

## Supporting Information

### Design and Optimization of Benzopiperazines as Potent Inhibitors of BET Bromodomains

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#### 1. BRD4 BD1 AlphaScreen Assay Conditions

The binding of exemplified compounds to BRD4 BD1 was assessed using 384-well AlphaScreen assay technology. His-epitope tagged BRD4 BD1<sub>144-168</sub> was cloned, expressed, and purified to homogeneity. BRD4 BD1 binding and inhibition was assessed by monitoring the engagement of biotinylated Histone H4 (1-21) K5/8/12/16 tetra-acetylated peptide with the targets using the AlphaScreen technology (PerkinElmer). Specifically, in a 384-well black or white flat bottom plate, BRD4 BD1 (50 nM final) was combined with peptide (50 nM final for BD1) in 50 mM HEPES (pH 7.3), 100 mM NaCl, 0.1% (w/v) BSA, and 0.01% (w/v) Triton X-100 either in the presence of DMSO (final 1.25% DMSO) or compound dilution series in DMSO. Alpha streptavidin donor beads and Nickel-chelate acceptor beads were added to a final concentration of 10 µg/ml each. After a minimum of 1 hour equilibration, plates were read on a BMG PHERAstar FS multi-label reader (BMG LabTech). The half maximal inhibitory concentration (IC<sub>50</sub>) values were calculated using IDBS Activity Base software with a four parameter logistic curve fit by the equation  $y = A + ((B - A) / (1 + ((C/x)^D)))$ , wherein A denotes the bottom plateau of the curve, B denotes the top plateau of the curve, C denotes the x value at the middle of the curve, D denotes the slope factor, x denotes the original known x values, and y denotes the original known y values. Data was fitted using the Levenburg Marquardt algorithm. (+)-JQ1 was used as control compound in this assay system and has a mean IC<sub>50</sub> of 0.17 µM.

## 2. MV-4-11 Anti-Proliferative Assay Conditions

The impact of exemplified compounds on cancer cell proliferation was determined using the acute myelocytic leukemia (AML) cell line MV-4-11 (ATCC) in a 3-day proliferation assay. MV-4-11 cells were maintained in RPMI 1640 media supplemented with 10% FBS at 37 °C and an atmosphere of 5% CO<sub>2</sub>. For compound testing, compound dilutions series were prepared in DMSO via a 3-fold serial dilution from 2 mM to 0.001 mM in 384-well white flat bottom plates. The final compound concentrations in the wells were 10, 3.3, 1.1, 0.37, 0.12, 0.041, 0.013 and 0.0045 μM. MV-4-11 cells were plated at a density of 3000 cells/well in final volume of 50 μl culture media and incubated for 72 hours. The amounts of viable cells were determined using the CellTiter-Glo Luminescent Cell Viability Assay kit (Promega) according to the manufacturer suggested protocol. Luminescent signal from the CellTiter-Glo assay was read on an EnVision multilabel plate reader (PerkinElmer). Values for the concentration that inhibited cell growth by 50% (GI<sub>50</sub>) between the DMSO control and background control (no cells) were determined using IDBS Activity Base software with a four parameter logistic curve fit by the equation  $y = A + ((B-A)/(1+((C/x)^D)))$ , wherein A denotes the bottom plateau of the curve, B denotes the top plateau of the curve, C denotes the x value at the middle of the curve, D denotes the slope factor, x denotes the original known x values, and y denotes the original known y values. Data was fitted using the Levenburg Marquardt algorithm. (+)-JQ1 was used as control compound in this assay system and has a mean GI<sub>50</sub> of 0.06 μM.

## 3. Mouse PO PK Experiment Conditions

Female Athymic nude mice (Hsd:Athymic Nude-Foxn1nu) were received at 4-5 weeks of age. All mice were acclimated prior to handling. Compound **FT001** was administered as a solution in PEG400/ ethanol (9:1, 10 mL/kg) via the oral route as a single dose of 5 mg/kg (N = 3). Blood was collected at the time points indicated in lithium heparin containing tubes, and plasma levels determined on a AB Sciex QTrap 5500 Mass Spectrometer (Table S1). At 24 hours, only one mouse had a plasma concentration below the lower limit of quantitation (i.e., <0.500 ng/mL), but was included to assist in defining the elimination phase. It should be noted that one of the 8 hour results was inconsistent with the other two results and was not included in the non-compartmental analysis (NCA). This sample was repeated in Run S0012 and found to be consistent with the original observation (0.202 vs 0.316 ng/mL). All procedures were carried out under the institutional guidelines of Translational Drug Development Institutional Animal Care and Use Committee (Protocol # 14083).

Table S1. Mouse plasma concentrations following a 5 mg/kg PO dose of compound **FT001**

Replicate	Mouse Plasma Concentrations of compound <b>FT001</b> (ng/mL)							
	0.00 hrs	0.25 hrs	0.50 hrs	1.0 hrs	2.0 hrs	4.0 hrs	8.0 hrs	24.0 hrs
1	BLQ	424	571	622	805	625	287	0.648
2	BLQ	351	619	642	568	650	444	0.412*
3	BLQ	454	569	631	763	912	0.316*	1.15
Mean	N/A	410	586	632	712	729	244	0.737
Std Dev	N/A	53.0	28.3	10.0	126	159	225	0.377
RSD (%)	N/A	12.9	4.83	1.59	17.8	21.8	92.3	51.2

BLQ = Below Limit of Quantitation (<0.500 ng/mL); N/A = Not Applicable; \* BLQ

The data was then analyzed in Phoenix-WinNonLin and the Pharmacokinetic (PK) parameters calculated (Table S2).

Table S2. PK parameters for 5 mg/kg PO dose

Matrix	Half-life (hr)	Tmax (hr)	Cmax (ng/mL)	AUC <sub>0-8</sub> (ng*hr/mL)	AUC INF (ng*hr/mL)	AUC % Extrapolated (%)	V/F (mL/kg)	Cl/F (mL/hr/kg)
Plasma	1.79	4.0	729	4782	7714	.03	1815	648

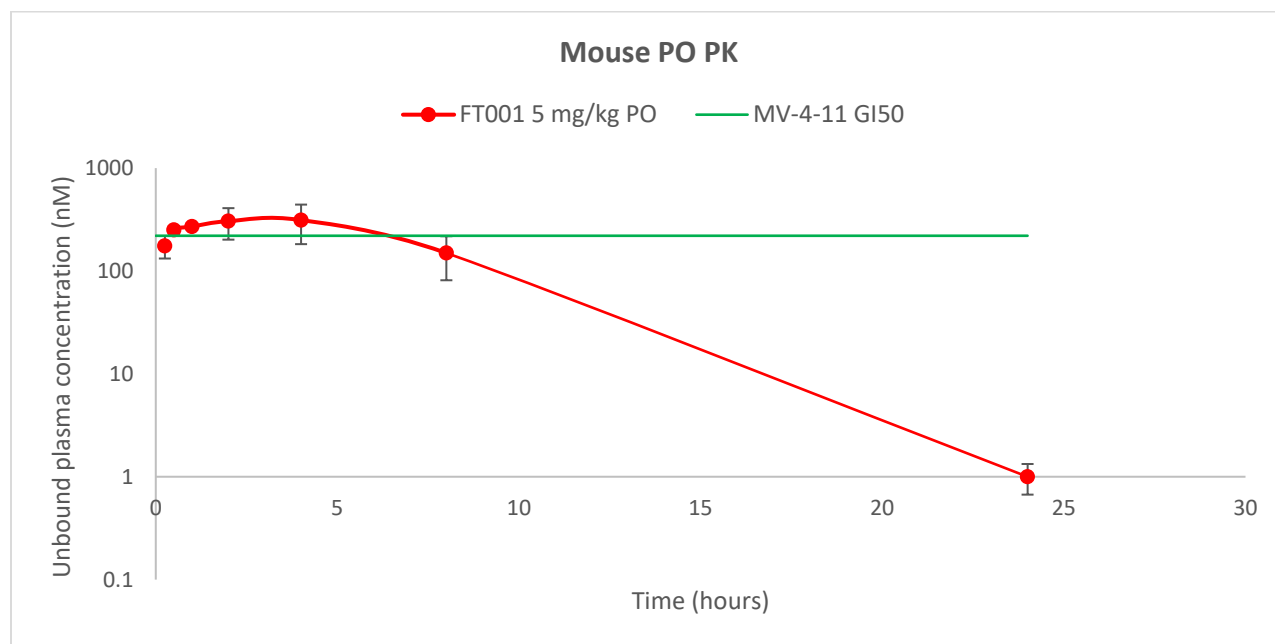


Figure S1. PK Profile for Compound **FT001** after a 5 mg/kg oral dose.

