Supporting Information for
Discovery of potent BET bromodomain 1 stereoselective inhibitors using DNA-encoded chemical library selections

CONFIDENTIAL
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1. Syntheses of Compounds

1a. Materials and instrumentation. All reactions involving air-sensitive reagents were carried out in anhydrous solvents under an atmosphere of nitrogen. Reagents and solvents purchased from commercial supplies were used as received. Reactions were monitored by thin-layer chromatography (TLC) on Bakerflex® silica gel plates (IB2-F) using UV-light (254 and 365 nm) detection or high-performance liquid chromatography/mass spectrometry (HPLC-MS). Column chromatography was carried out using Teledyne ISCO CombiFlash system equipped with either a silica or C-18 column. NMR spectra were recorded at room temperature using a Bruker Avance III HD 600 MHz spectrometer ([1H NMR at 600 MHz and 13C NMR at 150 MHz) or a Bruker Avance III HD 800 MHz spectrometer ([13C NMR at 200 MHz). Chemical shifts (δ) are reported in parts per million (ppm) with reference to solvent signals [1H-NMR: DMSO-d6 (2.50 ppm); 13C-NMR: DMSO-d6 (39.51 ppm)]. Signal patterns are reported as s (singlet), d (doublet), t (triplet), q (quartet), h (heptet), m (multiplet) and br (broad). Coupling constants (J) are given in Hz. HRMS measurements were performed using ThermoFisher Scientific Q Exactive instrument. Abbreviations presented in experimental procedures are referred to the following definitions: DIEA, N,N-diisopropylethylamine; DME, 1,2-dimethoxyethane; DMF, N,N-dimethylformamide; EtOAc, ethyl acetate; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; NaOH(aq), aqueous sodium hydroxide solution; Na2CO3(sat), saturated aqueous sodium carbonate solution; Na2SO4, sodium sulfate.

1b. Typical method for the preparation of compounds 2a
To a solution of 2-(((9H-fluoren-9-yl) methoxy) carbonyl)amino)-3-(1-(tert butoxycarbonyl) piperidin-4-yl)propanoic acid (1) (494.58 mg, 1 mmol, 1 equiv.), methylamine HCl (202.5 mg, 3 equiv.), and HATU (1140 mg, 3 mmol, 3 equiv.) in anhydrous DMF (10 mL) was added DIEA (522 μL, 3 mmol, 3 equiv.) under nitrogen. The reaction mixture was stirred at room temperature for 1 h and then quenched by the addition of water. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na2SO4, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH3OH/CH2Cl2, 1:99 to 5:95) to afford N1-(2-methoxy-5-nitrophenyl)-N4-methylterephthalamide (507 mg, 98%) as a sticky oil.

The compounds (2a–2c) were prepared in a way similar to the procedure described above.

1c.1. Synthesis of intermediate N1-(2-methoxy-5-nitrophenyl)-N4-methylterephthalamide (2a):

\[
\begin{align*}
\text{HN} & \quad \text{Fmoc} \\
\text{O} & \quad \text{NH} \\
\text{\_} & \quad \text{N} \\
\text{\_} & \quad \text{Boc}
\end{align*}
\]

Sticky oil; yield: 98%1H NMR (600 MHz, CD3OD) δ 7.80 (d, J = 7.5 Hz, 2H), 7.66 (t, J = 7.9 Hz, 2H), 7.39 (t, J = 7.3 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 4.47 (dd, J = 10.6, 6.7 Hz, 1H), 4.37 (dd, J = 10.6, 6.5 Hz, 1H), 4.21 (t, J = 6.5 Hz, 1H), 4.11 (dd, J = 9.9, 4.9 Hz, 1H), 4.04–4.02 (m, 2H), 2.72–2.65 (m, 5H),
1.72–1.47 (m, 5H), 1.44 (s, 9H), 1.13–1.00 (m, 2H); HRMS (ESI) m/z calcd for C_{29}H_{37}N_{3}O_{5} [M + H]+ 508.2806, found 508.2895.

Tert-butyl (R)-4-(((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-3-(methylamino)-3oxopropyl)piperidine-1-carboxylate (2b):

![Chemical structure of 2b]

White solid; yield: 98%; \(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 7.80 (d, \(J = 7.5\) Hz, 2H), 7.66 (t, \(J = 7.9\) Hz, 2H), 7.39 (t, \(J = 7.4\) Hz, 2H), 7.31 (t, \(J = 7.4\) Hz, 2H), 4.48 (dd, \(J = 10.6, 6.8\) Hz, 1H), 4.37 (dd, \(J = 10.6, 6.5\) Hz, 1H), 4.22 (t, \(J = 6.5\) Hz, 1H), 4.11 (dd, \(J = 9.9, 4.9\) Hz, 1H), 4.04–4.02 (m, 2H), 2.71–2.65 (m, 5H), 1.73–1.47 (m, 5H), 1.44 (s, 9H), 1.13–1.00 (m, 2H); HRMS (ESI) m/z calcd for C\(_{29}\)H\(_{37}\)N\(_{3}\)O\(_{5}\) [M + H]+ 508.2806, found 508.2794.; [\(\alpha\)]\(_D\)\(^{25}\) +2.7±1°, c = 1% in DMF

Tert-butyl (S)-4-(((((9H-fluoren-9-yl) methoxy)carbonyl)amino)-3-(methylamino)-3oxopropyl)piperidine-1-carboxylate (2c):

![Chemical structure of 2c]

Sticky oil; yield: 98%; \(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 7.80 (d, \(J = 7.5\) Hz, 2H), 7.66 (t, \(J = 8.0\) Hz, 2H), 7.39 (t, \(J = 7.4\) Hz, 2H), 7.31 (t, \(J = 7.4\) Hz, 2H), 4.48 (dd, \(J = 10.6, 6.8\) Hz, 1H), 4.37 (dd, \(J = 10.6, 6.4\) Hz, 1H), 4.22 (t, \(J = 6.5\) Hz, 1H), 4.11 (dd, \(J = 9.9, 4.9\) Hz, 1H), 4.04–4.02 (m, 2H), 2.71–2.65 (m, 5H), 1.72–1.47 (m, 5H), 1.44 (s, 9H), 1.13–1.00 (m, 2H); HRMS (ESI) m/z calcd for C\(_{29}\)H\(_{37}\)N\(_{3}\)O\(_{5}\) [M + H]+ 508.2806, found 508.2800.; [\(\alpha\)]\(_D\)\(^{25}\) -2.3±1°, c = 1% in DMF

1c.2 Typical method for the preparation of compound 4a

To a mixture of 2a (407 mg, 1 mmol, 0.6 eq) in dioxane (20 mL) was added HCl/dioxane (4 M, 4 mL, 1.5 eq.) dropwise via syringe at 20 °C, and the resulting reaction mixture stirred for 1 h. After completion of the reaction all solvents were removed in vacuo and kept overnight dry in high vacuo. To the same vessel mixture of DIEA (522 μL, 3 mmol, 2 eq), 2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetic acid (3a) (325 mg, 1.5 mmol, 1 eq) and HATU (1140 mg, 3 mmol, 3 equiv.) added in the solution of anhydrous DMF (10 ml) under nitrogen and stir for 12h. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated. The residue was purified by column chromatography on silica gel (CH\(_3\)OH/CH\(_2\)Cl\(_2\), 1:99 to 5:95) to afford 4a (510 mg, 84%) as a white solid.
The compounds (4a–4d) were prepared in a way similar to the procedure described above.

(9H-fluoren-9-yl) methyl (3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) carbamate (4a):

White solid; yield: 84%; ¹H NMR (600 MHz, DMSO-d₆) δ 11.57 (s, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.85–7.84 (m, 1H), 7.72 (t, J = 6.6 Hz, 2H), 7.53 (s, 1H), 7.51 (t, J = 8.7 Hz, 1H), 7.42–7.40 (m, 2H), 7.36–7.30 (m, 3H), 7.24 (d, J = 8.3 Hz, 1H), 6.39 (s, 1H), 6.28 (s, 1H), 4.36–4.28 (m, 2H), 4.25–4.20 (m, 2H), 3.99–3.96 (m, 2H), 3.76 (brs, 2H), 2.97–2.87 (m, 1H), 2.59–2.54 (m, 4H), 2.39 (s, 3H), 1.66–1.59 (m, 2H), 1.51–1.43 (m, 2H), 0.99–0.85 (m, 2H); HRMS (ESI) m/z calcd for C₃₆H₃₈N₄O₅ [M + H]+ 607.2915, found 607.2903.

