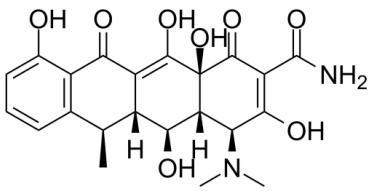
Data Sheet

Product Name:	Doxycycline (hyclate)
Cat. No.:	CS-2890
CAS No.:	24390-14-5
Molecular Formula:	C22H24N2O8.1/2C2H6O.ClH.1/2H2O
Molecular Weight:	512.94
Target:	Bacterial; MMP
Pathway:	Anti-infection; Metabolic Enzyme/Protease
Solubility:	H2O : 30 mg/mL (58.49 mM; Need ultrasonic)



$0.5H_2O$
$0.5C_2H_6O$
HCl

BIOLOGICAL ACTIVITY:

Doxycycline (hyclate) (Doxycycline hydrochloride hemiethanolate hemihydrate), an antibiotic, is an orally active and broad-spectrum metalloproteinase (**MMP**) inhibitor[1]. **In Vitro:** Doxycycline (hyclate) (Doxycycline hydrochloride hemiethanolate hemihydrate) affects growth of glioma cells only under high concentrations[2].

Doxycycline (hyclate) decreases MT-CO1 protein content with concentrations of 1 μg/mL and higher in SVG cells[2].

In Vivo: Doxycycline (hyclate) (Doxycycline hydrochloride hemiethanolate hemihydrate) (oral; 200 or 800 mg/kg/day; for 3 months) reduces active MMP-9 in untreated HT mice in a dose-dependent manner[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]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) bromphenol 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 Assay:** Doxycycline is diluted in culture medium at a concentration of 10 μg/mL.[4]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-^{3}H]-proline (9.62×10^{11} Bq/mmol). After 48 h 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 15 min 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 $CaCl_2$ 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 15 min 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. **Animal Administration:** Doxycycline is prepared in food diet.[1]Two groups of 6-month-old female heterozygous (HT) Col3a1 knockout mice

are treated for 3 months with doxycycline. Treatment is provided with food containing 200 or 800 mg/kg of doxycycline. Because preliminary measured food intake of these mice is averaged at 3.5 g/day and the average body weight of animals is 25 g, the average drug dose for low- and high-dose groups is 25 (Doxy25) or 100 (Doxy100) mg/kg per day, respectively. Untreated (wild type) WT and HT mice are maintained on a regular diet (NIH-07 mouse/rat diet) and served as controls. After 3 months, under general inhalation anesthesia (2% of isoflurane in oxygen) and aseptic conditions, the abdominal aortas are surgically exposed and stressed by the following technique: the blood flow is stopped by occluding the abdominal aorta against the spinal column with a sterile cotton-tip applicator pressed at the level of the renal arteries. After 30 s a second applicator is pressed at the level of iliac bifurcation and the first applicator is abruptly released, followed by release of the second applicator. The abdominal incision is sutured closed, and mice are returned to home cages. The treatment is continued after the intervention. One week after intervention mice are euthanized by an overdose of isoflurane. Blood is collected, and aortas and segments of colon and skin are harvested.

References:

[1]. 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.

[2]. Luger AL, et al. Doxycycline Impairs Mitochondrial Function and Protects Human Glioma Cells from Hypoxia-Induced Cell Death: Implications of Using Tet-Inducible Systems. Int J Mol Sci. 2018 May 17;19(5).

CAIndexNames:

2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, hydrochloride, (4S,4aR,5S,5aR,6R,12aS)-, compd. with ethanol, hydrate (2:2:1:1)

SMILES:

O=C(C(C1=O)=C(O)[C@@H](N(C)C)[C@]2([H])[C@@H](O)[C@]3([H])[C@@H](C)C4=C(C(C3=C(O)[C@@]21O)=O)C(O)=CC=C4)N.Cl.[0.5H2O].[0.5C2H6O]

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