#### 4. Pharmacodynamic Experiment (*MYC* mRNA suppression) Conditions

Female Athymic nude mice (Hsd:Athymic Nude-Foxn1nu) were received at 4-5 weeks of age. All mice were acclimated prior to handling. Female mice were inoculated subcutaneously in the right flank with 0.1 mL of a 50% IMDM / 50% Matrigel™ (BD Biosciences; Bedford, MA) mixture containing a suspension of MV-4-11 acute myeloid leukemia cells (approximately  $5 \times 10^6$  cells/mouse). At time of inoculation, the mice were 6-7 weeks old. When tumors reached 140 mm<sup>3</sup> a single oral dose of 12.5 mg/kg was administered (solution formulation in PEG400/ ethanol, 9:1; 10 mL/kg) (N = 5). Animals were sacrificed four hours post dose and tumors collected. All procedures were carried out under the institutional guidelines of Translational Drug Development Institutional Animal Care and Use Committee (Protocol # 14090).

Tumor *MYC* mRNA evaluation by qRT-PCR was performed following homogenization. RNA purification was performed using the RNeasy Plus Mini Kit (Qiagen) on a QIAcube instrument. *MYC* and *GAPDH* (loading control reference) mRNA levels were measured using the one-step QuantiTect Multiplex RT-PCR Kit (Qiagen) and TaqMan Gene Expression Assay primer/probe sets (Life Technologies). The qRT-PCR data analysis method used was the comparative Ct method, also known as the 2<sup>-[delta][delta]Ct</sup> method. *MYC* mRNA levels were normalized to *GAPDH* mRNA levels in each sample, and normalized gene expression

levels were expressed relative to Vehicle control levels, where Vehicle control = 100% *MYC* mRNA (Table S3, Figure S2).

Table S3. *MYC* mRNA determination in MV-4-11 tumors following a 12.5 mg/kg single PO dose of compound **FT001**

Replicate	Vehicle	12.5 mg/kg Compound <b>FT001</b>
1	59.70	23.91
2	131.61	19.85
3	122.17	20.05
4	121.97	28.42
5	64.57	29.17
Mean	100.00	24.28
Std Dev	34.83	4.44
SEM	15.58	1.98

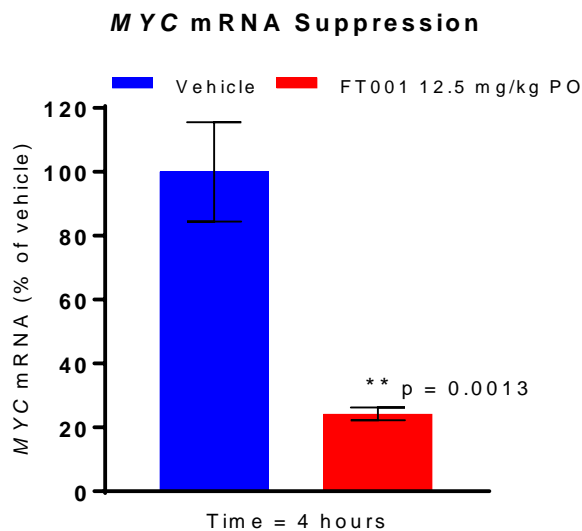


Figure S2. Suppression of *MYC* mRNA in an MV-4-11 mouse xenograft study measured 4 h after a single oral dose of **FT001** at 12.5 mg/kg. The *P* value (two-tailed) was calculated by using the unpaired *t* test. Error bars represent +/- SEM.

### 5. MV-4-11 Mouse Xenograft Efficacy Experiment

Female Athymic nude mice (Hsd:Athymic Nude-Foxn1nu) were received at 4-5 weeks of age. All mice were acclimated prior to handling. Female mice were inoculated subcutaneously in the right flank with 0.1 mL of a 50% IMDM / 50% Matrigel™ (BD Biosciences; Bedford, MA) mixture containing a suspension of MV-4-11 acute myeloid leukemia cells (approximately  $5 \times 10^6$  cells/mouse). At time of inoculation, the mice

were 6-7 weeks old. When tumors reached 140 mm<sup>3</sup> an oral dose of 12.5 mg/kg of compound **FT001** was administered twice daily for 20 days (solution formulation in PEG400/ ethanol, 9:1; 10 mL/kg) (N = 8). Tumor sizes (Table S4) and body weights (Figure S3) were measured twice weekly. Tumor volumes were calculated utilizing the formula: Tumor volume (mm<sup>3</sup>) = (a x b<sup>2</sup>)/2 where 'b' is the smallest diameter and 'a' is the largest diameter on the day tumors were measured. Mean tumor growth inhibition (TGI) was calculated on day 20 utilizing the formula:

$$\text{TGI} = \left[ 1 - \frac{(\bar{\chi}_{\text{Treated (Final)}} - \bar{\chi}_{\text{Treated (Day1)}})}{(\bar{\chi}_{\text{Vehicle Control (Final)}} - \bar{\chi}_{\text{Vehicle Control (Day1)}})} \right] \times 100\%$$

All procedures were carried out under the institutional guidelines of Translational Drug Development Institutional Animal Care and Use Committee (Protocol # 14089).

Table S4. MV-4-11 tumor sizes for treatment with vehicle (A) and compound **FT001** (B)

A

Mouse	Day of treatment with vehicle						
	1	3	8	12	15	19	20
1	123	255	443	953	1,119	1,330	1,533
2	135	367	629	856	1,293	2,664	2,741
3	151	332	498	1,221	1,748	2,914	3,026
4	203	208	243	601	842	1,616	1,946
5	136	197	219	473	828	1,242	1,401
6	101	200	240	492	688	1,347	1,457
7	123	176	216	470	671	1,368	1,481
8	152	224	318	684	1,098	1,813	2,032
Mean	<b>140.4</b>	<b>244.9</b>	<b>350.7</b>	<b>718.6</b>	<b>1,036.0</b>	<b>1,786.7</b>	<b>1,952.1</b>
Std Dev	<b>30.13</b>	<b>69.12</b>	<b>155.09</b>	<b>271.12</b>	<b>362.36</b>	<b>648.60</b>	<b>624.52</b>
Std Err	<b>10.65</b>	<b>24.44</b>	<b>54.83</b>	<b>95.86</b>	<b>128.11</b>	<b>229.32</b>	<b>220.80</b>

B

Mouse	Day of treatment with 12.5 mg/kg compound <b>FT001</b>						
	1	3	8	12	15	19	20
1	113	152	227	208	197	202	207
2	176	282	296	282	240	291	277
3	141	164	187	102	86	0	0
4	131	133	57	55	FD	FD	FD
5	119	138	152	83	34	0	0
6	146	216	161	106	69	55	29
7	133	141	101	93	89	87	74
8	163	282	296	256	284	276	273
Mean	<b>140.1</b>	<b>188.3</b>	<b>184.6</b>	<b>148.2</b>	<b>142.6</b>	<b>130.1</b>	<b>122.9</b>

Std Dev	FT001.22	63.22	85.80	86.97	96.44	124.90	125.62
Std Err	7.50	22.35	30.33	30.75	36.45	47.FT001	47.48