(9H-fluoren-9-yl) methyl (R)-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) carbamate (4b):

White solid; yield: 84%; ¹H NMR (600 MHz, DMSO-d₆) δ 11.57 (s, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.85–7.83 (m, 1H), 7.72 (t, J = 6.6 Hz, 2H), 7.53 (s, 1H), 7.50 (t, J = 8.7 Hz, 1H), 7.43–7.40 (m, 2H), 7.36–7.30 (m, 3H), 7.24 (d, J = 8.3 Hz, 1H), 6.39 (s, 1H), 6.28 (s, 1H), 4.36–4.30 (m, 2H), 4.25–4.20 (m, 2H), 3.98–3.96 (m, 2H), 3.76 (brs, 2H), 2.97–2.87 (m, 1H), 2.58–2.54 (m, 4H), 2.39 (s, 3H), 1.66–1.59 (m, 2H), 1.51–1.40 (m, 2H), 0.97–0.84 (m, 2H); HRMS (ESI) m/z calcd for C₃₆H₃₈N₄O₅ [M + H]+ 607.2915, found 607.2912; [α]₂⁵/D +1.7±1°, c = 1% in DMF

(9H-fluoren-9-yl) methyl (S)-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) carbamate (4c):

Sticky oil; yield: 84%; ¹H NMR (600 MHz, DMSO-d₆) δ 11.57 (s, 1H), 7.89 (d, J = 7.5 Hz, 2H), 7.84–7.83 (m, 1H), 7.72 (t, J = 6.6 Hz, 2H), 7.53 (s, 1H), 7.50 (t, J = 8.7 Hz, 1H), 7.42–7.39 (m, 2H), 7.35–7.30 (m, 3H), 7.24 (d, J = 8.3 Hz, 1H), 6.38 (s, 1H), 6.27 (s, 1H), 4.35–4.29 (m, 2H), 4.24–4.19 (m, 2H), 3.99–3.93 (m, 2H), 3.75 (brs, 2H), 2.96–2.86 (m, 1H), 2.57–2.55 (m, 4H), 2.38 (s, 3H), 1.67–1.60 (m, 2H), 1.50–1.38 (m, 2H), 0.96–0.84 (m, 2H); HRMS (ESI) m/z calcd for C₃₆H₃₈N₄O₅ [M + H]+ 607.2915, found 607.2902; [α]₂⁵/D -1.0±1°, c = 1% in DMF
(9H-fluoren-9-yl) methyl (R)-(1-(methylamino)-1-oxo-3-(1-(2-(2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl) propan-2-yl) carbamate(4d):

White solid; yield: 84%; $^1$H NMR (600 MHz, CD$_3$OD) $\delta$ 7.88−7.87 (m, 1H), 7.75 (d, $J = 7.1$ Hz, 2H), 7.61 (brs, 2H), 7.49 (brs, 1H), 7.42 (d, $J = 6.6$ Hz, 2H), 7.37−7.26 (m, 5H), 6.6 (m, 1H), 4.52−4.44 (m, 2H), 4.37−4.33 (m, 1H), 4.18 (t, $J = 6.4$ Hz, 1H), 4.11−4.08 (m, 1H), 3.96 (brs, 1H), 3.80 (s, 2H), 3.03−2.93 (m, 1H), 2.70 (s, 3H), 2.63−2.55 (m, 1H), 1.78−1.52 (m, 5H), 1.10−0.87 (m, 2H); HRMS (ESI) m/z calcd for C$_{35}$H$_{36}$N$_4$O$_5$ [M + H]$^+$ 593.2758, found 593.2754; $[\alpha]_{25}^D +1.3\pm1\,^\circ$, c = 1% in DMF

1c.3 Typical method for the preparation of compounds 6a

Compound 4a (322 mg, 0.84 mmol, 1 eq) was dissolved in DMF (3 ml) containing piperidine (10% v/v) and stirred 1h in room temperature. Resulting mixture is evaporated to The SM was dissolved in DMF containing piperidine, and all solvents were removed after 20 min. After completing reaction all solvents were removed in vacuo and kept overnight dry in high vacuo. To the same vessel mixture of DIEA (439 µL, 2.5 mmol, 3 eq), 5-(2,4-dimethylphenyl) picolinic acid (5a) (140 mg, 0.7 mmol, 0.8 eq) and HATU (957.6 mg, 2.5 mmol, 3 equiv.) added in the solution of anhydrous DMF (8 ml) under nitrogen. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH$_3$OH/CH$_2$Cl$_2$, 1:99 to 5:95) to afford 4a (327 mg, 68%) as a white solid.

The compounds (6a–6o) were prepared in a way similar to the procedure described above.

Synthesis of 5-(2,4-dimethylphenyl)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) picolinamide (6a): CDD-724

White solid; yield: 60%; $^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 11.56 (s, 1H), 8.66 (t, $J = 9.4$ Hz, 1H), 8.63 (s, 1H), 8.10−8.08 (m, 2H), 7.98 (dd, $J = 7.9, 2.1$ Hz, 1H), 7.53 (d, $J = 7.9$ Hz, 1H), 7.34 (d, $J = 8.3$ Hz, 1H), 7.18−7.17 (m, 2H), 7.14 (d, $J = 7.8$ Hz, 1H), 6.38 (s, 1H), 4.58−4.56 (m, 1H), 4.36−4.31 (m, 1H), 3.99−3.93 (m, 1H), 3.75 (s, 2H), 2.97−2.91 (m, 1H), 2.6 (d, $J = 4.5$ Hz, 2H), 2.50−2.46 (m, 1H), 2.39 (d, $J = 3.1$ Hz, 3H), 2.33 (s, 3H), 2.23 (s, 3H), 1.8 (brs, 1H), 1.69−1.52 (m, 4H), 0.97−0.94 (m, 2H); 13C NMR (150 MHz, DMSO-$d_6$) $\delta$ 171.8, 168.4, 163.1, 161.5, 148.3, 147.6, 147.6, 139.6, 138.0, 137.8, 137.2, 134.9, 134.0, 131.4, 131.3, 129.7, 129.4, 126.9, 124.7, 121.5, 120.9, 119.4, 115.3, 50.3, 45.4, 41.3, 32.6, 32.1, 31.9, 31.5, 30.9, 25.6, 20.7, 19.9, 18.5; HRMS (ESI) m/z calcd for C$_{35}$H$_{39}$N$_5$O$_4$ [M + H]$^+$ 594.3075, found 594.3068.
(R)-5-(2,4-dimethylphenyl)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) picolinamide (6b): CDD-787

White solid; yield: 60%; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.56 (s, 1H), 8.66 (t, \(J = 9.4\) Hz, 1H), 8.63 (s, 1H), 8.10–8.08 (m, 2H), 7.98 (dd, \(J = 7.9, 2.1\) Hz, 1H), 7.53 (brs, 1H), 7.34 (d, \(J = 7.9\) Hz, 1H), 7.23 (d, \(J = 8.3\) Hz, 1H), 7.19–7.18 (m, 2H), 7.14 (d, \(J = 7.8\) Hz, 1H), 6.38 (s, 1H), 4.58–4.56 (m, 1H), 4.36–4.31 (m, 1H), 3.99–3.93 (m, 1H), 3.75 (s, 2H), 2.98–2.91 (m, 1H), 2.6 (d, \(J = 4.5\) Hz, 2H), 2.50–2.46 (m, 1H), 2.39 (d, \(J = 3.1\) Hz, 3H), 2.23 (s, 3H), 1.8 (brs, 1H), 1.71–1.52 (m, 4H), 0.99–0.95 (m, 2H); 13C NMR (150 MHz, DMSO-\(d_6\)) \(\delta\) 171.8, 168.5, 163.2, 161.5, 148.4, 147.7, 139.6, 138.0, 137.8, 137.2, 134.9, 134.0, 131.3, 129.7, 129.4, 126.9, 124.7, 121.5, 120.9, 119.4, 115.3, 50.3, 45.4, 41.3, 32.6, 32.1, 31.9, 31.6, 30.9, 25.6, 20.7, 19.9, 18.5; HRMS (ESI) m/z calcd for C\(_{35}\)H\(_{39}\)N\(_5\)O\(_4\) [M + H]+ 594.3075, found 594.3072; [\(\alpha\)]\(\text{D}\)\(\text{+}\)16.2±1°, c = 1% in DMF

(S)-5-(2,4-dimethylphenyl)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) picolinamide (6c): CDD-786

White solid; yield: 60%; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.55 (s, 1H), 8.65 (t, \(J = 9.4\) Hz, 1H), 8.63 (s, 1H), 8.09–8.08 (m, 2H), 7.98 (dd, \(J = 7.9, 2.1\) Hz, 1H), 7.53 (brs, 1H), 7.34 (d, \(J = 7.9\) Hz, 1H), 7.23 (d, \(J = 8.3\) Hz, 1H), 7.18–7.17 (m, 2H), 7.13 (d, \(J = 7.8\) Hz, 1H), 6.38 (s, 1H), 4.59–4.54 (m, 1H), 4.36–4.31 (m, 1H), 3.98–3.92 (m, 1H), 3.75 (s, 2H), 2.98–2.89 (m, 1H), 2.59 (d, \(J = 4.5\) Hz, 2H), 2.54–2.46 (m, 1H), 2.38 (d, \(J = 3.1\) Hz, 3H), 2.33 (s, 3H), 2.22 (s, 3H), 1.8 (brs, 1H), 1.72–1.52 (m, 4H), 0.99–0.93 (m, 2H); 13C NMR (150 MHz, DMSO-\(d_6\)) \(\delta\) 171.8, 168.5, 163.2, 161.5, 148.4, 147.7, 139.6, 138.0, 137.8, 137.2, 134.9, 134.0, 131.3, 129.7, 129.4, 126.9, 124.7, 121.5, 120.9, 119.4, 115.3, 50.3, 45.4, 41.3, 32.6, 32.1, 31.9, 31.6, 30.9, 25.6, 20.7, 19.9, 18.5; HRMS (ESI) m/z calcd for C\(_{35}\)H\(_{39}\)N\(_5\)O\(_4\) [M + H]+ 594.3073, found 594.3072; [\(\alpha\)]\(\text{D}\)\(-\)16.7±1°, c = 1% in DMF

(R)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl)5-phenylpicolinamide (6d): CDD-956

White solid; yield: 60%; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.54 (s, 1H), 8.98 (s, 1H), 8.65 (t, \(J = 9.0\) Hz, 1H), 8.29 (dd, \(J = 8.1, 2.1\) Hz, 1H), 8.12–8.09 (m, 2H), 7.80 (d, \(J = 7.3\) Hz, 2H), 7.56–7.53 (m, 3H), 7.4
(t, J = 7.3 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 6.38 (s, 1H), 4.61–4.56 (m, 1H), 4.36–4.32 (m, 1H), 3.98–3.93 (m, 1H), 3.77–3.72 (m, 2H), 2.97–2.89 (m, 1H), 2.61 (d, J = 4.5 Hz, 1H), 2.54–2.46 (m, 1H), 2.38 (d, J = 2.5 Hz, 3H), 1.80 (s, 1H), 1.73–1.52 (m, 4H), 0.99–0.93 (m, 2H); 13C NMR (150 MHz, DMSO-d6) δ 171.7, 168.4, 163.0, 161.4, 148.1, 147.5, 146.5, 138.1, 137.1, 136.1, 135.5, 131.2, 129.3, 129.2, 128.7, 124.6, 122.0, 120.8, 119.3, 115.2, 50.2, 45.3, 41.2, 32.5, 32.0, 31.8, 31.5, 30.9, 25.5, 18.4; HRMS (ESI) m/z calcd for C33H35N5O4 [M + H]+ 566.2762, found 566.2762; [α]25/D +19.1±1°, c = 1% in DMF

