†Electronic Supplementary Information (ESI)

S.1.Synthesis of N-(3-(2-(2-(3-aminopropoxy) ethoxy) ethoxy) propyl) isonicotinamide

In the first step, mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine was synthesized. Breifly, 4,7,10-trioxa-1,13-tridecanediamine (6g, 29.7 mM) was dissolved in methanol (200 mL) along with triethyl amine (6 g, 60 mM). Di-tert-butyl dicarbonate (1.3 g, 6 mM) in dichloromethane (1 mL) was added dropwise to the reaction mixture under continuous stirring (1200 rpm). The reaction was carried out at room temperature for 12 hours and the solvents were evaporated under vacuum using rotary flash evaporator (IKA 50, Germany). The residue was extracted between saturated sodium bicarbonate (50 mL) dichloromethane (150 mL) mixture using separating flask. The organic layer was removed and the aqueous layer was once again extracted with dichloromethane (150 mL) . The combined organic layers were dried using anhydrous sodium sulphate and evaporated to obtain mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine. Conjugation of isonicotinic acid to mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine was done as follows. Isonicotinic acid (1.5 g, 12.2 mM) was dissolved in dichloromethane (25 mL) followed by addition of N-hydroxy succinimide (1.65 g, 14.3 mM). After mixing the components for 15 minutes at room temperature, EDC.HCl (2.75 g, 14.3 mM) and triethylamine (2.15 g, 21.3mM) were added, and the reaction was continued for overnight. Dichloromethane was evaporated and the crude mixture was extracted three times between saturated sodium bicarbonate (50 mL) dichloromethane (150 mL). The combined organic layers were dried over anhydrous sodium sulphate and the solvent was evaporated to obtain tert-butyl (1-oxo-1-(pyridin-4-yl)-6,9,12-trioxa-2-azapentadecan-15-yl) carbamate. The Boc groups were then cleaved by treating with dichloromethane:trifluoroacetic acid mixture at 1: 1 ratio for 3 hours .

S.2.Conjugation of propargylamine to isonicotinic acid grafted PSMA

Free carboxylic acid groups in the N-(3-(2-(2-(3-aminopropoxy) ethoxy) ethoxy) propyl) isonicotinamide grafted PSMA were functionalized with propargylamine. Briefly, 1g N-(3-(2-(2-(3-aminopropoxy) ethoxy) ethoxy) propyl) isonicotinamide grafted PSMA (approx. 3.533 mM of monomer units) was activated with N-Hydroxysuccinimide (0.35g, 3 mM) for 20 minutes in DMF (25 mL) followed by addition of EDC.HCl (0.58g, 3 mM). After 30 minutes of reaction in ice bath propargyl amine (0.21 g, 3.81 mM) and NEt_3 (0.385g, 3.85 mM) were added to the reaction mixture. The reaction was allowed to continue for 24 hrs at room temperature. DMF was then evaporated using rotary flash evaporator and the propargyl amine functionalized polymer was purified by precipitating and dialyzing with water (4L, dialysis membrane with a molecular weight cutoff 3500 Da). Water was

changed every 6 hours and dialysis was continued for 2 days. The product was then lyophilized and recovered.

S.3.Modification of 4,7,10-trioxa-1,13-tridecanediamine with azide and Fmoc-Arg-pbf-OH

Azidoacetic acid (1 g, 9.8 mM) was dissolved in dichloromethane (25 mL) and stirred for 15 minutes in an ice bath, under nitrogen. The carboxylic acid was activated with NHS (1.4 g, 12.2 mM) and EDC.HCl (2.26 g, 11.8 mM) for 15 minutes, successively. Mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine (1g, 3.1 mM) was added along with triethylamine (2.35 g, 23.5 mM) to the reaction mixture and the reaction was allowed to continue in ice bath for 4 h and then at room temperature for 24 h. The solvent was evaporated and residue was extracted between saturated sodium bicarbonate (150 mL) and dichloromethane (50 mL) for three times. Combined organic layers were dried using anhydrous sodium sulphate and dried to obtain azide functionalized mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine.

Coupling of Fmoc-Arg-pbf-OH to the azide functionalized glycol amine was done using EDC NHS. Breifly, Fmoc-Arg-pbf-OH (1 g, 1.54 mM) was activated with NHS (0.4 g, 3.5 mM) and EDC.HCl (0.65 g, 3.4 mM) , successively for 15 minutes, in dichloromethane (50 mL). Azide functionalized mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine (1.75g, 4.16 mM) was deprotected from Boc by reacting with mixture of DCM and TFA (1:1) for 3 h and the obtained product along with triethylamine (2 g, 20 mM) were added to the activated Fmoc-Arg-pbf-OH, under stirring. After 24 h, the solvent was evaporated and the residue was extracted between water (150 mL) and DCM (50 mL). The extractions were carried out thrice and the organic layers were dried using anhydrous sodium sulphate. DCM was evaporated to obtain Fmoc-Arg-pbf-OH and azide functionalized 4,7,10-trioxa-1,13-tridecanediamine.

S.4.Conjugation of L-lysine to the polymer

Fmoc-Arg-pbf-OH grafted polymer was dissolved in DMF: piperidine mixture (50:50 volume ratio) and reacted for 3 hours to remove Fmoc. The polymer was purified by washing with ethylacetate and coupled with Fmoc-Lys-Boc-OH using HBTU. Briefly, Fmoc-Lys-Boc-OH (0.5 g, 1 mM) was dissolved in dry DMF (50 mL) and reacted with HBTU (0.485g, 1.2 mM) in ice bath for 15 minutes. Arg-pbf-OH grafted polymer (0.6 g, 0.4 mM) and DIPEA (0.275 g, 2.1 mM) were added to the reaction mixture and reaction was allowed to continue for overnight at room temperature. DMF was evaporated and the residue was precipitated with ethylacetate and acetonitrile successively, to obtain purified polymer. The lysine grafted polymeric derivative (5) was dissolved in DMF: piperidine mixture (1:1 v/v) and reacted for 3 hours to remove Fmoc. The polymer was then purified by precipitating with ethylacetate. Dried polymer (0.5 g) was dissolved in dry DMF in the presence of triethylamine (0.2 g, 2 mM) followed by slow addition of acetic anhydride (0.35g, 3.4 mM). The

reaction was continued for overnight at room temperature. The solvent was then evaporated and residue was repeatedly washed with ethylacetate. The obtained product was treated with 15 mL of TFA: triisopropylsilane: phenol mixture in the volume ratio 95:2.5:2.5, for 3 hours, to remove pbf and Boc. The polymer was then purified by precipitating with ethylacetate.

S.5.Determination of CMC of the polymeric micelles

Aliquots of pyrene stock solution (0.1 mg/mL) in chloroform were added to clean glass vials. Chloroform was then allowed to evaporate in dark. Polymeric micelles in HEPES buffer, pH 7.4 were incubated with the dried pyrene for overnight, at 37ºC in a shaking incubator. Final concentrations of polymeric micelles examined were in the range of 1-50 µg/mL and the concentration of pyrene was 6.5×10^{-7} M in all the vials.

S.6.Interaction of polymer with plasma proteins

Human plasma was obtained from blood bank, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Polymeric particles (10 mg) were uniformly dispersed in 1 mL of phosphate buffer saline (PBS), pH 7.4 by probe sonication (100 W, 15 seconds). The dispersions were diluted with 4 mL of human plasma and incubated for 1 h at 37 º C. Following incubation, the dispersions were centrifuged at 20000 g for 1 h. Residues were washed thrice with PBS, dried by lyophilization and treated with urea-thiourea buffer (2M thiourea, 7 M urea and 4 % CHAPS detergent) to elute adsorbed proteins. Protein content was quantified using Bradford reagent (Biorad). BSA was used as standard and calibration curve was prepared as per the manufacturers protocol.

S.7 Haemolysis assay

Heparinized human blood was obtained from blood bank, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Whole blood (1 mL) was centrifuged at 1500 rpm for 10 minutes. The settled red blood cells (RBC s) were washed thrice with phosphate buffer saline (PBS), pH 7.4. RBC stock solution was prepared by diluting 50 µL of RBC s to 10 mL with PBS. Polymeric nanoparticles in PBS (100 µL) were incubated with equal volumes of RBC stock solution at 37 °C for 1 h. Final concentrations of nanoparticles were in the range of 125 - 2500 µg mL. After incubation, the mixtures were centrifuged at 1500 rpm for 10 minutes and haemoglobin released was quantified by recording the absorbance of supernatants at 540 nm using micro plate spectrophotometer (PowerWave XS2, Bio Tek Instruments, USA). PBS and 1 % Triton X-100 were used as negative and positive controls respectively. % haemolysis at pH 5.5 was determined, in the same way as that at pH 7.4, using PBS pH 5.5 as the incubation media for nanoparticles and RBC s. % haemolysis was calculated using the following equation

$$\% \text{ haemolysis} = \frac{\{\text{Absorbance of sample} - \text{Absorbance of negative control}\} \times 100}{\{\text{Absorbance of positive control} - \text{Absorbance of negative control}\}}$$

S.8 Evaluation of nanoparticles for ROS generation and genotoxicity

For evaluation of ROS generation, cells were seeded in 96 well plate at a density of 5000cells/well and incubated for 24 hours at 37 °C and 5 % CO_2. Polymeric nanoparticles were added to the wells at final concentrations ranging from 50 to 1000 µg/mL and incubated for 48 hours at 37 °C and 5 % CO_2. After incubation, wells were supplemented with fresh media followed by addition of 20 µL of DCFH-DA dye at a concentration of 10mM. Fluorescence in each well was measured, after 30 minutes incubation, at 480 nm excitation using a microplate reader. Untreated cells were kept as control.

Genotoxicity was evaluated using comet assay reagent kit (trevigen). The cells (MCF-7) were seeded in T-25 culture flask and experiments were performed once 80 % confluency was achieved. Polymeric nanoparticles were incubated with the cells at a concentration of 1 mg/ml for 48 h. Following the incubation, alkaline comet assay was performed according to manufacturers protocol.

S.9.Real time PCR analysis

Total RNA from the harvested cells were isolated using Trizol regent. Briefly, 5×10^5 cells were incubated with 500 µL of trizol reagent for 5 minutes. 100 µL of chloroform was added to the mixture and shaken vigorously for 30 sec. The dispersions were centrifuged at 12500 rpm for 15 minutes. Aqueous layers were separated and mixed with 250 µL of isopropanol. After incubating at - 20 º C for 15-20 minutes, the separated aqueous layers were again centrifuged at 12500 rpm for 15 minutes. Supernatants were then discarded and the pellets were washed with 75 % ethanol. The washed pellets were dried and dissolved in RNase free water and incubated at 65 º C for 15 minutes. Isolated RNAs were purified from any genomic DNA by treating them with DNase (1 U). cDNA was prepared from the RNA contents using cDNA kit (Biorad). Briefly, RNA solutions were mixed with appropriate amounts of 10 x cDNA reaction buffer and reverse transcriptase enzyme according to the manufacturers protocol. Final volume was reconstituted with nuclease free water to make the cDNA reaction buffer concentration 1x. PCR cycles employed were 65 ºC for 5 minutes, 42 ºC for 30 minutes and 70 ºC for 15 minutes. The obtained cDNA samples were stored at 4 ºC till they were used for real time PCR analysis. For real time PCR and cDNA, primers (forward and reverse) were mixed with q PCR master mix (QuantiNova SYBR Green PCR Kit) at final primer concentrations of 10 µM.

plk 1 expression was normalized with that of GAPDH. Delta delta Ct (2 ΔΔ Ct) method was used to determine the fold change in gene expression. Primer sequences of plk 1 and GAPDH primers used are as follows.

plk 1 forward : 5' - CCCATCTTCTGGGTCAGCAAG

plk 1 reverse : 5' - AAGAGCACCCCCACGCTGTT

GAPDH forward: 5' - TGCACCACCAACTGCTTAGC

GAPDH reverse: 5' - GGCATGGACTGTGGTCATGAG

S.10.Cellular uptake studies

MCF-7 cells in complete media (supplemented with 10% fetal bovine serum) were added to confocal plates and incubated for 24 hours, at 37 °C and 5 % CO_2, to attain 70-80 % confluency. Doxorubicin and FAM-labelled siRNA loaded polymeric nanoparticles were incubated with the cells for 3 hours. Final concentrations of doxorubicin and FAM-labelled siRNA were 5 µg/mL and 100 nM respectively. After incubation, cells were washed thrice with PBS and treated with Nuc Blue for 10 minutes. Fluorescence of doxorubicin, FAM-labelled siRNA and Nuc Blue were visualized, using confocal laser scanning microscope (CLSM, Olympus IX 81 under DU897 mode), at respective emission wavelengths of 561 nm, 488 nm and 405 nm.

For evaluating the nuclear localization of doxorubicin loaded nanoparticles, MCF- 7 cells in complete media were seeded in to 24 well plates containing spherical glass slides. After obtaining ~70-80 % confluency, cells were treated with doxorubicin (10 µg/mL) and plk-1 siRNA (100 nM) loaded polymeric micelles. Post incubation, cells were washed thrice with PBS and stained with lysotracker green (invitrogen), at a concentration of 100 nM, for 25 minutes. Nuclear staining was performed in the end using DAPI dihydrochloride (2 µg/mL) for 5 minutes. Repeated washing of the cells with phosphate buffer saline (pH 7.4) was performed after each staining. The cells were fixed with 4 % paraformaldehyde solution and visualized using confocal laser scanning microscope (Olympus, Fluoview FV1000).

S.11.Cell proliferation assay and live/dead staining of treated cells

Cells were seeded in to 96 well plate at a density of 10^4 cells/well and incubated for 24 hours at 37 °C under 5 % CO_2. Polymeric micelles loaded with doxorubicin and scrambled siRNA (100 nM) at various concentrations were added to one set of wells. Another set of wells were treated with polyplexes containing 100 nM plk-1 siRNA along with different concentrations of doxorubicin. The cells were incubated with the samples up to 48 hours. The wells were then replaced with fresh media (200 µL) and incubated with 10 µL of MTT solution (5 mg/ml) for 3 hours. The crystals formed were then dissolved in 200 µL of DMSO and absorbance was recorded at 540 nm using microplate spectrophotometer (PowerWave XS2, Bio Tek Instruments, USA).

For determining the live/dead ratio of cells treated with formulations, cells were seeded in to confocal plates and incubated at 37 ºC under 5 % CO_2**,** till ~ 80 % confluency was attained. The plates were then treated with either polymeric micelles bearing doxorubicin scrambled siRNA or doxorubicin plk-1 siRNA at siRNA concentration of 100 nM. After 48 hours of incubation with the samples, cells were stained with calcein/PPI (according to manufacturers protocol) and images were recorded using CLSM at 4x magnification.

S.12. Determination of primary amine content of the polymer by TNBS assay

The primary amine content of polymer was determined using TNBS assay according to previously reported method, with slight modification. Briefly, lyophilized nanoparticles were dispersed in double distilled water (400 µL) and mixed with 400 µL of 4 % sodium bicarbonate solution. 400 µL of aqueous TNBS solution (0.1 %) was added to the mixture and the reaction was carried out at 37 ° C for 2 h, at 1200 rpm. Final concentration of nanoparticles was kept at 100 µg/mL. Gamma amino butyric acid was used as standard. Known concentrations of the amino acid were treated with TNBS in the same manner as that of the nanoparticles and absorbances were recorded at 349 nm using micro plate spectrophotometer (PowerWave XS2, Bio Tek Instruments, USA). Experiments were carried out in triplicate.

Primary amine content of the polymeric nanoparticles was found to be 76.33 ± 6.97 µM 100 µg of nanoparticles.

S.13. Determination of average molecular weight of the click modified polymer by GPC

The polymeric derivative was dissolved in HPLC grade DMF for molecular weight determination Average molecular weight of the polymer was determined by gel permeation chromatography (Waters, USA, Styragel HR column) using Waters 2414 RI detector equipped with DMF coloumn.

S.14.Toxicological evaluation of the cationic micelles

The mice were randomly grouped in to two groups (n = 4) for performing repeated dose toxicity study. Dispersions of polymeric micelles in PBS, pH 7.4 (0.3 mL) were administered in one group (on days 1, 4 and 7) at a dose of 100 mg kg, intraperitoneally. The second group was similarly injected with blank PBS. On day 8, blood samples were collected from the mice by retro orbital venous puncture for evaluation of serum biochemistry and hematology analysis. The animals were then sacrificed by cervical dislocation and vital organs were collected in 4 % formalin for histopathological analysis.

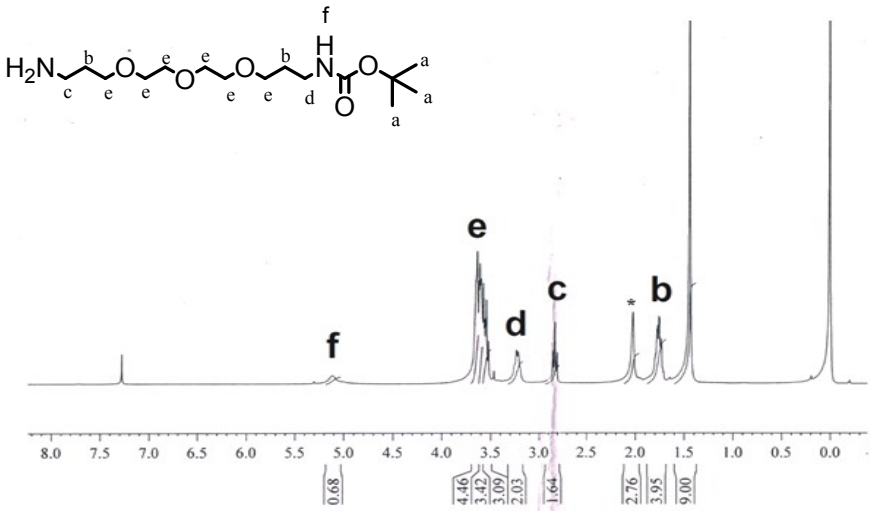
S.15. *In vivo* anti cancer efficacy

Various polymeric formulations were evaluated for their anti cancer efficacy using Ehrlich ascites tumor (EAT) model. EAT cell line was obtained as a gift from INMAS, New Delhi, India. 150 µL of EAT cell suspension (containing ~ 2×10^7 cells) was injected subcutaneously on the dorsal side of female swiss albino mice. Tumor volumes were estimated twice in a week, by measuring the length and breadth of tumors using Vernier caliper, following the inoculation. Volumes were calculated using the formula π 6 (length) (width) (height), where length was measured as the longest diameter across the tumor. Mice bearing solid tumors with an average volume of 300 mm^3 were divided in to five groups (4 animals each). Group I was treated with doxorubicin and plk 1 siRNA carrying polyplexes.

