

An anthracene based fluorescent probe for the selective and sensitive detection of Chromium (III) ions in an aqueous medium and its practical application

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Abstract: An anthracene based fluorescent probe, integrated with thiophene moiety, exhibited selective and sensitive detection of chromium (III) ions over other metal ions. Its synthesis was achieved by simple mixing of two commercially available compounds, 2-aminoanthracene, and 2-thiophenecarboxaldehyde, in onestep without the needed complex purification process. The probe molecule (**ANT-Th**) offered exceptional features such as “turn-on” fluorescence response, low detection limit (0.4 μM), and fast response time (<1 min) via $\text{C}=\text{N}$ bond hydrolysis. Also, a simple test paper system was developed for the rapid detection of chromium (III) ions with the naked eye.

Key words: Anthracene, thiophene, “turn-on” chemodosimeter, chromium (III) probe

1. Introduction

Among the heavy metal ions, chromium (III) ion has great impacts on the various systems such as chemical, biological and environmental systems. It is a crucial trace element in human nutrition to balance the “glucose tolerance factor”[1] and also acts as an essential role in the metabolism of proteins, nucleic acids, carbohydrates, and fats [2,3]. The National Research Council of United State has highly recommended taking 50–200 $\mu\text{g d}^{-1}$ as an important amount of chromium (III) ion. Intake deficiency of chromium (III) ion can cause different types of diseases such as diabetes, cardiovascular disease, and impaired immune function [4,5]. However, the higher level of chromium (III) ion can lead to an abnormal effect on enzymatic activities and damage cellular structures [6,7]. Besides, chromium can also be revealed in the environment as a pollutant during some manufacturing processes such as tanning, steelworks, chromate, and chrome pigment production [8–10]. Therefore, sensitive, effective, and practical detection methods for chromium (III) ions are highly demanded.

Recently, fluorescence sensing methods have been very popular for the detection of trace metal analysis compared to the current traditional techniques such as atomic emission spectroscopy (AES), atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS) [11–16]. Because they have many advantages such as operational simplicity, low-cost equipment, real-time detection, high sensitivity, and reproducibility. Some chromium (III) ion-selective chemical sensors have been developed by exploiting different types of fluorophore units including rhodamine [17–19], BODIPY [20,21], coumarin [22], anthracene [23,24]. However, some of these have still some drawbacks such as crosssensitivity toward other trivalent metal cations (especially Al^{3+} , Fe^{3+}), slow response, low water solubility, and high detection limit. Therefore, it seems

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that there is a demand to develop new fluorescent sensors for chromium (III) ions which can overcome these problems.

Among the known fluorescent molecules, anthracene has attracted a great deal of our attention due to its unique properties such as its chemical stability, high quantum yield, simple structure, and easily chemical modification. Thus, anthracene and its derivatives have been used as molecular probes for the detection of pH, metal ions, and small organic molecules [25–32].

In this study, a facile one-step synthesis of a Schiff base probe (**ANT-Th**) is presented to detect chromium ions in aqueous media. This probe molecule offers distinct properties such as fast “turn-on” response, operability in the water medium, low detection limit, and the ability to differentiate chromium (III) from chromium (VI).

2. Experimental details

2.1. General methods

All reagents were purchased from the commercial suppliers (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany, and Merck KGaA, Darmstadt, Germany) and used without further any purification. ^1H NMR and ^{13}C NMR were measured on a VNMRJ 600 nuclear magnetic resonance spectrometer (Varian Inc., Palo Alto, CA, USA). Mass analyses were conducted with Thermo Q-Exactive Orbitrap device (Thermo Fisher Scientific Inc., Waltham, MA, USA). Fluorescence emission spectra were obtained using a Varian Cary Eclipse fluorescence spectrophotometer (Varian Inc.).

2.2. Preparation fluorescence emission measurement solutions

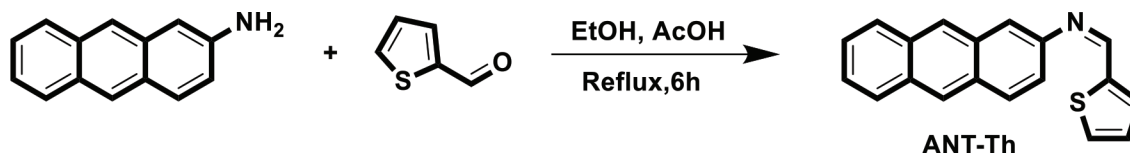
The stock solution of probe molecule **ANT-Th** (1 mM) was prepared in CH_3CN and stock solutions of metal ion salts (20 mM) were prepared in triple distilled deionized water. During the measurements, the metal ion solution was added into the probe solution (2 mL) using a micropipette. For fluorescence measurements, samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 390 nm, the emission spectra were integrated over a range of 410–700 nm (both excitation and emission slit a width of 5 nm/5 nm). All measurements were conducted in triplicate at least.

2.3. Synthesis of **ANT-Th**

2-aminoanthracene (200.0 mg, 1.03 mmol) and 2-thiophenecarboxaldehyde (116.1 mg, 1.03 mmol) were mixed in 10 mL ethanol in the presence of catalytic amount (2–3 drops) acetic acid (AcOH). The solution mixture was refluxed for 6 h under the nitrogen atmosphere. The obtained solid was filtered and recrystallized in an EtOH- CH_2Cl_2 mixture (3:1 v/v) to get the desired product **ANT-Th** as a dark green solid (67%). $^1\text{H-NMR}$ (600 MHz, d-DMSO): δ ppm 9.00 (s, 1H, N=C-H), 8.55 (d, $J = 12.6$ Hz, 2H, Ar-H), 8.12 (d, $J = 9$ Hz, 1H, Ar-H), 8.06 (t, $J = 8.4$ Hz, 2H, Ar-H), 7.87 – 7.84 (m, 2H, Ar-H), 7.74 – 7.73 (m, 1H, thiophene-H), 7.57 (d, $J = 9$ Hz, 1H, thiophene-H), 7.58 – 7.48 (m, 2H, Ar-H), 7.25 (t, $J = 4.2$ Hz, 1H, thiophene-H). APT $^{13}\text{C-NMR}$ (150 MHz, d-DMSO): δ ppm 157.0, 151.0, 145.7, 137.0, 134.9, 134.9, 134.5, 134.1, 133.1, 132.5, 131.5, 131.3, 130.9, 129.2, 129.1, 129.0, 128.6, 124.5, 121.4. HRMS: m/z : Calcd. for ($\text{C}_{19}\text{H}_{13}\text{NS}$) $[\text{M}+\text{H}^+]$: 287.08470; found, 288.08493.

