产品名称：GSK583
产品别名：GSK583

生物活性：

Description	GSK583 is a highly potent, orally active and selective inhibitor of **RIP2 Kinase**, with **IC_{50}** of 5 nM. GSK583 inhibits both TNF-α and IL-6 production with an IC_{50} value of 200 nM.
IC_{50} & Target	IC50: 5 nM (RIP2K)[1]
In Vitro	GSK583 (1 μM) exhibits excellent selectivity in a panel of 300 kinases, including p38α and VEGFR2. GSK583 potently and dose dependently inhibits MDP-stimulated tumor necrosis factor-alpha (TNFα) production with an IC50 of 8 nM. GSK583 demonstrates only a modest reduction in potency when profiled in a similar MDP-induced TNFα production assay in human whole blood (IC50 = 237 nM) and rat whole blood (IC50 = 133 nM)[1].
In Vivo	GSK583 (0.1, 1, and 10 mg/kg, p.o.) inhibits serum KC (the rodent orthologue of IL-8) levels in rats in a dose-dependent manner, with an IC50 derived from rat blood concentrations of 50 nM (or 20 ng/mL). Similarly, GSK583 inhibits serum KC levels and recruitment of neutrophils into the peritoneal cavity in mice in a dose-dependent manner, with an IC50 of 37 nM (15 ng/mL) derived from mouse blood concentration[1].
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 37 mg/mL (92.86 mM)** * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5097 mL	12.5486 mL	25.0972 mL
	5 mM	0.5019 mL	2.5097 mL	5.0195 mL
	10 mM	0.2510 mL	1.2549 mL	2.5097 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 (6.27 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.27 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 (6.27 mM); Clear solution

	此方案可获得 ≥ 2.5 mg/mL (6.27 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]. Haile PA et al. The Identification and Pharmacological Characterization of 6-(tert-Butylsulfonyl)-N-(5-fluoro-1H-indazol-3-yl)quinolin-4-amine (GSK583), a Highly Potent and Selective Inhibitor of RIP2 Kinase. J Med Chem, 2016 May 26, 59(10):4867-80.

实验参考：

Animal Administration	Mice[1] Female C57Bl/6 mice (for cytokine analyses) or male Balb/c mice (for peritoneal neutrophil analyses) (n=10/treatment group) are dosed orally 15 min prior to MDP challenge with vehicle or GSK583 (0.1, 1, or 10 mg/kg). For peritoneal neutrophil analysis, mice are sacrificed at 4 h post-MDP challenge (30 μg, i.p.) and peritoneal fluid is collected by lavage. Peritoneal neutrophils are quantified by FACS analysis. Rats[1] Female Crl:CD(SD) rats (n=8/treatment group) are dosed orally with vehicle or GSK583 15 min prior to MDP challenge (150 μg/rat, IV). At 2 h post MDP challenge, rats are sacrificed and terminal serum is prepared from blood collected via cardiac stick. Serum cytokine levels (IL-6, IL-8 or KC, IL-1β, and TNFα) are quantified by the MSD platform.
Kinase Assay	A fluorescent polarization based binding assay is developed to quantitate interaction of novel test compounds at the ATP binding pocket of RIP2K by competition with a fluorescently labeled ATP competitive ligand. Full length FLAG His tagged RIP2K is purified from a baculovirus expression system and is used at a final assay concentration of twice the KD apparent. A fluorescent labeled ligand that is reversible and competitive with the inhibitors is used at a final assay concentration of 5 nM. Both the enzyme and ligand are prepared in solutions in 50 mM HEPES pH 7.5, 150 mM NaCl, 10 mM MgCl2, 1 mM DTT, and 1 mM CHAPS. Test compounds are prepared in 100% DMSO, and 100 nL is dispensed to individual wells of a multiwell plate. Next, 5 μL of RIP2K is added to the test compounds at twice the final assay concentration and incubated at room temperature for 10 min. Following the incubation, 5 μL of the fluorescent labeled ligand solution is added to each reaction at twice the final assay concentration and incubated at room temperature for at least 10 min. Finally, samples are read on an instrument capable of measuring fluorescent polarization. Test compound inhibition is expressed as percent (%) inhibition of internal assay controls. For concentration response experiments, normalized data are fit using the following four parameter logistic equation: y = A + ((B-C))/(1+(10x)/(10C)D), where y is the % activity (% inhibition) at a specified compound concentration, A is the minimum % activity, B is the maximum % activity, C = log10(IC50), D = Hill slope, x = log10(compound concentration [M]), and pIC50 = (−C). [1]
References	[1]. Haile PA et al. The Identification and Pharmacological Characterization of 6-(tert-Butylsulfonyl)-N-(5-fluoro-1H-indazol-3-yl)quinolin-4-amine (GSK583), a Highly Potent and Selective Inhibitor of RIP2 Kinase. J Med Chem, 2016 May 26, 59(10):4867-80.