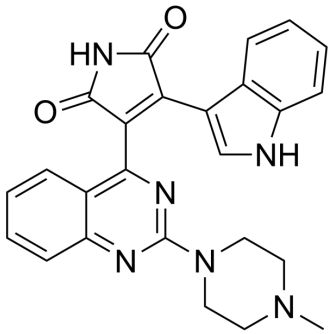
# Sotrastaurin



| | |
|---|---|
| **Cat. No.:** | HY-10343 |
| **CAS No.:** | 425637-18-9 |
| **Molecular Formula:** | $C_{25}H_{22}N_6O_2$ |
| **Molecular Weight:** | 438.48 |
| **Target:** | PKC |
| **Pathway:** | Epigenetics; TGF-beta/Smad |
| **Storage:** | Powder -20°C 3 years<br>4°C 2 years<br>In solvent -80°C 1 year<br>-20°C 6 months |

## SOLVENT & SOLUBILITY

**In Vitro**

DMSO : ≥ 50 mg/mL (114.03 mM)

* "≥" means soluble, but saturation unknown.

| Preparing Stock Solutions | Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 2.2806 mL | 11.4030 mL | 22.8061 mL |
| | 5 mM | 0.4561 mL | 2.2806 mL | 4.5612 mL |
| | 10 mM | 0.2281 mL | 1.1403 mL | 2.2806 mL |

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.70 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.70 mM); Clear solution

## BIOLOGICAL ACTIVITY

**Description**

Sotrastaurin (AEB071) is a potent and orally-active pan-PKC inhibitor, with $K_i$s of 0.22 nM, 0.64 nM, 0.95 nM, 1.8 nM, 2.1 nM and 3.2 nM for PKCθ, PKCβ, PKCα, PKCη, PKCδ and PKCε, respectively[1].

**$IC_{50}$ & Target**

| | | | |
|---|---|---|---|
| PKCθ<br>0.22 nM (Ki) | PKCβI<br>0.64 nM (Ki) | PKCα<br>0.95 nM (Ki) | PKCη<br>1.8 nM (Ki) |
| PKCδ<br>2.1 nM (Ki) | PKCε<br>3.2 nM (Ki) | | |

| | |
|---|---|
| **In Vitro** | In cell-free kinase assays Sotrastaurin (AEB071) inhibits PKC, with $K_i$ values in the subnanomolar to low nanomolar range. When Sotrastaurin is tested on a selected panel of kinases, the only enzyme on which Sotrastaurin displays an $IC_{50}$value below 1 μM is glycogen synthase kinase 3β[1]. Sotrastaurin (AEB071) inhibits p-MARCKS, a PKC substrate, and pS6 in all the cell lines, independently of the mutational status. There is a slight inhibition of pERK at lower doses also in the GNA11 mutant cells, but not in the WT cells at any concentrations. This is consistent with previous reports indicating that Sotrastaurin inhibits ERK1/2 phosphorylation in GNAQ mutant cell lines[2].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **In Vivo** | The combination therapy results in a significantly enhanced reduction in tumor volume when compared to either Sotrastaurin (AEB071) or BYL719 alone (p=0.049 vs. BYL719 and p=0.022 vs. Sotrastaurin at day 26). There is even a greater effect when compared to vehicle control (p=0.016)[2]. Sotrastaurin (STN) treatment of liver donors and orthotopic liver transplantation (OLT) recipients (Gr.I) or of OLT recipients alone (Gr.II) prolongs animal survival, as 9 out of 10 rats in Gr. I, and 6 out of 6 rats in Gr.II survive >14 days. In contrast, only 4 out of 10 control OLT recipients remain alive at day 14 (p<0.01)[3].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

| | |
|---|---|
| **Kinase Assay [1]** | Classical and novel PKC isotypes are assayed by scintillation proximity assay technology. In brief, the assay is performed in 20 mM Tris-HCl buffer, pH 7.4, and 0.1% bovine serum albumin by incubating 1.5 μM of the peptide substrate with 10 μM [$^{33}$P]ATP, 10 mM $Mg(NO_3)_2$, 0.2 mM $CaCl_2$, and PKC at a protein concentration varying from 25 to 400 ng/mL, and lipid vesicles containing 30 mol% phosphatidylserine, 5 mol% diacylglycerol (DAG), and 65 mol% phosphatidylcholine at a final lipid concentration of 0.5 μM. Incubation is performed for 60 min at room temperature. The reaction is stopped by adding 50 μL of a mixture containing 100 mM EDTA, 200 μM ATP, 0.1% Triton X-100, and 0.375 μg/well streptavidin-coated scintillation proximity assay beads in PBS without $Ca^{2+}$ and $Mg^{2+}$. Incorporated radioactivity is measured in a MicroBetaTrilux counter for 1 min. PKCζ is assayed. In situ Thr-219 autophosphorylation status analysis of PKCθ is done by a phospho-site-specific antibody[1].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Cell Assay [2]** | Cells are plated in a 96-well plate and treated with Sotrastaurin, BYL719 or DMSO at indicated concentrations for a period of 5 days. Viability is assessed using Cell Counting Kit. The Combination Index values are calculated using the CompuSyn software. Briefly explained, the plots generated by the CompuSyn software demonstrate the Y-axis combination index values, where CI<1, =1, and >1 indicate synergism, additive effect, and antagonism, respectively. The X-Axis represents the fractional activity, which reflects the fraction of cells inhibited by the treatments relative to vehicle control. For combination index studies, the concentrations tested included Sotrastaurin (0, 125, 250, 500, 1000 nM) and BYL719 (0, 250, 500, 1000, 2000 nM)[2].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration [2][3]** | Mice[2]<br>6-8 week nu/nu SCID female mice bearing subcutaneously injected 92.1 tumors (7 mice/group) of 100$mm^3$ diameter are treated with vehicle, Sotrastaurin (80mg/kg/d) TID and or BYL719 orally (50mg/kg/d) QD as single agents and in combination, 5 days/week for 2 weeks. After 2 weeks, two animals from each group are sacrificed and tumors are collected to analyze for Western blot. For Omm1 xenogratfs, 6-8 weeks athymic female mice bearing subcutaneously injected Omm1 tumors (7 mice/group) of 100 $mm^3$ diameter are treated with vehicle, Sotrastaurin (80mg/kg/d) TID and or BYL719 orally (50mg/kg/d) QD as single agents and in combination, 5 days/week for 3 weeks. Tumors are homogenized with grinding resins kits. Tumors are collected to analyze for H&E, and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. Tumors are measured every 2 to 3 days with calipers, and tumor volumes are calculated. Toxicity is monitored by weight loss.<br>Rats[3]<br>Male Sprague-Dawley (SD) rats (230-250g) are used throughout.Livers from SD rats are stored at 4C in UW solution for 30h, and then transplanted to SD rats with revascularization. Sotrastaurin (30mg/kg b.i.d. via oral gavage) is used in two treatment protocols. In Gr. I (n=10), liver Sotrastaurin is given to liver donors (90min prior to organ harvest) and OLT |

recipients (90min prior to the transplant, and for three days post-OLT). In Gr. II (n=6), Sotrastaurin is administered to OLT recipients only (according to Gr. I protocol). Gr. III controls are treated with PBS (n=10). OLT survival is assessed at day 14. Separate cohorts in Gr. I (n=3-4/gr) are sacrificed at 6h and 24h; OLT and peripheral blood samples are collected for analyses.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Sci Transl Med. 2018 Jul 18;10(450):eaaq1093.
- Cancer Res. 2015 Nov 1;75(21):4538-47.
- Cell Syst. 2018 Apr 25;6(4):424-443.e7.
- Biomed Pharmacother. 2019 Sep;117:109165.
- Antioxidants (Basel). 2021, 10(12), 1898.

See more customer validations on www.MedChemExpress.com

## REFERENCES

[1]. Evenou JP, et al. The potent protein kinase C-selective inhibitor AEB071 (sotrastaurin) represents a new class of immunosuppressive agents affecting early T-cell activation. J Pharmacol Exp Ther. 2009 Sep;330(3):792-801.

[2]. Musi E, et al. The phosphoinositide 3-kinase α selective inhibitor BYL719 enhances the effect of the protein kinase C inhibitor AEB071 in GNAQ/GNA11-mutant uveal melanoma cells. Mol Cancer Ther. 2014 May;13(5):1044-53

[3]. Kamo N, et al. Sotrastaurin, a protein kinase C inhibitor, ameliorates ischemia and reperfusion injury in rat orthotopic liver transplantation. Am J Transplant. 2011 Nov;11(11):2499-507.



Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA