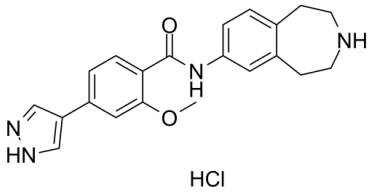
Data Sheet

Product Name:	JNJ-47117096 hydrochloride
Cat. No.:	CS-0011272
CAS No.:	1610536-69-0
Molecular Formula:	$C_{21}H_{23}ClN_4O_2$
Molecular Weight:	398.89
Target:	FLT3; MELK
Pathway:	PI3K/Akt/mTOR; Protein Tyrosine Kinase/RTK
Solubility:	DMSO : ≥ 250 mg/mL (626.74 mM); H2O : 3.33 mg/mL (8.35 mM; ultrasonic and warming and heat to 60°C)



BIOLOGICAL ACTIVITY:

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. IC50 & 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].

PROTOCOL (Extracted from published papers and Only for reference)

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]. **Cell Assay:** JNJ-47117096 (MELK-T1) is dissolved in DMSO.[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].

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.

CAIndexNames:

Benzamide, 2-methoxy-4-(1H-pyrazol-4-yl)-N-(2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)-, hydrochloride (1:1)

SMILES:

O=C(NC1=CC=C2CCNCCC2=C1)C3=CC=C(C4=CNN=C4)C=C3OC.Cl

Tel: 610-426-3128 Fax: 888-484-5008 E-mail: sales@ChemScene.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA