SUPPORTING INFORMATION

Structure-based design of an *in vivo* active selective BRD9 inhibitor

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Synthesis of Compounds

List of abbreviations

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

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

General Methods

Unless otherwise indicated all reactions were carried out in standard commercially available glassware using standard synthetic chemistry methods. Air-sensitive and moisture-sensitive reactions were performed under an atmosphere of dry nitrogen or argon with dried glassware. Commercial starting materials were used without further purification. Solvents used for reactions were of commercial "dry"- or "extra-dry" or "analytical" grade. All other solvents used were reagent grade.

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

NMR experiments were recorded on Bruker Avance 400 MHz and 500 MHz spectrometers at 298K. Samples were dissolved in 600µL DMSO-d6 or $CDCl_3$ and TMS was added as an internal standard. 1D 1H spectra were acquired with 30° excitation pulses and an interpulse delay of 4.2 sec with 64k datapoints and 20 ppm sweep width. 1D 13C spectra were acquired

with broadband composite pulse decoupling (WALTZ16) and an interpulse delay of 3.3 sec with 64k datapoints and a sweep width of 240 ppm. Processing and analysis of 1D spectra was performed with Bruker Topspin 2.0 software. No zero filling was performed and spectra were manually integrated after automatic baseline correction. Chemical shifts are reported in ppm on the δ scale.

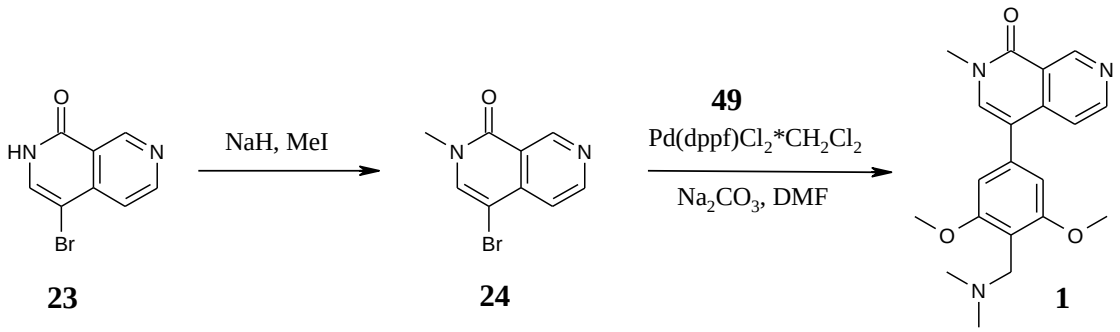
Analytical LC/MS data [**LC/MS(BAS1)**] were measured on an Agilent HPLC 1100 Series with Agilent LC/MSD SL detector using a Waters X-Bridge C18, 2.5 µm, 2.1x20mm column (Part.No. 186003201) and solvent A [20mM aqueous NH_4HCO_3/ NH_3 (pH 9)] and solvent B [acetonitrile HPLC grade] as eluent (additional settings: flow 1mL/min; injection volume 5 µl; column temp. 60 °C). Standard gradient: 0.00 min:10 % B; 0.00 – 1.50 min: 10 % -> 95 % B; 1.50 – 2.00 min: 95 % B; 2.00 – 2.10 min: 95 % -> 10 % B. For some intermediates analytical LC/MS data was measured using different methods: **LC/MS(31)** was measured on a Shamadzu HPLC LC-20AB, SPD-M20A 190-370 nm system using a Luna C18(2), 5 µm, 50x2 mm column and solvent A [H_2O containing 0.0375 % TFA] and solvent B [acetonitrile HPLC grade containing 0.018 % TFA] as eluent (additional settings: flow 0.8 mL/min, column temp. 40 °C). Standard gradient: 0.00 min:10 % B; 0.00 – 4.00 min: 10 % -> 80 % B; 4.00 – 4.90 min: 80 % B; 4.90 – 4.92 min: 80 % B -> 10 % B; 4.92 – 5.50 min: 10 % B. **LC/MS(32)** was measured on an Agilent HPLC 1200 Series (DAD 200-400 nm) with an Agilent 6120 MS system using a Luna C18(2), 3 µm, 30x2mm column and solvent A [H_2O containing 0.0375 % TFA] and solvent B [acetonitrile HPLC grade containing 0.018 % TFA] as eluent (additional settings: flow 1.0 mL/min, column temp. 50 °C). Standard gradient: 0.00 min:10 % B; 0.00 – 1.15 min: 10 % -> 80 % B; 1.15 – 1.55 min: 80 % B; 1.55 – 1.56 min: 80 % B -> 10 % B; 1.56 – 2.99 min: 10 % B. **LC/MS(45**) was measured on an Agilent HPLC 1100/1200 Series (DAD 200-400 nm) with an Agilent LC/MSD SL MS system using a Waters X-Bridge C18 , 2.5 µm, 2.1 x 30 mm column and solvent A [0,1% NH_4HCO_3/0,1% NH_3 in H_2O] and solvent B [acetonitrile HPLC grade] as eluent (additional settings: flow 1.4 mL/min, column temp. 45 °C). Standard gradient: 0.00 – 1.00 min: 10 % -> 95 % B; 1.00 – 1.30 min: 95 % B. **LC/MS(51**) and **LC/MS(52**) were measured on an Agilent HPLC 1100/1200 Series (DAD 200-400 nm) with an Agilent LC/MSD SL MS system using a YMC Triart C18, 3.0 µm, 2.0 x 30 mm column and solvent A [0.1% formic acid in water] and solvent B [0.1% formic acid in Acetonitrile (HPLC grade)] as eluent (additional settings: flow 1.4 mL/min, column temp. 45 °C). Standard gradient: 0.00 – 1.00 min: 15 % -> 100 % B; 1.00 – 1.1 min: 100 % B.

HRMS data were recorded using a Thermo Scientific Orbitrap Elite Hybrid Ion Trap/Orbitrap Spectrometer system with an Ultimate 3000 Series LPG-3400XRS Pump system. The mass calibration was performed using the Pierce LTQ Velos ESI positive ion calibration solution from Thermo Scientific (Lot PF200011, Product Nr. 88323).

All biologically evaluated compounds exist in >95% purity as shown by LC/MS, additionally for **1** and **2** purity > 95% was shown by Q-NMR.

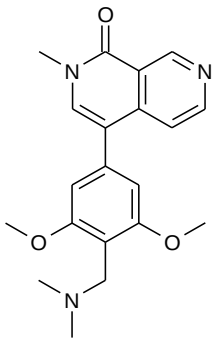
Compound **3** is commercially available from e.g. ChemDiv.

Synthesis of compound 1[1]



Supplementary Scheme 14: Synthesis of compound (**1**)

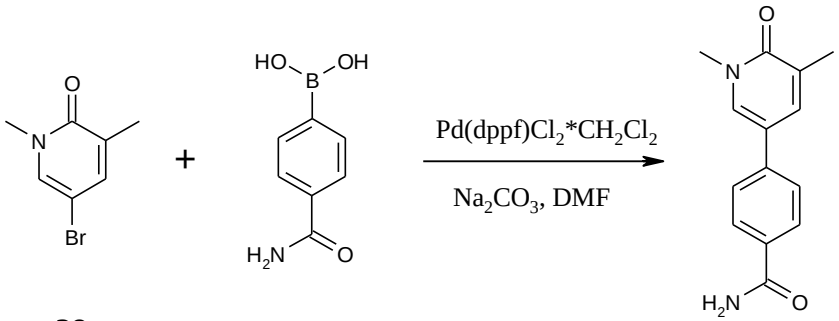
4-{4-[(dimethylamino)methyl]-2,6-dimethoxyphenyl}-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (1)



24 (200 mg; 837 µmol) and {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**49**) (375 mg; 1.26 mmol) and Pd(dppf)Cl_2·DCM (70.4 mg; 86.2 µmol) are suspended in DMF (2.0 mL) under argon. A degassed Na_2CO_3-solution (2N; 1.05 mL; 2.10 mmol) is subsequently added and the resulting mixture is heated at 80 °C for 1 h. After cooling to rt, DMF is evaporated and a the residue is purified by flash chromatography on silica gel using a DCM/MeOH gradient as eluent (0:100 --> 90:10; Meoh made basic with 0.1% NH_3) to give pre-purified material. Subsequent preparative RP-HPLC

chromatography yields highly pure 4-{4-[(dimethylamino)methyl]-2,6-dimethoxy-phenyl}-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (**1**) (210 mg; 594 µmol; 71 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.44 (s, 1H), 8.72 (d, J = 5.7 Hz, 1H), 7.86 (s, 1H), 7.56 (d, J = 5.7 Hz, 1H), 6.72 (s, 2H), 3.80 (s, 6H), 3.60 (s, 3H), 3.46 (s, 2H), 2.13 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ 160.9, 159.4 (2C), 151.4, 150.9, 141.5, 138.3, 135.5, 120.2, 117.8, 116.5, 113.9, 105.8 (2C), 56.3 (2C), 50.0, 45.5 (2C), 36.9; HRMS (CI+): calculated for $C_{20}H_{24}N_3O_3$ (MH+) 354.1812, found 354.1808, Δ - 1.1 ppm; LC/MS (BAS1): $[M+H]^+$ = 354; t_R = 0.91 min.

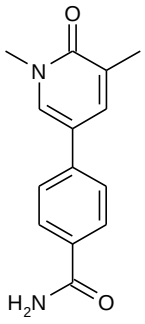
Synthesis of compound 4



28 **29** **4**

Supplementary Scheme 1: Synthesis of compound (**4**)

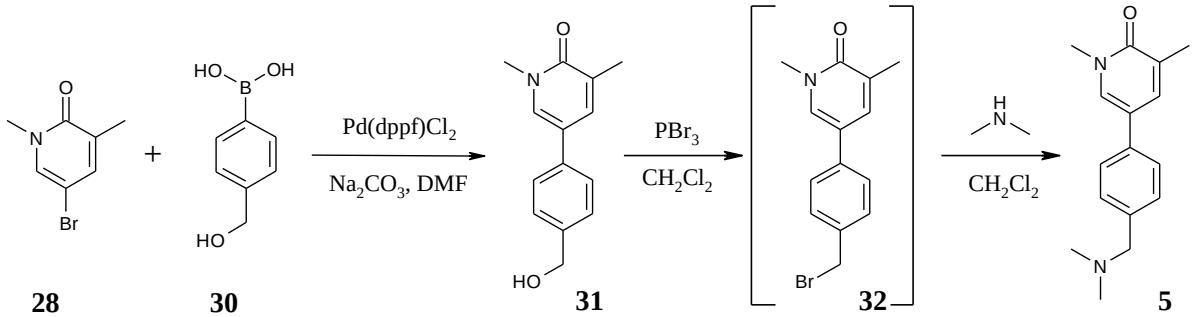
4-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)benzamide (4)



5-bromo-1,3-dimethyl-1,2-dihydropyridin-2-one (**28**) (83.3 mg; 412 µmol; commercial from AlfaAesar) and (4-carbamoylphenyl)boronic acid (**29**) (66.0 mg; 400 µmol; commercial from ABCR) and Pd(dppf)Cl_2·DCM (32.7 mg; 40.0 µmol) are suspended in DMF (800 µL) under argon. A degassed Na_2CO_3-solution (2N; 500 µL; 1.00 mmol) is subsequently added and the resulting mixture is heated at 100 °C for 3 h. After cooling to rt, water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: Sunfire C-18

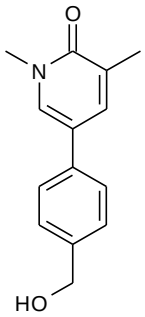
30x50 mm) using a MeCN/water gradient under acidic conditions. The product containing fractions are freeze dried. Further purification is achieved by automated silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 90:10; MeOH). The product containing fractions are evaporated, dissolved in MeCN/water and freeze dried to give 4-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)benzamide (**4**) (38.6 mg; 159 µmol; 40 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.11 (s, 1H), 7.99 (s, 1H), 7.92 (d, J = 7.9 Hz, 2H), 7.82 (s, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.34 (s, 1H), 3.53 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 167.9, 162.2, 139.5, 136.0, 135.6, 132.6, 128.6 (2C), 128.3, 125.1 (2C), 116.7, 37.8, 17.5; HRMS (CI+): calculated for $C_{14}H_{15}N_2O_2$ (MH+) 243.11280, found 243.11235, Δ -1.85 ppm; LC/MS (BAS1): $[M+H]^+$ = 243; t_R = 0.39 min.

Synthesis of compound 5



Supplementary Scheme 2: Synthesis of compound (**5**)

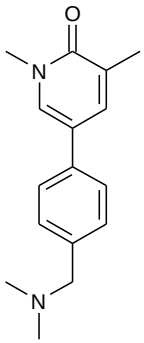
5-[4-(hydroxymethyl)phenyl]-1,3-dimethyl-1,2-dihydropyridin-2-one (31)



5-bromo-1,3-dimethyl-1,2-dihydropyridin-2-one (**28**) (9.70 g; 48.0 mmol; commercial from AlfaAesar), [4-(hydroxymethyl)phenyl]boronic acid (**30**) (10.9 g; 71.7 mmol; commercial from ABCR) and Cs_2CO_3 (46.9 g; 144 mmol) are dissolved in a 5:1 mixture of 1,4-dioxane

and water (200 mL). Pd(dppf)Cl_2 (3.84 g; 5.25 mmol) is added under an inert atmosphere (argon) and the mixture is heated at 90 °C for 12 h. After cooling, 1,4-dioxane is removed under reduced pressure, water is added and the mixture is extracted three times with DCM. The combined organic layer is washed with brine, dried over Na_2SO_4, filtered and evaporated. The crude product is purified by flash chromatography on SiO_2 using a PE/EA gradient (5:1 --> 2:1). The product containing fractions are evaporated to give pure 5-[4-(hydroxymethyl)phenyl]-1,3-dimethyl-1,2-dihydropyridin-2-one (**31**) (4.40 g; 19.2 mmol; 40 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.99 (s, 1H), 7.74 (s, 1H), 7.53 (d, J = 8.3 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 5.21 (s, 1H), 4.51 (s, 2H), 3.52 (s, 3H), 2.09 (s, 3H); LC/MS (BAS1): $[M+H]^+$ = 230; t_R = 0.69 min.

5-{4-[(dimethylamino)methyl]phenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (5)

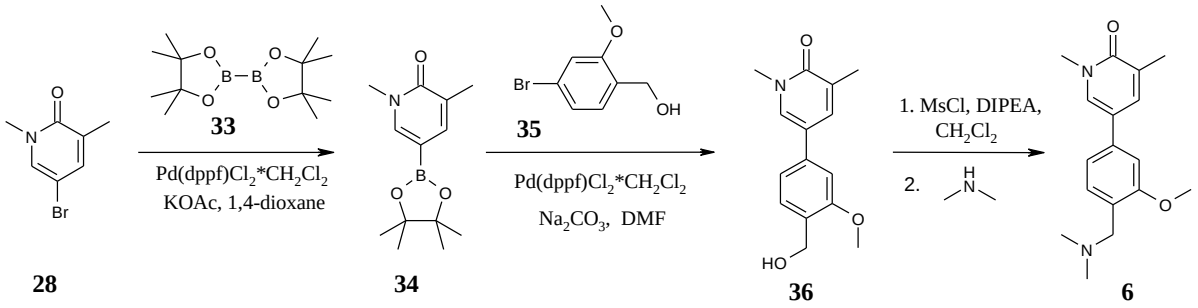


31 (500 mg; 2.18 mmol) is dissolved in DCM (10 mL) and cooled to 0 °C in an ice bath. Phosphorous tribromide (295 mg; 1.09 mmol) is added dropwise and the resulting mixture is stirred for 30 min. at 0 °C. After completion, water is added and the mixture is extracted three times with DCM. The combined aqueous layer is washed with brine, dried over Na_2SO_4, filtered and evaporated to give crude 5-[4-(bromomethyl)phenyl]-1,3-dimethyl-1,2-dihydropyridin-2-one (**32**), which is used without further purification.

Crude **32** is dissolved in DCM (5 mL). Dimethylamine (5.45 mL; 10.9 mmol, 2M solution in MeOH) is added and the resulting mixture is stirred at rt for 12 h. All solvents are removed under reduced pressure and the residue is taken-up in a small amount of MeCN and purified by preparative RP HPLC. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]phenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**5**) (40.0 mg; 156 µmol; 7.2 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.97 (s, 1H), 7.73 (s, 1H), 7.52 (d, J = 7.8 Hz, 2H), 7.31 (d, J = 7.8 Hz, 2H), 3.52 (s, 3H), 3.39 (s, 2H), 2.15 (s, 6H), 2.08 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 162.1, 137.9, 136.3, 135.4, 134.7, 129.7 (2C), 128.1, 125.6 (2C),

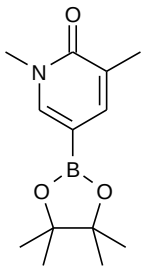
117.5, 63.5, 45.4 (2C), 37.7, 17.5; HRMS (CI+): calculated for $C_{16}H_{21}N_2O$ (MH+) 257.16483, found 257.16484, Δ – 0.1 ppm; LC/MS (BAS1): $[M+H]^+$ = 257; t_R = 0.93 min.

Synthesis of compound 6



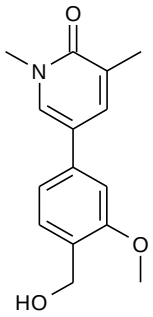
Supplementary Scheme 3: Synthesis of compound (**6**)

1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (34)



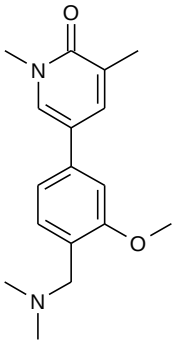
5-bromo-1,3-dimethyl-1,2-dihydropyridin-2-one (**28**) (10.0 g; 49.5 mmol), bis(pinacolato)-diboron (**33**) (17.2 g; 67.7 mmol, commercial from CombiBlocks), Pd(dppf)Cl_2·DCM (2.00 g; 2.45 mmol) and potassium acetate (9.42 g; 96.0 mmol) are suspended in 1,4-dioxane (100 mL) under argon and the resulting mixture is heated at 90 °C for 3 h. After cooling to rt the reaction mixture is filtered through Celite and washed with 1,4-dioxane (2x 100 mL). The filtrate is concentrated under reduced pressure, the residue is taken-up in DCM (200 mL) and washed with water (100 mL). The organic layer is dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product is purified by silica gel chromatography (Combiflash; column: Redisep RF, 330g) using a cHex/EA gradient as eluent (100:0 --> 0:100). The product containing fractions are evaporated to give 1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**34**) (8.35 g; 33.5 mmol; 68 %) as a dark yellow oil, which slowly crystallizes. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.87 (s, 1H), 7.39 (s, 1H), 3.46 (s, 3H), 1.98 (s, 3H), 1.26 (s, 12H); LC/MS (BAS1): $[M+H]^+$ = 250; t_R = 0.87 min.

5-[4-(hydroxymethyl)-3-methoxyphenyl]-1,3-dimethyl-1,2-dihydropyridin-2-one (36)



(4-bromo-2-methoxyphenyl)-methanol (**35**) (1.05 g 4.84 mmol; commercial from Aldrich), **34** (1.20 g; 4.82 mmol) and Pd(dppf)Cl_2·DCM (393 mg; 481 µmol) are suspended in DMF (5 mL) under argon. A degassed solution of sodium carbonate (2N; 6.02 mL; 12.0 mmol) is added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt water is added and the mixture is extracted three times with DCM (30 mL each). The combined organic layer is dried over Na_2SO_4, filtered and evaporated. The crude product is purified by silica gel chromatography (Combiflash; column: Redisep RF, 40g) using a cHex/EA gradient as eluent (100:0 --> 0:100). The product containing fractions are evaporated to give 5-[4-(hydroxymethyl)-3-methoxyphenyl]-1,3-dimethyl-1,2-dihydropyridin-2-one (**36**) (0.97 g; 3.74 mmol; 77 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.00 (d, J = 2.5 Hz, 1H), 7.77 (d, J = 2.6 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.14 (dd, J = 7.9, 1.7 Hz, 1H), 7.10 (d, J = 1.7 Hz, 1H), 4.98 (t, J = 5.5 Hz, 1H), 4.50 (d, J = 5.5 Hz, 2H), 3.86 (s, 3H), 3.53 (s, 3H), 2.10 (s, 3H); LC/MS (BAS1): $[M+H]^+$ = 260; t_R = 0.95 min.

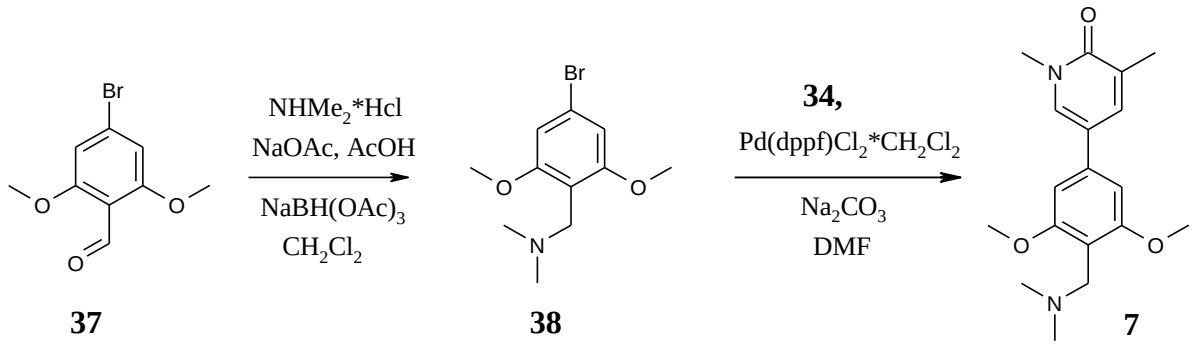
5-{4-[(dimethylamino)methyl]-3-methoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (6)



36 (100 mg; 386 µmol) is dissolved in DCM (1.0 mL). DIPEA (149 mg; 1.16 mmol) and methansulfonyl chloride (66.2 mg; 578 µmol) is added drop wise and the mixture is stirred at rt for 16 h. Dimethylamine hydrochloride (94.6 mg; 1.16 mmol) is added and stirring is

continued for 8 h. The reaction mixture is concentrated under reduced pressure, dissolved in DMSO (1.0 mL) and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried. Further purification is achieved by preparative RP-HPLC (column: Sunfire C-18 30x50 mm) using a MeCN/water gradient under acidic conditions. The product containing fractions are freeze dried to give pure 5-{4-[(dimethylamino)methyl]-3-methoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**6**) (18.1 mg; 63.2 µmol; 16 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.32 (s, 1H, ammonium ion), 8.12 (d, J = 2.7 Hz, 1H), 7.86 – 7.81 (m, 1H), 7.44 (d, J = 7.8 Hz, 1H), 7.29 (d, J = 1.8 Hz, 1H), 7.25 (dd, J = 7.9, 1.7 Hz, 1H), 4.25 (s, 2H), 3.95 (s, 3H), 3.54 (s, 3H), 2.74 (s, 6H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, DMSO, 1 C missing) δ 162.2, 158.8, 140.2, 136.2, 135.6, 133.5, 128.2, 117.7, 116.8, 108.6, 56.4, 55.3, 42.7 (2C), 37.8, 17.5; HRMS (CI+): calculated for $C_{17}H_{23}N_2O_2$ (MH+) 287.17540, found 287.17523, Δ -0.60 ppm; LC/MS (BAS1): $[M+H]^+$ = 287; t_R = 0.91 min.

Synthesis of compound 7



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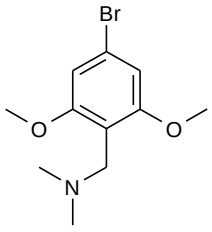
Supplementary Scheme 4: Synthesis of compound (**7**)

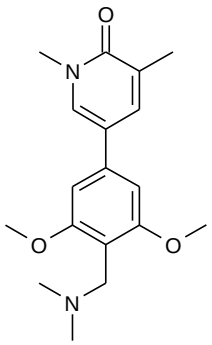
[(4-Bromo-2,6-dimethoxyphenyl)methyl]dimethylamine (38)



A mixture of NaOAc (17.7 g; 216 mmol), AcOH (8.65 g; 144 mmol) and dimethylamine hydrochloride (17.6 g; 216 mmol) in DCM (600 mL) is stirred for 10 min at rt. 4-Bromo-2,6-

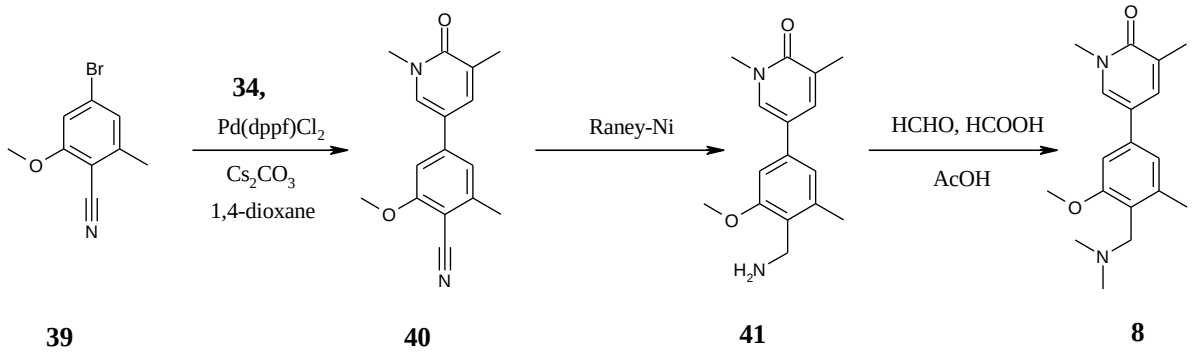
dimethoxybenzaldehyde (**37**) (35.3 g; 144 mmol, commercial from Activate) is added and stirring is continued. After 30 min sodium triacetoxyborohydride (63.1 g; 298 mmol) is added in one portion and the reaction mixture is stirred at rt for 16 h. Saturated $NaHCO_3$ solution is added and the layers are separated. The aqueous layer is extracted three times with DCM. The combined organic layer is dried over $MgSO_4$, filtered and evaporated to give pure [(4-bromo-2,6-dimethoxyphenyl)methyl]dimethylamine (**38**) (27.3 g; 99.6 mmol; 69 %). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.70 (s, 2H), 3.81 (s, 6H), 3.48 (s, 2H), 2.26 (s, 6H); LC/MS (BAS1): $[M+H]^+$ = 274/276; t_R = 1.11 min.

5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (7)



38 (110 mg; 401 µmol), 1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**34**) (100 mg; 401 µmol) and Pd(dppf)Cl_2·DCM (32.7 mg; 40.1 µmol) are suspended in DMF (800 µL) under argon. A degassed solution of sodium carbonate (2N, 500 µL; 1.00 mmol) is added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt, water is added and the mixture is purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**7**) (48.3 mg; 153 µmol; 38 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.04 (d, J = 2.8 Hz, 1H), 7.81 (dd, J = 2.6, 1.3 Hz, 1H), 6.79 (s, 2H), 3.83 (s, 6H), 3.54 (s, 3H), 3.39 (s, 2H), 2.10 (s, 3H), 2.09 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ 162.2, 159.6 (2C), 137.4, 136.5, 135.1, 127.9, 118.0, 112.9, 101.6 (2C), 56.3 (2C), 49.9, 45.4 (2C), 37.7, 17.5; HRMS (CI+): calculated for $C_{18}H_{25}N_2O_3$ (MH+) 317.18597, found 317.18558, Δ -1.26 ppm; LC/MS (BAS1): $[M+H]^+$ = 317; t_R = 0.88 min.

