Supplementary Information

Design, Synthesis, and Biological Application of A-D-A Type Boranil Fluorescent Dyes

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1. General methods

1.1 Instruments and materials

^{1}H NMR and ^{13}C NMR spectra were conducted on a Bruker 400 spectrometer. Mass spectra were obtained on a Bruker Daltonics micro-TOF-Q II mass spectrometer. Absorption spectra were recorded using a UV-2450 spectrometer (Shimadzu). Fluorescence spectra were performed on a F-7000 fluorometer (Hitachi). Fluorescence imaging experiments on cells and zebrafish were performed on an Olympus FV3000 confocal laser scanning microscope. HeLa cells were obtained from Xiangya Hospital at Central South University, China. Zebrafish were purchased from Nanjing Eze-Rinka Biotechnology Co., Ltd., China. All reagents were used as received, and deionized water was used in all experiments. All the tests were conducted at room temperature.

1.2 Fluorescence quantum yield measurements

The fluorescence quantum yield in solution was measured on a Hitachi F-7000 spectrophotometer using a standard reference (fluorescein, Φ_f = 0.92 in 0.1 M NaOH at 25 °C). The solution of the compound was adjusted to an absorbance of ca.0.05 and calculated from the following equation:

$$\Phi_u = \Phi_s * \frac{F_u}{F_s} * \frac{A_s}{A_u} * \frac{n_u^2}{n_s^2}$$

Φ denotes the fluorescence quantum yield; F means the integral intensity of fluorescence; A refers to the absorbance at the excitation wavelength and n is the refraction index of solvents. u and s represent the testing sample and the standard sample, respectively.

1.3 Theoretical calculations

The Density functional theory (DFT) calculations were performed on **CSU-BF-R** dyes using the Gaussian 09 program, whose geometries were optimized under B3LYP/6-31G level, and the frontier energy levels (HOMO and LUMO) were calculated.

1.4 Cell and zebrafish culture

Hela cells were used for imaging and cytotoxicity experiments. HeLa cells were cultured using DMEM (GIBCO, Invitrogen) medium with 10% fetal bovine and 1% dual antibody (streptomycin and penicillin) at 37 °C in a humidified 5% CO_2 incubator.

Zebrafish was cultured at 28 °C in E3 embryo media, consisting of 15 mM NaCl, 0.5 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$ and 1% methylene blue, pH = 7.5. Three-day old zebrafish were selected for the imaging experiments.

1.5 Cytotoxicity assay

MTT assays were performed on HeLa cells to determine the toxicity of **CSU-BF-OCH$_3$**. HeLa Cells were added to a 96-well plate and incubated in 5% CO_2 atmosphere for 24 h at 37 °C. The **CSU-BF-OCH$_3$** dissolved in DMSO with a specific concentration (0, 5, 10, 15, 20, 25, 30 μM, respectively) was added to each well and incubated for 24 h.

2. **Photophysical properties of CSU-BF-R dyes**

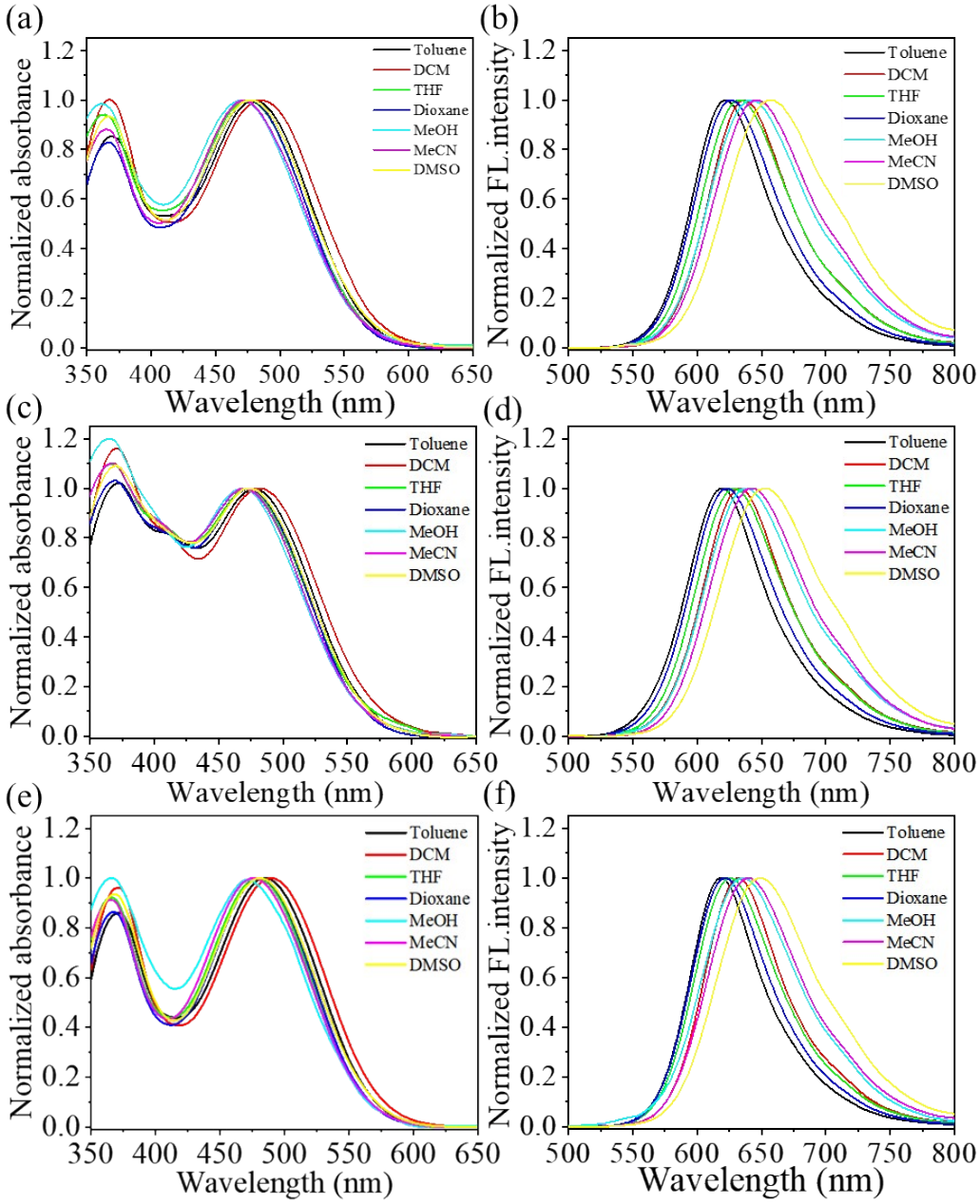

Fig. S1 Normalized absorption and emission spectra of **CSU-BF-H** (a and b), **CSU-BF-CH$_3$** (c and d), **CSU-BF-OCH$_3$** (e and f) in different solvents (λ_{ex} = 470 nm).

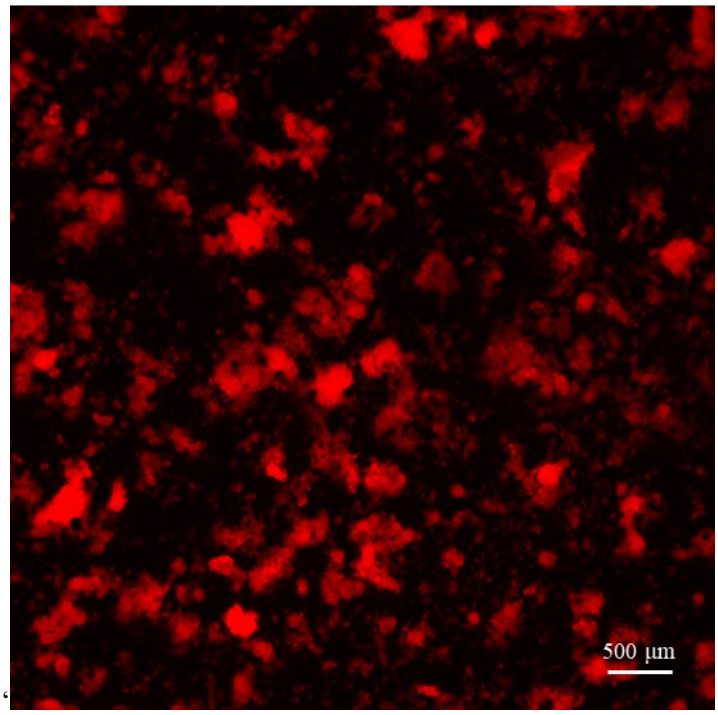

Fig. S2 Fluorescence photographs of **CSU-BF-OCH$_3$** powder. Scale bar: 500 μm.

3. **Detection of HClO by CSU-BF-OCH$_3$**

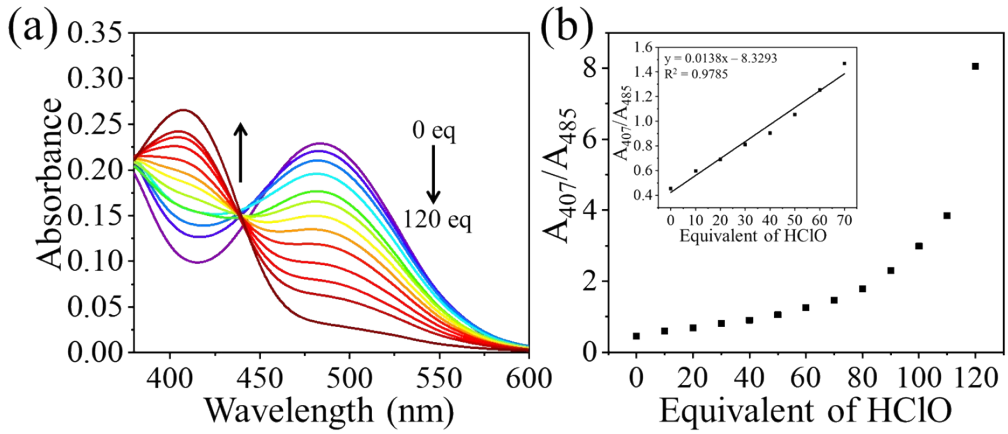

Fig. S3 (a) The absorption spectra of **CSU-BF-OCH$_3$** (10.0 μM) treated with various concentrations of HClO (0.0-120.0 equiv.) in THF/PBS buffer (10 mM, v/v, 3/7, pH = 7.40). (b) The linearity between the ratio of the absorbance at 407 nm and 485 nm (A_{407}/A_{485}) and the concentration of HClO.

