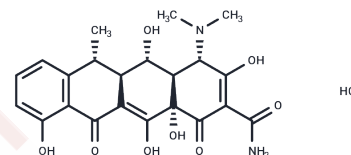


Doxycycline hydrochloride

Chemical Properties

CAS No. :	10592-13-9
Formula:	C ₂₂ H ₂₄ N ₂ O ₈ ·HCl
Molecular Weight:	480.9
Storage:	Store under nitrogen Powder: -20°C for 3 years In solvent: -80°C for 1 year <i>Actual storage temperature shall be subject to the COA.</i>



Biological Description

Description	Doxycycline hydrochloride (Hyclate) is an orally active tetracycline antibiotic with broad-spectrum matrix metalloproteinase (MMP) inhibitory activity, possessing both antibacterial and anti-cancer cell proliferation effects, and can be used to induce gene expression in ON-OFF systems.
Targets(IC50)	MMP, Antibacterial, Antibiotic
In vitro	In Wistar rats, daily administration of Doxycycline (25 mg) effectively inhibits the structural degradation of aortic elastin, diminishes the activity of matrix metalloproteinases MMP-2 and MMP-9 in the arterial wall, and consequently reduces the replication of smooth muscle cells in the intima.
In vivo	Doxycycline (50 μM) downregulates the expression of MMP-8 at both mRNA and protein levels in rheumatoid synovial fibroblasts and endothelial cells while completely inhibiting the degradation of type II collagen. Additionally, in corneal epithelial cells, doxycycline suppresses the increase in mRNA and protein levels of IL-1β induced by lipopolysaccharide.
Kinase Assay	Gelatin (0.1% (w/v)) is added to standard Laemmli acrylamide polymerization mixture. Tissue extract is mixed 1:2 with sample buffer [250 mM Tris-Cl pH 6.8, 10% (w/v) SDS, 20% (v/v) glycerol, 0.005% (w/v) bromophenol blue]. Serum is diluted 1:10 with electrophoresis buffer (2.5 mM Tris, 20 mM glycine, 0.005% SDS) and mixed 1:2 with sample buffer. Twenty μLs are loaded after 10-min incubation at room temperature without boiling. After electrophoresis at 90 V, the gels are soaked in 2.5% (w/v) Triton X-100, incubated 2 to 3 days at 37°C in gelatin digestion buffer [50 mM Tris-Cl, pH 8.0, 8 mM CaCl ₂ , 10 mM ZnSO ₂ , 0.02% (w/v) NaN ₃], stained in 0.05% Coomassie blue R-250 in acetic acid/methanol/water (1:4.5:4.5 by volume), destained in 10% acetic acid and 5% methanol, and scanned for lysis band intensity. The lysis band intensity is proportional to gelatinase activity and is quantified densitometrically by using One-Dimensional Scan software. The result, a number between 0.07 and 3.75, is normalized to the protein content by dividing the densitometry result with the relative optical density from the BCA protein assay kit result. The result is used for the analysis as the arbitrary unit. For the total MMP activity results of lysis bands of pro-MMP-9, active MMP-9, pro-MMP-2, and active MMP-2 are added. A protein size marker is used to determine the correct size.

Cell Research	<p>Doxycycline is diluted in culture medium at a concentration of 10 µg/mL. All in vitro treatments are performed in SMC cultures at 90% confluence, when ECM synthesis in SMC starts to be evident. Doxycycline (20 nM) is diluted in culture medium at a concentration of 10 µg/mL (20 nM), at which no toxicity or variation in primary cultured SMC proliferation has been reported, as well as in other cell lines and the incubation time is 48 h. SMC-C and SMC-Ch are seeded at equal cell density in 6-well plates and, when confluence reaches 90%, 1 mL culture medium is added to each well containing 3.7×10^4 Bq L-[5-H3]-proline (9.62×10^{11} Bq/mmol). After 48h incubation, cells are lysed with 0.5 mL 0.5 mol/L NaOH for 1 h. The resulting solution is neutralized with an equal amount of 0.5 mol/L HCl, and 50 µL are used to measure total proteins with the Bradford method. One volume of 10% TCA is added to the remaining 250 µL and centrifuged at 13,000 g for 15min at 4°C. The resulting precipitate is dissolved in 100 µL 0.2 mol/L NaOH, and then neutralized with 1 mol/L HCl. The solution is incubated with collagenase buffer (Tris-HCl, pH 7.6 20 mM, and CaCl₂ 250 mM final concentration) and 10 units of collagenase at 37°C overnight. Then, 150 µL 10% TCA are added and centrifuged at 13000 g for 15min at 4°C. The resulting supernatant is added to 4 mL of scintillation fluid and the radioactivity is measured in a liquid scintillation counter LS 600 TA.</p>
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Solubility Information

Solubility	DMSO: 10 mM, Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
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Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	2.0794 mL	10.3972 mL	20.7943 mL
5 mM	0.4159 mL	2.0794 mL	4.1589 mL
10 mM	0.2079 mL	1.0397 mL	2.0794 mL
50 mM	0.0416 mL	0.2079 mL	0.4159 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Wilfried Briest, et al. Doxycycline ameliorates the susceptibility to aortic lesions in a mouse model for the vascular type of Ehlers-Danlos syndrome. *J Pharmacol Exp Ther.* 2011 Jun;337(3):621-7.

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Trajano VC, et al. Osteogenic activity of cyclodextrin-encapsulated doxycycline in a calcium phosphate PCL and PLGA composite. *Mater Sci Eng C Mater Biol Appl.* 2016 Jul 1;64:370-5.

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