Electronic Supplementary Information (ESI)

A Highly Sensitive and Selective "Turn-on" Fluorescent Probe for Hypochlorous Acid Monitoring

Ye-Xin Liao, Mao-Die Wang, Kun Li, * Zhao-Xuan Yang, Ji-Ting Hou, Ming-Yu Wu, Yan-Hong Liu, and Xiao-Qi Yu*

Key Laboratory of Green Chemistry and Technology (Ministry of Education), College of Chemistry, Sichuan University, Chengdu, 610064, P.R. China.

Contents

1. General experimental section

1.1 Materials:

Unless otherwise indicated, all reagents and solvents were obtained from commercial suppliers and used without further purification. Column chromatography was performed on silica gel (Qingdao haiyang) 200-300 mesh or basic aluminum oxide (for chromatography use). All solvents used in spectra test systems were chromatographically pure. Ultrapure water was used throughout. Stock solutions of compound **3** and **1** were prepared by dissolving the corresponding compound in chromatographically pure DMSO. H_2O_2 stock solution was prepared by diluting 30% H_2O_2 solution, and the concentration was determined from absorption at λ=240 nm (ε=43.6 $M^{-1}\cdot cm^{-1}$). ^tBuOOH was prepared by diluting 70% ^tBuOOH solution. ·OH was generated by Fenton reaction between $FeSO_4$ and H_2O_2, and concentration of ·OH was determined by Fe^{2+}. $^tBuOO\cdot$ was generated by reaction between $FeSO_4$ and ^tBuOOH, and concentration of $^tBuOO\cdot$ was determined by Fe^{2+}. $^1O_2$ was generated by the reaction of H_2O_2 with Na_2MoO_4[1]. $O_2^{-}\cdot$ was generated from KO_2 solid diluted in DMSO. $ONOO^-$ was generated by the reaction of H_2O_2 and $NaNO_2$[2] and stocked at -20 °C, the concentration was determined from absorption at λ=302 nm (ε=1670 $M^{-1}\cdot cm^{-1}$) in 0.1 M NaOH solution[3].ClO^- was prepared by diluting 5% NaClO aqueous solution, and the concentration was determined from absorption at λ=292 nm (ε=350 $M^{-1}\cdot cm^{-1}$)[3]. The fluorescence quantum yields in solution were calculated using Fluorescein as a standard material.

1.2 Instruments

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AMX-400 with chemical shifts expressed in parts per million (in deuteriochloroform or DMSO-d_6, Me_4Si as internal standard). Fluorescence spectra were determined using a FluoroMax-4 Spectrofluoro photometer (HORIBA Jobin Yvon). UV/Vis absorption spectra were determined by a Hitachi PharmaSpec UV-1900 UV-Vis spectrophotometer. Mass spectral data were recorded on a Finnigan LCQDECA and a Bruker Daltonics Bio TOF mass spectrometer. High performance liquid chromatography (HPLC) were perform on a Waters e2695 Separatins Module using Waters 2998 PDA detector equipped with an Symmetry C18 column(4.6 X 150 mm, 5 μm), CH_3OH:H_2O=95:5(v/v) were used as eluents with a flow rate of 1 ml/min. 280nm was used as wavelength. TLC analyses were performed on silica gel GF 254. The pH values were determined by a Leici pH3c (digital display) pH meter.

2. Synthetic procedures

2.1 Preparation of **1-(benzo[d]thiazol-2-yl)naphthalen-2-ol** (**1**)

2-aminothiophenol (15.6 mmol, 1.95 g) and 2-hydroxy-1-naphthaldehyde (15.8 mmol, 2.72 g) were dissolved in DMF (50 mL), then sodium metabisulfite (16.0 mmol, 3.05 g) was added and the reaction mixture was reflux for 2 h. After cooling to room temperature, the mixture was poured into 100 mL water, and then extracted with ethyl acetate (EA) for three times. EA layer was collected and dried by anhydrous Na_2SO_4, solvent was removed under reduced pressure to give the crude product. Then crude product was purified by column chromatography using silica gel (200-300 mesh) and DCM/petroleum ether as a fluent to give a yellow green solid (0.80 g, yield 18.5%). ^1H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1 H), 8.27-8.25 (d, *J*=8.56 Hz, 1 H), 8.19-8.14 (m, 2 H), 8.01-7.98 (d, *J*=8.96 Hz, 1 H), 7.92-7.90 (d, *J*=8.08 Hz, 1 H), 7.60-7.57 (m, 1 H), 7.53-7.48 (m, 2 H), 7.40-7.34 (m, 2 H).^{13}C NMR (100 MHz, DMSO-d_6) δ 163.75, 154.83, 152.22, 135.37, 132.41, 132.35, 128.35, 127.86, 127.63, 126.06, 125.17, 123.91, 123.38, 122.64,

121.79, 118.28, 112.09. HRMS (ESI) calcd for $C_{17}H_{12}NOS^+$, $[M+H]^+$, m/z 278.0634 ; found, m/z 278.0617.

2.2 Preparation of **2-(2-(4-nitrophenoxy)naphthalen-1-yl)benzo[d]thiazole** (**2**)

60% NaH (220 mg) was added in portion into a solution of **1** (5 mmol, 1.14 g) in anhydrous DMF (2 mL) in an ice-bath. And the resulting solution was kept and stirred for 30 min. Then 4-chloronitrobenzene (5 mmol, 0.79 g) was added and the mixture was heated to 120 °C and stirred overnight. After cooling to room temperature, 50 mL water was poured into the reaction mixture and extracted by DCM thrice. Organic layer was collected, washed with saturated brine and then dried by anhydrous Na_2SO_4. Solvent was removed under reduced pressure. The residue was then purified by column chromatography using silica gel (200-300 mesh) using DCM/petroleum ether as the fluent and finally yielded a yellow solid (0.26 g, yield 12.6%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.24-8.21 (d, *J*=8.88 Hz, 1 H), 8.24-8.13 (m, 5 H), 8.02-8.00 (d, *J*=7.44 Hz, 1 H), 7.66-7.50 (m, 5 H), 7.20-7.18 (d, *J*=8.92 Hz, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.41, 161.04, 152.67, 150.08, 142.56, 135.55, 133.39, 132.15, 131.06, 128.54, 128.33, 126.44, 126.15, 125.78, 125.02, 123.19, 122.19, 121.63, 120.30, 117.53. HRMS (ESI) calcd for $C_{23}H_{15}N_2O_3S^+$, $[M+H]^+$, m/z 399.0798; found, m/z 399.0796.

2.3 Preparation of **4-((1-(benzo[d]thiazol-2-yl)naphthalen-2-yl)oxy)aniline** (**3**)

Compound **2** (398 mg, 1 mmol) and 20% (w/w) Pd/C powder (Pd, 10 wt% on carbon powder) was dissolved into 20 mL CH_3OH. The resulting solution was stirred at ambient temperature overnight in hydrogen atmosphere. After the reaction finished, Pd/C was removed by using a pad of Celite, and the filtrate was concentrated in vacuo to afford crude product. The crude product was further purified using basic aluminum oxide with DCM/petroleum ether as the fluent and finally yielded a pale yellow solid (327 mg, yield 89%).^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20-8.17 (m, 2 H), 8.11-8.09 (d, *J*=9.12 Hz, 1 H), 8.06-8.04 (d, *J*=8.40 Hz, 1 H), 8.01-7.99 (d, *J*=7.40 Hz, 1 H), 7.62-7.51 (m, 4 H), 7.18-7.16 (d, *J*=9.08 Hz, 1 H), 6.82-6.80 (m, 2 H), 6.61-6.58 (m, 2 H), 5.03 (s, 2 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.48, 154.32, 152.67, 146.05, 145.61, 135.68, 132.27, 132.12, 129.34, 128.33, 127.95, 126.18, 125.48, 124.98, 124.49, 122.99, 121.99, 120.25, 117.75, 117.63, 114.85. HRMS (ESI) calcd for $C_{23}H_{17}N_2OS^+$, $[M+H]^+$, m/z 369.1056; found, m/z 369.1059.

3. Effect of different solvents

Different solvents including water, THF, CH_3CN, CH_3OH, DMF, DMSO and water with 1 mM CTAB were used as the test solvents and fluorescence emission intensity at 465 nm were recorded.

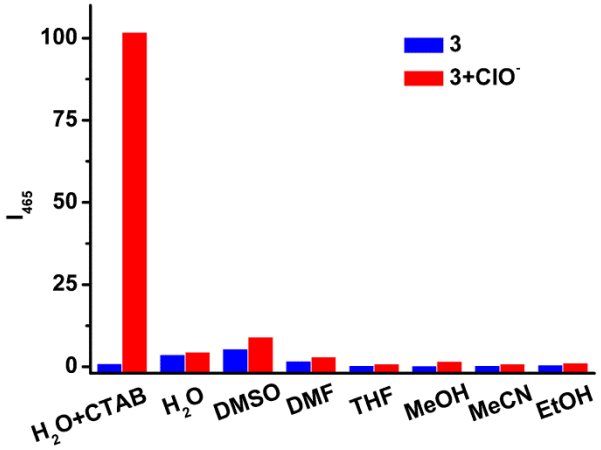

Figure S1 Flourescence responds of the reaction between **3** (5 μM) and ClO^- (50 μM) in HEPES buffer (0.5% DMSO, 20 mM, pH 7.40) at ambient temperature for 30 min. Fluorescence intensity was collected at 465 nm. λ_{ex}=350 nm, slits: 5/5 nm.

4. UV-visible Absorbance and Fluorescence Emission Measurements

UV spectra (Figure 2a) of **1** (20 μM), **3** (20 μM) and the reaction solution of **3** (20 μM) and ClO^- (200 μM**)** were recorded in DMSO/water (2/98, v/v) buffered by 20 mM HEPES at pH 7.40 (Figure S2a). Fluorescence emission spectra of 1 (5 μM) and the reaction solution of **3** (5 μM) and ClO^- (50 μM**)** were collected in DMSO/water (1/99, v/v) buffered by 20 mM HEPES at pH 7.40 (Figure S2b). All the reaction solution was placed at room temperature for 30 min.

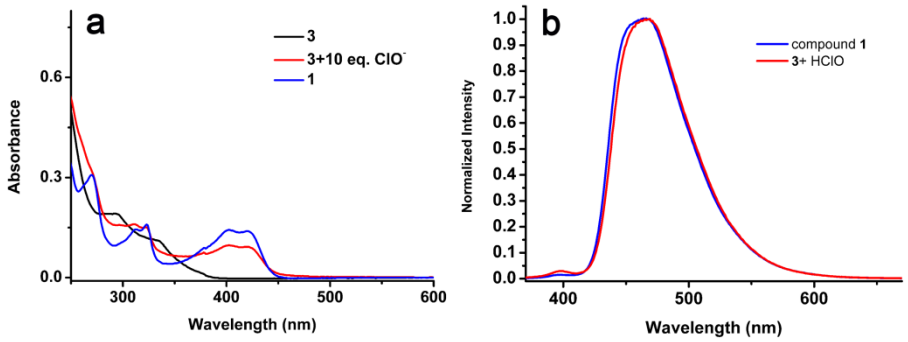

Figure S2 a) Absorption spectra of **1** (blue line), **3** (black line) and reaction between **3** and HClO (red line) at ambient temperature for 30 min. b) Fluorescence emission spectra of compound 1 (blue line) and the reaction of 3 and HClO (red line).

5. High resolution mass spectrum (HRMS) of reaction system between 3 and HClO/ClO^-

25 mL PBS solution (with 2% DMSO, v/v) with probe **3** (20 μM), HClO/ClO^- (200 μM) and CTAB (1 mM) was prepare and kept at ambient temperature for 30 min. Then the reaction solution was extracted by DCM for thrice. DCM layer was collected and solvent was removed. Residue was resolved by DCM: MeOH= 10: 1 (v/v) and flowed on a pad of silica gel (200-300 mesh), and the effluent was collected. Solvent was removed and the residue was analyzed directly by HRMS. The ES- result was shown as follow:

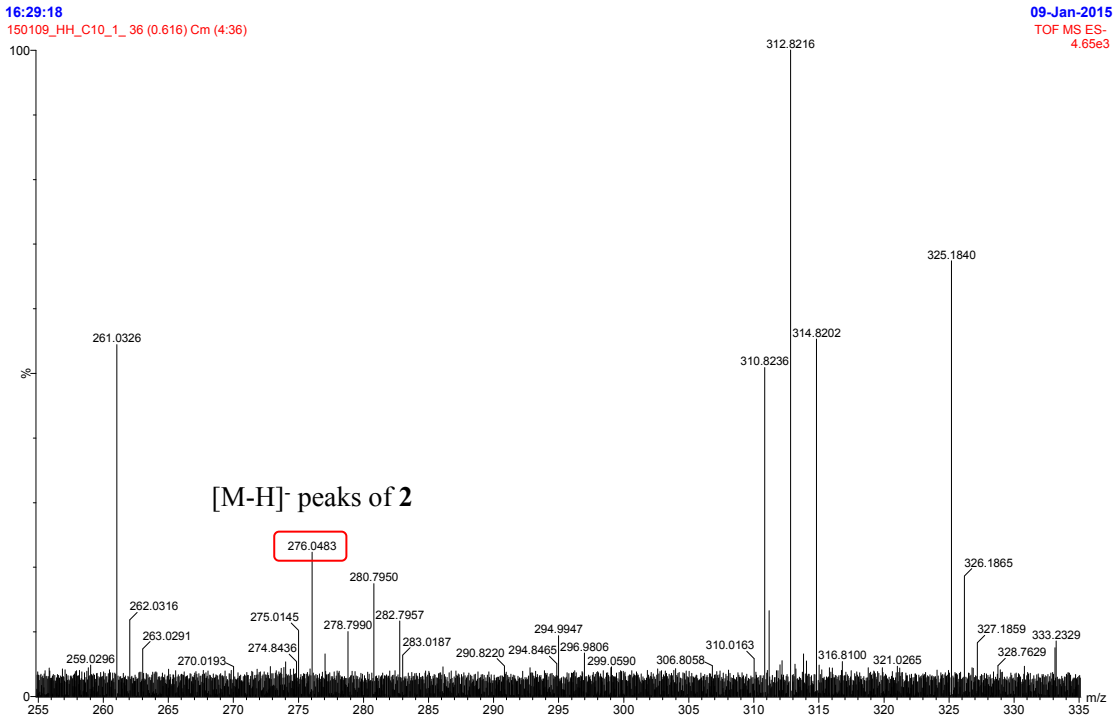

Figure S3 HRMS result of the reaction between **3** and HClO/ClO$^-$.

Effect of pH value

Various pH solutions were prepared by adjusting the pH values of HEPES buffer (20 mM) using 50% NaOH solution and 20% HCl solution. The solutions of **1**, **3** and the reaction between **3** and ClO$^-$ in different pH conditions (including: 3.02, 4.02, 5.26, 6.01, 6.52, 6.99, 7.40, 8.01, 8.53, 9.01, 10.15, 10.91 and 12.03) were prepared and kept at ambient temperature for 30 min, and spectra were recorded then by excited at 350 nm, using 5/5 nm as the excitation and emission slits.

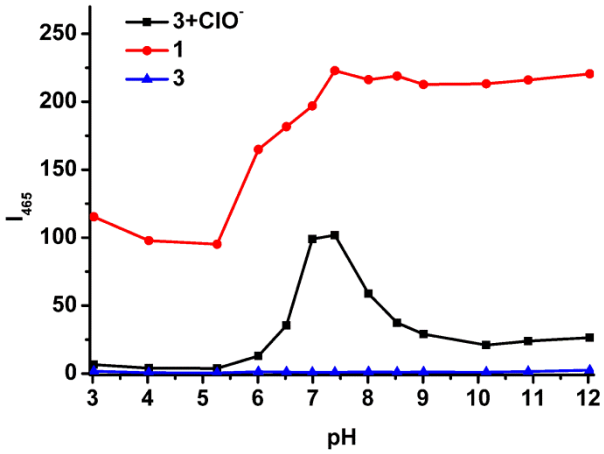

Figure S4 Effect of pH values to the relative fluorescence intensity of 5 μM **1** (●), 5 μM **3** (▲) and reaction between 5 μM **3** and 50 μM ClO- in water (0.5% DMSO in volum) buffered by 20 mM HEPES at pH 7.40 (■) for 30 min at ambient temperature. (The investigated pH values include 3.02, 4.02, 5.26, 6.01, 6.52, 6.99, 7.40, 8.01, 8.53, 9.01, 10.15, 10.91 and 12.03).

