产品名称：**PP1**
产品别名：**PP1**

## 生物活性：

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| **Description** | PP1 is a potent, and **Src** family-selective tyrosine kinase inhibitor with **$IC_{50}$** of 5 and 6 nM for Lck and Fyn, respectively. |
| **$IC_{50}$ & Target** | IC50: 5 nM (Lck), 6 nM (Fyn), 250 nM (EGFR), >50 μM (JAK2)[1] |
| **In Vitro** | PP1 inhibits Lck (IC50=5 nM) and FynT (IC50=6 nM) in vitro at concentrations significantly lower than those required to inhibit ZAP-70 (IC50>100 μM), JAK2 (IC50>50 μM), the EGFR kinase, and protein kinase A. PP1 inhibits whole cell tyrosine phosphorylation and proliferation in T cells stimulated with anti-CD3 and mitogens. PP1 selectively inhibits IL-2 gene expression over GM-CSF and IL-2R gene induction in human T cells[1]. |

**Solvent&Solubility**

***In Vitro:***

**DMSO : 28 mg/mL (99.52 mM;** Need ultrasonic)

| Preparing Stock Solutions | Solvent / Mass / Concentration | 1 mg | 5 mg | 10 mg |
|---|---|---|---|---|
| | 1 mM | 3.5542 mL | 17.7708 mL | 35.5417 mL |
| | 5 mM | 0.7108 mL | 3.5542 mL | 7.1083 mL |
| | 10 mM | 0.3554 mL | 1.7771 mL | 3.5542 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: ≥ 1.67 mg/mL (5.94 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (5.94 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 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: ≥ 1.67 mg/mL (5.94 mM); Clear solution

此方案可获得 ≥ 1.67 mg/mL (5.94 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 16.699999 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。

3.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 1.67 mg/mL (5.94 mM); Clear solution

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| | 此方案可获得 ≥ 1.67 mg/mL (5.94 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。<br>以 1 mL 工作液为例，取 100 μL 16.699999 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。 |
| **References** | [1]. Hanke JH, et al. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. J Biol Chem. 1996 Jan 12;271(2):695-701. |

**实验参考：**

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| **Cell Assay** | Inhibition of anti-CD3-stimulated tyrosine phosphorylation in purified human peripheral blood T cells is measured as follows. All incubations are carried out at 37°C in an Eppendorf Thermomixer 5436 at a mixing setting of 11. Cells ($1\times10^6$ in 100 μL of RPMI 1640 medium) are incubated for 15 min with drug prior to a 6-min incubation with 1 μg of anti-CD3/mL (anti-leu4, 100 μg/mL). The final volume of the reaction is 115 μL. Reactions are terminated by the addition of 57.5 μL of 3× solubilization buffer incubated at 100°C prior to its addition. Samples are mixed, boiled for 5 min, and stored at -70°C. Western blots of these cell lysates, run on 10% SDS-polyacrylamide gels, are probed with a polyclonal anti-phosphotyrosine antibody, and immune complexes are detected with I-labeled protein A (ICN). For quantitation, films are scanned using a Molecular Dynamics laser scanner, and the optical densities of the major substrate band, p70, are quantitated in the presence of anti-CD3 (in the presence and absence of drug). Percent inhibition is calculated as follows: (1-(p70 optical density units in presence of drug/p70 units in absence of drug))×100. $IC_{50}$ equals the concentration of compound at which 50% inhibition is measured [1]. |
| **Kinase Assay** | Protein A-Sepharose beads (prepared as a 50% (w/v) suspension) are added to the antibody/lysate mixture at 250 μL/mL and allowed to incubate for 30 min at 4°C. The beads are then washed twice in 1 mL of lysis buffer and twice in 1 mL of kinase buffer (25 mM HEPES, 3 mM $MnCl_2$, 5 mM $MgCl_2$, and 100 μM sodium orthovanadate) and resuspended to 50% (w/v) in kinase buffer. Twenty-five microliters of the bead suspension is added to each well of the enolase-coated 96-well high protein binding plate together with an appropriate concentration of compound and [$\gamma$-$^{32}$P]ATP (25 μL/well of a 200 μCi/mL solution in kinase buffer). After incubation for 20 min at 20°C, 60 μL of boiling 2× solubilization buffer containing 10 mM ATP is added to the assay wells to terminate the reactions. Thirty microliters of the samples is removed from the wells, boiled for 5 min, and run on a 7.5% SDS-polyacrylamide gel. The gels are subsequently dried and exposed to Kodak X-AR film. For quantitation, films are scanned using a Molecular Dynamics laser scanner, and the optical density of the major substrate band, enolase p46, is determined. Concentrations of compound that causes 50% inhibition of enolase phosphorylation (IC50) are determined from a plot of the density versus concentration of compound. In companion experiments for measuring the activity of compounds against Lck, the assay plate is washed with two wash cycles on a Skatron harvester using 50 mM EDTA, 1 mM ATP. Scintillation fluid (100 μL) is then added to the wells, and P incorporation is measured using a Pharmacia Biotech micro-β-counter. Concentrations of compound that causes 50% inhibition of enzyme activity ($IC_{50}$) are determined from a plot of the percent inhibition of enzyme activity versus concentration of compound [1]. |
| **References** | [1]. Hanke JH, et al. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor. Study of Lck- and FynT-dependent T cell activation. J Biol Chem. 1996 Jan 12;271(2):695-701. |