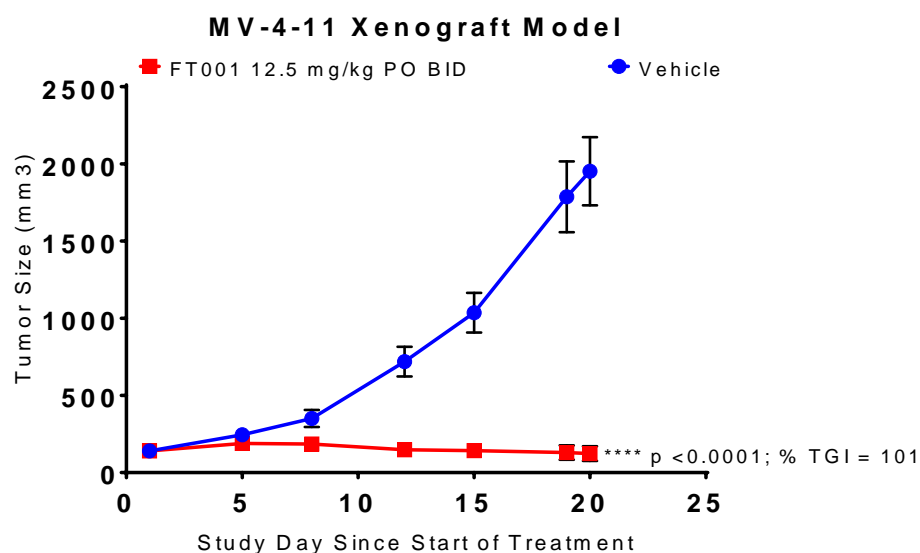


Figure S3. Tumor growth inhibition of an MV-4-11 mouse xenograft following 20 days continuous twice daily oral dosing of **FT001** at 12.5 mg/kg. The *P* value was calculated relative to Vehicle group on day 20 by using RM-ANOVA. Error bars represent +/- SEM.

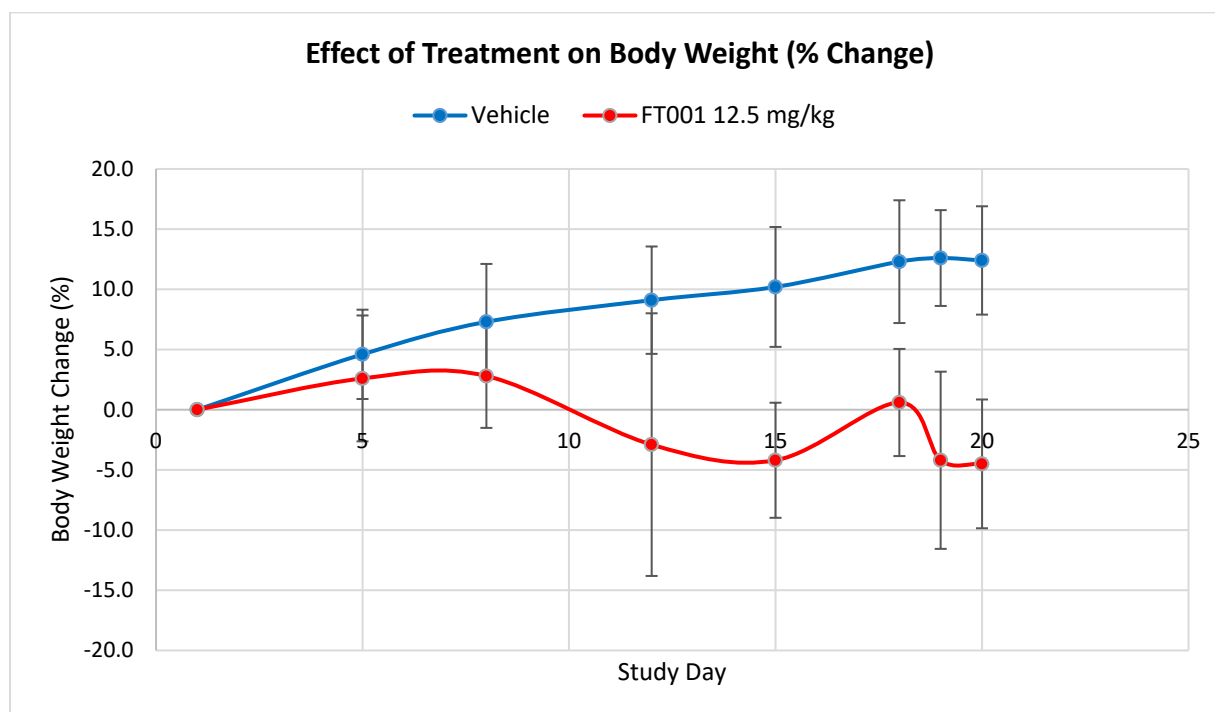


Figure S4. Effect of compound **FT001** (5 mg/kg) treatment on body weight following 20 days continuous twice daily PO dosing

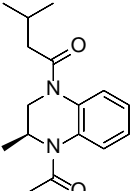
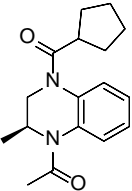
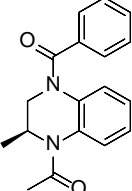
## 6. Characterization of described compounds and Synthesis and Characterization of FT001

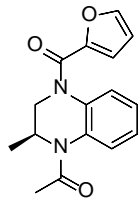
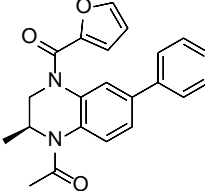
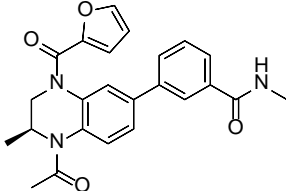
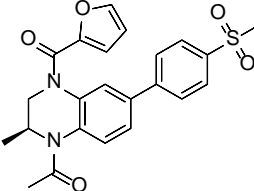
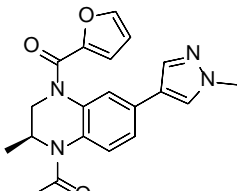
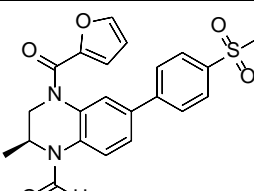
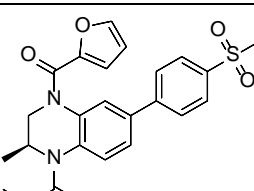
Unless otherwise noted, all materials were obtained from commercial suppliers and were used without further purification. Anhydrous solvents were obtained from Sigma-Aldrich (Milwaukee, WI) and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen atmosphere. Unless otherwise noted, mass-triggered HPLC purification and/or purity and low resolution mass spectral data were measured using either: (1) Waters Acquity ultra performance liquid chromatography (UPLC) system (Waters Acquity UPLC with Sample Organizer and Waters Micromass ZQ Mass Spectrometer) with UV detection at 220 nm and a low resonance electrospray positive ion mode (ESI) or (2) Waters HT2790 Alliance high performance liquid chromatography (HPLC) system (Waters 996 PDA and Waters ZQ Single Quad Mass Spectrometer) with UV detection at 220 nm and 254 nm and a low resonance ESI (positive/negative) mode. All compounds were purified to  $\geq 85\%$  purity as judged by LCMS. High resolution mass spectral (HRMS) data was measured on a Waters Xevo G2 Q-TOF with UPLC I class.  $^1\text{H}$  and  $^{13}\text{C}$  NMR was measured on a 400 MHz Varian Mercury 400 spectrometer at ambient temperature.

Experimental details and characterization of all described compounds are reported in a FORMA Therapeutics patent application (WO2015074081).<sup>1</sup>

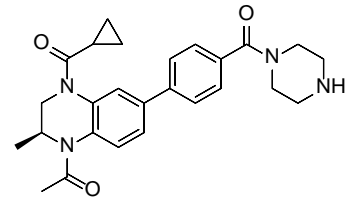
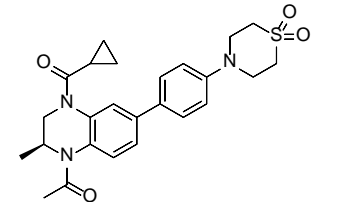
All described compounds were characterized by low resolution mass spectrometry (LRMS) (Table S5) and **FT001** was characterized by  $^1\text{H}$  and  $^{13}\text{C}$  NMR, LRMS and high resolution mass spectrometry (HRMS) (see below).