3. Results and discussion

The sensor molecule **ANT-Th** was obtained via facile one-step acid-catalyzed condensation reaction of readily available 2-aminoanthracene and 2-thiophenecarboxaldehyde without applying column chromatography for the purification process (Scheme 1). The chemical identity of **ANT-Th** was confirmed by nuclear magnetic resonance (NMR) spectroscopy and high-resolution mass spectroscopy (HRMS) techniques, as depicted in the Supporting Information (SI).



Scheme 1. Synthesis pathway of ANT-Th.

Firstly, it was investigated as the most efficient reaction medium for sensing events. Since **ANT-Th** structure hardly dissolves in completely aqueous media, the reasonable organic co-solvent is needed to increase the solubility, so the organic cosolvent should be miscible in water. Most proper solvent combinations were determined as EtOH-H₂O and CH₃CN-H₂O mixtures yet sensing media was chosen as CH₃CN: H₂O due to the high fluorescence intensity changes. Then, all percent combinations (9:1, 8:2, 7:3, etc.) were tried and the best ratio was found as CH₃CN: H₂O (6:4 v/v) (Figure S1). However, it was realized that the pH of the sensing medium was effective for the detection of chromium (III) (Cr³⁺) ions. As can be seen in Figure 1, variance in the pH value led to obvious fluorescence intensity changes in the system. Thus, the pH of the sensing medium was adjusted to pH = 7.0 with HEPES buffer.

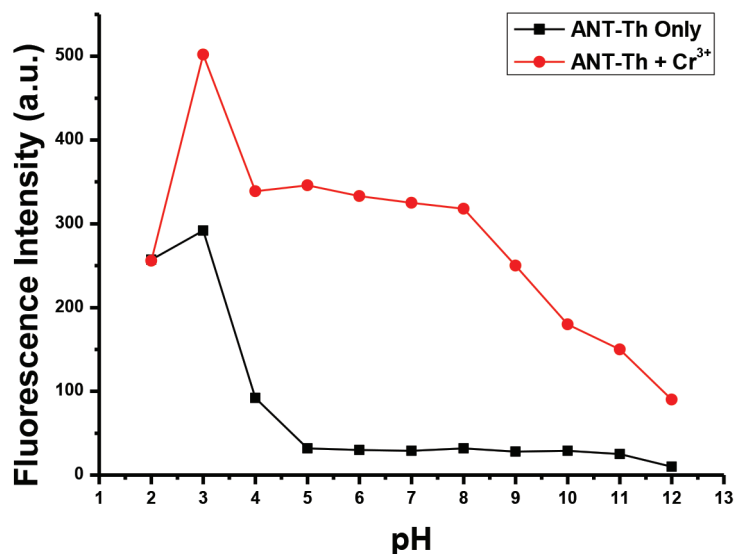


Figure 1. Effect of pH on the fluorescence intensity of ANT-Th (10 μ M) in 6:4 CH₃CN/HEPES in the absence (black line) and presence (red line) of Cr³⁺ (3.0 equiv.).

As anticipated, the free **ANT-Th** was the fluorescence-off mode ($\Phi_F = 0.01$) due to the photoinduced electron transfer (PET) process in which the lone pair electrons on the nitrogen atom are transferred to the

anthracene unit and as a result, its fluorescence ability diminishes. Upon the addition of the Cr^{3+} ions into the solution, a new strong emission peak appeared at 500 nm in a very short time ($\Phi_F = 0.38$). The emission intensity reached its maxima when 7 equiv. of Cr^{3+} ions were added, with a 30-fold enhancement (Figure 2). The detection limit (LOD) of the sensor molecule (**ANT-Th**) for Cr^{3+} ions was calculated as 0.4 μM (21 ppb) based on a signal-to-noise ratio ($S/N = 3$) (Figure S2). The spectroscopic response of **ANT-Th** was quite fast (<1 min), and complete saturation was observed after 5 min (Figure S3). Comparison of the **ANT-Th** with some other Cr^{3+} sensitive fluorescent probes (Table S1) [22,24,33–42] clearly shows the superiority of this method. The LOD value of **ANT-Th** was lower than the other Schiff bases based fluorescent probe, as indicated in Table S1 [22,34,36,41,42]. Similarly, the response time of **ANT-Th** was quite rapid than other works [24,35,38–40,42]. Moreover, our probe can be the cheapest method as it involves a facile one-step reaction with commercially available chemicals without further complex purification process (Table S1).

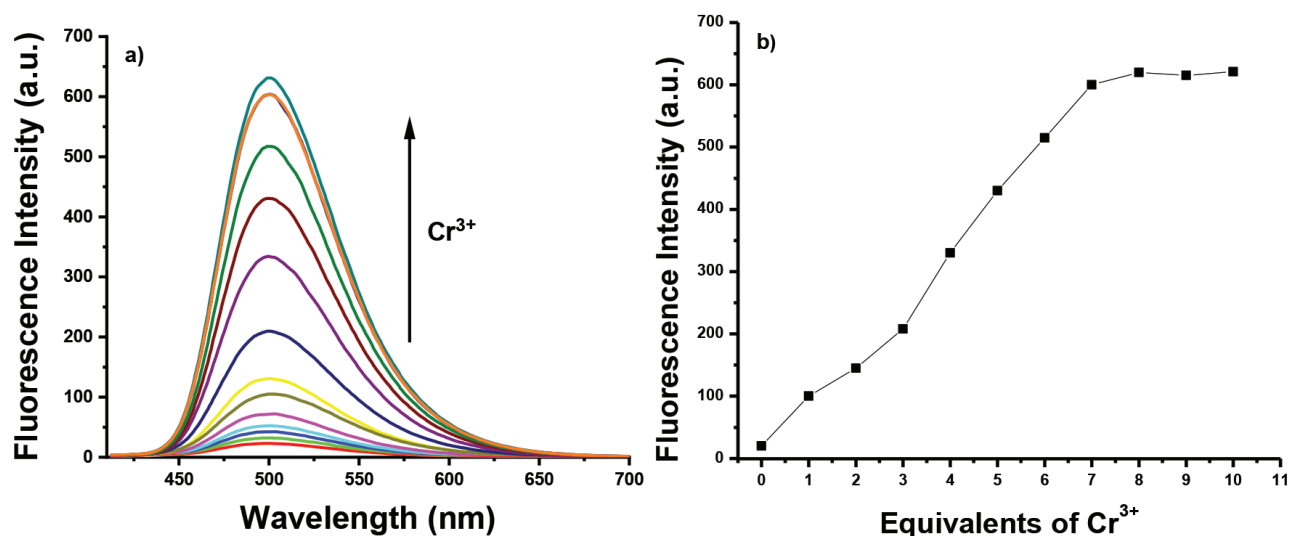


Figure 2. (a) Fluorescence titration spectra of **ANT-Th** (10 μM) in 6:4 $\text{CH}_3\text{CN}/\text{HEPES}$ at $\text{pH} = 7.0$ in the presence of Cr^{3+} , (b) Fluorescence intensity changes depending on the number of equivalents of Cr^{3+} (mole equivalents = 0–10 equiv.).

The selectivity study of **ANT-Th** was investigated by screening fluorescence intensity changes towards other metal ions including Na^+ , K^+ , Li^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Ag^+ , Hg^{2+} , Zn^{2+} , Pb^{2+} , Ni^{2+} , Cd^{2+} , Co^{2+} , Cr^{6+} , Fe^{3+} , Al^{3+} , and Ce^{3+} . Fortunately, no significant alteration was observed in the presence of other metal ions except for Fe^{3+} and Al^{3+} . These two metal species caused a similar fluorescence intensity change, but their intensities were relatively lower than that induced by Cr^{3+} even if their concentrations (250 μM) were much more than Cr^{3+} (50 μM) ions concentration (Figure 3a). It was further assessed the interference of other metal ions for the detection of Cr^{3+} by competitive experiments. The results showed that **ANT-Th** can properly detect Cr^{3+} ions in the mixture of other related metal ions (Figure 3b).

To understand the sensing mechanism reversible or not, an excess amount of EDTA was added into the reaction medium containing **ANT-Th** probe and Cr^{3+} ions. Interestingly, the solution preserved its emission intensity, verifying that the sensing mechanism could be irreversible chemical reaction triggered by Cr^{3+} ions. The final product of the sensing event could be easily monitored by thin-layer chromatography (TLC). The

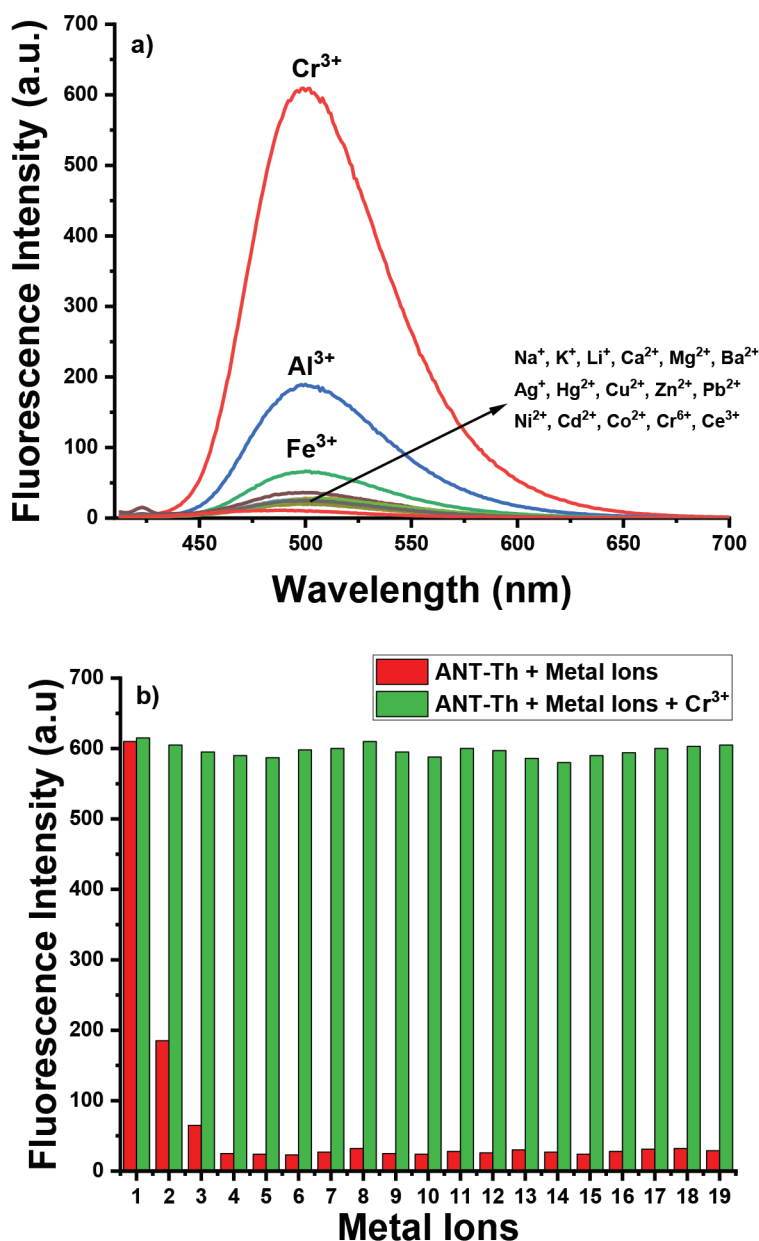
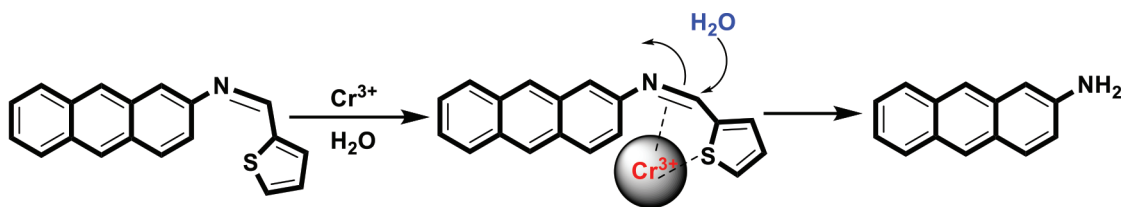


