Alisertib (MLN8237)

Technical Data

Molecular Weight (MW)	518.92
Formula	$C_{27}H_{20}ClFN_4O_4$
CAS No.	1028486-01-2
Synonyms	N/A
Solubility (25°C) *** <1 mg/ml means slightly soluble or insoluble**	DMSO 27 mg/mL Water <1 mg/mL Ethanol <1 mg/mL
Storage	3 years -20℃Powder 6 months-80℃in DMSO
Chemical Name	Benzoic acid, 4-[[9-chloro-7-(2-fluoro-6-methoxyphenyl)-5H-pyrimido[5,4-d][2]benzazepin-2-yl]amino]-2-methoxy-

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Biological Activity

Description	Alisertib (MLN8237) is a selective **Aurora A** inhibitor with **IC50** of 1.2 nM. It has >200-fold higher selectivity for Aurora A than Aurora B. Phase 3.
Targets	Aurora A
IC50	1.2 nM [1]
In vitro	MLN8237 shows >200-fold higher selectivity for Aurora A than the structurally related Aurora B with an IC50 of 396.5 nM, and does not have any significant activity against 205 other kinases. [1] MLN8237 (0.5 μM) treatment inhibits the phosphorylation of Aurora A in MM1.S and OPM1 cells, without affecting the Aurora B mediated histone H3 phosphorylation. MLN8237 significantly inhibits cell proliferation in multiple myeloma (MM) cell lines with IC50 values of 0.003-1.71 μM. MLN8237 displays more potent anti-proliferation activity against primary MM cells and MM cell lines in the presence of BM stroma cells, as well as IL-6 and IGF-1 than against MM cells alone. MLN8237 (0.5 μM) induces 2- to 6-fold increase in G2/M phase in primary MM cells and cell lines, as well as significant apoptosis and senescence, involving the up-regulation of p53, p21 and p27, as well as PARP, caspase 3, and caspase 9 cleavage. In addition, MLN8237 shows strong synergistic anti-MM effect with dexamethasone, as well as additive effect with doxorubicin and bortezomib. [2] MLN8237 (0.5 μM) treatment causes the inhibition of colony formation of FLO-1, OE19, and OE33 esophageal adenocarinoma cell lines, and induces a significant increase in the percentage of polyploid cells, and subsequently an increase in the percentage of cells in the sub-G1 phase, which can be further enhanced in combination with cisplatin (2.5 μM), involving the higher induction of TAp73β, PUMA, NOXA, cleaved caspase-3, and cleaved PARP as compared with a single-agent treatment. [3]
In vivo	MLN8237 significantly reduces the tumor burden with tumor growth inhibition (TGI) of 42% and 80% at 15 mg/kg and 30 mg/kg, respectively, and prolongs the survival of mice compared with the control. [2]

Features	First orally available inhibitor of Aurora A.

Protocol (Only for Reference)

Kinase Assay: [1]

Aurora A radioactive Flashplate enzyme assay	Aurora A radioactive Flashplate enzyme assay is conducted to determine the nature and degree of MLN8237-mediated inhibition in vitro. Recombinant Aurora A is expressed in Sf9 cells and purified with GST affinity chromatography. The peptide substrate for Aurora A is conjugated with biotin (Biotin-GLRRASLG). Aurora A kinase (5 nM) is assayed in 50 mM Hepes (pH 7.5), 10 mM $MgCl_2$, 5 mM DTT, 0.05% Tween 20, 2 μM peptide substrate, 3.3 μCi/mL [γ-^{33}P]ATP at 2 μM, and increasing concentrations of MLN8237 by using Image FlashPlates.

Cell Assay: [2]

Cell lines	MM1.S, MM.1R, LR5, RPMI 8226, DOX40, OPM1, OPM2, INA6, and U266
Concentrations	Dissolved in DMSO, final concentrations ~10 μM
Incubation Time	24, 48, and 72 hours
Method	Cells are exposed to various concentrations of MLN8237 for 24, 48, and 72 hours. Cells viability is measured using MTT assay, and cell proliferation is measured using ^{3}[H]-thymidine incorporation. For cell cycle analysis, cells are permeabilized by 70% ethanol at -20 °C, and incubated with 50 μg/mL PI and 20 units/mL RNase-A. DNA content is analyzed by flow cytometry using BDFACS-Canto II and FlowJo software. For the detection of apoptosis and senescence, cells are stained with fluorescein isothiocyanate-annexin V and PI. Apoptotic cells are determined by flow cytometric analysis using BDFACS-Canto II and FlowJo software.

