# Leflunomide

**Cat. No.:** HY-B0083

**CAS No.:** 75706-12-6

**Molecular Formula:** $C_{12}H_9F_3N_2O_2$

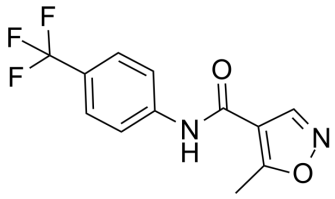
**Molecular Weight:** 270.21

**Target:** Dihydroorotate Dehydrogenase; Endogenous Metabolite; Bacterial

**Pathway:** Metabolic Enzyme/Protease; Anti-infection

**Storage:**

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



## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : ≥ 50 mg/mL (185.04 mM)

* "≥" means soluble, but saturation unknown.

**Preparing Stock Solutions**

| Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|
| 1 mM | 3.7008 mL | 18.5041 mL | 37.0083 mL |
| 5 mM | 0.7402 mL | 3.7008 mL | 7.4017 mL |
| 10 mM | 0.3701 mL | 1.8504 mL | 3.7008 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: 2.5 mg/mL (9.25 mM); Suspended solution; Need ultrasonic and warming
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (9.25 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (9.25 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

Leflunomide is a pyrimidine synthesis inhibitor, inhibiting dihydroorotate dehydrogenase (DHODH), and acts as a disease-modifying antirheumatic agent.

**In Vitro**

Leflunomide is actually a prodrug that has been shown to inhibit proliferation of mononuclear and T-cells. Leflunomide is an inhibitor of several protein tyrosine kinases, with $IC_{50}$ values between 30 mM and 100 mM in vitro cellular and enzymatic assays[1].

Leflunomide is capable of inhibiting anti-CD3- and interleukin-2 (IL-2)-stimulated T cell proliferation. Leflunomide is able to inhibit p59fyn and p56lck activity in in vitro tyrosine kinase assays. Leflunomide also inhibits $Ca^{2+}$ mobilization in Jurkat cells stimulated by anti-CD3 antibody but not in those stimulated by ionomycin. Leflunomide also inhibits distal events of anti-CD3 monoclonal antibody stimulation, namely, IL-2 production and IL-2 receptor expression on human T lymphocytes. Leflunomide also inhibits tyrosine phosphorylation in CTLL-4 cells stimulated by IL-2[2].
Leflunomide is an immunomodulatory drug that may exert its effects by inhibiting the mitochondrial enzyme dihydroorotate dehydrogenase (DHODH), which plays a key role in the de novo synthesis of the pyrimidine ribonucleotide uridine monophosphate (rUMP). Leflunomide prevents the expansion of activated and autoimmune lymphocytes by interfering with the cell cycle progression due to inadequate production of rUMP and utilizing mechanisms involving p53[3].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## PROTOCOL

**Kinase Assay** [1]

DHODase activity is measured by the DCIP colorimetric assay. This is a coupled assay in which oxidation of DHO and subsequent reduction of ubiquinone are stoichiometrically equivalent to the reduction of DCIP. Reduction of DCIP is accompanied by a loss of absorbance at 610 nm (ε=21500 M/cm). The assay is performed in a 96-well microtiter plate at ambient temperature (ca. 25°C). Stock solutions of 10 mM leflunomide and A771726 are prepared in dimethyl sulfoxide (DMSO) and these are diluted with reaction buffer (100 mM Tris and 0.1 % Triton X-100, pH 8.0) to prepare working stocks of the inhibitors at varying concentrations. For each reaction, the well contained 10 nM DHODase, 68 μM DCIP, 0.16 mg/mL gelatin, the stated concentration of ubiquinone, 10 μL of an inhibitor working stock to give the stated final concentration, and reaction buffer. After a 5-min equilibration period, the reaction is initiated by addition of DHO to the stated final concentrations. The total volume of reaction mixture for each assay is 150 μL, and the final DMSO concentration is ≤ 0.01% (v/v). The reaction progress is followed by recording the loss of absorbance at 610 nm over a 10-min period (during which the velocity remained linear). Velocities are reported as the change in absorbance at 610 nm per minute, and each reported value is the average of three replicates. In experiments where the DHO or ubiquinone concentration is varied, the other substrate is held constant at 200 μM. To determine the inhibitor potency of leflunomide and A771726, the effects of varying concentrations of the two compounds on the initial velocity of the DHODase reaction is measured over a concentration range of 0.01−1.0 μM. In these experiments the DHO and ubiquinone concentrations are held constant at 200 and 100 μM, respectively.
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Haematologica. 2018 Sep;103(9):1472-1483.
- Cancer Lett. 2018 Mar 28;417:21-34.
- Drug Des Devel Ther. 2020 May 18;14:1897-1908.
- Biotechnol Bioeng. 2021 Sep 3.
- Chin J Integr Med. 2021 Jul 28.

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## REFERENCES

[1]. Davis JP, et al. The immunosuppressive metabolite of leflunomide is a potent inhibitor of human dihydroorotate dehydrogenase. Biochemistry. 1996 Jan 30;35(4):1270-3.

[2]. Xu X, et al. Inhibition of protein tyrosine phosphorylation in T cells by a novel immunosuppressive agent, leflunomide. J Biol Chem. 1995 May 26;270(21):12398-403.

[3]. Fox RI, et al. Mechanism of action for leflunomide in rheumatoid arthritis. Clin Immunol. 1999 Dec;93(3):198-208.



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