Table S5. LRMS for described compounds

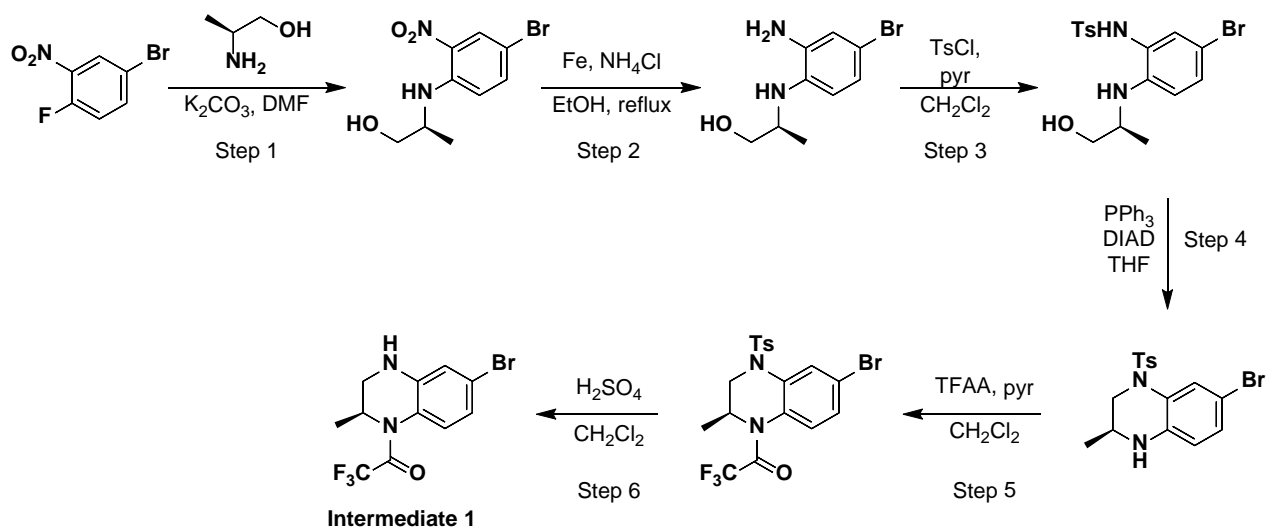
Example	Structure	Molecular Formula	Exact Mass	MS (ESI, $m/z$ ) [M+H] <sup>+</sup>
3		C <sub>16</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	274.17	275
4		C <sub>17</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub>	286.17	287
5		C <sub>18</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	294.14	295

Example	Structure	Molecular Formula	Exact Mass	MS (ESI, $m/z$ ) [M+H] <sup>+</sup>
6		C <sub>16</sub> H <sub>16</sub> N <sub>2</sub> O <sub>3</sub>	284.12	285
7		C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub>	360.15	361
8		C <sub>24</sub> H <sub>23</sub> N <sub>3</sub> O <sub>4</sub>	417.17	418
9		C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>5</sub> S	438.12	439
10		C <sub>20</sub> H <sub>20</sub> N <sub>4</sub> O <sub>3</sub>	364.15	365
11		C <sub>22</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> S	424.11	425
12		C <sub>24</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub> S	452.14	453

Example	Structure	Molecular Formula	Exact Mass	MS (ESI, $m/z$ ) [M+H] <sup>+</sup>
13		C <sub>25</sub> H <sub>24</sub> N <sub>2</sub> O <sub>5</sub> S	464.14	465
14		C <sub>23</sub> H <sub>22</sub> N <sub>2</sub> O <sub>6</sub> S	454.12	455
15		C <sub>22</sub> H <sub>26</sub> N <sub>2</sub> O <sub>5</sub> S	430.16	431
16		C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>4</sub> S	412.15	413
17		C <sub>21</sub> H <sub>26</sub> N <sub>4</sub> O <sub>2</sub>	366.21	367
18		C <sub>21</sub> H <sub>24</sub> N <sub>4</sub> O <sub>3</sub>	380.18	381
19		C <sub>21</sub> H <sub>25</sub> N <sub>5</sub> O <sub>2</sub>	379.20	380

Example	Structure	Molecular Formula	Exact Mass	MS (ESI, $m/z$ ) [M+H] <sup>+</sup>
20		C <sub>26</sub> H <sub>30</sub> N <sub>4</sub> O <sub>3</sub>	446.23	447
FT001		C <sub>25</sub> H <sub>29</sub> N <sub>3</sub> O <sub>4</sub> S	467.19	468

### Intermediate 1. (S)-1-(6-bromo-2-methyl-3,4-dihydroquinoxalin-1(2H)-yl)-2,2,2-trifluoroethanone



#### Step 1. (S)-2-(4-bromo-2-nitrophenylamino)propan-1-ol

A 1-L, three-necked, round bottomed flask fitted with a nitrogen inlet, overhead stirrer, thermocouple, and condenser was charged with 4-bromo-1-fluoro-2-nitrobenzene (46 g, 209 mmol), DMF (230 mL), potassium carbonate (31.8 g, 230 mmol), and (S)-2-aminopropan-1-ol (17.9 mL, 230 mmol). The reaction mixture was heated to 60 °C for 6 h and then cooled to rt and stirred overnight. The mixture was slowly diluted with water (690 mL), and the resulting slurry stirred at rt for 3 h and was then filtered. The solids were washed with water (120 mL) and dried under house vacuum (ca 17 Torr) overnight to afford (S)-2-(4-bromo-2-nitrophenylamino)propan-1-ol (72.98 g) as a yellow-orange solid which contained excess water, and was used in next step without further drying or purification. LRMS (ESI, pos. ion)  $m/z$  275 [M+1]<sup>+</sup>.

#### Step 2. (S)-2-(2-amino-4-bromophenylamino)propan-1-ol

A 2-L, three-necked, round bottomed flask fitted with a nitrogen inlet, overhead stirrer, thermocouple, and condenser was charged with iron powder (117 g, 2090 mmol), ammonium chloride (1.118 g, 20.90 mmol), ethanol (375 mL), and water (201 mL). The mixture was heated to 80 °C, and a solution of (S)-2-(4-bromo-2-nitrophenylamino)propan-1-ol (57.5 g, 209 mmol) in ethanol (200 mL) was slowly added. The mixture stirred at 80 °C for 4 h. The reaction mixture was filtered through a pad of Celite and washed with ethanol (100 mL). The filtrate was concentrated under reduced pressure to remove the ethanol, and the resulting aqueous slurry stirred at rt for 3 h. The slurry was then filtered, and the solids were washed with water (100 mL) and dried under a nitrogen atmosphere overnight to afford (S)-2-(2-amino-4-bromophenylamino)propan-1-ol (47.55 g, 89 %) as an off-white solid. LRMS (ESI, pos. ion) m/z 245 [M+1]<sup>+</sup>.

**Step 3. (S)-N-(5-bromo-2-(1-hydroxypropan-2-ylamino)phenyl)-4-methylbenzene sulfonamide**

A 1-L, round bottomed flask fitted with a nitrogen inlet, magnetic stir bar, and thermocouple was charged with (S)-2-(2-amino-4-bromophenylamino)propan-1-ol (47.55 g, 194 mmol), and pyridine (475 mL). The mixture was cooled to 0 °C and *p*-toluene sulfonyl chloride (37.0 g, 194 mmol) was added in portions of 12.6 and 24.4 g. The internal temperature rose from 1.5 to 15.6 °C following each addition. The reaction mixture stirred at 0 °C for 1.5 h and was then warmed to room temperature. The pyridine was removed under reduced pressure, and the residue was diluted with ethyl acetate (300 mL) and washed with water (100 mL). The combined organic phases were washed with half saturated brine (100 mL) and concentrated under reduced pressure. The residue was dissolved in toluene (250 mL), and the solution concentrated under reduced pressure (to remove some residual pyridine). The crude product was purified by column chromatography on silica gel (eluting with 1:1 hexanes–ethyl acetate). The product-containing fractions were combined and concentrated under reduced pressure. Toluene (250 mL) was added, and the mixture was concentrated under reduced pressure to removed trace pyridine. The material was dried under vacuum (ca 17 Torr) to afford (S)-N-(5-bromo-2-(1-hydroxypropan-2-ylamino)phenyl)-4-methylbenzenesulfonamide (88 g, 110%) as a viscous yellow oil that contains 11 wt% toluene. LRMS (ESI, pos. ion) m/z 399 [M+1]<sup>+</sup>.