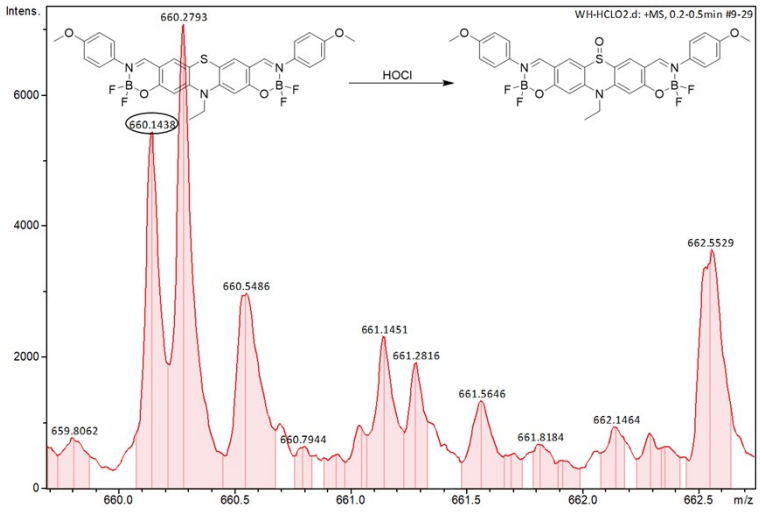

Fig. S4 HRMS spectrum of the solution of **CSU-BF-OCH$_3$** with HClO.

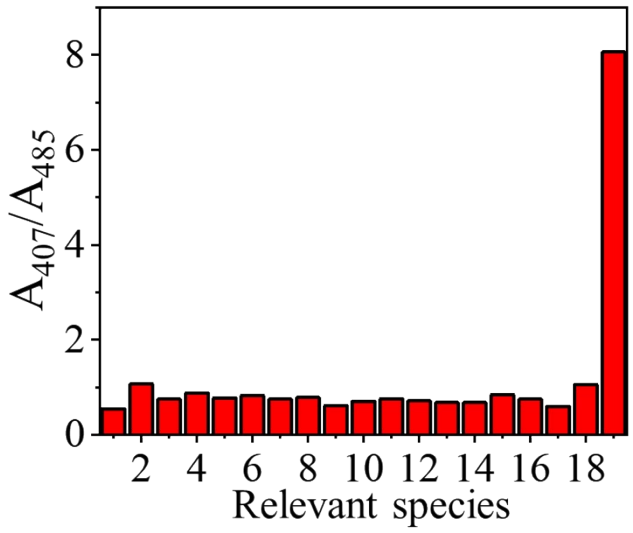

Fig. S5 The absorption ratio (A_{407}/A_{485}) of **CSU-BF-OCH$_3$** (10.0 μM) in THF/PBS buffer (10 mM, v/v, 3/7, pH = 7.40) with different relevant species (120.0 equiv.): 1, Blank; 2, Ca^{2+}; 3, K^{+}; 4, Mg^{2+}; 5, NH_4^{+}; 6, S^{2-}; 7, CO_3^{2-}; 8, SO_3^{2-}; 9, TBHP; 10, F^{-}; 11, NO_2^{-}; 12, Hcy; 13, Cys; 14, GSH; 15, NO·; 16, ROO·; 17, H_2O_2; 18. HO·; 19, HClO.

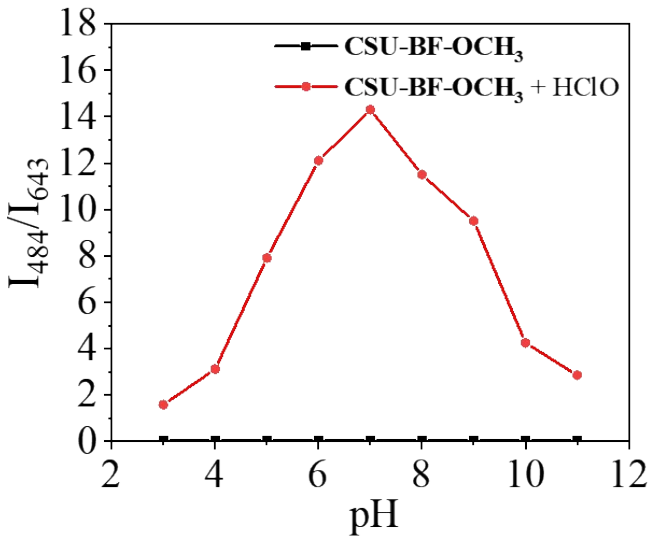

Fig. S6 The ratio of I_{484}/I_{643} of **CSU-BF-OCH$_3$** in the presence and absence of HClO (120.0 equiv.) at different pH values.

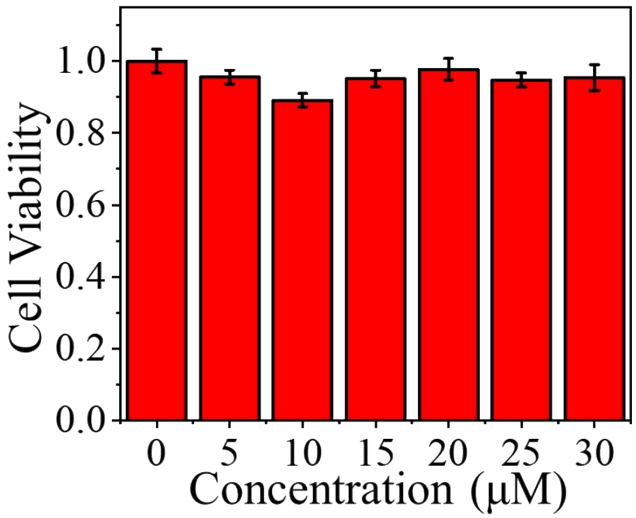

Fig. S7 The MTT assay of Hela cells incubated with different concentrations of **CSU-BF-OCH$_3$** for 24 h.

4. **Characterization of Boranil dyes and intermediates**

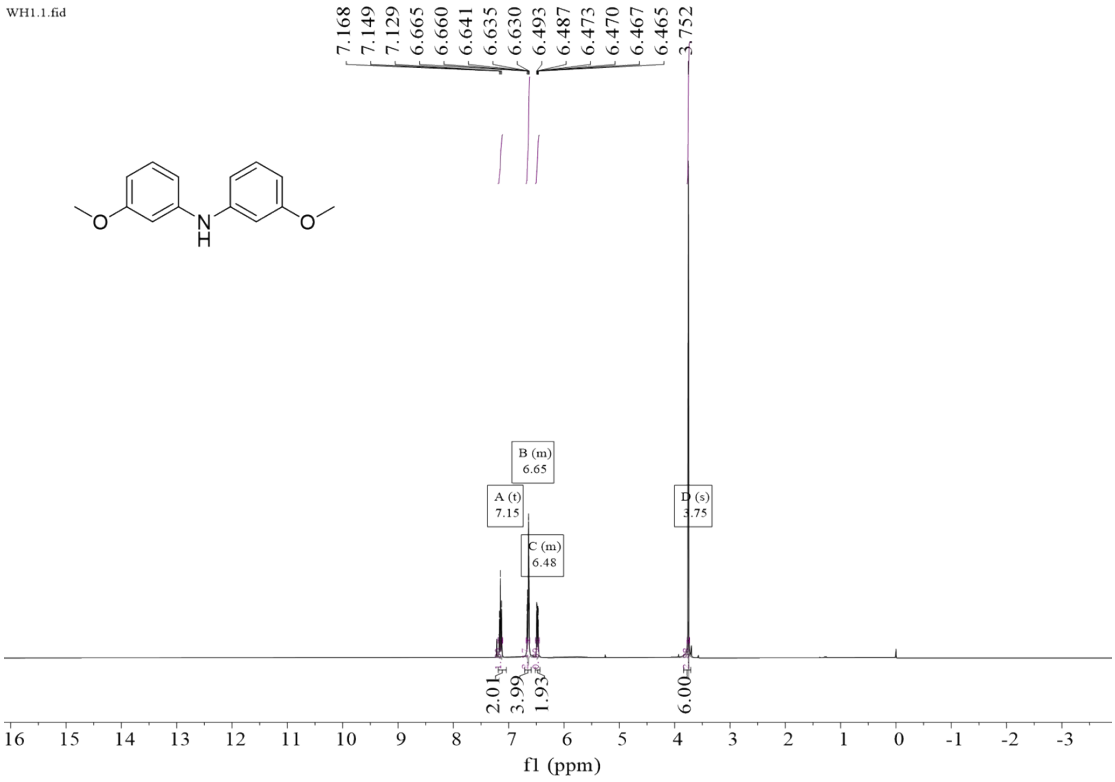

Fig. S8 ^{1}H NMR spectrum of compound **1** in $CDCl_3$.

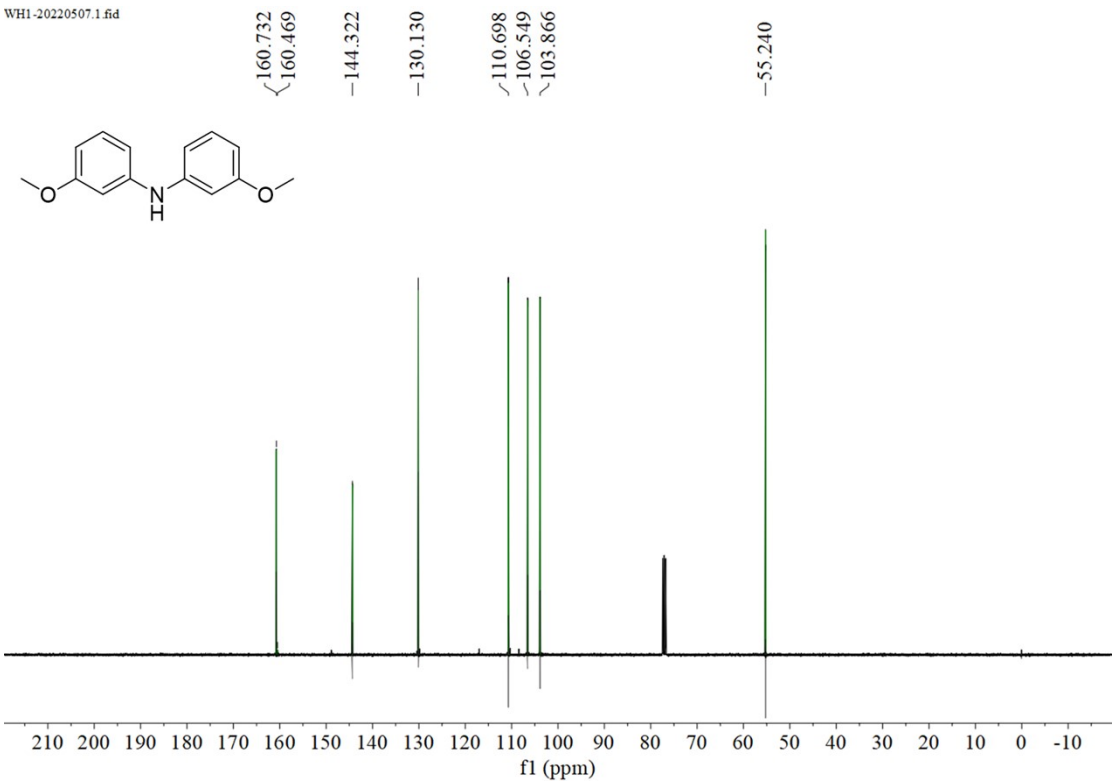

Fig. S9 ^{13}C NMR spectrum of compound **1** in $CDCl_3$.

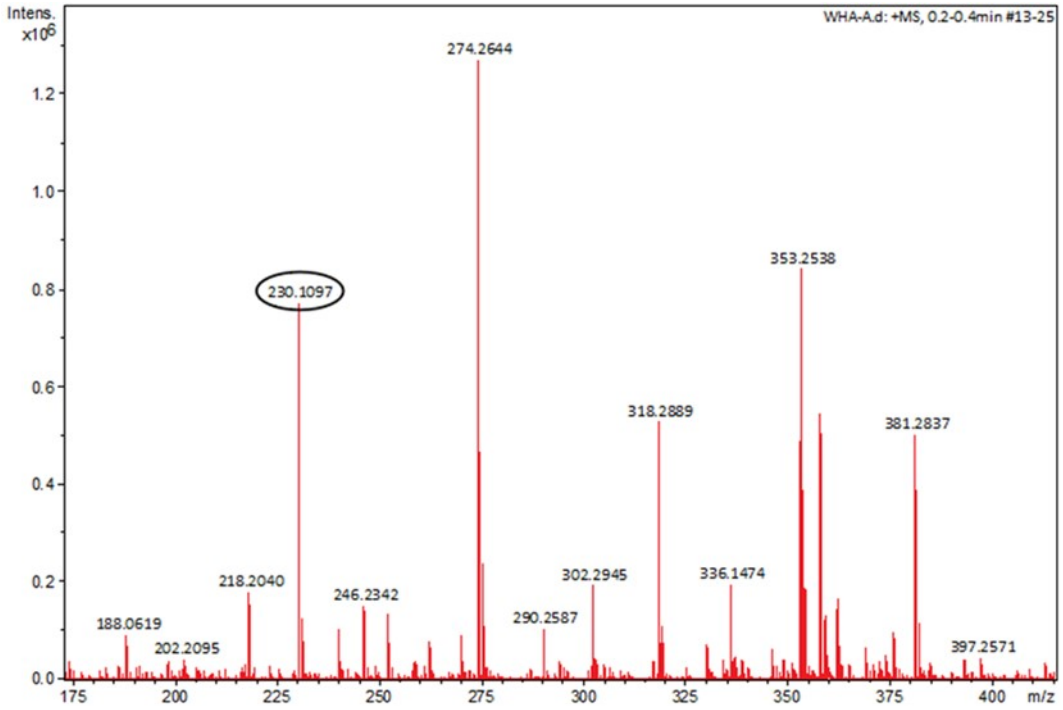

Fig. S10 HRMS spectrum of compound **1**.

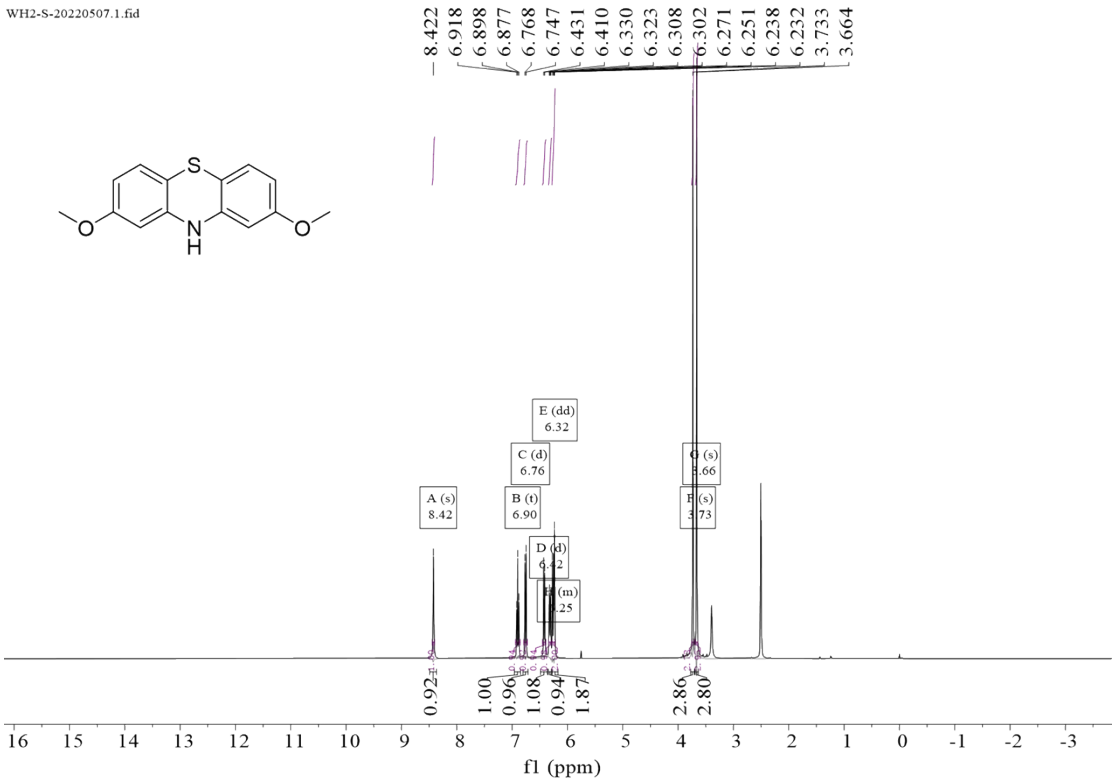

Fig. S11 ^{1}H NMR spectrum of compound **2** in DMSO-d_6.

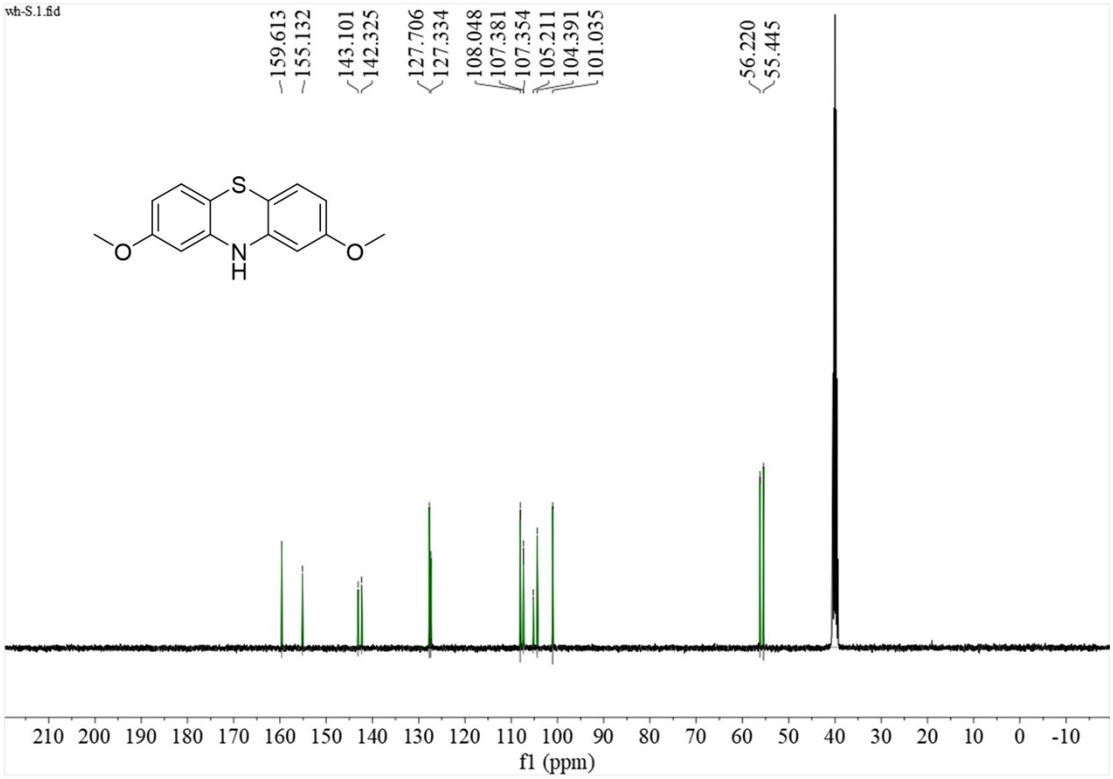

Fig. S12 ^{13}C NMR spectrum of compound **2** in DMSO-d_6.

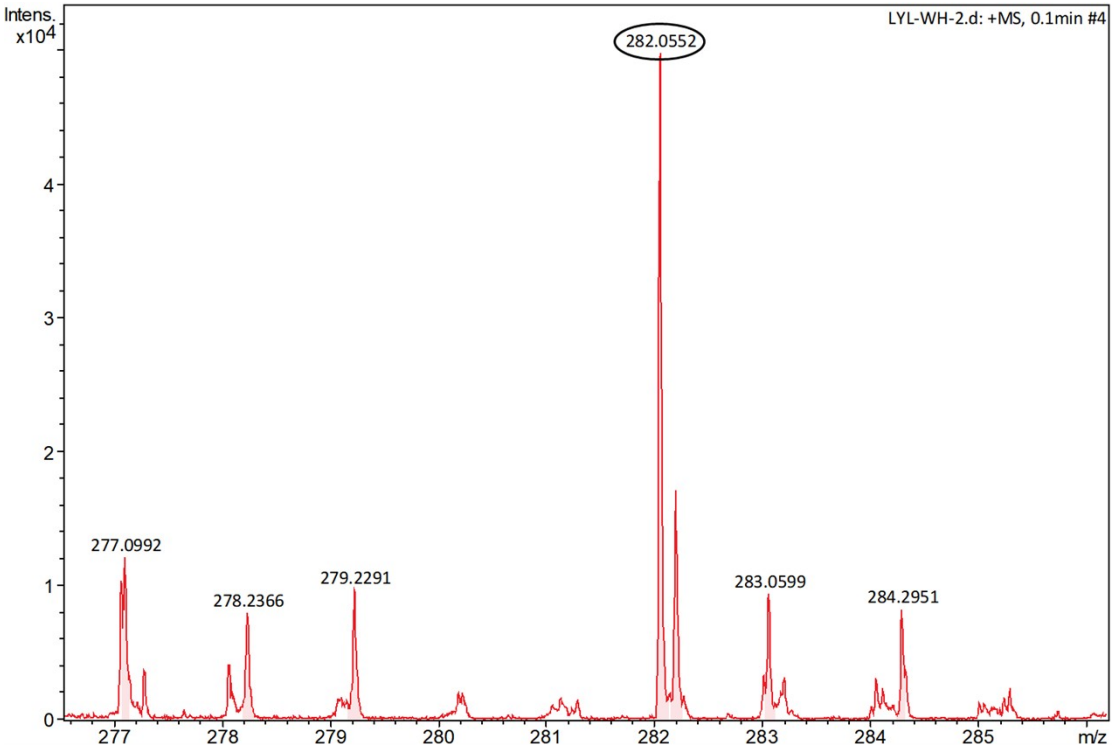

Fig. S13 HRMS spectrum of compound **2**.

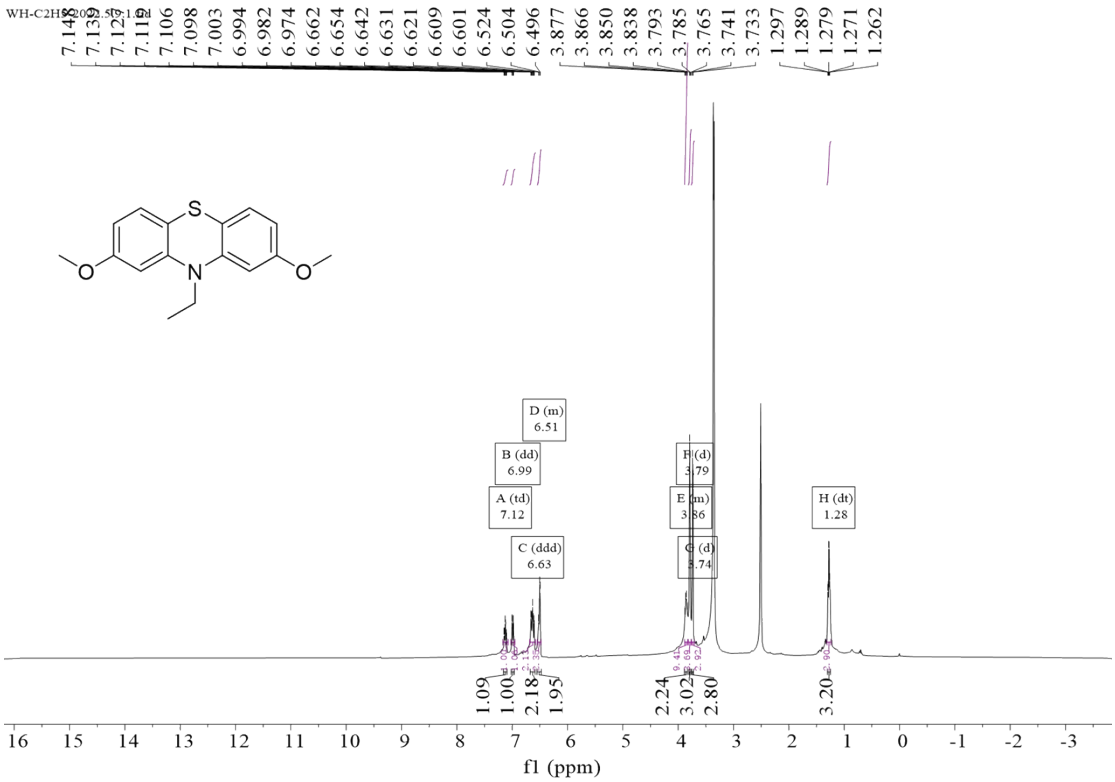

Fig. S14 [1]H NMR spectrum of compound **3** in DMSO-d_6.

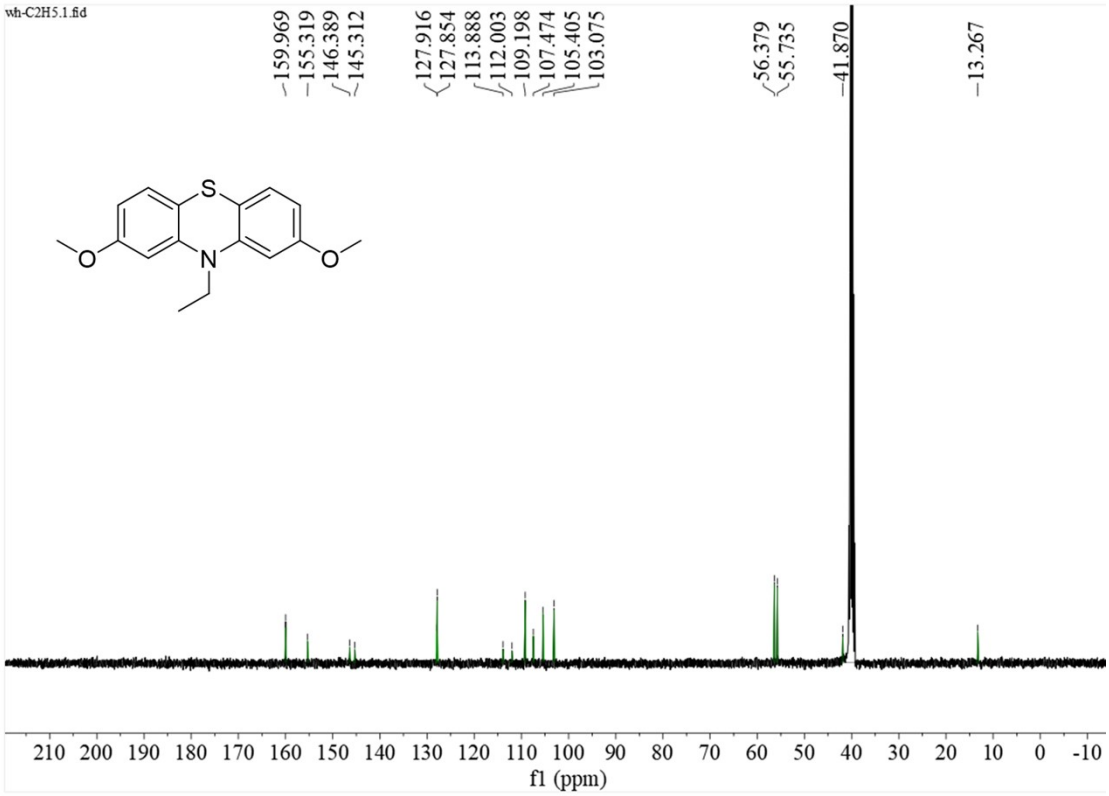

Fig. S15 [13]C NMR spectrum of compound **3** in DMSO-d_6.

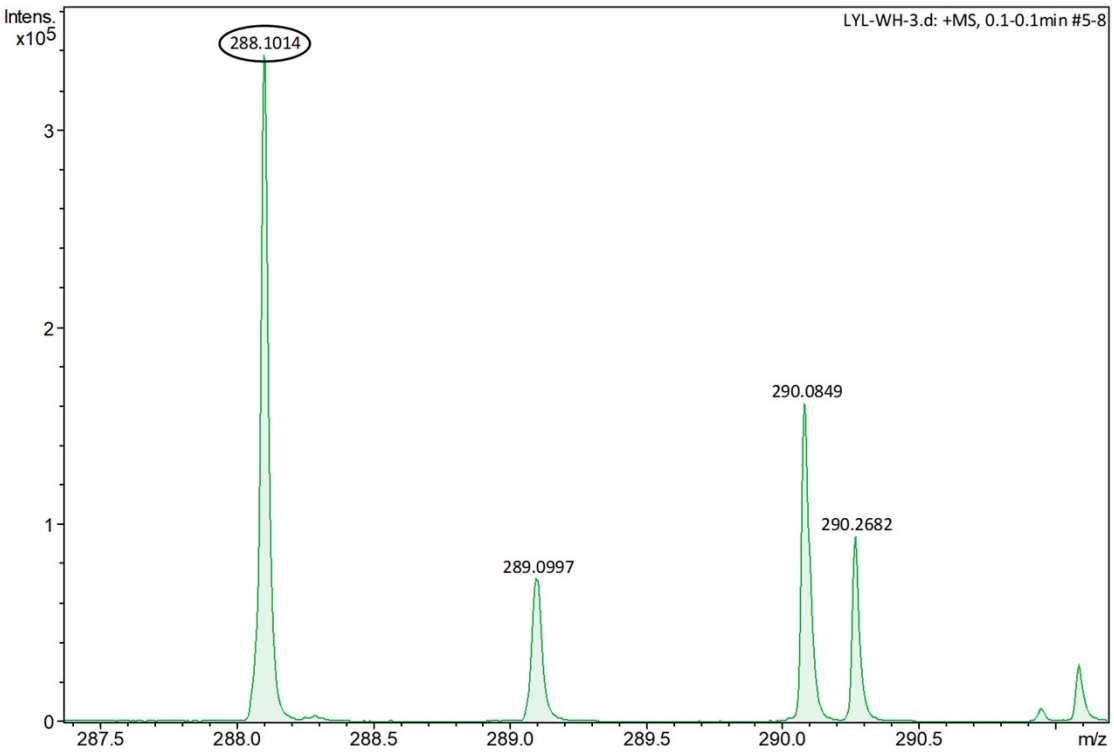

Fig. S16 HRMS spectrum of compound **3**.

