Discovery of a Potent, Cell Penetrant and Selective p300/CBP-Associated Factor (PCAF)/General Control Nonderepressible 5 (GCN5) Bromodomain Chemical Probe

Supporting Information

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Compound	PCAF pIC_{50}	BRD4 BD1 pIC_{50}	BRD9 pIC_{50}	Chrom Log$D_{pH7.4}$	Chrom LogP	Solubility (μM CLND)
8	4.8 ± 0.07 (6)	4.8 ± 0.26 (5)	5.8 ± 0.02 (4)	3.3	3.4	≥502
10	4.9 ± 0.08 (4)	4.7 ± 0.18 (4)	5.7 ± 0.09 (9)	3.2	3.2	≥585
11	5.2 ± 0.1 (30)	4.6 ± 0.1 (44/48)[1]	6.0 ± 0.09 (8)	3.8	3.8	≥431
12	5.2 ± 0.11 (30)	4.7 ± 0.27 (49/51)[1]	6.0 ± 0.1 (8)	3.4	3.4	≥593
13	5.1 ± 0.11 (6)	<4.3 (3)	5.9 ± 0.07 (7)	1.2	1.2	≥563
14	5.0 ± 0.06 (7)	4.5 ± 0.05 (3)	5.6 ± 0.11 (5)	3.1	3.1	≥615
15	4.7 ± 0.1 (28)	3.9 ± 0.55(4)[2]	4.6 ± 0.18 (10)	0.4	0.4	≥686
16	4.5 ± 0.02 (4)	<4.3 (4)	<4.3 (5)	2.2	2.1	≥534
18	5.2 ± 0.04 (6)	4.8 ± 0.25 (3)	5.9 ± 0.05 (5)	3.8	3.9	≥704
19	6.0 ± 0.11 (4)	<4.3 (3)	n.d.	0.3	2.1	≥395
20	6.5 ± 0.11 (16)	<4.3 (12)	5.7 ± 0.11 (25)	1.7	2.8	≥660
21	4.8 ± 0.08 (4)	<4.3 (4)	n.d.	–0.1	0.7	≥599
22	4.8 ± 0.04 (4)	<4.3 (4)	<4.3 (5)	–0.3	1.2	354
23	5.9 ± 0.05 (6)	<4.3 (4)	<4.3 (8)	0.6	n.d.	≥538
(*R*)-23	6.3 ± 0.04 (8)	<4.3 (7/8)[3]	<4.3 (6)	0.6	1.5	394
(*S*)-23	5.2 ± 0.05 (6)	<4.3 (3)	<4.3 (6)	0.6	1.5	≥550
27	4.4 ± 0.05 (3)	<4.3 (4)	n.d.	4.8	5.1	398
(*R*,*R*)-31	7.1 ± 0.02 (4)	<4.3 (4)	4.5 ± 0.16 (4)	3.6	4.0	328
(*S*,*S*)-31	4.6 ± 0.06 (4)	<4.3 (3)	<4.3 (4)	3.6	4.0	429
(*R*,*R*)-32	7.4 ± 0.11 (8)	<4.3 (6/8)[4]	5.1 ± 0.08 (4)	3.8	4.1	395
(*S*,*S*)-32	4.9 ± 0.50 (8)	<4.3 (8)	4.5 ± 0.13 (3/4)[5]	3.8	4.1	363

Table S1. Full data from Tables 1-5, including mean pIC_{50}, standard deviation, and the number of test occasions in the PCAF, BRD4 BD1 and BRD9 assays. n.d.=not determined

[1] Some pIC_{50} results below the curve-fitting threshold could not be included in mean and SD. In these cases, the number included in mean / number of times tested are both shown.
[2] Only high-concentration curve data included (dose response up to 1 mM compound).
[3] Data is pIC_{50} 4.8 (1/8) test occasions
[4] Data is pIC_{50} 4.4 (2/8) test occasions
[5] Data is pIC50 <4.3 (1/4) test occasions

Bromodomain	(*R*,*R*)-32 (GSK4027) K_i (nM)	(*R*,*R*)-32 (GSK4027) pK_i	Δ
PCAF	1.4	8.9	-
GCN5	1.4	8.9	0
ATAD2A	>30000	<4.5	>4.4
ATAD2B	>30000	<4.5	>4.4
BAZ2A	20000	4.7	4.2
BAZ2B	840	6.1	2.8
BRD1	110	6.9	2
BRD2 BD1	>30000	<4.5	>4.4
BRD2 BD2	>30000	<4.5	>4.4
BRD3 BD1	>30000	<4.5	>4.4
BRD3 BD2	26000	4.6	4.3
BRD4 BD1	>30000	<4.5	>4.4
BRD4 BD2	>30000	<4.5	>4.4
BRD4 BD1 + BD2	>30000	<4.5	>4.4
BRD4 (full length)	>30000	<4.5	>4.4
BRD7	1500	5.8	3.1
BRD9	1400	5.9	3.0
BRDT BD1	>30000	<4.5	>4.4
BRDT BD2	>30000	<4.5	>4.4
BRPF1	140	6.9	2.0
BRPF3	100	7.0	1.9
CECR2	11000	4.9	4.0
CREBBP	>30000	<4.5	>4.4
EP300	>30000	<4.5	>4.4
FALZ	130	6.9	2.0
PBRM1 BD2	>30000	<4.5	>4.4
PBRM1 BD5	>30000	<4.5	>4.4
SMARCA2	>30000	<4.5	>4.4
TAF1 BD2	>30000	<4.5	>4.4
TAF1L BD2	>30000	<4.5	>4.4
TRIM24 (Bromo)	>30000	<4.5	>4.4
TRIM24 (PHD, Bromo)	>30000	<4.5	>4.4
TRIM33 (PHD, Bromo)	>30000	<4.5	>4.4
WDR9 BD2	>30000	<4.5	>4.4

Table S2. Selectivity profile of **(*R*,*R*)-32** (GSK4027) in the BROMO*scan* panel (DiscoveRx Corp). Δ is the selectivity of **(*R*,*R*)-32** (GSK4027) expressed in log units for PCAF over each bromodomain

Assay	(*R*,*R*)-32 (GSK4027) XC_{50} (nM)	(*R*,*R*)-32 (GSK4027) pXC_{50}	Δ
PCAF TR-FRET	40	7.4	-
AChEase Inh	3200	5.5 (2)	1.9
Adenosine 2a Ag	>100000	<4 (2)	>3.4
Adrenergic α1b Ant	4000	5.4 (2)	2
Adrenergic α2c Ag	>100000	<4 (2)	>3.4
Adrenergic β2 Ag	>100000	<4 (2)	>3.4
Adrenergic β2 Ant	>100000	<4 (2)	>3.4
CB2 Ag	12600	4.9 (2)	2.5
Dopamine 1 Ant	>100000	<4 (2)	>3.4
Dopamine 2 Ant	10000	5 (2)	2.4
Dopamine 2 Ag	>100000	<4 (2)	>3.4
Histamine 1 Ant	>25100	<4.6 (2)	>2.8
hERG IonWorks Ant	39800	4.4 (3)	3.0
Muscarine 1 Ag	>50100	<4.3 (2)	>3.1
Muscarine 1 Ant	>50100	<4.3 (2)	>3.1
Muscarine 2 Ag	>50100	<4.3 (2)	>3.1
Muscarine 2 Ant	>50100	<4.3 (2)	>3.1
Neurokinin 1 Ant	>25100	<4.6 (2)	>2.8
μ Opioid Ag	>100000	<4 (2)	>3.4
K Opioid Ag	>100000	<4 (2)	>3.4
Serotonin 1B Ag	>100000	<4 (2)	>3.4
Serotonin 1B Ant	>100000	<4 (2)	>3.4
Serotonin 2A Ag	>25100	<4.6 (2)	>2.8
Serotonin 2A Ant	20000	4.7 (2)	2.7
Serotonin 2C Ag	>25100	<4.6 (2)	>2.8
Serotonin 2C Ant	>25100	<4.6 (2)	>2.8
Vasopressin 1a Ant	>50100	<4.3 (2)	>3.1
Monoamine oxidase A Inh	>100000	<4 (2)	>3.4
COX2 Block	>100000	<4 (2)	>3.4
PDE4B Ant	>100000	<4 (2)	>3.4
PDE3A Inh	>100000	<4 (2)	>3.4
GABA-A α1 Ag	>100000	<4 (2)	>3.4
KCNQ1/minK Block	>25100	<4.6 (2)	>2.8
Kv1.5 Block	>50100	<4.3 (2)	>3.4
L-type Ca channel (CaV 1.2) Block	>100000	<4 (2)	>3.4
NaV1.5 Block	>100000	<4 (2)	>3.4
Serotonin 3 Open	>50100	<4.3 (2)	>3.1
Serotonin 3 Block	>50100	<4.3 (2)	>3.1
α1 nicotonic AChR Open	>50100	<4.3 (2)	>3.1
α1 nicotonic AChR Block	10000	5 (2)	2.4
NMDA/NR2B Block	>50100	<4.3 (2)	>3.1
CYP3A4 Ant	>39800	<4.4 (2)	>3
Aurora B (STK12) Ant	>31600	<4.5 (1)	>2.9
LCK Ant	>31600	<4.5 (2)	>2.9
PI3Kγ Ant	>31600	<4.5 (1)	>2.9
Norepinephrine Ant	5000	5.3 (2)	2.1
OATP1B1 Inh	>50100	<4.3 (2)	>3.1
Serotonin Ant	20000	4.7 (2)	2.7
PXR Ag	25100	4.6 (2)	2.8
AhR Ag	>100000	<4 (2)	>3.4
Cell Health Mitochondrial Integrity	>199500	<3.7 (2)	>3.7
Cell Health Membrane Permeability	>199500	<3.7 (2)	>3.7
Cell Health Nucleus (size)	>199500	<3.7 (2)	>3.7
Phospholipidosis Accum	>100000	<4 (3)	>3.4

Table S3. Cross screening panel for **(*R*,*R*)-32** (GSK4027), the number of independent test occasions is shown in brackets. Δ is the selectivity of **(*R*,*R*)-32** (GSK4027) expressed in log units for PCAF over each assay result.

General experimental

All solvents were purchased from Sigma Aldrich (Hy-Dry anhydrous solvents) and commercially available reagents were used as received. All reactions were followed by TLC analysis (TLC plates GF254, Merck) or LCMS (liquid chromatography mass spectrometry) using a Waters ZQ instrument.

NMR spectra were recorded at ambient temperature unless otherwise stated using standard pulse methods on any of the following spectrometers and signal frequencies: Bruker AV-400 (^{1}H = 400 MHz, ^{13}C = 100.6 MHz), Bruker AV-500 (^{1}H = 500 MHz, ^{13}C = 125.8 MHz), Bruker AVII+ 600 (^{1}H = 600 MHz, ^{13}C = 150.9 MHz). Chemical shifts are reported in ppm and are referenced to tetramethylsilane (TMS) or the following solvent peaks: $CDCl_3$ (^{1}H = 7.27 ppm, ^{13}C = 77.00 ppm), DMSO-d_6 (^{1}H = 2.50 ppm, ^{13}C = 39.51 ppm) and CD_3OD (^{1}H = 3.31 ppm, ^{13}C = 49.15 ppm). Coupling constants are quoted to the nearest 0.1 Hz and multiplicities are given by the following abbreviations and combinations thereof: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Column chromatography was performed on pre-packed silica gel columns (30-90 mesh, IST) using a biotage SP4.

High resolution mass spectra (HRMS) were recorded on a Micromass Q-Tof Ultima hybrid quadrupole time-of-flight mass spectrometer, with analytes separated on an Agilent 1100 Liquid Chromatograph equipped with a Phenomenex Luna C18(2) reversed phase column (100 mm x 2.1 mm, 3 µm packing diameter). LC conditions were 0.5 mL/min flow rate, 35 °C, injection volume 2 - 5 µL. Gradient elution with (A) H_2O containing 0.1% (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. Gradient conditions were initially 5% B, increasing linearly to 100% B over 6 min, remaining at 100% B for 2.5 min then decreasing linearly to 5% B over 1 min followed by an equilibration period of 2.5 min prior to the next injection.

LCMS analysis was carried out on a H_2Os Acquity UPLC instrument equipped with a BEH column (50 mm x 2.1 mm, 1.7 µm packing diameter) and H_2Os micromass ZQ MS using alternate-scan positive and negative electrospray. Analytes were detected as a summed UV wavelength of 210 – 350 nm. Three liquid phase methods were used:

Formic – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) H_2O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

High pH – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

TFA – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) H_2O containing 0.1% volume/volume (v/v) TFA and (B) acetonitrile containing 0.1% (v/v) TFA. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

Mass-directed automatic purification (MDAP) was carried out using a H_2Os ZQ MS using alternate-scan positive and negative electrospray and a summed UV wavelength of 210 – 350 nm. Two liquid phase methods were used:

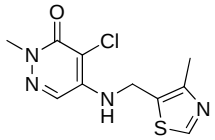
Formic – Sunfire C18 column (100 mm x 19 mm, 5 µm packing diameter, 20 mL/min flow rate) or Sunfire C18 column (150 mm x 30 mm, 5 µm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) H_2O containing 0.1% volume/volume (v/v) formic acid and (B) acetonitrile containing 0.1% (v/v) formic acid.

High pH – Xbridge C18 column (100 mm x 19 mm, 5 µm packing diameter, 20 mL/min flow rate) or Xbridge C18 column (150 mm x 30 mm, 5 µm packing diameter, 40 mL/min flow rate). Gradient elution at ambient temperature with the mobile phases as (A) 10 mM aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile.

Synthetic procedures

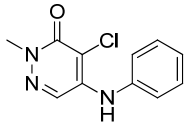
Compounds **8, 10–12** are commercially available and were purchased.

4-Chloro-2-methyl-5-(((4-methylthiazol-5-yl)methyl)amino)pyridazin-3(2H)-one (13)



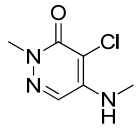
(4-Methylthiazol-5-yl)methanamine dihydrochloride salt (169 mg, 0.84 mmol) was added in a single portion to a stirred solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (100 mg, 0.56 mmol) in DMSO (1.5 mL) at rt. DIPEA (0.20 mL, 1.12 mmol) was then added in a single portion. The vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, the solution was diluted with DMSO (0.3 mL) and purified by formic MDAP. The combined fractions were evaporated under reduced pressure to give **13** as a white solid (37 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1H), 7.55 (s, 1H), 4.97 (br.s, 1H), 4.68 (d, *J* = 5.9 Hz, 2H), 3.78 (s, 3H), 2.51 (s, 3H); LCMS (formic acid) $(M+H)^+$ = 271.0, 273.0, R_t = 0.54 min (100%).

4-Chloro-2-methyl-5-(phenylamino)pyridazin-3(2H)-one (14)



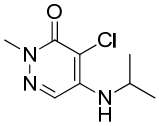
Aniline (0.07 mL, 0.84 mmol) was added in a single portion to a stirred solution 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (100 mg, 0.56 mmol) in DMSO (1.5 mL) at rt. The vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, additional aniline (0.07 mL, 0.84 mmol) was added and the vial reheated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, further aniline (0.07 mL, 0.84 mmol) was added and the vial reheated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, the solution was diluted with DMSO (0.3 mL) and purified by formic MDAP. The combined fractions were evaporated under reduced pressure to give **14** as a grey solid (32 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.46–7.42 (m, 2H), 7.31–7.27 (m, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 6.41 (br.s, 1H), 3.78 (s, 3H); LCMS (formic acid) $(M+H)^+$ = 236.0, 238.0, R_t = 0.78 min (100%).

4-Chloro-2-methyl-5-(methylamino)pyridazin-3(2H)-one (15)



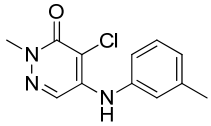
Methanamine (79 mg, 33% by weight in EtOH, 0.84 mmol) was added in a single portion to a stirred solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (100 mg, 0.56 mmol) in DMSO (2 mL) at rt. The vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, the solution was purified by formic MDAP. The combined fractions were evaporated under reduced pressure to give **15** as a white solid (61 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 4.71 (br.s, 1H), 3.78 (s, 3H), 3.19 (d, *J* = 5.4 Hz, 3H); LCMS (formic acid) $(M+H)^+$ = 174.0, 176.0, R_t = 0.45 min (98%).

4-Chloro-5-(isopropylamino)-2-methylpyridazin-3(2H)-one (16)



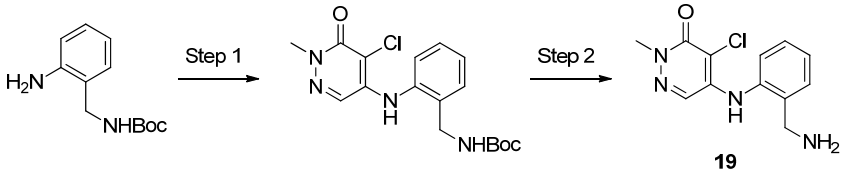
Propan-2-amine (0.07 mL, 0.84 mmol) was added in a single portion to a stirred solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (100 mg, 0.56 mmol) in DMSO (2 mL). Upon cooling to rt, additional propan-2-amine (0.14 mL, 1.68 mmol) was added and the vial reheated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, the solution was purified by formic MDAP. The combined fractions were evaporated under reduced pressure to give **16** as a white solid (60 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 4.49 (br.s, 1H), 3.83–3.76 (m, 4 H), 1.32 (d, *J* = 6.4 Hz, 6H); LCMS (formic acid) $(M+H)^+$ = 202.1, 204.0, R_t = 0.65 min (100%).

4-Chloro-2-methyl-5-(m-tolylamino)pyridazin-3(2H)-one (18)



m-Toluidine (0.15 mL, 1.40 mmol) was added in a single portion to a stirred solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (100 mg, 0.56 mmol) in DMSO (1.5 mL) at rt. The vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, additional *m*-toluidine (0.151 mL, 1.40 mmol) was added and the vial reheated in a Biotage Initiator microwave to 120 °C for 1 h. This process was repeated two more times. Upon cooling to rt, the solution was purified by formic MDAP. The combined fractions were evaporated under reduced pressure to give **18** as a grey solid (41 mg, 30%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.64 (s, 1H), 7.30–7.26 (m, 1H), 7.06–7.01 (m, 3H), 3.61 (s, 3H), 2.31 (s, 3H); LCMS (formic acid) $(M+H)^+$ = 250.1, 252.1, R_t = 0.88 min (100%).

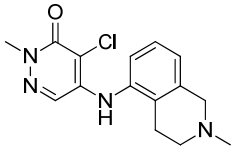
5-((2-(Aminomethyl)phenyl)amino)-4-chloro-2-methylpyridazin-3(2H)-one hydrochloride salt (19)



Step 1: *tert*-Butyl 2-aminobenzylcarbamate (99 mg, 0.44 mmol), 4-chloro-5-iodo-2-methylpyridazin-3(2*H*)-one (**17**)[1] (100 mg, 0.37 mmol), palladium (II) acetate (11 mg, 0.05 mmol), cesium carbonate (205 mg, 0.63 mmol) and (±)-BINAP (41 mg, 0.07 mmol) were suspended in toluene (3 mL) and heated to 80 °C under microwave conditions for 4 h. Upon cooling to rt, the reaction mixture was partitioned between EtOAc (10 mL) and water (3 mL) and the aqueous layer removed. The organic layer was washed (1x water [3 mL], 1x brine [3 mL]), dried over $MgSO_4$ and evaporated *in vacuo* to give a brown gum. The residue was purified by silica gel chromatography (10–50% EtOAc in cyclohexane). The appropriate fractions were combined and evaporated under reduced pressure to give *tert*-butyl 2-((5-chloro-1-methyl-6-oxo-1,6-dihydropyridazin-4-yl)amino)benzylcarbamate as a pale brown solid (44 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (br.s, 1H), 7.42–7.29 (m, 4H), 7.28–7.17 (m, 2H), 4.11 (d, *J* = 6.0 Hz, 2H), 3.59 (s, 3H), 1.35 (s, 9H); LCMS (TFA) $(M+H)^+$ = 365.3, 367.2, R_t = 0.95 min (100%).

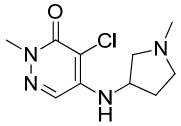
Step 2: A solution of 5 M HCl in IPA (3.00 mL, 15.0 mmol) was added to *tert*-butyl 2-((5-chloro-1-methyl-6-oxo-1,6-dihydropyridazin-4-yl)amino)benzylcarbamate (42 mg, 0.12 mmol) and the suspension stirred for 2 h at rt. The resulting solution was evaporated to dryness, triturated with *t*-BuOMe (2 mL) and then filtered. The collected solid was washed (1x TBME [2 mL]) and dried *in vacuo* to give **19** as a pale brown solid as the HCl salt (28 mg, 73%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (s, 1H), 8.31 (br.s, 3H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.52–7.38 (m, 2H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.27 (s, 1H), 4.05 (s, 2H), 3.61 (s, 3H); LCMS (TFA) $(M+H)^+$ = 264.9, 266.8, R_t = 0.46 min (99%).

4-Chloro-2-methyl-5-((2-methyl-1,2,3,4-tetrahydroisoquinolin-5-yl)amino)pyridazin-3(2H)-one (20)



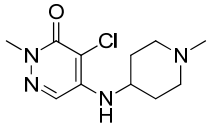
2-Methyl-1,2,3,4-tetrahydroisoquinolin-5-amine (70 mg, 0.43 mmol), 4-chloro-5-iodo-2-methylpyridazin-3(2*H*)-one (**17**)[1] (117 mg, 0.43 mmol), palladium (II) acetate (13 mg, 0.06 mmol), cesium carbonate (239 mg, 0.73 mmol) and (±)-BINAP (48 mg, 0.08 mmol) were suspended in toluene (3 mL) and heated to 80 °C under microwave conditions for 4 h. Upon cooling to rt, the reaction mixture was partitioned between EtOAc and water and the aqueous layer removed. The organic layer was washed (1x sat. aq. sodium hydrogencarbonate, 1x brine) and then evaporated *in vacuo* to a brown gum. The residue was then purified by MDAP (high pH). The combined fractions were evaporated under reduced pressure to give **20** as a white solid (19 mg, 13%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (br.s, 1H), 7.22 (app.t, *J* = 8.0 Hz, 1H), 7.12 (s, 1H), 7.10–7.05 (m, 2H), 3.59 (s, 3H), 3.52 (s, 2H), 2.66 (t, *J* = 5.5 Hz, 2H), 2.56 (t, *J* = 5.5 Hz, 2H), 2.33 (s, 3H); LCMS (high pH) $(M+H)^+$ = 305.2, 307.2, R_t = 0.79 min (100%).

4-Chloro-2-methyl-5-((1-methylpyrrolidin-3-yl)amino)pyridazin-3(2H)-one (21)



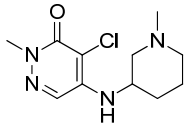
1-Methylpyrrolidin-3-amine (86 mg, 0.86 mmol) was added in a single portion to a stirred solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (103 mg, 0.57 mmol) and DIPEA (0.30 mL, 1.72 mmol) in DMSO (2 mL). The vial was sealed and then heated in a Biotage Initiator microwave to 130 °C for 1 h. Upon cooling to rt, the solution was purified by MDAP (high pH). The combined fractions were evaporated under reduced pressure to give **21** as a brown gum (36 mg, 26%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.84 (s, 1H), 4.37–4.33 (m, 1H), 3.72 (s, 3H), 2.89-2.83 (m, 2H), 2.67 (dd, *J* = 10.2, 4.2 Hz, 1H), 2.55–2.40 (m, 5H), 1.85–1.80 (m, 1H); LCMS (high pH) $(M+H)^+$ = 243.2, 245.2, R_t = 0.58 min (100%).

4-Chloro-2-methyl-5-((1-methylpiperidin-4-yl)amino)pyridazin-3(2H)-one (22)



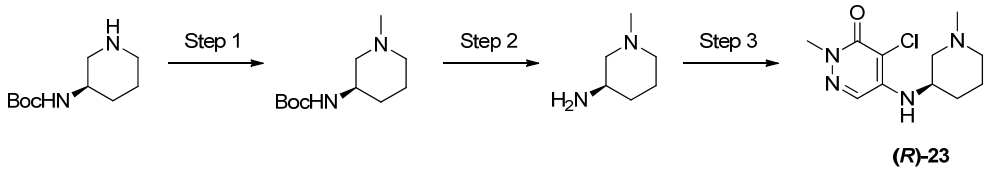
1-Methylpiperidin-4-amine (23 mg, 0.20 mmol) was added to a solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (24 mg, 0.13 mmol) in DMF (0.4 mL). DIPEA (0.04 mL, 0.20 mmol) was added, the vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 30 min. Upon cooling to rt, the solution was purified by MDAP (high pH). The combined fractions were evaporated under reduced pressure to give **22** (4 mg, 12%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.90 (s, 1H), 6.10 (d, *J* = 8.7 Hz, 1H), 3.57–3.55 (m, 4H), 2.73 (d, *J* = 11.7 Hz, 2H), 2.15 (s, 3H), 2.06–1.86 (m, 2H), 1.74 (d, *J* = 10.4 Hz, 2H), 1.69–1.58 (m, 2H); LCMS (formic) $(M+H)^+$ = 257.1, 259.1, R_t = 0.31 min (100%).

