产品名称：**PF-8380**
产品别名：**PF-8380**

生物活性：

Description	PF-8380 is a potent autotaxin inhibitor with an IC_{50} of 2.8 nM in isolated enzyme assay and 101 nM in human whole blood.
IC_{50} & Target	Autotaxin
	2.8 nM (IC_{50}, In isolated enzyme assay)
In Vitro	PF-8380 also inhibits rat autotaxin with an IC50 of 1.16 nM with FS-3 substrate. Potency of PF-8380 is maintained when using enzyme produced from fetal fibroblasts used in combination with lysophosphatidyl choline (LPC) as a substrate. In human whole blood incubated with PF-8380 for 2 h, autotaxin is inhibited with an IC50 of 101 nM[1]. Autotaxin (ATX), an enzyme with lysophospholipase D (lysoPLD) activity, catalyzes the production of lysophosphatidic acid (LPA) from lysophosphatidylcholine (LPC). Pre-treatment of GL261 and U87-MG cells with 1 μM PF-8380 followed by 4 Gy irradiation results in decreased clonogenic survival, decreases migration (33% in GL261; P=0.002 and 17.9% in U87-MG; P=0.012), decreases invasion (35.6% in GL261; P=0.0037 and 31.8% in U87-MG; P=0.002), and attenuates radiation-induced Akt phosphorylation[2].
In Vivo	The pharmacokinetic profile of PF-8380 is evaluated at an intravenous dose of 1 mg/kg and oral doses of 1 to 100 mg/kg out to 24 h. PF-8380 has mean clearance of 31 mL/min/kg, volume of distribution at steady state of 3.2 L/kg, and effective $t_{1/2}$ of 1.2 h. Oral bioavailability is moderate, ranging from 43 to 83%. Plasma concentrations increased with single oral escalating doses, but C_{max} increased at a rate that is approximately proportional to dose from 1 to 10 mg/kg and less than proportional to dose from 10 to 100 mg/kg. PF-8380 exposures estimated by area under the curve are approximately proportional to dose and linear up to 100 mg/kg. Plasma C16:0, C18:0, and C20:0 LPA levels are measured immediately after collection. Maximal reduction of LPA levels is observed by the 3 mg/kg dose at 0.5 h with all LPA returning at or above baseline at 24 h[1]. Treatment with 10 mg/kg PF-8380 increases tumor-associated vascularity modestly by 20% (P=0.497). When compared to control, treatment of PF-8380 45 min before 4 Gy irradiation decreases vascularity by nearly 48% when compared to control (P=0.031) and by 65% when compared to mice that received radiation alone (P=0.011) [2].
Solvent&Solubility	***In Vitro:***
	DMSO : ≥ 56 mg/mL (117.07 mM)
	* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	**1 mg**	**5 mg**	**10 mg**
	1 mM	2.0906 mL	10.4530 mL	20.9061 mL
	5 mM	0.4181 mL	2.0906 mL	4.1812 mL
	10 mM	0.2091 mL	1.0453 mL	2.0906 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。

In Vivo:

1. PF-8380 is dissolved in PEG 400[3].

References	[1]. Gierse J, et al. A novel autotaxin inhibitor reduces lysophosphatidic acid levels in plasma and the site of inflammation. J Pharmacol Exp Ther. 2010 Jul;334(1):310-7. [2]. Bhave SR, et al. Autotaxin Inhibition with PF-8380 Enhances the Radiosensitivity of Human and Murine Glioblastoma Cell Lines. Front Oncol. 2013 Sep 17;3:236. [3]. Cao P, et al. Autocrine lysophosphatidic acid signaling activates β-catenin and promotes lung allograft fibrosis. J Clin Invest. 2017 Apr 3;127(4):1517-1530.

实验参考：

Cell Assay	HUVEC (1×10^6) and bEnd.3 cells (1×10^6) are plated in 100 mm plates and after 24 h, U87-MG (2×10^6) and GL261 (2×10^6) cells are plated onto transwell inserts. After co-culture for 24 h, cells are treated with 1 μM of PF-8380 or vehicle control DMSO for 45 min prior to irradiation with 0, 2, 4, 6, or 8 Gy. After the treatments as co-culture with either PF-8380 or DMSO calculated numbers of U87-MG and GL261 cells are plated to enable normalization for plating efficiencies. After 7 to 10 day incubation plates are fixed with 70% EtOH and stained with 1% methylene blue. Colonies consisting of >50 cells are counted by viewing the plates under a microscope. The survival fractions are calculated as (number of colonies/number of cells plated)/(number of colonies for corresponding control/number of cells plated). Survival curves are analyzed by curve fitting to the alpha/beta model calculating D_0 and n [2].
Animal Administration	Rats[1] Male Lewis rats weighing 275 to 300 g are used and acclimated to their surroundings for approximately 1 week with food and water provided ad libitum. A minimum of 1 day before study, animals are anesthetized with isoflurane (to effect) and implanted with Culex vascular catheters in the carotid artery. Animals are acclimated in Culex cages overnight before dosing. Patency of the carotid artery catheter is maintained by using the "tend" function of the Culex automated blood sampler. Animals are dosed with PF-8380 at 1, 3, 10, 30, and 100 mg/kg by oral gavage after an overnight fast. Blood collections are obtained from the carotid artery and performed by the Culex automated blood sampler at 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after administration. Blood is centrifuged, and plasma is collected for analysis of PF-8380 and LPA concentrations. Mice[2] GL261 cells (1×10^6) are injected into the right hind limb of nude mice. Once tumors are palpable the mice are serpentine sorted into groups of six to seven animals representing similar distributions of tumor sizes (range=240 mm^3). Tumor bearing mice are injected intraperitoneally with vehicle (DMSO) or PF-8380 at 10 mg/kg body weight once daily for five consecutive days. Forty five minutes after drug injection, mice are anesthetized with isoflurane and positioned in the RS2000 irradiator. They are then irradiated with 2 Gy daily for five consecutive days for a total of 10 Gy. Lead blocks (10 mm thick) are used to shield the head, thorax, and abdomen. Tumor size is monitored longitudinally using an external traceable digital caliper. Mice are sacrificed by cervical dislocation once the tumors reached a volume of approximately 10 mm3 or when ulceration becomes apparent.
Kinase Assay	FS-3 substrate is solubilized in assay buffer at 500 μM and frozen at -20°C in single-use aliquots for up to 4 weeks. Recombinant autotaxin is diluted in Tris-buffered saline (140 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 50 mM Tris, pH 8.0) and incubated with compound in DMSO or DMSO alone (final 1% DMSO) for 15 min at 37°C, and the reaction is started with the addition of FS-3 at a final concentration of 1 μM. The reaction is allowed to proceed at 37°C for 30 min and monitored at 520 nm until the uninhibited control compared with a no-enzyme control gave a $Z'\geq0.5$. IC_{50}s are determined in triplicate by using a four-parameter fit [1].

References	[1]. Gierse J, et al. A novel autotaxin inhibitor reduces lysophosphatidic acid levels in plasma and the site of inflammation. J Pharmacol Exp Ther. 2010 Jul;334(1):310-7. [2]. Bhave SR, et al. Autotaxin Inhibition with PF-8380 Enhances the Radiosensitivity of Human and Murine Glioblastoma Cell Lines. Front Oncol. 2013 Sep 17;3:236. [3]. Cao P, et al. Autocrine lysophosphatidic acid signaling activates β-catenin and promotes lung allograft fibrosis. J Clin Invest. 2017 Apr 3;127(4):1517-1530.