Supporting Information

Visible and NIR-Light Photoactivatable *o*-Hydroxycinnamate System for Efficient Drug Release with Fluorescence Monitoring

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1. Instruments and Methods

1.1 Thin layer chromatography (TLC) and column chromatography

TLC was carried out on aluminium plates coated with silica gel mixed with fluorescent indicator. The purification of synthesized compounds was performed with silica gel (60-120 mesh) column chromatography.

1.2 NMR spectroscopy

^{1}H and ^{13}C NMR spectra were acquired on a Bruker 500 MHz spectrometer in $CDCl_3$ or DMSO-d_6 at ambient temperature with tetramethylsilane (TMS) as an internal standard. NMR standards used were as follows: (^{1}H-NMR) $CDCl_3$ = 7.260 ppm; DMSO-d_6 = 2.50 ppm. CD_3OD = 3.31 ppm (^{13}C-NMR) $CDCl_3$ = 77.00 ppm; DMSO-d_6 = 39.520 ppm. All chemical shifts (δ) are reported in ppm relative to TMS. Spin multiplicities were reported as a singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), triplet of doublets (td), doublet of doublet of doublets (ddd), multiplet (m) and broad (br) with coupling constant (*J*) reported in Hz.

1.3 Mass spectrometry

Electrospray ionization mass spectra (ESI-MS) were obtained using a Waters make ESI-MS model synapt G2 high-definition mass spectrometry.

1.4 FT-IR spectroscopy

Fourier transform-Infrared (FT-IR) spectra were measured using IR Affinity-1S (Shimadzu, Kyoto, Japan) FT-IR spectrophotometer equipped with a single reflection attenuated total reflectance (ATR) accessory. The IR spectra were recorded from 4000 to 450 cm^{-1} using a resolution of 4 cm^{-1} with 45 scans. In IR absorption spectra, the shapes and signal intensities (height) of peaks (bands) are denoted by the following abbreviations: br = broad, vs = very strong, s = strong, m = medium and w = weak.

1.5 UV-vis spectroscopy

UV-vis absorption spectra were recorded using a SpectraMax M2 plate reader (Molecular Devices) at 298 K from 800 to 200 nm.

1.6 Emission spectroscopy

Emission spectra were measured on SpectraMax M2 plate reader (Molecular Devices).

2. Characterization

2.1 UV-visible spectra

UV-visible absorption spectra were recorded using SpectraMax M2 plate reader (Molecular Devices) at 298 K from 250 to 600 nm. The absorption spectra of compound **2** (10 µM), and ***o*HC-GMC** (10 µM) were measured in 1:9 DMSO and PBS (100 mM, pH 7.3) at room temperature. All UV-visible

spectroscopic measurements were performed in a quartz cuvette with an optical path length of 10 mm. The wavelength was reported in nanometers (nm).

2.2 Emission spectra

Emission spectra were recorded using a SpectraMax M2 plate reader (Molecular Devices). Fluorescence emission spectra were recorded in a quartz cuvette with an optical path length of 10 mm. The emission spectra of ***o*HC-GMC** (50 μM) upon photoirradiation was determined in 1:9 DMSO and PBS (100 mM, pH 7.3) at 25 ºC with an excitation wavelength of 385 nm (λ_{em} = 475 nm).

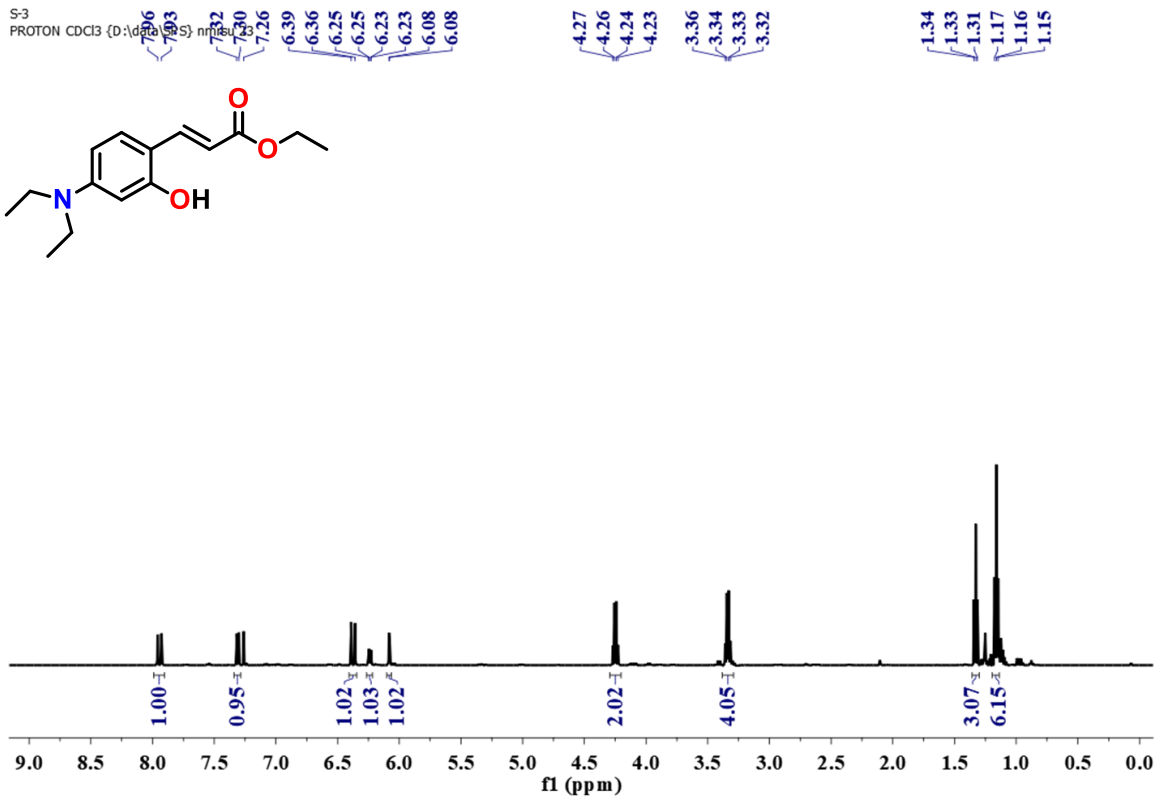
2.3 Determination of lipophilicity

The lipophilicity (log $P_{O/W}$) of ***o*HC-GMC** was determined by the standard flask-shaking method as reported in the literature.[1,2] Here, log $P_{O/W}$ = log (C_O/C_W) is defined as the logarithmic ratio of the ***o*HC-GMC** concentration in n-octanol to that in the water phase. Briefly, 0.4 mg of ***o*HC-GMC** was taken in 3 mL of n-octanol and water (1:1 v/v) and mixed vigorously for 24 h. The mixture was then kept in a stationary state for an additional 24 h to separate both n-octanol and water phases and then centrifuged at 2500 rpm for 10 min and the supernatant was isolated. The concentration of ***o*HC-GMC** was then determined by UV-vis spectroscopy in both the n-octanol (C_O) and water (C_W) phases to estimate the log $P_{O/W}$ values.

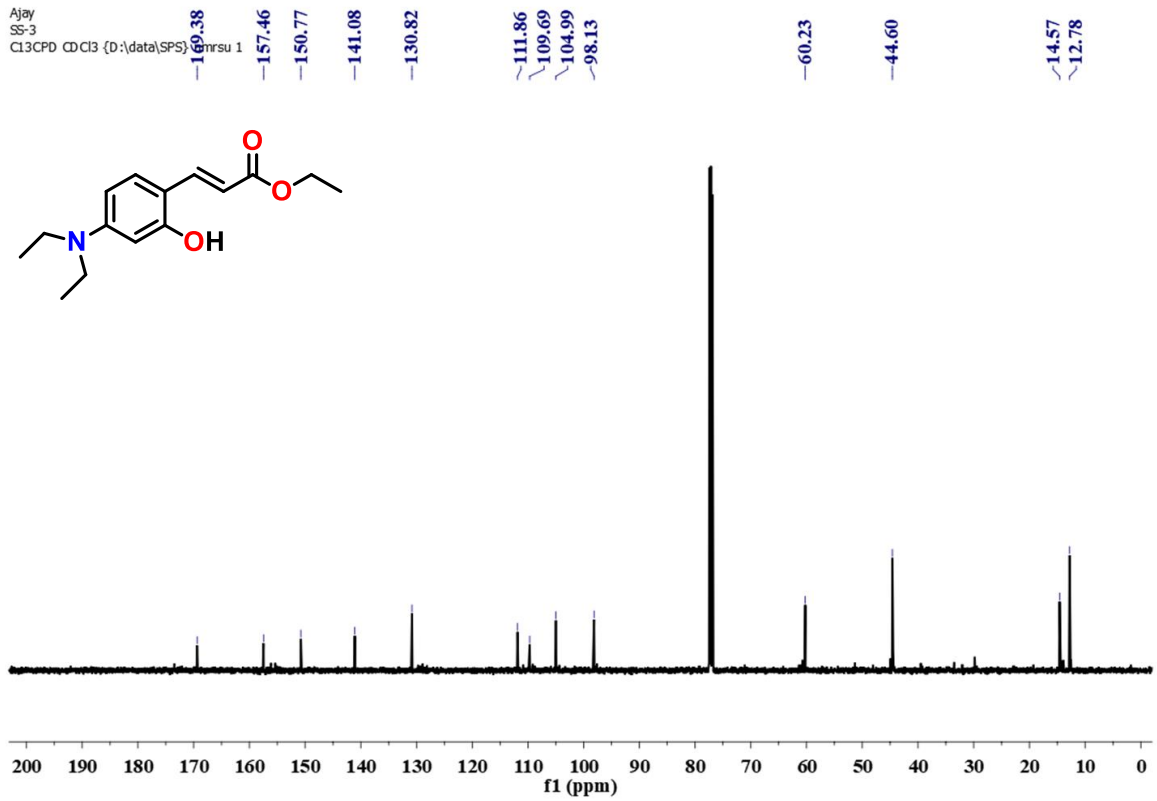
Table S1 Photophysical and photochemical data of ***o*HC-GMC**

Compound	λ_{abs} [a] /nm ($\varepsilon/10^3$ M^{-1} cm^{-1})	Φ_u [b]	δ_u(GM) [c] 800 nm	Log $P_{o/w}$ [d]
***o*HC-GMC**	266 (24), 380 (75)	0.028	1.61	2.11

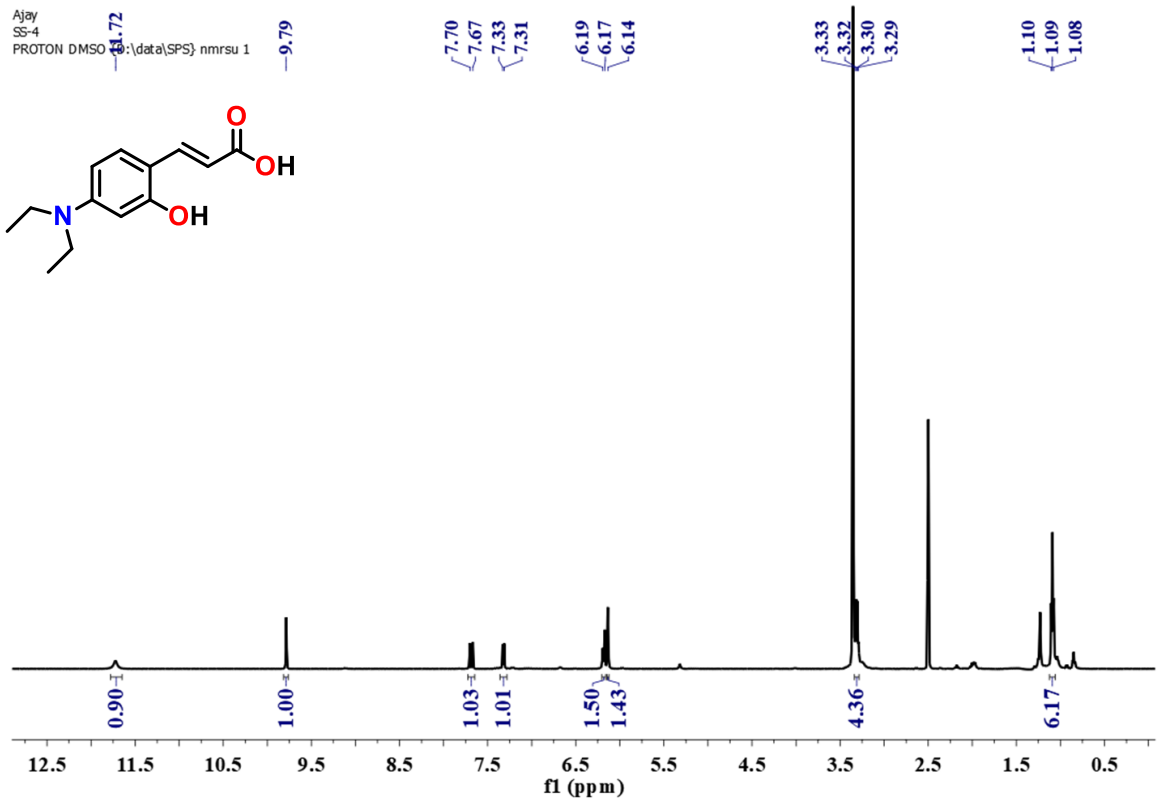
[a]Absorption spectra were recorded in 1:9 DMSO/phosphate buffer (100 mM, pH 7.3). [b]Photochemical quantum yield of (*E*) to (*Z*) photoisomerization after one-photon excitation at visible light (400-700 nm, 11 mW). [c]Two-photon uncaging cross section for (*E*) to (*Z)* photoisomerization with two-photon excitation at 800 nm. [d]Lipophilicity was determined by measuring the partition coefficient of the compound in n-octanol/water.



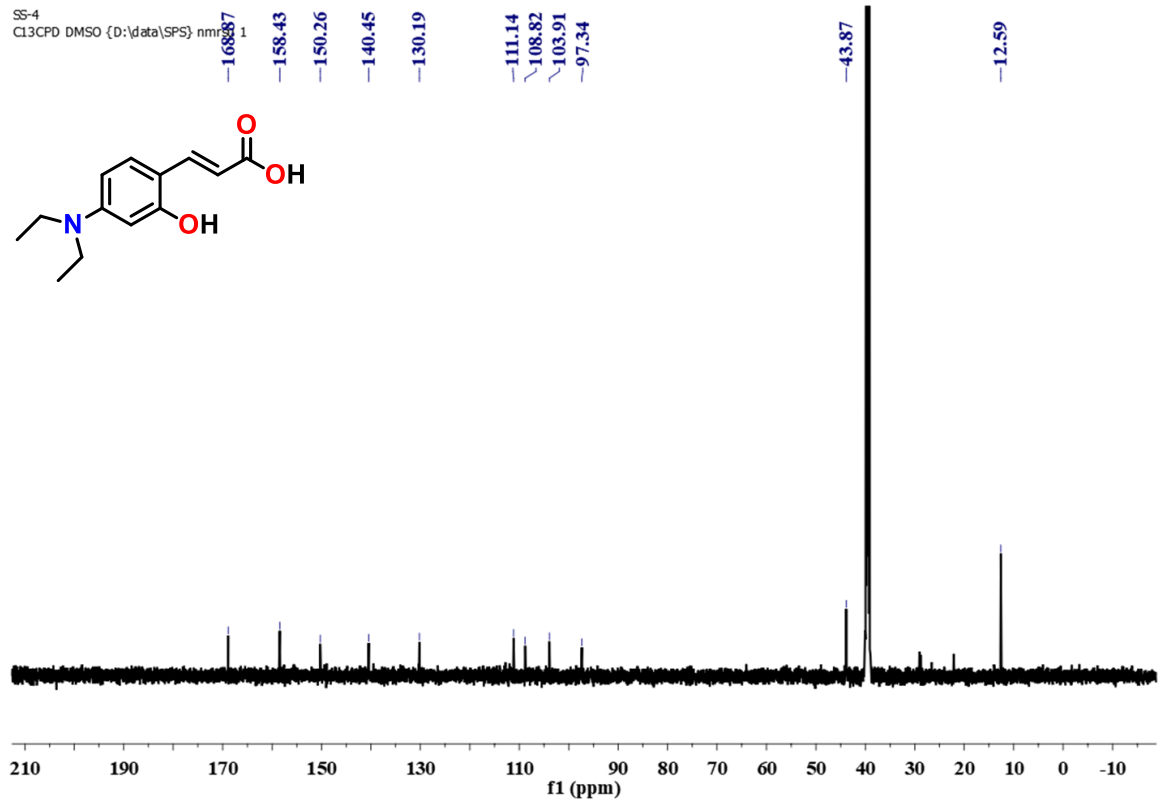
^{1}H-NMR spectrum of compound **1** in $CDCl_3$ at 298K.



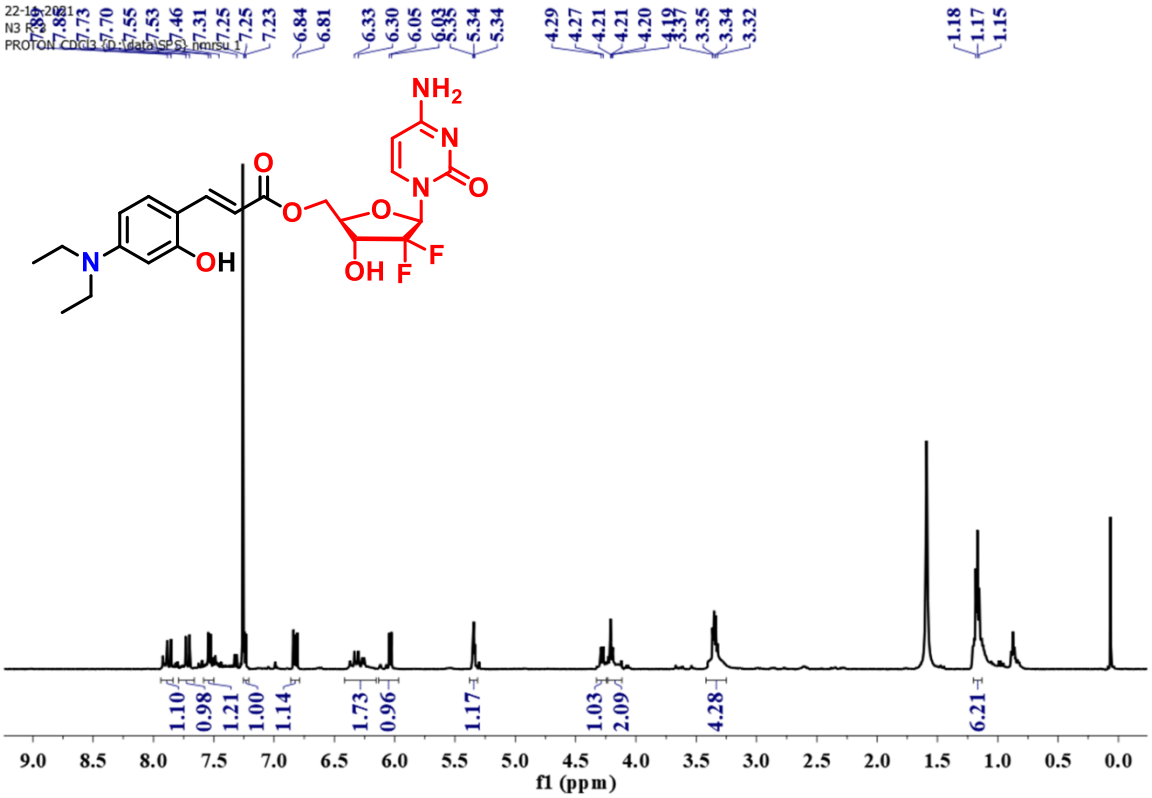
^{13}C-NMR spectrum of compound **1** in $CDCl_3$ at 298K.



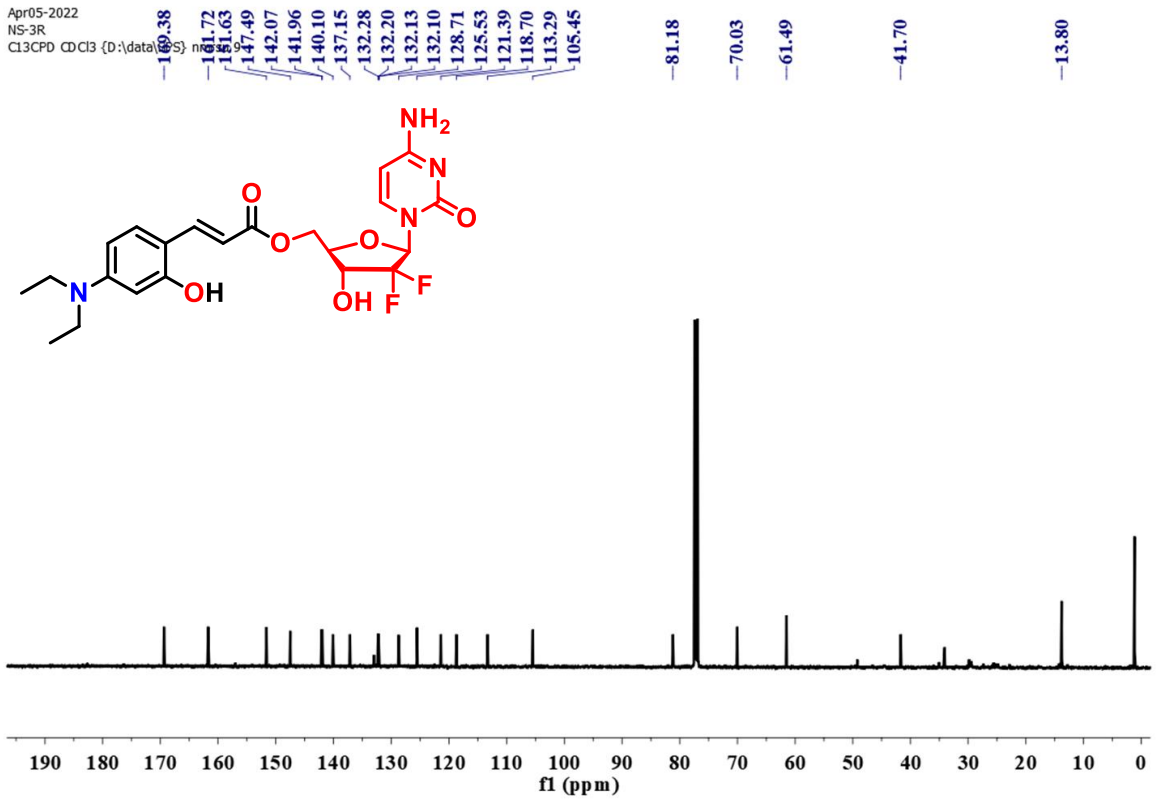
^{1}H-NMR spectrum of compound **2** in DMSO-d_6 at 298K.



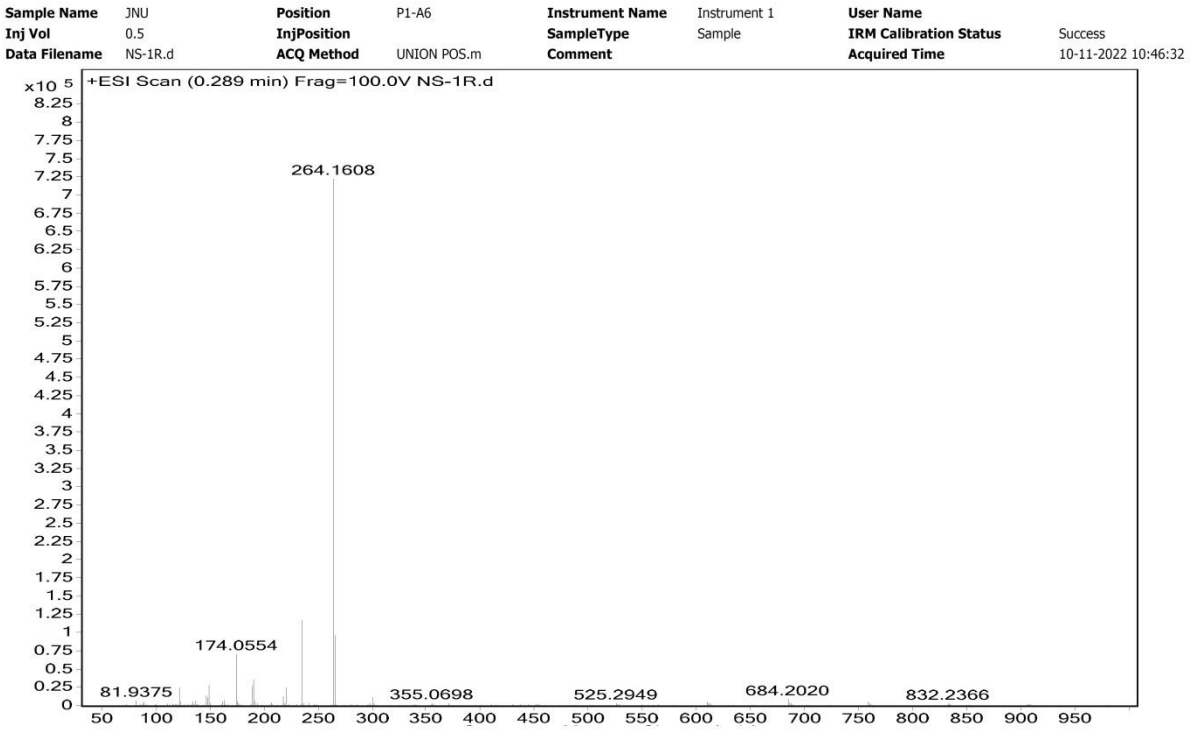
^{13}C-NMR spectrum of compound **2** in DMSO-d_6 at 298K.



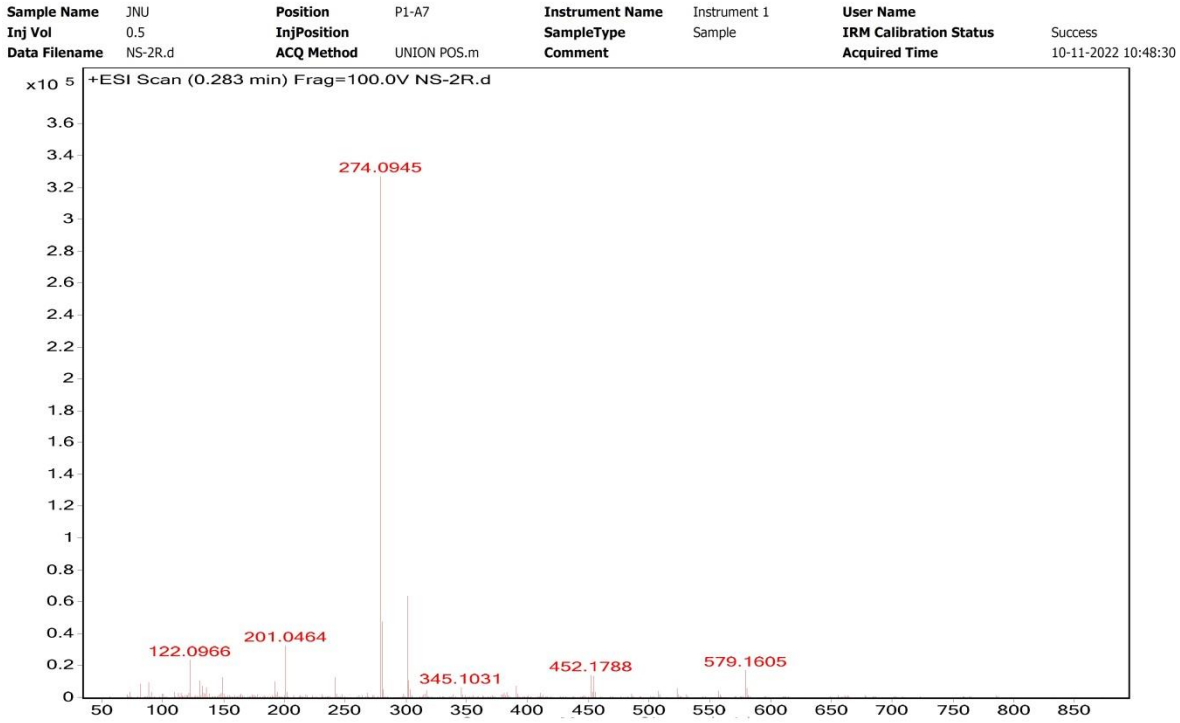
^{1}H-NMR spectrum of ***o*HC-GMC** in $CDCl_3$ at 298K.



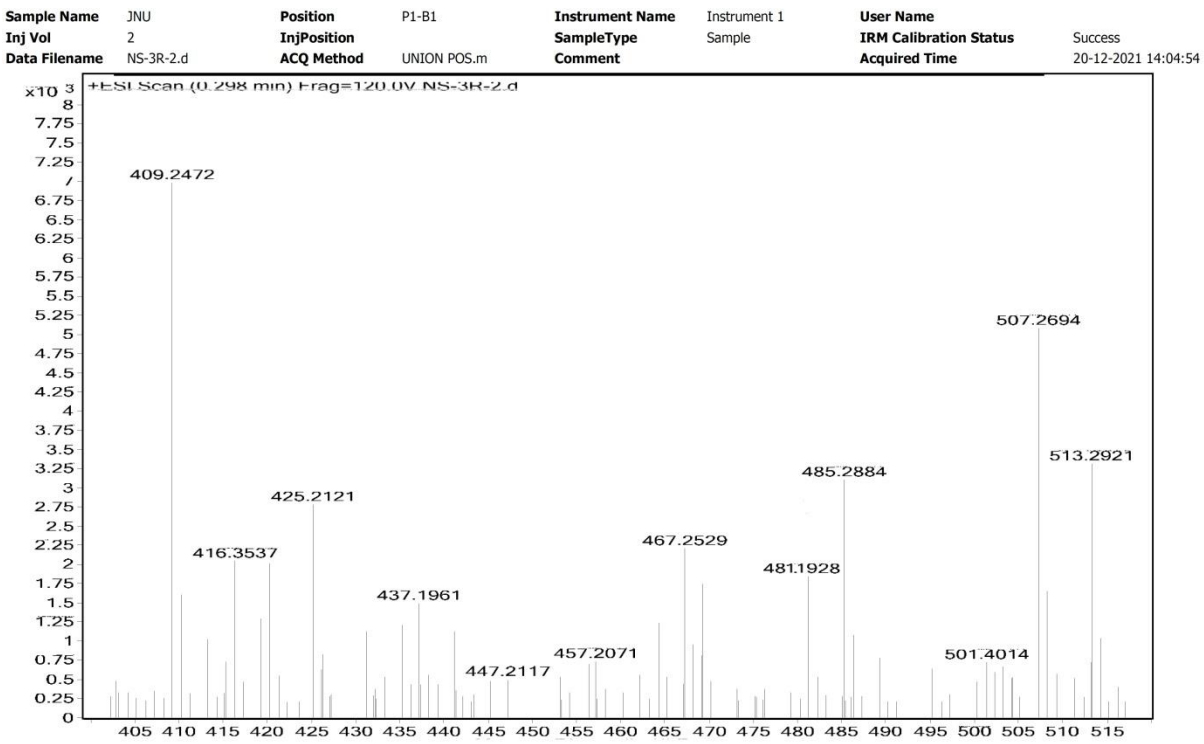
^{13}C-NMR spectrum of ***o*HC-GMC** in $CDCl_3$ at 298K.



ESI-HRMS spectrum of **1** in DCM/MeOH showing the peak at 264.1608 (*m/z*) assignable to $[M+H]^+$ at 298K.



ESI-HRMS spectrum of **2** in DCM/MeOH showing the peak at 274.0945 (*m/z*) assignable to $[M+K]^+$ at 298K.



