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 2011-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)
• Properly stored. Avoid repeated freeze-thaw cycles.

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. 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. 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. In addition, it has been reported that VX-765 inhibits pyroptosis.


CHEMICAL PROPERTIES

CAS number: 273404-37-8
Solubility: 100 mg/ml (200 mM) in DMSO or ethanol
Formula: C24H33ClN4O6
Molecular weight: 509 g/mol

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
InvivoGen Hong Kong: +852 3622-34-80
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PROTOCOLS

For reference only; as described in the indicated publications.

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

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

Animal Study
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

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
</tr>
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<tbody>
<tr>
<td>Ac-YVAD-cmk</td>
<td>Caspase -1 inhibitor</td>
<td>inh-yvad</td>
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<td>LPS-EK</td>
<td>LPS from E. coli K12</td>
<td>tlrl-eklp</td>
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<tr>
<td>Nigericin</td>
<td>Inflammasome inducer</td>
<td>tlrl-nig</td>
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<td>MSU Crystals</td>
<td>Inflammasome inducer</td>
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<tr>
<td>Poly(dAdT)</td>
<td>Inflammasome inducer</td>
<td>tlrl-patn</td>
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<tr>
<td>THP1-Null2 Cells</td>
<td>Human monocytes</td>
<td>thp-nullz</td>
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<tr>
<td>HEK-Blue™ IL-1β cells</td>
<td>IL-1β reporter cells</td>
<td>hkb-il1b</td>
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