Supporting Information

The Nuclear Targeted Type-I Photosensitizer for Anti-Tumor Therapy

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1 Experimental Procedures

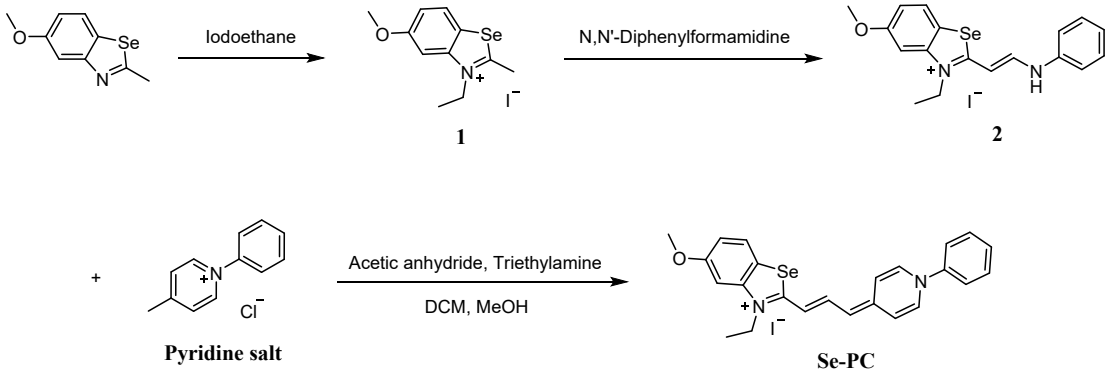
1.1 Materials and Reagents

All chemical reagents and solvents for synthesis and characterization were purchased from Aladdin, Energy Chemical, Bidepharm and Makclin and were used without further purification. Deoxyribonucleic acid from calf thymus (CT DNA, dsDNA) and ribonucleic acid from torula yeast (yeast RNA, ssRNA) were purchased from Solarbio. PBS buffer was purchased from VivaCell. Oligonucleotides were purchased from Sangon Biotech. Distilled water was used after passing through a water ultra-purification system. Fetal bovine serum (FBS) and penicillin/streptomycin were obtained from Biological Industries and Beijing Solarbio Science&Technology Co., Ltd. respectively. Dulbecco's Modified Eagle Medium (DMEM) was purchased from Beijing Solarbio Science&Technology Co., Ltd. The MCF-7, 4T1, L929 and HeLa cells were purchased from Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences. DNase I, RNase A, Dihydroethidium (DHE), DCFH-DA, Mito-Tracker Green, N-acetyl-L-cysteine (NAC), Calcein/PI Cell Viability/Cytotoxicity Assay Kit, Annexin V-FITC/Propidium Iodide (PI) Detection Kit, BeyoGel™ Agarose Precast Gel, TAE (50X) (DNase, RNase & Protease free, Sterile), DEPC-treated Water, DNA Damage Assay Kit by γ-H2AX Immunofluorescence and Universal Genomic DNA Purification Mini Spin Kit were purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, P. R. China). 6 × DNA Loading Buffer and Trans15K DNA Marker were purchased from TransGen Biotech. 2, 7-dichlorodihydrofluorescein (DCFH) was purchased from Innochem.

1.2 Instruments

UV-vis spectra and fluorescent spectra were conducted on a Fluorescence and Absorbance Spectrometer (Duetta, HORIBA). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Ascend^{TM} 400 spectrometer using TMS as the internal reference. High Resolution Mass Spectrometric (HR-MS) data were obtained on a Quadrupole Time-of-Flight Liquid Chromatograph Mass Spectrometer (LCMS-9030 or Agilent G6224A). The cell imaging experiments were measured by Leica Confocal Laser Scanning Microscope (STELLARIS 5). Small animals' fluorescence imaging was carried out by NightOWL II LB983 living imaging system. 635 nm LED light was employed as the light source for in vitro and vivo experiments. The output power of the laser was measured by a power meter (CEL-FA-A, Beijing Normal University Optoelectronic Instrument Factory).

1.3 Synthesis and Characterization



Scheme S1. Synthetic routes of **Se-PC**.

Synthesis of **compound 1**:

An oven-dried 25 mL pressure resistant tube was charged with 5-methoxy-2-methylbenzoselenazole (500 mg, 2.21 mmol, 1.0 equiv.) and Iodoethane (1.03 g, 6.63 mmol, 3 equiv.). The reaction mixture was stirred for 12 h at 120°C, then cooled to room temperature. At this stage, ethyl acetate (10 mL) was added and stirring was continued for another 1 h. The resulting suspension was filtered and washed with ethyl acetate (3 × 5 mL), and dried. The resulting grey green solid was used without any further purification (700 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 9.0 Hz, 1H), 7.76 (d, J = 2.3 Hz, 1H), 7.36 (dd, J = 9.0, 2.3 Hz, 1H), 4.73 (q, J = 7.2 Hz, 2H), 3.95 (s, 3H), 3.19 (s, 3H), 1.43 (t, J = 7.2 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 188.73, 160.03, 143.30, 128.54, 123.69, 117.45, 102.38, 56.85, 46.01, 20.06, 13.50. HR-MS: *m/z* calcd for $C_{11}H_{14}NOSe^{+}$ [M]$^{+}$: 256.0236, found: 256.0275.

Synthesis of **compound 2**:

Under nitrogen atmosphere, the mixture of **compound 1** (660 mg, 1.73 mmol, 1 equiv.) and N, N'-Diphenylformamidine (407 mg, 2.07 mmol, 1.2 equiv.) were stirred at 140°C for 90 min and then cool to room temperature. The crude product obtained and crystallized by a mixed solvent of ethanol and ethyl acetate. Then the crude product was purified on silica gel column chromatography to afford **compound 2** as yellow solid (560 mg, 67%).HR-MS: *m/z* calcd for $C_{18}H_{19}N_2OSe^{+}$ [M]$^{+}$: 359.0658, found: 359.0661.

Pyridine salt was synthesized according to the previous literature.[1]

Synthesis of **Se-PC**:

An oven-dried 25 mL round-bottom flask was charged with **compound 2** (150 mg, 0.309 mmol, 1.0 equiv.), **Pyridine salt** (76 mg, 0.371 mmol, 1.2 equiv.), 0.2 mL N, N-Diisopropylethylamine, 0.1 mL acetic anhydride and 1.8 mL anhydrous pyridine. The reaction mixture was stirred for

2 h at room temperature. Then the reaction mixture was dropwise added to 20 mL of anhydrous ether solution (20 mL). The resulting suspension was filtered and purified on silica gel column chromatography to afford **Se-PC** as purple black solid (152 mg, 88%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (d, *J* = 7.0 Hz, 2H), 7.89 (t, *J* = 13.3 Hz, 1H), 7.81 – 7.72 (m, 5H), 7.67 (t, *J* = 7.7 Hz, 2H), 7.61 (t, *J* = 7.2 Hz, 1H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.83 (dd, *J* = 8.7, 2.2 Hz, 1H), 6.40 (d, *J* = 11.9 Hz, 1H), 6.30 (d, *J* = 13.8 Hz, 1H), 4.21 (q, *J* = 7.0 Hz, 2H), 3.84 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.30, 160.23, 153.17, 144.99, 144.29, 142.69, 140.73, 130.61, 130.08, 126.42, 123.91, 119.64, 114.71, 114.14, 110.64, 100.56, 99.74, 56.27, 41.52, 12.43. HR-MS: *m/z* calcd for $C_{24}H_{23}N_2OSe^+$ $[M]^+$: 435.0971, found: 435.0976.

1.4 In vitro characterizations

Absorption spectra

For absorption measurements, **Se-PC** (4 μM) with or without CT DNA (100 μg/mL) were mixed in 10 mM PBS buffer (300 mM NaCl, pH 7.4) and absorbance spectra were recorded in the range of 400-800 nm.

Fluorescence spectra

Fluorescence spectra of **Se-PC** (4 μM) with or without CT DNA (100 μg/mL) in 10 mM PBS buffer (300 mM NaCl, pH 7.4) were recorded. Excitation wavelength: 612 nm, emission wavelength: 500-800 nm.

