Supporting Information

A Bacteria-Based Bioorthogonal Platform Disrupt the Flexible Lipid Homeostasis for Potent Metabolic Therapy

Jiadai Yi, [a, b] *Huan Wang,*[a, *] *Qingqing Deng,*[a, b] *Congcong Huang,*[a, b] *Lu Zhang,*[a] *Mengyu Sun,*[a, b] *Jinsong Ren,*[a, b, *] *and Xiaogang Qu*[a, b, *]

Methods

Materials

1,3,5-Triformylbenzene, 1,4-diaminobenzene and polyvinylpyrrolidone (PVP-K30) were obtained from Maklin. Acetic acid and acetonitrile were purchased from BeiJing chemical works. Sodium borohydride ($NaBH_4$) was purchased from Aladdin. $PdCl_2$ (99.9%) was purchased from TCI. O-Benzyl-L-serine (BenSer) and Calcein-AM were purchased from Sigma-Aldrich. The Free fatty Acids (FFA) Content Assay Kit was purchased from Beijing Solarbio Science & Technology Co. LTD, China. The Glutamine (Gln) Colorimetric Assay Kit was purchased from Elabscience Biotechnology Inc. All chemicals were used as received without further purification. Ultrapure water for experiments was produced by the Milli-Q-Plus system.

Characterization

Scanning electron microscope (SEM) images were recorded on a Hitachi S-4800 FESEM. The content of Pd was analyzed on the Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES, X Series 2, Thermo Scientific, USA). The UV-Vis absorption spectra were recorded using a JASCO V550 UV-Visible spectrophotometer. Fluorescence measurements were carried out on a JASCO FP-6500 spectrofluorometer. ^{1}H NMR spectrum was recorded on a Bruker-600 MHz NMR

instrument. The flow cytometry data were obtained by BD LSRFortessa™ Cell Analyzer. TEM imaging was done on the FEI Tecnai G^2F20 high-resolution transmission electron microscope at an acceleration voltage of 200 kV. FT-IR measurements were carried out on a BRUKER Vertex 70 FT-IR spectrometer, and 32 scans were taken with a spectral resolution of 2 cm^{-1}. The zeta potential measurements were performed on Malvern Nano ZS-90 at 25°C. XPS measurements were performed on a Thermo Fisher Scientific ESCALAB 250Xi XPS system. Confocal imaging was carried out on a Nikon A1R confocal microscope. Powder X-ray diffraction (XRD) measurement was conducted by a BRUKER D8 ADVANCE X-ray diffractometer equipped with CuKα radiation (λ=0.15406 nm).

Synthesis of COF

Firstly, 1,3,5-Triformylbenzene (12.5 mg) and 1,4-diaminobenzene (15 mg) were dissolved in 10 mL of acetonitrile in a 16 mL capped vial, and then, acetic acid (0.05 mL) was added. When the solution turned yellow, polyvinylpyrrolidone (0.1 g mL^{-1} in acetonitrile) was added, and the mixture was stirred at room temperature for 24 h. The product was obtained by centrifugation, washing, and subsequent drying.

Synthesis of CP

The preparation of H_2PdCl_4: A 10 mM H_2PdCl_4 aqueous solution was prepared by completely dissolving 44.5 mg $PdCl_2$ in 25 ml of 20 mM HCl in a boil water bath.

The CP was prepared according to the literature with slight modifications. [1] Then, the as-synthesized COF (10 mg) was dispersed in deionized water under sonication. Subsequently, the H_2PdCl_4 solution (1 mL) was added with stirring, and the resulting mixture was stirred for 1 h. A freshly prepared $NaBH_4$ (500 μL; 0.2 M) was added into the above aqueous solution under vigorous stirring. [2] After mixture, the resulting suspension was stirred for 3 h. The obtained product was dispersed after washing.

Bacteria culture

Dry *Lactobacillus rhamnosus* GG were cultured in the Man Rogosa Sharpe (MRS) Broth with continuous shaking at 37°C for 18 h. Then, the bacteria were harvested, washed with pH 7.4 PBS (10 mM) solution, and suspended in PBS solution (pH=7.4, 10 mM).

Synthesis of BCP

The bacterial culture was centrifuged at 5000 rpm for 3 minutes and washed twice. Afterward, the bacterial pellet was re-suspended in PBS by gentle pipetting. EDC and NHS were used for activation for 30 minutes, followed by centrifugation at 5000 rpm to remove free EDC and NHS. Subsequently, the bacteria were uniformly dispersed in PBS, and the dispersed CP solution was added. The mixture was stirred overnight, followed by centrifugation at 5000 rpm for washing. The final product, BCP, was obtained and re-dispersed in PBS for further use.

Catalytic efficacy of BCP in vial.

The catalytic efficacy of BCP in vial was validated by catalyzing the allylcarbamate cleavage of N-allyloxycarbonyl coumarin (N-alloc-cumarin). Briefly, the BCP with 20 μM palladium and 10 μM N-alloc-cumarin (40 mM in dimethyl sulfoxide (DMSO)) were mixed in PBS under 37℃. At different expected time point, the mixed solution was centrifuged and the supernatant was collected, and fluorescence intensity was detected via a fluorescence spectrofluorometer. Analytical HPLC with absorption detection at λ=280 nm. Eluent A: 0.05 M ammonium acetate containing acetate acid (0.1 %); Eluent B: acetonitrile, formic acid (0.1 %) rt=3.5 min (A/B = 70:30 to 5:95 in 13 min, isocratic 1 min, 5:95 to 70:30 in 5 min, isocratic 1 min).

Cell cultures

CT26 and 3T3 cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% heat-inactivated FBS in an atmosphere of 5% CO_2 at 37 °C. The medium was replaced once a day, and the cells were digested with trypsin and resuspended in a fresh complete medium before plating.

In vitro cytotoxicity assay

MTT assay was used to determine the cytotoxicity of BCP particles. Briefly, 3T3 cells were randomly seeded into 96-well plates at a density of 5000 cells per well (100 μL) for 24 h. Different concentrations of BCP particles were added to the wells. The cells were subsequently incubated for 48 h in the incubator. Then, MTT was added to each well to a final volume of 100 μL. After that, the plate was placed in the incubator for another 4 h. The media was then removed and DMSO (100 μL) was added to each well. Absorbance values were determined with a Bio-Rad model-680 microplate reader at 570 nm.

