

Electronic Supplementary Information

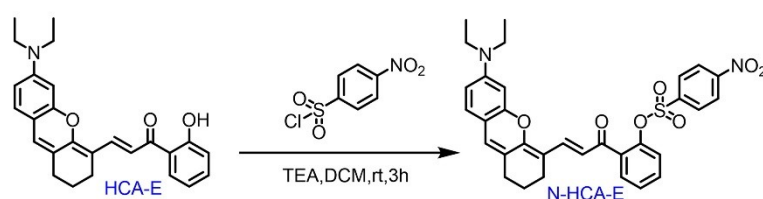
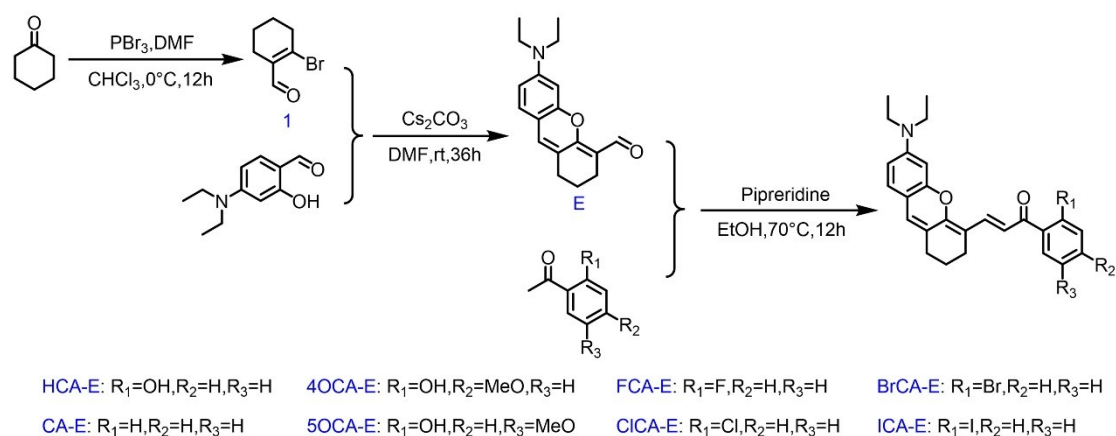
Developing large Stokes shift NIR xanthene-chalcone fluorophores for fluorescence imaging

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1. Synthesis and characterization of compounds



Scheme S1. The synthetic procedure of compounds.

Synthesis of compound E

The DMF (1.2 mL, 15.5 mmol) and CHCl₃ (10 mL) mixture was placed in a flask and PBr₃ (0.9 mL, 9.5 mmol) was slowly added under ice bath conditions. After stirring the mixture for 1 h, cyclohexanone (0.4 mL, 3.9 mmol) was added, followed by continued stirring at 25°C for 12 h. Upon completion of the reaction, the mixture was poured into ice water to quench any remaining PBr₃, and sodium hydroxide was added to adjust the pH=7. The organic layer was then subjected to multiple washes with methylene chloride and water before being separated and dried using anhydrous magnesium sulfate. The solvent was removed through vacuum distillation resulting in compound **1** as a yellow oil (0.6 g, 80 % yield).

The compounds **1** (0.4 g, 2.1 mmol), p-4-(diethylamino)-2-hydroxybenzaldehyde (0.39 g, 2 mmol) and Cs₂CO₃ (1.95 g, 6 mmol) were dissolved in 10 ml of anhydrous DMF and the mixture was stirred at 25°C for 36 h. After the reaction, the filtrate was filtered, collected and concentrated by spin steaming. The concentrate was washed several times

with methylene chloride and water, the organic layer was separated and dried with anhydrous magnesium sulfate. The solvent is removed by vacuum distillation and the crude product is obtained. After further purification by column chromatography, compound E was finally obtained as an orange-yellow solid (0.21 g, 37 %). ¹H NMR (600 MHz, Chloroform-*d*) δ 10.24 (s, 1H), 6.97 (d, *J* = 8.6 Hz, 1H), 6.60 (s, 1H), 6.39 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.33 (d, *J* = 2.1 Hz, 1H), 3.36 (q, *J* = 7.1 Hz, 4H), 2.52 – 2.49 (t, 2H), 2.42 (t, *J* = 6.0 Hz, 2H), 1.69 – 1.65 (m, 2H), 1.18 (t, *J* = 7.1 Hz, 6H). ¹³C NMR (151 MHz, Chloroform-*d*) δ 187.15, 162.15, 154.22, 149.65, 128.29, 127.68, 123.10, 111.34, 110.32, 107.85, 97.18, 77.37, 44.65, 29.88, 21.68, 20.72, 12.65.

Synthesis of xanthene-chalcone fluorophores

The synthesis methods of xanthene-chalcone fluorophores were similar. Acetophenone (**CA-E**, 0.58 g, 1.5 mmol), 2-hydroxyacetophenone (**HCA-E**, 0.60 g, 1.5 mmol), 2-hydroxy-4-methoxyacetophenone (**4OCA-E**, 0.65 g, 1.5 mmol), 2-hydroxy-5-methoxyacetophenone (**5OCA-E**, 0.65 g, 1.5 mmol), 2-fluoroacetophenone (**FCA-E**, 0.61 g, 1.5 mmol), 2-chlorophenone (**CICA-E**, 0.63 g, 1.5 mmol), 2-bromoacetophenone (**BrCA-E**, 0.70 g, 1.5 mmol), 2-iodoacetophenone (**ICA-E**, 0.77 g, 1.5 mmol) were respectively dissolved in 10 mL anhydrous ethanol, slowly added with 8 drops of piperidine. After the reaction was refluxed at 70°C for 12 h, the solvent was removed by vacuum distillation. The crude product was separated and purified by column chromatography to obtain the xanthene-chalcone fluorophores.

CA-E

Fuchsia solid (0.14 g, 24.1 %). ¹H NMR (400 MHz, Chloroform-*d*) δ 8.53 (d, *J* = 15.4 Hz, 1H), 8.01 (d, *J* = 7.4 Hz, 2H), 7.51 (d, *J* = 6.7 Hz, 1H), 7.47 (d, *J* = 7.4 Hz, 2H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 14.0 Hz, 1H), 6.45 (s, 1H), 6.41 (d, *J* = 4.5 Hz, 1H), 6.34 (s, 1H), 3.42 – 3.34

(q, 4H), 2.91 – 2.81 (m, 4H), 1.57 (m, $J = 6.9$ Hz, 2H), 1.39 – 1.33 (m, 6H). HR-MS (ESI, m/z) calcd $[M+H]^+$ for $[C_{26}H_{28}NO_2]^+$: 386.2115, found: 386.2109.

4OCA-E

Fuchsia solid (0.21 g, 32.3 %). 1H NMR (400 MHz, Chloroform-*d*) δ 14.13 (s, 1H), 8.54 (d, $J = 14.5$ Hz, 1H), 7.78 (d, $J = 9.0$ Hz, 1H), 6.92 (d, $J = 8.6$ Hz, 1H), 6.79 (d, $J = 14.7$ Hz, 1H), 6.47 (s, 1H), 6.43 (m, $J = 9.1$ Hz, 3H), 6.35 (d, $J = 7.9$ Hz, 1H), 3.83 (s, 3H), 3.38 (q, $J = 7.0$ Hz, 4H), 2.51 (s, 4H), 1.83 – 1.77 (m, 2H), 1.20 (t, $J = 7.1$ Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 191.65, 166.38, 165.33, 155.47, 155.03, 149.52, 139.93, 130.76, 127.20, 126.34, 124.21, 114.88, 112.94, 110.82, 109.18, 107.45, 107.16, 101.13, 97.61, 77.48, 55.59, 44.67, 29.84, 25.19, 21.15, 12.82. HR-MS (ESI, m/z) calcd $[M+H]^+$ for $[C_{27}H_{30}NO_4]^+$: 432.2169, found: 432.2164.

5OCA-E

Fuchsia solid (0.19 g, 29.2 %). 1H NMR (600 MHz, Chloroform-*d*) δ 13.06 (s, 1H), 8.59 (d, $J = 14.7$ Hz, 1H), 7.35 (d, $J = 3.0$ Hz, 1H), 7.06 (dd, $J = 9.0, 3.0$ Hz, 1H), 6.94 (dd, $J = 8.8, 5.0$ Hz, 2H), 6.81 (d, $J = 14.7$ Hz, 1H), 6.52 (s, 1H), 6.46 (s, 1H), 6.36 (s, 1H), 3.82 (s, 3H), 2.53 (d, $J = 6.2$ Hz, 4H), 1.84 – 1.81 (m, 2H), 1.58 (s, 2H), 1.21 (t, $J = 7.1$ Hz, 6H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 192.66, 157.81, 156.19, 155.10, 151.60, 149.70, 141.06, 127.33, 126.98, 124.16, 122.31, 120.83, 119.07, 113.11, 112.64, 110.89, 109.40, 107.72, 97.63, 77.37, 56.30, 44.71, 29.83, 25.21, 21.14, 12.83. HR-MS (ESI, m/z) calcd $[M+H]^+$ for $[C_{27}H_{30}NO_4]^+$: 432.2169, found: 432.2164.

FCA-E

Fuchsia solid (0.17 g, 27.9 %). 1H NMR (400 MHz, Chloroform-*d*) δ 8.47 (d, $J = 14.8$ Hz, 1H), 7.84 – 7.80 (m, 1H), 7.44 (d, $J = 7.8$ Hz, 1H), 7.21 (d, $J = 7.3$ Hz, 1H), 7.12 (d, $J = 10.6$ Hz, 1H), 6.91 (d, $J = 8.6$ Hz,

1H), 6.67 (d, $J = 15.0$ Hz, 1H), 6.45 (s, 1H), 6.40 (s, 1H), 6.34 (d, $J = 6.4$ Hz, 1H), 3.38 (q, $J = 7.1$ Hz, 4H), 2.52 – 2.45 (m, 4H), 1.80 – 1.76 (m, 2H), 1.20 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 188.24, 162.29, 159.78, 155.37, 154.95, 149.41, 140.31, 132.77, 130.99, 127.13, 125.93, 124.33, 118.95, 116.48, 116.25, 110.78, 109.16, 107.29, 97.62, 77.48, 44.71, 29.82, 25.02, 21.14, 12.80. HR-MS (ESI, m/z) calcd $[\text{M}+\text{H}]^+$ for $[\text{C}_{26}\text{H}_{27}\text{FNO}_2]^+$: 404.2020, found: 404.2015.

CICA-E

Fuchsia solid (0.18 g, 28.6 %). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.27 (d, $J = 15.3$ Hz, 1H), 7.49 (d, $J = 5.8$ Hz, 1H), 7.42 (d, $J = 6.9$ Hz, 1H), 7.33 (dd, $J = 7.3, 3.2$ Hz, 2H), 6.91 (d, $J = 8.6$ Hz, 1H), 6.46 (s, 1H), 6.40 (d, $J = 15.2$ Hz, 1H), 6.34 (d, $J = 6.2$ Hz, 1H), 6.29 (s, 1H), 3.37 (q, $J = 7.1$ Hz, 4H), 2.49 (t, $J = 5.9$ Hz, 2H), 2.44 (t, $J = 6.0$ Hz, 2H), 1.78 – 1.75 (m, 2H), 1.19 (t, $J = 7.1$ Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 192.76, 155.41, 154.82, 149.41, 141.28, 141.19, 131.24, 130.45, 130.14, 129.39, 127.17, 126.64, 126.16, 124.20, 119.03, 110.72, 108.91, 107.37, 97.47, 77.48, 44.72, 29.71, 24.85, 21.10, 12.76. HR-MS (ESI, m/z) calcd $[\text{M}+\text{H}]^+$ for $[\text{C}_{26}\text{H}_{27}\text{ClNO}_2]^+$: 420.1725, found: 420.1734.

BrCA-E

Fuchsia solid (0.17 g, 24.3 %). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.25 (d, $J = 15.2$ Hz, 1H), 7.62 (d, $J = 8.0$ Hz, 1H), 7.45 (d, $J = 7.5$ Hz, 1H), 7.37 (t, $J = 7.4$ Hz, 2H), 6.92 (d, $J = 8.9$ Hz, 1H), 6.47 (s, 1H), 6.36 (d, $J = 15.1$ Hz, 2H), 6.29 (s, 1H), 3.37 (q, $J = 7.0$ Hz, 4H), 2.51 (d, $J = 3.0$ Hz, 2H), 2.44 (d, $J = 2.8$ Hz, 2H), 1.79 – 1.75 (m, 2H), 1.19 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 193.61, 155.44, 154.82, 149.42, 143.33, 141.42, 133.29, 130.47, 129.22, 127.17, 127.10, 126.22, 124.20, 119.66, 118.75, 110.72, 108.95, 107.38, 97.47, 77.48, 44.73, 29.70, 24.84, 21.10, 12.77. HR-MS (ESI, m/z) calcd $[\text{M}+\text{H}]^+$ for $[\text{C}_{26}\text{H}_{27}\text{BrNO}_2]^+$: 464.1220, found: 464.1213.

ICA-E

Fuchsia solid (0.14 g, 18.2 %). ^1H NMR (400 MHz, Chloroform-*d*) δ 8.28 (d, $J = 15.2$ Hz, 1H), 7.91 (d, $J = 7.9$ Hz, 1H), 7.41 (d, $J = 7.3$ Hz, 2H), 7.09 (t, $J = 7.2$ Hz, 1H), 6.92 (d, $J = 8.5$ Hz, 1H), 6.48 (s, 1H), 6.35 (s, 1H), 6.34 – 6.29 (m, 2H), 3.41 – 3.34 (q, 4H), 2.51 – 2.49 (t, 2H), 2.47 – 2.43 (t, 2H), 1.80 – 1.75 (m, 2H), 1.20 (t, $J = 7.0$ Hz, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 194.87, 155.49, 154.84, 149.44, 146.84, 141.68, 139.92, 130.51, 128.48, 127.85, 127.19, 126.30, 124.18, 118.06, 110.73, 109.06, 107.40, 97.71, 92.71, 77.48, 44.74, 29.71, 24.85, 21.11, 12.78. HR-MS (ESI, m/z) calcd $[\text{M}+\text{H}]^+$ for $[\text{C}_{26}\text{H}_{27}\text{INO}_2]^+$: 512.1081, found: 512.1077.