To elucidate whether the fluorescence activation mechanism of **Se-PC** depends on specific DNA binding, different treatments were conducted (1: **Se-PC** + DNA, 2: **Se-PC** + DNA + DNase, 3: **Se-PC** + DNA + RNase). For **Se-PC** + DNA groups: **Se-PC** (4 μM) with CT DNA (100 μg/mL) were mixed in PBS buffer. For **Se-PC** + DNA + DNase groups: **Se-PC**-CT DNA in PBS buffer was treated with DNase I (100 U/mL) for 2 h at 37°C. For **Se-PC** + DNA + RNase groups: **Se-PC**-CT DNA in PBS buffer was treated with RNase I (100 μg/mL) for 2 h at 37°C. And fluorescence spectra were recorded. Excitation wavelength: 612 nm, emission wavelength: 500-800 nm.

For oligonucleotides selectivity studies, **Se-PC** (1 μM) was incubated with varying oligonucleotides (4 μM). And fluorescence spectra were recorded respectively by microplate reader (Synergy H1, BIOTEK).

To determine the ability to produce total ROS, **Se-PC** (4 μM) was incubated with DCFH (10 μM). The mixtures were irradiated with an LED light (635 nm, 0.1 mW/cm^2). For dynamic monitoring of ROS production, the mixtures were irradiated for different times and fluorescence spectra for the DCFH was acquired. As control, DCFH solution without **Se-PC** was

subjected to irradiation. Excitation wavelength: 488 nm, emission wavelength: 500-700 nm.

To determine the ability to produce total $^1O_2$, **Se-PC** (4 μM) was incubated with SOSG (0.5 μM). The mixtures were irradiated with an LED light (635 nm, 0.1 mW/cm^2). For dynamic monitoring of $^1O_2$ production, the mixtures were irradiated for different times and fluorescence spectra for the SOSG was acquired. As control, SOSG solution without **Se-PC** was subjected to irradiation. Excitation wavelength: 504 nm, emission wavelength: 515-750 nm.

To determine the ability to produce total $O_2^{\bullet-}$, **Se-PC** (4 μM) with CT DNA (100 μg/mL) were incubated with DHE (10 μM). The mixtures were irradiated with an LED light (635 nm, 0.1 mW/cm^2). For dynamic monitoring of $O_2^{\bullet-}$ production, the mixtures were irradiated for different times and fluorescence spectra of the DHE was acquired. As control, DHE without **Se-PC**, **Se-PC** + DNA or DHE + **Se-PC** aqueous solution was subjected to irradiation. Excitation wavelength: 300 nm, emission wavelength: 400-750 nm.

To determine the ability to produce total •OH, **Se-PC** (4 μM) was incubated with HPF (10 μg/mL). The mixtures were irradiated with an LED light (635 nm, 0.1 mW/cm^2). For dynamic monitoring of •OH production, the mixtures were irradiated for different times and fluorescence spectra for the HPF was acquired. As control, HPF aqueous solution without **Se-PC** was subjected to irradiation. Excitation wavelength: 490 nm, emission wavelength: 500-750 nm.

Computational details

For density functional theory calculations, all density functional theory calculations were performed using the Gaussian 09 program package. The ground state geometries were optimized at the B3LYP/6-31G* (d, p) level. The excitation state geometries were optimized at the B3LYP/TZVP (d, p) level. The solvent (water) effect was included in all calculations using the solvation model based on the density (PCM).

For molecular docking calculations, AutoDockTools platform were implemented to investigate the interaction between **Se-PC** and dsDNA. The crystal structures of dsDNA from PDB database (DNA PDB ID: 7YZ7, 111D and 2GYX) were selected as the research model. The final visualization of the calculation results was conducted using Pymol software.

EPR analysis

EPR spectra for the detection of $^1O_2$ using TEMP (water) as a spin trapper. Reaction mixture was containing TEMP (10 mM) and **Se-PC** (10 μM). The spectra of spin were obtained before and after the corresponding solutions were irradiated by LED light (635 nm, 0.1 mW/cm^2) for

12 min. EPR analysis was performed to confirm the generation of $O_2^{\bullet-}$ utilizing DMPO as spin trap agent. The spectra of spin were obtained before and after the corresponding solutions containing DMPO (10 mM) and **Se-PC** (10 μM) in MeOH were irradiated by LED light (635 nm, 0.1 mW/cm^2) for 12 min. The generation of •OH was confirmed in H_2O by EPR analysis with the similar experiment conditions.

1.5 Cellular assays

Cell Culture

MCF-7, 4T1, L929 and HeLa cells were cultured in Dulbecco's modified eagle's medium in high glucose (DMEM; Gibco) containing 10% fetal bovine serum (FBS; Gibco), and 1% penicillin/streptomycin (P/S; Gibco). All cells were cultured in a 5% CO_2 humidified incubator at 37°C.

Confocal Fluorescence Imaging

MCF-7, 4T1, L929 and HeLa cells were grown in a 35-mm Petri dish with a 10-mm glass bottom well. After 12 h, the cells were washed with 1 mL × PBS for three times and incubated in cell culture medium at 37°C before imaging. For living cells imaging assays, MCF-7, 4T1, L929 and HeLa cells were incubated with **Se-PC** (1 μM) for 60 min. And then confocal images were acquired using the following conditions: Excitation wavelength: 610 nm, emission collection: 620 nm-720 nm.

To clarify the cell uptake behaviors of **Se-PC**, MCF-7, 4T1, L929 and HeLa cells were incubated with **Se-PC** (1 μM), meanwhile the intracellular fluorescence was tracked by a confocal laser scanning microscope (CLSM) at different times (5, 30, 60, 90, 120, and 150 min). Excitation wavelength: 610 nm, emission collection: 620 nm-720 nm.

For living cells colocalization assays with **MTG**, MCF-7, 4T1 and HeLa cells were incubated with **Se-PC** (1 μM) for 120 min, and then **MTG** (1 μM) was added and incubated for another 30 min. Confocal images were acquired using the following conditions: Excitation wavelength for **Se-PC**: 610 nm, emission collection: 620 nm-720 nm; Excitation wavelength for **MTG**: 495 nm, emission collection: 505 nm-580 nm.

To test the ability for **Se-PC** to stain DNA in cells, the MCF-7 cells were fixed in cold ethanol for 15 min to ensure cell integrity and allow macromolecular enzymes or antibodies to enter the cells. After washing with PBS twice, the cells were respectively incubated with different conditions for 2 h at 37°C including Dnase I (100 U/mL), Rnase A (100 μg/mL) or PBS, and washed again with PBS twice. Then the cells were separately incubated with **Se-PC** (1 μM) for

120 min. And confocal images were acquired using the following conditions: Excitation wavelength: 610 nm, emission collection: 620 nm-720 nm.

Intracellular ROS detection

To determine the ability of **Se-PC** to generate total ROS in cells, MCF-7 cells were randomly divided into four groups (1: control, 2: control + light, 3: **Se-PC**, 4: **Se-PC** + light). For control groups: MCF-7 cells were incubation with 5 μM of DCFH-DA for 30 min. For control + light groups: MCF-7 cells were incubation with 5 μM of DCFH-DA for 30 min and then irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 5 μM of DCFH-DA for 30 min. For **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 5 μM of DCFH-DA for 30 min and then irradiated with an LED light. To test the ability of **Se-PC** to produce ROS in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and incubated with DCFH-DA and irradiated. To quench the effect of ROS, the cells were pretreated with N-acetylcysteine (NAC, 5.0 mM) for 4 h before staining with **Se-PC** and DCFH-DA. And confocal images were acquired using the following conditions: Excitation wavelength: 489 nm, emission collection: 494 nm-739 nm.

To determine the ability of **Se-PC** to generate $^1O_2$ in cells, MCF-7 cells were randomly divided into three groups (1: control + light, 2: **Se-PC**, 3: **Se-PC** + light). For control + light groups: MCF-7 cells were incubation with 5 μM of SOSG for 30 min and then irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 5 μM of SOSG for 30 min. For **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 5 μM of SOSG for 30 min and then irradiated with an LED light. To test the ability of **Se-PC** to produce $^1O_2$ in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and incubated with SOSG and irradiated. And confocal images were acquired using the following conditions: Excitation wavelength: 488 nm, emission collection: 504 nm-600 nm.

To determine the ability of **Se-PC** to generate $O_2^{\bullet-}$ in cells, MCF-7 cells were randomly divided into three groups (1: control + light, 2: **Se-PC**, 3: **Se-PC** + light). For control + light groups: MCF-7 cells were incubation with 1X of DHE for 30 min and then irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 1X of DHE for 30 min. For **Se-PC** + light

groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 1X of DHE for 30 min and then irradiated with an LED light. To test the ability of **Se-PC** to produce $O_2^{\bullet-}$ in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and incubated with DHE and irradiated. And confocal images were acquired using the following conditions: Excitation wavelength: 535 nm, emission collection: 545 nm-645 nm.

To determine the ability of **Se-PC** to generate •OH in cells, MCF-7 cells were randomly divided into three groups (1: control + light, 2: **Se-PC**, 3: **Se-PC** + light). For control + light groups: MCF-7 cells were incubation with 5 μM of HPF for 30 min and then irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 5 μM of HPF for 30 min. For **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min, followed by incubation with 5 μM of HPF for 30 min and then irradiated with an LED light. To test the ability of **Se-PC** to produce •OH in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and incubated with HPF and irradiated. And confocal images were acquired using the following conditions: Excitation wavelength: 488 nm, emission collection: 504 nm-600 nm.

Live/dead cell assay

MCF-7 cells were randomly divided into four groups (1: control, 2: control + light, 3: **Se-PC**, 4: **Se-PC** + light). For control groups: MCF-7 cells did not perform any preprocessing. For control + light groups: MCF-7 cells did not perform any preprocessing but irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min. For **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min and then irradiated with an LED light. To test the ability of **Se-PC** to damage cells in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and irradiated. To quench the effect of ROS, the cells were pretreated with N-acetylcysteine (NAC, 5.0 mM) for 4 h before staining with **Se-PC** and irradiated. After different treatments, Calcein AM and Propidium Iodide co-staining were performed according to the manufacture instruction. And confocal images were acquired using the following conditions: Excitation wavelength for Calcein AM: 488 nm, emission collection for Calcein AM: 499 nm-585 nm. Excitation wavelength for PI: 488 nm, emission collection for PI: 554 nm-740 nm.

1.6 Cytotoxicity assay

To validate the cytotoxicity of **Se-PC** with or without LED light, MCF-7, 4T1, L929 or HeLa cells (1×10^4 cells/well) in DMEM (supplemented with 10% FBS, 100 μL) were seeded in 96-well plate and incubated at 37°C for 12 h and another 6 h under normoxic (21% O_2) or hypoxic (2% O_2) atmosphere. Then, the medium was changed and cells were treated with **Se-PC** at a serial of concentrations (0, 0.1, 0.2, 0.4 and 0.8 μM) in DMEM (100 μL) at 37°C with 5% CO_2 humidified incubator and cultured for another 120 min under normoxic or hypoxic atmosphere. The cells were irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min) or not and cultured for another 12 h before addition of MTT. Subsequently, the cell viability was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay based on the conversion of MTT into formazan crystals by living cells. To quench the effect of ROS, the cells were pretreated with N-acetylcysteine (NAC, 5.0 mM) for 4 h before treating with **Se-PC** and irradiated.

1.7 Agarose gel electrophoresis assay

MCF-7 cells were randomly divided into five groups (1: control, 2: control + light, 3: **Se-PC**, 4: 1 μM **Se-PC** + light, 5: 1.5 μM **Se-PC** + light). For control groups: MCF-7 cells did not perform any preprocessing. For control + light groups: MCF-7 cells did not perform any preprocessing but irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min. For 1 μM **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min and then irradiated with an LED light. After continuing to incubate for 120 min. For 1.5 μM **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1.5 μM) for 120 min and then irradiated with an LED light. All conditions were continuing to incubate for 2 h. After different treatments, the cell DNA was purified using Universal Genomic DNA Purification Mini Spin Kit (Beyotime) according to the manufacture instruction. The purified DNA was mixed with the DNA Loading Buffer (6X), and then subjected to agarose gel electrophoresis.

1.8 γ-H_2AX immunofluorescence assay

MCF-7 cells were randomly divided into three groups (1: control, 2: **Se-PC**, 3: **Se-PC** + light). For control groups: MCF-7 cells did not perform any preprocessing. For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min. For 1 μM **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min and then irradiated with an LED light. All conditions were continuing to incubate for 2 h. After different treatments, the cells were

treated according to the DNA Damage Assay Kit (Beyotime). The confocal fluorescence imaging was performed and images were collected. The excitation wavelength of DAPI and γ-H_2AX were 405 nm and 495 nm, and emission signals were collected from 420 to 618 nm with blue fluorescence and from 500 to 739 nm with green fluorescence, respectively.

1.9 Mitochondrial membrane potential assay

MCF-7 cells were randomly divided into three groups (1: **Se-PC**, 2: **Se-PC** + light, 3: CCCP). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min. For 1 μM **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min and then irradiated with an LED light. After different treatments, For CCCP groups: MCF-7 cells were pretreated with CCCP (10 μM) for 20 min. After different treatments, the cells were treated according to the Mitochondrial membrane potential assay kit with JC-1 (Beyotime). In viable cells, a high mitochondrial membrane potential leads to JC-1 existing in an aggregate state, resulting in red fluorescence. Conversely, in apoptotic cells, mitochondrial damage and a lower mitochondrial membrane potential cause JC-1 to primarily exist in a monomer state, emitting green fluorescence. Due to an overlap in the emission spectrum of **Se-PC** with the red fluorescence of J-aggregates (Ex: 585 nm, Em: 595-655 nm), we focused solely on the emission of JC-1 monomers (Ex: 490 nm, Em: 500-600 nm).

1.10 Annexin V-FITC/PI co-staining assay

MCF-7 cells were randomly divided into three groups (1: control, 2: **Se-PC**, 3: **Se-PC** + light). For control groups: MCF-7 cells did not perform any preprocessing. For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min. For 1 μM **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min and then irradiated with an LED light. To test the ability of **Se-PC** to damage cells in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and irradiated. After different treatments, the cell apoptosis was detected using Annexin V-FITC Apoptosis Detection Kit (Beyotime) according to the manufacture instruction. The excitation wavelength of Annexin V-FITC and PI were 488 nm and 535 nm, and emission signals were collected from 499 to 525 nm with green fluorescence and from 600 to 635 nm with red fluorescence, respectively.

1.11 Flow cytometry analysis

To investigate the cell killing mechanism of **Se-PC**, MCF-7 cells were cultured in cell plates for 24 h. Then, MCF-7 cells were randomly divided into four groups (1: control, 2: control + light, 3: **Se-PC**, 4: **Se-PC** + light). For control groups: MCF-7 cells did not perform any preprocessing.

For control + light groups: MCF-7 cells were irradiated with an LED light (635 nm, 5 mW/cm^2, 5 min). For **Se-PC** groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min. For **Se-PC** + light groups: MCF-7 cells were pretreated with **Se-PC** (1 μM) for 120 min and then irradiated with an LED light. To test the ability of **Se-PC** to damage cells in hypoxic conditions, the cells were cultured in 2% oxygen atmosphere, treated with **Se-PC** and irradiated. After different treatments, the cell apoptosis was detected using Annexin V-FITC Apoptosis Detection Kit (Beyotime) according to the manufacture instruction. Flow cytometry was performed using the Attune NxT Acoustic Focusing Cytometer (ThermoFisher). Annexin V-FITC: 488nm, 530/30 nm; PI: 561nm, 585/16 nm. 10,000 cells were analyzed for each sample and flow cytometry data were analyzed with FlowJoTM 10.

1.12 In vivo antitumor evaluation

Female Balb/c mice (about 6 weeks aged) were purchased from Liaoning Changsheng biotechnoiogy Co. Experiments on mice were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health. The animal protocol was approved by the local research ethics review board of the Animal Ethic Committee of Dalian University of Technology (DUT20230322).

For tumor model establishment, 4T1 cells (1×10^6) were injected subcutaneously into the right armpit to establish the 4T1 tumor model of Balb/c mice. About 7 days after inoculation, the tumor volume of about 90-100 mm^3 could be used for experiments.

For in vivo tumor accumulation, **Se-PC** (100 μL, 50 μM) was injected into tumor bearing mice via the intratumoral injection, and fluorescence imaging was observed at different post-injection time by small animals' fluorescence imaging system.

For PDT evaluation in vivo, the tumor-bearing mice were randomly divided into four groups (each group contained five mice) and performed with the following different treatments:

1) Contro Group: intratumoral injection of PBS (100 μL) only,

2) Light Group: after intratumoral injection of PBS (100 μL) for 2 h, the tumor sites were irradiated with 635 nm light (50 mW/cm^2, 10 min),

3) **Se-Pc** Group: intratumoral injection of **Se-PC** (100 μL, 100 μM) only,

4) **Se-Pc** + Light Group: after intratumoral injection of **Se-PC** (100 μL, 100 μM) for 2 h, the tumor sites were irradiated with 635 nm light (50 mW/cm^2, 10 min) The tumor volume and body weight of all mice were measured every two days in each group of mice during the

treatment period. The greatest longitudinal diameter (length) and the greatest transverse diameter (width) were used to calculate the tumor volume.