(S)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)-1-(methylamino)-1-oxo propan-2-yl)-5-phenylpicolinamide (6e): CDD-2107

White solid; yield: 55%; 1H NMR (600 MHz, CD3OD) δ 8.88 (s, 1H), 8.18 (dd, J = 8.0, 1.5 Hz, 1H), 8.14–8.12 (m, 1H), 7.69 (d, J = 7.3 Hz, 2H), 7.66 (s, 1H), 7.51 (t, J = 7.5 Hz, 2H), 7.44 (t, J = 8.3 Hz, 2H), 7.31 (d, J = 8.4 Hz, 1H), 6.49 (s, 1H), 4.69–4.64 (m, 1H), 4.52–4.51 (m, 1H), 4.05–4.03 (m, 1H), 3.89–3.85 (m, 2H), 3.07–2.99 (m, 1H), 2.74 (s, 3H), 2.65–2.57 (m, 1H), 2.49 (s, 3H), 1.92–1.65 (m, 5H), 1.18–0.95 (m, 2H); 13C NMR (150 MHz, CD3OD) δ 174.6, 171.6, 166.2, 164.8, 151.4, 149.1, 148.0, 138.2, 137.9, 136.8, 132.8, 131.5, 130.3, 129.9, 128.3, 125.9, 125.9, 123.4, 121.9, 120.9, 117.4, 52.3, 47.5, 43.4, 40.6, 33.9, 33.3, 32.9, 32.3, 26.4, 19.1; HRMS (ESI) m/z calcd for C33H35N5O4 [M + H]+ 566.2762, found 566.2748; [α]25/D -17.7±1°, c = 1% in DMF

(R)-5-(4-chlorophenyl)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) picolinamide (6f): CDD-981

White solid; yield: 55%; 1H NMR (600 MHz, CD3OD) δ 8.82 (s, 1H), 8.14–8.09 (m, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.61 (s, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 6.45 (s, 1H), 4.69–4.64 (m, 1H), 4.53–4.50 (m, 1H), 4.04–4.02 (m, 1H), 3.87–3.80 (m, 2H), 3.05–2.97 (m, 1H), 2.74 (s, 3H), 2.64–2.56 (m, 1H), 2.45 (s, 3H), 1.91–1.64 (m, 5H), 1.16–0.95 (m, 2H); 13C NMR (150 MHz, CD3OD) δ 174.6, 171.5, 166.0, 164.7, 151.5, 149.4, 147.9, 139.4, 138.1, 136.6, 136.6, 136.1, 132.7, 131.3, 130.4, 129.8, 125.9, 123.4, 121.8, 121.0, 117.3, 52.3, 47.4, 43.3, 40.6, 40.2, 33.9, 33.3, 32.9, 32.3, 26.4, 19.0; HRMS (ESI) m/z calcd for C33H35ClN5O4 [M + H]+ 600.2372, found 600.2343; [α]25/D +16.5±1°, c = 1% in DMF

(R)-5-(4-chloro-3-fluorophenyl)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) picolinamide (6g): CDD-982

White solid; yield: 55%; 1H NMR (600 MHz, CD3OD) δ 8.82 (s, 1H), 8.14–8.09 (m, 2H), 7.65 (d, J = 8.5 Hz, 2H), 7.61 (s, 1H), 7.47 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.4 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 6.45 (s, 1H), 4.69–4.64 (m, 1H), 4.53–4.50 (m, 1H), 4.04–4.02 (m, 1H), 3.87–3.80 (m, 2H), 3.05–2.97 (m, 1H), 2.74 (s, 3H), 2.64–2.56 (m, 1H), 2.45 (s, 3H), 1.91–1.64 (m, 5H), 1.16–0.95 (m, 2H); 13C NMR (150 MHz, CD3OD) δ 174.6, 171.5, 166.0, 164.7, 151.5, 149.4, 147.9, 139.4, 138.1, 136.6, 136.1, 132.7, 131.3, 130.4, 129.8, 125.9, 123.4, 121.8, 121.0, 117.3, 52.3, 47.4, 43.3, 40.6, 40.2, 33.9, 33.3, 32.9, 32.3, 26.4, 19.0; HRMS (ESI) m/z calcd for C33H35ClN5O4 [M + H]+ 600.2372, found 600.2343; [α]25/D +16.5±1°, c = 1% in DMF

(R)-5-(4-chloro-3-fluorophenyl)-N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl) picolinamide (6g): CDD-982

S8
White solid; yield: 58%; $^{1}$H NMR (600 MHz, CD$_3$OD) δ 8.87 (s, 1H), 8.18 (dd, J = 8.1, 1.0 Hz, 1H), 8.13–8.11 (m, 1H), 7.63–7.61 (m, 2H), 7.58 (t, J = 7.9 Hz, 1H), 7.51 (dd, J = 8.3, 1.4 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 6.4 (brs, 1H), 4.69–4.64 (m, 1H), 4.53–4.51 (m, 1H), 4.05–4.03 (m, 1H), 3.88–3.81 (m, 2H), 3.64 (s, 1H), 3.55–3.51 (m, 1H), 3.06–2.99 (m, 1H), 2.74 (s, 3H), 2.64–2.57 (m, 1H), 2.47 (s, 3H), 1.92–1.65 (m, 5H), 1.17–0.96 (m, 2H); 13C NMR (150 MHz, CD$_3$OD) δ 174.6, 171.6, 165.9, 164.8, 160.6, 158.9, 151.2, 149.8, 147.9, 138.8, 138.2, 136.8, 132.5, 131.4, 125.9, 125.0, 123.4, 122.4, 121.8, 121.0, 117.3, 116.5, 52.3, 43.4, 40.6, 40.2, 33.9, 33.3, 32.6, 26.4, 19.0; HRMS (ESI) m/z calcd for C$_{33}$H$_{33}$ClFN$_{5}$O$_{4}$ [M + H]$^+$ 618.2278, found 618.2275; [α]$^{25}$D $+15.8 \pm 1^\circ$, c = 1% in DMF

(R)-N-(1-(methylamino)-1-oxo-3-(1-(2-(2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)propan-2-yl)-5-phenylpicolinamide (6h): CDD-986

White solid; yield: 58%; $^{1}$H NMR (600 MHz, CD$_3$OD) δ 8.88 (s, 1H), 8.19–8.12 (m, 2H), 7.9 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 7.3 Hz, 2H), 7.52–7.49 (m, 3H), 7.45–7.42 (m, 3H), 7.45–7.41 (m, 2H), 7.31 (d, J = 9.0 Hz, 1H), 6.59 (d, J = 9.4 Hz, 1H), 4.69–4.64 (m, 1H), 4.53–4.47 (m, 1H), 4.01 (t, J = 8.3 Hz, 1H), 3.97–3.92 (m, 1H), 3.74 (s, 2H), 2.96–2.88 (m, 1H), 2.59 (d, J = 4.5 Hz, 3H), 2.54 (s, 2H), 2.39 (brs, 3H), 1.78–1.76 (m, 1H), 1.69–1.50 (m, 4H), 1.01–0.90 (m, 2H); 13C NMR (150 MHz, CD$_3$OD) δ 174.6, 171.4, 166.2, 165.1, 149.1, 148.0, 142.5, 140.9, 138.5, 137.9, 136.7, 132.8, 131.5, 130.3, 129.0, 128.3, 123.4, 122.0, 121.4, 116.9, 52.3, 47.5, 43.3, 40.5, 40.3, 33.9, 33.2, 32.9, 32.2, 26.4; HRMS (ESI) m/z calcd for C$_{32}$H$_{33}$N$_{5}$O$_{4}$ [M + H]$^+$ 552.2605, found 552.2601; [α]$^{25}$D $+10.7 \pm 1^\circ$, c = 1% in DMF

N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl)picolinamide (6i): CDD-784

White solid; yield: 72%; $^{1}$H NMR (600 MHz, DMSO-d$_6$) δ 11.58 (s, 1H), 8.67 (d, J = 4.5 Hz, 1H), 8.63 (t, J = 8.5 Hz, 1H), 8.08–8.00 (m, 3H), 7.64–7.62 (m, 1H), 7.53 (s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 6.38 (brs, 1H), 4.56–4.53 (m, 1H), 4.35–4.30 (m, 1H), 3.97–3.92 (m, 1H), 3.74 (s, 2H), 2.96–2.88 (m, 1H), 2.59 (d, J = 4.5 Hz, 3H), 2.54 (s, 2H), 2.39 (brs, 3H), 1.78–1.76 (m, 1H), 1.69–1.50 (m, 4H), 1.01–0.90 (m, 2H); 13C NMR (150 MHz, DMSO-d$_6$) δ 172.2, 168.9, 162.0, 149.7, 148.9, 138.4, 137.6, 131.8, 129.9, 127.2, 125.1, 122.4, 121.2, 119.9, 116.6, 50.7, 45.8, 41.7, 40.9, 33.0,
32.5, 32.3, 32.0, 31.4, 26.0, 18.9; HRMS (ESI) m/z calcd for C_{27}H_{31}N_{5}O_{4} [M + H]^+ 490.2449, found 490.2451.

