KU-55933

Cat. No.: HY-12016

CAS No.: 587871-26-9

Molecular Formula: $C_{21}H_{17}NO_3S_2$

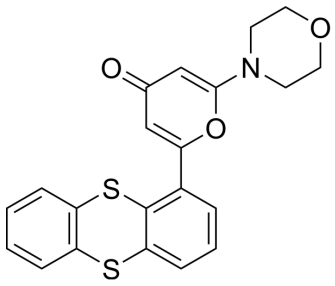
Molecular Weight: 395.49

Target: ATM/ATR; Autophagy

Pathway: Cell Cycle/DNA Damage; PI3K/Akt/mTOR; Autophagy

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 : 50 mg/mL (126.43 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.5285 mL	12.6425 mL	25.2851 mL
5 mM	0.5057 mL	2.5285 mL	5.0570 mL
10 mM	0.2529 mL	1.2643 mL	2.5285 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 (6.32 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (6.32 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.32 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

KU-55933 is a potent ATM inhibitor with an IC_{50} and K_i of 12.9 and 2.2 nM, respectively, and is highly selective for ATM as compared to DNA-PK, PI3K/PI4K, ATR and mTOR.

IC_{50} & Target

ATM	DNA-PK	mTOR	PI3K
12.9 nM (IC_{50})	2500 nM (IC_{50})	9300 nM (IC_{50})	16600 nM (IC_{50})

In Vitro

KU-55933 (10 μM) blocks the ionizing radiation-induced p53 serine 15 phosphorylation. KU-55933 has a dose-dependent

effect in inhibiting this ATM-dependent phosphorylation event with an estimated IC_{50} of 300 nM. KU-55933 ablates the ionizing radiation-induced phosphorylation of these ATM substrates. KU-55933 specifically inhibits ATM but not the other DNA damage-activated PIKKs, ATR, and DNA-PK[1]. KU-55933 induces pATM, p53, E2F1 and pATR, noticeably upregulates the nuclear fraction of E2F1 at the 0.5 h time point[2].

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

PROTOCOL

Kinase Assay [1]

ATM for use in the in vitro assay is obtained by immunoprecipitation with rabbit polyclonal antiserum raised to the COOH-terminal 400 amino acids of ATM in buffer containing 25 mM HEPES (pH 7.4), 2 mM $MgCl_2$, 250 mM KCl, 500 μM EDTA, 100 μM Na_3VO_4, 10% v/v glycerol, and 0.1% v/v Igepal. ATM-antibody complexes are isolated from nuclear extract by incubating with protein A-Sepharose beads for 1 hour and then through centrifugation to recover the beads. In the well of a 96-well plate, ATM-containing Sepharose beads are incubated with 1 μg of substrate glutathione S-transferase-p53N66 (NH_2-terminal 66 amino acids of p53 fused to glutathione S-transferase) in ATM assay buffer [25 mM HEPES (pH 7.4), 75 mM NaCl, 3 mM $MgCl_2$, 2 mM $MnCl_2$, 50 μM Na_3VO_4, 500 μM DTT, and 5% v/v glycerol] at 37°C in the presence or absence of inhibitor. After 10 minutes with gentle shaking, ATP is added to a final concentration of 50 μM and the reaction continued at 37°C for an additional 1 hour. The plate is centrifuged at 250×g for 10 minutes (4°C) to remove the ATM-containing beads, and the supernatant is removed and transferred to a white opaque 96-well plate and incubated at room temperature for 1.5 hours to allow glutathione S-transferase-p53N66 binding. This plate is then washed with PBS, blotted dry, and analyzed by a standard ELISA technique with a phospho-serine 15 p53 antibody. The detection of phosphorylated glutathione S-transferase-p53N66 substrate is performed in combination with a goat antimouse horseradish peroxidase-conjugated secondary antibody. Enhanced chemiluminescence solutionis used to produce a signal and chemiluminescent detection is carried out via a TopCount plate reader.

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

Cell Assay [1]

1BR or AT4 cells are seeded in 10-cm Petri dishes and treated on day 2 (80 to 90% confluence). Cells are preincubated for 1 hour with KU-55933 or vehicle control and then exposed to 5 Gy of ionizing radiation. Time courses of cell cycle distribution are performed, and the optimal time for discrimination of populations is selected as 16 hours. All subsequent experiments are performed at the 16-hour time point. Cells are stained with propidium iodide according to standard protocols and analyzed by FACS with a FACScalibur. Exponentially growing (50-70% confluent) SW620 cells in 60 mm dishes are exposed to KU-55933 or DMSO for 1 h before addition of etoposide (final concentration of 0.1 and 1 μM) for 16 h before harvesting, propidium iodide staining and analysis as above.

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

CUSTOMER VALIDATION

- Cancer Cell. 2021 Apr 12;39(4):566-579.e7.
- Nat Commun. 2019 Aug 21;10(1):3761.
- Mol Cell. 2022 Apr 14:S1097-2765(22)00290-8.
- Nucleic Acids Res. 2023 Jan 18;gkac1269.
- Nucleic Acids Res. 2020 Sep 18;48(16):9109-9123.

See more customer validations on www.MedChemExpress.com

REFERENCES

[1]. Hickson I, et al. Identification and characterization of a novel and specific inhibitor of the ataxia-telangiectasia mutated kinase ATM. Cancer Res. 2004 Dec 15;64(24):9152-9

[2]. Khalil HS, et al. Pharmacological inhibition of ATM by KU55933 stimulates ATM transcription.Exp Biol Med (Maywood). 2012 Jun;237(6):622-34. Epub 2012 Jun 22.

[3]. Kim YD, et al. Orphan nuclear receptor SHP negatively regulates growth hormone-mediated induction of hepatic gluconeogenesis through inhibition of STAT5 transactivation.J Biol Chem. 2012 Sep 12.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA