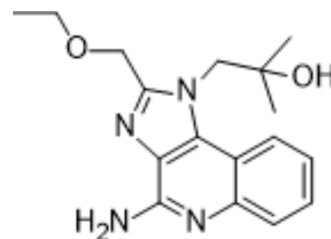


Resiquimod

Cat. No.:	HY-13740		
CAS No.:	144875-48-9		
Molecular Formula:	C ₁₇ H ₂₂ N ₄ O 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)
 H₂O : 0.1 mg/mL (0.32 mM; Need ultrasonic)
 * "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		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

- 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
- 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
- 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
- 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 .	
IC₅₀ & Target	TLR7	TLR8
In Vitro	<p>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].</p> <p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>	
In Vivo	<p>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.</p> <p>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].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>	

PROTOCOL

Kinase Assay ^[3]	<p>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.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Cell Assay ^[3]	<p>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.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Animal Administration ^[1]	<p>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.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

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 IFN α to TNF α 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- κ B-dependent signaling in the antiviral immunity of Japanese flounder (*Paralichthys olivaceus*). *Dev Comp Immunol.* 2015 Mar;49(1):113-20.

Caution: Product has not been fully validated for medical applications. For research use only.

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