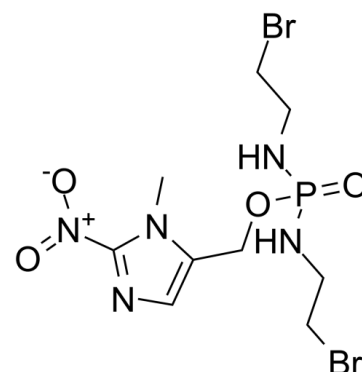


## Data Sheet

Product Name:	Evofosfamide
Cat. No.:	CS-0616
CAS No.:	918633-87-1
Molecular Formula:	C <sub>9</sub> H <sub>16</sub> Br <sub>2</sub> N <sub>5</sub> O <sub>4</sub> P
Molecular Weight:	449.04
Target:	Apoptosis
Pathway:	Apoptosis
Solubility:	DMSO : 94 mg/mL (209.34 mM; Need ultrasonic and warming)



### BIOLOGICAL ACTIVITY:

Evofosfamide (TH-302) is a **hypoxia**-activated prodrug with IC<sub>50</sub> of 10 μM and 1000 μM in hypoxia (N<sub>2</sub>) and normoxia (21% O<sub>2</sub>), respectively. IC<sub>50</sub> & Target: Hypoxia-activated prodrug<sup>[1]</sup> **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<sub>2</sub>/M cells. Inhibition of Chk1 by PF47736 in cells treated with Evofosfamide reduces Evofosfamide (TH-302)-mediated G<sub>2</sub>/M arrest under both normoxia and hypoxia<sup>[1]</sup>. **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<sup>trans</sup> 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<sup>[2]</sup>. 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<sub>2</sub>)<sup>[3]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Cell Assay:** TH-302 is dissolved in DMSO and stored, and then diluted with appropriate media before use<sup>[1],[11]</sup> Cells are treated with 0.1 μM of either PF477736 or AZD7762 and Evofosfamide (TH-302) for 2 h under either normoxia (21% O<sub>2</sub>) or hypoxia (N<sub>2</sub>). 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<sub>2</sub>) or hypoxia (N<sub>2</sub>). 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<sup>[1]</sup>. **Animal Administration:** TH-302 is prepared in saline (NaCl) (Mice and Rat).<sup>[2][3]</sup> Mice<sup>[2]</sup>

Female SCID mice of age 5-6 weeks are inoculated with SU.86.86, Hs766t or Mia-PaCa2 cells (5×10<sup>6</sup>) subcutaneously on the left hind leg. Tumors are allowed to grow for an average of three weeks to an average size of ~150 mm<sup>3</sup>. 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<sup>3</sup>.

Rats<sup>[2]</sup>

Syngeneic rhabdomyosarcoma R1 tumors (1 mm<sup>3</sup>) are implanted subcutaneously in the lateral flank of adult WAG/Rij rats.

Experiments are started upon a mean tumor volume of 4.2 cm<sup>3</sup>(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 [<sup>18</sup>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<sub>2</sub>; 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<sub>2</sub>; 2.5 L/minute) is given for 4 hours with the NaCl/Evofosfamide injection after the first 2 hours. The injection of the [<sup>18</sup>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 [<sup>18</sup>F]HX4 Hypoxia PET Imaging. Clin Cancer Res. 2015 Jul 1;21(13):2984-92.

### CAIndexNames:

Phosphorodiamidic acid, N,N'-bis(2-bromoethyl)-, (1-methyl-2-nitro-1H-imidazol-5-yl)methyl ester

### SMILES:

O=P(NCCBr)(OCC1=CN=C(N1C)[N+](O-)=O)NCCBr

**Caution: Product has not been fully validated for medical applications. For research use only.**

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