Tofacitinib citrate (CP-690550 citrate) Datasheet

**Biological Activity**

**Description**
Tofacitinib citrate (CP-690550) is a potent JAK3 inhibitor with IC50 of 1 nM.

**Targets**
JAK3

**IC50**
1 nM[1]

**In vitro**
Tofacitinib citrate inhibits IL-2-mediated human T cell blast proliferation and IL-15-induced CD69 expression with IC50 of 11 nM and 48 nM, respectively. Tofacitinib citrate prevents mixed lymphocyte reaction with IC50 of 87 nM. Tofacitinib citrate treatment of murine factor-dependent cell Paterson–erythropoietin receptor (FDCP-EpoR) cells harboring human wild-type or V617F JAK2 leads to prevention of cell proliferation with IC50 of 2.1 μM and 0.25 μM, respectively. Tofacitinib citrate inhibits interleukin-6-induced phosphorylation of STAT1 and STAT3 with IC50 of 23 nM and 77 nM, respectively. Moreover, Tofacitinib citrate generates a significant pro-apoptotic effect on murine FDCP-EpoR cells carrying JAK2V617F, whereas a lesser effect was observed for cells carrying wild-type JAK2. This activity is coupled with the inhibition of phosphorylation of the key JAK2V617F-dependent downstream signaling effectors signal transducer and activator of transcription (STAT3), STAT5, and vakti human thymoma viral oncogene homolog (AKT).[2] Additionally, Tofacitinib citrate prevents IL-15-induced CD69 expression in human and cynomolagus monkey NK and CD8+ T cells in vitro.[3]

**In vivo**
Tofacitinib citrate decrease a delayed-type hyper-sensitivity response and extended cardiac allograft survival in murine models. Furthermore, Tofacitinib citrate treatment of ex-vivo-expanded erythroid progenitors from JAK2V617F-positive PV patients results in specific, antiproliferative (IC50 = 0.2 μM) and pro-apoptotic activity. In contrast, expanded progenitors from healthy controls are less sensitive to Tofacitinib citrate in proliferation (IC50 > 1.0 μM), and apoptosis assays.[2] During 2 weeks of Tofacitinib citrate dosing at 10 and 30 mg/kg, a significant, time-dependent decrease in NK cell numbers relative to vehicle treatment is observed. Effector memory CD8+ cell numbers in the Tofacitinib citrate-treated group are 55% less than those observed in animals treated with vehicle.[2]

**Clinical Trials**
Tofacitinib citrate is under a Phase II clinical trial in the treatment of chronic plaque psoriasis.

**Features**

**Protocol (Only for Reference)**

**Kinase Assay**[1]

**Enzyme assays**
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 is polymutated acid-tyrosine [PGT (4:1)], coated onto Nunc Maxi Sorp plates at 100 μg/mL overnight at 37 °C. The plates are washed three times, and JAK enzyme is added to the wells, which contained 100 μL of kinase buffer (50 mM HEPES, pH 7.3, 125 mM NaCl, 24 mM MgCl2), 1 mM ATP, 1 mM sodium orthovanadate). For Tofacitinib citrate, it is also added for kinase assay at different doses. After incubation at room temperature for 30 min, the plates are washed three times. The level of phosphorylated tyrosine in a given well is determined by standard ELISA assay utilizing an antiphosphotyrosine antibody.

**Cell Assay**[2]

**Cell Lines**

FDCP-EpoR JAK2WT and JAK2V617F cell lines

**Concentrations**
0-4 μM

**Incubation Time**
72 hours

**Methods**
Determination of growth inhibition by Tofacitinib citrate is performed using identical culture conditions for both FDCP-EpoR JAK2WT and JAK2V617F cell lines. Briefly, 1 × 10^5 cells/mL are cultured in 96-well flat-bottom plates at 37 °C in a humidified 5% CO2 atmosphere using RPM 1640 supplemented with 1.25% FCS, and 5% WEHI supernatant. Decreased FCS concentration is necessary to prevent binding between Tofacitinib citrate and serum proteins. Growth inhibition assays are terminated by addition of 20 μL CellTiter96 One Solution Reagent. Flat-bottom plates are incubated for an additional 3 hours for MTT assay. Absorbance is determined at 595 nm on a BioTek Synergy-HT microplate reader. Results are the average ± standard deviation of three independent determinations.

