Supporting information

Quinoline H2S donor Decorated Fluorescent Carbon Dots: Visible Light Responsive H2S Nanocarriers

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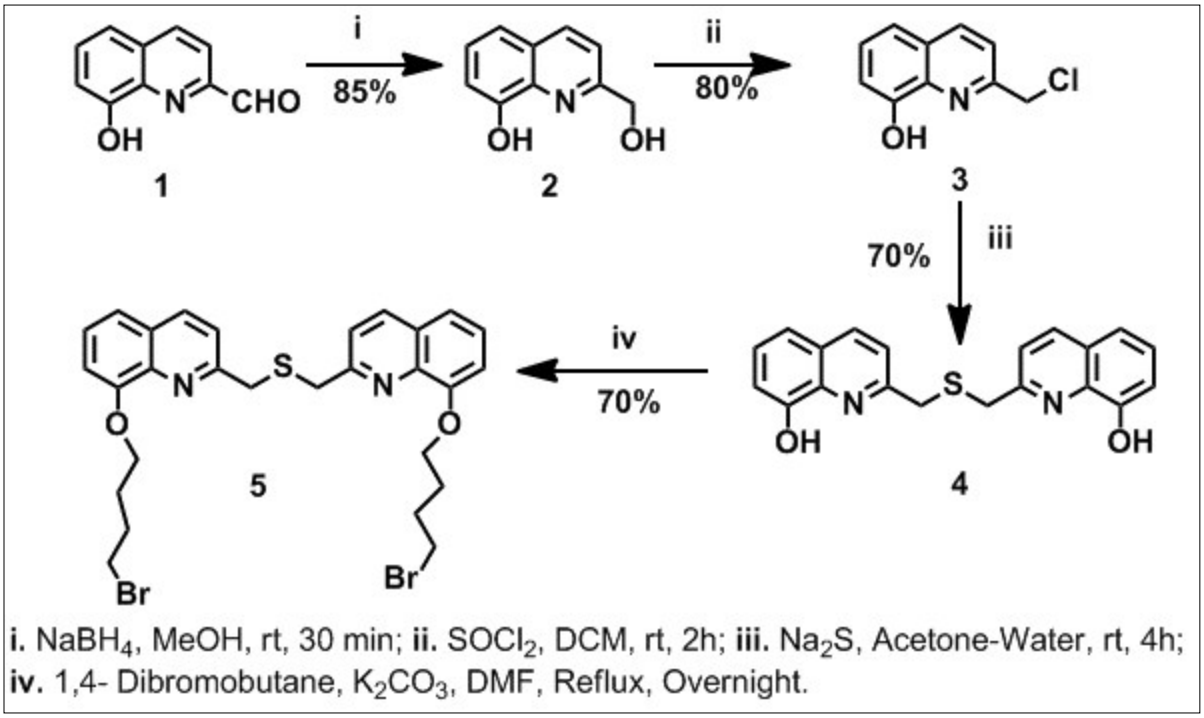
1. General scheme for the synthesis of the Quinoline H_2S donor (5).



i. $NaBH_4$, MeOH, rt, 30 min; **ii.** $SOCl_2$, DCM, rt, 2h; **iii.** Na_2S, Acetone-Water, rt, 4h; **iv.** 1,4- Dibromobutane, K_2CO_3, DMF, Reflux, Overnight.

Scheme S1 Synthetic scheme of the quinoline H_2S donor **QuH_2S** (**5**).

