Electronic Supplementary Material (ESI) for Nanoscale.
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# Electronic supplementary information (ESI)

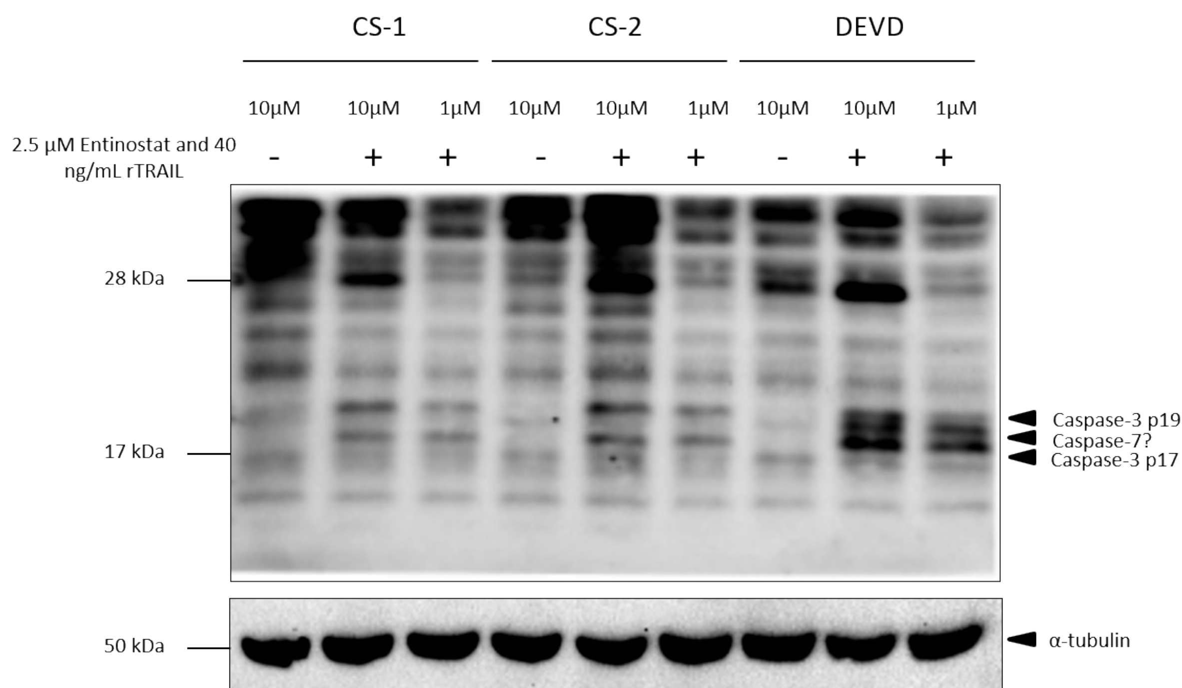


***Figure S1.*** *Complete blot presented in figure 3A. Cropping was performed to allow direct comparison of bands obtained by DEVD and CS1 probes. Another probe under evaluation (CS2) was not taken forward for this project.*

