Electronic Supplementary Material (ESI) for Chemical Communications

Photoresponsive Quinoline Tethered Fluorescent Carbon dots for Regulated Drug Delivery

S. Karthik,[a] Biswajit Saha,[b] Sudip Kumar Ghosh*[b] and N. D. Pradeep Singh*[a]

[a] Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur–721302, India.
[b]Department of Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur–721302, India.

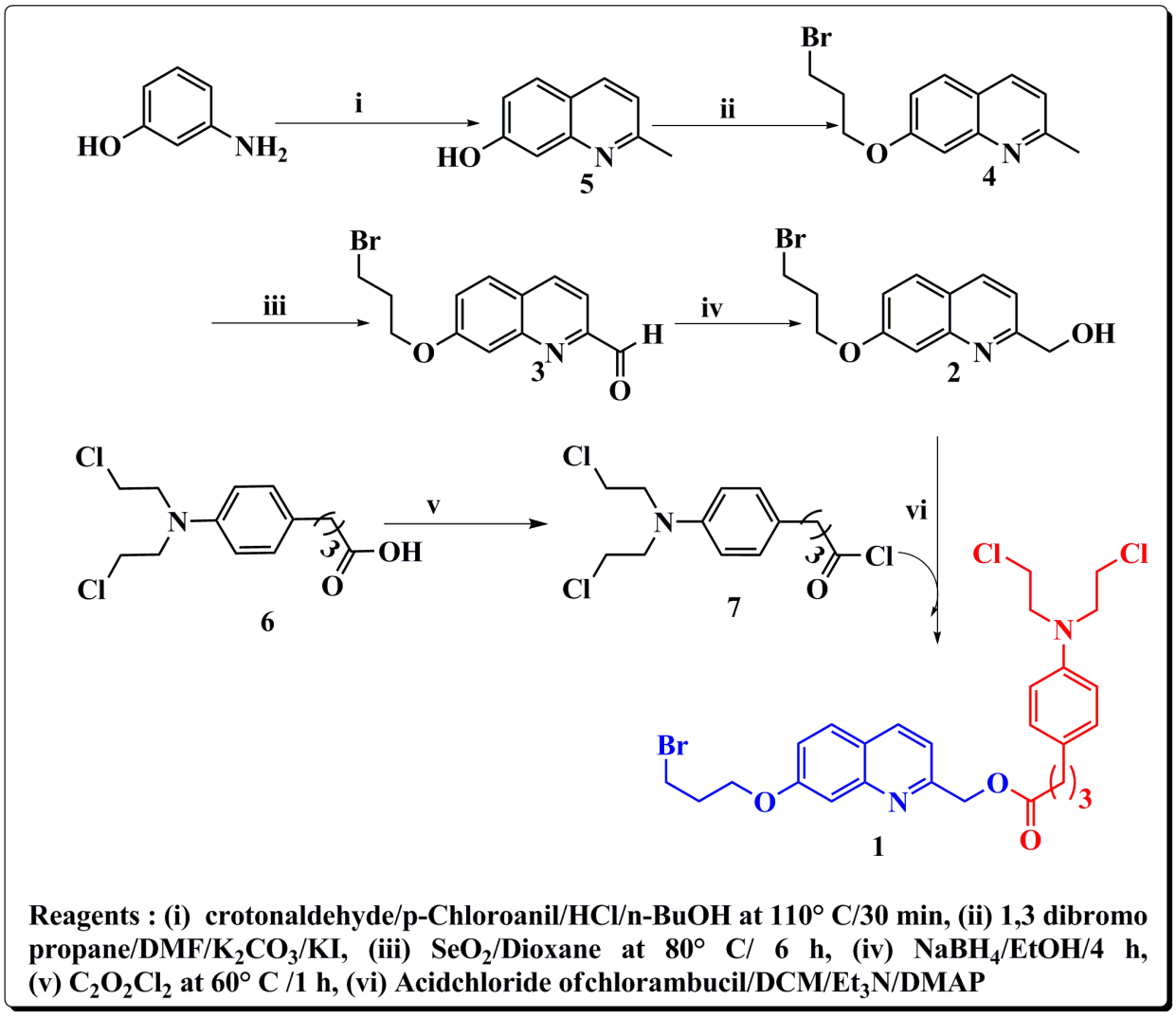
Supporting Information

1. Experimental

1.1. Materials and method

All reagents were purchased from Sigma Aldrich and used without further purification. Acetonitrile and dichloromethane were distilled from CaH_2 before use. ^1H NMR spectra were recorded on a BRUKER-AC 200 MHz spectrometer. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 7.26 ppm). Data are reported as follows: chemical shifts, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (Hz). ^{13}C NMR (50 MHz) spectra were recorded on a BRUKER-AC 200 MHz Spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as the internal standard (deuterochloroform: 77.0 ppm). UV/vis absorption spectra were recorded on a Shimadzu UV-2450 UV/vis spectrophotometer, fluorescence emission spectra were recorded on a Hitachi F-7000 fluorescence spectrophotometer, FT-IR spectra were recorded on a Perkin Elmer RXI spectrometer and HRMS spectra were recorded on a JEOL-AccuTOF JMS-T100L mass spectrometer. Zeta potential were measured by ZetaSizer Nano (Malvern instrument Ltd.,). Transmission Electron Microscopy (TEM) was measured on a FEI Tecnai G220S-Twin at 200 kV. The TEM sample was prepared by dispersing compounds in water and dropping on the surface of a copper grid. Photolysis of all the ester conjugates were carried out using 125 W medium pressure Hg lamp supplied by SAIC (India). Chromatographic purification was done with 60-120 mesh silica gel (Merck). For reaction monitoring, precoated silica gel 60 F254 TLC sheets (Merck) were used. RP-HPLC was taken using mobile phase acetonitrile, at a flow rate of 1mL / min (detection: UV 254 nm).

2. Synthesis of Phototrigger Quinoline-chlorambucil conjugates:

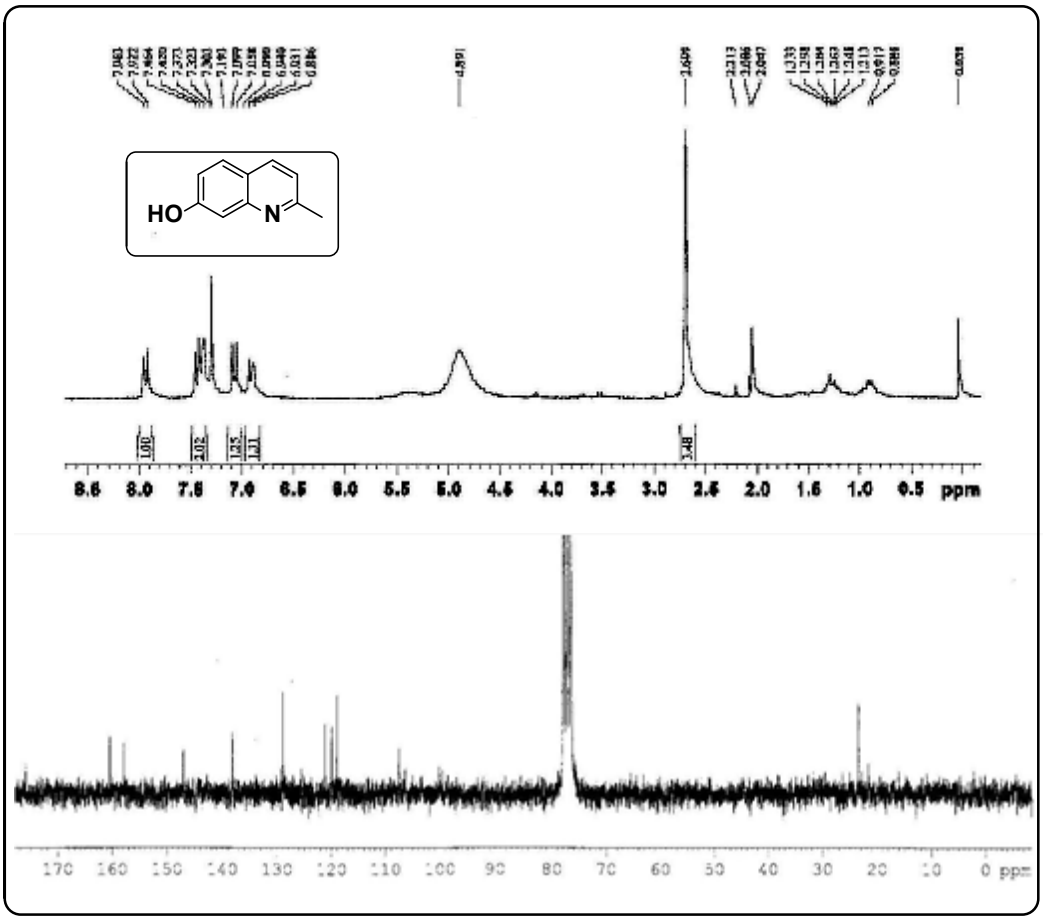


Reagents : (i) crotonaldehyde/p-Chloroanil/HCl/n-BuOH at 110° C/30 min, (ii) 1,3 dibromo propane/DMF/K_2CO_3/KI, (iii) SeO_2/Dioxane at 80° C/ 6 h, (iv) $NaBH_4$/EtOH/4 h, (v) $C_2O_2Cl_2$ at 60° C /1 h, (vi) Acidchloride ofchlorambucil/DCM/Et_3N/DMAP

Scheme S1: Synthesis of Quinoline chlorambucil conjugate.

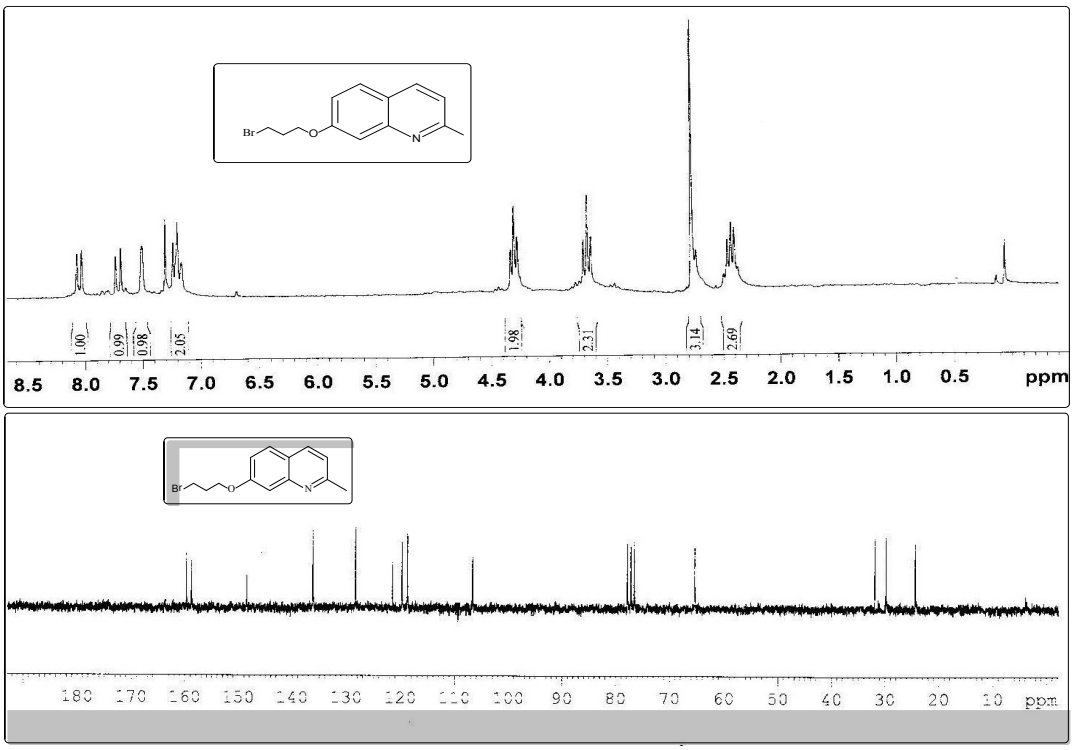
7-hydroxy quinoline (5): m-Aminophenol (775 mg, 7.1 mmol) was dissolved in 2 mL of concentrated hydrochloric acid (12 N), and then p-chloranil (1.75 g, 7.1 mmol) and 1-butanol (2.5 mL) were added. The mixture was stirred and heated to reflux (105°C) at which point a solution of crotonaldehyde (0.8 mL, 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).

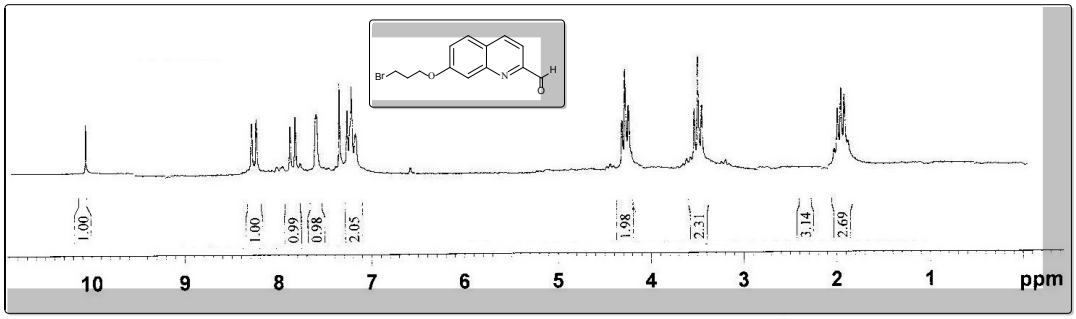


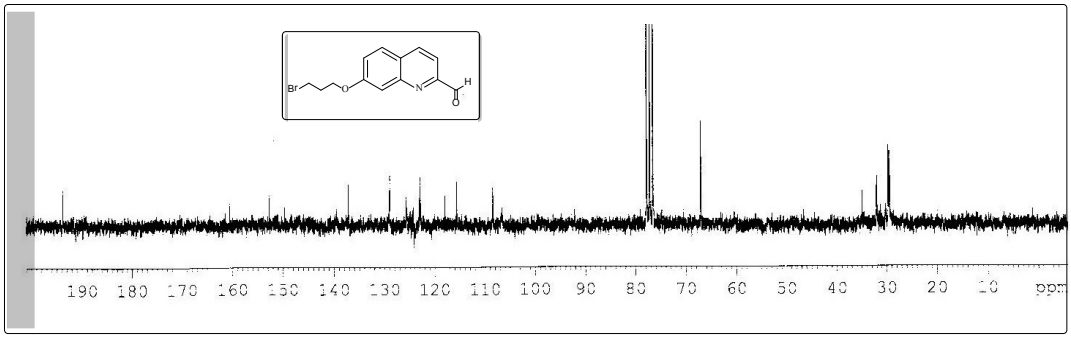
7-(3-bromopropoxy)-2-methylquinoline (4): 7-hydroxyquinoline (493 mg, 3.1 mmol) and K_2CO_3 and KI were dissolved in DMF (6 mL). Dibromopropane (1.37 mL, 5.3 mmol) was added dropwise to the mixture, and it was stirred for 6 h at room temperature under nitrogen. The reaction mixture was extracted with 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).

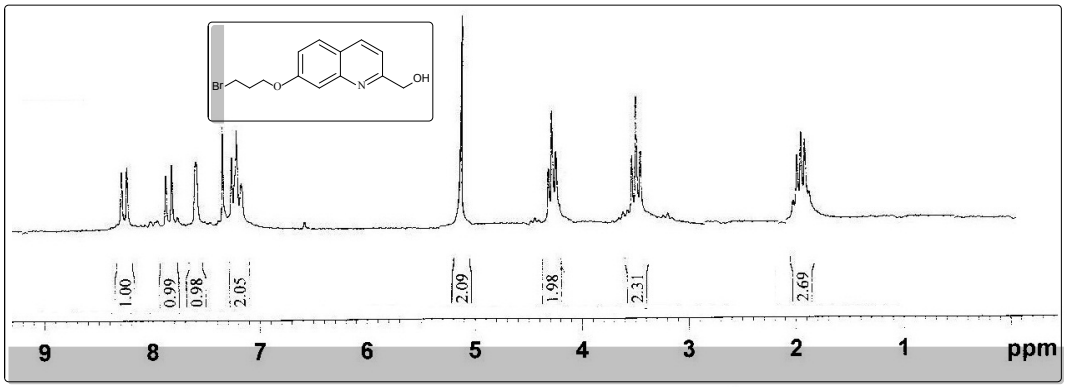


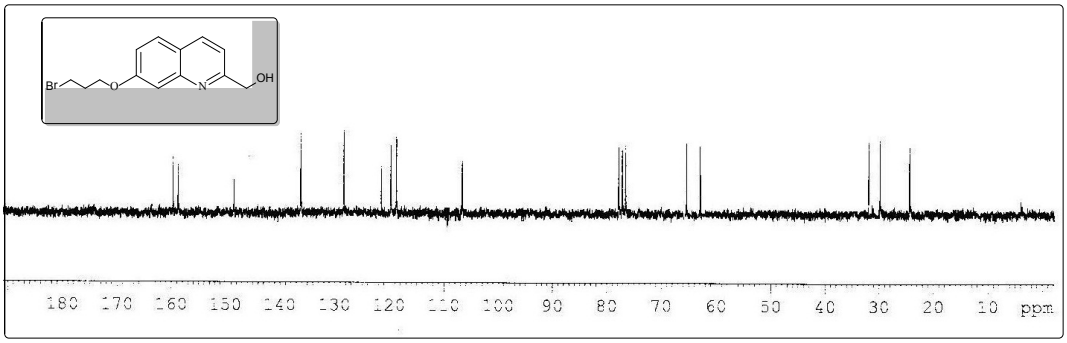
7-(3-bromopropoxy)-quinoline-2-carbaldehyde (3): A solution of 7-(3-bromopropoxy)-2-methylquinoline (873 mg, 2.12 mmol) in 1,4-dioxane (2.5 mL) was added under nitrogen to a suspension of selenium dioxide (240 mg, 2.16 mmol) in 1,4-dioxane (4 mL) at 60 °C. The temperature was raised to 80 °C, and the 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).



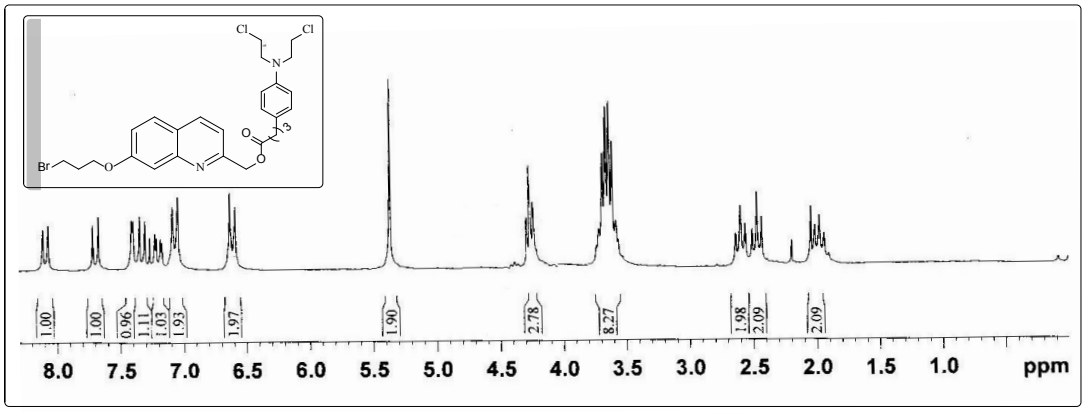


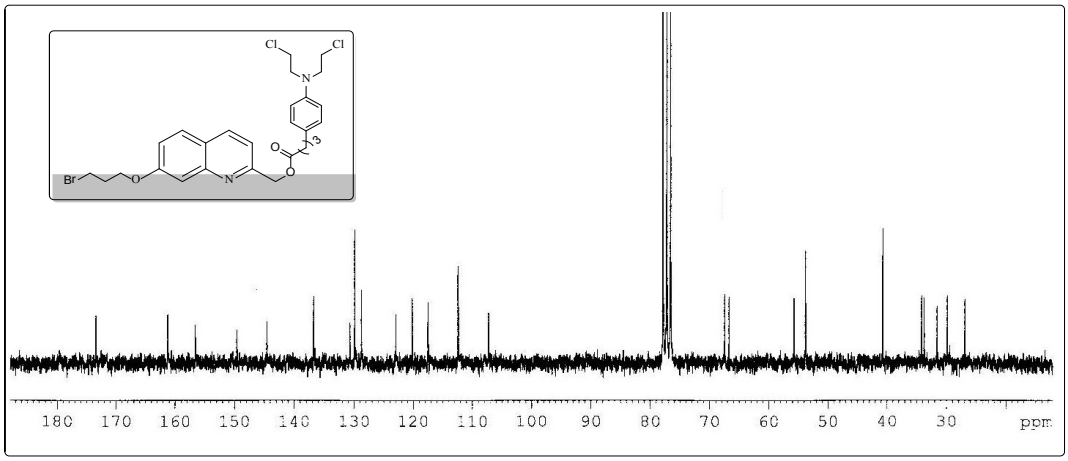
7-(3-bromopropoxy)-2-quinolylmethanol (2): Sodium borohydride (21 mg, 0.56 mmol) was added to an ice-cooled solution of 7-(3-bromopropoxy)-quinoline-2-carbaldehyde (762 mg, 1.85 mmol) in absolute ethanol (10 mL). After stirring for 4 hours, the solvent was removed by rotary evaporation. The residue was 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%. ^{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=8.2Hz), 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).





2.1. Synthesis of Carbon dots (Cdots)

Fluorescent Nitrogen containing carbon nanodots were synthesized according to the literature procedure (Songnan Qu, Xiaoyun Wang, Qipeng Lu, Xingyuan Liu, and Lijun Wang Angew. Chem. Int. Ed. 2012, 51, 1 – 5). First, citric acid (1.5 g) and urea (1.5 g) were added to distilled water (5 mL) to form a transparent solution. The solution was then heated in a domestic 750 W microwave oven for 4 mins, during which the solution changed from being a colorless liquid to a brown and finally dark-brown clustered solid, indicating the formation of carbon dots. This solid was then transferred to a vacuum oven and heated at 600°C for 1 h to remove the residual small molecules. An aqueous solution of the carbon dots was purified in a centrifuge (3000 r min^{-1}, 20 min) to remove large or agglomerated particles. Carbon dots were precipitated and rinsed with anhydrous ethanol twice, and vacuum-dried at ambient temperature to collect 0.98 g of carbon dots.

2.2. Synthesis of Quinoline chlorambucil loaded Carbon dots (Qucbl-Cdots):

The Cdots (100 mg) was dissolved in Dry THF (3 mL) under a N_2 atmosphere and potassium tertiary butoxide (75 mg) were added into the solution. After stirring for 1 h at room temperature, 7-(3-bromopropoxy)-2-quinolylmethyl chlorambucil was added dropwise to the mixture and the resulting solution was stirred for overnight at room temperature in the dark. The solvent was removed by rotatry evaporater and CDs was precipitated and rinsed with anhydrous ethanol twice, and vacuum-dried at ambient temperature to collect a final quinoline chlorambucil loaded carbon dots (Qucbl-Cdots).

3. Charaterization of carbon dots (Cdots)*, 7-methoxy quinoline, *Quinoline chlorambucil (Qucbl) and Quinoline chlorambucil loaded carbon dots (Qucbl-Cdots)

a) Absorption and Emission spectra of Carbon dots and Quinoline chlorambucil loaded Carbon dots.

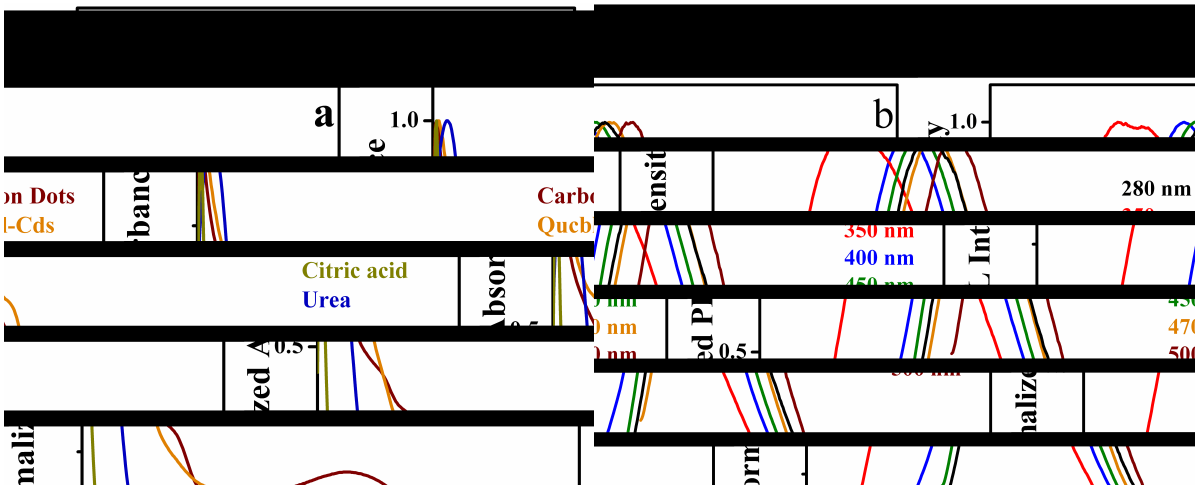


Figure S1: (a) Absorption spectra of carbon dots, citric acid, urea and quinoline chlorambucil loaded carbon dots (b) Tunable emission spectra of Carbon dots.

b) Absorption and fluorescence spectra of 7-methoxy quinoline:

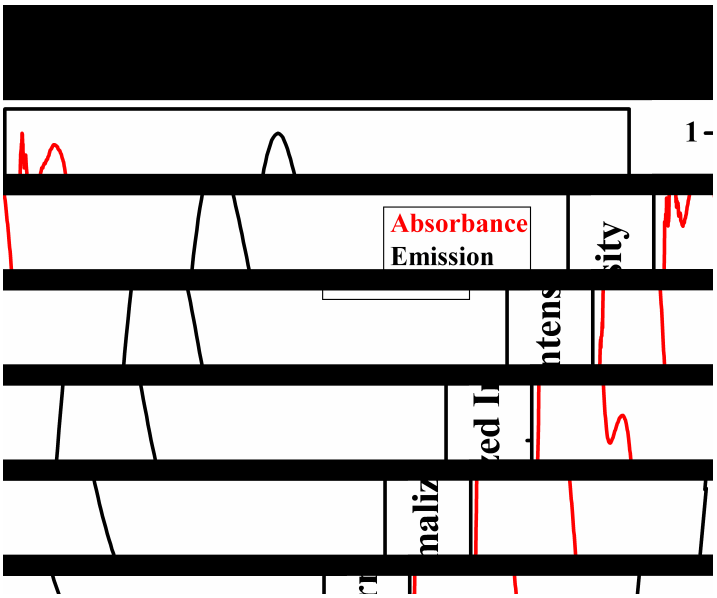


Figure S2 : (a)Absorption and emission spectra of 7-methoxy quinoline.

c) Absorption and Emission spectra of carbon dots and Qucbl-Cdots in Human serum albumin.

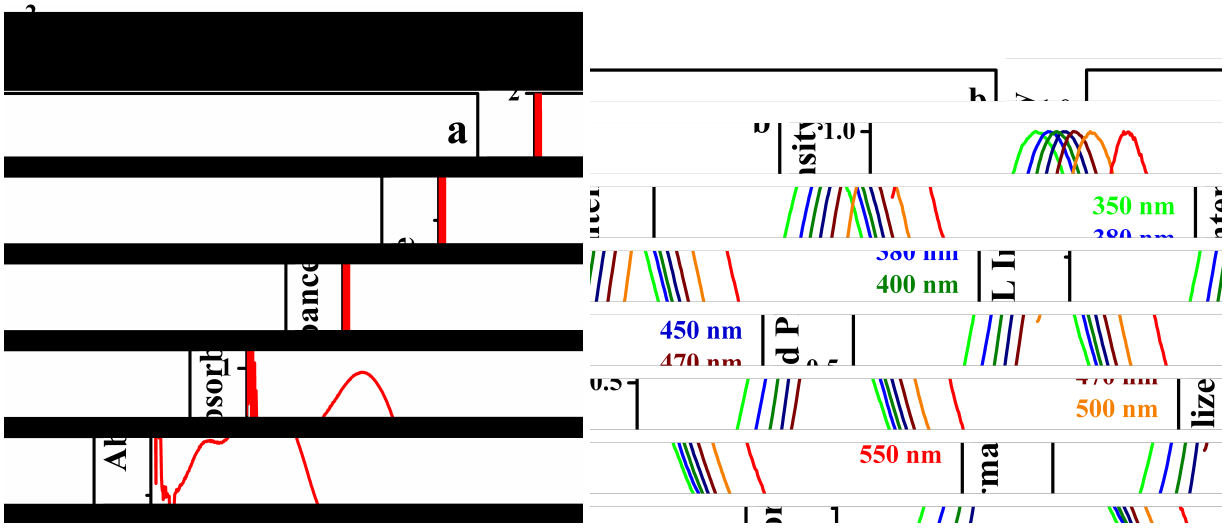


Figure S3: Absorption and emission spectra of carbon dots in HSA (a) Absorption spectra (b) Tunable emission spectra.

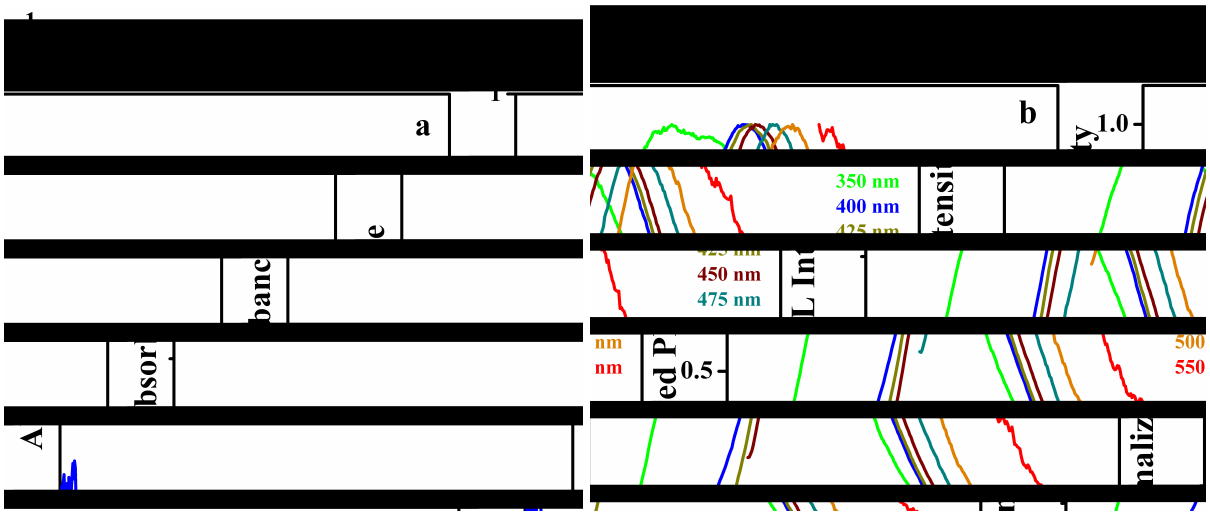


Figure S4: Absorption and emission spectra of Qucbl-Cdots in HSA a) Absorption spectra b) Emission spectra.

d) Measurement of fluorescence quantum yields[1]

The quantum yield of the Carbon Dots (CDs) was determined by reference point method.[1] Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield: 54%) was used as a standard sample to calculate the QY of Cdots and Qucbl-Cdots. which were dissolved in ultra pure water. The absorbance values of the solutions at the excitation wavelength were measured with UV–Vis

spectrophotometer. Photoluminescence (PL) emission spectra of all the sample solutions were recorded by Hitachi F-7000 fluorescence spectrophotometer at an excitation wavelength of 360 nm.

$$\frac{\phi_S}{\phi_R} = \frac{A_S}{A_R}\frac{(Abs)_R}{(Abs)_S}\frac{\eta_S^2}{\eta_R^2}$$

Where **Φ** represents quantum yield, **Abs** represents absorbance, **A** represents area under the fluorescence curve, and **η** is refractive index of the medium. The subscripts **S** and **R** denote the corresponding parameters for the sample and reference, respectively.

Table S1: Quantum yield of the fluorescent Cdots and Qucbl-cdots

Sample	Intergrated emission intensity (I)	Abs. At 360 nm (A)	Refractive index of solvent (η)	Quantum yield at 360nm (Q)
Quinine sulfate	55337.365	0.0717	1.33	54% (known)
Cdots	12394.66	0.0619	1	14.0 %
Qucbl-Cdots	8418.37	0.0595	1	9.9 %

Fluorescence quantum yield (excitation wavelength 360 nm, error limit within ± 5%).

e) FT-IR Overlay spectra of carbon dots, quinoline chlorambucil loaded Carbon dots and quinoline chlorambucil

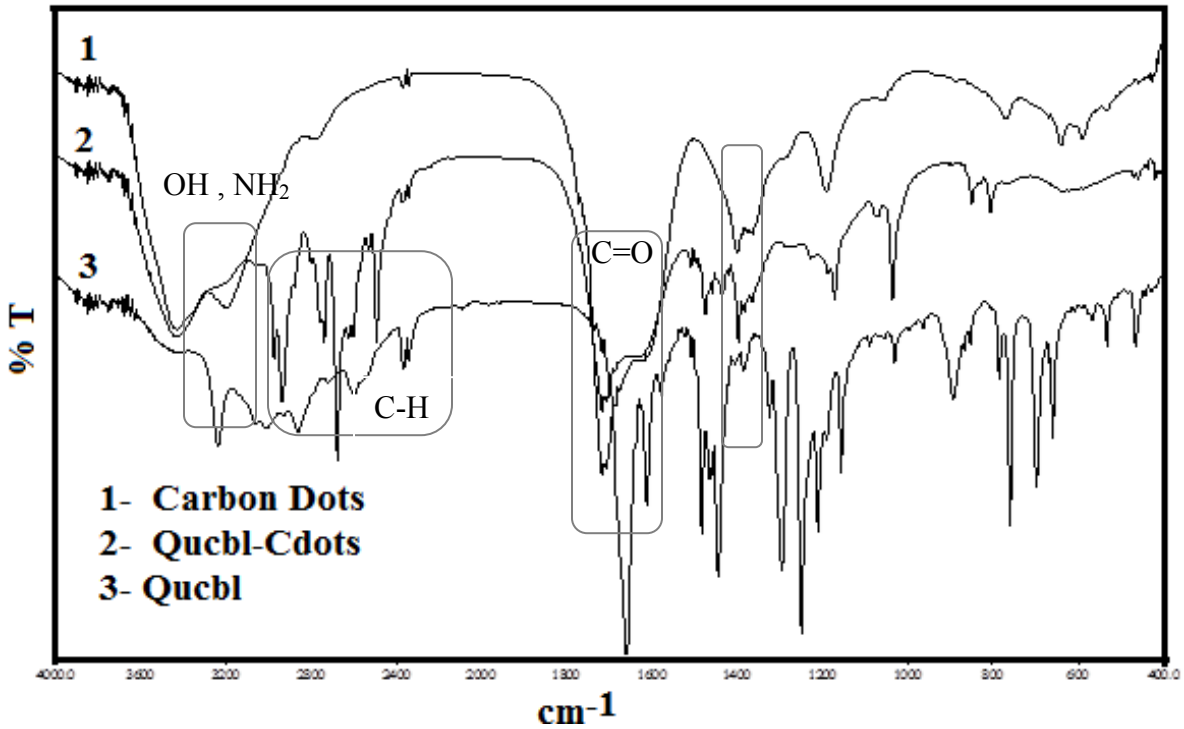
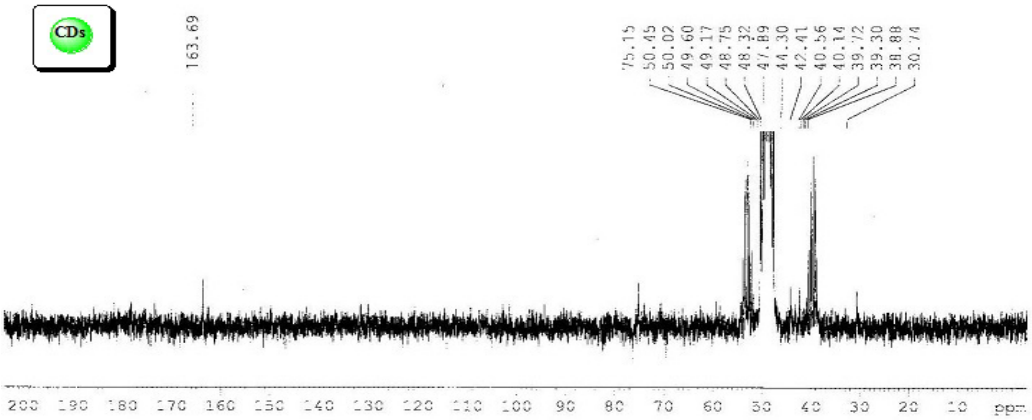


Figure S5: Overlay FT-IR spectra of Carbon dots (Cdots), Quinoline chlorambucil(Qucbl) and Quinoline chlorambucil loaded carbon dots(Qucbl-Cdots)**.**

f) Carbon NMR of carbon dots, quinoline chlorambucil and quinoline chlorambucil loaded carbon dots



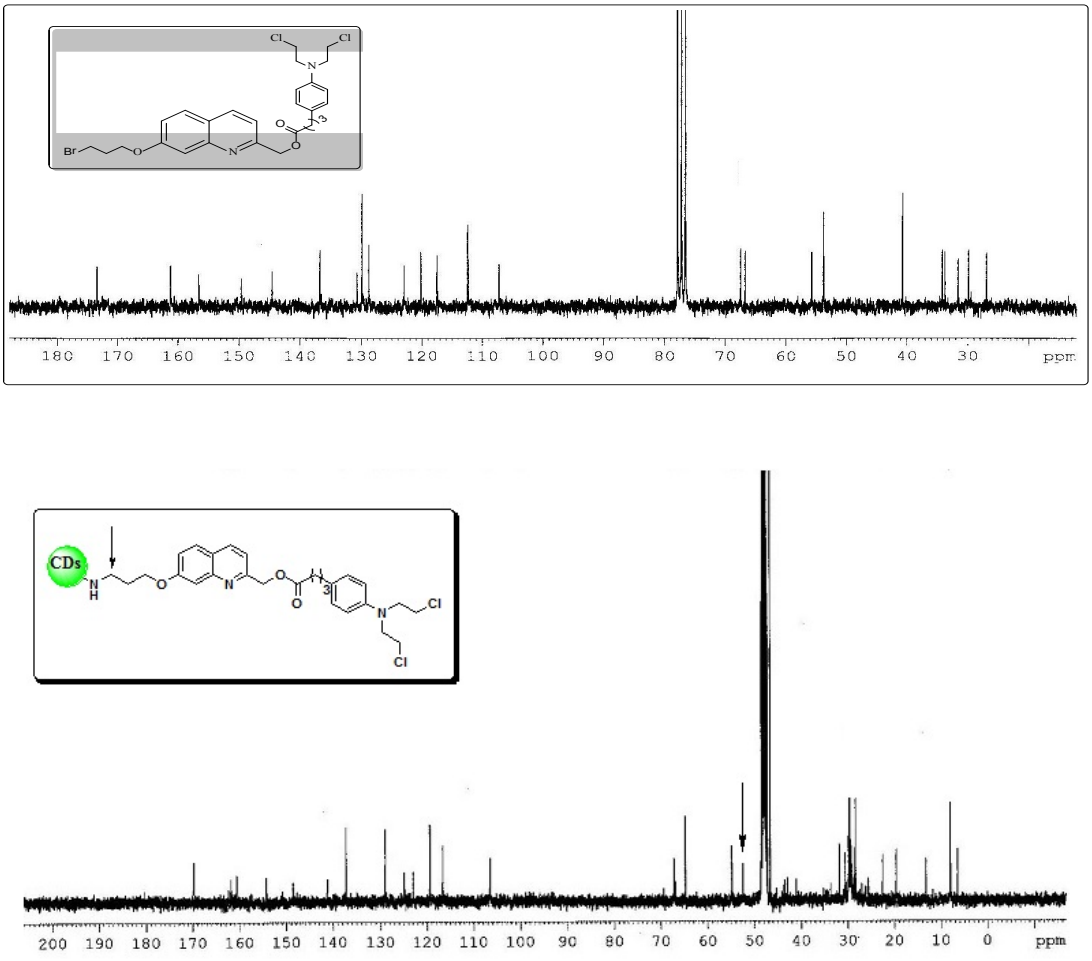


Figure S6: ^{13}C NMR spectra of Cdots and Qucbl-Cdots in 100 MHz

g) HPLC of carbon dots, quinoline chlorambucil and quinoline chlorambucil loaded carbon dots.

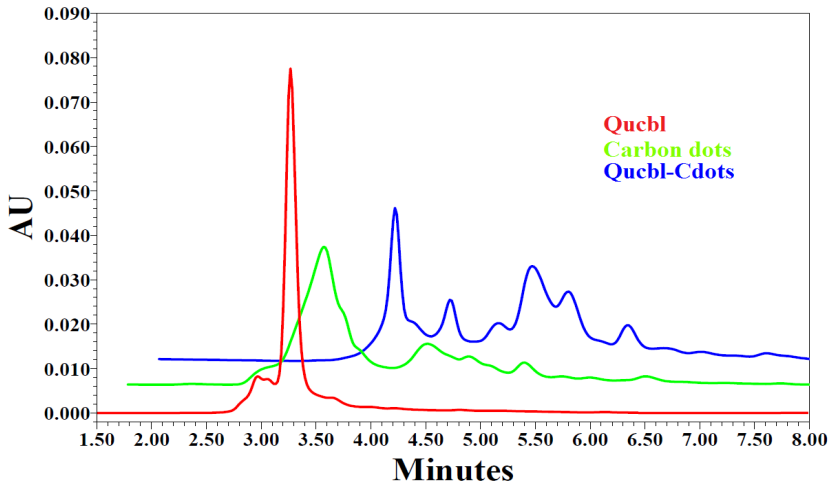


Figure S7: Overlay HPLC profile of Quinoline chlormbucil, Carbon dots and Quinoline chlorambucil loaded carbon dots in reverse phase HPLC using methanol/ water (8:2 v/v)), at a flow rate of 1 mL / min.

h) MALDI-TOF of Qucbl-Cdots and HRMS of Cdots

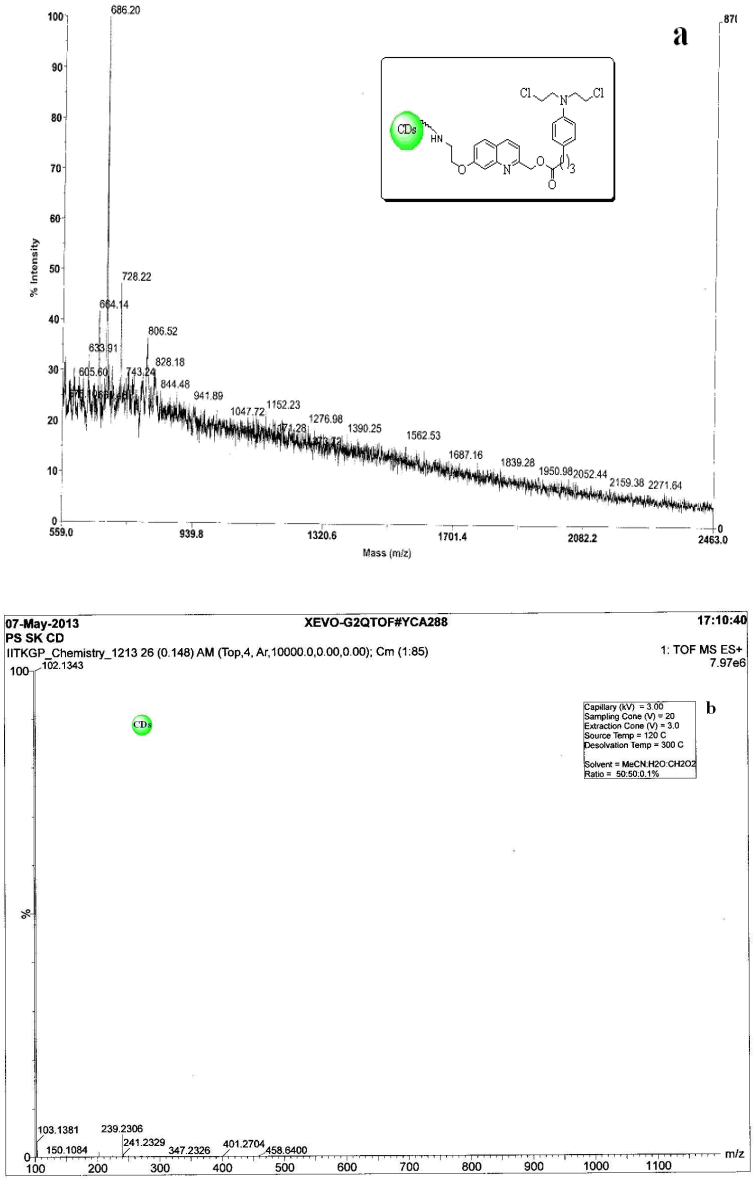
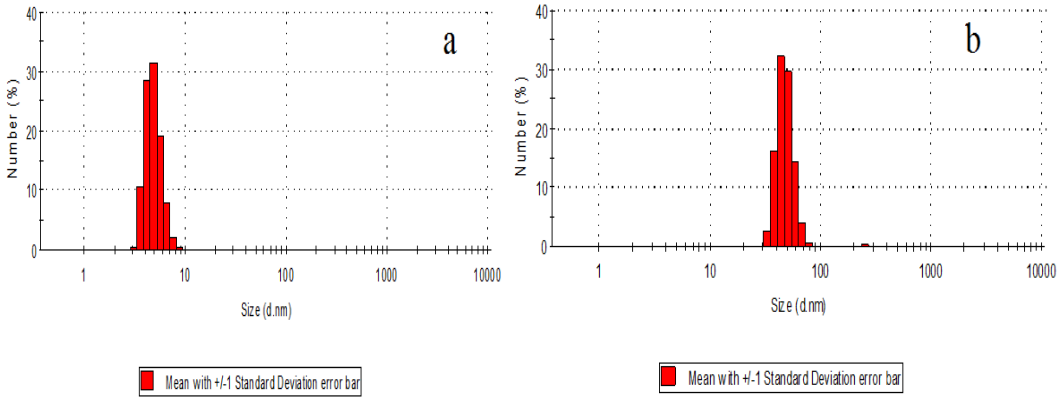


Figure S8: a)MALDI-TOF of Qucbl-Cdot, b) HRMS of Cdots.

4. Physicochemical properties of Carbon dots and Qucbl-Cdots

a) Dynamic Light Scattering (DLS) of Carbon dots and Qucbl-Cdots



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.

