Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

PEG-PEI modified gated N-doped mesoporous carbon nanospheres for pH/NIR light-triggered drug release and cancer phototherapy

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Supporting Information

Characterization

Hydrodynamic size, surface charge of the material was recorded by Nano ZS-90 Malvern zeta size analyzer. Topographic details, microstructural behavior of the material were surveyed by FEI Novanano SEM 450 and Transmission electron microscopy, Tecnai G2 TF30-ST at an accelerating voltage of 300 kV respectively. Crystallographic orientation of material was verified through WITec XMB3000-3000 PL micro RAMAN spectrometer. Specific surface area and pore size distribution of the material was collected using Quantachrome BET surface area analyzer. Photophysical measurements were done using Shimadzu UV2650 spectrophotometer and Horiba Quantamaster fluorimeter. The structural confirmation of linker molecules recorded using Bruker, Avance III Nuclear Magnetic Resonance Spectrometer. The presence of surface functionality was investigated through IRAffinity-1S, Shimadzu spectrophotometer with the KBr pellet technique ranging from 400 to 4000 cm^{-1}. Apoptosis of cell was analysed using BD ACCURI C6 Flow Cytometer and Live-cell images were captured under Epifluorescent Microscope (Olympus IX71, Olympus, Tokyo, Japan).

Stability of material with time

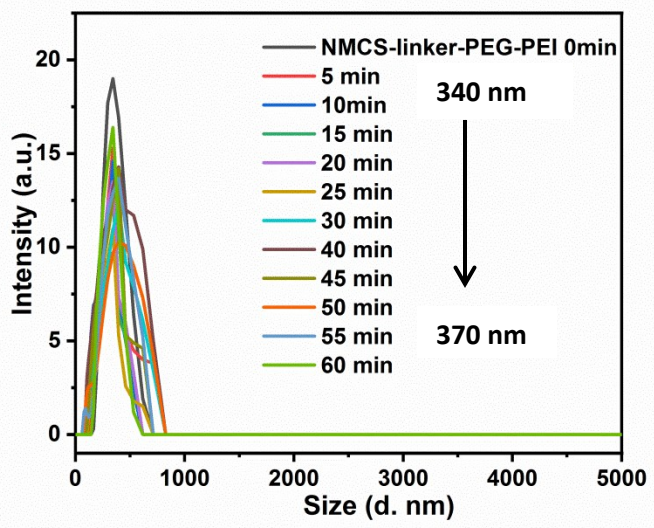


Figure S1 Hydrodynamic stability of NMCS on PEG-PEI polymer coatingwith respect to time

Synthesis of photocleavable linker

Synthesis of compound A-C

Compound A-C is synthesized following the previously reported protocol.[1]The supportive NMR datafor each compound are given below. Compound A: ^{1}H NMR (400 MHz, CDCl3) δ = 1.23 (t, 3H), 2.16 (m, 2H), 2.52 (t, 2H), 2.53 (s, 3H), 3.88 (s, 3H), 4.07-4.16 (m, 4H), 6.87 (d, *J*= 8 Hz, 1H), 7.47-7.55 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.78, 172.99, 152.61, 149.23, 130.45, 123.20, 111.23, 110.40, 67.76, 60.46, 55.95, 30.55, 26.19, 24.27, 14.19. Compound B: ^{1}H NMR (400 MHz, $CDCl_3$) δ= 1.27 (t, *J*= 8 Hz, 3H), 2.20 (m, 2H), 2.50 (t, 2H), 2.53 (s, 3H), 3.96 (s, 3H), 4.14-4.19 (m, 4H), 6.74 (s, 1H), 7.61 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.15, 172.83, 154.31, 148.87, 132.88, 108.75, 107.99, 68.48, 60.64, 56.61, 30.47, 24.18, 14.24. Compound C: ^{1}H NMR (400 MHz, $CDCl_3$ plus several drops of DMSO-d6) δ 1.38 (d, 3H), 2.09- 1.98 (m, 2H), 2.40 (t, *J*= 8 Hz, 2H), 3.86 (s, 3H), 4.00 (t, *J*= 8 Hz, 2H), 4.40 (bs, 1H), 5.41 (q, 1H), 7.30 (s, 1H), 7.45 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 200.25, 172.92, 154.30, 148.85, 138.31, 132.80, 108.74, 107.99, 68.47, 60.63, 56.60, 30.52, 30.38, 24.17, 14.20.

