

产品名称: **XL388**

产品别名: **XL388**

生物活性:				
Description	XL388 is a highly potent and ATP-competitive mTOR inhibitor with an IC_{50} of 9.9 nM. XL388 simultaneously inhibits both mTORC1 and mTORC2 .			
IC ₅₀ & Target	mTOR	mTORC1	mTORC2	DNA-PK
	9.9 nM (IC ₅₀)			8.831 μ M (IC ₅₀)
In Vitro	XL388 (Compound 28) also inhibits DNA-PK with an IC ₅₀ of 8.831 μ M. XL388 inhibits cellular phosphorylation of mTOR complex 1 (p-p70S6K, pS6, and p-4E-BP1) and mTOR complex 2 (pAKT (S473)) substrates. XL388 acts in an ATP-competitive manner, with a linear increase in IC ₅₀ values with increasing ATP concentration[1]. XL388 shows a dose-dependent effect in promoting MG-63 cell apoptosis. XL388 (100 nM) induces apoptosis in other two OS cell lines (U2OS and SaOs-2), but not in non-cancerous MC3T3-E1 cells. XL388 potently inhibits activation of both mTORC1 and mTORC2 in MG-63 cells. The effect of XL388 on mTORC1/2 activation is again dose-dependent. Further, mTORC1/2 activation is almost blocked in XL388 (100 nM)-treated U2OS cells, SaOs-2 cells and primary human OS cells[2].			
In Vivo	To assess the pharmacodynamic effects of XL388 (Compound 28) on the mTOR pathway signaling, athymic nude mice bearing PC-3 prostate tumors are dosed orally at 100 mg/kg of XL388. Rapamycin is also administered intraperitoneally at 5 mg/kg as a reference. Plasma and tumor samples are collected at 1, 4, 8, 16, 24, and 32 h for XL388 and at 4 h for Rapamycin after dosing and homogenized with buffer. Tumor lysates from each animal (n=5) are then pooled for each group and analyzed by immunoblot for levels of phosphorylated p70S6K, S6, 4E-BP1, and AKT. XL388 has moderate terminal elimination half-life ($t_{1/2}$ =1.35 h, 0.45 h, 6.11 h and 0.86 h for mouse (10 mg/kg, iv), rat (3 mg/kg, iv), dog (3 mg/kg, iv), monkey (3 mg/kg, iv))[1].			
Solvent&Solubility	In Vitro: DMSO : 50 mg/mL (109.77 mM; Need ultrasonic)			
		Solvent Concentration	Mass	
	Preparing	1 mM	2.1954 mL	10.9769 mL
	Stock Solutions	5 mM	0.4391 mL	2.1954 mL
		10 mM	0.2195 mL	1.0977 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			

	<p>Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (5.49 mM, 饱和度未知) 的澄清溶液。</p> <p>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。</p> <p>2.请依序添加每种溶剂: 10% DMSO\rightarrow 90% (20% SBE-β-CD in saline)</p> <p>Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (5.49 mM, 饱和度未知) 的澄清溶液。</p> <p>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。</p>
References	<p>[1]. Takeuchi CS, et al. Discovery of a novel class of highly potent, selective, ATP-competitive, and orally bioavailable inhibitors of the mammalian target of rapamycin (mTOR). J Med Chem. 2013 Mar 28;56(6):2218-34.</p> <p>[2]. Zhu YR, et al. The anti-cancer activity of the mTORC1/2 dual inhibitor XL388 in preclinical osteosarcoma models. Oncotarget. 2016 Aug 2;7(31):49527-49538.</p>
实验参考:	
Cell Assay	<p>U2OS, SaOs-2 and MG-63 OS cell lines as well as the murine calvaria-derived osteoblastic MC3T3-E1 cells are maintained and culture. The OB-6 human osteoblastic cells are cultured. For primary culture of murine osteoblasts, the trimmed calvariae of neonatal mice are digested with 0.1% collagenase I and 0.25% dispase. The resolving cell suspensions are neutralized with complete culture medium and are filtered. The calvarial osteoblasts are then resuspended in 10 mL α-MEM containing 15% FBS, and are cultured. Cells (5×10^4/well) are suspended in 1 mL of DMEM with 1% agar, 10 % FBS and with indicated XL388 (5, 25, 100 and 200nM) treatment. The cell suspension is then added on top of a pre-solidified 1% agar in a 100 mm culture dish. The drug containing medium is refreshed every 2 days. After 10-day incubation, the number of remaining colonies are stained and manually counted[2].</p>
Animal Administration	<p>Mice, Rats, Dogs and Monkeys[1]</p> <p>Pharmacokinetic studies of XL388 are determined in female athymic nude mice, female CD rats, male beagle dogs, and male cynomolgus monkeys. XL388 is administered intravenously and by oral gavage at 10 mg/kg as a solution formulated in EPW (5% ethanol/45% PEG400/water+1:2 HCl (m/m)) to mice, 3 mg/kg as a solution formulated in EPW (5% ethanol/45% PEG400/water+1:2 HCl (m/m)) to CD rats and male beagle dogs, and 3 mg/kg as a solution formulated in EPW (5% ethanol/45% PEG400/water+1:1.5 HCl (m/m)) to male cynomolgus monkeys. The plasma levels of XL388 are monitored over a 24 h period.</p>
Kinase Assay	<p>The measurement of mTOR enzyme activity is performed in an ELISA format following the phosphorylation of 4E-BP1 protein. All experiments are performed in the 384-well format. Generally, 0.5 μL of DMSO containing varying concentrations of the test compound is mixed with 15 μL of the enzyme solution. Kinase reactions are initiated with the addition of 15 μL of a solution containing the substrate. The assay conditions are as follows: 0.2 nM mTOR, 10 μM ATP, and 50 nM NHis-tagged 4E-BP1 in 20 mM Hepes, pH 7.2, 1 mM DTT, 50 mM NaCl, 10 mM MnCl_2, 0.02 mg/mL BSA, 0.01% CHAPS, 50 mM β-glycerophosphate. Following an incubation of 120 min at ambient temperature, 20 μL of the reaction mixture is transferred to a Ni-chelate-coated 384-well plate. The binding step of the 4E-BP1 protein proceeded for 60 min, followed by washing four times each with 50 μL of</p>

	<p>Tris-buffered saline solution (TBS). Anti-phospho-4E-BP1 rabbit immunoglobulin G (IgG; 20 μL, 1:5000) in 5% BSA-TBST (0.2% Tween-20 in TBS) is added, and the reaction mixture is further incubated for 60 min. Incubation with a secondary horseradish peroxidase (HRP)-tagged anti-IgG is similarly performed after the primary antibody is washed off (four washes of 50 μL). Following the final wash step with TBST, 20 μL of SuperSignal ELISA Femto is added and the luminescence measured using an EnVision plate reader. Data are reported as the mean ($n \geq 2$) [1].</p>
References	<p>[1]. <u>Takeuchi CS, et al. Discovery of a novel class of highly potent, selective, ATP-competitive, and orally bioavailable inhibitors of the mammalian target of rapamycin (mTOR). J Med Chem. 2013 Mar 28;56(6):2218-34.</u></p> <p>[2]. <u>Zhu YR, et al. The anti-cancer activity of the mTORC1/2 dual inhibitor XL388 in preclinical osteosarcoma models. Oncotarget. 2016 Aug 2;7(31):49527-49538.</u></p>



源叶生物