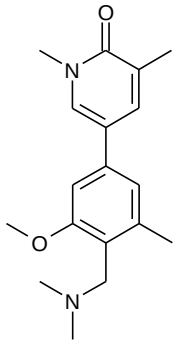
Synthesis of compound 8



39 **40** **41** **8**

Supplementary Scheme 5: Synthesis of compound (**8**)

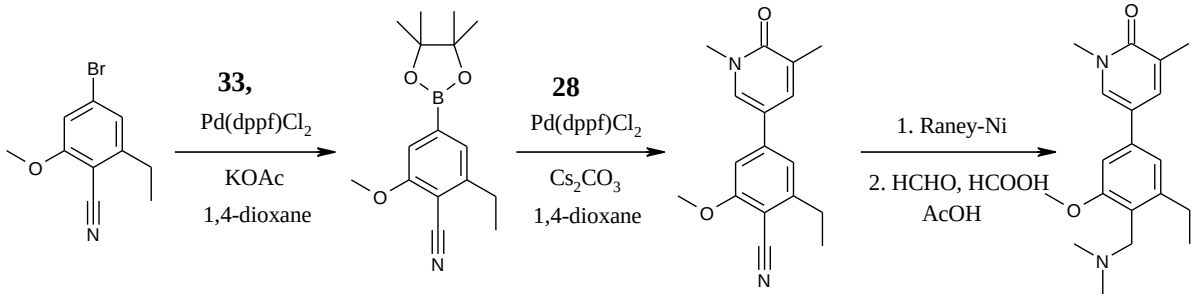
5-{4-[(dimethylamino)methyl]-3-methoxy-5-methylphenyl}-1,3-dimethyl-1,2-dihydro-pyridin-2-one (8)



To a solution of 4-bromo-2-methoxy-6-methyl-benzonitrile (**39**) (1.00 g; 4.42 mmol; commercial from ArkPharm) and 1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**34**) (1.10 g; 4.42 mmol) in 1,4-dioxane (50 mL) and water (2 mL) is added cesium carbonate (4.50 g; 13.9 mmol) and Pd(dppf)Cl_2 (200 mg; 0.27 mmol). The reaction mixture is stirred at 90 °C for 2 h. The reaction mixture is then filtered through celite. The filtrate is concentrated, dissolved in EA and washed with brine. The combined organic layer is dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue is purified by preparative RP-HPLC using a MeCN/water gradient as eluent to give 4-(1,5-dimethyl-6-oxo-1,6-dihydro-pyridin-3-yl)-2-methoxy-6-methylbenzonitrile (**40**) (480 mg; 1.79 mmol; 40 %) which is directly used for the next step. LC/MS(**40**): (M+H)+ =269; t_R = 1.16 min. To a solution of **40** (200 mg; 745 µmol) in MeOH (15 mL) is added aqueous ammonia (500 µL) and Raney-Ni (200 mg). The mixture is degassed and refilled with H_2 twice and stirred at rt under 50 Psi for 16 h. The reaction mixture is filtered and the residue is washed with THF/MeOH. The filtrated is then concentrated to give 5-{4-[(dimethylamino)methyl]-3-methoxy-5-methylphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**41**) (150 mg; 550 µmol; 74 %), which is directly used in the next step. LC/MS(**41**):

(M+H)+ =273; t_R = 0.8 min. A mixture of **41** (50.0 mg; 184 µmol), aqueous formaldehyde (50 µL), formic acid (50 µL) and acetic acid (1.0 mL) is heated at reflux for 16 h. After cooling to rt, the reaction mixture is concentrated under reduced pressure and the residue is dissolved in MeOH and purified by preparative RP-HPLC using a McCN/water gradient as eluent. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]-3-methoxy-5-methylphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**8**) (28.0 mg; 93.2 µmol; 51 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.38 (s, 1H, prot. amine), 8.11 (s, 1H), 7.82 (s, 1H), 7.14 (s, 1H), 7.12 (s, 1H), 4.26 (d, J = 5.6 Hz, 2H), 3.93 (s, 3H), 3.54 (s, 3H), 2.72 – 2.78 (m, 6H), 2.44 (s, 3H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 162.2, 159.2, 140.8, 139.2, 136.2, 135.5, 128.1, 120.1, 116.9, 115.9, 106.0, 56.5, 52.5, 42.9 (2C), 37.8, 20.0, 17.5; HRMS (CI+): calculated for $C_{18}H_{25}N_2O_2$ (MH+) 301.19105, found 301.19069, Δ -1.21 ppm; LC/MS (BAS1): [M+H]+ = 301; t_R = 1.02 min.

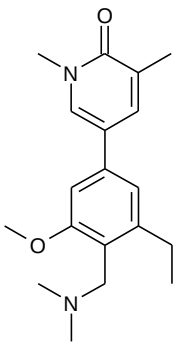
Synthesis of compound 9



42 **43** **44** **9**

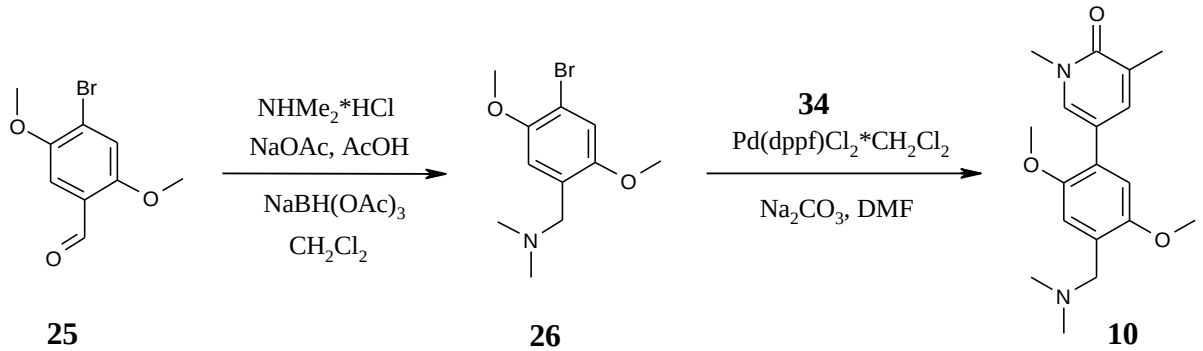
Supplementary Scheme 6: Synthesis of compound (**9**)

5-{4-[(dimethylamino)methyl]-3-ethyl-5-methoxyphenyl}-1,3-dimethyl-1,2-dihydro-pyridin-2-one (9)



To a solution of 4-bromo-2-ethyl-6-methoxybenzonitrile (**42**) (480 mg; 2.00 mmol; commercial from FCHGroup) and bis(pinacolato)diboron (**33**) (558 mg; 2.20 mmol) in 1,4-dioxane (9.0 mL) is added Pd(dppf)Cl_2 (146 mg; 0.20 mmol) and potassium acetate (588 mg; 6.00 mmol) and the mixture is refluxed for 24 h. After cooling to rt, EA and water is added, the layers are separated and the aqueous layer is extracted three times with EA. The combined organic layer is dried over Na_2SO_4, filtered and evaporated. The crude material is purified by silica gel chromatography to give 2-ethyl-6-methoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)benzonitrile (**43**) (570 mg; 1.98 mmol; 99 %) which is directly used for the next step. LC/MS(**43**): (M+H)+ =288; t_R = 1.86 min. To a solution of **43** (570 mg; 1.99 mmol) and 5-bromo-1,3-dimethyl-1,2-dihydropyridin-2-one (**28**) (440 mg; 2.18 mmol) in 1,4-dioxane (20 mL) and water (1.0 mL) is added Cs_2CO_3 (2.00 g; 6.15 mmol) and Pd(dppf)Cl_2 (100 mg; 137 µmol). The mixture is heated to 90 °C for 2 h. The reaction is filtered over a pad of celite and the filtrate is concentrated. The residue is dissolved in EA, washed with brine. The combined organic layer is dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material is purified by preparative RP-HPLC to give 4-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-2-ethyl-6-methoxybenzonitrile (**44**) (350 mg; 1.24 mmol; 62 %), which is directly used for the next step. LC-MS(**44**): $(M+H)^+$ = 283; t_R = 1.36 min. To a solution of **44** (350 mg; 1.24 mmol) in MeOH (20.0 mL) is added aq. NH_3 (1.0 mL) and Raney nickel (300 mg). The mixture is degassed and refilled with H_2 twice and stirred under 50 Psi at rt for 16 h. The reaction mixture is filter, washed with THF/MeOH and concentrated to give 5-[4-(aminomethyl)-3-ethyl-5-methoxyphenyl]-1,3-dimethyl-1,2-dihydropyridin-2-one, which is without further purification dissolved in AcOH (2.0 mL). Aqueous formaldehyde (100 µl) and formic acid (100 µL) is added and the mixture is heated to reflux for 24 h. After cooling to rt the reaction mixture is evaporated, taken-up in MeOH and purified by preparative RP-HPLC to give 5-{4-[(dimethylamino)methyl]-3-ethyl-5-methoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**9**) (32.8 mg; 104 µmol; 8.4 % for last two steps). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.15 (s, 1H; prot. amine), 8.12 (s, 1H), 7.83 (s, 1H), 7.15 (s, 1H), 7.12 (s, 1H), 4.28 (d, J = 5.8 Hz, 2H), 3.93 (s, 3H), 3.54 (s, 3H), 2.73 – 2.79 (m, 8H), 2.10 (s, 3H), 1.18 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO) δ 162.2, 159.1, 146.6, 139.5, 136.2, 135.5, 128.1, 118.4, 117.0, 115.1, 106.0, 56.5, 52.1, 42.9 (2C), 37.7, 25.8, 17.5, 16.3; HRMS (CI+): calculated for $C_{19}H_{27}N_2O_2$ (MH+) 315.20670, found 315.20627, Δ -0.43 ppm; LC/MS (BAS1): [M+H]+ = 315; t_R = 1.09 min.

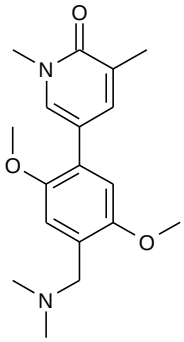
Synthesis of compound 10



25 **26** **10**

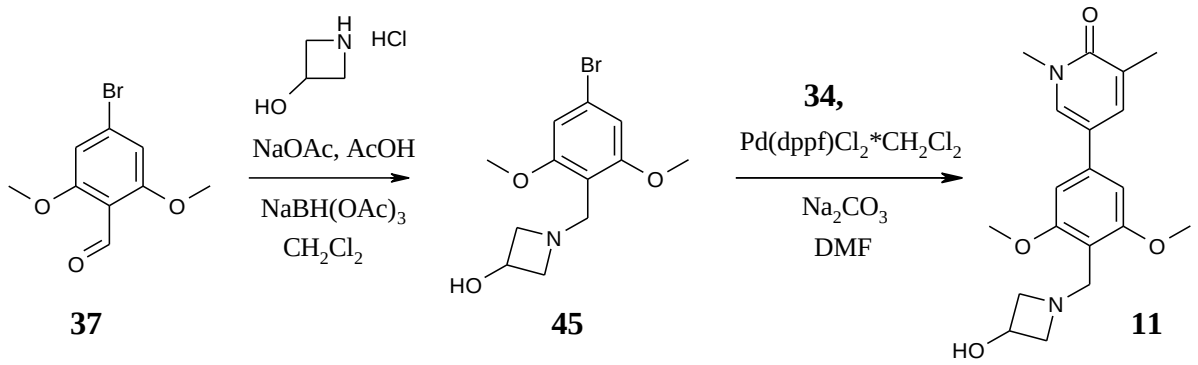
Supplementary Scheme 7: Synthesis of compound (**10**)

5-{4-[(dimethylamino)methyl]-2,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (10)



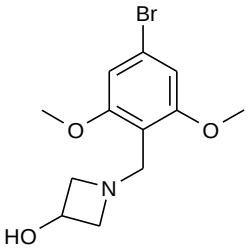
26 (120 mg; 438 µmol), 1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**34**) (120 mg; 482 µmol), and Pd(dppf)Cl_2·DCM (36.9 mg; 45.2 µmol) are suspended in DMF (1.0 mL) under argon. A degassed solution of sodium carbonate (2N, 438 µL; 876 µmol) is added and the resulting mixture is heated at 80 °C for 1 h. After cooling to rt water is added and the mixture is purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]-2,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**10**) (48.0 mg; 152 µmol; 35 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.73 (s, 1H), 7.53 (s, 1H), 7.02 (s, 1H), 6.88 (s, 1H), 3.77 (s, 3H), 3.72 (s, 3H), 3.49 (s, 3H), 3.39 (s, 2H), 2.18 (s, 6H), 2.05 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 161.9, 151.8, 150.4, 139.1, 136.6, 127.0, 126.8, 124.9, 115.7, 114.2, 113.1, 57.0, 56.6, 56.5, 45.7 (2C), 37.6, 17.5; HRMS (CI+): calculated for $C_{18}H_{25}N_2O_3$ (MH+) 317.18597, found 317.18577, Δ -0.62 ppm; LC/MS (BAS1): [M+H]+ = 317; t_R = 0.98 min.

Synthesis of compound 11



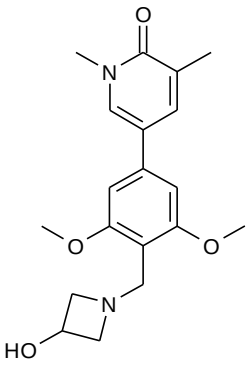
Supplementary Scheme 8: Synthesis of compound (**11**)

1-[(4-bromo-2,6-dimethoxyphenyl)methyl]azetidin-3-ol (45)



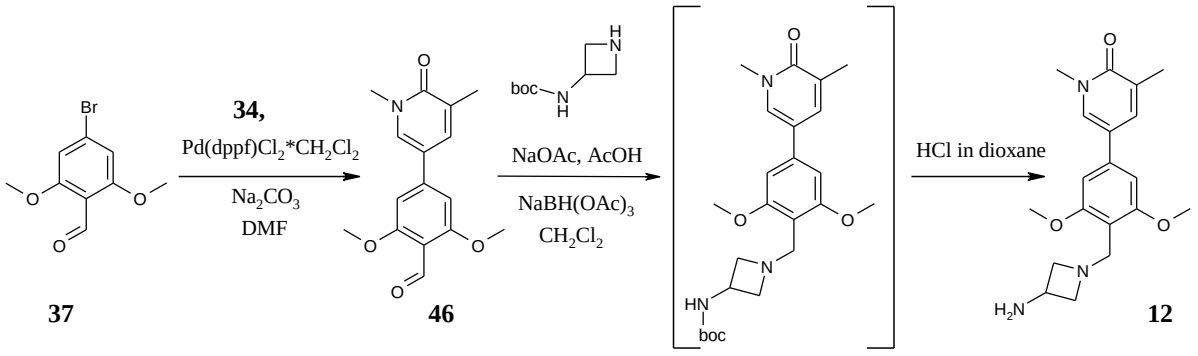
A mixture of NaOAc (95.4 mg; 1.16 mmol), AcOH (46.6 mg; 776 µmol) and azetidin-3-ol (134 mg; 1.22 mmol) in DCM (2.0 mL) is stirred for 10 min at 0 °C. 4-Bromo-2,6-dimethoxybenzaldehyde (**37**) (200 mg; 816 µmol) is added and stirring is continued. After 30 min sodium triacetoxyborohydride (339 mg; 1.60 mmol) is added in one portion and the reaction mixture is stirred at rt for 16 h. Saturated $NaHCO_3$ solution is added and the layers are separated. The aqueous layer is extracted three times with DCM. The combined organic layer is dried over $MgSO_4$, filtered and evaporated to give 1-[(4-bromo-2,6-dimethoxyphenyl)methyl]azetidin-3-ol (**45**) (220 mg; 99.6 mmol; 89 %). LC/MS(45): $[M+H]^+$ = 302/304; t_R = 0.49 min

5-{4-[(3-hydroxyazetidin-1-yl)methyl]-3,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (11)



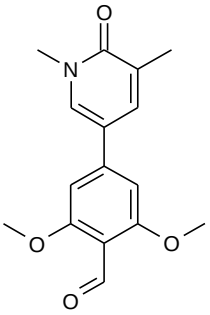
45 (80.0 mg; 264 µmol), 1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**34**) (100 mg; 401 µmol) and Pd(dppf)Cl_2·DCM (21.6 mg; 26.4 µmol) are suspended in DMF (800 µL) under argon. A degassed solution of sodium carbonate (2N; 331 µL; 662 µmol) is added and the resulting mixture is heated to 100 °C for 1 h. After cooling to rt water is added and the mixture is purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 5-{4-[(3-hydroxyazetidin-1-yl)methyl]-3,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**11**) (22.7 mg; 65.9 µmol; 25 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.04 (d, J = 2.7 Hz, 1H), 7.81 (s, 1H), 6.79 (s, 2H), 5.12 (d, J = 6.4 Hz, 1H), 3.98 – 4.06 (m, 1H), 3.83 (s, 6H), 3.51 – 3.55 (m, 5H), 3.31 (s, 3H), 2.79 (ddd, J = 6.2, 6.2, 2.2 Hz, 2H), 2.10 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 162.2, 159.4 (2C), 137.4, 136.5, 135.1, 127.9, 118.0, 112.3, 101.6 (2C), 63.4 (2C), 61.1, 56.2 (2C), 48.2, 37.7, 17.5; HRMS (CI+): calculated for $C_{19}H_{25}N_2O_4$ (MH+) 345.18088, found 345.18048, Δ -1.17 ppm; LC/MS (BAS1): $[M+H]^+$ = 345; t_R = 0.81 min.

Synthesis of compound 12



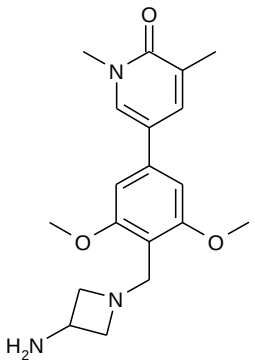
Supplementary Scheme 9: Synthesis of compound (**12**)

4-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-2,6-dimethoxybenzaldehyde (46)



4-bromo-2,6-dimethoxybenzaldehyde (**37**) (518 mg; 2.11 mmol), 1,3-dimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**34**) (500 mg; 2.01 mmol) and Pd(dppf)Cl_2·DCM (169 mg; 207 µmol) are suspended in DMF (4.0 mL) under argon. A degassed solution of sodium carbonate (2N, 2.51 mL; 5.02 mmol) is added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt, water is added and the mixture is filtered and purified by by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 4-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-2,6-dimethoxy-benzaldehyde (**46**) (460 mg; 1.60 mmol; 80 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.33 (s, 1H), 8.25 (d, J = 2.5 Hz, 1H), 7.95 – 7.90 (m, 1H), 6.91 (s, 2H), 3.92 (s, 6H), 3.56 (s, 3H), 2.11 (s, 3H). LC/MS (BAS1): $[M+H]^+$ = 288; t_R = 0.84 min.

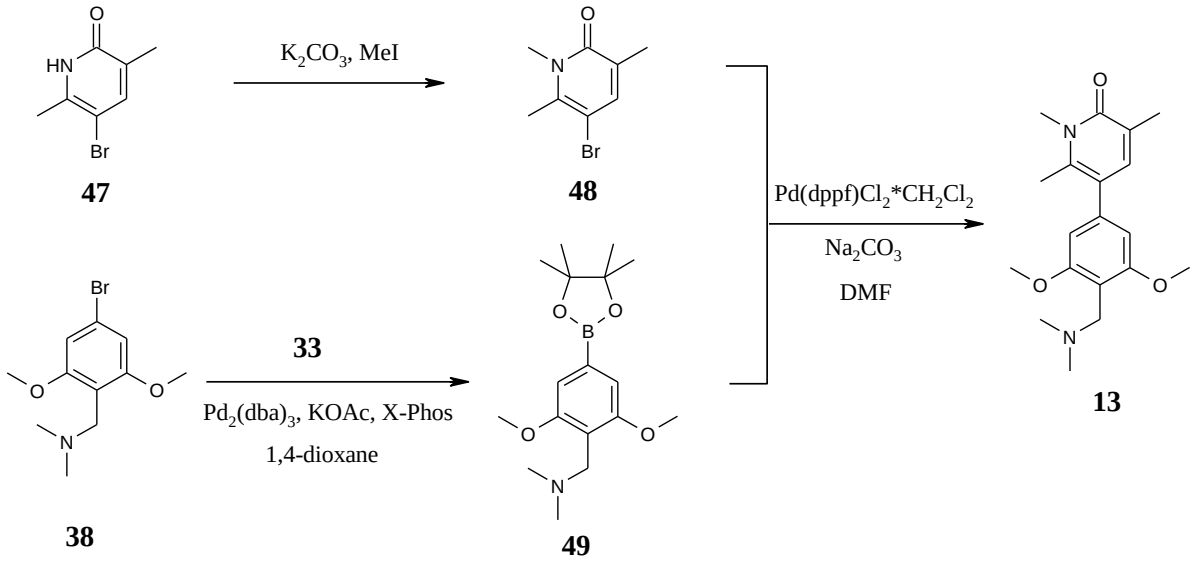
5-{4-[(3-aminoazetidin-1-yl)methyl]-3,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (12)



A mixture of NaOAc (40.7 mg; 496 µmol), AcOH (19.9 mg; 331 µmol) and tert-butyl N-(azetidin-3-yl)carbamate (85.4 mg; 496 µmol; commercial from Chontech) in DCM (2.0 mL) is stirred for 10 min at 0 °C. **46** (100 mg; 348 µmol) is added and stirring is continued. After 30 min sodium triacetoxyborohydride (144 mg; 697 µmol) is added in one portion and the reaction mixture is stirred at rt for 16 h. Saturated $NaHCO_3$ solution is added and the layers

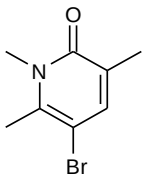
are separated. The aqueous layer is extracted three times with DCM. The combined organic layer is dried over Na_2SO_4, filtered and evaporated. The crude material is purified by silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 90:10). The product containing fractions are evaporated to give tert-butyl N-(1-{[4-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-2,6-dimethoxyphenyl]-methyl}azetidin-3-yl)carbamate (90.0 mg; 203 µmol; 58 %), which is directly dissolved in DCM (10 mL). HCl in 1,4-dioxane (4N solution; 507 µl; 2.03 mmol) is added and the reaction mixture is stirred at rt for 16 h. All volatiles are removed *in vacuo* and the residue is re-dissolved in MeOH. The free base of 5-{4-[(3-aminoazetidin-1-yl)methyl]-3,5-dimethoxyphenyl}-1,3-dimethyl-1,2-dihydropyridin-2-one (**12**) (60.0 mg; 175 µmol; 86 %) is generated using a SPX-cartridge. ^{1}H NMR (400 MHz, DMSO-d6, 1H under DMSO signal) δ 8.07 (d, J = 2.8 Hz, 1H), 7.83 (d, J = 2.6 Hz, 1H), 6.81 (s, 2H), 3.85 (s, 6H), 3.62 (s, 2H), 3.54 (s, 3H), 3.42 – 3.47 (m, 2H), 2.95 (t, J = 6.0 Hz, 2H), 2.10 (s, 3H). HRMS (CI+): calculated for $C_{19}H_{26}N_3O_3$ (MH+) 344.19687, found 344.19660, Δ -0.77 ppm; LC/MS (BAS1): $[M+H]^+$ = 344; t_R = 0.94 min.

Synthesis of compound 13[1]



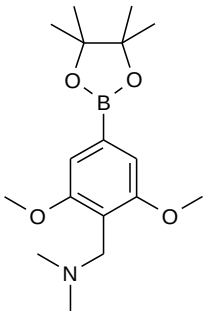
Supplementary Scheme 10: Synthesis of compound (**13**)

5-bromo-1,3,6-trimethyl-1,2-dihydropyridin-2-one (48)



To a suspension of 5-bromo-3,6-dimethyl-1,2-dihydropyridin-2-one (**47**) (2.54 g; 12.6 mmol) and potassium carbonate (4.13 g; 29.9 mmol) in THF (25 mL), iodomethane (811 µL; 13.1 mmol) is added and the resulting mixture is stirred at 80 °C for 16 h. Ammonia (10% aqueous solution; 30 mL) is added followed by water (50 mL). THF is removed under reduced pressure and the aqueous residue is extracted three times with DCM. The combined organic layer is dried over Na_2SO_4 and concentrated *in vacuo* to give crude 5-bromo-1,3,6-trimethyl-1,2-dihydropyridin-2-one (**48**) (2.58 g; 11.9 mmol; 95 %) which is used without further purification. For analytical purposes a small amount was purified by silica gel chromatography. ^{1}H NMR (400 MHz, DMSO-d6) δ 7.47 (s, 1H), 3.51 (s, 3H), 2.47 (s, 3H), 1.98 (s, 3H); LC/MS (BAS1): $[M+H]^+$ = 216/218; t_R = 0.84 min.

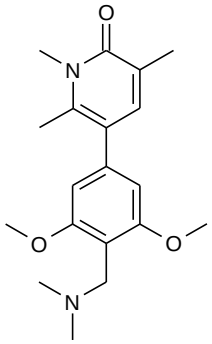
{[2,6-Dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}dimethylamine (49)



[(4-bromo-2,6-dimethoxyphenyl)methyl]dimethylamine (**38**) (15.7 g; 57.3 mmol) and bis(pinacolato)diboron (**33**) (43.6 g; 1.86 mol) are dissolved/suspended in 1,4-dioxane (300 mL) under N_2. Potassium acetate (17.0 g; 58.8 mmol), Pd_2dba_3 (1.00 g; 1.09 mmol) and 2-dicyclohexyl-phosphino-2′,4′,6′-triisopropylbiphenyl (1.00 g; 2.10 mmol) is added and the mixture is stirred at 90° C for 8 h. After cooling to rt the mixture is concentrated and the residue is taken-up in DCM. Water is added, the layers are separated and the aqueous phase is extracted two times with DCM. The combined organic layer is dried over Na_2SO_4, filtered and evaporated. The crude product is purified by preparative RP-HPLC using a MeCN/water (0.2 % TFA added to the water) gradient as eluent to give the TFA salt of {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**49**) which is

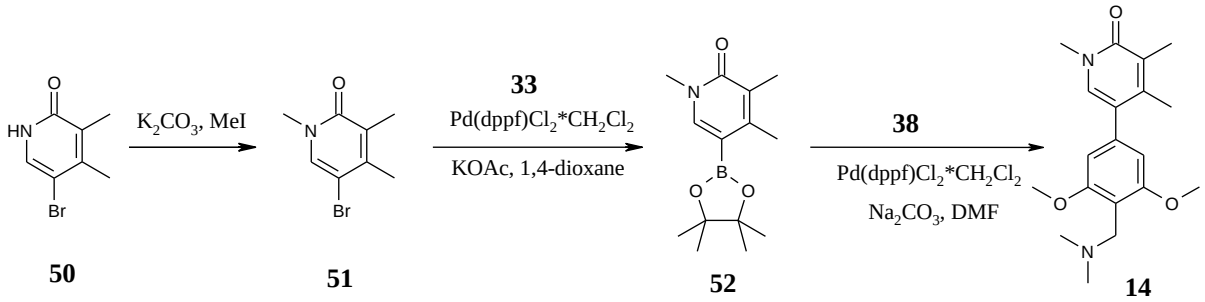
transferred into the corresponding hydrochloride by dissolving and stirring in HCl/MeOH for 30 min (5.45 g; 17.0 mmol; 30 %). ^{1}H-NMR (500 MHz, DMSO-d6) δ 9.44 (s, 1H), 6.95 (s, 2H), 4.21 (d, J = 5.3 Hz, 2H), 3.87 (s, 6H), 2.70 (d, J = 5.0 Hz, 6H), 1.32 (s, 12H); LC/MS (BAS1): $[M+H]^+$ = 240 (ester cleaved under basic conditions); t_R = 0.20 min.

5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-1,3,6-trimethyl-1,2-dihydro-pyridin-2-one (13)



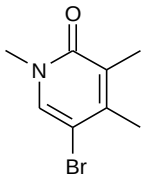
48 (100 mg; 462 µmol), **49** (216 mg; 672 µmol) and Pd(dppf)Cl_2·DCM (38.8 mg; 47.5 µmol) are suspended in DMF (2.0 mL) under argon. A degassed Na_2CO_3-solution (2N; 576 µL; 1.15 mmol) is subsequently added and the resulting mixture is heated to 100 °C for 1 h. After cooling to rt water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried. Further purification is achieved by automated silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 90:10; MeOH made basic with 0.1% NH_3). The product containing fractions are evaporated, dissolved in MeCN/water and freeze dried to give 5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-1,3,6-trimethyl-1,2-dihydro-pyridin-2-one (**13**) (46.0 mg; 139 µmol; 30 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.29 (s, 1H), 6.53 (s, 2H), 3.77 (s, 6H), 3.53 (s, 3H), 3.47 (s, 2H), 2.32 (s, 3H), 2.16 (s, 6H), 2.04 (s, 3H); ^{13}C NMR (125 MHz, DMSO, 1 C missing) δ 162.7, 159.0 (2C), 141.9, 140.5, 138.7, 124.2, 118.9, 105.9 (2C), 56.2 (2C), 50.0, 45.3 (2C), 31.9, 18.5, 17.4; HRMS (CI+): calculated for $C_{19}H_{27}N_2O_3$ (MH+) 331.20162, found 331.20123, Δ -1.17 ppm; LC/MS (BAS1): $[M+H]^+$ = 331; t_R = 0.92 min.

Synthesis of compound 14



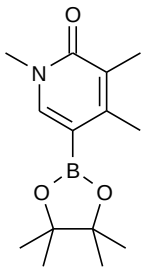
Supplementary Scheme 11: Synthesis of compound (**14**)

5-bromo-1,3,4-trimethyl-1,2-dihydropyridin-2-one (51)



To a suspension of 5-bromo-3,4-dimethyl-1,2-dihydropyridin-2-one **(50)** (2.50 g; 12.4 mmol) and potassium carbonate (4.28 g; 31.0 mmol) in THF (25 mL), iodomethane (840 µL; 13.6 mmol) is added and the resulting mixture is stirred at rt for 16 h. The reaction mixture is evaporated and the crude material is purified by silica gel chromatography (Combiflash; column: Redisep RF, 40g) using a cHex/EA gradient as eluent (100:0 --> 50:50). Product containing fractions are evaporated to give 5-bromo-1,3,4-trimethyl-1,2-dihydropyridin-2-one (**51**) (2.30 g; 10.6 mmol; 86 %). LC/MS (51): $[M+H]^+$ = 216/218; t_R = 0.42 min

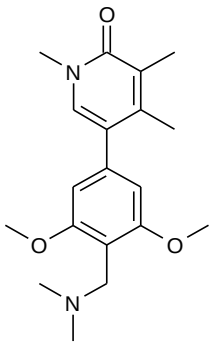
1,3,4-trimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (52)



51 (1.00 g; 4.63 mmol), bis(pinacolato)diboron (**33**) (1.76 g; 6.94 mmol), Pd(dppf)Cl_2·DCM (378 mg; 46.3 µmol) and potassium acetate (908 mg; 9.26 mmol) are suspended in 1,4-dioxane (5.0 mL) under argon and the resulting mixture is heated to 80 °C for 16 h. After cooling to rt, the reaction mixture is concentrated under reduced pressure, the residue is taken-up in DCM/MeOH and purified by silica gel chromatography (Combiflash; column: Redisep

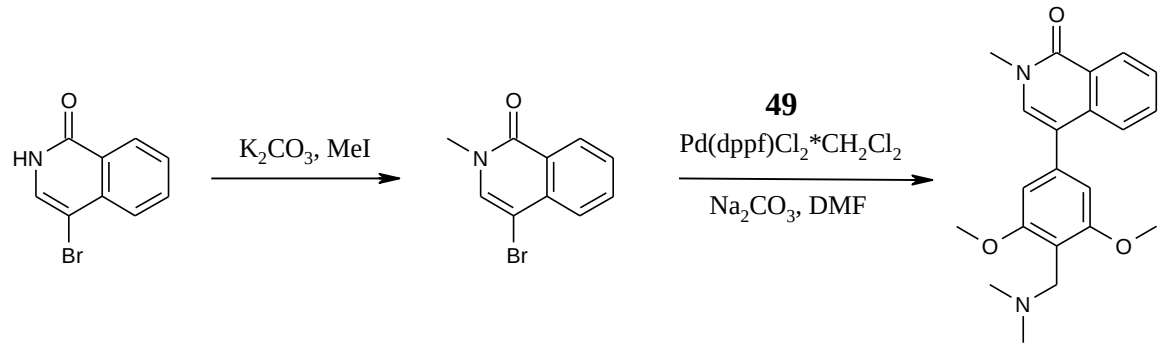
RF, 40 g) using a chex/EA gradient as eluent (80:20 --> 20:80). The product containing fractions are evaporated to give 1,3,4-trimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**52**) (1.20 g; 4.56 mmol; 98 %). LC/MS(52): $[M+H]^+$ = 264; t_R = 0.64 min

5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-1,3,4-trimethyl-1,2-dihydro-pyridin-2-one (14)



52 (100 mg; 380 µmol), [(4-bromo-2,6-dimethoxyphenyl)methyl]dimethylamine (**38**) (104 mg; 379 µmol) and Pd(dppf)Cl_2·DCM (31.0 mg; 38.0 µmol) are suspended in DMF (800 µL) under argon. A degassed Na_2CO_3-solution (2N; 475 µL; 950 µmol) is subsequently added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]-3,5-dimethoxy-phenyl}-1,3,4-trimethyl-1,2-dihydropyridin-2-one (**14**) (22.8 mg; 69.0 µmol; 18 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.52 (s, 1H), 6.53 (d, J = 1.4 Hz, 2H), 3.77 (d, J = 1.4 Hz, 6H), 3.46 (d, J = 1.4 Hz, 3H), 3.41 (s, 2H), 2.12 – 2.04 (m, 12H); ^{13}C NMR (125 MHz, DMSO) δ 161.6, 158.9 (2C), 144.7, 138.4, 135.0, 124.9, 121.6, 113.2, 106.0 (2C), 56.2 (2C), 50.0, 45.5 (2C), 37.3, 18.0, 13.5. HRMS (CI+): calculated for $C_{19}H_{27}N_2O_3$ (MH+) 331.20162, found 331.20101, Δ -1.83 ppm; LC/MS (BAS1): $[M+H]^+$ = 331; t_R = 1.02 min.

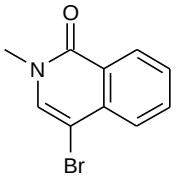
Synthesis of compound 15[1]



53 **54** **15**

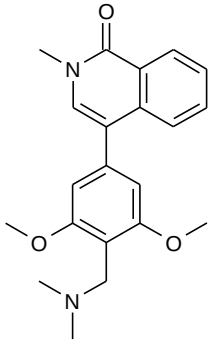
Supplementary Scheme 12: Synthesis of compound (**15**)

4-bromo-2-methyl-1,2-dihydroisoquinolin-1-one (54)



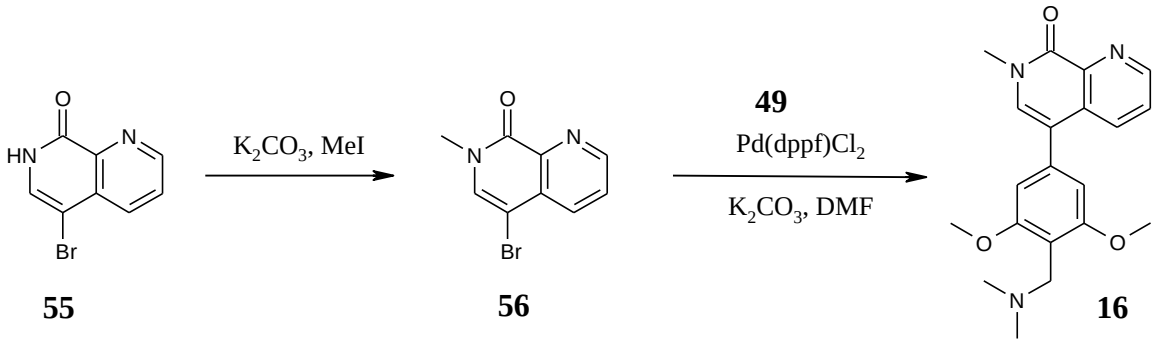
To a suspension of 4-bromo-1,2-dihydroisoquinolin-1-one (**53**) (1.00 g; 4.46 mmol) and potassium carbonate (1.17 g; 8.48 mmol) in THF (10 mL), iodomethane (323 µL; 5.09 mmol) is added and the resulting mixture is stirred at rt for 16 h. Since HPLC-MS of the reaction mixture indicates incomplete conversion, additional iodomethane (100 µl; 1.57 mmol) is added and stirring is continued for 5h. Ammonia (10% aqueous solution; 30 mL) is added followed by water (50 mL). THF is removed under reduced pressure whereupon a precipitation occurs. The solid is collected by filtration, washed with cold water and dried *in vacuo* to give 4-bromo-2-methyl-1,2-dihydroisoquinolin-1-one (**54**) (1.00 g; 4.20 mmol; 94 %) as a yellow solid which is used without further purification. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.28 (d, J = 8.0 Hz, 1H), 7.98 (s, 1H), 7.86 (t, J = 7.6 Hz, 1H), 7.76 (d, J = 8.1 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 3.52 (s, 3H); LC/MS (BAS1): $[M+H]^+$ = 238/240; t_R = 1.02 min.

4-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-2-methyl-1,2-dihydroisoquinolin-1-one (15)



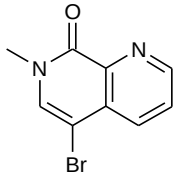
54 (130 mg; 546 µmol), {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl]methyl}-dimethylamine (**49**) (284 mg; 884 µmol) and Pd(dppf)Cl_2·DCM (44.6 mg; 54.6 µmol) are suspended in DMF (2.0 mL) under argon. A degassed Na_2CO_3-solution (2N; 683 µL; 1.37 mmol) is subsequently added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried. Further purification is achieved by automated silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 80:20; MeOH made basic with 0.1% NH_3). The product containing fractions are evaporated, dissolved in MeCN/water and freeze dried to give 4-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-2-methyl-1,2-dihydroisoquinolin-1-one (**15**) (84.8 mg; 240 µmol; 44 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.34 (d, J = 7.1 Hz, 1H), 7.72 (dd, J = 8.3, 7.1, 1H), 7.65 (d, J = 7.9 Hz, 1H), 7.58 – 7.54 (m, 2H), 6.71 (s, 2H), 3.80 (s, 6H), 3.58 (s, 3H), 3.49 (s, 2H), 2.16 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ 161.3, 159.3 (2C), 137.0, 136.3, 133.3, 132.8, 127.8, 127.1, 125.5, 124.8, 118.2, 113.4, 106.1 (2C), 56.3 (2C), 50.0, 45.4 (2C), 36.8; HRMS (CI+): calculated for $C_{21}H_{25}N_2O_3$ (MH+) 353.18597, found 353.18568, Δ -0.82 ppm; LC/MS (BAS1): $[M+H]^+$ = 353; t_R = 1.05 min.

Synthesis of compound 16



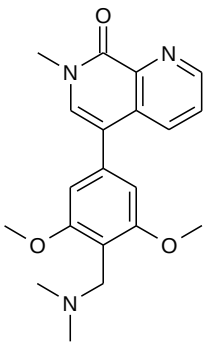
Supplementary Scheme 13: Synthesis of compound (**16**)

4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (56)



To a suspension of 5-bromo-7,8-dihydro-1,7-naphthyridin-8-one (**55**) (900 mg; 4.00 mmol, commercial from Princeton) and potassium carbonate (1.11 g; 8.03 mmol) in THF (40 mL), iodomethane (430 µL; 6.78 mmol) is carefully added and the resulting mixture is stirred at rt for 16 h. Since HPLC-MS of the reaction mixture indicates incomplete conversion additional iodomethane (400 µl; 6.31 mmol) is added and stirring is continued for 24h. Ammonia (10% aqueous solution; 30 mL) is added followed by water (50 mL). THF is removed under reduced pressure and the aqueous layer is extracted three times with DCM (30 mL each). The combined organic layer is dried over Na_2SO_4, filtered and evaporated to give 4-bromo-2-methyl-1,2-dihydro-2,7-naphthyridin-1-one (**56**) (860 mg; 3.69 mmol; 90 %) which is used without further purification. LC/MS (BAS1): $[M+H]^+$ = 239/241; t_R = 0.87 min.

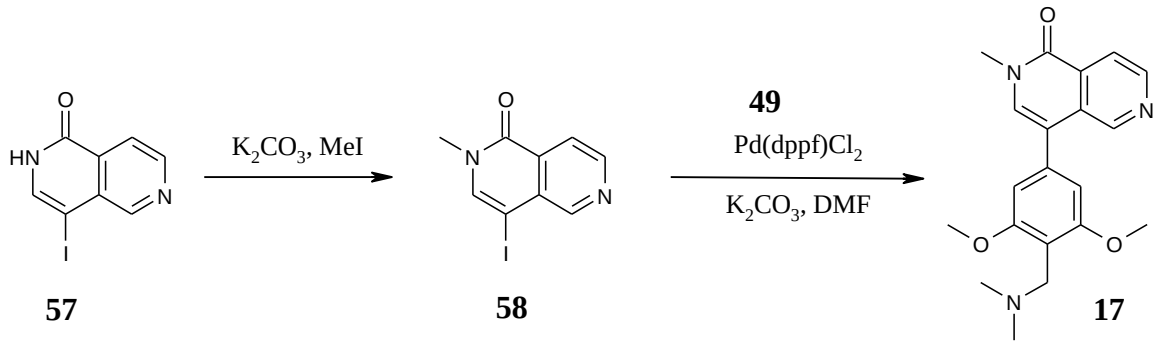
5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-7-methyl-7,8-dihydro-1,7-naphthyridin-8-one (16)



56 (80.0 mg 418 µmol), {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**49**) (134 mg; 418 µmol) and $Pd(dppf)Cl_2$ (61.0 mg; 83.4 µmol) are suspended in 1,4-dioxane (10 mL) and water (1 mL) under N_2. Potassium carbonate (173 mg; 1.25 mmol) is added and the resulting mixture is heated at 80 °C for 1 h. After cooling to rt water is added and the mixture is extracted three times with EA. The combined organic layer is washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue is purified by preparative RP-HPLC using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-7-methyl-7,8-dihydro-1,7-naphthyridin-8-one (**16**) (33.0 mg; 93.4 µmol; 22 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.83 (d, J = 4.2 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.74 – 7.65 (m, 2H), 6.74 (s, 2H), 3.81 (s, 6H), 3.59 – 3.65 (m, 5H), 2.28 (s, 6H); ^{13}C NMR (125 MHz, DMSO, 1 C missing) δ 160.2, 159.4 (2C), 149.6, 141.3, 136.6, 134.1, 133.3, 132.6, 127.1, 116.3, 106.0 (2C), 56.4 (2C), 50.0, 45.1 (2C), 37.3; HRMS (CI+): calculated for

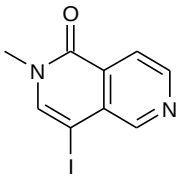
$C_{20}H_{24}N_3O_3$ (MH+) 354.18122, found 354.18067, Δ -1.55 ppm; LC/MS (BAS1): $[M+H]^+$ = 354; t_R = 0.91 min.

Synthesis of compound 17



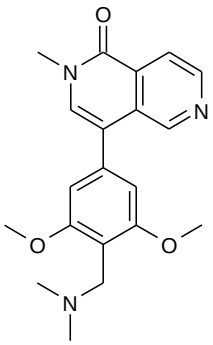
Supplementary Scheme 15: Synthesis of compound (**17**)

4-Iodo-2-methyl-1,2-dihydro-2,6-naphthyridin-1-one (58)



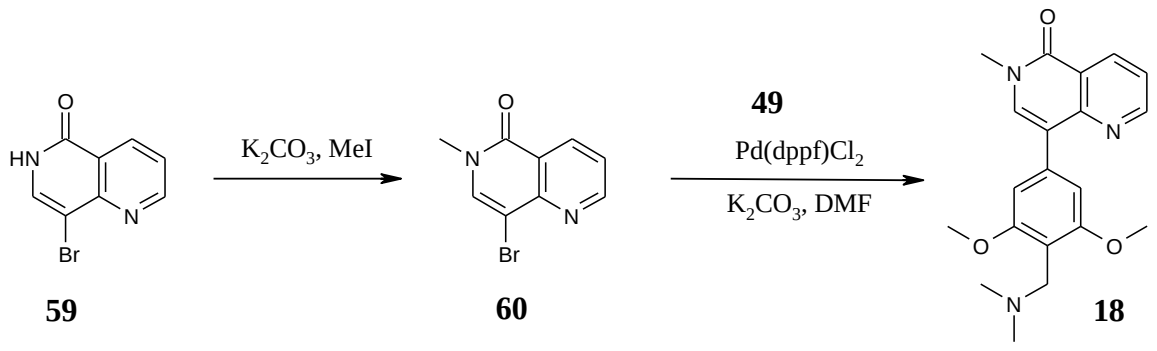
To a suspension of 4-iodo-1,2-dihydro-2,6-naphthyridin-1-one (**57**) (1.00 g; 3.68 mmol, commercial from FCHGroup) and potassium carbonate (1.52 g; 11.0 mmol) in DMF (50 mL), iodomethane (1.04 g; 7.32 mmol) is carefully added and the resulting mixture is stirred at rt for 24 h. Water and EA are added, the layers are separated and the aqueous layer is extracted three times with EA. The combined organic layer is washed with brine, dried over Na_2SO_4, filtered and evaporated to give 4-iodo-2-methyl-1,2-dihydro-2,6-naphthyridin-1-one (**58**) (450 mg; 1.57 mmol; 43 %) which is used without further purification. LC/MS(**58**): (M+H)+ =287; t_R = 0.57 min.

4-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-2-methyl-1,2-dihydro-2,6-naphthyridin-1-one (17)



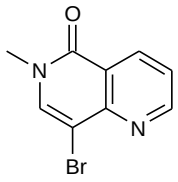
58 (100 mg; 350 µmol), {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**49**) (84.0 mg; 351 µmol) and Pd(dppf)Cl_2 (51.0 mg; 69.8 µmol) are suspended in 1,4-dioxane (10 mL) and water (1 mL) under N_2. Potassium carbonate (145 mg; 1.05 mmol) is added and the resulting mixture is heated at 80 °C for 1 h. After cooling to rt water is added and the mixture is extracted three times with EA. The combined organic layer is washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue is purified by preparative RP-HPLC using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 4-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-2-methyl-1,2-dihydro-2,6-naphthyridin-1-one (**17**) (30.0 mg; 84.9 µmol; 24 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.02 (s, 1H), 8.71 (d, J = 5.2 Hz, 1H), 8.14 (d, J = 5.2 Hz, 1H), 7.73 (s, 1H), 6.81 (s, 2H), 3.83 (s, 6H), 3.68 (s, 2H), 3.62 (s, 3H), 2.31 (s, 6H); ^{13}C NMR (125 MHz, DMSO, 1 C missing) δ 160.3, 159.4 (2C), 148.3, 146.5, 136.2, 135.2, 130.5, 130.0, 120.0, 116.4, 106.1 (2C), 56.4 (2C), 50.0, 45.0 (2C), 37.1; HRMS (CI+): calculated for $C_{20}H_{24}N_3O_3$ (MH+) 354.18122, found 354.18061, Δ -1.71 ppm; LC/MS (BAS1): $[M+H]^+$ = 354; t_R = 0.96 min.

Synthesis of compound 18



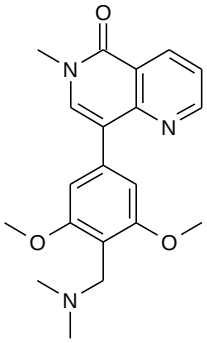
Supplementary Scheme 16: Synthesis of compound (**18**)

8-Bromo-6-methyl-5,6-dihydro-1,6-naphthyridin-5-one (60)



To a suspension of 8-bromo-5,6-dihydro-1,6-naphthyridin-5-one (**59**) (2.00 g; 8.89 mmol, commercial from TCI) and potassium carbonate (3.68 g; 26.6 mmol) in DMF (50 mL), iodomethane (2.52 g; 17.7 mmol) is carefully added and the resulting mixture is stirred at rt for 24 h. Water and EA are added, the layers are separated and the aqueous layer is extracted three times with EA. The combined organic layer is washed with brine, dried over Na_2SO_4, filtered and evaporated to give 8-bromo-6-methyl-5,6-dihydro-1,6-naphthyridin-5-one (**60**) (1.50 g; 6.27 mmol; 71 %) which is used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, J = 3.6 Hz, 1H), 8.73 (d, J = 8.0 Hz, 1H), 7.69 (s, 1H), 7.40 – 7.50 (m, 1H), 3.65 (s, 3H); LC/MS(**60**): (M+H)+ =239/241; t_R = 0.52 min.

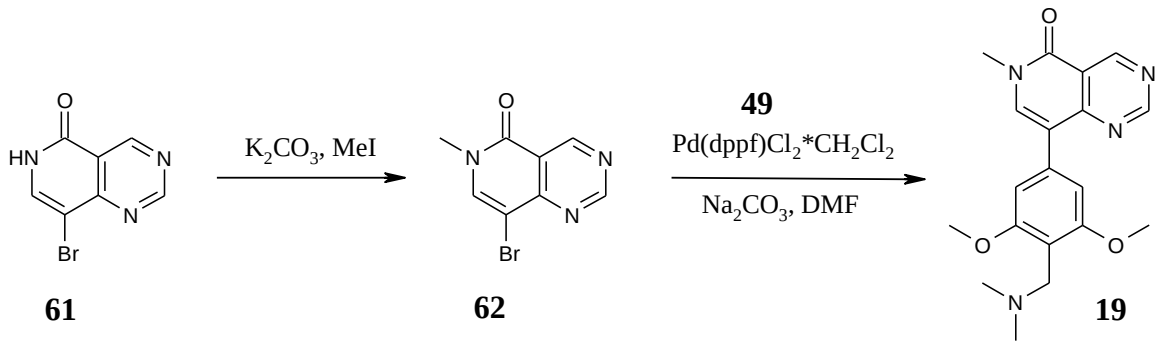
8-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-6-methyl-5,6-dihydro-1,6-naphthyridin-5-one (18)



60 (100 mg; 418 µmol), {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**49**) (134 mg; 418 µmol) and Pd(dppf)Cl_2 (61.0 mg; 83.4 µmol) are suspended in 1,4-dioxane (10 mL) and water (1 mL) under N_2. Potassium carbonate (173 mg; 1.25 mmol) is added and the resulting mixture is heated at 80 °C for 1 h. After cooling to rt water is added and the mixture is extracted three times with EA. The combined organic layer is washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue is purified by preparative RP-HPLC using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 8-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-6-methyl-5,6-dihydro-1,6-naphthyridin-5-one (**18**) (35.0 mg; 99.0 µmol;

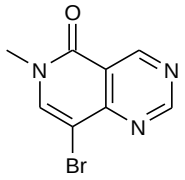
24 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.40 (s, 1H, prot. amine), 8.96 (d, J = 4.5 Hz, 1H), 8.65 (d, J = 8.1 Hz, 1H), 8.00 (s, 1H), 7.60 (dd, J = 8.1, 4.5 Hz, 1H), 7.03 (s, 2H), 4.24 (s, 2H), 3.88 (s, 6H), 3.63 (s, 3H), 2.76 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ 161.6, 158.7 (2C), 154.5, 151.6, 139.7, 137.7, 136.4, 122.5, 121.1, 118.3, 106.5 (2C), 105.2, 56.6 (2C), 49.9, 43.0 (2C), 36.9; HRMS (CI+): calculated for $C_{20}H_{24}N_3O_3$ (MH+) 354.18122, found 354.18067, Δ -1.54 ppm; LC/MS (BAS1): $[M+H]^+$ = 354; t_R = 0.96 min.

Synthesis of compound 19



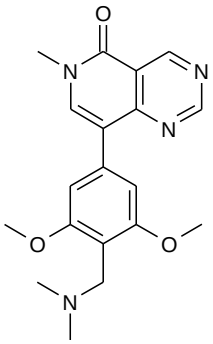
Supplementary Scheme 17: Synthesis of compound (**19**)

8-Bromo-6-methyl-5H,6H-pyrido[4,3-d]pyrimidin-5-one (62)



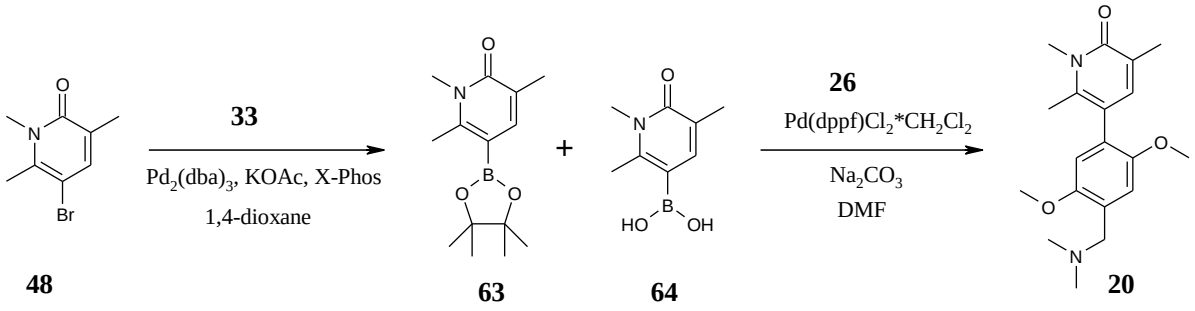
To a suspension of 8-bromo-5H,6H-pyrido[4,3-d]pyrimidin-5-one (**61**) (1.00 g; 4.42 mmol, commercial from FCHGroup) and potassium carbonate (1.19 g; 8.61 mmol) in THF (40 mL), iodomethane (430 µL; 6.78 mmol) is carefully added and the resulting mixture is stirred at rt for 16 h. Since HPLC-MS of the reaction mixture indicates incomplete conversion additional iodomethane (400 µl; 6.31 mmol) is added and stirring is continued for 24h. Ammonia (10% aqueous solution; 30 mL) is added whereupon precipitation occurs. The solid is filtered, washed with water and dried *in vacuo* to give 8-bromo-6-methyl-5H,6H-pyrido[4,3-d]pyrimidin-5-one (**62**) (890 mg; 3.71 mmol; 84 %) which is used without further purification. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.48 (s, 1H), 9.47 (s, 1H), 8.54 (s, 1H), 3.54 (s, 3H); LC/MS (BAS1): $[M+H]^+$ = 240/242; t_R = 0.29 min.

8-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-6-methyl-5H,6H-pyrido[4,3-d]pyrimidin-5-one (19)



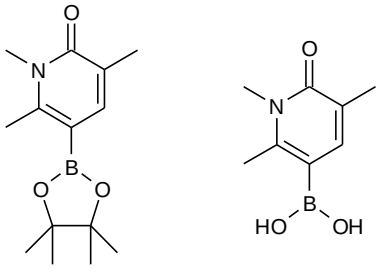
62 (100 mg; 417 µmol), {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**49**) (170 mg; 529 µmol) and Pd(dppf)Cl_2·DCM (34.0 mg; 41.6 µmol) are suspended in DMF (800 µL) under argon. A degassed Na_2CO_3-solution (2N; 521 µL; 1.04 mmol) is subsequently added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt, water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried. Further purification is achieved by automated silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 80:20; MeOH made basic with 0.1% NH_3). The product containing fractions are evaporated, dissolved in MeCN/water and freeze dried to give 8-{4-[(dimethylamino)methyl]-3,5-dimethoxyphenyl}-6-methyl-5H,6H-pyrido[4,3-d]pyrimidin-5-one (**19**) (35.4 mg; 99.9 µmol; 24 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 9.55 (s, 1H), 9.39 (s, 1H), 8.24 (s, 1H), 6.91 (s, 2H), 3.80 (s, 6H), 3.63 (s, 3H), 3.46 (s, 2H), 2.14 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ 160.8, 160.6, 159.0, 158.8 (2C), 156.5, 142.8, 134.8, 117.9, 117.3, 113.5, 106.3 (2C), 56.2 (2C), 50.0, 45.4 (2C), 37.0; HRMS (CI+): calculated for $C_{19}H_{23}N_4O_3$ (MH+) 355.17647, found 355.17598, Δ -1.36 ppm; LC/MS (BAS1): $[M+H]^+$ = 355; t_R = 0.88 min.

Synthesis of compound 20



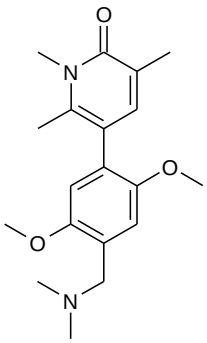
Supplementary Scheme 18: Synthesis of compound (**20**)

1,3,4-Trimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (63) and (1,2,5-trimethyl-6-oxo-1,6-dihydropyridin-3-yl)boronic acid (64)



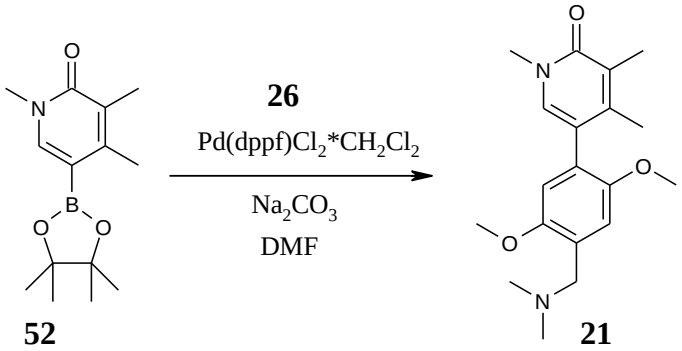
5-Bromo-1,3,6-trimethyl-1,2-dihydropyridin-2-one **(48)** (6.30 g; 29.2 mmol), bis(pinacolato)diboron (**33)** (29.6 g; 117 mmol), $Pd_2(dba)_3$ (0.60 g; 0.66 mmol) and 2-dicyclohexyl-phosphino-2′,4′,6′-triisopropylbiphenyl (0.60 g; 1.26 mmol) (378 mg; 46.3 µmol) and potassium acetate (9.00 g; 91.7 mmol) are suspended in 1,4-dioxane (80 mL) under N_2 and the resulting mixture is heated at 60 °C for 48 h. After cooling to rt the reaction mixture is concentrated under reduced pressure, the residue is taken-up in EA, water is added and the layers are separated. The aqueous layer is extracted three times with EA. The combined organic layer is dried over Na_2SO_4, filtered and evaporated. The crude material is purified by silica gel chromatography and subsequently by preparative RP-HPLC using a MeCN/water gradient as eluent to give an inseparable mixture of 1,3,4-trimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**63**) and (1,2,5-trimethyl-6-oxo-1,6-dihydropyridin-3-yl)boronic acid (**64**) (5.40 g; 20.5 mmol; 70 %), which is used for the next step. ^{1}H NMR (500 MHz, DMSO-d6, 3H from **64** covered under DMSO signal) δ 7.88 (s, 2 H, **64**), 7.39 (s, 1H, **63** and **64**), 3.46 (s, 3H, **63** and **64**), 2.57 (s, 3H, **63**), 1.96 (s, 3H, **63** and **64**), 1.27 (s, 12H, **63**); LC/MS (BAS1): $[M+H]^+$ = 264; t_R = 1.16 min (Ester).

5-{4-[(Dimethylamino)methyl]-2,5-dimethoxyphenyl}-1,3,6-trimethyl-1,2-dihydropyridin-2-one (20)



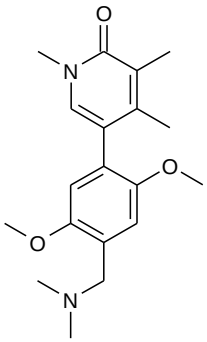
[(4-bromo-2,5-dimethoxyphenyl)methyl]dimethylamine (**26**) (313 mg; 1.14 mmol), inseparable mixture of **63** and **64** (300 mg; 1.14 mmol) and Pd(dppf)Cl_2·DCM (192 mg; 235 µmol) are suspended in DMF (3.0 mL) under argon. A degassed Na_2CO_3-solution (2N; 1.43 mL; 2.86 mmol) is subsequently added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt, water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried. Further purification is achieved by automated silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 90:10; MeOH made basic with 0.1% NH_3). The product containing fractions are evaporated, dissolved in MeCN/water and freeze dried to give 5-{4-[(dimethylamino)-methyl]-2,5-dimethoxyphenyl}-1,3,6-trimethyl-1,2-dihydro-pyridin-2-one (**20**) (8.30 mg; 25.1 µmol; 2.2 %). ^{1}H NMR (400 MHz, DMSO-d6) δ 7.14 (s, 1H), 7.02 (s, 1H), 6.72 (s, 1H), 3.71 (2, 3H), 3.67 (s, 3H), 3.51 (s, 2H), 2.19 (s, 6H), 2.11 (s, 3H), 2.00 (s, 3H); LC/MS (BAS1): $[M+H]^+$ = 331; t_R = 1.00 min.

