

Product Data Sheet

Product Name: Enzastaurin (LY317615)

Cat. No.: GC11499

Chemical Properties

Cas. No. 170364-57-5

Chemical Name 3-(1-methylindol-3-yl)-4-[1-[1-(pyridin-2-ylmethyl)piperidin-4-yl]indol-3-yl]pyrrole-2,5-dione

SMILES CN1C=C(C2=CC=CC=C21)C3=C(C(=O)NC3=O)C4=CN(C5=CC=CC=C54)C6CCN(CC6)CC7=CC=CC=N7

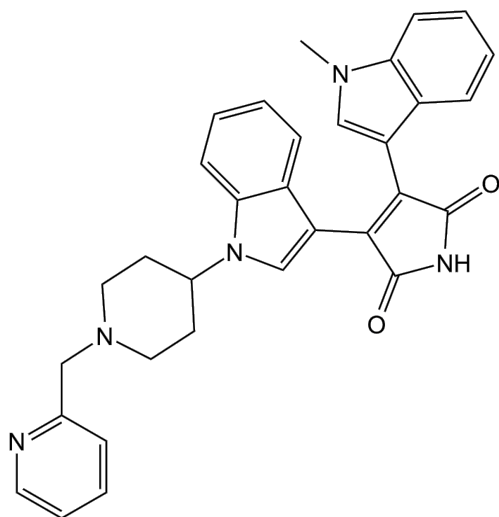
Formula $C_{32}H_{29}N_5O_2$ M.Wt 515.61

Solubility ≥ 8.6 mg/mL in DMSO 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

Kinase experiment [1]:

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The inhibition of PKC β II kinase activity by Enzastaurin was determined using a filter plate assay format measuring ^{33}P incorporation into myelin basic protein substrate. Reactions were done in 100 μl reaction volumes in 96-well polystyrene plates with final conditions as follows: 90mM HEPES (pH 7.5), 0.001% Triton X-100, 4% DMSO, 5mM MgCl_2 , 100 μM CaCl_2 , 0.1mg/ml

phosphatidylserine, 5 $\mu\text{g/ml}$ diacetyl glycerol, 30 μM ATP, 0.005 $\mu\text{Ci}/\mu\text{l}$ ^{33}ATP ,

Preparation Method 0.25mg/ml myelin basic protein, serial dilutions of Enzastaurin (1, 10, 50, 100, 1000, and 2000nM), and recombinant human PKC β II enzyme (390pM).

Reactions were started with enzyme addition, incubated at room temperature for 60 minutes, quenched with 10% H_3PO_4 , transferred to multiscreen anionic phosphocellulose 96-well filter plates, incubated 60 minutes, filtered, and washed with 4 volumes of 0.5% H_3PO_4 on a vacuum manifold. The scintillation cocktail was added, and the plates were read on a scintillation counter.

Reaction Conditions 1, 10, 50, 100, 1000, and 2000nM; 1h

Applications Enzastaurin treatment significantly inhibited the kinase activity of PKC β II in a dose-dependent manner.

Cell experiment [2]:

Cell lines SNU-620 cells

SNU-620 cells were grown in RPMI medium with 10% (v/v) fetal bovine serum (FBS), 100 $\mu\text{g/ml}$ streptomycin, and 100U/ml penicillin at 37°C in 5%

Preparation Method CO_2 /atmosphere. SNU-620 cells were seeded on 96-well plates, incubated for 24h, and then then treated with different concentrations of Enzastaurin (0.1, 1, 10, 100, and 1000 μM) at 37°C. After 72h, cell viability was measured.

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Reaction Conditions 0.1, 1, 10, 100, and 1000μM; 72h

Applications Enzastaurin treatment significantly reduced the cell viability of SNU-620 cells in a concentration-dependent manner.

Animal experiment [3]:

Animal models Athymic nude mice

Preparation Method Athymic nude mice (5 weeks old) were housed singly in a standard environment with food and water ad libitum. A suspension of 2×10^6 H460 cells in 50μl volume was injected subcutaneously into the left hindlimb of mice using a 1-cc syringe with 27.5-gauge needle. Tumors were grown for 1 week until average tumor volume reached 0.28cm^3 . Treatment groups consisted of vehicle control (5% DMSO) and Enzastaurin (80mg/kg). Each treatment group contained five mice. Enzastaurin at a dose of 80mg/kg was administered by oral gavage twice daily for 7 consecutive days. Tumors were measured three times weekly in three perpendicular dimensions.

Dosage form 80mg/kg; twice daily for 7 days; p.o.

Applications Enzastaurin treatment significantly inhibited the tumor growth in mice with H460 xenografts.

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- [1] Graff J R, McNulty A M, Hanna K R, et al. The protein kinase C β -selective inhibitor, enzastaurin (LY317615. HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts[J]. Cancer research, 2005, 65(16): 7462-7469.
- [2] Lee K W, Kim S G, Kim H P, et al. Enzastaurin, a protein kinase C β inhibitor, suppresses signaling through the ribosomal S6 kinase and bad pathways and induces apoptosis in human gastric cancer cells[J]. Cancer research, 2008, 68(6): 1916-1926.
- [3] Willey C D, Xiao D, Tu T, et al. Enzastaurin (LY317615), a protein kinase C beta selective inhibitor, enhances antiangiogenic effect of radiation[J]. International Journal of Radiation Oncology* Biology* Physics, 2010, 77(5): 1518-1526.

Background

Enzastaurin (LY317615) is an ATP-competitive and selective inhibitor of PKC β with an IC₅₀ value of 6nM [1]. Enzastaurin reduces phosphor-Cdc25C levels, resulting in G2/M-checkpoint abrogation, and inhibits Akt and GSK3 β phosphorylation as well as the secretion of VEGF [2]. Enzastaurin has been widely used to inhibit the phosphorylation level of GSK3 β and to restrain the growth of various cancer cells[3].

In vitro, Enzastaurin treatment for 72 hours significantly inhibited the viability of SNU-620 cells and SNU-1 cells, with IC₅₀ values of 3.56 μ M and 3.78 μ M, respectively [4]. Treatment with 20 μ M Enzastaurin for 48 hours significantly induced apoptosis in BCWM.1 cells, accompanied by an increase in the cleavage of caspase-8 and PARP[5]. Treatment with 10 μ M Enzastaurin for 24 hours significantly inhibited the migration and invasion of A549 cells, and led to a significant downregulation of the HIF1 α , VEGFC, and u-PAR genes[6].

In vivo, Enzastaurin treatment via oral administration at a dose of 80mg/kg twice daily for one week significantly enhanced the vascular damage induced by 2Gy radiation in the H460 xenograft mouse model and inhibited tumor growth[7]. Daily subcutaneous injection of a 25mg/kg dose of Enzastaurin for three consecutive days impaired the memory and learning ability of middle-aged rats[8].

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- [2] Giovannetti E, Honeywell R, Hanauske A R, et al. Pharmacological aspects of the enzastaurin-pemetrexed combination in non-small cell lung cancer (NSCLC)[J]. Current Drug Targets, 2010, 11(1): 12-28.
- [3] Nakajima E, Helfrich B, Chan D, et al. Enzastaurin a protein kinase C β -selective inhibitor, inhibits the growth of SCLC and NSCLC cell lines[J]. Journal of Clinical Oncology, 2006, 24(18_suppl): 13138-13138.
- [4] Lee K W, Kim S G, Kim H P, et al. Enzastaurin, a protein kinase C β inhibitor, suppresses signaling through the ribosomal S6 kinase and bad pathways and induces apoptosis in human gastric cancer cells[J]. Cancer research, 2008, 68(6): 1916-1926.
- [5] Moreau A S, Jia X, Ngo H T, et al. Protein kinase C inhibitor enzastaurin induces in vitro and in vivo antitumor activity in Waldenström macroglobulinemia[J]. Blood, The Journal of the American Society of Hematology, 2007, 109(11): 4964-4972.
- [6] Körner A, Mudduluru G, Manegold C, et al. Enzastaurin inhibits invasion and metastasis in lung cancer by diverse molecules[J]. British journal of cancer, 2010, 103(6): 802-811.
- [7] Willey C D, Xiao D, Tu T, et al. Enzastaurin (LY317615), a protein kinase C β selective inhibitor, enhances antiangiogenic effect of radiation[J]. International Journal of Radiation Oncology* Biology* Physics, 2010, 77(5): 1518-1526.
- [8] Willeman M N, Mennenga S E, Siniard A L, et al. The PKC- β selective inhibitor, Enzastaurin, impairs memory in middle-aged rats[J]. PloS one, 2018, 13(6): e0198256.

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