Resiquimod

Cat. No.: HY-13740
CAS No.: 144875-48-9
Molecular Formula: C₁₇H₂₂N₄O₂
Molecular Weight: 314.38
Target: Toll-like Receptor (TLR)
Pathway: Immunology/Inflammation
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 : ≥ 30 mg/mL (95.43 mM)
* “≥” means soluble, but saturation unknown.

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>Mass 1 mg</th>
<th>Mass 5 mg</th>
<th>Mass 10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td></td>
<td>3.1809 mL</td>
<td>15.9043 mL</td>
<td>31.8086 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>0.6362 mL</td>
<td>3.1809 mL</td>
<td>6.3617 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>0.3181 mL</td>
<td>1.5904 mL</td>
<td>3.1809 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 (7.95 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (7.95 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.95 mM); Clear solution

**BIOLOGICAL ACTIVITY**

**Description**
Resiquimod is a Toll-like receptor 7 and 8 (TLR7/TLR8) agonist that induces the upregulation of cytokines such as TNF-α, IL-6 and IFN-α.

**In Vitro**
Resiquimod (R-848) induces both hapten- and allergen-specific circulating T cells, including TH2 effectors, to produce IFN-γ and even to lose the ability to produce IL-4[2]. Resiquimod (R848) enhances PBL proliferation in a dose-
dependent manner, and increases the number of BrdU-positive cells in BrdU incorporation assay. Cells treated with R848 exhibits significantly increased (3.5-fold) luciferase (a reporter of NF-κB activity) activity\[^3\].

| In Vivo | Resiquimod (R-848) (50 μg/bird, i.m. route) significantly up-regulates the expression of IFN-α, IFN-β, IFN-γ, IL-1β, IL-4, iNOS and MHC-II genes in SPF chicken\[^1\]. |

**PROTOCOL**

**Kinase Assay**\[^3\]

For luciferase assay, FG-9307 cells are transfected with the firefly NF-κB-specific luciferase reporter vector pNFκB-Met-Luc2. Transfection efficiency is monitored by co-transfection with the pSEAP2 control vector, which constitutively expresses the human secreted enhanced alkaline phosphatase (SEAP). Then the cells are treated with Resiquimod (R848, 1 μg/mL), CQ (10 μM), CQ plus R848 or PBS and incubated at 22°C for 24 h. The culture medium of the transfectants is then analyzed for luciferase activity and SEAP activity using Luciferase Assay Kit and the Great EscPe™ SEAP Chemiluminescence Detection Kit, respectively. The assay is performed three times.

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

**Cell Assay**\[^3\]

For inhibition of lysosomal acidification, cells are incubated with 10 μM CQ for 1 h before Resiquimod (R848) treatment. After treatment, 20 μL of 5 mg/mL MTT is added to the plate. The plate is incubated at 22°C for 4 h, and 200 μL dimethyl sulfoxide is added to the plate to dissolve the reduced formazan. The plate is then read at 490 nm with a microplate reader. To determine the effect of Myd88 inhibition on R848-induced cell proliferation, the Myd88 inhibitor Pepinh-MYD and the control peptide Pepinh-Control are added to PBL at the concentration of 50 μM, and the plate is incubated at 22°C for 6 h. After incubation, the cells are treated with R848 and subjected to MTT assay as above. To determine the effect of NF-κB inactivation on R848-induced cell proliferation, BAY-11-7082, an irreversible inhibitor of IκB-α phosphorylation, is added to the cells at the concentration of 1 μM, and the plate is incubated at 22°C for 1 h. After incubation, the cells are treated with R848 and subjected to MTT assay as earlier. All experiments are performed three times.

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**Animal Administration**\[^1\]

A total of 40 SPF chickens of two-week old are allotted to one of the following four experimental groups (n=10/group): Group A: PBS control; Group B: inactivated NDV vaccine; Group C: commercial oil adjuvanted inactivated NDV vaccine prepared from lentogenic strain and Group D: combination of inactivated NDV vaccine and R-848 (50 μg/bird). Vaccine or PBS is administered by intramuscular route in the thigh muscle. A booster dose is given 14-day post immunization (d.p.i). Two weeks post-booster, experimental SPF birds are challenged with velogenic strain of NDV (10⁵ ELD₅₀ per bird) intramuscularly. Clinical signs and mortality are observed daily till 14 day post-challenge (d.p.c). Cloacal swabs (n=6/group) are collected from the birds on day 0, 4, 7 and 14 post-challenge and inoculated into 10-day old embryonated chicken eggs (n=3 eggs/sample) through intra-allantoic route. Three day post-inoculation, the allantoic fluid is checked for the NDV growth by spot haemagglutination using 10% chicken RBC.

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**CUSTOMER VALIDATION**

REFERENCES

