, DMSO-d_6): δ= 2.83 (s, 3 H), 4.23 (s, 2 H), 6.29 (s, 1 H), 6.35 (d, *J*=8.36 Hz, 1 H), 6.47 (d, *J*=8.36 Hz, 1 H), 8.73 (s, 1 H); 13C NMR (DMSO-d_6): δ= 33.7, 50.1, 110.7,

113.6, 113.9, 118.2, 127.6, 142.8, 153.9; HRMS [M+H] for $C_9H_{11}N_3O$, calcd., 178.0975, found, 178.0977; LCMS [M+H] = 178, 99% (t=1.33 min).

3-methyl-6-(pyrrolidin-1-ylsulfonyl)-3,4-dihydroquinazolin-2(1H)-one (12): To a suspension of compound **9** (200 mg, 0.767 mmol, 1.0 equiv) in dichloromethane (5 mL) was added pyrrolidine (0.63 mL, 7.67 mmol, 10.0 equiv). The reaction became homogenous and was stirred at room temperature under nitrogen atmosphere for 30 minutes. The reaction was judged complete via LCMS. The reaction was concentrated to a crude product that was stirred in aqueous 2 M HCl. The resulting suspension was filtered and washed with water. The resulting solid was dried under vacuum to provide the product as a white solid (160 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.52 - 1.75 (m, 4 H), 2.84 (s, 3 H), 3.01 - 3.16 (m, 4 H), 4.47 (s, 2 H), 6.90 (d, *J*=8.40 Hz, 1 H), 7.49 - 7.59 (m, 2 H), 9.63 (s, 1 H); HRMS [M+H] for $C_{13}H_{18}N_3O_3S$, calcd., 296.1063, found, 296.1069; LCMS [M+H] = 296, >99% (t=1.53 min).

N-ethyl-3-methyl-2-oxo-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (13): To a solution of ethylamine in THF (2 M, 20 mL, 40 mmol, 47.4 equiv) was added compound **9** (220 mg, 0.844 mmol, 1.0 equiv) under nitrogen atmosphere. The reaction was stirred for 10 minutes at room temperature and the reaction was judge complete via LCMS. The reaction was concentrated *in vacuo* to a crude product that was suspended in aqueous 2 M HCl. The resulting suspension was filtered and the solid was rinsed thoroughly with water and dried under high vacuum to provide a white solid. The solid was stirred in dichloromethane then filtered to provided the desired material as a white solid (140 mg, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.97 (t, *J*=7.22 Hz, 3 H), 2.59 - 2.92 (m, 2 H), 4.47 (s, 3 H), 6.89 (d, *J*=8.40 Hz, 1 H), 7.31 (br. s., 1 H), 7.45 - 7.64 (m, 2 H), 9.60 (s, 1 H); HRMS [M+Na] for $C_{11}H_{15}N_3O_3SNa$, calcd., 292.0726, found, 292.0728; LCMS [M+H] = 270, 95% (t=1.98 min).

3-methyl-2-oxo-N-phenyl-1,2,3,4-tetrahydroquinazoline-6-sulfonamide (14): To a solution **9** (200 mg, 0.767 mmol, 1.0 equiv) in dichloromethane (2 mL) was added pyridine (0.2 mL, 2.5 mmol, 3.2 equiv) followed by aniline (200 mg, 0.2 mL, 2.2 mmol, 2.9 equiv). The reaction was stirred overnight at room temperature under nitrogen. The reaction was judged complete by LCMS. The reaction was concentrated *in vacuo* to a crude product that was suspended in aqueous 2M HCl. The resulting suspension was filtered and the solid was rinsed thoroughly with water and dried under high vacuum to provide the desired material as a solid (195 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.81 (s, 3 H), 4.40 (s, 2 H), 6.80 (d, *J*=8.40 Hz, 1 H), 6.95 - 7.11 (m, 3 H), 7.18 - 7.26 (m, 2 H), 7.41 - 7.56 (m, 2 H), 9.59 (s, 1 H), 10.07 (s, 1 H); HRMS [M+H] for $C_{15}H_{16}N_3O_3S$, HRMS [M+H] for $C_{15}H_{16}N_3O_3S$, calcd., 318.0907, found, 318.0905; LCMS [M+H] = 318, >99% (t=2.44 min).

