Electronic Supplementary Material (ESI) for ChemComm.


# Stepwise dual stimuli triggered dual drug release by a single naphthalene based two-photon chromophore to reverse MDR for alkylating agents with dual surveillance in uncaging steps

Biswajit Roy,[a] Moumita Kundu,[b] Amit Kumar Singh,[a] Tara Singha,[c] Sayantan Bhattacharya,[c]

Prasanta Kumar Datta,[c] Mahitosh Mandal,[b] N. D. Pradeep Singh*[a]

[a]Department of Chemistry, Indian Institute of Technology Kharagpur, Kharagpur-721302, India.

[b]School of Medical Science and Technology, Indian Institute of Technology Kharagpur,

Kharagpur-721302, India.

[c]Department of Physics, Indian Institute of Technology Kharagpur, Kharagpur-721302, India.

## Supporting Information

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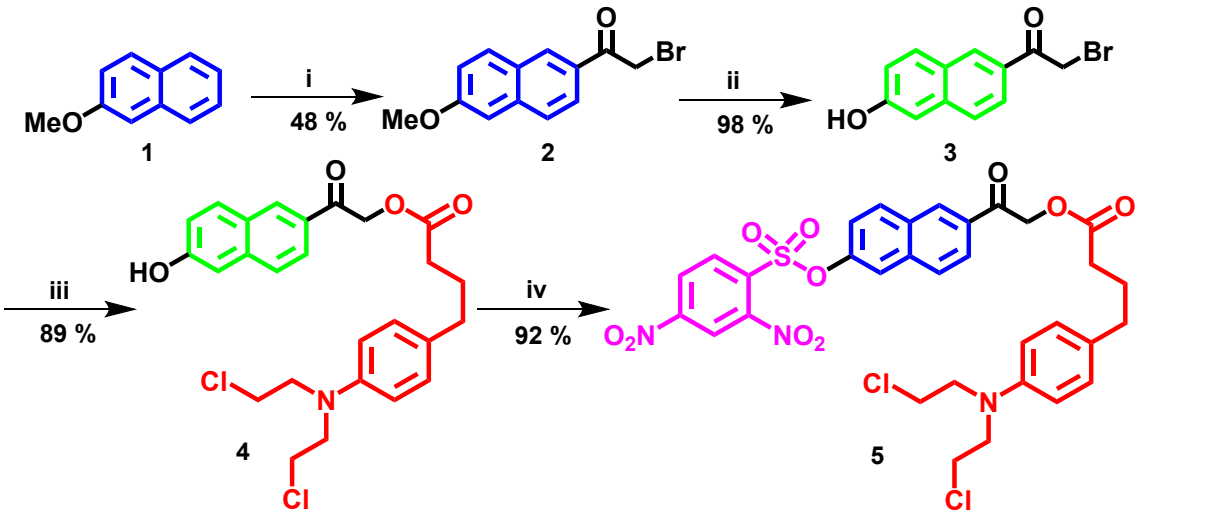
**1. General experimental details:**

Glassware used in reactions was thoroughly oven-dried. All commercial grade reagents were used without further purification and solvents were dried prior to use following standard protocol. Reactions were monitored by thin layer chromatography (TLC) using Merck silica gel 60 F254 pre-coated plates (0.25 mm) and the spots were visualized by exposure to UV light and/or by dipping into $KMnO_4$ solution. Silica gel of particle size 230–400 mesh and petroleum ether/ethyl acetate as eluent were used for column chromatographic purification. $^{1}H$ and $^{13}C$ NMR spectra for all the compounds were recorded at 400/600 and 100/150 MHz (Bruker Ultrashield$^{TM}$ 400, Ascend$^{TM}$ 600), respectively. The spectra were recorded in deuterochloroform ($CDCl_3$), deuterated acetonitrile ($CD_3CN$-$d_3$) and deuterated water ($D_2O$) as solvent at room temperature. Chemical shifts are reported in ppm from tetramethylsilane with the solvent resonance as internal standard ($CDCl_3$: $\delta_H$ = 7.26, $\delta_C$ = 77.16 ppm and $CD_3CN$-$d_3$: $\delta_H$ = 1.94, $\delta_C$ = 1.32, 118.26 ppm). Data for $^{1}H$ NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublet q: quartet, dt: doublet of triplets, br: broad.), coupling constant (Hz), integration. Data for $^{13}C$ NMR are reported as chemical shift. UV/vis absorption spectra were recorded on Shimadzu UV-2405 UV/vis spectrophotometer and Shimadzu UV-2600 UV/vis spectrophotometer. Fluorescence emission spectra were recorded on Hitachi F-7000 fluorescence spectrophotometer and Shimadzu RF-

6000 spectrofluorophotometer. HRMS spectra were recorded on a JEOL-AccuTOF JMS-T100L mass spectrometer. Photolysis was carried out using 125 W medium pressure mercury lamp supplied by SAIC (India). RP-HPLC was taken using acetonitrile/water as mobile phase (detection: UV 254 nm).

**2. Synthesis of DNs-Naph-Cbl:**

We have synthesized our DDS, (DNs-Naph-Cbl) in four steps (Scheme S1) starting from commercially available 2-methoxynaphthalene. Friedel-Crafts acylation on methoxynaphthalene **1** with bromoacetyl bromide and followed by deprotection of the methyl ether using excess anhydrous aluminium chloride in dry benzene furnished Compound **3**. Then Compound **3** was esterified with chlorambucil in the presence of $K_2CO_3$ in acetonitrile gave compound **4** (Naph-Cbl). Finally, free hydroxyl group of Naph-Cbl was protected by 2,4-dinitrophenylsulfonyl chloride in the presence of triethylamine in DCM yielded our DDS **5**.




**Reaction condition: i) bromoacetyl bromide, $AlCl_3$, dry DCM, 0 °C, 5 h; ii) $AlCl_3$, dry benzene, reflux, 3 h; iii) chlorambucil, $K_2CO_3$, acetonitrile, rt, 3 h; iv) 2,4-Dinitrobenzenesulfonyl chloride, $Et_3N$, 0 °C, 30 min.**

**Scheme S1** Synthesis of dinitrophenylsulphonyl-naphthalene-chlorambucil conjugate **5** (**DNs-Naph-Cbl**).

**3. Experimental procedure and Spectroscopic data:**

**2-bromo-1-(6-methoxynaphthalen-2-yl)ethanone (2)**: To a stirred solution of $AlCl_3$ (465 mg, 3.48 mmol) in 50 mL dry DCM, bromoacetyl bromide (312 µL, 3.48 mmol) was added dropwise at 0 °C. Stirring was continued for 1h, and then a solution of 2-methoxy naphthalene (500 mg, 3.16 mmol) in 10 mL dry DCM was added. After 5h of stirring at 0 °C, water was added to the system slowly maintaining the temperature constant. After usual work-up with DCM and water, the crude product was further purified by silica gel column chromatography using 3% EtOAc in hexane to afford 2-bromo-1-(6-methoxynaphthalen-2-yl)ethanone (424 mg, 1.52 mmol) compound **2** in 48 % yield. The compound is known.[1a] $^1$H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 7.79 (d, *J* = 8.6 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 7.17 (s, 1H), 4.55 (s, 2H), 3.96 (s, 3H).

**2-bromo-1-(6-hydroxynaphthalen-2-yl)ethanone (3)** : 2-bromo-1-(6-methoxynaphthalen-2-yl)ethanone **2** (300 mg, 1.075 mmol) was dissolved in 30 mL dry benzene and anhydrous $AlCl_3$ (1.43 g, 10.75 mmol) was added. The reaction mixture was heated to reflux for 3h, then cooled to 0 °C and water was added. Organic solvent was partially evaporated in rotary evaporator and the water part was extracted three times with EtOAc. The combined organic part was washed with brine, dried over anhydrous $Na_2SO_4$ and concentrated. The crude product was triturated

with hexane to afford compound **3** (279 mg, 1.054 mmol) in almost quantitative yield 98 %. The compound is known.[1a] $^1$H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.89 (d, *J* = 9.3 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.19 (s, 2H), 5.66 (br, 1H), 4.55 (s, 2H).

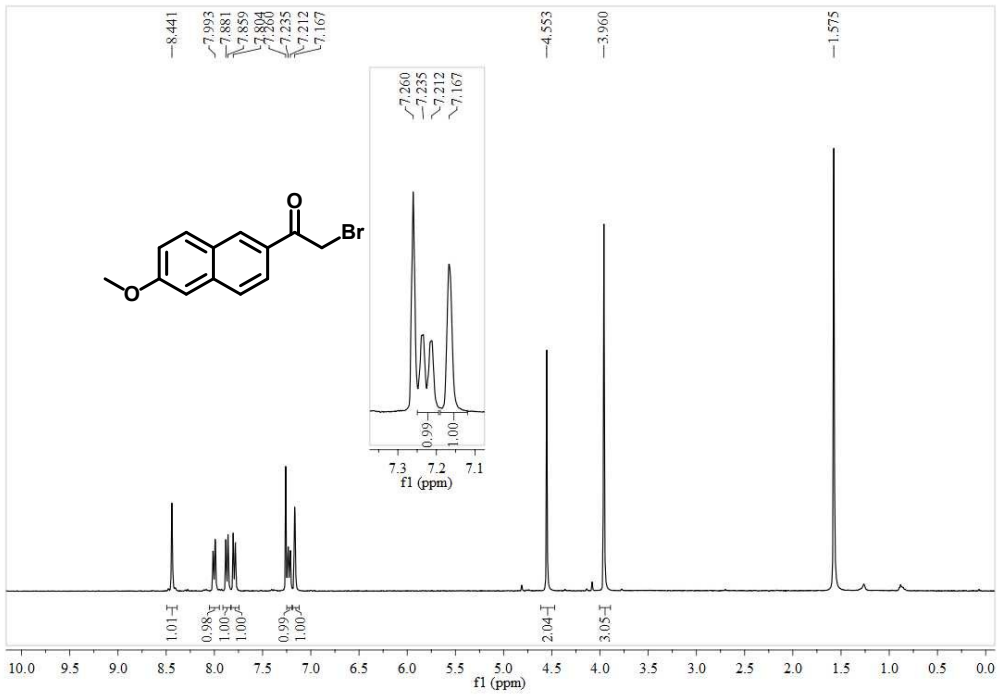
**2-(6-hydroxynaphthalen-2-yl)-2-oxoethyl 4-(4-(bis(2-chloroethyl)amino)phenyl)butanoate (4)** :

To a 25 mL round-bottom flask containing (115 mg, 0.377 mmol) chlorambucil in 10 mL acetonitrile, $K_2CO_3$ (104 mg, 0.75 mmol) was added and stirred for 20 min. To the reaction mixture compound **3** (100 mg, 0.377 mmol) in 5 mL acetonitrile was added. Stirring was continued for 3h, then solvent was evaporated. Water was added to the mixture and extracted with EtOAc three times. The combined organic layer was washed with aqueous sodium bicarbonate, brine and dried over $Na_2SO_4$ and concentrated. Further purification was achieved by silica gel column chromatography using 30% EtOAc in hexane to afford (424 mg, 1.52 mmol) compound **4** (163 mg, 0.335 mmol) in 89 % yield. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.88 (d, *J* = 8.6 Hz, 1H), 7.80 (d, *J* = 8.7 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 2H), 7.10 (d, *J* = 7.9 Hz, 2H), 6.68 (d, *J* = 7.6 Hz, 2H), 6.01 (br, 1H), 5.44 (s, 2H), 3.69 (d, *J* = 6.3 Hz, 4H), 3.63 (d, *J* = 5.8 Hz, 4H), 2.64 (t, *J* = 7.4 Hz, 2H), 2.53 (t, *J* = 7.3 Hz, 2H), 2.08 – 1.92 (m, 2H). $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$) δ 191.9, 173.4, 156.3, 143.7, 137.6, 131.7, 129.9, 129.5, 129.4, 127.5, 127.1,

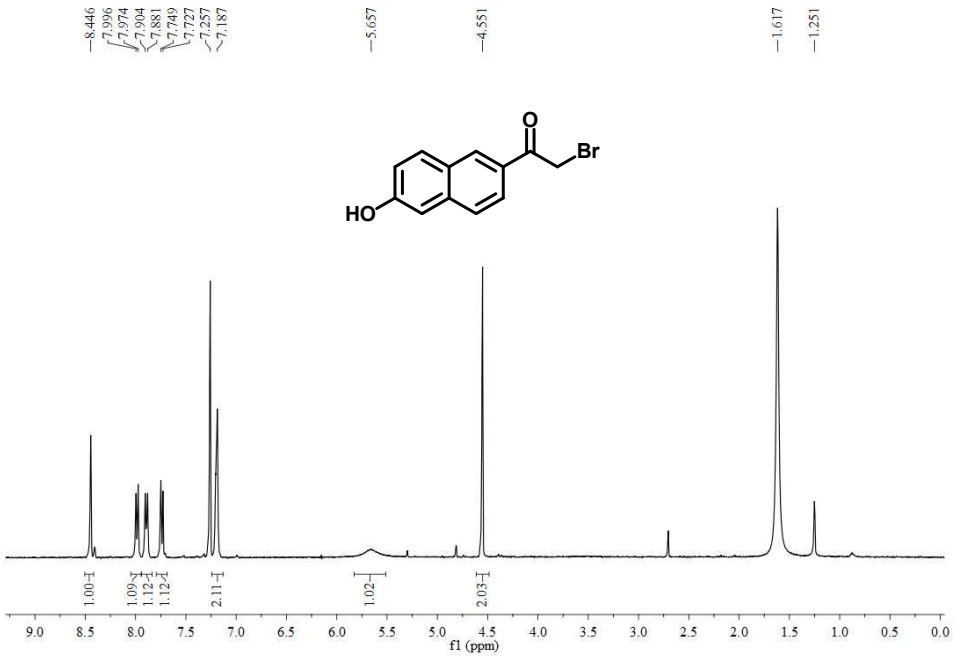
124, 119.2, 112.8, 109.7, 66, 54, 40.3, 33.9, 33.3, 26.7; HRMS (ESI-TOF): calculated for $C_{26}H_{28}Cl_2NO_4$ $[M+H]^+$ 488.1395; found 488.1398.

**2-(6-(((2,4-dinitrophenyl)sulfonyl)oxy)naphthalen-2-yl)-2-oxoethyl 4-(4-(bis(2-chloroethyl)amino)phenyl)butanoate (5)** : To a stirred solution of compound **4** (50 mg, 0.102 mmol) along with 2,4-Dinitrobenzenesulfonyl chloride (33 mg, 0.123 mmol) in 10 mL dry DCM, triethylamine (30 µL, 0.21 mmol) was added drop wise at 0 °C. Catalytic amount of DMAP was added to the solution and stirred for 30 min. at the same temperature. Water was added to the solution and extracted three times with DCM. The combined organic part was washed with brine and dried over $Na_2SO_4$ and concentrated. The crude product was triturated with hexane to afford almost pure compound **5** (68 mg, 0.094 mmol) in 92 % yield. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.49 (d, *J* = 8.6 Hz, 1H), 8.43 (s, 1H), 8.24 (d, *J* = 8.6 Hz, 1H), 8.03 – 7.99 (m, 2H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.75 (s, 1H), 7.43 (d, *J* = 8.9 Hz, 1H), 7.12 (d, *J* = 8.3 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 5.46 (s, 2H), 3.72 (t, *J* = 7.0 Hz, 4H), 3.64 (t, *J* = 7.0 Hz, 4H), 2.64 (t, *J* = 7.5 Hz, 2H), 2.54 (t, *J* = 7.4 Hz, 2H), 2.09 – 1.90 (m, 2H). $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$) δ 191.9, 173.1, 151, 149, 148.2, 144.4, 136, 133.9, 133.4, 132.5, 132.4, 131.3, 130.5, 129.8, 129.2, 129, 126.7, 124.9, 121.6, 120.5, 119.9, 112.1, 77.1, 66, 53.6, 40.6, 33.8, 33.2, 26.8; HRMS (ESI-TOF): calculated for $C_{32}H_{30}Cl_2N_3O_{10}S$ $[M+H]^+$ 718.1029; found 718.1038.

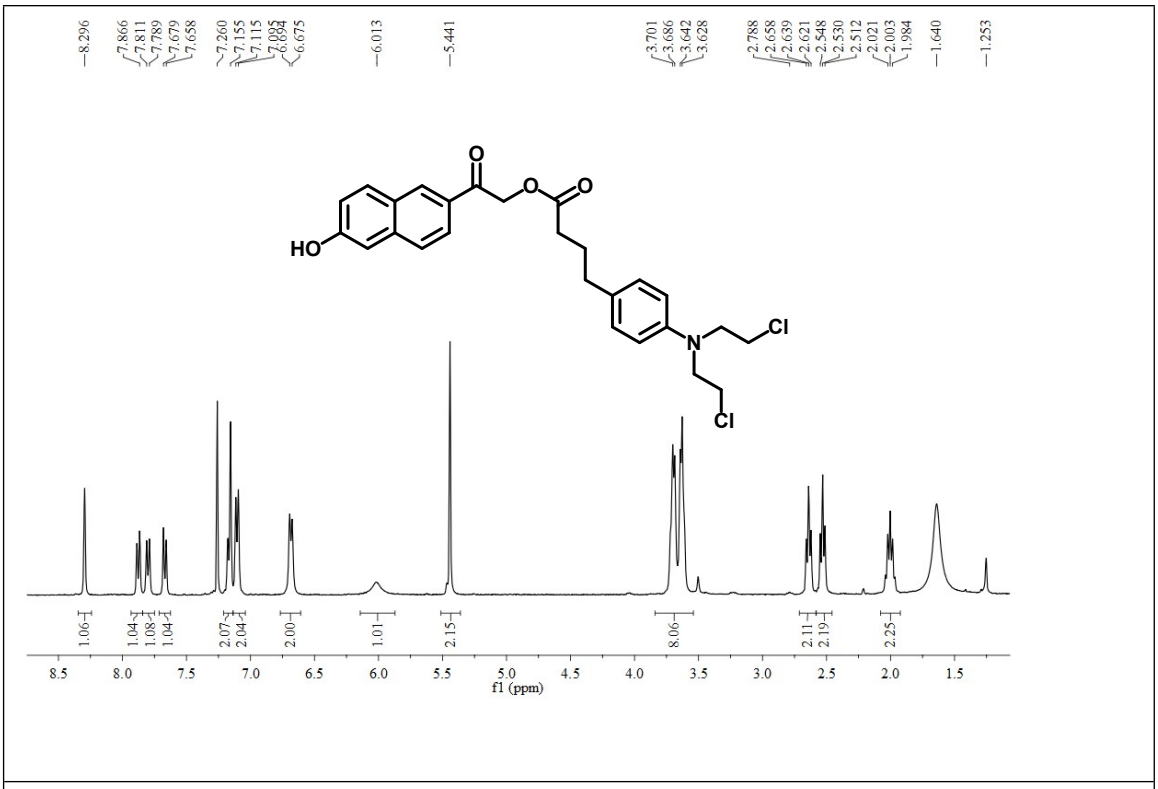
## 4. $^{1}H$ and $^{13}C$ NMR Spectra:



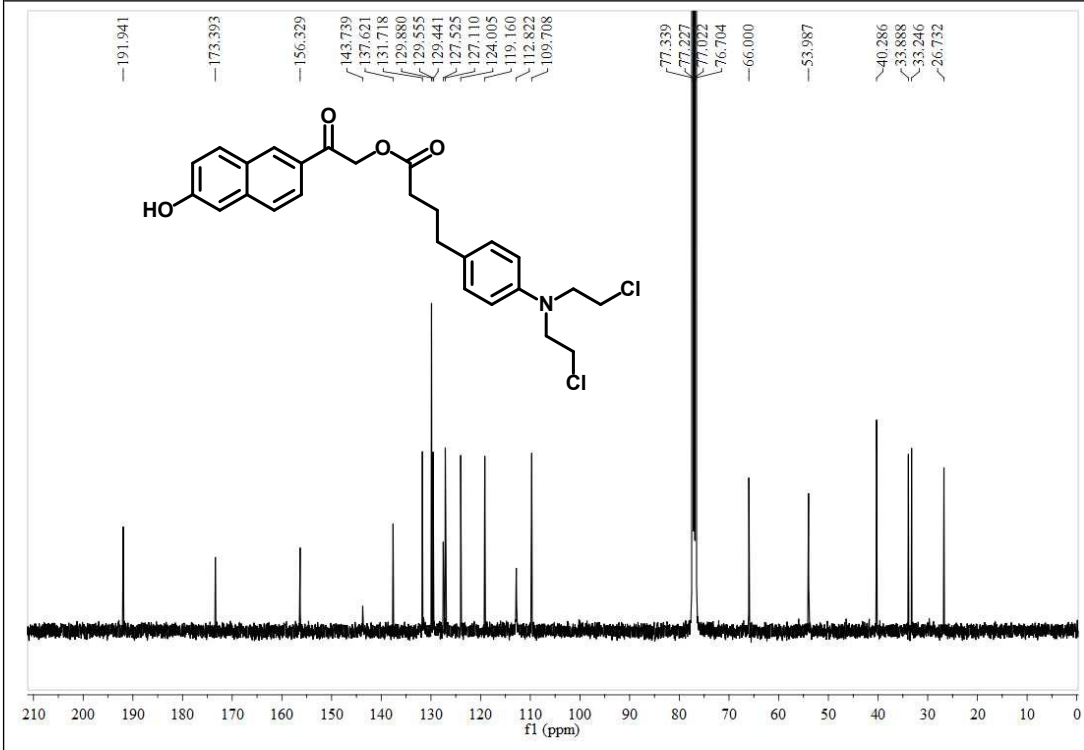

$^{1}H$ NMR ($CDCl_3$, 400 MHz) spectrum of compound **2**



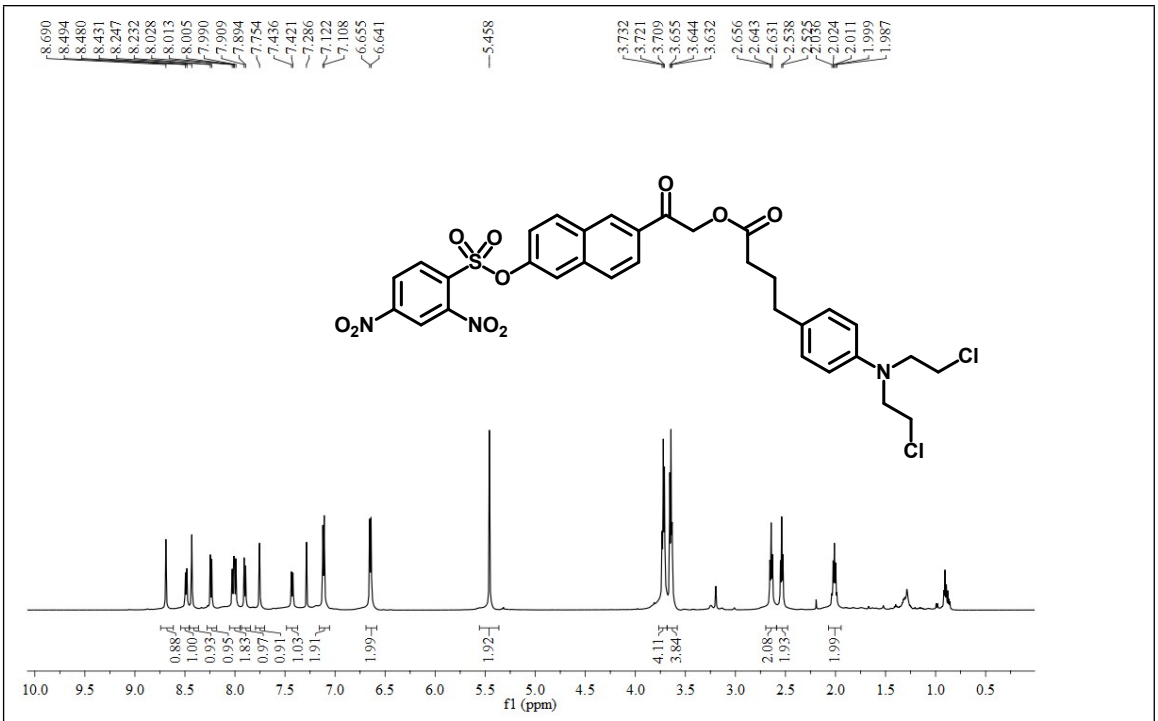

$^{1}H$ NMR ($CDCl_3$, 400 MHz) spectrum of compound **3**



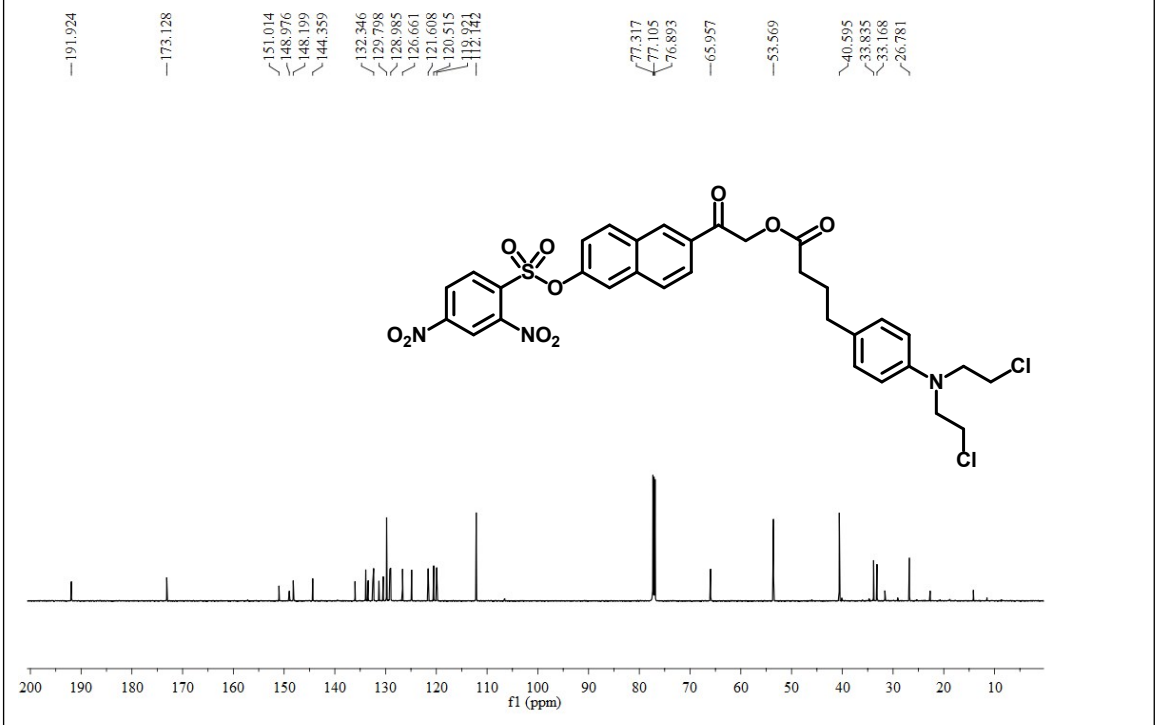

$^{1}H$ NMR ($CDCl_3$, 400 MHz) spectrum of compound **4**




$^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 100 MHz) spectrum of compound **4**




$^{1}$H NMR ($CDCl_3$, 600 MHz) spectrum of compound **5**




$^{13}$C{$^{1}$H} NMR ($CDCl_3$, 150 MHz) spectrum of compound **5**

**5. Photophysical properties Naph-Cbl:**

The absorption and emission spectra of Naph-Cbl in different solvents were recorded on a UV-Vis and fluorescence spectrophotometer respectively. In each case, a ($1 \times 10^{-4}$ M) solution was prepared with different solvent and after degassing the solution the spectra were taken.

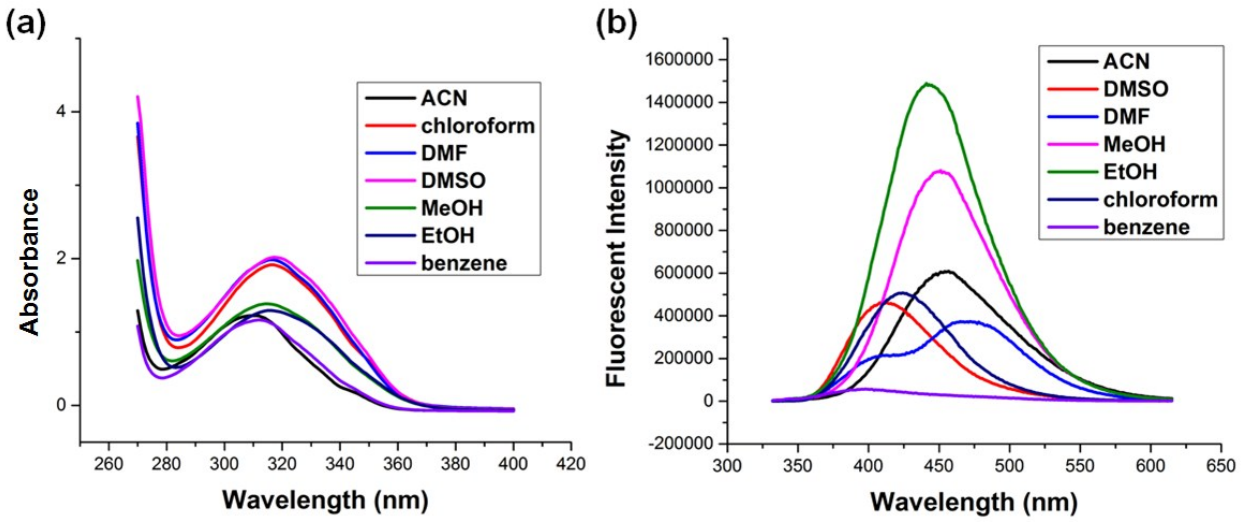



**Fig. S1** (a) normalized UV-Vis spectrum and (b) fluorescence spectrum of Naph-Cbl in different solvent

The presence of two bands in the emission spectrum of Naph-Cbl is due to the existences of both enol form and exited state ionized form (Influence of solvent on the excited state ionisation is also noted) [1b].

**6. Photophysical properties DNs-Naph-Cbl & Naph-Cbl in experimental condition and biological medium:**

The absorption and emission spectra of both the DDS was recoded in experimental condition and biological condition with (1 × $10^{-4}$ M) solutions and recorded on a UV-Vis and fluorescence spectrophotometer respectively.

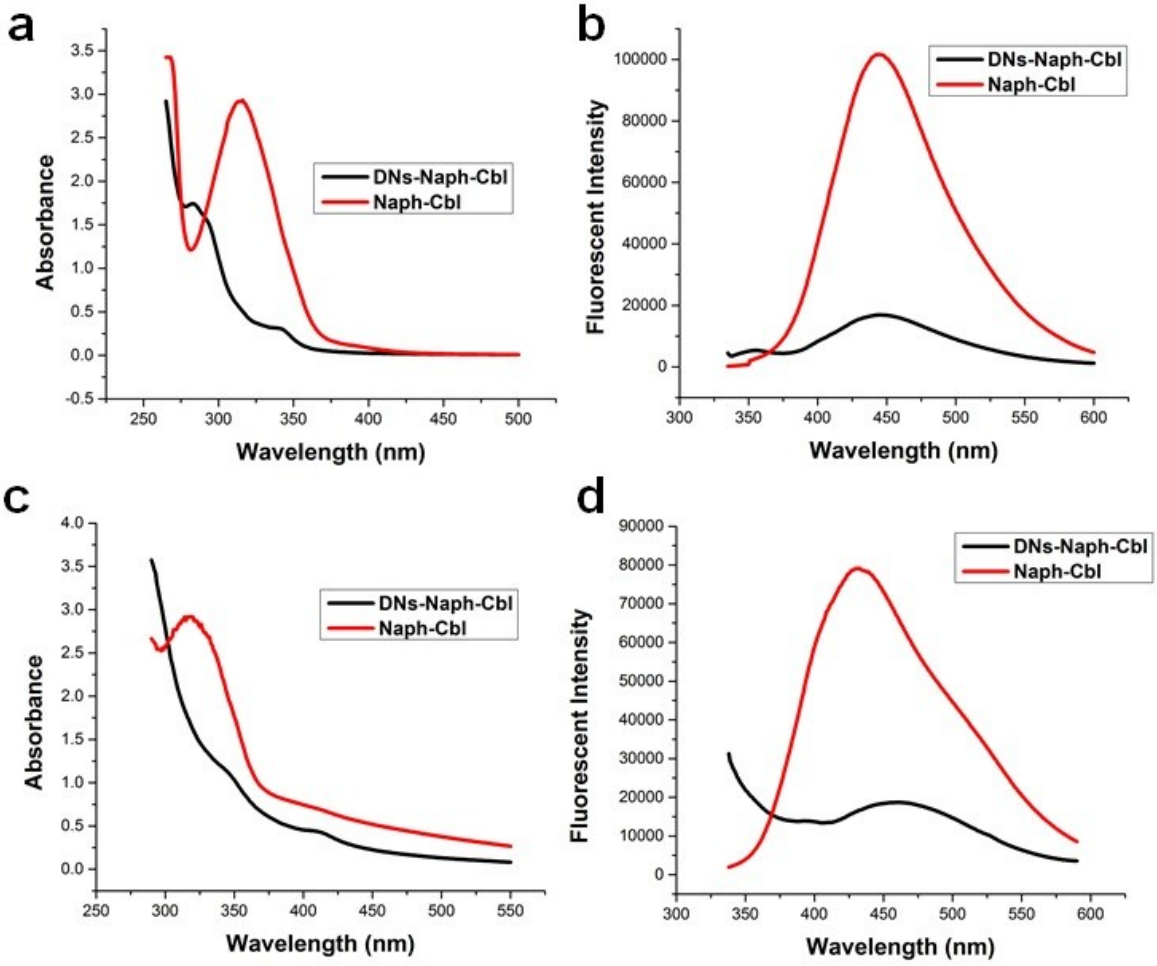



**Fig. S2** (a) UV-Vis absorption spectra and (b) fluorescence spectra of DNs-Naph-Cbl and Naph-Cbl of a 1 × $10^{-4}$ M solution in acetonitrile/PBS (3:7); (c) UV-Vis absorption spectra and (d) fluorescence spectra of DNs-Naph-Cbl and Naph-Cbl of a 1 × $10^{-4}$ M solution concentration in DMSO/PBS/fetal bovine serum (1:8:1).

**7. Z-scan measurement:**

An open aperture z-scan measurement was performed using 650 nm and 700 nm output of an optical parametric amplifier (OPA) (TOPAS-Prime) pump with an 808 nm, 50fs, 1kHz laser pulse from a Ti:Sapphire regenerative amplifier laser system (Coherent: Libra). Laser spot is focused at the beam waist of 48μm with a 20 cm plano-convex lens. To a solution of DNs-Naph-Cbl ($1 \times 10^{-5}$ M) in $CH_3CN$/PBS buffer of pH 7.4 (3:7) was added 2 equivalent GSH and it was kept in a 2 mm path length quartz cuvette with a constant magnetic stirring system. The sample is moved across the focus through a motorized translational stage (Newport, GTS-150). Rayleigh range ($Z_0$) is coming out to be 1.1 cm. Input pulse energy is maintained at 0.35 μJ using a circular neutral density filter. The transmitted laser beam is collected with another plano-convex lens of 15 cm and detected with an amplified Si-photodiode (Thorlabs PDA100A-EC). Data is taken through a lock-in amplifier (7225 DSP, Signal Recovery) for better signal to noise ratio. The lock-in amplifier is triggered by a 1 kHz TTL signal from the laser. Both the Lock-in amplifier and motorized translational stage are incorporated in LabVIEW 2012 environment for automation and averaging of data. The experimental curve was fitted with transmission equation involving two-photon absorption equation.[2] The two-photon absorption cross section δa was calculated to be 77 GM (1 GM = $10^{-50}$ $cm^4$ s/photon) at 650 nm and 34 GM at 700 nm.

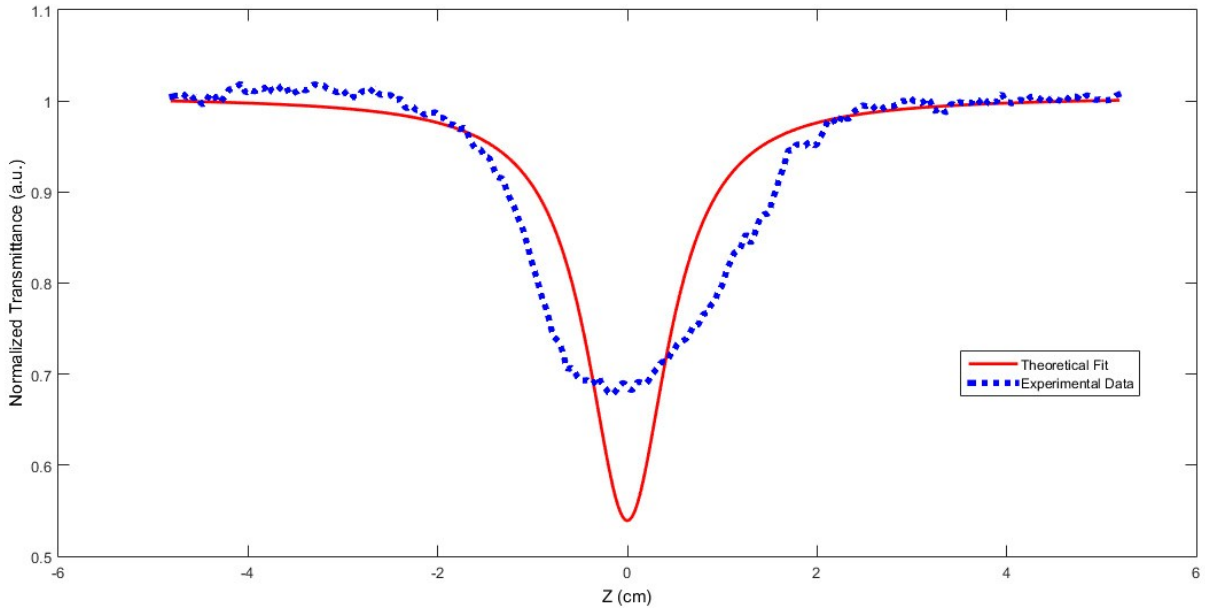



**Fig. S3** Open aperture (OA) Z-scan measurement (at 650 nm) of Naph-Cbl: Normalized transmittance. The dotted line represents experimental data and the solid line represents theoretical fitting.

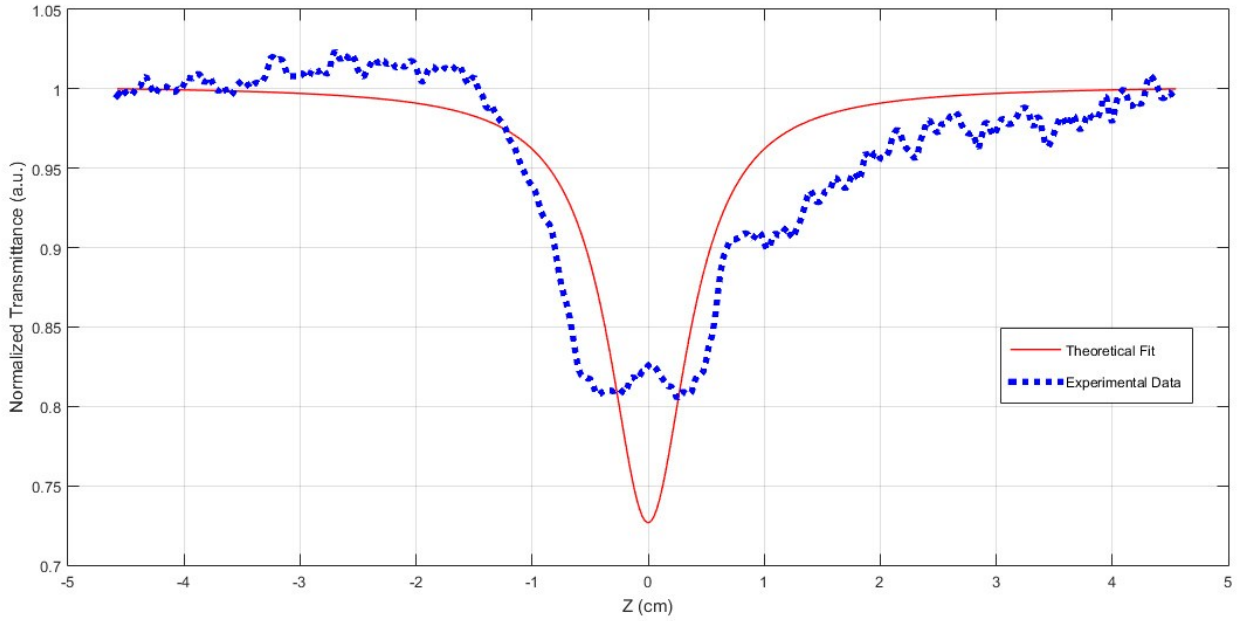
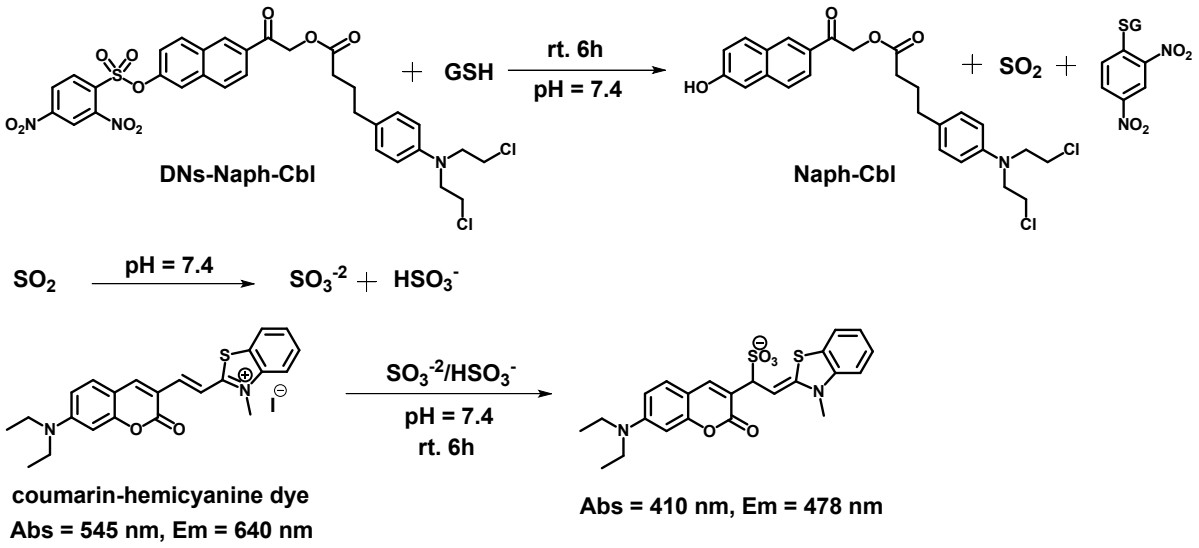



**Fig. S4** Open aperture (OA) Z-scan measurement (at 700 nm) of Naph-Cbl: Normalized transmittance. The dotted line represents experimental data and the solid line represents theoretical fitting.

The signal is not symmetrical with respect to positive and negative Z, can be attributed to the photouncaging of Naph-Cbl as it has very good photochemical quantum yield ($\varphi_u$ = 0.28).

**8. $SO_2$ sensing study:**

We have synthesized the coumarin-hemicyanine dye[3] for sensing $SO_2$ by following known literature report.[3] DNs-Naph-Cbl (1 X $10^{-4}$ M) was taken in acetonitrile/water (3:7) mixture in different vials (4 ml) and a solution of GSH in acetonitrile/water (3:7) mixture was added in different ratio (0.2, 0.4,……1.0) to the vials, independently. The glass vials were then sealed and stored at room temperature. After 6 h, coumarin-hemicyanine dye (1 X $10^{-5}$ M) was added to each of these sealed vials via syringe and further stored for another 6h at room temperature. Next, we sonicated the above prepared solutions for 30 minutes and recorded their emission spectrum. From Fig. S5, we observed gradual decrease in the intensity of the emission maximum of coumarin-hemicyanine dye, which confirmed the presence of hydrated $SO_2$ derivatives.




**Scheme S2** GSH triggered $SO_2$ release and sensing of hydrated $SO_2$ derivatives by coumarin-hemicyanine dye.

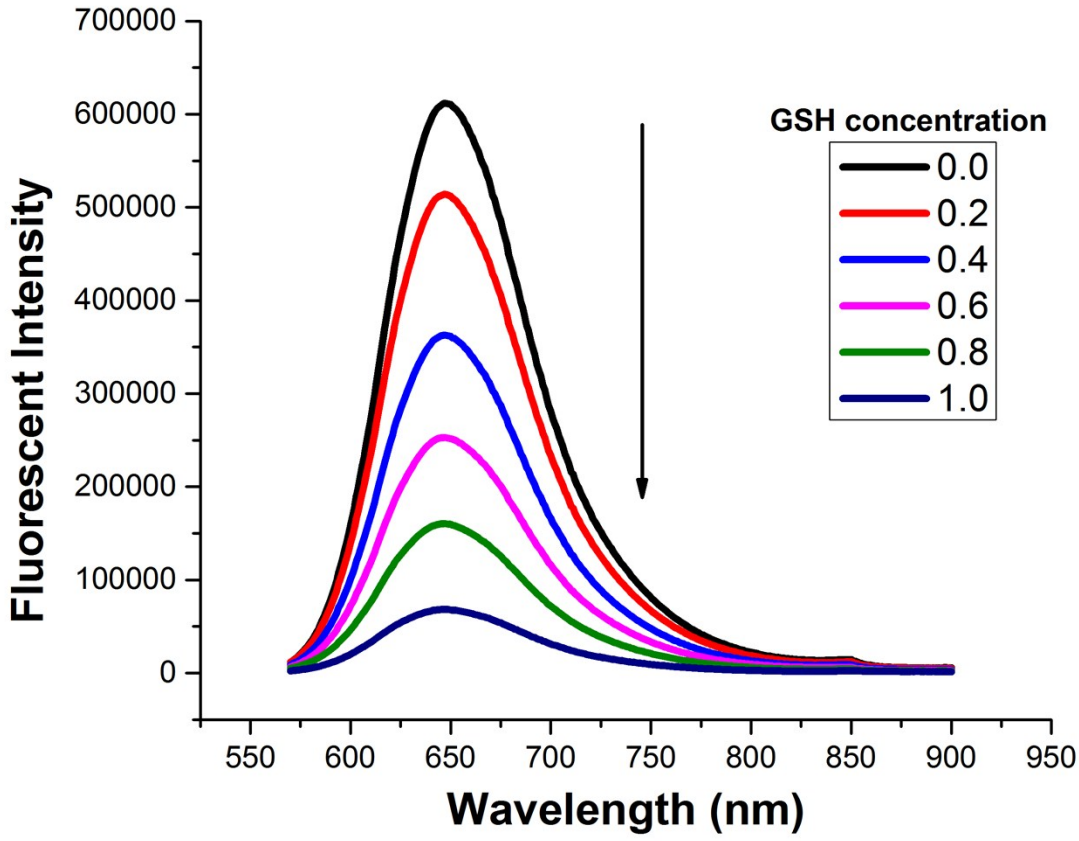



**Fig. S5** Change in the fluorescent spectral profile of coumarin-hemicyanine dye upon reacting with hydrated $SO_2$ derivatives generated from the reaction of DNs-Naph-Cbl and GSH. Exitation wavelength (445 nm).

**9. Quantification of released Naph-Cbl and $SO_2$:**

To quantify the amount of $SO_2$ release we measured it indirectly from the amount of Naph-Cbl released. As it is a ratiometric reaction, the amount of $SO_2$ release is equivalent to Naph-Cbl release. The experiment was carried out with a solution of DNs-Naph-Cbl ($1 \times 10^{-4}$ M) in $CH_3CN$/PBS buffer of pH 7.4 (3:7) was added GSH from 0 to 1 equivalent in increasing amounts. 15 minutes after each addition an aliquot of 25 μL was injected in HPLC. The released drug (Naph-Cbl) was quantified from the HPLC peak area in comparison with the injected authentic sample. The result (Fig. S6) shows that upon addition of GSH, it ratiometrically react with DNs-Naph-Cbl and released almost quantitatively one equivalent $SO_2$ and light activable prodrug Naph-Cbl.

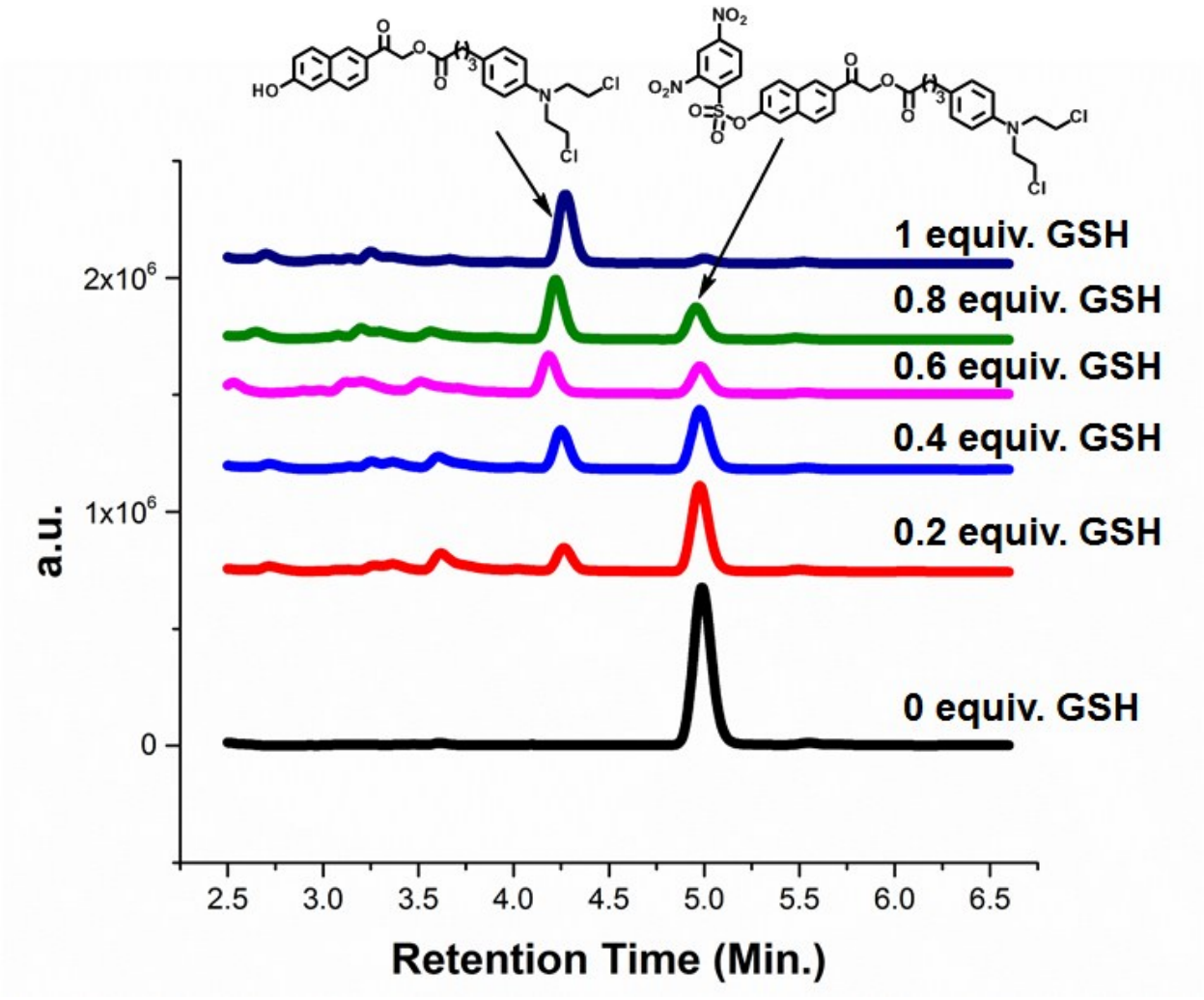



**Fig. S6** HPLC overlay chromatogram of DNs-Naph-Cbl in the presence of different equivalent of GSH from 0 to 1 equivalent. All spectra were acquired 30 min. after addition of the GSH.

**10. Stability of prodrugs:**

**Table S1:** Percentage of decomposition under dark condition[a] of DNs-Naph-Cbl and Naph-Cbl in biological medium and in different pH at 37 °C.

| | | Biological medium | Different pH | |
|---|---|---|---|---|
| Prodrug | Time | 10% fetal bovine serum pH = 7.4 | pH = 5.5 | pH = 8 |
| **DNs-Naph-Cbl** | 7 days | 4 | 4 | 7 |
| **Naph-Cbl** | 7 days | < 1 | < 1 | 2 |

(a) As examined by RP-HPLC.

**11. Photolysis of Naph-Cbl:**

To check the ability of Naph-Cbl as a photoresponsive drug delivery system, We added 2 equivalent GSH in a 30 mL (1 x $10^{-4}$ M) solution of Dns-Naph-Cbl in $CH_3CN$/PBS buffer of pH 7.4 (3:7) and kept for 30 minutes prior to expose to a medium pressure mercury lamp (125 W) as the source of light (λ ≥ 365 nm) using 1(M) aqueous solution of $CuSO_4$ UV cut-off filter.

**12. Procedure for the quantification of the released drug from Naph-Cbl:**

30 mL (1 x $10^{-4}$ M) solution of DNs-Naph-Cbl in $CH_3CN$/PBS buffer of pH 7.4 (3:7) was treated with 2 equivalent GSH and kept for 30 minutes to uncage the light activable prodrug Naph-Cbl. Then the solution was exposed to a medium pressure mercury lamp (125 W) as the source of light (λ ≥ 365 nm) using 1(M) aqueous solution of $CuSO_4$ UV cut-off filter.

Individual compounds are characterized by ESI-MS spectroscopy (Fig. S7 – S9).

The quantification of the released drug from Naph-Cbl with respect to time was carried out by exposing the solution of GSH-pretreated DNs-Naph-Cbl to the visible-light in a regular interval of time (Fig. S10a). An aliquot of 25 µL was injected in HPLC in each interval. The released drug was quantified from the HPLC peak area in comparison with injected authentic sample.

To demonstrate the precise control over the drug delivery, the solution was exposed to light and dark condition, periodically (Fig. S10b). Aliquots of 25 µL were injected in each case in the HPLC.

The released drug was quantified from the HPLC peak area in comparison with the injected authentic sample.

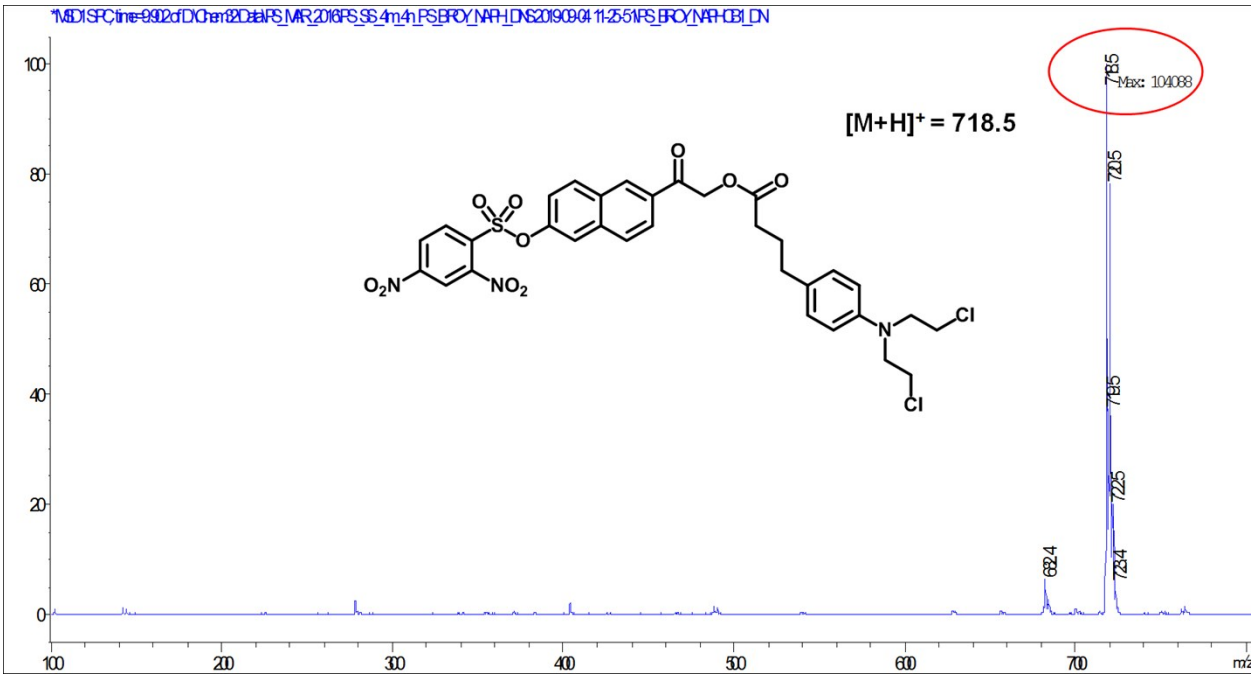



**Fig. S7** ESI-MS spectrum of DNs-Naph-Cbl at $t_R$ 5 min collected in the HPLC analysis (before GSH treatment).

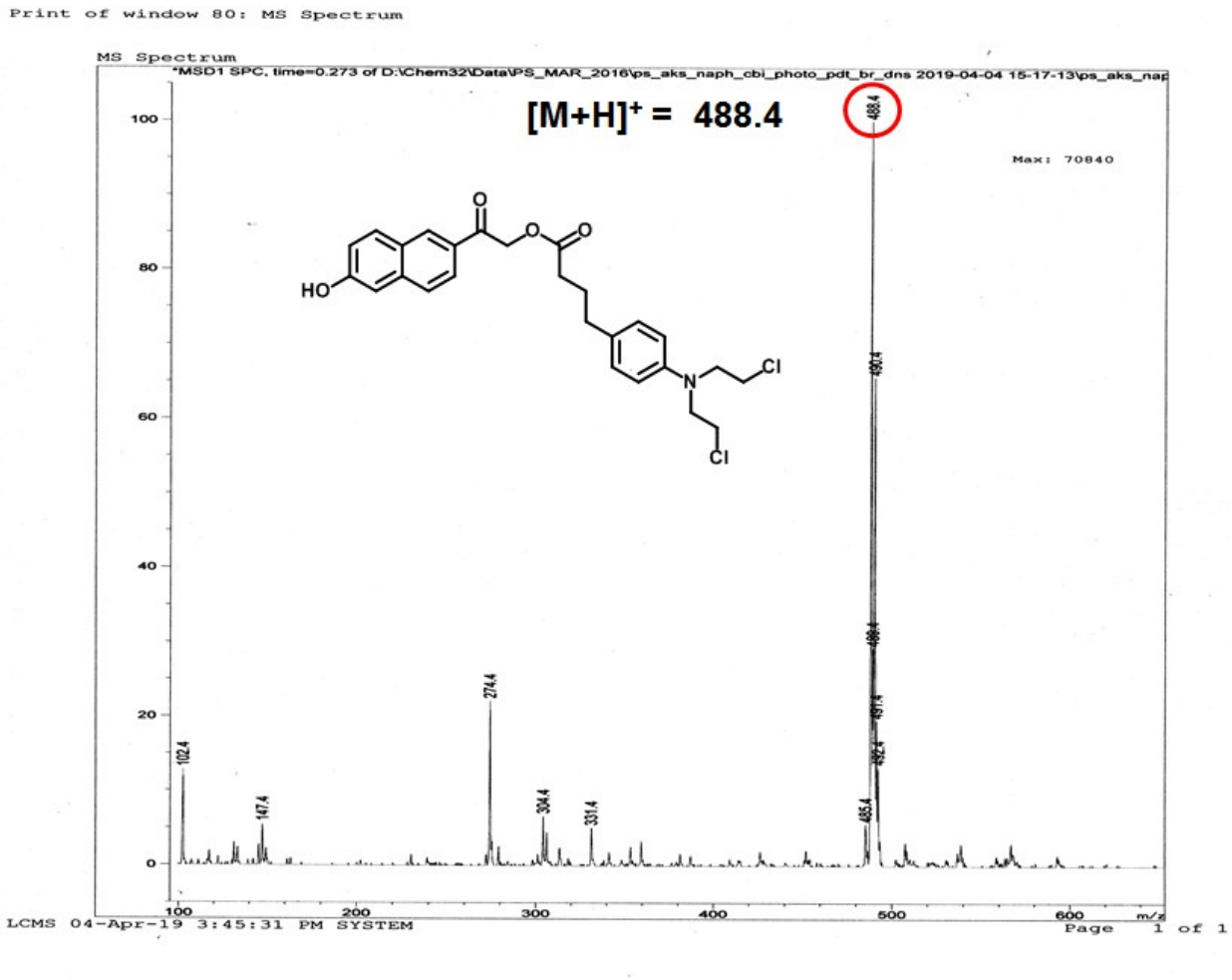



**Fig. S8** ESI-MS spectrum of Naph-Cbl at $t_R$ 4.3 min collected in the HPLC analysis (after GSH treatment).

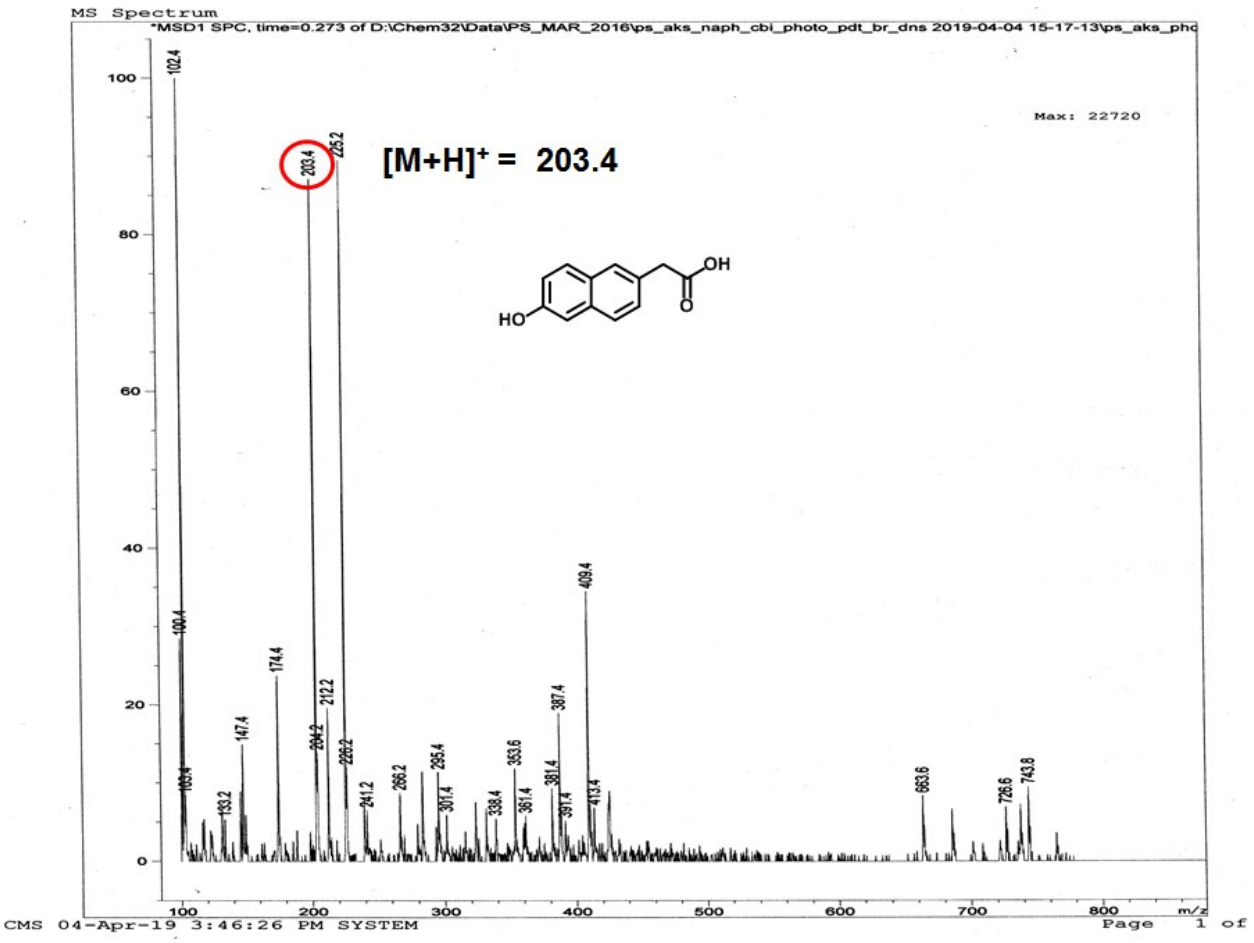



**Fig. S9** ESI-MS spectrum of photoproduct at $t_R$ 3 min collected in the HPLC analysis (after irradiation for 30 min.).

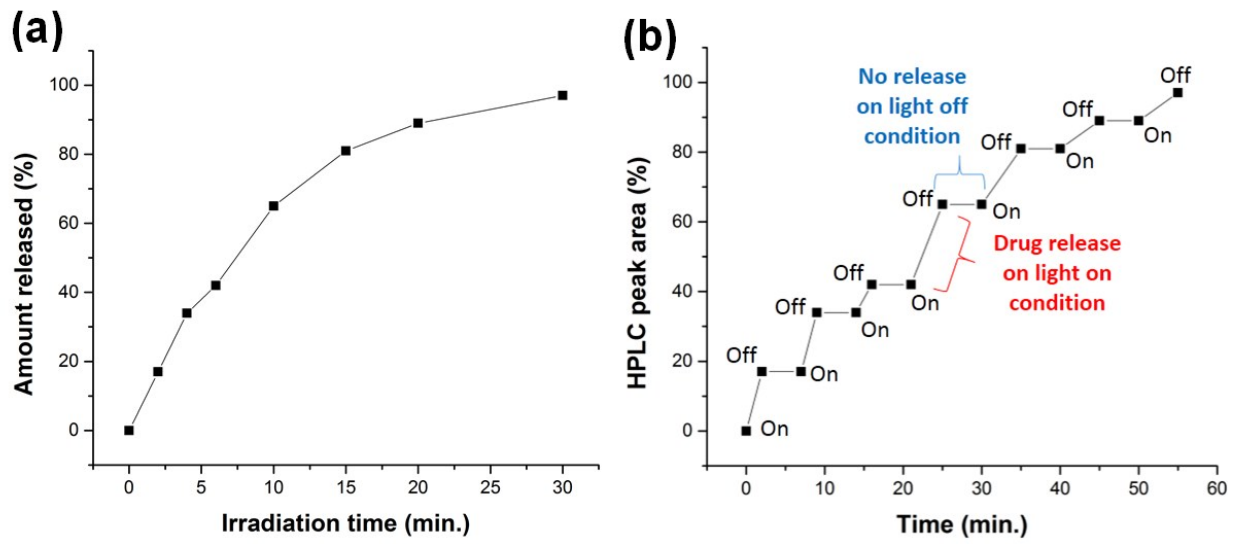



**Fig. S10** (a) The amount of chlorambucil released from Naph-Cbl on photolysis (≥ 365 nm) at different time intervals. (b) Release of chlorambucil under light and dark conditions. “On” and “Off” implies the switching on and off of the light source, respectively.

**13. Fluorescent spectral changes durring both the drug delivery steps in experimental condition:**

The emission spectra of DNs-Naph-Cbl, Naph-Cbl and the photoproduct were recorded in experimental condition. In each case, a ($1 \times 10^{-4}$ M) solution was prepared in acetonitrile/PBS (3:7) and recorded on a fluorescence spectrophotometer.

