Dual-targeted and Viscosity-sensitive of infrared AIE photosensitizer based on tumor microenvironmental response for Photodynamic Cancer Therapy

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Experimental Part

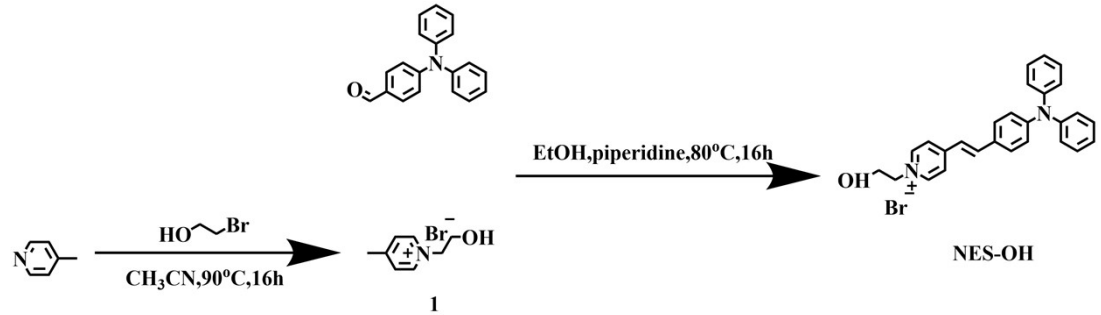
Reagents and instruments

Diphenylamino-4-benzaldehyde, 4-methylpyridine, 2-bromoethanol, ascorbic acid, 2,7-dichlorofluorescein diacetate (DCFH-DA) and 9,10-anthracenediyl-bis(methylene)dipropanedioic acid (ABDA) were purchased from Aladdin Reagent (Shanghai, China). Acetonitrile, anhydrous ethanol, dichloromethane, methanol, glycerol, dimethylsulfoxide and sodium hydroxide were from Tianjin Ltd. (China). Acetonitrile, dichloromethane, methanol, glycerol, dimethyl sulfoxide and sodium hydroxide were purchased from Tianjin Komeo Reagent Company. TEMP was purchased from Merck (Sigma) Reagent Co. Mitochondrial green, calcineurin, propidium iodide and reactive oxygen species detection kits were purchased from Shanghai Biyuntian Biotechnology Co. Ltd.

Fluorescence spectra was carried out on a FS5 spectrophotometer (Edinburgh, UK). The Ultraviolet-visible (UV-vis) absorption spectra was recorded using a TU-1901 UV/vis spectrophotometer (Pukeno General Instrument Co., Ltd., Beijing, China). ^{1}H NMR and ^{13}C NMR spectra were obtained from a nuclear magnetic resonance AVANCE III HD 600 MHz (Bruker). High resolution mass spectra were recorded on a Thermo Fisher TSQ ALTIS mass spectrometer. EPR spectra were obtained from an electron paramagnetic resonator A300-10/12 (Bruker, Germany). Fluorescence microscopy images were obtained using a confocal laser scanning microscope (AXR Nikon).

Synthesis of AIE photosensitizer

Synthesis routes to the NES-OH



Scheme S1 The synthetic routine of **NES-OH.**

Synthesis of Compound 1. A solution of 4-methylpyridine (2.6 mL, 26.88 mmol, 1 eq.) and 2-bromoethanol (1.89 mL, 26.88 mmol, 1 eq.) in CH_3CN (50 mL) was stirred at 90 ℃ for 16 h under nitrogen. After cooling to room temperature, the solvent was evaporated under vacuum to afford compound 1 (5.91 g, crude) as a yellow oil, which was used for the next step without further purification.^{1}H NMR (600 MHz, DMSO-*d6*) δ 8.86 (d, J =6.6 Hz, 2H), 7.92 (d, J = 6.4 Hz, 2H), 5.11 (s, 1H), 4.61-4.57 (m, 2H), 3.74-3.71 (m, 2H), 2.50 (s, 3H).

Synthesis of NES-OH: A mixture of compound 1 (200 mg, 0.733 mmol, 1 eq.), diphenylamino-4-benzaldehyde (159 mg, 0.733 mmol, 1 eq.) and 2 drops of piperidine were stirred in EtOH (30 mL) at 90 ℃ for 16 h under nitrogen. The solvent was evaporated under vacuum. The residue was purified by flash column chromatography (DCM/MeOH =8:1) to afford NES-OH as a red solid. ^{1}H NMR (600 MHz, DMSO-*d6*) δ 8.80 (m, J=6.8 Hz, 2H), 8.17 (m, 2H), 7.96 (d, J = 16.2 Hz, 1H), 7.64 (m, J=8.7 Hz 2H), 7.39 (m, J=7.9Hz, 4H), 7.33 (d, J=16.2 Hz, 1H), 7.17 (m, 2H), 7.12 (m, 4H), 6.96 (m, J=8.7Hz, 2H), 5.24 (t, J=5.3 Hz, 1H),4.55-4.47 (m, 2H), 3.84 (d, J=4.7 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-*d6*) δ 153.77, 149.96, 130.33, 130.19, 128.47, 125.87, 124.99, 123.34, 121.20, 120.98, 62.35, 60.52. HRMS (ESI, m/z): calcd for $C_{20}H_{25}N_2$ $(M\text{-}Br)^+$: 393.24106, found:393.24117.

