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产品名称：TAK-593
产品别名：TAK-593

生物活性：

Description	TAK-593 is a potent VEGFR and PDGFR family inhibitor with IC50s of 3.2, 0.95, 1.1, 4.3 and 13 nM for VEGFR1, VEGFR2, VEGFR3, PDFGRα and PDFGRβ, respectively.

IC_{50} & Target	VEGFR1	VEGFR2	VEGFR3	PDGFRα	PDGFRβ	$PDGFR\alpha^{V561D}$
	3.2 nM (IC_{50})	0.95 nM (IC_{50})	1.1 nM (IC_{50})	4.3 nM (IC_{50})	13 nM (IC_{50})	1 nM (IC_{50})

In Vitro	TAK-593 inhibits growth of HUVEC with an IC50 of 0.30 nM. It shows potent inhibitory activity against VEGFR (VEGFR1-3: IC50=3.2, 0.95, 1.1 nM) and PDGFR (PDGFRα, β: IC50=4.3, 13 nM) families. Against other kinases, the IC50 values of TAK-593 are above 100 nM, except for Fms (IC50=10 nM) and Ret (IC50=18 nM) kinases[1]. TAK-593 potently inhibits VEGF- and PDGF-stimulated cellular phosphorylation and proliferation of human umbilical vein endothelial cells and human coronary artery smooth muscle cells. TAK-593 also potently inhibits VEGF-induced tube formation of endothelial cells co-cultured with fibroblasts[2].
In Vivo	TAK-593 inhibits growth of HUVEC with an IC50 of 0.30 nM. It shows potent inhibitory activity against VEGFR (VEGFR1-3: IC50=3.2, 0.95, 1.1 nM) and PDGFR (PDGFRα, β: IC50=4.3, 13 nM) families. Against other kinases, the IC50 values of TAK-593 are above 100 nM, except for Fms (IC50=10 nM) and Ret (IC50=18 nM) kinases[1]. TAK-593 potently inhibits VEGF- and PDGF-stimulated cellular phosphorylation and proliferation of human umbilical vein endothelial cells and human coronary artery smooth muscle cells. TAK-593 also potently inhibits VEGF-induced tube formation of endothelial cells co-cultured with fibroblasts[2].

Solvent&Solubility

In Vitro:

DMSO : ≥ 48.5 mg/mL (108.87 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2448 mL	11.2241 mL	22.4482 mL
5 mM	0.4490 mL	2.2448 mL	4.4896 mL
10 mM	0.2245 mL	1.1224 mL	2.2448 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:

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: ≥ 2.67 mg/mL (5.99 mM); Clear solution 此方案可获得 ≥ 2.67 mg/mL (5.99 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 26.7 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: ≥ 2.67 mg/mL (5.99 mM); Clear solution 此方案可获得 ≥ 2.67 mg/mL (5.99 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 26.7 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: ≥ 2.67 mg/mL (5.99 mM); Clear solution 此方案可获得 ≥ 2.67 mg/mL (5.99 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 26.7 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。
References	[1]. Miyamoto N, et al. Discovery of N-[5-({2-[(cyclopropylcarbonyl)amino]imidazo[1,2-b]pyridazin-6-yl}oxy)-2-methylphenyl]-1,3-dimethyl-1H-pyrazole-5-carboxamide (TAK-593), a highly potent VEGFR2 kinase inhibitor. Bioorg Med Chem. 2013 Apr 15;21(8):2333-2345. [2]. Awazu Y, et al. Anti-angiogenic and anti-tumor effects of TAK-593, a potent and selective inhibitor of vascular endothelial growth factor and platelet-derived growth factor receptor tyrosine kinase. Cancer Sci. 2013 Apr;104(4):486-94.

实验参考：

Cell Assay	HUVECs are seeded into a 96-well plate at 3000 cells/well in Human Endothelial-SFM Growth Medium (Invitrogen) containing 3% fetal bovine serum (FBS) and are incubated overnight at 37 C in a 5% CO_2 incubator. Various concentrations of the test compounds (TAK-593) are added in the presence of 60 ng/mL VEGF, and the cells are cultured for a further 5 days. Cellular proliferation is determined by the WST-8 formazan assay using Cell Counting Kit-8[1].
Animal Administration	Rats: The iv administration in rats is conducted under anesthesia with diethyl ether. At 5, 10 (only for iv dosing), 15, 30 min, and 1, 2, 3, 4, 6, 8, 12, 24, 32 (only for monkeys) and 48 h (only for monkeys) after dosing, blood is taken from the tail vein in rats or from the femoral vein in monkeys. Then, the blood is centrifuged to obtain the plasma fraction. The plasma is kept frozen at 20°C until analysis. The concentration of TAK-593 in plasma is determined by the high-performance liquid chromatography with a fluorescence detector. The excitation and emission are 346 and 420 nm, respectively. Mice: Test compounds are administered at a dose of 10 mg/kg as a cassette dosing to nonfasted mice (BALB/cAJcl; female). After oral administration, blood samples are collected. The blood samples are centrifuged to obtain the plasma fraction. The plasma samples are deproteinized with acetonitrile containing an internal standard. After centrifugation, the supernatant is diluted with a

	mixture of 0.01 M ammonium formate solution and acetonitrile (9:1, v/v) and centrifuged again. The compound concentrations in the supernatant are measured by LC/MS/MS[1].
Kinase Assay	Enzyme reactions are performed in 50 mM TrisHCl pH 7.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 0.01% Tween-20 and 2 mM DTT, containing 10 μM ATP, 0.1 μg/mL biotinylated polyGluTyr (4:1) and 0.1 nM of VEGFR2. Prior to catalytic initiation with ATP, compound (TAK-593) and enzyme are incubated for 5 min at room temperature (preincubation). The reactions are quenched by the addition of 25 μL of 100 mM EDTA, 10 μg/mL AlphaScreen streptavidine donor beads and 10 μg/mL acceptor beads in 62.5 mM HEPES pH 7.4, 250 mM NaCl, and 0.1% BSA. Plates are incubated in the dark overnight and then read by plate reader[1].
References	[1]. Miyamoto N, et al. Discovery of N-[5-({2-[(cyclopropylcarbonyl)amino]imidazo[1,2-b]pyridazin-6-yl}oxy)-2-methylphenyl]-1,3-dimethyl-1H-pyrazole-5-carboxamide (TAK-593), a highly potent VEGFR2 kinase inhibitor. Bioorg Med Chem. 2013 Apr 15;21(8):2333-2345. [2]. Awazu Y, et al. Anti-angiogenic and anti-tumor effects of TAK-593, a potent and selective inhibitor of vascular endothelial growth factor and platelet-derived growth factor receptor tyrosine kinase. Cancer Sci. 2013 Apr;104(4):486-94.