

## Cyclophosphamide hydrate

### Chemical Properties

CAS No. : 6055-19-2

Formula: C<sub>7</sub>H<sub>17</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>P·H<sub>2</sub>O

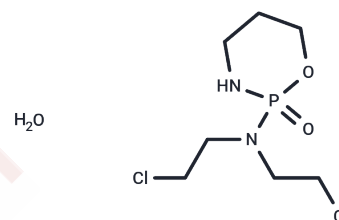
Molecular Weight: 279.10

Storage:

The compound is unstable in solution. Please use soon

Powder: -20°C for 3 years

Actual storage temperature shall be subject to the COA.



### Biological Description

Description	Cyclophosphamide hydrate is a synthetic alkylating agent structurally related to nitrogen mustards, with antitumor and immunosuppressive activities. It is widely used in the treatment of various cancers and is also commonly employed to induce animal models of premature ovarian failure, myelosuppression, cystitis, and myocarditis.
Targets(IC50)	DNA,DNA Alkylator/Crosslinker,MRP
In vitro	<p><b>METHODS:</b> Macrophage Raw 264.7 was treated with Cyclophosphamide hydrate (10-250 µg/mL) for 48 h. Cell viability was measured by MTT assay.</p> <p><b>RESULTS:</b> Cyclophosphamide hydrate was cytotoxic to Raw 264.7 with an IC<sub>50</sub> of 145.44 µg/mL. [1]</p> <p><b>METHODS:</b> Human breast cancer cells MDA-MB-231 and MDA-MB-435S were treated with Cyclophosphamide hydrate (0.25-1 mM) for 4-24 h, and cell migration was detected by Wound healing assay.</p> <p><b>RESULTS:</b> The increase in the number of migrating MDA-MB-231 cells was dependent on the concentration of Cyclophosphamide, while migration to MDA-MB-435S cells was significantly reduced and independent of Cyclophosphamide concentration. [2]</p>
In vivo	<p><b>METHODS:</b> To test the antitumor activity in vivo, Cyclophosphamide hydrate (50-150 mg/kg) was administered as a single intraperitoneal injection to BALB/c mice bearing mouse colorectal tumor CT26.</p> <p><b>RESULTS:</b> Cyclophosphamide hydrate induced a significant reduction in tumor volume. [3]</p> <p><b>METHODS:</b> Cyclophosphamide hydrate (140 mg/kg) was administered orally to Balb/c mice bearing mouse mammary carcinoma tumor 4T1 every six days for eighteen days to determine the antitumor activity in vivo.</p> <p><b>RESULTS:</b> Cyclophosphamide hydrate treatment significantly inhibited tumor growth in mice. [4]</p>
Kinase Assay	9L cells are treated with drug for the times indicated in each experiment. Floating and attached cells are collected, pooled, resuspended in lysis buffer (10 mM HEPES buffer, pH 7.4, containing 2 mM EDTA, 0.1% CHAPS detergent, 5 mM DTT, 350 ng/mL phenylmethylsulfonyl fluoride, 10 ng/mL pepstatin A, 10 ng/mL aprotinin, and 20 ng/mL leupeptin) and lysed by three freeze-thaw cycles (alternating between a dry ice isopropanol bath and a 37°C water bath). Lysates are spun in a bench top centrifuge at

Kinase Assay	full speed for 15 min and the supernatant (cell extract) fraction transferred to a new tube. Cell extracts (20 $\mu$ L) are assayed for caspase 9, caspase 8, and caspase 3 activity by incubation at 37°C for either 1 h (caspase 3) or 3 h (caspase 9 and caspase 8) in 500 $\mu$ L of reaction buffer (10 mM HEPES, pH 7.4, 2 mM EDTA, 0.1% CHAPS, and 5 mM DTT) containing 50 $\mu$ M caspase form-selective substrate: Ac-LETD-AFC for caspase 8; Ac-LEHD-AFC for caspase 9; and Ac-DEVD-AMC for caspase 3. Background activity is determined for each sample as follows. Cell extracts are preincubated for 15 min at room temperature, with or without caspase form-selective inhibitor: 1 $\mu$ M z-LETD-FMK for caspase 8, 1 $\mu$ M z-LEHD-FMK for caspase 9, and 5 $\mu$ L of Casputin for caspase 3. Caspase activity measured in the absence of inhibitor is divided by the background caspase activity measured in the presence of inhibitor. A value of 1 is subtracted from each measured activity, such that a caspase activity of 0 corresponds to no increase in the specific caspase activity with drug treatment. Fluorescence of the caspase product (excitation at 395 nm and emission at 525 nm for AFC substrates, and excitation at 380 nm and emission at 460 nm for the AMC substrate) is measured using a Shimadzu model RF-1501 spectrofluorophotometer and the manufacturer's PC-1501 software package.
Cell Research	9L/pBabe, 9L/Bax, and 9L/Bcl-2 cells are treated with 12, 24, or 50 $\mu$ M MFA for 72 h. Cells remaining on the plates at 0, 24, 48, and 72 h are washed twice with cold PBS and then stained for 5 min with crystal violet [1.25 g of crystal violet dissolved in a solution containing 50 mL of 37% formaldehyde and 450 mL of methanol]. The stained cells are washed three times in tap water and the plates are allowed to dry. The stain is eluted from the cells with 70% ethanol and the absorbance is then read at 595 nm. The staining intensity of each drug-treated sample ( $A_{595}$ ) is then graphed as a percentage of the staining intensity at the 0-h time point.

### Solubility Information

Solubility	DMSO: 250.00 mg/mL (895.74 mM),The compound is unstable in solution. Please use soon. H2O: 7.00 mg/mL (25.08 mM),Sonication is recommended. The compound is unstable in solution, please use soon. Ethanol: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 5.00 mg/mL (17.91 mM),Sonication is recommended. <i>Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.</i>

### Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.5829 mL	17.9147 mL	35.8295 mL
5 mM	0.7166 mL	3.5829 mL	7.1659 mL
10 mM	0.3583 mL	1.7915 mL	3.5829 mL
50 mM	0.0717 mL	0.3583 mL	0.7166 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

### Reference

- Yadav A, et al. In Vitro Cytotoxicity Study of Cyclophosphamide, Etoposide and Paclitaxel on Monocyte Macrophage Cell Line Raw 264.7. *Indian J Microbiol.* 2020 Dec;60(4):511-517.
- Lv M, Chen M, Zhang R, et al. Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. *Cell Research.* 2020, 30(11): 966-979.
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- Hung CM, et al. Cyclophosphamide promotes breast cancer cell migration through CXCR4 and matrix metalloproteinases. *Cell Biol Int.* 2017 Mar;41(3):345-352.
- Malvicini M, et al. A novel synergistic combination of cyclophosphamide and gene transfer of interleukin-12 eradicates colorectal carcinoma in mice. *Clin Cancer Res.* 2009 Dec 1;15(23):7256-65.
- Shi H, et al. Cyclophosphamide Induces the Ferroptosis of Tumor Cells Through Heme Oxygenase-1. *Front Pharmacol.* 2022 Feb 21;13:839464.
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