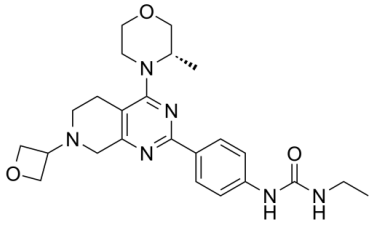
Data Sheet

Product Name:	GDC-0349
Cat. No.:	CS-0700
CAS No.:	1207360-89-1
Molecular Formula:	C24H32N6O3
Molecular Weight:	452.55
Target:	Autophagy; mTOR
Pathway:	Autophagy; PI3K/Akt/mTOR
Solubility:	DMSO : ≥ 100 mg/mL (220.97 mM)



BIOLOGICAL ACTIVITY:

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. IC50 & Target: Ki: 3.8 nM (mTOR)[1]

mTORC1, mTORC2[1] **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].

PROTOCOL (Extracted from published papers and Only for reference)

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].

Animal Administration: The dimesylate salt of GDC-0349 (Compound 8h) is formulated in 0.5% methylcellulose/0.2% Tween 80 (MCT) (Mice)[1].[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.

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.

CAIndexNames:

Urea, N-ethyl-N'-[4-[5,6,7,8-tetrahydro-4-[(3S)-3-methyl-4-morpholinyl]-7-(3-oxetanyl)pyrido[3,4-d]pyrimidin-2-yl]phenyl]-

SMILES:

O=C(NCC)NC(C=C1)=CC=C1C2=NC3=C(CCN(C4COC4)C3)C(N5[C@@H](C)COCC5)=N2

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