Supplementary Information

Discovery of a Pyridophenoselenazinium-based Photosensitizer for Breast Cancer Treatment

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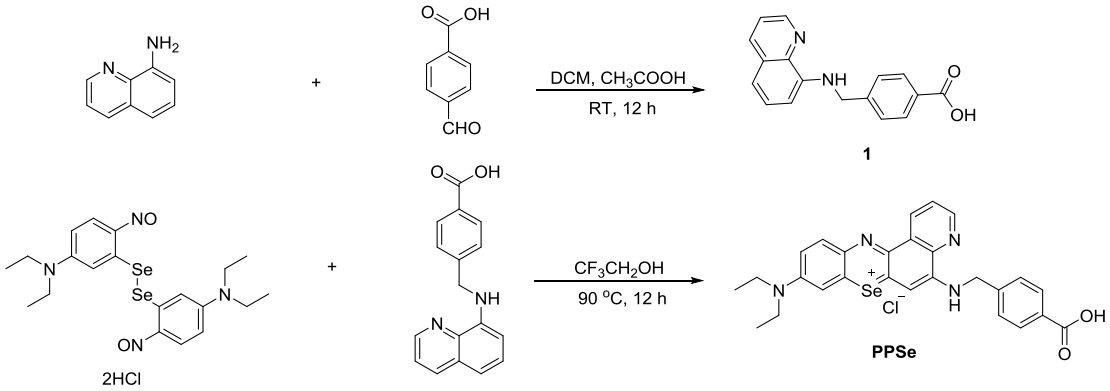
S1. Materials and Instruments

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers without further purification. Thin layer chromatography (TLC) was conducted using silica gel 60 F254, and column chromatography was carried out over silica gel (200-300 mesh). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-400 (400 MHz), chemical shift (δ) was reported as ppm in DMSO-d6 with TMS as the internal standard. The mass spectra were obtained on a Varian QFT-ESI mass spectrometer. The absorption spectra and fluorescent spectra were measured on UV-vis spectrophotometer and fluorescence spectrophotometer (Fluoromax-4, HORIBA).

S2. Synthesis route of PPSe



Scheme S1. Synthesis route of **PPSe.**

（1）Synthesis of 4-((quinolin-8-ylamino)methyl)benzoic acid (1)

4-formylbenzoic acid (300 mg, 2 mmol) and 8-amine-quinolin (346 mg, 2.4 mmol) were placed in a flask containing CH_2Cl_2 (40mL), and the mixture stirred for 15 min at room temperature when two drops of CH_3COOH were added. Then triacetoxyhydroborate sodium (835 mg, 3 mmol) was added and the solution stirred overnight at room temperature. The reaction mixture was poured into H_2O and extracted with ethyl acetate. The combined organic layer was dried with Na_2SO_4 and concentrated under vacuum. After purified by the silica gel chromatography (DCM/MeOH, 20:1), compound 1 was obtained as an orange solid, (430 mg, 77.2 %). ^{1}H-NMR (400 MHz, DMSO-d6) δ 12.84 (s, 1H), 8.78 (dd, J = 4.1, 1.5 Hz, 1H), 8.22

(dd, J = 8.3, 1.5 Hz, 1H), 7.89 (d, J = 8.2 Hz, 2H), 7.58 - 7.46 (m, 3H), 7.34 - 7.23 (m, 2H), 7.06 (d, J = 7.7 Hz, 1H), 6.51 (d, J = 7.5 Hz, 1H), 4.63 (d, J = 6.3 Hz, 2H). ^{13}C-NMR (100 MHz, DMSO-d6) δ 167.70, 147.47, 145.87, 144.59, 138.05, 136.44, 129.86, 129.86, 128.79, 128.10, 127.48, 122.22, 113.99, 105.31, 46.41. LRMS (m/z) calc. for $C_{17}H_{14}N_2O_2[M-H]^-$:278.3, found:277.3.