BCP-mediated catalytic reactions in living cells

Confocal microscopy imaging. CT26 cells were seeded on sterilized coverslips for 24 h in 6-well plates. The N-alloc-courmain (10 mM in DMSO) were incubated with the cells for 8 h. After that, cells were rinsed with PBS for 3 times. Next, the catalysts BCP was added to the culture at a concentration of 10 μM Pd and incubated for 24 h. Thereafter, the media was removed and cells were washed three times with PBS to eliminate extracellular particles. The fluorescence images were taken with a Nikon A1R confocal microscope.

Flow cytometric analysis. CT26 cells were plated in 6-well plates for 24 h. N-alloc-courmain (10 mM in DMSO) was added to the culture at a final concentration of 40 μM and incubated for 8 h. After that, cells were rinsed with PBS 3 times. Next, the catalysts BCP at a concentration of 10 μM Pd were incubated with the cells for 24 h. Thereafter, the media was removed and cells were washed three times with PBS to eliminate extracellular particles. Then, the cells were harvested using trypsin and resuspended in PBS. The intracellular fluorescence was analyzed using flow cytometry.

FFA content dectection

Add the extraction solution to the cells in a ratio of 10^4 cells to 500-1000 μL, followed by cell disruption using ice-cold ultrasonication (300 W power, 2 seconds on, 3 seconds off, total time 3 min). After sonication, samples were shaked for 15 min, then centrifuged at 5000 rpm for 5 min at 4°C, and the organic phase was collected for analysis.

In vitro anticancer experiments

The toxicity of BenSer and Pro-BenSer (PB) was studied by cell viability test. CT26 cells were seeded into 96-well plates at a density of 10,000 cells per well. The tumor cells were treated with BenSer or PB at various concentrations. After 60 h, the cell viability was evaluated by MTT assay. Change the dosed medium daily. Although BenSer showed toxicity as its concentration increased, PB maintained high cellular viability within 20 mM.

The antitumor efficacy of PB + BCP was further studied by live/death staining. CT26 cells were incubated in a 6-well plate and incubated at 37 °C for 24 h. Old media were removed and replaced by fresh media containing BCP (Pd: 10 $\mu g \cdot mL^{-1}$) and incubated for another 24 h. The excess of extracellular particles was removed by washing with PBS three times. Prodrug PB (10 mM in DMSO) was diluted in fresh media to give a final concentration of 10 mM. After different treatments, the cell medium was discarded and added with Annexin V-FITC (5 $\mu g \cdot mL^{-1}$) and 7-AAD (10 $\mu g \cdot mL^{-1}$). After incubating for 20 min, the cells were washed with PBS and observed by a Nikon A1R confocal microscope. Besides, apoptosis experiments were carried out by Annexin V-FITC/7-AAD staining via flow cytometry analysis.

Animal experiments.

Female Balb/c mice (about 16-20 g) were purchased from the Laboratory Animal Center of Jilin University (Changchun, China). All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Changchun Institute of Applied Chemistry Chinese Academy of Sciences and approved by Institutional Animal Care and Use Committee of Changchun Institute of Applied Chemistry Chinese Academy of Sciences (Permit Number: 20210016).

For biocompatibility evaluation, female Kunming mice were randomly divided into three groups and intravenously injected with PBS, PB, or BCP (20 $mg \cdot kg^{-1}$, 200 μL). Pd content in CP-treated group was the same as that in BCP-treated group. The body weight of mice was measured every 3 days to evaluate the *in vivo* biosafety. At the indicated time points, the mice were euthanized and the blood

samples were collected to perform blood biochemistry and hematology analysis. The major organs including heart, liver, spleen, lung, and kidney were harvested, fixed in 10% paraformaldehyde, processed into paraffin, sectioned at ~4 μm, and stained with hematoxylin and eosin (H&E).

For *in vivo* therapeutic evaluation, CT26 tumor-bearing Balb/c mice were randomly divided into 6 groups (n = 5). When the tumor volume reached about ~50 mm^3, the mice were intravenously injected with PBS or PB (1.2 $mg \cdot kg^{-1}$, 200 μL), and intratumoral injected with PBS(50 μL), CP or BCP (20 $mg \cdot kg^{-1}$, 50μL), in which Pd content in CP-treated groups was the same as that in BCP-treated groups. The tumor volume and bodyweight were recorded every other day. The tumor volume was calculated as follows: $V = W^2 \times L/2$, where W and L represented the tumor width and length, respectively. The mice were sacrificed after 2 weeks posttreatment, and the tumors were collected and taken photos. For histology analysis, the tumor tissues in each group were harvested from mice after the treatment. The tumor tissues were dissected, fixed in 10 % paraformaldehyde, embedded in paraffin, sectioned into ~4 μm, and then used for H&E staining assay. Moreover, the major organs were dissected to make a paraffin section for further H&E staining.

Histology

For histological analyses, harvested mouse tissues were fixed with paraformaldehyde (10%) after washing with 0.9% NaCl solution, and dehydrated. Subsequently, organ samples were embedded in paraffin, sectioned, and stained with H&E. Then, stained slides were observed on an Olympus BX-51 optical system.

Statistical Analysis

All data were expressed in this article as mean ± standard deviation (s.d.). The statistical analysis was performed by using Origin 8.0 software. Statistical evaluation was performed using unpaired Student's two-sided t test analysis. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Figures

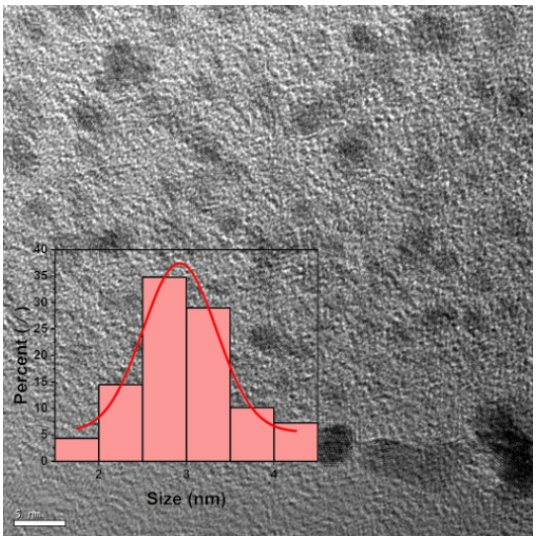


Figure S1. The TEM image of ultrasmall Pd nanoparticles loaded in CP and their corresponding size distributions.

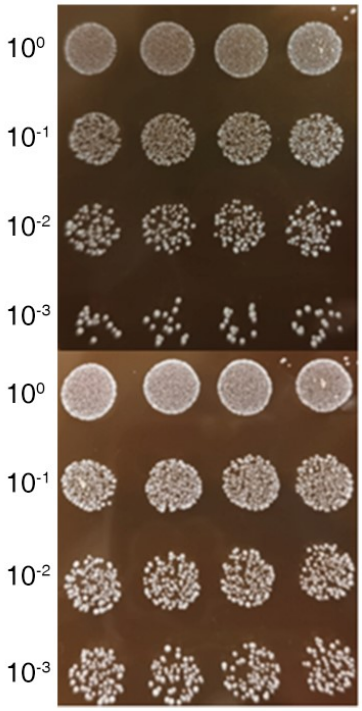


Figure S2. Photographs of the MRS agar plates before (top) and after (bottom) the overnight incubation of the bacteria with CP in different groups.

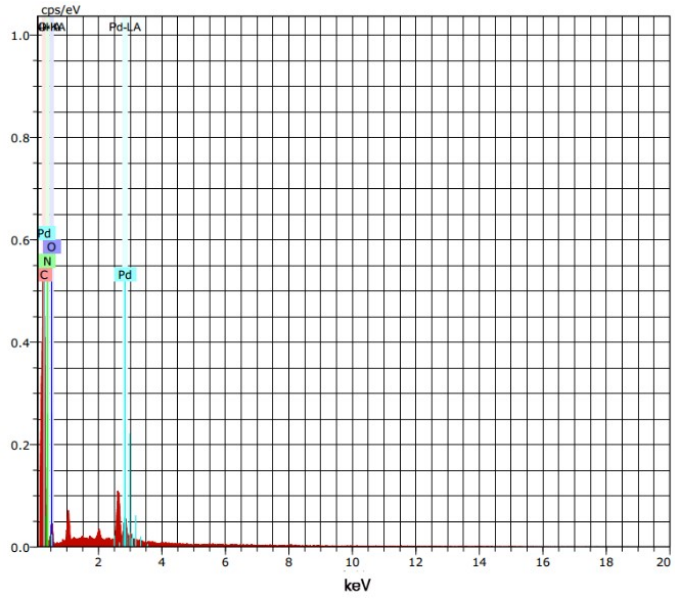


Figure S3. EDS spectrum of the BCP.

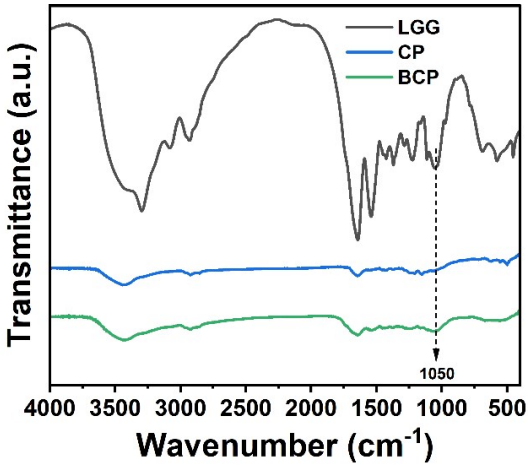


Figure S4. The FT-IR spectra of LGG, CP, and BCP.

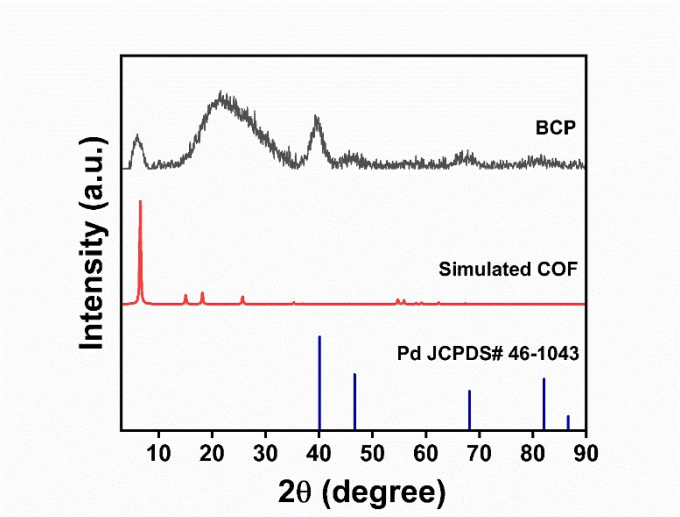


Figure S5. The XRD pattern of BCP.

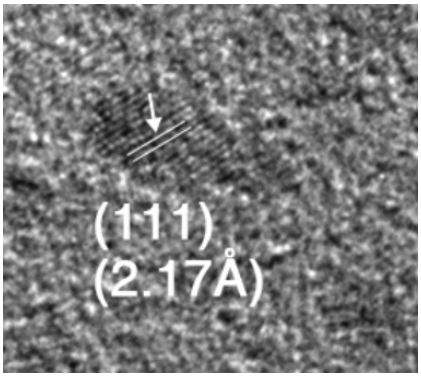


Figure S6. The HR-TEM image of Pd on the COF.

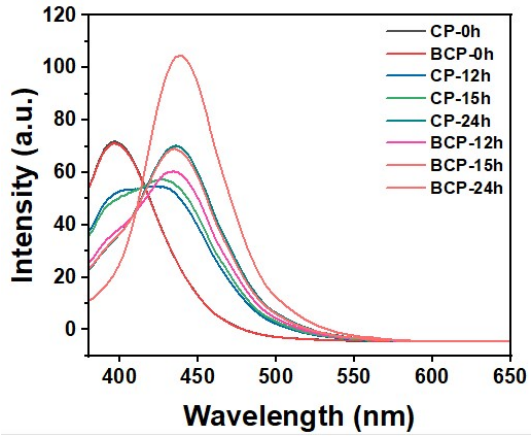


Figure S7. Fluorescence spectra of N-alloc-coumarin catalyzed by different materials with the same Pd concentration.

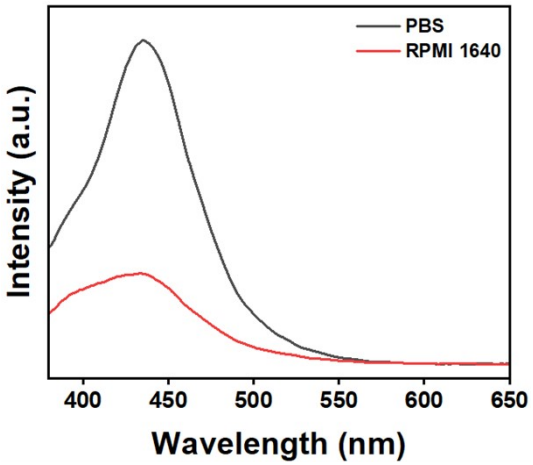


Figure S8. The fluorescence spectra of N-alloc-cumarin after reacting with CP in PBS or in RPMI 1640.

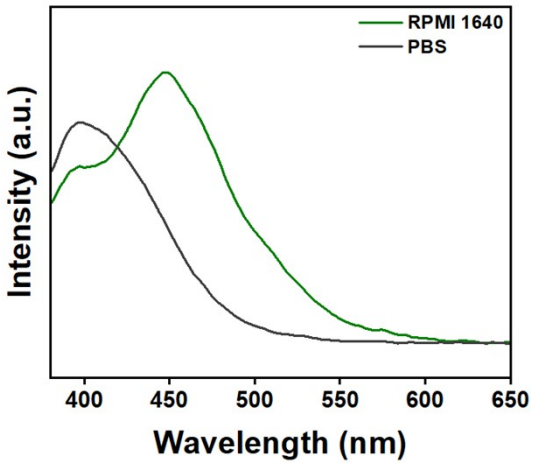


Figure S9. The fluorescence spectra of N-alloc-cumarin after reacting with BCP in PBS or in RPMI 1640.

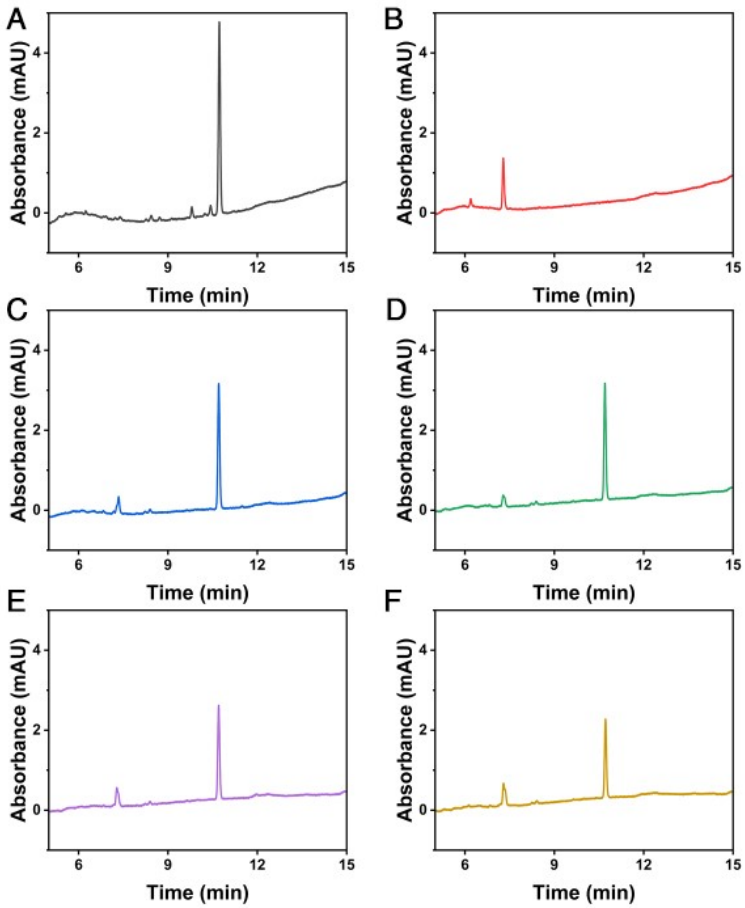


Figure S10. The HPLC analysis of the reaction process catalyzed by BCP at different time points. (A), (B) The standard examples of coumarin and N-alloc-coumarin. The examples of the catalytic reaction at 3 h (C), 6h (D), 9h (E), and 12h (F).

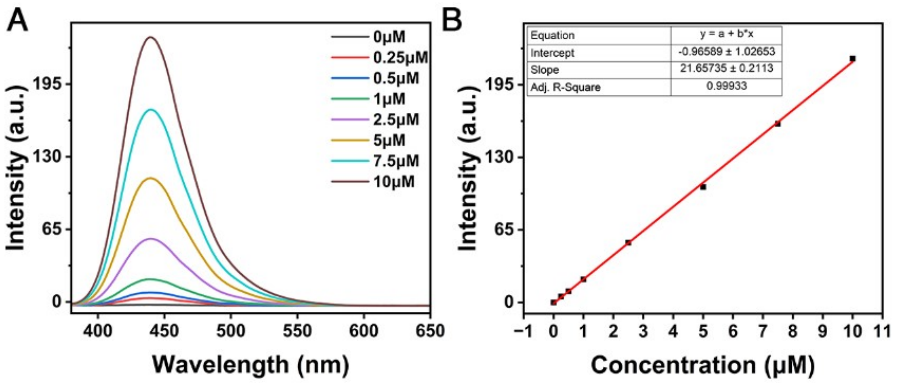


Figure S11. (A, B) The standard curve of coumarin.

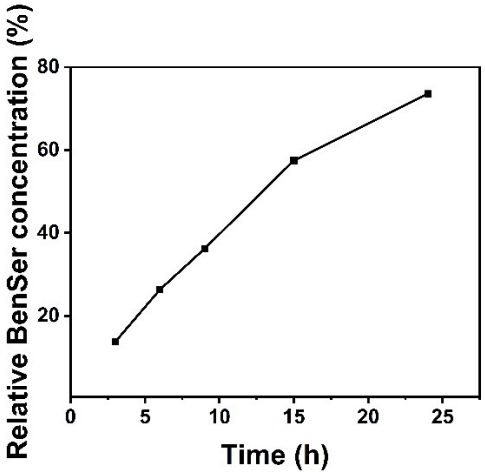


Figure S12. Time-dependent conversion efficacy of the Pro-BenSer (PB) upon incubation with BCP catalysts at 37 ℃ (pH 7.4), detected by HPLC technology. Briefly, the BCP with 20 μM palladium and 10 μM PB (40 mM in DMSO) were mixed in PBS under 37 ℃. At various expected time points, the mixed solution was centrifuged, the supernatant was collected, and the UV absorption was detected via Thermo Scientific Ultimate 3000 UHPLC system. Analytical HPLC with absorption detection at λ=217 nm. Eluent A: 0.025 M ammonium acetate; Eluent B: acetonitrile (A/B = 95:5 for 5 min, 95:5 to 5:95 in 10 min, isocratic 1 min, 5:95 to 95:5 in 5 min, isocratic 10 min).

