

VX-765

Caspase-1 & caspase-4 inhibitor - InvitroFit™

Catalog code: inh-vx765i-1, inh-vx765i-5

<https://www.invivogen.com/vx765>

For research use only

Version 23114-MM

PRODUCT INFORMATION

Contents

- VX-765 - InvitroFit™. Product is available in two quantities:
 - 10 mg VX-765 (#inh-vx765i-1)
 - 5 x 10 mg VX-765 (#inh-vx765i-5)

Storage and stability

- VX-765 is provided as a translucent film and shipped at room temperature.
- Upon receipt, VX-765 should be stored at -20°C.
- Resuspended product is stable for at least 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity ≥97% (UHPLC)
- The inhibitory activity has been validated using in-house 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

VX-765 is an orally available pro-drug converted by plasma esterases into an active peptidomimetic metabolite, VRT-043198. This metabolite is known to potentially inhibit caspase-1 and caspase-4¹. Both caspase-1 and 4 belong to a family of inflammatory caspases that are crucial in regulating inflammation and cell death. Notably, inflammasome induction drives the self-cleavage and activation of caspase-1 which in turn cleaves the pro-inflammatory cytokines interleukin-1 beta (IL-1β)/IL-18 and the pore-forming protein Gasdermin D (GSDMD) into their active forms. Additionally, the activation of the inflammasome also leads to alarmin secretion and pyroptosis, a form of immunogenic cell death.

Specifically, this inhibitor acts by covalent modification of the catalytic cysteine residue in the active site of caspase-1². Through its inhibitory activity, it has been demonstrated that VX-765 reduces the production of IL-1β and IL-18 in models of inflammatory disease both *in vitro* and *in vivo*^{1,3}. In addition, it has been reported that VX-765 inhibits pyroptosis⁴.

1. Wannamaker W. *et al.*, 2007. (S)-1-((S)-2-[[1-(4-amino-3-chloro-phenyl)-methanoyl]-amino]-3,3-dimethyl-butanoyl)-pyrrolidine-2-carboxylic acid ((2R,3S)-2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide (VX-765), an orally available selective interleukin (IL)-converting enzyme/caspase-1 inhibitor, exhibits potent anti-inflammatory activities by inhibiting the release of IL-1β and IL-18. *J Pharmacol Exp Ther.* 321(2):509-16. 2. Boxer M.B. *et al.*, 2010. A small molecule inhibitor of Caspase 1. *Probe Reports from the NIH Molecular Libraries Program.* Bethesda (MD). 3. McKenzie B.A. *et al.*, 2018. Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. *PNAS.* 115(26):E6065-E6074. 4. Doitsh G. *et al.*, 2014. Cell death by pyroptosis drives CD4⁺ T-cell depletion in HIV-1 infection. *Nature.* 505(7484):509-14. 5. Wany Y. *et al.*, 2017. Inflammasome activation triggers caspase-1-mediated cleavage of cGAS to regulate responses to DNA virus infection. *Immunity.* 46(3):393-404.

CHEMICAL PROPERTIES

CAS number: 273404-37-8

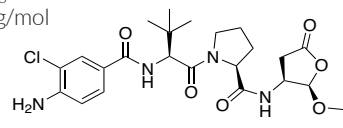
Solubility: 100 mg/ml (200 mM) in DMSO or ethanol

Formula: C₂₄H₃₃ClN₄O₆

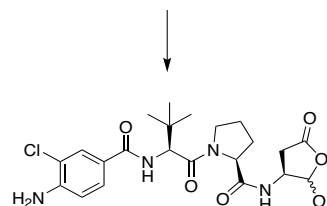
Molecular weight: 509 g/mol

Structure:

VX-765



VRT-043198



METHOD

Preparation of stock solution

1. Add the appropriate volume of DMSO to 10 mg of VX-765:
 - for a 10 mg/ml stock solution, add 1 ml DMSO
 - for a 25 mM stock solution, add 786 µl DMSO
2. Mix by vortexing. Prepare further dilutions using endotoxin free water or phosphate-buffered saline (PBS).

Working concentration: 0.1-50 µg/ml (200 nM-100 µM) as described below and in the protocols listed on the next page.

In vitro inhibition of caspase-1:

The following protocol describes the monitoring of caspase-1 inhibition in human **THP1-Null2 cells** by assessing the inhibition of IL-1β production.

1. Prepare a THP1-Null2 cell suspension and add 3 x 10⁵ cells per well in a 96-well plate.
2. Prime cells by adding 1 µg/ml **LPS-EK** for 3 hours at 37°C in 5% CO₂.
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 VX-765 (0.1-50 µg/ml).
5. Incubate from 6 hours to overnight at 37°C in 5% CO₂.
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.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

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PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay³

Cells: CD4⁺ T cells

Working concentration: 10 μ M

Pre-incubation time: 4 hours

Method: Fluorescent labelled inhibitors of caspases probes

Cell Culture Assay³

Cells: THP-1 cells

Working concentration: 20 μ M

Pre-incubation time: 2 hours

Method: Immunoblotting and type I interferons bioassays

Animal Study¹

Animal model: Naive male CD-1 mice

Dose: 10, 21, 43, and 84 mg/kg

Administration: Oral gavage

Solubility: 25% Cremophor EL in water

RELATED PRODUCTS

Product	Description	Cat. Code
Ac-YVAD-cmk	Caspase -1 inhibitor	inh-yvad
LPS-EK	LPS from <i>E. coli</i> 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-1 β cells	IL-1 β reporter cells	hkb-il1bv2
Z-VAD-FMK	Pan-caspase inhibitor	tlrl-vad

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