产品名称：**Sotrastaurin**
产品别名：**Sotrastaurin**

生物活性：

Description	Sotrastaurin is a potent pan-PKC inhibitor, with K_is of 0.22 nM, 0.64nM, 0.95 nM, 1.8 nM, 2.1 nM and 3.2 nM for PKCθ, PKCβ, PKCα, PKCη, PKCδ and PKCε, respectively.

IC_{50} & Target	PKCθ	PKCβI	PKCα	PKCη	PKCδ	PKCε
	0.22 nM (Ki)	0.64 nM (Ki)	0.95 nM (Ki)	1.8 nM (Ki)	2.1 nM (Ki)	3.2 nM (Ki)

In Vitro	In cell-free kinase assays Sotrastaurin (AEB071) inhibits PKC, with Ki 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 IC50value 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].
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].

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

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.70 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.70 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；

	向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (5.70 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.70 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。
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.

实验参考：

Cell Assay	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].
Animal Administration	Mice[2] 6-8 week nu/nu SCID female mice bearing subcutaneously injected 92.1 tumors (7 mice/group) of 100mm^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. Rats[3] 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.
Kinase Assay	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].
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.