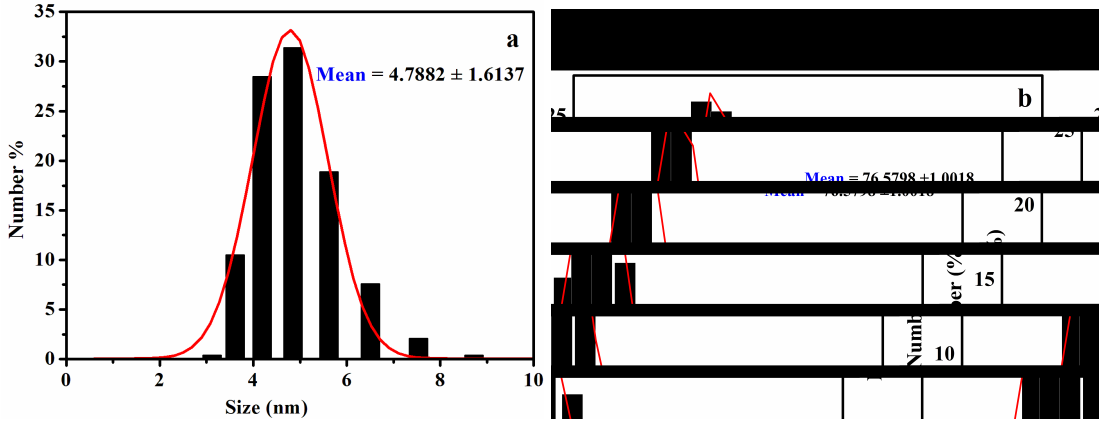


Figure S9: particle size distribution graph of (a) Carbon dots (b) Qucbl loaded Carbon dots as revealed by DLS.

b) XRD Spectra of Carbon-dots (Cdots)

The XRD patterns of the Cdots also displayed a broad peak centered at 27.5°, which is also attributed to highly disordered carbon atoms. Similar to the graphite lattice spacing.[2]

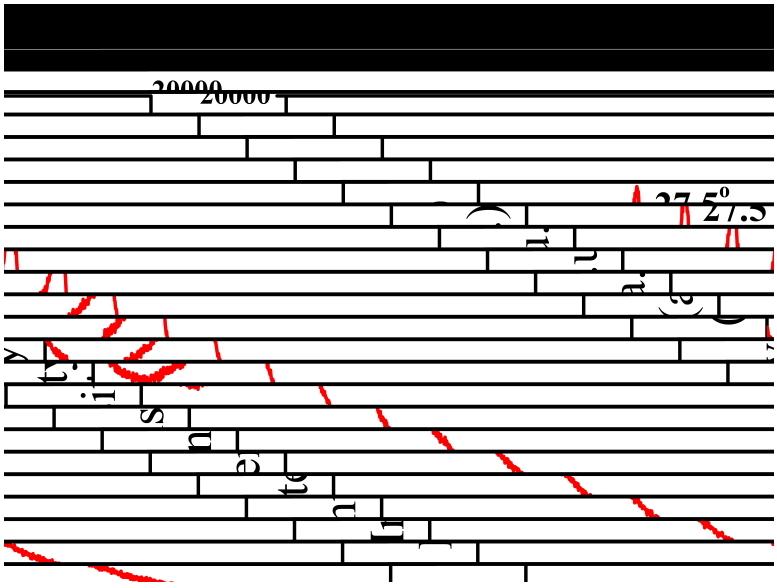


Figure S10: The XRD patterns of the Cdots.

c) TGA of Carbon dots and quinoline chlorambucil loaded carbon dots (Qucbl-Cdots)

By comparing the thermograms, it is evident that Qucbl-Cdots volatilizes at a higher temperature compared to Carbon dots indicating that Qucbl is loaded on the carbon dots.

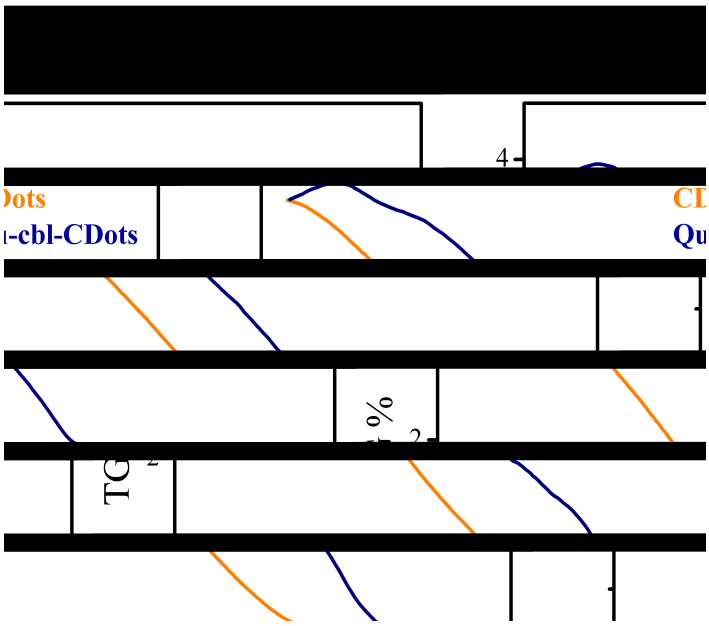


Figure S11: TGA spectra of Cdots and Qucbl-Cdots.

d) Zeta potential of carbon dots and quinoline chlorambucil loaded carbon dots in water

The Zeta potential of as prepared nitrogen functionalized carbon dots in aqueous solution were +65.3 mV, owing to the presence of abundant N-containing groups on the surface.[1] Once the quinoline chlorambucil is attached with the carbon dots the zeta potential is reduced to + 31.12 mV, because of the less available N-containing group on the surface, indicating attachment of Qu-Cbl on the surface of carbon dots

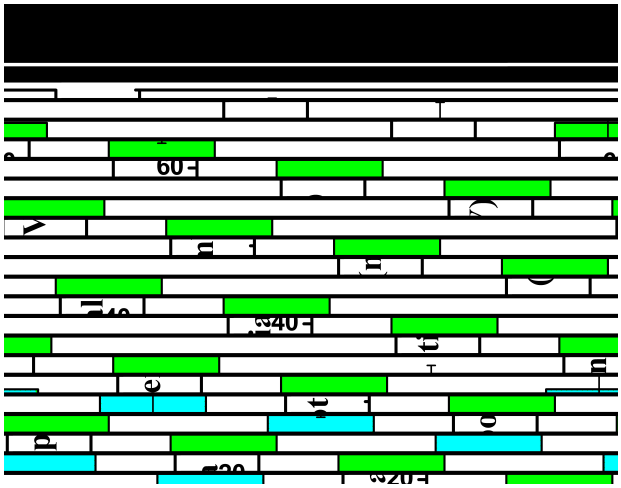
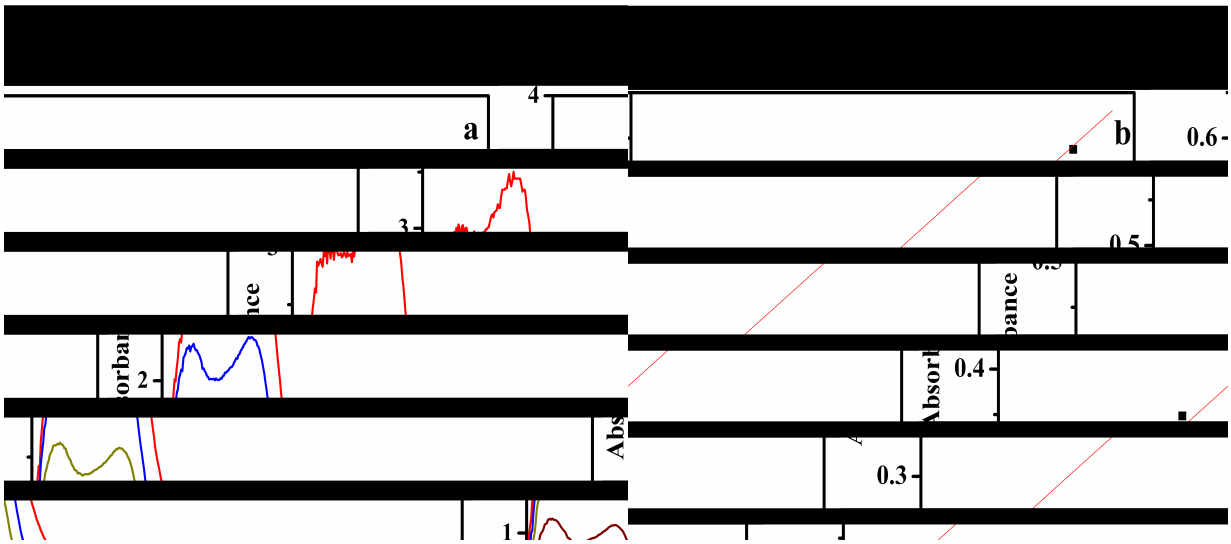


Figure S12: Zeta potential of carbon dots and Qucbl-Cdots

e. Loading of Quinoline chlorambucil on Carbon dot by absorption spectra.