Monitoring of intracellular mitochondrial and lysosomal viscosity

(1) **living cells photobleaching imaging**: HeLa cells were cultured in DMEM medium with 10% (v/v) fetal bovine serum and 1% penicillin-streptomycin solution for 24 h at 37 ℃ in an incubator with 5% CO_2. Then the cells were dealt with NES-OH (10 μM) for 30 mins. Finally, the cells were washed three times with PBS (pH=7.4). The fluorescence imaging of the HeLa cells was observed using a confocal microscope with a 480 nm laser, then continuous scan 280 times.

(2) **living cells photostability imaging:** The cultured HeLa cells were incubated with NES-OH (10 μM) for 30 min and then the cells were washed with PBS (pH=7.4), and the fluorescence imaging was observed by a confocal microscopy over a period of 200 min.

(3) **living cells co-localization imaging:** The cultured HeLa cells were incubated

with NES-OH (10 μM) and commercial Mito-Tracker Green (1 μM) or Lyso-Tracker Blue (1 μM) for 30 min, and their co-targeting ability was observed by fluorescence imaging on a confocal microscope with a green channel (λ_{ex} = 488 nm, λ_{em} = 490−530 nm) for MitoTracker Green or LysoTracker blue and a blue channel (λ_{ex} = 405 nm, λ_{em} = 419-470 nm) for NES-OH, respectively.

(4) monitoring the mitochondrial and lysosomal viscosity: The cultured HeLa cells were treated with the NES-OH (10 μM) for 30 min and then incubated with nystatin (10 μM) for another 30 min. The HeLa cells were dealt with the NES-OH (10 μM) for 30 min and then incubated with LPS (100 μg mL^{-1}) for 30 min. The HeLa cells were dealt with the NES-OH (10 μM) for 30 min and then incubated with chloroquine (CQ) for 30 min The fluorescence images were observed using a confocal microscope.

(5) Detection and differentiation of normal and cancer cells: Cancer cells (HeLa cells) and normal cells (HL-7702) were incubated with the NES-OH (10 μM) and commercial Lyso-Tracker Blue (1 μM) for 30 min, separately. The fluorescence images were carried out using a confocal microscope.

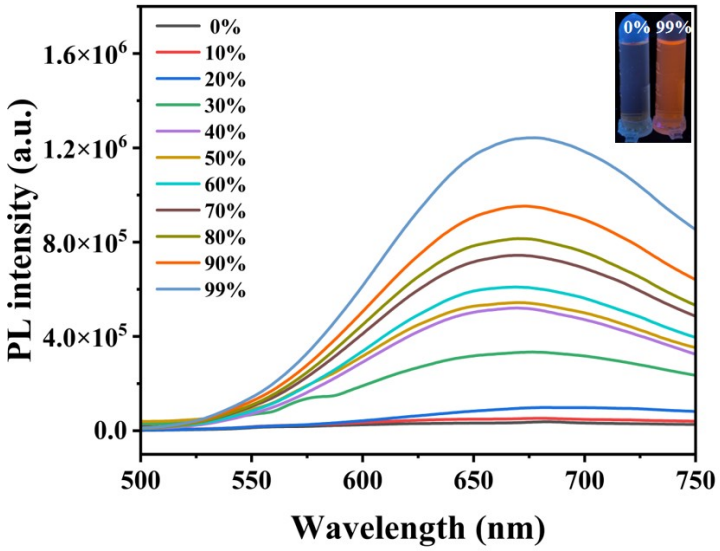


Fig. S1 fluorescence spectra of NES-OH in mixed solvents of isopropanol and water with different proportions.

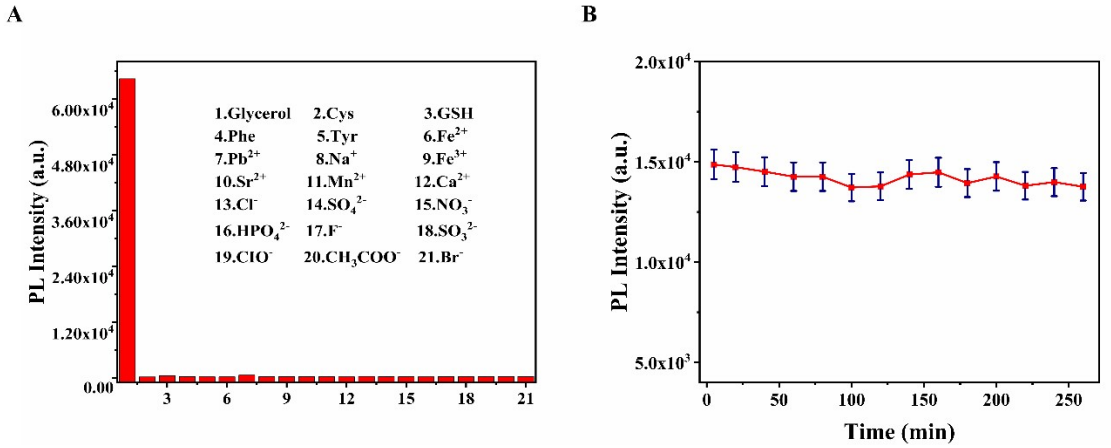


Fig. S2 FL intensity of NES-OH (5 μM) in PBS buffer (100 μM, pH 7.4) with various species.

