产品名称：GDC-0349
产品别名：GDC-0349

生物活性：

Description	GDC-0349 is a potent and selective ATP-competitive **mTOR** inhibitor with a K_i of 3.8 nM. GDC-0349 inhibits of both **mTORC1** and **mTORC2**complexes.

IC_{50} & Target	mTOR	mTORC1	mTORC2	Autophagy
	3.8 nM (Ki)			

In Vitro	GDC-0349 (Compound 8h) is a remarkably selective mTOR inhibitor, with less than 25% inhibition of 266 kinases, including all isoforms of PI3K when tested at 1 μM[1].
In Vivo	When dosed orally once daily in athymic mice in a MCF7-neo/Her2 tumor xenograft model (PI3K mutation), GDC-0349 (Compound 8h) inhibits tumor growth in a dose-dependent manner, achieving stasis (99% TGI) at the maximum tolerated dose. Body weight change is less than 10% up to the highest dose. GDC-0349 is also efficacious in other xenograft models, including PC3 (PTEN null) and 786-0 (VHL mutant). Similar levels of tumor growth inhibition are achieved when GDC-0349 is administered once every three days at higher doses compared to once every day. GDC-0349 has ~10-fold reduced free plasma clearance in both mice (100 mL/min/kg) and rats (171 mL/min/kg in rat)[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 100 mg/mL (220.97 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.2097 mL	11.0485 mL	22.0970 mL
	5 mM	0.4419 mL	2.2097 mL	4.4194 mL
	10 mM	0.2210 mL	1.1049 mL	2.2097 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.52 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.52 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 2.5 mg/mL (5.52 mM); Clear solution

	此方案可获得 ≥ 2.5 mg/mL (5.52 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.52 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.52 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Pei Z, et al. Discovery and Biological Profiling of Potent and Selective mTOR Inhibitor GDC-0349. ACS Med Chem Lett. 2012 Nov 29;4(1):103-7.

实验参考：

Animal Administration	Mice[1] Human breast cancer cells (MCF7 neo/HER2; modified ATCC variant) are implanted subcutaneously into the mammary fat pad of female NCR nude mice (5×10^6 cells/100 uL of 1:1 mixture of Hank's Balanced Salt Solution (HBSS)/Matrigel). To support estrogen dependent growth, recipient animals are pre-implanted with 0.36 mg estrogen pellets. Tumors are monitored until they reached a mean tumor volume of approximately 200-225 mm^3, then similarly sized tumors are randomly assigned to treatment cohorts (n=5-10). Human 786-O renal adenocarcinoma cells are implanted subcutaneously into the right hind flank of female nu/nu mice (1×10^7 cells/200 uL in 1:1 PBS/Matrigel). Tumors are monitored until they reached a mean tumor volume of approximately 205 mm^3, then similarly sized tumors are randomly assigned to treatment cohorts (n=10). Human prostate cancer NCI-PC3 cells are resuspended in Hank's Balanced Salt Solution and implanted subcutaneously into the right hind flanks of 120 female NCR nude mice. Each mouse is injected with 5×10^6 cells. Tumors are monitored until they reached a mean tumor volume of approximately 200-250 mm^3. The dimesylate salt of GDC-0349 is dosed daily or every third day by oral gavage (100 uL dose /25 gm animal) for 14-21 days.Tumor volume and body weight measurements are collected twice weekly. Tumor volumes are calculated.
Kinase Assay	The kinase activity of mTOR enzyme is assessed by incubating purified recombinant enzyme (mTOR(1360-2549)+GBL, prepared in-house) in a reaction mixture containing ATP, $MnCl_2$, and a fluorescently labeled mTOR substrate, e.g., GFP-4E-BP1. The reaction is stopped by an addition of a Terbium-labeled phospho-specific antibody, e.g., Tb-labeled anti-p4E-BP1 T37/T46, EDTA, and TR-FRET buffer solution. Product formation is detected by way of time-resolved fluorescence resonance energy transfer (TR-FRET), which occurs when the phosphorylated substrate and labeled antibody are in close proximity due to phosphospecific binding. Enzymatic activity is measured as an increase in TR-FRET signal using a Perkin Elmer Envision plate reader. The assay is performed in a 384-well Proxiplate Plus using the following protocol: Compound activity is tested in 10 point dose curves starting at the highest final concentration of 10 uM. They are serially diluted in 100% DMSO prior to further dilution with assay buffer. The reaction mixture (8μL) containing 0.25 nM mTOR+GBL enzyme, 400 nM GFP-4E-BP1, 8 uM ATP, 50 mM Hepes pH 7.5, 0.01% Tween 20, 10 mM $MnCl_2$, 1 mM EGTA, 1 mM DTT, 1% DMSO (±compound) is incubated at room temperature for 30 minutes. 8 μL of solution containing 2 nM Tb-anti-p4E-BP1 antibody & 10 mM EDTA diluted TR-FRET buffer is then added and incubated for 30 minutes to stop the reaction. The plate is

	scanned with the Envision plate reader. K_i values are calculated in Assay Explorer using the Morrison ATP-competitive tight binding equation for K_i apparent determination [1].
References	[1]. Pei Z, et al. Discovery and Biological Profiling of Potent and Selective mTOR Inhibitor GDC-0349. ACS Med Chem Lett. 2012 Nov 29;4(1):103-7.