Electronic Supplementary Material (ESI) for Analytical Methods.

A Mitochondria-specific NIR Fluorescence Probe for Dual-detection of Sulfur Dioxide and Viscosity in Living Cells and Mice

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Supporting Information

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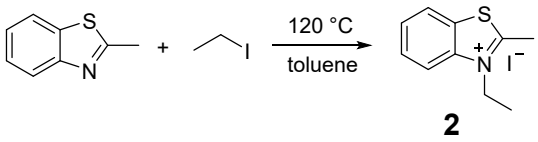
I. Synthesis and Characterization



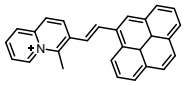
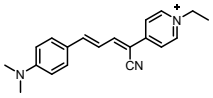
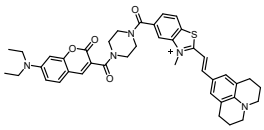
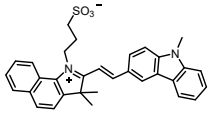
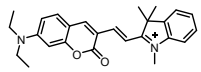
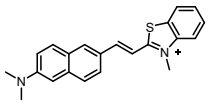
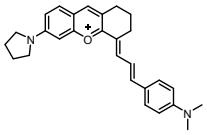
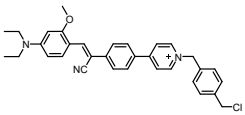
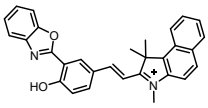
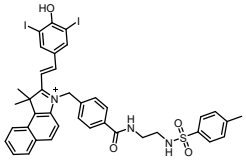
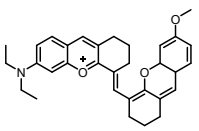
2

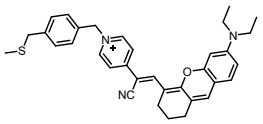
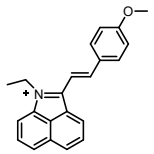
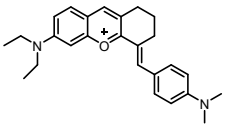
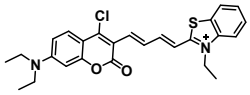
Scheme S1 Schematic route for compound **2**

Compound **2**: 2-methylbenzothiazole (2.98 g, 0.02 mol) and iodoethane (4.65 g, 0.03 mol) were added to 20 mL toluene, then the mixture was stirred for another 2 h at 120 °C. The reaction mixture was cool to room temperature, filtered, washed thoroughly with cold EtOH and dried under vacuum to obtain the product as a white solid (4.8 g, yield 80%). ^{1}H NMR (600 MHz, CD_3OD-d_4) δ 8.31 (d, J = 8.2 Hz, 1H), 8.28 (d, J = 8.6 Hz, 1H), 7.92 (t, J = 7.9 Hz, 1H), 7.82 (t, J = 7.7 Hz, 1H), 4.85 – 4.83(q, J = 7.4 Hz, 2H), 3.30 (s, 3H), 1.59 (t, J = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 177.51, 141.06, 129.96, 129.74, 128.65, 125.26, 117.26, 45.26, 17.23, 13.75. ESI–MS: calcd. for $C_{10}H_{12}INS$ 178.07 (M – I-), found 178.08 (M – I-).

II. Supporting Tables

Table S1. Comparison of **CMBT** with reported fluorescent probes for SO_2 and Viscosity

Ref.	probes	detection of SO_2 or Viscosity	λ_{em}	Aqueous Solubility	Targetability	Imaging application
3		Viscosity	600 nm	---	Mitochondria (0.98)	HeLa Cells
4		Viscosity	685 nm	---	Mitochondria (0.939)	HepG2 Cells
8		SO_2 and Viscosity	625 nm	PBS/DMSO (9/1)	Lysosome (0.92)	HepG2 Cells
11		SO_2	630 nm	PBS	Mitochondria (0.94)	HeLa Cells
12		SO_2	650 nm	EtOH/PBS (25/75)	---	HeLa Cells
13		SO_2	690 nm	PBS	Mitochondria (0.953)	HeLa Cells
18		SO_2 and Viscosity	680 nm	PBS/DMSO (7/3)	Lysosome (0.92)	A549 Cells
19		Viscosity	650 nm	---	Mitochondria (0.94)	HeLa Cells
21		SO_2 and Viscosity	605 nm	HEPES/DMSO (1/1)	Mitochondria (0.85)	C6 Cells
37		Viscosity	620 nm	---	Endoplasmic Reticulum (0.95, 0.93)	MCF-7,4T1 Mice
38		SO_2	800 nm	PBS/DMSO (1/1)	---	CCK-8, Mice