Tumor volume = width × width × length/2.

Moreover, after 14 days post-treatment, the mice were euthanized, tumor tissues were harvested for histological and immunohistochemical analysis by means of hematoxylin-eosin (H&E) staining or immunohistochemical staining (Ki-67). Meanwhile, the major organs (heart, liver, spleen, lung and kidney) were also dissected from the mice for further H&E analysis.

2. Supplemental Tables

Table S1. Calculated $\lambda_{max,\ calc.}$ determined by the theoretical HOMO-LUMO gap (eV).

Compounds	λ_{abs}/nm	f	S_1/eV	T_1/eV	T_2/eV	ΔE_{ST}/eV	SOC/cm^{-1}
BPC1	592	1.951	2.09	1.39	2.38	0.70	0.046
Se-PC	623	1.720	1.99	1.35	2.40	0.64	0.55

Table S2. Sequences for RNA and DNA oligonucleotides used in this study (ss: single strand, ds: double strand).

Structure	Names	Sequences(5'to3')
Duplex DNA extracted from calf thymus	calf thymus DNA	NA
All RNA extracted from yeast	yeast RNA	NA
DNA	$ds(A\text{-}T)_{20}$	AAAAAAAAAAAAAATTTTTTTTTTTTTT
	$ds(G\text{-}C)_{20}$	GGGGGGGGGGGGGGCCCCCCCCCCCCCC
	$dsDNA_{mix}$	CGATAAGCGCTTATCGAGTCGACTCGATAAGCG CTTATCG
	$ssDNA_{mix}$	ATGCTCAGACTGTCAGAGTC
	c-MYC	TGAGGGTGGGTAGGGTGGGTAA
	Tel26	AAAGGGTTAGGGTTAGGGTTAGGGAA
	hTelo_G4	TTAGGGTTAGGGTTAGGGTTAGGGTTA
	Mito 0.5-22	GGGGATGGCCATGGCTAGG
	Mito 0.5-22cs	CCTAGCCATGGCCATCCCC
	Mito	ACACTGATGCACTTTGTCTTCC
RNA	NC	UUGUACUACACAAAAGUACUG
	$ssRNA_{mix}$	AUGUACCUAGUCUGACCUAG

Table S3. The summary of IC_{50} values is shown in Figure 3e-h and Figure S10.

IC_{50} (μM)	**Dark**	**Light**
MCF-7 (21% O_2)	16.57	0.33
MCF-7 (2% O_2)	/	0.86
MCF-7 (21% O_2, 5 mM NAC)	19.44	0.59
4T1 (21% O_2)	5.38	0.38
4T1 (2% O_2)	/	0.67
HeLa (21% O_2)	11.19	0.29
HeLa (2% O_2)	/	0.43
L929 (21% O_2)	8.75	0.49
L929 (2% O_2)	/	0.94

3. Supplemental Figures

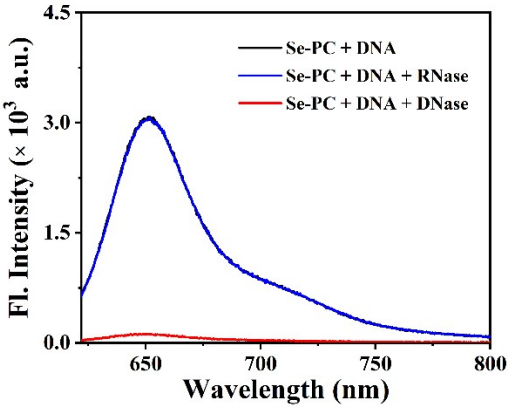


Figure S1. Fluorescence spectra of Se-PC-CT DNA in the presence of DNase I or RNase A in 10 mM PBS buffer (300 mM NaCl, pH 7.4).

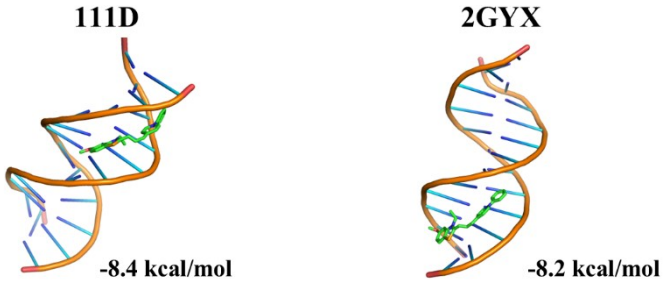


Figure S2. Molecular docking calculation of Se-PC with dsDNA (111D, and 2GYX).

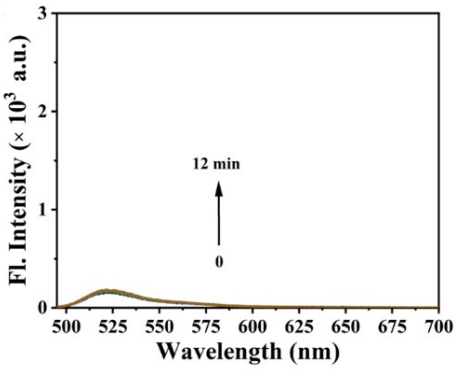


Figure S3. Fluorescence spectra of DCFH (10 μM) after irradiation for different time periods.

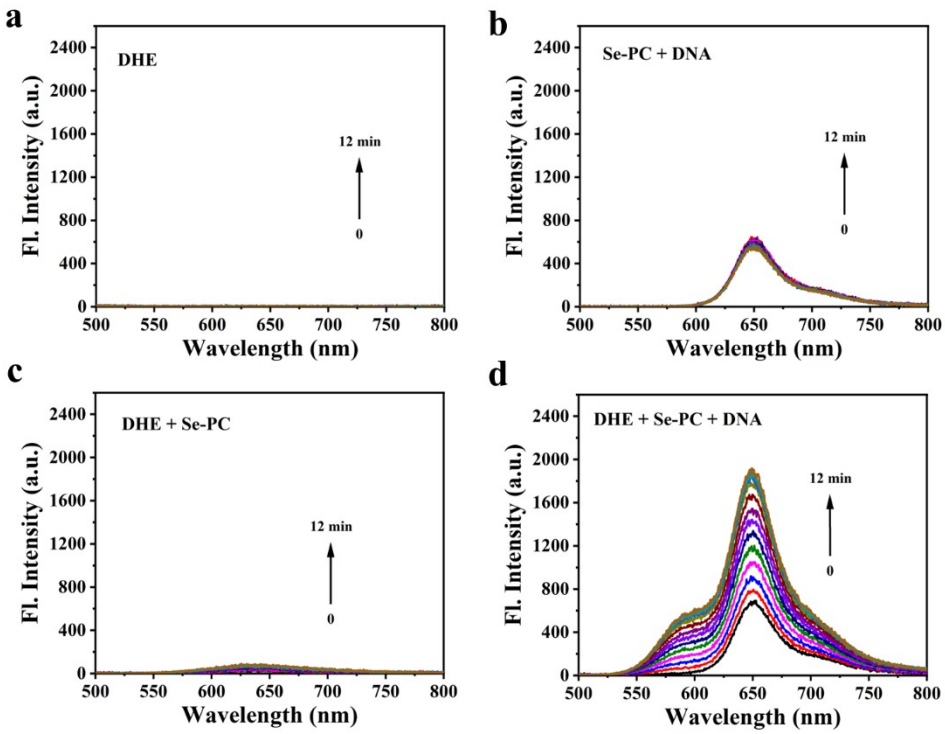


Figure S4. Fluorescence spectra of DHE (10 μM) with different treatments after irradiation for different time periods.