6. Detection limit of 3 to HClO/ClO$^-$

The detection limit (D) of **3** to HClO/ClO$^-$ was calculated base on the equation as follow: D=3σ/k, σ stands for the standard deviation of the blank measurement of **3**'s fluorescence intensity at 465

nm within 10 times; And k stands for a slope between fluorescence intensity versus HClO/ClO^- concentration in the fluorescence titration curve of **3** in the present of HClO/ClO^- (0-20 μM).

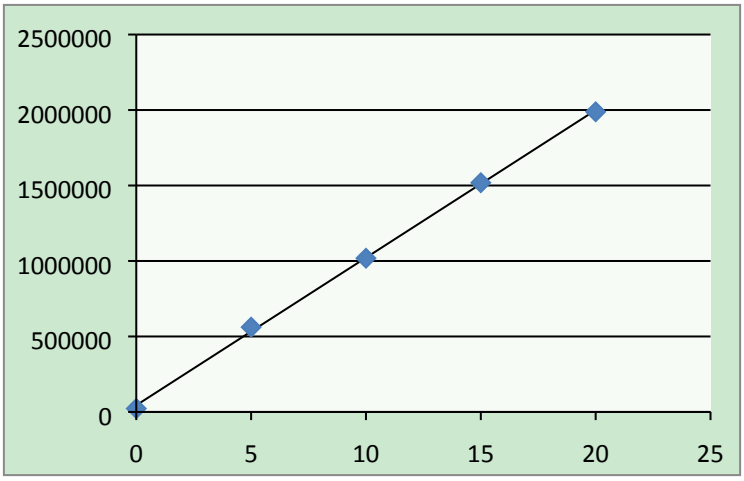

Figure S5 Fluorescence changes with various concentrations of HClO/ClO^- at 465 nm in the reaction between **3** and HClO/ClO^- at ambient temperature.

7. Kinetic property of the reaction between 3 and ClO^-

Kinetic property of the reaction between **3** (5 μM) and ClO^- (50 μM) was studied. Intensity was collected at 465 nm while the excitation wavelength λ_{ex}=350 nm, slits: 5/5 nm. Solution was kept at ambient temperature.

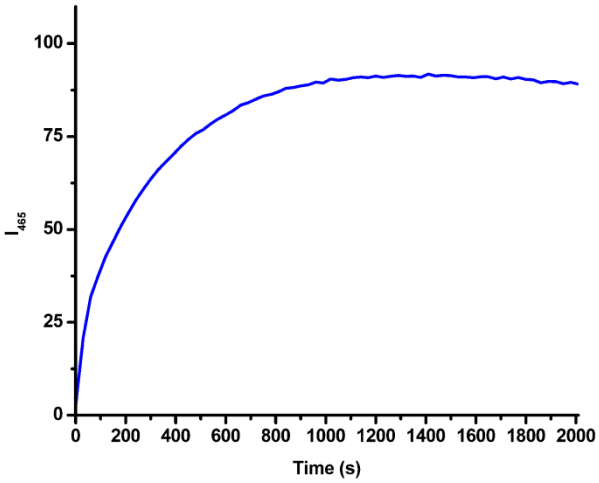

Figure S6. Time-dependent fluorescence changes of **3** (5 μM) in present of HClO (50 μM) at ambient temperature. λ_{ex}=350 nm, slits: 5/5 nm, emission intensity was collected at λ_{em}=465 nm.

8. Fluorescence quantum yields

Fluorescein in 0.1 N NaOH aqueous solution was used as the standard (quantum yield Φ_s=0.85). The quantum yields of **3** and the reaction solution between **3** and HClO/ClO^- in water (HEPES buffer, 20 mM, pH 7.40, 0.5% DMSO) was calculated based on the equation followed:

$$\Phi_u = \Phi_s * \frac{F_u}{F_s} * \frac{A_s}{A_u}$$

Where Φ is fluorescence quantum yield; F is integrated area under the corrected emission spectra; *A* is the absorbance at the excitation wavelength; the subscript s stands for the standard and u

stands for unknown.

9. Cells culture and imaging

HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum and 1% Antibiotic-Antimycotic at 37 °C in a 5% CO_2/95% air incubator. For fluorescence imaging, cells (4×10^3/well) were passed on a culture dish and incubated for 24h. Cells were washed twice with PBS, and then incubated with 5 μM **3** (PBS buffer, 1 mM CTAB) for 30 min at 37 °C. After washed twice with PBS, cells were incubated with 50 μM NaClO (PBS buffer as solvent) for another 20 min. Finally cells were washed three times and the confocal fluorescent images were captured, excitation wavelength: 405 nm, fluorescence emission was collected from 440 nm to 500 nm.

References

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3 M. Abo, Y. Urano, K.Hanaoka, T. Terai, T. Komatsu, T. Nagano, *J. Am. Chem. Soc.*, 2011, **133**, 10629-10637.