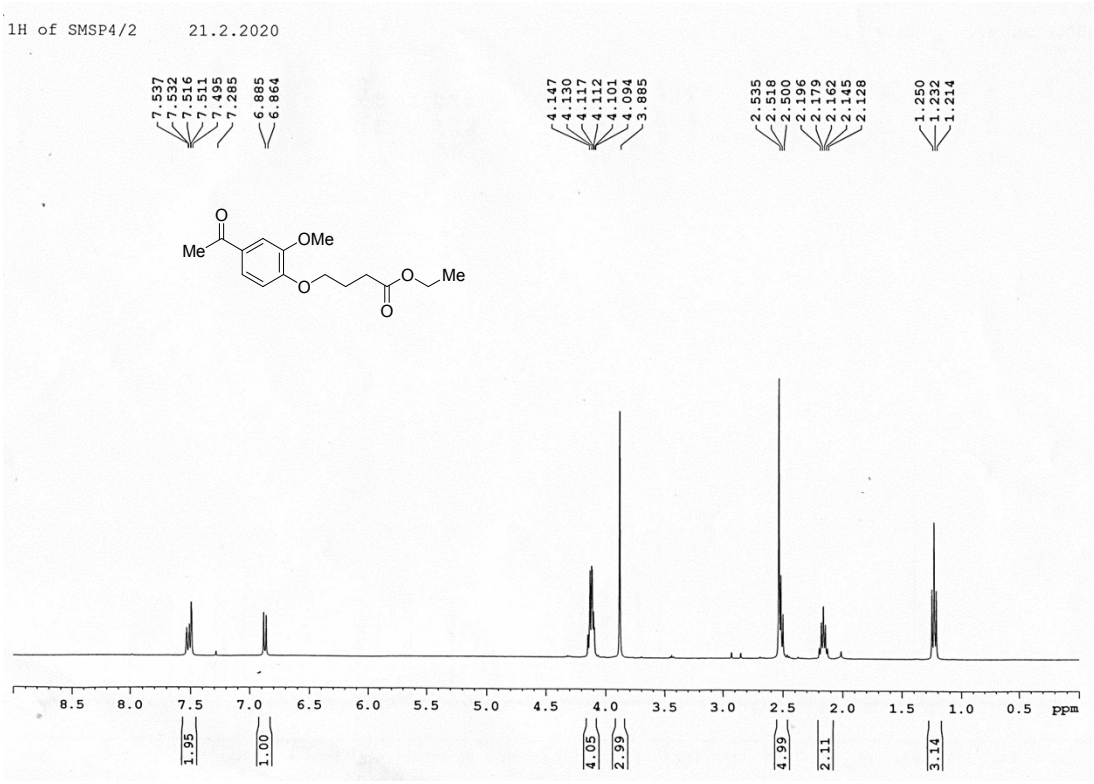
Synthesis of compound D

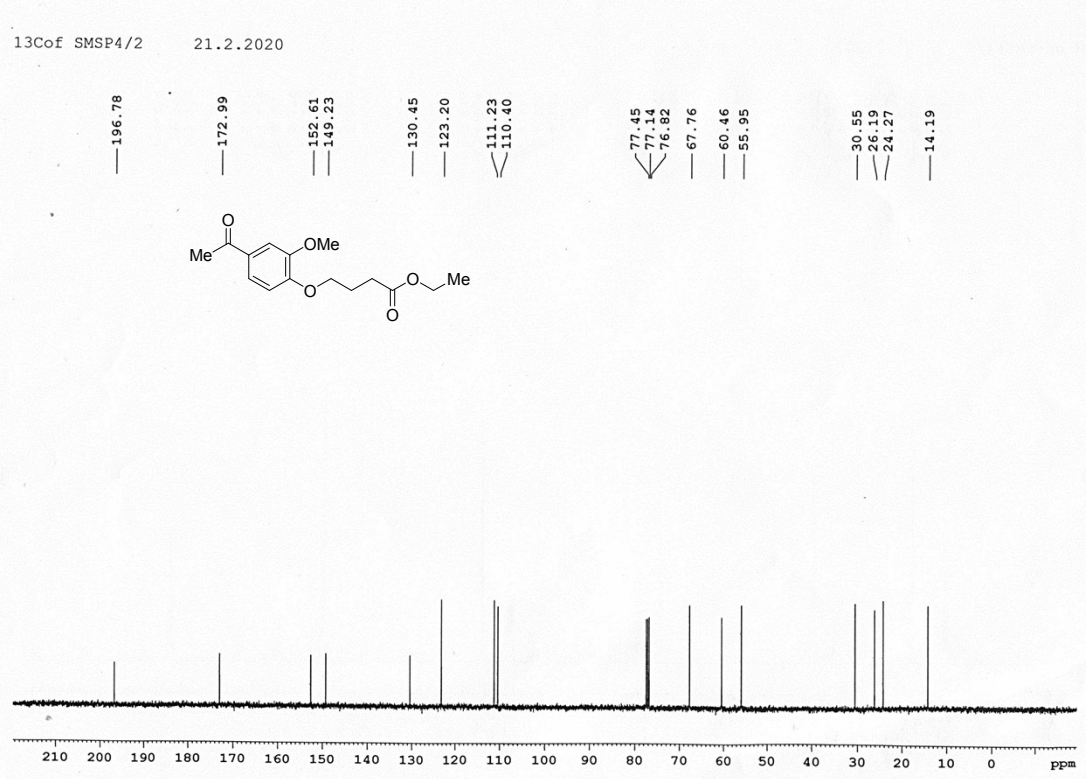
1 gm of compound C was mixed in 10 ml of DMF and solution temperature maintained at 0^{o}C. EDC.HCl followed by NHS (1.5 eq each) was added an continued stirring in chilled condition for 1h under inert and dark atmosphere, the reaction extended for next 12h at RT. After completion of reaction, the excess DMF was washed out through ethylacetate-water phase separation, dried over $MgSO_4$ followed by reduced pressure drying. To the above resulted –NHS linked compound (500 mg) in 5 mL dimethyl formamide, Et_3N (2 eq., 70mg, 1.26 mmol) was steadily added during stirring, consecutively propargylamine (2 eq., 70 mg, 1.26 mmol) was added. The reaction mixture was magnetically stirred overnight at RT under inert/ dark condition. On completion of reaction duration, DMF was removed with excess water washing from ethyacetate phase and dried using rotary evaporator. The solvent was concentrated to yield pale white solid.[21]H NMR (400 MHz, DMSO-d6) δ 1.36 (d, 3H), 1.91-1.98 (m, 2H), 2.27 (t, *J*= 8 Hz, 2H), 2.5 (s, 1H), 3.845-3.86 (m, 2H), 3.90 (s, 3H), 4.00 (t, *J*= 8 Hz, 2H), 5.23-5.28 (m, 1H), 5.52 (br, S, 1H), 7.35 (s, 1H), 7.52 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d6): δ 171.77, 153.86, 146.67, 139.32, 138.49, 81.73, 73.33, 68.60, 64.38, 56.51, 31.72, 30.42, 28.26, 25.62, 24.94, 24.48.

Synthesis of compound E

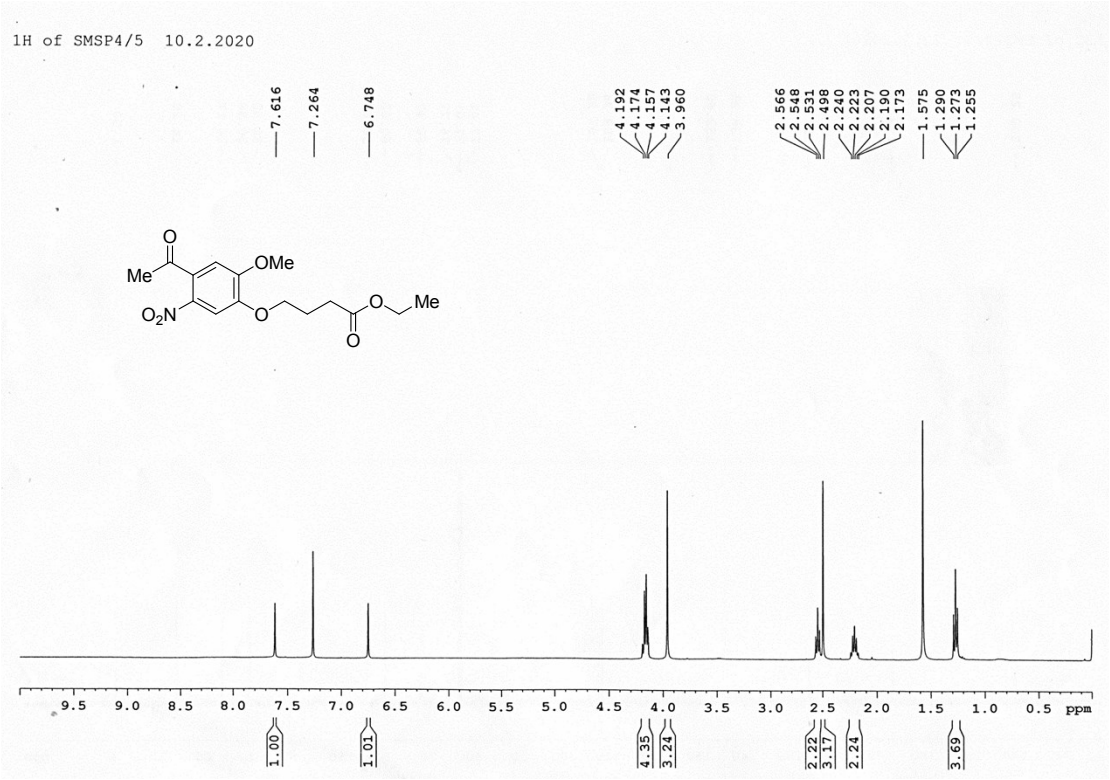
Propargylamine (198 mg, 3.6 mmol) was slowly added over a solution of Succinic anhydride (300 mg, 3 mmol) in 3 mL of THF. The mixture was stirred at RT for 24h and then was added CH_2Cl_2 (20 mL). The resulting precipitate was filtered to give the desired product (4-oxo-4-(prop-2-yn-1-ylamino)butanoic acid). The above product undergoes esterification with compound D (1:1) in dry CH_2Cl_2 in presence of 1.2 eq. of DCC and 4 mol% of DMAP for 6 h.[3] The crude product was filtered over celite and the solvent was evaporated to obtain our final photocleavable linker compound E. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (d, 3H), 1.99(s, 2H), 2.08-2.15 (m, 2H), 2.19 (t, *J*= 2.4Hz, 2H), 2.4 (t, *J*= 7.2 Hz, 2H), 3.90 (s, 3H), 3.95- 3.97 (m, 2H), 4.03 (t, *J*= 3.2, 2H), 4.05-4.08 (m, 2H), 5.44-5.49 (m, 2H), 6.70 (br, S, 1H), 7.28 (s, 1H), 7.45 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.32, 171.36, 162.71, 153.91, 146.53, 139.17, 137.88, 108.89, 79.66, 71.40, 68.37, 65.39, 56.28, 36.59, 32.48, 31.47, 29.63, 29.30, 29.04, 24.73, 24.5.

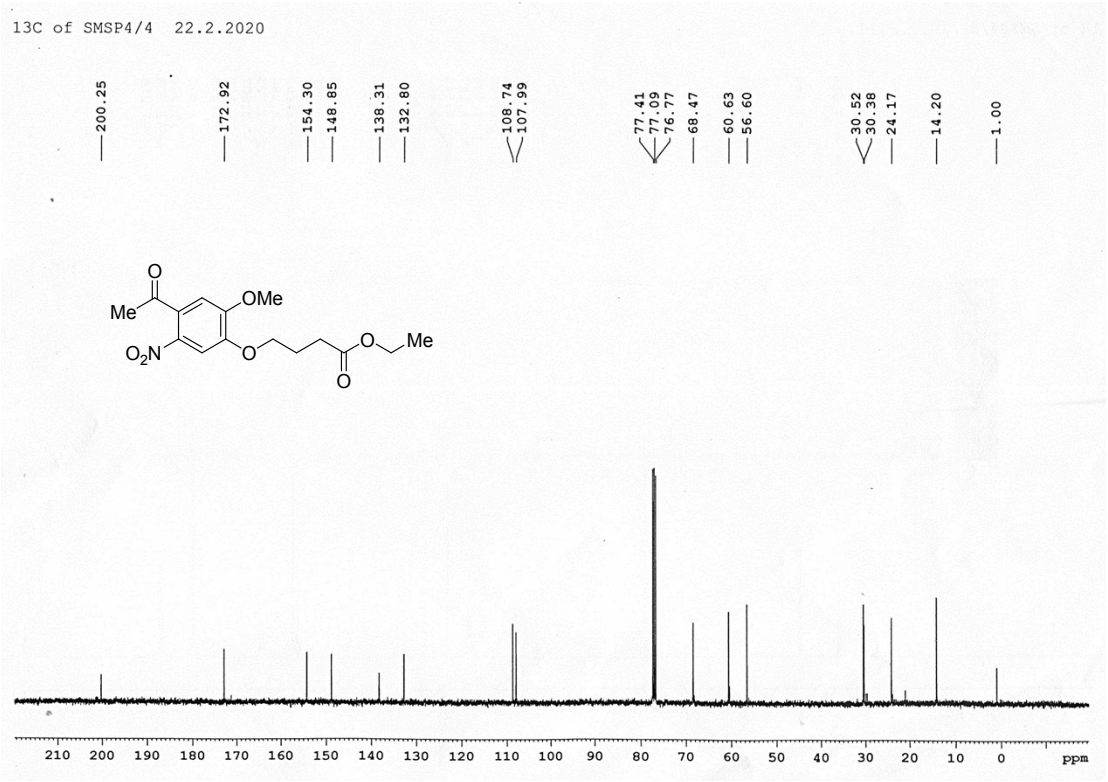
Compound A: (S2)



