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Supporting Information

Discovery of a Chemical Probe to Study Implications of BPTF Bromodomain Inhibition in Cellular and *in vivo* Experiments

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Supplementary Table 1. Data collection and refinement statistics

	BPTF BI-7190	**BRD9 BI-7189**
Data collection		
Resolution Limit defined by	STARANISO	STARANISO
Space group	P 2_1	P2_1 2_1 2
Cell dimensions		
a, *b*, *c* (Å)	27.24, 65.79, 39.73	68.88, 125.20, 29.46
α, β, γ (°)	90.00, 102.51, 90.00	90.00, 90.00, 90.00
Resolution (Å)	38.784-1.025 (1.091-1.025)[a]	62.600-1.504 (1.672-1.504)[a]
R_{merge}	8.5	7.52
$I / \sigma I$	11.1 (1.8)	19.4 (1.9)
Completeness (ellipsoidal) (%)	87.8 (40.4)[b]	91.3 (69.4)[b]
Completeness (spherical) (%)	79.9 (23.5) [b]	59.5 (11.1)[b]
CC(1/2)	0.995 (0.652)	0.999 (0.828)
Multiplicity	5.9 (2.7)	6.3 (4.7)
Refinement		
Resolution (Å)	38.78-1.025	62.6-1.504
No. reflections	54717	25083
R_{work} / R_{free}	15.4 / 17.1	21.1 /23.0
No. atoms		
Protein	1000	1827
Ligand/ion	28	48
Water	240	286
B-factors		
Protein	8.27	36.44
Ligand/ion	6,96	29.35
Water	23.42	46.01
R.m.s. deviations		
Bond lengths (Å)	0.010	0.008
Bond angles (°)	0.94	0.76

[a] highest reolution shell in parentheses
[b] resulting completeness after STARANISO for ellipsoidal / spherical shells

Supplementary Table 2. Bromodomain selectivity panel Discoverix (%ctrl)

Bromodomain	%CTL 10 µM BI-7190	%CTL 10 µM BI-4827
ATAD2A	46.0	70.0
ATAD2B	20.0	100.0
BAZ2A	27.0	66.0
BAZ2B	21.0	72.0
BRD1	0.0	52.0
BRD2(1)	40.0	38.0
BRD2(2)	56.0	33.0
BRD3(1)	31.0	40.0
BRD3(2)	50.0	25.0
BRD4(1)	49.0	39.0
BRD4(2)	58.0	27.0
BRD7	0.5	26.0
BRD9	0.0	16.0
BRDT(1)	34.0	56.0
BRDT(2)	76.0	76.0
BRPF1	0.3	41.0
BRPF3	16.0	68.0
CECR2	0.0	38.0
CREBBP	0.0	49.0
EP300	1.1	56.0
FALZ	0.0	67.0
GCN5L2	0.2	100.0
PBRM1(2)	89.0	100.0
PBRM1(5)	49.0	98.0
PCAF	9.7	83.0
SMARCA2	44.0	23.0
SMARCA4	58.0	82.0
TAF1(2)	23.0	53.0
TAF1L(2)	32.0	91.0
TRIM24(PHD,BROMO	19.0	80.0
TRIM33(PHD,BROMO.)	n.d.	86.0
WDR9(2)	74.0	58.0

Supplementary Table 3. Bromodomain selectivity panel DiscoveRx (K_d follow-up)

Bromodomain	%CTL 10 µM BI-7190	%CTL 10 µM BI-4827
FALZ@DRX	0.0029	-
BRD9@DRX	0.0260	
BRD7@DRX	0.0590	-
BRPF1@DRX	0.0620	-
CECR2@DRX	0.3900	-
EP300@DRX	1.1000	-
CREBBP@DRX	1.2000	-
BRD1@DRX	1.4000	-
PCAF@DRX	2.8000	-
TRIM24(PHD,BROMO@DRX	3.4000	-
BRPF3@DRX	3.6000	-
GCN5L2@DRX	>10.0000	-

Supplementary Table 4. Kinase selectivity panel INVITROGEN (%ctrl)

Kinase assay	%INHB @ 10 μM BI-7190
PDK1DIRECT	2.0
TBK1	1.0
STK3	-15.0
SRPK2	-2.0
ROCK2	1.0
PRKACA	1.0
PAK4	3.0
NEK2	-11.0
MYLK2	-3.0
MAPKAPK2	1.0
LCK	17.0
IGF1R	0.0
GSK3B	-4.0
FRAP1 (MTOR)	0.0
FGFR1	6.0
EPHB2	2.0
EGFR	3.0
CSNK2A1	0.0
CSNK1A1	1.0
CHEK1	1.0
CDK2/CYCLINA	2.0
CAMK1D	1.0
STK6	0.0
AMPK A1B1G1	-6.0
AKT2	5.0
ACVR1B	7.0
ABL1	0.0
RAF1	-4.0
MAPK14	-34.0
MAP3K8	3.0
MAP2K1	7.0

