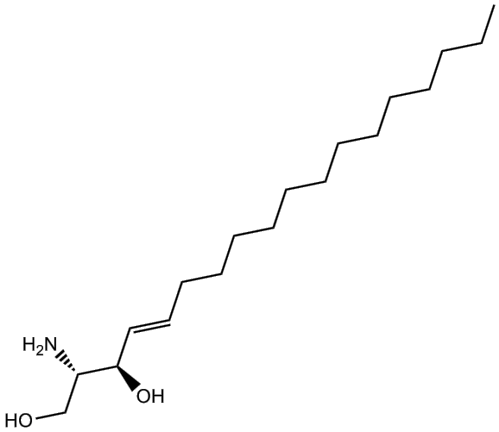
Product Data Sheet

Product Name: D-erythro-Sphingosine (synthetic)
Cat. No.: GC17591

Chemical Properties

Cas. No.	123-78-4		
Chemical Name	(2S,3R,E)-2-aminooctadec-4-ene-1,3-diol		
SMILES	O[C@H](/C=C/CCCCCCCCCCCCC)[C@H](CO)N		
Formula	$C_{18}H_{37}NO_2$	M.Wt	299.5
Solubility	≥ 14.9mg/mL in Ethanol	Storage	Store 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

Cell experiment [1]:	
Cell lines	Molt-4 cells (human lymphoblastic leukemia cell line)

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Preparation Method	Molt-4 cells were maintained in RPMI-1640 medium supplemented with L-glutamine and 2% fetal bovine serum at 37°C, 5% CO_2. Molt-4 cells were treated with D-erythro-Sphingosine at 100-2500nM for 1h, 2h, or 4h. After treatment, cell lysates were prepared and Rb phosphorylation status was analyzed by Western blotting; cell cycle distribution was analyzed by propidium iodide/RNase staining and flow cytometry after 24h treatment with 500nM D-erythro-Sphingosine.
Reaction Conditions	100-2500nM; 1-24h
Applications	D-erythro-Sphingosine induced time- and concentration-dependent dephosphorylation of retinoblastoma protein (Rb), with initial dephosphorylation observed at 1h, peak shift to hypophosphorylated Rb at 4h, significant effect at 500nM and maximal at 1-2.5μM. D-erythro-Sphingosine arrested cells in G0/G1 phase of the cell cycle. D-erythro-Sphingosine did not induce Rb dephosphorylation via PKC inhibition, cAMP/PKC activation, calcium ionophore, or serine/threonine phosphatase inhibition.
Animal experiment [2]:	
Animal models	Male CF-1 mice (6-8 weeks old) with cecal ligation and puncture (CLP)-induced polymicrobial sepsis

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Preparation Method	Mice were subjected to non-lethal CLP surgery. 48h after CLP, mice were intranasally inoculated with 10^6CFU *Pseudomonas aeruginosa* strain 762 (20μL). 2.5h after infection, mice were restrained in an inhalation tower and inhaled 333μL/mouse of 125μM D-erythro-Sphingosine (diluted in normal saline, sonicated 10min) for 15min at -0.25cm H_2O pressure with 0.25L/min/mouse inflow. Core body temperature was monitored via intra-abdominal Anipill implant every 15min; survival was tracked up to 20h post-infection; bronchoalveolar lavage (BAL) fluid was collected 4h post-infection (90min post-inhalation) for bacterial CFU counting on TSA plates.
Dosage form	125μM; 333μL/mouse; single dose
Applications	D-erythro-Sphingosine inhalation reduced pulmonary bacterial load in BAL fluid, and reduced infection-associated mortality.

References:

[1] Chao R, Khan W, Hannun YA. Retinoblastoma protein dephosphorylation induced by D-erythro-sphingosine. Journal of Biological Chemistry. 1992 Nov 25;267(33):23459-23462.

[2] Beckmann N, Pugh AM, Auteri NJ, et al. Therapeutic Inhaled Sphingosine for Treating Lung Infection in a Mouse Model of Critical Illness. Cellular Physiology and Biochemistry. 2020;54:1054-1067.

Background

D-erythro-Sphingosine (synthetic) is a bioactive activator of p32 kinase (EC_{50}=8μM). D-erythro-Sphingosine can be produced from ceramide by ceramidase hydrolysis and serves as the preferred substrate of SphK1 to be phosphorylated into S1P. D-erythro-Sphingosine regulates cell apoptosis, proliferation, differentiation, and inflammatory responses. D-erythro-Sphingosine can be used in research on cancer, neurodegenerative diseases, inflammation, sphingolipid metabolism, and

autophagy-related signaling pathways[1-4].

In vitro, treatment of RD, Rh18, Rh36, and Rh41 cells with 10μM D-erythro-Sphingosine for 0-24 hours dose-dependently induced apoptosis, accompanied by PARP cleavage and processing of caspase-3, caspase-8, and caspase-9[5]. Treatment of human lymphoblastic leukemia Molt-4 cells with 100-2500nM D-erythro-Sphingosine for 1-24 hours induced retinoblastoma protein (Rb) dephosphorylation and caused cell cycle arrest at G0/G1[6]. Treatment of human cisplatin-resistant A549/DDP cells with 5-10μM D-erythro-Sphingosine for 24 hours significantly upregulated p32 protein expression and upregulated mitochondrial fusion protein OPA1 expression[7].

In vivo, CF-1 mice subjected to cecal ligation and puncture on day 2 were intranasally infected with *Pseudomonas aeruginosa* strain 762. After 2.5 hours, a single inhalation of 125μM D-erythro-Sphingosine (333μL/mouse) reduced pulmonary airway bacterial load to levels found in healthy infected mice and decreased infection-associated mortality[8]. C57BL/6 mice with pulmonary *Pseudomonas aeruginosa* infection received a single nebulized inhalation of 1mL of 125μM D-erythro-Sphingosine, reduced lung bacterial burden[9]. CF-1 mice with 28% total body surface area full-thickness burn injury and *Pseudomonas aeruginosa* infection received a single nebulized inhalation of 800μL of 125μM D-erythro-Sphingosine, reduced mouse mortality[10].

References:

[1] Milne JE, Jarowicki K, Kocienski PJ, et al. Synthesis of D-erythro-sphingosine and D-erythro-ceramide. Chem Commun (Camb). 2002 Mar 7;(5):426-7.

[2] Pushkareva MYu, Bielawska A, Menaldiv D, et al. Regulation of sphingosine-activated protein kinases: selectivity of activation by sphingoid bases and inhibition by non-esterified fatty acids. Biochem J. 1993 Sep 15;294 (Pt 3)(Pt 3):699-703.

[3] Nakamura H, Hirabayashi T, Someya A, et al. Inhibition of arachidonic acid release and cytosolic phospholipase A2α activity by D-erythro-sphingosine. European Journal of Pharmacology. 2004 Jan 19;484(1):9-17.

[4] Wu Y, Liu Y, Gulbins E, et al. The Anti-Infectious Role of Sphingosine in Microbial Diseases. Cells. 2021 May 4;10(5):1105.

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[5] Phillips DC, Martin S, Doyle BT, et al. Sphingosine-Induced Apoptosis in Rhabdomyosarcoma Cell Lines Is Dependent on Pre-Mitochondrial Bax Activation and Post-Mitochondrial Caspases. Cancer Research. 2007 Jan 15;67(2):756-764.

[6] Chao R, Khan W, Hannun YA. Retinoblastoma protein dephosphorylation induced by D-erythro-sphingosine. Journal of Biological Chemistry. 1992 Nov 25;267(33):23459-23462.

[7] Yu C, Peng Z, Wang T, et al. p32/OPA1 axis-mediated mitochondrial dynamics contributes to cisplatin resistance in non-small cell lung cancer. Acta Biochimica et Biophysica Sinica. 2024;56(1):34-43.

[8] Beckmann N, Pugh AM, Auteri NJ, et al. Therapeutic Inhaled Sphingosine for Treating Lung Infection in a Mouse Model of Critical Illness. Cellular Physiology and Biochemistry. 2020;54(5):1054-1067.

[9] Rice TC, Pugh AM, Seitz AP, et al. Sphingosine Rescues Aged Mice from Pulmonary Pseudomonas Infection. Journal of Surgical Research. 2017 Nov;219:354-359.

[10] Rice TC, Seitz AP, Edwards MJ, et al. Frontline Science: Sphingosine rescues burn-injured mice from pulmonary Pseudomonas aeruginosa infection. Journal of Leukocyte Biology. 2016 Dec;100(6):1233-1237.

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