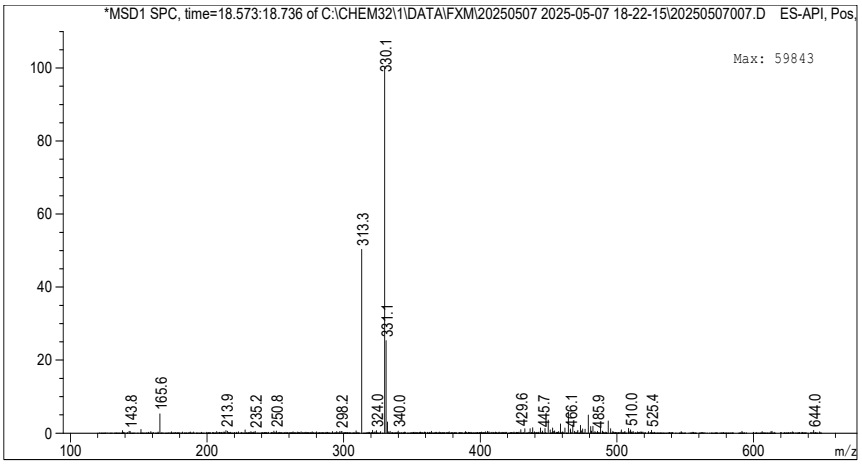


Figure S5. LCMS spectrum of DHE and **Se-PC** mixture after illumination.

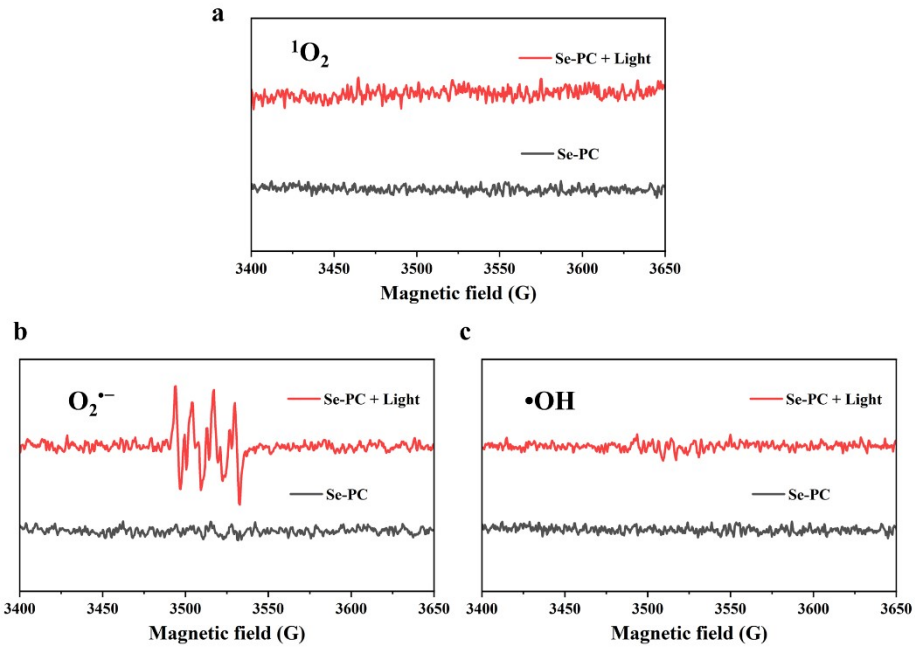


Figure S6. ESR spectra of Se-PC with or without light irradiation for (a) $^1O_2$ using TEMP (water) as a spin trapper, (b) $O_2^{\bullet-}$ using DMPO (methanol) as a spin trapper and •OH using DMPO (water) as a spin trapper.

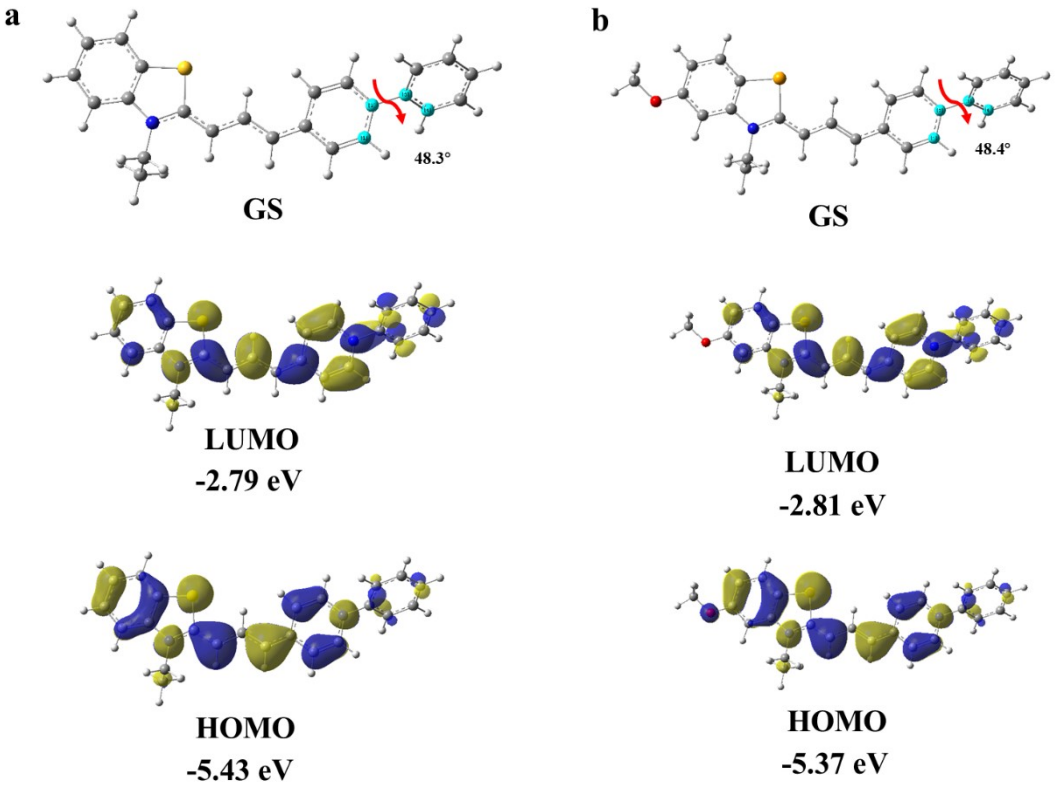


Figure S7. Optimized geometry and HOMO/LUMO of (a) **S-PC** and **Se-PC** in the ground state at the B3LYP/6-31G* level.

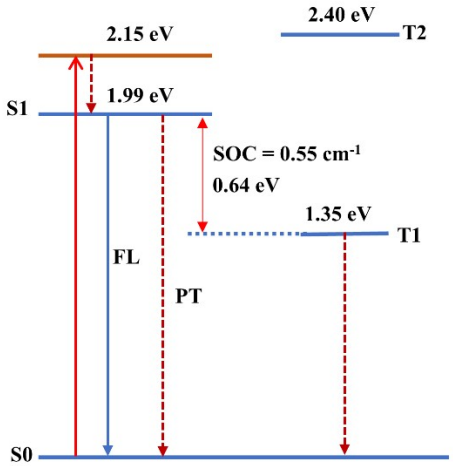


Figure S8. The intersystem crossing formation mechanism of **Se-PC** from TD-DFT calculations.

