Optimization of a Fragment-Based Screening Hit towards Potent DOT1L Inhibitors Interacting in an Induced Binding Pocket

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Supporting Information

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Figures S1 and S2

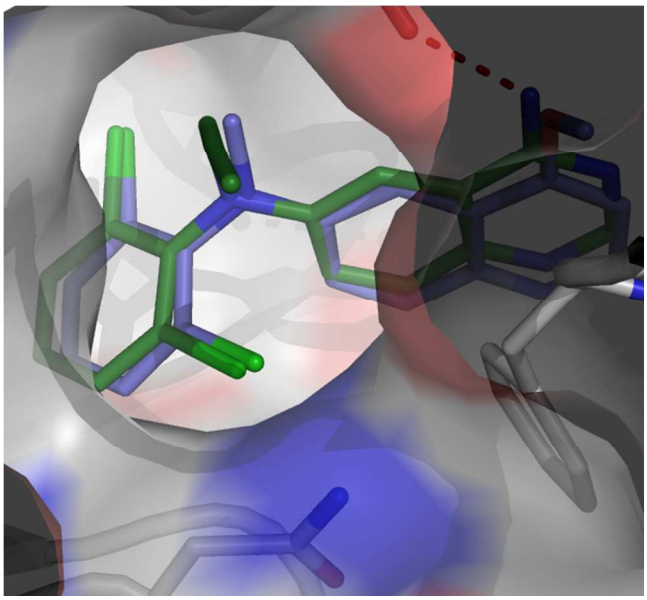


Figure S1. Docking of compound **6** (green) in the co-crystal structure of DOT1L.**5** (grey/blue) demonstrates the potential of the propargyl linker to extend through the narrow hydrophobic channel

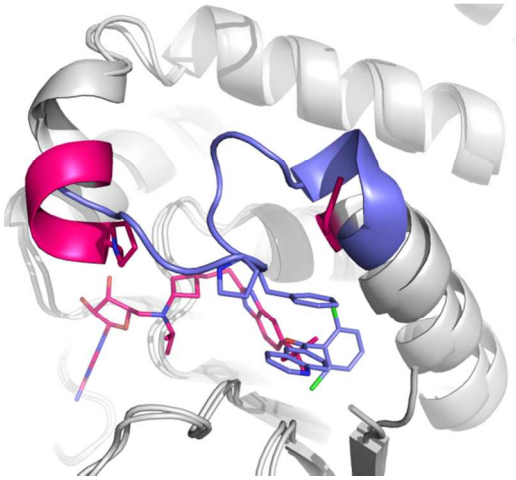


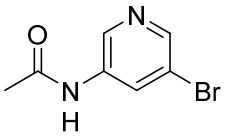
Figure S2. Superimposition of EPZ-5676 bound DOT1L co-crystal structure (PDB 4HRA) and compound **3** bound DOT1L co-crystal structure (PDB 5DTQ). The ligand and lid loop of the SAM binding pocket (residues 126-140, Pro130 and Phe131 shown as sticks) are coloured in blue for Dot1L.**3** and magenta for Dot1L.EPZ-5676. The flexible loop 296-309 at the crystal interface is omitted for clarity.

Experimental procedures for the synthesis of **2**-**10**

General Methods. All reagents and solvents were of commercial quality and used without further purification. Normal phase column chromatography purifications were carried out in the indicated solvent system (volume:volume) using pre-packed Silica RediSepRf® cartridges for use on the ISCO CombiFlash®. Nuclear Magnetic Resonance spectra were recorded on Bruker 400 MHz or 600 MHz / 54mm, UltraShield™ Plus, long hold time spectrometers. Spectra were taken in the indicated solvent at

ambient temperature and the chemical shifts are reported in parts per million (ppm (δ)) relative to the lock of the solvent used. Resonance patterns are recorded with the following notations: s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The LC-MS analyses (Rt = retention time) were performed with a Waters Acquity UPLC BSM, coupled to a Waters Acquity SQD (Single Quadrupole Detector) mass spectrometer with a range of ESI +/- : 100-1200 m/z, utilizing a Waters Acquity HSS T3 1.8 µm 2.1 x 50 mm column with 60°C column temperature, a flow of 1 mL/min and a solvent gradient 5 to 98% B over 1.4 min, followed by 0.4 min at 98% B; solvent A: water + 0.05% formic acid + 3.75 mM ammonium acetate, solvent B: acetonitrile + 0.04% formic acid. The injection volume was 1 µl and the solvent was typically MeOH. MS analyses (m/z) were performed on the same machine with flow injection: 0.4 mL/min in 70% B. HPLC analyses were performed with an Agilent 1100 Series HPLC utilizing a column Chromolith Performance RP-18-e 100-4.6 with 29°C column temperature, a flow of 2 mL/min and a solvent gradient 2% to 100% B over 4.5 min, followed by 1 min at 100% B; solvent A: water with 0.1% trifluoroacetic acid, solvent B: acetonitrile with 0.1% trifluoroacetic acid, and UV-detection at 215 nm. Preparative HPLC was done on a Gilson system with DAD-detector utilizing a 100x30 mm Waters Sunfire Prep C18 OBD^{TM} 5 µm column with a flow of 30 mL/min and a specified solvent gradient using solvent A: water with 0.1% trifluoroacetic acid, solvent B: acetonitrile.

Step 2-1 N-(5-bromopyridin-3-yl)acetamide



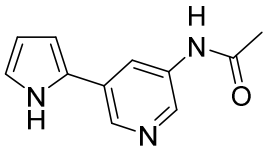
To a solution of 3-amino-5-bromopyridine (250 mg, 1.40 mmol), anhydrous CH_2Cl_2 (8 mL) and Et_3N (234 µL, 1.68 mmol) was added dropwise acetyl chloride (105 µL, 1.48 mmol) at rt under stirring. The mixture was stirred at rt for 1 h. The reaction mixture was diluted with sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 then with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under vacuum to afford 307 mg as a beige solid of the title compound (quantitative yield).

