Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Water-soluble thiophene-croconaine dye with high molar extinction coefficient for NIR fluorescence imaging-guided synergistic photothermal/photodynamic therapy of cancer

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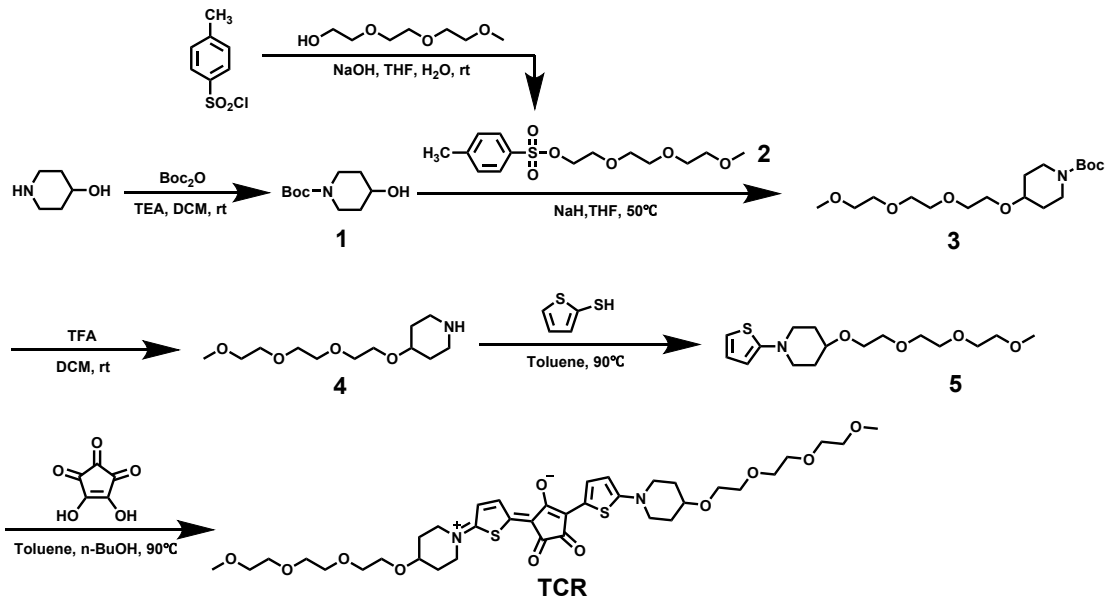


Scheme S1. Synthetic route of **TCR**.

Synthesis of compound 1. 4-Hydroxypyridine (2.0 g, 20.0 mmol) and triethylamine (3.6 mL, 30.0 mmol) were dissolved in 25 mL DCM under an ice bath. Then Boc anhydride (3.8 g, 20.0 mmol) was added into the mixture and stirred at room temperature for 24 h. The reaction solution was poured into 100 mL water. After adjusting the pH to neutral with 1 M HCl solution, the solution was extracted with EA (3 x 50 mL). The organic phase was dried with anhydrous Na_2SO_4 and concentrated under a reduced pressure. The crude product was purified by silica gel column chromatography (PE: EA = 1:1) to give compound **1** as a white solid (3.0 g, 75%).

Synthesis of compound 2. Triglyceride monomethyl ether (3.2 g, 20.0 mmol) was dissolved in 10 mL THF. Then NaOH solution (10 mL, 6 M) and *p*-toluenesulfonyl chloride (4.6 g, 24 mmol) in 10 mL THF were added into the reaction solution successively under an ice bath. After reacting for 2 h, the mixture was warmed up to room temperature and reacted for 48 h. To the reaction solution, 1 M HCl solution was added to adjust the pH to neutral and extracted with EA (3 x 50 mL). The organic phase was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give compound **2** as pale-yellow liquid (6.0 g, 94%).

Synthesis of compound 3. To a solution of compound **1** (1.5 g, 7.5 mmol) in 10 mL anhydrous DMF was added NaH (0.36 g, 14.9 mmol) under an ice bath and argon atmosphere. After stirring for 30 min, compound **2** (4.75 g, 14.9 mmol) was added into

the above mixture and stirred at 50 °C overnight. After cooling to room temperature, the reaction solution was poured into 50 mL water. After adjusting the pH to neutral with 1 M HCl solution, the solution was extracted with EA (3 x 50 mL). The organic phase was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (PE: EA = 2:1) to give compound **3** as yellow oily liquid (1.6 g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 – 3.58 (m, 11H), 3.57 – 3.53 (m, 2H), 3.37 (s, 3H), 3.30 (ddd, *J* = 13.5, 10.0, 3.8 Hz, 2H), 3.09 (dt, *J* = 12.7, 4.8 Hz, 2H), 2.04 – 1.89 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.75, 70.60, 70.51, 70.41, 70.27, 70.21, 67.61, 58.86, 40.11, 27.03. HRMS (ESI) m/z $[C_{12}H_{25}NO_4+H]^+$ calculated: 248.1856, found: 248.1936.

Synthesis of compound 4. Compound **3** (500.0 mg, 1.4 mmol) was dissolved in 3 mL DCM, then 3 mL trifluoroacetic acid was added dropwise under an ice bath. After stirring overnight at room temperature, the reaction solution was poured into 100 mL of 20% KOH solution and was extracted with EA (3 x 60 mL). The organic phase was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM: MeOH = 10:1) to give compound **4** as yellow oily liquid (290.0 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 5.5, 3.7 Hz, 1H), 6.57 (dd, *J* = 5.5, 1.3 Hz, 1H), 6.10 (dd, *J* = 3.7, 1.3 Hz, 1H), 3.71 – 3.62 (m, 10H), 3.54 (dd, *J* = 5.7, 3.7 Hz, 2H), 3.49 (dq, *J* = 8.4, 4.1 Hz, 1H), 3.45 – 3.38 (m, 2H), 3.37 (s, 3H), 2.93 (ddd, *J* = 12.3, 9.2, 3.4 Hz, 2H), 2.03 – 1.94 (m, 2H), 1.76 (dtd, *J* = 12.9, 8.8, 4.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.41, 126.13, 112.10, 105.46, 74.47, 71.96, 70.86, 70.70, 70.65, 70.54, 67.37, 59.02, 49.71, 30.55. HRMS (ESI) m/z $[C_{16}H_{27}NO_4S+Na]^+$ calculated: 352.1553, found: 352.1596.

Synthesis of compound 5. Under argon protection, 2-thiophene thiol (100.0 mg, 0.86 mmol) and compound **4** (298.0 mg, 1.2 mmol) were dissolved in 3 mL of toluene. After stirring at 90 °C for 4 h, the reaction solution was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (PE: EA = 2:1) to give compound **5** as yellow oily liquid (140.0 mg, 49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (dd, *J* = 5.5, 3.7 Hz, 1H), 6.57 (dd, *J* = 5.5, 1.3 Hz, 1H), 6.10 (dd, *J* = 3.7, 1.3 Hz, 1H), 3.71 – 3.62 (m, 10H), 3.54 (dd, *J* = 5.7, 3.7 Hz, 2H), 3.49 (dq, *J* = 8.4, 4.1 Hz,

1H), 3.45 – 3.38 (m, 2H), 3.37 (s, 3H), 2.93 (ddd, J = 12.3, 9.2, 3.4 Hz, 2H), 2.03 – 1.94 (m, 2H), 1.76 (dtd, J = 12.9, 8.8, 4.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.41, 126.13, 112.10, 105.46, 74.47, 71.96, 70.86, 70.70, 70.65, 70.54, 67.37, 59.02, 49.71, 30.55. HRMS (ESI) m/z $[C_{16}H_{27}NO_4S+Na]^+$ calculated: 352.1553, found: 352.1596.

Synthesis of TCR. Compound **5** (100.0 mg, 0.30 mmol) and ketonic acid (21.0 mg, 0.15 mmol) were dissolved in toluene (2 mL) and *n*-butanol (2 mL). After stirring at 90 °C for 30 min, the solvent was removed with rotary evaporation. The crude product was purified by silica gel column chromatography (DCM: MeOH = 40:1) to give compound **TCR** as a brownish black solid (49.0 mg, 42%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88-8.65 (m, 2H), 6.56 (s, 2H), 3.84 (d, J = 13.4 Hz, 4H), 3.74 (p, J = 2.6 Hz, 2H), 3.71-3.57 (m, 24H), 3.57-3.52 (m, 4H), 3.38 (s, 6H), 1.96 (dp, J = 13.4, 5.4, 4.7 Hz, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.48, 183.04, 173.28, 140.77, 135.84, 123.82, 112.90, 72.20, 71.89, 70.73, 70.64, 70.59, 70.50, 67.76, 59.04, 48.23, 30.25. HRMS (ESI) m/z $[C_{37}H_{52}N_2O_{11}S_2]^+$ calculated: 764.3013, found: 764.3001.

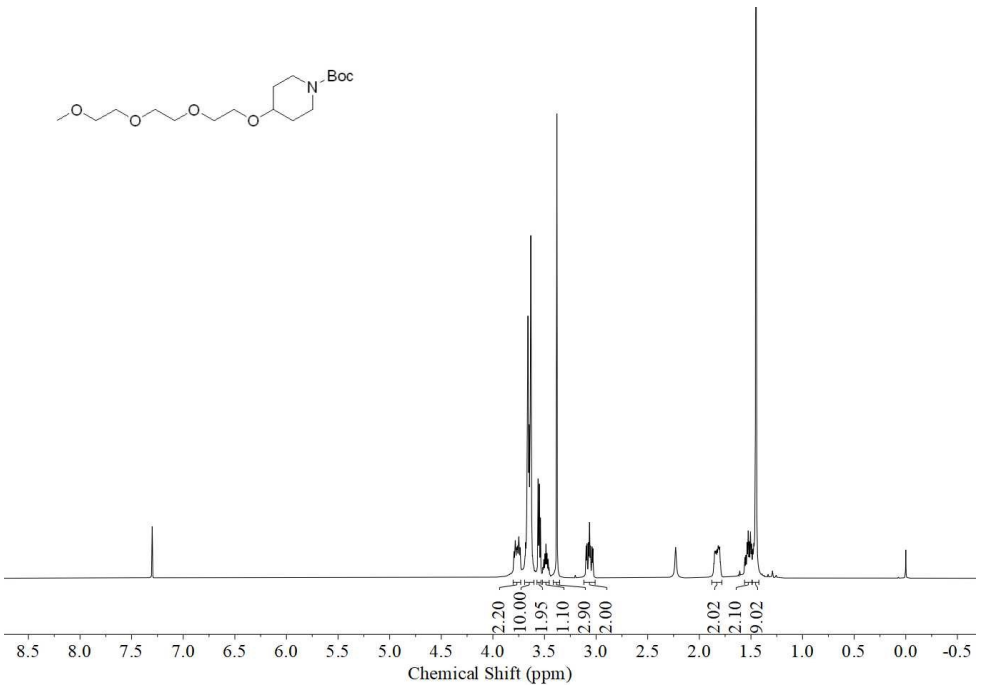


Figure S1. ^{1}H NMR spectrum of compound **3** in $CDCl_3$.

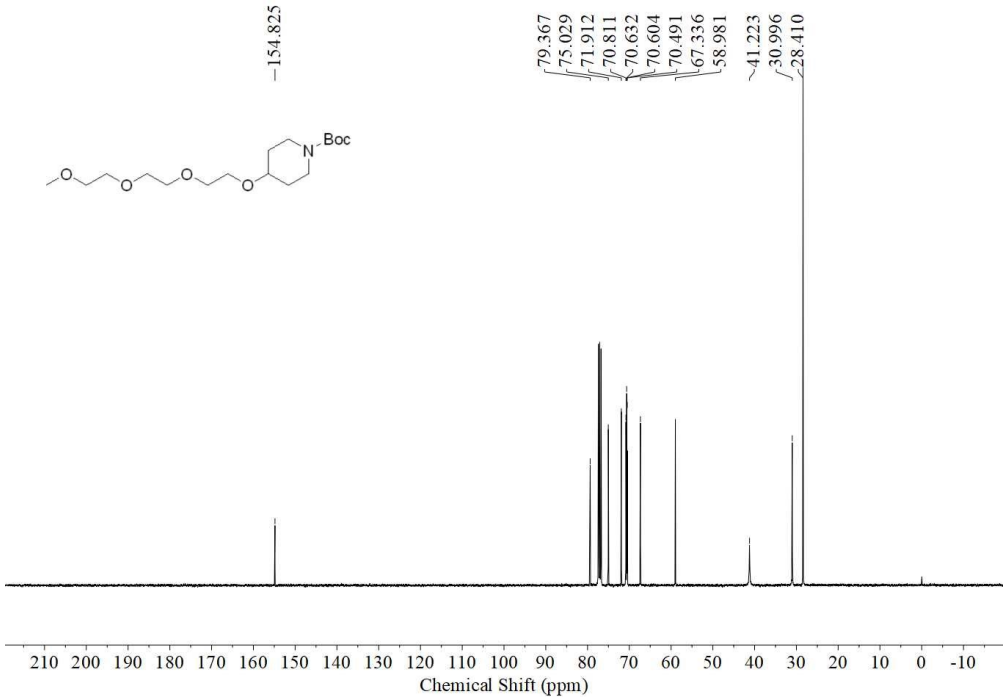


Figure S2. ^{13}C NMR spectrum of compound **3** in $CDCl_3$.

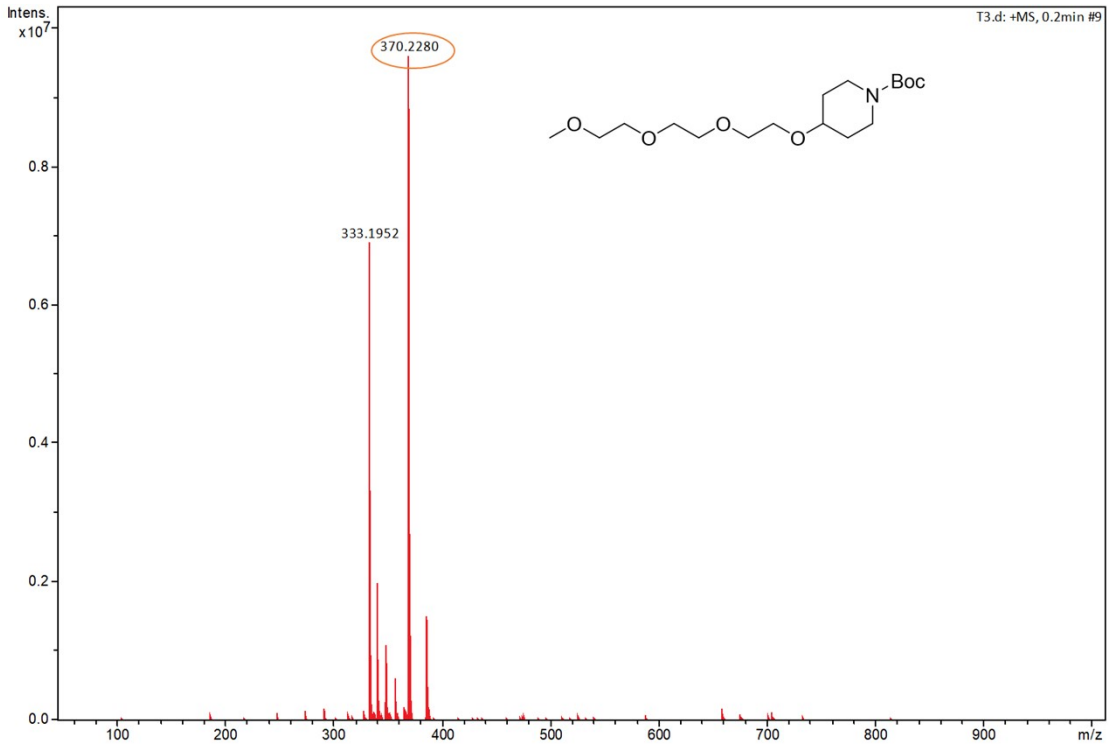


Figure S3. HRMS spectrum of compound **3** in CH_3OH.

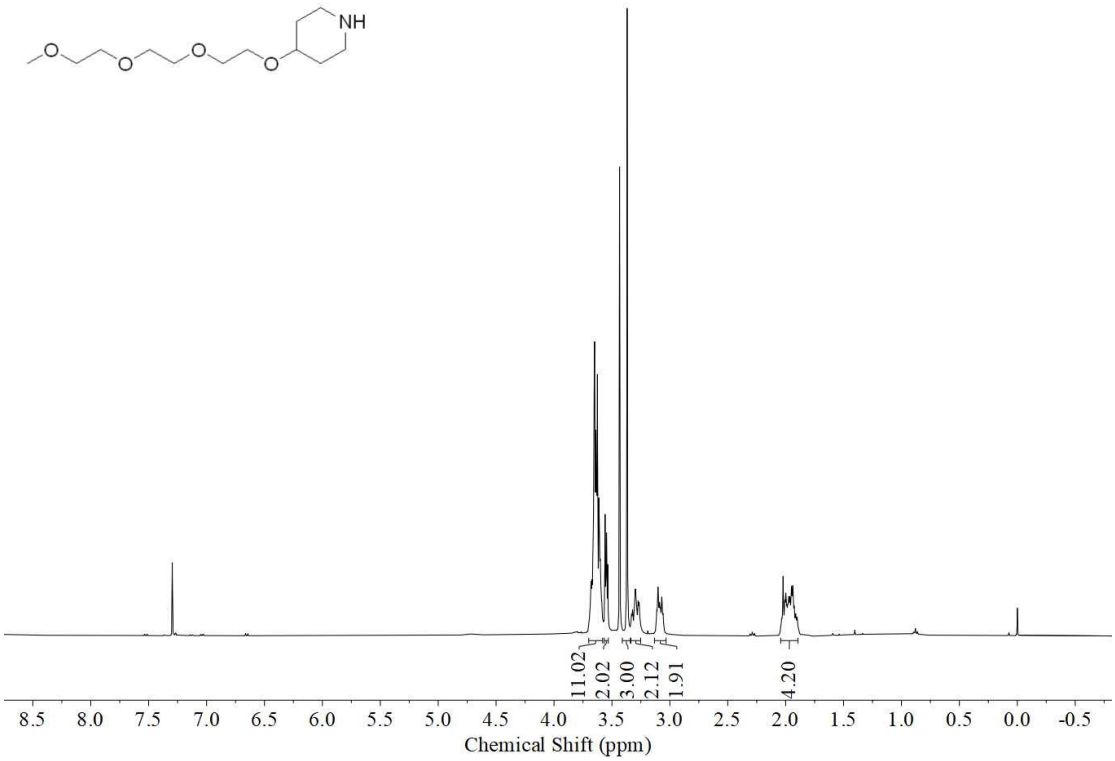


Figure S4. ^{1}H NMR spectrum of compound **4** in $CDCl_3$.

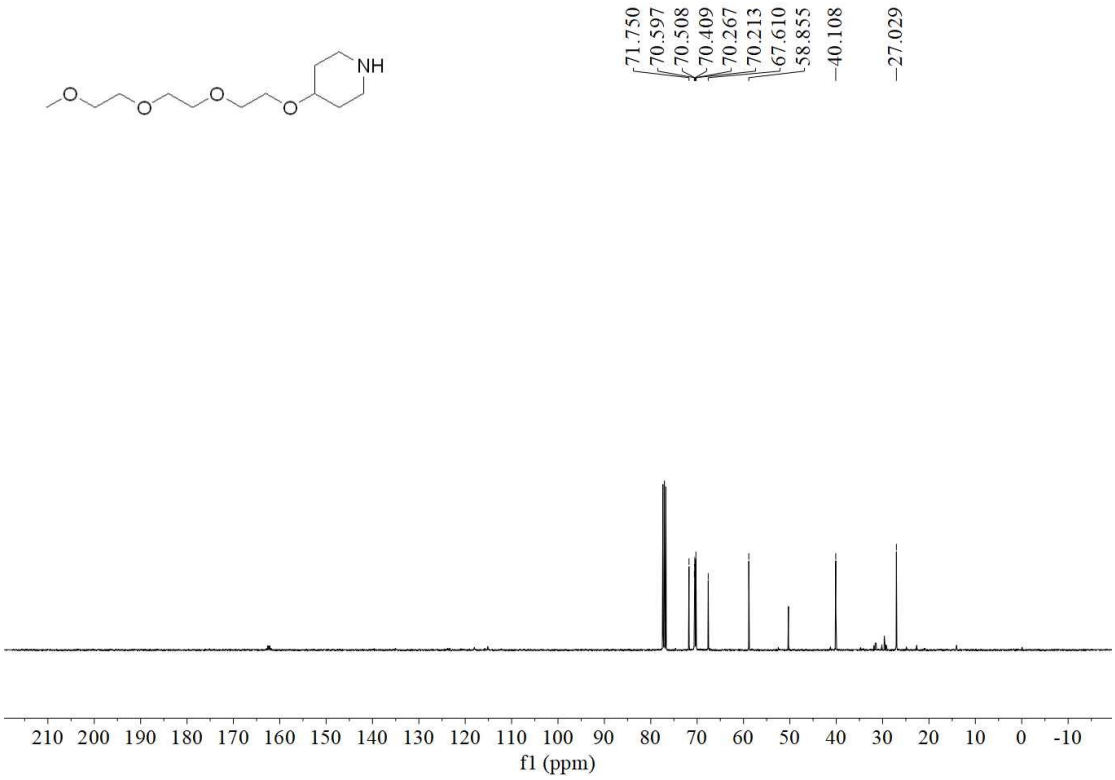


Figure S5. ^{13}C NMR spectrum of compound **4** in $CDCl_3$.

Figure S6. HRMS spectrum of compound **4** in CH_3OH.

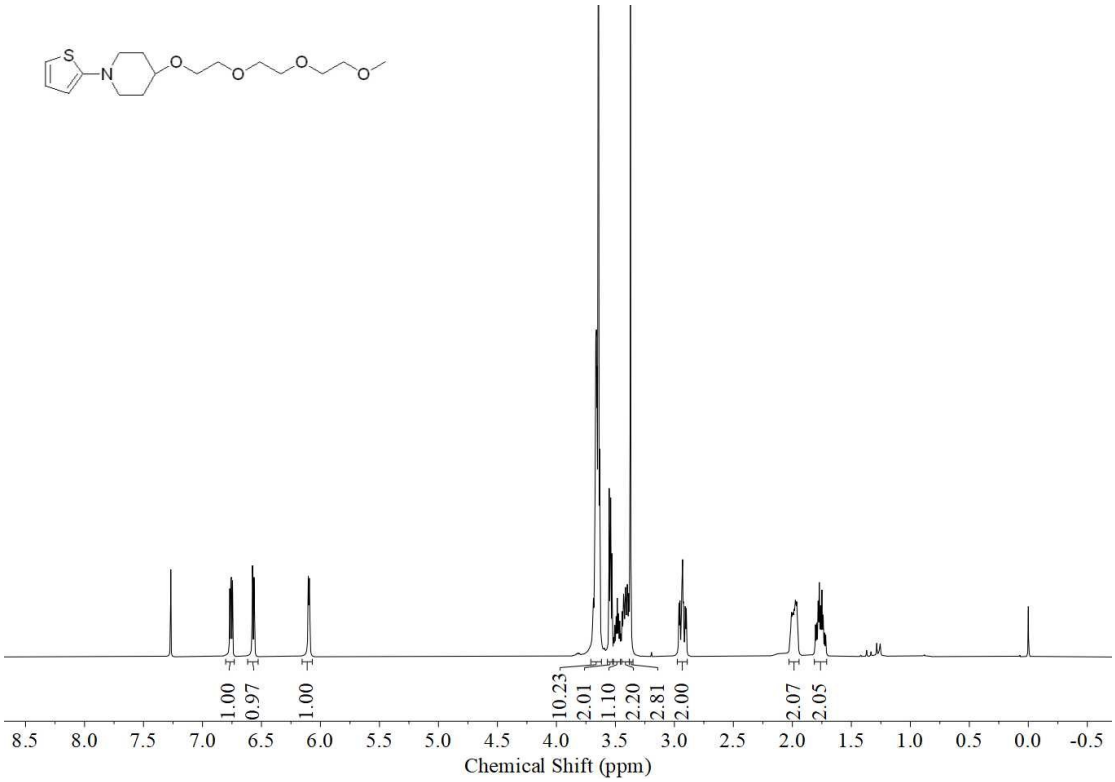


Figure S7. ^1H NMR spectrum of compound **5** in $CDCl_3$.

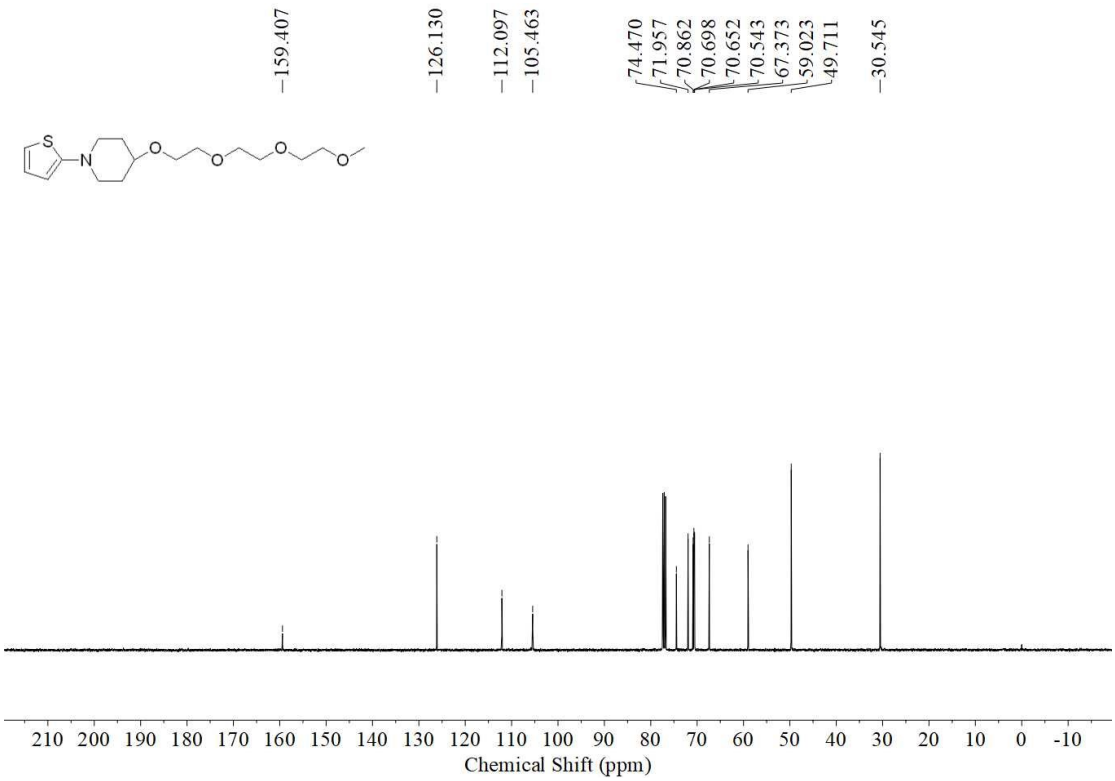


Figure S8. ^{13}C NMR spectrum of compound **5** in $CDCl_3$.

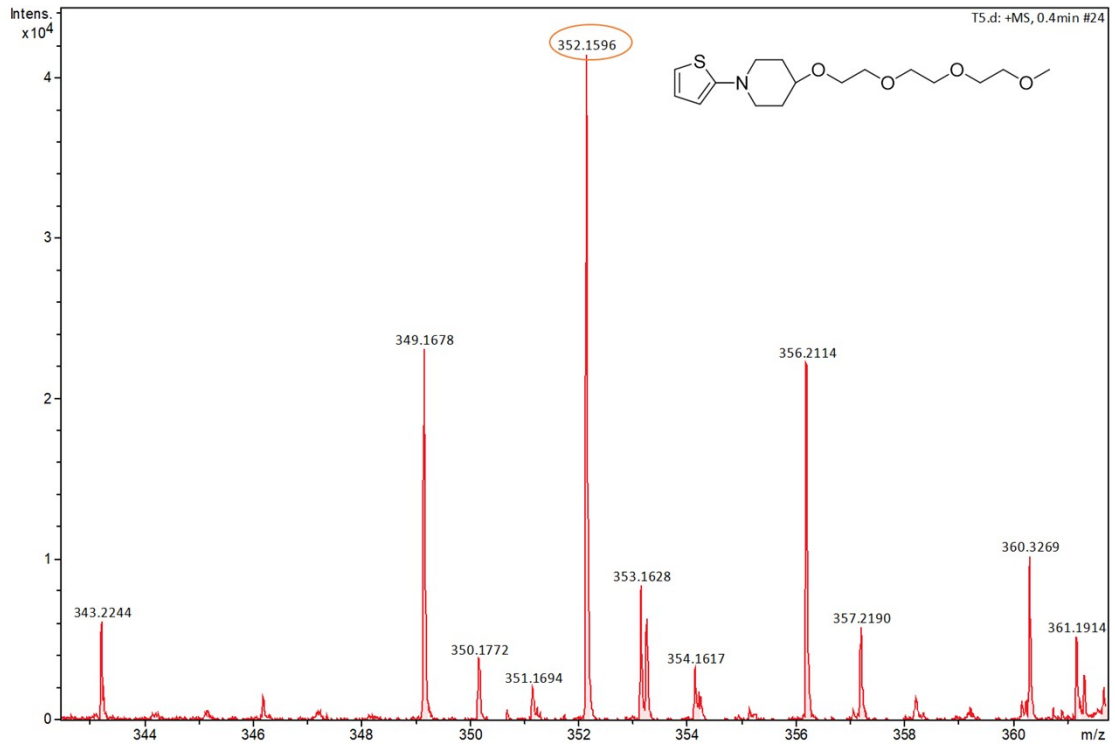


Figure S9. HRMS spectrum of compound **5** in CH_3OH.

