cAIM(PS)$_2$ Difluor (Rp/Sp)

cAIMP bisphosphorothioate and difluorinated: a STING ligand

Catalog code: tlrl-nacairs-05

https://www.invivogen.com/caimps2-rs

For research use only

Version 24A15-MM

PRODUCT INFORMATION

Contents

• 5 x 100 µg of cAIM(PS)$_2$ Difluor (Rp/Sp) provided lyophilized

Notes:

- This product is sterile filtered prior to lyophilization.

- cAIM(PS)$_2$ Difluor (Rp/Sp) is a mixture of Rp/Sp diastereoisomers.

• 1.5 ml endotoxin-free water

Storage and stability

- Product is shipped at room temperature and should be stored at -20 °C.
- Upon resuspension, prepare aliquots of cAIM(PS)$_2$ Difluor (Rp/Sp) and store at -20 °C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity and structure has been determined by LC/MS and NMR: ≥ 95 %
- The biological activity has been confirmed using 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

cAIM(PS)$_2$ Difluor (Rp/Sp) is composed of Rp/Sp-isomers of the bisphosphorothioate derivative of cAIMP, an analog of the bacterial cyclic dinucleotide (CDN) 3'3'-cGAMP[1]. cAIMP and cAIM(PS)$_2$ Difluor (Rp/Sp) are novel STING (stimulator of interferon genes)-activating synthetic CDNs. Unlike natural CDNs, whose constituent nucleosides are guanosine and/or adenine, cAIMP and its derivatives contain one adenine nucleoside and one inosine nucleoside. cAIM(PS)$_2$ Difluor (Rp/Sp) is composed of two 2'-deoxynucleosides with a fluorine atom at 2' position of each nucleoside. As STING agonists are being studied for their potential in immunotherapy and vaccination, an obstacle to their therapeutic utility is their lability to enzymatic hydrolysis by various nucleases and phosphodiesterases. The replacement of phosphodiester linkages with phosphorothioate linkages is a well-known strategy for improving resistance to enzymatic cleavage[2]. Moreover, fluorine atoms were incorporated into this analog as a means to improve its stability[3]. Indeed when compared to STING agonists such as 2'3'-cGAMP, this analog (referred to as compound 53 by Lioux *et al.*[1]) is not only more resistant to enzymatic cleavage but also more potent[1].

STING ligands such as cAIM(PS)$_2$ Difluor (Rp/Sp) induce production of type I interferons (IFNs) through IRFs and of proinflammatory cytokines through the NF-κB pathway. To facilitate their study, InvivoGen has developed stable reporter cells in two well established immune cell models: THP-1 human monocytes and RAW 264.7 murine macrophages. These cells express inducible SEAP and/or Lucia luciferase reporter genes under the control of an IRF-inducible or NF-κB-inducible promoter.

1. Lioux T. et al., 2016. Design, synthesis, and biological evaluation of novel cyclic adenosine-inosine monophosphate (cAIMP) analogs that activate stimulator of interferon genes (STING). J Med Chem. 59:10253-10267. **2. Yan H. et al., 2008.** Synthesis and immunostimulatory properties of the phosphorothioate analogues of cdiGMP. Bioorg. Med. Chem. Lett. 18, 5631–5634. **3. Böhm HJ. et al., 2004.** Fluorine in medicinal chemistry. Chembiochem. 5:637-43. **4. Zhang Z. et al., 2011.** The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nat Immunol.12:959-65. **5. Lippmann J. et al., 2010.** IFN beta responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). Cell Microbiol. 10:2579-88.

CHEMICAL PROPERTIES

Source: Synthetic

Synonyms: (Rp/Sp) c-[2'FdAM(PS)-2'FdIM(PS)] sodium salt

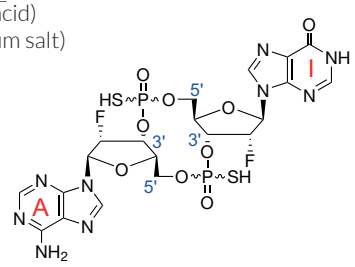
CAS number: 1951464-79-1

Formula: $C_{20}H_{21}F_2N_9O_9P_2S_2$ •2Na

Molecular weight: 695.5 (free acid)
739.5 (sodium salt)

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (500 µg/ml):

Stimulation of STING can be achieved with 100 ng -30 µg/ml cAIM(PS)$_2$ Difluor (Rp/Sp).

1. Briefly centrifuge the vial before opening to dislodge any lyophilized material that may be dispersed on the wall or cap of the vial. Carefully open the vial lid to avoid any loss of product.
2. Add 200 µl of endotoxin-free water to 100 µg of cAIM(PS)$_2$ Difluor (Rp/Sp).
3. Vortex until completely dissolved.

Induction of type I IFNs in THP1-Blue™ ISG cells

Induction of type I IFNs with cAIM(PS)$_2$ Difluor (Rp/Sp) can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the cytosolic DNA sensors[4], with the exception of DAI[5]. A protocol for the induction of type I IFNs using THP1-Blue™ ISG cells, an IRF-luciferase reporter cell line, is given below:

1. Resuspend cAIM(PS)$_2$ Difluor (Rp/Sp) as described above.
2. Stimulate cells with 100 ng -30 µg/ml cAIM(PS)$_2$ Difluor (Rp/Sp) for 16-48 h.
3. Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP (secreted embryonic alkaline phosphatase) in the cell culture supernatant using QUANTI-Blue™, Solution, a SEAP detection reagent.

RELATED PRODUCTS

Product	Catalog Code
STING ligands	
2'3'-cGAMP	tlrl-nacga23
DMXAA	tlrl-dmx
STING reporter cells	
RAW-Blue™ (IRF-SEAP) ISG cells	raw-isg
THP1-Blue™ (IRF-SEAP) ISG cells	thp-isg
THP1-Dual™ (NF-κB-SEAP & IRF-Luc) cells	thpd-nfis

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 Asia: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com