AZD9056 hydrochloride

Cat. No.: HY-19427A
CAS No.: 345303-91-5
Molecular Formula: C₂₄H₃₆Cl₂N₂O₂
Molecular Weight: 455.46
Target: P2X Receptor
Pathway: Membrane Transporter/Ion Channel
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: 50 mg/mL (109.78 mM; Need ultrasonic)

H₂O: 1.67 mg/mL (3.67 mM; ultrasonic and warming and heat to 60°C)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM</td>
<td></td>
<td>2.1956 mL</td>
<td>10.9779 mL</td>
<td>21.9558 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>0.4391 mL</td>
<td>2.1956 mL</td>
<td>4.3912 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>0.2196 mL</td>
<td>1.0978 mL</td>
<td>2.1956 mL</td>
</tr>
</tbody>
</table>

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.49 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
AZD9056 hydrochloride is a selective orally active inhibitor of P2X7 which plays a significant role in inflammation and pain-causing diseases.

In Vitro
The antagonist AZD9056 blocks P2X7 receptors with an IC₅₀ of 11.2 nM in HEK-hP2X7 cell line, indicating a high selectivity of the antagonist for the P2X7 receptor. The P2X7-receptor antagonist AZD9056 has a clear inhibitory effect (IC₅₀=1-3 μM) in mouse microglia BV2 cells[1]. AZD9056 is an inhibitor of BCRP and weakly inhibits BCRP-mediated transport of methotrexate
(IC$_{50}$≈92 μM)$^2$. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**In Vivo**

Treatment with AZD9056 exerts pain-relieving and anti-inflammatory effects. The upregulated expression of interleukin (IL)-1β, IL-6, tumor necrosis factor-α (TNF-α), matrix metalloproteinase-13 (MMP-13), substance P (SP) and prostaglandin E2 (PGE2) which is induced by MIA in cartilage tissues is reversed by AZD9056$^3$. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**PROTOCOL**

**Cell Assay**$^1$

AZD9056 is used as a stock solution in DMSO. Final DMSO concentrations in experiments does not exceed 1.0% (v/v). The effect of agonists on cell viability is assessed in parental HEK293 cells and HEK–hP2X7 cells using the CellTiter-Blue assay. For inhibition experiments, AZD9056 is added to the cells at concentrations up to 10 μmol/L 5 min prior to the addition of ATP (2.5 mM) or BzATP (0.25 mM). After incubation for 30 min at 37°C, an aliquot (20 μL) of the prewarmed CellTiter-Blue reagent is added. Samples are incubated for 1 h at 37°C. Fluorescence signals are measured$^1$. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Animal Administration**$^3$

Rats: To reveal the molecular mechanisms of action of P2X7R in articular cartilage in OA-induced pain and inflammation, the antagonist of P2X7R AZD9056 is used. Wistar rats are administered (by intra-articular injection) monosodium iodoacetate (MIA), and the rats with OA are then treated with the P2X7R antagonist, AZD9056$^3$. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**REFERENCES**

