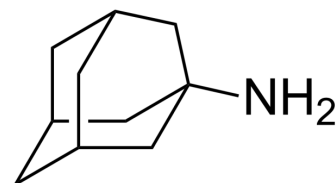


## Amantadine

<b>Cat. No.:</b>	HY-B0402
<b>CAS No.:</b>	768-94-5
<b>Molecular Formula:</b>	C <sub>10</sub> H <sub>17</sub> N
<b>Molecular Weight:</b>	151.25
<b>Target:</b>	Influenza Virus; Orthopoxvirus; SARS-CoV; Apoptosis; CDK; Bcl-2 Family
<b>Pathway:</b>	Anti-infection; Apoptosis; Cell Cycle/DNA Damage
<b>Storage:</b>	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

<b>In Vitro</b>	DMSO : 6.48 mg/mL (42.84 mM); ultrasonic and warming and adjust pH to 4 with 1 M HCL and heat to 60°C																					
	<table border="1"> <thead> <tr> <th rowspan="2">Solvent</th> <th rowspan="2">Mass</th> <th colspan="3">Concentration</th> </tr> <tr> <th>1 mg</th> <th>5 mg</th> <th>10 mg</th> </tr> </thead> <tbody> <tr> <td rowspan="3">Preparing Stock Solutions</td> <td>1 mM</td> <td>6.6116 mL</td> <td>33.0578 mL</td> <td>66.1157 mL</td> </tr> <tr> <td>5 mM</td> <td>1.3223 mL</td> <td>6.6116 mL</td> <td>13.2231 mL</td> </tr> <tr> <td>10 mM</td> <td>0.6612 mL</td> <td>3.3058 mL</td> <td>6.6116 mL</td> </tr> </tbody> </table>	Solvent	Mass	Concentration			1 mg	5 mg	10 mg	Preparing Stock Solutions	1 mM	6.6116 mL	33.0578 mL	66.1157 mL	5 mM	1.3223 mL	6.6116 mL	13.2231 mL	10 mM	0.6612 mL	3.3058 mL	6.6116 mL
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	Please refer to the solubility information to select the appropriate solvent.																					
<b>In Vivo</b>	<ol style="list-style-type: none"> <li>Add each solvent one by one: 10% DMSO &gt;&gt; 40% PEG300 &gt;&gt; 5% Tween-80 &gt;&gt; 45% saline Solubility: ≥ 1 mg/mL (6.61 mM); Clear solution</li> <li>Add each solvent one by one: 10% DMSO &gt;&gt; 90% (20% SBE-β-CD in saline) Solubility: ≥ 1 mg/mL (6.61 mM); Clear solution</li> <li>Add each solvent one by one: 10% DMSO &gt;&gt; 90% corn oil Solubility: ≥ 0.5 mg/mL (3.31 mM); Clear solution</li> </ol>																					

### BIOLOGICAL ACTIVITY

<b>Description</b>	Amantadine (1-Adamantanamine) is an orally active and potent antiviral agent with activity against influenza A viruses. Amantadine inhibits several ion channels such as NMDA and M2, and also inhibits Coronavirus ion channels. Amantadine also has anti-orthopoxvirus and anticancer activity. Amantadine can be used for Parkinson's disease, postoperative cognitive dysfunction (POCD) and COVID-19 research <sup>[1][2][3][4][5][6]</sup> .		
<b>IC<sub>50</sub> &amp; Target</b>	CDK2	Bcl-2	Bax
<b>In Vitro</b>	Amantadine (0-500 μM, 26 h) inhibits SARS-CoV-2 replication, with IC <sub>50</sub> concentrations between 83 and 119 μM <sup>[4]</sup> . Amantadine (0-100 μg/mL, 24-72 h) markedly inhibits the proliferation of HepG2 and SMMC7721 cells <sup>[6]</sup> .		

Amantadine (0-75 µg/mL, 48 h) arrests the cell cycle at the G0/G1 phase and induces apoptosis<sup>[6]</sup>.

Amantadine (0-75 µg/mL, 48 h) reduces the levels of the cell cycle-related genes and proteins (cyclin D1, cyclin E and CDK2), reduces Bcl-2 and increases the Bax protein and mRNA levels<sup>[6]</sup>.

