Supplemental Information

Identification of a chemical probe for family VIII bromodomains through optimization of a fragment hit

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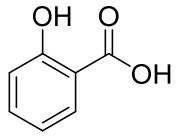
Chemistry Synthetic Methods and Compound Characterization

Proton (^{1}H NMR), carbon (^{13}C NMR), and fluorine (^{19}F NMR) magnetic resonance spectra where obtained in DMSO-d_6 at 400, 100, 376 MHz and respectively unless otherwise noted. The following abbreviations were utilized to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet and m = multiplet. High-resolution mass measurements were obtained on an Agilent ToF mass spectrometer. All air and moisture sensitive reactions were carried out under an atmosphere of dry nitrogen using heat-dried glassware and standard syringe techniques. Tetrahydrofuran (THF) and acetonitrile were purchased from EMD as anhydrous and were used without further drying. Silica gel chromatography was performed using an Analogix Intelliflash 280 or Biotage SP1 purification system with Sepra Si 50 silica gel using ethyl acetate/heptane mixtures as solvent unless otherwise indicated. HPLC was carried out on an Agella Venusil ASB C18 column (21.2 x 150 mm, 5 μm). A flow rate of 0.5-150 mL/min was used with mobile phase A: water + 0.1% modifier (v/v) and B: acetonitrile + 0.1% modifier (v/v). The modifier was formic acid, trifluoroacetic acid, ammonia acetate, or hydrochloric acid.

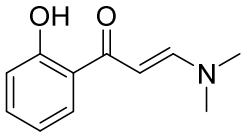
Quality control (QC) analysis was performed using a LCMS method. Acidic runs were carried out on a Shimadzu XB-C18 (2.1 x 30 mm, 5 μm), X-Bridge (50 x 4.6 mm, 5 μm), Gemini NX C18 (50 x 4.6, 3 μm), or Gemini NX C18 (50 x 4.6, 5 μm). A flow rate of 1.0-1.2 mL/min was used with mobile phase A: water +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

Compounds prepared/sourced by non-library chemistry methods

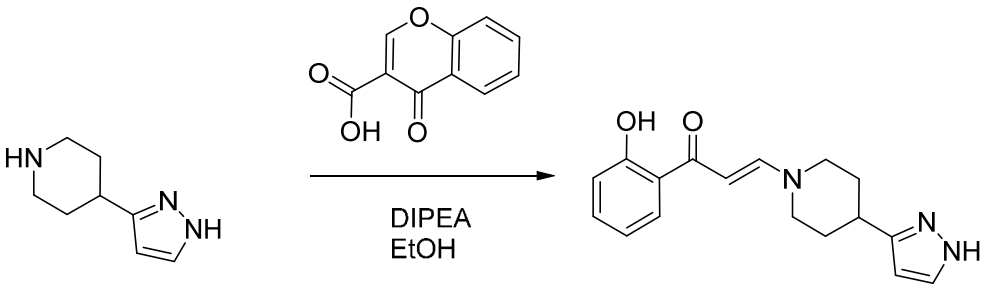
2-Hydroxybenzoic acid (Salicylic acid) (1)[1]



(2*E*)-3-(dimethylamino)-1-(2-hydroxyphenyl)prop-2-en-1-one (2)[2]

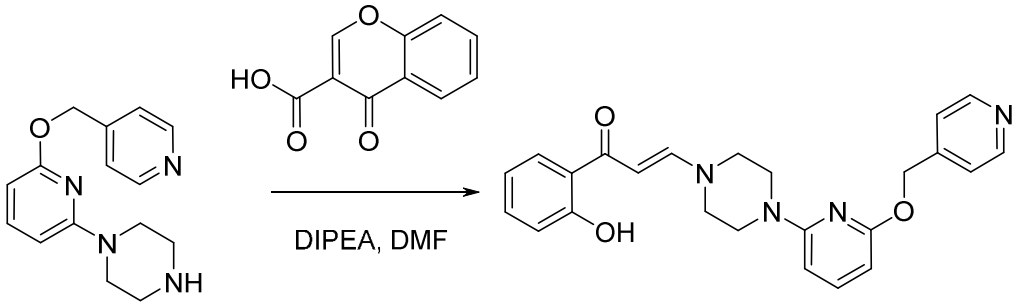


(*E*)-3-(4-(1H-pyrazol-3-yl)piperidin-1-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (4)



To a solution of 4-(1H-pyrazol-3-yl)piperidine (50 mg, 0.26 mmol) in ethanol (3.00 mL) was added dipropylethylamine (68.0 mg, 0.526 mmol) and chromone-3-carboxylic acid (47.7 mg, 0.316 mmol) at room temperature. The reaction was then stirred for another 10 hours at room temperature. LCMS indicated that the reaction was complete. The solution was concentrated and purified by prep-HPLC to give **4** (26.8 mg, 34.3%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.72 - 1.90 (m, 2 H), 2.04 - 2.20 (m, 2 H), 3.01 (t, *J*=11.29 Hz, 1 H), 3.14 (br. s., 1 H), 3.44 (br. s., 1 H), 3.69 (br. s., 1 H), 3.86 (br. s., 1 H), 5.93 (d, *J*=12.05 Hz, 1 H), 6.14 (d, *J*=1.51 Hz, 1 H), 6.82 (t, *J*=7.28 Hz, 1 H), 6.93 (d, *J*=8.03 Hz, 1 H), 7.29 - 7.39 (m, 1 H), 7.52 (d, *J*=1.00 Hz, 1 H), 7.68 (d, *J*=7.53 Hz, 1 H), 7.87 - 7.97 (m, 1 H), 13.98 (br. s., 1 H); ^{13}C NMR (126 MHz, DMSO) δ 191.0, 162.9, 154.5, 134.2, 129.3, 120.6, 118.4, 117.9, 101.9, 89.3, 54.2, 45.8, 32.9, 31.5, 1.63;HPLC = 95.01%, t = 4.97 min; HRMS [M+H] for $C_{17}H_{19}N_3O_2$, calc., 298.1550, found, 298.1553

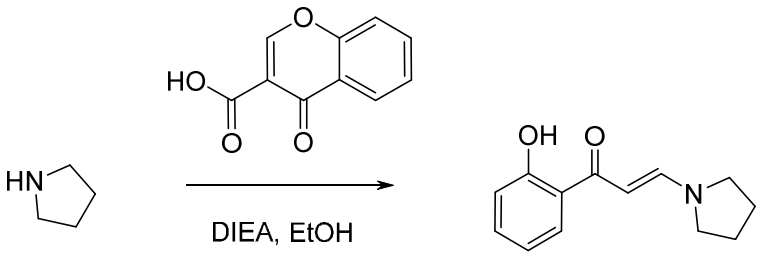
(*E*)-1-(2-hydroxyphenyl)-3-(4-(6-(pyridin-4-ylmethoxy)pyridin-2-yl)piperazin-1-yl)prop-2-en-1-one (5)



To a solution of 1-(6-(pyridin-4-ylmethoxy)pyridin-2-yl)piperazine (145 mg, 2.28 mmol) in ethanol (7 mL) was added diisopropylethylamine (0.795 ml, 4.57 mmol). To the reaction was added chromone-3-carboxylic acid (300 mg, 0.761 mmol). The reaction was stirred at room temperature and followed by LCMS and TLC. The reaction was judged complete after 18 hours and the reaction was concentrated to an oily residue. The residue was purified via silica gel column chromatography (80g, 20% ethyl acetate in heptane to 100% over 10 column volumes) to provide the desired material **5** as a yellow solid (230 mg, 73%). ^{1}H NMR (400 MHz, DMSO-

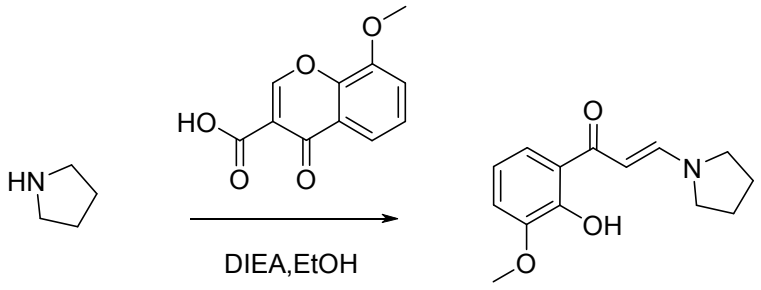
d_6) δ = 3.49 - 3.67 (m, 8 H) 5.35 (s, 9 H) 5.73 (s, 1 H) 6.15 - 6.24 (m, 2 H) 6.41 (d, *J*=8.00 Hz, 1 H) 6.78 - 6.85 (m, 2 H) 7.30 - 7.36 (m, 1 H) 7.36 - 7.40 (m, 2 H) 7.51 (t, *J*=8.00 Hz, 1 H) 7.87 - 8.03 (m, 2 H) 8.45 - 8.61 (m, 2 H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 192.3, 163.1, 162.0, 157.3, 153.1, 150.0, 147.4, 141.0, 134.4, 128.4, 121.7, 120.4, 118.5, 118.2, 100.0, 99.2, 90.4, 77.3, 76.8, 65.5; HPLC = 99%, t = 1.23 min; HRMS [M+H] for $C_{24}H_{25}N_4O_3$, calc., 417.1921, found, 417.1916.

(*E*)-1-(2-hydroxyphenyl)-3-(pyrrolidin-1-yl)prop-2-en-1-one (9)



To a solution of pyrroloidine (50 mg, 0.26 mmol) in ethanol (3.00 mL) was added diisopropylethylamine (68.0 mg, 0.526 mmol) and chromone-3-carboxylic acid (18.7 mg, 0.263 mmol) at room temperature, then stirred for another 10 hours. LCMS indicated that the reaction was complete. The solution was concentrated and purified by prep-HPLC to give **9** (53.9 mg, 94%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.74 - 1.93 (m, 2 H), 1.93 - 2.07 (m, 2 H), 3.36 (t, *J*=6.78 Hz, 2 H), 3.65 (t, *J*=6.78 Hz, 2 H), 5.89 (d, *J*=12.05 Hz, 1 H), 6.75 - 6.94 (m, 2 H), 7.37 (td, *J*=7.65, 1.76 Hz, 1 H), 7.89 (dd, *J*=8.03, 1.51 Hz, 1 H), 8.09 (d, *J*=12.05 Hz, 1 H), 14.54 (s, 1 H); ^{13}C NMR (100 MHz, DMSO) δ 200.0, 189.6, 162.4, 151.2, 133.7, 177.9, 177.5, 90.2, 52.4, 47.2, 24.6; HPLC = 99.63%, t = 3.05 min; HRMS [M+H] for $C_{13}H_{16}NO_2$, calc., 218.1176, found, 218.1175.

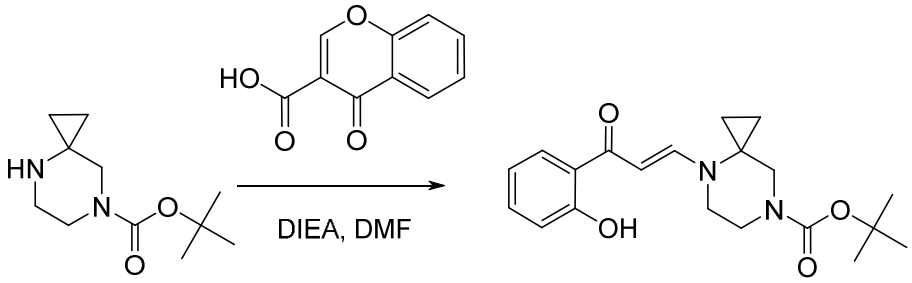
(*E*)-1-(2-hydroxy-3-methoxyphenyl)-3-(pyrrolidin-1-yl)prop-2-en-1-one (10)



To a solution of compound pyrrolidine (50 mg, 0.23 mmol) in ethanol (3.00 mL) was added diisopropylamine (58.7 mg, 0.454 mmol) and 8-methoxy-4-oxo-4H-chromene-3-carboxylic acid (19.4 mg, 0.273 mmol) at room temperature, then stirred for another 10 hours. LCMS indicated

that the reaction was complete. The solution was concentrated and purified by prep-HPLC to give **10** (33.9 mg, 60%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.89 - 2.14 (m, 4 H), 3.32 (t, *J*=6.78 Hz, 2 H), 3.59 (t, *J*=6.78 Hz, 2 H), 3.89 (s, 3 H), 5.72 (d, *J*=12.05 Hz, 1 H), 6.63 - 6.82 (m, 1 H), 6.94 (d, *J*=7.53 Hz, 1 H), 7.30 (d, *J*=8.03 Hz, 1 H) 8.09 (d, *J*=12.55 Hz, 1 H) 14.50 (s, 1 H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 140.8, 103.0, 100.0, 98.5, 69.8, 69.2, 66.4, 64.1, 40.7, 26.6, 5.5, 2.2, -3.4, -25.4; HPLC = 99.46%, t = 4.90 min; HRMS [M+H] for $C_{14}H_{17}NO_3$, calc., 248.1281, found, 248.1290.

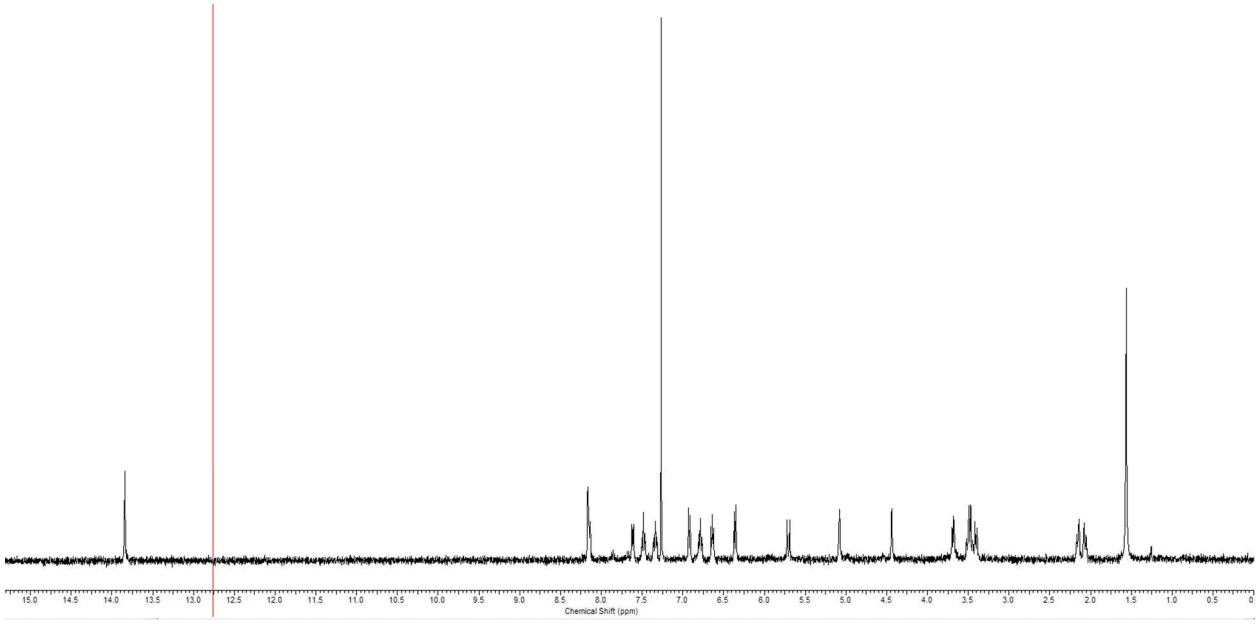
(*E*)-*tert*-butyl 4-(3-(2-hydroxyphenyl)-3-oxoprop-1-en-1-yl)-4,7-diazaspiro[2.5]octane-7-carboxylate (15)



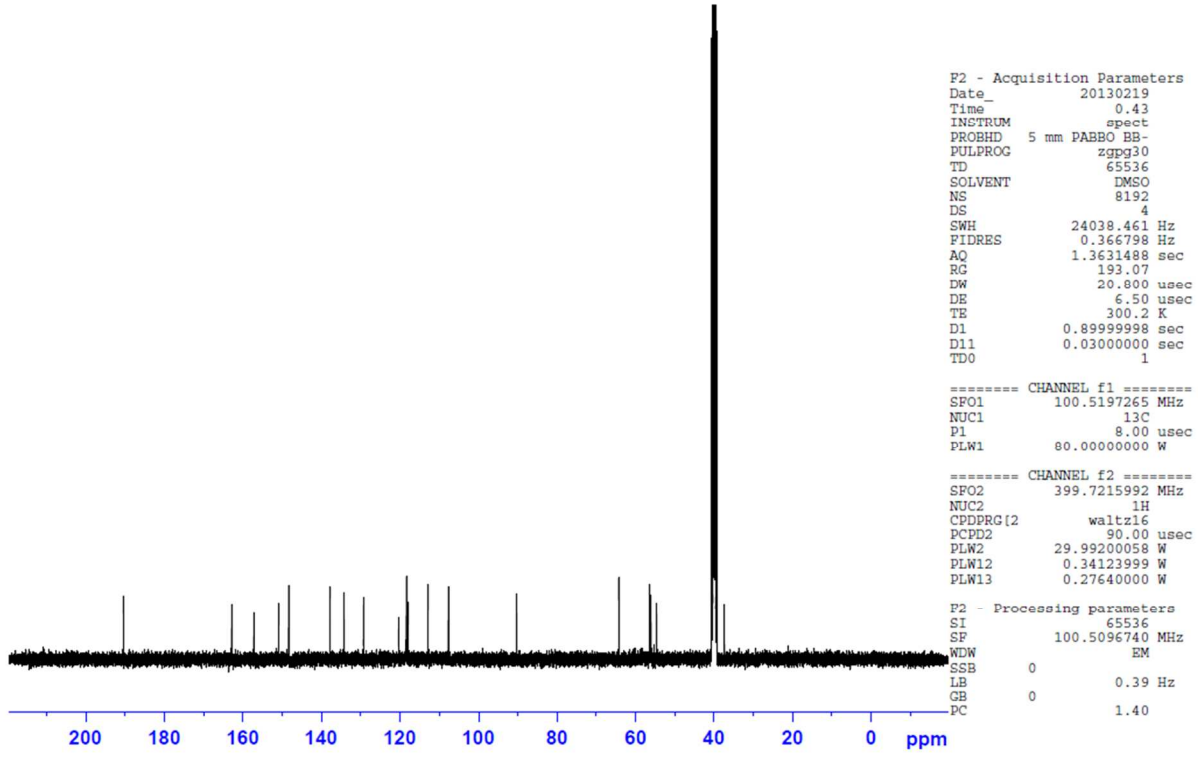
To a solution of *tert*-butyl 4,7-diazaspiro[2.5]octane-7-carboxylate (50.00 mg, 0.26 mmol) and chromone-3-carboxylic acid (55.8 mg, 0.263 mmol) in dimethylformamide (3.00 mL) was added diisopropylethylamine (170 mg, 1.31 mmol). Then mixture was stirred at 80 ºC for 3h. LCMS indicated that desired product was generated and the reaction was concentrated to a residue. The residue was purified by prep-HPLC to give **15** (14 mg, 15%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 0.75 - 1.14 (m, 4 H), 1.47 (s, 9 H), 3.39 (s, 2 H), 3.46 - 3.72 (m, 4 H), 5.62 - 6.19 (m, 1 H), 6.83 (t, *J*=7.53 Hz, 1 H), 6.94 (d, *J*=8.53 Hz, 1 H), 7.32 - 7.43 (m, 1 H), 7.66 (d, *J*=7.53 Hz, 1 H), 7.81 - 8.01 (m, 1 H), 13.68 (s, 1 H); ^{13}C NMR ($CDCl_3$) = 12.1, 28.3, 44.6, 44.8, 46.9, 80.5, 91.5, 118.1, 118.3, 120.2, 128.3, 134.4, 150.3, 154.6, 163.0, 192.2; HPLC = 99.62%, t = 5.28 min; HRMS [M+H] for $C_{20}H_{27}N_2O_4$, calc., 359.1965, found, 359.1973.

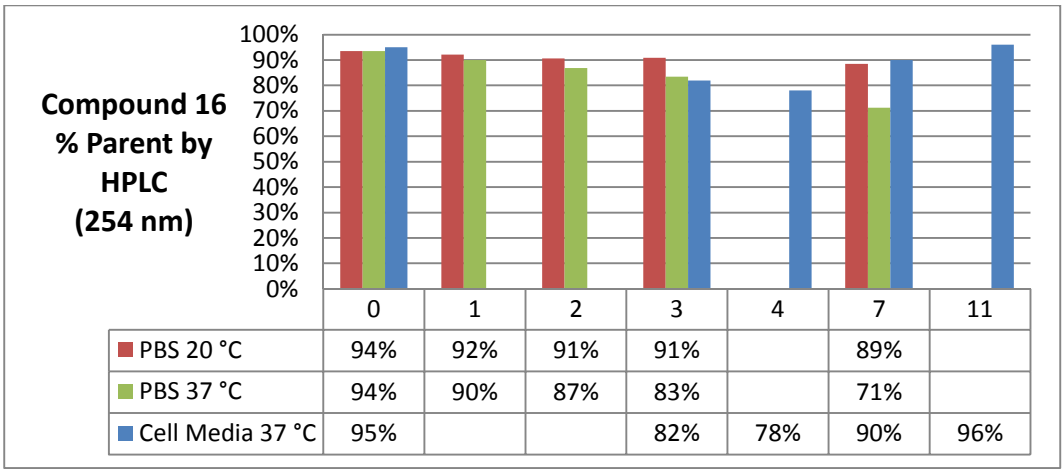
Spectral data for compound **16**

^{1}H-NMR of **16** (PFI-3) in $CDCl_3$



^{13}C-NMR of **16** (PFI-3) in DMSO-d6

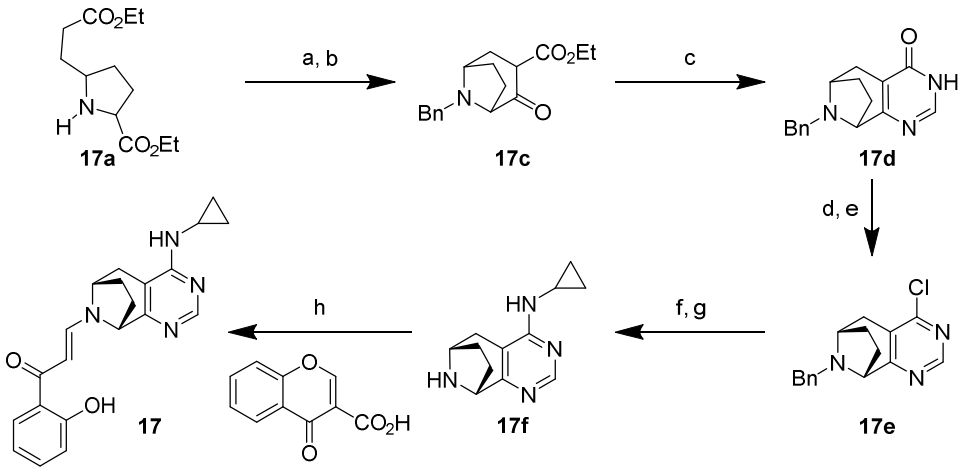




	0	1	2	3	4	7	11
PBS 20 °C	94%	92%	91%	91%		89%	
PBS 37 °C	94%	90%	87%	83%		71%	
Cell Media 37 °C	95%			82%	78%	90%	96%

Table T1

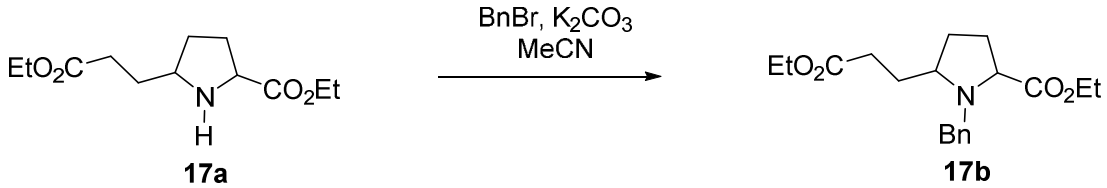
Table T1: HPLC analysis of % parent remaining for compound **16** in selected aqueous media and temperatures over time.



Scheme S1

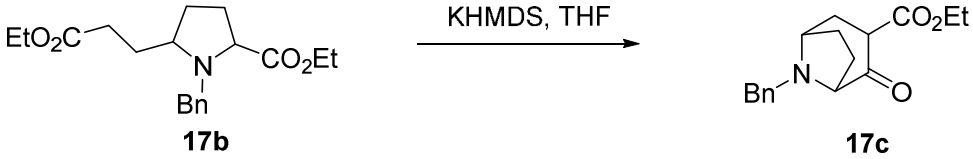
Scheme S1: Reaction sequence for synthesis of enamide (**17**). (a) BnBr, K_2CO_3, MeCN, 25°C, 89%; (b) KHMDS, THF, -78°C, 81%; (c) formamidine acetate, NaOMe, MeOH, 90°C, 86%; (d) chiral SFC column chromatography, 41% desired enantiomer, 31% undesired enantiomer; (e) $POCl_3$, 110°C, 10 hours, 60%; (f) cyclopropylamine, dioxane, 130°C, 72%; (g) Pd/C, 30 psi H_2, MeOH, 25°C, 58%; (h) chromone-3-carboxylic acid, DIPEA, EtOH, 25 to 50°C, 66%.

Ethyl 1-benzyl-5-(3-ethoxy-3-oxopropyl)pyrrolidine-2-carboxylate (17b)



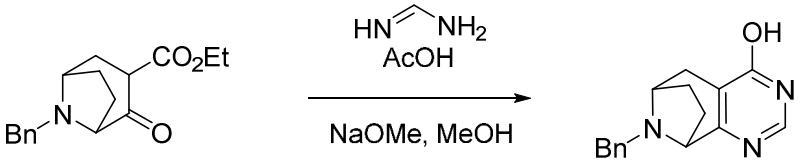
To a solution of compound **17a**[3] (3.40 g, 12.2 mmol) in MeCN (100 mL) was added K_2CO_3 (5.04 g, 36.5 mmol) and benzyl bromide (2.49 g, 14.6 mmol) at 26 °C. The reaction mixture was stirred at 26°C for 16 h. The reaction mixture was cloudy. TLC (petroleum ether/EtOAc = 10/1, UV) showed the reaction was complete. The reaction solution was filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (80 g, petroleum ether/EtOAc = 1/0~10/1) to afford compound **17b** (3.6 g, 89%) as colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.23 (m, 5H), 4.09 (q, *J* = 7.1 Hz, 2H), 3.97 – 3.82 (m, 3H), 3.65 (d, *J* = 13.9 Hz, 1H), 3.28 (dd, *J* = 8.6, 6.0 Hz, 1H), 2.77 (m, 1H), 2.42 (ddd, *J* = 15.3, 9.3, 5.6 Hz, 1H), 2.27 (ddd, *J* = 15.9, 9.2, 6.8 Hz, 1H), 2.07 – 1.76 (m, 4H), 1.75 – 1.48 (m, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.10 (t, *J* = 7.2 Hz, 3H); LCMS = 90%, m/z = 333.9 (M+H); HRMS [M+H] for $C_{19}H_{28}NO_4$, calc., 334.2013, found, 334.202.

Ethyl 8-benzyl-2-oxo-8-azabicyclo[3.2.1]octane-3-carboxylate (17c)



To a solution of compound **17b** (3.6 g, 10.8 mmol) in THF (50 mL) at -78°C was added KHMDS (1.0M in THF, 4.31 g, 21.6 mmol, 21.6 mL). The yellow reaction mixture was stirred at -78°C for 3 hours under N_2. TLC (petroleum ether/EtOAc=10/1, UV) showed the reaction was complete. Then the reaction mixture was poured into an ice-cooled mixture of EtOAc (200 mL) and pH~7.0 phosphate buffer (100 mL). The resulting mixture was separated; the aqueous phase was extracted with EtOAc (200 mL x 2). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silical gel (80 g, petroleum ether/EtOAc = 1/0~20/1) to give **17c** (2.5 g, 81%) as colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ ^{1}H NMR (400 MHz, Chloroform-*d*) δ 11.89 (s, 1H), 7.43 – 7.17 (m, 6H), 4.23 (dt, *J* = 7.6, 6.8 Hz, 2H), 3.77 – 3.66 (m, 2H), 3.47 – 3.40 (m, 0H), 3.40 – 3.30 (m, 2H), 2.78 – 2.68 (m, 0H), 2.64 (dd, *J* = 16.0, 4.8 Hz, 1H), 2.19 – 2.08 (m, 2H), 1.99 (t, *J* = 9.3 Hz, 1H), 1.87 (dd, *J* = 15.9, 1.1 Hz, 1H), 1.68 (s, 1H), 1.59 – 1.49 (m, 1H), 1.32 (t, *J* = 7.1 Hz, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.76, 172.59, 139.18, 128.88, 128.41, 127.11, 92.14, 77.31, 60.39, 60.10, 55.57, 52.89, 32.99, 29.57, 27.61, 14.45; LCMS = 94%, m/z = 287.9 (M+H); HRMS [M+H] for $C_{17}H_{22}NO_3$, calc., 288.1594, found, 288.1596.

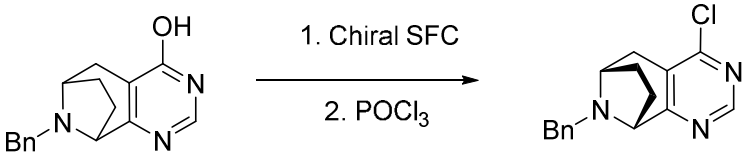
10-Benzyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-ol (17d)



17c **17d**

To a solution of **17c** (2.50 g, 8.70 mmol) in MeOH (30 mL) was added formamidine acetate (1.09 mg, 10.47 mmol) and fresh sodium methoxide (1.41 g, 26.1 mmol) at 26°C. The reaction mixture was stirred at 90°C for 2h. The reaction mixture was cloudy. The desired mass (Retention time: 0.235 min [M+1] = 268.0) was detected by LC-MS although **17c** was still remained. The reaction mixture was concentrated *in vacuo*. H_2O (20 mL) was added into the residue, and the pH of which was adjusted to 3 with 4N HCl (aq.). The solution was lyophilized. The residue was purified by chromatography on silica gel (40 g, DCM/MeOH=1/0~10/1) to give compound **17d** (2.0 g, 86%) as a yellow solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.16 (s, 1H), 7.72 – 7.35 (m, 6H), 4.49 – 4.24 (m, 4H), 3.11 (d, *J* = 18.2 Hz, 1H), 2.79 – 2.52 (m, 3H), 2.37 (d, *J* = 12.2 Hz, 1H), 2.04 (t, *J* = 9.5 Hz, 1H); LCMS = 97%, m/z = 268.1 (M+H); HRMS [M+H] for $C_{16}H_{18}N_3O$, calc., 268.1444, found, 268.1449.

(6*S*,9*R*)-10-benzyl-4-chloro-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidine (17e)

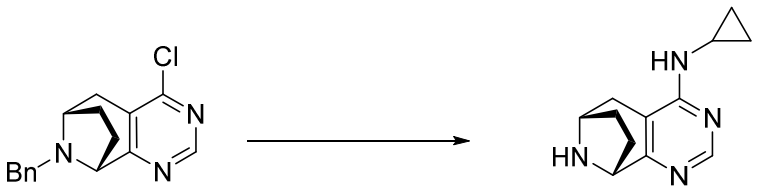


17d **17e**

The racemic 10-benzyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-ol **17d** (1.6 g, 6.0 mmol) was purified by preparative chiral SFC (Column: AD (250 mm x 50 mm x 10 um, Mobile phase: 40%MeOH-NH_3H_2O 200mL/min Workup: Lyophilization). After SFC, the undesired enantiomer was identified as (6*R*,9*S*)-10-benzyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-ol (650 mg, 41%, peak 1) and the desired enantiomer was assigned as (6*S*,9*R*)-10-benzyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-ol (500 mg, 31%, peak 2) were obtained. Peak 1: Chiral HPLC (Chiralpak AD-3 150 x 4.6 mm I.D., 3 um) e.e. = 100% (t = 4.297); $[a]_D^{20}$ 93.5 (*c* 0.150, MeOH). Peak 2: Chiral HPLC (Chiralpak AD-3 150 x 4.6 mm I.D., 3 um) e.e. = 100% (t = 6.179); $[a]_D^{20}$ –109.6 (*c* 0.160, MeOH). A mixture of (6*S*,9*R*)-10-benzyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-ol (2.0 g, 7.5 mmol) in $POCl_3$ (30 mL) was stirred at 110 ˚C for 10h. TLC (petroleum ether:EtOAc = 3:1, UV) showed the reaction mixture was complete. The reaction solution was concentrated *in vacuo*. EtOAc (100 mL) was added to the residue followed by ice-water (50 mL). The solution was adjusted with sat. aq. Na_2CO_3 to pH=7 and extracted with EtOAc (100 mL x 2). The combined organic layers were washed with brine (50 mL x 2), dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by chromatography on silica gel (petroleum

ether:EtOAc = 1:0 to 3:1) to afford compound **17e** (1.3 g, 60% yield) as yellow oil. LCMS = 99.9%, m/z = 285.8 (M+H).

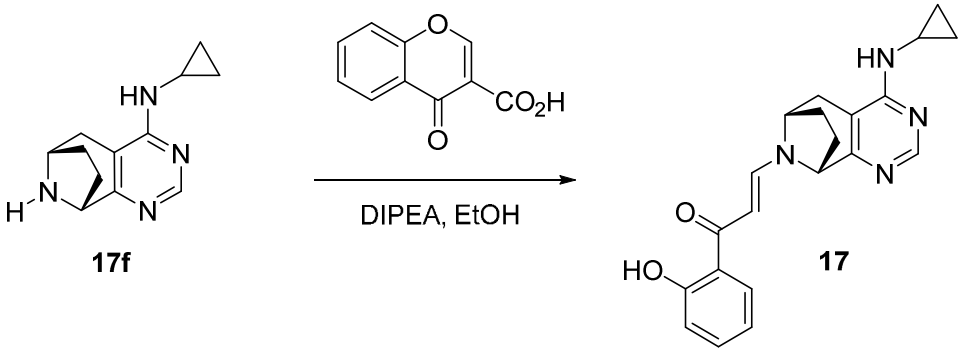
(6*S*,9*R*)-N-Cyclopropyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-amine (17f)



17e **17f**

To a solution of compound **17e** (1.3 g, 4.55 mmol, 1.0 equiv) in dioxane (20 mL) was added cyclopropylamine (1.3 g, 22.7 mmol, 5.0 equiv) at 20 ˚C. Then the reaction mixture was stirred at 130 ˚C for 10 h. The reaction mixture was yellow. TLC (Petroleum ether/EtOAc = 3/1, UV) showed starting material **17e** still remained, so additional cyclopropylamine (1.30 g, 22.7 mmol, 5.0 equiv) was added. The reaction solution was stirred at 130˚C for another 10h. Upon completion, the solution was concentrated *in vacuo* and purified by flash column chromatography on silica gel (CH_2Cl_2:MeOH = 1:0 to 20:1) to afford (6*S*,9*R*)-10-benzyl-N-cyclopropyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-amine (1.0 g, 72% yield) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (s, 1H), 7.26 (m, 5H), 4.64 (s, 1H), 3.85 (d, *J* = 6.0 Hz, 1H), 3.64 (s, 2H), 3.57 (t, *J* = 5.9 Hz, 1H), 2.95 – 2.78 (m, 1H), 2.70 (dd, *J* = 15.8, 5.0 Hz, 1H), 2.32 – 2.22 (m, 2H), 1.98 – 1.86 (m, 1H), 1.82 (d, *J* = 15.9 Hz, 1H), 1.56 – 1.43 (m, 1H), 1.01 – 0.79 (m, 2H), 0.65 – 0.47 (m, 2H).; ^{13}C NMR (100 MHz, Methanol-*d4*) δ 163.9, 163.1, 156.5, 139.6, 130.2, 129.4, 128.4, 109.9, 63.4, 57.8, 54.7, 33.9, 30.3, 29.2, 24.7, 7.5, 7.4; LCMS = 99.8%, m/z = 307.1 (M+H). Next, (6*S*,9*R*)-10-benzyl-N-cyclopropyl-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-4-amine (0.500 g, 1.63 mmol, 1.0 equiv) was dissolved in MeOH (50 mL) and Pd/C (10mol%, 17.4 mg, 0.163 mmol, 0.1 equiv) was added. The reaction mixture was stirred at 25˚C for 20h under 30 psi of H_2. TLC (DCM/MeOH = 10/1, UV) showed the reaction was complete. The reaction mixture was filtered and concentrated *in vacuo*. The residue was purified by prep-HPLC to afford **17f** (0.204 g, 58% yield) as a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.49 (s, 1H), 4.65 (s, 1H), 4.13 (dd, *J* = 4.9, 1.7 Hz, 1H), 4.02 – 3.87 (m, 1H), 2.91 – 2.76 (m, 1H), 2.66 (dd, *J* = 15.7, 5.1 Hz, 1H), 2.22 – 1.81 (m, 4H), 1.62 – 1.37 (m, 1H), 0.93 – 0.72 (m, 2H), 0.63 – 0.35 (m, 2H); ^{13}C NMR (126 MHz, Methanol-*d4*) δ 165.1, 163.5, 156.1, 109.7, 59.9, 54.2, 46.5, 35.8, 32.5, 30.8, 24.7, 7.4, 7.3; LCMS = 100%, m/z = 217.1 (M+H).