(2) Synthesis of 5-((4-carboxybenzyl) amino)-9-(diethylamino)pyrido[3,2-a]phenoselenazin-7-ium chloride **(PPSe)**

3,3'-diselanediylbis (N, N-dimethyl-4-nitrosoaniline) hydrochloride salt (262 mg, 0.40 mmol) and 4-((quinolin-8-ylamino) methyl) benzoic acid (140 mg, 0.5 mmol) were mixed in CF_3CH_2OH (5 mL), and the mixture was stirred at 90 ºC overnight. The reaction progress was monitored by TLC. The reaction mixture was poured into saturated NaCl solution after cooling down to room temperature and extracted with ethyl acetate. The combined organic layer was dried with Na_2SO_4 and concentrated under vacuum. After purified by the silica gel chromatography (DCM/MeOH, 10:1), 9-(diethylamino)-5-(ethylamino) pyrido[3,2-*a*] phenoselenazin-7-ium chloride (**PPSe**) was obtained as a blue solid (117 mg, 53%). ^{1}H-NMR (400 MHz, CD_3OD/DMSO-d6) δ 9.23 (d, *J* = 8.5 Hz, 1H), 8.98 (d, *J* = 3.9 Hz, 1H), 8.04 (d, *J* = 8.1 Hz, 2H), 7.84 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.68 (s, 1H), 7.54 (d, *J* = 8.6 Hz, 2H), 7.35 (d, *J* = 9.5 Hz, 1H), 4.97 (s, 2H), 3.69 (dd, *J* = 14.0, 6.9 Hz, 2H), 1.46 - 1.18 (m, 6H); LRMS (m/z) calc. for $C_{27}H_{25}N_4O_2SeCl$ $[M-Cl+H]^+$: 517.5, found: 517.6; UV-vis: 690 nm; HPLC conditions: C_{18} column, MeOH/ H_2O: 80/20 to 0:100, flow rate = 1.0 mL min-1, t_R = 14.97 min.

S3. HPLC Analysis

Sample **PPSe** were analyzed by HPLC using a C18 analytical column (250 mm × 3 mm). The solvents used for HPLC analysis were of HPLC grade. The conditions of HPLC were set as follows: solvent A = methyl alcohol (MeOH), solvent B = deionized water, and elution mode =gradient: from [80% A] and [20% B] to [0% A] and [100% B]. The total elution time was 30 min. The flow rate was fixed at 1.0 mL·min^{-1}.

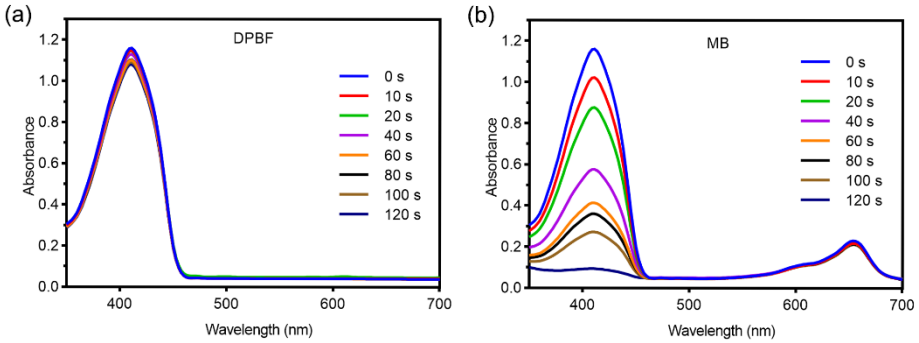


Figure S1. (a) Absorbance changes of the DPBF in EtOH under 660 nm light irradiation (2 mW/cm^2). (b) Absorbance changes of the DPBF used as a probe for the production of $^1O_2$ of Methylene blue (MB) detected in EtOH under 660 nm light irradiation (2 mW/cm^2).

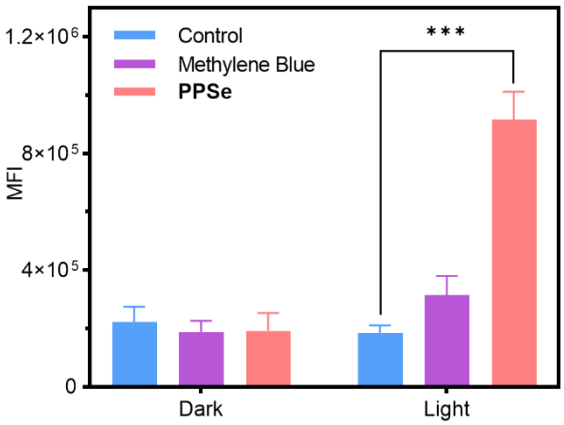


Figure S2. Flow cytometry analysis of ROS generation in 4T1 cells treated with **PPSe** (0.25 μM) and Methylene Blue (0.25 μM) with or without light irradiation (660 nm, 12 J/cm^2).