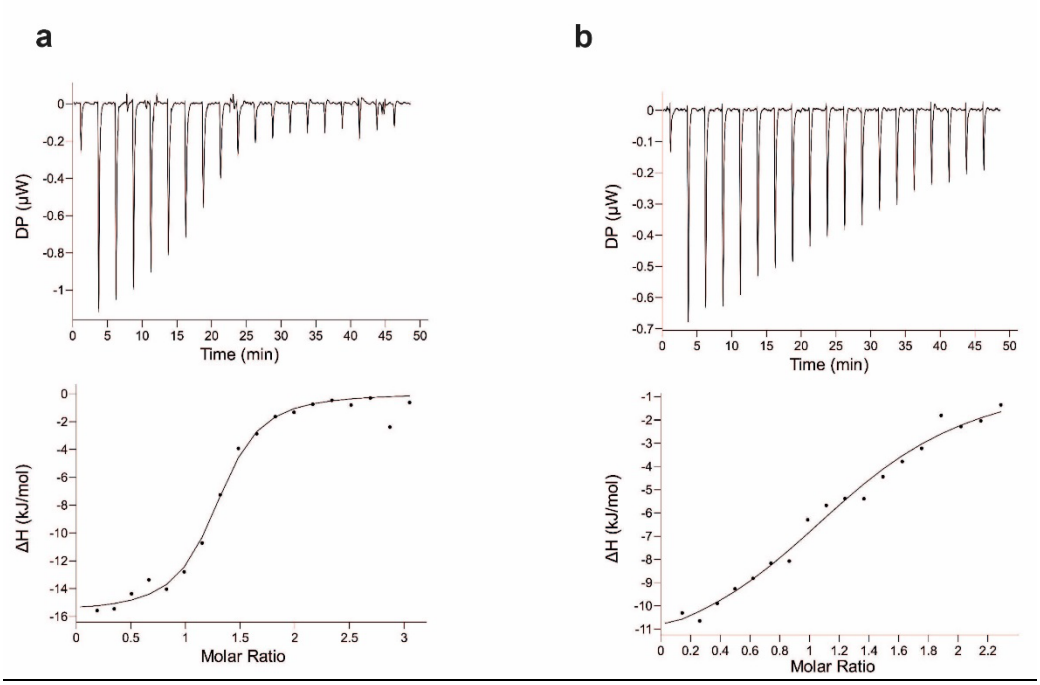
Supplementary Table 5: SafetyScreen44™

CEREP Assay	%INHB @ 10 µM BI-7190	%INHB @ 10 µM BI-4827
5HT1A/H	11.0	14.0
5HT1B(H)@CE	-5.0	-2.0
5HT2AH_AGON	-1.0	2.0
5HT2B/H AG	7.0	-8.0
5HT3/H	3.0	12.0
A2A/H	-2.0	-1.0
ACE(HU_AMTCH400)	-3.0	-4.0
ALPHA1AH_ANTAG	-1.0	-5.0
ALPHA2A/HU	36.0	-7.0
ANDROGEN/H	-11.0	-4.0
BETA1/HUM	4.0	7.0
BETA2/HUM	3.0	-3.0
BZD/CENTR/R	-7.0	-8.0
CA+/DHPSI/R	25.0	-6.0
CB1(HU)_AGON@CE	15.0	1.0
CB2/PERIPH/H	-9.0	7.0
CCKA/H	-6.0	-4.0
COX-1@CE	3.0	1.0
COX-2@CE	18.0	16.0
D1/H	6.0	13.0
D2SH_AGON	30.0	7.0
DATRANS/HUM	13.0	9.0
DELTA2/H	16.0	5.0
ETA/H	4.0	3.0
GCORTICOID/H	-3.0	0.0
H1/PYRIL/HS	9.0	2.0
H2/APT/HS	-4.0	-13.0
HERG_DOFETILIDE	3.0	11.0
K+/VOLT/RA	-6.0	-3.0
KAPPA(KOP)_HU@CE	22.0	12.0
LCK_CE	-14.0	16.0
M1/H	18.0	23.0
M2/H	14.0	7.0
M3/H	-7.0	-8.0
MAO-A_ANTAG	10.0	13.0
MU/H	6.0	3.0
N_NEURO_A4B2	-12.0	-15.0
NA+/SITE2/R	18.0	2.0
NEUP/H	-3.0	-3.0
NMDA/R	-4.0	2.0
PDE3A	8.0	6.0
PDE4D2	-5.0	-3.0
SLC6A4/H	16.0	-8.0
V1A/HUM	-4.0	0.0

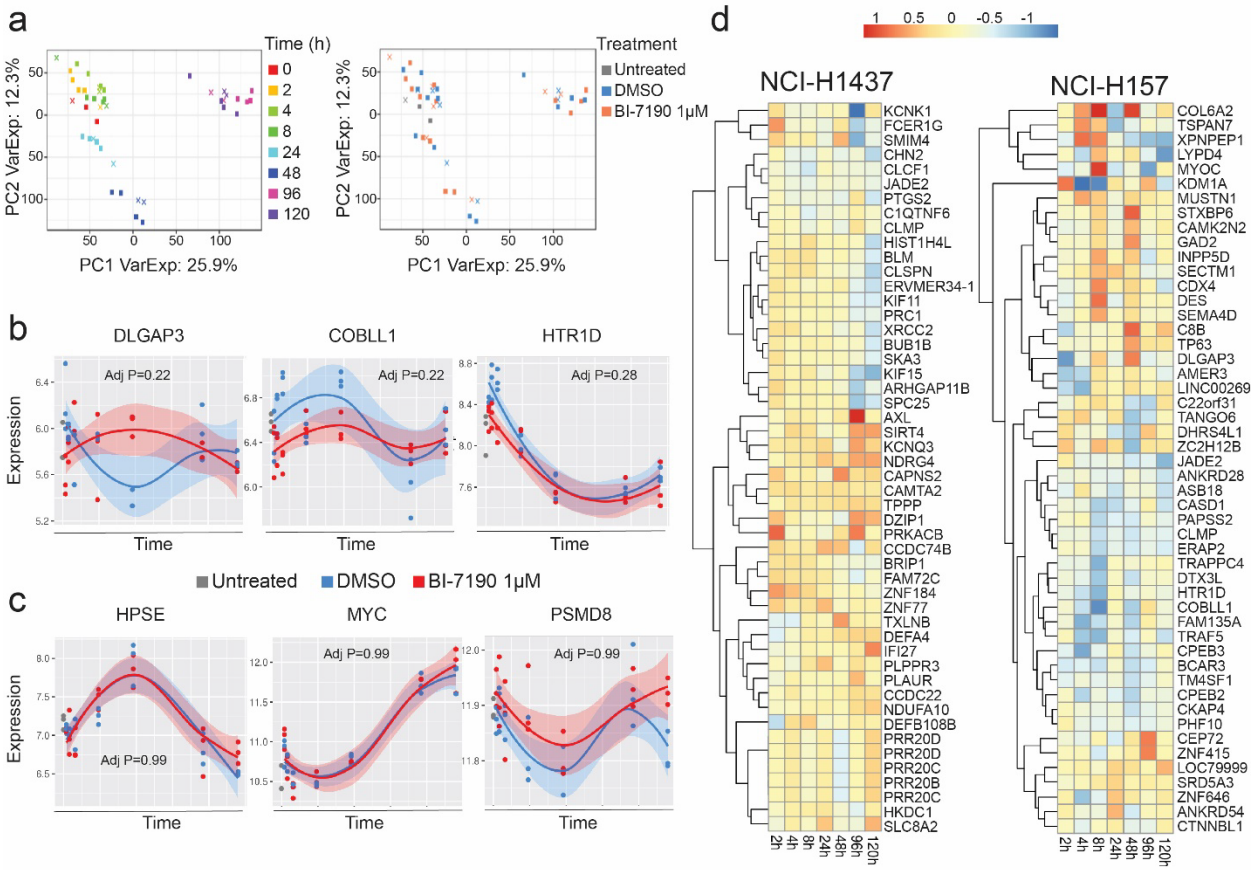
Supplementary Table 6: Effect of BI-7190 on cell viability. The indicated cell lines were treated with **BI-7190** for 7 days. The resulting IC_{50} is shown. BPTF expression (transcripts per million read – TPM) is based on CCLE data and BPTF dependency is taken from the DRIVE and AVANA depletion screenings.

