Supporting Information

1. Experimental Section

1.1 Instruments and materials

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 MHz NMR spectrometer. Chemical shifts were expressed in ppm (in chloroform-d ($CDCl_3$) and DMSO-d_6; TMS as an internal standard) and coupling constants (J) in Hz. Electrospray ionization was determined using a Waters Micromass LCT mass spectrometer. Fluorescence spectra were recorded using a Horiba Fluoromax-4 spectrofluorometer. Absorption spectra were recorded on a P Varian Cary 500 UV-vis spectrophotometer. Fluorescence images were captured using an Olympus FV-1000 laser scanning confocal fluorescence microscope.

All reagents were bought from commercial sources (Energy Chemical, Sigma-Aldrich, Adamas-beta) and used without further processing. All solvents were purified and dried before using by standard methods. The solvents used in spectrum analysis were of HPLC grade. The solutions for analytical studies were prepared with deionized water treated using a Milli-Q System (Billerica, MA, USA). The carboxylesterase was purchased from Jiangsu Meimian Industrial Co. Ltd.

Determination of the detection limit (*C*) of BPYM towards addition of Esterase

Based on the linear fitting in Fig. 1E, *C* is estimated as follows:

$$C = 3\sigma/B$$

Where σ is the standard deviation obtained from three individual fluorescence measurements (I_{631}) of **BPYM** (10 μM) without Esterase, B is the slope obtained after linear fitting the titration curves within certain ranges.

1.2 In vitro esterase response

In the study of the fluorescence reaction of the probe BPYM to esterase, BPYM and esterase were first added to a PBS solution containing 2% THF for an initial reaction period ranging from 0 to 60 minutes. After the reaction was completed, THF was added to the cuvette to a final volume fraction of 25%, and then the fluorescence spectroscopy test was performed.

1.3 Cell culture

KPC1199 cells (mouse pancreatic cancer cell line) and NIH-3T3 cells (mouse embryonic fibroblasts) were cultured in DMEM medium with 10 % fetal bovine serum (FBS), 1 % penicillin G streptomycin in 5 % CO_2 incubator at 37 °C. Similarly, BXPC3 cells (human pancreatic cancer cell line) were cultured in RPMI 1640 medium with 10 % FBS and 1 % penicillin G streptomycin in a continuous 37 °C incubator with 5 % CO_2.

1.4 Detection of dark cytotoxicity in vitro

KPC1199 cells, BXPC3 cells and mouse embryonic fibroblasts NIH-3T3 cells using a 96-well

plate were cultivated at 37 °C for 24 h. The cells were then co-cultured with **BPYM** or **BPY** at different concentrations (0 μM, 5 μM,10 μM,20 μM,40 μM,80 μM) for 24 h. The dark cytotoxicity was detected with Cell Counting Kit-8 (Yeason, China) assay. 10 μL of CCK-8 reagent mixed with 90 μL fresh culture medium was added into the well, and after a 2h incubation, the absorbance was recorded at 450 nm using a microplate reader.

1.5 Evaluation of photodynamic toxicity in vitro

The cells were co-cultured with a suite of different calculated concentrations (0 μM,5 μM,10 μM,20 μM,40 μM,80 μM) of **BPYM** or **BPY** for 4 h. Then, the cells were irradiated with white light (30 mW/cm^2) at different time (0 min, 10 min, 20 min) . After incubation for another 24 h, CCK8 assay was performed to evaluate the cell viability.

1.6 Cellular Uptake

KPC1199 cells、BXPC3 cells and NIH-3T3 cells using a Confocal culture dish were cultivated at 37 °C for 24 h. Subsequently, 1 mL fresh culture medium containing 20 μM **BPYM** was used to incubate the cells. After 0 min, 15 min, 30 min，60 min，90 min, 120 min and 150 min, the cells were washed with PBS and replaced with fresh medium. Finally, confocal laser scanning microscopy (CLSM) was used for cell imaging. Fluorescence intensity reflects the extent of the cytolactone enzyme reaction. Meanwhile, the cells were also dealt with 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), an esterase inhibitor, for 1 h. The fluorescence intensity was also observed by CLSM.

1.7 In vitro Cell Apoptosis Measurement

KPC1199 cells and BXPC3 cells using a 6-well plate were cultivated for 24 h, which were grouped into PBS, PBS+L, **BPY**, **BPY**+L, **BPYM**, **BPYM**+L. Subsequently, they were cocultured with culture medium containing **BPYM** or **BPY** for 12 h. In the light groups, each well was treated by white light (30 mW/cm^2) for 10 min. After 12 h, cell apoptosis measurement was performed using the apoptosis assay kit by flow cytometry.

1.8 Intracellular ROS Measurement

The procedure for the culture and treatment of KPC1199 cells and BXPC3 cells was described in the cell apoptosis experiment. DCFH-DA was subsequently added to the coculture for 10 minutes. The light groups in this case referred to the experiment conducted in the 6-well plates as explained above. After irradiation and washing, fluorescence imaging was performed with CLSM.

1.9 Western blotting analysis

To extract whole-cell lysates, RIPA buffer (including PMSF and phosphatase inhibitor) was utilized. The bicinchoninic acid (BCA) protein quantification test was used to quantify total protein isolated from cell lines or tissues. Total proteins were separated by SDS–PAGE, which were subsequently transferred to polyvinylidene difluoride membranes (Millipore, Darmstadt, Germany)

at 100 V for 100 minutes. The membranes were soaked in serum-free blot blocking solution (NCM Biotech, Suzhou, China) for 15 minutes before being labelled with primary antibodies (Caspase-3, Bcl-2, BAX, Cleaved caspase-3, β-actin) at a dilution of 1:1000 and incubated overnight at 4 ºC. The membranes were then incubated at room temperature for 1 h with a goat anti-mouse/anti-rabbit secondary antibody (1: 5000, Yeason, Shanghai, China) before identifying the protein bands with an ECL chemiluminescence kit (SB-WB012 or SB-WB011, Share Bio, Shanghai, China). For analysis of immunoblot density, ImageJ (National Institutes of Health) was used, with β-actin serving as an internal control for sample loading.

1.10 Detection of ICD effect

KPC1199 cells and BXPC3 cells were seeded in the 6-well plate overnight, and the cells were replaced with **BPYM** or **BPY** for 4 h. The cells were then irradiated with white light for 10 minutes at an intensity of 30 mW/cm^2. After an additional 2 hours of incubation, the cells were fixed and washed. The control group consisted of cells incubated with PBS and either **BPYM** or **BPY** without exposure to light.

1.10.1 In vitro CRT exposure

The cells were treated as described above. Then, KPC1199 cells and BXPC3 cells were investigated with Alexa Fluor®488 Anti Calreticulin antibody (CST, #62304) and Hoechst 33342 according to the manufacturer's instructions and detected by CLSM.

1.10.2 In vitro distribution of HMGB1

HMGB1 triggered by **BPYM** or **BPY** was identified by confocal laser scanning microscopy (CLSM) after immunofluorescent staining (HMGB1 antibody (CST, #3935S)). The KPC1199 cells and BXPC3 cells were operated as aforementioned and incubated with HMGB1 antibody for 1 h at room temperature. Then, Alexa Fluor 488-conjugated secondary antibody (CST, #4412) and Hoechst 33342 were stained as manufacturer's instructions. Finally, the cells were observed by CLSM.

1.11 Animal Experiment

Female C57BL/6J mice with 4-6 weeks of age were purchased from Jiangsu Jicui Pharmachem Biotechnology Co. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Medical School of Shanghai Jiao Tong University and approved by the Animal Ethics Committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University (Ethics number: **RJ2023-129A**). 2×10^6 KPC1199 cells were suspended in 100 μL PBS and injected subcutaneously into the back of 4-6 weeks old female C57BL/6J mice.

1.11.1 In vivo Imaging

The tumor grew to ~ 500 mm^3 before imaging. **BPYM** (33.3 μM, 100 μL) was injected into tumor-bearing mice for fluorescence imaging. Fluorescence images were acquired at different time

post-injection (0, 2, 4, 6, 8, 12, 24, 36 h). A tumor-bearing mice was euthanized after 8 hours injection, and tumors and organs were collected and imaged.

1.11.2 Evaluation of In vivo Antitumor Effect for BPYM and BPY

A unilateral KPC1199 tumor model was established to the effect of treatment in vivo. When tumor volume reached approximately 100 mm^3, 30 mice were randomly divided into 6 groups, including I) Control, II) Control + L, III) **BPYM** and IV) **BPYM** + L, V) **BPY**, VI) **BPY** + L. L: 50 mW/cm^2, white light, 10 minutes. Mice were injected with PBS, **BPYM**, **BPY** (33.3 μM), then the tumor site were irradiated with white light 4 h after injection. The weight of mice was recorded and the tumor volume was measured. The volume of the tumor was determined according to the equation $V= ab^2 \times 1/2$, where a and b were separately the longest and shortest diameters of the tumors. At 15 days after the initial treatment, tumor-bearing mice were euthanized. The major organs (heart, liver, spleen, lung, kidney) and tumor tissues were sectioned for staining hematoxylin-eosin (H&E). Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) was employed for tumor apoptosis detection. Ki-67 is applied for cell proliferation analysis.

A bilateral KPC1199 tumor model was constructed to assess the immunological effect of treatment in vivo. After primary tumor growth to ~100 mm^3, 18 mice were randomly divided into 6 groups, including I) Control, II) Control + L, III) **BPYM** and IV) **BPYM** + L，V) **BPY**，VI) **BPY** + L . L: 50 mW/cm^2, white light, 10 minutes. Mice were injected with PBS，**BPYM**，**BPY** (33.3 μM) , and then the tumor site were irradiated with white light 4 h after injection.. The weight of mice was recorded and the tumor volume was measured. Mouse weight and tumor volume were measured using the same method described above. After 12 days of treatment, 3 mice in each group were sacrificed for blood analysis, staining analysis of main organs (heart, liver, spleen, lung, kidney), tumor tissue and immune-related staining analysis.

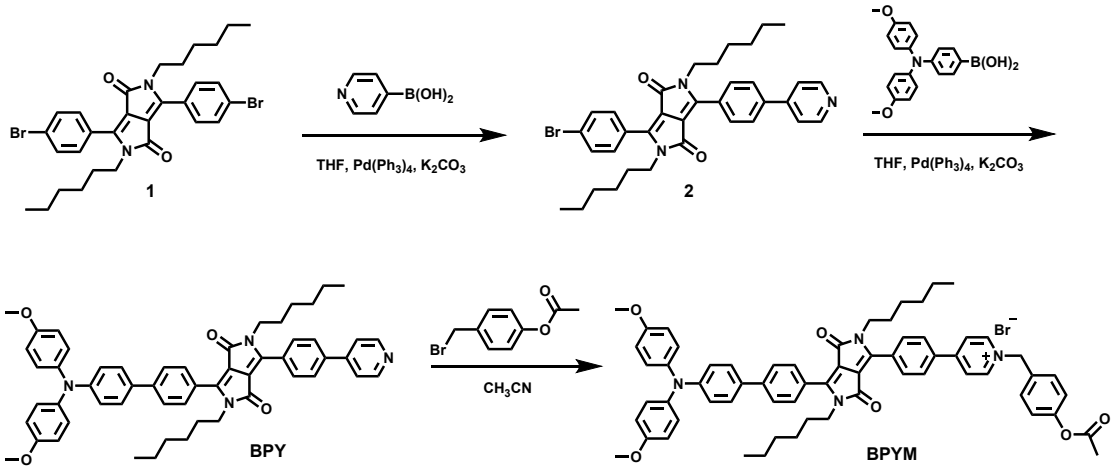
1.12 Analysis of Antitumor Immunity

For immune-related analysis, serum samples of three mice were obtained in each group and the immune stimulating cytokines were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA). The primary and distant tumor tissues were cut and treated with DMEM culture medium containing collagenase (type IV, 1 mg mL−1), hyaluronidase (0.1 mg mL−1), and Dnase (0.1 mg mL−1) at 37 °C for 1 h. After the digestion, the cells were separated from the remaining tissue debris by centrifugation and subsequent washing. The tumor cells obtained were then divided into two groups for further analysis. In one group, the cells were incubated with specific fluorescently-labeled antibodies: CD45-PerCP-CY5.5, CD86-PE, CD80-APC, and CD11c FITC for 30 minutes. The other group was stained with CD3-FITC, CD8-PE, and CD4-APC for 30 minutes, and then flow cytometry was used for measurement and analysis. Tumor tissue was also used for immunofluorescence staining and immunohistochemistry analysis.

1.13 Statistical Analysis

The data presented in this study are expressed as the means ± standard errors of the means (SEM). To assess the significant differences between groups, we performed one-way ANOVA with Tukey's multiple comparisons and the unpaired Student's t-test using GraphPad Prism 8. Each experiment included a minimum of three parallel groups, and please see graphic legend for details. Values of $P < 0.05$ were considered statistically significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$. Values of $p > 0.05$ were not significant.

2. Synthesis and characterization



Scheme S1. Synthesis routes

Compound **1**, compound **2** and **BPY** were synthesized following the previously reported procedures[1].

Synthesis of BPYM.

4-(Bromomethyl)phenyl acetate (114 mg, 0.5 mmol) and **BPY** (209 mg, 0.25 mmol) was refluxed under nitrogen in dry acetonitrile (20 mL) at 80 °C for 24 h. After removal of the solvent under vacuum, the crude product was purified by column chromatography (silica gel; dichloromethane/ ethanol, 10/1, v/v) to give **BPYM** (148 mg, 60 %) as a purple solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 9.31 (d, J = 6.6 Hz, 2H), 8.65 (d, J = 6.7 Hz, 2H), 8.29 (d, J = 8.3 Hz, 2H), 8.09 (d, J = 8.3 Hz, 2H), 7.94 (d, J = 8.3 Hz, 2H), 7.85 (d, J = 8.3 Hz, 2H), 7.67 (d, J = 4.4 Hz, 2H), 7.65 (d, J = 4.6 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 7.11 (d, J = 8.8 Hz, 4H), 6.96 (d, J = 8.9 Hz, 4H), 6.84 (d, J = 8.6 Hz, 2H), 5.87 (s, 2H), 3.79 (d, J = 7.2 Hz, 4H), 3.76 (s, 6H), 2.28 (s, 3H), 1.45 (p, J = 8.0, 7.4 Hz, 4H), 1.17 – 1.10 (m, 12H), 0.79 – 0.74 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-d) δ

169.32, 162.81, 162.17, 156.18, 151.80, 150.19, 148.94, 145.21, 143.70, 140.41, 134.78, 132.03, 131.18, 130.84, 130.75, 130.09, 129.39, 128.12, 127.57, 127.02, 126.97, 126.28, 125.47, 122.95, 119.99, 114.83, 114.77, 111.17, 109.35, 55.55, 55.44, 31.18, 29.41, 29.23, 26.37, 22.46, 22.43, 13.98. HR-MS (m/z): Calculated for $[M]^+$, 985.4904; Found, 985.4897.

