RIPA-56

Cat. No.: HY-101032  
CAS No.: 1956370-21-0  
Molecular Formula: C₁₃H₁₉NO₂  
Molecular Weight: 221.3  
Target: RIP kinase  
Pathway: Apoptosis  
Storage:
- Powder: -20°C for 3 years, 4°C for 2 years, In solvent: -80°C for 6 months, -20°C for 1 month

Solvent & Solubility

In Vitro 10 mM in DMSO

Preparing Stock Solutions

<table>
<thead>
<tr>
<th>Solvent Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>4.5188 mL</td>
<td>22.5938 mL</td>
<td>45.1875 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.9038 mL</td>
<td>4.5188 mL</td>
<td>9.0375 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.4519 mL</td>
<td>2.2594 mL</td>
<td>4.5188 mL</td>
</tr>
</tbody>
</table>

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

BIOLOGICAL ACTIVITY

Description  
RIPA-56 is a highly potent, selective, and metabolically stable inhibitor of receptor-interacting protein 1 (RIP1) with an IC₅₀ of 13 nM.

IC₅₀ & Target  
IC₅₀: 13 nM (RIP1)[¹]

In Vitro  
RIPA-56 has a half-life of 128 min in human liver microsomal stability assays and an EC₅₀ of 28 nM in TSZ-induced HT-29 necrosis assay. RIPA-56 also demonstrates potency in protection of murine L929 cells from TZ-induced necrosis (EC₅₀=27 nM). RIPA-56 shows efficient inhibition of RIP1 kinase activity, with an IC₅₀ of 13 nM and no inhibition of RIP3 kinase activity at a 10 μM concentration. RIPA-56 could form tight hydrophobic interactions with RIP1 through both the phenyl group and the 2,2-dimethylbutyl group, and form two important hydrogen bonds[¹].

In Vivo  
In the SIRS mice disease model, RIPA-56 efficiently reduces tumor necrosis factor alpha (TNFα)-induced mortality and multi-organ damage. Compared to known RIP1 inhibitors, RIPA-56 is potent in both human and murine cells, is much more stable in vivo, and is efficacious in animal model studies. RIPA-56 has an impressive PK profile in mice with a 3.1
**PROTOCOL**

| Kinase Assay [1] | The RIP1 kinase assay is performed in white 384-well plate. RIP1 is first incubated with RIPA-56 or DMSO control for 15 min, then ATP/MBP substrate mixture is added to initiate the reaction. The final concentration of ATP is 50 μM, and MBP 20 μM. After 90 min reaction at room temperature, the ADP-Glo reagent and detection solution are added. The RIP3 kinase assay conditions are almost identical to that of RIP1 assay, except the assay buffer contained 5 mM MgCl₂ instead of 20 mM MgCl₂ and 12.5 mM MnCl₂. The luminescence is measured on PerkinElmer Enspire[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| Cell Assay [1] | Cell necrosis assay is performed in 96-well cell culture plate. 3,000 cells are plated in each well and cultured at 37°C overnight. HT-29 cells are treated with 20 ng/mL TNFα/100 nM Smac Mimetics/20 μM z-VAD-FMK and RIPA-56 for 24 h. L929 cells are treated with 20 ng/mL TNFα/20 μM z-VAD-FMK and RIPA-56 for 6 h. The cell survival ratio is determined using the Cell Titer-Glo Luminescent Cell Viability Assay kit[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| Animal Administration [1] | Mice: Following intravenous (IV), intraperitoneal (IP), or oral administration (PO) of RIPA-56 to C57BL/6 mice (n=3), blood is sampled through eye puncture at various time points. Compound concentrations in the plasma samples are analyzed by LCMS/MS. Pharmacokinetic parameters are determined from individual animal data using noncompartmental analysis in phoenix 64[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

**REFERENCES**