Synthesis of compound 21



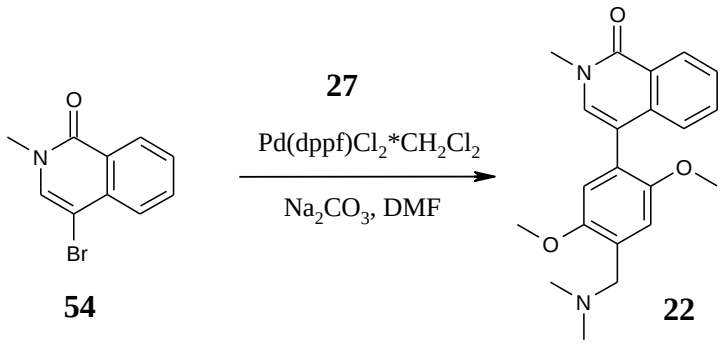
Supplementary Scheme 19: Synthesis of compound (**21**)

5-{4-[(Dimethylamino)methyl]-2,5-dimethoxyphenyl}-1,3,4-trimethyl-1,2-dihydro-pyridin-2-one (21)



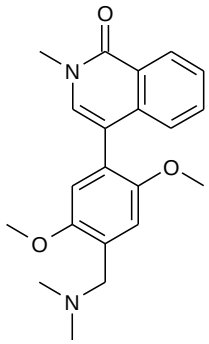
1,3,4-trimethyl-5-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridin-2-one (**52**) (80.0 mg; 304 µmol), [(4-bromo-2,5-dimethoxyphenyl)methyl]dimethylamine (**26**) (83.3 mg; 304 µmol) and Pd(dppf)Cl_2·DCM (24.8 mg; 30.4 µmol) are suspended in DMF (800 µL) under argon. A degassed Na_2CO_3-solution (2N; 380 µL; 760 µmol) is subsequently added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt, water (several drops) is added, the reaction mixture is filtered and purified by preparative RP-HPLC chromatography (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried to give 5-{4-[(dimethylamino)methyl]-2,5-dimethoxyphenyl}-1,3,4-trimethyl-1,2-dihydropyridin-2-one (**21**) (3.50 mg; 10.6 µmol; 3.5 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 7.38 (s, 1H), 7.01 (s, 1H), 6.75 (d, J = 1.5 Hz, 1H), 3.73 (d, J = 1.5 Hz, 3H), 3.67 (d, J = 1.4 Hz, 3H), 3.39 - 3.44 (m, 5H), 2.19 (d, J = 1.6 Hz, 6H), 2.03 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (125 MHz, DMSO) δ 161.8, 151.4, 151.3, 146.0, 134.9, 127.5, 125.3, 124.1, 118.2, 114.9, 113.0, 57.1, 56.4, 56.1, 45.7 (2C), 37.3, 17.4, 13.4; HRMS (CI+): calculated for $C_{19}H_{27}N_2O_3$ (MH+) 331.20162, found 331.20108, Δ -1.62 ppm; LC/MS (BAS1): $[M+H]^+$ = 331; t_R = 1.02 min.

Synthesis of compound 22



Supplementary Scheme 20: Synthesis of compound (**22**)

4-{4-[(Dimethylamino)methyl]-2,5-dimethoxyphenyl}-2-methyl-1,2-dihydroisoquinolin-1-one (22)



4-bromo-2-methyl-1,2-dihydroisoquinolin-1-one (**54**) (77.8 mg; 327 µmol), {[2,6-dimethoxy-4-(tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methyl}-dimethylamine (**27**) (150 mg; 467 µmol) and Pd(dppf)Cl_2·DCM (27.5 mg; 33.7 µmol) are suspended in DMF (1.0 mL) under argon. A degassed Na_2CO_3-solution (2N; 409 µL; 818 µmol) is subsequently added and the resulting mixture is heated at 100 °C for 1 h. After cooling to rt, water is added (several drops) and the mixture is filtered and purified by preparative RP-HPLC (column: X-Bridge C-18 30x50 mm) using a MeCN/water gradient under basic conditions. The product containing fractions are freeze dried. Further purification is achieved by automated silica gel chromatography (Combiflash; column: Redisep RF, 12g) using a DCM/MeOH gradient as eluent (100:0 --> 90:10; MeOH made basic with 0.1% NH_3). The product containing fractions are evaporated, dissolved in MeCN/water and freeze dried to give 4-{4-[(dimethylamino)-methyl]-2,5-dimethoxyphenyl}-2-methyl-1,2-dihydroiso-quinolin-1-one (**22**) (35.1 mg; 99.6 µmol; 30 %). ^{1}H NMR (500 MHz, DMSO-d6) δ 8.29 (d, J = 7.9 Hz, 1H), 7.63 (t, J = 7.6 Hz, 1H), 7.50 (t, J = 7.6 Hz, 1H), 7.44 (s, 1H), 7.14 (d, J = 8.1 Hz, 1H), 7.11 (s, 1H), 6.90 (s, 1H), 3.74 (s, 3H), 3.61 (s, 3H), 3.56 (s, 3H), 3.47 (s, 2H), 2.23 (s, 6H); ^{13}C NMR (125 MHz, DMSO) δ 161.5, 151.6, 151.6, 136.9, 133.5, 132.3, 127.9, 127.3, 126.8, 125.5, 125.2, 123.7,

115.3, 115.2, 113.4, 57.2, 56.4, 56.2, 45.8 (2C), 36.8; HRMS (CI+): calculated for $C_{21}H_{25}N_2O_3$ (MH+) 353.18597, found 353.18538, Δ -1.68 ppm; LC/MS (BAS1): $[M+H]^+$ = 353; t_R = 1.11 min.

ONLINE METHODS

BRD9 SPR-based fragment screen

The fragment screen was performed on a Biacore T200 instrument (GE Healthcare). His-tagged BRD9 (X-ray crystallography construct with an additional amino-terminal hexahistidine-tag[2]) was immobilized onto a Biacore NTA-chip (GE Healthcare) as described in the literature.[3] Briefly, the BRD9 protein was diluted to a concentration of 0.2 mg/ml in HBS-P+ buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 0.05 % P20) and injected over flow cell 4 of a Biacore NTA-chip, pre-loaded with Ni^{2+} and activated with EDC/NHS according to the manufacturer's instructions (T = 20°C). A second hexahistidine-tagged bromodomain protein, distantly related to BRD9, was immobilized on flow cell 3 as a control. Carbonic anhydrase II (SIGMA Aldrich) was used as a negative control and immobilized onto flow cell 2 (EDC/NHS activation, but no Ni^{2+}-loading). Buffer was injected over flow cell 1 to generate a blank reference surface. Approximately 8000 RU of each of the three proteins were immobilized. The buffer was then switched to screening buffer (HBS-P+; 1% DMSO).

The chip was equilibrated with running buffer for several hours. After 10 startup cycles (buffer injections) the fragment compounds were injected at a concentration of 100 μM from 384-well plates and the response was recorded for each compound. Positive (a bromodomain binder identified internally in a different bromodomain inhibitor program) and negative (buffer) controls were included at regular intervals to monitor the performance of the assay over the complete screening experiment. It turned out that the proteins on the chip were stable for the time required to analyse 384 compounds. After this a new chip was prepared as described above and the next 384 compounds were analysed. To correct for the excluded volume effect a DMSO calibration series was prepared as detailed in the instrument manufacturer's instructions and the calibration samples were measured at the beginning and end of each run.

The data were evaluated using BiaEvaluation (GE Healthcare) software. Briefly, all SPR responses were corrected for differences in bulk solvent refractive index and contributions from binding of compounds to the blank reference flow cell were subtracted. In this way the response (in response units, RUs) for each compound was determined. Fractional surface occupancy (FSO) normalized to the response for the reference compound was then calculated on a plate basis.[4] Based on our measured FSO the Z' value[5] of our assay over all plates was calculated to be 0.91 which is well above the 0.5 threshold which is considered to be the minimum requirement for a valid screening assay.

Any compounds showing abnormal binding behavior, were identified by visual inspection of the sensorgrams and removed from the list. Equally, any compound which showed binding to the reference protein carbonic anhydrase II was also disqualified. The MEDIAN and the standard deviation (STDEV) over all screened samples were calculated. All compounds showing a FSO $\geq$ MEDIAN + 3 x STDEV were classified as hits and subsequently subjected to SPR Kd measurements as described below.

BRD9 SPR K_D assay

His-tagged BRD9 was immobilized to a density of 2000 - 4000 RUs on flow cells 3 and 4 of a Biacore NTA-chip as described above. Carbonic anhydrase II was immobilized at a similar density on flow cell 2 and a blank reference surface was generated on flow cell 1.

The buffer was then switched to assay buffer (HBS-P+ = 10 mM HEPES, pH 7.4, 150 mM NaCl, 0.05 % P20 + 5 % DMSO) and the chip equilibrated for several hours before use for K_D determinations. To be able to correct for differences in bulk solvent refractive index caused by small variations in the DMSO concentration solvent correction samples were included at the beginning and end of the run as detailed in the instrument manufacturer's instructions.

Compounds were injected in concentration series (1:1 dilutions, 7 different concentrations), starting with a maximum concentration that was approximately 10-20-fold higher than the expected K_D. The concentration series were prepared in 96-well plates. In the case that the dilution window chosen for a particular compound did not appropriately bracket the K_D of the compound the measurement was repeated with an optimized starting concentration. Positive and negative control samples were included at regular intervals to be able to monitor the performance of the assay. CBS was used as a positive control for carbonic anhydrase II to check for integrity of the reference protein at regular intervals. To correct for the excluded volume effect a DMSO calibration series was prepared as detailed in the instrument manufacturer's instructions and the calibration samples were measured at the beginning and end of each run. Kd values were determined using Biaevaluation software by either performing a global fit of the double-referenced association and dissociation data to a 1:1 interaction model or by fitting the steady-state responses at each concentration to a 1:1 interaction model as appropriate. K_Ds from the two flow cells were averaged.

Isothermal Titration Calorimetry (ITC) assay

Protein were cloned, expressed and purified as previously described[6].

Calorimetric experiments were performed on an ITC200 or VP-ITC micro-calorimeter (MicroCalTM, LLC Northampton, MA). Protein solutions were buffer exchanged by dialysis into buffer 20 mM Hepes pH 7.5, 150 mM NaCl, and 0.5 mM TCEP. All measurements were carried out at 293.15 K while stirring at 1000 or 286 rpm. The micro syringe was loaded with protein solutions ranging from 250 to 320 μM, compound solutions were prepared at concentrations between 15 to 30 μM and 200 μl or 2 mL for the cells. All injections were performed using an initial injection of 0.5 μl followed by 30 injections of 1 μl with a duration

of 2 sec per injection and a spacing of 150 sec between injection for the ITC200; for the VP-ITC it was injected 2 µl followed by 34 injections of 8 µl with a duration of 16 sec per injection and a spacing of 240 sec between injections. The data were analysed with the MicroCal ORIGIN software package employing a single binding site model. The first data point was excluded from the analysis. Thermodynamic parameters were calculated ($\Delta G = \Delta H - T\Delta S = -RTlnKB$ where ΔG, ΔH and ΔS are the changes in free energy, enthalpy and entropy of binding, respectively).

Differential Scanning Fluorimetry (DSF)

DSF experiments were carried out in a Bio-Rad CFX384 Real-Time System (C1000Touch Thermal Cycler) in sealed Hard-Shell PCR 384 well plates (#HSP3805; PCR Sealers; #MSB1001; Bio-Rad) and a total volume of 10µl. The assay was optimized regarding protein consumption and SYPRO Orange dye excess (5000x concentration in DMSO, Invitrogen) to obtain a reliable fluorescence signal. Compound dilutions in assay buffer (25 mM HEPES pH 7.5, 150 mM NaCl, 1 mM TCEP) were prepared by a Hamilton Microlab Star pipetting robot. The reaction mixtures contained 8 µl compound dilution and 2µl of the BRD9 SYPRO Orange stock mix to yield final concentrations of 10µM BRD9, 25x SYPRO Orange and 400µM fragment at a DMSO concentration of 2%. Samples were heated at 1°C/min from 25°C to 95°C with fluorescence readings every 0.5°C. The entire BI generic fragment library (1697 compounds) was tested in duplicates. Melting curves were analyzed with the Bio-Rad CFX Manager-Data Analysis software in which T_m values were determined as the minimum of the first derivative of the recorded fluorescence intensity versus temperature plot. The T_m of native BRD9 was determined 200 times (47.2±0.5°C) and an in house positive control 60

times (53.5±0.5°C) from which a Z´ of 0.55 could be calculated. Thermal shifts of $\Delta T_m \geq 1°C$ ($\Delta T_m = T_{m,frag} - T_{m,DMSO}$) were assumed significant and defined as primary FBS hits.

Microscale Thermophoresis (MST)

MST is a fluorescence-based biophysical method which exploits the alteration of the mobility of molecules in a temperature gradient upon binding. The local temperature gradient is induced by an infrared laser and a detailed description of the technique is published elsewhere.[7] Fluorescence labeling of BRD9 with the NT647 dye was performed according to the manufacturer's protocol of the Monolith NT.115 Protein Labeling Kit RED-NHS (NanoTemper Technologies, Munich, Germany). Assay development comprised the optimization of protein concentration, buffer conditions, MST capillaries as well as the strength of the temperature gradient (IR laser power) using an in house positive control to achieve a reliably detectable change in the thermophoretic mobility (ΔF_{norm}) of labeled BRD9. Measurements were carried out in 25mM HEPES pH 7.5, 150 mM NaCl, 1 mM TCEP, 0.05% Tween-20 with 200 nM NT647-labeled BRD9 in the presence of 500 μM compound and 5% (v/v) d6-DMSO at 298K. Monolith NT.115 hydrophilic capillaries, an IR laser power of 40% with a laser on time of 30 seconds and an LED intensity of 60% were used.

For the in house implementation of fully automated sample preparation and MST data acquisition a Monolith NT.015 was modified in collaboration with NanoTemper Technologies and combined with a Hamilton Microlab Star pipetting system equipped with an in house developed tilting station. Individual capillaries were filled by dipping into each well of the vertically tilted 384-well plate (Greiner PP, small volume, deep well from Greiner Bio-One, Frickenhausen, Germany) and transferred in the detection device using a pneumatic gripper (Schunk, Lauffen/Neckar, Germany) attached to a pipetting channel.

Compounds were diluted in 30µl assay buffer with a Hamilton Microlab Star liquid handling system and 10µl of labeled BRD9 was added just-in-time prior to data acquisition to achieve equal incubation times. In one acquisition cycle 16 capillaries could be measured in which capillary 1 and 16 were a DMSO negative control and capillaries 2-14 contained seven fragments in duplicates.

Data was analyzed with the NanoTemper Analysis software version 1.2.205 and exported fluorescence as well as ΔF_{norm} values ($\Delta F_{norm}=F_{hot}/F_{cold}$) further evaluated. All MST traces were inspected manually and irregular traces (e.g. fluorescence quenching, protein aggregation) were discarded. Mean values of the duplicates were calculated and compared to either the mean value of the DMSO negative control in the individual acquisition cycle or the mean value of the DMSO control of the respective 384-well screening plate. An in house positive control was used to monitor integrity of labeled BRD9 throughout screening. Fragments were classified as hits if $\Delta\Delta F_{norm} \geq \Delta F_{norm}$(2sd DMSO) with $\Delta\Delta F_{norm} = \left|\Delta F_{norm}(\text{compound})-\Delta F_{norm}(\text{DMSO})\right|$.

BRD9 NMR spectroscopy

Confirmation of primary FBS hits obtained from DSF, SPR and MST was performed using two-dimensional $^{1}H/^{15}N$ HSQC NMR spectra[8] collected on a Bruker Avance III 600 MHz spectrometer equipped with a 5mm z-gradient TCI cryo-probe and a Bruker Sample Rail. Samples were freshly prepared just-in-time by a Tecan Freedom Evo pipetting robot in house customized for NMR sample tube filling before fully automated data acquisition[9]. Each sample contained 75 µM ^{15}N labeled BRD9 in 20 mM Tris-d11 pH 7.5, 200 mM NaCl, 1 mM TCEP and 8% (v/v) D_2O and was incubated with 500 µM fragment in a 2.5mm NMR tube at 298 K and a d6-DMSO concentration of 1%. Spectra were recorded with 48 transients and 96

data point in the indirect dimension. Identification of BRD9 binders was performed by manual comparison of spectra in the presence of a single fragment and the BRD9 reference spectrum in the presence of 1% (v/v) d6-DMSO using data processed by Bruker Topspin 3.0. For a more quantitative approach chemical shift perturbation was automatically evaluated using the Autoscreen module of Felix 2004 (FelixNMR Inc., San Diego, USA) for which either the entire spectrum or the region of interest (22 cross peaks defined by an in house positive control to identify the preferred binding site) was analyzed[10]. All three analysis methods showed > 90% overlap between confirmed BRD9 binding fragments, which were prioritized for Xray follow-up (see method description "BRD9 X-ray follow-up of confirmed primary FBS hits").

Protein purification and crystallization

The bromodomain of human BRD9 (residues 14-134 of isoform 5, Uniprot identifier Q9H8M2-1) was obtained from the SGC (Structural Genomics Consortium) and has been expressed and purified as previously described[2].

Protein crystallization was done using the hanging drop method by mixing 2.0 µL of apo BRD9 (10 mg/mL in 25 mM HEPES pH 7.5, 300 mM NaCl, 0.5 mM TCEP) with 2 µL of reservoir solution (30 % glycerol ethoxylate, 100 mM Tris pH 8.3) at 4 °C. Crystals grew within a few days to a final size of 150-200 µm). Apo crystals were transferred to a soaking buffer containing 33 % glycerol ethoxylate and soaked overnight by adding 0.1 µL of a 100 mM DMSO stock solution of **1**. Crystals were frozen in liquid nitrogen and data were collected at the SLS beam line X06SA (Swiss Light Source, Paul Scherrer Institute) at a wavelength of 1 Å using the PILATUS 6M detector. The crystals belonged to space group P21212 and contained 2 monomers per asymmetric unit. Images were processed with

autoPROC.[11] The resolution limits were set using default autoPROC settings. The structures were solved by molecular replacement using the BRD9 structure 3HME as a search model. Subsequent model building and refinement was done using standard protocols using CCP4,[12] COOT [13] and autoBUSTER (Bricogne, G. *et al.* (2011) BUSTER v.2.11.2. http://www.globalphasing.com).

For **compound 1** [1] the unit cell parameters were a = 70.80 Å, b = 125.34 Å, c = 29.92 Å and α, β, γ = 90° data and the structure was refined to Rwork and Rfree values of 17.8 % and 19.2 %, respectively, with 100% of the residues in Ramachandran favoured regions as validated with Molprobity.[14]

Compound 3 (unit cell: a = 70.92 Å, b = 125.41 Å, c = 29.53 Å, α, β, γ = 90°, resolution = 1.80 Å) was refined to R/Rfree = 20.3/22.0% with 100 % of the residues in Ramachandran favoured regions.

Compound 4 (unit cell: a = 71.04 Å, b = 125.02 Å, c= 29.94 Å, α, β, γ = 90°, resolution = 1.68 Å) was refined to R/Rfree = 18.9/20.5 %) with 100 % of the residues in Ramachandran favoured regions.

Compound 11 (unit cell: a = 70.31 Å, b= 125.17 Å, c = 30.02 Å, resolution: 2.3 Å) was refined to R/Rfree = 19.2/20.9 % with 99.55 % of the residues in Ramachandran favoured and 0.45 % in Ramachandran allowed regions.

Compound 2 (unit cell: a = 70.03 Å, b = 125.36 Å, c = 29.68 Å, α, β, γ = 90°, resolution = 1.82 Å) was refined to R/Rfree of 19.1/20.2% with 100 % of the residues in Ramachandran favoured regions.

Statistics for data collection and refinement can be found in **Supplementary Table 2**.

Stereo images (wall-eye stereo and cross-exe stereo) can be found in **Supplementary Fig. 5-14**.

BRD9 X-ray follow-up of confirmed primary FBS hits

Protein crystallisation in the FBS setting has been done by the hanging drop method at 20 °C. 1.2 µl of a 14 mg/ml apo protein solution (in 25 mM HEPES pH 7.5, 300 mM NaCl, 0.5 mM TCEP) have been mixed with 1 µl of the reservoir solution (28-33% Glycerol Ethoxylate, 100 mM Tris pH 8.1- 8.7). The resulting crystals were transferred into soaking and cryoprotectant solution (30% Glycerol Ethoxylate, 100 mM Tris pH 8.5) in which compound was dissolved at 100 mM. After overnight incubation crystals were flash frozen in liquid nitrogen. Diffraction data was collected at X06SA and X06DA beamlines of the Swiss Light Source (Paul Scherrer Institute, Switzerland). All following steps have been performed as described in the above section "Protein purification and crystallization".

BRD9 Bromodomain Virtual Screening

Ligand preparation of HiCOS was carried out using Schrodinger's ligprep module. The initial docking was done in Schrodinger's Glide Suite 2012. The standard precision docking mode was employed. The docking protein grid was derived from the in-house X-ray crystal structure of BRD9, including two constraints: one with hydrogen bond donor from the terminal amido_$N^{\delta 2}H$ of Asparigine-100 and the other with the OH of conserved water-106. Follow-up pharmacophore mapping was performed in OpenEye's vROCS 2012. The BRD9 specific pharmacophore model was derived from the binding modes of ligands selected from three in-house X-ray co-crystal structures. ShapeTan and Combifit scoring methods in vROCS were used in combination to select the compounds for further consideration.

BRD9-H3 AlphaScreen assay

This assay is used to identify compounds which inhibit the interaction of the bromodomain of BRD9 with a tetra-acetylated peptide based on the sequence of histone H3 (H3 K9/14/18/23Ac (1-28)). GST-BRD9 protein corresponding to amino acids 130 - 259 that contains the bromodomain of BRD9 (accession number NM_023924.4) was expressed in E. coli with an amino-terminal GST tag. The sequence of the H3 K9/14/18/23Ac(1-28) peptide is Biotin- ARTKQTARK(Ac)STGGK(Ac)APRK(Ac)QLATK(Ac)AARKS, MW: 3392.

Assay concentrations: 4 nM GST-BRD9 protein (aa 130 - 259) and 12 nM biotinylated H3 K9/14/18/23Ac(1-28) peptide are used in the BRD9 H3 AlphaScreen assay.

BRD7 H3 AlphaScreen assay

This assay is used to identify compounds which inhibit the interaction of the bromodomain of BRD7 with a tetra-acetylated peptide based on the sequence of histone H3 (H3 K9/14/18/23Ac (1-28)). GST-BRD7 protein corresponding to amino acids 129 - 236 that contains the bromodomain of BRD7 (accession number NM_013263.4) was expressed in E. coli with an amino-terminal GST tag. The sequence of the H3 K9/14/18/23Ac(1-28) peptide is Biotin- ARTKQTARK(Ac)STGGK(Ac)APRK(Ac)QLATK(Ac)AARKS, MW: 3392.

Assay concentrations: 8 nM GST-BRD7 protein (aa 129 - 236) and 12 nM biotinylated H3 K9/14/18/23Ac(1-28) peptide are used in the BRD7 H3 AlphaScreen assay.

BRD4-BD1 H4 AlphaScreen assay

This assay is used to identify compounds which inhibit the interaction of the bromodomain 1 of BRD4 with a tetra-acetylated peptide based on the sequence of histone H4 (K5/8/12/16(1-

18)). GST-BRD4-1 protein corresponding to amino acids 44 - 168 that contains the bromodomain 1 of BRD4 (accession number NP_490597.1) was expressed in E. coli with an amino-terminal GST tag. Tetra-acetylated histone H4 is a synthetic peptide, containing amino acids 1-18 of Histone H4 followed by a GSGS linker and biotinylated lysine (SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RH-GSGSK-biotin) MW = 2518.7Da.

Assay concentrations: 10 nM GST-BRD4-1 protein (aa 44 - 168) and 5 nM biotinylated H4 (K5/8/12/16(1-18) peptide are used in the BRD4-BD1 H4 AlphaScreen assay.

BRD4-BD2 AlphaScreen assay

This assay is used to identify compounds which inhibit the interaction of the bromodomain 2 of BRD4 with a tetra-acetylated peptide based on the sequence of histone H4 (K5/8/12/16(1-18)). GST-BRD4-2 protein corresponding to amino acids 333 - 460 that contains the bromodomain 2 of BRD4 (accession number NP_490597.1) was expressed in E. coli with an amino-terminal GST tag. Tetra-acetylated histone H4 is a synthetic peptide, containing amino acids 1-18 of Histone H4 followed by a GSGS linker and biotinylated lysine (SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RH-GSGSK-biotin) MW = 2518.7 Da.

Assay concentrations: 50 nM GST-BRD4-2 protein (aa 333 - 460) and 25 nM biotinylated H4 (K5/8/12/16(1-18) peptide are used in the BRD4-BD2 H4 AlphaScreen assay.

BRD2-BD1 AlphaScreen assay

This assay is used to identify compounds which inhibit the interaction of the bromodomain 1 of BRD2 with a tetra-acetylated peptide based on the sequence of histone H4 (K5/8/12/16(1-18)). GST-BRD2-1 protein corresponding to amino acids 71 - 194 that contains the

bromodomain 1 of BRD2 (accession number NP_005095.1) was expressed in E. coli with an amino-terminal GST tag. Tetra-acetylated histone H4 is a synthetic peptide, containing amino acids 1-18 of Histone H4 followed by a GSGS linker and biotinylated lysine (SGRGK(Ac)GGK(Ac)GLGK(Ac)GGAK(Ac)RH-GSGSK-biotin) MW = 2518.7Da.

Assay concentrations: 20 nM GST-BRD2-1 protein (aa 333 - 460) and 10 nM biotinylated H4 (K5/8/12/16(1-18) peptide are used in the BRD4-BD2 H4 AlphaScreen assay.

All AlphaScreen assays are done in a darkened room below 100 Lux. Compounds are dispensed onto assay plates (Proxiplate-384 PLUS, white, PerkinElmer) using an Access Labcyte Workstation with the Labcyte Echo 550 from a DMSO solution. For the chosen highest assay concentration of 100 μM, 150 nL of compound solution are transferred from a 10 mM DMSO compound stock solution. A series of 11 concentrations is transferred for each compound at which each concentration is fivefold lower than the previous one. DMSO is added such that every well has a total of 150 nL compound solution. 10 μL of a mix containing protein and peptide with the assay specific concentrations are prepared in assay buffer (50 mM HEPES pH=7.3; 25 mM NaCl; 0,1% Tween 20; 0.1% bovine serum albumin (BSA); 2 mM dithiothreitol (DTT)) and 5 μL of bead mix (AlphaLISA Glutathione Acceptor Beads and AlphaScreen Streptavidin Donor Beads mixed in assay buffer at a concentration of 10 μg/ml each) are added to the assay plate that contain 150 nL of the compound solution. After 60 minutes at room temperature the signal is measured in a PerkinElmer Envision HTS Multilabel Reader using the AlphaScreen specifications from PerkinElmer. Each plate contains negative controls where assay specific peptide and protein are left out and replaced by assay buffer. Negative control values are entered as low basis for normalization. IC50 values are calculated using a four parameter non-linear regression model.

BROMOscan

Bromodomain profiling was provided by DiscoveRx Corp. using their BROMOScan platform (http://www.discoverx.com/services/drug-discovery-development-services/epigeneticprofiling/Bromoscan). The BROMOscan screen accounted for the determination of the single concentration binding interaction (percent of control - % ctrl) for compound **1**, or **2**, and each of the 32 DNA tagged bromodomains included in the assay by binding competition against a reference immobilized ligand. BromoKdELECT accounted for the determination of the K_D between compound **1**, or **2**, and selected DNA tagged bromodomains by binding competition against a reference immobilized ligand.

Fluorescence Recovery After Photobleaching (FRAP)

FRAP studies were performed essentially as described[15] (1). In brief, U2OS cells were transfected (Fugene HD; Roche) with mammalian over-expression constructs encoding GFP fused to the N-terminus of full length BRD9, Brd7 or CECR2, respectively. Mutant proteins mutating the conserved Asn to Phe or Ala were generated as described in (1). The following mutations were introduced: N140A for human CECR2, N211F for mouse Brd7 and N163F for human BRD9. The imaging system consisted of a Zeiss LSM 710 laser-scanning and control system (Zeiss) coupled to an inverted Zeiss Axio Observer.Z1 microscope equipped with a high-numerical-aperture (N. A. 1.3) 40 x oil immersion objective (Zeiss). Samples were placed in an incubator chamber in order to maintaining temperature and humidity. FRAP and GFP fluorescence imaging were both carried out with an argon-ion laser (488 nm) and with a PMT detector set to detect fluorescence between 500-550 nm. Once an initial scan had been taken, a region of interest corresponding to approximately 50 % of the entire GFP positive nucleus was empirically selected for bleaching. A time lapse series was then taken to

record GFP recovery using 1% of the power used for bleaching. The image datasets and fluorescence recovery data were exported from ZEN 2009, the microscope control software, into Origin to determine the average half-time for full recovery for 10-20 cells per treatment point. Data were analysed using one-way ANOVA with Tukey's multiple comparisons test.

Cell lines and proliferation assays

Cells were grown in 50 µl medium as specified by the supplier for 7 days starting with 500 and with 1000 cells per well of a 384 well plate in the presence of varying concentrations of compound before measuring viability via cellular ATP levels using the cell titer glow assay (Promega).

MYC assay

To 750,000 MV-4-11 cells in 250 µl growth medium (in IMDM, 10 % FBS, GlutaMAX, 25 mM HEPES and 0,1% 2-Mercaptoethanol) per well compound was added at the desired concentration from a 10 mM stock solution using the HP D300 Digital Dispenser (Hewlett Packard). After 2 hours of incubation with the compound, cells were collected by centrifugation, washed in ice cold PBS and lysed in 15 µl of cell extraction buffer (Life Technologies #FNN0011 containing 1 mM PMSF and 1x Halt protease inhibitor cocktail (Thermo)). After 30 minutes on ice, nucleic acids were disrupted by sonication. cMYC levels were measured using the human c-Myc (Total) ELISA Kit (Life Technologies #KHO2041)

Animal handling

Mice were housed under pathogen-free conditions (AAALAC accredited facility) and treated according to the institutional, governmental and European Union guidelines (GV-SOLAS, Felasa, Austrian Animal Protection Laws). All animal studies were reviewed and approved by the internal ethics committee of Boehringer Ingelheim and the local governmental committee (Amt der Wiener Landesregierung, Magistratsabteilung 58, Vienna, Austria).

Pharmacokinetic and efficacy studies in mice

For evaluation of PK properties non-tumor bearing BomTac:NMRI-Foxn1nu mice (Taconic, Ry, Denmark) were treated once with an intravenous bolus dose formulated with 25 % HP-β-CD (dosing volume 5 mL/kg), or orally as a suspension formulated with 0.5% Natrosol^{TM} Hydroxyethylcellulose (dosing volume 10 mL/kg). EDTA-blood was sampled from the *Vena saphena* and plasma was obtained by centrifugation.

Female CIEA-NOG mice (NOD.Cg-*Prkdc*^{scid}*IL2rg*^{tm1Sug}/JicTac; Taconic, Ry, Denmark) were engrafted intravenously with 1×10^7 EOL-1 AML cells stably expressing luciferase and GFP. Following injection of the cells animals were randomized based on body weight (n=10/group). Treatment started on day 5 with either 0.5% Natrosol or **2** formulated with 0.5% Natrosol. All doses were calculated relative to the mouse body weight on the treatment day. **2** and the vehicle control were administered orally with a dosing volume of 10 mL/kg body weight. **2** was administered daily from day 5 until 17 and from day 20 until 22. Dosing was interrupted on day 18 for two days as one mouse in the treatment group reached -15% body weight loss. Tumour load was measured 2-3 times weekly based on bioluminescence imaging as described previously.[16] The following scoring system was used: score 0, no clinical signs; score 1, tail or hind limb weakness. Animals were sacrificed based on severity criteria including appearance of paralysis score 1 and/or body weight loss exceeding -18%. In

this tumor mouse model body weight changes can occur due to increased tumor load or due to intolerability.

Determination of physicochemical and *in vitro* DMPK parameters

Aqueous solubility was determined from 10 mM stock solutions of the compounds in DMSO diluted with aqueous McIlvaine buffer at pH 6.8, or with acetonitrile/water (1:1) as a reference. Samples were shaken for 24 h at room temperature in 96-well plates (Whatman Uniplate® 96 wells, 750 µL, polypropylene, round bottom). The plate was then centrifuged at 3,000 rpm for 2 min. 250 µL of each sample were transferred to a Millipore MultiScreenHTS filter plate with a polycarbonate membrane, pore size 0.45 µm. Filtrates were collected by centrifugation at 3,000 rpm for 2 min. The dissolved concentrations were determined by UPLC/UV on a Waters ACQUITY UPLC® SQD system equipped with a Waters ACQUITY UPLC® BEH 2.1x50 mm C18 column, particle size 1.7 µm, using a short gradient with water/0.1% formic acid as solvent A and acetonitrile/0.1 % formic acid as solvent B (5 to 95 % B with 1.7 min total cycle time). Compound signals were measured with a photodiode array UV detector operated at 254 nm. Solubility was determined with a one point calibration by comparing peak areas relative to the reference standard using Waters Empower software.

In vitro predictions of hepatic metabolic (CL) based on incubations with cryopreserved hepatocytes were carried out with an automated assay in a 24-well plate format on a Tecan robotic system at a test compound concentration of 1 µM. Cryopreserved hepatocytes (donor pools) were supplied by Celsis IVT. Cryopreserved cells were thawed according to protocols provided by the vendor and suspended in DMEM supplemented with insulin (5 µg/mL), glucagon (7 ng/mL), hydrocortisone (7.5 µg/mL) and serum of the respective species (50% of total volume). Test compounds were added after a 30 min pre-incubation. Suspensions of

$1x10^6$ cells/mL were incubated and continuously shaken in a Thermo Scientific Cytomat™ for 4 h at 85-95% relative humidity and 5-10% CO_2 at 37 °C. Aliquots were taken from the medium at 0, 0.5, 1, 1.5, 2.5 und 4 h and concentrations of the test compounds quantified by HPLC/MS/MS on a BIOCIUS Life Sciences RapidFire® system coupled to a Thermo Scientific™ TSQ Vantage™ triple-quadrupole mass spectrometer. Predicted clearances were calculated using the well-stirred model.

In vitro plasma protein binding (PPB) was determined by a semi-automated equilibrium dialysis assay on a Tecan robotic system at a test compound concentration of 3 µM. Dialysis chambers in custom-made Teflon devices for multiple parallel incubations were separated by a 18x18 cm dialysis membrane with molecular weight cut-off of 5,000 Da (Dianorm No. 5214). Plasma of the respective species spiked with test compound was dialyzed against Soerensen buffer (pH 7.4) for 3 h at 37 °C at 12 rpm on an overhead rotator. PPB was calculated based on test compound concentrations in the plasma and buffer compartments quantified by HPLC/MS/MS.

Caco-2 and *in vitro* cytochrome P450 inhibition assays have been carried out as described elsewhere.[17]

Determination of pharmacokinetic properties

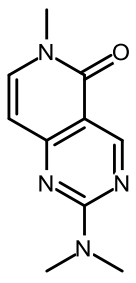
Compound concentrations from aliquots of 10 µL plasma were quantified by HPLC-MS/MS at unit mass resolution with ESI+ ionization. The BI proprietary compound BIBI1355BS was added to all samples as internal standard. Calibration and quality control samples were prepared using blank plasma from untreated animals as matrix. Calibration standards were prepared by serial dilutions in twelve steps by manual dilutions or using a Perkin-Elmer Janus automated liquid handling system. Pre-analytical sample preparation was carried out by

liquid/liquid extraction in a 96-well plate format with t-butyl methyl ether (TBME) or ethyl acetate under basic conditions, or by acetonitrile precipitation and centrifugation. Extracted plasma samples or supernatants of precipitated samples were evaporated to dryness under N_2, redissolved in 25% methanol/75% water/0.01% formic acid and injected to the HPLC/MS/MS system with a CTC PAL HTS-xt autosampler (injection volume 5 µL for *i.v.* and 1 µL for *p.o.* studies).

Quantitative analyses were performed with Agilent HP1200 analytical HPLC systems equipped with a Waters XBridge BEH C18 reversed phase HPLC column at room temperature (particle size 2.5 µm, column dimension 2.1 x 50 mm), applying a HPLC gradient with 5 mM ammonium acetate (pH 4.0) as solvent A and acetonitrile with 0.1% formic acid as solvent B with a cycle time of 2.0 min per sample. Solvent B was increased from 5 to 95% over 1 min, and then kept constant at 95% B from 1.0 - 1.3 min, before returning to 5% B and column re-equilibration from 1.4 to 2.0 min. The HPLC systems were coupled to SCIEX API5000 triple quadrupole or 4000 QTRAP® hybrid triple quadrupole/linear ion trap mass spectrometers operated in MRM mode with ion transitions of 354.2 to 309.0 for **1** and **2**, and 467.3 to 98.1 for BIBI1355BS (dwell times: 70 ms). Declustering potential (DP) and collision energy (CE) settings were automatically optimized using the SCIEX DiscoveryQuantTM 2.1 software. The source temperature of the Turbo Ion Spray source was set to 600°C. Chromatograms were integrated and peak areas were determined with Analyst® 1.5.1 (SCIEX). Pharmacokinetic parameters were calculated by non-compartmental analysis using the Boehringer Ingelheim proprietary software ATLAS. AUC values were determined by the linear trapezoidal rule and with C(0) = C(t1) for intravascular application and with C(0) = 0 for extravascular application.

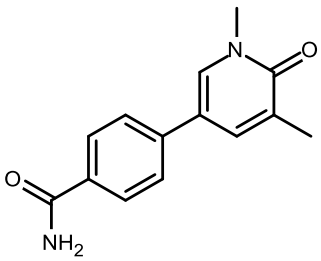
Supplementary Figure 1. Screening cascades leading to the identification of compounds **3** and **4** as validated hits

Triage Assay	**Prospective Criteria**	**Generic FBS Library***			**Virtual Screening of HiCos compounds****	
Total compounds screened		1697			208	
Screening Assay (fixed conc.)		**DSF** dTm ≥1°C (400 µM)	**SPR** BR ≥20% (100 µM)	**MST** ΔMST (DMSO, Cpd) ≥ MST(2sd DMSO) (500 µM)	**DSF** dTm≥1°C (500 µM)	**SPR** BR ≥8% (100 µM)
		36	45	124	25	23
Orthogonal Assay	Validation	34	38	38	11	23
		77 (^{15}N HSQC NMR)			23 (SPR Kd)	
X ray co-crystal structure		55			11	
Screening Assay SPR	K_D<100µM	12			13	
Resynthesis/ Verification	Activity /Structure confirmation	12			12	
Unwanted Fragment					1	
Validated Hits with K_D(SPR)<100µM		**12**			**11**	



3

K_D(SPR)=37.5 µM



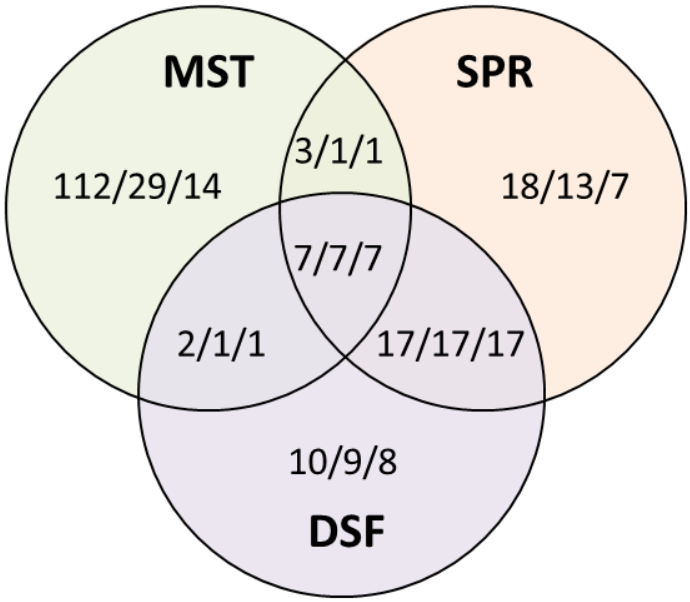
4

K_D(SPR)=9.1 µM

*** Generic FBS library** consists of a diverse set of compounds fulfilling the following constraints: MW 90-270 Da, clogP 0-3, TPSA 20-120, H-bond donors ≤3, H-bond acceptors ≤6, rotatable bonds ≤4, unwanted fragment excluded, DMSO solubility >50mM, solubility buffer pH 7.4 >100µM, LC/MS purity >80%, NMR purity >80%

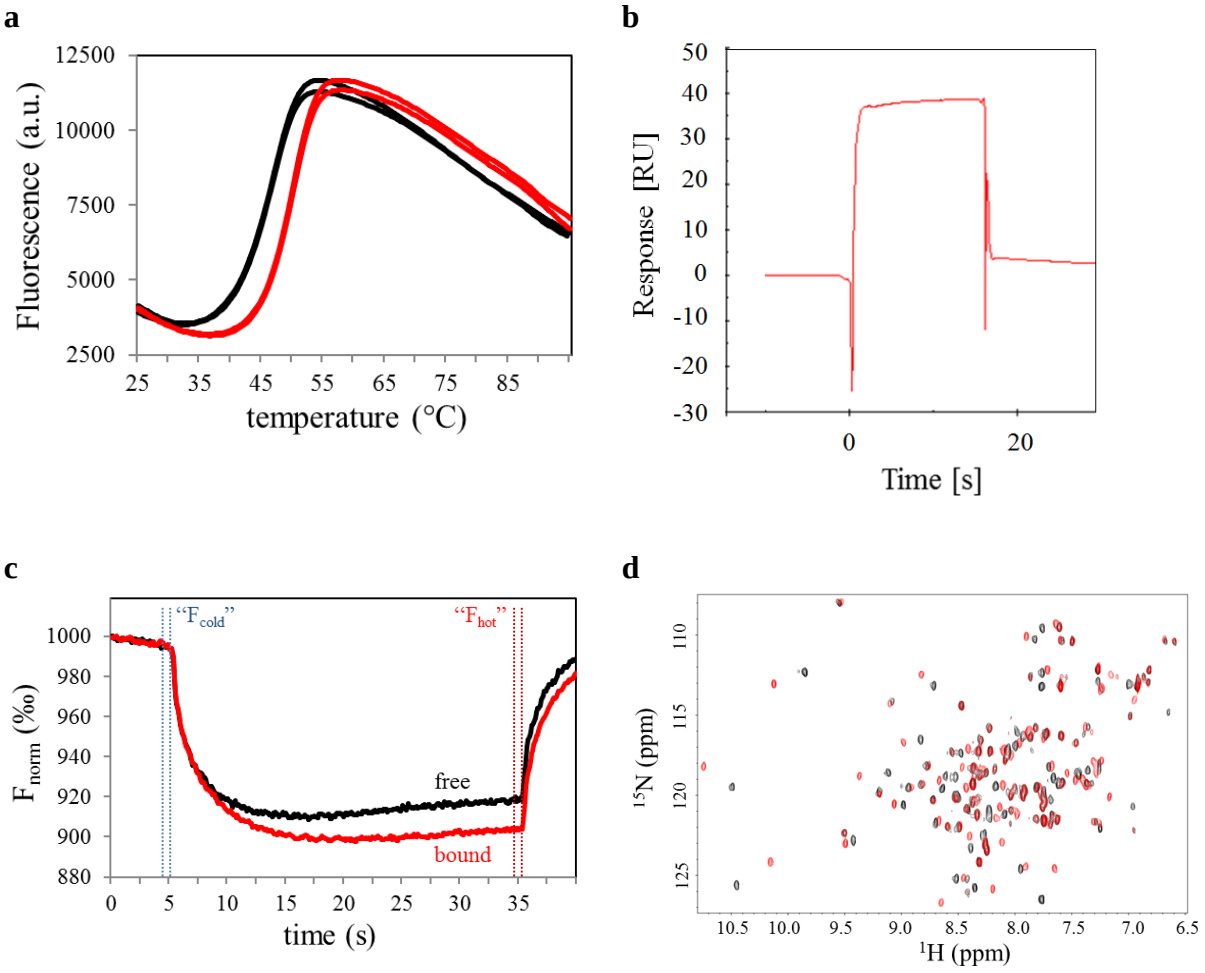
** **HiCos library** consists of a diverse set of compounds fulfilling the following constraints: MW <300 Da, clogP <6, TPSA <150, H-bond donors + H-bond acceptors >0, rotatable bonds <12, unwanted fragment excluded, stock solution 50mg/mL

Supplementary Figure 2. Overlap analysis of the primary fragment screening as well as follow up results (primary hits / 2D $^{1}H/^{15}N$ NMR confirmed / X-ray co-crystal structure) for the "generic FBS library"

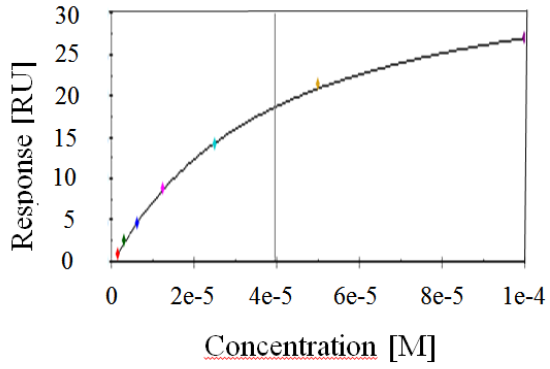


Supplementary Figure 3. Experimental screening data for compound **3**.

a) DSF melting curves (SYPRO Orange fluorescence emission as a function of temperature) of the DMSO negative control (black, T_m=47°C) and compound **3** (red) in duplicates reveals a stabilisation of BRD9 of 2.9°C by compound **3**. 10µM BRD9 was incubated with 400µM compound **3** and 25x SYPRO Orange at a DMSO concentration of 2%; b) SPR primary screen data ([**3**]=100 µM). Compound **3** shows a robust fast on/off response; c) MST screening traces of the DMSO negative control (black) and compound **3** (red). A $\Delta\Delta F_{norm}$ of 22.1 ‰ with a laser power of 40% and a laser on-time of 30s depicts a clear binding signal for compound **3**. 200nM NT647-labeled BRD9 was incubated with 500µM compound **3** at a final DMSO concentration of 5%; d) Confirmation of primary FBS hits bei 2D $^{1}H/^{15}N$ HSQC NMR. Significant chemical shift perturbation observed which is indicative for site specific binding of compound **3** to the bromodomain of BRD9 (75µM ^{15}N labeled protein in the presence of 500µM compound **3** and 1% d6DMSO). Alterations in the complex spectrum are very similar to those obtained for an in-house positive control suggesting that compound **3** binds to the acetyl-lysine binding site; e) SPR Kd data for **3**. The equilibrium binding data can be fitted to a 1:1 binding model, yielding a Kd of 39 µM.



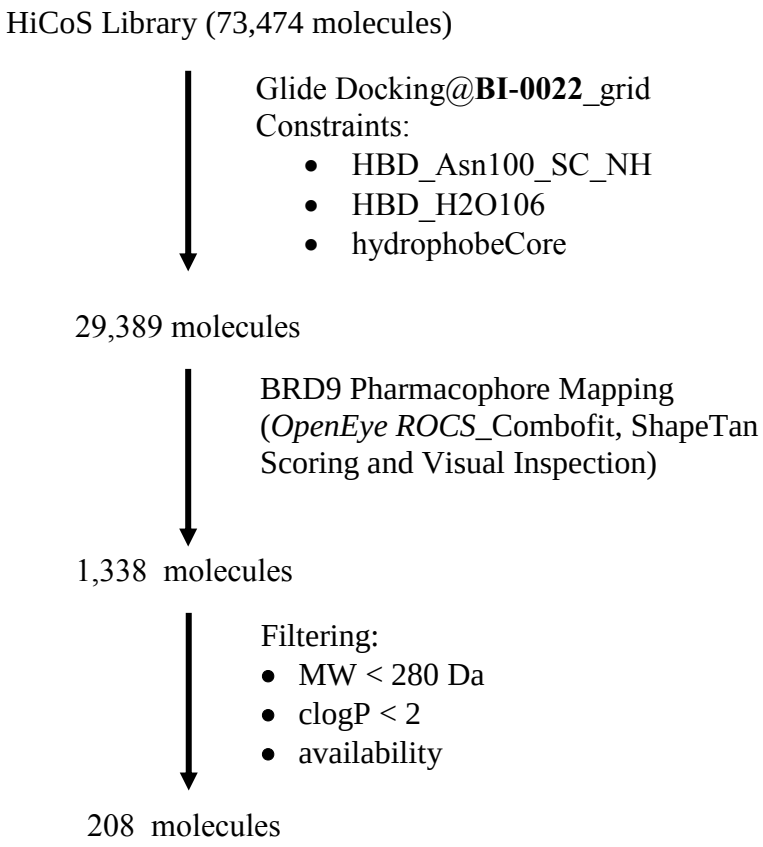
e



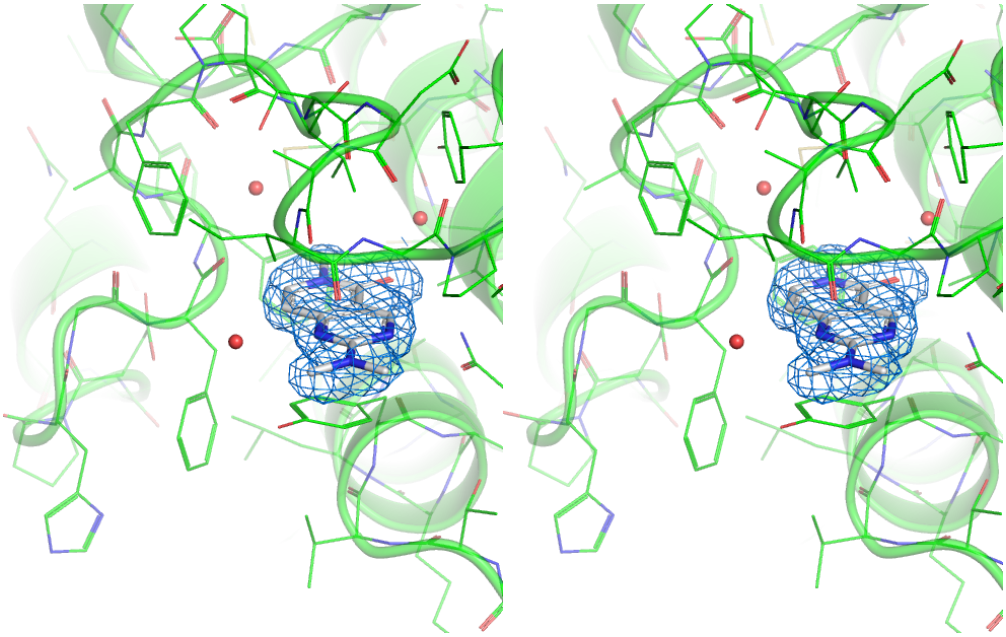
Report | Parameters

KD (M)	Rmax (RU)	offset (RU)	Chi² (RU²)
3.959E-5			0.0806
	38.57	-0.6238	

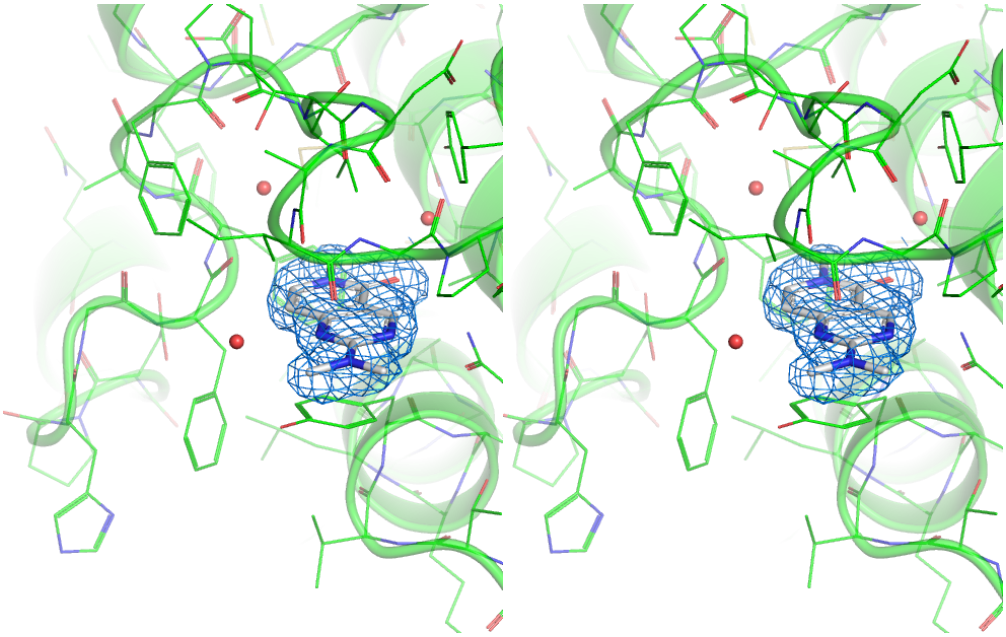
Supplementary Figure 4. Virtual Screening of HiCoS compounds cascade leading to the selection of 208 molecules: docking in BRD9 BD, followed by pharmacophore shape-based mapping.



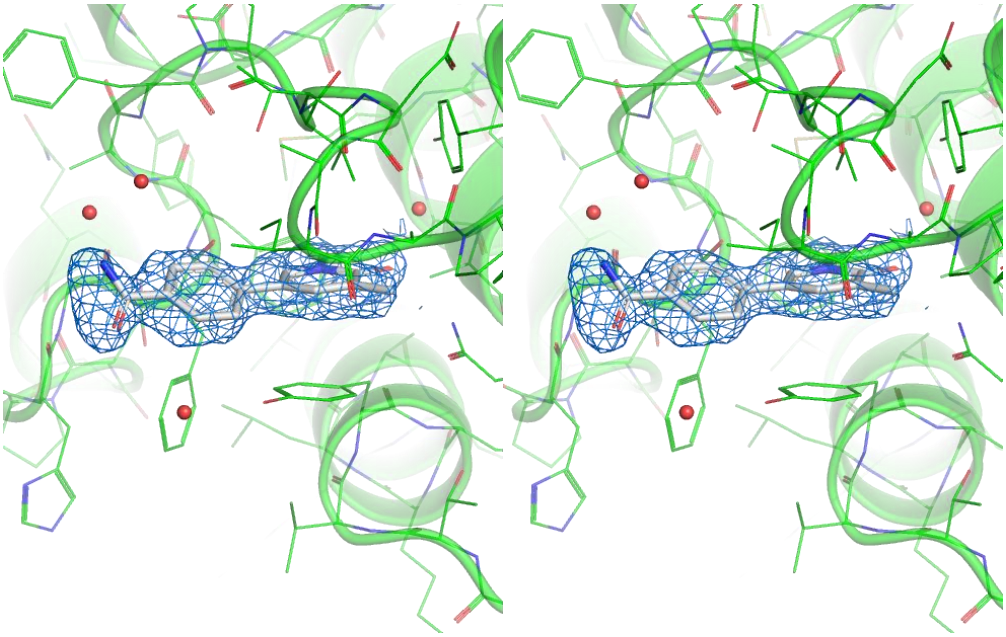
Supplementary Figure 5. Stereo image of **compound 3** (PDB code 5F2P) bound to BRD9 (wall-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



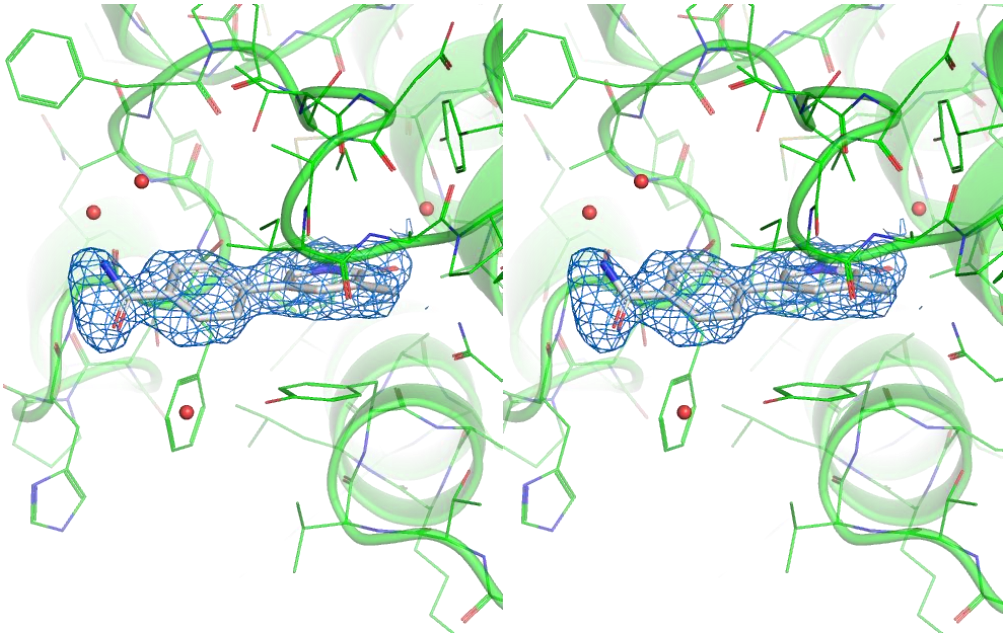
Supplementary Figure 6. Stereo image of **compound 3** (PDB code 5F2P)bound to BRD9 (cross-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



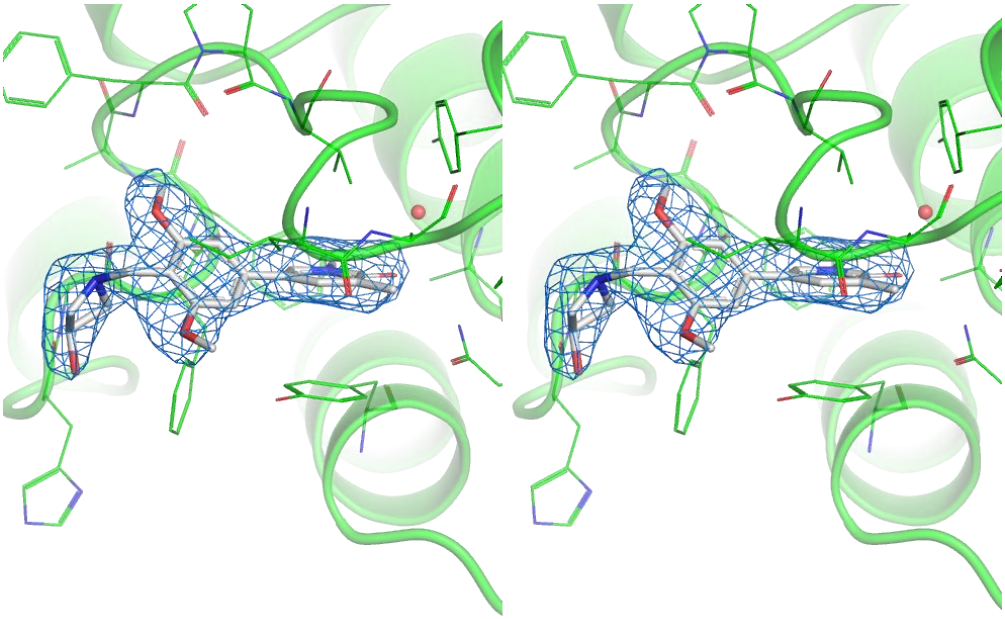
Supplementary Figure 7. Stereo image of **compound 4** (PDB code 5F25) bound to BRD9 (wall-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



