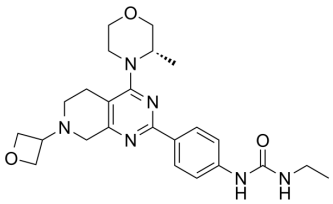
GDC-0349

Cat. No.:	HY-15248
CAS No.:	1207360-89-1
Molecular Formula:	$C_{24}H_{32}N_6O_3$
Molecular Weight:	452.55
Target:	mTOR; Autophagy
Pathway:	PI3K/Akt/mTOR; Autophagy
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



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

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.52 mM); Clear solution

BIOLOGICAL ACTIVITY

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].

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

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].

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PROTOCOL

Kinase Assay [1]

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].

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Animal Administration [1]

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.

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CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- Front Pharmacol. 2020 Nov 11;11:580407.

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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.

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