Experimental Section

Ultrasound treatment. All of the sonication for the sample tests were performed on an NSE-UPH-1 sonicator (Suzhou Nasonic Ultrasonic Technology Co., Ltd., China). The transducer frequency of the sonicator was 1.0 MHz, and the power density was set to 0.8 W/cm^2.

SPR studies. The binding between ERα and **TBEP** was measured by surface plasmon resonance (SPR) using a Biacore 1K instrument. Recombinant human ERα was immobilized on a CM5 biosensor chip at 2700 RU using a standard primary amine coupling chemistry at pH 4.5. A reference flow cell was prepared similarly without injecting protein. Multi-cycle kinetics protocol was employed with **TBEP** concentrations. Data were fitted into a 1:1 steady-state binding model using Biacore 1K evaluation software, and the equilibrium constant K_D was derived.

Density function theory calculations. The molecular structures of **TBEP** were constructed using GaussView5.0 software. Geometry optimization was performed at the B3LYP/6-311G(d) level of theory within the Gaussian09 program.

Molecular docking and dynamic simulations. Molecular docking and molecular dynamic simulations were performed in a Molecular Simulation Workstation (Guizhou Qianshuo Technology Co., Ltd.) equipped with Intel Xeon 8369B CPUs and RTX4090 GPUs. Atomic coordinates of ERα were obtained from the RCSB protein data bank (PDB ID: 1err). The binding pose of **TBEP** was searched by Sybyl 8.1 software. The best-fitted pose was selected for molecular dynamic simulations. AMBER19SB force field was applied for the proteins. The compounds were parametrized using the GAFF force field and RESP charge.

ROS detection. For the assessment of $^1O_2$, the specific capture agent ABDA was employed. The compounds (dissolved in DMSO, final concentration = 2 μM) was mixed with ABDA (dissolved in water, final concentration = 50 μM). The mixture was then treated with ultrasound for a period. The UV-Vis absorbance spectra of the mixture were recorded. For the assessment of O_2^{-}, the fluorescence probe dihydrorhodamine 123 (DHR 123) was utilized to monitor the generation of O_2^{-} in the solution. Specifically, **TBEP** (2 μM) was dispersed into a DMSO solution containing DHR 123 (5 μM). The resulting mixture was then treated with ultrasound irradiation for a period. The fluorescence of the samples was immediately measured with an excitation wavelength of 488 nm. The •OH generation was detected using hydroxyphenyl fluorescein (HPF) as indicator, which can emit green fluorescence at 515 nm upon reaction with •OH. In brief, **TBEP** (2 μM) was dispersed in ultrapure water containing HPF (2 μM). After ultrasound irradiation, the fluorescence of the sample was immediately measured with an excitation wavelength of 488 nm.

Evaluation of $^1O_2$ quantum yield. ABDA was used as a $^1O_2$-trapping agent while rose bengal (RB) was used as a reference. of A LED light (400-700 nm, 40 mW cm^{-2}) was used as the light source. The ABDA solution was (50 μM) mixed with **TBEP** (2 μM), and irradiated with the LED light for 5 min. The absorption of ABDA at different times was recorded to obtain the decay rate. The $^1O_2$ quantum yield of the samples (Φ_{sam}) in water was calculated by the formula: $\emptyset_{sample} = \emptyset_{RB} \times (K_{sample} \times A_{RB})/(K_{RB} \times A_{sample})$, where K_{sample} and K_{RB} are the decomposition rate constants of ABDA by the tested sample and

RB, respectively. A_{sam} and A_{RB} represent respectively the light absorbed by the tested sample and RB, determined by integrating the optical absorption bands in the wavelength range of 400-700 nm. Φ_{RB} is the $^1O_2$ quantum yield of RB, with $\Phi_{\mathrm{RB}} = 0.75$ in water.

Cell proliferation assays. The MCF-7 and MCF10A cells were obtained from ATCC. Cells were maintained in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS). The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. MCF-7 and MCF10A cells in the exponential growth phase were seeded in a 96-well plate (10000 cells per well). After incubation overnight, the compounds were added to each well. The plate was incubated for 72 h, and the cell viability was determined by a typical MTT method. For the antiproliferation studies with ultrasound, the cells were treated with sonication at 6 h after the addition of the compounds. The cell viability was measured after incubating for 72 h.

In-vitro ROS detection. MCF-7 cells were incubated with the compounds for 6 h, and exposed to ultrasound. The cells were incubated with DCFH-DA (0.5 μM) for 30 min. Then the fluorescence of cells was analyzed by a BD-C6 flow cytometry or visualized by a fluorescent microscopy.

Annexin V-FITC/PI staining. The MCF-7 cells with different treatment were collected in PBS containing ANNEXIN V-FITC and PI (50 μg/mL). The suspensions were incubated in dark for 20 min. After that, the cells were washed with PBS. The intracellular fluorescence was determined by a BD-C6 plus flowcytometry.

Western blotting assays. MCF-7 cells were incubated with the compound for 6 h, and exposed to ultrasound for up to 5 min. After incubation for 12 h, the whole protein was extracted. Cell lysates were separated by 8% SDS-PAGE gels. After that, the gel was electroblotted onto a polyvinylidene fluoride membrane, and incubated with primary antibody. The membranes were then wash with 0.1% Tween-20 in TBS, and detected by ECL. Quantitative analysis of the bands was performed on ImageJ software.

ELISA-based assay to qualify IL-6 and TNF-α. Human IL-6 ELISA kit and TNF-α ELISA kit were obtained from Wuhan Saipei Biotech Co., Ltd. The cell culture supernatant and blood sample were centrifuged with 1000 g for 20 min. The supernatant was added to the microplate coated with antibodies of succinic acid. After adding the enzyme-linked immunosorbent agent, the protein level was measured by reading the absorbance at 450 nm and a standard curve of pure proteins.

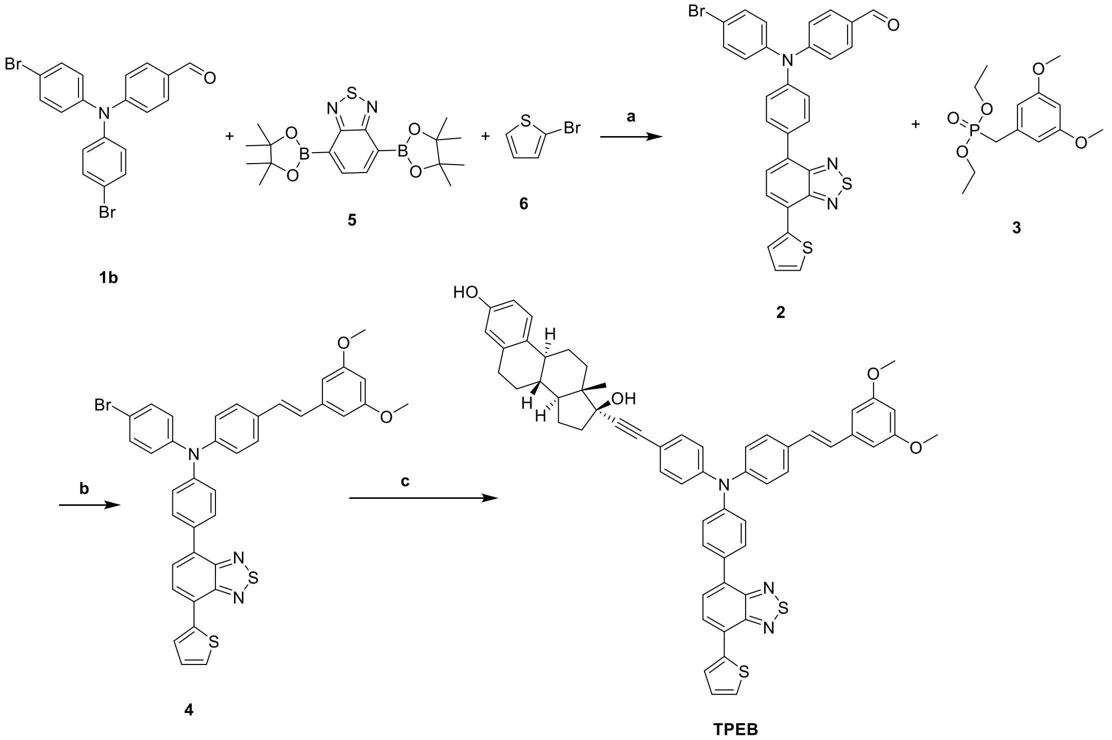
In vivo antitumor studies. Female Balb/c mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. All animal procedures were carried out with the consent of the Principles of Laboratory Animal Care and Use Committee of Guizhou University (No. EAE-GZU-2023-T020). The mice were injected with MCF7 cells on the back. When the tumors grew int ~4 mm in diameter, the mice were divided in to 5 groups (six mice in each group), including I) the vehicle control, II) only ultrasound treatment, III) 5 mpk **TBEP**, IV) 5 mpk Faslodex, V) 5 mpk **TBEP** and ultrasound treatment. The compounds were dissolved in saline and intravenously injected to the mice. For group II and group V, US irradiation of the tumor site was performed with an NSE-UPH-1 sonicator (US condition was kept at 1.0 MHz, duty cycle 50%, 2 min) at 6 h after the injection. The length and width of the tumors were recorded every two days. At the end of study, the tumor and major organs were dissected.

H&E staining. Tissue sections were immersed in hematoxylin solution for 5 minutes to stain nuclei blue, then were rinsed under running tap water for 2 minutes. After that, the cytoplasm and extracellular matrix were stained by immersing the slides in eosin Y solution for 2 minutes, followed by dehydration through ascending ethanol concentrations (70%, 95%, 100% for 1 minute each). Finally, the sections were mounted the with a coverslip and examined under a microscope.

Statistical Analysis. Data were shown as mean ± standard deviation. Significance of two groups was calculated by the Student's t-test in Origin 8 software. *$p < 0.05$, **$p < 0.01$.

1. Synthetic procedures and characterization data

Scheme 1. Synthesis of compound **TPEB**. Reagents and conditions: a) $Pd(PPh_3)_4$, Na_2CO_3, THF, 90 °C, 16h; b) t-BuOK, THF, 0 °C to room temperature, 2h; c) Ethinyl Estradiol, $PdCl_2(PPh_3)_2$, CuI, THF, Et_3N, 50 °C, 12h.



The synthesis of 4-((4-bromophenyl)(4-(7-(thiophen-2-yl)benzo[c][1,2,5]thiadiazol-4-yl)phenyl)amino)benzaldehyde (2)

4-(Bis(4-bromophenyl)amino)benzaldehyde (400 mg, 0.92 mmol), compound **7** (360 mg, 0.92 mmol), $Pd(PPh_3)_4$ (70 mg, 0.06 mmol), Na_2CO_3 (492 mg, 4.64 mmol) were mixed in a round flask under argon atmosphere, and anhydrous THF (30 ml) was added. The mixture was stirred and refluxed under an argon atmosphere at 90 °C for 30 minutes, then 2-Bromothiophene (181 mg, 1.11 mmol) was added. The mixture was stirred and refluxed for 16 hours. The reaction solution was cooled to room temperature and concentrated, then ethyl acetate was added to the concentrated reaction mixture, which was then washed with water. The organic phase was concentrated under vacuum and purified by flash column chromatography (PE/EA 7:1) to give compound **2** as a yellow solid. The yield was 63%. ^1H NMR (600 MHz, Chloroform-*d*) δ 9.86 (s, 1H), 8.15 (d, *J* = 3.9 Hz, 1H), 7.96 (t, *J* = 7.7 Hz, 3H), 7.74 (dd, *J* = 8.1, 2.9 Hz, 3H), 7.50 – 7.45 (m, 3H), 7.29 (d, *J* = 8.5 Hz, 2H), 7.24 – 7.22 (m, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H).

The synthesis of (E)-4-bromo-N-(4-(3,5-dimethoxystyryl)phenyl)-N-(4-(7-(thiophen-2-yl)benzo[c][1,2,5]thiadiazol-4-yl)phenyl)aniline (4)

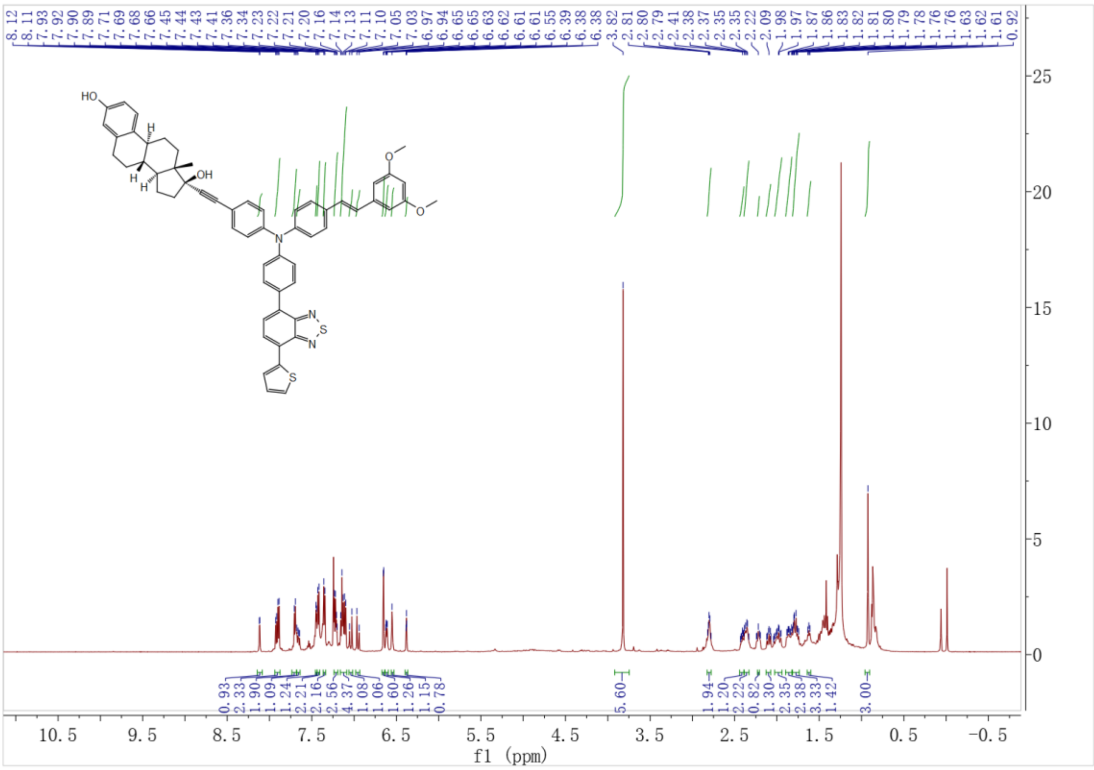
A solution of t-BuOK (0.96 g, 8.5 mmol) in THF (5 mL) was slowly added to a mixture of compound **2** (1.0 g, 2.8 mmol), diethyl phenylphosphonate (0.98 g, 3.4 mmol) and THF (30 mL) for 30 min at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The mixture was quenched into water (50 mL) and then extracted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4. The solvent was concentrated under vacuum and purified by flash column chromatography (PE/EA 10:1) to afford compound **4a** as a yellow solid, yield 86%. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.95 (s, 1H), 7.77 (d, *J* = 7.1 Hz, 1H), 7.46 (d, *J* = 18.9 Hz, 5H), 7.30 (s, 6H), 7.19 (d, *J* = 7.7 Hz, 2H), 7.13 (d, *J* = 8.5 Hz, 2H), 7.09 (s, 2H), 6.69 (d, *J* = 26.3 Hz, 2H), 6.44 (s, 1H), 3.88 (s, 6H).

