Costunolide inhibits FPTase with IC50 of 20 μM and also inhibits telomerase with IC50 of 90 μM and 65 μM in MCF-7 and MDA-MB-231 cells.

In vitro
Costunolide inhibits the growth and telomerase activity of MCF-7 and MDA-MB-231 cells in a concentration- and time-dependent manner. Costunolide also inhibits the farnesylation process of human lamin-B by farnesyl-proteintransferase (FPTase), in a dose dependent manner. Continuously treatment of Costunolide for 48 hours will significantly decrease proliferation of human tumor cells (A549, SK-OV-3, SK-MEL-2, XFP48 and HCT-1) in a dose-dependent manner. Costunolide induces apoptosis by ROS-mediated mitochondrial permeability transition and cytochrome C release to the cytosol in HL-60 human leukemia cells. A recent study indicates that Costunolide shows significant antifungal activity, including Trichophyton mentagrophytes, T. simii, T. rubrum, and so on.

In vivo
Costunolide inhibits angiogenic response by blocking the angiogenic factor signaling pathway. In a mouse corneal micropocket assay, Costunolide reduces VEGF-stimulated neovascularization in mice.

Description
Costunolide inhibits FPTase with IC50 of 20 μM and also inhibits telomerase with IC50 of 90 μM and 65 μM in MCF-7 and MDA-MB-231 cells.

Telomerase activity assay
The telomerase activity is measured by the TRAP assay using the TRAPez Telomerase Detection Kit, which includes primers of a 36-bp internal control (IC) for quantifying the amplification of telomerase activity within a linear range close to 2.5 logs. For RNase treatment, 10μL of extract are incubated with 1μg of RNase at 37 °C for 20 minutes. The products of the TRAP assay are resolved by electrophoresis in a nondenaturing12% PAGE in a buffer containing 0.5 × Tris–borate EDTA and detected by autoradiograph. For quantification of TRAP products, the dried gels are exposed to Fuji Imaging Plate at room temperature. Results are corrected for background, and a standard value of 100 is given to the untreated control cell signal. Signal intensities of Costunolide-treated cells are compared to the standard and are expressed as a fraction of the maximum value of 100.

FPTase assay
FPTase is partially purified from rat brain by ammonium sulfate fractionation and Mono Q column chromatography. A human lamin-B carboxyl-termius sequence peptide (biotin-TRASNRSCAIM) as a substrate of FPTase is supplied. The FPTase assay is performed. Briefly, the standard reaction mixture (25μL) containing [3H]farnesyl pyrophosphate, biotinylated TRANSRCASM, partially purified FPTase, reaction buffer and the indicated concentrations of test material is incubated at 37 °C for 20 minutes. The FPTase activity is determined by measuring the incorporation of the [3H]farnesyl group from [3H]farnesyl pyrophosphate into the substrate peptide using a liquid scintillation counter. The 1-HFP (1-hydroxyfarnesyl phosphonate) is used as a reference drug for the enzyme inhibition (IC50 1.0 μM). The FPTase activity is calculated as mean of three distinct experiments.

Cell Assay: [1]

Cell Lines
MCF-7 and MDA-MB-231 cells

Concentrations
0-100 μM

Incubation Time
48 hours

Methods
1) Plate 500-10,000 cells in 200 μL media per well in a 96 well plate. Leave 8 wells empty for blank controls. 2) Incubate (37 °C, 5% CO2) overnight to allow the cells to attach to the wells. 3) Add 2 μL of Costunolide dissolved in DMSO to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the samples into the media. 5) Incubate (37 °C, 5% CO2) for 48 hours to allow Costunolide to take effect. 6) Make 2 mL or more of MTT solution per 96 well plate at 5 mg/mL in PBS. Do not make a stock as MTT in solution is not stable long-term. 7) Add 20 μL MTT solution to each well. Place on a shaking table, 150 rpm for 5 minutes, to thoroughly mix the MTT into the media. 8) Incubate (37 °C, 5% CO2) for 1-5
Animal Study:[5]

| Animal Models | Hydron N containing VEGF are implanted into mouse cornea. |
| Formulation   | Costunolide is dissolved in dimethylsulfoxide (DMSO). |
| Doses         | 100 mg/kg |
| Administration| Intraperitoneal injection once daily. |

References

PLEASE KEEP THE PRODUCT UNDER -20°C FOR LONG-TERM STORAGE.

NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE

Specific storage and handling information for each product is indicated on the product datasheet. Most Selleck products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality, but save your shipping charges by using the most economical storage conditions for an overnight shipment. Upon receipt of the product, follow the storage recommendations on the product datasheet.