上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：WH-4-023
产品别名：Dual LCK/SRC inhibitor

生物活性：

Description	WH-4-023 is a potent and selective dual Lck/Src inhibitor with IC_{50} of 2 nM/6 nM for Lck and Src kinase respectively; little inhibition on p38α and KDR.
IC_{50} & Target	IC50: 2 nM (Lck), 6 nM (Src)[1]
In Vitro	WH-4-023 shows a similar potency increase on Lck as 2-substituted variants[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 46.8 mg/mL (82.30 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7585 mL	8.7924 mL	17.5849 mL
	5 mM	0.3517 mL	1.7585 mL	3.5170 mL
	10 mM	0.1758 mL	0.8792 mL	1.7585 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: ≥ 0.77 mg/mL (1.35 mM); Clear solution

此方案可获得 ≥ 0.77 mg/mL (1.35 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 7.7 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% corn oil

Solubility: ≥ 0.77 mg/mL (1.35 mM); Clear solution

此方案可获得 ≥ 0.77 mg/mL (1.35 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。

以 1 mL 工作液为例，取 100 μL 7.7 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。

References	[1]. Martin MW, et al. Novel 2-aminopyrimidine carbamates as potent and orally active inhibitors of Lck: synthesis, SAR, and in vivo antiinflammatory activity. J Med Chem. 2006 Aug 10;49(16):4981-91.

实验参考：

Cell Assay	The purpose of this assay is to test the potency of T cell receptor (TCR; CD3) and CD28 signaling pathway inhibitors in human T cells. T cells are purified from human peripheral blood lymphocytes (hPBL) and preincubated with or without compound prior to stimulation with a combination of an anti-CD3 and an anti-CD28 antibody in 96-well tissue culture plates (1×10^5 T cells/well). Cells are cultured for ~20 h at 37°C in 5% CO_2 and then secreted IL-2 in the supernatants is quantified by cytokine ELISA. The cells remaining in the wells are then pulsed with ^{3}H-thymidine overnight to assess the T cell proliferative response. Cells are harvested onto glass fiber filters and ^{3}H-thymidine incorporation into DNA is analyzed by liquid scintillation counter. For comparison purposes, phorbol myristic acid (PMA) and calcium ionophore are used in combination to induce IL-2 secretion from purified T cells. Potential inhibitor compounds are tested for inhibition of this response as described above for anti-CD3 and -CD28 antibodies. Human whole-blood anti-CD3/CD28-induced IL-2 secretion assays are run in a similar fashion as described above using whole blood from normal volunteers diluted 50% in tissue culture medium prior to stimulation[1].
Kinase Assay	The Lck HTRF kinase assay involves ATP-dependent phosphorylation of a biotinylated substrate peptide of gastrin in the presence or absence of inhibitor compound. The final concentration of gastrin is 1.2 μM. The final concentration of ATP is 0.5 μM (K_m app =0.6±0.1 μM), and the final concentration of Lck (a GST-kinase domain fusion (AA 225−509)) is 250 pM. Buffer conditions are as follows: 50 mM HEPES pH=7.5, 50 mM NaCl, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 2 mM DTT, 0.05% BSA. The assay is quenched and stopped with 160 μL of detection reagent. Detection reagents are as follows: Buffer made of 50 mM Tris, pH=7.5, 100 mM NaCl, 3 mM EDTA, 0.05% BSA, 0.1% Tween20. Prior to reading, Streptavidin allophycocyanin (SA-APC) is added at a final concentration in the assay of 0.0004 mg/mL, along with europilated anti-phosphotyrosine Ab (Eu-anti-PY) at a final conc of 0.025 nM. The assay plate is read in a Discovery fluorescence plate reader with excitation at 320 nm and emission at 615 and 655 nm[1].
References	[1]. Martin MW, et al. Novel 2-aminopyrimidine carbamates as potent and orally active inhibitors of Lck: synthesis, SAR, and in vivo antiinflammatory activity. J Med Chem. 2006 Aug 10;49(16):4981-91.