4-Chloro-2-methyl-5-((1-methylpiperidin-3-yl)amino)pyridazin-3(2H)-one (23)



DIPEA (0.29 mL, 1.70 mmol) was added to a solution of 1-methylpiperidin-3-amine (96 mg, 0.84 mmol) and 4,5-dichloro-2-methylpyridazin-3(2*H*)-one (**9**) (100 mg, 0.56 mmol) in DMSO (2 mL). The vial was sealed and then heated in a Biotage Initiator microwave to 130 °C for 1 h. Upon cooling to rt, the solution was purified by high pH MDAP. The combined fractions were evaporated under reduced pressure to give **23** as a yellow solid (37 mg, 26%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 5.89 (d, *J* = 9.0 Hz, 1H), 3.94–3.80 (s, 1H), 3.58 (s, 3H), 2.60–2.52 (m, 1H), 2.42–2.11 (m, 6H), 1.72–1.39 (m, 4H); LCMS (formic) $(M+H)^+$ = 257.2, 259.2, R_t = 0.34 min (100%).

(*R*)-4-Chloro-2-methyl-5-((1-methylpiperidin-3-yl)amino)pyridazin-3(2H)-one [(*R*)-23]



(*R*)-23

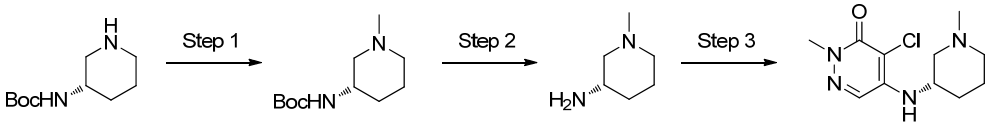
Step 1: Formic acid (1.94 mL, 50.5 mmol) was added to a solution of (*R*)-*tert*-butyl piperidin-3-ylcarbamate (5.06 g, 25.3 mmol) and 37% w/v formaldehyde in water (3.88 mL, 52.1 mmol) in 2-MeTHF (135 mL). The resulting solution was stirred for 1 h at rt, heated to 80 °C for 30 min and then cooled to rt. The solution was evaporated under reduced pressure to give a colourless oil. The residue was purified by silica gel chromatography (0–10% 2 M NH_3 in MeOH, in CH_2Cl_2). The appropriate fractions were combined and evaporated under reduced pressure to give (*R*)-*tert*-butyl (1-methylpiperidin-3-yl)carbamate as a white solid (3.71 g, 69%). ^{1}H NMR (400 MHz, DMSO-d_6, 120 °C) δ 6.65 (d, *J* = 8.0 Hz, 1H), 3.52–3.34 (m, obscured by water peak), 2.68 (dd, *J* = 10.0, 3.0 Hz, 1H), 2.55 (d, *J* = 11.0 Hz, 1H), 2.13 (s, 3H), 1.81–1.71 (m, 1H), 1.71–1.54 (m, 3H), 1.48–1.32 (m, 10H), 1.14–1.02 (m, 1H).

Step 2: A solution of 5 M HCl in IPA (55.0 mL, 275 mmol) was added to (*R*)-*tert*-butyl (1-methylpiperidin-3-yl)carbamate (3.70 g, 17.3 mmol) and the resulting suspension heated to 80 °C for 1 h. The resulting

suspension was cooled to rt and *t*-BuOMe (55 mL) added. The suspension was filtered, the collected solid washed (2 × *t*-BuOMe [30 mL]) and dried *in vacuo* to give (*R*)-1-methylpiperidin-3-amine dihydrochloride as a white solid (3.21 g, 100%). ^{1}H NMR (400 MHz, DMSO-d_6, 120 °C) δ 3.65 (tt, *J* = 4.5, 9.5 Hz, 1H), 3.54–3.45 (m, 1H), 3.31–3.19 (m, 1H), 3.18–3.08 (m, 1H), 3.02–2.89 (m, 1H), 2.75 (s, 3H), 2.18–2.02 (m, 1H), 2.01–1.84 (m, 2H), 1.81–1.61 (m, 1H), exchangeable protons not observed.

Step 3: DIPEA (3.28 mL, 18.8 mmol) was added to a suspension of 4,5-dichloro-2-methyl-3(2*H*)-pyridazinone (**9**) (800 mg, 4.47 mmol) and (*R*)-1-methylpiperidin-3-amine dihydrochloride (1.00 g, 5.36 mmol) in DMSO (2.4 mL). The mixture was heated to 130 °C for 4 h under microwave conditions. Upon cooling to rt, the reaction mixture was partitioned between CH_2Cl_2 (20 mL) and sat. aq. $NaHCO_3$ (20 mL). The organic layer was removed and the aqueous layer extracted (3 × CH_2Cl_2 [20 mL]). The organic portions were combined, dried over $MgSO_4$ and evaporated under reduced pressure to give an orange oil. The residue was purified by silica gel chromatography (0–5% 2 M NH_3 in MeOH, in CH_2Cl_2). The appropriate fractions were combined and evaporated under reduced pressure to give **(*R*)-23** as a crystalline brown solid (600 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 5.24 (br.s, 1H), 3.79–3.67 (m, 4H), 2.67–2.52 (m, 1H), 2.52–2.36 (m, 2H), 2.36–2.22 (m, 4H), 1.84–1.66 (m, 2H), 1.65–1.47 (m, 2H); LCMS (TFA) $(M+H)^+$ = 257.1, 259.0, R_t = 0.38 min (100%).

(*S*)-4-Chloro-2-methyl-5-((1-methylpiperidin-3-yl)amino)pyridazin-3(2H)-one [(*S*)-23]



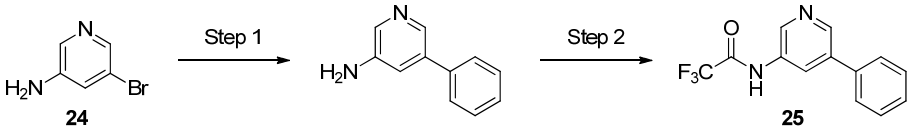
(*S*)-23

Step 1: Formic acid (0.39 mL, 10.20 mmol) was added to a suspension of (*S*)-*tert*-butyl piperidin-3-ylcarbamate (1.02 g, 5.09 mmol) and 37% w/v formaldehyde in water (0.83 mL, 10.2 mmol) in 2-MeTHF (50 mL). The suspension was heated to 80 °C for 45 min and cooled to rt. The reaction mixture was evaporated under reduced pressure to give a colourless oil. The residue was purified by silica gel chromatography (0–8% 2 M NH_3 in MeOH, in CH_2Cl_2). The appropriate fractions were combined and evaporated under reduced pressure to give (*S*)-*tert*-butyl (1-methylpiperidin-3-yl)carbamate as a white solid (985 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6, 120 °C) δ 6.65 (d, *J* = 8.0 Hz, 1H), 3.50–3.34 (m, obscured by water peak), 2.68 (dd, *J* = 10.0, 3.0 Hz, 1H), 2.55 (d, *J* = 11.0 Hz, 1H), 2.13 (s, 3H), 1.81–1.71 (m, 1H), 1.71–1.54 (m, 2H), 1.48–1.32 (m, 10H), 1.14–1.02 (m, 1H).

Step 2: A solution of 5 M HCl in IPA (15 mL, 75 mmol) was added to (*S*)-*tert*-butyl (1-methylpiperidin-3-yl)carbamate (985 mg, 4.60 mmol) and the mixture heated to 50 °C for 5 min and cooled to 20 °C. The resulting suspension was allowed to stand for 1 h, diluted with *t*-BuOMe (20 mL) and filtered. The collected solid was washed (2 × *t*-BuOMe [5 mL]) and dried *in vacuo* to give the (*S*)-1-methylpiperidin-3-amine dihydrochloride as a white solid (709 mg, 84%): ^{1}H NMR (DMSO-d_6, 400 MHz, 120 °C) δ 3.65 (tt, *J* = 4.5, 9.5 Hz, 1H), 3.54–3.45 (m, 1H), 3.31–3.19 (m, 1H), 3.18–3.08 (m, 1H), 3.02–2.89 (m, 1H), 2.75 (s, 3H), 2.18–2.02 (m, 1H), 2.01–1.84 (m, 2H), 1.81–1.61 (m, 1H), exchangeable protons not observed.

Step 3: DIPEA (0.41 mL, 2.4 mmol) was added to a stirred suspension of 4,5-dichloro-2-methylpyridazin-3(2*H*)-one (**9**) (100 mg, 0.56 mmol), and (*S*)-1-methylpiperidin-3-amine dihydrochloride (136 mg, 0.73 mmol) in DMSO (0.4 mL). The resulting suspension was heated to 130 °C for 5 h under microwave conditions. Upon cooling to rt, the reaction mixture was diluted with MeOH (2 mL) and loaded on to a preconditioned SCX cartridge in MeOH. The cartridge was eluted with MeOH (40 mL), followed by 2 M methanolic ammonia (50 mL). The basic fractions were evaporated under reduced pressure to give a brown oil. The residue was purified by MDAP (high pH). The combined fractions were evaporated under reduced pressure to give **(*S*)-23** as a brown solid (13 mg, 9%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (s, 1H), 5.89 (d, *J* = 9.0 Hz, 1H), 3.94–3.80 (s, 1H), 3.58 (s, 3H), 2.60–2.52 (m, 1H), 2.42–2.11 (m, 6H), 1.72–1.39 (m, 4H); LCMS (high pH) $(M+H)^+$ = 257.2, 259.2, R_t = 0.66 min (100%).

2,2,2-Trifluoro-*N*-(5-phenylpyridin-3-yl)acetamide trifluoroacetic acid salt (25)



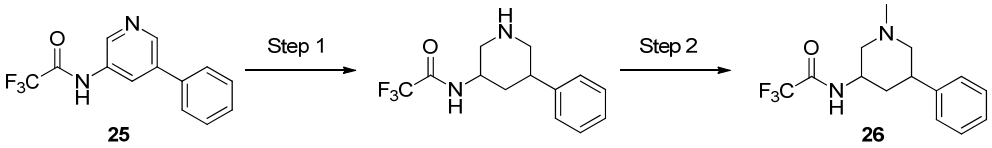
24 **25**

Step 1: $Pd(PPh_3)_4$ (0.668 g, 0.578 mmol) was added in a single portion to a stirred suspension of 5-bromopyridin-3-amine (5.00 g, 28.9 mmol), phenylboronic acid (4.23 g, 34.7 mmol) and potassium carbonate

(11.98 g, 87 mmol) in toluene (50 mL) and EtOH (50 mL) at rt under N_2. The resultant suspension was evacuated under vacuum and back-filled with N_2 three times. The resultant suspension was then heated to 100 °C for 1 h under N_2. Upon cooling to rt, the solvent was evaporated under reduced pressure to give a brown solid. The solid was dissolved in EtOAc (100 mL) and H_2O (50 mL). The separated organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give a brown solid. The solid was dissolved in MeOH (20 mL) and silica (~10 g) was added. The solvent was evaporated under reduced pressure and the resultant solid was directly purified by silica gel chromatography (0–10% 2 M NH_3 in MeOH, in CH_2Cl_2). The appropriate fractions were combined and evaporated under reduced pressure to give 5-phenylpyridin-3-amine as an orange solid (4.85 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 2 Hz, 1H), 8.11 (d, *J* = 2.7 Hz, 1H), 7.57–7.55 (m, 2H), 7.49–7.45 (m, 2H), 7.41–7.40 (m, 1H), 7.18–7.17 (m, 1H), 3.78 (br.s, 2H); LCMS (formic) $(M+H)^+$ = 171.1, R_t = 0.49 min (99%).

Step 2: Trifluoroacetic anhydride (5.23 mL, 37.0 mmol) was added dropwise over 3 min to a stirred solution of 5-phenylpyridin-3-amine (4.85 g, 28.5 mmol) in CH_2Cl_2 (100 mL) at rt. The resultant solution was stirred at rt for 15 min and then the solvent was evaporated under reduced pressure to give an orange foam. CH_2Cl_2 (10 mL) and Et_2O (50 mL) were added which resulted in a white preciptate forming. Upon standing for 10 min, the solid was filtered, washed with Et_2O (50 mL) and then air dried under vacuum for 10 min to give **25** as a white solid (6.12 g, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.61 (br.s, 1H), 8.86 (d, *J* = 2.2 Hz, 1H), 8.78 (d, *J* = 1.7 Hz, 1H), 8.36 (s, 1H), 7.73–7.71 (m, 2H), 7.56–7.47 (m, 3H); LCMS (formic) $(M+H)^+$ = 267.0, R_t = 0.95 min (100%).

2,2,2-Trifluoro-*N*-(1-methyl-5-phenylpiperidin-3-yl)acetamide formic acid salt (26)

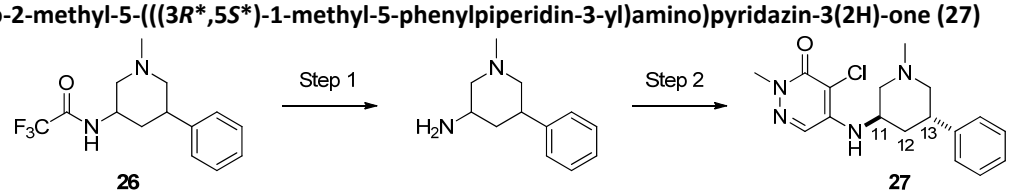


25 **26**

Step 1: A solution of 2,2,2-trifluoro-*N*-(5-phenylpyridin-3-yl)acetamide trifluoroacetic acid salt (**25**) (1.00 g, 2.63 mmol) in AcOH (15 mL) was hydrogenated using the H-cube (settings: 90 °C, 70 bar H_2, 1 mL / min flow rate) and 10% Pd/C CatCart 30 as the catalyst for 3 h. The solvent was evaporated under reduced pressure to give a colourless oil. The oil was dissolved in MeOH (10 mL) and then loaded onto an SCX cartridge (20 g) that had been prewashed with MeOH (2 CV). The cartridge was then eluted with MeOH (2 CV), followed by 2 M NH_3 in MeOH (2 CV). The methanolic ammonia fractions were combined and evaporated under reduced pressure to give a colourless oil. The oil was loaded in CH_2Cl_2 and purified by silica gel column chromatography using a gradient of 0-5% 2 M NH_3 in MeOH / CH_2Cl_2. The appropriate fractions were combined and evaporated under vacuum to give 2,2,2-trifluoro-N-(5-phenylpiperidin-3-yl)acetamide as a colourless oil (201 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (br.s, 1H), 7.35–7.31 (m, 2H), 7.27–7.21 (m, 3H), 4.31 (br.s, 1H), 3.23–3.20 (m, 1H), 3.05–3.02 (m, 1H), 2.97–2.89 (m, 2H), 2.74–2.69 (t, J = 11 Hz, 1H), 2.23–2.17 (m, 1H), 1.92–1.79 (m, 2H); LCMS (formic) $(M+H)^+$ = 273.1, R_t = 0.61 min (67%).

Step 2: Formic acid (0.057 mL, 1.48 mmol) was added in a single portion to a stirred solution of 2,2,2-trifluoro-N-(5-phenylpiperidin-3-yl)acetamide (201 mg, 0.74 mmol) and formaldehyde (0.11 mL, 37% by weight in water, 1.48 mmol) in 2-MeTHF (5 mL) at rt. The resultant solution was stirred at rt for 10 min and then warmed to 80 °C for 45 min. Upon cooling to rt, the solvent was evaporated under reduced pressure to give **26** as a colourless oil as the formic acid salt (210 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (br.s, 1H), 7.38–7.29 (m, 3H), 7.22–7.20 (m, 2H), 4.97–4.89 (m, 1H), 4.62 (br.s, 1H), 3.59–3.50 (m, 3H), 2.87 (dd, *J* = 13, 3.4 Hz, 1H), 2.75 (s, 3H), 2.57 (t, *J* = 11.6 Hz, 1H), 2.32 (d, *J* = 14.2 Hz, 1H), 1.96 (td, *J* = 13.6, 4 Hz, 1H); LCMS (formic) $(M+H)^+$ = 287.1, R_t = 0.61 min (80%).

4-Chloro-2-methyl-5-(((3*R,5*S**)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one (27)**



26 **27**

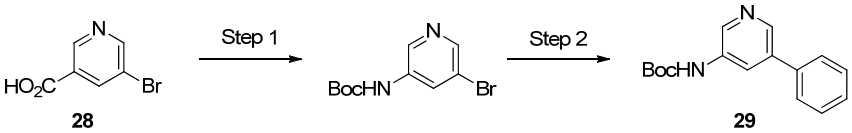
Step 1: Potassium carbonate (406 mg, 2.93 mmol) was added in a single portion to a stirred solution of 2,2,2-trifluoro-*N*-(1-methyl-5-phenylpiperidin-3-yl)acetamide formic acid salt (**26**) (210 mg, 0.63 mmol) in MeOH (2.5 mL) and H_2O (0.5 mL) at rt. The resultant solution was then allowed to stand at rt for 72 h and then was heated to 60 °C for 2 h. Upon cooling to rt, the solvent was evaporated under reduced pressure to give a white solid. The solid was dissolved in EtOAc (20 mL) and H_2O (10 mL). The separated aqueous phase was

extracted with EtOAc (2 × 10 mL), the combined organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give 1-methyl-5-phenylpiperidin-3-amine as a colourless oil (120 mg, 99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31–7.18 (m, 5H), 3.65–3.11 (m, 2H), 2.90–2.88 (m, 1H), 2.72–2.69 (m, 1H), 2.26–2.12 (m, 6H), 2.04–1.99 (m, 1H), 1.82–1.69 (m, 2H); LCMS (high pH) $(M+H)^+$ = 191.3, R_t = 0.66 min (70%).

Step 2: A solution of 1-methyl-5-phenylpiperidin-3-amine (120 mg, 0.63 mmol) in DMSO (2 mL) was added to a microwave vial containing 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (85 mg, 0.48 mmol). DIPEA (0.166 mL, 0.95 mmol) was added in a single portion and the resultant solution was heated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, the solution was purified by MDAP (high pH). The solvent was evaporated under vacuum to give a colourless oil. The oil was dissolved in MeOH (10 mL) and loaded onto an amino propyl column (2 g) that had been prewashed with MeOH (2 CV). The column was eluted with MeOH (2 CV) and the appropriate fractions were combined and evaporated under reduced pressure to give **27** as a cream solid (42 mg, 27%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.92 (s, 1 H), 7.31–7.23 (m, 4 H), 7.22–7.17 (m, 1H), 5.78 (d, *J* = 8.6 Hz, 1 H), 4.20 (dquin, *J* = 8.6, 3.0, 3.0, 3.0, 3.0 Hz, 1 H), 3.59 (s, 3 H), 2.93 (dddd, *J* = 13.0, 11.0, 4.0, 3.0 Hz, 1 H), 2.91–2.83 (m, 2 H), 2.31 (dd, *J* = 11.6, 2.2 Hz, 1 H), 2.27 (s, 3 H), 2.02 (t, *J* = 11.0 Hz, 1 H), 1.90 - 1.85 (m, 1 H), 1.72 (td, *J* = 13.0, 3.0 Hz, 1 H); LCMS (high pH) $(M+H)^+$ = 333.1, 335.2, R_t = 1.05min (100%).

Relative stereochemical determination: Axial-equatorial assignments and associated coupling constants (e.g.^{3}J12ax-13ax=11.5Hz & ^{3}J12ax-11eq=3Hz) indicate that the piperidine ring has *trans*-relative stereochemistry.

***t*ert-Butyl (5-phenylpyridin-3-yl)carbamate (29)**

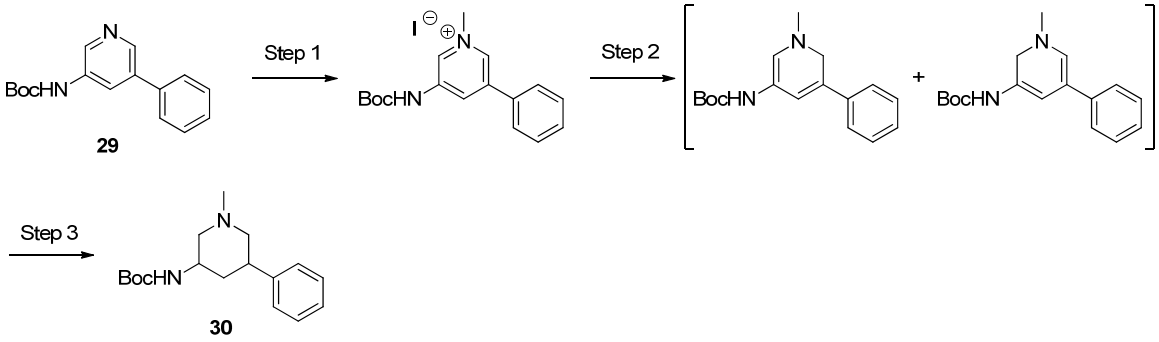


28 **29**

Step 1: Diphenyl phosphorylazide (40.0 mL, 186 mmol) was added dropwise over 15 min to a stirred solution triethylamine (51.5 mL, 371 mmol) and 5-bromonicotinic acid (**28**) (25 g, 124 mmol) in toluene (250 mL) and *tert*-butanol (50 mL) at rt under N_2. The resultant solution was stirred at rt for 20 min and then heated to 100 °C for 2 h. Upon cooling to rt, sat. aq. $NaHCO_3$ (200 mL) and EtOAc (200 mL) were added. The separated aqueous phase was extracted with EtOAc (2 × 100 mL). The combined organic phase was washed with brine (100 mL), passed through a hydrophobic frit and evaporated under reduced pressure to give an orange solid. The solid was loaded in CH_2Cl_2 and purified by silica gel chromatography using a gradient of 0-15% EtOAc / cyclohexane. The appropriate fractions were combined and evaporated under vacuum to give *tert*-butyl (5-bromopyridin-3-yl)carbamate as a white solid (29.81 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 8.30 (app. s, 2H), 6.68 (br.s, 1H), 1.53 (s, 9H); LCMS (formic) $(M+H)^+$ = 273.0, 275.0, R_t = 1.04 min (100%).

Step 2: $Pd(PPh_3)_4$ (2.52 g, 2.18 mmol) was added in a single portion to a stirred suspension of *tert*-butyl (5-bromopyridin-3-yl)carbamate (29.81 g, 109 mmol), phenylboronic acid (15.97 g, 131 mmol) and potassium carbonate (45.3 g, 327 mmol) in toluene (250 mL) and EtOH (250 mL) at rt under N_2. The resultant suspension was evacuated under vacuum and then back-filled with N_2 three times. The suspension was then heated to 100 °C for 1 h. Upon cooling to rt, EtOAc (300 mL) and H_2O (200 mL) were added. The separated aqueous phase was extracted with EtOAc (2 × 100 mL), the combined organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give an orange solid. The solid was mostly dissolved in CH_2Cl_2 (100 mL) and loaded onto a silica plug (15 cm x 30 cm). The plug was eluted with EtOAc (1000 mL). The eluent was combined and evaporated under reduced pressure to give a yellow solid. The solid was suspended in Et_2O (100 mL) and filtered. The collected solid was washed with Et_2O (200 mL) and air dried under vacuum for 10 min to give **29** as a cream solid (24.29 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 2.0 Hz, 1H), 8.40 (d, *J* = 2.4 Hz, 1H), 8.25 (br.s, 1H), 7.62–7.59 (m, 2H), 7.48–7.38 (m, 3H), 6.73 (br.s, 1H), 1.54 (s, 9H); LCMS (formic) $(M+H)^+$ = 271.1, R_t = 0.95 min (100%).

***tert*-Butyl-1-methyl-5-phenylpiperidin-3-yl)carbamate (30)**

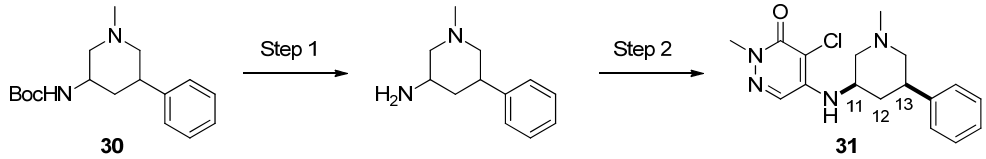


Step 1: MeI (6.74 ml, 108 mmol) was added dropwise over 2 min to a stirred solution of *tert*-Butyl (5-phenylpyridin-3-yl)carbamate (**29**) (24.29 g, 90 mmol) in DMF (125 ml) at rt. The resultant solution was stirred at rt for 16 h by which time a suspension had formed. The suspension was diluted with Et_2O (100 mL) and filtered. The collected solid was washed with Et_2O (2 × 100 mL) and air dried under vacuum for 10 min to give 3-((*tert*-butoxycarbonyl)amino)-1-methyl-5-phenylpyridin-1-ium iodide as a pale yellow solid (34.36 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (s, 1H), 9.08 (d, *J* = 7.3 Hz, 2H), 8.50 (s, 1H), 7.79–7.76 (m, 2H), 7.66–7.59 (m, 3H), 4.41 (s, 3H), 1.54 (s, 9H); LCMS (formic) $(M)^+$ = 285.1, R_t = 0.78 min (100%).

Step 2: Sodium borohydride (0.529 g, 13.97 mmol) was added portionwise over 1 min to a stirred suspension of 3-((*tert*-butoxycarbonyl)amino)-1-methyl-5-phenylpyridin-1-ium iodide (1.44 g, 3.49 mmol) in EtOH (30 mL) at rt. The resultant suspension was stirred at rt for 20 h and then sat. aq. $NaHCO_3$ (50 mL) was added, followed by EtOAc (50 mL). The separated aqueous phase was extracted with EtOAc (2 × 50 mL), the combined organic phase was passed through a hydrophobic frit and evaporated under reduced pressure to give a crude pale yellow oil (890 mg) which was used without further purification. LCMS (formic) $(M+H)^+$ = 287.2, R_t = 0.69 min (33%).

Step 3: A solution of the pale yellow oil (890 mg) in MeOH (20 mL) was hydrogenated using the H-cube (settings: 60 °C, 50 bar H_2, 1ml/min flow rate) and 10% Pd/C CatCart 30 as the catalyst. The solution was recycled through the H-cube for 24 h. The solvent was then evaporated to give a colourless oil. The oil was loaded in CH_2Cl_2 and purified by silica gel column chromatography using a gradient of 0-10% 2 M NH_3 in MeOH / CH_2Cl_2. The appropriate fractions were combined and evaporated under vacuum to give **30** as a colourless oil (405 mg, 40% over two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.29 (m, 2H), 7.24–7.20 (m, 3H), 4.38 (br.s, 1H), 3.83 (br.s, 1H), 3.16–3.15 (m, 1H), 2.98–2.92 (m, 1H), 2.33 (s, 3H), 2.26–2.20 (m, 1H), 1.97–1.91 (m, 1H), 1.71–1.45 (m, 11 H), 1.30–1.21 (m, 1H); LCMS (formic) $(M+H)^+$ = 291.2, R_t = 0.71 min (81%).

4-Chloro-2-methyl-5-(((3*R,5*R**)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one (31)**



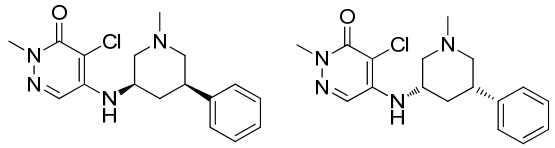
Step 1: TFA (1.07 mL, 13.95 mmol) was added in a single portion to a stirred solution of *tert*-butyl (1-methyl-5-phenylpiperidin-3-yl)carbamate (**30**) (405 mg, 1.40 mmol) in CH_2Cl_2 (10 mL) at rt. The resultant solution was stirred at rt for 30 min. The solvent was evaporated under reduced pressure to give a dark orange oil. The oil was dissolved in MeOH (10 ml) and then loaded onto an aminopropyl column (5 g) that had been prewashed with MeOH (2 CV). The colunm was eluted with MeOH (2 CV). The appropriate fractions were combined and evaporated under reduced pressure to give a yellow foam (315 mg) which was used without further purification. LCMS (high pH) $(M+H)^+$ = 191.2, R_t = 0.70 min (63%).

Step 2: DIPEA (0.44 mL, 2.51 mmol) was added in a single portion to a microwave vial containing a solution of 4,5-dichloro-2-methylpyridazin-3(2H)-one (**9**) (225 mg, 1.26 mmol) and the yellow foam (crude 1-methyl-5-phenylpiperidin-3-amine) (315 mg) in DMSO (3 mL) at rt. The vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 1 h. Upon cooling to rt, sat. aq. NH_4Cl (10 mL) and EtOAc (20 mL) were added. The separated aqueous phase was extracted with EtOAc (2 × 10 mL), the combined organic phase was then evaporated under reduced pressure to give an orange oil. The oil was loaded in CH_2Cl_2 and purified by silica gel column chromatography using a gradient of 0-10% 2 M NH_3 in MeOH / CH_2Cl_2. The appropriate fractions were combined and evaporated under vacuum to give an orange oil. The oil was dissolved in DMSO (4 mL) and purified by MDAP (high pH). The solvent was evaporated under vacuum to give **31** as a white solid

(44 mg, 11% over two steps based on (**9**) as yield limiting reagent). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.95 (s, 1 H), 7.35–7.29 (m, 2 H), 7.27–7.24 (m, 2 H), 7.24–7.19 (m, 1 H), 6.10 (d, *J* = 8.8 Hz, 1 H), 3.94 (qt, *J* = 10.0, 4.0 Hz, 1 H), 3.58 (s, 3 H), 2.98–2.90 (m, 2 H), 2.83 (dd, *J* = 11.0, 3.3 Hz, 1 H), 2.24 (s, 3 H), 2.01 (d, *J* = 12.5 Hz, 1 H), 1.96 (t, *J* = 10.6 Hz, 1 H), 1.88 (t, *J* = 11.2 Hz, 1 H), 1.68 (q, *J* = 12.1 Hz, 1 H); LCMS (high pH) $(M+H)^+$ = 333.2, 335.1, R_t = 0.90 min (100%).

Relative stereochemical determination: Axial-equatorial assignments and associated coupling constants (e.g. ^{3}J12ax-11ax=11Hz & ^{3}J12ax-13ax=11Hz) indicate that the piperidine ring has *cis*-relative stereochemistry as depicted.

4-Chloro-2-methyl-5-(((3*R*,5*R*)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one [(*R*,*R*)-31] and 4-chloro-2-methyl-5-(((3*S*,5*S*)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one [(*S*,*S*)-31]



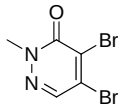
(*R*,*R*)-31 **(*S*,*S*)-31**

4-Chloro-2-methyl-5-(((3*R**,5*R**)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one (**31**) (118 mg) was dissolved in EtOH/heptane (1:1, 2 mL). The solution was purified by preparative chiral HPLC, 40% EtOH/heptanes, 30 mL / min, wavelength 215 nm, 30mm x 25 cm Chiralpak AD-H (Lot No. ADH12143-01). The appropriate fractions were evaporated under reduced pressure to give **(*R*,*R*)-31** (48 mg) and **(*S*,*S*)-31** (47 mg) as white solids.

(*R*,*R*)-31: Analytical data as **31** above; HPLC (Chiralpak AD-H column, 4.6 mm x 25 cm, 40% EtOH/heptanes, 1 mL/min): 6.7 min (major enantiomer), 12.9 min (minor enantiomer), >99% ee.

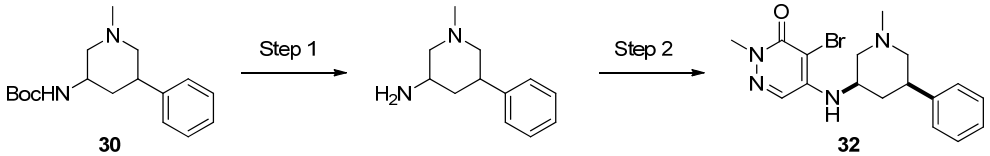
(*S*,*S*)-31: Analytical data as **31** above; HPLC (Chiralpak AD-H column, 4.6 mm x 25 cm, 40% EtOH/heptanes, 1 mL/min): 6.7 min (minor enantiomer), 12.9 min (major enantiomer), >99% ee.

4,5-Dibromo-2-methylpyridazin-3(2H)-one (33)



Methylhydrazine (1.75 mL, 38.8 mmol) was added dropwise over 5 min to a stirred solution of 3,4-dibromo-5-hydroxyfuran-2(5H)-one (10 g, 38.8 mmol) in EtOH (45 mL) at 0 °C. Following stirring at 0 °C for 1 h, the flask was removed from the cooling bath and heated to 80 °C for 4 h. Upon cooling to rt, the resultant solution was allowed to stand at rt for 12 h by which time a suspension had formed. The suspension was filtered, washed with EtOH (100 mL) and then dried under vacuum for 20 min to give 4,5-dibromo-2-methylpyridazin-3(2H)-one (**33**) as a pale yellow solid (5.32 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (s, 1H), 3.67 (s, 3H); LCMS (formic) $(M+H)^+$ = 266.7, 268.7, 270.8, R_t = 0.71 min (100%).

4-Bromo-2-methyl-5-(((3*R,5*R**)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one (32)**



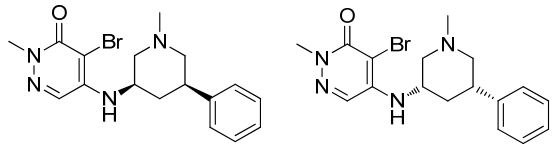
30 **32**

Step 1: TFA (1.85 mL, 24.04 mmol) was added portionwise over 30 s to a stirred solution of *tert*-butyl (1-methyl-5-phenylpiperidin-3-yl)carbamate (**30**) (698 mg, 2.40 mmol) in CH_2Cl_2 (20 mL) at rt. Following stirring at rt for 1 h the solvent was evaporated under reduced pressure to give an orange oil. The oil was dissolved in MeOH (20 mL) and then passed through an amino propyl column (10 g) that had been prewashed with MeOH (2 CV). The column was then further eluted with MeOH (2 CV). The appropriate fractions were combined and evaporated under reduced pressure to give crude 1-methyl-5-phenylpiperidin-3-amine as a yellow foam (580 mg) which was used without further purification.

Step 2: DIPEA (0.72 mL, 4.11 mmol) was added in a single portion to a stirred solution of 4,5-dibromo-2-methylpyridazin-3(2H)-one (**33**) (550 mg, 2.05 mmol) and the yellow foam (crude 1-methyl-5-phenylpiperidin-3-amine) (580 mg) in DMSO (10 mL) at rt. The vial was sealed and then heated in a Biotage Initiator microwave to 120 °C for 7 h. Upon cooling to rt, the vial was reheated in a Biotage Initiator microwave to 130

°C for 3 h. Upon cooling to rt, the solution was purified by MDAP (high pH). The solvent was evaporated under vacuum to give **32** as a pale yellow solid (141 mg, 18% over two steps based on (**33**) as the yield limiting reagent). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (s, 1H), 7.33–7.20 (m, 5H), 5.81 (d, *J* = 9 Hz, 1H), 3.96–3.90 (m, 1H), 3.59 (s, 3H), 2.97–2.92 (m, 2H), 2.84 (d, *J* = 10.8 Hz, 1H), 2.24 (s, 3H), 2.03–1.87 (m, 3 H), 1.73 (q, *J* = 12 Hz, 1H); LCMS (high pH) $(M+H)^+$ = 377.2, 379.2, R_t = 0.94 min (100%).