N-(3-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)-1-(methylamino)-1-oxopropan-2-yl)-5-phenylpicolinamide (6j): CDD-1146

White solid; yield: 62%; \(^{1}\)H NMR (600 MHz, DMSO-\(d_{6}\)) δ 11.56 (s, 1H), 8.98 (s, 1H), 8.66 (t, \(J = 8.8\) Hz, 1H), 8.29–8.27 (m, 1H), 8.12–8.10 (m, 2H), 7.8 (d, \(J = 7.5\) Hz, 2H), 7.55–7.53 (m, 3H), 7.49–7.46 (m, 1H), 7.34 (d, \(J = 8.3\) Hz, 1H), 7.24 (d, \(J = 8.3\) Hz, 1H), 6.38 (s, 1H), 4.60–4.58 (m, 1H), 4.36–4.32 (m, 1H), 3.98–3.93 (m, 1H), 3.74 (s, 2H), 2.97–2.88 (m, 1H), 2.61 (d, \(J = 4.5\) Hz, 3H), 2.5–2.46 (m, 1H), 2.38 (brs, 3H), 1.80 (brs, 1H), 1.73–1.52 (m, 4H), 1.02–0.92 (m, 2H); \(^{13}\)C NMR (150 MHz, DMSO-\(d_{6}\)) δ 172.2, 168.9, 163.5, 162.0, 148.6, 148.0, 147.0, 138.6, 137.6, 136.6, 136.0, 131.7, 131.8, 129.9, 129.7, 129.2, 127.6, 125.1, 122.5, 121.3, 119.9, 115.8, 50.7, 45.8, 41.7, 40.9, 33.0, 32.6, 32.3, 32.1, 31.4, 26.0, 18.9; HRMS (ESI) m/z calcd for C_{33}H_{35}N_{5}O_{4} [M + H]^+ 566.2762, found 566.2766.

1c.4. Typical method for the preparation of compounds 8 tert-butyl 4-(2-(5-(2,4-dimethylphenyl)picolinamido)propyl)piperidine-1-carboxylate (8a):

To a solution of tert-butyl 5-(2,4-dimethylphenyl)picolinic acid (5a) (228 mg, 1mmol, 1equiv.), tert-butyl 4-(2-aminopropyl)piperidine-1-carboxylate (7a) (242 mg, 1mmol, 1equiv.), and HATU (570 mg, 1.5 mmol, 1.5 equiv.) in anhydrous DMF (5 mL) was added DIEA (175 μL, 1.5 mmol, 1.5 equiv.) under nitrogen. The reaction mixture was stirred at room temperature for 3 h and then quenched by the addition of water. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na_{2}SO_{4}, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH_{3}OH/CH_{2}Cl_{2}, 1:99 to 5:95) to afford tert-butyl 4-(2-(5-(2,4-dimethylphenyl)picolinamido)propyl)piperidine-1-carboxylate (507 mg, 98%) as a sticky solid.

Sticky solid; yield: 98%; \(^{1}\)H NMR (600 MHz, DMSO-\(d_{6}\)) δ 8.58 (d, \(J = 1.5\) Hz, 1H), 8.51 (d, \(J = 9.1\) Hz, 1H), 8.08 (d, \(J = 8.4\) Hz, 1H), 7.95 (dd, \(J = 8.0, 2.2\) Hz, 1H), 7.17–7.16 (m, 2H), 7.13 (d, \(J = 7.8\) Hz, 1H), 4.21–4.16 (m, 1H), 3.89 (s, 2H), 2.33 (s, 3H), 2.22 (s, 3H), 1.77–1.75 (m, 1H), 1.67–1.62 (m, 1H), 1.59–1.57 (m, 1H), 1.48–1.42 (m, 1H), 1.37–1.31 (m, 11H), 1.17 (d, \(J = 6.5\) Hz, 3H), 1.04–1.57 (m, 2H); HRMS (ESI) m/z calcd for C_{27}H_{37}N_{3}O_{3} [M + H]^+ 452.2908, found 452.2912.

1c.5 Typical method for the preparation of compound 5-(2,4-dimethylphenyl)-N-(1-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)propan-2-yl)picolinamide (9a): CDD-906

To a mixture of 8a (451 mg, 1mmol, 1eq.), in dioxane (20 mL) was added HCl/dioxane (4 M, 4 mL, 1.5 eq.) dropwise via syringe at 20 °C, and the resulting reaction mixture stirred for 1 h. After completion of
the reaction all solvents were removed in vacuo and kept overnight dry in high vacuo. To the same vessel mixture of DIEA (263 μL, 1.5 mmol, 1.5 eq), 2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetic acid (3a) (217 mg, 1 mmol, 1 eq), and HATU (570 mg, 1.5 mmol, 1.5 equiv.) added in the solution of anhydrous DMF (10 ml) under nitrogen. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH₃OH/CH₂Cl₂, 1:99 to 5:95) to afford 9a (510 mg, 84%) as a white solid.

White solid; yield: 84%; ¹H NMR (600 MHz, DMSO-d₆) δ 11.57 (s, 1H), 8.55 (s, 1H), 8.51−8.49 (m, 1H), 8.08 (dd, J = 7.9, 3.1 Hz, 1H), 7.9 (dd, J = 7.9, 2.0 Hz, 1H), 7.5 (s, 1H), 7.33 (d, J = 8.3 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.13−7.12 (m, 2H), 7.08 (d, J = 7.9 Hz, 1H), 6.37 (s, 1H), 4.37−4.33 (m, 1H), 4.18−4.17 (m, 1H), 3.96−3.92 (m, 1H), 3.73 (s, 2H), 3.39 (s, 3H), 2.95−2.85 (m, 1H), 2.49−2.43 (m, 1H), 2.37 (d, J = 7.3 Hz, 1H), 2.29 (s, 3H), 2.18 (s, 3H), 1.80−1.78 (m, 1H), 1.63−1.57 (m, 2H), 1.51−1.50 (m, 1H), 1.29−1.27 (m, 1H), 1.15 (d, J = 6.3 Hz, 3H), 0.98−0.88 (m, 2H); 13C NMR (150 MHz, DMSO-d₆) δ 168.9, 164.4, 162.0, 150.7, 148.0, 146.4, 138.2, 137.6, 131.8, 130.8, 129.9, 129.6, 129.2, 128.5, 128.4, 125.1, 121.3, 119.9, 119.1, 115.8, 45.9, 41.8, 40.5, 39.5, 36.9, 36.2, 33.4, 32.6, 31.9, 18.9 (2 x); HRMS (ESI) m/z calcd for C₃₄H₃₈N₄O₃ [M + H]+ 551.3017, found 551.3004.

1c.6 Typical method for the preparation of compound tert-butyl (2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl) ethyl) carbamate (11a):
To a solution of tert-butyl (2-(piperidin-4-yl) ethyl) carbamate (10a) (228 mg, 1 mmol, 1 eq.), 2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetic acid (3a) (217 mg, 1 mmol, 1 eq.), and HATU (570 mg, 1.5 mmol, 1.5 equiv.) in anhydrous DMF (5 mL) was added DIEA (175 μL, 1.5 mmol, 1.5 equiv.) under nitrogen. The reaction mixture was stirred at room temperature for 3 h and then quenched by the addition of water. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel (CH₃OH/CH₂Cl₂, 1:99 to 5:95) to afford tert-butyl (2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl) ethyl) carbamate (11a) (507 mg, 98%) as a sticky oil.

The compounds (11a–11b) were prepared in a way similar to the procedure described above.

Sticky solid; yield: 84%; ¹H NMR (600 MHz, CD₃OD) δ 7.65 (s, 1H), 7.43 (dd, J = 8.4, 1.4 Hz, 1H), 7.29 (d, J = 8.4 Hz, 1H), 6.49 (s, 1H), 4.54−4.51 (m, 1H), 4.05−4.03 (m, 1H), 3.89−3.83 (m, 2H), 3.07−3.03
tert-butyl (2-(4-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperazin-1-yl)ethyl)carbamate (11b):

White solid; yield: 92%; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.56 (s, 1H), 7.58–7.53 (m, 1H), 7.40–7.39 (m, 1H), 7.34 (dd, \(J = 8.4, 1.5\) Hz, 1H), 7.25 (t, \(J = 8.4\) Hz, 1H), 6.65 (s, 1H), 6.38 (s, 1H), 3.76 (s, 2H), 3.64 (s, 1H), 3.49–3.45 (m, 4H), 2.40–2.39 (m, 4H), 2.33–2.31 (m, 6H), 1.36 (s, 9H); HRMS (ESI) m/z calcd for C\(_{23}\)H\(_{32}\)N\(_4\)O\(_4\) [M + H]+ 429.2496, found 429.2495.

1c.7 Typical method for the preparation of compound 5-(2,4-dimethylphenyl)-N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl)piperidin-4-yl)ethyl)picolinamide (12a):

Compound 11a (536 mg, 1 mmol, 1 eq) was dissolved in DMF (3 ml) containing piperidine (10% v/v) and stirred 1h in room temperature. Resulting mixture is evaporated to The SM was dissolved in DMF containing piperidine, and all solvents were removed after 20 min. After completing reaction all solvents were removed in vacuo and kept overnight dry in high vacuo. To the same vessel mixture of DIEA (439 \(\mu\)L, 2.5 mmol, 3 eq), 5-(2,4-dimethylphenyl) picolinic acid (5a) (140 mg, 0.7 mmol, 0.8 eq) and HATU (957.6 mg, 2.5 mmol, 3 equiv.) added in the solution of anhydrous DMF (8 ml) under nitrogen. The aqueous layer was extracted twice with EtOAc, and the combined organic extracts were washed with brine, dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated. The residue was purified by column chromatography on silica gel (CH\(_3\)OH/CH\(_2\)Cl\(_2\), 1:99 to 5:95) to afford 12a (327 mg, 68%) as a white solid.

The compounds (12a–12g) were prepared in a way similar to the procedure described above.

5-(2,4-dimethylphenyl)-N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetyl) piperidin-4-yl)ethyl)picolinamide (12a): CDD-813

Sticky oil; yield: 84%; \(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 11.56 (s, 1H), 8.82 (t, \(J = 5.9\) Hz, 1H), 8.57 (d, \(J = 1.5\) Hz, 1H), 8.07 (d, \(J = 8.0\) Hz, 1H), 8.07 (d, \(J = 8.0\) Hz, 1H), 7.94 (dd, \(J = 7.9, 2.14\) Hz, 1H), 7.54 (s, 1H), 7.35 (dd, \(J = 7.9, 2.14\) Hz, 1H), 7.25 (d, \(J = 8.3\) Hz, 1H), 7.17–7.15 (m, 2H), 7.11 (d, \(J = 8.3\) Hz, 1H), 6.38 (s, 1H), 4.39–4.39 (m, 1H), 3.76–3.76 (m, 2H), 3.98–3.96 (m, 1H), 3.76 (s, 2H), 2.96–2.94 (m, 1H), 2.54–2.50 (m, 1H), 2.39 (s, 3H), 2.32 (s, 3H), 2.21 (s, 3H), 1.73–1.70 (m, 2H), 1.55–1.45 (m, 3H), 0.97–0.91 (m, 2H); 13C NMR (150 MHz, DMSO-\(d_6\)) \(\delta\) 148.9, 148.6, 148.0, 139.6, 138.3, 138.2, 137.6, 135.4, 134.6, 131.8, 131.7, 130.1, 131.7, 129.9, 127.3, 125.1, 121.8, 121.3, 119.9, 115.7, 54.0, 46.0, 41.9,
36.7, 36.2, 33.3, 32.6, 33.3, 32.6, 31.9, 21.1, 20.4, 18.9; HRMS (ESI) m/z calcd for C$_{33}$H$_{36}$N$_4$O$_3$ [M + H]$^+$ 537.2860, found 537.2858.

N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)ethyl) picolinamide (12b): CDD-767

![Structural diagram](image)

White solid; yield: 80%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 11.55 (s, 1H), 8.77 (t, $J = 5.9$ Hz, 1H), 8.62 (d, $J = 2.1$ Hz, 1H), 8.05–8.02 (m, 1H), 7.99–7.96 (m, 1H), 7.59–7.57 (m, 1H), 7.53 (s, 1H), 7.34 (dd, $J = 8.4$, 1.8 Hz, 1H), 7.24 (d, $J = 8.3$ Hz, 1H), 6.38 (s, 1H), 4.37–4.35 (m, 1H), 3.97–3.95 (m, 1H), 3.75 (s, 2H), 3.34–3.31 (m, 2H), 2.97–2.93 (m, 1H), 2.54–2.50 (m, 1H), 2.38 (s, 3H), 1.70–1.69 (m, 2H), 1.53–1.43 (m, 3H), 0.97–0.89 (m, 2H); 13C NMR (150 MHz, DMSO-$d_6$) δ 168.5, 163.7, 161.5, 150.1, 148.3, 147.6, 137.7, 137.2, 131.4, 129.4, 126.3, 124.7, 121.8, 120.9, 119.4, 115.3, 45.5, 41.4, 39.0, 36.2, 35.7, 32.9, 32.1, 31.4, 18.4; HRMS (ESI) m/z calcd for C$_{25}$H$_{28}$N$_4$O$_3$ [M + H]$^+$ 433.2234, found 433.2225.

N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)ethyl) benzamide (12c): CDD-779

![Structural diagram](image)

White solid; yield: 80%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 11.55 (s, 1H), 8.41 (t, $J = 5.4$ Hz, 1H), 7.82 (d, $J = 4.2$ Hz, 1H), 7.54 (s, 1H), 7.52–7.49 (m, 1H), 7.47–7.43 (m, 2H), 7.35 (dd, $J = 8.4$, 1.5 Hz, 1H), 7.24 (d, $J = 8.3$ Hz, 1H), 6.38 (s, 1H), 4.39–4.37 (m, 1H), 3.98–3.96 (m, 1H), 3.76 (s, 2H), 3.30–3.27 (m, 2H), 2.98–2.94 (m, 1H), 2.55–2.50 (m, 1H), 2.39 (s, 3H), 1.70 (t, $J = 11.4$ Hz, 2H), 1.56–1.51 (m, 1H), 1.45–1.42 (m, 2H), 0.98–0.90 (m, 2H); 13C NMR (150 MHz, DMSO-$d_6$) δ 168.5, 166.0, 161.5, 150.5, 147.5, 139.6, 137.2, 134.6, 131.3, 130.9, 128.5, 127.0, 124.7, 120.9, 120.3, 119.4, 115.3, 45.5, 41.4, 39.0, 36.6, 35.6, 32.9, 32.2, 31.4, 18.4; HRMS (ESI) m/z calcd for C$_{26}$H$_{29}$N$_3$O$_3$ [M + H]$^+$ 432.2282, found 432.2275.

N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)ethyl) quinoline-2-carboxamide (12d): CDD-855

![Structural diagram](image)

White solid; yield: 80%; $^1$H NMR (600 MHz, DMSO-$d_6$) δ 11.58 (s, 1H), 8.92 (t, $J = 6.0$ Hz, 1H), 8.52 (d, $J = 8.4$ Hz, 1H), 8.15 (d, $J = 8.4$ Hz, 1H), 8.12 (d, $J = 8.4$ Hz, 1H), 8.04 (d, $J = 8.0$ Hz, 1H), 7.85–7.82 (m, 1H), 7.70–7.67 (m, 1H), 7.52 (s, 1H), 7.34 (dd, $J = 8.9$, 2.0 Hz, 1H), 7.25 (d, $J = 8.4$ Hz,
1H), 6.38 (s, 1H), 4.38–4.36 (m, 1H), 3.96–3.94 (m, 1H), 3.75 (s, 2H), 3.41–3.37 (m, 1H), 2.96–2.92 (m, 1H), 2.53–2.49 (m, 1H), 2.37 (s, 3H), 1.71–1.69 (m, 1H), 1.54–1.47 (m, 3H), 0.98–0.91 (m, 2H); 13C NMR (150 MHz, DMSO-d$_6$) δ 168.9, 162.9, 161.5, 148.4, 148.0, 147.5, 139.2, 137.8, 137.7, 137.2, 134.9, 134.1, 131.2, 129.6, 129.4, 126.8, 124.6, 121.4, 119.4, 115.3, 45.5, 41.8, 41.4, 32.4, 31.8, 31.2, 20.6, 19.8, 18.4; HRMS (ESI) m/z calcd for C$_{29}$H$_{30}$N$_4$O$_3$ [M + H]+ 483.2391, found 483.2382.

N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)ethyl)isoquinoline-3-carboxamide (12e): CDD-854

White solid; yield: 81%; $^1$H NMR (600 MHz, DMSO-d$_6$) δ 11.54 (s, 1H), 9.37 (s, 1H), 8.91 (t, J = 5.9 Hz, 1H), 8.54 (s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 8.1 Hz, 1H), 7.87 (t, J = 4.0 Hz, 1H), 7.80 (t, J = 4.0 Hz, 1H), 7.54 (s, 1H), 7.35 (dd, J = 8.4, 1.5 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 6.38 (s, 1H), 4.38–4.36 (m, 1H), 3.99−3.96 (m, 1H), 3.76 (s, 2H), 3.41–3.37 (m, 2H), 2.99−2.95 (m, 1H), 2.56–2.50 (m, 1H), 2.39 (s, 3H), 1.74 (s, 2H), 1.57–1.47 (m, 3H), 0.98–0.94 (m, 2H); 13C NMR (150 MHz, DMSO-d$_6$) δ 168.4, 161.9, 161.4, 147.5, 143.9, 137.1, 135.3, 131.3, 131.2, 129.4, 129.1, 128.9, 128.9, 127.9, 127.7, 124.6, 120.8, 119.5, 119.3, 115.2, 45.5, 41.8, 41.4, 35.8, 32.9, 32.1, 18.4, 18.0, 16.7; HRMS (ESI) m/z calcd for C$_{29}$H$_{30}$N$_4$O$_3$ [M + H]+ 483.2391, found 483.2387.

N-(2-(1-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperidin-4-yl)ethyl)isoquinoline-1-carboxamide (12f): CDD-853

White solid; yield: 84%; $^1$H NMR (600 MHz, DMSO-d$_6$) δ 11.57 (s, 1H), 8.91 (d, J = 8.5 Hz, 1H), 8.83 (t, J = 5.8 Hz, 1H), 8.52 (d, J = 5.5 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 5.5 Hz, 1H), 7.81–7.79 (m, 1H), 7.72–7.70 (m, 1H), 7.54 (s, 1H), 7.35 (dd, J = 8.4, 1.5 Hz, 1H), 7.25 (d, J = 8.3 Hz, 1H), 6.38 (s, 1H), 4.40–4.38 (m, 1H), 3.99–3.97 (m, 1H), 3.76 (s, 2H), 3.40–3.37 (m, 2H), 2.98–2.94 (m, 1H), 2.55–2.50 (m, 1H), 2.38 (s, 3H), 1.74–1.72 (m, 2H), 1.59–1.55 (m, 1H), 1.51–1.47 (m, 2H), 1.00–0.93 (m, 2H); 13C NMR (150 MHz, DMSO-d$_6$) δ 168.5, 166.0, 161.5, 151.4, 147.5, 140.7, 137.1, 136.5, 131.3, 130.5, 129.4, 128.2, 127.0, 126.5, 125.4, 124.6, 123.1, 120.8, 119.4, 115.3, 45.5, 41.8, 41.4, 36.3, 35.6, 32.9, 32.1, 18.4, 18.0, 16.6; HRMS (ESI) m/z calcd for C$_{23}$H$_{30}$N$_4$O$_3$ [M + H]+ 483.2391, found 483.2389.

