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A Novel 1, 10-Phenanthroline-Based Fluorescent Probe for Selective Detection of D-3-HB

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Abstract

A new probe, 4-(1H-imidazo[4,5-f] [1,10] phenanthrolin-2-yl) nitrobenzene (1), has been synthesized and characterized by ^{1}H-NMR, ^{13}C-NMR, EAs, FTIR, ESI-MS. UV-Vis and fluorescence spectrum results shown that probe 1 was highly selective to D-3-HB in DMSO/H_2O=1:1 (v:v=1:1), instead of common anions and metal ions. The absorbance intensity and the colour of probe 1 solution increased gradually with the increase of D-3-HB concentration and two new absorption bands appeared at 294 nm and 360 nm. " It is revealed that probe 1 can be a good candidate for simple, rapid and sensitive probe for the detection of D-3-HB.

Keywords: ***Diabetic ketoacidosis (DKA); Fluorescent; D-3-hydroxybutyric acid (D-3-HB); Fluorescence***

Introduction

For patients with diabetes, multiple factors contribute to the risk for poor glycemic control, which could be result in increased production of ketones [1-4]. Excess ketones can lead to diabetic ketoacidosis (DKA), which is a potentially life-threatening disorder characterized by hyperglycemia, ketonemia, and metabolic acidosis [5]. Although the overall mortality of DKA has improved over recent decades, the incidence and financial burden of DKA remain high. Among the total ketone body, such as D-3-hydroxybutyric acid (D-3-HB), acetone, acetoacetic acid and D-3-hydroxybutyric acid (D-3-HB) is the major, nearly 78% [6]. So, it is a reliable Method to detect the D-3-HB diagnosis of diabetic ketoacidosis.

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The chemical sensor method has drawn more and more attention due to its advantages of simplicity, high sensitivity and selectivity. Numbers of fluorescent chemo-sensors have been designed for the detection of metal ions, anions, amino acid and so on [7-18]. But there is very little report on the detection of D-3-HB by the fluorescence method. Up to now, only a novel Tb^{3+} complex based on benzoic acid can recognize D-3-HB in CH_3OH/H_2O was reported by prof. Chen [19]. Our early reported that the probe 4-(2-hydroxybenzylicene) thiosemicarbazide shown a peculiar OFF-ON fluorescent response to D-3-HB in Tris-HCl (pH=6.0) [20]. So, develop new probe for the detection of D-3-HB is a right way.

In this paper, a new probe named 4-(1H-imidazo[4,5-f] [1,10] phenanthrolin-2-yl) nitrobenzene (probe 1), was designed and synthesized and developed to detect D-3-HB via the luminescence and UV-Vis methods. Interestingly, probe 1 can high selectively recognize D-3-HB.

Experimental Details

Materials

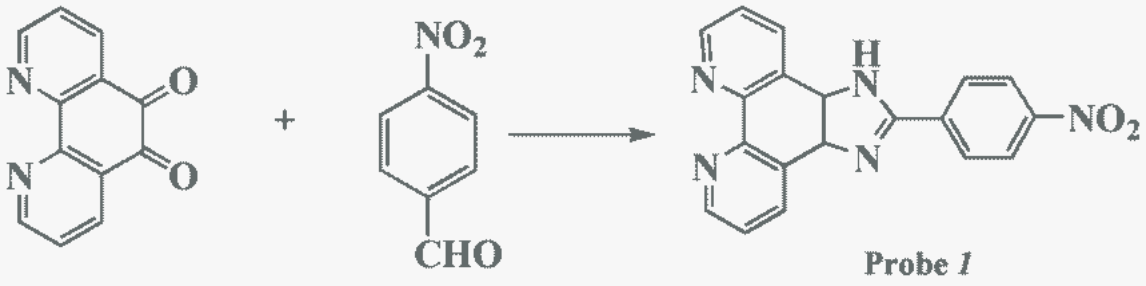
The salts solutions of metal ions such as NaCl, KCl, $MgCl_2·6H_2O$, $CaCl_2$, $BaCl_2$, $CrCl_3·6H_2O$, $CoCl_2·6H_2O$, $MnCl_2·4H_2O$, $FeCl_3·6H_2O$, $NiCl_2·6H_2O$, $CuCl_2·2H_2O$, $CdCl_2·6H_2O$, $ZnCl_2$, SrCl, $AlCl_3$ and the salts solutions of anions such as Na_3PO_4, Na_2CO_3, NaAC, NaBr, $Na_2C_2O_4·H_2O$, NaCl, NaF, $NaNO_3$, $NaNO_2$, NaH_2PO_4, Na_2HPO_4, $Na_2P_2O_7$, $Na_2B_4O_7$, Na_2SO_4, $NaClO_4$, NaCN, $NaHCO_3$, $NaHSO_4$ were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). D-3-HB was purchased from Sigma. All other chemicals used were of analytical grade. Deionized water was used to prepare all aqueous solutions.

Instruments

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-400 spectrometer with DMSO as the internal standard. Electrospray ionization mass spectra (ESI-MS) were measured on a Triple TOF^{TM} 5600$^+$ system. UV-Vis spectra were recorded on a Hewlett Packard HP-8453 spectrophotometer. Fluorescence spectra were recorded on a RF-5301 fluorescence spectrophotometer.

Synthesis of probe 4-(1H-imidazo[4,5-f] [1,10] phenanthrolin-2-yl) nitrobenzene

Probe 1 was prepared according to the reported methods from phen [21,22]. A solution of phenanthrene-9, 10-dione (0.42 g, 2.0 mmol), ammonium acetate (3.88 g, 50 mmol) and 4-nitrobenzaldehyde (0.24 g, 2.0 mmol) in 10 mL glacial acetic acid was refluxed for 1 h. The cooled deep red solution was diluted with 40 mL water, and neutralized with ammonium hydroxide. Then the mixture was filtered and the precipitates were washed with water and acetone. At last the products were dried and purified by chromatography over 60 mesh SiO_2 by using absolute ethanol as eluent, and the obtained yield was 0.24 g (35%). Calc. for $C_{19}H_{12}N_5O_2$: C, 66.66; H, 3.53; N, 20.46. Found: C: 66.70; H: 3.62; N: 20.45%. IR (cm^{-1}, s strong, m medium, w weak): 3436 m, ν(N-H); 2988 m, ν(C-H); 1638s ν(C=N). ^{1}H-NMR (400 MHz, DMSO), δ (ppm): 6.978-8.894 (m, 10H). ^{13}C-NMR (75 MHz, DMSO), δ (ppm): 161.617, 158.007, 153.135, 150.231, 142.526, 141.324, 136.107, 134.278, 130.780, 128.358, 127.012, 125.567, 124.198, 120.024, 116.116, 115.110, 114.243, 113.003, 112.641. Exact mass for 1: 341.09, ESI-MS (positive mode) $[1 + H^+]^+$ (m/z, 342.1116).



SCHEME. 1. **Synthesis of the probe 1.**

Results and Discussion

Synthesis and structural characterization

As shown in SCHEME 1, the target probe 1 was obtained from the reaction of phenanthrene-9, 10-dione, ammonium acetate and 4-nitrobenzaldehyde in glacial acetic acid. Its chemical structure was determined by ^{1}H-NMR, ^{13}C-NMR, Elemental analyses (EAs), electrospray ionization mass spectra (ESI-MS) and FT-IR spectra (IR) analysis.

UV–vis spectra for D-3-HB

FIG. 1 showed the change on the UV-vis spectra of the probe 1 (10 μM) upon addition of D-3-HB in DMSO/H_2O (v:v=1:1) solution. With the increase of the concentration of D-3-HB, the absorption peaks at 310 nm to 375 nm gradually increased and three new absorption peaks at 290 nm and 413 nm emerged. Three well-defined isosbestic points were noted at 285 nm, 294 nm and 308 nm. All of the results indicated the formation of a new species between probe 1 and D-3-HB. Meanwhile, the solution of probe 1-D-3-HB showed a dramatic color change from colorless to light yellow which could easily be detected by the naked-eye.

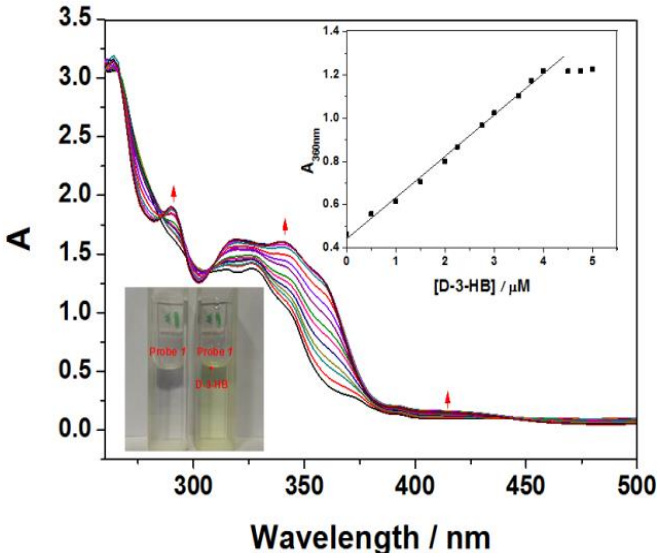


FIG. 1. **UV-vis spectra changes of the probe 1 (10 μM) after addition difference concentration of D-3-HB at room temperature. Inset: Linear range of D-3-HB concentration (0 μM to 4 μM). Photograph showing the color change of free probe 1 (10 μM) and in the presence of D-3-HB (4 μM).**

