产 品 说 明 书

MG132

MG-132(Z-Leu-Leu-Leu-al)是一种 26S 蛋白酶体抑制剂(IC50=100 nM)，具有细胞渗透性、可逆性。MG-132 可作为自噬激活剂，可诱导凋亡。

化学性质

分子量	CAS 号	储存条件(自收到货起)
475.62	133407-82-6	3 年 -20° C powder
		1 年 -80° C in solvent

溶液配置表

可选溶剂	浓度 / 体积 / 质量	1 mg	5 mg	10mg	25mg
Ethanol/DMSO	**1 mM**	2.1025 mL	10.5126 mL	21.0252 mL	52.563 mL
	5 mM	0.4205 mL	2.1025 mL	4.205 mL	10.5126 mL
	10 mM	0.2103 mL	1.0513 mL	2.1025 mL	5.2563 mL
	20 mM	0.1051 mL	0.5256 mL	1.0513 mL	2.6281 mL
	50mM	0.0421 mL	0.2103 mL	0.4205 mL	1.0513 mL
Ethanol	**100mM**	0.021 mL	0.1051 mL	0.2103 mL	0.5256 mL

推荐的实验操作

产品描述	MG-132 (Z-Leu-Leu-Leu-al) is a 26S proteasome inhibitor (IC50=100 nM) that is cell-permeable and reversible. MG-132 acts as an autophagy activator and also induces apoptosis.
靶点活性	20S proteasome:100 nM (cell free), Calpain:1.2 μM (cell free)
体外活性	**方法：**人宫颈癌细胞 HeLa 用 MG-132 (0.5-30 μM) 处理 24 h，使用 MTT 方法检测细胞生长抑制情况。 **结果：**MG-132 剂量依赖性地抑制 HeLa 细胞生长，IC50 约为 5 μM。 **方法：**人间皮瘤细胞 NCI-H2452 用 MG-132 (0.25-2 μM) 处理 36 h，使用 Western Blot 方法检测靶点蛋白表达水平。 **结果：**MG-132 处理诱导 NCI-H2052 细胞中 caspases 3、caspases 7、Bid 和 PARP 的切割，诱导 caspase 依赖性凋亡。 **方法：**人类黑色素瘤细胞 MeWo 用 MG-132 (0.01-1 μM) 处理 24 h，使用 Flow Cytometry 方法分析细胞周期情况。 **结果：**MG-132 诱导 MeWo 细胞的细胞周期阻滞在 G2 期。
体内活性	**方法：**为检测体内抗肿瘤活性，将 MG-132 (1 mg/kg) 静脉注射给携带人宫颈癌肿瘤 HeLa、CaSki 或 C33A 的 C.B-17/lcr-scid/scidJcl 小鼠，每周两次，持续四周。 **结果：**MG-132 治疗显著抑制人宫颈癌肿瘤的生长，表明在体内具有抗肿瘤活性。 **方法：**为研究 MG-132 长期治疗对心肌肥大的影响及其相关分子机制，将 MG-132 (0.1 mg/kg) 腹腔注射给具有腹主动脉束带（AAB）的大鼠，每天一次，持续八周。 **结果：**MG-132 治疗显著减弱了 AAB 大鼠的左心室肌细胞面积、左心室重量/体重和肺重量/体重比，降低了左心室舒张直径和壁厚，并增加了缩短分数。MG-132 治疗可显著逆转 AAB 大鼠 ERK1/2 和 JNK1 磷酸化水平的升高。

激酶实验	Inhibitory activities of ZLLa1 and ZLLLal against m-calpain and 20S proteasome were measured by previously described methods.For the m-calpain inhibitory assay,the 0.5 ml reaction mixture contained 0.24% alkali-denatured casein,28 mM 2-mercaptoethanol,0.94 unit of m-calpain,ZLLal or ZLLLal,6 mM CaCl2,and 0.1M Tris-HC1 (pH 7.5).The reaction was started by the addition of m-calpain solution and stopped by the addition of 0.5 ml of 10% trichloroacetic acid after incubation at 30℃ for 15 min.After centrifugation at 1,300×g for 10 min,the absorbance of the supernatant at 280 nm was measured.The reaction mixture for the 20S proteasome inhibitory assay contained 0.1 M Tris-acetate,pH 7.0,20S proteasome,ZLLa1 or ZLLLal,and 25 μM substrate dissolved in dimethyl sulfoxide in a final volume of 1 ml.After incubation at 37℃ for 15 min,the reaction was stopped by the addition of 0.1 ml of 10% SDS and 0.9 ml of 0.1 M Tris-acetate,pH 9.0.The fluorescence of the reaction products was measured.To determine the IC50s against m-calpain and 20S proteasome,various concentrations of the synthetic peptide aldehydes were included in the assay mixture .
细胞实验	The effect of MG132 on HeLa cell growth was determined by trypan blue exclusion cell counting or measuring MTT dye absorbance of living cells as previously described. In brief, cells (5x10^5 cells per well) were seeded in 24-well plates for cell counting, and cells (5x10^4 cells per well) were seeded in 96-well microtiter plates for the MTT assay. After exposure to indicated amounts of MG132 for 24 h, cells in 24-well plates or 96-well plates were collected with trypsin digestion for trypan blue exclusion cell counting or were used for the MTT assay. Twenty microliters of MTT solution (2 mg/ml in PBS) was added to each well of 96-well plates. The plates were again incubated for 4 h at 37?C. MTT solution in the medium was aspirated off and 200 μl of DMSO was added to each well to solubilize the formazan crystals formed in viable cells. Optical density was measured at 570 nm using a microplate reader. Each plate contained multiple wells at a given experimental condition and multiple control wells. This procedure was replicated for 2-4 plates per condition .
动物实验	Male Sprague–Dawley rats (8 weeks old, 180 – 230 g) were used to establish a pressure-overload model as described previously. All animals were separated into four groups (10 rats per group): (i) vehicle-treated sham group; (ii) MG132-treated sham group; (iii) vehicle-treated abdominal aortic banding (AAB) group; and (iv) MG132-treated AAB group. Under intraperitoneal pentobarbital (50 mg/kg) anesthesia, AAB was created using a 5-0 suture tied twice around the abdominal aorta in which. a 21-gauge needle was inserted. The needle was then retracted yielding a 70 – 80% constriction with an outer aortic diameter of 0.8 mm. In the sham surgery rats, the same surgery was performed as described above except the aorta was constricted. At Day 3 after the surgery, MG132-treated rats were intraperitoneally injected with 0.1 mg/kg/day of MG132 for 8 weeks. All control animals were injected with a corresponding volume of vehicle only (0.1% DMSO) . Sixteen-week-old male CD1 mice were used for all our experiments. Thirty minutes before the immobilization procedure, 0.1 mg/kg of buprenorphine was administrated IP. The mice were then anesthetized using isoflurane. The right hindlimb was immobilized as previously described. Briefly, the hindlimb was immobilized 7 days by stapling the foot exploiting normal dorso-tibial flexion using an Autosuture Royal 35W skin stapler. One tine was inserted close to the toe at the plantar portion of the foot while the other was inserted in the distal portion of the gastrocnemius. The other hindlimb was used as a control. During the immobilization period, the mice were injected subcutaneously with MG132 (7.5 mg/kg/dose) or vehicle (DMSO) twice daily. DMSO containing or not MG132 was diluted in sterile pure corn oil (1:100, injected volume 150 μL). After 7 days, the tibialis anterior (TA) muscles of immobilized and non-i