Supporting Information

Metal-Free Carbon Monoxide-Releasing Micelles Undergo Tandem Photochemical Reactions for Cutaneous Wound Healing

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Experimental Section

Materials. 3-Hydroxy-2-naphthoic acid, methyllithium, terephthalaldehyde, *o*-nitrobenzyl bromide, and sodium borohydride ($NaBH_4$) were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as received unless otherwise noted. Nile red (NR) was purchased from J&K Scientific Ltd. Tricarbonyldichlororuthenium(II) dimer (CORM-2), Lipopolysaccharide (LPS), and Griess Reagent were purchased from Sigma-Aldrich. All other reagents were of analytical grade or above and were obtained from domestic suppliers and used as received. Organic solvents, including toluene, dichloromethane (DCM), and acetonitrile (MeCN) were purified in a Solvent Purification System (Pure Solv^{TM}), and other solvents were used directly unless otherwise stated. Water was deionized on a Milli-Q® Direct Water system to reach a specific resistance of 18.2 MΩ cm. Allyl-Flu,[1] tricarbonylchloro(glycinato) ruthenium(II) (CORM-3),[2] and poly(ethylene oxide) (PEO)-based macroRAFT agent[3] were synthesized according to the literature procedures.

Sample Synthesis: Synthetic schemes employed for the preparation of compounds **A**-**C**, CO-releasing **FNM** monomer, and PEO-*b*-PFNM diblock copolymer are shown in Scheme 1.

Synthesis of 4-Hydroxymethylbenzaldehyde. 4-Hydroxymethylbenzaldehyde was synthesized according to literature procedure with a slight modification.[4] Briefly, terephthalaldehyde (2.68 g, 20 mmol) was dissolved in THF (60 mL) and was stirred at 0 ºC. $NaBH_4$ (190 mg, 5 mmol) was dissolved in 10 mL of THF and 10 mL of ethanol, which was slowly added to the THF solution of terephthalaldehyde at 0 ºC. After the addition, the mixture was stirred at room temperature for 6 h. After that, HCl (2 M) was added, and the solution pH was adjusted to 2. The solvent was then removed under reduced pressure, and the residues were washed with deionized water. The aqueous phase was then extracted with ethyl acetate (50 mL × 3), wash with saturated brine. After drying over anhydrous magnesium sulfate ($MgSO_4$), the crude product was purified by flash column chromatography (eluent: petroleum ether/ethyl acetate = 6:1) to give the targeted product (2.3 g, yield: 83%). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, TMS): 9.99 (s, 1H), 7.87 (d, J = 6.7 Hz, 2H), 7.53 (d, J = 7.9 Hz, 2H), 4.80 (s, 2H).

Synthesis of Compound A. Sodium hydroxide (5.4 mL, 5 M, 27 mmol) was added to a suspension of 1-(3-hydroxynaphthalen-2-yl)ethan-1-one (1.0 g, 5.37 mmol) in ethanol (15 mL), and the reaction was allowed to stir for 30 min at room temperature. 4-

Hydroxymethylbenzaldehyde (748 mg, 5.5 mmol) was added to the reaction mixture, and the resulting mixture was stirred for 5 h, resulting in the formation of a dark red solution. The mixture was then cooled to 0 °C in an ice-water bath, hydrogen peroxide (H_2O_2; 2 mL, 30%) was added dropwise, and the resulting mixture was stirred overnight at room temperature. After that, the mixture was acidified with HCl (0.5 M) to pH 6.5 with the formation of a bright yellow precipitate. The crude product was isolated by filtration, washed with ethanol, and used for the next step without further purification (1.15 g, yield: 67%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, TMS; Figure S1a): 9.61 (s, 1H), 8.84 (s, 1H), 8.34 – 8.18 (m, 4H), 8.07 (d, J = 8.5 Hz, 1H), 7.67 (t, J = 7.6 Hz, 1H), 7.57 (t, J = 8.1 Hz, 3H), 5.40 (t, J = 5.6 Hz, 1H), 4.71 – 4.55 (m, 2H). ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm; Figure S1b): 174.30 , 151.28 , 146.52 , 145.30 , 138.08 , 135.78 , 130.26 , 129.84 , 129.81 , 129.17 , 128.15 , 127.64 , 126.78 , 126.28 , 121.32 , 114.60 , 63.02. ESI-MS (Figure S1c): m/z calc. for $C_{20}H_{159}O_4$ $[M + H]^+$: 319.0965, found: 319.0960.

Synthesis of Compound B (Scheme 1a). To a mixture of compound **A** (318 mg, 1 mmol) and anhydrous K_2CO_3 (150 mg, 1.08 mmol) in 20 mL of DMF, and 2-nitrobenzyl bromide (240 mg, 1.12 mmol) was added and stirred at room temperature 8 h. The reaction mixture was filtered, and the filtrate was precipitated into 200 mL of deionized water. The residue was filtered and washed with ethanol (402 mg, yield: 89%). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm; Figure S2a): 8.81 (s, 1H), 8.30 (s, 1H), 8.25 (d, J = 8.3 Hz, 1H), 8.09 (d, J = 8.1 Hz, 2H), 8.02 (d, J = 7.7 Hz, 2H), 7.92 (d, J = 7.6 Hz, 1H), 7.78 (t, J = 7.5 Hz, 1H), 7.70 (t, J = 7.2 Hz, 1H), 7.60 (t, J = 7.4 Hz, 2H), 7.47 (d, J = 7.7 Hz, 2H), 5.47 (s, 2H), 5.38 (s, 1H), 4.60 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm; Figure S2b): 175.01 , 157.11 , 151.47 , 147.32 , 146.46 , 138.33 , 135.91 , 134.46 , 133.28 , 130.07 , 129.89 , 129.42 , 129.37 , 128.89 , 127.75 , 126.66 , 125.02 , 123.11 , 114.90 , 70.26 , 62.89. ESI-MS (Figure S2c): m/z calc. for $C_{27}H_{19}NO_6$ $[M + H]^+$: 454.1291, found: 454.1285. The 2D cosy NMR of compound B is shown in Figure S3.

Synthesis of FNM Monomer (Scheme 1a). Compound **B** (227 mg, 0.5 mmol) and 2 drops of dibutyltin dilaurate (DBTL) were dissolved in 30 mL of anhydrous toluene. The trace amount of water was removed by azeotropic distillation. After that, 2-isocyanatoethylmethacrylate (93 mg, 0.6 mmol) in 5 mL of *N*,*N*-dimethylformamide (DMF) was added dropwise and the reaction mixture was stirred overnight at room temperature. Afterwards, the mixture was precipitated

into 300 mL of water. The sediment was collected by filtration and was dried under vacuum overnight. The named monomer was purified by recrystallization in dichloromethane (DCM) and hexane (225 mg, yield: 73%).^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm; Figure S4a) 8.85 (s, 1H), 8.16 – 8.04 (m, 5H), 8.01 (s, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.71 (td, *J* = 7.6, 1.2 Hz, 1H), 7.63 (ddd, J = 8.2, 6.7, 1.2 Hz, 1H), 7.54 (ddd, *J* = 8.0, 6.9, 1.1 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 3H), 6.12 (s, 1H), 5.59 (s, 1H), 5.49 (s, 2H), 5.16 (d, *J* = 13.7 Hz, 3H), 4.26 (t, *J* = 5.3 Hz, 2H), 3.54 (q, *J* = 5.6 Hz, 2H), 1.94 (s, 3H). ^{13}C NMR(101 MHz, $CDCl_3$, δ, ppm; Figure S4b): 175.64, 167.32, 156.75, 156.14, 151.56, 146.70, 139.52, 138.73, 135.92, 133.94, 130.58, 130.12, 129.62, 129.43, 128.92, 128.26, 127.92, 127.23, 127.02, 126.18, 126.10, 124.72, 122.98, 114.26, 70.64, 66.14, 63.69, 40.35, 18.34. HPLC analysis (Figure S4c): elution peak at 3.26 min (17:5, v/v, CH_3OH/H_2O; λ = 270 nm). ESI-MS (Figure S4d): m/z calc. for $C_{34}H_{28}N_2O_9$ $[M+Na]^+$: 631.1693, found: 631.1685.

Synthesis of CO-Releasing PEO$_{45}$-b-PFNM$_{12}$ (OM) Amphiphilic Diblock Copolymer Through Reversible Addition-Fragmentation Chain Transfer (RAFT) Polymerization. Briefly, **FNM** monomer (183 mg, 0.3 mmol), AIBN (0.3284 mg, 0.002 mmol), and PEO-based macroRAFT agent (45 mg, 0.02 mmol) were dissolved in 600 μL of dimethyl sulfone (DMSO) in a reaction tube equipped with a magnetic stirring bar. The reaction tube was degassed by three freeze-pump-thaw cycles and then sealed under vacuum. After being thermostated at 70 ℃ in an oil bath for 8 h, the reaction was quenched into liquid nitrogen and opened, and the mixture was precipitated into 50 mL of diethyl ether three times. The sediment was filtered and dried under vacuum overnight, PEG-*b*-PFNM (OM) diblock copolymer was obtained as a yellow solid. The degree of polymerization (DP) of PFNM block was determined to be 12 based on the 1H NMR analysis (Figure S11a). Thus, the synthesized diblock copolymer was denoted as PEG_{45}-*b*-$PFNM_{12}$ (abbreviated as **OM**, Scheme 1b). The number average molecular weight (M_n) of **OM** diblock copolymer was 7.1 kDa and the molecular weight distribution (M_w/M_n) was determined to be 1.08 (Figure S11b).

Self-Assembly of OM Diblock Copolymer. OM diblock copolymer (1 mg) was dissolved in 1 mL of THF. The THF solution was then injected into 9 mL of DI water in one shot, and the mixture was vigorously stirred for 5 min at 25 ºC. After that, the resulting dispersion was transferred into a dialysis tube (MWCO = 30 kDa) and was dialyzed against DI water for 12 h

to remove the organic solvent. Fresh deionized water was replaced every 3 h.

Preparation of Nile Red-Loaded OM Micelles. Nile red (NR) was used as a fluorescent probe to investigate the polarity transition within the micelles and to mimic the drug release behavior from the micelles under visible light irradiation. Briefly, 0.1 mg of NR and 1 mg of **OM** diblock copolymer were dissolved in 1 ml of THF, which was then quickly injected into 9 mL of DI water in one shot. The mixture was stirred at room temperature for 5 min before dialysis against deionized water for 12 h (MWCO = 30 kDa); fresh DI water was replaced every 3 h, and NR-loaded OM micelles were obtained and stored at 4 ºC for further characterization.

