产品名称：**ETP-46464**
产品别名：**ETP-46464**

生物活性：

Description	ETP-46464 is an effective **mTOR** and **ATR** inhibitor with **IC_{50}**s of 0.6 and 14 nM, respectively.

IC_{50} & Target

mTOR	ATR	ATM	DNA-PK	PI3Kα
0.6 nM (IC_{50})	14 nM (IC_{50})	545 nM (IC_{50})	36 nM (IC_{50})	170 nM (IC_{50})

In Vitro

ETP-46464 (ATRi) also inhibits DNA-PK, PI3Kα and ATM with IC50s of 36 nM, 170 nM and 545 nM, respectively[1]. Platinum-sensitive and -resistant ovarian, endometrial and cervical cancer cell lines are treated with varying levels of Cisplatin (0-50 μM) with or without the ETP-46464 (5.0 μM) and/or the KU55933 (10.0 μM) for 72 h. Single-agent dose response analyses of ETP-46464 and KU55933 in a subset of cell lines reveal a wide LD50 range of 10.0±8.7 and 38.3±7.6 μM respectively. Co-treatment doses are chosen based on these studies and previously published evidence of phospho-Chk1 (Ser345) and phospho-ATM (Ser1981) inhibition following ionizing radiation exposure and dose response treatments with ETP-46464 and KU55933. Treatment with ETP-46464 significantly increases the response of Cisplatin in all cell lines tested, resulting in 52-89% enhancement in activity and are synergistic. The combined inhibition of ATR and ATM enhances the response of Cisplatin to a level equivalent to that observed using ETP-46464 alone. These effects are independent of p53 status, and are observed in all gynecologic (GYN) cancer cells tested. Treatment with ETP-46464, but not KU55933, not only sensitizes these GYN cancer cell lines to Cisplatin, but also enhances the response of Carboplatin[2].

Solvent&Solubility

In Vitro:

DMSO : 5 mg/mL (**10.63 mM**; Need ultrasonic)

H_2O : < 0.1 mg/mL (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1253 mL	10.6265 mL	21.2531 mL
	5 mM	0.4251 mL	2.1253 mL	4.2506 mL
	10 mM	0.2125 mL	1.0627 mL	2.1253 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: ≥ 0.5 mg/mL (1.06 mM); Clear solution

此方案可获得 ≥ 0.5 mg/mL (1.06 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 5.0 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]. Toledo LI, et al. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. Nat Struct Mol Biol. 2011 Jun;18(6):721-7. [2]. Teng PN, et al. Pharmacologic inhibition of ATR and ATM offers clinically important distinctions to enhancing platinum or radiation response in ovarian, endometrial, and cervical cancer cells. Gynecol Oncol. 2015 Mar;136(3):554-61.

实验参考：

Cell Assay	Cells are trypsinized with 0.25% Trypsin-EDTA and counted with 0.4% Trypan Blue using an automated cell counter and plated in 96-well plates at 5000 cells per well for KLE, HEC1B and HELA and 10,000 cells per well for OVCAR3, A2780, A2780-CP20 and SIHA. After cells attach and reach approximately 60% confluency (24-48 h post seeding), media is removed and replaced with fresh media containing Cisplatin (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 or 50 μM) or Carboplatin (0, 1.56, 3.13, 6.25, 12.5, 25, 50 or 100 μM) in 0.15% DMSO, 5 μM ETP-46464, 10 μM KU55933, or a combination of 5 μM ETP-46464 and 10 μM KU55933 and incubated for 72 h. Final concentrations of ETP-46464 and KU55933 utilized are based on prior evidence indicating inhibition of ATR and ATM signaling, respectively. Single-agent dose response analyses of ETP-46464 and KU55933 in a subset of cell lines revealed a wide LD50 range (10.0±8.7 and 38.3±7.6 μM, respectively). Similarly, cells are treated with fresh media containing Cisplatin (0, 0.78, 1.56, 3.13, 6.25, 12.5, 25 or 50 μM) in 0.08% DMSO and 5 μM VE-821. Cell viability is assessed using the MTS CellTiter 96 Aqueous One Solution Cell Proliferation Assay. After a 2 h incubation at 37°C, absorbance is measured at 490 nm using a microplate spectrophotometer. Three biological replicates are performed for each cell line where each inhibitor(s)/Cisplatin concentration is assayed in triplicate for each experiment[2].
Kinase Assay	Compounds (e.g., ETP-46464) and control inhibitors are delivered directly to cell media (100 μL per well) with a multi-well pipette at a final concentration of 10 μM. Media content is homogenized by carefully vortexing plates at 500 rpm. Prior to 4-hydroxy-tamoxifen (4-OHT) addition, Compounds (e.g., ETP-46464) are incubated at 37ºC for 15 minutes. Next, to induce ATR activity, 4-OHT is added to all wells and incubated for 60 minutes at 37ºC. Finally, cells are fixed with paraformaldehyde and processed for IF. Every compound (e.g., ETP-46464) is analyzed at least in three independent experiments[1].
References	[1]. Toledo LI, et al. A cell-based screen identifies ATR inhibitors with synthetic lethal properties for cancer-associated mutations. Nat Struct Mol Biol. 2011 Jun;18(6):721-7. [2]. Teng PN, et al. Pharmacologic inhibition of ATR and ATM offers clinically important distinctions to enhancing platinum or radiation response in ovarian, endometrial, and cervical cancer cells. Gynecol Oncol. 2015 Mar;136(3):554-61.