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产品名称：Peficitinib
产品别名：ASP015K; JNJ-54781532

生物活性：

Description	Peficitinib is an oral JAK inhibitor, with IC50s of 3.9, 5.0, 0.7 and 4.8 nM for JAK1, JAK2, JAK3 and Tyk2, respectively.

IC_{50} & Target	JAK3	JAK1	JAK2	Tyk2
	0.7 nM (IC_{50})	3.9 nM (IC_{50})	5 nM (IC_{50})	4.8 nM (IC_{50})

In Vitro	Peficitinib is an oral JAK inhibitor, with IC50s of 3.9, 5.0, 0.7 and 4.8 nM for JAK1, JAK2, JAK3 and Tyk2, respectively. Peficitinib inhibits IL-2-induced T cell proliferation with an IC50 of 10 nM. Peficitinib also suppresses the IL-2-induced STAT5 phosphorylation in rat and human whole blood, with mean IC50s of 124 nM and 127 nM, respectively[1].
In Vivo	Peficitinib (20 mg/kg, p.o.) suppresses IL-2-induced STAT5 phosphorylation by 78% in the rat model of adjuvant-induced arthritis (AIA). Peficitinib potently inhibits the increase in paw volume (≥1 mg/kg) with an ED50 of 2.7 mg/kg, significantly reduces the bone destruction score (≥ 10 mg/kg) and almost fully ameliorates both paw swelling and bone destruction scores (30 mg/kg)[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 60 mg/mL (183.83 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	3.0638 mL	15.3191 mL	30.6382 mL
	5 mM	0.6128 mL	3.0638 mL	6.1276 mL
	10 mM	0.3064 mL	1.5319 mL	3.0638 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 (7.66 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.66 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 (7.66 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.66 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]. Ito M, et al. A novel JAK inhibitor, peficitinib, demonstrates potent efficacy in a rat adjuvant-induced arthritis model. J Pharmacol Sci. 2017 Jan;133(1):25-33.

实验参考：

Cell Assay	Splenocytes from male Lewis rats are suspended in RPMI1640 supplemented with 10% fetal bovine serum and 50 μM 2-mercaptoethanol at a density of 1.5 × 10^6 cells/mL. Rat splenocytes are cultured with Concanavalin A for 24 h at 37°C to induce IL-2 receptor expression. Splenocytes are then incubated with IL-2 and Peficitinib or tofacitinib at designated concentrations in 96-well tissue culture plates. After 3-day incubation, alamarBlue® is added to each of the test wells, followed by 4-6 h incubation. Fluorescence intensity is measured at an excitation wavelength of 545 nm and an emission wavelength of 590 nm. All experiments are performed in triplicate, and experiments are performed either four times or once for assays using Peficitinib or tofacitinib, respectively. For each individual, wells cultured with cells and medium alone are prepared for the blanks, and IL-2 stimulated cells without JAK inhibitors are prepared for the controls. To calculate the % inhibition of JAK inhibitors, blanks and controls are designated as 100% and 0% inhibition, respectively[1].
Animal Administration	Rats[1] Seven-weeks-old female Lewis rats are used for the adjuvant-induced arthritis (AIA) model. Body weight and left hind paw volume of each rat are measured (MK-101PR volume meter), and the values are used to assign animals to one of six groups (n = 10). Arthritis is induced on day 0 in five of these groups by injecting a suspension of Mycobacterium tuberculosis H37 RA strain (0.5 mg/rat) in liquid paraffin into the right hind foot pad. The remaining group is not injected with adjuvant (normal group, n = 10). For the oral administration regimen, four of the adjuvant-injected groups receive Peficitinib (1, 3, 10, and 30 mg/kg) dissolved in 0.5% methylcellulose (MC) once daily. Rats in the normal and control groups receive 0.5% MC alone[1].
Kinase Assay	Human JAK1, JAK2, JAK3, TYK2-domains assays performed using streptavidin-coated 96-well plates. Reaction mixture contained 15 mM Tris-HCl (pH 7.5), 0.01% Tween 20, 2 mM dithiothreitol, 10 mM $MgCl_2$, 250 nM Biotin-Lyn-Substrate-2 (for JAK1, 2 and 3) or Biotin-IRS1-Substrate (for TYK2), and ATP (at final concentrations of 200 μM [JAK1], 10 μM [JAK2], 8 μM [JAK3], and 4 μM [TYK2]). Peficitinib or tofacitinib is dissolved in DMSO. The reaction is initiated by adding the kinase domain, followed by incubation at room temperature for 1 h. Kinase activity is measured as the rate of phosphorylation of Biotin-Lyn-Substrate-2 or Biotin-IRS-Substrate using HRP-conjugated anti-phosphotyrosine antibody (HRP-PY-20) using a phosphotyrosine-specific ELISA. TYK2 kinase assay of Peficitinib is performed with the ATP concentration of 10 μM[1].
References	[1]. Ito M, et al. A novel JAK inhibitor, peficitinib, demonstrates potent efficacy in a rat adjuvant-induced arthritis model. J Pharmacol Sci. 2017 Jan;133(1):25-33.