

Supporting information

Manipulating the Subcellular Localization and Anti-cancer Effect of Benzophenothiaziniums by Minor Alteration of N-Alkylation

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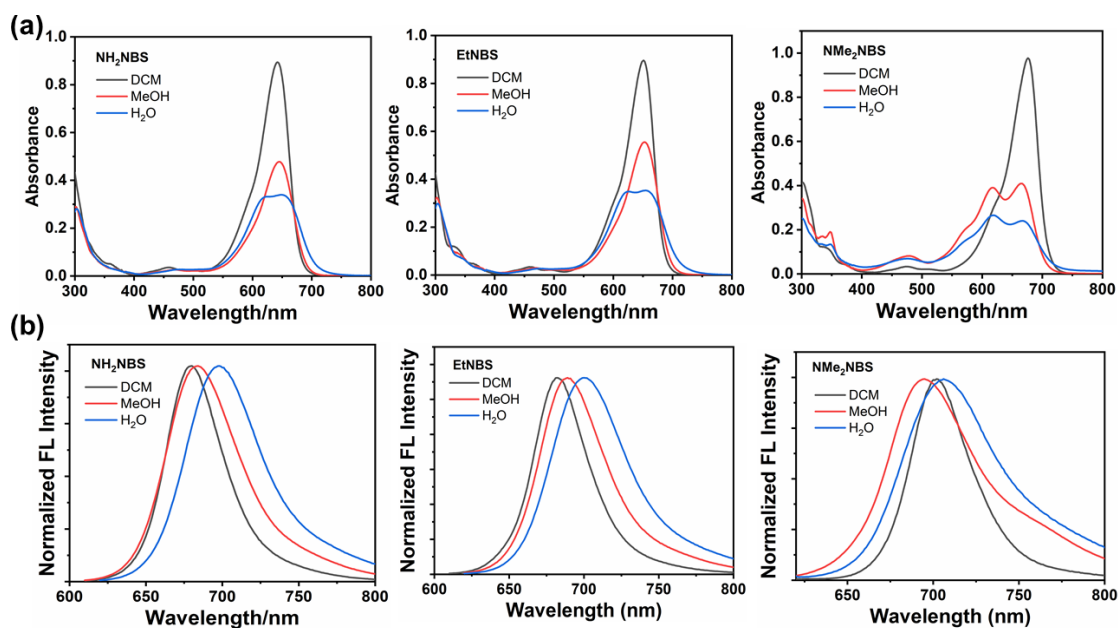
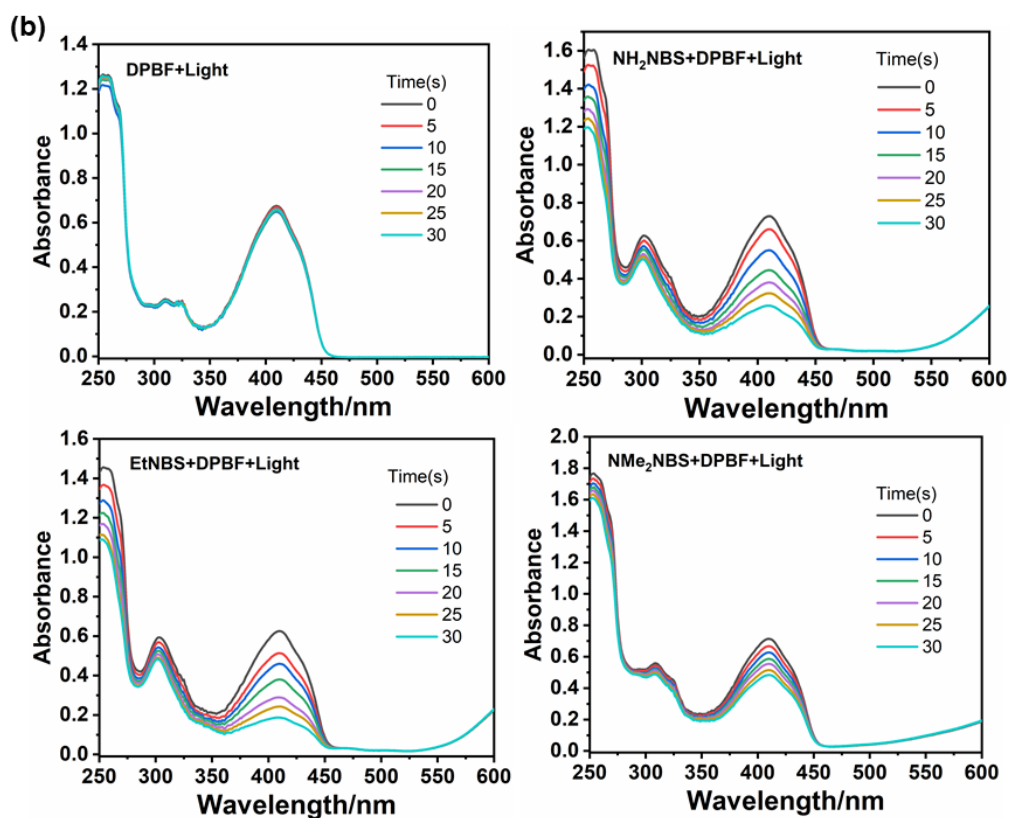
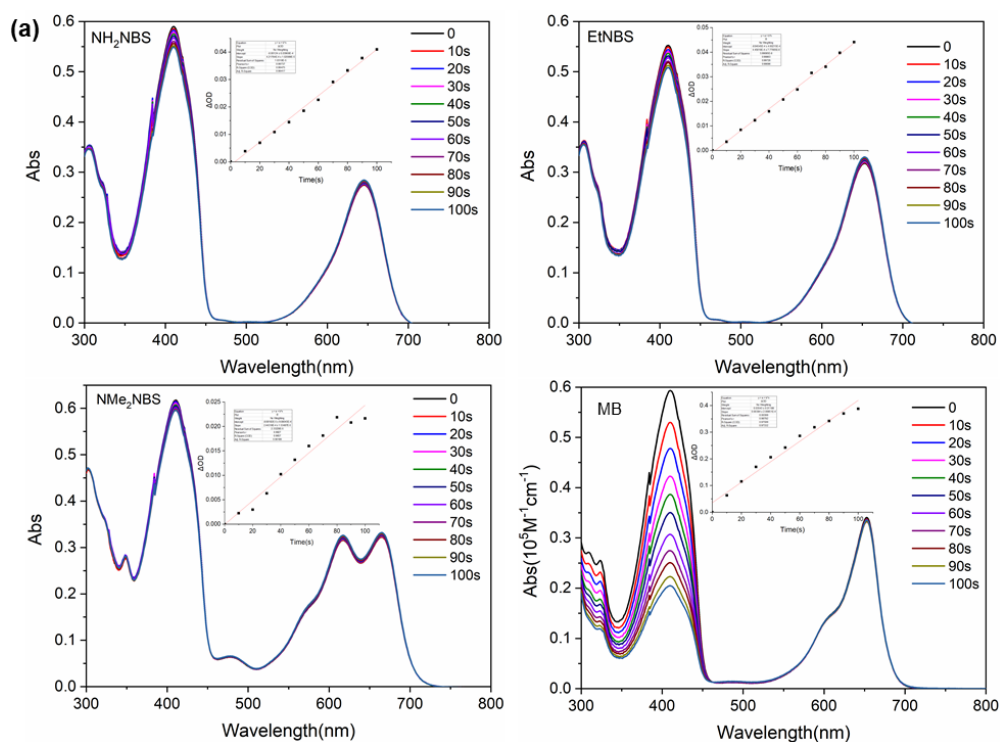


