

产品名称: **LFM-A13**

产品别名: **LFM-A13**

生物活性:

生物活性:				
Description	LFM-A13 is a potent BTK, JAK2, PLK inhibitor, inhibits recombinant BTK, Plx1 and PLK3 with IC50s 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		
	10 μM (IC ₅₀)	61 μM (IC ₅₀)		
	BRK	BMX		
	267 μM (IC ₅₀)	281 μM (IC ₅₀)		
	FYN	Hepatocyte growth factor receptor kinase (Met)		
	240 μM (IC ₅₀)	215 μM (IC ₅₀)		
	BTK			
	2.5 μM (IC ₅₀)			
In Vitro	LFM-A13 significantly inhibits BTK activity with an IC50 of 6.2 ± 0.3 μg/mL (= 17.2 ± 0.8 μM). The calculated Kis 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 IC50 of 10 μM; also inhibits BRK, BMX, FYN and with IC50s of 267, 281, 240 and 215 μM[4].			
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 DMBA-induced mammary tumorigenesis in mice. LFM-A13 alone or in combination with paclitaxel shows marked effect on the DMBA-induced 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].			
	<i>In Vitro:</i>			
	DMSO : ≥ 42 mg/mL (116.67 mM)			
	H₂O : < 0.1 mg/mL (insoluble)			
	* "≥" means soluble, but saturation unknown.			
Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.7778 mL	13.8889 mL	27.7778 mL
	5 mM	0.5556 mL	2.7778 mL	5.5556 mL
	10 mM	0.2778 mL	1.3889 mL	2.7778 mL
*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液。一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。				
储备液的保存方式和期限 -80℃, 6 months; -20℃, 1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃				

<p>Solvent&Solubility</p>	<p>储存时，请在 1 个月内使用。</p> <p><i>In Vivo:</i></p> <p>请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：</p> <p>——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶</p> <p>1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: ≥ 2.5 mg/mL (6.94 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.94 mM，饱和度未知) 的澄清溶液。</p> <p>以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。</p> <p>2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (6.94 mM); Suspended solution; Need ultrasonic</p> <p>此方案可获得 2.5 mg/mL (6.94 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。</p> <p>以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。</p> <p>3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (6.94 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.94 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。</p> <p>以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。</p>
<p>References</p>	<p>[1]. Mahajan S, et al. Rational design and synthesis of a novel anti-leukemic agent targeting Bruton's tyrosine kinase (BTK), LFM-A13 [α-cyano-β-hydroxy-β-methyl-N-(2,5-dibromophenyl)propenamide]. J Biol Chem. 1999 Apr 2;274(14):9587-99.</p> <p>[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.</p> <p>[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. "</p> <p>[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.</p>
<p>实验参考：</p>	
	<p>Mice^[4]</p> <p>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 (mm³). Tumor volumes are calculated using the formula for the volume of a prolate spheroid, $V = 4/3 \times 3.14 \times \text{length}/2 \times \text{width}/2 \times \text{depth}/2$. Due to the large heterogeneity in transgenic tumor volumes on day 0,</p>

<p>Animal Administration</p>	<p>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.</p> <p>Rats^[4]</p> <p>Lewis rats are kept in microisolator 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:xylozine 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.</p>
<p>Kinase Assay</p>	<p>Purified His6-Plx1 (250 ng) is added to a 20 μL reaction mixture containing 1\times kinase buffer (10 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, and 1 mM TT), 25 μM cold ATP, and 1 μCi [γ-³²P]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\times 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-³²P]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 glutathione-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-³²P]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-³²P-ATP] (specific activity approx. 500 cpm/pmol, concentration as required). The reaction is initiated by the addition of the</p>

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

源叶生物