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产品名称：LDE225 (Diphosphate)
产品别名：Erismodegib diphosphate；Sonidegib diphosphate

生物活性：

Description	Erismodegib diphosphate (Sonidegib diphosphate) is a potent and selective Smo antagonist with IC50 of 1.3 nM and 2.5 nM for mouse and human Smo in binding assay, respectively.
IC_{50} & Target	IC50: 1.3 nM (mSmo), 2.5 nM (hSmo)[1]
In Vitro	The IC_{50} values for Erismodegib diphosphate (Sonidegib diphosphate) for the major human CYP450 drug metabolizing enzymes is greater than 10 μM[1]. Erismodegib diphosphate, a small molecule, clinically investigated SMO inhibitor, used alone and in combination with Nilotinib, inhibits the Hh pathway in $CD34^+$ chronic phase (CP)-chronic myeloid leukaemia (CML) cells, reducing the number and self-renewal capacity of CML leukaemia stem cell (LSC). Erismodegib interacts directly with SMO, in a similar fashion to cyclopamine, to reduce expression of downstream Hh signaling targets. Primary $CD34^+$ CP-CML cells are cultured in serum free media (SFM)±Erismodegib for 6, 24 and 72 hours (h). At 72 h, while there is variability between the biological samples, *GLI1* is significantly downregulated following exposure to Erismodegib (10 nM; 0.78-fold and 100 nM; 0.73-fold, respectively ($p<0.01$)[2].
In Vivo	Erismodegib diphosphate (Sonidegib diphosphate) is a weak base with a measured pK_a of 4.2 and exhibits relatively poor aqueous solubility. In the subcutaneous $Ptch^{+/-}p53^{-/-}$ medulloblastoma allograft mouse model, Erismodegib diphosphate demonstrates dose-related antitumor activity after 10 days of oral administration of a suspension of the diphosphate salt. At a dose of 5 mg/kg/day qd, Erismodegib diphosphate significantly inhibits tumor growth, corresponding to a T/C value of 33% ($p<0.05$ as compared to vehicle controls). When dosed at 10 and 20 mg/kg/day qd, Erismodegib affords 51 and 83% regression, respectively[1]. Bone marrow cells and spleen cells from a subset of treated mice are transplanted into secondary recipient mice. Transplantation of either bone marrow (BM) or spleen cells from mice treated with Erismodegib diphosphate+ Nilotinib results in reduced white cell count (WCC) and reduces leukaemia development in secondary recipients compared to Erismodegib or Nilotinib alone[2].

In Vitro:

DMSO : 100 mg/mL (**146.74 mM;** Need ultrasonic)

H_2O : 0.25 mg/mL (**0.37 mM;** Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.4674 mL	7.3369 mL	14.6737 mL
5 mM	0.2935 mL	1.4674 mL	2.9347 mL
10 mM	0.1467 mL	0.7337 mL	1.4674 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：

Solvent&Solubility	——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 (3.67 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (3.67 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 (3.67 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (3.67 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 (3.67 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (3.67 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]. Pan S, et al. Discovery of NVP-LDE225, a Potent and Selective Smoothened Antagonist. ACS Med Chem Lett. 2010 Mar 16;1(3):130-4. [2]. Irvine DA, et al. Deregulated hedgehog pathway signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia. Sci Rep. 2016 May 9;6:25476. [3]. Ma W, et al. Reduced Smoothened level rescued Aβ-induced memory deficits and neuronal inflammation in animal models of Alzheimer's disease. J Genet Genomics. 2018 May 20;45(5):237-246.

实验参考：

Cell Assay	CD34$^+$ CP-CML cells are seeded in SFM alone±Erismodegib±Nilotinib and cultured for 24-72 h prior to assessment. Proliferation is measured using colorimetric assessment of BrDU incorporation. Proportion of viable cells versus those in early and late apoptosis is assessed by flow cytometry using annexin V-FITC and 7-amino-actinomycin D (7-AAD, Via-Probe solution). Cell cycle status is assessed using Ki67 (FITC) expression and 7-AAD incorporation. [2]
	Mice[2] The transgenic EGFP$^+$/SCLtTA/TRE-BCR-ABL mouse model is used to investigate the effect of Erismodegib treatment on CML LSC in vivo. *Scl-tTa-BCR-ABL* mice in the FVB/N background are crossed with transgenic GFP-expressing mice. Bone marrow cells are obtained 4 weeks post induction, GFP$^+$ cells are selected by flow cytometry and transplanted by tail vein injection (10^6 cells/mouse) into wild-type FVB/N recipient mice, irradiated at 900 cGy, generating a large cohort of mice with similar time of onset of leukemia. Blood samples obtained 4 weeks post transplantation confirmed a neutrophilic leukocytosis in recipient mice. Mice are treated with

Animal Administration	Nilotinib (50 mg/kg by gavage, daily), Erismodegib (80 mg/kg by gavage, daily), Erismodegib+Nilotinib, or with vehicle alone (control). After 3 weeks of treatment, animals are euthanised and marrow content of femurs and tibiae, spleen cells and blood obtained. Total white cell count (WCC), GFP-expressing WCC, myeloid cells, and GFP+ progenitors and stem cells are measured by flow cytometry. Survival is assessed in a subset of mice for 120d post discontinuation of treatment. Spleen and BM cells from a subset of mice in each arm are pooled and 5×10^6 cells/mouse (8 mice/condition) are transplanted into wild-type FVB/N recipient mice irradiated at 900 cGy. Engraftment is monitored by drawing peripheral blood (PB) every 4 weeks. The percentage of GFP^+ cells in PB is analyzed by flow cytometry.
References	[1]. Pan S, et al. Discovery of NVP-LDE225, a Potent and Selective Smoothened Antagonist. ACS Med Chem Lett. 2010 Mar 16;1(3):130-4. [2]. Irvine DA, et al. Deregulated hedgehog pathway signaling is inhibited by the smoothened antagonist LDE225 (Sonidegib) in chronic phase chronic myeloid leukaemia. Sci Rep. 2016 May 9;6:25476. [3]. Ma W, et al. Reduced Smoothened level rescued Aβ-induced memory deficits and neuronal inflammation in animal models of Alzheimer's disease. J Genet Genomics. 2018 May 20;45(5):237-246.