产品名称：**TW-37**
产品别名：**TW-37**

生物活性：

Description	TW-37 is a potent Bcl-2 inhibitor with K_i values of 260, 290 and 1110 nM for Mcl-1, Bcl-2 and Bcl-xL, respectively.		
IC_{50} & Target	Mcl-1	Bcl-2	Bcl-xL
	260 nM (Ki)	290 nM (Ki)	1110 nM (Ki)
In Vitro	TW-37 (TW37) is a novel nonpeptide small-molecule inhibitor designed using a structure-based design strategy. TW-37 targets the BH3-binding groove in Bcl-2 where proapoptotic Bcl-2 proteins, such as Bak, Bax, and Bid bind. In fluorescence polarization-based binding assays using recombinant Bcl-2 and Bcl-xL proteins, TW-37 binds to Bcl-2 and Bcl-xL with Ki values of 290 and 1110 nM, respectively. TW-37 has an IC50 of 1.8 μM for endothelial cells but shows no cytotoxic effects for fibroblasts at concentrations up to 50 μM. The mechanism of TW-37-induced endothelial cell death is apoptosis, in a process mediated by mitochondrial depolarization and activation of caspase-9 and caspase-3. The effect of TW-37 on endothelial cell apoptosis is not prevented by coexposure to the growth factor milieu secreted by tumor cells. Inhibition of the angiogenic potential of endothelial cells (i.e., migration and capillary sprouting assays) and expression of the angiogenic chemokines CXCL1 and CXCL8 are accomplished at subapoptotic TW-37 concentrations (0.005-0.05 μM)[1]. TW-37 is a potent Bcl-2 and Mcl-1 inhibitor. In fluorescence polarization-based binding assays using recombinant Bcl-2, Bcl-xL, and Mcl-1 proteins, TW-37 binds to Bcl-2, Bcl-xL, and Mcl-1 with Ki values of 290, 1,110 and 260 nM, respectively[2].		
In Vivo	A murine model of humanized vasculature is used to investigate the biological effect of TW-37 (TW37) on human microvascular endothelial cell in vivo. Using this model, a significant decrease is observed in total blood vessel number ($P<0.05$) comparing both 3 and 30 mg/kg TW-37 against vehicle control. In addition to reduction in total number of blood vessels, an unusual number of occluded vessels are occurring in the treated groups. The levels of vessel occlusion are assessed by counting completely blocked vessels and determining their number as a percentage of total vessel number. TW-37 concentration mediates a significant increase in the number of occluded vessels when compared with control[1].		
Solvent&Solubility	***In Vitro:*** **DMSO : ≥ 42 mg/mL (73.21 mM)** * "≥" means soluble, but saturation unknown.		

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.7431 mL	8.7154 mL	17.4307 mL
	5 mM	0.3486 mL	1.7431 mL	3.4861 mL
	10 mM	0.1743 mL	0.8715 mL	1.7431 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: ≥ 2.5 mg/mL (4.36 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.36 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 25.0 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。
References	[1]. Zeitlin BD, et al. Antiangiogenic effect of TW37, a small-molecule inhibitor of Bcl-2. Cancer Res. 2006 Sep 1;66(17):8698-706. [2]. Mohammad RM, et al. Preclinical studies of TW-37, a new nonpeptidic small-molecule inhibitor of Bcl-2, in diffuse large cell lymphoma xenograft model reveal drug action on both Bcl-2 and Mcl-1. Clin Cancer Res. 2007 Apr 1;13(7):2226-35.

实验参考：

Cell Assay	The sulforhodamine B (SRB) cytotoxicity assay is used. Briefly, optimal cell density for cytotoxicity assay, 2×10^4 to 3×10^4 cells per well, is determined by growth curve analysis. HDMECs are seeded at 2.5×10^4 per well in a 96-well plate and allowed to adhere overnight. Drug or control is diluted in EGM2-MV and layered onto cells, which are allowed to incubate for times as indicated in the figures. Alternatively, HDMECs are coincubated with TW-37 and 0 to 100 ng/mL recombinant human VEGF $(rhVEGF)_{165}$ or 0 to 100 ng/mL recombinant human CXCL8. Cells are fixed on the plates by addition of cold trichloroacetic acid (10% final concentration) and incubation for 1 hour at 4°C. Cellular protein is stained by addition of 0.4% SRB in 1% acetic acid and incubation at room temperature for 30 minutes. Unbound SRB is removed by washing with 1% acetic acid and the plates are air dried. Bound SRB is resolubilized in 10 mM unbuffered Tris-base and absorbance is determined on a microplate reader at 560 nm. Test results are normalized against initial plating density and drug-free controls. Data are obtained from triplicate wells per condition and are representative of at least three independent experiments[1]
Animal Administration	Mice[1] Porous poly L-lactic acid scaffolds (6×6×1 mm) with an average pore diameter of 180 μm are fabricated. Just before implantation, scaffolds are seeded with 1×10^6 HDMECs in a 1:1 Matrigel/EGM2-MV mix. Male severe combined immunodeficient (SCID) mice (CB.17.SCID) are anesthetized with ketamine and xylazine, and two scaffolds are implanted s.c. in the dorsal region of each mouse. At 10 days after transplantation, six mice per treatment are treated with 3 mg/kg or 30 mg/kg TW-37 (in vehicle: PBS/Tween 80/ethanol) or vehicle alone i.v. for 5 consecutive days. At the end of the treatment period, mice are euthanized, and the scaffolds are retrieved, fixed overnight in 10% buffered formaldehyde at 4°C, and mounted on glass slides. Immunohistochemistry is done for Factor VIII and microvessels are counted in 6 fields per scaffold and 12 scaffolds per treatment at ×200 magnification. Alternatively, sections are stained with H&E and occluded blood vessels are counted.
References	[1]. Zeitlin BD, et al. Antiangiogenic effect of TW37, a small-molecule inhibitor of Bcl-2. Cancer Res. 2006 Sep 1;66(17):8698-706. [2]. Mohammad RM, et al. Preclinical studies of TW-37, a new nonpeptidic small-molecule inhibitor

	of Bcl-2, in diffuse large cell lymphoma xenograft model reveal drug action on both Bcl-2 and Mcl-1. Clin Cancer Res. 2007 Apr 1;13(7):2226-35.