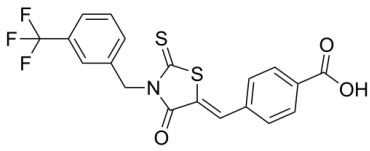
Data Sheet

Product Name:	CY-09
Cat. No.:	CS-8057
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
Solubility:	DMSO : ≥ 150 mg/mL;H_2O : < 0.1 mg/mL



BIOLOGICAL ACTIVITY:

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]. IC50 & Target: NLRP3[1] ***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]. ***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].

PROTOCOL (Extracted from published papers and Only for reference)

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]. **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]. **Animal Administration:** For the ***in vivo*** experiments, CY-09 is formulated in a vehicle containing 10% DMSO, 10% Solutol HS 15, and 80% saline.[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].

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.

CAIndexNames:

Benzoic acid, 4-[[4-oxo-2-thioxo-3-[[3-(trifluoromethyl)phenyl]methyl]-5-thiazolidinylidene]methyl]-

SMILES:

OC(C1=CC=C(/C=C2SC(N(CC3=CC=CC(C(F)(F)F)=C3)C\2=O)=S)C=C1)=O

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