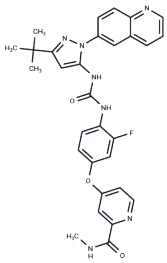
Data Sheet (Cat.No.T2640)

TargetMol

Rebastinib

Chemical Properties



CAS No. : 1020172-07-9

Formula: C30H28FN7O3

Molecular Weight: 553.59

Storage: Store at low temperature
Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	DCC-2036 (Rebastinib (DCC-2036)) is a conformational control Bcr-Abl inhibitor for Abl1 (WT, IC50: 0.8 nM) and Abl1(T315I, IC50: 4 nM), also inhibits LYN, SRC, HCK, FGR, FLT3, KDR, and Tie-2, and low activity to c-Kit. Rebastinib aimed at the Angiopoietin2-Tie2 pathway.
Targets(IC50)	Apoptosis,FLT,Bcr-Abl,Src
In vitro	In a xenograft mouse model bearing Ba/F3-BCR-ABL1T315I leukemia cells, DCC-2036 (100 mg/kg/day, p.o.) significantly inhibits BCR-ABL1 signaling and notably prolongs the lifespan of the mice.
In vivo	DCC-2036 exhibits antiproliferative activity against Ba/F3 cells expressing either wild-type or mutant BCR-ABL1, with IC50 values ranging from 2 to 150 nM. It also inhibits the proliferation of the Ph+ cell line K562 (IC50: 5.5 nM) and effectively induces apoptosis in Ba/F3 and K562 cells expressing BCR-ABL1. Notably, DCC-2036 selectively inhibits BCR-ABL positive cells by significantly suppressing CML cell lines compared to non-CML leukemia cell lines. It demonstrates potent non-ATP-competitive inhibition against unphosphorylated and phosphorylated ABL1 (IC50: 0.8/2 nM), unphosphorylated and phosphorylated mutant ABL1T315I (IC50: 1.4/5 nM), and the activation loop mutant ABL1H396P (IC50: 4 nM). Additionally, DCC-2036 inhibits SRC family kinases SRC/LYN/FGR/HCK and receptor TKs KDR/FLT3/TIE2 (IC50: 34/29/38/40/4/2/6 nM).
Kinase Assay	Assay of Abl1 kinase isoforms and determination of inhibitor potency: Activity of u-Abl1native is determined by following the production of ADP from the kinase reaction through coupling with the pyruvate kinase/lactate dehydrogenase system. In this assay, the oxidation of NADH (measured as a decreased A340 nM) is continuously monitored spectrophotometrically. The final reaction mixture (100 µL, in a 384-well Corning plate) is prepared as follows: An Abl1 kinase/coupled assay components mixture is prepared containing u-Abl1 kinase (1 nM), Abltide (EAIYAAPFAKKK, 0.2 mM), MgCl2 (9 mM), pyruvate kinase (~ 4 units), lactate dehydrogenase (~ 0.7 units), phosphoenol pyruvate (1 mM), and NADH (0.28 mM) in 90 mM Tris containing 0.1 % octyl-glucoside and 1 % DMSO, pH 7.5. Separately, an inhibitor mixture is prepared containing DCC-2036 serially diluted 3-fold in DMSO followed by dilution into buffer composed of 180 mM Tris, pH 7.5, containing MgCl2 (18 mM) and 0.2 % octyl-glucoside. Fifty µL of the inhibitor mixture is mixed with 50 µL of the above Abl1 kinase/coupled assay components mixture, which is then incubated at 30 °C for 2 hours before 2 µL of 25 mM ATP (500 µM, final) is added to

Kinase Assay	start the reaction. The reaction is recorded every 2 minutes for 2.5 hours at 30 °C on a Polarstar Optima or Synergy2 plate reader. Reaction rate (slope) is calculated using the 1 to 2 hour time frame with reader's software. Percent inhibition is obtained by comparison of reaction rate with that of a DMSO control. IC50 values are calculated from a series of percent inhibition values determined at a range of inhibitor concentrations using GraphPad Prism. The kinase assay for Abl1T315I, p-Abl1native or Abl1H396P is assayed the same as above except that 2.2 nM Abl1T315I, 1 nM p-Abl1 native or 1.3 nM Abl1H396P is used. The above assay format is also used for kinases other than Abl1 with the exception of TIE2, for which a fluorescence polarization/Transcreener format is used.The assay conditions are the same as described above except that PolyE4Y (final 1 mg/mL) is used as the substrate and one hour preincubation is used.
Cell Research	Ba/F3 cells or primary Ph+ leukemia cells are plated in triplicate in 96-well plates containing test compounds. After 72 hours, viable cells are quantified by Resazurin or MTT assay. Cells are diluted in medium to be added to each well of a 96-well tissue culture-treated plate. All cells are incubated overnight and maintained in a humidified atmosphere at 37 °C and 5% CO2. Cells are treated the following day. Serum-free medium is used during treatment with DCC-2036. MTT is used to assess the viability of cells following treatment. Aliquots of 20 mL of stock MTT solution are added to each well containing 200 mL of medium (10% final solution) and incubated with the cells for 2 hours. Following incubation the medium is removed and 200 mL of dimethylsulfoxide added to solubilize the formazan crystals. The absorbance is read on the plate reader at 550 and 690 nm. A subtraction analysis of the dual wavelength is performed (D550 to D690) to increase accuracy of the measuremen(Only for Reference)

Solubility Information

Solubility	Ethanol: 13 mg/mL (23.48 mM),Sonication is recommended. DMSO: 5.54 mg/mL (10.01 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 1 mg/mL (1.81 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.8064 mL	9.032 mL	18.0639 mL
5 mM	0.3613 mL	1.8064 mL	3.6128 mL
10 mM	0.1806 mL	0.9032 mL	1.8064 mL
50 mM	0.0361 mL	0.1806 mL	0.3613 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.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Chan WW, et al. Cancer Cell. 2011, 19(4), 556-568.
Cheng S, Jin P, Li H, et al. Evaluation of CML TKI Induced Cardiovascular Toxicity and Development of Potential Rescue Strategies in a Zebrafish Model. Frontiers in Pharmacology. 2021: 2866.
Gillen J, Richardson D, Moore K. Angiopoietin-1 and Angiopoietin-2 Inhibitors: Clinical Development. Curr Oncol Rep. 2019 Feb 26;21(3):22.

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