Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A naphthalimide derivative can release COS and form H_2S in a light controlled manner and protect cells against ROS with real time monitoring ability

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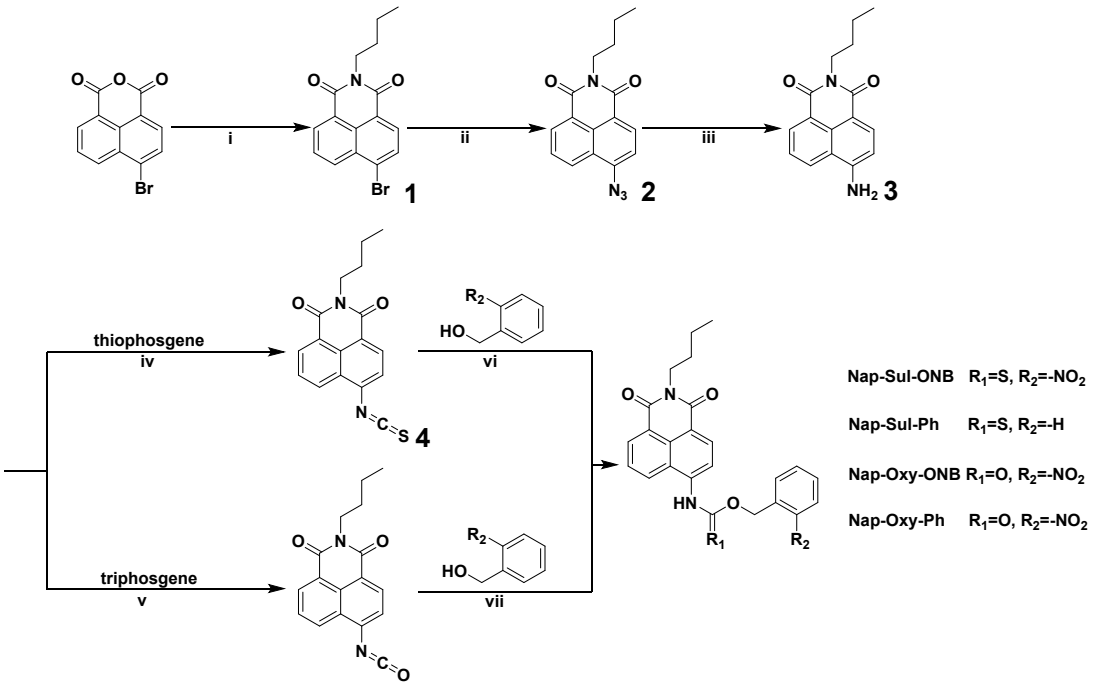
1. Experimental section



Scheme S1. Synthetic procedure of the targeted compounds. i. n-butylamine, EtOH, reflux, overnight. ii. NaN_3, DMF, 100 ℃, overnight. iii.

Na_2S, DMF, 4 h. iv. THF, DMAP, DIPEA. v. toluene, DMAP, DIPEA. vi. NaH, THF, 0 ℃-r.t., overnight. vii. DIPEA, toluene, 0 ℃-r.t., overnight.

UV-Vis photolysis experiments: All the tested compounds were dissolved in a mixed solvent (10 μM in 0.01 mM PBS, pH=7.4, containing 10 % DMSO). The UV-Vis absorption spectrum were recorded after samples were irradiated by a 365 nm LED (6.30 mW/cm^2) for every 2 min and the total light dose was 7.56 J/cm^2.

Fluorescence emission photolysis experiments: The sample of Nap-Sul-ONB and Nap-Oxy-ONB (10 μM in 0.01 mM PBS, pH=7.4, containing 10 % DMSO, respectively) were irradiated by a 365 nm LED (6.30 mW/cm^2) for every 2 min after which the emission spectrum were recorded. The total light dose was 7.56 J/cm^2. For comparing experiment, the power density was down-regulated as 3.15 mW/cm^2. Excitation wavelength: 435 nm. Ex slit=5 nm. Em slit=5 nm.

Methylene blue (MB) assay: The experiment methods was conducted according to previous reports[1,2] with some modification. Briefly, for each phial filled with 5 mL of PBS (0.01 mM, pH=7.4, containing 25 μg/mL of CA and 0.5 % of zinc acetate), 25 μL of Nap-Sul-ONB or Nap-Oxy-ONB (20 mM in DMSO, respectively) was added and mixed slowly. After irradiated by a 365 nm LED (6.30 mW/cm^2) for varied durations, 2.5 mL of the solution was taken out from each phial and mixed with 2.5 mL of MB reagent (a mixture of 1.25 mL of 30 mM $FeCl_3$ in 1.2 M HCl and

1.25 mL of 20 mM N,N-dimethyl-p-phenylene diamine in 7.2 M HCl), respectively. The solution was incubated for 2 h at 37 ℃. Then the absorption spectrum was recorded. The calibration curve was also obtained following the previous reports[1,2]. The maximal light dose was 7.56 J/cm^2.

Cell culture methods: MCF-7 cells were cultured in 5 % CO_2 at 37 °C with RPMI-1640 containing penicillin/streptomycin (Beyotime, Nantong, China) and 10% fetal bovine serum (FBS) (Hyclone, Lifescience, USA).

Cellular signal response/cellular accumulation experiments: MCF-7 cells were cultured in a glass bottom cell-culture dish with a culture medium (0.1% DMSO, v/v) containing 20 μM of Nap-Sul-ONB for 4 h. Then the cells were taken to LCSM to obtain the cell images after indicated period time of irradiation (365 nm LED, 6.30 mW/cm^2, 2 or 30 min). The light dose was 0.76 and 11.34 J/cm^2, respectively. λ_{EX}= 405 nm, λ_{EM}= 500-580 nm.

Cell protective effect against exogenous ROS: [3,4] MCF-7 cells were cultured with a culture medium containing 50 μM of H_2O_2 for 30 min. After that, the cells were incubated with 20 μM of Nap-Sul-ONB for 4 h and irradiated by 365 nm light (LED, 6.30 mW/cm^2) for 30 min, which the light dose was 11.34 J/cm^2. Next, the culture medium was removed and the cells were incubated with 0.5 mL of DCFH-DA work solution (5 μM) for 30 min. After washed with PBS (0.01 mM, pH=7.4, 1 mL×3), the cells were

taken to LCSM to detected the fluorescence signal of ROS. λ_{EX}= 488 nm, λ_{EM}= 520-550 nm.

Cell protective effect against endogenous ROS:[4] MCF-7 cells were cultured in a glass bottom cell-culture dish with a culture medium (no serum, containing1μg/mL of PMA) for 1 h. After that, the cells were incubated with 20 μM of Nap-Sul-ONB for 4 h and irradiated by 365 nm light (LED, 6.30 mW/cm^2) for 30 min with a light dose of 11.34 J/cm^2. Then the culture medium was removed and the cells were incubated with 0.5 mL of DCFH-DA work solution (5 μM) for 30 min. After washed with PBS (0.01 mM, pH=7.4, 1 mL×3), the cells were taken to LCSM to detected the fluorescence signal of ROS. λ_{EX}= 488 nm, λ_{EM}= 520-550 nm.

Cytotoxicity assay:[4,5] MCF-7 cells were plated in 96-well plates at a density of approximately 10^5/ml per well. The cells were exposed to 1μg/mL of PMA in serum-free growth media for 1 h. Then the cells were administrated with varied concentrations of Nap-Sul-ONB (in cell culture media) for 4 h and irradiated with 365 nm light (LED, 6.30 mW/cm^2) for 30 min (Light dose=11.34 J/cm^2). After incubated for further 24 h at 37 ℃, culture media were removed. To every well, 100 μL of MTT (0.5 mg/mL) was added and the cells were incubated for 4 h at 37 ℃ in 5 % CO_2. Next, the supernate was removed carefully and 100 μL of DMSO was added into each well. Finally, the absorption at 490 nm was measured by microplate reader.[5]

Live/dead cell co-staining assay: MCF-7 cells were exposed to 1μg/mL of PMA in serum-free growth media for 1 h. Then the cells were incubated with 20 μM of Sul-Nap-ONB for 4 h and irradiated with 365 nm light (LED, 6.30 mW/cm^2) for 5 or 30 min (The light dose was 1.89 or 11.34 J/cm^2.). After that, the cells were washed with PBS (0.01 mM, pH =7.4, 1 mL ×3) and further treated with calcein AM and propidium iodide (5 μM respectively) for 30 min, followed by washing with PBS. Finally the samples were measured by LSCM. Red: λ_{EX}= 561 nm, λ_{EM}= 600–640 nm. Green: λ_{EX}= 488 nm, λ_{EM}= 500–540 nm.

