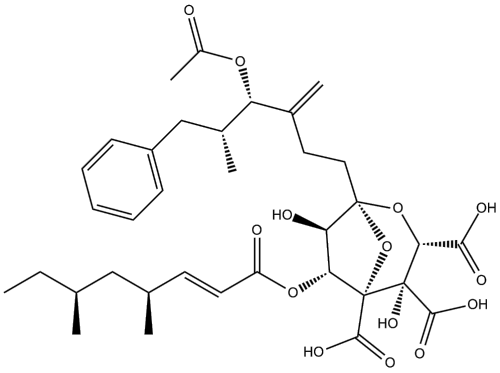
Product Data Sheet

Product Name: Zaragozic Acid A
Cat. No.: GC12351

Chemical Properties

Cas. No.	142561-96-4		
Chemical Name	(1S,3S,4S,5R,6R,7R)-1-((4S,5R)-4-acetoxy-5-methyl-3-methylene-6-phenylhexyl)-6-(((4S,6S,E)-4,6-dimethyloct-2-enoyl)oxy)-4,7-dihydroxy-2,8-dioxabicyclo[3.2.1]octane-3,4,5-tricarboxylic acid		
SMILES	O[C@@H]1[C@H]([C@@]2([C@](C(O)=O)([C@H](O[C@@]1(CCC([C@H]([C@@H](CC3=CC=CC=C3)C)OC(C)=O)=C)O2)C(O)=O)O)C(O)=O)OC(/C=C/[C@H](C[C@H](CC)C)C)=O		
Formula	$C_{35}H_{46}O_{14}$	M.Wt	690.73
Solubility	H_2O : 1 mg/mL (1.45 mM; Need ultrasonic and warming)	Storage	Store at -20°C,protect from light
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]:

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Cell lines	Human primary skin fibroblasts (HPSFs)
Preparation Method	Approximately 4×10^7 HPSF cells were cultured in DMEM medium containing low glucose (5mM) for 72 hours, and 2% fetal bovine serum and 100μM Zaragozic Acid A or DMSO were added to the medium as negative controls. The cells were incubated in DMEM medium containing 0.5mM glucose and 125μCi [^{3}H]Man for 30 minutes for labeling. Dol-P-Man and short-chain lipid-linked oligosaccharide (LLO) were extracted once with chloroform/methanol (2:1), and twice with chloroform/methanol (3:2). The organic phases were combined, dried, and washed. The silica gel 60 thin-layer chromatography method was used with chloroform/methanol/water (65:25:4) as the developing agent for separation. After enhancing the signal with spray, the chromatogram plate was analyzed by X-ray photography. The region containing Dol-P-Man was scraped and counted in a liquid scintillation counter.
Reaction Conditions	100μM; 72h
Applications	Zaragozic Acid A treatment notably increased Dol-P-Man levels in HPSFs.

Animal experiment [2]:

Animal models	Female Swiss Webster mice

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Preparation Method	Female Swiss Webster mice weighing approximately 25g were divided into five groups, three mice per group, and housed in metabolic cages. Groups I and II were treated as controls. Group I was dosed subcutaneously with saline (50μl) and 30min later gavaged with 10μCi of R,S-[5-^{3}H]mevalonolactone. Similar to group I, group II was administered saline subcutaneously and 30min later gavaged with 25μCi of R,S-[5-^{3}H]mevalonolactone containing 375nmol of unlabeled R,S-mevalonolactone. Groups III and IV were dosed with Zaragozic acid A (3mg/kg/day) subcutaneously. Thirty minutes after the Zaragozic acid A treatment, group III was gavaged with 25μCi of R,S-[5-^{3}H]mevalonolactone containing 375nmol of unlabeled R,S-mevalonolactone and group IV was gavaged with 10μCi of R,S-[5-^{3}H]mevalonolactone alone. Group V was gavaged with 5μCi of [1-^{14}C]farnesol containing 45μmol of unlabeled farnesol. Urine from each group was collected for 72h. The dicarboxylic acid fractions were extracted and analyzed by TLC as free acids or converted to methyl esters and characterized by C18 reverse-phase column using HPLC.
Dosage form	3mg/kg/day for 3 days; s.c.
Applications	Zaragozic acid A treatment caused massive production of Farnesol-derived dicarboxylic acids in urine of mice.

References:

[1] Haeuptle M A, Welti M, Troxler H, et al. Improvement of dolichol-linked oligosaccharide biosynthesis by the squalene synthase inhibitor zaragozic acid[J]. Journal of Biological Chemistry, 2011, 286(8): 6085-6091.
[2] Vaidya S, Bostedor R, Kurtz M M, et al. Massive production of farnesol-derived dicarboxylic acids in mice treated with the squalene synthase inhibitor zaragozic acid A[J]. Archives of biochemistry and biophysics, 1998, 355(1): 84-92.

Background

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Zaragozic Acid A is a competitive inhibitor of squalene synthase, with a K_i value of 78pM [1]. Zaragozic Acid A inhibits hepatic cholesterol synthesis, with an IC_{50} value of 6μM[2]. Zaragozic Acid A impairs staphyloxanthin formation and is a potent biofilm inhibitor in *S. aureus* and *Bacillus subtilis* by targeting CrtM enzyme without causing cell death[3]. Zaragozic Acid A has been widely used to regulate the cholesterol levels within cells and to inhibit the transformation of bronchial fibroblasts into myofibroblasts[4].

In vitro, Zaragozic Acid A treatment (100μM) for 72 hours significantly reduced the cholesterol level and increased the dolichol (Dol)-P-Man levels in human primary skin fibroblasts (HPSFs) with congenital disorders of glycosylation (CDG)[5]. Treatment with 10μg/ml Zaragozic Acid A for 48 hours would impair the maintenance of the Tat2p protein and the absorption of tryptophan in the tryptophan auxotrophic (trp1) yeast cells[6]. Treatment with 10μM Zaragozic Acid A for 48 hours significantly inhibited the replication of live Dengue virus type 2 (DEN-2 NGC) in K562 cells, with an EC_{50} value of 8.3μM[7].

In vivo, Zaragozic acid A treatment (3mg/kg/day; s.c.) caused massive production of Farnesol-derived dicarboxylic acids in urine of female Swiss Webster mice [8].

References:
[1] Bergstrom J D, Kurtz M M, Rew D J, et al. Zaragozic acids: a family of fungal metabolites that are picomolar competitive inhibitors of squalene synthase[J]. Proceedings of the National Academy of Sciences, 1993, 90(1): 80-84.
[2] Ponpipom M M, Girotra N N, Bugianesi R L, et al. Structure-activity relationships of C1 and C6 side chains of zaragozic acid A derivatives[J]. Journal of medicinal chemistry, 1994, 37(23): 4031-4051.
[3] Liu C I, Jeng W Y, Chang W J, et al. Binding modes of zaragozic acid A to human squalene synthase and staphylococcal dehydrosqualene synthase[J]. Journal of Biological Chemistry, 2012, 287(22): 18750-18757.
[4] Michalik M, Soczek E, Kosińska M, et al. Lovastatin-induced decrease of intracellular cholesterol level attenuates fibroblast-to-myofibroblast transition in bronchial fibroblasts derived from asthmatic patients[J]. European Journal of Pharmacology, 2013, 704(1-3): 23-32.
[5] Haeuptle M A, Welti M, Troxler H, et al. Improvement of dolichol-linked oligosaccharide

biosynthesis by the squalene synthase inhibitor zaragozic acid[J]. Journal of Biological Chemistry, 2011, 286(8): 6085-6091.
[6] Daicho K, Maruyama H, Suzuki A, et al. The ergosterol biosynthesis inhibitor zaragozic acid promotes vacuolar degradation of the tryptophan permease Tat2p in yeast[J]. Biochimica et Biophysica Acta (BBA)-Biomembranes, 2007, 1768(7): 1681-1690.
[7] Rothwell C, LeBreton A, Ng C Y, et al. Cholesterol biosynthesis modulation regulates dengue viral replication[J]. Virology, 2009, 389(1-2): 8-19.
[8] Vaidya S, Bostedor R, Kurtz M M, et al. Massive production of farnesol-derived dicarboxylic acids in mice treated with the squalene synthase inhibitor zaragozic acid A[J]. Archives of biochemistry and biophysics, 1998, 355(1): 84-92.