HPLC : Rt = 1.96 min

MS : $[M+H]^+$ 215.0/217.0., $[M-H]^-$ 212.9/214.9 (Br pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.62 (d, J = 1.5 Hz, 1H), 8.45 – 8.26 (m, 2H), 2.09 (s, 3H).

Step 2-2 N-(5-(1H-pyrrol-2-yl)pyridin-3-yl)acetamide



To a solution of N-(5-bromopyridin-3-yl)acetamide (Step 2-1) (200 mg, 0.91 mmol), 1-Boc-pyrrole-2-boronic acid pinacol ester (303 mg, 1.00 mmol), THF (6 mL) and 2.4 M aq. Cs_2CO_3 (1.14 mL, 2.73 mmol)

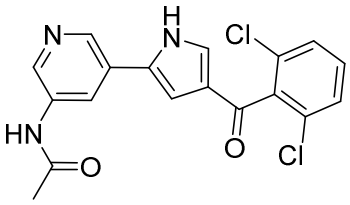
flushed with argon was added 1,1' bis(di-tert-butylphosphino)ferrocene palladium dichloride (36 mg, 0.055 mmol). The vial was sealed and the reaction mixture was stirred at 50°C for 13 h. The reaction mixture was diluted with EtOAc and extracted with brine. The organic layer was dried (Na_2SO_4), filtered and concentrated to dryness. The resulting brown oil (HPLC : Rt = 2.58 min, MS : $[M+H]^+$ 302.1, $[M-H]^-$ 300.1), without further purification, was treated in TFA (3.5 ml) at rt for 1 h. The reaction mixture was taken in H_2O and extracted with CH_2Cl_2. The aq. layer was neutralized (10 M aq. NaOH), basified ($NaHCO_3$) and saturated with NaCl, before being extracted twice with EtOAc. The EtOAc organic layers were combined, washed with brine, dried (Na_2SO_4), filtered and concentrated to dryness to afford 190 mg as a grey solid of the title compound (98% yield).

HPLC : Rt = 1.95 min

MS : $[M+H]^+$ 202.1, $[M-H]^-$ 200.0

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.46 (s, 1H), 10.13 (s, 1H), 8.54 (d, J = 1.9 Hz, 1H), 8.45 (d, J = 2.1 Hz, 1H), 8.21 – 8.16 (m, 1H), 6.94 – 6.88 (m, 1H), 6.54 – 6.49 (m, 1H), 6.19 – 6.12 (m, 1H), 2.09 (s, 3H).

Compound **2** N-(5-(4-(2,6-dichlorobenzoyl)-1H-pyrrol-2-yl)pyridin-3-yl)acetamide



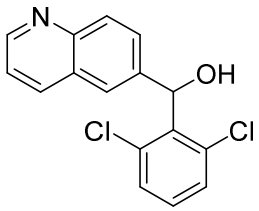
To a solution of 2,6-dichlorobenzoyl chloride (88 µL, 0.61 mmol) in CH_2Cl_2 (5 mL) was added $AlCl_3$ (126 mg, 0.94 mmol) and the suspension was stirred at rt for 1 h. A suspension of N-(5-(1H-pyrrol-2-yl)pyridin-3-yl)acetamide (Step 2-2) (100 mg, 0.47 mmol) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt for 1h30. $AlCl_3$ (126 mg, 0.94 mmol) was added and the mixture was stirred at rt for 24 additional hours. The reaction mixture was quenched by addition of ice, basified (sat. aq. $NaHCO_3$ /H_2O: 2/1) and extracted with EtOAc. The combined organic layers were washed with H_2O, sat. aq. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated to dryness. The residue was dissolved in DMA and purified by prep. HPLC (Gradient : 3% to 55% B in 20 min.). Fractions were collected, basified ($NaHCO_3$) and concentrated. The resulting suspension was cooled in the fridge then filtered. The resulting residue was washed with H_2O and dried under high vaccum to afford 15 mg of a beige solid as the title compound (8% yield).

HPLC : Rt = 2.64 min

MS : $[M+H]^+$ 374.0/376.1, $[M-H]^-$ 372.0/374.0 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.42 (s, 1H), 10.19 (s, 1H), 8.63 (d, J = 1.8 Hz, 1H), 8.54 (d, J = 1.9 Hz, 1H), 8.31 – 8.26 (m, 1H), 7.66 – 7.48 (m, 3H), 7.39 (s, 1H), 6.88 (s, 1H), 2.10 (s, 3H).

Step 3-1 (2,6-dichlorophenyl)(quinolin-6-yl)methanol

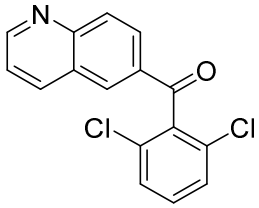


To a solution of 6-bromoquinoline (291 mg, 1.37 mmol) in THF (7 mL) at -78°C were added 4-methylmorpholine (142 mg, 1.37 mmol), then 1.6 M N-butyllithium in hexane (857 µl, 1.37 mmol) dropwise over 30 min. The reaction mixture was stirred at -78°C for 30 min. Magnesium bromide diethyl etherate (396 mg, 1.49 mmol) was added and the reaction mixture was stirred at -78°C for 90 min. A suspension of 2,6-dichlorobenzaldehyde (200 mg, 1.14 mmol) in THF (2 mL) was added dropwise over 45 min and the reaction mixture was stirred at -78°C for 3.5 h. Sat. aq. NH_4Cl (10 mL) was added and the reaction mixture was allowed to warm to 0°C, then extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2/EtOH, 98:2) to provide after evaporation and drying under vacuum 89 mg of the title compound (24% yield).

LC-MS : Rt = 0.85 min, $[M+H]^+$ 304.0/306.0 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (dd, J = 4.1, 1.5 Hz, 1H), 8.36 (d, J = 7.7 Hz, 1H), 7.95 (d, J = 8.8 Hz, 1H), 7.91 (s, 1H), 7.58 (dd, J = 8.8, 1.8 Hz, 1H), 7.55 – 7.44 (m, 3H), 7.42 – 7.33 (m, 1H), 6.69 (d, J = 4.8 Hz, 1H), 6.47 (d, J = 4.8 Hz, 1H).

