

Supporting Information

Tumor cell-discrimination photodynamic therapy through RNA-targeting ability of photosensitizer

Yang Tan ^{2, †}, Yuan Xu ^{1, 3, †}, Xiuqin Ma², Xiaoyi Jin ², Miao Li^{2,*}, Ye Tian^{1, 3*}

¹ College of Marine Technology and Environment, Dalian Ocean University, Dalian 116023, China; xuyuan81699@163.com (Y. X.)

² School of Biological Engineering, Dalian Polytechnic University, Dalian 116023, China; tanyang9808@163.com (Y. T.); maxiuqin012@163.com (M. X.); jinxiaoyi519@163.com (J. X.)

³ Key Laboratory of Environment Controlled Aquaculture, Ministry of Education, Dalian 116023, China; xuyuan81699@163.com

* Correspondence: tianye@dlou.edu.cn (Y.T.); lim@mail.dlut.edu.cn (M.L.); Tel.: +86-0411-8476-3255 (Y.T.); +86-0411-8632-2228 (M.L.)

[†] These authors contributed equally to this work.

1. Synthesis of QICY and Intermediates

1.1 Synthesis of Compound Q1

This compound Q1 was prepared according to the published procedure.¹ The solution of 4-iodoaniline (10.95 g, 49.99 mmol) and hydrochloric acid solution was cooled to -10°C and then NaNO₂ aqueous solutions (3.45 g, 49.99 mmol, 15 mL) was added drop wise with continuous stirring. At -10°C, the reaction mixture was stirred for 0.5 h and remained stationary for further 0.5 h. After reaction, the precipitate was washed with water and poured into 100 mL H₂O. Then, the solution was adjusted to pH=13 with saturated NaOH solution. The product filtered from the solution was further purified through a column chromatography using CH₂Cl₂ to collect the brown needles, **Q1** (6.50 g, 55.55%).

¹H NMR (400 MHz, CDCl₃) δ 7.49 (d, *J* = 8.8 Hz, 2H), 6.62 (d, *J* = 8.8 Hz, 2H), 5.18 (s, 1H), 3.55 (s, 2H).

HRMS-ESI: *m/z* calcd [M+H]⁺ for C₆H₈IN₂⁺, 234.9724; found, 234.9727.

1.2 Synthesis of Compound Q3

This compound **Q3** was prepared according to the published procedure.² The mixture of *iso*-propylmethyl ketone (0.66 g, 7.63 mmol) and *para*-iodophenyl hydrazine (1.05 g, 4.49 mmol) was refluxing under the catalysis of acetic acid (10 mL) in the N₂ atmosphere for 5 h. After reaction, the

solution diluted with H₂O was neutral to pH=7 by NaHCO₃. Then, the indole **Q2** was extracted with ethyl acetate, yielding a brown oil (1.10 g, 3.86 mmol, 86% crude). And then **Q2** reacted with iodomethane (0.87 g, 6.10 mmol) in acetonitrile (10 mL) under refluxing at 70°C overnight. The resulting precipitate was filtered, washed with diethyl ether to yield **Q3** as a pink solid (0.29 g, 67%).

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.29 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 3.93 (s, 3H), 2.73 (s, 3H), 1.52 (s, 6H).

HRMS-ESI: *m/z* calcd [M]⁺ for C₁₂H₁₅IN⁺, 300.0244; found, 300.0242.

1.3 Synthesis of Compound **Q4**

Mixing lepidine (2.00 g, 13.97 mmol) with methyl iodide (9.91 g, 69.84 mmol) in 20 mL toluene under refluxing for 8 h can obtain 1-methyl-4-methylquinolinium iodide **Q4**.³ After cooling the solution, the solid product was firstly crushed, then washed several times with ethyl ether, and then dried under vacuum to give yellow product **Q4**.

¹H NMR (500 MHz, DMSO-*d*₆) δ 9.36 (d, *J* = 6.0 Hz, 1H), 8.54 (d, *J* = 9.1 Hz, 1H), 8.49 (d, *J* = 8.9 Hz, 1H), 8.28 (t, *J* = 7.9 Hz, 1H), 8.07 (dd, *J* = 13.0, 6.7 Hz, 2H), 4.58 (s, 3H), 3.01 (s, 3H).

HRMS-EI: *m/z* calcd [M]⁺ for C₁₁H₁₂N⁺, 158.0964; found, 158.0964.

1.4 Synthesis of Compound **Q5**

Compound **Q4** (1.80 g, 6.32 mmol) reacted with N,

N'-diphenylformamidine (1.24 g, 6.32 mmol) at 160°C for 30 min.³ Then, the resulting precipitate was washed several times with ethyl ether. And then the dry powder was further crystallized from ethanol to obtain **Q5** in fair purity (1.79 g, 4.61 mmol, 73% yield).

¹H NMR (500 MHz, DMSO-*d*₆) δ 10.94 (d, *J* = 11.9 Hz, 1H), 8.89 (t, *J* = 12.2 Hz, 1H), 8.66 (d, *J* = 7.0 Hz, 1H), 8.32 (d, *J* = 8.5 Hz, 1H), 8.14 (dd, *J* = 7.7, 4.9 Hz, 2H), 8.10 – 8.05 (m, 1H), 7.84 (t, *J* = 7.6 Hz, 1H), 7.48 – 7.39 (m, 4H), 7.13 (t, *J* = 7.1 Hz, 1H), 6.75 (d, *J* = 12.4 Hz, 1H), 4.24 (s, 3H).

HRMS-ESI: *m/z* calcd [M]⁺ for C₁₈H₁₇N₂⁺, 261.1386; found, 261.1384.

1.5 Synthesis of Compound **QICY**

Firstly, **Q3** (426.84 mg, 1.00 mmol) and **Q 5** (388.05 mg, 1.00 mmol) were dissolved in the mixed solutions of CH₂Cl₂/CH₃OH (*V*₁/*V*₂=1:1). Then, 1 mL of (Ac)₂O and 1 mL N(Et)₃ were added as the catalyst in to the solutions with stirring at room temperature for 1.5 h. After reaction, the precipitated dye **QICY** was washed by high volumes of ethyl ether. And the crude dye **QICY** was further purified by silica column chromatography using the mixture of CH₂Cl₂/CH₃OH (*V*₁/*V*₂=200:1200/) mixture as eluent. Meanwhile, the blue fraction was collected, yielding green solid (209.50 mg, 0.35 mmol, 35% yield).

¹H NMR (500 MHz, DMSO-*d*₆) δ 8.65 (dd, *J* = 15.5, 7.7 Hz, 2H), 8.29 (t, *J* = 13.2 Hz, 1H), 8.18 (dd, *J* = 10.7, 8.0 Hz, 2H), 8.09 (t, *J* = 7.4 Hz, 1H), 7.88 – 7.82 (m, 2H), 7.63 (d, *J* = 9.8 Hz, 1H), 7.34 (d, *J* = 13.8 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 6.17 (d, *J* = 12.5 Hz, 1H), 4.30 (s, 3H), 3.32 (s, 3H), 1.68 (s, 6H).

^{13}C NMR (101 MHz, DMSO- d_6) δ 166.65, 151.77, 144.76, 143.36, 142.49, 142.12, 138.79, 136.48, 133.85, 130.61, 127.63, 125.21, 124.65, 118.59, 113.28, 111.85, 111.26, 100.03, 85.41, 47.17, 45.73, 42.99, 29.88, 27.94.

HRMS-ESI: m/z calcd $[\text{M}]^+$ for $\text{C}_{24}\text{H}_{24}\text{IN}_2^+$, 467.0979; found, 467.0974.

2. Structure Analyzing and Confirmation ^1H -NMR, ^{13}C -NMR, and TOF-MS Spectra of QICY and Intermediates

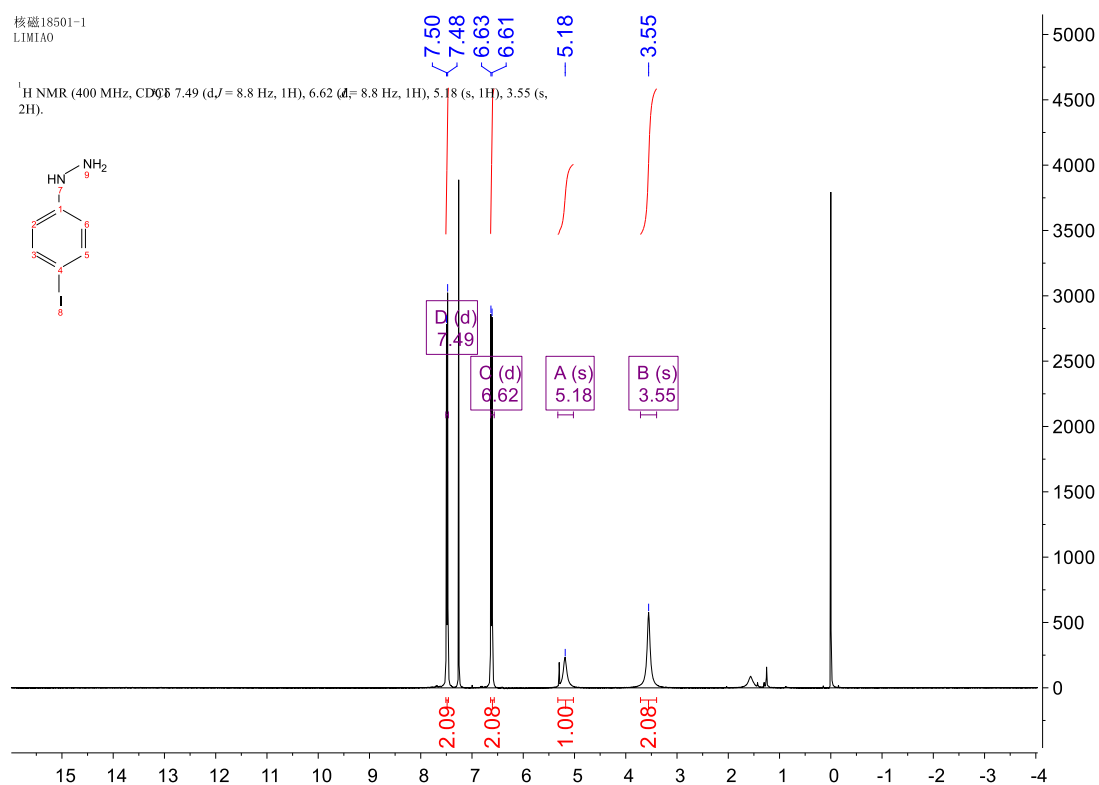


Figure S1. ^1H NMR spectrum of compound **Q1** recorded in CDCl_3 .

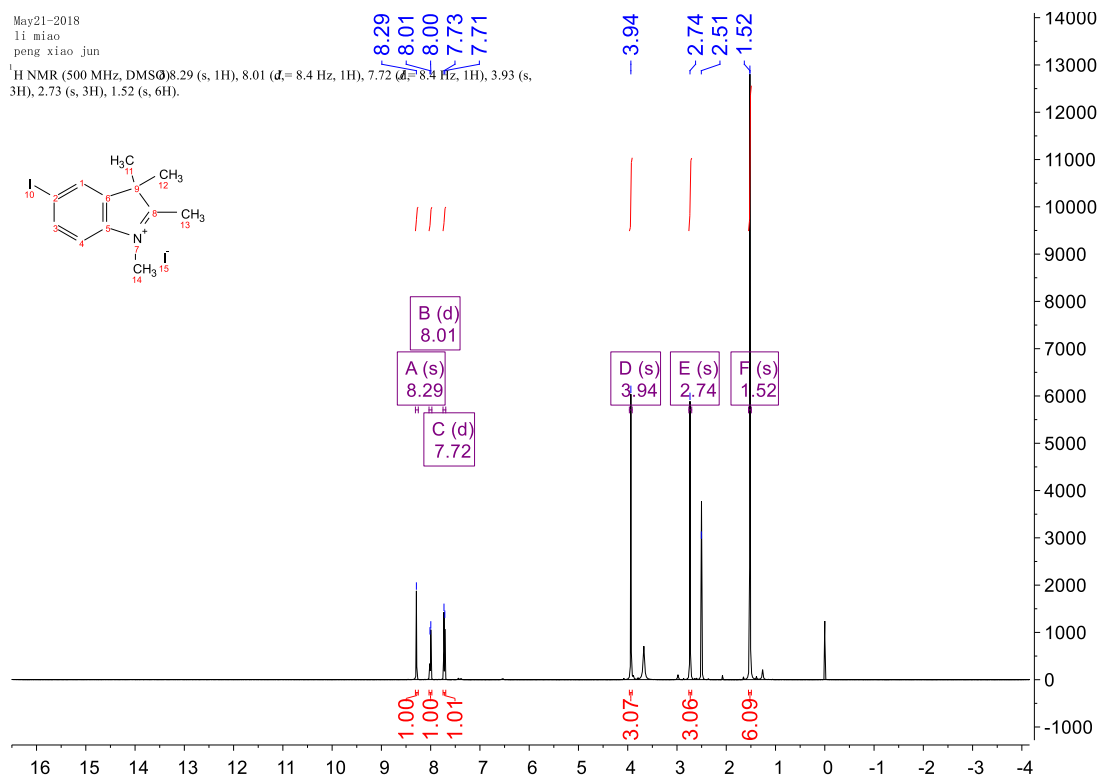


Figure S2. ¹H NMR spectrum of compound **Q3** recorded in DMSO-*d*₆.

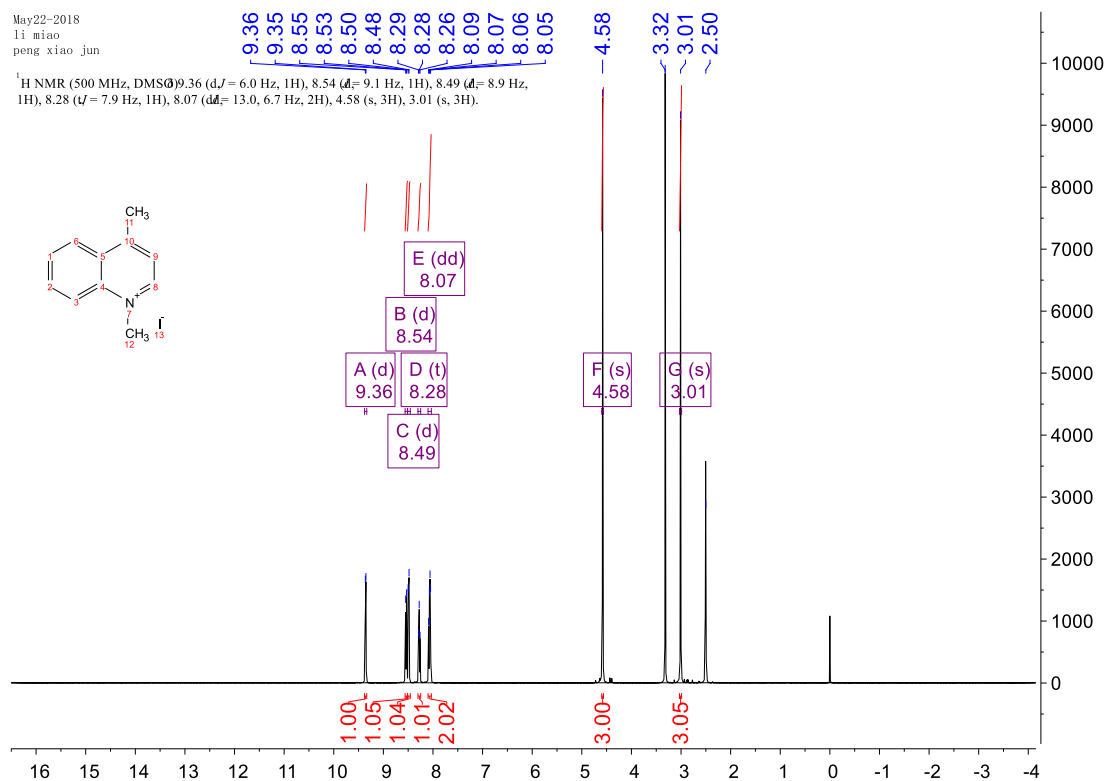


Figure S3. ¹H NMR spectrum of compound **Q4** recorded in DMSO-*d*₆.

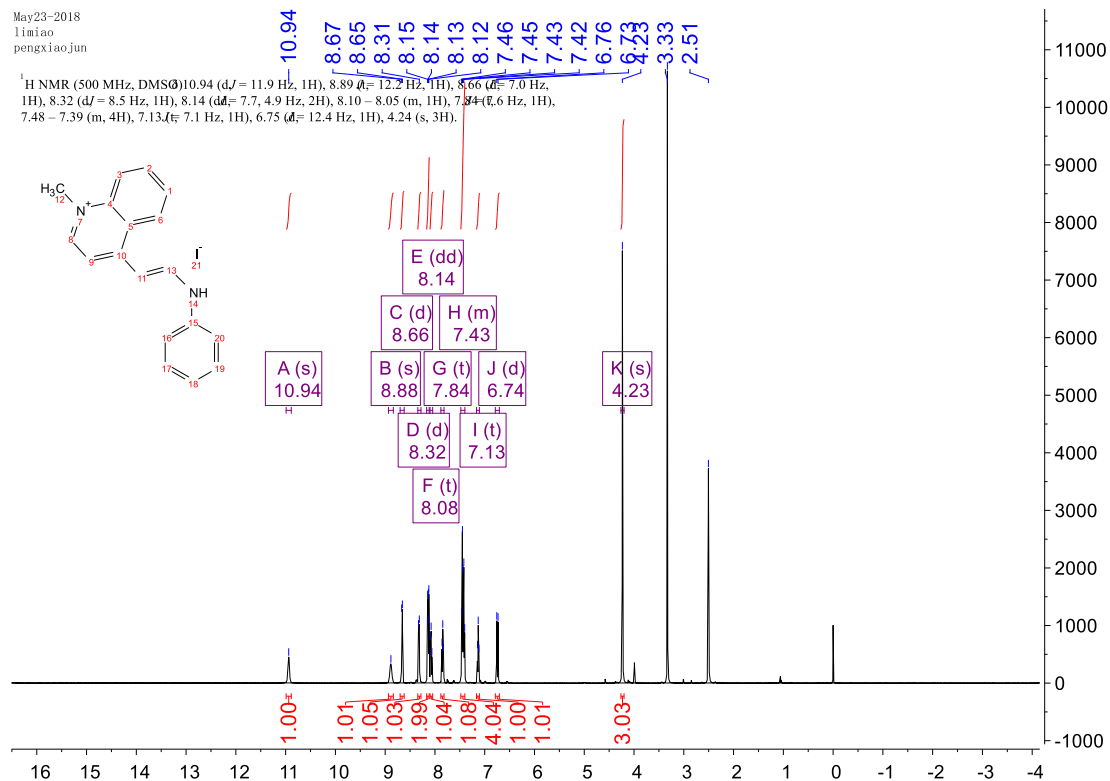


Figure S4. ¹H NMR spectrum of compound **Q5** recorded in DMSO-*d*₆.

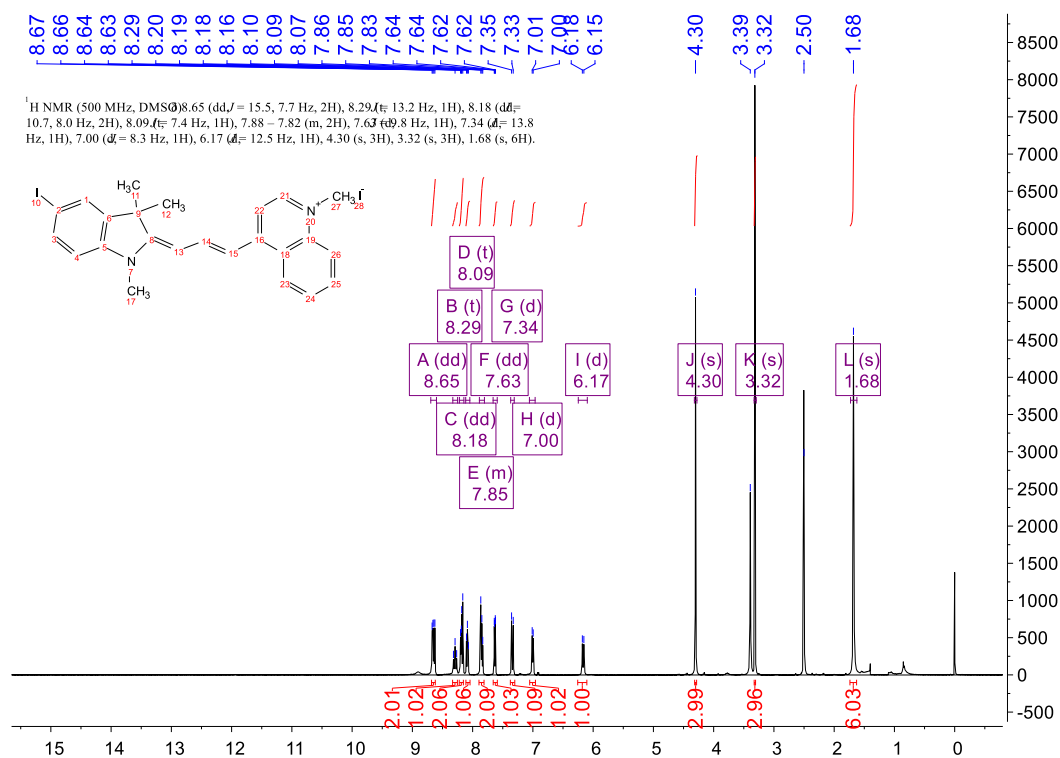


Figure S5. ¹H NMR spectrum of **QICY** recorded in DMSO-*d*₆.

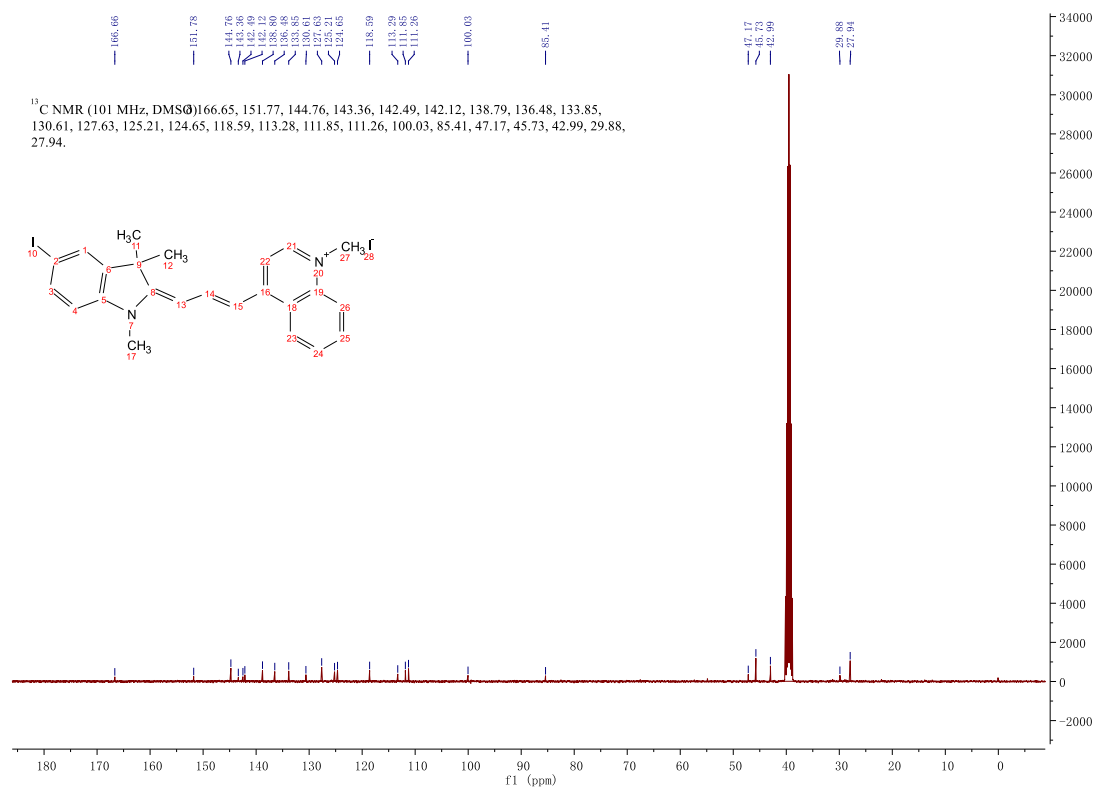


Figure S6. ¹³C NMR spectrum of QICY recorded in DMSO-*d*₆.

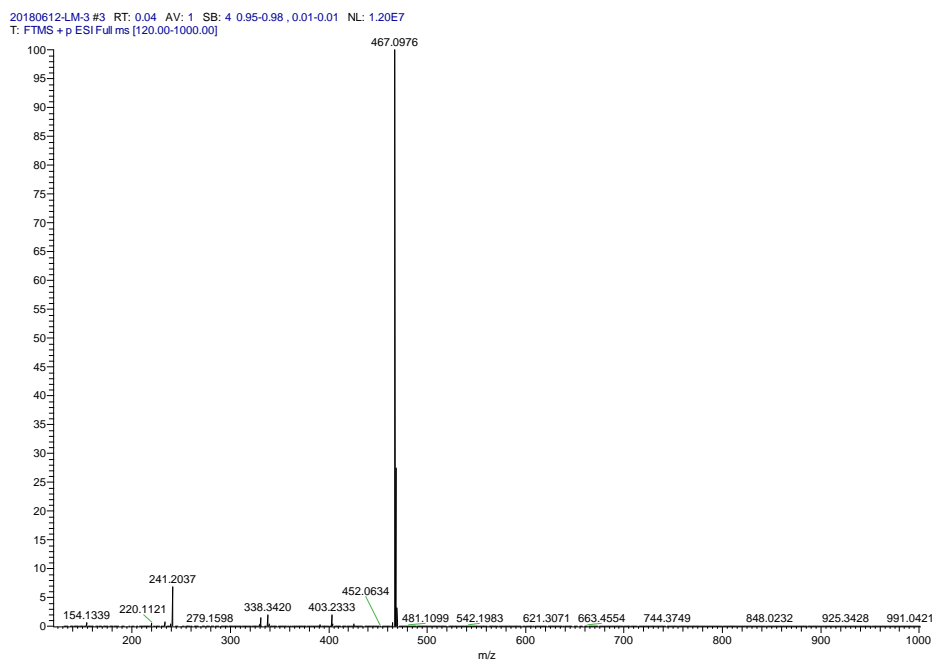


Figure S7. ESI mass spectrum of compound QICY.

3. Subcellular Localization Assay

The investigation of QICY subcellular localization were firstly explored by the co-localization assays with commercial organelle dyes Mito-tracker® Green FM and Hoechst 33342 for organelle localization, and then with SYTO® RNASelect™ green fluorescent cell stain and Hoechst 33342 for component localization. SYTO® RNASelect™ green fluorescent cell stain and Hoechst 33342 are selective for intracellular RNA and dsDNA separately. QICY and the commercial organelle trackers were successively added to the same dish and cultured with cells at 37°C for different duration in culture medium. The culture concentrations and working procedures of commercial trackers can be acquired from production instruction books provided by reagent companies. After incubation, cells were washed with PBS three times after discarding the culture medium, and 1 mL serum-free medium was finally added before imaging. Cells were imaged with a 100× oil-immersion objective lens in an inverted-type scanning microscope (Olympus FV3000). To avoid the cross-color interference, the fluorescence of QICY, RNA stain and Hoechst 3342 were excited and collected in the different phases of confocal laser scanning microscope. The laser wavelengths to excite QICY, commercial RNA stain and Hoechst 3342 and the corresponding collection ranges of fluorescence emission are listed in the Table S1.

Table S1. Imaging conditions of QICY, RNA stain, and Hoechst 3342 in CLSM

	λ_{ex} (nm)	λ_{em} (nm)
QICY	640	650- 750
Mito-tracker® Green FM	488	510-540
SYTO®RNASelect™	488	520- 600
Hoechst3342	405	430- 470

4. Experimental results

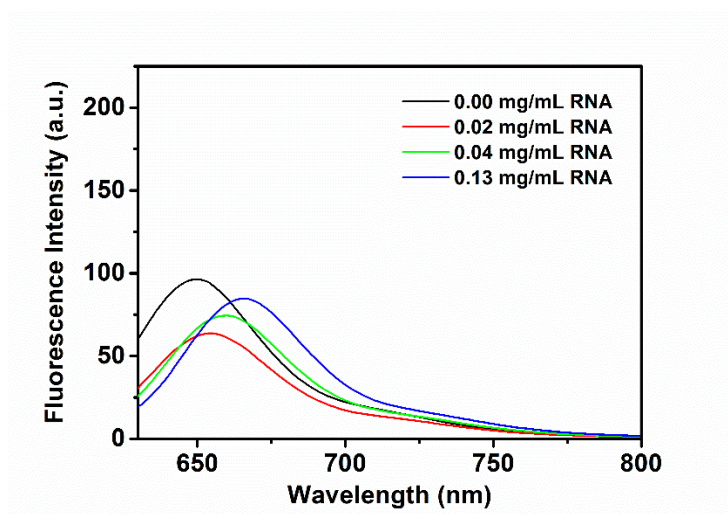


Figure S8. Influence of RNA titration on the fluorescence emission spectra of QICY (1.3 μM) in ultrapure water.

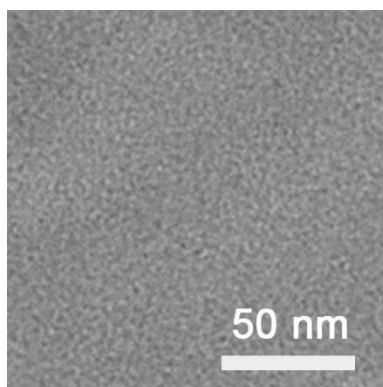


Figure S9. No QICY aggregates observed by TEM before interaction with RNA in ultrapure water.

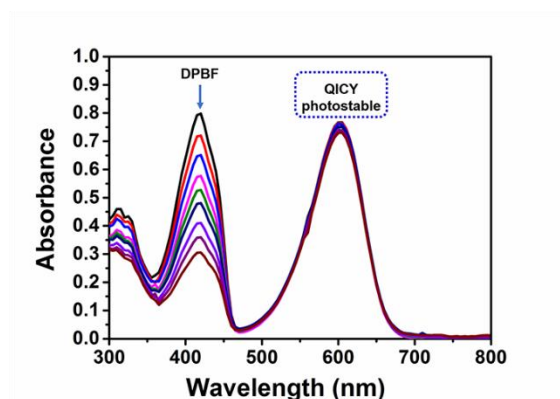


Figure S10. Absorbance change of QICY and DPBF in DMSO with irradiation time.

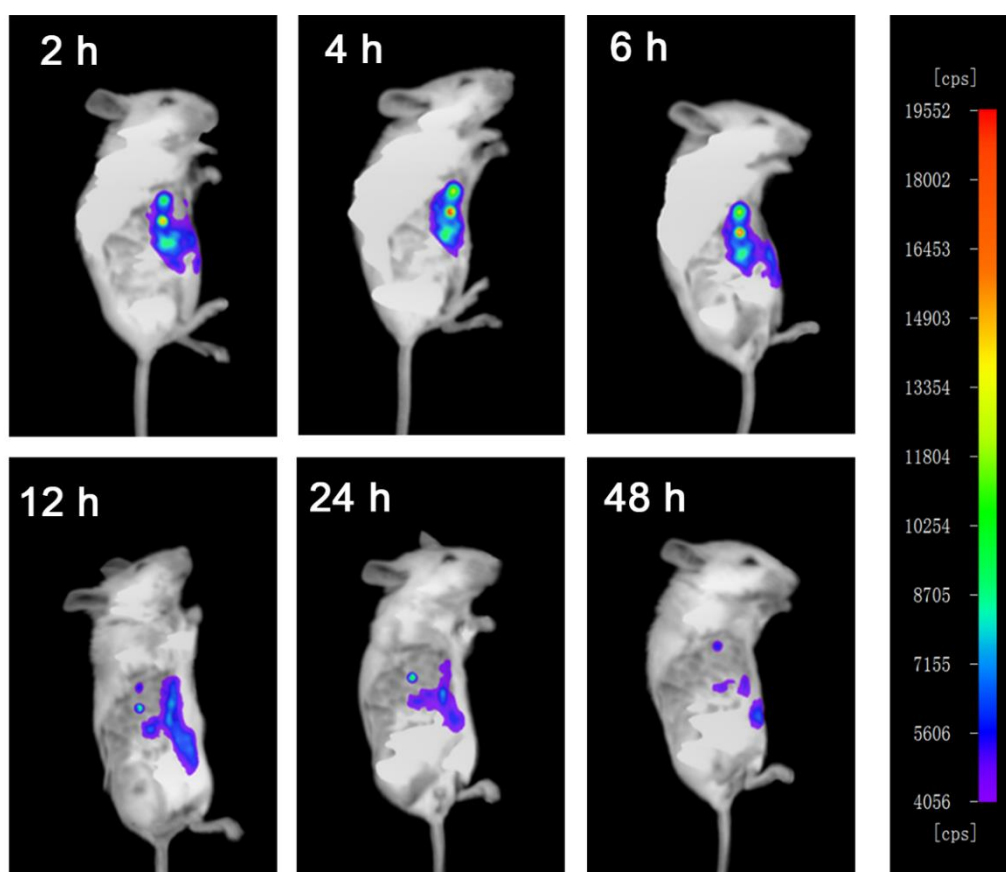


Figure S11. *In vivo* fluorescence imaging of 4T1 tumor-bearing BALB/c mice after the intravenous injection of QICY at the concentration of 400 μ M. Ex. = 630 nm/ Em. = 700 nm.

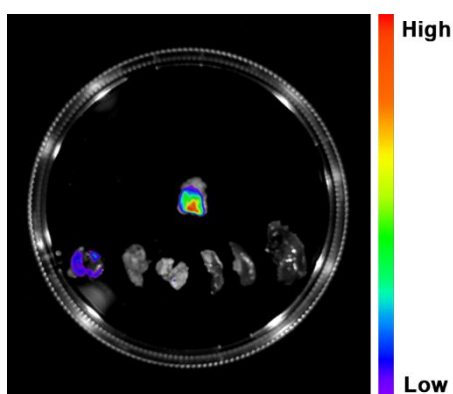


Figure S12. Fluorescence imaging of various organs (from top to bottom, left to right: tumor, kidney, colon, lung, heart, spleen, and liver) at 4 h after the intravenous injection of QICY (tail vein injection, 400 μ M, 100 μ L, 1.73 mg/kg).

References

- (1) Atchison, J.; Kamila, S.; Nesbitt, H.; Logan, K. A.; Nicholas, D. M.; Fowley, C.; Davis, J.; Callan, B.; McHale, A. P.; Callan, J. F. Iodinated cyanine dyes: a new class of sensitisers for use in NIR activated photodynamic therapy (PDT). *Chem. Commun.* **2017**, 53, 2009-2012.
- (2) Klotz, E. J. F.; Claridge, T. D. W.; Anderson, H. L. Homo- and hetero- 3 rotaxanes with two pi-systems clasped in a single macrocycle. *J. Am. Chem. Soc.* **2006**, 128, 15374-15375.
- (3) Peng, X. J.; Wu, T.; Fan, J. L.; Wang, J. Y.; Zhang, S.; Song, F. L.; Sun, S. G. An Effective Minor Groove Binder as a Red Fluorescent Marker for Live-Cell DNA Imaging and Quantification. *Angewandte Chemie-International Edition* **2011**, 50, 4180-4183.