

RU.521

cGAS inhibitor - InvitroFit™

Catalog code: inh-ru521-2, inh-ru521-5

<https://www.invivogen.com/ru521>

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents RU.521 is available in two quantities:

- inh-ru521-2: 2 x 2 mg RU.521 - InvitroFit™
- inh-ru521-5: 5 x 2 mg RU.521 - InvitroFit™

Note: This product is a tautomeric mixture (see Chemical properties).

Storage and stability

- RU.521 is shipped at room temperature. Store at -20°C.
- Upon resuspension of RU.521, 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 ≥ 95% (UHPLC)
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.
- The inhibitory activity has been validated using cellular assays.

DESCRIPTION

RU.521 is described as a small molecule inhibitor of cGAS (cyclic GMP-AMP synthase, cGAMP synthase)^{1,2}. It was identified by *in vitro* high-throughput screening of >100,000 compounds and further derived by structural and kinetic studies performed with murine cGAS¹. The presence of two modified chlorines in RU.521 allows it to target residues deep in the catalytic pocket of cGAS. The development of cGAS inhibitors is an important first step in finding new treatments for certain autoimmune diseases. In a healthy individual, cGAS senses the abnormal presence of cytosolic DNA such as during bacterial or viral infection. In some autoimmune diseases, cGAS can be constitutively activated by mislocalized self-DNA leading to high type I interferon (IFN) production. RU.521 has been demonstrated to reduce IFN production *in vitro* and *in vivo* in models of autoimmune disease, such as Aicardi-Goutières^{1,2}. RU.521 more potently inhibits the activity of murine cGAS compared to human cGAS². Of note, this inhibitor may not be as effective or specific as previously described¹.

1. Vincent J. et al., 2017. Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. Nat Commun. 28(1):750. 2. Zhou W. et al., 2018. Structure of the human cGAS-DNA complex reveals enhanced control of immune surveillance. Cell. 174(2):300-311.

CHEMICAL PROPERTIES

Solubility: 2 mg/ml (4.82 mM) in DMSO

Formula: C₁₉H₁₂Cl₂N₄O₃

Molecular weight: 415.23 g/mol

Structure:



3-(1-(6,7-dichloro-1H-benzimidazol-2-yl)-5-hydroxy-3-methyl-1H-pyrazol-4-yl)isobenzofuran-1(3H)-one

2-(4,5-dichloro-1H-benzimidazol-2-yl)-5-methyl-4-[(1R)-3-oxo-1,3-dihydro-2-benzofuran-1-yl]-1,2-dihydro-3H-pyrazol-3-one

METHODS

Preparation of 2 mg/ml (4.82 mM) stock solution

1. Add 1 ml of DMSO to 2 mg RU.521. Mix by vortexing.
2. Use immediately or store aliquots at -20°C.
3. Prepare further dilutions using sterile endotoxin-free water or an aqueous buffer.

Working concentration range: 200 ng/ml (482 nM) to 20 µg/ml (48.2 µM) for cell culture assays

Note: RU.521 displays cytotoxicity at concentrations >40 µg/ml in the cell lines tested such as RAW-Lucia™ ISG cells.

cGAS inhibition assay

To activate cGAS (located in the cytoplasm) nucleic acids must be delivered intracellularly by an appropriate method, such as cationic lipid transfection. The **choice of an effective method** for the delivery of nucleic acids to the cytoplasm is very important. The following protocol describes the monitoring of cGAS inhibition by RU.521 in RAW-Lucia™ ISG cells. These cells were generated from the RAW 264.7 murine macrophage cell line by stable integration of an IFN regulatory factor (IRF)-inducible Lucia luciferase reporter construct. RAW-Lucia™ ISG cells allow the monitoring of IRF activation by determining the activity of Lucia luciferase.

For more information, visit <https://www.invivogen.com/raw-lucia-isg>.

1. Add 20 µl of RU.521 at 200 ng/ml -20 µg/ml (final concentration) per well of a flat-bottom 96-well plate.
2. Add 160 µl of cell suspension (~100,000 cells) per well.
3. Incubate at 37°C for 3 hours.
4. Add 20 µl of a test sample or a cGAS ligand such as double-stranded DNA (dsDNA) complexed to a transfection reagent at 1 µg/ml (final concentration) per well of a flat-bottom 96-well plate.

Note: The transfection reagent used may affect the activity of RU.521.

5. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 hours.
6. Monitor Lucia luciferase reporter protein production using a luciferase detection reagent, such as QUANTI-Luc™ 4 Lucia/Gaussia.

RELATED PRODUCTS

Product	Description	Cat. Code
2'3'-cGAMP	STING agonist	tlrl-nacga23
G3-YSD	Y-form DNA, cGAS agonist	tlrl-ydna
ODN TTAGGG (A151)	TLR9, AIM2 & cGAS inhibitor	tlrl-ttag151
QUANTI-Luc™ 4 Lucia/Gaussia	Luciferase detection reagent	rep-qlc4lg1
RAW-Lucia™ ISG Cells	Macrophage reporter cells	rawl-isg
RAW-Lucia™ ISG-KO cGAS Cells	cGAS knockout reporter cells	rawl-kocgas

TECHNICAL SUPPORT

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