Datasheet

Carfilzomib (PR-171)

Product Name	Carfilzomib (PR-171)
Catalogue Number	BSV-S2853
Chemical Formula	$C_{40}H_{57}N_5O_7$
Function	Proteasome inhibitor
CAS No.:	868540-17-4

Description:

Carfilzomib (PR-171) is an irreversible **proteasome** inhibitor with **IC50** of <5 nM in ANBL-6 cells, displayed preferential in vitro inhibitory potency against the ChT-L activity in the β5 subunit, but little or no effect on the PGPH and T-L activities.

Product Details:

Target: Proteasome [1] (ANBL-6 cells)

Chemical name: L-Phenylalaninamide, (αS)-α-[[2-(4 morpholinyl)acetyl]amino] benzenebutanoyl-L-leucyl-N-[(1S)-3-methyl-1-[[(2R)-2-methyl-2-oxiranyl]carbonyl]butyl]-

Formula: $C_{40}H_{57}N_5O_7$

Molecular weight: 719.91

Purity: **99.49% (HPLC)**

Solubility: 100 mg/mL (DMSO)

Storage: 3 years -20°C powder, 2 years -80°C in solvent

Regulatory/ Restrictions: For laboratory use only.

Biological Activity:

In vitro:
Carfilzomib inhibits proliferation in a variety of cell lines and patient-derived neoplastic cells, including multiple myeloma, and induced intrinsic and extrinsic apoptotic signaling pathways and activation of c-Jun-N-terminal kinase (JNK). Carfilzomib reveals enhanced anti-MM activity compared with bortezomib, overcome resistance to bortezomib and other agents, and acts synergistically with dexamethasone (Dex). Carfilzomib shoes preferential in vitro inhibitory potency against the ChT-L activity in the β5 subunit, with over 80% inhibition at doses of 10 nM. Short exposure to low-dose Carfilzomib leads to preferential binding specificity for the β5 constitutive 20S proteasome and the β5i immunoproteasome subunits. Measurement of caspase activity in ANBL-6 cells pulsed with Carfilzomib reveals substantial increases in caspase-8, caspase-9, and caspase-3 activity after 8 hours, giving a 3.2-, 3.9- and 6.9-fold increase, respectively, over control cells after 8 hours. In carfilzomib pulse-treated cells, the mitochondrial membrane integrity is decreased to 41% (Q1 + Q2), compared with 75% in vehicle-treated control cells. [1] In another study, Carfilzomib has also shown preclinical effectiveness against hematological and solid malignancies. [2] Carfilzomib directly inhibits osteoclasts formation and bone resorption. [3]

In vivo:
Carfilzomib moderately reduces tumor growth in an in vivo xenograft model. Carfilzomib effectively decreases multiple myeloma cell viability following continual or transient treatment mimicking. Carfilzomib increases trabecular bone volume, decreases bone resorption and enhances bone formation in non-tumor bearing mice. [3]

Preparing stock solutions

ConcentrationMass	1 mg	5 mg	10 mg
1 mM	1.3891 mL	6.9453 mL	13.8906 mL
5 mM	0.2778 mL	1.3891 mL	2.7781 mL
10 mM	0.1389 mL	0.6945 mL	1.3891 mL
50 mM	0.0278 mL	0.1389 mL	0.2778 mL

Protocol (only for reference)

Kinase Assay: [1]	
Enzyme-linked immunosorbent assay for subunit profiling of carfilzomib	ANBL-6 cells (2 × 10^6/well) are plated in 96-well plates and treated with Carfilzomib doses from 0.001 to 10 μM for 1 hour. Cells are then lysed (20 mM Tris-HCl, 0.5 mM EDTA), and cleared lysates are transferred to polymerase chain reaction (PCR) plates. A standard curve is generated using untreated ANBL-6 cell lysates starting at a concentration of 6 μg protein/μL. The active site probe [biotin-(CH2)4-Leu-Leu-Leu-epoxyketone; 20 μM] is added and incubated at room temperature for 1 hour. Cell lysates are then denatured by adding 1% sodium dodecyl sulfate (SDS) and heating to 100°C, followed by mixing with 20 μL per well streptavidin-sepharose high-performance beads in a 96-well multiscreen DV plate and incubated for 1 hour. These beads are then washed with enzyme-linked immunosorbent assay (ELISA) buffer (PBS, 1% bovine serum albumin, and 0.1% Tween-20), and incubated overnight at 4°C on a plate shaker with antibodies to proteasome subunits. Antibodies used included mouse monoclonal anti-β1, anti-β2, anti-β1i, and anti-β5i, goat polyclonal anti-β2i, and rabbit polyclonal anti-β5 (affinity-purified antiserum against KLH-CWIRVSSDNVADLHDKYS peptide). The beads are washed and incubated for 2 hours with horseradish peroxidase-conjugated secondary goat antirabbit, goat antimouse or rabbit antigoat antibodies. After washing, the beads are developed using the supersignal ELISA picochemiluminescence substrate. Luminescent detection is performed. Raw luminescence is converted to μg/mL by comparison with the standard curve and expressed as the % inhibition relative to vehicle control. Curve fits are generated using the following nonsigmoidal dose-response equation: Y = Bottom + (Top-Bottom)/(1 + 10^((LogEC50 − X) × HillSlope)), where X is the logarithm of concentration, Y is the % inhibition, and EC50 is the dose showing 50% effect.

Cell Assay: [1]	
Cell lines	WST-1, ANBL-6 cells
Concentrations	100 nM
Incubation Time	1 hour
Method	WST-1 is used to determine the effects of proteasome inhibitor Carfilzomib on cell proliferation. The inhibition of proliferation is calculated in relation to parallel control cells that receives vehicle alone. A linear spline function is used to interpolate the median inhibitory concentration (IC50) using XLfit 4 software. The degree of resistance (DOR) is calculated using the formula:

	DOR = IC50(resistant cells)/IC50(sensitive cells). ANBL-6 cells pulsed with 100 nM carfilzomib are washed and suspended in PBS containing 5 μg/mL of JC-1, which exhibits potential-dependent accumulation in mitochondria. Analysis of the mitochondrial membrane potential-dependent color shift from 525 to 590 nm is carried out on a FacScan, and the data are analyzed with CellQuest software.
Animal Study: [4]	
Animal Models	Beige-nude-XID mice
Formulation	10% sulfobutylether β-cyclodextrin in 10 mmol/L citrate buffer pH 3.5,
Dosages	2.0 mg/kg
Administration	i.v.

References:

[1] Kuhn DJ, et al. Blood. 2007, 110(9), 3281-3290.
[2] Kuhn DJ, et al. Curr Cancer Drug Targets. 2011, 11(3), 285-295.
[3] Hurchla MA, et al. Leukemia. 2012.
[4] Dasmahapatra G, et al. Mol Cancer Ther. 2011, 10(9), 1686-1697.