Fragment-based discovery of CPI-637, a selective and cell-active benzodiazepinone CBP/EP300 bromodomain inhibitor

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Supporting Information

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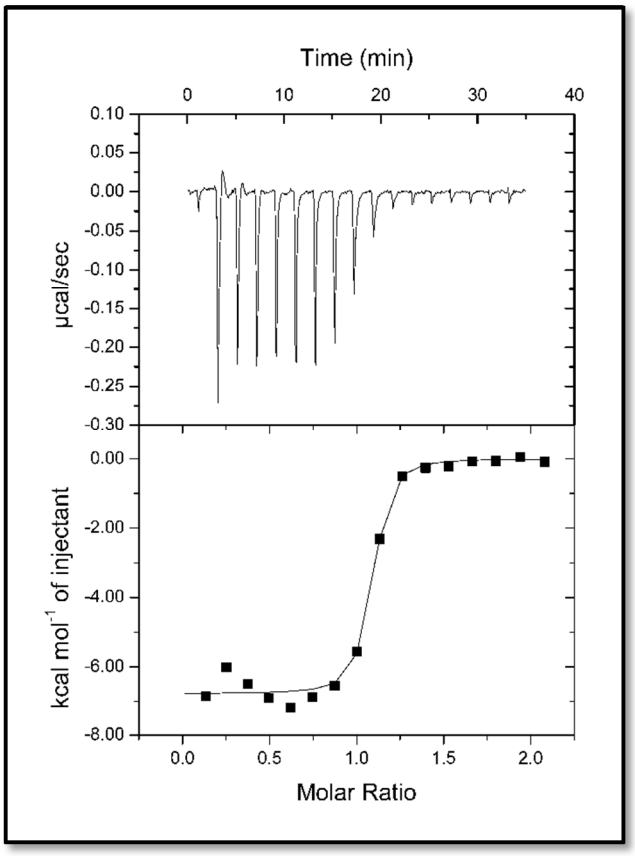
1. Thermal shift assay protocol. All assays were carried out in 384 well plates. In a conical tube CBP (4 μM) was combined with SYPROOrange (Life Technologies) to a final dye concentration of 5X in 50 mM Tris, 1 mM dithiothreitol (DTT), pH 8.5. The tube was centrifuged briefly to remove precipitate and the protein:dye solution was then added to a black Optiplate™ plates (Greiner), spun briefly (1 min, 900xg) and then 23 μL transferred to either DMSO controls or fragments plated from 100 mM DMSO stocks into clear bottom Fluotrac200™ plates (Greiner) to a final compound concentration of 800 μM (0.8% $^{v}/_{v}$ DMSO). Subsequently samples (15 μL) were transferred to LightCycler® 480 plates (Roche Diagnostics), spun (2 min, 900 x *g*) and analyzed on a Roche Lightcycler 480 II using a temperature gradient of 20-85 ^{o}C and a scanning rate of 1.2 ^{o}C/min. The midpoint of the melting transitions (T_m) were assessed using an application developed in-house measuring the first derivative of the rate of fluorescence change as a function of temperature. Compound induced changes in the melting temperature, ΔT_m, were calculated relative to DMSO controls within the same plate.

2. Isothermal calorimetry protocol. ITC measurements were made on a MicroCal ITC_{200} instrument (GE Healthcare). CBP was dialyzed overnight against 50 mM HEPES (pH 7.5), 150 mM NaCl, 0.2% v/v DMSO, clarified by passage through a 0.22 μm Spin-X tube (Corning, Inc), and the protein concentration determined by A_{280} on a NanoDrop instrument (Thermo Scientific). **CPI-637** was equilibrated in the cell (17 μM, 200 μL volume, 25.0^{o}C, stirrer speed 1000 rpm, 11 μcal/sec reference power) and CBP was added (170 μM) in the same buffer in a series of seventeen 2.4 μL injections (4.8 s each) with an equilibration time of 120 s between each injection. The resulting data were fitted using the instrument's Origin 7.0 software.



SI Figure 1. ITC thermodynamic data for compound **CPI-637**: K_d = 0.03 ± 0.01 µM, N=0.968, ΔH = 7000 ± 100 cal/mol, ΔS = 10.9 cal/mol/deg.

Protein info: Q92793, cbp_human, K1082-G1197

MHHHHHHGSLVPRGSMDYKDDDDKENLYFQ\GSKKIFKPEELRQALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYF
DIVKNPMDLSTIKRKLDTGQYQEPWQYVDDVWLMFNNAWLYNRKTSRVYKFCSKLAEVFEQEIDPVMQSLG

Uncut (aa)	mw	pI	Ext. Coeff.	$Abs_{280}^{1mg/ml}$
148	17605.9	5.64	28420	1.614

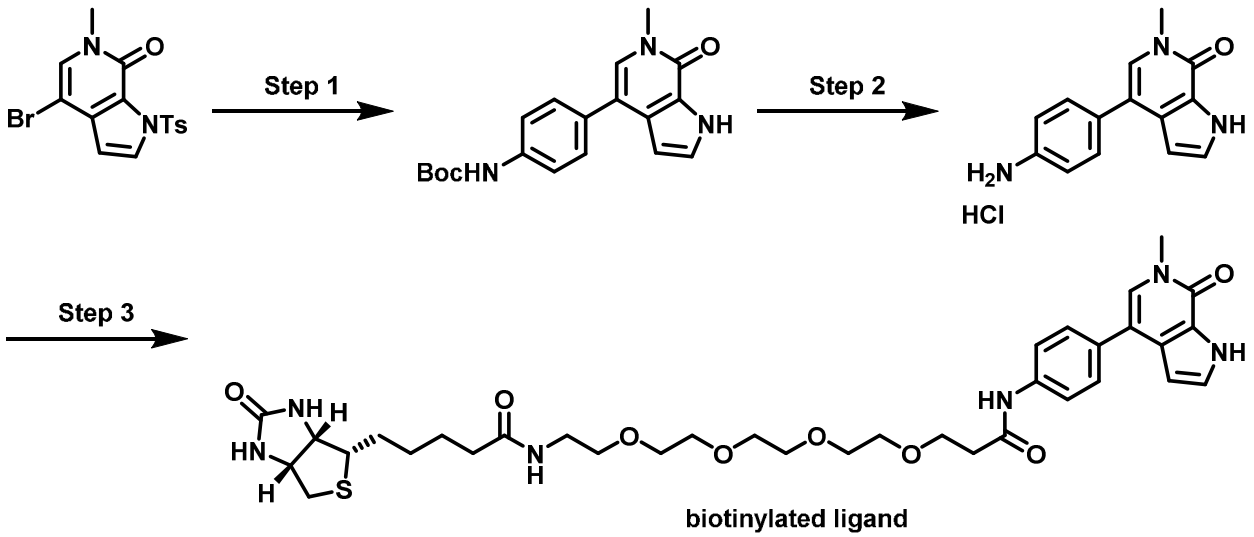
3. Biochemical assay protocols including synthesis of biotinylated probes. Compound potencies were evaluated in a panel of biochemical bromodomain binding assays. Binding of biotinylated small-molecule ligand or biotinylated histone H3K14 peptide ligand (BAZ2B) to recombinant His-tagged bromodomains was assessed by time-resolved fluorescence resonance energy transfer (TR-FRET). Test compounds that compete with the biotinylated ligand for bromodomain binding reduce the TR-FRET

signal. Assays were conducted in a total volume of 15 µL in white 384-well plates with the DMSO concentration held constant at 0.2%. All reagents were prepared in assay buffer (50 mM HEPES pH 7.5, 1 mM TCEP, 0.069 mM Brij-35, 50 mM NaCl, and 0.1 mg/mL bovine serum albumin). Compounds in DMSO were added to empty assay plates using an Echo 550 acoustic dispenser (Labcyte, Santa Clara, CA). Bromodomain was added followed by biotinylated ligand, and the plates were incubated for 10 minutes after each addition (20 minutes for BAZ2B). Subsequently, the TR-FRET detection reagents, anti-His-europium and streptavidin-allophycocyanin (PerkinElmer, Waltham, MA) were added and incubated for an additional 40 minutes. Compounds were evaluated as 10-point titrations with N = 2. Each compound was assayed in at least 3 independent assays. Results were analyzed with XLFit (IDBS, London, UK) beginning with a 4-parameter Hill fit and constraining one or more parameters if necessary to generate a suitable fit. The data are reported as IC_{50} values in units of micromolar.

SI Table 1. Reagents and conditions for the biochemical assays described in the text.

Reagent	CBP	EP300	BRD4-BD1	BRD4-BD2	CECR2	TAF1-BD1	TAF1-BD2	BAZ2B
[Bromodomain] (µM)	0.002	0.002	0.0015	0.002	0.0012	0.015	0.006	0.008
[Biot-Ligand] (µM)	0.025	0.025	0.0125	0.015	0.004	0.03	0.015	0.045 (H3K14 peptide)
[SA-APC] (µM)	0.05	0.05	0.1	0.1	0.0125	0.05	0.025	0.1
[Anti-His-Eu] (nM)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

Synthesis of the biotinylated ligand used for TR-FRET assays:



Step 1: A 50 mL vial was charged with a magnetic stir bar, 4-bromo-6-methyl-1-tosyl-1H-pyrrolo[2,3-c]pyridin-7(6H)-one (0.281 g, 0.737 mmol) (prepared as described in WO 2014206150), 1,4-dioxane (3.69 ml, 0.737 mmol), water (0.5 ml, 27.8 mmol), potassium carbonate (0.306 g, 2.211 mmol), 4-(tert-butoxycarbonylamino)phenylboronic acid (0.227 g, 0.958 mmol), and $Pd(PPh_3)_4$ (0.085 g, 0.074 mmol). The vial was purged, placed under an atmosphere of nitrogen and heated to 95 °C with stirring for 12 h before being allowed to cool to room temperature. The reaction was then diluted with water (20 mL). A precipitated formed which was collected via vacuum filtration using a Buchner funnel. The solids were washed with additional water (2 x 25 mL), dried, and collected. This material was suspended in methanol (~ 5 mL) and treated with potassium hydroxide (200 mg). After 2 h the methanol was removed *in vacuo* and the crude material was suspended in water (~20 mL), and the resulting solids were collected via vacuum filtration using a Buchner funnel. The solids were washed with additional water, collected, and dried *in vacuo* to afford tert-butyl 4-(6-methyl-7-oxo-6,7-dihydro-1H-pyrrolo[2,3-c]pyridin-4-yl)phenylcarbamate (362 mg, 0.907 mmol, 123 % yield) as a light yellow solid.

Step 2: A 25 mL round bottom flask was charged with a magnetic stir bar, tert-butyl 4-(6-methyl-7-oxo-6,7-dihydro-1H-pyrrolo[2,3-c]pyridin-4-yl)phenylcarbamate (105 mg, 0.309 mmol), methanol (1.547 mL, 0.309 mmol), and hydrochloric acid (0.62 mL, 2.48 mmol) 4 N in dioxane. The reaction was stirred at ambient temperature for 12 h and then concentrated *in vacuo* to afford 4-(4-aminophenyl)-6-methyl-1H-pyrrolo[2,3-c]pyridin-7(6H)-one (65 mg, 0.244 mmol, 79 % yield).

Step 3: A 25 mL vial was charged with a magnetic stir bar, 4-(4-aminophenyl)-6-methyl-1H-pyrrolo[2,3-c]pyridin-7(6H)-one (0.038 g, 0.159 mmol), anhydrous dimethylformamide (0.794 ml, 0.159 mmol), diisopropylethylamine (0.139 ml, 0.794 mmol), 17-oxo-21-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-4,7,10,13-tetraoxa-16-azahenicosan-1-oic acid (0.078 g, 0.159 mmol), and HATU (0.075 g, 0.199 mmol). The crude reaction mixture was directly purifed via reverse phase HPLC (eluting with 0.1% trifluoroacetic acid in water and acetonitrile). Lyophilization afforded N-(4-(6-methyl-7-oxo-6,7-dihydro-1H-pyrrolo[2,3-c]pyridin-4-yl)phenyl)-1-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)-3,6,9,12-tetraoxapentadecan-15-amide (31 mg, 0.041 mmol, 26.0 % yield). LRMS *m/z* 357 $(M+2H)^{2+}$; ^{1}H NMR: complex but roughly consistent with product structure.

