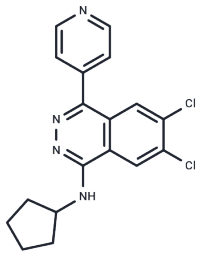
Data Sheet (Cat.No.T4343)

TargetMol
YOUR TARGET MOLECULES

A-196

Chemical Properties



CAS No. : 1982372-88-2

Formula: C18H16Cl2N4

Molecular Weight: 359.25

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	A-196 is a potent and selective inhibitor of SUV420 h1 and SUV420 h2 with IC50 values of 0.025 and 0.144 μM, respectively; more than 100-fold selective over other histone methyltransferases and non-epigenetic targets.
Targets(IC50)	Histone Methyltransferase
In vitro	A-196 potently inhibits SUV420 h1 and SUV420 h2 at both 1 and 10 μM, but has no activity at either concentration against any of the other PKMTs in the panel, including the other H4K20-modifying enzyme, PR-SET7, and those that utilize H3K4, H3K9, H3K27, and H3K79 as substrates. In cells, A-196 induces a global decrease in H4K20me2 and H4K20me3 and a concomitant increase in H4K20me1, but has no effect on any of the other histone modifications. A-196 inhibits 53BP1 foci formation upon ionizing radiation and reduces NHEJ-mediated DNA-break repair but does not affect the homology-directed repair.
Kinase Assay	Briefly, Hsp90 from porcine spleen extract is isolated by affinity capture on a purine-affinity media. The Hsp90 loaded media is then challenged with test compound (PF-04929113) at a given concentration, ranging from 0.8 to 500 μM, and the amount of Hsp90 liberated at each concentration is determined. The resulting IC50 values are corrected for the ATP ligand concentration and presented as apparent Kd values.
Cell Research	U2OS cells are seeded on 6-well plates with 3 μM A-196 or DMSO as a control, and incubated for 48 h. The cells are washed once in 1 X PBS and then lysis buffer (20 mM Tris-HCl pH 7.5, 0.5% Triton X-100, 150 mM NaCl, 1 mM EDTA, 10 mM MgCl2, PMSF, protease inhibitors, benzonase) is added to half the cells to create whole cell extract (WCE). The remaining cells are subjected to sequential cellular fractionation. First the cell pellet is resuspended in hypotonic buffer A (10 mM HEPES pH 7.5, 10 mM KCl, 1.5 mM MgCl2, 0.3 M sucrose, 1 mM DTT and protease inhibitors) and then 0.1% triton X-100 is added. The cells are incubated for 15 min on ice and pelleted by centrifugation at 1,500 g. The supernatant is clarified by centrifugation at max speed and saved as the cytoplasmic fraction. The pellet is resuspended in buffer B (3 mM EDTA, 3 mM EGTA, 1 mM DTT and protease inhibitors) and incubated on ice for 40 min and then centrifuged at 1,500 g for 5 min. The supernatant is clarified and saved as the nucleoplasmic fraction. The pellet is resuspended in lysis buffer and incubated for 5 min at room temperature before being resuspended in 4× loading dye. The final lysate contains the solubilized chromatin fraction.

Solubility Information

Solubility	DMSO: 7.2 mg/mL (20 mM), (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.7836 mL	13.9179 mL	27.8358 mL
5 mM	0.5567 mL	2.7836 mL	5.5672 mL
10 mM	0.2784 mL	1.3918 mL	2.7836 mL
50 mM	0.0557 mL	0.2784 mL	0.5567 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

Bromberg KD,etal.The SUV4-20 inhibitor A-196 verifies a role for epigenetics in genomic integrity.Nat Chem Biol. 2017 Mar;13(3):317-324.