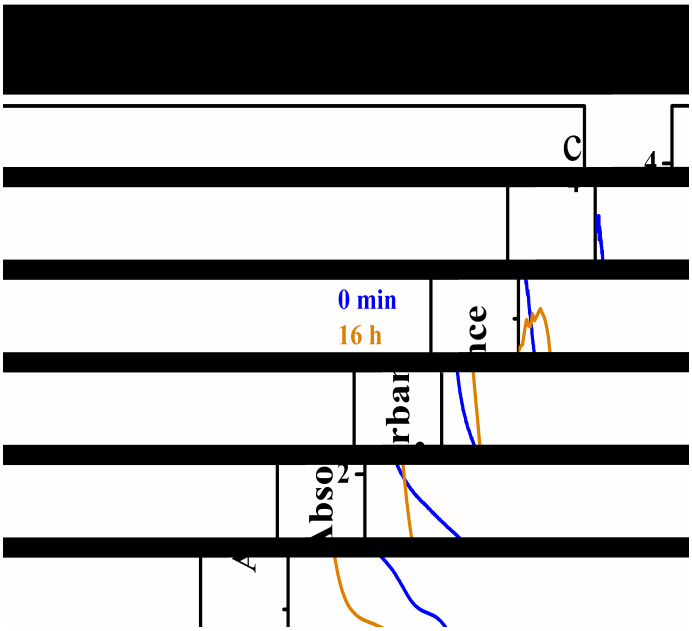


Figure S13: (a) Standard UV-vis absorption spectra of Quinoline chlorambucil (b) calibration curve for the concentration of quinoline chlorambucil. (c) UV-vis absorption spectra of Quinoline chlorambucil loaded on carbon dots.

The concentration remain in the reaction mixture was calculated by the absorption spectra = 0.29190×10^{-5}

Initial concentration of the reaction mixture = 3.4364×10^{-4}

Quinoline chlorambucil loaded on carbon dots

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

= $3.4364 \times 10^{-4} - 0.29190 \times 10^{-5}$ mol/mL

= 3.1445×10^{-4} mol/mL X M.wt of Qucbl

= 0.1830×10^{-4} g of Qucbl in 50 mg of carbon dots

= 3.6601×10^{-5} g in 1mg of carbon dots

~ 36µg/mg of qucbl loaded on the carbon dot.

5. Hydrolytic Stability of Qucbl-Cdots at pH 7.4:

1 mL of 2×10^{-4} M solution of Qucbl-Cdots was added in PBS containing 10% fetal bovine serum with pH = 7.4. All the tubes were kept in ultrasonic for 10 min to make the solutions homogeneous and stored at 37 °C in dark condition for 96 h. Then all the solutions were analyzed by reverse phase HPLC to examine the remaining percentage of the Qucbl-Cdots.

Photoresponsive nanocarrier	Time (day)	% of Qucbl-Cdots depleted	
		(pH 7.4) PBS	(pH 7.4) FBS
Qucbl- Cdots	4	2	4

Table S2: The remaining percentage of Qucbl- Cdots in dark condition at pH 7.4

6. Photolysis of Qucbl-Cdots using soft UV irradiation (≥ 365 nm) and He-Ne laser (632 nm):

a) Photolysis of Qucbl-Cdots using soft UV irradiation (≥ 365 nm):

Determination of photochemical quantum yield of Qu-cbl and Qucbl-Cdots (Φp)[3]. These experiments were carried out using a previously described method. 1 mg of Qucbl and Qucbl-Cdots was dissolved in 1 mL of methanol / water (1:1 v/v) mixture in quartz cuvettes, individually. They were irradiated under UV light by 125 W medium pressure Hg vapor lamp using a suitable filter 1 M $CuSO_4$ solution in 0.1 N H_2SO_4, the transmittance for the above filter = 365 to 500 nm). We have incorporated the above information in the revised manuscript. At regular interval of time, 20 µL of the aliquots was taken and analyzed by RP-HPLC using mobile phase methanol, at a flow rate of 1 mL/min (detection: UV 254 nm). Peak areas were determined by RP-HPLC, which indicated gradual decrease of the caged compound with time, and the average of three runs. The reaction was followed until the consumption of the caged compound is less than 5% of the initial area. Based on HPLC data for each caged compounds, we plotted normalized [A] (HPLC peak area) versus irradiation time.We observed an exponential correlation for the disappearance of the caged compounds, which suggested a first order reaction. Further, the quantum yield for the photolysis of caged compounds was calculated using below equation.

$$\phi_p = \frac{(k_p)_{CP}}{I_0 (F_{CP})}$$

where, the subscript 'CP' denotes caged compound. Fp is the photolysis quantum yield, kp is the photolysis rate constant, and I_0 is the incident photon flux and F is the fraction of light absorbed. Potassium ferrioxalate was used as an actinometer.

Cage compound	Quantum yield (Φp)
Qucbl	0.29
Qucbl-Cdots	0.17

Photochemical quantum yield (error limit within ± 5%).

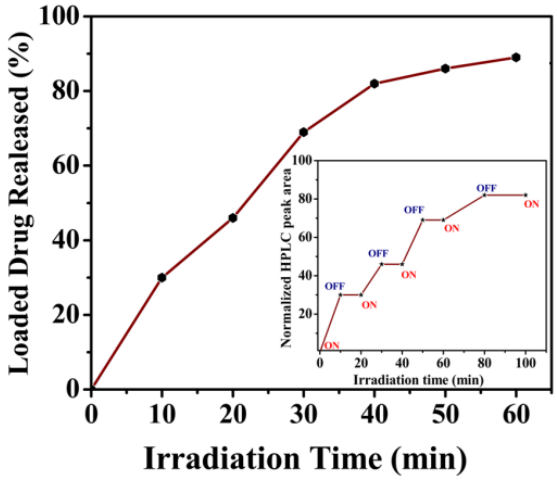


Figure S14. HPLC profiles of percentage of loaded chlorambucil released from Qucbl-Cdots under UV light (≥365 nm), Inset is the partial progress for the release of chlorambucil under bright and dark conditions. "ON" indicates the beginning of light irradiation; "OFF" indicates the ending of light irradiation

b) Photolysis of Qucbl-Cdots using Red laser: 1 mg of Qucbl-Cdots was dissolved in 1ml of methanol / water (1:1 v/v) mixture. Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated using He-Ne laser (5 mW/ cm^2). At regular time intervals, a small aliquot (100 µL) of the suspension was taken out and analyzed by reversed-phase HPLC using mobile phase methanol/ water (8:2 v/v), at a flow rate of 1 mL / min.

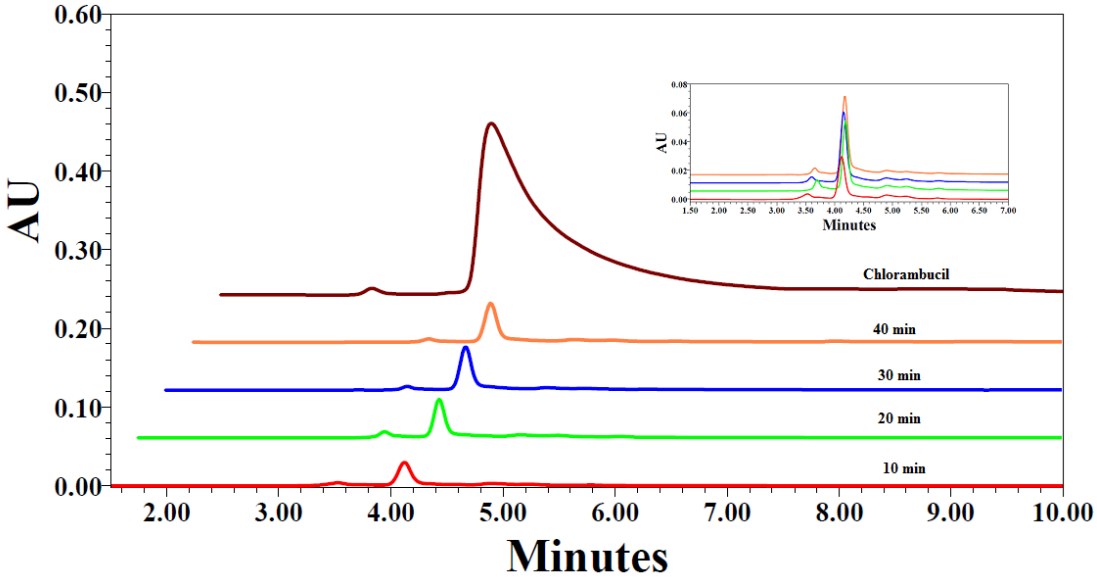


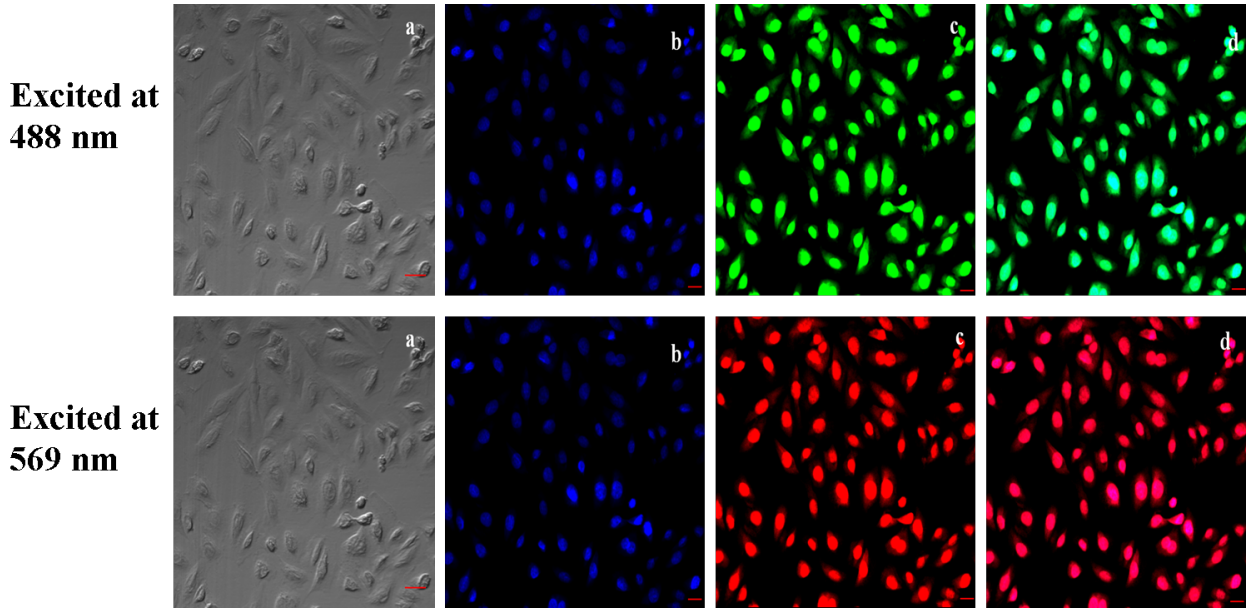
Figure S14a:HPLC profile of Chlorambucil release from Qucbl-Cdot using Red leaser. The *y-axes* were offset by 40 mAU units and the *x-axes* were offset by 10 s, to facilitate better visualization.

