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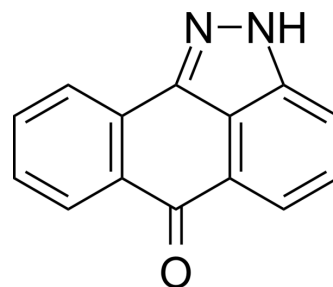
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## SP600125

Cat. No.:	HY-12041
CAS No.:	129-56-6
Molecular Formula:	C <sub>14</sub> H <sub>8</sub> N <sub>2</sub> O
Molecular Weight:	220.23
Target:	JNK; Autophagy; Apoptosis; Ferroptosis
Pathway:	MAPK/ERK Pathway; Autophagy; Apoptosis
Storage:	<div> <div>Powder</div> <div>-20°C    3 years</div> <div>4°C    2 years</div> </div> <div> <div>In solvent</div> <div>-80°C    2 years</div> <div>-20°C    1 year</div> </div>



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 12.5 mg/mL (56.76 mM; Need ultrasonic)					
	Preparing Stock Solutions	<div>Solvent</div> <div>Concentration</div>	Mass	1 mg	5 mg	10 mg
		1 mM		4.5407 mL	22.7035 mL	45.4071 mL
		5 mM		0.9081 mL	4.5407 mL	9.0814 mL
		10 mM		0.4541 mL	2.2704 mL	4.5407 mL
Please refer to the solubility information to select the appropriate solvent.						
In Vivo	1. Add each solvent one by one: 1% CMC-Na/saline water Solubility: 3.33 mg/mL (15.12 mM); Suspended solution; Need ultrasonic					
	2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: 2.08 mg/mL (9.44 mM); Suspended solution; Need ultrasonic					
	3. Add each solvent one by one: corn oil Solubility: 1 mg/mL (4.54 mM); Suspended solution; Need ultrasonic and warming and heat to 80°C					

### BIOLOGICAL ACTIVITY

Description	SP600125 is an orally active, reversible, and ATP-competitive JNK inhibitor with IC <sub>50</sub> s of 40, 40 and 90 nM for JNK1, JNK2 and JNK3, respectively. SP600125 is a potent ferroptosis inhibitor. SP600125 induces the transformation of bladder cancer cells from autophagy to apoptosis <sup>[1][2][3]</sup> .			
IC <sub>50</sub> & Target	JNK1 40 nM (IC <sub>50</sub> )	JNK2 40 nM (IC <sub>50</sub> )	JNK3 90 nM (IC <sub>50</sub> )	Autophagy

<b>In Vitro</b>	<p>SP600125 is an ATP-competitive inhibitor of JNK2 with a <math>K_i</math> value of <math>0.19 \pm 0.06 \mu\text{M}</math>. SP600125 inhibits the phosphorylation of c-Jun with <math>\text{IC}_{50}</math> of <math>5 \mu\text{M}</math> to <math>10 \mu\text{M}</math> in Jurkat T cells. In <math>\text{CD4}^+</math> cells, such as Th0 cells isolated from either human cord or peripheral blood, SP600125 blocks cell activation and differentiation and inhibits the expression of inflammatory genes COX-2, IL-2, IL-10, IFN-<math>\gamma</math>, and TNF-<math>\alpha</math>, with <math>\text{IC}_{50}</math> of <math>5 \mu\text{M}</math> to <math>12 \mu\text{M}</math><sup>[1]</sup>.</p> <p>In a mouse beta cells MIN6, SP600125 (<math>20 \mu\text{M}</math>) induces the phosphorylation of p38 MAPK and its downstream CREB-dependent promoter activation<sup>[2]</sup>.</p> <p>In HCT116 cells, SP600125 (<math>20 \mu\text{M}</math>) blocks the G2 phase to mitosis transition and induces endoreplication. This ability of SP600125 is independent of JNK inhibition, but due to its inhibition of CDK1-cyclin B activation upstream of Aurora A and Polo-like kinase 1<sup>[3]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
<b>In Vivo</b>	<p>Administration of SP600125 at 15 or 30 mg/kg i.v. significantly inhibits TNF-<math>\alpha</math> serum levels, whereas oral administration dose-dependently blocks TNF-<math>\alpha</math> expression with significant inhibition observed at 30 mg/kg per os<sup>[1]</sup>.</p> <p>SP600125 attenuates LPS-induced ALI in rats in vivo. The expression levels of TNF-<math>\alpha</math> and IL-6 in the BALF in rats in the SP600125 group are significantly decreased<sup>[4]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## PROTOCOL

<b>Cell Assay</b> <sup>[1]</sup>	<p>Determination of mRNA half-life is performed essentially, except that <math>\text{CD14}^+</math> cells are stimulated with (bacterial) lipopolysaccharide (LPS; <math>50 \text{ ng/mL}</math>) for 2 h before addition of actinomycin D (<math>5 \mu\text{g/mL}</math>). SP600125 (<math>25 \mu\text{M}</math>) or vehicle (0.5% DMSO vol/vol) is added immediately following the actinomycin D. Analysis is performed by using real-time reverse transcription (RT)-PCR. Total RNA is extracted with an RNeasy Mini kit. TNF mRNA is measured by real time RT-PCR, using a TNF Taqman probe. All data are normalized by using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. The TNF-<math>\alpha</math> forward primer is 5'-CTGGCCCAGGCAGTCAGAT-3' and the reverse primer is 5'-TATCTCTCAGCTCCACGCCATT-3'. The Taqman probe sequence is 5'-FAM-CCTGTAGCCCATGTTGTAGCAAACCTCA-TAMRA-3'<sup>[1]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
<b>Animal Administration</b> <sup>[1][4]</sup>	<p><b>Mice</b><sup>[1]</sup></p> <p>Female CD-1 mice (8-10 weeks of age) are dosed i.v. or per os with SP600125 in PPES vehicle (30% PEG-400/20% polypropylene glycol/15% Cremophor EL/5% ethanol/30% saline), final volume of <math>5 \text{ mL/kg}</math>, 15 min before i.v. injection with LPS in saline (<math>0.5 \text{ mg/kg}</math>). At 90 min, a terminal bleed is obtained from the abdominal vena cava, and the serum is recovered. Samples are analyzed for mouse TNF-<math>\alpha</math> by using an ELISA.</p> <p><b>Rats</b><sup>[4]</sup></p> <p>A total of 40 male Wistar rats are randomly divided into four groups (<math>n=10</math>): the control group, LPS group, normal saline group (NS) and the SP600125 group. Acute lung injury (ALI) is induced via intratracheal injection of LPS. Normal saline or SP600125 is administered via intraperitoneal injection (<math>15 \text{ mg/kg}</math>) 10 min after the LPS injection.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## CUSTOMER VALIDATION

- Signal Transduct Target Ther. 2024 Mar 25;9(1):74.
- Mil Med Res. 2023 Nov 25;10(1):56.
- Protein Cell. 2019 Mar;10(3):161-177.
- Nat Metab. 2023 Mar 6.
- Nat Metab. 2021 Oct 18.

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## REFERENCES

- [1]. Bennett BL, et al. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci U S A*, 2001, 98(24), 13681-13686.
- [2]. Vaishnav D, et al. SP600125, an inhibitor of c-jun N-terminal kinase, activates CREB by a p38 MAPK-mediated pathway. *Biochem Biophys Res Commun*, 2003, 307(4), 855-860.
- [3]. Kim JA, et al. SP600125 suppresses Cdk1 and induces endoreplication directly from G2 phase, independent of JNK inhibition. *Oncogene*, 2010, 29(11), 1702-1716.
- [4]. Zheng Y, et al. JNK inhibitor SP600125 protects against lipopolysaccharide-induced acute lung injury via upregulation of claudin-4. *Exp Ther Med*. 2014 Jul;8(1):153-158.
- [5]. Zhang H, et al. SP600125 Suppresses Keap1 Expression and Results in NRF2-mediated Prevention of Diabetic Nephropathy. *J Mol Endocrinol*. 2018 Feb;60(2):145-157.
- [6]. Yatsushige H, et al. Role of c-Jun N-terminal kinase in cerebral vasospasm after experimental subarachnoid hemorrhage. *Stroke*. 2005 Jul;36(7):1538-43.
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