Figure 3. (a) Fluorescence intensities of ANT-Th (10 μM) in 6:4 $\text{CH}_3\text{CN}/\text{HEPES}$ at $\text{pH}=7.0$ in the presence of Cr^{3+} (5.0 equiv.) and other metal ions (25.0 equiv.), (b) Fluorescence intensities of ANT-Th (10 μM) in 6:4 $\text{CH}_3\text{CN}/\text{HEPES}$ at $\text{pH} = 7.0$ in the presence of Cr^{3+} (5.0 equiv.) and 25.0 equiv. other metal ions 1, Cr^{3+} ; 2, Al^{3+} ; 3, Fe^{3+} ; 4, Na^+ ; 5, K^+ ; 6, Li^+ ; 7, Ca^{2+} ; 8, Mg^{2+} ; 9, Ba^{2+} ; 10, Ag^+ ; 11, Hg^{2+} ; 12, Cu^{2+} ; 13, Zn^{2+} ; 14, Pb^{2+} ; 15, Ni^{2+} ; 16, Cd^{2+} ; 17, Co^{2+} ; 18, Ce^{3+} ; 19, Cr^{6+} .

appearance of the green emissive spot on the TLC plate with the same R_f value and the emission color of 2-aminoanthracene proved that the hydrolysis reaction of the **ANT-Th** probe occurred (Figure S4). With the help of HRMS analysis of the probe solution (**ANT-Th** + Cr^{3+}) revealed that a main molecular ion peak at m/z 194.09 pointed out the exact molecular weight of the 2-aminoanthracene. As shown in Scheme 2, the sensing mechanism was proposed to proceed through the simultaneous coordination of Cr^{3+} ions with nitrogen

atom on C=N moiety and S atom in the thiophene part. This event induced an attack of the water molecule to imine moiety leading the formation of hydrolysis product, 2-aminoanthracene (Scheme 2).



Scheme 2. Proposed mechanism for the detection of Cr^{3+} ions.

To investigate the practical application, **ANT-Th** (10 μM) in 6:4 CH_3CN /HEPES solution at $\text{pH} = 7.0$ was added onto circular test papers and then they were dried in the air. Test papers did not show any emission under UV light at 366 nm without adding any metal species. As can be seen in Figure 4, only Cr^{3+} ions exhibited a “turn-on” response and gave brilliant green emission. Thus, it was concluded that these test papers can be used as a simple and fast tool for recognizing Cr^{3+} in a different area without using any sophisticated instrument.

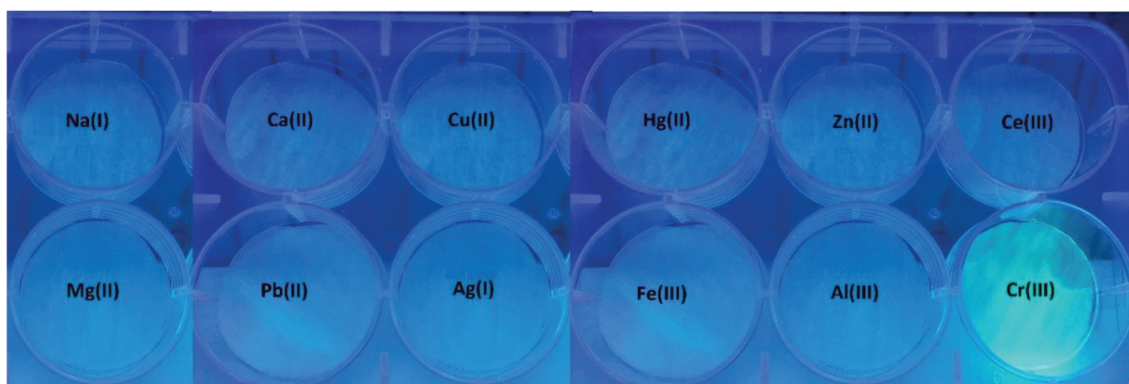


Figure 4. Detection of different metal ions (10 μM) with **ANT-Th** (10 μM) on circular test paper fluorescence images under UV light at 366 nm.

4. Conclusion

In sum, it was exhibited the facile synthesis and spectroscopic behaviors of a “turn-on” type fluorescent probe (**ANT-Th**) for the detection of Cr^{3+} ions in an aqueous environment. The probe molecule demonstrated high selectivity with high fluorescence enhancement, low detection limit, and quick response time. Moreover, the monitoring of Cr^{3+} ions in solid-state with the naked eye as a practical application was successfully achieved.

Acknowledgment

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Supporting Information

1. Determination of Quantum Yields

Fluorescence quantum yields of **ANT-Th** and **ANT-Th** + Cr^{3+} solutions were determined by using optically matching solutions of Rhodamine B ($\Phi_{\text{F}}=0.31$ in water) as a standard. The quantum yield was calculated according to the equation;

$$\Phi_{\text{F}(\text{X})} = \Phi_{\text{F}(\text{S})} (A_{\text{S}}F_{\text{X}}/A_{\text{X}}F_{\text{S}}) (n_{\text{X}}/n_{\text{S}})^2$$

Where Φ_{F} is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

2. Effect of Water Content

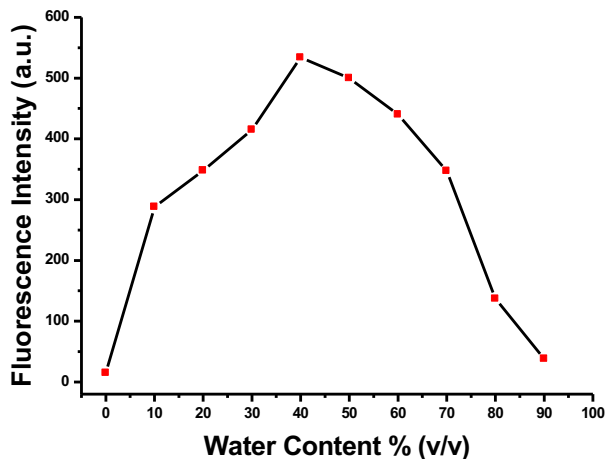


Figure S1 Effect of water content on the fluorescence intensity of **ANT-Th** (10 μM) in the presence of Cr^{3+} (60 μM) at $\text{pH}=7.0$

3. Determination of Detection Limit of Cr^{3+}

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **ANT-Th** (10 μM) without Cr^{3+} was measured by 10 times and

the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Cr^{3+} concentration could be obtained in the 0 – 10 μM . The detection limit is then calculated with the equation: detection limit = $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 0.4 μM (21 ppb) at $S/N = 3$.