N-(2-(4-(2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl)acetyl)piperazin-1-yl)ethyl) picolinamide (12g): CDD-778
White solid; yield: 81%; $^1$H NMR (600 MHz, CD$_3$OD) \( \delta \) 8.63 (d, \( J = 4.4 \) Hz, 1H), 8.11 (d, \( J = 7.8 \) Hz, 1H), 7.99–7.96 (m, 1H), 7.68 (s, 1H), 7.58–7.96 (m, 1H), 7.46 (dd, \( J = 8.4, 1.6 \) Hz, 1H), 7.33 (d, \( J = 8.4 \) Hz, 1H), 6.52 (s, 1H), 3.95 (s, 2H), 8.63 (d, \( J = 4.4 \) Hz, 1H), 3.73–3.35 (m, 6H), 3.31–3.30 (m, 4H), 2.51 (s, 3H); 13C NMR (150 MHz, DMSO-\( d_6 \)) \( \delta \) 172.1, 168.1, 164.8, 151.5, 150.3, 149.8, 138.9, 138.3, 133.1, 130.7, 128.1, 126.4, 123.3, 121.9, 120.9, 117.4, 58.0, 53.09 (2x), 43.8, 39.9, 35.2, 19.08 (2x); HRMS (ESI) m/z calcd for C$_{24}$H$_{27}$N$_5$O$_4$ [M + H]$^+$ 434.2187, found 434.2186.
2) $^1$H and $^{13}$C NMR spectra of most selective compounds, 6a (CDD-724), 6c (CDD-787), 6d (CDD-956), 6f (CDD-981), 6g (CDD-982), 6h (CDD-986), and 6j (CDD-1146).

$^1$H NMR (DMSO-$d_6$, 600 MHz) and zoom-in (0–5 ppm) spectra of 6a (CDD-724)
$^1$H NMR (DMSO-$d_6$, 600 MHz) and zoom-in (6–9 ppm) spectra of 6a (CDD-724)

$^{13}$C NMR (DMSO-$d_6$, 150 MHz) spectrum of 6a (CDD-724)
$^1$H NMR (DMSO-$d_6$, 600 MHz) and zoom-in (0–5 ppm) spectra of 6c (CDD-787)
$^1$H NMR (DMSO-$d_6$, 600 MHz) and zoom-in (6–9 ppm) spectra of 6c (CDD-787)

$^{13}$C NMR (DMSO-$d_6$, 150 MHz) spectrum of 6c (CDD-787)
$^1$H NMR (DMSO-$d_6$, 600 MHz) and zoom-in (0–6 ppm) spectra of 6d (CDD-956)
NMR (DMSO-$d_6$, 600 MHz) and zoom-in (6–9 ppm) spectra of 6d (CDD-956)

$^{13}$C NMR (DMSO-$d_6$, 150 MHz) spectrum of 6d (CDD-956)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (0–5 ppm) spectra of 6f (CDD-981)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (6–9 ppm) spectra of 6h (CDD-981)

$^{13}$C NMR (CD$_3$OD, 150 MHz) spectrum of 6f (CDD-981)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (0–5 ppm) spectra of 6g (CDD-982)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (6–9 ppm) spectra of 6g (CDD-982)

$^{13}$C NMR (CD$_3$OD, 150 MHz) spectrum of 6g (CDD-982)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (0–5 ppm) spectra of 6h (CDD-986)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (6–9 ppm) spectra of 6h (CDD-986)

$^{13}$C NMR (CD$_3$OD, 150 MHz) spectrum of 6h (CDD-986)
$^1$H NMR (DMSO-d$_6$, 600 MHz) and zoom-in (0–5 ppm) spectra of 6j (CDD-1146)
$^1$H NMR (DMSO-d$_6$, 600 MHz) and zoom-in (6–10 ppm) spectra of 6j (CDD-1146)

$^{13}$C NMR (DMSO-d$_6$, 150 MHz) spectrum of 6j (CDD-1146)
3. Tier 2 SAR analysis

Scheme S1. Syntheses of DEC-Tec selection hits and Tier 2 SAR 9 and 12a-12g. Reagents and conditions: i) different substituted picolinic acids, HATU, DIEA, DMF, 12h; ii) 4M HCl/dioxane, 1h; iii) 2-(4-methyl-2-oxo-1,2-dihydroquinolin-6-yl) acetic acid, HATU, DIEA, DMF, 12h; iv) different quinolines amines, HATU, DIEA, DMF, 12h.
Table S1. Structures and activities of analogues 6h, 6i, and 6j. ND, Not determined.

| Compound (CDD) | R<sup>1</sup> | R<sup>2</sup> | *Chirality | BRDT-BD1 IC<sub>50</sub> nM | BRDT-BD2 IC<sub>50</sub> nM | BRDT-BD1 vs. BRDT-BD2 fold | BRD4-BD1 IC<sub>50</sub> nM | BRD4-BD2 IC<sub>50</sub> nM | BRD4-BD1 vs. BRD4-BD2 fold |
|----------------|--------------|--------------|----------------|-----------------|-----------------|---------------------------|-----------------|-----------------|---------------------------|
| 6h (CDD-986)   | H            |              | R             | 5.4             | 20,930          | 3,875                     | 7.1             | 16,701          | 2,352                     |
| 6i (CDD-784)   | CH<sub>3</sub> |              | Rac           | 20.2            | 19,400          | 960                       | 15.7            | 32,640          | 2,078                     |
| 6j (CDD-1146)  | CH<sub>3</sub> |              | Rac           | 7.6             | ND              | ND                        | ND              | ND              | ND                        |
Table S2. Structures and activities of analogues 9, 12a-12g. NE, Not efficient.

| Compound | R² | R³ | X | BRDT -BD1 IC₅₀ nM | BRD T- BD2 IC₅₀ nM | BRDT -BD1 vs. BRDT -BD2 fold | BRD4- BD1 IC₅₀ nM | BRD4- BD2 IC₅₀ nM | BRD4- BD1 vs. BRD4- BD2 fold |
|----------|----|----|---|-------------------|-------------------|---------------------------|-------------------|-------------------|-----------------------------|
| 9 (CDD-906) | | | | 31 | 23,449 | 756 | 52.4 | 20,000 | 381 |
| 12a (CDD-813) | CH | | | 33.7 | 67,836 | 2012 | 27.8 | 8,689 | 312 |
| 12b (CDD-767) | CH | | | 35.4 | 6,800 | 192 | 13.8 | 75,200 | 5,449 |
| 12c (CDD-779) | CH | | | 148.2 | 10,400 | 70 | 47.9 | NE | NE |
| 12d (CDD-855) | CH | | | 81.3 | NE | NE | 122.4 | NE | NE |
| 12e (CDD-854) | CH | | | 115.7 | NE | NE | 119.4 | NE | NE |
| 12f (CDD-853) | CH | | | 638.1 | NE | NE | 341.4 | NE | NE |
| 12g (CDD-778) | N | | | 5200 | 71800 | 13.8 | 1300 | NE | NE |
4. Mosher Method.

We utilized the Mosher method to determine the absolute stereochemistry of α-chiral carbon of piperidine propanoic acid 3R. Two possible isomers R and S are possible at the α-carbon; to identify this, we have synthesized MTPA adducts 4 (R, R) (R-MTPA amide) and 4 (R, S) (S-MTPA amide) and carried out chiral analysis by determining $\Delta \delta SR$ (ppm). Detailed synthetic steps depicted in Scheme 3. Substituted piperidine propanoic acids (1R) undergo sequential addition of O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) mediated amide coupling produce intermediate (2R). Subsequent deprotection of fluorenylmethyloxycarbonyl followed by amide coupling reaction furnished desired products 4 (R, R) (R-MTPA amide) and 4 (R, S) (S-MTPA amide). After making MTPA amides with 3R, we found that there was clear difference in the chemical shift at positions 3, 4, 5 and 9, which are affected by shielding effect of phenyl group of MTPA. Detailed chemical shifts, $\Delta \delta SR$ (ppm) and $\Delta \delta SR$ (Hz) are depicted in Table 4. Protons on position 3 and 4, adjacent to the α-carbon have shown positive values of $\Delta \delta SR$ (ppm) and $\Delta \delta SR$ (Hz), which indicates below the plane. Whereas protons on position 10 shows above the plane and shown negative values of $\Delta \delta SR$ (ppm) and $\Delta \delta SR$ (Hz). Detailed H1 NMR spectra of MTPA amides are mentioned in supporting data. From this experiment it is clear that compound 3R is R isomer and its corresponding most active compounds CDD-787 and CDD-956 were also R isomers, which are also in agreement with our positive optical rotation.

**Scheme S2.** Scheme for the synthesis of MTPA amide from 1R. Reagents and conditions: i) Methylamine HCl, O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU), N,N-diisopropylethylamine (DIEA), N,N-dimethylformamide (DMF), room temperature (r.t), 1 h; ii) 10% Piperidine, DMF, 1h; iii) (R)-(+)-MTPA, 1,3-dicyclohexylcarbodiimide (DCC), anhydrous CH2Cl2 (DCM), 4-N,N (dimethylamino)pyridine (DMPA), r.t, 1 h; iv) (S)-(−)-MTPA, DCC, DCM, DMPA, r.t, 1 h.
Table S3. $\Delta \delta^{SR} (=\delta_S-\delta_R)$ data for the 4 ($R$, $R$) ($R$-MTP amide) and 4 ($R$, $S$) ($S$-MTP amide).

| Position | $\delta$ R-amide | $\delta$ S-amide | $\Delta \delta^{SR}$ (ppm) | $\Delta \delta^{SNR}$ (Hz) |
|----------|------------------|------------------|---------------------------|--------------------------|
| 1        | 3.57, s          | 3.39, s          | -0.18                     | -108                     |
| 2        | 4.53, m          | 4.53, m          |                           |                          |
| 3        | 1.65, m          | 1.75, m          | +0.1                      | +60                      |
| 4        | 1.50, m          | 1.69, m          | +0.19                     | +114                     |
| 5,9a     | 1.31, m          | 1.63             | +0.32                     | +192                     |
| 5,9b     | 1.17, m          | 1.52             | +0.35                     | +210                     |
| 5,9c     | 0.99, m          | 1.10, m          | +0.11                     | +66                      |
| 6,8a     | 3.97, m          | 4.05, d (13.4)   | +0.08                     | +48                      |
| 6,8b     | 2.50, brs        | 2.68, brs        | +0.18                     | +108                     |
| 7        | 1.44             | 1.44             |                           |                          |
| 10       | 2.74, s          | 2.72, s          | -0.02                     | -12                      |
Mosher Method Spectra of 4 (R, R) (R-MTPA amide) and 4 (R, S) (S-MTPA amide)