Detection of CO Release by Myoglobin (Mb) Assays. The release of CO from **OM** micelles was assessed spectrophotometrically by measuring the conversion of deoxymyoglobin (deoxy-Mb) to carbonmonoxy myoglobin (MbCO). The amount of MbCO formed was quantified using the following eq 1.[5, 6]

$$\frac{[\mathrm{MbCO}]}{[\mathrm{Mb}]+[\mathrm{MbCO}]} = \left(\frac{\varepsilon_d}{\varepsilon_i} - \frac{A_{542}}{A_i}\right) \cdot \frac{\varepsilon_i}{\varepsilon_d - \varepsilon_c} \qquad (1)$$

where A_{542} and A_i are the absorbance values at 542 and 552 nm ε_d, ε_c, and ε_i are the extinction coefficients of Mb at 542 nm, MbCO at 542 nm, and Mb and MbCO at 552 nm (the isosbestic point), respectively. [Mb] and [MbCO] are the concentrations of Mb and MbCO. $\varepsilon_d/\varepsilon_i$ and $\varepsilon_c/\varepsilon_i$ are 0.836 and 1.227,[5] respectively. Briefly, fresh myoglobin solutions (28.4 μM) were prepared by dissolving myoglobin in 0.01 M phosphate buffer (pH 7.4). The solution was deoxygenated by bubbling with nitrogen for 15 min. Sodium dithionite (0.1 wt%) was added to reduce myoglobin to deoxy-Mb. In a small vial with a rubber septum, **OM** micelles were deoxygenated by bubbling with nitrogen for 15 min. This solution was quickly transferred to a cuvette and then overlaid with 500 μL of light mineral oil (sigma) to prevent CO leakage. After irradiation of the **OM** micelles with a hand-held lamp (410 nm, 31.5 mW/cm^2), an aliquot of stock solution of deoxy-Mb was added. The absorption was recorded at room temperature on a UV-vis spectrometer.

Assessment of Photo-Mediated CO Release of the OM Micelles. The CO release contents were monitored by a commercially available CO detector (Drager Pac6500).[7] Briefly, the measurements were carried out in a closed system equipped with a Pac6500 CO detector and a test vial containing 0.1 g/L of **OM** micelle solutions (in DI water, PBS buffer, or phenol red-

free DMEM) were placed into the test vial with a magnetic stirring bar. The lid was then closed and the micelle solution was irradiated or not irradiated with a hand-held LED lamp (410 nm, 31.5 mW/cm^2). The gas-phase CO concentration was read by the CO detector and the data were recorded every 5 min. Assuming that the gas and liquid phases reach equilibrium and the pressure in the desicator stays at 1 atm, the amount of released CO (N_{co}) using the following eq 2.[7]

$$Nco = \frac{pV_g}{RT} + cV_l = p\left(\frac{V_g}{RT} + \frac{V_l}{k}\right) \quad (2)$$

Where p is the partial pressure of CO; V_g and V_l are the volumes of the gas phase (350 mL) and liquid phase (10 mL); R is the gas contant (0.0821 atm*L*mol^{-1}K^{-1}); T is the temperature (298.15 K); c is the CO concentration in the liquid phase, and k is Henry's law constant of CO in water (1052.63 atm*L*mol^{-1} at 298.15 K).

Cytotoxicity of OM Micelles, OC Micells, and Released Small Molecules. **OC** micelles were prepared by the irradiation of **OM** micelles for 30 min. After irradiation, the irradiated **OM** micelles were subjected to dialysis against deionized water. Both the samples inside (**OC** micelles) and outside (released small molecules) the dialysis bag were collected. RAW 264.7 cells were cultured in DMEM medium with 10% fetal bovine serum (FBS) and antibiotics (100 units/mL penicillin and 0.1 mg/mL streptomycin) at 37 ºC in a humidified atmosphere with 5% CO_2, and the cell viability was determined by standard MTT assay. RAW 264.7 cells were seeded on a 96-well plate at a density of 10,000 cells/well in 100 μL of DMEM medium. After 24 h incubation, DMEM medium was replaced by 100 μL of fresh medium containing (0, 0.01, 0.02, 0.05, 0.1, 0.2 g/L) **OM** micelles, **OC** micelles, or the released small molecules. After 24 h incubation, MTT reagent in 10 μL of PBS buffer (5 mg/mL) was added into each well, and the cells were further incubated with 5% CO_2 for 4 h at 37 ºC. The culture medium in each well was removed and replaced by 100 μL of DMSO. The absorbance values were recorded at the wavelength of 490 nm by a microplate reader (Thermo Fisher Scientific). The cell viability was calculated as $(A_{490,\ treated} - A_{490,\ blank})/(A_{490,\ control} - A_{490,\ blank}) \times 100\%$, where $A_{490,\ treated}$ and $A_{490,\ control}$ are the absorbance values in the presence and absence of **OM** micelles and $A_{490,\ blank}$ is the absorbance value of the plate with identical volume of MTT solution without cells, respectively.

Cellular Uptake of OM Micelles and Intracellular CO Release Detected by All-Flu

Probe. RAW264.7 macrophages were seeded on a 4-well plate (50,000 cells/well) in 500 μL of culture medium and incubated for 24 h at 37 ºC under 5% CO_2. The **OM** micelles (0.1 g/L) was added to the cultured cells in 500 μL of culture medium. After incubation for 2, 4, 6 h, the culture plates were rinsed with PBS buffer three times. After that, phenol red-free DMEM medium was added. The intracellular uptake was observed by a Leica SP-5 confocal laser scanning microscope. The blue channels were excited at 405 nm and collected at 415-500 nm.

Photo-Mediated Intracellular CO Release. The intracellular CO release was monitored by a previously reported CO probe, i.e., Allyl-Flu, exhibiting turn-on emission in the presence of CO.[1] RAW264.7 cells were seeded on a 4-well plate at a density of 50,000 cells/well in 500 μL of DMEM medium and were allowed to adhere to the plate for 24 h. After that, cells were treated with or without **OM** micelles (0.1 g/L in DMEM medium) for 4 h. Afterward, Allyl-Flu probe and $PdCl_2$ (final concentration: 5 μM) were added to each well. After the addition, the cells were further incubated at 37 ºC for 1 h. The culture plate was rinsed with PBS buffer three times. After that, phenol red-free DMEM medium was added, and the plate was exposed or not exposed to 410 nm (31.5 mW/cm^2) for 15 min. After illumination, the cells were further incubated at 37 ºC for 1 h before images were taken on a Leica SP-5 confocal laser scanning microscope. The blue and red channels were excited at 405 nm and collected at 415-500 nm and 550-650 nm, respectively; the green channel was excited at 488 nm and collected at 500-580 nm.

Quantification of the Intracellular Nitrite Levels. The accumulation of nitrite in the cell culture medium was measured by a microplate assay method based on the Griess reaction. Briefly, RAW264.7 cells were seeded in 24–well plates at a density of 50,000 cells per well and were cultured for 24 h. After that, cells were further incubated for 4 h with medium supplemented with PBS, lipopolysaccharides (LPS) (1 μg/mL), L-NAME (1 mM)/LPS (1 μg/mL), **OC** micelles (0.1 mg/mL)/LPS (1 μg/mL), **OM** micelles without light irradiation (0.1 mg/mL)/LPS (1 μg/mL), and **OM** micelles with light irradiation for 15 min (0.1 mg/mL)/LPS (1 μg/mL). After the treatment, the cells were incubated for another 20 h. The culture medium was collected and placed into a 96-well plate (50 μL per well), to which 50 μL of Griess reagent was added. The plate was gently shaken for 10 min and the absorbance at 550 nm was recorded. The nitrite levels were determined against a standard calibration curve.

Determination of Intracellular TNF-α Concentrations. RAW264.7 cells were seeded in 24–well plates at a density of 50,000 cells per well and were cultured for 24 h before the experiment. On the day of the experiment, the cell were incubated with L-NAME (1 mM), **OC** micelles (0.1 mg/mL), and **OM** micelles (0.1 mg/mL) for 4 h, respectively. After that, one of the **OM** micelle-treated group was illuminated with an LED lamp (410 nm, 31.5 mW/cm^2) for 15 min. The other OM-micelle-treated plate was left in the dark under the same incubation time. Thereafter, LPS was added to a final concentration of 1 μg/mL. The plates were then incubated at 37 °C for 1 h. The cell culture supernatant (100 μL) was then collected and the TNF-α concentration in each well was determined by using a commercial ELISA kit (TNF-α Mouse Instant ELISA^{TM} kit; Beyotime^{TM}) following the provided protocol. Cells without LPS treatment were used as the control.

Photo-Mediated in vivo CO Release. All the animal studies were approved by the Committee on the Ethics of Animal Experiments of the University of Science and Technology of China (USTC) and were performed in strict accordance with the Animal Care and Use Committee of USTC. Dorsal hair of BALB/c mice (6-8 weeks, Experimental Animal Center of Anhui Medical University) were shaved. **OM** micelles (0.1 g/L; 100 μL) or PBS buffer (100 μL) were injected into left and right hind limbs, respectively. The mice were exposed or were not exposed to an LED lamp (410 nm, the irradiation intensity on the skin surface was 31.5 mW/cm^2). After the treatment, fluorescence was recorded on an IVIS® Lumina III imaging system. The excitation filter was set at 420 nm, and the emission filter was set at 620 nm, respectively.

In vivo Wound Healing. Dorsal hair of BALB/c mice (6-8 weeks, Experimental Animal Center of Anhui Medical University) were shaved, and full-thickness cutaneous wound (5 mm in diameter) were created as described in previous literature reports (n = 4).[8] After that, 50 μL of PBS buffer, **OM** micelles without or with light irradiation, **OC** micelles, or CORM-3 was applied to the wounds every day. On days 0, 1, 3, 7, 10, and 12, wounds were digitally photographed by a hand-held Dino-Lite microscope (AF4915ZT). Wound areas were quantified using ImageJ software (NIH, USA). One mouse per group was sacrificed at days 3 and 12, respectively. Both the wound and surrounding tissues (~ 5 mm) were collected for histological evaluation.

Hematoxylin and Eosin (H&E) and Sirius Red Staining. 10% Neutral buffered formalin was used to fix the wound and surrounding tissues. The fixed tissues were embedded in paraffin and sequentially sectioned at 5 μm using a Finess ME microtome. Skin sections were stained with hematoxylin and eosin (H&E) for the evaluation of granulation tissue formation and wound maturity, while Sirius red staining was used to assess the extents of collagen deposition in the healed tissues during wound healing. All histological analyses were performed at least 4 independent wounds per group, and images displayed are representatives of all replicates. Images of entire sections were achieved on an IX71 microscope (Olympus) (Figure 4).

CD31 Immunofluorescent Staining. An antibody directed against the murine endothelial cell surface marker (CD31) was employed to evaluate the extents of endothelial cell proliferation in the wound section during wound healing. Briefly, skin sections embedded in paraffin were sliced into 5 μm thickness. After the treatment of blocking solution (5%, w/v, BSA in deionized water) to minimize non-specific binding sites, the primary antibody was applied for 1.5 h at room temperature, followed by washing five times with PBS buffer. A secondary antibody conjugated to goat anti-rabbit IgG-FITC was then added and incubated for 1 h to label the antigen. Finally, sections on a coverslip were mounted in the dark at 4 ^{o}C, and fluorescence images were taken on a Leica SP-5 confocal laser scanning microscope. The images were representative one of the local areas of wound sections stained with CD31 (Figure 4).

Statistical Analysis. Data are presented as mean ± standard deviations and were analyzed using Prism 8.0 software (GraphPad, San Diego, California, USA) and student's *t*-test. The level of significance was set at $p < 0.05$.

Characterization. ^{1}H and ^{13}C NMR spectra were taken on a 400 MHz Bruker nuclear magnetic resonance (NMR) spectrometer. Deuterated chloroform ($CDCl_3$) and dimethylsulfoxide (DMSO-d_6) were used as the solvents. HRMS spectra were taken on LCMS-2020 LC/MS system (Shimadzu). HPLC analysis was performed with a Shimadzu HPLC system, equipped with a LC-20AP binary pump, an SPD-20A UV-Vis detector, and a Symmetry C18 column. Fluorescence spectra were recorded on F-4600 (Hitachi) spectrofluorometer. UV-Vis absorption spectra were acquired on a TU-1910 double-beam UV-Vis spectrophotometer (Puxi General Instrumental Company, China). Molecular weights (MW) and molecular weight

distributions were determined by gel permeation chromatography (GPC) equipped with Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30 ^{o}C). It used a series of three linear Styragel columns at an oven temperature of 50 ^{o}C. The eluent was DMF at a flow rate of 1.0 mL/min. Transmission electron microscopy (TEM) was conducted on a JEOL 2010 electron microscope at an acceleration voltage of 200 kV. Dynamic light scattering (DLS) measurements were conducted using ALV-7004 with 632.8 nm laser light set at a scattering angle of 90^{o}. Hematoxylin and Eosin (H&E) and Sirius Red Staining images were obtained on an IX71 fluorescence microscope (Olympus, Japan). Confocal laser scanning microscopy (CLSM) images were acquired using a Leica TCS SP5 microscope. *In vivo* fluorescence imaging was acquired on an IVIS® Lumina III imaging system. The following parameter were applied for the fluorescence imaging, excitation filter: 420 nm, emission filter: 640 nm, field of view (FOV) 12.8 cm, F-stop 2, acquisition time: 0.5 s).

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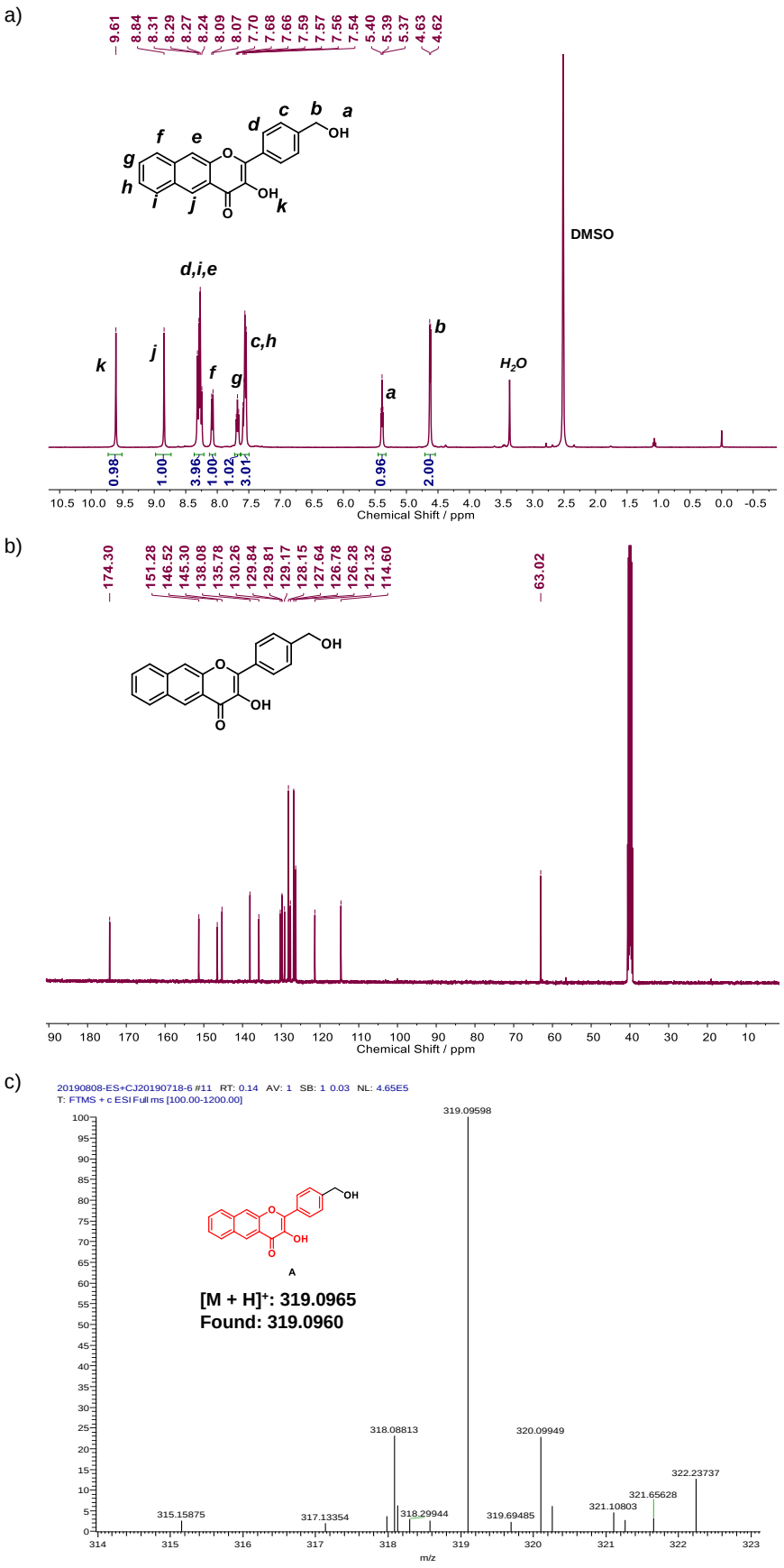


Figure S1. (a) ^{1}H and (b) ^{13}C NMR recorded in DMSO-d_6 and (c) ESI mass spectra for compound **A**, respectively.

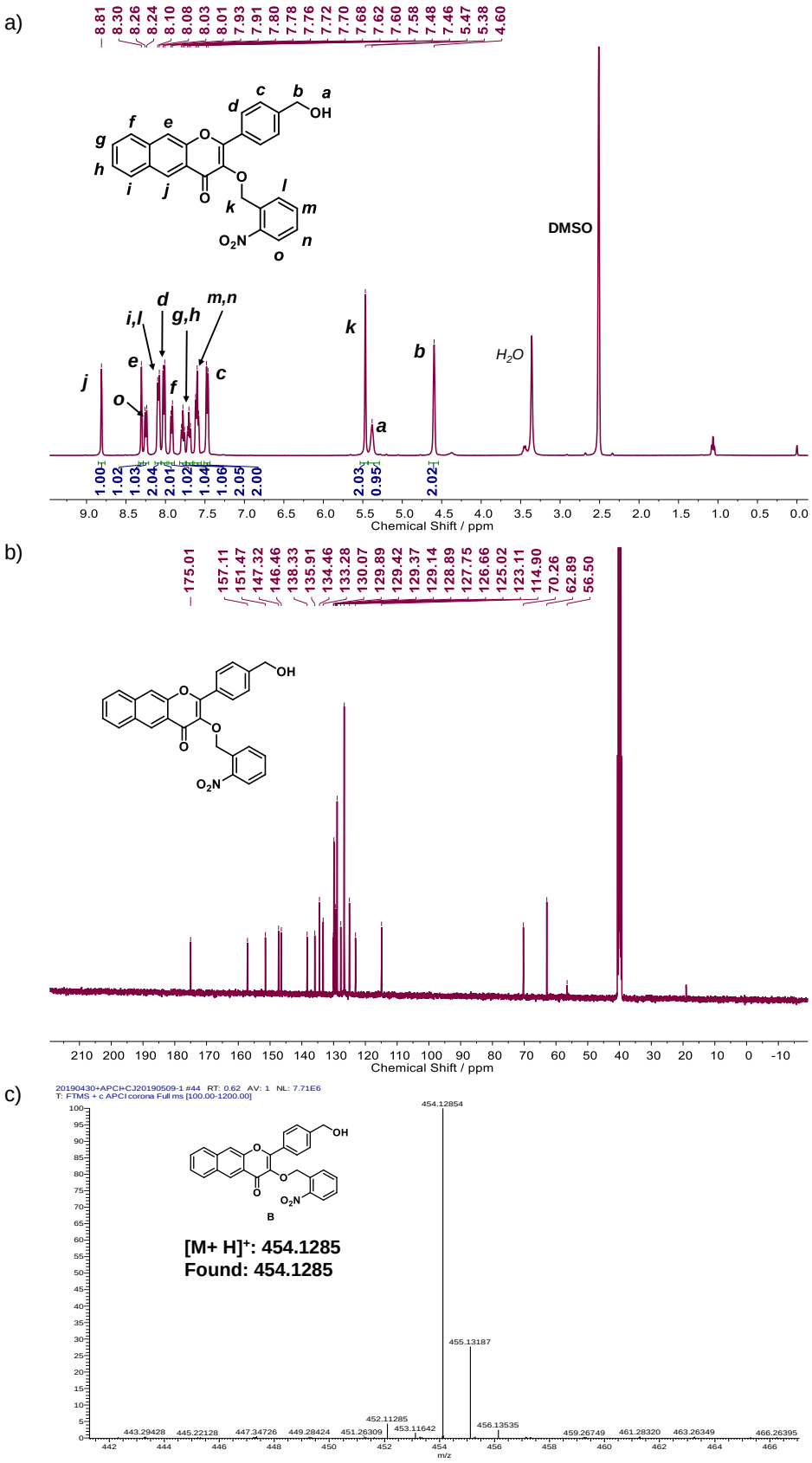


Figure S2. (a) ^{1}H and (b) ^{13}C NMR spectra recorded in DMSO-d_6 and (c) HRMS result for compound **B**, respectively.