40		Viscosity	720 nm	---	Mitochondria (0.94)	HeLa Cells
43		SO_2 and Viscosity	625 nm	Water	Mitochondria (0.926)	HeLa Cells
45		SO_2 and Viscosity	740 nm	PBS	---	HeLa Cells
This work		SO_2 and Viscosity	690 nm	PBS	Mitochondria (0.97)	HeLa Cells, Mice

III. Supporting Figures

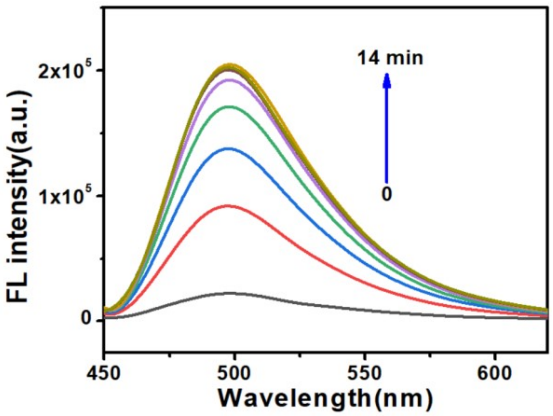


Fig. S1 The time-dependent changes in the fluorescence spectra of **CMBT** (10 μM) treated with 10 eq. SO_3^{2-}.

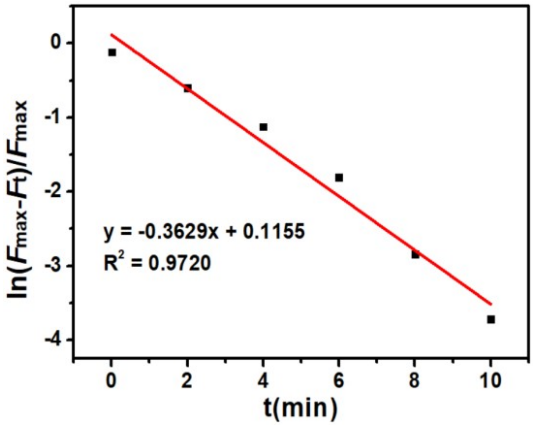


Fig. S2 The pseudo-first-order kinetic plot of the reaction of **CMBT** (10 μM) treated with 10 eq. SO_3^{2-}.

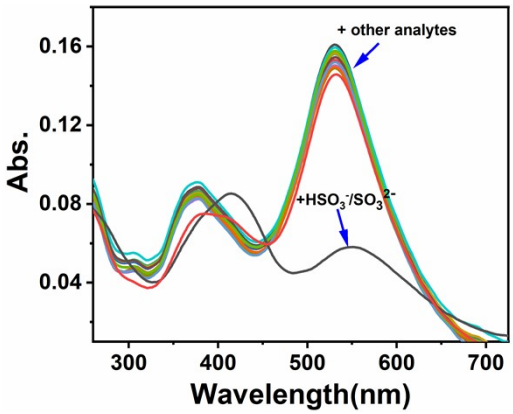


Fig. S3 UV-Vis spectra of probe **CMBT** (10 μM) in the presence of various anions (100 μM) in PBS buffer (pH 7.4). (1) Cys; (2) GSH; (3) Hcy; (4) NO_3^-; (5) AcO^-; (6) S_2^-; (7) CO_3^{2-}; (8) Cl^-; (9) I^-; (10) ClO^-; (11) H_2O_2; (12) NO_2^-; (13) ClO_4^-; (14) SO_3^{2-}.

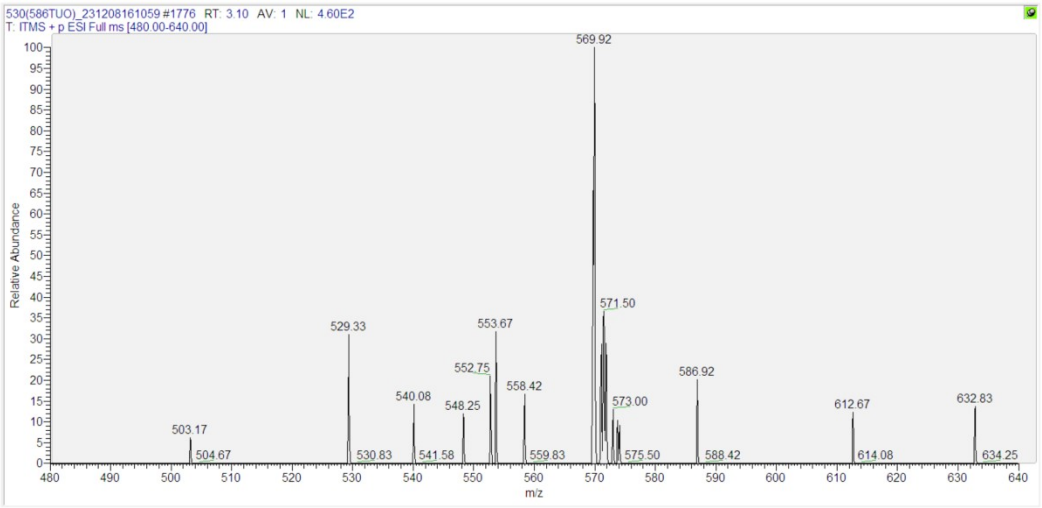


Fig. S4 The ESI-MS of product obtained by reaction between **CMBT** and SO_3^{2-}.

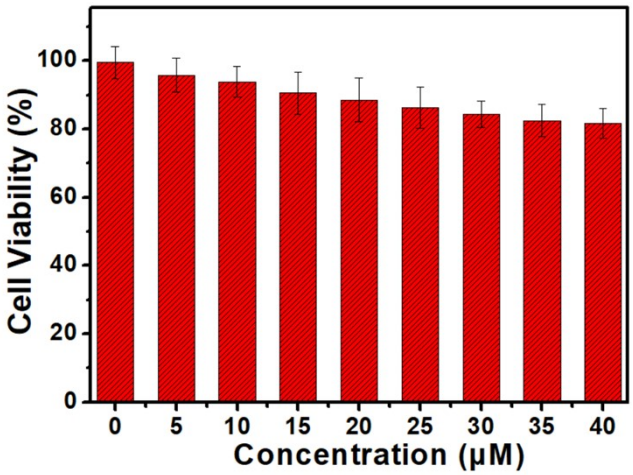


Fig. S5 Cytotoxicity assays of probe **CMBT** at different concentrations (0−40 μM) for HeLa cells.

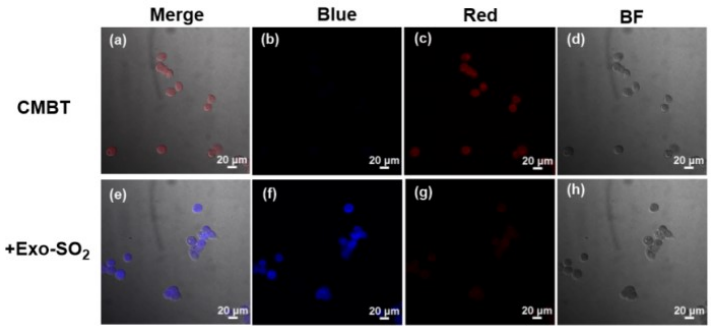


Fig. S6 Confocal images of exogenous SO_2 in living cells. (a–d) Cells stained with probe **CMBT**; (e–h) Cells incubated with probe **CMBT** and Na_2SO_3. Blue channel: λex = 405 nm, λem = 490–530 nm. Red channel: λex = 488 nm, λem = 600–700 nm. Scale bar = 20 μm.

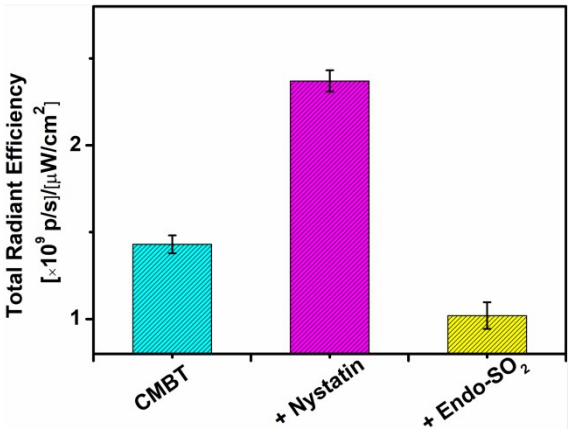


Fig. S7 The average of fluorescent intensity of tumor site post-injection of **CMBT**, **CMBT** and nystatin, **CMBT** and endo-SO_2. Values are means ± 3.

