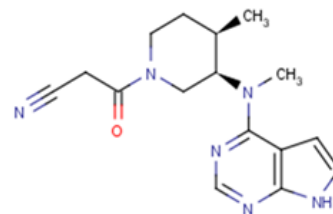


Tofacitinib

Chemical Properties

CAS No. : 477600-75-2
Formula: C₁₆H₂₀N₆O
Molecular Weight: 312.37
Appearance: solid
Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year



Biological Description

Description	Tofacitinib (Tasocitinib) is an oral, small molecule inhibitor of Janus kinases that is used to treat moderate-to-severe rheumatoid arthritis.
Targets(IC50)	ROCK,Apoptosis,JAK,Src
In vitro	Administering 1.5-15 mg/kg of CP-690550 daily over an extended period to mice and using flow cytometry for monitoring, it was observed that lymphocyte subpopulations underwent dose- and time-dependent changes. Notably, after 21 days, the most significant change detected was a 96% reduction in spleen NK1.1+TCRb cell count. Subsequent treatment with CP-690550 (1.87-30 mg/kg, s.c.) led to a dose-dependent decrease in delayed-type hypersensitivity (DTH) reactions in sensitized mice. In a model using ectopic heart transplants (from DBA2 donors to C57/BL6 hosts), CP-690550 treatment resulted in a dose-dependent increase in graft survival, with an EC ₅₀ (concentration required to maintain transplant survival in 50% of the mice for >28 days) of approximately 60 ng/mL. Additionally, CP-690550 effectively prevented the rejection of allogeneic kidney transplants in non-human primates (NHPs, macaca fascicularis), with mean survival times (MST) in the experimental groups receiving 50 to 100 ng/ml and 200 to 400 ng/ml doses being 62 and 83 days, respectively.
In vivo	CP-690550 effectively inhibits IL-2-induced proliferation, exhibiting a potency 30 times greater than its effect on GM-CSF-induced proliferation. This compound efficaciously suppresses the murine mixed lymphocyte reaction (MLR) with an IC ₅₀ value of 91 nM. On murine B cells, CP-690550 potently inhibits the IL-4-induced upregulation of CD23 (IC ₅₀ = 57 nM) and the expression of Class II Major Histocompatibility Complex (MHCII) (IC ₅₀ = 71 nM). CP-690550 is a specific oral inhibitor of JAK3, with 20 and 100 times lower effectivity on JAK2 and JAK1, respectively. Studies demonstrate that low doses of CP-690550 accelerate the onset of experimental autoimmune encephalomyelitis by promoting Th17 differentiation.
Kinase Assay	JAK3 Kinase Assay: A fragment encoding the catalytic domain of human JAK3 (785aa to 1125aa, JH1 catalytic domain) is amplified by PCR from the full length cDNA and cloned into the EcoRI site of the baculovirus transfer vector pVL1393. Recombinant baculovirus is used to infect Sf9 (Spodoptera frugipedra) cells and recombinant GSTJAK3 fusion protein is isolated on glutathione sepharose. The fusion protein is eluted with reduced glutathione and stored in buffer containing 50 mM Tris, pH 7.5, 10 mM DTT and 10% glycerol. JAK3 kinase activity is measured by ELISA as follows: Plates are coated overnight with a random L-glutamic acid and tyrosine co-polymer (4:1) (100 ug/mL).

The plates are washed and recombinant JAK3 JH1:GST (100 ng/well) with or without inhibitors is incubated at room temperature for 30 minutes, after which HRP-conjugated PY20 anti-phosphotyrosine antibody (ICN) is added and developed by TMB (3,3',5,5'-tetramethylbenzidine). Other kinases (Table 1) are produced in E. coli or in insect cells, depending upon what is found to be optimal for the given kinase. The catalytic activity of tyrosine kinases is measured using the aforementioned ELISA, whereas serine/threonine kinases are assayed using radioactive enzyme assays.

Cell Research

To measure IL-2-dependent proliferation, isolated lymphocytes are resuspended to a cell density of $1-2 \times 10^6$ /mL in complete RPMI medium (RPMI 1640 containing 10% (w/v) fetal calf serum (FCS), 1%(w/v) penicillin and streptomycin). Phytohemagglutinin (PHA) is added to a final concentration of 10 mg/mL, and the culture incubated for 3 days at 37 °C in a humidified 5% (v/v) CO₂ incubator to upregulate IL-2R and JAK3 expression. IL-2 (200U/mL), with or without CP-690,550 is then added and the cells are incubated for 72 hours at 37 °C in a humidified 5% (v/v) CO₂ incubator, after which 50 mL of 3H-thymidine (5mCi/mL) is added. The plates are incubated for an additional 18 hours, harvested with a 96-well harvester, and counted on a scintillation counter. HUO3 cells are maintained in culture with granulocyte-macrophage colony stimulating factor and human foreskin fibroblasts are maintained in culture with 10% fetal calf serum. CP-690550 is added to freshly plated cells and cultured for 4 days. 3Hthymidine is added during the last 18 hours of the culture period. (Only for Reference)

Solubility Information

Solubility

H₂O: < 1 mg/mL (insoluble or slightly soluble),
DMSO: 58 mg/mL (185.7 mM),
Ethanol: < 1 mg/mL (insoluble or slightly soluble),
< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.2013 mL	16.0067 mL	32.0133 mL
5 mM	0.6403 mL	3.2013 mL	6.4027 mL
10 mM	0.3201 mL	1.6007 mL	3.2013 mL
50 mM	0.064 mL	0.3201 mL	0.6403 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.

Reference

Si H, Wang J, He R, et al. Identification of U937/JAK3-M511I Acute Myeloid Leukemia Cells as a Sensitive Model to JAK3 Inhibitor. *Frontiers in oncology*. 2021, 11: 807200-807200.

Inhibitor · Natural Compounds · Compound Libraries · Recombinant Proteins

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