产品名称：TH-302
产品别名：Evofosfamide；艾伏磷酰胺

生物活性：

Description	Evofosfamide (TH-302) is a hypoxia-activated prodrug with IC_{50} of 10 μM and 1000 μM in hypoxia (N_2) and normoxia (21% O_2), respectively.
IC_{50} & Target	Hypoxia-activated prodrug[1]
In Vitro	Evofosfamide (TH-302) induces γH2AX and apoptosis. Evofosfamide displays hypoxia-selective and concentration-dependent cytotoxic activity that is comparable in both p53-proficient and -deficient cells. Treatment with Evofosfamide (TH-302) alone causes an accumulation of G2/M cells. Inhibition of Chk1 by PF47736 in cells treated with Evofosfamide reduces Evofosfamide (TH-302)-mediated G2/M arrest under both normoxia and hypoxia[1].
In Vivo	Evofosfamide (TH-302) is a hypoxia-activated prodrug known to activate selectively under the hypoxic conditions commonly found in solid tumors. The mean values of normalized K^{trans} decrease 69.2% for Evofosfamide (TH-302)-treated mice in Hs766t tumors, decrease 46.1% for Mia PaCa-2 tumors and increase 4.9% in SU.86.86 tumors. Both changes for Hs766t and Mia PaCa-2 treatment groups are statistically significant (P<0.01) when compare to their own control group[2]. A significant reduction in the hypoxic fraction (HF) to 2.1%±4.7% is seen after 95% oxygen breathing (P<0.001), whereas 7% oxygen breathing significantly increase the HF to 29.5%±14.7% (P=0.029). Exposing rhabdomyosarcoma-bearing rats to increasing oxygen conditions abolish the effect of TH-302 and reduce the T4×SV from 20.4±3.5 to 15.3±2.5 days (P=0.007), whereas control animals have an increased T4×SV. Upon combination with radiotherapy, the T4×SV of TH-302-treated tumors decrease from 30.8±5.9 (Evofosfamide (TH-302)+radiotherapy) to 25.7±2.9 days (Evofosfamide (TH-302)+radiotherapy+95% O_2) [3].
Solvent&Solubility	***In Vitro:*** **DMSO : 94 mg/mL (209.34 mM;** Need ultrasonic and warming)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2270 mL	11.1349 mL	22.2697 mL
5 mM	0.4454 mL	2.2270 mL	4.4539 mL
10 mM	0.2227 mL	1.1135 mL	2.2270 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 (5.57 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.57 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 (5.57 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.57 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 (5.57 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.57 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]. Meng F, et al. Enhancement of hypoxia-activated prodrug TH-302 anti-tumor activity by Chk1 inhibition. BMC Cancer. 2015 May 21;15:422. [2]. Zhang X, et al. MR Imaging Biomarkers to Monitor Early Response to Hypoxia-Activated Prodrug TH-302 in Pancreatic Cancer Xenografts. PLoS One. 2016 May 26;11(5):e0155289. [3]. Peeters SG, et al. TH-302 in Combination with Radiotherapy Enhances the Therapeutic Outcome and Is Associated with Pretreatment [^{18}F]HX4 Hypoxia PET Imaging. Clin Cancer Res. 2015 Jul 1;21(13):2984-92.

实验参考：

Cell Assay	Cells are treated with 0.1 μM of either PF477736 or AZD7762 and Evofosfamide (TH-302) for 2 h under either normoxia (21% O_2) or hypoxia (N_2). Following wash, cells are cultured for additional 22 h in the presence of Chk1 inhibitor under normoxia. Cells are fixed in 75% ethanol and cell cycle distribution is determined using cell cycle reagent and Guava flow cytometry. HT-29 cells are exposed to Evofosfamide (TH-302)e (8 nM, 40 nM, 200 nM, 1 μM, and 5 μM) and 0.1 μM of AZD7762 for 2 h under either normoxia (21% O_2) or hypoxia (N_2). After wash, cells are continuously cultured for additional 46 h in the presence of 0.1 μM of AZD7762. Luminescence-based caspase activity assay is performed[1].
Animal Administration	Mice[2] Female SCID mice of age 5-6 weeks are inoculated with SU.86.86, Hs766t or Mia-PaCa2 cells (5×10^6) subcutaneously on the left hind leg. Tumors are allowed to grow for an average of three weeks to an average size of ~150 mm^3. Mice are then randomized and placed into cohorts and treated with saline (control) or Evofosfamide (TH-302) (50 mg/kg) injected intraperitoneally. A total of 34 mice underwent MR imaging studies. The SU.86.86 group consist of 5 TH-302 treated and 5 control animals; Mia-PaCa2 consist of 6 Evofosfamide treated and 5 control animals; Hs766t consist of 7 Evofosfamide treated and 6 control animals. Animals are sacrificed when tumors reach 2000 mm^3. Rats[2] Syngeneic rhabdomyosarcoma R1 tumors (1 mm^3) are implanted subcutaneously in the lateral flank of adult WAG/Rij rats. Experiments are started upon a mean tumor volume of 4.2 cm^3(range, 2.0-8.1) to ensure a stable HF. Treatment is administered on 4 consecutive days and consist of an intraperitoneal injection (i.p.; QD×4) with either NaCl or Evofosfamide (TH-302) (25, 50, or 75

	mg/kg). Before the start of treatment, a PET scan is made using [^{18}F]HX4. Radiotherapy is applied in a single dose of 0, 4, 8, or 12 Gy on day 3 of the treatment, 3 hours after NaCl or Evofosfamide (TH-302) injection, 1 hour after oxygen modification. During both PET imaging and radiotherapy, rats are anesthetized using a mixture of ketamine/xylazine (i.p; 66.7 and 6.7 mg/kg, respectively). During the 5 days of treatment (1 day PET imaging, 4 days of injections with Evofosfamide or vehicle), animals are exposed to modified oxygen concentrations for 4 hours per day in order to alter the HF of the tumor. The combination oxygen modification of nicotinamide (i.p. 500 mg/kg) and carbogen (95% oxygen, 5% CO_2; 5 L/minute) consist of a nicotinamide injection and 30 minutes later the exposure to carbogen breathing for 3.5 hours. In the middle of the nicotinamide/carbogen treatment, NaCl/Evofosfamide is administered. Reduced oxygen breathing (7%, residual N_2; 2.5 L/minute) is given for 4 hours with the NaCl/Evofosfamide injection after the first 2 hours. The injection of the [^{18}F]HX4 PET tracer [mean 18.8 MBq, range 7.1-25.1 MBq; lateral tail vein using an intravenous line (Venoflux 0.4 mm G27) flushed with 10% heparine)] is given 2 hours before the end of the oxygen modification. PET imaging is performed 3 hours after tracer injection.
References	[1]. Meng F, et al. Enhancement of hypoxia-activated prodrug TH-302 anti-tumor activity by Chk1 inhibition. BMC Cancer. 2015 May 21;15:422. [2]. Zhang X, et al. MR Imaging Biomarkers to Monitor Early Response to Hypoxia-Activated Prodrug TH-302 in Pancreatic Cancer Xenografts. PLoS One. 2016 May 26;11(5):e0155289. [3]. Peeters SG, et al. TH-302 in Combination with Radiotherapy Enhances the Therapeutic Outcome and Is Associated with Pretreatment [^{18}F]HX4 Hypoxia PET Imaging. Clin Cancer Res. 2015 Jul 1;21(13):2984-92.