Figure S1. Normalized absorption and emission spectra of NH_2NBS , EtNBS , NMe_2NBS in PBS solvents. The concentration used for each compound was $10 \mu\text{M}$.

Table S1. Physical and photophysical properties.

Solvent	Dye	λ_{abs} (nm)	$\epsilon(\text{M}^{-1}\text{cm}^{-1})$	λ_{em} (nm)	Φ_{fl}	Φ_{Δ}
DCM	NH_2NBS	642	89351	680	0.24	N.D.
	EtNBS	651	89585	682	0.40	N.D.
	NMe_2NBS	676	97661	702	0.20	N.D.
MeOH	NH_2NBS	645	47781	684	0.10	0.057
	EtNBS	653	55495	689	0.14	0.060
	NMe_2NBS	665	40942	695	0.04	0.034
H_2O	NH_2NBS	649	33957	698	0.02	N.D.
	EtNBS	654	35314	700	0.03	N.D.
	NMe_2NBS	667	24061	706	0.015	N.D.



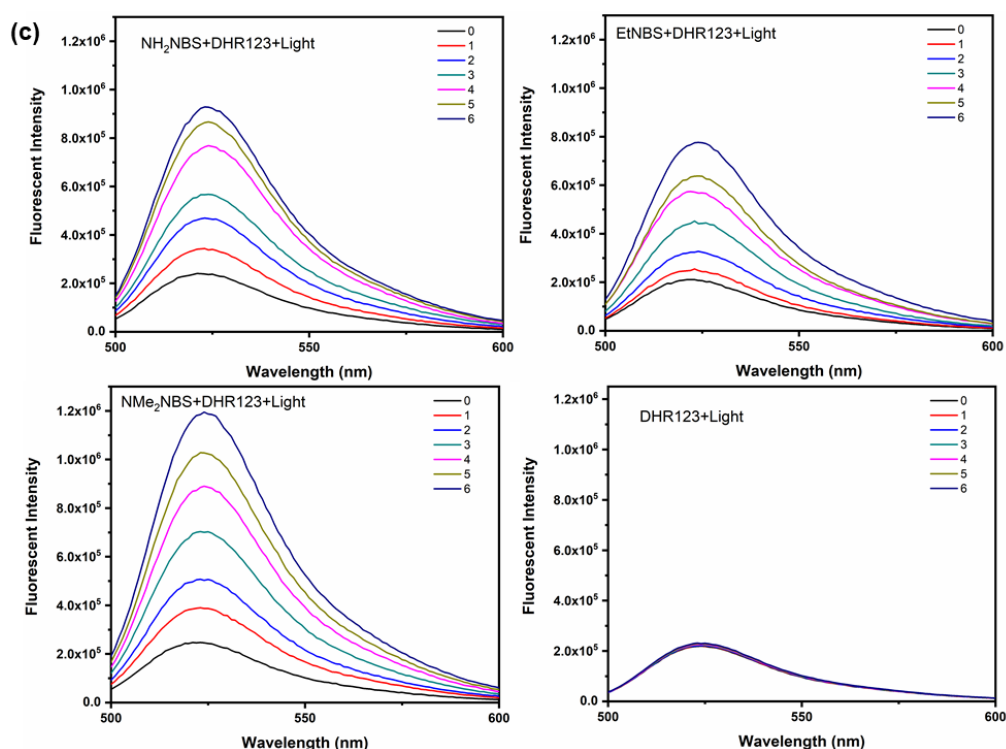


Figure S2. (a) The singlet oxygen quantum yields of the three PSs were determined in MeOH using DPBF bleaching assay (after 635 nm irradiation for 100 seconds, Dye concentration: 10 μ M). (b) Photoinduced $^1\text{O}_2$ production was determined in MeOH (after 635 nm, 20 mW cm^{-1} , irradiation for 30s, Dye concentration: 10 μ M) (c) Photoinduced $\text{O}_2^{\bullet-}$ radical generation was determined in PBS solution using DHR 123 (after 635 nm, 20 mW cm^{-1} , irradiation for 6 min, Dye concentration: 10 μ M).

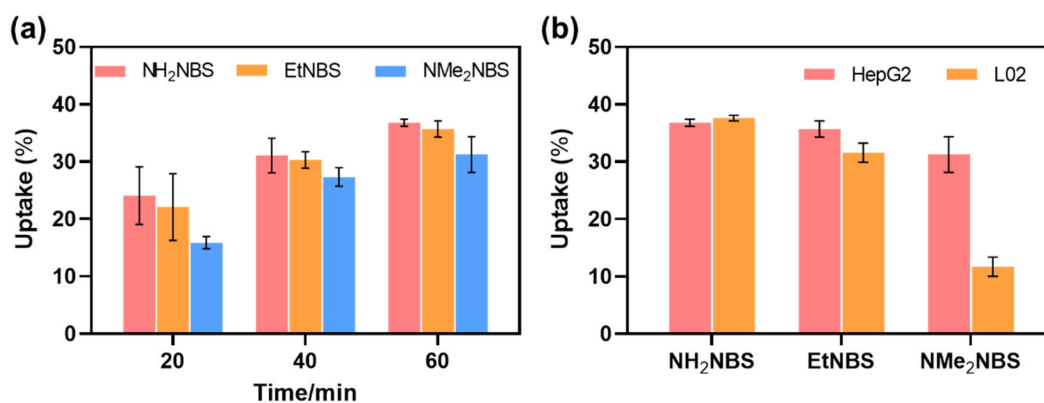


Figure S3. (a) Uptake of three PSs by HepG2 cells at different time intervals. (b) Uptake of three PSs by HepG2 cells and L02 cells after incubation for one hour.

Table. S2 Phototoxicity and dark toxicity of NH_2NBS , EtNBS , NMe_2NBS against L02 cells.

Compound	$\text{IC}_{50}(\mu\text{M})$		
	Dark	Light (N^a)	PI^c
NH_2NBS	3.00 ± 0.40	0.039 ± 0.001	77
EtNBS	4.69 ± 0.27	0.050 ± 0.010	94
NMe_2NBS	57.04 ± 2.86	1.15 ± 0.35	50

