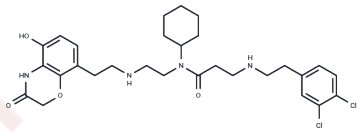
Data Sheet (Cat.No.TQ0100)

TargetMol

AZ505

Chemical Properties



CAS No. : 1035227-43-0

Formula: C29H38Cl2N4O4

Molecular Weight: 577.54

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	AZ505 is an effective and specific SMYD2 inhibitor (IC50: 0.12 μM).
Targets(IC50)	Histone Methyltransferase
In vitro	The IC50 of AZ505 for SMYD2 is 0.12 μM, which is >600-fold greater than the IC50s of AZ505 for other histone methyltransferases, such as SMYD3 (IC50>83.3 μM), DOT1L (IC50>83.3 μM) and EZH2 (IC50>83.3 μM) [1]. AZ505 fails to inhibit the enzymatic activities of a panel of protein lysine methyltransferases. AZ505 is nominated for an ITC binding study with Kd of 0.5 μM. In contrast, the calculated Kd for the p53 substrate peptide is 3.7 μM [2].
Kinase Assay	SMYD2 is expressed in insect cells and purified. Methylation (12 μL) reactions are carried out in TDT buffer (50 mM Tris-HCl [pH 9.0], 2 mM DTT, and 0.01% Tween 20) at room temperature using 1.25 nM SMYD2 protein, 200 nM SAM, and 100 nM biotinylated p53 peptide substrate (Biotin-aminohexanoyl-GSRAHSSHLKSKKGQSTSRH) in low volume 384-well plates. Following a 75 min incubation period, reactions are quenched by the addition of 5 μL of detection solution (20 mM HEPES [pH 7.4], 1.7 mg/mL BSA, 340 mM NaCl, 680 μM SAH, 0.04 mg/mL Streptavidin-coated AlphaScreen donor, and Protein A-coated acceptor beads), and 1 nM of a custom p53K370me1 polyclonal antibody. Reaction plates are incubated overnight in the dark at room temperature and read using an Envision 2101 Multi-label Reader. Compounds showing >50% inhibition of SMYD2 are nominated for concentration dose-response determination and are also subjected to an artifact assay. Seven compound concentrations are selected beginning at 30 μM with six half-log dilution steps. The artifact assay conditions are identical to those in the SMYD2 enzymatic activity assay, except for the absence of SMYD2 protein and the presence of 1 nM methylated p53 peptide. IC50 values are calculated from dose-response data using in-house software [2].

Solubility Information

Solubility	DMSO: 145 mg/mL (251.06 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 10 mg/mL (17.31 mM),Solution. 10% DMSO+90% Saline: < 10 mg/mL (17.31 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.7315 mL	8.6574 mL	17.3148 mL
5 mM	0.3463 mL	1.7315 mL	3.463 mL
10 mM	0.1731 mL	0.8657 mL	1.7315 mL
50 mM	0.0346 mL	0.1731 mL	0.3463 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Komatsu S, et al. Overexpression of SMYD2 contributes to malignant outcome in gastric cancer. Br J Cancer. 2015 Jan 20;112(2):357-64.

Ferguson AD, et al. Structural basis of substrate methylation and inhibition of SMYD2. Structure. 2011 Sep 7;19(9):1262-73.