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Supplementary Material to

Platin-C Nanoparticles: A Recipe for the Delivery of Curcumin-Cisplatin Combination Chemotherapeutics to Mitochondria

Materials and Instruments

All chemicals were used as received without further purification unless otherwise noted. Cisplatin, N, N′-dicyclohexylcarbodiimide (DCC), hydrogen peroxide solution (30 wt.% in H_2O) was purchased from Sigma Aldrich. Acid terminated poly lactide-co-glycolide (dL/g, 0.15 to 0.25) was procured from Lactel. Amine terminated polyethylene glycol (NH_2-PEG2000-NH_2) was procured from JenKem Technology, China. $CDCl_3$ and DMSO-d6 were purchased from Cambridge Isotope Laboratories Inc. Regenerative cellulose membrane amicon ultra centrifugal 100 kDa filters were purchased from Merck Millipore Ltd. NucBlue® live cell stain ready probe (Cat. No. R37605) and MitoTracker® Red FM (Cat. No. M22425) were procured from Life Technologies. Copper grids for transmission electron microscopy (TEM) were purchased from Electron Microscopy Sciences. Glutamine, penicillin/streptomycin trypsin-EDTA solution, HEPES buffer (1M), sodium pyruvate, oligomycin (catalog number 75351), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP-catalog number C2920), antimycin (catalog number A8674), and rotenone R8875) were procured from Sigma Life Sciences. Roswell Park Memorial Institute (RPMI) medium and fetal bovine serum (FBS) were purchased from Gibco Life Technologies. NF-$\kappa\beta$ (Catalog number ab16502) and TNF-α (Catalog number ab183218), β-Actin (Catalog number ab8226) and citrate synthase assay kit (catalog number ab239712) were purchased from Abcam. RIPA buffer (catalog number 89900) was purchased from Fischer Scientific. Tris/glycine/SDS buffer (Catalog number 161-0732), SDS-PAGE gel preparation kit TGX stain-freeTM fast castTM acrylamine 10% (Catalog number 161-0182), and Clarity™ western ECL substrate (Catalog number 170-5060) were purchased from Bio-Rad Inc. Nitrocellulose membrane (catalog number 88018), and tween-20 was purchased from Fisher Bioreagents. The primer sequence for β-actin gene was forward 5' GCATCCTCACCCTGAAGTAC 3' and reverse 5' GATAGCACAGCCTGGATAGC 3'. The forward and reverse primer sequence was 5'TGCACTGAAGTTCAATGGTGG3' and 5' CTTCCAGCAACTCCCCTTTG3', respectively, for the SOD protein gene. The primers for IL6 gene were purchased from Sigma-Aldrich (5'AATTCGGTACATCCTCGACGG3' and 5'GGTTGTTTTCTGCCAGTGCCand3', forward and backward). The primers for TNF-α and NF-$\kappa\beta$ genes were purchased from Sigma-Aldrich. The forward and reverse primer sequence for TNF-α gene was 5'CCTGGAAAGGACACCATGAGC3' and 5'CCCCTCAGCTTGAGGGTTTG3'. The forward and reverse primer sequence for NFκβ gene was 5'GATCCGCCAGGTGAAGGG3' and 5' GCAATTTCTGGCTGGTTGG 3'. Primers were purchased from integrated DNA technologies (IDT). Lipopolysaccharides (LPS, catalog number: L7895) was purchased from Millipore sigma.

Distilled water was purified by passage through a Millipore Milli-Q Biocel water purification system (18.2 MΩ) containing a 0.22 μm filter. ^1H and ^{13}C spectra were recorded on 400 MHz Varian NMR spectrometer and ^{195}Pt NMR spectra were recorded on a 500 MHz Varian NMR spectrometer using K_2PtCl_4 (δ = -1628 ppm) as an external standard. Electrospray ionization mass spectrometry (ESI-MS) and high-resolution mass spectrometry (HRMS)-ESI were recorded on Perkin Elmer SCIEX API 1 plus and Thermo scientific ORBITRAP ELITE instruments, respectively. Dynamic light

scattering (DLS) measurements were carried out using a Malvern Zetasizer Nano ZS system. Transmission electron microscopy (TEM) images were acquired using a Philips/FEI Tecnai 20 microscope. Gel permeation chromatographic (GPC) analyses were performed on Shimadzu LC20-AD prominence liquid chromatographer equipped with a refractive index detector and Waters columns; molecular weights were calculated using a conventional calibration curve constructed from narrow polystyrene standards using dimethylformamide (DMF) as an eluent at a temperature of 40 °C. High performance liquid chromatography (HPLC) analyses were made on an Agilent 1200 series instrument equipped with a multi-wavelength UV-visible and a fluorescence detector. Cells were counted using Countess® automated cell counter procured from Invitrogen life technology. Real-Time PCR (RT-PCR) studies were carried out using CFX Connect System from BIO-RAD. Reverse Transcription Supermix for RT-qPCR kit was obtained from Bio-Rad. Real time PCR reaction was carried out using SsoAdvanced Universal SYBR® Green Supermix in 20 μL reactionPlate reader analyses were performed on a Bio-Tek Synergy HT microplate reader. Optical measurements were carried out on a NanoDrop 2000 spectrophotometer. Inductively coupled plasma mass spectrometry (ICP-MS) studies were performed on an Agilent 7900 ICP-MS instrument. Biorad's ChemiDoc imaging system used for western blot analysis. Mitochondrial bioenergetics assays were performed on XFe96 Extracellular Flux Analyzers (Agilent Seahorse Biosciences).

Cell Lines and Cell Culture

Cisplatin resistant human ovarian carcinoma cell line A2780/CP70 was kindly provided by Thomas Hamilton (Fox Chase Cancer Center, Jenkintown, PA). PC-3 (catalog number: CRL-1435) and A2780 cells were purchased from ATCC. All the cell lines were grown at 37 °C in 5% CO_2 in RPMI 1640 medium supplemented with 10% FBS, 1% L-glutamine, 1% sodium pyruvate, 1% HEPES, and 1% penicillin/streptomycin. Cells were passed every 3 to 4 days and restarted from frozen stocks after 20 passages.

Methods

Synthesis of 5-(4-((1*E*,6*E*)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-1-yl)-2-methoxyphenoxy)-5-oxopentanoic acid

The synthesis of this compound was carried out as per a previously reported method[1]. A solution of curcumin (2.010 g, 5.46 mmol) and DMAP (112 mg, 0.92 mmol) in 100 mL tetrahydrofuran (THF) was prepared and triethylamine (1.33 mL, 10.5 mmol) was added. Glutaric anhydride (685 mg, 6 mmol) in 5 mL of THF was added slowly dropwise into the reaction mixture. The reaction was refluxed overnight at 50°C and evaporated. The resulting residue was dissolved with 55 mL of ethyl acetate followed by 15 mL of 1M HCl and stirred for 10 min. The reaction was extracted with nanopure water 3 times, and the organic solvent was evaporated. The crude product was purified by column chromatography using DCM/MeOH mixture as eluent. 27% yield ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm, 7.61 (q, 2H), 7.12 (q, 2H), 7.04 (d, 2H), 6.93 (q, 2H), 6.52 (q, 2H), 5.84 (d, 1H), 3.97 (s, 3H), 3.87 (s, 3H), 2.71 (t, 2H), 2.57 (t, 2H), 2.11 (q, 3H). ^{13}C NMR ($CDCl_3$, 200 MHz): δ 203.58, 184.49, 181.78, 177.83, 170.74, 151.27, 147.97, 146.78, 141.13, 141.08, 139.36, 134.11, 127.54, 123.17, 123.03, 120.96, 114.83, 111.36, 109.64, 101.51, 55.95, 55.87, 32.89, 32.55, 19.90 ppm.