**Solubility (25°C)**

- DMOS: 63.0 mg/mL
- Water: <1 mg/mL
- Ethanol: 63 mg/mL

**Storage**

- 2 years, -20°C, Powder
- 2 weeks, 4°C in DMOS
- 6 months, -80°C in DMOS

**Return Policy**
Selleck Chemicals wishes you the best possible online shopping experience with our 365 day unconditional Return Policy. If you are not satisfied with your purchase, either for protocol related or product related problems, you may return any item(s) within 365 days from the original purchase date. Please see the following instructions when you return products.

1. All requests for returns should be communicated to Selleck Chemicals prior to shipping. Any items returned to Selleck Chemicals should be in the original packaging and in the same condition as originally purchased.
2. When returning purchased goods, please inform us of the purchase order number or package tracking number.
3. Return shipping is absolutely FREE.
4. This offer is only valid for products purchased directly from Selleck and its authorized distributors.
5. Once your return request is received and approved, your refund will be processed or automatically applied to your credit card within 7 days. Please note that depending on your credit card company, it may take additional 2-10 business days for us to post the refund to your account.

**Toll Free:**
(877) 796-6397
-- USA and Canada only--

**Fax:**
+1-713-796-9816

**Orders:**
+1-832-582-8158
sales@selleckchem.com

**Tech Support:**
technical@selleckchem.com
(biology support)
technical@selleckchem.com

**Website:**
www.selleckchem.com

---

[1] Tofacitinib citrate-treated group are 55% less than those observed in animals treated with vehicle.

[2] Moreover, Tofacitinib citrate generates a significant pro-apoptotic effect on murine FDCP-EpoR cells carrying JAK2V617F, whereas a lesser effect was observed for cells carrying wild-type JAK2. This activity is coupled with the inhibition of phosphorylation of the key JAK2V617F-dependent downstream signaling effectors signal transducer and activator of transcription (STAT3), STAT5, and vakti human thymoma viral oncogene homolog (AKT).


---

www.selleckchem.com/datasheet/CP-690550-Datasheet.html
**Animal Study:**

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>Mauritius-origin adult cynomolgus monkeys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>0.5% methylcellulose in distilled water</td>
</tr>
<tr>
<td>Doses</td>
<td>10, 30 mg/kg/d</td>
</tr>
<tr>
<td>Administration</td>
<td>Oral gavage</td>
</tr>
</tbody>
</table>

**References**


**Customer Reviews**

Data independently produced by Dr. Akihiko Yoshimura of Akihiko Yoshimura. Tofacitinib citrate (CP-690550 citrate) purchased from Selleck.

The STAT3 inhibitor CP690,550 inhibits arthritis in vivo and the expression of IL-6 cytokine family in vitro.

(A) Whole-cell lysates from MC3T3-E1 cells stimulated with IL-1β (10 ng/ml) plus CP690,550 at the indicated concentrations were analyzed by immunoblotting to detect pSTAT3 and STAT3. Actin served as an internal control. (B) 6-week-old DBA/1 male mice were given an initial injection of type 2 collagen on day -21, and arthritis was induced with a second injection on day 0. Vehicle or CP690,550 (15 mg/kg/day) was administered intraperitoneally once daily for 2 weeks from day 7 (n = 4 per group). Arthritis scores were measured three times a week. (C and D) Total RNA was prepared from primary osteoblasts treated with IL-1β (10 ng/ml), TNFα (10 ng/ml) or OSM (50 ng/ml) with (+) or without (-) CP690,550 (100 nM) for 24 hours, and IL-6 expression relative to β-actin was analyzed by quantitative real-time PCR. Data are means ± SD of IL-6/β-actin. (P < 0.001; n = 3). (E) IL-6 protein levels in the supernatant of osteoblasts treated with IL-1β (left panel) or TNF (right panel) plus indicated concentrations of CP690,550 for 24 hours were assessed by ELISA. Data are means ± SD of IL-6 (pg/ml).
Specific storage and handling information for each product is indicated on the product datasheet. Most Selleck products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality, but save your shipping charges by using the most economical storage conditions for an overnight shipment. Upon receipt of the product, follow the storage recommendations on the product datasheet.