# Pemetrexed

**Cat. No.:** HY-10820

**CAS No.:** 137281-23-3

**Molecular Formula:** $C_{20}H_{21}N_5O_6$

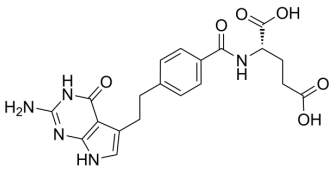
**Molecular Weight:** 427.41

**Target:** Antifolate; Autophagy

**Pathway:** Cell Cycle/DNA Damage; Autophagy

**Storage:**

| | | |
|---|---|---|
| Powder | -20°C | 3 years |
| | 4°C | 2 years |
| In solvent | -80°C | 6 months |
| | -20°C | 1 month |



## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : 250 mg/mL (584.92 mM; Need ultrasonic)

$H_2O$ : < 0.1 mg/mL (ultrasonic;warming;heat to 60°C) (insoluble)

**Preparing Stock Solutions**

| Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|
| 1 mM | 2.3397 mL | 11.6984 mL | 23.3967 mL |
| 5 mM | 0.4679 mL | 2.3397 mL | 4.6793 mL |
| 10 mM | 0.2340 mL | 1.1698 mL | 2.3397 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: ≥ 2.08 mg/mL (4.87 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (4.87 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (4.87 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

Pemetrexed (LY231514) is an antifolate, the $K_i$ values of the pentaglutamate of Pemetrexed (LY231514) are 1.3, 7.2, and 65 nM for inhibits thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), respectively[1].

**$IC_{50}$ & Target**

Ki: 1.3 nM (TS), 7.2 nM (DHFR), 65 nM (GARFT)[1]

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| **In Vitro** | Pemetrexed (LY231514) disodium is a novel classical antifolate, the antitumor activity of which may result from simultaneous and multipie inhibition of several key folate-requiring enzymes via its polyglutamated metabolites. Pemetrexed (LY231514) is one of the best substrates that is known for the enzyme FPGS ($K_m$=1.6 μM and $V_{max}/K_m$=621). It is likely that polyglutamation and the polyglutamated metabolites of LY231514 play profound roles in determining both the selectivity and the antitumor activity of this novel agent. Whereas LY23l5l4 only moderately inhibits TS ($K_i$=340 nM, recombinant mouse), the pentaglutamate of LY23l5l4 is 100-fold more potent ($K_i$=3.4 nM), making LY231514 one of the most potent folate-based TS inhibitors[1].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **In Vivo** | The group of mice treated with PC61 plus Pemetrexed demonstrates statistically longer survival than other groups. In a survival analysis, significantly better survival is observed in the group of mice treated with PC61 plus Pemetrexed compare with those treated with PC61 alone, rat IgG plus Pemetrexed, or no treatment[2].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

| | |
|---|---|
| **Kinase Assay** [1] | AICARFT inhibition assays are carried out at room temperature by monitoring the formation of [6S]-5,6,7,8-tetrahydrofolate from 10-formyl-[6R,S]-5,6,7,8-tetrahydrofolate at $A_{298}$. All solutions are purged with $N_2$ gas prior to use. The reaction solution contains 33 mM Tris-Cl, pH 7.4, 25 mM KCl, 5 mM 2-Mercaptoethanol, 0.05 mM AICA ribonucleotide, and 16 nM (2 milliunits/mL) of AICARFT. 10-Formyl-[6R,S]-5,6,7,8-tetrahydrofolate concentrations of 0.037, 0.074, and 0.145 mM are used (0.61, 1.23, and 2.45 times its $K_m$ value, respectively). LY231514 is tested as an inhibitor at 0.08-0.8 mM (four concentrations). When the tri- and pentaglutamates of LY231514 are used as inhibitors, the concentrations are 0.0005-0.009 mM (eight concentrations). Enzyme assays are initiated by the addition of enzyme. Data is analyzed using the ENZFITTER program for competitive inhibition.<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Cell Assay** [1] | Dose-response curves are generated to determine the concentration required for 50% inhibition of growth ($IC_{50}$). Pemetrexed 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 at a final concentration of $4.8\times10^4$ cells/well in a total volume of 2 mL. Test compounds at various concentrations are added to duplicate wells so that the final volume of DMSO is 0.5%. The plates are incubated for 72 h 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. Control wells usually contain $4\times10^5$ to $6\times10^5$ cells at the end of the incubation. For several studies, $IC_{50}$s 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[1].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration** [2] | Mice[2]<br>Female CBA mice and female NOD/SCID mice (NOD.CB17-Prkdc$^{scid}$) at 6-8 wk of age are used. Premetrexed (100 mg/kg) is given i.p. from days 4-8 (5 consecutive d) to tumor-bearing mice to explore the synergistic effect when combined with anti-CD25 Ab or IgG control. The dose and schedule used for Pemetrexed in the current study is determined based on previous studies in mice.<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## CUSTOMER VALIDATION

- Mol Cell. 2019 Dec 5;76(5):838-851.e5.
- J Clin Invest. 2021 Aug 2;131(15):e138022.
- Cell Rep Med. 2023 Jan 10;100911.

- Theranostics. 2020 May 15;10(13):6048-6060.
- J Control Release. 2022 Dec 9;353:490-506.

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## REFERENCES

[1]. Shih C, et al. LY231514, a pyrrolo[2,3-d]pyrimidine-based antifolate that inhibits multiple folate-requiring enzymes. Cancer Res. 1997 Mar 15;57(6):1116-23.

[2]. Anraku M, et al. Synergistic antitumor effects of regulatory T cell blockade combined with pemetrexed in murine malignantmesothelioma. J Immunol. 2010 Jul 15;185(2):956-66.



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