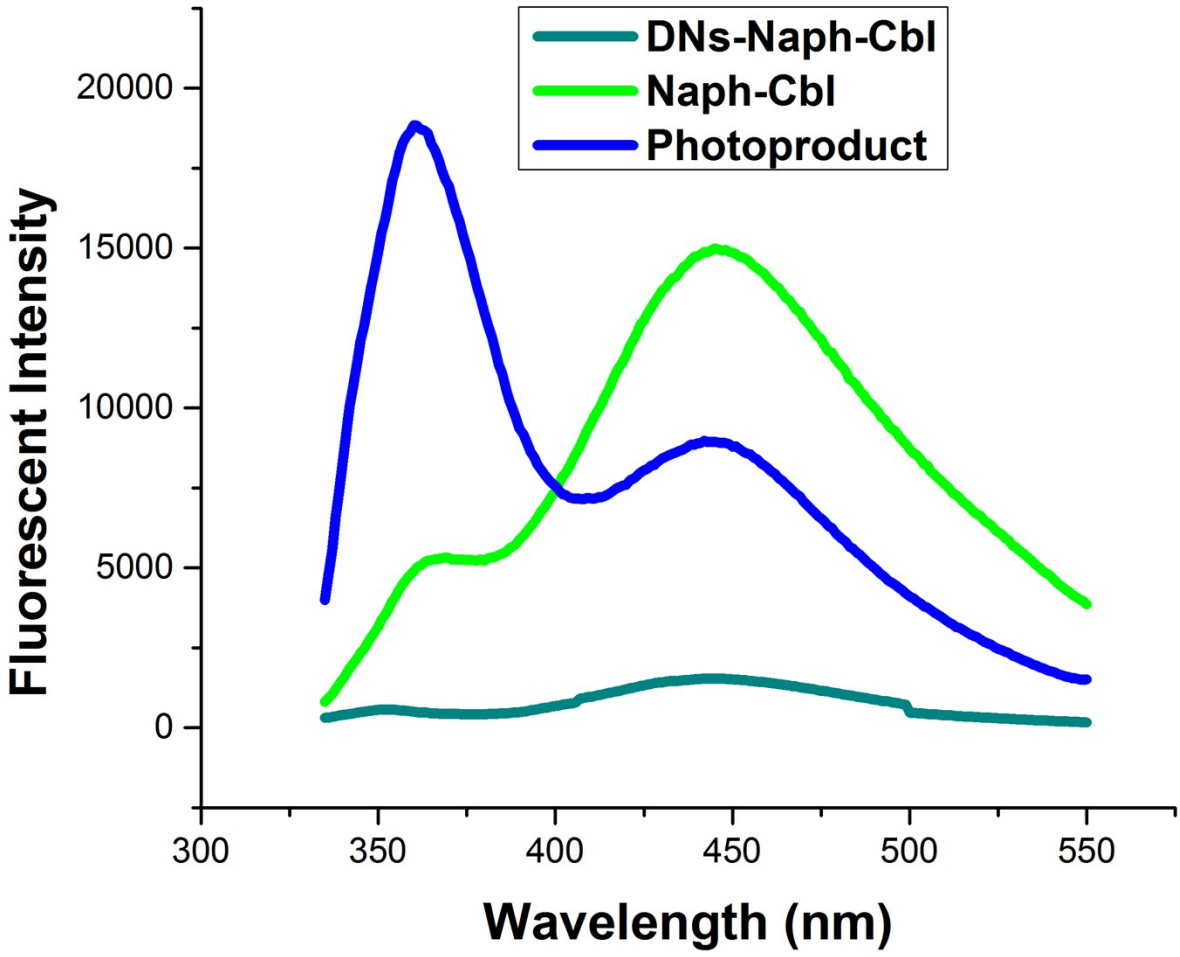



**Fig. S11** Fluorescence spectra of DNs-Naph-Cbl, Naph-Cbl and the photoproduct were recorded with $1 \times 10^{-4}$ M solution in acetonitrile/PBS (3:7) in each case.

**14. Two-photon photolysis:**

A 1 mL (1 x $10^{-5}$ M) solution of DNs-Naph-Cbl in $CH_3CN$/PBS buffer of pH 7.4 (3:7) was added 2 equivalent GSH and it was taken in a 1mm quartz cuvette and irradiated with a laser of wavelength 650nm with 100 fs pulses at a 1KHz rate with a 60 µm diameter beam spot. During the photolysis after regular time intervals, small aliquots (25 µL) were taken out from the solution for HPLC study. The released drug was quantified from the HPLC peak area in comparison with injected authentic sample. We have found that after 2h of photolysis 17 % of the drug got released.

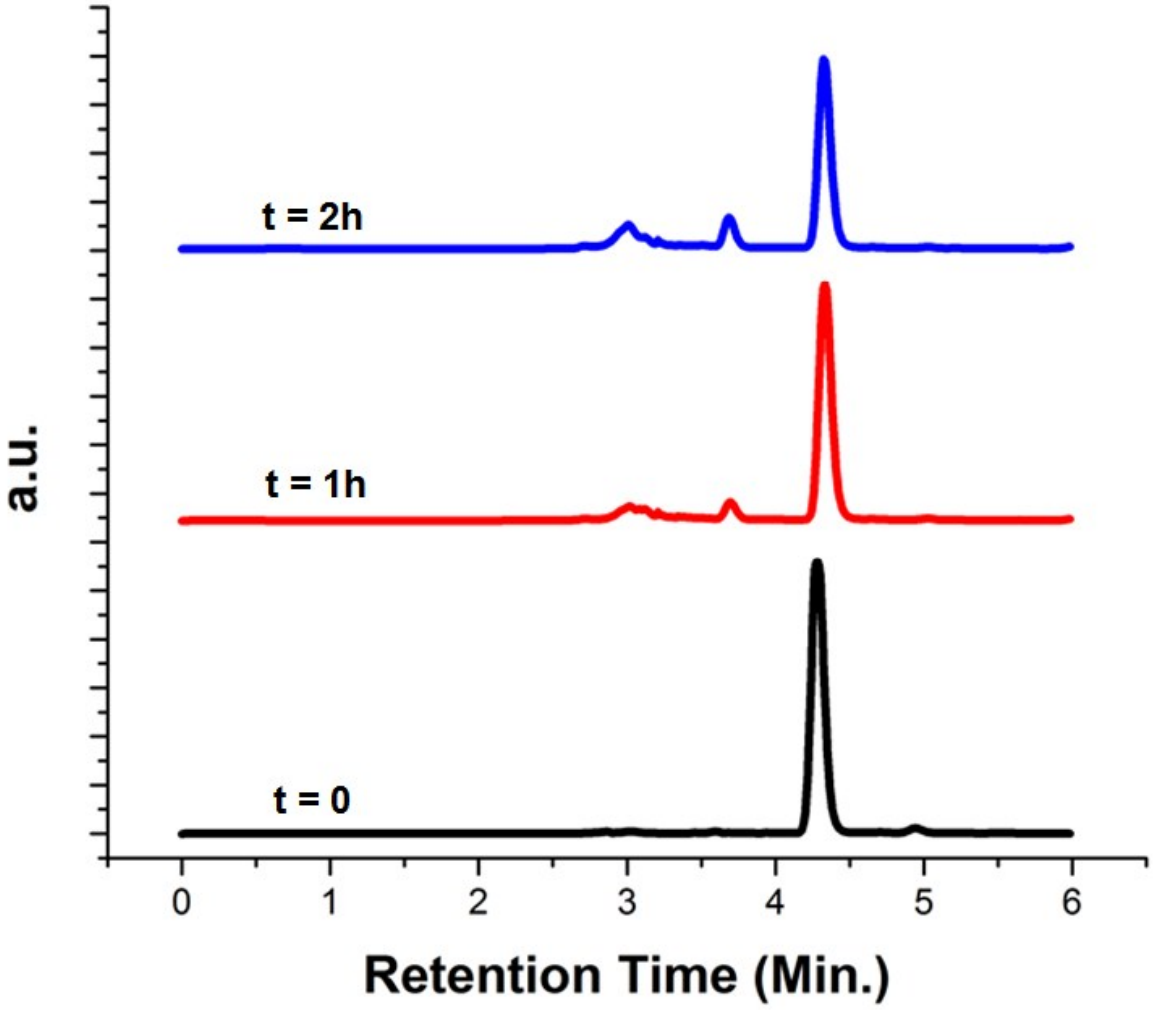



**Fig. S12** HPLC overlay chromatogram of Naph-Cbl at different time intervals of light irradiated with a laser of wavelength 650nm with 100 fs pulses at a 1 KHz rate.

**15. Mechanism of photorelease:**

The mechanism of $SO_2$ release by GSH from 2,4-dinitrophenyl sulfonyl protected compound is known in the literature.[4] To postulate the photorelease mechanism for our DDS, we carried out certain supporting experiments. First, to demonstrate the mechanism of the photorelease proceeds through Photo-Favorskii reaction, we monitored the photolysis of our DDS by $^1$H NMR at regular intervals (Fig. S13).

**15.1. $^1$H NMR study of the photorelease of chlorambucil:**

2 mg of Naph-Cbl was taken in 0.5 mL $CD_3CN/D_2O$ (1:1) mixed solvent into an NMR tube and was exposed to UV light ($\lambda \geq 365$ nm) for a regular interval of time (0-120 min, time interval = 60 min). NMR analysis also carried out during these intervals.

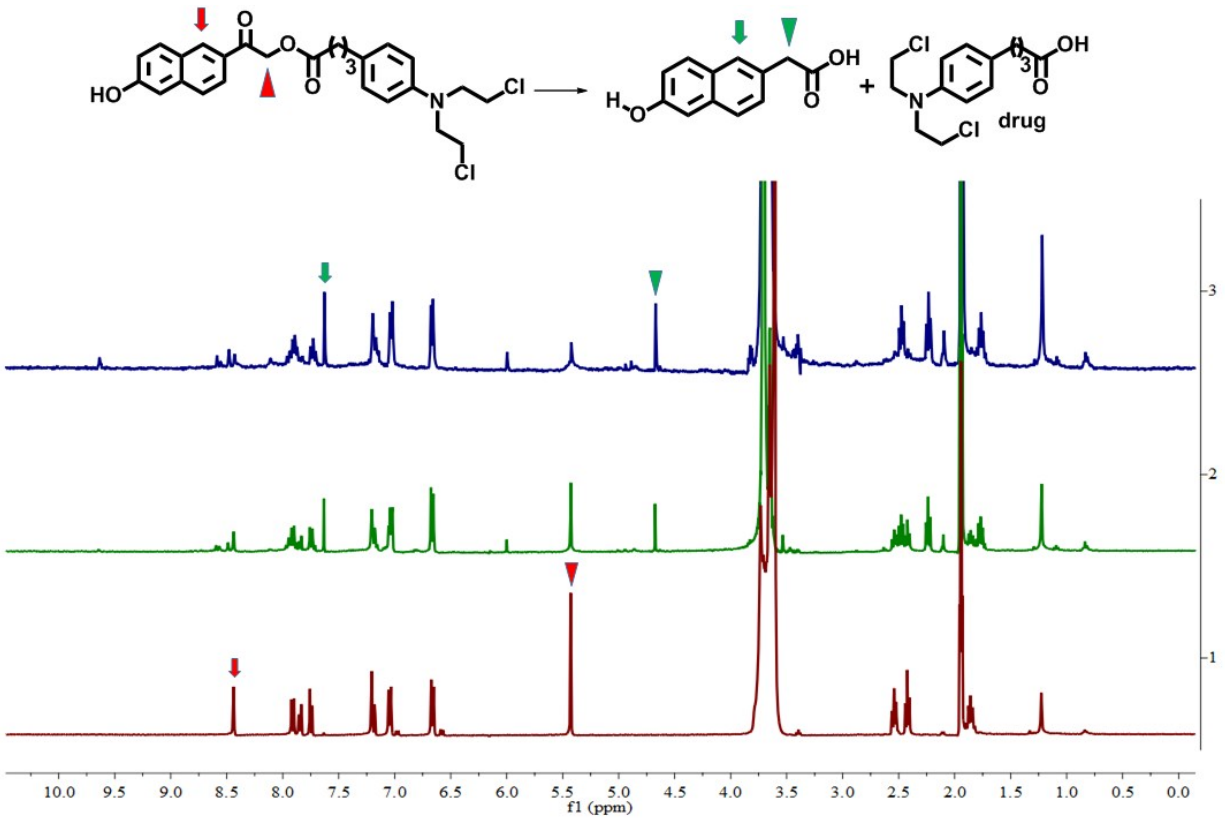



**Fig. S13** $^{1}H$ NMR study of Naph-Cbl in $CD_3CN/D_2O$ (1:1) during photolysis at regular interval of time (0-120 min, time interval = 60 min).

The $^{1}H$ NMR study shows that the characteristic peak at 5.43 δ corresponds to the methylene proton and singlet *peri*-hydrogen at 8.43 δ of Naph-Cbl (Fig. S13) was gradually decreased upon irradiation and a subsequent increase of new peaks at 4.67 δ and 7.63 δ was observed, which may be attributed to the methylene proton of the photoproduct 2-hydroxy-6-naphthylacetic acid. Similar rearranged product is found with p-hydroxyphenacyl (*p*HP) i.e. *p*-hydroxyphenylacetic acid *via* Photo-Favorskii rearrangement. We have also synthesized other possible α-cleavage photoproduct to compare (Fig. S14).

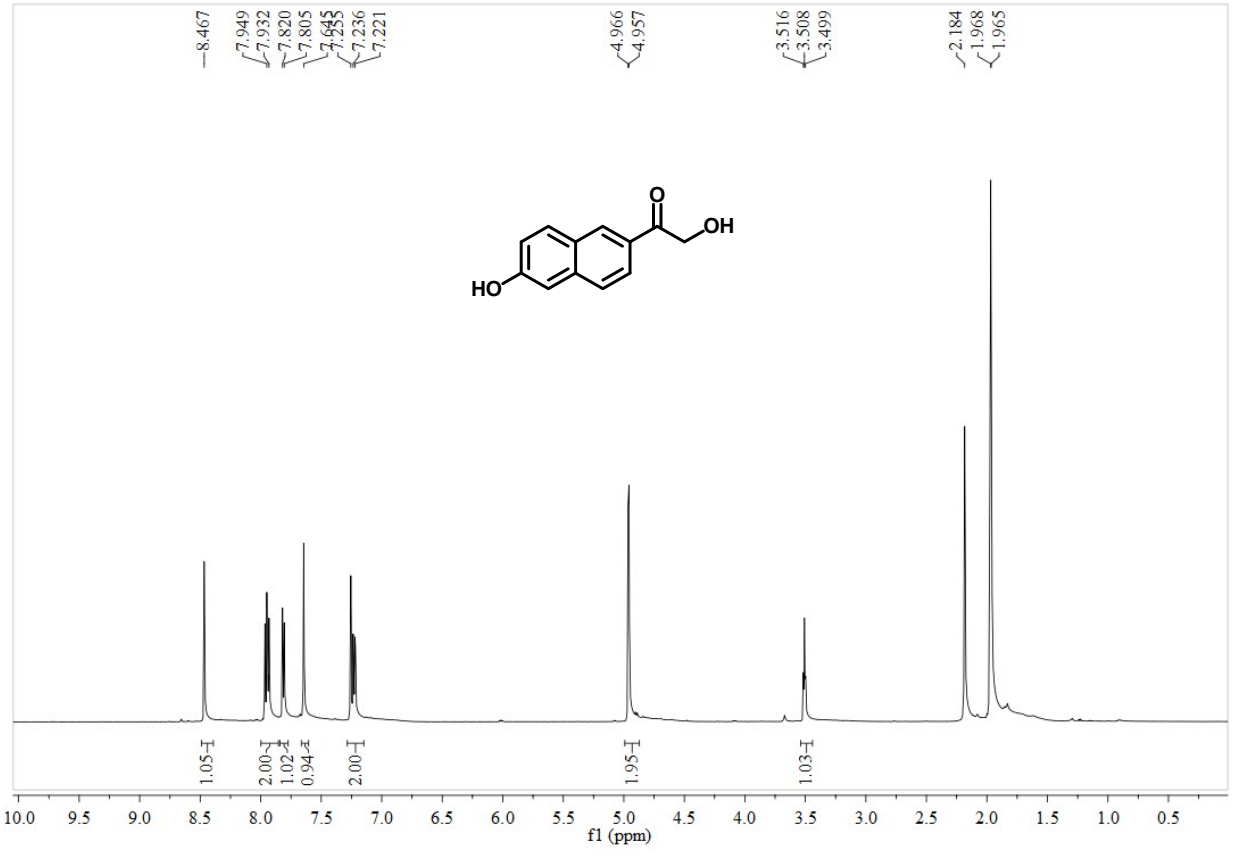



**Fig. S14** Possible α-cleavage photoproduct

Secondly, our pH study indicated as we move towards lower pH the uncaging quantum yield of Naph-Cbl increases (Fig. S15 & Table S2), which is a unique feature of Photo-Favorskii rearrangement.[5]

**15.2. Quantum yield of photolysis of Naph-Cbl at different pH:**

Photolysis of Naph-Cbl ($1\times 10^{-4}$ M) in $CH_3CN$/PBS buffer (3:7) of different pH (5.5, 7.4, 8) was carried out at different time of irradiation (0, 2, 4, 6, 10, 15, 20, 30 min). after each interval 25 µL aliquots were collected and analyzed by RP-HPLC using mobile phase acetonitrile and water (90:10), at a flow rate of 1mL/min (detection: UV 250 nm). Peak areas were determined by RP-HPLC. HPLC peaks area of caged compound versus irradiation time were plotted. Quantum yield of Naph-Cbl was calculated at different pH (Table S2) by using potassium ferrioxalate as an actinometer.

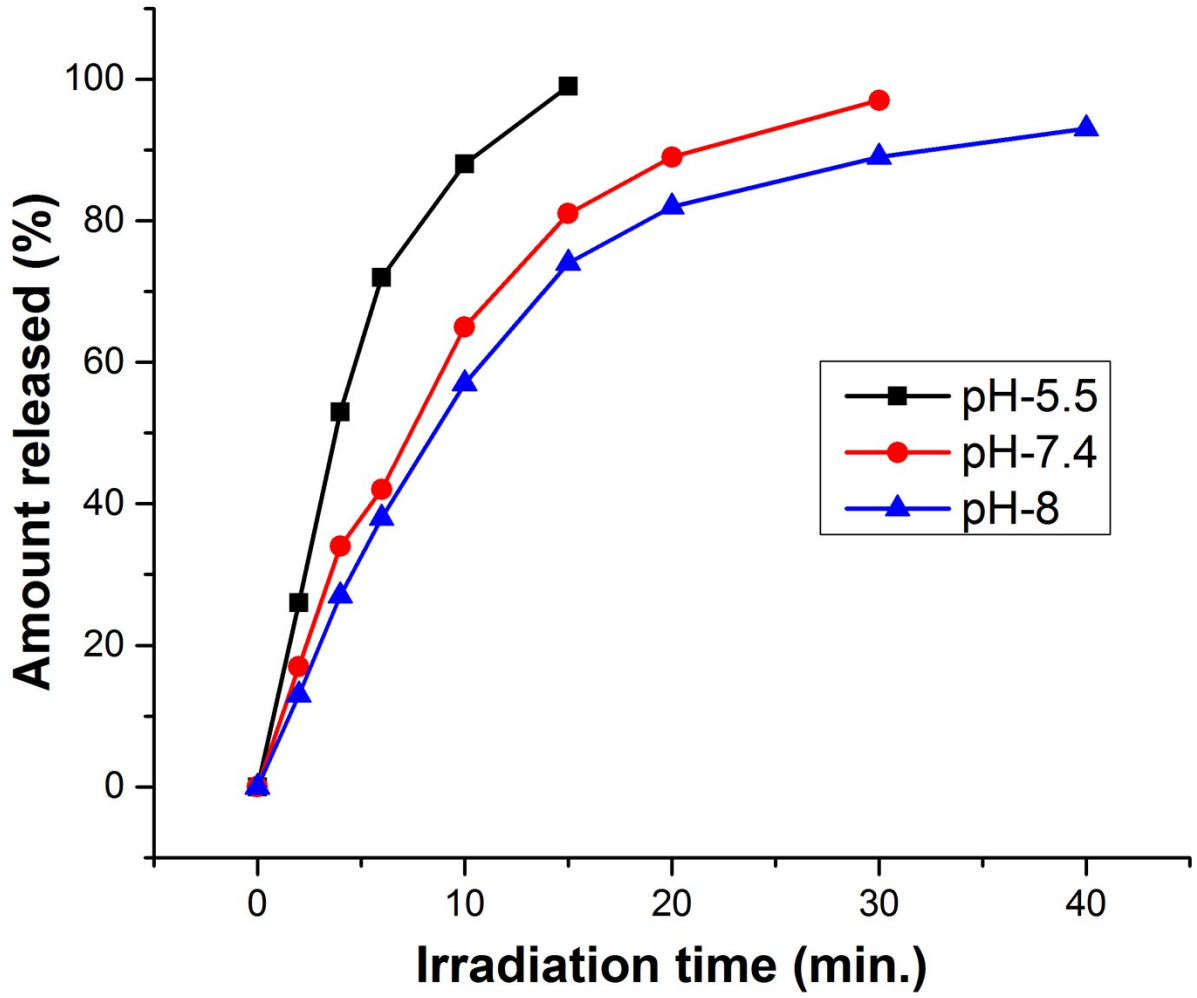



**Fig. S15** The amount of chlorambucil released from Naph-Cbl on photolysis (≥ 365 nm) at different pH.

| pH | Time taken for 90% release | Quantum yield |
|---|---|---|
| 5.5 | 11 | 0.49 |
| 7.4 | 21 | 0.28 |
| 8 | 34 | 0.17 |

**Table S2:** Quantum yield and time taken for 90% uncaging of chlorambucil from Naph-Cbl on photolysis (≥ 365 nm) at different pH.

Third To check that our photorelease proceeds via the triplet excited state, the photolysis of Naph-Cbl was carried out in the presence of the triplet quencher potassium sorbate.[6] We found that the drug release by Naph-Cbl was almost completely arrested. It clearly indicates that the photorelease occurs from the triplet excited state (Fig. S16).

**15.3. Quenching studies in the presence of triplet state quencher (potassium sorbate):**

An experiment was performed to ensure whether the photorelease occurs from singlet or triplet state, in which photolysis of Naph-Cbl (1× 10 –4 M) in $CH_3CN$/PBS buffer of pH 7.4 (3:7) was carried out in presence and absence of 200 μM of triplet quencher potassium sorbate (PoS). At different time of irradiation (0, 2, 4, 6, 10, 15, 20, 30 min) aliquots were collected and analyzed by RP-HPLC using mobile phase acetronitrile and water (90:10), at a flow rate of 1mL/min (detection: UV 250 nm). Peak areas were determined by RP-HPLC. HPLC peaks area of caged compound (from HPLC peak area) versus irradiation time were plotted. The result showed that drug release by Naph-Cbl was almost completely arrested in the presence of 200 μM PoS, clearly indicating that photorelease occurs from the triplet excited state (Fig. S16).

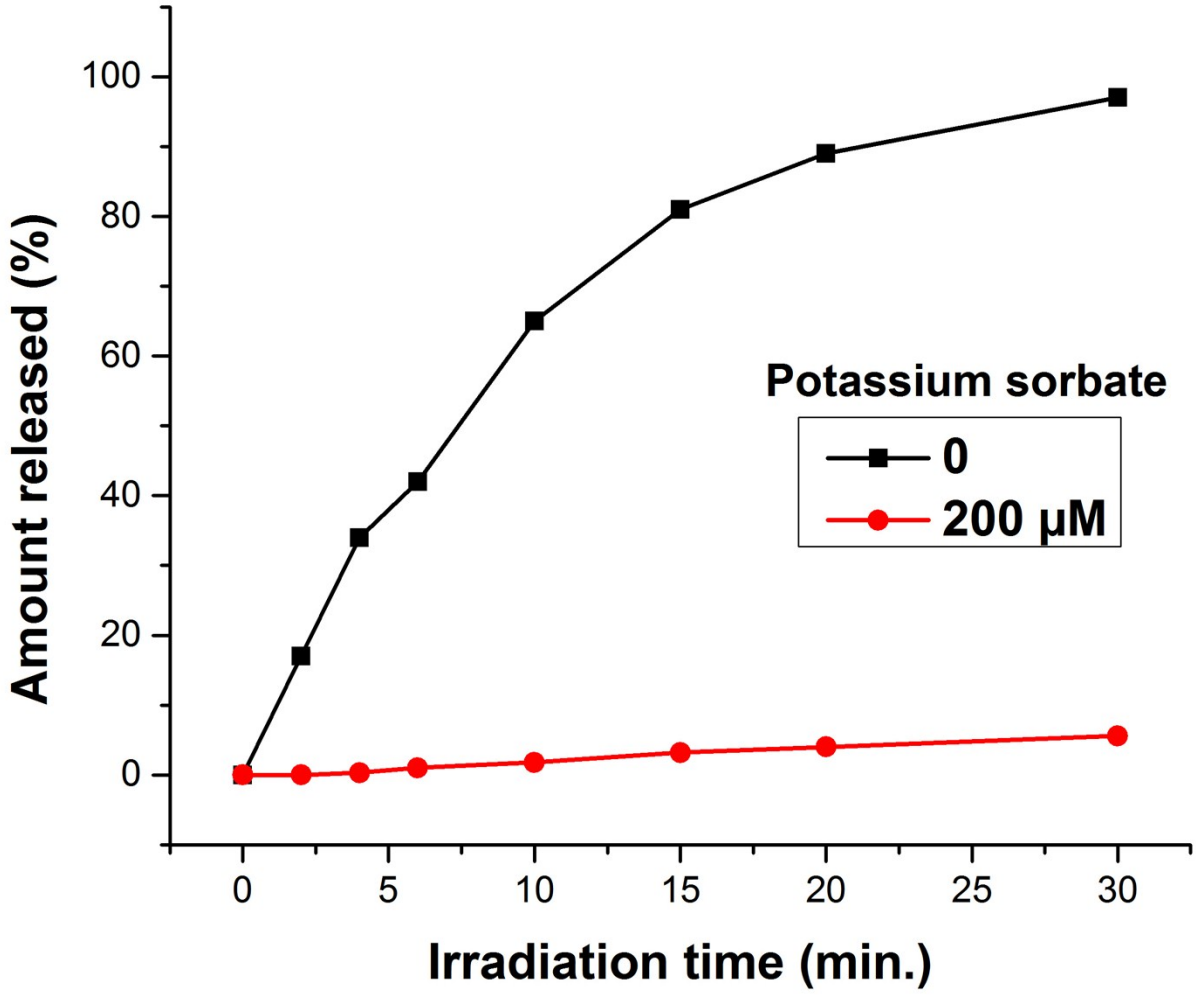
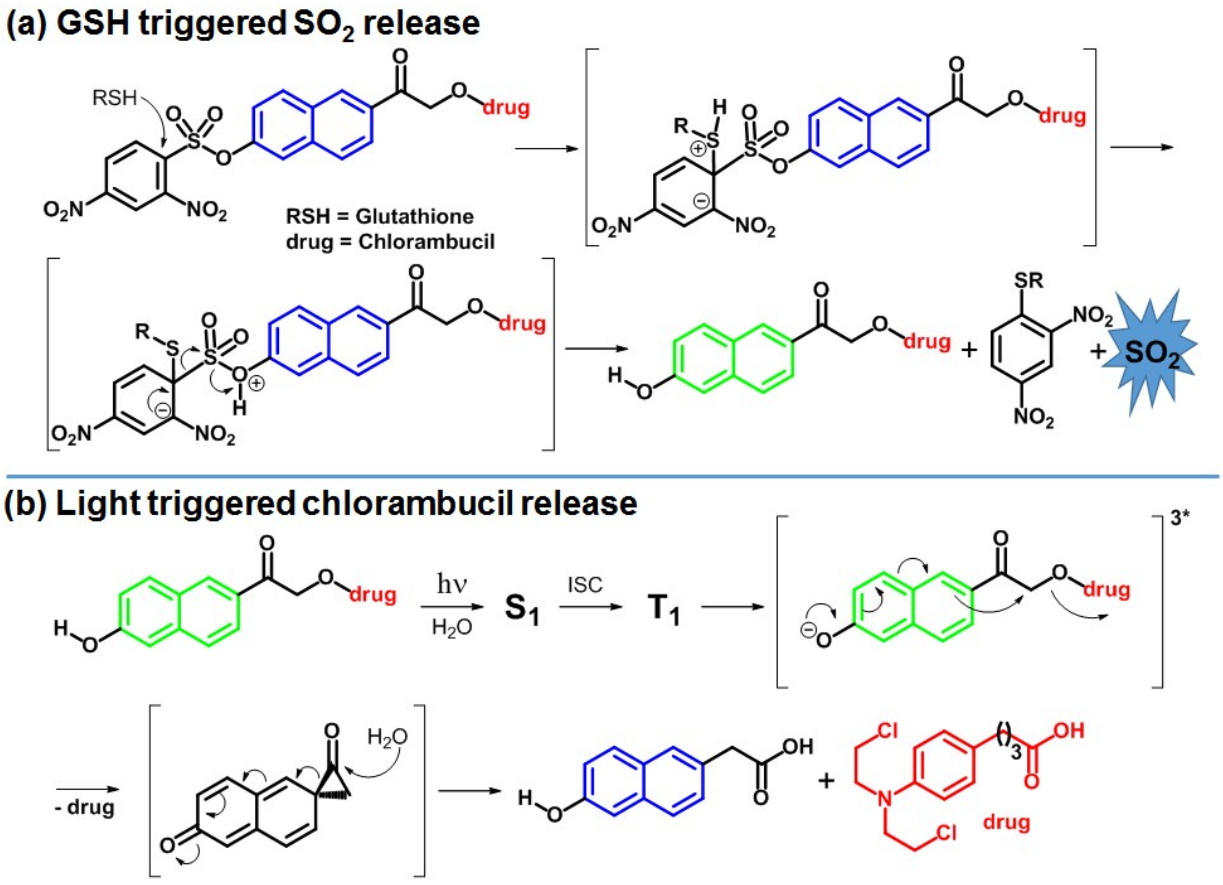



