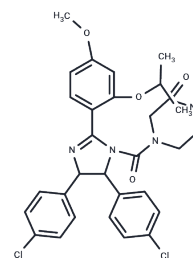


## Nutlin-3

## Chemical Properties

CAS No. :	548472-68-0
Formula:	C <sub>30</sub> H <sub>30</sub> Cl <sub>2</sub> N <sub>4</sub> O <sub>4</sub>
Molecular Weight:	581.49
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	Nutlin-3 is an MDM2 antagonist that inhibits MDM2-p53 interaction (K <sub>i</sub> =90 nM) and activates p53. Nutlin-3 binds preferentially to the p53-binding pocket of MDM2, leading to stabilization of p53 and activation of the p53 pathway. Nutlin-3 has antitumor activity.
Targets(IC50)	E1/E2/E3 Enzyme,MDM-2/p53
In vitro	<p><b>METHODS:</b> 22RV1 (p53 WT), DU145 (p53 Mut) and PC-3 (p53 Null) cells were treated with Nutlin-3 (2-10 μM) for 48 h. Apoptosis was detected by Hoechst 33342 staining.</p> <p><b>RESULTS:</b> Nutlin-3 induced a significant increase in apoptosis in 22RV1 cells, whereas apoptosis was not significantly increased in p53-deficient DU145 or PC-3 cells. Nutlin-3 preferentially increased apoptosis in cells expressing WT p53. [1]</p> <p><b>METHODS:</b> H460 and HCT116 p53+/+ cells were treated with Nutlin-3 (0-100 μM) and Inauhzin (0-100 μM) for 72 h. Cell viability was measured by WST assay.</p> <p><b>RESULTS:</b> Inauhzin and Nutlin-3 showed significant synergistic effects in inhibiting the growth of both tumor cell lines, as the CI values were significantly less than 1 in the effective dose range, especially when lower doses of Nutlin-3 were used. [2]</p>
In vivo	<p><b>METHODS:</b> To assay antitumor activity in vivo, Nutlin-3 (150 mg/kg, EtOH: Tween: 5% Glucose = 5:5:90; administered orally twice daily for four days and stopped for two days) and Inauhzin (15 mg/kg, intraperitoneally once daily) were administered to SCID mice bearing HCT116 p53+/+ xenografts for 21 days.</p> <p><b>RESULTS:</b> Each compound alone reduced mean tumor volume by less than 20% after 21 days of treatment. However, when both treatments were combined at the same dose, the final average tumor volume was reduced by 60%. Inauhzin and Nutlin-3 activated p53 and inhibited the growth of xenograft tumors in the animals. [2]</p>
Kinase Assay	Biacore study: Competition assay is performed on a Biacore S51. A Series S Sensor chip CM5 is utilized for the immobilization of a PentaHis antibody for capture of the His-tagged p53. The level of capture is ~200 response units (1 response unit corresponds to 1 pg of protein per mm <sup>2</sup> ). The concentration of MDM2 protein is kept constant at 300 nM. Nutlin-3 is dissolved in DMSO at 10 mM and further diluted to make a concentration series of Nutlin-3 in each MDM2 test sample. The assay is run at 25°C in running buffer (10 mM HEPES, 0.15 M NaCl, 2% DMSO). MDM2-p53 binding in the presence of Nutlin-3 is calculated as a percentage of binding in the absence of Nutlin-3 and IC <sub>50</sub> is calculated

Cell Research	Nutlin-3 (NUT) is dissolved with DMSO (100 mM) and diluted with appropriate media[2]. Human non-small-cell lung carcinoma wild type p53-containing H460 and A549, human non-small-cell lung carcinoma p53-null H1299, and human colon cancer HCT116 (p53+/+ and p53-/-) cells are used. Cells (1.5×10 <sup>5</sup> ) are plated into 6-well plates, and incubated at 37°C overnight. After treatment of Inauhzin and Nutlin-3 at the indicated concentrations for 48 h, cells are harvested, fixed in 70% ice-cold ethanol overnight at -20°C, resuspended in propidium iodide-solution (50 µg/mL PI, 0.1 mg/mL RNase A, 0.05% Tritin X-100 in PBS) for 40 min at 37°C, then analyzed for DNA content using a flow cytometer and proprietary software[2].
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### Solubility Information

Solubility	DMSO: 230 mg/mL (395.54 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 (17.2 mM),Solution. 10% DMSO+90% Saline: < 10 mg/mL (17.2 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	1.7197 mL	8.5986 mL	17.1972 mL
5 mM	0.3439 mL	1.7197 mL	3.4394 mL
10 mM	0.172 mL	0.8599 mL	1.7197 mL
50 mM	0.0344 mL	0.172 mL	0.3439 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

- Supiot S, et al. Nutlin-3 radiosensitizes hypoxic prostate cancer cells independent of p53. *Mol Cancer Ther.* 2008 Apr;7(4):993-9.
- Guo Y, Li Q, Zhao G, et al. Loss of TRIM31 promotes breast cancer progression through regulating K48- and K63-linked ubiquitination of p53. *Cell Death & Disease.* 2021, 12(10): 1-13.
- Yang S, Jiang L, Deng L, et al. Chaperone-Mediated Autophagy Alleviates Cerebral Ischemia-Reperfusion Injury by Inhibiting P53-Mediated Mitochondria-Associated Apoptosis. *Neurochemical Research.* 2025, 50(1): 29.
- Zhang Y, et al. Inauhzin and Nutlin3 synergistically activate p53 and suppress tumor growth. *Cancer Biol Ther.* 2012 Aug;13(10):915-24.

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