Electronic Supplementary Information (ESI)

Photoactivatable dihydroalkaloids for cancer cell imaging and chemotherapy with high spatiotemporal resolution

Xia Ling,[‡ab] Letao Huang,[‡b] Youzhen Li,[a] Qing Wan,[b] Zhiming Wang,[b] Anjun Qin,[b] Meng Gao,*[ab] Ben Zhong Tang*[bc]

[a] National Engineering Research Center for Tissue Restoration and Reconstruction, Key Laboratory of Biomedical Engineering of Guangdong Province, Key Laboratory of Biomedical Materials and Engineering of the Ministry of Education, Innovation Center for Tissue Restoration and Reconstruction, South China University of Technology, Guangzhou 510006, China School of Medicine, South China University of Technology, Guangzhou 510006, China. E-mail: msmgao@scut.edu.cn

[b] AIE Institute, Center for Aggregation-Induced Emission, Key Laboratory of Luminescence from Molecular Aggregates of Guangdong Province, State Key Laboratory of Luminescent Materials and Devices, South China University of Technology, Guangzhou 510640, China

[c] Department of Chemistry and Hong Kong Branch of Chinese National Engineering Research Center for Tissue Restoration and Reconstruction, The Hong Kong University of Science & Technology, Clear Water Bay, Kowloon, Hong Kong, China. E-mail: tangbenz@ust.hk

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Materials and Chemicals

DHCHE and DHSAN were prepared according to a modified literature method.[1] Chelerythrine and sanguinarine were purchased from Chengdu Herbpurify Co., Ltd; D_2O, $CDCl_3$ and DMSO-*d*$_6$ were purchased from Sigma-Aldrich; Cucurbit[7]uril was purchased from ShangHai SuperLan Chemical Tech Centre; H_2O_2 (30 wt.%), MeOH and other solvents were purchased from Guangzhou Chemical Reagent Factory without further purification. NaN_3 was purchased from Tianjin Fuchen Chemical Reagents Factory. THF was distilled from sodium under dry nitrogen prior to use.

Dulbecco's Modified Essential Medium (DMEM) was purchased from Gibco (Life Technologies). Ultra pure water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Hoechst 33342 was purchased from Sigma. Phosphate buffered saline (PBS), fetal bovine serum (FBS), Nile Red, penicillin and streptomycin were purchased from Thermo Fisher Scientific. ER-Tracker Red was purchased from Beyotime Biotechnology.

Equipment and Methods

The UV-Vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer, medium scanning rate, and quartz cuvettes of 1 cm path length. The photoluminescence spectra were recorded on a Horiba Fluoromax-4 spectrofluorometer. The absolute fluorescence quantum yield was measured using a Hamamatsu quantum yield spectrometer C11347 Quantaurus_QY. The fluorescence lifetime was measured using a Hamamatsu Compact Fluorescence Lifetime Spectrometer C11367. The ^1H and ^{13}C NMR spectra were measured on a Bruker AV 400 NMR spectrometer. The high resolution mass spectra (HRMS) were recorded on a Bruker maxis impact mass

spectrometer operated in ESI model. The single crystal of CHE was grown from hexane/MeOH via solute solution diffusion method, the single crystals of DHCHE, DHSAN were grown from hexane/dichloromethane via solute solution diffusion method. Single crystal X-ray diffraction intensity data were collected on a Bruker–Nonices Smart Apex CCD diffractometer with graphite monochromated MoKα radiation. Processing of the intensity data was carried out using the SAINT and SADABS routines, and the structure and refinement were conducted using the SHELTL suite of X-ray programs (version 6.10). Confocal lasing scanning microscopic (CLSM) images were obtained on the confocal microscope (Zeiss Laser Scanning Confocal Microscope; LSM710). Automated cell counter (Countess II) was employed for cell counting. The cell viability assay was conducted on a microplate reader (Tecan Infinite M200 PRO).

NMR and HRMS data of CHE

^{1}H NMR (D_2O, 400 MHz): δ 9.32 (s, 1H), 7.79 (dd, J_1 = 25.2 Hz, J_2 = 9.2 Hz, 2H), 7.55 (d, J = 8.8 Hz, 1H), 7.41 (s, 1H), 7.35 (d, J = 8.8 Hz, 1H), 6.72 (s, 1H), 6.16 (s, 2H), 4.50 (s, 3H), 4.14 (s, 3H), 4.03 (s, 3H); ^{13}C NMR (D_2O, 100 MHz): δ 148.6, 148.2, 147.9, 147.2, 145.8, 131.2, 130.9, 129.3, 125.7, 124.1, 119.7, 118.6, 117.1, 116.1, 108.1, 105.5, 105.2, 103.2, 102.9, 52.0. HRMS (ESI): m/z $[M]^+$ calcd for $C_{21}H_{18}NO_4$, 348.1236; found, 348.1240.

NMR and HRMS data of SAN

^{1}H NMR (D_2O, 400 MHz): δ 9.28 (s, 1H), 7.66 (dd, J_1 = 14.0 Hz, J_2 = 5.6 Hz, 2H), 7.61 (d, J = 8.8 Hz, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.38 (s, 1H), 6.90 (s, 1H), 6.44 (s, 2H),

6.21 (s, 2H), 4.46 (s, 3H), 3.79 (s, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 148.6, 148.2, 147.9, 147.2, 145.8, 131.2, 130.9, 129.3, 125.7, 124.1, 119.7, 118.6, 117.1, 116.1, 108.2, 105.5, 105.2, 103.2, 102.9, 52.0. HRMS (ESI): m/z $[M]^+$ calcd for $C_{20}H_{14}NO_4$, 332.0923; found, 332.0923.

Synthesis of DHCHE

Chelerythrine (120 mg, 0.31 mmol) was first dissovled in MeOH (20 mL) and then added with $NaBH_4$ (100 mg, 2.7 mmol), the mixture was stirred at room temperature for 30 min. After completion of the reaction, the precipitation was filtered, washed with MeOH and H_2O, and dried under vacuum to yield DHCHE as a white solid (102 mg, 94% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.78 (d, J = 8.4 Hz, 1H), 7.61-7.53 (m, 3H), 7.31 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 6.14 (s, 2H), 4.18 (s, 2H), 3.87 (s, 3H), 3.78 (s, 3H), 2.51 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 152.1, 147.8, 147.2, 145.5, 142.0, 130.4, 125.6, 125.3, 125.2, 123.8, 123.7, 120.0, 118.7, 111.6, 104.2, 101.2, 99.7, 60.5, 55.6, 48.2, 41.0. HRMS (ESI): m/z $[M]^+$ calcd for $C_{21}H_{19}NO_4$, 350.1348; found, 350.1396.

Synthesis of DHSAN

Sanguinarine (100 mg, 0.27 mmol) was first dissovled in MeOH (20 mL) and then added with $NaBH_4$ (40 mg, 1.1 mmol), the mixture was stirred at room temperature for 30 min. After completion of the reaction, the precipitation was filtered, washed by MeOH and H_2O, and dried under vacuum to yield DHSAN as a white solid (50 mg, 55% yield). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.77 (d, J = 8.4 Hz, 1H), 7.55 (t, J_1 = 18.0 Hz, J_2 = 8.4 Hz, 2H), 7.40 (d, J = 7.7 Hz, 1H), 7.31 (s, 1H), 6.94 (d, J = 8.0 Hz,

1H), 6.12 (d, *J* = 13.2 Hz, 4H), 4.12 (s, 2H), 2.51 (s, 3H); ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ 147.9, 147.3, 147.9, 144.3, 141.9, 130.4, 126.4, 125.7, 123.9, 123.8, 120.2, 116.3, 112.8, 107.2, 104.2, 101.3, 101.2, 99.7, 48.9, 41.3. HRMS (ESI): m/z $[M]^+$ calcd for $C_{20}H_{15}NO_4$, 334.1035; found, 334.1080.

Photoactivatable fluorescence measurement in solution

The stock solution of DHCHE and DHSAN was prepared by dissolving in DMSO (10 mM). 30 μL of their stock solution was respectively added into H_2O (2970 μL), and the mixture was shaken on a vortex mixter for 1.0 min. The mixture were then irradiated at 365 nm for different time under portable UV lamp and the PL spectra were measured on a fluorescence spectrophotometer with λ_{ex} = 450 nm or 330 nm.

Singlet oxygen ($^1O_2$) quenching

The mixture of DHCHE (200 μM)/DHSAN (100 μM) and NaN_3 (0, 1.0, 10, 20 mM) in DMSO/PBS solution (v : v = 1 : 99) were irradiated under portable UV lamp at 365 nm for 0-20 min and the PL spectra were measured on a microplate reader with λ_{ex} = 450 nm.

Cell Culture

HeLa, A549 and NIH-3T3 cells were cultured in DMEM containing 1% penicillin-streptomycin and 10% FBS at 37 °C in a humidity atmosphere with 5% CO_2.

Cell Viability Measurement

The cells were seeded in a 96-well plate with a density of 1×10^5 cells/mL. After incubation for 24 h, different concentrations of DHCHE and DHSAN were added and further incubation for 30 min. The cells were then irradiated at 365 nm for 5 min and

further incubated for 24 h. After washing with PBS buffer for twice, freshly prepared MTT solution (0.5 mg/mL, 100 μL) was added. After incubation at 37 ^{o}C for 4 h, the MTT solution was removed and washed twice with PBS buffer. DMSO (100 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates. The absorbance of sample and control wells at 570 nm was then measured by a microplate reader. Cell viability was then calculated by the ratio of the absorbance of sample wells to control cells.

Photoactivatable cell imaging

The HeLa cells in culture media were first treated with DHCHE or DHSAN (100 μM) for 30 min, and then the CLSM images were taken under confocal microscope through continuous irradiation at 405 nm. Green channel: 420-550 nm; Red channel: 580-697 nm. Laser power: 10% for DHCHE and 5% for DHSAN.

***In situ* monitoring of the cell morphology changes under photoactivation**

The HeLa cells in culture media were first treated with DHCHE or DHSAN (100 μM) for 30 min, and the CLSM images were taken after continuous irradiation at 405 nm for 3 min. The cells were then incubated under dark and the CLSM images were taken at 5 and 10 min to monitor the fluorescence imaging and cell morphology changes. Green channel: 420-550 nm; Red channel: 580-697 nm. Laser power: 10% for DHCHE and 5% for DHSAN.

Co-localization with Hoechst 33342

The HeLa cells in cell culture medium were first treated with DHCHE or DHSAN (100 μM) for 30 min and then irradiated under 405 nm for 3 min. The cells were then treated

with Hoechst 33342 (5 μg/mL) for 1 min and 3 min, respectively. The fluorescence images for DHCHE and DHSAN after photoactivation were taken with λ_{ex} = 488 nm and λ_{em} = 580-700 nm. For Hoechst 33342, λ_{ex} = 405 nm and λ_{em} = 420-550 nm. Laser power: 5%.

Co-localization of DHSAN with ER-Tracker Red

The HeLa cells were first incubated with DHSAN (100 μM) and ER-Tracker Red (1 μM) for 30 min at 37 ºC, and then the CLSM images were taken under confocal microscope. For DHSAN, λ_{ex} = 405 nm and λ_{em} = 420-550 nm, Laser power: 10.0%. For ER-Tracker Red, λ_{ex} = 543 nm and λ_{em} = 580-700 nm, Laser power: 4.0%.

Co-localization of DHCHE with Nile Red

The HeLa cells were first incubated with DHCHE (100 μM) and Nile Red (5 μM) for 30 min at 37 ºC, and then the CLSM images were taken under confocal microscope. For DHCHE, λ_{ex} = 405 nm, λ_{em} = 420-550 nm, Laser power: 5.0%. For Nile Red, λ_{ex} = 543 nm and λ_{em} = 580-650 nm, Laser power: 10.0%.

Selective imaging and killing of HeLa cells in the presence of NIH-3T3 cells

HeLa cancer cells and NIH-3T3 normal cells were co-cultured in a 35 mm Petri dish with a coverslip. The cells were then treated with DHCHE or DHSAN (100 μM) for 30 min. The selected HeLa cells were then continuously irradiated with a bleaching model (405 nm, 2% laser power) to activate fluorescence. Then, the whole observation window of multi-cells was imaged. This process was repeated to activate all the selected HeLa cells. The emission filter was 580-697 nm for both DHCHE and DHSAN.

Table S1. The photophysical properties of compounds CHE and SAN.

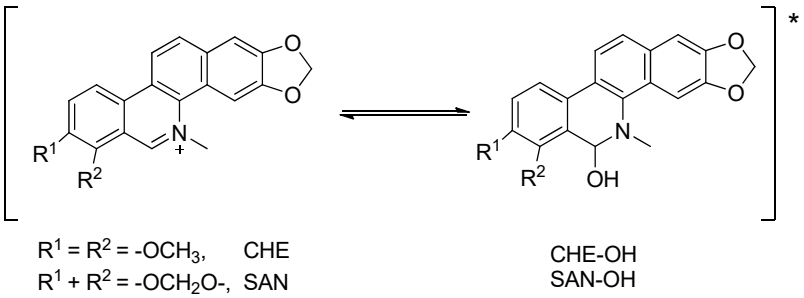
		λ [nm][d]	λ [nm][e]	Φ_f [%][f]	τ (ns)[g]	k_r [10^7 s^{-1}][h]	k_{nr} [10^8 s^{-1}][i]
CHE	Soln[a]	402	589	1.1	0.97	1.13	10.20
	Solid[b]	330	584	18.2	11.81	1.54	0.69
	Soln with CB[7][c]	427	540	35.6	15.95	2.23	0.40
SAN	Soln[a]	471	606	5.4	2.47	2.19	3.83
	Solid[b]	330	603	5.0	3.55	1.41	2.68
	Soln with CB[7][c]	392	555	39.6	16.98	2.33	3.56

a) CHE and SAN in water with a concentration of 10 μM; b) Thin solid film; c) CHE and SAN in water with cucurbit[7]uril (CB[7]), [CB[7]] = 20 μM, [CHE] = [SAN] = 10 μM; d) Maximum absorption wavelength; e) Maximum emission wavelength; f) Absolute quantum yield; g) Average fluorescence lifetime; h) Radiative relaxation rate $k_r = \Phi/\tau$; i) Non-radiative relaxation rate $k_{nr} = (1-\Phi)/\tau$.

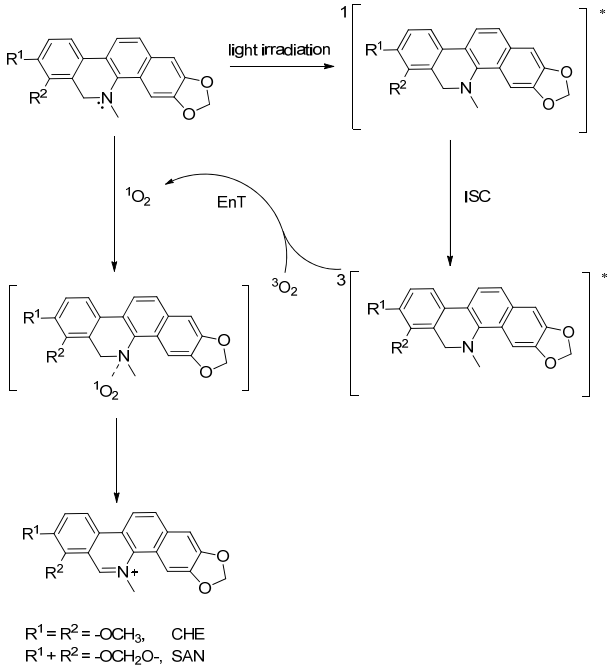
Table S2. The photophysical properties of compounds DHSAN and DHCHE.

		λ [nm][c]	λ [nm][d]	Φ_f [%][e]	τ (ns)[f]	k_r [10^7 s^{-1}][g]	k_{nr} [10^8 s^{-1}][h]
DHCHE	Soln[a]	350	445	18.1	2.87	6.30	2.85
	Solid[b]	330	427	24.5	2.90	8.45	2.60
DHSAN	Soln[a]	335	450	16.3	2.58	6.32	3.24
	Solid[b]	330	435	12.8	2.64	4.85	3.30

a) DHCHE and DHSAN in THF solution with a concentration of 10 μM; b) Thin solid film; c) Maximum absorption wavelength; d) Maximum emission wavelength; e) Absolute quantum yield; f) Average fluorescence lifetime; g) Radiative relaxation rate $k_r = \Phi/\tau$; h) Non-radiative relaxation rate $k_{nr} = (1-\Phi)/\tau$.



Scheme S1 The equilibrium beween quaternary cation and pseudobase forms of CHE and SAN at excited state.[1]



Scheme S2 The proposed reaction mechanism for photo-triggered transformation from DHCHE/DHSAN to CHE/SAN. ISC, intersystem crossing; EnT, engery transfer.[2]

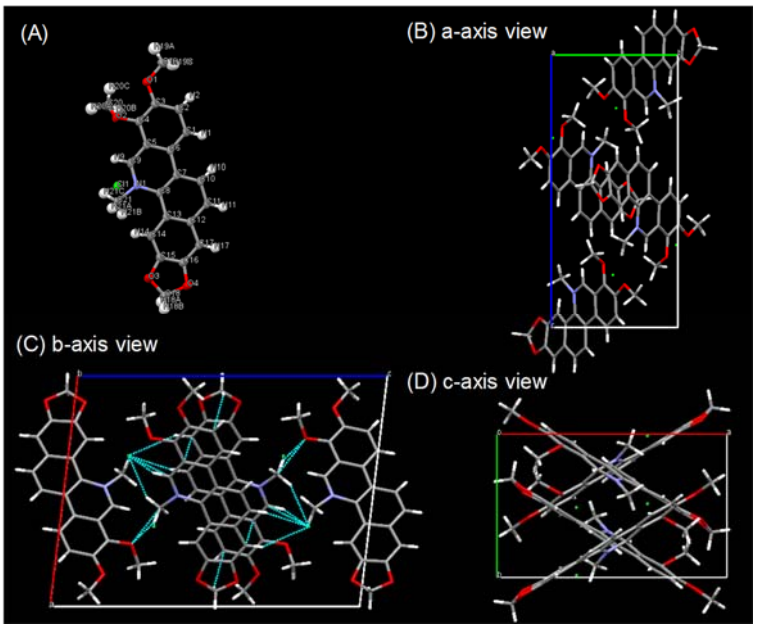


Fig. S1 (A) The ORTEP drawing of compound CHE with Cl^- as the counter anion. (B-D) The view along with a-axis, b-axis, and c-axis, respectively.

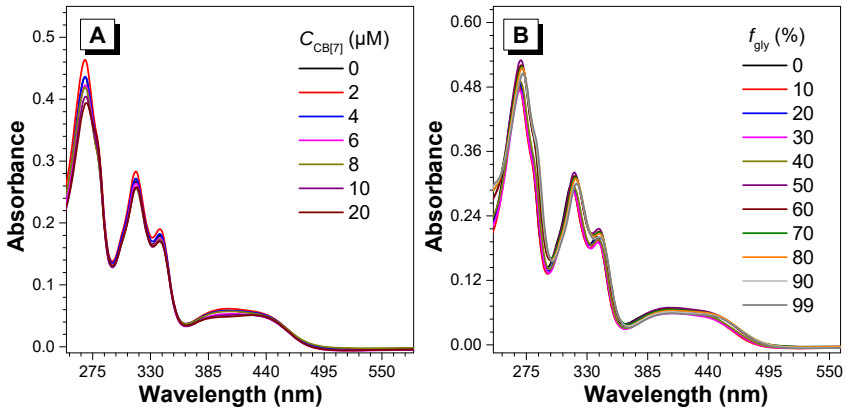


Fig. S2 (A) The UV-Vis absorption spectra of CHE in water treated with different concentrations of cucurbit[7]uril ($C_{CB[7]}$); (B) The UV-Vis absorption spectra of (B) CHE in water/glycerol mixture with different glycerol fractions (f_{gly}); [CHE] = 10 μM.

