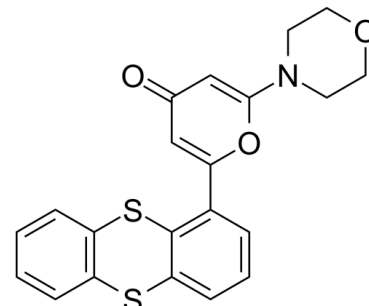


## Data Sheet

<b>Product Name:</b>	KU-55933
<b>Cat. No.:</b>	CS-0146
<b>CAS No.:</b>	587871-26-9
<b>Molecular Formula:</b>	C <sub>21</sub> H <sub>17</sub> NO <sub>3</sub> S <sub>2</sub>
<b>Molecular Weight:</b>	395.49
<b>Target:</b>	ATM/ATR; Autophagy
<b>Pathway:</b>	Autophagy; Cell Cycle/DNA Damage; PI3K/Akt/mTOR
<b>Solubility:</b>	DMSO : 80 mg/mL (202.28 mM; Need ultrasonic)



### BIOLOGICAL ACTIVITY:

KU-55933 is a potent **ATM** inhibitor with an **IC<sub>50</sub>** and **K<sub>i</sub>** 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<sub>50</sub> & Target:** IC<sub>50</sub>: 12.9 nM (ATM)<sup>[1]</sup> **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<sub>50</sub>** 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<sup>[1]</sup>. KU-55933 induces pATM, p53, E2F1 and pATR, noticeably upregulates the nuclear fraction of E2F1 at the 0.5 h time point<sup>[2]</sup>. Metformin increases ATM and AMPK phosphorylation, as well as SHP protein level in primary hepatocytes, and this stimulatory effect of metformin is repressed by a specific ATM kinase inhibitor KU-55933<sup>[3]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** <sup>[1]</sup>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<sub>2</sub>, 250 mM KCl, 500 μM EDTA, 100 μM Na<sub>3</sub>VO<sub>4</sub>, 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<sub>2</sub>-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<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 50 μM Na<sub>3</sub>VO<sub>4</sub>, 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 solution is used to produce a signal and chemiluminescent detection is carried out via a TopCount plate reader. **Cell Assay:** KU-55933 is prepared as 10 mM stock solutions in DMSO.<sup>[1]</sup>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.

## 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.

## CAIndexNames:

4H-Pyran-4-one, 2-(4-morpholinyl)-6-(1-thianthrenyl)-

## SMILES:

O=C1C=C(OC(C2=C3SC4=C(SC3=CC=C2)C=CC=C4)=C1)N5CCOCC5

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

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