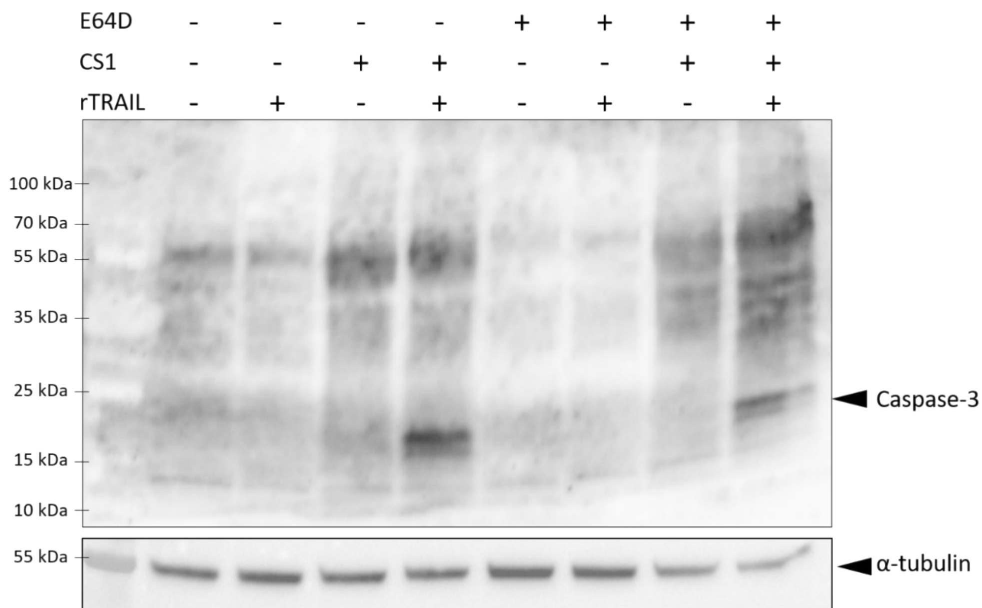


***Figure S2.*** *Western blot analysis of HCT116 cell lysates incubated with or without CS1 probe and E64d confirm the ability of CS1 to label caspase-3, avoiding labelling of cysteine cathepsins. HCT116 cells were treated with 10 ng/mL of rTRAIL for 5 hr, cell lysates were incubated with 10 μM of E64-d for 30 min at 37°C, shaking, following by incubation with 10 μM of CS1 for 1 hr, at 37°C, shaking.*