Cell Line	IC_{50} [µM]	Tumor Type	BPTF TPM	BPTF dependency DRIVE	BPTF dependency AVANA
RI1	2.471	hematopoietic/lymphoma	87.50	N.A.	N.A.
MV-4-11	2.871	hematopoietic/leukemia	48.14	N.A.	-0.903916
MOLM-13	3.743	hematopoietic/leukemia	30.83	-1.928	-0.9136062
CAL-85-1	3.945	breast carcinoma	32.49	-0.424	N.A.
NOMO-1	4.273	hematopoietic/leukemia	21.02	N.A.	-0.5363579
G-402	4.517	renal cancer other	40.90	-3.597	-0.37710807
MFE-280	4.753	uterus carcinoma	53.79	-0.489	N.A.
THP-1	6.773	hematopoietic/leukemia	36.09	-0.817	-0.6302416
SK-N-DZ	6.952	neuroblastoma	78.28	-2.36	-0.6102058
G-401	7.618	renal cancer other	47.91	-0.991	-0.5739901
RH41	8.591	rhabdomyosarcoma	55.97	-1.962	N.A.
RCC-MF	10.564	renal carcinoma	53.45	N.A.	N.A.
EBC-1	11.354	NSCLC	34.34	-1.171	-0.6775747
A-204	11.472	rhabdomyosarcoma	34.21	-1.075	N.A.
SK-BR-3	12.203	breast carcinoma	94.82	N.A.	-0.84194267
KM-H2	14.249	hematopoietic/lymphoma	46.10	N.A.	N.A.
HCC1954	14.297	breast carcinoma	28.40	-0.345	-0.46980155
NCI-H520	14.531	NSCLC	36.88	N.A.	-0.6592013
NCI-H2342	15.445	NSCLC	35.88	N.A.	N.A.
NCI-H1385	16.22	NSCLC	60.60	N.A.	N.A.
NCI-H2196	16.775	SCLC	68.59	N.A.	N.A.
NCI-H522	16.797	NSCLC	125.12	-3.315	-1.151013
CAL-12T	17.073	NSCLC	40.33	N.A.	-0.7186407
NCI-H1623	18.107	NSCLC	31.64	N.A.	N.A.
CAMA-1	18.522	breast carcinoma	42.25	-2.024	-0.824505
MHHES1	19.339	bone sarcoma	84.45	N.A.	-0.90669304
OV90	19.445	ovarian carcinoma	49.44	-2.975	-0.41407254
MCF7	20.458	breast carcinoma	119.14	-0.839	-0.9352039
CAPAN-1	21.35	pancreas carcinoma	36.74	N.A.	N.A.
NCI-H1437	21.536	NSCLC	30.22	-0.692	-0.37582883
A-704	22.297	renal carcinoma	22.80	N.A.	N.A.
HS 852.T	23.133	melanoma	40.39	-0.704	-0.44500682
ABC-1	23.435	NSCLC	39.67	-0.362	-0.6654938
NCI-H1703	24.527	NSCLC	34.87	-4.411	-0.64254373
A-673	24.8	NSCLC	46.68	-1.119	-0.57518625
LS411N	24.992	colon carcinoma	21.47	-0.485	N.A.
NCI-H1299	25.235	NSCLC	52.37	-2.393	-0.4974135

RCC-FG2	26.234	renal carcinoma	18.53	N.A.	N.A.
MDA-MB-415	27.528	breast carcinoma	28.60	-0.675	-0.8647817
NCI-H2172	27.557	NSCLC	41.34	-0.466	-0.34749064
SK-OV-3	28.212	ovarian carcinoma	40.54	N.A.	-0.71049577
SK-UT-1	28.213	sarcoma/soft tissue	59.49	N.A.	N.A.
HCT 116	28.62	colon carcinoma	57.67	-2.928	N.A.
NCI-H661	30.266	NSCLC	54.23	-3.391	-0.468839
TOV-21G	31.54	ovarian carcinoma	48.27	N.A.	-0.33642498
A549	36.05	sarcoma/soft tissue	55.33	-1.409	-0.6542769



