产品名称：Degrasyn (WP1130)
产品别名：Degrasyn

生物活性：

Description	Degrasyn (WP1130) is a cell-permeable deubiquitinase (DUB) inhibitor, directly inhibiting DUB activity of USP9x, USP5, USP14, and UCH37. Degrasyn has been shown to downregulate the antiapoptotic proteins Bcr-Abl and JAK2.
IC_{50} & Target	IC50: 1.8 μM (Bcr-Abl, in K562, BV-173 cells)[1] Deubiquitinase[2]
In Vitro	Degrasyn, a small molecule that specifically and rapidly down-regulates both wild-type and mutant Bcr/Abl protein without affecting bcr/abl gene expression in chronic myelogenous leukemia (CML) cells. Degrasyn is more effective in inducing apoptosis of myeloid and lymphoid tumor cells (IC50 0.5 to 2.5 μM) than normal $CD34^+$ hematopoietic precursors, dermal fibroblasts, or endothelial cells (IC50 ~5 to 10 μM). Degrasyn reduces Bcr/Abl protein levels through a unique mechanism that is not affected by mutations that interfere with imatinib mesylate binding. Degrasyn inhibits phosphorylation of both the wild-type and the T315I mutant Bcr/Abl proteins, as demonstrated by the rapid disappearance (within 1 hour) of phosphotyrosyl-Bcr/Abl in both BV173 and BV173R cells. Degrasyn-induced down-regulation of Bcr/Abl is accompanied by apoptosis of CML cells[1]. Treatment with Degrasyn yields an anti-proliferative effect across all cell lines tested in a dose-dependent manner. Notably, treatment with Degrasyn results in marked anti-proliferative activity and morphological changes in NCH644 and NCH421K glioma stem-like cells. Treatment with Degrasyn results in a dose-dependent and probably compensatory increase of Mcl-1 mRNA levels after 6 h and only a minor decrease after 24 h. Similar findings are observed for Usp9X levels. These data suggest that Degrasyn down-regulates Mcl-1 and Usp9X through a post-transcriptional mechanism[2].
In Vivo	Degrasyn suppresses the growth of K562 heterotransplanted tumors as well as both wild-type Bcr/Abl and T315I mutant Bcr/Abl-expressing BaF/3 cells transplanted into nude mice. To assess the possible therapeutic activity of Degrasyn against CML, nude mice received a subcutaneous transplant with K562 cells and are treated with 30 mg/kg Degrasyn every other day for 9 days after palpable tumor formation (10 days). This dose and schedule are well tolerated and effective in other tumor models. Antitumor activity is compared with that after daily Imatinib mesylate treatment (50 mg/kg). Nineteen days after tumor inoculation, tumors in the control group reached maximum allowable dimension, and the effect of therapy is assessed. Degrasyn treatment suppresses K562 tumor growth to an extent comparable to that observed in imatinib mesylate-treated animals, suggesting that Degrasyn is active in reducing the growth of established K562 tumors[1].

In Vitro:

DMSO : 100 mg/mL (260.23 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.6023 mL	13.0117 mL	26.0234 mL
5 mM	0.5205 mL	2.6023 mL	5.2047 mL
10 mM	0.2602 mL	1.3012 mL	2.6023 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

Solvent&Solubility	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 (6.51 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.51 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% corn oil Solubility: ≥ 2.5 mg/mL (6.51 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.51 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]. Bartholomeusz GA, et al. Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. Blood. 2007 Apr 15;109(8):3470-8.

实验参考：

Cell Assay	CML cells are seeded in 96-well plates (2×10^4 cells/well) and Degrasyn, Imatinib mesylate, or Dasatinib (0.08-10 μM) are added in a final volume of 100 μL medium. Plates are incubated at 37°C for 72 hours, after which 20 μL of MTT reagent (stock 5 mg/mL) is added, and the plates are incubated at 37°C for another 2 hours. Cells are lysed with 100 μL lysis buffer (20% SDS in 50% N, N-dimethylformamide adjusted to pH 4.7 with 80% acetic acid and 1 M hydrochloric acid; final concentration of acetic acid is 2.5% and hydrochloric acid is 2.5%) and incubated for 6 hours. The optical density of each sample at 570 nm is determined with a SPECTRA MAX M2 plate reader[1].
Animal Administration	Mice[1] To assess activity against T315I Bcr/Abl mutant cells, BaF/3 cells transfected with wild-type Bcr/Abl or the T315I mutant are transplanted into female Swiss nude mice (5-6 weeks old). BaF/3wt cells are suspended (5×10^7cells/mL) in RPMI medium, and 0.1 mL of this suspension is injected intravenously into 10 mice. The same procedure is used to inoculate 10 mice with BaF/3/T315I cells. One day after tumor cell inoculation, mice are randomly assigned to 1 of 3 groups: one group received Degrasyn (40 mg/kg, intraperitoneally) in 50 μL DMSO/PEG300 (vehicle) every other day (7 injections); another received imatinib mesylate (100 mg/kg, intraperitoneally) in vehicle daily (14 injections); and the third received vehicle alone daily (14 injections). Spleens from each mouse are harvested, photographed, and weighed 15 days after tumor cell inoculation.
References	[1]. Bartholomeusz GA, et al. Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. Blood. 2007 Apr 15;109(8):3470-8.