

ESIPT-based fluorescence probe for the ratiometric detection of superoxide

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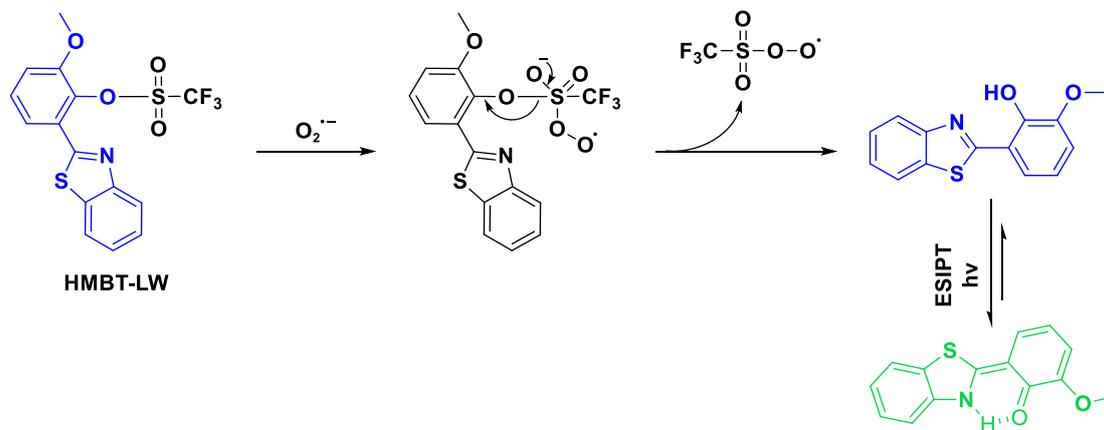
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1. Reaction mechanism



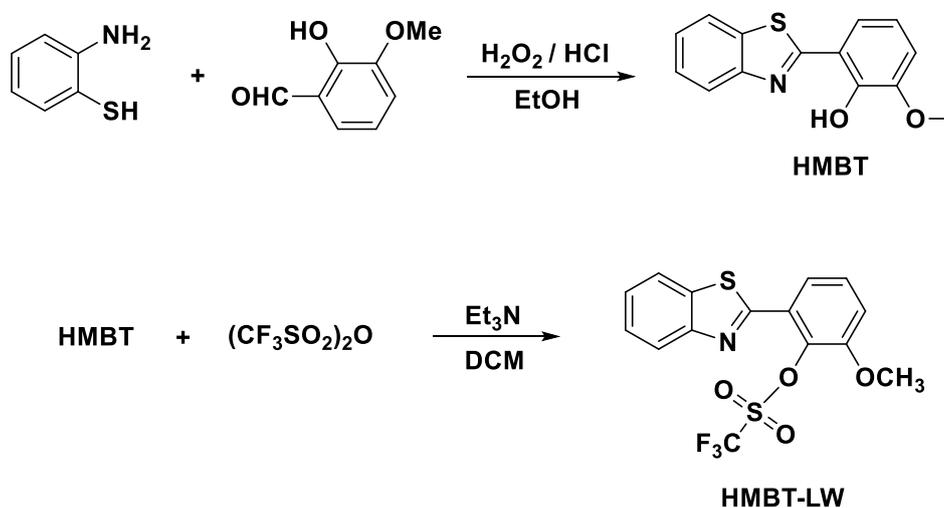
Scheme S1. Reaction mechanism of **HMBT-LW** with superoxide.

2. Experimental

2.1. Synthetic experiments: material and apparatus

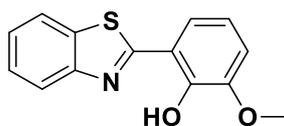
All chemical reagents and solvents were purchased from commercial sources and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel plates and visualized by UV. Column chromatography was performed using silica gel (Sigma-Aldrich) 200-400 mesh. ^1H , ^{13}C and ^{19}F NMR spectra were recorded employing a Bruker AV-500 spectrometer with chemical shifts expressed in parts per million (CDCl_3 , Me_4Si as internal standard). Electrospray ionization (ESI) mass spectrometry was performed in a Bruker MicrTOF spectrometer.

2.2. Synthetic experiments: synthetic procedures



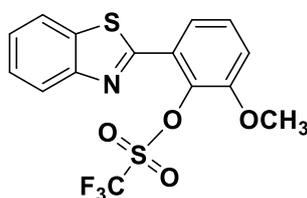
Scheme S2. Synthesis of target probe HMBT-LW.

2-(Benzo[d]thiazol-2-yl)-6-methoxyphenol (HMBT).



HMBT was synthesized according to literature procedures.¹ A solution of 2-aminothiophenol (0.90 mL, 12.6 mmol) and *o*-vanillin (1.44 g, 9.45 mmol) in EtOH (30 mL), aq H₂O₂ (30%, 56.8 mmol) and aq HCl (32% HCl, 28.35 mmol) was stirred at room temperature for 2 h. The solution was quenched by 35 mL H₂O. The precipitate was filtered, dried and recrystallized from EtOH to afford the title compound as a light yellow solid (2.20 g, 68%). M.p 158-165 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.02 (d, *J* = 8.1 Hz, 1H), 7.94 – 7.87 (m, 1H), 7.52 (ddd, *J* = 8.3, 7.4, 1.2 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.35 (dd, *J* = 7.9, 1.3 Hz, 1H), 6.99 (dt, *J* = 7.8, 3.9 Hz, 1H), 6.94 – 6.89 (m, 1H), 3.97 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 169.31 (s), 151.66 (s), 148.96 (s), 148.22 (s), 132.63 (s), 126.64 (s), 125.50 (s), 122.17 (s), 121.42 (s), 119.96 (s), 119.09 (s), 116.77 (s), 114.07 (s), 56.22 (s). HRMS (ES⁺): calc. for C₁₄H₁₁NO₂S [M+Na]⁺ 280.0403, found 280.0435.

2-(Benzo[d]thiazol-2-yl)-6-methoxyphenyl trifluoromethanesulfonate (HMBT-LW).



To a solution of **HMBT** (77.1 mg, 0.3 mmol) in dry CH₂Cl₂ (10 mL) at -78 °C was added trifluoromethanesulfonic anhydride (240 μL) dropwise under an argon atmosphere. The resulting mixture was stirred at -78 °C for 10 min and then triethylamine (150 μL) was added to the solution and stirred for 30 min at room temperature. The solvent was removed under vacuum and the residues were purified through silica column chromatography to obtain **HMBT-LW** as a pale-yellow solid. M.p 77-80 °C. ¹H NMR (500 MHz, CDCl₃) δ 8.16 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 8.4 Hz, 1H), 7.57 – 7.50 (m, 2H), 7.47 – 7.40 (m, 2H), 7.16 (dd, *J* = 8.3, 1.5 Hz, 1H), 3.97 (s, 3H). ¹³C NMR (125 MHz, CDCl₃) δ 161.62 (s), 153.58 (s), 152.29 (s), 136.25 (s), 135.59 (s), 128.86 (s), 128.51 (s), 126.53 (s), 125.81 (s), 123.90 (s), 122.87 (s), 122.27 (s), 121.45 (s), 119.72 (s), 117.17 (s), 114.70 (s), 114.62 (s), 56.47 (s). ¹⁹F NMR (470 MHz, CDCl₃) δ -73.72 (s).

3. Generation of various ROS

ROO•

ROO• was generated from 2,2'-azobis (2-amidinopropane) dihydrochloride. AAPH (2, 2' azobis (2-amidinopropane) dihydrochloride, 1 M) was added into deionizer water, and then stirred at 37 °C for 30min.

•O₂⁻

Superoxide was generated from KO₂. KO₂ and 18-crown-6 ether was dissolved in DMSO to afford a KO₂ stock solution.

•HO

Hydroxyl radical was generated by the Fenton reaction. To prepare •OH solution, hydrogen peroxide (H₂O₂, 10 eq) was added to Fe(ClO₄)₂ in deionised water.

¹O₂: NaMoO₄ (20 mM) and H₂O₂ (20 mM) were prepared in deionized water. Equal aliquots of these two solutions were then mixed to yield ¹O₂ of 10 mM.

ONOO⁻

Simultaneously, 0.6 M KNO₂, 0.6 M in HCl, 0.7 M in H₂O₂ was added at to a 3 M NaOH solution at 0 °C. The concentration of peroxynitrite was estimated by using extinction co-efficient of 1670 cm⁻¹ M⁻¹ at 302 nm in 0.5 M sodium hydroxide aqueous solutions.

•OCl

The concentration of •OCl was determined from the absorption at 292 nm ($\epsilon = 350 \text{ M}^{-1} \text{ cm}^{-1}$).

H₂O₂

The concentration of H₂O₂ was determined from the absorption at 240 nm ($\epsilon = 43.6 \text{ M}^{-1} \text{ cm}^{-1}$).

4. UV-Vis and fluorescence analysis of HMBT-LW

HMBT-LW was made into a 5 mM stock solution in anhydrous DMSO. Superoxide anion was prepared by dissolving KO_2 in DMSO with the help of 18-crown-6. In titration experiments, different volumes of KO_2 diluents were added to DMSO (1.5 mL) containing 3 μl of **HMBT-LW** stock solution, and the corresponding volumes were made up to 3 ml in cuvette.

The fluorescence quantum yield was determined on an Absolute PL Quantum Yield Spectrometer (HAMAMATSU C11347). The PL Quantum Yield (Φ) determined is expressed as the ratio of the number of photons emitted from molecules (PN_{em}) to that absorbed by molecules (PN_{abs}).

$$\Phi = \text{PN}_{em} / \text{PN}_{abs}$$

The fluorescence quantum yield of 2-(2'-hydroxy-3'-methoxyphenyl)benzothiazole (**HMBT**) 5 μM was 0.508 in PBS buffer solution (10 mM, V/V, DMSO/PBS = 1/1, pH = 7.4).

