# (Z)-LFM-A13

**Cat. No.:** HY-18009

**CAS No.:** 244240-24-2

**Molecular Formula:** $C_{11}H_8Br_2N_2O_2$

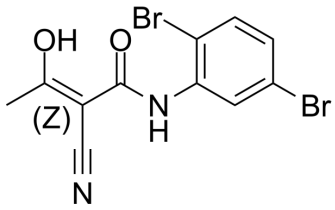
**Molecular Weight:** 360.01

**Target:** Btk; Polo-like Kinase (PLK); JAK

**Pathway:** Protein Tyrosine Kinase/RTK; Cell Cycle/DNA Damage; Epigenetics; JAK/STAT Signaling; Stem Cell/Wnt

**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 : ≥ 42 mg/mL (116.66 mM)

* "≥" means soluble, but saturation unknown.

**Preparing Stock Solutions**

| Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|
| 1 mM | 2.7777 mL | 13.8885 mL | 27.7770 mL |
| 5 mM | 0.5555 mL | 2.7777 mL | 5.5554 mL |
| 10 mM | 0.2778 mL | 1.3889 mL | 2.7777 mL |

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

**In Vivo**

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.94 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (6.94 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.94 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

LFM-A13 is a potent BTK, JAK2, PLK inhibitor, inhibits recombinant BTK, Plx1 and PLK3 with $IC_{50}$s of 2.5 μM, 10 μM and 61 μM; LFM-A13 shows no effects on JAK1 and JAK3, Src family kinase HCK, EGFR and IRK.

**$IC_{50}$ & Target**

| Plx1 | PLK3 | BRK | BMX |
|---|---|---|---|
| 10 μM ($IC_{50}$) | 61 μM ($IC_{50}$) | 267 μM ($IC_{50}$) | 281 μM ($IC_{50}$) |

| | | | |
|---|---|---|---|
| | FYN<br>240 μM ($IC_{50}$) | Hepatocyte growth factor receptor kinase (Met)<br>215 μM ($IC_{50}$) | BTK<br>2.5 μM ($IC_{50}$) |

| | |
|---|---|
| **In Vitro** | LFM-A13 significantly inhibits BTK activity with an $IC_{50}$ of 6.2 ± 0.3 μg/mL (= 17.2 ± 0.8 μM). The calculated $K_i$s of LFM-A13 for BTK, JAK1, JAK3, IRK, EGFR and HCK are 1.4, 110, 148, 31.6, 166 and 214 μM. LFM-A13 (200 μM) markedly increases the chemosensitivity of ALL-1 cells to ceramide-induced apoptosis[1]. LFM-A13 (100 μM) suppresses Epo-induced phosphorylation of EpoR, Jak2, Btk, Stat5 and Erk1/2 in R10 cells. LFM-A13 (100 μM) inhibits auto-phosphorylation of Jak2, Tec and Btk rather than Lyn kinase auto-phosphorylation in COS cells[2]. LFM-A13 potently inhibits Plx1 with $IC_{50}$ of 10 μM; also inhibits BRK, BMX, FYN and with $IC_{50}$s of 267, 281, 240 and 215 μM[4].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **In Vivo** | LFM-A13 (25, 50 and 100 mg/kg) shows no apparent toxicity to rats. LFM-A13 (50 mg/kg, three times a week, i.p.) attenuates mammary tumorigenesis in mice. LFM-A13 alone or in combination with paclitaxel shows marked effect on the breast tumor incidence, mean tumor numbers, average tumor weight, and size in BALB/c mice. LFM-A13 (50 mg/kg, three times a week, i.p.) significantly decreases PLK1, cyclin D1, CDK-4, P53 and Bcl-2 expression, but increases the expression of p21, IκB, Bax and caspase 3 expression in mice[3]. LFM-A13 (200 mg/kg) does not cause hematologic toxicity in rats. LFM-A13 (10 or 50 mg/kg, i.p.) exhibits anti-tumor effects dose dependently in the MMTV/Neu transgenic mouse model of breast cancer[4].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