The synthesis of (8R,9S,13S,14S,17S)-17-((4-((4-((E)-3,5-dimethoxystyryl)phenyl)(4-(7-(thiophen-2-yl)benzo[c][1,2,5]thiadiazol-4-yl)phenyl)amino)phenyl)ethynyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (TBEP)

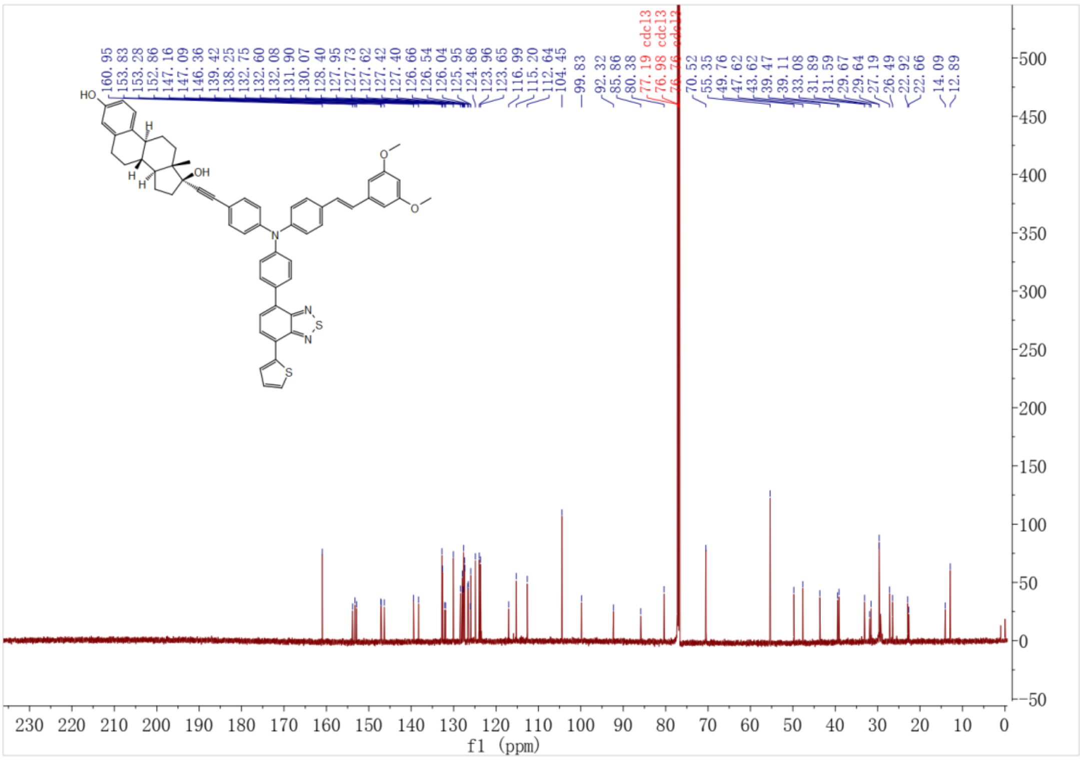
Compound **4** (200 mg, 0.41 mmol), ethinyl estradiol (146 mg, 0.49 mmol), $PdCl_2(PPh_3)_2$ (14.4 mg, 0.02 mmol), CuI (23.5 mg, 0.12 mmol) were mixed in a round flask under argon atmosphere, and anhydrous THF was added with anhydrous Et_3N (1:1). The mixture was stirred under an argon atmosphere at 50 °C for 12 h. The reaction solution was concentrated and ethyl acetate was added to the concentrated reaction mixture, which was then washed with water. The organic phase was concentrated under vacuum and purified by flash column chromatography (PE/EA 3:1)to give compound **TBEP** as a light yellow solid. The synthesis of compound **TBEP** was the same as that of compound **TBEP**. Red solid, yield 45%. ^{1}H NMR (600 MHz, Chloroform-*d*) δ 8.12 (d, *J* = 3.6 Hz, 1H), 7.91 (dd, *J* = 16.6, 7.9 Hz, 2H), 7.70 (d, *J* = 7.4 Hz, 2H), 7.68 – 7.64 (m, 1H), 7.45 (d, *J* = 5.2 Hz, 1H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.24 – 7.19 (m, 3H), 7.13 (dt, *J* = 18.3, 8.7 Hz, 4H), 7.04 (d, *J* = 16.2 Hz, 1H), 6.95 (d, *J* = 16.2 Hz, 1H), 6.65 (d, *J* = 2.1 Hz, 2H), 6.62 (dd, *J* = 8.5, 2.9 Hz, 1H), 6.55 (s, 1H), 6.38 (d, *J* = 2.2 Hz, 1H), 3.82 (s, 6H), 2.80 (p, *J* = 8.3, 6.9 Hz, 2H), 2.41 (td, *J* = 8.8, 4.5 Hz, 1H), 2.36 (dt, *J* = 14.2, 8.8 Hz, 2H), 2.22 (s, 1H), 2.13 – 2.07 (m, 1H), 1.98 (ddd, *J* = 17.7, 13.5, 6.4 Hz, 2H), 1.90 – 1.82 (m, 2H), 1.82 – 1.73 (m, 3H), 1.62 (t, *J* = 7.6 Hz, 1H), 0.92 (s, 3H). ^{13}C NMR (151 MHz, cdcl$_3$) δ 160.95, 153.83, 153.28, 152.86, 147.16, 147.09, 146.36, 139.42, 138.25, 132.75, 132.60, 132.08, 131.90, 130.07, 128.40, 127.95, 127.73, 127.62, 127.42, 127.40, 126.66, 126.54, 126.04, 125.95, 124.86, 123.96, 123.65, 116.99, 115.20, 112.64, 104.45, 99.83, 92.32, 85.86, 80.38, 77.19, 76.98, 76.76, 70.52, 55.35, 49.76, 47.62, 43.62, 39.47, 39.11, 33.08, 31.89, 31.59, 29.67, 29.64, 27.19, 26.49, 22.92, 22.66, 14.09, 12.89. ESI-HRMS m/z calcd for $C_{58}H_{51}N_3O_4S_2Na^+$ 940.3219, found 940.3216 [M + Na]$^+$. HPLC purity > 95%.

