产品名称：**JNK-IN-8**
产品别名：**JNK-IN-8**

生物活性：

Description

JNK-IN-8 is a potent **JNK** inhibitor with **IC_{50}**s of 4.7 nM, 18.7 nM, and 1 nM for **JNK1**, **JNK2**, and **JNK3**, respectively.

IC_{50} & Target

JNK3	JNK1	JNK2
1 nM (IC_{50})	4.7 nM (IC_{50})	18.7 nM (IC_{50})

In Vitro

JNK-IN-8 inhibits phosphorylation of c-Jun, a direct substrate of JNK kinase. JNK-IN-8 inhibits c-Jun phosphorylation in HeLa and A375 cells with EC50 of 486 nM and 338 nM, respectively. JNK-IN-8 also exhibits exceptional selectivity based upon KinomeScan and enzymatic profiling. Cumulatively these combined profiling technologies demonstrate that both JNK-IN-8 and JNK-IN-12 are remarkably selective covalent JNK inhibitors and are appropriate for interrogating JNK-dependent biological phenomena[1]. JNK-IN-8, a selective pan-JNK inhibitor, is discovered to inhibit JNK kinase by broad-base kinase selectivity profiling of a library of acrylamide kinase inhibitors based on the structure of imatinib using the KinomeSca approach. JNK-IN-8 possess distinct regio-chemistry of the 1,4-dianiline and 1,3-aminobenzoic acid substructures relative to imatinib and uses an N,N-dimethyl butenoic actemide warhead to covalently target Cys154. JNK-IN-8 adopts an L-shaped type I binding conformation to access Cys 154 located towards the lip of the ATP-binding site[2].

Solvent&Solubility

In Vitro:

DMSO : ≥ 35 mg/mL (68.95 mM)

H2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.9701 mL	9.8505 mL	19.7009 mL
5 mM	0.3940 mL	1.9701 mL	3.9402 mL
10 mM	0.1970 mL	0.9850 mL	1.9701 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: ≥ 2.5 mg/mL (4.93 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.93 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 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: 2.5 mg/mL (4.93 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (4.93 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (4.93 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.93 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Zhang T, et al. Discovery of potent and selective covalent inhibitors of JNK. Chem Biol. 2012 Jan 27;19(1):140-54. [2]. Liu Q, et al. Developing irreversible inhibitors of the protein kinase cysteinome. Chem Biol. 2013 Feb 21;20(2):146-59.

实验参考：

Cell Assay	HEK-293 cells stably expressing Interleukin Receptor 1 (HEK293-IL1R) are cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FBS, 2 mM glutamine and 1×antimycotic/antibiotic solution. Cells are serum starved for 18 h before incubation with DMSO or JNK-IN-8, stimulated with 2 μM Anisomycin for 1h and lysates are clarified by centrifugation for 10 min at 16000 g and 4°C[1].
Kinase Assay	A375 cells are pre-treated with 1 μM JNK-IN-8 for the indicated amounts of time. Remove the medium and wash 3 times with PBS. Resuspend the cell pellet with 1 mL Lysis Buffer (1% NP-40, 1% CHAPS, 25 mM Tris, 150 mM NaCl, Phosphatase Inhibitor Cocktail, and Protease Inhibitor Cocktail). Rotate end-to-end for 30 min at 4°C. Lysates are cleared by centrifugation at 14000 rpm for 15 min in the Eppendorf. The cleared lysates gel filtered into Kinase Buffer (0.1% NP-40, 20 mM HEPES, 150 mM NaCl, Phosphatase Inhibitor Cocktail, Protease Inhibitor Cocktail) using Bio-Rad 10DG colums. The total protein concentration of the gel-filtered lysate should be around 5-15 mg/mL. Cell lysate is labeled with the probe from ActivX at 5 μM for 1 hour. Samples are reduced with DTT, and cysteines are blocked with iodoacetamide and gel filtered to remove excess reagents and exchange the buffer. Add 1 volume of 2X Binding Buffer (2% Triton-100, 1% NP-40, 2 mM EDTA, 2X PBS) and 50 μL streptavidin bead slurry and rotate end-to-end for 2 hours, centrifuge at 7000 rpm for 2 min. Wash 3 times with 1X Binding Buffer and 3 times with PBS. Add 30 μL 1X sample buffer to beads, heat samples at 95°C for 10 min. Run samples on an SDS-PAGE gel at 110V. After transferred, the membrane is immunoblotted with JNK antibody[1].
References	[1]. Zhang T, et al. Discovery of potent and selective covalent inhibitors of JNK. Chem Biol. 2012 Jan 27;19(1):140-54. [2]. Liu Q, et al. Developing irreversible inhibitors of the protein kinase cysteinome. Chem Biol. 2013 Feb 21;20(2):146-59.