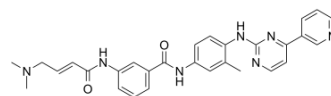


JNK-IN-8

Cat. No.:	HY-13319		
CAS No.:	1410880-22-6		
Molecular Formula:	C ₂₉ H ₂₉ N ₇ O ₂		
Molecular Weight:	507.59		
Target:	JNK		
Pathway:	MAPK/ERK Pathway		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



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 Concentration	Mass		
		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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
Solubility: ≥ 2.5 mg/mL (4.93 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
Solubility: 2.5 mg/mL (4.93 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.5 mg/mL (4.93 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

JNK-IN-8 (JNK Inhibitor XVI) is a potent JNK inhibitor with IC₅₀s of 4.7 nM, 18.7 nM, and 1 nM for JNK1, JNK2, and JNK3, respectively^[1].

IC₅₀ & 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[1]

A375 cells are pre-treated with 1 μ M 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 columns. 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 μ M 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 μ L 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 μ L 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[1]

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 \times antimycotic/antibiotic solution. Cells are serum starved for 18 h before incubation with DMSO or JNK-IN-8, stimulated with 2 μ M Anisomycin for 1h and lysates are clarified by centrifugation for 10 min at 16000 g and 4°C^[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nat Commun. 2020 Jan 3;11(1):71.
- Cell Death Differ. 2020 May;27(5):1569-1587.
- J Exp Clin Cancer Res. 2018 May 4;37(1):99.
- J Biol Chem. 2014 Oct 17;289(42):28753-64.
- Mol Cell Biol. 2021 Jan 4;MCB.00333-20.

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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.

Caution: Product has not been fully validated for medical applications. For research use only.

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