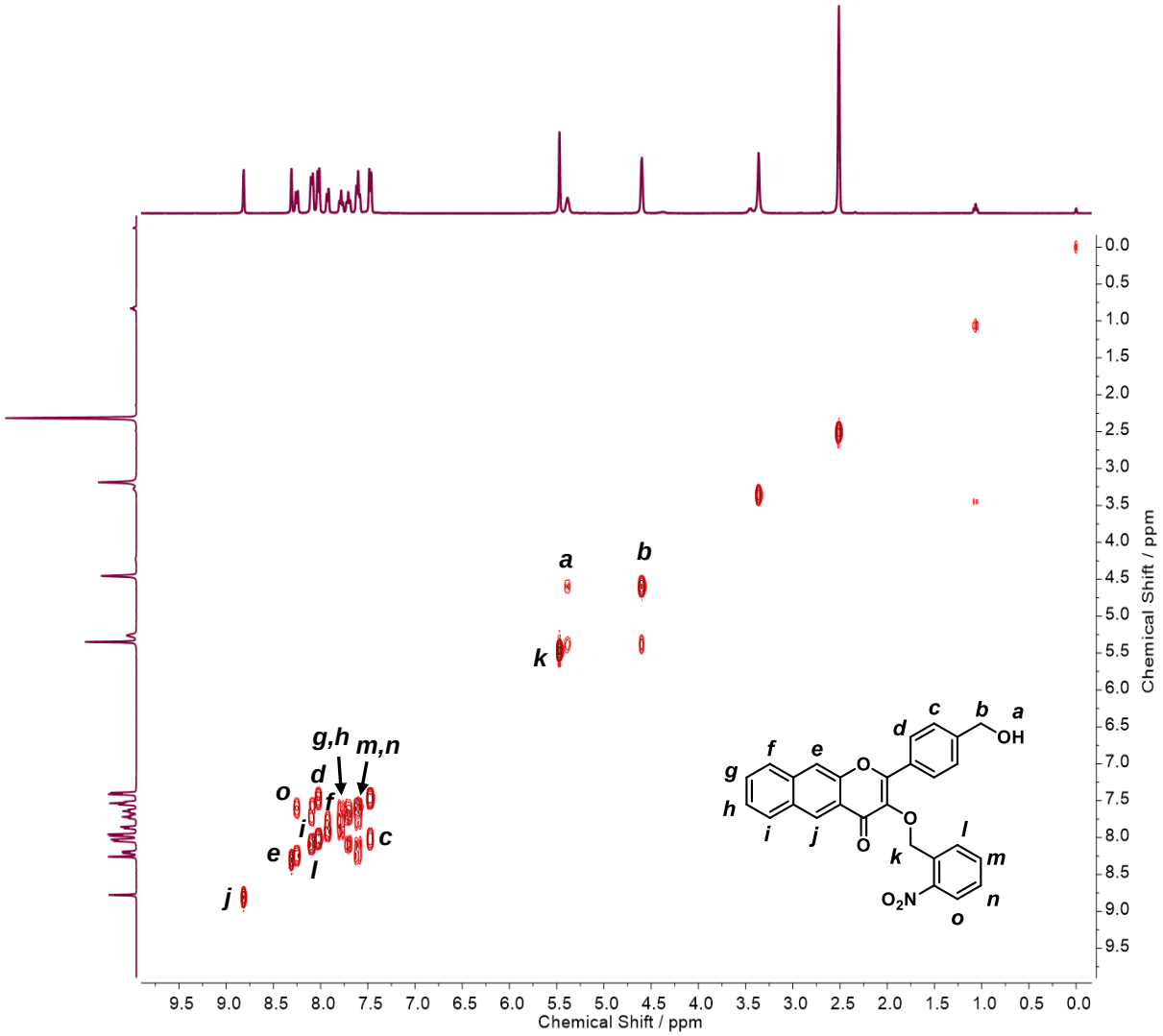


Figure S3. 2D ^{1}H-^{1}H COSY NMR spectrum recorded in DMSO-d_6 for compound **B**.

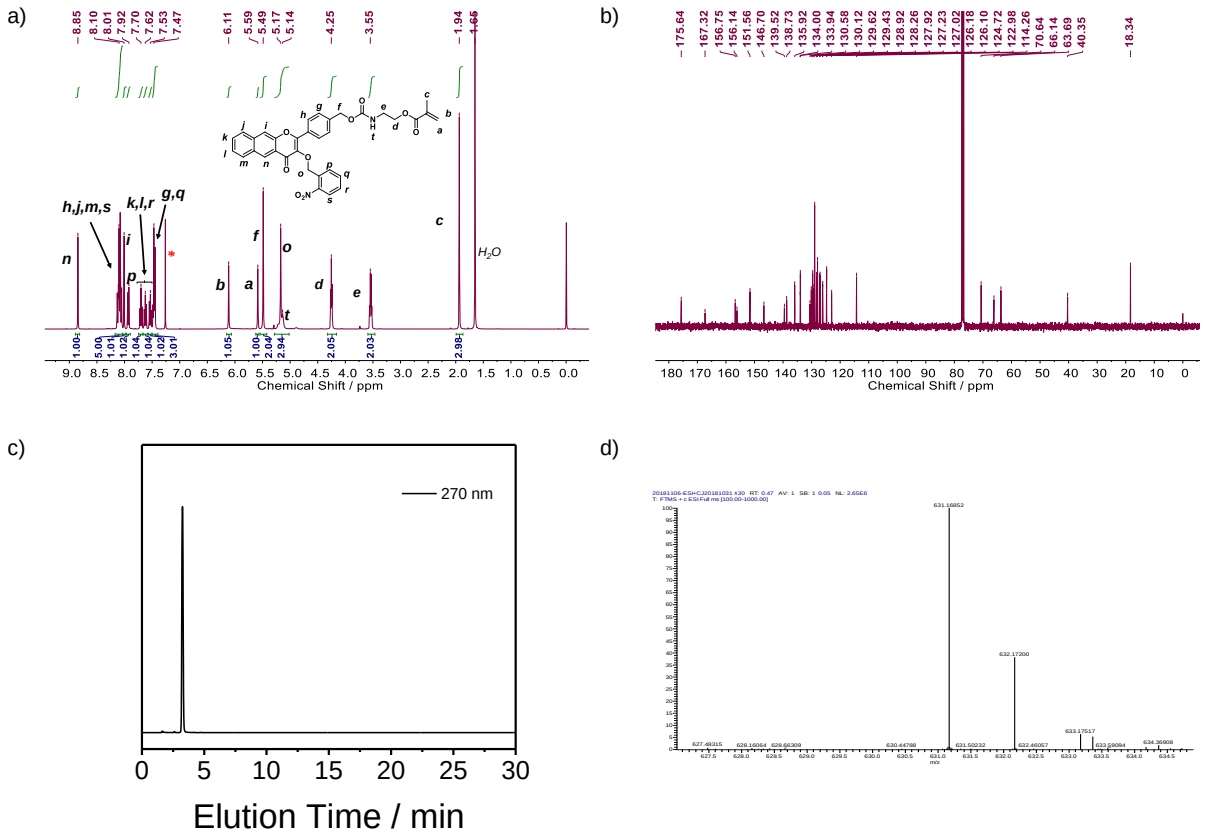


Figure S4. (a) ^{1}H and (b) ^{13}C NMR spectra recorded in $CDCl_3$ for FNM monomer. (c) HPLC elution profile detected at 270 nm for FNM monomer using MeOH/H_2O = 17/5 (*v*/*v*) as the eluent. (d) ESI-MS analysis for FNM monomer.

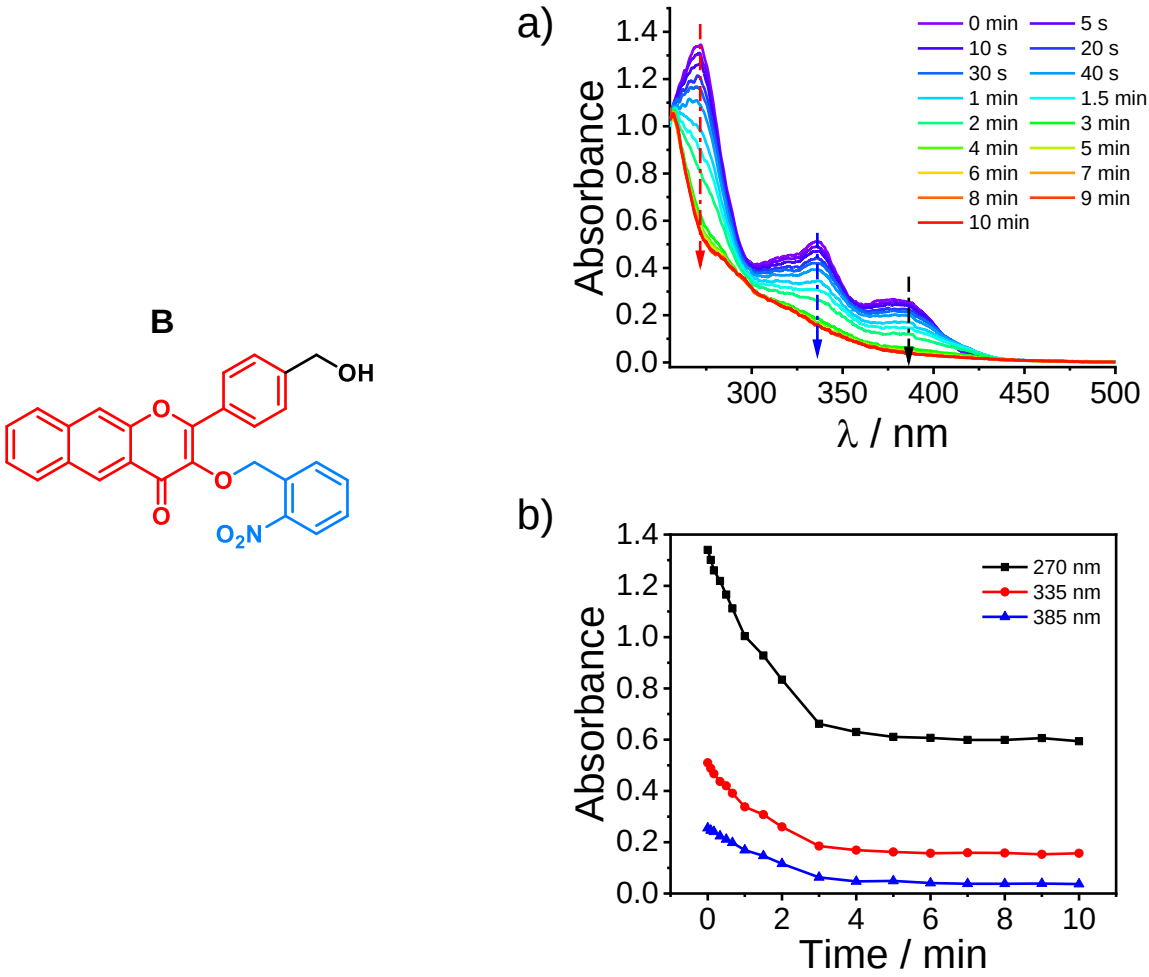


Figure S5. (a) UV-vis absorbance spectra and (b) absorbance intensity changes of mixed solutions (40 μM, DMSO/H_2O = 6/4, *v*/*v*) of compound **B** under 410 nm light irradiation (31.5 mW/cm^2).

