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A novel OFF-ON-OFF fluorescence probe based on coumarin for Al^{3+} and F^- detection and bioimaging in living cells

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Abstract

A novel fluorescence probe L2 based on coumarin has been designed and synthesized. The probe L2 can be used for relay recognition of metal ions Al^{3+} and anion F^- in the aqueous HEPES buffer (0.05 M, pH=7.4), and build a OFF-ON-OFF detection system. The probe showed high selectivity and sensitivity to target ions in the process of relay recognition, and the corresponding detection limit could be as low

as 0.014 μM (Al^{3+}) and 0.03 μM (F^{-}). Besides, the geometry optimizations of probe L2 and [L2+Al^{3+}] complex were carried out using the Gaussian 16 program based on DFT, and the identification mechanism of the probe was also discussed by the mass spectrometry and theoretical calculations. Moreover, the probe have also been successfully applied to detection of target ions in living cells.

Keywords: Fluorescence probe, coumarin, Al^{3+}, F^{-}, cell imaging

1.Introduction

It is well known that the aluminum is the most abundant metal element in nature and its reserves account for about 8% of the earth's crust [1, 2]. At the same time, the aluminum has the advantages of small density, good thermal conductivity and good ductility. So it has also been widely used in various fields, such as industrial production, aerospace materials, medical equipment, food additives, drinking-water purification, and so on [3-6]. But the Al^{3+} is not a necessary trace element, and the intake of Al^{3+} ions will remain in various organs of the body. First, Al^{3+} ions are very easily deposited in brain tissue, which may cause brain damage and serious memory loss. The existence of excessive Al^{3+} ions can also lead to a series of biochemical reactions, which will affect the normal metabolism of human cells. Meanwhile, Al^{3+} ions can directly damage the activity of osteoblasts, thus inhibiting bone matrix synthesis [7-9]. Therefore, the harm of Al^{3+} ions to human body is not to be underestimated.

Similarly, the fluorine also play a very important role in many chemical and biomedical industry [10,11]. The fluoride is the common ingredient of the anesthetic,

hypnotic, psychotropic drugs and military nerve gases. Meanwhile, fluorinated compounds also can be used to prevent dental caries, and they are usually added to some oral hygiene products. Fluorine is widely distributed in natural water, and fluorine is also found in all tissues of human body, mainly accumulated in teeth and bone tendons. A proper amount of F^- ions is essential for the human body, but excessive intake of fluoride is also harmful to people's health. The fluorosis is a typical symptom caused by chronic fluorosis [12, 13]. High concentration of fluoride can also cause serious pulmonary edema, pulmonary hemorrhage, arrhythmia, nausea and strong irritation in the eyes and upper respiratory tract. Even more, it will also endanger human life [14, 15]. From the point of view of environment and human health, it is very necessary to develop effective detection methods for fluoride.

In recent years, fluorescence sensing detection technology has attracted wide attention. Compared with the traditional detection technology, the fluorescence detection technology can show better detection results and have better application value and potential. Therefore, the construction of fluorescent probes with specific recognition performance has become one of the most popular research projects at home and abroad [16, 17]. For the fluorescent probes that had been reported, most of them were some metal ions fluorescent probes and anion fluorescent probes. And many of them have some probes can be used for specific detection and identification of Al^{3+} or F^- [18-29]. However, many probes are usually limited by lengthy synthetic routes or high detection limits, which cannot be applied to practical detection applications. Most importantly, most of those probes are single response, although the

selectivity of probes to the target ions is good, but the utilization ratio is not high compared to the relay response or the multi response probe. Therefore, the design and synthesis of fluorescent probes with high selectivity, high sensitivity and low cost, which can break through a single response type, is still a challenging subject.

In this paper, coumarin was used as the fluorescent group, a novel fluorescent probe (L2) with simple structure and good fluorescence response performance was designed and synthesized. The probe L2 can be used for relay recognition of metal ions Al^{3+} and anion F^{-}, and build a OFF-ON-OFF detection system. The optical properties and detection properties of probe L2 were systematically studied through a series of UV-Vis spectra and fluorescence emission spectra. At the same time, the influences of other common anion and cation on the detection effect of probe L2 were also discussed. Finally, probe L2 was used for fluorescence imaging analysis of target ions in living cells.

2. Experimental

2.1 Materials and Instruments

Unless otherwise noted, all the solvents and inorganic salts were purchased from Sinopharm Chemical Reagent Ltd., The 4-hydroxy-4-biphenylcarbonitrile, hexamethylenetetramine, Rhodamine B and hydrazine hydrate (85%) were purchased from Aladdin Chemical Reagent Ltd., and used without further purification. The ^{1}H NMR spectra were recorded on an AVANCE Ⅱ 400 MHz spectrometer (Bruker BioSpin) and ^{13}C NMR spectra were recorded on 101 MHz spectrometers. Mass Spectrometry (MS) were measured by a Liquid Chromatography-Ion Trap Mass