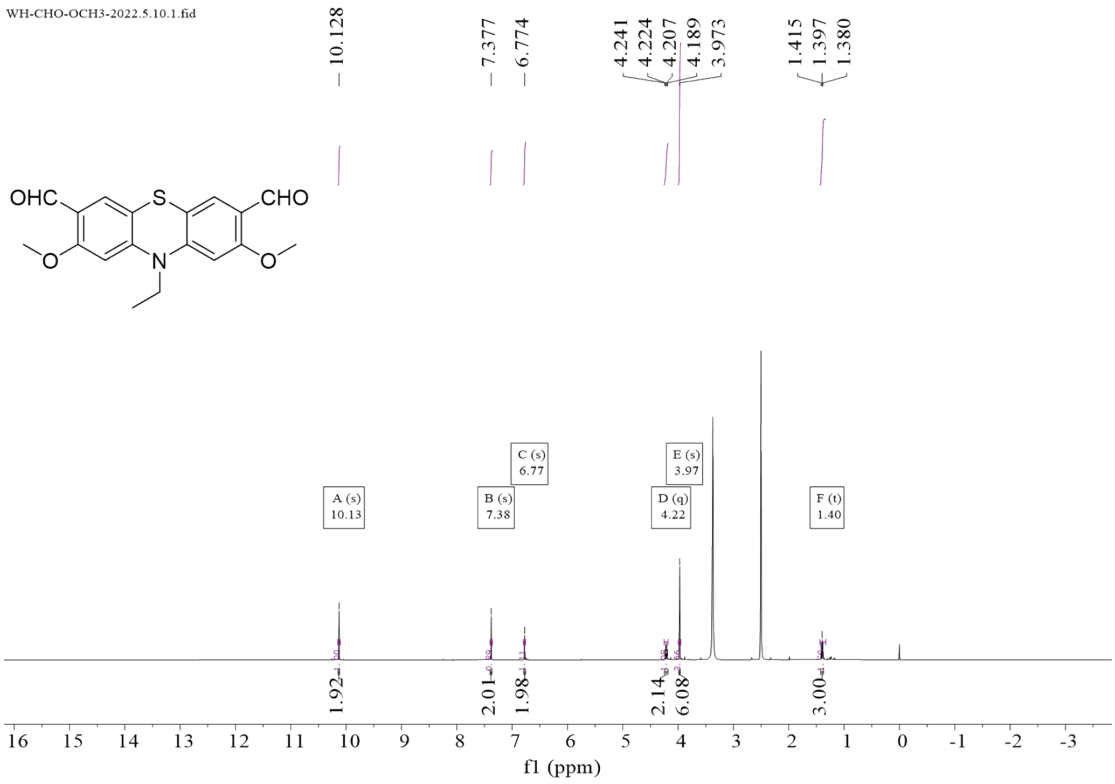

Fig. S17 ^{1}H NMR spectrum of compound **4** in DMSO-d_6.

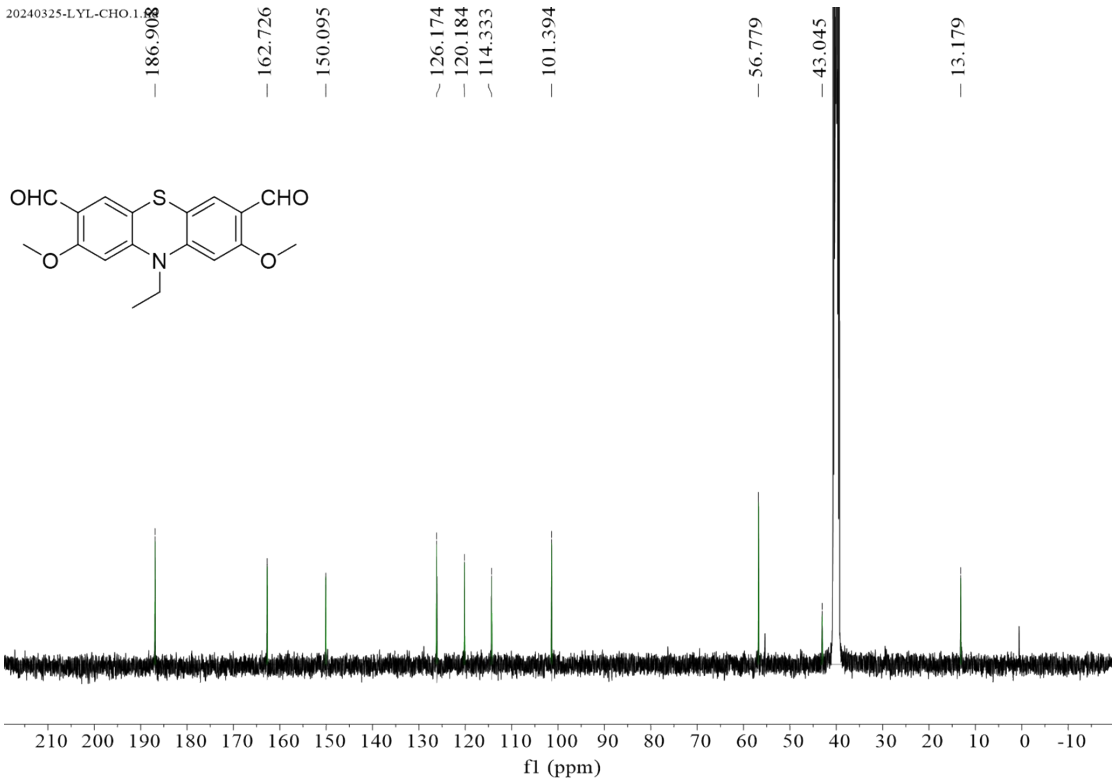

Fig. S18 ^{13}C NMR spectrum of compound **4** in DMSO-d_6.

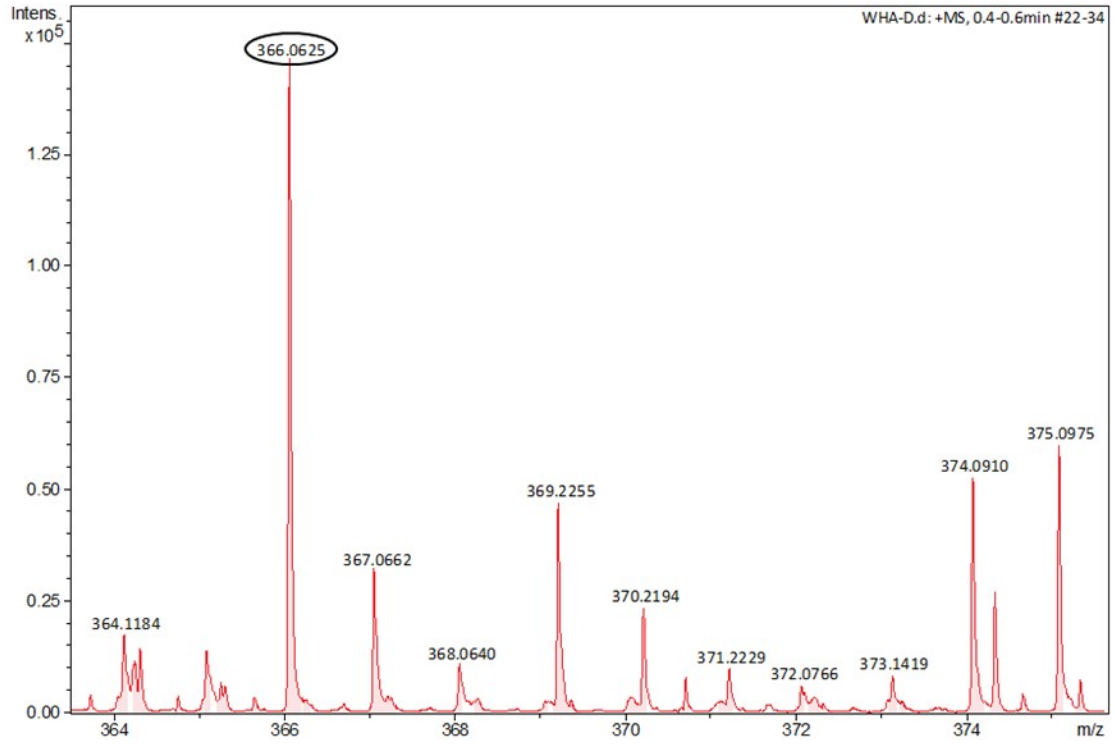

Fig. S19 HRMS spectrum of compound **4**.

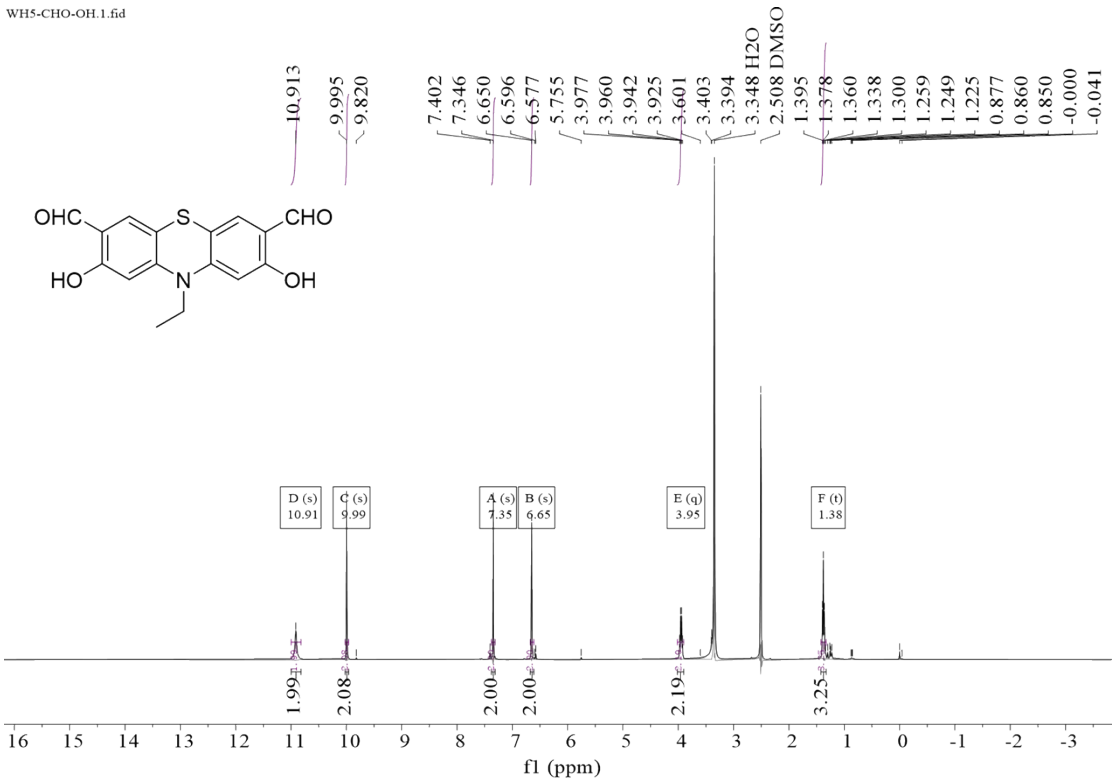

Fig. S20 ^{1}H NMR spectrum of compound **5** in DMSO-d_6.

