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产品名称：Danusertib (PHA-739358)
产品别名：达鲁舍替

生物活性：

Description	Danusertib is a pyrrolo-pyrazole and aurora kinase inhibitor with IC50 of 13, 79, and 61 nM for Aurora A, B, and C, respectively.

IC_{50} & Target	Aurora A	Aurora B	Aurora C
	13 nM (IC_{50})	79 nM (IC_{50})	61 nM (IC_{50})

In Vitro	Danusertib (0.01 to 50 μM) significantly decreases viability of C13 and A2780cp cells. The IC50s are 10.40 and 1.83 μM for C13 cells, and 19.89 and 3.88 μM for A2780cp cells after 24- and 48-h treatment. Danusertib induces cell cycle arrest in G2/M phase in C13 and A2780cp cells. Danusertib treatment results in a marked increase in the percentage of cells arrested in G2/M phase and an accumulation of polyploidy in C13 and A2780cp cells. Danusertib demotes the expression of CDK1/CDC2 and cyclin B1 but promotes the expression of p21 Waf1/Cip1, p27 Kip1, and p53. Danusertib induces autophagy in C13 and A2780cp cells with the involvement of PI3K/Akt/mTOR signaling pathway[1]. PHA-739358 strongly inhibits proliferation of all leukemic cell lines tested, with IC50 values ranging from 0.05 μM to 3.06 μM. PHA-739358 induces antiproliferative effects in BaF3-p210 cells, including IM-resistant M351T, E255K, and T315I mutants. PHA-739358 (5 μM) reduces phosphorylation of CrkL in BaF3-p210 wt cells and IM-resistant mutants[2]. Danusertibsertib leads to cell-cycle arrest and completely inhibits cell proliferation of the GEP-NET cells in vitro[3].
In Vivo	PHA-739358 (15 mg/kg twice a day, i.p.) and IM are well tolerated, and significantly inhibit proliferation of K562 cells andvirtually suppressed tumor growth during the 10-day treatment period[2]. In a subcutaneous murine xenograft model, danusertibsertib (2×15 mg/kg/d, i.p.) significantly reduces tumor growth in vivo compared with controls or mice treated with streptozotocine/5-fluorouracil[3].

Solvent&Solubility

In Vitro:

DMSO : 50 mg/mL (105.36 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1073 mL	10.5363 mL	21.0726 mL
	5 mM	0.4215 mL	2.1073 mL	4.2145 mL
	10 mM	0.2107 mL	1.0536 mL	2.1073 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→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (5.27 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.27 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。 2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.27 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.27 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]. Zi D, et al. Danusertib Induces Apoptosis, Cell Cycle Arrest, and Autophagy but Inhibits Epithelial to Mesenchymal Transition Involving PI3K/Akt/mTOR Signaling Pathway in Human Ovarian Cancer Cells. Int J Mol Sci. 2015 Nov 13;16(11):27228-51. [2]. Gontarewicz A, et al. Simultaneous targeting of Aurora kinases and Bcr-Abl kinase by the small molecule inhibitor PHA-739358 is effective against imatinib-resistant BCR-ABL mutations including T315I. Blood. 2008 Apr 15;111(8):4355-64. [3]. Fraedrich K, et al. Targeting Aurora Kinases with Danusertib (PHA-739358) Inhibits Growth of Liver Metastases from Gastroenteropancreatic Neuroendocrine Tumors in an Orthotopic Xenograft Model. Clin Cancer Res. 2012 Sep 1;18(17):4621-32. Epub 2012 Jul 2.

实验参考：

Cell Assay	The MTT assay is performed to examine the effect of danusertib on the viability of C13 and A2780cp cells. Briefly, cells are seeded in 96-well culture plates at a density of 8×10^3 cells/well. After cells are attached, the cells are treated with danusertib at different concentrations (0.01-50 μM). The control cells receive the vehicle only. After 24-h incubation, 10 μL MTT (5 g/L) is added to each well and cultured for another 4 h. Then, the media is carefully aspirated and 100 μL DMSO is added. The absorbance at the 450 nm wavelength is measured with a Synergy H4 Hybrid microplate reader. The IC_{50} values are determined using the relative viability over danusertib concentration curve using GraphPad Prism 6.0. [1]
Animal Administration	To evaluate the efficacy and toxicity of PHA-739358 in vivo, a subcutaneous animal model for CML is used; 5×10^7K562 cells are injected into the flanks of female SCID mice and tumor growth is monitored daily by palpation. On day 7, when tumors reach an estimated weight of 100 to 150 mg, animals are assigned to 3 experimental groups by random selection and receive the following treatment for a period of 10 days: group 1, control, vehicle solution (7 mice); group 2, PHA-739358 twice a day intraperitoneally at a dose of 15 mg/kg (7 mice); and group 3, IM twice a day per os at 100 mg/kg. Tumor growth is assessed by caliper, and tumor weight is calculated according to the following formula: Tumor weight=[length (mm) × $width^2$ (mm)]/2. Toxicity is monitored by changes in body weight and vitality of the animals. [2]
	[1]. Zi D, et al. Danusertib Induces Apoptosis, Cell Cycle Arrest, and Autophagy but Inhibits

References	Epithelial to Mesenchymal Transition Involving PI3K/Akt/mTOR Signaling Pathway in Human Ovarian Cancer Cells. Int J Mol Sci. 2015 Nov 13;16(11):27228-51. [2]. Gontarewicz A, et al. Simultaneous targeting of Aurora kinases and Bcr-Abl kinase by the small molecule inhibitor PHA-739358 is effective against imatinib-resistant BCR-ABL mutations including T315I. Blood. 2008 Apr 15;111(8):4355-64. [3]. Fraedrich K, et al. Targeting Aurora Kinases with Danusertib (PHA-739358) Inhibits Growth of Liver Metastases from Gastroenteropancreatic Neuroendocrine Tumors in an Orthotopic Xenograft Model. Clin Cancer Res. 2012 Sep 1;18(17):4621-32. Epub 2012 Jul 2.