JNK-IN-8

Cat. No.: HY-13319

CAS No.: 1410880-22-6

Molecular Formula: $C_{29}H_{29}N_7O_2$

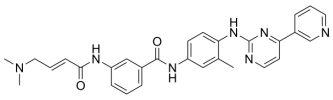
Molecular Weight: 508

Target: JNK

Pathway: MAPK/ERK Pathway

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 35 mg/mL (68.90 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Concentration \ Solvent \ Mass	1 mg	5 mg	10 mg
1 mM	1.9685 mL	9.8425 mL	19.6850 mL
5 mM	0.3937 mL	1.9685 mL	3.9370 mL
10 mM	0.1969 mL	0.9843 mL	1.9685 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (4.92 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (4.92 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.92 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

JNK-IN-8 (JNK Inhibitor XVI) 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[1].

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 EC_{50} 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 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 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 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]. 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×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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nat Nanotechnol. 2021 Jul;16(7):830-839.
- Mil Med Res. 2023 Jun 5;10(1):25.
- Nat Commun. 2020 Jan 3;11(1):71.
- J Clin Invest. 2024 Jan 9:e174984.
- Cell Death Differ. 2020 May;27(5):1569-1587.

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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.

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