Synthesis of *c,c,t*-[Pt(NH_3)$_2$$Cl_2(OH)_2$]

Adopting previously reported method[2] with minor changes; Hydrogen peroxide (30 wt%, 60 mL) was added drop wise to a round bottom flask containing cisplatin (1.0 g, 3.33 mmol). The reaction mixture was heated to 75 °C for 5 h. The bright yellow solution was kept at room temperature in the dark overnight to allow crystallization of the product. Yellow crystals were separated by filtration, washed with cold water and dried to get 554 mg of compound. Yield 50%. IR (KBr) υ_{max} (cm^{-1}): 3803 (w), 3515 (w), 3458 (br, OH), 3269 (w), 1582 (s), 1442(s), 1378(s), 1074 (m, Pt-OH), 860 (br), 542 [br, Pt-N(O)].

Synthesis of *c,c,t*-[Pt(NH_3)$_2$$Cl_2$(OH)($OCOCH_2CH_2COOH$)]

Suitably modified method from an earlier report[2] was adopted for this synthesis as per which, a mixture of *c,c,t*-[Pt(NH_3)$_2$$Cl_2(OH)_2$] (250 mg, 0.748 mmol) and succinic anhydride (74.8 mg, 0.748 mmol) were dissolved in 20 mL of DMSO and stirred overnight at room temperature. Once the reaction solution became clear, the reaction mixture was washed with diethyl ether to get rid of the majority of DMSO in it. Once 2-3 mL of the reaction mixture is left, it was gently added to excess of acetone to afford the product as yellow crystalline precipitate. It was then centrifuged (6000 rpm, 10 min, 5°C) and lyophilized. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm, 7.61 (q, 2H), 7.12 (q, 2H), 7.04 (d, 2H), 6.93 (q, 2H), 6.52 (q, 2H), 5.84 (d, 1H), 3.97 (s, 3H), 3.87 (s, 3H), 2.71 (t, 2H), 2.57 (t, 2H), 2.11 (q, 3H).

Synthesis of 5-(4-((1*E*,6*E*)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-1-yl)-2-methoxyphenoxy)-5-oxopentanoic anhydride

A solution of 5-(4-((1*E*,6*E*)-7-(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-1-yl)-2-methoxyphenoxy)-5-oxopentanoic acid (0.686 g, 1.42 mmol) in dry CH_2Cl_2 was prepared and a solution of DCC (0.146 g, 0.71 mmol) in 3 mL of CH_2Cl_2 was added. The reaction mixture was stirred overnight at room temperature. The byproduct, dicyclohexylurea (DCU), was filtered off in a glass filter and washed with a small amount of CH_2Cl_2. The solvent was evaporated, and the resulting residue was taken up in ethyl acetate. The residual DCU was removed by filtering the resulting suspension through a glass filter. The filtrate was evaporated to give anhydride. Yield 0.694 g. This product was used without further purification for the synthesis of Platin-*C*.

Synthesis of Platin-*C*

Mixture of curcumin anhydride (808 mg, 0.847 mmol) and *cis,cis,trans,*-diamminedichlorohydroxosuccinatoplatinum(IV) (183 mg, 0.423 mmol) was dissolved in DMF and stirred at RT for 72 h. It was then filtered and evaporated to dryness. Product was re-dissolved in acetonitrile and little volume of DMF. It was then re-precipitated with diethyl ether three times. Dichloromethane was added to precipitant and sonicated three times to get rid of curcumin-glutaric acid. It was then dissolved in acetone and centrifuged. Supernatant was reprecipitated with diethyl ether, centrifuged and lyophilized. 45% yield (172 mg produced) ^{1}H NMR ($DMSO_6$, 400 MHz): δ ppm, 7.62 (m, 4H), 7.33 (m, 4H), 7.13 (q, 4H), 7.06 (d, 4H), 6.93 (q, 4H), 6.80 (q, 4H), 6.56 (broad, 2.23H), 3.84 (d, 12H), 2.74 (t, 4H), 2.61 (t, 4H), 2.37 (q, 4H). Elemental analysis calculated (%) for $C_{30}H_{36}Cl_2N_2O_{13}Pt.H_2O$: C 39.31, H 4.18, N 3.06; found: C 39.19, H 4.20, N 2.95. ^{195}Pt (DMSO-d_6, 107.6 MHz): δ ppm 1230.3.

Synthesis of PLGA-*b*-PEG-NH_2

The non-targeted polymer, PLGA-*b*-PEG-NH_2, was synthesized by using an amide coupling reaction. Amine terminated polyethylene glycol, NH_2-PEG-NH_2, (1 g, 0.5 mmol), PLGA-COOH (0.975 g, 0.167 mmol), and 4-dimethylaminopyridine (20.3 mg, 0.167 mmol) in 20 mL dry CH_2Cl_2 were stirred at 0°C. DCC (103 mg, 0.5 mmol) in 3 mL dry CH_2Cl_2 was added drop wise to the solution. The solution was warmed to room temperature and stirred overnight. It was then filtered to remove the dicyclohexylurea byproduct, precipitated using a mixture of 1:1 methanol-diethyl ether, isolated via centrifugation (5000 rpm, 4 °C, 10 min), and lyophilized overnight. PLGA-*b*-PEG-NH_2 was isolated as a white solid. 38% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm, 5.24 (m, 35H), 4.81 (m, 70H), 3.68 (s, 126H), 1.58 (m, 106H), ^{13}C NMR ($CDCl_3$, 200 MHz): δ 169.41, 169.29, 166.42, 166.33, 70.54, 69.17, 69.01, 60.80, 16.67, 16.63 ppm. GPC molecular weight: M_n = 21,200 g/mol, PDI = 1.61 in DMF.

Synthesis of PLGA-*b*-PEG-TPP

To synthesize the mitochondria-targeted polymer, 6-hexanoic TPP acid (133 mg, 0.294 mmol) and N-hydroxysuccinimide (NHS) (33.8 mg, 0.294 mmol) were dissolved in 5 mL of DCM and stirred at 0˚C. DCC (60 mg, 0.294 mmol) was dissolved in 1 mL of CH_2Cl_2, added drop wise into the reaction mixture and stirred overnight at room temperature. DCU was filtered off, and the solution was evaporated. It was then redissolved with 10 mL of CH_2Cl_2 and PLGA-PEG-NH_2 (471 mg, 0.0589 mmol) and triethylamine (Et_3N) (8.3 μL) were added. The reaction was stirred overnight and reprecipitated with 10% methanol in diethyl ether 3 times and lyophilized. 59% yield (290 mg produced) ^{1}H NMR ($CDCl_3$, 400 MHz): δ ppm, 7.74 (m, 16H), 5.21 (m, 35H), 4.83 (m, 63H), 3.64 (s, 122H), 1.57 (m, 103H) ^{13}C NMR ($CDCl_3$, 200 MHz): δ 169.29, 166.33, 133.76, 133.66, 133.56, 130.54, 130.42, 70.54, 69.00, 60.80, 16.67 ppm. GPC molecular weight: M_n = 22,600 g/mol, PDI = 1.81 in DMF.

Synthesis and Stability of Platin-*C* Nanoparticles

5 mg/mL of PLGA-*b*-PEG-NH_2 (for NT-Platin-*C*-NPs) or PLGA-*b*-PEG-TPP (for T-Platin-*C*-NPs) and Platin-*C* were dissolved in DMF. Platin-*C* was added in a different ratio varying from 10 % to 50 % feed with respect to polymer weight (5 mg/mL), which was added drop wise into 10 mL of water with constant stirring and the stirring was continued at room temperature for another 2 h post the completion of addition. The solution was then washed 3 times with nanopure water with Amicon ultracentrifugation filtration membranes with a molecular weight cutoff of 100 kDa (3000 rpm, 4°C). NPs were finally suspended in 1 mL nanopure water. The Platin-*C* loading was determined using ICP-MS and HPLC. Using ICP-MS, platinum content was obtained which further used to calculate % loading and %encapsulation efficiency (EE) of Platin-*C* in the nanoparticles. HPLC studies were carried out using an Agilent 1200 series instrument. The solution from each dialysis tube (20 μL) was taken in acetonitrile containing 0.1% trifluoroacetic acid and was injected into HPLC. A Zorbax C18 column was used as a solid phase and a 50:50 acetonitrile: isopropyl alcohol solution served as the mobile phase. The wavelength of detection used for these experiments was 283 nm and the elution time of the platin-C was 13.3 mins. The % loading and % encapsulation efficiency were determined using below provided formulae.

