SCIENCE CHINA
Chemistry

Supporting Information

Specific Staining and Precise Killing of Cancer Cells through Fusion of Pharmaceutical Chemistry and π-bridge Effect Design Philosophy

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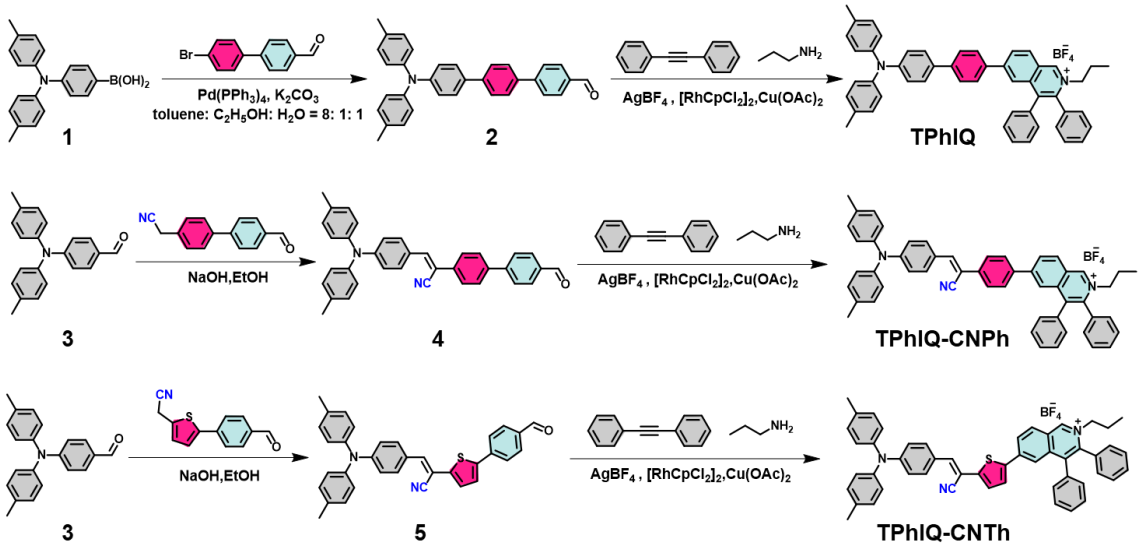
Synthesis and characterization



Scheme S1. Molecular structures and synthetic routes of the TPhIQ derivatives.

Preparation of TPhIQ: Firstly, 4-[bis (4-tolyl) amine] phenyl] boric acid (1) was added to 250 mL double-mouth flask, and the intermediate product 2 was simply prepared according to the method shown in Scheme S1. Then a mixture of compound 2 (0.30 mmol), $[RhCp^*Cl_2]_2$ (2.0 mol%), $AgBF_4$ (0.30 mmol), $Cu(OAc)_2$ (0.30 mmol) and diphenylacetylene (0.64 mmol) were put into the 25 mL schlenk reaction tube. The reaction tube was vacuumed and ventilated with nitrogen for three times with double rows of tubes to ensure that the reaction environment was in an anhydrous and oxygen-free condition. After the above operations were completed, propylamine (0.45mmol) and tert-amyl alcohol (dissolved, a small amount is better) were added and heated and stirred at 110℃ under nitrogen at 110℃ for 6 h. When the reaction was complete, the mixture was cooled and diluted with CH_2Cl_2 (10 mL), then filtered through a Celite pad and the Celite pad was washed with CH_2Cl_2 (30 mL) and MeOH (20 mL). The residents were purified by silica column chromatography, using CH_2Cl_2/MeOH (50:1, v/v) as eluent to give pure products. The solids were further precipitated from CH_2Cl_2/PE mixtures under sonication and filtered to obtain orange powder-like products TPhIQ with a yield of 72%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 10.27 (s, 1H), 8.71 (d, J = 8.7 Hz, 1H), 8.56 – 8.50 (m, 1H), 7.80 (d, J = 8.3 Hz, 2H), 7.74 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 7.61 (d, J = 8.7 Hz, 2H), 7.55 – 7.48 (m, 2H), 7.45 – 7.40 (m, 3H), 7.39 – 7.30 (m, 4H), 7.15 (d, J = 8.1 Hz, 4H), 6.96 (t, J = 8.2 Hz, 6H), 4.42 (t, J = 7.9 Hz, 2H), 2.28 (s, 6H), 1.86 – 1.79 (m, 2H), 0.72 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (TMS, ppm) 144.84, 133.31,

130.95, 130.64, 128.83, 128.54, 127.99, 127.45, 125.27, 122.39, 121.85, 32.72, 20.90, 19.35, 13.50. HRMS /MALDI-TOF : *m/z* (cation) 671.3218 [M^+, calcd 671.3421].

Preparation of TPhIQ-CNPh: 4-di-toluidine benzaldehyde (3) was added to 250 mL double-mouth flask, and the intermediate product 4 was prepared according to the method reported in the references.[1] Then the intermediate product 4 (0.30 mmol), $[RhCp^*Cl_2]_2$ (2.0 mol%), $AgBF_4$ (0.30 mmol), $Cu(OAc)_2$ (0.30 mmol) and diphenylacetylene (0.64 mmol) were put into 25 mL schlenk reaction tube. The yellow product TPHIQ-CNPh was obtained by the reaction method similar to that of synthetic TPhIQ, and the yield was 72%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 10.29 (s, 1H), 8.73 (d, J = 8.7 Hz, 1H), 8.52 (dd, J = 8.7, 1.5 Hz, 1H), 7.97 (s, 1H), 7.85 (dd, J = 13.7, 8.8 Hz, 4H), 7.79 (d, J = 8.6 Hz, 2H), 7.69 (s, 1H), 7.51 (dd, J = 6.5, 3.0 Hz, 2H), 7.44 – 7.41 (m, 2H), 7.37 (s, 3H), 7.32 (d, J = 1.9 Hz, 2H), 7.20 (d, J = 8.2 Hz, 4H), 7.05 (d, J = 8.3 Hz, 4H), 6.87 (d, J = 8.9 Hz, 2H), 5.76 (s, 1H), 4.46 – 4.35 (m, 2H), 2.30 (s, 6H), 1.84 (dt, J = 14.9, 7.4 Hz, 2H), 0.80 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ(TMS, ppm): 150.68, 144.93, 143.77, 143.64, 138.10, 134.77, 131.52, 130.89, 130.64, 130.45, 128.85, 128.79, 128.75, 126.81, 126.39, 122.87, 118.99, 104.81, 24.20, 20.98, 10.98. HRMS /MALDI-TOF : *m/z* (cation) 722.3559 [M^+, calcd 722.3530].

Preparation of TPhIQ-CNTh. The synthesis method of TPhIQ-CNTh is similar to that of TPhIQ-CNPh, and the synthesis method of intermediate 5 is similar to that of intermediate 4. The intermediate product 4 in the raw material of TPhIQ-CNPh reaction is changed into intermediate product 5, and similar operations are carried out to obtain the red product TPhIQ-CNTh with a yield of 69%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm) 10.17 (s, 1H), 8.63 (d, J = 8.8 Hz, 1H), 8.51 (dd, J = 8.8, 1.8 Hz, 1H), 7.89 (d, J = 4.1 Hz, 1H), 7.81 – 7.73 (m, 3H), 7.57 – 7.54 (m, 1H), 7.50 – 7.44 (m, 3H), 7.43 – 7.35 (m, 6H), 7.31 – 7.27 (m, 2H), 7.21 (d, J = 8.0 Hz, 4H), 7.10 – 7.03 (m, 4H), 6.84 (d, J = 8.9 Hz, 2H), 4.34 (t, J = 7.7 Hz, 2H), 2.30 (s, 6H), 1.82 (q, J = 7.5 Hz, 2H), 0.79 (t, J = 7.4 Hz, 3H). HRMS /MALDI-TOF : *m/z* (cation) 728.3942 [M^+, calcd 728.3094].

Supporting Figures

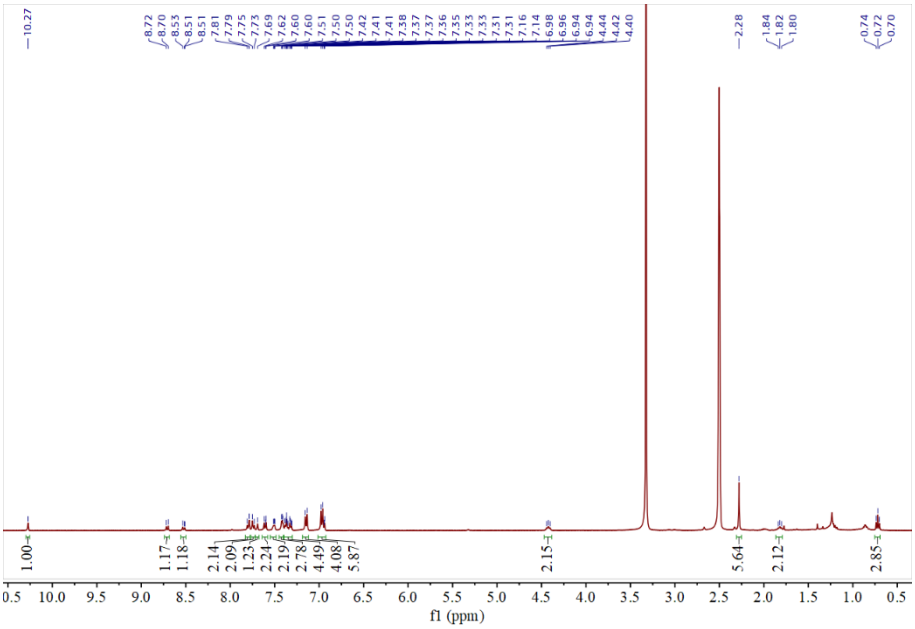


Figure S1. ^{1}H NMR spectrum of TPhIQ in DMSO-d_6.

