AP-III-a4

Cat. No.: HY-15858
CAS No.: 1177827-73-4
Molecular Formula: C₃₁H₄₃FN₈O₃
Molecular Weight: 594.72
Target: Enolase; Apoptosis
Pathway: Metabolic Enzyme/Protease; Apoptosis
Storage: 4°C, protect from light
* In solvent: -80°C, 6 months; -20°C, 1 month (protect from light)

SOLVENT & SOLUBILITY

In Vitro
DMSO: 180 mg/mL (302.66 mM; Need ultrasonic)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent 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></td>
<td>1.6815 mL</td>
<td>8.4073 mL</td>
<td>16.8146 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td></td>
<td>0.3363 mL</td>
<td>1.6815 mL</td>
<td>3.3629 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td></td>
<td>0.1681 mL</td>
<td>0.8407 mL</td>
<td>1.6815 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 (4.20 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (4.20 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (4.20 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
AP-III-a4 (ENOblock) is a nonsubstrate analogue enolase inhibitor with an IC₅₀ of 0.576 μM. AP-III-a4 can be used for the research of cancer and diabetic[1].

IC₅₀ & Target
IC₅₀: 0.576 μM (enolase)[1]

In Vitro
AP-III-a4 (ENOblock) (0-10 μM; 24 h) inhibits HCT116 cell viability in a dose-dependent manner[1].
AP-III-a4 directly binds to enolase and inhibits its activity[1].
AP-III-a4 (0-10 μM; 24 or 48 h) inhibits cancer cell migration and invasion, induces cancer cell apoptosis[1].
AP-III-a4 (10 μM; 24 h) can induce glucose uptake and inhibit phosphoenolpyruvate carboxykinase (PEPCK) expression in...
hepatocytes and kidney cells\textsuperscript{[1]}. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Cell Viability Assay\textsuperscript{[1]}**

<table>
<thead>
<tr>
<th>Cell Line:</th>
<th>HCT116</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration:</td>
<td>1.25, 2.5, 5 and 10 μM</td>
</tr>
<tr>
<td>Incubation Time:</td>
<td>24 h</td>
</tr>
<tr>
<td>Result:</td>
<td>Induced higher levels of HCT116 colon cancer cell death in hypoxic conditions compared to normoxia.</td>
</tr>
</tbody>
</table>

**Western Blot Analysis\textsuperscript{[1]}**

<table>
<thead>
<tr>
<th>Cell Line:</th>
<th>HCT116</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration:</td>
<td>1.25, 2.5, 5 and 10 μM</td>
</tr>
<tr>
<td>Incubation Time:</td>
<td>24 h for AKT, 48 h for Bcl-Xl</td>
</tr>
<tr>
<td>Result:</td>
<td>Bound to enolase in cell lysate and bound to purified enolase. Decreased the expression of AKT and Bcl-Xl, which are negative regulators of apoptosis.</td>
</tr>
</tbody>
</table>

**Cell Invasion Assay\textsuperscript{[1]}**

<table>
<thead>
<tr>
<th>Cell Line:</th>
<th>HCT116</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration:</td>
<td>0.156, 0.312, 0.625, 1.25 and 2.5 μM</td>
</tr>
<tr>
<td>Incubation Time:</td>
<td>24 h</td>
</tr>
<tr>
<td>Result:</td>
<td>Significantly inhibits cancer cell invasion at a treatment concentration of 0.625 μM.</td>
</tr>
</tbody>
</table>

**Cell Migration Assay\textsuperscript{[1]}**

<table>
<thead>
<tr>
<th>Cell Line:</th>
<th>HCT116</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration:</td>
<td>0.625, 1.25 and 2.5 μM</td>
</tr>
<tr>
<td>Incubation Time:</td>
<td>24 h</td>
</tr>
<tr>
<td>Result:</td>
<td>Inhibited cell migration dose-dependently.</td>
</tr>
</tbody>
</table>

**RT-PCR\textsuperscript{[1]}**

<table>
<thead>
<tr>
<th>Cell Line:</th>
<th>Huh7 and HEK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration:</td>
<td>10 μM</td>
</tr>
<tr>
<td>Incubation Time:</td>
<td>24 h</td>
</tr>
<tr>
<td>Result:</td>
<td>Induced glucose uptake and inhibited PEPCK expression.</td>
</tr>
</tbody>
</table>

**In Vivo**

AP-III-a4 (ENOblock) (10 μM; 96 h) inhibits cancer cell metastasis and suppresses the gluconeogenesis regulator PEPCK in zebrafish\textsuperscript{[1]}. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
<table>
<thead>
<tr>
<th>Animal Model:</th>
<th>The zebrafish cancer cell HCT116 xenograft model[1]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage:</td>
<td>10 μM</td>
</tr>
<tr>
<td>Administration:</td>
<td>96 h</td>
</tr>
<tr>
<td>Result:</td>
<td>Reduced cancer cell dissemination. Inhibited PEPCK expression and induced glucose uptake. Inhibited adipogenesis and foam cell formation.</td>
</tr>
</tbody>
</table>

**CUSTOMER VALIDATION**
- SSRN. 2023 Feb 10.
- Research Square Print. November 14th, 2022

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**REFERENCES**