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  3 years  
  4°C  2 years  
- In solvent  
  -80°C  6 months  
  -20°C  1 month

**SOLVENT & SOLUBILITY**

### In Vitro

DMSO : ≥ 100 mg/mL (451.88 mM)  
* "≥" means soluble, but saturation unknown.

#### Preparing Stock Solutions

<table>
<thead>
<tr>
<th>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.

### In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
   Solubility: ≥ 2.5 mg/mL (11.30 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
   Solubility: ≥ 2.5 mg/mL (11.30 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil  
   Solubility: ≥ 2.5 mg/mL (11.30 mM); Clear solution

**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. RIPA-56 can be used for the treatment of systemic inflammatory response syndrome[1].

**IC₅₀ & Target**  
IC₅₀: 13 nM (RIP1)[1]
**In Vitro**

RIPA-56 shows efficient inhibition of RIP1 kinase activity, with an IC\textsubscript{50} of 13 nM and no inhibition of RIP3 kinase activity at a 10 μM concentration. RIPA-56 also demonstrates potency in protection of murine L929 cells from TNFα/z-VAD-FMK (TZ)-induced necrosis (EC\textsubscript{50}=27 nM)[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 h half-life, 22% oral bioavailability (P.O.), and 100% bioavailability from intraperitoneal injection (I.P.)[1].

**PROTOCOL**

**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**