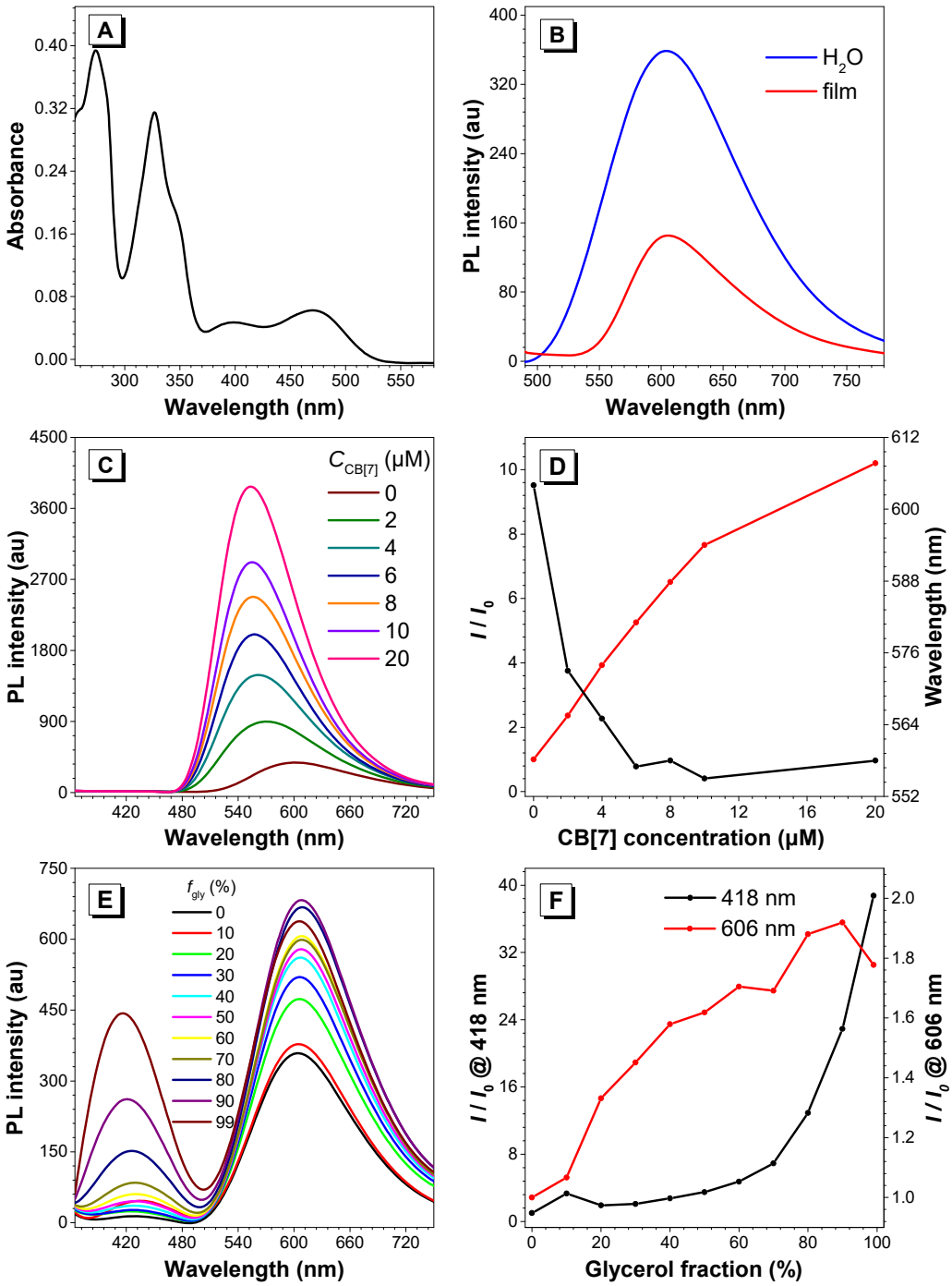


Fig. S3 (A) The UV-Vis absorption spectra of SAN in water. (B) The PL spectra of SAN in water (blue line) and in the film state (red line). (C) The PL spectra of SAN in water in the presence of different concentrations of CB[7]. (D) The plots of relative maximum emission intensity (I/I_0) (red line) and wavelength of maximum emission (black line) of SAN in water in the presence of different concentrations of CB[7]. (E) The PL spectra of SAN in water/glycerol mixture with different glycerol fractions. (F) The plots of relative emission intensity (I/I_0) of SAN at 418 nm (black line) and 606 nm (red line) in water/glycerol mixture with different glycerol fractions. [SAN] =10 μM; λ_{ex} = 330 nm.

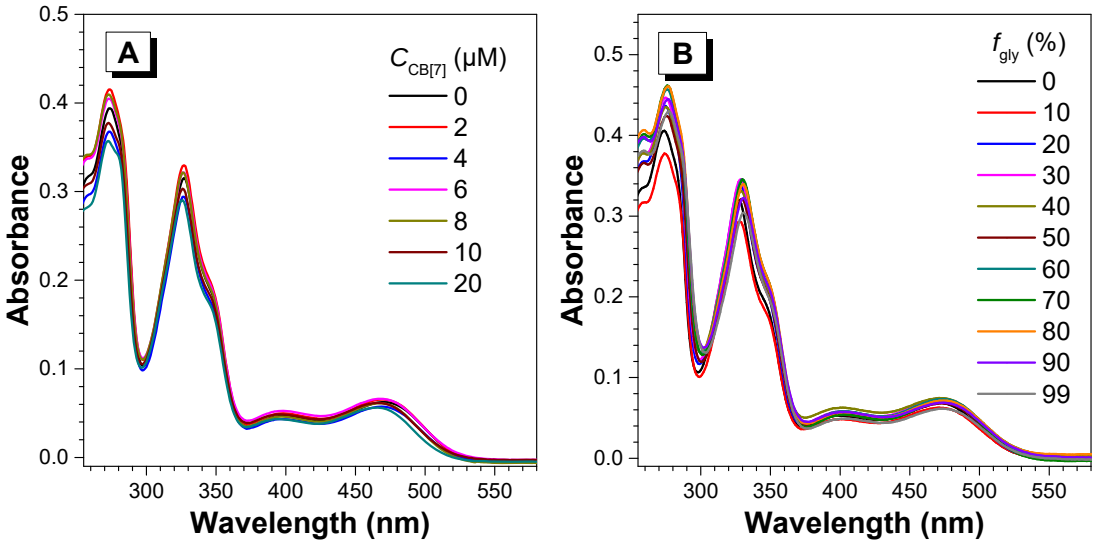


Fig. S4 (A) The UV-Vis absorption spectra of SAN in water treated with different concentrations of cucurbit[7]uril ($C_{CB[7]}$); (B) The UV-Vis absorption spectra of SAN in water/glycerol mixture with different glycerol fractions (f_{gly}); [SAN] = 10 μM.

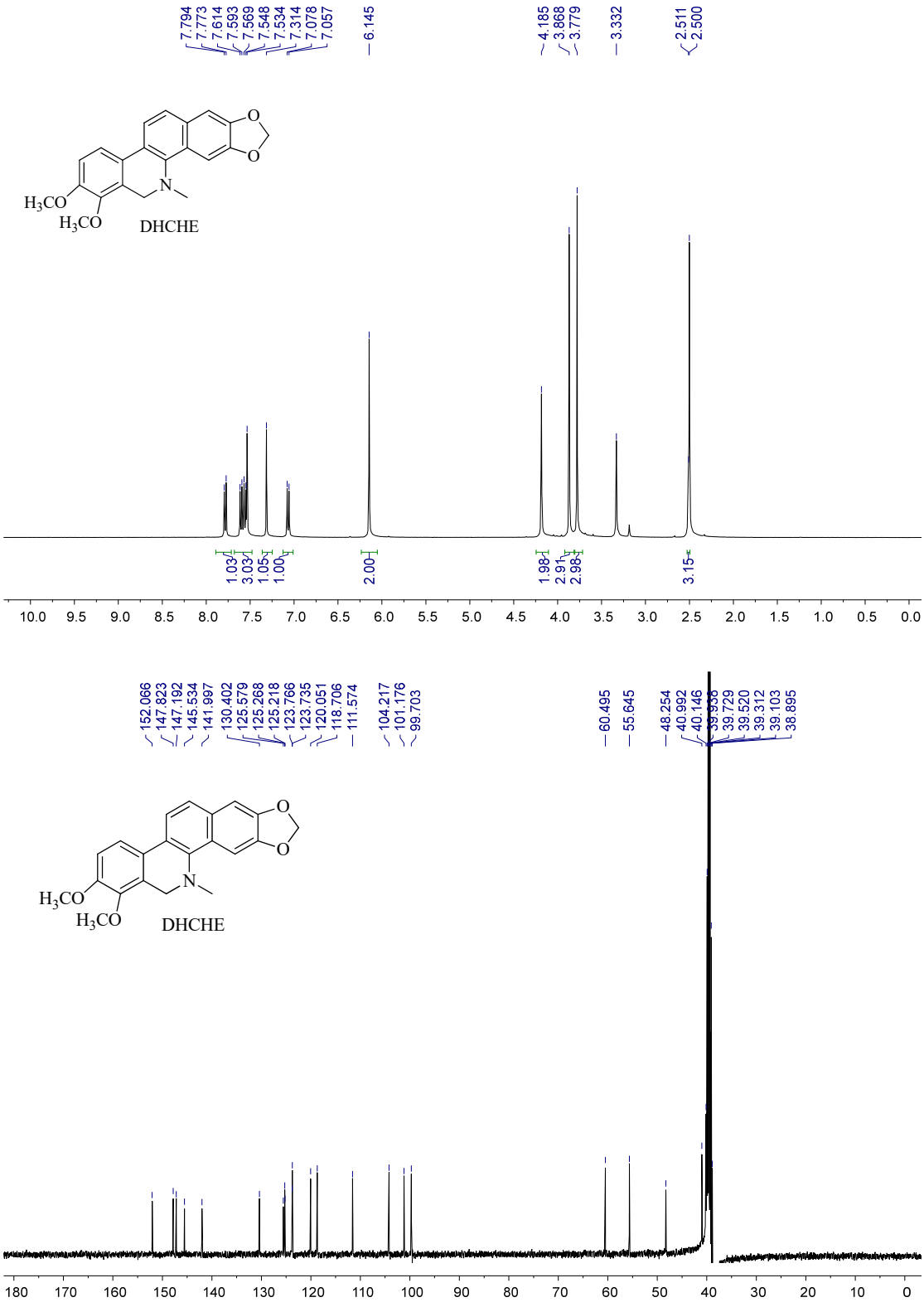


Fig. S5 The ^{1}H and ^{13}C NMR spectra of DHCHE in DMSO-*d*$_6$.

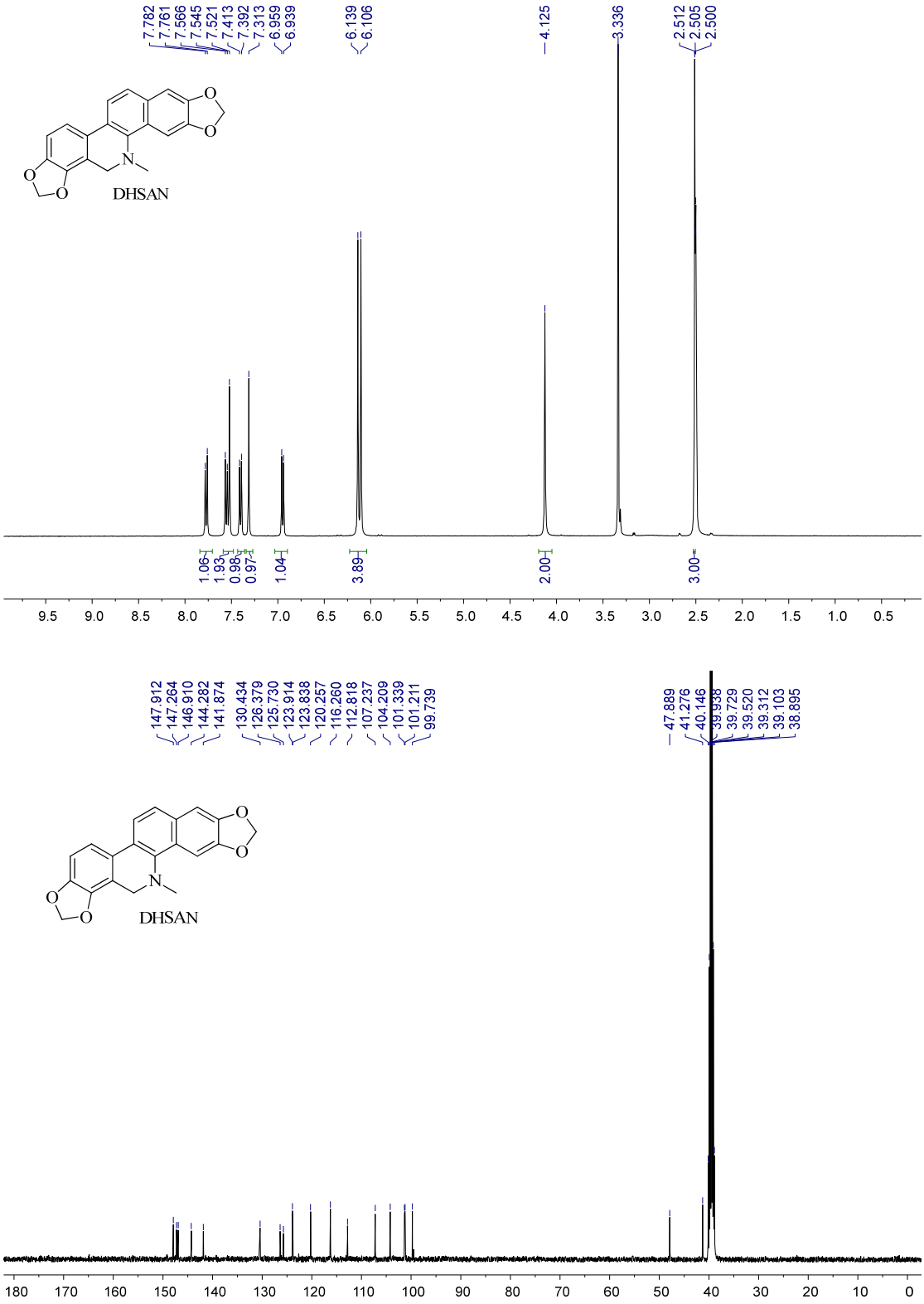


Fig. S6 The ^{1}H and ^{13}C NMR spectra of DHSAN in DMSO-*d*$_6$.

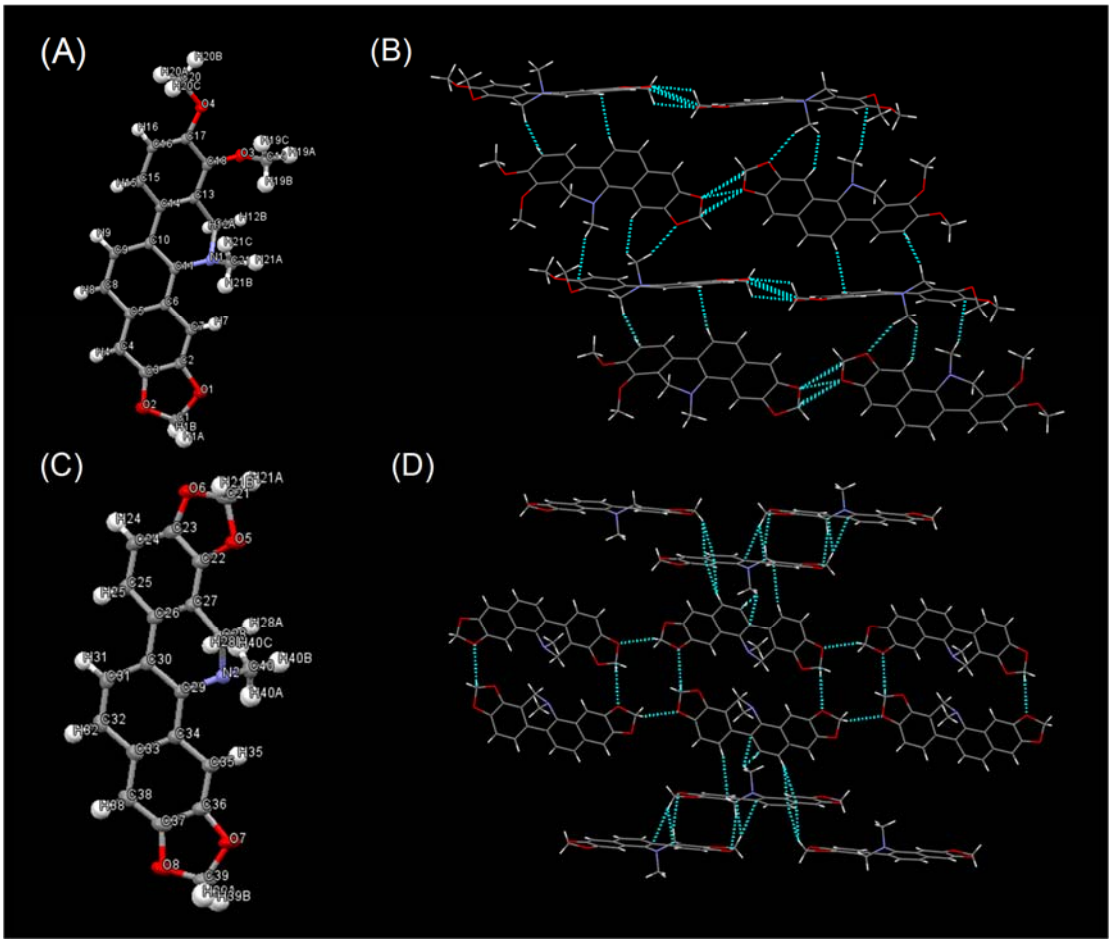


Fig. S7 (A, C) The ORTEP drawing of DHCHE and DHSAN. (B，D) The crystal packing structure of DHCHE and DHSAN.

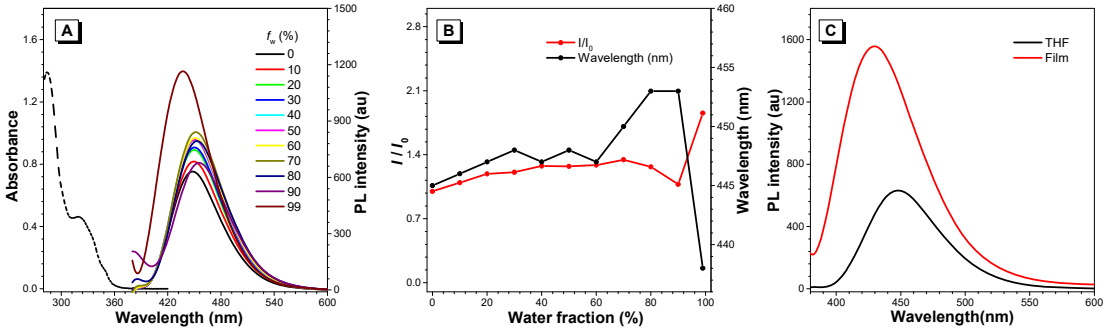


Fig. S8 (A) The UV-Vis absorption spectra of DHCHE in THF (dashed line) and PL spectra of DHCHE (solid line) in THF/water mixture with different water fractions (f_w). (B) Plots of relative maximum emission intensity (red, I/I_0) and maximum emission wavelength (black) of DHCHE in THF/water mixture with different water fractions (f_w). (C) The PL spectra of DHCHE in THF solution and in the film state. [DHCHE] = 10 μM; λ_{ex} = 330 nm.

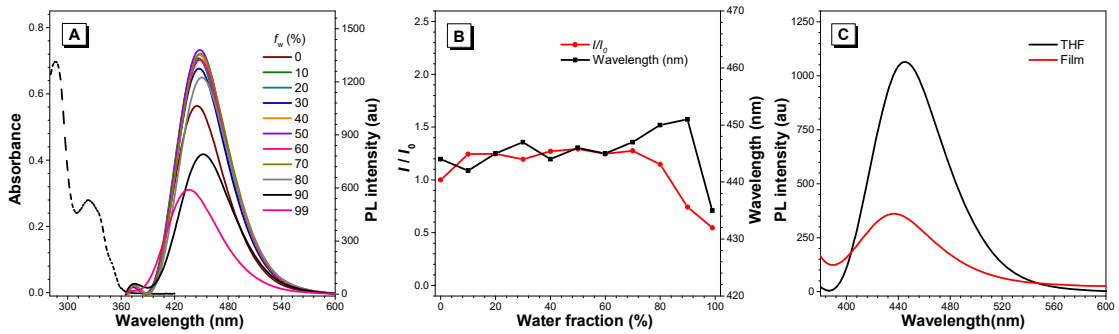


Fig. S9 (A) The UV-Vis absorption spectra of DHSAN in THF (dashed line) and PL spectra of DHSAN (solid line) in THF/water with different water fractions (f_w). (B) Plots of relative maximum emission intensity (red, I/I_0) and maximum emission wavelength (black) of DHSAN in THF/water mixture with different water fractions (f_w). (C) The PL spectra of DHSAN in THF solution and in the film state. [DHSAN] = 10 μM; λ_{ex} = 330 nm.

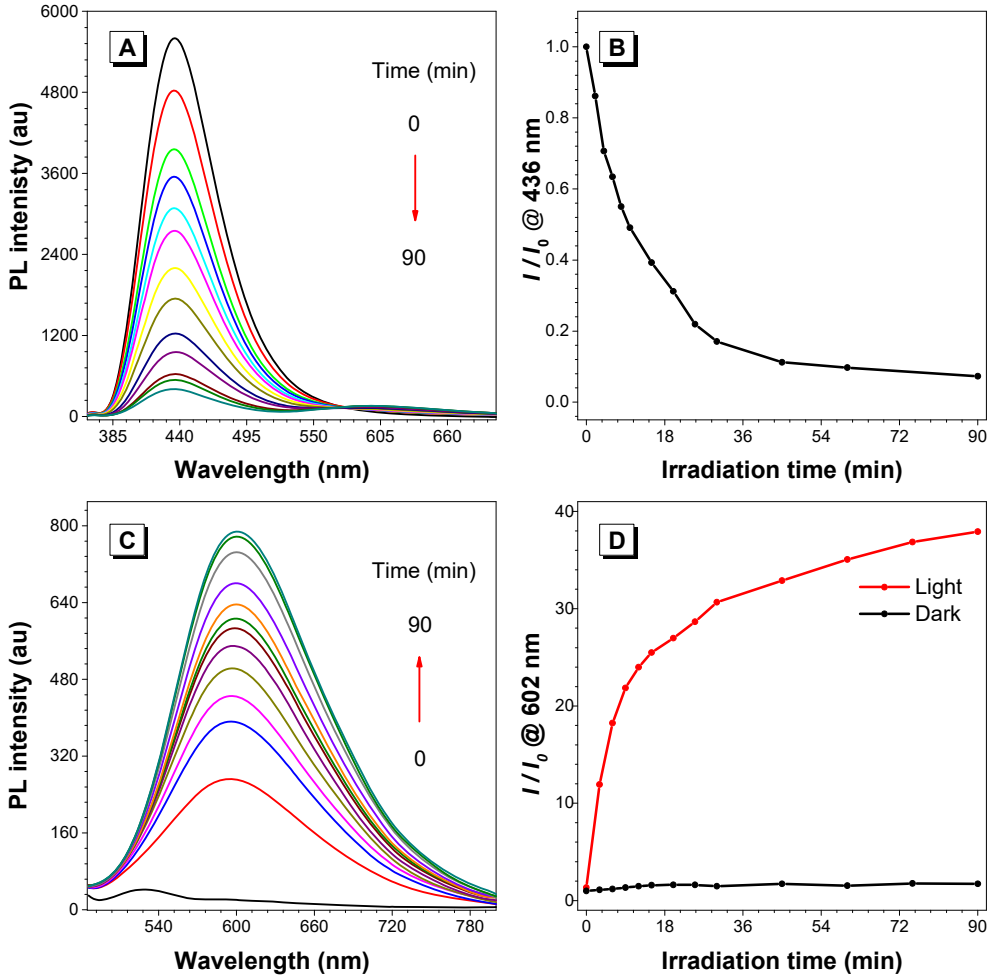


Fig. S10 The PL spectra and plots of maximum emission intensity changes (I/I_0) of DHSAN in co-solvent of DMSO/water (v : v = 1 : 99) under irradiation at 365 nm for 0-90 min. [DHSAN] = 100 μM; (A, B) λ_{ex} = 330 nm and (C, D) 450 nm.

