CY-09

Cat. No.: HY-103666

CAS No.: 1073612-91-5

Molecular Formula: $C_{19}H_{12}F_3NO_3S_2$

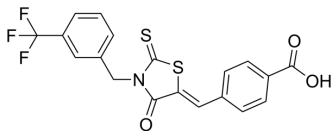
Molecular Weight: 423.43

Target: NOD-like Receptor (NLR)

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 : ≥ 150 mg/mL (354.25 mM)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.3617 mL	11.8083 mL	23.6167 mL
5 mM	0.4723 mL	2.3617 mL	4.7233 mL
10 mM	0.2362 mL	1.1808 mL	2.3617 mL

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 (5.90 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.90 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

CY-09 is a selective and direct NLRP3 inhibitor. CY-09 directly binds to the ATP-binding motif of NLRP3 NACHT domain and inhibits NLRP3 ATPase activity, resulting in the suppression of NLRP3 inflammasome assembly and activation[1].

IC_{50} & Target

NLRP3

In Vitro

CY-09 exhibits a dose-dependent inhibitory effect on monosodium urate (MSU), nigericin, ATP-induced caspase-1 activation and IL-1β secretion at the doses of 1 to10 μM in LPS-primed bone marrow-derived macrophages (BMDMs). Cytosolic LPS-induced noncanonical NLRP3 activation in BMDMs can also be blocked by CY-09 treatment. CY-09 specifically inhibits NLRP3

	inflammasome activation and has no effect on LPS-induced priming effects. CY-09 treatment remarkably suppresses nigericin-induced ASC oligomerization. It is found that CY-09 treatment inhibits the interaction of Flag-NLRP3 and mCherry-NLRP3 in HEK-293T cells, suggesting that CY-09 blocks NLRP3 oligomerization[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	CY-09 treatment in vivo efficiently suppresses monosodium urate (MSU) injection-induced IL-1β production and neutrophil influx, suggesting that CY-09 can block MSU-induced NLRP3 inflammasome activation in vivo. CY-09 treatment also increases the survival of NLRP3 mutant mice up to days 30 to 48 even after treatment is stopped at day 25. The caspase-1 cleavage observed in adipose tissue of high-fat diet (HFD)-treated mice is also suppressed by CY-09[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	For ATPase activity assay, purified recombinant human proteins are incubated at 37°C with indicated concentrations of CY-09 for 15 min in the reaction buffer. ATP (25 μm) is then added, and the mixture is further incubated at 37°C for another 40 min. The amount of ATP converted into adenosine diphosphate (ADP) is determined by luminescent ADP detection with ADP-Glo Kinase Assay kit according to the manufacturer's protocol. The results are expressed as percentage of residual enzyme activity to the vehicle-treated enzyme. For ATP binding assay, purified NLRP3 proteins are incubated with ATP binding agarose for 1 h and then different concentrations of CY-09 are added and incubated for 2 h with motion at 4°C. Beads are washed and boiled in loading buffer. Samples are subjected to immunoblotting analysis[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	To induce NLRP3 inflammasome activation, 5×10^5/mL BMDMs and 6×10^6/mL PBMCs are plated in 12-well plates. The following morning, the medium is replaced, and cells are stimulated with 50 ng/mL LPS or 400 ng/mL Pam3CSK4 (for noncanonical inflammasome activation) for 3 h. After that, CY-09 or other inhibitors are added into the culture for another 30 min, and then the cells are stimulated for 4 h with monosodium urate (MSU) (150 μg/mL), Salmonella typhimurium (multiplicity of infection) or for 30 min with ATP (2.5 mM) or nigericin (10 μM). Cells are transfected with poly(dA:dT) (0.5 μg/mL) for 4 h or LPS (500 ng/mL) overnight. Cell extracts and precipitated supernatants are analyzed by immunoblot[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	WT or $Nlrp3^{-/-}$ mice at the age of 6 wk, with similar plasma glucose levels and body weights are randomized into different groups. For generation of high-fat diet (HFD)-induced diabetic mice, mice are fed with HFD for 14 wk. The diabetic mice are treated with CY-09 (i.p.) at a dose of 2.5 mg/kg once a day for 6 wk. The mice are maintained with HFD when used for CY-09 treatment and the subsequent experiments[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Blood. 2020 Jul 23;136(4):501-515.
- Chem Eng J. 1 May 2022, 135115.
- Cancer Lett. 2023 Sep 21;216403.
- Biomed Pharmacother. 2022 Jul;151:113098.
- Free Radic Biol Med. 2022 Jan 29;181:29-42.

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REFERENCES

[1]. Jiang H, et al. Identification of a selective and direct NLRP3 inhibitor to treat inflammatory disorders. J Exp Med. 2017 Nov 6;214(11):3219-3238.

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