Supplementary Figure 1: Raw data of ITC analysis of **BI-7190** (a) BRD9 (K_d = 810 ± 200 nM ΔH =-15.8 kJ/mol, ΔG=-34.6, TΔS =-18.8 kJ/mol (b) BRD7 (K_d = 5720 ± 1130nM ΔH =-12.7 kJ/mol, ΔG=-29.7, TΔS =-17.0kJ/mol



Supplementary Figure 2: Treatment with BI-7190 induces minor transcriptional modulation in NCI-H1437 and NCI-H157 cells over 120h. (a) Principal Component plots of NCI-H157 cells showing all samples colored by time point (left) or by treatment (right). (b) Expression of the top differentially expressed genes over time in vehicle- or BI-7190-treated NCI-H157 cells. Basal values in untreated cells are shown for reference. (c) Expression of the indicated BPTF targets as in (b). (d) Heatmap of the top 50 most differentially expressed genes in BI-7190-treated compared to vehicle-treated NCI-H1437 and NCI-H157 cells.

Supplementary Methods

Protein purification

The construct for expression of BPTF protein (residues 2917-3037, uniprot ID: Q12830) containing a N-terminal GST-tag vector with TEV cleavage site is based on pdb entry (PMID: = 22464331). The plasmid was used to transform Escherichia coli, strain BL21 (DE3) cells (Invitrogen). For protein expression, an overnight culture in LB-medium supplemented with ampicillin (100 µg/mL) at 37 °C was prepared and diluted the next day to fresh TB-medium. At OD_{600} the culture was cooled to 22 °C. The expression was induced by the addition of 0.5 mM IPTG and incubated for 20 h. Cell pellets obtained by centrifugation at 5000 rpm were stored at -20 °C. Cells were solubilized in lysis buffer (20 mM Tris-HCl; 500 mM NaCl; 5 % Glycerol; 2 mM TCEP; cOmplete™ Protease Inhibitor Cocktail; pH 7.5) and disrupted by sonication on ice. After 15 min the sonicated lysate was clarified by centrifugation (50 min, 13500 rpm, 4 °C). The clear supernatant was mixed with 5 mL Glutathione Sepharose® 4B resin (GE Healthcare) slurry and after a washing step in lysis buffer stired for 3h on ice. The beads were spun down and loaded into a XK16 column cartridge and washed with lysis buffer until the baseline was stable. The protein was eluted with 100 % elution buffer (20 mM Tris-HCl; 500 mM NaCl; 5 % Glycerol; 2 mM TCEP; 25 mM L-Glutathione reduced; pH 7.5) and the GST tag was cleaved with TEV protease at 4 °C overnight. After an desalting step into 20 mM Tris-HCl; 200 mM NaCl; 2 mM TCEP; 5 % Glycerol; pH 7.5 by using a HiPrep 26/10 Desalting column (GE Healthcare), the cleaved BPTF protein was collected in the flow-through fraction of a second GST run (same beads used as before). As running buffer 20 mM Tris-HCl; 200 mM NaCl; 2 mM TCEP; 5 % Glycerol; pH 7.5 is used and the same buffer supplemented with 25 mM L-Glutathione reduced; pH 7.5 as elution buffer. After concentration using an Amicon© Ultra 15 mL Centrifugal Filter (Merck

Millipore), the solution was laded on a HiLoad Superdex S75 column (GE Healthcare) with 20 mM Tris-HCl; 200 mM NaCl; 2 mM TCEP; 5 % Glycerol; pH 7.5 as running buffer at a flow rate of 2 mL/min. Peak fractions were analyzed by SDS-PAGE using 4-12 % gradient gel (Invitrogen). The protein containing fractions were pooled and concentrated to 3 mg/mL with an Amicon© Ultra 15 mL Centrifugal Filter (Merck Millipore).

Synthetic procedures:

List of abbreviations

AcOH	Acetic acid
MeCN	Acetonitrile
Boc	*tert*.butoxy carbonyl; di-*tert*-butyl dicarbonate
cHex	Cyclohexane
DAD	Diode array detector
DCM	Dichloromethane, CH_2Cl_2
dppf	1,1'-Bis(diphenylphosphino)ferrocene
DIPEA	Diisopropylethyl amine
DME	1,2-Dimethoxyethane
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulphoxide
EtOAc or EA	Ethyl acetate
EtOH	Ethanol
h	Hour(s)
Hex	Hexane
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectroscopy
INT	Intermediate
KOAc	Potassium acetate
LC	Liquid Chromatography
M	Molar (mol/L)
MeOH	Methanol
µL	Microliter
µm	Micrometer
min	Minute(s)
mL	Milliliter

mm	Millimeter
MS	Mass spectrometry
MsCl	Methanesulfonyl chloride
nm	Nanometer
N	Normal
NMR	Nuclear magnetic resonance
PE	Petrolether
Pd_2dba_3	Tris(dibenzylideneacetone)dipalladium(0)
$Pd(dppf)Cl_2$	[1,1′-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)
Ppm	Parts per million
prot.	Protonated
RP	Reversed phase
rt	Room temperature (20 to 25°C)
SM	Starting material
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
t_R	Retention time [min]
XPhos	2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

General Methods

If not specifically defined herein, compounds were obtained from commercially suppliers such as Sigma-Aldrich. Unless otherwise indicated all reactions were carried out in standard commercially available glassware using standard synthetic chemistry methods. Air-sensitive and moisture-sensitive reactions were performed under an atmosphere of dry nitrogen or argon with dried glassware. Commercial starting materials were used without further purification. Solvents

used for reactions were of commercial “dry”- or “extra-dry” or “analytical” grade. All other solvents used were reagent grade.