4. CBP BRET assay protocols. Inhibition of bromodomain binding to histone H3 was evaluated in transiently transfected HEK293 cells using Bioluminescence Resonance Energy Transfer (BRET, Promega Corporation, Madison, WI). Cells were plated in 10 cm dishes and cultured overnight. On day 2, they were transiently transfected with Halotag-Histone H3.3 and CBP-Nanoluc vectors with the aid of FuGENE HD and cultured overnight. On day 3, the transfected cells were harvested and resuspended in assay medium +/- HaloTag® NanoBRET™ 618 Ligand. Cells were added to white 384-well TC-treated assay plates (Corning, Corning, NY, Cat. #4513) and allowed to recover for 4 h. Compound dilutions in DMSO were prepared with the Echo 550 and were further diluted with Opti-MEM (Life Technologies, Grand Island, NY) before transfer into the assay plates. The plates were incubated overnight. On day 4, Nano-Glo® Luciferase Assay Substrate diluted in Opti-MEM was added to each well, and within 10 min, plates were read on the Envision plate reader (PerkinElmer) with excitation at 450 nm and emission at 610 nm. All incubations were conducted at 37 °C and 5% CO_2. Assay controls included MIN (high concentration of inhibitor), MAX (no compound), and no NanoBRET ligand. Raw NanoBRET ratios were obtained by dividing the acceptor emission (610 nm) by the donor emission (450 nm). Background correction was achieved by subtracting the NanoBRET ratio for the no NanoBRET ligand control from the NanoBRET ratio for the other wells. Compounds were evaluated as 9-point titration curves with N=2 in each of two plates. The final concentration of DMSO was 0.4%. IC_{50} values were generated as described for the bromodomain binding assays.

5. *MYC* cellular assay protocol. AMO-1 cells (DSMZ) were plated at 20,000 cells/well in 96-well plates in Iscove's Modified Dulbecco's Medium supplemented with 10% fetal calf serum. Cells were treated for 6 h with a dose titration of **CPI-637** starting from a 5 µM top concentration. After 6 h, cells were lysed and processed for QuantiGene Plex expression analysis according to the manufacturer's instructions (Affymetrix/eBioscience), and data were collected on a MAGPIX multiplex Luminex instrument (EMD Millipore). Median Fluorescence Intensity (MFI) for *MYC* was normalized to the geometric mean of the MFI for *GAPDH* and *PP1B* for each dose. Data are reported as fold expression ± SD. EC_{50} was generated by interpolation of a non-linear regression with GraphPad Prism 6.

6. Protein crystallography protocols and data. The CREBBP bromodomain was overexpressed in *E. coli* and purified by Ni^{2+}-affinity chromatography, followed by removal of its histidine purification tag with TEV protease. The protein was then passed through ion exchange chromatography and another round of Ni^{2+}-affinity chromatography, and finally desalted and concentrated to ~20 mg/mL for use in crystallization. Alternatively, in the cases of **23** and **CPI-637**, the CREBBP bromodomain-PHD construct was overexpressed in *E. coli* and purified by $Ni2^{+}$-affinity chromatography, followed by removal of the histidine purification tag with TEV protease. The protein was then passed through a second Ni2+-affinity column, and the flow-through was collected and purified further by size exclusion chromatography. The purity of the samples was checked by SDS-PAGE before concentration to ~15 mg/mL for use in crystallization.

The co-crystal structures of **1**, **12**, **14**, **27** bound to CREBBP bromodomain (Gly-Ser-Lys1083-Gly1198) were determined from crystals grown at 4 °C using the sitting drop technique. Crystals were grown using CREBBP bromodomain protein at ~1.5 mM concentration that was equilibrated against: 0.2 M magnesium chloride, 0.1 M Bis-Tris pH 6.5, and 27% polyethylene glycol (PEG) 3350 (**1**), 0.2 M potassium thiocyanate, 0.1 M Bis-Tris pH 5.5, 5% v/v ethylene glycol, and 23% PEG 3350 (**12** and **14**), 0.2 M calcium acetate hydrate and 20% w/v Polyethylene glycol 3,350 (**27**) and then cryo-protected using a 1:1 mixture of paratone and paraffin oils before flash freezing in liquid nitrogen.

The co-crystal structures of **23** and **CPI-637** were obtained using a longer construct that included both the bromodomain and the PHD domain of CREBBP (residues R1081-L1312). These crystals were grown at 4^{o}C using the hanging drop vapor diffusion technique by mixing protein at ~0.5 mM with an equal volume of well solution containing 0.1 M Bis-Tris at pH 8, 0.2 M sodium thiocyanate and 19-23% PEG 3350, the crystals were then soaked with compound at a concentration of 1 mM for 24 hours. Data were collected at the Advanced Photon Source for **1** (21-ID-D). **27** (21-ID-F), and **CPI-637** (17-ID), the Canadian Light Source (CMCF-08ID) for **12** and **14,** and the Shanghai Synchrotron Radiation Facility (beamline 17U1) **23.** The structures were refined using refmac5 and the CCP4 suite of programs. Coordinates are deposited with accession codes: 4YK0 (**1**), 5I83 (**12**), 5I86 (**14**), 5I8B (**23**), 5I89 (**27**), 5I8G (**CPI-637**).

Compound **1** has been deposited to the PDB, cf. 4YK0.

SI Table 2. Data Collection and Refinement for CBP Bromodomain Complexes

	CBP-BrD/12	**CBP-BrD/14**	**CBP-BrD/27**
Data collection	CMCF-08ID	CMCF-08ID	APS 21-ID-F
space group	$P2_12_12_1$	P1	C2
unit cell (Å,˚)	***a***= 33.8, ***b***= 50.9, ***c***= 80.5 α=β=γ= 90.0	***a***= 34.0, ***b***= 42.0, ***c***= 49.8 α=96.8, β=109.7, γ= 99.4	***a***= 94.3, ***b***= 34.3 ***c***= 40.0 α=γ= 90.0, β=106.8
Resolution (Å)	50-1.35 (1.40-1.35)	50-1.05 (1.09-1.05)	50-1.07 (1.11-1.07)
Rsym[a,b]	0.080 (0.63)	0.032 (0.376)	0.069 (0.207)
Completeness (%)[b]	99.8 (100.0)	92.7 (87.8)	99.0 (97.3)
I/σI[b]	17.4 (3.4)	25.9 (2.6)	17.6 (6.8)
Refinement			
Resolution (Å)	31.58 – 1.35	40.71 – 1.05	45.13 – 1.07
No. reflections (F>0σ(F))	29268	103515	50979
R_{work}/R_{Free}	0.144, 0.188	0.139, 0.161	0.117, 0.137
No. atoms	1235	2474	1319
Mean B-factor (Å^2)	20.69	15.54	13.40
Rmsd bonds (Å)	0.010	0.010	0.009
Rmsd angles (˚)	1.463	1.583	1.380
Ramachandran (%) (favored/outliers)	100/0	100/0	100/0

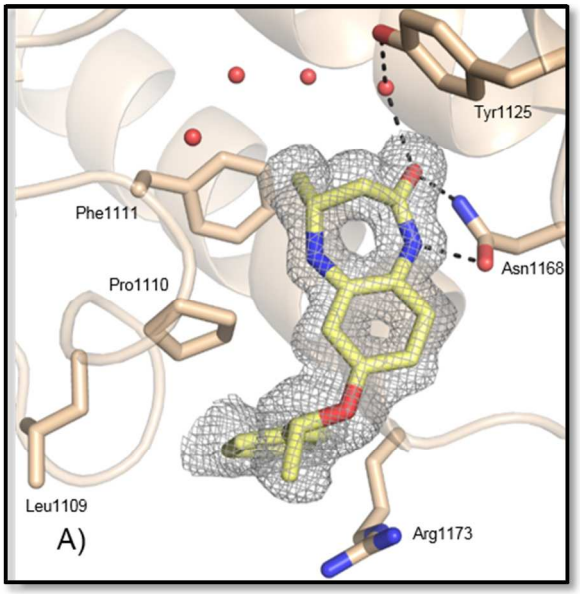
[a]In parenthesis, for the highest resolution shell.

[b] Rsym = $\Sigma||I| - |\langle I\rangle||/\Sigma|\langle I\rangle|$, where I is the intensity of a single observation and <I> the average intensity for symmetry equivalent observations.
[c] R = $\Sigma|Fo-Fc|/\Sigma|Fo|$, where Fo and Fc are observed and calculated structure factor amplitudes, respectively. R_{FREE} is calculated as R for reflections omitted from refinement.

SI Table 3. Data Collection and Refinement for CBP Bromodomain-PHD Complexes

	CBP-BrD-PHD/ 23	**CBP-BrD-PHD/ CPI-637**
Data collection	SSRF 17U1	APS 17-ID
space group	C2	C2
unit cell (Å,˚)	***a***= 92.2, ***b***= 60.6, ***c***= 54.1 β= 102.8, α=γ=90	***a***= 92.1, ***b***= 60.1, ***c***= 54.1 β= 102.7, α=γ=90
Resolution (Å)	50.0-1.52 (1.56-1.52)	38.67-1.41 (1.45-1.41)
Rsym[a,b]	0.08 (0.50)	3.3 (55.8)
Completeness (%)[b]	98.5 (97.2)	99.6 (99.4)
I/σI[b]	14.4 (2.0)	18.0 (2.73)
Refinement		
Resolution (Å)	45.02-1.52	38.7-1.41
No. reflections (F>0σ(F))	41810	50634
R_{work}/R_{Free}	0.205/0.218	20.19/22.05
No. atoms	2139	2220
Mean B-factor (Å^2)	34.4	33.2
Rmsd bonds (Å)	0.006	0.006
Rmsd angles (˚)	1.22	1.198
Ramachandran (%) (favored/outliers)	100/0	100/0

[a] In parenthesis, for the highest resolution shell.
[b] Rsym = $\Sigma||I| - |\langle I\rangle||/\Sigma|\langle I\rangle|$, where I is the intensity of a single observation and <I> the average intensity for symmetry equivalent observations.
[c] R = $\Sigma|Fo-Fc|/\Sigma|Fo|$, where Fo and Fc are observed and calculated structure factor amplitudes, respectively. R_{FREE} is calculated as R for reflections omitted from refinement.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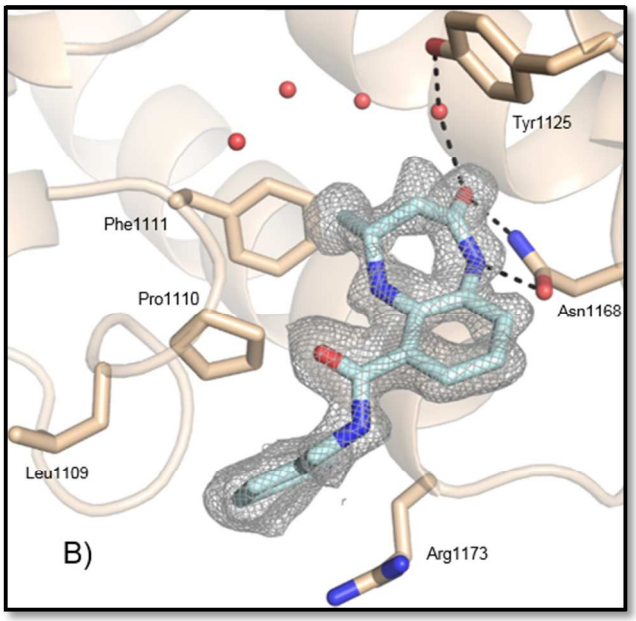
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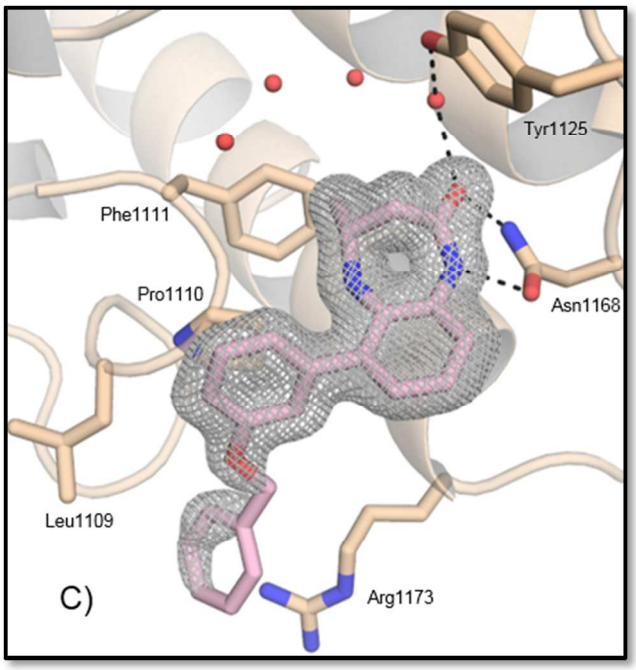
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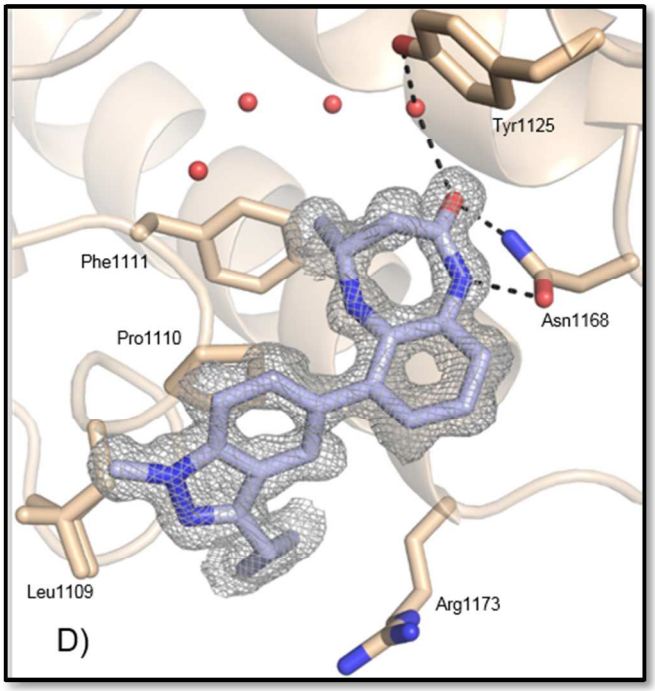
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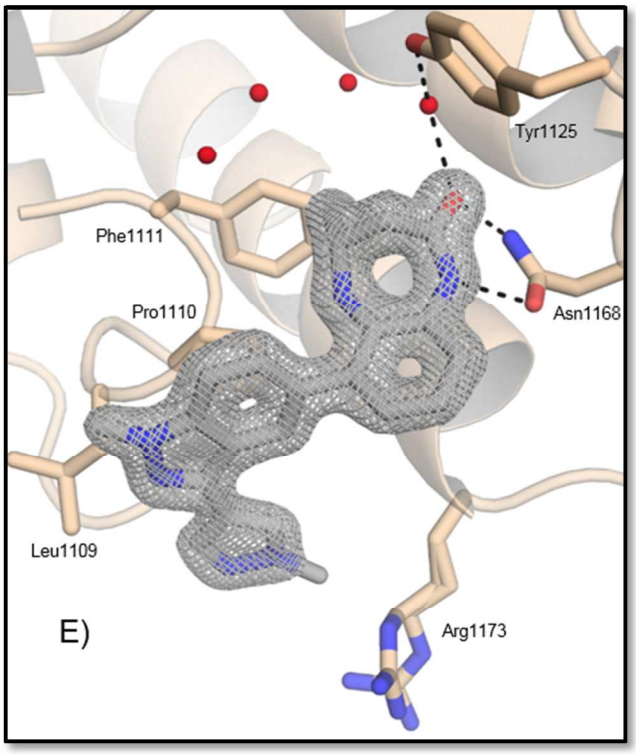
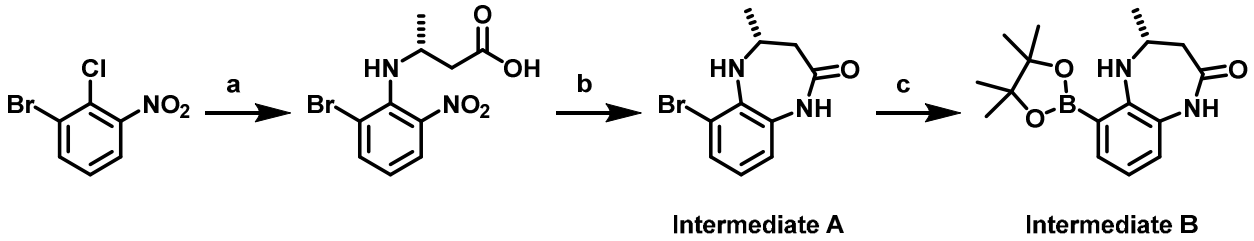


SI Figure 2. Co-crystal structures of compounds in the CBP bromodomain, showing $2F_o$-F_c composite omit electron density contoured at 1.0σ (grey mesh). A) **12**. B) **14**. C) **23**. D) **27**. E) **CPI-637**.

7. Compound synthesis and characterization

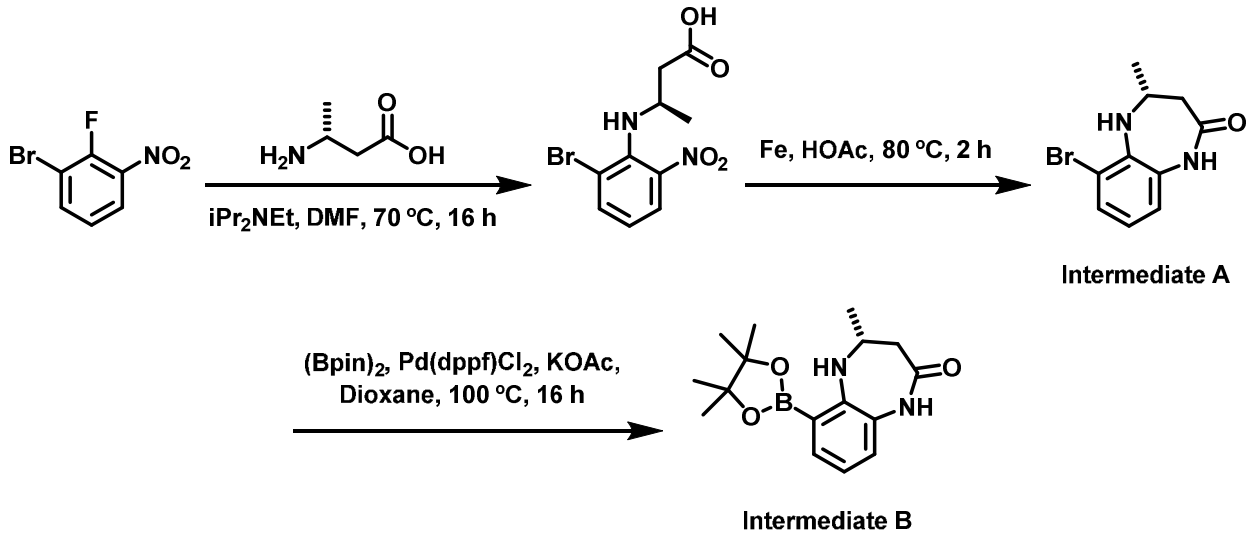
Most of the compounds in these studies were prepared through a nucleophilic aromatic substitution, reduction/cyclization sequence to produce two key compounds, **Intermediates A** and **B** (SI Scheme 1). For example, 1-bromo-2-chloro-3-nitrobenzene underwent substitution with methyl (*R*)-3-aminobutanoic acid (or its enantiomer) to afford the corresponding nitrobenzene benzodiazepinone precursor. Subsequent iron-mediated reduction of the nitro group and cyclization under the reaction conditions established the benzodiazepinone core **Intermediate A**, which could be transformed through Pd-catalyzed Suzuki coupling into the elaborated candidate compounds. Alternatively, **Intermediate A** could be converted into boronic acid ester **Intermediate B** for use as the nucleophile in similar Suzuki reactions. Compounds with alternative substitution on the benzene core were prepared by incorporating reactive handles (*i.e.* bromide or latent phenol) into the initial nitroarene building blocks.

SI Scheme 1. Synthesis of key benzodiazepinone intermediates.



Reagents: (a) (*R*)-3-aminobutyric acid, iPr_2NEt, DMF, 70 °C, 90%; (b) Fe, AcOH, 80 °C, 51%; (c) $(Bpin)_2$, $Pd(dppf)Cl_2$, KOAc, dioxane, 100 °C.

General procedure for Intermediates A & B



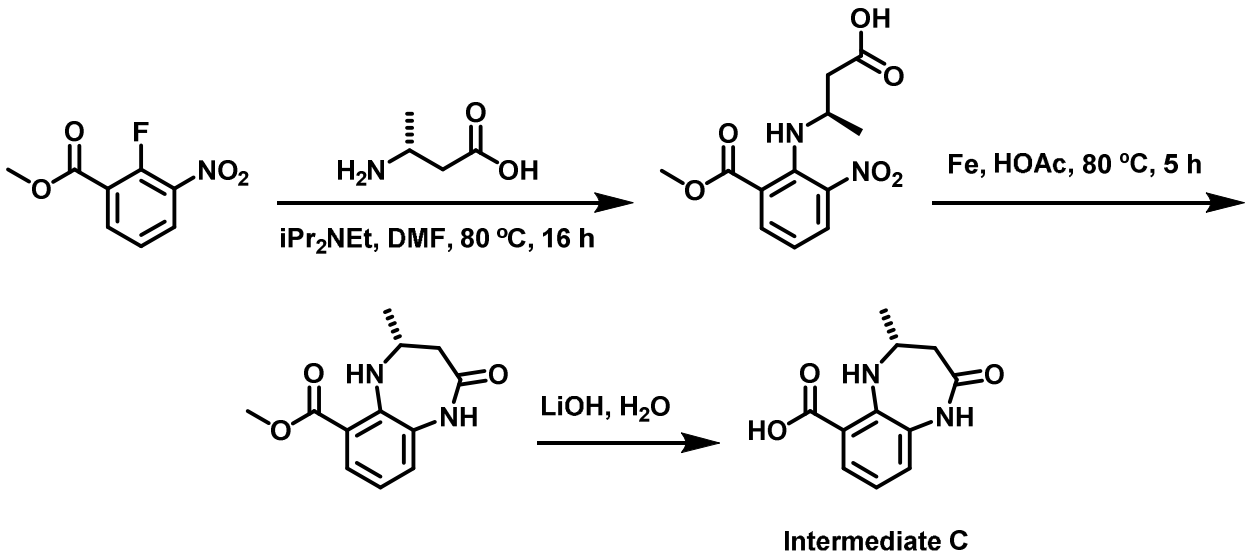
Step 1: To a solution of 1-bromo-2-fluoro-3-nitrobenzene (3.0 g, 13.64 mmol) in DMF (50 mL) was added *N*-ethyl-*N*-isopropylpropan-2-amine (5.3 g, 40.91 mmol) and (*R*)-3-aminobutanoic acid (1.7 g, 16.36 mmol) portion-wise. The resulting mixture was heated to 80 ºC for 10 h. After cooling the reaction to room temperature, water (30 mL) was added and the mixture was acidified with HCl (1N) to pH 6 and then extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the title compound (3.7 g, 90%) as a yellow solid that required no further purification.

Step 2: To a solution of (*R*)-3-((2-bromo-6-nitrophenyl)amino)butanoic acid (7.5 g, 24.74 mmol) in acetic acid (50 mL) was added Fe powder (7.0 g, 0.125 mol). The mixture was heated to 100 °C for 1 h. After cooling the reaction to room temperature, the reaction mixture was filtered and the filtrate was

concentrated *in vacuo*. Water (30 mL) was added and the mixture was extracted with EtOAc (60 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (petroleum ether / EtOAc = 3 / 1) to give the title compound (**Intermediate A**, 3.2 g, 51%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.63 (s, 1H), 7.26 (d, J = 8.0 Hz, 1H), 6.87 (d, J = 7.6 Hz, 1H), 6.71 – 6.67 (m, 1H), 4.58 (s, 1H), 3.98 – 3.97 (m, 1H), 2.40 – 2.41 (m, 1H), 2.21 – 2.18 (m, 1H), 1.20 (d, J = 6.0 Hz, 3H).

Step 3: To a solution of (*R*)-6-bromo-4-methyl-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (1.6 g, 6.27 mmol) in dioxane (25 mL) was added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (2.3 g, 9.41 mmol), KOAc (1.8 g, 18.82 mmol) and [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (0.5 g, 0.68 mmol). The mixture was heated to 100 °C for 16 h under nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was filtered and concentrated *in vacuo* to give the title compond (**Intermediate B**, 1.8 g, crude) as a brown solid that required no further purification.

General procedure for Intermediate C



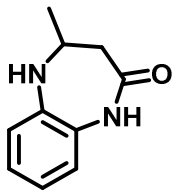
Step 1: To a solution of methyl 2-fluoro-3-nitrobenzoate (4.0 g, 20.09 mmol) in DMF (80 mL) was added *N*-ethyl-*N*-isopropylpropan-2-amine (7.8 g, 60.35 mmol) and (*R*)-3-aminobutanoic acid (2.3 g, 22.30 mmol). The resulting mixture was heated to 80 °C for 15 h. After cooling the reaction to room temperature, water (50 mL) was added and the mixture was extracted with EtOAc (100 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give the title compound (4.1 g, 73%) as a yellow solid that required no further purification.

Step 2: A mixture of (*R*)-3-((2-(methoxycarbonyl)-6-nitrophenyl)amino)butanoic acid (4.1 g, 14.53 mmol) and Fe powder (4.2 g, 75.21 mol) in acetic acid (50 mL) was heated to 100 °C for 1 h. After cooling the reaction to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo*. Water (20 mL) was added and the mixture was extracted with EtOAc (80 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue

was purified by silica gel chromatography (petroleum ether/EtOAc = 3/1) to give the title compound (1.5 g, 44%) as a white solid.

Step 3: To a solution of (*R*)-methyl 4-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-6-carboxylate (1.3 g, 5.55 mmol) in DMF/H_2O (40 mL/10 mL) was added LiOH (720 mg, 30.06 mmol). The resulting mixture was heated to 80 °C for 15 h. After cooling the reaction to room temperature, the solvent was concentrated *in vacuo*. Water (20 mL) was added and the mixture was extracted with EtOAc (30 mL x 3). The aqueous phase was acidified with HCl (1N) to pH 3. The resulting precipitate was collected by filtration to give the title compound (**Intermediate C**, 1.0 g, 82%) as a white solid.

4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (1)



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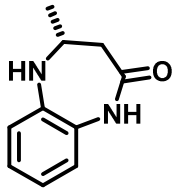
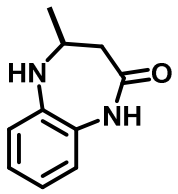
(R)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (2)



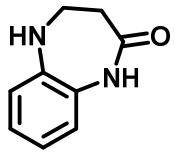
Separated from **1** (380 mg, 2.16 mmol) by SFC-80-(8) AS(250mm*30mm,10um), Base-EtOH Mobile phase B: Gradient 45%. (182.00 mg, 1.03 mmol, 47.82% yield, 100% purity, ee% 100%) was obtained as a white solid. LCMS $(M + H)^+$ M/Z 176.8. ^{1}H NMR (400MHz, $CDCl_3$) δ= 7.64 (s, 1H), 7.03-7.00 (m, 1H), 6.89-6.88 (m, 2H), 6.80-6.78 (d, *J*=8 Hz, 1H), 4.06-4.02 (m, 1H), 2.68-2.63 (m, 1H), 2.48-2.43 (m, 1H), 1.36-1.33 (d, *J*=12.8 Hz, 3H).

(S)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (3)



Separated from **1** (380 mg, 2.16 mmol) by SFC-80-(8) AS(250mm*30mm,10um), Base-EtOH Mobile phase B: Gradient 45%. (176.00 mg, 0.999 mmol, 46.24% yield, 100% purity, ee% 100%) was obtained as a white solid. LCMS (M + H) $^{+}$ M/Z 176.8. ^{1}H NMR (400MHz, $CDCl_3$) δ= 7.44 (s, 1H), 7.03-6.99 (m, 1H), 6.90-6.85 (m, 2H), 6.79-6.77 (d, *J*=8 Hz,1H), 4.07-3.99 (m, 1H), 2.96-2.62 (m, 1H), 2.47-2.41 (m, 1H), 1.34-1.32 (d, *J*=6.4 Hz, 3H).

1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (4)



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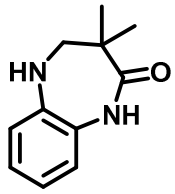
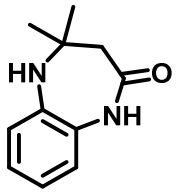
3,3-dimethyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (5)



Commercially available.

4,4-dimethyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (6)



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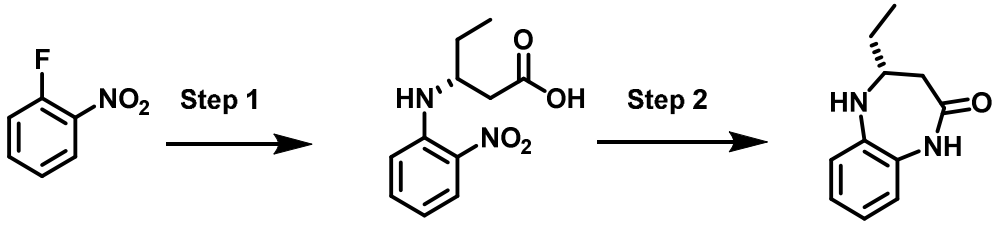
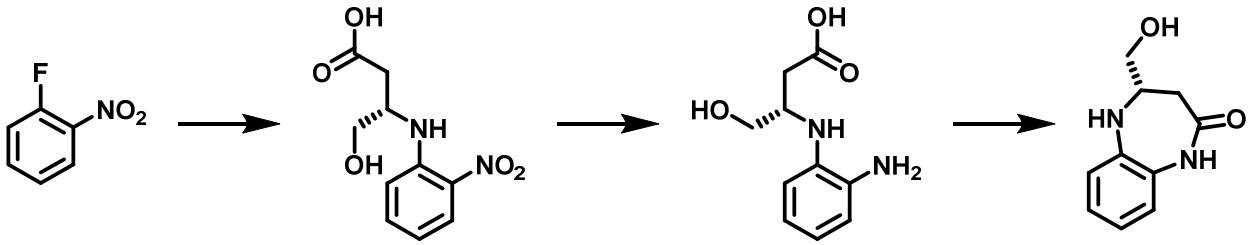
(R)-4-ethyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (7)



Step 1: A round bottomed flask was charged with a stirbar, 1-fluoro-2-nitrobenzene (0.405 g, 2.87 mmol, 1 equiv), (R)-3-aminopentanoic acid (0.504 g, 4.30 mmol, 1.5 equiv), potassium carbonate (0.793 g, 5.74 mmol, 2 equiv) and dimethylsulfoxide (10 mL). The flask was heated to 100 °C and stirred 1 h before being cooled and poured into 1M aqueous HCl (20 mL). The mixture was washed with ethyl acetate (3 X 20 mL). The combined ethyl acetate washes were dried (Na_2SO_4), filtered and concentrated. The residue was purified by silica gel chromatography (eluting with methanol/dichloromethane) to provide (R)-3-((2-nitrophenyl)amino)pentanoic acid as an orange oil. LCMS M/Z $(M+H)^+$ 239.

Step 2: A disposable reaction tube was charged with (*R*)-3-(2-nitrophyenylamino)pentanoic acid (0.511 g, 2.15 mmol, 1 equiv), a stirbar, ethanol (12 mL) and acetic acid (2.55 ml, 42.9 mmol, 20 equiv). Iron (0.958 g, 17.2 mmol, 8 equiv) was added, and the orange suspension was stirred at 110 °C 3 h. The mixture was cooled, filtered through celite, and concentrated *in vacuo* to provide a residue that was purified by silica gel chromatography (eluting with methanol/dichloromethane), then lyophilized to yield (*R*)-4-ethyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (26 mg, 0.136 mmol, 6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.44 (s, 1H), 6.82 - 6.92 (m, 3H), 6.65 - 6.72 (m, 1H), 5.32 (br. s., 1H), 3.53 - 3.63 (m, 1H), 2.43 (dd, *J* = 3.74, 13.29 Hz, 1H), 2.22 - 2.31 (m, 1H), 1.45 - 1.61 (m, 2H), 0.90 (t, *J* = 14.70 Hz, 3H). LCMS M/Z $(M+H)^+$ 191.

(S)-4-(hydroxymethyl)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (8)

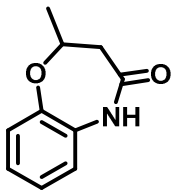


Step 1: A disposable reaction tube was charged with a stirbar, (*S*)-3-amino-4-hydroxybutanoic acid (400 mg, 3.36 mmol), 1-fluoro-2-nitrobenzene (710 mg, 5.0 mmol), potassium phosphate (2.1 g, 10 mmol), and dimethylacetamide (14 mL). The reaction was heated to 150 °C for 20 min before the mixture was cooled and purified (without aqueous workup) by silica gel chromatography (methanol/dichloromethane 0:10 to 2:8) to provide (*S*)-4-hydroxy-3-((2-nitrophenyl)amino)butanoic acid (180 mg, 22% yield). LCMS M/Z $(M+H)^+$ 241.

Step 2: A vial equipped with a septum was charged with a stirbar, (*S*)-4-hydroxy-3-(2-nitrophenylamino)butanoic acid (180 mg, 0.749 mmol), 10% (w/w) palladium on carbon (40 mg), and methanol (2 mL). The headspace was flushed with hydrogen, and the reaction was stirred at room temperature under an atmosphere of hydrogen (1 atm) for 2 h. The mixture was filtered through celite, and the filtrate was concentrated *in vacuo.* Residual methanol was removed by a series of azeotropic distillations with dichloromethane to provide (*S*)-3-((2-aminophenyl)amino)-4-hydroxybutanoic acid in an adequate purity for the next step. LCMS M/Z $(M+H)^+$ 211.

Step 3: A vial equipped with a septum was charged with a stirbar, (*S*)-3-(2-aminophenylamino)-4-hydroxybutanoic acid (60 mg, 0.29 mmol), COMU (180 mg, 0.43 mmol), and dimethylformamide (5 mL). The reaction was stirred at room temperature for 2.5 h before it was purified (without aqueous workup) by silica gel chromatography twice (hexane/ ethyl acetate 10:0 to 0:10 and methanol/dichloromethane 0:10 to 2:8) to provide the title compound as a tan solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (s, 1H), 6.88 (d, *J* = 3.79 Hz, 2H), 6.84 (d, *J* = 7.36 Hz, 1H), 6.68 - 6.76 (m, 1H), 5.14 (br. s., 1H), 4.90 (t, *J* = 5.46 Hz, 1H), 3.59 - 3.69 (m, 1H), 3.36 - 3.44 (m, 1H), 2.34 - 2.43 (m, 1H), 2.23 - 2.34 (m, 1H). LCMS M/Z $(M+H)^+$ 193.

2-methyl-2,3-dihydrobenzo[b][1,4]oxazepin-4(5H)-one (9)



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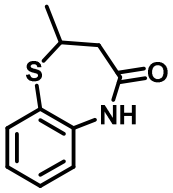
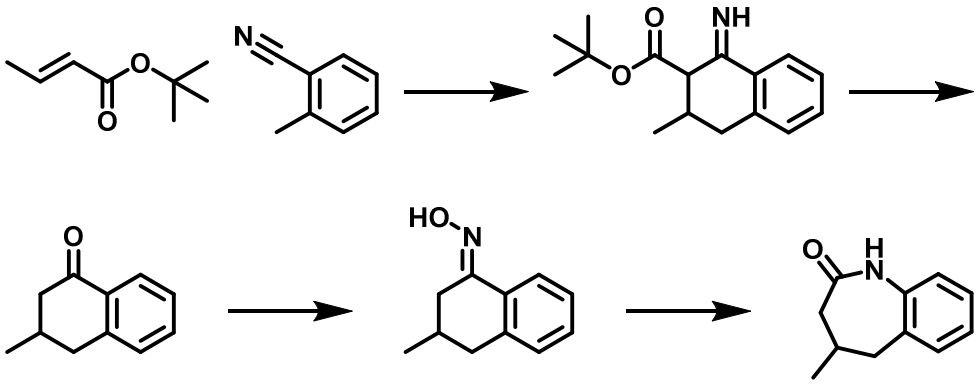
2-methyl-2,3-dihydrobenzo[b][1,4]thiazepin-4(5H)-one (10)



Commercially available from Sigma-Aldrich.