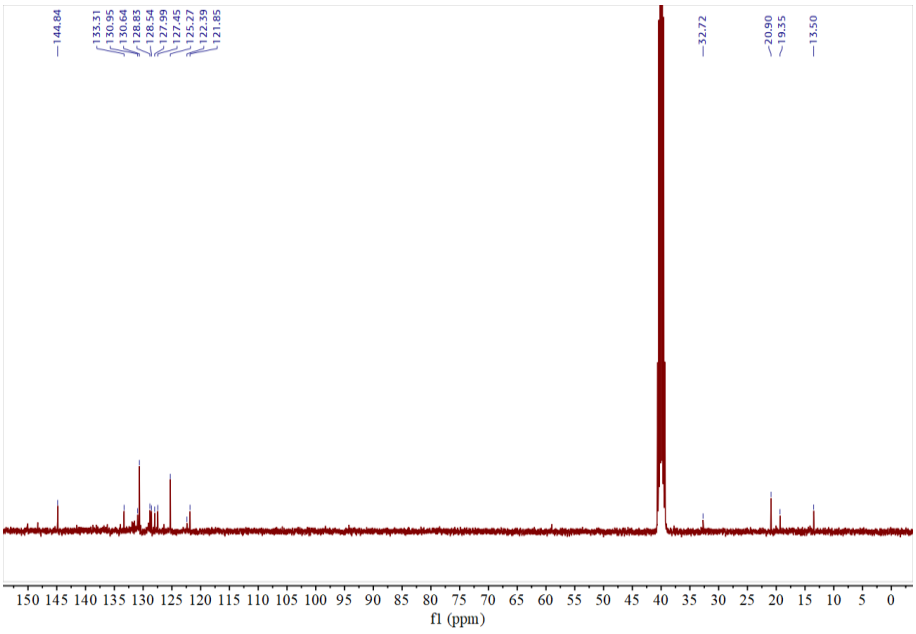


Figure S2. ^{13}C NMR spectrum of TPhIQ in DMSO-d_6.

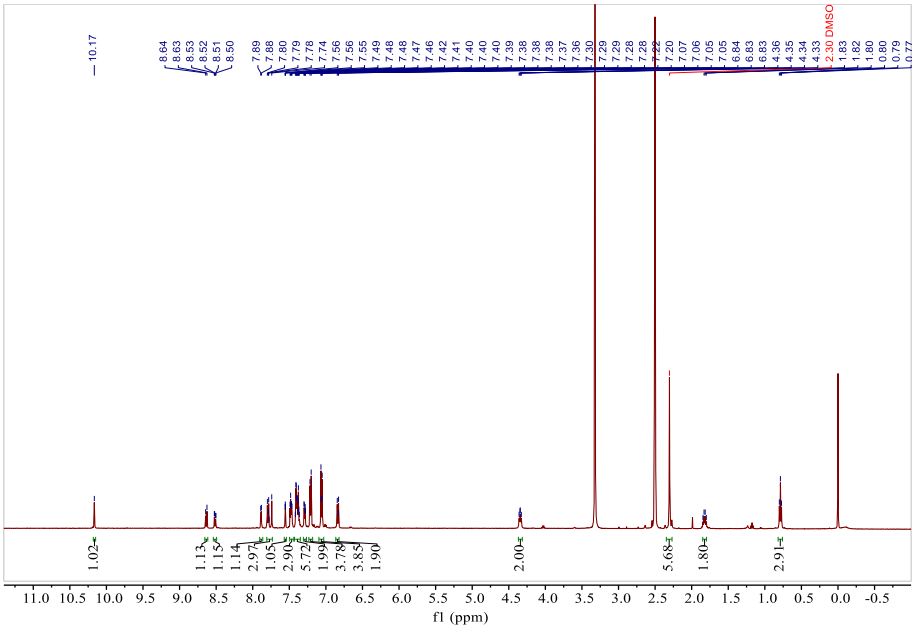


Figure S3. ^{1}H NMR spectrum of TPhIQ-CNPh in DMSO-d_6.

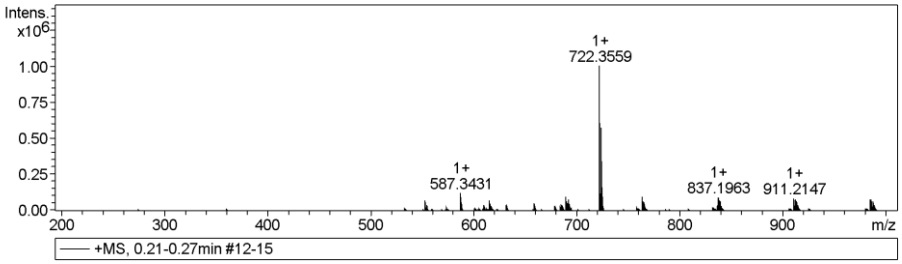


Figure S4. HRMS spectrum of TPhIQ-CNPh.

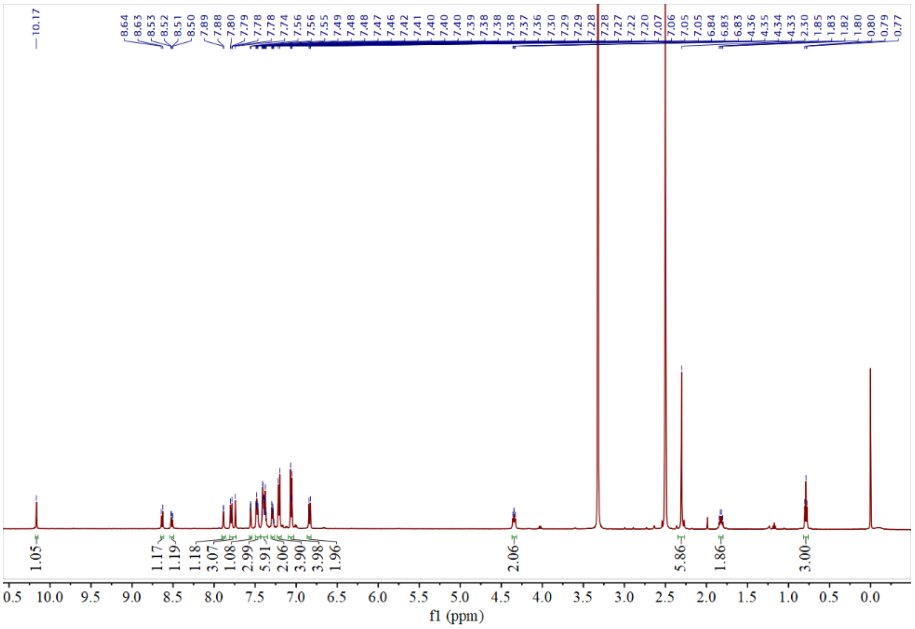


Figure S5. ^{1}H NMR spectrum of TPhIQ-CNTh in DMSO-d_6.

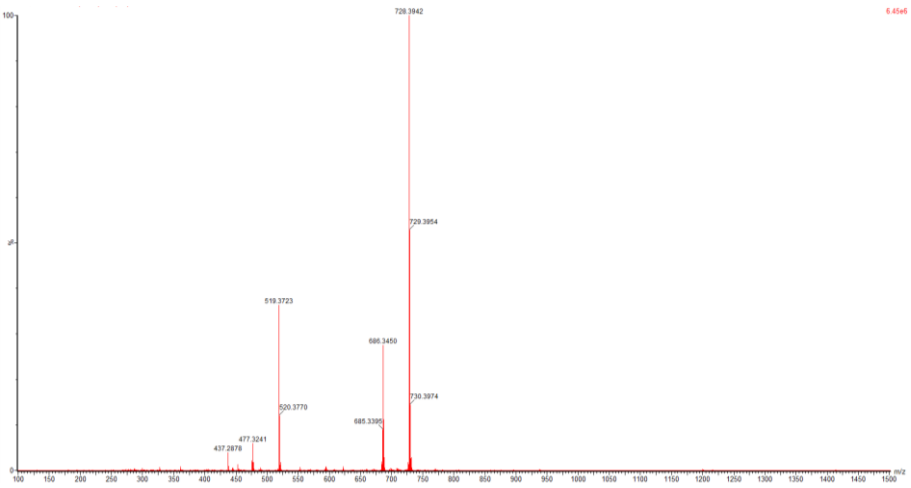


Figure S6. TOF spectrum of TPhIQ-CNTh.

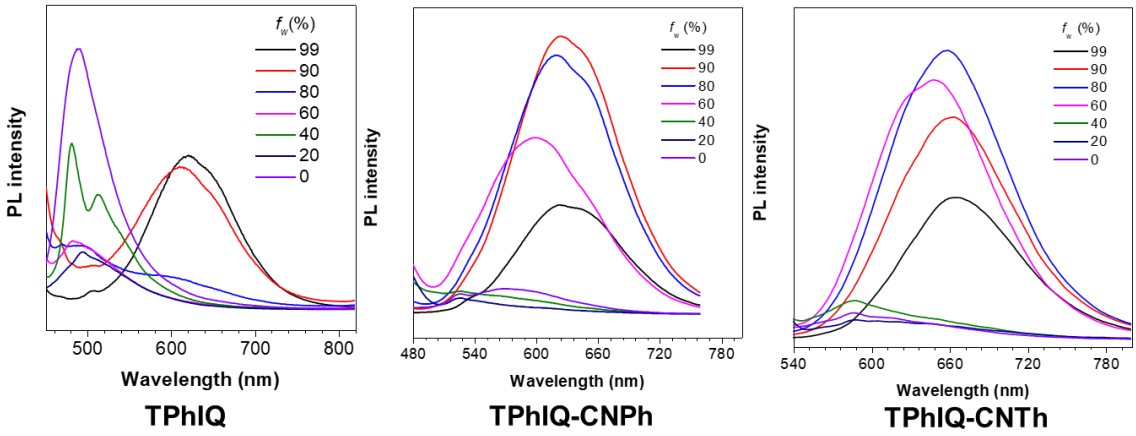


Figure S7. PL spectra of TPhIQs in THF/H_2O mixtures with different water fractions (f_w) under the same excitation conditions.

