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产品名称：AS601245
产品别名：AS601245

生物活性：

Description	AS601245 is a cell-permeable JNK Inhibitor with IC_{50}s of 150, 220, and 70 nM for three JNK human isoforms (hJNK1, hJNK2, and hJNK3), respectively.		
IC_{50} & Target	hJNK1	hJNK2	hJNK3
	150 nM (IC_{50})	220 nM (IC_{50})	70 nM (IC_{50})
In Vitro	AS601245 inhibits isolated hJNK3 in an ATP-competitive manner. Selectivity of AS601245 is tested against a large panel of kinases. AS601245 exhibits 10- to 20-fold selectivity over c-src, CDK2, and c-Raf and more than 50- to 100-fold selectivity over a range of Ser/Thr- and Tyr-protein kinases[1]. The effects of AS601245 and Clofibrate alone or in association are analysed on proliferation, apoptosis, differentiation and the gene expression profile of CaCo-2 human colon cancer cells. Reduction of cell proliferation, accompanied by the modulation of p21 expression is observed in HepG2 cells, also. 5 μM Clofibrate, 0.1 μM AS601245, and the combined treatment with the two substances do not cause acute toxicity in HepG2 cells. All treatments reduce cell proliferation starting from 48 hours after the beginning of experiments, and the inhibitory effect reaches the maximum at 72 hours[2].		
In Vivo	AS601245 is a potent inhibitor of LPS-induced TNF-α release in mice. Administered orally at 0.3, 1, 3, and 10 mg/kg, AS601245 decreases the TNF-α release in a dose-dependent manner. AS601245 (40, 60, and 80 mg/kg) administered i.p. provides significant protection against the delayed loss of hippocampal CA1 neurons in a gerbil model of transient global ischemia. This effect is mediated by JNK inhibition and therefore by c-Jun expression and phosphorylation. A significant neuroprotective effect of AS601245 administered either by i.p. injection (6, 18, and 60 mg/kg) or as i.v. bolus (1 mg/kg) followed by an i.v. infusion (0.6 mg/kg/h) is also observed in rats after focal cerebral ischemia[1].		

Solvent&Solubility

In Vitro:

DMSO : 10 mg/mL (26.85 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.6849 mL	13.4246 mL	26.8492 mL
5 mM	0.5370 mL	2.6849 mL	5.3698 mL
10 mM	0.2685 mL	1.3425 mL	2.6849 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 mg/mL (2.68 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.68 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 10.0 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% corn oil Solubility: ≥ 1 mg/mL (2.68 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.68 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 10.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Carboni S, et al. AS601245 (1,3-benzothiazol-2-yl (2-[[2-(3-pyridinyl) ethyl] amino]-4 pyrimidinyl) acetonitrile): a c-Jun NH2-terminal protein kinase inhibitor with neuroprotective properties. J Pharmacol Exp Ther. 2004 Jul;310(1):25-32. [2]. Cerbone A, et al. AS601245, an Anti-Inflammatory JNK Inhibitor, and Clofibrate Have a Synergistic Effect in Inducing Cell Responses and in Affecting the Gene Expression Profile in CaCo-2 Colon CancerCells. PPAR Res. 2012;2012:269751. [3]. Cicenas J, et al. JNK, p38, ERK, and SGK1 Inhibitors in Cancer. Cancers (Basel). 2017 Dec 21;10(1). pii: E1.