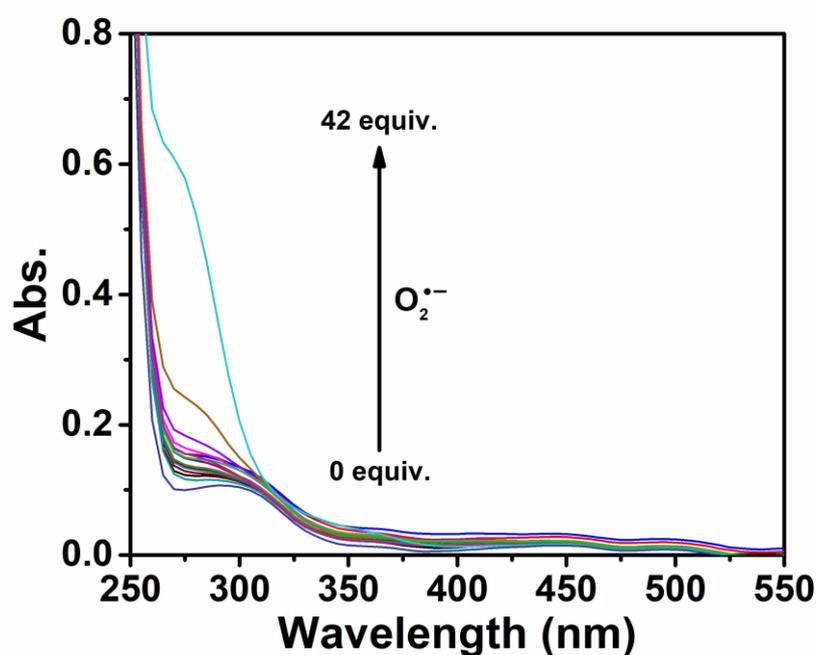


Figure S1. UV spectra of **HMBT-LW** (5 μM), with (42 equiv.) and without $\text{O}_2^{\bullet-}$ in PBS buffer solution (10 mM, V/V, DMSO/PBS = 1/1, pH = 7.4).

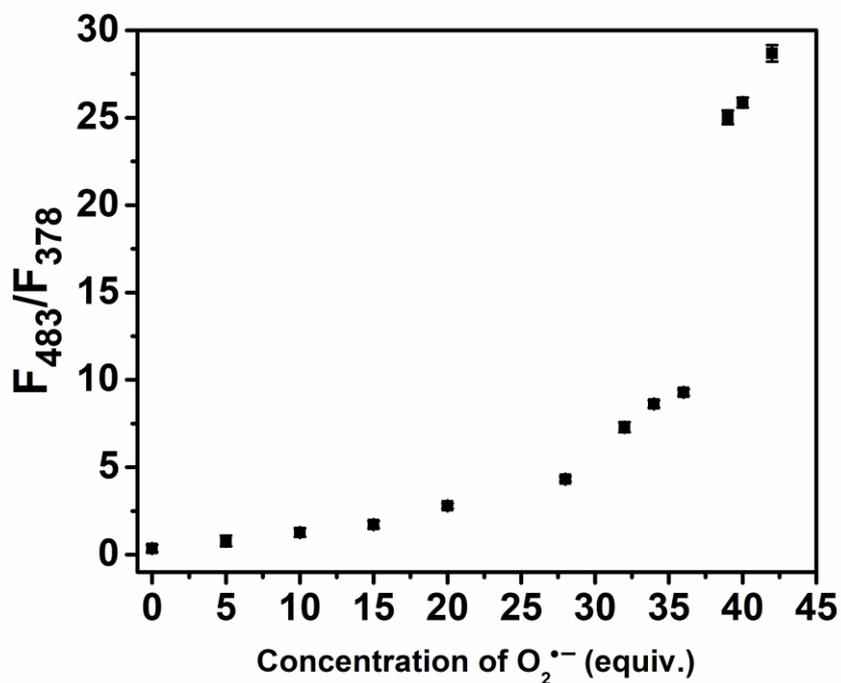


Figure S2. A plot of fluorescence intensity ratio changes (based on the peak heights at the maxima, 378 and 483 nm respectively) depending on O₂^{•-} concentration. **HMBT-LW** (5 μM) in PBS buffer solution (10 mM, V/V, DMSO/PBS = 1/1, pH = 7.4). Error bar represents s.d. λ_{ex} = 310 nm. Slit widths: ex = 8 nm, em = 5 nm.

