

Product Data Sheet

Product Name: PKR-IN-C16

Cat. No.: GC17925

Chemical Properties

Cas. No. 608512-97-6

Chemical Name 6,8-dihydro-8-(1H-imidazol-5-ylmethylene)-7H-pyrrolo[2,3-g]benzothiazol-7-one

SMILES O=C1NC2=CC=C3C(SC=N3)=C2/C1=C/C4=CNC=N4

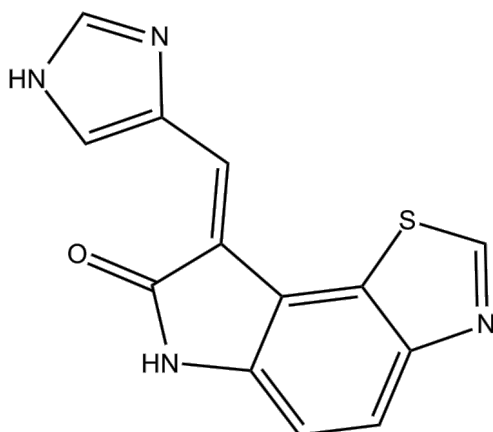
Formula $C_{13}H_8N_4OS$ M.Wt 268.3

Solubility $\leq 2.5\text{mg/ml}$ in DMSO; 0.5mg/ml in dimethyl formamide 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

Cell experiment [1]:

Cell lines HCT116 cells

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Product Data Sheet

HCT116 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin. The cells were cultured at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air. The medium was replaced three times per week. Cell viability in vitro was determined by MTS assay. The HCT116 cells treated with various concentrations (0, 0.1, 0.5, 1 and 2μM) of PKR-IN-C16 were seeded into 96-well plates at a density of 2×10^3 cells per well. The entire experiment lasted for 3 days. At each time point, the cells were incubated with MTS reagent and then further incubated for 120min. The absorbance was measured at 450nm. The cells treated with DMSO were used as the controls. The wells were imaged by a microscope.

Reaction Conditions 0, 0.1, 0.5, 1 and 2μM; 3 days

Applications PKR-IN-C16 inhibited the proliferation of HCT116 cells in vitro in a dose-dependent manner.

Animal experiment [2]:

Animal models BALB/c-nu/nu mice

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Preparation Method For tumor xenograft analysis, Huh7 cells in the logarithmic phase in vitro were trypsinized, collected, washed, and resuspended in Dulbecco's modified Eagle's medium with matrix gel for tumor implantation. Female 5-week-old BALB/c-nu/nu mice were used. Subsequently, 3×10^6 Huh7 cells were injected subcutaneously into the right flank of nude mice. After successful implantation (about 10mm in diameter), the mice were randomized into two groups: control (n=6) and PKR-IN-C16 injection (n=6). The PKR-IN-C16 group was injected i.p. every day for 4 weeks with the PKR-IN-C16 (300µg/kg), and the control group was injected i.p. with phosphate-buffered saline. For tumor growth analysis, the tumor size was measured every day using a sliding caliper, and the tumor volume was calculated as $(\text{longest diameter}) \times (\text{shortest diameter})^2 / 2$. Four weeks after transplantation, the mice were sacrificed using CO₂, and the xenograft tumors were collected for immunohistochemical staining.

Dosage form 300µg/kg/day for 4 weeks; i.p.

Applications PKR-IN-C16 treatment led to a slow growth rate of tumors in mice, a reduction in tumor volume, decreased expression of CD31, and inhibition of angiogenesis.

References:

- [1] Hashimoto Y, Tokumoto Y, Watanabe T, et al. C16, a PKR inhibitor, suppresses cell proliferation by regulating the cell cycle via p21 in colorectal cancer[J]. Scientific reports, 2024, 14(1): 9029.
[2] Watanabe T, Ninomiya H, Saitou T, et al. Therapeutic effects of the PKR inhibitor C16 suppressing tumor proliferation and angiogenesis in hepatocellular carcinoma in vitro and in vivo[J]. Scientific reports, 2020, 10(1): 5133.

