Supplementary Information

ESIPT-based fluorescence probe for the rapid detection of hypochlorite (HOCl/ClO$^-$)

Luling Wu[a], Qingye Yang[b], Liyuan Liu[a], Adam C. Sedgwick[a]*, Alexander J. Cresswell[a]*, Steven D. Bull[a]*, Chusen Huang[b]* and Tony D. James[a,c]*

[a]Department of Chemistry, University of Bath, Bath, BA2 7AY, U.K.

[b]The Education Ministry Key Laboratory of Resource Chemistry, Shanghai Key Laboratory of Rare Earth Functional Materials, and Shanghai Municipal Education Committee Key Laboratory of Molecular Imaging Probes and Sensors, Department of Chemistry, Shanghai Normal University, 100 Guilin Road, Shanghai 200234, China.

[c]Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554 Japan.

Email: T.D.James@bath.ac.uk, A.J.Cresswell@bath.ac.uk, A.C.Sedgwick@bath.ac.uk, S.D.Bull@bath.ac.uk, huangcs@shnu.edu.cn

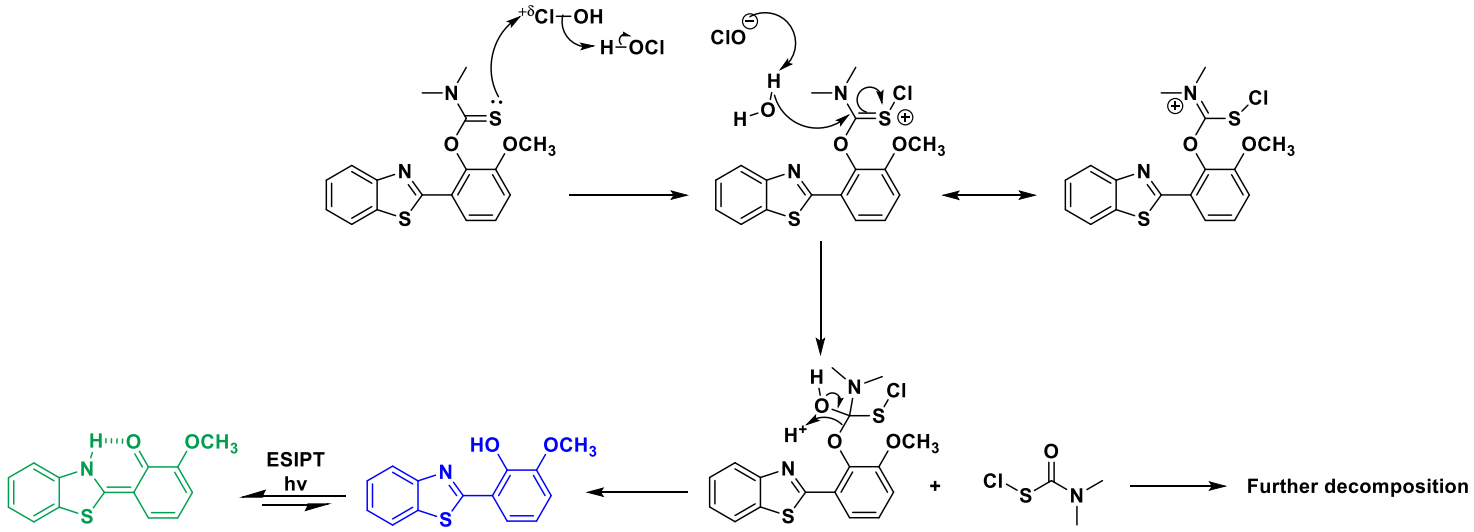
Content

1. **Reaction mechanisms**
2. **Methods**
3. **UV analysis**
4. **Fluorescence analysis**
5. **Mass spec analysis**
6. **HClO/ClO$^-$ detection in water samples**
7. **Cell imaging experiments**
8. **Synthetic experiments**
9. **NMR**
10. **References**

1. Reaction mechanisms

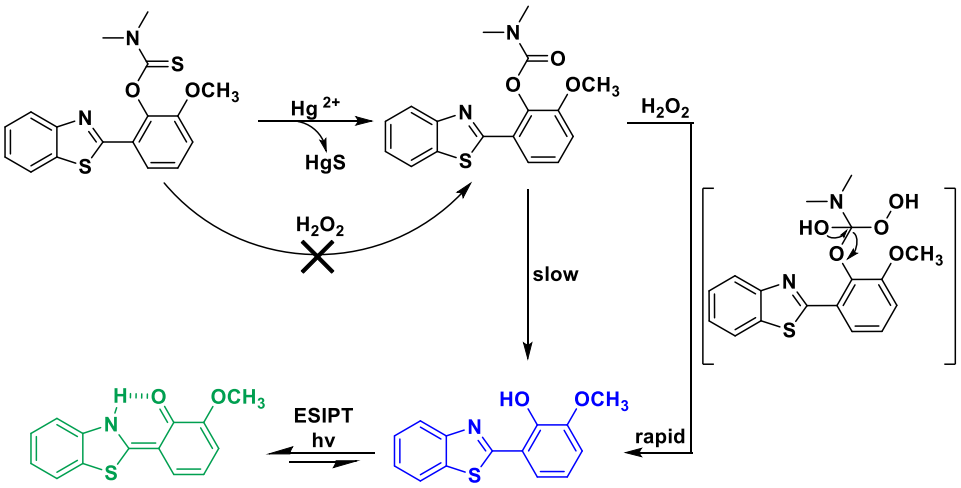
The mechanism for the hydrolysis of the thiocarbamate of **TCBT-OMe** with hypochlorite involves the sulfur of the thiocarbonyl reacting with an electrophilic halogen atom.[1-3] The charged intermediate then undergoes base-catalysed nucleophilic attack by H_2O to afford a tetrahedral intermediate, which results in the elimination of the ESIPT fluorophore producing a fluorescence response.

Addition of **HOCl/ClO$^-$** to **TCBT-OMe:**



Scheme S1. Reaction mechanism of **TCBT-OMe** with HClO

Addition of **Hg^{2+}** and **H_2O_2** to **TCBT-OMe:**



Scheme S2. Reaction of **TCBT-OMe** with **Hg^{2+}** or **Hg^{2+}** and **H_2O_2**

2. Methods

2.1 Spectroscopic Materials and Methods

All spectroscopic measurements except pH titration were performed in 0.1 M phosphate buffer (pH 7.4, 1% DMSO, v/v, 1 mM CTAB) at room temperature. Absorption spectra were recorded using a CARYWINUV UV-Visible spectrophotometer. Fluorescence spectra were recorded using a perkin-elmer LS50 scanning spectrofluorometer. Samples for absorption and fluorescence measurements were contained in 1 cm×1 cm quartz cuvettes (3.5 mL volume). pH effect on the fluorescence intensity of **TCBT-OMe** toward HOCl was tested in water solution (containing 1% DMSO, 1 mM CTAB).

2.2 Generation of various ROSs

ROO•

$ROO^{\cdot}$ 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;

$^{-}O_2$

Superoxide was generated from KO_2. KO_2 and 18-crown-6 ether (2.5 eq) was dissolved in DMSO to afford a 0.25 M solution.

•HO

Hydroxyl radical was generated by the Fenton reaction. To prepare •OH solution, hydrogen peroxide (H_2O_2, 10 eq) was added to $Fe(ClO_4)_2$ in deionized water.

$ONOO^{-}$

Simultaneously, 0.6 M KNO_2, 0.6 M in HC1, 0.7 M in H_2O_2 was added at to a 3 M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using extinction co-efficient of 1670 $cm^{-1}M^{-1}$ at 302 nm in 0.1 M sodium hydroxide aqueous solutions.

^{-}OCl

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

H_2O_2

The concentration of H_2O_2 was determined from the absorption at 240 nm (ε = 43.6 $M^{-1}cm^{-1}$).

3. UV Analysis

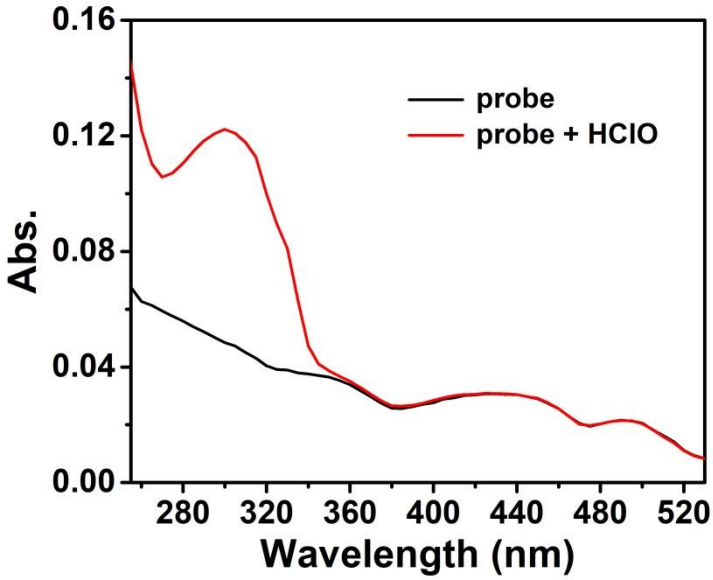


Fig. S1. UV spectra of **TCBT-OMe** (5 μM), with and without HClO/ClO^- (10 μM) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB.

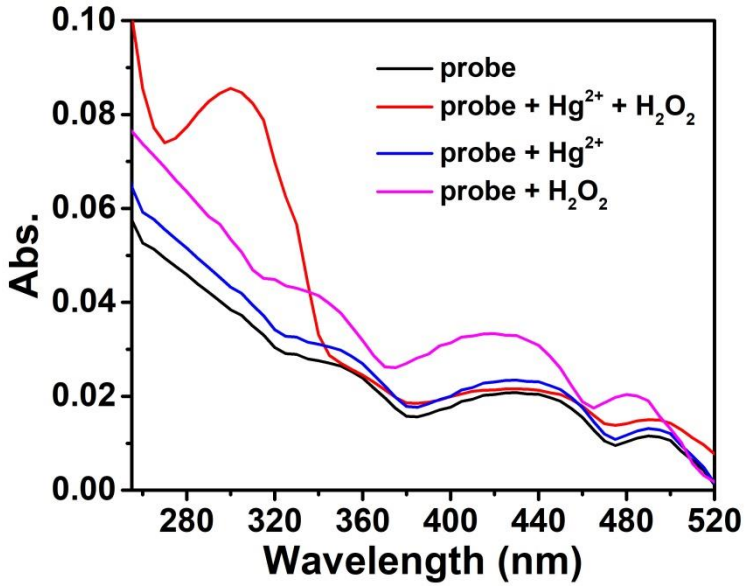


Fig. S2. UV spectra of **TCBT-OMe** (5 μM) with and without the addition of Hg^{2+} (15 μM) and H_2O_2 (120 μM) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB. Each measurement was taken 14 min after Hg^{2+} addition.

4. Fluorescence analysis

4.1 Fluorescence response of TCBT-OMe to HOCl

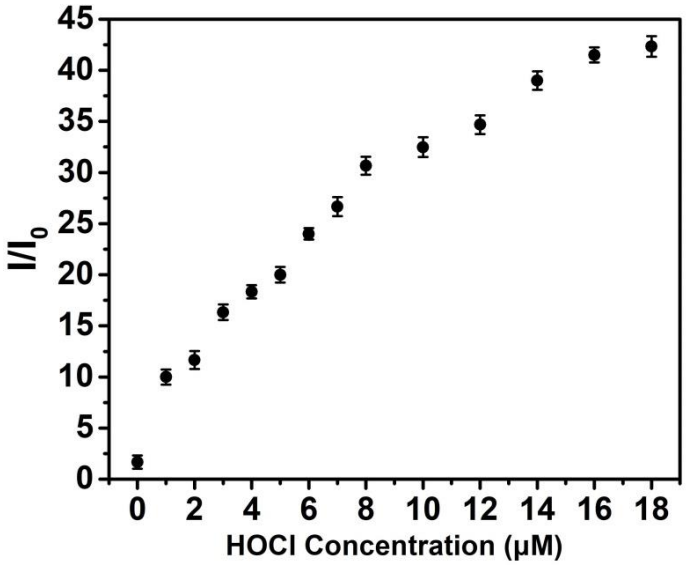


Fig. S3. Fluorescence intensity changes (I/I_{HOCl}) for **TCBT-OMe** (5 μM) with the addition of HOCl (0 - 18 μM) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB at 25 ºC. Fluorescence intensities were measured with λex = 310 nm, λem = 472 nm.