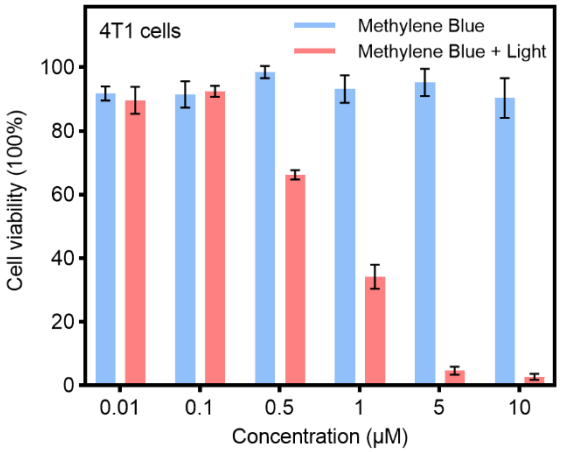


Figure S3. Cell viabilities of 4T1 cells treated with varied concentrations of Methylene Blue with or without light irradiation (660 nm, 12 J/cm^2).

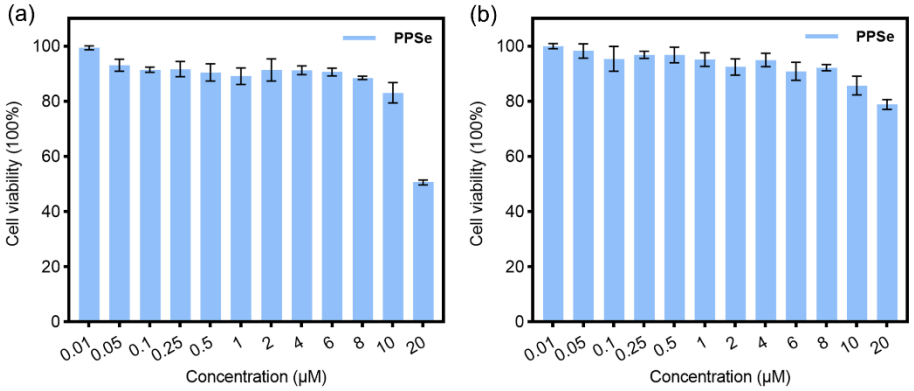


Figure S4. (a-b) Cell viabilities of 4T1 and MDA-MB-231 cells treated with varied concentrations of **PPSe**.

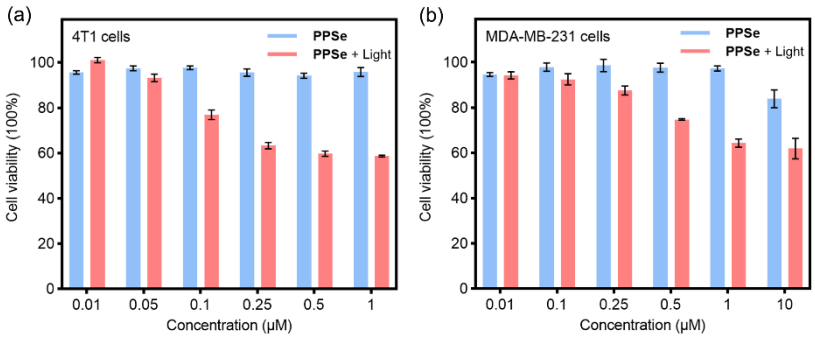


Figure S5. Cell viabilities of 4T1 and MDA-MB-231 cells treated with varied concentrations of **PPSe** under hypoxia (1% O_2).

Table S1. Cytotoxicity (IC_{50}, μM) of **PPSe** against cancerous (4T1, MDA-MB-231, MCF-7, MDA-MB-468 and MDA-MB-453) cell lines for 24 h in the absence and presence of 660 nm (20 mW/cm^2, 10 min) irradiation. [a]

Cells	Dark	Light
4T1	＞10	0.17 ± 0.010
MDA-MB-231	＞10	0.16 ± 0.010
MCF-7	＞10	0.71 ± 0.061
MDA-MB-468	＞10	0.12 ± 0.014
MDA-MB-453	3.6 ± 0.061	0.29 ± 0.012

[a] Date is presented as the means ± standard deviations (SD) of three repeated measurements.