Background

PKR-IN-C16 is an ATP-binding site-directed small molecule that inhibits RNA-dependent protein kinase (PKR) autophosphorylation, with an IC₅₀ value of $0.21 \pm 0.04 \mu\text{M}$ ^[1]. PKR-IN-C16 has been widely used in the research of neuroprotection for brain injuries, as well as in the preparation of new

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mRNA vaccine formulations to enhance the translation and expression of mRNA^[2-3].

In vitro, PKR-IN-C16 treatment at 0.5μM for 24 hours can significantly reduce the phosphorylation of eIF2α in DYT-*PRKRA* lymphoblasts and inhibit cell apoptosis^[4]. PKR-IN-C16 treatment (6μM; 2.5h) significantly stimulated the integrated stress response (ISR) in HeLa cells, accompanied by the enhanced expression of PERK and GCN2 proteins^[5]. Treatment with 2μM PKR-IN-C16 for 7 days significantly inhibited the proliferation of HCT116 cells and reduced cell division^[6].

In vivo, PKR-IN-C16 treatment (300μg/kg/day; i.p.) reduced the tumor growth rate, decreased tumor volume, inhibited CD31 expression, and suppressed angiogenesis in the mouse xenograft tumor model^[7]. A day after injecting PKR-IN-C16 into the abdominal cavity at a dose of 100μg/kg, the volume of the infarct after ischemia and the apoptosis rate were notably decreased in the hypoxic-ischemic rat model^[8]. Intraperitoneal injection of 600μg/kg dose of PKR-IN-C16 within 24 hours in the model of toxic neuroinflammation in rats can prevent PKR-induced neuronal loss and reduce the inflammatory response^[9].

References:

- [1] Jammi N V, Whitby L R, Beal P A. Small molecule inhibitors of the RNA-dependent protein kinase[J]. Biochemical and biophysical research communications, 2003, 308(1): 50-57.
- [2] Couturier J, Paccalin M, Lafay-Chebassier C, et al. Pharmacological inhibition of PKR in APP^{swe}PS1^{dE9} mice transiently prevents inflammation at 12 months of age but increases Aβ₄₂ levels in the late stages of the Alzheimer's disease[J]. Current Alzheimer research, 2012, 9(3): 344-360.
- [3] Zhang W, Liu Y, Chin J M, et al. Sustained release of PKR inhibitor C16 from mesoporous silica nanoparticles significantly enhances mRNA translation and anti-tumor vaccination[J]. European Journal of Pharmaceutics and Biopharmaceutics, 2021, 163: 179-187.
- [4] Frederick K, Patel R C. Luteolin protects DYT-PRKRA cells from apoptosis by suppressing PKR activation[J]. Frontiers in Pharmacology, 2023, 14: 1118725.
- [5] Szaruga M, Janssen D A, de Miguel C, et al. Activation of the integrated stress response by inhibitors of its kinases[J]. Nature communications, 2023, 14(1): 5535.
- [6] Hashimoto Y, Tokumoto Y, Watanabe T, et al. C16, a PKR inhibitor, suppresses cell proliferation by regulating the cell cycle via p21 in colorectal cancer[J]. Scientific reports, 2024, 14(1): 9029.
- [7] Watanabe T, Ninomiya H, Saitou T, et al. Therapeutic effects of the PKR inhibitor C16

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suppressing tumor proliferation and angiogenesis in hepatocellular carcinoma in vitro and in vivo[J]. Scientific reports, 2020, 10(1): 5133.

[8] Xiao J, Tan Y, Li Y, et al. The specific protein kinase R (PKR) inhibitor C16 protects neonatal hypoxia-ischemia brain damages by inhibiting neuroinflammation in a neonatal rat model[J]. Medical science monitor: international medical journal of experimental and clinical research, 2016, 22: 5074.

[9] Tronel C, Page G, Bodard S, et al. The specific PKR inhibitor C16 prevents apoptosis and IL-1 β production in an acute excitotoxic rat model with a neuroinflammatory component[J]. Neurochemistry international, 2014, 64: 73-83.

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