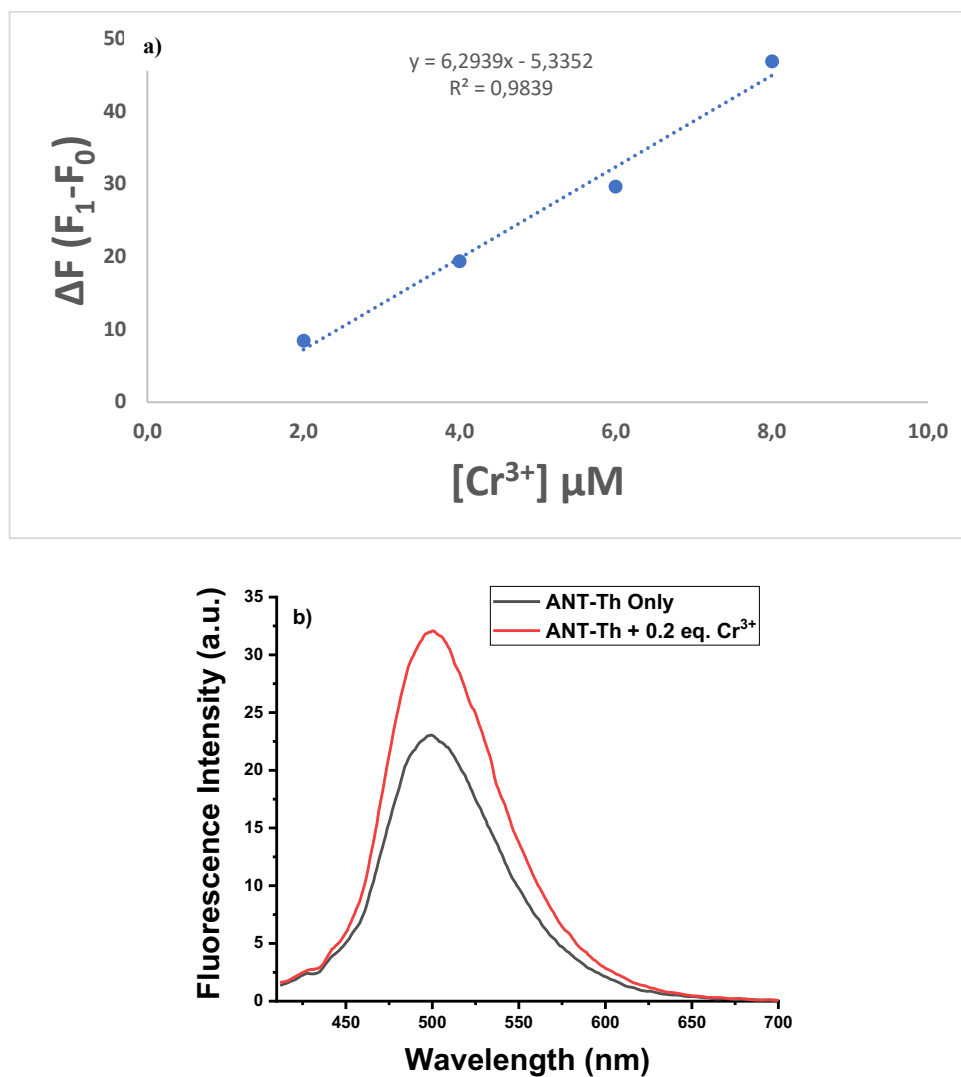


Figure S2 (a) Fluorescence changes of **ANT-Th** (10 μM) upon addition of Cr^{3+} (2.0 to 10.0 μM , 0.2 to 1.0 equiv.) (b) Fluorescence spectra of **ANT-Th** (10 μM) in the presence of Cr^{3+} (2.0 μM , 0.2 equiv.) in 6:4 $\text{CH}_3\text{CN}/\text{HEPES}$ at $\text{pH}=7.0$

4. Time-dependent Fluorescence Change of ANT-Th

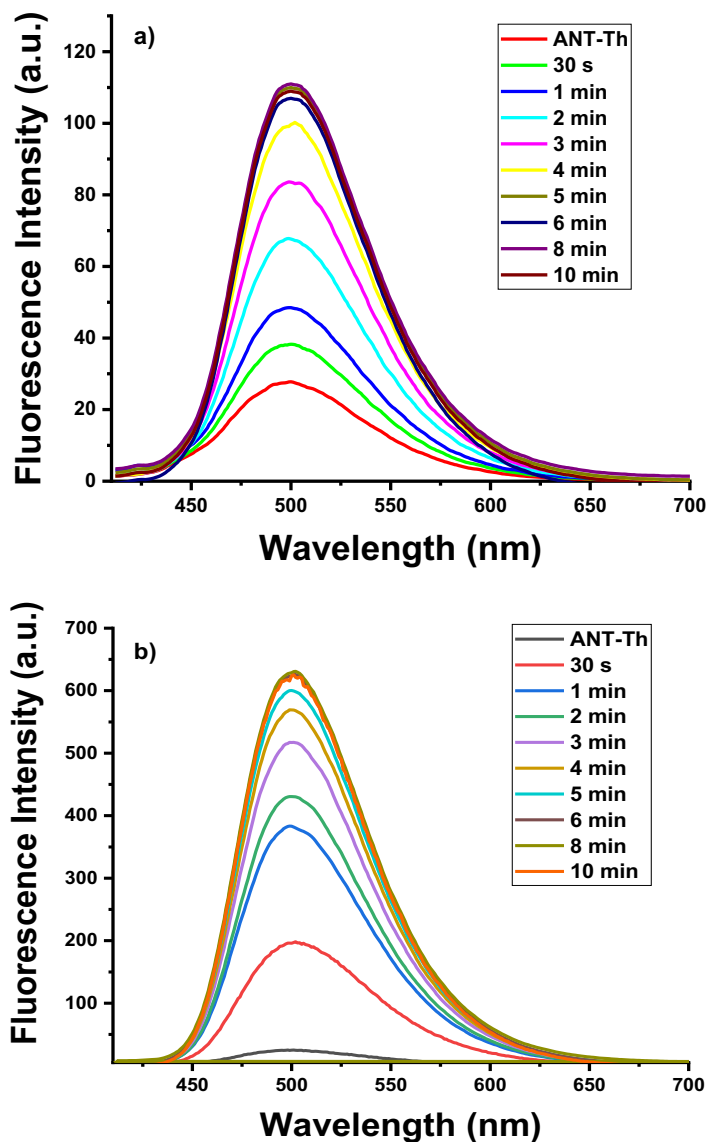


Figure S3 Time-dependent fluorescence change of ANT-Th (10 μM) in the presence of (a) 1.0 equivalent (b) 5.0 equivalent of Cr^{3+} measured in 6:4 $\text{CH}_3\text{CN}/\text{HEPES}$ at $\text{pH}=7.0$