**Step 4. (S)-7-bromo-3-methyl-1-tosyl-1,2,3,4-tetrahydroquinoxaline<sup>2</sup>**

A 3-L, three-necked, round bottomed flask fitted with a nitrogen inlet, overhead stirrer, and thermocouple was charged with (S)-N-(5-bromo-2-(1-hydroxypropan-2-ylamino)phenyl)-4-methylbenzenesulfonamide (77.0 g, 193 mmol), THF (1925 mL), and triphenylphosphine (60.7 g, 231 mmol). The solution was cooled to 0 °C, and diisopropyl azodicarboxylate (DIAD) (41.2 mL, 212 mmol) was slowly added while maintaining the internal temperature below 7 °C. The reaction mixture stirred at 0 °C for 1.5 h and was then warmed to room temperature. The mixture was concentrated and the residue was purified by column chromatography on silica gel (eluting with dichloromethane). The product-containing fractions were combined and concentrated under reduced pressure. The material was treated with a mixture of 4:1 hexanes – dichloromethane, and the slurry was stirred for 3 h and then filtered. The solids were rinsed with cold (ca. 0 °C) solution of 4:1 hexanes – dichloromethane (50 mL) and dried to afford (S)-7-bromo-3-methyl-1-tosyl-1,2,3,4-tetrahydroquinoxaline (61.8 g, 84%) as a white solid. LRMS (ESI, pos. ion) m/z 381 [M+1]<sup>+</sup>.

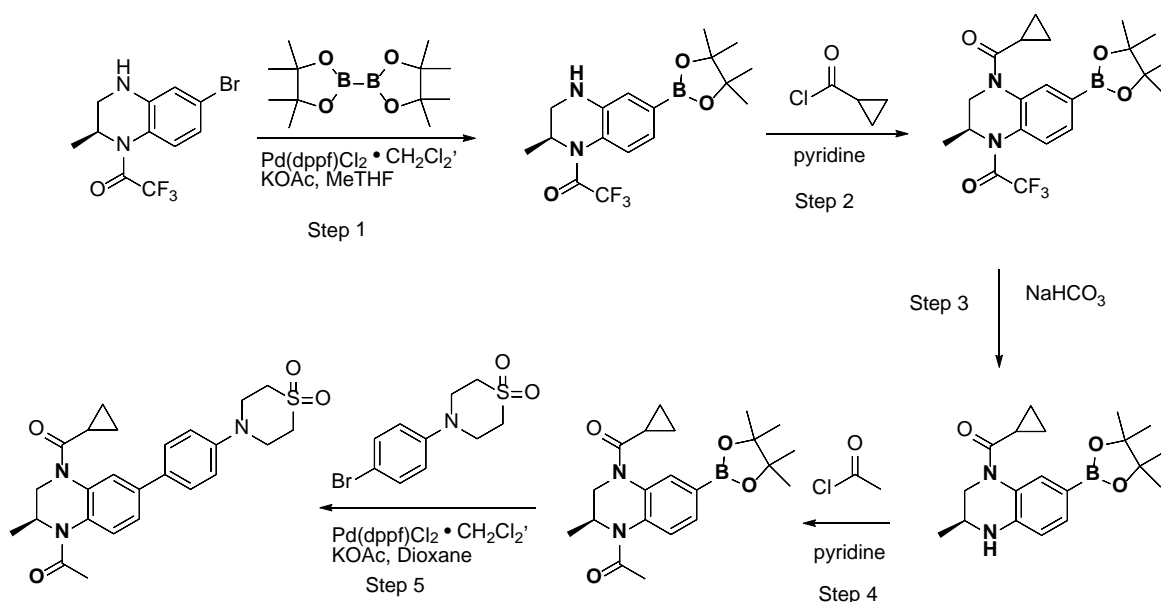
**Step 5. (S)-1-(6-bromo-2-methyl-4-tosyl-3,4-dihydroquinoxalin-1(2H)-yl)-2,2,2-trifluoro ethanone**

A 3-L, three-necked, round bottomed flask fitted with a nitrogen inlet, overhead stirrer, and thermocouple was charged with (S)-7-bromo-3-methyl-1-tosyl-1,2,3,4-tetrahydroquinoxaline (90.43 g, 237 mmol),

dichloromethane (1720 mL), and pyridine (28.8 mL, 356 mmol). The solution was cooled to 0 °C, and 2,2,2-trifluoroacetic anhydride (40.2 mL, 285 mmol) was slowly added while maintaining the internal temperature below 10 °C. Once the addition was complete, the reaction mixture was stirred at 0 °C for 30 min and then warmed to rt. After 1.5 h at rt, the reaction mixture was washed with water (200 mL), 1 M aqueous HCl solution (200 mL), and water (200 mL). The combined organic layers were concentrated under reduced pressure. The crude product was further dried under vacuum (ca 17 Torr) at rt during which time the product crystallized to afford (*S*)-1-(6-bromo-2-methyl-4-tosyl-3,4-dihydroquinoxalin-1(2*H*)-yl)-2,2,2-trifluoroethanone (113 g, 99%) as a tan solid. LRMS (ESI, pos. ion)  $m/z$  477[M+1]<sup>+</sup>.

**Step 6. (*S*)-1-(6-bromo-2-methyl-3,4-dihydroquinoxalin-1(2*H*)-yl)-2,2,2-trifluoroethanone**

A 3-L, three-necked, round bottomed flask fitted with a nitrogen inlet, magnetic stir bar, and thermocouple was charged with (*S*)-1-(6-bromo-2-methyl-4-tosyl-3,4-dihydroquinoxalin-1(2*H*)-yl)-2,2,2-trifluoroethanone (106.81 g, 224 mmol), dichloromethane (1070 mL), and sulfuric acid (18 M, 119 mL, 2238 mmol). The reaction mixture stirred at rt for 1 h and was then cooled to 0 °C. Water (1070 mL) was slowly added in 100 mL portions, while maintaining the internal temperature at ca. 0 °C. (Note: the internal temperature rose from ca. 2.6 °C to 23.8 °C with each addition). After the final water addition, the internal temperature was 34 °C, and the mixture was cooled to rt. The aqueous layer was separated extracted with dichloromethane (700 mL). The combined organic layers were washed with water (700 mL) and then concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluting with 100% dichloromethane). The product-containing fractions were combined and concentrated under reduced pressure. Further drying under vacuum (ca. 17 Torr) at rt afforded (*S*)-1-(6-bromo-2-methyl-3,4-dihydroquinoxalin-1(2*H*)-yl)-2,2,2-trifluoroethanone (71.8 g, 99 %) as a brown solid. LRMS (ESI, pos. ion)  $m/z$  323[M+1]<sup>+</sup>.



**Step 1. (*S*)-2,2,2-trifluoro-1-(2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2*H*)-yl)ethanone**

A 500-mL round bottom flask fitted with a nitrogen inlet, magnetic stir bar, and condenser was charged with (*S*)-1-(6-bromo-2-methyl-3,4-dihydroquinoxalin-1(2*H*)-yl)-2,2,2-trifluoroethanone (10 g, 30.9 mmol),

2-methyltetrahydrofuran (200 mL), potassium acetate (7.59 g, 77.0 mmol), and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (15.72 g, 61.9 mmol). Nitrogen was bubbled through the mixture for 30 min and then PdCl<sub>2</sub>(dppf)•CH<sub>2</sub>Cl<sub>2</sub> adduct (0.253 g, 0.309 mmol) was added. The mixture was heated to 75 °C and monitored by LC/MS for reaction completion. Upon reaction completion (ca. 24 h) the mixture was cooled to room temperature, filtered and solids washed with 2-methyltetrahydrofuran (100 mL). The filtrate was washed with half saturated brine (50 mL). The organic layer was concentrated under reduced pressure and purified by flash chromatography on silica gel (eluting with 5:1 hexane-ethyl acetate and ending with 2:1 hexane-ethyl acetate) to afford (S)-2,2,2-trifluoro-1-(2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)ethanone (10.62 g, 93%) as a foam. LRMS (ESI, pos. ion) m/z 371 [M+1]<sup>+</sup>.