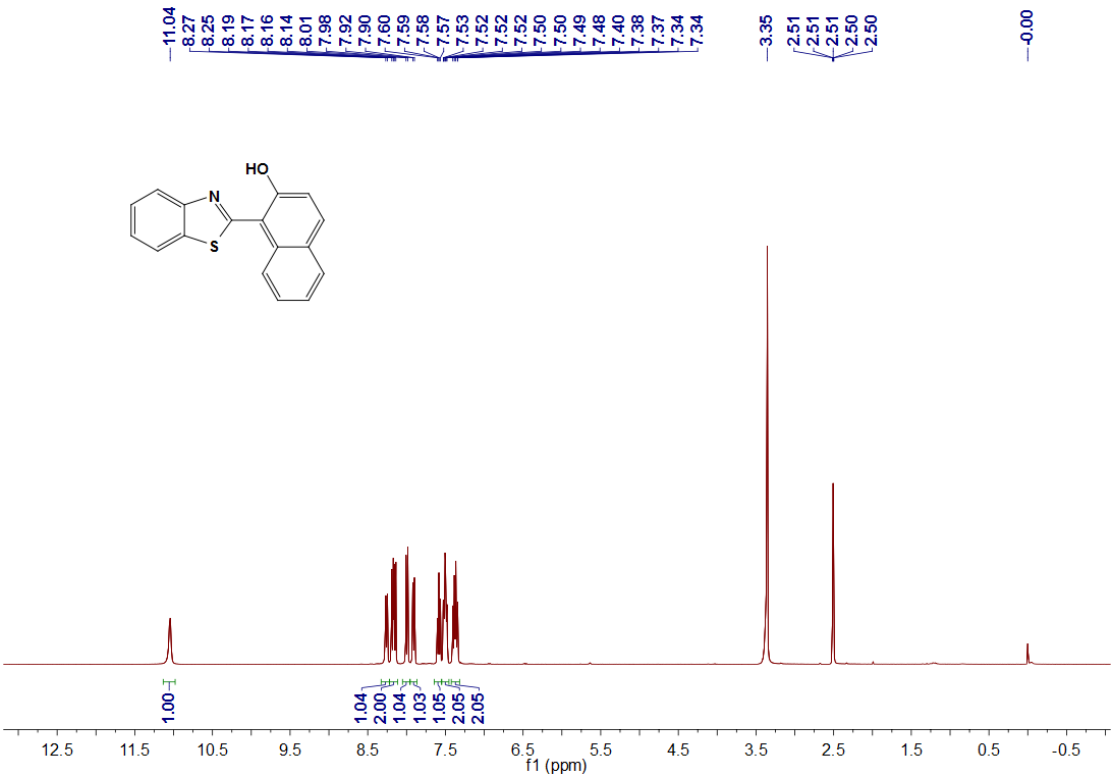

Figure S7. ^{1}H NMR spectrum of **1** in DMSO-d_6.

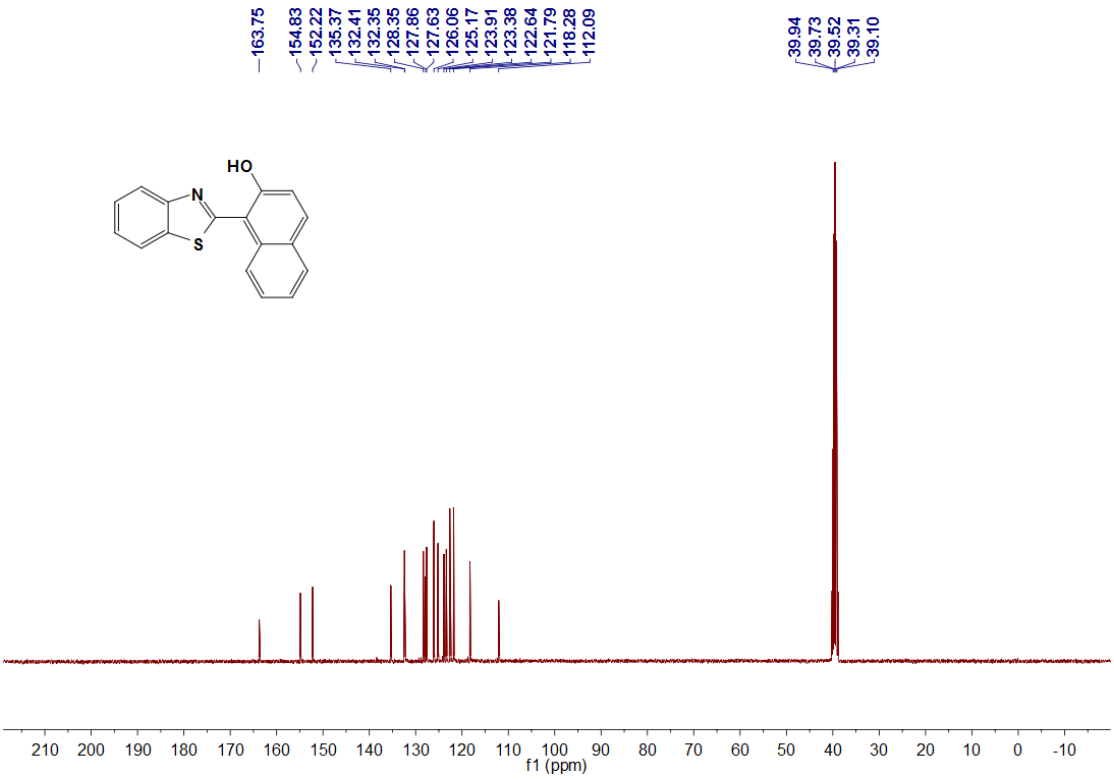

Figure S8. ^{13}C NMR spectrum of **1** in DMSO-d_6.

