

Product Data Sheet

Product Name: PIK-75
Cat. No.: GC16904

Chemical Properties

Cas. No. 372196-77-5

Chemical Name N-[(E)-(6-bromoimidazo[1,2-a]pyridin-3-yl)methylideneamino]-N,2-dimethyl-5-nitrobenzenesulfonamide;hydrochloride

SMILES CC1=C(C=C(C=C1)[N+](=O)[O-])S(=O)(=O)N(C)N=CC2=CN=C3N2C=C(C=C3)Br.Cl

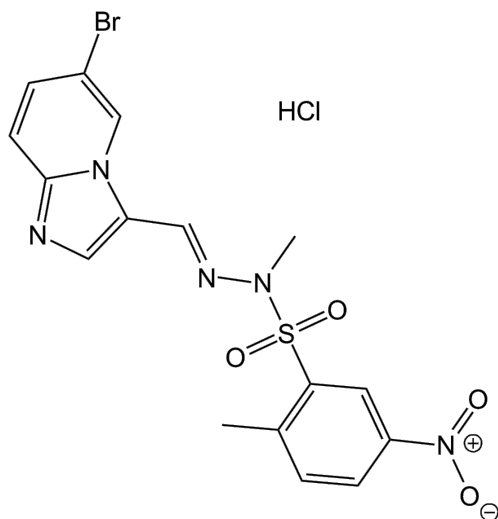
Formula $C_{16}H_{14}BrN_5O_4S.HCl$ M.Wt 488.74

Solubility $\geq 8.15\text{mg/mL}$ in DMSO with gentle 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:**

PI3K enzyme activity is determined in 50 μ L of 20 mM HEPES, pH 7.5, and 5 mM $MgCl_2$ containing 180 μ M phosphatidyl inositol, with the reaction starts by the addition of 100 μ M ATP (containing 2.5 μ Ci of [γ - ^{32}P]ATP). After a 30-min incubation at room temperature, the enzyme reaction is stopped by the addition of 50 μ L of 1 M HCl. Phospholipids are then extracted with 100 μ L of chloroform/methanol [1:1 (v/v)] and 250 μ L of 2 M KCl followed by liquid scintillation counting. Inhibitors (e.g., PIK75)) are diluted in 20% (v/v) DMSO to generate a concentration versus inhibition of enzyme activity curve, which is then analyzed with the use of Prism version 5.00 for Windows to calculate the IC_{50} [2].

Cell experiment:

MIA PaCa-2 cells are maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (HI-FBS), 2.5% horse serum (HS) and 100 U/mL Penicillin/Streptomycin. AsPC-1 cells are cultured in RPMI-1640 media supplemented with 20% HI-FBS, 100 U/mL Penicillin/Streptomycin and 1 mM sodium pyruvate. A total of 2,000 human pancreatic cancer cells (MIA PaCa-2 or AsPC-1) per well are plated in 96-well flat-bottom plates and then treated with either Gemcitabine, PIK-75 alone (0.1 μ M, 0.3 μ M and 1 μ M) or in combination of both drugs with indicated concentrations. At the indicated times, 20 μ L of 1 mg/mL MTT in PBS is added to each well and further incubated for ~4 h. After centrifugation and removal of the medium, 150 μ L of DMSO is added to each well to dissolve the formazan crystals. The absorbance is measured at 562 nm using an ELx808 absorbance microplate reader. Absorbance of untreated cells is designated as 100%, and the relative viable cells are expressed as a percentage of this value[3]

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

**Animal
experiment:**

Mice[3]MIA PaCa-2 cells ($\sim 1.7 \times 10^6$ cells/mouse) mixed with Matrigel are injected subcutaneously into the flank of male athymic nude (Foxn1nu) mice aged 6-weeks. Gemcitabine (50 mg/mL) is dissolved in PBS and PIK-75 (20 mg/mL) is dissolved in DMSO. Injection solution is made as 10% of Cremophor EL and 3% of poly(ethylene glycol) 400 in sterile water. Before administration of compounds, Gemcitabine is further diluted in PBS and DMSO or PIK-75 is further diluted in the injection solution and sterilized by 0.2 μ m filter unit. These diluents are mixed with 1:1 ratio and administered into peritoneal cavity of the mouse. Gemcitabine (20 mg/kg) or Gemcitabine (20 mg/kg)/PIK-75 (2 mg/kg) combination is administered twice per week and vehicle control and PIK-75 (2 mg/kg) are administered 5 times per week. The body weights and tumor sizes are measured 3 times per week. Tumor volumes are calculated.

