TPEN

Cat. No.: HY-100202

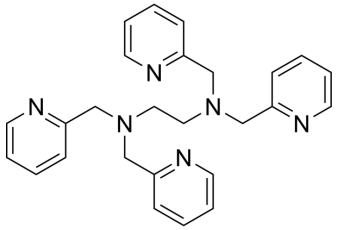
CAS No.: 16858-02-9

Molecular Formula: $C_{26}H_{28}N_6$

Molecular Weight: 424.54

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 20 mg/mL (47.11 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.3555 mL	11.7775 mL	23.5549 mL
	5 mM	0.4711 mL	2.3555 mL	4.7110 mL
	10 mM	0.2355 mL	1.1777 mL	2.3555 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 mg/mL (4.71 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2 mg/mL (4.71 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2 mg/mL (4.71 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

TPEN (TPEDA) is a specific cell-permeable heavy metal chelator. TPEN has a higher affinity for Zn^{2+}, but a lower affinity for Mg^{2+} and Ca^{2+}. TPEN induces DNA damage and increases intracellular ROS production. TPEN also inhibits cell proliferation and induces apoptosis[1][2][3].

In Vitro

Heavy metal chelator TPEN attenuates fura-2 fluorescence changes induced by cadmium, mercury and methylmercury. TPEN, a cell-permeable chelator for heavy metal cations with a low affinity for Ca^{2+}. In cells stimulated with 10 or 30 μM cadmium chloride, the addition of TPEN at 3 hr after exposure significantly decreases the elevated fura-2 fluorescence ratio to the basal levels within 10 min (119.6±2.4% or 109±1.5% decrease in ΔRatio (F340/F380) induced by 10 or 30 μM cadmium chloride, respectively), suggesting that a cadmium chloride-induced increase in the fura-2 fluorescence ratio is dependent

on an increase in intracellular heavy metal cations but not intracellular Ca^{2+}[1].
TPEN is a metal chelator, which targets colon cancer cells through redox cycling of copper. TPEN reduces cell viability in a dose- and time-dependent manner. TPEN-induced cell death is also dependent on the redox cycling of copper since the copper chelator neocuproine inhibited DNA damage and reduced pChk1, γ-H2AX, and ATM protein expression. Cell death by low TPEN concentrations, involved ATM/ATR signaling in all 3 cell lines, since pre-incubation with specific inhibitors of ATM and DNA-PK led to the recovery of cells from TPEN-induced DNA damage[2].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay [1]

Human neuroblastoma cell line SH-SY5Y, are grown in Dulbecco's Modified Eagle's Medium (DMEM) mixed 1:1 with Ham's F-12 nutrient mixture containing 10% fetal bovine serum, 100 unit/mL penicillin and 100 μg/mL streptomycin at 37°C in a humidified 5% CO2 atmosphere. Two days before experimentation, cells are seeded at a density of 7×10^4 cells/cm^2 in a 96-well plate. Cells in a 96-well plate are serum-starved for 4 hr; calcium indicator fura-2 is then loaded into the cells by using Calcium kit II fura-2. In brief, SH-SY5Ycells are incubated with 5 μM fura-2/AM in the presence of 0.04% Pluronic F-127, a dispersing agent to improve the efficiency of loading with fura-2, and 1.25 mM probenecid, a blocker of organic anion transport to prevent leakage of fura-2 from cells. After 1 hr incubation at 37°C, fura-2 fluorescence is measured at 500 nm emission after excitation at 340 nm (F340) or 380 nm (F380) using an Infinite M200 plate reader at 37°C.The change in $[Ca^{2+}]_i$ is reflected by the ratio of F340 and F380. To determine the changes in fura-2 fluorescence ratio induced by heavy metal compounds, cells are treated with manganese chloride, lead acetate, cadmium chloride , mercuric chloride and MeHg chloride dissolved in distilled water. We confirmed that the cells adhered to the bottom of the plate after 6 hr exposure to heavy metal compounds. The cells are also treated with three Ca^{2+} channel blockers, lanthanum chloride dissolved in distilled water, verapamil and 2-APB dissolved in DMSO, 30 min before heavy metal exposure. The heavy metal chelator TPEN is dissolved in DMSO and added 3 hr after the stimulation with heavy metals to determine the contribution of endogenous and exogenous heavy metals on fura-2 fluorescence changes. We measured the effect of TPEN (20 μM) on the fura-2 fluorescence ratio after a 10 min treatment with TPEN, since our preliminary experiments showed that the effect of TPEN on fura-2 fluorescence reached maximum and stabilized within 10 min of the treatment[1].
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- J Nanobiotechnology. 2023 Sep 4;21(1):316.
- Food Chem. 2024 Jan 11:442:138386.
- Br J Pharmacol. 2021 Jan;178(2):346-362.
- Food Funct. 7th July 2021.
- Front Pharmacol. 2022 Feb 23;13:816133.

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Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com
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