**Step 2. (S)-1-(4-(cyclopropanecarbonyl)-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)-2,2,2-trifluoroethanone**

A 250-mL round bottom three neck flask fitted with nitrogen inlet, magnetic stir bar, and thermocouple was charged with (S)-2,2,2-trifluoro-1-(2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)ethanone (9.88 g, 26.7 mmol), and pyridine (3.24 mL, 40.0 mmol). The reaction was cooled to 0 °C and cyclopropanecarbonyl chloride (2.91 mL, 32.0 mmol) was added slowly, maintaining the temperature below 7 °C. The mixture was stirred cold for 20 min and then allowed to warm to rt and monitored for completion by LC/MS. Upon completion (ca. 1 h) the mixture was diluted with 1,2-dichloroethane (100 mL) and washed with 1M HCl (100 mL) and water (100 mL). The organic layer was dried with magnesium sulfate, filtered and washed with 1,2-dichloroethane (50 mL). The filtrate was concentrated under reduce pressure and further dried on house vacuum (ca. 17 torr). This afforded (S)-1-(4-(cyclopropanecarbonyl)-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)-2,2,2-trifluoroethanone (13.7 g, 117%) as a white solid containing 13 wt. % DCE. LRMS (ESI, pos. ion) m/z 439 [M+1]<sup>+</sup>.

**Step 3. (S)-cyclopropyl(3-methyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)methanone**

A 500-mL, three-necked round bottom flask fitted with a nitrogen inlet, magnetic stir bar, thermocouple, and condenser was charged with (S)-1-(4-(cyclopropanecarbonyl)-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)-2,2,2-trifluoroethanone (11.7 g, 26.7 mmol), ethanol (150 mL), and half-saturated aqueous sodium bicarbonate solution (82 mL, 422 mmol). The reaction mixture was heated to 78 °C and monitored by LC/MS for completion. Upon reaction completion (ca. 3 h), the mixture was cooled to rt, and the ethanol removed under reduced pressure. The aqueous layer was then extracted with dichloromethane (2 × 125 mL), and the combined organic layers were concentrated under reduced pressure. This afforded (S)-cyclopropyl(3-methyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)methanone (8.75 g, 96%) as an oil, which was used as is for the next step. LRMS (ESI, pos. ion) m/z 343 [M+1]<sup>+</sup>.

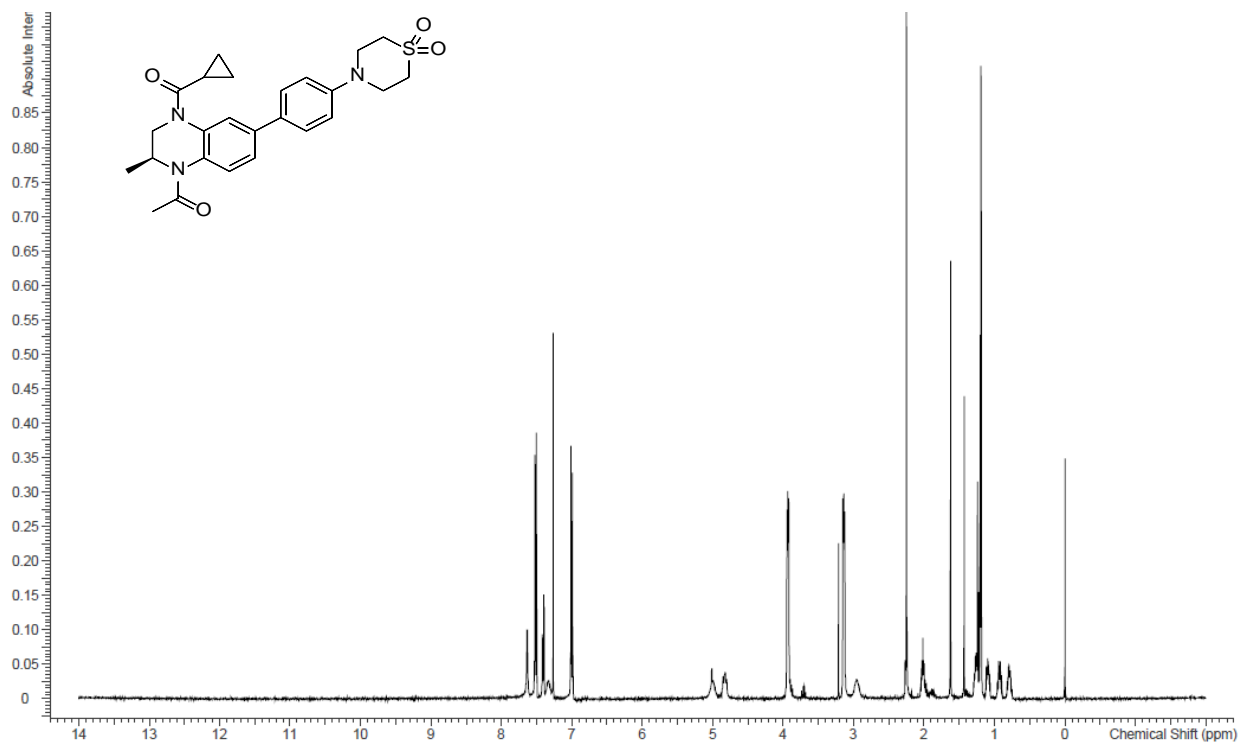
**Step 4. (S)-1-(4-(cyclopropanecarbonyl)-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)ethanone**

A 250-ml three neck round bottom flask fitted with nitrogen, magnetic stir bar, and thermocouple was charged with (S)-cyclopropyl(3-methyl-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)methanone (8.75 g, 25.6 mmol), dichloromethane (90 mL), and pyridine (3.10 mL, 38.4 mmol). The reaction was cooled to 0 °C and acetyl chloride (2.363 mL, 33.2 mmol) was added slowly, maintaining the temperature below 7 °C. The mixture was stirred cold for 30 min and then allowed to warm to rt and monitored for completion by LC/MS. Upon completion, the mixture was washed water (3x50 mL). The organic layer was concentrated under reduced pressure and purified by flash chromatography on silica gel (eluting with 1:1 hexane–ethyl acetate) to afford (S)-1-(4-(cyclopropanecarbonyl)-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)ethanone (8.35 g, 85%) as a solid that contained 9.4 wt.% ethyl acetate. LRMS (ESI, pos. ion) m/z 385 [M+1]<sup>+</sup>.

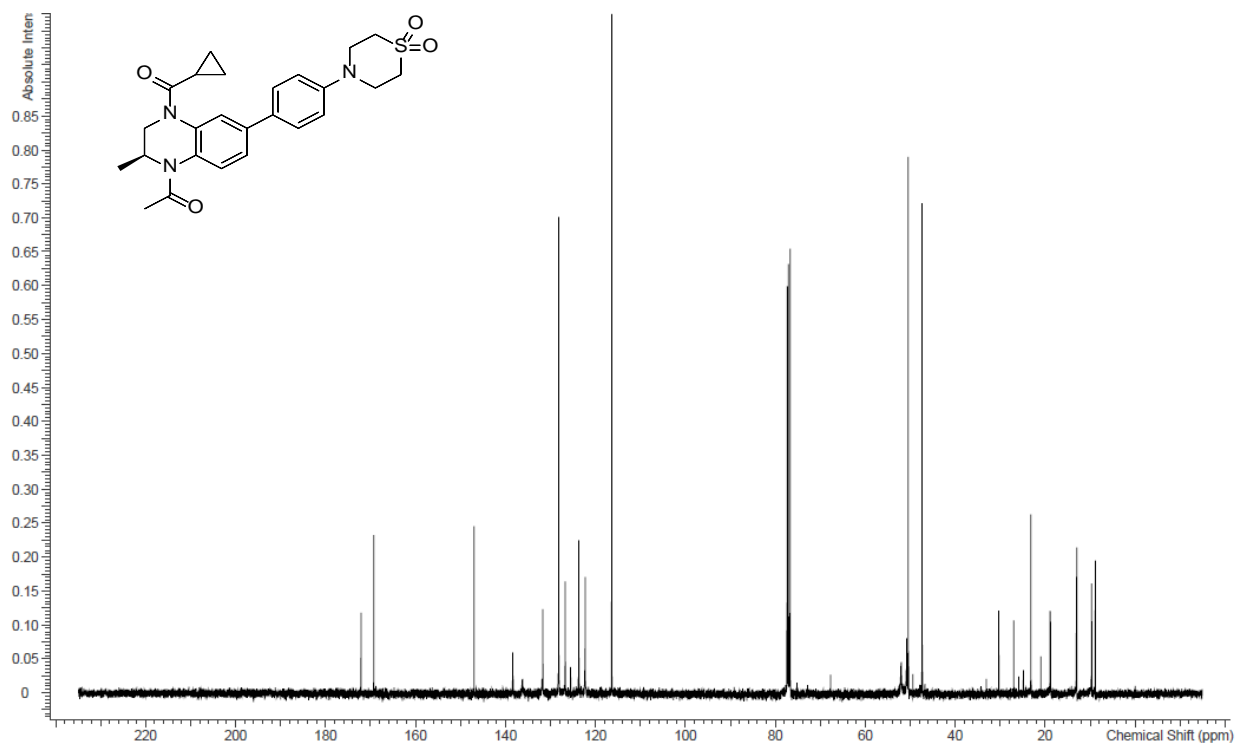
**Step 5: (S)-1-(4-(cyclopropanecarbonyl)-6-(4-(1,1-dioxidothiomorpholino)phenyl)-2-methyl-3,4-dihydroquinoxalin-1(2H)-yl)ethan-1-one, FT001**

