**Supporting Information**

# Polydiacetylene/lipid-coated red-emissive silica nanorods for sustainable release and ameliorated anticancer efficacy of Ru(arene) complex bearing piperlongumine natural product

Wickneswaran Ishaniya,[a] Chezhiyan Sumithaa,[a] Muthuraman Subramani,[b] Aswathy Karanath-Anilkumar,[c,d] Ganesh Munuswamy-Ramanujam,[a,c] Arumugam Madan Kumar,[e] Saravanakumar Rajendran,[b] and Mani Ganeshpandian[a*]

[a]*Department of Chemistry, SRM Institute of Science and Technology, Kattankulathur 603 203, Tamil Nadu, India*
[b]*Chemistry division, School of Advanced Sciences, Vellore Institute of Technology, Chennai-600127, Tamilnadu, India*
[c]*Molecular Biology and Immunobiology Division, Interdisciplinary Institute of Indian System of Medicine, SRM IST, Kattankulathur, Tamil Nadu 603203, India*
[d]*Department of Biotechnology, Faculty of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur 603 203, Tamil Nadu, India*
[e]*Cancer Biology Lab, Molecular and Nanomedicine Research unit, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India.*

*To whom correspondence should be addressed, e-mail: ganeshpm@srmist.edu.in

## Instrumentation

Microanalysis was carried out with a Vario EL elemental analyzer. UV-Vis spectroscopy was analysed using Cary-60 UV-Vis spectrophotometer using cuvettes of 1cm path length. NMR spectra were recorded on a Bruker 500 MHz NMR spectrometer. High resolution mass spectrometry was performed on a QTOF – HRMS equipped with an ESI source. Fluorescent microscopy image was taken using Leica DM6 Fluorescent Microscope. Emission intensity measurements were carried out using an FLS-1000 Spectrofluorometer. HR-TEM analysis was carried out using JEM-2100 Plus. TGA analysis was carried out using STA 2500 REGULUS-TGA-DSC Thermal analyser. BET analyses were taken using Quantachrome Instruments, Autosorb IQ series. Particle size distribution and zeta potential measurements were recorded on Horiba Model SZ-100Z V2, Nano Particle Size, and Zeta Potential Analyser, Japan. ICP-MS analysis was taken using inductively coupled plasma mass spectrometry (ICP-MS, NexION 2000C, Perkin Elmer). Powder X-Ray Diffractometer (XRD) images were taken using Bruker USA D8 Advance Davinci. FTIR spectra were analysed using Shimadzu, IRtracer 100.

## MTT assay

Cell viability of Ru(pip) and cis-platin was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. MCF-7 human breast carcinoma cells, THP-1 human leukemia monocytic carcinoma cells, HepG2 liver carcinoma and normal embryonic kidney cells (HEK-239) were procured from National Centre for Cell Science (NCCS), Pune. MCF-7, HEPG2 and HEK-239 cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) culture media supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) (Invitrogen Corporation, CA, USA) whereas THP-1 cells were cultured in RPMI supplemented with 10% inactivated FBS, penicillin (100 IU/ml), streptomycin (100 mg/ml) and amphotericin B (5 mg/ml) in a humidified atmosphere of 5% $CO_2$ at 37˚C until confluent. MCF-7 cells were trypsinized and plated at a density of ~20, 000 cells/well in 96-well plate and incubated at 37 °C in the $CO_2$ incubator. The half inhibitory concentration ($IC_{50}$) values were determined using the MTT assay after 24 h incubation of different concentrations of complex in both the cell lines. The stock solutions of the compounds were prepared in 1% DMSO/10 mM PBS mixture immediately prior to dilution. Different

concentrations of complex solution in μM and nanoformulation in μg/ml were prepared by the dilution of the stock solution using culture media in triplicate. The MTT insoluble formazan was dissolved in DMSO and the MTT reduction was quantified by measuring the absorbance at 570 nm (Multiskan Spectrophotometer, USA). The obtained data were plotted and fitted using GraphPad Prism software to calculate the 50% viability ($IC_{50}$) value. The data were obtained for three biological replicates each and used to calculate the mean. The $IC_{50}$ values provided are mean ± standard deviation. The same procedure was carried out in THP-1 cells without trypsinization.

