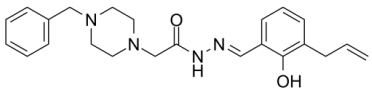
Data Sheet

Product Name:	PAC-1
Cat. No.:	CS-3498
CAS No.:	315183-21-2
Molecular Formula:	C23H28N4O2
Molecular Weight:	392.49
Target:	Apoptosis; Autophagy; Caspase
Pathway:	Apoptosis; Autophagy
Solubility:	DMSO : 50 mg/mL (127.39 mM; Need ultrasonic); H2O : < 0.1 mg/mL (insoluble)



BIOLOGICAL ACTIVITY:

PAC-1 is an activator of **procaspase-3** induces apoptosis in cancer cells with **EC_{50}** of 2.08 μM. IC50 & Target: EC50: 2.08 μM (procaspase-3)[1] **In Vitro:** PAC-1 activates procaspase-3 with an EC_{50} of 2.08 μM. PAC-1 exhibits an enhanced zinc chelating ability (EC_{50}= 7.08 μM). PAC-1 induces leukemia cell death with IC_{50} 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 IC_{50}s ranging from 4.03 to 53.44μM. The overall mean IC_{50} 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 IC_{50}=412.34 μM) than PAC-1 (mean IC_{50}=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 mm^3, 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].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]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]. **Cell Assay:** PAC-1 is dissolved in DMSO and stored, and then diluted with appropriate medium before use[1].[1]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:** [1]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 $diameter^2$×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.

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.

CAIndexNames:

1-Piperazineacetic acid, 4-(phenylmethyl)-, 2-[[2-hydroxy-3-(2-propen-1-yl)phenyl]methylene]hydrazide

SMILES:

O=C(N/N=C/C1=CC=CC(CC=C)=C1O)CN2CCN(CC3=CC=CC=C3)CC2

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