4-Bromo-2-methyl-5-(((3*R*,5*R*)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one [(*R*,*R*)-32, (GSK4027)] and 4-bromo-2-methyl-5-(((3*S*,5*S*)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one [(*S*,*S*)-32, (GSK4028)]



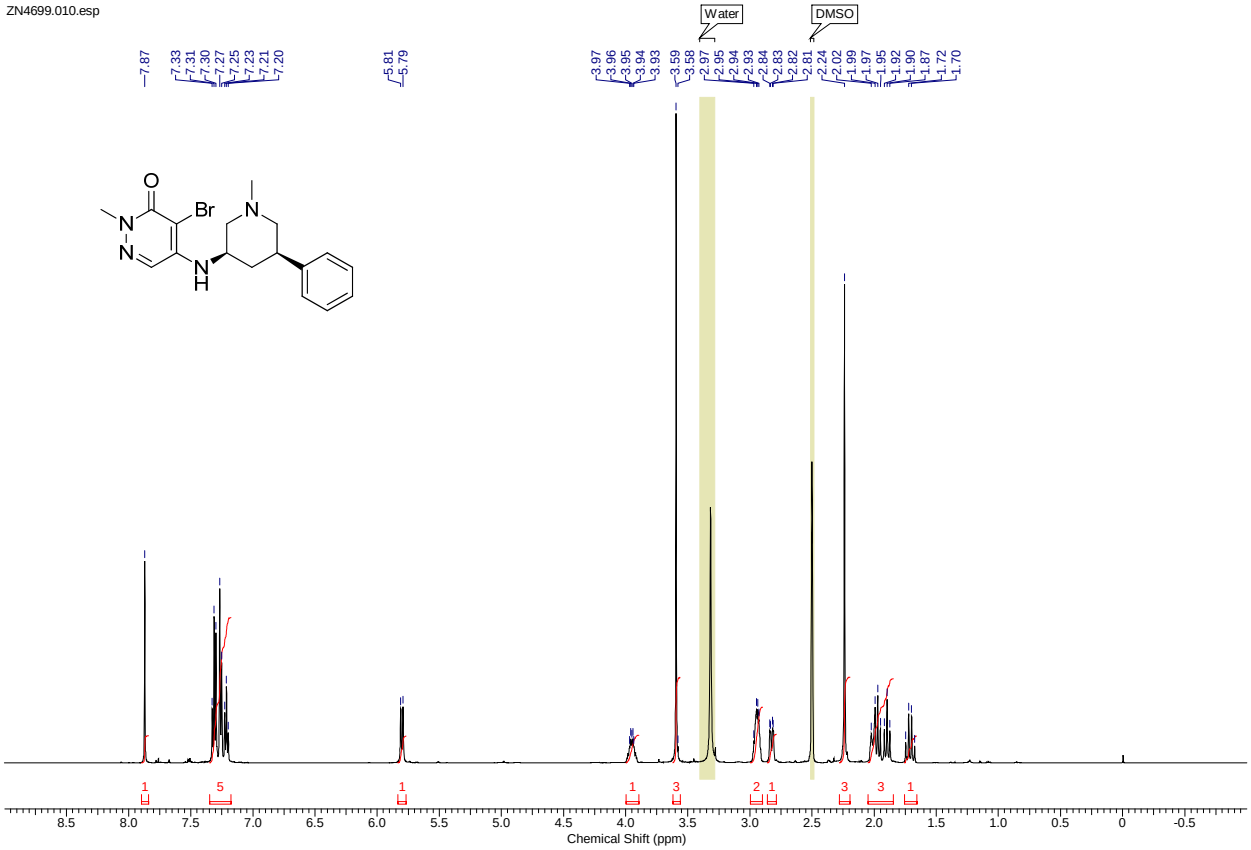
(*R*,*R*)-32 GSK4027 **(*S*,*S*)-32 GSK4028**

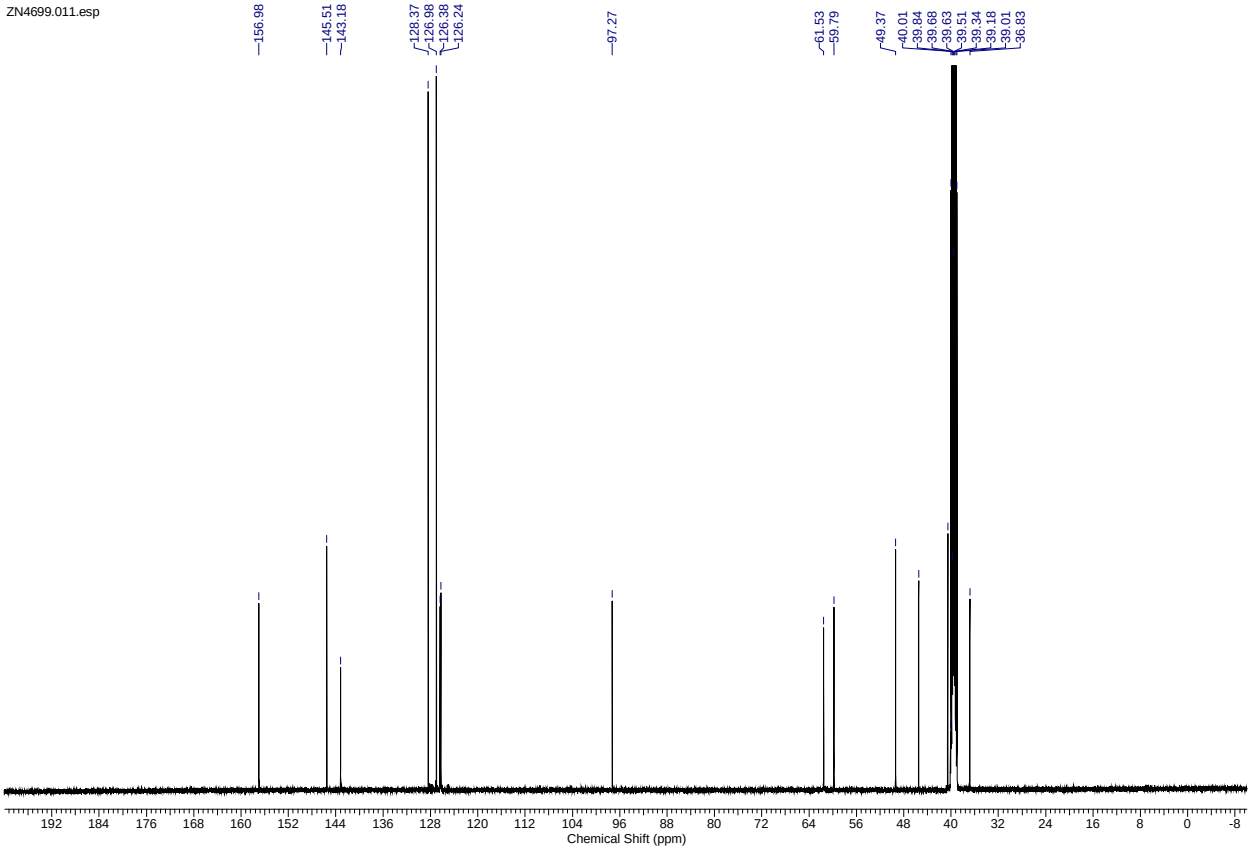
4-Bromo-2-methyl-5-(((3*R**,5*R**)-1-methyl-5-phenylpiperidin-3-yl)amino)pyridazin-3(2H)-one (**32**) (140 mg) was dissolved in EtOH/heptane (1:1, 2 mL). The solution was purified by preparative chiral HPLC, 30% EtOH/heptanes, 30 mL / min, wavelength 215 nm, 30mm x 25 cm Chiralpak AD-H (Lot No. ADH12143-01). The appropriate fractions were evaporated under reduced pressure to give **(*R*,*R*)-32** (GSK4027) (46 mg) and **(*S*,*S*)-32** (GSK4028) (47 mg) as white solids.

(*R*,*R*)-32 (GSK4027): m.p. 86–88 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (s, 1H), 7.33–7.20 (m, 5H), 5.81 (d, *J* = 9.1 Hz, 1H), 3.97–3.93 (m, 1H), 3.59 (s, 3H), 2.97–2.93 (m, 2H), 2.84 (dd, *J* = 10.8, 3.2 Hz, 1H), 2.24 (s, 3H), 2.02–1.87 (m, 3 H), 1.73 (q, *J* = 12.1 Hz, 1H); ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 157.0, 145.5, 143.2, 128.4, 127.0, 126.4, 126.2, 97.3, 61.5, 59.8, 49.4, 45.5, 40.6, 39.6, 36.8; HRMS $(M+H)^+$ calculated for $C_{17}H_{22}BrN_4O$ 377.0972; found 377.0971; LCMS (high pH) $(M+H)^+$ = 377.2, 379.2, R_t = 0.94 min (100%); HPLC (Chiralpak AD-H column, 4.6 mm x 25 cm, 30% EtOH/heptanes, 1 mL/min): 8.1 min (major enantiomer), 13.6 min (minor enantiomer), >99% ee.

(*S*,*S*)-32 (GSK4028): Analytical data as **32** above; HPLC (Chiralpak AD-H column, 4.6 mm x 25 cm, 30% EtOH/heptanes, 1 mL/min): 8.1 min (minor enantiomer), 13.6 min (major enantiomer), 97.4% ee.

^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125.8 MHz, DMSO-d_6) spectra for (*R*,*R*)-32 (GSK4027)





TR-FRET Assays

The BRD4 BD1 and BRD9 TR-FRET assays have been described previously.[2]

PCAF TR-FRET Assay

Ligand preparation: A solution of Alexa Fluor 647 hydroxysuccinimide ester in DMF was added to a 1.29 fold excess of a proprietary bromodomain binding small molecule containing a pendant primary amine, also in DMF, and when thoroughly mixed, the solution was basified by the addition of a 1.32 fold excess of diisopropylethylamine. Reaction progress was followed by electrospray LC/MS and when judged complete, GSK3103956A was isolated and purified by reverse-phase C18 HPLC. GSK3103956A was characterised by mass spectroscopy and analytical reverse-phase HPLC.

Protocol: Compound competition for binding to the bromodomain of Flag-6His-Halo-PCAF (715-831) with the Alexa647 fluorophore-labelled small molecule-based ligand GSK3103956A was monitored using a Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) micro-titre plate-based biochemical assay. Prior to compound competition studies, Flag-6His-Halo-PCAF was covalently labelled through the HaloTag® with the Tag-lite® HaloTag-Lumi4®-Terbium (Cisbio, SHALOTBC) substrate, through a haloalkane dehalogenase reaction. This labelling was carried out with Flag-6His-Halo-PCAF in excess, leading to 100% utilisation of all Tag-lite® HaloTag-Lumi4®-Terbium substrate and ~ 5% labelling of PCAF bromodomain protein with Terbium cryptate. Compounds were titrated from 10 mM in 100% DMSO and 100 nL transferred to a low volume black 384 well micro titre plate using a Labcyte Echo 555. A Thermo Scientific Multidrop Combi was used to dispense 10 µL of 2 nM labelled PCAF protein and 50 nM GSK3103956A in an assay buffer of 50 mM HEPES, 50 mM NaCl, 5% glycerol, 1mM DTT and 1 mM CHAPS, pH 7.4, to the compounds in the 384 well microplates. After equilibrating the assay reagents with compound for 30 mins in the dark at room temperature, TR-FRET was then measured on a TRF laser equipped Perkin Elmer Envision 2104 multimode plate reader using the appropriate protocol (excitation = 337 nm; emission 1 Terbium = 545 nm; emission 2 Alexa647 = 665 nm). TR-FRET ratio was calculated using the following equation: Ratio = ((Acceptor fluorescence at 665 nm) / (Donor fluorescence at 545 nm)) * 1000. All TR-FRET ratio data was normalised to give a percent inhibition, by using the robust mean of 16 high and 16 low control wells on each microplate. Subsequently, a four parameter curve fit of the following form was then applied to the percent inhibition values to calculate an IC_{50} for each of the test compounds.

$$y = \frac{a-d}{1+\left(\frac{x}{c}\right)^b} + d$$

Where ‘a’ is the minimum, ‘b’ is the Hill slope, ‘c’ is the IC_{50} and ‘d’ is the maximum.

NanoBRET assay

Full curve protocol: HEK293 cells (8 x 10^5) were plated in each well of a 6-well plate and co-transfected with Histone H3.3-HaloTag (NM_002107) and NanoLuc-PCAF (Q92831). Twenty hours post-transfection cells were collected, washed with PBS, and exchanged into media containing phenol red-free DMEM and 4% FBS in the absence (control sample) or the presence (experimental sample) of 100nM NanoBRET 618 fluorescent ligand (Promega). Cell density was adjusted to 2 x 10^5 cells/ml and then re-plated in a 96-well assay white plate (Corning Costar #3917). Inhibitors were then added directly to media at final concentrations between 0-33μM and the plates were incubated for 18hrs at 37^{o}C in the presence of 5% CO_2. NanoBRET Nano-Glo substrate (Promega) was added to both control and experimental samples at a final concentration of 10μM. Readings were performed within 5 minutes using the CLARIOstar (BMG) equipped with 450/80 nm bandpass and 610 nm longpass filters with a 0.5sec reading setting. A corrected BRET ratio was calculated and is defined as the ratio of the emission at 610 nm/450 nm for experimental samples (i.e. those treated with NanoBRET fluorescent ligand) subtracted by and the emission at 610 nm/450 nm for control samples (not treated with NanoBRET fluorescent ligand). BRET ratios are expressed as milliBRET units (mBU), where 1 mBU corresponds to the corrected BRET ratio multiplied by 1000.