Table S1. Fluorescence quantum yields of probes.

	soln [a)]	film
TPhIQ	0.9%	6.6%
TPhIQ-CNPh	0.9%	9.4%
TPhIQ-CNTh	0.9%	12.4%

[a)] solution: measured in THF solution;

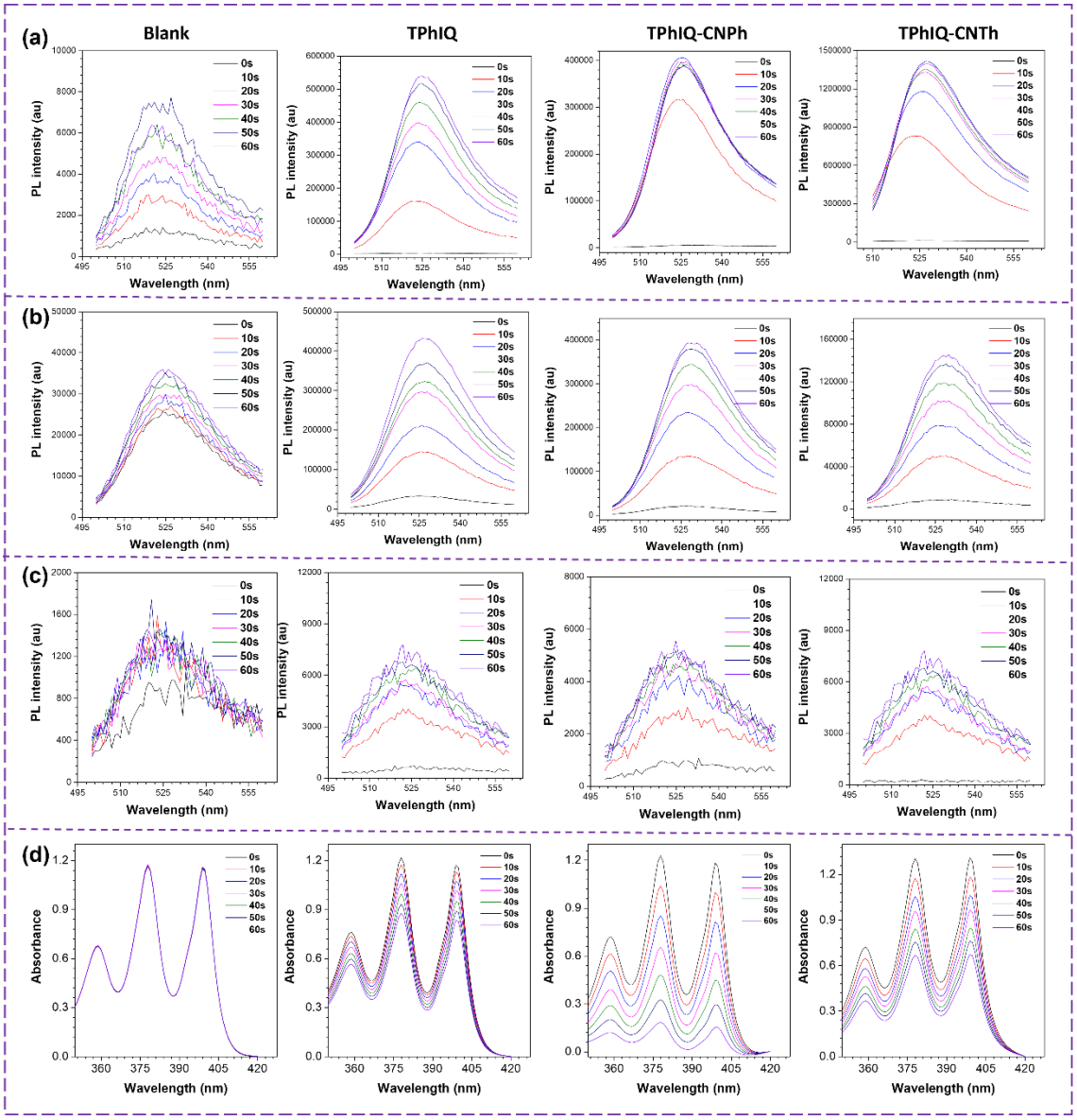


Figure S8. ROS production of TPhIQ derivatives (A) total ROS; (B) $O_2^{\bullet -}$; (C) •OH; (D) $^1O_2$

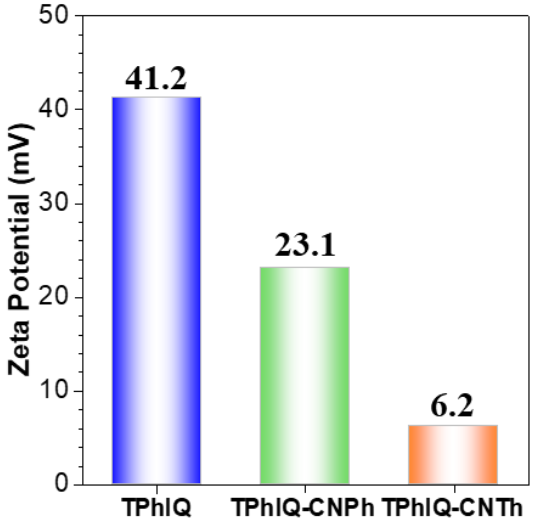


Figure S9. Zeta potentials of TPhIQ Nano particles.

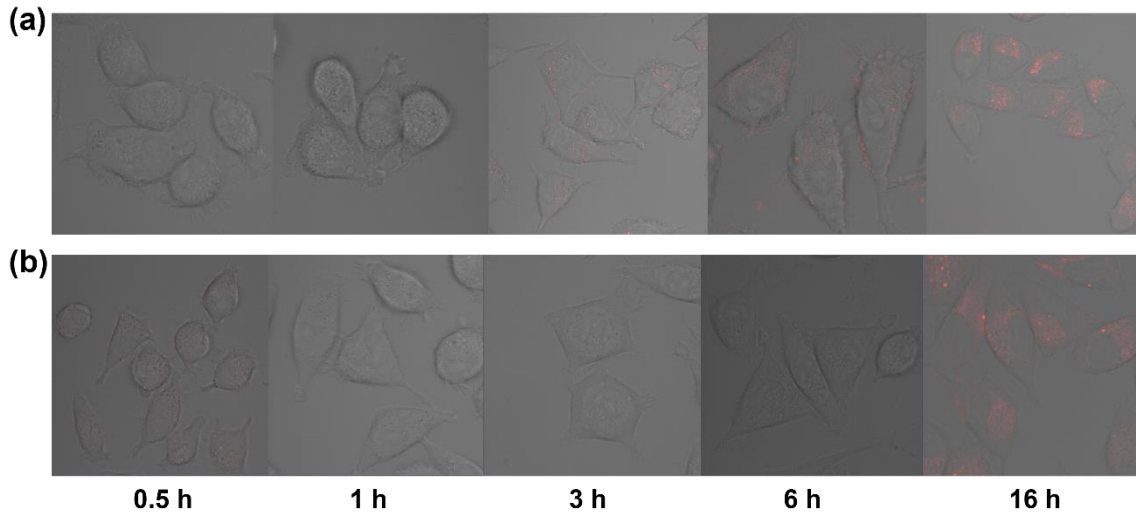


Figure S10. (A) TPhIQ-CNPh and (B) TPhIQ-CNTh uptaken by L929 cells.

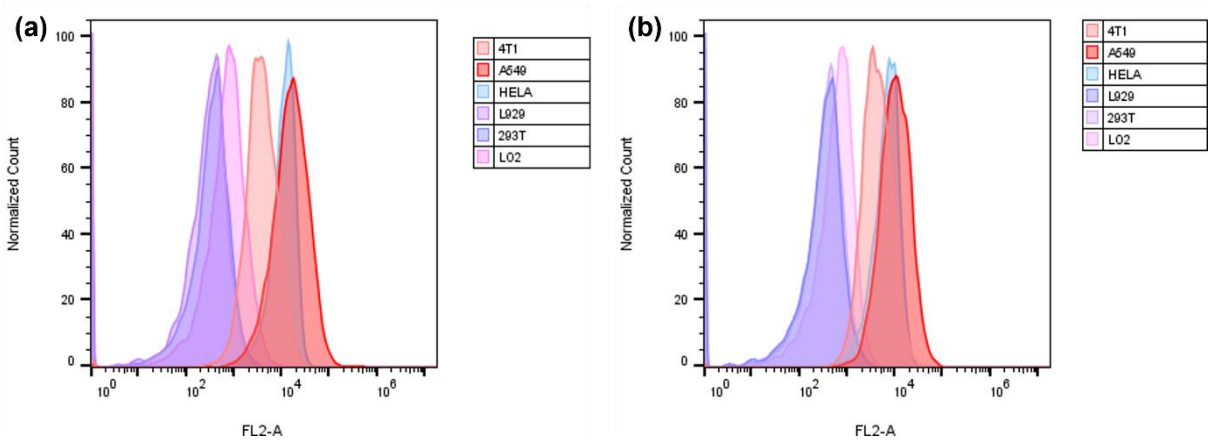


Figure S11. Flow cytometry histograms of different normal cells (L929 cells, 293 cells, and LO2 cells) and cancer cells (HeLa cells, 4T1 cells, and A549 cells) incubated with 2 μM TPhIQ-CNPh and TPhIQ-CNTh for 2h, respectively.