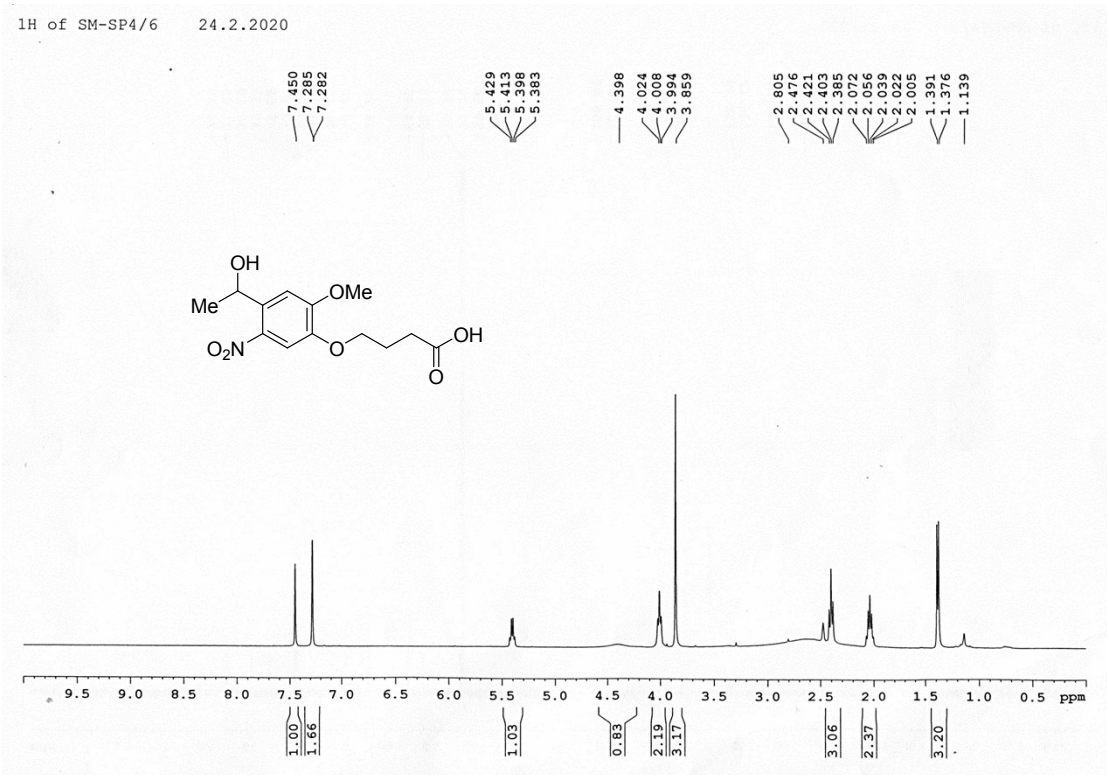


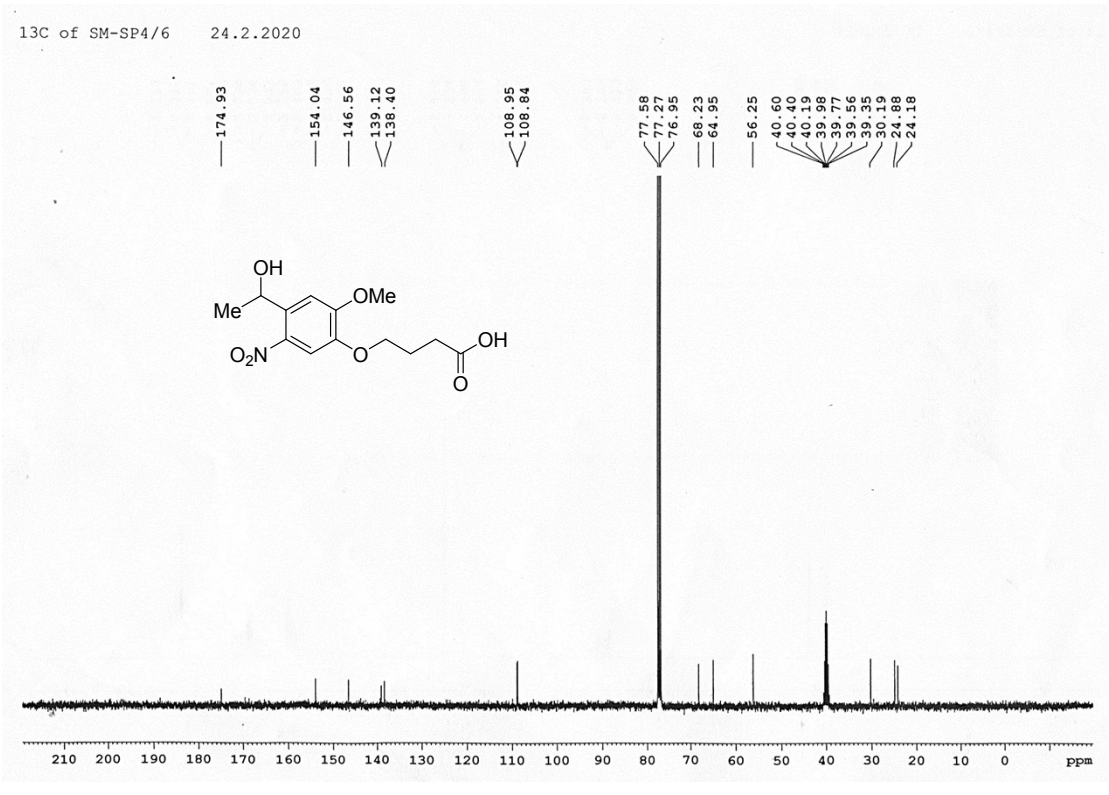
Compound B: (S3)



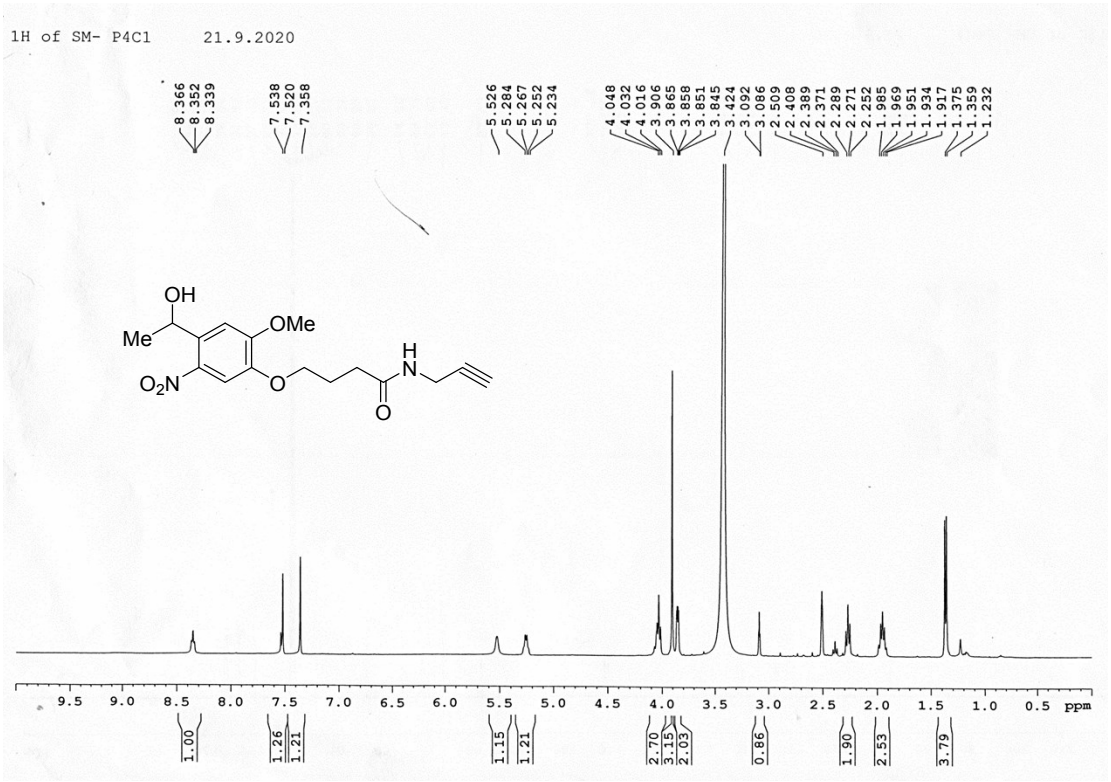


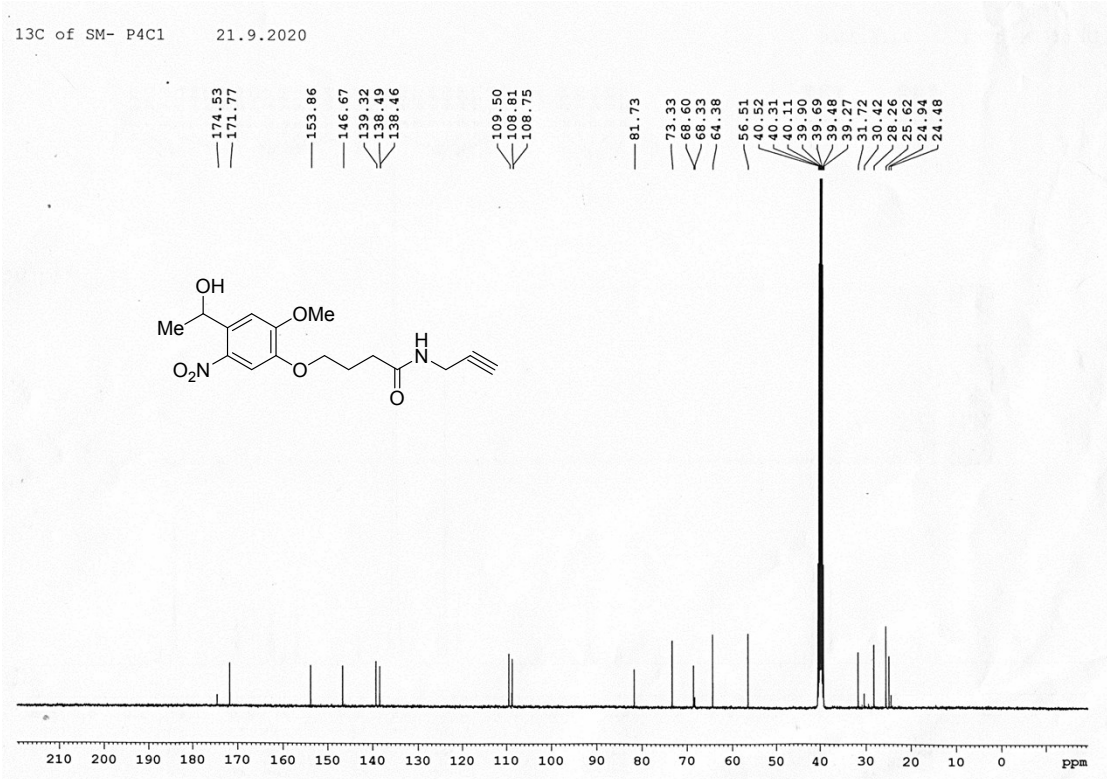
Compound C: (S3)



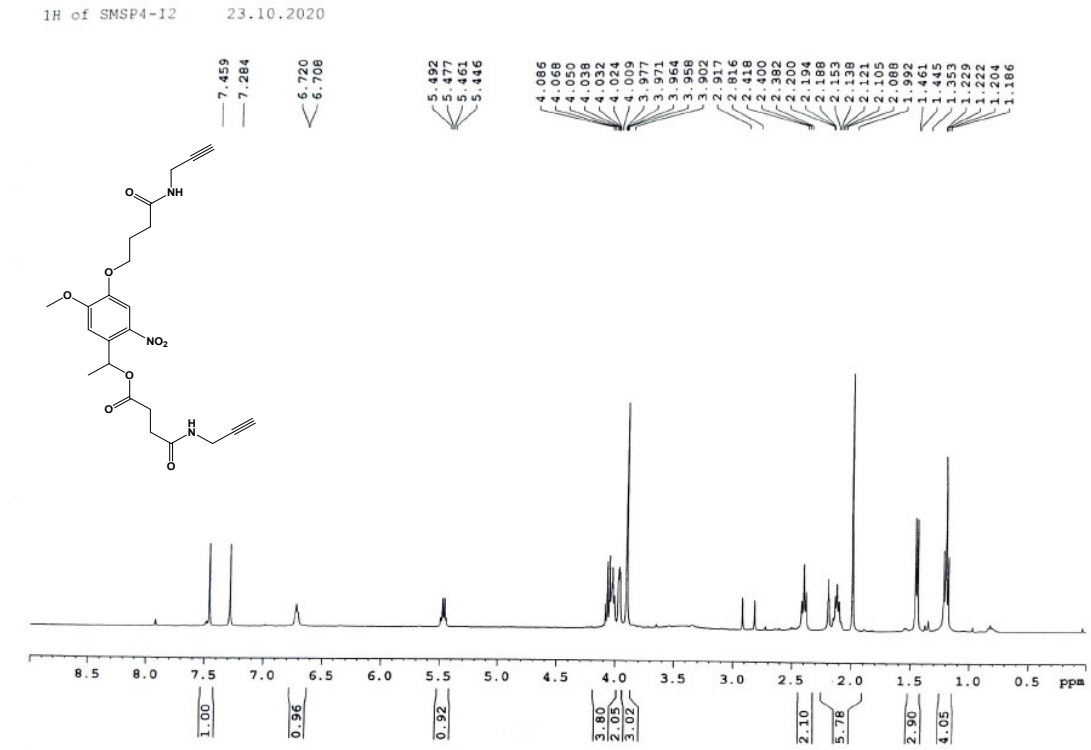


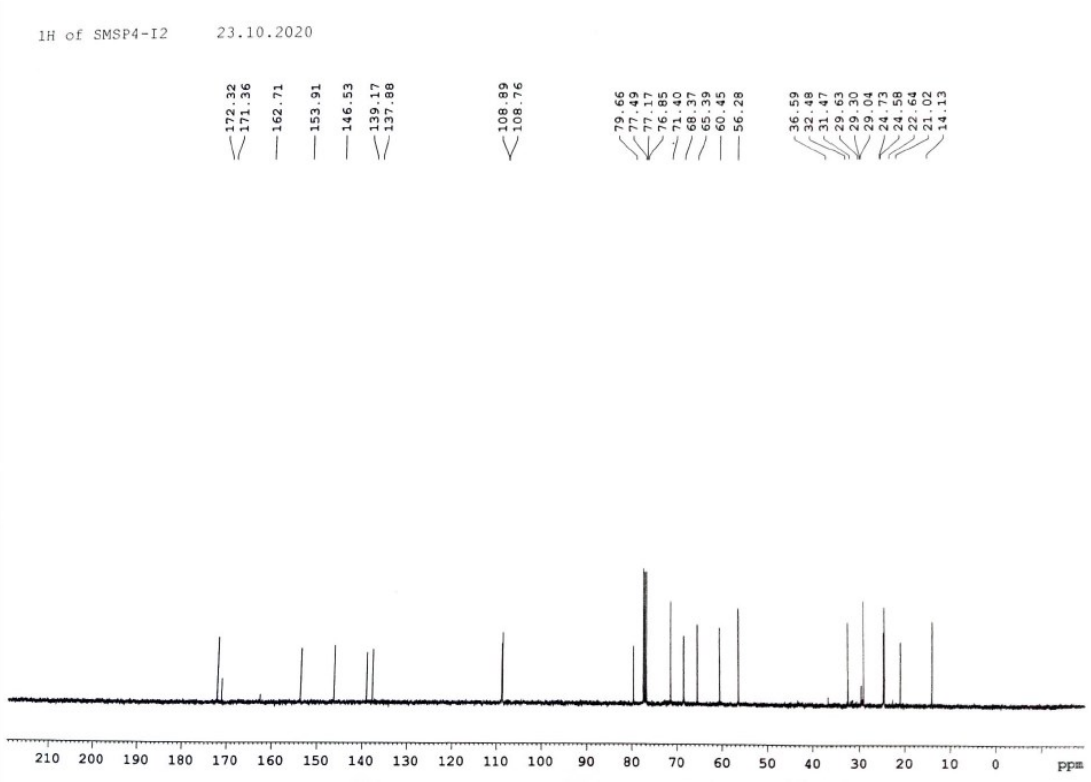
Compound D: (S4)



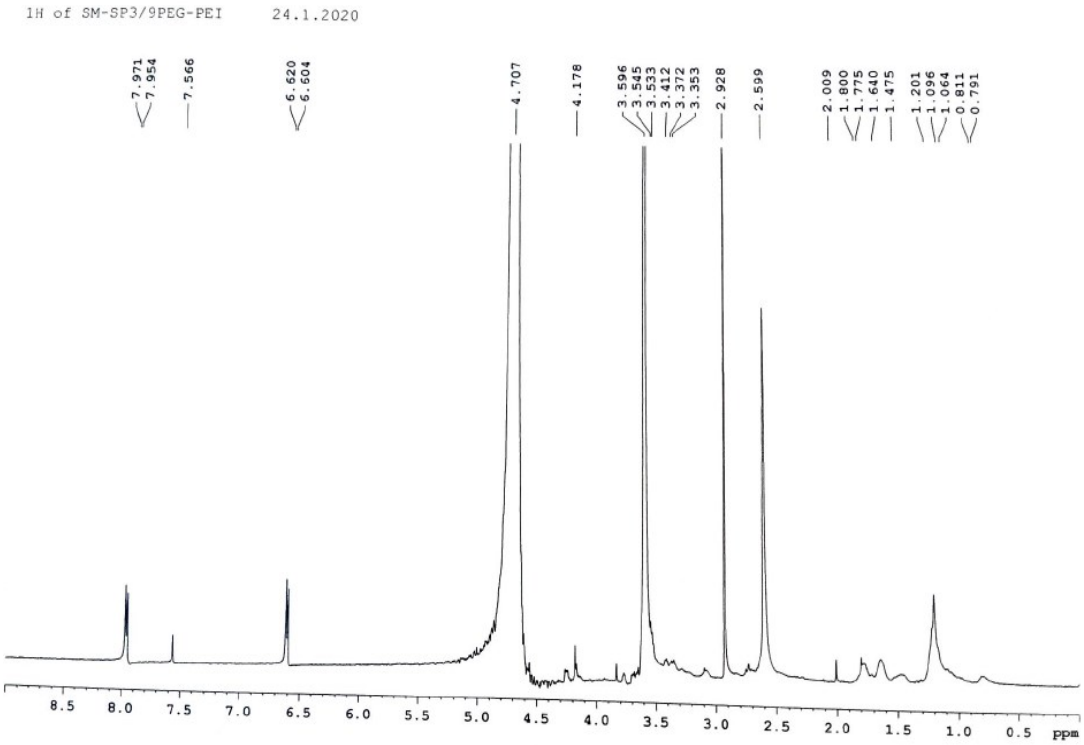


Compound E: (S5)





PEG-PEI polymer: (S6)



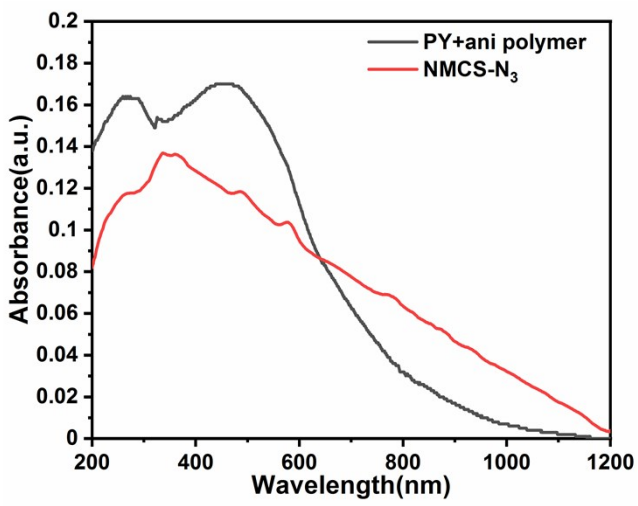


Figure S7 UV-VIS-DRS spectra of PYANI polymer before and after carbonization.

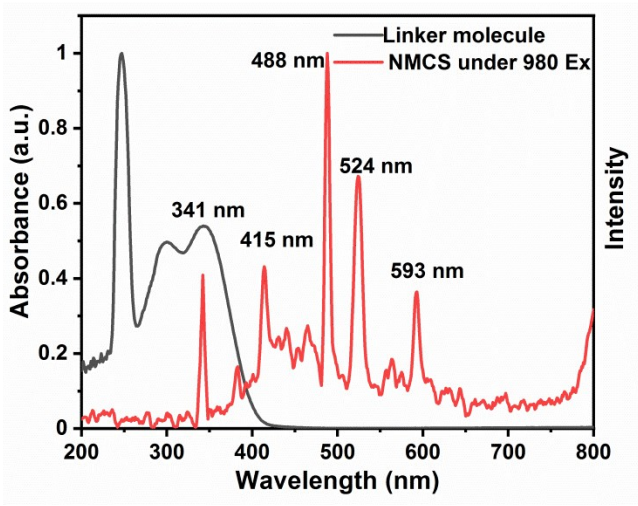


Figure S8 Spectral overlap between UV absorption of linker molecule and PL emission spectra of NMCS under 980 nm laser.

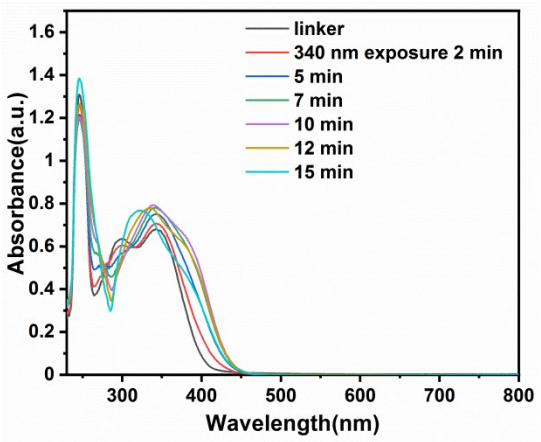


Figure S9 Degradation of linker molecule under 340 nm UV light

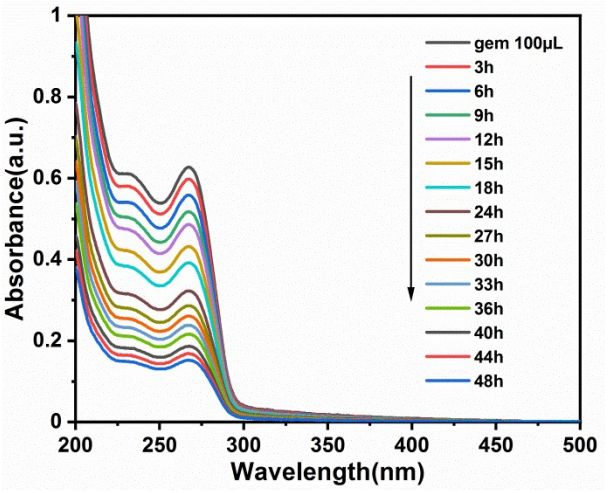


Figure S10 Time dependent UV-VIS spectra of supernatant during gemcitabine loading on NMCS

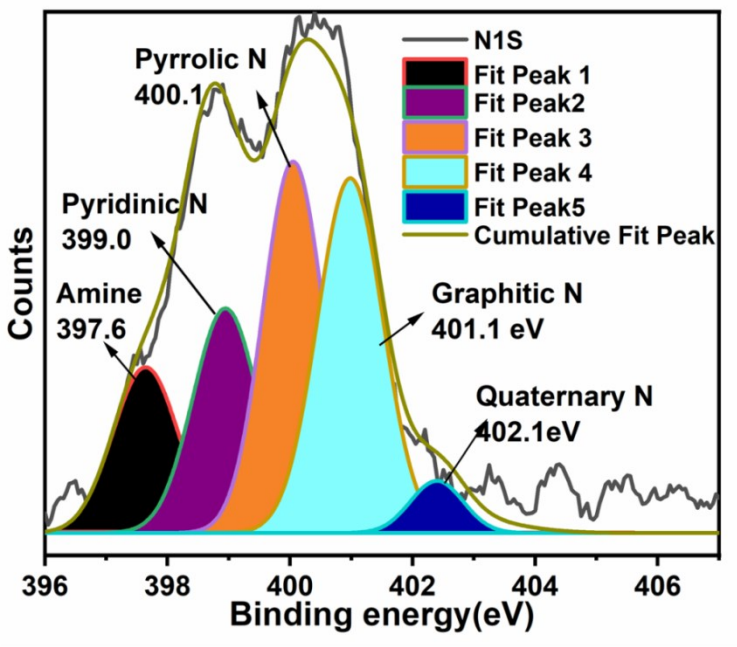


Figure S11 Deconvoluted high resolution N1s spectrum

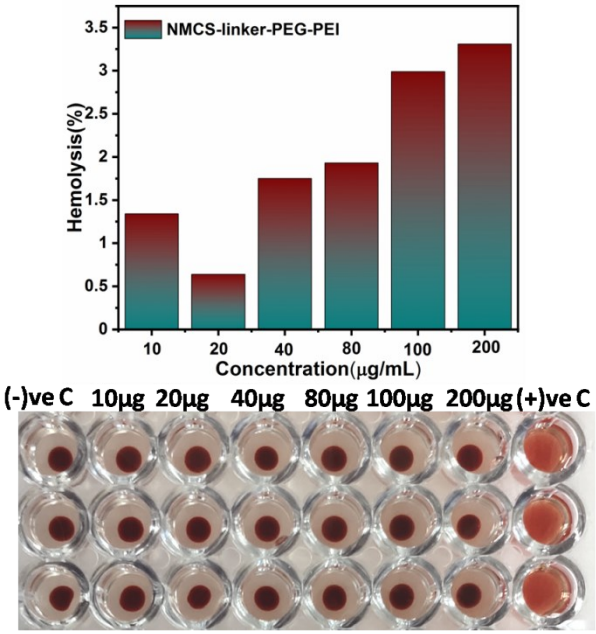


Figure S12 Hemolytic assay of synthesized NMCS-linker-PEG-PEI

Comparative IC50 value of free gemcitabine, and gemcitabine loaded on therapeutic material

Sample	Incubation Time(h)	IC50
Free Gemcitabine	0.5	7.12 mg
NMCS-PEG-PEI	24	200 µg
NMCS@linker-PEG-PEI-gem	24	60 µg (20 µg loaded)

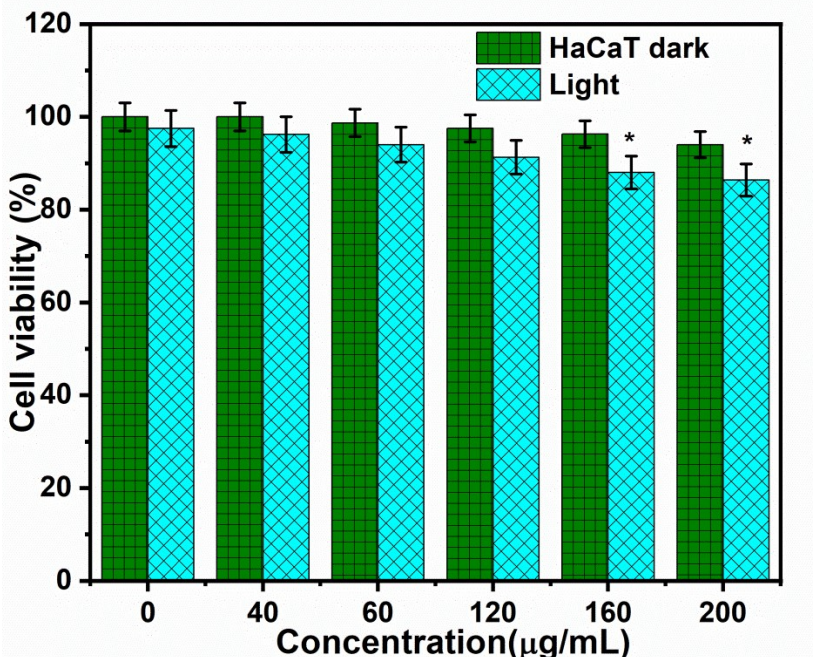


Figure S13 Cytotoxicity assay of PEG-PEI-MCS in HaCaT cell line in absence and presence of 980 nm laser

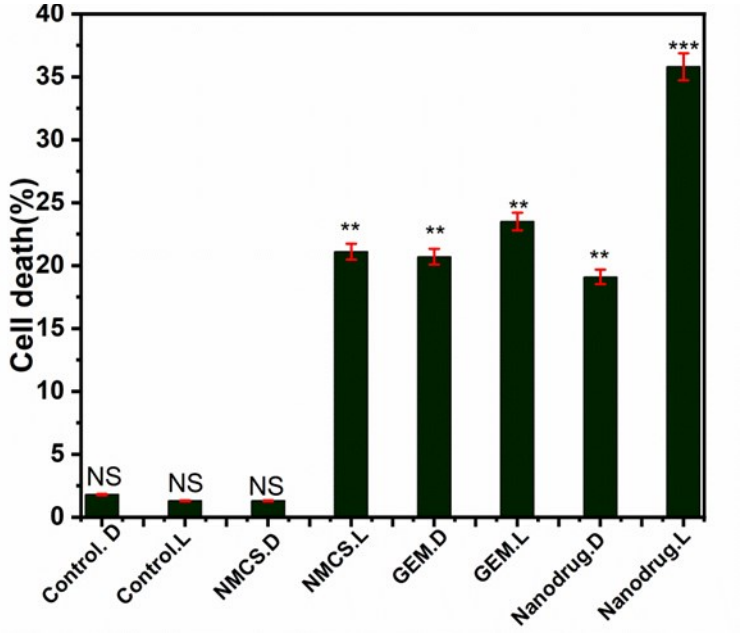


Figure S14 Statistical presentation of cell death percentage induced by NMCS-linker-PEG-PEI-GEM nanodrug under dark (D) and light (L) as investigated by flow cytometry

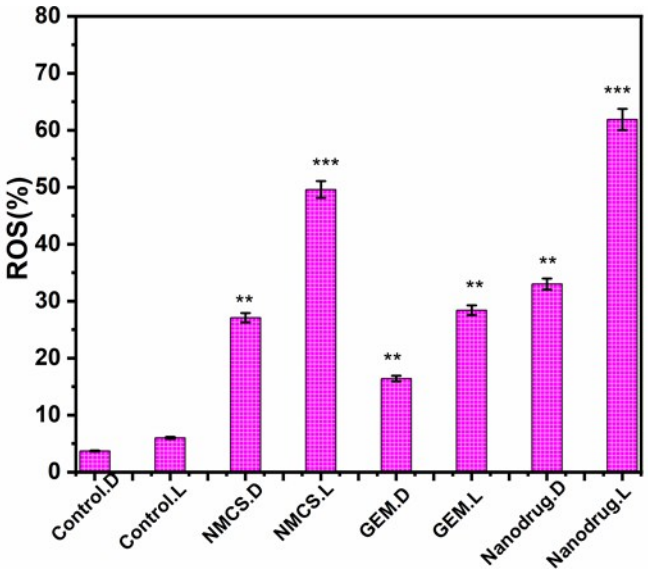


Figure S15 percentage of ROS as detected by flow cytometry

Statistical analysis

Data were obtained from at least three independent xperiments;and the results were quantified as the mean ± SD. Experimental data were analyzed by Student's t-test. The IC50 values were calculated by using the program GraphPad Prism 4 (GraphPad Software, San Diego, California, USA). The level of significance was regarded as P <.05 and for obtained for treatment comparedto control.