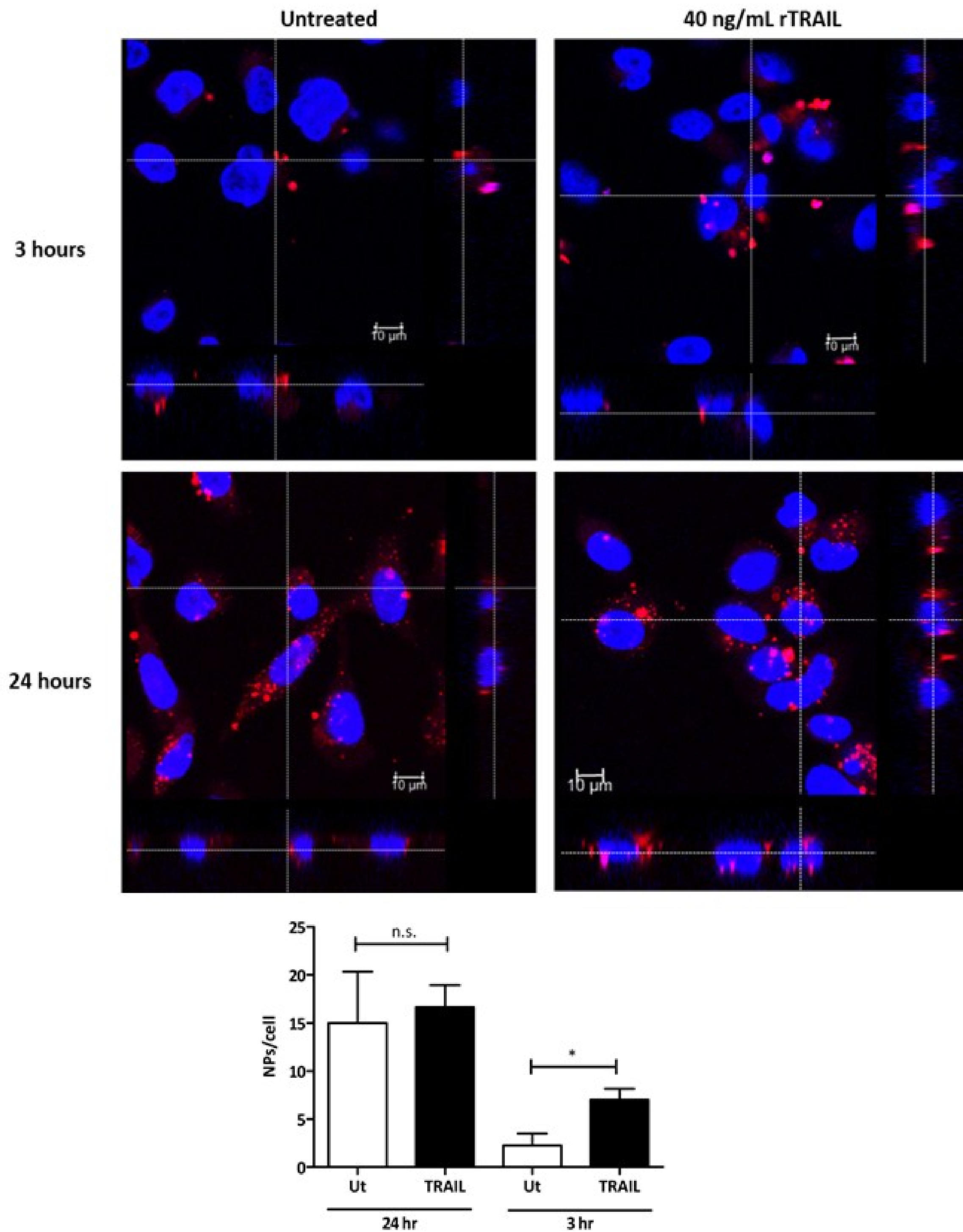


***Figure S3.*** *Confocal images with Z-stack showing different nanoparticle uptake in PC3 cells between untreated cells and apoptotic cells. Nuclei stained with DAPI come up in blue, nanoparticles loaded with nile-red come up in red. Only cells presenting nanoparticles uptake were taken into consideration for quantification.*

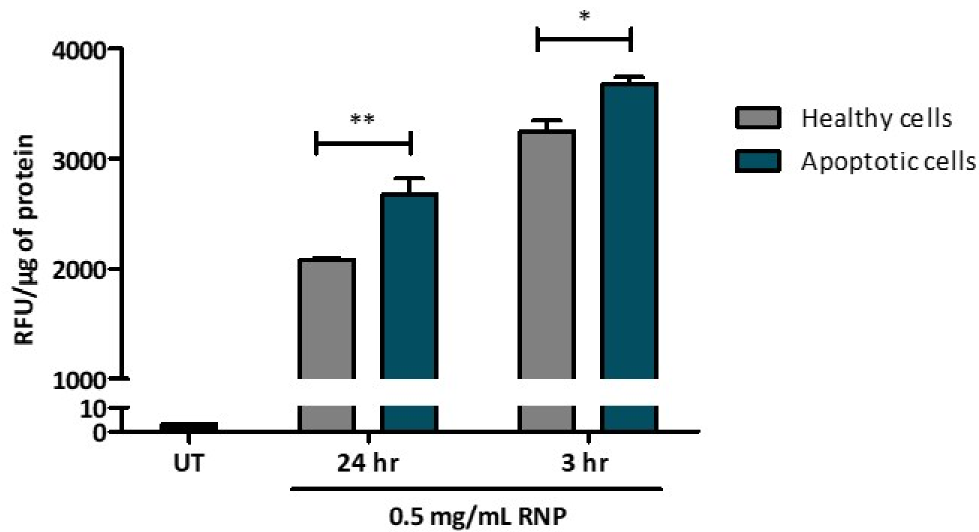


***Figure S4.*** *Nanoparticles uptake assay on PC3 cells. Cells were seeded in 6-well plate and treated with Rhodamine-6G loaded NPs for 24 and 3 hr, with or without pro-apoptotic stimuli, consisting in 2.5 µM entinostat for 24 hr and 40 ng mL$^{-1}$ rTRAIL 3 hr. Cells were washed with PBS (3x) and lysed. Following protein quantification, lysates were added to a 96-well black plate in triplicate and volume adjusted with PBS. Fluorescence from internalised RNPs was* read at $516/20_{Ex}$ $557/20_{Em}$ using Cytation5 plate reader (Biotek).

