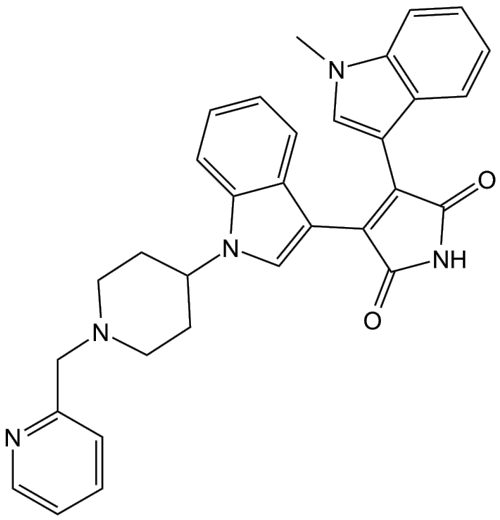
Product Data Sheet

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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Preparation Method	The inhibition of PKCβII 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 100µl 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 $MgCl_2$, 100µM $CaCl_2$, 0.1mg/ml phosphatidylserine, 5µg/ml diacetyl glyerol, 30µM ATP, 0.005µCi/µl ^{33}ATP, 0.25mg/ml myelin basic protein, serial dilutions of Enzastaurin (1, 10, 50, 100, 1000, and 2000nM), and recombinant human PKCβII 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 PKCβII in a dose-dependent manner.

Cell experiment [2]:

Cell lines	SNU-620 cells
Preparation Method	SNU-620 cells were grown in RPMI medium with 10% (v/v) fetal bovine serum (FBS), 100µg/ml streptomycin, and 100U/ml penicillin at 37°C in 5% 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 1000µM) 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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References:
[1] Graff J R, McNulty A M, Hanna K R, et al. The protein kinase Cβ–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 Cβ 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 PKCβ with an IC_{50} value of 6nM [1]. Enzastaurin reduces phosphor-Cdc25C levels, resulting in G2/M-checkpoint abrogation, and inhibits Akt and GSK3β phosphorylation as well as the secretion of VEGF [2]. Enzastaurin has been widely used to inhibit the phosphorylation level of GSK3β 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 IC_{50} values of 3.56μM and 3.78μM, respectively [4]. Treatment with 20μM 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 10μM Enzastaurin for 24 hours significantly inhibited the migration and invasion of A549 cells, and led to a significant downregulation of the HIF1α, 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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References:

[1] Graff J R, McNulty A M, Hanna K R, et al. The protein kinase Cβ–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] 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 Cbeta-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 Cβ 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 beta 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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