

Product Data Sheet

Product Name: Nonactin
Cat. No.: GC12090

Chemical Properties

Cas. No. 6833-84-7

Chemical Name (1R,2R,5R,7R,10S,11S,14S,16S,19R,20R,23R,25R,28S,29S,32S,34S)-2,5,11,14,20,23,29,32-octamethyl-4,13,22,31,37,38,39,40-octaoxapentacyclo[32.2.1.17,10.116,19.125,28]tetracontane-3,12,21,30-tetrone

SMILES O=C([C@H](C)[C@@H]1O[C@H]2CC1)O[C@H](C)C[C@@H]3O[C@H]([C@H](C)C(O[C@@H](C)C[C@H]4O[C@@H]([C@@H](C)C(O[C@H](C)C[C@@H]5O[C@H]([C@H](C)C(O[C@@H](C)C2)=O)CC5)=O)CC4)=O)CC3

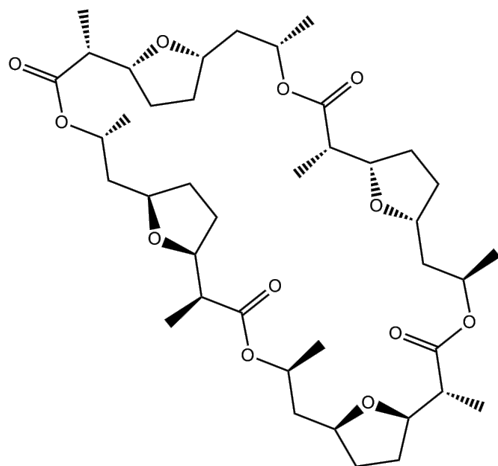
Formula C₄₀H₆₄O₁₂ M.Wt 736.94

Solubility 1mg/mL in ethanol, 0.25mg/mL in DMSO, 10mg/mL in DMF Storage Desiccate at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

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

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Cell experiment [1]:

Cell lines MTC-derived TT cells

Preparation Method The MTC-derived TT cells were cultured in DMEM/F12 medium enriched with 15% heat-inactivated fetal bovine serum (FBS) and maintained under a humidified atmosphere with 5% CO₂ at 37°C. Cells were plated at 7,500 cells per well in a 96-well plate and allowed to adhere overnight. Following this, they were exposed to various concentrations of Nonactin (0.12-0.5μM) for up to 72h. To evaluate cell viability, a solution of 0.33mg/mL MTS dye mixed with 200μg/mL phenazine methosulfate (PMS) was used. Absorbance readings were taken at 490nm. Caspase-3 activity in TT cells was evaluated utilizing the ApoAlert Caspase Fluorescent Assay kit. And cells were collected for PCR and Western blot analyses.

Reaction Conditions 0.12-0.5μM; 72h

Applications Treatment of medullary thyroid carcinoma TT cells with Nonactin reduces RET protein and mRNA level, suppresses pAKT/pmTOR signaling, lowers cyclin D1/E, E2F1 and BCL-2, increases γH2AX and cleaved PARP, and elevates caspase-3/7 activity.

Animal experiment [2]:

Animal models BALB/cA Jcl-nu mice

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Preparation Method Specific pathogen-free female nude mice (BALB/cA Jcl-nu) were used in this experiment. β -catenin-mutant HCT 116 cells (2×10^6 cells/mL) were inoculated subcutaneously into the axillar region of the nude mice on Day 0. Nonactin was dissolved in 10% dimethylacetamide, 10% NIKKOL HCO60, and saline and given daily to the animals by intraperitoneal administration (100mg/kg). Tumor-bearing nude mice were randomly grouped (six mice/group), and administration of nonactin started on Day 9 to Day 12. Tumor volumes were calculated using a microgauge according to the following equations: Tumor volume (mm^3) = $1/2 \times (\text{tumor length}) \times (\text{tumor width})^2$. TUNEL staining of xenograft tumor tissue was performed using the FragEL DNA Fragmentation Detection Kit according to the manufacturer's protocol. Tissue sections were viewed at 100 $\times$ magnification, and images were captured with a digital camera.

Dosage form 100mg/kg/day; i.p.; 4 days

Applications Nonactin (100mg/kg/day; i.p.; 4 days) induced marked tumor regression and robust TUNEL-positive apoptosis in β -catenin-mutant HCT 116 xenograft mice without significant body-weight loss^[7].

References:

- [1] Alqahtani T, Alsubait A, Aloumi M, et al. A novel role for nonactin: interfering with G-quadruplex in RET-driven medullary thyroid cancer. *BMC Cancer*. 2024;24(1):1569.
[2] Shikata Y, Kiga M, Futamura Y, et al. Mitochondrial uncoupler exerts a synthetic lethal effect against β -catenin mutant tumor cells. *Cancer Sci*. 2017;108(4):772-784.

Background

Nonactin is a macrotetrolide antibiotic and mitochondrial oxidative phosphorylation uncoupler^{[1][2]}. Nonactin inhibits recombinant human adenine nucleotide translocase (ANT) isoforms ANT1–4 with

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IC₅₀ values of 4.4, 3.3, 4.7, and 3.3 μM, respectively^[3]. Nonactin is commonly used in studies of oxidative-phosphorylation uncoupling, ionophore mechanisms, and apoptosis in β-catenin-mutant tumors^[4].

In vitro, treatment of BHK cells with Nonactin (5 μg/ml; 12-14h) blocks cell-surface expression of NDV-HN and VSV-G glycoproteins without reducing their total synthesis, causes intracellular accumulation of VSV-G, inhibits syncytium formation and lowers infectious-virus yield^[5]. Treatment of medullary thyroid carcinoma TT cells with Nonactin (0.12-0.5 μM; 72h) reduces RET protein and mRNA level, suppresses pAKT/pmTOR signaling, induces G1 arrest, lowers cyclin D1/E, E2F1 and BCL-2, increases γH2AX and cleaved PARP, and elevates caspase-3/7 activity^[6].

In vivo, Nonactin (100mg/kg/day; i.p.; 4 days) induced marked tumor regression and robust TUNEL-positive apoptosis in β-catenin-mutant HCT 116 xenograft mice without significant body-weight loss^[7].

References:

- [1] Pampaloni F, Mayer B, Kabat Vel-Job K, et al. A Novel Cellular Spheroid-Based Autophagy Screen Applying Live Fluorescence Microscopy Identifies Nonactin as a Strong Inducer of Autophagosomal Turnover. *SLAS Discov.* 2017;22(5):558-570.
- [2] Martínez-Haya B, Avilés-Moreno JR, Hamad S, Elguero J. On the ionophoric selectivity of nonactin and related macrotetrolide derivatives. *Phys Chem Chem Phys.* 2017;19(2):1288-1297.
- [3] Zhang Y, Tian D, Matsuyama H, et al. Human Adenine Nucleotide Translocase (ANT) Modulators Identified by High-Throughput Screening of Transgenic Yeast. *J Biomol Screen.* 2016;21(4):381-390.
- [4] Hu J, Zhang J, He J. Structures, Synthesis and Biological Activities of Nonactic Acid and Its Derivatives. *Curr Med Chem.* 2021;28(42):8673-8691.
- [5] Lee JM, Kim JG, Kim TH, et al. Nonactin hinders intracellular glycosylation in virus-infected baby hamster kidney cells. *Mol Med Rep.* 2010;3(1):115-119.
- [6] Alqahtani T, Alsubait A, Aloumi M, et al. A novel role for nonactin: interfering with G-quadruplex in RET-driven medullary thyroid cancer. *BMC Cancer.* 2024;24(1):1569.
- [7] Shikata Y, Kiga M, Futamura Y, et al. Mitochondrial uncoupler exerts a synthetic lethal effect against β-catenin mutant tumor cells. *Cancer Sci.* 2017;108(4):772-784.

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