产品名称：Pazopanib HCl (GW786034 HCl)
产品别名：盐酸帕唑帕尼 ；Pazopanib Hydrochloride

生物活性：

Description	Pazopanib Hydrochloride (GW786034 Hydrochloride) is a novel multi-target inhibitor of VEGFR1, VEGFR2, VEGFR3, PDGFRβ, c-Kit, FGFR1, and c-Fms with an IC_{50} of 10, 30, 47, 84, 74, 140 and 146 nM, respectively.

IC_{50} & Target [1]

VEGFR1	VEGFR2	VEGFR3	PDGFRβ	FGFR1	c-Kit
10 nM (IC_{50})	30 nM (IC_{50})	47 nM (IC_{50})	84 nM (IC_{50})	140 nM (IC_{50})	74 nM (IC_{50})
c-Fms					
146 nM (IC_{50})					

In Vitro

Pazopanib shows good potency against all the human VEGFR receptors with an IC50 of 10, 30, and 47 nM for VEGFR-1, -2, and -3, respectively. Significant activity is also seen against the closely related tyrosine receptor kinases PDGFRβ, c-Kit, FGF-R1, and c-fms with IC50s of 84, 74, 140, and 146 nM, respectively. In cellular assays, in addition to inhibiting the VEGF-induced proliferation of HUVECs, Pazopanib potently inhibits VEGF-induced phosphorylation of VEGFR-2 in HUVEC cells with an IC50 of ~8 nM. Pazopanib possesses good pharmacokinetics in rat, dog, and monkey with low clearances (1.4-1.7 mL/min/kg) and good oral bioavailabilities (72, 47, 65%) dosed at 10, 1, and 5 mg/kg, respectively. The cytochrome P450 profile is also improved with inhibition >10 μM against the isozymes tested, with the exception of 2C9 (7.9 μM)[1].

In Vivo

Treatment of mice with 100 mg/kg of Pazopanib twice daily for five days results in significant inhibition in the degree of vascularization. The antiangiogenic activity of Pazopanib is examined in mice bearing established human xenografts (200−250 mm^3) using HT29 (colon carcinoma), A375P (melanoma), and HN5 (head and neck carcinoma) tumors following a standard three-week course of therapy. The HN5 and HT29 xenografts responded better at all doses compared to the A375P model, which is historically more resistant to VEGFR-2 inhibitors. As support that the observed inhibition of xenograft growth is working through an antiangiogenic rather than antitumor mechanism, no antiproliferative activity is observed below 10 μM for Pazopanib against these human tumor lines (HT29, HN5, A375P) growing in serum-containing media. No significant effect on the body weight of mice is observed, and the animals appeared healthy and active throughout the study duration[1]. The quantity of adherent leukocytes in the Pazopanib eye drops group is less than untreated diabetic animals and more than the healthy animals. Average leukocytes adhered to the retinal vasculature in healthy animals is 37.2±7.8, whereas diabetic animals have an average value of 102±15.6, approximately 3-fold higher than healthy animals. Animals treated with 0.5 % w/v Pazopanib suspension demonstrate 69.5±9.5 leukocytes adhered in their retinal vasculature, which is found to be significantly lower than diabetic animals[2].

In Vitro:

DMSO : 10 mg/mL (21.10 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.1098 mL	10.5490 mL	21.0979 mL
5 mM	0.4220 mL	2.1098 mL	4.2196 mL
10 mM	0.2110 mL	1.0549 mL	2.1098 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。

Solvent&Solubility	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:*** 请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂： ——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶 1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: ≥ 1 mg/mL (2.11 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.11 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 10.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: ≥ 1 mg/mL (2.11 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.11 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 10.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 1 mg/mL (2.11 mM); Clear solution 此方案可获得 ≥ 1 mg/mL (2.11 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 10.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Harris PA, et al. Discovery of 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-benzenesulfonamide (Pazopanib), a novel and potent vascular endothelial growth factor receptor inhibitor. J Med Chem. 2008, 51(15), 4632-4640. [2]. Thakur A, et al. Pazopanib, a multitargeted tyrosine kinase inhibitor, reduces diabetic retinal vascular leukostasis and leakage. Microvasc Res. 2011 Nov;82(3):346-50.