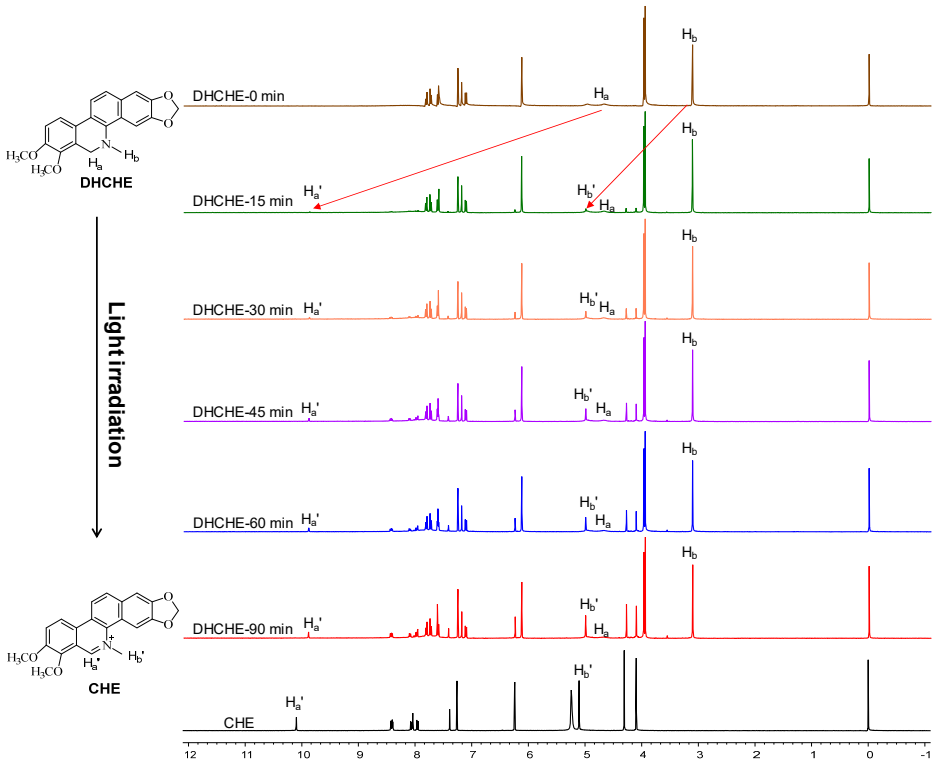


Fig. S11 The ^{1}H NMR stacking spectra of DHCHE in "$CDCl_3$ + CF_3CO_2D" under light irradiation at 365 nm.

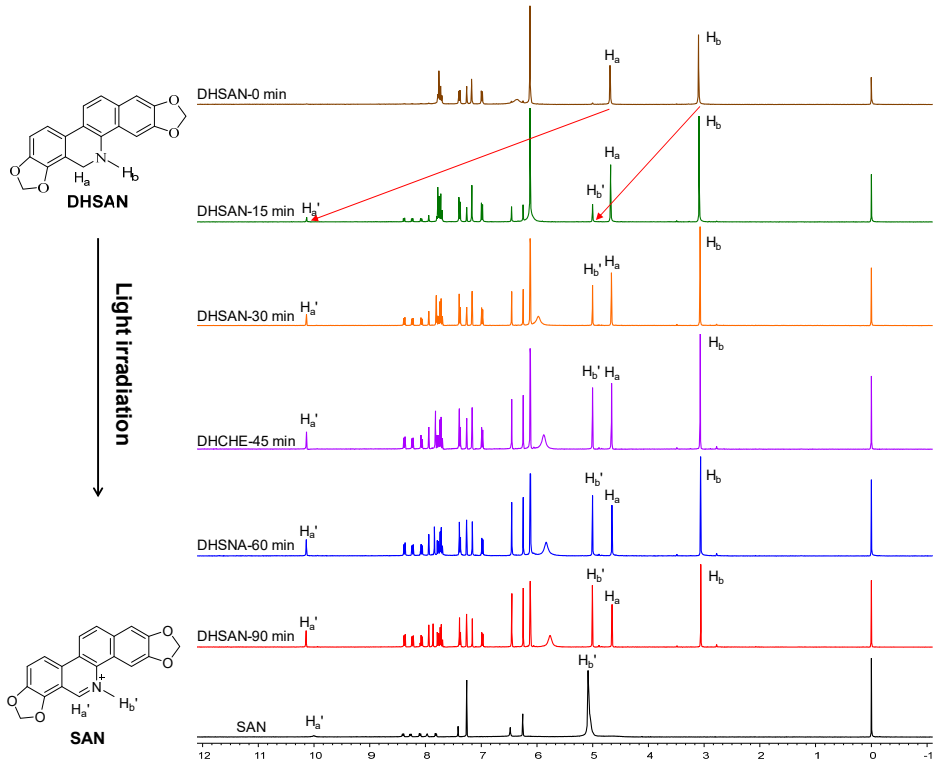


Fig. S12 The ^{1}H NMR stacking spectra of DHSAN in "$CDCl_3$ + CF_3CO_2D" under light irradiation at 365 nm.

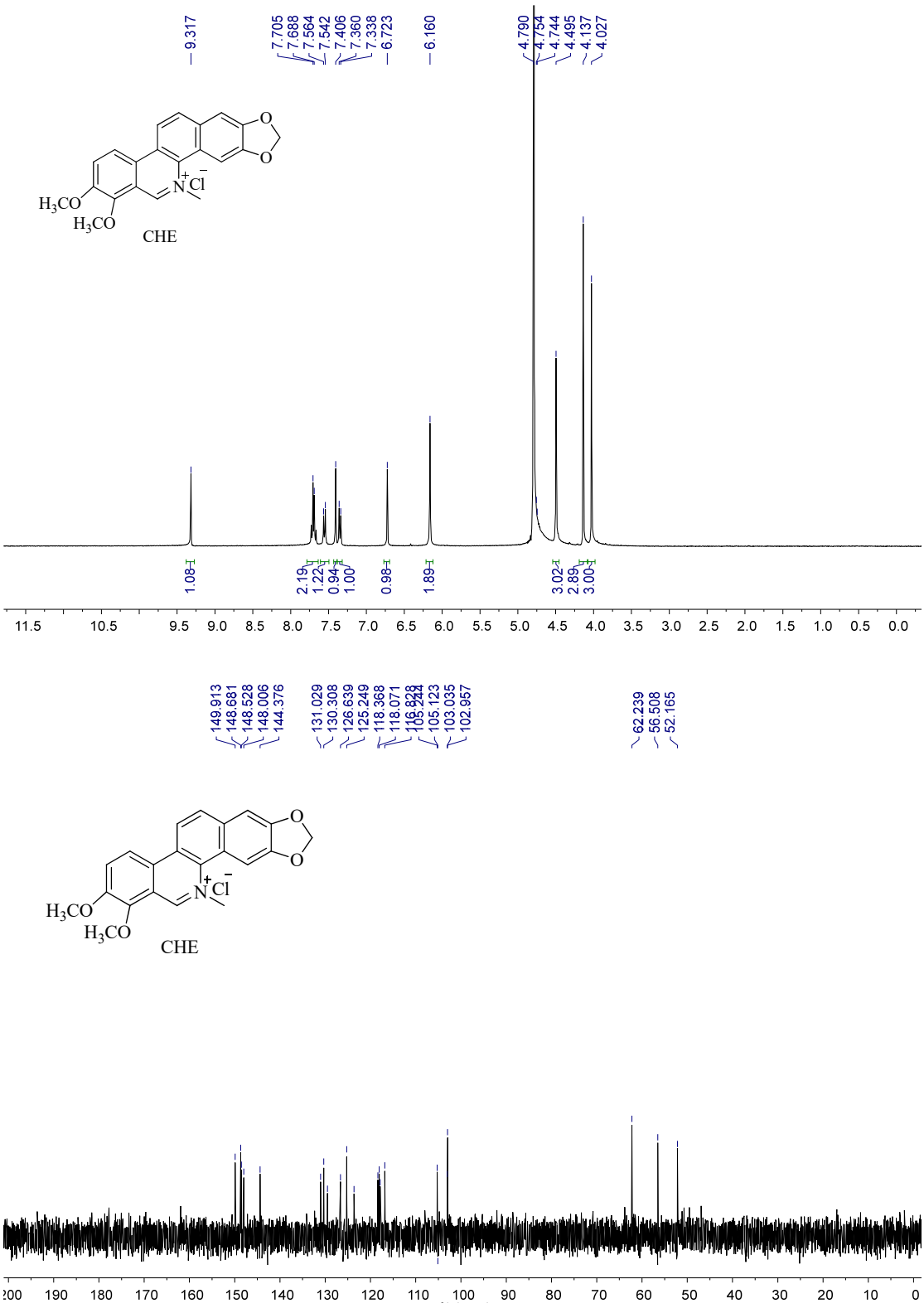


Fig. S13 The ^{1}H and ^{13}C NMR spectra of CHE in D_2O.