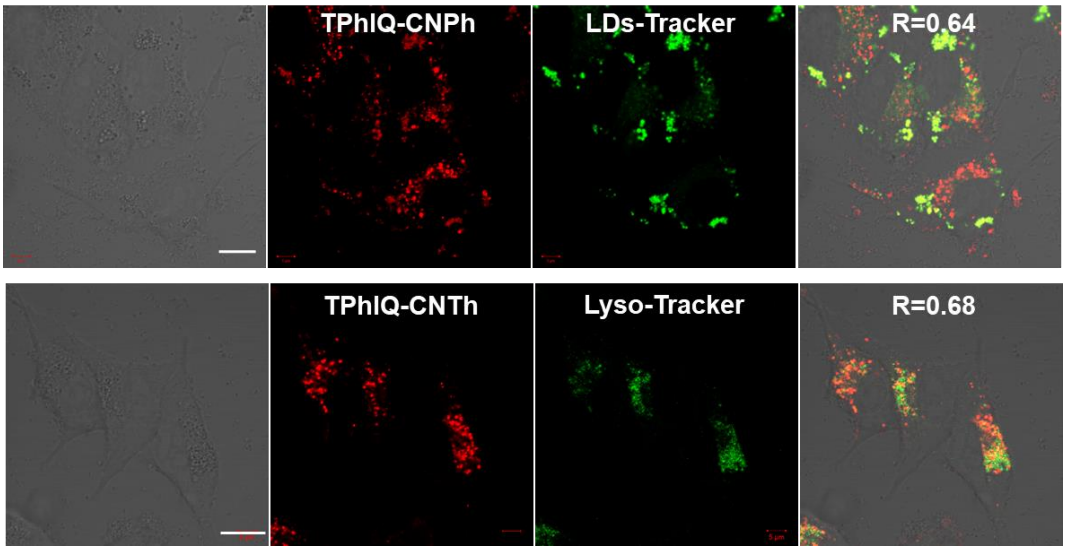


Figure S12. Co-location imaging with commercial dyes of TPhIQ-CNPh and TPhIQ-CNTh.

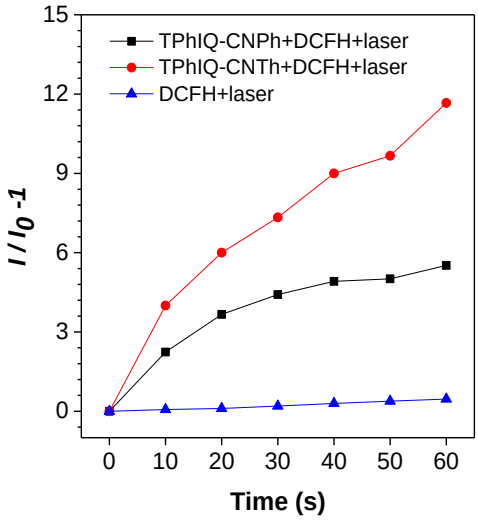


Figure S13. Intracellular ROS production under irradiation of 488 nm laser.

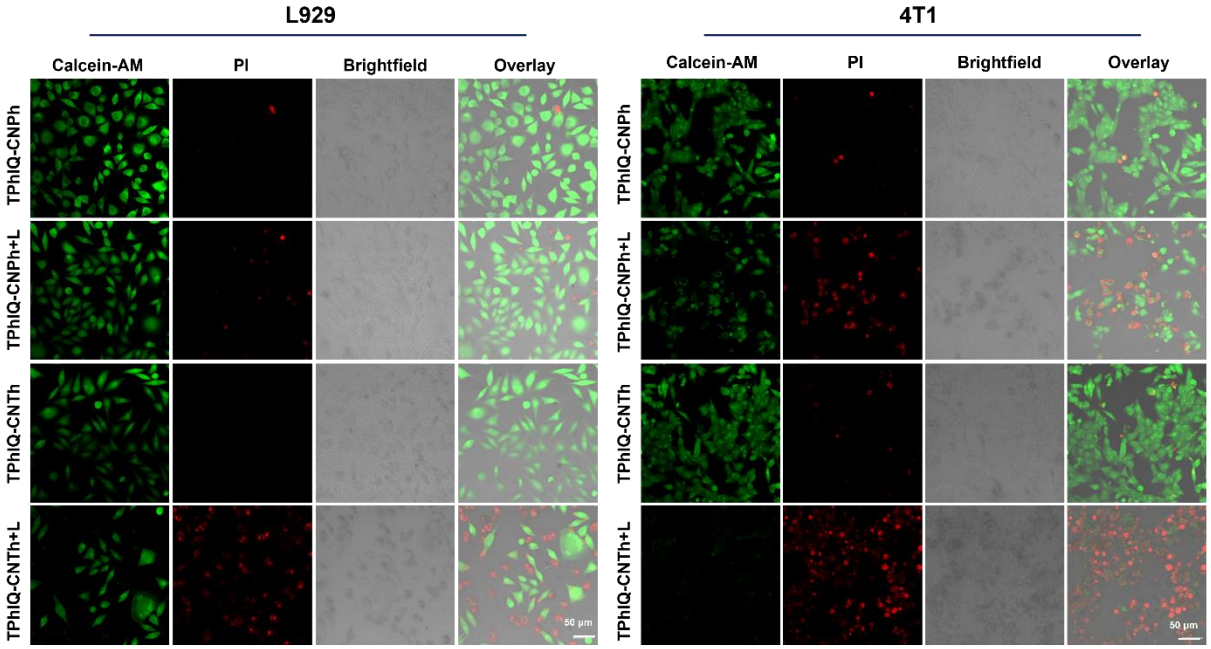


Figure S14. Live/dead cells (L929 and 4T1) costaining assay by using calcein-AM with green fluorescence for live cells and PI with red fluorescence for dead cells after treatment by PDT using 2 μM TPhIQ-CNPh and TPhIQ-CNTh as the PS (white light irradiation of 40 mW cm^{-2} for 10 min).

