Bicyclic 2-Pyridone, with Fused Heterocyclic Rings, a Facile Core for Pure Type I Photosensitizers

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Materials

All materials were used directly without further purification. citric acid, (1S,2S) -(-)-1,2-Diphenyl-1,2-ethanediamine, Thionyl chloride, N-Bromosuccinimide, Sodium Methoxide, Silver carbonate, Potassium phosphate, Tetrakis(triphenylphosphine)palladium, 4-(Diphenylamino)phenylboronic acid and were purchased from Energy Chemical. methanol, petroleum ether, ethyl acetate, 1,4-dioxane and dichloromethane (DCM), dimethyl sulfoxide (DMSO), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), rose bengal (RB) and tetrabutylammonium hexafluorophosphate ($(n\text{-}Bu)_4N^+PF_6^-$) were purchased from BT Reagent. Pluronic F127 was purchased from Bidepharm.

Penicillin-streptomycin was purchased from Macgene (China). RPMI-1640 and FBS were purchased from Gibco (USA). Calcein-AM/PI Live-Dead Cell Staining Kit, Reactive Oxygen Species Assay Kit and Lyso-Tracker Green were purchased from Beijing Solarbio Science and Technology Co., Lta. The 2',7'-dichlorofluorescein diacetate (DCFH-DA), dihydrorhodamine 123 (DHR123) and 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) required for the active oxygen test were purchased from Shanghai McLean Biochemical Technology Co., Ltd.

Equipment

^1H and ^{13}C NMR spectra were recorded on a Bruker AV 400 spectrometer. Mass spectra (MS) were collected using a Finnigan Biflex III mass spectrometer. The ultraviolet-visible spectra were collected on the Thermo Fisher UV-2700

spectrophotometer. The PL emission spectra were collected on the HORIBA FluoroMax-4 spectrofluorometer. Measure the particle size of nanoparticles using a BeNano 180 Zeta particle size analyzer. Measure cytotoxicity using BIO-RAD enzyme-linked immunosorbent assay (ELISA) reader. Record cell imaging using a confocal laser PL microscope (Zeiss LSM 980). *In vivo* imaging was measured on IVIS lumina series III.

Preparation of BCP-TPA NPs

Dissolve 1 mg BCP-TPA and 10 mg Pluronic F127 in 1 mL of DMSO solution, and poured it into 9 mL ultrapure water under ultrasonic conditions for 10 min. Centrifuge at 5000 rpm for 30 min using ultrafiltration centrifuge tubes to concentrate and obtain nanoparticles. It is named BCP-TPA NPs. The standard curve was obtained through the ultraviolet absorption test, and then the final concentration of BCP-TPA NPs was calculated to be 2.85×10^{-3} mol/L. Ultrapure water was added to prepare a solution with a certain concentration for subsequent biological applications.

Total ROS detection by indicator DCFH

First, 1 mM DCFH-DA in ethanol and 10 mM NaOH aqueous solution were prepared, then 2 mL of NaOH solution was mixed with 0.5 mL of DCFH-DA solution, and the reaction was carried out in the dark for 30 min. After the reaction, 10 mL of PBS solution was added to obtain a DCFH solution with a concentration of 40 μM. Subsequently, an accurately weighed of the PSs was dissolved in DMSO to prepare a stock solution at a concentration of 1 mM. Finally, the experimental procedure was divided into three groups: Group illumination blank: DCFH-DA (50 μL, 40 μM) + PBS (1950 μL); Group illumination experimental: PSs (20 μL, 1 mM) + DCFH-DA (50 μL, 40 μM) + PBS (1930 μL); Group dark control: PSs (20 μL, 1 mM) + DCFH-DA (50 μL, 40 μM) + PBS (1930 μL), maintained in light-absent conditions. All reagents in the above three groups of experiments were prepared by ready-to-use preparation to ensure the activity of the solutions. The lighting condition of the illumination group was white light (2 mW cm^{-2}). The fluorescence intensity of the DCF at the maximum wavelength of 520 nm was detected.

Detection of $^1O_2$ Generation by indicator ABDA

Firstly, weigh 1 mg of ABDA solid powder and dissolve it in 244 μL of DMSO to prepare a 10 mM ABDA solution Liquid. Secondly, an accurately weighed of the PSs was dissolved in DMSO to prepare a stock solution at a concentration of 1 mM. Then, taking RB as the control, weigh 1 mg of Bengal Rose Red (RB) and dissolve it in 0.98 mL of deionized water to prepare a 1 mM RB solution. Finally, the experimental procedure was divided into two groups: Group RB control: ABDA (20 μL, 10 mM) + RB (20 μL, 1 mM) + deionized water (1960 μL); Group illumination experimental: ABDA (20 μL, 10 mM) + PSs (20 μL, 1 mM) + deionized water (1960 μL). The lighting condition of the illumination group was white light (2 mW cm^{-2}). The intensity of the UV absorption peak of ABDA was detected in the wavelength range of 300 nm - 500 nm.

Detection of O_2^{-}• Generation by indicator DHR123

Firstly, weigh 1 mg of DHR123 solid powder and dissolve it in 5 mL of DMSO to prepare a 40 μM DHR123 solution. Subsequently, an accurately weighed of the PSs was dissolved in DMSO to prepare a stock solution at a concentration of 1 mM. Finally, the experimental procedure was divided into two groups: Group illumination blank: DHR123 (50 μL, 40 μM) + PBS (1950 μL); Group illumination experimental:30 μL DHR123 (40 μM) + 20 μL PSs (10 μM) + 1950 μL PBS, after irradiated by white light irradiation of 2 mW cm^{-2}, the fluorescence signal of indicator was monitored to indicate the O_2^{-}• generation rate with the excitation wavelength of 488 nm and the emission wavelength of 525 nm. All reagents in the above three groups of experiments were prepared by ready-to-use preparation to ensure the activity of the solutions.

Cell Culture

4T1 cells (mouse breast cancer cell line) were obtained from National Infrastructure of Cell Line Resource (NICR), and were cultured in RPMI-1640 media containing 10% FBS and 2% penicillin-streptomycin at 37 ℃ in a humidified atmosphere of 5% CO_2.

In Vitro Cytotoxicity

4T1 cells were seeded in 96-well plates at a density of 5 × 10^3 cells/well and incubated for 24 h. Then the cells were incubated with different concentrations of BCP-TPA NPs in fresh medium. The cells were exposed to white light of 5 mW cm^{-2} for 10 min after 24 h incubation. At the same time, the BCP-TPA NPs incubated cells without laser irradiation were also conducted for the dark cytotoxicity study. After further incubation for 24 h, the medium was removed and washed with PBS for three times. Cells were then incubated with fresh serum-free medium containing 10% MTT for 4 h in the dark. Then, all the media were removed and 150 μL DMSO was added. Finally, the absorbance of the products was measured at a wavelength of 570 nm by a microplate reader. The results were expressed as the viable percentage of cells after different treatments relative to the control cells without any treatment. The following steps of MTT test were the same as the above procedures.

Colocalization Imaging in 4T1 Cell

4T1 cells were seeded in Φ20 mm glass bottom cell culture dishes (1.0 ± 0.05 × 10^6 cells in each dish). After overnight culture in a humidified incubator at 37 ℃ with 5% CO_2, culture medium was removed and cells were stained with BCP-TPA NPs (10 μM) for 15 min. After washed by PBS for 3 times, 4T1 cells were fixed with 4% fixative solution for 10 min. Before imaging, each dish was washed by PBS for 3 times. For co-localization with LysoTracker, the fixed cells were stained with LysoTracker (50 nM) for 10 min at 37 ℃.

Live/Dead Cell Staining

First, 4T1 cells were seeded and cultured in glass bottom dish for 24 h, then exposed to different following treatments: 1) Blank; 2) the cells were exposed to white light of 5 mW cm^{-2} for 10 min; 3) incubated with BCP-TPA NPs for 30 min; 4) incubated with BCP-TPA NPs for 30 min and exposed to white light of 5 mW cm^{-2} for 10 min. Then successively stained with Calcein-AM in PBS for 20 min, PI in PBS for 5 min. Subsequently, the cells were gently washed and then imaged by CLSM. Conditions: excitation wavelength: 488 nm for Calcein-AM and 543 nm for PI; emission filter: 500-550 nm for Calcein-AM and 550-650 nm for PI.

Intracellular ROS Generation

4T1 cells were primarily seeded and cultured in glass bottom dish for 24 h. The original culture medium was then replaced with 1 mL of fresh one with or without BCP-TPA NPs, followed by incubation of 30 min. Then the cells were washed with PBS for three times, and incubated with 1 mL fresh FBS-free medium containing 10 mM DCFH-DA for another 20 min. The BCP-TPA NPs loaded cells were subsequently exposed to white light of 5 mW cm^{-2} for 10 min. After further incubation at 37 °C, the cells were imagedby CLSM with the excitation at 488 nm and emission was collected from 500-550 nm.

Animals

BALB/c (female, 4-6 weeks) mice were purchased from Chengdu Dashuo Experimental Animal Co., Ltd.

Statement of ethical approval: All mice were housed in designated animal facilities, fed ad libitum and inspected regularly. All animal studies were performed in accordance with the Regulations for Care and Use of Laboratory Animals and Guideline for Ethical Review animals (China, GB/T 35892-2018). All animal studies were approved by the Sichuan University Animal Charity Protection and Treatment Committee and performed in accordance with humane care and use of research animals. All animal procedures were reviewed and approved by Ethical Committees of West China School of Stomatology, Sichuan University (WCHSIRB-D-2017-042).

Feeding conditions: all the animals were submitted to controlled temperature conditions (22 ~ 26 ^{o}C), humidity (50 ~ 60%) and light (12 h light/12 h dark, 15 ~ 20 LX).

Fluorescence imaging of tumors and major organs

BALB/c mice (female, 5 weeks, 20 in total) bearing 4T1 tumors (≈100 mm^3) were divided into 6groups according to different circulation times: 3 h, 6 h, 9 h, 12 h, 24 h, and 48 h. BCP-TPA NPs (10 μM,100 μL) were injected into different groups of mice by tail vein injection. The enrichment of photosensitizers in tumors at different time were detected, respectively. The enrichment of BCP-TPA NPs in heart, liver, spleen, lung, kidney and tumor were also compared at 48 hours. Fluorescent images of tumors and organs were captured by IVIS Lumina Series III.

In Vivo Therapeutic Studies

BALB/c mice (female, 5 weeks, 20 in total) bearing 4T1 tumors were randomly divided into four groups (5 in each group), group PBS, administration with PBS (100 μL) alone; group Light+PBS Only, PBS administration (100 μL) followed by wight light (200 mW cm^{-2} , 10 min); group BCP-TPA NPs Only, administration with BCP-TPS NPs (10 μM, 100 μL); group BCP-TPA NPs + light, administration with BCP-TPA NPs (10 μM, 100 μL) and followed by wight light (200 mW cm^{-2}, 10 min). During the treatment period, the tumor volume of all mice was measured every two days using a vernier caliper. Then, the greatest longitudinal diameter (length) and the greatest transverse diameter (width) were used to calculate the tumor volume. Tumor volume V = length × $width^2$ /2. After 14 days post-treatment, tumors in all groups were harvested and weighed. For histological analysis, the hematoxylin-eosin (H&E) staining of tumor slices was carried out. Meanwhile, the fresh blood samples were collected for serum biochemistry text. The healthy mice without any treatment were used as control.

