Inhibition of CDC25B Phosphatase Through Disruption of Protein-Protein Interaction

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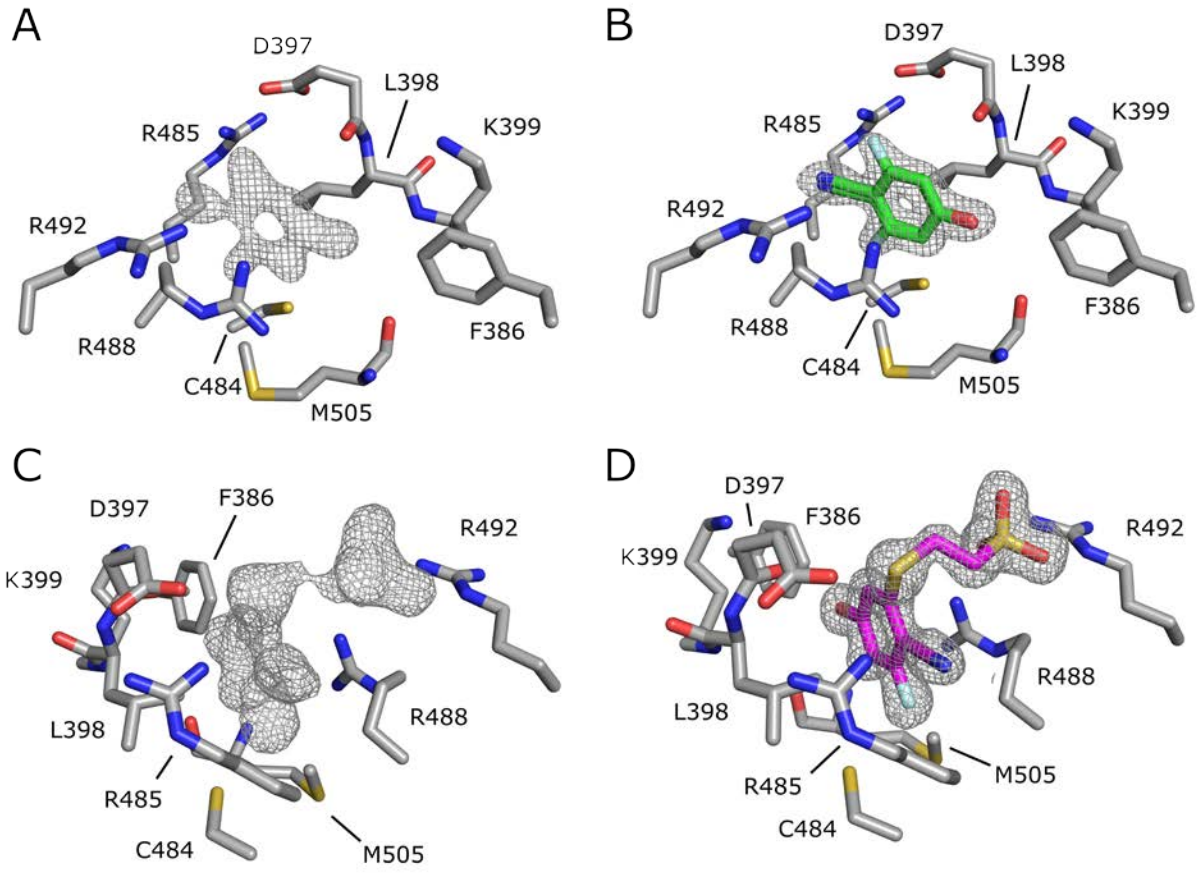
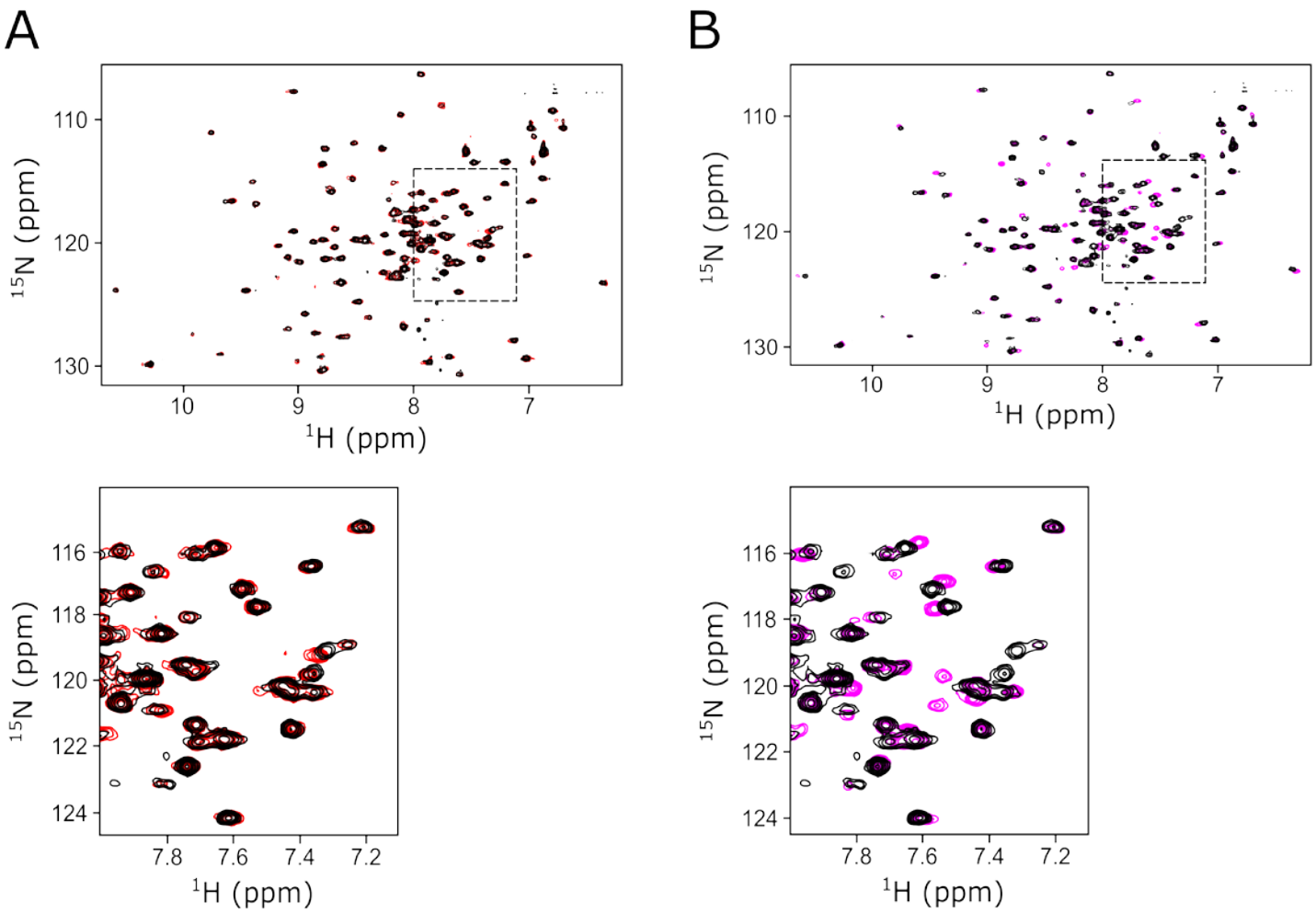
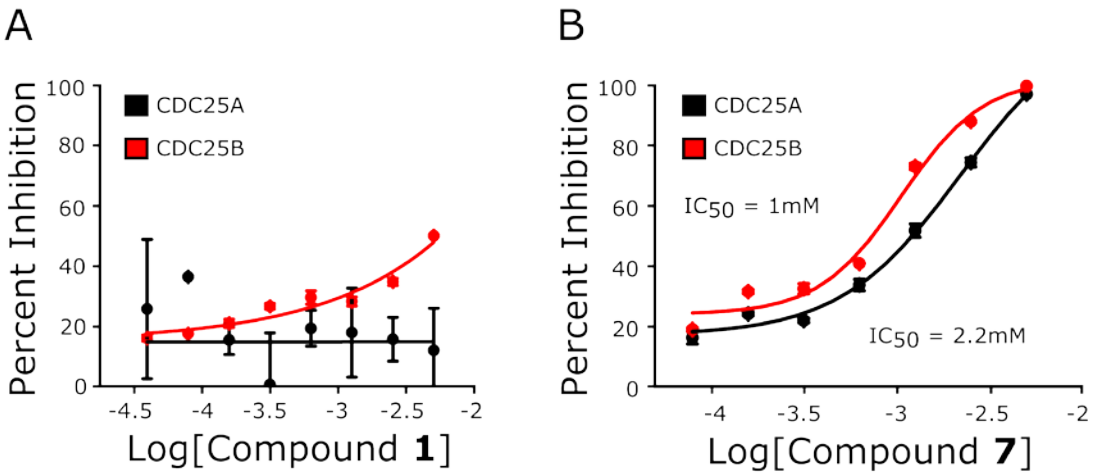
Supplemental Figure 1. Electron density for compounds 1 and 7 before and after refinement. A) Fo-Fc electron density map displayed at 3.0 σ for compound **1** prior to molecular replacement. B) 2Fo-Fc map displayed at 1.5 σ for compound **1** after refinement. C) Fo-Fc map displayed at 1.5 σ for compound **7** prior to molecular replacement. D) 2Fo-Fc map displayed at 1.0 σ for compound **7** after refinement. The occupancy for the ligand is 0.8.



Supplemental Figure 2. Compound 1 and 7 interact with CDC25A. A) Chemical shift perturbations induced by compound **1**. The portion of the spectrum showing the largest perturbations is shown below. B) Chemical shift perturbations induced by compound **7**. The same portion of the spectrum shown for compound **1** is shown below.



Supplemental Figure 3. Inhibition of protein-protein interactions by compound 1 and 7. A) Effect of titration with compound **1** on the interactions of CDC25A and CDC25B with CDK2/CycA. B) Same plot as in A for compound **7**.



Compound Synthesis

All solvents and reagents (including compounds **1**-**6**) were used as obtained from commercial sources unless otherwise indicated. The ^{1}H NMRs were taken on a Bruker Avance III 600MHz or Varian MR400. Chemical shifts were reported in ppm relative to tetramethylsilane or residual solvent signal. The mass measurements were determined on a Micromass LCT time-of-flight mass spectrometer using positive mode and electrospray ionization. The exact mass measurements were determined on Agilent Q-TOF time-of-flight mass spectrometer using positive or negative ion mode and electrospray ionization. Analytical TLC was performed on Merck TLC aluminum plates precoated with F_{254} silica gel 60 (UV, 254 nm, and iodine). Infrared (IR) spectra were recorded on Perkin-Elmer FT-IR Spectrum BX on neat powder and only major characteristic signals are reported.

2,6-difluoro-4-(methoxymethoxy)benzonitrile (6)
In 10 mL of DCM were dissolved 1 g of 2,6-difluoro-4-hydroxybenzonitrile (6.45 mmol, 1 eq) and 1 g of DIPEA (7.74 mmol, 1.2 eq). Then 0.571 g of MOMCl (7.09 mmol, 1.1 eq) were added, and the reaction mixture was allowed to stand at r.t. overnight. Next day the mixture was filtered through 20 g of silica gel, eluting with DCM, and the solvent was evaporated.

Yield 1.28 g (100%). Colorless oil.
^{1}H NMR (499.68 MHz, $CDCl_3$): δ = 3.49 (s, 3H, MeO), 5.21 (s, 2H, CH_2), 6.72 (d, 2H, $^3J_{(H-F)}$ = 9.95 Hz).
^{19}F NMR (470.12 MHz, $CDCl_3$): δ = 103.0 (d, $^3J_{(H-F)}$ = 9.95 Hz).
^{13}C NMR (150.92 MHz, $CDCl_3$): δ = 56.75 (OMe), 85.44 (t, $^2J_{(C-F)}$ = 19.8 Hz, C-1), 94.75 (OCH_2O), 100.8 (dd, $^2J_{(C-F)}$ = 23.1 Hz, $^4J_{(C-F)}$ = 2.2 Hz, CH_{Ar}-3, CH_{Ar}-5), 109.6 (CN), 162.9 ($^3J_{(C-F)}$ = 13.2 Hz, C-4), 164.2 (dd, $^1J_{(C-F)}$ = 259.1 Hz, $^1J_{(C-F)}$ = 6.7 Hz).
IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3098 (w), 2942 (w), 2836 (w), 2240 (w), 2156 (w), 2082 (w), 2008 (w), 1634 (s), 1574 (m), 1496 (m), 1454 (m), 1350 (m), 1308 (w), 1218 (m), 1158 (m), 1140 (s), 1076 (s), 1044 (s), 1004 (s), 920 (s), 842 (m), 716 (m), 688 (w).

