Product Data Sheet

Product Name: NVP-BSK805
Cat. No.: GC13229

Chemical Properties

Cas. No.	1092499-93-8		
Chemical Name	4-[[2,6-difluoro-4-[3-(1-piperidin-4-ylpyrazol-4-yl)quinoxalin-5-yl]phenyl]methyl]morpholine;dihydrochloride		
SMILES	C1CNCCC1N2C=C(C=N2)C3=NC4=C(C=CC=C4N=C3)C5=CC(=C(C(=C5)F)CN6CCOCC6)F.Cl.Cl		
Formula	$C_{27}H_{28}F_2N_6O$	M.Wt	490.55
Solubility	≥ 20.95 mg/mL in DMSO, ≥ 4.75 mg/mL in EtOH with ultrasonic and warming, ≥ 3.45 mg/mL in Water with ultrasonic and warming	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	NVP-BSK805		

Protocol

Kinase experiment [1]:

The human JAK2 kinase domain (amino acids 840 ~ 1132) was contained in plasmid construct pAcG2TtevJAK2. The plasmid constructs for JAK3 (813 ~ 1124), TYK2 (888 ~ 1187) and JAK1 (866 ~ 1154) were designed. The generation of the recombinant baculoviruses with BD BaculoGoldTM Bright linearized DNA, plaque assay, and virus amplification from single plaques was performed according to the manual (BD Biosciences Pharmingen). Janus kinase domains were expressed in Sf9 cells in 400 mL shake flasks with 100 mL ExCell420 culture medium (JRH Biosciences Ltd) with Penicillin/Streptomycin solution for 48 hrs at 27°C. Suspension culture cells were infected at a density of 1 × 106 and the multiplicity of infection (MOI) for each virus was optimized for yield of soluble protein. The kinase domain of human JAK2 and of JAK1, JAK3, and TYK2 was expressed at an MOI of 1 and 0.5, respectively. Time of expression at 27°C was 48 hrs for JAK2 and 48 hrs or 72 hrs for JAK1, JAK3, and TYK2. Forty-eight or seventy-two hrs post-infection, the cells from a 100 mL expression culture were harvested by centrifugation at 3000 × g for 5 mins and lysed with 12 mL lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1 mM DTT, 1 mM sodium orthovanadate, 1 % Triton X-100, 10 % glycerol, 1 × EDTA-free complete protease inhibitor cocktail (Roche Diagnostics), and 12.5 U/mL Benzonase) for 30

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Enzymatic Assays	mins at 4°C, followed by centrifugation at 14,000 × g for 45 mins to pellet insoluble material. For GST-tag affinity purification of kinase domain proteins, all steps were performed at 4°C. The cleared lysates were incubated with 0.2 mL of a 50 % slurry of washed Glutathione Sepharose 4B for 2 hrs at 4°C, followed by 5 washes with 1 mL of 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1 % Triton X-100, 1 mM DTT, and 10 % glycerol. Bound protein was eluted in 5 aliquots each starting with a 10 mins incubation with 0.25 mL elution buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1 % Triton X-100, 1 mM DTT, 10 % glycerol, 10 mM reduced L-glutathione). Eluates were concentrated about 5-fold with Amicon Ultra-4 spin columns. After addition of Brij35 to 0.1 % final concentration, the protein was snap frozen in small aliquots and stored at -80°C. In these conditions, kinase activities were stable for at least 6 months. The JAK kinase domain enzymes were incubated for 30 mins at room temperature in a medium containing 0.1 μM [γ33P]-ATP, 1 mM MnCl2, 5 mM MgCl2, 30 μM of synthetic peptide substrate EQEDEPEGDYFEWLE, 1 mM DTT, 1 % DMSO, 50 μg/mL BSA, 0.01 % Brij35, and 50 mM Tris-HCl pH 7.5. The ATP concentration was below the Km for all proteins. Curves were fitted by non-linear regression using the logistic equation and the global fit function of XLfit? (model 205). Expression and characterization of full-length wild type and V617F mutant JAK2 as well as kinase assay conditions had been described elsewhere. Kinase selectivity of NVP-BSK805 was assessed in an internal kinase panel: In the Caliper assays, kinase reactions were carried out with peptide substrates that migrate with different velocities in an electrical field when phosphorylated. The peptides carried a fluorescent label in order to allow the detection and quantification of the peptides in a capillary system. Peptide fluorescence intensities were quantified using the LC3000 instrument (Caliper Life Sciences, Hopkinton, MA, USA). Kinase activity was measured by quantifying the amount of ATP remaining in solution following a kinase reaction. In the LanthaScreen? TR-FRET kinase assays, terbium was used as the lanthanide chelate combined with an antibody directed against the phosphorylated substrate.

Cell experiment [1]:

Cell lines	Ba/F3 cells
Preparation method	Soluble in DMSO. General tips for obtaining a higher concentration: Please warm the tube at 37℃ for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20℃ for several months.

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Reaction Conditions	100 nM; 72 hrs
Applications	NVP-BSK805 inhibited the growth of JAK2V617F cells (Ba/F3) and induced apoptosis with a GI50 at concentrations < 100 nM.
Animal experiment [1]:	
Animal models	RhEpo-induced polycythemia model in female BALB/c mice
Dosage form	50, 75 and 100 mg/kg; p.o.; q.d.
Applications	At the doses of 25, 50 and 100 mg/kg, NVP-BSK805 suppressed rhEpo-induced STAT5 phosphorylation as well as rhEpo-mediated polycythemia and splenomegaly in BALB/c mice.
Other notes	Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.

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References:

[1]. Baffert F, Régnier CH, De Pover A, et al. Potent and selective inhibition of polycythemia by the quinoxaline JAK2 inhibitor NVP-BSK805. Mol Cancer Ther, 2010, 9(7): 1945-1955.

Background

NVP-BSK805 is a potent and selective inhibitor of JAK2 with IC50 value of 0.58 nM [1].
Janus kinase 2 (JAK2) is a member of the JAK family and is a non-receptor tyrosine kinase. JAK2 regulates signal transduction in the cell nucleus via activation of signal transducers and activators of transcription proteins (STATs), which form dimers upon phosphorylation and migrate into the nucleus to regulate the activation of target genes [2].
In JAK radiometric filter binding kinase assays, NVP-BSK805 inhibited full-length JAK2 wild-type and JAK2V617F enzymes with IC50 values of 0.58 nM and 0.56 nM, respectively [1]. In CHRF-288-11, SET-2 and HEL cells which expressed JAK2, NVP-BSK805 inhibited STAT5a phosphorylation [2].
In SCID beige mice injected with JAK2V617F-dependent Ba/F3 cells, NVP-BSK805 (150 mg/kg) suppressed STAT5 phosphorylation in spleen extracts by nearly 50% relative to vehicle-treated controls at the 6- and 12-hour time. In rats injected with recombinant human erythropoietin (rhEpo), which induced transient polycythemia and splenomegaly, NVP-BSK805 suppressed rhEpo-induced STAT5 phosphorylation in spleen in a dose-dependent way [1].

References:
[1]. Baffert F, Régnier CH, De Pover A, et al. Potent and selective inhibition of polycythemia by the quinoxaline JAK2 inhibitor NVP-BSK805. Mol Cancer Ther, 2010, 9(7): 1945-1955.
[2]. Ringel F, Kaeda J, Schwarz M, et al. Effects of Jak2 type 1 inhibitors NVP-BSK805 and NVP-BVB808 on Jak2 mutation-positive and Bcr-Abl-positive cell lines. Acta Haematol, 2014, 132(1): 75-86.

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