CYT387
JAK1/JAK2 & TBK-1/IKK-e inhibitor
Catalog # inh-cy87
http://www.invivogen.com/cyt387
For research use only
Version # 17E15-MM

PRODUCT INFORMATION
Contents:
- 10 mg CYT387

Storage and stability:
- CYT387 is shipped at room temperature. Store at -20°C.
- Upon resuspension, prepare aliquots of CYT387 and store at -20°C. Resuspended CYT387 is stable for 6 months when properly stored.

Quality control:
- Purity ≥97% (UHPLC)
- The inhibitory activity of CYT387 has been assessed using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION
CYT387, also known as Momelotinib, is a potent ATP-competitive inhibitor of Janus kinases JAK1 and JAK2, thereby interrupting signaling via the JAK-STAT (signal transducers and activators of transcription) pathway. CYT387 is significantly less active against other kinases, including JAK3. In addition, CYT387 is a potent inhibitor of the noncanonical IkB kinases IKK-e and TANK-binding kinase 1 (TBK1). As a result, CYT387 prevents both NF-kB and STAT activation. Consequently, this multi-target inhibitor disrupts the expression of pro-tumorigenic cytokines, induces apoptosis and suppresses proliferation of many cell types, in particular cells harboring the JAK2V617F mutation which is associated with blood cancers.1,3,4


CHEMICAL PROPERTIES
Solubility: 70 mg/ml (168.9 mM) in DMSO
CAS number: 1056634-68-4
Formaldehyde: C23H22N6O2
Molecular weight: 414.47
Structure:

RUXOLITINIB
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METHODS
Preparation of 10 mg/ml (24.1 mM) stock solution
- Add 1 ml of DMSO to 10 mg CYT387. Mix by vortexing.
- Prepare further dilutions with sterile, endotoxin-free water.

Working concentration: 100 ng/ml - 30 μg/ml (241.2 nM - 72.4 μM) for cell culture assays

Inhibition assay:
Described below is a protocol to study the JAK/STAT pathway in the murine B16 melanoma reporter cells, B16-Blue™ ISG cells.
1. Prepare a B16-Blue™ ISG cell suspension at ~470,000 cells/ml.
2. Add 160 μl of cell suspension (~75,000 cells) per well.
3. Add 20 μl of CYT387 100 ng/ml -30 μg/ml (final concentration) 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: Murine pro-B cells Ba/F3-JAK2V617F & human erythroleukemia cells
Working concentration: 100 nM - 5 μM (41.5 ng/ml - 2.075 μg/ml)
Incubation time: 2 - 72 hours
Methods: Inhibition assay and Western blot (STAT-3 & STAT-5)

Cell Culture Assay:
Cells: Murine macrophages RAW 264.7 & human carcinoma cells A549
Working concentration: 10 nM - 10 μM (4.15 ng/ml - 4.15 μg/ml)
Incubation time: 1 - 72 hours (10 days for clonogenic assay)
Methods: Cell viability, clonogenic assay, qRT-PCR (CCL5 & IL6 mRNA), and Western blot (IKK-ε, TBK1 & STAT-3)

Animal Study:
Animal model: Mice with Kras-driven lung cancer
Dose: 100 mg/kg
Administration: oral gavage once daily

Animal Study:
Animal model: Balb/c mice with myeloproliferative neoplasms
Dose: 25 - 50 mg/kg
Administration: oral gavage twice daily

RELATED PRODUCTS

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<td>a549d-difs</td>
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<tr>
<td>AG490</td>
<td>JAK2 Inhibitor</td>
<td>tlr-ag4</td>
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<tr>
<td>B16-Blue™ ISG Cells</td>
<td>SEAP reporter cells</td>
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<td>TBK1 &amp; IKK-e inhibitor</td>
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<td>CP-690550</td>
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<td>Ruxolitinib</td>
<td>JAK1 &amp; JAK2 inhibitor</td>
<td>tlr-rux</td>
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