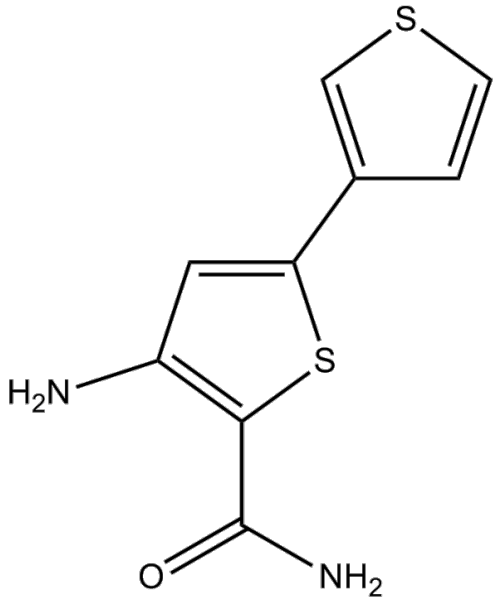
Product Data Sheet

Product Name: SC-514
Cat. No.: GC12372

Chemical Properties

Cas. No.	354812-17-2		
Chemical Name	3-amino-5-thiophen-3-ylthiophene-2-carboxamide		
SMILES	C1=CSC=C1C2=CC(=C(S2)C(=O)N)N		
Formula	$C_9H_8N_2OS_2$	M.Wt	224.3
Solubility	≥ 50.4mg/mL in DMSO	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

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Kinase experiment: IKK complexes are immunoprecipitated from IL-1β-treated RASF cell lysates (0.5-2 mg) using a NEMO antibody (3-10 μg) followed by the addition of protein A-agarose beads. Antibody complexes are pelleted by centrifugation and washed 3 times with 1 mL of cold whole-cell lysis buffer followed by 2 washes in kinase buffer (25 mM HEPES, pH 7.6, 2 mM MgCl2, 2 mM MnCl2, 10 mM NaF, 5 mM DTT, and 1 mM phenylmethylsulfonyl fluoride). 100-200 μg of immunoprecipitated IKK is analyzed for kinase activity in a reaction containing 10 μM biotinylated IκBα peptide as substrate and 1 μM [γ-33P]ATP (2500 Ci/mmol). After incubation at room temperature for 30 min, 25 μL of the reaction mixture is withdrawn and added to a SAM 96 biotin capture plate. After successive wash steps the plate was allowed to air-dry, and 25 μL of scintillation fluid is added to each well. Incorporation of [γ-33P]ATP is measured using a Top-Count NXT[1].

Cell experiment: For crystal violet staining assay, melanoma cell lines (1×104) are seeded in 60 mm dishes, and then untreated or pretreated with SC-514 (50 μM) and/or Fotemustine. Then, cells are formalin-fixed and stained with crystal violet. Cell numbers are measured as the optical density at 595 nm (OD595) of solubilized crystal violet from formalin-fixed cells. Cytotoxicity are also determined by the MTT reduction assay[2].

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Animal experiment: Rats[1] SC-514 or vehicle (2% Me2SO in saline) is administered either by oral gavage (50 mg/kg) or intraperitoneally (10 and 50 mg/kg) to adult male Wistar rats that have been deprived of food overnight. Two hours after compound treatment, 1 mg/kg LPS (Escherichia coli) in saline is administered intraperitoneally 90 min after LPS administration; the animals are bled and serum TNFα levels analyzed by a rat-specific TNFα ELISA.Mice[2] Male nu/nu BALB/c mice (6 weeks old) are maintained in individual ventilated cages. A375 or G361 (5×106) cells are resuspended in 0.1 mL PBS and inoculated subcutaneously into the backs of nude mice and allowed to grow for 7 days. After that, mice are randomly assigned to 4 groups (n=6 for each group) and treated by intraperitoneal injection with 200 µL 30% PEG/5% Tween-80 solution as the vehicle control and 25 mg/kg SC-514 and/or 25 mg/kg Fotemustine daily for 13-15 consecutive days. Body weight and tumor volume are measured every 3 days. Tumor volumes are determined by a caliper and calculated. At the end of the experiment, mice are sacrificed and tumor xenografts are collected. Tumor tissues are stored at -80°C for Western blot analysis.

References:

[1]. Kishore N, et al. A selective IKK-2 inhibitor blocks NF-kappa B-dependent gene expression in interleukin-1 beta-stimulated synovial fibroblasts. J Biol Chem. 2003 Aug 29;278(35):32861-71.
[2]. Tse AK, et al. Sensitization of melanoma cells to alkylating agent-induced DNA damage and cell death via orchestrating oxidative stress and IKKβ inhibition. Redox Biol. 2017 Apr;11:562-576.

Background

SC-514 is a cell-permeable and selective inhibitor of IKK-2 with IC50 value of 3-12μM [1, 2].

SC-514 inhibits all forms of recombinant human IKK-2 with IC50 values in the 3–12μM range. It also inhibits the native IKK complex. SC-514 specifically binds at the ATP-binding site of IKK-2 and exerts a reversible and competitive inhibition with ATP. However, SC-514 shows non-competitive inhibition with the IκB site. As an inhibitor of IKK-2, SC-514 is found to block the phosphorylation and degradation of IκBα and reduce the translocation level of p65 into the nucleus in IL-1β-treated

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RASFs. Additionally, SC-514 shows dose-dependent inhibition in the transcription of NF-κB-induced genes, including IL-6, IL-8, and COX-2. Moreover, SC-514 shows efficacious in reduction of LPS-induced TNFα production in the acute model of inflammation. SC-514 is also reported to inhibit the osteoclastogenesis in BMM cells through attenuating RANKL-induced activation of NF-κB [1, 2].

References:

[1] Kishore N, Sommers C, Mathialagan S, Guzova J, Yao M, Hauser S, Huynh K, Bonar S, Mielke C, Albee L, Weier R, Graneto M, Hanau C, Perry T, Tripp CS. A selective IKK-2 inhibitor blocks NF-kappa B-dependent gene expression in interleukin-1 beta-stimulated synovial fibroblasts. J Biol Chem. 2003 Aug 29;278(35):32861-71.
[2] Liu Q, Wu H, Chim SM, Zhou L, Zhao J, Feng H, Wei Q, Wang Q, Zheng MH, Tan RX, Gu Q, Xu J, Pavlos N, Tickner J, Xu J. SC-514, a selective inhibitor of IKKβ attenuates RANKL-induced osteoclastogenesis and NF-κB activation. Biochem Pharmacol. 2013 Dec 15;86(12):1775-83.

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