**PRODUCT INFORMATION**

**Content:**
- 5 mg CP-690550

**Storage and stability:**
- CP-690550 is provided as a solid and shipped at room temperature. Store at -20°C. Solid product is stable for 2 years when properly stored.
- Upon resuspension in DMSO, prepare aliquots of CP-690550 and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6 months when properly stored.

**Quality control:**
- Purity: ≥96% (LC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) is confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

**DESCRIPTION**

CP-690550 (Tofacitinib) specifically inhibits JAK3, which has a pivotal role in cytokine signal transduction that governs lymphocyte survival, proliferation, differentiation, and apoptosis. In experimental models, treatment with CP-690550 decreases IL-6 production, a critical cytokine that drives inflammation. Moreover, it has been shown that CP-690550 also inhibits TNF-induced chemokine expression.


**CHEMICAL PROPERTIES**

**Synonym:** Tofacitinib
**CAS number:** 477600-75-2
**Formula:** C_{16}H_{20}N_{6}O
**Molecular weight:** 312.37
**Solubility:** 100 mg/ml in DMSO

**METHODS**

**Preparation of stock solution (20 mM)**
1. Add 800 µl DMSO to 5 mg CP-690550 and vortex until complete solubilization.
2. Prepare aliquots and store stock solution at -20°C. Further dilutions can be prepared using aqueous buffers. We do not recommend storing the aqueous solution for more than one day.

**Working concentration:** 10 nM - 4 µM (3 ng/ml - 1.25 µg/ml) for cell culture assays

**Inhibition assay**
Described below is a protocol to study the JAK/STAT pathway in the human THP-1 monocyte reporter cell line, THP1-Blue™ ISG cells.
1. Prepare a THP1-Blue™ ISG cell suspension at ~625,000 cells/ml.
2. Add 160 µl of cell suspension (~100,000 cells/ml) to each well.
3. Add 20 µl of CP-690550 at a final concentration of 10 nM - 4 µM and incubate at 37 °C for 1 hour.
4. Add 20 µl of sample per well of a flat-bottom 96-well plate. Note: We recommend using a positive control such as IFN-α at 100 IU/ml.
5. Incubate the plate at 37 °C in a 5% CO₂ incubator for 18-24 hours.
6. Monitor SEAP production using a SEAP detection assay, such as QUANTI-Blue™.

**PROTOCOLS**

For reference only; as described in the indicated publications.

**Cell Culture Assay**
- **Cells:** Human T cells
- **Working concentration:** 10 nM - 4 µM
- **Incubation time:** 72 hours

**Animal Study**
- **Animal model:** Male DBA/J1 mice
- **Dose:** 1.5 - 15 mg/kg/day
- **Administration:** Subcutaneous implantation of osmotic pumps

**RELATED PRODUCTS**

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<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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</thead>
<tbody>
<tr>
<td>AG490</td>
<td>JAK1 &amp; JAK2 inhibitor</td>
<td>tlr1-ag4</td>
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<tr>
<td>AZD1480</td>
<td>JAK1 &amp; JAK2 inhibitor</td>
<td>inh-ad14</td>
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<tr>
<td>Ruxolitinib</td>
<td>JAK1 &amp; JAK2 inhibitor</td>
<td>tlr1-rux</td>
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<tr>
<td>THP1-Blue™ ISG cells</td>
<td>SEAP reporter cells</td>
<td>thp-isg</td>
</tr>
</tbody>
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