ESI-HRMS spectrum of ***o*HC-GMC** in DCM/MeOH showing the peak at 481.1928 (*m/z*) assignable to $[M+H]^+$ at 298K.

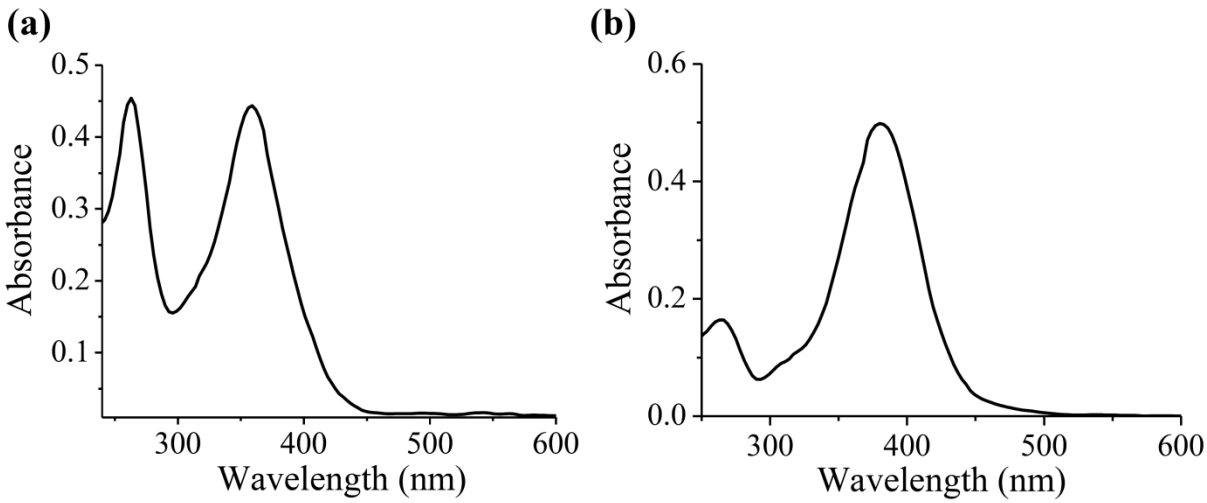


Fig. S1 Absorption spectra of **(a)** compound **2** (10 μM) and **(b)** ***o*HC-GMC** (10 μM) in 1:9 DMSO and phosphate buffer (100 mM, pH 7.3) at 25 ºC.

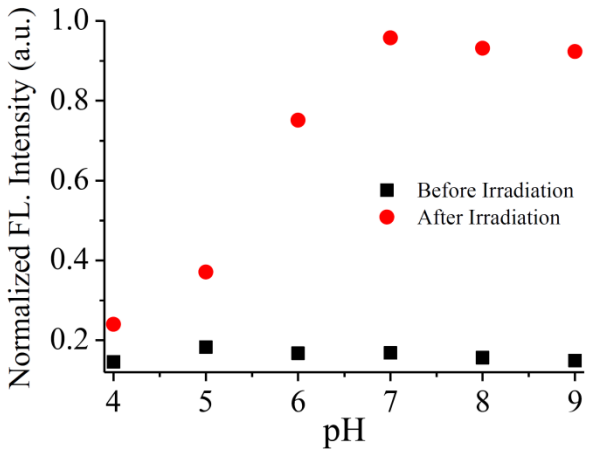


Fig. S2 Effect of pH for the drug release of ***o*HC-GMC** (50 μM) in 1:9 DMSO/PBS (100 mM) before (■) and after (●) photoirradiation for 10 min with visible light (400-700 nm, 11 mW). The emission intensity of formed coumarin reporter was determined at 475 nm upon excitation at 385 nm.

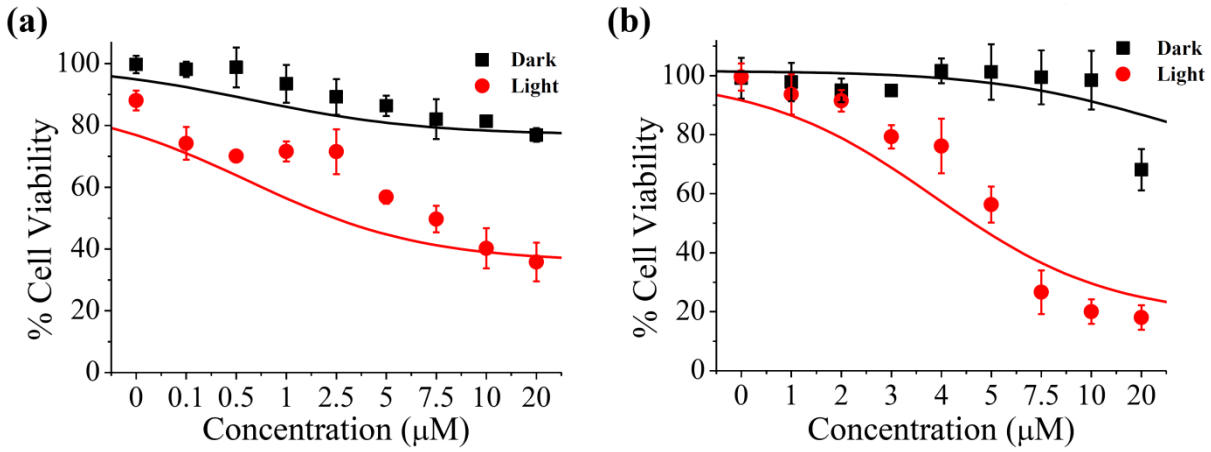


Fig. S3 Cell viability as determined by MTT assay in **(a)** A549 cells and **(b)** MCF7 cells treated with different concentrations of ***o*HC-GMC** prodrug in the absence (■) or presence (●) of visible light (400-700 nm, 11 mW) for 30 min irradiation.

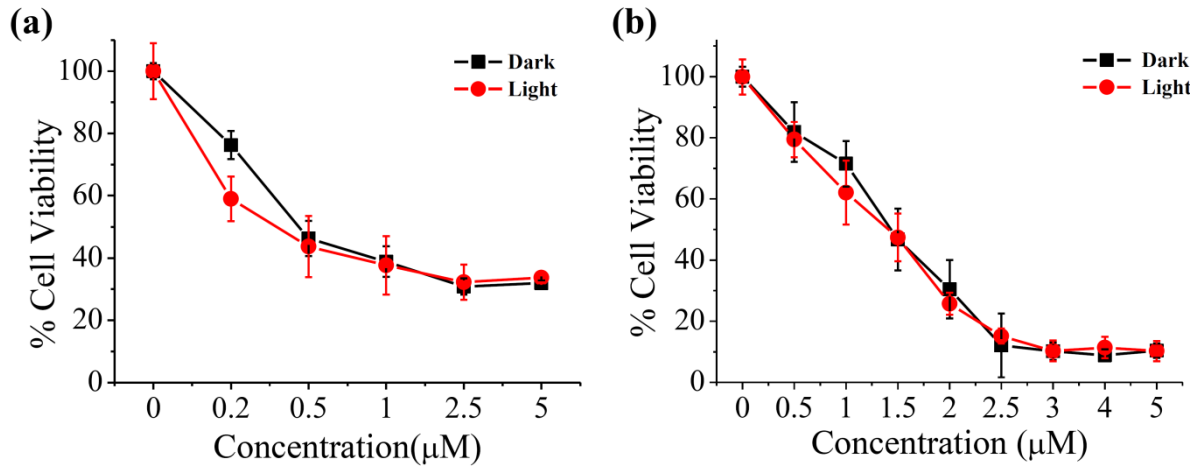


Fig. S4 Cell viability as determined by MTT assay in **(a)** A549 cells and **(b)** MCF7 cells treated with different concentrations of **GMC** in the absence (■) or presence (●) of visible light (400-700 nm, 11 mW) for 30 min irradiation.

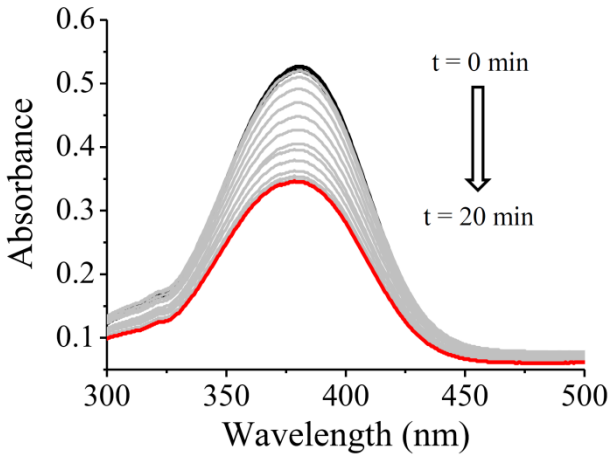


Fig. S5 Absorption spectra of ***o*HC-GMC** (10 μM) in 1:9 DMSO/PBS (100 mM, pH 7.3) upon irradiation with two-photon NIR femtosecond laser light (800 nm, 50 mW) at 25 ºC. The sample solution was exposed to NIR light each time before measuring the absorption spectra. The absorption spectra of ***o*HC-GMC**, before (black line) and after 20 min irradiation (red line), are highlighted.

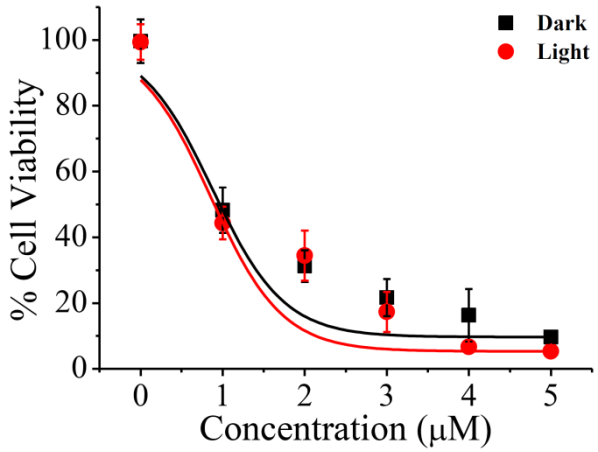


Fig. S6 Cell viability as determined by MTT assay in A549 cells treated with different concentrations of **GMC** in the absence (■) or presence (●) of two photon NIR light (800 nm, 50 mW) for 10 min irradiation.

References

1. N. Singh, A. Gupta, P. Prasad, R. K. Sah, A. Singh, S. Kumar, S. Singh, S. Gupta and P. K. Sasmal, *J. Med. Chem.*, 2021, **64**, 17813–17823.

2. A. Gupta, P. Prasad, S. Gupta and P. K. Sasmal, *ACS Appl. Mater. Interfaces*, 2020, **12**, 35967–35976.