JNJ-47117096 hydrochloride

Cat. No.: HY-12420

CAS No.: 1610536-69-0

Molecular Formula: $C_{21}H_{23}ClN_4O_2$

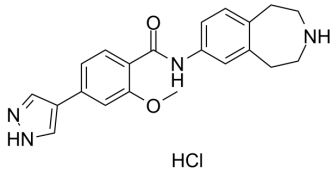
Molecular Weight: 398.89

Target: MELK; FLT3

Pathway: PI3K/Akt/mTOR; Protein Tyrosine Kinase/RTK

Storage: 4°C, sealed storage, away from moisture

* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 250 mg/mL (626.74 mM)

H_2O : 3.33 mg/mL (8.35 mM; ultrasonic and warming and heat to 60°C)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Concentration \ Solvent \ Mass	1 mg	5 mg	10 mg
	1 mM	2.5070 mL	12.5348 mL	25.0696 mL
	5 mM	0.5014 mL	2.5070 mL	5.0139 mL
	10 mM	0.2507 mL	1.2535 mL	2.5070 mL

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

BIOLOGICAL ACTIVITY

Description

JNJ-47117096 hydrochloride is potent and selective MELK inhibitor, with an IC_{50} of 23 nM, also effectively inhibits Flt3, with an IC_{50} of 18 nM.

IC_{50} & Target

IC50: 23 nM (MELK), 18 nM (Flt3)[1]

In Vitro

JNJ-47117096 hydrochloride is potent and selective MELK inhibitor, with an IC_{50} of 23 nM, also effectively inhibits Flt3, with an IC_{50} of 18 nM, and slighitly blocks CAMKIIδ, Mnk2, CAMKIIγ, and MLCK (IC_{50}, 810 nM, 760 nM, 1000 nM, 1000 nM). JNJ-47117096 (MELK-T1) suppresses the proliferation of Flt3-driven Ba/F3 cell lines, with an IC_{50} of 1.5 μM in the absence of IL-3, while no inhibitory activity is observed in the presence of IL-3. JNJ-47117096 does not inhibit the proliferation of Ba/F3 cell lines transfected with either FGFR1, FGFR3, or KDR, either in the presence or absence of IL-3[1]. JNJ-47117096 (MELK-T1, 10 μM) delays the progression of MCF-7 cells through S-phase. JNJ-47117096 inhibits MELK, and then exerts stalled replication forks and DNA double-strand breaks (DSBs). JNJ-47117096 activates the ATM-mediated DNA-damage response (DDR). JNJ-47117096 (3, 10 μM) results in a growth arrest and a senescent phenotype. Moreover, JNJ-47117096 induces a strong phosphorylation of p53, a prolonged up-regulation of p21 and a down-regulation of FOXM1 target genes[2].

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

PROTOCOL

Kinase Assay [1]

Inhibition of MELK kinase activity is measured using a radioactive filter binding assay. Briefly, each assay well contains 1.25 nM MELK (human, residues 1-340) 10 μM ATP, 6.7 uCi/mL γ^{33}P-ATP, 3 μM biotinylated ZIP-tide peptide (Biotin-KKLNRTLSFAEPG) in 30 μL reaction buffer (25 mM Tris pH 7.5, 10 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA, 0.1% Triton X100). Kinase reactions are performed for 25 minutes at room temperature before stopping with 40 μL 2% orthophosphoric acid. Unbound radioactivity is removed by filtering the reaction through a MAPH filter plate. The trapped ^{33}P labelled peptide is then washed twice with 200 μL 0.5% orthophosphoric acid, 20 μL Microscint-20 added per well and the amount of radioactivity determined by scintillation counting using a Topcount. To calculate compound IC_{50}, semi-log serial dilutions are used to produce 8-point dose-response curves in duplicate. IC_{50} values are then derived using the four parameter logistic fit method in GraphPad Prism 5.0[1].

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Cell Assay [1]

Compounds (JNJ-47117096) dissolved in DMSO are sprayed into 384-well plates (100 nL/well). A suspension of Ba/F3-Flt3 cells is added (20,000 cell/well), followed by the addition of 10 ng/mL IL3. The cells are incubated for 24 h at 37°C and 5% CO_2. Alamar Blue solution is added, and after 4 h incubation at 37°C, the fluorescent intensity is measured on a Fluorescence plate reader (540 nm excitation and 590 nm emission). The control experiment in the absence of IL3 is performed in the same way. To calculate compound IC_{50}, semi-log serial dilutions are used to produce 8- point dose-response curves in duplicate. A best-fit curve is fitted by a minimum sum of squares method to the plot of %Control vs. compound concentration. From this an IC_{50} value is calculated[1].

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

REFERENCES

[1]. Johnson CN, et al. Fragment-based discovery of type I inhibitors of maternal embryonic leucine zipper kinase. ACS Med Chem Lett. 2014 May 23;6(1):25-30.

[2]. Beke L, et al. MELK-T1, a small-molecule inhibitor of protein kinase MELK, decreases DNA-damage tolerance in proliferating cancer cells. Biosci Rep. 2015 Oct 2;35(6). pii: e00267.

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