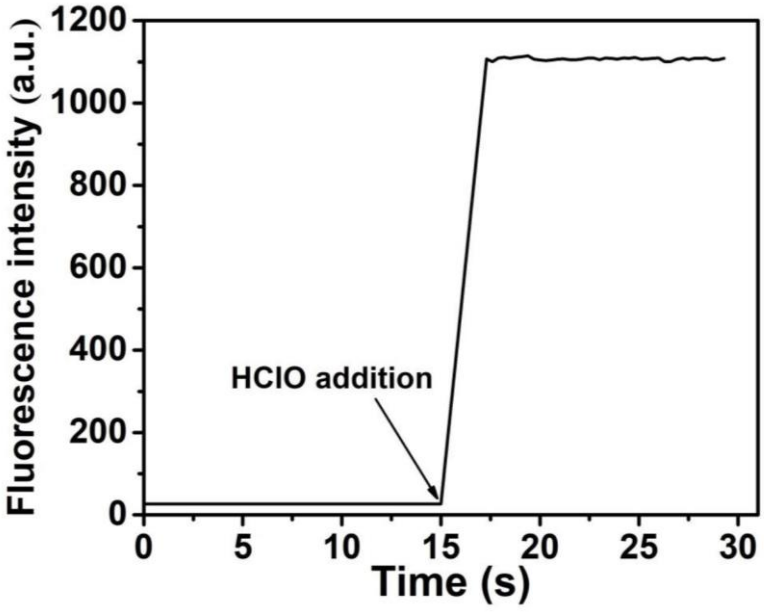


Fig. S4 - Fluorescence intensity over time of **TCBT-OMe** (5 μM) with the addition of HOCl (20 M) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB at 25 ◦C. λex = 310 nm/ λem = 472 nm

4.2 Determination of detection limit

The detection limit (CDL) was calculated by IUPAC assay. CDL = 3 sbm^{-1}(sb is the ratio of signal and noise, m is the slope of linear equation). The sb was determined through standard deviation (11 times) of F_{472} for **TCBT-OMe** at 5 μM without addition of HOCl or Hg^{2+}. According the linear equation (Y = 26.87312 + 71.48 X) of **TCBT-OMe** to HOCl concentration (from 0 to 0.67 μM, Fig. S4), which was calculated as about 0.16 nM. According the linear equation (Y = 26.17956 + 58.46 X) of **TCBT-OMe** to Hg^{2+} concentration (from 0 to 1.88 μM, Fig. S5), which was calculated as about 34.7 nM.

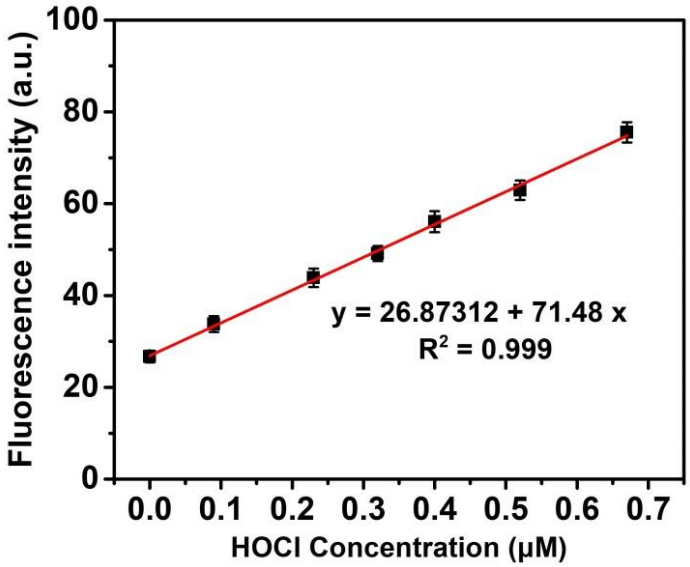


Fig. S5. Fluorescence intensity versus concentration of HClO for the calculation of the limit of detection for **TCBT-OMe** in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB at 25 °C. Fluorescence intensities were measured with λ_{ex} = 310 nm, λ_{em} = 472 nm. Error bar represents s.d. LOD is 0.16 nM.

4.3 Effects of pH on the fluorescence response of TCBT-OMe for HOCl

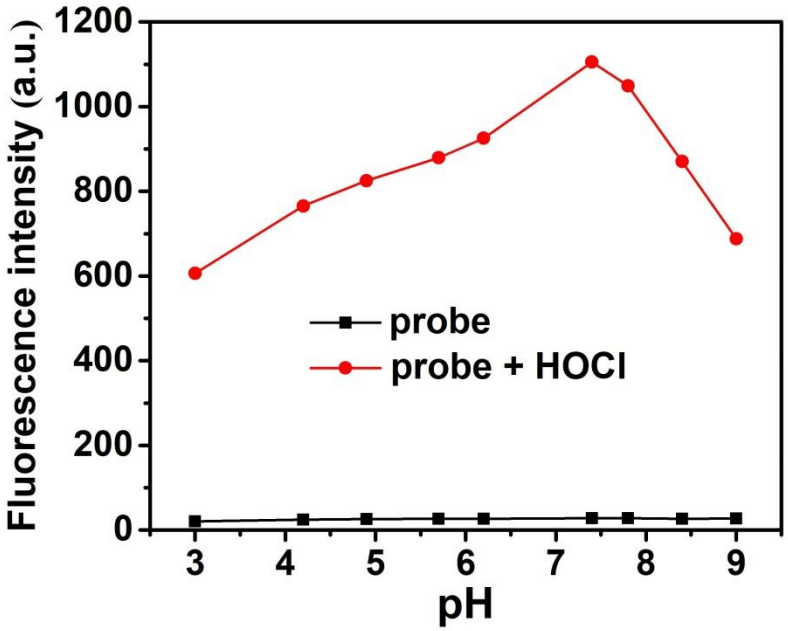


Fig. S6. Effects of pH on the fluorescence response of **TCBT-OMe** (5 μM) for HOCl (35 μM) in water (containing 1% DMSO, 1 mM CTAB). λ_{ex}= 410 nm, λ_{em}= 472 nm. Each measurement was acquired 15 min after HOCl addition. Slit widths: ex = 6 nm em = 4 nm.

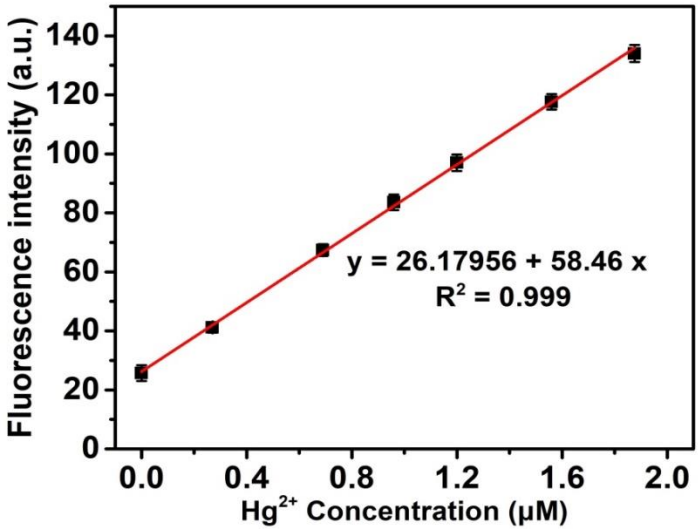


Fig. S7. Fluorescence intensity versus concentration of Hg^{2+} for the calculation of the limit of detection for **TCBT-OMe** in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB at 25 °C. Fluorescence intensities were measured with λ_{ex} = 310 nm, λ_{em} = 472 nm. Error bar represents s.d. LOD is 34.7 nM.

