Product Datasheet

Technical Data

Formula	$C_{20}H_{21}N_5O_6.5/2H_2O.2Na$		
Molecular Weight	516.42	**CAS No.**	357166-30-4
Solubility (25°C)*	In vitro	Water	100 mg/mL (193.64 mM)
		DMSO	Insoluble
		Ethanol	Insoluble

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Capsid Bio tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.
* Room temperature shipping (Stability testing shows this product can be shipped at 2-8°C for storage purposes.)

Preparing Stock Solutions

Concentration \ Volume / Mass	1 mg	5 mg	10 mg
1 mM	1.9364 mL	9.6820 mL	19.3641 mL
5 mM	0.3873 mL	1.9364 mL	3.8728 mL
10 mM	0.1936 mL	0.9682 mL	1.9364 mL
50 mM	0.0387 mL	0.1936 mL	0.3873 mL

Biological Activity

Description	Pemetrexed Disodium Hydrate (LY-231514) is a novel antifolate and antimetabolite for TS, **DHFR** and GARFT with **K_i** of 1.3 nM, 7.2 nM and 65 nM, respectively. This compound stimulates **autophagy** and **apoptosis**.
Targets	TS [1] / 1.3 nM(Ki); DHFR [1] / 7.2 nM(Ki); GARFT [1] / 65 nM(Ki)
In vitro	Pemetrexed disodium hydrate shows the antiproliferative activity in CCRF-CEM leukemia, GC3/C1 colon carcinoma, and HCT-8 ileocecal carcinoma cells with IC50 of 25 nM, 34 nM and 220 nM, respectively. [1] A recent study shows that cisplatin plus this compound combined with SOCS-1 gene delivery shows the antitumor effect by inhibition of cell proliferation, invasiveness, and induction of apoptosis in MPM cells infected with adenovirus-expressing SOCS-1 vector. [2]
In vivo	In the human H460 non-small cell lung carcinoma xenograft, Pemetrexed disodium hydrate produces a duration-dependent tumor growth delay (TGD). [3]

Protocol (from reference)

Kinase Assay:[1]

- **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[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 25 mM $MgC1_2$, 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, 30μM and 30 nM (1.7 milliunits/mL), respectively. At the 6R-MTHF concentration, an uninhibited reaction and six concentrations of inhibitor are assayed. $K_{i\ app}$ values are determined by fitting the data to the Morrison equation using nonlinear regression analysis with the aid of the program ENZFITTER. K_i values are calculated using the equation: $K_{i\ app} = K_i(1 + [S]/K_m)$, where [S] is equal to 30 μM and K_m 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 $K_{i\ app}$ values by fitting the data to the Morrison equation by nonlinear regression analysis. $K_{i\ app} = K_i(1 + [S]/K_m)$, where [S] is equal to the concentration of 7,8-dihydrofolate used and K_m 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, pH 7.5, at 25°C. The concentrations of substrates and enzyme used are 10 μM α,β-glycinamide ribonucleotide, 0-10 μM 10-formyl-5,8-dideazafolic acid, and 10 nM (1.9 milliunits/mL) GARFT. K_i values are calculated using the Enzyme Mechanism program of the Beckman DU640 spectrophotometer, which uses nonlinear regression analysis to fit data to the Michaelis-Menten equation for competitive inhibition.

Cell Assay:[1]

- **Cell lines**

CCRF-CEM leukemia, GC3/C1 colon carcinoma, and HCT-8 ileocecal carcinoma cells.

- **Concentrations**

0-30 μM

- **Incubation Time**

72 hours

- **Method**

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. This compound 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% CO_2 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% CO_2-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.

Animal Study:[3]

- **Animal Models**
  EMT-6 mammary carcinoma, the human HCT 116 colon carcinoma, and the human H460 non-small cell lung carcinoma are injected s.c. into the nude mice.
- **Dosages**
  100 mg/kg or 150 mg/kg
- **Administration**
  Administered via i.p.

References

https://pubmed.ncbi.nlm.nih.gov/9067281/ https://pubmed.ncbi.nlm.nih.gov/22532243/ https://pubmed.ncbi.nlm.nih.gov/10741729/

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