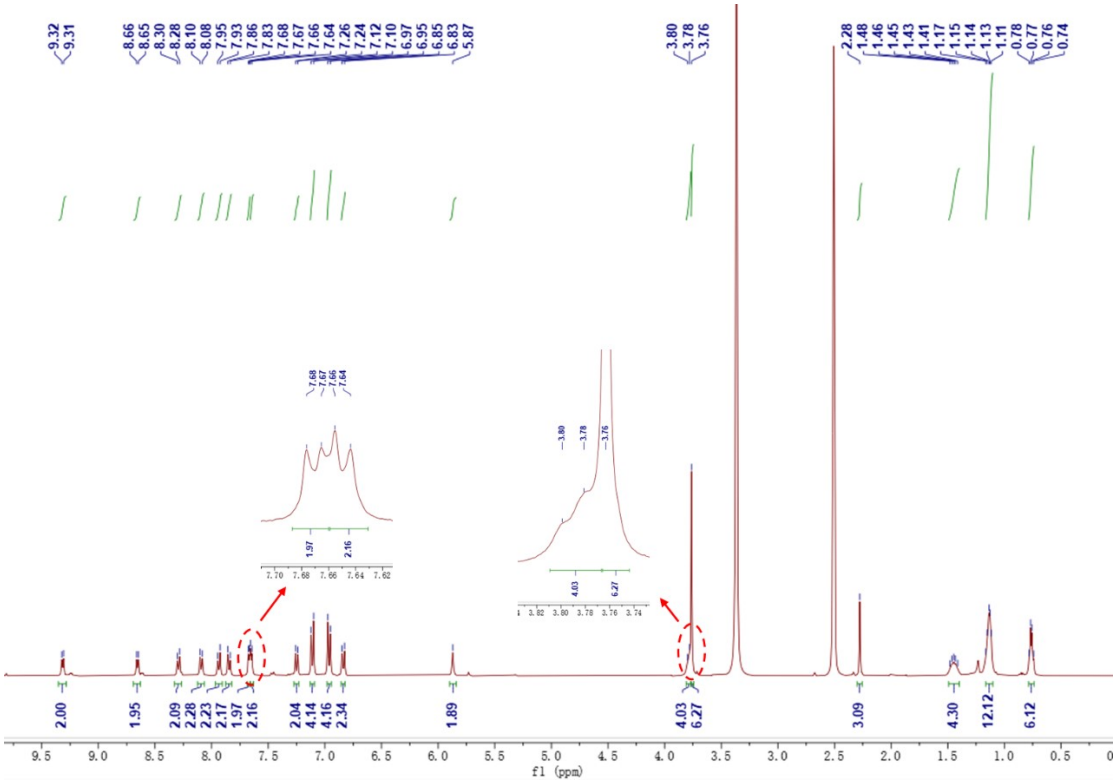


Figure S1. ^{1}H NMR spectrum of BPYM in DMSO-d_6.

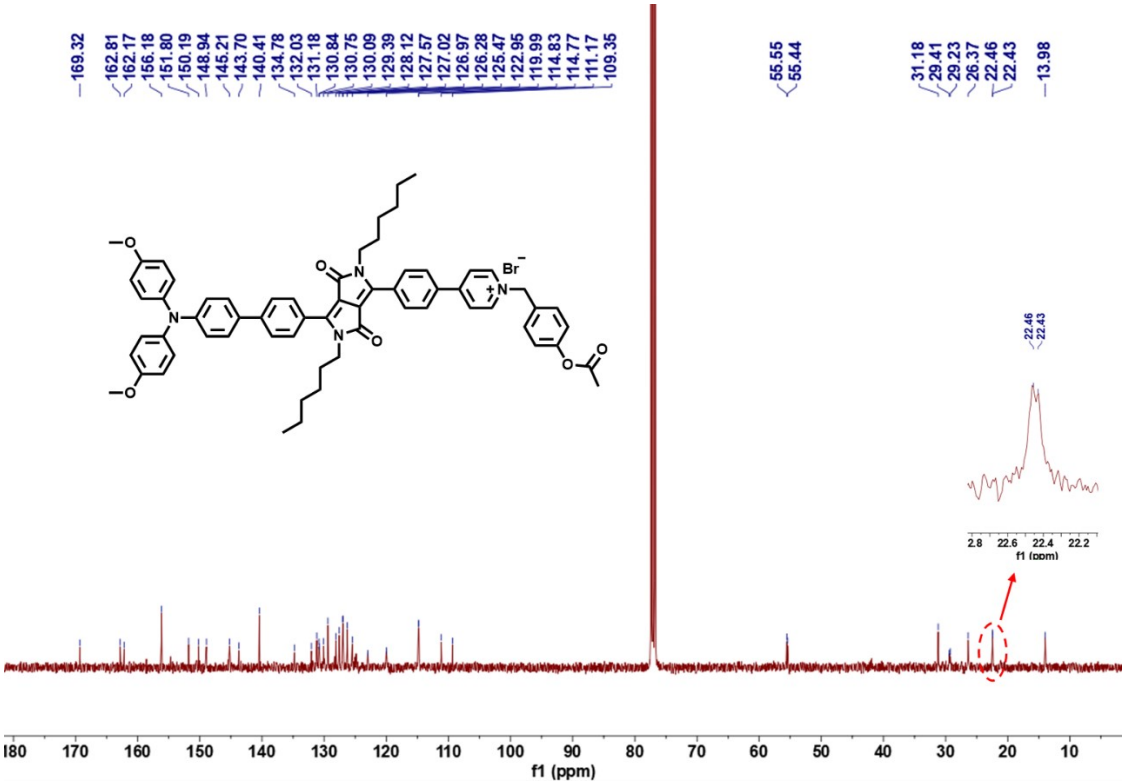


Figure S2. ^{13}C NMR spectrum of **BPYM** in $CDCl_3$.