Synthesis of compound iBCP-COOH: Add (1s, 2s) -1,2-diphenylethylenediamine (10.0 mmol, 2.1 g) and citric acid (10.0 mmol, 1.9 g) to the hydrothermal synthesis kettle. React at 140 °C for 4 h. After the reaction is completed, take out the crude product and place it in ethanol (40.0 mL). Heat it to 70 °C, stir for 0.5 h, filter while hot, dry the filter cake, and obtain the final product with a yield of 87%. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 13.29 (s, 1H), 8.42 (s, 1H), 7.47 – 7.37 (m, 5H), 7.37 – 7.31 (m, 3H), 7.27 – 7.20 (m, 2H), 6.01 (d, *J* = 1.5 Hz, 1H), 5.91 (d, *J* = 1.5 Hz, 1H), 5.32 (d, *J* = 2.7 Hz, 1H), 4.85 (d, *J* = 2.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ 175.0, 171.8, 167.2, 160.1, 154.7, 145.2, 142.1, 139.9, 129.5, 129.3, 128.8, 128.5, 126.2, 126.1, 105.8, 80.8, 72.9, 68.1, 66.6, 43.2. HRMS(ESI): calcd for $C_{20}H_{17}N_2O_3^+$(M+H)$^+$: 333.1242, found: 333.1239.

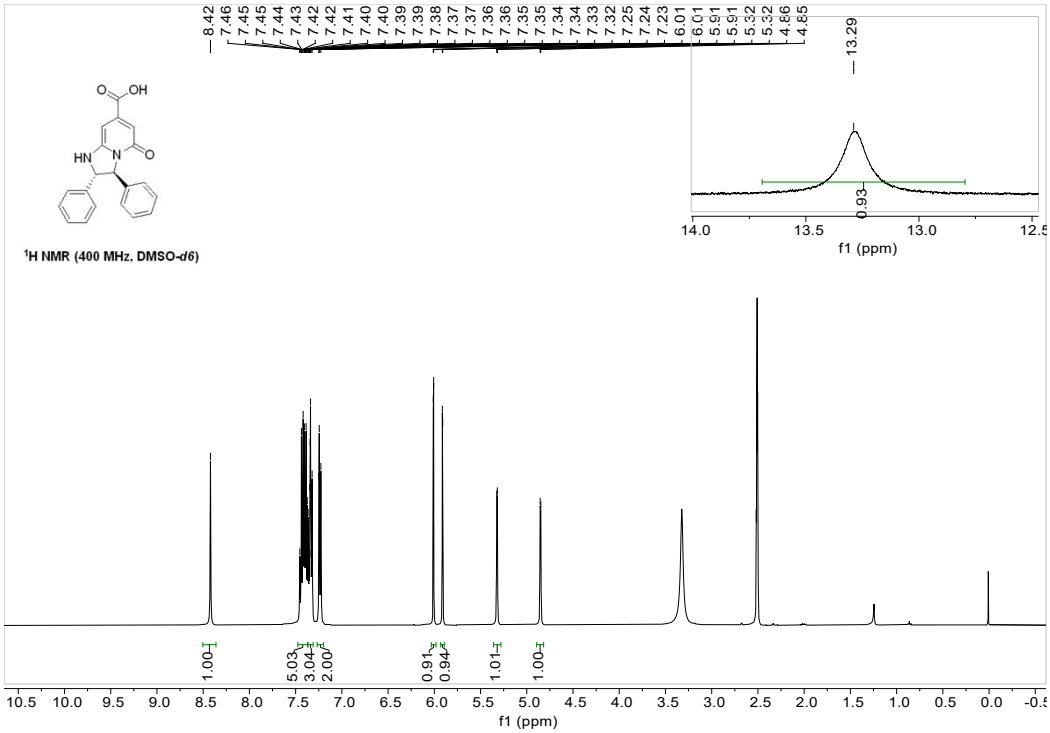

Fig. S1. ^{1}H NMR spectrum of compound **iBCP-COOH** in DMSO-*d6*.

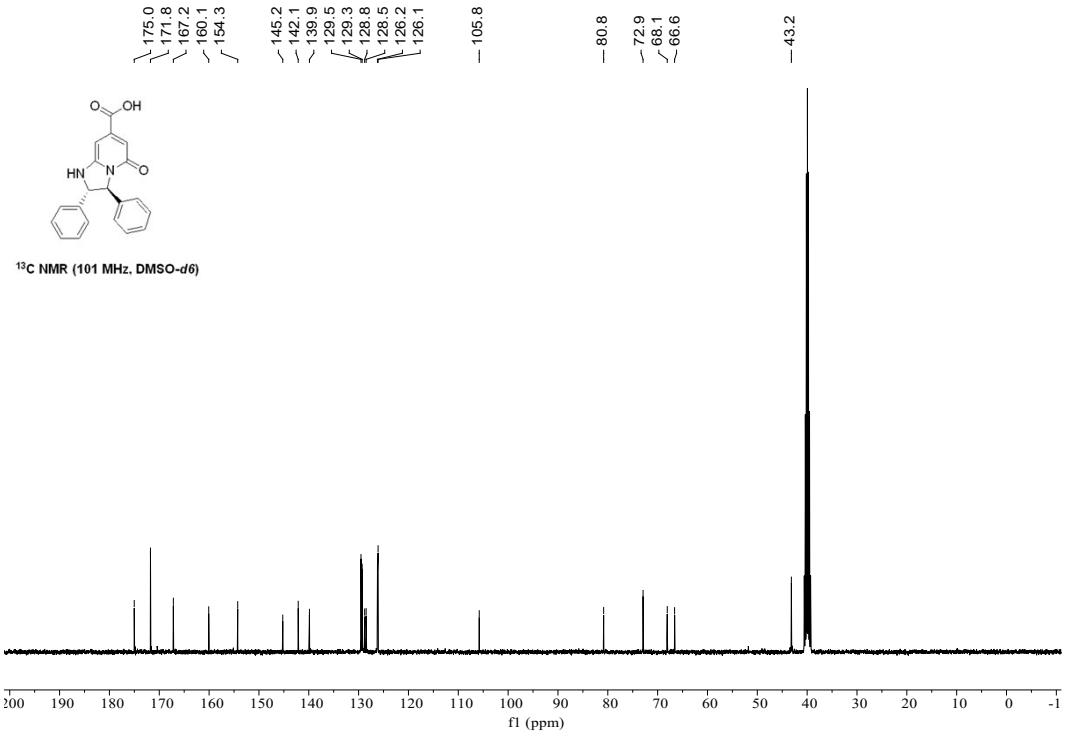

Fig. S2. ^{13}C NMR spectrum of compound **iBCP-COOH** in DMSO-*d6*.

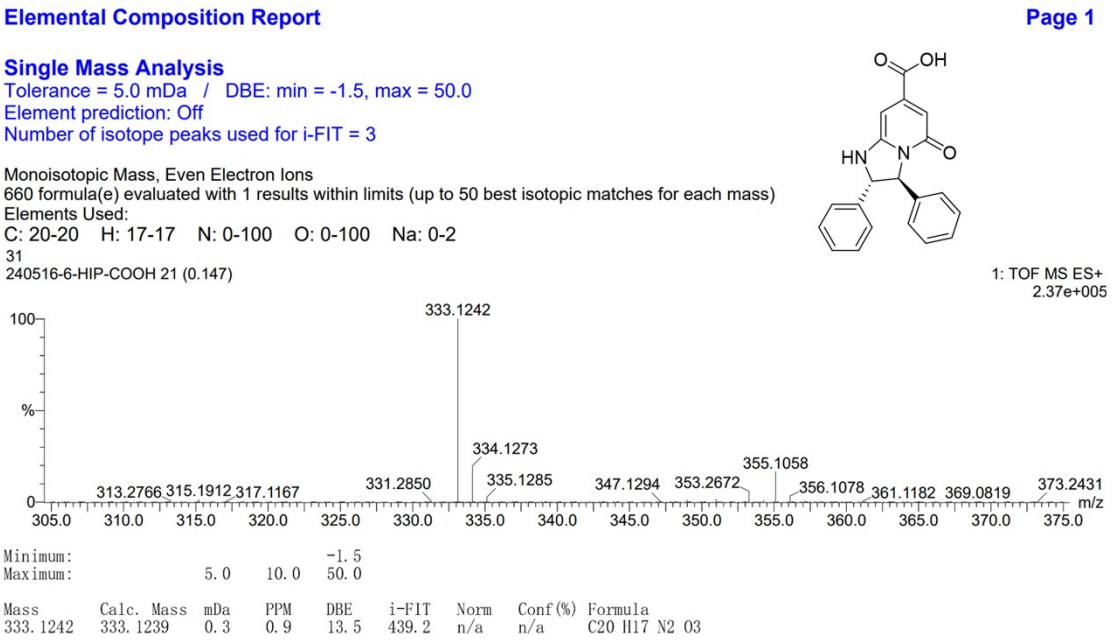

Fig. S3. HRMS spectrum of compound **iBCP-COOH**.

Synthesis of compound iBCP-COOME: Dissolve iBCP-COOH (2.0 mmol, 662.0 mg) in methanol (20.0 mL), add dichlorosulfoxide dropwise at 0 °C. After the dropwise addition, transfer the reaction solution to 70 °C and continue stirring for 4 h. After TLC detection, reduce the pressure of the reaction solution and concentrate it. Add water (30.0 mL), extract with ethyl acetate (3 × 30 mL), merge the organic layers, dry the organic layers with anhydrous $MgSO_4$, and concentrate under reduced pressure to obtain the crude product. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a dichloromethane /methanol mixture (10/1, V_{PE}/V_{EA}) as the eluent to give desired compound iBCP-COOME with 85.8% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (s, 1H), 7.46 – 7.35 (m, 6H), 7.35 – 7.32 (m, 2H), 7.26 – 7.21 (m, 2H), 6.03 (d, J = 1.6 Hz, 1H), 5.93 (d, J = 1.6 Hz, 1H), 5.34 (d, J = 2.7 Hz, 1H), 4.88 (d, J = 2.7 Hz, 1H), 3.84 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 166.1, 159.9, 154.4, 143.8, 142.0, 139.8, 129.5, 129.3, 128.8, 128.5, 126.2, 126.1, 105.7, 80.5, 68.1, 66.6, 53.1. **HRMS(ESI):** calcd for $C_{21}H_{19}N_2O_3^+$(M+H)$^+$: 347.1400, found: 347.1396.

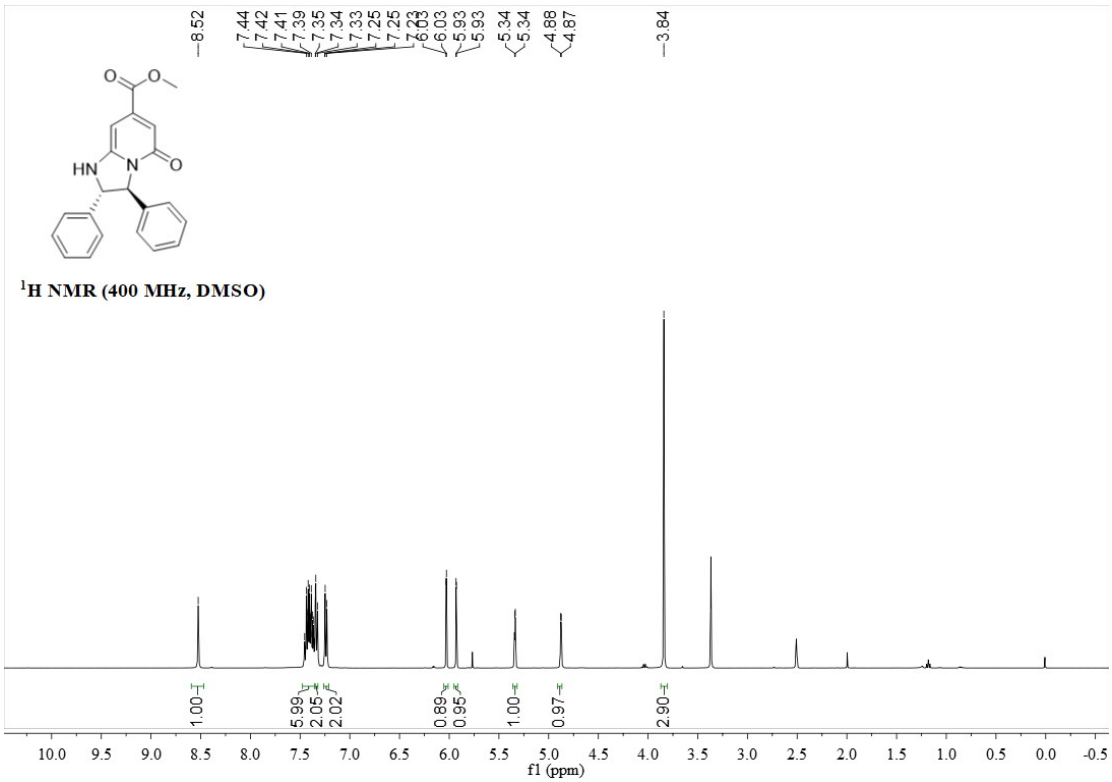

Fig. S4. ^{1}H NMR spectrum of compound **iBCP-COOME** in DMSO-*d6*.

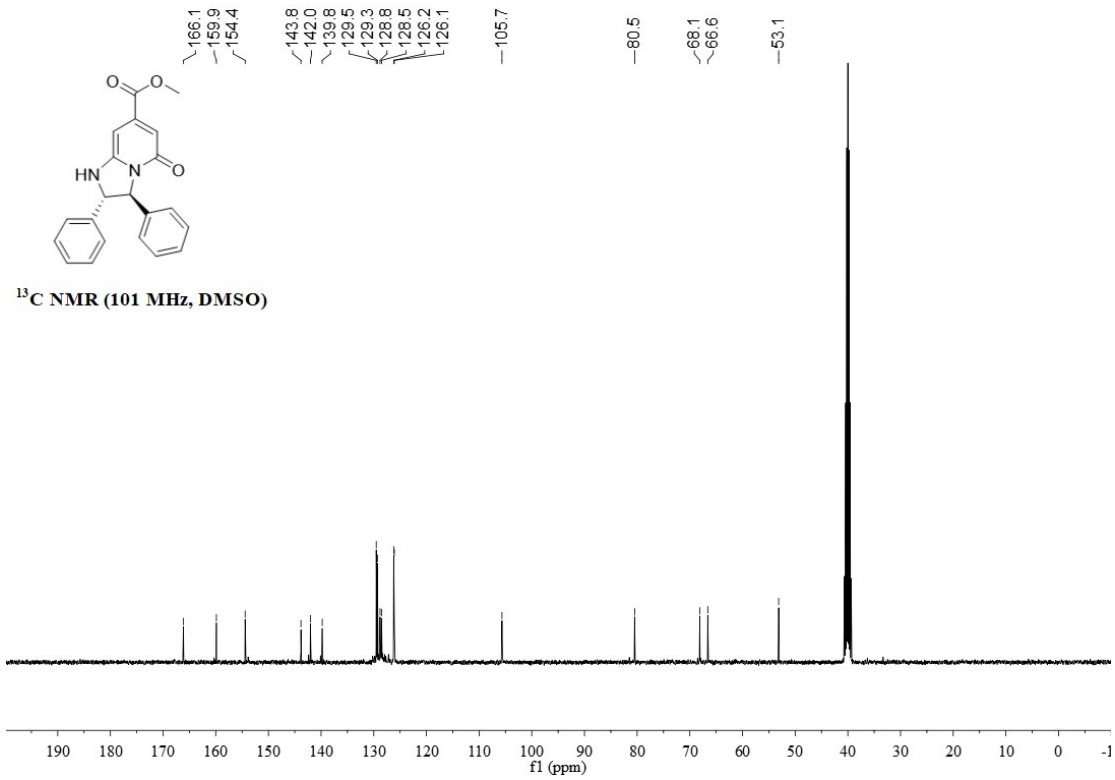

Fig. S5. ^{13}C NMR spectrum of compound **iBCP-COOME** in DMSO-*d6*.

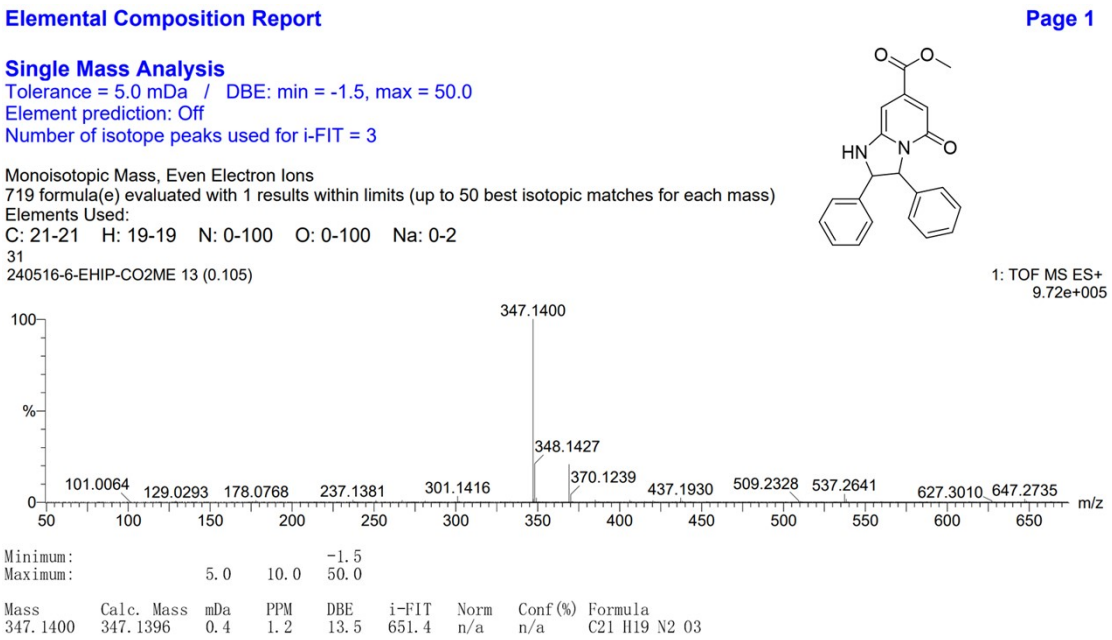

Fig. S6. HRMS spectrum of compound **iBCP-COOME**.

Synthesis of compound iBCP-Br: Dissolve compound iBCP-COOME (2.0 mmol, 692.0 mg) in dichloromethane (30.0 mL), then add N-bromosuccinimide (6.0 mmol, 1.1 g) and stir continuously at room temperature for 2 h until TLC detects the end of the reaction. Add water (30.0 mL), extract the reaction solution with dichloromethane (3 × 30 mL), merge the organic layers, dry the organic layers with anhydrous $MgSO_4$, and concentrate under reduced pressure to obtain the crude product. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a dichloromethane/methanol mixture (20/1, V_{PE}/V_{EA}) as the eluent to give desired compound iBCP-COOME with 89.8% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.03 (s, 1H), 7.48 – 7.38 (m, 6H), 7.36 – 7.32 (m, 2H), 7.28 – 7.24 (m, 2H), 5.46 (d, J = 4.0 Hz, 1H), 4.93 (d, J = 3.8 Hz, 1H), 3.92 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 165.5, 154.8, 151.3, 148.6, 141.0, 139.1, 129.6, 129.5, 129.1, 128.9, 126.5, 126.4, 94.0, 70.8, 67.9, 67.0, 53.7. **HRMS(ESI):** calcd for $C_{21}H_{16}N_2O_3Br_2Na^+$(M+Na)$^+$: 524.9415, found: 524.9425.

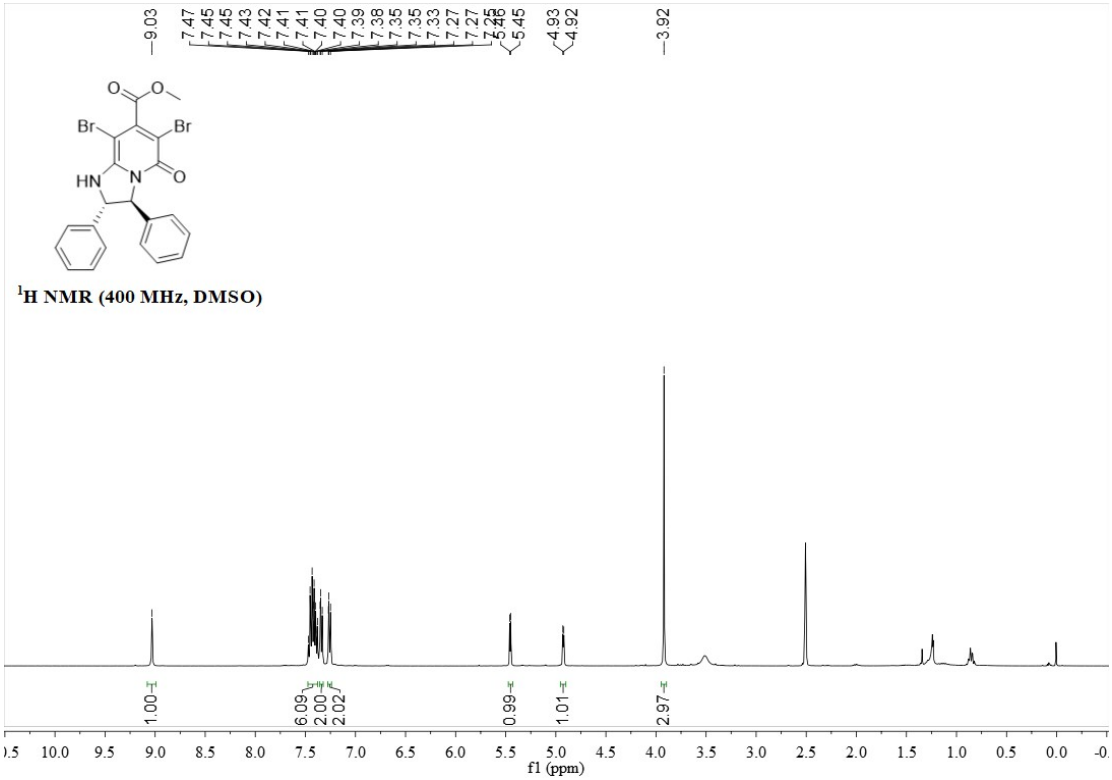

Fig. S7. [1]H NMR spectrum of compound **iBCP-Br** in DMSO-*d6*.

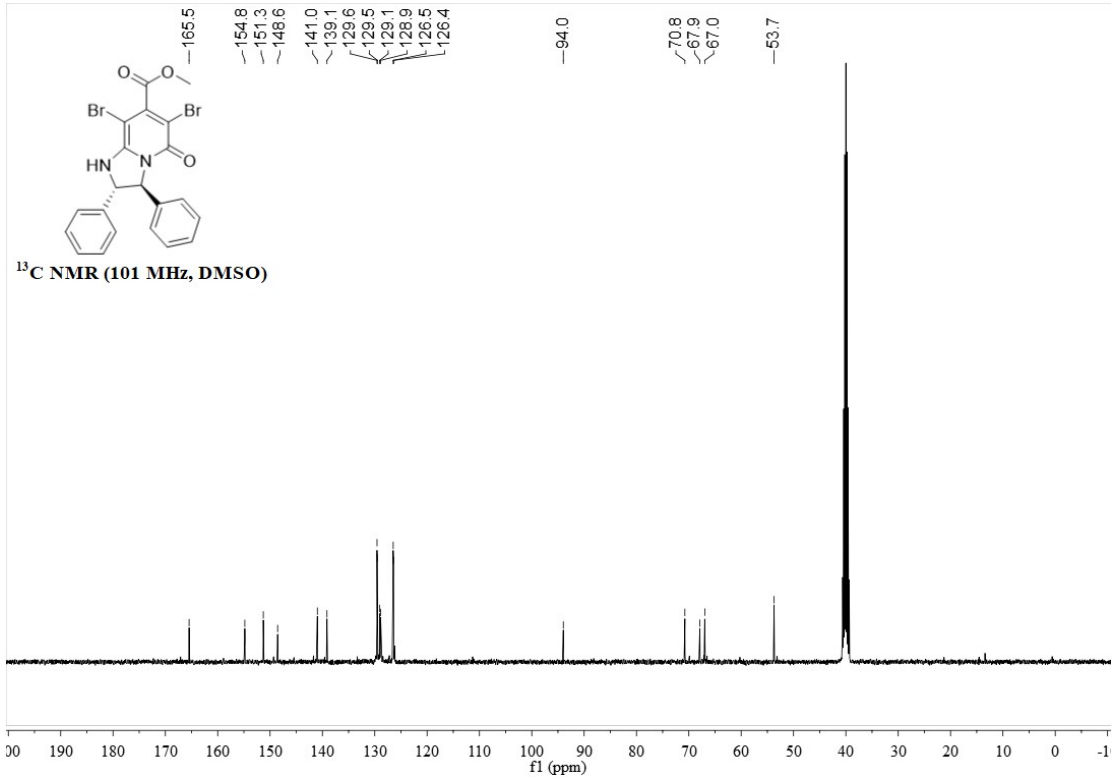

Fig. S8. [13]C NMR spectrum of compound **iBCP-Br** in DMSO-*d6*.

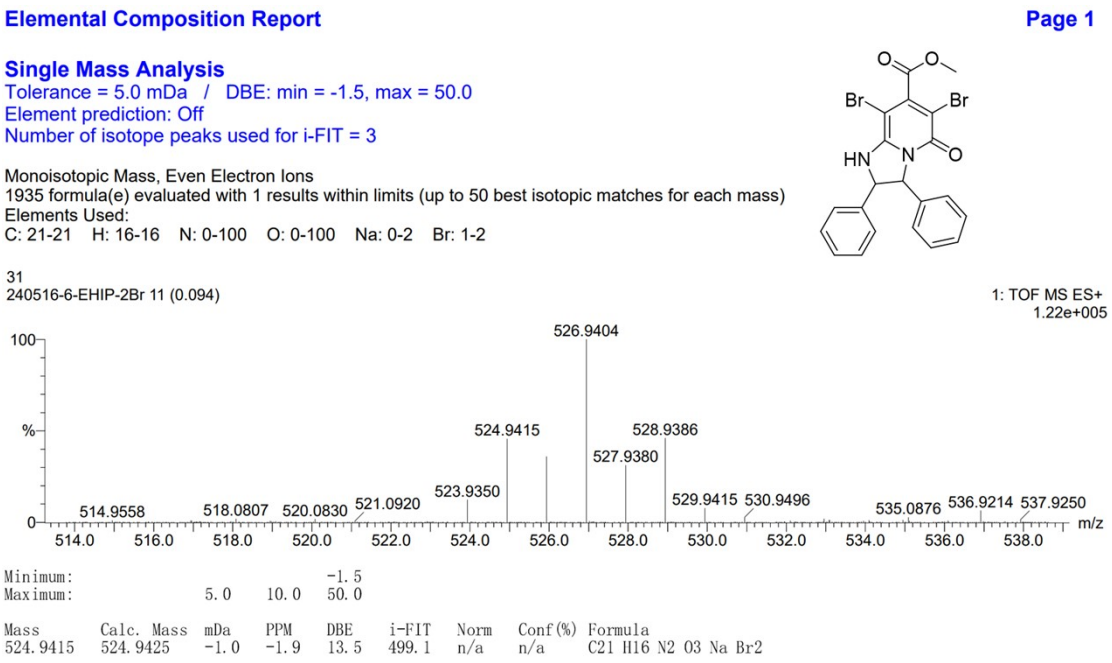

Fig. S9. HRMS spectrum of compound **iBCP-Br**.

Synthesis of compound BCP-Br： Dissolve compound iBCP-Br (2.0 mmol, 1.0 g) in methanol (30.0 mL), add silver carbonate (2.0 mmol, 551.5 mg) and sodium methoxide (8.0 mmol, 432.0 mg), and stir at 65 °C for 3 hours. After the TLC detection reaction is completed, adjust the pH of the reaction mixture to 3-4, filter out the filter residue, concentrate the filtrate under reduced pressure, add water (20.0 mL), then extract with dichloromethane (3 × 20 mL), merge the organic layers, dry the organic layers with anhydrous $MgSO_4$, and concentrate under reduced pressure to obtain the crude product. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a dichloromethane/methanol mixture (15/1, V_{PE}/V_{EA}) as the eluent to give desired compound BCP-Br with 7.5% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.09 (d, *J* = 7.3 Hz, 2H), 7.51 (dd, *J* = 6.1, 1.9 Hz, 3H), 7.38 (t, *J* = 7.8 Hz, 2H), 7.35 – 7.30 (m, 3H), 4.03 (s, 3H), 3.30 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 177.1, 164.6, 153.8, 151.3, 147.8, 134.1, 133.0, 129.8, 129.7, 129.0, 128.7, 128.5, 126.0, 114.4, 105.6, 88.8, 53.5, 52.7. **HRMS(ESI):** calcd for $C_{22}H_{16}N_2O_4Br_2Na^+$(M+Na) $^+$: 552.9380, found: 552.9375.

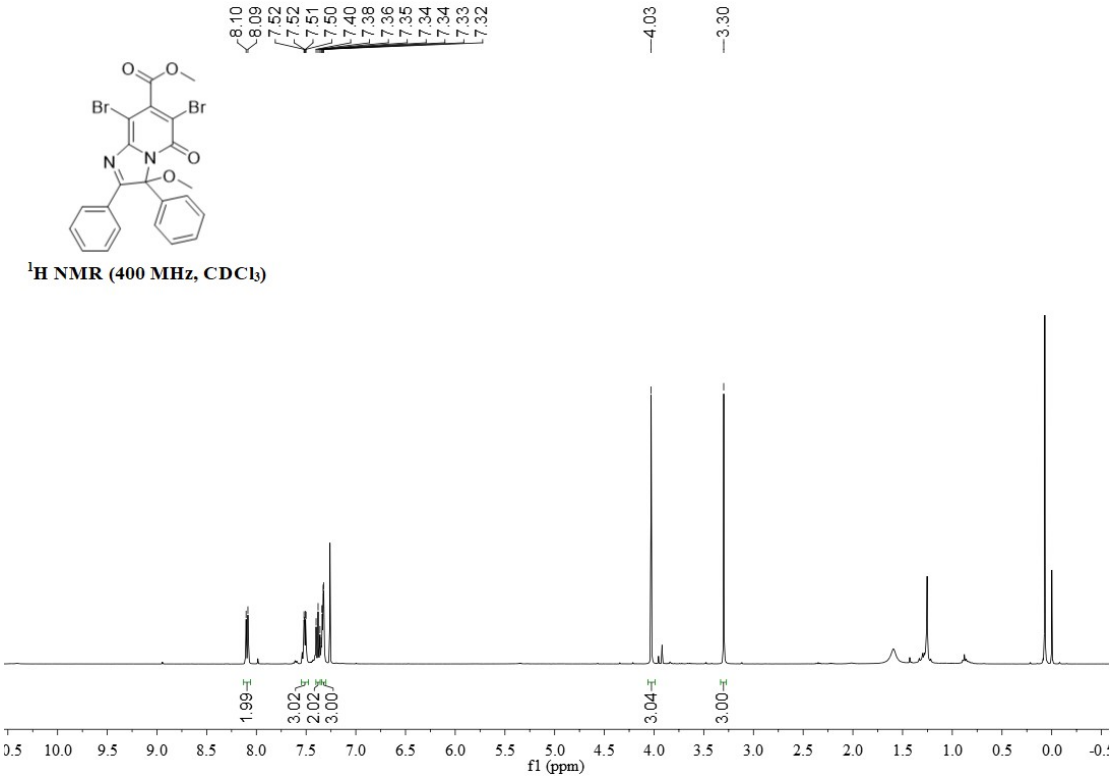

Fig. S10. [1]H NMR spectrum of compound **BCP-Br** in Chloroform-*d*.

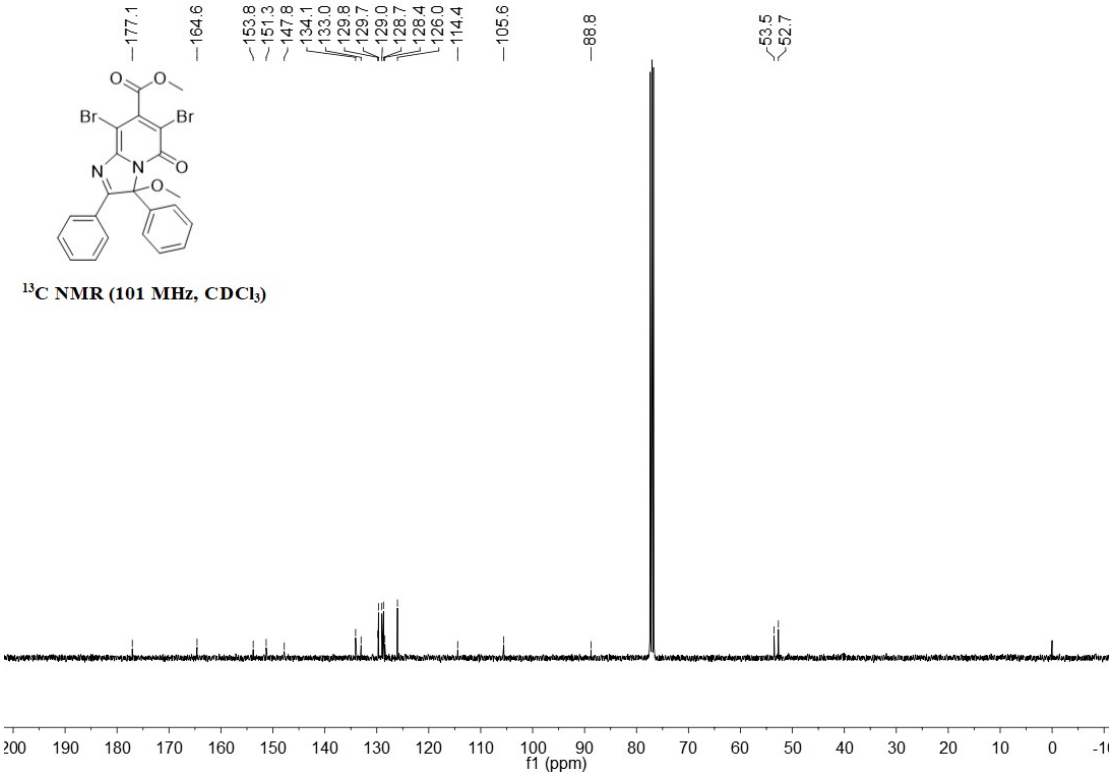

Fig. S11. [13]C NMR spectrum of compound **BCP-Br** in Chloroform-*d*.