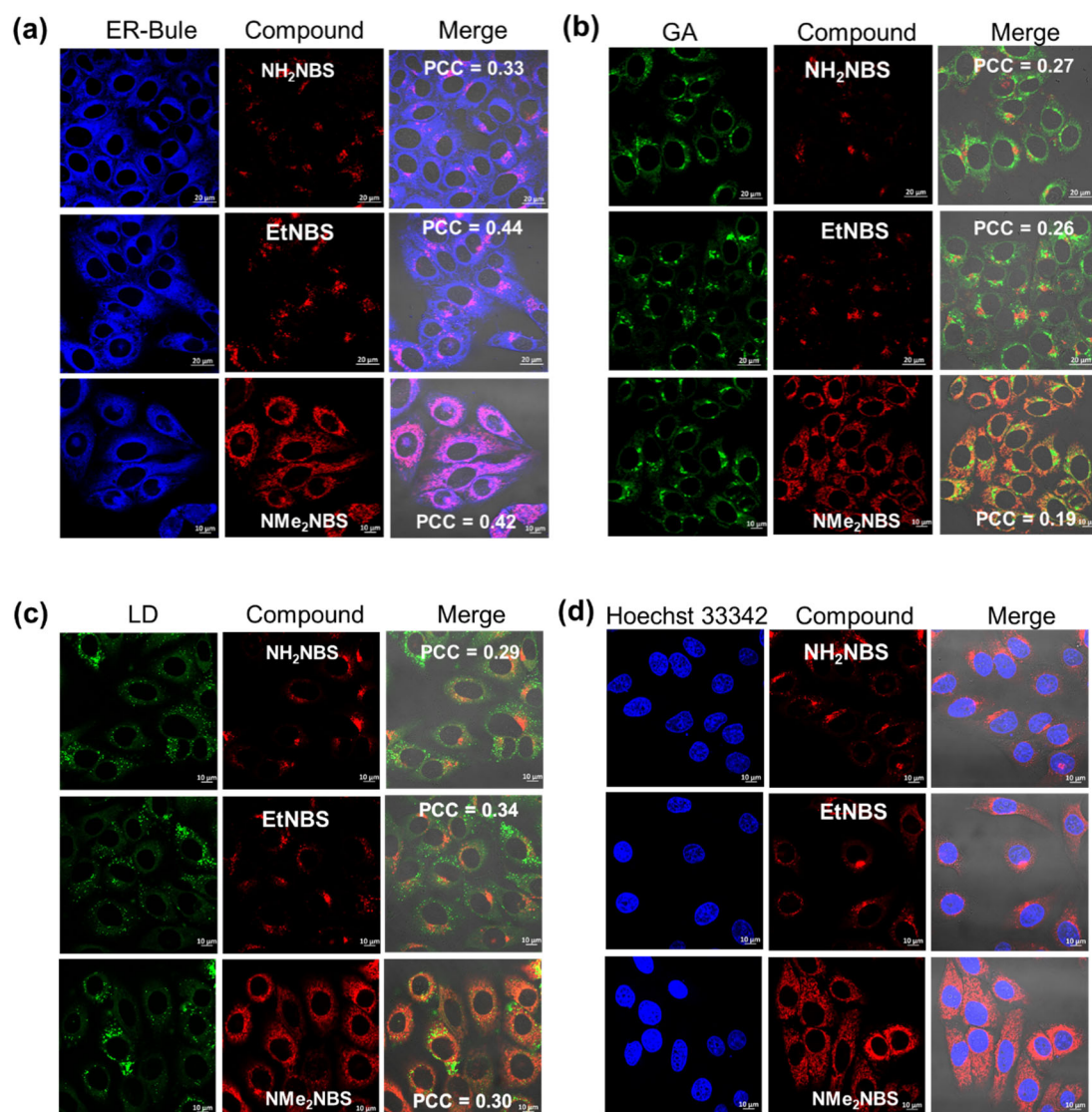


Figure S4. Subcellular localization of NH₂NBS (100 nM), EtNBS (100 nM), NMe₂NBS (100 nM), ER Blue white (1 μM), GA: Golgi apparatus Green (1 μM), LD: Lipid green (1 μM).

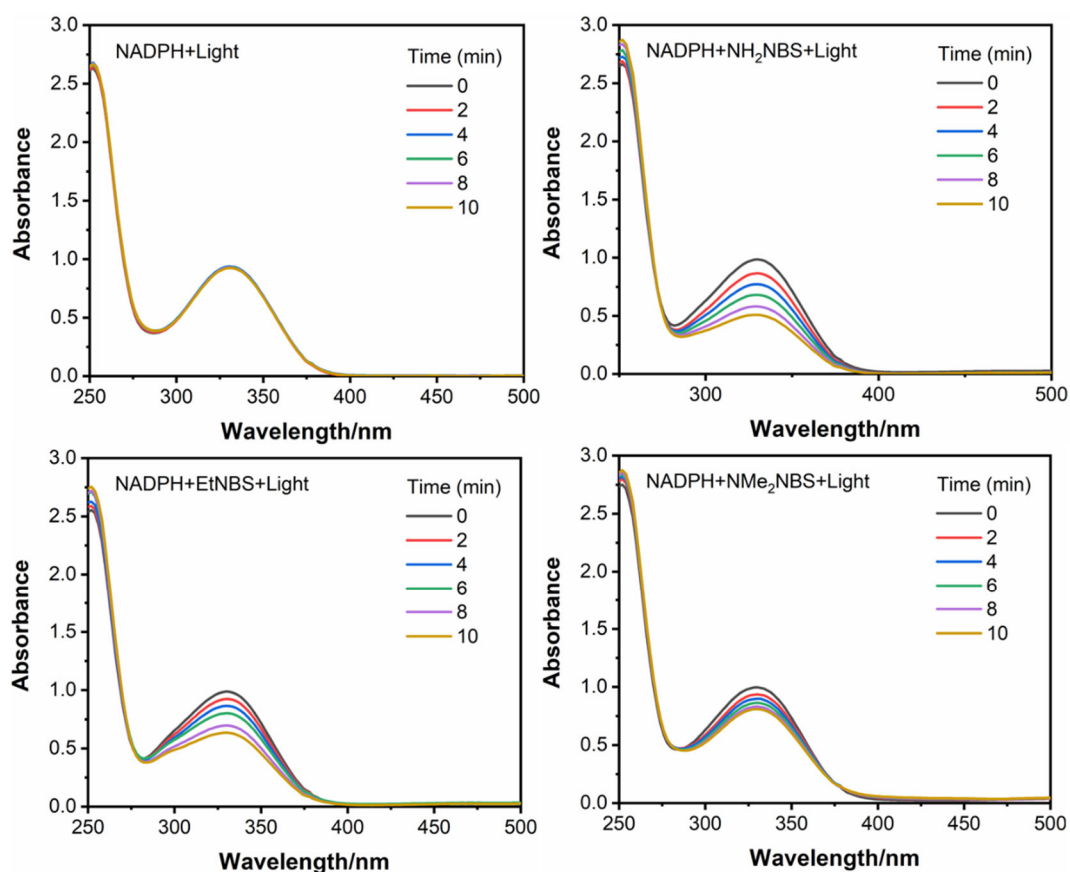


Figure S5. UV-Vis spectra of photocatalytic oxidation of three PSs (10 μM) with NADPH (160 μM) in PBS solution, after different time of 635 nm irradiation at a power density 20 mW/cm^2 .

