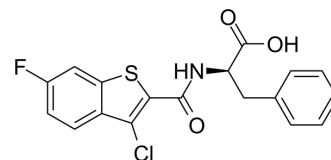


CU-CPT 4a

Cat. No.:	HY-108473		
CAS No.:	1279713-77-7		
Molecular Formula:	C ₁₈ H ₁₃ ClFNO ₃ S		
Molecular Weight:	377.82		
Target:	Toll-like Receptor (TLR)		
Pathway:	Immunology/Inflammation		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	6 months
		-20°C	1 month



SOLVENT & SOLUBILITY

In Vitro	DMSO : 100 mg/mL (264.68 mM; Need ultrasonic)				
		Solvent Concentration	Mass 1 mg	5 mg	10 mg
	Preparing Stock Solutions	1 mM	2.6468 mL	13.2338 mL	26.4676 mL
		5 mM	0.5294 mL	2.6468 mL	5.2935 mL
10 mM		0.2647 mL	1.3234 mL	2.6468 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	<ol style="list-style-type: none"> Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (6.62 mM); Clear solution Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (6.62 mM); Clear solution 				

BIOLOGICAL ACTIVITY

Description	CU-CPT 4a (TLR3-IN-1) is a potent, highly selective TLR3 signaling inhibitor. CU-CPT 4a represses the expression of downstream signaling pathways mediated by the TLR3/dsRNA complex, including TNF- α and IL-1 β ^[1] .
IC ₅₀ & Target	TLR3 3.44 μ M (IC ₅₀ , in RAW 264.7 cells)
In Vitro	CU-CPT 4a shows dose-dependent inhibitory effects blocking Poly (I:C)-induced TLR3 activation with an IC ₅₀ of 3.44 μ M ^[1] . CU-CPT 4a competes with dsRNA for binding to TLR3 with a K _i of 2.96 μ M ^[1] . MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Cell Assay ^[1]

RAW 264.7 cells are planted in 6-well plates in duplicate at 1,000,000 cells per well with 3 mL of medium (RPMI 1640 medium, supplemented with 10% FBS, Penicillin (100 U/mL) and Streptomycin (100 mg/mL)) and grown for 24 h at 37°C in a 5% CO₂ humidified incubator. After 24 h, non-adherent cells and media are removed and replaced with fresh RPMI 1640 medium (3 mL/well). Two wells of adherent macrophages are treated with Poly (I:C) (15 µg/mL). One of the two wells is treated with 27 µM CU CPT 4a. One additional well is treated with only CU CPT 4a (27 µM) only. Plates are then incubated for an additional 24 h. After removal of the medium, cells are washed with PBS (3×1 mL), the 6 well plate is put on ice, then 500 µL of lysis buffer is added in each well (Lysis Buffer: 120 µL 0.5M EDTA; 12 mL Mammalian Protein Extraction Reagent, 100 µL cocktail, 0.36 mL NaCl (5 M, aqueous)). After 5 min, the mixture is transferred into corresponding 1.5 mL tube, spun for 20 min at 13.2 K rpm in a cold room. Approximately 400 µL of supernatant are collected into new tubes, frozen at -80°C until ready for cytokine measurement^[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Yibo Wang, et al. Small-Molecule Modulators of Toll-like Receptors. *Acc Chem Res.* 2020 May 19;53(5):1046-1055.

Caution: Product has not been fully validated for medical applications. For research use only.

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