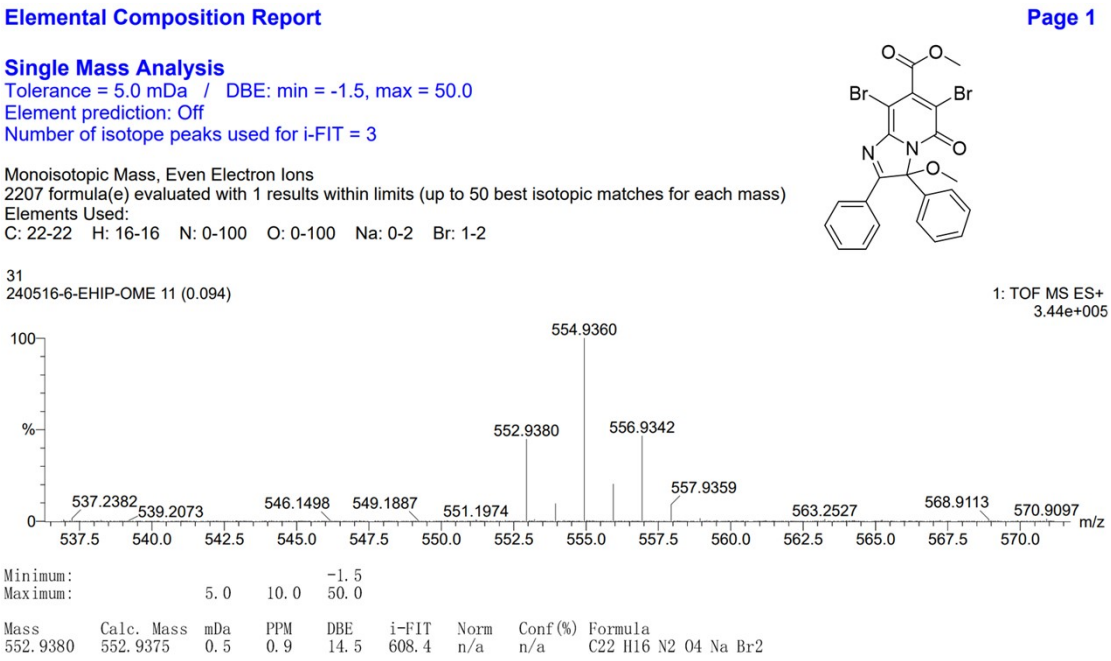

Fig. S12. HRMS spectrum of compound **BCP-Br**.

Synthesis of compound BCP-TPA： Dissolve compound BCP-Br (0.2 mmol, 106.0 mg) in 1,4-dioxane (15.0 mL), add potassium phosphate (0.6 mmol, 127.2 mg), tetratriphenylphosphine palladium (0.04 mmol, 46.2 mg), and 4-borate triphenylamine (1.0 mmol, 289.0 mg), and stir under argon protection at 100 °C for 12 h. After the TLC detection reaction is completed, the reaction solution is concentrated under reduced pressure, and water (20.0 mL) is added. Then, ethyl acetate (3 × 15 mL) is extracted and the organic layers are merged. The organic layers are dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to obtain the crude product. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography using a dichloromethane/methanol mixture (20/1, V_{PE}/V_{EA}) as the eluent to give desired compound BCP-TPA with 25.0% yield. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.05 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.0 Hz, 2H), 7.47 – 7.40 (m, 3H), 7.38 – 7.29 (m, 9H), 7.28 – 7.15 (m, 12H), 7.12 – 7.06 (m, 6H), 7.07 – 6.98 (m, 4H), 3.53 (s, 3H), 3.39 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 174.6, 167.5, 147.8, 147.6, 147.6, 147.5, 144.6, 134.5, 132.9, 130.9, 130.6, 129.4, 129.2, 128.7, 128.6, 127.2, 126.1, 124.9, 124.7, 123.3, 123.1, 122.3, 122.3, 103.1, 52.3, 52.1, 29.7, 26.9. **HRMS(ESI)**: calcd for $C_{58}H_{45}N_4O_4^+(M+H)^+$: 861.3442, found: 861.3441.

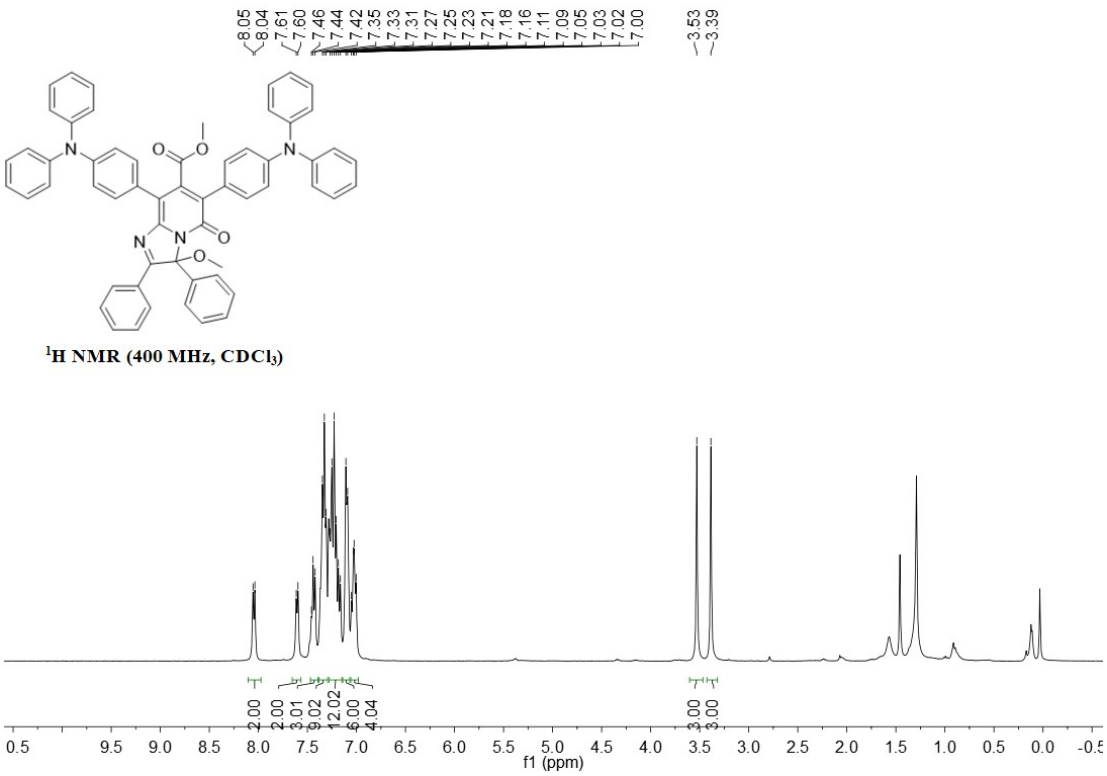

Fig. S13. [1]H NMR spectrum of **BCP-TPA** in Chloroform-*d*.

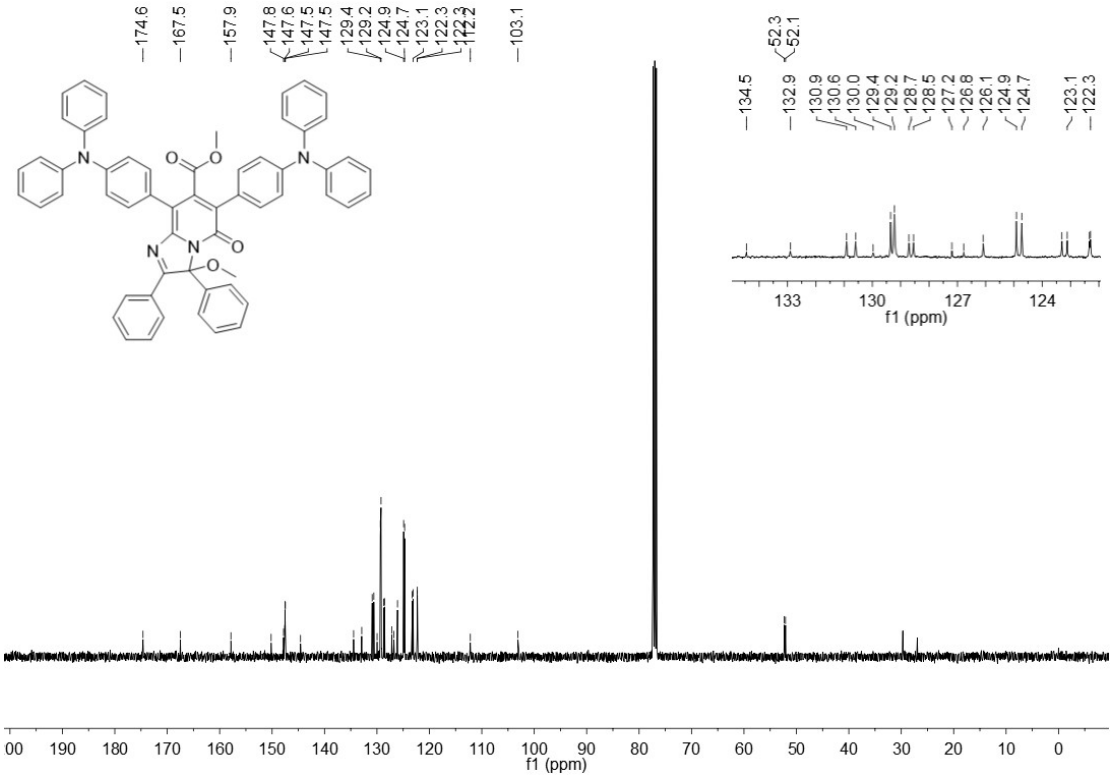

Fig. S14. [13]C NMR spectrum of **BCP-TPA** in Chloroform-*d*.