2-chloro-4-fluoro-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (15): To a solution of 2-chloro-4-fluromethylbenzene-1-sulfonyl chloride (144 mg, 0.631 mmol, 1.5 equiv) in pyridine (1.0 mL) was added 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv). The reaction was stirred for 5 minutes at room temperature and dichloromethane (5 mL) was added. The reaction was stirred for an additional 1 h. The reaction was quenched with N,N,N'-trimethylethylenediamine (0.1 mL, 0.92 mmol, 2.2 equiv) and concentrated to a residue. The residue was dissolved in methanol and passed through a SCX cartridge (5 g) eluting with methanol. The eluent was concentrated to a pale brown residue. The residue was partitioned between MTBE/ethyl acetate (1:1) and aqueous 2 M HCl. The organic layer was collected, washed with water and brine, dried over sodium sulfate, filtered, and concentrated to a yellow solid. The solid was triturated with MTBE and filtered to provide the desired product as a white solid (95 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.78 (s, 3 H), 4.26 (s, 2 H), 6.59 (d, *J*=8.20 Hz, 1 H), 6.76 - 6.86 (m, 2 H), 7.28 - 7.37 (m, 1 H), 7.66 (dd, *J*=8.79, 2.54 Hz, 1 H), 7.96 (dd, *J*=8.88, 5.96 Hz, 1 H), 9.08 (s, 1 H), 10.24 (s, 1 H); HRMS [M+H] for $C_{15}H_{14}ClFN_3O_3S$, calcd., 370.0423, found, 370.0430; LCMS [M+H] = 370, >99% (t=1.71 min).

2-methyl-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (16): To a stirred suspension of 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv) in dichloromethane (10 mL) was added pyridine (0.20 mL, 2.48 mmol, 5.9 equiv) followed by 2-methylbenzene-1-sulfonyl chloride (120 mg, 0.631 mmol, 1.5 equiv). After 2 h, the solvent was evaporated and the residue was partitioned between ethyl acetate and aqueous 2 M HCl. The organic layer was collected, washed with water and brine, dried over magnesium sulfate, filtered, and concentrated to a residue. The residue was dissolved in DMSO (~1 mL) and purified by automated HPLC to provided the desired material (50 mg, 36%). ^{1}H NMR (600 MHz, DMSO-d_6) δ = 2.54 - 2.58 (s, 3 H), 2.76 - 2.82 (s, 3 H), 4.26 (s, 2 H), 6.58 (d, *J*=8.35 Hz, 1 H), 6.74 - 6.82 (m, 2 H), 7.31 (t, *J*=7.25 Hz, 1 H), 7.36 (d, *J*=7.47 Hz, 1 H), 7.44 - 7.56 (m, 2 H), 7.71 - 7.89 (m, 1 H), 9.08 (s, 1 H), 10.00 (s, 1 H); HRMS [M+H] for $C_{16}H_{18}N_3O_3S$, calcd., 332.1063, found, 332.1071; LCMS [M+H] = 322, 95% (t=1.68 min).

2-methoxy-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (17, PFi-1): To a stirred suspension of 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv) in dichloromethane (10 mL) was added pyridine (0.20 mL, 2.48 mmol, 5.9 equiv) followed by 2-methoxybenzene-1-sulfonyl chloride (130 mg, 0.631 mmol, 1.5 equiv). After 2 h, the solvent was evaporated and the residue was partitioned between ethyl acetate and aqueous 2M HCl. The organic layer was collected, washed with water and brine, dried over magnesium sulfate, filtered, and concentrated to a residue. The residue was dissolved in DMSO (~1 mL) and purified by automated HPLC to provided the desired material (50 mg, 34%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.78 (s, 3 H), 3.91 (s, 3 H), 4.25 (s, 2 H), 6.59 (d, *J*=9.16 Hz, 1 H), 6.79-6.81 (m, 2 H), 6.97 (t, *J*=7.5 Hz, 1 H), 7.14 (d, *J*=8.2 Hz, 1 H), 7.52-7.56 (m, 1 H), 7.68 (dd, *J*=1.4, 7.8 Hz, 1 H), 9.09 (s, 1 H), 9.64 (s, 1 H); HRMS [M+H] for $C_{16}H_{18}N_3O_4S$, calcd., 348.1013, found, 348.1019; LCMS [M+H] = 384.1, >99% (t=1.23 min).

