

Glybenclamide

NLRP3 Inflammasome Inhibitor; Proton Pump Inhibitor

Catalog code: tlrl-gly

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

For research use only

Version 20114-MM

PRODUCT INFORMATION

Contents

- 1 g of glybenclamide (glyburide)

Storage and stability

- Glybenclamide is shipped at room temperature. Store at 15-25 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for at least 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity ≥ 98% (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

Glybenclamide, also known as glyburide, is an ATP-sensitive potassium channel inhibitor and an inhibitor of the NLRP3 (NOD-like receptor (NLR) pyrin domain-containing protein 3) inflammasome¹⁻³. The NLRP3 inflammasome is an innate immune sensor that is activated by a two-step process; a first signal ('priming') provided mainly by bacterial components or endogenous cytokines involves NF-κB induction, while the second signal provided by a wide array of stimuli including microbial toxins, endogenous molecules or crystalline substances and leads to inflammasome assembly and activation. This triggers inflammasome multimerization and caspase-1 activation with the subsequent cleavage of interleukin-1β (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, glybenclamide inhibits NLRP3 inflammasome activation by inducing the closure of ATP-sensitive K⁺ channels, and thereby raising the intracellular K⁺ concentration. This inhibitor works downstream of the ATP receptor P2X7, and upstream of NLRP3¹. Of note, its inhibitory activity appears to be specific for the NLRP3 inflammasome as it does not impact NLRC4- or NLRP1-mediated responses^{1,2}.

1. Zahid A. *et al.*, 2019. Pharmacological Inhibitors of the NLRP3 Inflammasome. *Front Immunol.* 10:2538. 2. Lamkanfi M. *et al.*, 2009. Glyburide inhibits the Cryopyrin/Nalp3 inflammasome. *J. Cell Biol.*, 187: 61-70. 3. Dostert C. *et al.*, 2009. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS One.* 4(8):e6510.

TECHNICAL SUPPORT

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CHEMICAL PROPERTIES

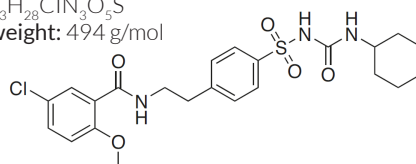
CAS number: 10238-21-8

Solubility: 25 mg/ml in DMSO

Formula: C₂₃H₂₈ClN₃O₅S

Molecular weight: 494 g/mol

Structure:



METHODS

Preparation of 25 mg/ml stock solution

1. Briefly spin the vial before opening the cap.
2. Add 200 µl of DMSO to 5 mg Glybenclamide. Mix by vortexing.
2. Use immediately or store aliquots at -20 °C.
3. Prepare further dilutions using sterile endotoxin-free water or sterile phosphate buffered saline (PBS).

Note: Addition of H₂O or PBS to the glybenclamide stock solution generates a white solution. Homogenize before use.

Working concentration range: 20 to 200 µg/ml for cell culture assays

In vitro inhibition of the NLRP3 inflammasome:

The following protocol describes the monitoring of NLRP3 inflammasome 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 glybenclamide (20-200 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.

RELATED PRODUCTS

Product	Description	Cat. Code
LPS-EK	LPS from <i>E. coli</i> K12	tlrl-eklps
MSU Crystals	Inflammasome inducer	tlrl-msu
THP1-Null2 Cells	Human monocytes	thp-nullz
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1b
VX-765	Caspase -1 and -4 inhibitor	inh-vx765i-1