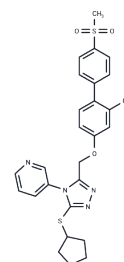


NMS-873

## Chemical Properties

CAS No. : 1418013-75-8  
 Formula: C<sub>27</sub>H<sub>28</sub>N<sub>4</sub>O<sub>3</sub>S<sub>2</sub>  
 Molecular Weight: 520.67  
 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	NMS-873 is a potent and selective allosteric inhibitor of VCP/p97.
Targets(IC50)	p97
In vivo	NMS-873 activates the unfolded protein response, disrupts autophagy, and induces cell death. Across various blood and solid tumor cell lines, it inhibits the sensitivity of p97 to protease digestion, preventing the degradation of the linker-D2 domain, thereby exhibiting antiproliferative activity.
Kinase Assay	Biochemical assay development and HTS: The ATPase activity and the kinetic parameters of recombinant wild-type VCP and its mutants are evaluated by monitoring ADP formation in the reaction, using a modified NADH-coupled assay. As ADP and NADH are ATP-competitive inhibitors of VCP ATPase activity, the standard protocol for the NADH-coupled assay is modified into a two-step procedure. In the first part, an ATP-regenerating system (40 U/ml pyruvate kinase and 3 mM phosphoenolpyruvate) recycles the ADP produced by VCP activity, keeps the substrate concentration constant (thus preventing product inhibition) and accumulates a stoichiometric amount of pyruvate. In the second part, the VCP enzymatic reaction is quenched with 30 mM EDTA and 250 μM NADH and stoichiometrically oxidized by 40 U/ml lactic dehydrogenase to reduce accumulated pyruvate. The decrease of NADH concentration is measured at 340 nm using a Tecan Safire 2 reader plate. The assay is performed in 96- or 384-well UV plates in a reaction buffer with 50 mM Hepes, pH 7.5, 0.2 mg/mL BSA, 10 mM MgCl <sub>2</sub> and 2 mM DTT. Experimental data are fitted with a cooperative equation obtaining a K <sub>s</sub> * of about 60 μM and a Hill coefficient (n) of 2.0 ± 0.1. The HTS campaign is performed against a 1-million-compound library using a miniaturized assay in 1,536-well format and a more sensitive ADP detection system, Transcreener ADP FP. A 20-min preincubation of 10 nM VCP and 10 μM inhibitor is performed, after which 10 μM ATP is added to the reaction, which is allowed to proceed for 90 min before quenching. The average Z' of the screening is 0.58, and the hit rate using 3× s.d. (38% inhibition) as cutoff is 1.7%. Primary hits with >60% inhibition at 10-μM concentration are pruned using physicochemical and structural filters to leave 7,516 compounds. At the end, reconfirmation is performed in duplicate on 3,988 primary hits, and 500 compounds are selected for a dose-response evaluation using the previously described NADH-modified coupled assay. The potency of the most interesting HTS hits is measured against both wild-type VCP and the C522T mutant. ATP concentrations that yielded the half-maximal

Kinase Assay	velocity (Ks*) for each enzyme, corresponding to 60 μM and 130 μM for the wild type and C522T mutant, respectively, are used in the assay. To explore the dependency of reversible inhibitors from substrate concentration, their potency is evaluated also at saturating ATP concentration (1 mM) and compared to the potency of a standard ATP competitive inhibitor (AMP-PNP).
Cell Research	Cells are seeded at 1,600 cells per well in 384-well white clear-bottom plates. Twenty-four hours after seeding, cells are treated with the compounds (eight dilution points, in duplicate, for each compound) and incubated for an additional 72 h at 37 °C under a 5% CO2 atmosphere. Cells are then lysed, and the ATP content in each well is determined using a thermostable firefly luciferase-based assay as a measure of cell viability. IC50 values are calculated using the percentage of growth of treated cells versus the untreated control. (Only for Reference)

### Solubility Information

Solubility	DMSO: 245 mg/mL (470.55 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: < 10 mg/mL (19.21 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. 10% DMSO+90% Corn oil: 10 mg/mL (19.21 mM),Solution. 10% DMSO+90% Saline: < 10 mg/mL (19.21 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. 10% DMSO+90% (20% SBE-β-CD in Saline): < 10 mg/mL (19.21 mM),Lower concentrations may be soluble, but exact solubility limit is unknown. <i>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.</i>

### Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.9206 mL	9.603 mL	19.206 mL
5 mM	0.3841 mL	1.9206 mL	3.8412 mL
10 mM	0.1921 mL	0.9603 mL	1.9206 mL
50 mM	0.0384 mL	0.1921 mL	0.3841 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

Magnaghi P, et al. Nat Chem Biol. 2013, 9(9), 548-556.

Du, Yuanjiao, et al. VPS13D interacts with VCP/p97 and negatively regulates ER-mitochondrial interactions. Molecular Biology of the Cell. (2021): mbc-E21

Xie S, Liu H, Zhu S, et al.Arsenic trioxide and p97 inhibitor synergize against acute myeloid leukemia by targeting nascent polypeptides and activating the ZAKα-JNK pathway.Cancer Gene Therapy.2024: 1-12.

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