Supplemental material:

Synthesis of batimastat-loaded nanoparticles (EL-NP-BB 94)

PLA (10 mg, average MW 75k-120k) (Sigma Aldrich, St. Louis, MO) was dissolved in 1 ml acetone (VWR International, Radnor, PA). 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol) - 2000] (2 mg, DSPE-PEG (2000) Maleimide , Avanti Polar Lipids, Inc., Alabaster, AL) and BB-94 (Sigma Aldrich, St. Louis, MO) were dissolved in dimethyl sulfoxide (DMSO) (Sigma Aldrich, St. Louis, MO), and this solution was then added to the PLA solution. The polymer solution was added drop-wise (16 µl/sec) to water and kept under sonication (Omni Ruptor 4000) for 20 minutes at 4°C. Following sonication, the particles were washed twice with distilled water by centrifugation at 14000x g for 30 minutes at 4°C and then re-suspended in distilled water. The non-solvent (water) to solvent (acetone) ratio was 1:15 for all experiments. Three different batches containing ratios of 5:1, 10:1, and 15:1 polymer to BB-94 were prepared in which the ratio between the two polymers (PLA: DSPE-PEG(2000) Maleimide) was 4:1.

Nanoparticles characterization

The NP suspension (1 mg) was diluted in HPLC-grade water, and the ζ-potential and particle size were measured with a 90Plus particle size analyzer (Brookhaven Instruments Co, Holtsville, NY). Transmission Electron Microscopy (TEM) was used to study NPs morphology. A drop of 0.1 mg/ml NPs-water suspension was placed on a formvar-coated copper grid and dried overnight in vacuum desiccator. Grid-mounted samples were imaged using a Hitachi H7600 TEM.

Nanoparticle degradation study as assessed by gel permeation chromatography (GPC)

Nanoparticles (5 mg) were suspended in 5 mL of DI water at stirred at 37°C. At week 1 and week 4, NPs were isolated by filtration and lyophilized. NPs were dissolved in chloroform at a concentration of 1mg/ml. The solution was filtered directly into chromatography vials using 0.2µm PTFE filters. Polymer molecular weight was assessed by Size Exclusion Chromatography/ Gel Permeation Chromatography(SEC/GPC) using a Shodex KF-804L column on a Waters HPLC/GPC system equipped with an Autosampler, column heater, and refractive index (RI) Detector. Chloroform was used as the mobile phase at a flow rate 0.65 ml/min, and the column was kept at 30°C during all separation runs. The injection volume for GPC analysis was 50 µL. Polystyrene standards of 9, 35, 50, 100 and 200 kDa were used as markers, and a control sample of PLA (Average M_W 75,000-120,000 kDa) was used to validate the calibration curve reliability.

Polymer nanoparticles as prepared showed one broad peak at retention time at ~9 min. With time, low molecular weight products (with retention time ~12-13 min) appeared suggesting polymer degradation (Figure I).

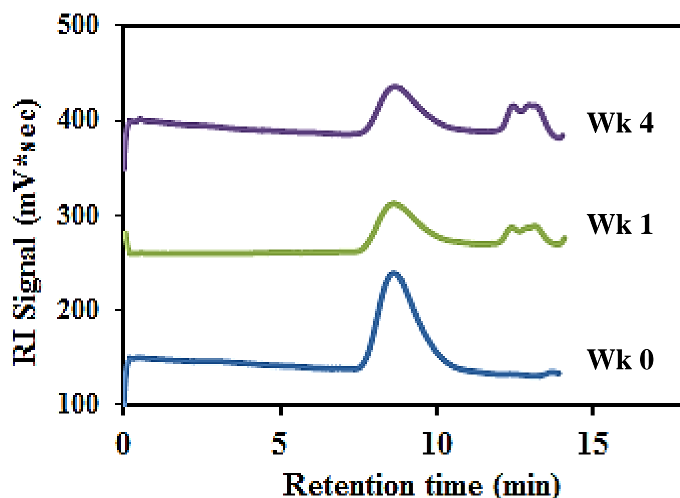

Figure I: GPC curves show peaks for degradation products at retention time (~13 min) at week 1 and 4.

Release profile of BB-94

To study release kinetics, a known amount of NPs was suspended in phosphate buffered saline (PBS) and incubated at 37°C on a shaker. Suspensions were centrifuged for 30 minutes at 10000×g at room temperature (RT). The supernatant was removed, and the sediment was freeze-dried and characterized for BB-94 concentration by dissolving it in 100 µl DMSO and measuring absorbance by UV spectrophotometry.

BB-94 activity

Briefly, NPs were dissolved in dichloromethane (Aldrich, MO) and DI water at a ratio of 3:1. This mixture was mixed vigorously and centrifuged for 10 minutes at 10000×g to extract BB-94 from the NPs. The DI water containing BB-94 was subsequently collected and lyophilized. The activity of the extracted BB-94 from the NPs was tested by two different methods. In the first approach, culture medium collected from rat aortic smooth muscle cell (RASMC) cultures was loaded into a SDS-PAGE zymography gel, and extracted BB-94 was added to the development buffer(500ng/ml) (50 mM Tris Base, 5 mM CaCl2•2H2O, 200 mM NaCl, 0.02% brij 35). Gels were stained with 0.5% coommasie blue for an hour at room temprature and were destained with 5% water, 40% methanol, 10% acetic acid. After this, the gels were photographed using epi-illuminated white light in a Bio-Rad Gel Doc instrument. Zymography bands were quantified using Image J software. In the second approach, BB-94 activity was tested in RASMCs cultures. Briefly, RASMCs were grown to 80% confluency at 37°C and 5% CO2 in DMEM containing 10% FBS and 1% penicillin-streptomycin (ScienCell Research Laboratories, Carlsbad, CA). After 24 hours, cells were treated with extracted BB-94 (500 nM) in serum-free medium for 24 hours. After treatment, conditioned medium was collected. The total protein of the culture medium was

quantified using Bicinchoninic Acid (BCA) protein assay (Pierce, Rockford, IL) with 10 μg total protein loaded per well and prestained molecular weight standards (Precision Plus Protein Standard, Bio-Rad, Hercules, CA). Gel zymography was performed as described in the previous section.

Reverse zymography for TIMP activity

After 24 hours incubation in the development buffer, gels were stained with 0.5% coommasie blue for an hour and were destained. The gel pictures were captured using epi-illuminated white light and a Bio-Rad Gel Doc instrument; bands were analyzed and quantified using Image J, and data was reported as RDU.

NP toxicity for rat aortic smooth muscle cells (RASMCs) and rat aortic endothelial cells (RAOEC)

RASMCs and RAOECs were cultured with NPs for 24 hours. MTT colorimetric assays (Sigma Aldrich, St. Louis, MO) were performed according to the manufacturer's protocol to confirm cell viability. Control and NP-treated cells were washed with PBS and incubated for 3 h with 55 μl of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml). Formazan crystals formation was detected at 570 nm and viability was shown as % MTT reduction compared to control. MTT activity was normalized to control.