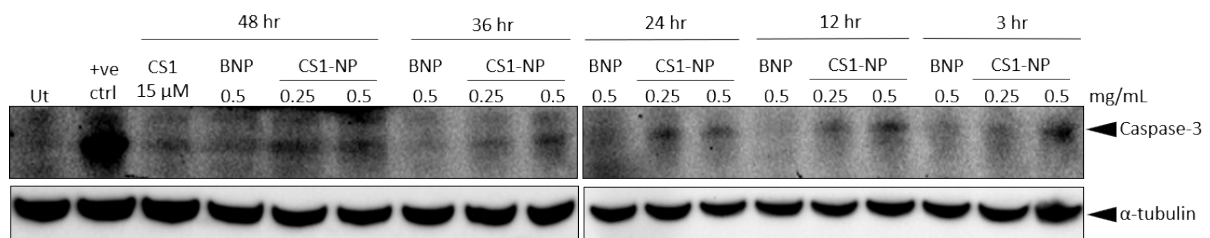


***Figure S5.*** *Caspase-3 labelling by CS1-NP up to 48 hours prior apoptosis stimulation with entinostat and rTRAIL. Positive control (+ve ctrl) was done by incubating apoptotic lysates with CS1 probe (1 hr, 37°C, shaking). Free CS1 was added to a final concentration of 15 µM, corresponding to the amount of CS1 in 0.5 mg mL$^{-1}$ of nanoparticles. Prior to stimulate the apoptotic process with rTRAIL, cells were washed of uninternalized NPs. Tubulin was used as loading control.*

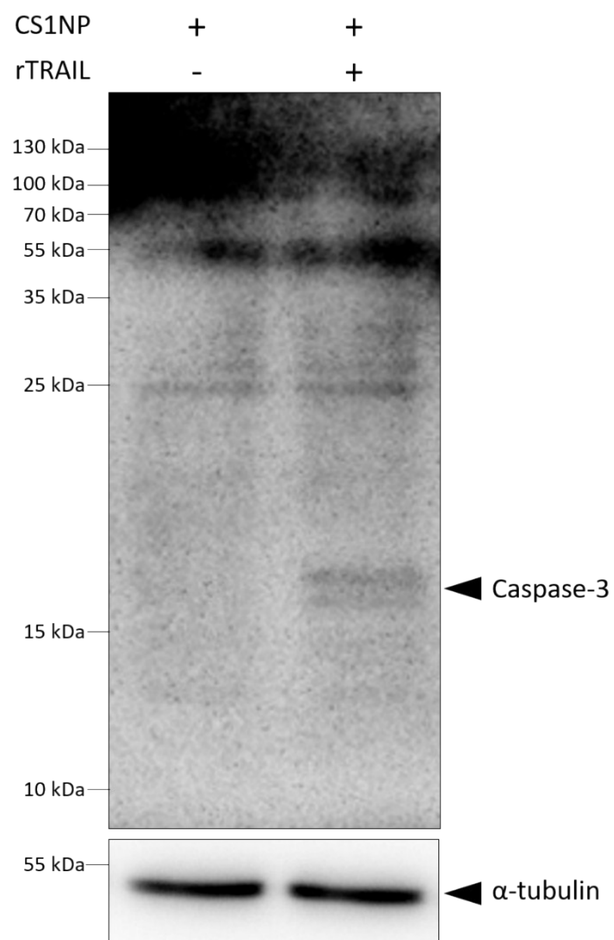


***Figure S6.*** *Western blot analysis of HCT116 cell incubated with 0.5 mg/mL CS1-NP for 24 hr, followed by pro-apoptotic stimulation with 10 ng/mL of rTRAIL for 5 hr.*

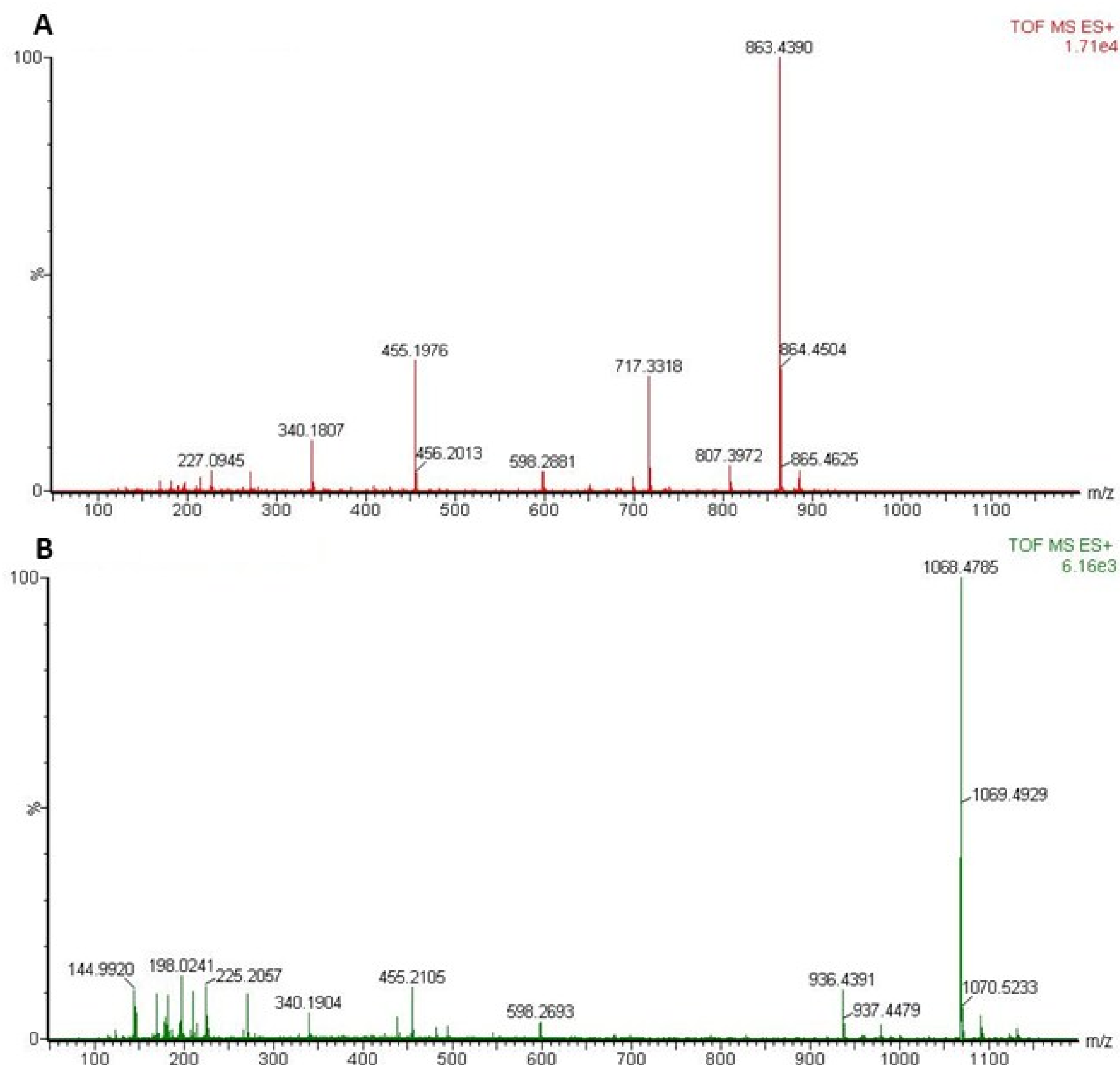


***Figure S7.*** *Mass spectrometry of biotin-Ahx-Asp(t-Bu)-Glu(Me)-Thr(Bzl)-OH peptide (A) and of final probe CS1 (B). The [m/z+H]$^+$ of the t-Bu-protected peptide is expected to be of 863.4219 Da, and MS analysis revealed a [m/z+H]$^+$ of 863.4390. The [m/z+H]$^+$ of the CS1 probe is expected to be 1068.4594 Da, consistent with the 1068.4785 [m/z+H]$^+$ value revealed by MS analysis.*

