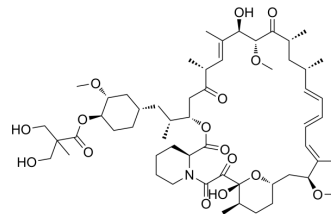


Temsirolimus

Cat. No.:	HY-50910
CAS No.:	162635-04-3
Molecular Formula:	C ₅₆ H ₈₇ NO ₁₆
Molecular Weight:	1030.29
Target:	mTOR; Autophagy; Apoptosis; Bacterial
Pathway:	PI3K/Akt/mTOR; Autophagy; Apoptosis; Anti-infection
Storage:	4°C, protect from light, stored under nitrogen * In solvent : -80°C, 1 year; -20°C, 6 months (protect from light, stored under nitrogen)



SOLVENT & SOLUBILITY

In Vitro

Ethanol : 200 mg/mL (194.12 mM; Need ultrasonic)
 DMSO : ≥ 100 mg/mL (97.06 mM)
 H₂O : < 0.1 mg/mL (insoluble)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	0.9706 mL	4.8530 mL	9.7060 mL
	5 mM	0.1941 mL	0.9706 mL	1.9412 mL
	10 mM	0.0971 mL	0.4853 mL	0.9706 mL

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

In Vivo

- Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline
Solubility: ≥ 5 mg/mL (4.85 mM); Clear solution
- Add each solvent one by one: 10% EtOH >> 90% corn oil
Solubility: ≥ 5 mg/mL (4.85 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
Solubility: ≥ 2.08 mg/mL (2.02 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.08 mg/mL (2.02 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Temsirolimus is an inhibitor of mTOR with an IC₅₀ of 1.76 μM. Temsirolimus activates autophagy and prevents deterioration of cardiac function in animal model^[8].

IC₅₀ & Target	mTOR 1.76 μ M (IC ₅₀)
In Vitro	<p>Temsirolimus potently inhibits mTOR kinase activity with IC₅₀ of 1.76 μM, similar to that of rapamycin with IC₅₀ of 1.74 μM in the absence of FKBP12. Temsirolimus (10 nM to <5 μM) displays a modest and selective antiproliferative activity via FKBP12-dependent mechanism, but can completely inhibit the proliferation of a broad panel of tumor cells at low micromolar concentrations (5-15 μM), involving FKBP12-independent suppression of mTOR signaling. Temsirolimus treatment at micromolar but not nanomolar concentrations (20 μM) causes a marked decline in global protein synthesis and disassembly of polyribosomes, accompanied by rapid increase in the phosphorylation of translation elongation factor eEF2 and the translation initiation factor eIF2A^[1]. Temsirolimus inhibits the phosphorylation of ribosomal protein S6, more potently in PTEN-positive DU145 cells than in PTEN-negative PC-3 cells, and inhibits cell growth and clonogenic survival of both cells in a concentration-dependent manner^[2]. Temsirolimus (100 ng/mL) potently inhibits proliferation and induces apoptosis in primary human lymphoblastic leukemia (ALL) cells^[3].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
In Vivo	<p>CCI-779 (20 mg/kg, i.p.) inhibits the growth of both prostate cancer xenografts, and the growth of PC-3 tumors is inhibited in a dose-dependent manner and growth inhibition is greater than for DU145 tumors^[2]. In the NOD/SCID xenograft models with human ALL, Temsirolimus treatment at 10 mg/kg/day produces a decrease in peripheral blood blasts and in splenomegaly^[3]. Administration of Temsirolimus (20 mg/kg, i.p. 5 days/week) significantly delays the growth of DAOY xenografts by 160% after 1 week and 240% after 2 weeks, compared with controls. Single high-dose of Temsirolimus (100 mg/kg, i.p) treatment induces 37% regression of tumor volume within 1 week. Temsirolimus treatment for 2 weeks also delays the growth of rapamycin-resistant U251 xenografts by 148%^[4]. Inhibition of mTOR by Temsirolimus improves performance on four different behavioral tasks and decreases aggregate formation in a mouse model of Huntington disease^[5]. Administration of Temsirolimus induces significant dose-dependent, antitumor responses against subcutaneous growth of 8226, OPM-2, and U266 xenografts with ED₅₀ of 20 mg/kg and 2 mg/kg for 8226 and OPM-2, respectively, which are associated with inhibited proliferation and angiogenesis, induction of apoptosis, and reduction in tumor cell size^[6].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

PROTOCOL

Kinase Assay ^[1]

The Flag-tagged wild-type human mTOR (Flag-mTOR) DNA constructs are transiently transfected into HEK293 cells. Protein extraction and purification of Flag-mTOR are carried out 48 hours later. In vitro kinase assays of purified Flag-mTOR in the presence of various concentrations of Temsirolimus without FKBP12 are performed in 96-well plate and detected by dissociation-enhanced lanthanide fluorescent immunoassay (DELFI) using His6-S6K1 as the substrate. Enzymes is first diluted in kinase assay buffer (10 mM Hepes (pH 7.4), 50 mM NaCl, 50 mM β -glycerophosphate, 10 mM MnCl₂, 0.5 mM DTT, 0.25 μ M microcystin LR, and 100 μ g/mL BSA). To each well, 12 μ L of the diluted enzyme is mixed briefly with 0.5 μ L Temsirolimus. The kinase reaction is initiated by adding 12.5 μ L kinase assay buffer containing ATP and His6-S6K to give a final reaction volume of 25 μ L containing 800 ng/mL FLAG-mTOR, 100 μ M ATP, and 1.25 μ M His6-S6K. The reaction plate is incubated for 2 hours (linear at 1-6 hours) at room temperature with gentle shaking and then terminated by adding 25 μ L Stop buffer (20 mM Hepes (pH 7.4), 20 mM EDTA, and 20 mM EGTA). The DELFIA detection of the phosphorylated (Thr-389) His6-S6K is performed at room temperature using a monoclonal anti-P(T389)-p70S6K antibody labeled with Europium-N1-ITC (Eu) (10.4 Eu per antibody). 45 μ L of the terminated kinase reaction mixture is transferred to a MaxiSorp plate containing 55 μ L PBS. The His6-S6K is allowed to attach for 2 hours after which the wells are aspirated and washed once with PBS. 100 μ L of DELFIA buffer with 40 ng/mL Eu-P(T389)-S6K antibody is added. The antibody binding is continued for 1 hour with gentle agitation. The wells are then aspirated and washed four times with PBS containing 0.05% Tween 20 (PBST). 100 μ L of DELFIA Enhancement solution is added to each well and the plates are read in a PerkinElmer Victor model plate reader.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay ^[2]

Survival of prostate cancer cells following various treatments is also determined in a colony-forming assay. Exponentially growing cells are exposed to varying doses of mitoxantrone or docetaxel for 24 hours, or to CCI-779 for 3 days. Following this treatment, the cells are washed and trypsinized. Serial dilutions are plated in 6-well plates in 5 mL medium. The plates are incubated for 10 days at 37°C in an atmosphere containing 5% CO₂ at 90% humidity. The plates are then stained with

methylene blue and colonies containing >50 cells are counted.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration [2]

For generation of xenografts, cells are implanted in matrigel; matrigel is stored at -20°C and then thawed on ice at 4°C for 3 hours before use. Cells are gently resuspended in 1 mL of PBS and incubated on ice for 5 minutes. A prechilled pipette is used to transfer cells to the tube containing 1 mL of matrigel, and the cell concentration is adjusted to $3 \times 10^7/\text{mL}$. The cells (3×10^6 in 0.1 mL) are injected s.c. into both flanks of mice using a 25-gauge needle. When xenografts grew to a size of about 5 mm in diameter, animals are assorted randomly into groups of 10 mice. The following experiments are conducted: Mice bearing PC-3 tumors are treated with CCI-779 (1, 5, 10, and 20 mg per kg per day), or vehicle solution for 3 or 5 days per week for 3 weeks. Mice bearing DU145 tumors are only treated with CCI-779 (20 mg per kg per day) or vehicle solution for 3 weeks. Mice bearing PC-3 tumors receive the following treatments: (a) control, vehicle solution for CCI-779; (b) chemotherapy alone, mitoxantrone 1.5 mg/kg or docetaxel 10 mg/kg is injected i.p. weekly for 3 doses; (c) CCI-779 alone, 5 or 10 mg/kg is injected i.p. daily, three times a week for 3 weeks; (4) chemotherapy followed by CCI-779.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- Autophagy. 2019 Jun;15(6):998-1016.
- Genome Med. 2016 Oct 31;8(1):116.
- J Exp Clin Cancer Res. 2024 Jan 11;43(1):18.
- PLoS Biol. 2019 May 21;17(5):e3000252.

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- [2]. Wu L, et al. Effects of the mammalian target of rapamycin inhibitor CCI-779 used alone or with chemotherapy on human prostate cancer cells and xenografts. *Cancer Res*, 2005, 65(7), 2825-2831.
- [3]. Teachey DT, et al. The mTOR inhibitor CCI-779 induces apoptosis and inhibits growth in preclinical models of primary adult human ALL. *Blood*, 2006, 107(3), 1149-1155.
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- [5]. Ravikumar B, et al. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. *Nat Genet*. 2004 Jun;36(6):585-95. Epub 2004 May 16.
- [6]. Frost P, et al. In vivo antitumor effects of the mTOR inhibitor CCI-779 against human multiple myeloma cells in a xenograft model. *Blood*. 2004 Dec 15;104(13):4181-7. Epub 2004 Aug 10.
- [7]. Dela Cruz FS, et al. A case study of an integrative genomic and experimental therapeutic approach for rare tumors: identification of vulnerabilities in a pediatric poorly differentiated carcinoma. *Genome Med*. 2016 Oct 31;8(1):116.
- [8]. Jason C. Choi, et al. Temsirolimus activates autophagy and ameliorates cardiomyopathy caused by lamin A/C gene mutation. *Sci Transl Med*. 2012 Jul 25; 4(144): 144ra102.

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

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