| | |
|---|---|
| **Kinase Assay [4]** | Purified His6-Plx1 (250 ng) is added to a 20 μL reaction mixture containing 1× kinase buffer (10 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, and 1 mM TT), 25 μM cold ATP, and 1 μCi [γ-$^{32}$P]ATP in the presence of different concentrations of LFM-A13 ranging from 5 μg/mL (13.9 μM) to 100 μg/mL (278 μM). The reaction mixtures are incubated at room temperature for 15-30 min and autophosphorylation is stopped by addition of 2× SDS-PAGE reducing sample buffer. A parallel experiment is performed in the presence of cold ATP. The kinase reactions are then subjected to immunoblotting using the commercially available anti-Plk antibodies. The immunoblots confirmed that the same amount of Plx1 protein is present in each reaction. In addition, we also examined the effects of LFM-A13 on substrate phosphorylation by Plx1. In brief, 250 ng of purified Plx1 is first incubated at room temperature for one hour with different concentrations of LFM-A13. After one hour of incubation, the tubes containing the reaction mixtures are put on ice and the substrate, GST-Cdc25 peptide (254-316) (200 ng), kinase buffer, and [γ-$^{32}$P]ATP are added and the kinase reaction allowed to proceed for 15 min at room temperature. Immunoblotting with anti-Cdc25 antibodies is used to confirm that equal amounts of the substrate peptide are present in each reaction mixture. Anti-Plk antibodies, the polyclonal antibodies to gluthathione-S-transferase (GST) and ECL kit are used in the assay. The mode of human PLK3 inhibition by LFM-A13 is examined in titration experiments using increasing concentrations of [γ-$^{32}$P]ATP and purified N-terminal His6-tagged recombinant human PLK3, residues 19-301, expressed by baculovirus in Sf21 insect cells. In brief, in a final reaction volume of 25 μL, PLK3 (h) (5-10 mU) is incubated with 8 mM MOPS, pH 7.0, 0.2 mM EDTA, 2 mg/mL casein, 10 mM Mg acetate, and [γ-$^{32}$P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the MgATP mix. After incubation for 40 min at room temperature, the reaction is stopped by the addition of 5 μL of a 3% phosphoric acid solution. Ten microliters of the reaction is then spotted onto a P30 filtermat and washed three times for 5 min in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting. The $K_i$ of PLK3 by LFM-A13 is calculated from the reciprocal plots of the intensity of phosphorylation of the substrate (1/v) versus the concentration of the inhibitor (i) (viz., LFM-A13). From this Dixon plot, the $K_i$ represents the dissociation constants of the EI complex, which is determined by the point of linear intersection[4].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration [4]** | Mice[4]<br>Neu transgenic mice carrying one or more tumors are randomLy placed in the study. For the evaluation of tumor kinetics, tumor-bearing mice are randomLy assigned to either vehicle control or treatment groups. Tumor growth is determined by the measurement of tumors with a caliper in three dimensions three days a week and expressed as tumor volume in cubic millimeters ($mm^3$). Tumor volumes are calculated using the formula for the volume of a prolate spheroid, V = 4/3 × 3. 14 × |

length/2 × width/2 × depth/2. Due to the large heterogeneity in transgenic tumor volumes on day 0, tumor growth for each mouse is normalized to the starting volume for that particular tumor. Therefore, each mouse also serves as its own control, and the tumor growth curves are generated to show the rate of change in tumor volumes. LFM-A13 (10 or 50 mg/kg) is administered by twice daily intraperitoneal injections on 5 consecutive days per week. Paclitaxel is administered intraperitoneally on days 1, 3, 5, 8, 10, and 12 at a dose level of 6.7 mg/kg. Gemcitabine is administered on days 1, 8, and 15 at a dose level of 33.7 mg/kg.

Rats[4]

Lewis rats are kept in microisolater cages containing autoclaved food, water, and bedding. Lewis rats are treated with i.v. injections of LFM-A13 at multiple dose levels. LFM-A13 is administered as a 0.5 mL bolus injection containing 10% DMSO as a vehicle. Animals are electively sacrificed on day 7 to determine the toxicity of LFM-A13 by evaluating multiple organs for the presence of toxic lesions. Blood is collected by intracardiac puncture following anesthesia with ketamine:xylazine and immediately heparinized. Blood counts (red blood cells [RBC], white blood cells [WBC], and platelets [Plt]) are determined using a HESKA Vet ABC-Diff Hematology Analyzer. Absolute neutrophil counts (ANC) and absolute lymphocyte counts (ALC) are calculated from WBC values after determining the percentages of neutrophils and lymphocytes by a manual differential count. Values for the laboratory parameters are pooled for vehicle controls and LFM-A13 treatments, and for each parameter differences between means are evaluated for statistical significance using Students t-test (vehicle vs LFM-A13 treatment, unequal variances, two-tailed). The calculations are performed in Excel spreadsheets. To determine significant effects, the p-values are adjusted using the Bonferroni method to control for random variation. For histopathologic studies, formalin fixed tissues are dehydrated and embedded in paraffin by routine methods. Glass slides with affixed 4-5 micron tissue sections are prepared and stained with hematoxylin and eosin.

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

## CUSTOMER VALIDATION

- Sci Immunol. 2022 Jan 21;7(67):eabj5501.
- Dig Dis Sci. 2019 Aug;64(8):2167-2176.
- Oncotarget. 2017 Jul 25;8(30):49238-49252.

See more customer validations on www.MedChemExpress.com

## REFERENCES


[1]. Mahajan S, et al. Rational design and synthesis of a novel anti-leukemic agent targeting Bruton's tyrosine kinase (BTK), LFM-A13 [alpha-cyano-beta-hydroxy-beta-methyl-N-(2, 5-dibromophenyl)propenamide]. J Biol Chem. 1999 Apr 2;274(14):9587-99.

[2]. van den Akker E, et al. The Btk inhibitor LFM-A13 is a potent inhibitor of Jak2 kinase activity. Biol Chem. 2004 May;385(5):409-13.

[3]. "Sahin K, et al. LFM-A13, a potent inhibitor of polo-like kinase, inhibits breast carcinogenesis by suppressing proliferation activity and inducing apoptosis in breast tumors of mice. Invest New Drugs. 2017 Nov 15."

[4]. Uckun FM, et al. Anti-breast cancer activity of LFM-A13, a potent inhibitor of Polo-like kinase (PLK). Bioorg Med Chem. 2007 Jan 15;15(2):800-14. Epub 2006 Oct 26.





Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA