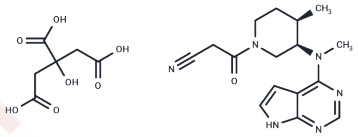
Data Sheet (Cat.No.T2398)

TargetMol

Tofacitinib Citrate

Chemical Properties



CAS No. : 540737-29-9

Formula: C22H28N6O8

Molecular Weight: 504.49

Storage: Store at low temperature,Keep away from moisture, Keep away from direct sunlight
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	Tofacitinib Citrate (CP-690550 citrate) is a a potent, cell-permeable inhibitor of JAK1/2/3 (IC50s: 1/20/112 nM).
Targets(IC50)	Apoptosis,Antibacterial,Antifungal,Influenza Virus,JAK
In vitro	Although Tofacitinib (CP-690,550) was highly potent for JAK3 inhibition (enzyme inhibitory potency of 1 nM), it was 20- to 100-fold less potent for JAK2 and JAK1, respectively. CP-690,550 inhibited IL-2-induced proliferation with 30-fold greater potency than its effects on GM-CSF-induced proliferation. CP-690,550 demonstrated potent inhibition in the mixed lymphocyte reaction using murine, monkey, or human cells. Consistent with its mechanism of action, these cellular activities correlated with the ability of CP-690,550 to block IL-2-induced phosphorylation of JAK3 and one of its key substrates, STAT5 [1]. CP-690,550 treatment of murine factor-dependent cell Patersen-erythropoietin receptor (FDCP-EpoR) cells harboring human wild-type or V617F JAK2 resulted in inhibition of cell proliferation with an IC50 of 2.1 microM and 0.25 microM, respectively. CP-690,550 treatment of ex-vivo-expanded erythroid progenitors from JAK2 (V617F)-positive PV patients resulted in specific, antiproliferative (IC50: 0.2 microM) and pro-apoptotic activity [2]. The pharmacological inhibition of JAK3 by tofacitinib synergistically enhanced the antitumor effects of IMA in CML cells [3].
In vivo	CP-690,550 treatment significantly prolonged graft survival as compared to vehicle. Four of 12 animals dosed with CP-690,550 (two from each dose group) survived to study termination with normal renal function and mild rejection as determined by histopathology [1]. Monotherapy of mice with tofacitinib quells Ab responses to an immunotoxin derived from the bacterial protein Pseudomonas exotoxin A, as well as to the model Ag keyhole limpet hemocyanin. Thousand-fold reductions in IgG1 titers to both Ags were observed 21 d post immunization. Tofacitinib treatment led to reduced numbers of CD127+ pro-B cells [4].
Kinase Assay	The JAK1, JAK2, and JAK3 kinase assays utilize a protein expressed in baculovirus-infected SF9 cells (a fusion protein of GST and the catalytic domain of human JAK enzyme) purified by affinity chromatography on glutathione sepharose. The substrate for the reaction was polyglutamic acid-tyrosine [PGT (4:1)], coated onto Nunc Maxi Sorp plates at 100 µg/mL overnight at 37 °C. The plates were washed three times, and JAK

Kinase Assay	enzyme was added to the wells, which contained 100 μL of kinase buffer (50 mM HEPES, pH 7.3, 125 mM NaCl, 24 mM MgCl2) + ATP + 1 mM sodium orthovanadate). After incubation at room temperature for 30 min, the plates were washed three times. The level of phosphorylated tyrosine in a given well was determined by standard ELISA assay utilizing an anti-phosphotyrosine antibody [5].
Cell Research	Apoptotic cells were detected by flow cytometry using recombinant human Annexin-V conjugated with allophycocyanin. Briefly, after exposure to CP-690,550 for different periods of time, cells were washed in Ca2+-free PBS and resuspended in 100 μL of binding buffer (10 mM HEPES pH 7.4; 0.15 M NaC1; 5 mM KCl; 1 mM MgCl2; 1.8 mM CaCl2) to which Annexin-V-APC had been previously added. Cells were incubated for 20 min in the dark at room temperature, washed and resuspended in 0.3 mL binding buffer. Cells were analyzed on a FACSCalibur flow cytometer equipped with the Cell Quest Pro software [2].
Animal Research	Mice received tofacitinib in PEG300 (100 mg/ml) or vehicle alone (PEG300) by osmotic pump infusion (Alzet Model 2004, 0.25 μl/hour, 28 days). Four days prior to immunization, mice were anesthetized and their dorsal surface was shaved. A one cm incision was made on the back to create a subcutaneous pocket and insert the pump. The incision site was closed with wound clips. Mice were injected weekly (i.p.) with SS1P recombinant immunotoxin (RIT; 5 μg/mouse) beginning on day 0; control mice received injections of saline alone. Every week before SS1P or vehicle immunization, ~50 μl of blood was drawn to obtain serum samples. Sera were stored at ?80°C until analyzed [4].

Solubility Information

Solubility	DMSO: 250 mg/mL (495.55 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: 2 mg/mL (3.96 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	1.9822 mL	9.911 mL	19.822 mL
5 mM	0.3964 mL	1.9822 mL	3.9644 mL
10 mM	0.1982 mL	0.9911 mL	1.9822 mL
50 mM	0.0396 mL	0.1982 mL	0.3964 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

Changelian PS, et al. Prevention of organ allograft rejection by a specific Janus kinase 3 inhibitor. Science. 2003 Oct 31;302(5646):875-8.

Manshouri T, et al. The JAK kinase inhibitor CP-690,550 suppresses the growth of human polycythemia vera cells carrying the JAK2V617F mutation. Cancer Sci. 2008 Jun;99(6):1265-73.

Yagi K, et al. Pharmacological inhibition of JAK3 enhances the antitumor activity of imatinib in human chronic myeloid leukemia. Eur J Pharmacol. 2018 Apr 15;825:28-33.

Onda M, et al. Tofacitinib suppresses antibody responses to protein therapeutics in murine hosts. J Immunol. 2014 Jul 1;193(1):48-55.

Flanagan ME, et al. Discovery of CP-690,550: a potent and selective Janus kinase (JAK) inhibitor for the treatment of autoimmune diseases and organ transplant rejection. J Med Chem. 2010 Dec 23;53(24):8468-84.