VX-765

Caspase-1 & caspase-4 inhibitor

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

https://www.invivogen.com/vx765

For research use only

Version 20I11-MM

PRODUCT INFORMATION

Contents

- VX-765 (provided as a translucent film) 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 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 potently inhibit caspase-1 and caspase-4[1]. 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-1[2]. 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 pyroptosis[4].

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-1beta 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

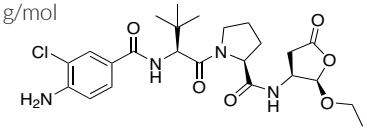
Solubility: 100 mg/ml (200 mM) in DMSO or ethanol

Formula: $C_{24}H_{33}ClN_4O_6$

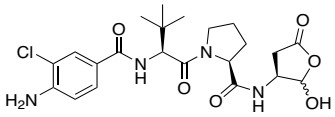
Molecular weight: 509 g/mol

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 10^5 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% CO_2.
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% CO_2.
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

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PROTOCOLS

For reference only; as described in the indicated publications.

Cell Culture Assay[3]
Cells: $CD4^+$ T cells
Working concentration: 10 µM
Pre-incubation time: 4 hours
Method: Fluorescent labelled inhibitors of caspases probes

Cell Culture Assay[3]
Cells: THP-1 cells
Working concentration: 20 µM
Pre-incubation time: 2 hours
Method: Immunoblotting and type I interferons bioassays

Animal Study[1]
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 *E. coli* 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-1β cells	IL-1β reporter cells	hkb-il1b
Z-VAD-FMK	Pan-caspase inhibitor	tlrl-vad