(*E*)-3-((6*S*,9*R*)-4-(Cyclopropylamino)-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-10-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one (17)

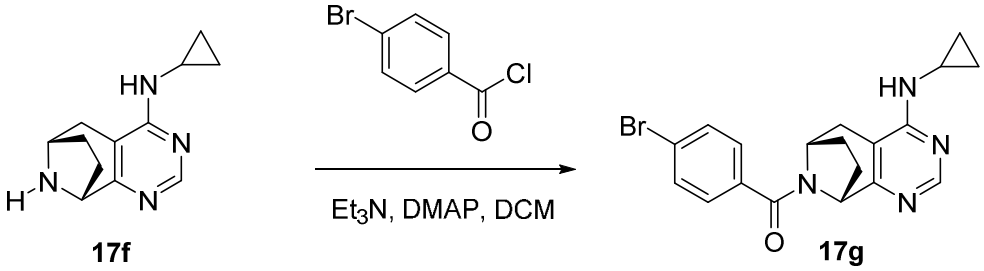


17f 17

To a solution of the **17f** (50 mg, 0.23 mmol, 1.0 equiv) in EtOH (1.16 mL) was added DIPEA (0.203 mL, 1.16 mmol, 5.0 equiv). The reaction was stirred and was homogenous. To the reaction was added chromone-3-carboxylic acid (52.7 mg, 0.277 mmol.) The reaction was stirred at room temperature for 4.5 hours then heated to 50°C. After 1 h, starting material remained by LCMS and chromone-3-carboxylic acid (22.0 mg, 0.115 mmol) was added. After 3 h stirring at 50°C, another addition of chromone-3-carboxylic acid (22.0 mg, 0.115 mmol) and DIPEA (40.6 uL, 0.23 mmol). After 2 hours, another addition of chromone-3-carboxylic acid (22.0 mg, 0.115 mmol) and DIPEA (40.6 uL, 0.23 mmol) was made. After 1.5 h, the reaction was complete by LCMS and concentrated *in vacuo*. The residue was purified via silica column (10% MeOH:CH_2Cl_2) to yield 66%, 55.0 mg of material isolated. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 13.65 (s, 1H), 8.55 (s, 1H), 7.97 (d, *J* = 12.4 Hz, 1H), 7.64 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.35 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1H), 6.92 (dd, *J* = 8.3, 1.2 Hz, 1H), 6.81 (ddd, *J* = 8.2, 7.2, 1.2 Hz, 1H), 6.03 (d, *J* = 12.5 Hz, 1H), 4.71 (br s, 2H), 4.45 (br s, 1H), 2.94 (br s, 1H), 2.83 (ddq, *J* = 9.5, 6.8, 3.5 Hz, 1H), 2.47 – 2.24 (m, 2H), 2.20 – 2.10 (m, 2H), 1.83 – 1.69 (m, 2H), 0.92 – 0.83 (m, 2H), 0.59 – 0.51 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.3, 163.0, 161.9, 156.8, 147.9, 134.5, 128.6, 120.3, 118.4, 118.3, 106.6, 33.9, 32.0, 29.4, 29.1, 24.1, 22.8, 14.2, 7.6, 7.4; LCMS = 100%, m/z = 363.4 (M+H).

Proof of stereochemistry derivative **17g**

(4-bromophenyl)((6*S*,9*R*)-4-(cyclopropylamino)-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-10-yl)methanone (17g)



17f 17g

To a solution of compound **17f** (43 mg, 0.17 mmol) in CH_2Cl_2 (0.17 mL) was added triethylamine (47.4 uL, 0.34 mmol), DMAP (1.0 mg, 0.008 mmol), and 4-bromobenzoyl chloride (56 mg, 0.26 mmol) at 25 ˚C. Then the reaction mixture was stirred at 25 ˚C for 72 h. Upon

completion, CH_2Cl_2 (5 mL) was added to the residue followed by saturated aqueous NH_4Cl (5 mL). The solution was extracted with CH_2Cl_2 (5 mL x 2). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by chromatography on silica gel (heptanes:EtOAc = 1:0 to 0:1) to afford (4-bromophenyl)((6*S*,9*R*)-4-(cyclopropylamino)-6,7,8,9-tetrahydro-5H-6,9-epiminocyclohepta[d]pyrimidin-10-yl)methanone (50 mg, 74% yield) as white solid. The solid was crystallized by dissolving in a small amount of EtOAc (~0.5 mL) and adding heptanes (~0.1 mL) until solid precipitated out, then heat to dissolve into solution and allow to cool slowly to generate crystals over a period of 2 days. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.52 (s, 1H), 7.52 (d, *J* = 7.9 Hz, 3H), 7.28 – 7.21 (m, 2H), 5.20 (s, 1H), 4.75 (d, *J* = 41.1 Hz, 2H), 3.08 (d, *J* = 15.6 Hz, 1H), 2.87 – 2.79 (m, 1H), 2.36 – 2.19 (m, 2H), 2.09 (dd, *J* = 22.7, 14.0 Hz, 2H), 1.80 – 1.51 (m, 3H), 0.93 – 0.84 (m, 2H), 0.57 (s, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 163.2, 162.1, 156.7, 134.3, 131.9, 129.3, 125.1, 107.8, 61.7, 50.9, 34.8, 30.5, 28.7, 24.1, 7.6, 7.5; LCMS = 100%, m/z = 400.3 (M+H).

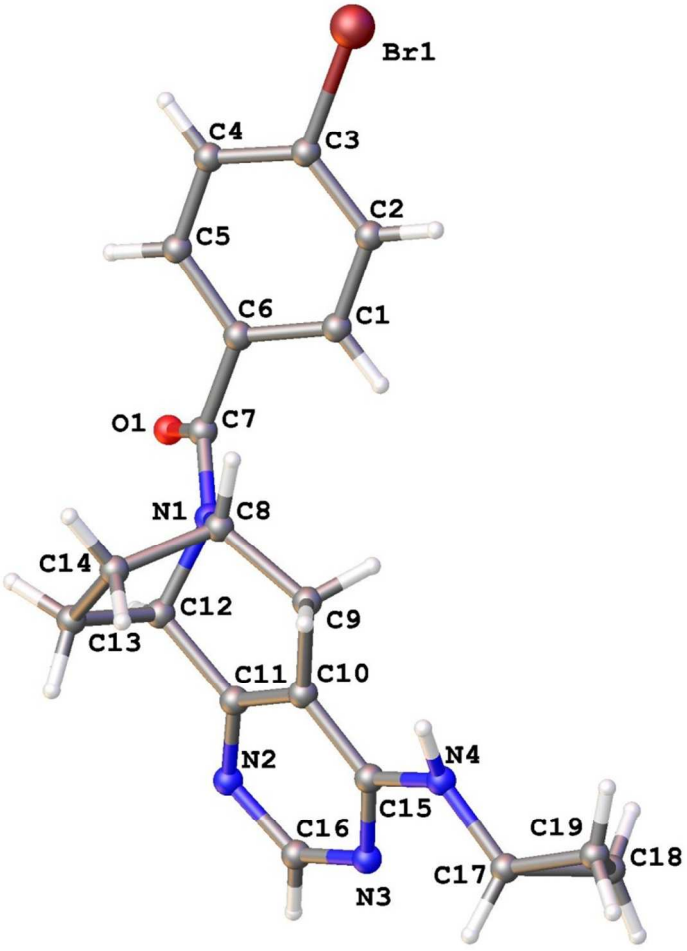
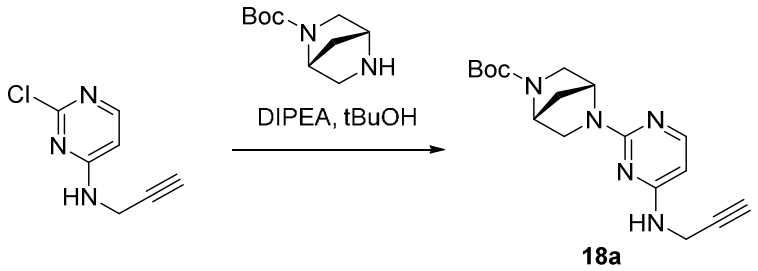


Figure F1

Figure F1 Proof of absolute stereochemistry for compound **17g**, a *p*-bromobenzamide derivative of **17f** which had been previously separated to high enantiopurity by chiral SFC. This served as further confirmation of stereochemistry for compound **17** to go alongside the protein X-ray structure of Figure 3D.

Negative control compound **18**

***tert*-Butyl (1*R*,4*R*)-5-(4-(prop-2-yn-1-ylamino)pyrimidin-2-yl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (18a)**



18a

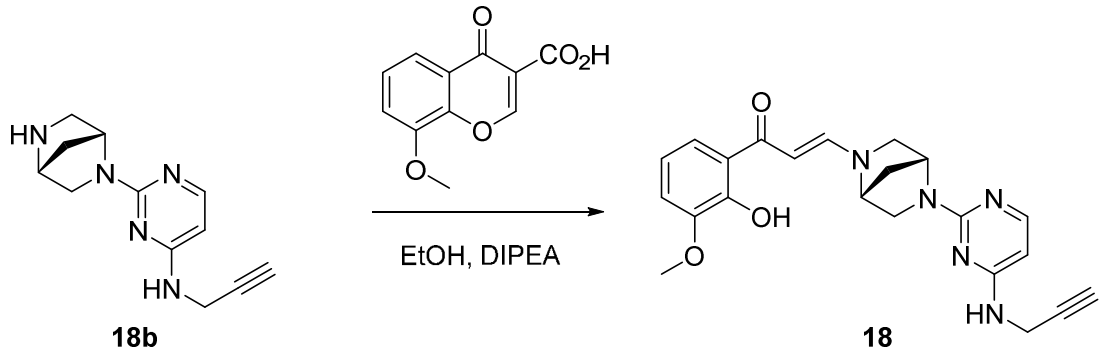
To a solution of compound chloro-N-(prop-2-yn-1-yl)pyrimidin-4-amine[4] (120 mg, 0.605 mmol, 1.0 eq.) in 2.0 mL of t-BuOH was added (1*R*,4*R*)-*tert*-butyl 2,5-diazabicyclo[2.2.1]heptane-2-carboxylate (122 mg, 0.726 mmol, 1.2 eq.), DIPEA (313 mg, 2.42 mmol, 4.00 eq.). The reaction mixture was stirred at 120°C for 2 hours in a Microwave under N_2. The reaction mixture was concentrated to get crude product, which was purified by ISCO flash chromatography on silica gel (PE:EA=100:1to 1:10) to provide desired product compound **18a** (100 mg, yield: 50 %) as a yellow solid. LCMS = 90.4%, m/z = 329.9 (M+H).

2-((1*R*,4*R*)-2,5-Diazabicyclo[2.2.1]heptan-2-yl)-N-(prop-2-yn-1-yl)pyrimidin-4-amine (18b)

18a **18b**

To a solution of compound **18a** (24 mg, 0.073 mmol, 1.0 eq.) in 6mL of dry CH_2Cl_2 was added TFA (2 mL). The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was concentrated to give the crude product compound **18b** (17 mg, yield: 100 %) as a crude yellow oil. LCMS = 77.0%, m/z = 229.9 (M+H).

(*E*)-1-(2-Hydroxy-3-methoxyphenyl)-3-((1*R*,4*R*)-5-(4-(prop-2-yn-1-ylamino)pyrimidin-2-yl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)prop-2-en-1-one (18)

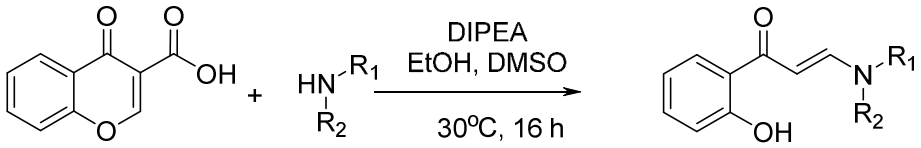


18b **18**

To a crude solution of 2-((1*R*,4*R*)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-N-(prop-2-yn-1-yl)pyrimidin-4-amine **18b** (8.0 mg, 0.035 mmol) in EtOH (10 mL) was added 8-methoxy-4-oxo-4H-chromene-3-carboxylic acid (10. mg, 0.0454 mmol) and DIPEA (18 mg, 0.140 mmol). The reaction mixture was stirred at 25˚C for 18 hours. Upon completion, the reaction mixture was concentrated. The crude product was purified by prep. HPLC to provide (*E*)-1-(2-hydroxy-3-methoxyphenyl)-3-((1*R*,4*R*)-5-(4-(prop-2-yn-1-ylamino)pyrimidin-2-yl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)prop-2-en-1-one **18** (10.2 mg, 72% yield) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 12.1 Hz, 1H), 7.89 (d, *J* = 5.8 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 7.9 Hz, 1H), 6.72 (t, *J* = 8.0 Hz, 1H), 5.79 (d, *J* = 5.8 Hz, 1H), 5.69 (d, *J* = 12.1 Hz, 1H), 5.09 (s, 1H), 4.84 (s, 1H), 4.38 (s, 1H), 4.14 (dd, *J* = 5.5, 2.6 Hz, 2H), 3.88 (s, 4H), 3.65 (s, 2H), 3.45 (s, 2H), 2.23 (s, 1H), 2.10 (d, *J* = 9.8 Hz, 1H), 2.01 (d, *J* = 10.1 Hz, 1H), 1.79 (s, 2H); ^{13}C NMR (101 MHz, DMSO) δ 191.7, 162.2, 160.2, 156.4, 153.6, 149.4, 149.2, 120.3, 119.9, 117.2, 115.0, 91.0, 80.2, 77.4, 71.5, 64.9, 56.6, 56.2, 55.2, 54.9, 37.9, 30.7; HRMS [M+H] for $C_{22}H_{24}N_5O_3$, calc., 406.1874, found, 406.1887.

Compounds Prepared by Parallel Medicinal Chemistry Library Methods

General synthetic protocol:



To a reaction vial was dispensed 4-oxo-4H-chromene-3-carboxylic acid (100 μmol) followed by addition of the selected amine monomer (300 μmol). The reactants were dissolved in ethanol (500 μL) and DMSO (500 μL) followed by the addition of DIPEA (300 μmol, 3.0 equiv). The vial were capped and agitated for 30^{o}C for 16 hours. The solvent was removed by Speedvac and the residue was purified via preparative HPLC to provide the desired products.

Purification and analytical process for library compounds:

All the compounds were dissolved in 100% DMSO. 10 μL from each well was transferred to a daughter plate and diluted with 200 μL DMSO. LCMS of crude compounds were done and

these were further purified on Waters Autopurification System. Prep fractions were collected in barcoded test tubes and evaporated in Thermo explorer (40°C, 15 Torr, 16 hrs). Each of the compounds were then dissolved in ethanol (1.6 ml) and transferred to pre-tared 2 drum glass vials. Finally they were dried in Genevac first for 1 hr (at 40°C, 10 mbar) and then for 12 hrs (at 40°C, 0 mbar). Weights were taken using Metller Balance equipped with weighing software. These compounds were then dissolved in calculated amount of DMSO to prepare 30 mM solution. 8 µL was removed for final QC analysis (diluted with 150 uL DMSO) on an analytical LC/MS. QC reports were generated to check identity and purity of each compound.

Preparative HPLC for library compounds:

Column: RESTEK C18 30 X 2.1 mm 3 micron using Solvent A = 0.05% Formic acid in Water; Solvent B = Acetonitrile. Detection via MS and ELSD.
Gradient and Flow Rate:

Time-min	Flow ml/min	%A	%B
0	1.50	98	2
0.75	1.50	98	2
1.00	1.50	90	10
2.00	1.50	2	98
2.25	1.50	2	98
2.90	1.50	98	2
3.00	1.50	98	2

Analytical Methods for library compounds:

Method PF-CD05
Column Xbridge C18 2.1×50mm 5µm
Temperature 50 ℃
Mobile Phase A 0.05% NH4OH in water
Mobile Phase B 100% acetonitrile
Gradient - Initial 5% B
Time 0.00 mins 5% B
Time 0.50 mins 5% B
Time 3.40 mins 100% B
Time 4.20 mins 100% B
Time 4.21 mins 5% B
Time 4.70 mins 5% B
Flow rate 0.8 ml / min
Injection volume 2 µl

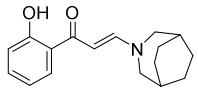
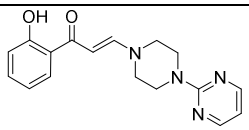
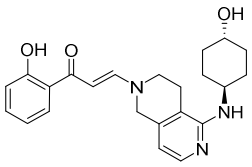
Agilent 1200 HPLC/1956 MSD/SEDEX 75 ELSD

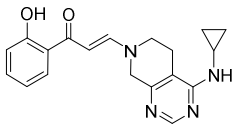
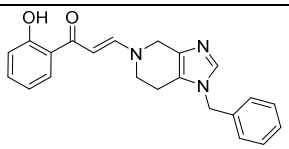
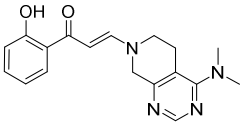
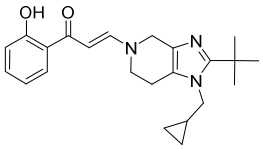
Ionization Mode API-ES
Polarity Negative

Method SP#4436
Column RESTEK C18(30x2.1) 3u
Temperature 500°C
Mobile Phase A .05% HCOOH in water
Mobile Phase B ACETONITRILE
Gradient
Time Initial 2% B
Time 0.75 mins 2 % B
Time 1.00 mins 10% B
Time 2.00 mins 98% B
Time 2.25 mins 98% B
Time 2.90 mins 2% B
Time 3.00 mins 2% B
Flow rate 1.5 ml / min
Injection volume 3 ul

WATERS ACQUITY UPLC/WATERS 3100 MSD/PL-ELS 2100 ICE ELSD
Ionization Mode API-ES
Polarity Positive/Negative

Data for compounds prepared by parallel medicinal chemistry methods

	Structure	Name	MWT	Obs MWt	RT (min)	QC Method	UV purity	Total amount made (mg)
6		(2E)-3-(3-azabicyclo[3.2.2]non-3-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one	271.360	272	3.18	PF-CD05	91	6.7
7		(2E)-1-(2-hydroxyphenyl)-3-[4-(pyrimidin-2-yl)piperazin-1-yl]prop-2-en-1-one	310.357	311	1.65	SP#4436	95.5	13.3
8		(2E)-3-{5-[(trans-4-hydroxycyclohexyl)amino]-3,4-dihydro-2,6-naphthyridin-2(1H)-yl}-1-(2-hydroxyphenyl)prop-2-en-1-one	393.487	394	2.58	PF-CD05	91	10.5

11		(2E)-3-[4-(cyclopropylamino)-5,8-dihydropyrido[3,4-d]pyrimidin-7(6H)-yl]-1-(2-hydroxyphenyl)prop-2-en-1-one	336.395	337	2.47	PF-CD05	95.9	360
12		(2E)-3-(1-benzyl-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridin-5-yl)-1-(2-hydroxyphenyl)prop-2-en-1-one	359.429	360	1.47	SP#4436	94.3	8.5
13		(2E)-3-[4-(dimethylamino)-5,8-dihydropyrido[3,4-d]pyrimidin-7(6H)-yl]-1-(2-hydroxyphenyl)prop-2-en-1-one	324.384	325	2.564	PF-CD05	98.9	6.7
14		(2E)-3-[2-tert-butyl-1-(cyclopropylmethyl)-1,4,6,7-tetrahydro-5H-imidazo[4,5-c]pyridin-5-yl]-1-(2-hydroxyphenyl)prop-2-en-1-one	379.504	380	3.022	PF-CD05	93	1.4

Small Molecule X-Ray Methods and Data for 17g:

Crystallographic information: A representative crystal was chosen from the lot of material, mounted on a Mitegen loop in immersion oil and collected at -150 ˚C. A trial solution was found using Intrinsic Phasing and then further refined. During refinement, a disordered moiety was found from the difference map near a special position and refined with 0.5 occupancy using a constrained model from [Guzei 2014]. The structure was solved in the tetragonal crystal system in the $P4_1$ space group with Z=1. The structure refined to final values of : R=5.0% with GOOF = 0.998. In this case, the absolute configuration was determined directly from the data, with the Hooft parameter reporting as -0.006 with an esd of 0.018 [Hooft 2008].

Data collection was performed on a Bruker APEX diffractometer at -150C. Data collection consisted of omega and phi scans. The structure was solved by direct methods using SHELX software suite in the space group $P4_1$. The structure was subsequently refined by the full-matrix least squares method. All non-hydrogen atoms were found and refined using anisotropic displacement parameters. The hydrogen atoms located on nitrogen were found from the Fourier difference map and refined with distances restrained. The remaining hydrogen atoms were placed in calculated positions and were allowed to ride on their carrier atoms. The final refinement included isotropic displacement parameters for all hydrogen atoms.

Analysis of the absolute structure using likelihood methods (Hooft 2008) was performed using PLATON (Spek 2010). Assuming the sample submitted is enantiopure, the results indicate that the absolute structure has been correctly assigned. The method calculates that the probability

that the structure is correctly assigned is 100.0. The Hooft parameter is reported as -0.021 with an esd of 0.016. The disorder treatment of the solvent molecule used a calculated geometry and refined this entity isotropic as half occupied near a special position (Guzei 2014). The final R-index was 4.75%. A final difference Fourier revealed a couple of higher than normal residuals near the disordered solvent molecule. Pertinent crystal, data collection and refinement are summarized in table 1. Atomic coordinates, Bond lengths, bond angles, Torsion angles and displacement parameters are listed in tables 2 –6.

Software used for structure solution and graphics and general references

SHELXTL, Version 5.1, Bruker AXS, **1997**
PLATON, Spek, A. L. *J. Appl. Cryst.* **2003**, 36, 7-13.
Guzei, I. A. *J. Appl. Cryst.* **2014**, 47, 806-809.
Hooft, R. W. W., Straver, L. H., Spek, A. L. *J. Appl. Cryst.* **2008**, 41, 96-103.
Flack, H. D. *Acta Cryst.* **1983**, A39, 867-881.

Identification code	Z610	
Crystallization	EtOAc-Heptane	
Empirical formula	C40 H42 Br2 N8 O3	
Formula weight	842.63	
Temperature	123(2) K	
Wavelength	1.54178 Å	
Crystal system	Tetragonal	
Space group	$P4_1$	
Unit cell dimensions	a = 21.3664(14) Å	α= 90°.
	b = 21.3664(14) Å	β= 90°.
	c = 8.8337(8) Å	γ = 90°.
Volume	4032.8(6) $Å^3$	
Z	4	
Density (calculated)	1.388 Mg/m^3	
Absorption coefficient	2.928 mm^{-1}	
F(000)	1728	
Crystal size	0.30 x 0.04 x 0.02 mm^3	
Theta range for data collection	2.068 to 57.629°.	
Index ranges	-23<=h<=23, -23<=k<=23, -9<=l<=9	
Reflections collected	35395	
Independent reflections	5361 [R(int) = 0.0993]	
Completeness to theta = 57.629°	97.8 %	
Absorption correction	Empirical	
Max. and min. transmission	1 and 0.7520	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	5361 / 3 / 469	
Goodness-of-fit on F^2	1.038	
Final R indices [I>2sigma(I)]	R1 = 0.0475, wR2 = 0.1200	
R indices (all data)	R1 = 0.0576, wR2 = 0.1265	
Absolute structure parameter	-0.020(17)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.402 and -0.466 e.$Å^{-3}$	

Table 2

Table 2 Crystal data and structure refinement for **17g**

	x	y	z	U(eq)
Br(1)	6533(1)	5076(1)	5461(1)	75(1)
Br(2)	10416(1)	11409(1)	7684(1)	64(1)
N(1)	4740(3)	2735(3)	8993(8)	41(2)
N(2)	3657(3)	1974(4)	11637(10)	58(2)
N(3)	2735(3)	2640(4)	11912(9)	52(2)
N(4)	2548(3)	3476(3)	10256(9)	47(2)
N(5)	7692(3)	10318(3)	4092(8)	43(2)
N(6)	6748(3)	9497(3)	1252(9)	46(2)
N(7)	7173(3)	8449(3)	965(8)	46(2)
N(8)	7904(3)	8036(3)	2664(9)	44(2)
O(1)	5721(2)	2534(3)	9943(7)	50(2)
O(2)	7854(2)	11248(2)	2862(7)	50(1)
C(1)	5371(4)	4001(4)	8297(9)	38(2)
C(2)	5643(4)	4499(4)	7475(10)	48(2)
C(3)	6166(4)	4387(4)	6583(10)	50(2)
C(4)	6436(4)	3787(4)	6468(11)	51(2)
C(5)	6167(4)	3303(4)	7277(10)	47(2)
C(6)	5631(3)	3385(3)	8200(10)	40(2)
C(7)	5370(4)	2852(4)	9117(10)	41(2)
C(8)	4306(3)	2940(3)	7742(11)	42(2)
C(9)	3723(4)	3233(4)	8524(10)	42(2)
C(10)	3493(4)	2844(4)	9871(10)	43(2)
C(11)	3838(3)	2331(4)	10442(11)	45(2)
C(12)	4446(4)	2154(4)	9621(12)	51(2)
C(13)	4304(4)	1787(4)	8117(11)	53(2)
C(14)	4153(4)	2311(4)	6911(12)	49(2)
C(15)	2924(4)	2980(4)	10675(10)	45(2)
C(16)	3112(4)	2157(5)	12306(13)	64(3)
C(17)	1956(4)	3596(4)	11024(11)	53(2)
C(18)	1933(5)	4080(5)	12307(13)	66(3)
C(19)	1655(5)	4214(5)	10737(13)	68(3)
C(20)	9106(4)	10530(4)	4821(10)	42(2)
C(21)	9642(4)	10676(4)	5672(10)	43(2)
C(22)	9677(4)	11229(4)	6460(10)	45(2)
C(23)	9193(4)	11665(4)	6470(10)	47(2)