Preparative RP-HPLC was carried out on Agilent or Gilson systems using columns from Waters (Sunfire C18 OBD, 5 or 10 µm, 20x50 mm, 30x50 mm or 50x150 mm; X-Bridge C18 OBD, 5 or 10 µm, 20x50, 30x50, or 50x150 mm) or YMC (Triart C18, 5 or 10 µm, 20x50 mm, or 30x50 mm). Unless otherwise indicated compounds were eluted with MeCN/water gradients using either acidic (0.2 % HCOOH or TFA) or basic water (5 mL 2 M NH_4HCO_3 + 2 mL NH_3 (32 %) made up to 1 L with water).

NMR experiments were recorded on a Bruker Avance500 MHz spectrometer equipped with a TCI cryoprobe at 298 K. Samples were dissolved in 600 µL DMSO-d6 and TMS was added as internal standard. 1D 1H spectra were acquired with 30° excitation pulses and an interpulse delay of 4.2 s with 64k data points and 20 ppm sweep width.

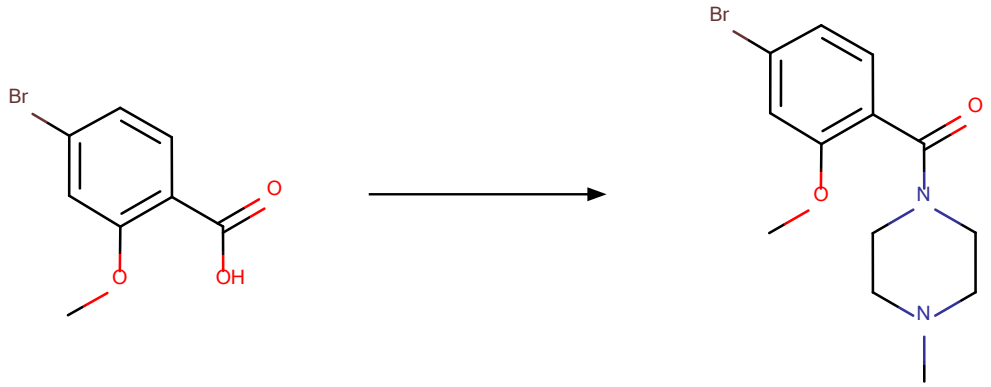
1D 13C spectra were acquired with broadband composite pulse decoupling (WALTZ16) and an interpulse delay of 3.3 sec with 64 k data points and a sweep width of 240 ppm. No zero filling was performed and spectra were manually integrated after automatic baseline correction. Chemical shifts are reported in ppm on the δ scale. Analysis of 1D and 2D spectra was performed with ACDlabs software.

Analytical LC/MS [LC/MS(BAS1)] data were measured on an Agilent HPLC 1200 Series with Agilent LC/MSD SL detector using a Waters X-Bridge C18, 2.5 µm, 2.1x20 mm column (Part.No. 186003201) and solvent A [20mM aqueous NH_4HCO_3/ NH_3 (pH 9)] and solvent B [acetonitrile HPLC grade] as eluent (additional settings: flow 1mL/min; injection volume 5 µl;

column temp. 60 °C). Gradient: 0.00 min:10 % B; 0.00 – 1.50 min: 10 % -> 95 % B; 1.50 – 2.00 min: 95 % B; 2.00 – 2.10 min: 95 % -> 10 % B.

Compound Syntheses

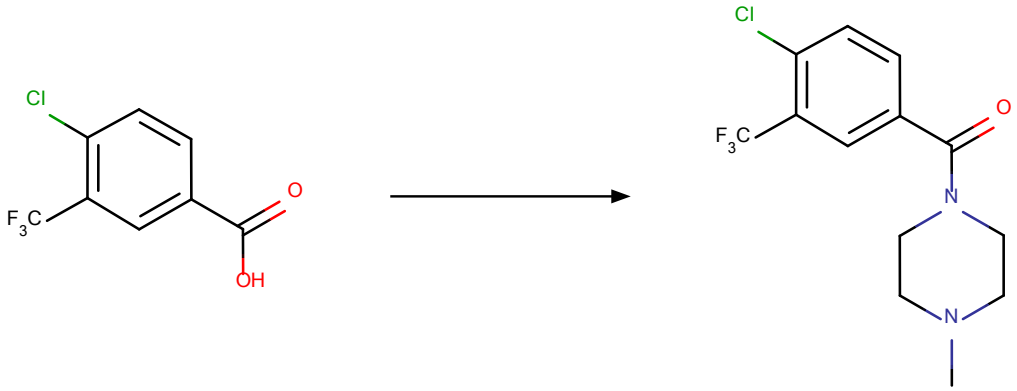
(4-Bromo-2-methoxy-phenyl)-(4-methyl-piperazin-1-yl)-methanone



4-Bromo-2-methoxy-benzoic acid (5.0 g; 21.641 mmol) is suspended in thionyl chloride (40.0 g; 336.217 mmol) and stirred at room temperature overnight. The excess thionyl chloride is removed under reduced pressure and the crude acid chloride is dissolved in dry DCM (50 mL) and TEA (3.44 mL; 23.805 mmol), cooled to 0°C. N-methylpiperazine (2.64 mL; 23.805 mmol) is added and the reaction mixture is stirred overnight at room temperature.

The reaction mixture is concentrated under reduced pressure, loaded onto isolute and purified by column chromatography over silica gel using MeOH/DCM (gradient 0-10% MeOH) as eluent to give (4-Bromo-2-methoxy-phenyl)-(4-methyl-piperazin-1-yl)-methanone XY (4.8 g; 15.326 mmol; 70.8%).

