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产品名称：IRAK inhibitor 4
产品别名：IRAK inhibitor 4

生物活性：

Description	IRAK inhibitor 4 is an interleukin-1 receptor associated kinase 4(IRAK4) inhibitor.
In Vitro	Lack of IRAK-4 impairs the production of proinflammatory mediators by macrophages and DCs in response to M. bovis and M. tuberculosis. IRAK-4$^{-/-}$ cells stimulated with E. coli LPS display delayed activation kinetics of all signaling proteins analyzed, and exhibit dramatically reduced p65 phosphorylation[1]. IRAK1/4 (20 μM) has an inhibitory effect on LPS mediated IL-6 production. IRAK1/4 inhibitor do not decrease p38 phosphorylation in AMs. Combination of IRAK1/4 and Rip2 inhibitors inhibits TLR2-mediated cytokine production in sarcoidosis PBMCs and AMs[2]. IRAK4 is overexpressed and activated in T-ALL. IRAK4 mRNA level is elevated in T-ALL cells from patients compared with the levels detected in thymic T cells or T cells from peripheral blood[3].
In Vivo	IRAK-4$^{-/-}$ mice exhibit a greatly reduced survival rate following aerosol infection compared with IRAK-4$^{+/+}$ or IRAK-4$^{+/-}$ mice. IRAK-4$^{-/-}$ mice show increased bacterial burden in all organs at 15, 30, and 60 d postinfection[1]. MCL1, but not BCL-xL, overrides the therapeutic effects of combinatorial IRAK1/4 inhibitor and ABT-737 therapy in vivo[3].

Solvent&Solubility

In Vitro:

DMSO : 12.5 mg/mL (20.14 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.6112 mL	8.0559 mL	16.1119 mL
	5 mM	0.3222 mL	1.6112 mL	3.2224 mL
	10 mM	0.1611 mL	0.8056 mL	1.6112 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: ≥ 1.25 mg/mL (2.01 mM); Clear solution

此方案可获得 ≥ 1.25 mg/mL (2.01 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 12.5 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: ≥ 1.25 mg/mL (2.01 mM); Clear solution 此方案可获得 ≥ 1.25 mg/mL (2.01 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 12.5 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Marinho FV, et al. Lack of IL-1 Receptor-Associated Kinase-4 Leads to Defective Th1 Cell Responses and Renders Mice Susceptible to Mycobacterial Infection. J Immunol. 2016 Sep 1;197(5):1852-63. [2]. Talreja J, et al. Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1β and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. J Immunol. 2016 Aug 15;197(4):1368-78. [3]. Li Z, et al. Inhibition of IRAK1/4 sensitizes T cell acute lymphoblastic leukemia to chemotherapies. J Clin Invest. 2015 Mar 2;125(3):1081-97.

实验参考：

Cell Assay	THP-1 cells are grown in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. For monocytic differentiation, cells are seeded in 24-well flat-bottom culture plates at a density of 5×10^5 cells/well and allowed to adhere and differentiate for 48 h at 37°C in the presence of 100 nM PMA. THP-1 cells are incubated with 0.1 or 1 μM IRAK-4 inhibitor (IRAK inhibitor 1) for 45 min and then stimulated with M. bovis BCG Moreau (MOI 5:1) or E. coli LPS (1 μg/mL). Culture supernatants are collected after 24 h of stimulation and assayed for the concentrations of human TNF-α or IL-12/23p40 by ELISA. For Western blot analysis, cells are incubated with IRAK-4 inhibitor, in the same concentrations described above, for 45 min and then stimulated with M. bovis BCG Moreau (MOI 5:1) or E. coli LPS (1 μg/mL) for 30 min. The cells are then processed for Western blot assay, as described below. [1]
Animal Administration	To evaluate IRAK-4 involvement in mycobacterial infection, IRAK-$4^{+/+}$ (wild-type), IRAK-$4^{+/-}$ (heterozygous), and IRAK-$4^{-/-}$ (IRAK-4-knockout) mice are used. Eight-week-old mice are infected i.v. with 1×10^6 CFU of M. bovis strain Moreau. The bacterial loads in the spleens, livers, and lungs are determined at 15, 30, and 60 d postinfection. Briefly, the organs are collected aseptically and homogenized in distilled water that contained 0.05% Tween 80. Serial dilutions of the resulting suspensions are plated on Middlebrook 7H11 agar medium supplemented with 10% oleic acid-albumin-dextrose-catalase, and CFU are counted following a 21-d incubation at 37°C and 5% CO_2. [1]
References	[1]. Marinho FV, et al. Lack of IL-1 Receptor-Associated Kinase-4 Leads to Defective Th1 Cell Responses and Renders Mice Susceptible to Mycobacterial Infection. J Immunol. 2016 Sep 1;197(5):1852-63. [2]. Talreja J, et al. Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1β and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. J Immunol. 2016 Aug 15;197(4):1368-78. [3]. Li Z, et al. Inhibition of IRAK1/4 sensitizes T cell acute lymphoblastic leukemia to chemotherapies. J Clin Invest. 2015 Mar 2;125(3):1081-97.