C(24)	8658(4)	11531(3)	5602(10)	43(2)
C(25)	8603(3)	10961(4)	4781(9)	38(2)
C(26)	8021(4)	10852(4)	3845(10)	41(2)
C(27)	7752(4)	9830(3)	5318(10)	41(2)
C(28)	7913(4)	9201(4)	4525(10)	41(2)
C(29)	7515(3)	9098(3)	3100(9)	38(2)
C(30)	7121(3)	9568(4)	2521(10)	44(2)
C(31)	7072(4)	10188(4)	3371(10)	46(2)
C(32)	6642(4)	10124(4)	4810(11)	55(2)
C(33)	7081(4)	9826(4)	6056(11)	50(2)
C(34)	7529(4)	8527(4)	2230(10)	40(2)
C(35)	6795(4)	8944(4)	570(11)	48(2)
C(36)	7927(4)	7455(4)	1791(12)	53(2)
C(37)	8463(4)	7014(4)	2051(12)	55(2)
C(38)	8403(4)	7354(4)	544(11)	53(2)
O(93)	4410(20)	-600(20)	3170(50)	350(30)
O(94)	4810(15)	121(13)	4670(40)	360(30)
C(91)	4510(30)	440(20)	2220(50)	480(90)
C(92)	4565(13)	-76(18)	3380(40)	280(40)
C(93)	4884(15)	-322(14)	5860(40)	141(13)
C(94)	5170(20)	20(20)	7200(40)	181(19)

Table 3

Table 3 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **17g**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	82(1)	70(1)	74(1)	19(1)	1(1)	-31(1)
Br(2)	55(1)	72(1)	65(1)	-2(1)	-12(1)	-18(1)
N(1)	31(4)	39(4)	54(4)	12(3)	-4(3)	-3(3)
N(2)	43(4)	54(5)	79(6)	33(4)	-6(4)	-11(3)
N(3)	38(4)	57(5)	61(5)	23(4)	-4(3)	-9(3)
N(4)	39(4)	51(4)	51(4)	13(4)	-3(4)	-4(3)
N(5)	41(4)	32(4)	56(4)	3(3)	-4(3)	-2(3)
N(6)	37(4)	48(4)	54(4)	3(4)	-9(3)	2(3)
N(7)	34(4)	55(4)	51(4)	-3(3)	-1(3)	-11(3)
N(8)	42(4)	31(3)	58(5)	-6(3)	-2(4)	-5(3)
O(1)	37(3)	45(3)	67(4)	8(3)	-14(3)	0(2)
O(2)	48(3)	39(3)	63(4)	12(3)	-3(3)	0(2)
C(1)	39(4)	38(4)	36(5)	-2(4)	-3(3)	1(3)
C(2)	61(5)	34(4)	49(5)	-4(4)	-9(4)	-5(4)
C(3)	49(5)	53(5)	49(6)	-1(4)	-8(4)	-15(4)
C(4)	43(5)	62(6)	48(5)	-2(5)	2(4)	-6(4)
C(5)	44(5)	47(5)	51(5)	-4(4)	-5(4)	0(4)
C(6)	34(4)	36(4)	52(5)	-5(4)	-9(4)	-1(3)
C(7)	31(4)	39(5)	53(5)	-3(4)	-7(4)	1(4)
C(8)	32(4)	38(4)	54(5)	10(4)	-4(4)	-4(3)
C(9)	32(4)	39(4)	56(5)	13(4)	-7(4)	-5(3)
C(10)	36(4)	36(4)	57(6)	14(4)	-13(4)	-6(3)
C(11)	35(4)	40(4)	60(5)	10(4)	-13(4)	-9(3)
C(12)	41(5)	34(4)	79(7)	19(4)	-7(4)	-2(3)
C(13)	50(5)	37(4)	73(7)	1(4)	-8(5)	-3(4)
C(14)	42(5)	43(5)	64(6)	-1(4)	-10(4)	2(4)
C(15)	38(4)	46(5)	52(5)	7(4)	-12(4)	-11(3)
C(16)	41(5)	70(6)	82(8)	40(6)	-3(5)	-14(4)
C(17)	38(5)	57(6)	63(6)	14(5)	3(4)	-3(4)
C(18)	48(5)	63(6)	85(8)	11(6)	8(5)	1(4)
C(19)	47(5)	67(6)	90(8)	28(6)	11(5)	10(4)
C(20)	41(5)	36(4)	50(5)	-4(4)	4(4)	-4(3)
C(21)	33(4)	45(5)	49(5)	8(4)	6(4)	1(3)
C(22)	44(5)	42(5)	50(5)	-2(4)	8(4)	-11(4)
C(23)	64(6)	32(4)	46(5)	-4(4)	1(4)	-12(4)
C(24)	53(5)	31(4)	45(5)	-2(4)	5(4)	2(3)
C(25)	35(4)	30(4)	50(5)	5(4)	4(4)	3(3)

C(26)	39(5)	35(5)	50(5)	1(4)	6(4)	5(3)
C(27)	43(4)	37(4)	42(5)	5(4)	-3(4)	-4(3)
C(28)	34(4)	31(4)	58(5)	4(4)	-4(4)	-4(3)
C(29)	37(4)	33(4)	44(5)	2(4)	0(4)	-5(3)
C(30)	31(4)	50(5)	53(5)	5(4)	-1(4)	-6(3)
C(31)	45(5)	34(4)	58(6)	3(4)	-10(4)	4(3)
C(32)	45(5)	47(5)	74(7)	-9(5)	1(5)	3(4)
C(33)	45(5)	45(5)	61(6)	5(4)	11(4)	-1(4)
C(34)	33(4)	36(4)	52(5)	0(4)	4(4)	-9(3)
C(35)	35(4)	61(6)	47(5)	3(5)	-4(4)	-8(4)
C(36)	49(5)	37(5)	73(6)	-8(4)	-2(5)	-9(4)
C(37)	59(6)	36(5)	69(6)	-5(4)	1(5)	0(4)
C(38)	56(5)	44(5)	60(6)	-4(5)	4(5)	2(4)

Table 4

Table 4 Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **17g**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	x	y	z	U(eq)
H(4)	2670(40)	3600(40)	9230(50)	56
H(8)	8120(40)	8070(40)	3660(60)	53
H(1)	5016	4075	8916	46
H(2)	5469	4907	7533	58
H(4A)	6794	3717	5852	61
H(5)	6350	2898	7211	57
H(8A)	4514	3246	7048	50
H(9A)	3829	3659	8878	51
H(9B)	3381	3270	7773	51
H(12)	4740	1919	10294	62
H(13A)	3941	1504	8252	64
H(13B)	4671	1536	7801	64
H(14A)	4417	2260	5999	59
H(14B)	3707	2296	6607	59
H(16)	2981	1917	13153	77
H(17)	1667	3230	11124	63
H(18A)	2327	4293	12588	79
H(18B)	1639	4004	13154	79
H(19A)	1194	4225	10644	82
H(19B)	1880	4514	10079	82
H(20)	9082	10147	4279	51
H(21)	9983	10390	5702	51
H(23)	9223	12040	7042	57
H(24)	8327	11827	5563	52
H(27)	8080	9949	6073	49
H(28A)	8362	9200	4243	49
H(28B)	7842	8852	5241	49

H(31)	6934	10538	2696	55
H(32A)	6280	9849	4599	66
H(32B)	6486	10539	5139	66
H(33A)	7075	10079	6996	60
H(33B)	6948	9394	6298	60
H(35)	6536	8883	-292	57
H(36)	7513	7249	1621	63
H(37A)	8371	6560	2070	66
H(37B)	8800	7150	2749	66
H(38A)	8702	7698	329	64
H(38B)	8273	7108	-350	64
H(91A)	4672	828	2648	716
H(91B)	4756	325	1323	716
H(91C)	4071	491	1936	716
H(93A)	5161	-668	5530	169
H(93B)	4473	-501	6145	169
H(94A)	5227	-278	8034	271
H(94B)	5577	190	6901	271
H(94C)	4892	356	7514	271

Table 5

Table 5 Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^3) for **17g**.

Cellular Biology Methods:

FRAP Assay

FRAP studies were performed as described in reference 18 of the main text. U2OS cells were transfected with expression plasmids for full-length PB1 or SMARCA2 protein, respectively, chimerized with an N-terminal eGFP. Six hours after transfection 2.5 μM SAHA was added. Inhibitor was added 1 hour before imaging, which was carried out 24 hours after transfection. Statistical significance was assessed using one-way ANOVA with Dunnett's multiple comparisons test.

BioMap® Assay

Diversity PLUS

System + Symbol	Human Primary Cells	Disease / Tissue Relevance	Biomarker Readouts
3C	Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, ICAM-1, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, SRB
4H	Venular endothelial cells	Asthma, Allergy, Autoimmunity	MCP-1, Eotaxin-3, VCAM-1, P-selectin, uPAR, SRB, VEGFRII
LPS	Peripheral blood mononuclear cells + Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, CD40, E-selectin, CD69, IL-8, IL1α, M-CSF, sPGE2, SRB, sTNFα
SAg	Peripheral blood mononuclear cells + Venular endothelial cells	Autoimmune Disease, Chronic Inflammation	MCP-1, CD38, CD40, E-selectin, CD69, IL-8, MIG, PBMC Cytotoxicity, Proliferation, SRB
BT	Peripheral blood mononuclear cells + B cells	Asthma, Allergy, Oncology, Autoimmunity	B cell Proliferation, PBMC Cytotoxicity, Secreted IgG, sIL17A, sIL17F, sIL-2, sIL-6, sTNFα
BF4T	Bronchial epithelial cells + Dermal fibroblasts	Asthma, Allergy, Fibrosis, Lung Inflammation	MCP-1, Eotaxin-3, VCAM-1, ICAM-1, CD90, IL-8, IL1α, Keratin 8/18, MMP-1, MMP-3, MMP-9, PAI-1, SRB, tPA, uPA
BE3C	Bronchial epithelial cells	Lung Inflammation, COPD	ICAM-1, uPAR, IP-10, I-TAC, IL-8, MIG, EGFR, HLA-DR, IL1α, Keratin 8/18, MMP-1, MMP-9, PAI-1, SRB, tPA, uPA
CASM3C	Coronary artery smooth muscle cells	Cardiovascular Inflammation, Restenosis	MCP-1, VCAM-1, TM, TF, uPAR, IL-8, MIG, HLA-DR, IL-6, LDLR, M-CSF, PAI-1, Proliferation, SAA, SRB
HDF3CGF	Dermal fibroblasts	Fibrosis, Chronic Inflammation	MCP-1, VCAM-1, ICAM-1, Collagen I, Collagen III, IP-10, I-TAC, IL-8, MIG, EGFR, M-CSF, MMP-1, PAI-1, Proliferation_72hr, SRB, TIMP-1, TIMP-2
KF3CT	Keratinocytes + Dermal fibroblasts	Psoriasis, Dermatitis, Skin Biology	MCP-1, ICAM-1, IP-10, IL-8, MIG, IL1α, MMP-9, PAI-1, SRB, TIMP-2, uPA
MyoF	Lung fibroblasts	Fibrosis, Chronic Inflammation, Wound Healing, Matrix Remodeling	a-SM Actin, bFGF, VCAM-1, Collagen-I, Collagen-III, Collagen-IV, IL-8, Decorin, MMP-1, PAI-1, TIMP-1, SRB
/Mphg	Venular endothelial cells + Macrophages	Cardiovascular Inflammation, Restenosis, Chronic Inflammation	MCP-1, MIP-1α, VCAM-1, CD40, E-selectin, CD69, IL-8, IL1α, M-CSF, sIL10, SRB, SRB-Mphg

Figure F2

Figure F2 Summary of cells, disease association and endpoints used in BioMap assay

Renal Cell Carcinoma Proliferation

Rcc4 and 786-O cell lines were obtained from Pugh group (Centre for cellular and molecular physiology, Nuffield Department of Medicine, Oxford University). Both cell lines were grown in DMEM media containing 1% Penicillin/Streptomycin, 10 % FBS and 1% L-Glutamine. The MTT assay was performed using the commercially available proliferation kit I (Roche, 11465007001) according to manufacturer's instructions. Briefly, cells were seeded in 96 well plates, in quadruplicate at a density of 4000 cells per well at time zero. Twelve hours after seeding, compound treatment (**16**, **17** and **18**) was performed at five different concentrations (0.1, 1.0, 2.5, 5.0 and 10 µM) for 24, 48 and 72 hours. Compounds were refreshed every 24 hours. Each day 10 µl of the labelling reagent (final concentration 0.5 mg/ml) was added to each well and the plates were incubated at 37°C and 5% CO_2 for 4 hours. 100 µl of solubilisation solution was then added to each well and the plates were incubated overnight at 37°C and 5% CO_2. The following day the plates were analysed using a spectrophotometer reader at 570 nm, with a reference wavelength of 650nm.

