Everolimus

Cat. No.: HY-10218

CAS No.: 159351-69-6

Molecular Formula: $C_{53}H_{83}NO_{14}$

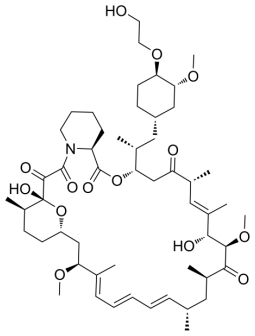
Molecular Weight: 958.22

Target: mTOR; FKBP; Autophagy; Apoptosis; Bacterial

Pathway: PI3K/Akt/mTOR; Apoptosis; Autophagy; Immunology/Inflammation; Anti-infection

Storage:

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



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (52.18 mM; ultrasonic and warming and heat to 60°C)

H_2O : < 0.1 mg/mL (ultrasonic;warming;heat to 60°C) (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.0436 mL	5.2180 mL	10.4360 mL
5 mM	0.2087 mL	1.0436 mL	2.0872 mL
10 mM	0.1044 mL	0.5218 mL	1.0436 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (2.61 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (2.61 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (2.61 mM); Clear solution
4. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: 2.5 mg/mL (2.61 mM); Suspended solution; Need ultrasonic
5. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (2.61 mM); Suspended solution; Need ultrasonic

BIOLOGICAL ACTIVITY

Description

Everolimus (RAD001) is a Rapamycin (HY-10219) derivative and a potent, selective and orally active mTOR1 inhibitor. Everolimus binds to FKBP-12 to generate an immunosuppressive complex. Everolimus inhibits tumor cells proliferation and

	induces cell apoptosis and autophagy. Everolimus has potent immunosuppressive and anticancer activities[1][2].
IC_{50} & Target	mTOR 5-6 nM (IC_{50})
In Vitro	Everolimus (RAD001) is an orally active derivative of rapamycin that inhibits the Ser/Thr kinase, mTOR[1]. In both the sensitive murine B16/BL6 melanoma (IC_{50}, 0.7 nM) and the insensitive human cervical KB-31 (IC_{50}, 1,778 nM), antiproliferative concentrations of Everolimus results in total dephosphorylation of S6K1 and the substrate S6 and a shift in the mobility of 4E-BP1, which is indicative of a reduced phosphorylation status[3]. Everolimus exhibits a dose-dependent inhibition in both the total cells and the stem cells from the BT474 cell line and the primary breast cancer cells, albeit with different degrees of growth inhibition. Compare with the total cells, Everolimus is less effective in growth inhibition in the stem cells at all tested concentrations ($P<0.001$). The IC_{50} values of Everolimus for BT474 and the primary CSCs are 2,054 and 3,227 nM, or 29 times and 21 times greater than the IC_{50} values for their corresponding total cells, respectively[4]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Everolimus is orally active in both mice and rats, producing an antitumor effect that is characterized by dramatic reduction in tumor growth rates as opposed to producing tumor regressions. In the rat CA20498 model, daily treatment with Everolimus (0.5 or 2.5 mg/kg) dose-dependently inhibits growth, and intermittent dosing using a higher dose of 5 mg/kg (once or twice per week) also shows similar antitumor efficacy. Inhibition by Everolimus is characterized by sustained suppression rather than regression and is not associated with any body weight loss[1]. The effect of Everolimus treatment (0.1-10 mg/kg/d) is selective and differ from the effects of PTK/ZK (100 mg/kg). With either growth factor, Everolimus dose-dependently increases the hemoglobin content (convert to blood equivalents and indicative of the number of vessels as well as vascular leakiness) but reduces the Tie-2 content (number of endothelial cells indicative of the number of vessels) and this is significant for VEGF stimulation but not bFGF stimulation. The pharmacokinetics of Everolimus in mice shows that maximum levels of only 0.1 μM are achieved in a human tumor xenograft following a single administration, whereas plasma levels reach 1 to 3 μM for ~4 h[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [2]	Tumor cells are plated into 96-well plates at densities ranging from 500 to 5,000/100 μL/well, with repeat experiments being done at an optimal cell number, typically 1,000 to 2,000 per well, and incubated overnight. Cells are exposed to Everolimus and incubated for 4 days and the cell number is determined by methylene blue staining. For this, 50 μL glutaraldehyde [20% (v/v)] is added to the wells incubated for 10 min at room temperature. The culture medium is aspirated, cells are washed with distilled water, and 100 μL methylene blue [0.05% (w/v) in water] is added and incubated for 10 min at 37°C. Stained cells are washed three times with water, 200 μL HCl [3% (v/v)] is added, and the plate shaken at room temperature for 20 min. The absorbance of each well is determined at 650 nm. The IC_{50} values are calculated using Softmax 2.0 software[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [2]	Mice[2] Everolimus, PTK/ZK, and their respective vehicles are prepared each day just before administration to animals and the administration volume is individually adjusted based on animal body weight. In C57/BL6 mice, Everolimus is administered at doses ranging from 0.1 to 10 mg/kg/d orally (10 mL/kg) and predominantly at 2.5 to 10 mg/kg because these doses provides the maximum effect. PTK/ZK is administered at 50 to 100 mg/kg/d orally. Rats[2] Wistar-Furth rats are divided into two equal groups based on body weight and treated either with vehicle or Everolimus (10 mg/kg/d orally in mice and 5 mg/kg three times per week orally in rats). Directly after the first measurement at baseline (day 0), Everolimus or vehicle is administered orally by gavage (10 mL/kg) for up to 7 days maximum with subsequent magnetic resonance measurements made within 30 min of the last dose. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Nat Med. 2016 Jul;22(7):723-6.
- Nature. 2016 Dec 1;540(7631):119-123.
- Signal Transduct Target Ther. 2021 May 28;6(1):188.
- Nat Cancer. 2023 Oct;4(10):1508-1525.
- Mil Med Res. 2023 Dec 20;10(1):68.

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REFERENCES

[1]. O'Reilly T, et al. Biomarker Development for the Clinical Activity of the mTOR Inhibitor Everolimus (RAD001): Processes,Limitations, and Further Proposals. Transl Oncol. 2010 Apr;3(2):65-79.

[2]. Lane HA, et al. mTOR inhibitor RAD001 (everolimus) has antiangiogenic/vascular properties distinct from a VEGFR tyrosine kinase inhibitor. Clin Cancer Res, 2009, 15(5), 1612-1622.

[3]. Zhu Y, et al. Antitumor effect of the mTOR inhibitor Everolimus on human breast cancer stem cells in vitro and in vivo. Tumour Biol. 2012 Oct;33(5):1349-62.

[4]. Kawata T, et al. Dual inhibition of the mTORC1 and mTORC2 signaling pathways is a promising therapeutic target for adult T-cell leukemia. Cancer Sci. 2018 Jan;109(1):103-111.

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