2. ^{1}H NMR and ^{13}C NMR Spectra of final compound

^{1}H NMR of compound **TBEP**



^{13}C NMR of compound **TBEP**

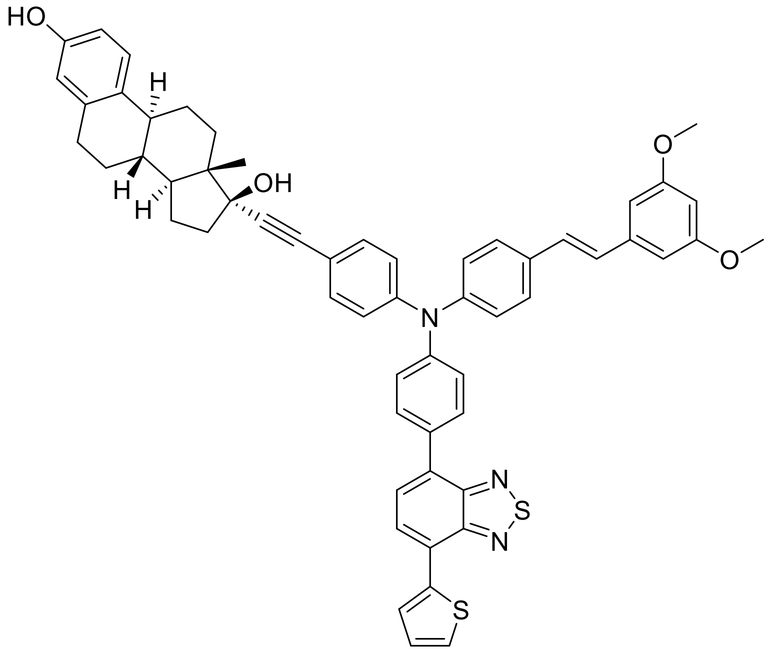


3. HPLC result and spectra

Table S1. HPLC results of final compounds

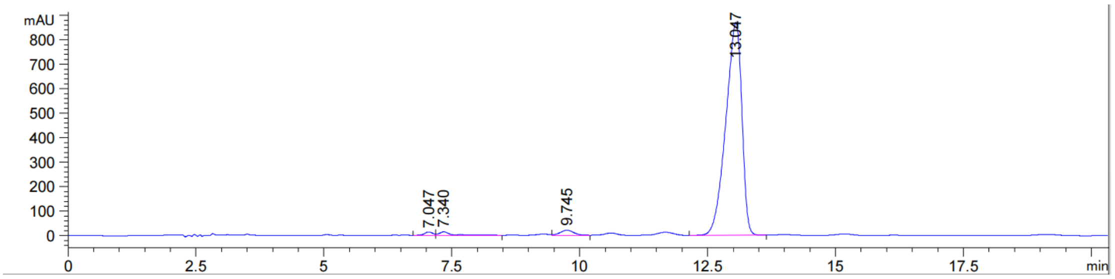
Cmpd	Reverse Phase	Ret. Time	Purity(%)
TBEP	100: 0 MeOH:H_2O	13.047	96.1893

All the HPLC were conducted on Agilent 1100 Series and all the results were obtained under the condition of UV 254nm.

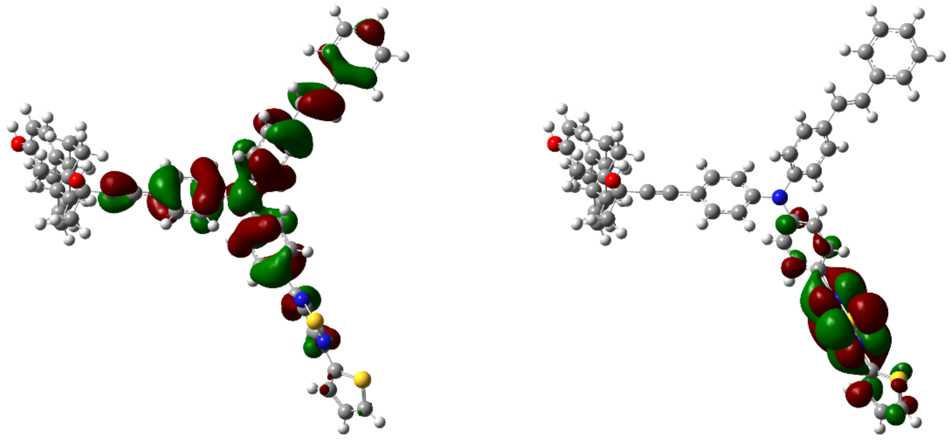


(8R,9S,13S,14S,17S)-17-((4-((4-((E)-3,5-dimethoxystyryl)phenyl)(4-(7-(thiophen-2-yl)benzo[c][1,2,5]thiadiazol-4-yl)phenyl)amino)phenyl)ethynyl)-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (**TBEP**)

Reverse Phase (Method 100:0 MeOH:H_2O, Flow rate 1.0 mL/min)



No.	Retention Time	Area	Peak height	Percent
1	7.047	153.23752	13.60137	0.7759
2	7.340	222.04515	14.18531	1.1243
3	9.745	377.32419	20.52789	1.9105
4	13.047	18997.3	869.74567	96.1893
Total				100.0000



HOMO (-0.70 eV)

LUMO (1.02 eV)

Figure S1. Frontier orbital distributions of TBEP calculated by DFT-B3LYP/6-311G(d) methods.

