MCC950

NLRP3 inflammasome inhibitor - InvitroFit™

Catalog code: inh-mcc, inh-mcc-5

https://www.invivogen.com/mcc950

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents MCC950 is available in two quantities:

- **inh-mcc:** 10 mg MCC950 - InvitroFit™
- **inh-mcc-5:** 5 x 10 mg MCC950 - InvitroFit™

Storage and stability:

- MCC950 is provided as a translucent film and shipped at room temperature. Store at -20 °C.
- Upon resuspension, prepare aliquots of MCC950 and store at -20 °C. Resuspended MCC950 is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control:

- Purity ≥98% (UHPLC)
- The inhibitory activity of the product has been validated using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

MCC950 (sulfonylurea-derived compound) is a potent and specific NLRP3 (NOD-like receptor (NLR) pyrin domain-containing protein 3) inhibitor that prevents NLRP3 inflammasome assembly in a reversible manner[1,2]. The NLRP3 inflammasome is an innate immune sensor that is activated by a two-step process; a first signal ('priming') is provided by microbial molecules such as lipopolysaccharide (LPS), while the second signal is provided by various stimuli including endogenous molecules, crystalline substances or bacterial toxins. Upon assembly, it activates caspase-1 and mediates the processing and release of interleukin-1β (IL-1β) and IL-18. Studies have demonstrated that MCC950 blocks the release of IL-1β induced by NLRP3 activators, such as ATP, MSU crystals, and nigericin[3,4]. Specifically, this small molecule directly targets the NLRP3 NATCH domain and interferes with the Walker B motif function that is crucial for ATP hydrolysis, a requirement for NLRP3 conformational change and oligomerization[1,2]. In research models of inflammation, such as cryopyrin-associated periodic syndromes (CAPS) and myocardial infarction, MCC950 effectively inhibited NLRP3-induced IL-1β production[3-5]. Importantly, it does not inhibit the AIM2, NLRC4, or NLRP1 inflammasomes[4].

1. Tapia-Abellán A. *et al.*, 2019. MCC950 closes the active conformation of NLRP3 to an inactive state. Nature Chemical Biology. 15(6):560-4. **2. Coll R.C. *et al.*, 2019.** MCC950 directly targets the NLRP3 ATP-hydrolysis motif for inflammasome inhibition. Nature Chemical Biology. 15(6):556-9. **3. Guo H. *et al.*, 2015.** Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med. 21(7):677-87. **4. Coll RC. *et al.*, 2015.** A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nature Med 21(3), 248-255. **5. van Hout GP. *et al.*, 2016.** The selective NLRP3-inflammasome inhibitor MCC950 reduces infarct size and preserves cardiac function in a pig model of myocardial infarction. Eur Heart J. ehw247.

CHEMICAL PROPERTIES

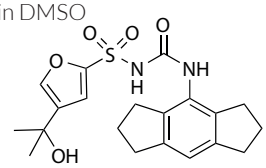
Solubility: 10 mg/ml (24.72 mM) in DMSO

CAS number: 210826-40-7

Formula: $C_{20}H_{24}N_2O_5S$

Molecular weight: 404.48 g/mol

Structure:



METHODS

Preparation of 20 mM (8.1 mg/ml) stock solution

- Add 1.235 ml of DMSO to 10 mg MCC950. Mix by vortexing.
- Prepare further dilutions by adding the appropriate amount using sterile endotoxin-free water or an aqueous buffer.

Working concentration: 300 nM (121.34 ng/ml) to 10 µM (4.04 µg/ml) for cell culture assays

***In vitro* inhibition of the NLRP3 inflammasome:**

1. Prepare a THP-1 cell suspension at 2 x 10^6 cells/ml and add 180 µl of this cell suspension per well of a 96-well plate (3 x 10^6 cells/well).
2. Prime cells by adding 1 µg/ml LPS-EK for 3 hours at 37 °C in 5% CO_2.
3. Gently remove medium and add 180 µl of fresh test medium.
4. Stimulate cells by adding IL-1β inducers, such as MSU crystals (100-200 mg/ml) in the presence or absence of MCC950 (300 nM-10 µM).
5. Incubate from 6 hours to overnight at 37 °C in 5% CO_2.
6. Determine caspase-1 inhibition by detecting mature IL-1β with InvivoGen's HEK-Blue™ IL-1β cells, which are specifically engineered to detect bioactive IL-1β.

PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay[4]

Cells: Bone marrow-derived macrophages and human peripheral blood mononuclear cells
Working concentration: 100 nM-10 µM
Incubation time: 16 hours
Method: Inflammasome activation assays and Western blotting

Animal Study[5]

Animal model: pigs
Dose: 6 or 3 mg/kg daily for 7 days
Administration: Intravenous (IV)

RELATED PRODUCTS

Product	Description	Cat. Code
Ac-YVAD-cmk	Caspase -1 inhibitor	inh-yvad-5
LPS-EK	LPS from *E. coli* K12	tlrl-eklps
Nigericin	Inflammasome inducer	tlrl-nig
MSU Crystals	Inflammasome inducer	tlrl-msu
Poly(dA:dT)	Inflammasome inducer	tlrl-patn
THP1-Null2 Cells	Human monocytes	thp-nullz
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1bv2
Z-VAD-FMK	Pan-caspase inhibitor	tlrl-vad

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