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.

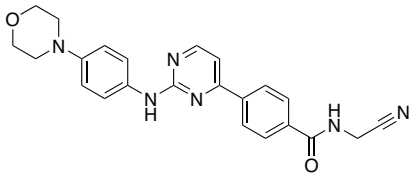
DESCRIPTION

CYT387, also known as Momelotinib, is a potent ATP-competitive inhibitor of Janus kinases JAK1 and JAK2[1], 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)[2]. 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 cells types, in particular cells harboring the JAK2V617F mutation which is associated with blood cancers[1, 3, 4].

1. Pardanani A. et al., 2009. CYT387, a selective JAK1/JAK2 inhibitor: In vitro assessment of kinase selectivity and preclinical studies using cell lines and primary cells from polycythemia vera patients. Leukemia 23:1441–5. **2. Zhu Z. et al., 2014.** Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. Cancer Discov. 4:452-65. **3. Tyner JW. et al., 2010.** CYT387, a novel JAK2 inhibitor, induces hematologic responses and normalizes inflammatory cytokines in murine myeloproliferative neoplasms. Blood. 15:5232-40. **4. Monaghan KA. et al., 2011.** The novel JAK inhibitor CYT387 suppresses multiple signalling pathways, prevents proliferation and induces apoptosis in phenotypically diverse myeloma cells. Leukemia. 25:1891-9.

CHEMICAL PROPERTIES

Solubility: 70 mg/ml (168.9 mM) in DMSO
CAS number: 1056634-68-4
Formula: C23H22N6O2
Molecular weight: 414.5
Structure:



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% CO_2 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[1]
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: Proliferation assay and Western blot (STAT-3 & STAT-5)

Cell Culture Assay[2]
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, kinase assay, qRT-PCR (CCL5 & IL6 mRNA), and Western blot (IKK-ε, TBK1 & STAT-3)

Animal Study[2]
Animal model: Mice with Kras-driven lung cancer
Dose: 100 mg/kg
Administration: oral gavage once daily

Animal Study[3]
Animal model: Balb/c mice with myeloproliferative neoplasms
Dose: 25 - 50 mg/kg
Administration: oral gavage twice daily

RELATED PRODUCTS

Product	Description	Cat. Code
A549-Dual™ Cells	Dual reporter cells	a549d-nfis
AG490	JAK2 Inhibitor	tlrl-ag4
B16-Blue™ ISG Cells	SEAP reporter cells	bb-ifnabg
BX795	TBK1 & IKK-e inhibitor	tlrl-bx7
CP-690550	JAK3 Inhibitor	tlrl-cp69
Ruxolitinib	JAK1 & JAK2 inhibitor	tlrl-rux

TECHNICAL SUPPORT
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