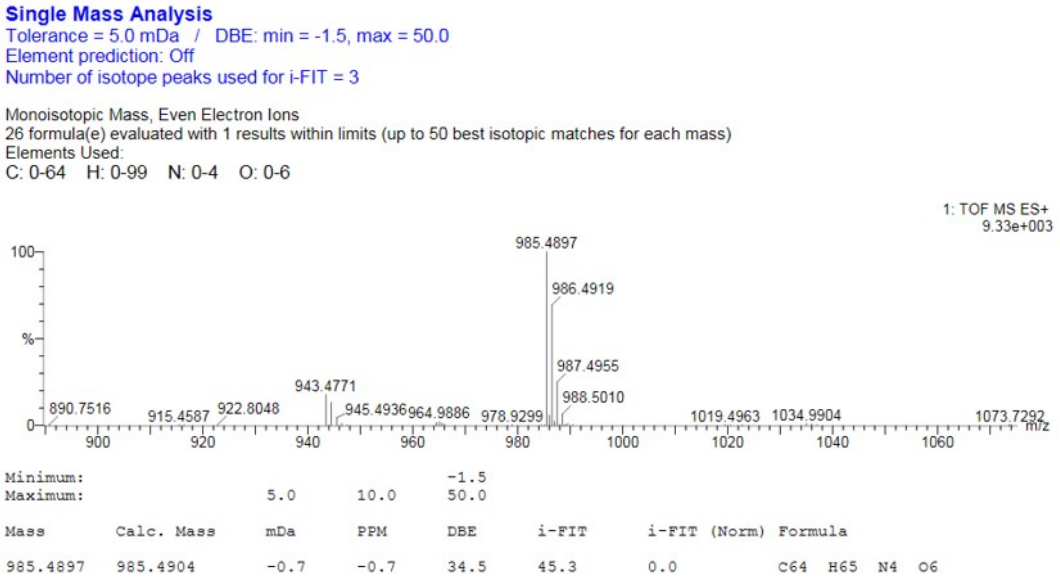


Figure S3. HR-MS spectra of **BPYM**.

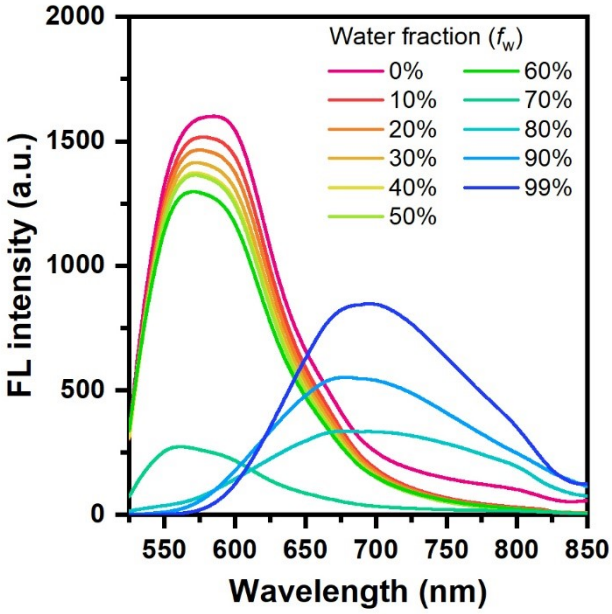


Figure S4. Fluorescence spectra of BPY (10 μ M) in THF/H_2O mixtures with varying water content (0-99%), λ_{ex} =505 nm.

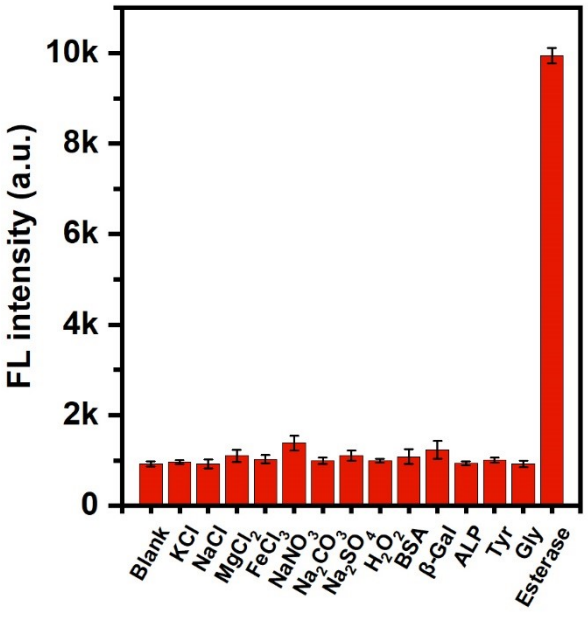


Figure S5. Fluorescence intensity at 631 nm of **BPYM** (10 μM) toward different analytes in THF-PBS solution (2.5:7.5, v/v, pH = 7.4) at 37.4 °C. KCl (1 mM), NaCl (1 mM), $MgCl_2$ (1 mM), $FeCl_3$ (1 mM), $NaNO_3$ (1 mM), Na_2CO_3 (1 mM), Na_2SO_4 (1 mM), H_2O_2 (1 mM), BSA (1 mg mL^{-1}), β-Gal (0.1 U mL^{-1}), ALP (0.1 U mL^{-1}), Tyr (200 μM), Gly (200 μM), Esterase (0.1 U mL^{-1}).

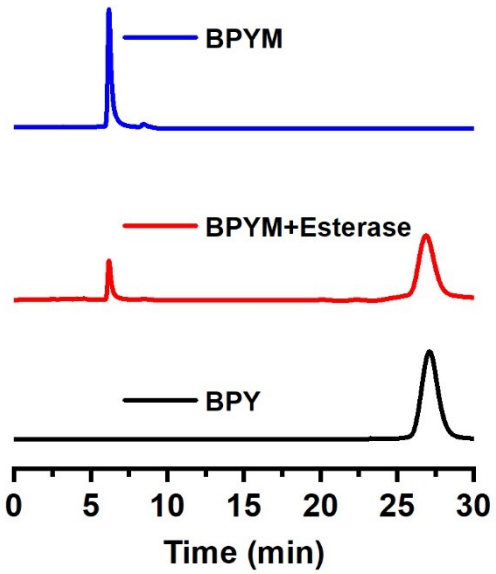


Figure S6. High-performance liquid chromatography (HPLC) experiments: 100% CH_3CN.

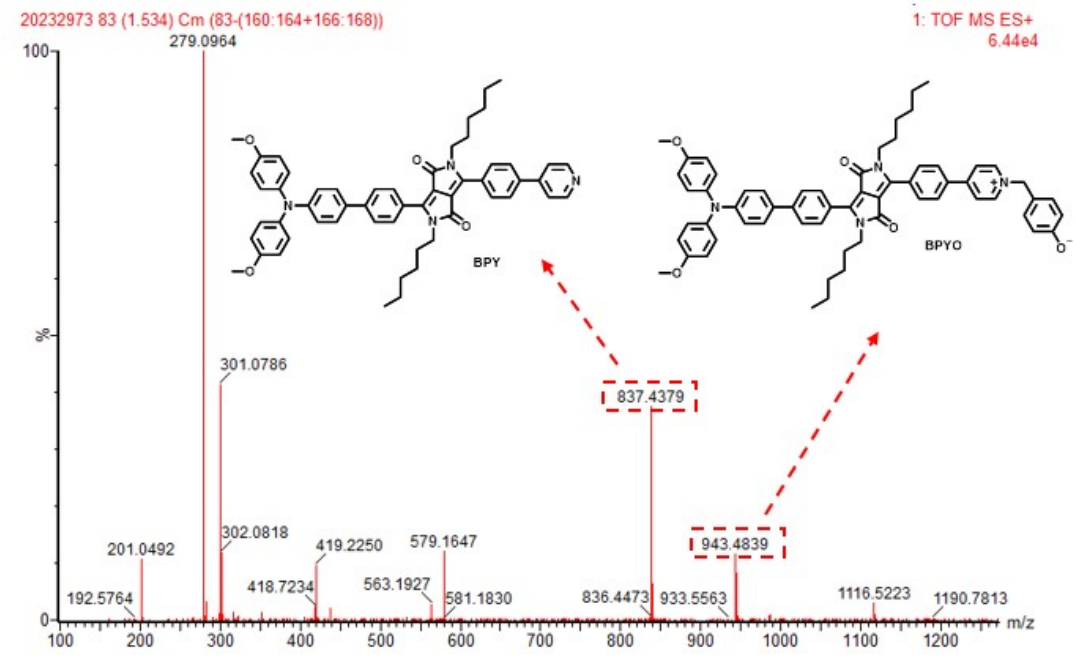


Figure S7. High-resolution mass spectrum (HRMS) experiments of **BPYM** with esterase.

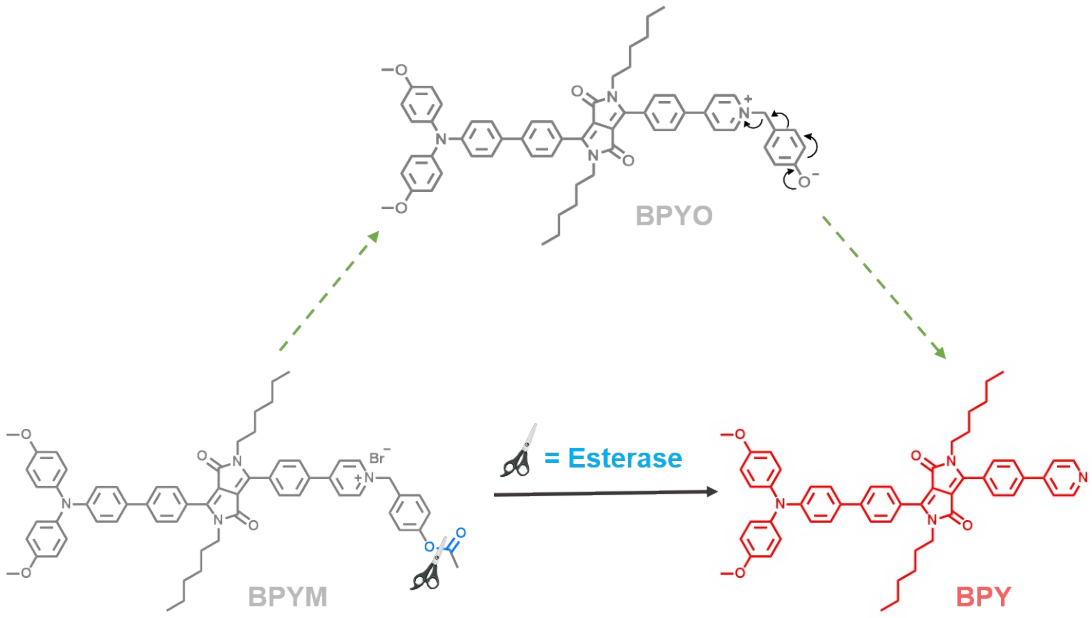


Figure S8. Proposed Reaction Mechanism for **BPYM** toward esterase.

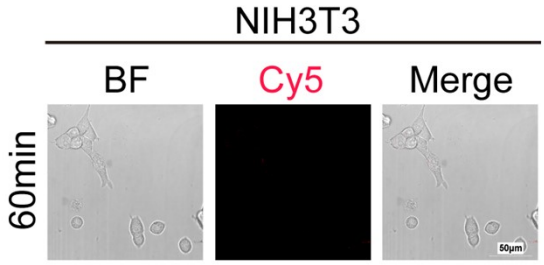


Figure S9. The CLSM images of NIH-3T3 in the control group treated with **BPYM** (20 μ M) for 60 min. Scale bars = 50 μ m.

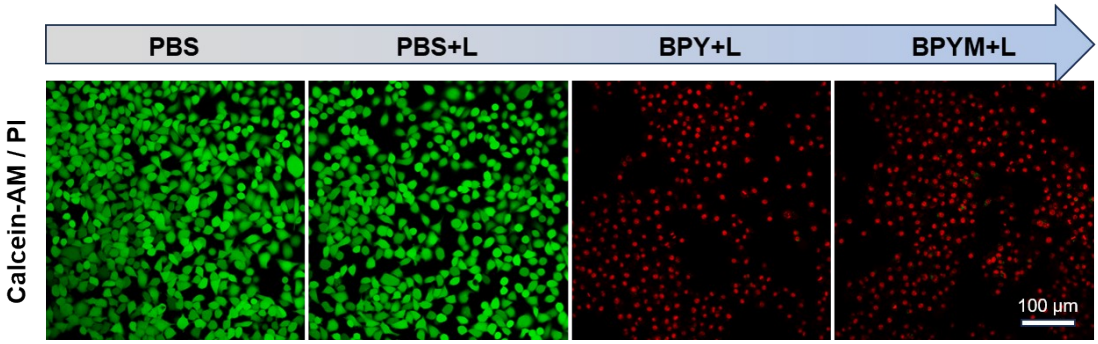


Figure S10. Live and dead cell staining of KPC1199 cells after various treatments. The green and red fluorescence mean live and dead cells, respectively. Scale bars =100 μm.

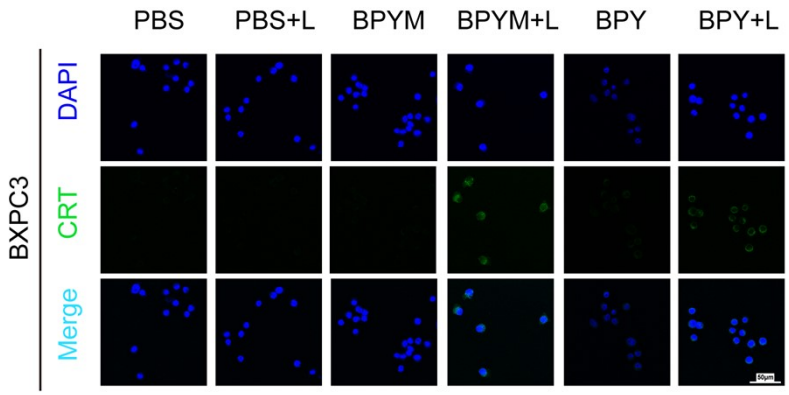


Figure S11. CLSM analysis of CRT in BXPC3 cells after treatment with PBS, **BPYM** or **BPY** by irradiation with/without white light (30 mW/cm^2, 10 min). Scale bars = 50 μm.

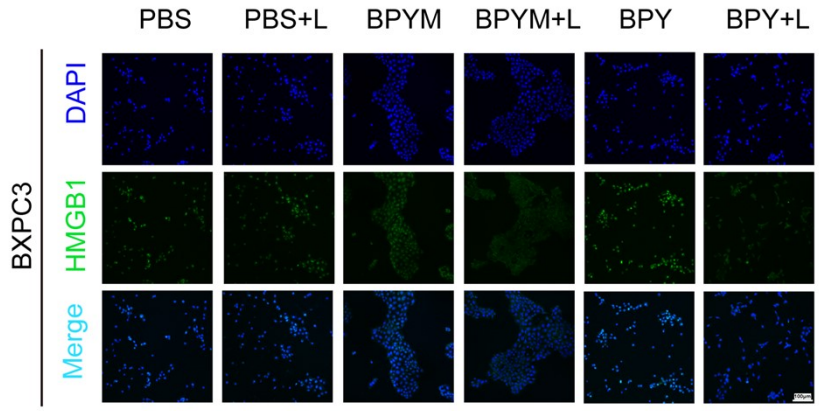


Figure S12. Immunofluorescence staining of HMGB1 (green) release from BXPC3 cells after treatment with PBS, **BPYM** or **BPY** by irradiation with white light (30 mW/cm^2, 10 min). Scale bars = 100 μm.

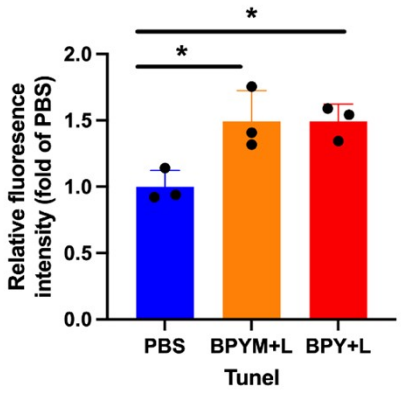


Figure S13. The corresponding quantitative analysis (n=3) of Tunel immunofluorescence staining. Data were presented as mean ± SD, *p < 0.05.