4.4. Fluorescence response of TCBT-OMe to Hg^{2+} with the addition of H_2O_2

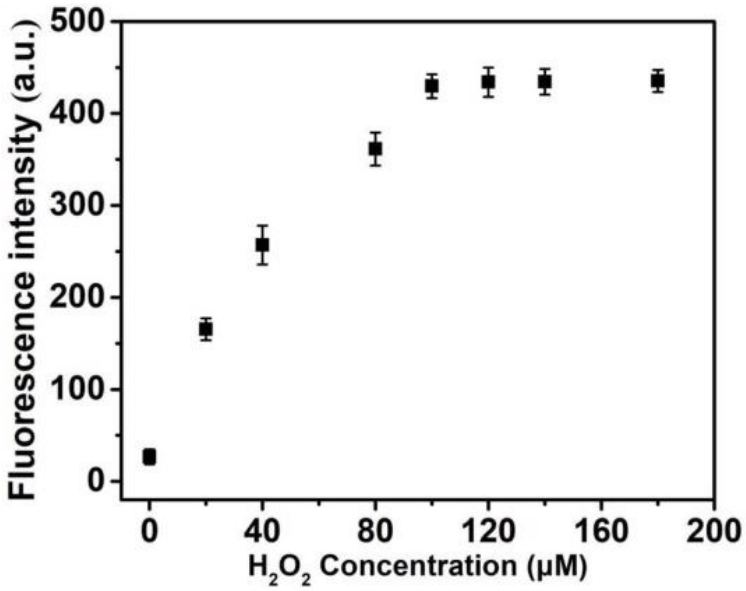


Fig. S8. Fluorescence spectra of **TCBT-OMe** (5 μM) in the presence of Hg^{2+} (9 μM) and increasing concentrations of H_2O_2 (final concentration: 0, 20, 40, 80, 100, 120, 140 μM and 180 μM) in PBS buffer (pH 7.4, containing 1% DMSO, 1 mM CTAB). 14 min wait before measurement in buffer solution. λ_{ex} = 310 nm/ λ_{em} = 472 nm. Error bars represent s.d.

4.5 Selectivity of TCBT-OMe for Hg^{2+}

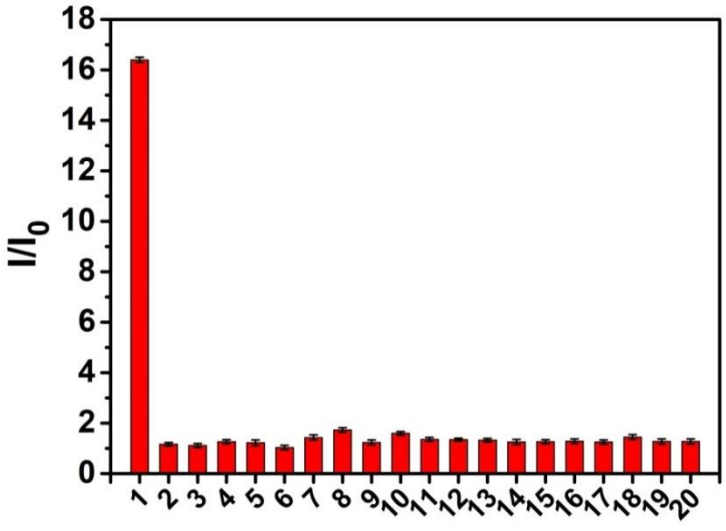


Fig. S9. Selectivity bar chart of **TCBT-OMe** in PBS pH 7.4, containing 120 μM H_2O_2, 1% DMSO, 1 mM CTAB with addition of Hg^{2+} (9 μM) and other metal cations. 1, Hg^{2+}; 2, blank; 3, Na^{+}; 4, K^{+}; 5, Mg^{2+}; 6, Ca^{2+}; 7, Fe^{2+}; 8, Fe^{3+}; 9, Al^{3+}; 10, Cu^{2+}; 11, Zn^{2+}; 12, Pb^{2+}; 13, Cd^{2+}; 14, Mn^{2+}; 15, Li^{+}; 16, Cr^{2+}; 17, Ag^{+}; 18, Ni^{2+}; 19, Cd^{2+}; 20, Rh^{3+}. Note: The concentration of **TCBT-OMe** and each interfering species are 5 μM and 15 μM respectively, 15 min wait before measurement in buffer solution. λ_{ex} = 310 nm/ λ_{em} = 472 nm Error bars represent s.d.

4.6 Time-dependent fluorescence response of TCBT-OMe to Hg^{2+}

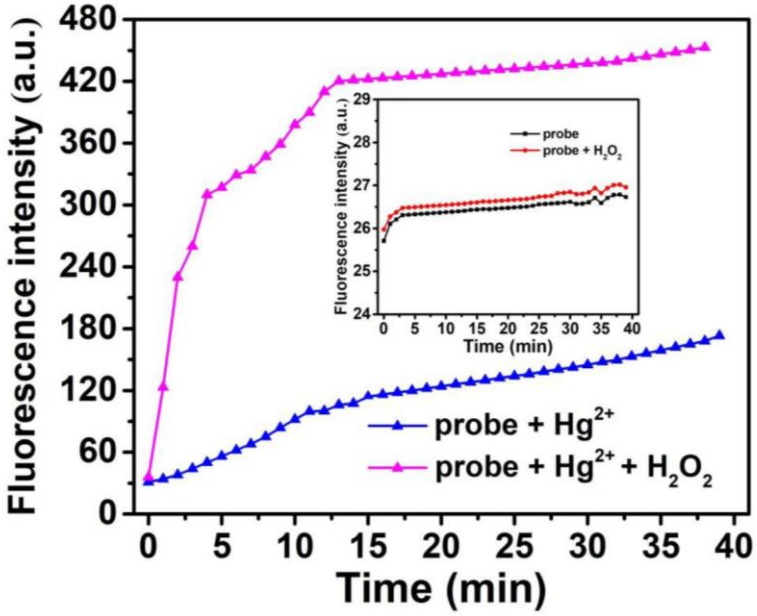
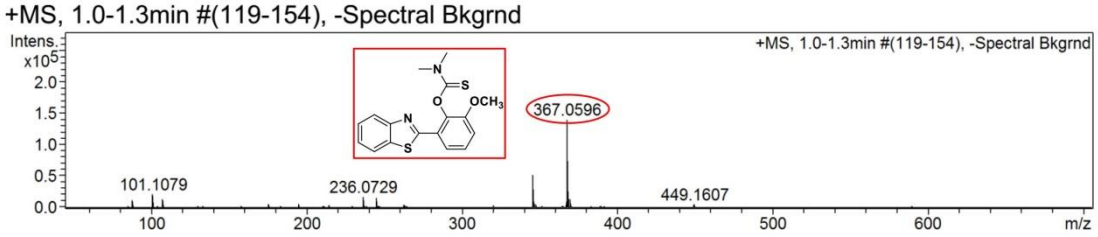