实验参考：

Cell Assay	CaCo-2 cell proliferation is evaluated by using the kit “CellTiter-Glo Luminescent Cell Viability Assay”. This highly sensitive assay detects the luminescence released by the metabolically active cells. Quantification of luminescence is expressed as RLU (relative light unit). For the proliferation experiments, treatments are performed by adding the drugs (e.g., 0.1 μM AS601245) to the CaCo-2 cells seeded at about 4,000 cells/well in a 96-well plate. HepG2 cellproliferation is analysed through the MTT method. Briefly, 1500 cells/well are seeded in 200 μL of serum-supplemented media and the following day treated with the drugs (e.g, 0.1 μM AS601245). 20 μL of 5 mg/mL thiazolyl blue tetrazolium bromide is subsequently added to the cells and removed 2 hours later. 100 μL of DMSO is added to the cells, and the absorbance is recorded at 570 nm through a 96 well plate ELISA reader. Viability is evaluated through Trypan blue exclusion test[2].
Animal Administration	Mice[1] C3H/HEN mice receive an oral treatment with AS601245 (0.3, 1, 3, or 10 mg/kg). Fifteen minutes later, Endotoxins (0.3 mg/kg) are i.p. injected. Heparinized whole blood is collected by retro orbital puncture under isoflurane anesthesia. TNF-α is determined in plasma using an enzyme-linked immunosorbent assay kit. Control animals receive 0.5% CMC/0.25% Tween 20 (10 mL/kg) as vehicle[1]. Rats[1] Wistar rats are randomly divided into a vehicle-treated control group, a reference compound-treated group, and a drug-treated group. The reference compound-treated group receive MK-801 (3 mg/kg i.p.) administered 1 h postischemia onset. The drug-treated group receive AS601245 (6, 18, or 60

	mg/kg) administered at the initiation of reperfusion and 5 h later. Control animals receive 0.9% saline (10 ml/kg i.p.). Wistar rats are randomly divided into three groups. The reference compound-treated group receive MK-801 (3 mg/kg i.p.) administered 1 h postischemia onset. The drug-treated group receive an intravenous bolus of AS601245 (1 mg/kg) injected at the initiation of reperfusion followed by an intravenous infusion with a flow of 0.6 mg/kg/h during 22 h. Control animals receive a bolus plus an intravenous infusion of 0.9% saline (10 mL/kg)[1].
Kinase Assay	Rat JNK3 assays are performed in 96-well low binding Corning MTT plates: 0.5 μg of recombinant, preactivated GST-JNK3 is incubated with 1 μg of recombinant, biotinylated GST-c-Jun and 2 μM [^{33}Pγ]ATP (2 nCi/μl), in the presence or absence of compounds according to formula I and in a reaction volume of 50 μL containing 50 mM Tris-HCl, pH 8.0; 10 mM $MgCl_2$, 1 mM Dithiothreitol, and 100 μM $NaVO_4$, for 120 min and at room temperature. The reaction is stopped by the addition of 200 μL of a solution containing 250 μg of Streptavidin-coated SPA beads, 5 mM EDTA, 0.1% Triton X-100, and 50 μM ATP, in phosphate saline buffer and further incubation at room temperature for 60 min. After incubation, beads are sedimented by centrifugation at 1500g for 5 min, resuspended in 200 μL of phosphate-buffered saline (PBS) containing 5 mM EDTA, 0.1% Triton X-100, and 50 μM ATP and the radioactivity is measured in a scintillation beta counter, following further sedimentation of the beads by settling down for 60 min at room temperature. Similar method is used to demonstrate inhibition of JNK1 and JNK2[1].
References	[1]. Carboni S, et al. AS601245 (1,3-benzothiazol-2-yl (2-[[2-(3-pyridinyl) ethyl] amino]-4 pyrimidinyl) acetonitrile): a c-Jun NH2-terminal protein kinase inhibitor with neuroprotective properties. J Pharmacol Exp Ther. 2004 Jul;310(1):25-32. [2]. Cerbone A, et al. AS601245, an Anti-Inflammatory JNK Inhibitor, and Clofibrate Have a Synergistic Effect in Inducing Cell Responses and in Affecting the Gene Expression Profile in CaCo-2 Colon CancerCells. PPAR Res. 2012;2012:269751. [3]. Cicenas J, et al. JNK, p38, ERK, and SGK1 Inhibitors in Cancer. Cancers (Basel). 2017 Dec 21;10(1). pii: E1.