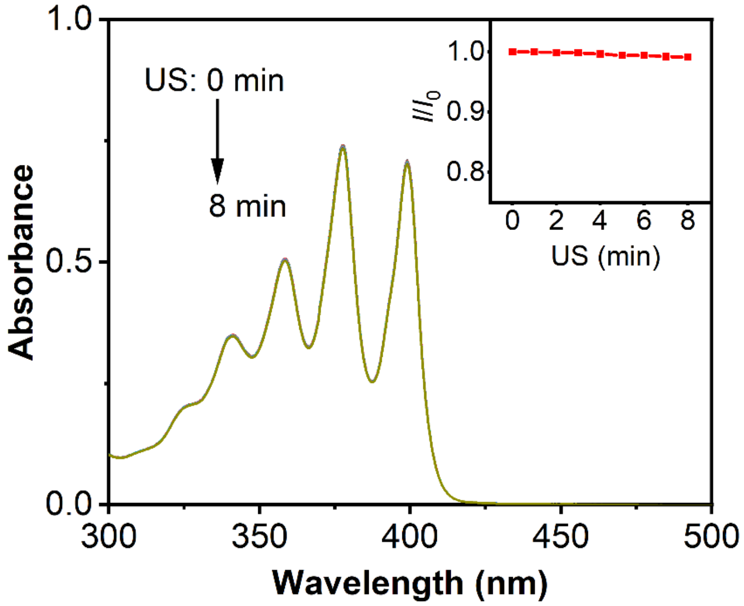


Figure S2. Time-dependent decrease in ABDA (50 μM) absorbance under ultrasound irradiation (1.0 MHz, 0.8 W/cm²) without the presence of **TBEP**.

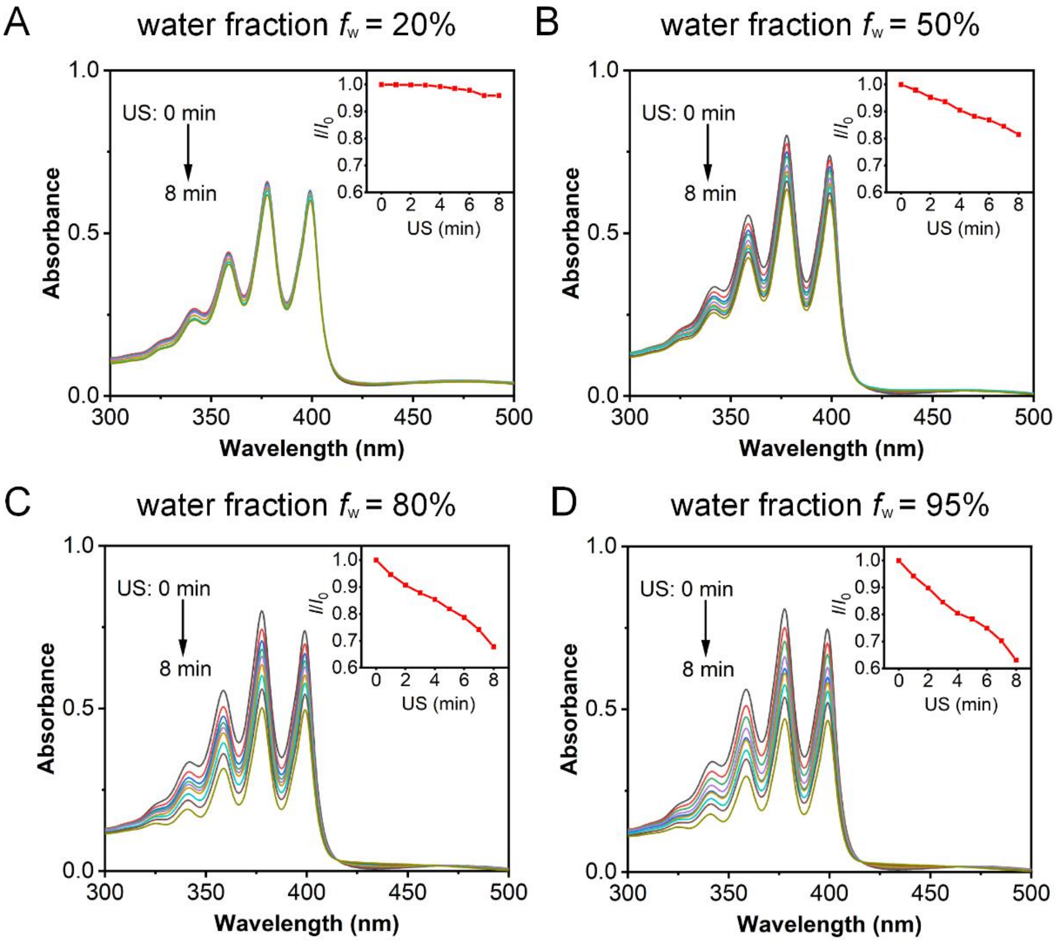


Figure S3. Time-dependent decrease in ABDA (50 μM) absorbance in water/DMSO mixtures under ultrasound irradiation (1.0 MHz, 0.8 W/cm²) in the presence of **TBEP** (2 μM). (A) Water fraction = 20%. (B) Water fraction = 50%. (C) Water fraction = 80%. (D) Water fraction = 95%.

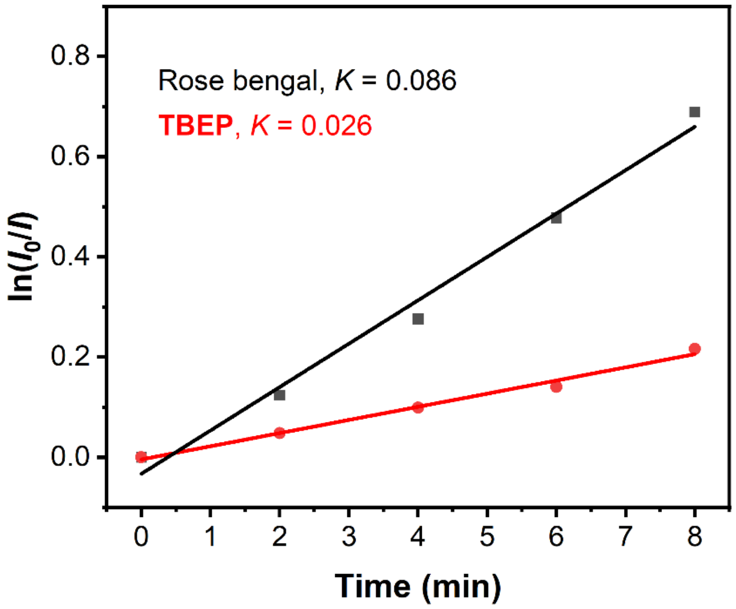


Figure S4. Decomposition rate constants of ABDA by rose Bengal and **TBEP** under white light irradiation (400-700 nm, 25 mW cm^{-2}). Solvent: water/DMSO mixture (f_{water} = 95%).

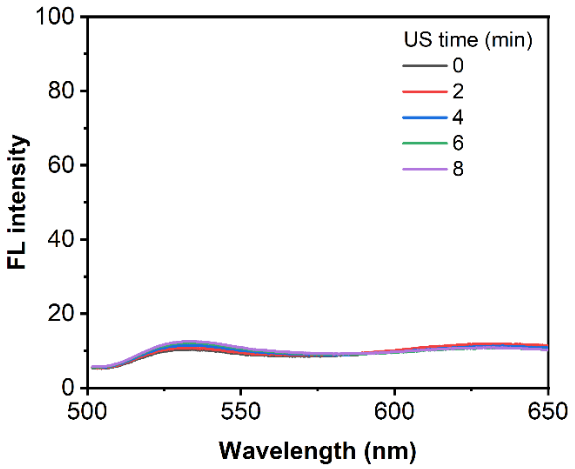


Figure S5. Generation of •OH by **TBEP** (2 μM) upon ultrasound detected fluorescence probe HPF (2 μM). Solvent: water/DMSO mixture (f_{water} = 50%).