***Reagents.*** All reagents were purchased from commercial suppliers and used without further purification. 2-chlorotrityl chloride resin, diisopropylcarbodiimide (DICI), *N,N*-diisopropylethylamine (DIPEA), piperidine, 1-[Bis(dimethylamino)methylene]1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU), trifluoroethanol (TFE), trifluoroacetic acid (TFA), triisopropylsilane (TIPS) were purchased from Iris Biotech. *N,N*-dimethylformamide (DMF), ninhydrin, dimethyl sulfoxide (DMSO), acetic acid (AcOH), acetonitrile, 2,4,6-trimethylpyridine (collidine), phosphorus pentoxide ($P_2O_5$), ethyl acetate, sodium bicarbonate ($NaHCO_3$), citric acid, sodium chloride (NaCl), magnesium sulphate, tetrahydrofuran (THF), hydrogen bromide in acetic acid (HBr 30 % (w/v) in $CH_3COOH$ (HBr/AcOH)), potassium fluoride (KF), biotin, 2,6-dimethylbenzoic acid (DMBA), 4-methylmorpholine (NMM), poly(lactic-co-glycolic) acid (MW 7000-17000 Da, lactic acid : glycolic acid ratio (LG) 50:50, resomer RG502H), Rhodamine 6G, Nile red, dichloromethane (DCM), 2-(*N*-morpholino)ethanesulfonic acid (MES), polyvinyl alcohol (PVA, MW 13000-23000, 87-89 % hydrolysed), **Tris(hydroxymethyl)aminomethane** (TRIS), tween-20, dithiothreitol (DTT), bovine serum albumin (BSA), Phenylmethylsulphonyl fluoride (PMSF), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), IGEPAL CA-630, Glycerol, *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED), ammonium persulfate (APS), (*S*)-(+)-Camptothecin (CPT), and entinostat (MS-275) were purchased from Sigma-Aldrich. *N*-hydroxybenzotriazole (HOBt), methanol (MeOH), Pierce BSA protein assay kit, Pierce ECL plus western blotting substrate, Dulbecco’s modified eagle medium (DMEM, high glucose), fetal bovine/calf serum (FBS/FCS), McCoy's 5A modified medium, Roswell Park Memorial Institute medium (RPMI), phosphate buffered saline tablets (PBS), 0.5 % trypsin-EDTA, penicillin-streptomycin (5000 U/mL), 30 % Acrylamide/Bisacrylamide (37.5:1) were purchased from Thermo Fisher Scientific. Methoxy poly(ethylene glycol)-b-poly(lactide-co-glycolide) (mPEG-PLGA, LG 50:50, mPEG MW ~5000 Da, PLGA MW ~10000 Da) was purchased from Akina Inc. Ethylenediaminetetraacetic acid (EDTA) and sodium dodecyl sulphate (SDS) were

purchased from Melford Laboratories. Sucrose was purchased from BDH laboratory supplies. Glycine was purchased from Avantor. N-Acetyl-Asp-Glu-Val-Asp-7-amido-4-Methylcoumarin (Ac-DEVD-AMC, caspase-3/7 fluorogenic substrate) was purchased from Enzo Scientific. Streptavidin-horseradish peroxidase (Strep-HRP, #3999S), FLIP mAb (D16A8, #8510), PARP mAb (46D11, #9532), and caspase-3 mAb (3G2, #9668) were purchased from Cell Signalling Technologies. Anti-tubulin antibody (YL1/2, #ab6160) and rabbit anti-rat IgG H&L-HRP conjugate (#ab6734) were purchased from Abcam. Goat anti-mouse IgG (H+L)-HRP conjugate (#1721011) and goat anti-rabbit IgG (H+L)-HRP conjugate (#1706515) were purchased from Biorad. Acridine orange was purchased from Life Technologies. DAPI was purchased from Vector laboratories. Recombinant human TNF-Related Apoptosis-Inducing Ligand (rTRAIL) was purchased from Calbiochem. Fmoc-Asp(tBu)-OH, Boc-Asp(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glu(Me)-OH, Fmoc-Val-OH, Fmoc-Thr(Bzl)-OH were all purchased from Combi-blocks.