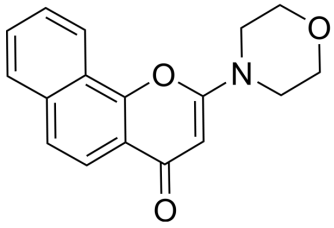
NU 7026

Cat. No.:	HY-15719
CAS No.:	154447-35-5
Molecular Formula:	$C_{17}H_{15}NO_3$
Molecular Weight:	281.31
Target:	DNA-PK; Apoptosis
Pathway:	Cell Cycle/DNA Damage; PI3K/Akt/mTOR; Apoptosis
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 1 year -20°C 6 months



SOLVENT & SOLUBILITY

In Vitro

DMSO : 2.5 mg/mL (8.89 mM; Need ultrasonic)

Preparing Stock Solutions

Concentration \ Solvent \ Mass	1 mg	5 mg	10 mg
1 mM	3.5548 mL	17.7740 mL	35.5480 mL
5 mM	0.7110 mL	3.5548 mL	7.1096 mL
10 mM	---	---	---

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

NU 7026 (LY293646) is a novel specific DNA-PK inhibitor with IC_{50} of 0.23 μM, also inhibits PI3K with IC_{50} of 13 μM.

IC_{50} & Target

DNA-PK	PI3K
0.23 μM (IC_{50})	13 μM (IC_{50})

In Vitro

NU7026 (10 μM) potentiates ionizing radiation (IR) cytotoxicity [potentiation factor at 90% cell kill (PF_{90})=1.51±0.04] in exponentially growing DNA-PK proficient but not deficient cells[1]. NU7026 synergistically sensitizes I83 cells to Chlorambucil (CLB) 3.5-fold[2].NU7026, a novel inhibitor of the DNA repair enzyme DNA-dependent protein kinase (DNA-PK). At a dose of 10 μM, which is nontoxic to cells per se, a minimum NU7026 exposure of 4 h in combination with 3 Gy radiation is required for a significant radiosensitisation effect in CH1 human ovarian cancer cells[3].

?Solution in vitro: NU7026 is dissolved in anhydrous DMSO. NU7026 is added to cells to a final concentration of 0.25% DMSO (v/v)[4].

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

In Vivo

NU7026, a novel inhibitor of the DNA repair enzyme DNA-dependent protein kinase (DNA-PK). Following intravenous

administration to mice at 5 mg/kg, NU7026 underwent rapid plasma clearance (0.108 L/h) and this is largely attributed to extensive metabolism. Bioavailability following interperitoneal (i.p.) and p.o. administration at 20 mg/kg is 20 and 15%, respectively[3].

?Solution in vivo:

?NU7026 is formulated in 10% DMSO and 5% Tween 20 in saline (i.p. and p.o.) (Mice)[3].

?NU7026 is formulated in 10% ethanol, 25% PEG 200 and 5% Tween 20 in saline (i.v.)[3].

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

PROTOCOL

Kinase Assay [1]

Mammalian DNA-PK (500 ng/μL) is isolated from HeLa cell nuclear extract after chromatography using Q-Sepharose, S-Sepharose, and Heparin agarose. DNA-PK (250 ng) activity is measured at 30°C, in a final volume of 40 μL, in buffer containing 25 mM HEPES (pH 7.4), 12.5 mM $MgCl_2$, 50 mM KCl, 1 mM DTT, 10% v/v Glycerol, 0.1% w/v NP-40, and 1 mg of the substrate GST-p53N66 (the NH2-terminal 66 amino acid residues of human wild-type p53 fused to glutathione S-transferase) in polypropylene 96-well plates. To the assay mix, varying concentrations of inhibitor (in DMSO at a final concentration of 1% v/v) are added. After 10 min of incubation, ATP is added to give a final concentration of 50 μM, along with a 30-mer double-stranded DNA oligonucleotide (final concentration of 0.5 ng/mL), to initiate the reaction. After 1 h with shaking, 150 μL of PBS are added to the reaction, and 5 μL are then transferred to a 96-well opaque white plate containing 45 μL of PBS per well, where the GSTp53N66 substrate is allowed to bind to the wells for 1 h. To detect the phosphorylation event on the serine 15 residue of p53 elicited by DNA-PK, a p53 phosphoserine-15 antibody is used in a basic ELISA procedure. An antirabbit horseradish peroxidase-conjugated secondary antibody is then used in the ELISA before the addition of chemiluminescence reagent to detect the signal as measured by chemiluminescent counting via a TopCount NXT[1].

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Cell Assay [2]

I83 cells are plated in RPMI 1640 medium with 10% FBS (1.5×10^5 cells/mL) and treated with vehicle (DMSO), 5 μM CLB, CLB IC_{50}, 10 μM NU7026, or the combination of both drugs for 0, 6, 24, and 48 h. Cell cycle distribution, apoptosis, DNA-PK phosphorylation, and γH2AX determination are determined, and they are expressed as a percentage of cells in each phase of the cycle. DNA content is analyzed with a FACSCalibur flow cytometer equipped with CellQuest software[2].

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Animal Administration [3]

Mice[3]

Female BALB/c mice are used. NU7026 is formulated in 10% DMSO and 5% Tween 20 in saline for i.p. and perorally (p.o.) administration at 20 and 50 mg/kg, respectively. For i.v. dosing at 5 mg/kg, NU7026 is formulated in 10% ethanol, 25% PEG 200 and 5% Tween 20 in saline. Control animals receive the vehicle alone. Groups of three mice are injected per time point. Blood is collected by cardiac puncture following transient anaesthesia with halothane at 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h post administration. Following centrifugation at 1500 g for 2 min to obtain plasma, samples are stored at −20°C until analysis. For urinary excretion studies, NU7026 is administered at 5 mg/kg i.v. Urine is collected over 24 h in metabolic cages, and stored at −20°C until required.

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

CUSTOMER VALIDATION

- Nat Commun. 2020 Dec 3;11(1):6182.
- Mol Cell. 2022 Apr 14:S1097-2765(22)00290-8.
- Nucleic Acids Res. 2023 Jan 18;gkac1269.
- Cell Syst. 2020 Jan 22;10(1):66-81.e11.
- Cell Death Dis. 2022 Apr 25;13(4):404.

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REFERENCES

[1]. Veuger SJ, et al. Radiosensitization and DNA repair inhibition by the combined use of novel inhibitors of DNA-dependent protein kinase and poly(ADP-ribose) polymerase-1. Cancer Res. 2003 Sep 15;63(18):6008-15.

[2]. Amrein L, et al. Chlorambucil cytotoxicity in malignant B lymphocytes is synergistically increased by 2-(morpholin-4-yl)-benzo[h]chomen-4-one (NU7026)-mediated inhibition of DNA double-strand break repair via inhibition of DNA-dependent protein kinase. J

[3]. Nutley BP, et al. Preclinical pharmacokinetics and metabolism of a novel prototype DNA-PK inhibitor NU7026. Br J Cancer. 2005 Oct 31;93(9):1011-8.

[4]. Ciszewski WM, et al. Interleukin-4 enhances PARP-dependent DNA repair activity in vitro. J Interferon Cytokine Res. 2014 Sep;34(9):734-40.

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