

M5049

TLR7/8 inhibitor - InvitroFit™

Catalog code: inh-m5049

<https://www.invivogen.com/m5049>

For research use only

Version 23118-MM

PRODUCT INFORMATION

Contents

- 5 mg of M5049 (Enpatoran) - InvitroFit™

Storage and stability

- M5049 is provided lyophilized and shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, store at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality Control

- Purity: ≥95% (UHPLC)
- The inhibition of human TLR7, human TLR8, and mouse TLR7 by M5049 has been confirmed 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

M5049 (also known as Enpatoran) is a small molecule that functions as a dual and selective inhibitor of TLR7 and TLR8¹. Its potency has been demonstrated in a range of *in vitro* assays and mouse lupus models *in vivo*¹. M5049 efficiently inhibits human (h)TLR7, mouse (m)TLR7, and hTLR8, but not mTLR8, as assessed using InvivoGen's reporter cell lines HEK-Blue™ hTLR7, mTLR7, hTLR8, and mTLR8 (see validation data sheet).

Structural studies have revealed that M5049 binds and stabilizes the hTLR8 dimer in its resting (inactive) state, antagonizing the binding of any TLR8 ligands¹. A similar mode of action has been suggested for TLR7 inhibition.

TLR7 and TLR8 are both activated by single-stranded (ss)RNA but also RNA molecules found in immune complexes with RNA protein-binding autoantibodies¹. Thus, these two TLRs play a beneficial role in clearing microbial infections, but they can also contribute to the pathogenesis of autoimmune diseases such as cutaneous and systemic lupus erythematosus (CLE/SLE)¹⁻³. In addition, it is postulated that TLR7/8 may play a role in the cytokine storm in severe coronavirus disease 2019 (COVID-19) pneumonia^{2,3}. M5049 is currently under investigation as an oral treatment for lupus (NCT04647708) and severe COVID-19 (NCT04448756).

1. Vlach J. *et al.*, 2020. Discovery of M5049: a novel selective Toll-Like Receptor 7/8 inhibitor for treatment of autoimmunity. *J Pharmacol Exp Ther.* 376:397. 2. Port A. *et al.*, 2021. Phase 1 study in healthy participants of the safety, pharmacokinetics, and pharmacodynamics of enpatoran (M5049), a dual antagonist of toll-like receptors 7 and 8. *Pharmacol Res Perspect* 9(5):e00842. 3. Klopp-Schulze I. *et al.* 2022. Applying Modeling and Simulations for Rational Dose Selection of Novel Toll-Like Receptor 7/8 Inhibitor Enpatoran for Indications of High Medical Need. *Clin Pharmacol Ther.* 112: 297-306.

CHEMICAL PROPERTIES

CAS number: 2101938-42-3

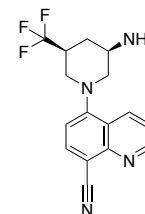
Synonyms: Enpatoran; 5-[(3R,5S)-3-amino-5-(trifluoromethyl)piperidin-1-yl]quinoline-8-carbonitrile

Formula: C₁₆H₁₅F₃N₄

Molecular weight: 320.32 g/mol

Solubility: 62.4 mM (20 mg/ml) in H₂O

Chemical structure:



METHODS

Preparation of 15.6 mM stock solution (5 mg/ml)

1. Add 1 ml of H₂O to 5 mg of M5049.
2. Vortex until completely resuspended.
3. Prepare aliquots and store at -20°C. Once M5049 has been resuspended, dilutions can be prepared with aqueous buffers.

PROTOCOLS

Below is a protocol using HEK-Blue™ hTLR7 cells for studying the specific inhibition of human TLR7 signaling by M5049. 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 TLR7 signaling can be assessed using QUANTI-Blue™ Solution, a SEAP detection reagent.

1. Add 20 µl of M5049 (10x conc.) per well of a flat-bottom 96-well plate.
2. Prepare a suspension of HEK-Blue™ hTLR7 cells (~310,000 cells per ml) in culture medium.
3. Add 160 µl of cell suspension (~50,000 cells) to each well.
4. Incubate the plate at 37°C in 5% CO₂ for 3 hours.
5. Add 20 µl (10x conc.) of an inducer of TLR7 signaling (e.g. Imiquimod) and incubate the plate at 37°C in 5% CO₂ for 18-24 hours.
6. Prepare QUANTI-Blue™ Solution and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

Product	Description	Cat.Code
HEK-Blue™ hTLR7 Cells	Human NF-κB reporter cells	hkb-htlr7v2
HEK-Blue™ mTLR7 Cells	Human NF-κB reporter cells	hkb-mtlr7
HEK-Blue™ hTLR8 Cells	Human NF-κB reporter cells	hkb-htlr78
R848	TLR7/8 agonist	tlrl-r848
Imiquimod	TLR7 agonist	tlrl-imqs
TL8-506	TLR8 agonist	tlrl-tl8506
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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