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产品名称：LY2801653 (dihydrochloride)
产品别名：Merestinib dihydrochloride

生物活性：

Description	Merestinib dihydrochloride (LY2801653 dihydrochloride) is a potent, orally bioavailable c-Met inhibitor (Ki=2 nM) with anti-tumor activities. Merestinib dihydrochloride also has potent activity against MST1R (IC50=11 nM), FLT3 (IC50=7 nM), AXL (IC50=2 nM), MERTK (IC50=10 nM), TEK (IC50=63 nM), ROS1, DDR1/2 (IC50=0.1/7 nM) and MKNK1/2 (IC50=7 nM)[1][2].
IC_{50} & Target	Ki: 2 nM (c-Met)[1] IC50: 11 nM (MST1R), 7 nM (FLT3), 2 nM (AXL), 10 nM (MERTK), 63 nM (TEK), 0.1/7 nM (DDR1/2), 7 nM (MKNK1/2)[1]
In Vitro	Merestinib (LY2801653) also inhibits MST1R (IC50=11 nM), AXL (IC50=2 nM), MERTK (IC50=10 nM), TYRO3 (IC50=28 nM), ROS1, PDGFRA (IC50=41 nM), FLT3 (IC50=7 nM), TEK (IC50=63 nM), DDR1/2 (IC50=0.1/7 nM) and MKNK1/2 (IC50=7 nM)[1]. Merestinib demonstrates effects on MET pathway-dependent cell scattering and cell proliferation. The mean IC50 value (n=6 determinations) of Merestinib for inhibition of MET auto-phosphorylation in HGF-stimulated H460 cells is 35.2±6.9 nM and the IC50 for MET auto-phosphorylation in S114 cells is 59.2 nM. Transfection with the MET variants confers growth-factor independence and treatment with Merestinib inhibits growth of these MET variant clones with an IC50 ranging from 3-fold more potent (V1092I) to approximately 6-fold less potent (L1195V) compare with the growth inhibition of cells with the MET wild-type sequence[1]. Merestinib (2, 5, and 10 μM) reduces the number of viable TFK-1 and SZ-1 cells in a dose and time dependent manner, and significant inhibits wound healing for TFK-1 and SZ-1 cell lines. Merestinib inhibits cell invasion in TFK-1 and SZ-1 cells in a concentration dependent manner[2].
In Vivo	Merestinib (LY2801653) demonstrates anti-tumor effects in MET amplified (MKN45), MET autocrine (U-87MG, and KP4) and MET over-expressed (H441) xenograft models; and in vivo vessel normalization effects. Merestinib (LY2801653) is a type-II ATP competitive, slow-off inhibitor of MET tyrosine kinase with a pharmacodynamic residence time (K_{off}) of 0.00132 min^{-1} and $t_{1/2}$ of 525 min. Merestinib (LY2801653) treatment inhibits MET phosphorylation with a composite TED50 (50 % target inhibition dose) of 1.2 mg/kg and a composite TED90 (90 % target inhibition dose) of 7.4 mg/kg[1]. Merestinib (LY2801653) (20 mg/kg) reduces TFK-1 tumor growth significantly relative to vehicle control. Merestinib (LY2801653) inhibits the growth of intra- and extrahepatic CCC xenograft tumors[2].

In Vitro:

DMSO : ≥ 100 mg/mL (159.88 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.5988 mL	7.9942 mL	15.9885 mL
	5 mM	0.3198 mL	1.5988 mL	3.1977 mL
	10 mM	0.1599 mL	0.7994 mL	1.5988 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

Solvent&Solubility	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 (4.00 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.00 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 (4.00 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.00 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 (4.00 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.00 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]. Yan SB, et al. LY2801653 is an orally bioavailable multi-kinase inhibitor with potent activity against MET, MST1R, and other oncoproteins, and displays anti-tumor activities in mouse xenograft models. Invest New Drugs. 2013 Aug;31(4):833-44. [2]. Barat S, et al. Targeting c-MET by LY2801653 for treatment of cholangiocarcinoma. Mol Carcinog. 2016 Jan 12.

实验参考：

Cell Assay	H460 cells are cultured in RPMI media supplemented with 10% FBS and plated (prior to becoming 70% confluent) in 96-well plates at 20,000 cells/well and are incubated overnight at 37°C. The next day, the cells are incubated with RPMI-1640 in low serum (0.5% FBS) for 2 hours prior to treatment with Merestinib. Thirty minutes after the addition of Merestinib (LY2801653), HGF at a final concentration of 100ng/mL is added. After a 10-minute incubation, cell lysates are prepared and pMET is quantified. Relative IC_{50} values are determined using MSD activity units by calculating the percentage of inhibition with respect to on-plate MIN (unstimulated) and MAX controls and then fitting the percentage-of-inhibition values and 10-point dose response data to a 4-parameter logistic

	equation using ActivityBase[1].
Animal Administration	S114 cells are implanted subcutaneously onto female athymic nude mice. For dose response evaluation, on day 8 after the implantation, Merestinib (LY2801653) is given at a range of 0.75 mg/kg to 100 mg/kg (n=8 per dose group). At 2 hours after dose, blood samples and tumors are collected and flash frozen. For time course study, Merestinib (LY2801653) is given at 12 mg/kg (n=10 per time point). Animals are sacrificed at 2, 8, 16, and 24 hours after dose, and blood samples and tumors are collected. pMET is measured in the S114 tumor lysates using the MSD ELISA assay. Lysates are prepared from pulverized frozen tumor tissue, and homogenized with Lysing Matrix D beads, with addition of RIPA lysis buffer containing phosphatase and protease inhibitors. Protein concentration is determined using the DC protein assay kit. The pMET MSD ELISA assay is performed. [1]
Kinase Assay	The K_i value and mode of inhibition of LY2801653 for the MET kinase activity are determined using a radiometric filter-binding assay. Reactions are carried out in 96-well plates in Enzyme dilution buffer (EDB) compose of 50 mM Tris HCl pH 7.5, 2 mM DTT, 0.005% Triton X-100, 10 mM $MgCl_2$, and 250 μM EDTA. Serially diluted LY2801653 (final concentration 250 to 0 nM) are followed by the addition of a series of 8 concentrations of ^{33}P-γ-ATP (final concentration 400 to 10 μM ATP), and 5 nM enzyme (final concentration). After a 2-hour incubation, PolyGluTyr synthetic protein substrate (final 150 μg/mL) is added to initiate the 30-minute kinase reaction. Reactions are quenched with 10% H_3PO_4, transfer to a pre-wetted Multiscreen anionic phosphocellulose 96-well filter plate, and washed; radioactivity is measured with a scintillation counter. The experimental data are fit to a global mix model inhibition equation using GraphPad Prism softwar to generate an alpha value to determine the modality of inhibition and to calculate the K_i value for LY2801653[1].
References	[1]. Yan SB, et al. LY2801653 is an orally bioavailable multi-kinase inhibitor with potent activity against MET, MST1R, and other oncoproteins, and displays anti-tumor activities in mouse xenograft models. Invest New Drugs. 2013 Aug;31(4):833-44. [2]. Barat S, et al. Targeting c-MET by LY2801653 for treatment of cholangiocarcinoma. Mol Carcinog. 2016 Jan 12.