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

#### Cell Viability Assay<sup>[4]</sup>

Cell Line:	Vero E6 cells
Concentration:	500 µM, 100 µM, 20 µM, 4 µM, and 8 nM
Incubation Time:	26 h
Result:	Caused a concentration-dependent reduction (IC <sub>50</sub> =83 µM) of viral nucleic acids in the supernatant 26 h after infection at 10-500 µM. Caused a concentration-dependent reduction (IC <sub>50</sub> =119 µM) of viral nucleic acids in the cytosol 26 h after infection.

#### Cell Proliferation Assay<sup>[6]</sup>

Cell Line:	Human HCC cell lines (HepG2 and SMMC-7721) and normal hepatocellular cells (L02 cells)
Concentration:	0, 1, 2, 5, 10, 25, 50 and 100 µg/mL
Incubation Time:	24, 48 and 72 h
Result:	Inhibited cellular proliferation in a time- and dose-dependent manner in HepG2 and SMMC-7721 cells.

#### Cell Cycle Analysis<sup>[6]</sup>

Cell Line:	HepG2 and SMMC-7721 cells
Concentration:	0, 10, 25, 50 and 75 µg/mL
Incubation Time:	48 h
Result:	Significantly increased the population of HepG2 and SMMC-7721 cells in the G0/G1 phase in a dose-dependent manner, and significantly decreased the number of HepG2 cells in the S phase.

#### Apoptosis Analysis<sup>[6]</sup>

Cell Line:	HepG2 and SMMC-7721 cells
Concentration:	0, 10, 25, 50 and 75 µg/mL
Incubation Time:	48 h
Result:	Markedly increased the percentage of apoptotic HepG2 and SMMC-7721 cells (early- and late-stage apoptosis) in a dose-dependent manner.

#### Western Blot Analysis<sup>[6]</sup>

Cell Line:	HepG2 and SMMC-7721 cells
Concentration:	0, 10, 25, 50 and 75 µg/mL
Incubation Time:	48 h
Result:	Showed downregulation of cyclin D1, cyclin E and CDK2, and showed a decrease in Bcl-2

levels and an increase of Bax levels in HepG2 and SMMC-7721 cells.

#### RT-PCR<sup>[6]</sup>

Cell Line:	HepG2 and SMMC-7721 cells
Concentration:	0, 10, 25, 50 and 75 µg/mL
Incubation Time:	48 h
Result:	Revealed an increase in Bax and decrease in Bcl-2 genes.

#### In Vivo

Amantadine (25 mg/kg, IP, once daily for 3 days) inhibits surgery induced neuroinflammation and learning and memory impairment<sup>[5]</sup>.

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

Animal Model:	Fischer 344 rats (Four-month old, male, 290-330 g, 15 rats each group) <sup>[5]</sup>
Dosage:	25 mg/kg
Administration:	IP, once daily for 3 days (the first dose at 15 min before surgery)
Result:	Inhibited surgery induced neuroinflammation and learning and memory impairment, increased GDNF (glial cell line-derived neurotrophic factor) that was co-localized with glial fibrillary acidic protein (an astrocytic marker) in the hippocampus.

## CUSTOMER VALIDATION

- Signal Transduct Target Ther. 2021 Mar 27;6(1):134.
- Int J Nanomedicine. 2019 Nov 27;14:9217-9234.
- Chem Phys Lipids. 2024 May 11:262:105397.

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## REFERENCES

- [1]. Donald F Smee, et al. A review of compounds exhibiting anti-orthopoxvirus activity in animal models. *Antiviral Res.* 2003 Jan;57(1-2):41-52.
- [2]. Fink K, et al. Amantadine Inhibits SARS-CoV-2 In Vitro. *Viruses.* 2021 Mar 24;13(4):539.
- [3]. Zhang J, et al. Amantadine alleviates postoperative cognitive dysfunction possibly by increasing glial cell line-derived neurotrophic factor in rats. *Anesthesiology.* 2014 Oct;121(4):773-85.
- [4]. Lan Z, et al. Amantadine inhibits cellular proliferation and induces the apoptosis of hepatocellular cancer cells in vitro. *Int J Mol Med.* 2015;36(3):904-910.
- [5]. Suzuki H, et al. Emergence of amantadine-resistant influenza A viruses: epidemiological study. *J Infect Chemother.* 2003;9(3):195-200.
- [6]. Hubsher G, et al. Amantadine: the journey from fighting flu to treating Parkinson disease. *Neurology.* 2012;78(14):1096-1099.

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

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