Supporting Information

Contents

A Novel Fluorescent Probe with ESIPT and AIE Effects: "Ratio-type" Fluorescent Probe for Co^{2+} and "Turn-Off" Fluorescent Probe for HClO

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1. Materials and instrumentations

Chemicals and Materials. Titanium tetrachloride, benzophenone and trifluoroacetic acid were obtained from Energy (Nanjing, China). 4-Hydroxybenzophenone and 2-Benzothiazolinone, hydrazone were obtained from Bidepharm (Nanjing, China). Zinc powder was obtained from Shanghai Peak Chemical Reagent (Shanghai, China). Methenamine was obtained from Nanjing Chemical Reagent (Nanjing, China). Inorganic metal salts including $FeCl_3$, $AlCl_3$, $Cd(NO_3)_2$, $MgSO_4$, $CuCl_2$, $CoCl_2$, $BaCl_2$, $Cr(NO_3)_3$, $MnCl_2$, $NiCl_2$ and $ZnCl_2$, KSCN, $NaNO_2$, Na_2SO_3, NaClO were obtained from Energy (Nanjing, China). Other anions NaCl, $CaCl_2$, CH_3COOK, were obtained from Shanghai Peak Chemical Reagent (Shanghai, China). H_2O_2 were obtained from Aladdin (Nanjing, China). Pure water was obtained from Hangzhou Wahaha Group.

General Instrumentation. Fluorescence spectra were recorded on Configuration Fluoromax-4C-L 1446D-0216-FM equipped with the scan speed was 600 nm min^{-1} and the band pass of excitation and emission was set as 5 and 5 nm. ESI-MS data were recorded on Alilent 6230. NMR spectra were recorded on Bruker-AV300 with DMSO-d_6 as the solvent and tetramethylsilane (TMS) as internal standard, Dynamic light scattering (DLS) were recorded on Malvern Zetasizer.

2. Optical studies process

Fluorescence detection conditions were set as follows: Fluoromax-4 fluorescence spectrophotometer, room temperature, excitation wavelength: 360 nm, slits: (5 nm/5 nm).

2.1 preparation of stock solution

7.0 mg of compound AE-3 was accurately weighed and transferred to a 15 mL centrifuge tube, followed by the addition of 10 mL of tetrahydrofuran solution to obtain the probe stock solution A (1 mM). Then 1 mL of the probe stock solution A (1 mM) was transferred with a pipette into another 15 mL centrifuge tube, 9 mL of THF was added to obtain the probe stock solution B (100 μM).

Using the preparation of Fe^{3+} stock solution (100 μM) as an example, 16.2 mg of $FeCl_3$ was accurately weighed into a 15 mL centrifuge tube, 10 mL of pure water was added, resulting in a concentration of 10^{-2} mol/L Fe^{3+} solution. Then 1 mL of the 10^{-2} mol/L Fe^{3+} solution was transferred with a pipette into another 15 mL centrifuge tube, 9 mL of phosphate buffer solution (50 mM, pH=7.4) was added, resulting in a concentration of 10^{-3} mol/L Fe^{3+} solution. Repeat the dilution with phosphate buffer solution (50 mM, pH=7.4) to obtain the Fe^{3+} stock solution (100 μM). The same method was used to prepare other metal stock solutions (100 μM) including Fe^{3+}, Al^{3+}, Cd^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+} and Fe^{2+}.

Using the preparation of SCN^- stock solution (1 mM) as an example, 9.7 mg of KSCN

was accurately weighed into a 15 mL centrifuge tube, 10 mL of pure water was added , resulting in a concentration of 10^{-2} mol/L SCN^- solution. Then 1 mL of the 10^{-2} mol/L SCN^- solution was transferred with a pipette into another 15 mL centrifuge tube, 9 mL of phosphate buffer solution (50 mM, pH=7.4) was added to obtain SCN^- stock solution (1 mM). The same method was used to prepare other analytes stock solutions (1 mM) including SCN^-, NO^{2-}, NO^{3-}, SO_4^{2-}, SO_3^{2-}, Cl^-, and $CH3COO^-$.

7.5 μL of 10% active chlorine solution was transferred with a pipette into a 15 mL centrifuge tube, phosphate buffer solution (50 mM, pH=7.4) was added to 10 mL to obtain HClO stock solution (1 mM). Then 33 μL of 30% mass fraction hydrogen peroxide solution was transferred with a pipette into a 15 mL centrifuge tube, phosphate buffer solution (50 mM, pH=7.4) was added to 10 mL to obtain H_2O_2 stock solution (1 mM).

2.2 AIE study

300 μL of probe stock solution B (100 μM) was successively pipetted into 10 individual 5 mL centrifuge tubes. Subsequently, 2700 μL, 2400 μL, 2100 μL, 1800 μL, 1500 μL, 1200 μL, 900 μL, 600 μL, 300 μL, and 0 μL of THF were added to the respective tubes. Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to 3 mL, yielding solutions with water content of 0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90%, respectively. Next, 30 μL of probe stock solution A (1 mM) was pipetted into 2 individual 5 mL centrifuge tubes. Subsequently, 120 μL and 0 μL of THF were added to the tubes, followed by the addition of phosphate buffer solution (50 mM, pH 7.4) to each tube to reach a final volume of 3 mL, resulting in solutions with water content of 95% and 99%, respectively. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis. The prepared solutions with water content of 10%, 50%, and 99% were to be tested with DLS (Dynamic Light Scattering).

2.3 analyse of HClO

HClO selectivity experiment process: 300 μL of probe stock solution B (100 μM) was

successively pipetted into 9 individual 5 mL centrifuge tubes. Subsequently, 150 μL of analytes stock solutions (1 mM, including SCN^-, NO_2^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, Cl^-, CH_3COO^-, H_2O_2 and HClO). Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis.

HClO interference resistance experiment process: 300 μL of probe stock solution B (100 μM) was successively pipetted into 8 individual 5 mL centrifuge tubes. Subsequently, 150 μL of analytes stock solutions (1 mM, including SCN^-, NO_2^-, NO_3^-, SO_4^{2-}, SO_3^{2-}, Cl^-, CH_3COO^-, H_2O_2). Then, 150 μL of HClO stock solution (1 mM) was added to each centrifuge tube, and phosphate buffer solution (50 mM, pH=7.4) was added to bring the total volume to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis.

HClO time response experiment process: 300 μL of probe stock solution B (100 μM) was successively pipetted into 13 individual 5 mL centrifuge tubes. Then, 150 μL of HClO stock solution (1 mM) was added to each centrifuge tube, and add phosphate buffer solution (50 mM, pH=7.4) to bring the total volume to 3 mL. After 10 min, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, 300 min, 360 min, 900 min and 960 min, the prepared solutions were transferred into quartz cuvettes for fluorescence spectroscopy analysis.

Determination of the detection limit: 300 μL of probe stock solution B (100 μM) was successively pipetted into 10 individual 5 mL centrifuge tubes. Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis. Calculate the standard deviation(σ) of the fluorescence intensity at 556 nm for 10 samples. 300 μL of probe stock solution B (100 μM) was successively pipetted into 6 individual 5 mL centrifuge tubes. Subsequently, 30 μL, 60 μL, 90 μL, 120 μL, 150 μL and 180 μL of HClO stock solution (1 mM) was added to the respective tubes. Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the

solution to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis. Calculate the slope(k) of the linear relationship between the fluorescence intensity at 556 nm and the concentration of HClO. The limit of detection (Lod) of probe to target analyte was calculated by the formula: detection limit = 3σ/k.

2.4 analyse of Co^{2+}

Co^{2+} selectivity experiment process: 300 μL of probe stock solution B (100 μM) was successively pipetted into 12 individual 5 mL centrifuge tubes. Subsequently, 300 μL of analytes stock solutions (100 μM, including Fe^{3+}, Al^{3+}, Cd^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+} and Fe^{2+}). Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis.

Co^{2+} interference resistance experiment process: 300 μL of probe stock solution B (100 μM) was successively pipetted into 11 individual 5 mL centrifuge tubes. Subsequently, 300 μL of analytes stock solutions (100 μM, including Fe^{3+}, Al^{3+}, Cd^{2+}, Mg^{2+}, Cu^{2+}, Ba^{2+}, Cr^{3+}, Mn^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+} and Fe^{2+}). Then, 300 μL of Co^{2+} stock solution (100 μM) was added to each centrifuge tube, and phosphate buffer solution (50 mM, pH=7.4) was added to bring the total volume to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis.

Co^{2+} time response experiment process: 300 μL of probe stock solution B (100 μM) was successively pipetted into 10 individual 5 mL centrifuge tubes. Then, 300 μL of Co^{2+} stock solution (100 μM) was added to each centrifuge tube, and phosphate buffer solution (50 mM, pH=7.4) was added to bring the total volume to 3 mL. After 10 min, 20 min, 30 min, 40 min, 50 min, 60 min, 70 min, 80 min, 90 min and 100 min, the prepared solutions were transferred into quartz cuvettes for fluorescence spectroscopy analysis.

Determination of the detection limit: 300 μL of probe stock solution B (100 μM) was successively pipetted into 10 individual 5 mL centrifuge tubes. Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to

3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis. Calculate the standard deviation(σ) of the F556nm fluorescence intensity for 10 samples. 300 μL of probe stock solution B (100 μM) was successively pipetted into 12 individual 5 mL centrifuge tubes. Subsequently, 30 μL, 60 μL, 90 μL, 120 μL, 150 μL, 180 μL, 210μL, 240μL, 270μL, 300μL, 330μL and 360μL of Co^{2+} stock solution (100 μM) was added to the respective tubes. Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to 3 mL. The prepared solutions were then transferred into quartz cuvettes for fluorescence spectroscopy analysis. Calculate the slope(k) of the linear relationship between the F556nm fluorescence intensity and the concentration of HClO. The limit of detection (Lod) of probe to target analyte is calculated by the formula: detectionlimit = 3σ/k.

Job's curve determination： 0 μL, 60 μL, 120 μL, 180 μL, 240 μL and 300 μL of probe stock solution B (100 μM) was added to 6 individual 5 mL centrifuge tubes. Subsequently, 600μL, 540 μL, 480 μL, 420 μL, 360 μL and 300 μL of Co^{2+} stock solution (100 μM) was added to the respective tubes. After that, 300 μL, 240μL, 180 μL, 120 μL, 60 μL and 0 μL of THF was successively pipetted into each centrifuge tubes. Then, phosphate buffer solution (50 mM, pH=7.4) was added to each tube to bring the total volume of the solution to 3 mL. Subsequently, 36 μL, 42 μL, 48 μL, 54 μL, 60 μL of probe stock solution A (1 mM) was added to other 5 individual 5 mL centrifuge tubes. Add 240 μL, 180 μL, 120 μL, 60 μL, 0 μL of Co^{2+} stock solution (100 μM) and 228 μL, 216 μL, 204 μL, 192 μL, 180 μL of THF into centrifuge tubes in sequence. Then, phosphate buffer solution (50 mM, pH=7.4) was also added to each tube to bring the total volume of the solution to 3 mL.

2.5 practical water detection

Sample sources: T-water, tap water from the experimental building of China Pharmaceutical University. B-water, tap water boiled and cooled to room temperature. L-water, water from the artificial lake at China Pharmaceutical University.

1. 300 μL of probe stock solution B (100 μM) was added to 5 mL centrifuge tubes，

300μL Co^{2+} stock solution (100 μM) and 2400μL phosphate buffer solution (50 mM, pH=7.4) to prepare the Co^{2+} target analyte.

2. 300 μL of probe stock solution B (100 μM) was added to 4 individual 5 mL centrifuge tubes. Then, T-water, B-water, L-water and phosphate buffer solution (50 mM, pH=7.4) were added to each tube to bring the total volume of the solution to 3 mL, resulting in three practical samples and blank probe sample.

3. 300μL of probe stock solution B (1mM) was added to 5 mL centrifuge tubes, 300μL HClO stock solution (1 mM), 300μL of THF and 2100μL phosphate buffer solution (50 mM, pH=7.4) were added to prepare the HClO target analyte.

4. 300 μL of probe stock solution B (1mM) was added to 4 individual 5 mL centrifuge tubes. Subsequently, 600μL of THF was added to the respective tubes. Then, T-water, B-water, L-water and phosphate buffer solution (50 mM, pH=7.4) were added to each tube to bring the total volume of the solution to 3 mL, resulting in three practical samples and blank probe sample.

3. Other fluorescence spectra

3.1 AIE effect

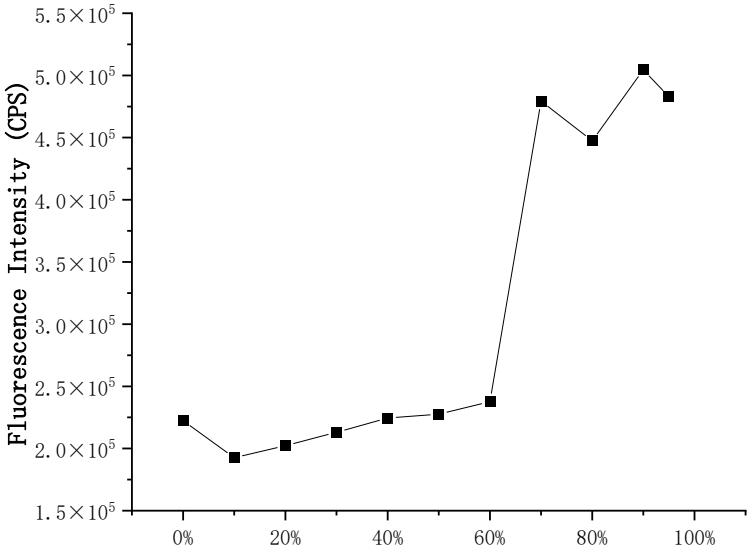

Figure S 1 The relationship between the fluorescence intensity of AE-3 (10 μM) at 556nm and different water content (0%-99%) in THF/phosphate buffer (50 mM, pH=7.4)

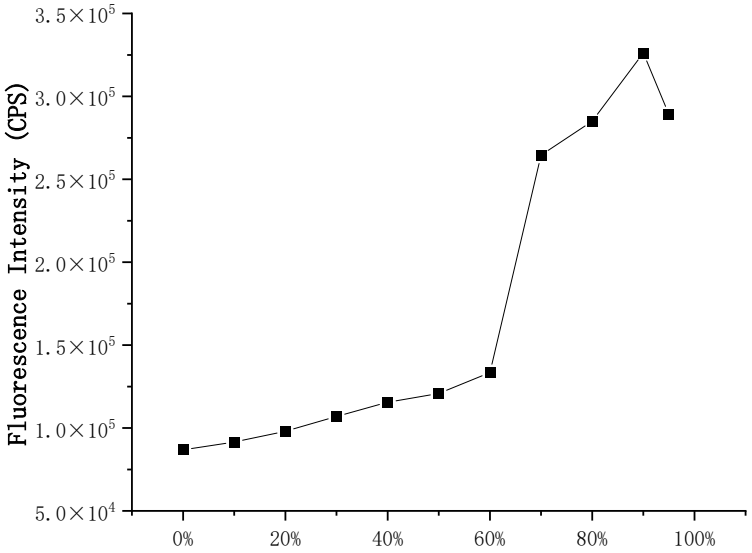

Figure S 2 The relationship between the fluorescence intensity of AE-3 (10 μM) at 474nm and different

water content (0%-99%) in THF/phosphate buffer (50 mM, pH=7.4)

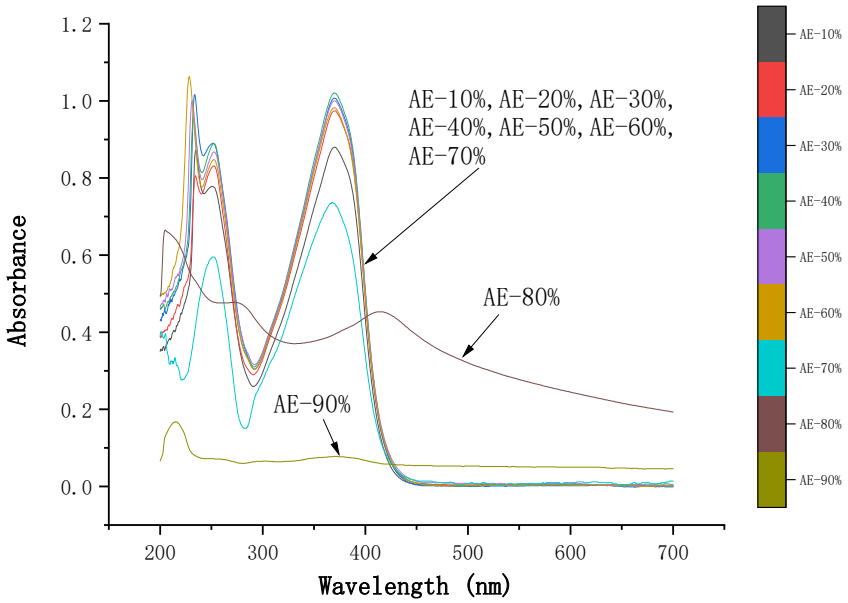

Figure S 3 the UV spectra of AE-3 (10 μM) in a THF/phosphate buffer (50 mM, pH=7.4) with different water content.

3.2 Co^{2+} fluorescence spectroscopy

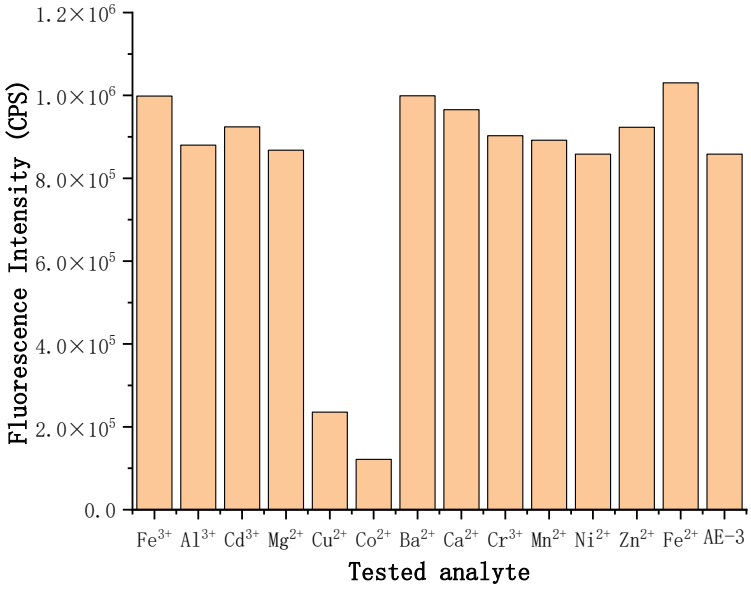

Figure S 4 The 556nm fluorescence intensity of the AE-3 (10 μM) in the presence of different analytes (10

μM) in THF/phosphate buffer (1/9, v/v, 50 mM, pH=7.4)

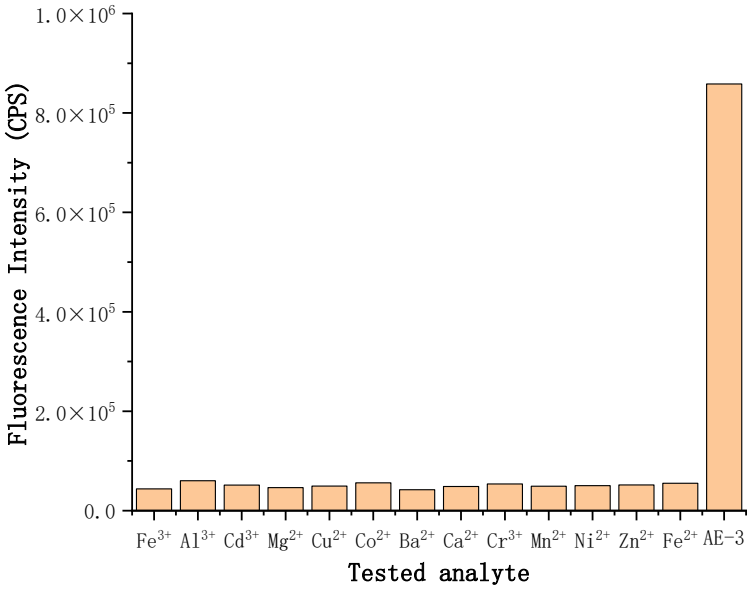

Figure S 5 The 556nm fluorescence intensity of the AE-3 (10 μM) and Co2+ (10 μM) in the presence of different analytes (10 μM) in THF/phosphate buffer (1/9, v/v, 50 mM, pH=7.4).

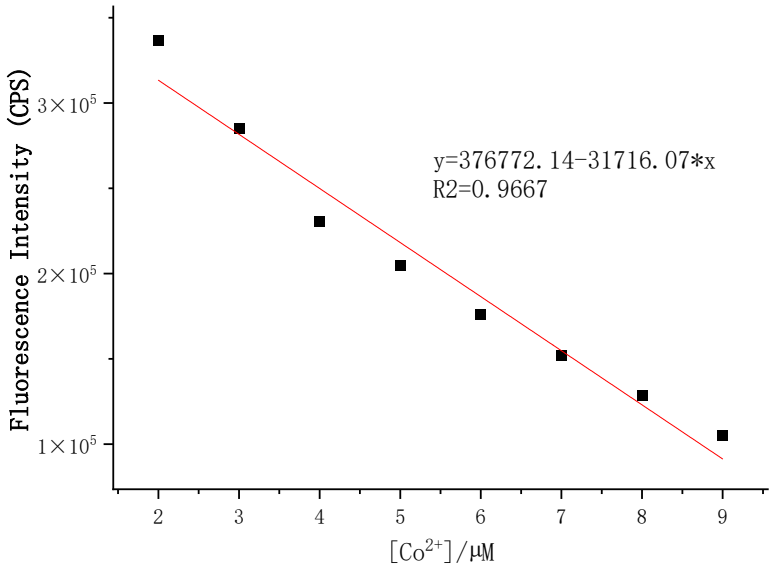

Figure S 6 The linear relationship between556nm fluorescence intensity of AE-3 (10 μM) and Co^{2+} (0.1-0.9eq).

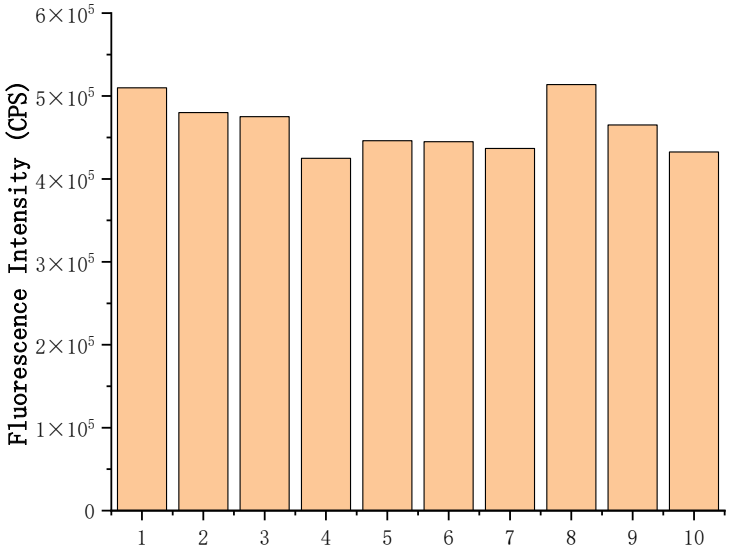

Figure S 7 The 556nm fluorescence intensity of 10 blank specimens AE-3 (10 μM) in THF/phosphate buffer (1/9, v/v, 50 mM, pH=7.4)

Table 1

Probe	**Detection Limit**	**Solvent**	**Ex/Em (nm)**	**Ref.**
1	100 μ M	DMSO	470/515	[1]
2	734 μ M	DMSO–water1:1(v/v)	-/520	[2]
3	0.65mM	Bis–trisbuffer(pH7)	-/420	[3]
4	0.19mM	DMSO/bis–trisbuffer solution	-/634	[4]
5	22.7 μ M	DMF/CDCl3/CD3CN, water	530/522	[5]
6	41 μ M	Ethanol, CH3Cl	350/474	[6]

7	7.06 μ M	Ethanol, DMSO,	240/445	[7]
8	100 μ M	DMF/H_2O	Emission at 336 and 442	[8]
9	2.823 μM	THF/phosphate buffer (1/9, v/v, pH=7.4)	360/556	This work

3.3 HClO fluorescence spectroscopy

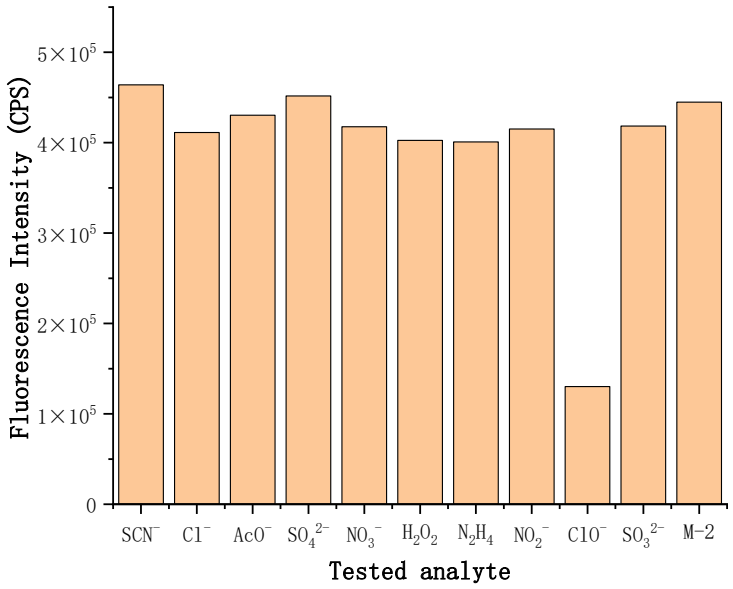

Figure S 8 The fluorescence intensity at 556nm bar graph of the AE-3 (10 μM) in the presence of different analytes (50 μM) in THF/phosphate buffer (1/9, v/v, 50 mM, pH=7.4)

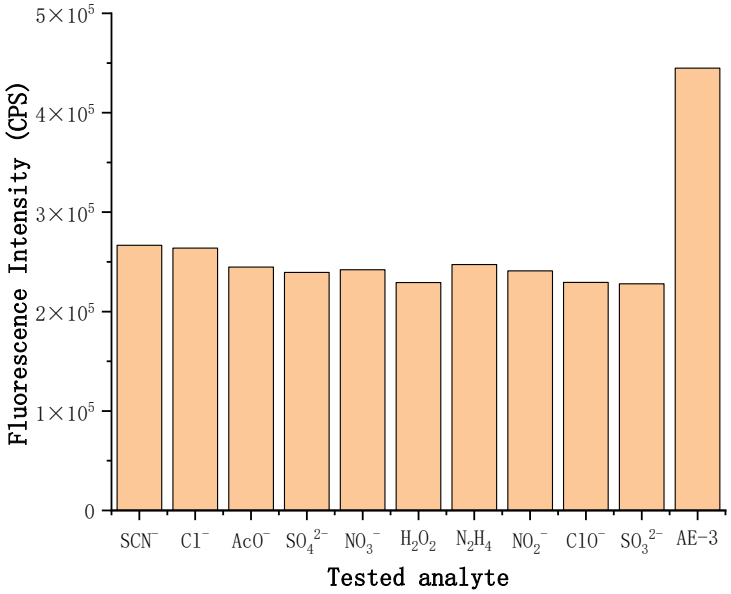

Figure S 9 The fluorescence intensity at 556nm bar graph of the AE-3 (10 μM) and HClO (50 μM) in the presence of different analytes (50 μM) in THF/phosphate buffer (1/9, v/v, 50 mM, pH=7.4)

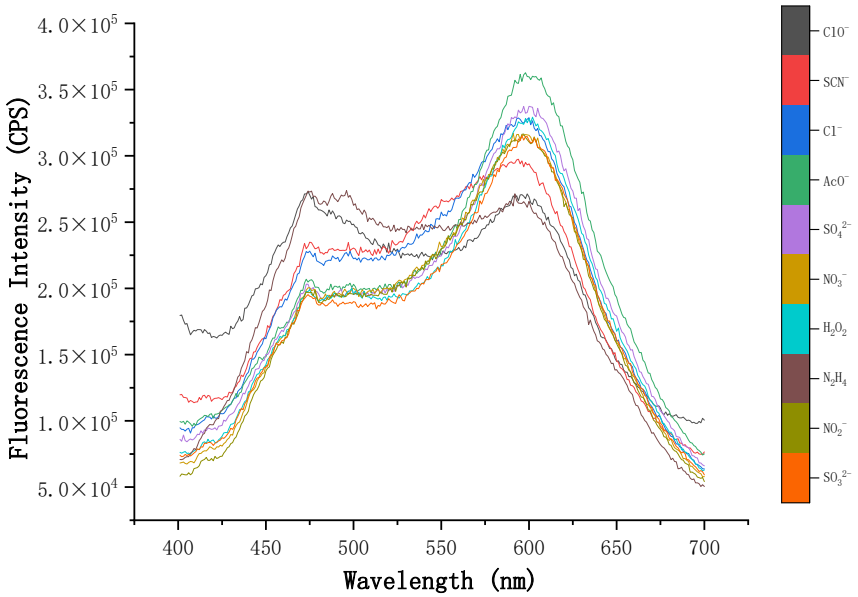

Figure S 10 The fluorescence spectra of AE-3 (10 μM) and HClO+ (50 μM) with addition of analyte (50 μM) in THF/phosphate buffer (1/9, v/v, 50 mM, pH=7.4)

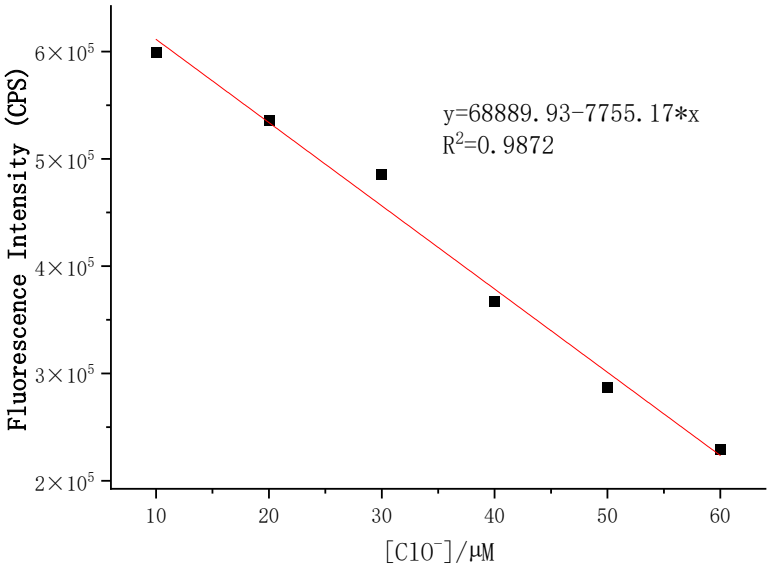

Figure S 11 The linear relationship between fluorescence intensity at 556nm of AE-3 (10 μM) and HClO (1-6eq)

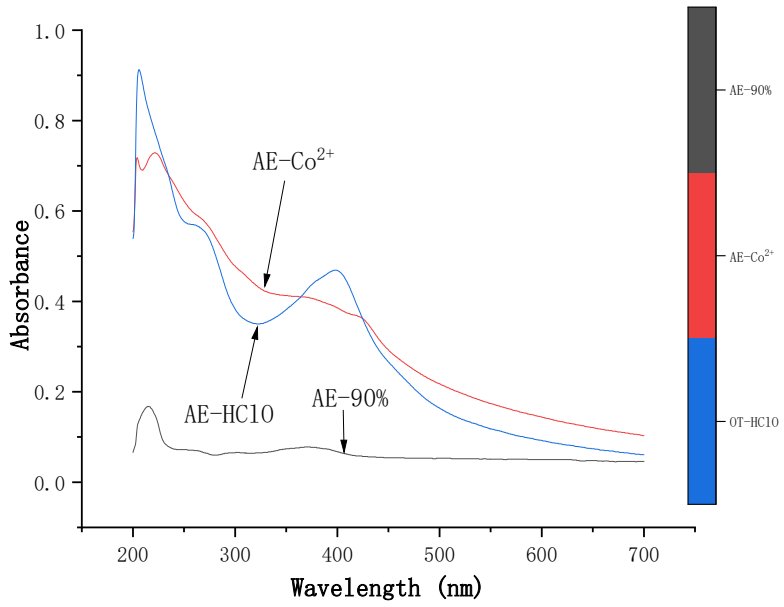

Figure S 12 UV spectra of the probe alone, with hypochlorous acid, and with cobalt ions in THF/phosphate buffer (50 mM, pH=7.4).

Table 2

Probe	Detection Limit	Solvent	Ex/Em (nm)	Ref.

9	0.8μM	10mMPBS,pH7.4	320/388	[9]
10	1.4μM	10mMHEPES-CH3CN(9/1,v/v),pH7.4	420/490	[10]
11	6.2μM	20mMHEPES-EtOH(6/4,v/v),pH7.0	540/583	[11]
12	5μM	PBS-DMSO(6/4,v/v),pH7.4	490/524	[12]
13	3.7μM	0.1MPBSwith1%EtOH,pH7.5	572/597	[13]
14	50μM	0.1MNa2CO3-NaHCO3buffer-DMFsolution(30/1,v/v),pH¼9.0	290/-	[14]
15	2μM	Waterwith1.5%DMF	470/-	[15]
17	11.55 μM	THF/phosphate buffer (1/9, v/v, pH=7.4)	360/556	This work

4. NMR Spectrum

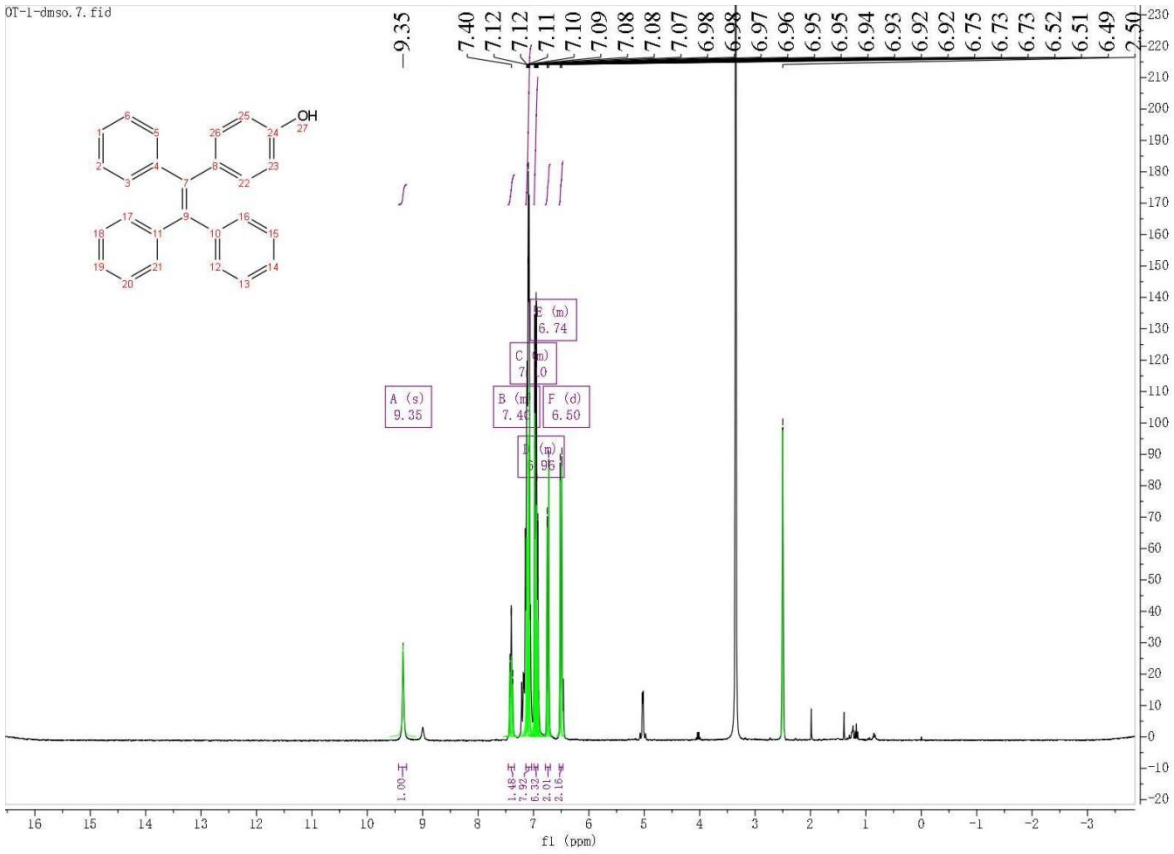

Figure S 13 The NMR Hydrogen Spectrum of AE-1

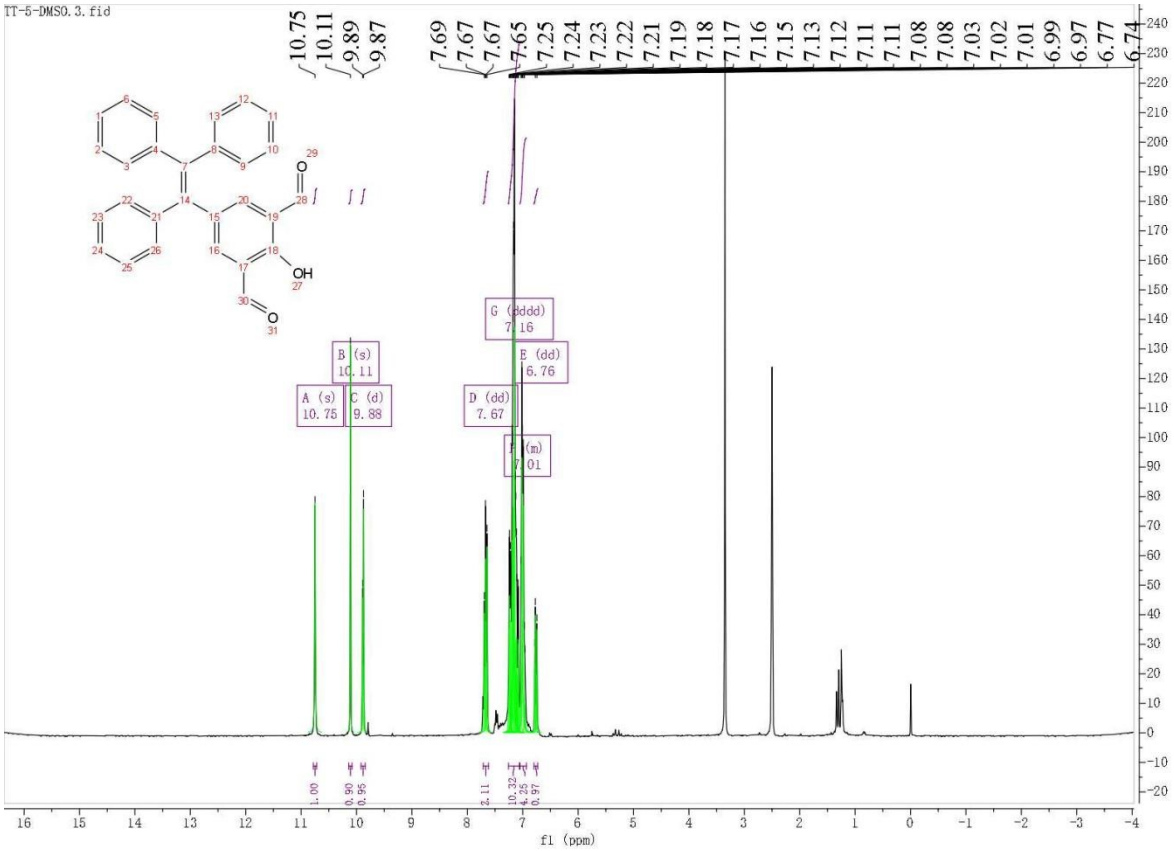

Figure S 14 The NMR Hydrogen Spectrum of AE-2

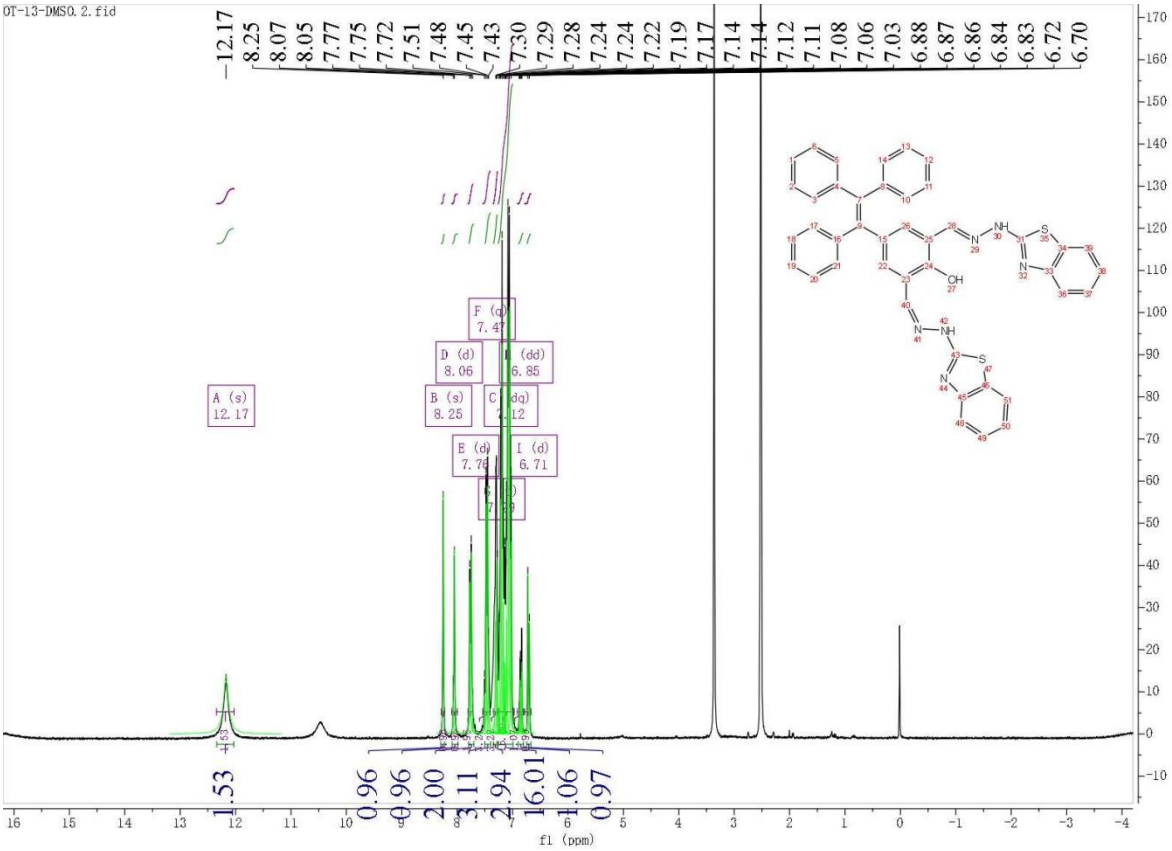

Figure S 15 The NMR Hydrogen Spectrum of AE-3

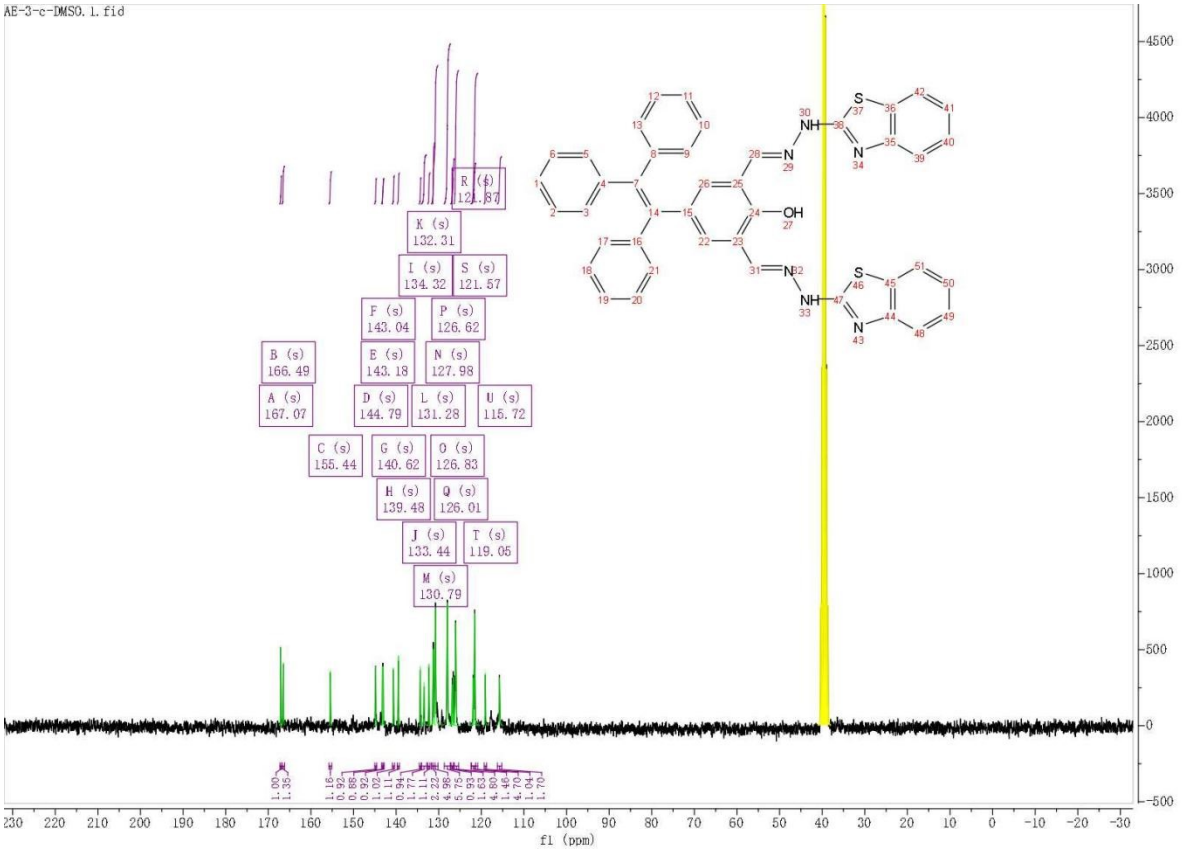

Figure S 16 The NMR Carbon Spectra of AE-3

5. Mass Spectrum

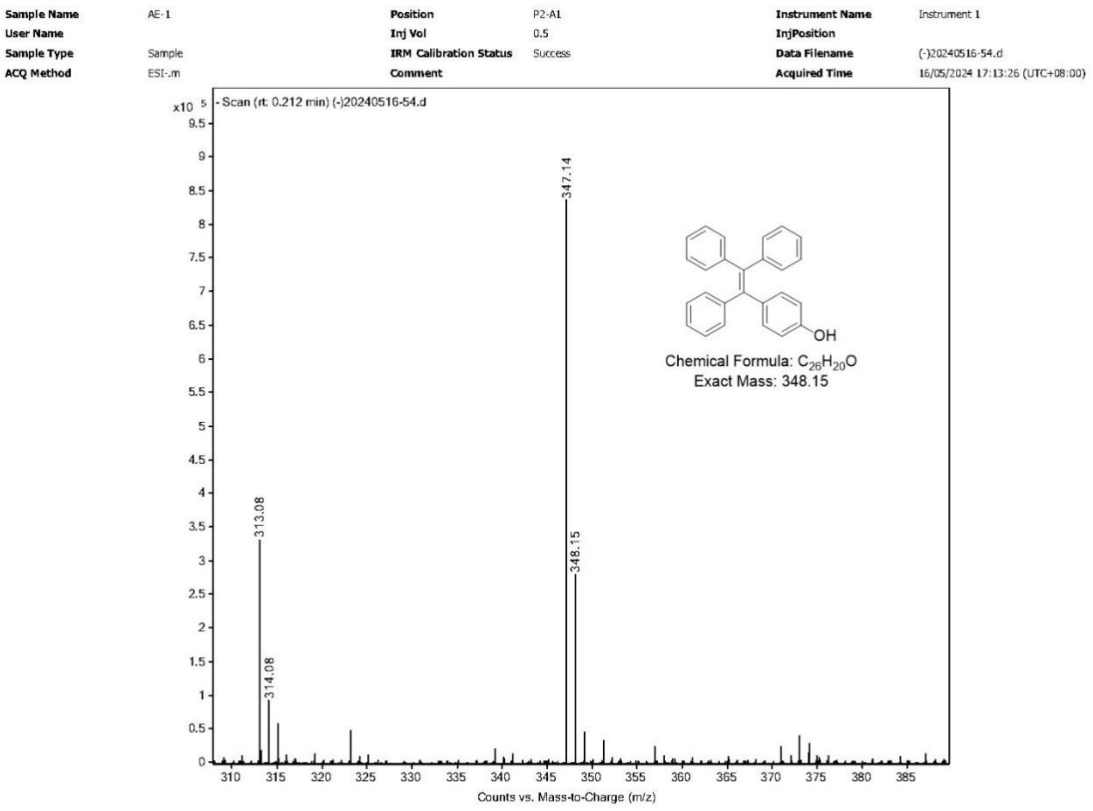

Figure S 17 Mass Spectrum of AE-1

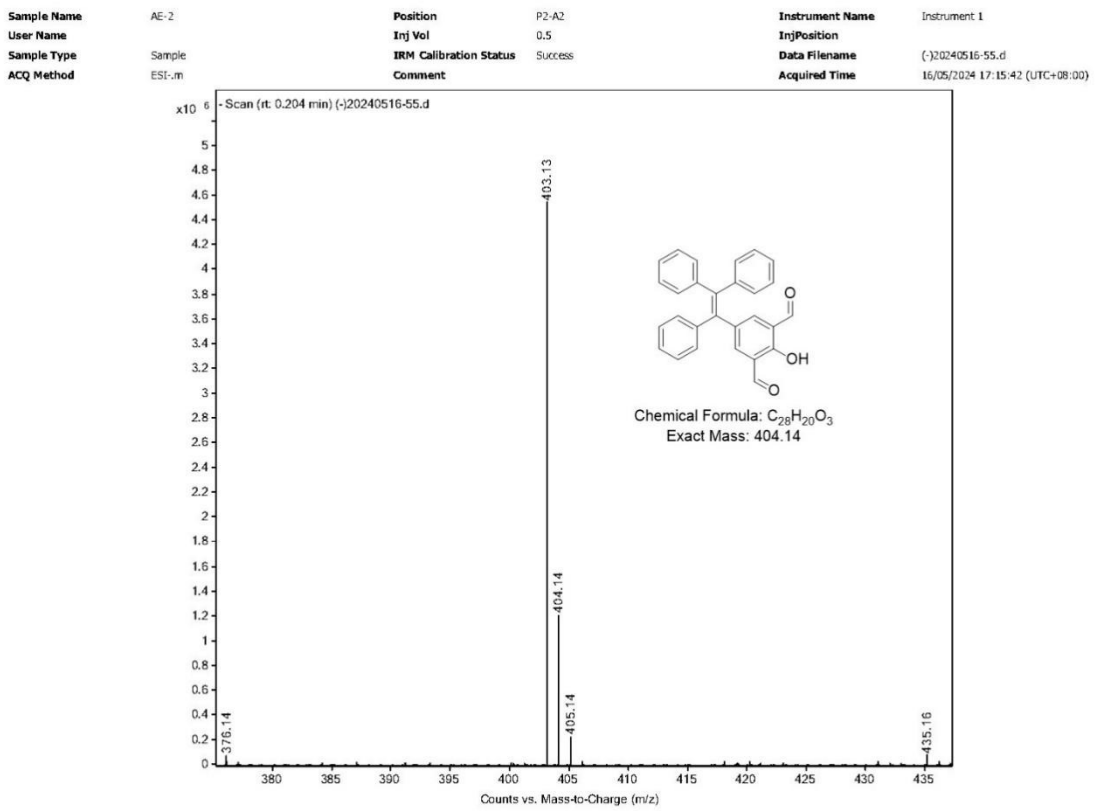

Figure S 18 Mass Spectrum of AE-2

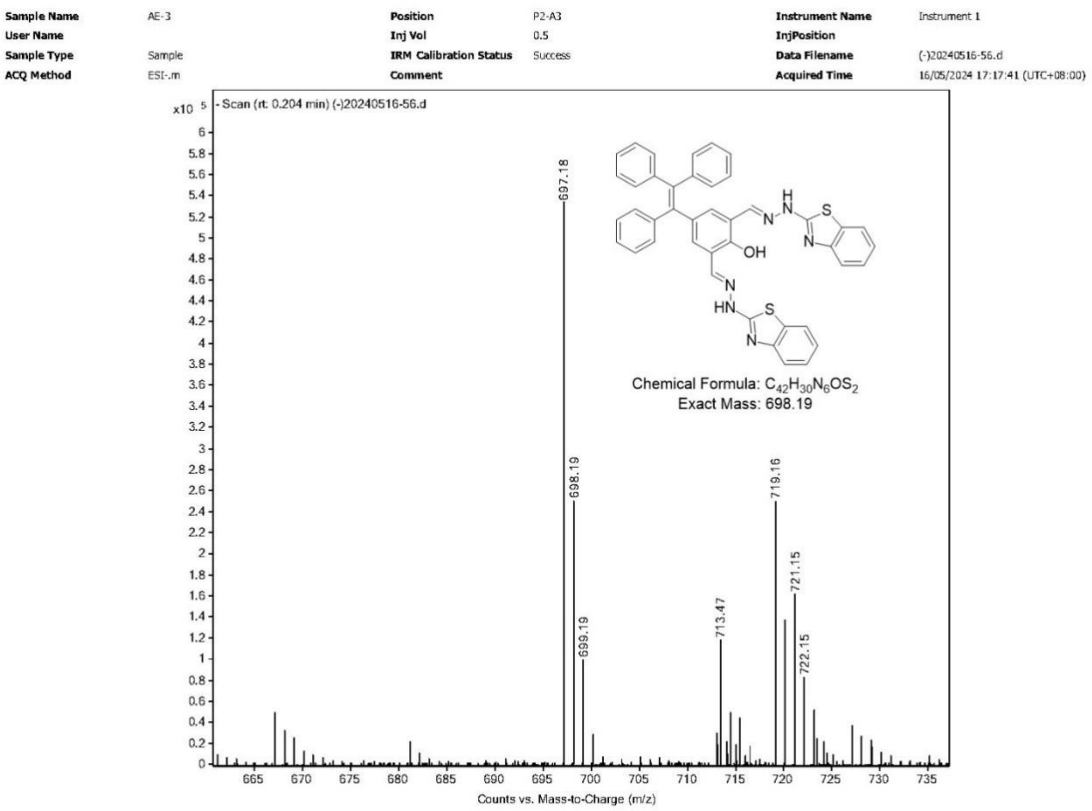

Figure S 19 Mass Spectrum of AE-3

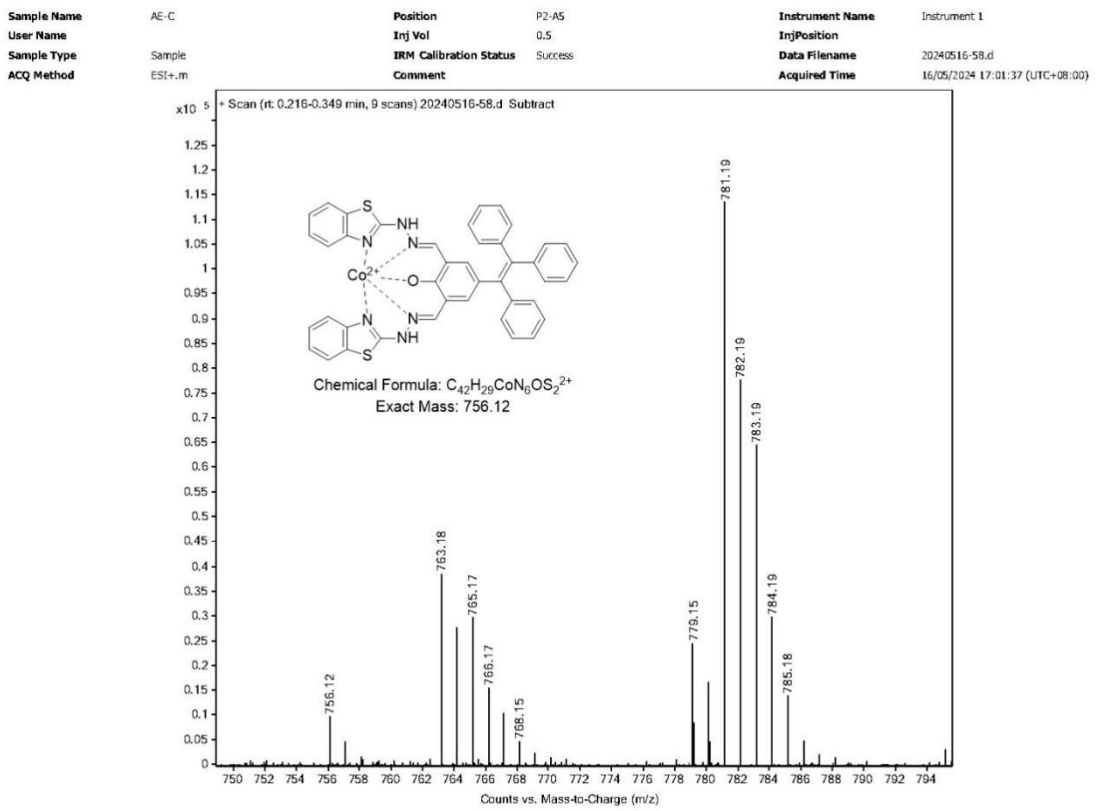

Figure S 20 The mass spectrum of compound AE-3 after chelation with cobalt ions

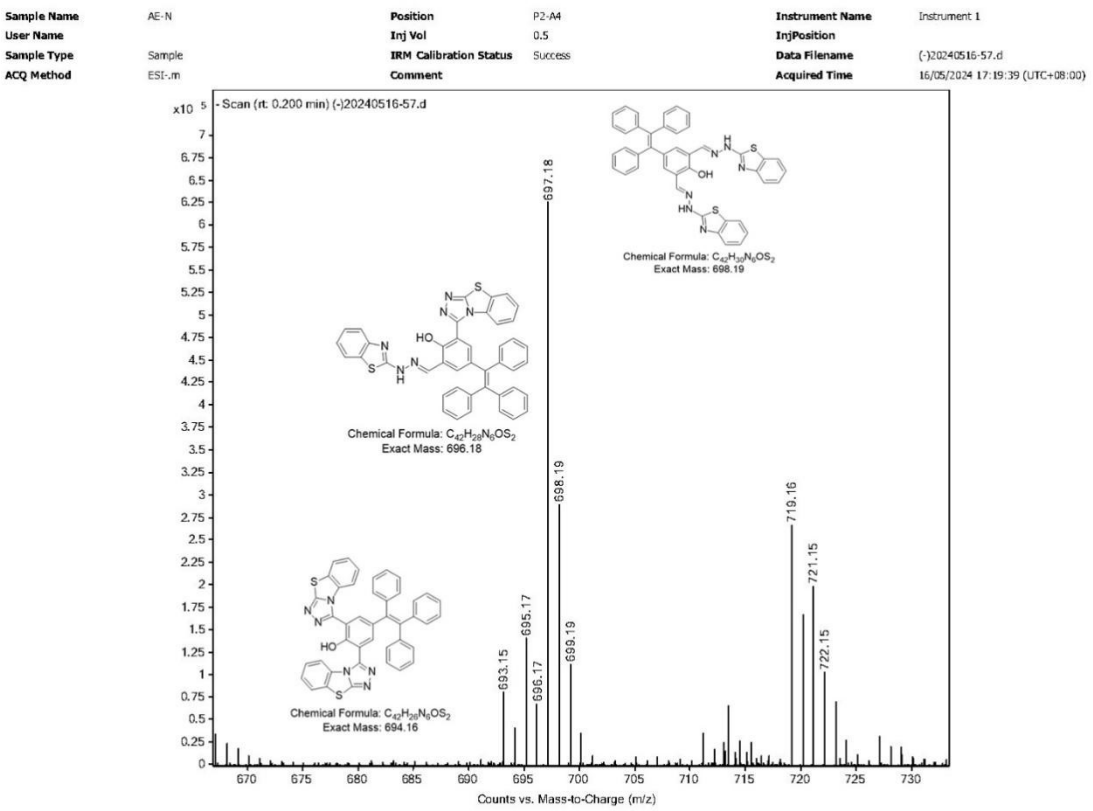

Figure S 21 The mass spectrum following the reaction of compound AE-3 with hydrazine.

References:

[1] ABEBE F A, ERIBAL C S, RAMAKRISHNA G, et al. A 'turn-on' fluorescent sensor for the selective detection of cobalt and nickel ions in aqueous media[J]. Tetrahedron Letters, 2011, 52(43): 5554-5558.

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