Fig. S10 Fluorescence intensity over time for **TCBT-OMe** (5 μM) in the presence of Hg^{2+} (9 μM) and H_2O_2 (120 μM) in PBS pH 7.4, containing 1% DMSO, 1 mM CTAB at 25 ˚C. λ_{ex} = 310 nm/ λ_{em} = 472 nm

5. Mass spec analysis



#	m/z	I	I %	Area	S/N
1	88.0217	11189	8.0	191	3606.8
2	101.1079	20658	14.9	470	4702.5
3	107.0478	14040	10.1	95	2819.8
4	236.0729	17367	12.5	468	1231.0
5	245.0386	15696	11.3	663	1078.1
6	345.0798	51403	37.0	2621	2133.3
7	346.0817	9771	7.0	509	395.2
8	367.0596	139074	100.0	7784	3676.3
9	368.0613	25790	18.5	1397	670.6
10	369.0557	13391	9.6	738	342.7

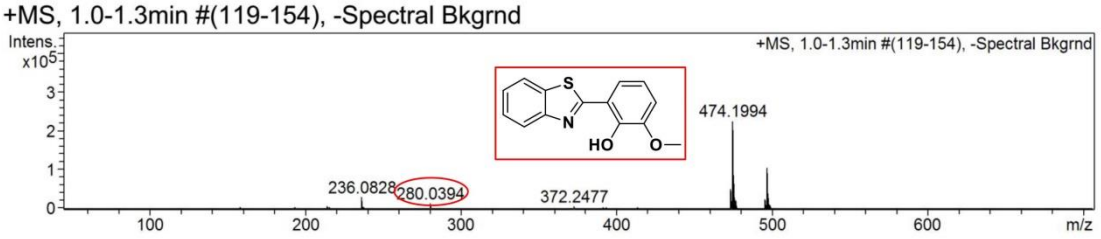
Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C17H16N2O2S2 **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	345.0798	345.0726	21.00	0.0141	C 17 H 17 N 2 O 2 S 2
1	367.0596	367.0545	13.90	0.0144	C 17 H 16 N 2 Na 1 O 2 S 2

Fig. S11. HRMS spectrum of **TCBT-OMe** before the addition of specific analyte



#	m/z	I	I %	Area	S/N
1	236.0828	28861	12.8	587	1485.2
2	280.0394	12856	5.7	593	1076.1
3	473.2001	50645	22.4	4465	1031.3
4	474.1994	225811	100.0	19204	4655.1
5	475.1984	73219	32.4	5979	1528.2
6	476.1963	22349	9.9	1843	472.3
7	495.1806	24482	10.8	2161	680.8
8	496.1774	104641	46.3	9721	2958.8
9	497.1800	32248	14.3	2798	927.5
10	498.1779	9402	4.2	827	275.1

Generate Molecular Formula Parameters

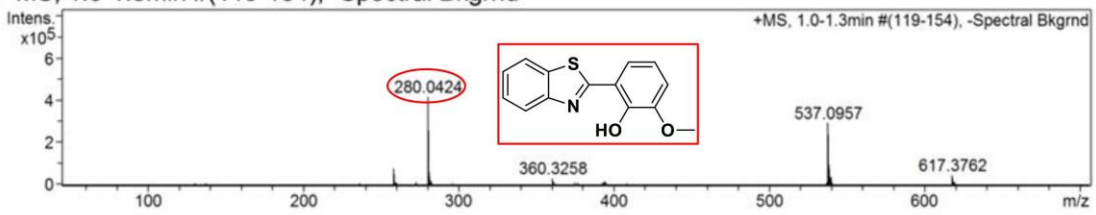
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C14H11NO2S **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	280.0394	280.0403	-2.90	0.0376	C 14 H 11 N 1 Na 1 O 2 S 1

Fig. S12. HRMS spectrum of **TCBT-OMe + HClO**.

+MS, 1.0-1.3min #(119-154), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	258.0597	78311	18.9	3729	2818.8
2	280.0424	414406	100.0	21860	9123.5
3	281.0457	61666	14.9	3018	1333.9
4	282.0396	18760	4.5	865	398.9
5	360.3258	32009	7.7	1839	1574.0
6	537.0957	290149	70.0	30765	7093.7
7	538.0959	97771	23.6	10008	2325.3
8	539.0931	42064	10.2	4573	974.0
9	617.3762	46419	11.2	5354	2814.8
10	618.3791	18083	4.4	2193	1085.0

Generate Molecular Formula Parameters

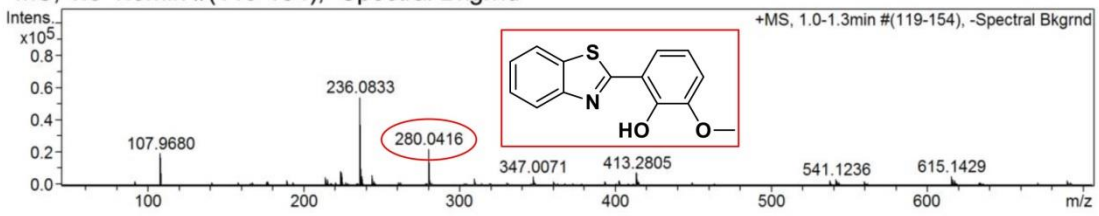
Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C14H11NO2S **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	258.0597	258.0583	5.20	0.0050	C 14 H 12 N 1 O 2 S 1
1	280.0424	280.0403	7.40	0.0110	C 14 H 11 N 1 Na 1 O 2 S 1

Fig. S13. HRMS spectrum of **TCBT-OMe + Hg^{2+}**.