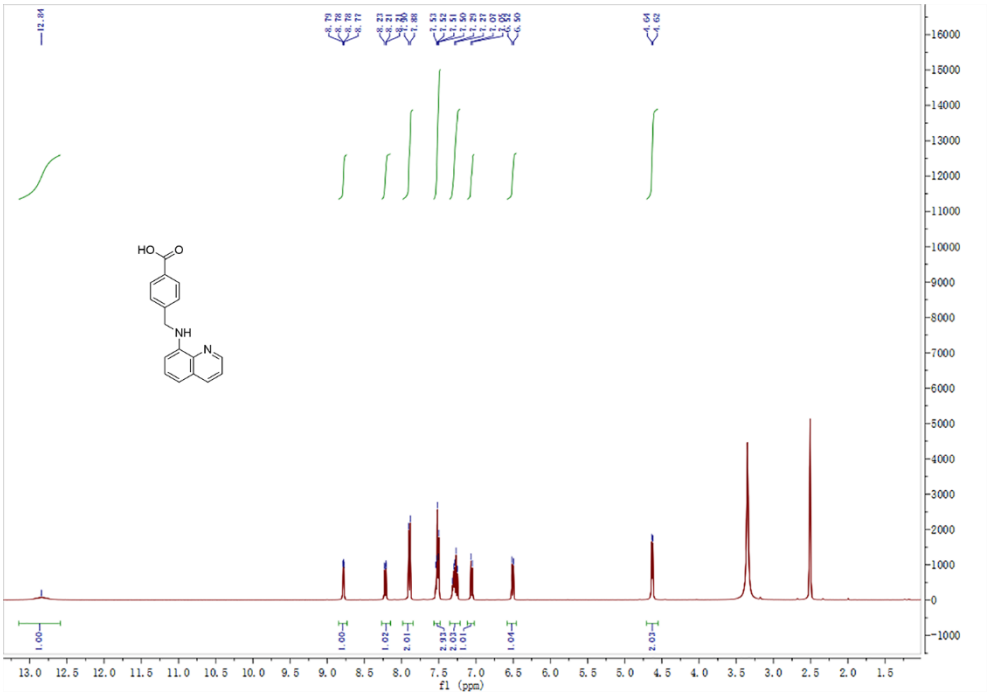


Figure S6. ^{1}H-NMR spectrum (400 MHz) of compound **I** in DMSO-d_6.

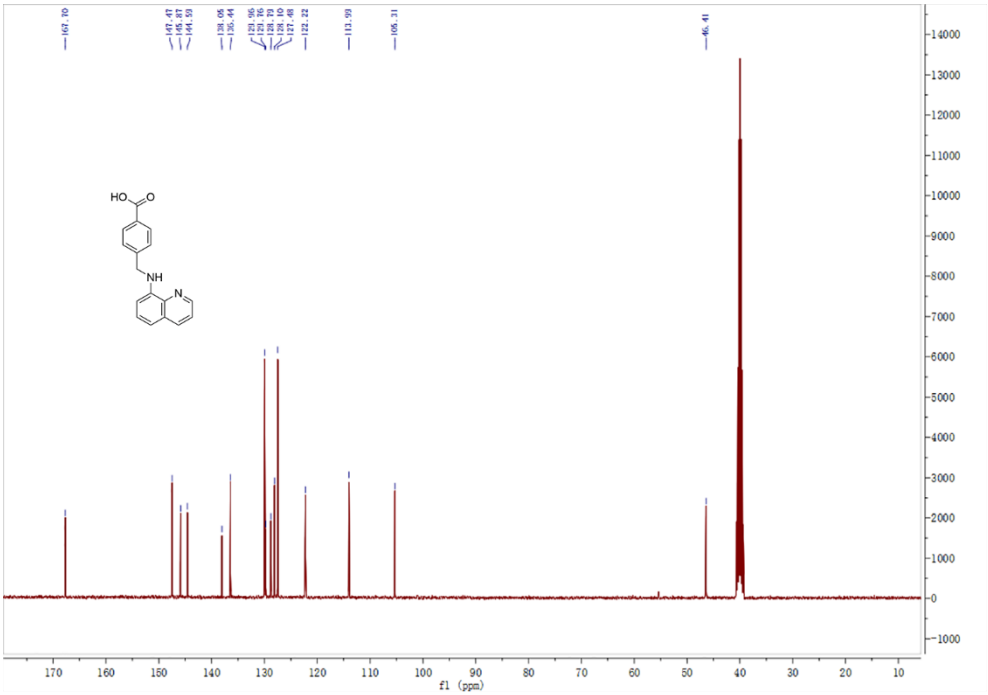


Figure S7. ^{13}C-NMR spectrum (100 MHz) of compound **I** in DMSO-d_6.