A 250-ml three neck round bottom flask fitted with nitrogen inlet, magnetic stir bar, and thermocouple was charged with (S)-1-(4-(cyclopropanecarbonyl)-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroquinoxalin-1(2H)-yl)ethanone (7.56 g, 19.67 mmol), 4-(4-bromophenyl)thiomorpholine 1,1-dioxide (5.71 g, 19.67 mmol), dioxane (80 mL), and sodium hydrogen carbonate (1M in water, 59.0 mL, 59.0 mmol). The mixture was purged with nitrogen and then PdCl<sub>2</sub>(dppf)•CH<sub>2</sub>Cl<sub>2</sub> adduct (0.803 g, 0.984 mmol) was added. The mixture was heated to 80 °C and monitored for completion by LC/MS. Upon completion (ca. 5 h), the reaction mixture was cooled to rt and filtered through celite, washing with ethyl acetate (75 mL). Volatiles were removed under reduced pressure and the residue was further diluted with ethyl acetate (200 mL). The aqueous layer was separated and washed with fresh ethyl acetate (100 mL). The combined organic layers were concentrated under reduced pressure and purified by flash chromatography on silica gel (eluting with 4:1 ethyl acetate-hexanes) to afford (S)-1-(4-(cyclopropanecarbonyl)-6-(4-(1,1-dioxidothiomorpholino)phenyl)-2-methyl-3,4-dihydroquinoxalin-1(2H)-yl)ethan-1-one **FT001** (4.55 g, 49.5%) as an off white solid containing 2.3 wt.% 2-methyltetrahydrofuran and 2.0 wt.% MTBE. LRMS (ESI, pos. ion) m/z 468 [M+1]<sup>+</sup>. HRMS Exact mass for C<sub>25</sub>H<sub>29</sub>N<sub>3</sub>O<sub>4</sub>S required: 467.19; mass observed: [M+H]<sup>+</sup> 468.1959. [α]<sub>D</sub><sup>20</sup> +154.1° (c 0.3 g/100 mL, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ ppm 0.72 - 0.98 (m, 2 H) 1.01 - 1.15 (m, 2 H) 1.15 - 1.20 (m, 4 H) 1.20 - 1.33 (m, 3 H) 1.96 - 2.08 (m, 1 H) 2.24 (s, 3 H) 2.87 - 3.03 (m, 1 H) 3.09 - 3.17 (m, 4 H) 3.86 - 3.98 (m, 4 H) 4.73 - 4.89 (m, 1 H) 4.93 - 5.08 (m, 1 H) 6.95 - 7.05 (m, 2 H) 7.28 - 7.37 (m, 1 H) 7.37 - 7.44 (m, 1 H) 7.46 - 7.56 (m, 2 H) 7.58 - 7.68 (m, 1 H).

### FT001 <sup>1</sup>H NMR (CDCl<sub>3</sub>)



### FT001 <sup>13</sup>C NMR (CDCl<sub>3</sub>)



## 7. BET Selectivity Data for Compound FT001

The BET selectivity assay was run at DiscoverX against 40 bromodomains in BROMOscan™.

T7 phage strains displaying bromodomains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (5,000 x g) and filtered (0.2µm) to remove cell debris. Streptavidin-coated magnetic beads were treated with biotinylated small molecule or acetylated peptide ligands for 30 minutes at room temperature to generate affinity resins for bromodomain assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT) to remove unbound ligand and to reduce non-specific phage binding. Binding reactions were assembled by combining bromodomains, liganded affinity beads, and **FT001** in 1x binding buffer (17% SeaBlock, 0.33x PBS, 0.04% Tween 20, 0.02% BSA, 0.004% Sodium azide, 7.4 mM DTT). **FT001** was prepared as 1000X stock in 100% DMSO. Kds were determined using an 11-point 3-fold compound dilution series with one DMSO control point.

**FT001** was distributed by acoustic transfer (non-contact dispensing) in 100% DMSO and then diluted directly into the assays such that the final concentration of DMSO was 0.09%. All reactions were performed in polypropylene 384-well plates. Each was a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS, 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS, 0.05% Tween 20, 2 µM non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR.

Binding constants (Kds) were calculated with a standard dose-response curve using the Hill equation:

$$\text{Response} = \text{Background} + (\text{Signal} - \text{Background}) / (1 + (\text{Kd}^{\text{Hill Slope}} / \text{Dose}^{\text{Hill Slope}}))$$

The Hill Slope was set to -1.

Curves were fitted using a non-linear least square fit with the Levenberg-Marquardt algorithm.

Kds were determined for each bromodomain in duplicate and presented as the mean (Table S6).

Table S6. Kds for each bromodomain following incubation with compound **FT001**.

DiscoverX Gene Symbol	Modifier	Kd (nM)
ATAD2A	>	10000
ATAD2B	>	10000
BAZ2A	>	10000
BAZ2B	>	10000
BRD1	>	10000
BRD2(1)	=	58
BRD2(1,2)	=	71
BRD2(2)	=	77

BRD3(1)	=	24
BRD3(1,2)	=	32
BRD3(2)	=	37
BRD4(1)	=	69
BRD4(1,2)	=	31
BRD4(2)	=	31
BRD4(full-length,short-iso.)	=	33
BRD7	=	1100
BRD8(1)	>	10000
BRD8(2)	>	10000
BRD9	>	10000
BRDT(1)	=	47
BRDT(1,2)	=	85
BRDT(2)	=	53
BRPF1	>	10000
BRPF3	>	10000
CECR2	=	5100
CREBBP	=	3300
EP300	>	10000
FALZ	>	10000
GCN5L2	>	10000
PBRM1(2)	>	10000
PBRM1(5)	>	10000
PCAF	>	10000
SMARCA2	>	10000
SMARCA4	>	10000
TAF1(2)	=	7100
TAF1L(2)	>	10000
TRIM24(Bromo.)	>	10000
TRIM24(PHD,Bromo.)	>	10000
TRIM33(PHD,Bromo.)	>	10000
WDR9(2)	=	8600