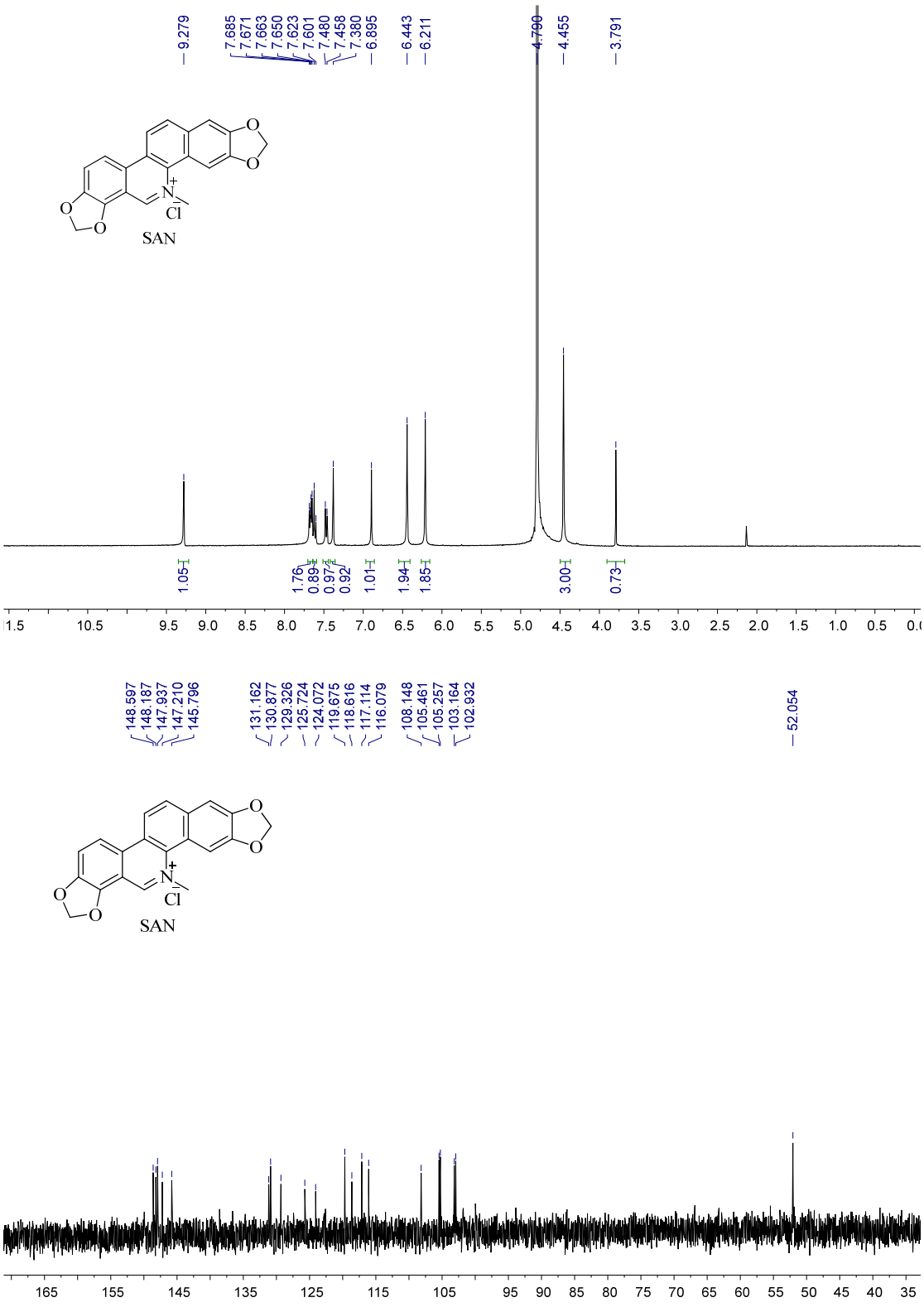


Fig. S14 The ^{1}H and ^{13}C NMR spectra of SAN in D_2O.

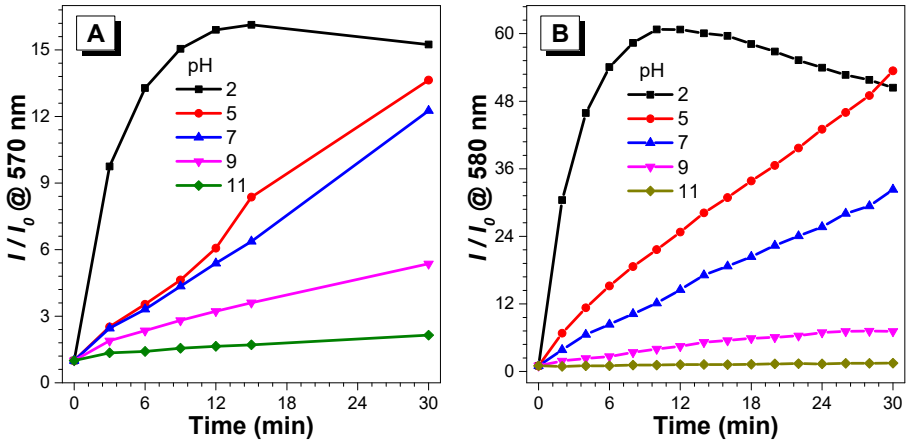


Fig. S15 Plots of relative maximum emission intensity (I/I_0) of (A) DHCHE and (B) DHSAN under irradiation at 365 nm for 0-30 min in co-solvent of DMSO/water (v : v = 1 : 99) at different pH. [DHCHE] = [DHSAN] = 100 μM; λ_{ex} = 450 nm. For DHCHE, λ_{em} = 580 nm; For DHSAN, λ_{em} = 570 nm.

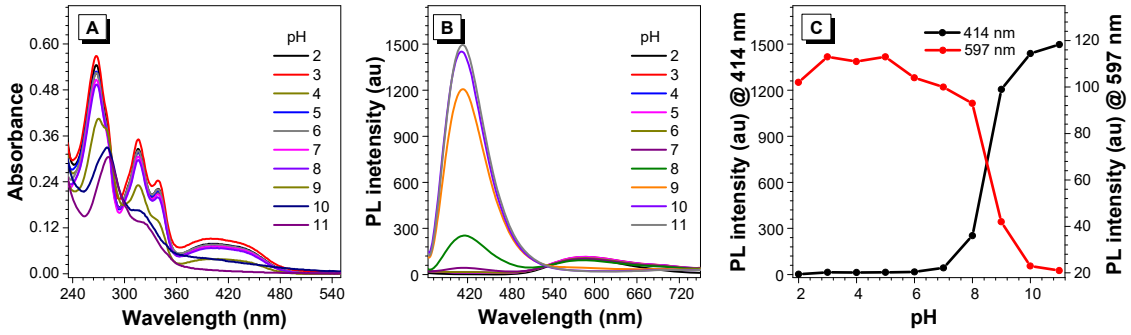


Fig. S16 (A) The UV-Vis absorption and (B) PL spectra of CHE in Britton-Robinson buffer solution at different pH, λ_{ex} = 330 nm. (C) The plots of emission intensity changes of CHE at 414 nm (black) and 597 nm (red) in Britton-Robinson buffer solution at different pH. [CHE] = 10 μM.

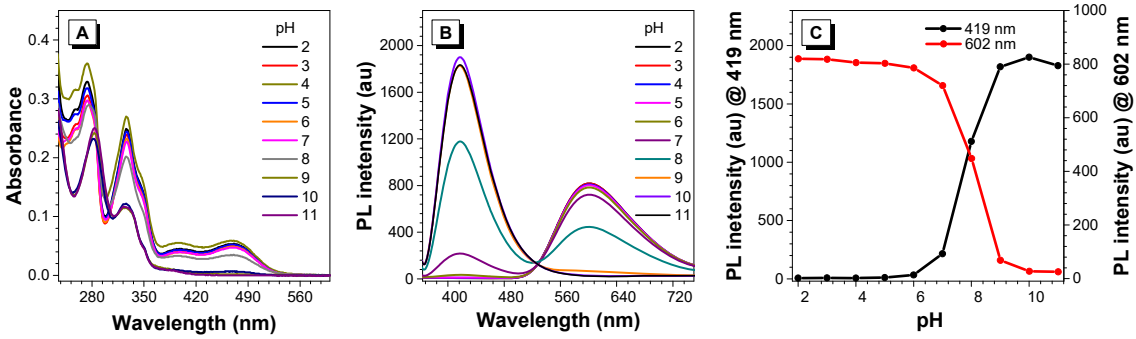


Fig. S17 (A) The UV-Vis absorption and (B) PL spectra of SAN in Britton-Robinson buffer solution at different pH, λ_{ex} = 330 nm. (C) The plots of emission intensity changes of SAN at 419 nm (black) and 602 nm (red) in Britton-Robinson buffer solution at different pH. [SAN] = 10 μM.

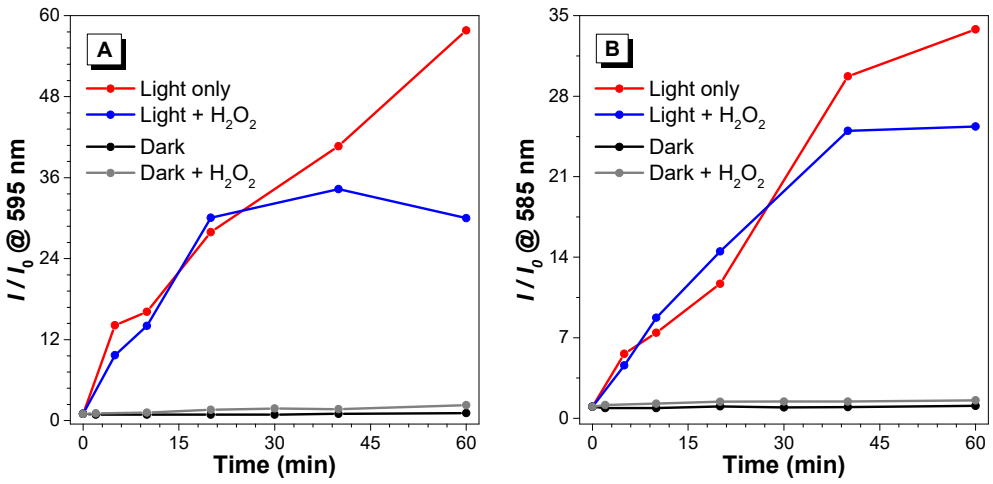


Fig. S18 The plots of relative maximum emission intensity (I/I_0) of DHCHE (A) and DHSAN (B) in co-solvent of DMSO/water (v : v = 1 : 99) with or without H_2O_2 (3 wt%) under dark or light irradiation at 365 nm for 0-60 min. For DHCHE, λ_{ex} = 450 nm, λ_{em} = 585 nm. For DHSAN, λ_{ex} = 450 nm, λ_{em} = 595 nm. [DHCHE] = [DHSAN] = 100 μM.

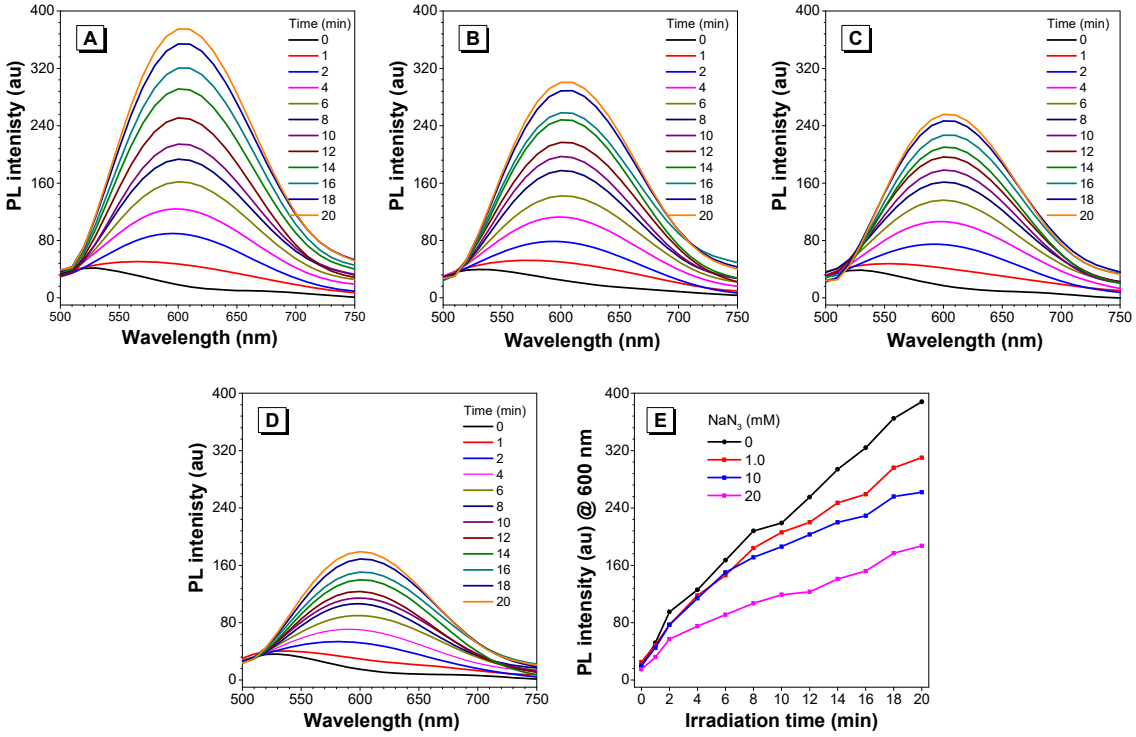


Fig. S19 The PL spectra (A-D) and plots of maximum emission intensity changes (E) of DHSAN in co-solvent of DMSO and PBS (v : v = 1:99) with different concentrations of NaN_3 under 365 nm irradiation for 0-20 min. [DHSAN] = 100 μM, [NaN_3] = 0 (A), 1.0 (B), 10 (C), and 20 mM (D), λ_{ex} = 450 nm.