**Acridine Orange (AO)/ Ethidium Bromide (EB) staining assay**

MCF-7 and THP-1 cells were seeded on 24 well plates with 10% of FBS medium, around 50,000 cells in each well. Using ICP-MS data, the stock solution of complex-loaded NPs was prepared and then the nanoformulation was diluted to reach the $IC_{50}$ concentration of the complex. The apoptotic assay was performed after treatment with $IC_{50}$ concentrations of complex and nanoformulation for 24 h. After that, the cells were pelleted and the supernatant was discarded. These cells were then treated with PBS containing 5 μL of both acridine orange (AO) and ethidium bromide (EB). MCF-7 cells were viewed under an inverted fluorescence microscope (EVOS FL digital inverted fluorescence microscope (AMG)) and THP-1 cells were visualized under (Confocal Laser Scanning Microscopy (CLSM)). The result was recorded with the green, orange, and red fluorescent emitting cells based on the live, apoptosis and necrosis cells respectively. The experiment was performed in triplicates and each experiment was repeated three times to obtain mean values and the standard error of the mean.

**ROS scavenging assay**

Intracellular Reactive Oxygen Species (ROS) was measured using $H_2$DCF-DA fluorescent dye in THP-1 cells using a standardized method with minor modifications. The cells were cultured in a 6-well plate at a density of $7x10^4$cells/well. Cells were treated with peroxide followed by the treatment of Ru(pip) and PL-Ru(pip)@MSNRs at their $IC_{50}$ concentrations. $H_2O_2$ alone treated cells were used as positive control, and all the groups were incubated for 24 h. At the end of incubation, the cells were exposed to 10 μM of $H_2$DCF-DA for 30 min. Cells were

then harvested and fluorescence intensity was measured using BD FACS calibur (Ex/Em – 488 nm/530 nm). The analysis was done using BD Cell Quest Pro software.

**Cell cycle analysis**

With the aid of FACS, PI-based cycle analysis was carried out to ascertain this. 1 ml of THP-1 cells were treated with 100 µl of $IC_{50}$ concentration of the samples and were incubated for 24 hours. It was then centrifuged at 1,500 rpm for 5 min and the supernatant was discarded. Then the cells were fixed with 70 % ice-cold ethanol (500 µl) and incubated overnight. PBS was used to rinse ethanol-fixed cells before treating it with 100 µl of RNase for 30 min followed by staining with PI for 15 minutes in the dark. PI signals were collected using 585/42 band pass filter and analysed with a BD FACS Flow Cytometer. The same procedure was carried out for MCF-7 cells after trypsinization. The experiment was performed in triplicates and each experiment was repeated three times to obtain mean values and the standard error of the mean.

**Determination of the Ruthenium Content in Ru(pip)@MSNRs**

The quantification of ruthenium content was done by the digestion of loaded mesoporous silica nanorods. 1 mg of Ru(pip)@MSNRs were suspended in 0.8 mL of concentrated nitric acid and the solution was vigorously stirred for 48h. The suspension was then given 0.2 mL of concentrated hydrofluoric acid, and it was stirred for a further 24 h. The resultant solution was centrifuged, filtered, and examined in triplicate by ICP-MS. The standard ruthenium solution was used for comparison to determine the ruthenium content present in Ru(pip)@MSNRs.

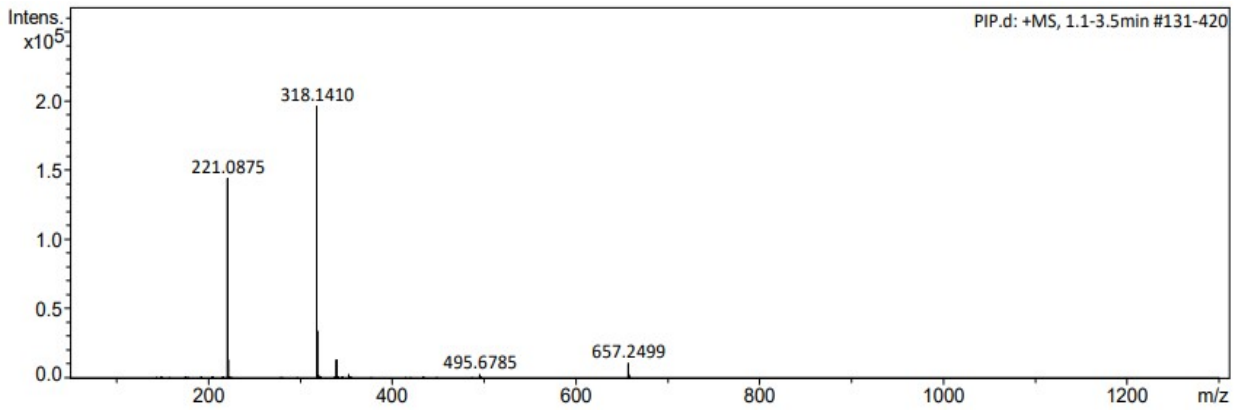


**Figure S1**: ESI-HRMS spectrum of piperlongumine in methanol.

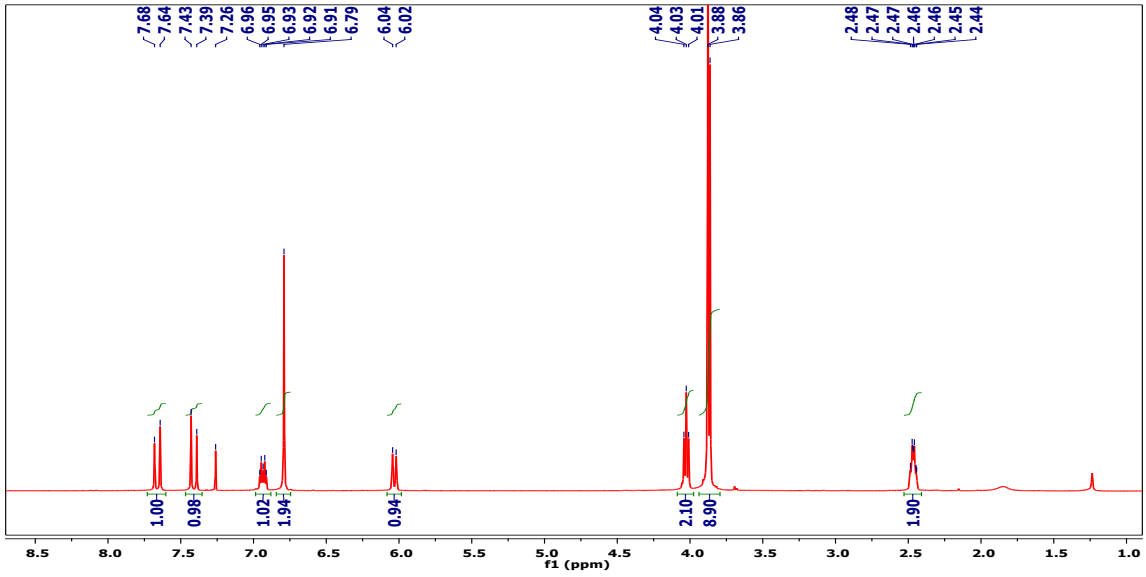


**Figure S2**: $^{1}$H-NMR spectrum of piperlongumine in $CDCl_3$

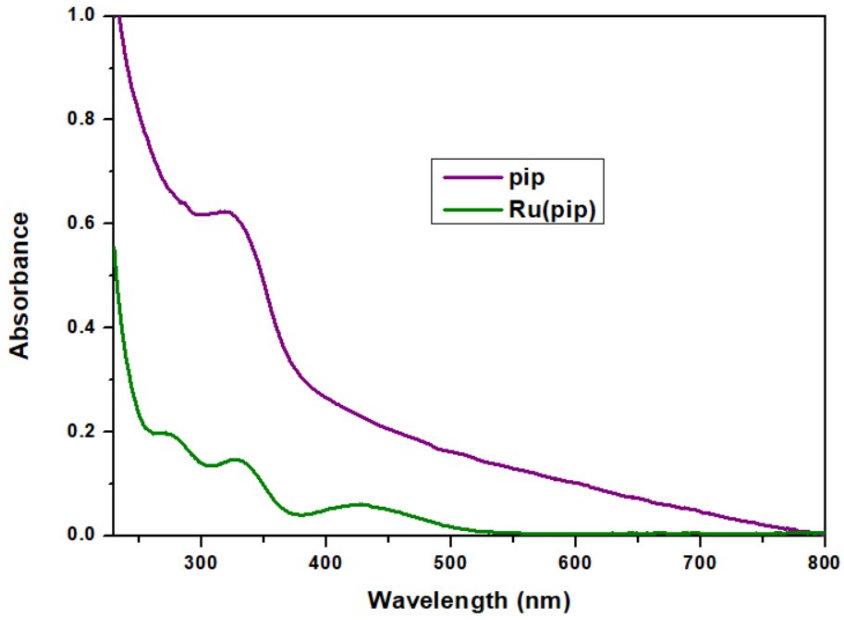


**Figure S3**: UV-Vis absorption spectra of piperlongumine and Ru(pip) in methanol.

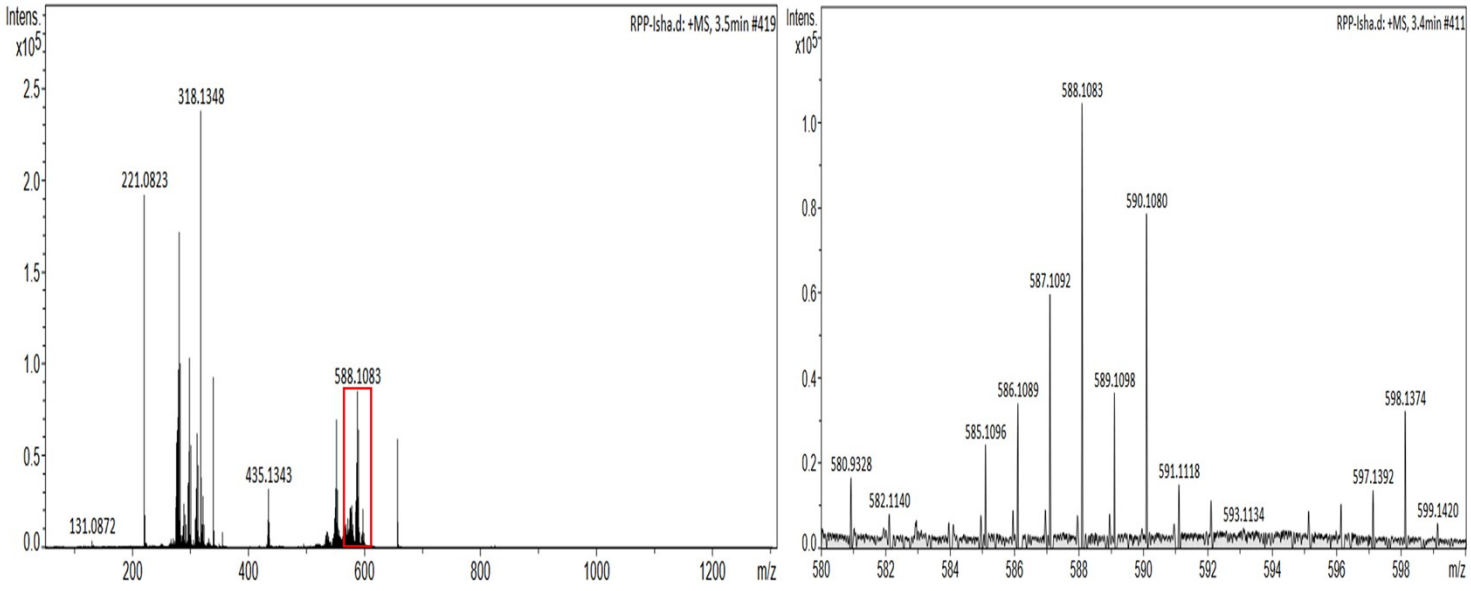


**Figure S4**: ESI-HRMS spectra of Ru(pip) in methanol

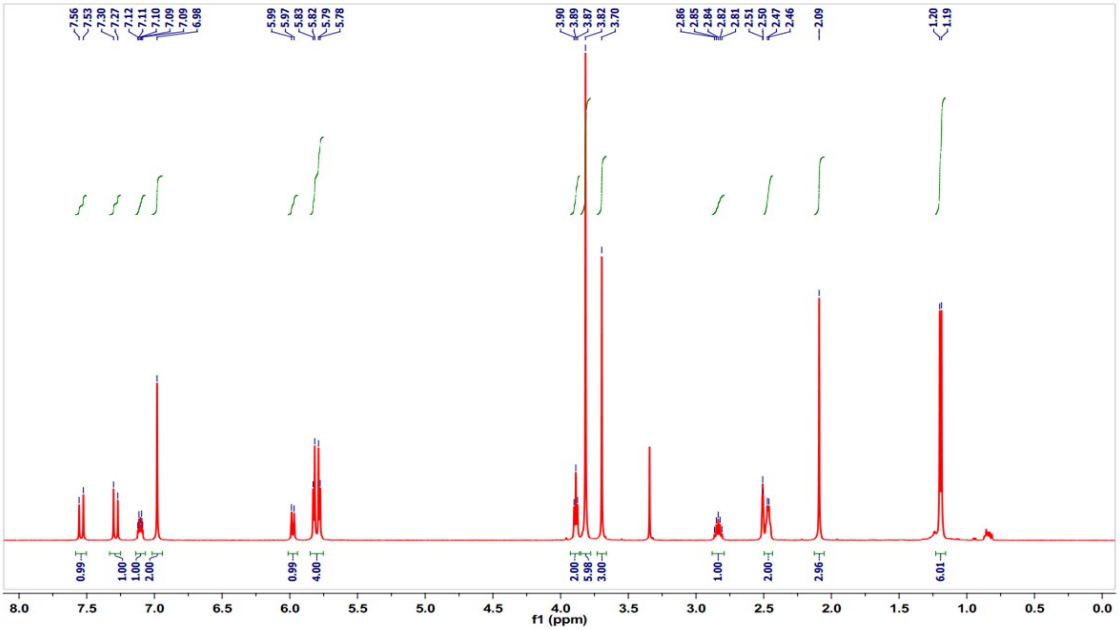


**Figure S5**: $^{1}$H-NMR spectrum of Ru(pip) in DMSO-$d_6$

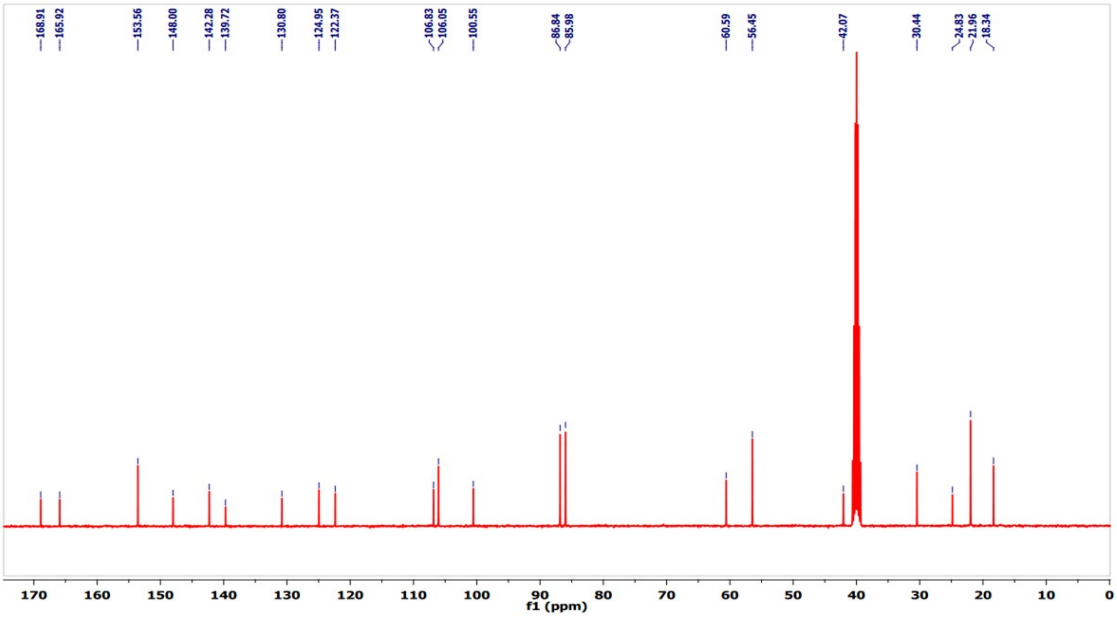


**Figure S6**: $^{13}$C-NMR spectrum of Ru(pip) in DMSO-$d_6$

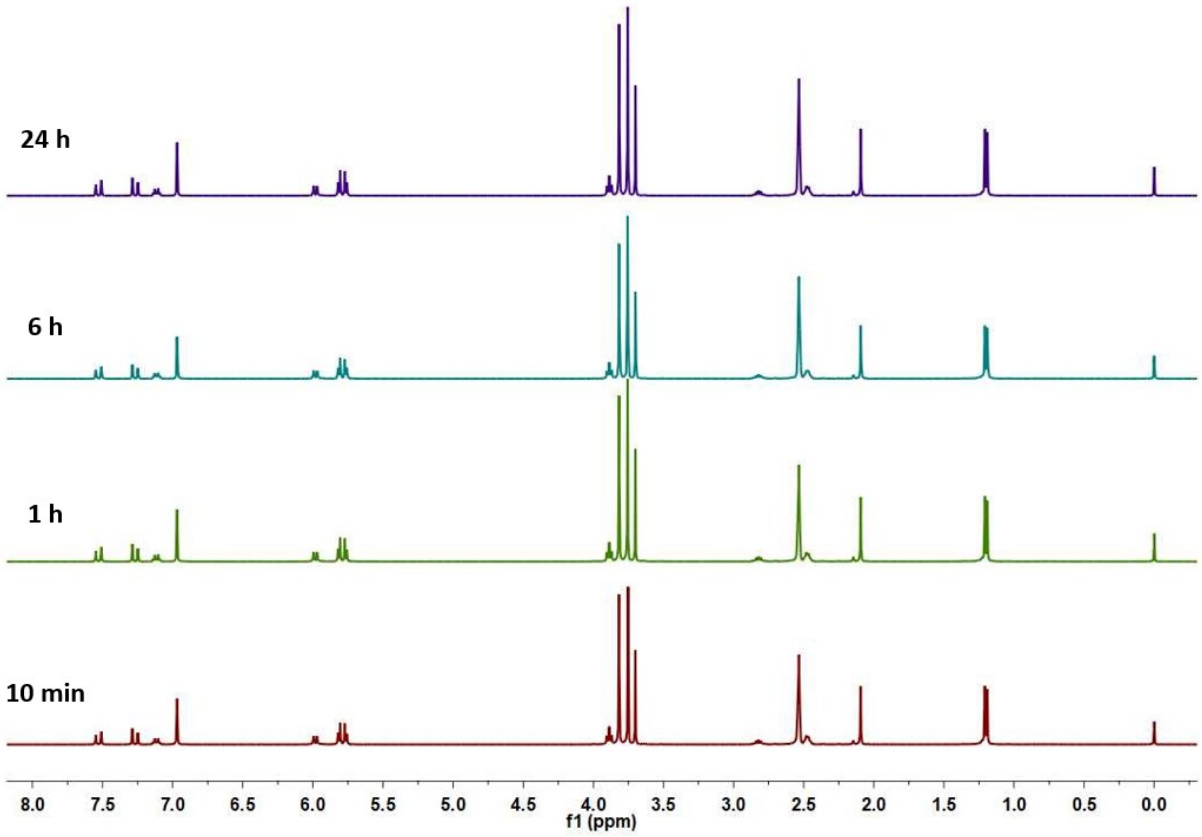


**Figure S7**: $^{1}$H NMR spectra of the stability of Ru(pip) in DMSO-$d_6$ up to 24 h.

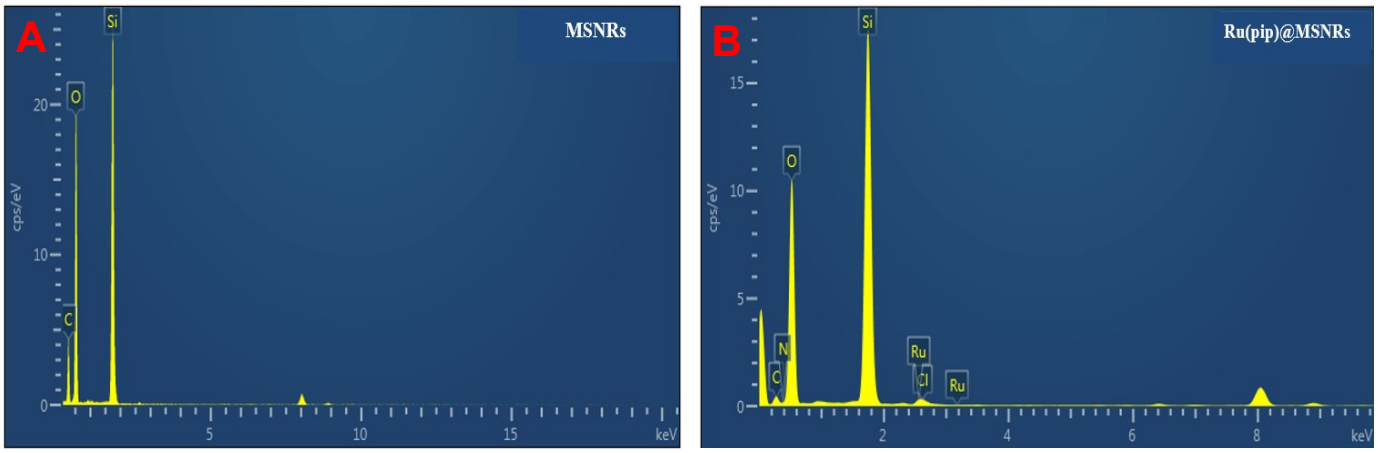


**Figure S8**: EDX- analyses of (A) MSNRs and (B) Ru(pip)@MSNRs

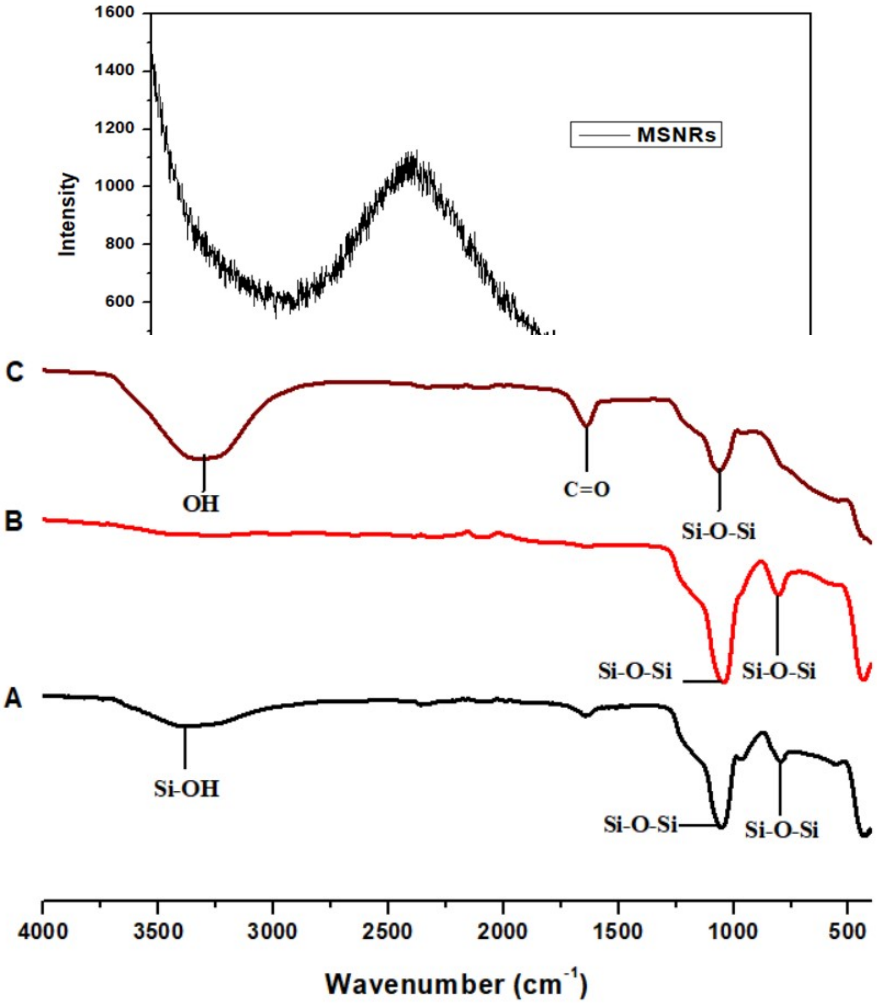


**Figure S9**: Wide-angle XRD pattern of MSNRs

**Figure S10**: FTIR spectra of MSNRs (A), Ru(pip)@MSNRs (B) and PL-Ru(pip)@MSNRs (C)

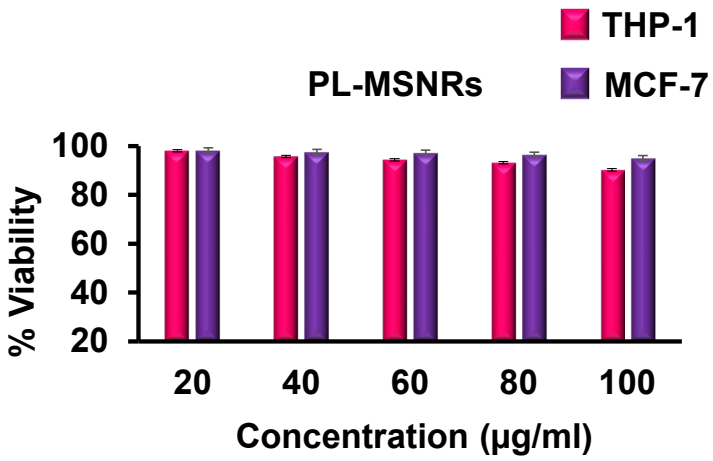


**Figure S11:** Viability of THP-1 and MCF-7 after treatment for 24 h with different concentrations of PL-MSNRs