产品名称：**R406**
产品别名：**R406**

生物活性：

Description	R406 is a competitive Syk inhibitor for ATP binding with a K_i of 30 nM, potently inhibits Syk kinase activity in vitro with an IC_{50} of 41 nM, measured at an ATP concentration corresponding to its K_m value.
IC_{50} & Target	Ki: 30 nM (Syk)[1] IC50: 41 nM (Syk)[1]
In Vitro	R406 also exhibits antagonistic activity for adenosine A_3 receptor with an IC50 estimated to be 93 nM[1]. In Ramos B lymphoma cells, B-cell receptor (BCR) crosslinking induces robust phosphorylation of B-cell linker protein (BLNK), which is ablated by addition of the Syk inhibitor R406. Additionally, R406 significantly reduces constitutive Syk signaling in EBV+ cell lines derived from patients with Post-transplant lymphoproliferative disorder (PTLD), termed SLCL. Therefore, R406 inhibits Syk activation[2].
In Vivo	Prophylactic treatment of mice with R406 administers 1 h before immune complex challenge reduces the cutaneous reverse passive Arthus reaction by approximately 72 and 86% at 1 and 5 mg/kg, respectively, compared with the vehicle control. The net optical density reading of extravasated dye extracted after treatment with R406 at 1 or 5 mg/kg R406 is reduced from 0.14 (vehicle) to 0.04 or 0.02, respectively ($p<0.01$)[1].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 61 mg/mL (97.04 mM)** **H_2O : < 0.1 mg/mL** (insoluble) * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.5908 mL	7.9538 mL	15.9076 mL
5 mM	0.3182 mL	1.5908 mL	3.1815 mL
10 mM	0.1591 mL	0.7954 mL	1.5908 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 (3.98 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (3.98 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 (3.98 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (3.98 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 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]. Braselmann S, et al. R406, an orally available spleen tyrosine kinase inhibitor blocks fc receptor signaling and reduces immune complex-mediated inflammation. J Pharmacol Exp Ther, 2006, 319(3), 998-1008. [2]. Hatton O, et al. Syk-induced phosphatidylinositol-3-kinase activation in Epstein-Barr virus posttransplant lymphoproliferative disorder. Am J Transplant. 2013 Apr;13(4):883-90. [3]. Kitai M, et al. Effects of a spleen tyrosine kinase inhibitor on progression of the lupus nephritis in mice. J Pharmacol Sci. 2017 May;134(1):29-36.

实验参考：

Cell Assay	Cells (0.5-1×10^6 cells/mL) are plated in serial dilutions of small molecule inhibitors R406, LY294002, or PD98059 (0-10 μM), or equivalent amounts of vehicle (DMSO, 0-1:1000). Drug and media are replenished after 48 h of a total of 96 h in culture at 37°C. Cell cycle analysis using propidium iodide. The percentage of apoptotic cells is determined using Annexin V-enhanced GFP (EGFP) apoptosis detection kits. Data is analyzed on a FACScan flow cytometer[2]
Animal Administration	Mice[1] Mice are challenged intravenously with 1% ovalbumin (OVA) in saline (10 mg/kg) containing 1% Evans blue dye. Ten minutes later, mice are anesthetized with isofluorane and shaved dorsolaterally. The rabbit anti-OVA IgG (50 μg/25 μL) is injected intradermally on the left side of the back at three adjacent locations. Three injections of rabbit polyclonal IgG (50 μg/25 μL) on the opposite side of the same animal served as controls. R406 (1 and 5 mg/kg) or vehicle (67% PEG 400) is administered to animals 60 min before antibody/antigen challenge. Four hours after challenge, the animals are euthanized, and skin tissue is assessed for edema and inflammation by measuring dye extravasation into the surrounding tissue. Punch biopsy of the injection sites (8 mm) are incubated in 2 mL of formamide at 80°C overnight. The concentration of the extravasated Evans blue dye is measured spectrophotometrically at OD610.
Kinase Assay	The fluorescence polarization reactions are performed. For $K_i$determination, duplicate 200 μL reactions are set up at eight different ATP concentrations from 200 μM (2-fold serial dilutions) in the presence of either DMSO or R406 at 125, 62.5, 31.25, 15.5, or 7.8 nM. At different time points, 20 μL of each reaction is removed and quenched to stop the reaction. For each concentration of R406, the rate of reaction at each concentration of ATP is determined and plotted against the ATP concentration to determine the apparent K_m and V_{max} (maximal rate). Finally the apparent K_m (or apparent K_m/V_{max}) is plotted against the inhibitor concentration to determine the K_i. All data analysis is performed using Prism and Prism enzyme kinetics programs[1]
References	[1]. Braselmann S, et al. R406, an orally available spleen tyrosine kinase inhibitor blocks fc receptor signaling and reduces immune complex-mediated inflammation. J Pharmacol Exp Ther, 2006, 319(3), 998-1008. [2]. Hatton O, et al. Syk-induced phosphatidylinositol-3-kinase activation in Epstein-Barr virus posttransplant lymphoproliferative disorder. Am J Transplant. 2013 Apr;13(4):883-90. [3]. Kitai M, et al. Effects of a spleen tyrosine kinase inhibitor on progression of the lupus nephritis in

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