产品名称：Decernotinib
产品别名：VX-509; VRT-831509

生物活性：

Description	Decernotinib is a potent, orally active JAK3 inhibitor, with K_is of 2.5, 11, 13 and 11 nM for JAK3, JAK1, JAK2, and TYK2, respectively.

IC_{50} & Target						
	JAK3	JAK1	Tyk2	JAK2	FLT3	ROCK I
	2.5 nM (Ki)	11 nM (Ki)	11 nM (Ki)	13 nM (Ki)	1 μM (Ki)	1.5 μM (Ki)
	GSK3β	CDK2/CycA	PknB			
	1.8 μM (Ki)	2.6 μM (Ki)	8 μM (IC_{50})			

In Vitro	Decernotinib (VX-509) is a potent JAK3 inhibitor, with Kis of 2.5, 11, 13 and 11 nM for JAK3, JAK1, JAK2, and TYK2, respectively. Decernotinib potently blocks T-cell proliferation with a mean IC50 of 170 ± 101 nM, and inhibits IL-2-stimulated T-cell proliferation (IC50, 140 and 400 nM). VX-509 is also cytotoxic to B-cell in response to CD40L and IL-4 (IC50, 50 nM)[1].
In Vivo	Decernotinib (VX-509, 10, 25, or 50 mg/kg, p.o.) significantly and dose-dependently inhibits the increases in ankle diameter and paw weight occuring in response to collagen injections in rats. Decernotinib potently alleviates cartilage damage and bone resorption in rats. Decernotinib (10, 25, or 50 mg/kg, p.o., b.i.d.) suppresses ear edema in a mouse model of delayed-type hypersensitivity[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 50 mg/mL (127.43 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5485 mL	12.7427 mL	25.4855 mL
	5 mM	0.5097 mL	2.5485 mL	5.0971 mL
	10 mM	0.2549 mL	1.2743 mL	2.5485 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

References	[1]. Mahajan S, et al. VX-509 is a potent and selective Janus kinase 3 (JAK3) inhibitor that attenuates inflammation in animal models of autoimmune disease. J Pharmacol Exp Ther. 2015 Mar 11. pii: jpet.114.221176.

实验参考：

Cell Assay	Whole-blood samples from healthy volunteers are used to collect peripheral blood mononuclear cells, which are plated in T75 tissue culture flasks at a density of 1 × 10^6/mL. Cells are stimulated with 10 μg/mL phytohemagglutinin at 37°C for 72 hours. After 72 hours, cells are detached from the flask by scraping, washed, and plated at a density of 1 × 10^5/well in a 96-well plate. Decernotinib (9.7 nM to 10 μM) is added, and plates are incubated for 30 minutes at 37°C, followed by stimulation with human IL-2. In two rows, only DMSO is added; one row is not stimulated with IL-2, and one row is stimulated with IL-2 to serve as the proliferation control. Plates are incubated at 37°C for 2 days. On day 2, cells are pulsed with 20 μCi/mL methyl-[^{3}H]thymidine for 18-24 hours and harvested onto

	filters for radiographic determination. Data are analyzed to generate an IC_{50} value using Softmax pro software[1]
Animal Administration	Rat[1] The collagen-induced arthritis (CIA) rat model is used to evaluate the effects of oral Decernotinib [10 mg/kg b.i.d., 25 mg/kg b.i.d., 50 mg/kg b.i.d., 50 mg/kg q.d., or 100 mg/kg q.d.] on joint inflammation and histopathology. Female Lewis rats (157-187 g) are anesthetized with isoflurane and injected with 300 μL Freund's incomplete adjuvant, containing 2 mg/mL bovine type II collagen, at the base of the tail and two sites on the back on days 0 and 6. The rats are randomized to study groups at the onset of paw swelling (arthritis), which occurs between days 10 and 11. Dosing of either Decernotinib or vehicle via oral gavage is initiated on the first day of established arthritis and continued to day 6 of arthritis. Dosing volume is 5 mL/kg. Groups are controls (no collagen injection plus vehicle; n = 4), collagen plus vehicle (n = 5), collagen plus Decernotinib 10 mg/kg b.i.d. (n = 8); collagen plus Decernotinib 10 mg/kg b.i.d. (n = 8); collagen plus (n = 8); collagen plus (n = 8); collagen plu (n = 8); and collagen plus (n = 8); collagen plu (n = 8); all treatments are administered for 6 days. An additional group of rats is given collagen plus 10 mg/kg subcutaneous etanercept, a human tumor necrosis factor-α antagonist, on study days 11 and 14. Caliper measurements of normal (baseline) ankle joints begin on day 9 and continue through the last day of study. Differences in mean ankle diameter are tested for significance using Student's t test, with significance set at $P \leq 0.05$. The rats are euthanized on day 7 of arthritis, which is study day 17 or 18 depending on when animals are randomized to groups; paws and knees are harvested to determine paw weight and to conduct a histopathological analysis of inflammation (knee and ankle), pannus formation (ankle), cartilage destruction (knee), and bone resorption (knee and ankle). Scores range from 0 (normal) to 5 (severe pathology) and are assigned by a veterinary pathologist. Percent inhibition is calculated using the following formula: [(mean of treatment group) − (mean of control)] ÷ [(mean of collagen + vehicle) − (mean of control)]. Kruskal-Wallis one-way analysis of variance nonparametric tests are used to determine statistical significance among the histopathology groups, with significance set at $P \leq 0.05$[1]
Kinase Assay	The effect of Decernotinib on JAK3 activity is assessed by measuring the residual kinase activity of the recombinantly expressed JAK3 kinase domain using a radiometric assay. The final concentrations of the components in the assay are as follows: 100 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT), 0.01% BSA, 0.25 nM JAK3, 0.25 mg/mL polyE4Y, and 5 μM ^{33}P-γ-ATP (200 μCi/μmol). A 10 mM stock solution of Decernotinib is prepared in DMSO, from which additional dilutions are prepared. A substrate mixture (100 mM HEPES, 10 mM $MgCl_2$, 0.5 mg/mL polyE4Y, and 10 μM ^{33}P-γ-ATP) is added and mixed with Decernotinib stock solution. The reaction is initiated by the addition of an enzyme mixture [100 mM HEPES (pH 7.5), 10 mM $MgCl_2$, 2 mM DTT, 0.02% BSA, 0.5 nM JAK3]. After 15 minutes, the reaction is quenched with 20% trichloroacetic acid (TCA). The quenched reaction is transferred to the GF/B filter plates and washed three times with 5% TCA. Following the addition of Ultimate Gold scintillant (50 μL), the samples are counted in a Packard TopCount gamma counter. In this procedure, the radioactivity trapped is a measure of the residual JAK3 kinase activity. From the activity versus concentration of Decernotinib titration curve, the K_i value is determined by fitting the data to an equation for competitive tight binding inhibition kinetics[1]
References	[1]. Mahajan S, et al. VX-509 is a potent and selective Janus kinase 3 (JAK3) inhibitor that attenuates inflammation in animal models of autoimmune disease. J Pharmacol Exp Ther. 2015 Mar

	11. pii: jpet.114.221176.