2. Figures

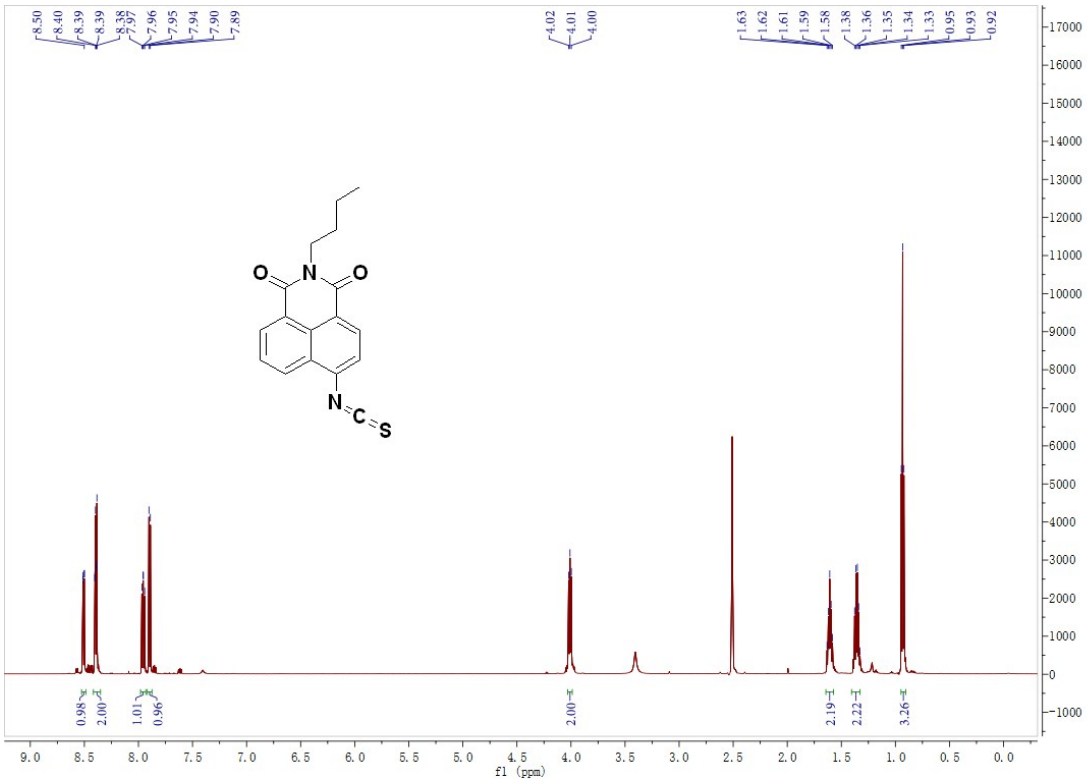


Fig. S1 ^{1}H NMR spectrum of Intermeidate **4**. (DMSO-d6)

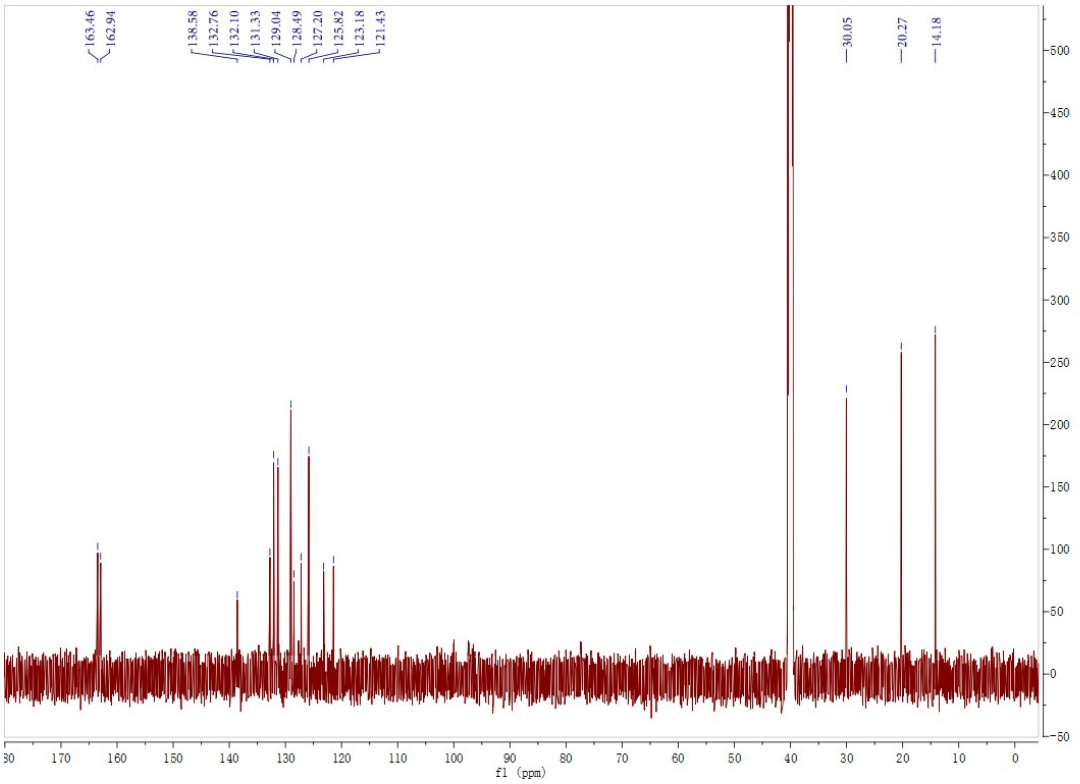


Fig. S2 ^{13}C NMR spectrum of Intermediate **4**. (DMSO-d6)

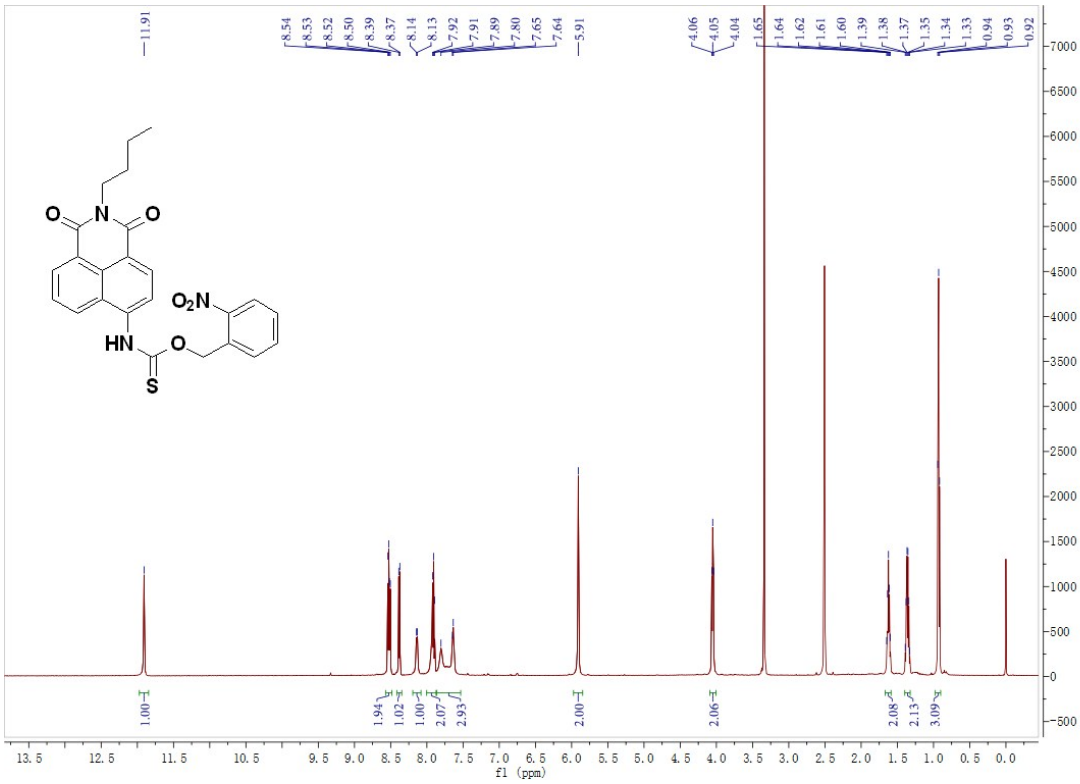


Fig. S3 ^{1}H NMR spectrum of **Nap-Sul-ONB**. (DMSO-d6)