Compound **3** (2,6-dichlorophenyl)(quinolin-6-yl)methanone

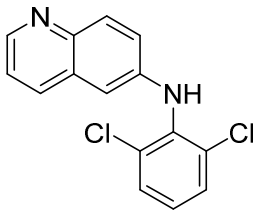


To a solution of (2,6-dichlorophenyl)(quinolin-6-yl)methanol (Step 3-1) (71 mg, 0.23 mmol) in $CHCl_3$ (4 mL) at rt was added manganese dioxide (204 mg, 2.34 mmol) and the mixture was stirred at rt for 20 h. Manganese dioxide (204 mg, 2.34 mmol) was added and the mixture was stirred at rt 74 additional hours. The reaction mixture was filtered over Celite. The resulting solid was washed with a warm solution of CH_2Cl_2/EtOAc/MeOH (4:1:1). The resulting solution was concentrated under reduced pressure to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2/EtOH, 98:2). The residue was triturated in CH_2Cl_2/pentane 1:7, filtered and dried under vacuum to provide 27 mg as the title compound in 36% yield.

LC-MS : Rt = 1.07 min, $[M+H]^+$ 301.9/303.9 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.19 – 8.89 (m, 1H), 8.61 (d, J = 8.2 Hz, 1H), 8.40 (s, 1H), 8.23 – 8.08 (m, 2H), 7.82 – 7.41 (m, 4H)

Step 4-1 N-(2,6-dichlorophenyl)quinolin-6-amine



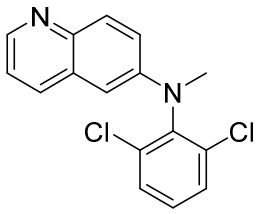
To a solution of 6-bromoquinoline (300 mg, 1.43 mmol), 2,6-dichloroaniline (236 mg, 1.43 mmol) and Cs_2CO_3 (698 mg, 2.14 mmol) in dioxane (7 mL) under argon were added tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (37 mg, 0.036 mmol) and 4,5-bis(diphenyl-phosphino)-9,9-dimethylxanthene (62 mg, 0.11 mmol). The reaction mixture was sealed and stirred at 100°C for 18 h. The reaction mixture was taken in water and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to dryness. The residue was purified by silica gel column chromatography (EtOAc/heptane, 0:100 to 50:50 in 25 min) to provide after evaporation and drying under vacuum 336 mg as a yellow solid of the title compound (81% yield).

HPLC : Rt = 2.70 min

MS : $[M+H]^+$ 289.0/291.0 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 – 8.53 (m, 1H), 8.33 (s, 1H), 7.99 (d, J = 8.5 Hz, 1H), 7.82 (d, J = 9.1 Hz, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.37 – 7.26 (m, 3H), 6.52 (d, J = 2.5 Hz, 1H).

Compound **4** N-(2,6-dichlorophenyl)-N-methylquinolin-6-amine



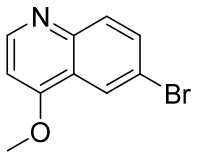
To a solution of N-(2,6-dichlorophenyl)quinolin-6-amine (Step 4-1) (40 mg, 0.14 mmol) in DMF (1.3 mL) under argon was added 55% NaH in oil (8 mg, 0.18 mmol) and the mixture was stirred at rt for 30 min. Iodomethane (10 µL, 0.16 mmol) was added and the mixture was stirred at rt for 15 h. The reaction mixture was quenched with one drop of water and diluted in DMF to be purified by prep. HPLC (Gradient : 15% to 95% B in 20 min). Fractions were collected, basified ($NaHCO_3$) and concentrated. The residue was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under vacuum to afford 31 mg as an oil of the title compound (75% yield).

HPLC : Rt = 2.95 min

MS : $[M+H]^+$ 303.0/305.0 (2 Cl pattern)

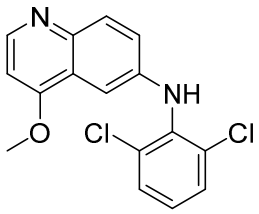
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (dd, J = 4.2, 1.6 Hz, 1H), 8.11 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 9.2 Hz, 1H), 7.67 (d, J = 8.1 Hz, 2H), 7.51 – 7.42 (m, 1H), 7.36 (dd, J = 8.3, 4.2 Hz, 1H), 6.93 (s, 1H), 6.80 (d, J = 8.4 Hz, 1H), 3.29 (s, 3H)

Step 5-1 6-bromo-4-methoxyquinoline



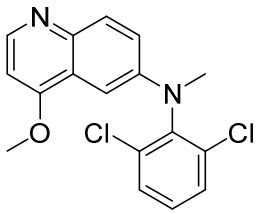
To a solution obtained by reacting sodium (28 mg, 1.22 mmol) in MeOH (5 mL) under argon was added 6-bromo-4-chloroquinoline (270 mg, 1.10 mmol) and the mixture was heated with microwave irradiation at 100°C for 2.5 h. The reaction mixture was quenched with H_2O and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated to dryness to afford 261 mg as an off-white solid of the title compound (96% yield).
HPLC : Rt = 2.31 min
MS : $[M+H]^+$ 238.0/240.0 (Br pattern)
^1H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 5.3 Hz, 1H), 8.27 (d, J = 2.1 Hz, 1H), 7.96 – 7.82 (m, 2H), 7.10 (d, J = 5.2 Hz, 1H), 4.06 (s, 3H)

Step 5-2 N-(2,6-dichlorophenyl)-4-methoxyquinolin-6-amine



To a solution of 6-bromo-4-methoxyquinoline (Step 5-1) (252 mg, 1.03 mmol), 2,6-dichloroaniline (170 mg, 1.03 mmol) and Cs_2CO_3 (502 mg, 1.54 mmol) in dioxane (5 mL) under argon were added tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (27 mg, 0.026 mmol) and 4,5-bis(diphenyl-phosphino)-9,9-dimethylxanthene (45 mg, 0.077 mmol). The mixture was flushed with argon, sealed and stirred at 100°C for 18.5 h. The reaction mixture was quenched with H_2O and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered and concentrated to dryness. The residue was purified by silica gel column chromatography (EtOAc/heptane, 0:100 to 100:0 in 25 min) to provide after evaporation and drying under vacuum 232 mg as a yellow solid of the title compound (69% yield).
HPLC : Rt = 2.87 min
MS : $[M+H]^+$ 319.0/321.0 (2 Cl pattern)
^1H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, J = 5.1 Hz, 1H), 8.29 (s, 1H), 7.76 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 8.1 Hz, 2H), 7.32 (t, J = 8.1 Hz, 1H), 7.25 (dd, J = 9.1, 2.6 Hz, 1H), 6.84 (d, J = 5.1 Hz, 1H), 6.77 (d, J = 2.6 Hz, 1H), 3.89 (s, 3H)

Compound **5** N-(2,6-dichlorophenyl)-4-methoxy-N-methylquinolin-6-amine



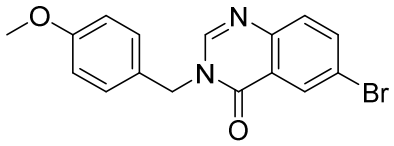
To a solution of N-(2,6-dichlorophenyl)-4-methoxyquinolin-6-amine (Step 5-2) (35 mg, 0.11 mmol) in DMF (1 mL) under argon was added 55% NaH in oil (7 mg, 0.15 mmol) and the mixture was stirred at rt for 30 min. Iodomethane (9 µL, 0.14 mmol) was added and the mixture was stirred at rt for 2 h. The mixture was quenched with few drops of H_2O, diluted with DMF and purified by prep. HPLC (Gradient : 5% to 95% B in 20 min). Fractions were collected, basified ($NaHCO_3$) and concentrated. The residue was extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under high vacuum to afford 26 mg as a yellow solid of the title compound (73% yield).

HPLC : Rt = 3.13 min

MS : $[M+H]^+$ 333.0/335.0 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.47 (d, J = 5.1 Hz, 1H), 7.75 (d, J = 9.2 Hz, 1H), 7.68 (d, J = 8.3 Hz, 2H), 7.52 – 7.43 (m, 1H), 7.00 (s, 1H), 6.90 (d, J = 5.1 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 3.98 (s, 3H), 3.29 (s, 3H).

Step 6-1 6-bromo-3-(4-methoxybenzyl)quinazolin-4(3H)-one



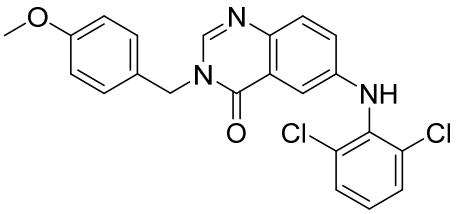
To a solution of 6-bromoquinazolin-4-ol (2.08 g, 9.15 mmol) in DMF (35 mL) under argon was added 55% NaH in oil (439 mg, 10.1 mmol) and the mixture was stirred at rt for 30 min. 4-Methoxybenzyl chloride (1.52 mL, 11.0 mmol) was added and the mixture was stirred at rt for 145 min. The reaction was quenched with H_2O and the precipitate was filtered, washed with water and dried under vacuum to give 3.05 g as an off-white solid of the title compound (95% yield).

HPLC : Rt = 3.33 min

MS : $[M+H]^+$ 345.1/347.1 (Br pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.23 (d, J = 2.3 Hz, 1H), 7.97 (dd, J = 8.6, 2.3 Hz, 1H), 7.64 (d, J = 8.7 Hz, 1H), 7.34 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.12 (s, 2H), 3.71 (s, 3H).

Step 6-2 6-((2,6-dichlorophenyl)amino)-3-(4-methoxybenzyl)quinazolin-4(3H)-one



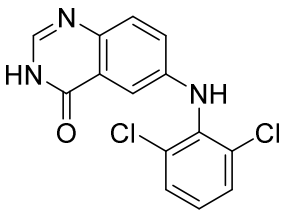
To a solution of 6-bromo-3-(4-methoxybenzyl)quinazolin-4(3H)-one (Step 6-1) (3.05 g, 8.75 mmol), 2,6-dichloroaniline (1.45 g, 8.75 mmol), Cs_2CO_3 (4.28g, 13.1 mmol) in dioxane (40 mL) under argon were added tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (226 mg, 0.219 mmol) and 4,5-bis(diphenyl-phosphino)-9,9-dimethylxanthene (380 mg, 0.656 mmol). The mixture was flushed with argon and stirred at 100°C for 19 h. The mixture was diluted with H_2O and extracted twice with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to dryness. The residue was purified by silica gel column chromatography (EtOAc/heptane, 0:100 to 50:50 in 30 min) to provide after evaporation and drying under vacuum 2.64 g as an off-white foam of the title compound (69% yield).

HPLC : Rt = 3.39 min

MS : $[M+H]^+$ 426.2/428.2 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.30 (s, 1H), 7.59 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.8 Hz, 1H), 7.33 (t, 1H), 7.30 – 7.25 (m, 2H), 7.19 (dd, J = 8.9, 2.8 Hz, 1H), 6.93 (d, J = 2.7 Hz, 1H), 6.88 – 6.83 (m, 2H), 5.03 (s, 2H), 3.69 (s, 3H).

Step 6-3 6-((2,6-dichlorophenyl)amino)quinazolin-4(3H)-one



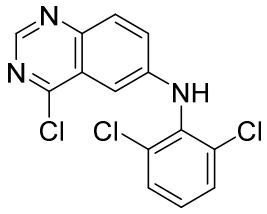
A solution of 6-((2,6-dichlorophenyl)amino)-3-(4-methoxybenzyl)quinazolin-4(3H)-one (Step 6-2) (2.63 mg, 6.05 mmol) in TFA/H_2O 20/1 (18.9 mL) was stirred at 90°C for 2 days. The mixture was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed with sat. aq. $NaHCO_3$. The organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 100:0 to 94:6 in 30 min) to provide after evaporation and drying under vacuum 1.64 g as a green solid of the title compound (88% yield).