5. TLC image of the hydrolysis reaction of ANT-Th with Cr (III) ion

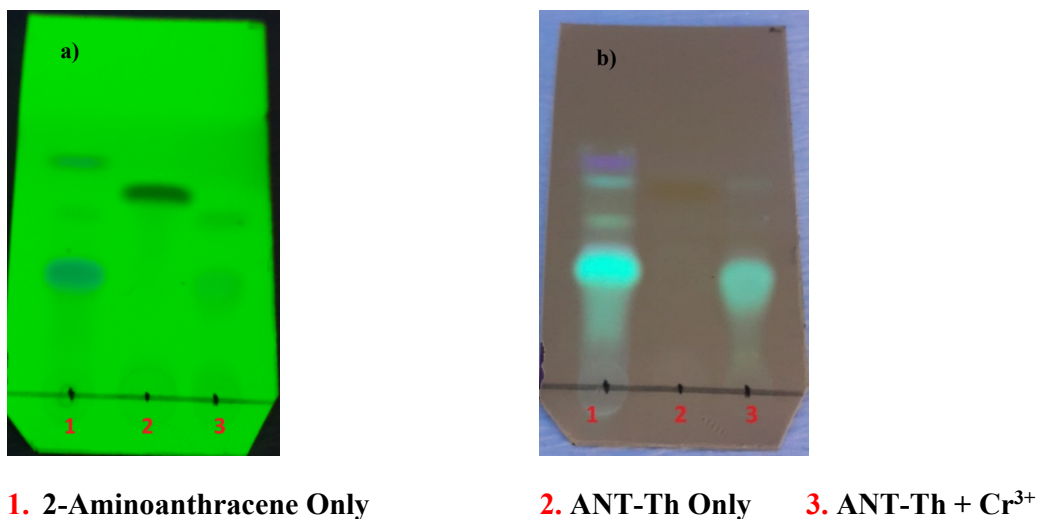
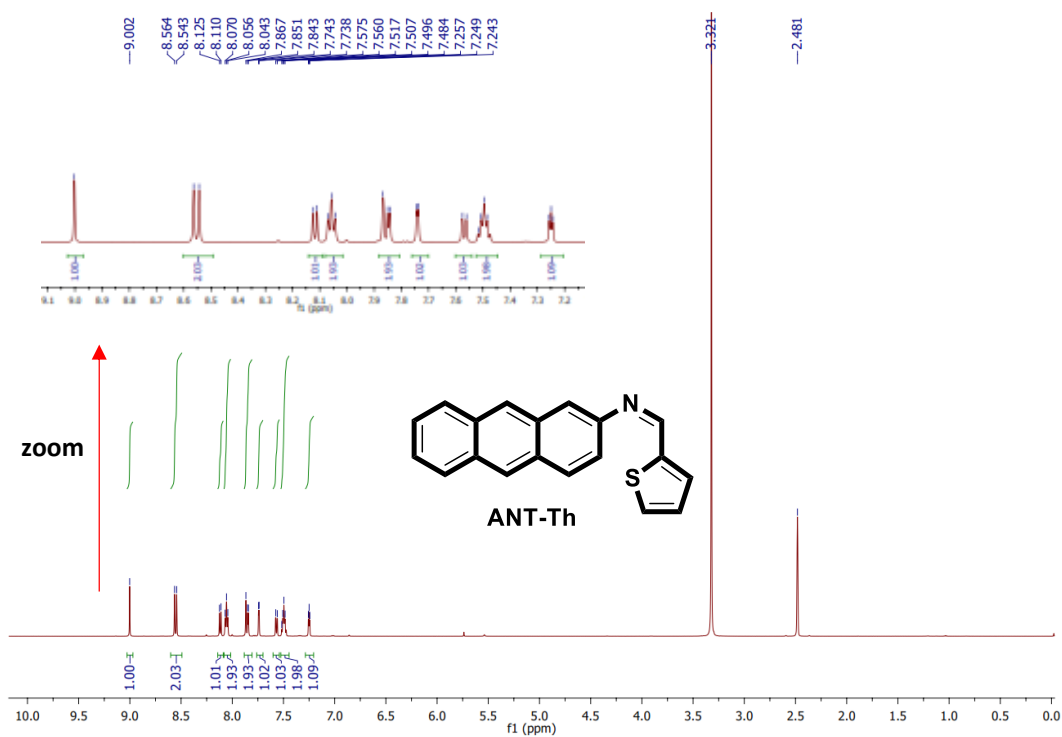
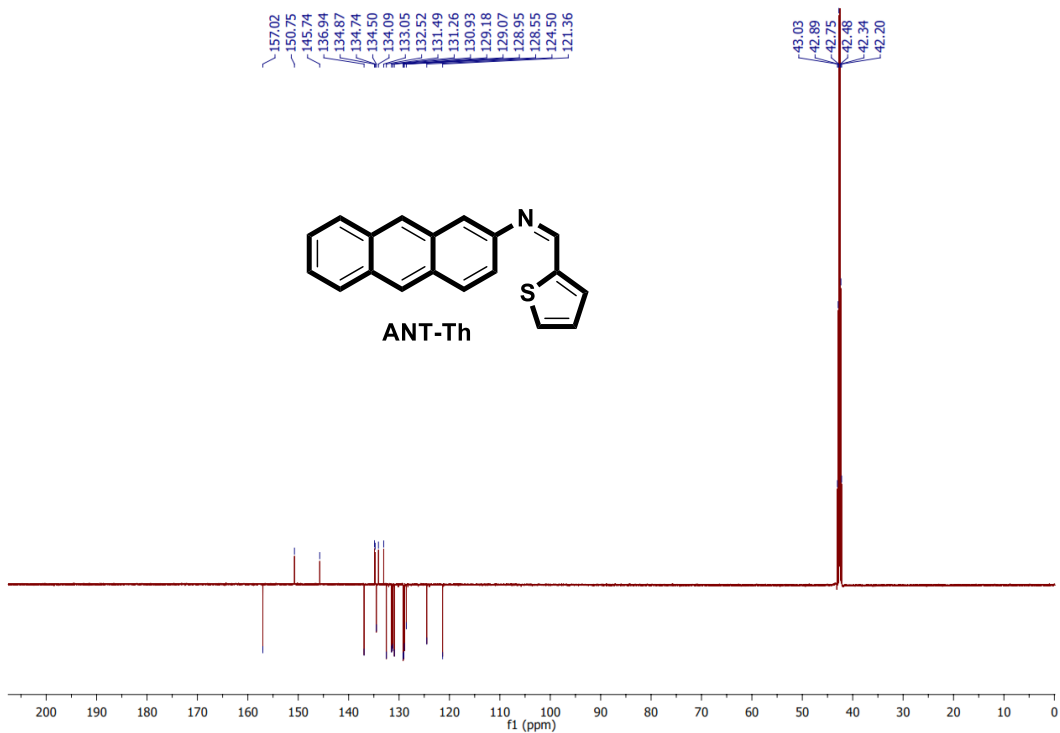


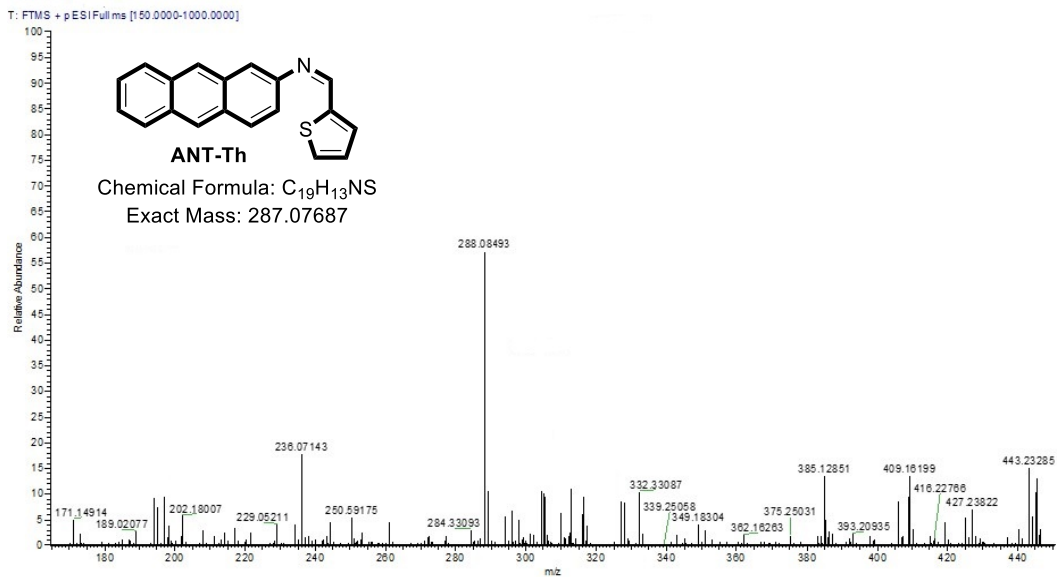
Figure S4. TLC image of the hydrolysis reaction of ANT-Th with Cr³⁺ ion under UV light **a)** 254 nm **b)** 366 nm

¹H-NMR and APT ¹³C-NMR Spectra of ANT-Th





HRMS Spectrum of ANT-Th and Hydrolysis Product



T: FTMS + pESI Full ms [150.0000-1000.0000]

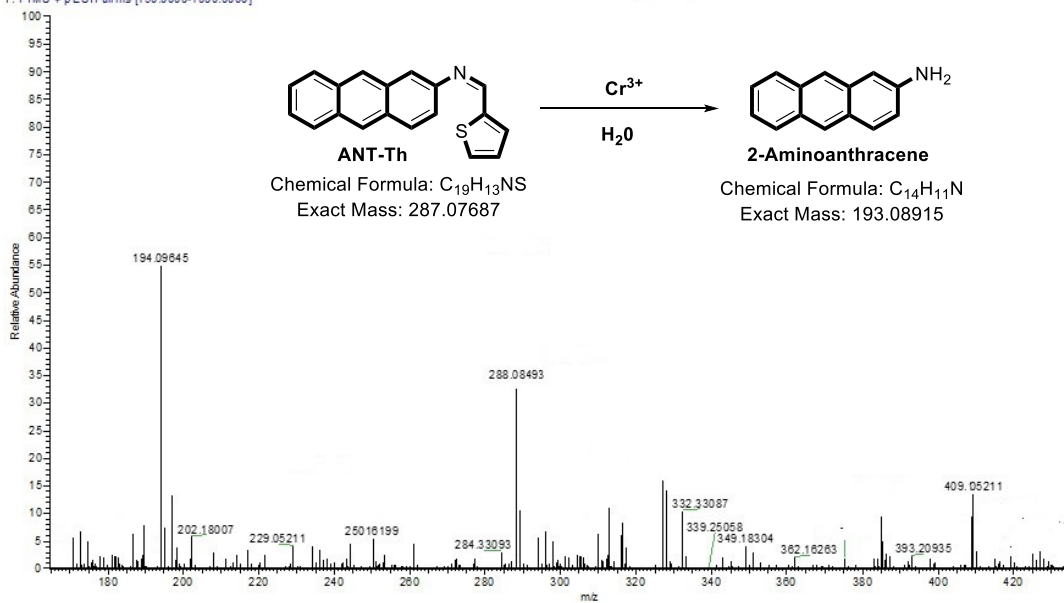


Table S1. Comparison of the present method with other reported Cr³⁺ selective fluorescence probes

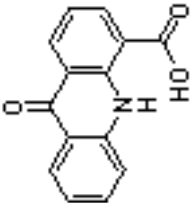
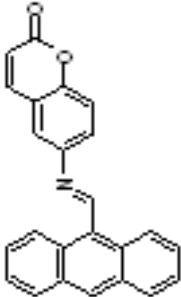
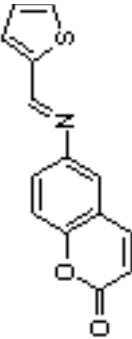
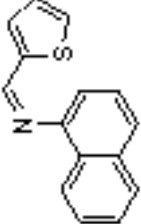
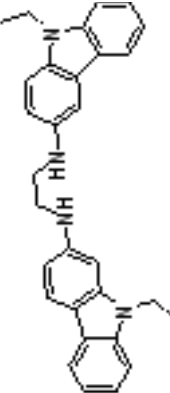
Compound	Synthesis Step	Mechanism	Solvent System	Response Time	LOD	Application	Ref. No
	2 steps	Turn-off	9:1 (v/v) DMF: H ₂ O	NA	1 µM	NA	33
	2 steps	Turn-on	9:1 (v/v) CH ₃ CN: H ₂ O	<2 min Saturation: 10 min	0.5 µM	YES	24
	1 step	Turn-on	4:6 (v/v) CH ₃ CN : Buffer (HEPES) pH = 7.4	NA	1 µM	YES	22
	1 step	Turn-on	9:1 (v/v) MeOH: H ₂ O	NA	0.15 µM	YES	34
	2 steps	Turn-off	9:1 (v/v) EtOH: Buffer (BR) pH= 7.4	2 min	0.1 µM	NA	35

Table S1. (Continued).

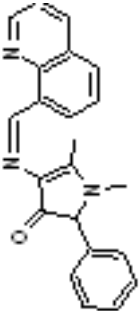
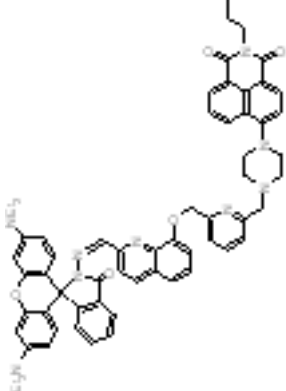
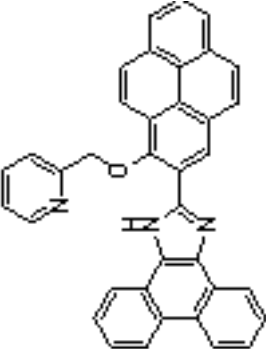
Compound	Synthesis Step	Mechanism	Solvent System	Response Time	LOD	Application	Ref. No
	2 steps	Turn-on	4:1 (v/v) EtOH: H ₂ O	NA	1.5 µM	NA	36
	7 steps	Turn-on Ring-opening	1:1 (v/v) CH ₃ CN: Buffer (Tris-HCl) pH = 7.4	NA	0.2 µM	YES	37
	3 steps	Ratiometric	1:1 (v/v) THF: Buffer (HEPES) pH = 7.0	<10 min Saturation: 120 min	0.23 µM	NA	38

Table S1. (Continued).

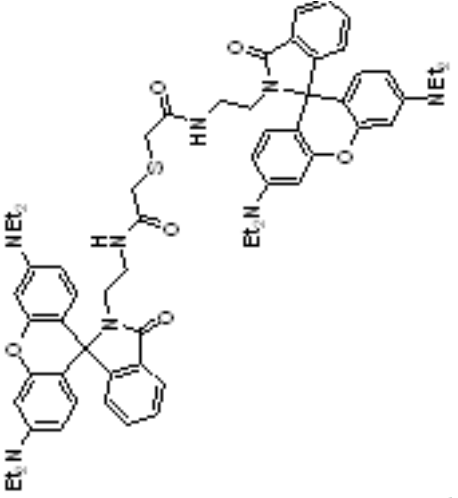
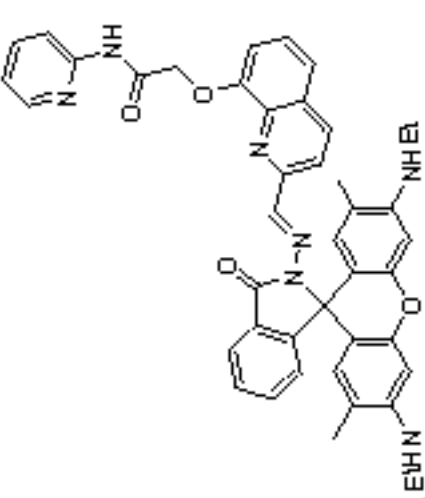
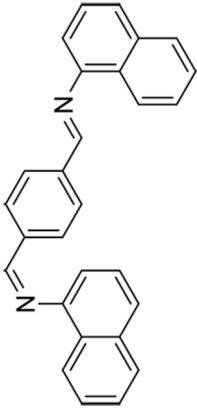
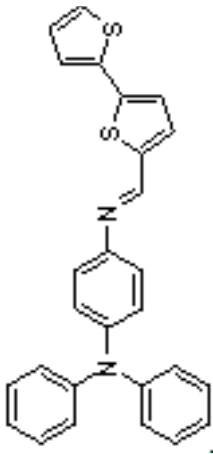
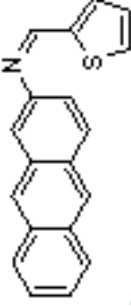
Compound	Synthesis Step	Mechanism	Solvent System	Response Time	LOD	Application	Ref. No
	2 steps	Turn-on Ring-opening	3:1 (v/v) MeOH: Buffer (HEPES) pH = 7.2	<10 min Saturation: 60 min	0.38 µM	NA	39
	5 steps	Turn-on Ring-opening	9:1 (v/v) CH ₃ CN: Buffer (Tris-HCl) pH = 7.4	3 min	5.6 µM	YES	40

Table S1. (Continued).

Compound	Synthesis Step	Mechanism	Solvent System	Response Time	LOD	Application	Ref. No
	1 step	Turn-off	1:4 (v/v) CH ₃ CN: Buffer (HEPES) pH = 7.4	NA	3.92 µM	YES	41
	3 steps	Turn-on	1:1 (v/v) THF: H ₂ O	5 min	1.5 µM	NA	42
	1 step	Turn-on Hydrolysis	6:4 (v/v) CH ₃ CN: Buffer (HEPES) pH = 7.0	<1 min Saturation: 5 min	0.4 µM	YES	This work