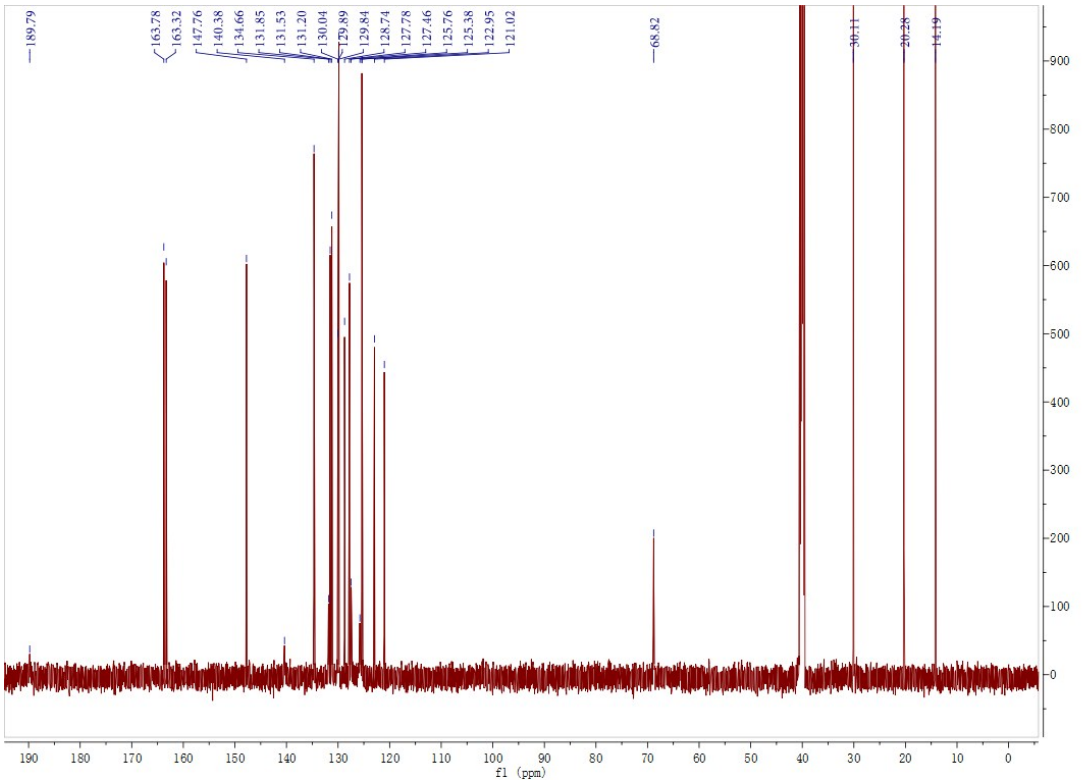


Fig. S4 ^{13}C NMR spectrum of **Nap-Sul-ONB**. (DMSO-d6)

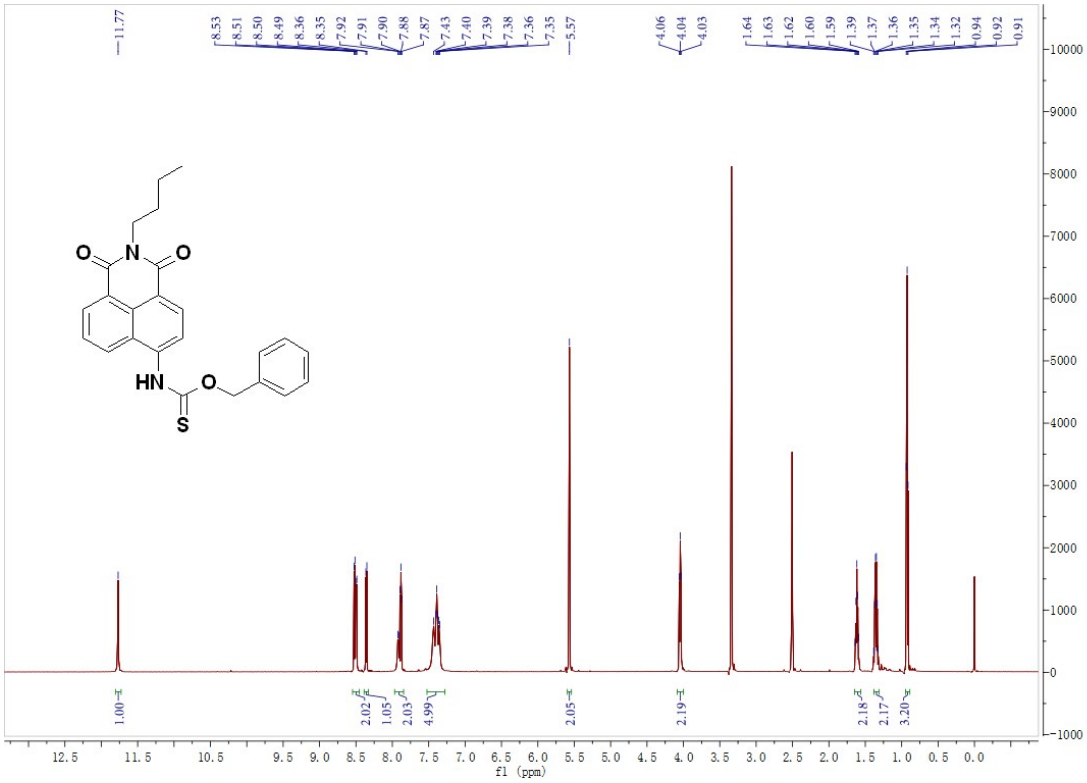


Fig. S5 ^{1}H NMR spectrum of **Nap-Sul-Ph**. (DMSO-d6)

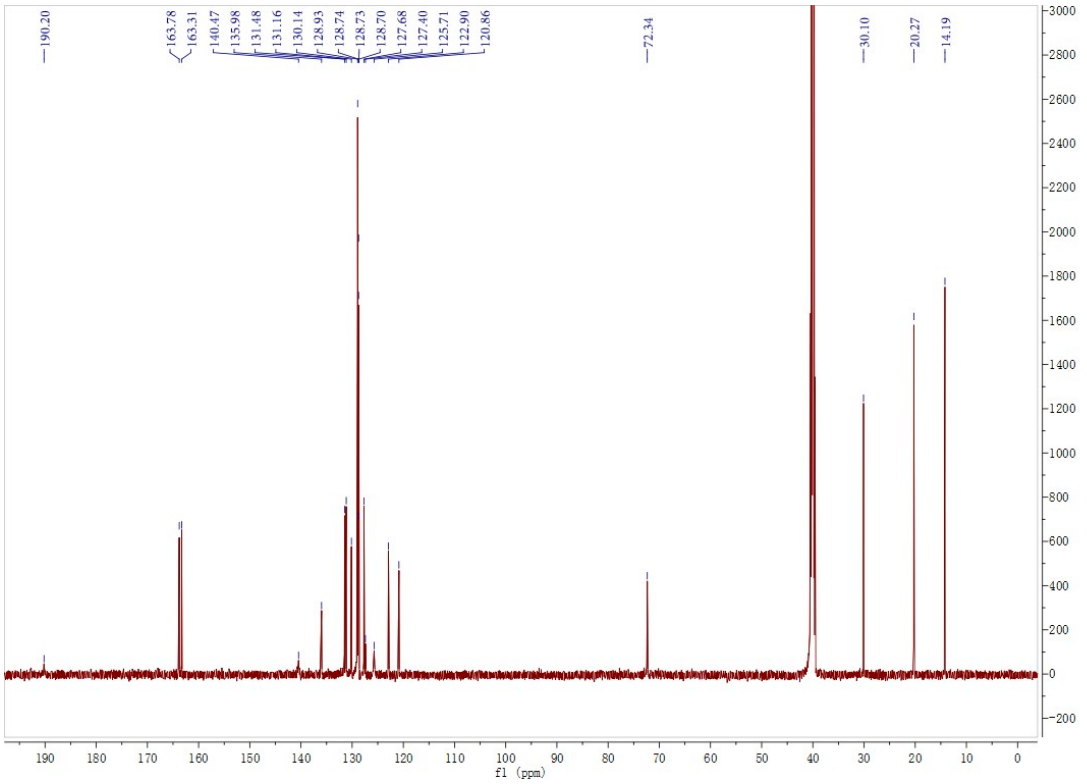


Fig. S6 ^{13}C NMR spectrum of **Nap-Sul-Ph**. (DMSO-d6)

