pp242

mTOR inhibitor

Catalog # inh-pp242

For research use only

Version # 16I13-MM

PRODUCT INFORMATION

Contents:

• 5 mg pp242

Storage and stability:

- pp242 is provided lyophilized and shipped at room temperature. Store at -20 °C. Lyophilized pp242 is stable for 2 years when properly stored.
- Upon resuspension, prepare aliquots of pp242 and store at -20 °C. Resuspended pp242 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

pp242 (also known as Torkinib) is a potent, selective and ATP-competitive inhibitor of mTOR (mammalian target of rapamycin) kinase, a key protein kinase of the PI3K/AKT signaling pathway that plays a central role in controlling cell growth, proliferation, and survival. mTOR is a major negative regulatory axis of autophagy. Thus, pp242 is an effective inducer of autophagy[1]. Specifically, pp242 is able to induce autophagy by inhibiting the two mTOR-containing complexes (mTORC1 and mTORC2), neither of which is fully blocked by classical mTOR inhibitors such as rapamycin[2-4].

1. 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. **2. Feldman, ME. et al., 2009.** Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. 7:371-83. **3. Shao H. et al., 2009.** Dual targeting of mTORC1/C2 complexes enhances histone deacetylase inhibitor-mediated anti-tumor efficacy in primary HCC cancer in vitro and in vivo. J. Hepatol. 56(1):176-83. **4. Janes MR. et al., 2010.** Effective and selective targeting of Ph+ leukemia cells using a TORC1/2 kinase inhibitor. Nat Med. 16(2):205-213.

CHEMICAL PROPERTIES

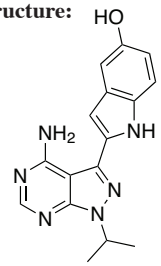
Solubility: 62 mg/ml (201.1 mM) in DMSO
18 mg/ml (58.4 mM) in ethanol

CAS number: 1092351-67-1

Formula: $C_{16}H_{16}N_6O$

Molecular weight: 308.3

Structure:



METHODS

Preparation of 20 mM (6.2 mg/ml) stock solution

- Add 810 µl of DMSO to 5 mg pp242. Mix by vortexing.
- Prepare further dilutions using sterile, endotoxin-free water or an aqueous buffer.

Working concentration: 250 nM (77.1 ng/ml) to 1 µM (308.3 ng/ml) for cell culture assays

Autophagy reporter assay:

Described below is a protocol to study the effects of pp242 in HeLa-Difluo™ hLC3b cells, autophagy reporter cells 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 pp242 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[1]

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

Cell Culture Assay[4]

Cells: Fibroblasts and solid tumor cell lines (SKOV3, PC-3, 786-O, U87)
Working concentration: 50-400 nM
Incubation time: 1.5-3 hours
Method: Immunoblotting and immunofluorescence analysis

Animal Study[4]

Animal model: Balb/cJ mice
Dose: 60 mg/kg daily for 9 days
Administration: Oral gavage

RELATED PRODUCTS

Product	Description	Cat. Code
HeLa-Difluo™ hLC3 cells	Autophagy reporter cells	heldf-hlc3b
Rapamycin	mTOR inhibitor	tlrl-rap
RAW-Difluo™ hLC3 cells	Autophagy reporter cells	rawdf-mlc3b
Torin 1	mTOR inhibitor	inh-tor1

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com