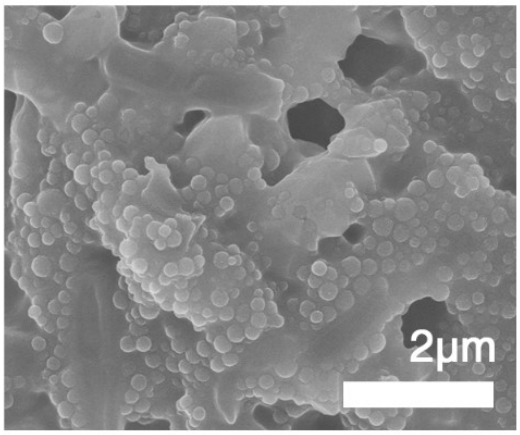


Figure S13. The SEM image of BCP after the catalytic reaction.

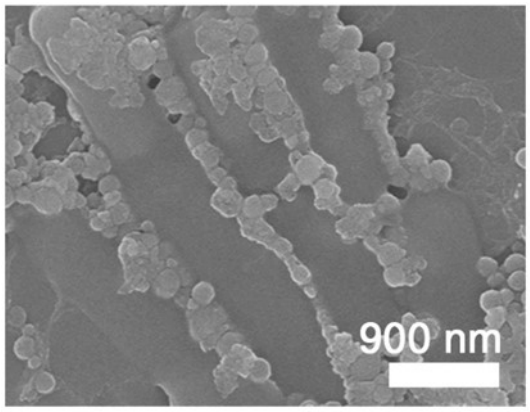


Figure S14. The SEM image of BCP after storing for 14 days.

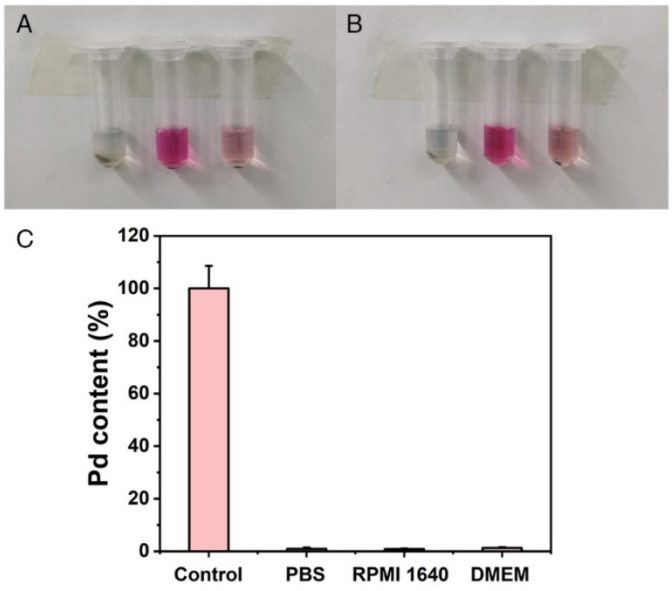


Figure S15. (A) Photograph of BCP in PBS, RPMI 1640, and DMEM media after centrifugation on day 0. (B) Photograph of BCP in PBS, RPMI 1640, and DMEM media after centrifugation on day 7. (C) Ion release of Pd from BCP in PBS, RPMI 1640, and DMEM media.

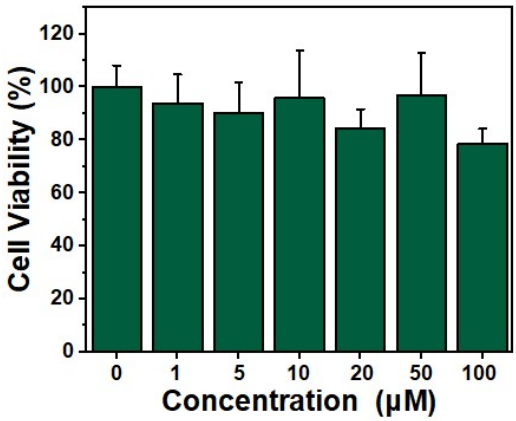


Figure S16. Cell viability of 3T3 cells after treating with various concentrations of Pd in BCP for 48 h. Error bars represent standard deviation from the mean (n = 3).

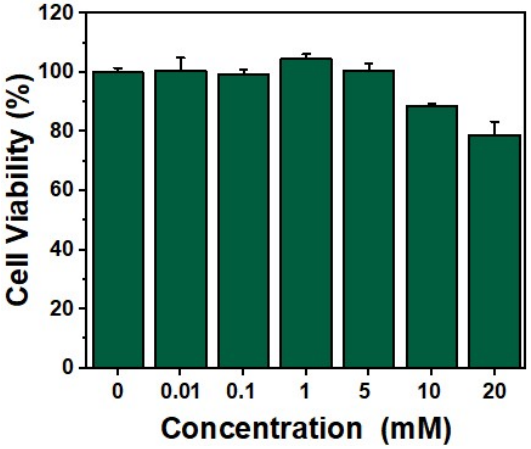


Figure S17. Cell viability of CT26 cells after treating with various concentrations of PB for 48 h. Error bars represent standard deviation from the mean (n = 3).

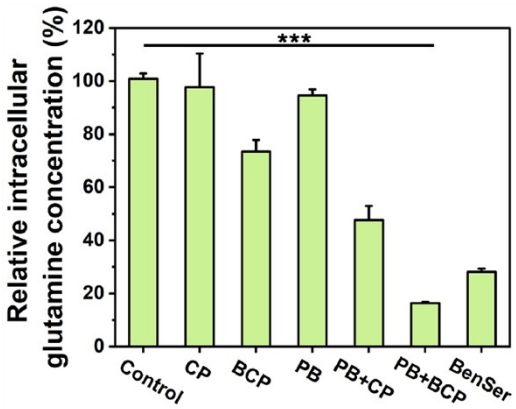


Figure S18. Relative intracellular glutamine levels in each group after different treatments (n = 3).

