Tofacitinib citrate

Cat. No.: HY-40354A

CAS No.: 540737-29-9

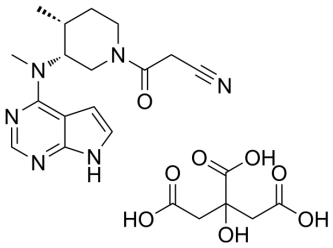
Molecular Formula: $C_{22}H_{28}N_6O_8$

Molecular Weight: 504.49

Target: JAK; Apoptosis; Bacterial; Fungal; Influenza Virus

Pathway: Epigenetics; JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Stem Cell/Wnt; Apoptosis; Anti-infection

Storage: 4°C, sealed storage, away from moisture and light
* In solvent : -80°C, 2 years; -20°C, 1 year (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 28.57 mg/mL (56.63 mM; Need ultrasonic)
H_2O : 3.33 mg/mL (6.60 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9822 mL	9.9110 mL	19.8220 mL
	5 mM	0.3964 mL	1.9822 mL	3.9644 mL
	10 mM	0.1982 mL	0.9911 mL	1.9822 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 (4.96 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (4.96 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.96 mM); Clear solution
4. Add each solvent one by one: 50% PEG300 >> 50% saline
   Solubility: 2.5 mg/mL (4.96 mM); Clear solution; Need ultrasonic
5. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: ≥ 1.43 mg/mL (2.83 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Tofacitinib citrate is an orally available JAK1/2/3 inhibitor with IC_{50}s of 1, 20, and 112 nM, respectively. Tofacitinib citrate has antibacterial, antifungal and antiviral activities.

IC_{50} & Target	JAK3 1 nM (IC_{50})	JAK2 20 nM (IC_{50})	JAK1 112 nM (IC_{50})	Rock-II 3400 nM (IC_{50})
	Lck 3870 nM (IC_{50})			

In Vitro

Tofacitinib (CP-690550) citrate binds potentially at JAK3 and JAK2 as 2.2 nM and 5 nM (K_d). The report includes additional binding for Tofacitinib at Camk1 (K_d of 5,000 nM), DCamkL3 (K_d of 4.5 nM), Mst2 (K_d of 4,300 nM), Pkn1 (K_d of 200 nM), Rps6ka2 (Kin.Dom.2-C-terminal) (K_d of 1,400 nM), Rps6ka6 (Kin.Dom.2-C-terminal) (K_d of 1,200 nM), Snark (K_d of 420 nM), Tnk1 (K_d of 640 nM) and Tyk2 (K_d of 620 nM)[1].

K562, KCL22, and THP-1 cells are exposed to different doses of STI571 or JAK inhibitors for 72 h to quantify the effects of tyrosine kinase inhibitor (TKI) activity. Cell growth inhibition is then evaluated using the MTT assay. The proliferation of K562 and KCL22 cells, but not THP-1 cells, is inhibited by IMA in a concentration-dependent manner. The IC_{50} value of IMA is 0.28 μM for K562 and 0.17 μM for KCL22. Although treatment with Tofacitinib (TOF) or INCB018424 alone does not suppress cell proliferation, both Tofacitinib and INCB018424 make the K562 and KCL22 more sensitive to IMA[4].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

Animals that are treated with Tofacitinib show a significantly lower production of anti-drug antibodies (ADAs) compare with PEG-treated control mice (for five weeks after initial immunization, $p<0.01$, n=8). Moreover ADAs become detectable earliest on day 28. A difference of 1000- to 200-fold in titers to SS1P is apparent from days 21 through 35, respectively. Compare to SS1P, mice injected with keyhole limpet hemocyanin (KLH) generate a more rapid antibody response. Yet, the administration of Tofacitinib reduces anti-KLH titers compare to controls ($p<0.05$ on day 21, $p<0.01$ on day 28, respectively, n=5). Reductions in titers ranged from 5000- to 250-fold from days 21 through 28, respectively[2]. Based on previous dose-response studies, a daily dose of Tofacitinib of 6.2 mg/kg is selected to provide 80% inhibition of hind paw volume and plasma exposure capable of suppressing the JAK1 and JAK3 signaling pathways for >4 hours[3].

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PROTOCOL

Cell Assay [4]

Cell survival assay is performed using MTT. Briefly, K562, KCL22, and THP-1 cells (1×10^5 cells/well) are plated onto 96-well microplates and treated with or without IMA (0.06-1.0 μM) and/or Tofacitinib (10-1,000 nM) or RUX (10-1,000 nM) for 72 h at 37°C in a humidified 5% (v/v) CO_2 atmosphere. The medium (200 μL) is then incubated with 10 μL of 5 mg/ml MTT solution for 4 h at 37°C. After being centrifuged at 352 g for 5 min, the culture medium is removed, and 100 μL of DMSO are added to each well to dissolve the formazan. Absorbance is measured at 570 nm using a microplate reader. The results are expressed as percentages[4].

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Animal Administration [2][3]

Mice[2]

Female BALB/c mice (6-8 weeks old) are used. Mice receive Tofacitinib in PEG300 (100 mg/mL) or vehicle alone (PEG300) by osmotic pump infusion (0.25 μL/hour, 28 days). Four days prior to immunization, mice are anesthetized and their dorsal surface is shaved. A one cm incision is made on the back to create a subcutaneous pocket and insert the pump. The incision site is closed with wound clips. Mice are 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 is drawn to obtain serum samples. Sera are stored at -80°C until analyzed.

Rats[3]

Adjuvant-induced arthritis (AIA) is induced in female Lewis rats. Rats are randomized according to hind paw volume and assigned to Tofacitinib or vehicle treatment regimens. Groups of 7-8 rats per treatment group, and normal naive rats (n=4 per group), are euthanized either 4 hours, 4 days, or 7 days after beginning treatment (days 16, 20, and 23 after immunization, respectively). Once-daily oral administration of vehicle or Tofacitinib (6.2 mg/kg) is initiated on day 16 following immunization and continued through day 23. Paw volumes are reassessed 4 and 7 days after the beginning of treatment (days 20 and 23 after immunization, respectively). For micro-computed tomography (micro-CT) imaging, as well as tartrate-resistant acid phosphatase (TRAP) staining in paw tissue, AIA is induced in a separate cohort of Lewis rats.

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CUSTOMER VALIDATION

- Ann Rheum Dis. 2021 Sep;80(9):1201-1208.
- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- J Allergy Clin Immunol. 2023 Dec 8:S0091-6749(23)02411-9.
- Nano Res. 2023 Apr 15.
- BMC Med. 2021 Oct 15;19(1):247.

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REFERENCES

[1]. Jiang JK, et al. Examining the chirality, conformation and selective kinase inhibition of 3-((3R,4R)-4-methyl-3-(methyl(7H-pyrrolo[2,3-d]pyrimidin-4-yl)amino)piperidin-1-yl)-3-oxopropanenitrile (CP-690,550). J Med Chem. 2008 Dec 25;51(24):8012-8.

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

[3]. LaBranche TP, et al. JAK inhibition with tofacitinib suppresses arthritic joint structural damage through decreased RANKL production. Arthritis Rheum. 2012 Nov;64(11):3531-42.

[4]. Yagi K, et al. Pharmacological inhibition of JAK3 enhances the antitumor activity of STI571 in human chronic myeloid leukemia. Eur J Pharmacol. 2018 Apr 15;825:28-33.

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