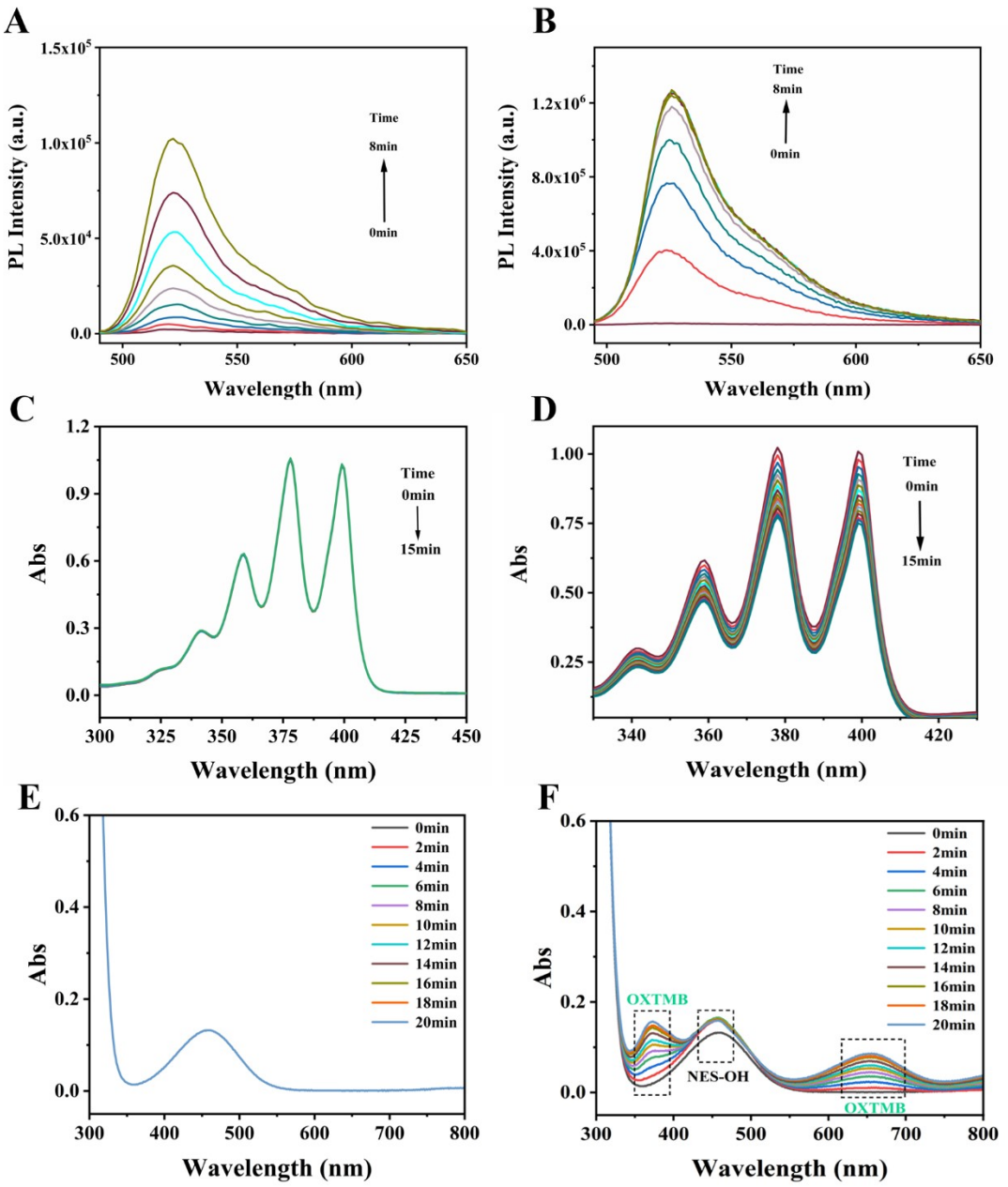


Fig. S3 (A) FL spectra of DCFH (10 μM) with different irradiation times; (B) FL spectra of DCFH (10 μM) containing NES-OH (5 μM) at different irradiation times; (C) Absorption spectra of ABDA (100 μM) at different irradiation times; (D) Absorption spectra of ABDA (100 μM) containing NES-OH (10 μM) at different irradiation times; (E) Absorption spectra of TMB (100 μM) with different irradiation times; (F) Absorption spectrum of TMB (100 μM) containing NES-OH (5 μM).

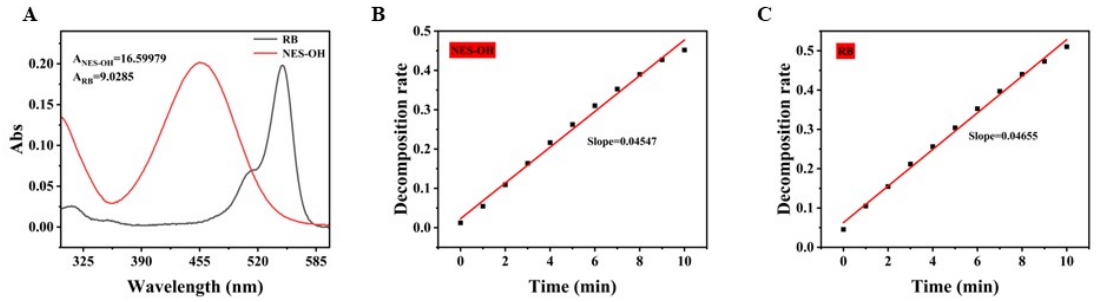


Fig. S4 UV-vis spectra of Red Bengal rose (RB) and NES-OH (A); decomposition rates of ABDA with NES-OH (B) and RB (C).

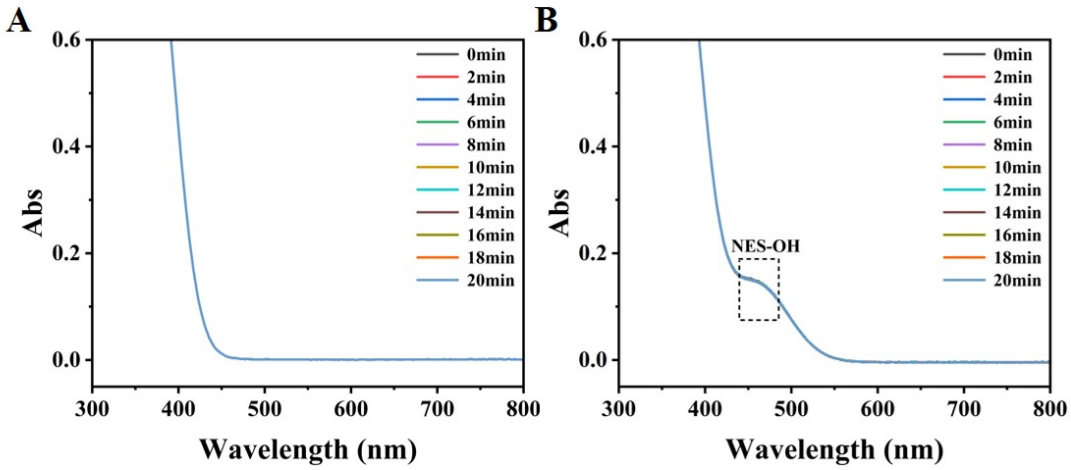


Fig. S5 (A) Absorption spectra of NBT (100 μM) at different irradiation times; (B) FL spectra of NBT (100 μM) containing NES-OH (5 μM).

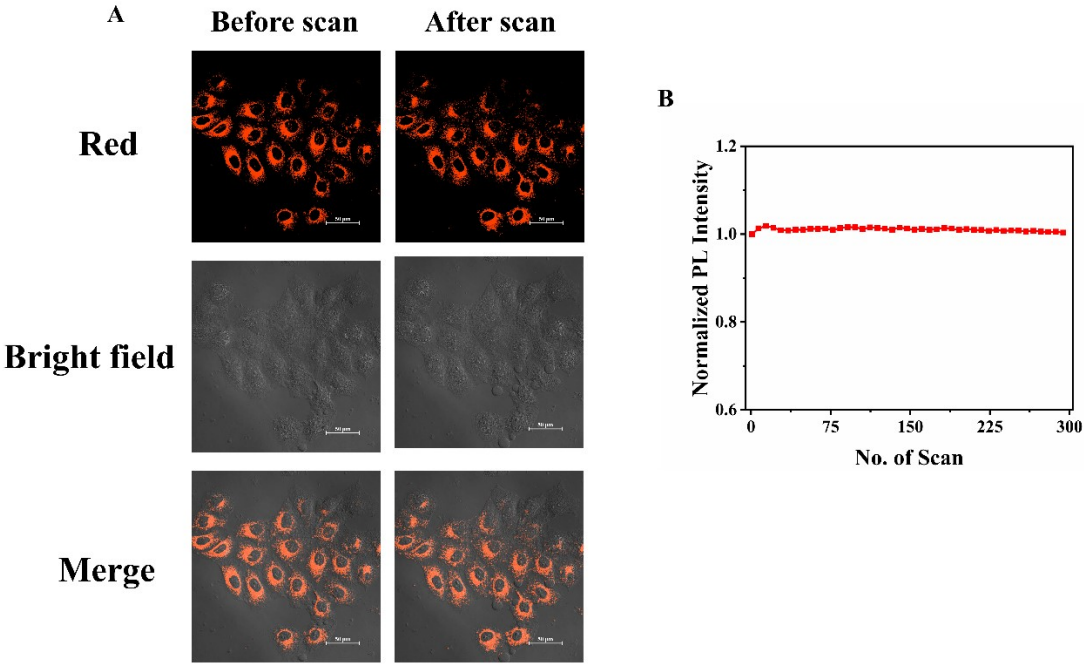


Fig. S6 (A) Fluorescence images of **NES-OH** (10 μM) in HeLa cells before and after irradiation; (B) Relative FL intensity of **NES-OH** in HeLa cells by continuous irradiation at 280 scans.

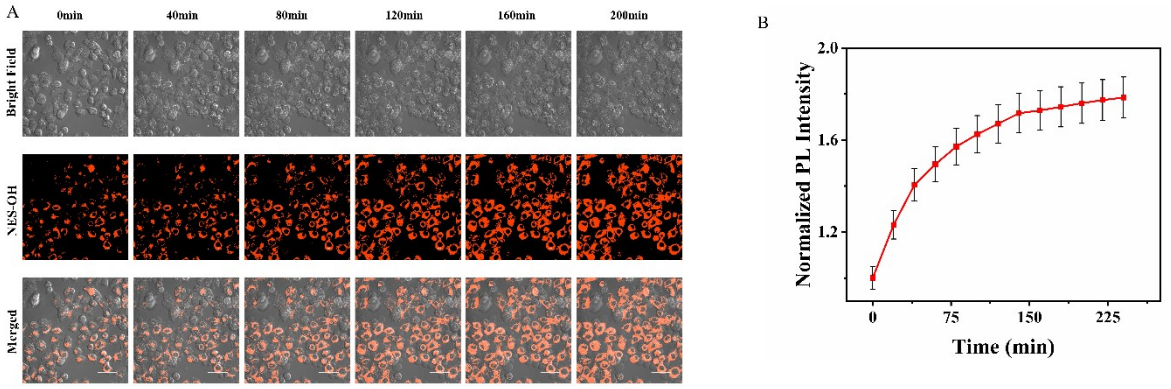


Fig. S7 (A) CLSM images of Hela cells incubated with **NES-OH** (10 μM) for 200 min; (B) Relative FL intensity of **NES-OH** in HeLa cells by continuous irradiation at 200 min.

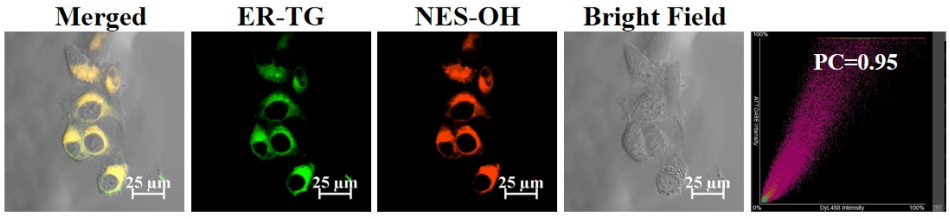


Fig. S8 Co-localized fluorescence imaging of ER-Tracker Green co-treated HeLa cells.

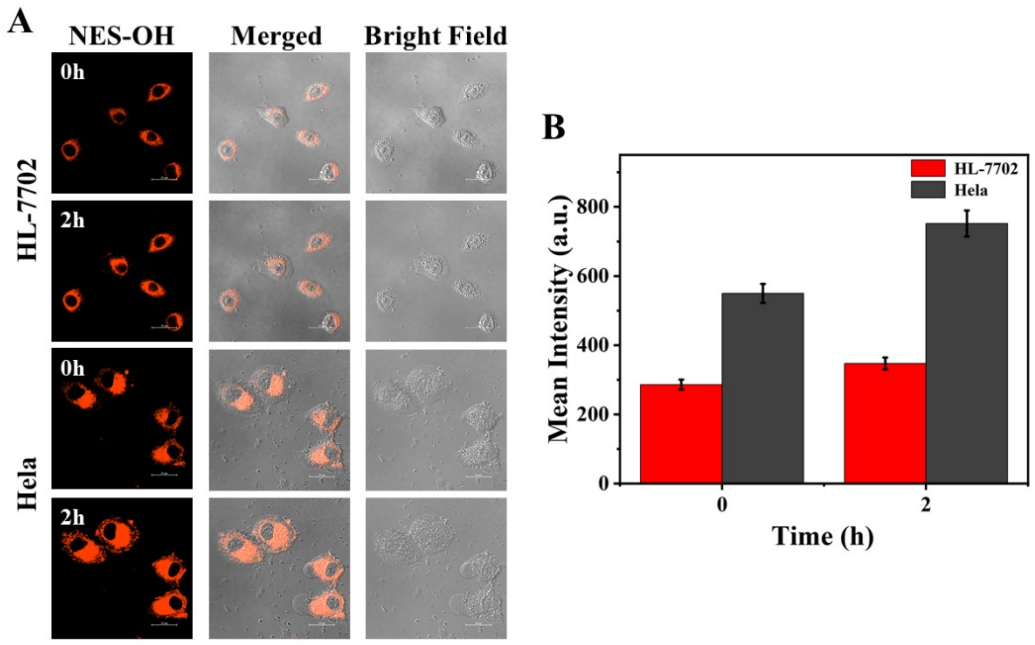


Fig. S9 (A) CLSM images of **NES-OH** (10 μM) stained cancer and normal cells. (B) Mean FL intensity obtained from (A).

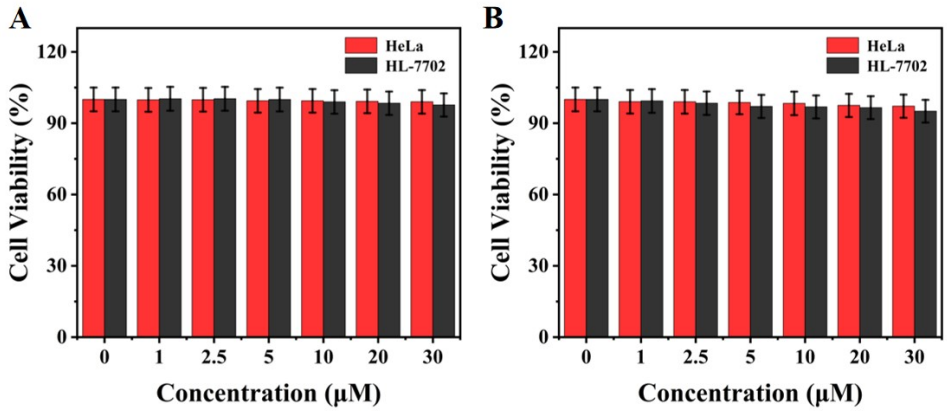


Fig. S10 The cytotoxicity of NES-OH in cancer cell and normal cells (A) 5 h; (B) 10 h.

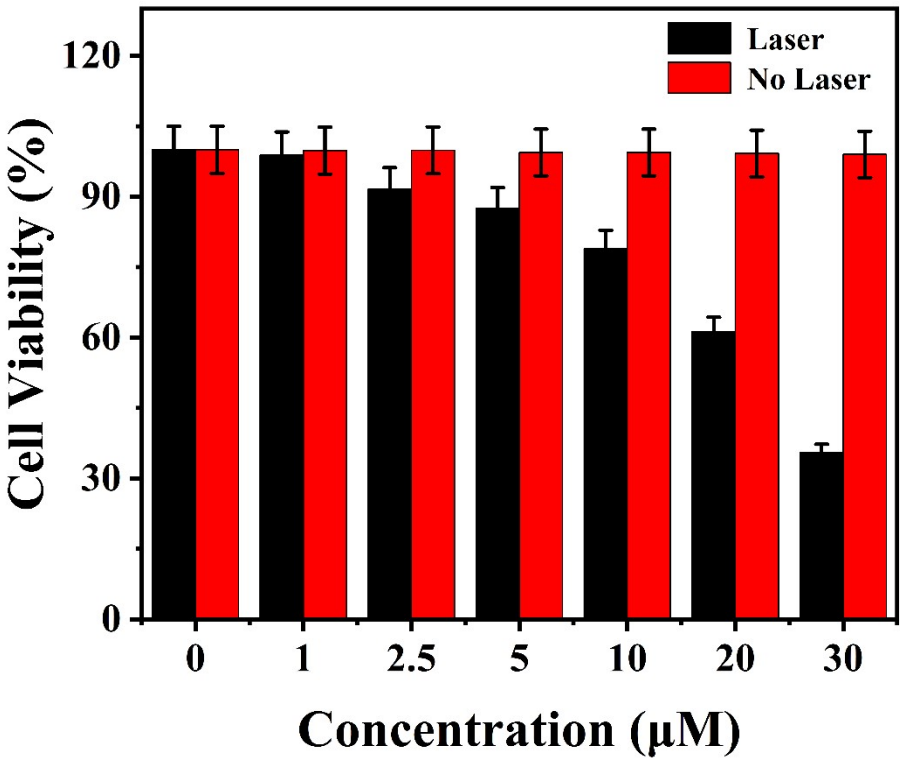


Fig. S11 Cell viability of Hela cells incubated with NES-OH upon white light irradiation.

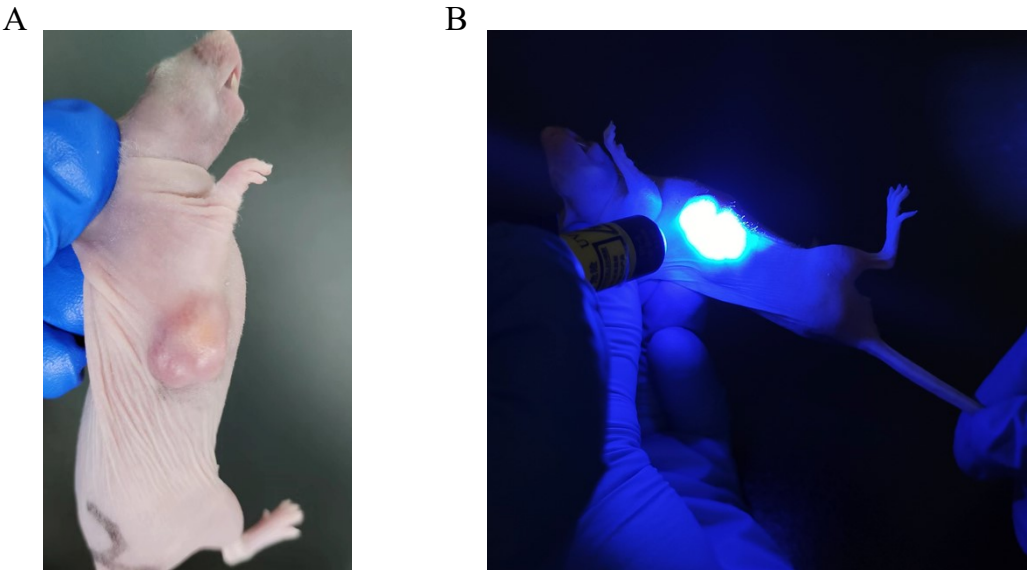


Fig. S12 (A) Photographs of tumor bearing mice and (B) Physical Photographs of tumor bearing mice under illumination on PDT (470 nm, 50 mW/cm^{-2}).

^{1}H NMR, ^{13}C NMR and HR-MS spectra analysis of Compound 1 and NES-OH.

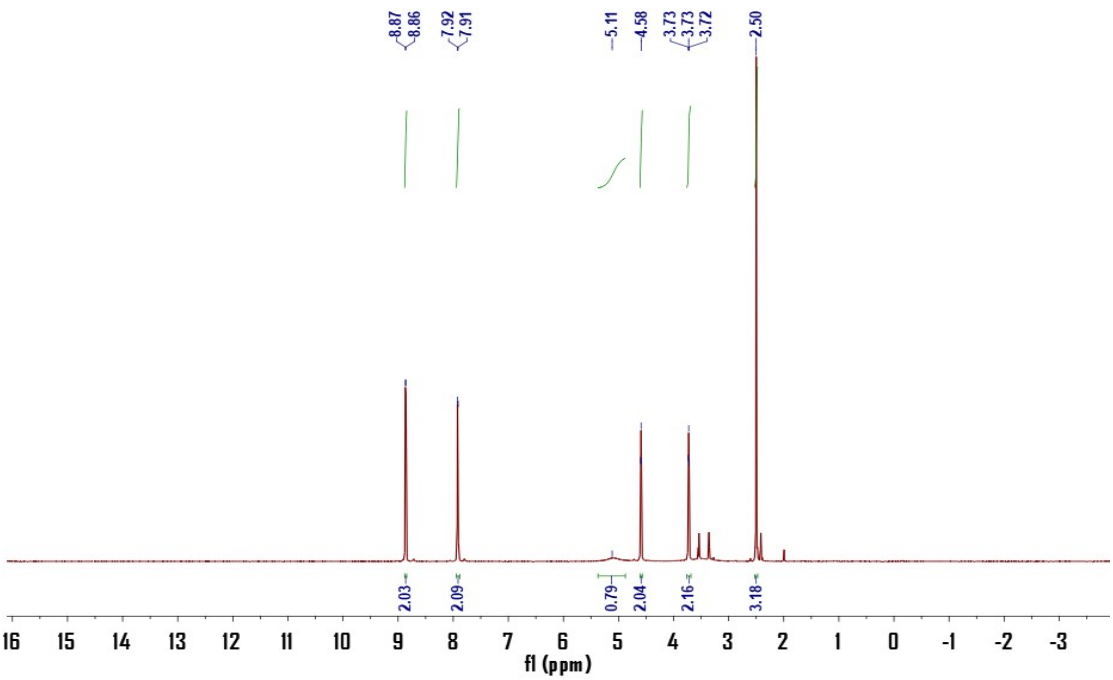


Fig. S13 [1]H NMR spectra of Compound 1.

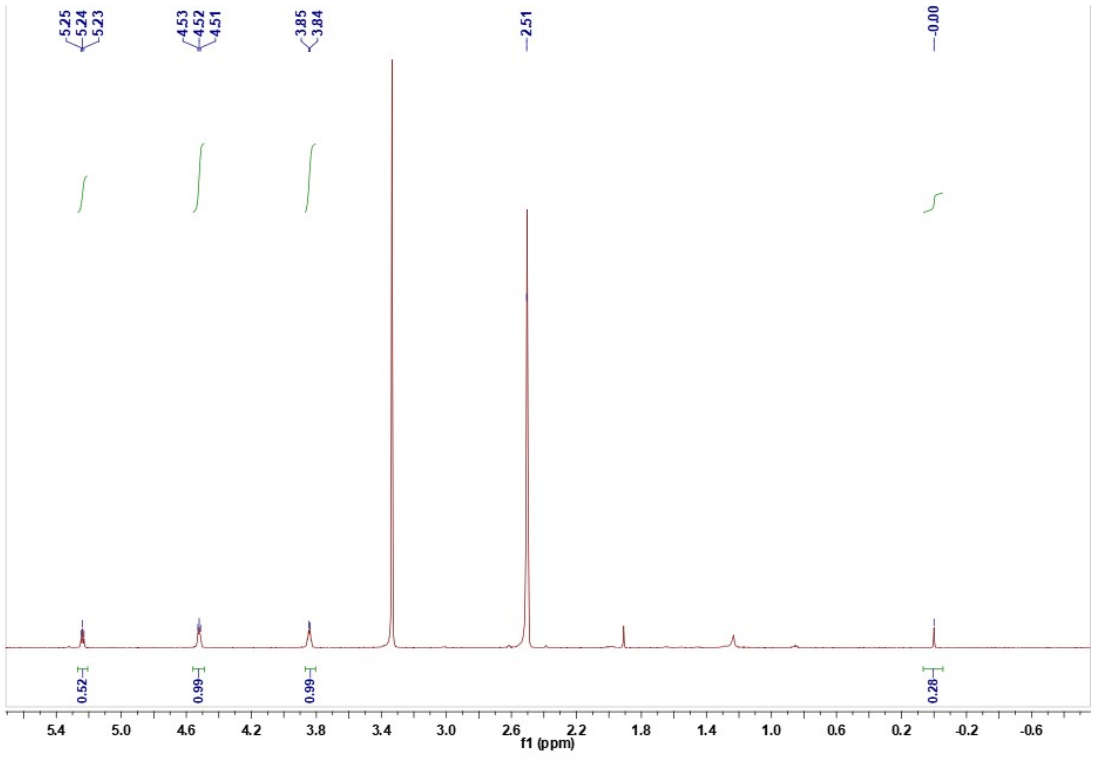


Fig. S14 [1]H NMR spectra of NES-OH.

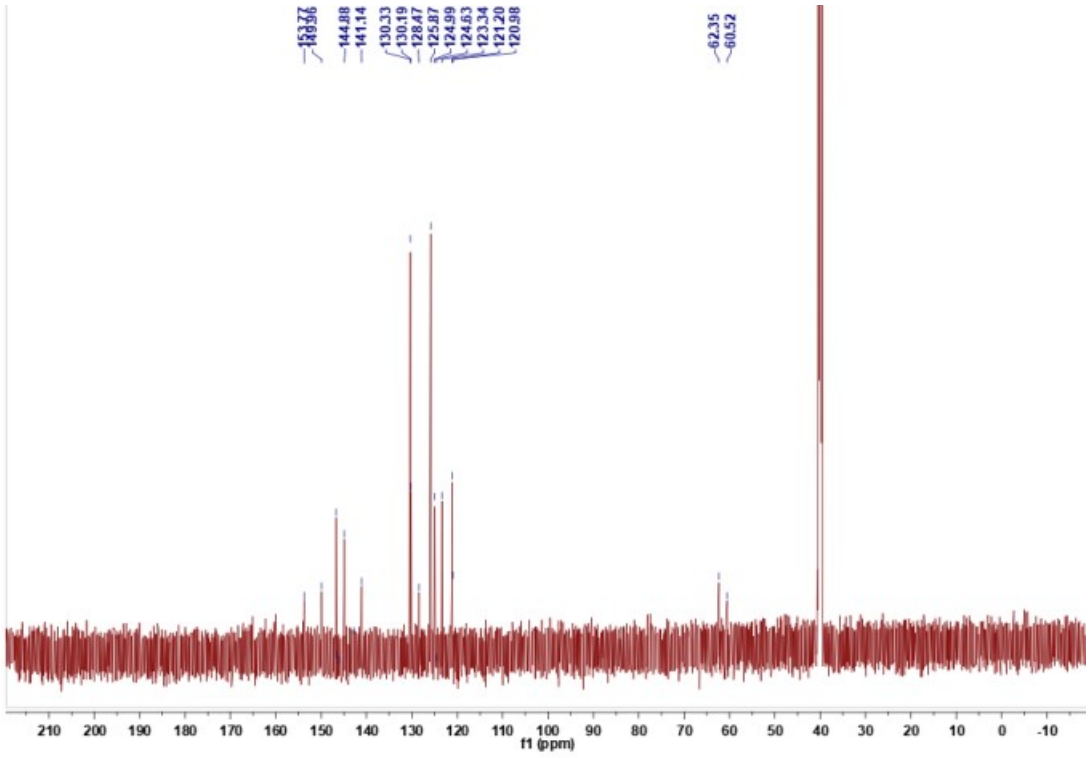


Fig. S15 ^{13}C NMR spectra of NES-OH.

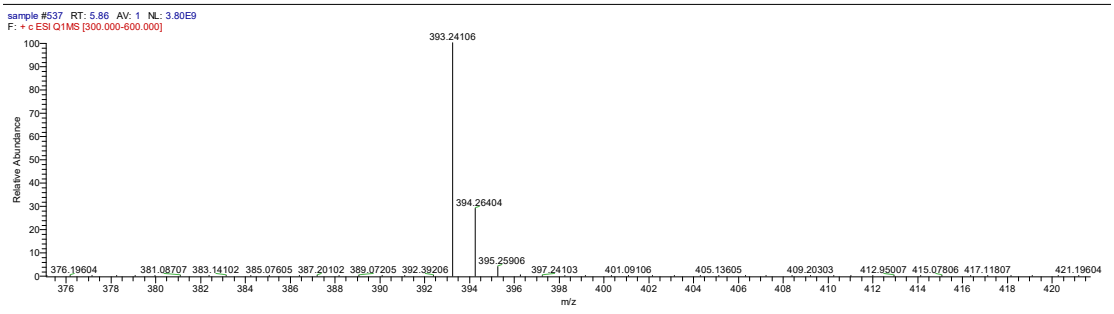


Fig. S16 HR-MS spectra of NES-OH