HPLC : Rt = 2.66 min

MS : $[M+H]^+$ 306.1/308.1, $[M-H]^-$ 304.0/306.1 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H), 8.39 (s, 1H), 7.85 (s, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.53 (d, J = 8.7 Hz, 1H), 7.35 (t, 1H), 7.21 (dd, J = 8.7, 2.8 Hz, 1H), 6.93 (d, J = 2.8 Hz, 1H).

Step 6-4 4-chloro-N-(2,6-dichlorophenyl)quinazolin-6-amine



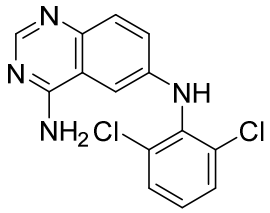
A solution of 6-((2,6-dichlorophenyl)amino)quinazolin-4(3H)-one (Step 6.3) (1.63 g, 5.27 mmol)and 1 drop of DMF in $POCl_3$ (25 mL, 268 mmol) under argon was stirred at 100°C for 3 h. The mixture was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and washed twice with sat. aq. $NaHCO_3$ and with brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under vacuum to afford 1.73 g as a green solid of the title compound (99% yield).

HPLC : Rt = 3.45 min

LC-MS : Rt = 1.15 min, $[M+H]^+$ 324.1/326.1 $[M-H]^-$ 322.0/324.0 (3 Cl pattern)

^1H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H), 8.78 (s, 1H), 7.93 (d, J = 9.1 Hz, 1H), 7.65 (d, J = 8.1 Hz, 2H), 7.55 (dd, J = 9.1, 2.6 Hz, 1H), 7.39 (t, 1H), 6.81 (d, J = 2.6 Hz, 1H).

Step 6-5 N6-(2,6-dichlorophenyl)quinazoline-4,6-diamine



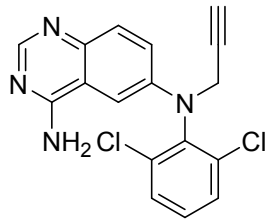
A solution of 4-chloro-N-(2,6-dichlorophenyl)quinazolin-6-amine (Step 6-4) (600 mg, 1.81 mmol) and 0.5 M ammonia in dioxane (28 mL, 14.0 mmol) under argon was splitted in two and heated with microwave irradiation at 170°C for 16 h. The reaction mixtures were diluted with EtOAc and washed with H_2O and brine. The combined aq. layers were extracted with EtOAc and the combined organic layers were dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under vacuum to afford 629 mg as a brown solid of the crude title compound (97% yield).

HPLC : Rt = 2.49 min

MS : $[M+H]^+$ 305.1/307.1, $[M-H]^-$ 303.1/305.1 (2 Cl pattern)

^1H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 8.12 (s, 1H), 7.56 (d, J = 8.1 Hz, 2H), 7.52 (d, J = 8.9 Hz, 1H), 7.37 – 7.23 (m, 4H), 6.86 (d, J = 2.5 Hz, 1H).

Compound **6** N6-(2,6-dichlorophenyl)-N6-(prop-2-yn-1-yl)quinazoline-4,6-diamine



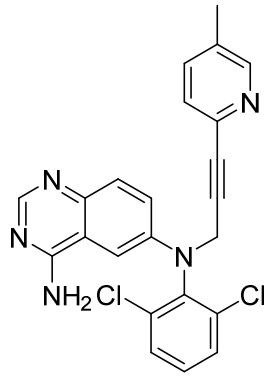
To a solution of N6-(2,6-dichlorophenyl)quinazoline-4,6-diamine (Step 6-5) (200 mg, 0.557 mmol) in DMF (3 mL) under argon was added 55% NaH in oil (29.2 mg, 0.668 mmol) and the mixture was stirred at rt for 20 min. 80% Bromopropargyl in toluene (0.078 mL, 0.724 mmol) was added and the mixture was stirred at rt for 105 min. The reaction was quenched with 3 drops of H_2O, filtered and purified by prep. HPLC (Gradient : 3% to 50% B in 20 min; 2 runs). Fractions were collected, basified ($NaHCO_3$) and concentrated. The product crystallized in H_2O. The solid was filtered, washed with water and dried under vacuum to afford 123 mg as a white solid of the title compound (64% yield).

HPLC : Rt = 2.76 min

MS : $[M+H]^+$ 343.1/345.1 (2 Cl pattern)

^1H NMR (400 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.66 (d, J = 8.1 Hz, 2H), 7.58 – 7.41 (m, 4H), 7.34 (d, J = 2.7 Hz, 1H), 6.76 (dd, J = 9.1, 2.6 Hz, 1H), 4.54 (d, J = 2.5 Hz, 2H), 3.21 (t, J = 2.5 Hz, 1H).

Compound **7** N6-(2,6-dichlorophenyl)-N6-(3-(5-methylpyridin-2-yl)prop-2-yn-1-yl)quinazoline-4,6-diamine



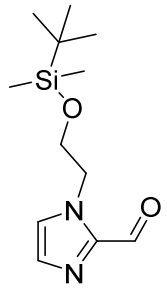
A solution of N6-(2,6-dichlorophenyl)-N6-(prop-2-yn-1-yl)quinazoline-4,6-diamine (compound **6**) (30 mg, 0.087 mmol), 2-bromo-5-methylpyridine (16 mg, 0.091 mmol), CuI (1.7 mg, 0.0089 mmol), $Pd(PPh_3)_2Cl_2$ (1.9 mg, 0.0027 mmol) in triethylamine (0.7 mL) and NMP (0.08 ml) under argon was stirred at rt for 17 h. The solvent was blown-off with N_2 and the residue was taken in DMF, filtered and purifed by prep. HPLC (Gradient : 3% to 70% B in 20 min). Fractions were collected, basified ($NaHCO_3$), concentrated and extracted twice with CH_2Cl_2. The organic layers were washed with brine, dried (Na_2SO_4), filtered, evaporated and dried under vacuum to afford 24 mg as an off-white foam of the title compound (63% yield).