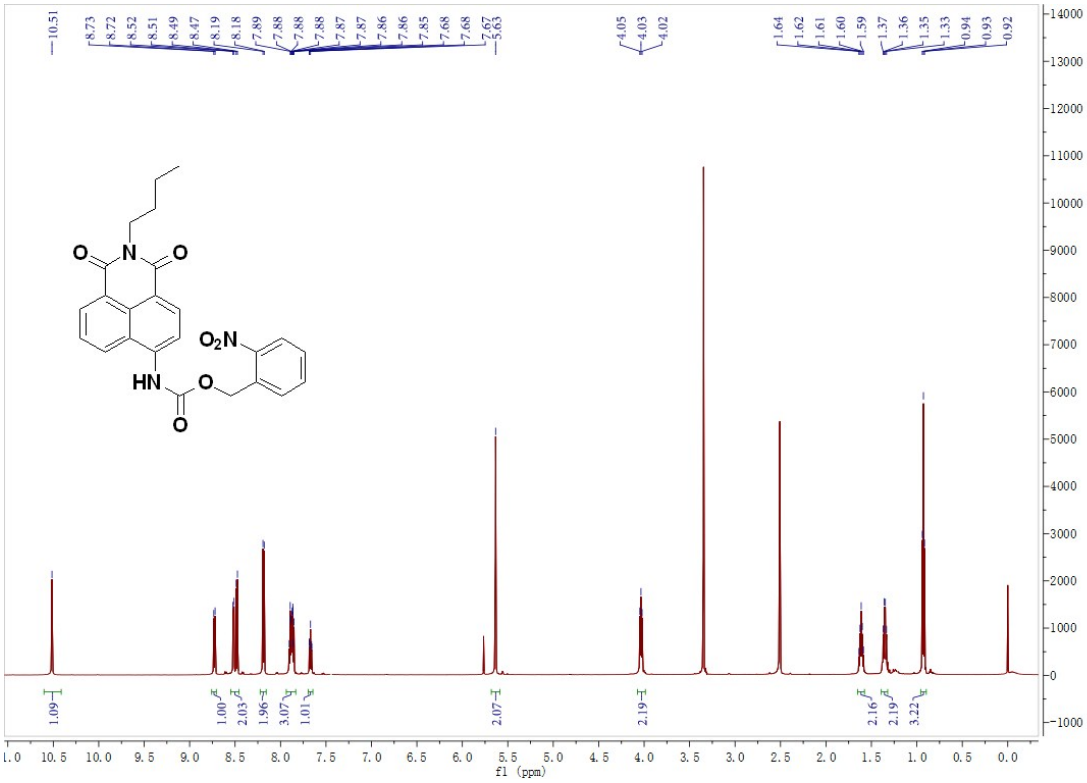


Fig. S7 ^{1}H NMR spectrum of **Nap-Oxy-ONB**. (DMSO-d6)

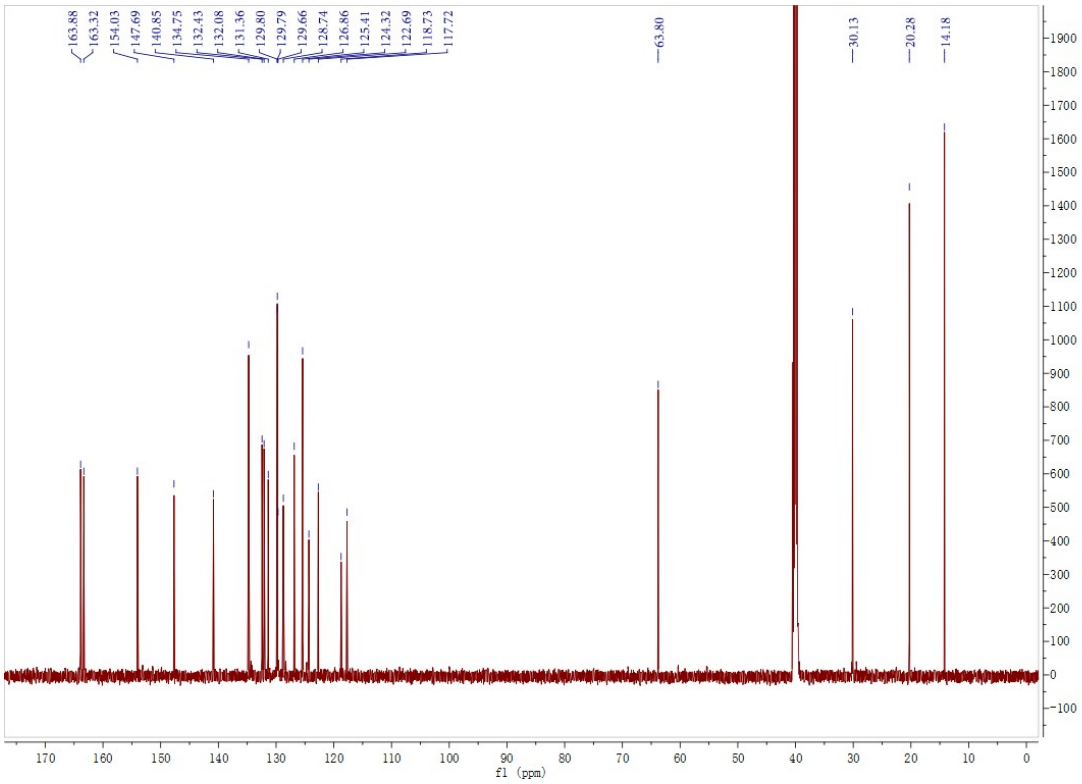


Fig. S8 ^{13}C NMR spectrum of **Nap-Oxy-ONB**. (DMSO-d6)

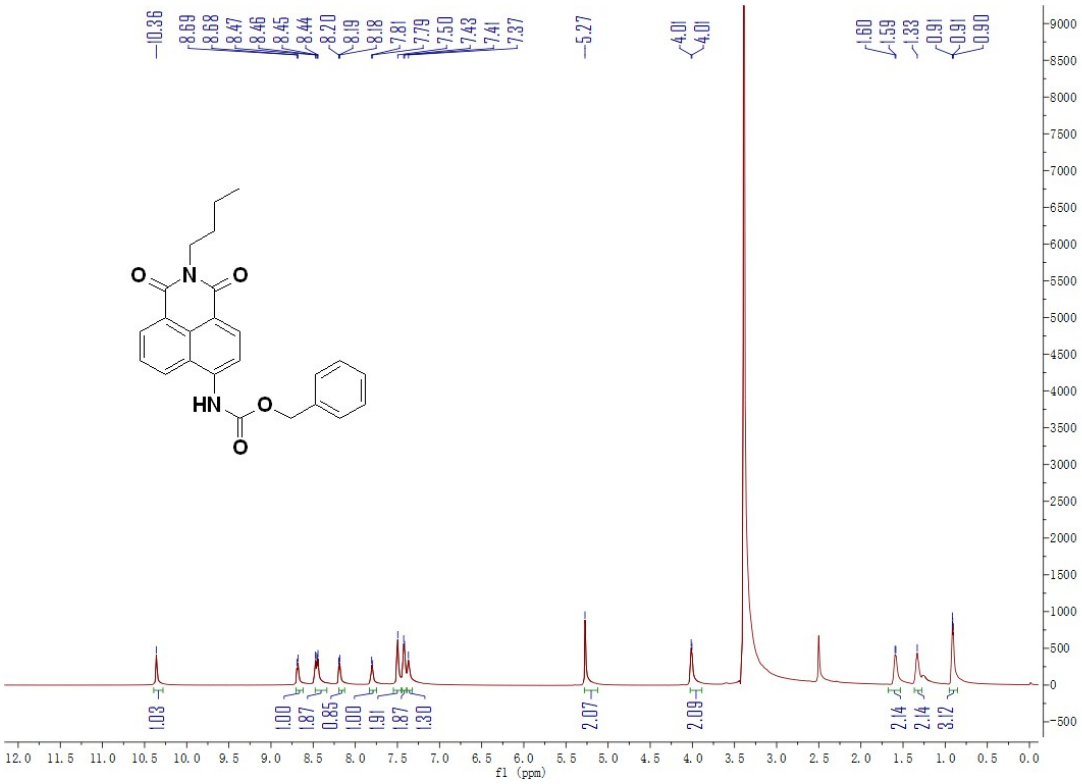


Fig. S9 ^{1}H NMR spectrum of **Nap-Oxy-Ph**. (DMSO-d6)

