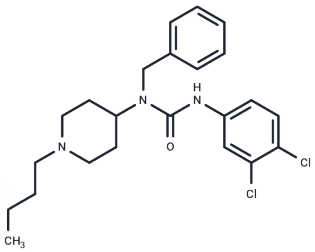
Data Sheet (Cat.No.T5374)

TargetMol

NAcM-OPT

Chemical Properties



CAS No. : 2089293-61-6

Formula: C23H29Cl2N3O

Molecular Weight: 434.4

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	NAcM-OPT is a specific, reversible inhibitor targeting N-Acetyl-UBE2M interaction with DCN1 (IC50: 79 nM).
Targets(IC50)	E1/E2/E3 Enzyme,NEDD8
In vitro	NAcM-OPT inhibits neddylation in and prevents anchorage-independent growth of a DCN1 amplified cell line without causing changes in protein homeostasis [1].
In vivo	NAcM-OPT has an oral bioavailability of 88% (calculated using IV dose at 1.5 mg/kg and PO dose at 50 mg/kg) [2].
Kinase Assay	TR-FRET assays were carried out in black 384-well microtiter plates at a final volume of 20 µL per well. To screen library compounds, the assay cocktail was prepared as a mixture of 50 nM Biotin-DCN1, 20 nM Ac-UBE2M12-AlexaFluor488, 2.5 nM Tb-Streptavidin in assay buffer (25 mM HEPES, 100 mM NaCl, 0.1% Triton X-100, 0.5 mM DTT, pH 7.5). The assay cocktail was incubated for 1 hour at room temperature and distributed using a WellMate instrument. Compounds to be screened were added to assay plates from DMSO stock solutions by pin transfer using 50SS pins. The assay mixture was incubated for 1 hour at room temperature prior to measuring the TR-FRET signal with a PHERAstar FS plate reader equipped with modules for excitation at 337 nm and emissions at 490 and 520 nm. The integration start was set to 100 µs and the integration time to 200 µs. The number of flashes was fixed at 100. The ratio of 520/490 was used as TR-FRET signal in calculations. Assay endpoints were normalized from 0% (DMSO only) to 100% inhibition (unlabeled competitor peptide) for hit selection and for curve fitting [1].
Cell Research	Exponentially growing cells were plated in 6-well plates at 0.4 × 10^6 cells/well in 2 ml of media and incubated overnight at 37 °C in a humidified 5% CO2 incubator. 24 and 48 hrs after plating, the media was aspirated and replenished with 2 ml fresh media containing either 4 µL of DMSO or a 500× compound DMSO stock solution. The cells were harvested after 72 hrs via trypsinization, thoroughly washed with PBS, pelleted, flash frozen in liquid N2, and stored at ?80 °C. Cell pellets were thawed on ice and lysed by harvested resuspension in 30-40 µL of lysis buffer [50 mM Tris, 150 mM NaCl, 0.5% NP-40, 0.1% SDS, 6.5 M Urea, 2 mM 1,10-orthophenanthroline, 1× Halt Protease and Phosphatase inhibitor cocktail, 0.25 kU Universal Nuclease, pH 7.5]. Cell suspensions were incubated on ice for 25 minutes with occasional mixing by pipetting up and down.

Cell Research	Lysates were cleared by centrifugation at 13,000 rpm for 20 minutes and the supernatant collected. The protein concentration of total cell lysate was determined by BCA assay using BSA as a control. Cell lysates were diluted into 2× SDS-PAGE sample buffer such that 25 µg of total protein was loaded per well. Samples were heated at 95 °C for 2 minutes, briefly cleared by pulse centrifugation, separated on 4–12% NuPAGE gels, and transferred to PVDF membranes at 100 V for 90 minutes at 4 °C. Membranes were blocked for 1 hour in blocking buffer consisting of 1× TBS, 0.1% Tween-20, and 5% Blotting grade non-fat dry milk. Primary antibodies were prepared in blocking buffer and incubated with membranes overnight at 4 °C with rocking, followed by extensive washing in 1× TBS, 0.1% Tween-20. Secondary antibodies were prepared in blocking buffer according to the manufactures recommendations and incubated with membranes for 1 hour at room temperature. After extensive washing, membranes were developed with SuperSignal West Pico Chemiluminescent substrate and developed by film exposure [1].

Solubility Information

Solubility	DMSO: 270 mg/mL (621.55 mM),Sonication is recommended. H2O: Insoluble, (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (4.6 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.302 mL	11.5101 mL	23.0203 mL
5 mM	0.4604 mL	2.302 mL	4.6041 mL
10 mM	0.2302 mL	1.151 mL	2.302 mL
50 mM	0.046 mL	0.2302 mL	0.4604 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Scott DC, et al. Blocking an N-terminal acetylation-dependent protein interaction inhibits an E3 ligase. Nat Chem Biol. 2017 Aug;13(8):850-857.

Hammill JT, et al. Discovery of an Orally Bioavailable Inhibitor of Defective in Cullin Neddylation 1 (DCN1)-Mediated Cullin Neddylation. J Med Chem. 2018 Apr 12;61(7):2694-2706.

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