2-fluoro-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (18): To a stirred suspension of 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv) in dichloromethane (10 mL) was added pyridine (0.20 mL, 2.48 mmol, 5.9 equiv) followed by 2-flurobenzene-1-sulfonyl chloride (123 mg, 0.631 mmol, 1.5 equiv). After 2 h, the solvent was evaporated and the residue was partitioned between ethyl acetate and aqueous 2M HCl. The organic layer was collected, washed with water and brine, dried over magnesium sulfate, filtered, and concentrated to a residue. The residue was dissolved in DMSO (~1 mL) and purified by automated HPLC to provided the desired material (50 mg, 35%). ^{1}H NMR (600 MHz, DMSO-d_6) δ = 2.79 (s, 3 H), 4.28 (s, 2 H), 6.60 (d, *J*=8.79 Hz, 1 H), 6.77 - 6.88 (m, 2 H), 7.32 (t, *J*=7.69 Hz, 1 H), 7.37 - 7.47 (m, 1 H), 7.67 (d, *J*=5.27 Hz, 1 H), 7.73 (t, *J*=7.47 Hz, 1 H), 9.11 (s, 1 H) 10.23 (br. s., 1 H); HRMS [M+H] for $C_{15}H_{15}FN_3O_3S$, calcd., 336.0813, found, 336.0817; LCMS [M+H] = 336.0, 95% (t=0.66 m).

4-methyl-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (19): To a solution of 4-methylbenzene-1-sulfonyl chloride (120 mg, 0.631 mmol, 1.5 equiv) in pyridine (1.0 mL) was added 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv). The reaction was stirred for 5 minutes at room temperature and dichloromethane (5 mL) was added. The reaction was stirred for an additional 1 h. The reaction was quenched with N,N,N'-trimethylethylenediamine (0.1 mL, 0.92 mmol, 2.2 equiv) and concentrated to a residue. The residue was dissolved in methanol and passed through a SCX cartridge (5 g) eluting with methanol. The eluent was concentrated to a pale brown residue. The residue was partitioned between MTBE/ethyl acetate (1:1) and aqueous 2M HCl. The organic layer was collected, washed with water and brine, dried over sodium sulfate, filtered, and concentrated to a yellow solid. The solid was triturated with MTBE and filtered to provide the desired product as a white solid (80 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.34 (s, 3 H), 2.80 (s, 3 H), 4.28 (s, 2 H), 6.59 (d, *J*=8.40 Hz, 1 H), 6.70 - 6.90 (m, 2 H), 7.32 (d,

J=8.01 Hz, 2 H), 7.57 (d, *J*=8.20 Hz, 5 H), 9.08 (s, 1 H), 9.82 (s, 1 H); HRMS [M+H] for $C_{16}H_{18}N_3O_3S$, calcd., 332.1063, found, 332.1067; LCMS [M+H] = 332, >99% (t=1.69 min).

N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)-2-(trifluoromethyl)benzenesulfonamide (20): To a stirred suspension of 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv) in dichloromethane (10 mL) was added pyridine (0.20 mL, 2.48 mmol, 5.9 equiv) followed by 2-(trifluoromethyl)benzene-1-sulfonyl chloride (154 mg, 0.631 mmol, 1.5 equiv). After 2 h, the solvent was evaporated and the residue was partitioned between ethyl acetate and aqueous 2M HCl. The organic layer was collected, washed with water and brine, dried over magnesium sulfate, filtered, and concentrated to a residue. The residue was dissolved in DMSO (~1 mL) and purified by automated HPLC to provided the desired material (50 mg, 31%). ^{1}H NMR (600 MHz, DMSO- d_6) δ = 2.79 (s, 3 H), 4.28 (s, 2 H), 6.60 (d, *J*=8.79 Hz, 1 H), 6.77 - 6.88 (m, 2 H), 7.32 (t, *J*=7.69 Hz, 1 H), 7.37 - 7.47 (m, 1 H), 7.67 (d, *J*=5.27 Hz, 1 H), 7.73 (t, *J*=7.47 Hz, 1 H), 9.11 (s, 1 H), 10.23 (br. s., 1 H); HRMS [M+H] for $C_{16}H_{14}F_3N_3O_3S$, calcd., 386.0781, found, 386.0787; LCMS [M+H] = 386.3, 96% (t=2.84 min).

3-methoxy-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (21): To a solution of 6-bromo-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**8**) (200 mg, 0.83 mmol, 1.0 equiv) in 1,4-dioxane (10 mL) was added 3-methoxybenzenesulfonamide (310 mg, 1.66 mmol, 2.0 equiv) followed by N,N'-Dimethylethylenediamine (0.089 mL,0.828 mmol, 1.0 equiv), copper iodine (158 mg, 0.828 mmol, 1.0 equiv), and 40% potassium fluoride on alumina (601 mg, 4.14 mmol, 5.0 equiv). The reaction was placed under nitrogen and heated at 110 °C for 18 h. The reaction was judged completed via LCMS. The reaction was filtered through celite which was rinsed thoroughly with ethyl acetate. The filtrate was concentrated to a residue that was dissolved in methanol. The solution was passed through a SCX cartridge (5g) eluting with methanol. The eluent was concentrated to a yellow solid. The solid was purified via column chromatography (15 g silica, 98:2 dichloromethane:methanol) to

provide the desired material as a white solid (220 mg, 69%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.79 (s, 3 H), 3.76 (s, 3 H), 4.27 (s, 2 H), 6.53 - 6.67 (m, 1 H), 6.75 - 6.87 (m, 2 H), 7.09 - 7.26 (m, 3 H), 7.42 (t, *J*=8.01 Hz, 1 H), 9.08 (s, 1 H) 9.87 (s, 1 H); HRMS [M+H] for $C_{16}H_{18}N_3O_4S$, calcd., 348.1013, found, 348.1020; LCMS [M+H] = 348.1, 95% (t=3.50 min).

4-methoxy-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (22): To a stirred solution of 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (500 mg, 2.82 mmol, 1.0 equiv) in pyridine (4 mL) was added 4-methoxybenzene-1-sulfonyl chloride (583 mg, 2.82 mmol, 1.0 equiv) dissolved in dichloromethane (1 mL) at 0 °C. The resulting solution was stirred at room temperature for 2 h. The reaction was judged complete via TLC (5% methanol in dichloromethane). The reaction was concentrated under reduced pressure to dryness and the resulting residue was diluted with dichloromethane. The organic phase was washed with water, brine, dried over sodium sulfate, and concentrated. The residue was purified by column chromatography on alumina eluting with 1% methanol in dichloromethane to provide the desired material as a light brown solid (450 mg, 46%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.77 (s, 3 H), 3.79 (s, 3 H), 4.28 (s, 2 H), 6.58 (d, *J*=8.3 Hz, 1 H), 6.76-6.79 (m, 2 H), 7.03 (d, *J*=8.8 Hz, 2 H), 7.60 (d, *J*=8.8 Hz, 2 H), 9.11 (s, 1 H), 9.77 (s, 1 H); HRMS [M+H] for $C_{16}H_{18}N_3O_4S$, calcd., 348.1013, found, 348.1018; LCMS [M+H] = 348.2, 96% (t=2.69 min).