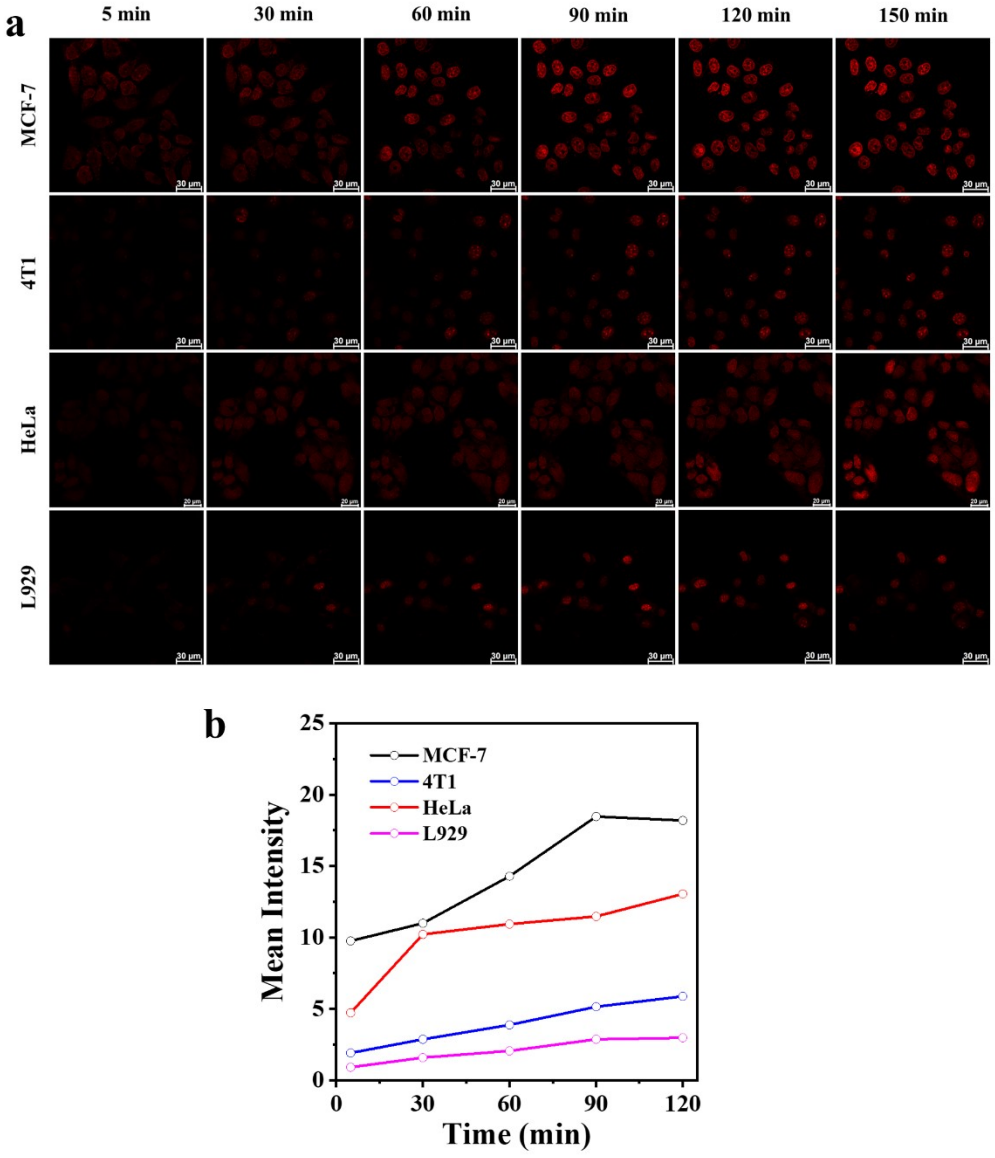


Figure S9. (a) Cellular uptake of **Se-PC** in MCF-7, 4T1, HeLa and L929 cells. (b) Mean fluorescence intensity of **Se-PC** in Figure S4a. Scale bar: 30 μm.

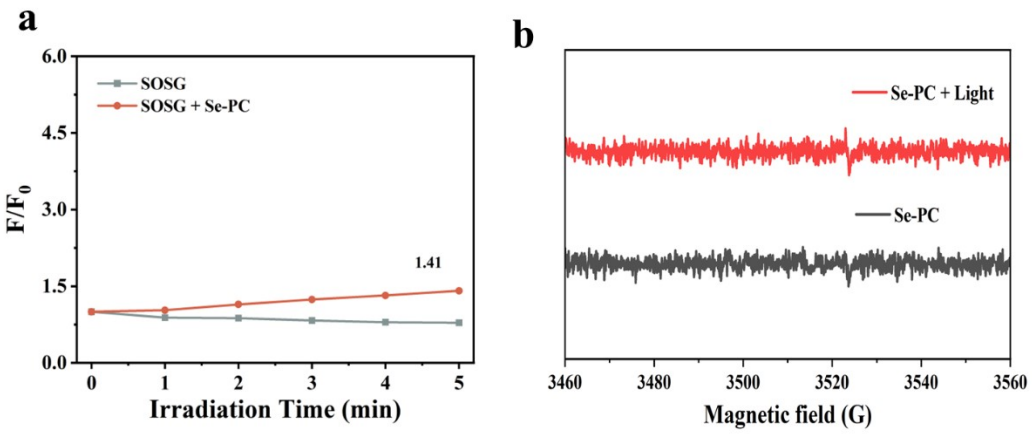


Figure S10. (a) Fluorescence intensities of SOSG (0.5 μM) at 525 nm with irradiation (5 mW/cm^2) or without irradiation for different time periods. (b) ESR spectra of **Se-PC** with or without light irradiation (5 mW/cm^2) for $^1O_2$ using TEMP (water) as a spin trapper.

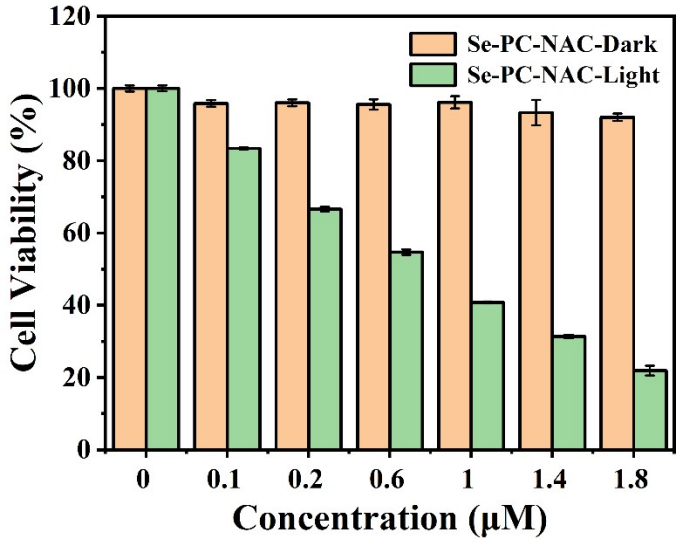


Figure S11. Cell viability of MCF-7 (5 mM NAC pretreatment) with **Se-PC** in the presence or the absence of LED light.

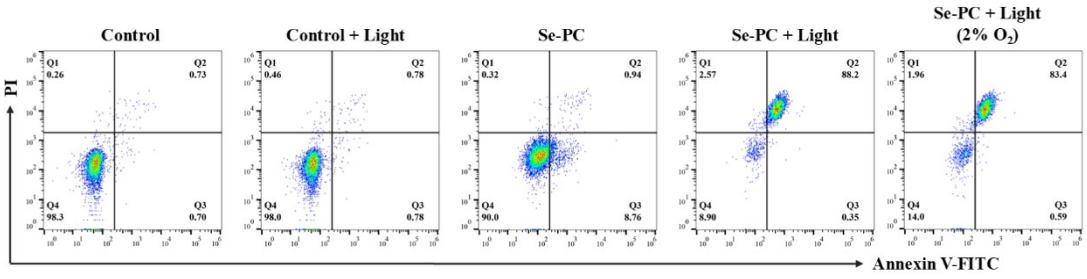


Figure S12. Flow cytometry profiles for MCF-7 cells treated with different treatments and stained with Annexin V-FITC and PI.

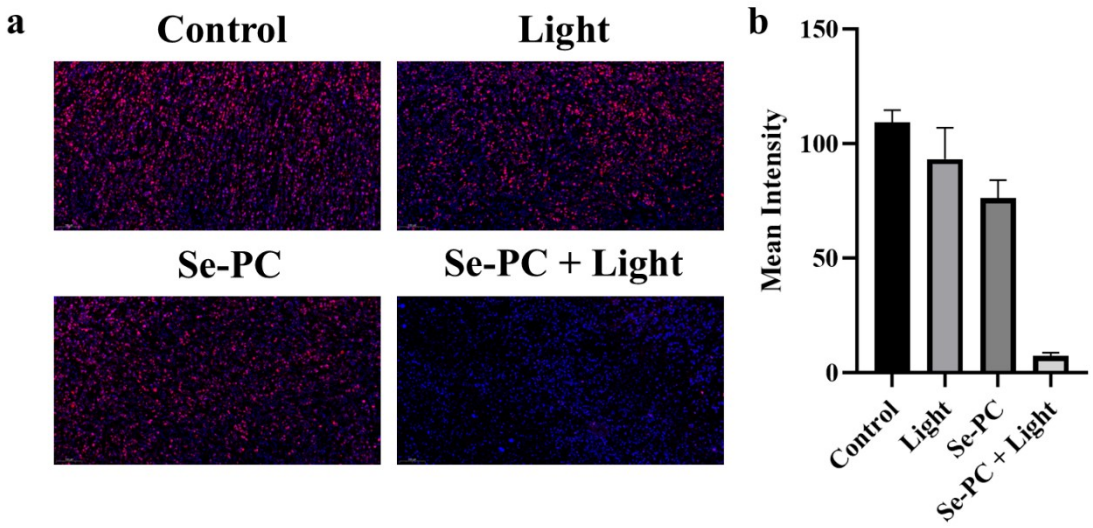


Figure S13. (a) Immunofluorescence staining analysis of Ki67 in tumor tissues after different treatments and (b) the corresponding semi-quantitative analysis.