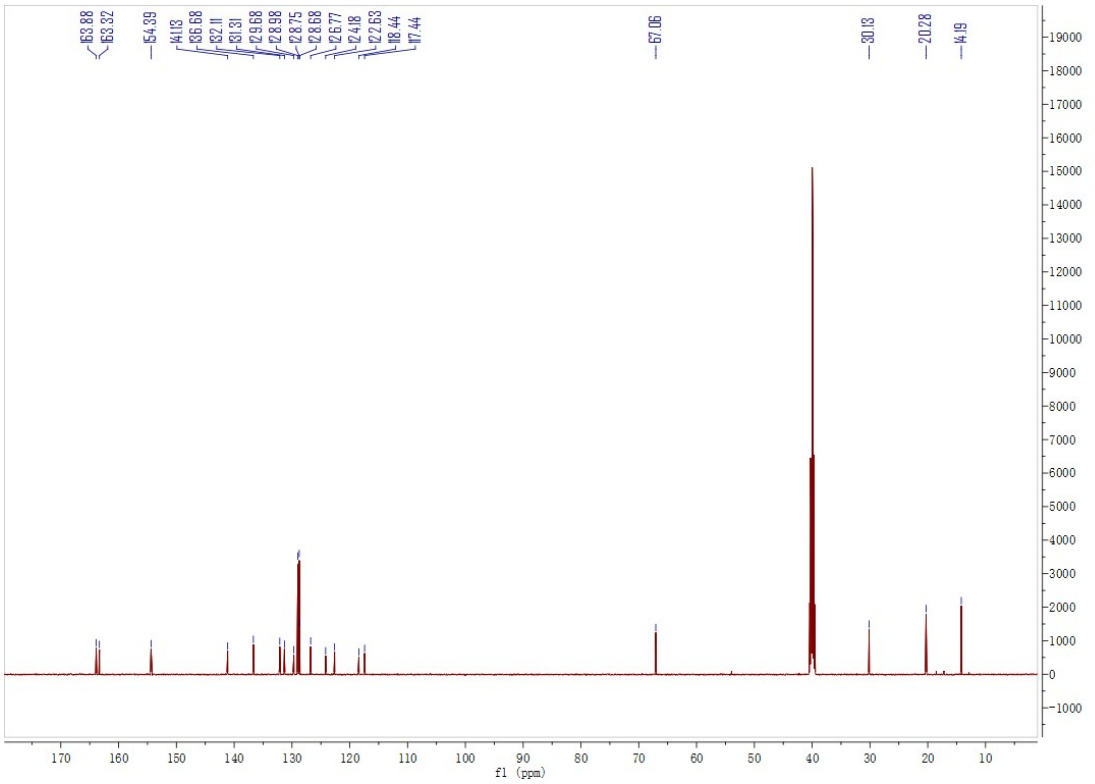


Fig. S10 ^{13}C NMR spectrum of **Nap-Oxy-Ph**. (DMSO-d6)

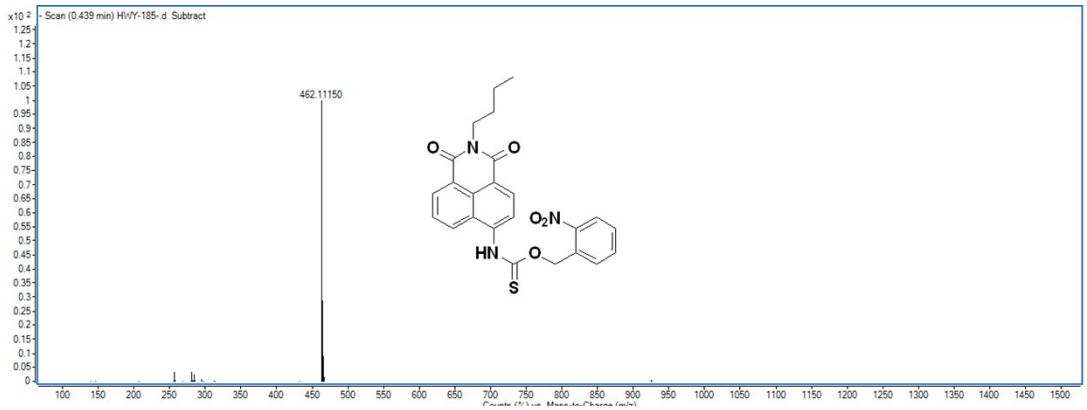


Fig. S11 ESI-HRMS diagram of **Nap-Sul-ONB**.

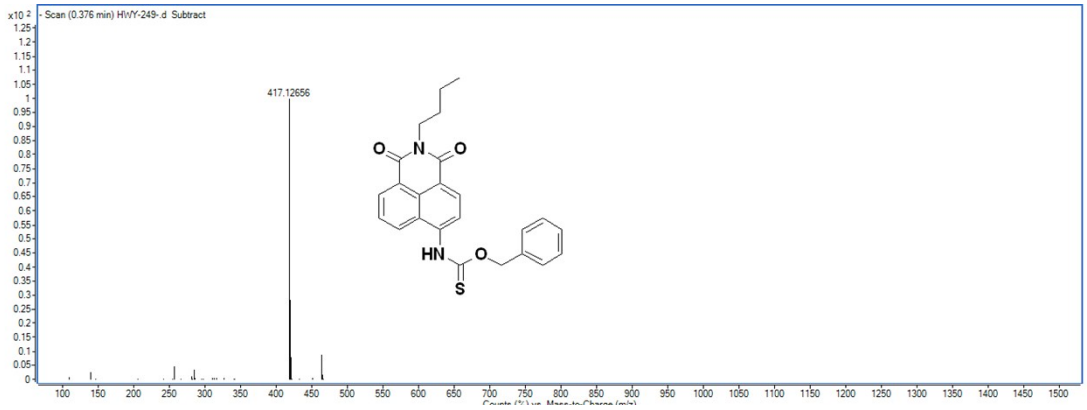


Fig. S12 ESI-HRMS diagram of **Nap-Sul-Ph**.

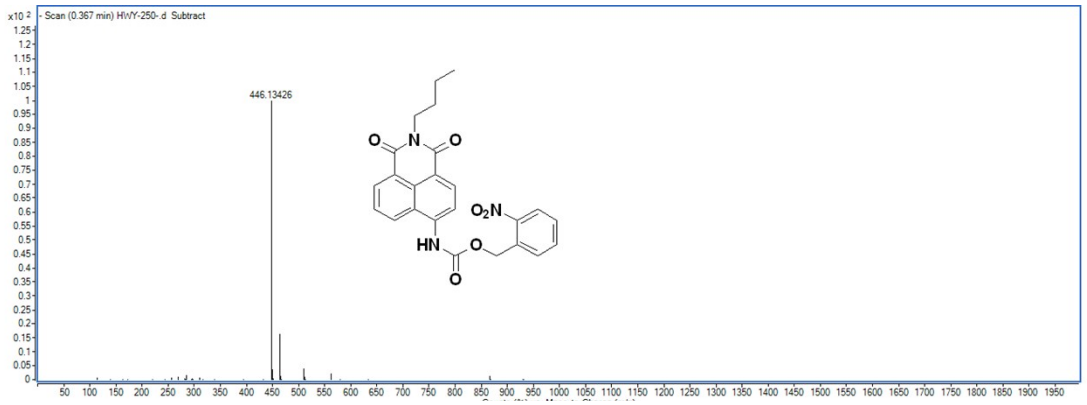


Fig. S13 ESI-HRMS diagram of **Nap-Oxy-ONB**.