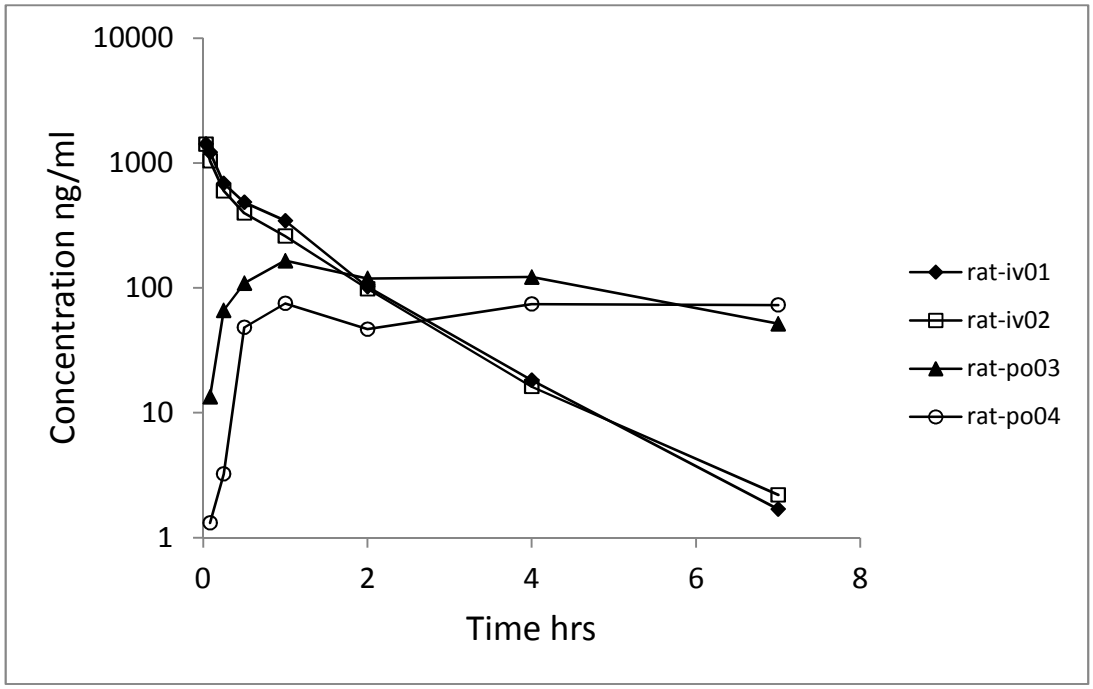
N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (23): To a stirred solution of 6-bromo-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**8**) (500 mg, 2.07 mmol, 1.0 equiv) and bezenesulfonamide (489 mg, 3.11 mmol. 1.5 equiv) in 1,4-dioxane (10 mL) was added Cs_2CO_3 (1.01 g, 3.11 mmol, 1.5 equiv) followed by degassing for 20 minutes. To the reaction mixture was then added 2-di-t-butylphosphino-2',4',6'-tri-i-propyl-1,1'-biphenyl (159 mg, 0.331 mmol, 0.16 equiv), and $Pd_2(dba)_3$ (209 mg, 0.227 mmol, 0.11 equiv). The reaction was heated to 100 °C for 16 h. The reaction was then cooled to room temperature and was diluted with ethyl acetate (100 mL) and filtered through celite. The

filtrate was concentrated to a residue that was purified via column chromatography to provide the desired material as a solid (250 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.80 (s, 3 H) 4.28 (s, 2 H), 6.58 (d, *J*=8.2 Hz, 1 H), 6.77-6.80 (m, 2 H), 7.51-7.70 (m, 5 H), 9.11 (s, 1 H), 9.93 (s, 1 H); HRMS [M+H] for $C_{15}H_{16}N_3O_3S$, calcd., 318.0907, found, 318.0915; LCMS [M+H] = 318.2, 98% (t=2.81 min).