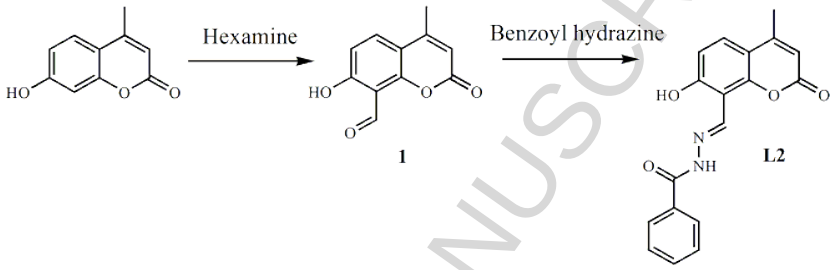
Spectrometry (Thermo LXQ). Fluorescence spectra measurements were performed on a Cary Eclipse fluorescence spectrometer (Variance, LTD, Australia) and the slit widths were 5 nm for emission. Cell imaging experiments were applied on a Leica DMI4000B inverted fluorescence microscope (Germany).

2.2 Synthesis of probe L2

Synthesis of compound **1**: the methylumbelliferone (0.03 mol) and hexamine (0.07 mol) in glacial acetic acid (50 mL) were refluxed for 8 h. Then added 75 mL 20% of hydrochloric acid and continue heating for 45 min. After cooling to room temperature, added 400 mL of ice water mixture and the product was extracted several times with ethyl ether. All organic phase was mixed, and the solution was dried over anhydrous sodium sulfate, filtered and the solvent was removed by vacuum distillation, the yellow solid powder was obtained finally (1.06g, 17.3%). ^{1}H NMR (400 MHz, 25℃, $CDCl_3$) δ 12.22 (s, 1H), 10.63 (s, 1H), 7.76 (s, 1H), 6.91 (s, 1H), 6.22 (s, 1H), 2.45 (s, 3H). ^{13}C NMR (101 MHz, 25℃, $CDCl_3$) δ 193.38 (s, 6H), 165.25 (s, 3H), 159.16 (s, 2H), 156.14 (s, 2H), 152.67 (s, 2H), 132.92 (s, 6H), 114.27 (s, 6H), 112.01 (d, *J*=8.4 Hz, 9H), 108.66 (s, 2H), 77.37 (s, 23H), 77.06 (s, 22H), 76.74 (s, 22H), 18.93 (s, 6H), 1.01 (s, 1H).

Synthesis of probe L2: 8-formyl-7-hydroxy-4-methylcoumarin (204 mg, 1.0 mmol) and benzoyl hydrazine (143 mg, 1.05 mmol) were dissolved in 30 mL ethyl alcohol, and the mixture was refluxed for 6 h. After the reaction completed, removed the solvent by vacuum distillation, and the crude products were washed with cold ethanol for two times and dried by vacuum drying, the yellow powder products were

obtained (277 mg, 86.6%). ^{1}H NMR (400 MHz, DMSO-d) δ 12.80 (s, 1H), 12.45 (s, 1H), 9.14 (s, 1H), 7.99 (d, J=7.4 Hz, 2H), 7.77–7.61 (m, 2H), 7.58 (t, J=7.4 Hz, 2H), 6.97 (d, J=8.8 Hz, 1H), 6.25 (s, 1H), 2.40 (s, 3H).^{13}C NMR (101 MHz, DMSO) δ 163.11, 161.65,159.83, 129.12, 154.16, 153.01, 144.02, 132.91, 132.78, 129.14, 128.43, 128.12, 114.04, 112.18, 111.20, 106.06, 18.76 ppm. MS: calcd for $C_{18}H_{14}O_4N_2$ $[M+Na]^+$ (m/z): 345.33; found: 345.52. (Scheme 1)



Scheme 1. Synthesis process of the probe L2.

2.3 Spectral measurement

A series of metal ions stock solutions (10 mM) and anions stock solutions (10 mM) were prepared from corresponding chlorine salts ($PbCl_2$, $CdCl_2$, $MgCl_2$, $CoCl_2$, $CuCl_2$, $NiCl_2$, $FeCl_2$, $MnCl_2$, $HgCl_2$, KCl, LiCl, NaCl, $AlCl_3$, $FeCl_3$, $CrCl_3$ and $ZnCl_2$) and corresponding sodium salts (NaCl, NaBr, NaI, NaF, $NaNO_2$, $NaNO_3$, $NaClO_4$, Na_2CO_3, $NaHCO_3$, Na_2SO_4, $NaHSO_4$ and CH_3COONa). The 1.0 mM probe L2 stock solution was prepared with dimethylsulfoxide and dilute the probe solution to 10 μM with the HEPES (0.05 M, pH=7.4) buffer solution. The corresponding fluorescence emission spectra of the probe system in the presence of different 10 equiv. metal ions were measured. The fluorescence response of the probe was discussed based on the measured results. And the emission spectra of the probe in the presence of different

concentrations of Al^{3+} were recorded. Besides, the interference experiments of common ions were also discussed. Meanwhile, the selectivity of [L2+Al^{3+}] complex system towards F^- was further studied by the optical spectrogram determination. The excitation wavelength was 334 nm.