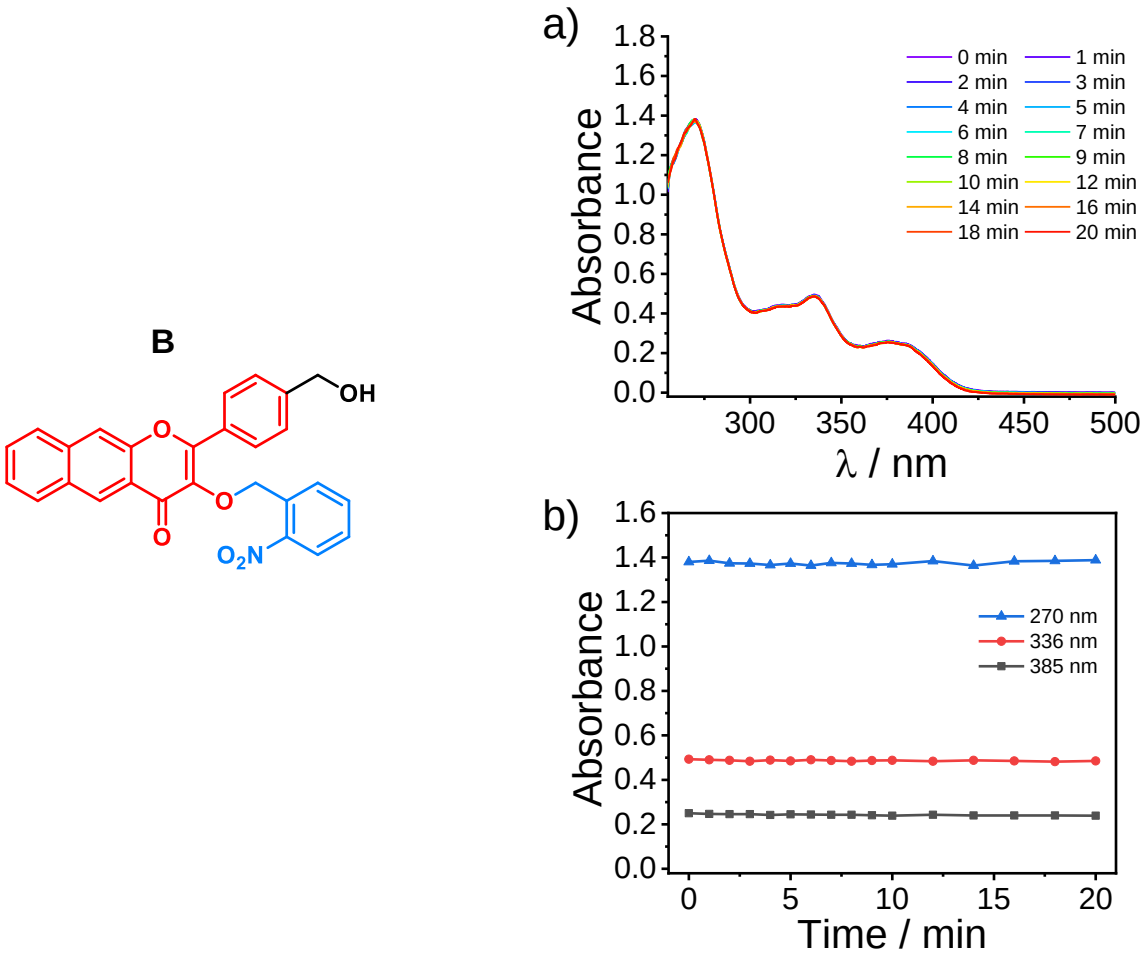


Figure S6. (a) UV-vis absorbance spectra and (b) absorbance intensity changes of mixed solutions (40 μM, DMSO/H_2O = 6/4, *v*/*v*) of compound **B** under dark condition.

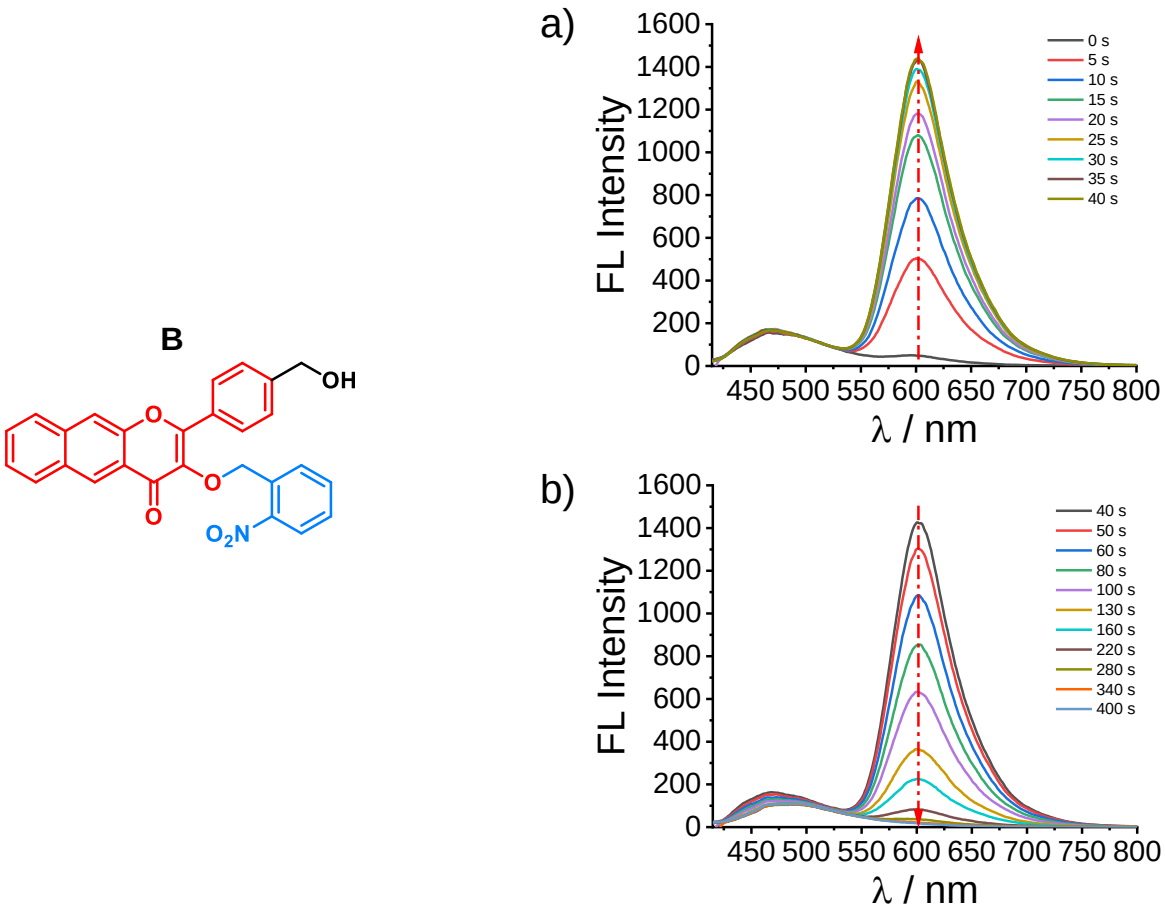


Figure S7. Fluorescence emission spectra (λ_{ex} = 405 nm; slit width: Ex. 5 nm, Em. 5 nm) recorded at (a) 0-40 s and (b) 40-400 s for mixed solutions (40 μM, DMSO/H_2O = 6/4, *v*/*v*) of compound **B** under 410 nm light irradiation (31.5 mW/cm^2).

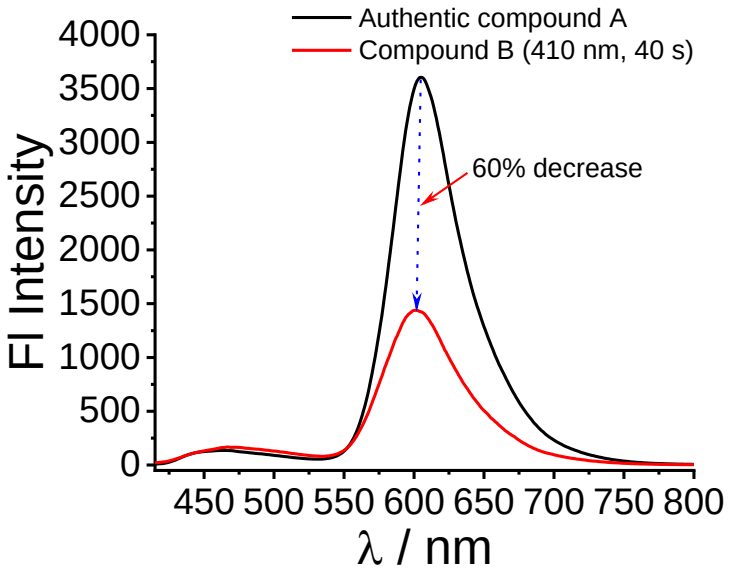


Figure S8. Fluorescence emission spectra (λ_{ex} = 405 nm; slit width: Ex. 5 nm, Em. 5 nm) of aqueous solutions (40 μM, DMSO/H_2O = 6/4, *v*/*v*) of authentic compound A and compound B with 410 nm irradiation for 40 s.

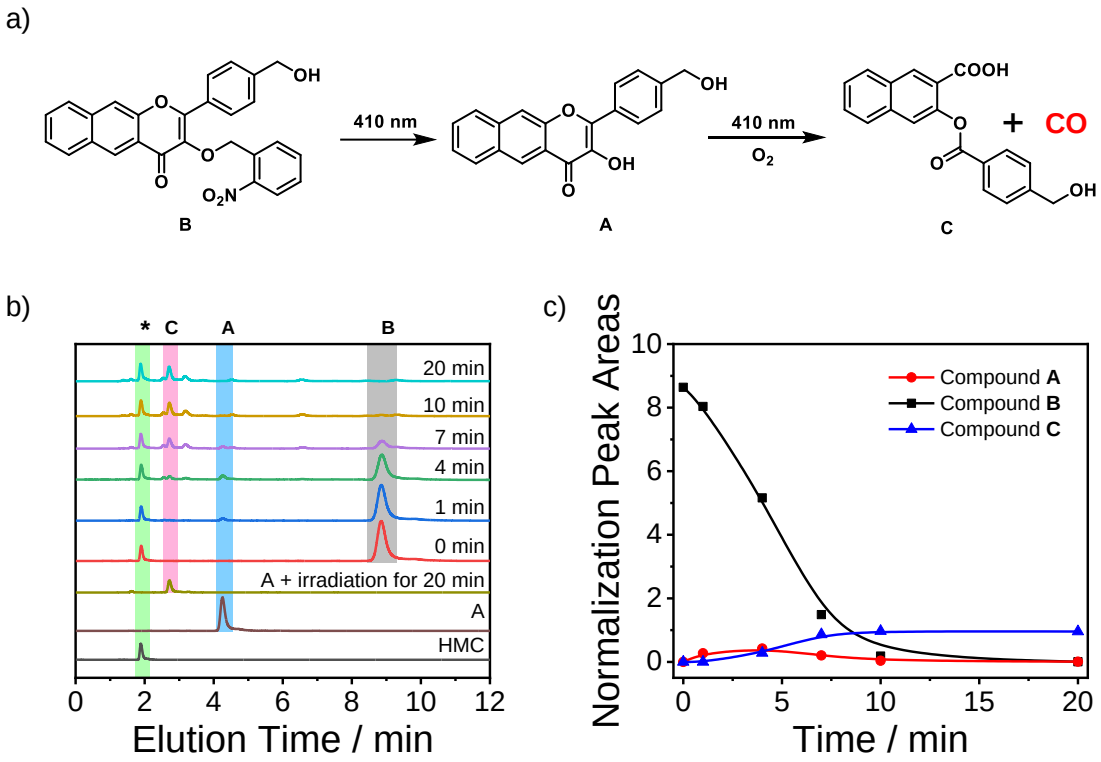


Figure S9. (a) Proposed CO release mechanism through photo-mediated tandem reactions of compound **B** bearing two photoresponsive moieties. (b) HPLC elution profiles of compound **B** under 410 nm light irradiation for varying times (0 min, 1 min, 4 min, 7 min, 10 min, and 20 min) using MeCN:H_2O = 6:4 (containing 0.1% TFA, v/v). Authentic compound **A** before and after irradiation with 410 nm light for 20 min was used for comparison. The asterisk represents the signal of 7-hydroxy-4-methylcoumarion (HMC), which was used as the internal standard. (c) Normalized peak areas against HMC internal standard of compounds **A**, **B**, and **C** under 410 nm irradiation quantified from HPLC results in (b).

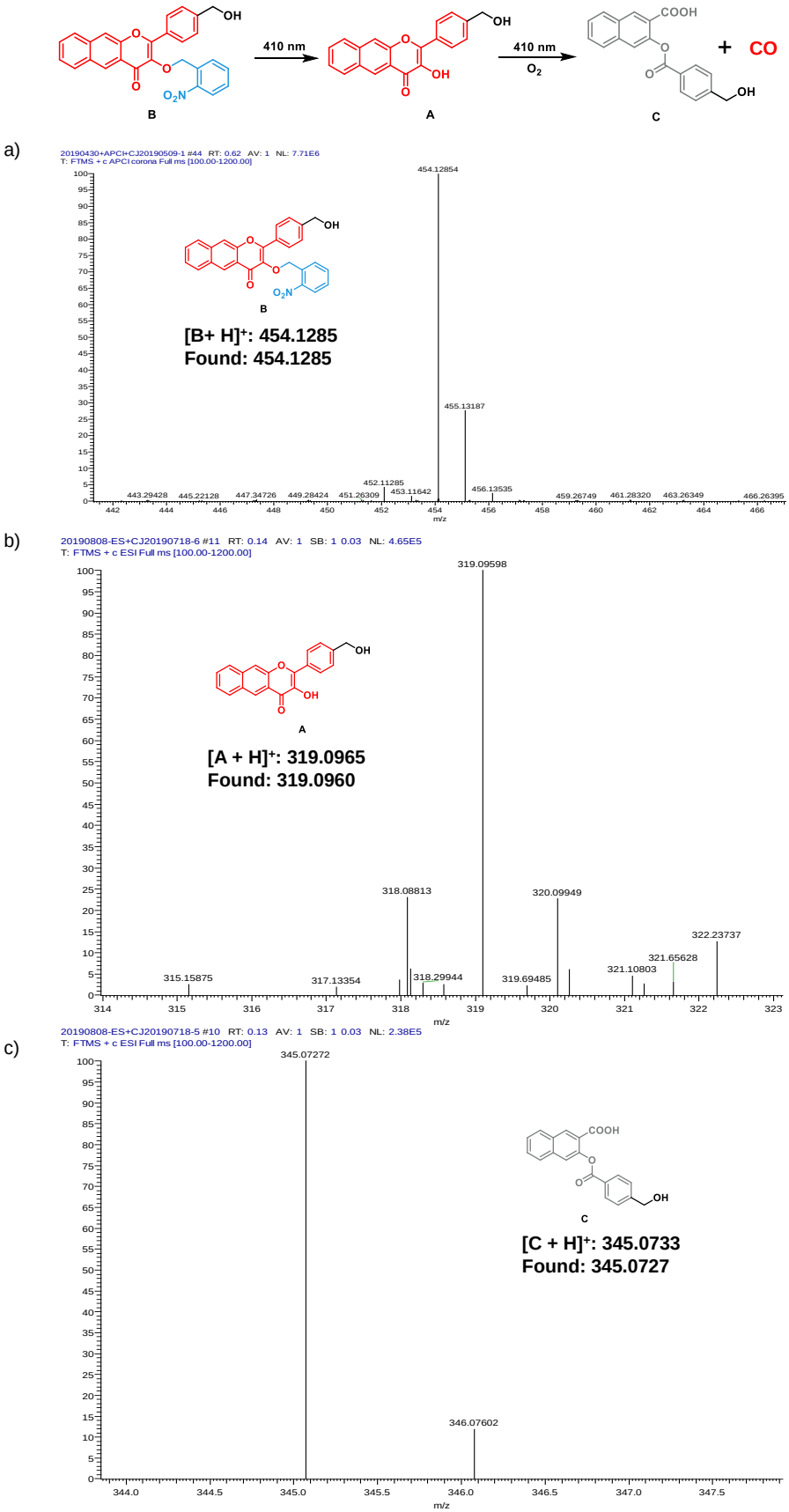


Figure S10. HRMS analysis of compound **B** under 410 nm light irradiation, revealing the formation of compound **A** intermediate and compound **C** with further photolysis.

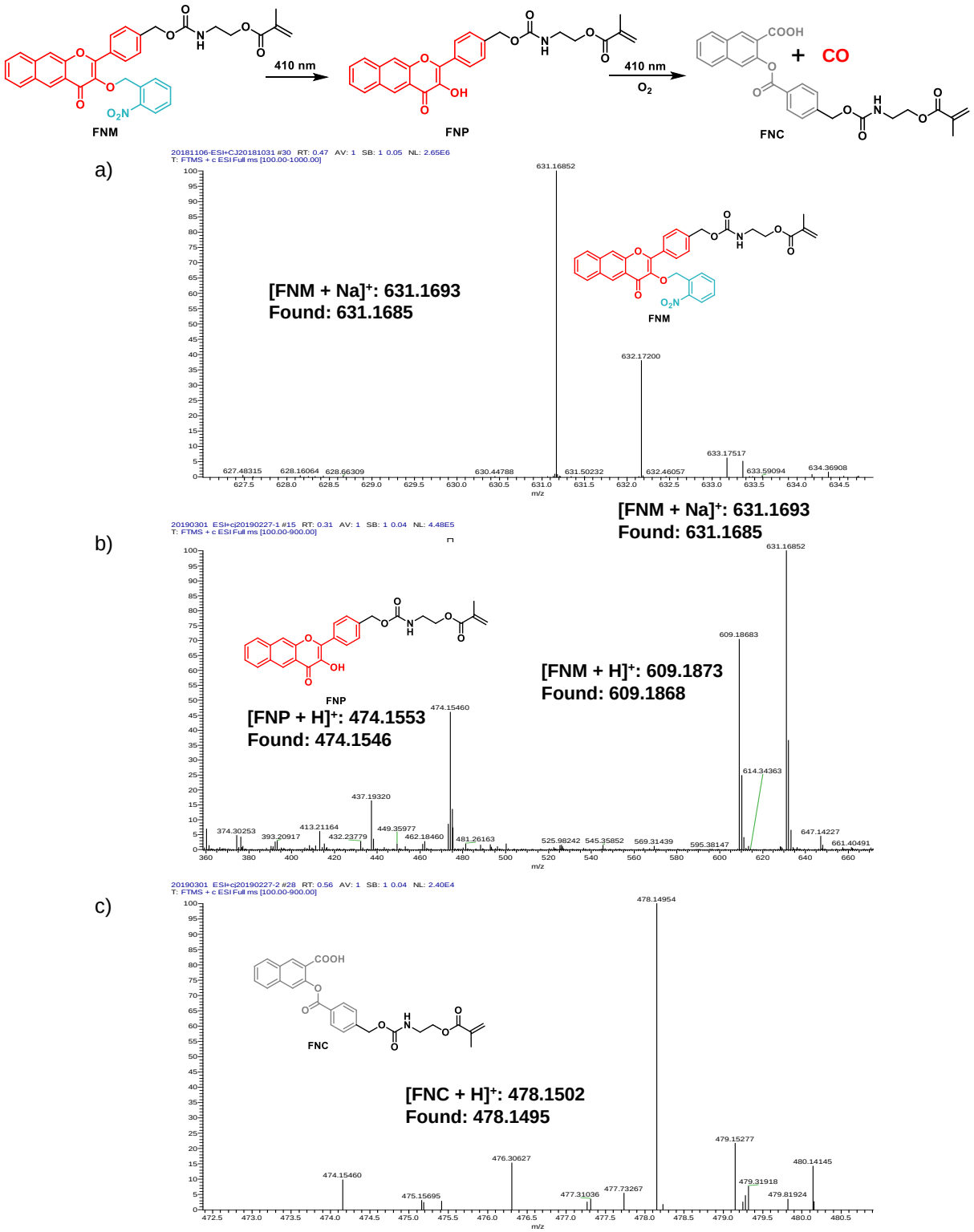
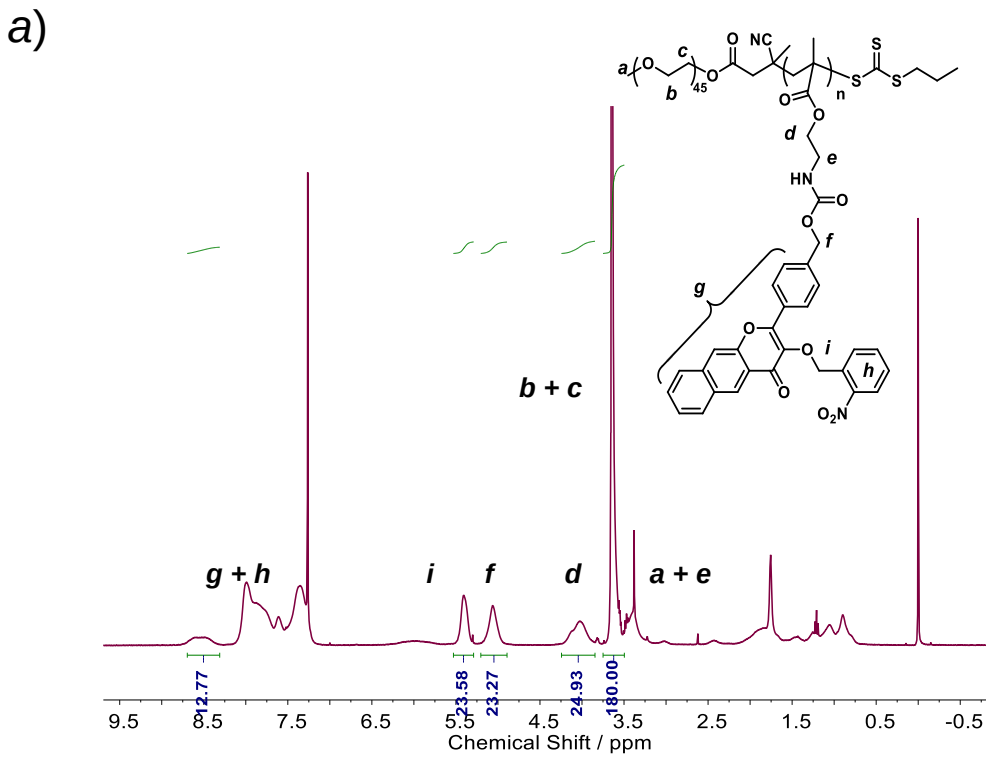


Figure S11. ESI-MS results of FNM monomer (a) before and (b, c) after 410 nm light irradiation for (b) 1 min and (c) 20 min, respectively.



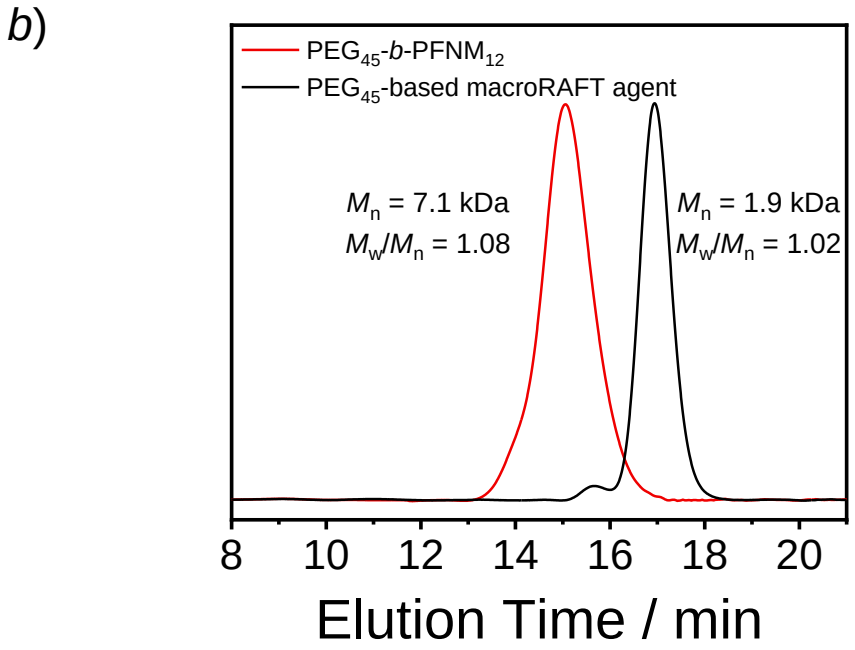


Figure S12. (a) ^{1}H NMR spectrum recorded in $CDCl_3$ for PEO_{45}-*b*-$PFNM_{12}$ (**OM**) diblock copolymer. (b) GPC elution profiles of PEO-based macroRAFT agent and OM diblock copolymer.

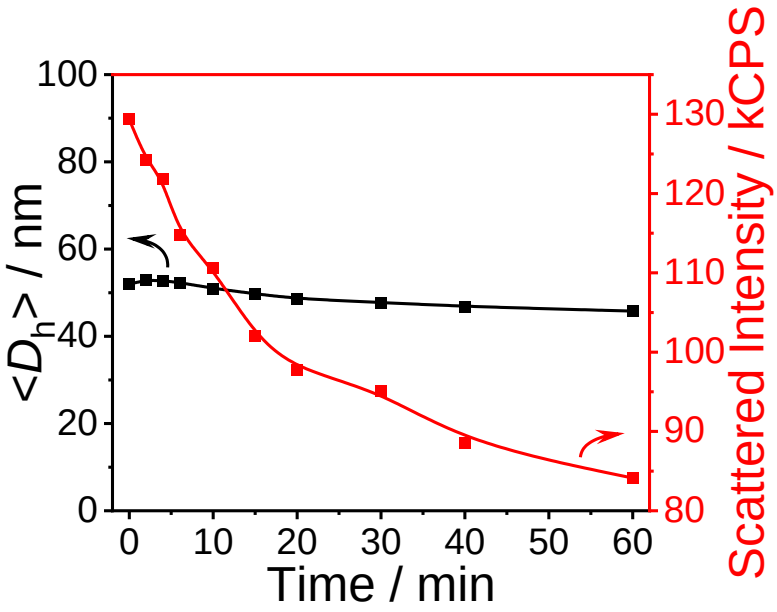


Figure S13. Evolution of intensity-average hydrodynamic diameters, $<D_h>$, and corresponding scattering intensities of **OM** micelles (0.1 g/L) under light irradiation (410 nm, 31.5 mW/cm^2).

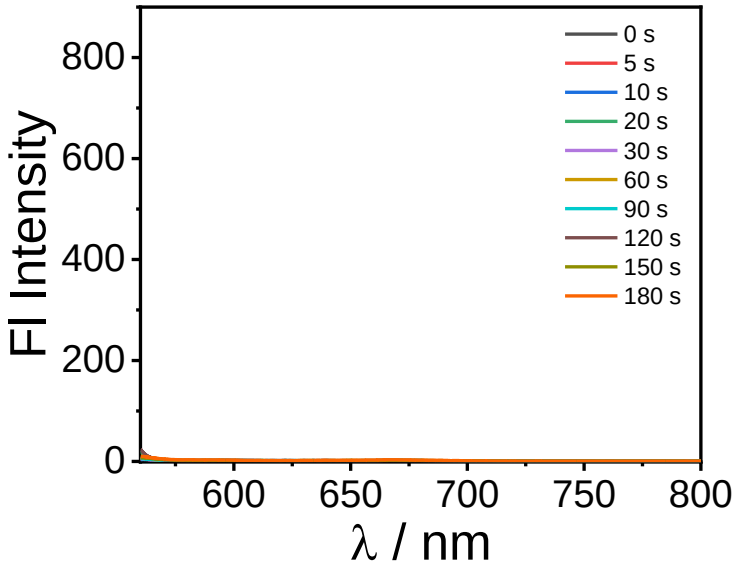


Figure S14. Fluorescence emission spectra (λ_{ex} = 550 nm; slit width: Ex. 5 nm, Em. 5 nm) of **OM** micelles under 410 nm light irradiation (31.5 mW/cm^2).

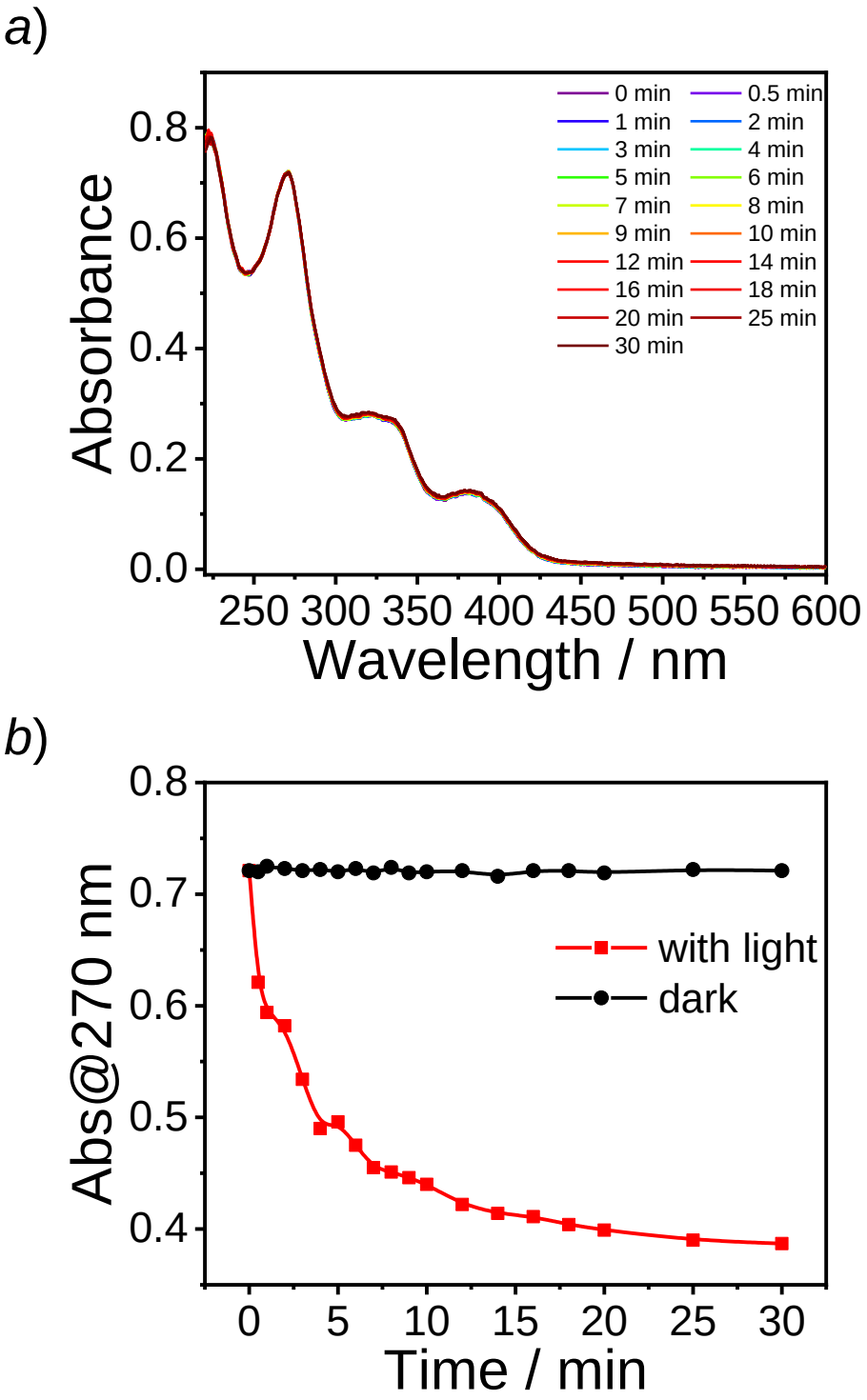


Figure S15. (a) UV-Vis absorbance spectra of aqueous dispersion (0.1 g/L, PBS buffer, 10 mM, pH 7.4, 25 ºC) of **OM** micelles without light irradiation. (b) Absorption intensity changes at 270 nm of aqueous dispersion (0.1 g/L, PBS buffer, 10 mM, pH 7.4, 25 ºC) of **OM** micelles with and without 410 nm light irradiation (31.5 mW/cm^2).

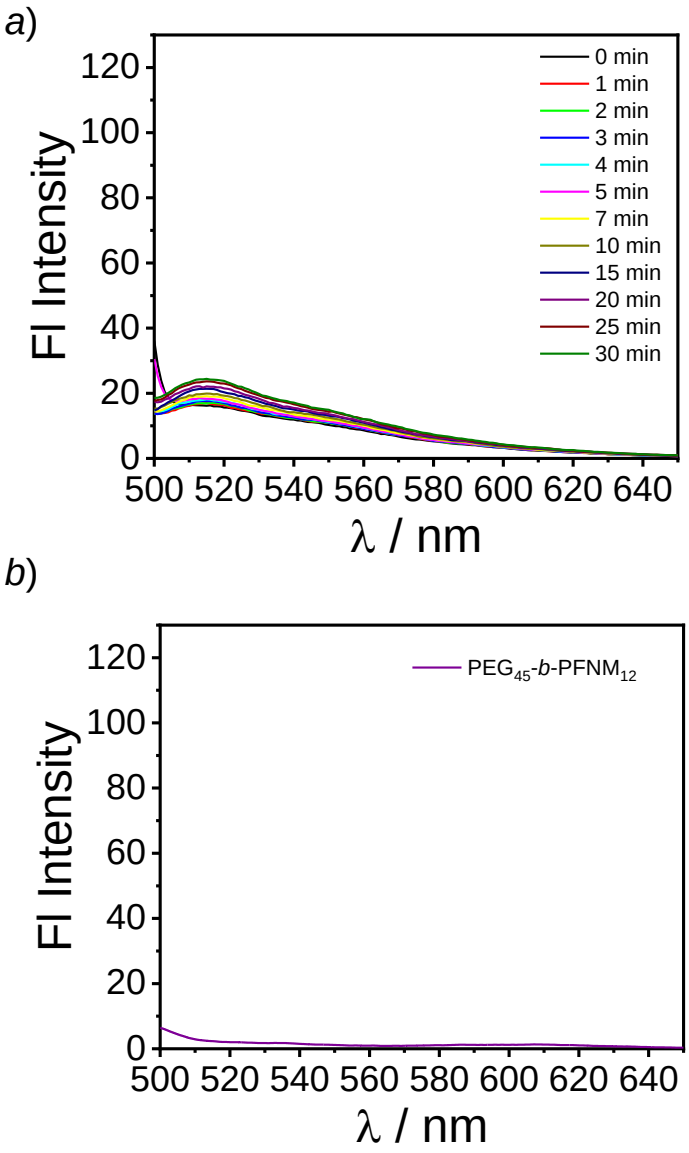


Figure S16. (a) Fluorescence emission spectra (λ_{ex} = 490 nm; slit width: Ex. 5 nm, Em. 5 nm) of aqueous solution (PBS buffer, 10 mM, pH 7.4, 25 ºC) of CO probe (5 μM) and $PdCl_2$ (5 μM) under 410 nm light irradiation (31.5 mW/cm^2). (b) Fluorescence emission spectra (λ_{ex} = 490 nm; slit width: Ex. 5 nm, Em. 5 nm) of aqueous dispersion of **OM** micelles (0.1 g/L, PBS buffer, 10 mM, pH 7.4, 25 ºC) after irradiation with 410 nm light for 20 s.

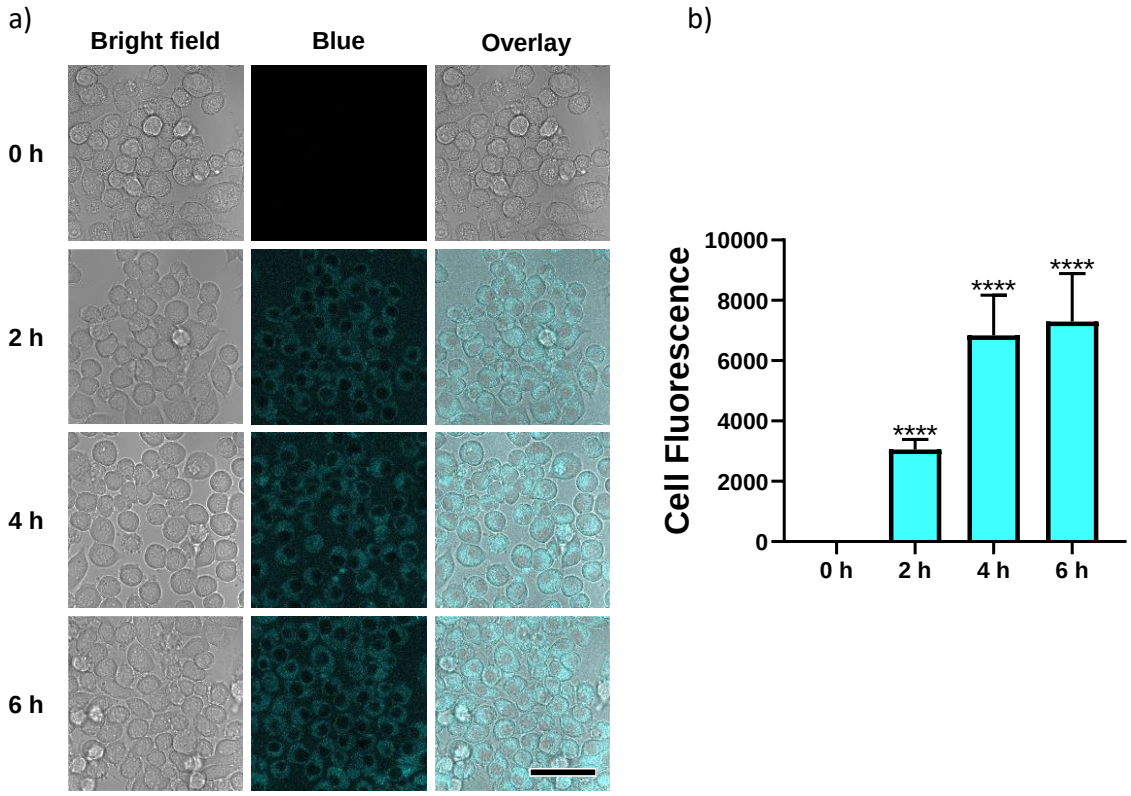


Figure S17. (a) Time-dependent CLSM images and (b) quantitative analysis of fluorescence intensities of RAW264.7 cells upon incubation with OM micelles (0.1 g/L). Scale bar: 50 μm. **** $p < 0.0001$ compared with the control group after incubation for 0 h.

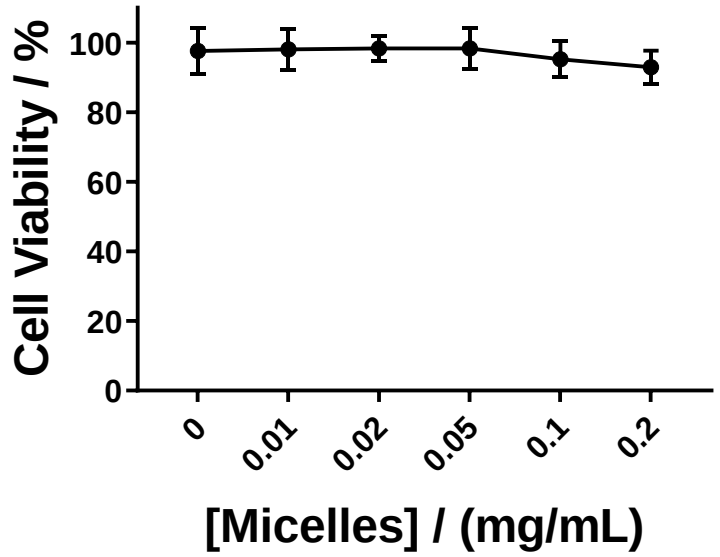


Figure S18. Cell viability as determined by MTT assay of the released small molecules from **OM** micelles after irradiation for 30 min (410 nm, 31.5 mW/cm^2).

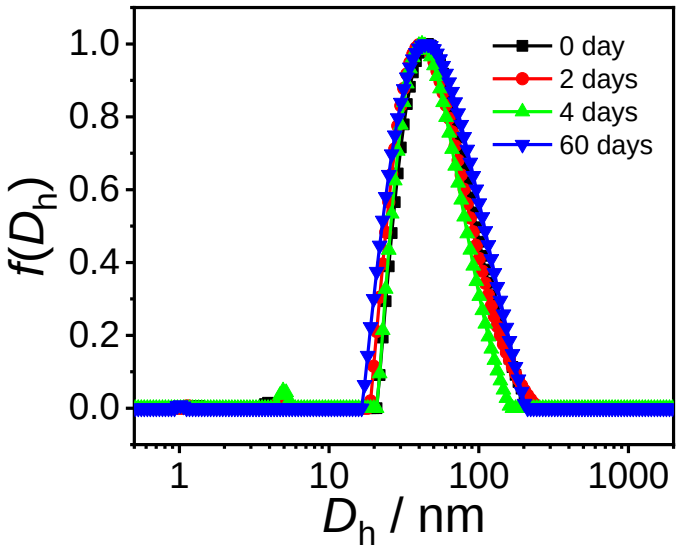


Figure S19. Time-dependent hydrodynamic diameter distributions of **OM** micelles (0.1 g/L) in PBS buffer without light irradiation.