Photolysis image of Qucbl-Cdots by using He-Ne leaser.

7. Cell Imaging and Cytotoxicity of quinoline chlorambucil loaded carbon dots and carbon dots on HeLa cell line:

a. Qucbl-Cdots for cell imaging studies using HeLa cell line: Cell imaging studies was carried out using the HeLa cell line obtained from National Centre for Cell Science (NCCS) which was maintained in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS) at 37 ^{o}C and 5 % CO_2. To study the cellular uptake of Qucbl-Cdots, briefly HeLa cells (5×10^4 cells/well) were plated on 12 well plates and allowed to adhere for 4–8 hours. Cells were then incubated with 3×10^{-5} M both the compounds separately in cell culture medium for 4 h at 37 ^{o}C and 5 % CO_2. Thereafter, cells were washed two times with PBS. Imaging was done in Olympus confocal microscope (FV1000, Olympus) using the respective filter.

b. Nuclear Co-localization Studies using Qucbl-Cdots and a nuclear staining dye DAPI (4,6 Di amidino-2-phenylindole, Dihydrochloride) : Cells, grown and plated as described above, were incubated for 4 h at 37 °C with 1 ml of in complete DMEM containing 30 µM of Qucbl-Cdots. Thereafter, cells were washed 3 times with 1X PBS at room temperature. The cells were counterstained with 5 µg/mL of DAPI at room temperature in the dark for 5 mins. After gentle washing in 10 X PBS for 3 times the cells were viewed under confocal microscope.



FigureS15: Confocal images of HeLa cells: (a) brightfield image (b) Cells nuclear were stained by DAPI, (c) showing the uptake of Qucbl-Cdots (λex = 488 nm shows Green emission and λex =569 nm shows red emission in cellular medium) (d) overlay image of b and c showing both Qucbl-Cdots and DAPI are located at the cell nuclei (Scale bar = 20 µm).

c. Cytotoxicity of quinoline chlorambucil loaded carbon dots and carbon dots on HeLa cell line

1. Cytotoxicity before photolysis: The cytotoxicity in vitro was measured using the MTT (3–(4,5–dimethylthiazol–2–yl)–2,5–diphenyltetrazolium bromide, a yellow tetrazole) assay on HeLa cell line. Briefly, cells growing in log phase were seeded into 96–well cell–culture plate at 1×10^4 cells/mL. Different concentration of Qucbl-Cdots, Cdots and chloambucil were added in the wells with an equal volume of PBS in the control wells. The cells were then incubated for 72 h at 37 °C in 5% CO_2. Thereafter, fresh media containing 0.40 mg/ml MTT were added to the 95

well plates and incubated for 4 h at 37 °C in 5% CO_2. Formazan crystals thus formed were dissolved in DMSO after decanting the earlier media and absorbance recorded at 595 nm.

2. Cytotoxicity after 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 different concentration (0.5, 1, 5, 10, 15 and 20 μM) of Qucbl-Cdots, Cdots and chloambucil 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 irradiated by UV light (≥365 nm) using 125 W medium pressure Hg lamp with a suitable filter (1 M $CuSO_4$ solution in 0.1 N H_2SO_4). After irradiation the cells were again incubated for 72 h. Then cytotoxicity was measured using the MTT assay as described in the section c.1.

3. Externally light regulated cytotoxicity: 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). The HeLa cells incubated with 2×10^{-5} M Qucbl-Cdots was incubated for 4 h at 37 ^oC and 5 % CO_2. Then the cells were irradiated for different time intervals (keeping the cell-culture plate 5 cm apart from the light source) using 125 W medium pressure Hg lamp as irradiation source (≥ 365nm) and 1M $CuSO_4$ solution in 0.1 N H_2SO_4 as UV cut-off filter. After irradiation the cells were again incubated for 72 h. Then cytotoxicity was measured using the MTT assay as described in the the section c.1. We found that the cytotoxicity toward HeLa cells at the irradiation time of 30 min showed the highest level of toxicity (about 15% in comparison to the control), indicating that most of the drugs were released from the Qucbl-Cdots (Figure S16).

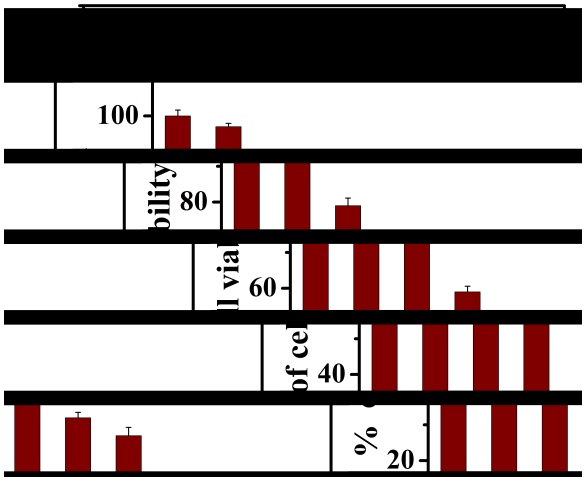


Figure S16: Cell viability test of Qucbl–Cdots in HeLa cell line after regular time intervals of irradiation in presence of 3×10^{-5} M Qucbl-Cdots.

b) Cell cycle analysis by FACS:

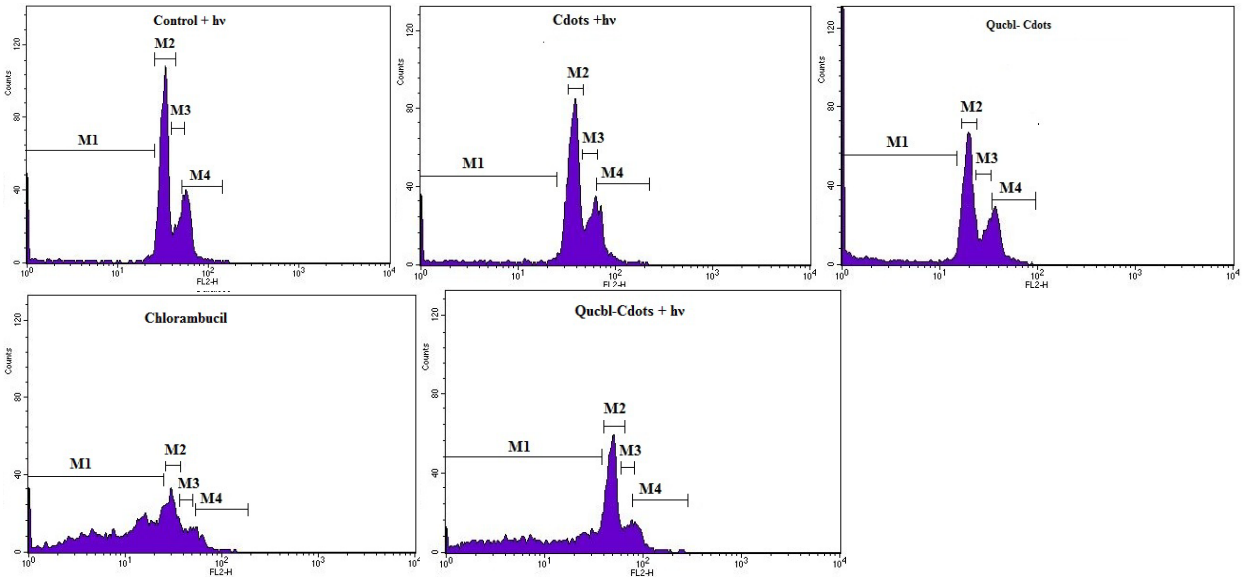


Figure S17: Cell cycle analysis by FACS calibur for 48 h after treatment to UV light for 30 min. Percentage of apoptotic cells are indicated as the proportion of cells that contained sub-G1 phase.

References:

1. S. Mandal, C. Ghatak, V.G. Rao, S.Ghosh, N. Sarkar. *J.Phys.Chem.C* **2012**, 116, 5585−5597. S. Zhu; Q. Meng; L. Wang; J. Zhang; Y. Song; H. Jin; K. Zhang; H. Sun; H. Wang; B.Yang. *Angew. Chem. Int. Ed*. **2013**, 52, 3953 –3957.
2. Pin-Che Hsu and Huan-Tsung Chang; *Chem. Commun*., **2012**, 48, 3984–3986
3. Yue Zhu, Christopher M. Pavlos, John P. Toscano,Timothy M. Dore. *J.Am.Chem.Soc.* **2006**, 128, 4267-4276; Jana, A.; Atta, S.; Sarkar, S. K.; Singh, Pradeep N. D. *Tetrahedron,* **2010,**52, ,9798-9807 **.**