

# BMS-986256

TLR7/8 inhibitor - InvitroFit™

Catalog code: inh-afi

<https://www.invivogen.com/tlr-7-8-inhibitor-bms986256-afimetonan>

For research use only

Version 24J07-NJ

## PRODUCT INFORMATION

### Contents

- 2 mg of BMS-986256 (Afimetonan) - InvitroFit™

### Storage and stability

- BMS-986256 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 1 month when properly stored. Avoid repeated freeze-thaw cycles.

### Quality Control

- Purity: ≥95% (UHPLC)
- The inhibition of human TLR7 by BMS-986256 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

BMS-986256 (also known as Afimetonan) is an indole-based small molecule that functions as a dual and selective inhibitor of TLR7 and TLR8<sup>1,2</sup>. Its potency has been demonstrated in various *in vitro* assays and a mouse lupus model *in vivo*<sup>1,2</sup>. BMS-986256 efficiently inhibits human (h)TLR7, mouse (m)TLR7, hTLR8, but not mTLR8, as assessed using InvivoGen's reporter cell lines HEK-Blue™ hTLR7, mTLR7, hTLR8, and mTLR8. BMS-986256 exerts inhibitory actions on both NF-κB and IRF pathways downstream hTLR7 and hTLR8 as assessed with THP1-Dual™ -derived cells.

The specific binding mechanism of BMS-986256 is not known yet. However, it is hypothesized to behave similarly to other TLR7/8 antagonists by maintaining the inactive conformation of TLR7 and TLR8 receptors<sup>2,3</sup>. Future research is needed to elucidate the exact mode of action of BMS-986256.

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>2</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 systemic lupus erythematosus (SLE) and Sjogren's syndrome<sup>2</sup>. BMS-986256 is currently under phase II clinical investigation in patients with SLE (NCT04895696)<sup>2,4</sup>.

1. Bristol-Myers Squibb company. 04.01.2018. WO 2018/005586 A1. [1,2,4] Triazolo [1,5-A] Pyridinyl substituted indole compounds. 2. Zheng H. et al., 2023. Recent Advances on Small-Molecule Antagonists Targeting TLR7. *Molecules* 28 (2), pp.634. [ff10.3390/molecules28020634](https://doi.org/10.3390/molecules28020634). [ffhal-04564284](https://doi.org/10.3390/molecules28020634). 3. 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. 4. Kalliokas. et al., 2021. Targeting TLR Signaling Cascades in Systemic Lupus Erythematosus and Rheumatoid Arthritis: An Update. *Biomedicines* 12(1):138.

## CHEMICAL PROPERTIES

CAS number: 2171019-55-7

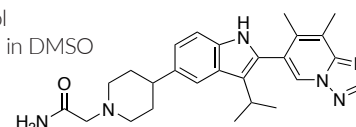
Synonyms: Afimetonan

Formula: C<sub>26</sub>H<sub>32</sub>N<sub>6</sub>O

Molecular weight: 444.57 g/mol

Solubility: 11.25 mM (5 mg/ml) in DMSO

Chemical structure:



## METHODS

### Preparation of 11.25 mM stock solution (5 mg/ml)

1. Add 400 µl of DMSO to 2 mg of BMS-986256.
2. Vortex until completely resuspended.
3. Prepare aliquots and store at -20°C. Once BMS-986256 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 BMS-986256. 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 BMS-986256 (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-htr7v2
HEK-Blue™ hTLR8 Cells	TLR8 reporter cells	hkb-htr8
Imiquimod	TLR7 agonist	tlr-imqs
TL8-506	TLR8 agonist	tlr-tl8506
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

## TECHNICAL SUPPORT

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