Animal Study: [2]

Animal Models	Severe combined immune-deficient (SCID) mice inoculated subcutaneously with MM1.S cells
Formulation	Formulated in 10% 2-hydroxypropyl-β-cyclodextrin/1% sodium bicarbonate
Dosages	~30 mg/kg/day
Administration	Orally

1

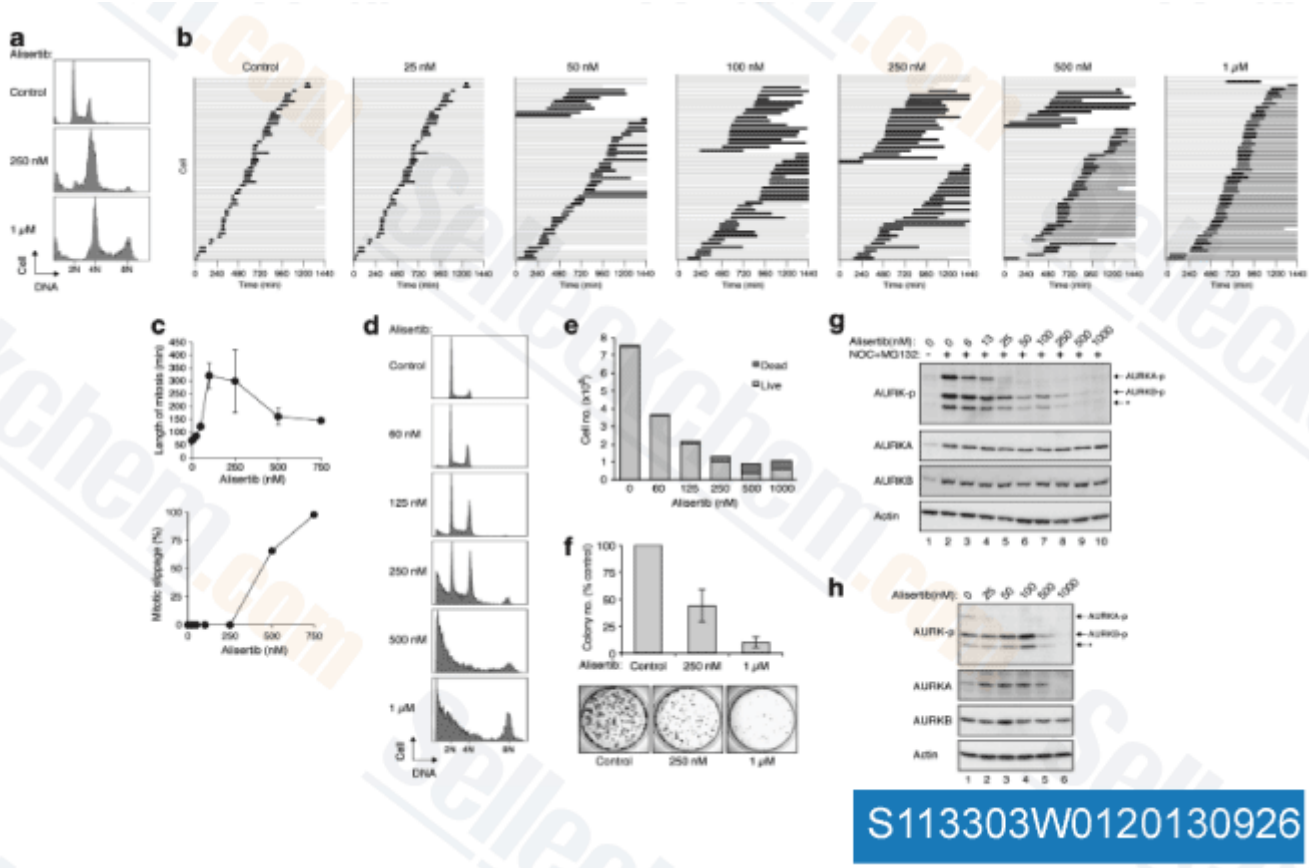
References

[1] Manfredi MG, et al. Clin Cancer Res, 2011, 17(24), 7614-7624.

