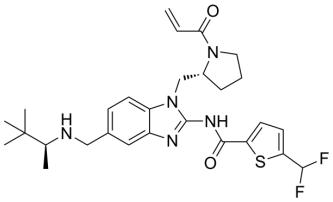
PRN694

Cat. No.:	HY-12680
CAS No.:	1575818-46-0
Molecular Formula:	$C_{28}H_{35}F_2N_5O_2S$
Molecular Weight:	543.67
Target:	Itk
Pathway:	Protein Tyrosine Kinase/RTK
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 125 mg/mL (229.92 mM; Need ultrasonic and warming)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.8394 mL	9.1968 mL	18.3935 mL
	5 mM	0.3679 mL	1.8394 mL	3.6787 mL
	10 mM	0.1839 mL	0.9197 mL	1.8394 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (3.83 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (3.83 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (3.83 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

PRN694 is an irreversible, highly selective and potent covalent interleukin-2-inducible T-cell kinase (ITK) and resting lymphocyte kinase (RLK) dual inhibitor with IC_{50}s of 0.3 nM and 1.4 nM, respectively. PRN694 exhibits extended target residence time on ITK and RLK, enabling durable attenuation of effector cells in vitro and in vivo[1].

IC_{50} & Target

IC50: 0.3 nM (ITK), 1.4 nM (RLK), 3.3 nM (TEC), 17 nM (BTK), 17 nM (BMX), 30 nM (JAK3), 125 nM (BLK)[1]

In Vitro

PRN694 inhibits TEC, BTK, BMX, BLK, JAK3 with IC_{50}s of 3.3, 17, 17, 125, 30 nM, respectively[1].

	?Immunoblot analysis of TCR activation pathways reveales that PRN694 blocks activation or nuclear translocation of NFAT1, JunB, pIκBα, and pERK. Results reveal inhibition of Ca^{2+} signaling with PRN694 at all concentrations above 1 nM. PRN694 significantly attenuates NK cell FcR-induced killing at concentrations exceeding 0.37 μM[1]. ?Day 6 flow cytometry analysis reveals that PRN694 significantly inhibits the anti-CD3/CD28-induced proliferation of both CD4 and CD8 T-cells ($p<0.01$)[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	The PRN694 occupancy of ITK is 98, 95, and 54% at 1, 6, and 14 h, respectively. The concentrations of PRN694 in the plasma are 2.8, 0.66, and 0.027 μM at 1, 6, and 14 h, respectively. At 14 h, the plasma level of PRN694 is over 10 fold lower than the IC_{50} in whole blood. RN694 treatment also results in significantly lower weights relative to vehicle ($p<0.05$)[1]. ?Colitis studies show reduced numbers of $CD4^+$ T cells present in the colonic epithelium of PRN694-treated mice compare with controls[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Recombinant ITK at a final concentration of 0.5 μM in 50 mM Hepes, pH 7.5, 10 mM $MgCl_2$, 0.01% Triton X-100, and 1 mM EGTA are combined with 1.5 μM PRN694 for 90 min to facilitate binding. The mixture is then diluted 50 fold to initiate dissociating of the ligand from the enzyme, and 10 μL is transferred to a Greiner 384-well black plate. Europium-coupled anti-His_6 antibody is added to each well and incubated for 5 min, followed by the addition of an ITK binding fluorescent tracer. The tracer binds to ITK as a function of ligand dissociation, and binding is detected by time-resolved FRET between the europium-coupled antibody and the tracer on a plate reader. Time points acquired are 0.25, 1, 3, 6, and 24 h[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	Cells are cultured in vitro at 37°C and 5% CO_2 using RPMI 1640 with 10% fetal calf serum. Cells are pretreated for 30 min with PRN694 or other inhibitors and then washed two times. T-cells are then stimulated for 6 h with 1 μg/mL soluble anti-CD3 for CD69 activation, which is detected by flow cytometry, or 45 min with plate-bound anti-CD3 (10 μg/mL plating concentration) and soluble anti-CD28 (1 μg/mL) for downstream signal analysis by immunoblotting. NK cells are stimulated for 6 h with plate-bound anti-CD52 for CD107a/b activation, detected by flow cytometry, or for 45 min for downstream signal analysis by immunoblotting[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	Mice are randomized by weight and sensitized with aliquots of 150 μL of 5% oxazolone in 3 parts ethanol and 1 part acetone on their shaved abdomens. Seven days after the sensitization, the mice are challenged with 10 μL of 3% oxazolone on the front and back of the right ears. The left ears are treated with the ethanol/acetone mixture. One hour prior to the challenge, the animals received either vehicle control (5% ethanol, 95% Captex 355 NP/EF, intraperitoneal injection at 5 mL/kg), 20 mg/kg PRN694 in 5% ethanol, 95% Captex (intraperitoneal injection at 5 mL/kg), or 0.5 mg/kg dexamethasone (intraperitoneal injection at 5 mL/kg). A control group of animals receive no oxazolone or drug treatment. Twenty-four hours after the oxazolone challenge, the mice are sacrificed, and a 7 mm disc is punched out of each ear and weighed to measure edema[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Zhong Y, et al. Targeting interleukin-2-inducible T-cell kinase (ITK) and resting lymphocyte kinase (RLK) using a novel covalent inhibitor PRN694. J Biol Chem. 2015 Mar 6;290(10):5960-78.

[2]. Cho HS, et al. A Small Molecule Inhibitor of ITK and RLK Impairs Th1 Differentiation and Prevents Colitis Disease Progression. J Immunol. 2015 Nov 15;195(10):4822-31.

Caution: Product has not been fully validated for medical applications. For research use only.

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