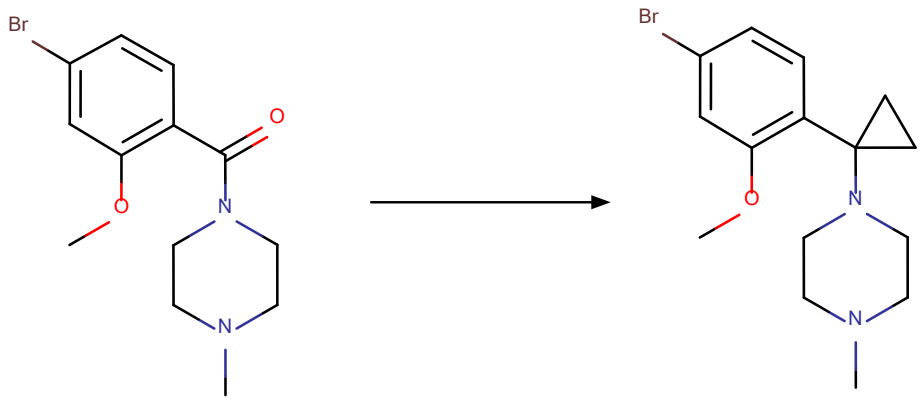
(4-Chloro-3-trifluoromethyl-phenyl)-(4-methyl-piperazin-1-yl)-methanone



4-Chloro-3-(trifluoromethyl)benzoic acid (150 mg; 0.666 mmol), HATU (304.773 mg; 0.802 mmol) and DIPEA (323.75 µL; 2.0 mmol) are dissolved in DMSO (0.8 mL) and stirred at room temperature for 15 min. N-Methylpiperazine (111.51 µL; 1.0 mmol) is added and the reaction mixture is stirred at room temperature overnight.

The reaction mixture is filtered, concentrated under reduced pressure and purified by RP-HPLC (column X-Bridge C-18 30x50 mm). The product containing fractions are combinded and concentrated under reduced pressure to yield (4-Chloro-3-trifluoromethyl-phenyl)-(4-methyl-piperazin-1-yl)-methanone XY (106.4 mg; 0.347 mmol; 51.9%).

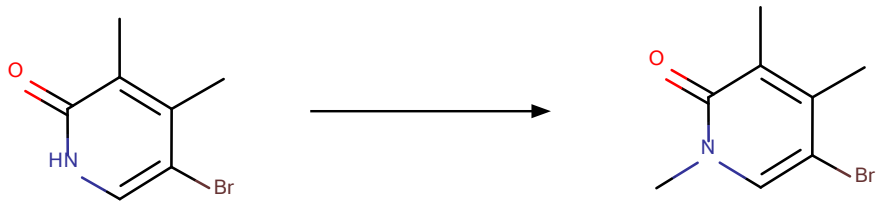
1-[1-(4-Bromo-2-methoxy-phenyl)-cyclopropyl]-4-methyl-piperazine



To a cooled solution of bis(cyclopentadienyl)zirconium dichloride (1.4 g; 4.789 mmol; commercial from Aldrich) in dry THF (15 mL), a 1M ethyl magnesium bromide solution (9.579 mL; 9.579 mmol) is added dropwise at -78°C and stirred for 15 min before allowed to warm to 0°C. To the orange-red solution a solution of (4-Bromo-2-methoxy-phenyl)-(4-methyl-piperazin-1-yl)-methanone (0.5 g; 1.596 mmol) in dry THF (10 mL) is added and the resulting mixture is stirred at room temperature for 2 hours.

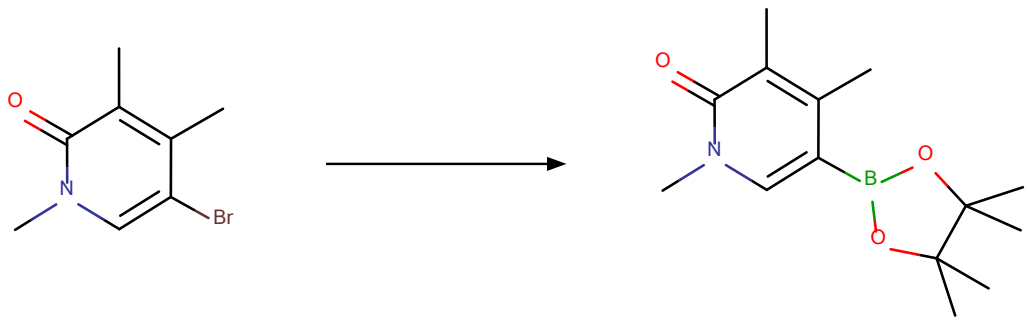
The reaction mixture is poured into water (100 mL), concentrated under reduced pressure and extracted with DCM (3*100 mL). The combined organic phases are dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude brownish oil is dissolved in DMF and purified by preparative RP-HPLC using a ACN/water gradient (20 to 60%) as eluent to give 1-[1-(4-Bromo-2-methoxy-phenyl)-cyclopropyl]-4-methyl-piperazine (75 mg; 0.231 mmol; 14.4%). HPLC method: LCMSBAS1: t_{ret} [min] = 1.29; $[M+H]^+$ = 325/327.