[2] G?rgün G, et al. Blood, 2010, 115(25), 5202-521

[3] Sehdev V, et al. Mol Cancer Ther, 2012, 11(3), 763-774.

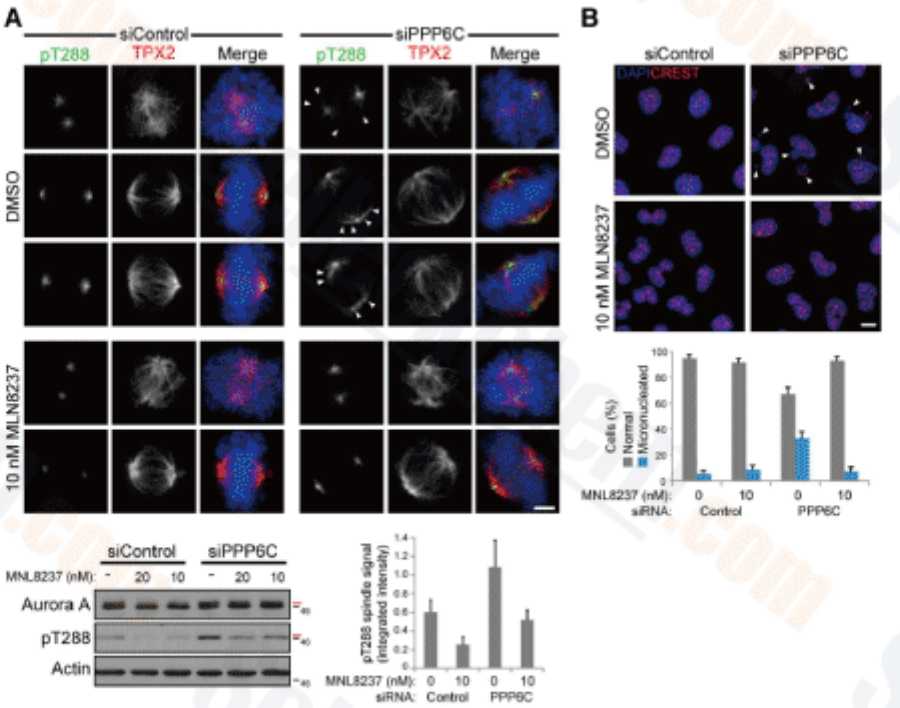
Customer Reviews



, , Oncogene. 2013 Aug 19
Alisertib (MLN8237) purchased from **Selleck**

Alisertib inhibits AURKA and AURKB in a concentration-dependent manner. (a) Alisertib induces G 2 /M delay or genome reduplication. HeLa cells were exposed to buffer or the indicated concentrations of Alisertib. After 24 h, the cells were harvested and analyzed with flow cytometry. The positions of 2N, 4N and 8N DNA contents are indicated. (b) Alisertib delays mitotic exit or induces slippage. HeLa cells stably expressing histone H2B-GFP were exposed to buffer or the indicated concentrations of Alisertib. Individual cells were then tracked for 24 h with time-lapse microscopy. Each horizontal bar represents one cell (n ¼ 50). Key: light gray ¼ interphase; black ¼ mitosis (from DNA condensation to anaphase or mitotic slippage); dark gray ¼ interphase after mitotic slippage; truncated bars ¼ cell death. (c) Different concentrations of Alisertib are involved in delaying mitotic exit and inducing slippage. Live-cell imaging of cells treated with Alisertib was described in panel (b). The duration of mitosis (mean±90% confidence interval) and the percentage of cells that underwent mitotic slippage during the imaging period was quantified. (d) Alisertib promotes apoptosis in a concentration-dependent manner. HeLa cells were incubated with the indicated concentrations of Alisertib for 48 h. The cells were then harvested and analyzed with flow cytometry. (e) Concentration-dependent cytotoxicity of Alisertib. HeLa cells were cultured in the presence of the indicated concentrations of Alisertib for 48 h. The number of live and dead cells was analyzed with trypan blue exclusion assay. (f) Concentration-dependent suppression of long-term survival by Alisertib. HeLa cells were seeded on 60-mm culture plates and grown in the presence of 250 n M or 1 m M of Alisertib. After 24 h, the cells were washed gently and propagated in normal medium for another 10–12 days. Colonies were fixed and