2. Characterization of synthesized molecules and photoproduct by NMR Spectroscopy:

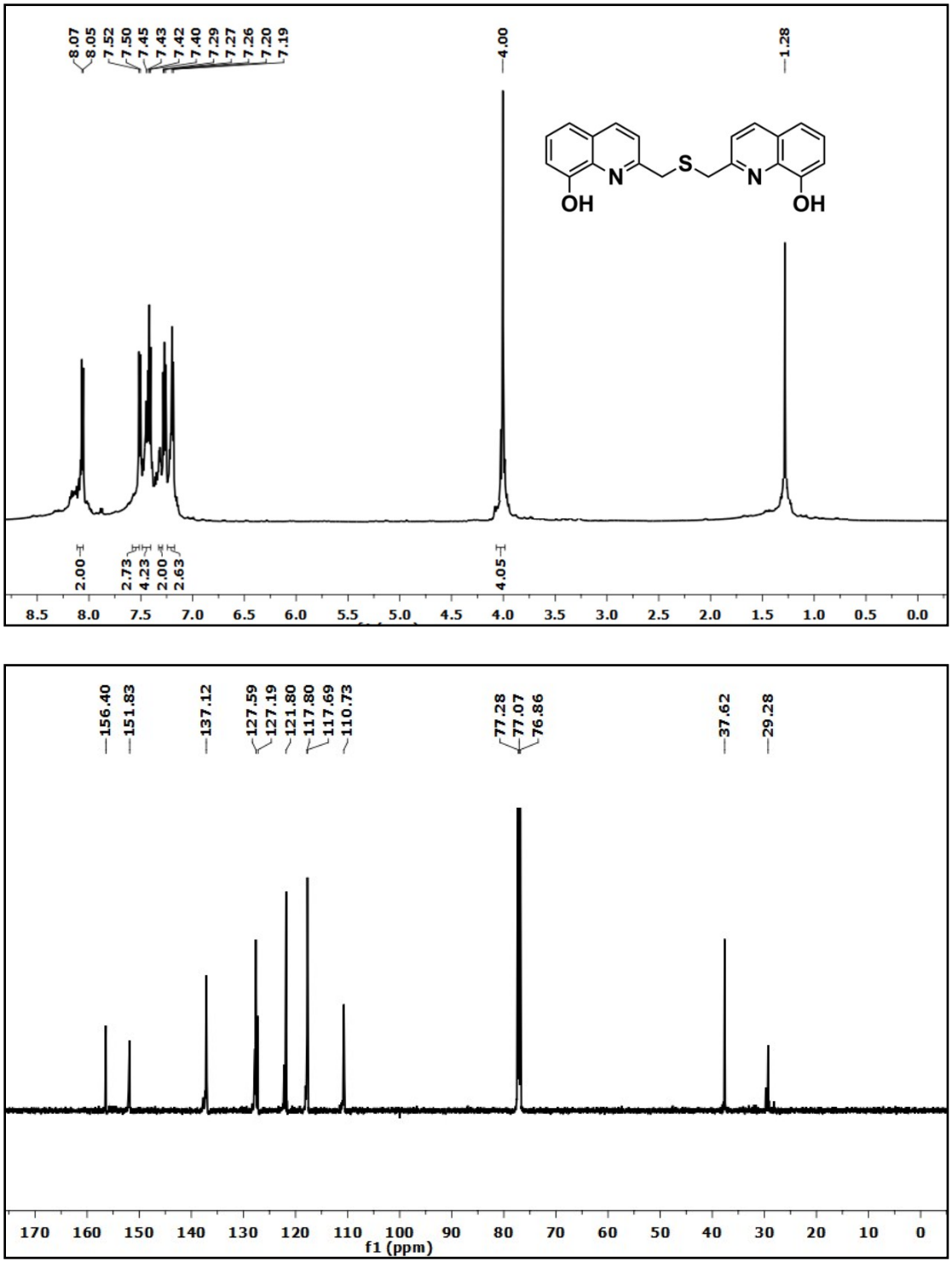


Fig. S1. ^{1}H and ^{13}C NMR spectra of **4** in $CDCl_3$.

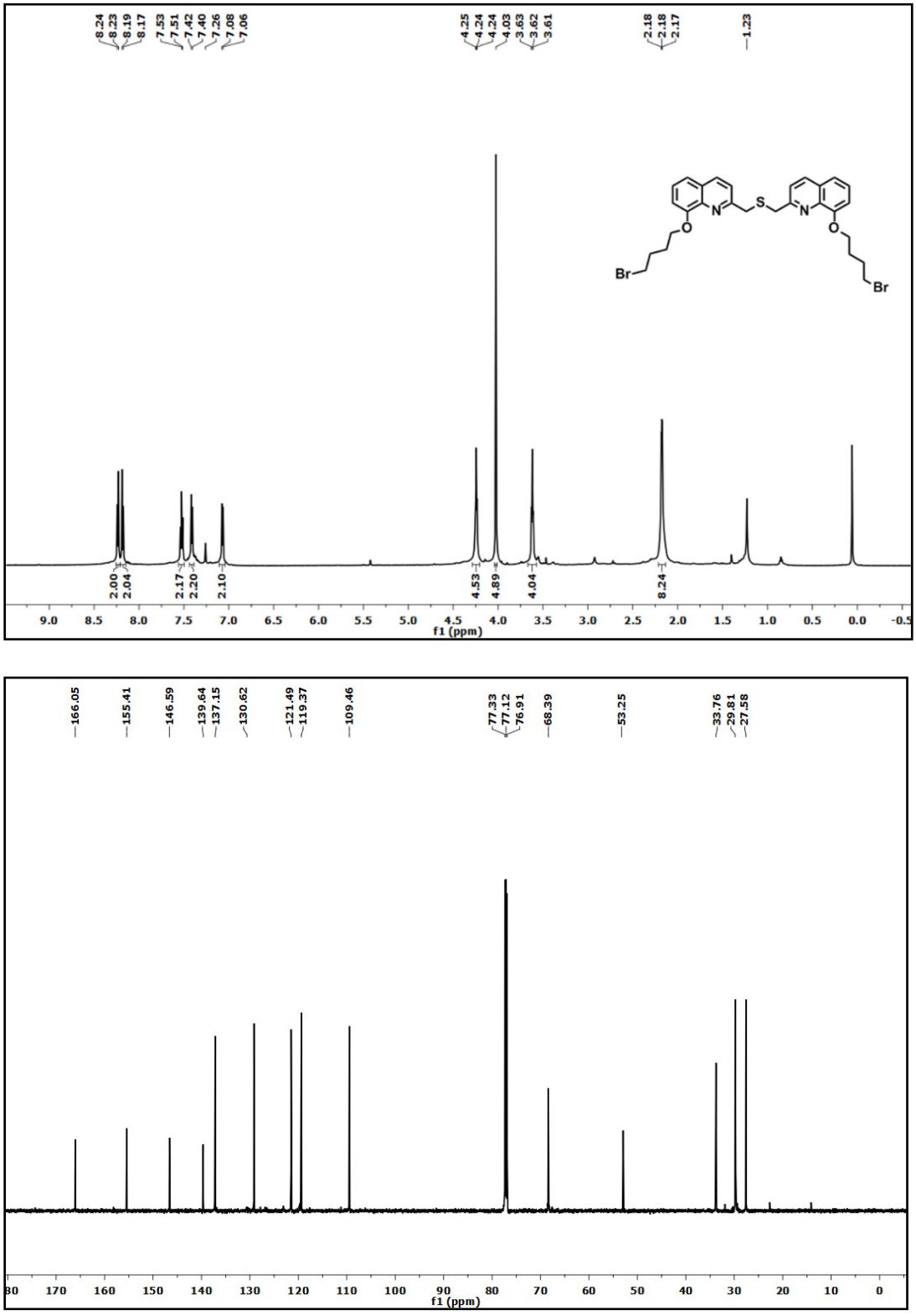


Fig. S2. ^{1}H and ^{13}C NMR spectra of **5** in $CDCl_3$.

3. Measurement of fluorescence quantum yield

The fluorescence quantum yield (QY) of the carbon dots (CDs) and QuH_2S-CDs were determined by the reference point method.[1] Quinine sulphate in 0.1 M H_2SO_4 (literature quantum yield: 54 %) was used as a standard sample to calculate the QY of CDs and QuH_2S-CDs, which were dissolved in methanol. The absorbance values of the solutions at the excitation wavelength were measured by 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 410 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 the area under the fluorescence curve, and **η** is the refractive index of the medium. The subscripts **S** and **R** denote the corresponding parameters for the sample and reference, respectively.

4. Determination of incident photon flux (I_0) of the UV lamp by potassium ferrioxalate actinometry:

Potassium ferrioxalate actinometry was used for the determination of incident photon flux (I_0) of the UV lamp used for irradiation. Solution of potassium ferrioxalate, 1,10- phenanthroline and the buffer solution were prepared following the literature procedure.[2] 0.006 M solution of potassium ferrioxalate was irradiated using 125 W medium pressure Hg lamp with 1 M $NaNO_2$ solution (UV cut-off filter), as visible light source (≥ 410 nm). At regular interval of time (3 min), 1 mL of the aliquots was taken out and to it 3 mL of 1,10-phenanthroline solution and 2 mL of the buffer solution were added and the whole solution was kept in dark for 30 min. The absorbance of red phenanthroline-ferrous complex formed was then measured spectrophotometrically at 510 nm. The amount of Fe^{2+} ion was determined from the calibration graph. The calibration graph was plotted by measuring the absorbance of phenanthroline-ferrous complex at several known concentration of Fe^{2+} ion in dark. From the slope of the graph the molar absorptivity of the phenanthroline-ferrous complex was calculated to be 1.10 × 104 M^{-1} cm^{-1} at 510 nm which is

found to be similar to reported value. Using the known quantum yield (1.188 ± 0.012) for potassium ferrioxalate actinometer at 406.7 nm, the number of Fe^{2+} ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident intensity (I_0) of the 125 W Hg lamp was determined as 2.886 x 10^{16} quanta s^{-1}.

5. Measurement of photochemical quantum yield for QuH_2S-CDs (6):

A solution of 4 mg of the H_2S donor **6** was prepared in 20 mL ACN/PBS buffer (3:7) and mixed with methylene blue cocktail (as described in "Methylene Blue Assay for H_2S Quantification" below). Half of the solution was kept in dark and to the remaining half nitrogen was passed and irradiated (keeping the quartz cuvette 5 cm apart from the light source) using 125 W medium pressure Hg lamp as UV light source (≥ 410 nm) and 1 M $NaNO_2$ solution as UV cut-off filter with continuous stirring for 50 min. At a regular interval of time (10 min), 2 mL of the aliquots were taken and analyzed by UV-Vis spectroscopy. The amount of H_2S released was calculated from the methylene blue assay. Based on it, we plotted the amount of H_2S released versus irradiation time. We observed an exponential correlation for the release of H_2S which suggested a first order reaction kinetics. Further, the photochemical quantum yield (Φ_p) was calculated using below equation (1).

$$(\Phi)_{CG} = (\Phi)_{act} \times [(k_p)_{CG}/(k_p)_{act}] \times [F_{act}/F_{CG}] \text{ ---------- (1)}$$

Where the subscript 'CS' and 'act' denotes caged substrate and actinometer respectively. Ferrioxalate was used as an actinometer. Φ_p is the photolysis quantum yield, k_p is the photolysis rate constant and F is the fraction of light absorbed.

6. Zeta Potentials of CDs and QuH_2S-CDs:

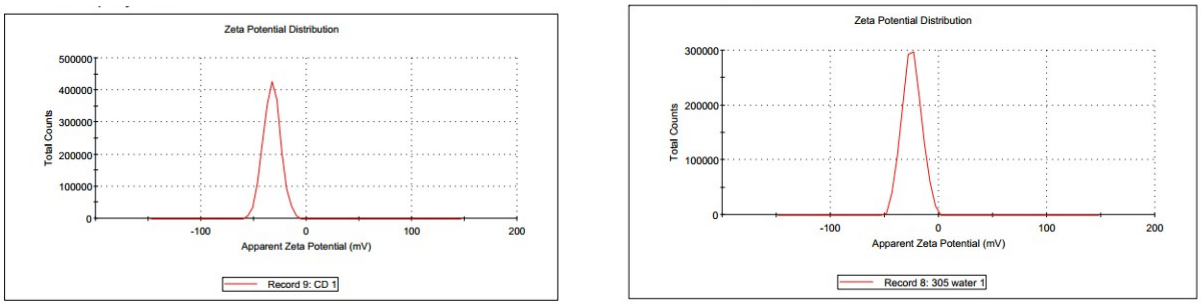


Fig. S3. Zeta Potentials of CDs and QuH_2S-CDs

7. Tunable Emission Property of Quinoline-Carbon Dots (QuH_2S-CDs) (6):

Aqueous suspensions of QuH_2S-CDs exhibited emission spectra in the visible region with the maxima strictly depending on the excitation wavelength **(Fig. S4).** [3]

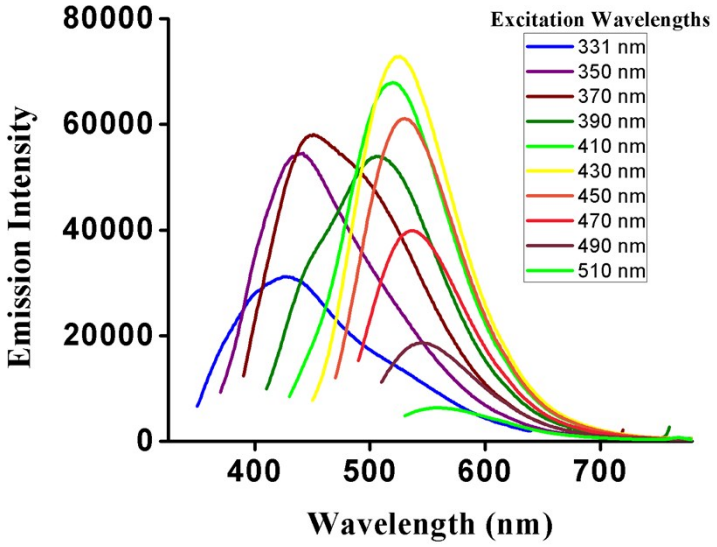


Fig. S4. Emission spectra of QuH_2S-CDs (**6**) with different excitation wavelength.

8. Control experiment for H_2S release in the presence of the H_2S selective fluorescent probe (D):

We performed the control experiment with CDs and QuH_2S in the presence of the H_2S selective fluorescent probe D (**Fig. S7**). We observed that CDs showed no effect and QuH_2S showed very little effect in the given condition i.e., in aqueous PSB buffer medium at $\lambda \geq 410$ nm. This proves that the effect shown by our system during H_2S detection experiment is really attributed to the QuH_2S-CDs. The control experiment with CDs and QuH_2S in the presence of the H_2S selective fluorescent probe D is provided as **Fig. S7** and discussed in the section ….

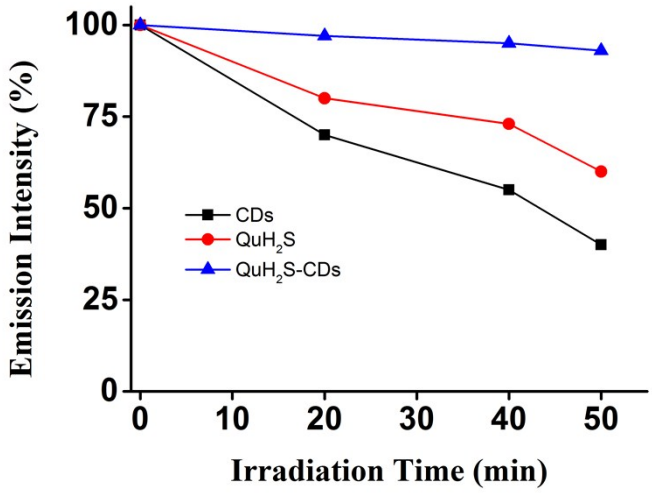


Fig. S5 Change in the fluorescence intensity of fluorescent probe D in presence of CDs, QuH_2S, QuH_2S-CDs at different irradiation ($\lambda \geq 410$ nm) time intervals in aqueous PBS buffer medium.

9. Hydrolytic stability of QuH_2S-CD in biological environment:

We thank the reviewer for this valuable suggestion. To check the stability of QuH_2S-CD in the presence of reactive cellular species, such as cysteine and glutathione, in the biological environment, we prepared the solution of QuH_2S-CD (5 mg/mL) in PBS buffer containing 10 % fetal bovine serum (FBS) at pH = 7.4 and added the solution of thiols (5 mM) (e.g; N-acetyl cysteine (NAC-SH) and GSH) separately to it. After 7 days, aliquots were taken from each sample and the UV-Vis absorption spectra were recorded. The results showed that the photodecomposition of QuH_2S-CD was negligible. Hence, in the presence of cellular thiols (cysteine and GSH) our designed QuH_2S-CD donors exhibited sufficient stability and could be suitable for biological applications.

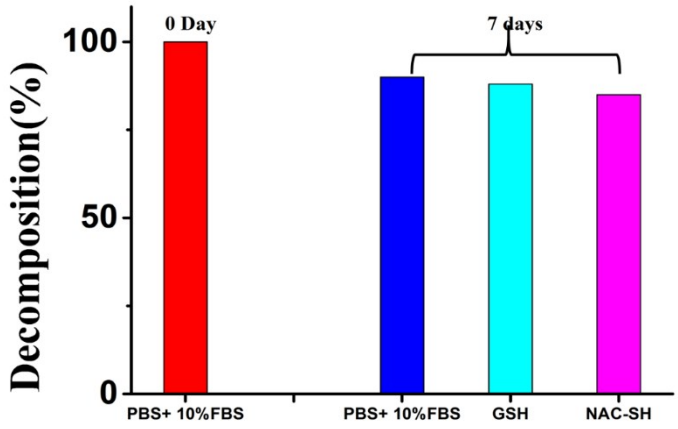


Fig. S6 Decomposition of QuH_2S-CD donors (1 mg/ mL) in PBS buffer containing 10 % fetal bovine serum (FBS) at pH = 7.4, NAC–SH and GSH (5 mM) at pH = 7.4 during testing the hydrolytic stability for 7 days.

10. Characterization of adduct (A) formed during H_2S detection:

We carried out the experiment using Na_2S as a standard H_2S donor with the dye (D) and observed the changes both in UV-Visible and emission spectroscopy. We isolated the adduct (A) and recorded the UV-Vis and emission spectra of the adduct A. We found the spectroscopic data of the adduct (A) matched with our experimental results.

