

Product Data Sheet

Product Name: GDC-0349

Cat. No.: GC12021

Chemical Properties

Cas. No. 1207360-89-1

Chemical Name 1-ethyl-3-[4-[4-[(3S)-3-methylmorpholin-4-yl]-7-(oxetan-3-yl)-6,8-dihydro-5H-pyrido[3,4-d]pyrimidin-2-yl]phenyl]urea

SMILES CCNC(=O)NC1=CC=C(C=C1)C2=NC3=C(CCN(C3)C4COC4)C(=N2)N5CCOCC5C

Formula $C_{24}H_{32}N_6O_3$

M.Wt 452.55

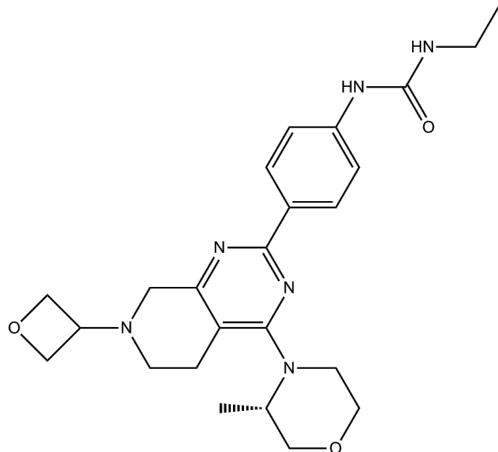
Solubility $\geq 4.55\text{mg/mL}$ in DMSO

Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Caution: Product has not been fully validated for medical applications. For research use only.

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**Kinase
experiment:**

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, MnCl₂, 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 μ M. They are serially diluted in 100% DMSO prior to further dilution with assay buffer. The reaction mixture (8 μ L) containing 0.25 nM mTOR+GBL enzyme, 400 nM GFP-4E-BP1, 8 μ M ATP, 50 mM Hepes pH 7.5, 0.01% Tween 20, 10 mM MnCl₂, 1 mM EGTA, 1 mM DTT, 1% DMSO ($\pm$ compound) is incubated at room temperature for 30 minutes. 8 μ L 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
experiment:**

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 μ L 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 mm³, 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 μ L in 1:1 PBS/Matrigel). Tumors are monitored until they reached a mean tumor volume of approximately 205 mm³, 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 mm³. The dimesylate salt of GDC-0349 is dosed daily or every third day by oral gavage (100 μ L 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.

Background

GDC-0349 is a potent, selective, ATP-competitive inhibitor of (Mammalian target of rapamycin) mTOR with Ki value of 3.8nM [1].

GDC-0349 is a remarkably selective mTOR inhibitor, with less than 25% inhibition of 266 kinases, including all isoforms of PI3K. GDC-0349 inhibited mTOR downstream markers , involving phospho-Akt(s473) and phospho-4EBP1 in an in vivo PK/PD study in mouse, consistent with an inhibition of mTORC1/mTORC2 complexes [1].

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GDC-0349 inhibited tumor growth in a dose-dependent manner when dosed orally once daily in athymic mice in a MCF7-neo/Her2 tumor xenograft model (PI3K mutation). GDC-0349 was also efficacious in other xenograft models, including PC3 (PTEN null) and 786-0 (VHL mutant). However, GDC-0349 showed only modest tumor growth delay in the A549 mouse xenograft model (KRas mutant, LKB1 deficient) [1].

References:

[1] Pei, Z.; Blackwood, E.; Liu, L.; Malek, S.; Belvin, M.; Koehler, M. F. T.; Ortwine, D. F.; Chen, H.; Cohen, F.; Kenny, J. R.; Bergeron, P.; Lau, K.; Ly, C.; Zhao, X.; Estrada, A. A.; Truong, T.; Epler, J. A.; Nonomiya, J.; Trinh, L.; Sideris, S.; Lesnick, J.; Bao, L.; Vijapurkar, U.; Mukadam, S.; Tay, S.; Deshmukh, G.; Chen, Y.-H.; Ding, X.; Friedman, L.; Lyssikatos, J. P. Discovery and biological profiling of potent and selective mTOR inhibitor GDC-0349 ACS Med. Chem. Lett. 2013, 4, 103– 107

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