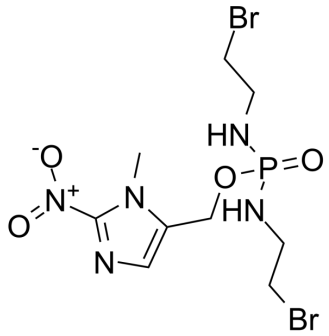
Evofosfamide

Cat. No.:	HY-10535
CAS No.:	918633-87-1
Molecular Formula:	$C_9H_{16}Br_2N_5O_4P$
Molecular Weight:	449.04
Target:	Apoptosis
Pathway:	Apoptosis
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 : 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

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 (5.57 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.57 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.57 mM); Clear solution

BIOLOGICAL ACTIVITY

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 G_2/M cells. Inhibition of Chk1 by PF47736 in cells treated with Evofosfamide reduces Evofosfamide (TH-302)-mediated G_2/M arrest under both normoxia and hypoxia[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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].

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PROTOCOL

Cell Assay [1]

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].

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Animal Administration [2][3]

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.

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CUSTOMER VALIDATION

- Front Oncol. 30 April 2021.
- ACS Med Chem Lett. 2015 Jun 22;6(8):948-52.
- SLAS Discov. 25 October 2021.

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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.

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