4-methyl-1,3,4,5-tetrahydro-2H-benzo[b]azepin-2-one (11)



Step 1: To a solution of *N*,*N*-diisopropylamine (5.79 mL, 41.0 mmol) in diglyme (1000 mL) was slowly added butyllithium (16.39 mL, 41.0 mmol) at -78 °C. The reaction was warmed to 0 °C for 10 minutes. Once the N,N-diisopropylamide had been generated in situ, the reaction was cooled to -78 °C, and 2-methylbenzonitrile (2.4 g, 20.49 mmol) was slowly added over 10 minutes, followed by the addition of (*E*)-tert-butyl but-2-enoate (2.91 g, 20.49 mmol) 5 minutes later. After stirring for 15 minutes at -78 °C, the reaction was warmed to 20 °C and stirred at this temperature for 45 minutes before it was quenched with a saturated aqueous solution of ammonium chloride. The product was extracted with diethylether (repeated 4 times), and the combined organic layers were dried over sodium sulfate, filtered and concentrated *in vacuo* to give tert-butyl 1-imino-3-methyl-1,2,3,4-tetrahydronaphthalene-2-carboxylate as a yellow oil. This crude material was used in the next step without further treatment. LCMS M/Z $(M+H)^+$ 260.

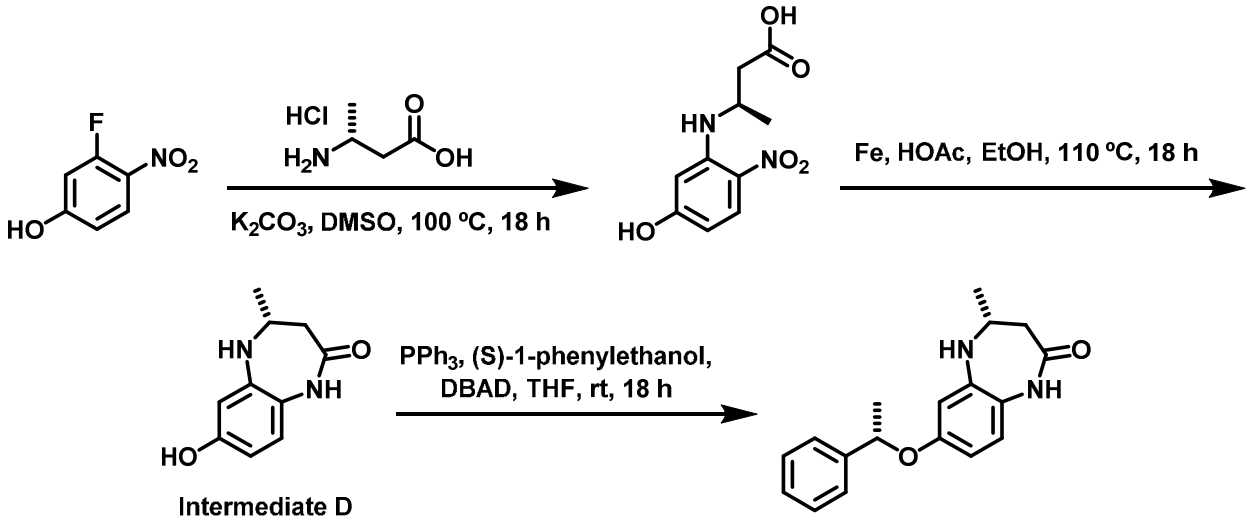
Step 2: To a solution of tert-butyl 1-imino-3-methyl-1,2,3,4-tetrahydronaphthalene-2-carboxylate (5 g, 19.28 mmol) in MeOH (20 mL) was added 6 M aqueous hydrochloric acid (20 mL, 120 mmol) at room

temperature. The reaction was heated to 70 °C (gas evolution was observed) for 1.5 hours before the reaction was diluted with water, and the product was extracted with diethylether (repeated 4 times). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography (Hexanes/diethylether 19:1 to 8:2) to give the 3-methyl-3,4-dihydronaphthalen-1(2H)-one intermediate (yield not determined). LCMS M/Z $(M+H)^+$ 161.

Step 3: To a solution of 3-methyl-3,4-dihydronaphthalen-1(2H)-one (500 mg, 3.12 mmol) and hydroxylamine hydrochloride (260 mg, 3.75 mmol) in methanol (20 mL) was added sodium hydroxide (150 mg, 3.75 mmol). After the reaction was stirred overnight at room temperature, additional hydroxylamine hydrochloride (108 mg, 1.560 mmol) and sodium hydroxide (62.4 mg, 1.560 mmol) were added. Then the reaction was heated to 70 °C for about 1.5 hours before it was concentrated *in vacuo*. The residue was suspended in a mixture of ethyl acetate and dichloromethane (9:1), filtered through celite and the filtrate was concentrated *in vacuo* to give the 3-methyl-3,4-dihydronaphthalen-1(2H)-one oxime intermediate as a white solid (547 mg, 100% yield). LCMS M/Z $(M+H)^+$ 176.

Step 4: Polyphosphoric acid (10 mL) was added to 3-methyl-3,4-dihydronaphthalen-1(2H)-one oxime (547 mg, 3.12 mmol) and the mixture was heated to 120 °C for 2 hours. Then the reaction was cooled to 50 °C before it was quenched with water (200 mL) (exothermic process) (preforming the quench a lower temperature was difficult due to the high viscosity of the reaction mixture). The product was extracted with dichloromethane (repeated 4 times), and the combined organic layers were washed with a saturated solution of sodium bicarbonate, dried over sodium sulfate, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (Hexanes/Ethyl acetate 85:15 to 65:35) to give 4-methyl-1,3,4,5-tetrahydro-1-benzazepin-2-one (**11**) as a white solid (425 mg, 78% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.53 (br. s., 1H), 7.17 - 7.26 (m, 2H), 7.07 (tt, *J* = 1.20, 7.50 Hz, 1H), 6.95 (d, *J* = 8.00 Hz, 1H), 2.81 (dd, *J* = 6.69, 13.38 Hz, 1H), 2.44 - 2.53 (m, 1H), 2.31 (dd, *J* = 6.58, 13.27 Hz, 1H), 2.21 (dd, *J* = 6.91, 12.26 Hz, 1H), 1.79 (dd, *J* = 6.91, 12.26 Hz, 1H), 0.99 (dd, *J* = 1.34, 6.69 Hz, 3H). LCMS M/Z $(M+H)^+$ 176.

(R)-4-methyl-7-((R)-1-phenylethoxy)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (12)

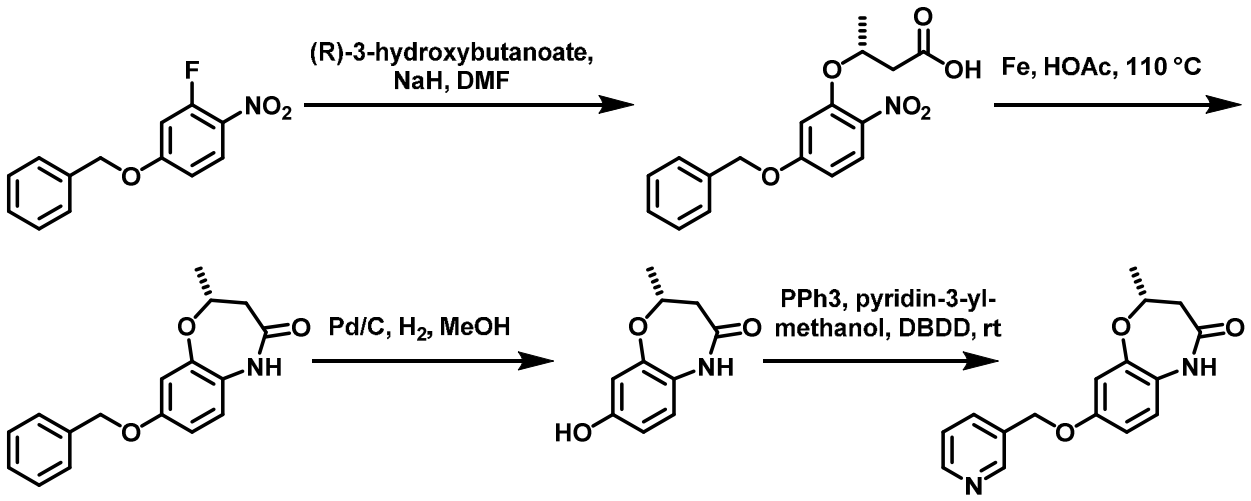


Step 1: A round bottomed flask was charged with a stirbar, 3-fluoro-4-nitrophenol (1.535 g, 9.77 mmol, 1 equiv), (R)-3-aminobutanoic acid, HCl (1.5 g, 10.75 mmol, 1.1 equiv), potassium carbonate (2.70 g, 19.54 mmol, 2 equiv) and dimethylsulfoxide (25 mL). The flask was heated to 100 °C and stirred 18 h before being cooled, filtered through celite, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (eluting with methanol/dichloromethane) to provide (R)-3-((5-hydroxy-2-nitrophenyl)amino)butanoic acid as an orange oil (900 mg, 3.75 mmol, 38%) clean product as orange oil. LCMS M/Z $(M+H)^+$ 241.

Step 2: A disposable reaction tube was charged with (R)-3-(5-hydroxy-2-nitrophenylamino)butanoic acid (0.433, 1.803 mmol, 1 equiv) , a stirbar, ethanol (10 mL) and acetic acid (2.064 ml, 36.1 mmol, 20 equiv). Iron (0.805 g, 14.42 mmol, 8 equiv) was added, and the orangish suspension was stirred at 110 °C 18 h. The mixture was cooled, filtered through celite, and concentrated *in vacuo* to provide a residue that was purified by silica gel chromatography (eluting with methanol/dichloromethane). (R)-7-hydroxy-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]-diazepin-2-one (**Intermediate D**) was isolated as an amorphous solid and used in subsequent reactions. LCMS M/Z $(M+H)^+$ 193.

Step 3: A disposable reaction tube was charged with polymer bound triphenylphosphine (0.134 g, 0.510 mmol, 2 equiv), (R)-7-hydroxy-4-methyl-4,5-dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one (0.049 g, 0.255 mmol, 1 equiv), a stirbar, (S)-1-phenylethanol (0.092 ml, 0.765 mmol, 3 equiv), and anhydrous tetrahydrofuran (2 mL). (E)-di-tert-butyl diazene-1,2-dicarboxylate (0.117 g, 0.510 mmol, 2 equiv), dissolved in 1 mL of tetrahydrofuran, was added dropwise and the resulting solution was stirred at room temperature 18 h. The mixture was filtered, concentrated *in vacuo*, and purified by HPLC (eluting with water/acetonitrile/0.1% trifluoroacetic acid) before being lyophilized to yield (4R)-4-methyl-7-[(1R)-1-phenylethoxy]-1,3,4,5-tetrahydro-1,5-benzodiazepin-2-one (7.3 mg, 0.0178 mmol, 7%) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.28 (s, 1H), 7.30 - 7.40 (m, 4H), 7.21 - 7.28 (m, 1H), 6.70 (d, *J* = 8.79 Hz, 1H), 6.52 (br. s., 1H), 6.36 (d, *J* = 6.59 Hz, 1H), 5.33 (q, *J* = 6.29 Hz, 1H), 3.82 (dd, *J* = 6.68, 10.71 Hz, 1H), 2.39 (dd, *J* = 4.12, 13.46 Hz, 1H), 2.13 (dd, *J* = 7.32, 13.37 Hz, 1H), 1.51 (d, *J* = 6.23 Hz, 3H), 1.14 (d, *J* = 6.41 Hz, 3H). LCMS M/Z $(M+H)^+$ 297.

(R)-2-methyl-8-(pyridin-3-ylmethoxy)-2,3-dihydrobenzo[b][1,4]oxazepin-4(5H)-one (13)



Step 1: To sodium (*R*)-3-hydroxybutanoate (1 g, 4.04 mmol, 1 equiv) in DMF at 0 °C was added sodium hydride (170 mg, 4.25 mmol, 1.05 equiv). The mixture was stirred at 0 °C for 30 min before 4-(benzyloxy)-2-fluoro-1-nitrobenzene (536 mg, 4.25 mmol, 1.05 equiv) was added and the mixture stirred for an additional 18 h. The mixture was diluted with EtOAc and washed with 1N HCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography (eluting with hexanes/EtOAc) to give the title compound (140 mg, 10%). LCMS M/Z (M+H) 332.

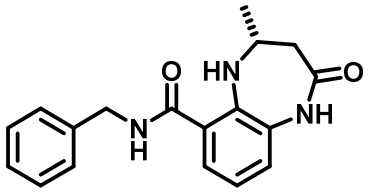
Step 2: A mixture of (*R*)-3-(5-(benzyloxy)-2-nitrophenoxy)butanoic acid (140 mg, 0.423 mmol) and Fe powder (189 mg, 3.38 mmol) in acetic acid (0.5 mL) was heated to 110 ^{o}C for 16 h. After cooling the reaction to room temperature, the reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (petroleum ether/EtOAc = 3/1) to give the title compond (75 mg, 63%) as a white solid. LCMS M/Z (M+H) 284.

Step 3: To a solution of (*R*)-8-(benzyloxy)-2-methyl-2,3-dihydrobenzo[*b*][1,4]oxazepin-4(5*H*)-one (75 mg, 0.265 mmol mmol) in MeOH (2 mL) was added Pd/C (5% wt, 28 mg). The mixture was stirred at 20 °C for 2 h under hydrogen atmosphere. The mixture was filtered and concentrated *in vacuo* to give the title compound (24 mg, 47%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.33 (s, 1H), 6.79 (d, *J* = 8.7 Hz, 1H), 6.47 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.41 (d, *J* = 2.7 Hz, 1H), 4.69 (m, 1H), 2.53 (m, 1H), 2.29 (d, *J* = 7.5 Hz, 1H), 1.28 (d, *J* = 6.2 Hz, 3H). LCMS M/Z (M+H) 194.

Step 4: A disposable reaction tube was charged with polymer bound triphenylphosphine (0.097 g, 0.311 mmol, 2 equiv), (R)-7-hydroxy-4-methyl-4,5-dihydro-1H-benzo[b][1,4]diazepin-2(3H)-one (0.030 g, 0.155 mmol, 1 equiv), a stirbar, pyridin-3-yl-methanol (0.051 g, 0.466 mmol, 3 equiv), and anhydrous tetrahydrofuran (2 mL). (E)-di-tert-butyl diazene-1,2-dicarboxylate (0.072 g, 0.311 mmol, 2 equiv), dissolved in 1 mL of tetrahydrofuran, was added dropwise and the resulting solution was stirred at room temperature 2h. The mixture was filtered, concentrated *in vacuo*, and purified by silica gel chromatography (eluting with hexane/ethyl acetate, then ethyl acetate/methanol). The product containing fractions were further purified by HPLC (eluting with water/acetonitrile/0.1% trifluoroacetic acid). Product containing fractions were combined and partitioned between ethyl acetate (20 mL) and 2M aqueous potassium carbonate solution (20 mL). The ethyl acetate layer was dried (Na_2SO_4), filtered

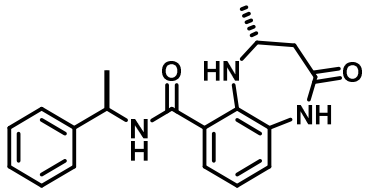
and concentrated to yield (R)-2-methyl-8-(pyridin-3-ylmethoxy)-2,3-dihydrobenzo[b][1,4]oxazepin-4(5H)-one (30 mg, 0.106 mmol, 68%) as an off-white amorphous solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.48 (s, 1H), 8.65 (br. s., 1H), 8.54 (d, J = 3.34 Hz, 1H), 7.85 (d, J = 7.80 Hz, 1H), 7.42 (dd, J = 4.79, 7.69 Hz, 1H), 6.93 (d, J = 8.47 Hz, 1H), 6.76 (dd, J = 2.79, 8.58 Hz, 1H), 6.71 (d, J = 2.90 Hz, 1H), 5.10 (s, 2H), 4.73 (m, 1H), 2.55 (dd, J = 4.46, 14.05 Hz, 1H), 2.35 (dd, J = 7.47, 14.16 Hz, 1H), 1.30 (d, J = 6.24 Hz, 3H). LCMS M/Z $(M+H)^+$ 285.

(R)-N-benzyl-4-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (14)



A disposable tube was charged with (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1Hbenzo[b][1,4]diazepine-6-carboxylic acid (**Intermediate D**) (78 mg, 0.354 mmol) and a stirbar. DMF (1 mL) was added, followed by benzylamine (46.4 µl, 0.425 mmol), HATU (148 mg, 0.390 mmol), and iPr_2NEt (124 µl, 0.708 mmol). Stirred at room temperature 4 h before being diluted with ethyl acetate, washed three times with brine, concentrated *in vacuo* with celite, and purified by silica gel chromatography (Biotage, eluting with methylene chloride/methanol/ammonium hydroxide). Following lyophilization from dioxane, the title compound was isolated as a white amorphous solid (76 mg, 0.25 mmol, 69%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 9.04 (t, *J* = 6.02 Hz, 1H), 7.30 - 7.36 (m, 4H), 7.24 (qd, *J* = 4.18, 8.64 Hz, 1H), 7.16 (br. s., 1H), 6.97 - 7.06 (m, 1H), 6.79 (t, *J* = 7.80 Hz, 1H), 4.37 - 4.53 (m, 2H), 3.90 (br. s., 1H), 2.89 (br. s., 1H), 2.69 (s, 2H), 2.41 (dd, *J* = 3.79, 13.38 Hz, 1H), 2.24 (dd, *J* = 8.36, 13.49 Hz, 1H), 1.13 (d, *J* = 6.24 Hz, 3H). LCMS M/Z $(M+H)^+$ 310.

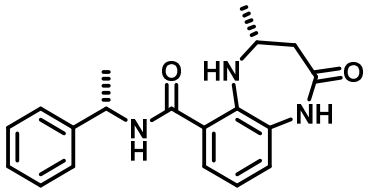
(R)-4-methyl-2-oxo-N-((R)-1-phenylethyl)-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (15)



A disposable tube was charged with (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1Hbenzo[b][1,4]diazepine-6-carboxylic acid (**Intermediate D**) (50 mg, 0.227 mmol) and a stirbar. DMF (1 mL) was added, followed by (*R*)-1-phenylethanamine (35.1 µl, 0.272 mmol), HATU (95 mg, 0.250 mmol), and iPr_2NEt (79 µl, 0.454 mmol). Stirred at room temperature 4 h before being diluted with ethyl acetate, washed three times

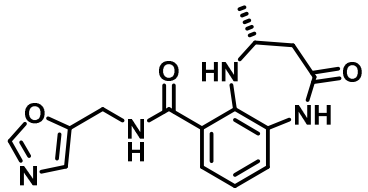
with brine, concentrated *in vacuo* with celite, and purified by silica gel chromatography (Biotage, eluting with methylene chloride/methanol/ammonium hydroxide). Lyophilization from dioxane afforded the title compound as an amorphous solid (56 mg, 0.17 mmol, 75%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.56 (s, 1H), 8.89 (d, *J* = 7.97 Hz, 1H), 7.34 - 7.41 (m, 3H), 7.31 (t, *J* = 7.55 Hz, 2H), 7.19 - 7.25 (m, 1H), 7.02 (d, *J* = 7.97 Hz, 1H), 6.85 (t, *J* = 7.83 Hz, 1H), 5.10 (quin, *J* = 7.21 Hz, 1H), 3.83 - 3.92 (m, 1H), 2.38 (dd, *J* = 3.98, 13.32 Hz, 1H), 2.17 (dd, *J* = 8.51, 13.46 Hz, 1H), 1.42 - 1.51 (m, 3H), 0.99 (d, *J* = 6.32 Hz, 3H). LCMS M/Z $(M+H)^+$ 324.

(R)-4-methyl-2-oxo-N-((S)-1-phenylethyl)-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (16)



A disposable tube was charged with (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1Hbenzo[b][1,4]diazepine-6-carboxylic acid (**Intermediate D**) (50 mg, 0.227 mmol) and a stirbar. DMF (1 mL) was added, followed by (*S*)-1-phenylethanamine (35.1 µl, 0.272 mmol), HATU (95 mg, 0.250 mmol), and iPr_2NEt (79 µl, 0.454 mmol). Stirred at room temperature 4 h before being diluted with ethyl acetate, washed three times with brine, concentrated *in vacuo* with celite, and purified by silica gel chromatography (Biotage, eluting with methylene chloride/methanol/ammonium hydroxide). Lyophilization from dioxane afforded the title compound as a white, amorphous solid (43 mg, 0.13 mmol, 59%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.86 (d, *J* = 8.03 Hz, 1H), 7.37 - 7.42 (m, 2H), 7.30 - 7.36 (m, 3H), 7.19 - 7.27 (m, 1H), 7.01 (dd, *J* = 1.34, 7.80 Hz, 1H), 6.82 (t, *J* = 7.80 Hz, 1H), 5.13 (quin, *J* = 7.25 Hz, 1H), 3.81 - 3.92 (m, 1H), 2.39 (dd, *J* = 3.79, 13.60 Hz, 1H), 2.22 (dd, *J* = 8.47, 13.38 Hz, 1H), 1.44 (d, *J* = 6.91 Hz, 3H), 1.13 (d, *J* = 6.24 Hz, 3H). LCMS M/Z $(M+H)^+$ 324.

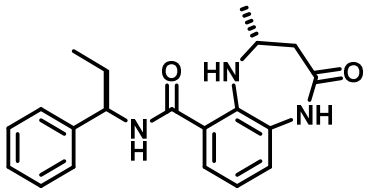
(R)-4-methyl-N-(oxazol-5-ylmethyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (17)



To a solution of (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-6-carboxylic acid (**Intermediate C**, 100 mg, 0.45 mmol) in DMF (5 mL) was added oxazol-5-ylmethanamine hydrochloride (67 mg, 0.49 mmol), triethylamine (230 mg, 2.27 mmol) and HATU (190 mg, 0.50 mmol). The mixture was allowed to stir at room temperature for 16 h. The mixture was concentrated *in vacuo*, and the crude

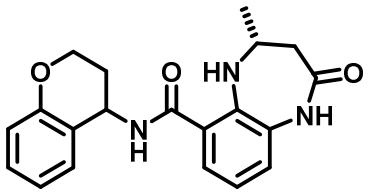
residue was purified by reverse phase chromatography (acetonitrile 40-70%/0.2% formic acid in water) to give the title compound (25 mg, 18%) as a white solid.[1]H NMR (400 MHz, CD_3OD) δ 8.14 (s, 1H), 7.33 – 7.31 (m, 1H), 7.07 – 7.05 (m, 2H), 6.90 – 6.86 (m, 1H), 4.65 – 4.55 (m, 2H), 4.04 – 3.99 (m, 1H), 2.53 – 2.49 (m, 1H), 2.34 – 2.28 (m, 1H), 1.21 (d, *J* = 6.4 Hz, 3H). LCMS M/Z $(M+H)^+$ 301.

(R)-4-methyl-2-oxo-N-((R)-1-phenylpropyl)-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (18)



To a solution of (*R*)-1-phenylpropan-1-amine (74 mg, 55 mmol), (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-6-carboxylic acid (**Intermediate C**, 100 mg, 0.45 mmol) in DMF (5 mL) was added HATU (190 mg, 0.50 mmol) and N-ethyl-N-isopropylpropan-2-amine (176 mg, 1.36 mmol). The resulting mixture was allowed to stir at room temperature for 15 h. The mixture was concentrated *in vacuo*, and the crude residue was purified by reverse phase chromatography (acetonitrile 23-43% / 0.2% formic acid in water) to give the title compound (85 mg, 55%) as a pink solid. [1]H NMR (400 MHz, CD_3OD) δ 7.37 – 7.28 (m, 5H), 7.22 – 7.20 (m, 1H), 7.06 – 7.05 (m, 1H), 6.97 – 6.96 (m, 1H), 4.91 – 4.88 (m, 1H), .3.97 – 3.92 (m, 1H), 2.46 – 2.41 (m, 1H), 2.27 – 2.22 (m, 1H), 1.91 – 1.87 (m, 2H), 1.01-0.98 (m, 6H). LCMS M/Z $(M+H)^+$ 338.