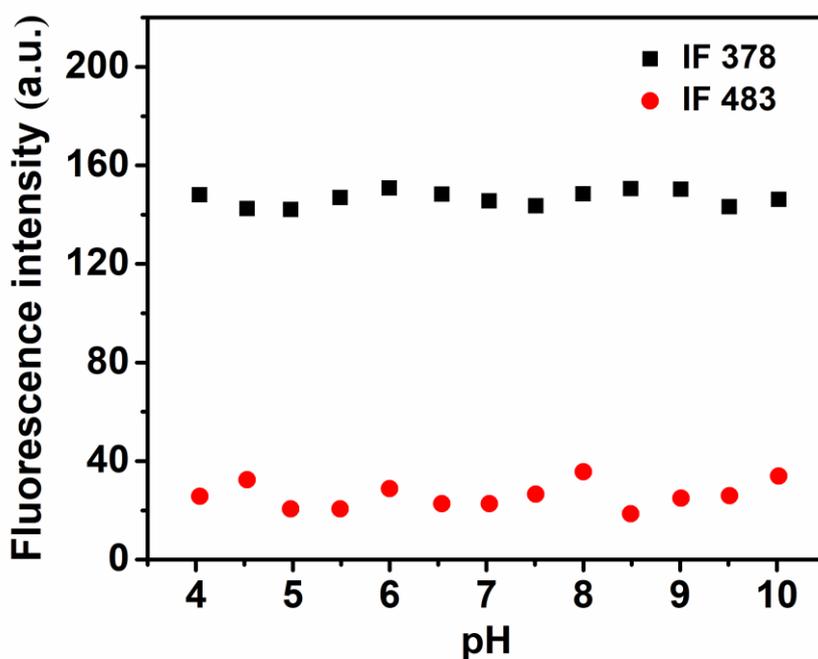


Figure S3. pH effect on the fluorescence intensity of **HMBT-LW** (5 μM) at 378 nm (in black) and 483 nm (in red) in solution (V/V, DMSO/water = 1/1). λ_{ex} = 310 nm. Slit widths: ex = 8 nm, em = 5 nm.

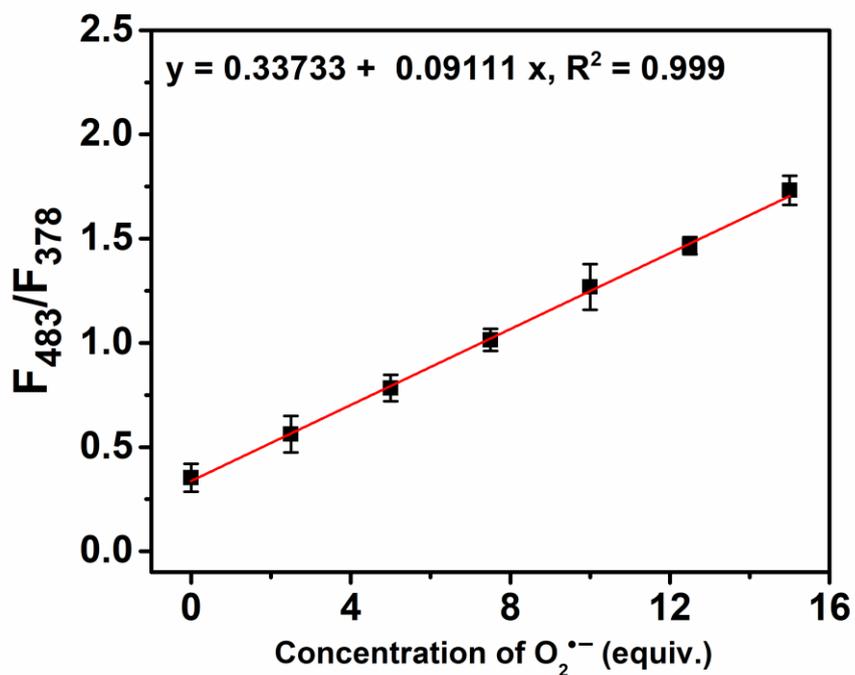


Figure S4. Fluorescence intensity versus concentration of $O_2^{\bullet-}$ for the calculation of the limit of detection for **HMBT-LW** ($5 \mu\text{M}$) in PBS buffer solution (10 mM, V/V, DMSO/PBS = 1/1, pH = 7.4) at 25 °C. Fluorescence intensities were measured with $\lambda_{\text{ex}} = 310 \text{ nm}$. Error bar represents s.d. The LOD is $7.4 \mu\text{M}$. Slit widths: $\text{ex} = 8 \text{ nm}$, $\text{em} = 5 \text{ nm}$.

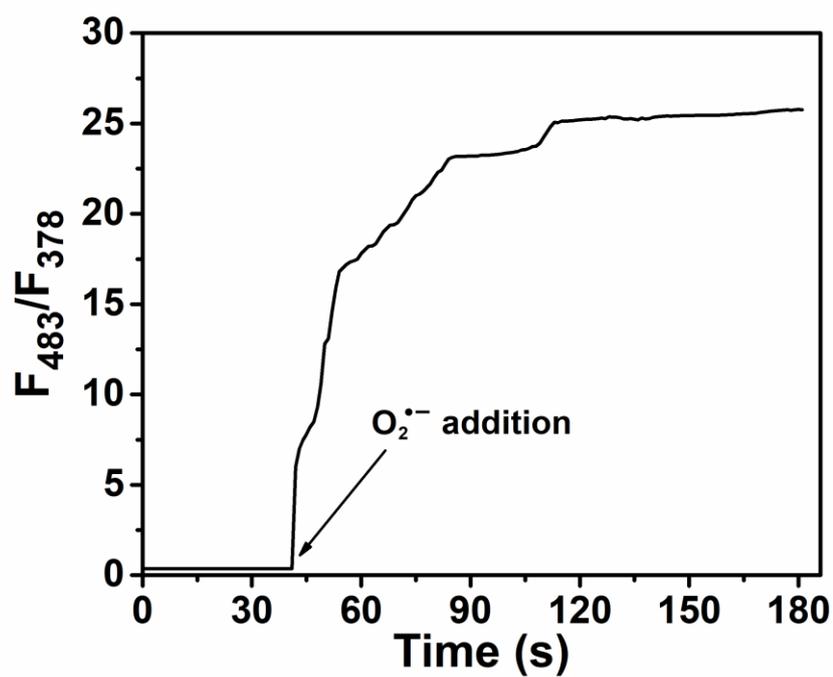


Figure S5. Time-dependent fluorescence ratio changes of **HMBT-LW** (5 μ M) towards 39 equiv. of superoxide anion in PBS buffer solution (10 mM, V/V, DMSO/PBS = 1/1, pH = 7.4) at 25 $^{\circ}$ C. λ_{ex} = 310 nm. Slit widths: ex = 8 nm, em = 5 nm.

5. Mass spec analysis of HMBT-LW

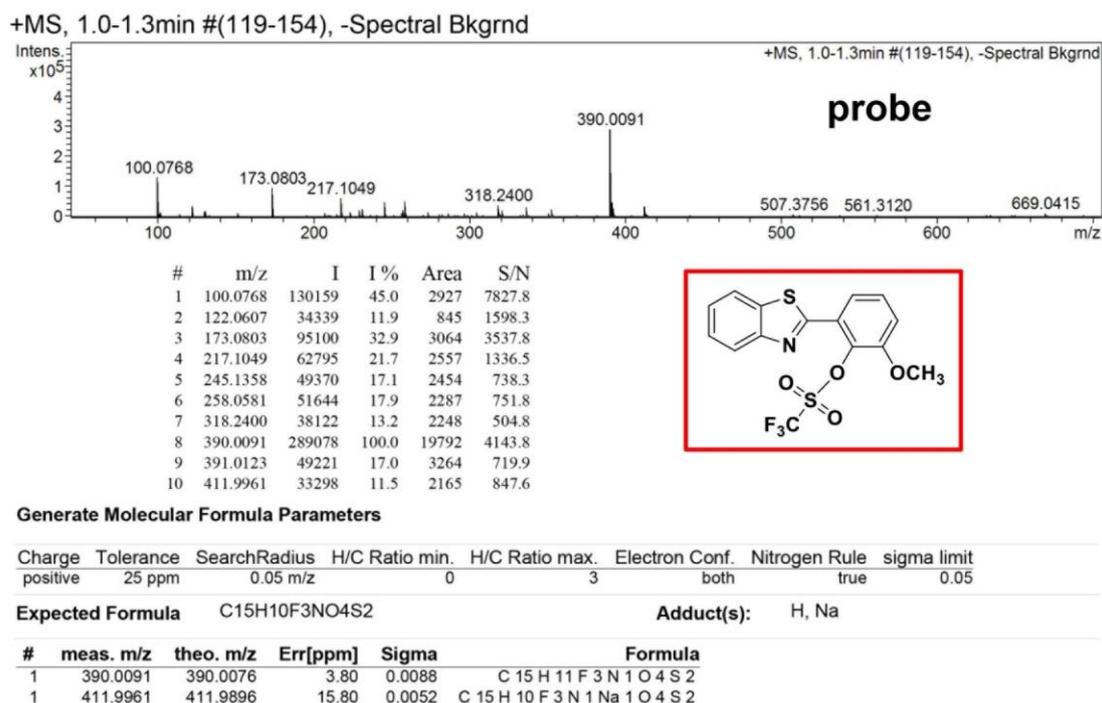


Figure S6. HRMS spectrum of HMBT-LW before the addition of superoxide.

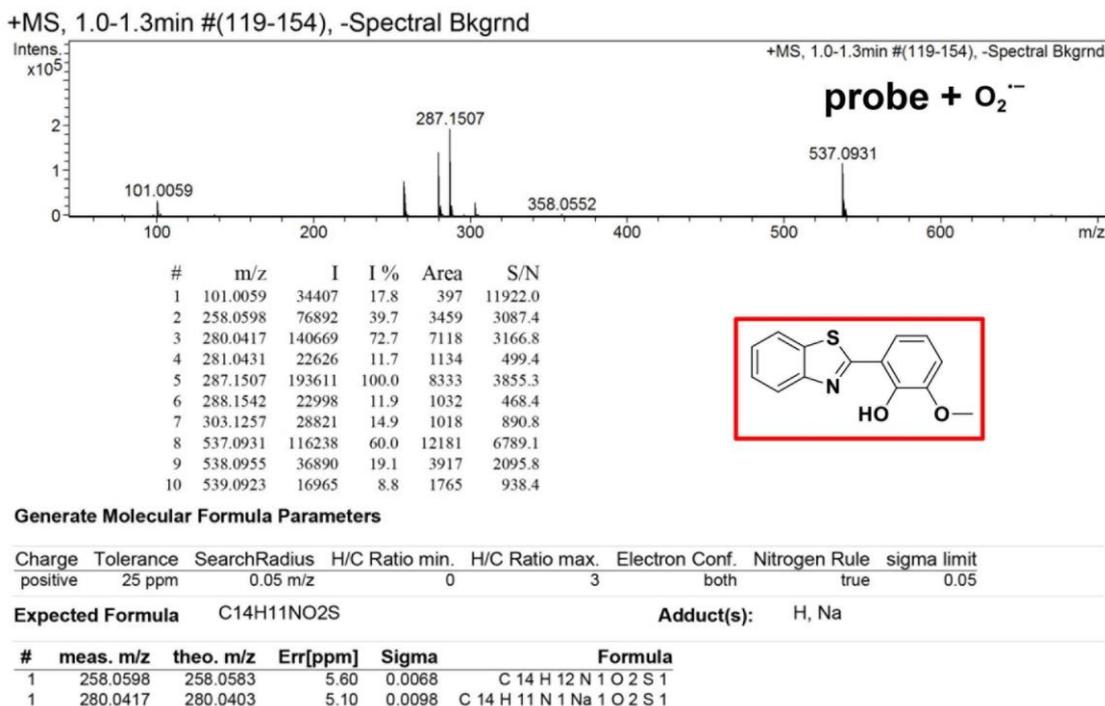


Figure S7. HRMS spectrum of HMBT-LW + O₂^{•-}.

6. NMR spectrum

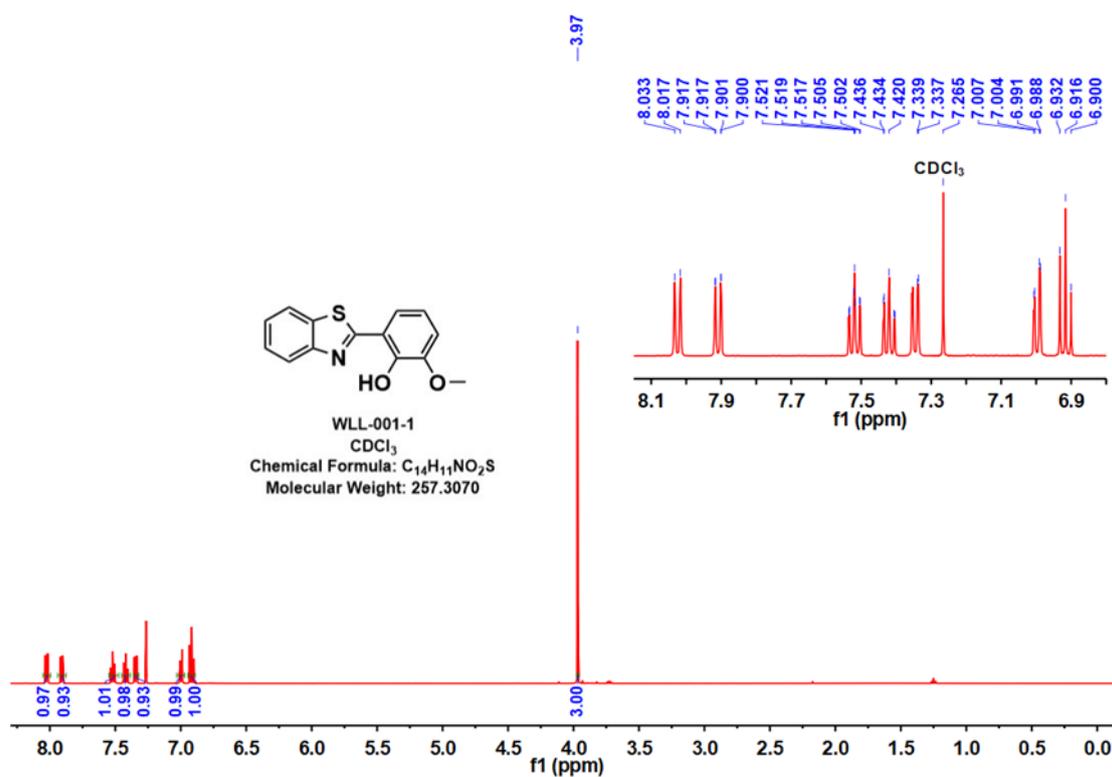


Figure S8. ¹H NMR of probe HMBT.

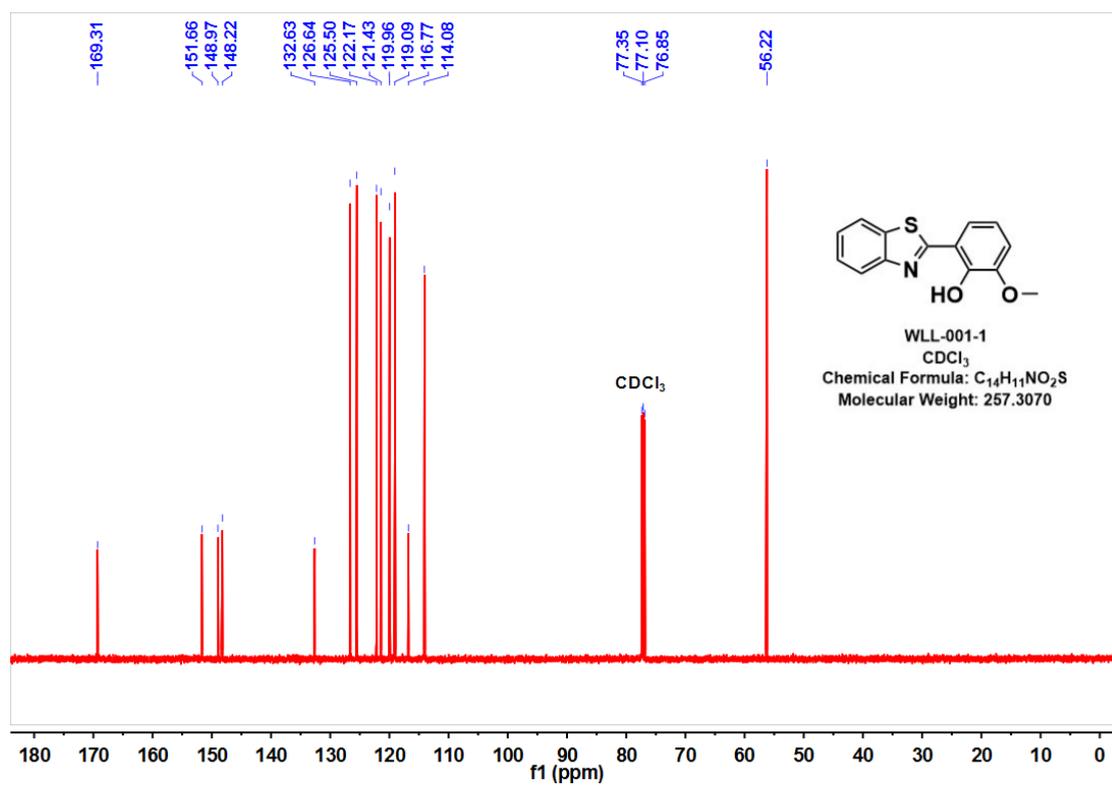


Figure S9. ¹³C NMR of probe HMBT.

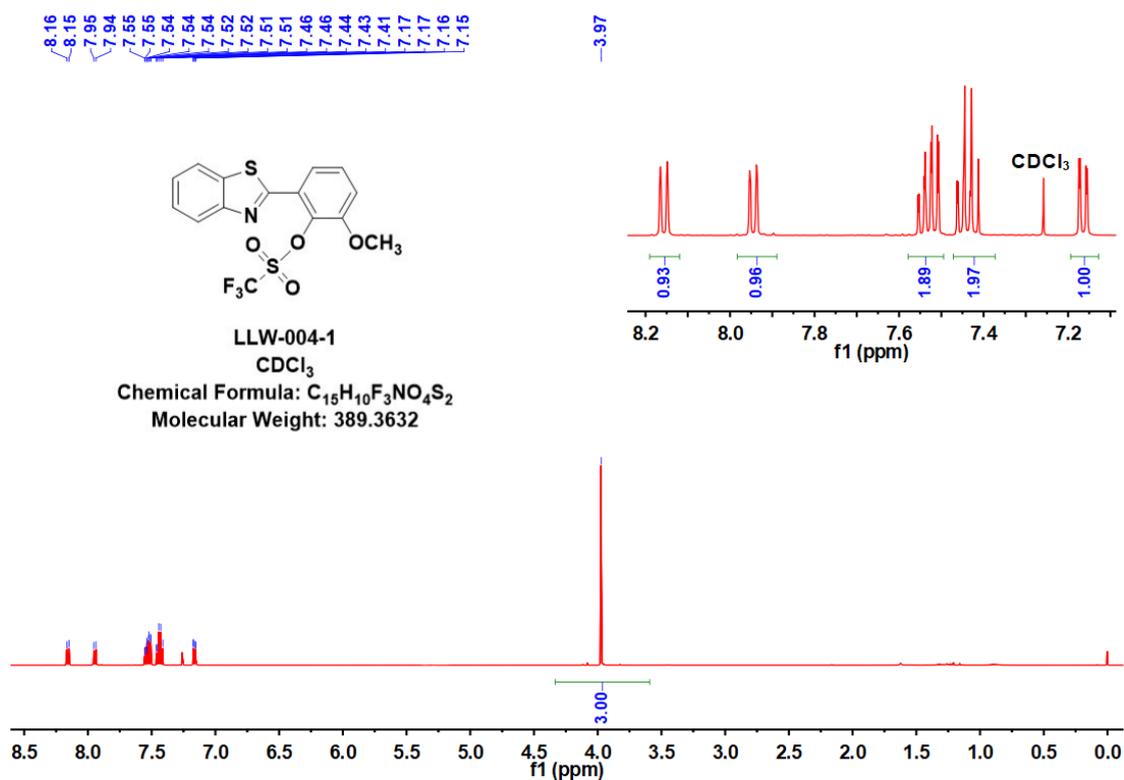


Figure S10. ¹H NMR of probe HMBT-LW.

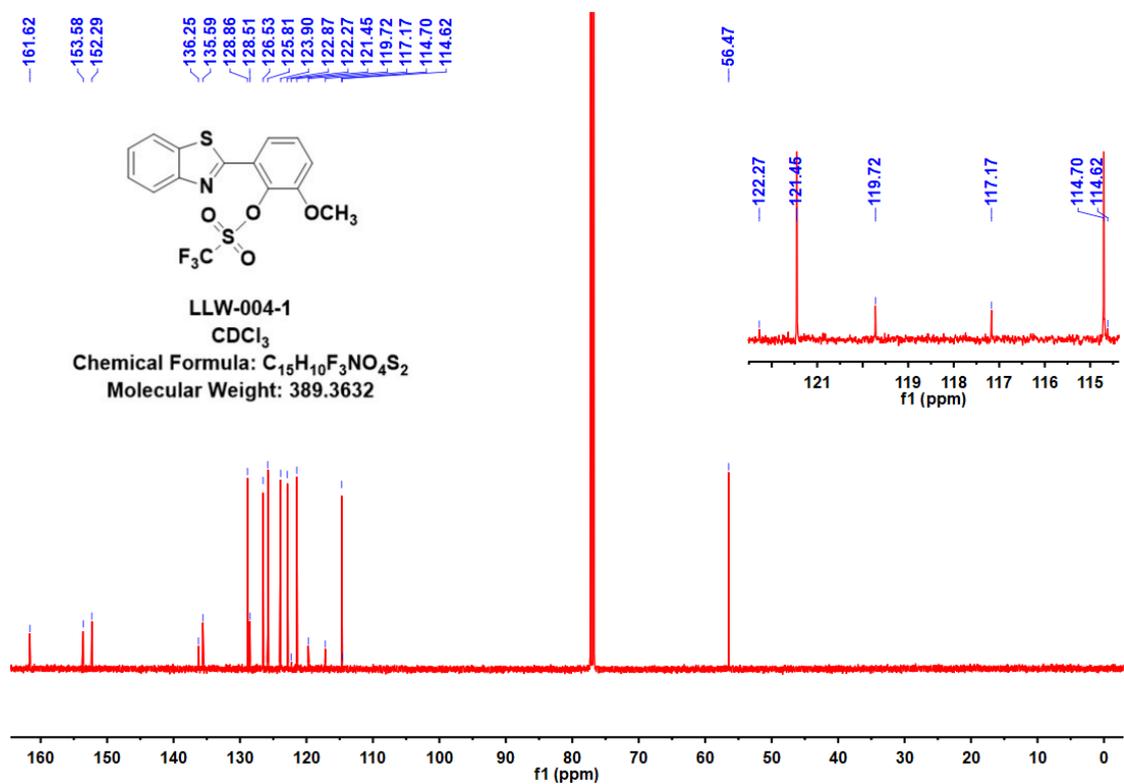


Figure S11. ¹³C NMR of probe HMBT-LW.

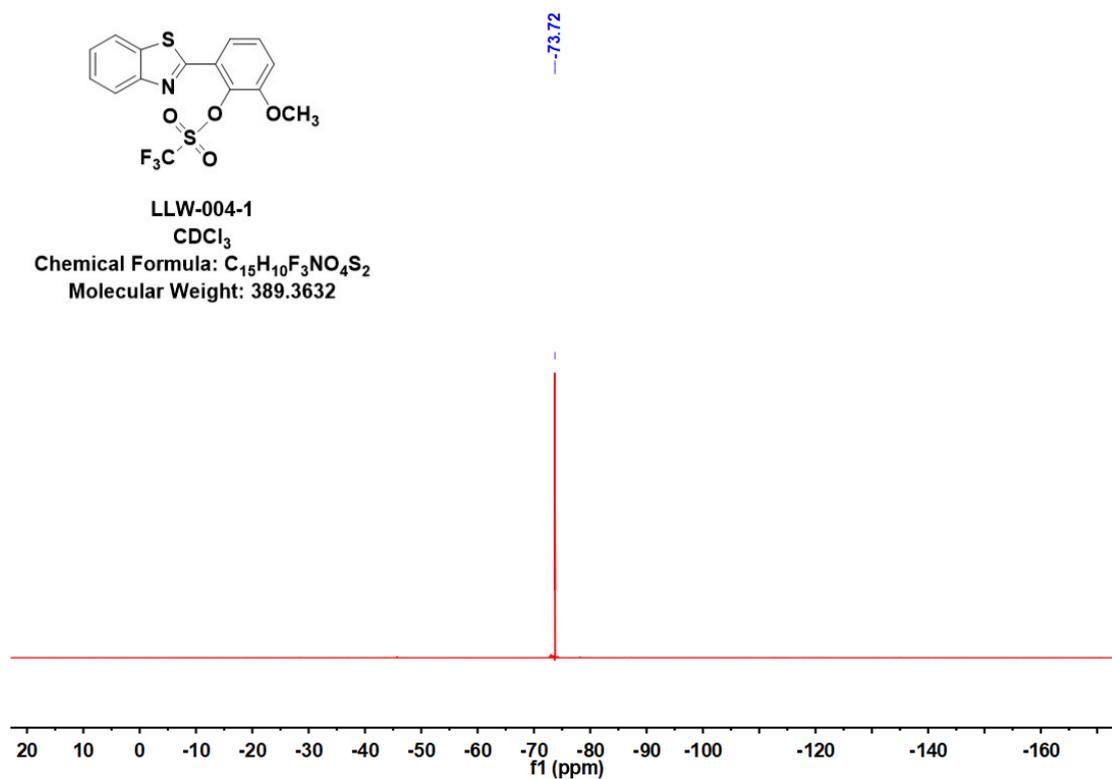


Figure S12. ¹⁹F NMR of probe **HMBT-LW**.

7. References

1. X. Yang, Y. Guo and R. M. Strongin, *Angew. Chem. Int. Ed.*, 2011, **50**, 10690-10693.