## 8. Crystallographic Data for Compound 8

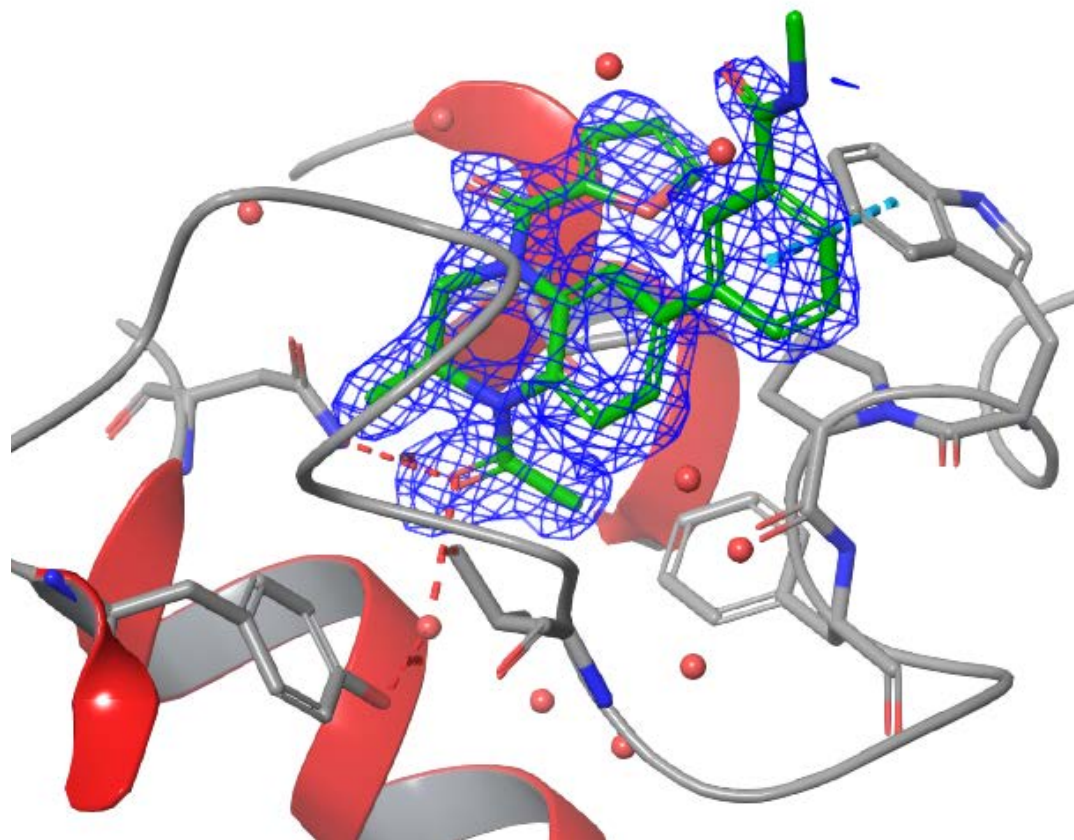


Figure S5. A co-crystal structure of **8** (green) bound to BRD4 BD1. PDB code: 5VOM. Key hydrogen bond interactions between **8**, Asn140, a highly conserved water molecule and Tyr97 are indicated (red dotted line). An edge-to-face interaction between **8** and Trp81 is also indicated (cyan dotted line).

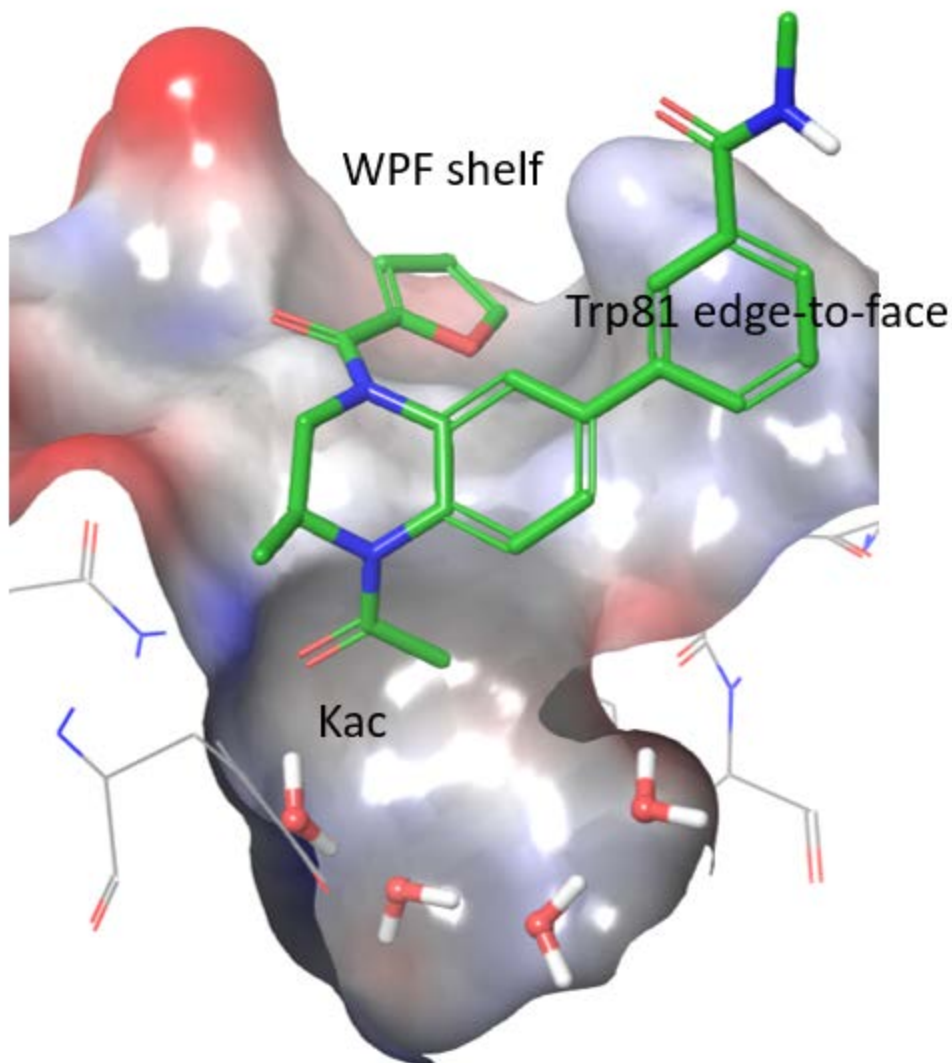


Figure S6. A co-crystal structure of **8** (green) bound to BRD4 BD1. PDB code: 5VOM. WPF shelf, Trp81 and Kac binding regions are indicated.

The co-crystal structure of **8** bound to BRD4 BD1 was determined from crystals grown at 18 °C using the hanging drop technique. The crystallographic protein construct is the same as the alpha screen assay construct (residues 44-168) except that the His tag was removed by TEV cleavage. Crystals were grown using untagged BRD4 BD1<sub>44-168</sub> protein at 10 mg/mL concentration that was equilibrated against 30% PEG 4000, 0.1 M MES pH 6.8 for 4 days and then cryoprotected using 15% glycerol added to reservoir solution and then flash frozen in liquid nitrogen. Data were collected at the Australian Synchrotron MX2 beamline ADSC Q315r, and the structure refined by using refmac5. Coordinates are deposited with accession code: 5VOM. (Table S7).

Table S7. Crystallographic data for 8

<b>BRD4 – Compound 8</b>	
<b>Data collection</b>	
Space group	P1
Cell dimensions	
<i>a, b, c</i> (Å)	30.22 39.37 55.53
$\alpha, \beta, \gamma$ (°)	95.05 104.19 91.58
Resolution (Å)	50.00 - 1.66 (1.77-1.66) <sup>a</sup>
R <sub>sym</sub> or R <sub>merge</sub>	8.9 (38.2) <sup>a</sup>
I / $\sigma$	13.6 (4.56) <sup>a</sup>
Completeness (%)	96.1 (90.7) <sup>a</sup>
<b>Refinement</b>	
Resolution (Å)	39.18 - 1.66
R <sub>work</sub> / R <sub>free</sub>	0.17513 / 0.21777
No. of atoms	2490
protein	2117
ligand	62
water	311
B-factors	
protein	11.66
ligand	13.55
water	19.84
R.m.s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.469

<sup>a</sup>Values in parentheses for highest-resolution shell.

## 9. References

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- Samanta, K.; Srivastava, N.; Saha, S.; Panda, G. Inter- and intramolecular Mitsunobu reaction and metal complexation study: synthesis of *S*-amino acids derived chiral 1,2,3,4-tetrahydroquinoxaline, benzo-annulated [9]-N<sub>3</sub> peraza, [12]-N<sub>4</sub> peraza-macrocycles. *Org. Biomol. Chem.*, **2012**, *10*, 1553-1564.