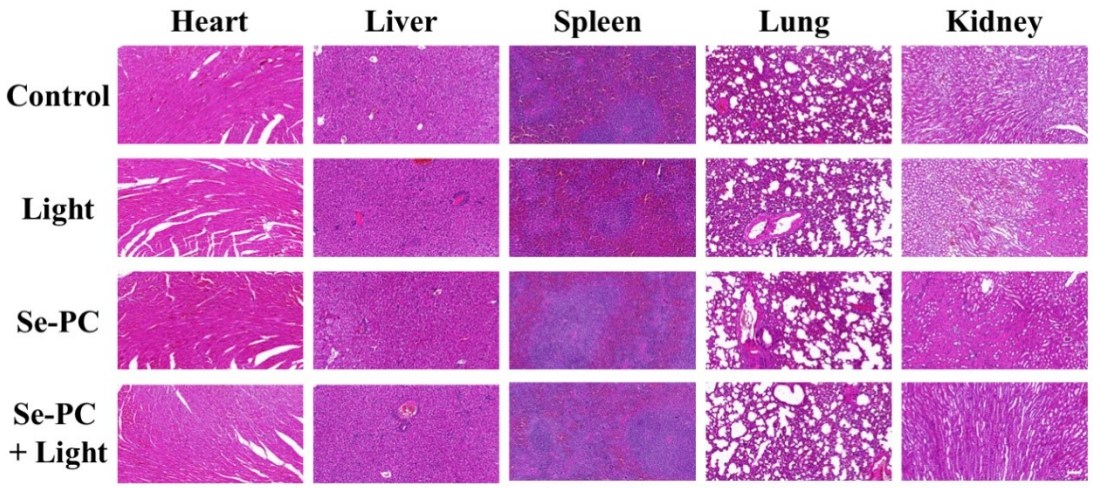


Figure S14. Images of H&E staining of main organ slides after different treatments; scale bars: 100 μm.

4. HR-MS and NMR spectra

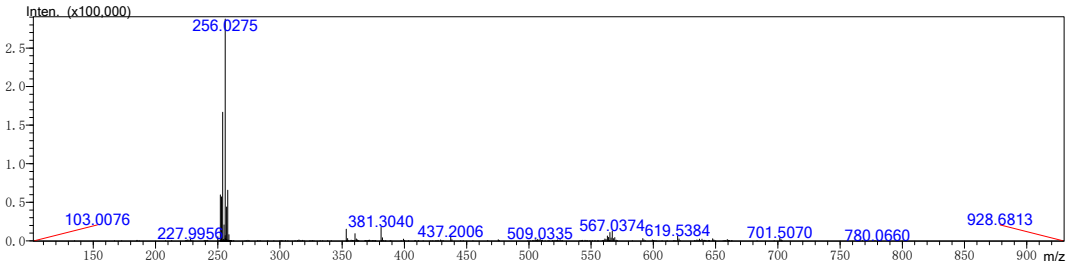


Figure S15. HR-MS spectrum of **compound 1**.

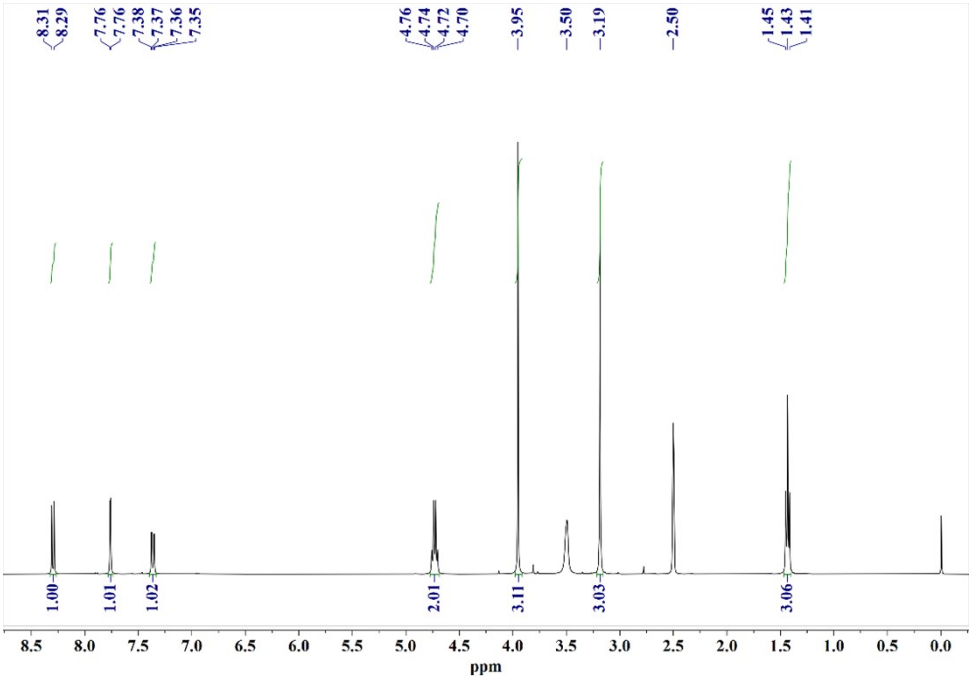


Figure S16. ^{1}H NMR spectrum of **compound 1** in DMSO-d_6.

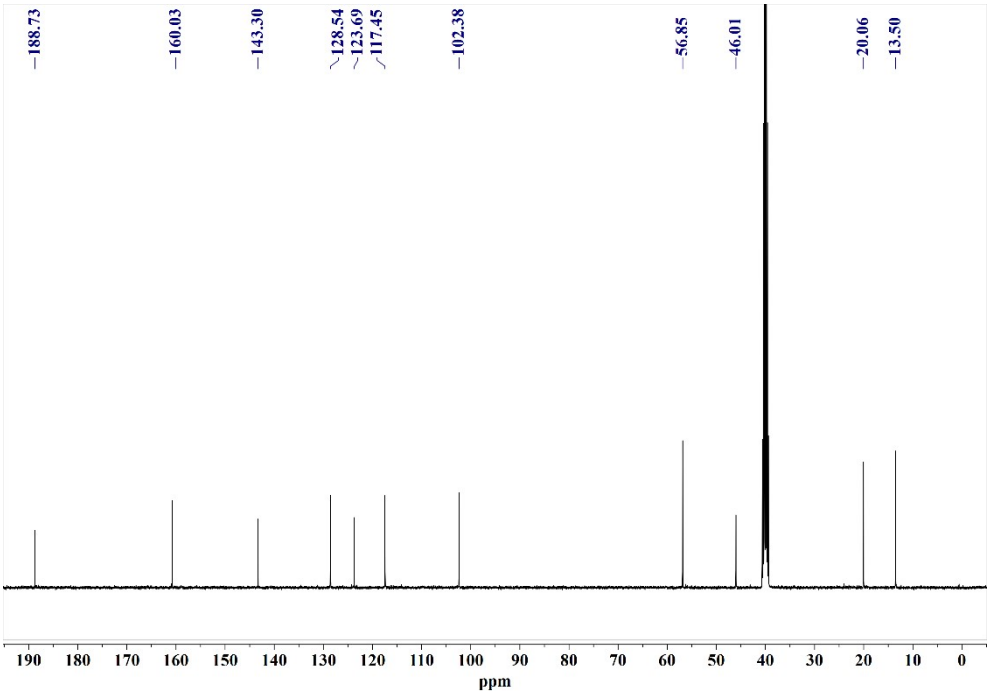


Figure S17. ^{13}C NMR spectrum of **compound 1** in DMSO-d_6.