**Fig. S16** Time course of photolysis of Naph-Cbl under irradiation of UV light (≥ 365 nm) in the presence of 200 μM triplet quencher potassium sorbate (PoS).

Based on the above experimental results and literature precedence[7] we proposed a possible mechanism for the photo-uncaging of Cbl from Naph-Cbl in aqueous solution (Scheme S3). Upon irradiation, Naph-Cbl is excited to the singlet state in presence of water then it undergoes efficient intersystem crossing (ISC) to its triplet excited state, which undergoes photo-Favorskii rearrangement to give a putative spirodione intermediate with the concomitant release of the

drug. The spirodione is then subject to hydrolytic ring opening to give 2-hydroxy-6-naphthylacetic acid.




**Scheme 3** (a) Possible mechanism of GSH triggered $SO_2$ release. (b) Possible photorelease mechanism of the Naph-Cbl.

**16. Measurement of fluorescence quantum yields:**

The fluorescence quantum yield (QY) of Naph-Cbl was determined by reference point method.[8] Quinine sulphate in 0.1 M H2SO4 (literature quantum yield: 0.547) was used as a standard sample to calculate the QY. Naph-Cbl was dissolved in $CH_3CN$/PBS buffer of pH 7.4 (3:7). The absorbance values of the solutions at the excitation wavelength were measured with UV–Vis spectrophotometer. Photoluminescence (PL) emission spectra were recorded by Hitachi F-7000 fluorescence spectrophotometer at an excitation wavelength of 350 nm and 320 for Quinine sulphate and Naph-Cbl respectively.

$$\frac{(\Phi)s}{(\Phi)r} = \frac{(A)s}{(A)r} X \frac{(Abs)r}{(Abs)s} X \frac{(\eta)s}{(\eta)r}$$

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

| **compounds** | **Fluorescent quantum yield ($\varphi_f$)** |
|---|---|
| DNs-Naph-Cbl | 0.035 |
| Naph-Cbl | 0.19 |
| Photoproduct<br><br>(2-hydroxy-6-naphthylacetic acid) | 0.23 |

**Table S3:** Fluorescence quantum yields of DNs-Naph-Cbl, Naph-Cbl and the photoproduct in 3:7 acetonitrile/PBS solvent system

**17. 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.[9,10] Solution (0.006 M) of potassium ferrioxalate was irradiated using 125W medium pressure Hg lamp as UV light source (≥365 nm) and 1M CuSO4 solution as UV cut-off filter. At regular interval of time (3 min), 1mL 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 at 510 nm, which is found to be similar to reported value. Using the known quantum yield for potassium ferrioxalate actinometer at 363.8 nm, the number of $Fe^{2+}$ ion formed during photolysis and the fraction of light absorbed by the actinometer, the incident photon flux ($I_0$) at 365 nm of the 125W Hg lamp was determined as $1.55 \times 10^{17}$ photons $s^{-1}$ $cm^{-2}$.

**18. Photochemical quantum yield determination for Naph-Cbl:**

A 20 mL solution of Naph-Cbl ($1\times10^{-4}$ M) was prepared in $CH_3CN$/PBS buffer of pH 7.4 (3:7). Nitrogen was passed throughout the solution for 30 min and irradiated using 125 W medium pressure Hg lamp as light source (≥ 410 nm) and 1M $NaNO_2$ solution as UV cut-off filter. At regular interval of time, 20μl of the aliquots was taken and analyzed by RP-HPLC using mobile phase acetonitrile , at a flow rate of 1mL/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 ester is less than 10% of the initial area. Based on HPLC data for caged compound, the natural logarithm of the concentration of caged compound (lnC) (from HPLC peak area) versus irradiation time was plotted. 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 compound was calculated using equation (1)

$$\mathbf{(\Phi)CG = (\Phi)act \times [(kp)CG/(kp)act]\times[Fact/FCG]} \text{ ---------- } (1)$$

Where, the subscript 'CG' and 'act' denote caged compound and actinometer, respectively. Φ is the relative photolysis quantum yield, kp is the photolysis rate constant and F is the fraction of light absorbed. Potassium ferrioxalate was used as an actinometer.[9]

**19. Antiproliferative activity assays:**

**19.1 Cell lines:**

Human breast carcinoma (MDA-MB-231) was obtained from the National Centre for Cell Science (NCCS), Pune, India, and chemo-resistant MDA-MB-231 was prepared by following our previous report.[11] Both the cell line maintained in dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Cells were cultured at 37 °C in a CO2 incubator (Thermo Fisher Scientific, USA). S-14

**19.2 Real-time cellular uptake and localization study:**

To study the cellular uptake and localization of DNs-Naph-Cbl conjugate, MDA-MB-231cells were first seeded in a 6-well plate and then incubated with 75µM of DNs-Naph-Cbl for 4 h at 37 °C in a CO2 incubator. Thereafter cells were fixed using 4% paraformaldehyde for 5-10 min and washed thrice with phosphate-buffer saline (PBS). Imaging was done using confocal microscopy (CLSM; Olympus FV 1000 attached to an inverted microscope 1X 81, Japan).

**19.3 In Vitro Cytotoxicity assay:**

**19.3.1 Before treatment:**

The cytotoxicities of DNs-Naph-Cbl,chlorambucil (Cbl) were determined without any treatment on MDA-MB-231cells using MTT assay. Cells (5000cells/well of a 96-well plate) were treated for 48 h with different concentrations (0.1–250 μM) and cell viability was determined by MTT assay (Mossman, 1983), measuring absorbance at 550nm using a microplate reader (Bio-Rad 550). Cell viability was calculated using the formula, Viability (%) = 100 × A2/A1; (Where A2 = Absorbance of the treated cell; A1 = Absorbance of the control cells).

**19.3.2 After GSH and light irradiation:**

Cancer cell line (5000 cells/well of a 96-well plate) was treated with different concentrations of DNs-Naph-Cbl, chlorambucil (Cbl) (0.1–250μM) and incubated for 4 h at 37 °C in $CO_2$ incubator. Then, cells pretreated with DNs-Naph-Cbl and chlorambucil (Cbl) were treated with GSH and incubated for 12 h. Thereafter, the cells were irradiated by light, (≥ 365 nm) for 20 min (keeping the culture plate 10 cm away from the light source) using UV-visible lamp (Bangalore GeneiPvt. Ltd.) under aseptic condition. After irradiation, the cells were again incubated for 24 h and cell viability was measured by MTT assay (Mossman, 1983). Cell viability was calculated as described above.

**19.4 MTT assay:**

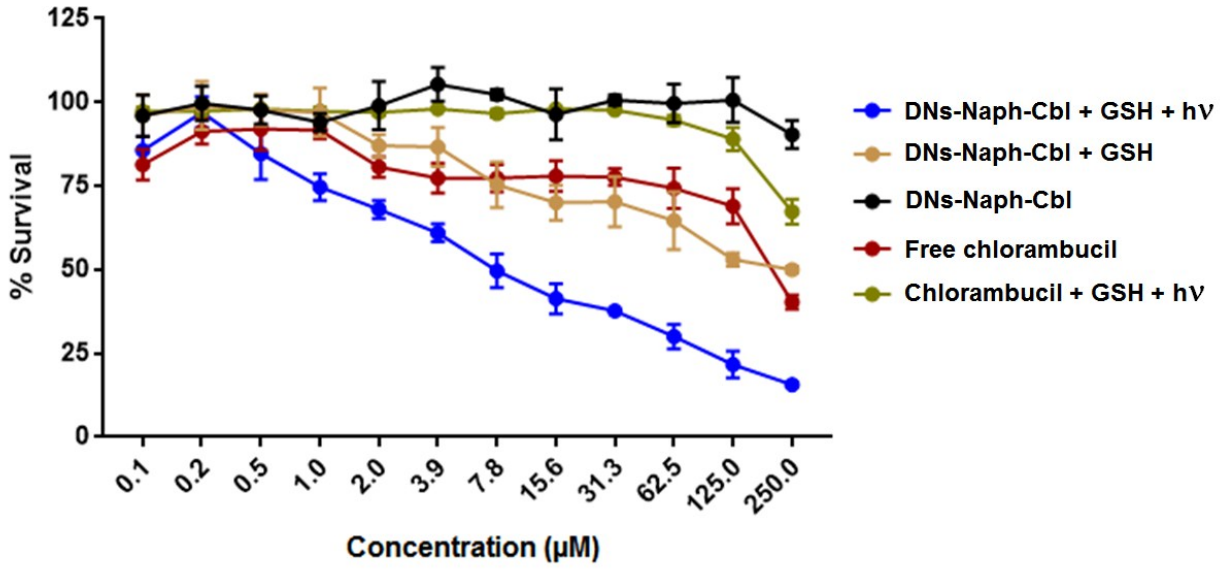



**Fig. S17** Cell cytotoxicity analysis for MDA-MB-231 cell line examined by MTT assay.

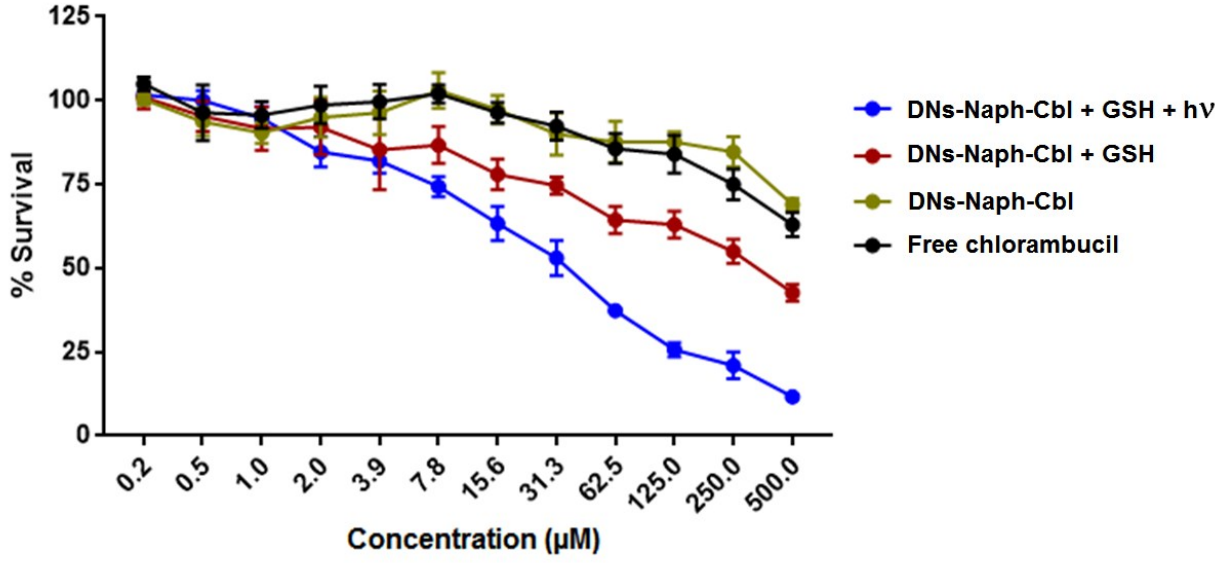



**Fig. S18** Cell cytotoxicity analysis for chemo-resistant MDA-MB-231 cell line examined by MTT assay.

**19.5 Fluorescence microscopy**

Orthogonal drug release from DNs-Naph-Cbl after GSH addition and light (≥ 365 nm) irradiation was also studied by fluorescence microscopy. MDA-MB-231 cells were treated with 75μM of ANPD-X and put aside for 4 h at 37 °C. After that, cells were treated with GSH. Then, cells were irradiated with UV-visible light (≥365 nm) for 20 min. Thereafter, the plate was incubated for 24 h at 37 °C in a CO2 incubator. Fluorescent images were captured using a high-performance charge-coupled device (CCD) camera (1X81, Olympus).

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