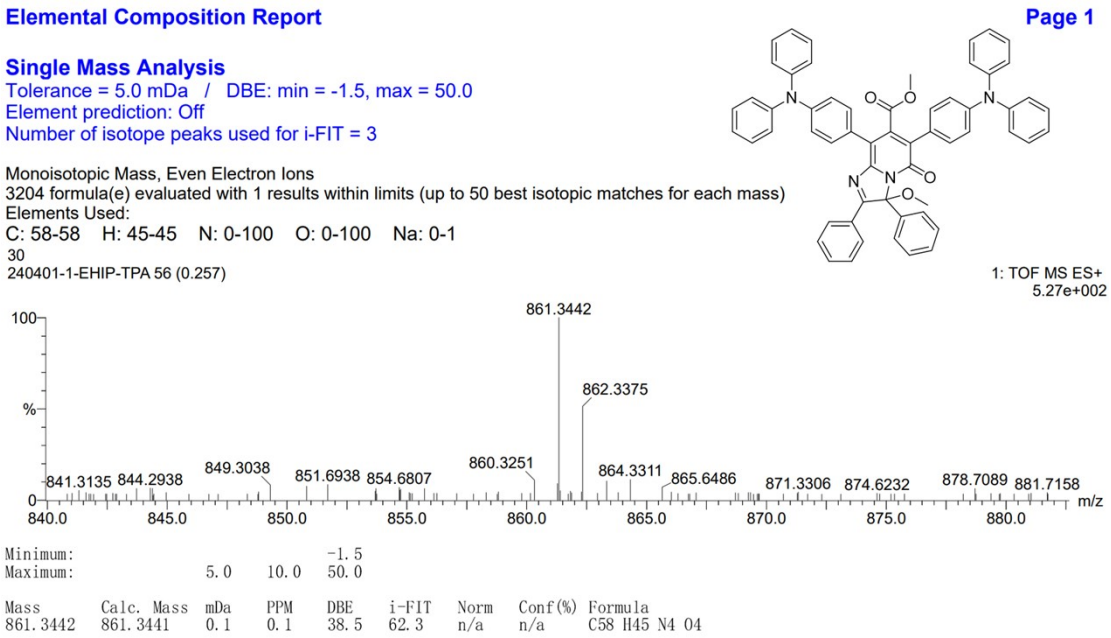

Fig. S15. HRMS spectrum of **BCP-TPA**.

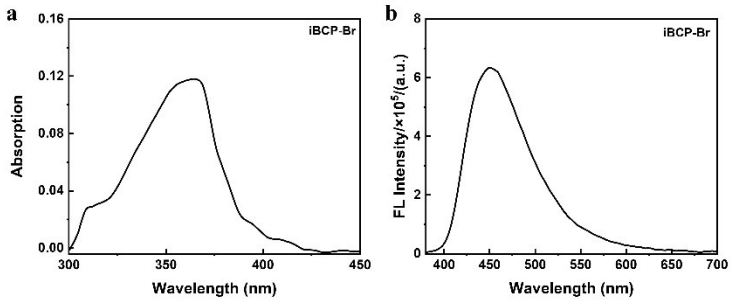

Fig. S16. (a) UV-vis absorption spectra and (b) PL spectra of iBCP-Br. [iBCP-Br]=10 μM

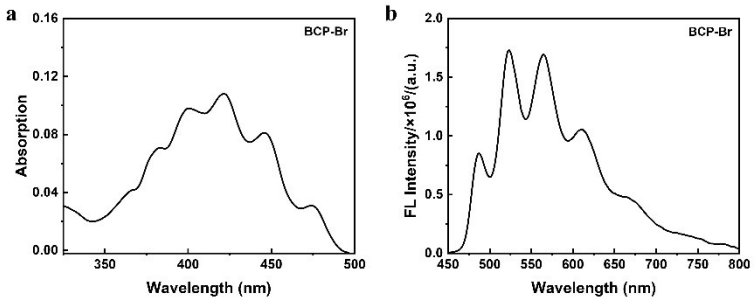

Fig. S17. (a) UV-vis absorption spectra and (b) PL spectra of BCP-Br. [BCP-Br]=10 μM

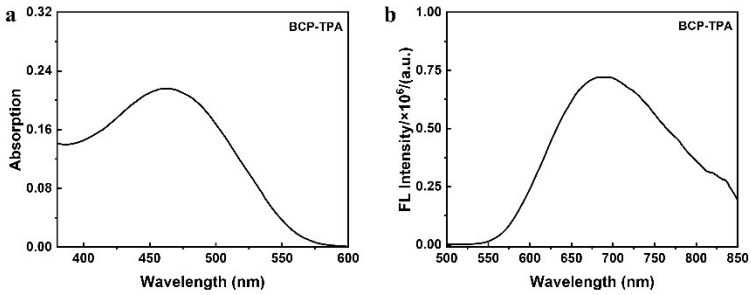

Fig. S18. (a) UV-vis absorption spectra and (b) PL spectra of BCP-TPA. [BCP-TPA]=10 μM

Table S1. λ_{abs} and λ_{em} of BCPs

	λ_{abs} (nm)	λ_{em} (nm)
iBCP-Br	360	455
BCP-Br	420	525
iBCP-TPA	460	696

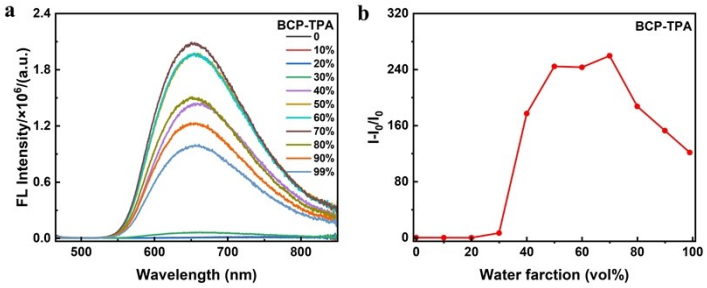

Fig. S19. (a) PL spectra of BCP-TPA in DMSO/H_2O mixture with different water fraction (f_W). (b) Plots of relative PL intensity (I/I_0) of BCP-TPA versus water fraction. [BCP-TPA]=10 μM

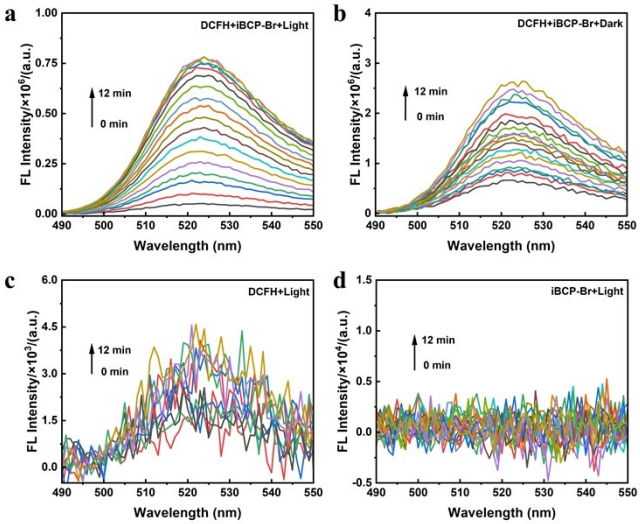

Fig. S20. PL spectra of (a) iBCP-Br with DCFH under white light irradiation (2 mW cm^{-2}), (b) iBCP-Br with DCFH solution in dark, (c) only DCFH in PBS solution under white light irradiation, (d) only iBCP-Br solution under white light irradiation (2 mW cm^{-2}) [iBCP-Br] = 10 μM, [DCFH]

= 40 μM.

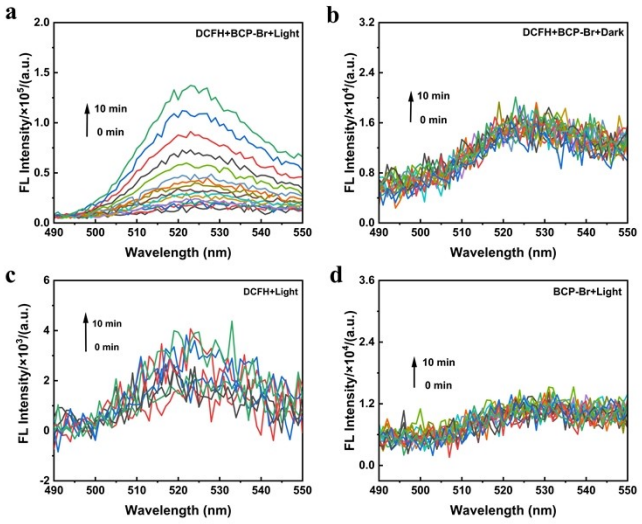

Fig. S21. PL spectra of (a) BCP-Br with DCFH under white light irradiation (2 mW cm^{-2}), (b) BCP-Br with DCFH solution in dark, (c) only DCFH in PBS solution under white light irradiation, (d) only iBCP-Br solution under white light irradiation (2 mW cm^{-2}) [BCP-Br] = 10 μM, [DCFH] = 40 μM.

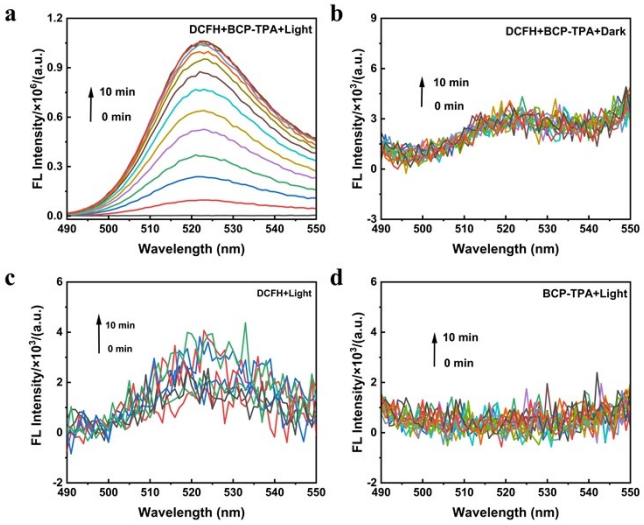

Fig. S22. PL spectra of (a) BCP-TPA with DCFH under white light irradiation (2 mW cm^{-2}), (b) BCP-TPA with DCFH solution in dark, (c) only DCFH in PBS solution under white light irradiation, (d) only BCP- TPA solution under white light irradiation (2 mW cm^{-2}) [BCP- TPA] = 10 μM, [DCFH] = 40 μM.

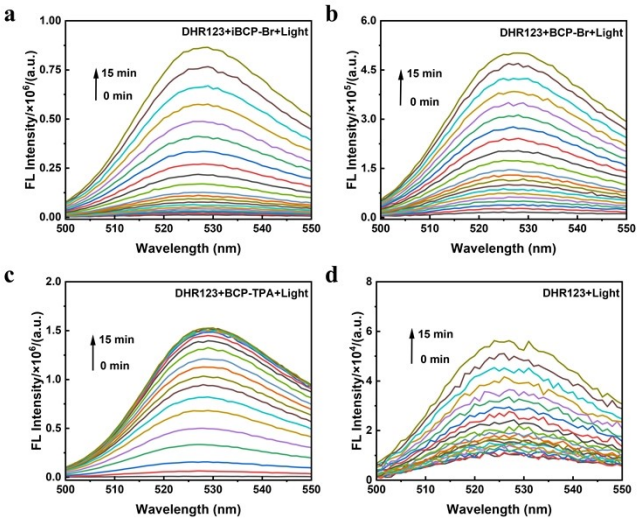

Fig. S23. PL spectra of DHR123 with (a) iBCP-Br, (c) BCP-Br, (c) BCP-TPA and (d) DHR123 only in PBS solution under white light irradiation (2 mW cm^{-2}). [iBCP-Br] = [BCP-Br] = [BCP-TPA] = 10 μM, [DHR123] = 40 μM.