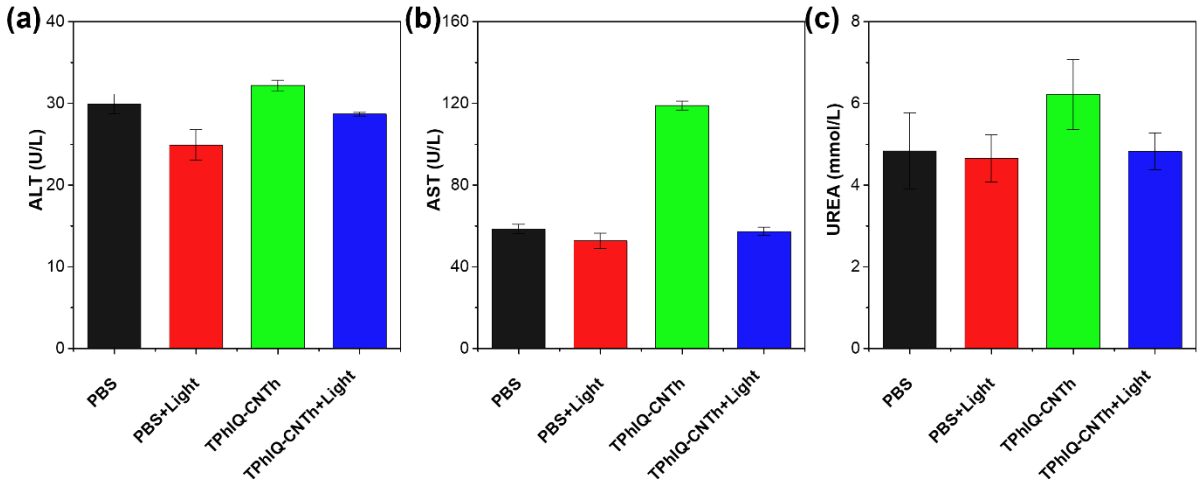


Figure S15. Blood tests for mice treated with PBS, PBS+L, TPhIQ-CNTh and TPhIQ-CNTh +L.

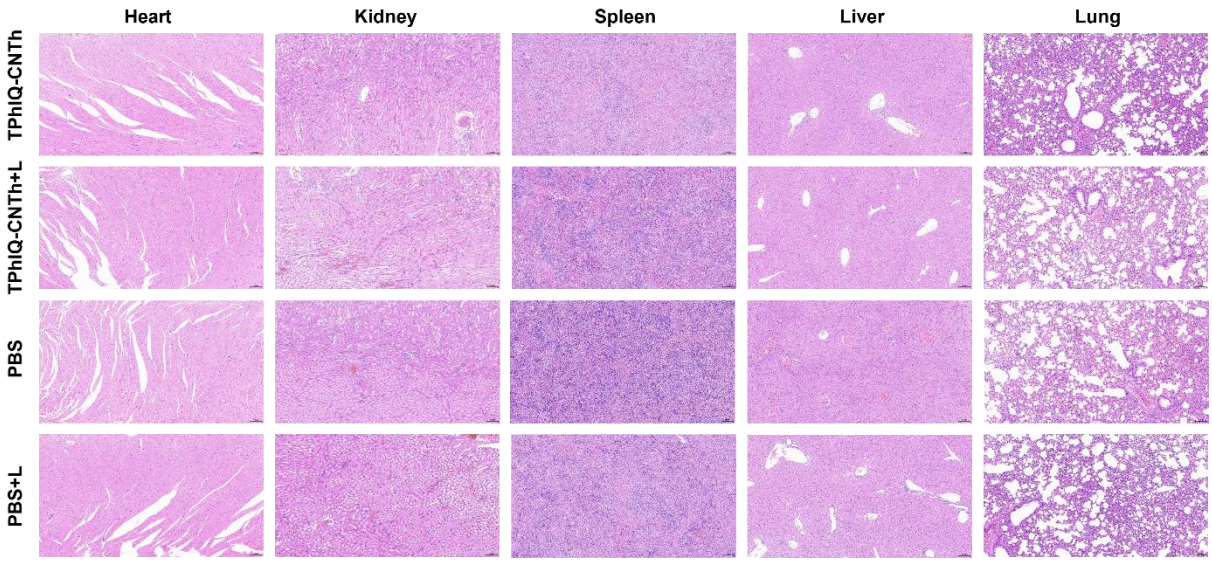


Figure S16. H&E staining images of the heart, liver, spleen, lung, kidney, and intestine after different treatments for 12 days. Scale bar: 100 μm.

References

1 Alam P, He W, Leung NLC, Ma C, Kwok RTK, Lam JWY, Sung HHY, Williams ID, Wong KS, Tang BZ. *Adv. Funct. Mater*, 2020, 30: 201909268