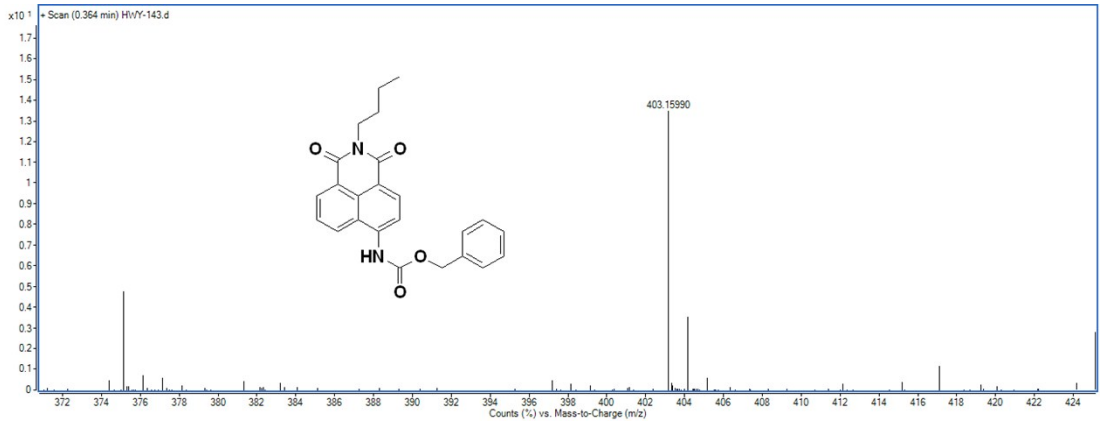


Fig. S14 ESI-HRMS diagram of **Nap-Oxy-Ph**.

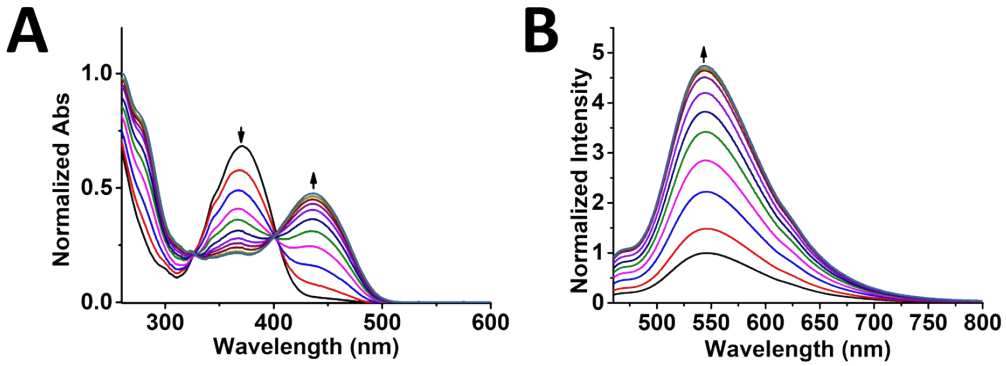


Fig. S15 UV-Vis absorption and fluorescence emission photolysis experiments of **Nap-Oxy-ONB**. (A) UV-Vis absorption photolysis of **Nap-Oxy-ONB**. (B) Fluorescence emission photolysis of **Nap-Oxy-ONB**. The excitation wavelength was 435 nm.

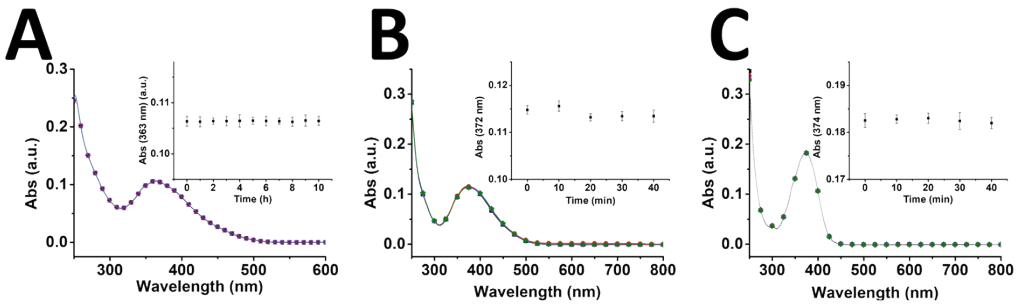


Fig. S16 UV-Vis absorption of (A) **Nap-Sul-ONB** in dark and photolysis experiments of (B) **Nap-Sul-Ph** and (C) **Nap-Oxy-Ph**. The data are representative of three independent experiments and shown as the mean ± S.D.

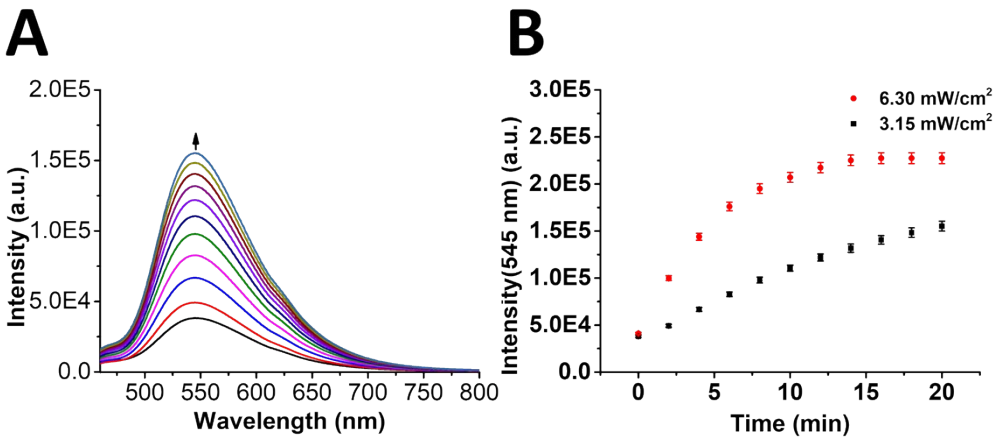


Fig. S17 (A) Fluorescence emission spectra response of **Nap-Sul-ONB** towards the irradiation of 365 nm light (3.15 mW/cm^2). (B) Fluorescence intensity response of **Nap-Sul-ONB** under the trigger of 365 nm light with different power density. The data are representative of three independent experiments and shown as the mean ± S.D.

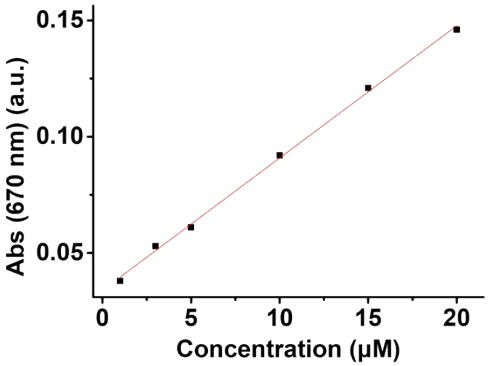


Fig. S18 Calibration curve of MB assay. Equation: y=0.03399+ 0.00569x, R-square=0.99777.

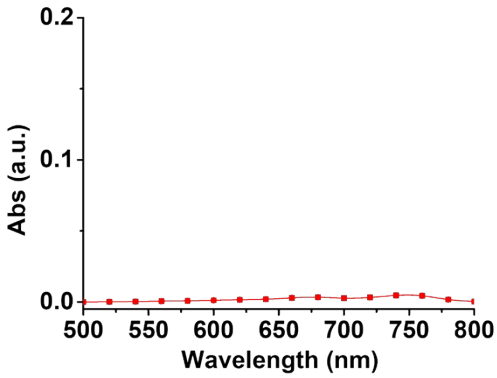


Fig. S19 UV-Vis absorption spectrum of MB assay for **Nap-Oxy-ONB**.

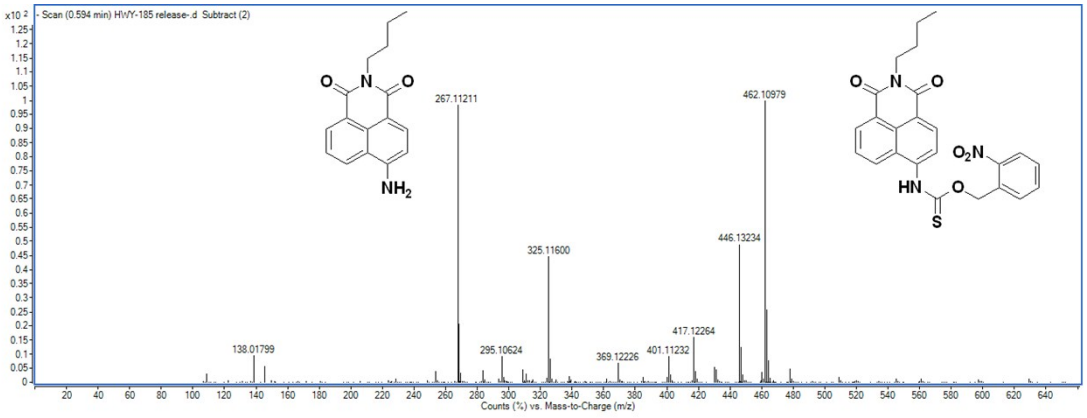


Fig. S20 ESI-MS diagram of the irradiated **Nap-Sul-ONB**.