$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (0–6 ppm) spectra of 4 (R, R)
$^1$H NMR (CD$_3$OD, 600 MHz) and zoom-in (1–6 ppm) spectra of 4 (R, S)

S-MTPA amide
5. Chiral analysis of compounds CDD-724, CDD-786, CDD-787, CDD-1146, CDD-2107, and CDD-956.

| Column              | Chiralpak_IH_3          |
|---------------------|-------------------------|
| Mobile phase        | Solvent A1: Heptane Solvent B2: Ethanol |
| Gradient            | Isocratic: Heptane/Ethanol(40/60) for 15 minutes |
| Flow rate           | 0.75ml/min               |
| Detection           | UV 254nm                 |
| Temperature         | ambient                  |
| Concentration       | 1mM (injection volume 5µl) |
| Instrument          | Analytical HPLC          |
| # | Time  | Area   | Height | Width | Area%  | Symmetry |
|---|-------|--------|--------|-------|--------|----------|
| 1 | 2.78  | 1722.5 | 244    | 0.175 | 1.919  | 0.701    |
| 2 | 5.137 | 88562.4| 210.2  | 0.6416| 98.081 | 0.588    |
| 1 | 2.78  | 1722.5 | 244    | 0.175 | 1.919  | 0.701    |
| 2 | 5.137 | 88562.4| 210.2  | 0.6416| 98.081 | 0.588    |
Figure S1. Four molecules captured in the BRDT-BD1/CDD-956 complex crystal.
A. Four BRDT-BD1 protein molecules are captured in the asymmetric unit with one CDD-956 bound to each protein molecule. They are colored in tan, magenta, gray, and red. Bound CDD-956 are shown in sticks. B. Superimposition of four BRDT-BD1/CDD-956 complexes in the asymmetric unit. Each of the four molecular units is nearly identical (showing RMSD values less than 0.5 Å between shared ~100 CA atoms). Each molecule is shown in cartoon with side chains of key CDD-956 interacting residues shown in sticks.
Figure S2. LIGPLOT diagram showing interactions between BRDT-BD1 and CDD-956.
### 6. Table S4. Data collection and refinement statistics.

*Statistics for the highest-resolution shell are shown in parentheses.*

| Protein:ligand complex | BRDT-BD1:CDD-956* |
|------------------------|--------------------|
| Wavelength             | 1.000              |
| Resolution range       | 48.37 - 1.82 (1.885 - 1.82) |
| Space group            | P 2₁ 2₁ 2₁         |
| Unit cell              | 61.69, 94.38, 112.7 90 90 90 |
| Total reflections      | 759141 (35916)     |
| Unique reflections     | 59693 (5882)       |
| Multiplicity           | 12.7 (6.1)         |
| Completeness (%)       | 99.98 (100.00)     |
| Mean I/sigma(I)        | 8.30 (0.72)        |
| Wilson B-factor        | 25.72              |
| R-merge                | 0.2041 (2.094)     |
| R-meas                 | 0.2122 (2.288)     |
| R-pim                  | 0.05729 (0.9129)   |
| CC1/2                  | 0.991 (0.306)      |
| CC*                    | 0.998 (0.684)      |
| Reflections used in refinement | 59690 (5882) |
| Reflections used for R-free | 1909 (189) |
| R-work                 | 0.1777 (0.3321)    |
| R-free                 | 0.1834 (0.3363)    |
| CC(work)               | 0.967 (0.641)      |
|                  |           |
|------------------|-----------|
| CC(free)         | 0.971 (0.548) |
| Number of non-hydrogen atoms | 4230 |
| macromolecules   | 3581 |
| ligands          | 178      |
| solvent          | 477      |
| Protein residues | 434      |
| RMS(bonds)       | 0.005    |
| RMS(angles)      | 0.84     |
| Ramachandran favored (%) | 98.83 |
| Ramachandran allowed (%) | 0.94 |
| Ramachandran outliers (%) | 0.23 |
| Rotamer outliers (%) | 0.51 |
| Clashscore       | 6.70     |
| Average B-factor | 33.91    |
| macromolecules   | 33.15    |
| ligands          | 29.71    |
| solvent          | 41.15    |
| Number of TLS groups | 26     |
7. Table S5. Matrix of CDD-956 screen in BROMOscan assays. Percent Control (%Ctrl): CDD-956 was screened at 1000 nM, and results for binding interactions are reported as '% Ctrl', where lower numbers indicate stronger hits in the matrix. %Ctrl Calculation: (CDD-956 signal - positive control signal)/(negative control signal - positive control signal)*100 negative control = DMSO(100%Ctrl), positive control = control compound (0%Ctrl).

| Compound Name     | DiscoveRx Gene Symbol | Entrez Gene Symbol | Percent Control | Compound Concentration (nM) |
|-------------------|-----------------------|--------------------|-----------------|-----------------------------|
| CDD-956 ATAD2A    | ATAD2                 | 100                | 1000            |
| CDD-956 ATAD2B    | ATAD2B                | 97                 | 1000            |
| CDD-956 BAZ2A     | BAZ2A                 | 100                | 1000            |
| CDD-956 BAZ2B     | BAZ2B                 | 100                | 1000            |
| CDD-956 BRD1      | BRD1                  | 96                 | 1000            |
| CDD-956 BRD2(1)   | BRD2                  | 0                  | 1000            |
| CDD-956 BRD2(2)   | BRD2                  | 2                  | 1000            |
| CDD-956 BRD3(1)   | BRD3                  | 0                  | 1000            |
| CDD-956 BRD3(2)   | BRD3                  | 26                 | 1000            |
| CDD-956 BRD4(1)   | BRD4                  | 0                  | 1000            |
| CDD-956 BRD4(2)   | BRD4                  | 78                 | 1000            |
| CDD-956 BRD7      | BRD7                  | 95                 | 1000            |
| CDD-956 BRD9      | BRD9                  | 90                 | 1000            |
| CDD-956 BRDT(1)   | BRDT                  | 0                  | 1000            |
| CDD-956 BRDT(2)   | BRDT                  | 68                 | 1000            |
| CDD-956 BRPF1     | BRPF1                 | 66                 | 1000            |
| CDD-956 BRPF3     | BRPF3                 | 100                | 1000            |
| CDD-956 CECR2     | CECR2                 | 100                | 1000            |
| CDD-956 CREBBP    | CREBBP                | 100                | 1000            |
| CDD-956 EP300     | EP300                 | 100                | 1000            |
| CDD-956 FALZ      | BPTF                  | 100                | 1000            |
| CDD-956 GCN5L2    | KAT2A                 | 99                 | 1000            |
| CDD-956 PBRM1(2)  | PBRM1                 | 100                | 1000            |
| CDD-956 PBRM1(5)  | PBRM1                 | 100                | 1000            |
| CDD-956 PCAF      | KAT2B                 | 100                | 1000            |
| CDD-956 SMARCA2   | SMARCA2               | 100                | 1000            |
| CDD-956 SMARCA4   | SMARCA4               | 100                | 1000            |
| CDD-956 TAF1(2)   | TAF1                  | 88                 | 1000            |
| CDD-956 TAF1L(2)  | TAF1L                 | 100                | 1000            |
| CDD-956 TRIM24(PhD,Bromo.) | TRIM24 | 100 | 1000 |
| CDD-956 TRIM33(PhD,Bromo.) | TRIM33 | 84 | 1000 |
| CDD-956 WDR9(2)   | BRWD1                 | 100                | 1000            |
8. Table S6. Viability EC₅₀ of selected compounds against AML cell line MV4;11. NE, Not efficient.

| Compound | MV4-11 EC₅₀ nM |
|----------|----------------|
| CDD-724  | NE             |
| CDD-787  | 8              |
| CDD-956  | 21             |
| CDD-981  | 58             |
| CDD-982  | 100            |
| CDD-986  | 143            |
| CDD-1146 | NE             |

9. Materials and Methods.

**Production of recombinant bromodomain proteins:** Recombinant His6-tagged BRDT-BD1 and BD2 were produced as previously described (1)(1)(1)(1)(1)(1). In brief, cDNA encoding human BRDT and BRD4 BD1 and BD2 bromodomains were subcloned into pET15b or pET28b bacterial vectors (Addgene, USA) with an N-terminal polyhistidine tag (6-His; linear) for expression and purification. His-tagged recombinant protein was isolated and eluted by immobilized metal affinity chromatograph followed by size exclusion gel filtration chromatography. Each BD was confirmed to be properly folded and active by a fluorescence thermal shift stability assay and AlphaScreen with biotinylated JQ1 as ligand, respectively.

**DEC-Tec affinity selection with bromodomain proteins.** To identify BRDT-BD1 selective compounds, we screened our DEC-Tec library pool. We had five screening conditions: 1) absence of bromodomain proteins (bead binding non-target control, NTC); 2) presence of His-BRDT-BD1 at 0.3 μM; 3) presence of His-BRDT-BD2 at 0.3 μM (a counter-screen for bromodomain selective compounds); 4) presence of His-BRDT-BD1 plus JQ1 at 100 μM; and 5) presence of His-BRDT-BD2 plus JQ1 at 100 μM. After three rounds of DEC-Tec selection, the DNA barcode from the last round of selection was PCR amplified. Following cleanup by Agencourt AMPure XP beads and quantitation with Agilent high sensitivity DNA kit using a Bioanalyzer, the DNA was sequenced in a single-read 105-cycle sequencing on an Illumina NextSeq 500 instrument.