Fluorescence titration and binding studies

To further investigate the chemosensing properties of probe 1, the relationship between probe 1 with D-3-HB was performed by fluorescence titration. As shown in FIG. 2 with the addition of increasing amounts of D-3-HB to a solution of probe 1 in DMSO/H_2O (v:v=1:1), the emission band at 506 nm increased gradually and the peak at 430 nm decreased gradually. Based on the use of a UV lamp (λ_{ex}=365 nm), in the presence of D-3-HB, the solution of probe 1 showed a dramatic color change from blue to light green which could easily be detected by the naked-eye (FIG. 2 inset).

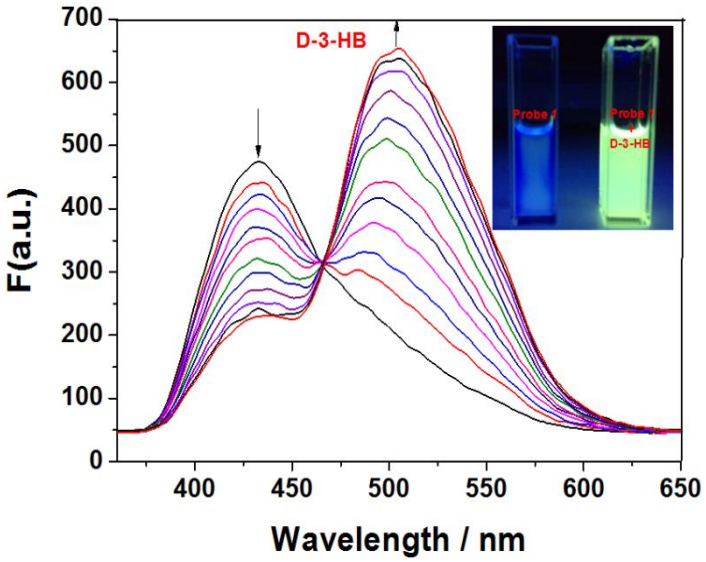


FIG. 2. **Fluorescence spectra of 1 (1.0 µM) in DMSO/H_2O (v:v=1:1) in the presence of different amounts of D-3-HB (0 µM to 5.0 µM). Inset: fluorescence changes of 1 (1.0 µM) upon addition of 5.0 µM D-3-HB.(λ_{ex}=350 nm).**

Selectivity of probe 1 over anions and metal ions

To value the selectivity of probe 1 for D-3-HB, it was treated with various relevant anions (10 equiv.) in DMSO/H_2O (v:v=1:1) solutions, then their fluorescence emission on fluorescence spectrophotometer was determinate. Excited to us that the D-3-HB treatment induces a large increase for the fluorescence intensity at 506 nm, where as other physiologically important anions, such as SO_4^{2-}, Ac^-, SO_3^{2-}, $S_2O_3^{2-}$, PO_4^{3-}, NO_3^-, I^-, HPO_4^{2-}, HCO_3^-, $H_2PO_4^-$, $P_2O_7^{2-}$, F^-, CrO_4^{2-}, $Cr_2O_7^{2-}$, CO_3^{2-}, Cl^-, $C_2O_4^{2-}$, Br^-, SCN^-, CN^-, caused a fluorescence increment at a slightly excess concentration. From FIG. 2, we could found that only D-3-HB addition induces a strong emission enhancement. Among these different anions, physiologically important anions which exist in living cells, only SO_4^{2-}, PO_4^{3-}, CO_3^{2-} could cause moderate intensity of fluorescence enhancement and they could be removed by adding Ca^{2+} to the system. All above results proved that the new system exhibits a high selectivity to D-3-HB in DMSO/H_2O (v:v=1:1). This method represents an extremely easy way to qualitatively and quantitatively determine the presence of D-3-HB. Moreover, metal ions existing in cells (such as Al^{3+}, Ba^{2+}, Na^+, K^+, Ca^{2+}, Ni^{2+}, Cr^{3+}, Sr^+, Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Sn^{2+}) were also determinate. The results shown that the metal ions have no remarkable interference on D-3-HB determination (FIG. 3).