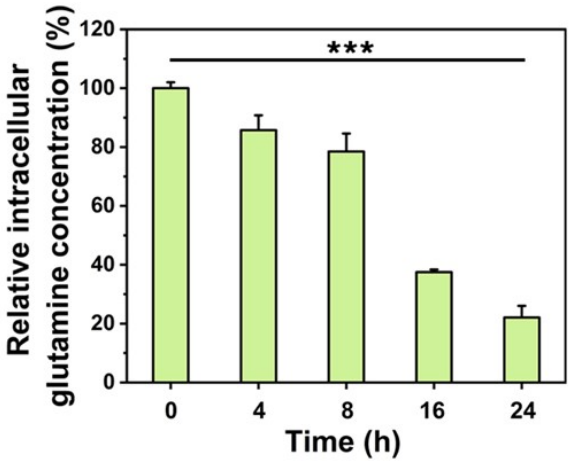


Figure S19. Relative intracellular glutamine levels in cells after PB+BCP treatment for the indicated time points (n = 3).

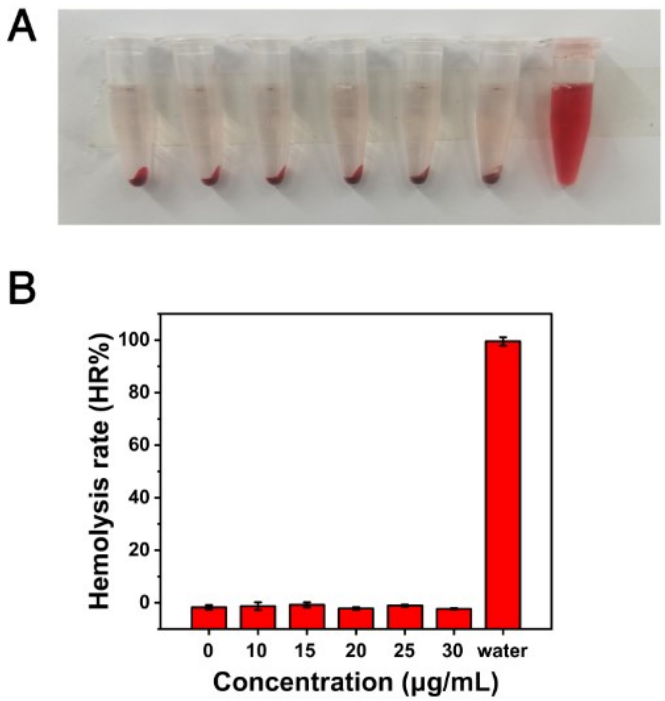


Figure S20. The hemolysis assay. (A) Typical digital photographs of concentration-dependent hemolysis of BCP. From left to right: hemolysis of BCP under various concentrations or H_2O (the positive control). (B) Concentration-dependent hemolysis of BCP. Error bars represent standard deviation from the mean (n = 3).

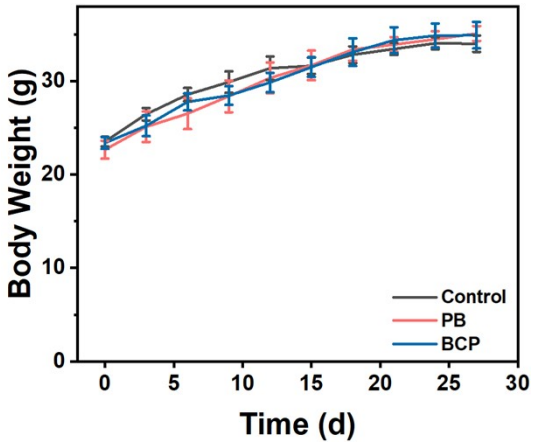


Figure S21. Bodyweight changes of mice after treating with the catalysts or the pro-drug, respectively. Error bars represent standard deviation from the mean (n = 5).

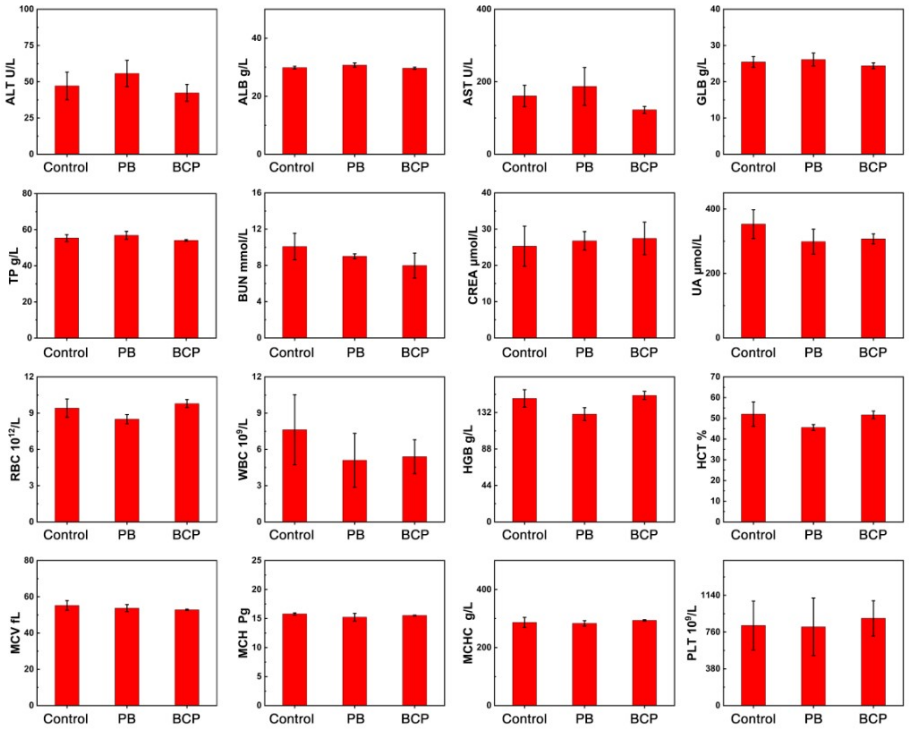


Figure S22. *In vivo* toxicity. Blood biochemistry assay and hematology assay of the mice after intravenously injection with BCP at 28 days post-injection. Error bars represent standard deviation from the mean (n = 3).

