

Product Data Sheet

Product Name: CP-466722

Cat. No.: GC16489

Chemical Properties

Cas. No. 1080622-86-1

Chemical Name 2-(6,7-dimethoxyquinazolin-4-yl)-5-pyridin-2-yl-1,2,4-triazol-3-amine

SMILES COC1=C(C=C2C(=C1)C(=NC=N2)N3C(=NC(=N3)C4=CC=CC=N4)N)OC

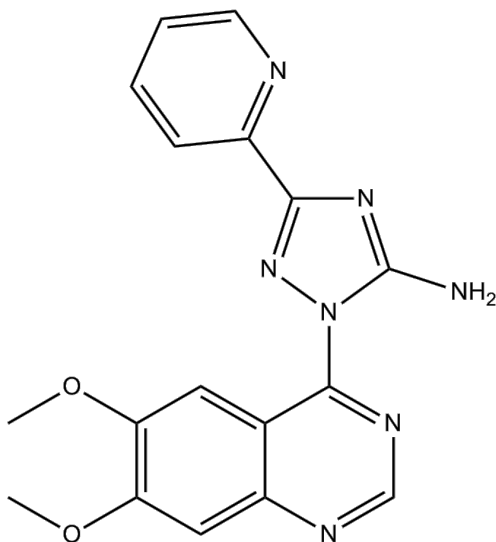
Formula $C_{17}H_{15}N_7O_2$ M.Wt 349.35

Solubility $\geq 4.36\text{mg/mL}$ in DMSO with ultrasonic and warming Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

**Kinase
experiment:**

To screen for small molecule inhibitors of ATM kinase activity, an in vitro kinase assay is carried out, and an ELISA assay develops which measures the phosphorylation status of the ATM downstream target p53. Recombinant GST-p53(1-101) and full-length Flag-tagged ATM & ATR are purified for use in the ELISA and in vitro kinase assays. Briefly, Nunc 96 well Maxisorp plates are coated overnight (4°C) with 2 µg of purified, recombinant GST-p53(1-101) in PBS. All subsequent incubations are performed at room temperature. The plates are washed (0.05% v/v-Tween/PBS) before addition of purified recombinant full-length ATM kinase (30-60 ng) in a final volume of 80 µL of reaction buffer (20 mM HEPES, 50 mM NaCl, 10 mM MgCl₂, 10 mM MnCl₂, 1 mM DTT and 1 µM ATP) in the presence or absence of compound. Compounds including CP-466722 (10 µM) are added to plates in duplicate and the kinase assay is incubated (90 min). Plates are washed (0.05% v/v-Tween/PBS), blocked (1 h, 1% w/v-BSA/PBS) and rinsed before anti-Phospho(Ser15)-p53 antibody (1:1000/PBS) is added to the plates and incubated (1 h). To reduce non-specific binding plates are washed (0.05% v/v-Tween/PBS) prior to incubation (1 h) with HRP-conjugated goat anti-rabbit IgG secondary antibody (1:5000/PBS). Secondary antibody that is linked to the phosphorylated GST-p53(1-101) protein is detected with TMB substrate reagent. Plates are developed (15-30 min) and the reaction is stopped (1M H₂SO₄ final concentration) before absorbance is determined (λ450 nm). Compounds that inhibit ATM kinase activity in ELISA assays, are characterized with respect to inhibition of ATM/ATR kinases using in vitro kinase assays. Western blotting using the anti-Phospho(Ser15)-p53 antibody is used as a readout of ATM/ATR inhibition[1].

Cell experiment: Cells are plated in triplicate (40,000 cells/plate), incubated as required before culture media and trypsinized cells are combined and viability determined: Vi-CELL™ XR cell viability analyzer[1].

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

References:

- [1]. Rainey MD, et al. Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. *Cancer Res.* 2008 Sep 15;68(18):7466-74.
- [2]. Guo K, et al. Development of a cell-based, high-throughput screening assay for ATM kinase inhibitors. *J Biomol Screen.* 2014 Apr;19(4):538-46.
- [3]. Węsierska-Gądek J, et al. Interactions Between Ataxia Telangiectasia Mutated Kinase Inhibition, Poly(ADP-ribose) Polymerase-1 Inhibition and BRCA1 Status in Breast Cancer Cells. *J Cancer Prev.* 2014 Jun;19(2):125-36.

Background

CP-466722 is a selective inhibitor of ATM kinase with IC₅₀ value of 0.41 μ M [1].

ATM (ataxia-telangiectasia, mutated) is a serine/threonine protein kinase and plays an important role in the cellular responses to DNA double-strand breaks (DSBs) [2] [3].

CP-466722 is a potent ATM inhibitor and is regarded as a promising drug to increase tumor cells sensitivity to IR. When tested with MCF7 cells, CP-466722 (10 μ M) showed inhibition on the pATM and pKAP1 signals which induced by etoposide pre-treatment (25 μ M) [1]. In GBM 12 cells, CP-466722 treatment significantly increased the cell sensitivity to TMZ and increased the cell apoptosis which had no effect on the TMZ-resistant GBM12 TMZ cells which indicated that CP-466722 (as an ATM inhibitor) may enhance the efficacy of TMZ in tumors that were inherently sensitive to TMZ [2]. When tested with Hela, MCF-7 and mouse cells pre-treated with IR which induced the increase in ATM-dependent phosphorylation events and then CP-466722 treatment resulted in the disruption of ATM-dependent phosphorylation events and inhibition of ATM-dependent p53 induction at the minimal dose of 6 μ M. Further, it was shown that CP-466722 treatment disrupted ATM-dependent cell cycle in increasing the percent in G2/M phase while decreasing the proportion in G1-phase without adverse effects [3].

References:

- [1]. Guo, K., et al., Development of a cell-based, high-throughput screening assay for ATM kinase inhibitors. *J Biomol Screen*, 2014. 19(4): p. 538-46.
- [2]. Nadkarni, A., et al., ATM inhibitor KU-55933 increases the TMZ responsiveness of only inherently TMZ sensitive GBM cells. *J Neurooncol*, 2012. 110(3): p. 349-57.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

[3]. Rainey, M.D., et al., Transient inhibition of ATM kinase is sufficient to enhance cellular sensitivity to ionizing radiation. Cancer Res, 2008. 68(18): p. 7466-74.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA