Supporting Information

Synthesis and *In Vitro* evaluation of Charge Reversal Photoresponsive Quinoline Tethered Mesoporous Silica for Targeted Drug Delivery

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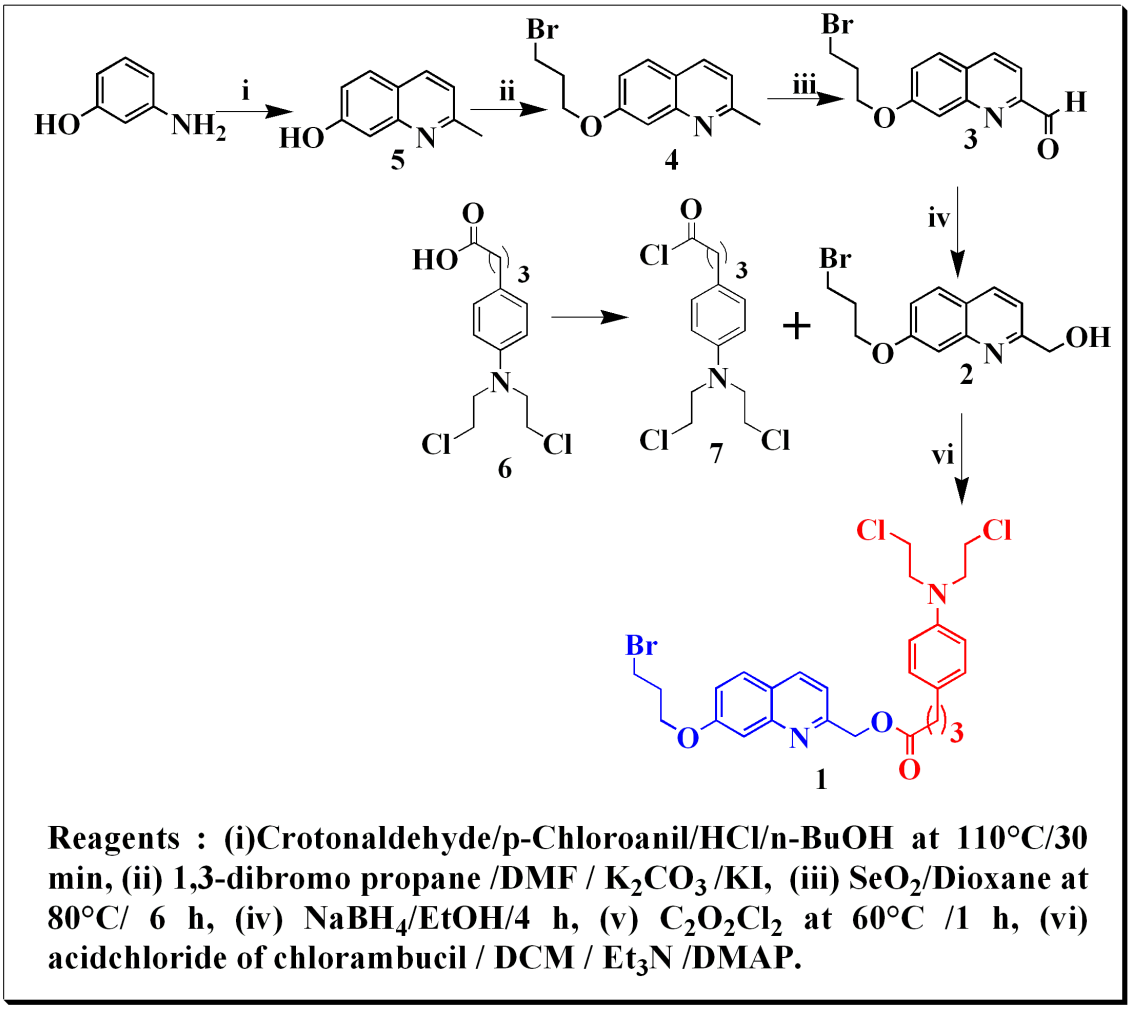
1) Synthesis of Photocage Quinoline–chlorambucil (Qucbl)

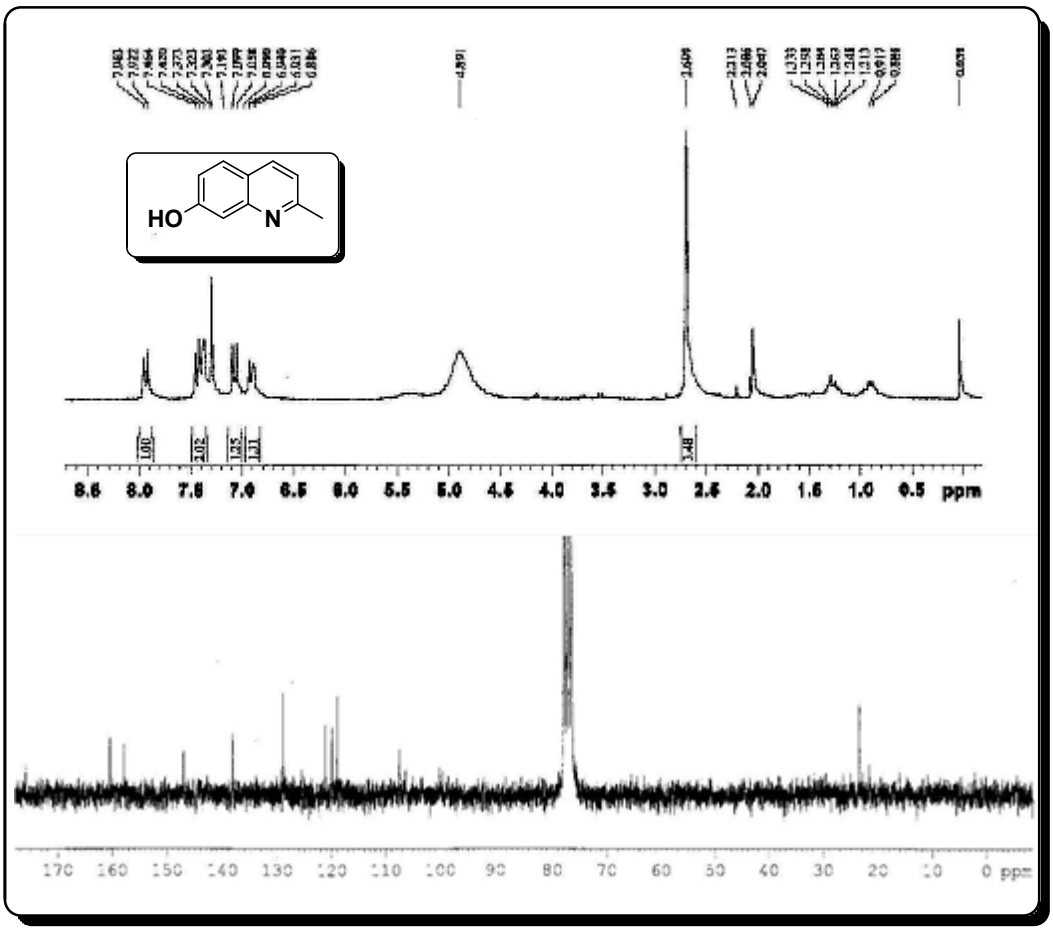


Reagents : (i)Crotonaldehyde/p-Chloroanil/HCl/n-BuOH at 110°C/30 min, (ii) 1,3-dibromo propane /DMF / K_2CO_3 /KI, (iii) SeO_2/Dioxane at 80°C/ 6 h, (iv) $NaBH_4$/EtOH/4 h, (v) $C_2O_2Cl_2$ at 60°C /1 h, (vi) acidchloride of chlorambucil / DCM / Et_3N /DMAP.

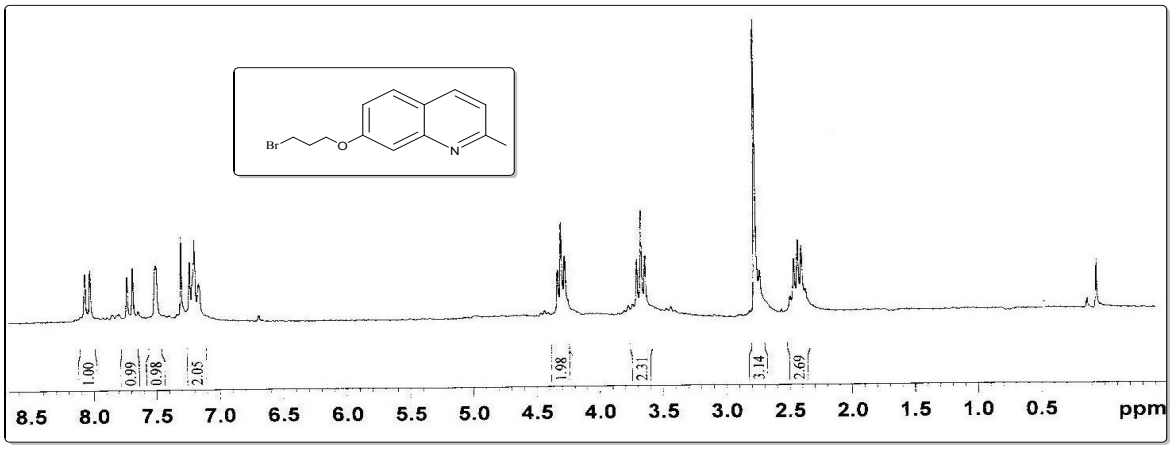
Scheme S1: Synthesis of Quinoline–Chlorambucil (QuCbl)

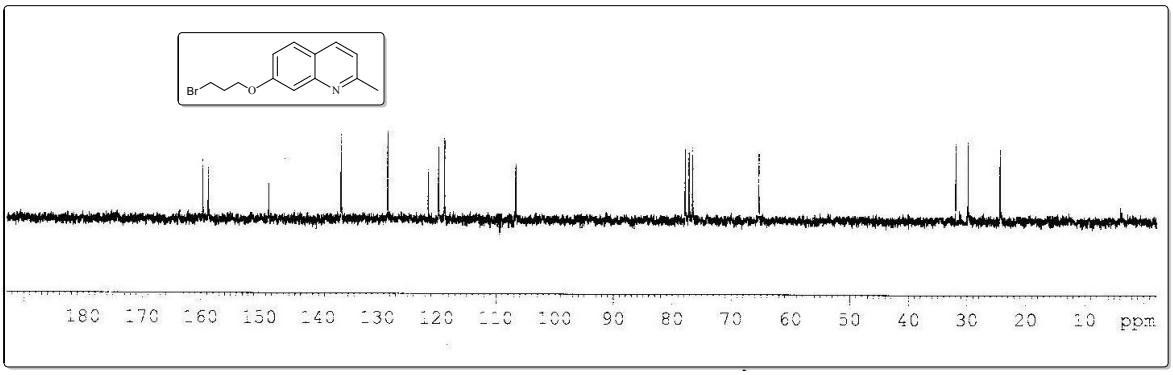
7–hydroxy quinoline (5): m–Aminophenol (775 mg, 7.1 mmol) was dissolved in 2 mL of concentrated hydrochloric acid (12 N), and then p–chloranil (1.75 g, 7.1 mmol) and 1–butanol (2.5 mL) were added. The mixture was stirred and heated to reflux (105°C) at that point a solution of crotonaldehyde (0.8 mL, 9.23 mmol) in 1–butanol (0.2 mL) was added to the refluxing solution dropwise over 20 min. After the addition was complete, the mixture was allowed to reflux for another 30 minutes. The 1–butanol was removed by rotary evaporation, and the residue was dissolved in water and washed with ether. The aqueous layer was neutralized with 10% NaOH and extracted with EtOAc. The EtOAc layer was dried over $MgSO_4$ and evaporated. The crude material was purified by column chromatography through silica gel using EtOAc/hexane (6:4) to yield 502 mg (3.15 mmol, 44%) of 1 as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.98 (1H, d, J= 8.2 Hz), 7.48 (1H, s), 7.44 (1H, d , J=8.2Hz), 7.16 (1H, d, J=8.2Hz), 6.9 (1H, d, J=8.2Hz), 4.55 (1H,

OH), 2.72 (3H, s) ; ^{13}C NMR ($CDCl_3$, 50 MHz): δ =160.5(1C), 157.9 (1C), 147.0 (1C), 138.2 (1C), 128.9 (1C), 121.2 (1C), 119.9 (1C), 119.0 (1C), 107.5 (1C), 23.5 (1C).

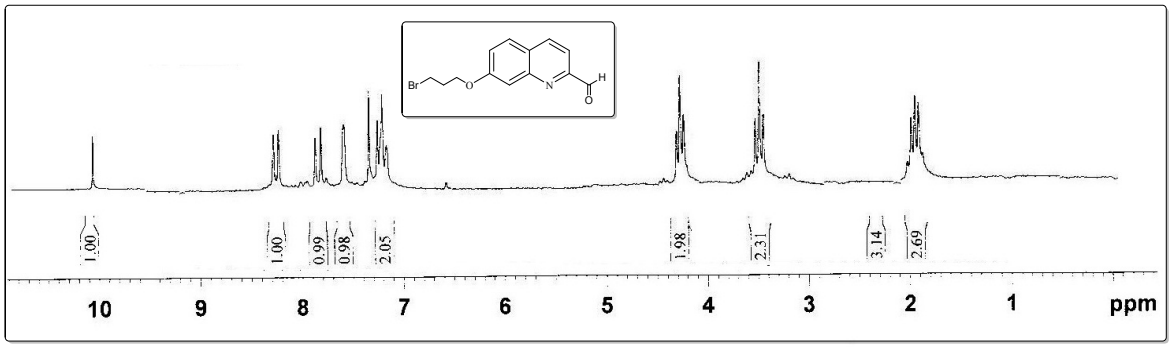


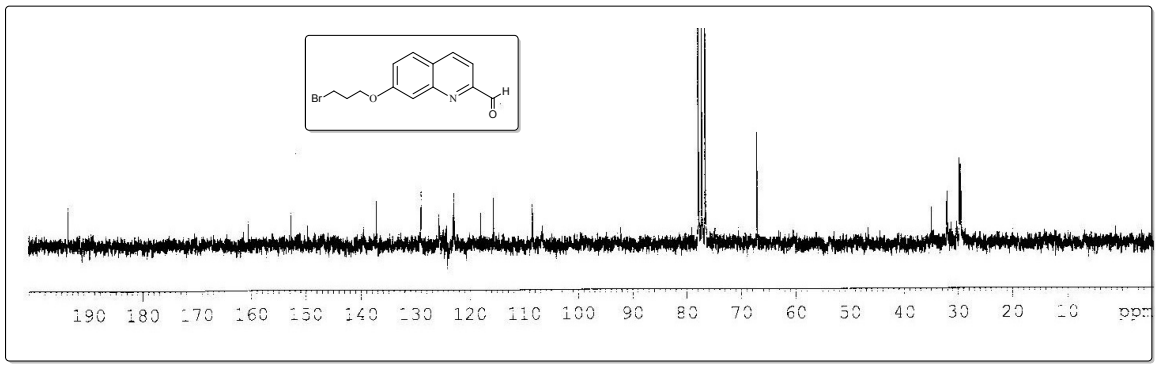
7–(3–bromopropoxy)–2–methylquinoline (4): 7–hydroxyquinoline (493 mg, 3.1 mmol) and K_2CO_3 and KI were dissolved in DMF (6 mL). Dibromopropane (1.37 mL, 5.3 mmol) was added dropwise to the mixture, and it was stirred for 6 h at room temperature under nitrogen. The reaction mixture was extracted with ethylacetate. The solvent was removed by rotary evaporation, leaving brown oil, which was purified over silica gel using EtOAc/hexane (3:7) to yield 986 mg of 4, 80%. . ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.98 (1H, d, J= 8.2 Hz), 7.48 (1H, s), 7.44 (1H, d , J=8.2Hz), 7.16 (1H, d, J=8.2Hz), 6.91 (1H, d, J=8.2Hz), 4.20 (2H, t, J=6), 3.56 (2H, t, J=6) 2.72 (3H, s)2.37(2H, t, J=6); ^{13}C NMR ($CDCl_3$, 50 MHz): δ =160.9(1C), 157.5(1C), 147.6 (1C), 136.4 (1C), 128.5 (1C), 121.2 (1C), 119.9 (1C), 118.9 (1C), 106.8 (1C), 65.5 (1C), 32.1(1C), 30.0(1C), 24.5 (1C).



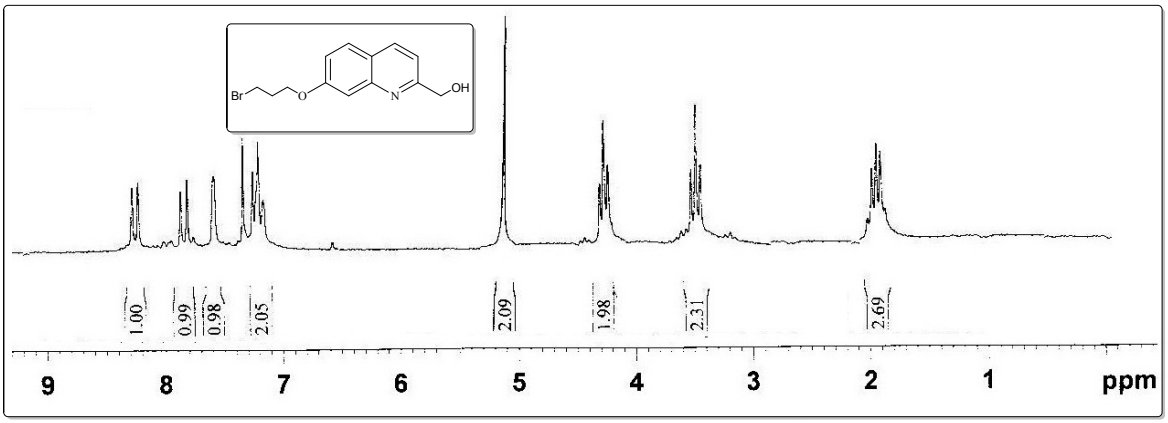


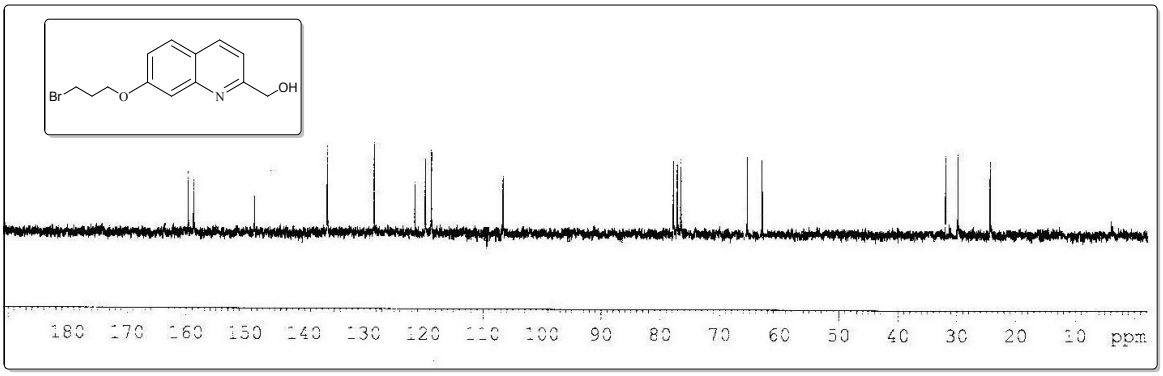
7–(3–bromopropoxy)–quinoline–2–carbaldehyde (3): A solution of 7–(3–bromopropoxy)–2–methylquinoline (873 mg, 2.12 mmol) in 1,4–dioxane (2.5 mL) was added under nitrogen to a suspension of selenium dioxide (240 mg, 2.16 mmol) in 1,4–dioxane (4 mL) at 60 °C. The temperature was raised to 80 °C, and the mixture was stirred for 8 h. After removal of the black precipitate by filtration, the filtrate was evaporated to a residue. The crude product was purified by flash column chromatography (EtOAc/hexane 2:8) to yield 762 mg of 3, 84%. ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 10.1 (1H, s), 7.94 (1H, d, J= 8.2 Hz), 7.45 (1H, s), 7.41 (1H, d , J=8.2Hz), 7.19 (1H, d, J=8.2Hz), 6.89 (1H, d, J=8.2Hz), 4.24(2H, t, J=6), 3.50 (2H, t, J=6), 2.39(2H, t, J=6); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 193.8 (1C), 160.9(1C), 157.5(1C), 147.6 (1C), 136.4 (1C), 128.5 (1C), 121.2 (1C), 119.9 (1C), 118.9 (1C), 106.8 (1C), 65.6 (1C), 32.9(1C), 31.2(1C), 23.9 (1C).



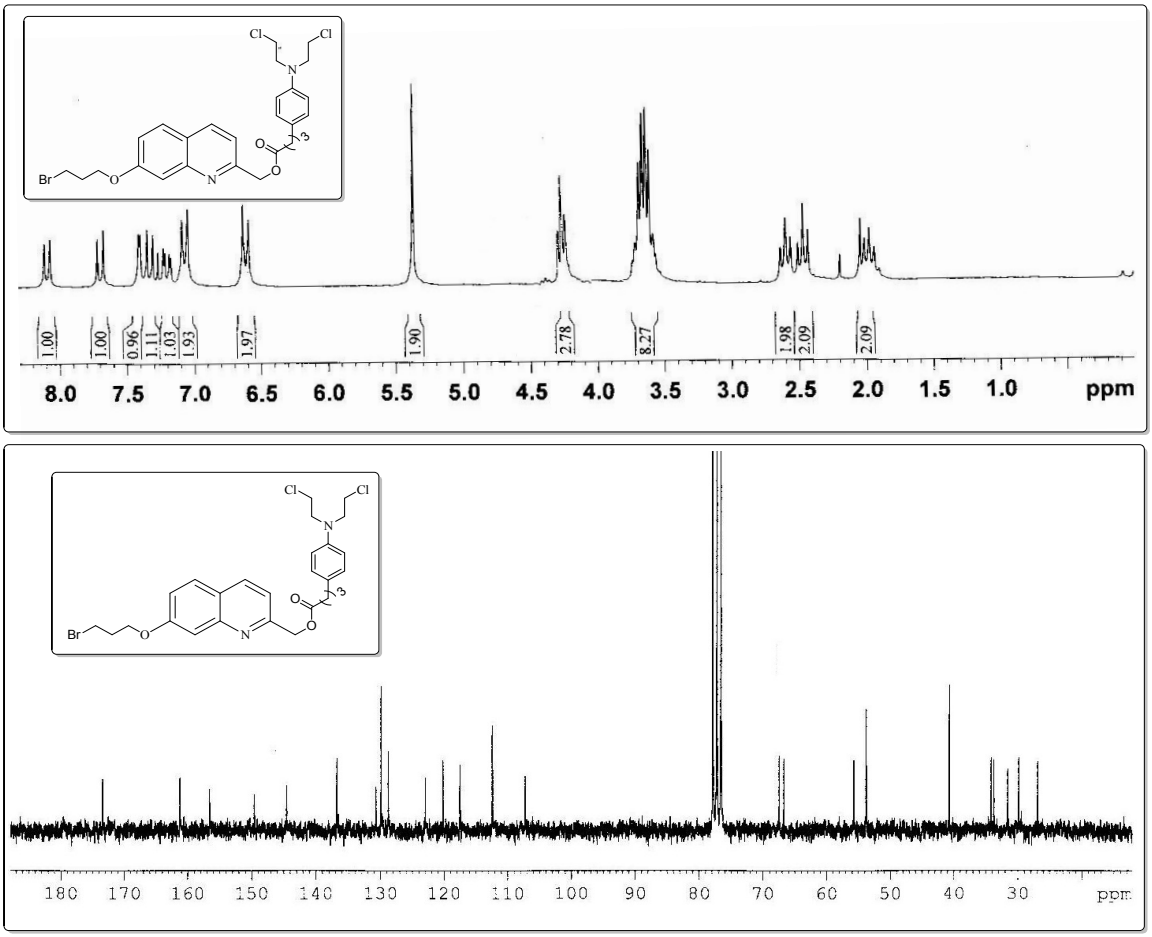


7–(3–bromopropoxy)–2–quinolylmethanol (2): Sodium borohydride (21 mg, 0.56 mmol) was added to an ice–cooled solution of 7–(3–bromopropoxy)–quinoline–2–carbaldehyde (762 mg, 1.85 mmol) in absolute ethanol (10 mL). After stirring for 4 hours, the solvent was removed by rotary evaporation. The residue was dissolved in water, and washed with diethyl ether. The organic layer was dried ($NaSO_4$) and evaporated and the crude product was purified by flash column chromatography (EtOAc/hexane 6:4) to yield 731 mg of product 2, 96%. ^1H NMR ($CDCl_3$, 200 MHz): δ = 7.94 (1H, d, J= 8.2 Hz), 7.45 (1H, s), 7.41 (1H, d , J=8.2Hz), 7.19 (1H, d, J=8.2Hz), 6.89 (1H, d, J=8.2Hz), 523(2H, s), 4.24(2H, t, J=6), 3.50 (2H, t, J=6), 2.39(2H, t, J=6); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 160.9(1C), 157.5(1C), 147.6 (1C), 136.4 (1C), 128.5 (1C), 121.2 (1C), 119.9 (1C), 118.9 (1C), 106.8 (1C), 65.6 (1C),63.2(1C), 32.9(1C), 31.2(1C), 23.9 (1C).





7–(3–bromopropoxy)–2–quinolylmethyl chlorambucil (1): Chlorambucil (0.088g, 0.29 mmol) was dissolved in 1 mL oxalyl chloride and was stirred for 1 h at 60 °C. Then oxalyl chloride was removed under vacuum to afford the acid chloride of chlorambucil as brown oil. Then the acid chloride was dissolved in dry DCM (5 mL) and the esterification reaction was carried out without further purification. To the solution of the acid chloride (5) (0.093 g, 0.29 mmol) in CH_2Cl_2 (5 mL) 7–(3–bromopropoxy)–2–quinolylmethanol (0.082 g, 0.29 mmol) was added followed by triethylamine (62 µL, 0.45 mmol). The mixture was stirred at room temperature for 12 h, and then the solvent was removed under reduced pressure. The crude reaction mixture was purified by column chromatography using 40% EtOAc in pet ether to give the compound 1 (0.132 mg, 80%). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.94 (1H, d, J= 8.2 Hz), 7.45 (1H, s), 7.41 (1H, d , J=8.2 Hz), 7.19 (1H, d, J=8.2 Hz), 7.09 (2H, d, J=8.8 Hz),6.63 (2H, d, J=8.8 Hz), 6.89 (1H, d, J=8.2 Hz), 5.37(2H, s), 4.24(2H, t, J=6), 3.69–3.52 (8H ,m) 3.48 (2H, t, J=6), 2.56–2.49 (2H, t, J = 7.2 Hz), 2.43–2.36 (2H, t, J = 7.4 Hz), 1.96–1.85 (2H, m); ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 173.4 (1C), 161.9(1C), 156.5(1C), 149.6 (1C), 14.5 (1C), 136.4 (1C), 128.5 (1C), 126.7 (2C), 121.2 (1C), 119.9 (1C), 118.9 (1C), 112.2 (2C), 107.2 (1C), 67.6 (1C), 66.2(1C), 53.6 (2C), 40.6 (2C), 33.9 (1C), 33.7 (1C), 32.9 (1C), 31.2 (1C), 26.8 (1C), 23.9 (1C). HRMS cal. For $C_{25}H_{27}BrCl_2N_2O_3$: 554.3064, found: 554.3068.



2) Charaterisation of MSNs

a)Nitrogen sorption isotherms analysis of MSNs material. A specific surface area of 302.69 m^2g^{-1} and an average mesopore diameter of 2.9 nm for the as-synthesized MSNs were observed by Brunauer-Emmett-Teller (BET) analysis.

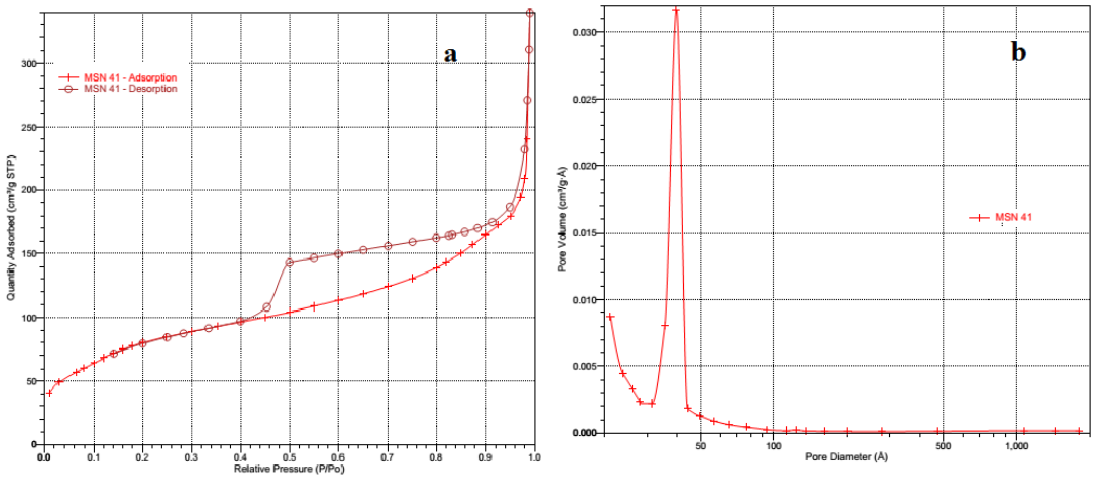


Figure S1. (a) BET nitrogen adsorption/desorption isotherms (b) pore size distribution of the MSNs materials.

b) Powder XRD profile of MSNs. Powder X-ray diffraction (XRD) analysis shows that the materials exhibit the hexagonal mesoporous structure, which is typical of MCM- 41 with the characteristic (100) peak between 2.10 and 2.20 degrees (2).

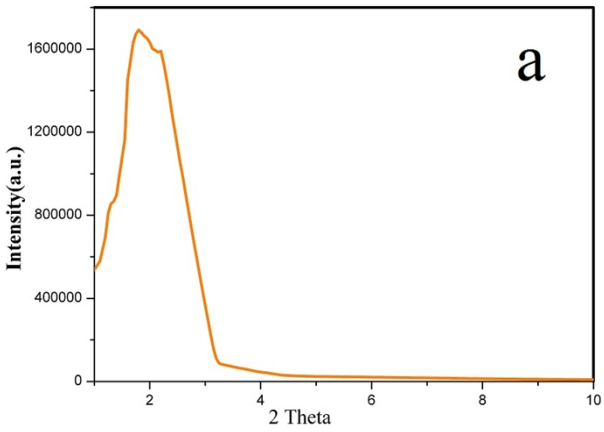


Figure S2. Powder XRD profile of MSNs

c) TEM image of MSNs and QuCbl–Fol–MSNs :

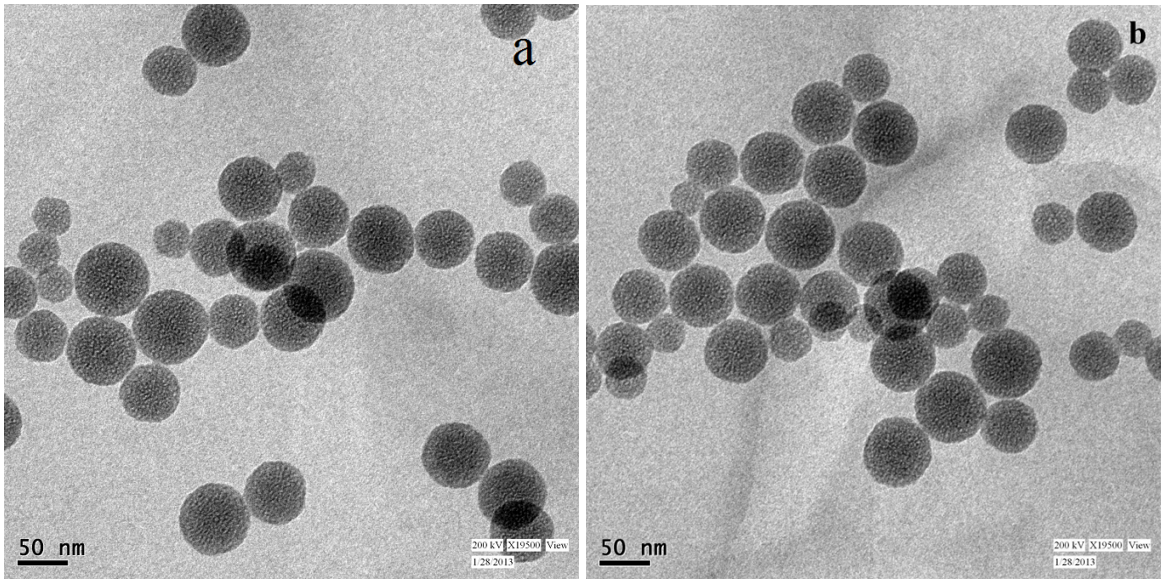
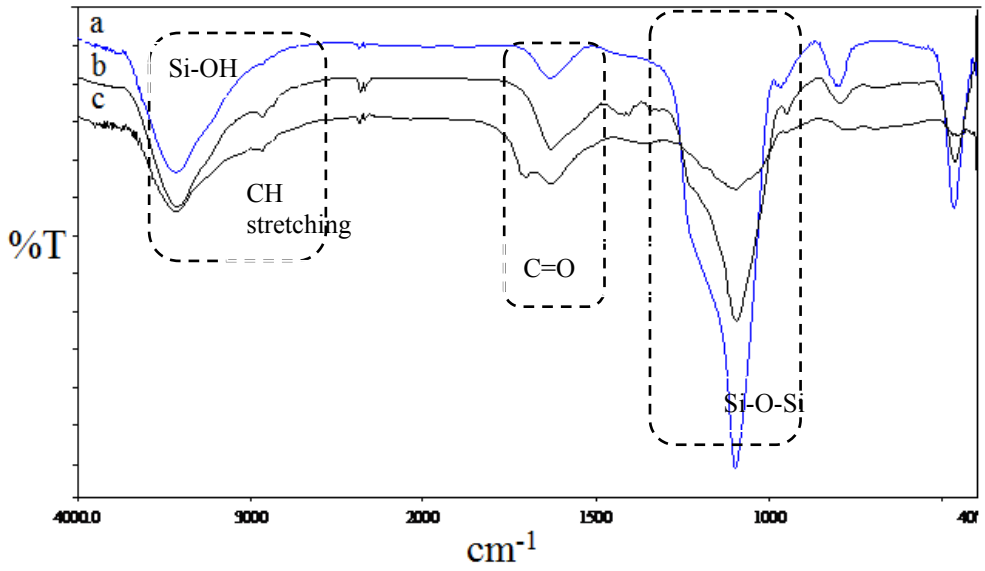


Figure S3. TEM image of (a-b) MSNs

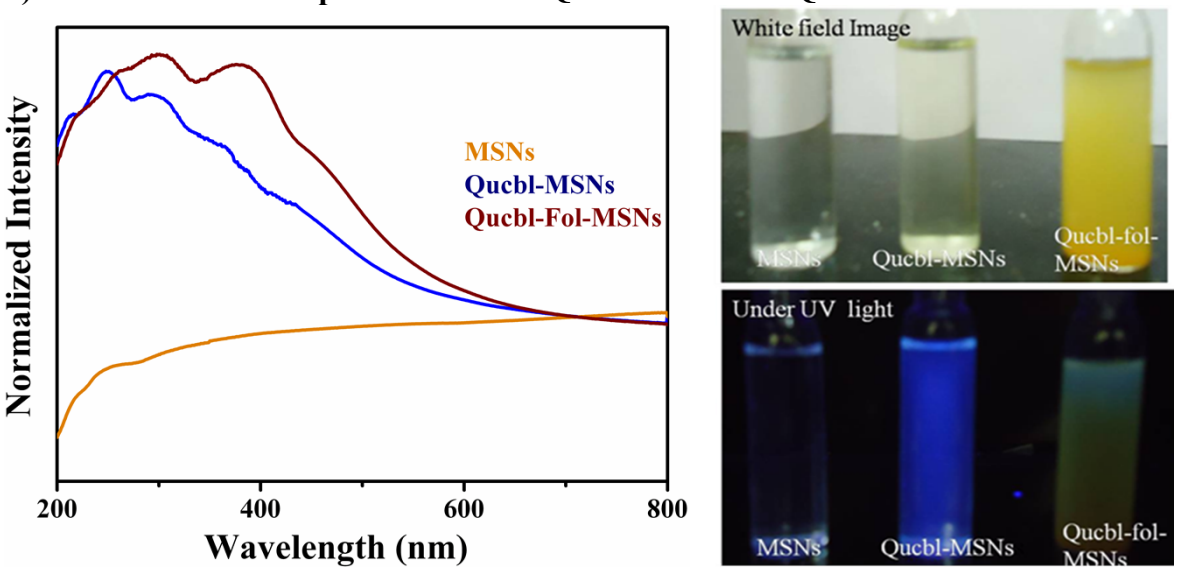
3) Charaterisation of Obtained MSN , Qucbl-MSNs and Qucbl–fol–MSNs material

i) Overlay FT-IR spectra of MSNs, QuCbl–MSNs and Qucbl–Fol–MSNs: The spectrum of the sample has high intensity peaks at 948 and 3450 cm^{-1} (SiO−H) and at 1087 cm^{-1} (Si−O−Si). The intensities of these peaks become lower in functionalized materials. New peak at 2900 cm^{-1}(C-H streaching) and at 1720 cm^{-1} (ester C=0) was observed in Sample C.



FigureS4. Overlay FT-IR spectra of (a) MSNs (b) Qucbl–MSNs (c) Qucbl–Fol–MSNs

ii) Solid state UV–Vis spectra of MSNs Qucbl-MSNs and Qucbl–Fol–MSNs :



FigureS5. Solid state UV–Vis spectra of MSNs, QuCbl–MSNs and Qucbl–Fol–MSNs

iii) Thermogravimetric analysis of MSNs and Qucbl–Fol–MSNs : The formation of the said surface-modified nanoparticles was further corroborated by TGA measurements. Step-wise weight loss at high temperature from the nanoparticles could be explained on the basis of decomposition of the surface functionalities introduced at various stages.

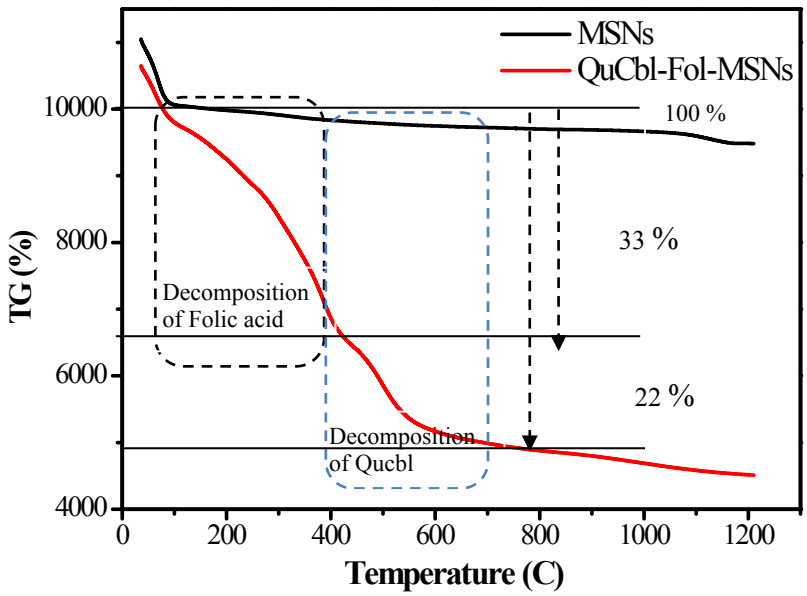
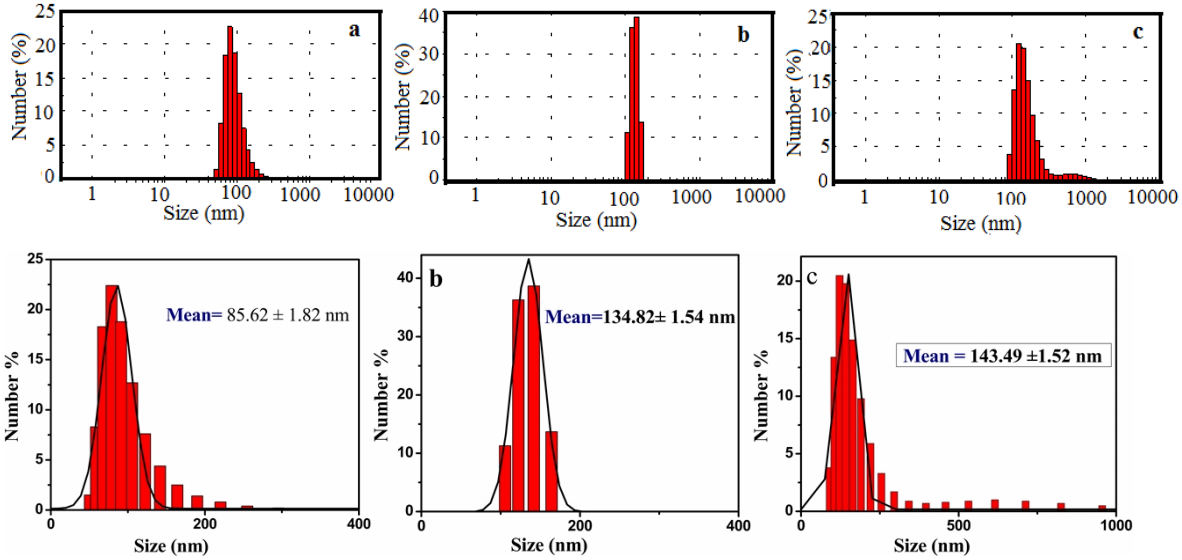


Figure S6. TGA data of MSNs and Qucbl–Fol–MSNs.

v) Dynamic light scattering profile of MSNs, Qucbl–MSNs and Qucbl–Fol–MSN: DLS measurement of the dispersed MSNs, Qucbl–MSNs and Qucbl–Fol–MSN was performed in water.



The mathematical fit of the DLS–based curves from the actual point was performed by Origin's curve fitting using the nonlinear least squares fitter which is based on the Levenberg–Marquardt algorithm

Figure S7. Dynamic light scattering spectra of MSNs in water (a) MSNs (b) Qucbl–MSNs (c) Qucbl–Fol–MSNs

4) UV–vis absorption spectra of Quinoline methanol Quinoline chlorambucil (Qucbl) and Quinoline chlorambucil loaded and folic acid decorated MSNs(Qucbl–Fol–MSNs)

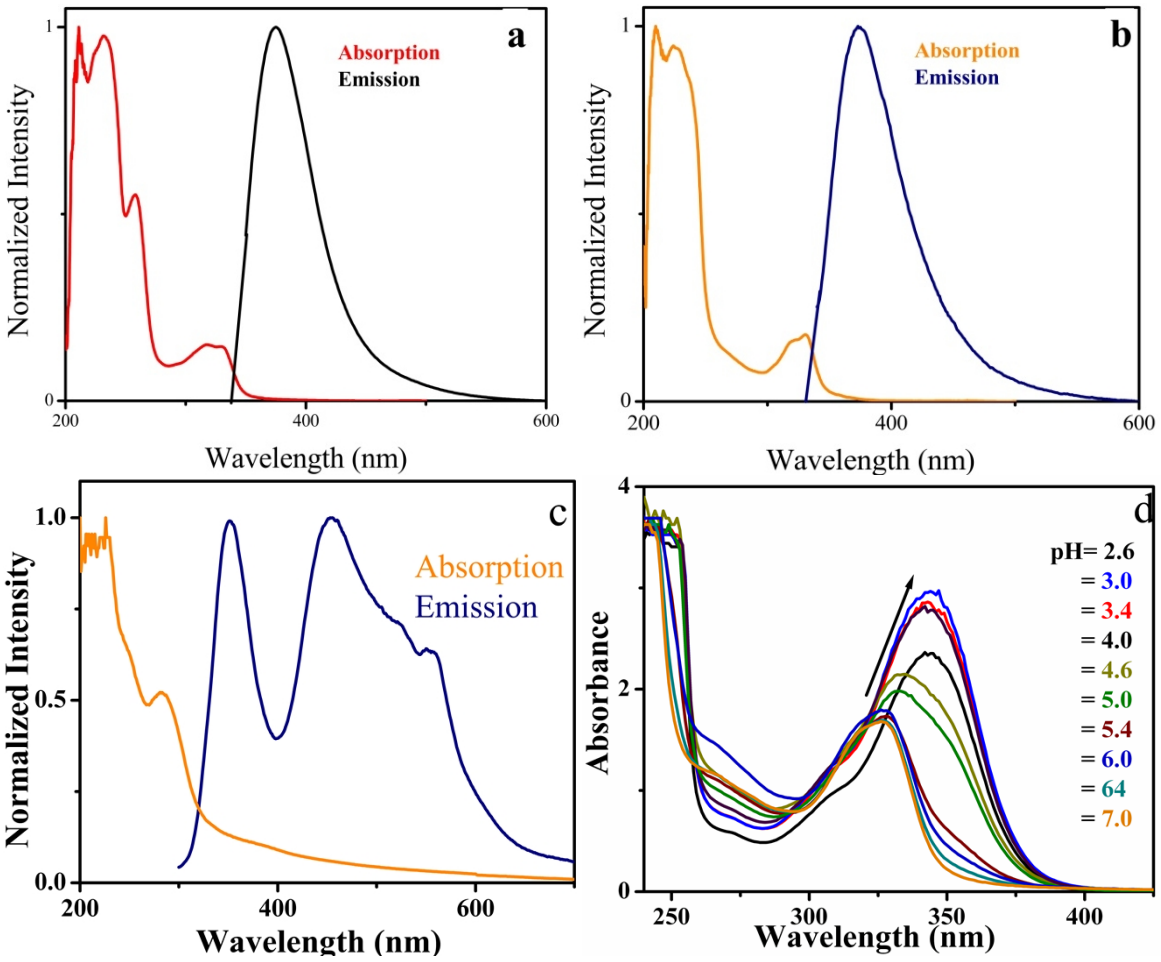


Figure S8. Normalized absorption and emission spectra: (a) Quinoline–Chlorambucil (Qucbl) congucate (b) quinolylmethanol (Qu–OH) (c) Normalized absorption and emission spectra of quinoline chlormbucil and folic acid decorated MSNs (d) pH responsive absorption spectra of Quinoline methanol conjugate.

a) Hydrolytic Stability of Qucbl–Fol–MSNs:

Table S1: The percentage of drug released from Qucbl–Fol–MSNs in dark condition at pH 7.4

Photoresponsive nanocarrier	Time (day)	% of drug depleted	
		(pH 7.4) PBS	(pH 7.2) FBS
Qucbl–Fol–MSNs	4	2	4

We observed insignificant (2-4%) release of the drug, which proves that the nanoparticles are quite stable under the dark condition and suitable for following biological application in vitro.

b) Calculation of Quinoline chlorambucil loaded on MSNs:

$$\text{Quinoline chlorambucil loaded on MSNs} = \frac{(\text{initial conc of Qucbl - final conc of Qucbl in reaction medium}) \text{ X M.Wt of Qucbl}}{\text{Amount of mesoporous silica taken}}$$

Initial concentration of Qucbl the reaction mixture (0 min) = 4.1258 X 10^{-4}mol/mL

The final concentration of Qucbl in the reaction mixture (20 h) was calculated from the absorption spectra = 3.741X10^{-4}mol/mL

Quinoline chlorambucil loaded on MSNs

= initial conc of Qucbl - final conc of Qucbl in reaction medium

= 4.1258 X10^{-4} – 3.7149X10^{-4}mol/mL

= 4.1099X10^{-5} mol/mL X M.wt of Qucbl (665.36)

= 2734.5630X10^{-5}g of Qucbl in 100 mg of MSNs

= 27.3456X10^{-5} g in 1mg of MSNs

~ 273 µg/mg of Qucbl loaded on the MSNs.

10) Cell Imaging and Cytotoxicity of MSNs, and Qucbl–Fol–MSNs on HeLa cell line:

a) Time dependent internalization studies of Qucbl–Fol–MSNs at pH 7.4: We followed the same procedure except the Qcbl–Fol-MSNs were dispersed in PBS of pH 4.8. we have carried out cell internalization of Qucbl-Fol-MSNs in HeLa cells using two different pH PBS buffers solution at 37^{o}C and 5 % CO_2. The cells were then incubated with 50 µg of Qucbl-Fol-MSNs (Q2) separately in both the cell culture mediums for 6 h at 37 ^{o}C and 5 % CO_2. It was clearly observed that pH of the buffer solution has no detectable effect on the cellular internalization. But with increase in the acidity of the buffer it was observed that our DDS internalized more in lysosome due to protonation of quinoline nitrogen.

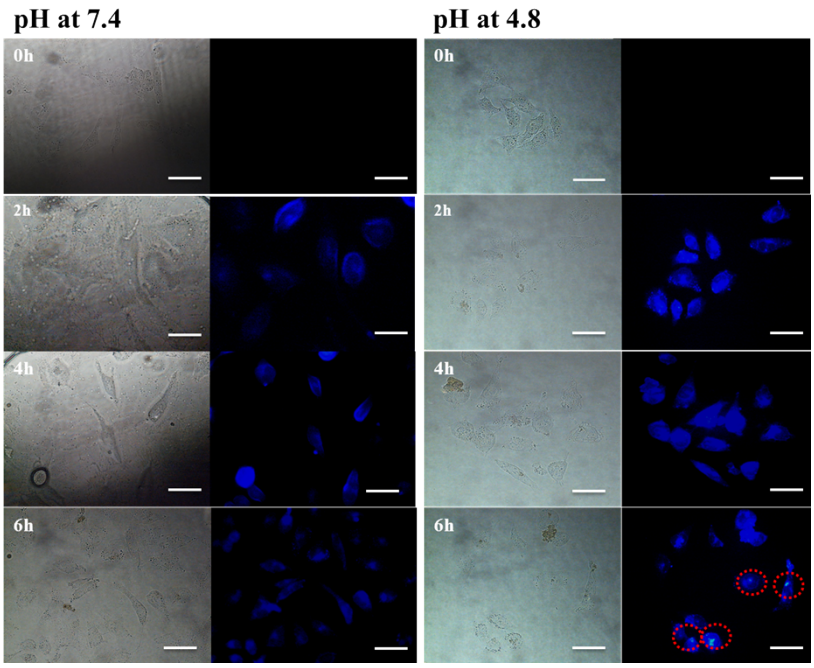


Figure S9: Time dependent CLSM images of HeLa cells incubated with Qucbl-Fol-MSNs under different pH (7.4 and 4.8). The blue fluorescence is from the Qucbl-Fol-MSNs pH 4.8 Qucbl-fol-MSNs internalized more when compare to pH 7.4. Scale bar: 40 μm.

11) **Photolysis of Qucbl–Fol–MSNs using soft UV irradiation (≥ 365 nm) and 675 nm laser diode:**

Photolysis of Qucbl–Fol–MSNs using Red laser: 1 mg of Qucbl–Fol–MSNs was dissolved in 1ml acetonitrile. Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated using 675 nm laser diode (15 mW/ cm^2). At regular time intervals, a small aliquot (100 μL) of the suspension was taken out and centrifuged (5000 r/min) for 10 min, the obtained transparent solution was analyzed by reverse phase HPLC using mobile phase acetonitrile, at a flow rate of 1 mL / min.

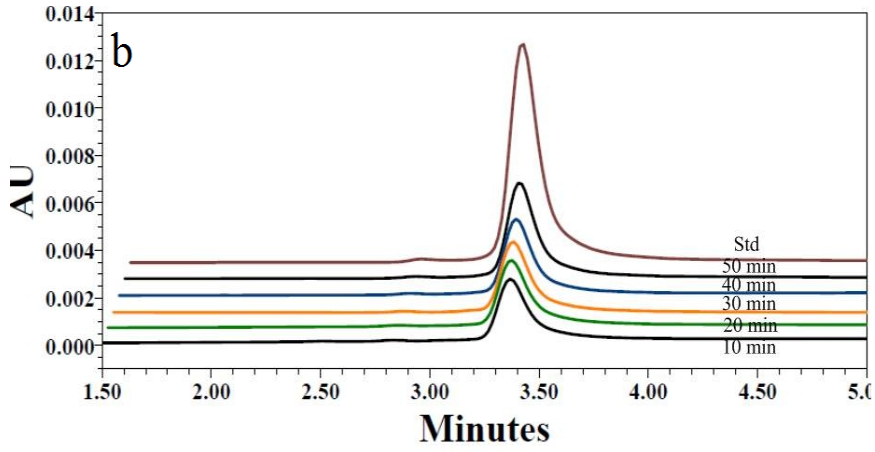


Figure S10: HPLC profile of Chlorambucil release from Qucbl–Fol–MSNs using 675 nm laser diode (15 mW/ cm^2). The y–axes were offset by 15 mAU units and the x–axes were offset by 5 s, to facilitate better visualization.

Cytotoxicity after two photon photolysis: HeLa cells maintained in minimum essential medium (in 96–well cell–culture plate at concentration of 1×10^4 cells/mL) containing 10% fetal bovine serum (FBS) and 50 µg concentration of Q2 was incubated for 4 h at 37 ^oC and 5 % CO_2. Then the cells were irradiated (keeping the cell–culture plate 5 cm apart from the light source) using 675 nm laser diode (15mW/cm^2). After irradiation the cells were again incubated for 72 h. Then cytotoxicity was measured using the MTT assay as described in the earlier section.

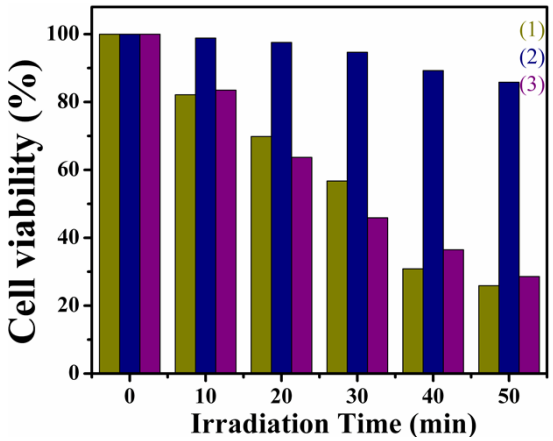


Figure S11. Cell viability test 50 µg of Qucbl-Fol-MSNs (Q2) under soft UV irradiation 1PE (≥365 nm) and 2PE (675 nm) in HeLa cell line. (1) Qucbl–Fol–MSNs + 1PE (@≥ 365 nm), (2) Qucbl–Fol–MSNs + 2PE (675 nm) (3) chlorambucil.

we have carried out cell internalization of Qucbl-Fol-MSNs in HeLa cells using two different buffer solutions (PBS (pH 7.4) and DMEM (pH= 7.2)) at 37^oC and 5 % CO_2. The cells were incubated with 50 µg of Qucbl-Fol-MSNs (Q2) separately in both the cell culture mediums for 6 h. It was clearly observed that pH of the buffer solution has no detectable effect on the cellular internalization.

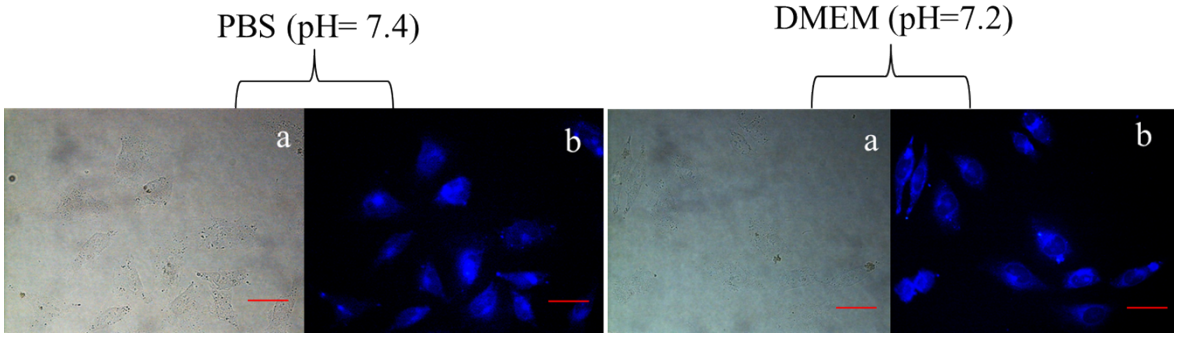


Figure S12: CLSM images of HeLa cells incubated with Qucbl-Fol-MSNs under different buffer solution (PBS and DMEM). The blue fluorescence is from the Qucbl-Fol-MSNs, Scale bar: 40 μm.