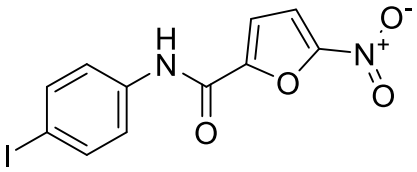
Product data sheet

MedKoo Cat#: 555342 Name: C-176 STING inhibitor CAS#: 314054-00-7 Chemical Formula: $C_{11}H_7IN_2O_4$ Exact Mass: 357.945 Molecular Weight: 358.0915	
Product supplied as:	Powder
Purity (by HPLC):	≥ 98%
Shipping conditions	Ambient temperature
Storage conditions:	Powder: -20°C 3 years; 4°C 2 years.
	In solvent: -80°C 3 months; -20°C 2 weeks.

1. Product description:
C-176 is a covalent, in vivo-active, small-molecule inhibitor of STING, attenuates STING-associated autoinflammatory disease in mice.

2. CoA, QC data, SDS, and handling instruction
SDS and handling instruction, CoA with copies of QC data (NMR, HPLC and MS analytical spectra) can be downloaded from the product web page under "QC And Documents" section. Note: copies of analytical spectra may not be available if the product is being supplied by MedKoo partners. Whether the product was made by MedKoo or provided by its partners, the quality is 100% guaranteed.

3. Solubility data

Solvent	Max Conc. mg/mL	Max Conc. mM
DMSO	72	201.07
Ethanol	2	5.59

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	2.79 mL	13.96 mL	27.93 mL
5 mM	0.56 mL	2.79 mL	5.59 mL
10 mM	0.28 mL	1.40 mL	2.79 mL
50 mM	0.06 mL	0.28 mL	0.56 mL

5. Molarity Calculator, Reconstitution Calculator, Dilution Calculator
Please refer the product web page under section of "Calculator"

6. Recommended literature which reported protocols for in vitro and in vivo study
In vitro study

1. Haag SM, Gulen MF, Reymond L, Gibelin A, Abrami L, Decout A, Heymann M, van der Goot FG, Turcatti G, Behrendt R, Ablasser A. Targeting STING with covalent small-molecule inhibitors. Nature. 2018 Jul;559(7713):269-273. doi: 10.1038/s41586-018-0287-8. Epub 2018 Jul 4. PMID: 29973723.

In vivo study

1. Haag SM, Gulen MF, Reymond L, Gibelin A, Abrami L, Decout A, Heymann M, van der Goot FG, Turcatti G, Behrendt R, Ablasser A. Targeting STING with covalent small-molecule inhibitors. Nature. 2018 Jul;559(7713):269-273. doi: 10.1038/s41586-018-0287-8. Epub 2018 Jul 4. PMID: 29973723.

7. Bioactivity
Biological target:

C-176 is a strong and covalent mouse STING inhibitor.

In vitro activity

To discover molecules that inhibit STING, a cell-based chemical screen was performed and the nitrofuran derivative C-176 was identified to strongly reduce STING-mediated, but not RIG-I- or TBK1-mediated, IFNß reporter activity (Fig. 1a, b and Extended

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Data Fig. 1a-d). Celluar washout experiments, native mass spectrometry assays on mmSTING (wild type and STING(C91S)) and top-down liquid-chromatography tandem mass spectrometry (LC-MS/MS) together confirmed that the Cys91 of mmSTING is covalently modified by C-176 (Fig. 2c, Extended Data Fig. 3 and Supplementary Information). It was found that within living cells C-176-AL, a C-176 derivative, effectively and specifically labelled mmSTIN G, whereas neither hsSTIN G nor mmSTING with a Cys91 substitution (either STING(C91A) or STING(C91S)) were targeted by the clickable compound (Fig. 2d, e and Extended Data Fig. 4c-e). The gel-based protein profiling approach was used to study the degree of molecular selectivity in the covalent interaction between the compounds and STING. When compared to iodoacetamide azide, which is a non-specific cross-linking probe, an azide-based clickable C-176 probe (C-176-AZ) showed markedly lower background proteome reactivity (Extended Data Fig. 4f). Furthermore, testing STING against a panel of proteins that contain hyper-reactive cysteine residues revealed that only STING was efficiently labelled by C-176-AZ23,24 (Extended Data Fig. 4g). It was also noted that the installation of an additional methyl group at the central amine moiety of C-176 completely abolished the inhibitory capacity of the compound (Extended Data Fig. 1e, f).

Reference: Nature. 2018 Jul;559(7713):269-273. https://doi.org/10.1038/s41586-018-0287-8

In vivo activity

The effects of pharmacological inhibition of STING in mice were studied with C-176. First it was verified that the compounds target STING by using an in vivo click-chemistry approach and the pharmacokinetic profile of C-176 on single-dose intraperitoneal injection (Extended Data Fig. 6a, b). We next evaluated whether C-176 can suppress the induction of type I IFNs triggered by the administration of CMA was assessed. Of note, pretreatment with C-176 markedly reduced the CMA-mediated induction of serum levels of type I IFNs and IL-6. (Fig. 4a and Extended Data Fig. 6c). Thus, C-176 is effective in mice and-as expected for a covalent inhibitor- the short serum half-life does not limit its in vivo inhibitory capacity. To assess the potential of C-176 to antagonize STING in a model of autoinflammatory disease, its efficacy in Trexl-/mice was investigated. Trexl-l - mice show signs of severe multi-organ inflammation caused by the persistent activation of the cyclic GMP-AMP synthase-STING pathway and recapitulate certain pathogenic features of Aicardi-Goutieres syndrome in humans. Having verified that C-178 suppresses interferon-stimulated genes in cells from Trexl - mice (Extended Data Fig. 7a), a two-week in vivo efficacy study with C-176 was performed. Notably, treatment of Trexl ~- mice with C-176 resulted in a significant reduction in serum levels of type I IFNs and in a strong suppression of inflammatory parameters in the heart (Extended Data Fig. 7b, c). Wild-type mice on a two-week treatment with C-176 showed no evident signs of overt toxicity (Extended Data Fig. 6d-g). A three-month trial with C-176 in Trexl4 – mice was conducted, which demonstrated marked amelioration of various signs of systemic inflammation (Fig. 4b, c and Extended Data Fig. 7e). Thus, C-176 attenuates STING-associated autoinflammatory disease in mice.

Reference: Nature. 2018 Jul;559(7713):269-273. https://doi.org/10.1038/s41586-018-0287-8