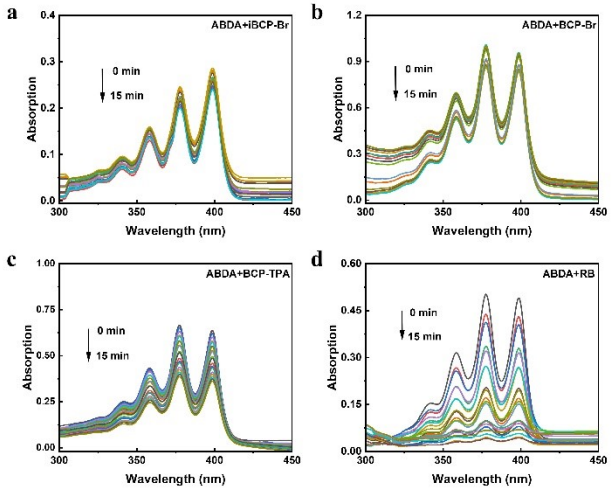

Fig. S24. UV absorption spectra of (a) iBCP-Br, (c) BCP-Br, (c) BCP-TPA, and (d) RB with ABDA in PBS solution under white light irradiation (2 mW cm^{-2}). [iBCP-Br] = [BCP-Br] = [BCP-TPA] = 10 μM, [ABDA] = 0.1 mM.

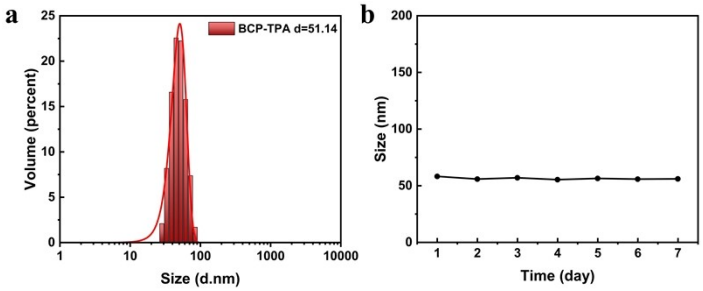

Fig. S25. (a) Dynamic laser scattering size of BCP-TPA NPs. (b) Stability of BCP-TPA NPs for 7 days.

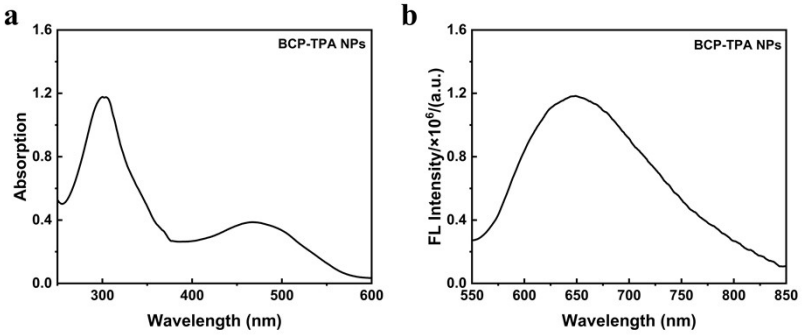

Fig. S26. (a) Normalized UV-vis absorption spectra and (b) Normalized PL of BCP-TPA NPs. [BCP-TPA NPs] = 10 μM

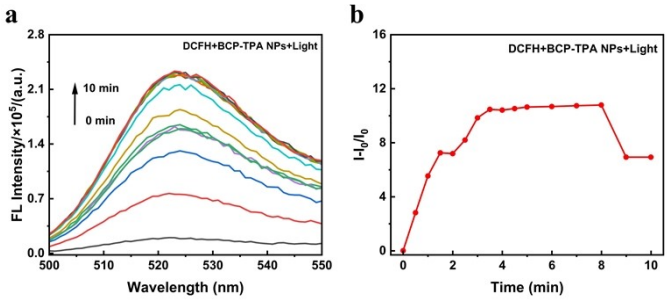

Fig. S27. (a) PL spectra and (b) Fluorescence intensity net change [$(I-I_0)/I_0$] at 532 nm of BCP-TPA NPs with DCFH under white light irradiation (2 mW cm^{-2}). [BCP-TPA NPs] = 10 μM, [DCFH] = 40 μM.

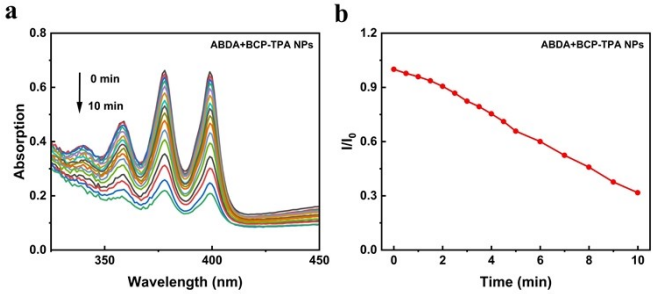

Fig. S28. (a) UV absorption spectra and (b) Absorbance intensity change (I/I_0) of BCP-TPA NPs with ABDA in PBS solution under white light irradiation (2 mW cm^{-2}). [BCP-TPA NPs] = 10 μM, [ABDA] = 0.1 mM.

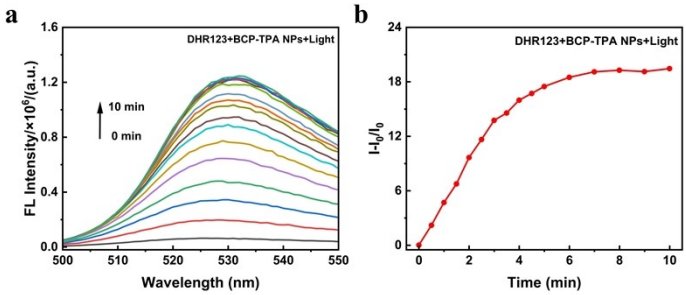

Fig. S29. (a) PL spectra and (b) Fluorescence intensity net change [$(I-I_0)/I_0$] at 525 nm of BCP-TPA NPs with DHR123 under white light irradiation (2 mW cm^{-2}). [BCP-TPA NPs] = 10 μM, [DHR123]

= 40 μM.

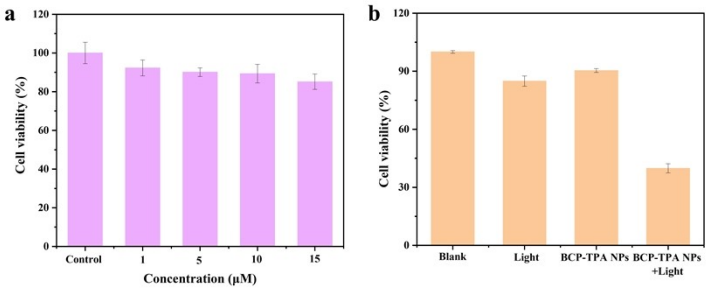

Fig. S30. Cell survival rate (a) without and (b) with BCP-TPA NPs upon white light irradiation. [BCP-TPA NPs] = 1.0×10^{-5} mol L^{-1}; laser irradiation (5 mW cm^{-2}, 10 min).

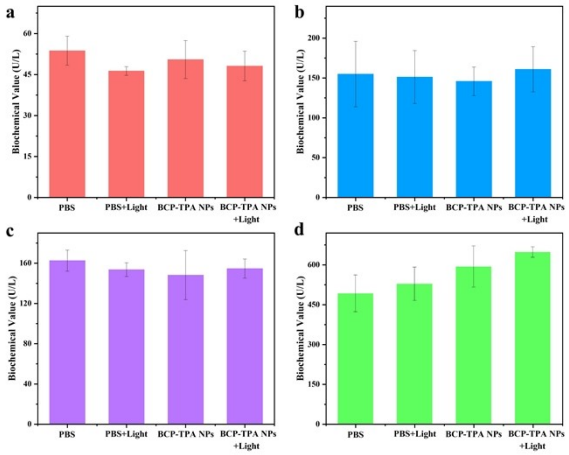

Fig. S31. Serum biochemical parameters analyzed with the specimens collected from the animals received various treatments. The levels of (a) aspartate alanine aminotransferase (ALT), (b) aminotransferase (AST), (c) alkaline phosphatase (ALP), and (d) lactate dehydrogenase (LDH) determined by an automated analyzer.

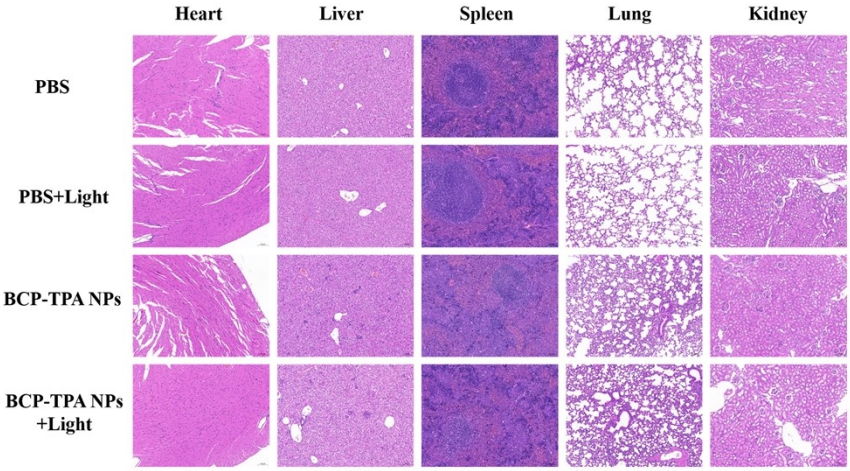

Fig. S32. Histological H&E staining for different organs collected from mice in group VI on the 14th day after the treatment.

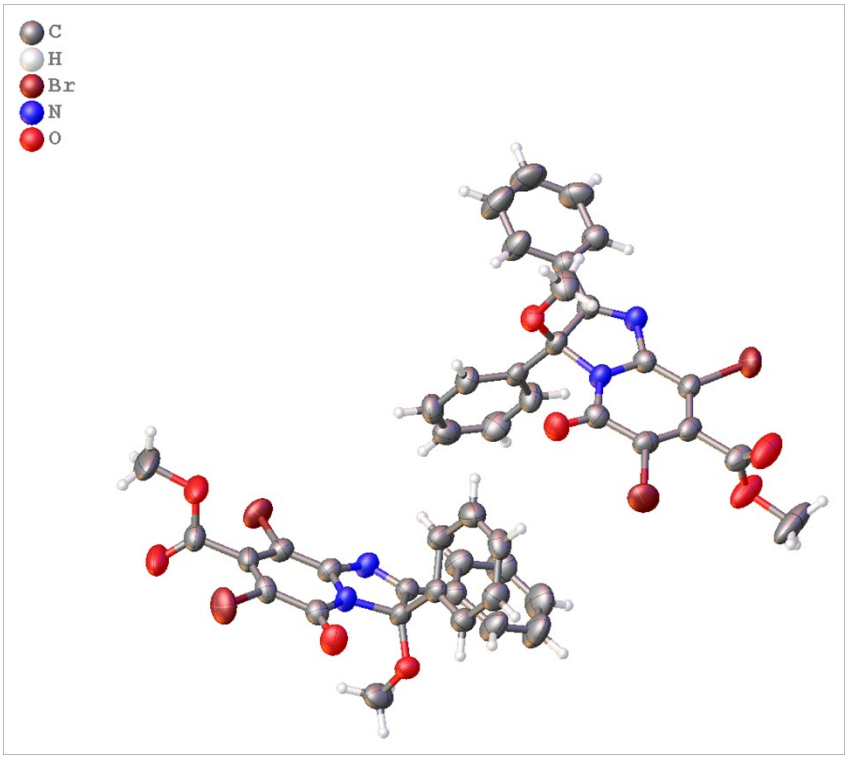

Fig. S33. Crystal structure of BCP-Br

Table 1 Crystal data and structure refinement for cu_20191015_FENCE_LUO_01_0ma_a.