% Loading of Platin-*C* = $\frac{100 \, X \, Wt_{de}}{(Wt_p + Wt_{de})}$

% Encapsulation Efficiency (EE) of Platin-*C* = $\frac{100 \, X \, Wt_{de}}{Wt_{du}}$

where,

Wt_{de} =Weight of encapsulated Platin-*C*

Wt_p = Weight of polymer in nanoparticles

Wt_{du} = Weight of Platin-C used

For stability studies, freshly prepared T-Platin-*C*-NPs were suspended in water (10 mg/mL of T-Platin-*C*-NP with respect to polymer concentration) and the NP size (diameter, nm), PDI, and surface charge (zeta potential, mV) were obtained from three independent measurements. The NPs were stored at 4 ºC. Every 48 h, targeted NPs were taken gently and diameter, PDI, and zeta potential were measured for 7 days to understand the stability of the targeted nanoparticles.

Release Kinetics of Platin-*C* from Nanoparticles

The release of Platin-*C* from T-Platin-*C*-NPs was studied using 1X PBS at physiological pH of 7.4 at 37 °C. The nanoparticle solutions were prepared in dialysis tube with MWCO of 10,000 at a concentration of 2 mg/mL of T-Platin-*C*-NP with respect to polymer. These tubes were then submerged in PBS at pH 7.4 and kept in the shaking incubator at 37 °C up to 72 h. For the first 6 h, buffer was replenished with fresh buffer every hour and later changed every 12 h. The samples were collected at predetermined time points and samples were analyzed by ICP-MS to quantify the remaining Pt in the dialysis tubes.

Cytotoxicity Assay

The cytotoxicity of T/NT-Platin-*C*-NPs, Platin-*C*, curcumin, cisplatin and a mixture of curcumin and cisplatin were tested in A2780/CP70 by MTT assay. A2780/CP70 cells (2000 cells/well/100 μL) were plated on a 96 well plate and allowed to grow 24 h at 37 °C in 5% CO_2. The media was changed and increasing concentrations of each formulation was added. For all the test articles, the media was changed after 24 h and the cells were further incubated in fresh media for an additional 48 h. After the given incubation time, MTT was added (5 mg/mL, 20 μL/well) and incubated for 5 h for MTT to be reduced to purple formazan by mitochondrial reductase of viable cells. The media was removed, and the cells were lysed with 100 μL of DMSO. To homogenize the formazan solution, the plates were subjected to 5 min of gentle shaking and the absorbance was read at 550 nm with a background reading at 800 nm with a plate reader. Cytotoxicity was expressed as mean percentage increase relative to the unexposed control ± SD. Control values were set at 0% cytotoxicity or 100% cell viability. Cytotoxicity data (where appropriate) was fitted to a sigmoidal curve and a three parameters logistic model used to calculate the inhibitory concentration-50 (IC_{50}) that is the concentration of chemotherapeutics causing 50% inhibition in comparison to untreated controls. The mean IC_{50} is the concentration of agent that reduces cell growth by 50% under the experimental conditions and is the average from at least three independent measurements that were reproducible and statistically significant. The IC_{50} values were reported at ±99% confidence intervals. These analyses were performed with GraphPad Prism (San Diego, U.S.A).

Confocal Microscopy

A2780/CP70 cells were seeded on microscope coverslips (1.0 cm) at a density of 6 x 10^7 cells/mL and grown overnight in DMEM. The medium was changed and Platin-*C* containing targeted and non-targeted NPs was added to a final Platin-*C* concentration of 10 μM. The cells were incubated for 4 h at 37 °C in 5% CO_2. Mitochondria and nuclei of the cells were stained using MitoTracker® Red FM and NucBlue stains respectively following manufacturer's protocols. The medium was removed, and the cells were fixed using 4% paraformaldehyde for 20 min. The coverslips were then rinsed with PBS, water, and mounted on slides using mounting media. Images were collected at 320 ms for the DAPI, FITC and Cy5 channels. Images were further analyzed with ImageJ.

Mito stress Assay

The real time oxygen consumption rate (OCR) values through mitochondrial OXPHOS in A2780 and A2780/CP70 cells upon treatment with NT-Platin-C-NPs and T-Platin-C-NP were determined by using Seahorse Analyzer. Different parameters of mitochondrial respiration such as basal respiration, maximal respiration, and ATP production were investigated using Seahorse XFe96 Analyzer. One day prior to the assay, XF sensor cartridges were hydrated using 200 μL of XF calibrant buffer and kept at 37 °C incubator without CO_2 overnight. Cells were plated at a density of 20,000 cells per well in 80 μL RPMI media (with 10% FBS) and the plate was incubated at 37 °C with 5% CO_2 for 4 h. Finally, 100 μL of fresh media was added to have total 180 μL per well and incubated for 16 h. Cells were treated with NT-Platin-*C*-NP or T-Platin-*C*-NP (both nanoparticles are with respect to 10 μM Platin-*C* concentration) for 4 h. After treatment, the media was replaced with fresh media and incubated for 20 h. Before conducting the Mitostress assay, Seahorse media (XF Assay Medium Modified DMEM) was reconstituted with glucose (1.8 mg/mL), sodium pyruvate (1%) and L-glutamine (1%) and adjusted for to pH 7.4 by using 0.1 N NaOH. The cells were washed thrice with freshly prepared seahorse medium and incubated at 37 °C in non-CO_2 incubator for 1 h. Meanwhile, various reagents were added to the ports of the sensor cartridge. The port A was filled with 20 μL of oligomycin (10 μM) port B with 22 μL of FCCP (10 μM) and port C with 25 μL of antimycin A/rotenone (10 μM) to have a final concentration of 1 μM in each well. The cartridge was calibrated for pH and O_2. After calibration, the experiment plate was run where 5 measurements were recorded for basal OCR and after addition of each reagent. After the assay, the media was aspirated and 20 μL of RIPA buffer was added to each well and incubated for 20 mins at 37 °C. Further BCA assay was performed to obtain protein values and OCR values were normalized with protein concentration. The statistical analysis was obtained one-way ANOVA test.

Citrate synthase activity

Citrate synthase activity assay was performed using citrate synthase activity assay kit (ab239712) and assay was carried as per protocol. Cells were plated at a density of 1X10^6 in a six well plate in 2 mL of RPMI media and kept at 37 °C incubator without CO_2 overnight. After 16 h, the cells were treated with cisplatin, curcumin, Platin-*C*, combination of cisplatin and curcumin at concentration of 10 μM, NT-Platin-*C*-NP, or T-Platin-*C*-NP at 10 μM concentration with respect to Platin-C. After 4 h of the treatment, the media was replaced with fresh media and incubated for 20 h. The cells were trypsined afterwards and 100 μL ice-cold CS assay buffer was added to the pellet and kept it on ice for 10 mins. After 10 mins, the cells were centrifuged at 10000xg at 4 for 5 mins and supernatant was collected. The supernatant (10 μL) was added to a 96-well plate and volume was adjusted to 50 μL with CS assay buffer. Simultaneously, GSH standard solutions were prepared. A 5 μL CS developer