2.4 Preparation of the real samples

The Black tea and toothpaste were purchase from the local supermarket. A series of pretreatment processes for the sample were carried out before testing. Samples of black tea were dried in an oven and ground into powder. Accurately weigh 0.2 g of the powder sample and ashing it in a ceramic crucible at high temperature. After cooling, the residue was dissolved with 0.5 mL hydrochloric acid, and then the volume was fixed to 5.0 mL with the HEPES buffer solution (pH=7.4). Shake well for reserve. Likewise, the 10 mg of toothpaste was dispersed in 5.0 mL of distilled water. The mixture was sonicated, centrifuged and then filtered. The filtrate was used for qualitative analysis of F^-.

2.5 Cell imaging

Firstly, the RAMOS cells were seeded at a 24-well plate and incubated in medium (supplementing with RPMI 1640, 10% fetal bovine serum) at 37 ◦C in a thermostat for 24 h [30]. Then the RAMOS cells were treated with 10 μM probe L2 for 30 min. After washing the cells with PBS buffer for three times, and the cells were further incubated with 50 μM Al^{3+} for 30 min. Then continue to incubated with 50 μM F^- for 30 min. The fluorescence imaging of cells in each stage were analyzed by the inverted fluorescence microscope (Germany，DM2500).

3. Results and discussion

3.1 The selectivity of probe L2 to Al^{3+}

The fluorescence respond of the probe L2 were investigated by measuring the fluorescence emission spectra in the presence of different metal ions including Pb^{2+}, Mg^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{2+}, Al^{3+}, Mn^{2+}, Hg^{2+}, K^{+}, Li^{+}, Na^{+}, Fe^{3+}, Cr^{3+} and Zn^{2+}. As shown in Fig.1, the single probe L2 presented an extremely weak fluorescence emission at 461 nm (Φ=0.02). When 10 equiv Al^{3+} was added, the emission peak of the probe system at 461 nm increased significantly (Φ=0.41). But under the same conditions, the addition of other metal ions did not cause large fluctuations in the fluorescence emission of the probe system. Similarly, the fluorescence emission of the corresponding probe mixed solution after the addition of different metal ions under the irradiation of 365 nm ultraviolet lamp showed that the addition of Al^{3+} ions induced the appearance of the visible blue fluorescence of the naked eyes. The above results showed that probe L2 has good selective fluorescence response to Al^{3+} ions. The Job's plot was used to predict the binding ratio between probe L2 and Al^{3+} and the results were shown in Fig.S5. The fluorescence intensity of the system reached the maximum when the molar ratio of probe L2 to Al^{3+} is 1:1. This conclusion indicated that the stoichiometric ratio of probe L2 to Al^{3+} is 1:1.

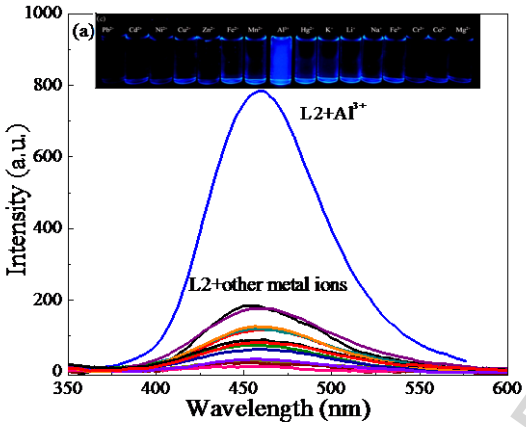


Fig.1 Fluorescence spectra of probe L2 (10 μM) under the injections of 10 equiv. different metal ions (Pd^{2+}, Cd^{2+}, Mg^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Fe^{2+}, Al^{3+}, Mn^{2+}, Hg^{2+}, K^{+}, Li^{2+}, Na^{+}, Fe^{3+}, Cr^{3+} and Zn^{2+}).

3.2 The anti-interference of the probe L2

The interference immunity of the probe to competing ions is an important parameter to evaluate the practical application of a probe L2. So the anti-interference performance of the probe system was tested when some common metal ions coexist. As shown in Fig.2, probe L2 still has excellent single selectivity for the identification of Al^{3+} in the presence of other interfering ions. The coexistence of other common ions can not interfere with the recognition process of probe L2, and the fluorescence intensity of system has not changed significantly. The above experimental phenomena showed that the probe L2 has excellent anti-interference ability for Al^{3+} ion recognition.

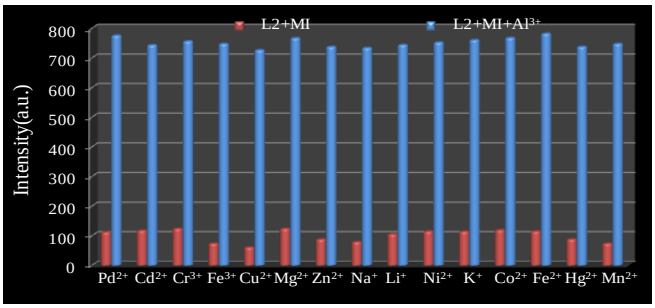


Fig.2 Fluorescence selectivity of L2 (10 μM) toward Al^{3+} (10 equiv.) in the presence of additional various metal ions (10 equiv.) in the same media.

