产品名称：卡纽替尼二盐酸盐
产品别名：Canertinib dihydrochloride

生物活性：

Description	Canertinib dihydrochloride (CI-1033 dihydrochloride) is a potent and irreversible EGFR inhibitor; inhibits cellular EGFR and ErbB2 autophosphorylation with IC_{50}s of 7.4 and 9 nM.	
IC_{50} & Target [1]	EGFR	ErbB2
	7.4 nM (IC_{50})	9 nM (IC_{50})
In Vitro	Canertinib dihydrochloride (CI-1033 dihydrochloride) significantly inhibits growth of cultured melanoma cells, RaH3 and RaH5, in a dose-dependent manner. IC50 is approximately 0.8 μM and by 5μM both cell lines are completely growth-arrested within 72 h of treatment. Incubation of exponentially growing RaH3 and RaH5 with 1 μM canertinib accumulated the cells in the G1-phase of the cell cycle within 24 h of treatment without induction of apoptosis. 1 μM canertinib inhibits ErbB1-3 receptor phosphorylation with a concomitant decrease of Akt-, Erk1/2- and Stat3 activity in both cell lines[2].	
In Vivo	Canertinib dihydrochloride (CI-1033 dihydrochloride) shows superior in vivo antitumor activity, giving growth delays in A431 xenografts exceeding 50 days following oral administration[1]. The growth of human malignant melanoma xenografts, RaH3 and RaH5, in nude mice is significantly inhibited by i.p. injections of 40 mg/kg/day canertinib (Fig. 4). The anti-proliferative effect on melanoma xenografts is visible already within 4 days of treatment and further increased throughout the treatment period as observed through the differences in tumor volumes, reaching statistical significance within 18 days of treatment[2].	

Solvent&Solubility

In Vitro:

DMSO : ≥ 155 mg/mL (277.35 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7894 mL	8.9468 mL	17.8936 mL
	5 mM	0.3579 mL	1.7894 mL	3.5787 mL
	10 mM	0.1789 mL	0.8947 mL	1.7894 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: ≥ 2.58 mg/mL (4.62 mM); Clear solution

此方案可获得 ≥ 2.58 mg/mL (4.62 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.8 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: ≥ 2.58 mg/mL (4.62 mM); Clear solution 此方案可获得 ≥ 2.58 mg/mL (4.62 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.8 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。
References	[1]. Smaill JB, et al. Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-d]pyrimidine-6-acrylamides bearing additional solubilizing functions. J Med Chem. 2000 Apr 6;43(7):1380-97. [2]. Djerf Severinsson EA, et al. The pan-ErbB receptor tyrosine kinase inhibitor canertinib promotes apoptosis of malignant melanoma in vitro and displays anti-tumor activity in vivo. Biochem Biophys Res Commun. 2011 Oct 28;414(3):563-8.

实验参考：

Cell Assay	RaH3 and RaH5 cells are treated with increasing concentrations (0-10 μM) of Canertinib for 72 h. The cells are suspended in buffer and counted[2].
Animal Administration	Mice: Canertinib treatment starts when the tumors show reliable growth. The mice are randomized into control and treatment groups. In the canertinib treated RaH3 group (n=4) and RaH5 group (n=7) each mouse receives i.p. injections of 1.2 mg canertinib (40 mg/kg/day) in 0.1 ml 0.15 M NaCl 5 days a week. The control RaH3 (n=3) and RaH5 (n=7) mice receive i.p. injections of vehicle only according to the same regimen. At the end of the treatment period, the mice are sacrificed by cervical dislocation where after the tumors are removed and weighed[2].
Kinase Assay	Enzyme assays for IC50 determinations are performed in 96-well filter plates. The total volume is 0.1 mL containing 20 mM Hepes, pH 7.4, 50 mM sodium vanadate, 40 mM magnesium chloride, 10 μM adenosine triphosphate (ATP) containing 0.5 mCi of [32P]ATP, 20 mg of polyglutamic acid/tyrosine, 10 ng of EGFR tyrosine kinase, and appropriate dilutions of inhibitor (Canertinib). All components except the ATP are added to the well and the plate is incubated with shaking for 10 min at 25°C. The reaction is started by adding [32P]ATP, and the plate is incubated at 25°C for 10 min. The reaction is terminated by addition of 0.1 mL of 20% trichloroacetic acid (TCA). The plate is kept at 4°C for at least 15 min to allow the substrate to precipitate. The wells is then washed five times with 0.2 mL of 10% TCA and 32P incorporation determined with a plate counter[1].
References	[1]. Smaill JB, et al. Tyrosine kinase inhibitors. 17. Irreversible inhibitors of the epidermal growth factor receptor: 4-(phenylamino)quinazoline- and 4-(phenylamino)pyrido[3,2-d]pyrimidine-6-acrylamides bearing additional solubilizing functions. J Med Chem. 2000 Apr 6;43(7):1380-97. [2]. Djerf Severinsson EA, et al. The pan-ErbB receptor tyrosine kinase inhibitor canertinib promotes apoptosis of malignant melanoma in vitro and displays anti-tumor activity in vivo. Biochem Biophys Res Commun. 2011 Oct 28;414(3):563-8.