Cytotoxicity, Myoblast Differentiation and Adipocyte Differentiation

Cell culture. C2C12 cells were purchased from American Type Culture Collection (CRL-1772). Differentiation of C2C12 cells was initiated by plating $4x10^4$ cells/well in 48-well plates, in

DMEM media containing 10% fetal bovine serum and incubated with increasing concentrations of compound 16 or 17 as indicated in the figures. After 48 h, cells reached confluence, the media was changed to DMEM containing 2% horse serum, and the cells were allowed to differentiate for 48 h. C3H10T1/2 mesenchymal cells were obtained from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. For adipocyte differentiation, $4x10^4$ cells/well were plated in 48-well plate in DMEM medium that contained increasing concentrations of compound 16 or 17 as indicated in the figures. Two-day post-confluent cells were differentiated in medium with a standard adipogenic cocktail (1 µg/ml insulin, 0.25 µg/ml dexamethasone, 0.5 mM isobutylmethylxanthine IBMX). After 48 h, cells were maintained in medium containing 1 µg/ml insulin for 2 days, finally cells were kept in growth medium for additional 2 days. Media was changed daily to ensure the viability of both compounds.

Immunocytochemistry. C2C12 cells grown on 48-well plates were stained with anti-myosin heavy chain (MF20) or anti-myogenin (F5D) antibodies obtained as hybridoma supernatants from the Developmental Studies Hybridoma Bank. After differentiation, cells were fixed in formalin, then incubated overnight with hybridoma supernatant at a 1:10 dilution along with 5% horse serum, 0.2% Triton X-100 in PBS. Secondary antibody binding and HRP staining were performed with the Vectastain ABC universal kit and developed using Vector® VIP Peroxidase Substrate according to the manufacturer's protocol (Vector Laboratories, INC, Burlingame, CA, USA).

Oil Red O staining: The differentiating cells were fixed with 10% phosphate-buffered formalin for 1 h. The cells were washed with PBS and 60% isopropanol (Gesta S, 2011). The cells were then stained with a working solution of 60% Oil Red O (60:40 stain-water) for 1 h and washed repeatedly with water to remove excess Oil Red O.

MTT assay. Cells were seeded in 48-well plates ($4x10^4$ cells/well) overnight prior to drug treatment, and were then maintained in presence of vehicle (DMSO) or increasing doses of compound **16** or **17** for 72 hours before addition of MTT solution (5 µg/mL). Plates were incubated for 4 hours in MTT solution, then the media was removed and plates were air-dried. Five hundred microliters of DMSO were added to each well and incubated at room temperature for 30 minutes with gentle shaking. The plates were read at OD540 in Synergy H4 Hybrid microplate reader (Bio Tek, Winooski, VT, USA).

Biophysical and Structural Biology Methods:

Protein purification

cDNA encoding human bromodomains were cloned, expressed and purified as previously described.[5] For purification of in vivo biotinylated protein expression the same construct boundaries (e.g. CBP residues R1081-G1198) were bromodomain subcloned into pNIC-BIO1 vector, a derivative from pNIC28-Bsa4 vector (Gene Bank: EF198106), containing a 10 His-tag and TEV protease cleavage site at the N-terminus and an in frame biotinylation sequence (SSKGGYGLNDIFEAQKIEWHE) inserted at the C-terminus. The constructs were transformed

into BL21 (DE3)-R3-BirA cell line (BL21 derivative co-expressing BirA using a pACYC co-expression vector). Cells were grown overnight at 37 ºC in 10 mL of Luria-Bertani medium with 50 μg/mL kanamycin and 34 μg/ml chloramphenicol (start-up culture). The start-up culture was diluted 1:1000 in fresh medium and cell growth was allowed at 37 ºC to an optical density of about ~1.0 (OD_{600}) before the temperature was decreased to 25 ºC. d-Biotine was dissolved into 10 mM bicine pH8.3 and added to the culture at 500 μM final. The protein expression was induced for 8 h at 25 ºC with 50 μM isopropyl-β-D-thiogalactopyranoside (IPTG). Proteins were purified using Ni-affinity chromatography and size exclusion chromatography.

Thermal Shift Assay

Thermal melting experiments were carried out using an Mx3005p Real Time PCR machine (Stratagene). Proteins were buffered in 10 mM HEPES pH 7.5, 500 mM NaCl and assayed in a 96-well plate at a final concentration of 2 μM in 20 μL volume. Compounds were added at a final concentration of 10 μM. SYPRO Orange (Molecular Probes) was added as a fluorescence probe at a dilution of 1:1000. Excitation and emission filters for the SYPRO-Orange dye were set to 465 nm and 590 nm, respectively. The temperature was raised with a step of 3°C per minute from 25°C to 96°C and fluorescence readings were taken at each interval.

Isothermal Titration Calorimetry (ITC)

Experiments were carried out on a VP-ITC microcalorimeter (MicroCal™). All experiments were performed at 15 °C in 50 mM HEPES pH 7.5, 150 mM NaCl. The titrations were conducted using an initial injection of 2 μl followed by 34 identical injections of 8 μl. The dilution heats were measured on separate experiments and were subtracted from the titration data. Thermodynamic parameters were calculated using $\Delta G = \Delta H - T\Delta S = -RT\ln K_B$, where ΔG, ΔH and ΔS are the changes in free energy, enthalpy and entropy of binding respectively. In all cases a single binding site model was employed.

Crystallization

Protein Expression and purification: Proteins were cloned, expressed and purified as previously described.[5]

Crystallization: Aliquots of the purified proteins were set up for crystallization using a mosquito® crystallization robot (TTP Labtech, Royston UK). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nl, 75+75 nl and 50+100 nl). Initial hits were optimized further scaling up the drop sizes. All crystallizations were carried out using the sitting drop vapor diffusion method at 4 °C. PB1(5) crystals with **2** were grown by mixing 100 nl of the protein (10.8 mg/ml and 2 mM final ligand concentration) with 200 nl of reservoir solution containing 0.20 M Na_2SO_4, 20 % PEG3350 and 10 % ethylene glycol. PB1(5) crystals with **3** were grown by mixing 150 nl of protein (8 mg/ml and 2 mM final ligand concentration) with 150 nl of reservoir solution

containing 0.2 M sodium malonate, 0.1 BT-propane pH 6.5, 20 % PEG6000 and 10 % ethylene glycol. In all cases diffraction quality crystals grew within a few days.

Data Collection and Structure solution: All crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data were collected in-house on a Rigaku FRE rotating anode system equipped with a RAXIS-IV detector at 1.52 Å (PB1(5)/**2**) or at Diamond, beamline I04.1 at a wavelength of 0.92 Å (PB1(5)/**3**). Indexing and integration was carried out using MOSFLM[6] and scaling was performed with SCALA[7]. Initial phases were calculated by molecular replacement with PHASER[8] using the known models of PB1(5) (PDB ID 3G0J). Initial models were built by ARP/wARP[9] followed by manual building in COOT[10]. Refinement was carried out in REFMAC5[11]. In all cases thermal motions were analyzed using TLSMD[12] and hydrogen atoms were included in late refinement cycles. Data collection and refinement statistics can be found in Supplemental Table 3. The models and structure factors have been deposited with PDB accession codes: 5E7D (PB1(5)/Compound **2**), 4Q0N (PB1(5)/Compound **3**), 5KDH (SMARCA2/Compound **17**).

Supplemental Biological Data

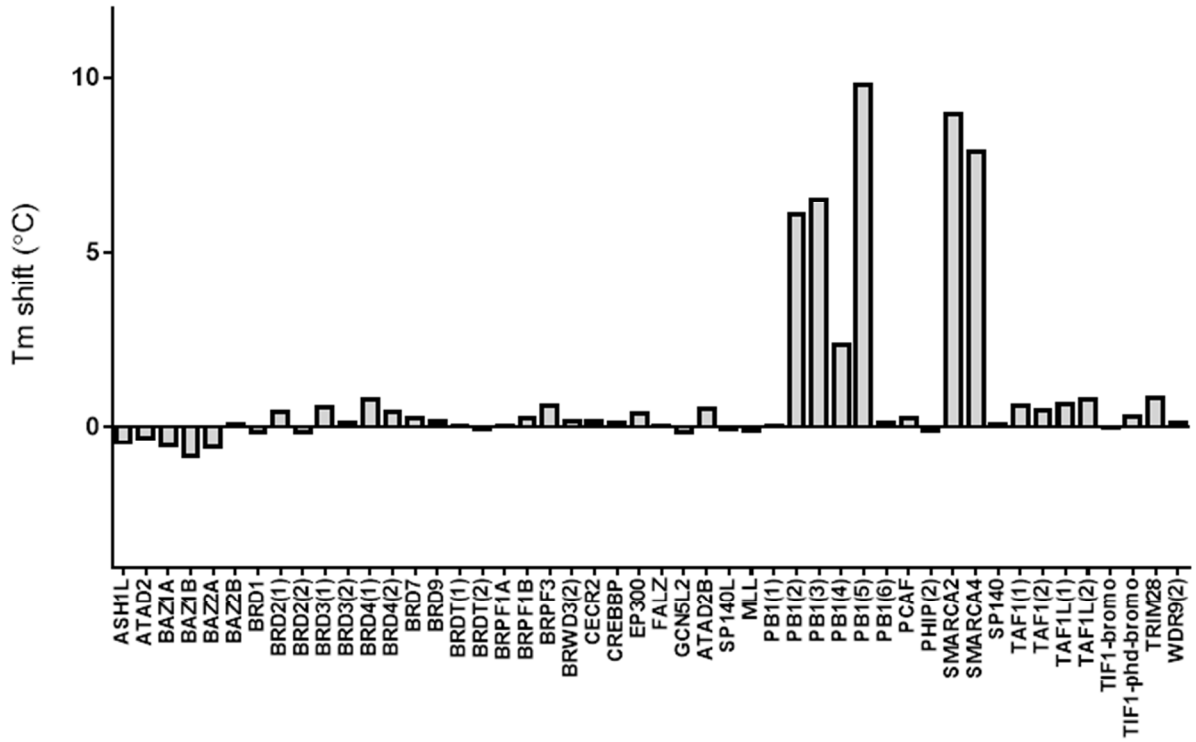


Figure F3

Figure F3: Graphical representation of bromodomain DSF panel screen for compound **17**

Protein	Delta Tm °C	St. Dev
ASH1L	-0.32	0.16
ATAD2	-0.49	0.26
BAZ1A	-0.16	0.56
BAZ1B	-0.29	0.78
BAZ2A	-0.23	0.50
BAZ2B	0.21	0.19
BRD1	0.17	0.42
BRD2(1)	0.62	0.27
BRD2(2)	0.11	0.34
BRD3(1)	0.49	0.12
BRD3(2)	0.24	0.18
BRD4(1)	0.80	0.04
BRD4(2)	0.28	0.24
BRD7	0.26	0.01
BRD9	-0.28	0.61
BRDT(1)	0.37	0.46
BRDT(2)	0.12	0.21
BRPF1A	0.13	0.13
BRPF1B	0.22	0.06
BRPF3	0.49	0.15
BRWD3(2)	0.28	0.17
CECR2	0.02	0.19
CREBBP	0.25	0.21
EP300	0.32	0.08
FALZ	0.31	0.38
GCN5L2	-0.01	0.16
ATAD2B	0.22	0.41
SP140L	0.20	0.32
MLL	-0.09	0.01
PB1(1)	-0.28	0.42
PB1(2)	5.85	0.31
PB1(3)	6.36	0.23
PB1(4)	2.08	0.38
PB1(5)	9.33	0.68
PB1(6)	0.33	0.27
PCAF	0.30	0.08
PHIP(2)	0.07	0.23
SMARCA2	8.47	0.71

SMARCA4	7.39	0.68
SP140	0.11	0.07
TAF1(1)	0.33	0.42
TAF1(2)	0.22	0.37
TAF1L(1)	0.23	0.62
TAF1L(2)	0.37	0.60
TIF1-bromo	-0.19	0.23
TIF1-phd-bromo	0.19	0.16
TRIM28	0.40	0.62
WDR9(2)	-0.05	0.26

Table T6

Table T6: Bromodomain DSF panel screen for compound **17**, raw data

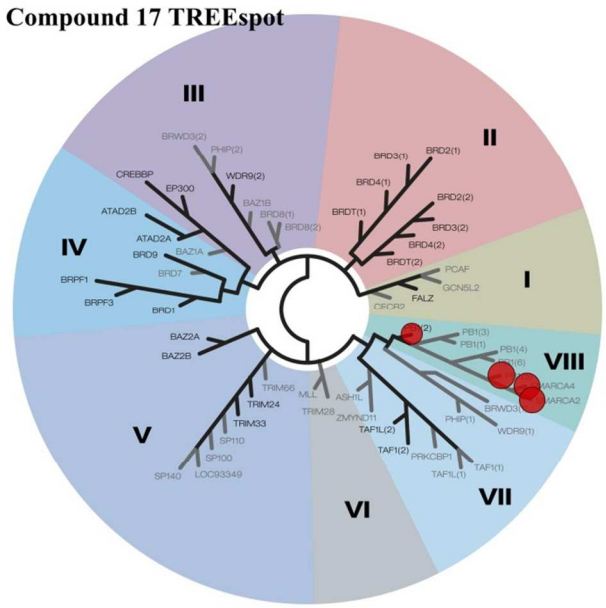


Figure F4

Figure F4: Compound **17** has increased PB1(2) activity compared to compound **16** as shown in this TREE*spot* image of its BROMOscan profile. Proteins in bold lines indicate compound was screened against it, proteins in grey indicate no bromodomain assay was run. Red dot indicates that compound **17** was sub-200nM Kd against that bromodomain. No dot indicates compound had a Kd of at least >20μM against the bromodomain.