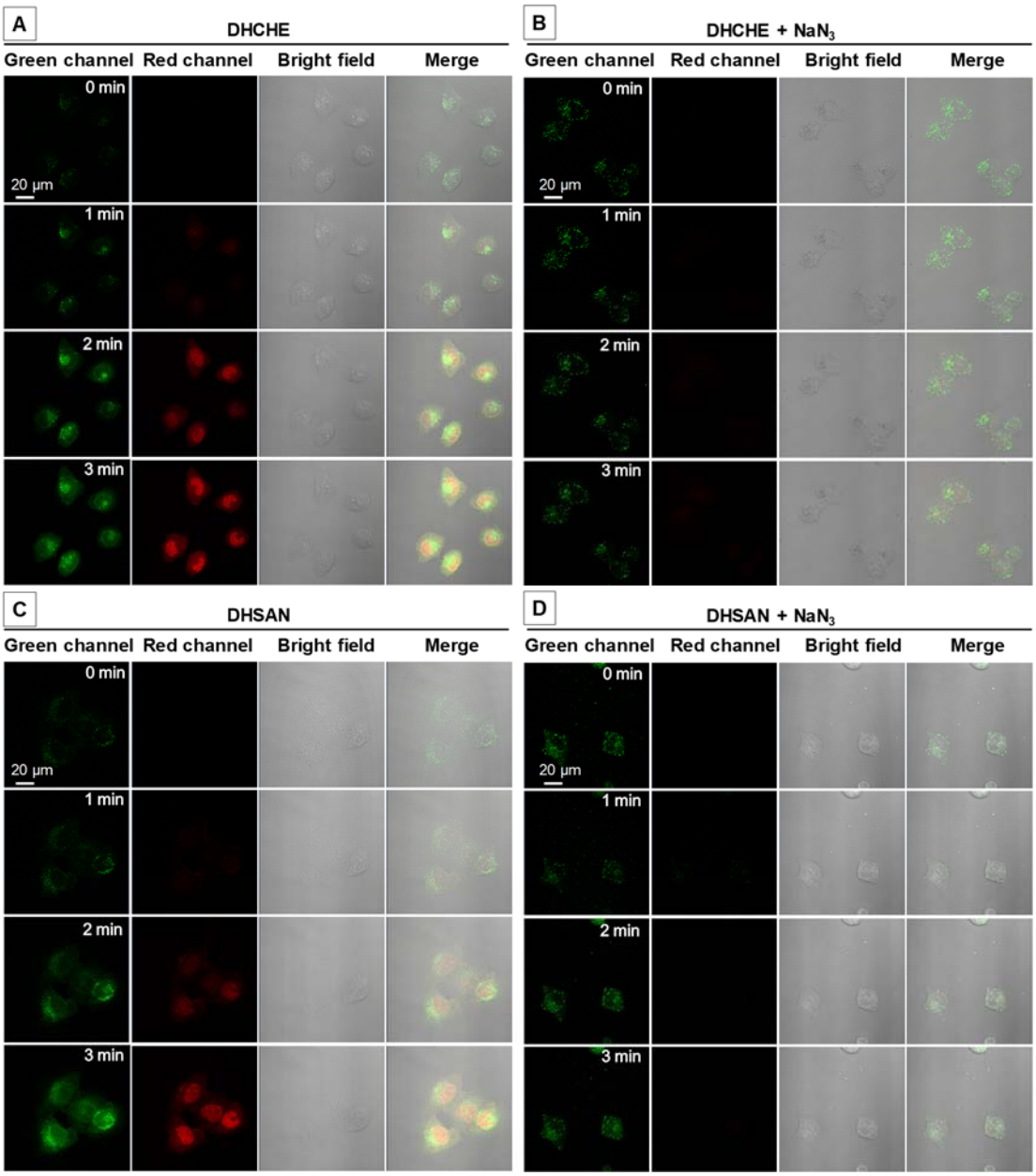


Fig. S20 (A-D) Fluorescence and bright field images of HeLa cells treated with DHCHE or DHSAN in the absence or presence of NaN_3. [DHCHE] = [DHSAN] = 100 μM; [NaN_3] = 50 mM. Green channel: λ_{ex} = 405 nm; λ_{em} = 420-550 nm, Red channel: λ_{ex} = 405 nm; λ_{em} = 580-697 nm.

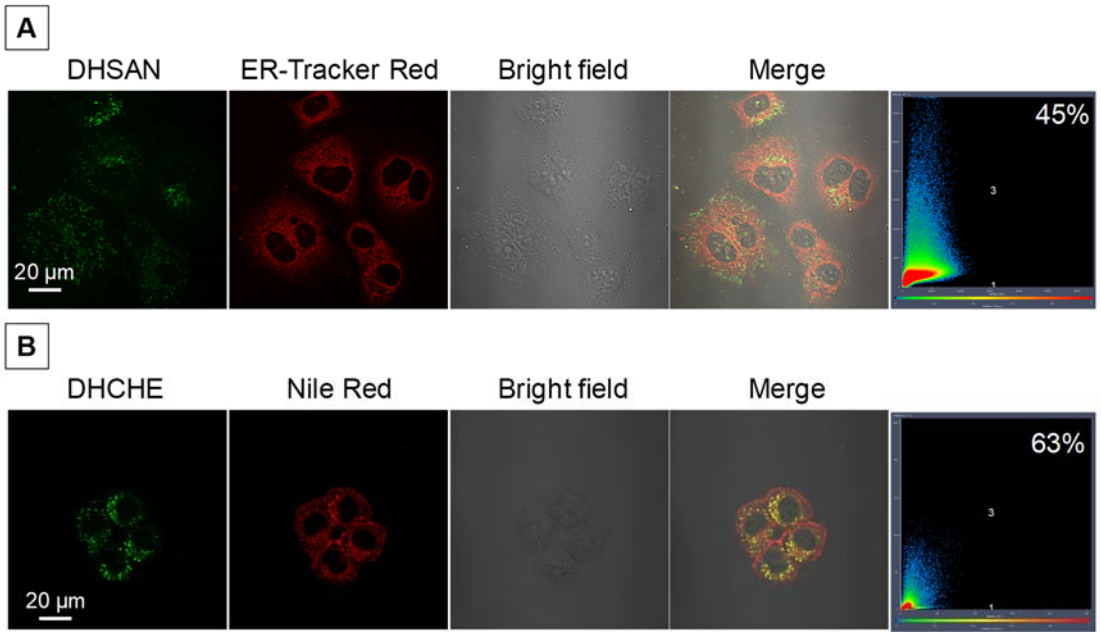


Fig. S21 (A) Fluorescence and bright field images of HeLa cells treated with DHSAN and ER-Tracker Red for 30 min. [DHSAN] = 100 μM; [ER-Tracker Red] = 1.0 μM. Green channel: λ_{ex} = 405 nm; λ_{em} = 420-550 nm. Red channel: λ_{ex} = 543 nm; λ_{em} = 580-700 nm. (B) Fluorescence and bright field images of HeLa cells treated with DHCHE and Nile Red for 30 min. [DHCHE] = 100 μM; [Nile Red] = 5 μM. Green channel: λ_{ex} = 405 nm; λ_{em} = 420-550 nm. Red channel: λ_{ex} = 543 nm; λ_{em} = 580-650 nm.

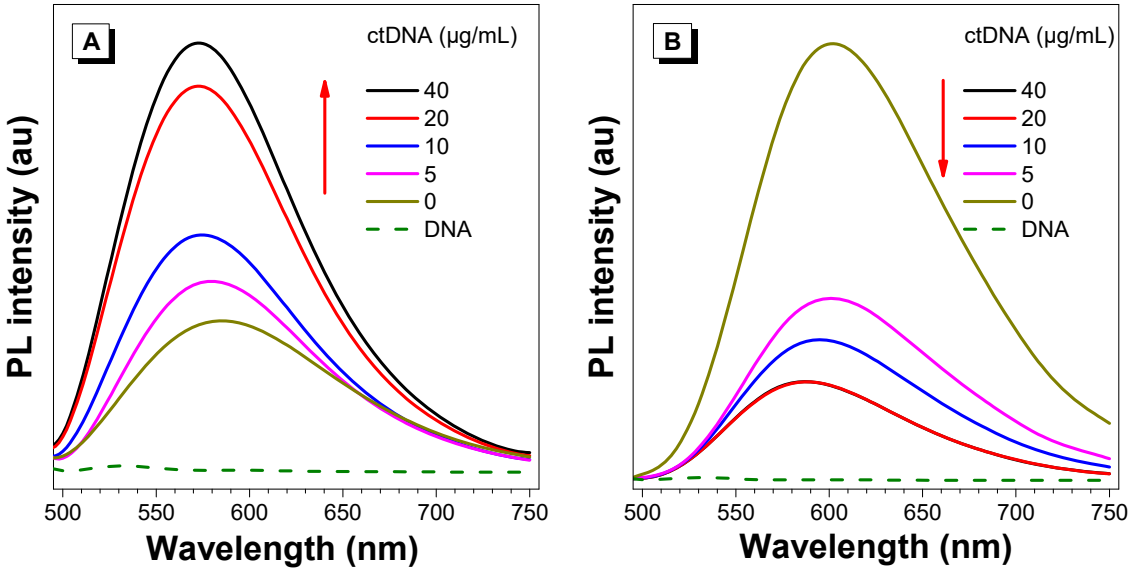


Fig. S22 The fluorescence spectra of (A) CHE and (B) SAN with different concentrations of calf thymus DNA (ctDNA) in PBS buffer solution, dashed line: the buffered solution of ctDNA, [ctDNA] = 50 μM. [CHE] = [SAN] = 10 μM, λ_{ex} = 450 nm.

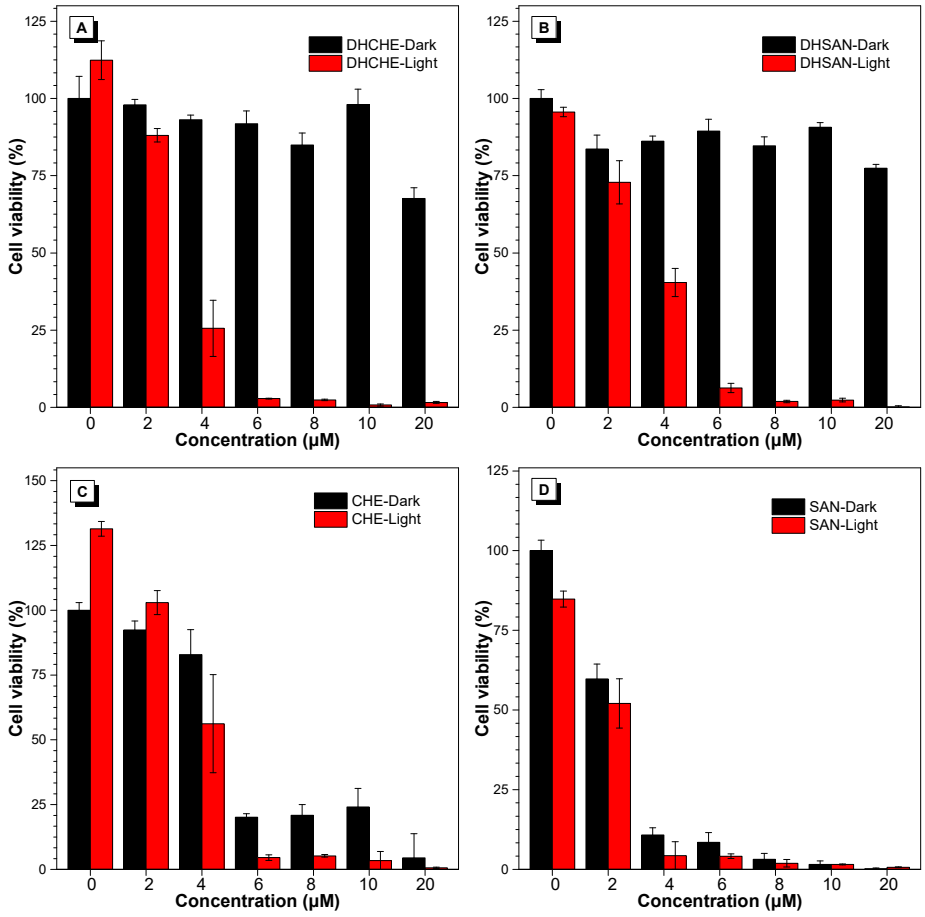


Fig. S23 The cell viabilities of A549 cancer cells treated with different concentrations of (A) DHCHE, (B) DHSAN, (C) CHE, and (D) SAN under dark or light irradiation (365 nm, 5 mW/cm^2, 5 min).

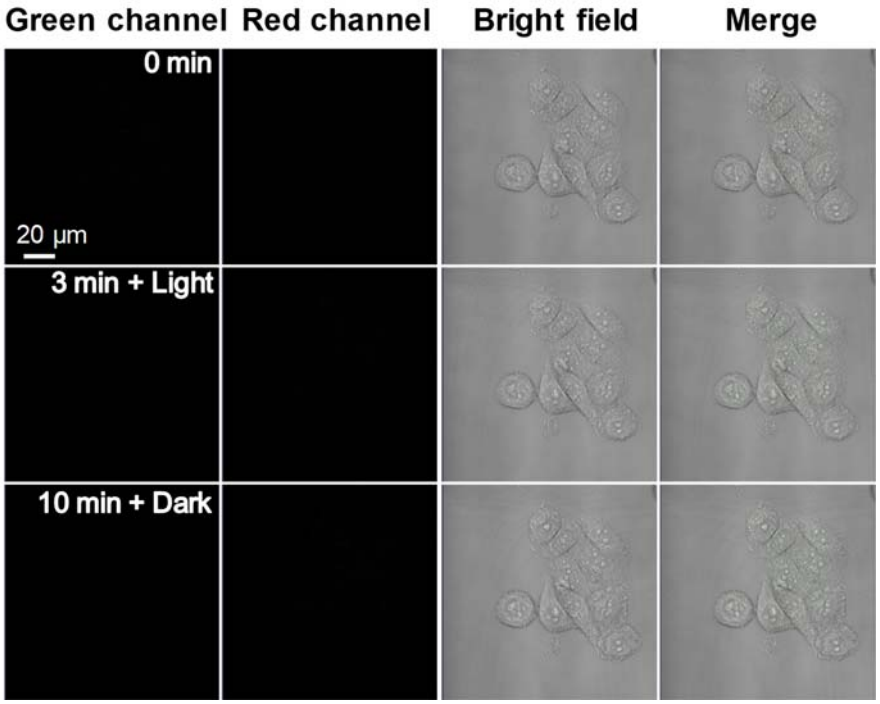


Fig. S24 CLSM images of live HeLa cells under control. λ_{ex} = 405 nm. Green channel: 420-550 nm; Red channel: 580-697 nm. Laser power: 10%.

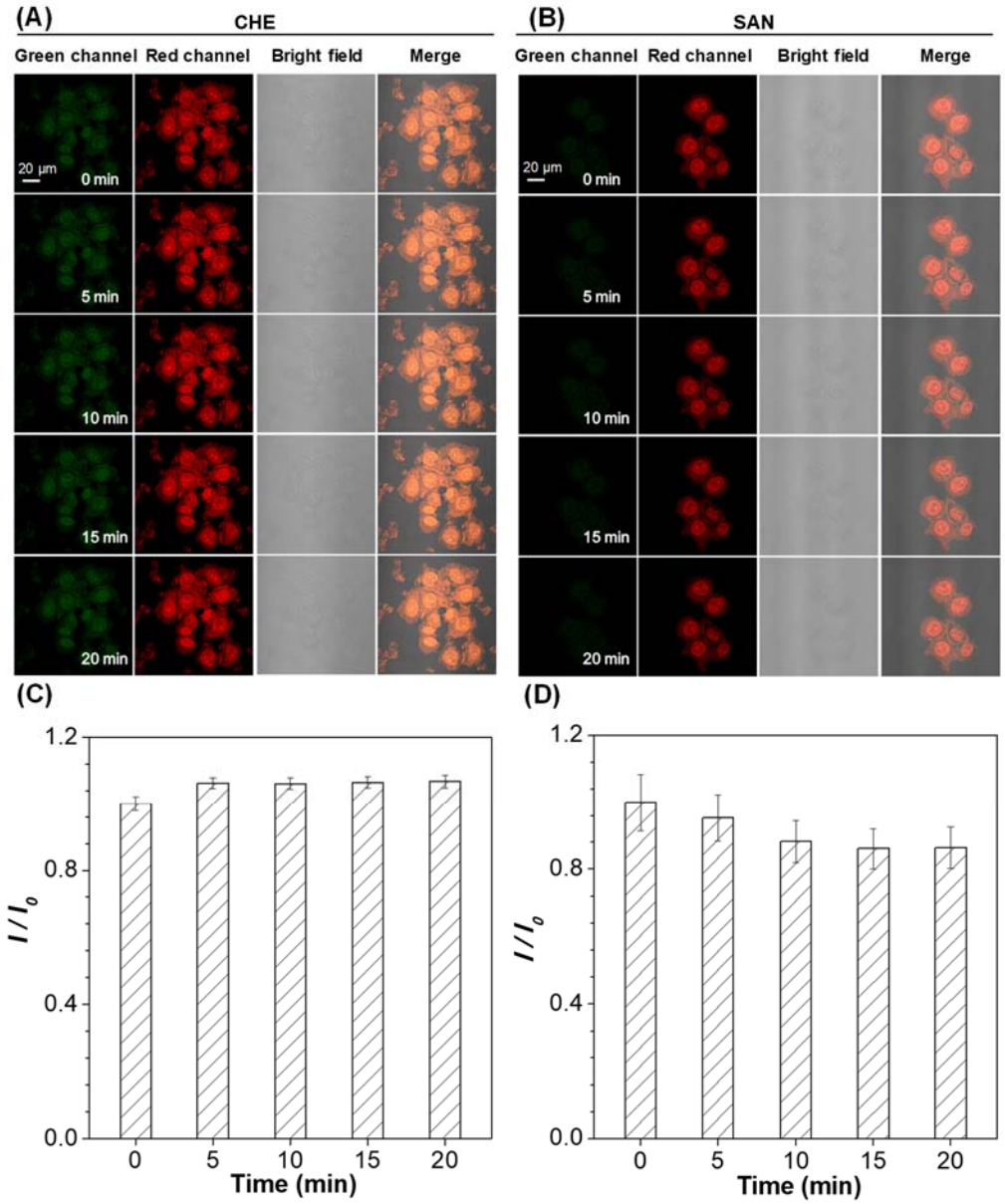


Fig. S25 (A) CLSM images of fixed HeLa cells stained with CHE under prolonged light irradiation at 405 nm for 20 min. Laser power: 10%. (B) CLSM images of fixed HeLa cells stained with SAN under prolonged light irradiation at 405 nm for 20 min. Laser power: 5%. (C) Plots of relative fluorescence intensity (I/I_0) changes of fixed HeLa cells treated with CHE under prolonged light irradiation at 405 nm for different time. (D) Plots of relative fluorescence intensity (I/I_0) changes of fixed HeLa cells treated with SAN under prolonged light irradiation at 405 nm for different time. [CHE] = [SAN] = 20 μM. λ_{ex} = 405 nm. Green channel: 420-550 nm; Red channel: 580-697 nm.

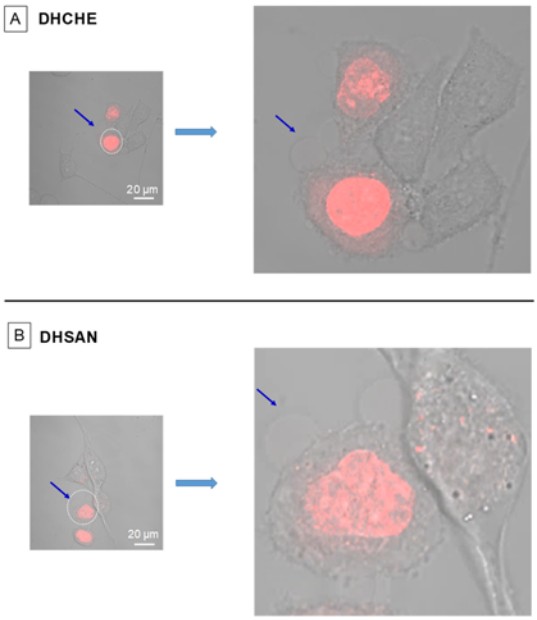


Fig. S26 The membrane blisters were indicated by blue arrows in the magnified merged images of Fig. 5A-B from the main text.

Reference

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2 K. Kundu, S. F. Knight, S. Lee, W. R. Taylor and N. Murthy, *Angew. Chem. Int. Ed. Engl.*, 2010, **49**, 6134-6138.