

Product Data Sheet

Product Name: 20(S)-Hydroxycholesterol

Cat. No.: GC15422

Chemical Properties

Cas. No. 516-72-3

Chemical Name (3S,8S,9S,10R,13S,14S,17S)-17-((S)-2-hydroxy-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-ol

SMILES O[C@](CCCC(C)C)(C)[C@@H]1[C@](CC2)(C)[C@@H](CC1)[C@H]3[C@H]2[C@](C[C@@H]4O)(C)C(C4)=CC3

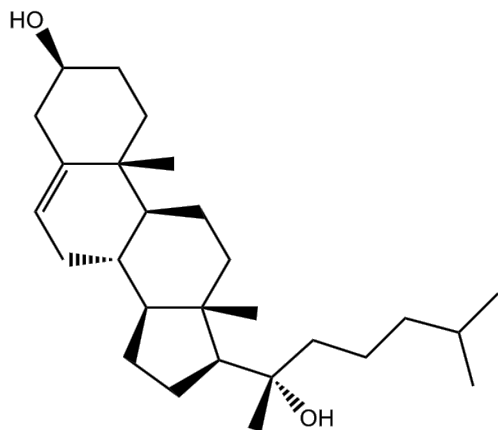
Formula $C_{27}H_{46}O_2$ M.Wt 402.65

Solubility Ethanol:50mg/ml 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 M2-10B4 mouse marrow stromal cells

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

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Preparation Method M2-10B4 cells were cultured in RPMI supplemented with 5% fetal bovine serum (FBS), 50µg/mL ascorbic acid and 3mM β-glycerophosphate. Cells were treated with 5µM of the osteogenic oxysterol cocktail 20(S)-hydroxycholesterol (20S) + 22(S)-hydroxycholesterol (22S) (SS, 1:1) for indicated time periods (10min to 8h), then washed twice and cultured in differentiation medium for the duration indicated for each experiment.

Reaction Conditions 5µM 20(S)-hydroxycholesterol; 10min to 8h

Applications 20(S)-hydroxycholesterol treatment for 10min significantly increased alkaline phosphatase (ALP) activity after 4 days. 20(S)-hydroxycholesterol treatment for 2h significantly increased mineralization after 14 days. 20(S)-hydroxycholesterol treatment for 2-8h increased osteocalcin (OCN) mRNA expression measured after 8 days. 20(S)-hydroxycholesterol treatment for 0.5-24h induced nuclear protein binding to an OSE2 site measured after 4 days.

References:

[1] Aghaloo TL, Amantea CM, Cowan CM, et al. Oxysterols enhance osteoblast differentiation in vitro and bone healing in vivo. J Orthop Res. 2007 Nov;25(11):1488-97.

Background

20(S)-Hydroxycholesterol is a selective allosteric activator of the Hedgehog pathway protein Smoothened ($EC_{50}=3\mu M$). 20(S)-Hydroxycholesterol binds to the extracellular cysteine-rich domain (CRD) of Smoothened in a stereoselective manner, activating downstream Gli transcription factors. 20(S)-Hydroxycholesterol also enhances osteogenic differentiation of bone marrow stromal cells and synergizes with simvastatin to activate the Raf/MEK/ERK pathway, promoting bone regeneration. 20(S)-Hydroxycholesterol can be used in research related to developmental biology, oncology, and bone and angiogenesis mechanisms^[1-4].

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In vitro, 20(S)-Hydroxycholesterol (5 μ M) treatment of cortical neurons cultured on reactive astrocytes for 24 hours significantly increased neurite outgrowth. Treatment of cortical neurons with 20(S)-Hydroxycholesterol (5 μ M) for 30 minutes significantly increased Y416-Src phosphorylation^[5]. A combination of 20(S)-hydroxycholesterol and 22(S)-hydroxycholesterol (1:1) (5 μ M) was used to treat M2-10B4 mouse bone marrow stromal cells for 10 minutes to 8 hours, followed by culture in differentiation medium for 4 to 14 days. This treatment induced alkaline phosphatase activity, increased mineralization, elevated osteocalcin mRNA expression, and promoted nuclear protein binding to the OSE2 site of the osteocalcin promoter^[6].

References:

- [1] Nachtergaele S, Mydock LK, Krishnan K, et al. Oxysterols are allosteric activators of the oncoprotein Smoothed. Nat Chem Biol. 2012 Jan 8;8(2):211-20.
- [2] Nedelcu D, Liu J, Xu Y, et al. Oxysterol binding to the extracellular domain of Smoothed in Hedgehog signaling. Nat Chem Biol. 2013 Sep;9(9):557-64.
- [3] Cheng YS, Zhang T, Ma X, et al. A proteome-wide map of 20(S)-hydroxycholesterol interactors in cell membranes. Nat Chem Biol. 2021 Dec;17(12):1271-1280.
- [4] Peyrot SM, Nachtergaele S, Luchetti G, et al. Tracking the subcellular fate of 20(s)-hydroxycholesterol with click chemistry reveals a transport pathway to the Golgi. J Biol Chem. 2014 Apr 18;289(16):11095-11110.
- [5] Berretta A, Gowing EK, Jasoni CL, et al. Sonic hedgehog stimulates neurite outgrowth in a mechanical stretch model of reactive-astrogliosis. Sci Rep. 2016 Feb 23;6:21896.
- [6] Aghaloo TL, Amantea CM, Cowan CM, et al. Oxysterols enhance osteoblast differentiation in vitro and bone healing in vivo. J Orthop Res. 2007 Nov;25(11):1488-97.

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