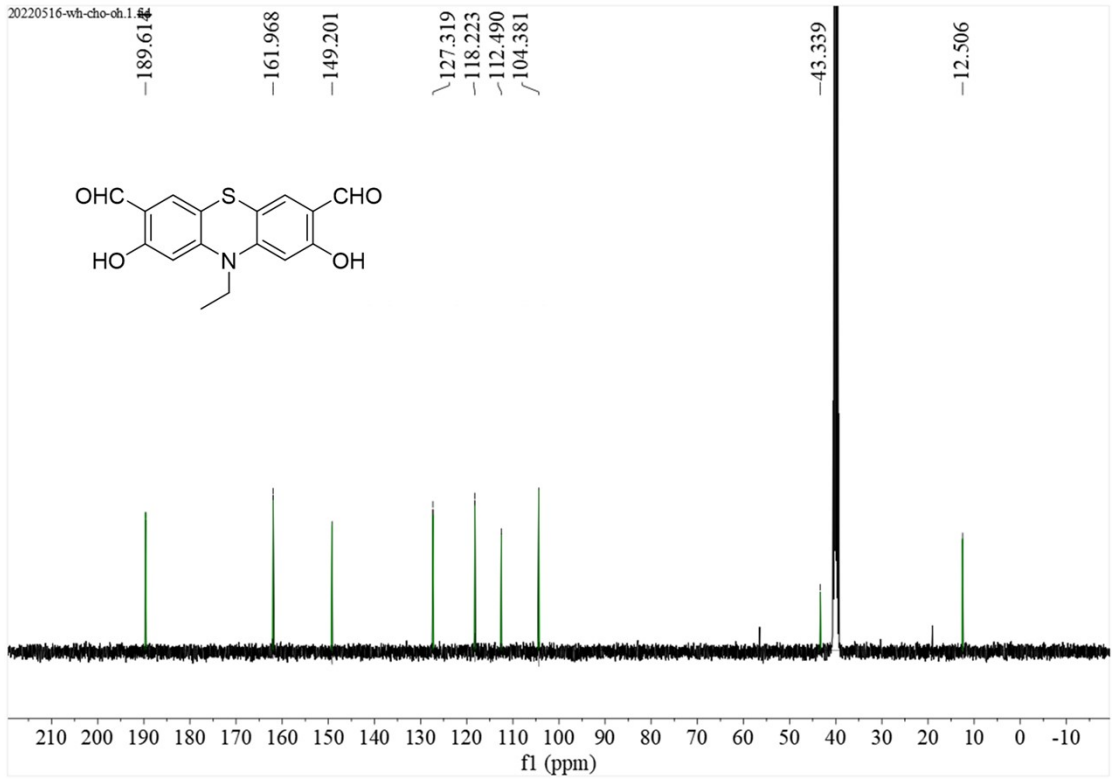

Fig. S21 ^{13}C NMR spectrum of compound **5** in DMSO-d_6.

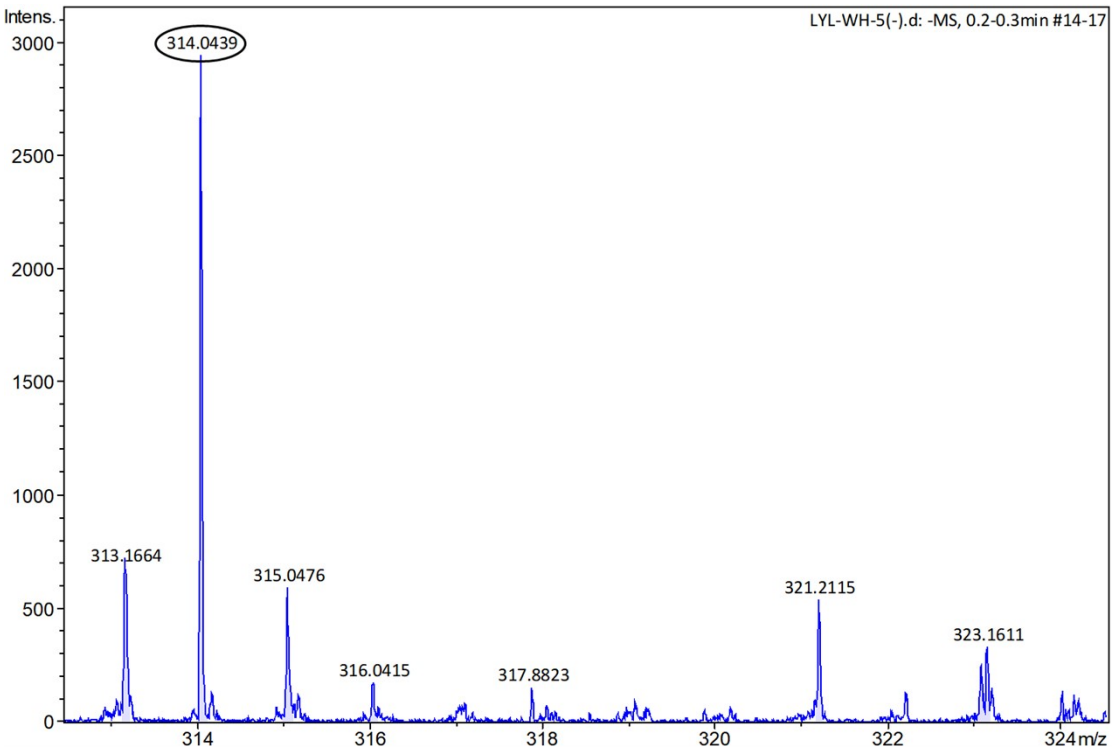

Fig. S22 HRMS spectrum of compound **5**.

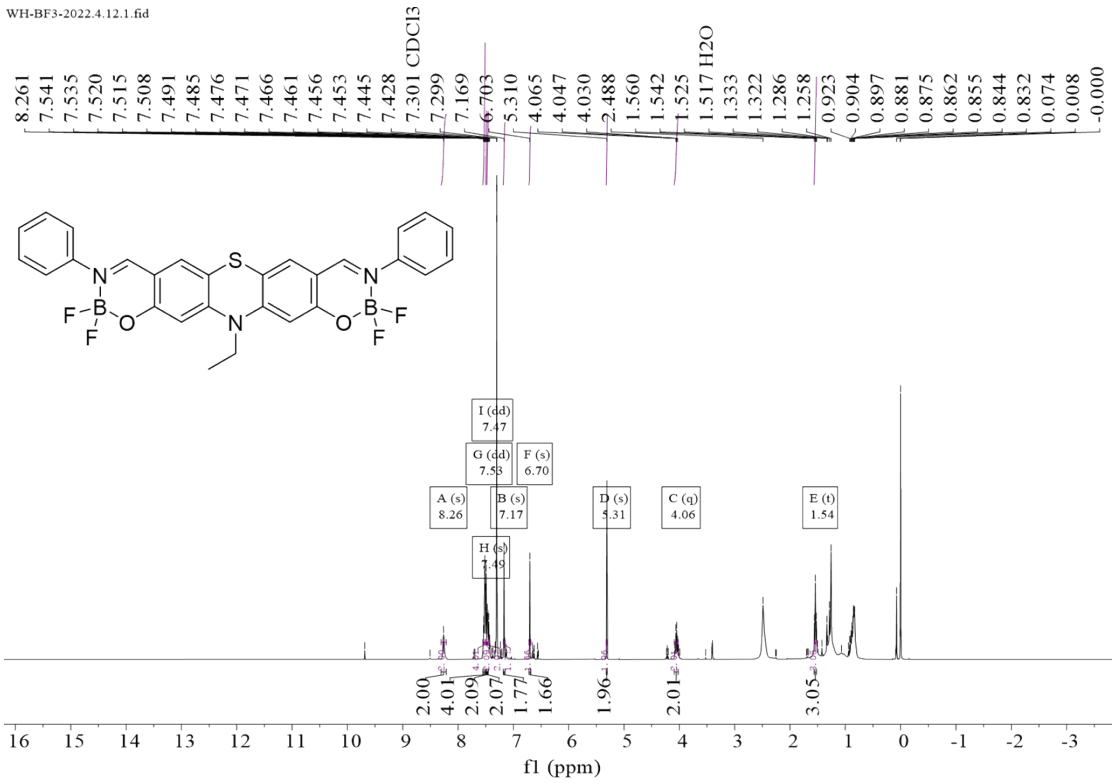

Fig. S23 ^{1}H NMR spectrum of **CSU-BF-H** in $CDCl_3$.

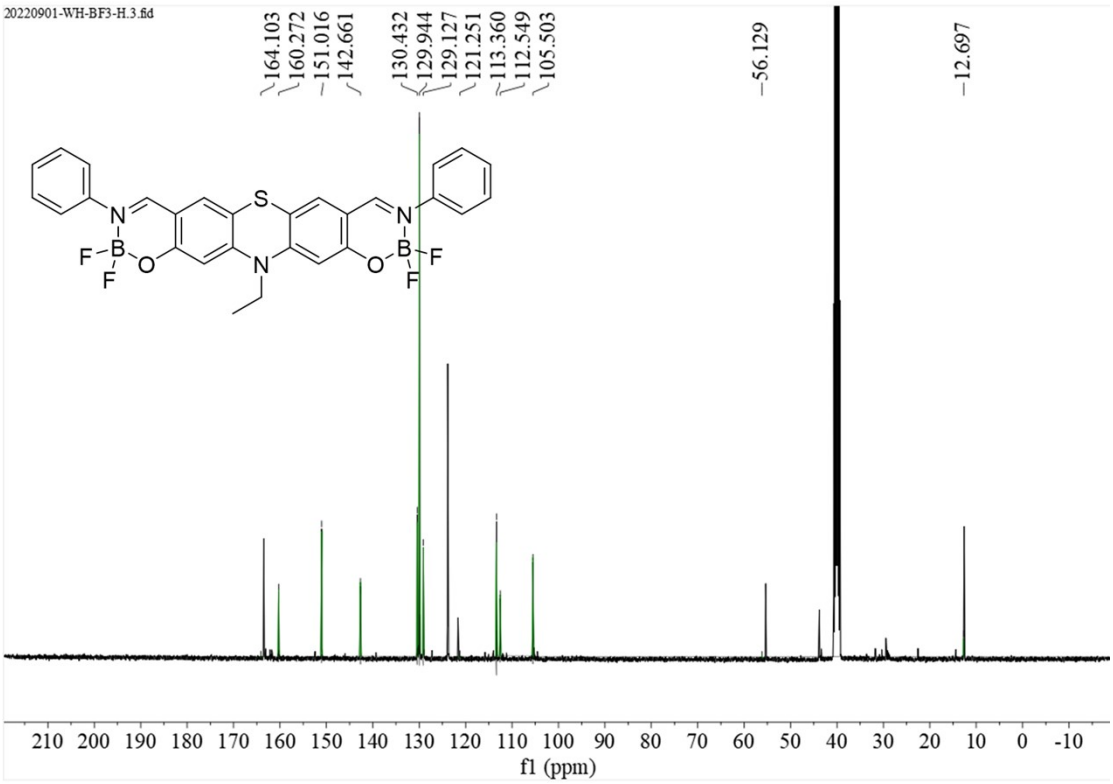

Fig. S24 ^{13}C NMR spectrum of **CSU-BF-H** in DMSO-d_6.

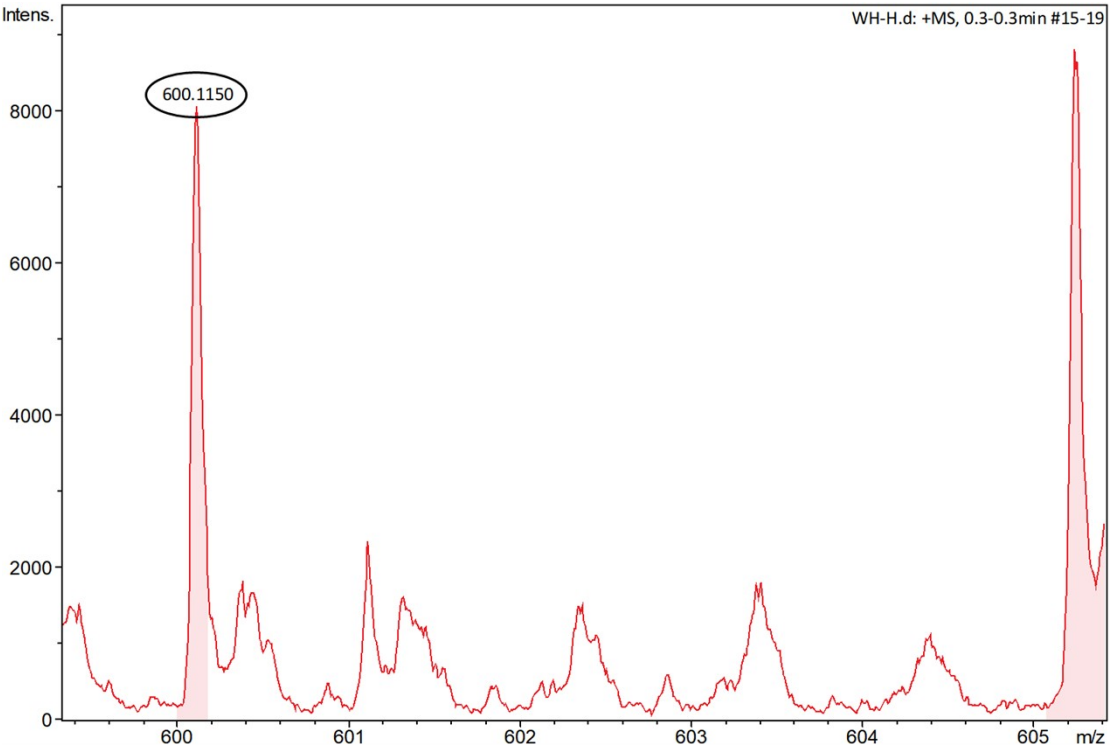

Fig. S25 HRMS spectrum of **CSU-BF-H**.

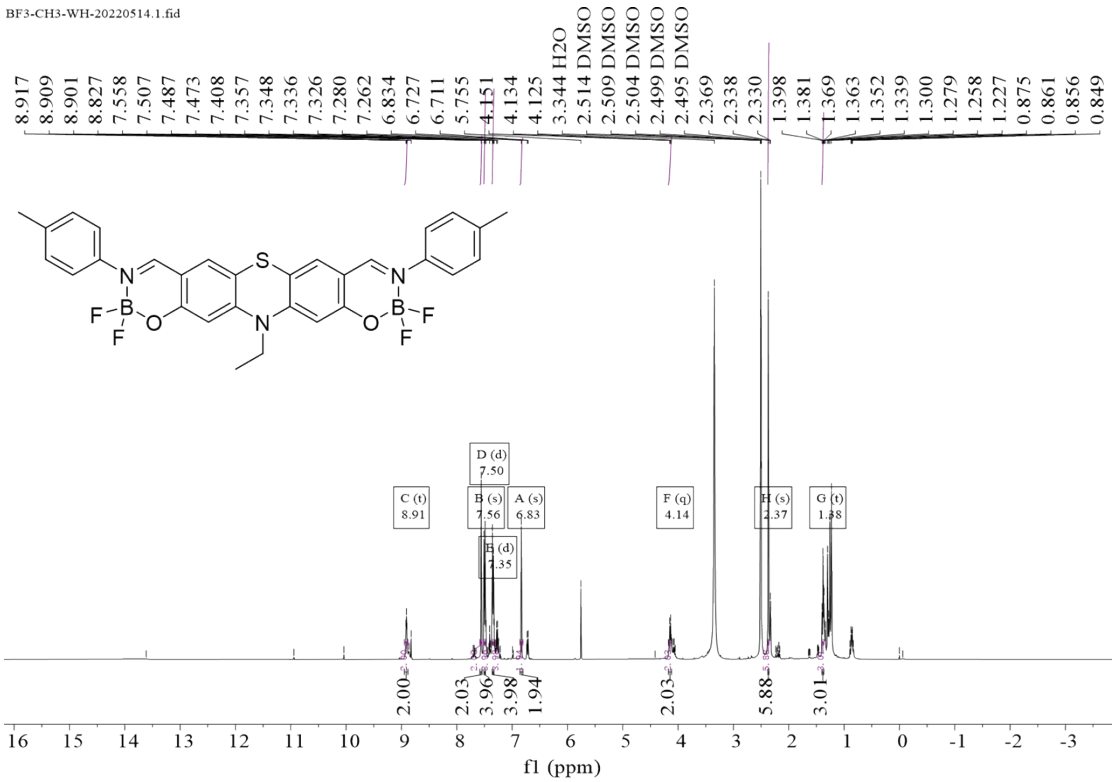

Fig. S26 [1]H NMR spectrum of **CSU-BF-CH$_3$** in DMSO-d_6.

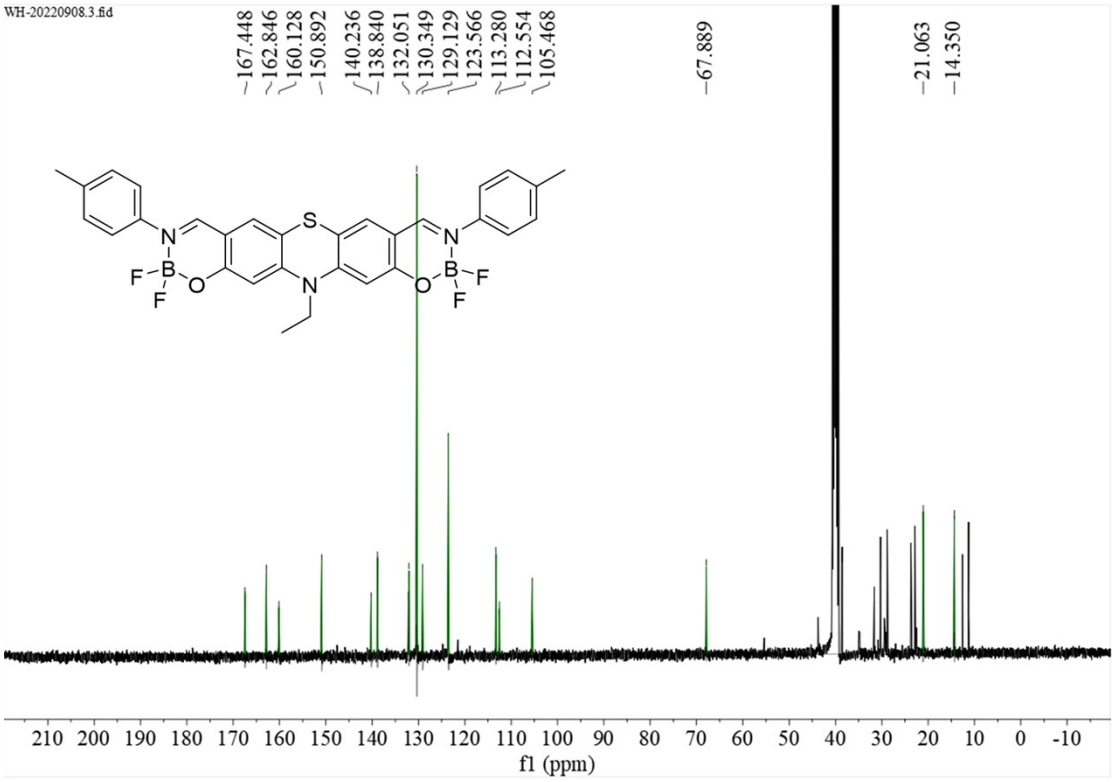

Fig. S27 [13]C NMR spectrum of **CSU-BF-CH$_3$** in DMSO-d_6.

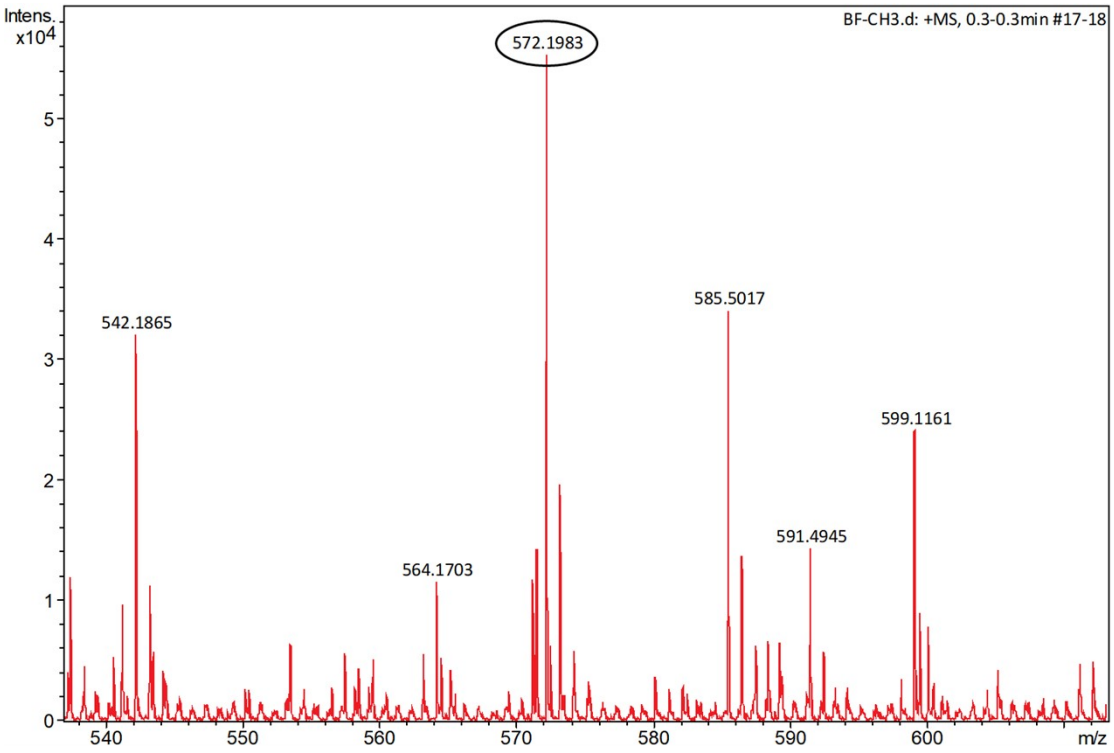

Fig. S28 HRMS spectrum of **CSU-BF-CH$_3$**.

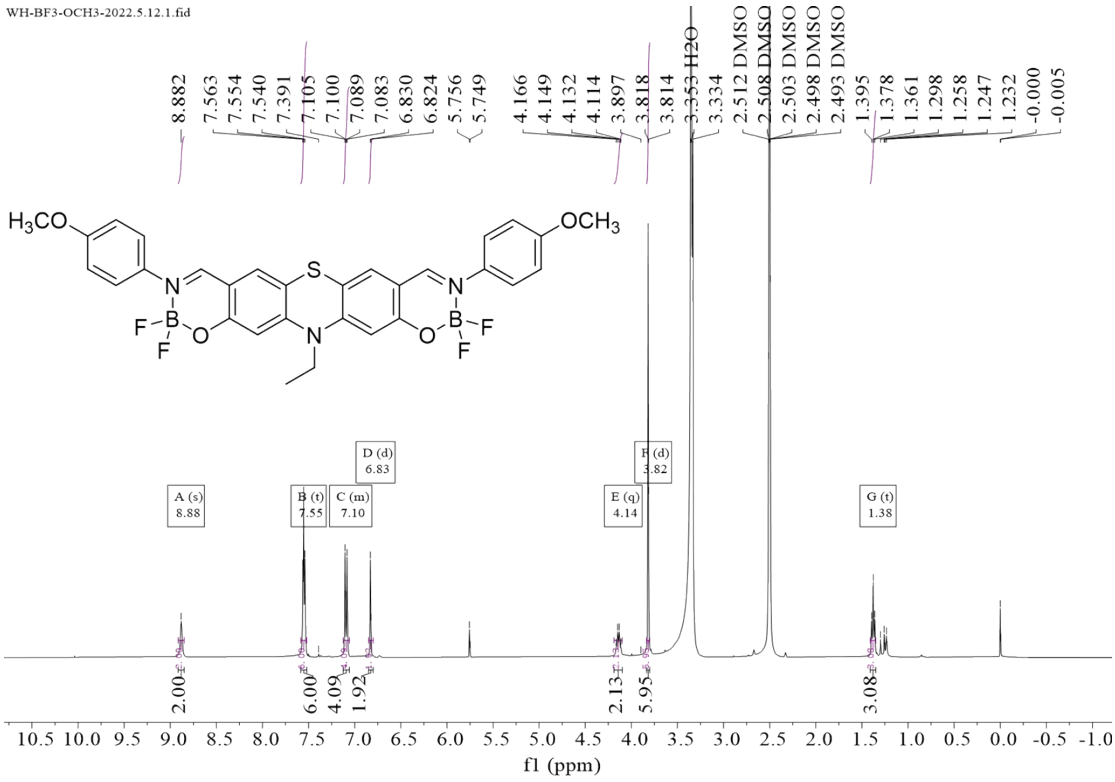

Fig. S29 ^{1}H NMR spectrum of **CSU-BF-OCH$_3$** in DMSO-d_6.

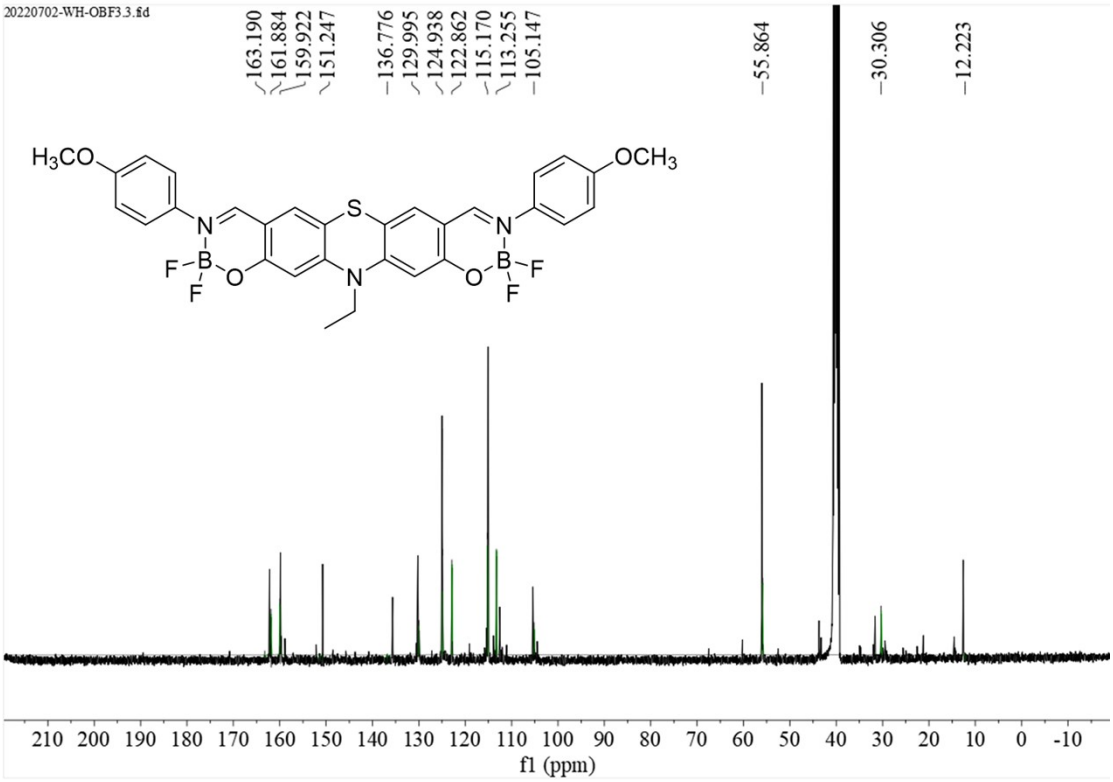

Fig. S30 ^{13}C NMR spectrum of **CSU-BF-OCH$_3$** in DMSO-d_6.

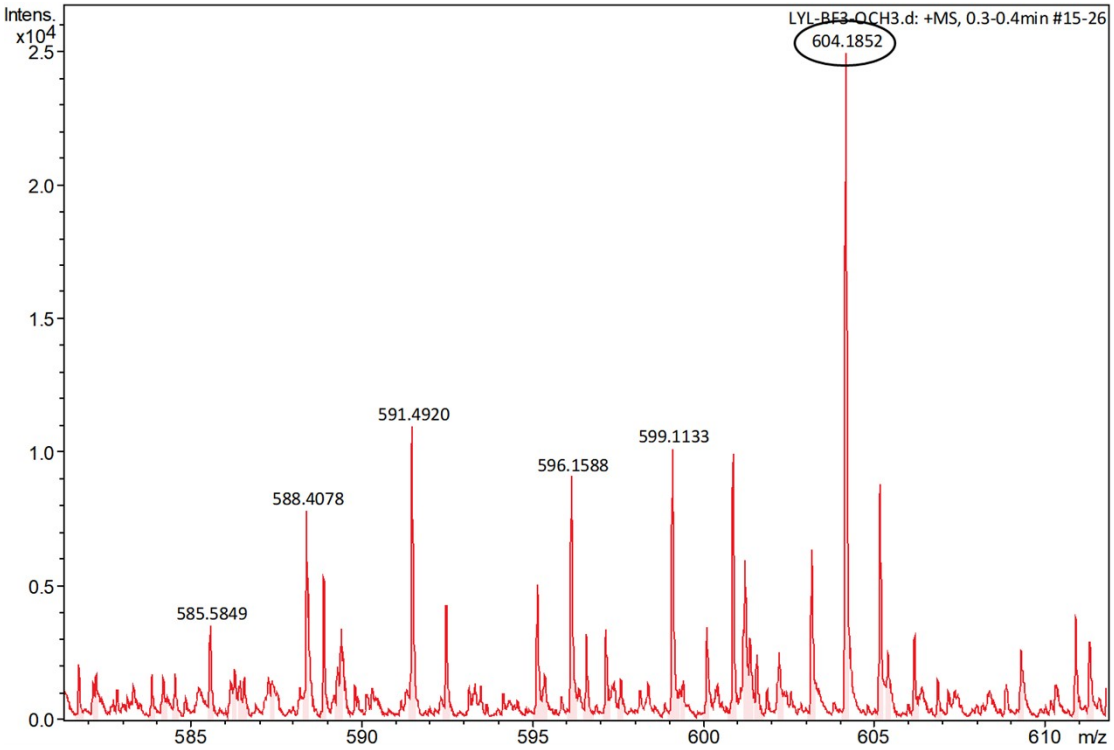

Fig. S31 HRMS spectrum of **CSU-BF-OCH$_3$**.