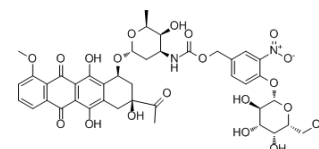


## Daun02

<b>Cat. No.:</b>	HY-13061		
<b>CAS No.:</b>	290304-24-4		
<b>Molecular Formula:</b>	C <sub>41</sub> H <sub>44</sub> N <sub>2</sub> O <sub>20</sub>		
<b>Molecular Weight:</b>	884.79		
<b>Target:</b>	Topoisomerase; ADC Cytotoxin		
<b>Pathway:</b>	Cell Cycle/DNA Damage; Antibody-drug Conjugate/ADC Related		
<b>Storage:</b>	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 (113.02 mM)  
 \* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent		1 mg	5 mg	10 mg
	Concentration	Mass			
	1 mM		1.1302 mL	5.6511 mL	11.3021 mL
	5 mM		0.2260 mL	1.1302 mL	2.2604 mL
	10 mM		0.1130 mL	0.5651 mL	1.1302 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: ≥ 2.5 mg/mL (2.83 mM); Clear solution

### BIOLOGICAL ACTIVITY

#### Description

Daun02 is a prodrug of the topoisomerase inhibitor Daunorubicin.

#### IC<sub>50</sub> & Target

Topoisomerase      Daunorubicins/Doxorubicins

#### In Vitro

Daun02 is a prodrug, which is converted by β-galactosidase to Daunorubicin, which has been shown to reduce calcium ion (Ca<sup>2+</sup>)-dependent action potentials in neuroblastoma cells<sup>[1]</sup>. Daunorubicin is a topoisomerase inhibitor<sup>[2]</sup>. Daun02 is a good substrate for β-galactosidase (β-gal). The concentration of Daun02 producing 50% (EC<sub>50</sub>) decrease in cell viability is 0.5 μM, 1.5 μM, and 3.5 μM for T47-D, Panc02, and MCF-7, respectively<sup>[3]</sup>.

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

#### In Vivo

Daun02 is a good substrate for β-gal with K<sub>m</sub> and V<sub>max</sub> values of 0.37 mM and 8.6 μmol/min/mg protein. At a concentration

of  $10^{-5}$  M, Daun02 is 79% bound to plasma protein compares to 94% for Daunomycin<sup>[3]</sup>.  
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## PROTOCOL

### Cell Assay <sup>[3]</sup>

Murine Panc02 cells are maintained as exponentially growing monolayer cultures in DMEM/F12 or RPMI-1640 medium supplemented with 10% FBS, 1% glutamine, penicillin, and streptomycin at 37°C. For cytotoxicity assay, the cells are seeded into 96-well microplates and incubated overnight. Initial experiments indicate that FBS contains low levels of intrinsic  $\beta$ -gal activity as evidenced by the slow conversion of Daun02 to Daunomycin; however, this is not evident for human serum. Therefore, prior to addition of Daun02, the FBS concentration is reduced from 10% to 1% for Panc02 cells. Human serum (10%) is used for the transduced human cell lines. The cells are incubated for 24 h and then MTT is added. Lysis buffer (20% SDS dissolved in 50% DMF) is added 4 h after the addition of MTT and the cells are incubated overnight. The optical density at 570 nm is determined using a BIO-RAD microplate reader. Cytotoxicity is expressed as the concentration of drug or prodrug that produced a 50% ( $EC_{50}$ ) reduction in cell viability<sup>[3]</sup>.  
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

### Animal Administration <sup>[3]</sup>

Mice<sup>[3]</sup>  
Male athymic BALB/c mice (nu/nu genotype, 18-20 g) are used. Daunomycin is administered at a dose of 20 mg/kg in 100  $\mu$ L normal saline solution into the tail vein. Daun02 is administered intraperitoneally at a dose of 200 mg/kg in 200  $\mu$ L vehicle. (This route is selected because the volume of drug solution, 200  $\mu$ L, is too great for tail vein administration.) Tumor volume is determined by caliper measurement in two dimensions and converted to tumor mass. Tumor growth is monitored over a period of 30 days or until the tumors has reached a mass of 5% of bodyweight (about 1 g). The animals are then killed by carbon dioxide asphyxiation.  
MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- Sci Rep. 2017 Jan 3;7:39817.

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## REFERENCES

- [1]. Koya E, et al. Targeted disruption of cocaine-activated nucleus accumbens neurons prevents context-specific sensitization. *Nat Neurosci.* 2009 Aug;12(8):1069-73.
- [2]. Lehmann M, et al. Activity of topoisomerase inhibitors daunorubicin, idarubicin, and aclarubicin in the *Drosophila* Somatic Mutation and Recombination Test. *Environ Mol Mutagen.* 2004;43(4):250-7.
- [3]. Farquhar D, et al. Suicide gene therapy using *E. coli* beta-galactosidase. *Cancer Chemother Pharmacol.* 2002 Jul;50(1):65-70.

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

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