产品名称：**IC 261**
产品别名：**IC261**

生物活性：

Description	IC261 is a selective, ATP-competitive CK1 inhibitor, with IC_{50}s of 1 μM, 1 μM, 16 μM for Ckiδ, Ckiε and Ckiα1, respectively.		
IC_{50} & Target	CKIδ	CKIε	CkIα1
	1 μM (IC_{50})	1 μM (IC_{50})	16 μM (IC_{50})
In Vitro	IC261 is a selective, ATP-competitive CK1 inhibitor, with IC_{50}s of 1 μM, 1 μM, 16 μM for Ckiδ, Ckiε and Ckiα1, respectively. IC261 is less active on PKA, $p34^{cdc2}$, and $p55^{fyn}$ (IC_{50}s > 100 μM)[1]. IC261 induces mitotic arrest, spindle defects and centrosome amplification in AC1-M88 cells. IC261 (1 μM) increases G2/M cells after 12 h, and causes cell death at 24 h in AC1-M88 cells. IC261 (1 μM) also induces apoptosis in the extravillous trophoblast hybrid cells[2]. IC261 (1.25 μM) suppresses the proliferation of several pancreatic tumour cell lines, including ASPC-1, BxPc3, Capan-1, Colo357, MiaPaCa-2, Panc1, Panc89, PancTu-1 and PancTu-2 cells. IC261 (1.25 μM) specifically enhances CD95-mediated apoptosis of pancreatic tumour cells[3].		
In Vivo	IC261 (20.5 mg/kg) inhibits tumor growth of PancTu-2 cells in SCID mice, downregulates several anti-apoptotic proteins, such as CK1δ/ε, KRAS, and IL6 and upregulates p21, ATM, CHEK1 and STAT1 in mice[3].		

Solvent&Solubility

In Vitro:

DMSO : ≥ 33 mg/mL (106.00 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.2120 mL	16.0601 mL	32.1203 mL
	5 mM	0.6424 mL	3.2120 mL	6.4241 mL
	10 mM	0.3212 mL	1.6060 mL	3.2120 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 (8.03 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (8.03 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。

References	[1]. Mashhoon N, et al. Crystal structure of a conformation-selective casein kinase-1 inhibitor. J Biol Chem. 2000 Jun 30;275(26):20052-60. [2]. St?ter M, et al. Inhibition of casein kinase I delta alters mitotic spindle formation and induces apoptosis in trophoblast cells. Oncogene. 2005 Dec 1;24(54):7964-75. [3]. Brockschmidt C, et al. Anti-apoptotic and growth-stimulatory functions of CK1 delta and epsilon in ductal adenocarcinoma of the pancreas are inhibited by IC261 in vitro and in vivo. Gut. 2008 Jun;57(6):799-806.

实验参考：

Cell Assay	Human extravillous trophoblast cells irreversibly leave the cell cycle and die when isolated from its natural extracellular matrix. The cell line AC1-M88 is employed in vitro experiments. This cell line is generated by fusion of extravillous trophoblasts with AC1-1. Cells are grown in DMEM (CV-1) or DMEM/F-12 (AC1-M88) medium supplemented with 10% fetal calf serum (FCS) at 37°C in a humidified 5% CO_2 atmosphere. Where indicated, cells are γ-irradiated with 5 Gy and harvested at the given time points for western blot analysis, treated with 1 μM IC261 or 0.4 μM nocodazole for 12 h and fixed for immunofluorescence analysis, or treated with 1 μM IC261 and fixed for flow cytometrical analysis or lysed for western blot analysis at the indicated time points. IC261 and nocodazole are dissolved in DMSO as stock solutions (25 and 10 mM, respectively), and control cells are treated with 0.004% DMSO. For immunocytochemistry, the cells are grown on coverslips and are treated with methanol (−20°C) for 5 min, followed by acetone (−20°C) for 20-30 s prior to being used for immunocytochemical detection[2].
Animal Administration	Five million PancTu-1 cells resuspended in 100 μL of a solution containing 50% Matrigel and 50% DMEM/RPMI-1640 (1:1) are injected into the dorsolateral site of 6-week-old C.B-17/IcrHsd-scid-bg mice. After 17 days, mice are randomised to the control group (n=5), the IC261 treatment group (n=5), the gemcitabine group (n=5) and to the IC261/gemcitabine group (n=5). Injection of dimethylsulfoxide (DMSO; control group), IC261 (20.5 mg/kg), gemcitabine (0.6 mg/kg) alone or in combination (20.5 mg/kg IC261/0.6 mg/kg gemcitabine) (treatment groups) is performed daily for 8 days. Mice are sacrificed by asphyxiation with CO_2 the day after the last treatment. Tumours are measured before and during treatment. Finally, the tumours are excised, measured, weighed and fixed in formalin or shock frozen. Tumour volume is calculated according to the formula for a rotational ellipsoid (length × height × width × 0.5236)[3].
Kinase Assay	Casein kinase activity is assayed at 37°C. The standard reaction (40 μL) contains 25 mM 2-(N-morpholino)ethanesulfonic acid, pH 6.5, 50 mM NaCl, 15 mM $MgCl_2$, 2 mg/mL casein, 2 mM EGTA, 100 μM [γ-^{32}P]ATP (100-400 cpm/pmol). Initial velocity measurements are carried out in duplicate with ATP as the varied substrate. Kinetic constants and their standard errors are calculated. For assay of inhibitor potency (IC_{50}), [γ -^{32}P]ATP is held constant (10 μM), whereas IC261 concentration is varied (0.1, 0.3, 1, 3, and 10 μM). To assess kinetic mechanism, inhibitors are held constant (IC261, 20 μM; IC3608, 100 μM), whereas [γ -^{32}P]ATP is varied as above. For screening small molecule libraries, CK1 isoforms (Ckiα1, δ, and ε) are assayed that casein is used at 10 mg/mL, [γ -^{32}P]ATP is held constant at 2 μM or 1 mM[1].
References	[1]. Mashhoon N, et al. Crystal structure of a conformation-selective casein kinase-1 inhibitor. J Biol Chem. 2000 Jun 30;275(26):20052-60. [2]. St?ter M, et al. Inhibition of casein kinase I delta alters mitotic spindle formation and induces apoptosis in trophoblast cells. Oncogene. 2005 Dec 1;24(54):7964-75.

	[3]. Brockschmidt C, et al. Anti-apoptotic and growth-stimulatory functions of CK1 delta and epsilon in ductal adenocarcinoma of the pancreas are inhibited by IC261 in vitro and in vivo. Gut. 2008 Jun;57(6):799-806.