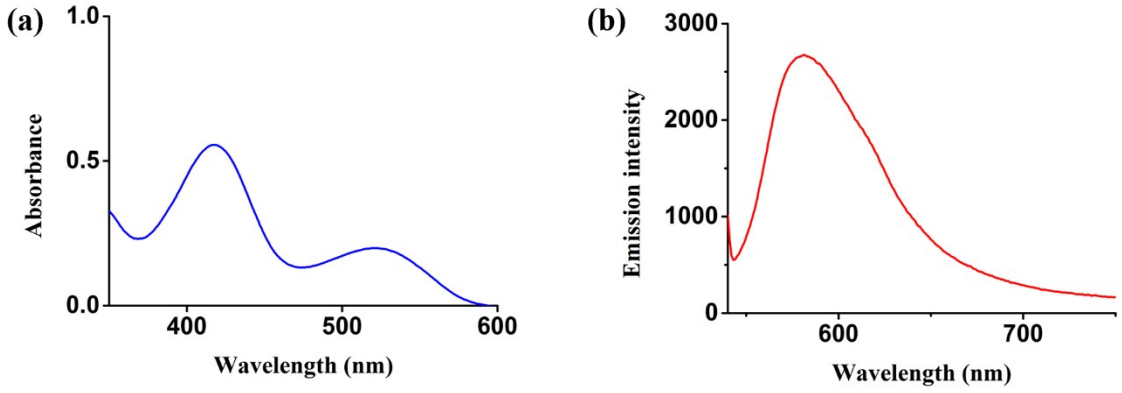


Fig. S7 UV-Visible (a) and emission (b) spectra of the Dye D.

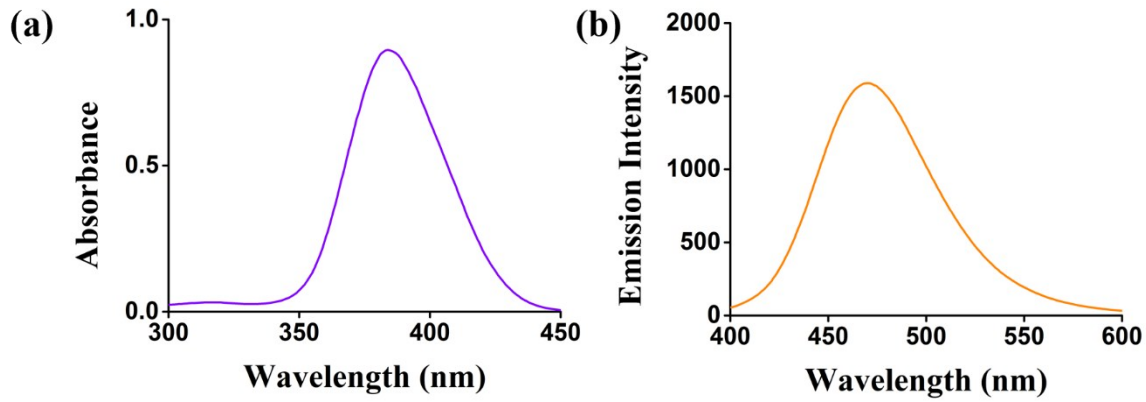


Fig. S8 UV-Visible (a) and emission (b) spectra of the Adduct A.

HRMS: calcd for the adduct (A) $C_{18}H_{22}N_2S_3$ $[M+ H]^+$, 362.0965; found: 362.0945.

11. Methylene Blue Assay for H_2S Quantification:

Methylene blue assay was carried out as described previously with some modifications.[4] A 5 mM solution of Na_2S in sodium phosphate buffer (20 mM, pH 7.4)/acetonitrile (HPLC grade) (7:3) was prepared ($Na_2S.9H_2O$, 120.20 mg in 100 mL volumetric flask) and used as the stock solution. Aliquots of 100, 200, 300, 500, 700, 1000 µL of the Na_2S stock solution were added into a 50 mL volumetric flask and dissolved in a mixture of sodium phosphate buffer/acetonitrile to obtain the standard solutions in 10, 20, 30, 50, 70, 100 µM, respectively. 1 ml aliquot of the respective solution was reacted with the methylene blue (MB+) cocktail: 30 mM $FeCl_3$ (400 µL) in 1.2 M HCl, 20 mM of N,N-dimethyl-1,4- phenylenediamine sulfate (400 µL) in 7.2 mM HCl, 1% w/v of $Zn(OAc)_2$ (100 µL) in H_2O at room temperature for at least 15 min (each reaction was performed in triplicate). The absorbance of methylene blue was measured at λ_{max} = 663 nm. To obtain the molar absorptivity of (MB+) a linear regression was plotted with the observed absorbance and concentration. Fig. S5. Standard Calibration curve with different concentration of Na_2S.

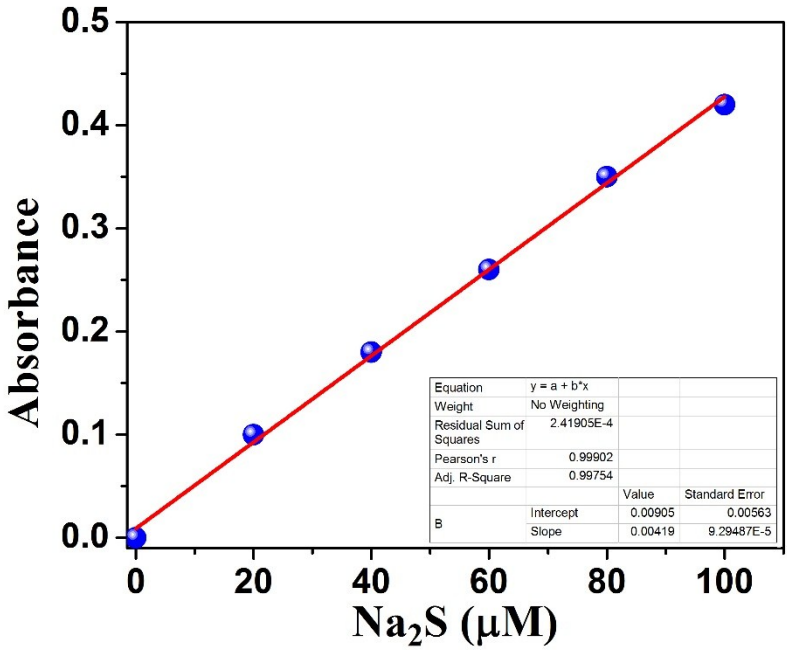


Fig. S9. Standard Calibration curve with different concentration of Na_2S.

In this experiment, a 100 μM solution (total volume 20 mL) of the compound **6** was prepared in a 7:3 solution of sodium phosphate buffer (20 mM, pH 7.4)/acetonitrile. This solution was placed in a 24 mL scintillation vial. The resulting reaction vessel was irradiated with a 125 W medium-pressure mercury lamp as the source of UV-Vis light ($\lambda \geq 365$ nm) using a suitable UV cut-off filter (1M $CuSO_4$ solution) with continuous stirring. The aliquot (1 mL) was collected at different time intervals (5, 10, 15, and 20 min) and was mixed immediately with the methylene blue cocktail: 30 mM $FeCl_3$ (200 μL) in 1.2 M HCl, 20 mM of N,N-dimethyl-1,4- 11 phenylenediamine sulfate (200 μL) in 7.2 mM HCl, 1% w/v of $Zn(OAc)_2$ (100 μL) in H_2O at room temperature for at least 20 min. The absorbance of methylene blue was measured at λ_{max}= 663 nm against a blank: 30 mM $FeCl_3$ (400 μL) in 1.2 M HCl, 20 mM of N,Ndimethyl-1,4- phenylenediamine sulfate (400 μL) in 7.2 mM HCl, 1% w/v of $Zn(OAc)_2$ (100 μL) in H_2O, ACN (500 μL), 20 mM sodium phosphate buffer pH 7.4 (500 μL).

12. Determination of the percentage of H_2S release from QuH_2S-CDs:

In our current study, we have quantified the amount of H_2S released from 0.2 mg/mL of QuH_2S-CDs by methylene blue assay using the below equation:

$$\text{Percentage of } H_2S \text{ released} = \frac{\text{Amount of } H_2S \text{ released}}{\text{Amount of } QuH_2S \text{ loaded on the surface of CDs}} X100$$

We found the amount of H_2S released from 0.2 mg/mL of QuH_2S-CDs by methylene blue assay was 40 µM.

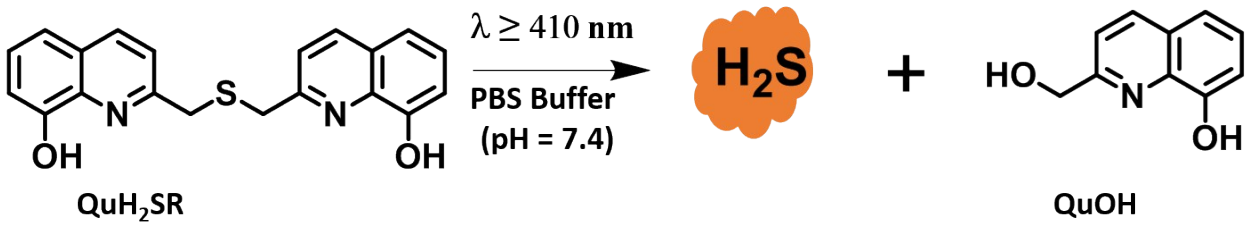
Also we carried out the loading study of QuH_2S on CDs using UV-Visible spectroscopy according to the literature procedure[5] and found 65 µg/mg of QuH_2S loaded on the carbon dot.