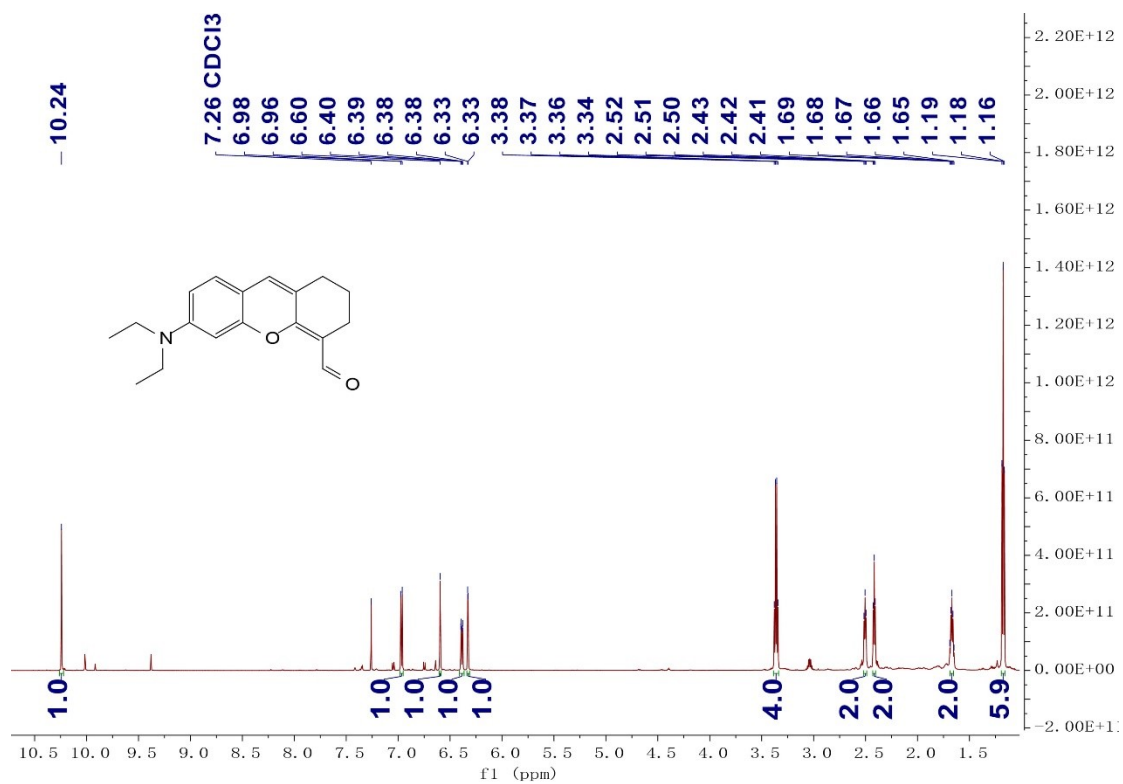


Fig. S1. ¹H NMR of compound E.

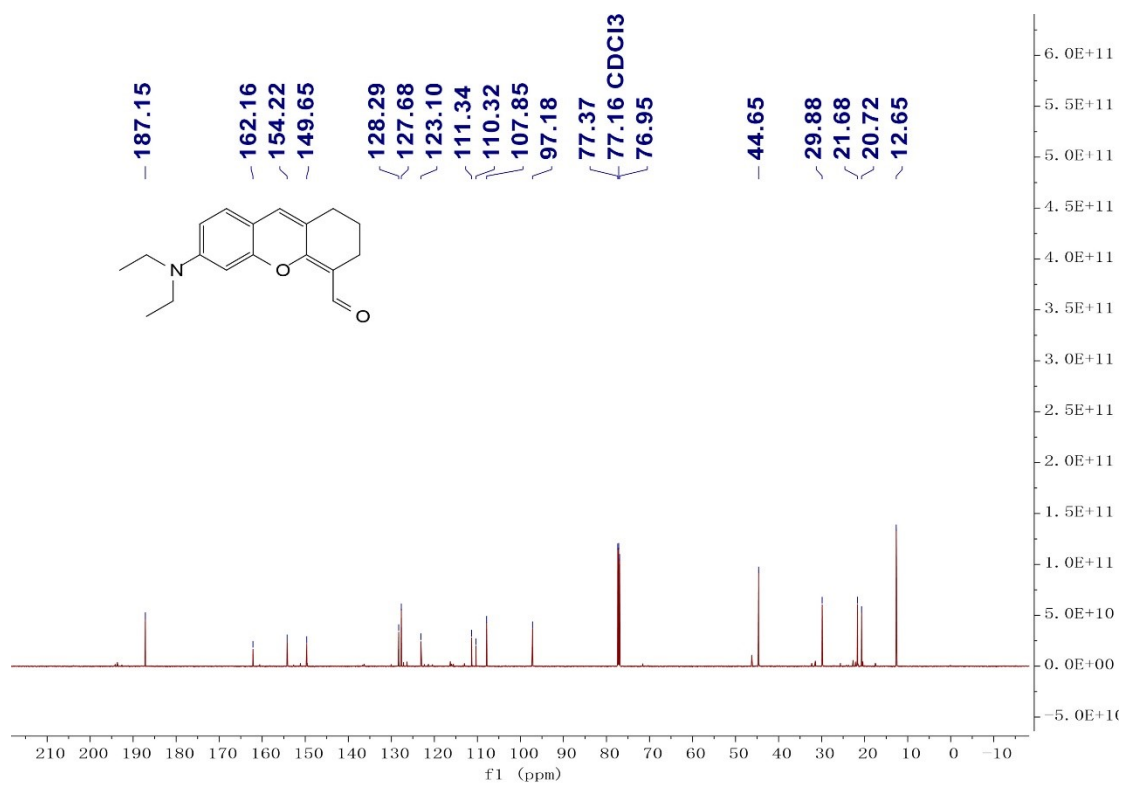


Fig. S2. ¹³C NMR of compound E.

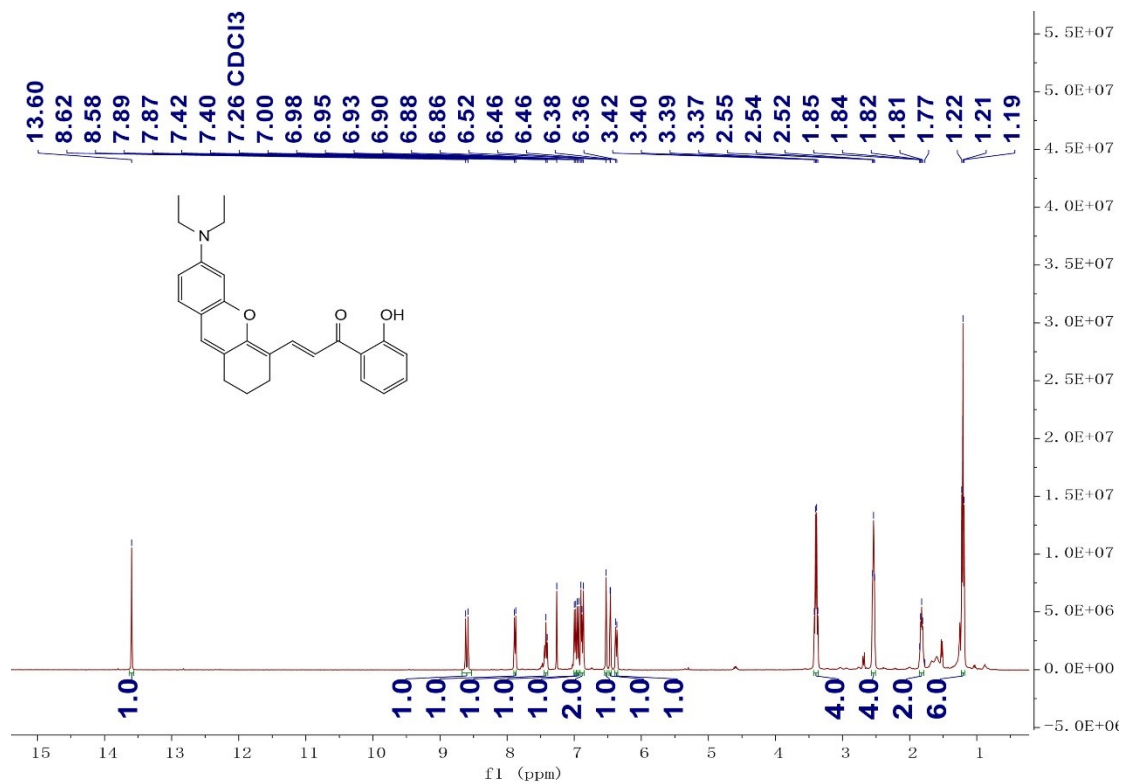


Fig. S3. ¹H NMR of HCA-E.

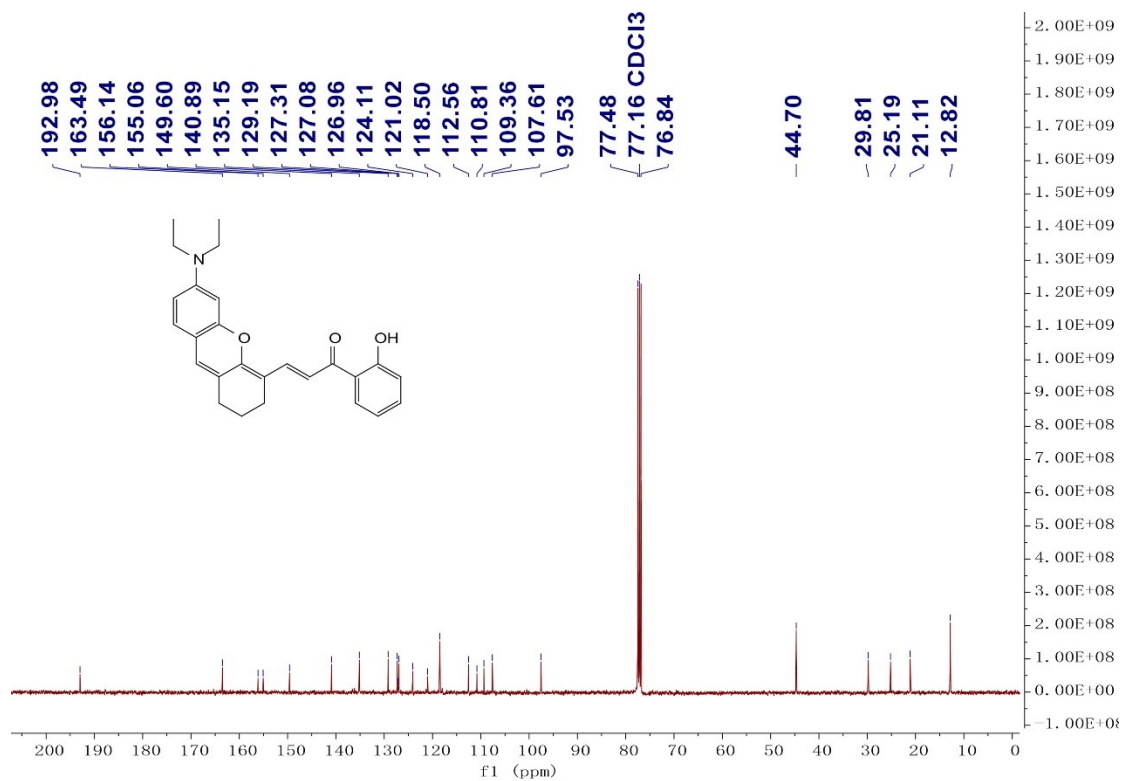


Fig. S4. ¹³C NMR of HCA-E.

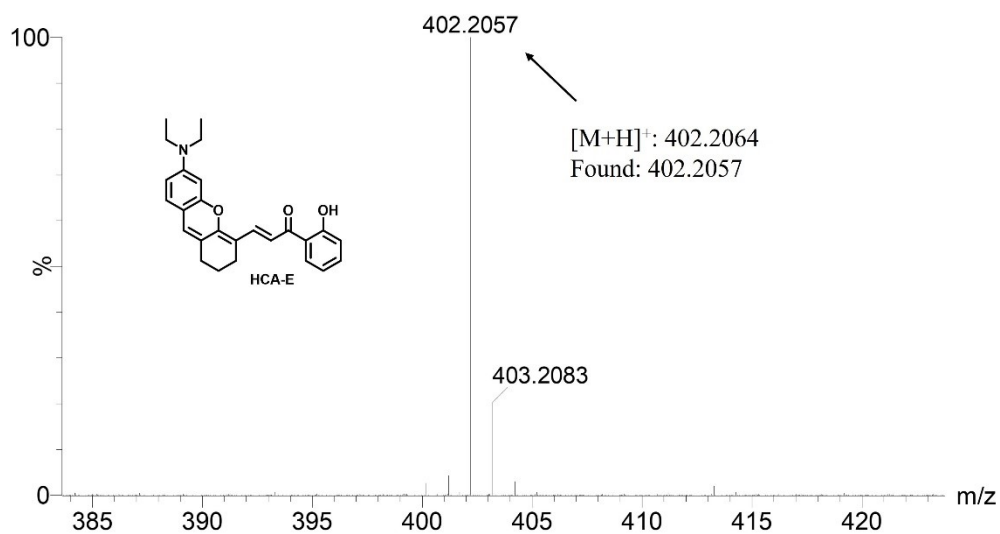


Fig. S5. HRMS of HCA-E.

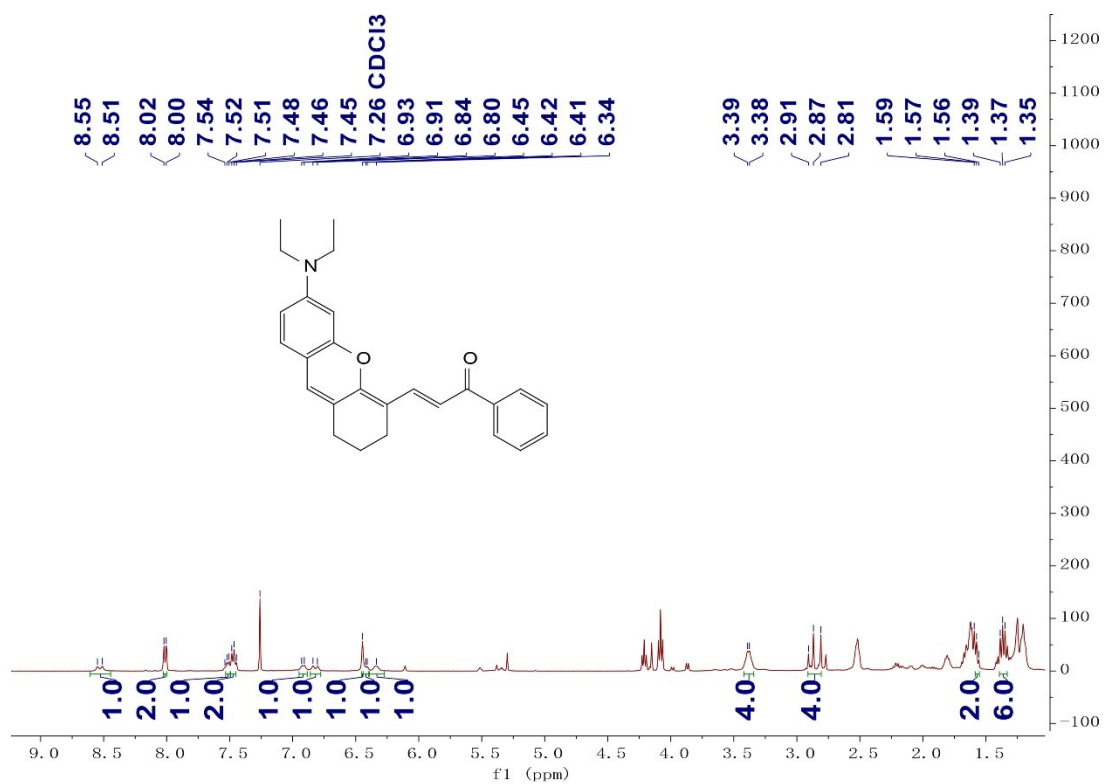


Fig. S6. ¹H NMR of CA-E.

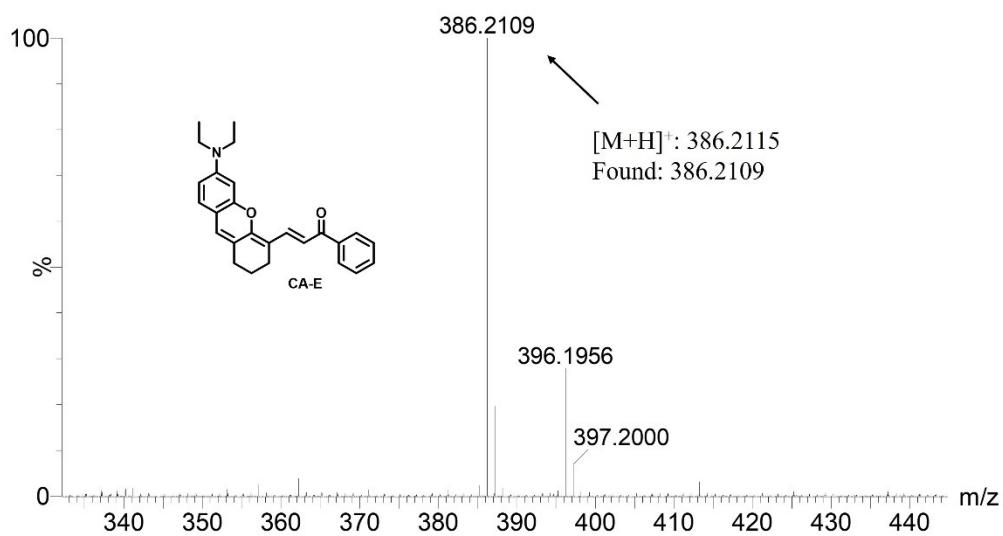


Fig. S7. HRMS of CA-E.

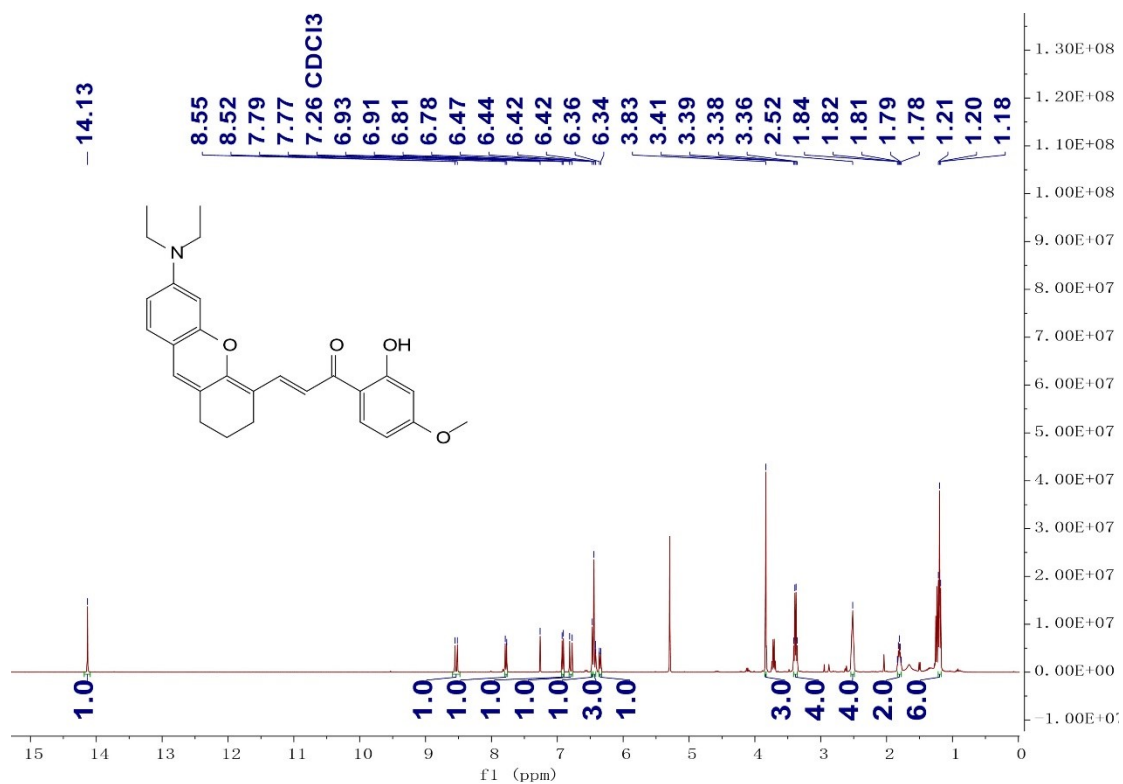


Fig. S8. ¹H NMR of 4OCA-E.



Fig. S9. ^{13}C NMR of 4OCA-E.

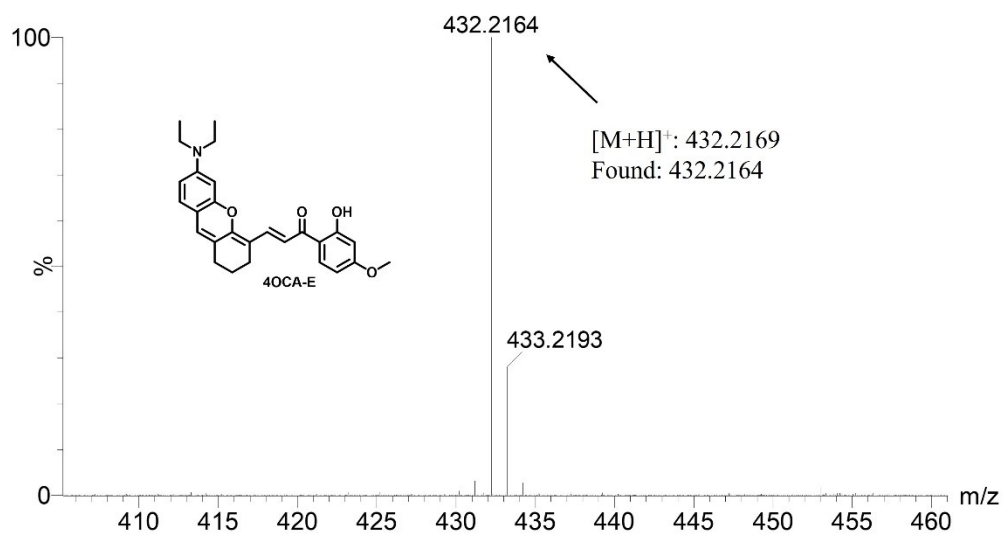


Fig. S10. HRMS of 4OCA-E.

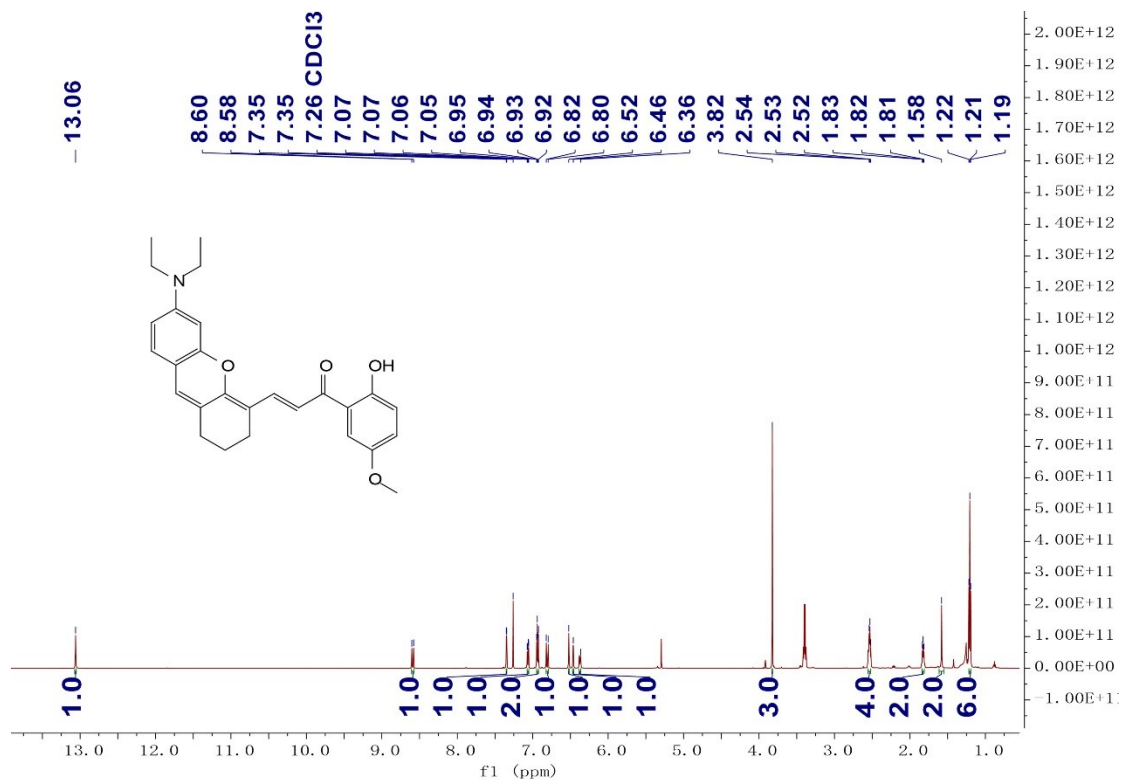


Fig. S11. ¹H NMR of 5OCA-E.

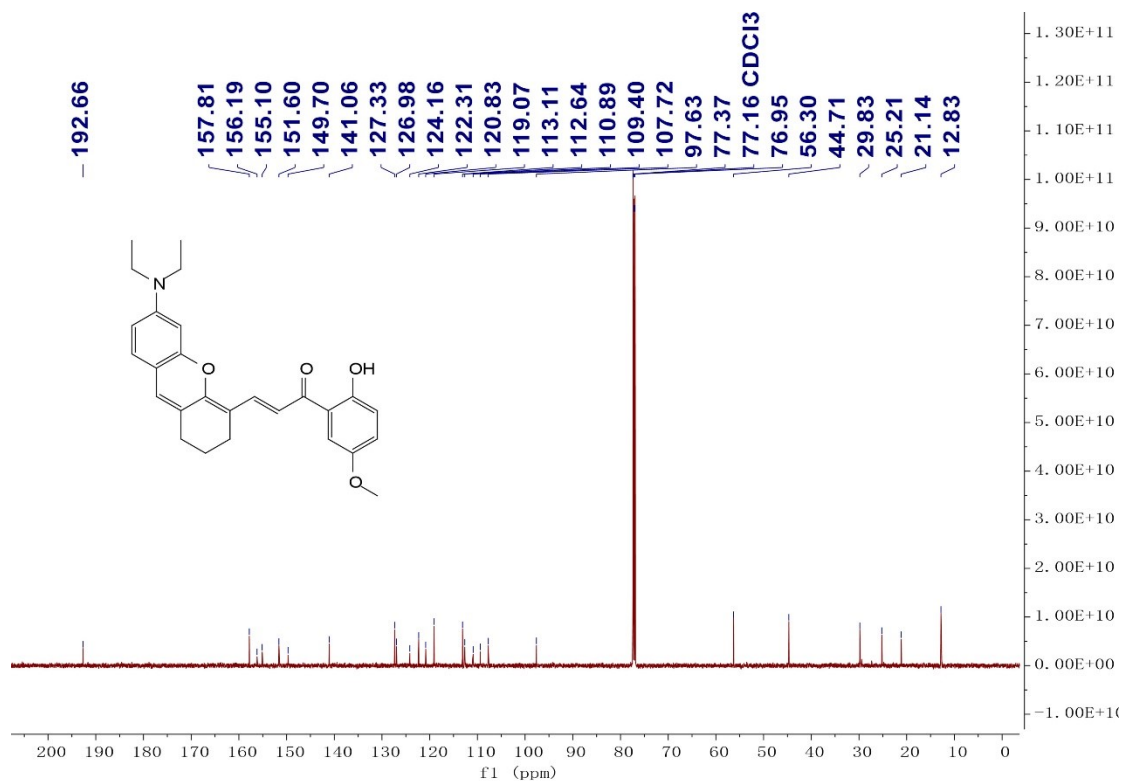


Fig. S12. ¹³C NMR of 5OCA-E.

