Product Data Sheet

Product Name: CC-401
Cat. No.: GC13529

Chemical Properties

Cas. No.	395104-30-0		
Chemical Name	3-[3-(2-piperidin-1-ylethoxy)phenyl]-5-(1H-1,2,4-triazol-5-yl)-1H-indazole		
SMILES	C1CCN(CC1)CCOC2=CC=CC(=C2)C3=NNC4=C3C=C(C=C4)C5=NC=NN5		
Formula	$C_{22}H_{24}N_6O$	M.Wt	388.47
Solubility	≥ 19.4mg/mL in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ 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	CC-401		

Protocol

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Cell experiment:

Human HK-2 proximal tubular epithelial cells are cultured in DMEM/F12 media supplemented with 10% FCS, 10 ng/mL EGF, and 10 μg/mL bovine pituitary extract. For Western blot studies, cells are seeded into six-well plates and allowed to adhere overnight, and medium is changed to DMEM/F12 supplemented with only 0.5% FCS for 24 h, by which time cells are confluent. CC-401 is prepared in citric acid (pH 5.5) and added to the confluent cells 1 h before the addition of 300 mM sorbitol, and cells are harvested 30 min later using urea-RIPA buffer. Three experiments are performed, each with two replicates per condition. For ELISA experiments, HK-2 cells are seeded into 24-well plates, allowed to adhere overnight, cultured in DMEM/F12 with 0.5% FCS for 24 h, and then incubated with CC-401 or vehicle for 60 min before stimulation with 1 μM Angiotensin II (AngII). Supernatants are harvested 48 h later and assayed for TGF-β1 content using a commercial ELISA kit. Three experiments are performed, each using six replicates per condition[1].

Mice[2] To assess the efficacy of JNK signaling inhibition by CC-401 in anti-angiogenic and Oxaliplatin combination therapy in a mouse xenograft model, adult (8-10 weeks of age) female severe combined immunodeficient mice (C.B.17 SCID) are used. To generate tumors, HT29 cells (1×106 cells) are injected subcutaneously into the left flank of the mice. When the tumors reached approximately 200 mm3, mice are divided into eight groups (eight mice per group) for treatment with Bevacizumab, Oxaliplatin, CC401, and the appropriate combinations of Bevacizumab, Oxaliplatin and CC-401. Mice in the Bevacizumab treatment group receive 5 mg/kg of Bevacizumab by intraperitoneal injection every 3 days for 21 days. The Oxaliplatin treatment group is injected intraperitoneally with 5 mg/kg Oxaliplatin per week for 2 weeks. The CC-401 treatment group is injected intraperitoneally 25 mg/kg for every 3 days. The combination treatment groups receive Bevacizumab (every 3 days, 5 mg/kg), Oxaliplatin (weekly for 2 weeks, 5 mg/kg), and CC-401

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Animal experiment:

(every 3 days, 25 mg/kg). The control group receive saline intraperitoneally. Tumor volume and body weight are measured every 3 days. Tumor volume is calculated. Tumor growth delay is calculated as the difference in the time for control and treated tumors to grow from 200 to 800 mm3. For tumor growth delay calculations, mice are continued to receive treatments till the tumor volume reached 800 mm3. For immunohistochemistry mice are sacrificed after treatments on day 9 for tumor processing and staining. Rats[3] Female WKY rats (180-220 g) are used. Groups of 9 or 10 rats are immunized by subcutaneous injection of 5 mg of sheep IgG in Freund's complete adjuvant followed 5 days later (termed day 0) by a tail vein injection of sheep anti-rat GBM serum. In this study, CC-401 (200 mg/kg/b.i.d. by oral gavage) or vehicle (sodium citrate) treatment is initiated in groups of 9 or 10 rats at 7 days after anti-GBM serum administration and continued twice daily thereafter until animals are killed at day 24. Additional groups of rats without treatment are killed at day 7 or day 24 after anti-GBM serum injection as controls. Animals are housed in metabolic cages for 22 hours to collect urine on days 5, 14, and 21. Blood is collected at the time of death. Analysis of serum creatinine and urinary protein are performed.

References:

[1]. Ma FY, et al. A pathogenic role for c-Jun amino-terminal kinase signaling in renal fibrosis and tubular cell apoptosis. J Am Soc Nephrol. 2007 Feb;18(2):472-84.

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[2]. Vasilevskaya IA, et al. Inhibition of JNK Sensitizes Hypoxic Colon Cancer Cells to DNA-Damaging Agents. Clin Cancer Res. 2015 Sep 15;21(18):4143-52.
[3]. Ma FY, et al. Blockade of the c-Jun amino terminal kinase prevents crescent formation and halts established anti-GBM glomerulonephritis in the rat. Lab Invest. 2009 Apr;89(4):470-84.

Background

CC-401 is a specific inhibitor of JNK with Ki values of 25-50nM [1].

CC-401 is a second generation ATP-competitive inhibitor of all three forms of JNK. It is selective against JNK over other kinases such as p38, ERK, IKK2 and ZAP70. CC-401 potently inhibits JNK in cell-based assay with concentration of 1 to 5μM. The activation of JNK signaling is identified in many immune-mediated kidney disease models. Thus, as the JNK inhibitor, CC-401 is found to be effective in these renal injury models. In the acute anti-GBM disease, CC-401 inhibits JNK activation and causes 50%-70% reduction of proteinuria. In addition, CC-401 is also used in liver injury models [1, 2 and 3].

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References:
[1] Ma F Y, Flanc R S, Tesch G H, et al. A pathogenic role for c-Jun amino-terminal kinase signaling in renal fibrosis and tubular cell apoptosis. Journal of the American Society of Nephrology, 2007, 18(2): 472-484.
[2] Flanc R S, Ma F Y, Tesch G H, et al. A pathogenic role for JNK signaling in experimental anti-GBM glomerulonephritis. Kidney international, 2007, 72(6): 698-708.
[3] Bogoyevitch M A, Arthur P G. Inhibitors of c-Jun N-terminal kinases—JuNK no more Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 2008, 1784(1): 76-93.

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