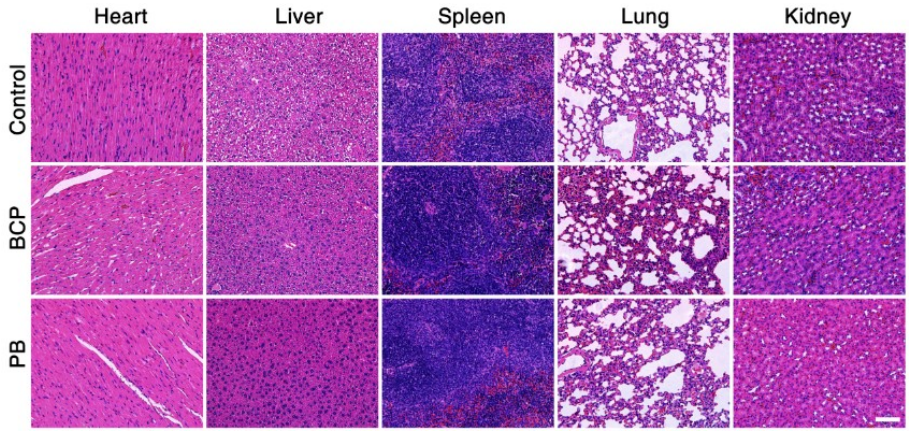


Figure S23. H&E-stained images of the major organs harvested from mice at 28 days' post-treatment. Scale bar is 50 μm.

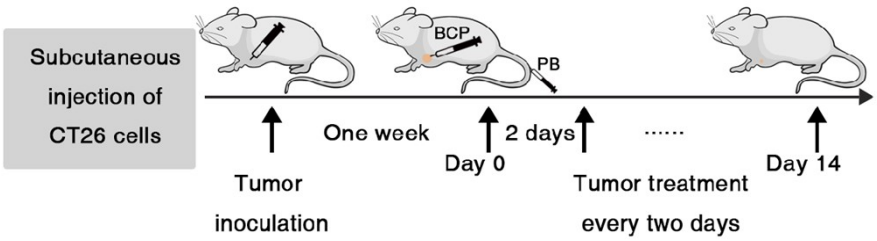


Figure S24. Schematic illustration of the *in vivo* antitumor experimental process.

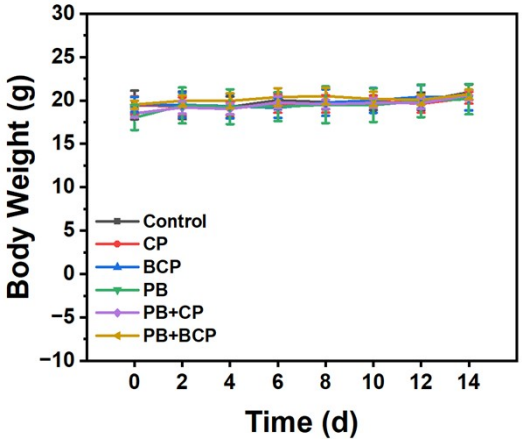


Figure S25. Bodyweight changes of the tumor-bearing mice in different groups during the experimental period. Error bars represent standard deviation from the mean (n = 5).

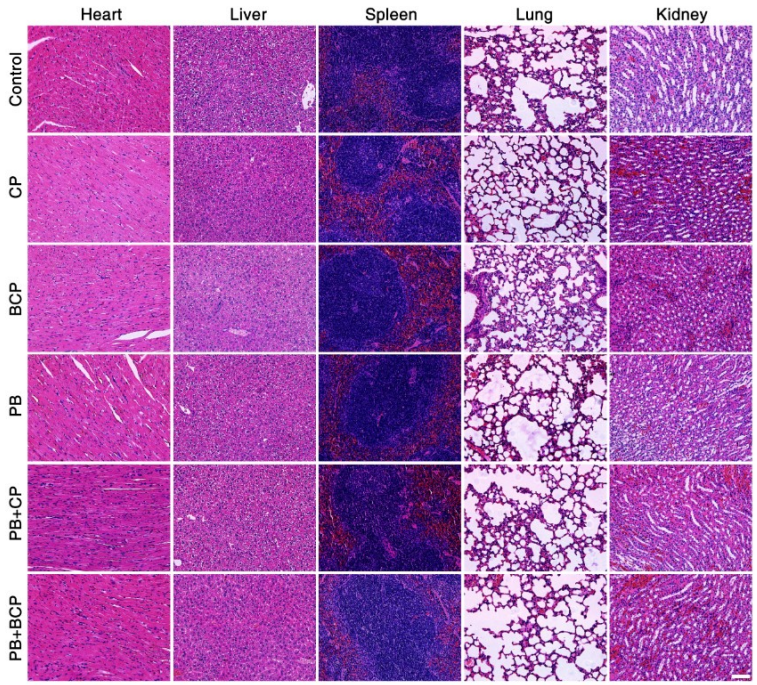
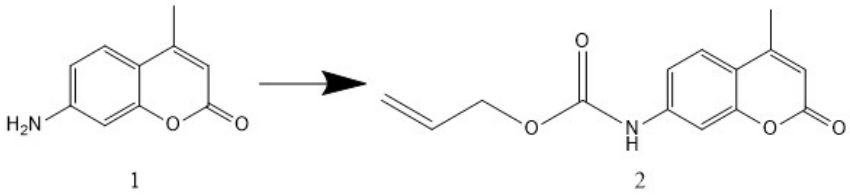


Figure S26. H&E-stained images of the major organs harvested from mice at 14 days' posttreatment. Scale bar is 50 μm.

Synthesis of N-allyloxycarbonyl coumarin



N-allyloxycarbonyl coumarin: allyl (4-methyl-2-oxo-2H-chromen-7-yl) carbamate was synthesized according to the literature with a little modification. In brief, 99.2 mg 7-Amino-4-methylcoumarin, 1, and 51.3 mg pyridine were dissolved in 3 mL DCM to obtain the yellow suspension, and then 84.1 mg allylchloroformate was added to the suspension. The mixture became a bright yellow suspension after stirring at 0 ℃ for 12 h. Then 40 mL 0.5 M HCl was added to the mixture, after filtering, washing by diethyl ether, and drying, N-alloc-coumarin was obtained, 2, and the product was used without further purification (Yield: 87%).

^{1}H NMR (600 MHz, DMSO) δ 10.24 (s, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.54 (d, J = 1.9 Hz, 1H), 7.41 (dd, J = 8.7, 2.0 Hz, 1H), 6.24 (d, J = 1.1 Hz, 1H), 6.00 (ddd, J = 22.7, 10.8, 5.5 Hz, 1H), 5.32 (ddd, J = 13.8, 11.8, 1.5 Hz, 2H), 4.65 (d, J = 5.5 Hz, 2H), 2.39 (d, J = 0.9 Hz, 3H).

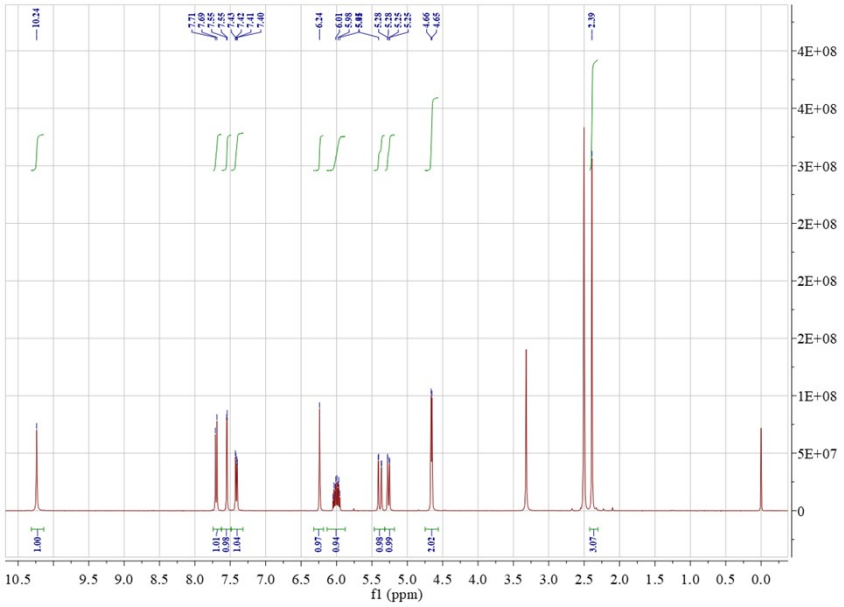
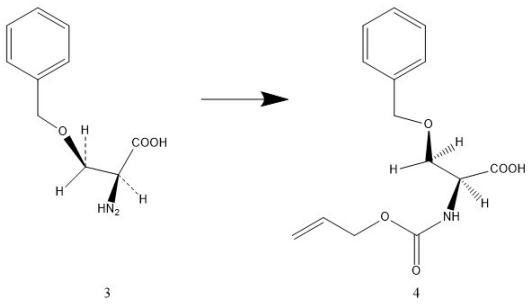


Figure S27. The ^{1}HNMR spectrum of N-alloc-coumarin, 2. (H_2O δ=3.3, DMSO-d6 δ=2.5)

Synthesis of Pro-BenSer



Prepare a 50 mL solution of 1N NaOH, dissolve O-benzyl-L-serine (976 mg, 5 mmol), and add chloromethyl acrylate (6 mmol). The mixture is stirred overnight at room temperature, then quenched by adding 100 mL of water to terminate the reaction. The aqueous phase is washed with ether, followed by adjusting the pH of the aqueous phase to 2 using cold 1N HCl. The water phase is then extracted multiple times with ethyl acetate, dried over anhydrous magnesium sulfate, and the solvent is evaporated under reduced pressure to obtain the product.

^{1}H NMR (600 MHz, DMSO) δ 7.35 – 7.26 (m, 5H), 5.91 (ddd, J=22.1, 10.5, 5.3, 1H), 5.62 (d, J=8.2, 1H), 5.27 (dd, J=38.7, 13.8, 2H), 4.59 – 4.54 (m, 4H), 3.94 (d, J=7.5, 1H), 3.72 (dd, J=9.4, 3.1, 1H).

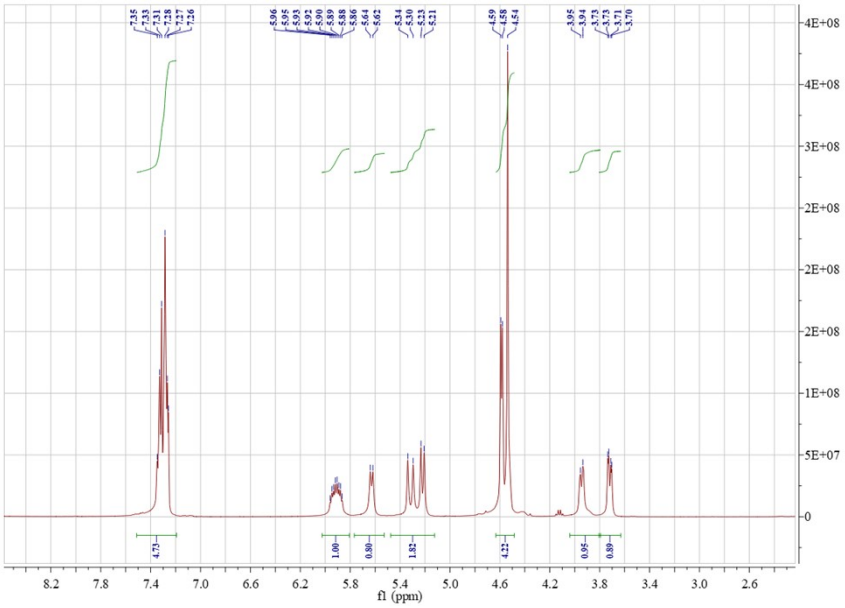


Figure S28. The ^{1}HNMR spectrum of PB, 4. (DMSO-d6 δ=2.5).

References

(1) Hu, C.; Cai, L.; Liu, S.; Pang, M. Integration of a highly monodisperse covalent organic framework photosensitizer with cation exchange synthesized Ag2Se nanoparticles for enhanced phototherapy. *Chem. Commun.* **2019**, *55* (62), 9164-9167.

(2) Zhang, L.; Sang, Y. J.; Liu, Z. Q.; Wang, W. J.; Liu, Z. W.; Deng, Q. Q.; You, Y. W.; Ren, J. S.; Qu, X. G. Liquid Metal as Bioinspired and Unusual Modulator in Bioorthogonal Catalysis for Tumor Inhibition Therapy. *Angew. Chem. Int. Ed.* **2023**, *62* (9), e202218159.