2-[(2-Cyano-3-fluoro-5-hydroxyphenyl)thio]ethanesulfonic acid, sodium salt (7)
Into a 25-mL round bottom flask were placed 0.150 g of 2,6-difluoro-4-(methoxymethoxy)benzonitrile 9 (0.753 mmol, 1 eq), 0.124 g of sodium 2-mercaptoethanesulfonate (0.753 mmol, 1 eq), 0.160 g of sodium carbonate (1.51 mmol, 2 eq) and 1.5 mL of DMF. The reaction mixture was stirred at 100 °C under argon for 3 days. Then DMF was evaporated, the residue was dissolved in water and treated with 0.78 mL of conc. HCl. After ½ h the acid was removed in vacuum, the residue was dissolved in methanol and evaporated with 2 g of silica gel. Column chromatography was performed with 7 g of silica gel, eluting first with pure EtOAc, then with EtOAc/EtOH 5:2.

Yield 0.068 g (30%). Off-white solid.
^{1}H NMR (600.13 MHz, DMSO-d_6): δ = 2.72 (t, 2H, ^3J = 8.16 Hz, CH_2-SO_3Na), 3.24 (t, 2H, ^3J = 8.16 Hz, CH_2-S), 6.61 (dd, 1H, $^3J_{(H-F)}$ = 11.33 Hz, ^4J = 1.59 Hz, H-4), 6.71 (d, 1H, ^4J = 1.59 Hz, H-6), 11.37 (br s, 1H, OH).
^{13}C NMR (150.92 MHz, DMSO-d_6): δ = 27.7 (CH_2-S), 50.0 (CH_2-SO_3Na), 89.3 (d, $^2J_{(C-F)}$ = 17,6 Hz, C-2), 100.4 (d, $^2J_{(C-F)}$ = 22.1 Hz, CH-4), 109.7 (CH-6), 112.9 (CN), 144.7 (d, $^3J_{(C-F)}$ = 2.2 Hz, C-1), 163.5 (d, $^3J_{(C-F)}$ = 13.2 Hz, C-5), 164.8 (d, $^1J_{(C-F)}$ = 239.5 Hz, C-3).
^{19}F NMR (282.38 MHz, DMSO-d_6): δ = –105.9 (d, 1F, $^3J_{(H-F)}$ = 11.33 Hz).
HRMS (ESI): Calcd. for $C_9H_7FNO_4S_2$ $[M-Na]^-$: 275.9806, found: 275.9813.

3-[(2-Cyano-3-fluoro-5-hydroxyphenyl)thio]propane-1-sulfonic acid, sodium salt (8)
This compound was prepared similarly to 2-[(2-Cyano-3-fluoro-5-hydroxyphenyl)thio]ethanesulfonic acid, sodium salt (7), starting from 0.150 g of 2,6-difluoro-4-(methoxymethoxy)benzonitrile (0.753 mmol, 1 eq) and 0.134 g of sodium 3-mercaptopropanesulfonate (0.753 mmol, 1 eq).

Yield 0.154 g (65%).
^{1}H NMR (600.13 MHz, DMSO-d_6): δ = 1.86-1.95 (m, 2H, CH_2), 2.56 (t, 2H, ^{3}J = 6.97 Hz, CH_2-SO_3Na), 3.14 (t, 2H, ^{3}J = 6.97 Hz, CH_2-S), 6.44 (d, 1H, $^{3}J_{(H-F)}$ = 9.54 Hz, H-4), 6.60 (s, 1H, H-6), 8.31 (br s, 1H, OH).
^{19}F NMR (470.12 MHz, DMSO-d_6): δ = 106.87 (s, 1F).
^{13}C NMR (150.92 MHz, DMSO-d_6): 24.7 (CH_2), 30.52 (CH_2-S), 49.87 (CH_2-SO_3Na), 87.1 (C-2), 100.5 (CH-4), 110.6 (CH-6), 113.6 (CN), 144.1 (C-1), 162.3 (C-5), 165.0 (d, $^{1}J_{(C-F)}$ = 253.1 Hz, C-3).
HRMS (ESI): Calcd. for $C_{10}H_9FNO_4S_2$ $[M-Na]^-$: 289.9963, found: 289.9979.