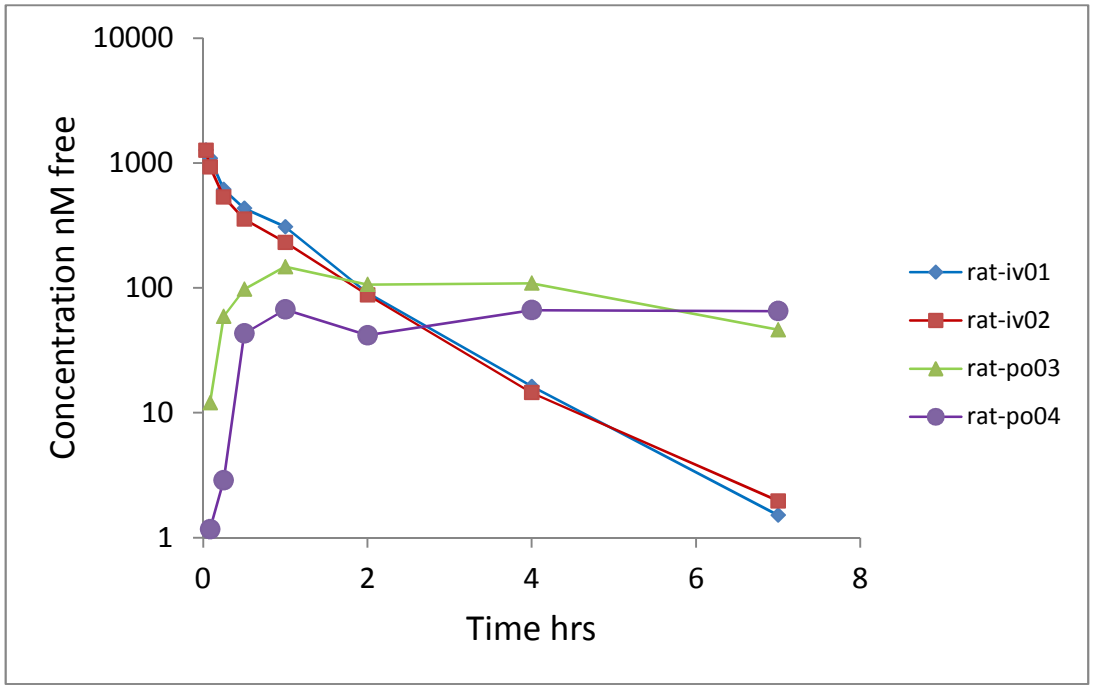
4-cyano-N-(3-methyl-2-oxo-1,2,3,4-tetrahydroquinazolin-6-yl)benzenesulfonamide (24): To a solution of 4-cyanobenzene-1-sulfonyl chloride (127 mg, 0.631 mmol, 1.5 equiv) in pyridine (1.0 mL) was added 6-amino-3-methyl-3,4-dihydroquinazolin-2(1H)-one (**11**) (100 mg, 0.421 mmol, 1.0 equiv). The reaction was stirred for 5 minutes at room temperature and dichloromethane (5 mL) was added. The reaction was stirred for an additional 1 h. The reaction was quenched with N,N,N'-trimethylethylenediamine (0.1 mL, 0.92 mmol, 2.2 equiv) and concentrated to a residue. The residue was partitioned between MTBE/ethyl acetate (1:1) and aqueous 2M HCl. The organic layer was collected, washed with water and brine, dried over sodium sulfate, filtered, and concentrated to a yellow solid. The solid was triturated with MTBE and filtered to provide the desired product as a white solid (36 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 2.81 (s, 3 H), 4.30 (s, 2 H), 6.62 (d, *J*=8.40 Hz, 1 H), 6.80 (s, 2 H), 7.71 - 7.88 (m, 2 H), 7.97 - 8.16 (m, 2 H), 9.14 (s, 1 H) 10.15 (s, 1 H); HRMS [M+H] for $C_{16}H_{15}N_4O_3S$, calcd., 343.0859, found, 343.0865; LCMS [M+H] = 343.1, >99% (t=1.54 min).