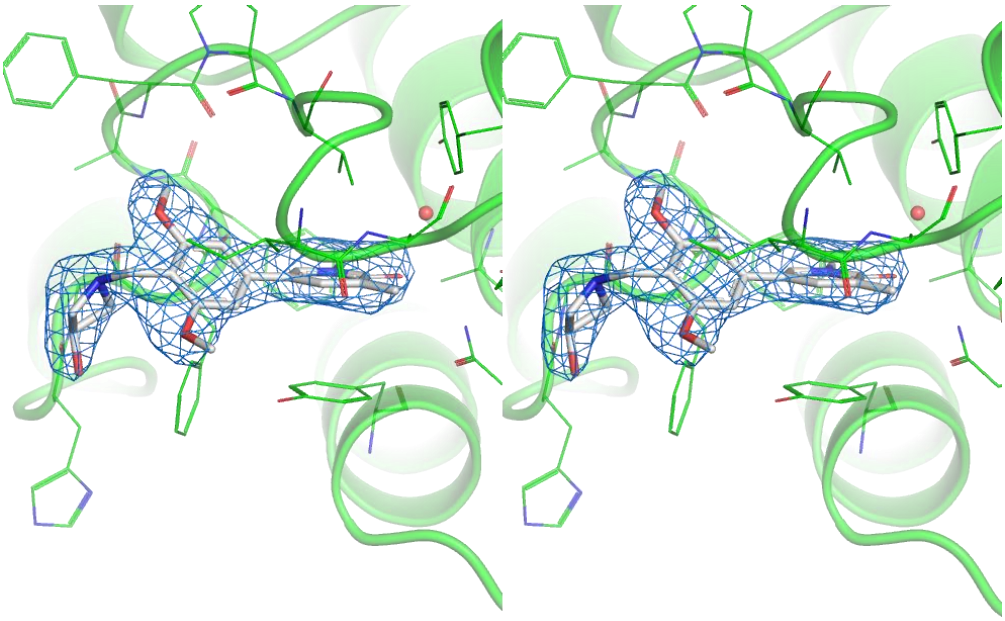
Supplementary Figure 8. Stereo image of **compound 4** (PDB code 5F25) bound to BRD9 (cross-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



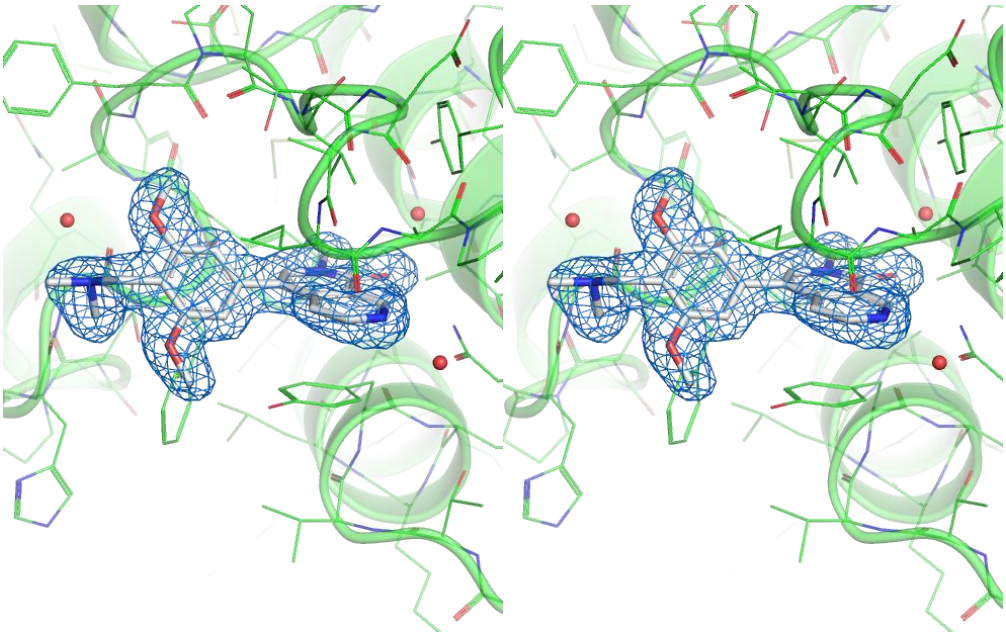
Supplementary Figure 9. Stereo image of **Compound 11** (PDB code 5F1L) bound to BRD9 (wall-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



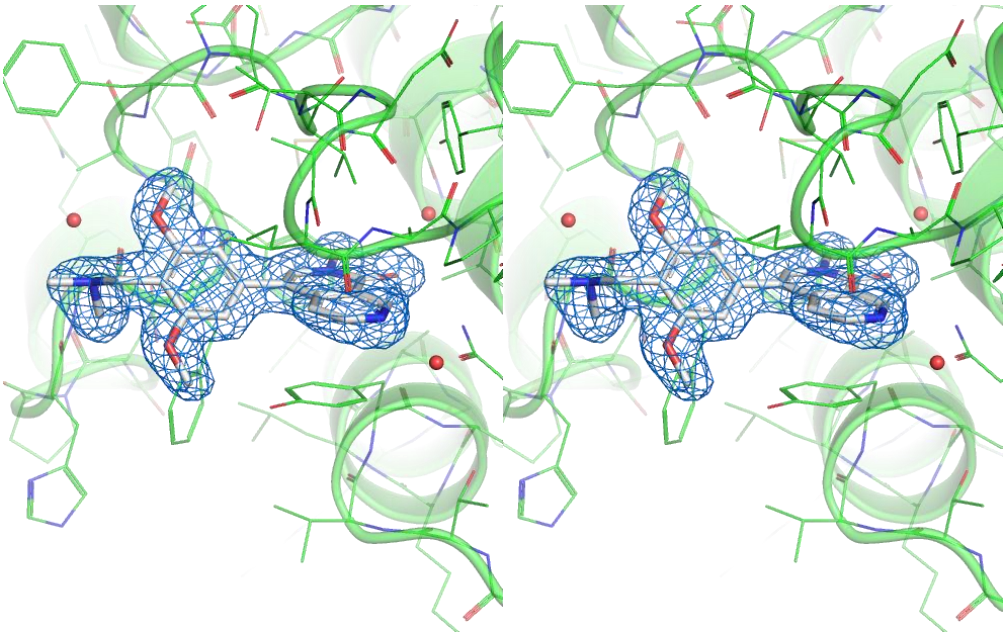
Supplementary Figure 10. Stereo image of **Compound 11** (PDB code 5F1L) bound to BRD9 (cross-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ



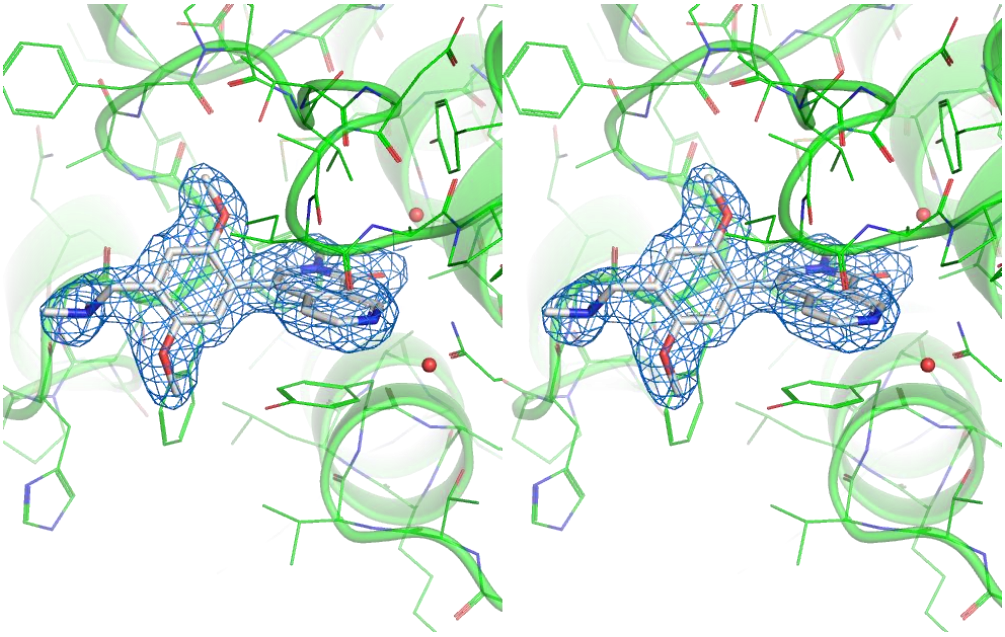
Supplementary Figure 11.[1] Stereo image of **1** (PDB code 5EU1) bound to BRD9 (wall-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



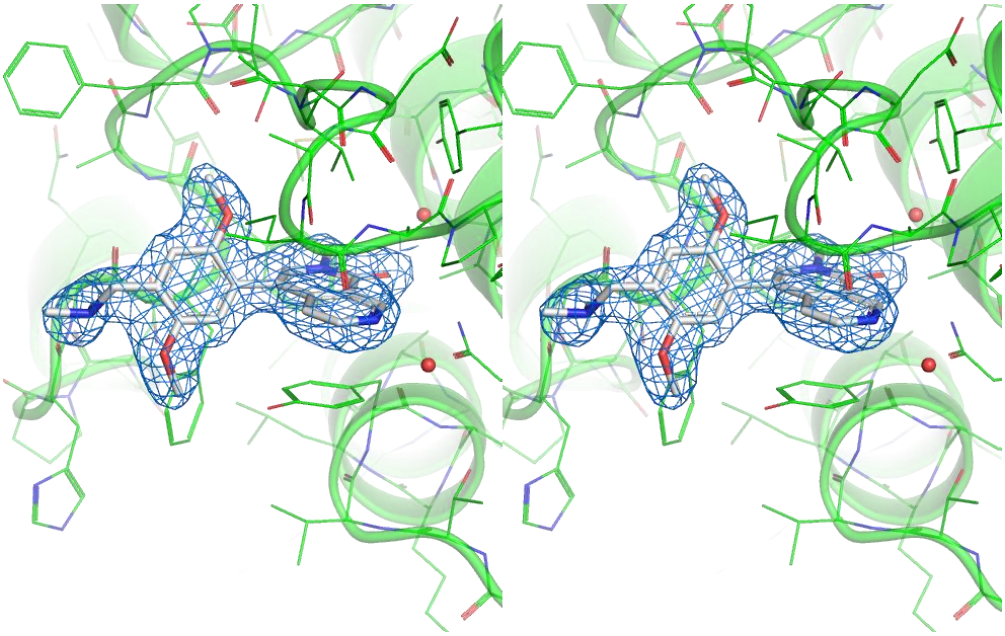
Supplementary Figure 12.[1] Stereo image of **1** (PDB code 5EU1) bound to BRD9 (cross-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



Supplementary Figure 13. Stereo image of **2** (PDB code 5F1H) bound to BRD9 (wall-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



Supplementary Figure 14. Stereo image of **2** (PDB code 5F1H) bound to BRD9 (cross-eye stereo). The refined $2F_o$-F_c electon density is countoured at 1 σ.



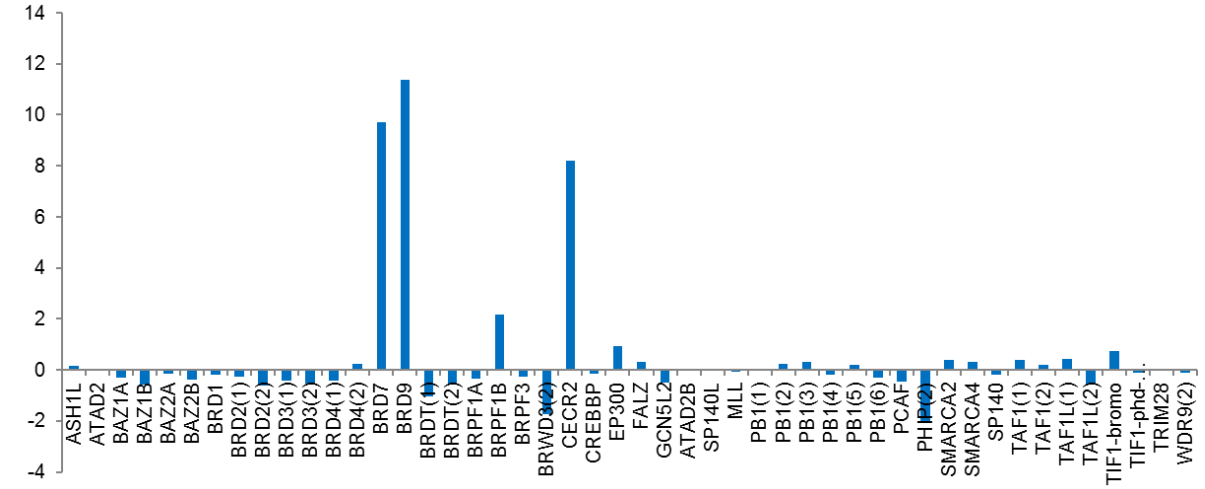
Supplementary Figure 15. Bromodomain selectivity profile for **1** using differential scanning fluorimetry against 48 bromodomains **a**) thermal shift difference upon treatment with **1** (ΔTm °C) **b)** histogram representation of the selectivity pattern using DSF.

1 showed binding to BRD9, BRD7 and CECR2 bromodomains. **1** is highly selective towards the BET family members.

a

target	
ASH1L	0.19
ATAD2	-0.04
BAZ1A	-0.3
BAZ1B	-0.57
BAZ2A	-0.14
BAZ2B	-0.38
BRD1	-0.17
BRD2(1)	-0.26
BRD2(2)	-0.6
BRD3(1)	-0.41
BRD3(2)	-0.51
BRD4(1)	-0.4
BRD4(2)	0.23
BRD7	9.74
BRD9	11.38
BRDT(1)	-1.03
BRDT(2)	-0.55
BRPF1A	-0.34
BRPF1B	2.2
BRPF3	-0.26
BRWD3(2)	-1.71
CECR2	8.22
CREBBP	-0.15
EP300	0.95
FALZ	0.33
GCN5L2	-0.48
ATAD2B	0
SP140L	0
MLL	-0.05
PB1(1)	0
PB1(2)	0.23
PB1(3)	0.31
PB1(4)	-0.18
PB1(5)	0.22
PB1(6)	-0.31
PCAF	-0.46
PHIP(2)	-2.05
SMARCA2	0.42
SMARCA4	0.32
SP140	-0.17
TAF1(1)	0.4
TAF1(2)	0.21
TAF1L(1)	0.43
TAF1L(2)	-0.56
TIF1-bromo	0.76
TIF1-phd-bromo	-0.08
TRIM28	0.06
WDR9(2)	-0.1

b


14
12
10
8
6
4
2
0
-2
-4
ASH1L
ATAD2
BAZ1A
BAZ1B
BAZ2A
BAZ2B
BRD1
BRD2(1)
BRD2(2)
BRD3(1)
BRD3(2)
BRD4(1)
BRD4(2)
BRD7
BRD9
BRDT(1)
BRDT(2)
BRPF1A
BRPF1B
BRPF3
CECR2
CREBBP
EP300
FALZ
GCN5L2
ATAD2B
SP140L
MLL
PB1(1)
PB1(2)
PB1(3)
PB1(4)
PB1(5)
PB1(6)
PCAF
SMARCA2
SMARCA4
SP140
TAF1(1)
TAF1(2)
TAF1L(1)
TAF1L(2)
TIF1-bromo
TRIM28
WDR9(2)

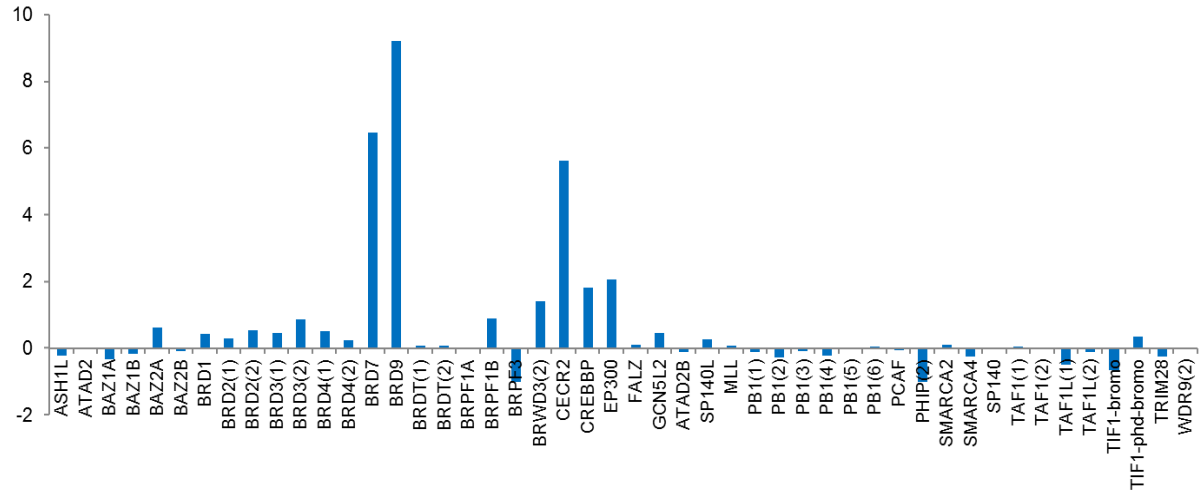
Supplementary Figure 16. Bromodomain selectivity profile for **2** using differential scanning fluorimetry against 48 bromodomains **a**) thermal shift difference upon treatment with **2** (ΔTm °C) **b)** histogram representation of the selectivity pattern using DSF**.**

2 showed binding to BRD9, BRD7 and CECR2 bromodomains. **2** is highly selective towards the BET family members.

a

Protein	
ASH1LA-p017	-0.23
ATAD2A-p023	0.01
BAZ1AA-p007	-0.34
BAZ1BA-p010	-0.17
BAZ2AA-p006	0.62
BAZ2BA-p028	-0.09
BRD1A-p020	0.43
BRD2A-p052	0.3
BRD2A-p058	0.54
BRD3A-p070	0.47
BRD3A-p071	0.87
BRD4A-p088	0.51
BRD4A-p093	0.23
BRD7A-p009	6.47
BRD9A-p022	9.21
BRDTA-p056	0.07
BRDTA-p057	0.09
BRPF1A-p020	-0.03
BRPF1B-p006	0.89
BRPF3A-p010	-1
BRWD3A-p010	1.4
CECR2A-p021	5.61
CREBBPA-p068	1.82
EP300A-p028	2.07
FALZA-p019	0.1
GCN5L2A-p013	0.45
KIAA1240A-p016	-0.1
LOC93349A-p026	0.28
MLLA-p016	0.07
PB1A-p096	-0.11
PB1A-p101	-0.28
PB1A-p102	-0.08
PB1A-p103	-0.23
PB1A-p104	0.01
PB1A-p106	0.06
PCAFA-p029	-0.06
PHIPA-p020	-1.01
SMARCA2A-p025	0.11
SMARCA4A-p020	-0.25
SP140A-p013	0.02
TAF1A-p023	0.05
TAF1A-p027	-0.03
TAF1LA-p036	-0.48
TAF1LA-p041	-0.11
TIF1A-p039	-0.66
TIF1A-p041	0.36
TRIM28A-p008	-0.24
WDR9A-p015	-0.02

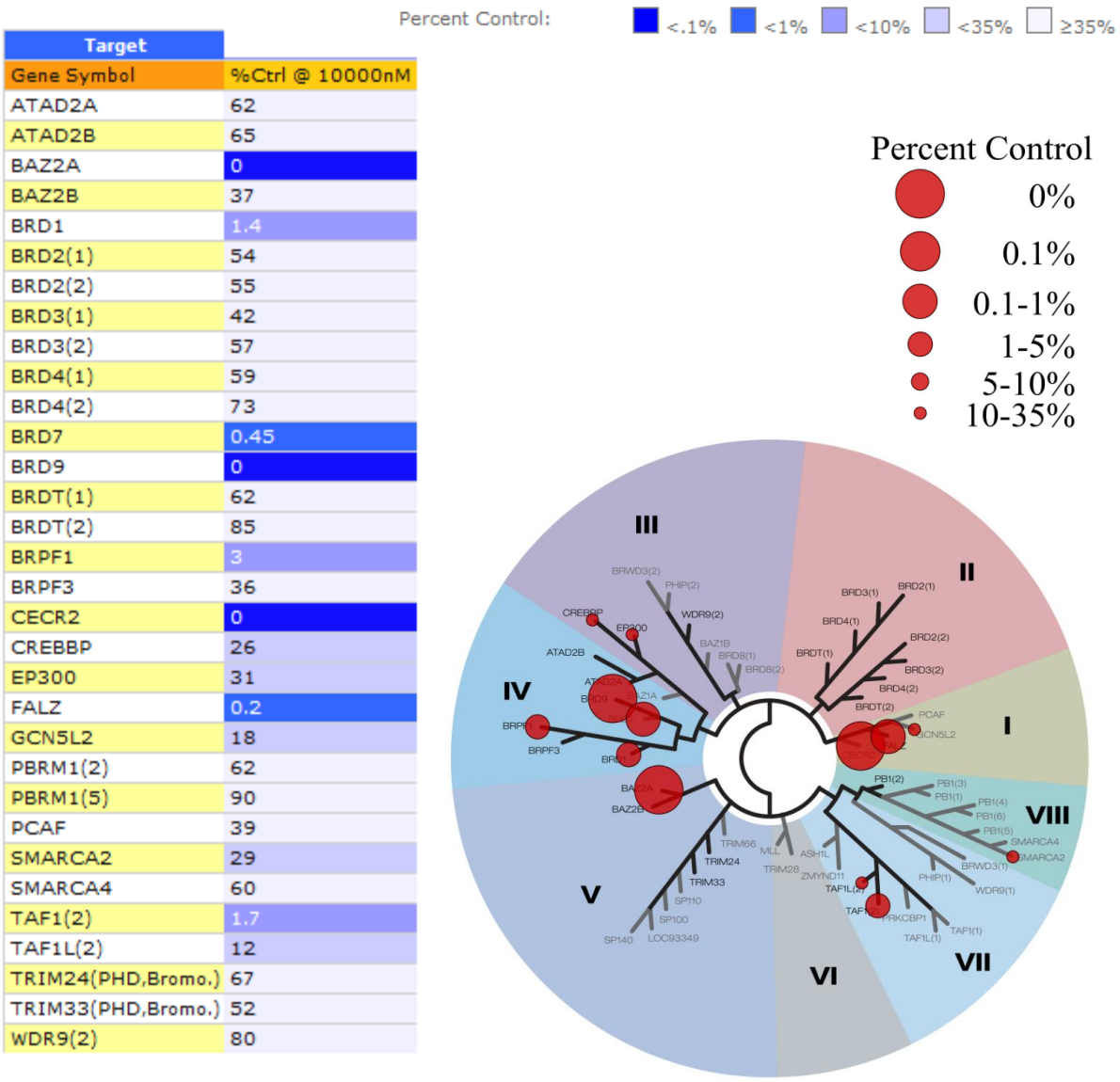
b


10
8
6
4
2
0
-2
ASH1L
ATAD2
BAZ1A
BAZ1B
BAZ2A
BAZ2B
BRD1
BRD2(1)
BRD2(2)
BRD3(1)
BRD3(2)
BRD4(1)
BRD4(2)
BRD7
BRD9
BRDT(1)
BRDT(2)
BRPF1A
BRPF1B
BRPF3
BRWD3(2)
CECR2
CREBBP
EP300
FALZ
GCN5L2
ATAD2B
SP140L
MLL
PB1(1)
PB1(2)
PB1(3)
PB1(4)
PB1(5)
PB1(6)
PCAF
PHIP(2)
SMARCA2
SMARCA4
SP140
TAF1(1)
TAF1(2)
TAF1L(1)
TAF1L(2)
TIF1-bromo
TIF1-phd-bromo
TRIM28
WDR9(2)

Supplementary Figure 17. DiscoveRx selectivity data for **1.**[1]
a) % ctrl bromoscan selectivity data at 10 µM (table and phylogenetic tree); **b**) BromoMax Kd for selected bromodomains

a

Target	
Gene Symbol	%Ctrl @ 10000nM
ATAD2A	62
ATAD2B	65
BAZ2A	0
BAZ2B	37
BRD1	1.4
BRD2(1)	54
BRD2(2)	55
BRD3(1)	42
BRD3(2)	57
BRD4(1)	59
BRD4(2)	73
BRD7	0.45
BRD9	0
BRDT(1)	62
BRDT(2)	85
BRPF1	3
BRPF3	36
CECR2	0
CREBBP	26
EP300	31
FALZ	0.2
GCN5L2	18
PBRM1(2)	62
PBRM1(5)	90
PCAF	39
SMARCA2	29
SMARCA4	60
TAF1(2)	1.7
TAF1L(2)	12
TRIM24(PHD,Bromo.)	67
TRIM33(PHD,Bromo.)	52
WDR9(2)	80



b

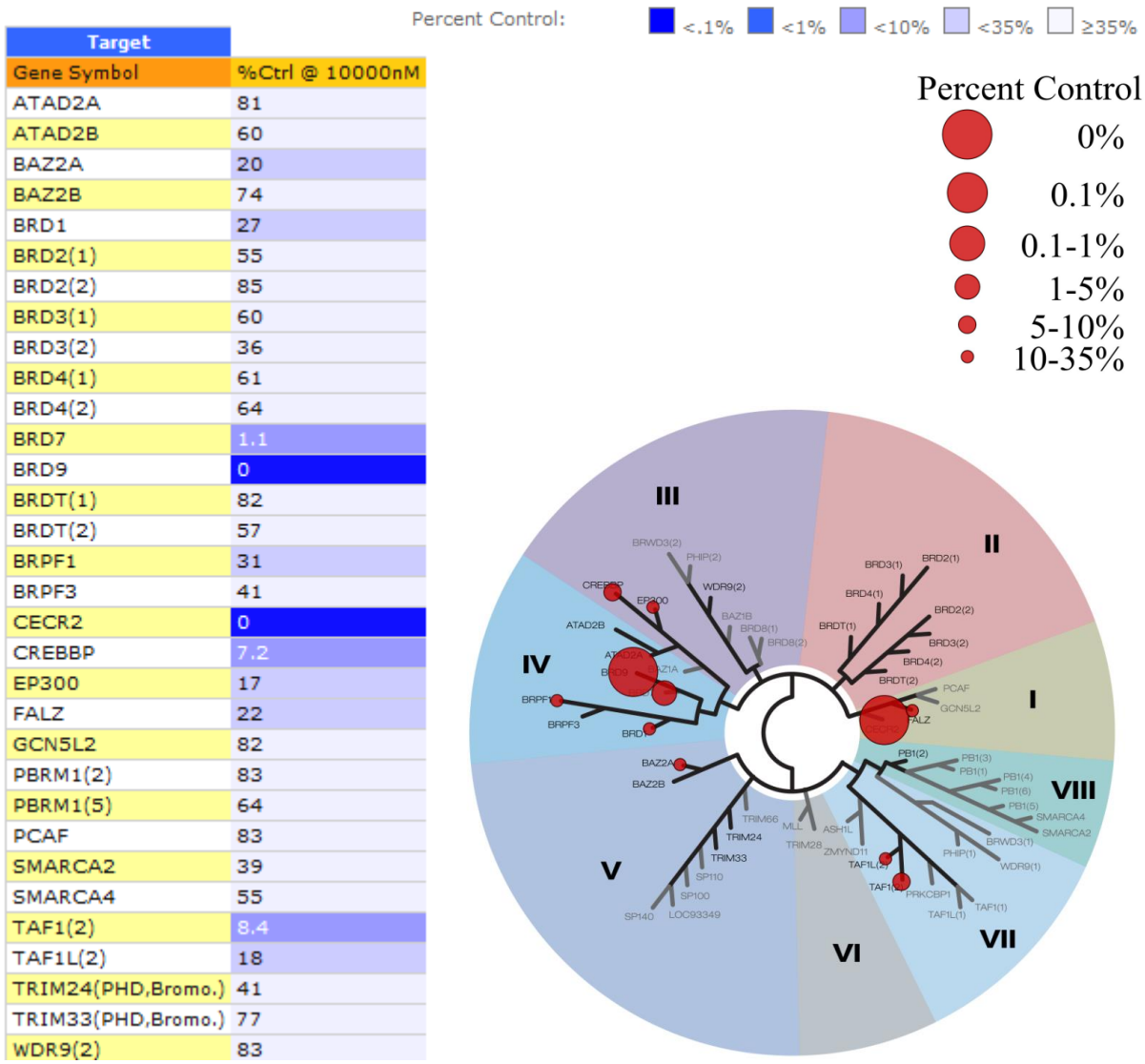
Targets	Kd (nM)
BAZ2A	>3,000
BRD1	2,600
BRD4-BD1	> 10,000
BRD7	0.3
BRD9	**0.75**
BRPF1	210

Targets	Kd (nM)
CECR2	8.8
CREBBP	8,600
EP300	10,000
FALZ	850
TAF1(2)	1,000
TAF1L(2)	1,200

Supplementary Figure 18. DiscoveRx selectivity data for **2.**
a) % ctrl bromoscan selectivity data at 10 µM (table and phylogenetic tree); **b**) BromoMax Kd for selected bromodomains

a

Target	
Gene Symbol	%Ctrl @ 10000nM
ATAD2A	81
ATAD2B	60
BAZ2A	20
BAZ2B	74
BRD1	27
BRD2(1)	55
BRD2(2)	85
BRD3(1)	60
BRD3(2)	36
BRD4(1)	61
BRD4(2)	64
BRD7	1.1
BRD9	0
BRDT(1)	82
BRDT(2)	57
BRPF1	31
BRPF3	41
CECR2	0
CREBBP	7.2
EP300	17
FALZ	22
GCN5L2	82
PBRM1(2)	83
PBRM1(5)	64
PCAF	83
SMARCA2	39
SMARCA4	55
TAF1(2)	8.4
TAF1L(2)	18
TRIM24(PHD,Bromo.)	41
TRIM33(PHD,Bromo.)	77
WDR9(2)	83

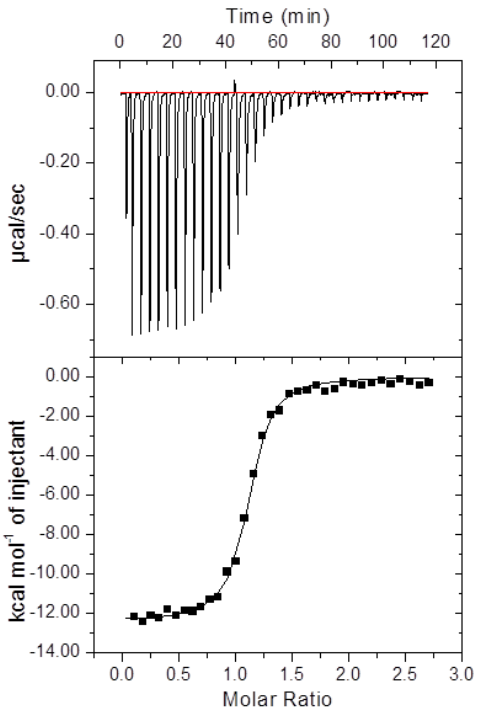


b

Targets	Kd (nM)
BRD4-BD1	> 10,000
BRD7	73
BRD9	**5.9**
BRPF1	790
CECR2	77

Targets	Kd (nM)
CREBBP	2,700
FALZ	>10,000
GCN5L2	>10,000
TAF1(2)	3,800
TAF1L(2)	4,100

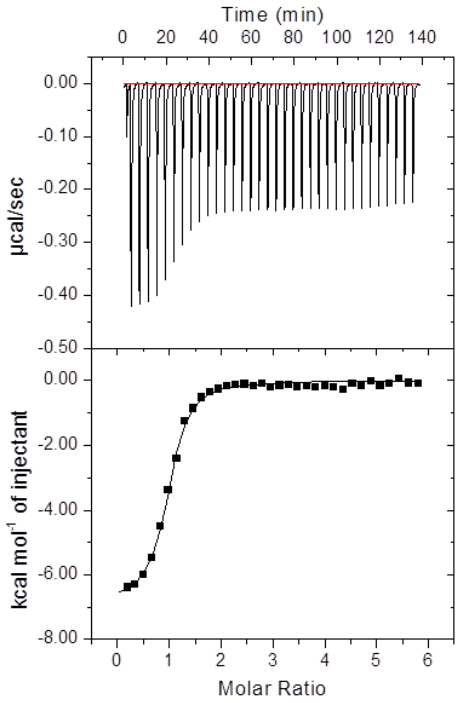
Supplementary Figure 19. ITC analysis of **1** in CECR2 (T=293.15K)
Compound **1** binds CECR2 with a K_D value of 187 nM (ΔH = -12.4 kcal/mol)



ITC type	Protein	K_A (10^6 M^{-1})	K_D (nM)	N	ΔH (kcal/mol)	TΔS (kcal/mol)	ΔG (kcal/mol)
VP-ITC	CECR2	5.36 ± 0.46	186.57	1.090 ± 0.005	-12.38 ± 0.089	-3.576	-8.804

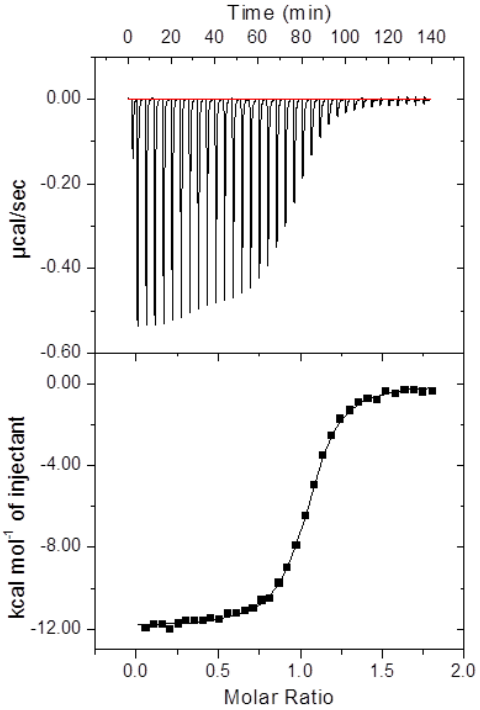
Supplementary Figure 20. ITC analysis of **2** in BRD7 and CECR2 (T=293.15 K)
a) Compound **2** binds BRD7 with a K_D value of 239 nM (ΔH = -6.9 kcal/mol); b) Compound **2** binds CECR2 with a K_D value of 200 nM (ΔH = -11.9 kcal/mol)

a



ITC type	Protein	K_A (10^6 M^{-1})	K_D (nM)	N	ΔH (kcal/mol)	TΔS (kcal/mol)	ΔG (kcal/mol)
VP-ITC	BRD7	4.18 ± 0.39	239.23	0.951 ± 0.011	-6.88 ± 0.103	1.879	-8.761

b

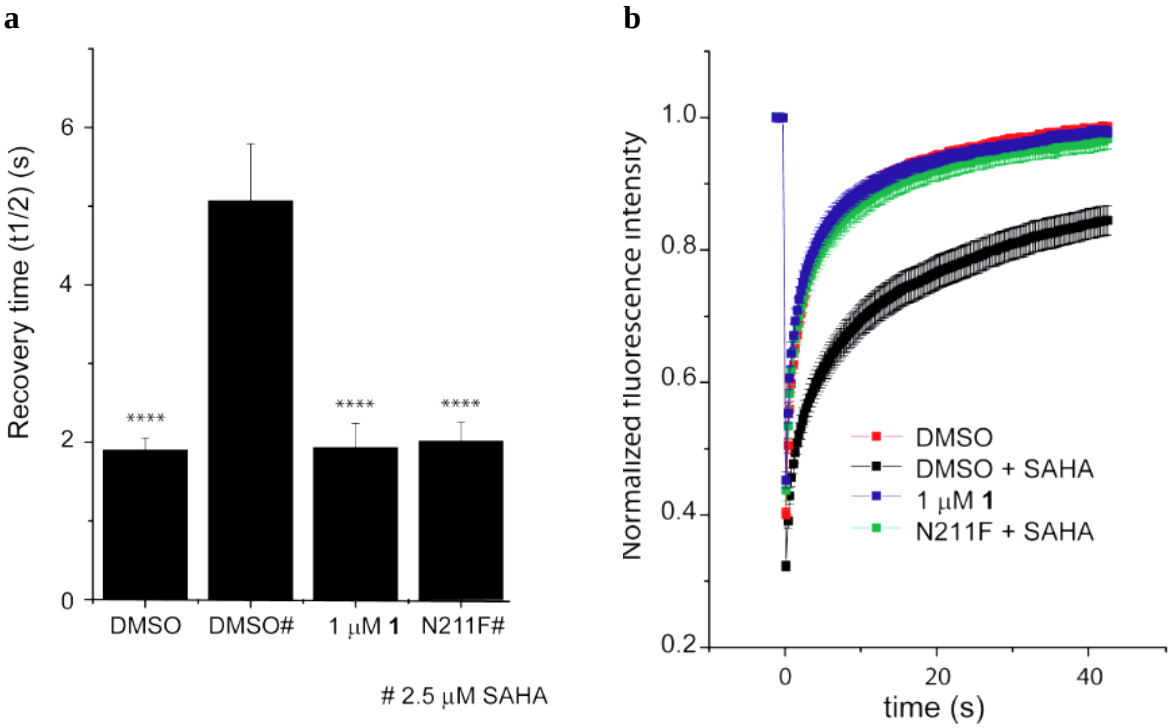


ITC type	Protein	K_A (10^6 M^{-1})	K_D (nM)	N	ΔH (kcal/mol)	TΔS (kcal/mol)	ΔG (kcal/mol)
VP-ITC	CECR2	4.99 ± 0.17	200.40	1.040 ± 0.002	-11.89 ± 0.032	-3.107	-8.783

Supplementary Figure 21. Recovery After Photobleaching (FRAP) assay U2OS cells transfected with GFP-BRD7 for **1**

(a) Influence of **1** on half recovery times of U2OS cells transfected with wild-type full-length GFP-BRD7 or the N211F mutant construct. Cells were treated with 2.5 μM SAHA (shown by "#") to increase the assay window. Bars indicate by * indicates $p < 0.05$ significant difference from wt treated with SAHA. (b) Time dependence of fluorescence recovery in the bleached area of cells expressing wt or mutant GFP-BRD7 with the corresponding treatment as in (a).

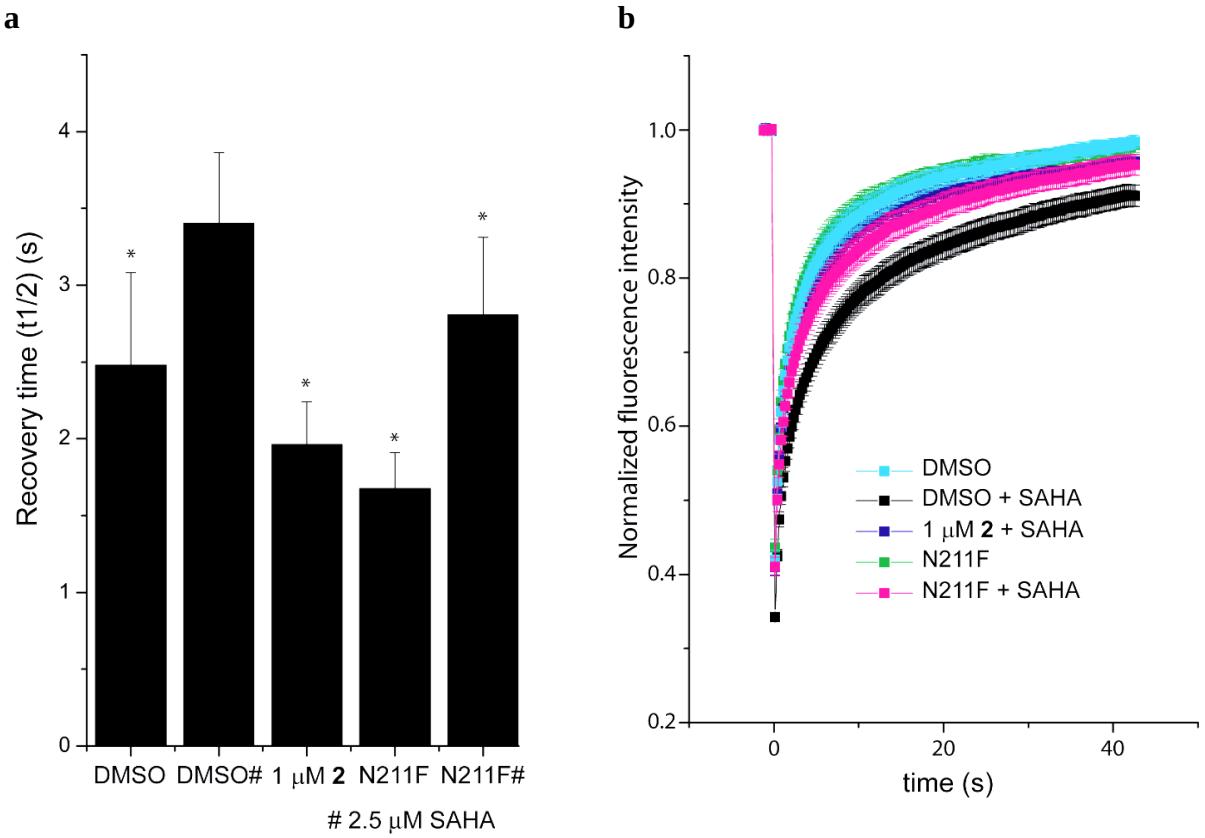
Curves represent averaged data of at least 20 replicates.



Supplementary Figure 22. Recovery After Photobleaching (FRAP) assay U2OS cells transfected with GFP-BRD7 for **2**

(a) Influence of **2** on half recovery times of U2OS cells transfected with wild-type full-length GFP-BRD7 or the N211F mutant construct. Cells were treated with 2.5 μM SAHA (shown by "#") to increase the assay window. Bars indicate by * indicates $p < 0.05$ significant difference from wt treated with SAHA. (b) Time dependence of fluorescence recovery in the bleached area of cells expressing wt or mutant GFP-BRD7 with the corresponding treatment as in (a).

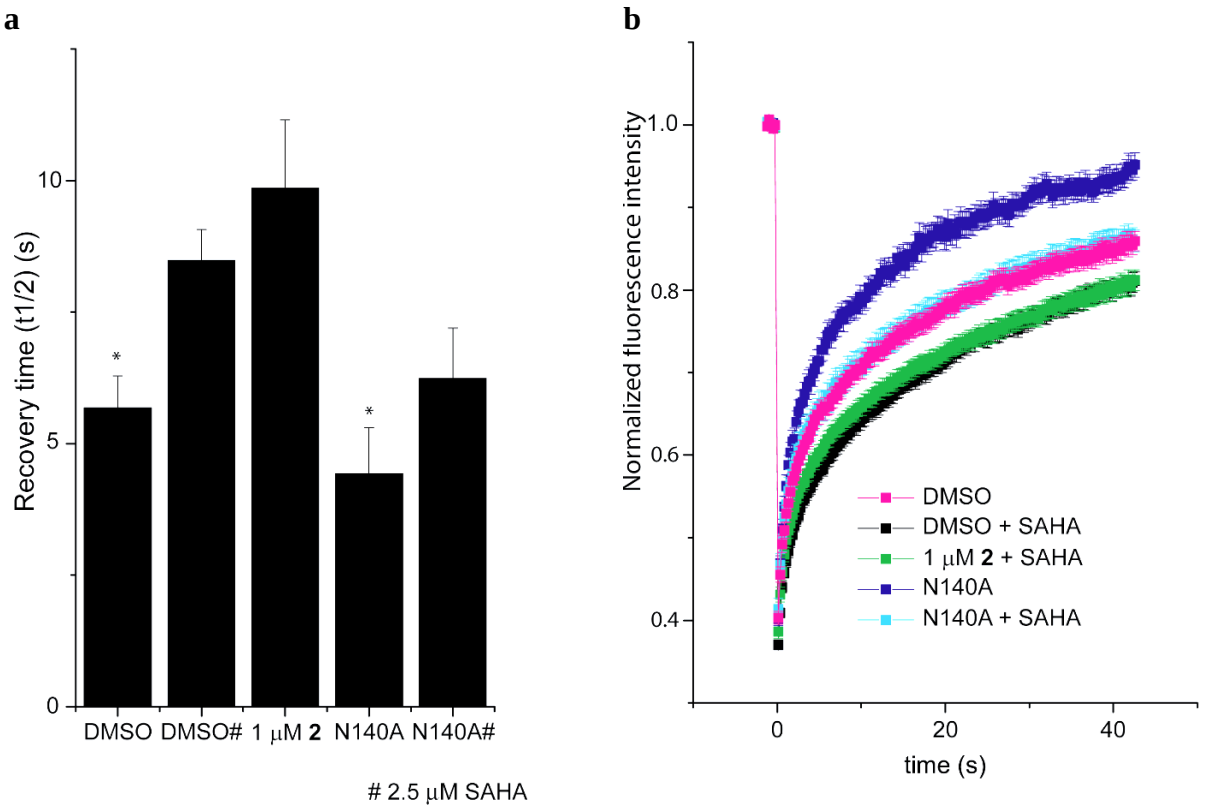
Curves represent averaged data of at least 20 replicates.



Supplementary Figure 23. Recovery After Photobleaching (FRAP) assay U2OS cells transfected with GFP-CECR2 for **2**

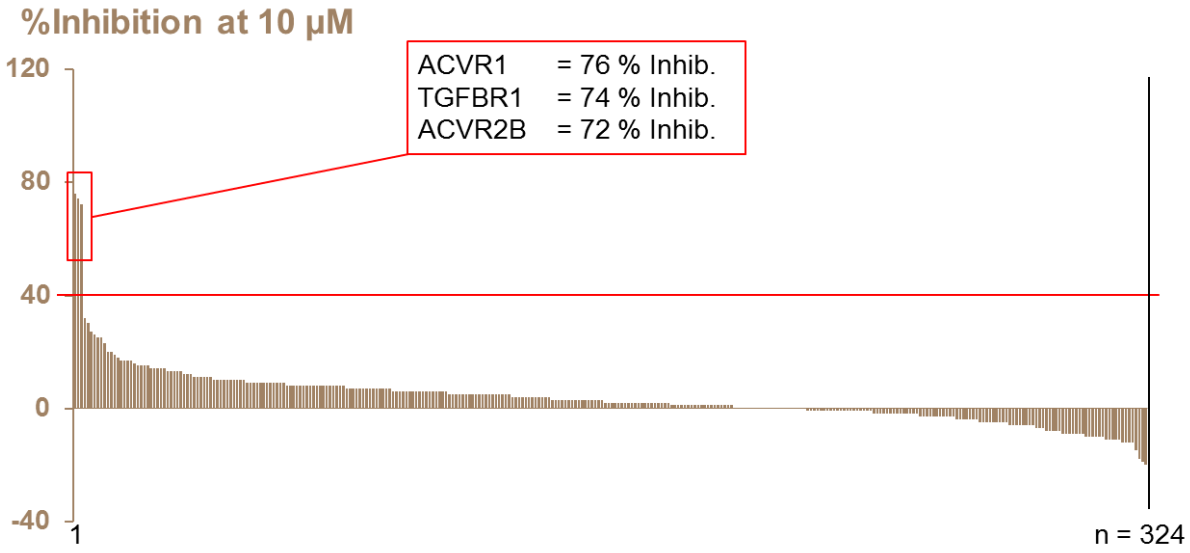
(a) Influence of **2** on half recovery times of U2OS cells transfected with wild-type full-length GFP-CECR2 or the N140F mutant construct. Cells were treated with 2.5 µM SAHA (shown by "#") to increase the assay window. Bars indicate by * indicates $p < 0.05$ significant difference from wt treated with SAHA. No cellular inhibition of CECR2 bromodomain was observed with 1µM **2** (b) Time dependence of fluorescence recovery in the bleached area of cells expressing wt or mutant GFP-CECR2 with the corresponding treatment as in (a).

Curves represent averaged data of at least 20 replicates.

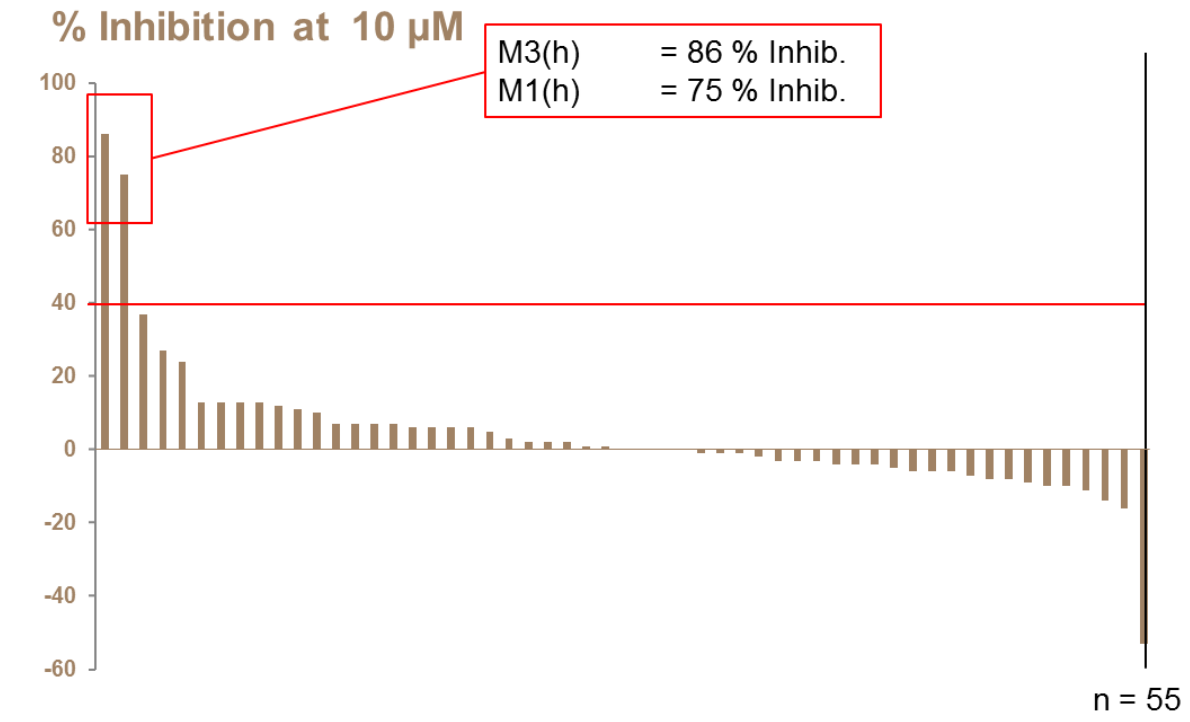


Supplementary Figure 24. Selectivity profile of **2** towards kinase and GPCR
a) kinase selectivity profile (% inhibition at 10 µM on 324 kinases). 282 kinases showed < 10% inhibition at 10 µM, 3 kinases showed > 40 % inhibition at 10 µM [IC_{50}(**2**, ACVR1)= 5090 nM, IC_{50}(**2**, TGFBR1)=5140 nM, IC_{50}(**2**, ACVR2B)=7680 nM] ; b) GPCR selectivity profile (% inhibition at 10 µM on 55 GPCR). 2 GPCR showed > 40 % Inhibition at 10 µM

a

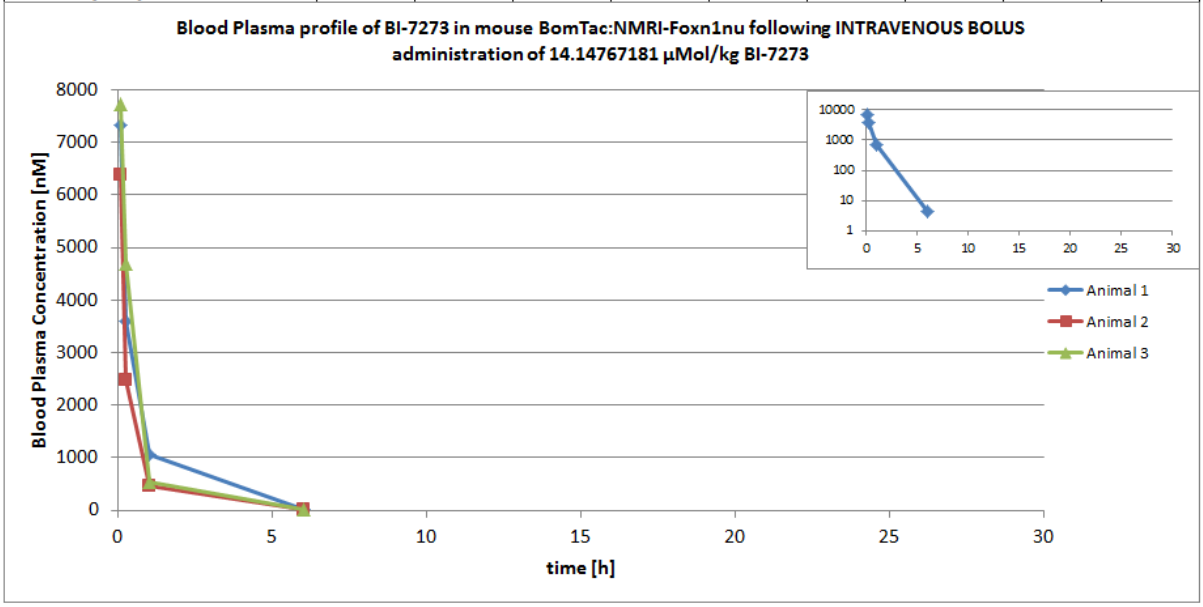


b



Supplementary Figure 25. PK profile of **1** in mice upon *i.v.* administration (5 mg/kg)

BI-7273		5 mg/kg	INTRAVENOUS BOLUS		Mus musculus (house mouse) BomTac:NMRI-Foxn1nu					
			1.02 mg BI-7273 + 2 µl HCl 1M + 814 µl HP-ß-CD 25 %							
			Blood Plasma Concentration [nM]							
		Time	Animal 1	Animal 2	Animal 3				Mean	Stddev
MW (Salt)	353 g/Mol	0,083	7330	6390	7720				7150	683
MW (Base)	353 g/Mol	0,250	3590	2470	4700				3590	1110
Dose (Base)	5 mg/kg	1,000	1060	456	532				683	330
	14,1 µMol/kg	6,000	4,56	3,82	5,23				4,54	0,708
		24,000								
LLOQ	3,8 nM									
QNMR	8 µM									
AUD Range			0h...06h	0h...06h	0h...06h					
AUD [nM*h]			5930	3520	4980				4810	1220
AUC [nM*h]			5930	3520	4990				4810	1220
MRT [h]			0,6	0,5	0,5				0,5	0,1
Clearance [ml/min/kg]			40	67	47				51	14
Vss [l/kg]			1,5	2,0	1,3				1,6	0,4
Clearance [%QH]									57	



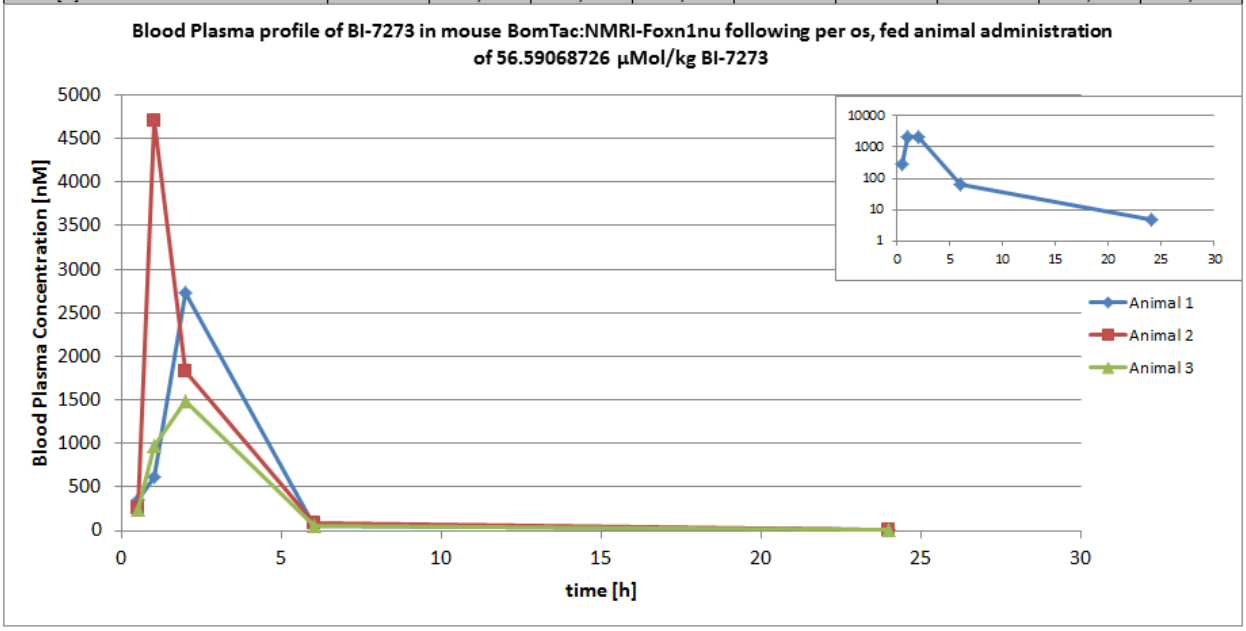
Supplementary Figure 26. PK profile of **1** in mice upon *p.o.* administration (20 mg/kg)

BI-7273 20 mg/kg per os, fed animal Mus musculus (house mouse)

BomTac:NMRI-Foxn1nu

4.08 mg BI-7273 + 1,632 µl Natrosol 0.5 %

			Blood Plasma Concentration [nM]							
		Time	Animal 1	Animal 2	Animal 3				Mean	Stddev
MW (Salt)	353 g/Mol	0,500	351	263	235				283	60,5
MW (Base)	353 g/Mol	1,000	611	4700	973				2090	2260
Dose (Base)	20 mg/kg	2,000	2730	1830	1490				2010	644
	56,6 µMol/kg	6,000	50,4	80,1	56				62,2	15,8
		24,000	3,59	3,2	7,92				4,9	2,62
LLOQ	2,56 nM									
QNMR	8 µM									
AUD Range			0h...24h	0h...24h	0h...24h					
AUD [nM*h]			8050	9130	5250				7470	2000
Cmax [nM]			2730	4700	1490				2970	1620
Tmax [h]			2,0	1,0	2,0				1,7	0,6