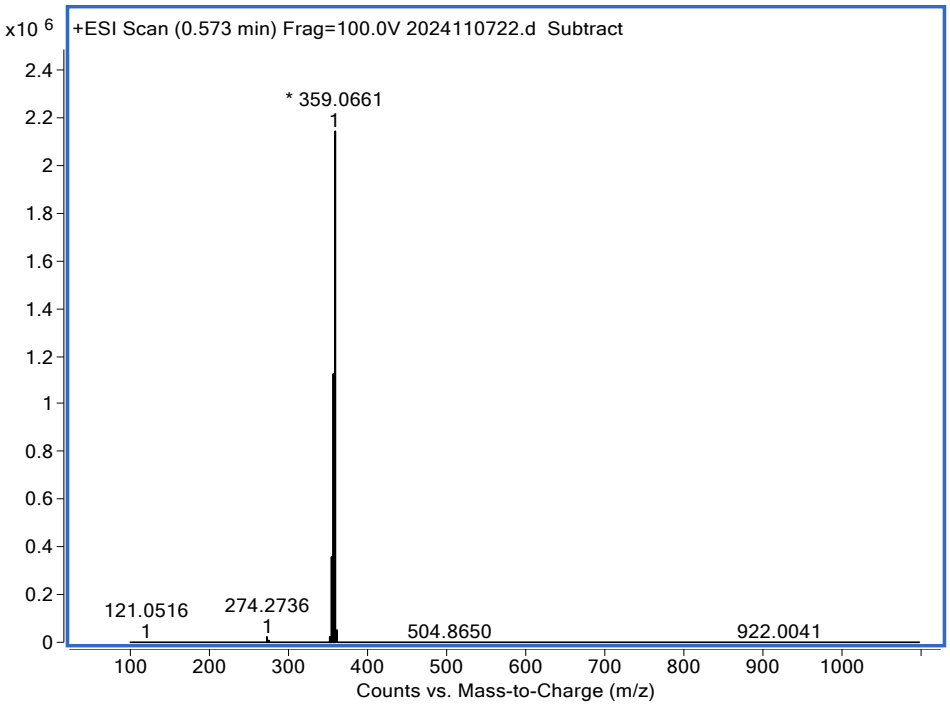


Figure S18. HR-MS spectrum of **compound 2**.

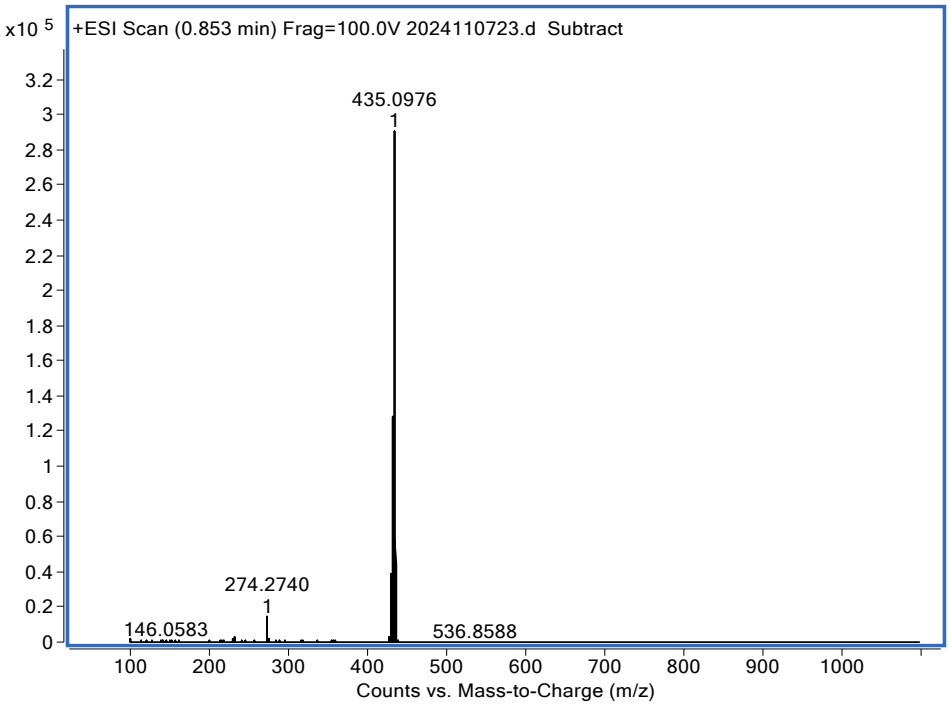


Figure S19. HR-MS spectrum of **Se-PC**.

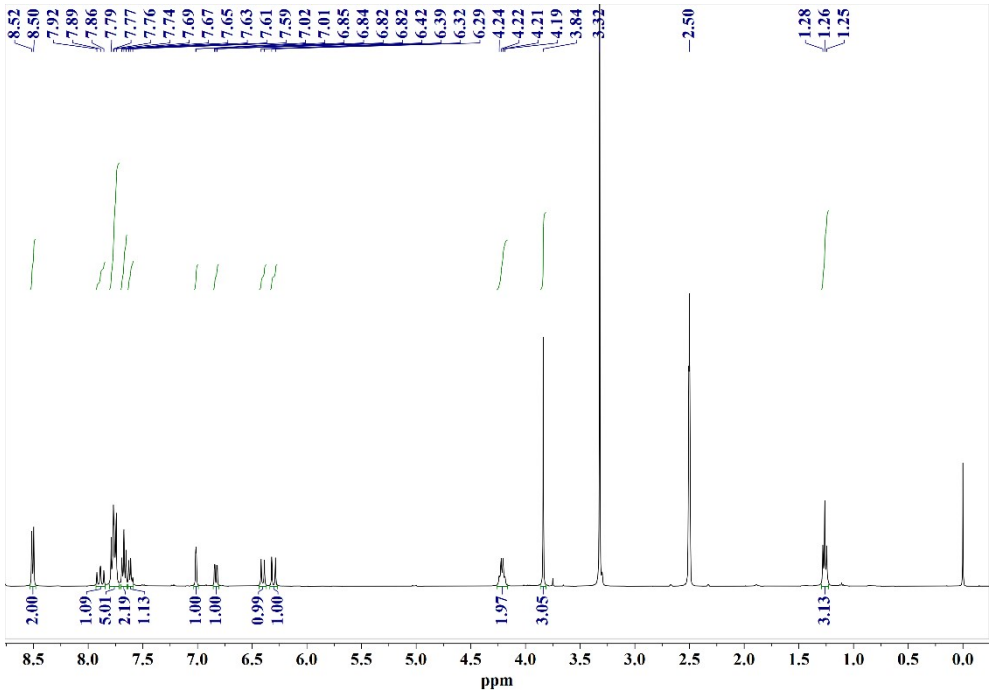


Figure S20. ^{1}H NMR spectrum of **Se-PC** in DMSO-d_6.

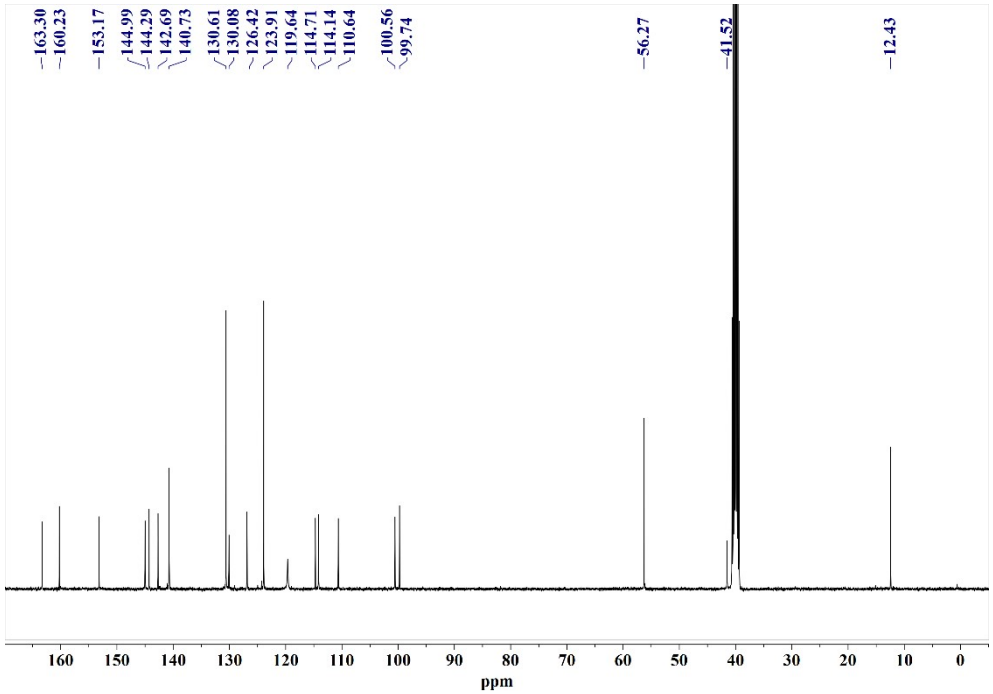


Figure S21. ^{13}C NMR spectrum of **Se-PC** in DMSO-d_6.

5. Supporting reference

[1] K. Uno, N. Sugimoto, Y. Sato, Nat. Commun. **2021**, 12, 2650.