Now by using the above equation, we have quantified that ~ 60% of H_2S released from 0.2 mg/mL of QuH_2S-CDs.

13. Characterization of photoproduct (QuOH-CD) via parallel experiment:

Our designed system QuH_2S-CDs is the light responsive H_2S releasing moiety, which produces QuOH as a photoproduct after the release of H_2S. But characterisation of the photoproduct is very difficult as because there also CD was decorated with the photoproduct.

So that we performed a parallel experiment with a similar type of model system QuH_2SR and monitor the photorelease experiment in the aqueous PBS buffer (pH ~7.4) medium as same reaction condition we used for the QuH_2S-CD. We isolated the photoproduct and characterized by 1H-NMR and HRMS. We found the photoproduct matched with QuOH which helps us understand the photorelease mechanism for our carbon dot attached quinoline system.



Scheme 2 Photorelease of H_2S from QuH_2S-CDs triggered by visible light in PBS buffer (pH~7.4) medium.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (d, J = 8.8 Hz, 1H), 7.62 (d, J = 8.8 Hz, 1H), 7.47 (t, J = 7.8Hz,1H), 7.34 (d, J = 8.3 Hz, 1H), 7.20 (d, J = 7.8 Hz, 1H), 4.83 (s, 2H).
HRMS (ESI+) calcd for $C_{10}H_9NO_2$ $[M+ H]^+$, 175.0633; found: 175.0640.

Hence the increase in the fluorescence of the products can be attributed to the increase in hydrophilicity of the system after the formation of the photoproduct, quinolin-2-ylmethanol-CDs (QuOH-CDs), due to the presence of hydroxyl (hydrophilic) group.

14. Confocal imaging for H_2S release using fluorescence probe:

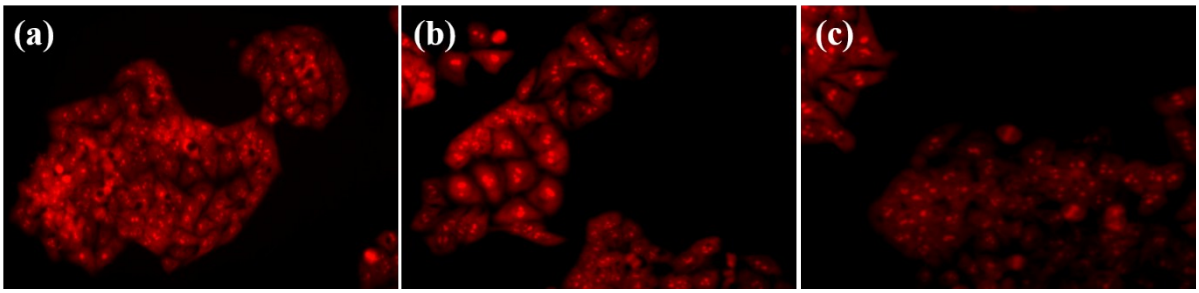


Fig. S10 Confocal microscopy images of H_2S release from QuH_2S-CDs. Gradual release of H_2S from QuH_2S-CDs was monitored using H_2S sensitive fluorescent probe (D) at different time intervals during irradiation with light ($\lambda \geq 410$ nm), (a) 0 min; (b) 25 min; (c) 50 min.

15. References:

1. a) S. Mandal, C. Ghatak, V. G. Rao, S. Ghosh, N. Sarkar. *J. Phys. Chem. C*, 2012, **116**, 5585−5597. (b) 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. E. T. Ryan, T. Xiang, K. P. Johnston, M. A. Fox, *J. Phys. Chem. A,* 1997, **101**, 1827–1835.
3. H. Guo, B. You, S. Zhao, Y. Wang, G. Sun, Y. Bai and L. Shi, *RSC Adv*., 2018, **8**, 24002
4. Y. Zhao, H. Wang and M. Xian, *J. Am. Chem. Soc*., 2011, **133**, 15.
5. S. Karthik, B. Saha, S. K. Ghosh and N. D. Pradeep Singh, *Chem. Commun.,* 2013, **49**, 10471—10473.