References:

- [1]. Knight ZA, et al. A pharmacological map of the PI3-K family defines a role for p110 α in insulin signaling. *Cell*. 2006 May 19;125(4):733-47.
- [2]. Duong HQ, et al. Inhibition of NRF2 by PIK-75 augments sensitivity of pancreatic cancer cells to gemcitabine. *Int J Oncol*. 2014 Mar;44(3):959-69.
- [3]. Zheng Z, et al. Isoform-selective inhibition of phosphoinositide 3-kinase: identification of a new region of nonconserved amino acids critical for p110 α inhibition. *Mol Pharmacol*. 2011 Oct;80(4):657-64.

Background

PIK-75 is a specific inhibitor of p110 α isoform of PI3K with IC₅₀ of 5.8 nM [1].

In acute myeloid leukemia cells, PIK-75 targeted the p110 α isoform of PI3K, which led to a loss of connection between Bcl-xL and Bak. PIK-75 also transiently decreased Cdk7/9, leading to loss of Mcl-1 protein, and alleviation of its inhibition of pro-apoptotic Bak. The simultaneous loss of Bcl-xL and Mcl-1 led to rapid apoptosis. [2] In human monocyte-endothelial cell, PIK-75 suppressed the events of downstream signaling, including AKT phosphorylation, IKK activation, and NF- κ B transcription. PIK-75 inhibited in vitro and in vivo production of TNF- α and IL-6, decreased the E-

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

selectin, ICAM-1, and VCAM-1 expression, and blocked cell adhesion [3]. In human smooth muscle cells, PIK-75 decreased TNF- α -induced CD38 expression in asthmatic and nonasthmatic cells [4]. In pancreatic β -cells, acute treatment with PIK-75 enhanced the glucose-induced insulin secretion [5].

In vivo, PIK-75 significantly suppressed the histological abnormalities associated with dextran sulfate sodium-induced murine colitis. PIK-75 can attenuate experimental inflammation in the colon [3].

References:

- [1]. Zheng Z, Amran SI, Thompson PE, Jennings IG. Isoform-selective inhibition of phosphoinositide 3-kinase: identification of a new region of nonconserved amino acids critical for p110 α inhibition. *Mol Pharmacol*. 2011 Oct;80(4):657-64.
- [2]. Thomas D, Powell JA, Vergez F, Segal DH, Nguyen NY, Baker A, Teh TC, Barry EF, Sarry JE, Lee EM, Nero TL, Jabbour AM, Pomilio G, Green BD, Manenti S, Glaser SP, Parker MW, Lopez AF, Ekert PG, Lock RB, Huang DC, Nilsson SK, Récher C, Wei AH, Guthridge MA. Targeting acute myeloid leukemia by dual inhibition of PI3K signaling and Cdk9-mediated Mcl-1 transcription. *Blood*. 2013 Aug 1;122(5):738-48.
- [3]. Dagia NM, Agarwal G, Kamath DV, Chetrapal-Kunwar A, Gupte RD, Jadhav MG, Dadarkar SS, Trivedi J, Kulkarni-Almeida AA, Kharas F, Fonseca LC, Kumar S, Bhonde MR. A preferential p110 α /gamma PI3K inhibitor attenuates experimental inflammation by suppressing the production of proinflammatory mediators in a NF-kappaB-dependent manner. *Am J Physiol Cell Physiol*. 2010 Apr;298(4):C929-41.
- [4]. Jude JA, Tirumurugan KG, Kang BN, Panettieri RA, Walseth TF, Kannan MS. Regulation of CD38 expression in human airway smooth muscle cells: role of class I phosphatidylinositol 3 kinases. *Am J Respir Cell Mol Biol*. 2012 Oct;47(4):427-35.
- [5]. Aoyagi K, Ohara-Imaizumi M, Nishiwaki C, Nakamichi Y, Ueki K, Kadowaki T, Nagamatsu S. Acute inhibition of PI3K-PDK1-Akt pathway potentiates insulin secretion through upregulation of newcomer granule fusions in pancreatic β -cells. *PLoS One*. 2012;7(10):e47381.

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