5-Azacytidine

Cat. No.: HY-10586  
CAS No.: 320-67-2  
Molecular Formula: C₈H₁₂N₄O₅  
Molecular Weight: 244.2  
Target: Nucleoside Antimetabolite/Analog; Autophagy; DNA Methyltransferase  
Pathway: Cell Cycle/DNA Damage; Autophagy; Epigenetics  
Storage: Powder  
-20°C 3 years  
4°C 2 years  
In solvent  
-80°C 6 months  
-20°C 1 month

SOLVENT & SOLUBILITY

In Vitro  
DMSO : ≥ 31 mg/mL (126.95 mM)  
* “≥” means soluble, but saturation unknown.

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>Mass (1 mg)</th>
<th>Mass (5 mg)</th>
<th>Mass (10 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td>4.0950 mL</td>
<td>20.4750 mL</td>
<td>40.9500 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.8190 mL</td>
<td>4.0950 mL</td>
<td>8.1900 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.4095 mL</td>
<td>2.0475 mL</td>
<td>4.0950 mL</td>
</tr>
</tbody>
</table>

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.5 mg/mL (10.24 mM); Clear solution

2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
Solubility: ≥ 2.5 mg/mL (10.24 mM); Clear solution

3. Add each solvent one by one: 10% DMSO >> 90% corn oil  
Solubility: ≥ 2.5 mg/mL (10.24 mM); Clear solution

BIOLOGICAL ACTIVITY

Description  
5-Azacytidine is a nucleoside analogue of cytidine that specifically inhibits DNA methylation by trapping DNA methyltransferases.

IC₅₀ & Target  
DNMT1  
Nucleoside Antimetabolite/Analog  
Autophagy
### In Vitro
Unmethylated CpG islands associated with a variety of genes become partially or fully methylated in tumors and can be reactivated by 5-Azacytidine\(^1\). 5-Azacytidine acts as weak inducers of erythroid differentiation of Friend erythroleukemia cells in the same concentration range where they affect DNA methyltransferase activity\(^2\). 5-Azacytidine inhibits L1210 cells with ID\(_{50}\) and ID\(_{90}\) values of 0.019 and circa 0.15 \(\mu\)g/mL, respectively\(^3\).

### In Vivo
TdR\(^{-3}H\) incorporation is significantly inhibited when the animals are exposed to 5-Azacitidine (100 mg/kg, i.p.) for 2 hr or longer\(^3\).

### PROTOCOL

#### Kinase Assay \(^3\)
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 mmole/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-jul 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/LPCA. After 1 hr, 12 mL of Diotol are added, and the radioactivity is determined.

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

#### Cell Assay \(^3\)
Twenty mL of cells (circa 1×10\(^4\) 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\(^3\) cells/mL) are incubated with the drug at 37°C for 3 days, and cell growth is determined.

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#### Animal Administration \(^3\)
For the in vivo experiments, leukemic mice (bearing circa 1×10\(^3\) 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\(^{-3}H\) or UR\(^{-3}H\), 10 /\mu Ci/12.5 \mu g). After 1 hr, animals (3 mice/group) are killed by cervical fracture, and the ascites are treated with heparin, collected, pooled, and then centrifuged immediately in a Sorvall refrigerated centrifuge Model R2C-B at 800×g for 10 min (4°C).

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REFERENCES