Vibrational Circular Dichroism

VCD spectra were acquired using a BioTools Chiral-2X FT-VCD spectrometer operating at 4 cm^{-1} resolution. Spectra were measured for samples in $CDCl_3$ (~ 10 mg/250 µL). Baseline artefacts were removed using the standard half-difference method (e.g., $VCD_{GSK4027}$ (corr'd) = ($VCD_{GSK4027}$ minus $VCD_{GSK4028}$)). Low energy conformations were identified using the LowMode search tool in MOE'09 (MMFF94x force field) with Born solvation (ε=40). Harmonic vibrational frequencies and intensities (VCD + IR) were calculated using Gaussian'09 model chemistry B3LYP/dgdzvp2 with PCM solvent modelling (ethanol dielectric). IR and VCD spectra for each conformational model were synthesized by fitting the frequency vs intensity line spectra with Lorentzian band shapes using a resolution factor of 8 cm^{-1} (HWHH). Conformationally-averaged VCD and IR spectra were synthesized using the fractional populations predicted for major conformers by Boltzmann statistics (QM free energies). Frequency scales were then aligned with solution-phase data using a uniform scale factor (0.9775).
Comparisons of VCD spectra are shown in Figure S1. The spectrum of **(*R*,*R*)-32** (GSK4027) is the mirror image with the spectrum calculated for a model with (3*S,5S*) absolute configuration, while the spectrum for **(*S*,*S*)-32** (GSK4028) is coincident with the spectrum calculated for a model with (3*S,5S*) absolute configuration (Figure S1).

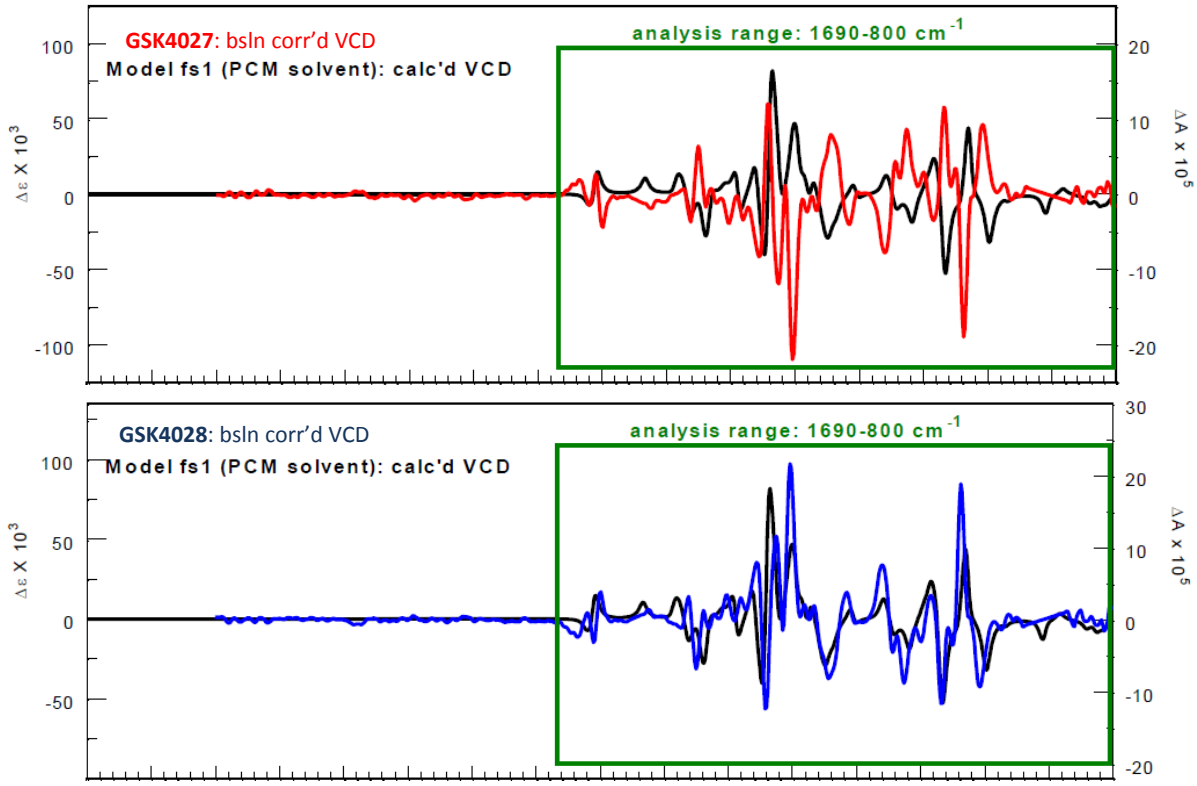


Figure S1. VCD spectra for **(*R*,*R*)-32** (GSK4027) and **(*S*,*S*)-32** (GSK4028) superimposed with the spectra calculated for a model with (3*S,5S*) absolute configuration

These data are consistent with **(*R*,*R*)-32** (GSK4027) being assigned with (3*R,5R*) absolute configuration and **(*S*,*S*)-32** (GSK4028) with (3*S,5S*) absolute configuration. The confidence limit in these assignments was estimated to be ≥99%, by CompareVOA™ (BioTools, Inc. Jupiter Beach, FLA, USA), based on similarity index statistics (ESI = 72.8; TNS = 70.9).

Crystallization and Crystallography Materials

PCAF Crystallography Methods

Crystal structure of PCAF/**15** complex: Human deHis-tagged N-terminal bromodomain of PCAF (715-831) was crystallised @10 mg/mL at 4°C in 200 nL + 200 nL sitting drops using a 96 well MRC plate with a well solution of Morpheus condition 37 (0.1 M morpheus buffer 1 pH 6.5 (imidazole, MES), 30% morpheus P550MMe_P20K (PEGMMe550, PEG20K), 0.12 M morpheus alcohol mixture (1,6-hexandiol, 1-butanol, 1,2-propandiol (racemic), 2-propanol, 1,4-butandiol, 1,3-propanediol). Crystals were soaked in the well solution containing nominally 2 mM compound (1% DMSO diluted from 200mM compound DMSO stock) for approximately 8 hours before being plunge-frozen in liquid nitrogen, no additional cryoprotectant was added.

Data from single crystals were collected at 100K on an in-house RIGAKU FR-E$^+$ SUPERBRIGHT/Saturn A200 detector/ACTOR robotic system and processed to 1.68 Å using XDS[3] and SCALA.[4]. A molecular replacement solution was determined with a previously collected in house structure. The H3 cell (α=β=90˚, γ=120˚, a=b=99.264 Å, c=100.378 Å) has two molecules in the ASU. Manual model building was performed using COOT[5] and refined using REFMAC[6] within CCP4 software package. There was clear difference density for the ligands in each of the acetylated lysine binding site, allowing the ligand to be ambiguously modelled. The binding mode was identical in both A and B chains in the ASU. The statistics for the data collection and refined co-ordinates are given in Table S4. The final crystal structure is deposited in the Protein Data Bank under the accession code 5mkx.

Mouse PCAF Crystallography Methods

Crystal structure of mouse PCAF/**(*R*)-23** complex: **(*R*)-23** was co-crystallised with deHis-tagged bromodomain of mouse PCAF (715-831). The complex was formed by adding 1 µL of 200 mM compound in 100% DMSO to 50 µl of protein @14.5 mg/mL, incubated on ice for 15 min prior to centrifugation to remove precipated compound. Diverse crystal screening at 20 °C with 100 nL + 100 nL sitting drops using a 96 well MRC plate gave crystals in Morpheus conditions consisting of 30% P550MME P20K, 0.1M Morpheus buffer 3 pH8.5, 10% morpheus amino acids. Crystals were briefly cryo-protected with the addition of 20% ethylene glycol before being plunge-frozen in liquid nitrogen.

Data from single crystals were collected at 100K on an in-house RIGAKU FR-E+ SUPERBRIGHT/Saturn A200 detector/ACTOR robotic system and processed to 1.64 Å using MOSFLM[7] and SCALA.[4] A molecular replacement solution was determined with a previously collected in house structure of the human protein . The H3 cell (α=β=90˚, γ=120˚, a=b=93.481 Å, c=32.783 Å) has one molecules in the ASU. Manual model building was performed using COOT[5] and refined using REFMAC[6] within CCP4 software package. There was clear difference density for the ligand in the acetylated lysine binding site, allowing the ligand to be ambiguously modelled. The statistics for the data collection and refined co-ordinates are given in Table S4. The final crystal structure is deposited in the Protein Data Bank under the accession code 5ml0.

GCN5 Crystallography Methods

Crystal structure of GCN5/**(*R*,*R*)-32** (GSK4027) complex: **(*R*,*R*)-32** (GSK4027) was co-crystallised with deHis-tagged bromodomain of human GCN5 (726-837 Delta L727K728). The complex was formed by adding 1.2 µL of 200mM compound in 100% DMSO to 60 µL of protein @ 12.12 mg/mL, incubated on ice for one hour prior to centrifugation to remove precipated compound. Diverse crystal screening at 4°C with 100 nL + 100 nL sitting drops using a 96 well MRC plate gave crystals in Index conditions consisting of 0.2 M ammonium acetate, 0.1 M Tris pH 8.5, 25% w/v polyethylene glycol 3,350. Crystals were briefly cryo protected with the addtion of 20% ethylene glycol before being plunge-frozen in liquid nitrogen.

Data from single crystals were collected at 100K at Diamond Synchrotron Radiation Facility (Oxford) and processed to 1.80 Å using XDS[3] and SCALA.[4] A molecular replacement solution was determined using the pdb entry 3D7C. The orthorhombic cell (α=β=γ=90˚, a=45.630 Å , b=74.000 Å, c=76.310 Å) has two molecules in the ASU. Manual model building was performed using COOT[5] and refined using REFMAC[6] within CCP4 software package. There was clear difference density for the ligand in the acetylated lysine binding site, allowing the ligand to be ambiguously modelled. The statistics for the data collection and refined co-ordinates are given in Table S4. The final crystal structure is deposited in the Protein Data Bank under the accession code 5mlj.

BRD9 Crystallography Methods

Crystal structure of BRD9/**20** complex: Apo crystals of TEV-cleaved 6His-Flag-Tev-Brd9 (134-239) were grown in Morpheus conditio n54 (0.1M morpheus buffer 2 pH 7.5, 30% morpheus_EDO_P8K, 0.1 M Morpheus ethylene glycols). Apo crystals were transferred to soaking buffer comprising well solution supplemented with the compound (from a stock solution dissolved in DMSO) at nominal soaking concentration of 2 mM. Crystals were soaked overnight then were briefly washed free from compound precipitate with well solution before being plunge-frozen into liquid nitrogen before loading in a puck for mounting with a sample collector. Data from single crystals were collected on an in-house RIGAKU FR-E+ SUPERBRIGHT/Saturn A200 detector/ACTOR robotic system. Data was processed and scaled using D*Trek to 1.67Å. Structures were solved by Fourier synthesis using REFMAC[6] (*via* CCP4) starting from a previously determined in house structure, model-building was performed using COOT[5] and refined using REFMAC via CCP4.

The P1 cell (α=68.21˚,β= 76.01˚,γ=73.37˚, a=24.551Å, b=34.076Å, c=39.750Å) has 1 molecule in the ASU. There was clear difference density for the ligand at the AcK binding site (OMIT maps showen in Table S6), allowing the ligand to be uniquely placed. The statistics for the data collection and refined co-ordinates are given in Table S4. The final model has been deposited to the protein data bank under the accession code 5mky.

BRD4-BD1 Crystallography Methods

Crystal structures of BRD4 complexes: Methods have been previously described.[8] Specific successful co-crystallization conditions, data collection details and refinement procedures for each complex are given below and in Supplementary Table S4. In all instances *E. coli* expressed tag cleaved His_6-TEV-tagged BRD4-BD1(44-168) was used.