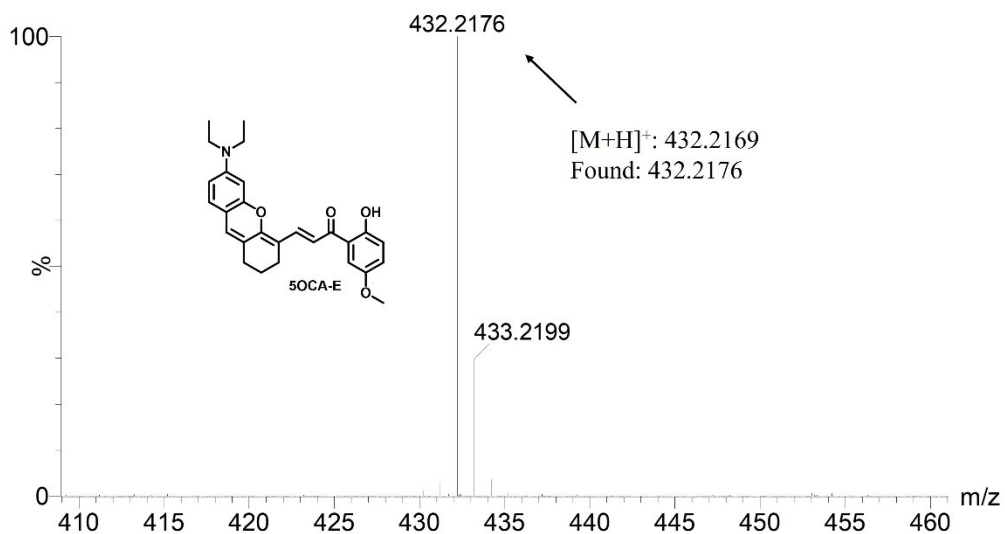


Fig. S13. HRMS of 5OCA-E.

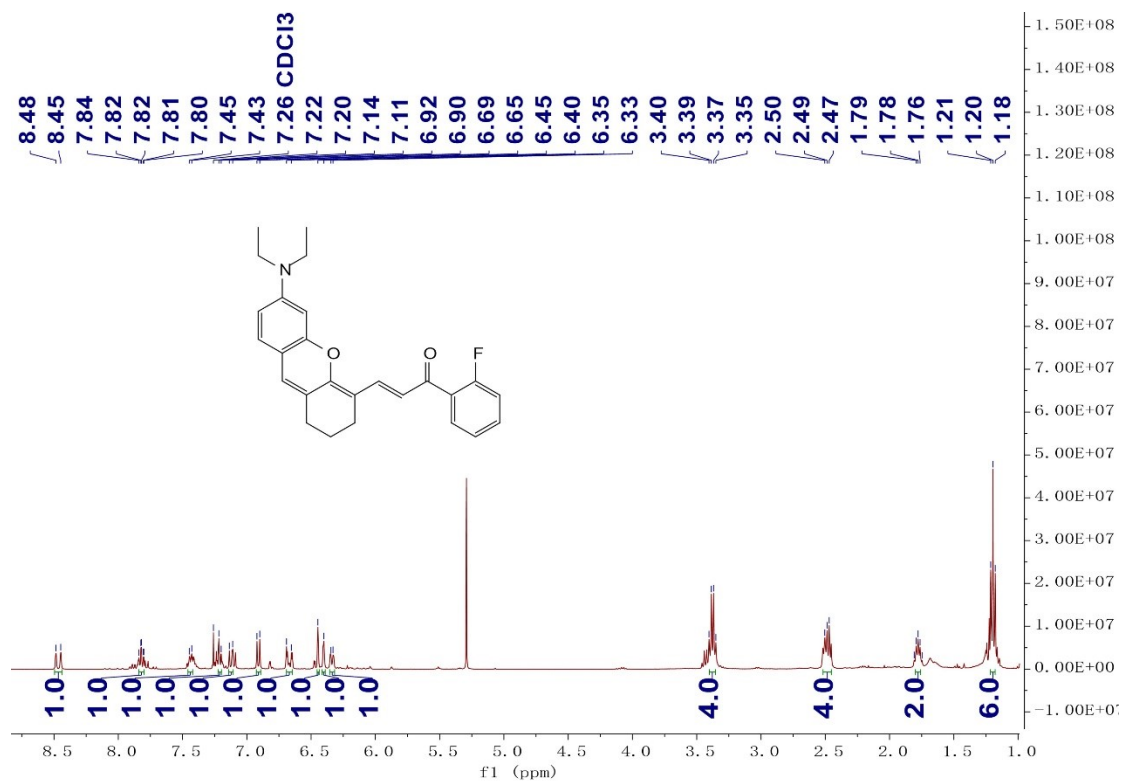


Fig. S14. ¹H NMR of FCA-E.

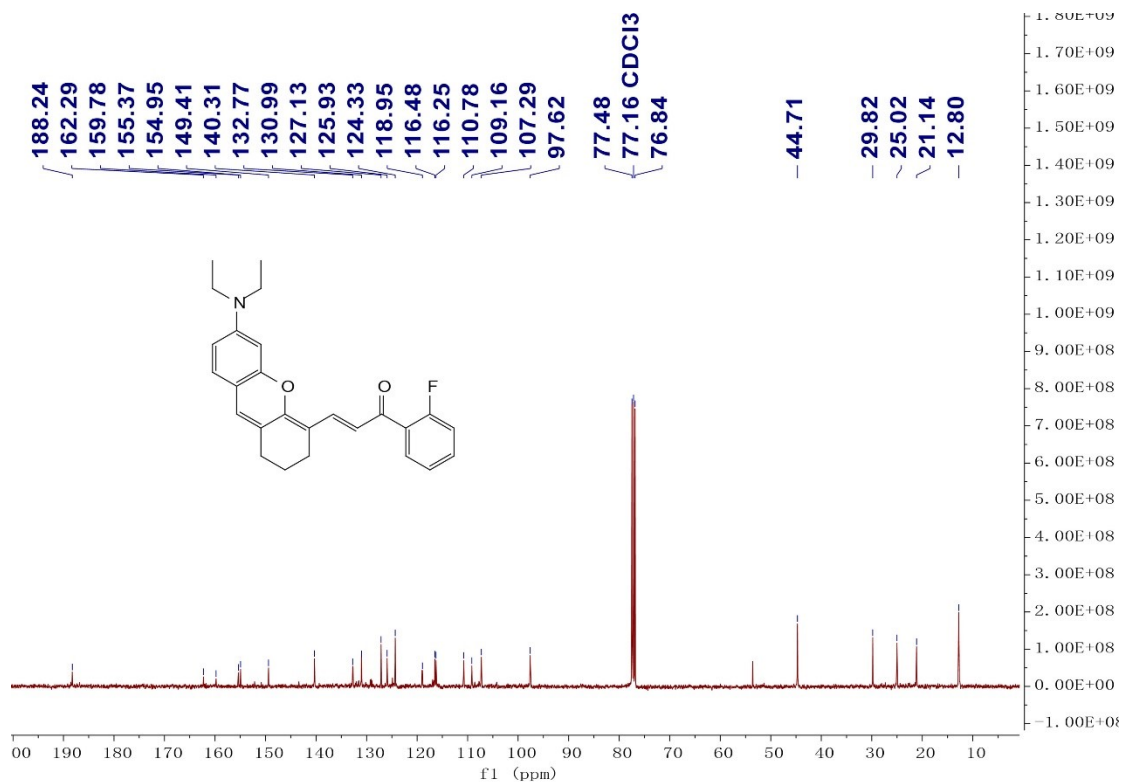


Fig. S15. ^{13}C NMR of FCA-E.

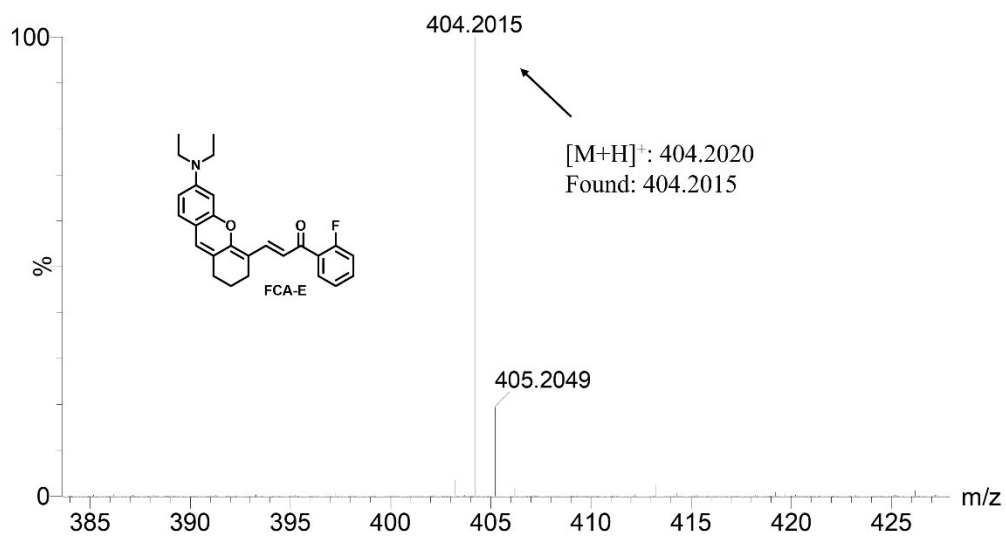


Fig. S16. HRMS of FCA-E.

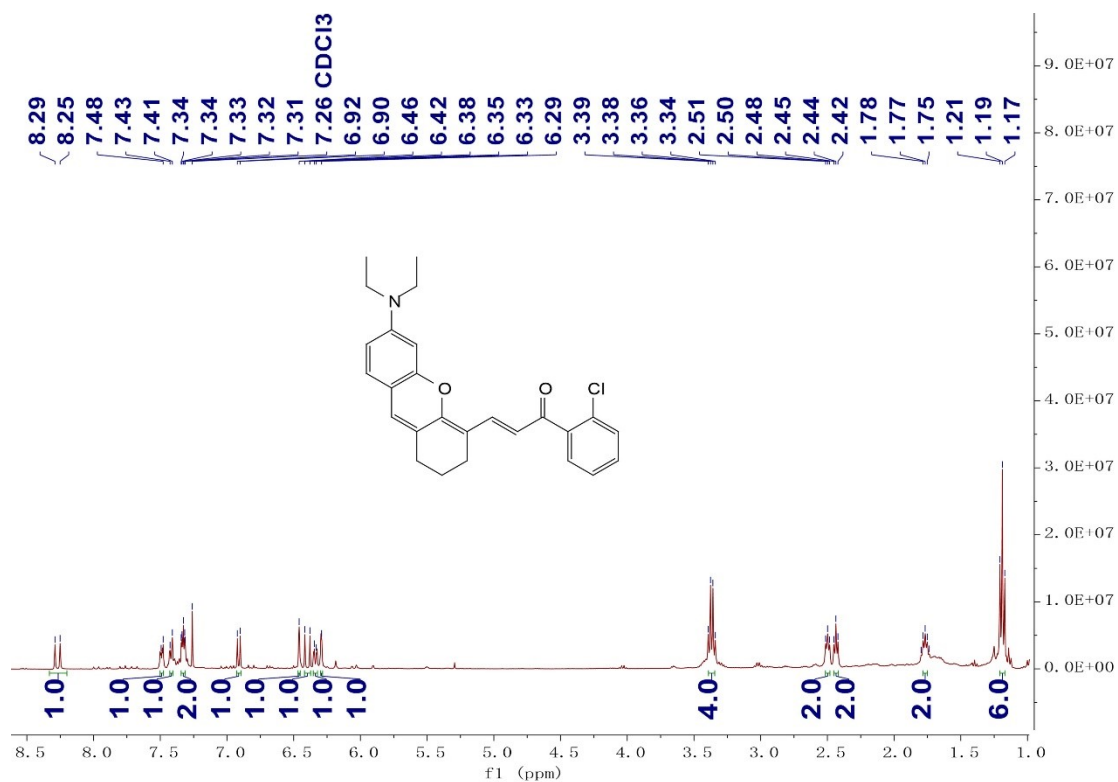


Fig. S17. ¹H NMR of CICA-E.

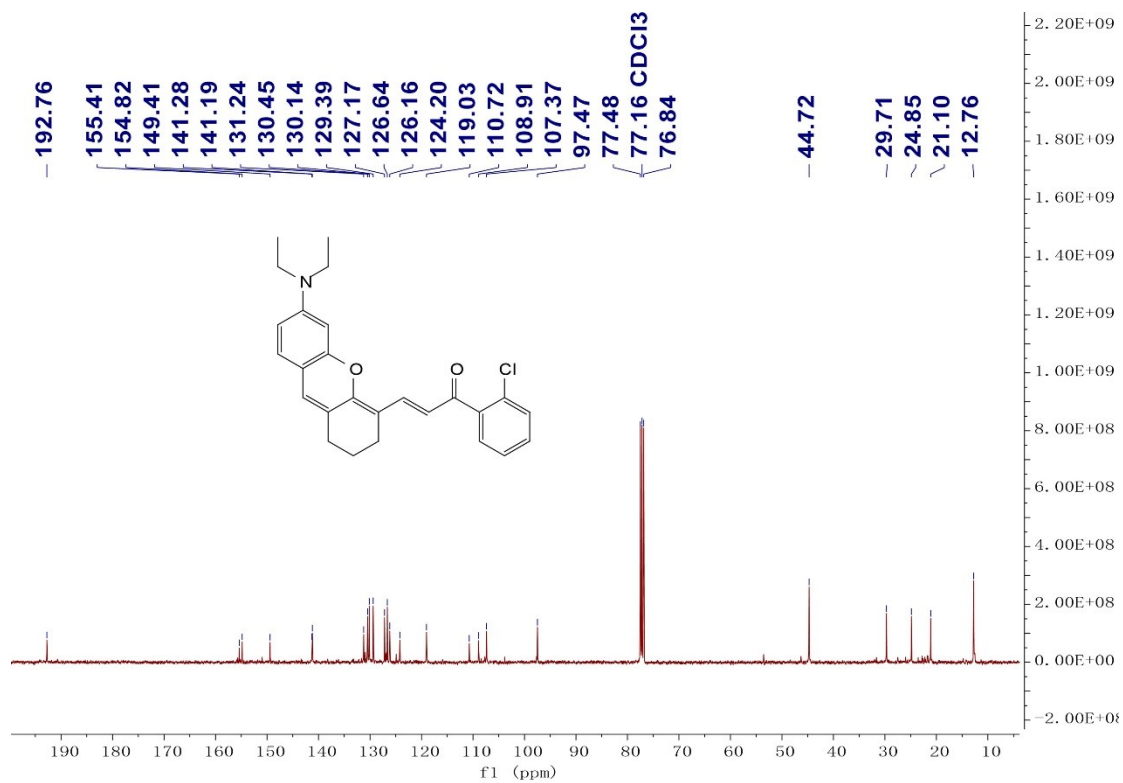


Fig. S18. ¹³C NMR of CICA-E.

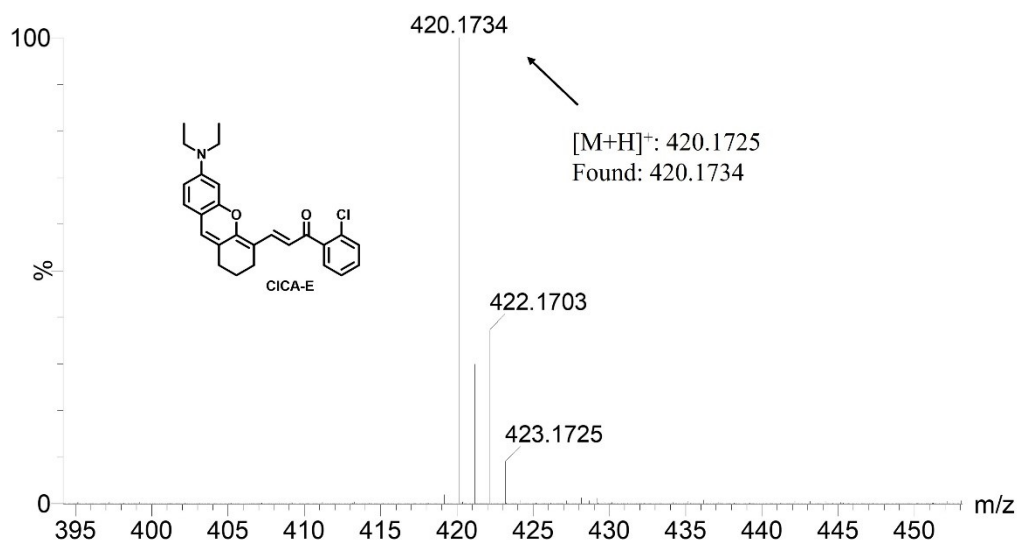


Fig. S19. HRMS of CICA-E.

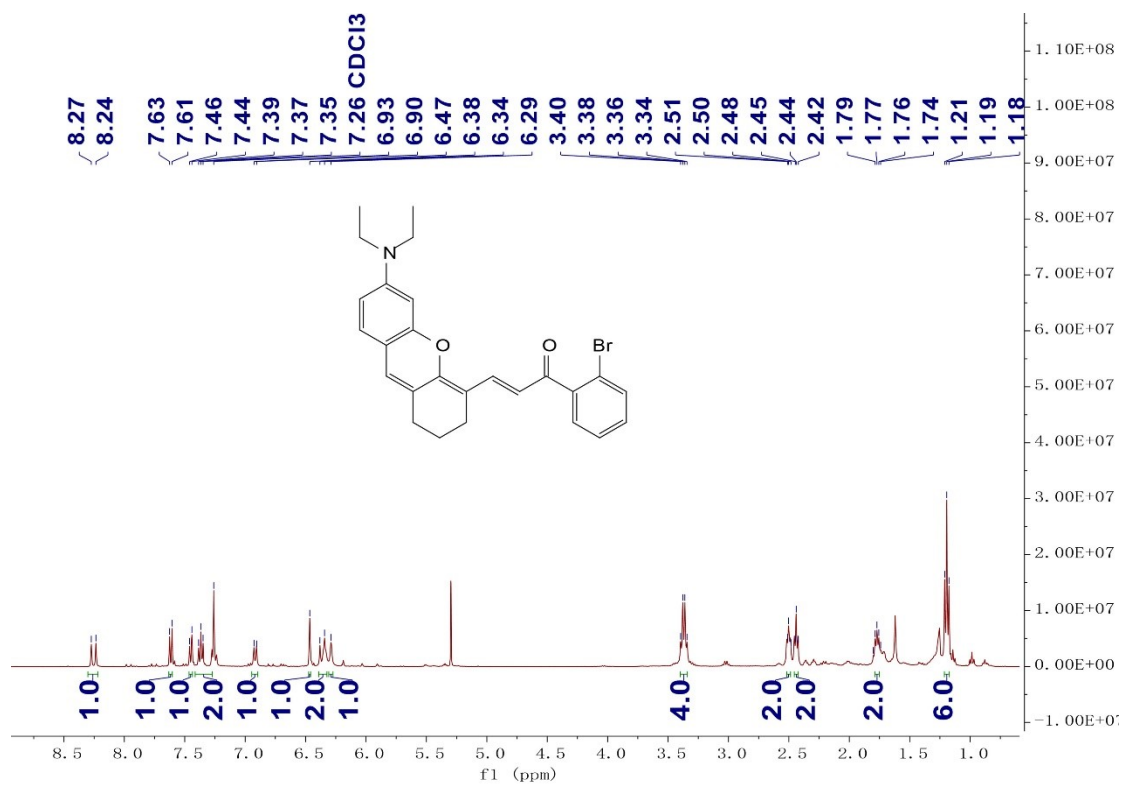


Fig. S20. ¹H NMR of BrCA-E.

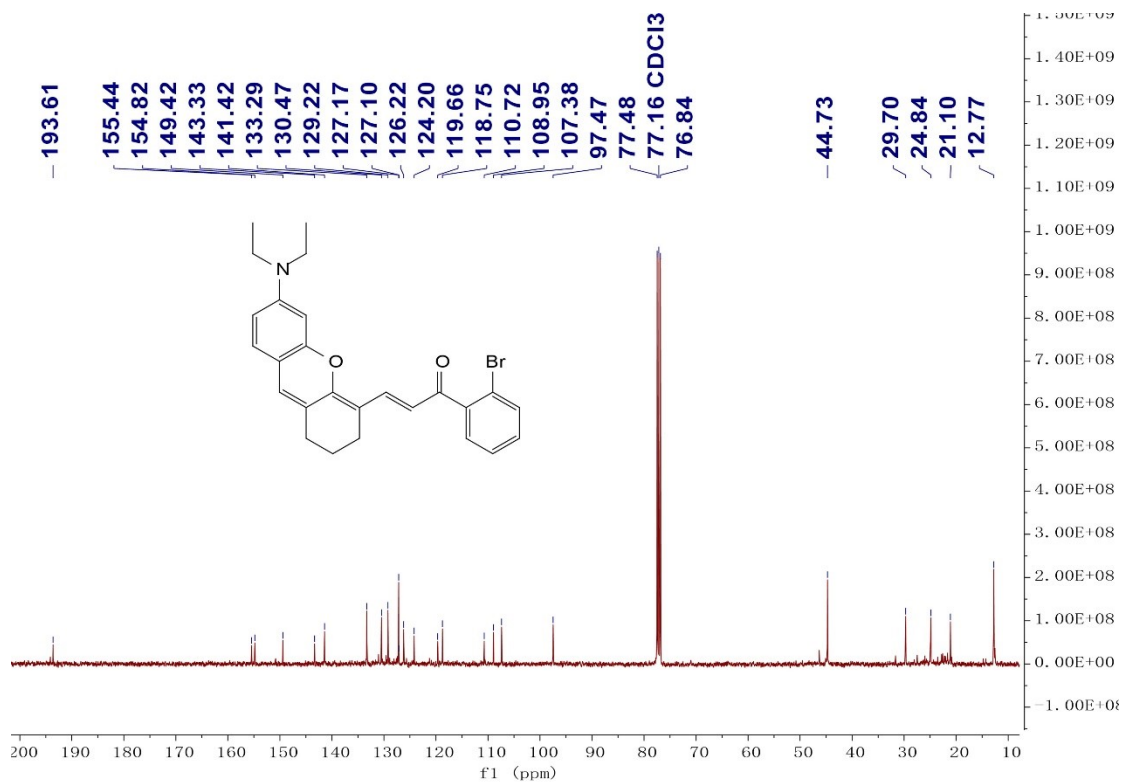


Fig. S21. ^{13}C NMR of BrCA-E.

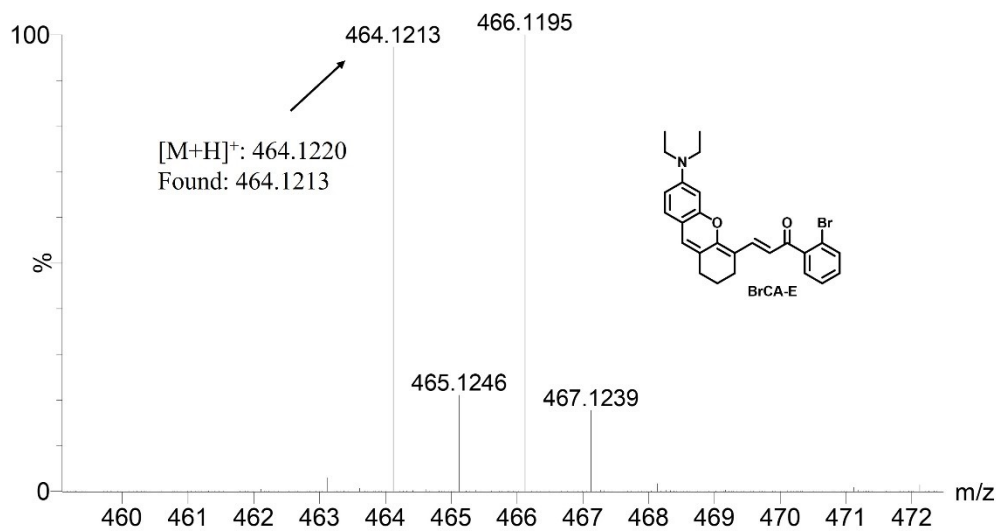


Fig. S22. HRMS of BrCA-E.

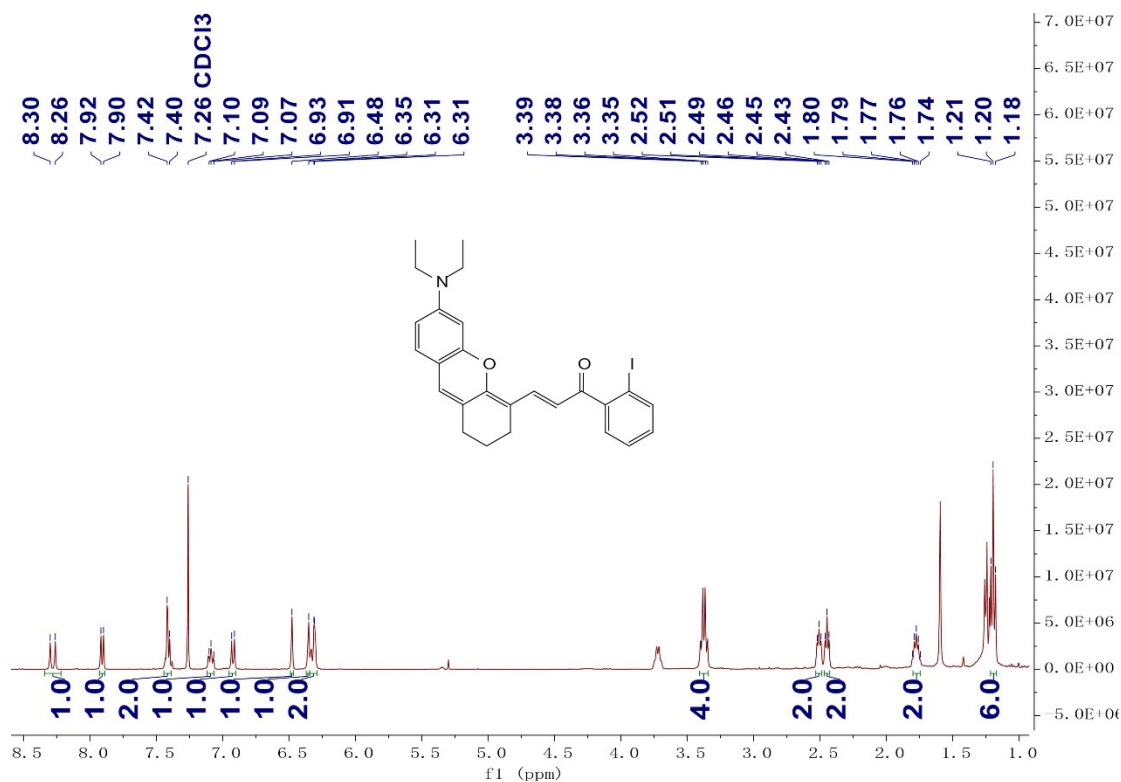


Fig. S23. ¹H NMR of ICA-E.

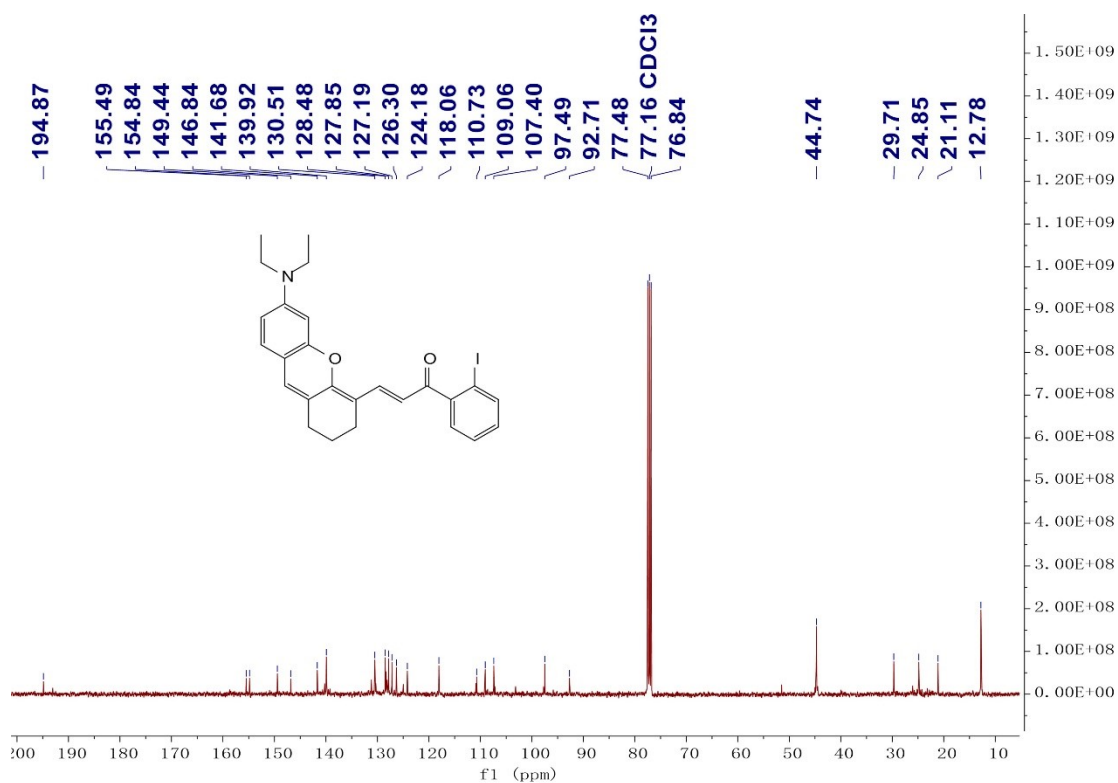


Fig. S24. ¹³C NMR of ICA-E.

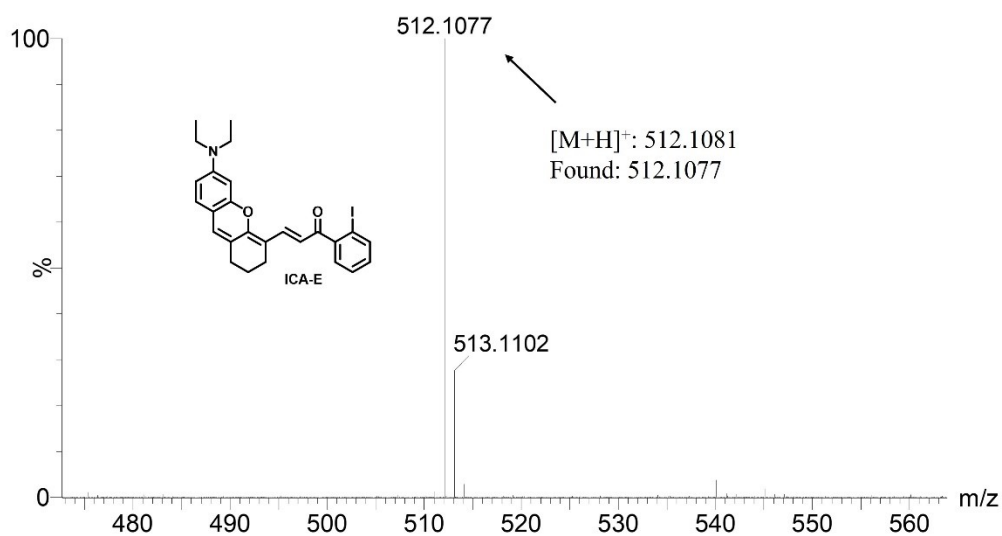


Fig. S25. HRMS of ICA-E.

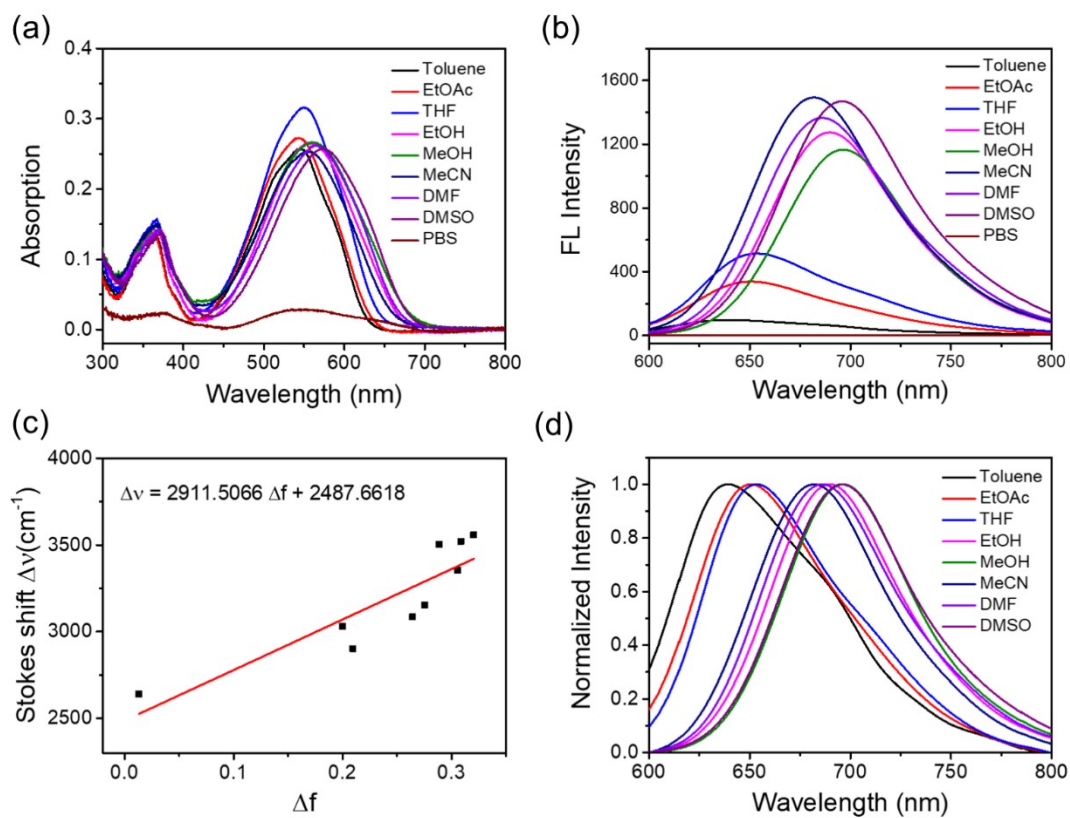


Fig. S26. Spectral properties of CA-E in different solvents.

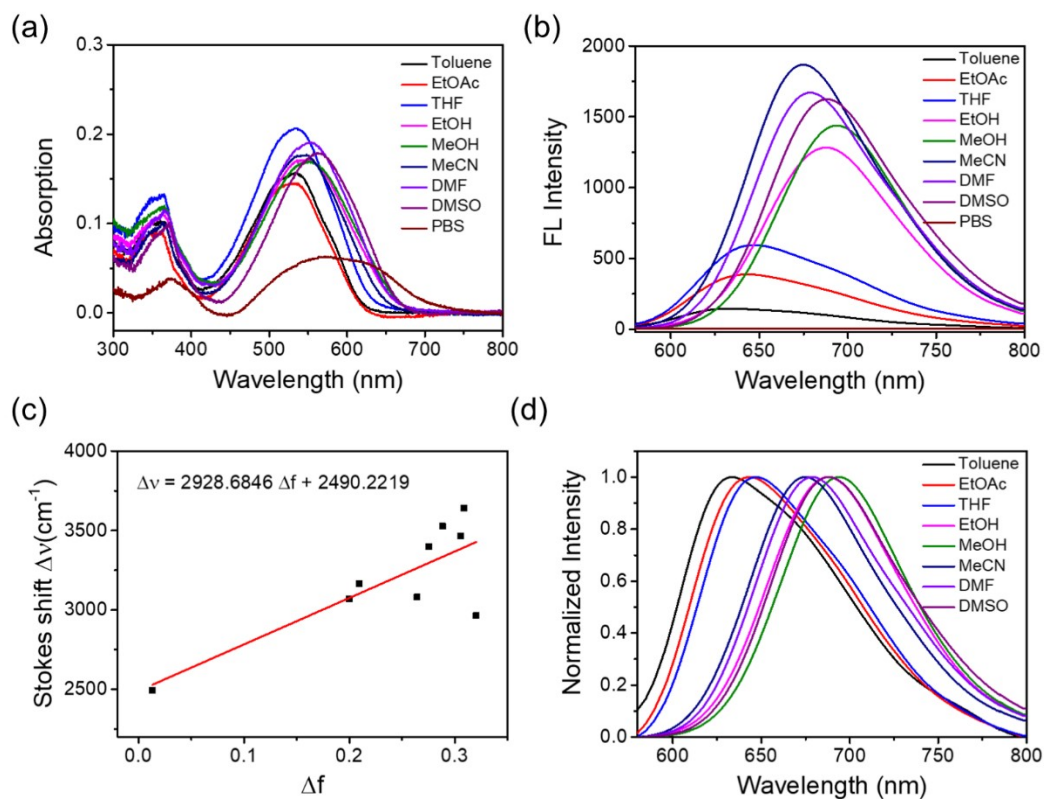


Fig. S27. Spectral properties of **4OCA-E** in different solvents.

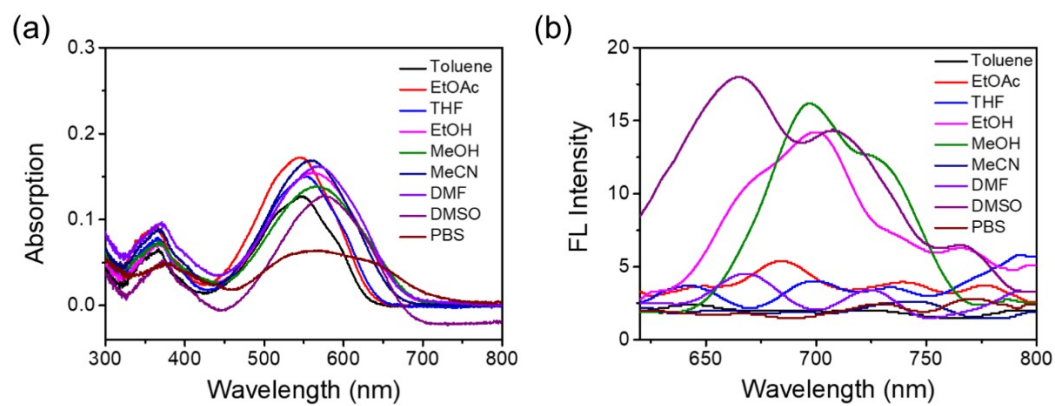


Fig. S28. Spectral properties of **5OCA-E** in different solvents.

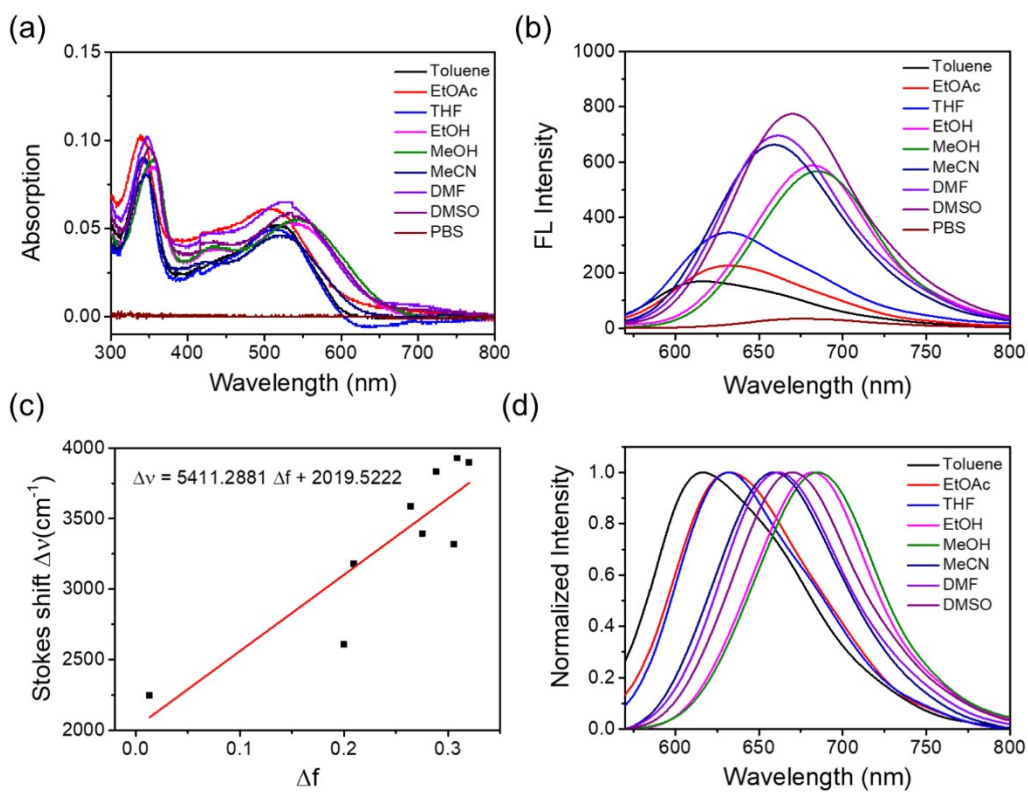


Fig. S29. Spectral properties of FCA-E in different solvents.

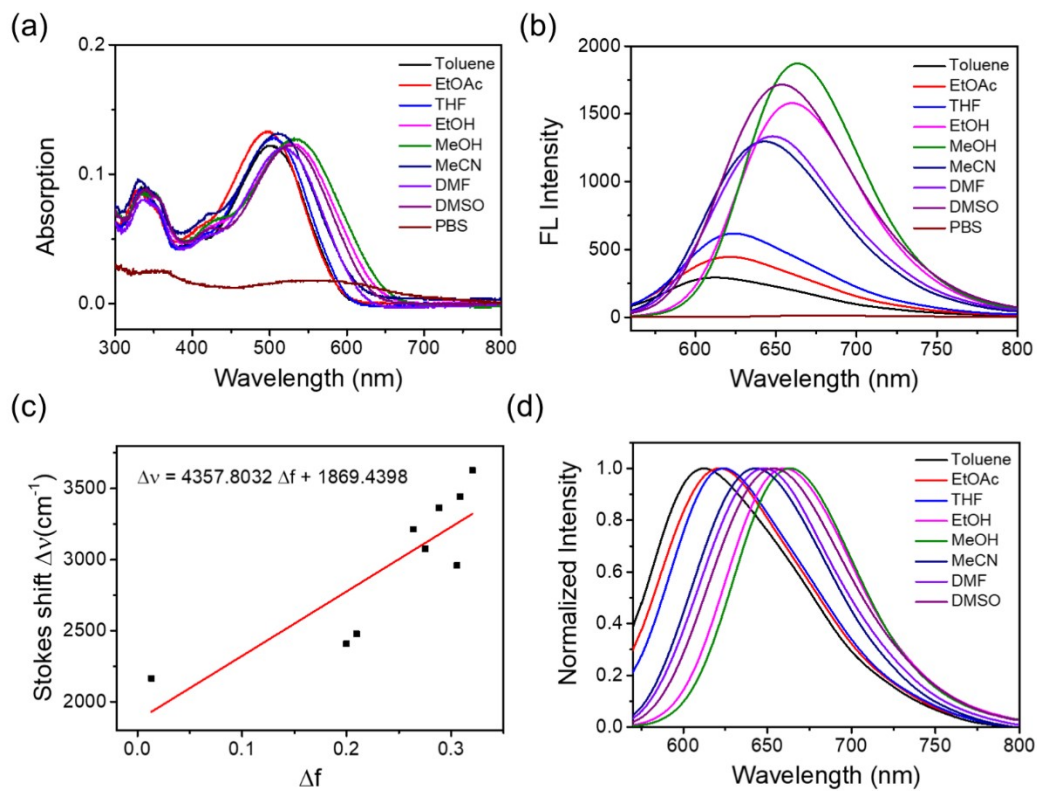


Fig. S30. Spectral properties of CICA-E in different solvents.

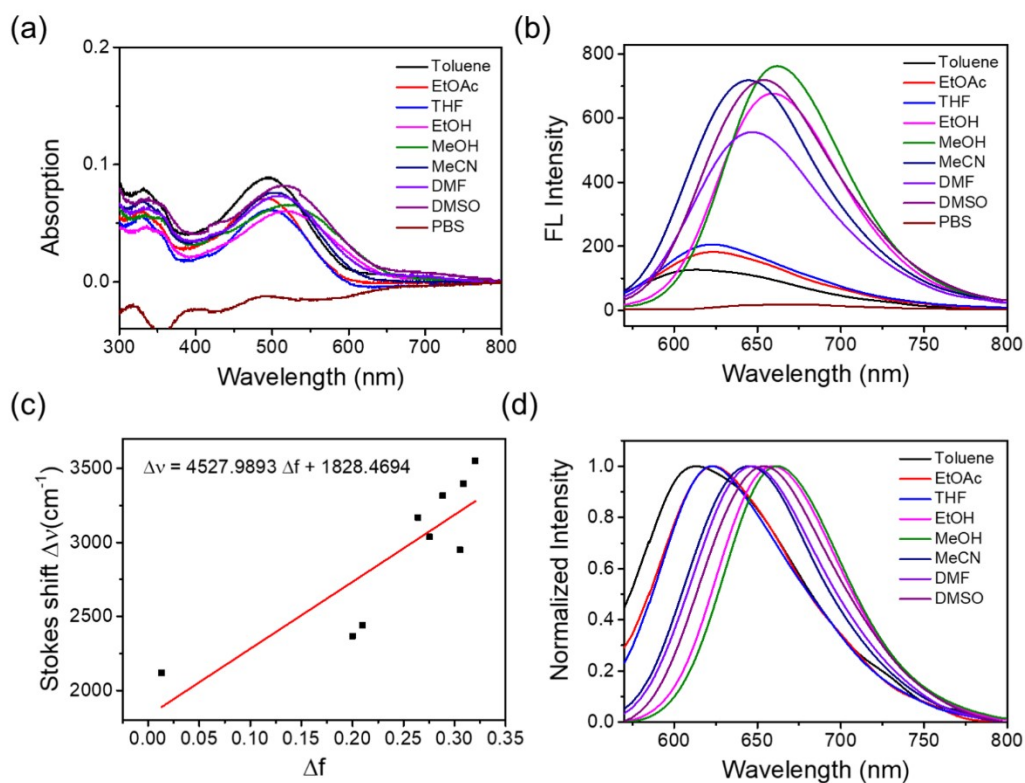


Fig. S31. Spectral properties of **BrCA-E** in different solvents.

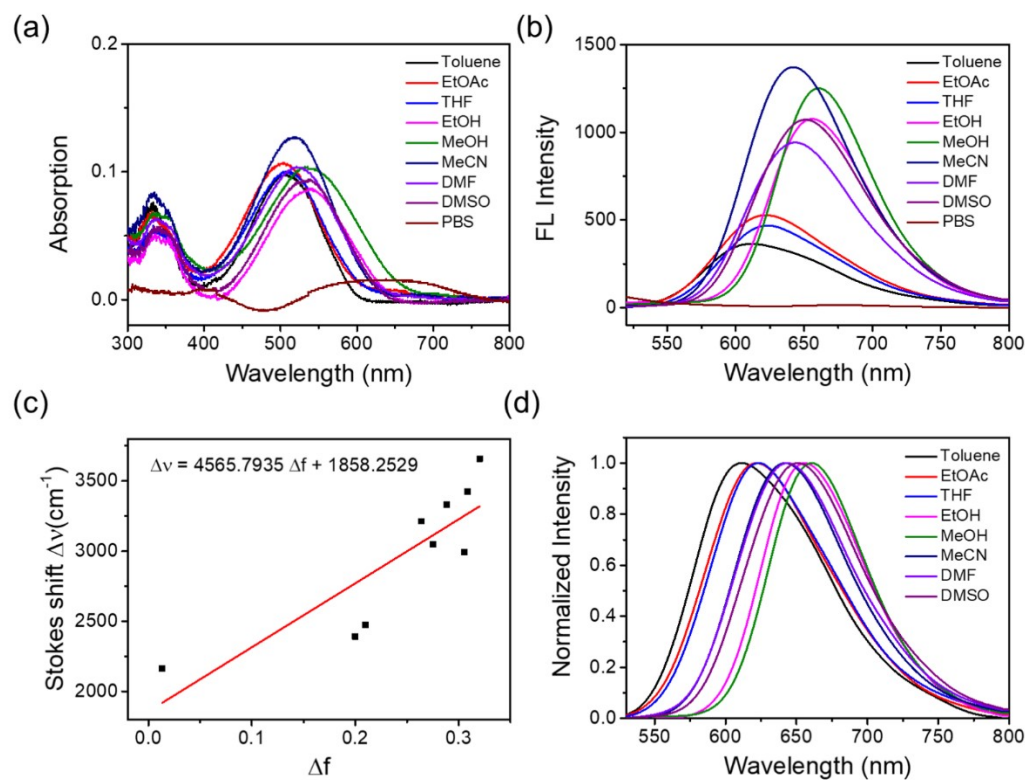


Fig. S32. Spectral properties of **ICA-E** in different solvents.

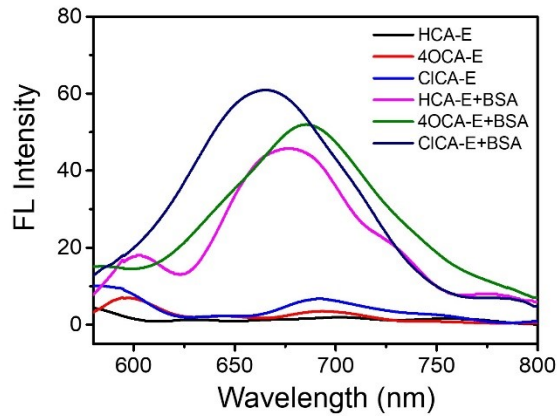


Fig. S33. Fluorescence spectra of **HCA-E**, **4OCA-E** and **CICA-E** in HEPES buffer with BSA.

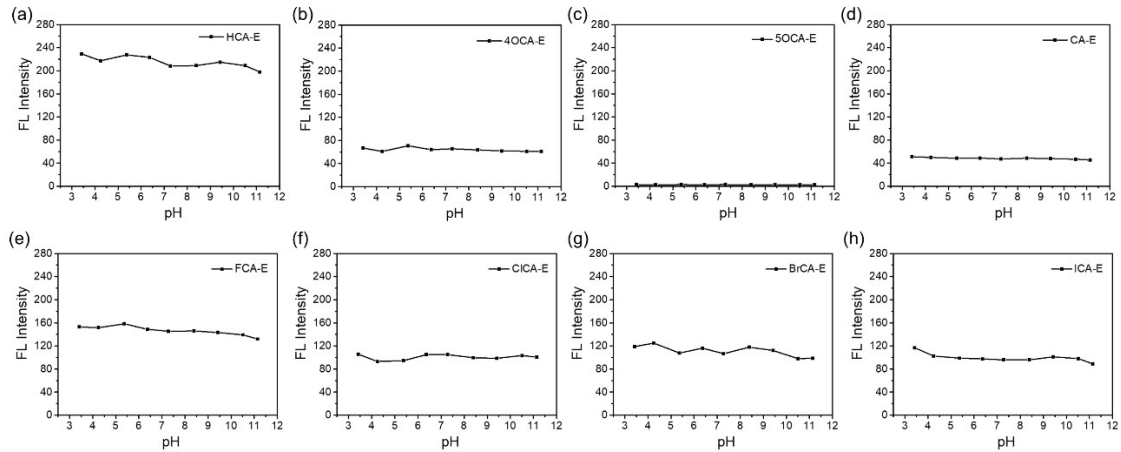


Fig. S34. Spectral intensity of xanthene-chalcone fluorophores in solvents with different pH.

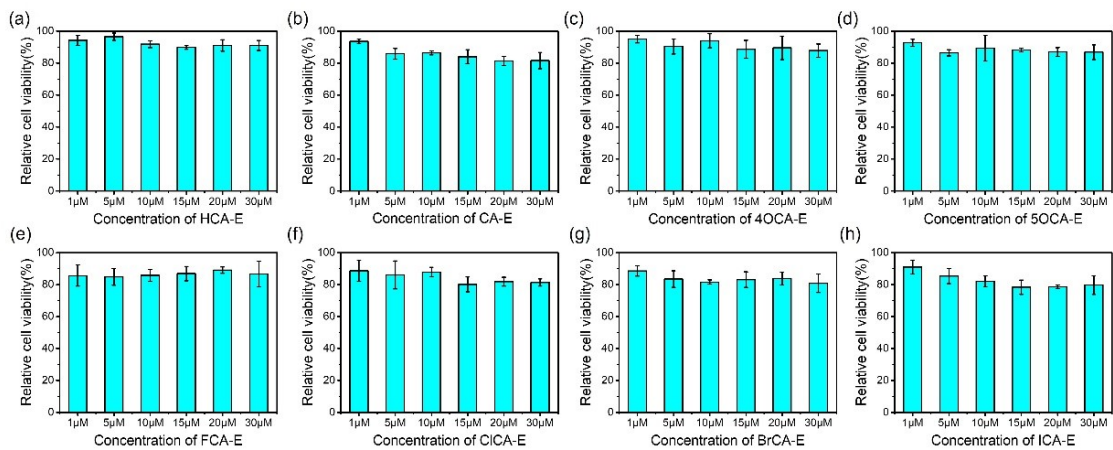


Fig. S35. Cytotoxicity of xanthene-chalcone fluorophores.

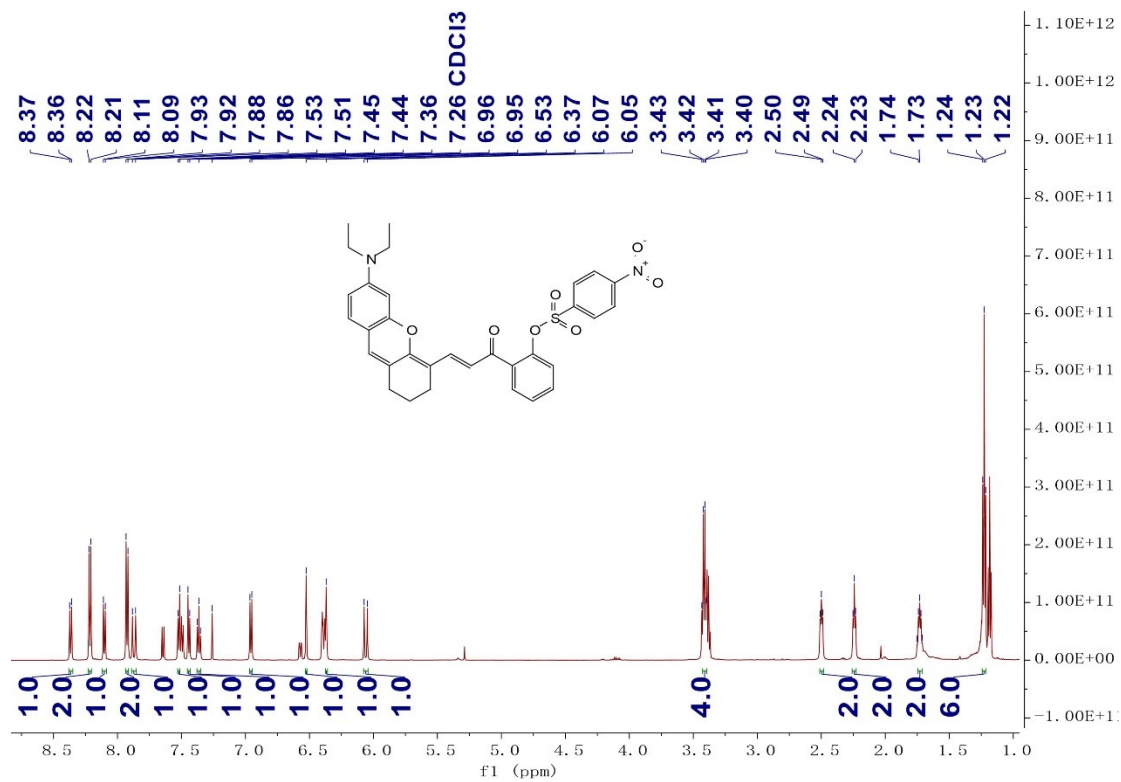


Fig. S36. ¹H NMR of N-HCA-E.

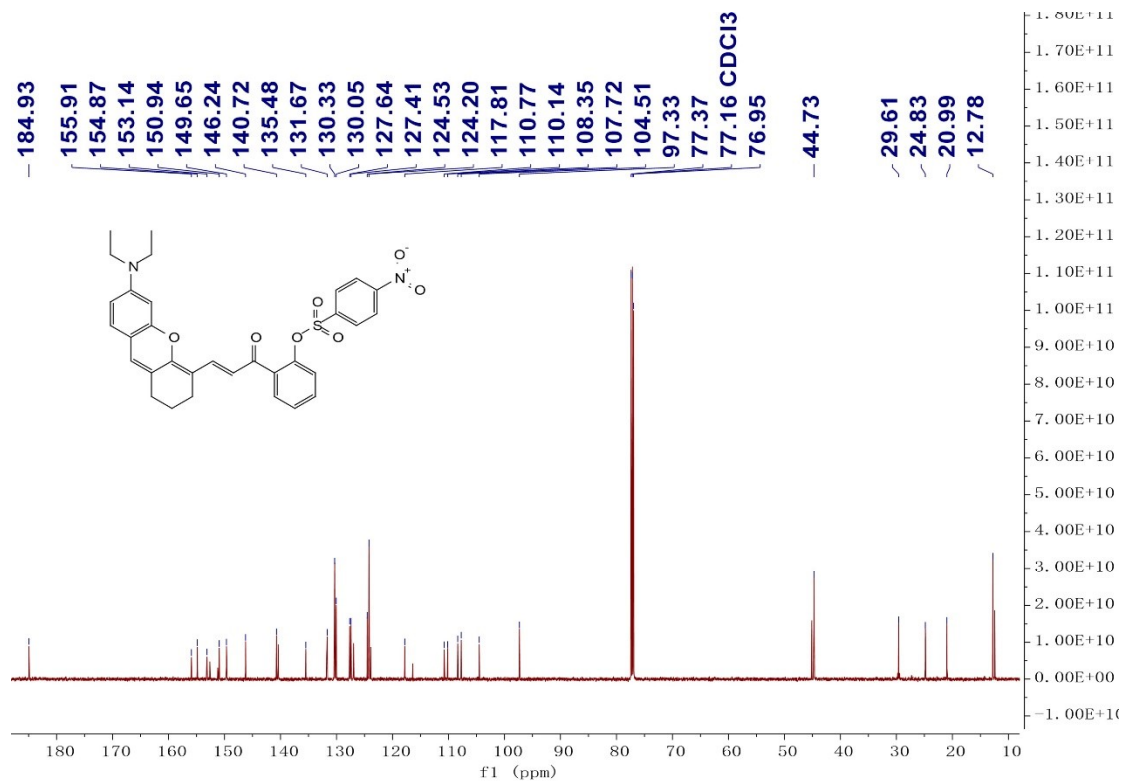


Fig. S37. ¹³C NMR of N-HCA-E.

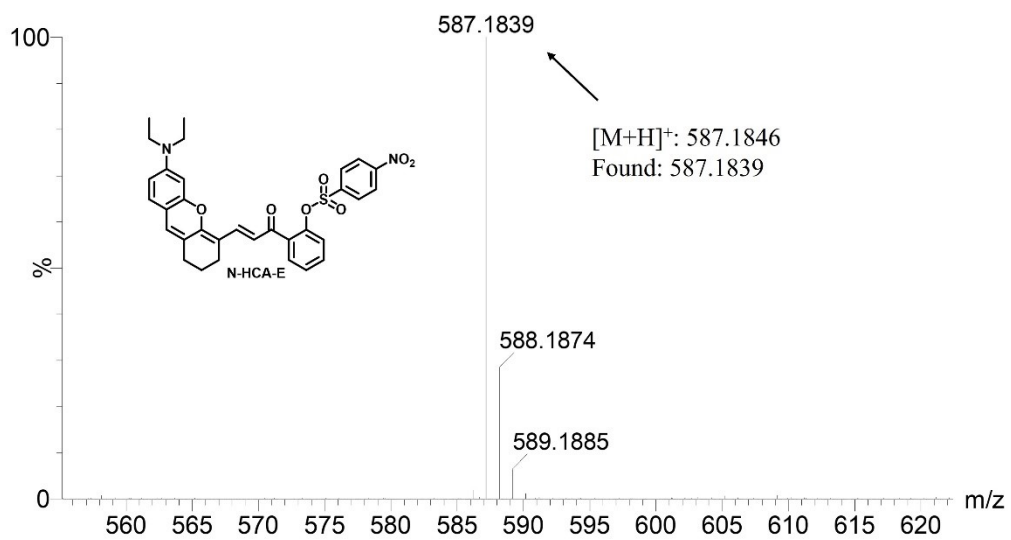


Fig. S38. HRMS of N-HCA-E.

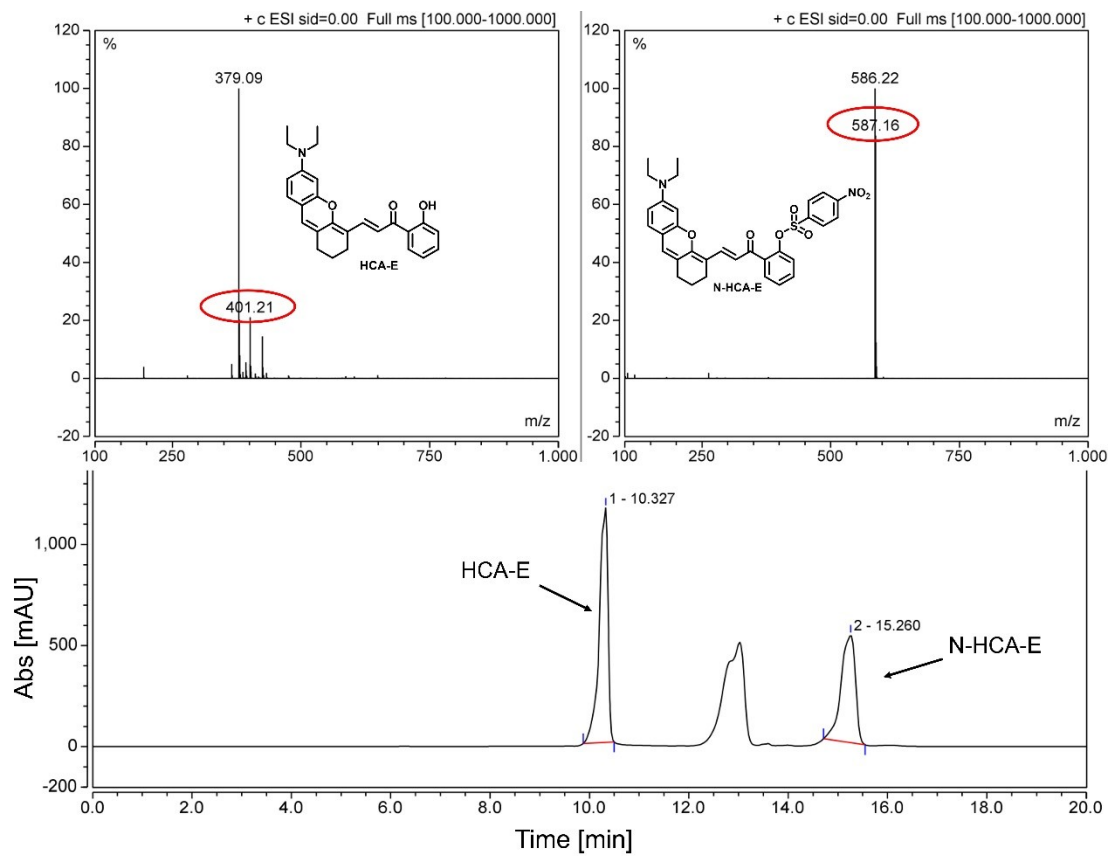


Fig. S39. LC-MS of N-HCA-E after responding to GSH.

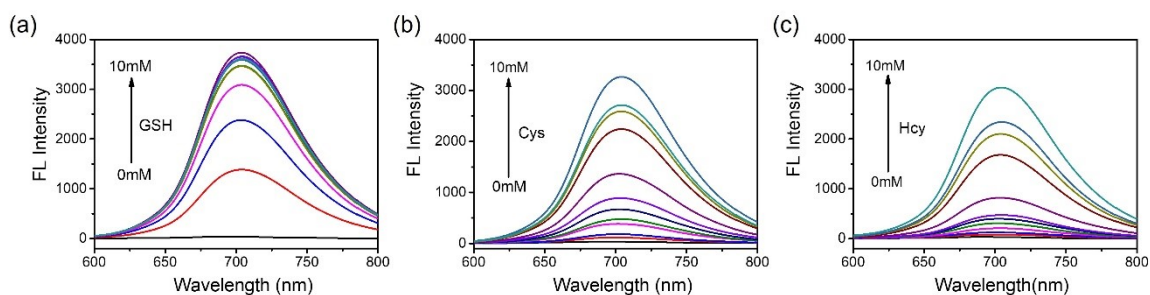


Fig. S40. Fluorescence spectra of probe N-HCA-E (10 μM) after adding different concentrations of GSH, Cys or Hcy (0-10 mM).

Table S1. Comparison of LOD and LOQ values with recently published works.

Analyte	LOD	LOQ	Ref
N-HCA-E	0.31 μM	1.04 μM	This work
SL1	0.06 μM	0.21 μM	[1]
Probe A	0.26 μM	0.88 μM	[2]
MITO-PQDNs	0.25 μM	/	[3]
QB-3	0.46 μM	/	[4]

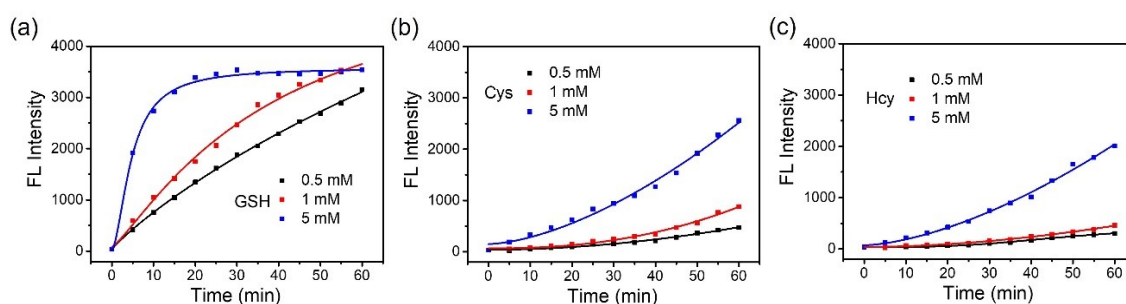


Fig. S41. The fluorescence trend of probe N-HCA-E (10 μM) at 709 nm within 0-60 min after adding GSH, Cys or Hcy (0.5 mM, 1 mM, 5 mM).

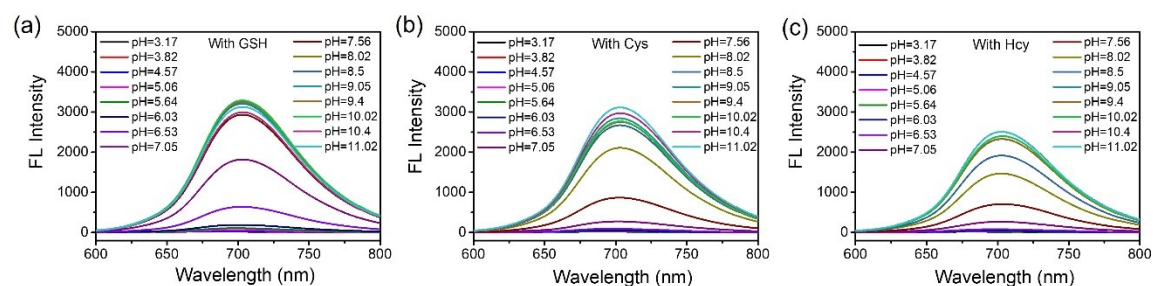


Fig. S42. The fluorescence spectra of probe N-HCA-E (10 μM) in the pH range of 3-11 with the addition of GSH, Cys and Hcy (1 mM).

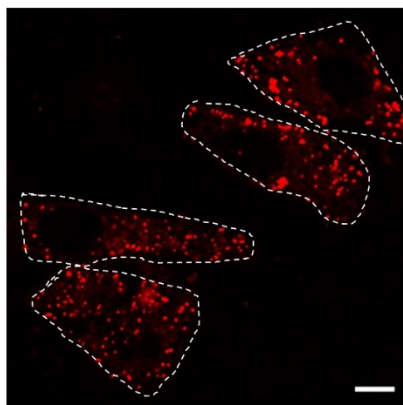


Fig. S43. Regions of cells for measurement of fluorescence intensity.

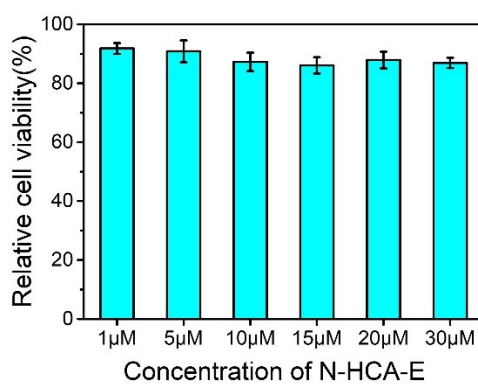


Fig. S44. Cytotoxicity of **N-HCA-E**.

Notes and references

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