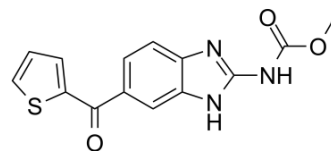


## Nocodazole

<b>Cat. No.:</b>	HY-13520												
<b>CAS No.:</b>	31430-18-9												
<b>Molecular Formula:</b>	C <sub>14</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S												
<b>Molecular Weight:</b>	301.32												
<b>Target:</b>	Microtubule/Tubulin; Bcr-Abl; CRISPR/Cas9; Autophagy; Apoptosis												
<b>Pathway:</b>	Cell Cycle/DNA Damage; Cytoskeleton; Protein Tyrosine Kinase/RTK; Autophagy; Apoptosis												
<b>Storage:</b>	<table border="0"> <tr> <td>Powder</td> <td>-20°C</td> <td>3 years</td> </tr> <tr> <td></td> <td>4°C</td> <td>2 years</td> </tr> <tr> <td>In solvent</td> <td>-80°C</td> <td>6 months</td> </tr> <tr> <td></td> <td>-20°C</td> <td>1 month</td> </tr> </table>	Powder	-20°C	3 years		4°C	2 years	In solvent	-80°C	6 months		-20°C	1 month
Powder	-20°C	3 years											
	4°C	2 years											
In solvent	-80°C	6 months											
	-20°C	1 month											



### SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 20 mg/mL (66.37 mM; Need ultrasonic)				
		Solvent Concentration	Mass		
	<b>Preparing Stock Solutions</b>		1 mg	5 mg	10 mg
		1 mM	3.3187 mL	16.5937 mL	33.1873 mL
		5 mM	0.6637 mL	3.3187 mL	6.6375 mL
	10 mM	0.3319 mL	1.6594 mL	3.3187 mL	
Please refer to the solubility information to select the appropriate solvent.					
<b>In Vivo</b>	1. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2 mg/mL (6.64 mM); Clear solution				

### BIOLOGICAL ACTIVITY

<b>Description</b>	Nocodazole (Oncodazole) is a rapidly-reversible inhibitor of microtubule. Nocodazole binds to β-tubulin and disrupts microtubule assembly/disassembly dynamics, which prevents mitosis and induces apoptosis in tumor cells. Nocodazole inhibits Bcr-Abl, and activates CRISPR/Cas9.			
<b>IC<sub>50</sub> &amp; Target</b>	Abl 91 nM (Kd)	ABL(E255K) 120 nM (Kd)	ABL(T315I) 170 nM (Kd)	BRAF 1.8 μM (Kd)
	BRAF(V600E) 1.1 μM (Kd)	c-KIT 1.6 μM (Kd)	MEK1 1.7 μM (Kd)	MEK2 1.6 μM (Kd)
	MET	PI3Ky	Microtubule/Tubulin	CRISPR/Cas9

	1.7 $\mu$ M (K <sub>d</sub> )	1.5 $\mu$ M (K <sub>d</sub> )	(K <sub>d</sub> )	(K <sub>d</sub> )
<b>In Vitro</b>	<p>Nocodazole exhibits good affinity toward c-KIT, with a K<sub>d</sub> value of 1.6 <math>\mu</math>M in highly malignant human cancer cells. Nocodazole displays good binding affinity toward the components of the mitogen-activated protein kinase (MAPK) pathway, such as BRAF (K<sub>d</sub>=1.8 <math>\mu</math>M), BRAF(V600E) (K<sub>d</sub>=1.1 <math>\mu</math>M), MEK1 (K<sub>d</sub>=1.7 <math>\mu</math>M), and MEK2 (K<sub>d</sub>=1.6 <math>\mu</math>M)<sup>[1]</sup>. Nocodazole has the highest affinity for <math>\alpha\beta_{IV}</math> and the lowest affinity for <math>\alpha\beta_{III}</math><sup>[2]</sup>.</p> <p>Nocodazole (1 nM) induces apoptosis of COLO 205 cancer cells<sup>[3]</sup>.</p> <p>Nocodazole (<math>\geq</math> 30 <math>\mu</math>g/mL) significantly increases the percentage of annexin-V-binding cells without significantly modifying average forward scatter of human erythrocytes<sup>[4]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>			
<b>In Vivo</b>	<p>Nocodazole (5 mg/kg/three times per week, i.p.) has antitumor effects in athymic mice bearing COLO 205 tumor xenografts. Nocodazole (1 nM) + R-41400 dramatically increase the levels of p21/CIP1 and p27/KIP1 protein in the tumor tissues<sup>[3]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>			

## PROTOCOL

### Cell Assay <sup>[3]</sup>

Proteins are loaded at 50  $\mu$ g/lane and separated by 12% (w:v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis, blotted, and probed with antibodies for cyclin E, p53, p21/CIP1, p27/KIP1, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), cyclin A, cyclin D1, cyclin D3, cyclin B, CDK2, CDK4, and cytochrome C. Immunoreactive bands are visualized by incubating with the colorigenic substrates nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate. The expression of GAPDH is used as the control for equal protein loading.

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

### Animal Administration <sup>[3]</sup>

COLO 205 cells are grown in RPMI 1640 supplemented with 10% FCS. Cells are harvested through two consecutive trypsinizations, centrifuged at 300 $\times$ g; for 5 min, washed twice, and resuspended in sterile phosphate-buffered saline (PBS). Cells (5 $\times$ 10<sup>5</sup>) in 0.1 mL are injected subcutaneously between the scapulae of each nude mouse. After transplantation, tumor size is measured with calipers, and the tumor volume is estimated. Once tumors reach a mean size of 200 mm<sup>3</sup>, animals receive intraperitoneal injections of DMSO (25  $\mu$ L), R-41400 (50 mg/kg), nocodazole (5 mg/kg), or R-41400 + nocodazole three times per week for 6 wk.

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

## CUSTOMER VALIDATION

- Adv Sci (Weinh). 2020 Jun 17;7(15):1903583.
- Nat Commun. 2019 Jun 28;10(1):2860.
- Nucleic Acids Res. 2020 Dec 16;48(22):e127.
- Autophagy. 2021 Mar 22;1-17.
- Theranostics. 2019 May 31;9(13):3732-3753.

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## REFERENCES

- [1]. Park H, et al. Nocodazole is a high-affinity ligand for the cancer-related kinases ABL, c-KIT, BRAF, and MEK. ChemMedChem. 2012 Jan 2;7(1):53-6.
- [2]. Keliang Xu, et al. Interaction of nocodazole with tubulin isotypes. Drug Development Research 2002

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[3]. Wang YJ, et al. R-41400 potentiates the antitumor effects of nocodazole: In vivo therapy for human tumor xenografts in nude mice. *Mol Carcinog.* 2002 Aug;34(4):199-210.

[4]. Signoretto E, et al. Nocodazole Induced Suicidal Death of Human Erythrocytes. *Cell Physiol Biochem.* 2016;38(1):379-92.

[5]. Zhang JP, et al. Efficient precise knockin with a double cut HDR donor after CRISPR/Cas9-mediated double-stranded DNA cleavage. *Genome Biol.* 2017 Feb 20;18(1):35.

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

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