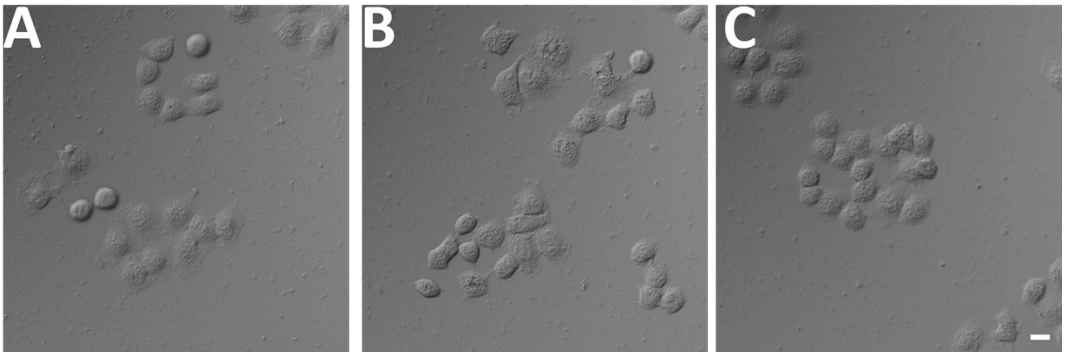


Fig. S21 Cellular accumulation of **Nap-Sul-ONB** in MCF-7 cells. (A) MCF-7 cells were incubated with 20 μM of **Nap-Sul-ONB** for 4 h in dark. (B) MCF-7 cells were incubated with 20 μM of **Nap-Sul-ONB** for 4 h followed by a further incubation for 20 min. (C) MCF-7 cells were incubated with 20 μM of **Nap-Sul-ONB** for 4 h followed by a further incubation for 40 min. λ_{ex}=405 nm. λ_{em}=500-580 nm. Scale bar: 20 μm.

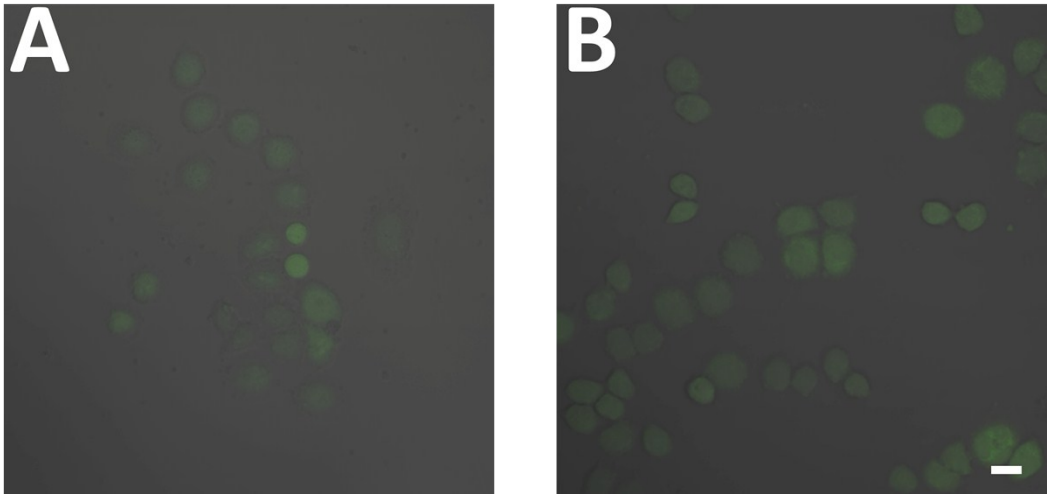


Fig. S22 Cell images of MCF-7 cells treated with H_2O_2 and DCFH-DA. (A) MCF-7 cells were incubated successively with 50 μM of H_2O_2 for 30 min and 5 μM of DCFH-DA for 30 min in dark. (B) MCF-7 cells were successively incubated with 50 μM of H_2O_2 for 30 min and 5 μM of DCFH-

DA for 30 min and irradiated by 365 nm light for 30 min. λ_{ex}=488 nm. λ_{em}=520-550 nm. Scale bar: 20 μm.

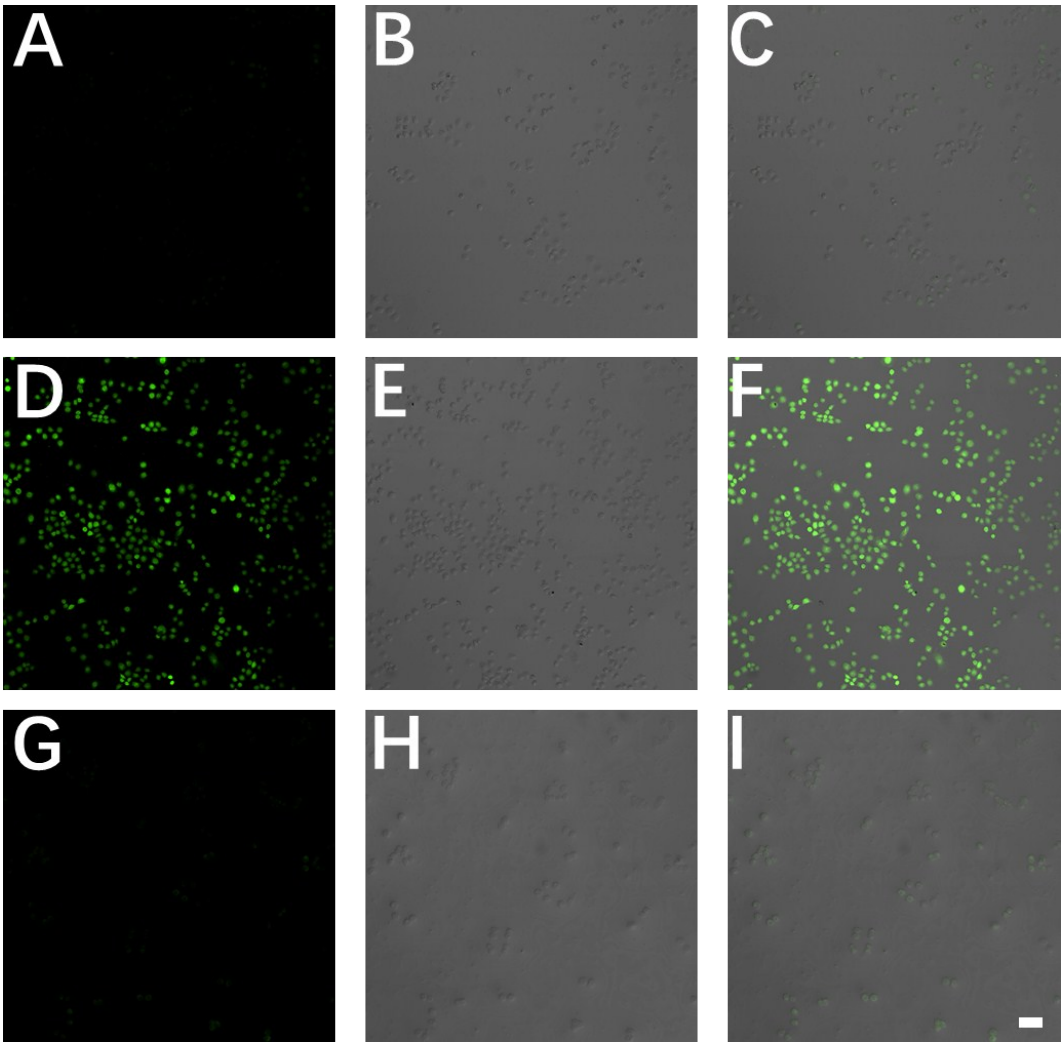


Fig. S23 Antioxidant activity of **Nap-Sul-ONB** against the ROS formation induced by H_2O_2. The cellular ROS level of MCF-7 cells was detected by DCFH-DA. (A-C) Control. (D-F) MCF-7 cells were pretreated with H_2O_2 for 30 min and then incubated with **Nap-Sul-ONB** for 4 h without light irradiation. (G-I) MCF-7 cells were pretreated with H_2O_2 for 30 min and then incubated with **Nap-Sul-ONB** for 4 h followed by 365 nm light irradiation for 30 min. (A, D, G) Green channel. (B, E, H) Bright field. (C,

F, I) Merged images. λ_{ex}=488 nm. λ_{em}=520-550 nm. Scale bar: 80 μm.

