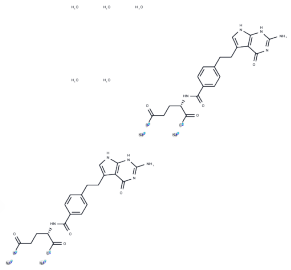
Data Sheet (Cat.No.T6226)

TargetMol

Pemetrexed disodium hemipenta hydrate

Chemical Properties



CAS No. : 357166-30-4

Formula: C20H21N5O6·2Na·5/2H2O

Molecular Weight: 518.43

Storage: 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	Pemetrexed disodium hemipenta hydrate (LY-231514 Disodium Hydrate) is a new-type antifolate and antimetabolite for TS, DHFR, and GARFT. The Ki of Pemetrexed Disodium Hydrate for TS, DHFR and GARFT is 1.3 nM, 7.2 nM and 65 nM, respectively.
Targets(IC50)	Apoptosis,Antifolate,Autophagy,DHFR,DNA/RNA Synthesis
In vitro	In the human H460 non-small cell lung carcinoma xenograft model, Pemetrexed disodium effectively inhibits tumor growth.
In vivo	In CCRF-CEM leukemia, GC3/C1 colon cancer, and HCT-8 ileocecal colon cancer cells, Pemetrexed disodium exhibited antitumor activity with IC50 values of 25 nM, 34 nM, and 220 nM, respectively.
Kinase Assay	Enzyme Assays and Methods.: TS activity is assayed using a spectrophotometric method,which involved monitoring the increase in absorbance at 340 nm resulting from formation of the product,7,8-dihydrofolate.The assay buffer contains 50 mM N-tris [hydroxymethyljmethyl-2-aminoethanesulfonic acid,25 mM MgC12,6.5 mM formaldehyde,1 mM EDTA,and 75 mM 2-mercaptoethanol,pH 7.4.The concentrations of deoxyuridylate monophosphate,6R-MTHF,and hIS are 100 μM,30 μM and 30 nM (1.7 milliunits/mL),respectively.At the 6R-MTHF concentration,an uninhibited reaction and six concentrations of inhibitor are assayed.Ki app values are determined by fitting the data to the Morrison equation using nonlinear regression analysis with the aid of the program ENZFITTER.Ki values are calculated using the equation: Ki app= Ki(1 + [S]/Km), where [S] is equal to 30 μM and Km is equal to 3 μM.DHFR activity is assayed spectrophotometrically by monitoring the dis appearance of the substrates NADPH and 7,8-dihydrofolate at 340 nm.The reaction takes place at 25°C in 0.5 mL of 50 mM potassium phosphate buffer,which contains 150 mM KC1 and 10 nM 2-mercaptoethanol, pH 7.5,and 14 nM (0.34 milliunitlmL) DHFR.The NADPH concentration is 10 μM and 7,8-dihydrofolate is varied at 5,10,or 15 μM.At each 7,8-dihydrofolate concentration,an uninhibited reaction and seven concentrations of inhibitor are assayed.The ENZFITI'ER microcomputer program is used to obtain Ki app values by fitting the data to the Morrison equation by nonlinear regression analysis.Ki app= Ki(1 + [S]/Km),where [S] is equal to the concentration of 7,8-dihydrofolate used and Km of 7,8-dihydrofolate is equal to 0.15 μM.GARFT activity is assayed spectrophotometrically by monitoring the increase of absorbance resulting from formation of the product 5,8-dideazafolate at 295 nm.The reaction solvent contains 75 mM HEPES,20% glycerol,and 50 mM a-thioglygerol,

Kinase Assay	pH 7.5,at 25°C.
Cell Research	Dose-response curves are generated to determine the concentration required for 50% inhibition of growth (IC50). Pemetrexed disodium is dissolved initially in DMSO at a concentration of 4 mg/mL and further diluted with cell culture medium to the desired concentration. CCRF-CEM leukemia cells in complete medium are added to 24-well Cluster plates in a total volume of 2.0 mL. Pemetrexed disodium at various concentrations are added to duplicate wells so that the final volume of DMSO is 0.5%. The plates are incubated for 72 hour at 37 °C in an atmosphere of 5% CO2 in air. At the end of the incubation, cell numbers are determined on a ZBI Coulter counter. For several studies, IC50s are determined for each compound in the presence of either 300 μM AICA, 5 μM thymidine, 100 μM hypoxanthine, or combination of 5 μM hymidine plus 100 μM hypoxanthine. For adherent tumor cells, a modification of the original MTT colorimetric assay is used to measure cell cytotoxicity. The human tumor cells are seeded in 100 μL assay medium/well in 96-well flat-bottomed tissue culture plates. The assay medium contains folic acid-free RPMI 1640 supplemented with 10% FCS and either 2 nM folinic acid or 2.3 μM folic acid as the sole folate source. Well 1A is left blank. Stock solutions of antifolates are prepared in Dulbecco's PBS at 1 mg/mL, and a series of 2-fold dilutions are subsequently made in PBS. Ten-μL aliquots of each concentration are added to triplicate wells. Plates are incubated for 72 hours at 37 °C in a humidified atmosphere of 5% CO2-in-air. MTT is dissolved in PBS at 5 mg/mL, 10 μL of stock MTF solution are added to each well of an assay, and the plates are incubated at 37 °C for 2 additional hours. Following incubation, 100 μL of DMSO are added to each well. After thorough formazan solubilization, the plates are read on a Dynatech MR600 reader, using a test wavelength of 570 nm and a reference wavelength of 630 nm.The IC50 is determined as the concentration of drug required to inhibit cell growth by 50% compared to an untreated controls.(Only for Reference)

Solubility Information

Solubility	H2O: 100 mg/mL (192.89 mM),Sonication is recommended. DMSO: Slightly soluble, (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.9289 mL	9.6445 mL	19.289 mL
5 mM	0.3858 mL	1.9289 mL	3.8578 mL
10 mM	0.1929 mL	0.9645 mL	1.9289 mL
50 mM	0.0386 mL	0.1929 mL	0.3858 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

Shih C, et al. Cancer Res. 1997, 57(6), 1116-1123.
Bi G, Liang J, Zhao M, et al. MiR-6077 promotes cisplatin/pemetrexed resistance in lung adenocarcinoma by targeting CDKN1A/cell cycle arrest and KEAP1/ferroptosis pathways. Molecular Therapy-Nucleic Acids. 2022
Iwahori K, et al. Int J Cancer. 2012, doi: 10.1002/ijc.27611.
Zhao G, Liang J, Shan G, et al.KLF11 regulates lung adenocarcinoma ferroptosis and chemosensitivity by suppressing GPX4.Communications Biology.2023, 6(1): 570.
Teicher BA, et al. Clin Cancer Res. 2000, 6(3), 12016-1023.
Zhao G, Liang J, Zhang Y, et al.MNT inhibits lung adenocarcinoma ferroptosis and chemosensitivity by suppressing SAT1.Communications Biology.2024, 7(1): 680.