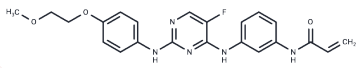
Data Sheet (Cat.No.T2603)

TargetMol

Spebrutinib

Chemical Properties



CAS No. : 1202757-89-8

Formula: C22H22FN5O3

Molecular Weight: 423.44

Appearance: no data available

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year

Biological Description

Description	Spebrutinib (LMK-435) is an orally bioavailable, selective inhibitor of Bruton's agammaglobulinemia tyrosine kinase (BTK), with potential antineoplastic activity.
Targets(IC50)	BTK,Src
In vitro	Oral administration of 3-30 mg/kg AVL-292 suppresses clinical symptoms of inflammation in a mouse model of collagen-induced arthritis, including the reduction of joint and paw swelling as well as visible redness in the affected paws.
In vivo	AVL-292 inhibits the activity of BTK, subsequently suppressing B-cell proliferation with an EC50 of 3 nM. Additionally, AVL-292 exerts an inhibitory effect on BTK expressed in Ramos cells, with an EC50 of 8 nM, demonstrating dose-dependency, and inhibits the downstream BCR pathway.
Kinase Assay	Procedures for BTK OMNIA Assay: The Omnia continuous read assay is performed essentially as described by the vendor. The assay conditions are: 40 μM ATP (1X KMATP), 10 μM Y5-Sox, and 10 nM BTK enzyme. Briefly, a substrate mix containing 1.13X ATP and the Y5 Sox substrate is first prepared in 1X Omnia Kinase Reaction Buffer (KRB) consisting of 20 mM Tris, pH 7.5, 5 mM MgCl2, 1 mM EGTA, 5 mM?β-glycerophosphate, 5% glycerol, and 0.2 mM DTT. For IC50 measurements, 5 μL of enzyme are incubated with serially diluted (3-fold) compounds prepared in 50% DMSO in a Corning (#3574) 384-well, white, non-binding surface microtiter plate at 25°C for 30 min. Kinase reactions are started with the addition of 45 μL of the ATP/Y5 substrate mix and monitored at λex360/λem485 in a Synergy 4 plate reader for 60 minutes. Progress curves from each well are examined for linear reaction kinetics and fit statistics. Initial velocity from each reaction is determined from the slope of a plot of relative fluorescence units versus time and then plotted against inhibitor concentration to estimate IC50 using the Response, Variable Slope model in GraphPad Prism from GraphPad Software.
Cell Research	A suspension of resting purified na?ve human B cells isolated by negative selection in RPMI is prepared at 0.4-0.5 × 106 cells/ml. Cells are mixed together with α-human IgM (final concentration of 5 μg/ml in each well) and vehicle (dimethyl sulfoxide) or AVL-292 (final concentrations of 0.01, 0.1, 1.0, 10.0, 100.0, or 1000 nM per well) and seeded in a 96-well plate. Cells are incubated for 56 hours in a humidified incubator maintained at 37°C and 5% CO2. 3H-Thymidine is added (final concentration of 1 μCi in each well) and cells are incubated overnight, harvested, and measured for 3H incorporation.

Experiments are performed in triplicate.(Only for Reference)

Solubility Information

Solubility	Ethanol: < 1 mg/mL (insoluble or slightly soluble), DMSO: 79 mg/mL (186.57 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.3616 mL	11.808 mL	23.6161 mL
5 mM	0.4723 mL	2.3616 mL	4.7232 mL
10 mM	0.2362 mL	1.1808 mL	2.3616 mL
50 mM	0.0472 mL	0.2362 mL	0.4723 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Reference

Evans E, et al. ASH Annual Meeting, 2011 San Diego, CA.
Jin X, Yang Y, Liu D, et al.Identification of a covalent NEK7 inhibitor to alleviate NLRP3 inflammasome-driven metainflammation.Cell Communication and Signaling.2024, 22(1): 565.
Evans EK, et al. J Pharmacol Exp Ther. 2013, 346(2), 219-228.