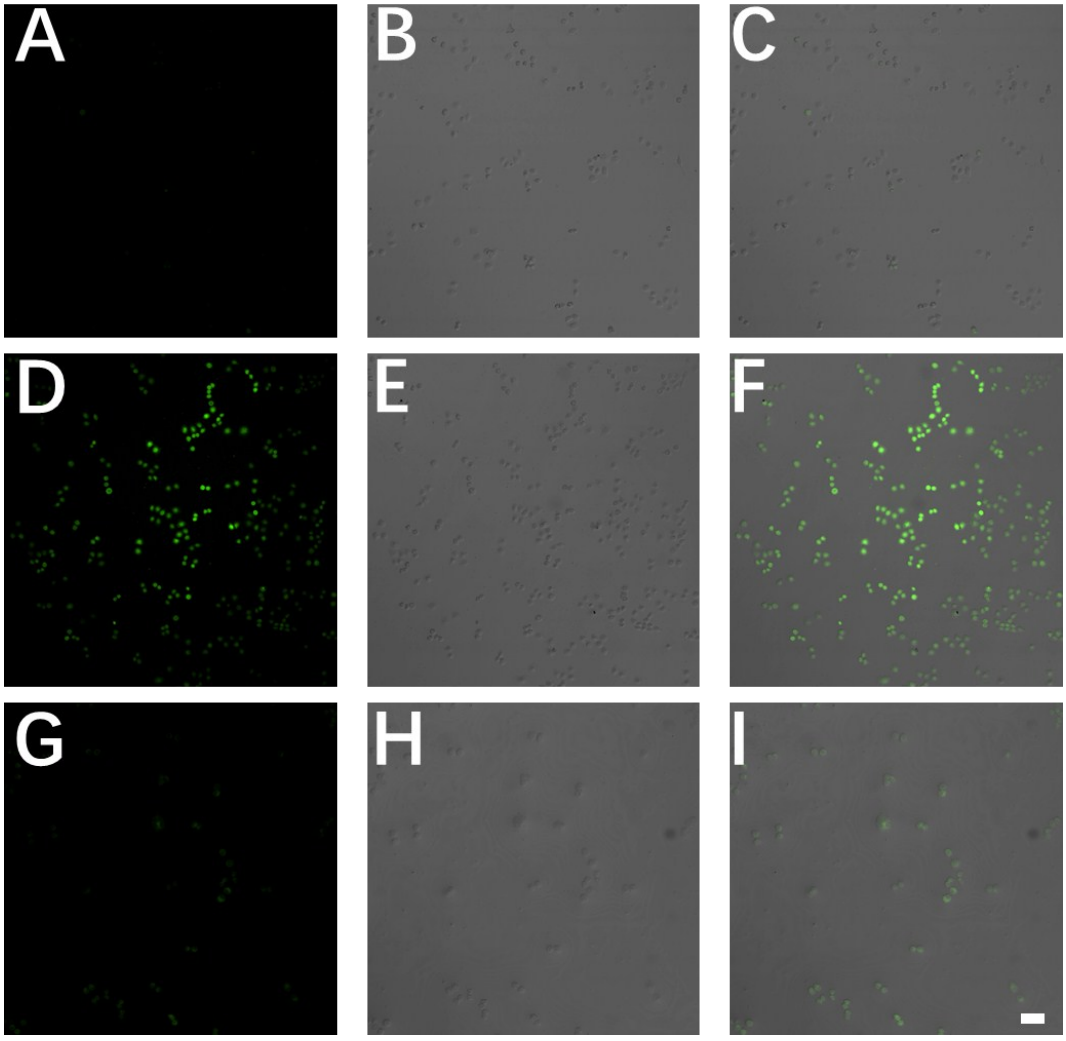


Fig. S24 Antioxidant activity of **Nap-Sul-ONB** against the ROS formation induced by PMA. The cellular ROS level of MCF-7 cells was detected by DCFH-DA. (A-C) Control. (D-F) MCF-7 cells were pretreated with PMA for 30 min and then incubated with **Nap-Sul-ONB** for 4 h without light irradiation. (G-I) MCF-7 cells were pretreated with PMA for 30 min and then incubated with **Nap-Sul-ONB** for 4 h followed by 365 nm light irradiation for 30 min. (A, D, G) Green channel. (B, E, H) Bright field. (C, F, I) Merged images. λ_{ex}=488 nm. λ_{em}=520-550 nm. Scale bar: 80 μm.

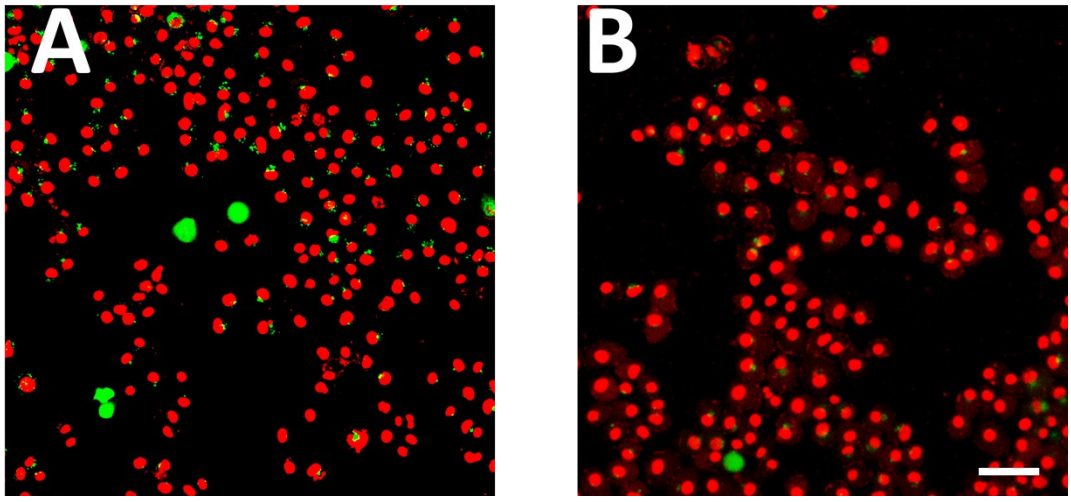


Fig. S25 Live/dead cell co-staining assay using calcein AM and propidium iodide as fluorescence probes. (A) MCF-7 cells were incubated with 1 μg/mL of PMA for 1 h. (B) MCF-7 cells were incubated with 1 μg/mL of PMA for 1 h and 20 μM of **Nap-Sul-ONB** for 4 h in dark. Scale bar: 80 μm.

Table S1. Photophysics parameters of the Nap-Sul-ONB before and after 365 nm light irradiation for 20 min.

	ε/(L*mol^{-1}*cm^{-1})			λ_{Em}/nm	I_L/I_D[a]
Dark	23681 (255 nm)	15679 (363 nm)	4645 (425 nm)	544	5.5
Light	23667 (255 nm)	7758 (363 nm)	8672 (425 nm)		

[a] I_L/I_D=(Fluorescence intensity of irradiated samples)/(Fluorescence intensity of samples without irradiation).

3. References

1. Y. Zhao, S. G. Bolton, M. D. Pluth, Org. Lett. 19 (2017) 2278-2281.

2. C. R. Powell, J. C. Foster, B. Okyere, M. H. Theus, J. B. Matson, J. Am. Chem. Soc. 138 (2016) 13477-13480.

3. S. Sestito, L. Pruccoli, M. Runfola, V. Citi, A. Martelli, G. Saccomanni, V. Calderone, A. Tarozzi, S. Rapposelli, Eur. J. Med. Chem. 184 (2019) 11745-11757.

4. Y. Hu, X. Li, Y. Fang, W. Shi, X. Li, W. Chen, M. Xian, H. Ma, Chem. Sci. 10 (2019) 7690-7694.

5. F. H. Chen, N. Lu, H. W. Zhang, L. Zhao, L. C. He, H. P. Sun, Q. D. You, Z. Y. Li, Q. L. Guo, Mol. Pharmacol. 82 (2012) 958-971.