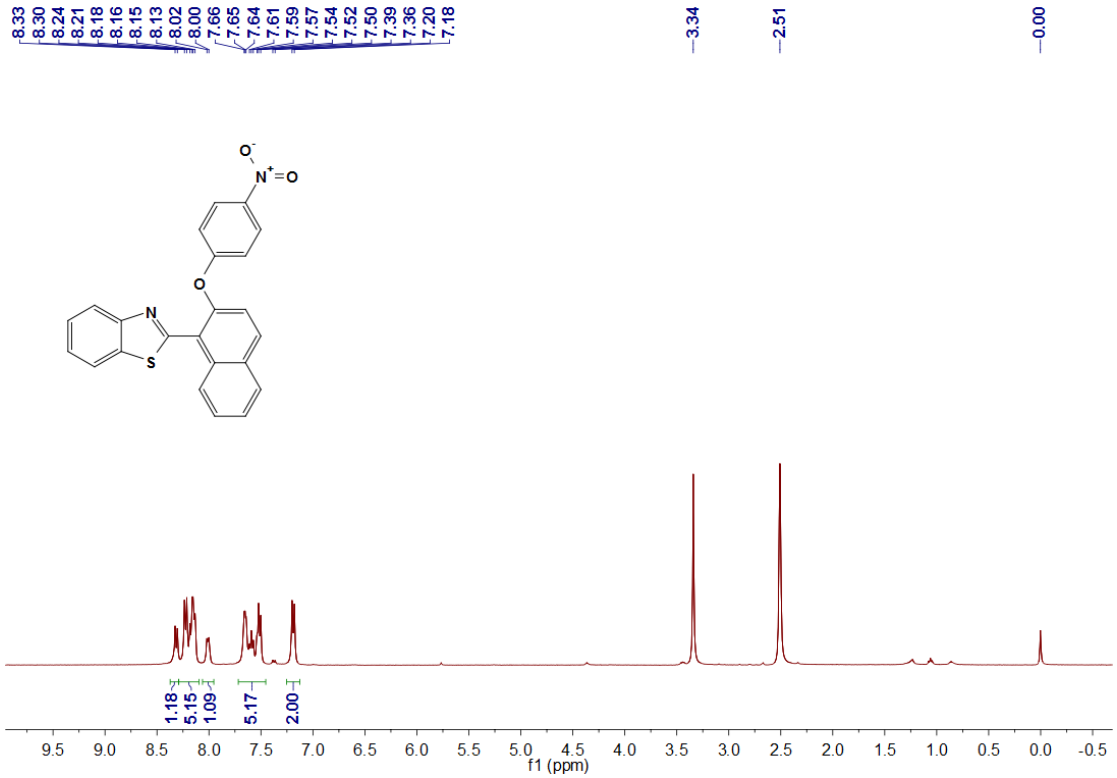

Figure S9. ^{1}H NMR spectrum of **2** in DMSO-d_6.

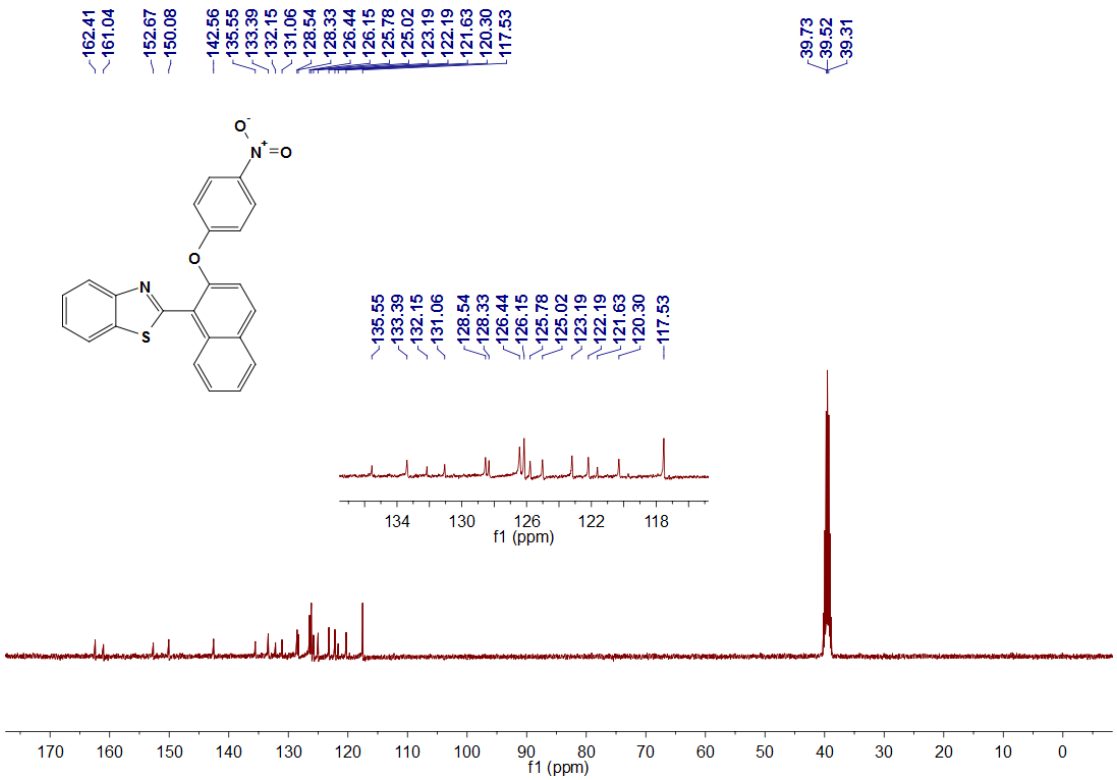

Figure S10. ^{13}C NMR spectrum of **2** in DMSO-d_6.

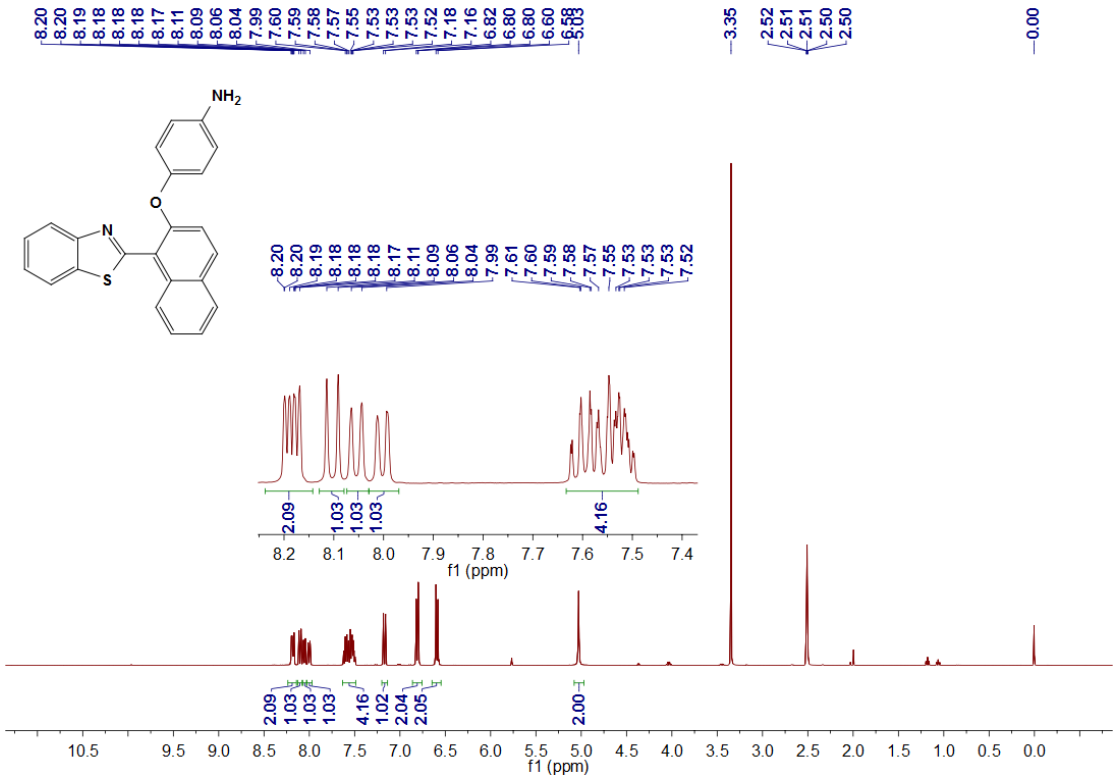

Figure S11. ^{1}H NMR spectrum of **3** in DMSO-d_6.

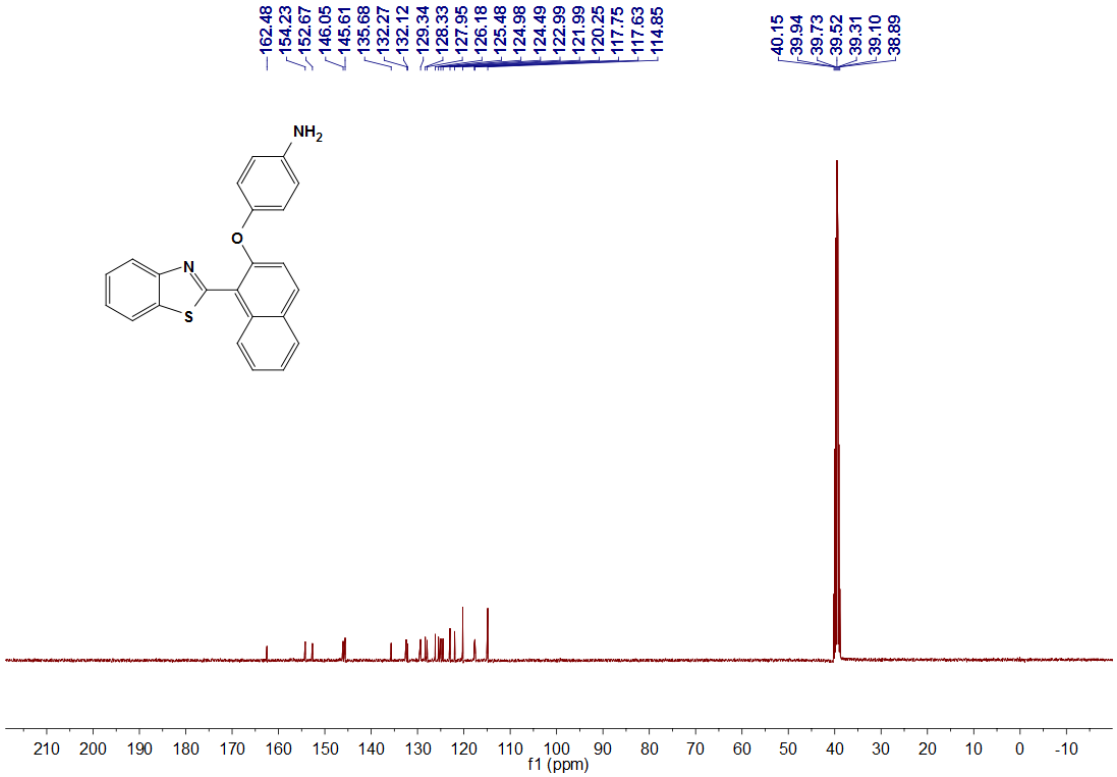

Figure S12. ^{13}C NMR spectrum of **3** in DMSO-d_6.

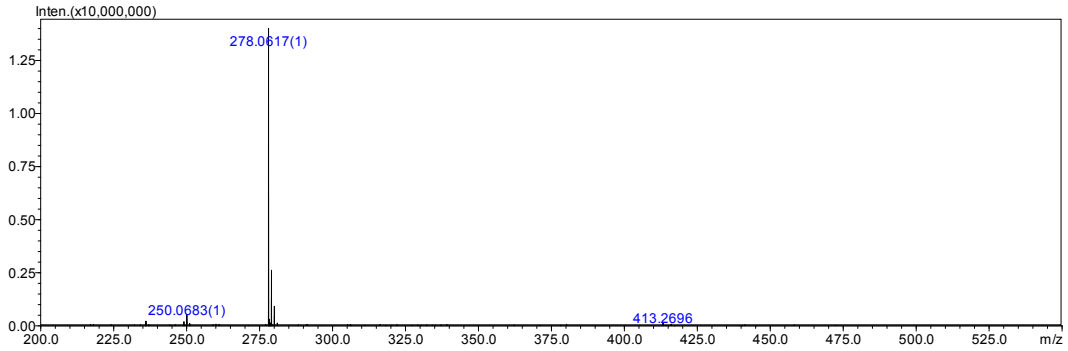

Figure S13. HRMS result of **1**.

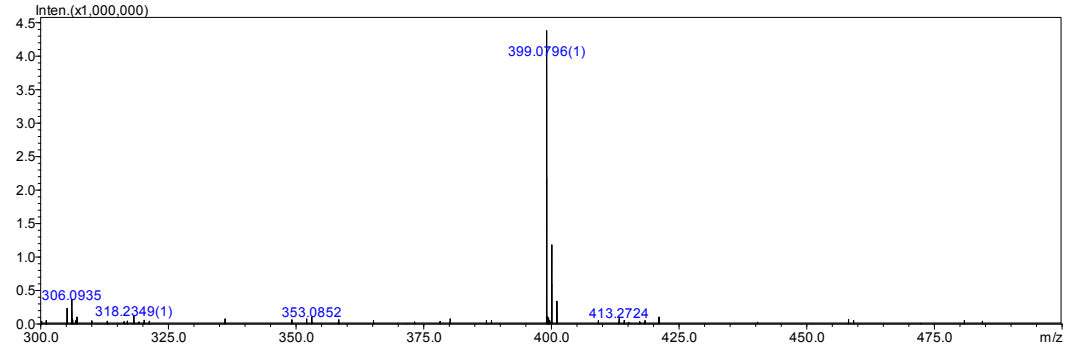

Figure S14. HRMS result of **2**.

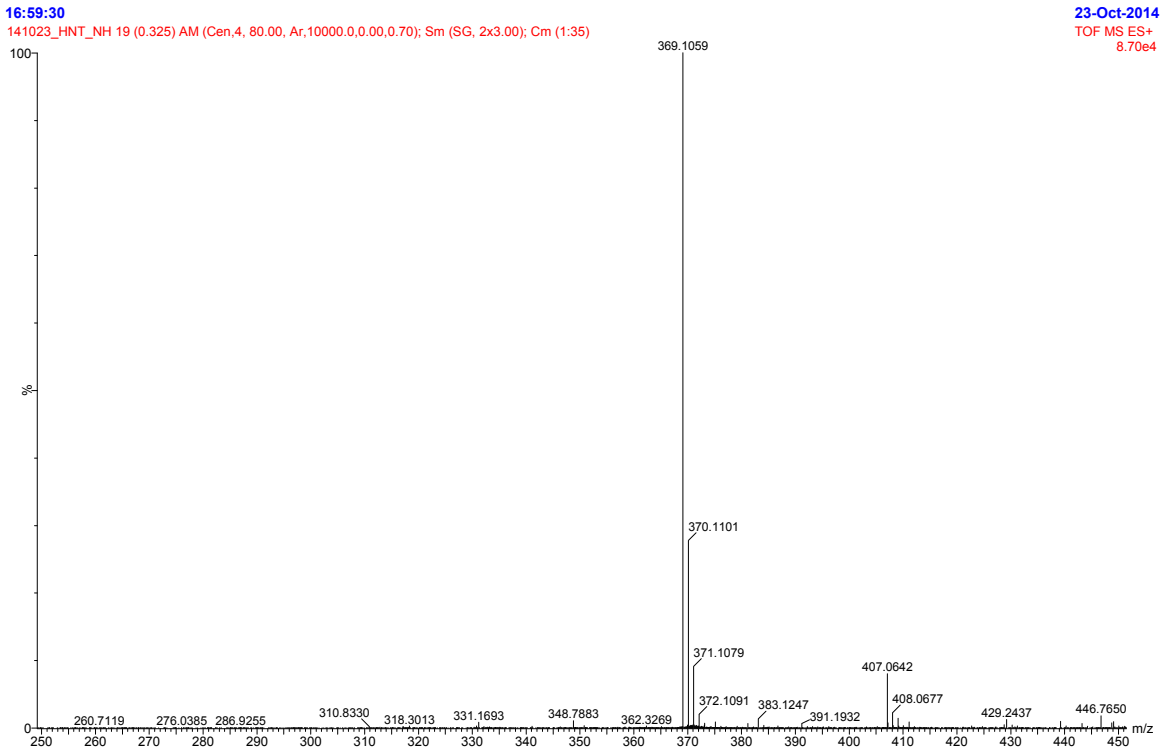

Figure S15 HRMS result of **3**.