CU-CPT9a

TLR8 inhibitor - InvitroFit™

Catalog code: inh-cc9a

https://www.invivogen.com/cucpt9a

For research use only

Version 23I14-MM

PRODUCT INFORMATION

Contents

- 10 mg CU-CPT9a - InvitroFit™
- 5 ml CU-CPT9a Diluent

Storage and stability

- CU-CPT9a is provided as a dried powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Diluent is provided as a clear solution. Upon receipt, store at 4 °C
- Upon resuspension of CU-CPT9a in DMSO prepare aliquots and store at -20 °C. Resuspended product is stable for up to 3 months when properly stored at -20 °C. Avoid repeated freeze-thaw cycles.

Quality control

- Purity: ≥95% (UHPLC)
- Inhibition of TLR8 by CU-CPT9a has been confirmed using cellular assays.
- Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and TLR4 cellular assays.

PRODUCT DESCRIPTION

CU-CPT9a is a potent and selective inhibitor of human Toll-like receptor 8 (TLR8)[1-4]. Normally upon the binding of a TLR8 agonist, such as R848 or ssRNA, two TLR8 protomers are brought in close proximity, which induces the necessary conformational change for activation and initiation of downstream signaling. CU-CPT9a binds to and stabilizes the TLR8 dimer in its resting state, thus preventing TLR8 activation. Therefore, it antagonizes binding of any TLR8 ligands. Importantly, CU-CPT9a blocks TLR8-induced expression of NF-κB in various cultured and primary cells without having any effect on other TLRs, especially the closely related TLR7[1-3]. Thereby, by using CU-CPT9a to specifically block TLR8 it has been established that TLR8 is a dominant sensor of pyrogenic Gram-positive bacteria (i.e. *S. aureus*) as well as having an important role in sensing Gram-negative bacteria (i.e. *E. coli* and *P. aeruginosa*)[4]. Additionally, CU-CPT9a has been shown to exert potent anti-inflammatory effects in samples from patients with inflammatory diseases such as osteoarthritis (OA), rheumatoid arthritis (RA), and adult-onset Still's disease (AOSD)[1].

Taken together, it seems that TLR8 plays an important role in both inflammatory conditions as well as in the sensing of bacterial infection. Hence, CU-CPT9a is a useful tool in furthering our understanding of the role of TLR8 in these pathologies.

1. Zhang, S. *et al.*, 2018. Small-molecule inhibition of TLR8 through stabilization of its resting state. Nat Chem Biol. 14(1):58-64. **2. Hu Z. *et al.*, 2022.** Protocol for evaluation and validation of TLR8 antagonists in HEK-Blue cells via secreted embryonic alkaline phosphatase assay. STAR Protoc. 3(1):101061. **3. Hu Z. *et al.*, 2018.** Small-molecule TLR8 antagonists via structure-based rational design. Cell Chem Biol. 25(10):1286-91. **4. Moen, S.H. *et al.*, 2019.** Human Toll-like Receptor 8 (TLR8) is an important sensor of pyrogenic bacteria, and is attenuated by cell surface TLR signaling. Front Immunol. 10:1209.

CHEMICAL PROPERTIES

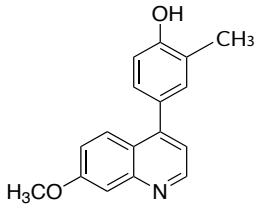
Synonyms: 2-Methyl-4-(7-methoxyl-4-quinolinyl)-phenol, Phenol,4-(7-methoxy-4-quinolinyl)-2-methyl

CAS Number: 2165340-32-7

Formula: $C_{17}H_{15}NO_2$

Molecular weight: 265.31 g/mol

Solubility: 100 mM DMSO



METHODS

Preparation of a 10 mM CU-CPT9a stock solution

1. Resuspend CU-CPT9a in 0.378 ml of DMSO (100 mM stock).
2. Prepare aliquots and store at -20 °C until required.
3. **On the day of use,** thaw a CU-CPT9a aliquot, and perform a 1:10 dilution into the provided CU-CPT9a Diluent.
4. Mix well and use immediately.

Note: Subsequent dilutions into the working concentration range can be performed with sterile water.

Working concentration range: 1 - 10 µM (for cell culture assays)

Specific inhibition of TLR8 by CU-CPT9a in cellular assays

Below is a protocol for using InvivoGen's HEK-Blue™ hTLR8 cells for studying the specific inhibition of human TLR8 (hTLR8) by CU-CPT9a. These cells express an inducible secreted embryonic alkaline phosphatase (SEAP) reporter to readily measure the activation of the NF-κB pathway. Changes in SEAP expression due to inhibition of TLR8 can be assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

Note: For more information regarding this cell line please visit https://www.invivogen.com/hek-blue-htlr8.

1. Add 20 µl CU-CPT9a (10-100 µM) per well of a flat bottom 96-well plate.
2. Prepare a suspension of HEK-Blue™ hTLR8 cells in culture medium.
3. Add 160 µl of the cell suspension (~50,000 cells) to each well.
4. Incubate the plate at 37°C in a CO_2 incubator for 3 hours.
5. Add 20 µl of an inducer of TLR8 signaling (such as R848 or TL8-506) and incubate the plate at 37°C in a CO_2 incubator for 24 hours.
6. Prepare QUANTI-Blue™ Solution and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

Product	Description	Cat. Code
R848	TLR7/8 agonist	tlrl-r848
TL8-506	TLR8 agonist	tlrl-tl8506
HEK-Blue™ hTLR8 cells	Human NF-κB reporter cells	hkb-htlr8
Quanti-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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