BRD4-BD1/**15** was co-crystallised with at least 3:1 excess of compound @9mg/mL in 120 nL+120 nL sitting drops using a 96 well MRC plate with a well solution of 0.1 M SPG buffer pH 6 25% (w/v) PEG 1500 at 20°C. Crystals were cryoprotected using well with 20% ethylene glycol prior to flash freezing in liquid nitrogen.

Data from a single crystal was collected at the Europeon Synchrotron Radiation Facility (Genoble) and processed to 1.63 Å using XDS[3] and SCALA.[4] A molecular replacement solution was determined with a previously collected in house structure. The $P2_12_12_1$ cell (α=β=γ=90˚, a=36.990 Å, b=41.860 Å, c=81.580 Å) has a single molecule in the ASU. Manual model building was performed using COOT (7) and refined using REFMAC[6] *via* CCP4. There was clear difference density for the ligands in the acetylated lysine binding site, allowing the ligand to be ambiguously modelled. The statistics for the data collection and refined co-ordinates are given in Table S4. The final crystal structure is deposited in the Protein Data Bank under the accession code 5mli.

BRD4-BD1/**12** was co-crystallised with at least 3:1 excess of compound @9.7mg/ml in 120 nL+120 nL sitting drops using a 96 well MRC plate with a well solution of 0.2 M Sodium nitrate 0.1 M Bis Tris propane pH 6.5 20% (w/v) PEG 3350 at 20°C. Crystals were cryoprotected using well with 25% glycerol prior to flash freezing in liquid nitrogen.

Data from a single crystal was collected at 100K on an in-house RIGAKU FR-E^+ SUPERBRIGHT/Saturn A200 detector/ACTOR robotic system and processed to 1.62 Å using MOSFLM[7] and SCALA.[4] A molecular replacement solution was determined with a previously collected in house structure. The $P2_12_12_1$ cell (α=β=γ=90˚, a= 37.594 Å, b= 44.238 Å, c= 77.726 Å) has a single molecule in the ASU. Manual model building was performed using COOT[5] and refined using REFMAC[6] *via* CCP4. There was clear difference density for the ligands in the acetylated lysine binding site, allowing the ligand to be ambiguously modelled. The statistics for the data collection and refined co-ordinates are given in Table S4. The final crystal structure is deposited in the Protein Data Bank under the accession code 5mkz.

(collection on a single crystal)	**Human PCAF/15**	**Human BRD4-BD1/15**	**Human BRD4-BD1/12**	**Human BRD9/20**	**Mouse PCAF/(*R*)-23**	**Human GCN5/ (*R*,*R*)-32 (GSK4027)**
Data collection						
Space group	H 3	$P2_12_12_1$	$P2_12_12_1$	P 1	H3	$P2_12_12_1$
Cell						
a, *b*, *c* (Å)	99.264 99.264 100.378	36.990 41.860 81.580	37.594 44.238 77.726	4.551 34.076 39.750	93.481 93.481 32.783	45.630 74.000 76.310
α, β, γ (°)	90.000 90.000 120.00	90.000, 90.000, 90.000	90.000, 90.000 90.000	68.21 76.01 73.37	90.000 90.000 120.000	90.000 90.000 90.000
Resolution (Å)	49.63-1.63 (1.77-1.68)	40.79-1.63 (1.72-1.63)	44.24-1.62 (1.70-1.62)	36.48-1.67 (1.73-1.67)	30.39-1.64 (1.73-1.64)	38.84-1. 80 (1.90-1.80)
R_{merge}	0.026 (0.203)	0.090 (0.520)	0.026 (0.106)	0.035 (0.119)	0.017 (0.039)	0.043 (0.069)
$I/\sigma I$	21.2(3.6)	13.6 (3.5)	34.8 (10.4)	18.6 (4.1)	50.7 (20.8)	18.2 (10.5)
Completeness (%)	94.2 (83.6)	99.3 (98.9)	97.3 (83.9)	88.3 (85.3)	97.0(84.5)	92.8 (73.0)
Redundancy	2.8 (2.1)	6.2 (6.3)	4.1(2.9)	2.19 (1.83)	3.5 (1.9)	2.6 (2.2)
Refinement						
Resolution (Å)	49.63-1.68	40.79-1.63	44.24-1.62	36.48 - 1.67	30.39-1.64	38.84-1.80
No. reflections	112548 (10854)	100261 (14436)	68927 (5938)	25399	44224 (3111)	58906 (2532)
No. uniq reflections	39507 (5104)	16289 (2309)	16730 (2068)	11611	12718(1631)	22545 (2532)
R_{work}/ R_{free}	0.182/0.207	0.160/0.190	0.142/0.169		0.179/0.222	0.169/0.211
No. atoms	2240	1370	1416	1096	1175	2229
Protein	1832	1080	1091	838	930	1909
Ligand/ion	22/26	11	17/18	42	17/8	46/8
Water	360	279	290	216	220	366
B-factors						
Protein	29.68	22.01	11.38	29.07	24.13	16.20
Ligand/ion	21.02/46.14	28.26	10.42/21.25	37.61	12.53/41.75	17.45
Water	9.35	36	28.49	41.89	26.31	27.9
R.m.s deviations						
Bond lengths (Å)	0.006	0.006	0.007	0.009	0.005	0.005
Bond angles (°)	1.042	1.1	1.291	1.114	1.012	0.978

*Highest resolution shell is shown in parenthesis.

Table S4. Data collection and refinement statistics for X-ray structures.

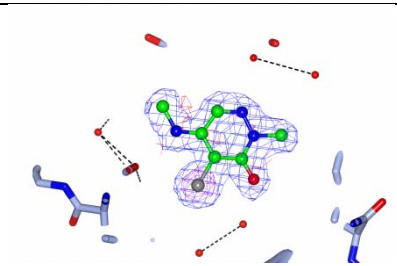
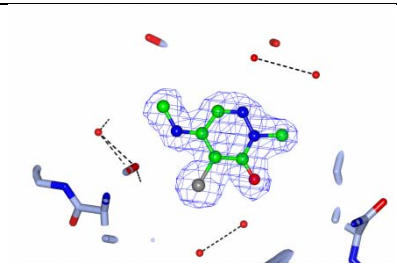
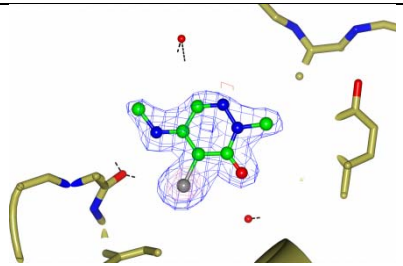
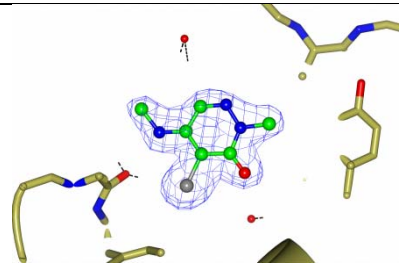
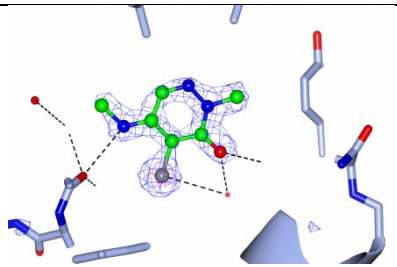
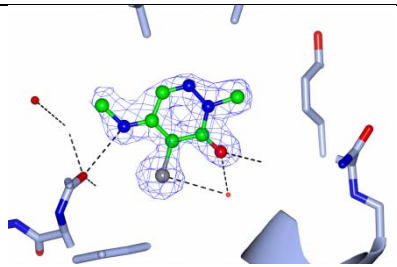
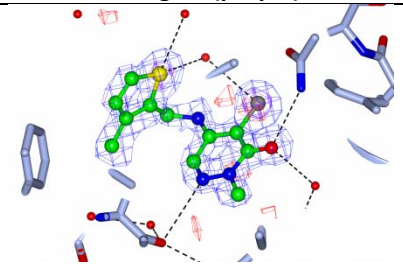
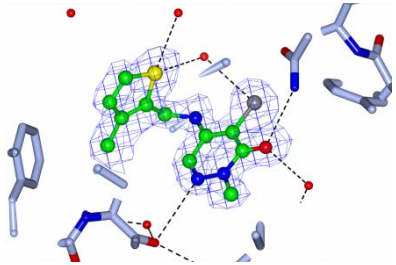
	Chain A		Chain B	
	OMIT (Fo-Fc) map ±3.5sigma (blue/red)	**OMIT (2Fo-Fc) map +1sigma (blue)**	**OMIT (Fo-Fc) map ±3.5sigma (blue/red)**	**OMIT (2Fo-Fc) map +1sigma (blue)**
Human PCAF/ 15	 **+12.0sigma (purple)**		 **+12.0sigma (purple)**	
Human BRD4-BD1/15	 **+9.0sigma (purple)**			
Human BRD4-BD1/12	 **+9.0sigma (purple)**			

Table S5. OMIT maps of X-ray structures.

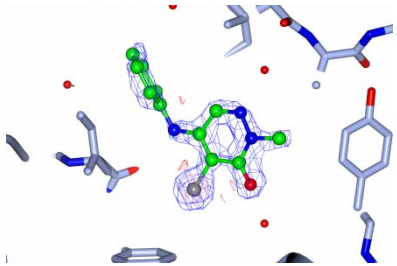
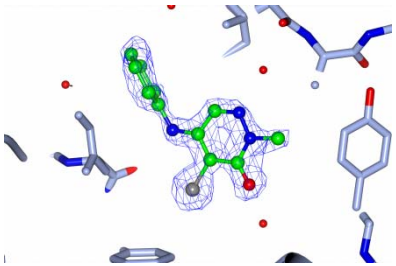
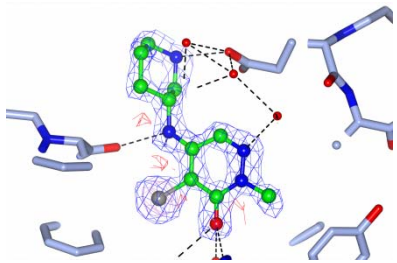
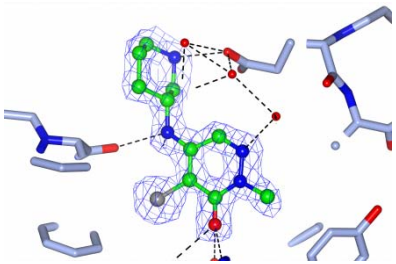
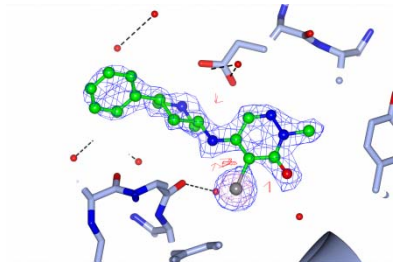
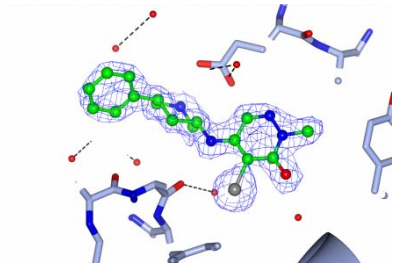
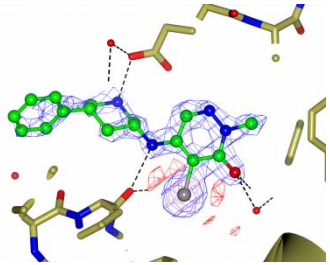
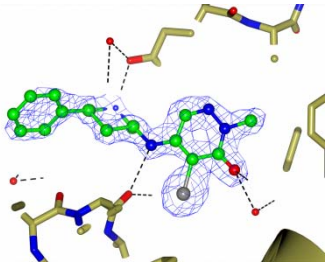
	Chain A		Chain B	
	OMIT (Fo-Fc) map ±3.5sigma (blue/red)	**OMIT (2Fo-Fc) map +1sigma (blue)**	**OMIT (Fo-Fc) map ±3.5sigma (blue/red)**	**OMIT (2Fo-Fc) map +1sigma (blue)**
Human BRD9/20	 **+9.0sigma (purple)**			
Mouse PCAF/(*R*)-23	 **+12.0sigma (purple)**			
Human GCN5/ (*R*,*R*)-32 (GSK4027)	 **+12.0sigma (purple)**		 **+12.0sigma (purple)**	

Table S6. OMIT maps of X-ray structures.

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