Target	Compound 17 TREEspot
Gene Symbol	Kd (nM)
ATAD2A	>30000
ATAD2B	>30000
BAZ2A	>30000
BAZ2B	>30000
BRD1	>30000
BRD2(1)	>30000
BRD2(1,2)	>30000
BRD2(2)	>30000
BRD3(1)	>30000
BRD3(1,2)	>30000
BRD3(2)	>30000
BRD4(1)	>25000
BRD4(1,2)	>30000
BRD4(2)	>30000
BRD4(full-length,short-iso.)	>30000
BRD7	>30000
BRD8(1)	>30000
BRD8(2)	>30000
BRD9	>30000
BRDT(1)	>30000
BRDT(1,2)	>25000
BRDT(2)	>30000
BRPF1	>30000
BRPF3	>30000
CECR2	>30000
CREBBP	>30000
EP300	>30000
FALZ	>30000
GCN5L2	>30000
PBRM1(2)	170
PBRM1(5)	36
PCAF	>30000
SMARCA2	49
SMARCA4	46
TAF1(2)	>30000
TAF1L(2)	>30000
TRIM24(Bromo.)	>30000
TRIM24(PHD,Bromo.)	>30000
TRIM33(PHD,Bromo.)	>30000
WDR9(2)	>30000

Table T7

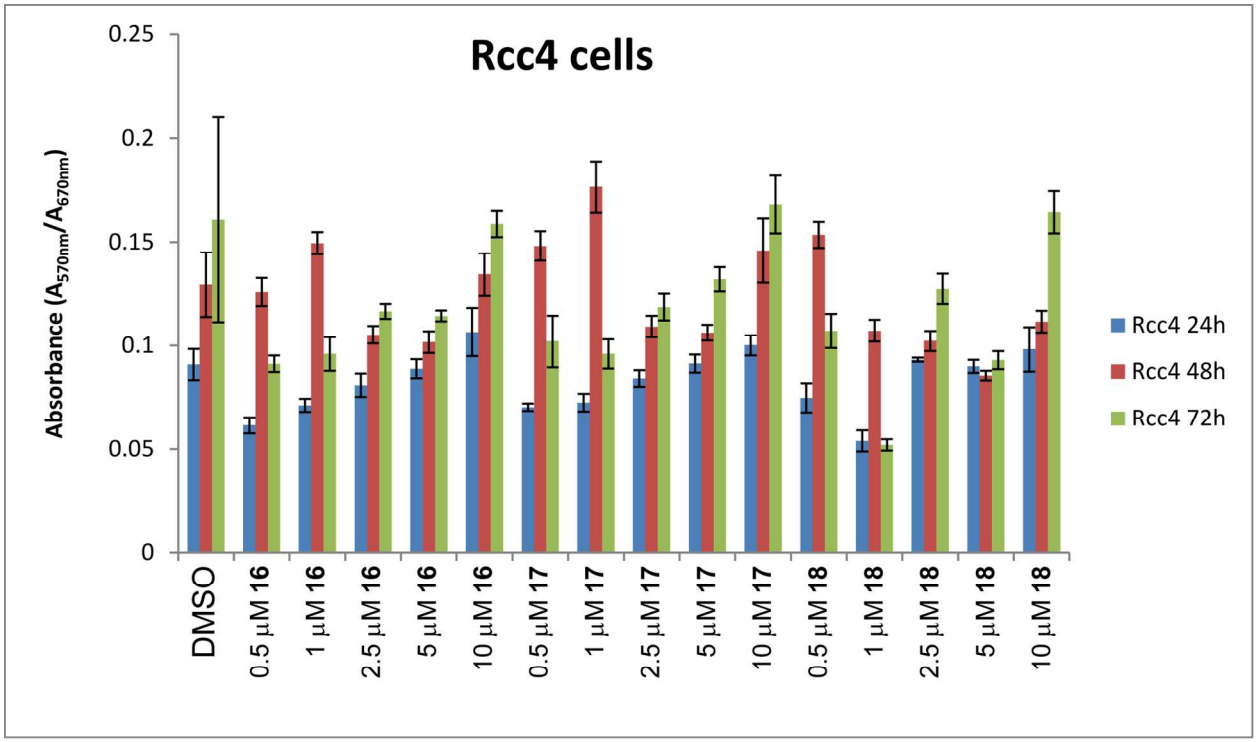
Table T7: Bromodomain BROMO*scan* panel screen for compound **17**, raw data

Compound	Protein	K_A (10^6 M^{-1})	K_D (nM)	N	ΔH (kcal/mol)	TΔS (kcal/mol)	ΔG (kcal/mol)
17	PB1 (2)	5.254 ± 0.237	190.33	0.829 ± 0.002	-7.855 ± 0.029	1.161	-9.016
	PB1 (3)	2.430 ± 0.287	411.52	0.857 ± 0.010	-8.946 ± 0.143	-0.381	-8.565
	PB1 (4)	0.72 ± 0.047	1396.64	0.931 ± 0.010	-6.403 ± 0.094	1.454	-7.857

	PB1 (5)	33.80 ± 1.670	29.58	0.922 ± 0.001	-14.510 ± 0.034	-4.426	-10.083
	SMARCA2	26.80 ± 1.960	37.31	0.929 ± 0.002	-13.280 ± 0.049	-3.313	-9.967
	SMARCA4	18.74 ± 1.630	53.36	0.926 ± 0.003	-12.000 ± 0.062	-2.243	-9.757

Table T8

Table T8: Dissociation constants and thermodynamic parameters from isothermal titration calorimetry assays for compound **17**


Rcc4 cells
Absorbance (A570nm/A670nm)
0.25
0.2
0.15
0.1
0.05
0
DMSO
0.5 µM 16
1 µM 16
2.5 µM 16
5 µM 16
10 µM 16
0.5 µM 17
1 µM 17
2.5 µM 17
5 µM 17
10 µM 17
0.5 µM 18
1 µM 18
2.5 µM 18
5 µM 18
10 µM 18
Rcc4 24h
Rcc4 48h
Rcc4 72h

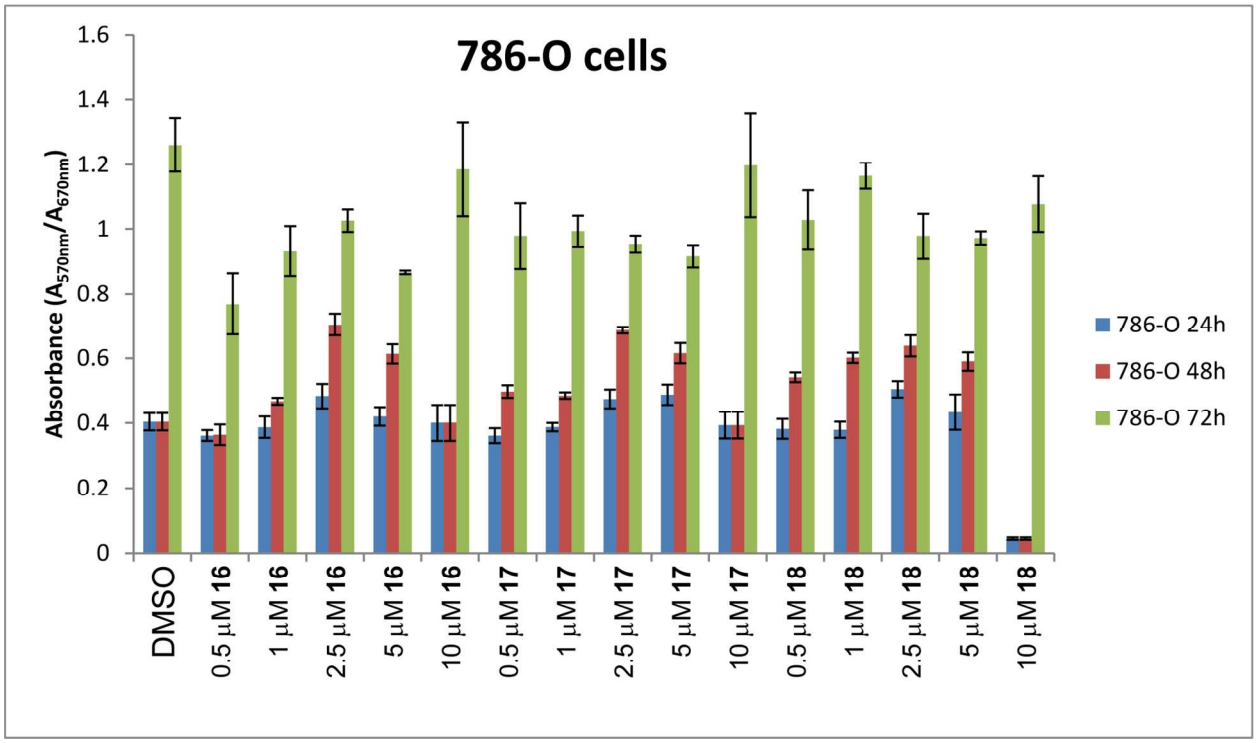

786-O cells
Absorbance (A570nm/A670nm)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
0.5 µM 16
1 µM 16
2.5 µM 16
5 µM 16
10 µM 16
0.5 µM 17
1 µM 17
2.5 µM 17
5 µM 17
10 µM 17
0.5 µM 18
1 µM 18
2.5 µM 18
5 µM 18
10 µM 18
786-O 24h
786-O 48h
786-O 72h

Figure F5

Figure F5: Representative image of MTT assay on 786-O and Rcc4 cell lines. Upon treatment with five different concentrations of compounds **16**, **17** and **18** no effect on cell proliferation was obesved in either cell line.

Data Collection			
PDB ID	5E7D	4Q0N	5DKH
Protein/Ligand	PB1(5)/**2**	PB1(5)/**3**	SMARCA2/**17**
Space group	$P2_1$	$P2_1$	$P3_1$
Cell dimensions: a, b, c (Å) α, β, γ (deg)	40.87 135.85 56.12 90.00 90.53 90.00	42.02 136.72 114.41 90.00 91.30 90.00	64.00 64.00 89.16 90.00 90.00 120.00
Resolution* (Å)	1.87 (1.97-1.87)	1.78 (1.88-1.78)	1.70 (1.73-1.70)
Unique observations*	44203 (5611)	119499 (17332)	45175 (2337)
Completeness* (%)	87.7 (76.7)	97.0 (96.6)	99.8 (96.8)
Redundancy*	4.4 (3.8)	2.6 (2.5)	10.4 (10.0)
Rmerge*	0.069 (0.632)	0.052 (0.484)	0.070 (1.325)
I/ σI*	12.1 (2.0)	11.8 (2.1)	18.6 (2.0)
Refinement			
Resolution (Å)	1.87	1.78	1.70
R_{work} / R_{free} (%)	20.3/24.8	17.6/21.4	19.9/24.1
Number of atoms (protein/other/water)	3636/60/232	7434/236/545	2792/68/177
B-factors (Å^2) (protein/other/water)	33.96/30.80/33.68	29.48/22.85/33.03	32.76/33.32/36.17
r.m.s.d bonds (Å)	0.015	0.015	0.016
r.m.s.d angles (^{o})	1.364	1.579	1.762
Ramachadran Favoured (%)	98.68	98.96	99.10
Allowed (%)	1.32	0.92	0.90
Disallowed (%)	0.00	0.12	0.00

* Values in parentheses correspond to the highest resolution shell.

Table T9

Table T9: Data collection and refinement statistics for protein crystallography

Supplementary Methods and Data References

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