

Product Data Sheet

Product Name: RKI-1447

Cat. No.: GC15437

Chemical Properties

Cas. No. 1342278-01-6

Chemical Name 1-[(3-hydroxyphenyl)methyl]-3-(4-pyridin-4-yl-1,3-thiazol-2-yl)urea

SMILES C1=CC(=CC(=C1)O)CNC(=O)NC2=NC(=CS2)C3=CC=NC=C3

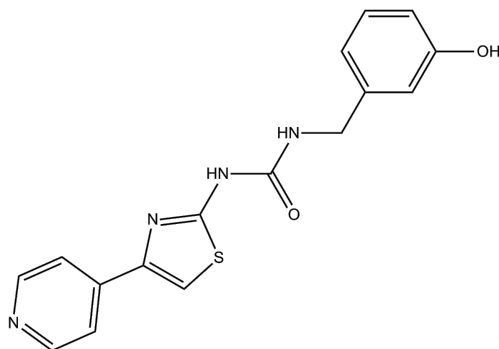
Formula $C_{16}H_{14}N_4O_2S$ M.Wt 326.37

Solubility ≥ 16.3 mg/mL in DMSO, ≥ 6.32 mg/mL in EtOH 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.

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**Kinase
experiment:**

Compounds are tested on three separate days with 8 point dilutions performed in duplicate to determine average IC₅₀ values. The assay conditions are optimized to 15 μ L of kinase reaction volume with 5 ng of enzyme in 50 mM HEPES (pH 7.5), 10 mM MgCl₂, 1 mM EGTA, and 0.01% Brij-35. The reaction is incubated for 1 h at room temperature in the presence of 1.5 μ M of peptide substrate with 12.5 μ M of ATP (for ROCK1) or 2 μ M of substrate with 50 μ M of ATP (for ROCK2). The reaction is then stopped and the ratio of phosphorylated to unphosphorylated peptides is determined by selective cleavage of only the unphosphorylated peptide. The ratio of the signals at 445 nm and 520 nm is measured. IC₅₀ values are determined using fitted curves with GraphPad Prism 5 software[1].

Cell experiment:

NIH 3T3 cells are plated at 8000 cells/well in 8-chamber slides in serum free media for 24 hours, and treated with vehicle, 1 μ M RKI-1447 or 1 μ M RKI-1313 for 1 hour. The cells are then stimulated with complete media, 10 μ M LPA, 200 ng/mL bradykinin or 30 ng/mL PDGF for 30 mins. After stimulation, the cells are fixed in 4% paraformaldehyde and stained with Texas-Red Phalloidin. Mounting medium containing DAPI is then added and at least 100 cells per well are observed[1].

**Animal
experiment:**

Mice: MMTV/neu transgenic mice are treated i.p. daily for 14 days with either Vehicle [20%-2-hydroxypropyl-beta-cyclodextrin (HPCD)] or 200 mpk RKI-1447 dissolved in freshly prepared HPCD. The percent change in volume is calculated on the basis of the tumor volume on the last day of treatment (V_f) relative to that on the day of initiation of treatment (V₀). The average percent change in tumor volume is then calculated for each treatment group[1].

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References:

[1]. Patel RA, et al. RKI-1447 is a potent inhibitor of the Rho-associated ROCK kinases with anti-invasive and antitumor activities in breast cancer. Cancer Res. 2012 Oct 1;72(19):5025-34.

Background

RKI-1447 is a potent inhibitor of ROCK 1 and 2 (Rho-associated protein kinase 1 and 2).

ROCK is a serine-threonine kinase, which regulates cell shape and movement via acting on cytoskeleton.

RKI-1447 specifically and dose-dependently inhibits ROCK1 and ROCK2. RKI-1447 inhibited the phosphorylation of the ROCK substrates MLC-2 and MYPT-1 in human cancer cells. However, it does not affect the phosphorylation of the AKT, MEK, and S6 kinase at a high concentrations of 10 μ mol/L. It indicated a high specificity. Additionally, it was also observed that RKI-1447 selectively suppressed ROCK-mediated cytoskeleton re-organization following LPA stimulation, but does not affect PAK-mediated lamellipodia and filopodia formation following PDGF and Bradykinin stimulation. RKI-1447 inhibited migration, invasion and anchorage-independent tumor growth of breast cancer cells [1]

In mouse model, tumors were monitored at the time of tumor onset and the beginning of treatment (once a day for 14 days) with vehicle (20% HPDC) or RKI-1447 (200 mg/kg/day), when tumor volumes reached 75 to 2,300 mm³. Tumors from mice treated with vehicle increased in average tumor volume of 68.3% but tumors from mice treated with the RKI-1447 increased in average tumor volume of only 8.8%. Thus, RKI-1447 inhibited mammary tumor growth by 87% [1].

Reference:

[1] Patel R A et al. , RKI-1447 Is a Potent Inhibitor of the Rho-Associated ROCK Kinases with Anti-Invasive and Antitumor Activities in Breast Cancer. Cancer Res, 2012, 72(19): 5025-5034.

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