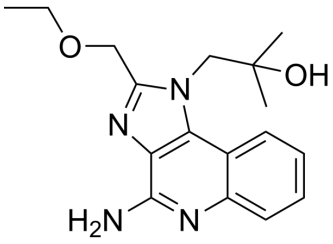
Resiquimod

Cat. No.:	HY-13740
CAS No.:	144875-48-9
Molecular Formula:	$C_{17}H_{22}N_4O_2$
Molecular Weight:	314.38
Target:	Toll-like Receptor (TLR); HCV
Pathway:	Immunology/Inflammation; Anti-infection
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 1 year -20°C 6 months



SOLVENT & SOLUBILITY

In Vitro

DMF : 50 mg/mL (159.04 mM; Need ultrasonic)
DMSO : ≥ 30 mg/mL (95.43 mM)
Methanol : 25 mg/mL (79.52 mM; Need ultrasonic)
H_2O : 0.1 mg/mL (0.32 mM; Need ultrasonic)
* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.1809 mL	15.9043 mL	31.8086 mL
5 mM	0.6362 mL	3.1809 mL	6.3617 mL
10 mM	0.3181 mL	1.5904 mL	3.1809 mL

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

In Vivo

1. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 2.5 mg/mL (7.95 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (6.62 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (6.62 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (6.62 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.
IC_{50} & Target	TLR7 TLR8
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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Resiquimod can be used in animal modeling to establish a model of cytokine release in rats and mice, and a model of immune-mediated cardiac tissue injury. 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]. The pharmacokinetic properties of Resiquimod is characterized by a short half-life, resulting in a low AUC and a concomitantly high C_{max} at relevant doses[5]. Induction of systemic Lupus erythematosus[4]. • Background Alterations in TLR signaling contributes to the initiation and/or exacerbation of lupus in humans and in murine models. Autoreactive B cells (in which TLR-7 activation occurs in response to RNA-containing antigens), in synergy with B cell receptor, undergo proliferation, isotype switching, and plasma cell differentiation, leading to the production of autoantibodies. • Specific Mmodeling Methods Mice: Wild-type FVB/N, BALB/c, and C57BL/6 mice?• female?• 7-9 weeks old (period: 4 weeks) Administration: 100 μg?• topical application to the right ear?•?three times a week for 4 weeks. • Modeling Indicators Metabolism changes: Elevates levels of autoantibodies to double-stranded DNA and multiple organ involvement, including glomerulonephritis, hepatitis, carditis. Histology analysis: Marked splenomegaly, and the liver revealed severe mononuclear cell infiltration around the portal veins and hepatocyte necrosis. • Correlated Product(s): Myosin H Chain Fragment, mouse (HY-P2464) • Opposite Product(s): Enpatoran (HY-134581) MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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 EscAPe™ SEAP Chemiluminescence Detection Kit, respectively. The assay is performed three times.

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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 (10^5 ELD_{50} 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.

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

CUSTOMER VALIDATION

- Nat Nanotechnol. 2023 Jan 12.
- Adv Mater. 2024 Jan 31:e2308155.
- Adv Mater. 2024 Jan 25:e2310421.
- Adv Mater. 2022 Nov 25;e2208782.
- Nat Biomed Eng. 2018 Aug;2(8):578-588.

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REFERENCES

[1]. Maki Yokogawa, et al. Epicutaneous application of toll-like receptor 7 agonists leads to systemic autoimmunity in wild-type mice: a new model of systemic Lupus erythematosus. Arthritis Rheumatol. 2014 Mar;66(3):694-706.

[2]. Manuel Keppler, et al. Imidazoquinolines with improved pharmacokinetic properties induce a high IFNα to TNFα ratio in vitro and in vivo. Front Immunol. 2023 Jun 20:14:1168252.

[3]. Sachan S, et al. Adjuvant potential of resiquimod with inactivated Newcastle disease vaccine and its mechanism of action in chicken. Vaccine. 2015 Aug 26;33(36):4526-32.

[4]. Brugnolo F, et al. The novel synthetic immune response modifier R-848 (Resiquimod) shifts human allergen-specific CD4+ TH2 lymphocytes into IFN-gamma-producing cells. J Allergy Clin Immunol. 2003 Feb;111(2):380-8.

[5]. Zhou ZX, et al. Immune effects of R848: evidences that suggest an essential role of TLR7/8-induced, Myd88- and NF-κB-dependent signaling in the antiviral immunity of Japanese flounder (Paralichthys olivaceus). Dev Comp Immunol. 2015 Mar;49(1):113-20.

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