3.3 Identification mechanism and binding mode

To verify that the probe L2 and Al^{3+} ions can form a stable complex with a ratio of 1:1, we analyzed the mass spectrum of the probe L2 system in the presence of Al^{3+}. As shown in Fig.3, there is a peak at m/z 345.55, which corresponded to the expected mass of single probe L2 (calcd. for 345.33 $[M+Na]^+$). The main peak located at m/z 456.61 (calcd. for 456.33 $[L2+Al^{3+}+3Cl+H]^+$), which should corresponded to the peak of the $[L2+Al^{3+}]$ complex. The results indicated that L2 and Al^{3+} formed a stable complex with a ratio of 1:1, which is consistent with the result of Job' plot.

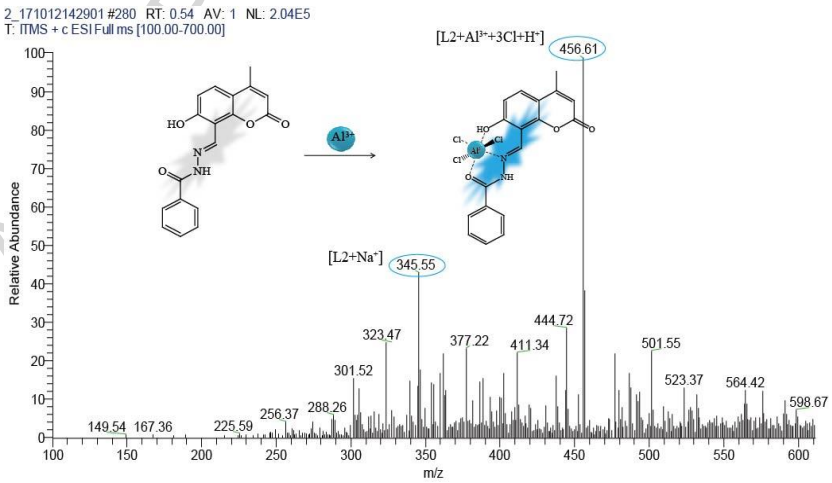


Fig.3 The MS spectrum of probe L2 system in the presence of Al^{3+}.

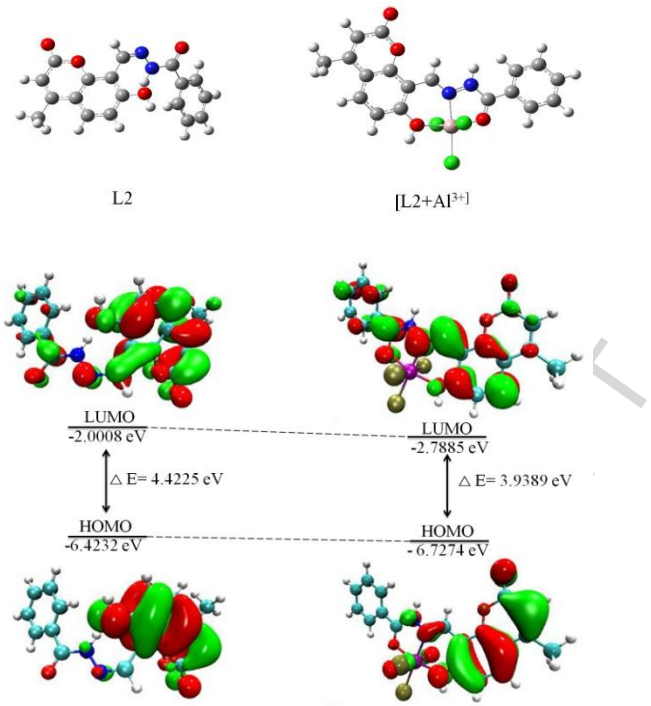


Fig.4 DFT optimised structures of the probe L2 and [L2+Al^{3+}] complex and the corresponding HOMO and LUMO.

Besides, the geometry optimizations of probe L2, [L2+Al^{3+}] complex were carried out based on DFT using the Gaussian 16 program [31], in which the B3LYP function and the def2-tzvp basis set were used. The optimized configurations and corresponding the LUMO and HOMO [32] are shown in Fig.4, the results were analyzed and the response mechanism of probe L2 to Al^{3+} was inferred as follows: the single probe L2 molecule hardly showed any fluorescence due to the effect of the internal charge transfer (ICT). The hydroxyl oxygen (-OH), acyl oxygen (C=O) and ammonia nitrogen (C=N) atoms of the probe molecule can provide the sites for binding of Al^{3+}. The enhancement in fluorescence after the probe L2 combined with Al^{3+} is probably due to the formation of a six member and a five member chelate ring with the metal ion, which not only enhances the conjugation in the ligand framework

but also increased the rigidity of the molecular assembly by restricting the free rotations of the azomethine carbon. Besides, the chelation of L2 with Al^{3+} suppressed the intramolecular charge transfer effect and the C=N double bond in the probe L2 structure also occurred the cis/trans isomeri-zation, which induced the chelation enhanced fluorescence effect (CHEF). So the system presented a strong blue fluorescence emission.

3.4 Relay identification

When a series of common anions were added to the [L2+Al^{3+}] system, it is surprising to found that the introduction of F^- led to a large degree of quenching of the fluorescence of the [L2+Al^{3+}] system (Φ=0.02). This may be due to the competitive combination of F^- with Al^{3+}, and to form the $[AlF_6]^{3-}$ complexes. The probe L2 returned to monomer form, so the fluorescence of the system disappeared. However, the addition of other common anions such as Cl^-, Br^-, I^-, NO_2^-, NO_3^-, ClO_4^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, HSO_4^- and CH_3COO^- did not cause the same optical change under the same condition (Fig.5a). This phenomenon indicated that the [L2+Al^{3+}] complex can be used as a new probe system for the in situ identification of F^-.

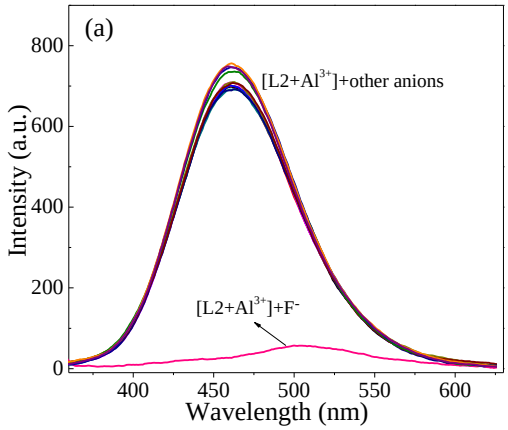


Fig.5 Fluorescence spectra of [L2+Al^{3+}] (10 μM) upon the addition of different anions. λem=461 nm.

Similarly, the anti-interference performance of the [L2+Al^{3+}] complex system as a probe for the detection of F^- was also discussed. As shown in Fig.6, the [L2+Al^{3+}] complex could still achieved a single identification of F^- in the presence of other equivalent anions (Cl^-, Br^-, I^-, NO_2^-, NO_3^-, ClO_4^-, CO_3^{2-}, HCO_3^-, SO_4^{2-}, HSO_4^- and CH_3COO^-). As a result, the [L2+Al^{3+}] complex has excellent single selectivity and anti-interference ability for F^-. In other words, the probe L2 could be used to build a OFF-ON-OFF relay recognition detection system.

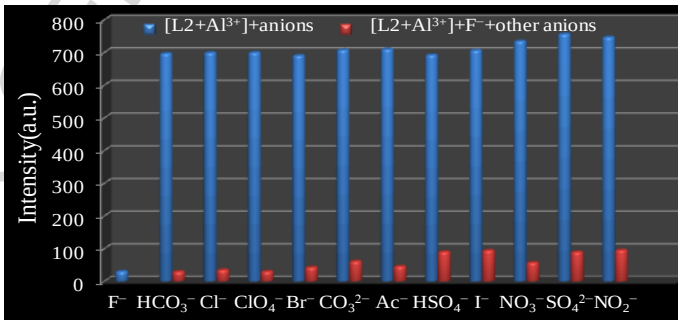


Fig.6 Fluorescence response of probe [L2+Al^{3+}] (10 μM) toward F^- (100 μM) in the presence of various anions.

3.5 Optimization of determination condition (pH and response time)

The fluorescence response of probe L2 (10 μM) toward Al^{3+} and the [L2+Al^{3+}]

toward F^- under different pH conditions was further studied, so as to determined the suitable pH condition for the detection of the Al^{3+} and F^- ions by the probe L2 and probe [L2+Al^{3+}] complex. As shown in Fig.7, the fluorescence emission intensity of probe L2 in pH=3~12 is not significantly changed without adding Al^{3+} ions. It can also be seen from the results that strong acid or strong base conditions had great influence on the detection of Al^{3+} by probe L2. And in the range of pH 5.0~9.0, the probe L2 system has the best effect on the detection of Al^{3+} and the fluorescence emission intensity of the system tends to be stable. Meanwhile, the experimental results that the detection effect of probe [L2+Al^{3+}] is not ideal for F^- under the condition of over acid (pH<5), This is due to the low rate of coordination reaction between Al^{3+} and F^- under acidic conditions. Under the condition of over alkali, some of Al^{3+} ions will form the $[Al(OH)_4]^-$ with hydroxyl groups, which seriously affected the accuracy of F^- content detection. Therefore, all the experiments were carried out in HEPES buffer solution (0.05M, pH=7.4).

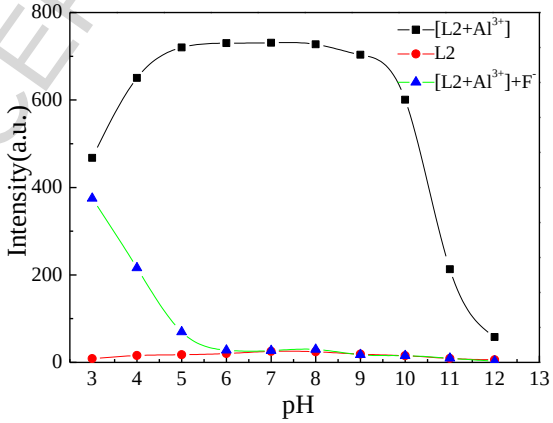


Fig. 7 Fluorescence intensity of L2 (10 μM) at various pH values from 3 to 12, under the absence and presence of Al^{3+} and F^- (λ_{ex}=334 nm, λ_{em}=461 nm).

The response time of probe L2 to Al^{3+} and [L2+Al^{3+}] complex system to F^- were

further studied. The intensity of fluorescence emission of the corresponding test solution were recorded every 10 s in the experiment. The results as shown in Fig.8, the fluorescence emission intensity of probe L2 did not change with time. With the addition of 10 μM Al^{3+}, the fluorescence emission intensity of the probe system at 461 nm increased instantaneously, and the fluorescence intensity of the system could reached the maximum after about 1 min, then the fluorescence intensity tended to be stable. When the 10 equiv. F^- was added to the [L2+Al^{3+}] complex system, the fluorescence intensity of the system decreased sharply, and the fluorescence quenching process of the system required only 2 min. The above experimental results showed that the probe L2 can realize fast relay recognition and detection for Al^{3+} and F^-. This study also provided a basis for the optimization of our detection process.

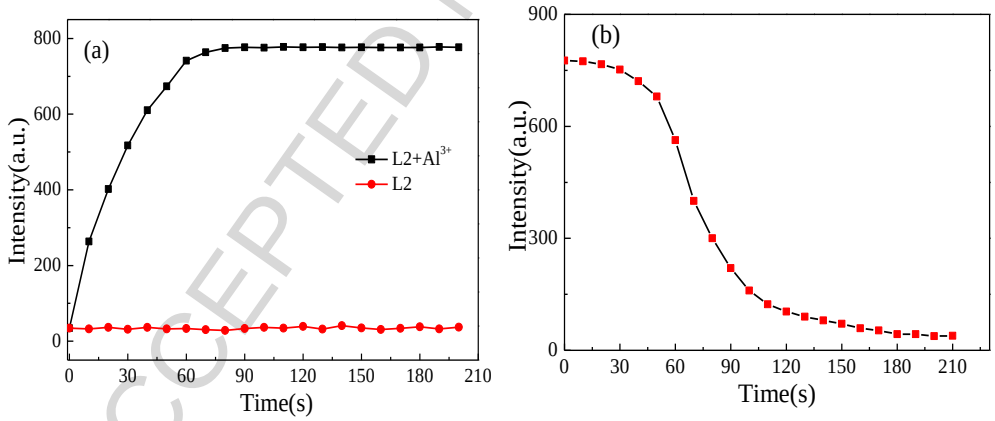


Fig.8 (a) Time course of compound L2 (10 μM) responding to Al^{3+} (100 μM); (b) Time course of ensemble [L2+Al^{3+}] (10 μM) responding to F^- (100 μM).

3.6 Fluorescent titration experiments (Al^{3+} and F^-)

To future study the fluorescence response sensitivity of probe L2 to Al^{3+}, the fluorescence titrations of probe L2 (10 μM) in the presence of different concentration of Al^{3+} (0~10 equiv.) were carried out. The results were shown in Fig.9(a), the

fluorescence emission intensity at 461 nm of the probe L2 system increased gradually with the increase of the addition of Al^{3+} concentration. And the fluorescence intensity of 461 nm was enhanced to the maximum when the amount of Al^{3+} ions is up to 1.0 equiv. With continuously increasing the amount of Al^{3+}, the fluorescence intensity of the system tended to be stable and no longer enhanced. Moreover, there are a good linear relationship (Fig.S6, R^2=0.998) between the fluorescence emission intensity and the concentration of Al^{3+} (0~1.0 equiv.). And the detection limit (L=3σ/slope, slope=1.671×10^7, σ=0.08) of probe L2 to Al^{3+} can be as low as 0.014 μM [33, 34]. Based on the linear relationship between 1/[Al^{3+}] and 1/[F-F_0] in Fig.S7 and Benesi-Hildebrand formula ($\frac{1}{F-F_0}=\frac{1}{k(F_{max}-F_0)c}+\frac{1}{F_{max}-F_0}$) [35], the binding constants (k) between probe L2 and Al^{3+} ion were calculated to be 5.453×10^4 M^{-1}. Compared with Al^{3+} fluorescent probe that has been reported (Table 1), the probe L2 showed lower detection limit and higher sensitivity to Al^{3+}. In contrast, the probe L2 seems to be more valuable.

Subsequently, the sensitivity of [L2+Al^{3+}] complex system to F^- was also determined by the fluorescent titration experiment. As shown in Fig.9(b), the fluorescence emission peak of [L2+Al^{3+}] system at 461 nm decreased linearly with the increase of F^- ions content. When the concentration of F^- ions reached 100 μM, the fluorescence emission of [L2+Al^{3+}] system is completely quenched. Through the linear curve between the concentration of F^- and fluorescence emission intensity in Fig.S8, the detection limit of [L2+Al^{3+}] to F^- can be calculated to be 0.03 μM. Its value is much lower than the maximum acceptable value of F^- (4.0 mg L^{-1}, 211 μM）

in drinking water provided by the US Environmental Protection Agency. [42]

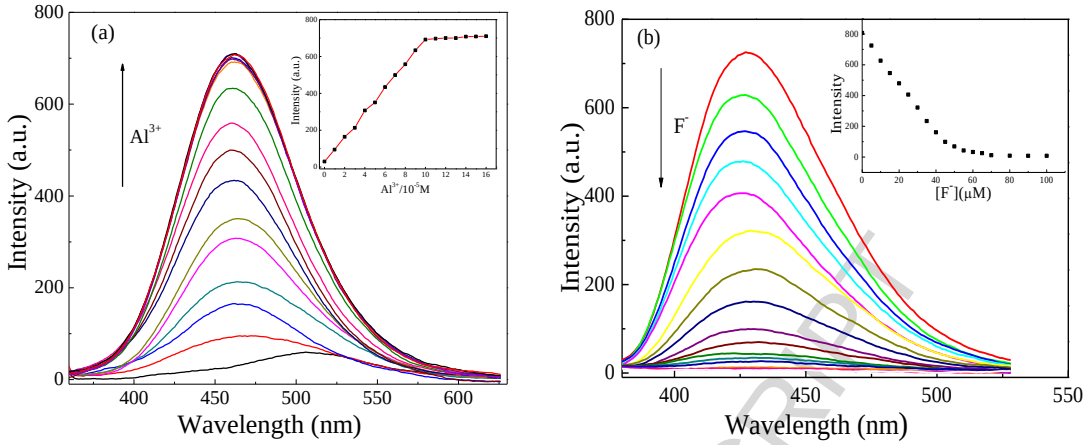
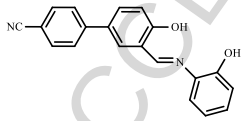
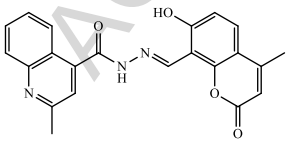
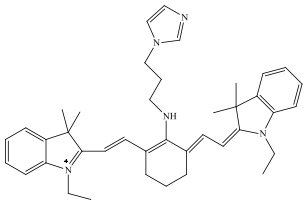
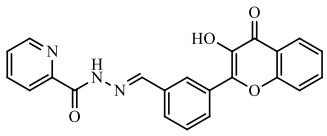
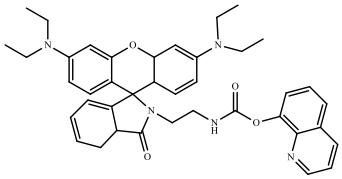
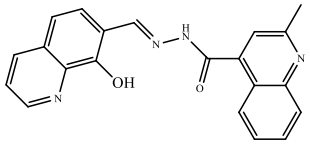
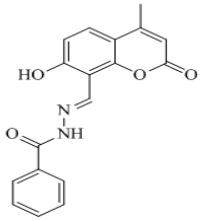


Fig.9 (a)Fluorescence titration of L2 (10 μM) in the presence of different concentration of Al^{3+} (0~10 equiv.), λ_{ex}=334 nm. Inset: fluorescence emission intensity at 461 nm changes with increasing concentration of Al^{3+}; (b) Fluorescence titration of [L2+Al^{3+}] (10 μM) in the presence of different concentration of F^- (0~10 equiv.), λ_{ex}=334 nm. Inset: fluorescence emission intensity at 461 nm changes with increasing concentration of F^-.

Table 1 Comparison of different fluorescent sensors for Al^{3+} detection.

Fluorescent probes	Detection limit（μM）	Reference
	1.37	Sens. Actuators B, 2014, [36]
	0.82	Spectrochim. Acta A, 2014, [37]
	0.0195	Anal. Chim. Acta, 2015, [38]

	21.7	Inorg. Chim. Acta, 2015, [39]
	8	RSC Adv, 2014, [40]
	5.2	Synthetic. Met, 2015, [41]
	0.014	This work

3.7. Analysis of real samples

In order to verify the practical application value of the probe L2, the detection of trace target ions in the real samples (Black tea and Tooth paste) was studied. Preparation of the samples to be analyzed was performed as described above in Section 2.4. The detection data of standard addition and recovery experiments were shown in Table 2. The content of Al^{3+} ions detected in Black tea samples is 0.07 μM, the recovery rate of standard addition can reach as high as 99.6%. Alike, The content of F^{-} ions detected in Tooth paste samples is 0.04 μM and the recovery rate can reach as 100.2%. This result showed that the probe can be applied to the detection of trace target ions in real samples.

Table 2 The measured data of target ions from the real samples.

