上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：AZ505 (ditrifluoroacetate)
产品别名：AZ505 ditrifluoroacetate

生物活性：

Description	AZ505 ditrifluoroacetate is a potent and selective SMYD2 inhibitor with IC50 of 0.12 μM.
IC_{50} & Target	IC50: 0.12 μM (SMYD2)[1]
In Vitro	AZ505 ditrifluoroacetate is highly selective and shows an activity at submicromolar concentrations in vitro. The IC50 of AZ505 ditrifluoroacetate for SMYD2 is 0.12 μM, which is >600-fold greater than the IC50s of AZ505 ditrifluoroacetate for other histone methyltransferases, such as SMYD3 (IC50>83.3 μM), DOT1L (IC50>83.3 μM) and AZ505 ditrifluoroacetate (IC50>83.3 μM)[1]. AZ505 ditrifluoroacetate is a potent and selective SMYD2 inhibitor with an IC50 of 0.12 μM. The human SMYD (SET and MYND domain-containing protein) family of protein lysine methyltransferases contains five members (SMYD1-5). Moreover, AZ505 ditrifluoroacetate fails to inhibit the enzymatic activities of a panel of protein lysine methyltransferases. AZ505 ditrifluoroacetate is nominated for ITC binding study with Kd of 0.5 μM. In contrast, the calculated Kd for the p53 substrate peptide is 3.7 μM. AZ505 ditrifluoroacetate binding to SMYD2 is driven primarily by entropy, which often suggests that binding is mediated by hydrophobic interactions with few specific hydrogen bonds[2].

Solvent&Solubility

In Vitro:

DMSO : ≥ 125 mg/mL (155.17 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.2413 mL	6.2066 mL	12.4133 mL
	5 mM	0.2483 mL	1.2413 mL	2.4827 mL
	10 mM	0.1241 mL	0.6207 mL	1.2413 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.08 mg/mL (2.58 mM); Clear solution

此方案可获得 ≥ 2.08 mg/mL (2.58 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 20.8 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

	2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.08 mg/mL (2.58 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (2.58 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.08 mg/mL (2.58 mM); Clear solution 此方案可获得 ≥ 2.08 mg/mL (2.58 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 20.8 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Komatsu S, et al. Overexpression of SMYD2 contributes to malignant outcome in gastric cancer. Br J Cancer. 2015 Jan 20;112(2):357-64. [2]. Ferguson AD, et al. Structural basis of substrate methylation and inhibition of SMYD2. Structure. 2011 Sep 7;19(9):1262-73.

实验参考：

Kinase Assay	SMYD2 is expressed in insect cells and purified. AlphaScreen technology is used to screen our chemical library for small molecule inhibitors of SMYD2. 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].
References	[1]. Komatsu S, et al. Overexpression of SMYD2 contributes to malignant outcome in gastric cancer. Br J Cancer. 2015 Jan 20;112(2):357-64. [2]. Ferguson AD, et al. Structural basis of substrate methylation and inhibition of SMYD2. Structure. 2011 Sep 7;19(9):1262-73.