Tofacitinib

Cat. No.: HY-40354

CAS No.: 477600-75-2

Molecular Formula: $C_{16}H_{20}N_6O$

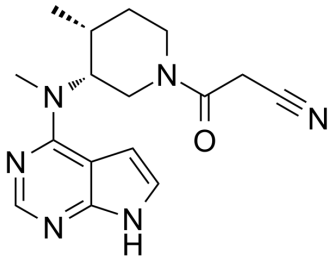
Molecular Weight: 312.37

Target: JAK; Apoptosis

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

Storage:

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



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (400.17 mM; Need ultrasonic)

Ethanol : 2.5 mg/mL (8.00 mM; Need ultrasonic)

H_2O : 0.15 mg/mL (0.48 mM; Need ultrasonic and warming)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 0.5% MC >> 0.5% Tween-80
   Solubility: 5 mg/mL (16.01 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 2.5 mg/mL (8.00 mM); Clear solution
3. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (8.00 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (6.66 mM); Clear solution
5. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (6.66 mM); Clear solution
6. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (6.66 mM); Clear solution

BIOLOGICAL ACTIVITY

Description	Tofacitinib is an orally available JAK3/2/1 inhibitor with IC_{50}s of 1, 20, and 112 nM, respectively.
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]. 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Kinase activity is recorded via a competition binding assay of selected kinases that are fused to a proprietary tag. Measurements of the amount of kinase binds to an immobilized, active-site directed ligand in the presence and absence of the test compound (e.g., Tofacitinib) provide a % of DMSO control for binding of ligand. Activities between 0 and 10 are selected for Kd determinations. Dendrogram representations are generated by an in-house visualization tool designated PhyloChem[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Human $CD4^+$ positive cells are enriched from peripheral blood mononuclear cells obtained from a healthy donor by magnetic separation ($CD4^+$ MACS beads). $CD4^+$ cells are activated for 3 days with plate bound anti-CD3 and anti CD28 antibodies (5 ug/mL each), and then expanded for another 4 days in the presence of IL-2 (50 U/mL). Cells are rested overnight in 1% RPMI, and pre-incubated with Tofacitinib or DMSO control for 1 hour at indicated concentrations (5 nM, 50 nM, 500 nM; DMSO concentration is equal in all preparations) and then activated with IL-2 (1000 u/mL) or IL-12 (100 ng/mL) for 15 minutes. Cells (10×10^6/condition) are lysed in 1% Triton-x lysis buffer and equal amounts of cell lysate are run in NuPage Bis-Tris gel (4-12% gradient). Proteins are transferred onto nitrocellulose membrane. Detection is done with indicated antibodies using Odyssey western blotting system[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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 (Alzet Model 2004, 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). Tofacitinib is suspended in 0.5% methylcellulose/0.025% Tween 20 for in vivo studies. 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.

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

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.
- Sci Adv. 2024 Mar 22;10(12):eadl0368.
- Nano Res. 2023 Apr 15.

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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]. Calama E, et al. Tofacitinib ameliorates inflammation in a rat model of airway neutrophilia induced by inhaled LPS. Pulm Pharmacol Ther. 2017 Apr;43:60-67.

Caution: Product has not been fully validated for medical applications. For research use only.

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