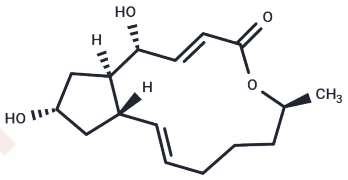
Data Sheet (Cat.No.T6062)

TargetMol

Brefeldin A

Chemical Properties



CAS No. : 20350-15-6

Formula: C16H24O4

Molecular Weight: 280.36

Storage: Store at low temperature,Keep away from moisture,
Store under nitrogen
Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	Brefeldin A (Cyanein) belongs to the class of macrolide antibiotics and is an ATPase inhibitor (IC50=0.2 μM). Brefeldin A can induce tumor cell differentiation and apoptosis, and also possesses autophagy inhibitory activity.
Targets(IC50)	ATPase,Mitophagy,Antibacterial,Antibiotic,Autophagy,HSV,CRISPR/Cas9
In vitro	**METHODS**: Tumor cells HL60, K562 and HT-29 were treated with Brefeldin A (2 μM) for 72 h. DNA fragments were detected by DNA filter elution assay. **RESULTS**: Brefeldin A induced DNA fragmentation with different kinetics. intact DNA fragments were observed in HL60 cells within 15 h, whereas 48-72 h was required for K562 and HT-29 cells. [1] **METHODS**: Human breast cancer cells MDA-MB-231 were treated with Brefeldin A (0.05-1 μg/mL) for 24 h, and the expression levels of target proteins were detected by Western Blot. **RESULTS**: PARP cleavage, a hallmark event of cell death, could be detected in Brefeldin A-treated suspension MDA-MB-231 cells. [2]
In vivo	In HF4.9 and HF28RA cells, Brefeldin A (25 ng/mL) completely inhibits cell growth. Similarly, in HF1A3 cells, Brefeldin A (75 ng/mL) fully inhibits cell growth.
Kinase Assay	ELISA-based active site binding assay: Samples (lysed cells or tissue homogenates) are treated for 1 h at room temperature with the biotinylated active site probe PR-584 (5-15 μM). Samples are denatured by addition of SDS (0.9% final) and heating to 100 °C for 5 min. The denatured samples are transferred to a 96-well or 384-well filter plat, mixed with streptavidin-sepharose beads (2.5-5 μL packed beads/well), and incubated for 1 h at room temperature on a plate shaker. The beads are washed 5 times with 100-200 μL /well of ELISA buffer (PBS, 1% bovine serum albumin, 0.1% Tween-20) by vacuum filtration. The beads are incubated overnight at 4 °C on a plate shaker with the following antibodies recognizing the six catalytic subunits diluted into ELISA buffer: β5, β1, and β2 diluted 1:3000, LMP7 and LMP2 diluted 1:5000, and MECL-1 diluted 1:1000. The beads are washed 5 times with 100-200 μL /well of ELISA buffer and incubated with HRP-conjugated secondary antibody diluted 1:5000 in ELISA buffer and incubated 2 h at room temperature on a plate shaker. The beads are washed 5 times with 100-200 μL /well of ELISA buffer and developed for chemiluminsecence signal using the supersignal

Kinase Assay	ELISA pico substrate following the manufacturer's instructions. Luminescence is measured on a plate reader and converted to ng of proteasome or μg/ml of lysate by comparison with 20S proteasome or untreated cell lysate standard curves. For proteasome inhibitor studies, active site probe binding values are expressed as the percent of binding relative to DMSO treated cells.
Cell Research	HF1A3, HF4.9 cell viability upon the treatments is tested using double staining of cells with YO-PRO 1/PI and SYTO16/PI probes. To access cell proliferation, cells are treated with 0-100 ng/mL Brefeldin A in complete medium for 20 hours before adding 1 μCi/mL [methyl-3H]-thymidine for additional 4 hours at 37 °C. The incorporated radioactive thymidine is quantified by scintillation counting with Microbeta counter. To examine long-term effects of Brefeldin A treatment, cells are seeded at initial concentration 105 cells/mL and treated with 0-75 ng/mL Brefeldin A for up to 5 days. At the time indicated, a sample of cells is removed and viable cell number is assessed by standard Trypan Blue exclusion assay.(Only for Reference)

Solubility Information

Solubility	Ethanol: 2.81 mg/mL (10.02 mM),Sonication is recommended. DMSO: 128.75 mg/mL (459.23 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 4 mg/mL (14.27 mM),Sonication is recommended. *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.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	3.5668 mL	17.8342 mL	35.6684 mL
5 mM	0.7134 mL	3.5668 mL	7.1337 mL
10 mM	0.3567 mL	1.7834 mL	3.5668 mL
50 mM	0.0713 mL	0.3567 mL	0.7134 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

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