Kinase Assay:

Protocol

Biological Activity

In vitro assay of mTOR catalytic activity

Storage

Formula

CAS No.

Synonyms

<table>
<thead>
<tr>
<th>Molecular Weight (MW)</th>
<th>1030.29</th>
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**Formula**

- 

**CAS No.**

- 162635-04-3, 343261-52-9, 1034922-90-1

**Synonyms**

- CCI-779

**Biological Activity**

**Description**

Temsirolimus (CCI-779, Torisel) is a specific mTOR inhibitor with IC50 of 1.76 μM.

**IC50**

- 1.76 μM

**In vitro**

- 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. Temsirolimus (100 ng/mL) potently inhibits proliferation and induces apoptosis in primary human lymphoblastic leukemia (ALL) cells. In the absence of FKBP12, Temsirolimus potently inhibits mTOR kinase activity with IC50 of 1.76 μM similar to that of rapamycin with IC50 of 1.74 μM. Temsirolimus treatment at nanomolar concentrations (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 eIF2 and the translation initiation factor eIF2A.

**In vivo**

- 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 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 48%. Inhibition of mTOR by Temsirolimus improves performance on four different behavioral tasks and decreases aggregate formation in a mouse model of Huntington disease.

**Clinical Trials**

- A Phase IV study of two different doses of Temsirolimus in patients with mantle cell lymphoma is currently ongoing.

**Features**

- **Protocol** (Only for Reference)
  
  - **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 are performed in the presence of various concentrations of Temsirolimus without FKBP12 are performed in 96-well plate and detected by dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA) 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 Mg-glycerophosphate, 10 mM MnCl2, 0.5 mM DTT, 0.25 mM microcin L and, 100 μg/mL BSA). To each well, 12 μl of the diluted enzyme is mixed briefly with 0.5 μL Temsirolimus. The kinase reaction is initiated by adding 12.5 μL kinase assay buffer containing ATP and His6-S6K to give a final reaction volume of 25 μL containing 600 ng/mL Flag-mTOR, 100 μM ATP, and 1.25 μM 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 μL Stop buffer (20 mM Hapes [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 μL of the terminated kinase reaction mixture is transferred to a MaxiSorp plate containing 55 μL PBS. The His6-S6K is allowed to attach for 2 hours after which the wells are aspirated and

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www.selleckchem.com/datasheet/Temsirolimus-DataSheet.html
washed once with PBS. 100 μL of DELFIA buffer with 40 ng/mL Eu-(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 μL of DELFIA Enhancement solution is added to each well and the plates are read in a PerkinElmer Victor model plate reader.

### Cell Assay:[1]

**Cell Lines**
- A549, H157, H460, H446, HCT116, HT29, SW480, DLD1, Caco2, LNCap, DU145, MDA468, MDA231, HEK293, and PC3-MM2

**Concentrations**
- Dissolved in DMSO, final concentrations ~20 μM

**Incubation Time**
- 72 hours

**Methods**
- Cells are exposed to various concentrations of Temsirolimus for 72 hours. After treatment, viable cell densities are determined by MTS dye conversion using CellTiter AQ assay kit.

### Animal Study:[2]

**Animal Models**
- Female athymic nude mice injected s.c. with DAOY, or U251 cells

**Formulation**
- Prepared in 100% EtOH as a 50 mg/mL stock solution, and diluted in 5% Tween 80 and 5% polyethylene glycol 400

**Doses**
- 20 mg/kg

**Administration**
- Injection daily 5 times per week

**References**

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**Customer Reviews**

Data independently produced by Dr. Zhang of Tianjin Medical University. Temsirolimus (Torisel) purchased from Selleck.

Breast cancer cells were pretreated with 100ng/ml EGF for 15 min and then treated with the indicated concentrations of Temsirolimus for 24 hours.

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NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE

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