Merestinib dihydrochloride

Cat. No.: HY-15514A

CAS No.: 1206801-37-7

Molecular Formula: $C_{30}H_{24}Cl_2F_2N_6O_3$

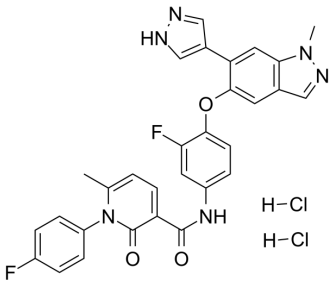
Molecular Weight: 625.45

Target: c-Met/HGFR; FLT3; ROS; Discoidin Domain Receptor

Pathway: Protein Tyrosine Kinase/RTK

Storage: 4°C, sealed storage, away from moisture

* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture)



SOLVENT & SOLUBILITY

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

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (4.00 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (4.00 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.00 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Merestinib dihydrochloride (LY2801653 dihydrochloride) is a potent, orally bioavailable c-Met inhibitor (K_i=2 nM) with anti-tumor activities. Merestinib dihydrochloride also has potent activity against MST1R (IC_{50}=11 nM), FLT3 (IC_{50}=7 nM), AXL (IC_{50}=2 nM), MERTK (IC_{50}=10 nM), TEK (IC_{50}=63 nM), ROS1, DDR1/2 (IC_{50}=0.1/7 nM) and MKNK1/2 (IC_{50}=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 (IC_{50}=11 nM), AXL (IC_{50}=2 nM), MERTK (IC_{50}=10 nM), TYRO3 (IC50=28 nM), ROS1,

PDGFRA (IC50=41 nM), FLT3 (IC_{50}=7 nM), TEK (IC50=63 nM), DDR1/2 (IC_{50}=0.1/7 nM) and MKNK1/2 (IC_{50}=7 nM)[1].
Merestinib demonstrates effects on MET pathway-dependent cell scattering and cell proliferation. The mean IC_{50} 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 IC_{50} 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 IC_{50} 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].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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

PROTOCOL

Kinase Assay [1]

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

Cell Assay [1]

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

Animal Administration [1]

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

CUSTOMER VALIDATION

- Cancer Discov. 2016 Dec;6(12):1334-1341.
- Clin Cancer Res. 2020 Jun 1;26(11):2615-2625.
- Methods Mol Biol. 2018;1711:351-398.
- Patent. WO2017019702A1.

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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.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA