Torin 1

Cat. No.: HY-13003

CAS No.: 1222998-36-8

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

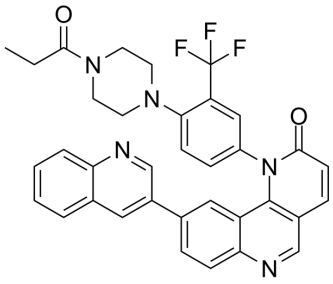
Molecular Weight: 607.62

Target: mTOR; Autophagy

Pathway: PI3K/Akt/mTOR; Autophagy

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 2 mg/mL (3.29 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.6458 mL	8.2288 mL	16.4577 mL
	5 mM	---	---	---
	10 mM	---	---	---

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

In Vivo

1. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 0.25 mg/mL (0.41 mM); Clear solution
2. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: ≥ 0.25 mg/mL (0.41 mM); Clear solution
3. Add each solvent one by one: 1% DMSO >> 99% saline
   Solubility: 0.05 mg/mL (0.08 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

Torin 1 is a potent inhibitor of mTOR with an IC_{50} of 3 nM. Torin 1 inhibits both mTORC1/2 complexes with IC_{50} values between 2 and 10 nM. Torin 1 is an effective inducer of autophagy.

IC_{50} & Target

mTOR 3 nM (IC_{50})	mTORC1 2-10 nM (IC_{50})	mTORC2 2-10 nM (IC_{50})	ATM 0.6 μM (IC_{50})
DNA-PK 1 μM (IC_{50})	PI3K-α 1.8 μM (IC_{50})	hVps34 3 μM (IC_{50})	Autophagy

In Vitro	Torin1 (250 nM) completely inhibits proliferation and causes a G1/S cell cycle arrest, and decreases cell size to a greater degree than 50 nM rapamycin in wild-type MEFs[1]. Torin1 has more than 800-fold selectivity between mTOR and PI3Kis, and is very selective relative to other PIKK family kinases with the exception of DNA-PK[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Torin1 (20 mg/kg, i.p.) is efficacious in a U87MG xenograft model, and demonstrates good pharmacodynamic inhibition of downstream effectors of mTOR in tumor and peripheral tissues[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	To produce soluble mTORC1, HEK-293T cell lines are generated that stably express N-terminally FLAG-tagged Raptor using vesicular stomatitis virus G-pseudotyped MSCV retrovirus. For mTORC2, HeLa cells are generated that stably express N-terminally FLAG-tagged Protor-1. Both complexes are purified by lysing cells in 50 mM HEPES, pH 7.4, 10 mM sodium pyrophosphate, 10 mM sodium β-glycerophosphate, 100 mM NaCl, 2 mM EDTA, 0.3% CHAPS. Cells are lysed at 4°C for 30 min, and the insoluble fraction is removed by microcentrifugation at 13,000 rpm for 10 min. Supernatants are incubated with FLAG-M2 monoclonal antibody-agarose for 1 h and then washed three times with lysis buffer and once with lysis buffer containing a final concentration of 0.5 mol/L NaCl. Purified mTORC1 is eluted with 100 μg/mL 3× FLAG peptide in 50 mM HEPES, pH 7.4, 100 mM NaCl. Eluate can be aliquoted and stored at -80°C. Kinase assays are performed for 20 min at 30°C in a final volume of 20 μL consisting of the kinase buffer (25 mM HEPES, pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, 500 μM ATP) and 150 ng of inactive S6K1 or Akt1 as substrates. Reactions are stopped by the addition of 80 μL of sample buffer and boiled for 5 min. Samples are subsequently analyzed by SDS-PAGE and immunoblotting. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	On Day 0, 96-well plates are seeded with 500 cells per well and grown overnight. On Day 1, cells are treated with the appropriate compounds and subsequently analyzed on Days 3-5. For analysis, plates are incubated for 60 min at room temperature; 50 μL of CellTiter-Glo reagent is added to each well, and plates are mixed on an orbital shaker for 12 min. Luminescence is quantified on a standard plate luminometer. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2]	For pharmacodynamic experiments, torin 1 powder is first dissolved at 25 mg/mL in 100% N-methyl-2-pyrrolidone and then diluted 1:4 with sterile 50% PEG400 prior to injection. Six-week old male C57BL/6 mice are fasted overnight prior to drug treatment. The mice are treated with vehicle (for 10 hr) or 26 (20 mg/kg for 2, 6 or 10 hr) by IP injection, and then refed 1 h prior to sacrifice (CO_2 asphyxiation). Tissues are collected and frozen on dry ice. The frozen tissue is thawed on ice and lysed by sonication in tissue lysis buffer (50 mM HEPES, pH 7.4, 40 mM NaCl, 2 mM EDTA, 1.5 mM sodium orthovanadate, 50 mM sodium fluoride, 10 mM sodium pyrophosphate, 10 mM sodium β-glycerophosphate, 0.1% SDS, 1.0% sodium deoxycholate and 1.0% Triton, supplemented with protease inhibitor cocktail tablets). The concentration of clear lysate is measured using the Bradford assay and samples are subsequently normalized by protein content and analyzed by SDS-PAGE and immunoblotting. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Metab. 2018 Jan 9;27(1):118-135.e8.
- Cell Stem Cell. 2022 Apr 7;29(4):545-558.e13.
- J Immunother Cancer. 2020 Sep;8(2):e000517.
- Autophagy. 2022 Jul 1;1-14.
- Autophagy. 2020 Oct;16(10):1786-1806.

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REFERENCES

[1]. Thoreen CC, et al, An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem, 2009, 284(12), 8023-8032.

[2]. Liu Q, et al. 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. J Med Che

[3]. Bi C, et al. Inhibition of 4EBP phosphorylation mediates the cytotoxic effect of mechanistic target of rapamycin kinase inhibitors in aggressive B-cell lymphomas. Haematologica. 2017 Apr;102(4):755-764.

[4]. Brandt M, et al. mTORC1 Inactivation Promotes Colitis-Induced Colorectal Cancer but Protects from APC Loss-Dependent Tumorigenesis. Cell Metab. 2018 Jan 9;27(1):118-135.e8.

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