HPLC : Rt = 2.72 min

LC-MS : Rt = 0.85 min, $[M+H]^+$ 434.2/436.2 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, J = 2.0 Hz, 1H), 8.23 (s, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.63 – 7.39 (m, 6H), 7.20 (d, J = 7.9 Hz, 1H), 6.81 (dd, J = 9.2, 2.2 Hz, 1H), 4.81 (s, 2H), 2.28 (s, 3H).

Step 8-1 1-(2-((tert-butyldimethylsilyl)oxy)-1H-imidazole-2-carbaldehyde



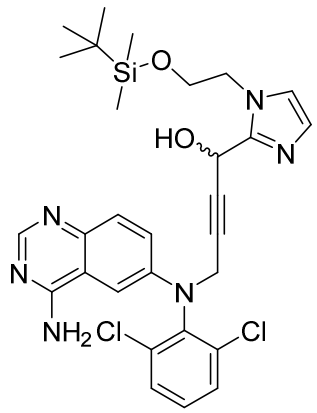
A solution of imidazole-2-carboxaldehyde (500 mg, 5.05 mmol), potassium carbonate (2.1 g, 15.2 mmol) and (2-bromoethoxy)-tert-butyldimethylsilane (1.3 mL, 6.06 mmol) in DMF (7 mL) was sealed and stirred at 55°C for 21 h. The reaction mixture was quenched with H_2O and extracted twice with EtOAc. The combined organic layers were washed with H_2O, twice with brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under vacuum. The residue was purified by silica gel column chromatography (heptane/EtOAc, 100:0 to 30:70 in 15 min) to provide after evaporation and drying under vacuum 800 mg as a yellow oil of the title compound (62% yield).

HPLC : Rt = 2.71 min

MS : $[M+H]^+$ 255.2

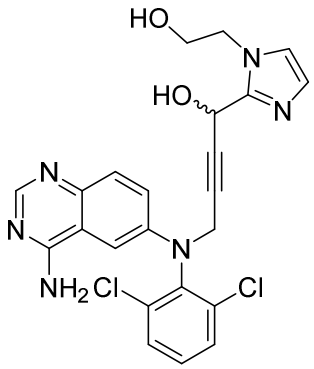
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (d, J = 0.8 Hz, 1H), 7.62 – 7.58 (m, 1H), 7.28 (d, J = 1.0 Hz, 1H), 4.48 (t, J = 5.3 Hz, 2H), 3.84 (t, J = 5.1 Hz, 2H), 0.78 (s, 9H), -0.12 (s, 6H).

Step 8-2 N6-(2,6-dichlorophenyl)-N6-(3-(5-methylpyridin-2-yl)prop-2-yn-1-yl)quinazoline-4,6-diamine



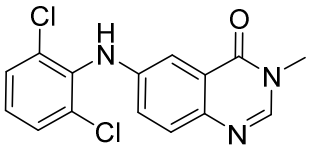
To a solution of N6-(2,6-dichlorophenyl)-N6-(prop-2-yn-1-yl)quinazoline-4,6-diamine (compound **6**) (30 mg, 0.087 mmol) and 0.5 ml anhydrous THF under argon was added a 1 M ethyl magnesium bromide solution in THF (0.35 ml, 0.35 mmol) and the reaction mixture was stirred at rt for 1 h. The reaction mixture was added onto 1-(2-((tert-butyldimethylsilyl)oxy)-1H-imidazole-2-carbaldehyde (step 8-1) (156 mg, 0.612 mmol) and stirred at rt for 20 min. The solvent was blown-off with N_2 and the residue was taken in DMF, filtered and separated by prep. HPLC (Gradient : 5% to 95% B in 20 min). Fractions containing product were collected, basified ($NaHCO_3$), concentrated and extracted twice with CH_2Cl_2. The organic layers were washed with brine, dried (Na_2SO_4), filtered, evaporated and dried under vacuum to afford 61 mg as a yellow sticky oil of the impure title compound (65% yield).
HPLC : Rt = 3.07 min
LC-MS : Rt = 0.92 min, $[M+H]^+$ 597.3/599.3, $[M-H]^-$ 595.2/597.2 (2 Cl pattern)

Compound **8** 4-((4-aminoquinazolin-6-yl)(2,6-dichlorophenyl)amino)-1-(1-(2-hydroxyethyl)-1H-imidazol-2-yl)but-2-yn-1-ol



A solution of N6-(2,6-dichlorophenyl)-N6-(3-(5-methylpyridin-2-yl)prop-2-yn-1-yl)quinazoline-4,6-diamine (step 8-2) (60 mg, 0.056 mmol) in THF (1 ml) was treated with HF-pyridine (0.6 ml, 6.9 mmol) at rt for 1.5 h. The reaction mixture was poured dropwise onto sat. aq. $NaHCO_3$ and then extracted twice with EtOAc. The combined organic layers were washed with sat. aq. $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, concentrated under reduced pressure and dried under vacuum. The residue was taken in DMF, filtered and purifed by prep. HPLC (Gradient : 3% to 50% B in 20 min). Fractions were collected, basified ($NaHCO_3$), concentrated and extracted twice with CH_2Cl_2 and twice with EtOAc. The combined organic layers were washed with brine, dried (Na_2SO_4), filtered, evaporated and dried under vacuum to afford 6 mg as an off-white solid of the title compound (22% yield).
HPLC : Rt = 2.22 min
MS : $[M+H]^+$ 483.2/485.2, $[M-H]^-$ 481.1/483.1 (2 Cl pattern)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.53 – 7.46 (m, 2H), 7.46 – 7.39 (m, 2H), 7.33 (d, J = 2.6 Hz, 1H), 7.06 (d, J = 1.2 Hz, 1H), 6.77 – 6.69 (m, 2H), 6.09 (d, J = 5.5 Hz, 1H), 5.47 – 5.41 (m, 1H), 4.90 (t, J = 5.0 Hz, 1H), 4.57 (t, J = 2.2 Hz, 2H), 4.20 – 4.08 (m, 1H), 3.90 – 3.79 (m, 1H), 3.55 (q, J = 5.5 Hz, 2H).