Sample	Target ions	Added (μM)	Found (μM)	Recovery (%)

Black tea	Al^{3+}	0	0.07±0.01	
		5.0	5.05±0.02	99.6%
Tooth paste	F^-	0	0.04±0.01	
		5.0	5.05±0.03	100.2%

3.8 Cell imaging

In order to verify the practical application value of the probe L2, the fluorescence imaging application of probe L2 in living cells was preliminarily explored. The cells used in the imaging experiments were human lymphocytic tumor cells (RAMOS). First, the cells were cultured by probe L2 and the cells imaging under the bright field and fluorescence field was observed under inverted fluorescence microscope. The cells were then treated with Al^{3+} and F^- ions in turn. The corresponding cells imaging were shown in Fig.10, the Fig.10a and Fig.10b are the imaging of cells after probe L2 treating under bright field and fluorescence field. At this time, the cells showed no obvious fluorescence phenomenon. After Al^{3+} treatment, the cells showed strong blue fluorescence emission (Fig.10c). But the fluorescence was quenched due to the subsequent addition of F^- (Fig.10d). This series of phenomena indicated that the probe L2 has good cell permeability and can be used for the identification and detection of target ions in biological systems. This is of great help in studying the physiological functions of Al^{3+} and F^- in organism.

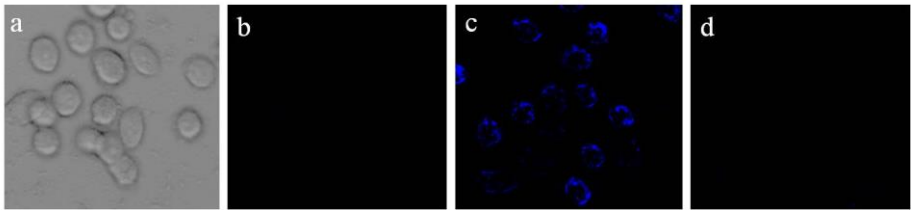


Fig.10 Images of RAMOS cells: (a) bright field and (b) fluorescence field images of cells incubated with probe L2 (10 μM); (c) fluorescence field image of cells further incubated with Al^{3+} (100 μM) (d) fluorescence field image of cells further incubated with F^{-} (100 μM).

4. Conclusions

A novel schiff base fluorescent probe (L2) based on coumarin fluorescence was designed and synthesized. The probe L2 can be successfully applied to relay recognition detection of Al^{3+} and F^{-}. First, the probe L2 has high selectivity and anti-interference ability for Al^{3+}. The main phenomenon is that the system changed from no fluorescence to strong blue fluorescence emission. And the titration experiments also showed that the probe L2 has excellent sensitivity to Al^{3+} and the minimum detection limit can be as low as 0.014 μM. In addition, the [L2+Al^{3+}] complex can also be used as a new probe for in-situ relay identification of F^{-} ions. The addition of F^{-} will cause the fluorescence emission quenching of [L2+Al^{3+}] complex system. The detection limit of probe [L2+Al^{3+}] for F^{-} can be as low as 0.03 μM. Meanwhile, the identification mechanism of the probe was also discussed by the mass spectrometry and DFT calculations. Moreover, the probe L2 can also be used as a target ions detector to successfully detect the Al^{3+} and F^{-} in living cells.

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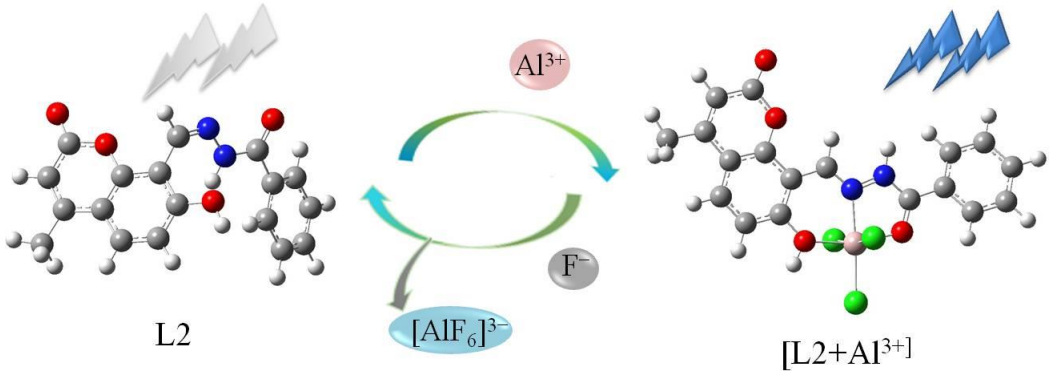
Graphical Abstract



Highlights

- A novel fluorescence probe L2 based on coumarin has been designed and synthesized.
- The probe L2 can be used for relay recognition of metal ions Al^{3+} and anion F^- and build a OFF-ON-OFF detection system
- The corresponding detection limit could be as low as 0.014 μM (Al^{3+}) and 0.03 μM (F^-)
- The probe have also been successfully applied to detection of target ions in living cells.