实验参考：

Cell Assay	The effect of Pazopanib on cell proliferation is measured using 5-bromo-2-deoxyuridine (BrdU) incorporation method using commercially available kits. HUVEC is seeded in medium containing 5% fetal bovine serum (FBS) in type 1 collagen coated 96-well plates and incubated overnight at 37°C, 5% CO_2. The medium is aspirated from the cells, and various concentrations of Pazopanib in serum-free medium are added to each well. After 30 min, either VEGF (10 ng/mL) or bFGF (0.3 ng/mL) is added to the wells. Cells are incubated for an additional 72 h and BrdU (10 μM) is added during the last 18 to 24 h of incubation. At the end of incubation, BrdU incorporation in cells is measured by ELISA. Data are fitted with a curve described by the equation, y=Vmax(1−(x/(K+x))), where K is equal to the IC50[1].
	Mice[1] Tumors are initiated by injection of tumor cell suspension in 8−12 week old nude mice. When tumors

Animal Administration	reach a volume of 100−200 mm^3, mice are randomized and divided into groups of eight. Pazopanib is administered once or twice daily at 10, 30, or 100 mg/kg. Animals are euthanized by inhalation of CO_2 at the completion of the study. Tumor volume is measured twice weekly by calipers, using the equation: tumor volume (mm^3)=(length×$width^2$)/2. Results are routinely reported as % inhibition=1−(average growth of the drug treated population/average growth of vehicle treated control population). Rats[2] Male Brown-Norway (BN; pigmented) rats weighing 200 to 250 g are acclimatized for at least two days prior to any experimental procedure. After overnight fasting for 12-16 h, an intraperitoneal injection of 30 mg/mL solution of Streptozotocin in 10 mM citrate buffer (pH 4.5) is administered (60 mg/kg body weight) to induce diabetes. After 3-4 h of Streptozotocin injection, animals are put on a regular diet and 24 h after Streptozotocin injection, blood sample (5-10 µL) is collected via tail vein. The blood glucose levels in the animals are determined with a glucose monitor. Animals with blood glucose levels greater than 250 mg/dL are considered diabetic. The animals are divided into three groups. Group 1: Healthy (n=12), Group 2: Diabetic (n=12) and Group 3: Diabetic+Treatment (n=12). Treatment is started immediately after diabetes induction. Both eyes are dosed twice daily for 30 days with 0.5 % w/v Pazopanib suspension (10 µL volume in each eye) and animals in all groups are sacrificed on day 31, 16-17 h after last dose on day 30.
Kinase Assay	VEGFR enzyme assays are initiated by the addition of 10 µL of activated VEGFR2 kinase solution [final concentration, 1 nM enzyme in 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, containing 0.1 mg/mL bovine serum albumin (BSA), 300 µM dithiothreitol (DTT)] to 10 µL substrate solution [final concentration, 360 nM peptide, (biotin-aminohexyl-EEEEYFELVAKKKK-NH2), 75 µM ATP, 10 µM $MgCl_2$], and 1 µL of titrated compound (Pazopanib) in DMSO. Plates are incubated at room temperature for 60 min, and then the reaction is quenched by the addition of 20 µL of 100 mM ethylene diamine tetraacetic acid (EDTA). After quenching, 20 µL HTRF reagents (final concentration, 15 nM Streptavidin-linked allophycocyanin, 1 nM Europium-labeled antiphosphotyrosine antibody diluted in 0.1 mg/mL BSA, 0.1 M HEPES, pH 7.5) is added and the plates incubated for a minimum of 10 min. The fluorescence at 665 nM is measured with a plate reader[1].
References	[1]. Harris PA, et al. Discovery of 5-[[4-[(2,3-dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methyl-benzenesulfonamide (Pazopanib), a novel and potent vascular endothelial growth factor receptor inhibitor. J Med Chem. 2008, 51(15), 4632-4640. [2]. Thakur A, et al. Pazopanib, a multitargeted tyrosine kinase inhibitor, reduces diabetic retinal vascular leukostasis and leakage. Microvasc Res. 2011 Nov;82(3):346-50.