Step 9-1 6-((2,6-dichlorophenyl)amino)-3-methylquinazolin-4(3H)-one



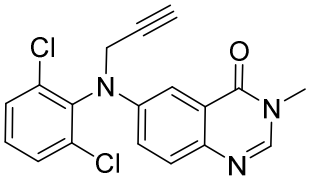
To a solution of 6-bromo-3-methylquinazolin-4(3H)-one (685 mg, 2.81 mmol), 2,6-dichloroaniline (500 mg, 3.09 mmol), Cs_2CO_3 (1.37 g, 4.21 mmol) in anhydrous dioxane (11 mL) were added tris(dibenzylideneacetone)dipalladium(0) chloroform adduct (73 mg, 0.071 mmol) and 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (122 mg, 0.21 mmol). The reaction mixture was flushed with argon, the vial was sealed and the mixture was stirred at 100°C for 17.25 h. The reaction mixture was diluted with EtOAc and washed with sat. aq. $NaHCO_3$. The organic layer was washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to dryness. The residue was taken in hot MeOH, sonicated then cooled in the fridge. The resulting suspension was filtered. The solid was washed with MeOH. The filtrate was concentrated. The residue was taken in hot CH_3CN, sonicated then cooled in the fridge and filtered. The resulting solid was washed with few CH_3CN. Both solids were recombined and dried to afford 663 mg as an off-white solid of the title compound (73% yield).

HPLC : Rt = 2.92 min

MS : $[M+H]^+$ 320.1/322.1 (2 Cl pattern)

^1H NMR (400 MHz, DMSO-d_6) δ 8.42 (s, 1H), 8.14 (s, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.54 (d, J = 8.8 Hz, 1H), 7.35 (t, J = 8.1 Hz, 1H), 7.21 (dd, J = 8.8, 2.8 Hz, 1H), 6.99 (d, J = 2.7 Hz, 1H), 3.44 (s, 3H).

Step 9-2 6-((2,6-dichlorophenyl)(prop-2-yn-1-yl)amino)-3-methylquinazolin-4(3H)-one



To a solution of 6-((2,6-dichlorophenyl)amino)-3-methylquinazolin-4(3H)-one (Step 9-1) (654 mg, 2.02 mmol) in anhydrous DMF (8 mL) was added 60% NaH in oil (97 mg, 4.23 mmol) and the mixture was stirred at rt for 30 min. 80% bromopropargyl in toluene (283 µL, 2.63 mmol) was added and the mixture was stirred at rt for 2 h. The reaction was quenched with H_2O, diluted with sat. aq. $NaHCO_3$ and extracted twice with EtOAc. The organic layers were washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to dryness. The residue was dissolved in CH_2Cl_2 and purified by silica gel column chromatography (CH_2Cl_2/MeOH, 100:0 to 94:6 in 25 min) to provide 671 mg as a beige solid of the title compound (92% yield).

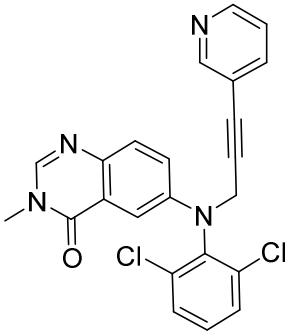
HPLC : Rt = 3.18 min

MS : $[M+H]^+$ 358.2/360.2 (2 Cl pattern)

^1H NMR (400 MHz, DMSO-d_6) δ 8.18 (s, 1H), 7.69 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 9.0 Hz, 1H), 7.50 (dd, J =

8.5, 7.8 Hz, 1H), 7.18 (d, J = 2.7 Hz, 1H), 7.00 (dd, 1H), 4.52 (d, J = 2.4 Hz, 2H), 3.46 (s, 3H), 3.29 (t, J = 2.4 Hz, 1H).

Compound **9** 6-((2,6-dichlorophenyl)(3-(pyridin-3-yl)prop-2-yn-1-yl)amino)-3-methylquinazolin-4(3H)-one



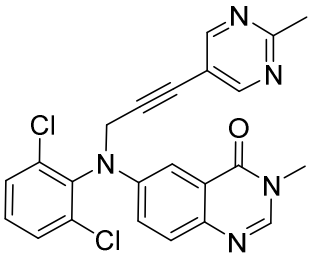
To a solution of 6-((2,6-dichlorophenyl)(prop-2-yn-1-yl)amino)-3-methylquinazolin-4(3H)-one (Step 9-2) (45 mg, 0.13 mmol), 3-iodopyridine (36 mg, 0.18 mmol), CuI (2 mg, 10.5 µmol), $Pd(PhCN)_2Cl_2$ (6 mg, 0.016 mmol) in dioxane (0.3 mL) were added tri-tert-butylphosphonium tetrafluoroborate (9 mg, 0.031 mmol) then diisopropylamine (24 µL, 0.17 mmol) and the mixture was stirred at rt under argon for 17.75 h. The reaction mixture was blown-off with N_2. The residue was dissolved in DMF, filtered and purified by prep. HPLC (Gradient: 5% to 95% B in 20 min). Fractions were collected, basified ($NaHCO_3$) and concentrated. The sticky oily suspension was separated, washed with water and dissolved in CH_2Cl_2. The organic phase was washed with brine and dried (Na_2SO_4), filtered and concentrated to dryness to afford 11 mg as an off-white foam of the title compound (20% yield).

