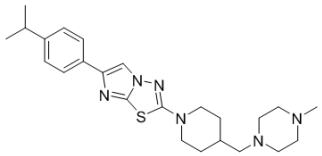
E260

Cat. No.:	HY-112097		
CAS No.:	1241537-79-0		
Molecular Formula:	$C_{24}H_{34}N_6S$		
Molecular Weight:	438.63		
Target:	Others		
Pathway:	Others		
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 : 1 mg/mL (2.28 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.2798 mL	11.3991 mL	22.7983 mL
	5 mM	---	---	---
	10 mM	---	---	---

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

In Vivo

1. Add each solvent one by one: 0.5% CMC-Na/saline water
   Solubility: 22 mg/mL (50.16 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 0.1 mg/mL (0.23 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 0.1 mg/mL (0.23 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 0.1 mg/mL (0.23 mM); Clear solution

BIOLOGICAL ACTIVITY

Description	E260 is a Fer/FerT kinase inhibitor.
IC_{50} & Target	Fer/FerT kinase[1]
In Vitro	E260 is a Fer and FerT inhibitor, which selectively evokes metabolic stress in cancer cells by imposing mitochondrial

dysfunction and deformation, and onset of energy-consuming autophagy which decreases the cellular ATP level. To demonstrate that E260 directly targets Fer and FerT, an in vitro kinase assay is performed using a purified kinase domain (KD)-containing fragment of these enzymes. This analysis demonstrates the direct inhibitory effect of E260 on this domain as reflected by the significantly decreased auto-phosphorylation level of the Fer/FerT KD when incubated with ATP and increasing concentrations of E260. Moreover, computational analysis of E260 docking in the modeled whole Fer protein reveals that the highest scored binding mode of E260 to Fer falls in the ATP-binding pocket of the enzyme's KD. To measure the dissociation constant (K_d) of E260 from Fer/FerT KD, a microscale thermophoresis (MST) test is performed using ascending concentrations of E260. This analysis corroborates the direct binding of E260 to Fer/FerT KD and determines a K_d of 0.85 μM. To examine the effect of the E260 micellar formulation on Fer in malignant cells, the kinase is immunoprecipitated from untreated and from E260-treated SW620 CC cells. When applied to metastatic grade IV SW620 CC cells, which are serum starved for 16 h and treated with 3 mM H_2O_2 to activate Fer, E260 exhibits inhibitory effects on the Fer-kinase activity as is reflected by suppressed auto-phosphorylation activity of the enzyme. To characterize the effect of E260 on malignant cells, metastatic SW620 cells are treated with E260 followed by analysis of viability. Onset of death is observed in the E260-treated cells, with an EC_{50} value of 400 nM after 24 h of treatment and an EC_{50} of 300 nM after 48 h. E260 exhibits an EC_{50} of 3.2 μM after 72 h treatment of non-metastatic PANC-1 cells, which are derived from a primary pancreatic ductal carcinoma. Moreover, the maximum death level of these cells after 72 h of treatment with E260 is about 70% following treatment with 4 μM E260. In comparison, SU.86.86 which are metastatic ductal carcinoma cells, prove to be more susceptible to E260 with an EC_{50} of 1.1 μM after 72 h of treatment and 100% death level imposed by 2 μM E260[1].

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

In Vivo

E260 suppresses xenografts progression in vivo. The pharmacokinetic (PK) profile of E260 is determined in mice. E260 exhibits a $T_{1/2}$ of 175 min in the blood, and a volume of distribution of 4244 mL/kg suggesting an efficient distribution of the compound in the animal tissues. To evaluate the efficacy of E260 on tumor growth, SW620 cells are subcutaneously introduced into immuno-compromised "Nude" mice. Administration of E260 leads to a significant attenuation of tumor progression throughout the experiment, and to a 10-fold decrease in average tumor volume after 22 days of treatment. To further demonstrate the anti-cancer activity of E260 in vivo, mice bearing SW48 cells derived xenografts are treated with E260 and the tumor progression profiles are determined. Mice treated with E260 demonstrate a 5-6-fold attenuation in tumors progression when compared to the control treated group[1].

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PROTOCOL

Kinase Assay [1]

To test the effect of E260 on the Fer/FerT KD auto-phosphorylation activity, 0.5 μg of the Fer/FerT KD protein is incubated in 0.5 mL kinase activity buffer (50 mM HEPES pH 7.5, 10 mM $MgCl_2$, 1 mM EGTA, 0.01% Brij-35) and 1 μM ATP. As a negative control, the KD protein is incubated in the same buffer without ATP. The KD and ATP containing mixture is incubated for 1 h at room temperature with ascending concentrations of E260 dissolved in DMSO or with DMSO alone. Following the incubation period, a sample from the incubated mixture is separated by SDS-PAGE and a WB analysis is performed using specific anti-Fer and anti-pY antibodies to evaluate the inhibitory effect of E260, as reflected by the diminished phosphorylation level of the Fer/FerT KD[1].

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Cell Assay [1]

Cells death level is determined using the MultiTox-Fluor Multiplex Cytotoxicity Assay. Briefly, SW620 CC cells are inoculated into black 96-well plate. After 24 h, when the cells are completely attached, 2 μM E260 or control solution are administrated at different concentrations and incubated for the desired period of time. Following the incubation period, the assay's fluorophore which ias used to determine the cell death levels is added to each well. The relative fluorescence intensity emitted by the fluorophore from each well is determined using an ELISA reader and is compared to the florescence intensity obtained from the standard curve drawn to translate it to cell death percentage and normalized to the non-treated cells which are also used in each analysis[1].

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Animal Administration [1]

Mice[1]

Nude mice are inoculated with 1.5×10^6 SW620 or SW48 CC cells and divided randomly into experimental groups. The mice

are transferred from ad libitum diet to a stricter diet 2 days before inoculation to lower blood glucose levels. At this point the mice are also housed one per cage to ensure an even consumption of food. The diet is comprised of 3 g/mouse/day of standard chow, given at the same time every day. The food is consumed within an average time of 2 h, consequently the mice are kept without food for the next 22 h until the daily ration. The mice are kept on this diet throughout the experiment. The mice are randomized 4 days after tumor inoculation and placed again each in a cage. Mice are injected intraperitoneally every 12 h for 22 days with 25 or 50 mg/kg of the micellar E260 formulation, and control mice are injected with empty micelles[1].

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

REFERENCES

[1]. Elkis Y, et al. A novel Fer/FerT targeting compound selectively evokes metabolic stress and necrotic death in malignant cells. Nat Commun. 2017 Oct 16;8(1):940.

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