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产品名称：BAY 1161909
产品别名：Empesertib

生物活性：

Description	Empesertib (BAY 1161909) is a potent Mps1 inhibitor, with an IC_{50} of < 1 nM.
IC_{50} & Target	Mps1
	1 nM (IC_{50})
In Vitro	BAY 1161909 is a potent Mps1 inhibitor, with an IC_{50} of < 1 nM. BAY 1161909 also shows anti-tumor effect, suppressing the proliferation of Hela cells, with an IC_{50} of < 400 nM[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 35 mg/mL (62.54 mM)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7870 mL	8.9348 mL	17.8696 mL
	5 mM	0.3574 mL	1.7870 mL	3.5739 mL
	10 mM	0.1787 mL	0.8935 mL	1.7870 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 (4.47 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (4.47 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 (4.47 mM); Suspended solution; Need ultrasonic

此方案可获得 2.5 mg/mL (4.47 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。

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 (4.47 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.47 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]. SCHULZE, Volker, et al. PRODRUG DERIVATIVES OF SUBSTITUTED TRIAZOLOPYRIDINES. WO2014198647A2

实验参考：

Cell Assay	Cultivated tumor cells are plated at a density of 5000 cells/ well (MCF7, DU145, HeLa-MaTu-ADR), 3000 cells/well (NCI-H460, HeLa-MaTu, HeLa), or 1000 cells/well (B16F10) in a 96-well multititer plate in 200 μL of their respective growth medium supplemented 10% fetal calf serum. After 24 hours, the cells of one plate (zero-point plate) are stained with crystal violet, while the medium of the other plates is replaced by fresh culture medium (200 μL), to which the test substances (BAY 1161909, etc.) are added in various concentrations (0 μM, as well as in the range of 0.01-30 μM; the final concentration of the solvent DMSO is 0.5%). The cells are incubated for 4 days in the presence of test substances. Cell proliferation is determined by staining the cells with crystal violet: the cells are fixed by adding 20 μL/measuring point of an 11% glutaric aldehyde solution for 15 minutes at room temperature. After three washing cycles of the fixed cells with water, the plates are dried at room temperature. The cells are stained by adding 100 μL/measuring point of a 0.1% crystal violet solution (pH 3.0). After three washing cycles of the stained cells with water, the plates are dried at room temperature. The dye is dissolved by adding 100 μL/measuring point of a 10% acetic acid solution. The IC_{50} values are determined by means of a 4 parameter fit. The compounds A1 (BAY 1161909), A2, A3, A4 and A5 are characterized by an IC_{50} determined in a HeLa-MaTu-ADR cell proliferation assay[1].
Kinase Assay	N-terminally GST-tagged human full length recombinant Mps-1 kinase is used. As substrate for the kinase reaction a biotinylated peptide of the amino-acid sequence PWDPDDADITEILG is used. For the assay 50 nL of a 100-fold concentrated solution of the test compounds (BAY 1161909, etc.) in DMSO is pipetted into a black low volume 384 well microtiter plate, 2 μL of a solution of Mps-1 in assay buffer [0.1 mM sodium-ortho-vanadate, 10 mM $MgCl_2$, 2 mM DTT, 25 mM Hepes pH 7.7, 0.05% BSA, 0.001 % Pluronic F-127] are added and the mixture is incubated for 15 min at 22°C to allow pre-binding of the test compounds to Mps-1 before the start of the kinase reaction. Then the kinase reaction is started by the addition of 3 μL of a solution of 16.7 adenosine-tri-phosphate and peptide substrate in assay buffer and the resulting mixture is incubated for a reaction time of 60 min at 22°C. The concentration of Mps-1 in the assay is adjusted to the activity of the enzyme lot and is chosen appropriate to have the assay in the linear range, typical enzyme concentrations are in the range of about 1 nM (final cone, in the 5 μL assay volume). The reaction is stopped by the addition of 3 μL of a solution of HTRF detection reagents (100 mM Hepes pH 7.4, 0.1 % BSA, 40 mM EDTA, 140 nM Streptavidin-XLent, 1 .5 nM anti-phospho(Ser/Thr)-Europium-antibody[1].
References	[1]. SCHULZE, Volker, et al. PRODRUG DERIVATIVES OF SUBSTITUTED TRIAZOLOPYRIDINES. WO2014198647A2