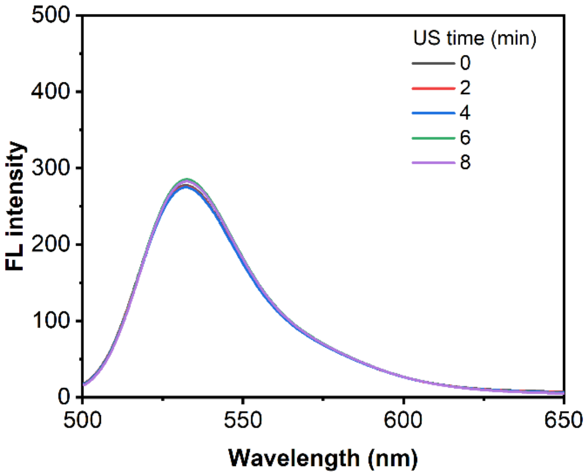


Figure S6. Generation of O_2^{-} by **TBEP** (2 μM) upon ultrasound detected fluorescence probe DHR123 (5 μM). Solvent: water/DMSO mixture (f_{water} = 50%).

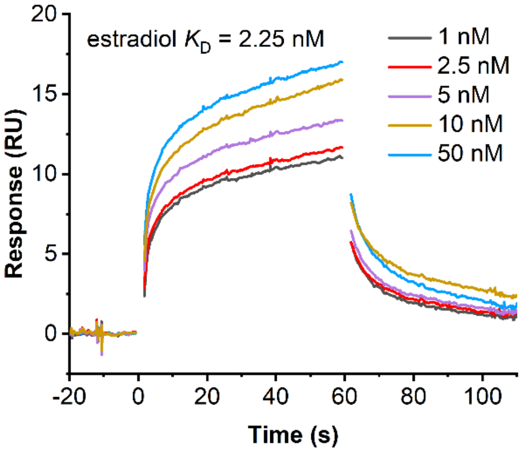


Figure S7. SPR sensorgram showing real-time binding kinetics between **estradiol** and immobilized ERα.

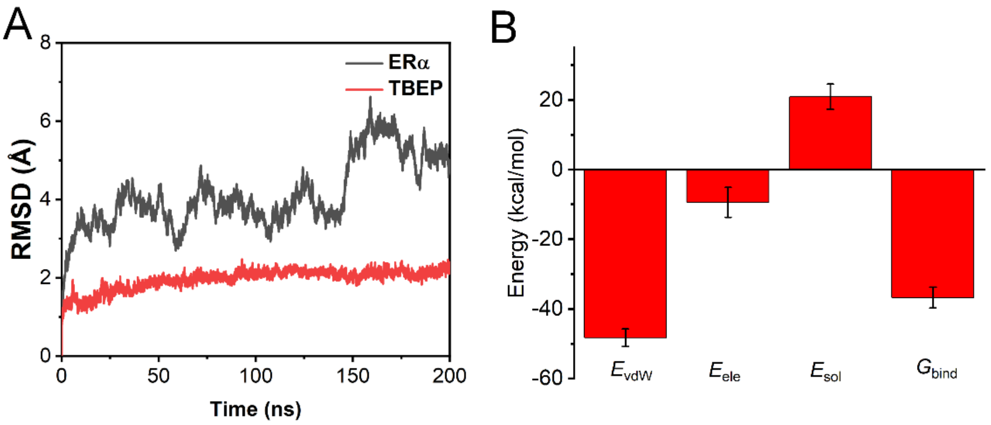


Figure S8. (A) Root mean square deviation of ERα and TBEP in 200 ns molecular dynamic simulations. (B) Van der Waals interactions (E_{vdW}), electric interactions (E_{ele}), solvation energy (E_{sol}), and binding free energies (G_{bind}) for the binding of ERα and TBEP calculated by MM-GBSA method.

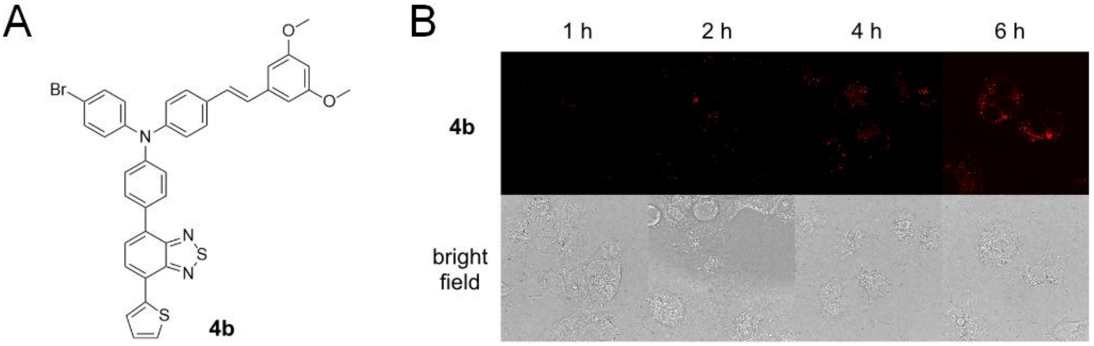


Figure S9. (A) Chemical structure of compound **4b**. (B) Confocal fluorescence images of MCF7 and MCF10A cells incubated with **4b** (5 µM) for up to 6 h.

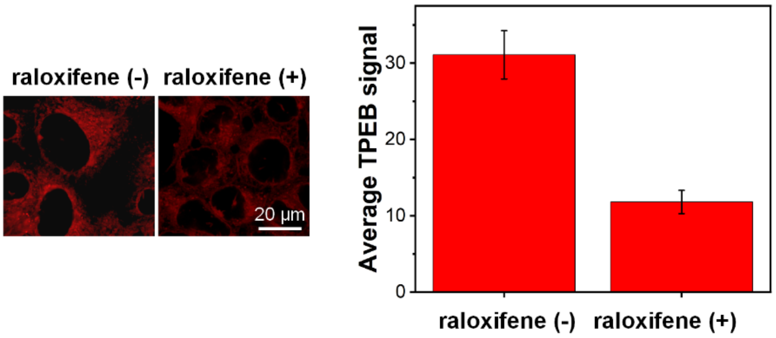


Figure S10. Fluorescent images of **TBEP** (5 µM) in MCF-7 cells with/without the presence of a ERα inhibitor, raloxifene (20 µM). MCF-7 cells were pretreated with a

ERα inhibitor, raloxifene (20 µM) for 2 h, and there treated with **TBEP** (5 µM) for 6 h.