+MS, 1.0-1.3min #(119-154), -Spectral Bkgrnd



#	m/z	I	I %	Area	S/N
1	107.9680	19314	35.8	440	10221.5
2	214.0901	4352	8.1	168	530.8
3	223.9732	8365	15.5	324	785.1
4	236.0833	53924	100.0	602	3946.9
5	237.0860	5133	9.5	86	369.0
6	243.9420	5572	10.3	224	363.9
7	280.0416	22359	41.5	951	1848.9
8	347.0071	5027	9.3	304	333.6
9	413.2805	7409	13.7	419	502.5
10	615.1429	5293	9.8	559	581.3

Generate Molecular Formula Parameters

Charge	Tolerance	SearchRadius	H/C Ratio min.	H/C Ratio max.	Electron Conf.	Nitrogen Rule	sigma limit
positive	25 ppm	0.05 m/z	0	3	both	true	0.05

Expected Formula C14H11NO2S **Adduct(s):** H, Na

#	meas. m/z	theo. m/z	Err[ppm]	Sigma	Formula
1	280.0416	280.0403	4.70	0.0233	C 14 H 11 N 1 Na 1 O 2 S 1

Fig. S14. HRMS spectrum of **TCBT-OMe + Hg^{2+} + H_2O_2**.

6. HClO/ClO^- detection in water samples

Table S1. Recovery of HClO/ClO^- in three water samples. (Sample A, tap water from University of Bath; Sample B, water from Avon River in Bath; Sample B, water from Roman Bath spa in Bath) in the presence of CTAB (1 mM) and **TCBT-OMe**

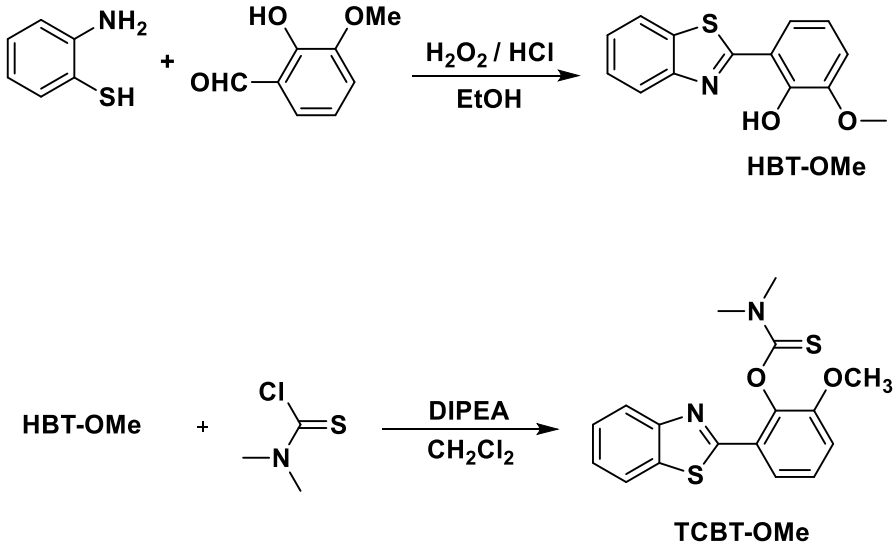
Type	Added (μM)	Found (μM)	R.S.D (%)	Recovery (%)
sample A	2.81	2.72±0.03	4.9	96.80
	5.97	5.89±0.01	5.3	98.66
	12.84	12.76±0.14	4.5	99.38
sample B	2.81	2.78±0.25	3.4	98.93
	5.97	5.86±0.09	4.5	98.15
	12.84	13.01±0.14	5.8	101.32
sample C	2.81	3.08±0.18	6.1	95.73
	5.97	6.24±0.21	4.7	102.85
	12.84	13.91±0.27	5.2	100.86

7. Cell imaging experiments

Cell imaging experiment method

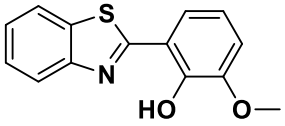
The HeLa cells were seeded into confocal Petri dishes in complete medium, and then incubated for 12 h under standard culture conditions. After the cells had attached, the cells were washed with DMEM for three times. (a) 10 μL of **TCBT-OMe** stock solution (8 mM) was loaded into the HeLa cells for 30 min; (b) HeLa cells pretreated with **TCBT-OMe** (40 μM) for another 30 min after preincubation with 1.2 μg mL^{-1} for 90 min; (c) HeLa cells pretreated with **TCBT-OMe** (40 μM) for another 30 min after preincubation with 250 μM ABAH for 70 min; (d) HeLa cells loaded with **TCBT-OMe** (40 μM) for 30 min followed by the addition of 8 μM NaOCl for 5 min. Finally, cells were washed twice with 2 mL of DMEM respectively, then 2 mL of fresh DMEM was added into the confocal dish as the culture medium for confocal microscopy study. Fluorescence images were collected by Leica TCS SP5 II confocal laser scanning microscopy using an HC× PLAPO 63× oil objective (NA: 1.40) and **TCBT-OMe** was excited at 405 nm and the emissions were collected in the range of 420-590 nm (yellow channel).

8. Synthetic experiments



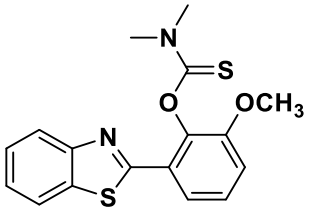
Scheme S3. Synthesis of **TCBT-OMe**

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



HBT-OMe was synthesized according to the similar procedures.[4] In brief, A solution of 2-aminothiophenol (0.9 mL, 12.6 mmol) and *o*-vanilin (1.44 g, 9.45 mmol) in EtOH (30 mL), aq H_2O_2 (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 H_2O. The precipitate was filtered, dried and recrystallized from EtOH to afford the title compound as a light yellow solid (2.20 g, 68%) ^{1}H NMR (500 MHz, $CDCl_3$) 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); ^{13}C NMR (500 MHz, $CDCl_3$) 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 $C_{14}H_{11}NO_2S$ $[M+Na]^+$ 280.0403, found 280.0435.

***O*-(2-(Benzo[d]thiazol-2-yl)-6-methoxyphenyl) dimethylcarbamothioate**



Dimethylthiocarbamoyl chloride (5.0 g, 14.4 mmol, 4 eq) was slowly added into the solution of **HBT-OMe** (0.94 g, 3.6 mmol, 1 eq) in dry CH_2Cl_2 (30 mL). *N*-diisopropylethylamine (1.25 mL, 7.2 mmol, 2 eqiv) was then added dropwise to the reaction mixture, which was then stirred at rt until the reaction reached completion. After the removal of CH_2Cl_2, the residues were purified through silica column chromatography to obtain **TCBT-OMe** as a light-yellow solid (180 mg, 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.09 (d, J = 8.7 Hz, 1H), 7.99 (d, J = 10.2 Hz, 1H), 7.91 (d, J = 7.5 Hz, 1H), 7.49 (ddd, J = 7.2, 4.7, 1.2 Hz, 1H), 7.42 – 7.34 (m, 2H), 7.11 (d, J = 8.2 Hz, 1H), 3.90 (s, 3H), 3.53 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 186.64 (s), 162.40 (s), 152.64 (s), 152.33 (s), 141.22 (s), 135.80 (s), 128.15 (s), 126.60 (s), 126.08 (s), 125.12 (s), 123.29 (s), 121.33 (s), 121.20 (s), 114.27 (s), 56.39 (s), 43.64 (s), 39.21 (s). HRMS (ES^+): calc. for $C_{17}H_{16}N_2O_2S_2$ $[M+H]^+$ 345.0726, found 345.0751.

9. NMR

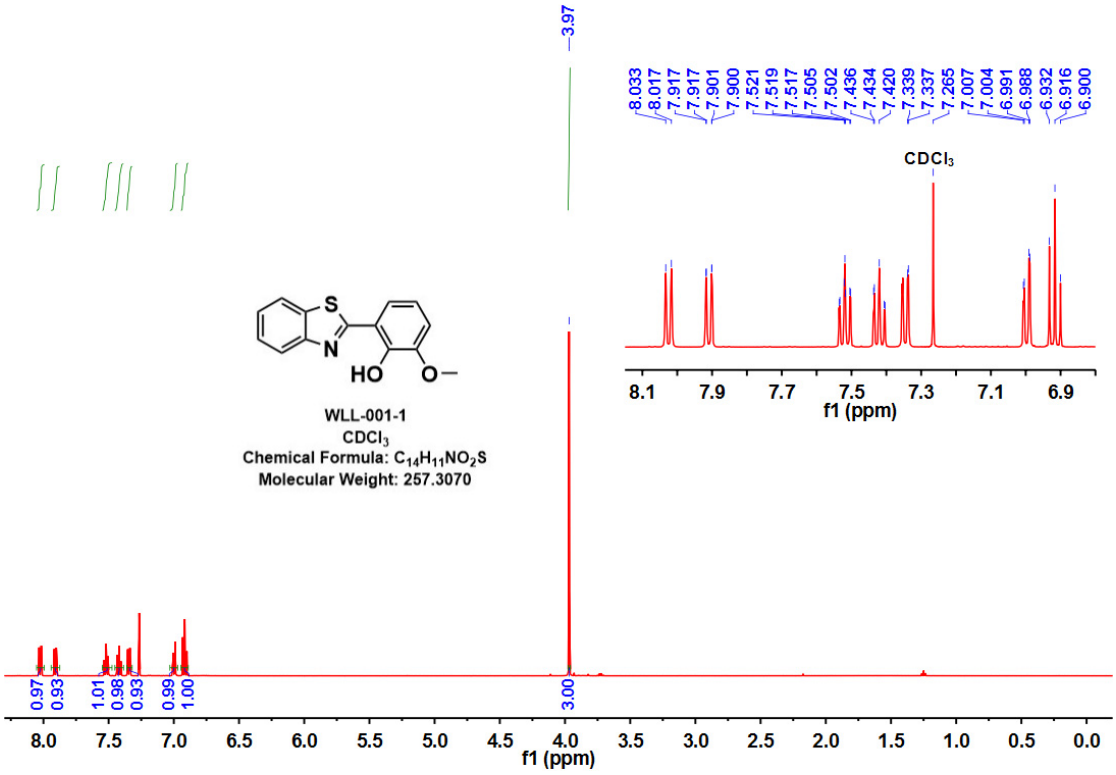


Fig. S15. [1]H NMR spectrum of **HBT-OMe**.

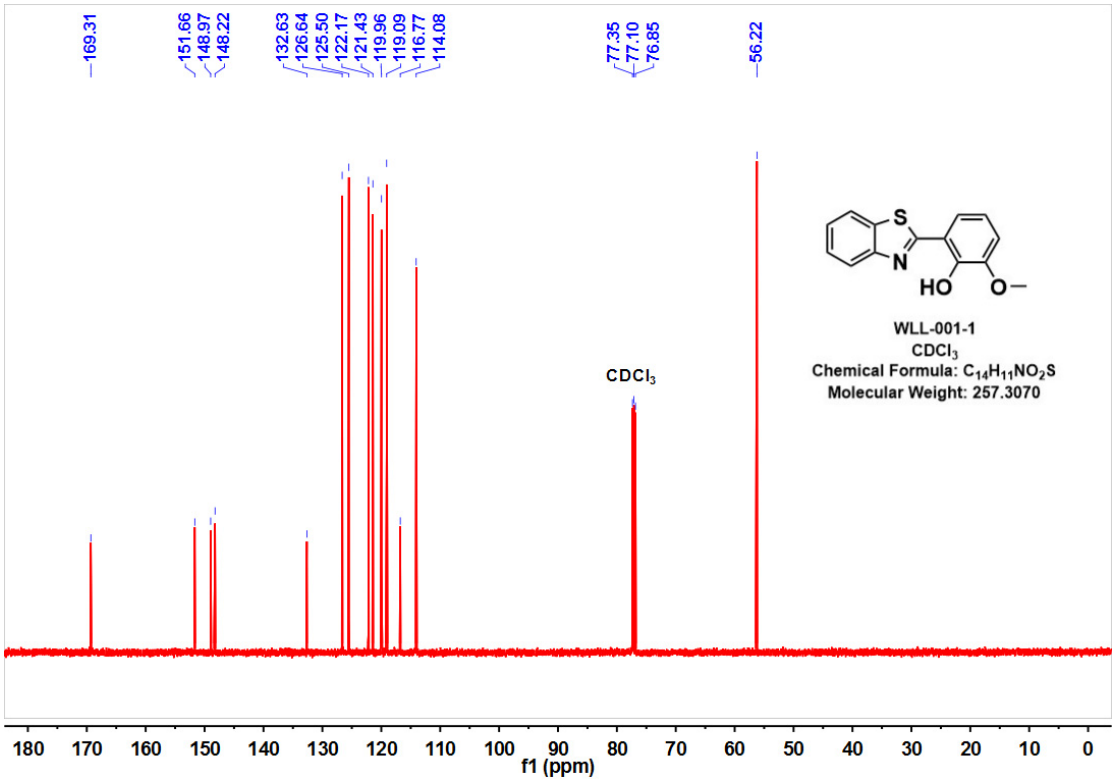


Fig. S16. [13]C NMR spectrum of **HBT-OMe**.

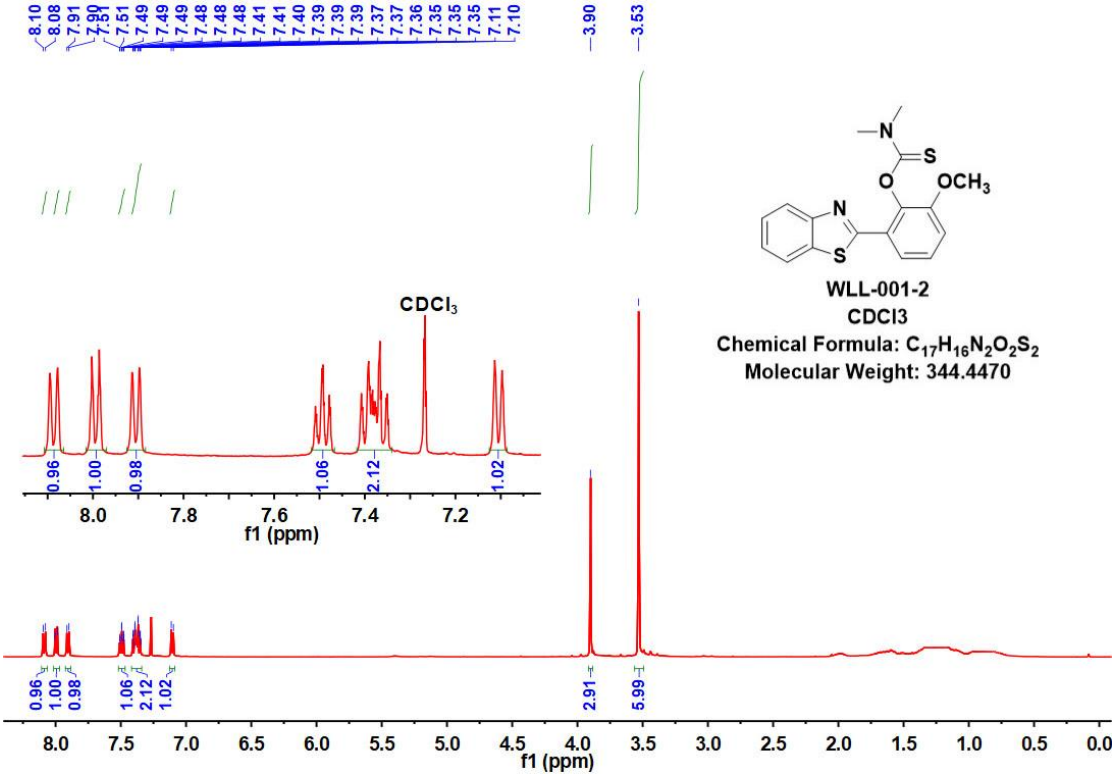


Fig. S17. [1]H NMR spectrum of **TCBT-OMe**.

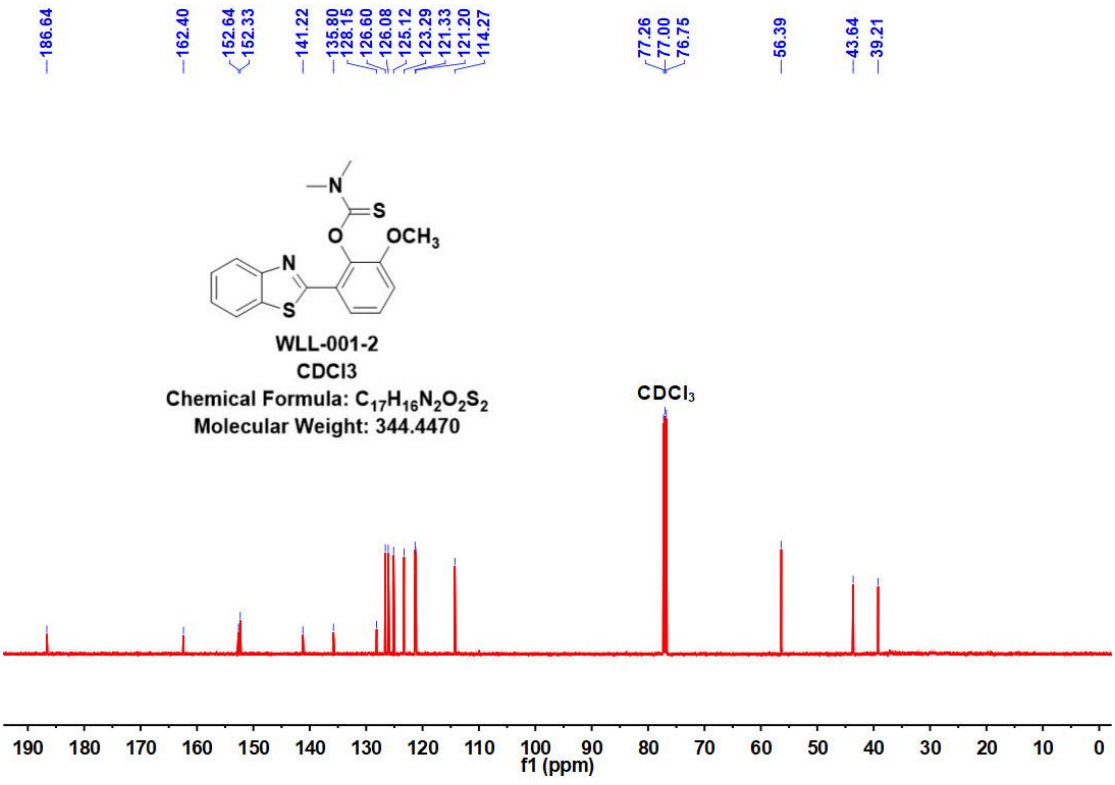


Fig. S18 . [13]C NMR spectrum of **TCBT-OMe**.

10. References

1. A. Corsaro and V. Pistara, *Tetrahedron*, 1998, **54**, 15027-15062.
2. H. Singh, P. Singh and N. Malhotra, *J. Chem. Soc.Perkin Trans. 1*, 1981, 2647-2649.
3. B. C. Zhu, P. Li, W. Shu, X. Wang, C. Y. Liu, Y. Wang, Z. K. Wang, Y. W. Wang and B. Tang, *Anal. Chem.*, 2016, **88**, 12532-12538.
4. X. Yang, Y. Guo and R. M. Strongin, *Angew, Chem, Int, Ed,*, 2011, **50**, 10690-10693.