Identification code	cu_20191015_FENCE_LUO_01_0ma_a
Empirical formula	C22H16Br2N2O4
Formula weight	532.19
Temperature/K	300.99
Crystal system	triclinic
Space group	P-1
a/Å	15.9326(10)
b/Å	9.1297(6)
c/Å	16.4070(10)
α /°	89.995(3)
β /°	117.183(2)
γ /°	90.000(2)
Volume/Å3	2123.0(2)
Z	4
ρ calcg/cm3	1.665
μ /mm-1	5.116
F(000)	1056.0
Crystal size/mm3	0.36 × 0.25 × 0.04
Radiation	CuKα (λ = 1.54178)
2Θ range for data collection/°	6.056 to 103.438
Index ranges	-16 ⩽ h ⩽ 16, -9 ⩽ k ⩽ 9, -16 ⩽ l ⩽ 16
Reflections collected	12526
Independent reflections	4618 [Rint = 0.0486, Rsigma = 0.0508]

Data/restraints/parameters4618/0/545

Goodness-of-fit on F2 1.075

Final R indexes [I>=2σ (I)] R1 = 0.0341, wR2 = 0.0949

Final R indexes [all data] R1 = 0.0407, wR2 = 0.1105

Largest diff. peak/hole / e Å-3 0.48/-0.38

checkCIF (basic structural check) running

Checking for embedded fcf data in CIF ...
Found embedded fcf data in CIF. Extracting fcf data from uploaded CIF, please wait . . .

checkCIF/PLATON (basic structural check)

Structure factors have been supplied for datablock(s) cu_20191015_fence_luo_01_0ma_a

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE,

No syntax errors found, CIF dictionary
Please wait while processing .... Interpreting this report

Structure factor report

Datablock: cu_20191015_fence_luo_01_0ma_a

Bond precision: C-C = 0.0073 A Wavelength=1.54178

Cell: a=15.9326(10) b=9.1297(6) c=16.407(1)
alpha=89.995(3) beta=117.183(2) gamma=90.000(2)

Temperature: 301 K

	Calculated	Reported
Volume	2123.0(2)	2123.0(2)
Space group	P -1	P -1
Hall group	-P 1	-P 1
Moiety formula	C22 H16 Br2 N2 O4	C22 H16 Br2 N2 O4
Sum formula	C22 H16 Br2 N2 O4	C22 H16 Br2 N2 O4
Mr	532.17	532.19
Dx, g cm-3	1.665	1.665
Z	4	4
Mu (mm-1)	5.115	5.116
F000	1056.0	1056.0
F000'	1053.10	
h, k, lmax	16, 9, 16	16, 9, 16
Nref	4715	4618
Tmin, Tmax	0.269, 0.815	0.469, 0.750
Tmin'	0.127	

Correction method= # Reported T Limits: Tmin=0.469 Tmax=0.750 AbsCorr = ?

Data completeness= 0.979 Theta(max)= 51.719

R(reflections)= 0.0341(4069) wR2(reflections)= 0.1105(4618)

S = 1.075 Npar= 545

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level A

THETM01_ALERT_3_A The value of sine(theta_max)/wavelength is less than 0.550
Calculated sin(theta_max)/wavelength = 0.5091
PLAT057_ALERT_3_A Correction for Absorption Required RT(exp) ... 3.03 Do !

Alert level B

SYMMS02_ALERT_1_B No unit-cell angle should be equal to 90 for a triclinic cell
Cell 15.9326 9.1297 16.4070
Angles 89.9950 117.1830 90.0000
PLAT112_ALERT_2_B ADDSYM Detects New (Pseudo) Symm. Elem a 100 %Fit
PLAT113_ALERT_2_B ADDSYM Suggests Possible Pseudo/New Space Group P21/c Check
Check Model Parameter Symmetry for Reflection Data Support
PLAT934_ALERT_3_B Number of (Iobs-Icalc)/Sigma(W) > 10 Outliers .. 2 Check
-6 -2 9, 2 1 13,

Alert level C

PLAT052_ALERT_1_C Info on Absorption Correction Method Not Given Please Do !
PLAT088_ALERT_3_C Poor Data / Parameter Ratio ..... 8.47 Note
PLAT155_ALERT_4_C The Triclinic Unit Cell is NOT Reduced ..... Please Do !
PLAT242_ALERT_2_C Low 'MainMol' Ueq as Compared to Neighbors of C8 Check
PLAT242_ALERT_2_C Low 'MainMol' Ueq as Compared to Neighbors of C30 Check
PLAT341_ALERT_3_C Low Bond Precision on C-C Bonds ..... 0.00732 Ang.
PLAT767_ALERT_4_C INS Embedded LIST 6 Instruction Should be LIST 4 Please Check
PLAT906_ALERT_3_C Large K Value in the Analysis of Variance ..... 5,732 Check
PLAT911_ALERT_3_C Missing FCF Refl Between Thmin & STh/L= 0.509 96 Report
2 -6 0, 4 -6 0, -7 8 1, -6 8 1, -4 8 1, 3 -9 1,
-13 -4 2, -4 9 2, -2 0 2, 2 -6 2, 10 6 2, -14 -1 3,
-8 -8 3, -4 0 3, -13 -2 4, -10 -7 4, -9 -7 4, -2 0 4,
0 -5 4, 4 -6 5, -1 -4 6, 1 -7 6, 2 -6 6, 4 -5 6,
6 -3 6, -13 -5 7, -12 -6 7, -6 -3 7, -5 -3 7, -1 -3 7,
0 0 7, 5 6 7, -14 -4 8, -11 -3 8, -10 -3 8, -8 -3 8,
-7 -3 8, -6 -3 8, -6 8 8, -5 -3 8, -4 8 8, -1 -3 8,
-1 -2 8, -1 -1 8, 0 -3 8, 0 7 8, -15 -3 9, -14 -3 9,
-12 -5 9, -10 -3 9, -9 -2 9, -8 -3 9, -7 -3 9, -6 -2 9,
-4 -2 9, -4 7 9, -3 -2 9, -2 -2 9, -2 0 9, -1 -2 9,
2 -1 9, 3 -3 9, -15 -3 10, -13 -5 10, -13 -4 10, -13 5 10,
-12 -2 10, -11 -2 10, -8 -5 10, -8 -2 10, -6 -2 10, -4 2 10,
-3 0 10, -7 -2 11, -5 -2 11, -3 2 11, -1 6 11, 2 -3 11,
2 -2 11, -13 -3 12, -9 -2 12, -8 -2 12, -1 5 12, 3 -2 12,
-2 -1 13, -1 -1 13, -1 4 13, 1 2 13, 1 3 13, 2 -3 13,
-9 -1 14, -8 -1 14, -2 3 14, 0 1 14, 1 -2 14, -8 4 15,
PLAT918_ALERT_3_C Reflection(s) with I(obs) much Smaller I(calc) . 2 Check

Alert level G

PLAT793_ALERT_4_G Model has Chirality at C5 (Centro SpGr) S Verify
PLAT793_ALERT_4_G Model has Chirality at C27 (Centro SpGr) S Verify
PLAT909_ALERT_3_G Percentage of I>2sig(I) Data at Theta(Max) Still 87% Note
PLAT913_ALERT_3_G Missing # of Very Strong Reflections in FCF .... 1 Note
-2 0 4,
PLAT941_ALERT_3_G Average HKL Measurement Multiplicity ..... 2.7 Low
PLAT969_ALERT_5_G The 'Henn et al.' R-Factor-gap value ..... 1,954 Note
Predicted wR2: Based on SigI**2 5,66 or SHELX Weight 10,28
PLAT978_ALERT_2_G Number C-C Bonds with Positive Residual Density. 1 Info
PLAT992_ALERT_5_G Repd & Actual _reflns_number_gt Values Differ by 2 Check

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2 **ALERT level A** = Most likely a serious problem - resolve or explain
4 **ALERT level B** = A potentially serious problem, consider carefully
10 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
8 **ALERT level G** = General information/check it is not something unexpected

2 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
5 ALERT type 2 Indicator that the structure model may be wrong or deficient
11 ALERT type 3 Indicator that the structure quality may be low
4 ALERT type 4 Improvement, methodology, query or suggestion
2 ALERT type 5 Informative message, check

It is advisable to attempt to resolve as many as possible of the alerts in all categories. Often the minor alerts point to easily fixed oversights, errors and omissions in your CIF or refinement strategy, so attention to these fine details can be worthwhile. In order to resolve some of the more serious problems it may be necessary to carry out additional measurements or structure refinements. However, the purpose of your study may justify the reported deviations and the more serious of these should normally be commented upon in the discussion or experimental section of a paper or in the "special_details" fields of the CIF. checkCIF was carefully designed to identify outliers and unusual parameters, but every test has its limitations and alerts that are not important in a particular case may appear. Conversely, the absence of alerts does not guarantee there are no aspects of the results needing attention. It is up to the individual to critically assess their own results and, if necessary, seek expert advice.

Publication of your CIF in IUCr journals

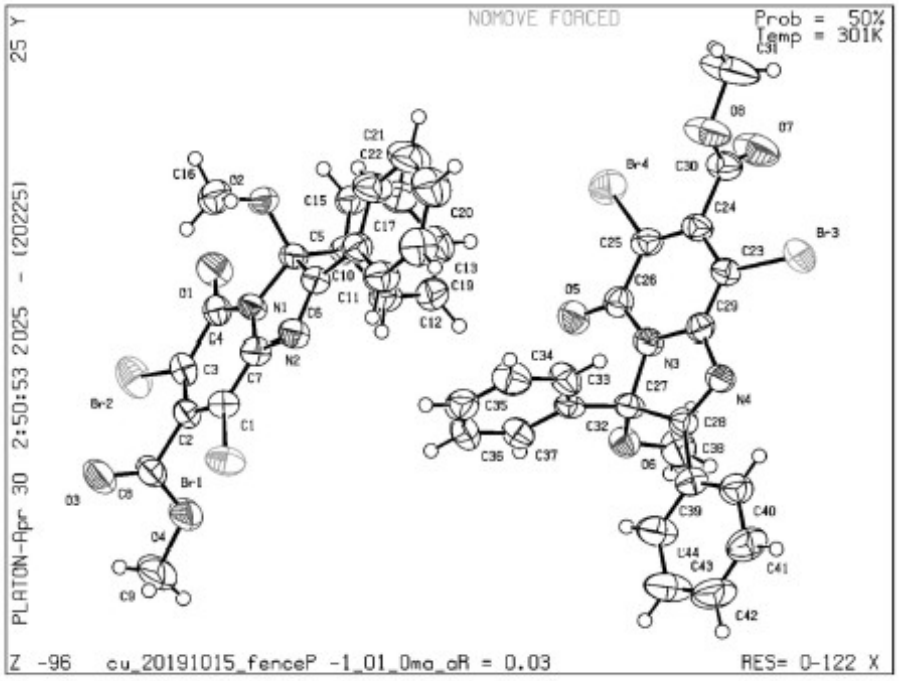
A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E* or *IUCrData*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

Publication of your CIF in other journals

Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF submission.

PLATON version of 02/02/2025; check.def file version of 02/02/2025

Datablock cu_20191015_fence_luo_01_0ma_a – ellipsoid plot



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