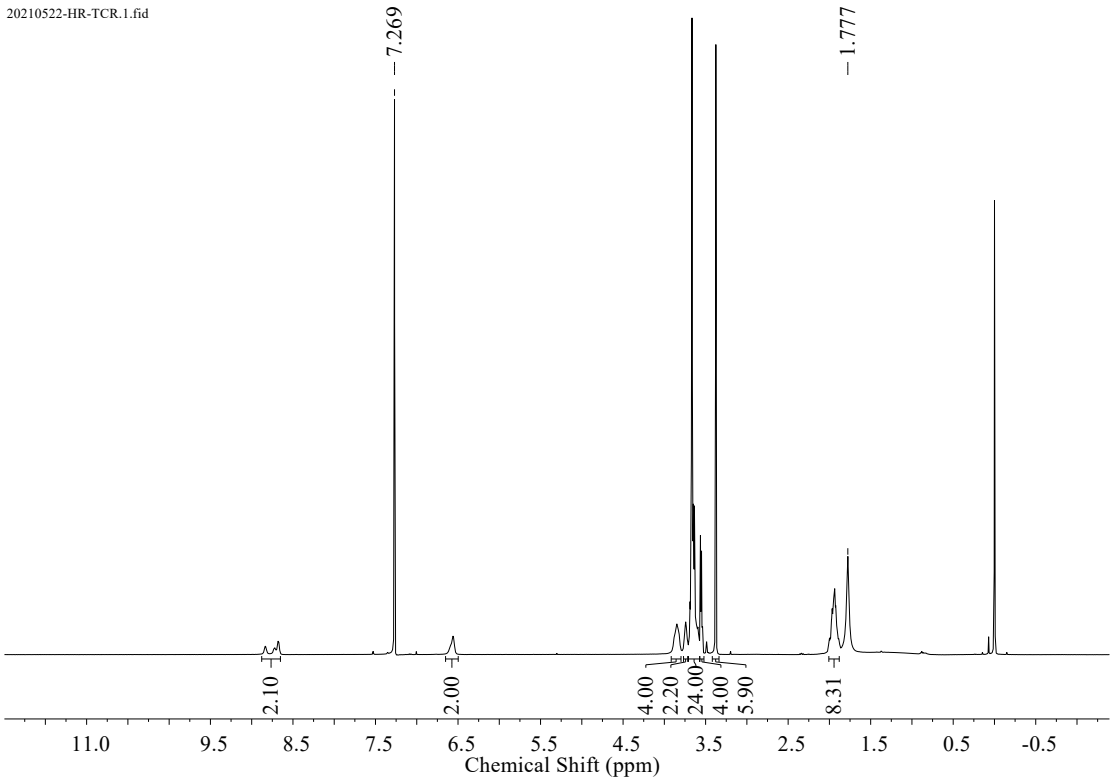


Figure S10. ^{1}H NMR spectrum of **TCR** in $CDCl_3$.

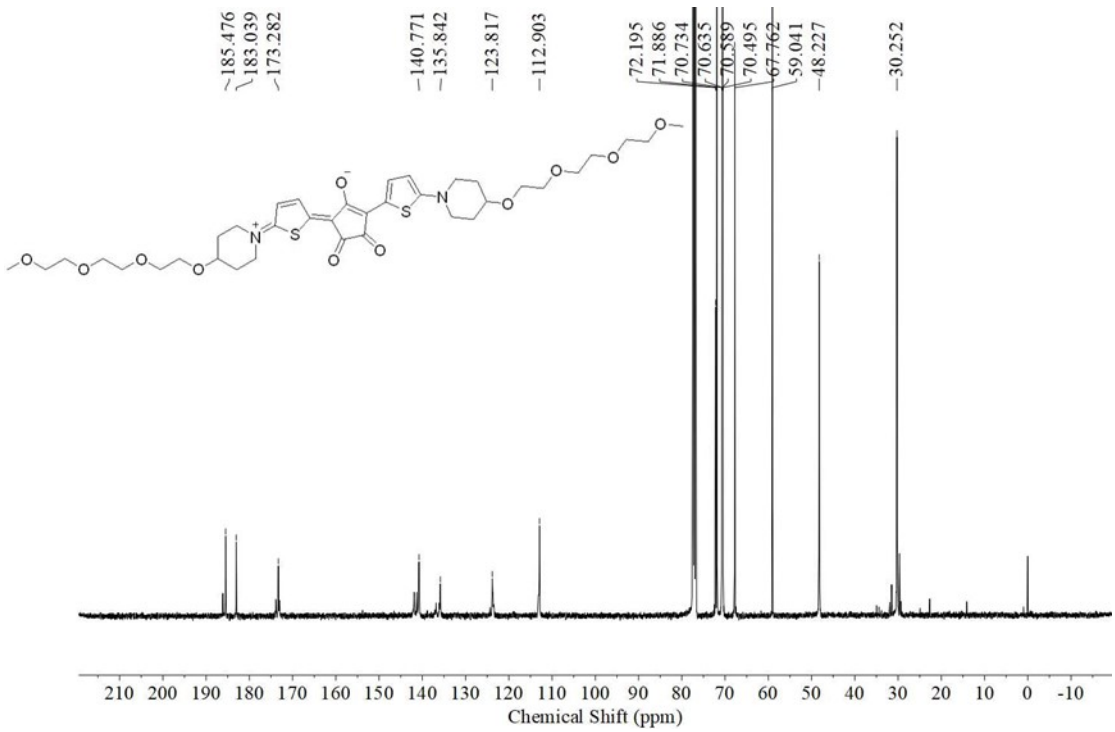


Figure S11. ^{13}C NMR spectrum of **TCR** in $CDCl_3$.

