

Product Data Sheet

Product Name: Seocalcitol

Cat. No.: GC14513

Chemical Properties

Cas. No. 134404-52-7

Chemical Name (1R,3S,5E)-5-[(2Z)-2-[(1R,3aS,7aR)-1-[(2R,3E,5E)-7-ethyl-7-hydroxynona-3,5-dien-2-yl]-7a-methyl-2,3,3a,5,6,7-hexahydro-1H-inden-4-ylidene]ethylidene]-4-methylidenecyclohexane-1,3-diol

SMILES CCC(CC)(C=CC=CC(C)C1CCC2C1(CCCC2=CC=C3CC(CC(C3=C)O)O)C)O

Formula C₃₀H₄₆O₃

M.Wt 454.7

Solubility DMF: 25 mg/mL, DMSO: 30 mg/mL, Ethanol: 30 mg/mL, Ethanol: PBS (pH 7.2) (1:2): 0.33 mg/mL

Storage 4°C, protect from light, stored under nitrogen

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.

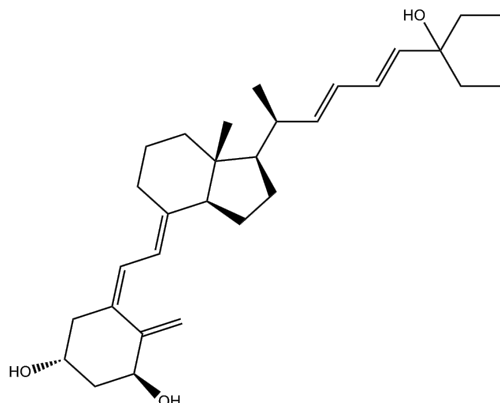
Caution: Product has not been fully validated for medical applications. For research use only.

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Structure



Protocol

Kinase experiment:

Vitamin D receptor protein is prepared from cultures of human osteosarcoma cell line MG-63. Suspensions of 5×10^7 cells/mL are homogenized, sonicated, and centrifuged at 30,000g for 1 h at 4°C. The presence of the $1\alpha,25(\text{OH})_2\text{D}_3$ receptor is verified by sucrose density gradient analysis. The supernatants are adjusted to 2 mg protein/mL and used for binding studies. Samples of 500 μL are incubated with 10,000 dpm [^3H] $1\alpha,25(\text{OH})_2\text{D}_3$ (180 Ci/mmol) and increasing concentrations of $1\alpha,25(\text{OH})_2\text{D}_3$ or vitamin D3 analogs are added. After incubation for 60 min at 22°C, bound and free [^3H] $1\alpha,25(\text{OH})_2\text{D}_3$ are separated on dextran-coated charcoal. Each compound is tested in three separate experiments[1].

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Cell experiment: This fluorescent dye allowed to determine ROS release in HL60 cells, untreated or treated VD3 or Seocalcitol. Briefly, after treatment, HL60 cells are washed and re-suspended at 106 cells/mL in RPMI-1640 without FCS and phenol red. Then, 10 μ M H₂-DCFDA probe is added to each plate at a final volume of 2 mL. Cells are incubated for 45 min at 37°C in the dark. A second wash is made before the fluorescence analysis using spectrometer at 488 nm intensity excitation λ_{ex} and 516 nm emission λ_{em} . Results, in arbitrary fluorescence units (AFU), are expressed according to the ratio $[(AFU\text{-treated cells})/(AFU\text{ control cells})] \times 100[3]$.

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**Animal
experiment:**

Mice[4] Six- to eight-week-old male BALB/c NU/Nu mice are inoculated subcutaneously with 106 SKHEP-1 cells into the right flank. Twenty-four hours after inoculation, mice are randomly assigned to a control group (n=10) or the treatment groups (n=10), receiving 0.02, 0.1 or 0.5 µg/kg per day of Seocalcitol (intraperitoneal or oral on alternate days). Control animals receive propylene glycol alone. Tumor size is measured using vernier calipers every third day and the volumes are estimated using the formula $0.5 \times \text{length} \times (\text{width})^2$. Animals receive sterile food and water. Rats[5] Newborn Sprague-Dawley rat pups are randomly assigned to 1 of 6 treatment groups, consisting of daily intraperitoneal injections of the vitamin D analogue Seocalcitol (0.38 or 1.25 µg/kg body weight) alone, or in combination with all-trans retinoic acid (RA; 500 µg/kg body weight) and/or Dexamethasone (DEX; 0.25 µg/day) in a 3×2×2 factorial design. Seocalcitol and RA injections are conducted on P3 through P12, whereas Dexamethasone is administered on P4 through P12. Seocalcitol is prepared for injections in a quantity sufficient for all injections by dilution from a stock solution (4 mM in isopropanol) into the carrier Solutol HS 15 (BASF). Solutol-diluted Seocalcitol is stored as aliquots in sealed glass vials under nitrogen gas at 4°C, with daily injections conducted with freshly unsealed aliquots of Seocalcitol. Stock solutions of RA (50 mg/mL DMSO) are stored under nitrogen at -80°C and prepared for injection by dilution of freshly thawed aliquots into cottonseed oil (2 µg/µL). A Dexamethasone stock solution (10 mg/mL ethanol) is stored under nitrogen at 4°C and prepared fresh for daily injections in 0.9% NaCl (0.25 µg/µL). All rats receive equivalent volumes of the 3 carrier solutions employed (solutol, cottonseed oil, 0.9% NaCl). Seocalcitol and RA are administered as a 2-phase solution via a 10 µL injection using a 20 µL glass-barreled syringe and a 28-gauge needle, whereas Dexamethasone is administered in a separate 10 µL injection.

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References:

- [1]. Wiberg K, et al. Studies on two new vitamin D analogs, EB 1089 and KH 1060: effects on bone resorption and osteoclast recruitment in vitro. *Bone*. 1995 Oct;17(4):391-5.
- [2]. Roy S, et al. Comparative effects of 1,25-dihydroxyvitamin D3 and EB 1089 on mouse renal and intestinal 25-hydroxyvitamin D3-24-hydroxylase. *J Bone Miner Res*. 1995 Dec;10(12):1951-9.
- [3]. Bondza-Kibangou P, et al. Antioxidants and doxorubicin supplementation to modulate CD14 expression and oxidative stress induced by vitamin D3 and seocalcitol in HL60 cells. *Oncol Rep*. 2007 Dec;18(6):1513-9.
- [4]. Ghous Z, et al. Inhibition of hepatocellular cancer by EB1089: in vitro and in vivo study. *Anticancer Res*. 2008 Nov-Dec;28(6A):3757-61.
- [5]. Ormerod AK, et al. The calcitriol analogue EB1089 impairs alveolarization and induces localized regions of increased fibroblast density in neonatal rat lung. *Exp Lung Res*. 2008 May;34(4):155-82.

Background

Seocalcitol (EB1089) Description: IC50: In SKHEP-1 cells, the IC50 for seocalcitol was 460.99 nM. Calcitriol receptor, also known as the vitamin D receptor (VDR), is a member of the nuclear receptor family of transcription factors. The VDR not only regulates transcriptional responses but also involved in microRNA-directed post transcriptional mechanisms. Seocalcitol (EB1089) is a vitamin D analog that has been extensively studied and shown to have profoundly reduced hypercalcemic effects.

In vitro: In a previous in vitro study, it was found that proliferation of Hep 3B, PLC/PRF/5 and SKHEP-1 HCC cells was significantly inhibited at all seocalcitol concentrations tested, while HTC cells only responded to 1,000 nM concentration of EB1089. However, proliferation of Novikoff cells was unaffected by the drug at all concentrations examined [1]. Another cell in-vitro study indicated that EB1089 significantly inhibited HEP-2 cell proliferation and increased p57 mRNA and protein levels; this was blocked by siRNA-p57 but not by siRNA-con [2].

In vivo: Seocalcitol effectively inhibited SKHEP-1 tumor growth without inducing hypercalcemia ($p < 0.05$). These results indicate that seocalcitol is an effective growth inhibitor of HCC tumors [1].

Clinical trial: 56 patients with inoperable advanced HCC were included in an uncontrolled study of oral Seocalcitol treatment for up to 1 year. Out of 33 patients evaluable for tumour response, two had complete response (CR), 12 stable disease and 19 progressive disease. The CRs appeared after 6 and 24 months of treatment, and lasted for 29 and at least 36 months. Seocalcitol was well tolerated; the most frequent toxicity was hypercalcaemia and related symptoms. Most patients tolerated a daily

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dose of 10 mg of Seocalcitol. These data showed that Seocalcitol may have an effect in the treatment of HCC, especially in early disease when a prolonged treatment can be instituted. The survival benefit with or without tumour response should be determined in controlled studies [3].

Reference:

- [1] Ghous Z, Akhter J, Pourgholami MH, Morris DL. Inhibition of hepatocellular cancer by EB1089: in vitro and in vivo study. *Anticancer Res.* 2008;28(6A):3757-61.
- [2] Lu L, Qiu J, Liu S, Luo W. Vitamin D3 analogue EB1089 inhibits the proliferation of human laryngeal squamous carcinoma cells via p57. *Mol Cancer Ther.* 2008;7(5):1268-74.
- [3] Dalhoff K1 Dancey J, Astrup L, Skovsgaard T, Hamberg KJ, Lofts FJ, Rosmorduc O, Erlinger S, Bach Hansen J, Steward WP, Skov T, Burcharth F, Evans TR. A phase II study of the vitamin D analogue Seocalcitol in patients with inoperable hepatocellular carcinoma. *Br J Cancer.* 2003 21;89(2):252-7.

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