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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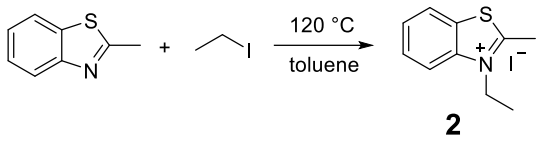
# I. Synthesis and Characterization



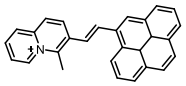
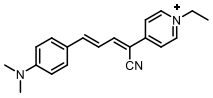
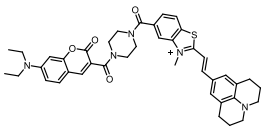
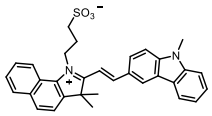
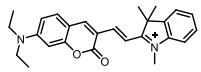
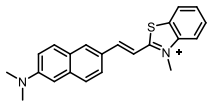
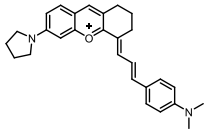
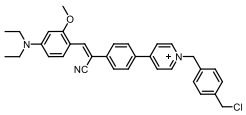
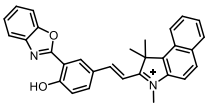
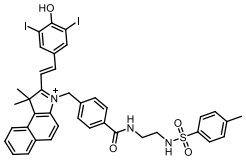
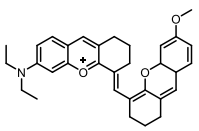

**2**

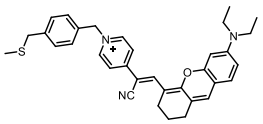
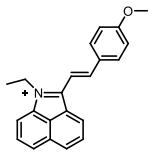
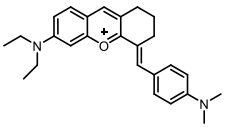
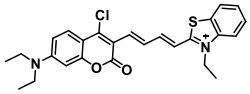
**Scheme S1** Schematic route for compound **2**

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 | $\lambda_{em}$ | 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

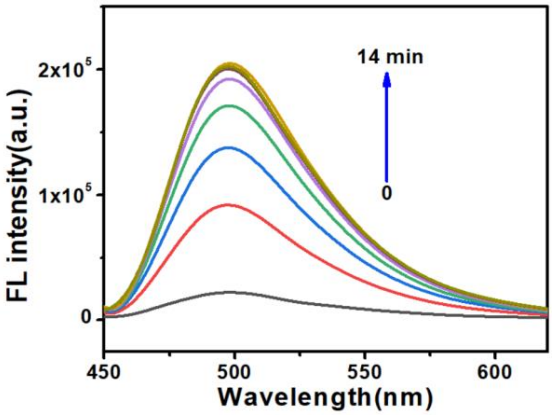



**Fig. S1** The time-dependent changes in the fluorescence spectra of **CMBT** (10 μM) treated with 10 eq. $SO_3^{2-}$.

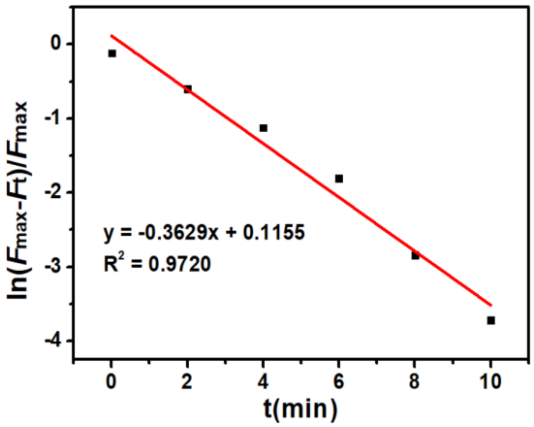



**Fig. S2** The pseudo-first-order kinetic plot of the reaction of **CMBT** (10 μM) treated with 10 eq. $SO_3^{2-}$.

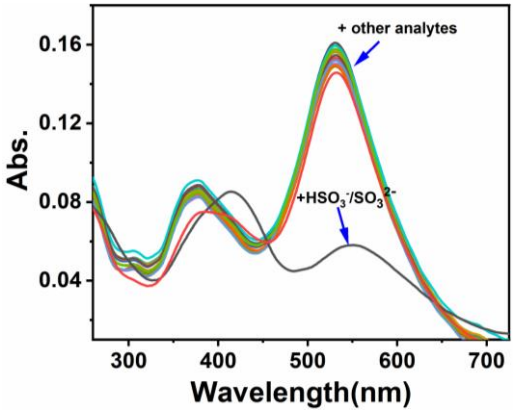



**Fig. S3** UV-Vis spectra of probe **CMBT** (10 μM) in the presence of various anions (100 μM) in PBS buffer (pH 7.4). (1) Cys; (2) GSH; (3) Hcy; (4) $NO_3^-$; (5) $AcO^-$; (6) $S_2^-$; (7) $CO_3^{2-}$; (8) $Cl^-$; (9) $I^-$; (10) $ClO^-$; (11) $H_2O_2$; (12) $NO_2^-$; (13) $ClO_4^-$; (14) $SO_3^{2-}$.

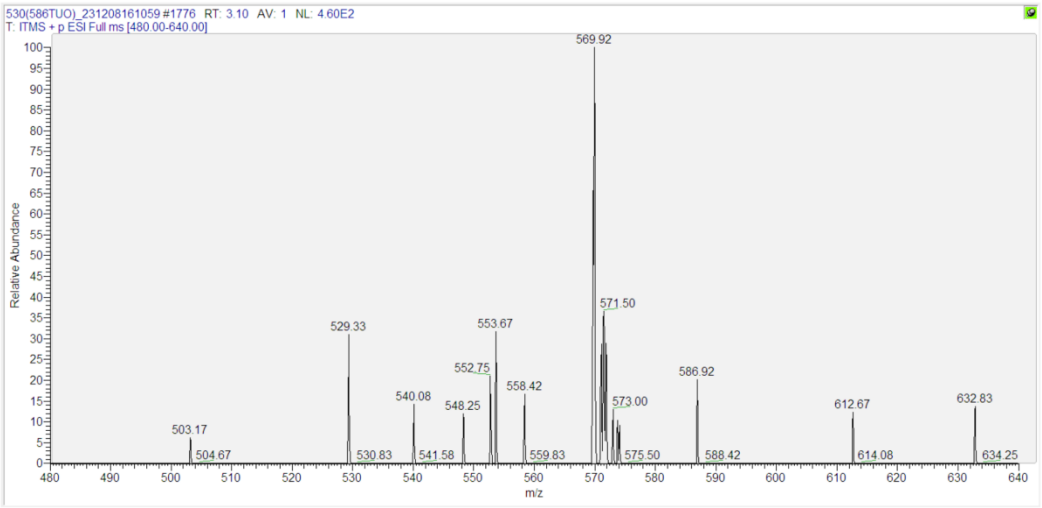



**Fig. S4** The ESI-MS of product obtained by reaction between **CMBT** and $SO_3^{2-}$.

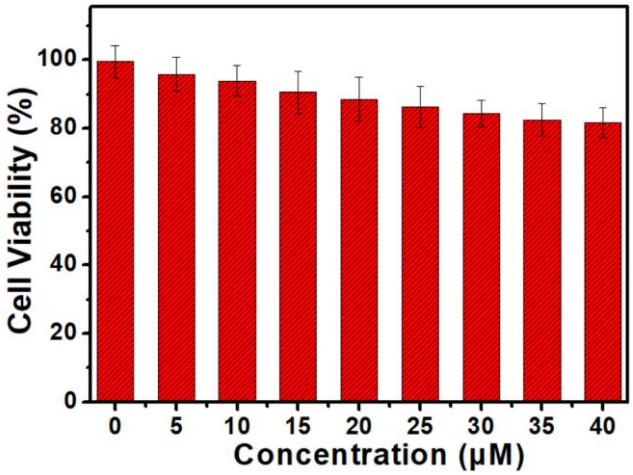



**Fig. S5** Cytotoxicity assays of probe **CMBT** at different concentrations (0−40 μM) for HeLa cells.

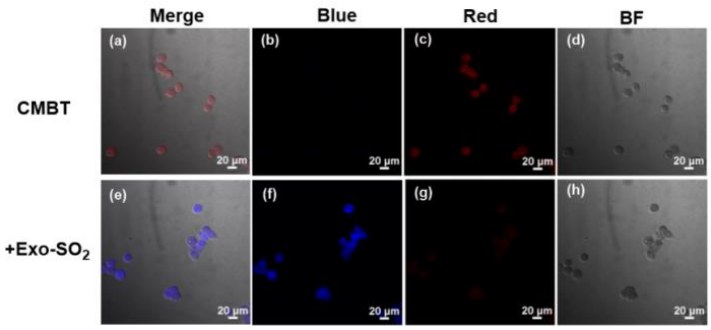



**Fig. S6** Confocal images of exogenous $SO_2$ in living cells. (a–d) Cells stained with probe **CMBT**; (e–h) Cells incubated with probe **CMBT** and $Na_2SO_3$. Blue channel: $\lambda$ex = 405 nm, $\lambda$em = 490–

530 nm. Red channel: $\lambda$ex = 488 nm, $\lambda$em = 600–700 nm. Scale bar = 20 μm.

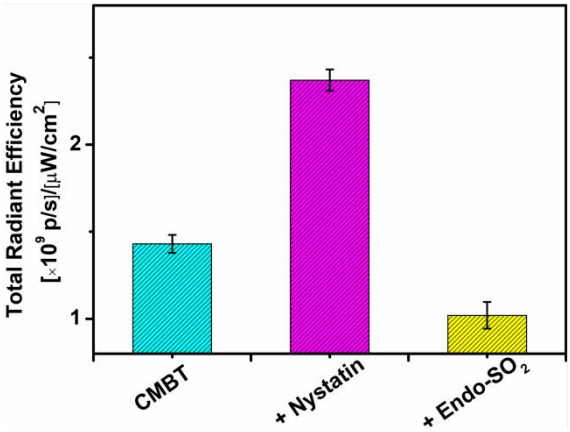



**Fig. S7** The average of fluorescent intensity of tumor site post-injection of **CMBT**, **CMBT** and nystatin, **CMBT** and endo-$SO_2$. Values are means ± 3.

# V. Spectra of Compounds

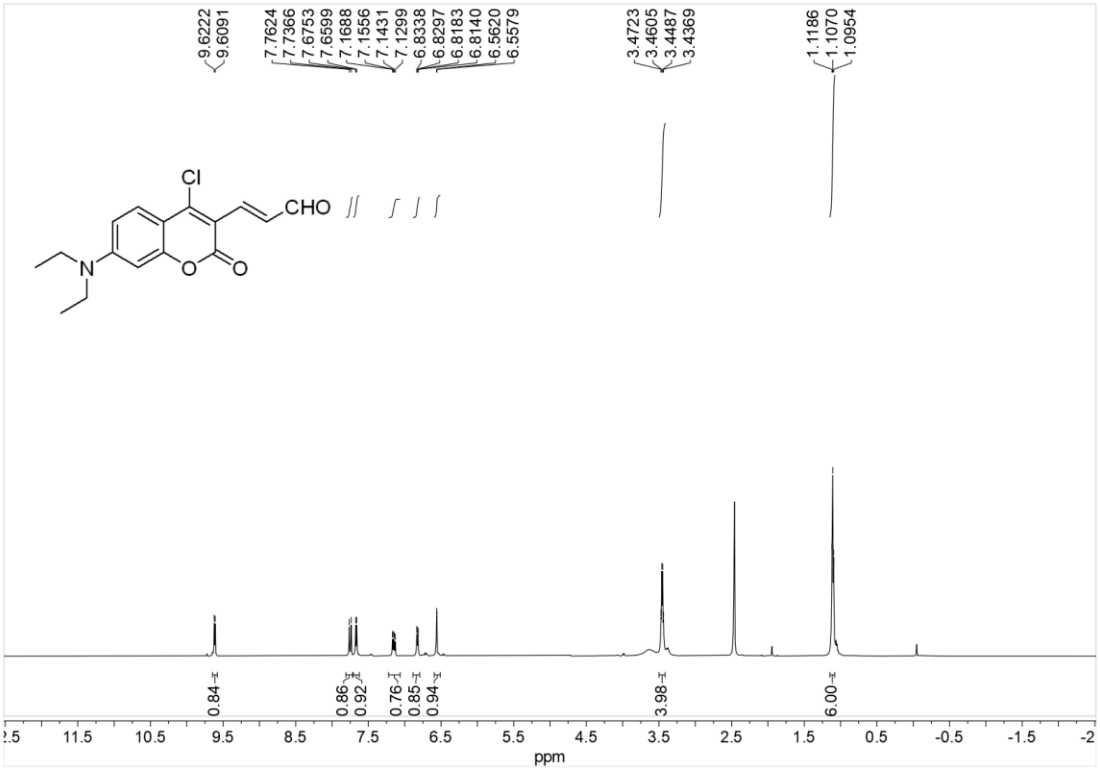



**Fig. S8** $^{1}$H NMR spectra of compound **1** in DMSO-$d_6$ (600 MHz, 298 K)

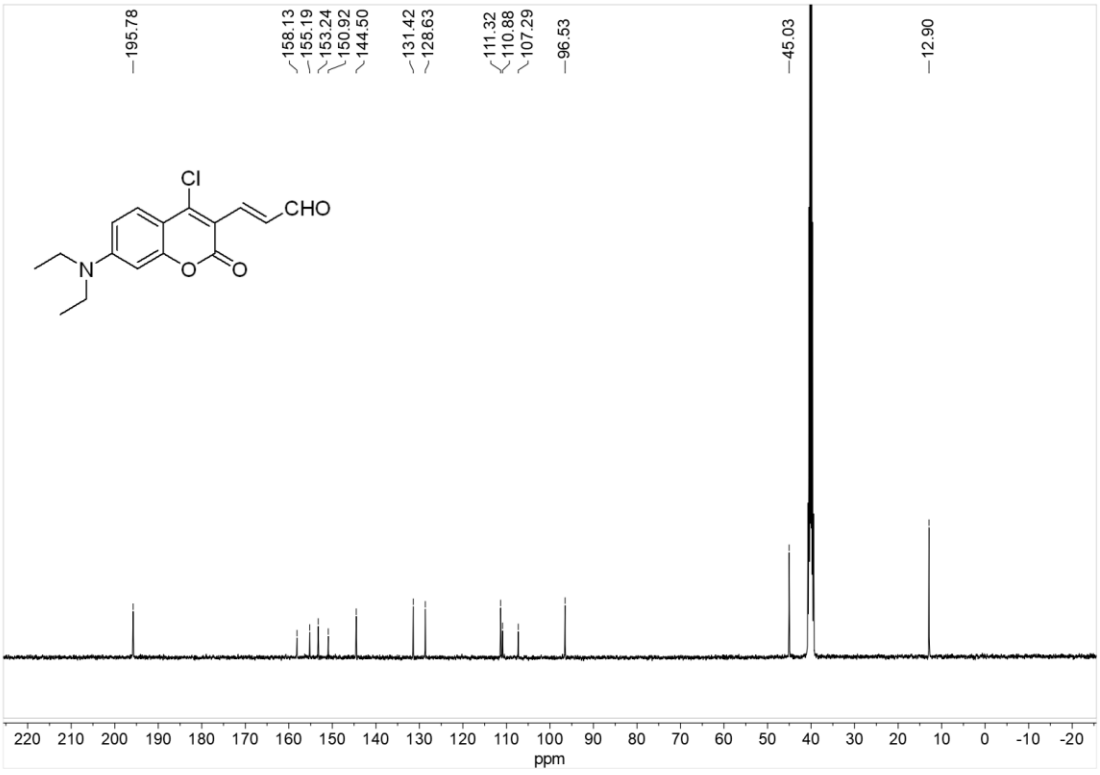



**Fig. S9** $^{13}$C NMR spectra of compound **1** in DMSO-$d_6$ (101 MHz, 298K)

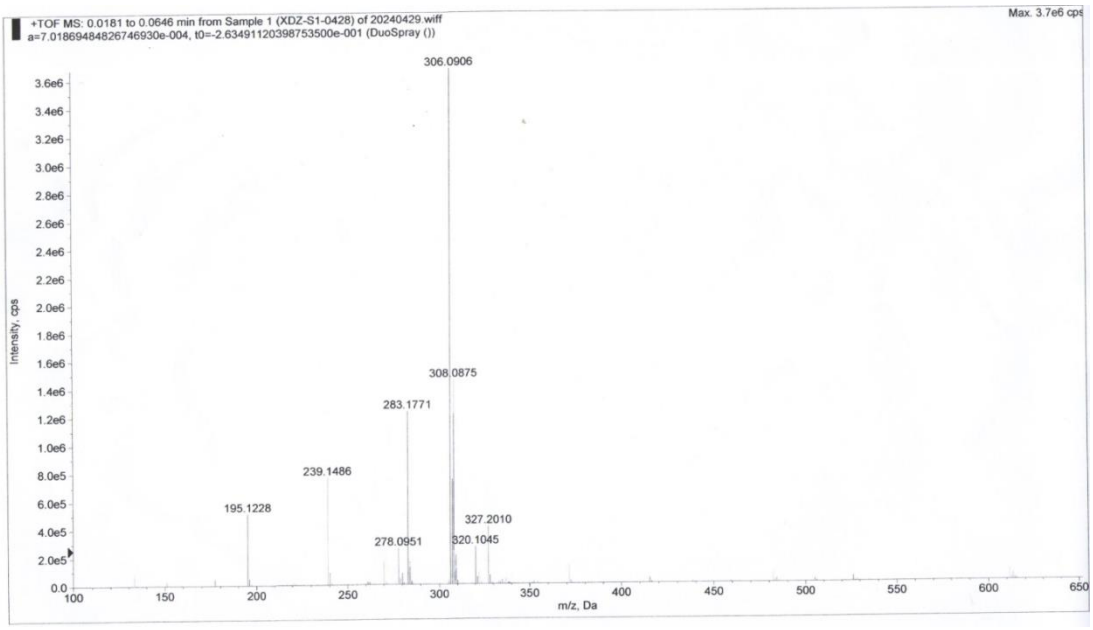



**Fig. S10** The ESI-MS spectra of compound **1**

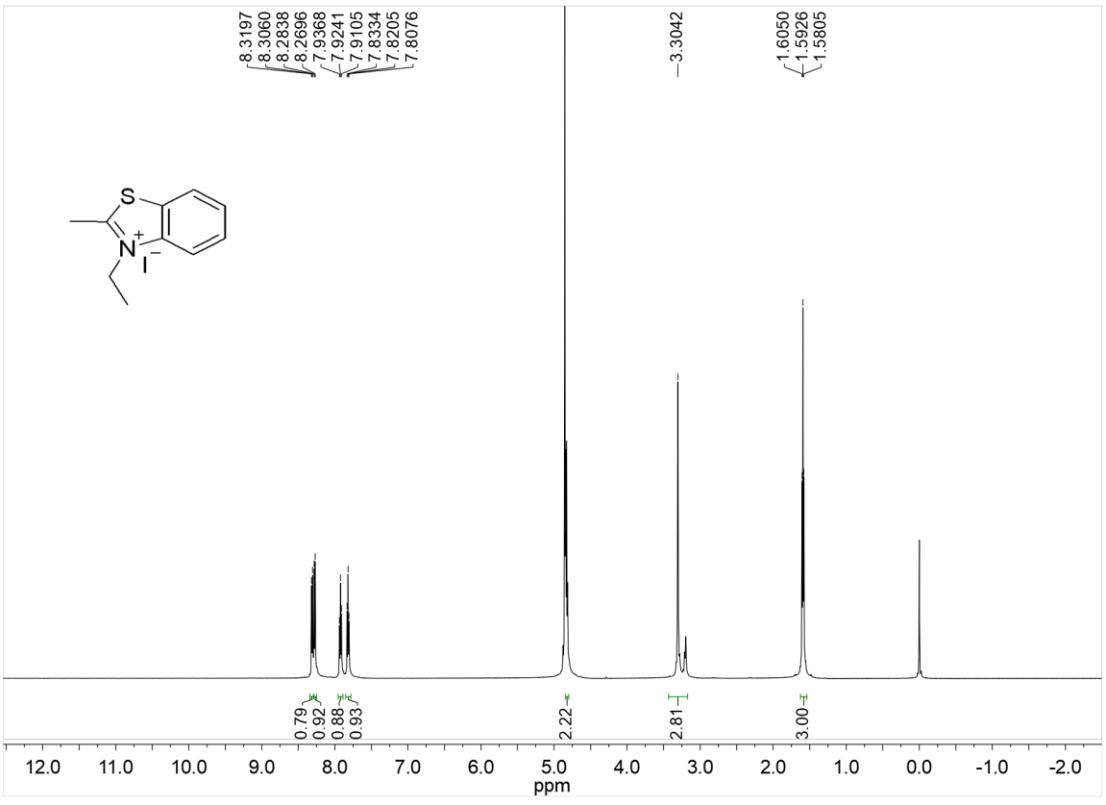



**Fig. S11** $^{1}$H NMR spectra of compound **2** in $CD_3OD$ (400 MHz, 298 K)

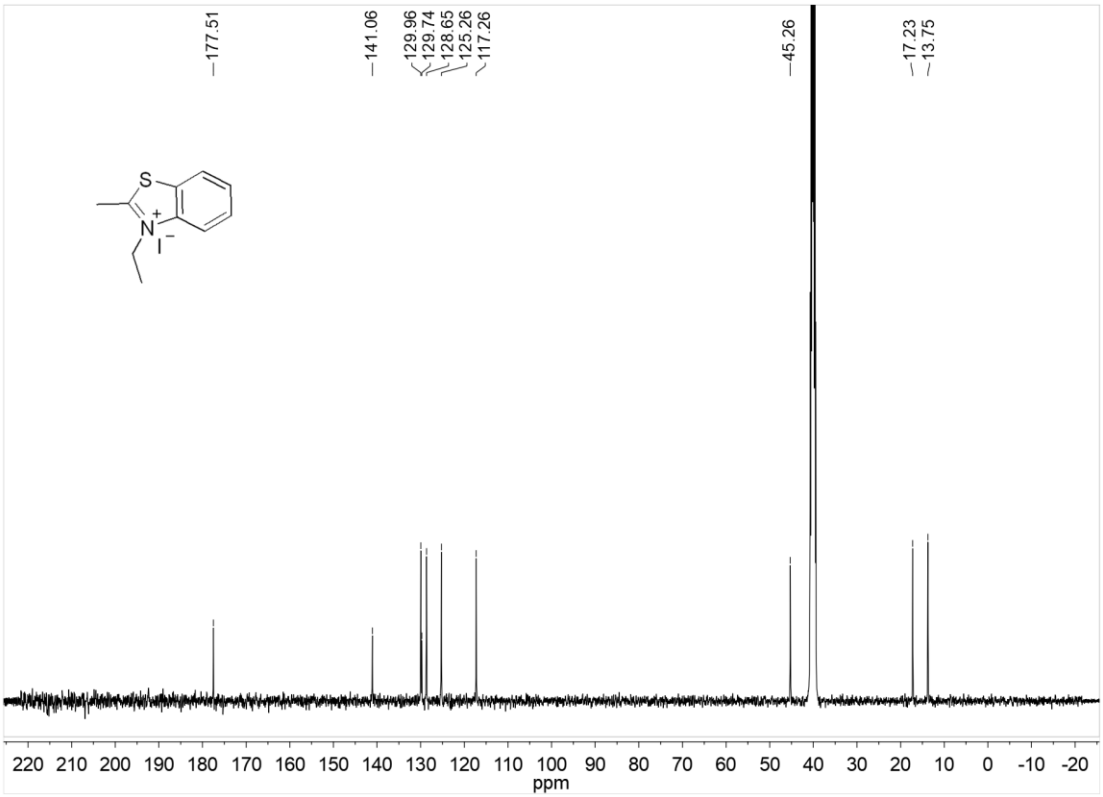



**Fig. S12** $^{13}$C NMR spectra of compound **2** in DMSO-$d_6$ (101 MHz, 298 K)

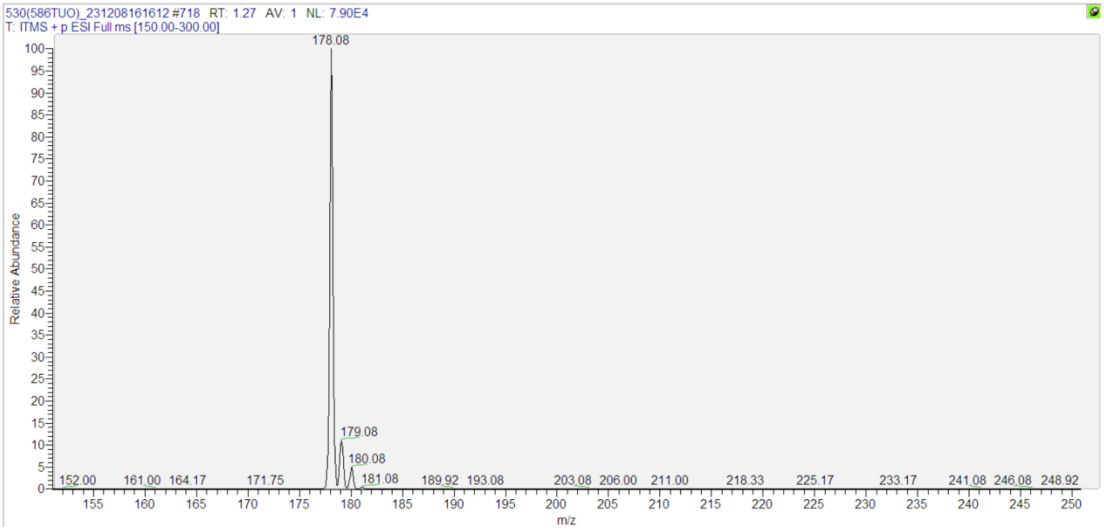



**Fig. S13** The ESI-MS spectra of compound **2**

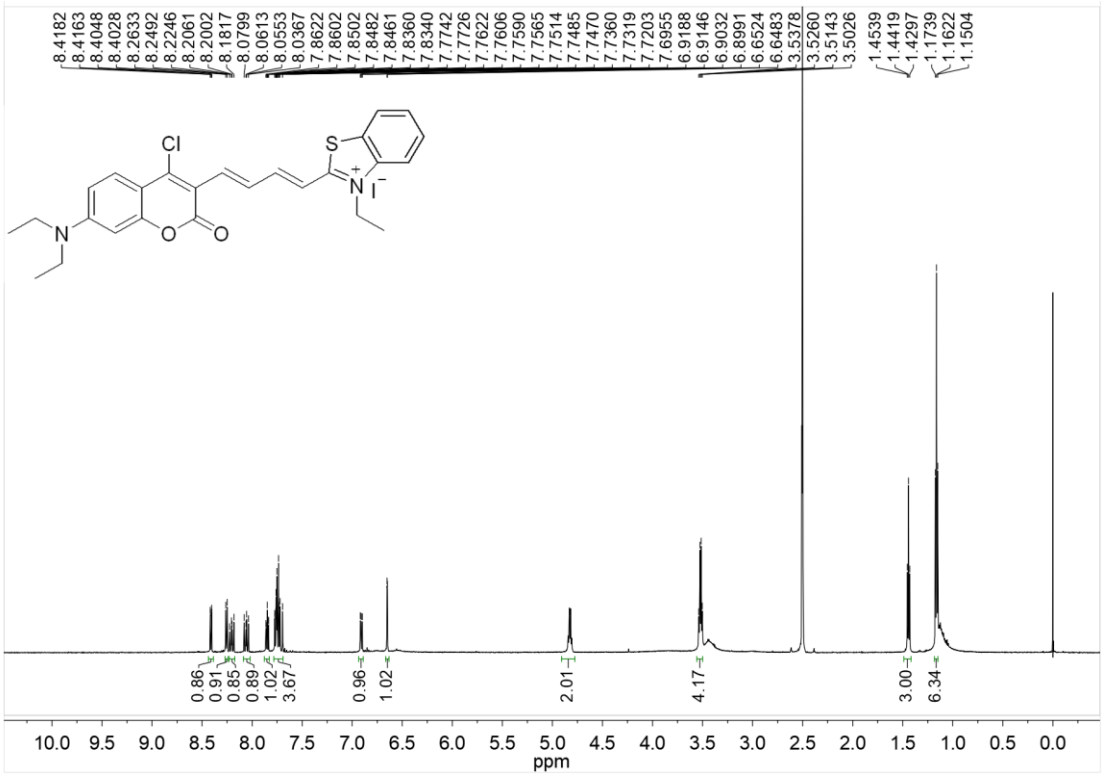



**Fig. S14** $^{1}$H NMR spectra of **CMBT** in DMSO-$d_6$ (600 MHz, 298 K)

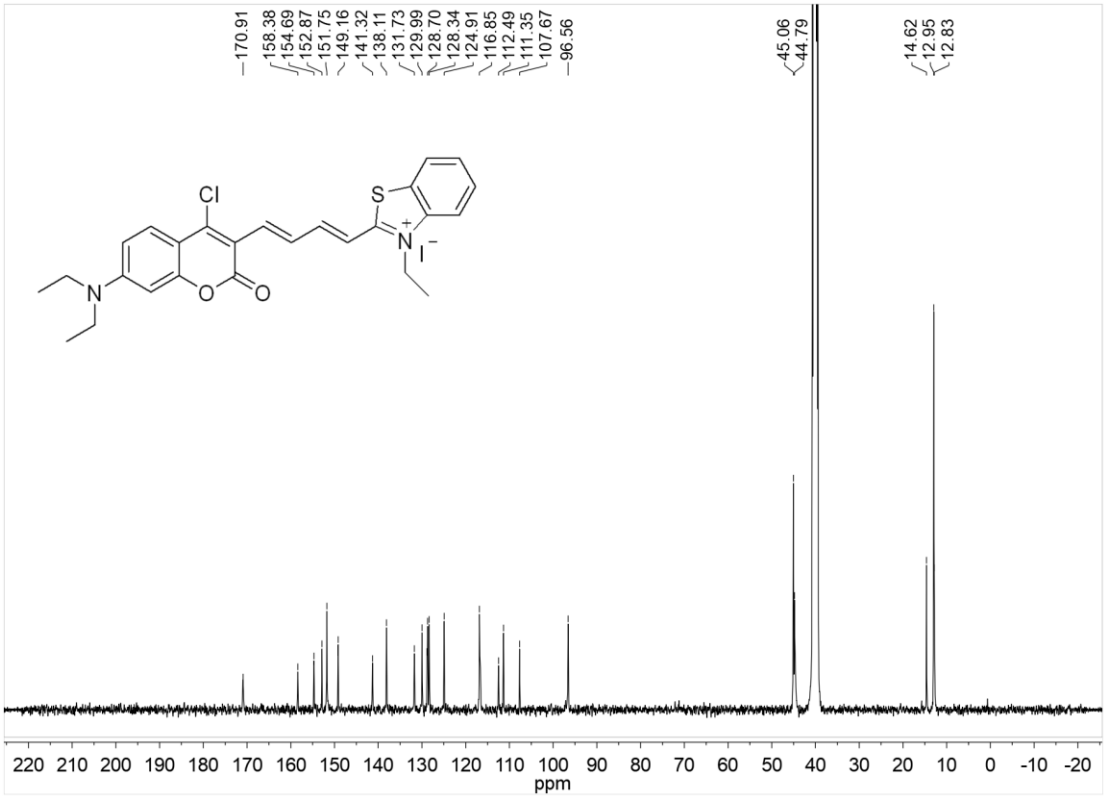



**Fig. S15** $^{13}$C NMR spectra of **CMBT** in DMSO-$d_6$ (101 MHz, 298 K)

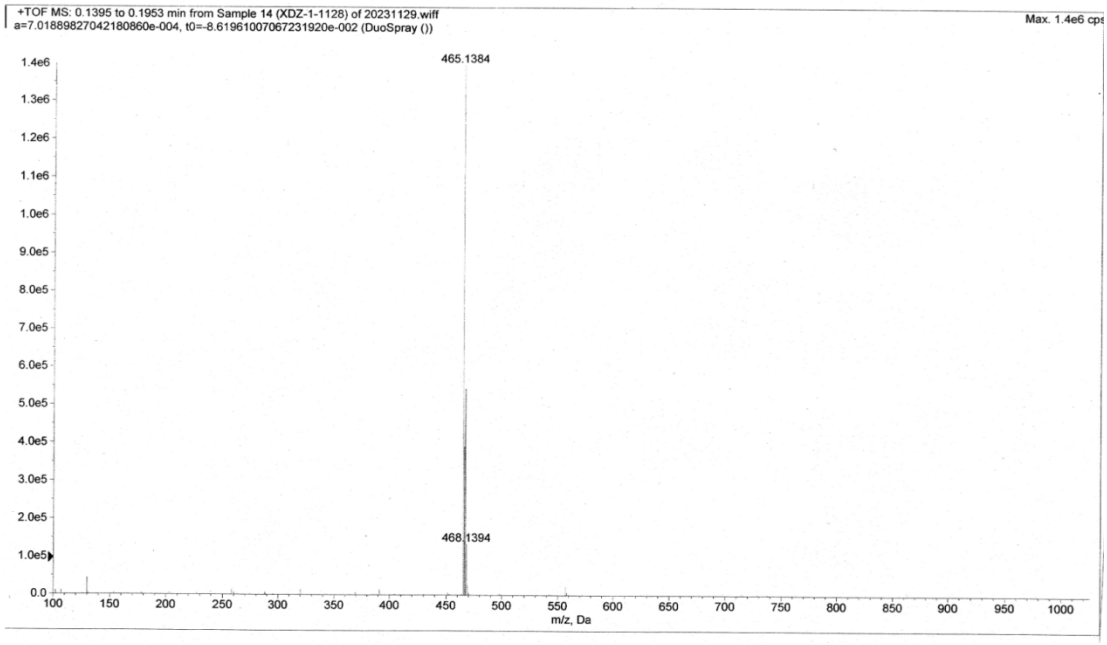



**Fig. S16** The HR-MS spectra of probe **CMBT**