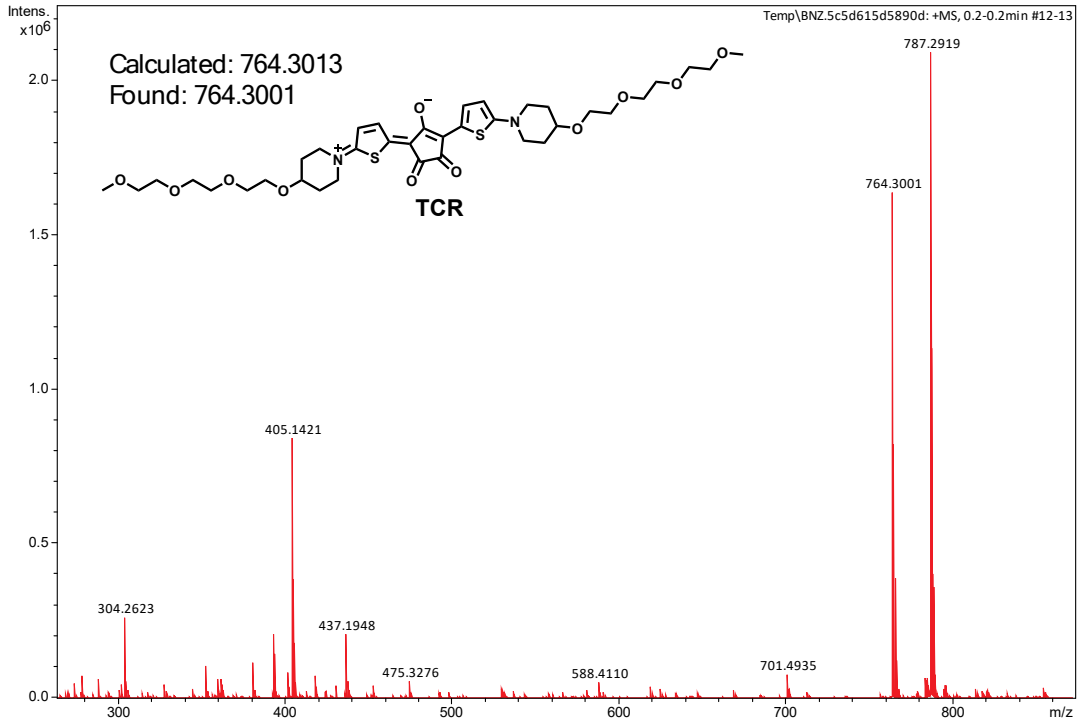


Figure S12. HRMS spectrum of **TCR** in CH_3OH.

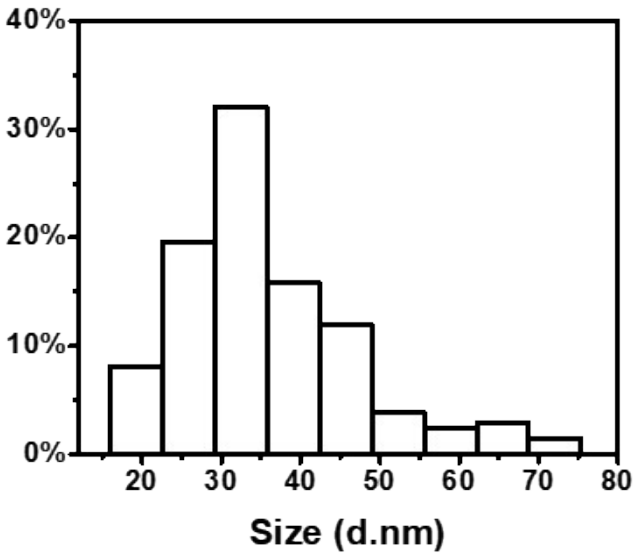


Figure S13. The size histogram of **TCR**.

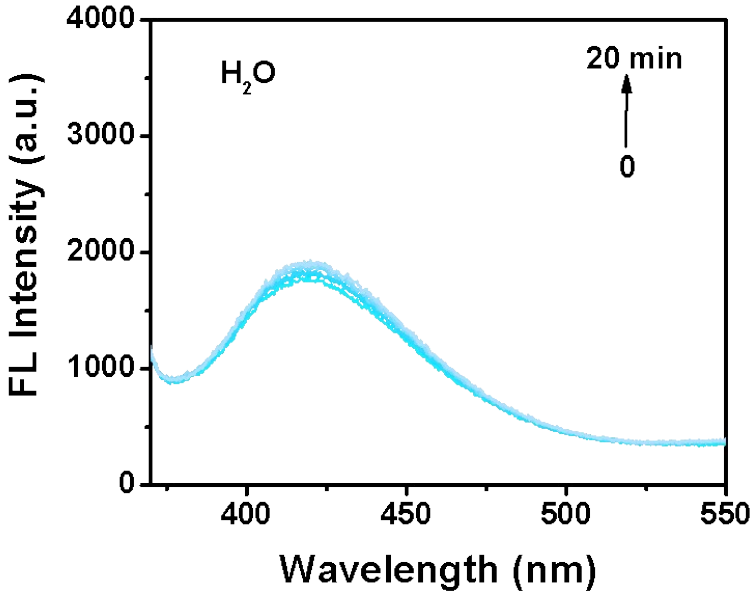


Figure S14. Time-dependent fluorescence spectra of TA aqueous solution under laser irradiation (λ_{ex} = 320 nm, 1.0 W cm^{-2}).

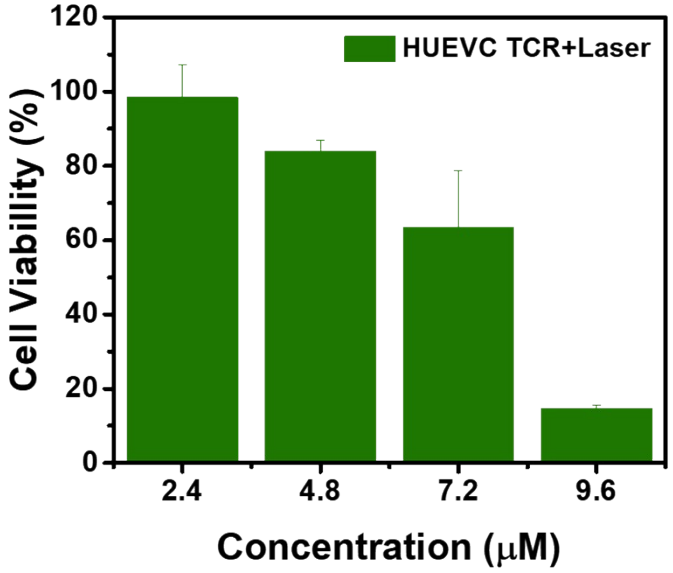


Figure S15. Cytotoxicity assay of **TCR** against HUEVC cells under laser irradiation.

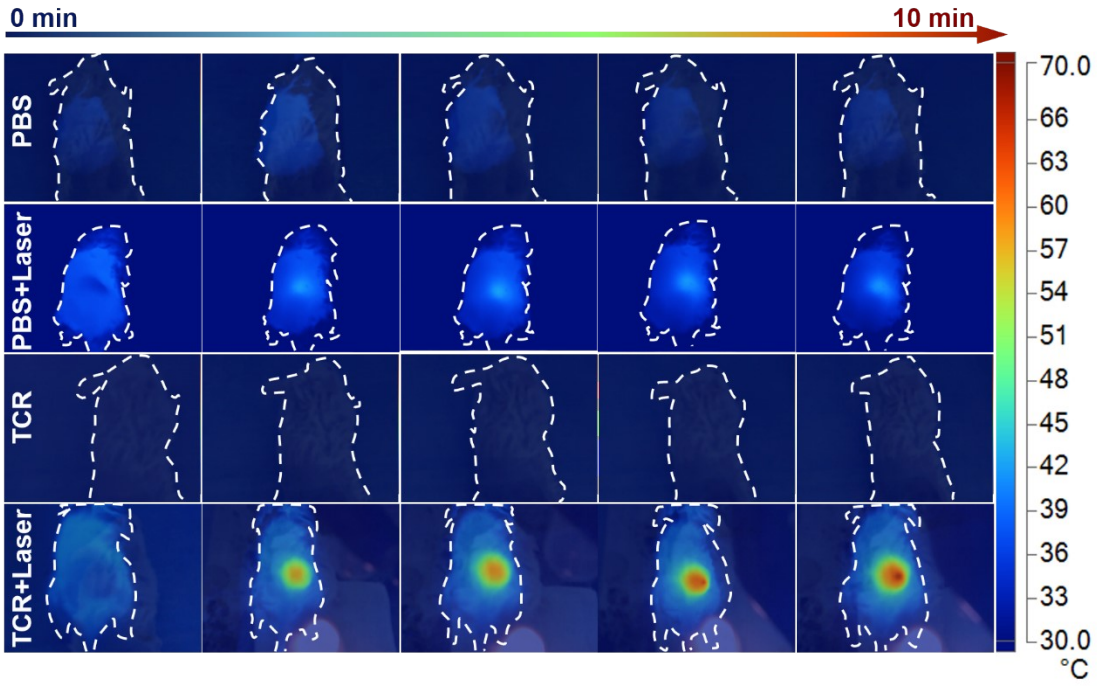


Figure S16. Time-dependent infrared thermography of mice under different conditions (735 nm, 1.0 W cm^{-2}).

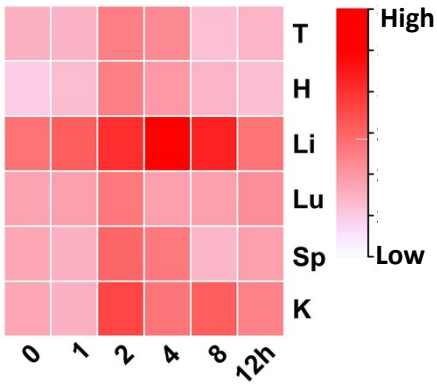


Figure S17. Heat map of fluorescent intensity of major organs (i.e., heart (H), liver (Li), lung (Lu), spleen (Sp), kidneys (K) and tumor (T)) of mice in vitro after intravenous **TCR** injection.

Table S1. Molecular structure and molar extinction coefficient of the recently reported organic phototheranostics.

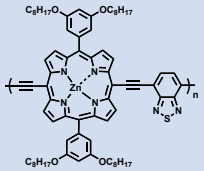
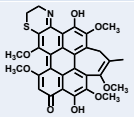
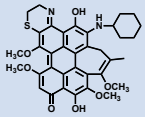
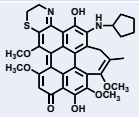
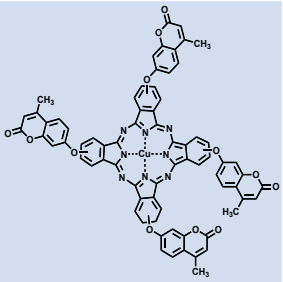
Organic dyes	Molecular structure	ε	Ref.
Porphyrin-PorCP		4.20×10^4 M^{-1} cm^{-1} at 800 nm	1
Hypocrellin-HD		1.62×10^4 M^{-1} cm^{-1} at 650 nm	2
Hypocrellin-HE		2.14×10^4 M^{-1} cm^{-1} at 640 nm	2
Hypocrellin-HF		2.40×10^4 M^{-1} cm^{-1} at 640 nm	2
CuPc-Cou		2.90×10^4 dm^3 mol^{-1} cm^{-1} at 683 nm	3

Table S2. The PCE of different CR dyes.

Material Name	PCE	References
CR780-PEG$_{5K}$	54.49%	4
Croc815	34.7%	5
Croc770	32.0%	5
CR760-PEG-RGD	45.37%	6
Cro-Fe@BSA	17.6%	7

Table S3. Liver and kidney function tests of 4T1 mice treated with saline or **TCR**.

Category	saline	TCR	Units
TP	53.5	50.8	g/L
ALB	26.5	27.5	
GLO	27	23.3	g/L
TBIL	2.83	2.97	umol/L
ALT	34	27	U/L
AST	234	167	
GGT	1.1	1.6	U/L
BUN	6.14	6.5	mmol/L
CRE	74	84	mmol/L
GLU	8.76	8.74	mmol/L

References

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