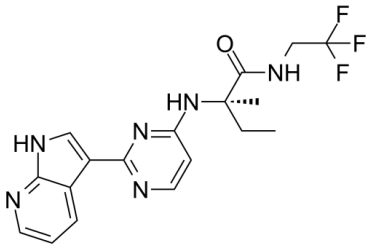
Data Sheet

Product Name:	Decernotinib
Cat. No.:	CS-3990
CAS No.:	944842-54-0
Molecular Formula:	C18H19F3N6O
Molecular Weight:	392.38
Target:	JAK
Pathway:	Epigenetics; JAK/STAT Signaling; Stem Cell/Wnt
Solubility:	DMSO : ≥ 50 mg/mL (127.43 mM)



BIOLOGICAL ACTIVITY:

Decernotinib is a potent, orally active **JAK3** inhibitor, with **K_i**s of 2.5, 11, 13 and 11 nM for **JAK3**, JAK1, JAK2, and TYK2, respectively. IC50 & Target: Ki: 2.5 (JAK3), 11 (JAK1), 13 (JAK2), 11 nM (TYK)[1] **In Vitro:** Decernotinib (VX-509) is a potent JAK3 inhibitor, with K_is of 2.5, 11, 13 and 11 nM for JAK3, JAK1, JAK2, and TYK2, respectively. Decernotinib potently blocks T-cell proliferation with a mean IC_{50} of 170 ± 101 nM, and inhibits IL-2-stimulated T-cell proliferation (IC_{50}, 140 and 400 nM). VX-509 is also cytotoxic to B-cell in response to CD40L and IL-4 (IC_{50}, 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].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]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].

Cell Assay: Decernotinib is serially diluted in DMSO as eight 1:3 dilutions starting from 10 mM, and then added to medium[1].[1] **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: Decernotinib is solubilized at 2, 5, and 10 mg/mL as an aqueous solution in 10% vitamin E d-α-tocophenyl polyethylene glycol 1000 succinate and 1% hydroxypropyl methylcellulose acetyl succinate[1].[1]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 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].

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.

CAIndexNames:

Butanamide, 2-methyl-2-[[2-(1H-pyrrolo[2,3-b]pyridin-3-yl)-4-pyrimidinyl]amino]-N-(2,2,2-trifluoroethyl)-, (2R)-

SMILES:

C[C@](C(NCC(F)(F)F)=O)(CC)NC1=NC(C2=CNC3=NC=CC=C32)=NC=C1

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