

# M5049

TLR7/8 inhibitor - InvitroFit™

Catalog code: inh-m5049, inh-m5049-5

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

For research use only

Version 25A31-MM

## PRODUCT INFORMATION

**Contents:** M5049 (Enpatoran) - InvitroFit™ is available in two quantities:

- **inh-m5049:** 5 mg
- **inh-m5049-5:** 25 mg (5 x 5 mg)

### 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 is confirmed using HEK-Blue™ hTLR7 cells.
- 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 quinoline-derivative that functions as a dual and selective inhibitor of TLR7 and TLR8<sup>1</sup>. Its potency has been demonstrated in a range of *in vitro* assays and mouse lupus models *in vivo*<sup>1</sup>. 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. M5049 exerts inhibitory actions on both NF-κB and IRF pathways downstream hTLR7 and hTLR8 as assessed with THP1-Dual™ -derived cells.

Structural studies have revealed that M5049 binds and stabilizes the hTLR8 dimer in its resting (inactive) state, antagonizing the binding of any TLR8 ligands<sup>1</sup>. 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 autoantibodies<sup>1</sup>. 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)<sup>1-3</sup>. In addition, it is postulated that TLR7/8 may play a role in the cytokine storm in severe coronavirus disease 2019 (COVID-19) pneumonia<sup>2,3</sup>. 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

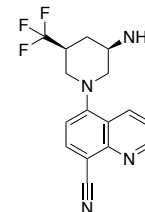
**Synonyms:** Enpatoran; 5-[[[(3R,5S)-3-amino-5-(trifluoromethyl)piperidin-1-yl]quinoline-8-carbonitrile

**Formula:** C<sub>16</sub>H<sub>15</sub>F<sub>3</sub>N<sub>4</sub>

**Molecular weight:** 320.32 g/mol

**Solubility:** 62.4 mM (20 mg/ml) in H<sub>2</sub>O

Chemical structure:



## METHODS

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

1. Add 1 ml of H<sub>2</sub>O 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% CO<sub>2</sub> 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% CO<sub>2</sub> 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	TLR7 reporter cells	hkb-htlr7v2
HEK-Blue™ mTLR7 Cells	TLR7 reporter cells	hkb-mtlr7
HEK-Blue™ hTLR8 Cells	TLR8 reporter cells	hkb-htlr8
R848	TLR7/8 agonist	tlr-r848-1
Imiquimod	TLR7 agonist	tlr-imqs-1
TL8-506	TLR8 agonist	tlr-tl8506
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

## TECHNICAL SUPPORT

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