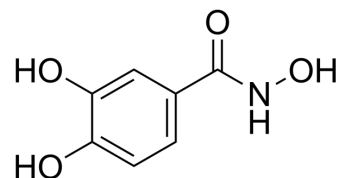


Didox

Cat. No.:	HY-19387		
CAS No.:	69839-83-4		
Molecular Formula:	C ₇ H ₇ NO ₄		
Molecular Weight:	169.13		
Target:	DNA/RNA Synthesis		
Pathway:	Cell Cycle/DNA Damage		
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 : 100 mg/mL (591.26 mM; Need ultrasonic)					
		Solvent Concentration	Mass	1 mg	5 mg	10 mg
	Preparing Stock Solutions	1 mM		5.9126 mL	29.5631 mL	59.1261 mL
		5 mM		1.1825 mL	5.9126 mL	11.8252 mL
		10 mM		0.5913 mL	2.9563 mL	5.9126 mL
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: ≥ 2.75 mg/mL (16.26 mM); Clear solution					
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.75 mg/mL (16.26 mM); Clear solution					
	3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.75 mg/mL (16.26 mM); Clear solution					

BIOLOGICAL ACTIVITY

Description	Didox (NSC-324360) is a synthetic ribonucleotide reductase (RR) inhibitor.
IC ₅₀ & Target	Ribonucleotide reductase ^[1]
In Vitro	Didox (NSC-324360) suppresses LPS-induced mRNA levels of iNOS, IL-6, IL-1, TNF-α, NF-κβ (p65), and p38-α, after 24 h of treatment. Treatment with Didox also suppresses the secretion of nitric oxide (NO), IL-6, and IL-10. Using mitochondrial dehydrogenase activity as a measure of cytotoxicity, the effects of Didox on cellular respiration in RAW264.7 are examined

over a range of concentrations for 24 h. Cells exposures to 200 μ M and below Didox, with and without LPS, do not exhibit significant cellular toxicity^[1]. Didox (NSC-324360) is active against all human and murine acute myeloid leukemia (AML) lines tested with IC₅₀ values in the low micromolar range (mean IC₅₀ 37 μ M [range 25.89-52.70 μ M])^[2].

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

In Vivo

Once engraftment is established by bioluminescent imaging, the animals receive daily administrations of Didox at 425 mg/kg via IP injection over 5 days. Didox (NSC-324360) treatment significantly reduces leukemic burden compared to vehicle treated controls ($p=0.0026$ and $p=0.0342$). More importantly, Didox (NSC-324360) provides a significant survival benefit ($p<0.0001$ and $p=0.0094$)^[2].

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

PROTOCOL

Cell Assay ^[1]

RAW264.7 macrophages are treated with Didox alone, with 0.1 μ g/mL LPS, or the two in combination. Cellular respiration, as an indication of cytotoxicity, is measured by the MTT assay, which quantifies mitochondrial dehydrogenase activity. Macrophages are plated into 96 well Costar plates at 10^5 cells per well in 100 μ L of DMEM media. After 4 h of incubation at 37°C for adherence, compounds and DMSO carrier control (0.01% final) are added in triplicate over serial dilutions beginning with 200 μ M per well in a total volume of 200 μ L, and the plates incubated for 24 h. Four h before termination of the assay, each well receives 20 μ L of a 5 mg/mL MTT solution in un-supplemented DMEM. After centrifugation, the supernatant for each well is discarded and cells containing reduced MTT are solubilized with 100 μ L of acidified isopropanol (4 mM HCl, 0.1% NP-40 in isopropanol). Following a brief period of shaking, the optical density (O.D.) for each well is recorded at 550 nm. Each experiment is repeated three times and the data averaged from each triplicate, then expressed as percentage of the control O.D. values for each experiment^[1].

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Animal Administration ^[2]

Mice^[2]

Luciferase-tagged leukemia cells are transplanted into 8- week old, sublethally irradiated (4.5 Gy) C57Bl/6 mice by tail vein injection of 1.0×10^6 cells per mouse. Mice are injected with 150 mg/kg D-Luciferin, anesthetised with Isoflurane, and imaged using the IVIS 100 imaging system. Mice begin treatment with Didox upon detection of clear signal. The animals are treated with daily administrations of Didox (NSC-324360) at 425 mg/kg Didox by intraperitoneal injection (IP) for 5 days. Control animals receive 5% dextrose water by IP injection. Repeat imaging is performed on the day following the final treatment^[2].

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

CUSTOMER VALIDATION

- Free Radic Biol Med. 2020 May 20;152:525-539.
- Phytother Res. 2022 Feb 7.
- Biomolecules. 2022 Feb 12;12(2):299.
- J Ethnopharmacol. 2021 Sep 30;114694.
- J Ethnopharmacol. 2018 Dec 5;227:166-175.

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REFERENCES

[1]. Matsebatlela TM, et al. 3,4-Dihydroxy-benzohydroxamic acid (Didox) suppresses pro-inflammatory profiles and oxidative stress in TLR4-activated RAW264.7 murine macrophages. Chem Biol Interact. 2015 May 25;233:95-105.

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

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