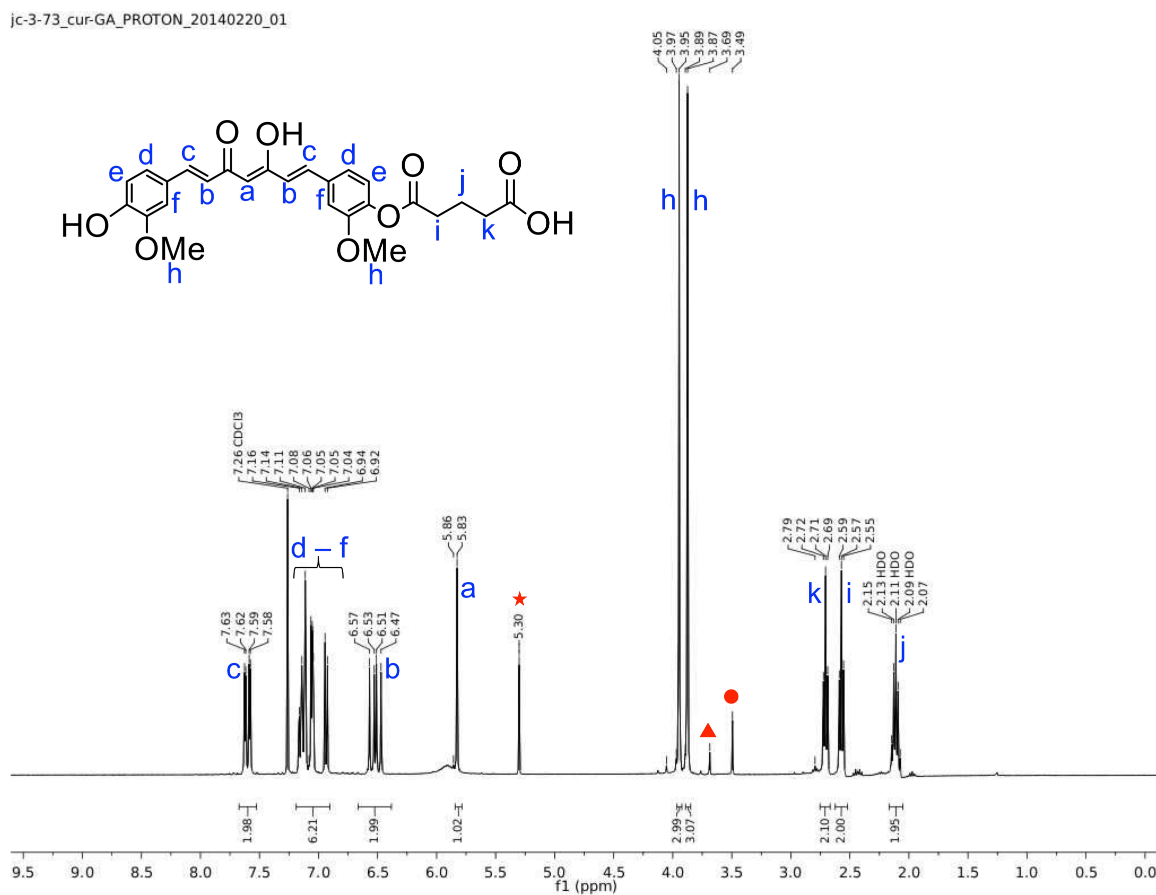
and 2 μL CS substate mix was added to each well and immediately absorbance was measured at 412 nm in kinetic mode for 50 mins. The background value was subtracted from each value CS activity by using GSH standard curve for time interval and the data were normalized with protein concentration as obtained from the BCA assay. The statistical analysis was obtained one-way ANOVA test.

Western Blot Analyses

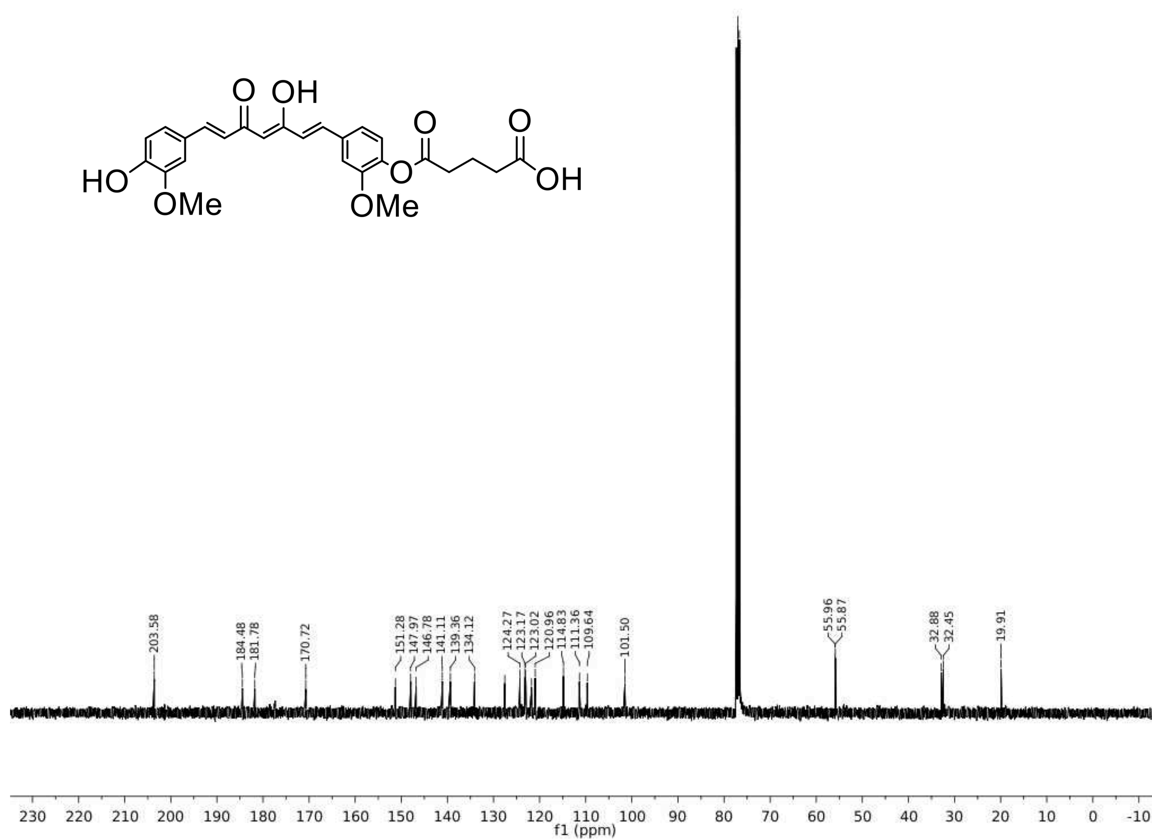
PC-3 cells were seeded in 6-well plate at a density of $1x10^6$ per well and 1 μg of LPS was added for 12 h and media was replenished by fresh media. Suspensions of NT-Platin-C-NP and T-Platin-C-NPs were added to cells at the concentration of 10 μM with respect to Platin-*C* for 24 h. The cells were lysed with RIPA buffer and the total cell lysate (60 μg) was resolved on a 4−20% gradient gel. Proteins were transferred to PVDF membrane and blocked with 5% non-fat dry milk (NFDM) in TBST for 1 hour at room temperature. Further the membranes were probed with TNF-α (1:1000), NF-$\kappa\beta$ (1:1000), or β-actin (1:1000) human reactive primary mouse-antibodies for overnight at 4 °C. Next day, the membranes were washed thrice with TBST and probed with HRP-conjugated anti-mouse secondary antibody (1:2000) for 1 h at room temperature. The membrane was washed 5 times with TBST and developed using western Clarity™ ECL substrate (Biorad) and imaged using ChemiDoc western blot imager.

RT-PCR Analysis

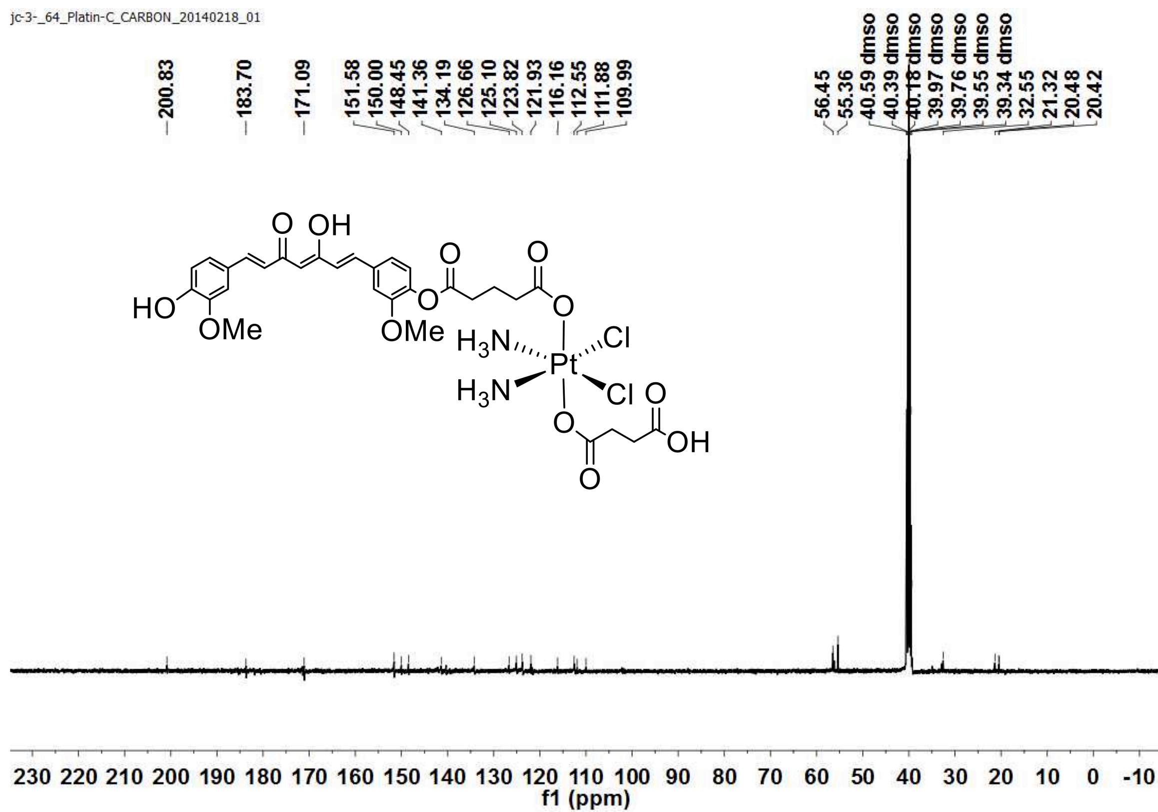
PC-3 cells were seeded in 6-well plate at a density of $1x10^6$ cells per well and 1 μg of LPS was added for 12 h and media was replenished by fresh media. The cells were treated with NT/T-Platin-C-NPs at a concentration of 10 μM with respect to Platin-C for 24 h. RNA was extracted using a kit from Qiagen. Briefly, cells were harvested with trypsin and lysed with buffer RLT. 1 volume of 70% ethanol was added to the cell lysate and mixed well. Lysate were transferred to RNeasy mini spin column and centrifuged for 1 minute at 8000 rpm. Flow-through was discarded. A solution of buffer RW1 (700 μL) was added to the mini spin column and centrifuged for 1 for minute at 8000 rpm. Flow-through was discarded. A solution of 500 μL of buffer RPE was added to the mini spin column and centrifuged for 1 minute at 8000 rpm. The RNA was recovered from mini spin column using RNase-free water. Purity and concentration of RNA was checked using Nanodrop. The ratio of A260/A280 was around 2.0 suggesting the purity of the RNA. Reverse transcription from each sample was carried out using 1 μg of RNA from each sample in a 20 μL reaction volume using iScript Reverse Transcription Supermix from Bio-Rad. Real time PCR reaction was carried out using SsoAdvanced Universal SYBR Green Supermix in 10 μL reaction using Bio-Rad machine. β-actin was used as an internal control. Data were analyzed using the comparative Ct value and expressed as fold change $2^{-\Delta\Delta CT}$. The primer sequence for β-actin gene was forward 5' GCATCCTCACCCTGAAGTAC 3' and reverse 5' GATAGCACAGCCTGGATAGC 3'. The forward and reverse primer sequence was 5'TGCACTGAAGTTCAATGGTGG3' and 5' CTTCCAGCAACTCCCCTTTG3', respectively, for the SOD protein gene. The primers for IL6 gene were purchased from Sigma-Aldrich (5'AATTCGGTACATCCTCGACGG3' and 5'GGTTGTTTTCTGCCAGTGCCand3', forward and backward). The primers for TNF-α and NF-$\kappa\beta$ genes were purchased from Sigma-Aldrich. The forward and reverse primer sequence for TNF-α gene was 5'CCTGGAAAGGACACCATGAGC3' and 5'CCCCTCAGCTTGAGGGTTTG3'. The forward and reverse primer sequence for NF-$\kappa\beta$ gene was 5'GATCCGCCAGGTGAAGGG3' and 5' GCAATTTCTGGCTGGTTGG 3'.



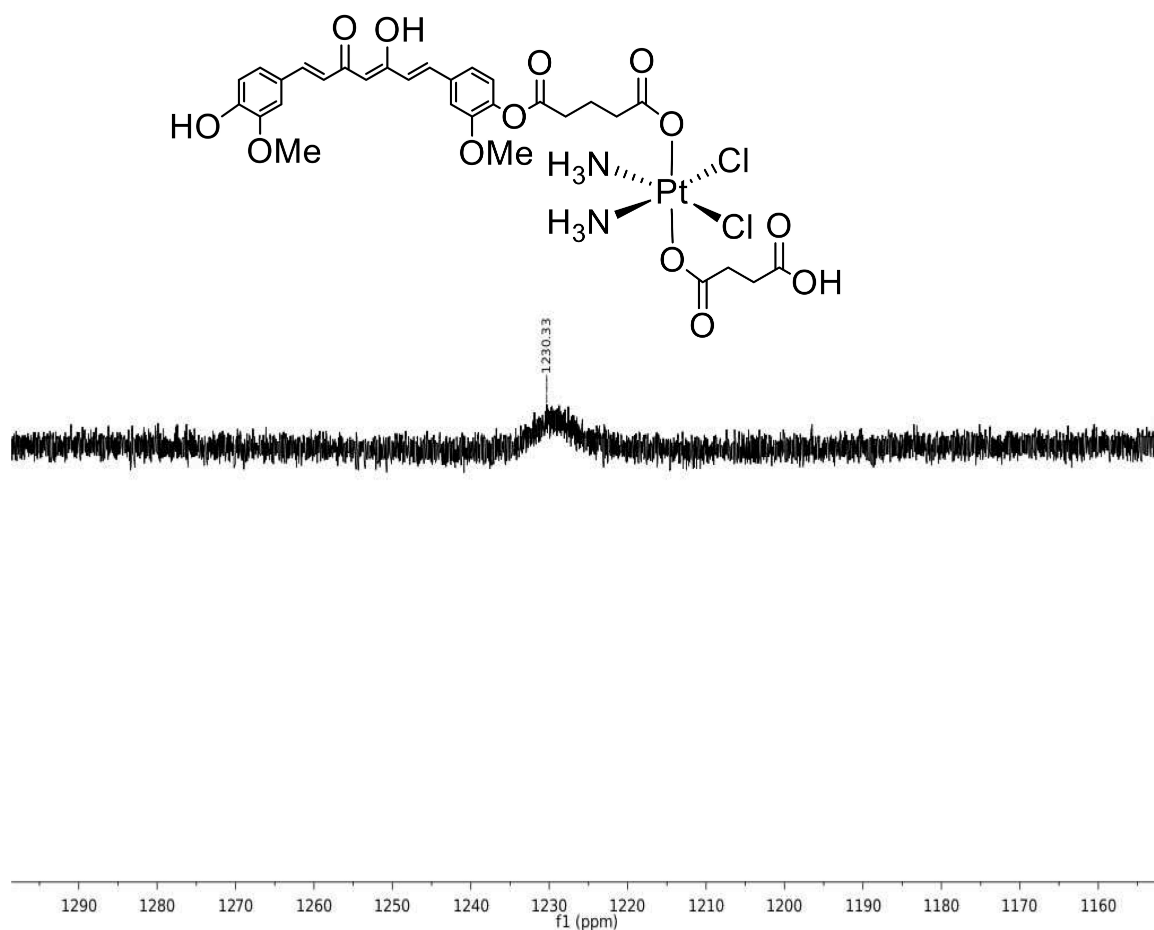
Supplementary Figure 1. ^{1}H NMR spectrum of curcumin-glutaric acid adduct recorded using a 400 MHz NMR spectrometer. Solvent residual peaks for dichloromethane, diethyl ether and water in $CDCl_3$ have been labeled using symbols.



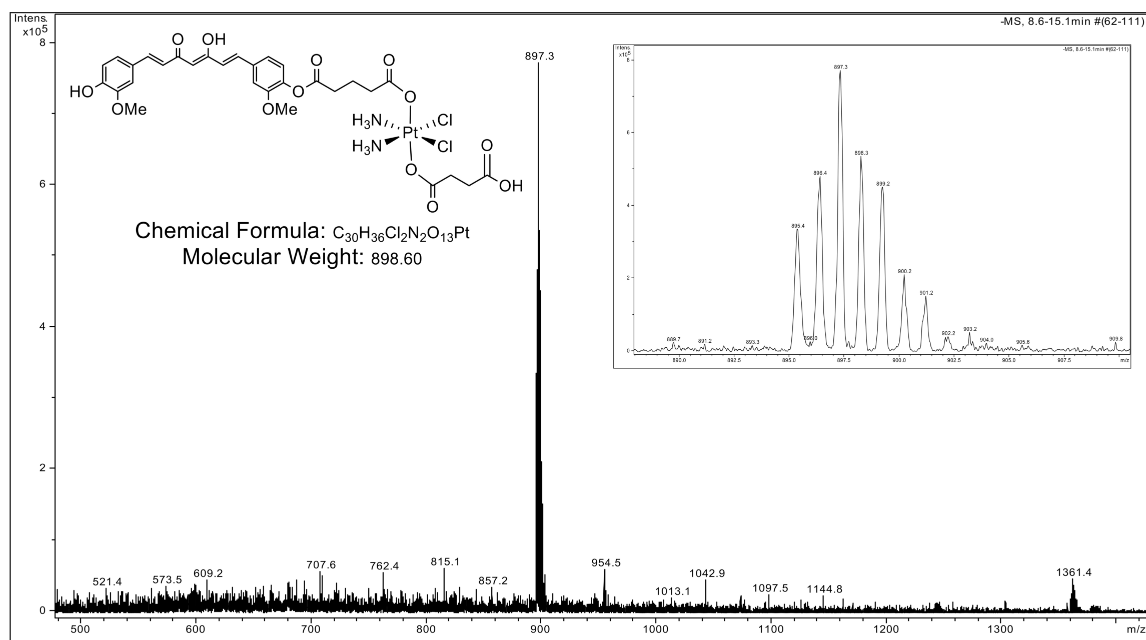
Supplementary Figure 2. ^{13}C NMR spectrum of curcumin-glutaric acid adduct in $CDCl_3$ recorded using a 400 MHz NMR spectrometer.



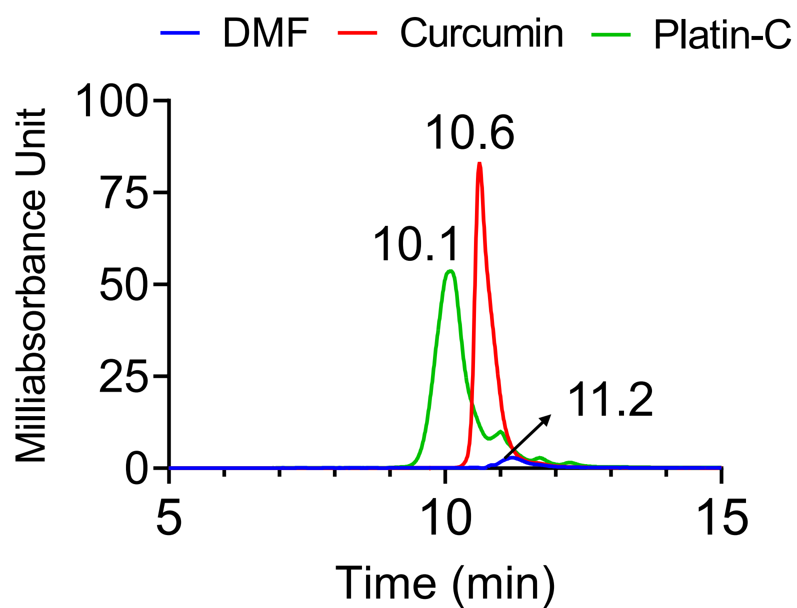
Supplementary Figure 3. ^{13}C NMR spectrum of Platin-*C* in DMSO-d_6 recorded using a 400 MHz NMR spectrometer.



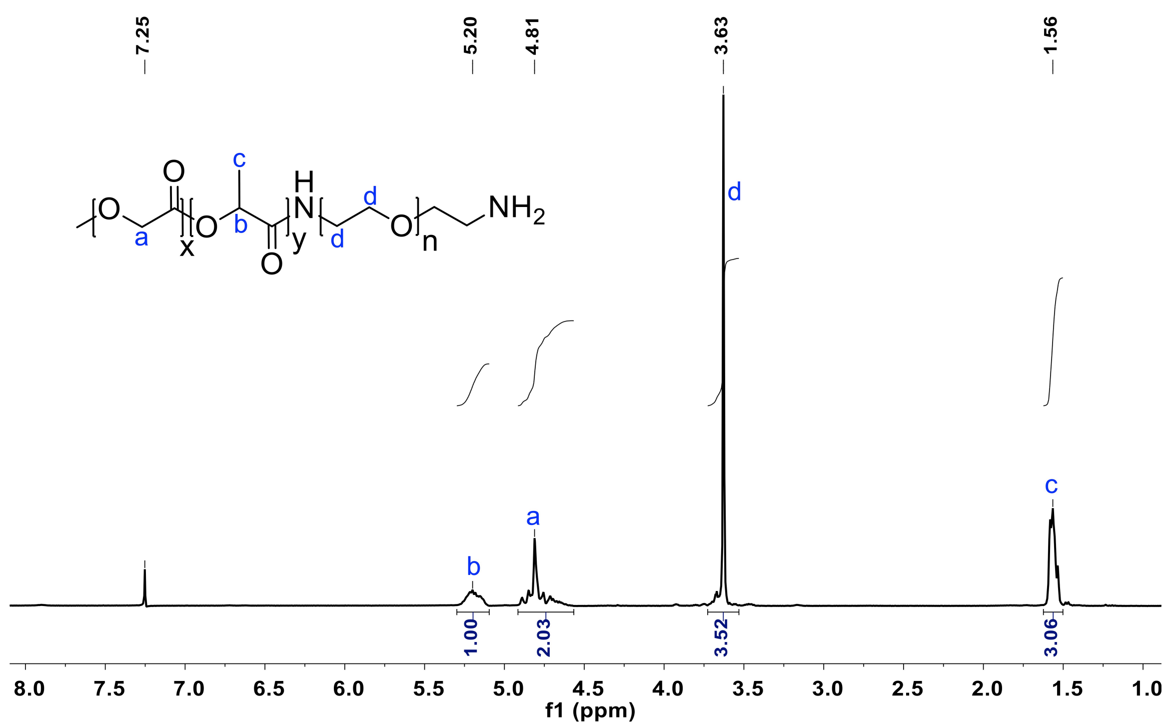
Supplementary Figure 4. ^{195}Pt NMR spectrum of Platin-*C* in DMSO-d_6 recorded using a 400 MHz NMR spectrometer.



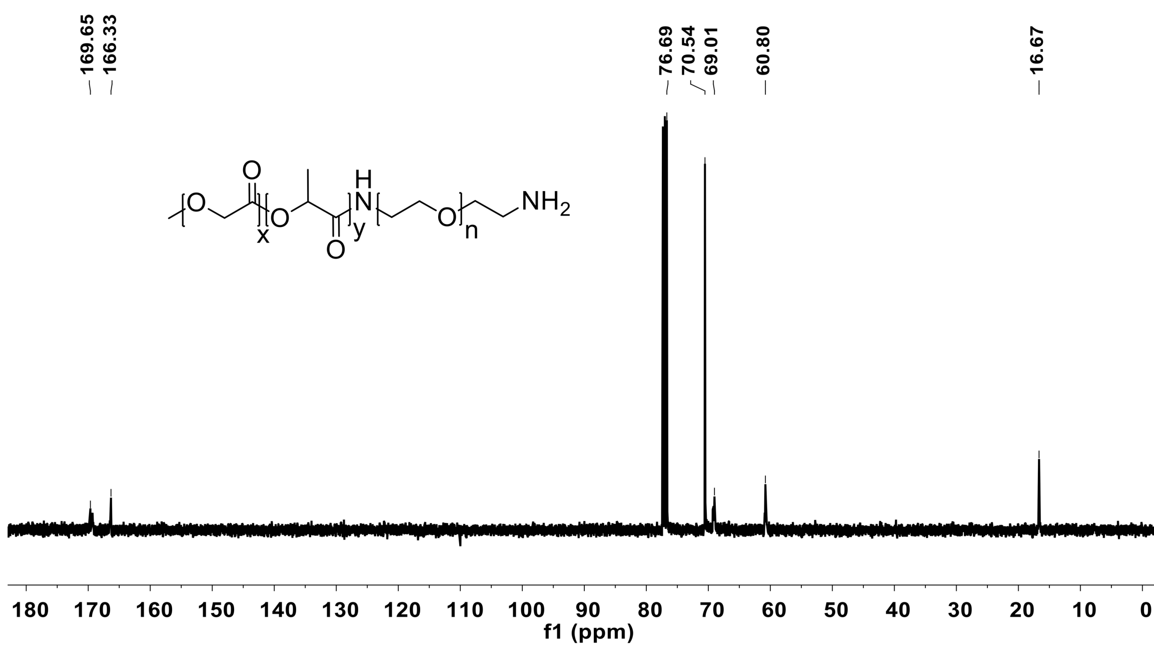
Supplementary Figure 5. ESI-MS spectrum of Platin-*C* showing $[M\text{-}H^{+}]^{-}$ peak at 897.3. Inset shows the corresponding isotopic distribution for the obtained peak showing the pattern characteristic for Platinum isotopes.



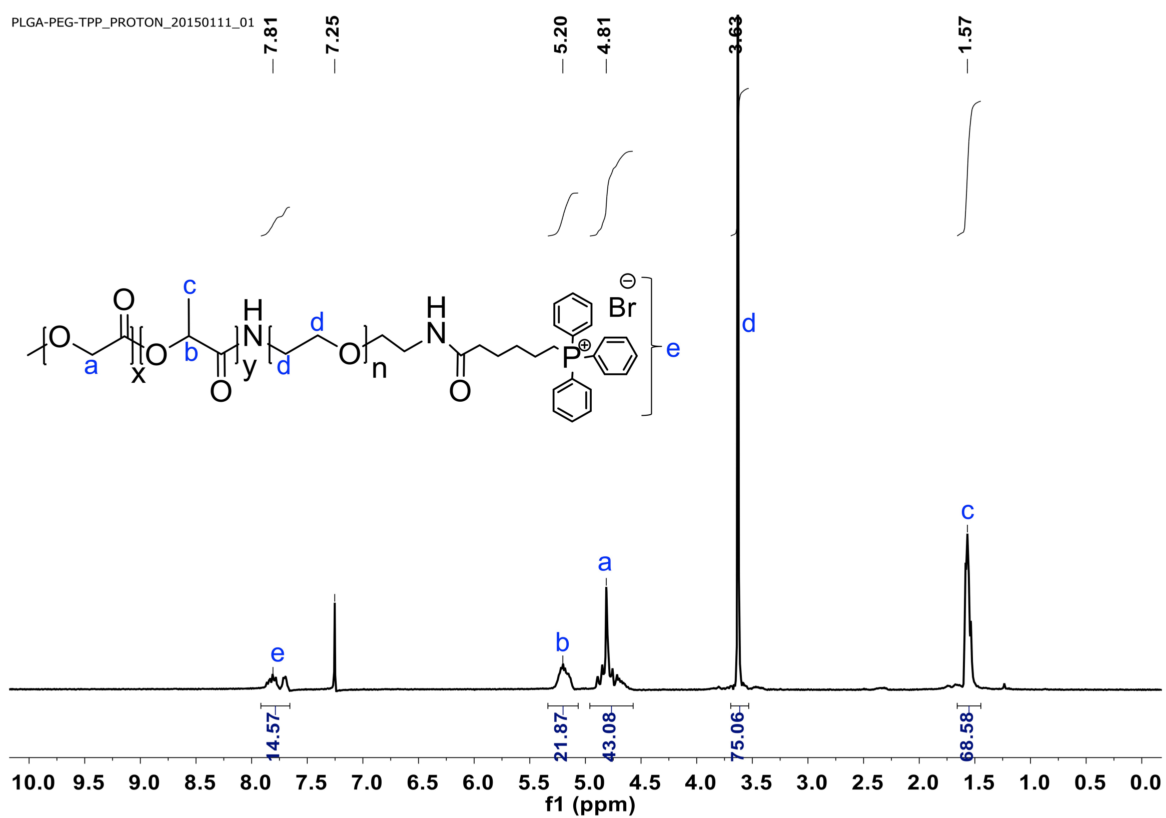
Supplementary Figure 6. HPLC chromatogram of Platin-*C* and curcumin confirming bulk purity of the synthesized complex. Detection wavelength = 290 nm.



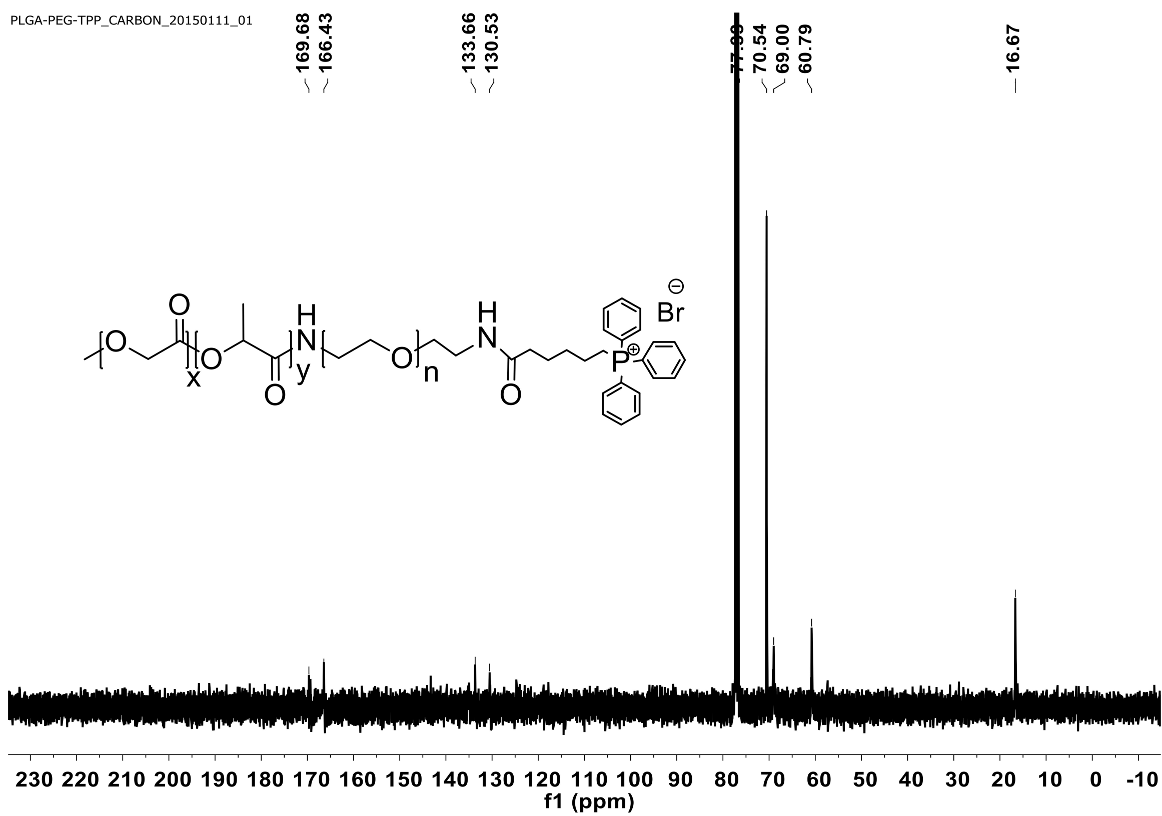
Supplementary Figure 7. ^{1}H NMR spectrum of the non-targeted PLGA-*b*-PEG-NH_2 polymer in $CDCl_3$ recorded using a 400 MHz NMR spectrometer.



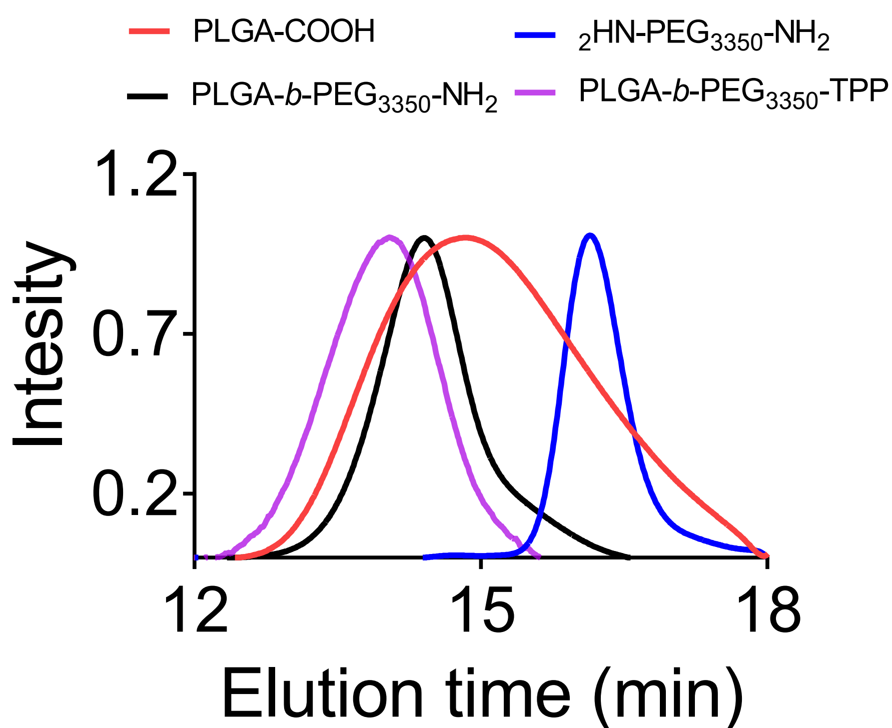
Supplementary Figure 8. ^{13}C NMR spectrum of the non-targeted PLGA-*b*-PEG-NH_2 polymer in $CDCl_3$ recorded using a 400 MHz NMR spectrometer.



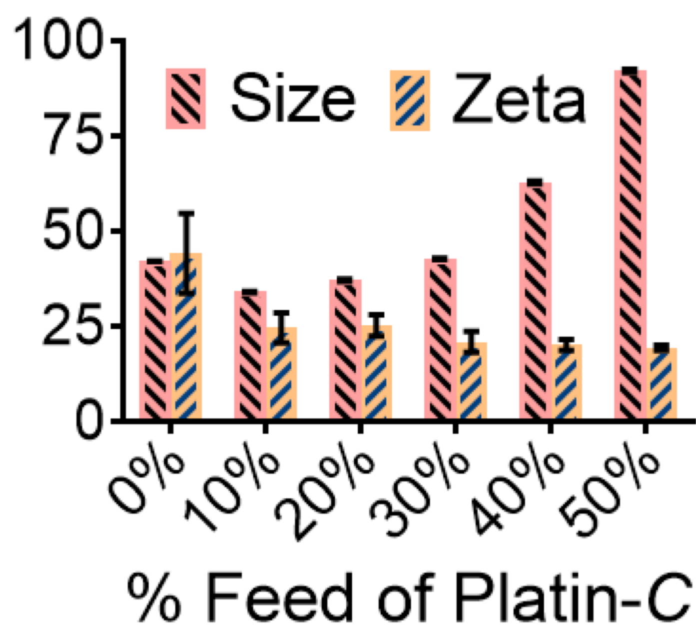
Supplementary Figure 9. ^{1}H NMR spectrum of the targeted PLGA-*b*-PEG-TPP polymer in $CDCl_3$ recorded using a 400 MHz NMR spectrometer.



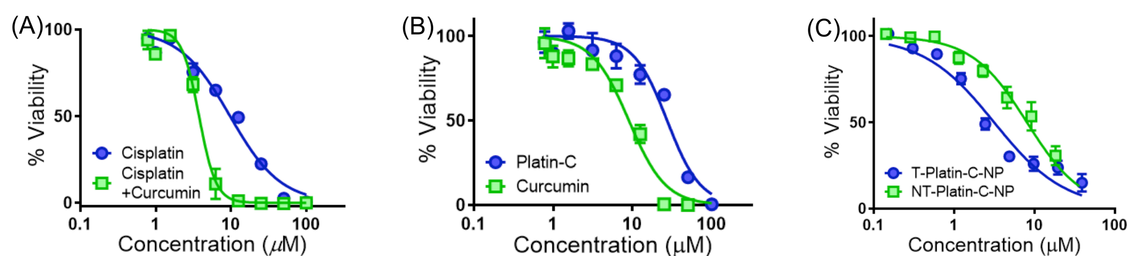
Supplementary Figure 10. ^{13}C NMR spectrum of the targeted PLGA-*b*-PEG-TPP polymer in $CDCl_3$ recorded using a 400 MHz NMR spectrometer.



Supplementary Figure 11. Gel Permeation Chromatograms of PLGA-COOH, H_2N-PEG-NH_2, PLGA-*b*-PEG-NH_2 and PLGA-*b*-PEG-TPP polymers.



Supplementary Figure 12. Hydrodynamic diameter and zeta potential of various Platin-*C* feed NT-Platin-*C*-NPs containing as determined by Dynamic Light Scattering (DLS) measurements.



Supplementary Figure 13. Representative plots for the MTT assays performed on A2780/CP70 cells using the various test articles as mentioned in the respective plots.

References:

(1) Shi, W.; Dolai, S.; Rizk, S.; Hussain, A.; Tariq, H.; Averick, S.; L'Amoreaux, W.; El Idrissi, A.; Banerjee, P.; Raja, K. *Organic Letters* **2007**, *9*, 5461.

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