
Product Data Sheet

Product Name: IRAK inhibitor 4

Cat. No.: GC16264

Chemical Properties

Cas. No. 1012104-68-5

Chemical Name 1-(4-hydroxycyclohexyl)-N-(3-methylbutyl)-2-[[5-[2-(trifluoromethoxy)phenyl]-1H-indazol-3-yl]amino]benzimidazole-5-carboxamide

SMILES CC(C)CCNC(=O)C1=CC2=C(C=C1)N(C(=N2)NC3=NNC4=C3C=C(C=C4)C5=CC=CC=C5OC(F)(F)F)C6CCC(CC6)OFormula C₃₃H₃₅F₃N₆O₃ M.Wt 620.66

Solubility DMSO : 12.5 mg/mL (20.14 mM; Need ultrasonic) Storage Store at -20°C

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure **Protocol****Cell experiment:**

THP-1 cells are grown in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. For monocytic differentiation, cells are seeded in 24-well flat-bottom culture plates at a density of 5×10⁵ cells/well and allowed to adhere and differentiate for 48 h at 37°C in the presence of 100 nM PMA. THP-1 cells are incubated with 0.1 or 1 µM IRAK-4 inhibitor (IRAK inhibitor 1) for 45 min and then stimulated with M. bovis BCG Moreau (MOI 5:1) or E. coli LPS (1 µg/mL). Culture supernatants are collected after 24 h of stimulation and assayed for the concentrations of human TNF-α or IL-12/23p40 by ELISA. For Western blot analysis, cells are incubated with IRAK-4 inhibitor, in the same concentrations described above, for 45 min and then stimulated with M. bovis BCG Moreau (MOI 5:1) or E. coli LPS (1 µg/mL) for 30 min. The cells are then processed for Western blot assay, as described below.

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

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Animal experiment:

To evaluate IRAK-4 involvement in mycobacterial infection, IRAK-4+/+ (wild-type), IRAK-4+/- (heterozygous), and IRAK-4-/- (IRAK-4-knockout) mice are used. Eight-week-old mice are infected i.v. with 1×10^6 CFU of *M. bovis* strain Moreau. The bacterial loads in the spleens, livers, and lungs are determined at 15, 30, and 60 d postinfection. Briefly, the organs are collected aseptically and homogenized in distilled water that contained 0.05% Tween 80. Serial dilutions of the resulting suspensions are plated on Middlebrook 7H11 agar medium supplemented with 10% oleic acid-albumin-dextrose-catalase, and CFU are counted following a 21-d incubation at 37°C and 5% CO₂.

References:

- [1]. Marinho FV, et al. Lack of IL-1 Receptor-Associated Kinase-4 Leads to Defective Th1 Cell Responses and Renders Mice Susceptible to Mycobacterial Infection. *J Immunol.* 2016 Sep 1;197(5):1852-63.
- [2]. Talreja J, et al. Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1 β and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. *J Immunol.* 2016 Aug

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15;197(4):1368-78.

[3]. Li Z, et al.

Inhibition of

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chemotherapies.

J Clin Invest.

2015 Mar

2;125(3):1081-

97.

Background

IRAK inhibitor 4 is an interleukin-1 receptor associated kinase 4 (IRAK4) inhibitor.

Lack of IRAK-4 impairs the production of proinflammatory mediators by macrophages and DCs in response to *M. bovis* and *M. tuberculosis*. IRAK-4^{-/-} cells stimulated with *E. coli* LPS display delayed activation kinetics of all signaling proteins analyzed, and exhibit dramatically reduced p65 phosphorylation[1]. IRAK1/4 (20 μM) has an inhibitory effect on LPS mediated IL-6 production. IRAK1/4 inhibitor do not decrease p38 phosphorylation in AMs. Combination of IRAK1/4 and Rip2 inhibitors inhibits TLR2-mediated cytokine production in sarcoidosis PBMCs and AMs[2]. IRAK4 is overexpressed and activated in T-ALL. IRAK4 mRNA level is elevated in T-ALL cells from patients compared with the levels detected in thymic T cells or T cells from peripheral blood[3].

IRAK-4^{-/-} mice exhibit a greatly reduced survival rate following aerosol infection compared with IRAK-4^{+/+} or IRAK-4^{+/-} mice. IRAK-4^{-/-} mice show increased bacterial burden in all organs at 15, 30, and 60 d postinfection[1]. MCL1, but not BCL-xL, overrides the therapeutic effects of combinatorial IRAK1/4 inhibitor and ABT-737 therapy in vivo[3].

References:

[1]. Marinho FV, et al. Lack of IL-1 Receptor-Associated Kinase-4 Leads to Defective Th1 Cell Responses and Renders Mice Susceptible to Mycobacterial Infection. *J Immunol.* 2016 Sep 1;197(5):1852-63.

[2]. Talreja J, et al. Dual Inhibition of Rip2 and IRAK1/4 Regulates IL-1β and IL-6 in Sarcoidosis Alveolar Macrophages and Peripheral Blood Mononuclear Cells. *J Immunol.* 2016 Aug 15;197(4):1368-78.

[3]. Li Z, et al. Inhibition of IRAK1/4 sensitizes T cell acute lymphoblastic leukemia to chemotherapies. *J Clin Invest.* 2015 Mar 2;125(3):1081-97.

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