BMS-345541 hydrochloride

Cat. No.: HY-10518
CAS No.: 547757-23-3
Molecular Formula: C₁₄H₁₈ClN₅
Molecular Weight: 291.78
Target: IKK
Pathway: NF-κB

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: 20 mg/mL (68.54 mM; Need ultrasonic)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Concentration</th>
<th>Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td>3.4272 mL</td>
<td>17.1362 mL</td>
<td>34.2724 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.6854 mL</td>
<td>3.4272 mL</td>
<td>6.8545 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.3427 mL</td>
<td>1.7136 mL</td>
<td>3.4272 mL</td>
<td></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 mg/mL (6.85 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2 mg/mL (6.85 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2 mg/mL (6.85 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
BMS-345541 hydrochloride is a selective inhibitor of the catalytic subunits of IKK (IKK-2 IC₅₀=0.3 μM, IKK-1 IC₅₀=4 μM). BMS-345541 binds at an allosteric site of IKK.

IC₅₀ & Target
- IKK-2
  0.3 μM (IC₅₀)
- IKK-1
  4 μM (IC₅₀)

In Vitro
BMS-345541 inhibits IKK-2 and IKK-1 in dose-dependent manner. BMS-345541 fails to inhibit a panel of both
serine/threonine and tyrosine kinases at concentrations as high as 100 μM. MS-345541 at concentrations as high as 100 μM fails to block both the anisomycin-stimulated phosphorylation of c-Jun and LPS-stimulated activation of MAPKAP K2 in THP-1 cells, as well as the EGF-stimulated phosphorylation of STAT3 in H292 cells[1]. BMS-345541 treatment results in a concentration-dependent inhibition of melanoma cell proliferation in SK-MEL-5, A375, and Hs 294T cells. BMS-345541 (0, 100 μM) shows apoptotic features as revealed by TUNEL staining and nuclear condensation[2].

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

| In Vivo | BMS-345541 (10 mg/kg, p.o.) results in prolonged serum drug levels, with concentrations sustained at or above 1 μM for many hours in mice. BMS-345541 dose-dependently inhibits the production of TNFα measured in the serum of animals challenged with an intraperitoneal administration of LPS[1]. BMS-345541 (0, 10, 25, and 75 mg/kg, p.o.) effectively inhibits SK-MEL-5 tumor growth in a dose-dependent manner in the mice. Tumor-bearing mice treated with 75 mg/kg of BMS-345541 show effective inhibition of growth of SK-MEL-5, A375, and Hs 294T tumors by 86±2.8%, 69±11% and 67±3.4%, respectively[2]. BMS-345541 (30 and 100 mg/kg, p.o.) is effective in blocking both clinical and histological endpoints of inflammation and injury in mice[3].

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**PROTOCOL**

### Kinase Assay [1]

Assays measuring the enzyme-catalyzed phosphorylation of GST-IκBα are performed by adding enzyme (IKK-2, IKK-1, or IKK-ε, typically to a final concentration of 0.5 μg/mL) at 30°C to solutions of 100 μg/mL GST-IκBα and 5 μM [33P]ATP in 40 mMTris·HCl, pH 7.5, containing 4 mM MgCl₂, 34 mM sodium phosphate, 3 mM NaCl, 0.6 mM potassium phosphate, 1 mM KCl, 1 mM dithiothreitol, 3% (w/v) glycerol, and 250 μg/mL bovine serum albumin. The specific activity of [33P]ATP used in the assay is 100 Ci/mmol. After 5 min, the kinase reactions are stopped by the addition of 2× LaemmLi sample buffer and heat-treated at 90°C for 1 min. The samples are then loaded on to NuPAGE 10% BisTris gels. After completion of SDS-PAGE, gels are dried on a slab gel dryer. The bands are then detected using a 445Si PhosphorImager, and the radioactivity is quantified using ImageQuant software. Under these conditions, the degree of phosphorylation of GST-IκBα is linear with time and concentration of enzyme.

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### Cell Assay [2]

Briefly, SK-MEL-5 cells are treated with BMS-345541 at different concentrations or for different time periods. The cells are collected by trypsinization, fixed in 70% ethanol for 2 hours on ice and stained with PI solution (PBS containing 2 μg/mL PI, 0.1% Triton X-100, and 125 units/mL RNase A) at 37°C for 30 minutes. Cell fluorescence is measured by flow cytometry with 488 nm excitation and 620 nm emission filters and resulting data are analyzed using the software program MultiCycle.

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### Animal Administration [1]

BMS-345541 is administered either by intravenous tail vein injection or by peroral gavage to groups of three 18-22-g female BALB/c mice. BMS-345541 is formulated as a 2 mg/mL solution in 3% Tween 80, water. Mice receive either a 2 mg/kg (1 mL/kg) intravenous bolus or a 10 mg/kg (5 mL/kg) peroral gavage. Whole blood samples are taken from individual mice by orbital bleed and cardiac puncture at 0, 0.05, 0.25, 0.5, 1.0, 3.0, 6.0, and 8.0 h after dosing. Whole blood is centrifuged at 20×10³ g for 5 min. Serum is stored at −20°C until analysis.

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

**CUSTOMER VALIDATION**

REFERENCES

