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产品名称：(-)-N-[4-(2,2-二甲基丙酰基)-5-[[2-(乙基氨基)乙磺酰胺]甲基]-5-苯基-4,5-二氢-1,3,4-噻二唑-2-基]-2,2-二甲基丙酰胺
产品别名：Litronesib；LY-2523355; KF-89617

生物活性：

Description	Litronesib is a selective mitosis-specific kinesin Eg5 inhibitor, with antitumor activity.
IC_{50} & Target	Eg5
In Vitro	Litronesib (LY2523355) is a selective Eg5 inhibitor. Litronesib (25 nM) induces cancer cells to death during mitotic arrest, and this needs sustained activation of spindle-assembly checkpoint (SAC)[1].
In Vivo	Litronesib (LY2523355; 1.1, 3.3, 10, and 30 mg/kg, i.v.) shows antitumor activity in a dose-dependently, and causes a dramatic increase in cancer cells immuno-positive for histone H3 phosphorylation in Colo205 xenograft tumors[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 50 mg/mL (97.71 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9543 mL	9.7713 mL	19.5427 mL
	5 mM	0.3909 mL	1.9543 mL	3.9085 mL
	10 mM	0.1954 mL	0.9771 mL	1.9543 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°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

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：

——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

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (4.89 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.89 mM，饱和度未知) 的澄清溶液。

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。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

Solubility: ≥ 2.5 mg/mL (4.89 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.89 mM，饱和度未知) 的澄清溶液。

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。

	3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (4.89 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.89 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。 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。
References	[1]. Ye XS, et al. A Novel Eg5 Inhibitor (LY2523355) Causes Mitotic Arrest and Apoptosis in Cancer Cells and Shows Potent Antitumor Activity in Xenograft Tumor Models. Mol Cancer Ther. 2015 Nov;14(11):2463-72.

实验参考：

Cell Assay	Cancer cells are plated in poly-d-lysine coated 96-well plates and incubated overnight. Cells are then treated with indicated concentrations of Litronesib for various time periods. Cells are then fixed with 3.7% formaldehyde in PBS for 45 minutes or 1× Prefer fixative solution for 30 minutes, at room temperature, and permeabilized with cold methanol for 10 minutes and then 0.2% Triton X-100 in PBS for 10 minutes. Cells are washed three times with PBS. Cells are then incubated with 100 μg/mL DNase-free RNase and 10 μg/mL of propidium iodide for 1 hour and scanned for mitotic index (MI) measurement based on DNA condensation as percentage of cells with condensed DNA. For MI based on histone H3 phosphorylation or apoptosis analysis, cells are incubated overnight at 4°C with anti-phospho-histone H3 antibody or anti-phospho-histone H2AX at 1:1,000 dilution with 5% bovine serum albumin (BSA) in PBS, respectively. Cells are washed three times with 0.2% Triton-X 100 in PBS and incubated with Alexa 488 secondary antibody (1:1,000 in PBS-2% BSA) for 60 minutes at room temperature. Cells are washed three times again with PBS and stained for 15 minutes in PBS containing 10 μg/mL propidium iodide and 100 μg/mL RNaseA. The stained cells are scanned with an Acumen Explorer eX3 microplate cytometer. The results are expressed as percentage of cells positive for phospho-histone H3-Ser10 or phospho-histone H2AX[1].
Animal Administration	Primary human-tumor xenograft models are established and maintained in nude mice. Antitumor efficacy in subcutaneous xenograft tumor-bearing mice with 10 mice per treatment group from either established cancer cell lines or fragments of human tumor explants is evaluated as tumor volume by serial caliper measurements and is calculated. The p388 syngeneic tumor model is developed for high-content imaging analysis using female BDF1 mice, weighing 20 to 23 g, which are acclimated in-house for one week before their use in experiments. p388 murine lymphocytic leukemia cells are authenticated by STR assay and maintained in RPMI1640 medium containing 10% FBS. For inoculation, the cells are washed with serum-free medium three times and 1.25 million cells are implanted by intraperitoneal injection into mice. On day 5 after implantation, mice are treated with Litronesib, via either intravenous bolus or intravenous infusion at appropriate doses and durations. Mice are euthanized and the ascitic (intraperitoneal) fluid containing the p388 tumor cells is drawn and analyzed by acumen, flow cytometry, and TUNEL assays for phospho-histone H3, G2-M, and apoptosis. For pharmacokinetic study, the blood samples are collected via cardiac puncture and generated plasma with EDTA and determined Litronesib exposure in the plasma[1].
	[1]. Ye XS, et al. A Novel Eg5 Inhibitor (LY2523355) Causes Mitotic Arrest and Apoptosis in Cancer

References	Cells and Shows Potent Antitumor Activity in Xenograft Tumor Models. Mol Cancer Ther. 2015 Nov;14(11):2463-72.