2. Rodent Pharmacokinetic profiles for Compound 17

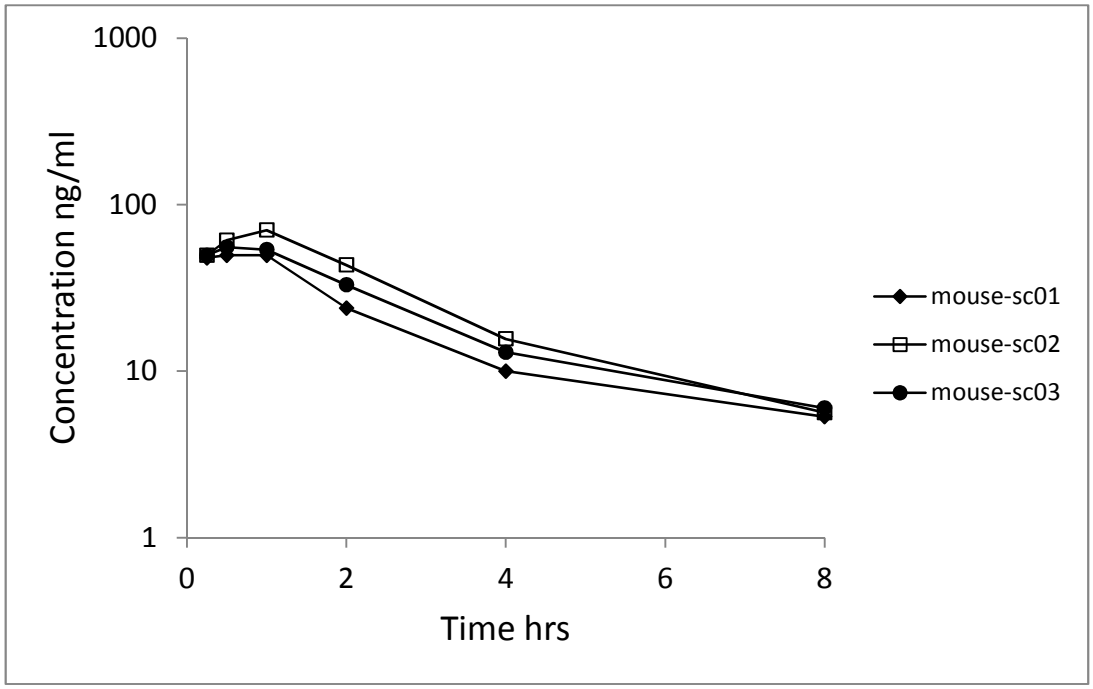
Supplementary Figure 1 Rat i.v./p.o. pharmacokinetic profile: Total drug concentration profile.



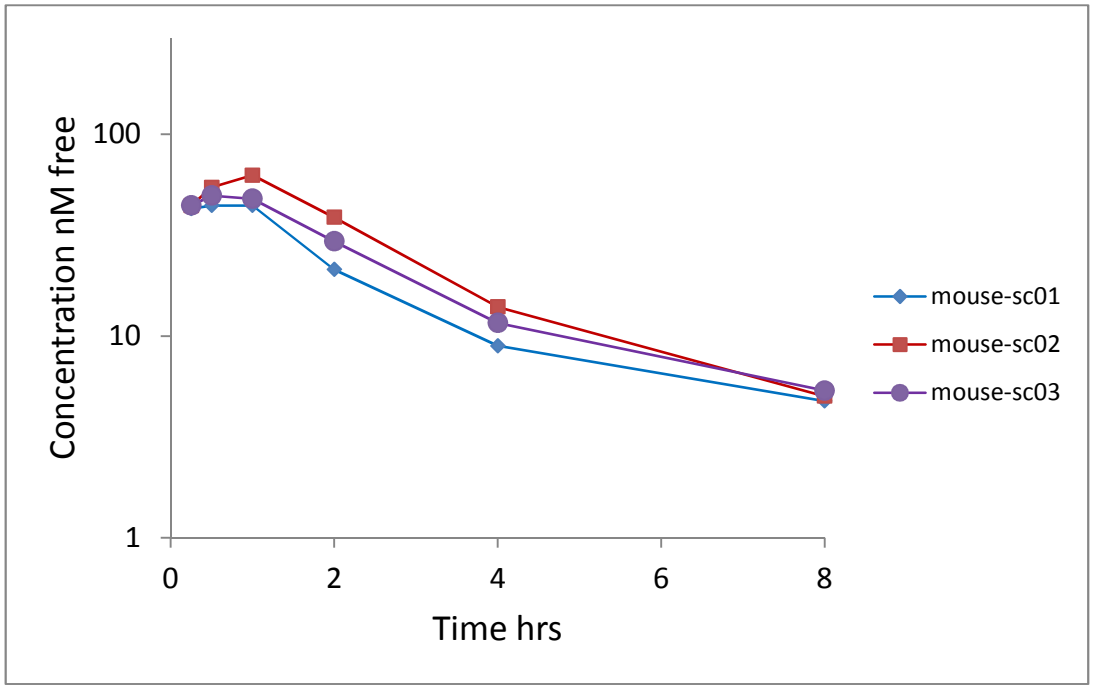
Supplementary Figure 2 Rat i.v./p.o. pharmacokinetic profile: Free drug concentrations.



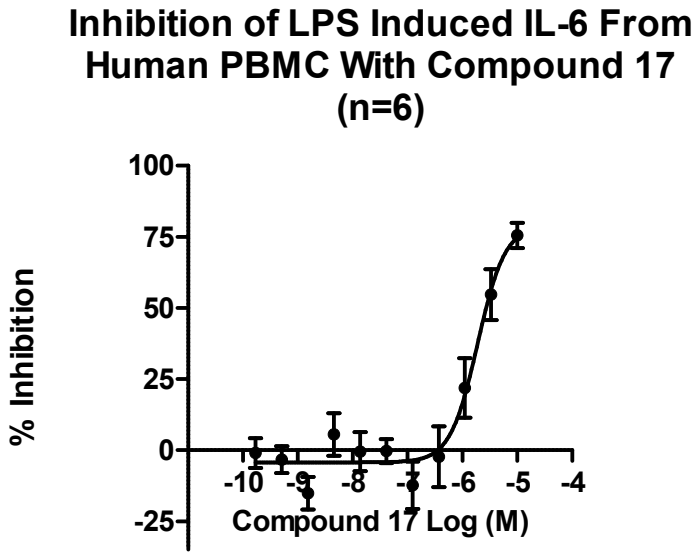
Supplementary Figure 3 Mouse s.c. pharmacokinetic profile: Total drug concentration profile.



Supplementary Figure 4 Mouse s.c. pharmacokinetic profile: Free drug concentration profile



3. Supplementary Figure 5 Inhibition of IL6 production by compound **17** in human PBMCs on stimulation by LPS



Bottom	-4.306
Top	79.00
LogEC50	-5.724
HillSlope	1.743
EC50	1.888e-006

4. T_m shift Bromodomain selectivity at 10μM

Supplemental Table 1

Protein	**ΔT_m [°C]**	**Bromdomain Family**
BRD2(1)	4.61	BET
BRD2(2)	5.32	BET
BRD3(1)	5.24	BET
BRD3(2)	5.45	BET
BRD4(1)	6.48	BET
BRD4(2)	3.79	BET
BRDT(1)	2.08	BET
PB1(5)	0.83	Non- BET
PCAF	0.81	Non- BET
BAZ2B	0.3	Non- BET
CREBBP	1.72	Non- BET

5. X-Ray Statistics

Supplemental Table 2

Data collection and refinement statistics for Figures 3 and 4

PDB ID	4HBV	4HBW	4HBY	4HBX
Ligand	**8**	**12**	**14**	**13**
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions: a, b, c (Å) α, β, γ (deg)	36.69 46.40 76.87 90.00 90.00 90.00	36.65 46.02 77.31 90.00 90.00 90.00	43.70 48.47 61.34 90.00 90.00 90.00	43.48 48.37 61.03 90.00 90.00 90.00
Resolution* (Å)	1.63 (1.72-1.63)	1.68 (1.78-1.68)	1.59 (1.67-1.59)	1.62 (1.71-1.62)
Unique observations*	15933 (1998)	15402 (2189)	18169 (2586)	16951 (2415)
Completeness* (%)	96.6 (85.7)	100.0 (100.0)	99.3 (98.3)	99.9 (97.1)
Redundancy*	3.9 (2.7)	4.5 (4.3)	4.6 (4.6)	4.4 (3.9)
Rmerge*	0.066 (0.354)	0.097 (0.592)	0.099 (0.654)	0.068 (0.572)
I/ σI*	12.4 (2.1)	9.1 (2.0)	8.5 (2.0)	11.6 (2.0)
Resolution (Å)	1.63	1.68	1.59	1.62
R_{work} / R_{free} (%)	20.6/24.0	17.7/22.8	18.5/23.9	17.9/20.9
Number of atoms (protein/other/water)	1050/21/115	1073/23/170	1057/70/131	1087/20/157
B-factors (Å^2) (protein/other/water)	24.20/24.50/30.25	19.17/18.00/31.22	25.93/27.66/31.96	20.78/18.84/30.51
r.m.s.d bonds (Å) r.m.s.d angles (^{o})	0.016 1.621	0.015 1.575	0.015 1.641	0.015 1.594
Ramachadran Favoured (%) Allowed (%) Disallowed (%)	96.80 3.20 0.00	98.36 0.82 0.82	97.58 2.42 0.00	99.17 0.83 0.00

* Values in parentheses correspond to the highest resolution shell.

6. Supplementary References

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