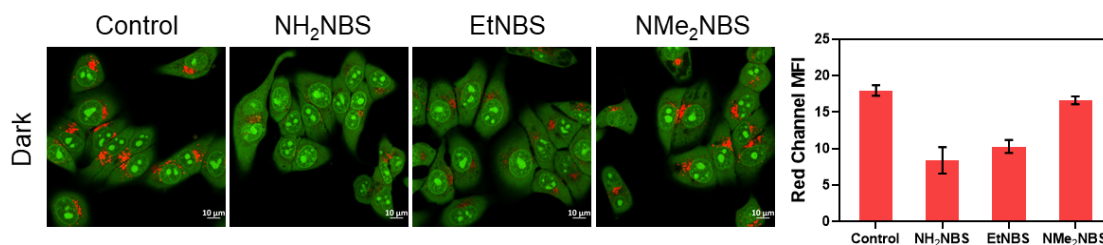


Figure S6. The confocal fluorescence images of AO staining after co-cultured HepG2 cells with high concentration of NH₂NBS, EtNBS and NMe₂NBS (1 μM).

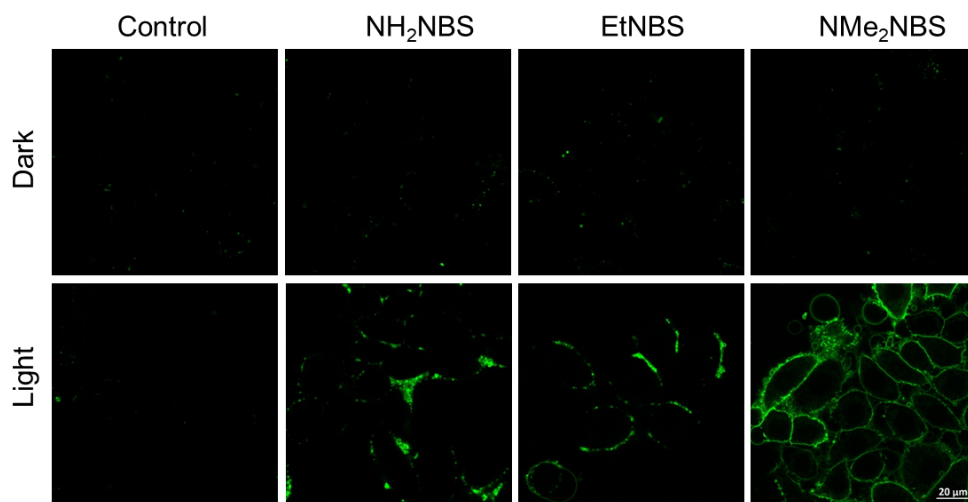


Figure S7. The confocal fluorescence images of Annexin V-FITC staining.

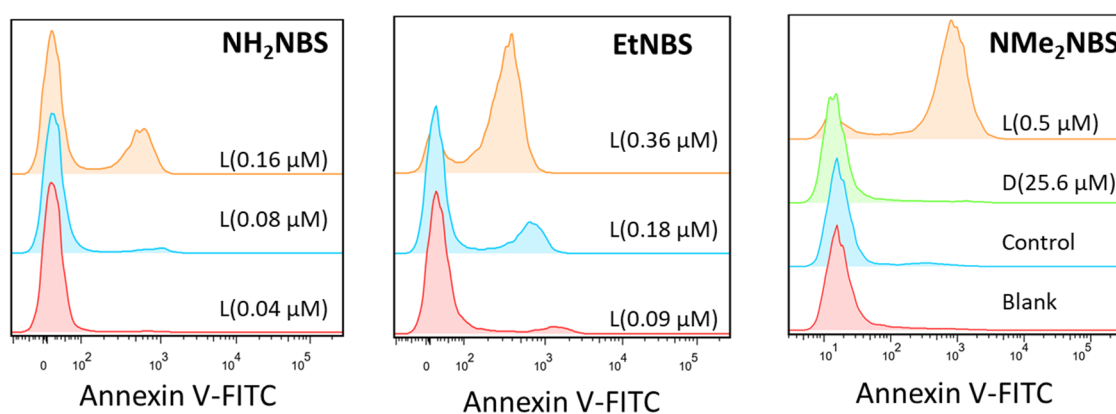


Figure. S8. Flow cytometric assay of photoirradiation-induced apoptosis for HepG2 cells incubated with different concentrations of NH_2NBS , EtNBS and NMe_2NBS . L: light, D: Dark.

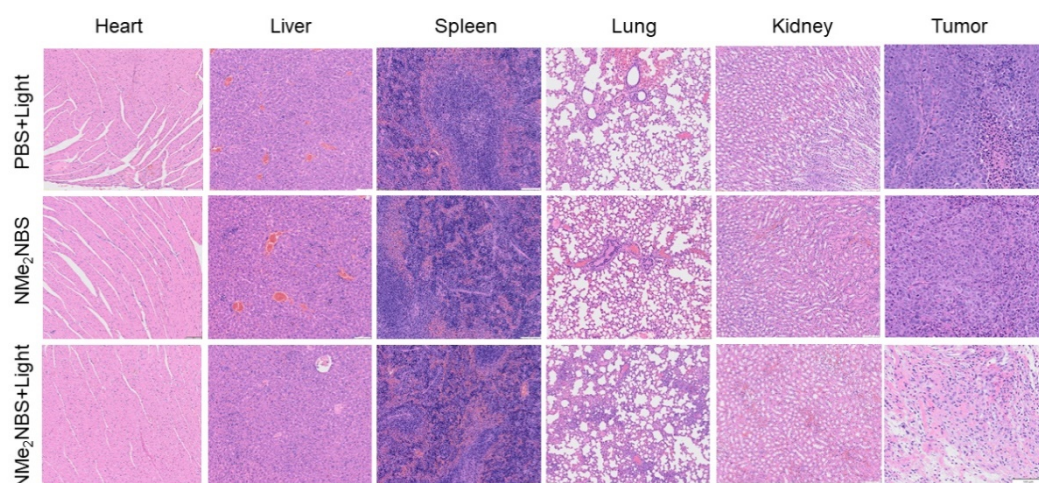


Figure S9. H&E staining of organ tissues and tumor tissue slices after four weeks treatment.

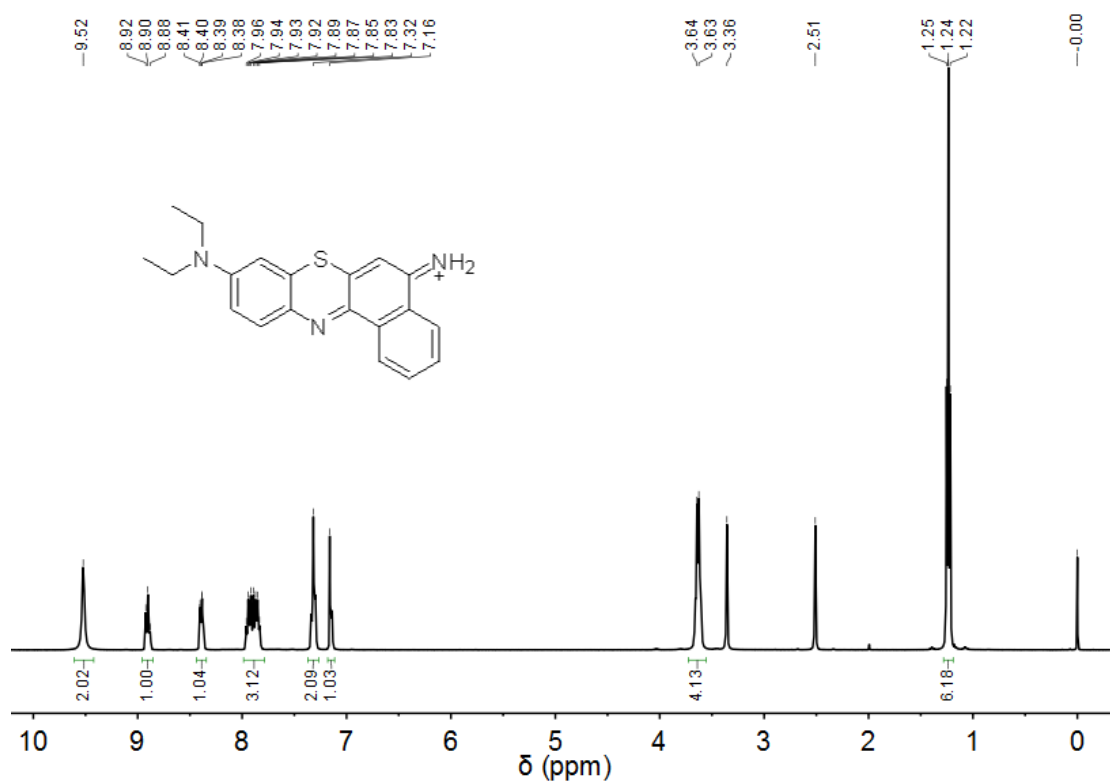


Figure S10. ^1H NMR of NH_2NBS in $\text{DMSO}-d_6$.

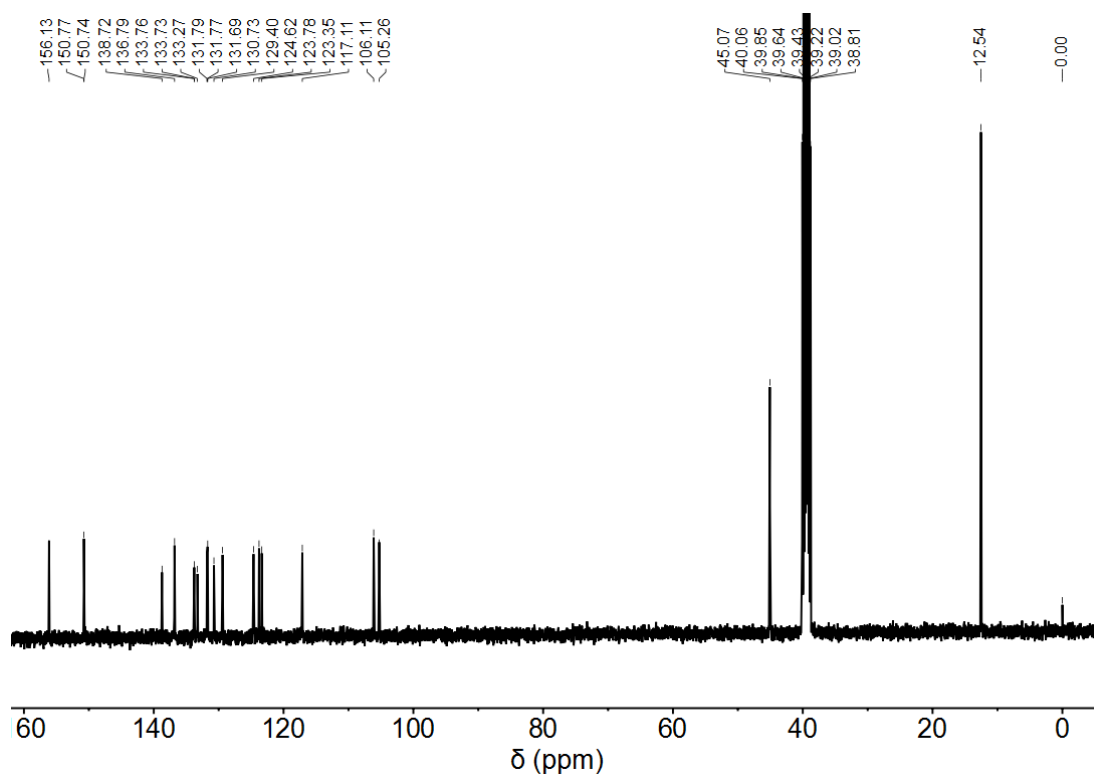
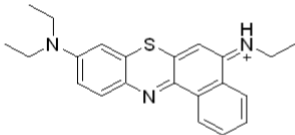


Figure S11. ^{13}C NMR of NH_2NBS in $\text{DMSO}-d_6$.



152.67
150.73
139.65
136.73
133.40
133.05
133.00
131.99
131.95
131.22
131.09
129.40
124.61
123.96
123.92
122.99
117.27
105.25
102.76
45.03
40.06
39.85
39.64
39.53
39.43
39.22
39.01
38.93
38.80
13.87
12.57
-0.00

δ (ppm)

Figure S13. ^{13}C NMR of EtNBS in DMSO- d_6 .

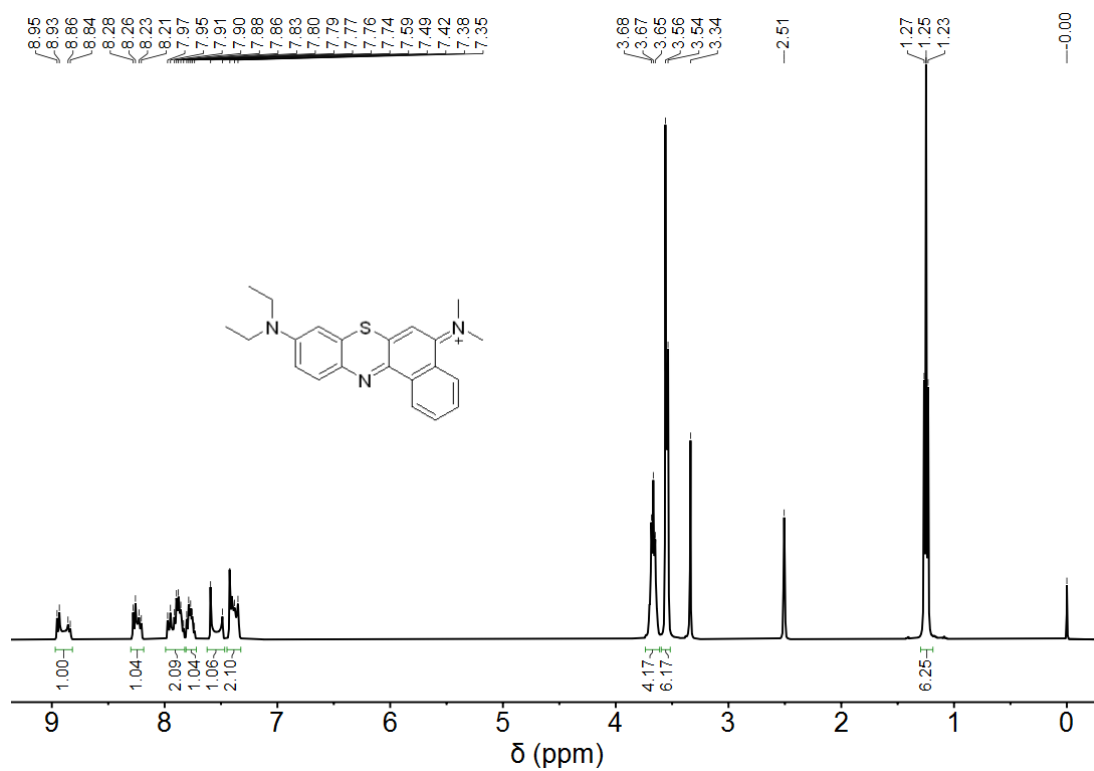


Figure S14. ¹H NMR of NMe₂NBS in DMSO-*d*₆.

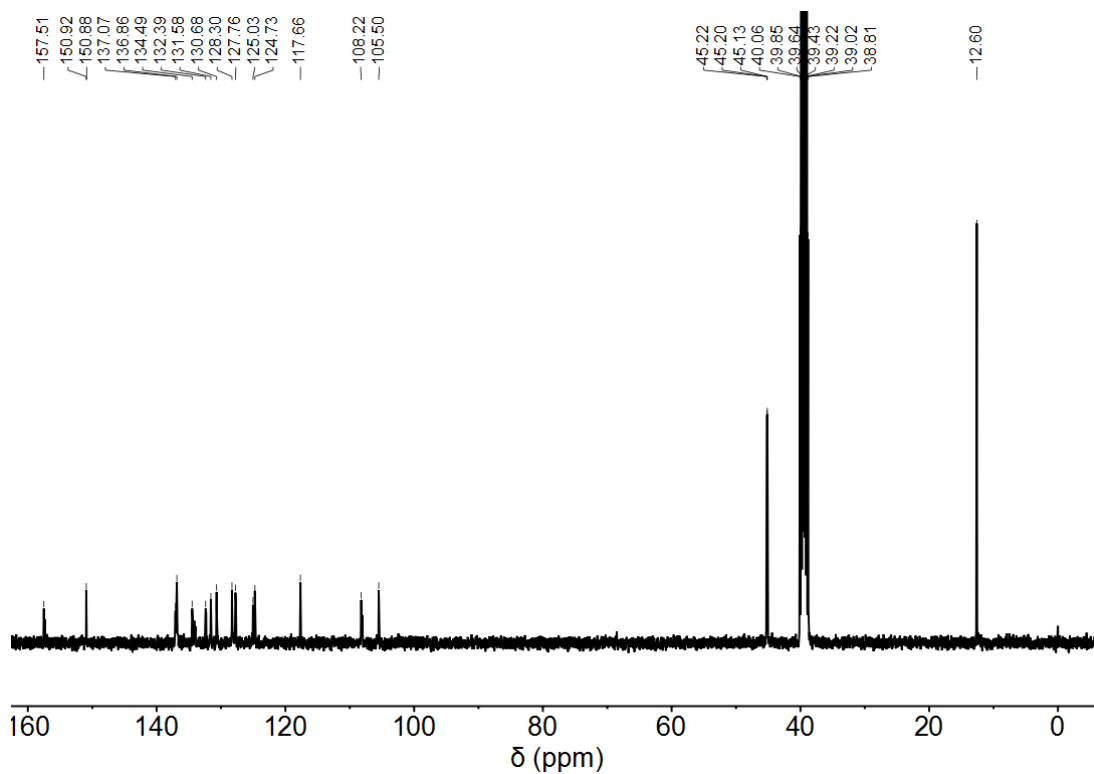


Figure S15. ¹³C NMR of NMe₂NBS in DMSO-*d*₆.

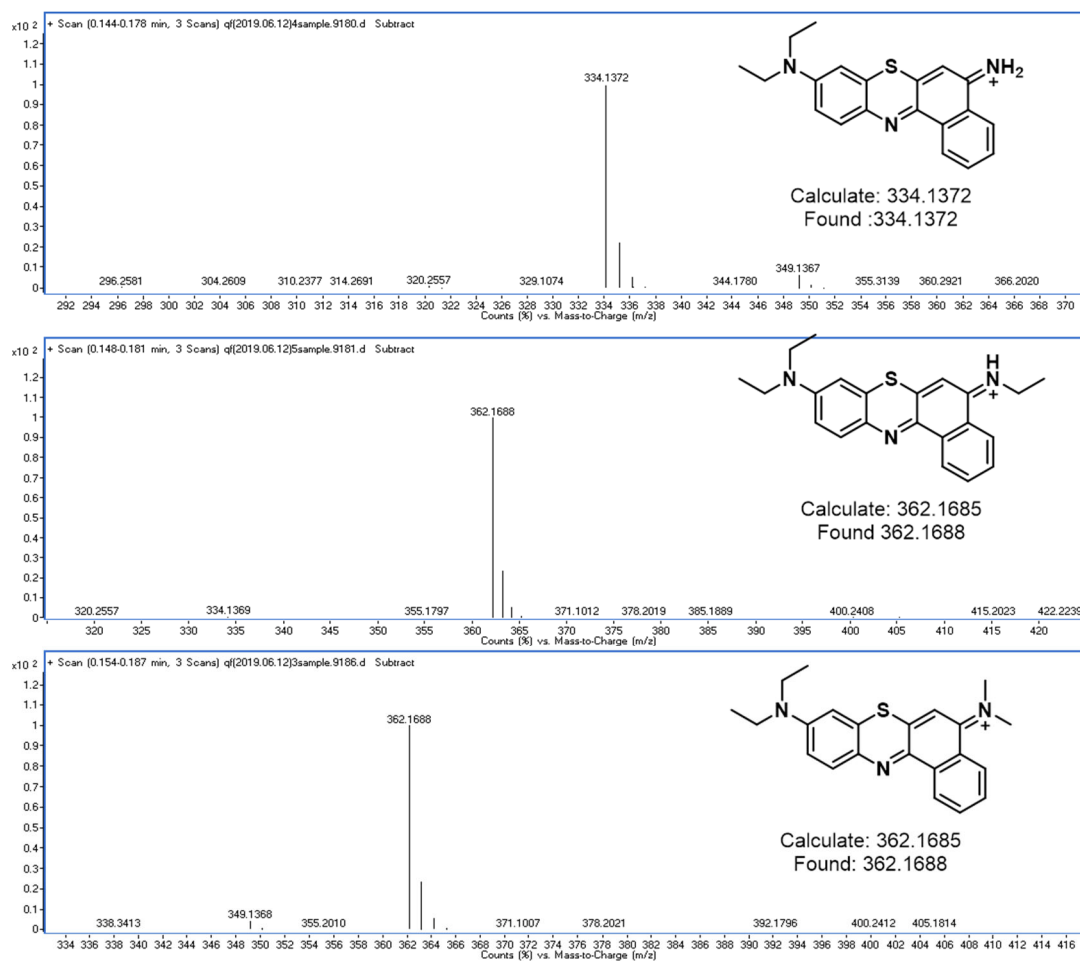


Figure S16. HRMS of NH₂NBS, EtNBS and NMe₂NBS.