5-Bromo-1,3,4-trimethyl-1H-pyridin-2-one



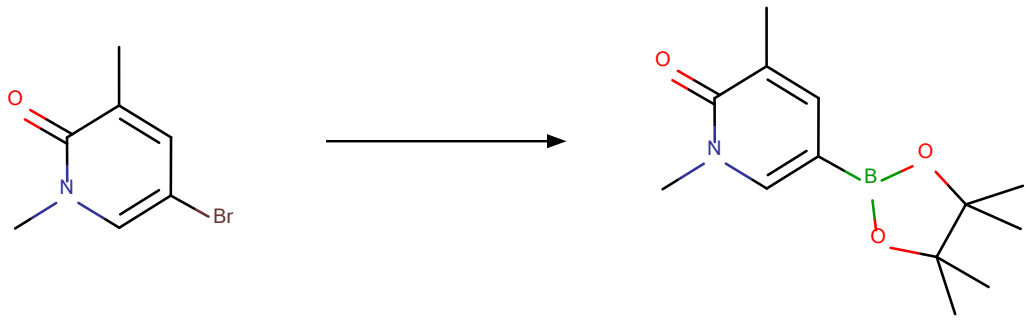
5-Bromo-3,4-dimethyl-1H-pyridin-2-one (2.5 g; 12.373 mmol) and potassium carbonate (4.275 g; 30.933 mmol) are dissolved in THF and stirred at room temperature for 10 min. Iodomethane (0.84 mL; 13.611 mmol) is added dropwise and the reaction mixture is stirred at room temperature for 3 hours. The reaction mixture is concentrated under reduced pressure, loaded onto isolute and purified by column chromatography over silica gel using a hexane/EtOAc gradient (0%-50%). The product containing fractions are combined and concentrated under reduced pressure to yield 5-Bromo-1,3,4-trimethyl-1H-pyridin-2-one (2.3 g; 10.644 mmol; 86.0%).

1,3,4-Trimethyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-pyridin-2-one



5-Bromo-1,3,4-trimethyl-1H-pyridin-2-one (1.0 g; 4.628 mmol), 4,4,5,5,4',4',5',5'-Octamethyl-[2,2']bi[[1,3,2]dioxaborolanyl] (1.763 g; 6.942 mmol), 1,1'-Bis(diphenylphosphino)ferrocene palladium(II) dichloride (0.78 g; 0.853 mmol) and potassium acetate (0.908 g; 9.256 mmol) are suspended in dioxane (5 mL) and stirred at 80°C overnight. The reaction mixture is filtered, the filtrate is concentrated under reduced pressure, dissolved in DCM/MeOH, loaded onto isolute and purified by column chromatography over silica gel using a cHex/EtOAc gradient (80%-20%). The product containing fractions are combined and concentrated under reduced pressure to yield 1,3,4-Trimethyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-pyridin-2-one (1.2 g; 4.56 mmol; 98.5%).

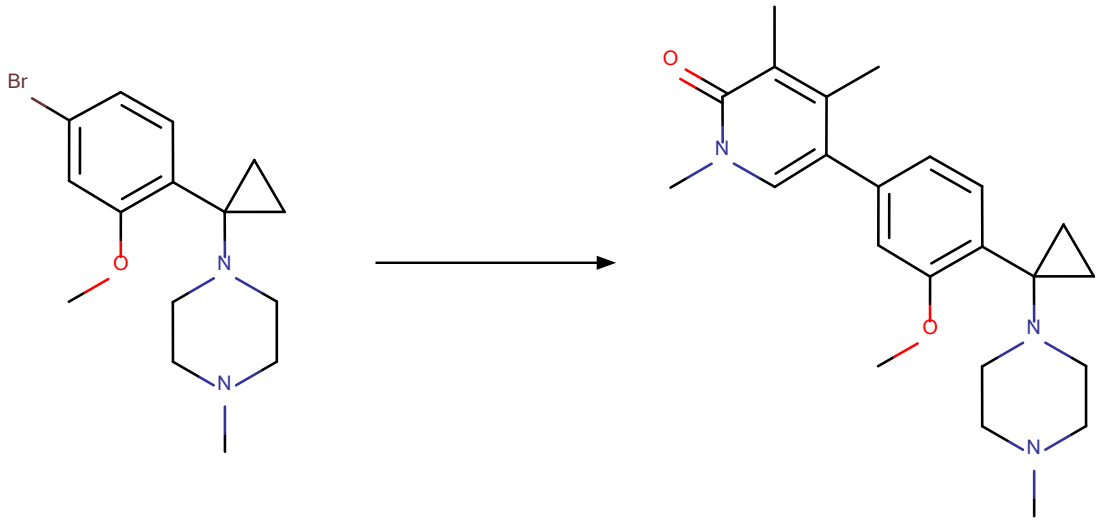
1,3-Dimethyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-pyridin-2-one



5-Bromo-1,3-dimethyl-1H-pyridin-2-one (0.2 g; 0.99 mmol), 4,4,5,5,4',4',5',5'-Octamethyl-[2,2']bi[[1,3,2]dioxaborolanyl] (276.5 mg; 1.089 mmol), Tris(dibenzyledenaceton)dipalladium(0) (90.6 mg; 0.1 mmol), Tricyclohexylphosphine (41.6 mg; 0.148 mmol) and potassium acetate (146 mg; 1.485 mmol) are suspended in dioxane (2.5 mL), flushed with argon and stirred at 95°C for 2 hours. The reaction mixture is cooled down to room temperature, filtered over a plug of

Celite and concentrated under reduced pressure. The crude product is dissolved in DCM, loaded onto isolute and purified by flash chromatography over silica gel using a cHex/EtOAc gradient (100%-0%). The product containing fractions are combined and concentrated under reduced pressure to yield 1,3-Dimethyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-pyridin-2-one (86 mg; 0.345 mmol; 34.9%).