Group II, III, IV and V were treated respectively with doxorubicin and scrambled siRNA loaded polymeric micelles, free Doxorubicin, plk 1 siRNA complexed polymeric micelles and blank PBS (designated as control).

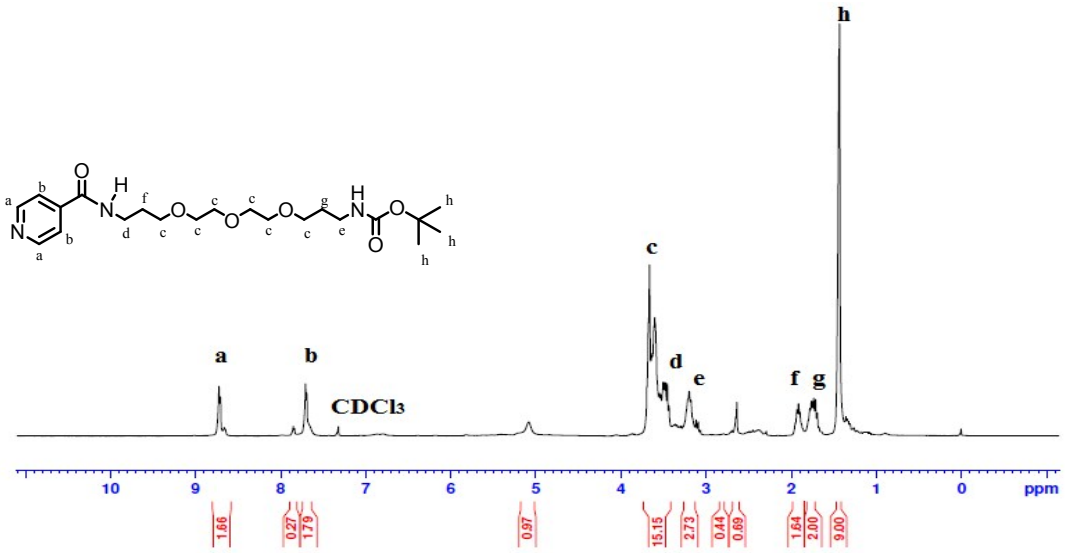
All the formulations were administered intratumorally. For intratumoral injection, 50 µL of various formulations (in PBS) were injected in the longitudinal direction from the edge to centre of the tumors. Each injection was administered slowly over 1 minute. Following each injection, the needle was kept at the injection site for further 5 minutes in order to prevent any leakage of the sample. Four injections were given over a period of 2 weeks. Doxorubicin and siRNA were respectively administered at 1.5 mg kg and 0.5 mg kg dose. At the end point of study, animals were sacrificed by cervical dislocation followed by collecting tumors, vital organs and blood. Tumors and vital organs were evaluated using histopathological analysis. Serum biochemical parameters of blood samples were also studied.

A



δ 1.43 (s, 9H, Boc C*H*$_3$), 1.75 (m, 4H, NH-CH$_2$-C*H*$_2$), 2.83 (t, J = 6.6 Hz, 2H, -O-C*H*$_2$), 3.22 (m, 2H, HN- C*H*$_2$), 3.5-3.8 (m, 12H, -O-C*H*$_2$), 5.15 (brs, 1H, N*H*)

B



δ 1.445 (s, 9H, Boc C*H*$_3$), 1.725 (m, 2H, NH-CH$_2$-C*H*$_2$), 1.879 (m, 2H, NH-CH$_2$-C*H*$_2$), 3.2 (m, 2H, HN- C*H*$_2$), 3.45 (m, 2H, HN- C*H*$_2$), 3.54-3.78 (m, 12H, -O-C*H*$_2$), 8.4 (d, J = 7.2 Hz, 2H,Ar C*H*), 9 (d, J = 7.2 Hz, 2H, Ar C*H*).

Figure S.1: ^{1}H NMR spectra (300 MHz, $CDCl_3$) of Mono Boc protected 4,7,10 – trioxa-1,13- tridecanediamine (A) and isonicotinic acid conjugate of Boc protected 4,7,10 – trioxa-1,13- tridecanediamine (B)

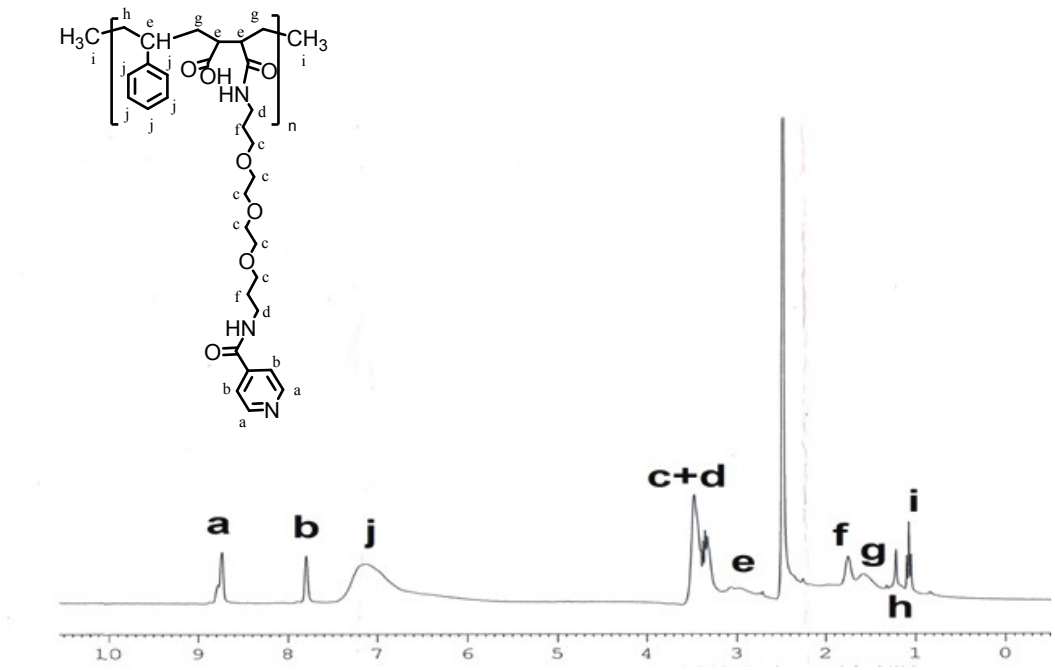


Figure S.2: ^{1}H NMR spectra (300 MHz, DMSO-*d*6) of isonicotinic acid grafted polymer (1)

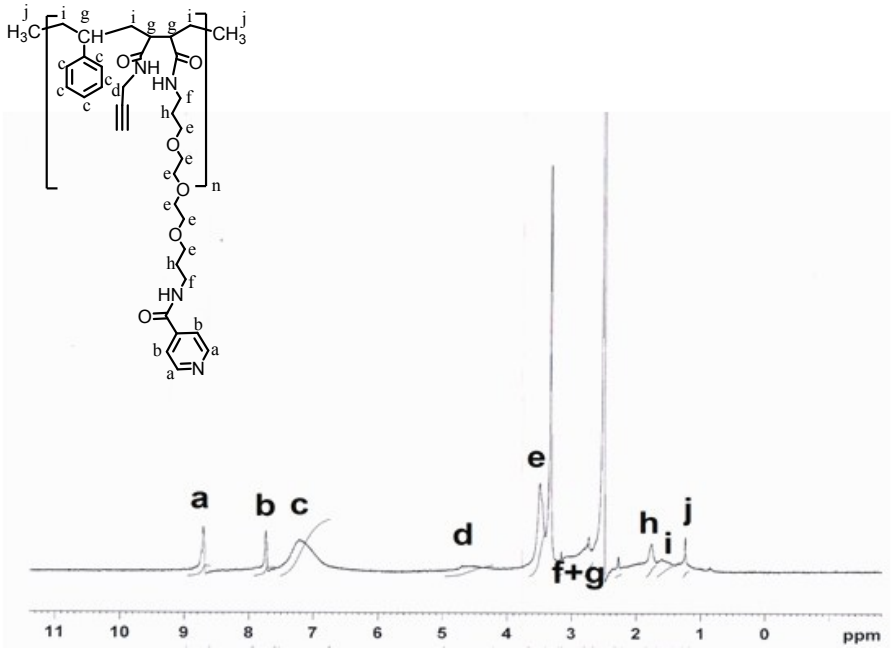


Figure S.3: ^{1}H NMR spectra (300 MHz, DMSO-*d*6) of alkyne functionalized polymer (2)

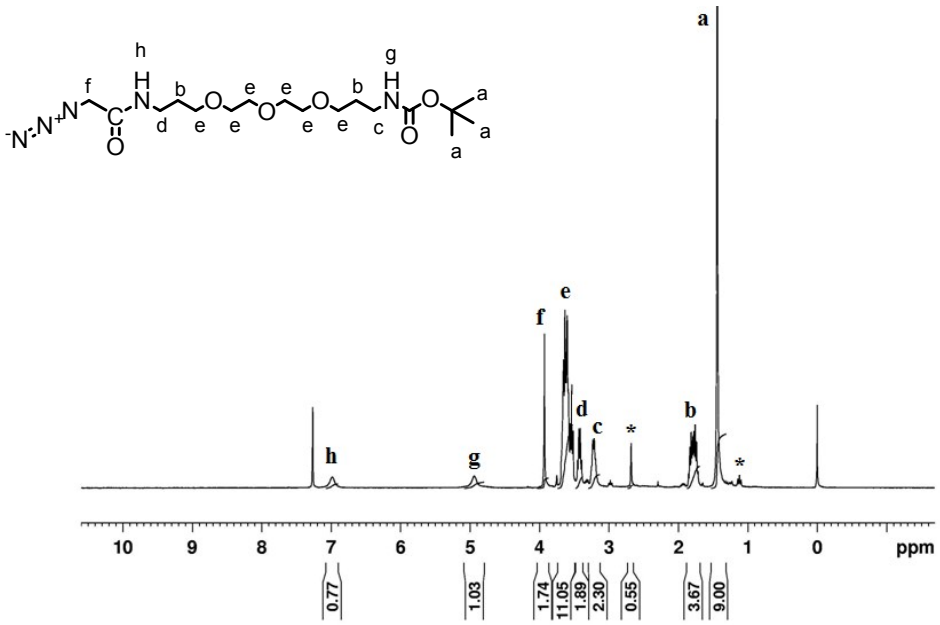
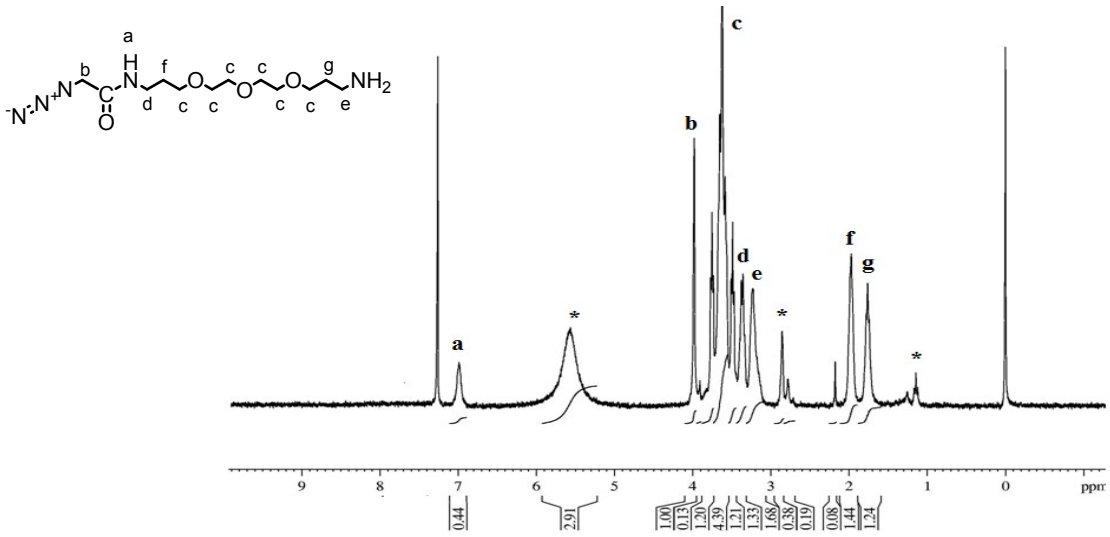


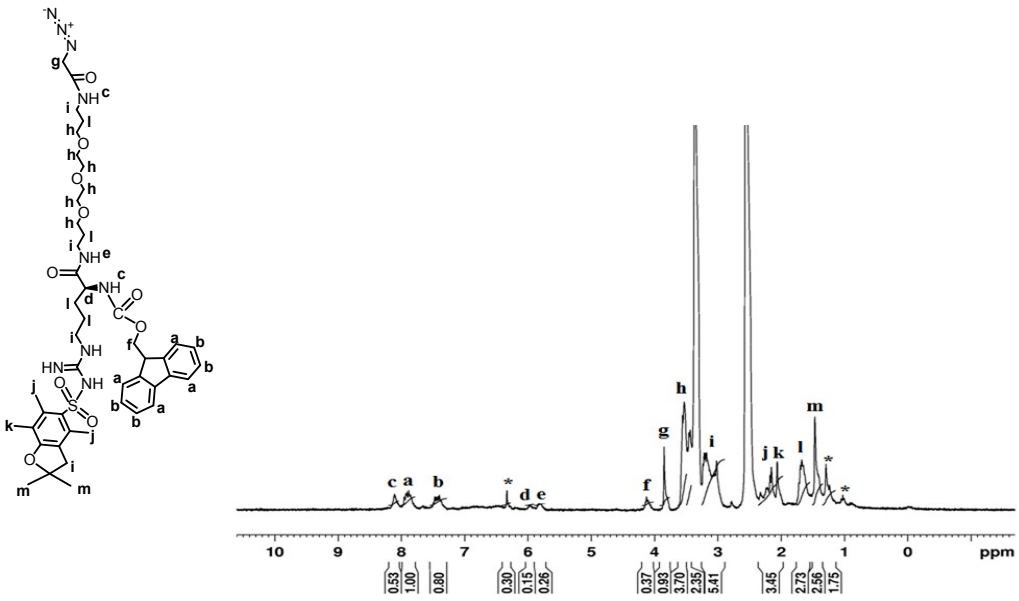
Figure S.4: ^{1}H NMR spectra (300 MHz, $CDCl_3$) of azide functionalized mono N- Boc protected 4,7,10-trioxa-1,13-tridecanediamine. δ 1.44 (s, 9H, Boc C*H*$_3$), 1.8 (m, 4H, NH-CH_2-C*H*$_2$), 3.2 (m, 2H, HN- C*H*$_2$), 3.4 (m, 2H, HN- C*H*$_2$), 3.51-3.75 (m, 12H, -O-C*H*$_2$), 3.93 (s, 2H, N- C*H*$_2$), 4.93 (brs, 1H, N*H*), 6.98 (brs, 1H, N*H*).

A



^{1}H NMR spectra (300 MHz, $CDCl_3$), 1.78 (m, 2H, NH-CH_2-C$\underline{H_2}$), 1.97(m, 2H, NH-CH_2-C$\underline{H_2}$), 3.35 (m, 2H, HN- C$\underline{H_2}$), 3.47 (m, 2H, HN- C$\underline{H_2}$), 3.5-3.77 (m, 12H, -O-C$\underline{H_2}$), 3.98 (s, 2H, N- C$\underline{H_2}$), 6.99 (brs, 1H, N$\underline{H}$).

B



^{1}H NMR spectra (300 MHz, DMSO-*d*6), 1.46 (s, C-C$\underline{H_3}$), 1.71(m, NH-CH_2-C$\underline{H_2}$), 2. 064 (s, Ar- C$\underline{H_3}$), 2.154 (s, Ar- C$\underline{H_3}$), 3.2-3.558 (m, -O-C$\underline{H_2}$), 3.85 (s, 2H, N- C$\underline{H_2}$), 5.8 (brs, N$\underline{H}$), 5.98 (brs, NH-C$\underline{H}$), 7.4 (m, Ar- C$\underline{H}$), 7.8 (m, Ar C$\underline{H}$), 8.1 (brs, N$\underline{H}$).

Figure S.5: ^{1}H NMR spectra of azide functionalized 4,7,10-trioxa-1,13-tridecanediamine (A) azide and Fmoc-Arg-pbf-OH coupled 4,7,10-trioxa-1,13-tridecanediamine (B).

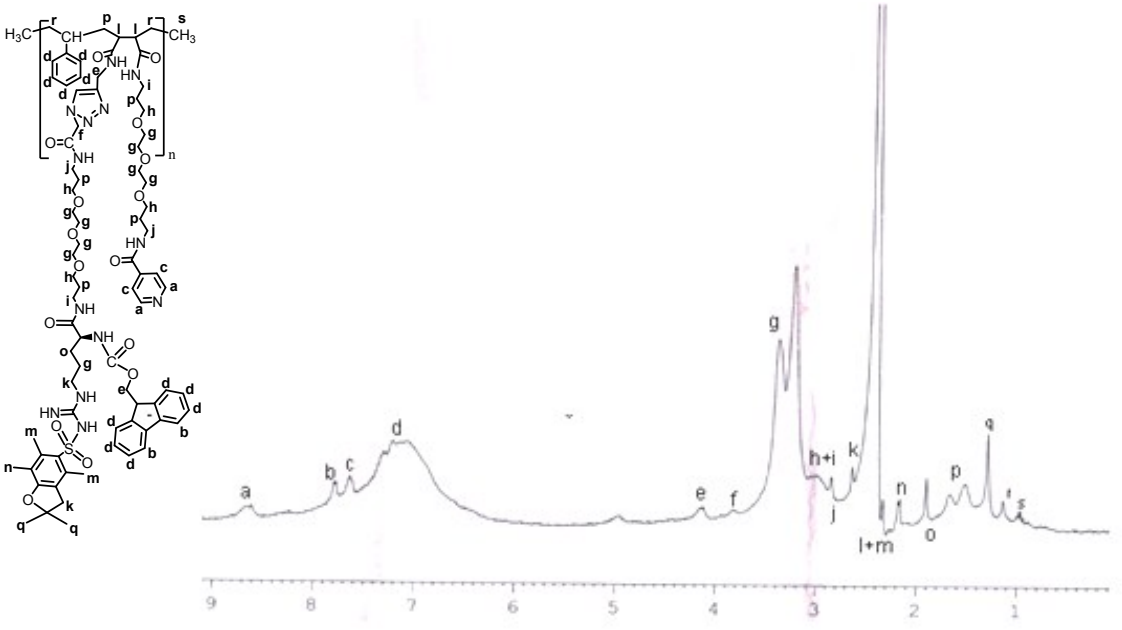


Figure S.6: ^{1}H NMR spectra (300 MHz, DMSO-*d*6) of click modified Fmoc-Arg-pbf-OH grafted polymer

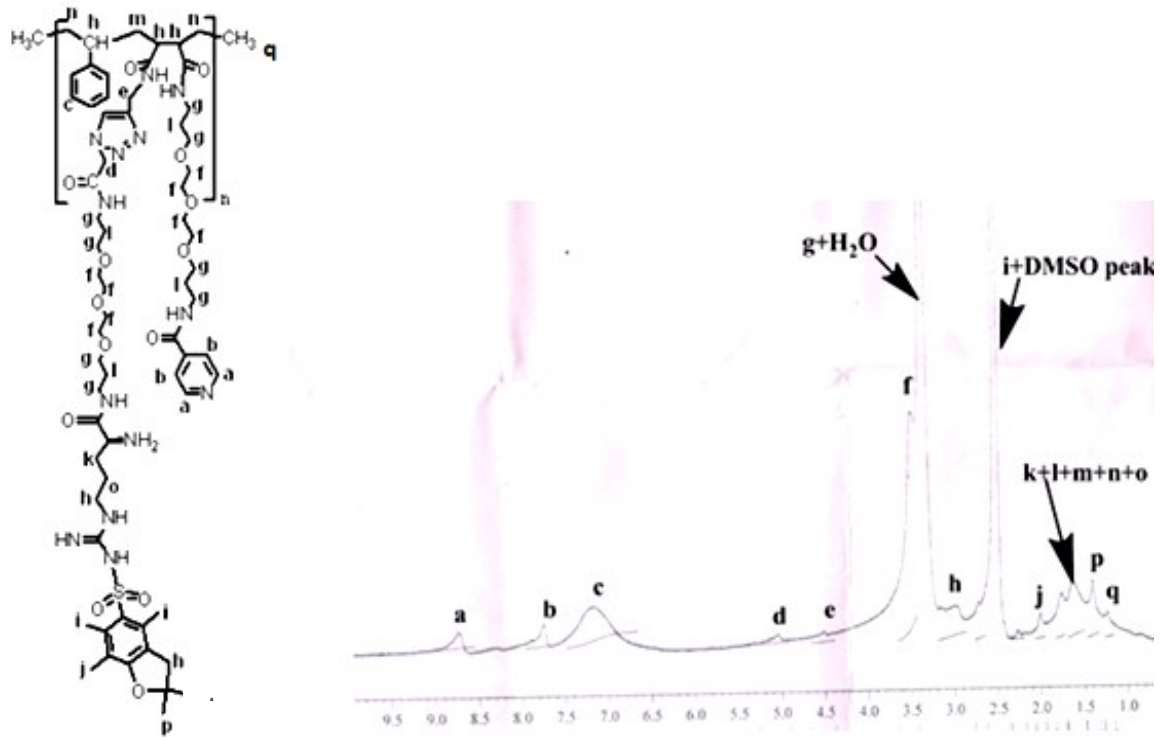


Figure S.7: ^{1}H NMR spectra (300 MHz, DMSO-*d*6) of click modified arginine grafted polymer after removal of Fmoc

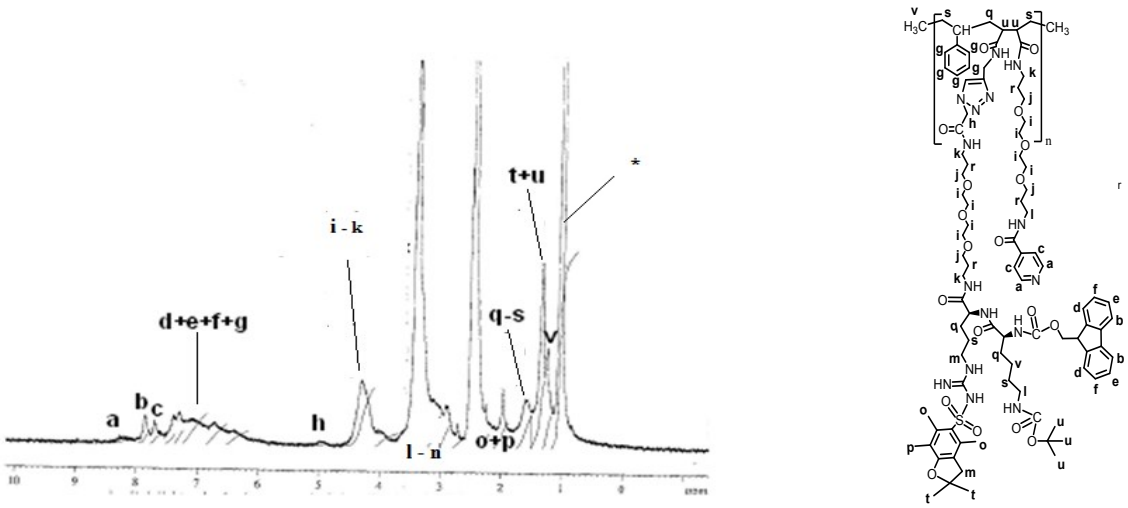


Figure S.8: [1]H NMR spectra (300 MHz, DMSO-*d*6) of click modified polymer after grafting of Fmoc-Lys-Boc-OH.

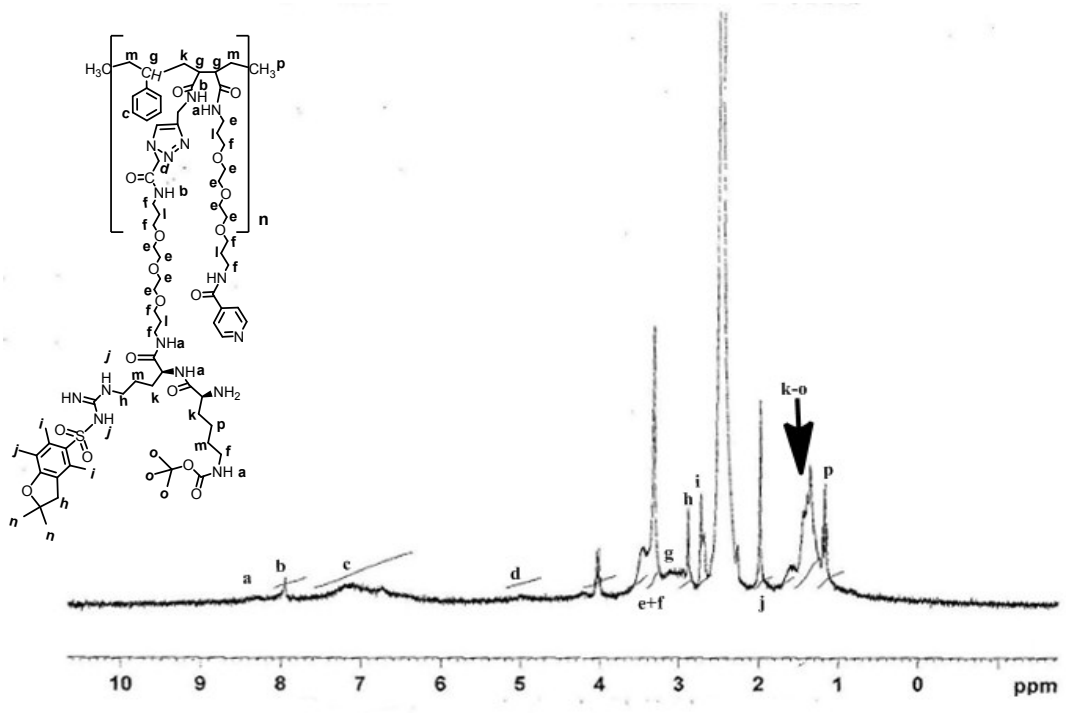


Figure S.9: [1]H NMR spectra (300 MHz, DMSO-*d*6) of arginine and lysine grafted polymer after removal of Fmoc

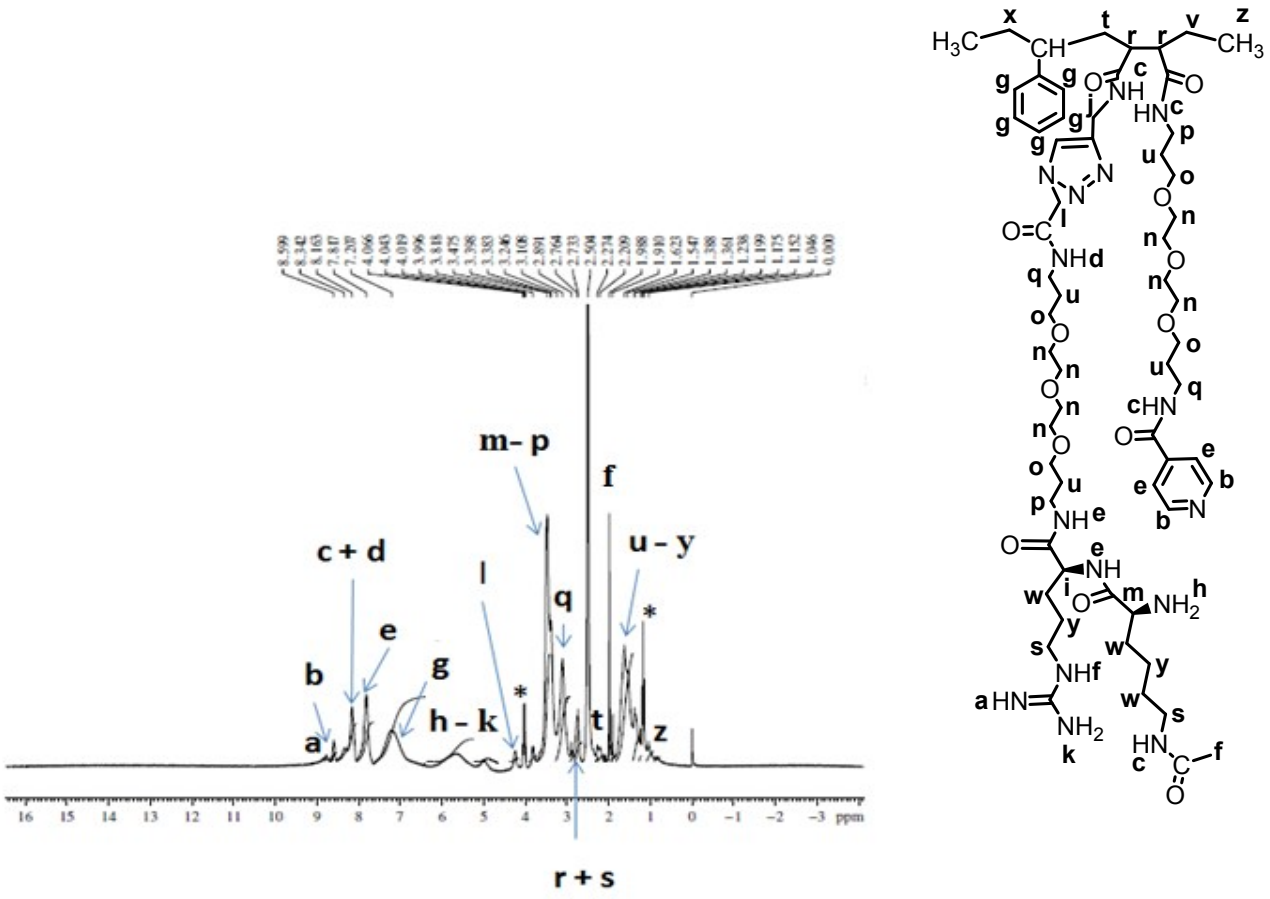


Figure S.10: [1]H NMR sectra (400 MHz, DMSO-*d*6) of arginine and lysine grafted polymer after removal of protecting groups

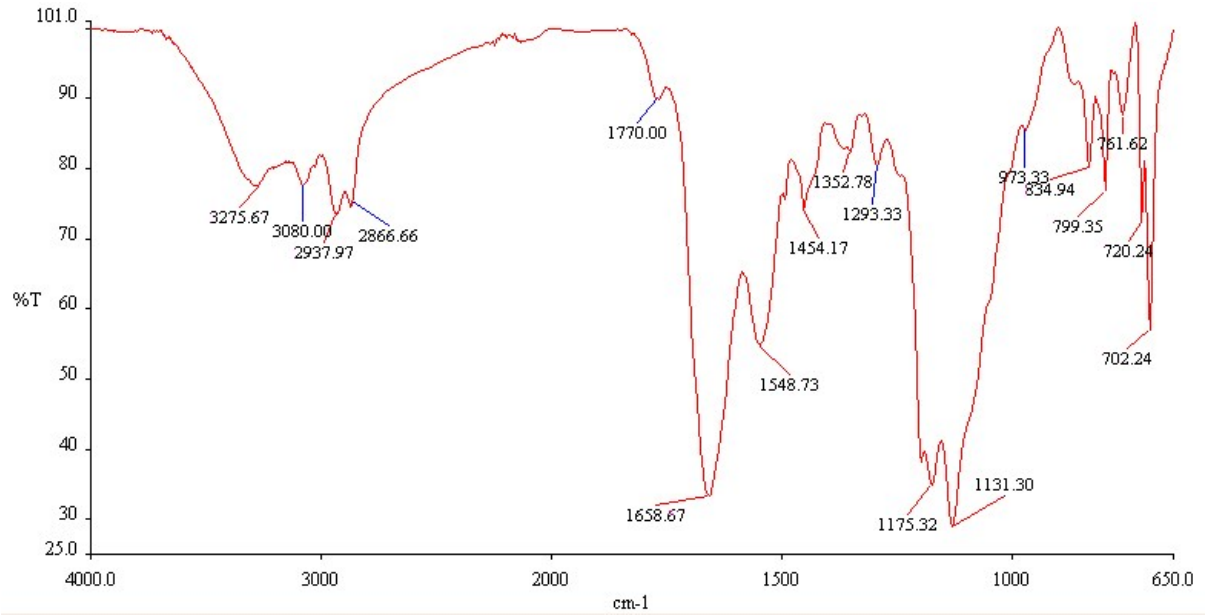


Figure S.11: ATR-FTIR spectra of the synthesized polymer

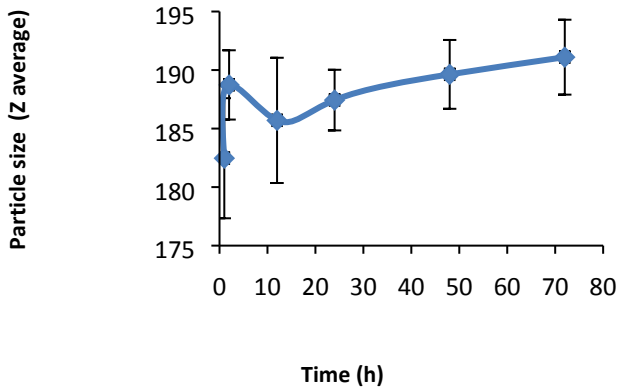


Figure S.12: Stability of the polymeric micelles in RPMI 1640 media supplemented with 10 % FBS.

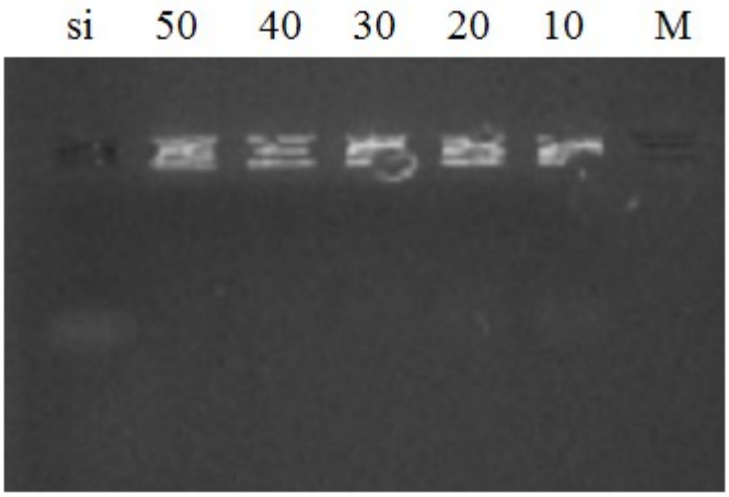


Figure S.13:. Agarose gel (1.2 %) electrophoresis of blank polyplexes (without drug). siRNA (negative control) and blank micelles are labelled by si and M, respectively. Polymer siRNA weight ratios are shown with numbers (10 - 50). The bands of siRNAs were visualized by immersing the gel in SYBR green solution for 1 h in dark and imaging using gel doc system. (Gel Doc XR, BIO RAD).

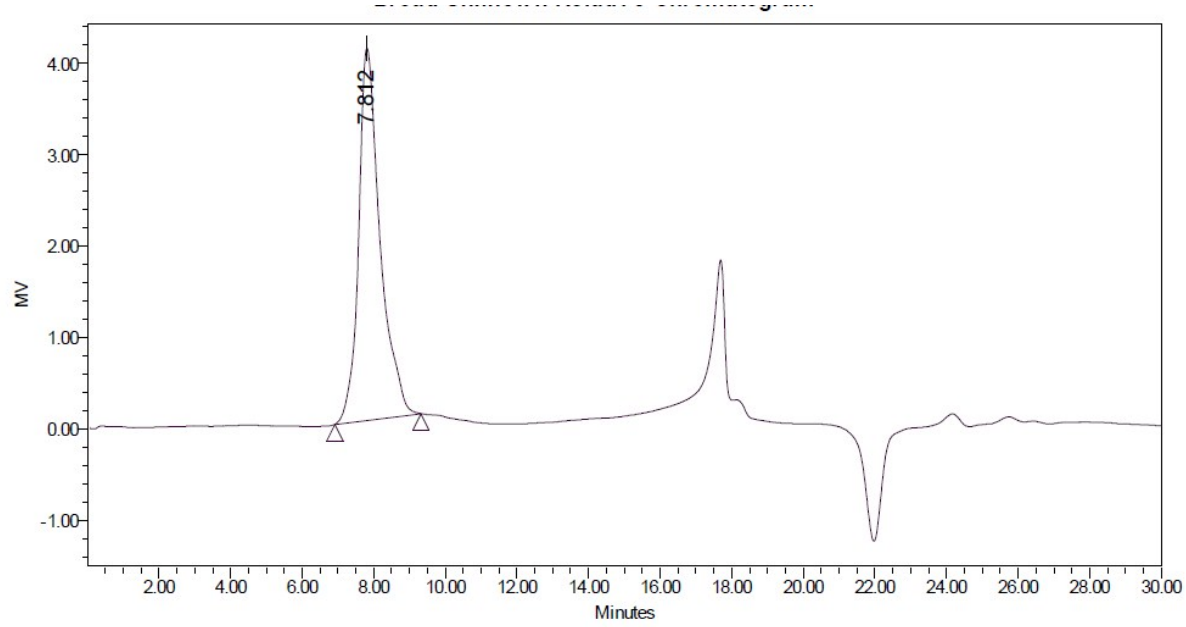


Figure S.14: Gel permeation chromatogram of arginine and acetyllysine grafted polymer.

Average molecular weight of the polymer (Mw) was determined from standard calibration curve obtained by polyethylene glycols of known molecular weights and calculated to be ~ 12.5 KDa.

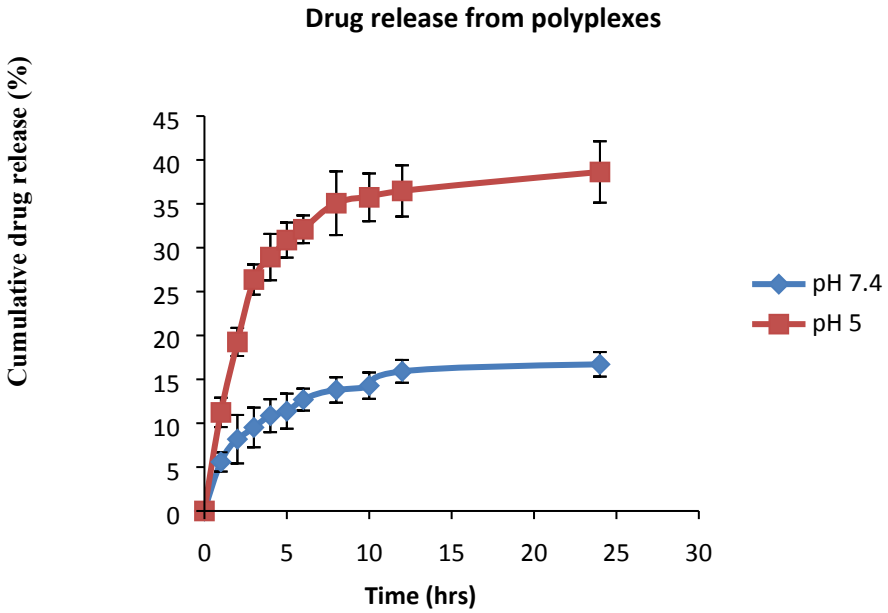


Figure S.15: In vitro drug release from the optimized micelles at pH 7.4 and 5

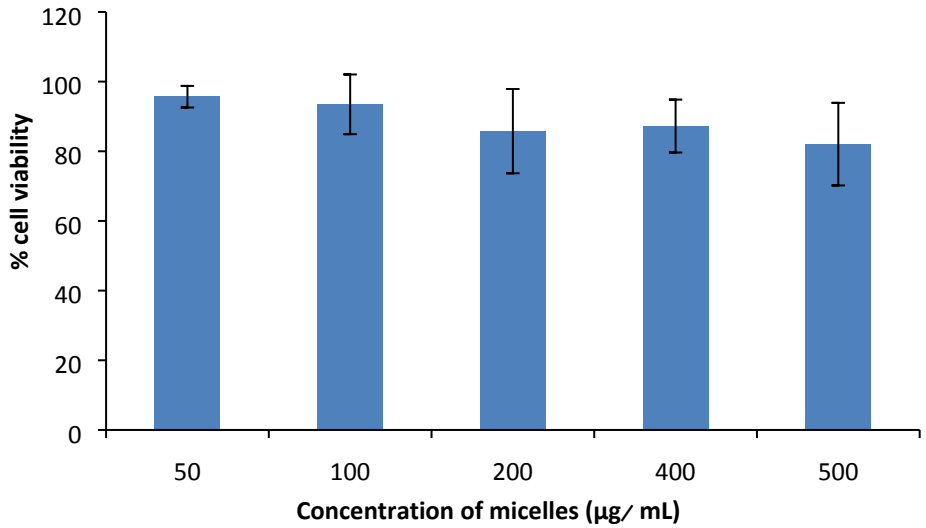


Figure S.16: MTT assay of blank polymeric micelles in MCF 7 cells after 48 h incubation

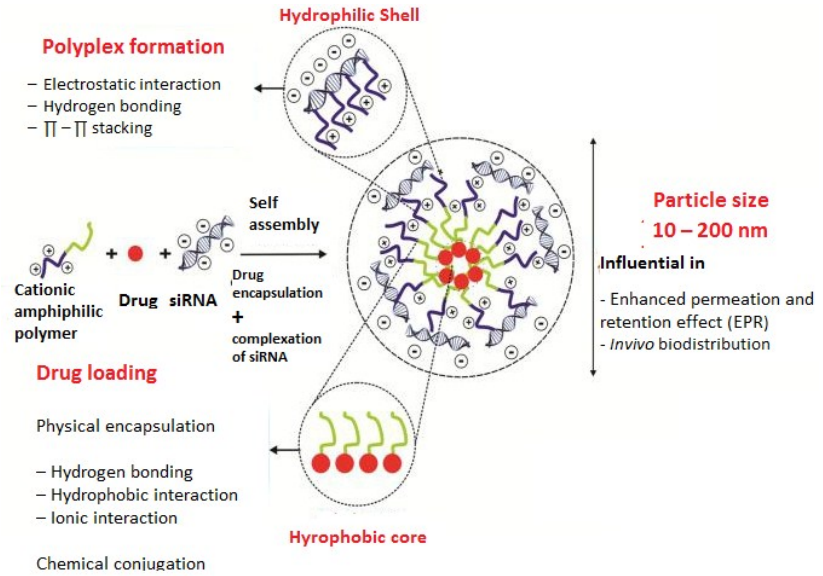


Figure S.17. Schemetic representation of the criteria for designing a suitable carrier for co-delivery of siRNAs and chemotherapeutics

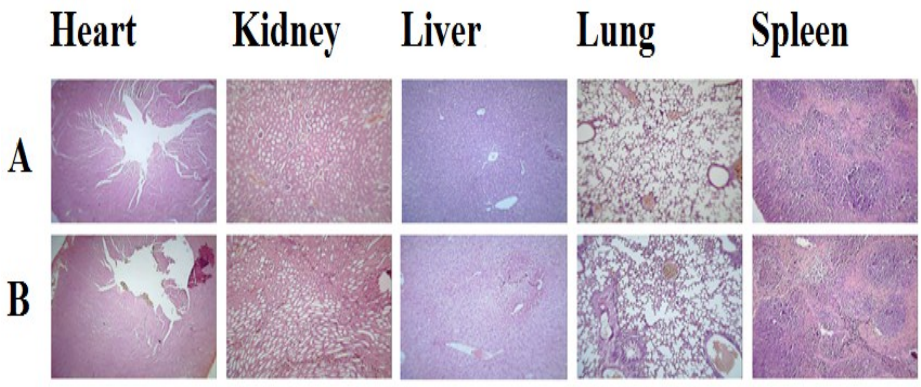


Figure S.18. Histopathological evaluation of vital organs of mice in repeated dose toxicity study. 'A' shows organs of control mice and 'B' represents that of nanoparticle treated mice.

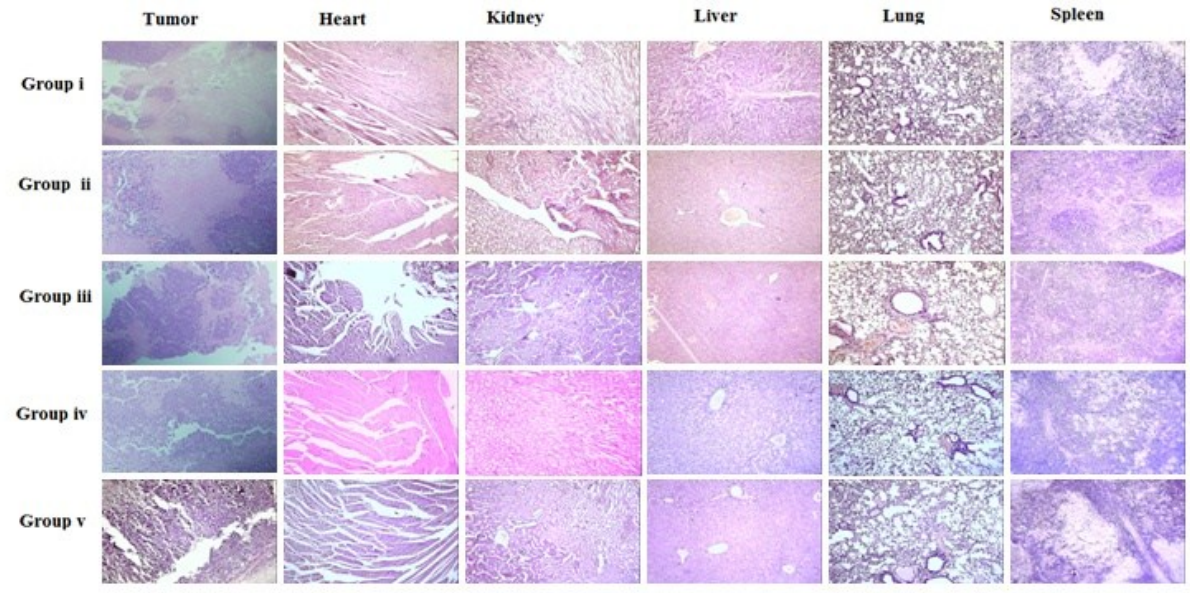


Figure S.19. Histopathological evaluation of vital organs and tumors of mice in tumor regression study.

Group i - Dox and siRNA loaded micelles

Group ii - Dox loaded micelles

Group iii - Free Dox

Group iv - siRNA loaded micelles

Group v - Control

Histopathological evaluation of the vital organs revealed negligible toxicity in all the groups. In case of heart, microscopic examination showed unremarkable tunica intima with medial layer showing presence of bundles of cardiac muscle fibres and hyalinised blood vessels. Kidney sections showed presence of healthy glomeruli, tubules, interstitium and blood vessels. Microscopic examination of spleen depicted lymphoid tissue, nucleated erytheroblasts, normal myelopoiesis and normal megakaryopoiesis. Lung sections were characterised by bronchial epithelium which was lined by stratified ciliated columnar epithelium. Bronchi and bronchioles walls were presented with smooth muscle fibres. Alveolar architecture exhibited emphysematous change. Alveolar space was empty and alveolar septa was expanded, edematous and filled with polymorphs and mononuclear cell infiltrate. No granuloma was seen in any of the groups. Liver sections showed polygonal cells with once cell plate thickness and uniform size in all three zones. Nuclear features were round to oval with regular nuclear margins. Eosinophilic cytoplasm with mild ballooning degeneration was observed. There was

no fibrosis, steatosis or pigment deposition present. All these histopathological observations were more or less similar in the control and treated groups indicating no toxicity

Tumor sections of control group was with more viable tumor cells than that of treated groups. Areas of light pink colour indicate necrotic areas and purple colour shows viable tumor cells. Dox and siRNA treated group exhibited the maximum necrosis compared to the other groups.

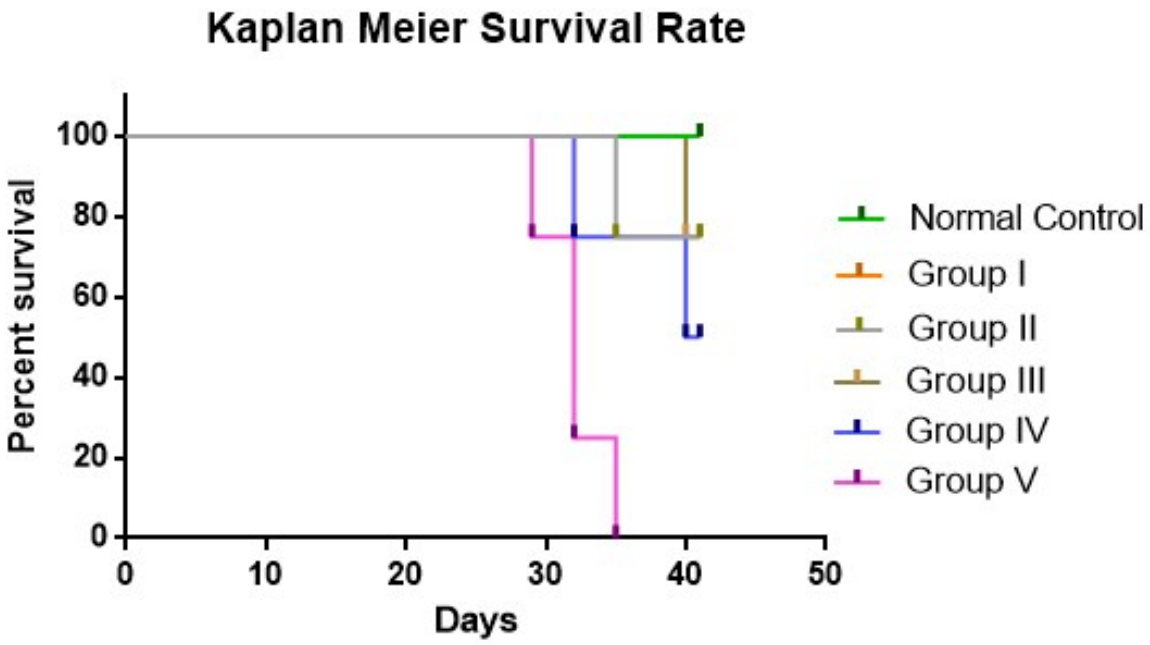


Figure S.20. Survival curve of the different groups of mice used in tumor regression study.

Group i - Dox and siRNA loaded micelles

Group ii - Dox loaded micelles

Group iii - Free Dox

Group iv - siRNA loaded micelles

Group v - Control

Normal control - Normal mice without induced tumor

Major serum biochemical parameters of various groups of tumor regression study are shown in table. S.2. There was only slight difference in the parameters of treated groups with respect to control. Even in free doxorubicin treated group, there was no significant alteration in the serum biochemical values. This could be due to the fact that route of administration chosen for the study was intratumoral and also due to low cumulative dose of doxorubicin (6 mg kg) administered, compared to that for intravenous administration.

Table. S.1 Hematology and serum biochemistry analysis from repeated dose (100 mg kg) toxicity study of cationic micelles in male swiss albino mice

Hematology and serum biochemistry parameters with units	**Control group (PBS)**	**Cationic micelles (100 mg kg)**
WBC (10^3 mm ^{3})	6.725 ± 2.866	5.325 ± .79
Lymphocytes (%)	55.45 ± 4.8	60.87 ± 7
Monocytes (%)	31.2 ± 8	27.2 ± 1.5
Granulocytes (%)	13.78 ± 4.39	11.5 ± 5.8
RBC (10^6 mm ^{3})	8.535 ± .59	9.23 ± .4
HGB	14.45 ± .65	14.5 ± 1.82
HCT	42.4 ± .94	44.2 ± 1.32
MCV	50.25 ± 1.91	48.75 ± 1.6
MCH	16.77 ± 1.11	15.46 ± .9
MCHC	33.51 ± .665	32.44 ± 1
RDW	14.52 ± .63	15.71 ± .83
PLT	916.75 ± 83.44	818.5 ± 85
MPV	5.17 ± .5	6.2 ± .7
PCT	.524 ± .1	.549 ± .12
PDW	5.5 ± .53	4 ± 1.44
SGOT (IU L)	101.86 ± 12.47	112.6 ± 12.76
SGPT (IU L)	42.5 ± 5.83	38.36 ± 2.76
Creatinine (mg dL)	0.34 ± 0. 06	0.4 ± 0. 04
Urea (mg dL)	37.22 ± 3	42.9 ± 3.8

Mean ± SD, n = 4

Table. S.2 Serum biochemistry analysis of EAT tumor bearing mice treated with various formulations

Serum biochemistry parameters with units	**Group i**	**Group ii**	**Group iii**	**Group iv**	**Group v**
SGPT (IU L)	47.41 ± 6.29	45.3 ± 1.41	49.47 ± 9	46.52 ± 3.24	41.43 ± 6.4
SGOT (IU L)	127 ± 4.1	128.66 ± 12.7	134 ± 8.8	119 ± 3.22	115± 13.9
Creatinine (mg dL)	0.46 ± 0. 06	0.455 ± 0. 05	0.49 ± 0. 09	0.42 ± 0. 04	0.4 ± .01
Urea (mg dL)	43.22 ± 8.1	42.46 ± 7.3	51.67±13.17	38.68 ± 1.37	37.96 ± 2.65
CK - MB (IU L)	2.4 ± 0.6	3.1 ± 0.45	3.8 ± .75	2.6 ± .48	2.2 ± .62

Mean ± SD, n = 4