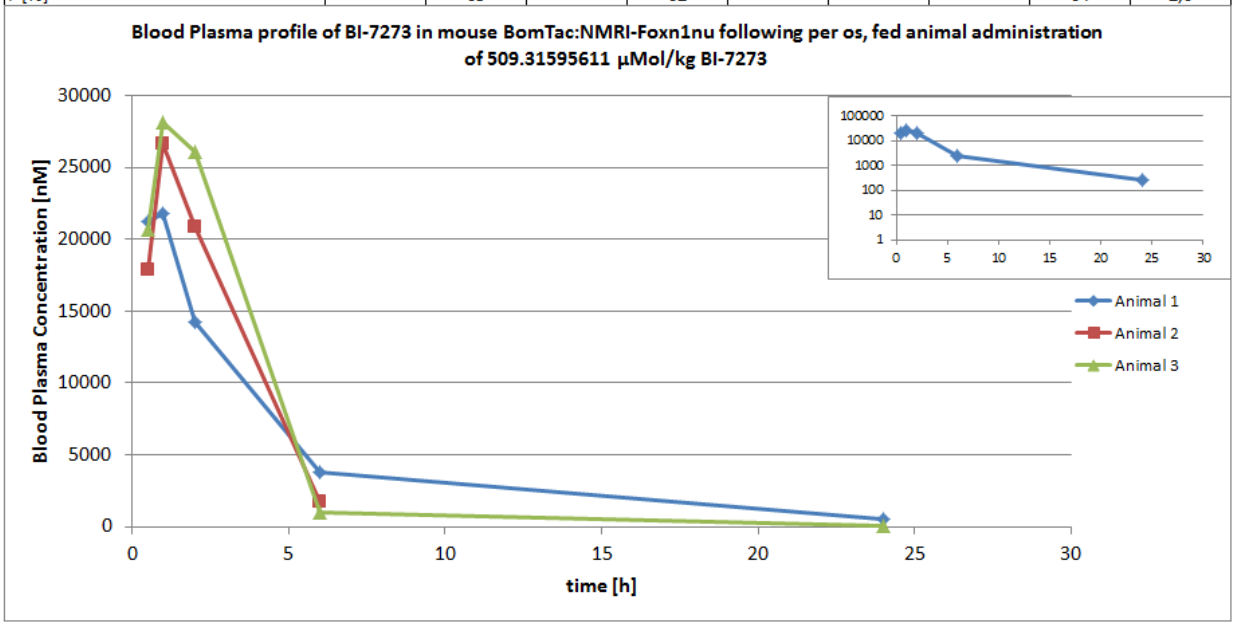
Supplementary Figure 27. PK profile of **1** in mice upon *p.o.* administration (180 mg/kg)

BI-7273 180 mg/kg per os, fed animal Mus musculus (house mouse)

BomTac:NMRI-Foxn1nu

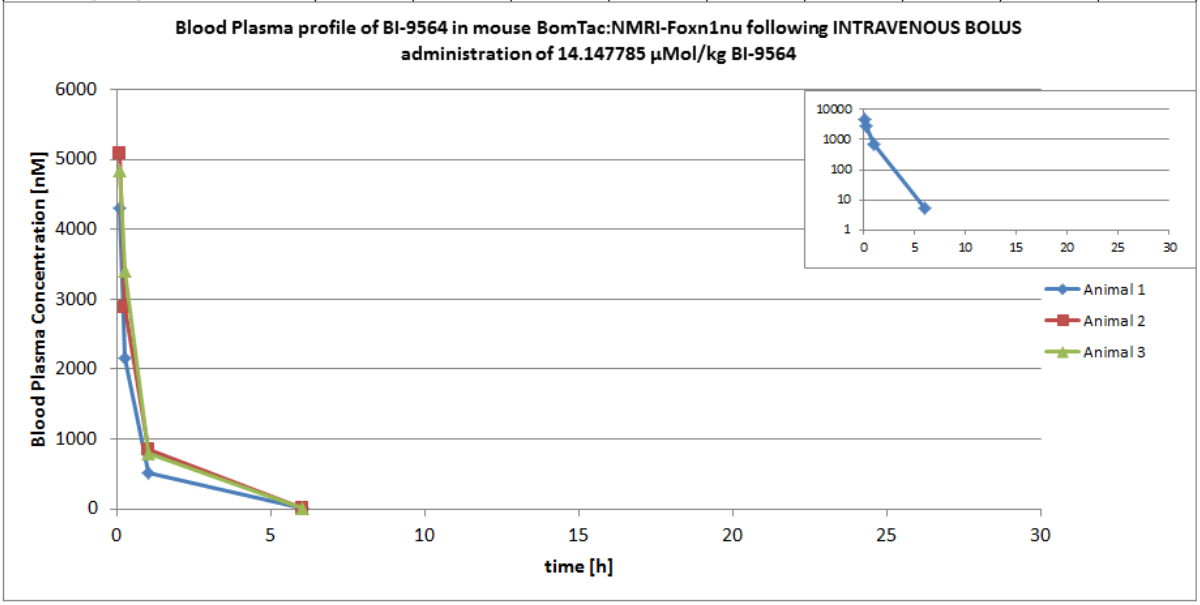
30.88 mg BI-7273 + 1,680 µl Natrosol 0.5 %

			Blood Plasma Concentration [nM]							
		Time	Animal 1	Animal 2	Animal 3				Mean	Stddev
MW (Salt)	353 g/Mol	0,500	21200	*17900*	20700				21000	340
MW (Base)	353 g/Mol	1,000	21800	*26600*	28100				25000	4440
Dose (Base)	180 mg/kg	2,000	14200	*20900*	26100				20100	8360
	509 µMol/kg	6,000	3760	*1700*	973				2370	1970
		24,000	532		31,4				282	354
LLOQ	19,7 nM									
QNMR	9,79 µM									
AUD Range			0h...24h		0h...24h					
AUD [nM*h]			109000		108000				108000	817
AUC [nM*h]			113000		108000				110000	3480
Cmax [nM]			21800		28100				25000	4440
Tmax [h]			1,0		1,0				1,0	0,0
MRT [h]			5,1		2,1				3,6	2,1
F [%]			65		62				64	2,0



Supplementary Figure 28. PK profile of **2** in mice upon *i.v.* administration (5 mg/kg)

BI-9564		5 mg/kg	INTRAVENOUS BOLUS		Mus musculus (house mouse) BomTac:NMRI-Foxn1nu					
			1.08 mg BI-9564 + 2 µl HCl 1M + 970 µl HP-ß-CD 25 %							
			Blood Plasma Concentration [nM]							
		Time	Animal 1	Animal 2	Animal 3				Mean	Stddev
MW (Salt)	353 g/Mol	0,083	4300	5090	4840				4740	404
MW (Base)	353 g/Mol	0,250	2150	2890	3400				2810	627
Dose (Base)	5 mg/kg	1,000	509	849	789				716	181
	14,1 µMol/kg	6,000	4,53	3,65	7,47				5,22	2
		24,000								
LLOQ	3,6 nM									
QNMR	9 µM									
AUD Range			0h...06h	0h...06h	0h...06h					
AUD [nM*h]			3180	4620	4650				4150	843
AUC [nM*h]			3180	4620	4660				4150	843
MRT [h]			0,6	0,6	0,6				0,6	0,0
Clearance [ml/min/kg]			74	51	51				59	14
Vss [l/kg]			2,6	1,9	1,9				2,1	0,4
Clearance [%QH]									65	



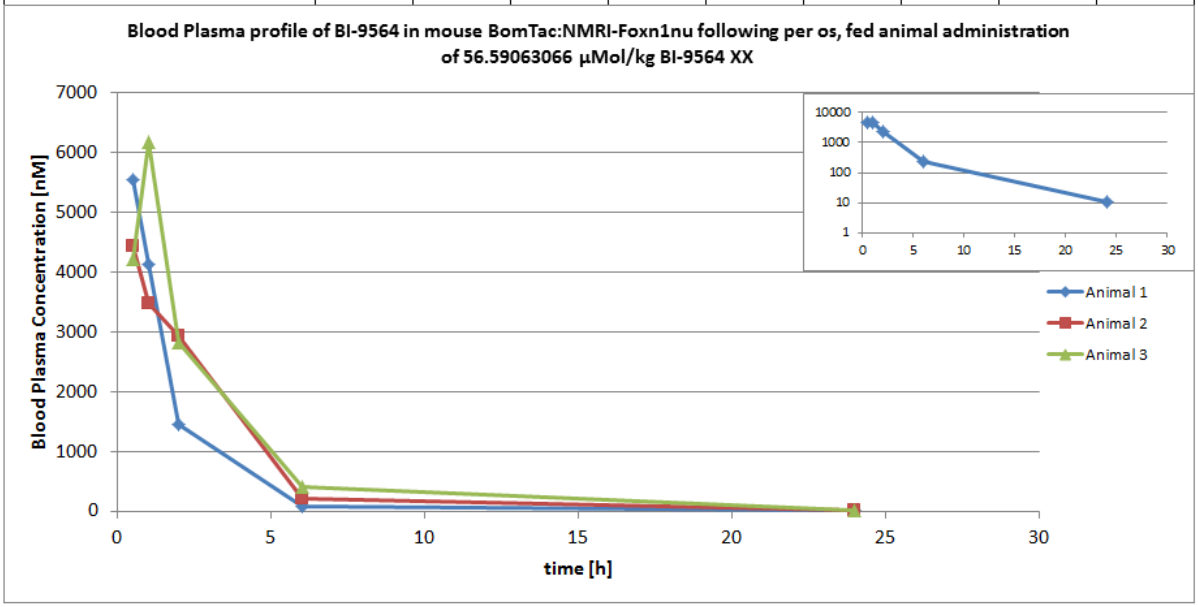
Supplementary Figure 29. PK profile of **2** in mice upon *p.o.* administration (20 mg/kg)

BI-9564 20 mg/kg per os, fed animal Mus musculus (house mouse)

BomTac:NMRI-Foxn1nu

4.12 mg BI-9564 + 1,854 µl Natrosol 0.5 %

			Blood Plasma Concentration [nM]							
		Time	Animal 1	Animal 2	Animal 3				Mean	Stddev
MW (Salt)	353 g/Mol	0,500	5550	4440	4220				4730	712
MW (Base)	353 g/Mol	1,000	4130	3480	6170				4590	1400
Dose (Base)	20 mg/kg	2,000	1440	2940	2830				2400	838
	56,6 µMol/kg	6,000	85,7	216	402				234	159
		24,000	5,69	18	8,4				10,7	6,48
LLOQ	5,6 nM									
QNMR	9 µM									
AUD Range			0h...24h	0h...24h	0h...24h					
AUD [nM*h]			10500	14700	18300				14500	3930
AUC [nM*h]			10500	14800	18300				14500	3930
Cmax [nM]			5550	4440	6170				5390	874
Tmax [h]			0,5	0,5	1,0				0,7	0,3
F [%]			63	89	110				88	24



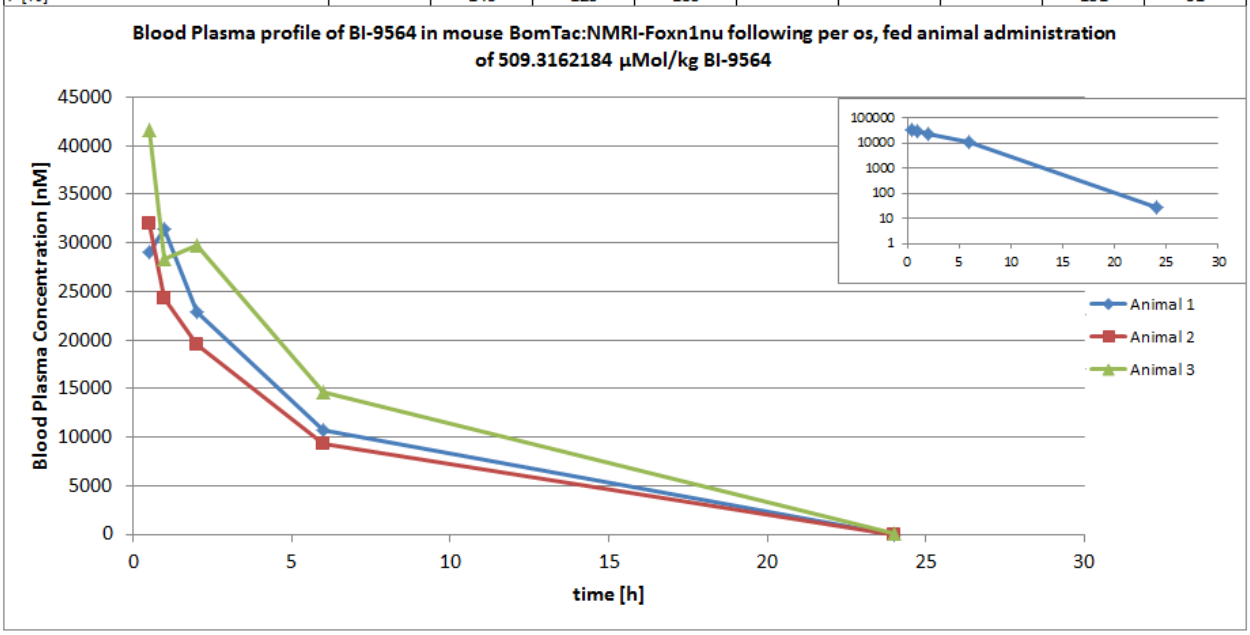
Supplementary Figure 30. PK profile of **2** in mice upon *p.o.* administration (180 mg/kg)

BI-9564 180 mg/kg per os, fed animal Mus musculus (house mouse)

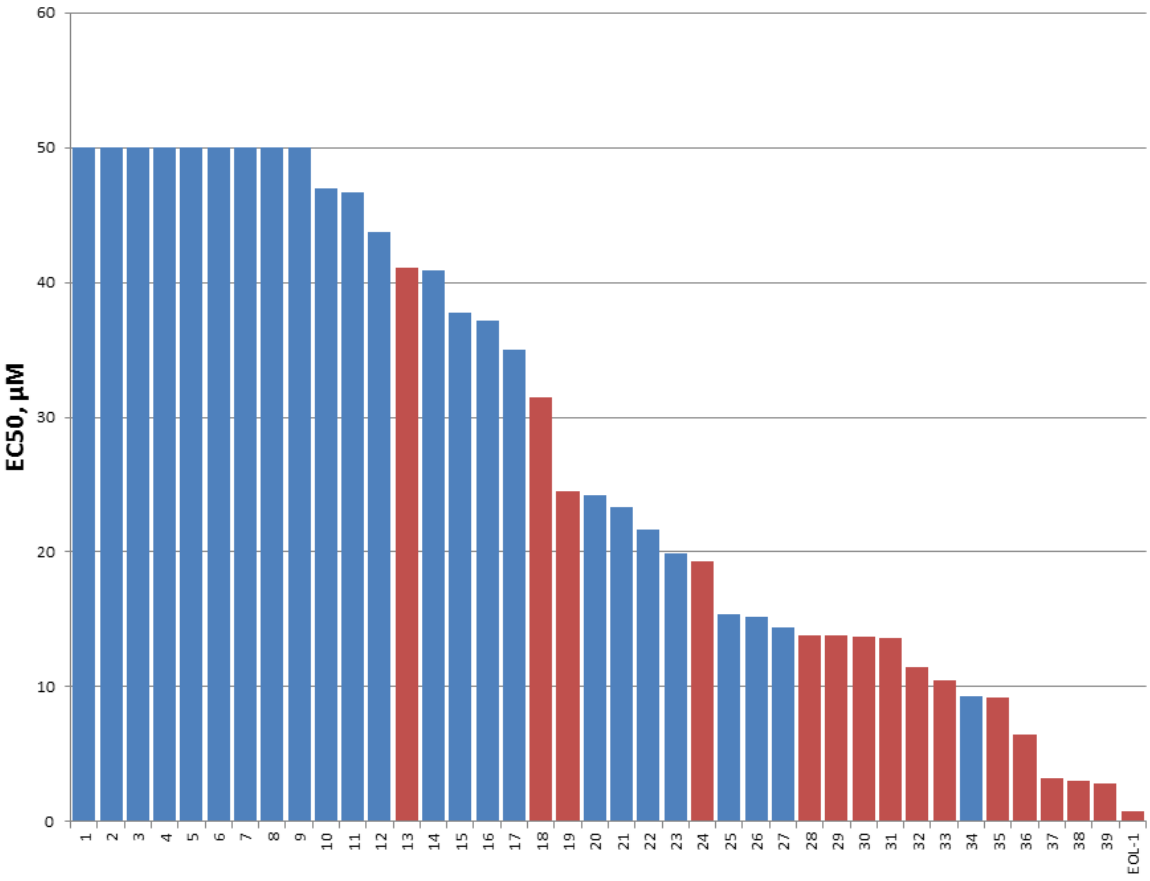
BomTac:NMRI-Foxn1nu

29.74 mg BI-9564 + 1,591 µl Natrosol 0.5 %

			Blood Plasma Concentration [nM]							
		Time	Animal 1	Animal 2	Animal 3				Mean	Stddev
MW (Salt)	353 g/Mol	0,500	29100	32000	41600				34200	6530
MW (Base)	353 g/Mol	1,000	31400	24300	28300				28000	3560
Dose (Base)	180 mg/kg	2,000	22900	19500	29700				24000	5210
	509 µMol/kg	6,000	10700	9390	14600				11600	2690
		24,000	11,1	14,1	55,5				26,9	24,8
LLOQ	19,8 nM									
QNMR	9,63 µM									
AUD Range			0h...24h	0h...24h	0h...24h					
AUD [nM*h]			213000	186000	277000				226000	46600
AUC [nM*h]			213000	186000	277000				226000	46600
Cmax [nM]			31400	32000	41600				35000	5720
Tmax [h]			1,0	0,5	0,5				0,7	0,3
MRT [h]			4,0	4,0	4,2				4,1	0,1
F [%]			143	125	185				151	31

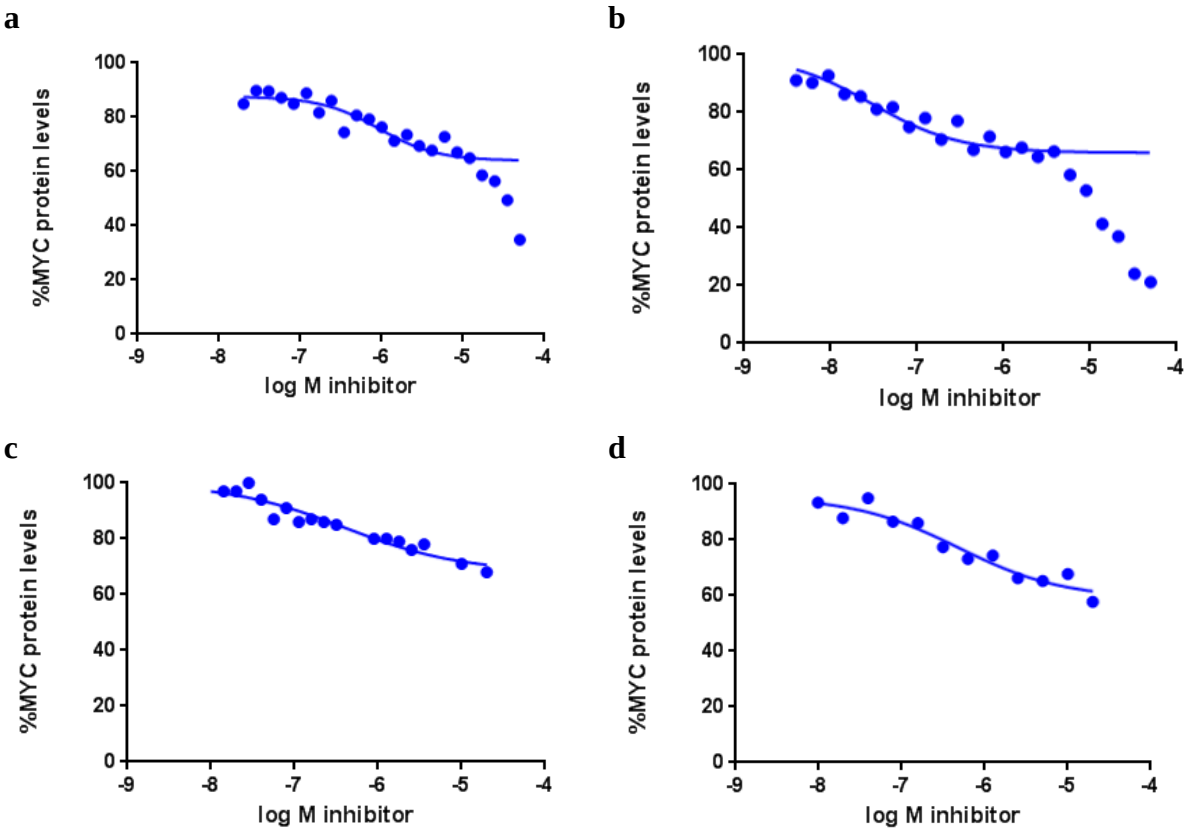


Supplementary Figure 31. Representation of the EC50s of **2** over various cell lines. (Red bars: AML cell lines).



Supplementary Figure 32. Dose dependent partial reduction in MYC levels by BRD9 inhibitors. MV-4-11 cells were treated for 2 hour with BRD9 inhibitors before analysis of MYC protein levels by ELISA

a) MYC reduction levels upon treatment with **13**, b) MYC reduction levels upon treatment with **15**, c) MYC reduction levels upon treatment with **1**, d) MYC reduction levels upon treatment with **2**



Supplementary Table 1. Small molecules screening data

Category	Parameter	Description	Description	Description
Assay	Type of assay	Thermal Shift Assay (DSF)	Surface Plasmon Resonance	Microscale Thermophoresis
	Target	BRD9	BRD9	BRD9
	Primary measurement	Protein Stability	Mass increase	Protein mobility in temperature gradient
	Key reagents	Fluorescent Dye	His-tagged protein	Fluorescent Dye
	Assay protocol	Anal Biochem. 2004 Sep 1;332(1):153-9.	J.Biomol. Screening 2009 14:337-49	ChemMedChem. 2015 Sep;10(9):1511-21
	Additional comments			
Library	Library size	1.697	1.697	1.697
	Library composition	Generic fragment set	Generic fragment set	Generic fragment set
	Source	BI pool	BI pool	BI pool
	Additional comments			
Screen	Format	384 well	384 well	384 well
	Concentration(s) tested	400 µM, 2% DMSO	100 µM, 1 % DMSO	500 µM, 5% DMSO
	Plate controls	Positive (in-house bromodomain binder) and negative (DMSO) controls	Positive (in-house bromodomain binder) and negative (buffer) controls	Positive (in-house bromodomain binder) and negative (DMSO) controls
	Reagent/ compound dispensing system	Hamilton Star system	CyBio System 6	Hamilton Star system
	Detection instrument and software	Bio-Rad CFX384 Real-Time System (C1000Touch Thermal Cycler;) / Bio-Rad CFX Manager-Data Analysis software	Biacore T200 / BiaEvaluation software	In house implemented automation of Nanotemper NT.015 / Nanotemper Analysis software

	Assay validation/QC	Z’ = 0.55	Z’ = 0.93	
	Correction factors		DMSO solvent correction	
	Normalization		Normalization to positive control	
	Additional comments			
Post-FBS analysis	Hit criteria	ΔT≥1°C	BR ≥20%	ΔMST(DMSO, Cpd) ≥ MST(2sd DMSO)
	Hit rate	2.1% Hit rate	2.7%	6.6%
	Additional assay(s)			
	Confirmation of hit purity and structure	Entire screening library QC’ed for purity and structural identity	Entire screening library QC’ed for purity and structural identity	Entire screening library QC’ed for purity and structural identity
	Additional comments	94.4% Hit confirmation by ^{15}N HSQC	72.2% Hit confirmation by ^{15}N HSQC	26 % Hit confirmation by ^{15}N HSQC

Supplementary Table 2. Crystallographic data collection and refinement statistics (molecular replacement)

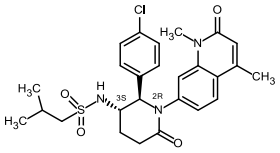
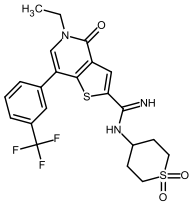
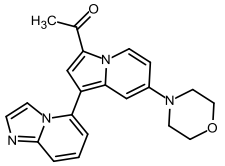
	Compound 3	Compound 4	Compound 11
Data collection			
Space group	P 2_1 2_1 2	P 2_1 2_1 2	P 2_1 2_1 2
Cell dimensions			
a, *b*, *c* (Å)	70.92, 125.41, 29.53	71.04, 125.02, 29.94	70.31, 125.17, 30.02
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	1.80 (1.97-1.80)*	1.68 (1.81 – 1.68) *	2.27 (2.54 – 2.27) *
CC1/2	0.999 (0.973)	1.000 (0.950)	0.999 (0.955)
Rmerge	4.4 (54.9)	3.0 (59.1)	4.6 (123.6)
$I / \sigma I$	20.3 (3.3)	30.9 (3.6)	24.7 (3.2)
Completeness (%)	99.7 (99.5)	99.9 (100.0)	98.9 (98.3)
Redundancy	6.0 (5.7)	6.3 (6.3)	6.3 (6.4)
Refinement			
Resolution (Å)	1.80	1.68	2.30
No. reflections	25074	31312	12218
R_{work} / R_{free} (%)	20.3/22.0	18.9/20.5	19.2/20.9
No. Atoms			
Protein	1835	1817	1852
Ligand/ion	30	36	50
Water	235	306	68
B-factors (Å$^{2)}$			
Protein	42.56	36.53	70.54
Ligand/ion	35.66	34.64	69.30
Water	48.12	46.45	62.57
Estimated Coordinate Error (Å)	0.301	0.253	0.400
R.m.s. deviations			
Bond lengths (Å)	0.008	0.008	0.009
Bond angles (°)	0.80	0.81	0.92

*Values in parentheses are for highest-resolution shell.

	Compound 1[1]	Compound 2
Data collection		
Space group	P 2_1 2_1 2	P 2_1 2_1 2
Cell dimensions		
a, *b*, *c* (Å)	70.80, 125.34, 29.92	70.03, 125.36, 29.68
α, β, γ (°)	90.00, 90.00, 90.00	90.00, 90.00, 90.00
Resolution (Å)	1.60 (1.79 – 1.605) *	1.82 (2.10- 1.82)
CC1/2	1.000 (0.924)	0.999 (0.961)
Rmerge	3.2 (101)	4.4 (88.6)
$I / \sigma I$	26.2 (3.1)	19.9 (3.4)
Completeness (%)	99.9 (98.7)	99.7 (99.7)
Redundancy	6.3 (6.3)	6.4 (6.4)
Refinement		
Resolution (Å)	1.60	1.82
No. reflections	35816	24186
R_{work} / R_{free} (%)	17.8/19.2	19.1/20.2
No. Atoms		
Protein	1851	1841
Ligand/ion	52	52
Water	352	208
B-factors (Å^2)		
Protein	35.75	49.63
Ligand/ion	32.13	43.47
Water	47.24	53.11
Estimated Coordinate Error (Å)	0.226	0.285
R.m.s. deviations		
Bond lengths (Å)	0.009	0.009
Bond angles (°)	0.81	0.83

*Values in parentheses are for highest-resolution shell.

Supplementary Table 3. Literature BRD9 inhibitors: **LP99** binds BRD9 anchor region Asn100 *via* its methyl quinolinone, **I-BRD9** binds BRD9 anchor region Asn100 via its ethylthienopyridone, **Compound 28** binds BRD9 anchor region Asn100 via the ketone.

			
	LP99[18]	**I-BRD9**[19]	**"compound 28"**[20]
BRD9-BD Affinity/Activity			
K_D(BRD9, ITC)	99 nM	n.a.	68 nM
K_D(BRD9, DiscoveRx)	n.a.	1.9 nM	n.a.
IC_{50}(BRD9)	n.a.	(TR-FRET) 5.1 nM	(Alpha Screen) 126 nM
Bromodomain Selectivity (48 bromodomains) T_M shift at 10μM	BRD9 > 9°C BRD7 +4-6°C All other bromodomains ≤ 1°C		BRD9 +4.5°C BRD7 +5.6°C BRD4-BD1 +1.3°C EP300 +2°, CREBBP+1.8°C BRPF1 +1.7 °C, FALZ +1.1°C
K_D(BRD7, ITC)	909 nM	n.a.	368 nM
K_D(BRD7, DiscoveRx)	n.a.	380 nM	n.a.
K_D(BRD4-BD1, DiscoveRx)	n.a.	1400nM	n.a.
IC_{50}(BRD4-BD1, TR-FRET)	n.a.	5.0μM	n.a.
Other K_D(DiscoveRx)	n.a.	CECR2= 140nM, CREBBP= 740nM, EP300=770nM BRDT-BD1=1500 nM, BRPF1=2100nM, BRD2-BD1=3200nM, BRD3-BD1=3000nM, TAF1 BD2= 7500nM All other BDs >10μM	n.a.
Other target Selectivity		n.a.	n.a.
% ctrl inhibition at 10μM	55 receptors and ion channels (CEREP panel) NK3 66% All other inactive		
IC_{50}	n.a.	IC_{50} >5μM vs. 49 unrelated protein (23 GPCR, 13 ion channel, 7 enzyme, 3 kinase, 2 transporter, 1 nuclear receptor)	n.a.
Target Engagement			
BRD9 FRAP assay	~90% inhibition at 800nM	n.a.	Full inhibition at 1μM
NanoBRET IC_{50} (BRD9/H3.3)	5.1 μM	158 nM	n.a.
NanoBRET IC_{50} (BRD9/H4)	6.2 μM	n.a.	n.a.
NanoBRET IC_{50} (BRD7/H3.3)	3.7 μM	n.a.	n.a.
NanoBRET IC_{50} (BRD7/H4)	3.3 μM	n.a.	n.a.
***In vitro* ADME Properties**			
Aqueous solubility (pH 6.8)	n.a.	359μL	n.a.
membrane permeability	n.a.	210nM/s	n.a.

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