stained with crystal violet solution (examples of the plates are shown). Average±s.d. from three independent experiments. (g) Both AURKA and AURKB are inhibited by Alisertib.Mitotic HeLa cells were obtained by exposure to nocodazole for 16 h followed by mechanical shake off. The cells were incubated with the indicated concentrations of Alisertib for 2 h. Lysates were then prepared and activated phospho-AURKAThr288 and AURKBThr232were detected with immunoblotting. The asterisk indicates the position of an AURKB-like protein (the same throughout this study). Uniform loading was confirmed by immunoblotting for actin. In this assay, nocodazole and MG132 (a proteasome inhibitor) were added to prevent the cells from exiting mitosis. Accordingly, the total AURKA and AURKB levels remained constant throughout the experiment. (h) Alisertib prevents activation of AURKA and AURKB. HeLa cells were incubated with the indicated concentrations of Alisertib for 8 h. Nocodazole was then added for another 6 h to trap cells that entered mitosis. Lysates were prepared and analyzed with immunoblotting. Actin analysis was included to assess loading and transfer.



, , J Cell Biol, 2010, 191(7), 1315-32
Alisertib (MLN8237) purchased from **Selleck**

Aurora A inhibition rescues the PPP6C depletion phenotype. (A) HeLa cells transfected for 48 h with control and PPP6C si08 duplexes were treated with 10 or 20 nM MLN8237 or a solvent control for 15 min before lysis in phosphatase inhibitor containing buffer or fixation. Total lysates were analyzed by Western blotting. The red and black lines indicate the hosphorylated and nonphosphorylated forms of Aurora A. Fixed cells were stained using DAPI to detect DNA and antibodies to α-tubulin and Aurora A pT288. The intensity of pT288 staining was integrated using ImageJ over

the spindle region defined by TPX2 staining and is plotted in the bar graph (n = 4). Arrowheads indicate micronuclei. Bar, 5 µm. (B) HeLa cells transfected for 48 h with control and PPP6C si08 duplexes were treated with 10 nM MLN8237 or a solvent control for 24 h before fixation and staining with DAPI to detect DNA.

Alisertib (MLN8237) has been referenced in 26 publications.

- Regulation of Embryonic and Induced Pluripotency by Aurora Kinase-p53 Signaling. [Lee DF, et al. Cell Stem Cell, 2012, 11(2):179-94]

  PubMed: 22862944

- Protein phosphatase 6 regulates mitotic spindle formation by controlling the T-loop phosphorylation state of Aurora A bound to its activator TPX2. [Zeng K, et al. J Cell Biol, 2010, 191(7):1315-32]

  PubMed: 21187329

- Early adipogenesis is regulated through USP7-mediated deubiquitination of the histoneacetyltransferase TIP60. [Gao Y, et al. Nat Commun, 2013, 4:2656]

  PubMed: 24141283

- A TACC3/ch-TOG/clathrin complex stabilises kinetochore fibres by inter-microtubule bridging. [Booth DG, et al. EMBO J, 2011, 30(5):906-19]

  PubMed: 21297582

- Targeting Sonic Hedgehog-Associated Medulloblastoma through Inhibition of Aurora and Polo-like Kinases. [Markant SL, et al. Cancer Res, 2013, 73(20):6310-22]

  PubMed: 24067506

- Targeting Aurora Kinase A Inhibits Hypoxia-mediated Neuroblastoma Cell Tumorigenesis. [Romain CV, et al. Clin Cancer Res, 2014, 34(5):2269-74]

  PubMed: 24778030

- p53 deficiency enhances mitotic arrest and slippage induced by pharmacological inhibition of Aurora kinases. [Marxer M, et al. Oncogene, 2014, 10.1038/onc.2013.325]

  PubMed: 23955083

- Uncovering new substrates for Aurora A kinase. [Sardon T, et al. EMBO reports, 2010, 11(12):977-84]

