**SB 203580**

**Cat. No.:** HY-10256  
**CAS No.:** 152121-47-6  
**Molecular Formula:** C₂₁H₁₆FN₃OS  
**Molecular Weight:** 377.43  
**Target:** p38 MAPK; Autophagy; Mitophagy  
**Pathway:** MAPK/ERK Pathway; Autophagy  
**Storage:**  
- Powder: -20°C 3 years, 4°C 2 years  
- In solvent: -80°C 6 months, -20°C 1 month

### SOLVENT & SOLUBILITY

#### In Vitro

**DMSO:** 100 mg/mL (264.95 mM; Need ultrasonic)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td>2.6495 mL</td>
<td>13.2475 mL</td>
<td>26.4950 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.5299 mL</td>
<td>2.6495 mL</td>
<td>5.2990 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.2649 mL</td>
<td>1.3247 mL</td>
<td>2.6495 mL</td>
</tr>
</tbody>
</table>

Please refer to the solubility information to select the appropriate solvent.

#### In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
   Solubility: ≥ 2.5 mg/mL (6.62 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
   Solubility: ≥ 2.5 mg/mL (6.62 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil  
   Solubility: ≥ 2.5 mg/mL (6.62 mM); Clear solution

### BIOLOGICAL ACTIVITY

**Description**  
SB 203580 (RWJ 64809) is a widely used p38 MAPK inhibitor with an IC₅₀ of 0.3-0.5 μM. SB 203580 (RWJ 64809) shows more than 100-fold selectivity over Akt (PKB), LCK, and GSK-3β.

| IC₅₀ & Target | p38 MAP kinase  
0.3-0.5 μM (IC₅₀ in IL-2-stimulated T cells) | PKB  
3-5 μM (IC₅₀) | Autophagy | Mitophagy |
In Vitro

SB 203580 inhibits IL-2-driven T cell proliferation with an IC\textsubscript{50} of 3-5 \(\mu\)M, SB 203580 is able to inhibit the activity of PDK1 in a dose-dependent manner with an IC\textsubscript{50} in the 3-10 \(\mu\)M range\[1\].

SB 203580 at a concentration of 1 \(\mu\)M is sufficient for inhibiting p38 kinase activity in TF-1 cells. SB 203580 at 5 and 10 \(\mu\)M enhances NF-\(\kappa\)B-mediated gene transcription independently of phosphorylation on the transactivation domains of the p65 subunit. SB203580-mediated increase in NF-\(\kappa\)B transcriptional activity is associated with enhanced phosphorylation of ERK1/2 and c-Jun N-terminal kinase (JNK), but not p38 kinase\[2\].

SB203580 treatment does not decrease the phosphorylation of p38 MAPK, but it significantly reduces the phosphorylation of MAPKAPK2, HSP27, and ATF2\[4\].

In Vivo

All animals challenged with NS (noninfected controls) and treated with either SB203580 or placebo survive. Compared with placebo, pretreatment with the highest dose of SB203580 (100 mg/kg) 1 hour before E. coli increases the hazards ratio of death. With E. coli, compared with placebo, at 48 hours, but not 24 hours, low and high dose SB203580 decrease phosphorylated p38 MAPK and the ratio of phosphorylated to total p38. High dose SB203580 decreases lung neutrophils on histology at 24 hours in a trend approaching significance (\(p = 0.09\)) and increases them significantly at 48 hours (\(p = 0.01\)) in patterns different over time\[3\]. SB 203580 is evaluated in several models of cytokine inhibition and inflammatory disease. It is demonstrated clearly to be a potent inhibitor of inflammatory cytokine production in both mice and rats with IC\textsubscript{50} values of 15 to 25 mg/kg\[4\].

PROTOCOL

Cell Assay \[2\]

Phosphorylation of p38, JNK1/2, and ERK1/2 is analysed by Western blotting. Briefly, TF-1 cells are cultured for 16 h in RPMI 1640 containing 0.1% FBS and subsequently stimulated for various periods of time with medium or OA (30 ng/mL) or SB 203580(1 \(\mu\)M, 5 \(\mu\)M, 10 \(\mu\)M) plus OA. After harvesting, total cell extracts are prepared by resuspending the cells in 500 \(\mu\)L 1x sample buffer (containing 2% SDS, 10% glycerol, 2% \(\beta\)-mercaptoethanol, 60 mM Tris-HCl (pH 6.8) and bromophenol blue) and lysing the cells by passing them through a 23G1 needle (three times). Cell extracts are directly boiled for 10 min and stored at -20°C. Before loading, samples are again boiled for 5 min and cell extracts are resolved by running 1/10th volume on a SDS/12.5%PAGE gel (acryla-mide:bisacrylamide is 173:1) and transferred to cellulosenitrate membrane. Immunoblotting with the antibodies is performed by standard procedures and detection is performed\[2\].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration \[3\]

Mice\[3\]

In survival studies, C57BL/6J mice weighing 20 g to 30 g are challenged with 0.05 mL of IT normal saline (NS, noninfected controls) or E. coli (15 \(\times\) 10\(^9\) CFU/kg). One hour before NS challenge, mice (\(n = 24\)) receive either intraperitoneal SB203580 (100 mg/kg in 0.25 mL) or diluent only (placebo).

Infected animals receive SB203580 in doses of 100, 10, 1, or 0.1 mg/kg or placebo 1 hour before IT E. coli (\(n = 241\)); SB203580 100 or 0.1 mg/kg or placebo 1 hour after E. coli (\(n = 121\)); or SB203580 100 mg/kg or placebo 12 hours after E. coli (\(n = 72\)). Animals are observed every 2 hours for the initial 48 hours, every 4 hours from 48 hours to 72 hours, every 8 hours from 72 hours to 96 hours, and then twice daily until study completion (168 hours)\[3\].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

REFERENCES