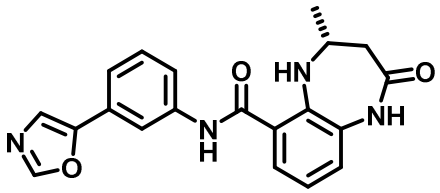
(4R)-N-(chroman-4-yl)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (19)



To a solution of (*R*)-chroman-4-amine (81 mg, 0.55 mmol), (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-6-carboxylic acid (**Intermediate C**, 100 mg, 0.45 mmol) in DMF (5 mL) was added HATU (131 mg, 0.55 mmol) and N-ethyl-N-isopropylpropan-2-amine (176 mg, 1.36 mmol). The resulting mixture was allowed to stir at room temperature for 15 h. The mixture was concentrated *in vacuo*, and the crude residue was purified by reverse phase chromatography (acetonitrile 23-43% / 0.2% formic acid in water) to give the title compound (30 mg, 18%) as a white solid. [1]H NMR (400 MHz, DMSO-d_6) δ 9.56 (s, 1H), 8.90 (d, *J* = 8.4 Hz, 1H), 7.29 (d, *J* = 6.4 Hz, 1H),7.22 – 7.14 (m, 2H), 7.00 (d, *J* = 6.8 Hz, 1H), 6.86 –

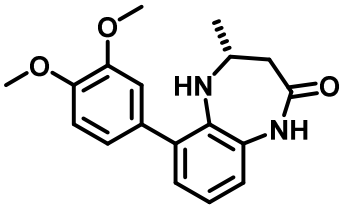
6.76 (m, 3H), 5.28 – 5.25 (m, 1H), 4.30 – 4.22 (m, 1H), 3.98 – 3.95 (m, 1H), 2.50 – 2.40 (m, 1H), 2.27 – 2.20 (m, 3H), 1.21 (d, *J* = 6.4 Hz, 3H). LCMS M/Z $(M+H)^+$ 352.

(R)-4-methyl-N-(3-(oxazol-5-yl)phenyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepine-6-carboxamide (20)



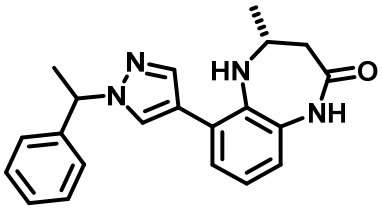
To a solution of (*R*)-4-methyl-2-oxo-2,3,4,5-tetrahydro-1*H*-benzo[*b*][1,4]diazepine-6-carboxylic acid (**Intermediate C**, 100 mg, 0.45 mmol) in DMF (5 mL) was added 3-(oxazol-5-yl)aniline (80 mg, 0.50 mmol), triethylamine (176 mg, 1.36 mmol) and HATU (190 mg, 0.50 mmol). The mixture was allowed to stir at room temperature for 16 h. The mixture was concentrated *in vacuo*, and the crude residue was purified by reverse phase chromatography (acetonitrile 40-70% / 0.2% formic acid in water) to give the title compound (15 mg, 9.1%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.29 (s, 1H), 8.15 (s, 1H), 7.56 – 7.54 (m, 1H), 7.52 – 7.48 (m, 4H), 7.16 – 7.14 (m, 1H), 7.02 – 6.99 (m, 1H), 4.11 – 4.06 (m, 1H), 2.63 – 2.58 (m, 1H), 2.40 – 2.35 (m, 1H), 1.31 (d, *J* = 6.4 Hz, 3H). LCMS M/Z $(M+H)^+$ 363.

(R)-6-(3,4-dimethoxyphenyl)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (21)



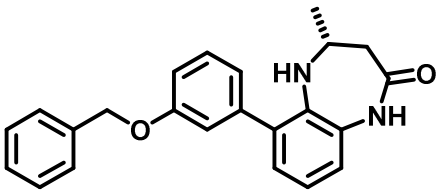
A disposable tube was charged with (3,4-dimethoxyphenyl)boronic acid (70 mg, 0.4 mmol), **Intermediate A** (0.2 mmol), XPhos precat. III (20 mg, 0.02 mmol), and a stirbar before being evacuated and backfilled with nitrogen three times. Acetonitrile (2 mL) was added, followed by sodium hydroxide (1 M, 0.22 mL, 0.22 mmol), and the solution was stirred at 90 ºC 30 min. The reaction mixture was concentrate in vacuo with celite and purified by silica gel chromatography (Biotage, eluting with hexanes/ethyl acetate). Lyophilization from dioxane afforded the title compound as a white amorphous solid (28 mg, 0.089 mmol, 45%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 7.05 (d, *J* = 8.25 Hz, 1H), 6.80 - 6.97 (m, 5H), 3.86 - 3.94 (m, 1H), 3.82 (d, *J* = 2.90 Hz, 1H), 3.80 (s, 3H), 3.77 (s, 3H), 2.61 (dd, *J* = 4.91, 13.16 Hz, 1H), 2.17 (dd, *J* = 5.46, 13.04 Hz, 1H), 1.12 (d, *J* = 6.24 Hz, 3H). LCMS M/Z $(M+H)^+$ 313.

(4R)-4-methyl-6-(1-(1-phenylethyl)-1H-pyrazol-4-yl)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (22)



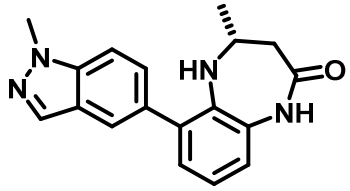
A disposable tube was charged with (*R*)-6-bromo-4-methyl-4,5-dihydro-1Hbenzo[b][1,4]diazepin-2(3H)-one (**Intermediate A**) (0.128 g, 0.5 mmol), 1-(1-phenylethyl)-4-(4,4,5,5-tetramethyl- 1,3,2-dioxaborolan-2-yl)-1H-pyrazole (0.298 g, 1.000 mmol), XPhos precat. (0.042 g, 0.050 mmol), and a stribar before being evacuated and backfilled with nitrogen three times. Acetonitrile (2 mL) was added, followed by sodium hydroxide (1 M, 1.0 ml, 1.0 mmol), and the solution was stirred at 65 °C 15 min. The reaction mixture was concentrated *in vacuo* with celite and purified by silica gel chromatography (Biotage, eluting with hexanes/ethyl acetate). Lyophilization from dioxane afforded the title compound as an off-white amorphous solid (153 mg, 0.44 mmol, 88%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.07 (d, *J* = 2.7 Hz, 1H), 7.63 (s, 1H), 7.38 - 7.24 (m, 5H), 6.98 (dd, *J* = 6.4, 2.8 Hz, 1H), 6.87 - 6.79 (m, 2H), 5.65 (d, *J* = 6.0 Hz, 1H), 3.99 (br s, 1H), 3.93 (br s, 1H), 2.55 - 2.50 (m, 1H), 2.16 (dd, *J* = 13.2, 6.0 Hz, 1H), 1.85 (d, *J* = 7.1 Hz, 3H), 1.13 (dd, *J* = 6.2, 1.8 Hz, 3H). LCMS M/Z $(M+H)^+$ 347.

(R)-6-(3-(benzyloxy)phenyl)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (23)



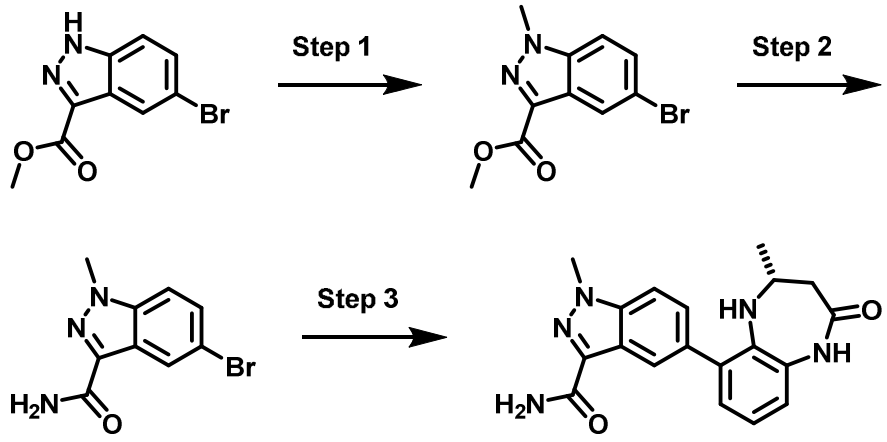
To a solution of (*R*)-6-bromo-4-methyl-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**Intermediate A**, 50 mg, 0.20 mmol) in dioxane (0.5 ml) was added 2-(3-benzyloxyphenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (161.3 mg, 0.52 mmol), 0.5 ml of 2 M aqueous potassium carbonate, and 1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (16 mg, 0.02 mmol, 65%). The mixture was then heated to 130 °C for 1 h. The mixture was diluted with EtOAc and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by reverse phase HPLC (acetonitrile 5–85% / 0.1% NH_4OH in water) to afford the title compound (48 mg, 0.13 mmol, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.54 (s, 1H), 7.51 – 7.25 (m, 6H), 7.15 – 7.00 (m, 1H), 7.00 – 6.82 (m, 5H), 5.15 (s, 2H), 3.84 – 3.74 (m, 2H), 3.30 (d, *J* = 7.9 Hz, 4H), 2.57 (dd, *J* = 13.2, 4.8 Hz, 1H), 2.16 (dd, *J* = 13.2, 5.8 Hz, 1H), 1.06 (d, *J* = 6.0 Hz, 3H). LCMS M/Z $(M+H)^+$ 359.

(R)-4-methyl-6-(1-methyl-1H-indazol-5-yl)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (24)



A mixture of (*R*)-6-bromo-4-methyl-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**Intermediate A**, 100 mg, 0.39 mmol), 1-methyl-1H-indazole-5-boronic acid (83 mg, 0.47 mmol), Cs_2CO_3 (255 mg, 0.78 mmol), and 1,1'-bis(diphenylphosphion) ferrocene dichloride palladium(II) (28 mg, 0.04 mmol) in dioxane (5 mL) and H_2O (1 mL) was irradiated in a microwave at 110 °C for 30 min. After cooling the reaction to room temperature the solvent was concentrated *in vacuo*. The crude residue was purified by reverse phase chromatography (acetonitrile 23-43%/0.2% formic acid in water) to give the title compound (45 mg, 38%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (s, 1H), 8.08 (s, 1H), 7.74 – 7.70 (m, 2H), 7.39 – 7.36 (m, 1H), 6.94 – 6.88 (m, 3H), 4.08 (s, 3H), 3.90 – 3.88 (m, 1H), 3.81 – 3.80 (m, 1H), 2.65 – 2.61 (m, 1H), 2.21– 2.18 (m, 1H), 1.07(d, *J* = 6.4 Hz, 3H). LCMS M/Z $(M+H)^+$ 307.

(R)-1-methyl-5-(4-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-6-yl)-1H-indazole-3-carboxamide (25)



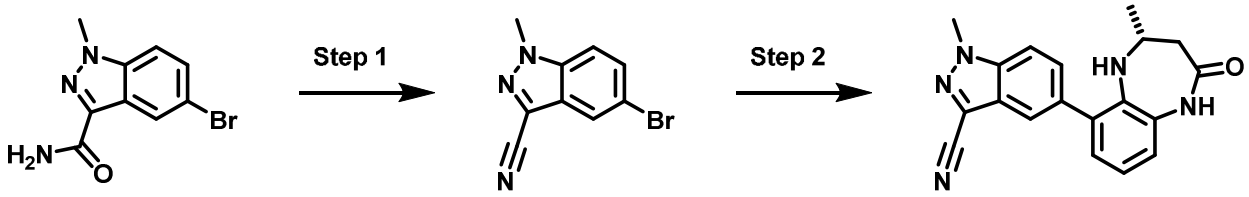
Step 1: To methyl 5-bromo-1*H*-indazole-3-carboxylate (762 mg, 3.0 mmol) in acetonitrile (20 ml) at 20 °C was added potassium carbonate (2.0 g, 15 mmol) and methyl iodide (1.1 ml, 15.5 mmol). The mixture was stirred at 20 °C for 10 h under nitrogen atmosphere. The mixture was concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (petroleum ether/EtOAc = 10/1 to 5/1) to give methyl 5-bromo-1-methyl-1H-indazole-3-carboxylate (600 mg, 75%) as a light yellow solid.

Step 2: To a mixture of 5-bromo-1-methyl-1*H*-indazole-3-carboxylate (320 mg, 1.2 mmol) in methanol (5 ml) was added aqueous ammonium hydroxide (5 mL). The mixture was heated to 90 °C for 10 h under

nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was concentrated *in vacuo* to give 5-bromo-1-methyl-1H-indazole-3-carboxamide (320 mg, crude) as a white solid that required no further purification. LCMS M/Z $(M+H)^+$ 254.

