产品名称：Tivantinib(ARQ 197)
产品别名：Tivantinib

生物活性：

Description	Tivantinib is a novel and highly selective c-Met tyrosine kinase inhibitor with K_i of 355 nM.
IC_{50} & Target	Ki: 355 nM (c-Met)[1]
In Vitro	Tivantinib (ARQ 197) selectively inhibits c-Met activity in cell-free and cell-based assays. c-Met-expressing cancer cell lines treated with Tivantinib display either a dose-dependent loss of proliferative capacity or caspase-dependent apoptosis that positively correlates with either ligand-dependent c-Met activity or constitutively active c-Met. To examine the biochemical mode of inhibition of Tivantinib, kinetic analyses are done using recombinant human c-Met in a filtermat-based assay. The K_m of ATP is 50.5±2.2 μM, which is similar to the K_m value of ATP. In these kinetic studies, Tivantinib inhibits human recombinant c-Met with a calculated inhibitory constant (K_i) of ~355 nM. In vitro exposure to Tivantinib inhibits constitutive c-Met phosphorylation in HT29 and MKN-45 cells, and HGF-induced c-Met phosphorylation in MDA-MB-231 and NCI-H441 cells with an IC_{50} of 100 to 300 nM[1]. Tivantinib is a low-molecular-weight compound, and is the first in class orally available selective inhibitor of c-Met[2]
In Vivo	Pharmacodynamically, the phosphorylation of c-Met in human colon xenograft tumors (HT29) is strongly inhibited by Tivantinib (ARQ 197), as assessed by a dramatic reduction of c-Met autophosphorylation 24 hours after a single oral dose of 200 mg/kg of Tivantinib. This same dosage in mice shows that tumor xenografts are exposed to sustained plasma levels of Tivantinib, consistent with the observed pharmacodynamic inhibition of c-Met phosphorylation and inhibition of proliferation of c-Met harboring cancer cell lines. A C_{max} of 5.73 μg/mL (13 μM), an area under the concentration-time curve of 12.1 μg/mL h, and a $t_{1/2}$ of 2.4 hours are measured. Plasma levels of Tivantinib 10 hours after dosing are determined to be 1.3 μM, >3-fold above the biochemical inhibitory constant of Tivantinib for c-Met[1]
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 100 mg/mL (270.69 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.7069 mL	13.5347 mL	27.0695 mL
	5 mM	0.5414 mL	2.7069 mL	5.4139 mL
	10 mM	0.2707 mL	1.3535 mL	2.7069 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 (6.77 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.77 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]. Munshi N, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther. 2010 Jun;9(6):1544-53. [2]. Bai YL, et al. Quantitative analysis of tivantinib in rat plasma using ultra performance liquid chromatography with tandem mass spectrometry. J Pharm Biomed Anal. 2016 Jul 15;126:98-102.

实验参考：

Cell Assay	HT29, MKN-45, MDA-MB-231, and NCI-H441 (lung cancer) human cancer cells are seeded in 96-well plates overnight in a medium with 10% FBS. Each cell line is optimized for seeding cell number to ensure a similar degree of confluence at the end of the experiment in nontreated (control) wells. The next day, cells are treated with different concentrations of Tivantinib for 24 hours at 37°C. After ARQ 197 treatment, the drug-containing medium is removed, and cells are washed twice with PBS and incubated in a drug-free medium for an additional 48 hours. Cells are then incubated and stained for 4 hours with the MTS reagent (final concentration of 0.5 mg/mL) per well and are lysed. The results are quantitated by spectrophotometry at λ=450 nm[1]
Animal Administration	Mice[1] Female athymic nude mice are acclimated to the animal housing facility for at least 1 week before the study. Efficacy studies are done in athymic mice bearing HT29, MKN-45, or MDA-MB-231 tumor xenografts to determine the effect of ARQ 197 on tumor growth. Tumor cells [5×10^6 (HT29) and 8×10^6 (MKN-45 and MDA-MB-231) cells/animal] are inoculated s.c. on day 0. Tumor dimensions are measured by a digital caliper and tumor volumes are calculated as length×width^2/2. When tumors reached a volume of ~100 mm^3, mice are randomized into groups and treated daily with orally administered vehicle control or 200 mg/kg Tivantinib formulated in polyethylene glycol 400/20% Vitamin E tocopheryl polyethylene glycol succinate (60:40) at 30 mg/mL, for 5 consecutive days, followed by a 2-day dosing holiday for four cycles. Therefore, each animal received a total of 20 doses. Results are expressed as mean tumor volume±SEM. To assess differences in tumor size between groups, a Mann-Whitney nonparametric t test is performed and significance is defined as $P<0.05$. Rats[2] Six male Sprague-Dawley rats (180-220 g) are used to study the pharmacokinetics of Tivantinib. Diet is prohibited for 12 h before the experiment but water is freely available. Blood samples (0.3 mL) are collected from the tail vein into heparinized 1.5 mL polythene tubes at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 h after oral administration of Tivantinib (10 mg/kg). The samples are immediately centrifuged at 4000g for 8 min. The plasma obtained (100 μL) is stored at -20°C until analysis. Plasma Tivantinib concentration versus time data for each rat is analyzed by DAS (Drug and statistics) software.
	Recombinant c-Met protein (50 ng) comprising residues 974 to 1390 is incubated in a reaction buffer [50 mM Tris-HCl (pH 7.5), 2 mM DTT, 0.1 mM Na_3VO_4, 10 mM $MgCl_2$, 1 mM EGTA, 0.02 mg/mL bovine serum albumin, and 10% glycerol] with various concentrations of Tivantinib or DMSO in a total volume of 20 μL. After incubating for 20 minutes at room temperature, 20 μL of 20 μM poly-Glu-Tyr substrate and 20 μL of increasing amounts of cold ATP containing 1.5 μCi of

Kinase Assay	[γ-^{33}P]ATP are added to initiate the reaction. The reaction is stopped after 5, 10, 20, 40, and 60 minutes by the addition of 10% phosphoric acid and 10 μL aliquots of the reaction mixture are spotted onto P30 filtermat in triplicate. The filters are washed thrice for 5 minutes with 0.75% phosphoric acid and once with methanol for 2 minutes. The level of radioactivity is determined by using a Wallac TriLux MicroBeta liquid scintillation counter. Nonspecific binding is determined by conducting the assay in the absence of enzyme and then subtracting the value from each of the experimental values. Reaction rates are determined in the linear range of each reaction and GraphPad Prism software is used to calculate the K_m and V_{max} values[1]
References	[1]. Munshi N, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther. 2010 Jun;9(6):1544-53. [2]. Bai YL, et al. Quantitative analysis of tivantinib in rat plasma using ultra performance liquid chromatography with tandem mass spectrometry. J Pharm Biomed Anal. 2016 Jul 15;126:98-102.