**Bromodomain proximity assay.** The AlphaScreen™ assay was performed following previous publication with minor modifications from the manufacturer’s protocol (PerkinElmer, USA). A 20-μL reaction was set up in a PerkinElmer 384-well AlphaPlate where His-bromodomain at 10 nM was
incubated with biotinylated JQ1 at 10 nM, nickel chelate acceptor beads at 12.5 μg/mL, and tested compound at various concentrations for 15 min at room temperature, followed by the addition of streptavidin donor beads at 12.5 μg/mL and another 60-min incubation at room temperature. The plate was read on a Tecan Infinite M1000 Pro plate reader.

**Thermal shift assay.** The dye SYPRO Orange (ThermoFisher Scientific, USA) was used to perform the protein thermal shift assay. The assay was set up on a 384-well Roche plate where His-bromodomain at a concentration of 2 μM was incubated with the test compound at various concentrations, and SYPRO Orange dye at 5 × in a 10-μL reaction. The melting curve experiment and data analysis was run on a Roche Lightcycler 480 real-time PCR instrument.

**Metabolic stability assay in liver microsomes.** CDD-787 and CDD-956 (2.0 μM) were incubated in the mouse or human liver microsomes (0.5 mg protein/mL) at 37 °C in the presence of NADPH (1.0 mM). The samples are collected at specific time-points 0, 5, 10, 20, 40 and 60 min in duplicate. The reactions are terminated by adding equivalent volume of ice-cold CH₃OH and vortexed. The reaction mixtures are centrifuged at 15,000 g for 15 min. Five μL of the supernatant was analyzed by ultra-high performance liquid chromatography (UHPLC)-MS/MS analysis (QQQ, Agilent 6490) equipped with 50 mm × 4.6 mm column (XDB C-18, Agilent Technologies, USA). LC-MS/MS (Agilent Technologies, QQQ, Santa Clara, CA) was operated in positive mode with electrospray ionization. Ultrapure nitrogen was applied as the sheath gas and the collision gas. The capillary gas temperature was set at 280 °C and the capillary voltage was set at 3.6 kV. The column temperature was maintained at 40 °C. The flow rate was at 0.3 mL/min with a 50% mobile phase (acetonitrile containing 0.1% formic acid) in a 6-min run. CDD-787 and CDD-956 were measured using multiple-reaction monitoring method with the mass transition m/z 594.3>318.1 for CDD-787; m/z 566.3>290.1 for CDD-956. JQ1 (mass transition: m/z 457.4>341.3) and alprazolam (m/z 309.2>281.3) were used as the short and long half-life control, respectively.

**BromoKdELECT assays.** Assays were performed at Eurofins DiscoverX (Fremont, CA) using the following procedures. T7 phage strains displaying bromodomains were grown in parallel in 24-well blocks in an E. coli host derived from the BL21 strain. E. coli were grown to log-phase and infected with T7 phage from a frozen stock (multiplicity of infection = 0.4) and incubated with shaking at 32°C until lysis (90-150 minutes). The lysates were centrifuged (5,000 x g) and filtered (0.2μm) to remove cell debris. Streptavidin coated magnetic beads were treated with biotinylated small molecule or acetylated peptide
ligands for 30 minutes at room temperature to generate affinity resins for bromodomain assays. The liganded beads were blocked with excess biotin and washed with blocking buffer (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, and 1 mM DTT) to remove unbound ligand and to reduce non specific phage binding. Binding reactions were assembled by combining bromodomains, liganded affinity beads, and test compounds in 1x binding buffer (16 % SeaBlock, 0.32x PBS, 0.02%SBA, 0.04 % Tween 20, 0.004% Sodium azide, and 7.9 mM DTT). Test compounds were prepared as 100X stocks in 100% DMSO and subsequently diluted 1:25 in monoethylene glycol (MEG). The compounds were then diluted directly into the assays such that the final concentrations of DMSO and MEG were 0.1% and 2.4%, respectively. All reactions were performed in polypropylene 384 well plates in a final volume of 0.02 ml. The assay plates were incubated at room temperature with shaking for 1 hour and the affinity beads were washed with wash buffer (1x PBS with 0.05% Tween 20). The beads were then re-suspended in elution buffer (1x PBS with 0.05% Tween 20 and 2 μM of non-biotinylated affinity ligand) and incubated at room temperature with shaking for 30 minutes. The bromodomain concentration in the eluates was measured by qPCR.

**Crystallization, data collection, and structure solution.** BRDT-BD1 was co-crystallized with CDD-956 by the hanging drop vapor diffusion method. For crystallization, purified BRDT-BD1 at 3.5 mg/ml was mixed with a 5 molar excess of CDD-956. The protein-inhibitor mixture was concentrated using Amicon Ultra-15 centrifugal filters (Millipore Sigma) to 24 mg/ml. Mosquito (TTP labtech) was used to dispense equal volumes of protein and reservoir (250 nl each) against 70 μl reservoir buffer in 96 wells crystallization tray (96-Well clear polystyrene microplate from SPT Labtech). Crystals of the BRDT-BD1/CDD-956 complex were observed after 2 days in drop containing 1.26 M Ammonium Sulfate, 0.1 M Tris (pH 8.5), and 0.2 M Lithium sulfate. The crystals were cryo-cooled in the same solution with 20 % glycerol. The diffraction data were collected at Advanced Photo Source (APS) (Lemont, Illinois, USA). The data was integrated and scaled by using iMosflm and SCALA (2, 3). The crystal structure of the BRDT-BD1/CDD-956 complex was determined by molecular replacement in PHENIX using a crystal structure of the human BRDT BD1 (PDB ID: 4FLP) as a search model (4). CDD-956 was fitted manually into electron density by using COOT. The final models have gone through several rounds of refinement using Phenix.refine (5) followed by manual model building using COOT (6). For all structural analysis and preparation of figures, the visualization program PyMOL was used (7).

**NanoBRET target engagement intracellular BET bromodomain assay.** The NanoLuc-BRDT-BD1 and NanoLuc-BRDT-BD2 vectors were constructed by subcloning the same BRDT bromodomain
sequences applied for protein expression into the NanoLuc-BRD4-BD1 and NanoLuc-BRD4-BD2 vectors (Promega, USA) to replace the corresponding BRD4 bromodomain sequences. The NanoBRET tracer competition assay was performed in transiently transfected HEK293 cells expressing each NanoLuc-bromodomain vector on a 384-well plate following the manufacturer’s protocol (Promega, USA). Tracer titration was performed for each NanoLuc fusion vector to determine the optimized tracer concentration. The fusion protein was allowed to express for 36 h. The cells were then preincubated with tested compounds at different concentrations for 2 h followed by 2 h of incubation with tracer. Freshly prepared NanoBRET Nano-Glo substrate plus extracellular NanoLuc inhibitor was then added to initiate the subsequent bioluminescence resonance energy transfer (BRET) measurements using a CLARIOstarPlus BMG LABTECH plate reader. Data analysis was done by measuring the ratio of acceptor emission to donor emission (BRET ratio) and normalized by subtracting no-tracer-control-background.

Cell culture. AML cell lines MV4;11, MOLM-13, THP-1, and Kasumi1 were graciously shared by Dr. Stevens at Texas Children’s Hospital. Cell lines were incubated at 37°C under 5% CO2, in RPMI-1640 plus 10% FBS and 1% Penicillin/Streptomycin. Mycoplasma testing was performed using the LookOut Mycoplasma qPCR Detection Kit (Sigma) at entry into the lab. Cell line identity was confirmed annually using STR fingerprinting at the Cytogenetics and Cell Authentication Core at MD Anderson Cancer Center. Cells used for experiments were <20 passages from thawing.

Cell viability testing. Dose-response testing of CDD hits were conducted on 40,000 cells/mL plated in 384-well plates with 4 ten-fold dilutions of tamibarotene and read out by CellTiterGlo (Promega) at 72 hours of treatment per manufacturer instructions. There were at least 3 biologic replicates for each condition, and experiments were independently repeated three times to confirm similar results. Analysis was done using GraphPad Prism normalizing to the CellTiterGlo signal of DMSO-treated samples as indicated.

Flow cytometry. Cells were harvested after 24 hours treatment for cell cycle analysis. After a PBS rinse, cells were fixed overnight in 70% methanol at -20 °C. The following day, samples were rinsed with 0.5% BSA in PBS, then incubated in 100 µg/mL RNase/PBS at 37 °C for 15 minutes. Then cells were resuspended in 50 µg/mL PI in PBS. The samples were then analyzed on the BD canto II flow cytometer. For apoptosis detection, cells were harvested at 72 hours of treatment. Media was aspirated to leave a residual of 300-400uL. DAPI and AlexaFluor 488-conjugated Annexin V antibody (1:200 dilution,
ThermoFisher A13201) was added to the tube in equal volume, and samples were analyzed in the LSR I flow cytometer. For both experiments, three biologic replicates were averaged for each condition, and experiments were all repeated three times to confirm results. Results were analyzed using FlowJo v10 and plotted in GraphPad Prism using an unpaired t-test to compare statistical significance.

**Quantitative RT-PCR analysis.** Expression of MYC and GAPDH were determined using real-time quantitative reverse transcriptase-polymerase chain reaction (qPCR). Three technical replicates per sample were used. Primers for MYC, GAPDH, and housekeeping control ACTB were ordered from Sigma-Millipore. Relative expression was calculated using the comparative \( \Delta\Delta Ct \) method normalizing to ACTB and DMSO. All results were plotted in GraphPad Prism using an unpaired t-test to determine statistical significance.

**Coordinates.** Atomic coordinates and structure factors of the BRDT BD1/CDD-956 complex have been deposited in the Protein Data Bank (www.pdb.org) under an identification number 7UBO.
10. Supporting References.

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