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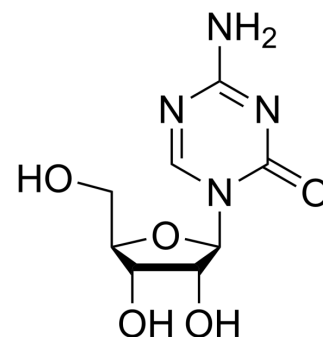
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## 5-Azacytidine

Cat. No.:	HY-10586
CAS No.:	320-67-2
Molecular Formula:	C <sub>8</sub> H <sub>12</sub> N <sub>4</sub> O <sub>5</sub>
Molecular Weight:	244.2
Target:	Autophagy; DNA Methyltransferase; Nucleoside Antimetabolite/Analog; Bacterial; Antibiotic; Organoid
Pathway:	Autophagy; Epigenetics; Cell Cycle/DNA Damage; Anti-infection; Stem Cell/Wnt
Storage:	Powder    -20°C    3 years 4°C    2 years In solvent   -80°C    2 years -20°C    1 year



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : ≥ 31 mg/mL (126.95 mM)  
 H<sub>2</sub>O : 25 mg/mL (102.38 mM; ultrasonic and warming and heat to 60°C)  
 \* "≥" means soluble, but saturation unknown.

	Solvent Concentration	Mass	1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM		4.0950 mL	20.4750 mL	40.9500 mL
	5 mM		0.8190 mL	4.0950 mL	8.1900 mL
	10 mM		0.4095 mL	2.0475 mL	4.0950 mL

Please refer to the solubility information to select the appropriate solvent.

#### In Vivo

- Add each solvent one by one: PBS  
Solubility: 20 mg/mL (81.90 mM); Clear solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.08 mg/mL (8.52 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
Solubility: ≥ 2.08 mg/mL (8.52 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil  
Solubility: ≥ 2.08 mg/mL (8.52 mM); Clear solution

### BIOLOGICAL ACTIVITY

#### Description

5-Azacytidine (Azacitidine; 5-AzaC; Ladakamycin) is a nucleoside analogue of cytidine that specifically inhibits DNA methylation. 5-Azacytidine is incorporated into DNA to covalently trap DNA methyltransferases and contributes to reverse

	epigenetic changes <sup>[1][2]</sup> . 5-Azacytidine induces cell autophagy <sup>[4]</sup> .		
IC <sub>50</sub> & Target	DNMT1	Nucleoside Antimetabolite/Analog	Autophagy
In Vitro	<p>Unmethylated CpG islands associated with a variety of genes become partially or fully methylated in tumors and can be reactivated by 5-Azacytidine<sup>[1]</sup>. 5-Azacytidine acts as weak inducers of erythroid differentiation of Friend erythroleukemia cells in the same concentration range where they affect DNA methyltransferase activity<sup>[2]</sup>. 5-Azacytidine inhibits L1210 cells with ID<sub>50</sub> and ID<sub>90</sub> values of 0.019 and circa 0.15 µg/mL, respectively<sup>[3]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>		
In Vivo	<p>TdR-<sup>3</sup>H incorporation is significantly inhibited when the animals are exposed to 5-Azacytidine (100 mg/kg, i.p.) for 2 hr or longer<sup>[3]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>		

## PROTOCOL

Kinase Assay <sup>[3]</sup>	<p>A crude cell-free extract is isolated from LI 210 cells in culture by suspension of the cells in a given volume of 0.05mol/L Tris-HCl buffer, pH 7.4, and sonic extraction with a Biosonik at 70% maximal output for 30 sec. The supernatant is collected after centrifugation at 105,000 × g for 60 min (4°C) in a Model L Spinco ultracentrifuge. The final protein concentration of the cell-free extracts is approximately 3 mg/mL. The extracts are used as the source of enzymes. Ribonucleotide reductase activity is measured. A unit of enzyme is defined as the amount that catalyzed dCMP synthesis at a rate of 1 µmole/hr. The assay systems for the measurement of pyrimidine nucleoside (CR) and deoxynucleoside (TdR, CdR) kinases are essentially those described by Chu and Fischer. However, reactions are terminated by heating for 2 min in a boiling water bath, and the phosphorylated derivatives are isolated according to the method of Bach. Fifty-µl aliquots are applied to 1-inch discs of diethylaminoethyl paper, which are then placed in counting vials and eluted with 0.5 mL of 0.5 mol/L PCA. After 1 hr, 12 mL of Diotol are added, and the radioactivity is determined.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Cell Assay <sup>[3]</sup>	<p>Twenty mL of cells (circa 1×10<sup>4</sup> cells/mL) are pipetted into sterilized culture tubes with screw caps and incubated at 37°C overnight. The experiment is initiated by the addition of 1 mL of 5-Azacytidine (5-azaCR) or medium for a given period (from 0 to 240 min) prior to the addition of 1 mL of metabolite (or medium). Cell growth is determined twice a day for 3 days by means of a Model A Coulter counter. To determine IDSO and ID90 values, 5 mL of L1210 cells (5×10<sup>3</sup> cells/mL) are incubated with the drug at 37°C for 3 days, and cell growth is determined.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
Animal Administration <sup>[3]</sup>	<p>For the in vivo experiments, leukemic mice (bearing circa 1×10<sup>3</sup> cells/animal) are given injections i.p. with 0.2 mL of 5-Azacytidine (5-azaCR) of a given concentration. Two hr later, the reaction is started by injecting 0.5 mL of labeled metabolite (TdR-<sup>3</sup>H or UR-<sup>3</sup>H, 10 /µCi/12.5 µg). After 1 hr, animals (3 mice/group) are killed by cervical fracture.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## CUSTOMER VALIDATION

- Mol Cancer. 2023 Dec 4;22(1):195.
- Adv Mater. 2023 Sep 8;e2302503.
- Nat Commun. 2023 Sep 19;14(1):5709.
- Nat Commun. 2022 May 13;13(1):2672.
- Mol Cell. 2022 Sep 29;S1097-2765(22)00896-6.

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## REFERENCES

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- [1]. Christman JK. 5-Azacytidine and 5-aza-2'-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene*. 2002 Aug 12;21(35):5483-95.
- [2]. Creusot F, et al. Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2'-deoxycytidine. *J Biol Chem*. 1982 Feb 25;257(4):2041-8.
- [3]. Li LH, et al. Cytotoxicity and mode of action of 5-azacytidine on L1210 leukemia. *Cancer Res*. 1970 Nov;30(11):2760-9.
- [4]. Marycz K, et al. 5-Azacytidine and Resveratrol Enhance Chondrogenic Differentiation of Metabolic Syndrome-Derived Mesenchymal Stem Cells by Modulating Autophagy. *Oxid Med Cell Longev*. 2019 May 12;2019:1523140.
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