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产品名称：Ensartinib
产品别名：X-376

生物活性：

Description	X-376 is a potent and highly specific **ALK** tyrosine kinase inhibitor (TKI) (**IC_{50}**=0.61 nM). X-376 is a less potent inhibitor of **MET** (**IC_{50}**=0.69 nM). X-376 displays potent anti-tumor activity[1].

IC_{50} & Target	ALK	MET
	0.61 nM (IC_{50})	0.69 nM (IC_{50})

In Vitro	The ability of X-376 to inhibit the growth of different cancer cell lines harboring ALK fusions or point mutations is tested. X-376 is potent in H3122 lung cancer cells harboring EML4-ALK E13;A20 (IC50: 77 nM). X-376 is also potent in H2228 lung cancer cells harboring EML4-ALK E6a/b; A20 (IC50: 57 nM). Furthermore, X-376 is potent in SUDHL-1 lymphoma cells harboring NPM-ALK (IC50: 32 nM). X-376 also inhibits SY5Y neuroblastoma cells harboring ALK F1174L, MKN-45 gastric carcinoma cells harboring MET dependent, HepG2 cells and PC-9 lung cancer cell lines harboring EGFR exon 19 del with IC50s of 142 nM, 150 nM, 15.137 μM and 3.062 μM, respectively[1].
In Vivo	The effects of X-376 in vivo against H3122 xenografts are examined. A pharmacokinetic study reveals that X-376 shows substantial bioavailability and moderate half-lives in vivo. Nude mice harboring H3122 xenografts are treated with X-376 at 50 mg/kg bid. X-376 significantly delays the growth of tumors compared to vehicle alone. In the xenograft experiments, X-376 appears well-tolerated in vivo. Mouse weight is unaffected by X-376 treatment. Drug-treated mice appear healthy and do not display any signs of compound related toxicity. To further assess potential side effects of X-376, additional systemic toxicity and toxico-kinetic studies are performed in Sprague Dawley (SD) rats. Following 10 days of repeated oral administration of X-376 at 25, 50, 100 mg/kg in SD rats, all animals survive to study termination. The no significant toxicity (NST) levels are determined to be 50 mg/kg for X-376. At NST levels, X-376 achieves an AUC of 41 μM×hr and a C_{max} of 5.04 μM[1].

Solvent&Solubility

In Vitro:

DMSO : 100 mg/mL (**182.68 mM**; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.8268 mL	9.1339 mL	18.2678 mL
	5 mM	0.3654 mL	1.8268 mL	3.6536 mL
	10 mM	0.1827 mL	0.9134 mL	1.8268 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 (4.57 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.57 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 (4.57 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.57 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 (4.57 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.57 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]. Lovly CM, et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinaseinhibitors. Cancer Res. 2011 Jul 15;71(14):4920-31.

实验参考：

Cell Assay	For viability experiments, cells are seeded in 96-well plates at 25%-33% confluency and exposed to drugs. The human lung adenocarcinoma cell lines H3122 and H2228 are treated with X-376 (10, 30, 100, 300 and 1000 nM). SUDHL-1 lymphoma cells are treated with X-376 (5, 10, 30, 100 and 300 nM). SY5Y neuroblastoma cells are treated with X-376 (30, 100, 300 and 1000 nM). At 72 hours post X-376 addition, Cell Titer Blue Reagent is added and fluorescence is measured on a Spectramax spectrophotometer. All experimental points are set up in hextuplicate replicates and are performed at least two independent times. IC50s are calculated using GraphPad Prism version 5 for Windows. The curves are fit using a nonlinear regression model with a log (inhibitor) vs. response formula[1].
Animal Administration	Mice[1] Nude mice (nu/nu) are injected with H3122 cells. Once tumors reach an average volume of 450 mm^3, a total of 27 athymic mice harboring H3122 tumors are randomized and dosed via oral gavage with 50 mg/kg X-376 or the control vehicle. Two, five, and fifteen hours after the single treatment (3 tumors/timepoint/group), mice are sacrificed and serum is collected for assessment of drug concentration using an LC-MS based bioanalytical method.
References	[1]. Lovly CM, et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinaseinhibitors. Cancer Res. 2011 Jul 15;71(14):4920-31.