HPLC : Rt = 2.96 min

MS : $[M+H]^+$ 435.2/437.2 (2 Cl pattern)

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (dd, J = 4.9, 1.6 Hz, 1H), 8.48 (d, J = 2.1, 0.7 Hz, 1H), 8.17 (s, 1H), 7.76 – 7.67 (m, 3H), 7.58 (d, J = 9.0 Hz, 1H), 7.51 (dd, J = 8.5, 7.8 Hz, 1H), 7.42 – 7.34 (m, 1H), 7.24 (d, J = 2.8 Hz, 1H), 7.06 (dd, J = 9.0, 2.8 Hz, 1H), 4.81 (s, 2H), 3.45 (s, 3H).

Compound **10** 6-((2,6-dichlorophenyl)(3-(2-methylpyrimidin-5-yl)prop-2-yn-1-yl)amino)-3-methylquinazolin-4(3H)-one



A solution of 6-((2,6-dichlorophenyl)(prop-2-yn-1-yl)amino)-3-methylquinazolin-4(3H)-one (step 9-2) (40 mg, 0.11 mmol), 5-bromo-2-methylpyrimidine (22 mg, 0.12 mmol), CuI (2 mg, 0.012 mmol) and $Pd(PPh_3)_2Cl_2$ (2.4 mg, 3.42 µmol) in Et_3N (0.8 mL) and NMP (100 µL) was stirred under argon at rt for 19.5 h. The reaction mixture was blown-off with N_2. The residue was dissolved in DMF, filtered and purified by prep. HPLC (Gradient: 5% to 95% B in 20 min). Fractions were collected, basified ($NaHCO_3$) and concentrated. The sticky oily suspension was separated , washed with water and dissolved in CH_2Cl_2. The organic phase was washed with brine and dried (Na_2SO_4), filtered and concentrated to dryness to afford 11 mg as an off-white foam of the title compound (22% yield).
HPLC : Rt = 3.30 min
MS : $[M+H]^+$ 450.2/452.1 (2 Cl pattern)
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 2H), 8.19 (s, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.60 (d, J = 9.0 Hz, 1H), 7.52 (t, J = 8.1 Hz, 1H), 7.24 (d, J = 2.5 Hz, 1H), 7.07 (dd, J = 8.9, 2.8 Hz, 1H), 4.85 (s, 2H), 3.46 (s, 3H), 2.62 (s, 3H).

Dot1L scintillation proximity assay (SPA)

For IC_{50} determination, compounds were pre-incubated with Dot1L(2-416) for 30 min before the reaction was started by addition of *S*-[methyl-^{3}H-] adenosyl–L-methionine (^{3}H-SAM) (Perkin Elmer, 18-20 Ci/mmol) and biotinylated nucleosomes (Wuxi Biortus Biosciences Ltd, China). Final concentrations in an assay volume of 10 µL were 0.5 nM Dot1L, 200 nM SAM (corresponds to SAM K_M) and 60 nM nucleosomes. The assay buffer contained 20 mM Tris pH 8, 10 mM $MgCl_2$ and 0.01% Tween-20. Following a reaction time of 90 min (180 min for assay containing 0.05 nM Dot1L), the reaction was stopped/quenched by the addition of 5 µL 4.5 mg/mL Streptavidin SPA Beads (Perkin Elmer, RPNQ0006) diluted in 300 mM MES (pH 6) and 600 µM SAM (A7007, Sigma). Plates were centrifuged and read on a Topcount NXT HTS (Packard) and IC50 data was analysed using XLFit 5.2 (idbs).

Dot1L surface plasmon resonance assay (SPR)

Ligand association and dissociation rates were determined by surface plasmon resonance (SPR) using a BiacoreTM T100 (GE Healthcare). Biotinylated hDot1L (2-416)-Avi was immobilized on a Streptavidin-coated sensorchip (GE Healthcare, BR-1005-31) to a density of 5000-8000 RU. The running buffer contained 25 mM HEPES, 100 mM NaCl, 0.05% Tween-20, 1 mM TCEP, 1% DMSO, pH 8. Experiments were carried out at 20 °C. SAH (Sigma A9384) was used as internal control resulting in K_D 100 nM under these conditions. Curve fitting was performed using the Biacore T100 Evaluation software.

Experimental procedure for the generation of the Dot1L cocrystal structures

Dot1L crystals were grown at 20 °C using the hanging drop vapor diffusion method. Purified Dot1L methyltransferase domain (aa2–aa332) at 8 mg/ml in 20 mM Tris pH 8.0, 200 mM NaCl, 1 mM TCEP, 1 mM EDTA was pre-incubated with inhibitor compounds at final concentrations between 1-2 mM resulting in a DMSO concentration of 4-10%. For crystallization the protein inhibitor complex was mixed with an equal volume of a reservoir solution. Seeding was applied prior to sealing the setups. Seed stocks were made out of previously obtained Dot1L crystals. Three different reservoir conditions have been identified

yielding Dot1L co-crystals. Condition one is 1.0-1.6 M potassium sodium tartrate tetrahydrate, 0.1 M Hepes pH 6.6-7.2. The second condition is 1.3-1.6 M lithium sulfate monohydrate, 0.1 M Hepes or Tris pH 7.0-8.5. Finally, the third reservoir composition is 0.9-1.4 M lithium sulfate monohydrate, 0.1-0.3 M ammonium sulfate, 0.1 M sodium citrate tribasic dihydrate. Prior to flash cooling the crystals in liquid nitrogen, they were rapidly soaked in reservoir solution containing 20-25% ethylene glycol. In some cases inhibitor compounds were added to the cryo buffer as well. During data acquisition, the crystal temperature was kept at 100 K. Diffraction data were collected at the Swiss Light Source (beamline X10SA) using a Pilatus pixel detector with an incident monochromatic X-ray beam. Raw diffraction data were processed and scaled using XDS/XSCALE[1] software. The structure was determined by molecular replacement with PHASER[2] using as search model the coordinates of Dot1L (PDB code 1NW3)[3] or other previously solved in-house structures of Dot1L. The program BUSTER[4] was used for full structure refinement. The refined coordinates of the complex structures have been deposited in the RCSB Protein Data Bank.

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