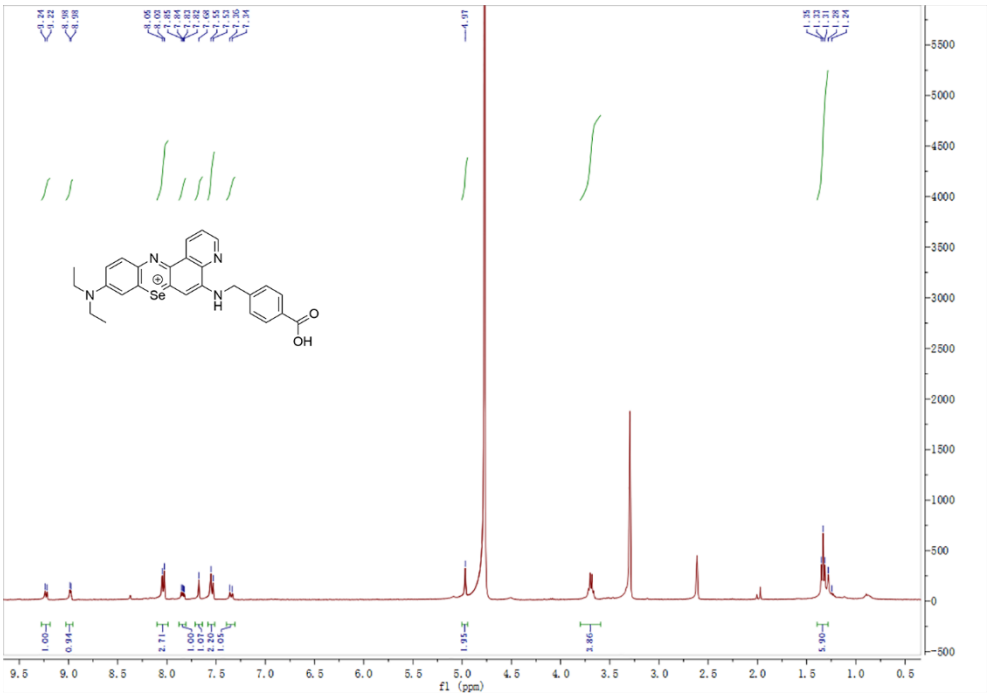
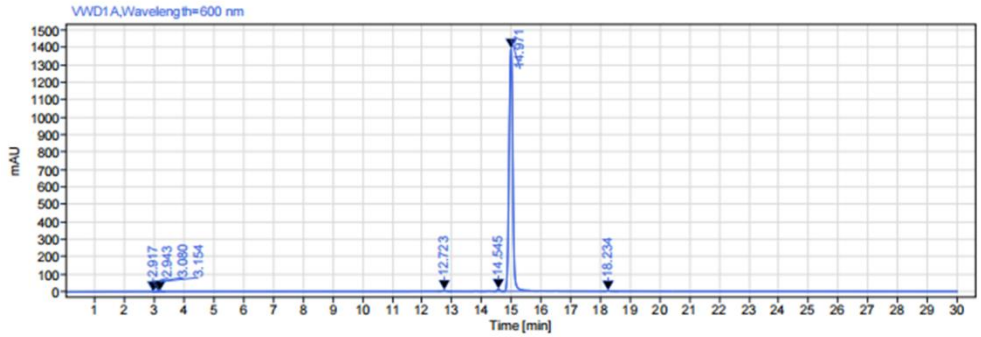


Figure S8. ^{1}H-NMR spectrum (400 MHz) of compound **PPSe** in CD_3OD/DMSO-d6.



Signal: VWD1A,Wavelength=600 nm

RT [min]	Type	Width [min]	Area	Height	Area%	Name
2.917	VV	0.03	3.26	2.21	0.03	
2.943	VB	0.04	4.82	2.87	0.04	
3.080	BV	0.16	37.57	5.56	0.30	
3.154	VV	0.20	30.84	4.64	0.25	
12.723	VB	0.50	48.84	6.57	0.39	
14.545	BB	0.33	81.18	12.78	0.65	
14.971	BV	1.15	12273.43	1391.93	98.10	
18.234	BV	0.51	30.66	2.61	0.25	
		Sum	**12510.60**			

Figure S10. HPLC purity spectra of **PPSe** in MeOH/H_2O, t_R = 14.971 min.