V. Spectra of Compounds

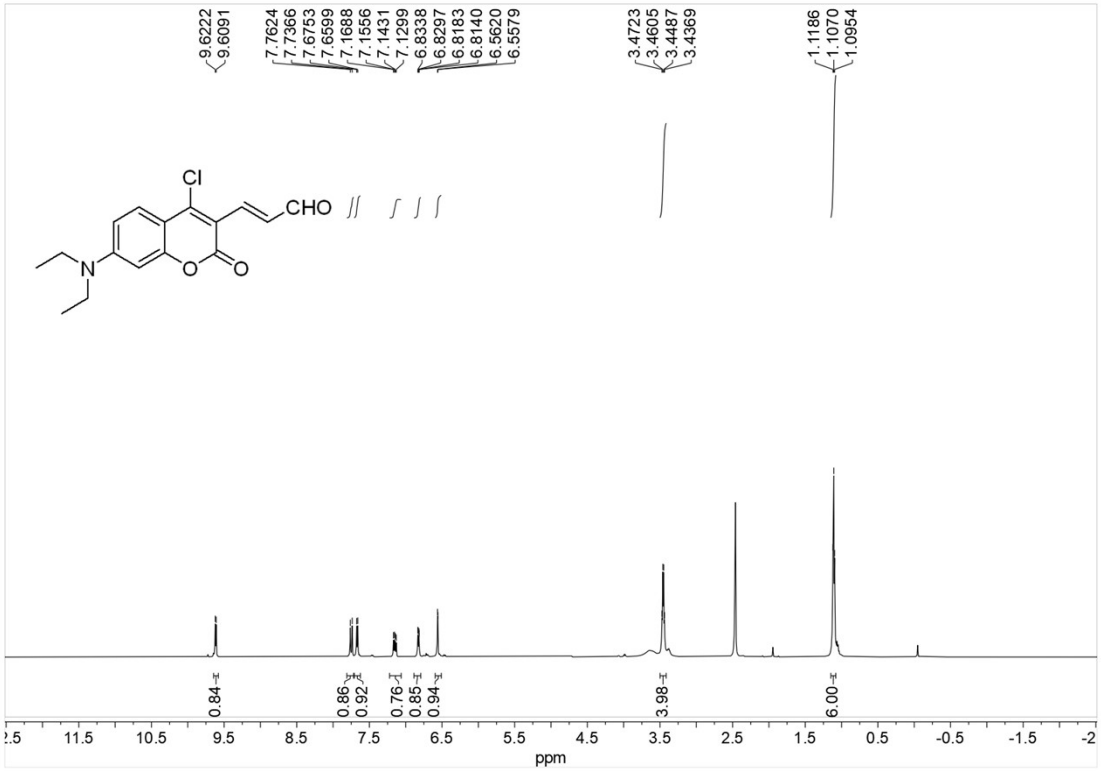


Fig. S8 ^{1}H NMR spectra of compound **1** in DMSO-d_6 (600 MHz, 298 K)

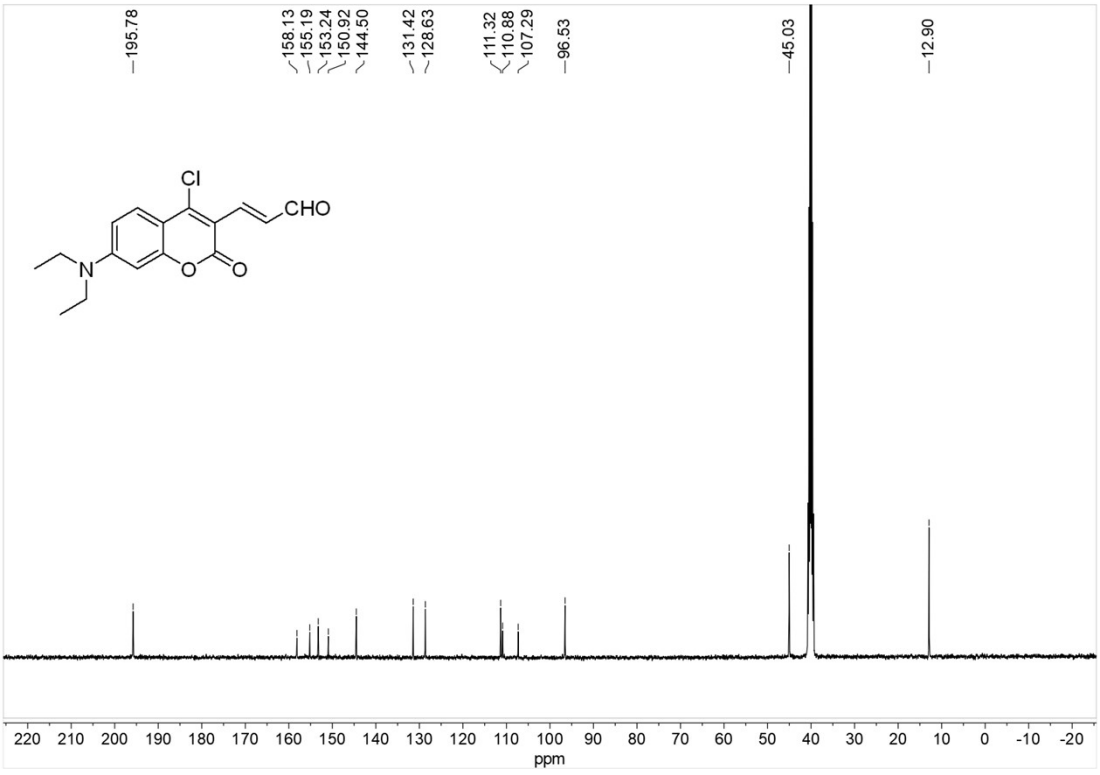


Fig. S9 ^{13}C NMR spectra of compound **1** in DMSO-d_6 (101 MHz, 298K)

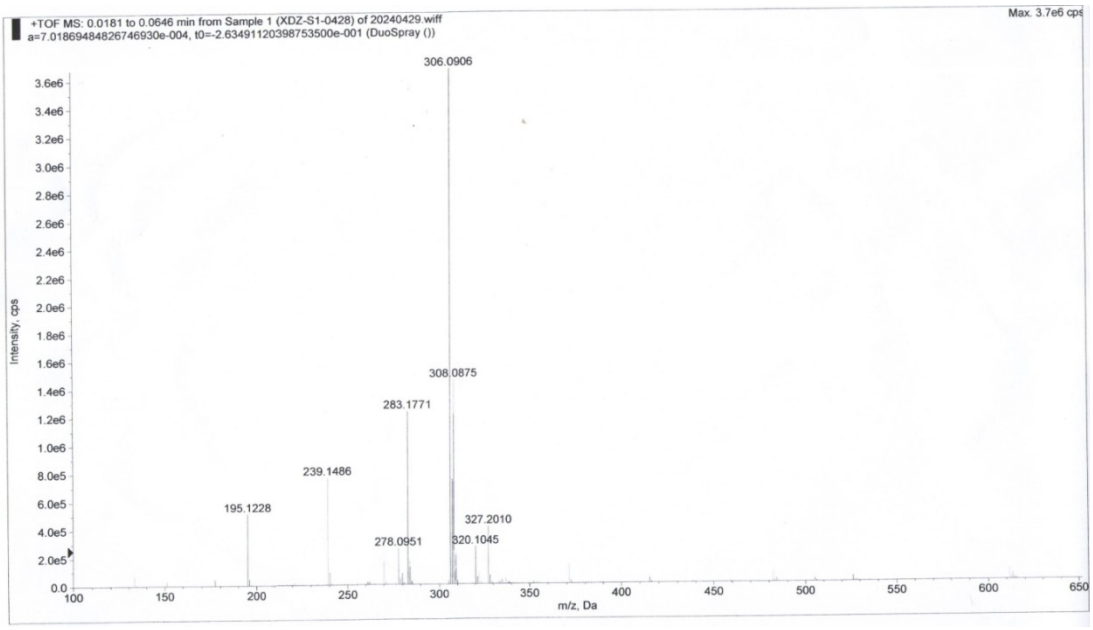


Fig. S10 The ESI-MS spectra of compound **1**

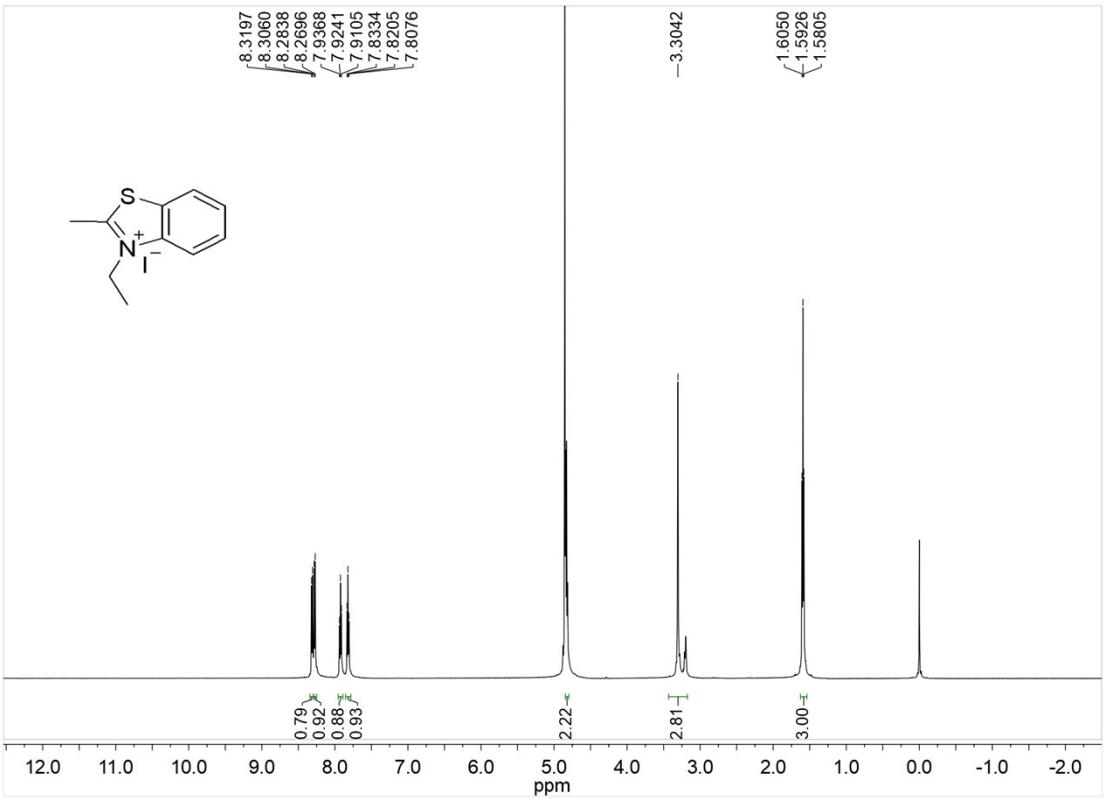


Fig. S11 ^{1}H NMR spectra of compound **2** in CD_3OD (400 MHz, 298 K)

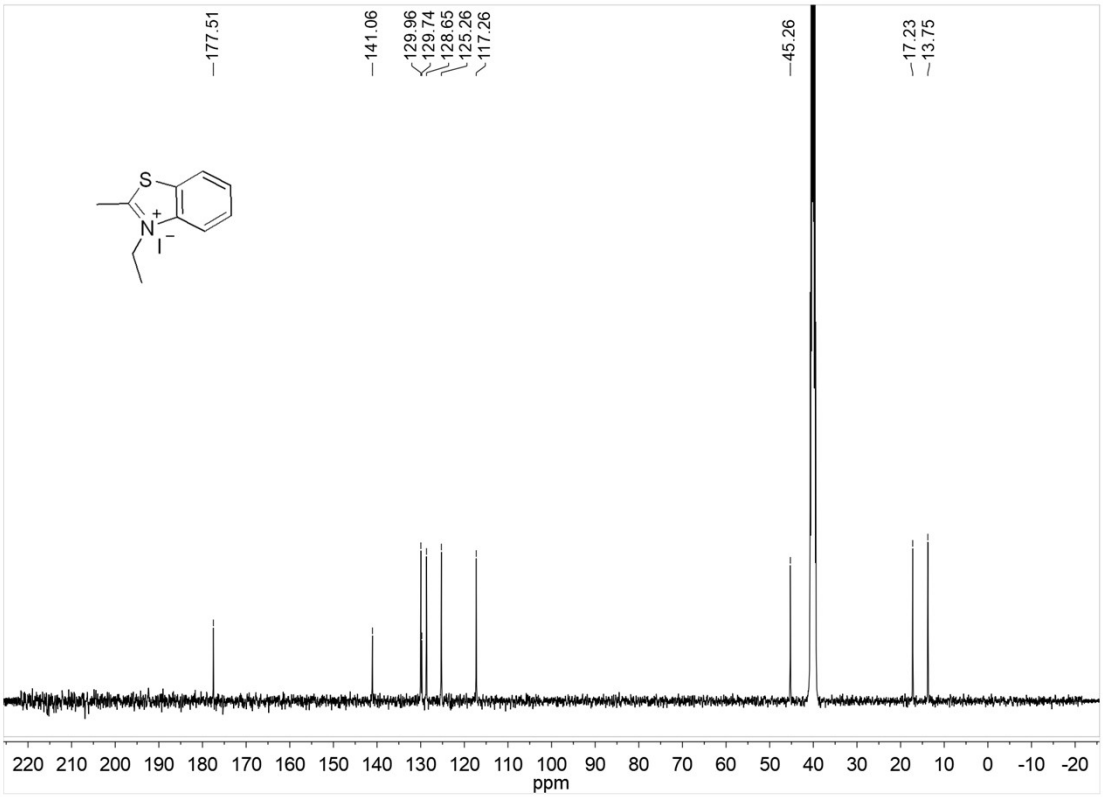


Fig. S12 ^{13}C NMR spectra of compound **2** in DMSO-d_6 (101 MHz, 298 K)

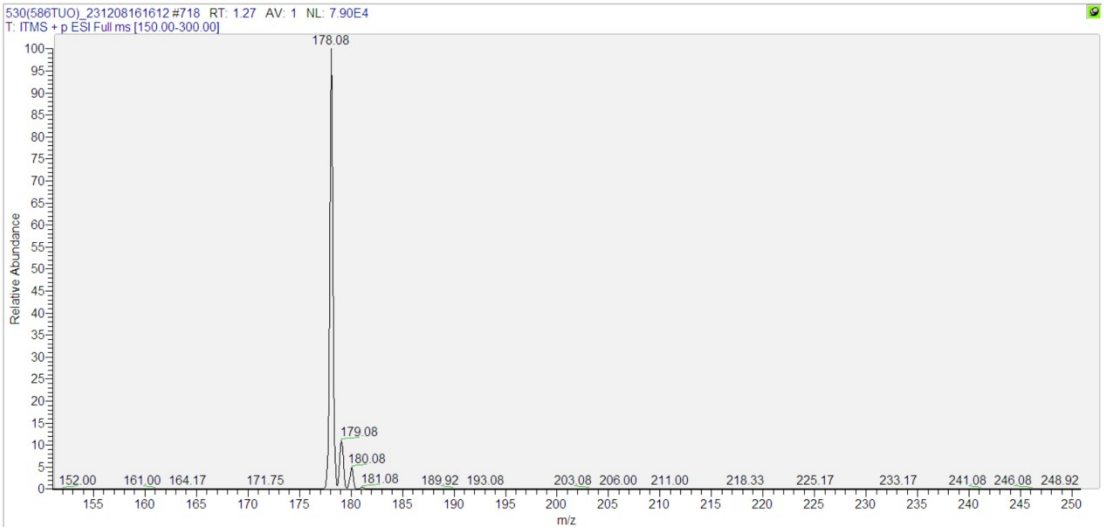


Fig. S13 The ESI-MS spectra of compound **2**

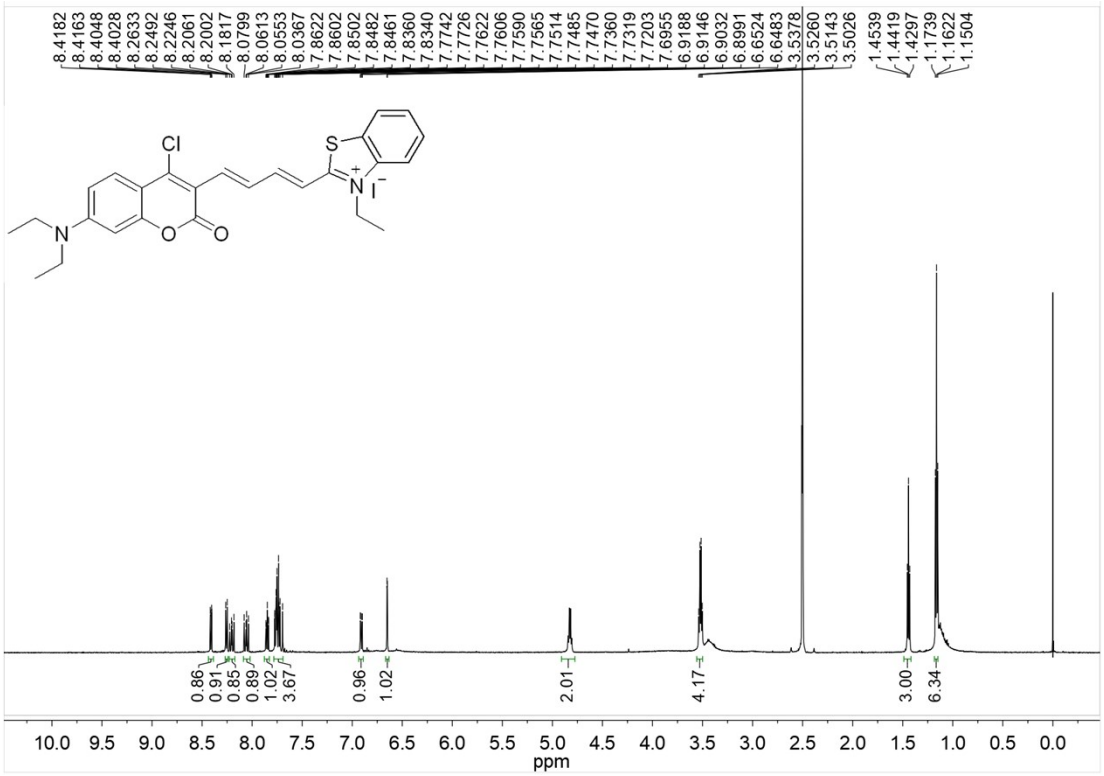


Fig. S14 ^{1}H NMR spectra of **CMBT** in DMSO-d_6 (600 MHz, 298 K)

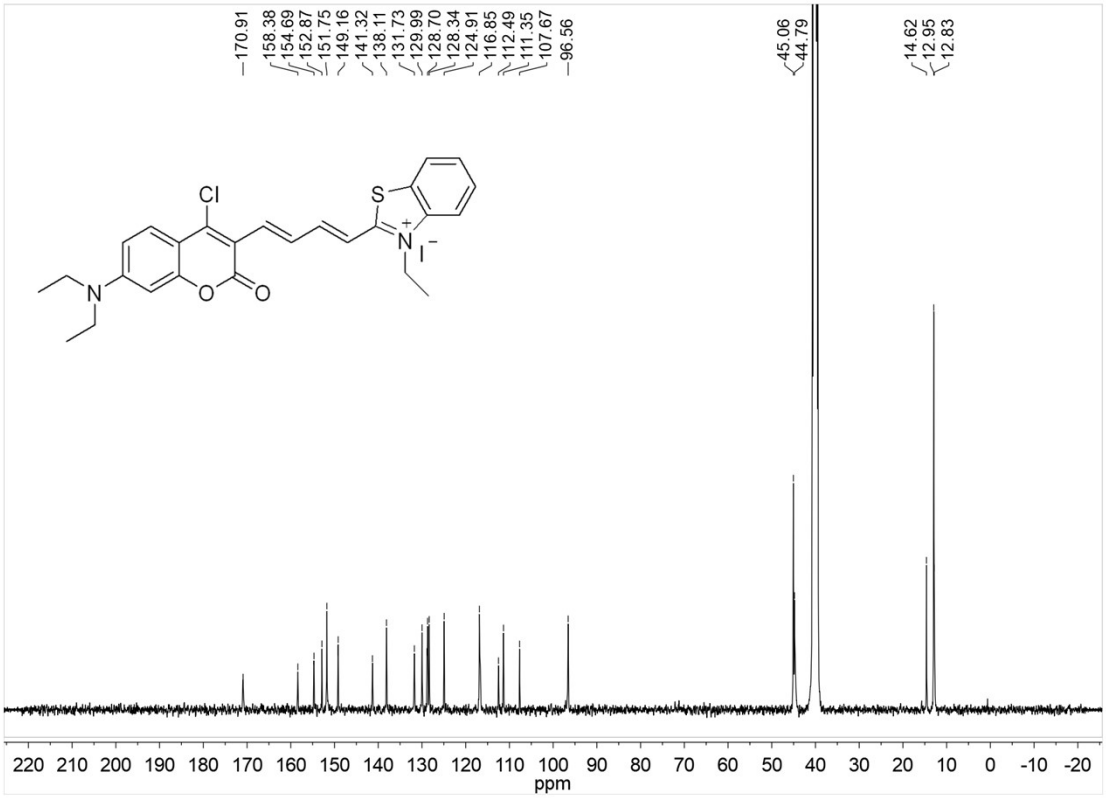


Fig. S15 ^{13}C NMR spectra of **CMBT** in DMSO-d_6 (101 MHz, 298 K)

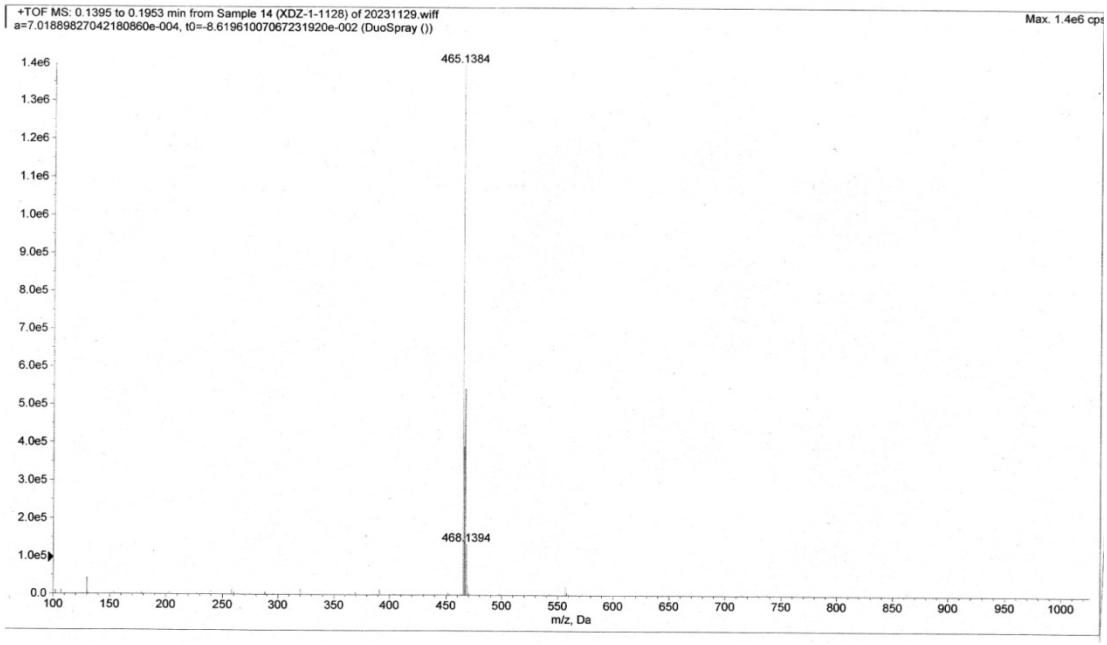


Fig. S16 The HR-MS spectra of probe **CMBT**