5-{3-Methoxy-4-[1-(4-methyl-piperazin-1-yl)-cyclopropyl]-phenyl}-1,3,4-trimethyl-1H-pyridin-2-one (BI-7190; XY)



1-[1-(4-Bromo-2-methoxy-phenyl)-cyclopropyl]-4-methyl-piperazine (37.0 mg; 0.114 mmol), 1,3,4-Trimethyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-pyridin-2-one (32.929 mg; 0.125 mmol) and 1,1'-Bis(diphenylphosphino)ferrocene palladium(II) dichloride (19.155 mg; 0.023 mmol) are dissolved in DMF (0.8 mL). A 2M sodium carbonate solution in water (0.114

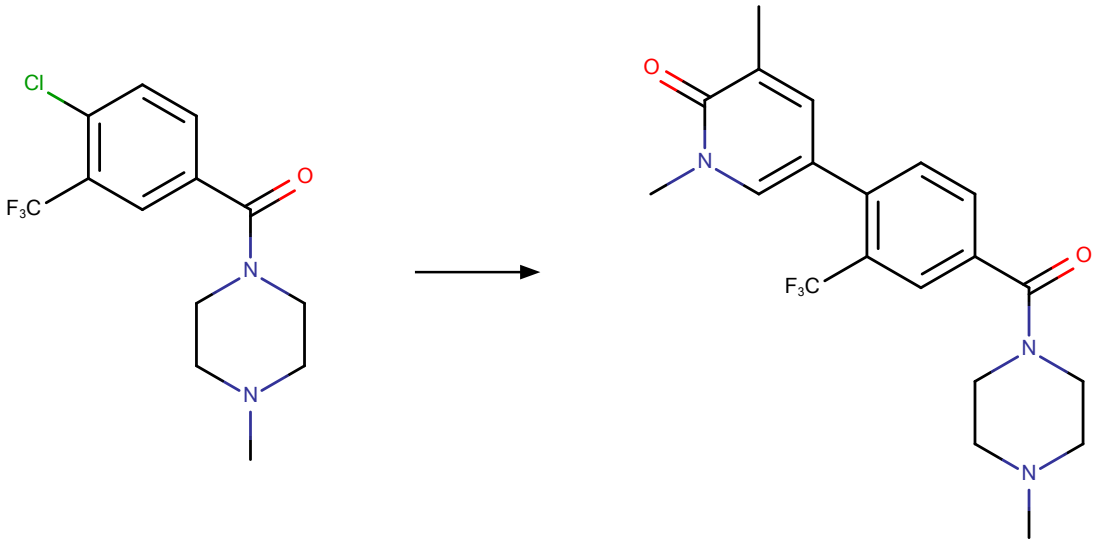
mL; 0.228 mmol) is added, the reaction flask purged with argon for 5 min and the reaction mixture is stirred at 80°C for 1 hour. The reaction mixture is filtered, the filtrate is concentrated under reduced pressure and purified by flash chromatography over silica gel using a DCM/MeOH gradient (0-10% MeOH) as eluent under basic conditions. The product containing fractions are combined and concentrated under reduced pressure to yield 5-{3-Methoxy-4-[1-(4-methyl-piperazin-1-yl)-cyclopropyl]-phenyl}-1,3,4-trimethyl-1H-pyridin-2-one (8 mg; 0.021 mmol; 18.4%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.51 (s, 1H), 7.18 (d, 1H, *J*=7.6 Hz), 6.87 (d, 1H, *J*=1.6 Hz), 6.81 (dd, 1H, *J*=1.6, 7.6 Hz), 3.78 (s, 3H), 3.44 (s, 3H), 2.3-2.5 (range, 4H), 2.1-2.3 (range, 4H), 2.05 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 0.7-0.9 (range, 2H), 0.7-0.9 (range, 2H)

^{13}C NMR (DMSO-d_6, 125 MHz) δ 161.6, 159.1, 144.6, 138.3, 135.2, 133.4, 124.9, 123.7, 121.2, 121.2, 113.1, 55.9, 55.7, 49.6, 46.3, 44.1, 37.3, 18.0, 15.4, 13.5

LC/MS (BAS1): $[M+H]^+$ = 382; t_R = 1.22 min.

1,3-Dimethyl-5-[4-(4-methyl-piperazine-1-carbonyl)-2-trifluoromethyl-phenyl]-1H-pyridin-2-one (BI-4827; XY)



(4-Chloro-3-trifluoromethyl-phenyl)-(4-methyl-piperazin-1-yl)-methanone (106.0 mg; 0.346 mmol), 1,3-Dimethyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1H-pyridin-2-one (90.0 mg; 0.361 mmol) and 1,1'-Bis(diphenylphosphino)ferrocene palladium(II) dichloride (32.75 mg; 0.040 mmol) are dissolved in DMF (0.8 mL). A 2M sodium carbonate solution in water (0.501 mL; 1.0 mmol) is added, the reaction flask purged with argon for 5 min and the reaction mixture is stirred at 100°C for 1 hour. After the reaction mixture is cooled down to room temperature one drop of water is added and the reaction mixture is filtered, the filtrate is concentrated under reduced pressure and purified by RP-HPLC using a ACN/water gradient as eluent to yield 1,3-Dimethyl-5-[4-(4-methyl-piperazine-1-carbonyl)-2-trifluoromethyl-phenyl]-1H-pyridin-2-one (35.6 mg; 0.09 mmol; 25.0%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.79 (d, 1H, *J*=0.9 Hz), 7.72 (d, 1H, *J*=7.9 Hz), 7.66 (d, 1H, *J*=2.5 Hz), 7.53 (d, 1H, *J*=7.9 Hz), 7.33 (s, 1H), 3.6-3.7 (range, 2H), 3.48 (s, 3H), 3.3-3.4 (range, 2H), 2.3-2.4 (range, 2H), 2.2-2.3 (range, 2H), 2.20 (s, 3H), 2.04 (s, 3H)

^{13}C NMR (DMSO-d_6, 125 MHz) δ 167.6, 162.0, 138.1, 137.9, 136.8, 136.3, 133.4, 131.2, 128.1, 127.4, 125.4, 124.2, 115.9, 55.0, 54.6, 47.6, 46.0, 42.0, 37.6, 17.3

LC/MS (BAS1): $[M+H]^+$ = 395; t_R = 0.95.