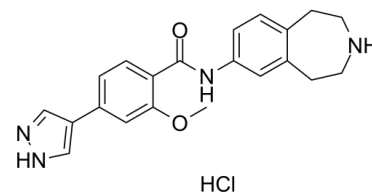


## Data Sheet

<b>Product Name:</b>	JNJ-47117096 hydrochloride
<b>Cat. No.:</b>	CS-0011272
<b>CAS No.:</b>	1610536-69-0
<b>Molecular Formula:</b>	C <sub>21</sub> H <sub>23</sub> CIN <sub>4</sub> O <sub>2</sub>
<b>Molecular Weight:</b>	398.89
<b>Target:</b>	FLT3; MELK
<b>Pathway:</b>	PI3K/Akt/mTOR; Protein Tyrosine Kinase/RTK
<b>Solubility:</b>	DMSO : ≥ 250 mg/mL (626.74 mM)



### BIOLOGICAL ACTIVITY:

JNJ-47117096 hydrochloride is potent and selective **MELK** inhibitor, with an **IC<sub>50</sub>** of 23 nM, also effectively inhibits **Flt3**, with an **IC<sub>50</sub>** of 18 nM. **IC<sub>50</sub> & Target:** IC<sub>50</sub>: 23 nM (MELK), 18 nM (Flt3)<sup>[1]</sup> **In Vitro:** JNJ-47117096 hydrochloride is potent and selective MELK inhibitor, with an **IC<sub>50</sub>** of 23 nM, also effectively inhibits Flt3, with an **IC<sub>50</sub>** of 18 nM, and slightly blocks CAMKIIδ, Mnk2, CAMKIIγ, and MLCK (**IC<sub>50</sub>**, 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<sub>50</sub>** 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<sup>[1]</sup>. 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<sup>[2]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** <sup>[1]</sup>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<sup>33</sup>P-ATP, 3 μM biotinylated ZIP-tide peptide (Biotin-KKLNRTLSTFAEPG) in 30 μL reaction buffer (25 mM Tris pH 7.5, 10 mM MgCl<sub>2</sub>, 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 <sup>33</sup>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<sub>50</sub>**, semi-log serial dilutions are used to produce 8-point dose-response curves in duplicate. **IC<sub>50</sub>** values are then derived using the four parameter logistic fit method in GraphPad Prism 5.0<sup>[1]</sup>. **Cell Assay:** JNJ-47117096 (MELK-T1) is dissolved in DMSO.<sup>[1]</sup>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<sub>2</sub>. 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<sub>50</sub>**, 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<sub>50</sub>** value is calculated<sup>[1]</sup>.

### 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=CCN=C4)C=C3OC.Cl

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

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