Torin 1

mTOR inhibitor

Catalog # inh-tor1

http://www.invivogen.com/torin1

For research use only

Version # 17E18-MM

PRODUCT INFORMATION

Contents:

• 10 mg Torin 1

Storage and stability:

- Torin 1 is shipped at room temperature. Store at -20 °C.
- Upon resuspension, prepare aliquots of Torin 1 and store at -20 °C. Resuspended Torin 1 is stable for 6 months when properly stored.

Quality control:

- Purity ≥97% (UHPLC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Torin 1 is a potent and selective ATP-competitive inhibitor of mTOR (mammalian target of rapamycin) kinase[1], the catalytic subunit of two functionally distinct complexes (mTORC1 and mTORC2) that coordinately promote cell growth, proliferation, and survival. Unlike classical mTOR inhibitors such as rapamycin, Torin 1 is able to effectively block phosphorylation of mTORC1 and mTORC2[2]. Torin 1 is an effective inducer of autophagy, as inhibition of mTOR mimics cellular starvation by blocking signals required for cell growth and proliferation[3,4]. Interestingly, Torin 1 has also been shown to have cytotoxic and cytostatic effects in rapamycin-resistant cells[5].

1. Liu Q. et al., 2010. Discovery of 1-(4-(4-propionylpiperazin-1-yl)-3-(trifluoromethyl) phenyl)-9-(quinolin-3-yl)benzo[h][1,6]naphthyridin-2(1H)-one as a highly potent, selective Mammalian Target of Rapamycin (mTOR) inhibitor for the treatment of cancer. Med Chem. 53(19):7146-55. **2. Thoreen CC. et al., 2009.** An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem. 284(12):8023-32. **3. Jung CH. et al., 2010.** mTOR regulation of autophagy. FEBS Lett. 584(7):1287-95. **4. Wang, R.C. et al., 2012.** Akt-mediated regulation of autophagy and tumorigenesis through Beclin 1 phosphorylation. Science 338(6109):956-9. **5. Park S. et al., 2016.** Rapamycin-resistant and torin-sensitive mTOR signaling promotes the survival and proliferation of leukemic cells. BMB Rep. 49(1): 63-68. **6. Zhou J. et al., 2013.** Activation of lysosomal function in the course of autophagy via mTORC1 suppression and autophagosome-lysosome fusion. Cell Res. 23(4):508-23.

CHEMICAL PROPERTIES

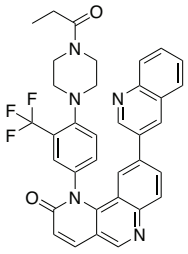
Solubility: 2 mg/ml (3.29 mM) in DMSO

CAS number: 1222998-36-8

Formula: $C_{35}H_{28}F_3N_5O_2$

Molecular weight: 607.6

Structure:



METHODS

Preparation of 3 mM (1.823 mg/ml) stock solution

- Weigh out 2 mg of Torin 1.
- Add 1.097 ml of DMSO to the 2 mg of Torin 1. Mix by vortexing.
- Prepare dilutions using sterile, endotoxin-free water or an aqueous buffer.

Working concentration: 250 nM (152 ng/ml) to 1 µM (607.6 ng/ml) for cell culture assays

Autophagy reporter assay:

Described below is a protocol to study the effects of Torin 1 in HeLa-Difluo™ hLC3b cells, an autophagy reporter cell line derived from the human epithelial carcinoma HeLa cell line. HeLa-Difluo™ hLC3b cells express two fluorescent reporter genes (RFP and GFP) fused to the N-terminal of the LC3 protein. The expression of this fusion protein enables the monitoring of autophagic flux in real time.

Day 1

1. Prepare a HeLa-Difluo™ hLC3b cell suspension at ~100,000 cells/ml.
2. Add 500 µl of cell suspension (~50,000 cells) per well of a 24-well plate.
3. Leave to incubate overnight at 37 °C in a 5% CO_2 incubator.

Day 2

1. Add 20 µl of Torin 1 at a final concentration of 250 nM to 1 µM and incubate at 37 °C for 30 min to 1 hour.
2. Perform image-based analysis for autophagy using a fluorescent microscope.

PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay[4]

Cells: HeLa expressing GFP-LC3
Working concentration: 250 nM
Incubation time: 2-4 hours
Method: Biochemical asssay and GFP-LC3 puncta

Cell Culture Assay[6]

Cells: MEF (Murine Embryonic Fibroblasts) expressing GFP-LC3
Working concentration: 1 µM
Incubation time: 3 hours
Method: Immunoblotting and GFP-LC3 puncta

Animal Study[1]

Animal model: Mice bearing U87MG xenografts
Dose: 20 mg/kg daily for 10 days
Administration: Intraperitoneal (IP)

RELATED PRODUCTS

Product	Description	Cat. Code
HeLa-Difluo™ hLC3 cells	Autophagy reporter cells	heldf-hlc3b
pp242	mTOR inhibitor	inh-pp242
Rapamycin	mTOR inhibitor	tlrl-rap
RAW-Difluo™ hLC3 cells	Autophagy reporter cells	rawdf-mlc3b

TECHNICAL SUPPORT
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