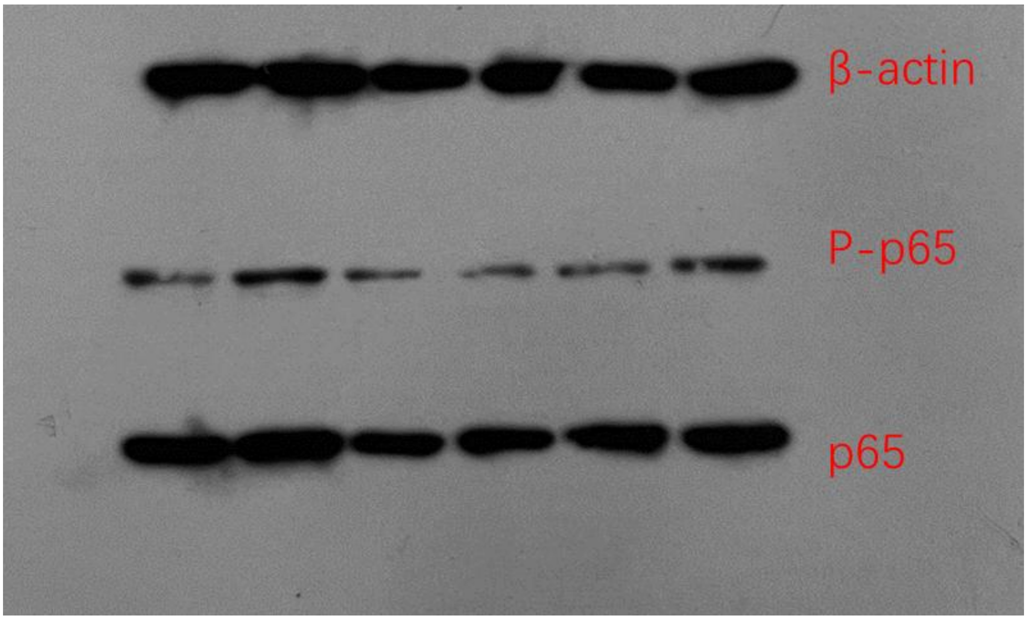


Figure S11. Uncropped raw figure for Figure 3C.

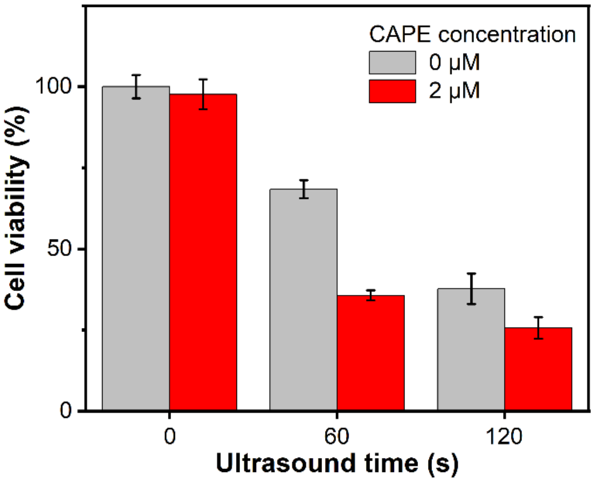


Figure S12. Synergetic effects of NF-κB inhibitor caffeic acid phenethyl ester (CAPE) and sonosensitizer PPIX (10 µM) against MCF7 cells.

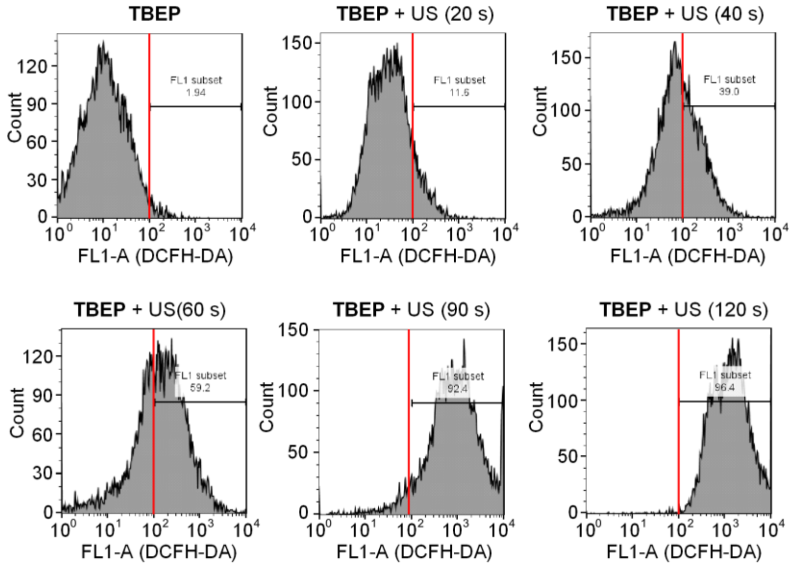


Figure S12. Intracellular ROS detection in MCF7 cells treated with **TBEP** (5 μM) and different period of ultrasound (0-120 s).

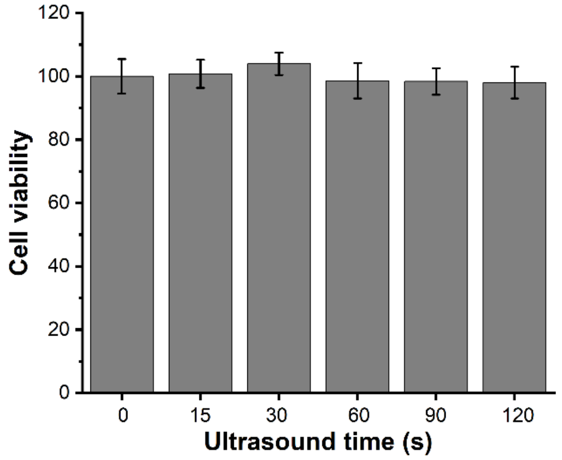


Figure S13. Viability of MCF7 cells treated with different period of ultrasound and then incubated for 72 h.

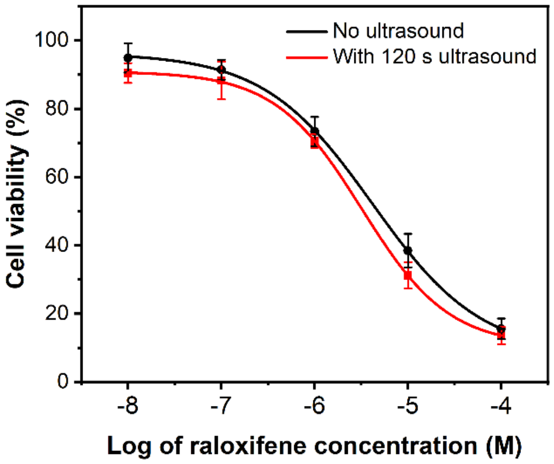


Figure S14. MCF7 cells were treated with raloxifene in the presence of ultrasound (120 s) or without the presence of ultrasound. After incubating for 72 h, the cell viability was determined by MTT method.

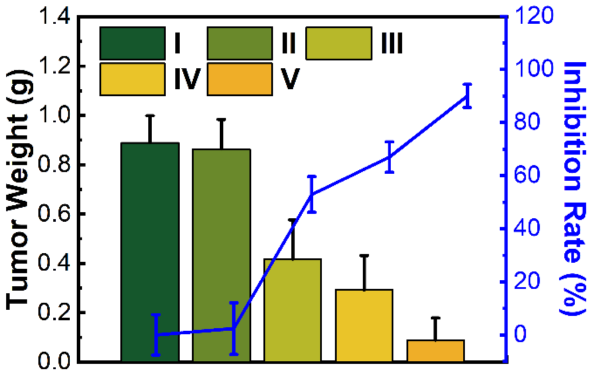


Figure S15. Weight of the dissected tumors for mice at the 21th day in each group.

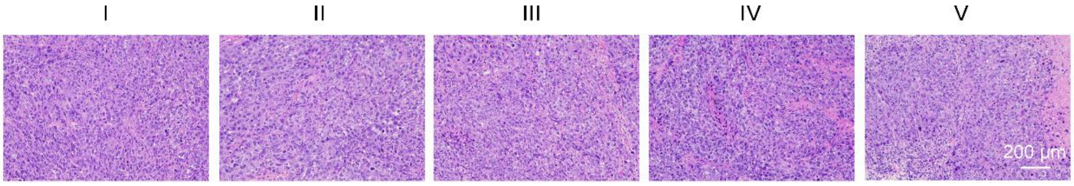


Figure S16. Hematoxylin and eosin staining results of dissected tumors at the 21th in each group.

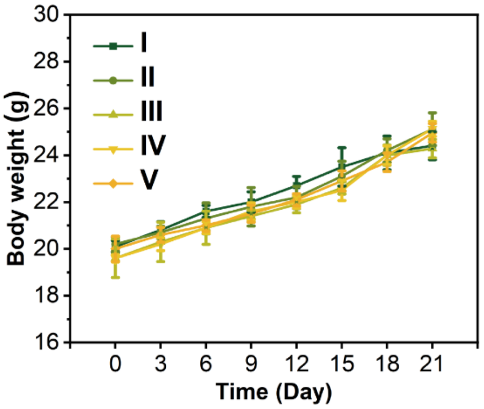


Figure S17. Curve of mice body weight in each group.

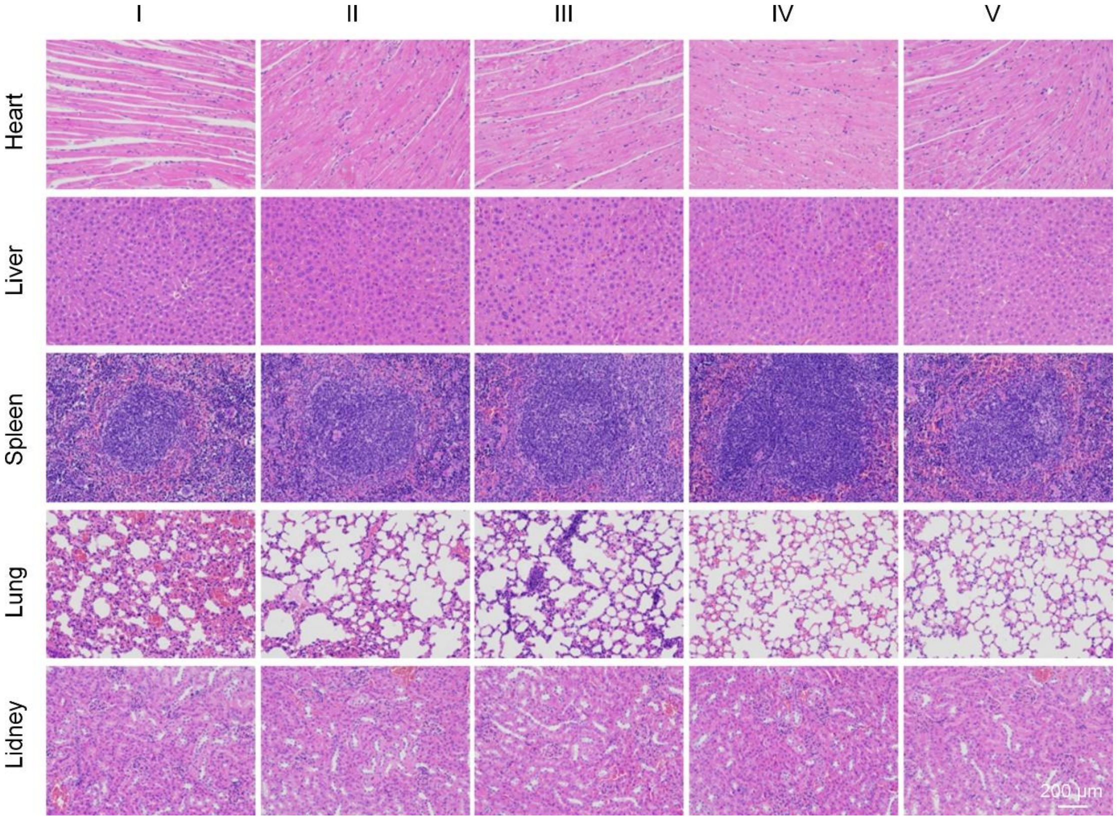


Figure S18. Hematoxylin and eosin staining results of dissected major organs at the 21th day.

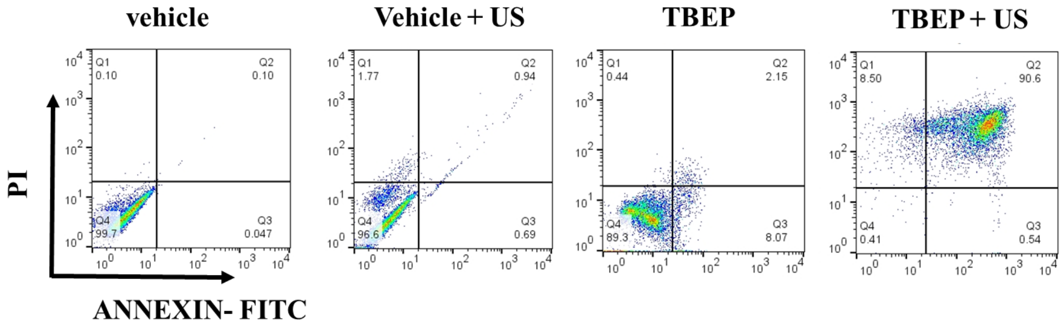


Figure S19. Raw data for Figure 4C.

Table S1. Association rate constant (k_a), dissociation rate constant (k_d), and dissociation constant (K_D) determined by surface plasmon resonance analysis.

compound	k_a (L·mol^{-1}·s^{-1})	k_d (s^{-1})	K_D (mol·L^{-1})
estradiol	1.22×10^{7}	2.75×10^{-2}	2.25×10^{-9}
TBEP	4.53×10^{5}	3.99×10^{-2}	8.81×10^{-8}