Step 3: To a mixture of 5-bromo-1-methyl-1*H*-indazole-3-carboxamide (70 mg, 0.28 mmol), cesium carbonate (182 mg, 0.56 mmol) and (*R*)-4-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**Intermediate B**, 84 mg, 0.28 mmol) in dioxane (20 ml)/H_2O (4 ml) was added [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (22 mg, 0.03 mmol). The resulting mixture was heated to 100 °C for 10 h under nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was concentrated *in vacuo*. The crude residue was purified by reverse phase chromatography (acetonitrile 10-40% / 0.2% formic acid in water) to give the title compound (24 mg, 24%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 1H), 7.77 - 7.75 (m, 1H), 7.50 - 7.48 (m, 1H), 7.11 - 7.09 (m, 1H), 7.02 - 7.01 (m, 2H), 4.21 (s, 3H), 4.04 - 4.00 (m, 1H), 2.78 - 2.74 (m, 1H), 2.59 - 2.31 (m, 1H), 1.17 - 1.16 (s, 3H). LCMS M/Z $(M+H)^+$ 350.

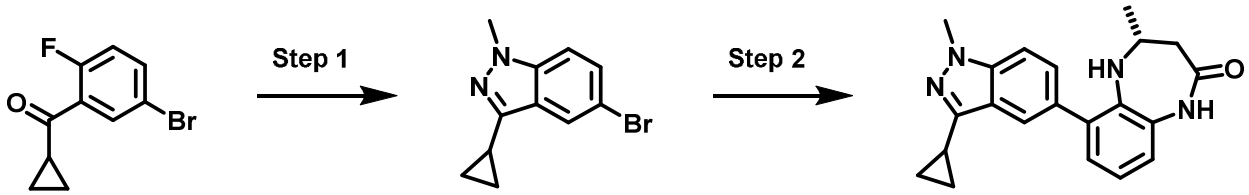
(R)-1-methyl-5-(4-methyl-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-6-yl)-1H-indazole-3-carbonitrile (26)



Step 1: To a solution of 5-bromo-1-methyl-1H-indazole-3-carboxamide (320 mg, crude) in THF (9 ml) was added trifluoromethanesulfonic anhydride (0.9 ml) and pyridine (0.9 ml). The mixture was stirred at 20 °C for 10 h. Water (10 mL) was added and the mixture was extracted with EtOAc (15 mL x 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give 5-bromo-1-methyl-1H-indazole-3-carbonitrile (320 mg, crude) as a yellow solid that required no further purification.

Step 2: To a mixture of 5-bromo-1-methyl-1*H*-indazole-3-carbonitrile (200 mg, crude), cesium carbonate (767 mg, 2.36 mmol) and (*R*)-4-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**Intermediate B**, 300 mg, 1.2 mmol) in dioxane (20 ml)/H_2O (4 ml) was added [1,1′-bis(diphenylphosphino)ferrocene] dichloropalladium(II) (87 mg, 0.12 mmol). The resulting mixture was heated to 100 °C for 10 h under nitrogen atmosphere. After cooling the reaction to room temperature, the mixture was concentrated *in vacuo*. The crude residue was purified by reverse phase chromatography (acetonitrile 39-59% / 0.2% formic acid in water) to give the title compound (46 mg, 12%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.88 (d, *J* = 8.4 Hz, 1H), 7.79 (s, 1H), 7.60 - 7.58 (m , 1H), 7.09 - 7.08 (m, 1H), 7.06 - 7.02 (m, 2H), 4.25 (s, 3H), 4.03 - 4.01 (m, 1H), 2.79 - 2.75 (m, 1H), 2.37 - 2.33 (m, 1H), 1.19 (s, 3H). LCMS M/Z (M+H) 332.

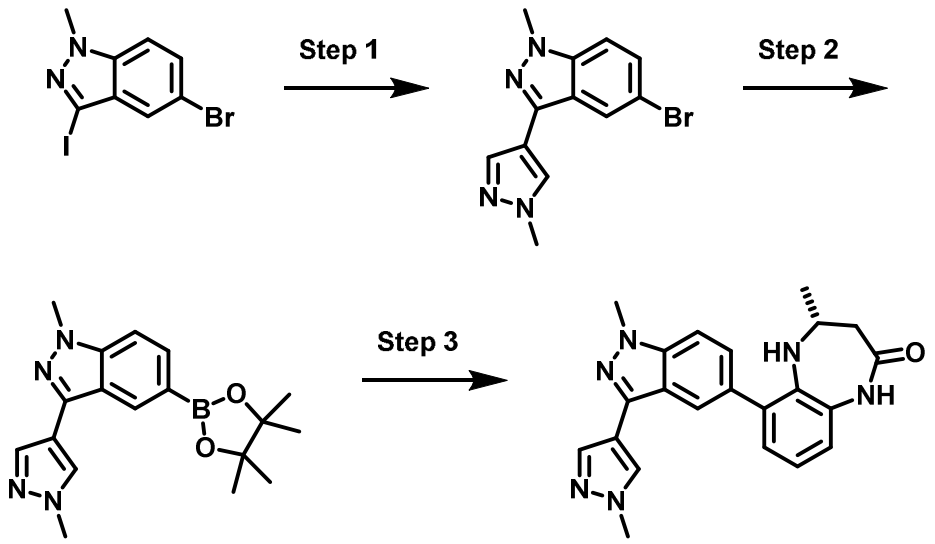
(R)-6-(3-cyclopropyl-1-methyl-1H-indazol-5-yl)-4-methyl-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (27)



Step 1: A mixture of (5-bromo-2-fluorophenyl)(cyclopropyl)methanone (5.0 g, 20.60 mmol), methylhydrazine (40% aq., 5.7 g, 123.01 mmol), CuO (82 mg, 1.00 mmol) and K_2CO_3 (5.7 g, 41.01 mmol) in DMF (25 mL) was heated to 110 °C for 16 h. After cooling the reaction to room temperature, the mixture was filtered and the filtrate was concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (petroleum ether/EtOAc = 10/1) to give 5-bromo-3-cyclopropyl-1-methyl-1H-indazole (2.7 g, 52%) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H), 7.40 (d, *J* = 8.8 Hz, 1H), 7.16 (d, *J* = 8.8 Hz, 1H), 3.93 (s, 3H), 2.14 – 2.09 (m, 1H), 1.01 – 1.00 (m, 4H).

Step 2: A mixture of 5-bromo-3-cyclopropyl-1-methyl-1*H*-indazole (199 mg, 0.79 mol), (*R*)-4-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**Intermediate B**, 200 mg, 0.66 mmol), potassium carbonate (273 mg, 1.78 mmol) and bis(triphenylphosphine)palladium(II) dichloride (49 mg, 0.07 mmol) in 1,4-dioxane (4 mL) and water (1 mL) was heated to 100 °C for 12 h under nitrogen atmosphere. After cooling the reaction mixture to 25 °C, water (20 mL) was added and the mixure extracted with EtOAc (20 mL x 2). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by reverse phase chromatography (acetonitrile 26-56% / 0.2% formic acid in water) to give the title compound (46 mg, 20%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.74 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.42 - 7.39 (m, 1H), 7.10 - 7.00 (m, 3H), 4.02 - 3.97 (m, 4H), 2.77 - 2.72 (m, 1H), 2.37 - 2.26 (m, 2H), 1.17 (d, *J* = 6.8 Hz, 3H), 1.07 - 1.03 (m, 4H). LCMS M/Z (M+H) 347.

(R)-4-methyl-6-(1-methyl-3-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-5-yl)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (CPI-637)



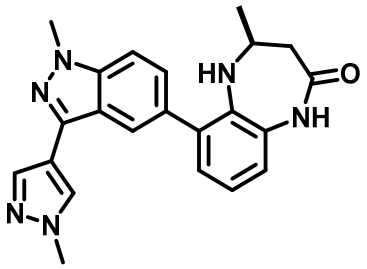
Step 1: A mixture of 5-bromo-3-iodo-1-methyl-1*H*-indazole (400 mg, 1.18 mmol), 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (295.4 mg, 1.42 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (86.2 mg, 0.118 mmol) and potassium carbonate (488.6 mg, 3.54 mmol) in dioxane/H_2O (5 mL /0.5 mL) was heated to 100 °C for 16 h under nitrogen atmosphere. After cooling the reaction to room temperature, water (5 mL) was added and the mixture was extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by silica gel chromatography (petroleum ether/EtOAc = 3/1 to 1/1) to give 5-bromo-1-methyl-3-(1-methyl-1H-pyrazol-4-yl)-1H-indazole (270 g, 78.1 %) as a white solid. LCMS M/Z (M+H) 291.

Step 2: A mixture of 5-bromo-1-methyl-3-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-indazole (270 mg ,0.798 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (243.2 mg, 0.957 mmol), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (58.3 mg, 0.0798 mmol) and potassium acetate (234.6 mg, 2.39 mmol) in dioxane (3 mL) was heated to 100 °C for 2 h under nitrogen atmosphere. After cooling the reaction to room temperature, water (5 mL) was added and the mixture was extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give 1-methyl-3-(1-methyl-1H-pyrazol-4-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole (320 mg, crude) as brown oil that required no further purification.

Step 3: A mixture of 1-methyl-3-(1-methyl-1*H*-pyrazol-4-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole (320 mg, 0.946 mmol), (*R*)-6-bromo-4-methyl-4,5-dihydro-1*H*-benzo[*b*][1,4]diazepin-2(3*H*)-one (**Intermediate A**, 193.1 mg, 0.757 mmol), bis(triphenylphosphine)palladium(II) dichloride (56 mg, 0.08 mmol) and potassium carbonate (313.4 mg, 2.271 mmol) in dioxane/H_2O (3 mL /0.3mL) was heated to 100 °C for 12 h under nitrogen atmosphere. After cooling the reaction to room temperature, water (5 mL) was added and the mixture was extracted with EtOAc (10 mL x 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The crude residue was purified by reverse phase chromatography (acetonitrile 26-56% / 0.2% formic acid in water) to give the title compound (28 mg, 10 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (s, 1 H), 7.93 - 7.89 (m, 1H), 7.83 - 7.81 (m, 1H), 7.53 - 7.49 (m, 1H), 7.43 - 7.39 (m, 1H), 7.13 - 7.07 (m, 1H), 7.01 - 6.92 (m, 2H), 4.15 (s, 3H), 4.01 (s, 3H), 3.99 - 3.96 (m, 1H), 2.82 - 2.75 (m, 1H), 2.51 - 2.45 (m, 1H),

1.19 (d, *J* = 6.27 Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 171.71, 139.95, 137.01, 136.90, 136.49, 133.80, 130.60, 128.42, 127.48, 127.25, 127.05, 121.53, 120.99, 120.84, 120.32, 114.69, 109.18, 54.51, 40.36, 38.62, 35.06, 23.06. Melting point: 280.26 °C. LCMS M/Z $(M+H)^+$ 387. EE: 99.2%; [α] in $CHCl_3$ = - 46°, @ c = 0.097 g/100 mL.

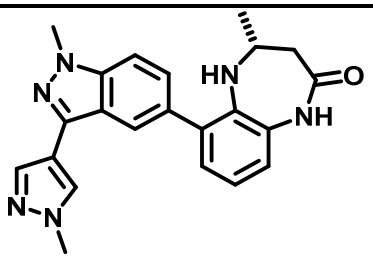
(S)-4-methyl-6-(1-methyl-3-(1-methyl-1H-pyrazol-4-yl)-1H-indazol-5-yl)-1,3,4,5-tetrahydro-2H-benzo[b][1,4]diazepin-2-one (enant-CPI-637)



The parent compound was prepared following the same procedure as for **CPI-637** but started with the opposite enantiomer of **Intermediate A** (prepared from (S)-3-aminobutanoic acid). 99.4% ee. [α] in $CHCl_3$ = +42°, @ c = 0.052 g/100 mL.

8. Off target bromodomain affinity of 28 (CPI-637).

SI Table 3. Off-target biochemical data for **28**.



28

Off target bromodomain	Biochemical IC_{50} (μM)
BAZ2B	>20
CECR2	7.7 ± 0.60

TAF1 BD1	>20
TAF1 BD2	17 ± 0.54
BRD4 BD1	11 ± 0.59
BRD4 BD2	13 ± 1.5
BRD9	0.73 ± 0.042
BRG1	>20
BRPF1	>20