产品名称：4-(苯基甲基)-1-哌嗪乙酸 2-[[2-羟基-3-(2-烯丙-1-基)苯基]亚甲基]酰肼
产品别名：半胱天冬酶原活化物 1；PAC-1；Procaspase activating compound 1

生物活性：

Description	PAC-1 is an activator of procaspase-3 induces apoptosis in cancer cells with EC_{50} of 2.08 μM.
IC_{50} & Target [1]	Procaspase-3
	2.08 μM (EC_{50})
In Vitro	PAC-1 activates procaspase-3 with an EC50 of 2.08 μM. PAC-1 exhibits an enhanced zinc chelating ability (EC50= 7.08 μM). PAC-1 induces leukemia cell death with IC50 of 4.03 μM, which is consistent with the values reported by other investigators. PAC-1 treatment also results in death of other malignant cells in a concentration-dependent manner with IC50s ranging from 4.03 to 53.44μM. The overall mean IC50 in the fifteen malignant cell lines is 0.88 mM for WF-210 and 19.40 μM for PAC-1. In contrast, the sensitivity of the normal human cells (PBL, L-02, HUVEC and MCF 10A) to WF-210 is 2.6-fold lower (mean IC50=412.34 μM) than PAC-1 (mean IC50=158.29 μM)[1]. Procaspase-activating compound-1 (PAC-1) is the first direct caspase-activating compound discovered. PAC-1 treatment upregulates Ero1α in multiple cell lines, whereas silencing of Ero1α significantly inhibits calcium release from ER and cell death[2].
In Vivo	To evaluate the in vivo effect of WF-210 on the growth of malignant tumors, we examined the ability of WF-210 to suppress tumor growth in mouse Hep3B and MDA-MB-435 xenograft models. These two cell lines express procaspase-3 at relatively high levels. Tumors induced by xenografts of the liver cancer cell Hep3B are allowed to develop and grow to a size of 100 mm3, after which WF-210 (2.5 mg/kg) or PAC-1 (5.0 mg/kg) is given daily for two weeks by intravenous (i.v.) administration. As shown in both PAC-1 and WF-210 significantly inhibits the growth of Hep3B tumor xenografts[1].
Solvent&Solubility	***In Vitro:*** **DMSO : 50 mg/mL** (**127.39 mM**; Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.5478 mL	12.7392 mL	25.4784 mL
	5 mM	0.5096 mL	2.5478 mL	5.0957 mL
	10 mM	0.2548 mL	1.2739 mL	2.5478 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 (6.37 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.37 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。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 2.5 mg/mL (6.37 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (6.37 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 25.0 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: ≥ 2.5 mg/mL (6.37 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.37 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Wang F, et al. A novel small-molecule activator of procaspase-3 induces apoptosis in cancer cells and reduces tumor growth in human breast, liver and gallbladder cancer xenografts. Mol Oncol. 2014 Dec;8(8):1640-52. [2]. Seervi M, et al. ERO1α-dependent endoplasmic reticulum-mitochondrial calcium flux contributes to ER stress and mitochondrial permeabilization by procaspase-activating compound-1 (PAC-1). Cell Death Dis. 2013 Dec 19;4:e968. [3]. Putt KS, et al. Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy. Nat Chem Biol. 2006 Oct;2(10):543-50.

实验参考：

Cell Assay	Cell viability is measured using the MTT method or the Cell Titer-Glo luminescent assay. For the MTT assay, the cells (1×10^5 cells/mL) are seeded into 96- well culture plates. After overnight incubation, cells are treated with various concentrations of agents (PAC-1, WF-210 or other agents) for 24 or 72 h. Then 10 mL MTT solution (2.5 mg/mL in PBS) is added to each well, and the plates are incubated for an additional 4 h at 37°C. After centrifugation (2500 rpm, 10min), the medium containing MTT is aspirated, and100mL DMSO is added. The optical density of each well is measured at 570 nm with a Biotek Synergy HT Reader. The Cell Titer-Glo kit is used to determine the relative levels of intracellular ATP as a biomarker for live cells[1].
Animal Administration	Mice[1] To determine the in vivo anti-tumor activity of WF-210, viable human gallbladder cancer GBC-SD cells (5×10^6/100 mL PBS per mouse), human breast cancer MDA-MB-435 cells (1×10^7/100 mL PBS per mouse), human liver cancer Hep3B cells (5×10^6/100 mL PBS per mouse) and human breast cancer MCF-7 cells (1×10^7/100 mL PBS per mouse) are subcutaneously (s.c.) injected into the right flank of 7- to 8-week old male SCID mice or Balb/c nude mice. Cell numbers are confirmed by trypan blue staining prior to injection. Specially, MCF-7 xenograft mice are also administered with the hormone 17-beta-estradiol (3 mg/kg) on alternate days. When the average s.c. tumor volume reached 100 mm^3, mice are randomly divided into various treatment and control groups (eight mice per group). Tumor size is measured once every two days with a caliper (calculated volume=shortest $diameter^2\times$longest diameter/2). Body weight, diet consumption and tumor size are recorded once every two days. After two or four weeks, mice are sacrificed and tumors are excised and stored at -80°C until further analysis.

Kinase Assay	Various concentrations of WF-210 or PAC-1 are added to procaspase-3 in buffer containing 50 mM HEPES, 0.1% CHAPS, 10% glycerol, 100 mM NaCl, 0.1 mM EDTA, 10 mM DTT pH 7.4,and incubated for 12 h at 37°C. The final volume is 10 mL and the final concentration of procaspase-3 is 1 mM. Then 40 mL of the substrate Ac-DEVD-pNA (final concentration 0.4 mM) in buffer containing 50 mM HEPES pH 7.4, 100 mM NaCl, 10 mM DTT, 0.1 mM EDTA disodium salt, 0.10% CHAPS, 10% glycerol is added and the absorbance of the plate is read at 405 nm for a total of 1 h. The slope of the linear portion for each well is determined as the enzyme activity[1].
References	[1]. Wang F, et al. A novel small-molecule activator of procaspase-3 induces apoptosis in cancer cells and reduces tumor growth in human breast, liver and gallbladder cancer xenografts. Mol Oncol. 2014 Dec;8(8):1640-52. [2]. Seervi M, et al. ERO1α-dependent endoplasmic reticulum-mitochondrial calcium flux contributes to ER stress and mitochondrial permeabilization by procaspase-activating compound-1 (PAC-1). Cell Death Dis. 2013 Dec 19;4:e968. [3]. Putt KS, et al. Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy. Nat Chem Biol. 2006 Oct;2(10):543-50.