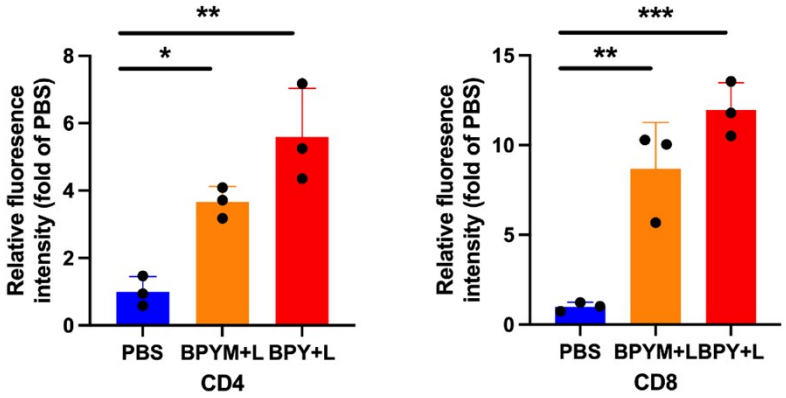


Figure S14. The corresponding quantitative analysis (n=3) of CD4 or CD8 immunofluorescence staining. Data were presented as mean ± SD, *p < 0.05, **p < 0.01, ***p < 0.001.

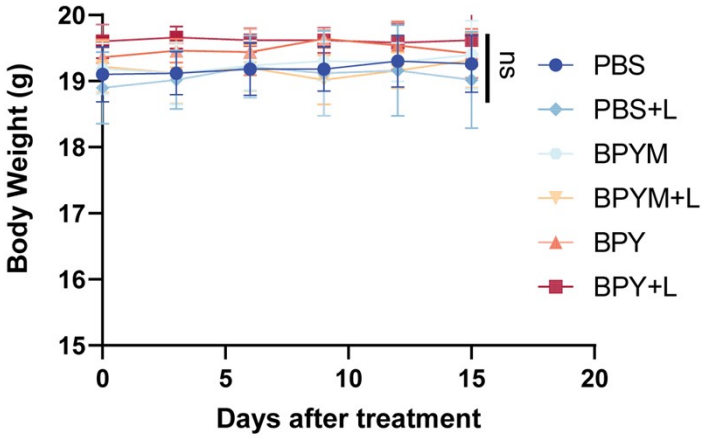


Figure S15. Changes in body weight of kpc1199 tumor-bearing mice during treatments in unilateral subcutaneous xenograft model for 15 days. Data were presented as mean ± SD, ns, $p > 0.05$.

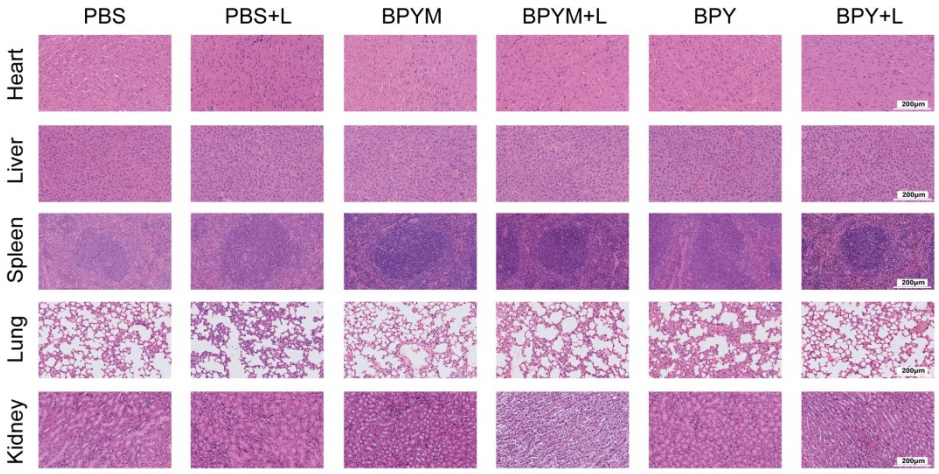


Figure S16. Histological H&E staining analysis of major organs including heart, liver, spleen, lung, and kidney in KPC1199 tumor-bearing mice after 15 days of different treatments in unilateral subcutaneous xenograft model. Scale bar: 200 μm.

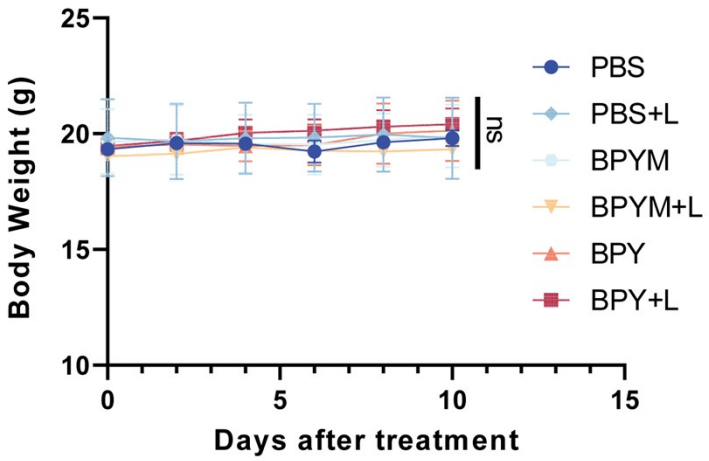


Figure S17. Changes in body weight of kpc1199 tumor-bearing mice during treatment in bilateral subcutaneous xenograft model for 10 days. Data were presented as mean ± SD (n=3), ns, $p > 0.05$.

1. Li X, Xu W, Yang Z, et al. A lipid droplet-targeted multifunctional AIE-active fluorescent probe for hydrogen peroxide detection and imaging-guided photodynamic therapy. *Sens. Actuators, B*, 2023;375.