产品名称：**SC-514**
产品别名：**SC-514**

生物活性：

Description	SC-514 is a selective IKK-2 inhibitor (IC_{50}=11.2±4.7 μM), which does not inhibit other IKK isoforms or other serine-threonine and tyrosine kinases.

IC_{50} & Target [1]	IKK-2	CDK2/A	AUR2	PRAK	MSK	
	11.2 μM (IC_{50})	61 μM (IC_{50})	71 μM (IC_{50})	75 μM (IC_{50})	123 μM (IC_{50})	

In Vitro	SC-514 inhibits the native IKK complex or recombinant human IKK-1/IKK-2 heterodimer with IC_{50}s of 6.1±2.2 μM and 2.7±0.7 μM, respectively. IKK-2 inhibition by SC-514 is selective, reversible, and competitive with ATP. SC-514 inhibits transcription of NF-κB-dependent genes in IL-1β-induced rheumatoid arthritis-derived synovial fibroblasts in a dose-dependent manner. SC-514 inhibits all forms of recombinant human IKK-2 including rhIKK-2 homodimer, rhIKK-1/rhIKK-2 heterodimer, as well as the constitutively active form of rhIKK-2 with comparable IC_{50} values in the 3-12 μM range[1]. To evaluate whether the reactive oxygen species (ROS)-inducing IKKβ inhibitor increases the sensitivity of melanoma cells to nitrosourea. The responses of melanoma cells are first assessed to SC-514/Fotemustine co-treatment. Melanoma cell lines are treated with 50 μM of SC-514 and Fotemustine alone and in combination for 48 h and growth inhibition is assessed. Co-treatment with SC-514 significantly enhances Fotemustine-induced cytotoxicity in all melanoma cell lines tested[2].
In Vivo	SC-514 is efficacious in an acute model of inflammation, namely LPS-induced serum TNFα production in the rat. SC-514 shows a dose-dependent inhibition of TNFα production, validating IKK-2 as a potential anti-inflammatory drug target in vivo[1]. To obtain in vivo evidence for the implication of SC-514 in the response of cancer cells to Fotemustine, the xenograft mouse model of melanoma is used. Nude mice engrafted with A375 or G361 tumors are treated with vehicle control and 25 mg/kg SC-514 and/or 25 mg/kg Fotemustine daily for 13-15 consecutive days and the tumor behavior is monitored. Fotemustine treatment with SC-514 shows a clear combined effect and reduces the size of tumors in mice[2].

Solvent&Solubility

In Vitro:

DMSO : 100 mg/mL (**445.83 mM;** Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	4.4583 mL	22.2916 mL	44.5831 mL
	5 mM	0.8917 mL	4.4583 mL	8.9166 mL
	10 mM	0.4458 mL	2.2292 mL	4.4583 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: ≥ mg/mL; Clear solution 此方案可获得 ≥ mg/mL 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 0.0 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: ≥ mg/mL; Clear solution 此方案可获得 ≥ mg/mL 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 0.0 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。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ mg/mL; Clear solution 此方案可获得 ≥ mg/mL 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 0.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Kishore N, et al. A selective IKK-2 inhibitor blocks NF-kappa B-dependent gene expression in interleukin-1 beta-stimulated synovial fibroblasts. J Biol Chem. 2003 Aug 29;278(35):32861-71. [2]. Tse AK, et al. Sensitization of melanoma cells to alkylating agent-induced DNA damage and cell death via orchestrating oxidative stress and IKKβ inhibition. Redox Biol. 2017 Apr;11:562-576.

实验参考：

Cell Assay	For crystal violet staining assay, melanoma cell lines (1×10^4) are seeded in 60 mm dishes, and then untreated or pretreated with SC-514 (50 μM) and/or Fotemustine. Then, cells are formalin-fixed and stained with crystal violet. Cell numbers are measured as the optical density at 595 nm (OD595) of solubilized crystal violet from formalin-fixed cells. Cytotoxicity are also determined by the MTT reduction assay[2].
Animal Administration	Rats[1] SC-514 or vehicle (2% Me_2SO in saline) is administered either by oral gavage (50 mg/kg) or intraperitoneally (10 and 50 mg/kg) to adult male Wistar rats that have been deprived of food overnight. Two hours after compound treatment, 1 mg/kg LPS (Escherichia coli) in saline is administered intraperitoneally 90 min after LPS administration; the animals are bled and serum TNFα levels analyzed by a rat-specific TNFα ELISA. Mice[2] Male nu/nu BALB/c mice (6 weeks old) are maintained in individual ventilated cages. A375 or G361 (5×10^6) cells are resuspended in 0.1 mL PBS and inoculated subcutaneously into the backs of nude mice and allowed to grow for 7 days. After that, mice are randomly assigned to 4 groups (n=6 for each group) and treated by intraperitoneal injection with 200 μL 30% PEG/5% Tween-80 solution as the vehicle control and 25 mg/kg SC-514 and/or 25 mg/kg Fotemustine daily for 13-15 consecutive days. Body weight and tumor volume are measured every 3 days. Tumor volumes are determined by a caliper and calculated. At the end of the experiment, mice are sacrificed and tumor xenografts are collected. Tumor tissues are stored at -80°C for Western blot analysis.
	IKK complexes are immunoprecipitated from IL-1β-treated RASF cell lysates (0.5-2 mg) using a NEMO antibody (3-10 μg) followed by the addition of protein A-agarose beads. Antibody complexes

Kinase Assay	are pelleted by centrifugation and washed 3 times with 1 mL of cold whole-cell lysis buffer followed by 2 washes in kinase buffer (25 mM HEPES, pH 7.6, 2 mM $MgCl_2$, 2 mM $MnCl_2$, 10 mM NaF, 5 mM DTT, and 1 mM phenylmethylsulfonyl fluoride). 100-200 μg of immunoprecipitated IKK is analyzed for kinase activity in a reaction containing 10 μM biotinylated IκBα peptide as substrate and 1 μM [γ-^{33}P]ATP (2500 Ci/mmol). After incubation at room temperature for 30 min, 25 μL of the reaction mixture is withdrawn and added to a SAM 96 biotin capture plate. After successive wash steps the plate was allowed to air-dry, and 25 μL of scintillation fluid is added to each well. Incorporation of [γ-^{33}P]ATP is measured using a Top-Count NXT [1].
References	[1]. Kishore N, et al. A selective IKK-2 inhibitor blocks NF-kappa B-dependent gene expression in interleukin-1 beta-stimulated synovial fibroblasts. J Biol Chem. 2003 Aug 29;278(35):32861-71. [2]. Tse AK, et al. Sensitization of melanoma cells to alkylating agent-induced DNA damage and cell death via orchestrating oxidative stress and IKKβ inhibition. Redox Biol. 2017 Apr;11:562-576.