  PubMed: 21072059

- DNA replication stress in CHK1-depleted tumour cells triggers premature (S-phase) mitosis through inappropriate activation of Aurora kinase B. [Zuazua-Villar P, et al. Cell Death Dis, 2014, 10.1038/cddis.2014.231]

  PubMed: 24853431

- PCM1 Recruits Plk1 to Pericentriolar Matrix to Promote Primary Cilia Disassembly before Mitotic Entry. [Wang G, et al. J Cell Sci, 2013, 126(Pt 6):1355-65]

  PubMed: 23345402

- Drug-resistant Aurora A mutants for cellular target validation of the small molecule kinase inhibitors MLN8054 and MLN8237. [Sloane D, et al. ACS Chem Biol, 2010, 5(6):563-76]

  PubMed: 20426425

- CH-01 is a hypoxia-activated prodrug that sensitizes cells to hypoxia/reoxygenation through inhibition of Chk1 and Aurora A. [Cazares-Körner C, et al. ACS Chem Biol, 2013, 8(7):1451-9]

  PubMed: 23597309

- A functional cooperativity between Aurora A kinase and LIM kinase1: Implication in the mitotic process. [Ritchey L, et al. Cell Cycle, 2012, 11(2):296-309]

  PubMed: 22214762

- Tetraploidization increases sensitivity to Aurora B kinase inhibition. [Marxer M, et al. Cell Cycle, 2012, 11(13):2567-77]

  PubMed: 22722494

- Aurora-A inactivation causes mitotic spindle pole fragmentation by unbalancing microtubule-generated forces. [Asteriti IA, et al. Mol Cancer, 2011, 10:131]

  PubMed: 22011530

- Prometaphase arrest-dependent phosphorylation of Bcl-
  2 family proteinsand activation of mitochondrial apoptotic pathway are associated wit
  h 17α-estradiol-induced apoptosis in human Jurkat T cells. [Han CR, et al. Biochim Biophys Acta, 2013, 1833(10):2220-32]

  PubMed: 23707954

- The centrosomal adaptor TACC3 and the microtubule polymerase chTOG interact via defined C-terminal subdomains in an Aurora-A kinase independent manner. [Thakur HC, et al. J Biol Chem, 2013, 289(1):74-88]

  PubMed: 24273164

- Furry Protein Promotes Aurora A-mediated Polo-like Kinase 1 Activation. [Ikeda M, et al. J Biol Chem, 2012, 287(33):27670-81]

  PubMed: 22753416

- Co-treatment with vorinostat synergistically enhances activity of Aurora kinase inhibitor against human breast cancer cells. [Fiskus W, et al. Breast Cancer Res Treat, 2012, 135(2):433-44]

  PubMed: 22825030

- Multimodal Effects of Small Molecule ROCK and LIMK Inhibitors on Mitosis, and Their Implication as Anti-Leukemia Agents. [Oku Y, et al. PLoS One, 2014, 9(3):e92402]

  PubMed: 24642638

- The Cytoskeletal Protein RHAMM and ERK1/2 Activity Maintain the Pluripotency of Murine Embryonic Stem Cells. [Jiang J, et al. PLoS One, 2013, 8(9):e73548]

  PubMed: 24019927

- Evolution of Resistance to Aurora Kinase B Inhibitors in Leukaemia Cells. [Failes TW, et al. PLoS One, 2012, 7(2):e30734]

  PubMed: 22359551

- Phosphorylation of multifunctional nucleolar protein nucleophosmin (NPM1) by aurora kinase B is critical for mitotic progression. [Shandilya J, et al. FEBS Lett, 2014, 10.1016/j.febslet.2014.05.014]

  PubMed: 24857377

- Targeting Aurora kinase A suppresses the growth of human oral squamous cell carcinoma cells in vitro and in vivo. [Tanaka H, et al. Oral Oncol, 2013, 49(6):551-9]

  PubMed: 23481312

- Emerging drugs for high-grade osteosarcoma. [Hattinger CM, et al. Expert Opin Emerg Drugs, 2010, 15(4):615-34]

  PubMed: 20690888

- CDKN1A-mediated Responsiveness of MLL-AF4-positive Acute Lymphoblastic Leukemia to Aurora Kinase-A Inhibitors. [Chen YP ,et al. Int J Cancer, 2013, 10.1002/ijc.28708]

  PubMed: 24382688

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