XL388

Cat. No.: HY-13806

CAS No.: 1251156-08-7

Molecular Formula: $C_{23}H_{22}FN_3O_4S$

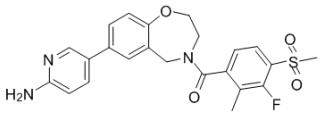
Molecular Weight: 455.5

Target: mTOR; Autophagy

Pathway: PI3K/Akt/mTOR; Autophagy

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (109.77 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1954 mL	10.9769 mL	21.9539 mL
	5 mM	0.4391 mL	2.1954 mL	4.3908 mL
	10 mM	0.2195 mL	1.0977 mL	2.1954 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution

BIOLOGICAL ACTIVITY

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 μM (IC_{50})

In Vitro

XL388 (Compound 28) also inhibits DNA-PK with an IC50 of 8.831 μM. 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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	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-glycerophophate. 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 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 mixuture 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≥2) [1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [2]	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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice, Rats, Dogs and Monkeys[1] 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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Oncotarget. 2017 May 2;8(18):30151-30161.
- Oncotarget. 2016 Aug 2;7(31):49527-49538.

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REFERENCES

[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.

[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.

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