上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：PNU-159682
产品别名：PNU-159682

生物活性：

Description	PNU-159682, a highly potent metabolite of the anthracycline nemorubicin (DNA topoisomerase II inhibitor) with outstanding cytotoxicity, is a potent ADCs cytotoxin.
IC_{50} & Target	Daunorubicins/Doxorubicins
In Vitro	PNU-159682 inhibits a panel of human tumor cell lines with IC_{70} values in the range of 0.07-0.58 nM, and is 2,360- to 790-fold and 6,420- to 2,100-fold more potent than MMDX and doxorubicin, respectively[1]. PNU-159682 (100 μM) weakly inhibits topoisomerase II unknotting activity. PNU-159682 (10 μM)-DNA adducts contain one or two drug molecules bound to double-stranded DNA[2]. PNU-159682 shows cytotoxic effect on CAIX-expressing SKRC-52 cells with IC50 of 25 nM[3].
In Vivo	PNU-159682 (15 μg/kg, i.v.) shows antitumor activity in mice bearing disseminated murine L1210 leukemia and in MX-1 human mammary carcinoma xenografts at 4 μg/kg[1]. PNU-159682 (25 nmol/kg) exhibits a potent antitumor effect in mice bearing SKRC-52 xenografted tumors[3].

Solvent&Solubility

In Vitro:

DMSO : ≥ 100 mg/mL (155.86 mM)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.5586 mL	7.7928 mL	15.5855 mL
	5 mM	0.3117 mL	1.5586 mL	3.1171 mL
	10 mM	0.1559 mL	0.7793 mL	1.5586 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (3.90 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (3.90 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline)

	Solubility: 2.5 mg/mL (3.90 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (3.90 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (3.90 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (3.90 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Quintieri L, et al. Formation and antitumor activity of PNU-159682, a major metabolite of nemorubicin in human liver microsomes. Clin Cancer Res. 2005 Feb 15;11(4):1608-17. [2]. Cazzamalli S, et al. Acetazolamide Serves as Selective Delivery Vehicle for Dipeptide-Linked Drugs to Renal Cell Carcinoma. Mol Cancer Ther. 2016 Dec;15(12):2926-2935. [3]. Scalabrin M, et al. Virtual Cross-Linking of the Active Nemorubicin Metabolite PNU-159682 to Double-Stranded DNA. Chem Res Toxicol. 2017 Feb 20;30(2):614-624.

实验参考：

Cell Assay	SKRC-52 cells are seeded in 96-well plates in RPMI added with 10% FCS (100 μL) at a density of 5000 cells per well and allowed to grow for 24 h. The medium is replaced with medium containing different concentrations of test substance (1:3 dilution steps) and plates are incubated under standard culture conditions. After 72 h the medium is removed, MTS cell viability dye (20 μL) in 150 μL of the medium is added, the plates are incubated for 2 h under culture conditions and the absorbance at 490 nm measured on a Spectra Max Paradigm multimode plate reader. Experiments are performed in triplicate and average cell viability calculated as measured background corrected absorbance divided by the absorbance of untreated control wells. IC_{50} values are determined by fitting data to the four-parameter logistic equation, using a Prism 6 software for data analysis.[3]
Animal Administration	Four- to six-week-old female CD-1 athymic nude mice are used for evaluation of the activity of PNU-159682 against MX-1 human mammary carcinoma xenografts. On day 0, animals (n=14) are grafted s.c. with MX-1 tumor fragments in the right flank. Eight days later, they are randomly assigned to the drug treatment group or control group (n=7 mice per group), and treatment is started. PNU-159682 is given i.v. (4 μg/kg) according to a q7dx3 (every 7 days for three doses) schedule; control animals receive saline injections. Tumor volume is estimated from measurements done with a caliper; where D and d are the longest and the shortest diameters, respectively. For ethical reasons, control animals are sacrificed on day 21 when the mean tumor volume in the group is appr 2,500 mm^3; animals receiving drug treatment are monitored up to day 50, at which point they are sacrificed. [1]
	The inhibition of topoisomerase II activity is tested by taking advantage of the ability of this enzyme to decatenate kinetoplast DNA (kDNA); the test is specific for both isoforms of topoisomerase II (α and β) because it relies on the conversion of catenated DNA to its decatenated form, which requires double strand cut and ligation uniquely performed by topoisomerase II. The DNA used in this test is

Kinase Assay	the mitochondrial kDNA of Crithidia fasciculata, a catenated network of DNA rings, most of which are 2.5 kb monomers. The kDNA networks are large relative to the monomers and do not migrate in the gel remaining in the well, while the minicircles can be easily resolved in agarose gel. Both the gel and the running buffer contain the intercalator ethidium bromide, which allows the monitoring of monomers appearance with a UV light source and the resolution of different DNA forms (linear, nicked circular DNA, and relaxed DNA monomers), helping to clearly distinguish the linear DNA from the nicked circular DNA. In this test, 200 ng of kDNAs is incubated for 1 h at 37°C with doxorubicin at 10, 1, or 0.1 μM, or with PNU-159682 at 100, 10, or 1 μM in the presence of 0.025 U of human topoisomerase IIα in a topoisomerase II reaction buffer (Tris-HCl pH 7.9 40 mM, KCl 80 mM, DTT 5 mM, BSA 15 μg/mL, ATP 1 mM, and $MgCl_2$ 10 mM). At the end of the incubation period, each sample is spiked with 3 μL of gel loading buffer (xylene cyanol 0.25%, blue bromophenol 0.25%, Ficoll 400 18%, and EDTA 6 mM) and then analyzed by agarose (1%) gel electrophoresis. Runs are performed in TBE buffer (Tris 89 mM, boric Acid 89 mM, EDTA 2 mM, pH 8.0) in the presence of ethidium bromide 0.5 μg/mL. Samples are run overnight at 1 V/cm. [2]
References	[1]. Quintieri L, et al. Formation and antitumor activity of PNU-159682, a major metabolite of nemorubicin in human liver microsomes. Clin Cancer Res. 2005 Feb 15;11(4):1608-17. [2]. Cazzamalli S, et al. Acetazolamide Serves as Selective Delivery Vehicle for Dipeptide-Linked Drugs to Renal Cell Carcinoma. Mol Cancer Ther. 2016 Dec;15(12):2926-2935. [3]. Scalabrin M, et al. Virtual Cross-Linking of the Active Nemorubicin Metabolite PNU-159682 to Double-Stranded DNA. Chem Res Toxicol. 2017 Feb 20;30(2):614-624.