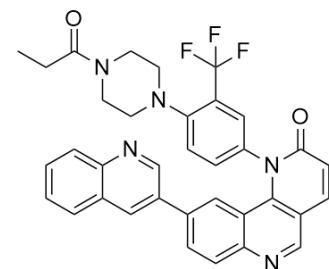


Torin 1

Cat. No.:	HY-13003		
CAS No.:	1222998-36-8		
Molecular Formula:	C ₃₅ H ₂₈ F ₃ N ₅ O 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)			
		Solvent Concentration	Mass	
			1 mg	5 mg
	Preparing Stock Solutions	1 mM	1.6458 mL	8.2288 mL
		5 mM	---	---
		10 mM	---	---
Please refer to the solubility information to select the appropriate solvent.				
In Vivo	1. Torin 1 is dissolved in 100% N-methyl-2-pyrrolidone and subsequently diluted with PEG400 and water at the ratio of 1:2:2 [3]. 2. Torin 1 is dissolved in NMP/PEG 400 (1:4) ^[4] .			

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.			
IC₅₀ & Target	mTOR	mTORC1	mTORC2	ATM
	3 nM (IC ₅₀)	2-10 nM (IC ₅₀)	2-10 nM (IC ₅₀)	0.6 μM (IC ₅₀)
	DNA-PK	PI3K-α	hVps34	Autophagy
	1 μM (IC ₅₀)	1.8 μM (IC ₅₀)	3 μM (IC ₅₀)	
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 MgCl₂, 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 (CO₂ 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.
- J Immunother Cancer. 2020 Sep;8(2):e000517.
- Autophagy. 2019 Nov 7:1-21.
- Cell Rep. 2019 Aug 20;28(8):1971-1980.e8.
- Haematologica. 2017 Apr;102(4):755-764.

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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 Chem. 2010 Oct 14;53(19):7146-55.
- [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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Caution: Product has not been fully validated for medical applications. For research use only.

India Contact:

Life Technologies (India) Pvt. Ltd.

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com