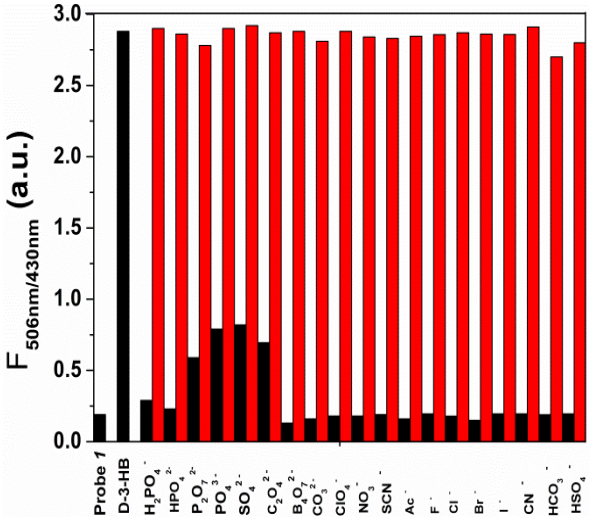


FIG. 3. **The fluorescence responses ($F_{506\ nm/430\ nm}$) of probe 1 (1.0 µM) with various anions (10 µM) in DMSO/H_2O (v:v=1:1). The final concentration for D-3-HB is 2.0 µM, for is 10 µM. (λ_{ex}=350 nm).**

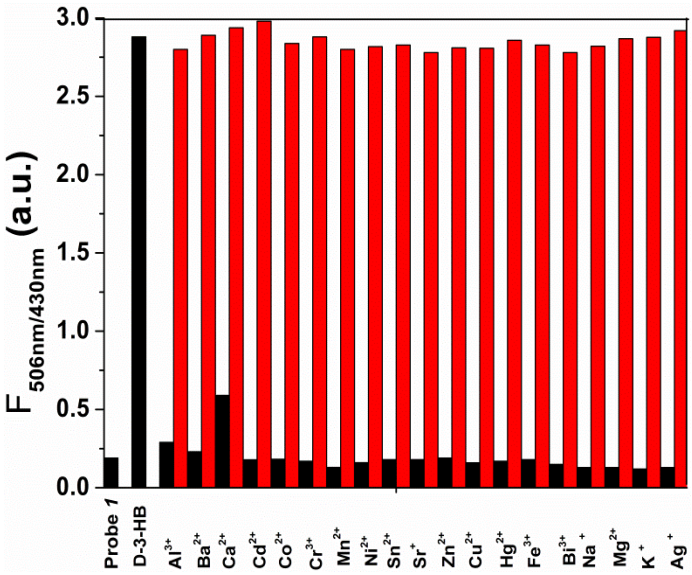


FIG. 4. **Emission intensity at $F_{506\ nm/430nm}$ of probe 1 (1.0 µM) in DMSO/H_2O (v:v=1:1) induced by indicated D-3-HB and metal ions. The final concentration for D-3-HB is 2.0 µM, for Al^{3+}, Ba^{2+}, Ca^{2+}, Ni^{2+}, Cr^{3+}, Sr^{+}, Na^{+}, K^{+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Co^{2+}, Sn^{2+} is 10 µM. λ_{ex}=350 nm.**

Effect of reaction time

We further examined the time of the fluorescence intensities of the probe 1 in the presence of 4.0 equiv. D-3-HB in DMSO/H_2O (v:v=1:1) solution. As shown in FIG. 4, the fluorescence response of the probe 1 was very fast, reaching a stable

value within 20s and the maximal fluorescence signal was reached within 30s, it is the same with the early reported for detection of Al^{3+} [22,23].

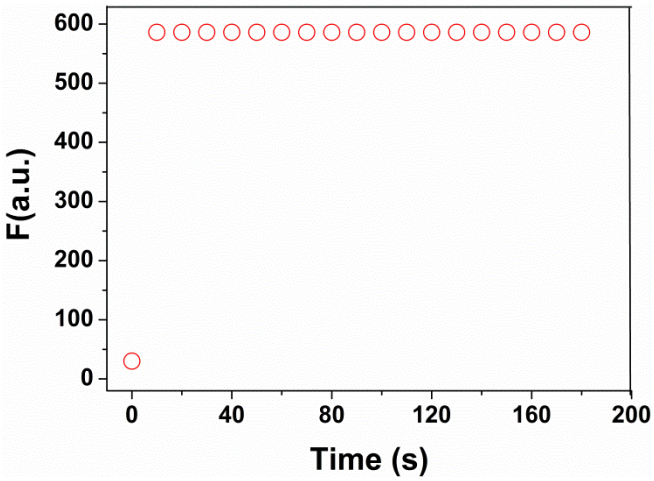
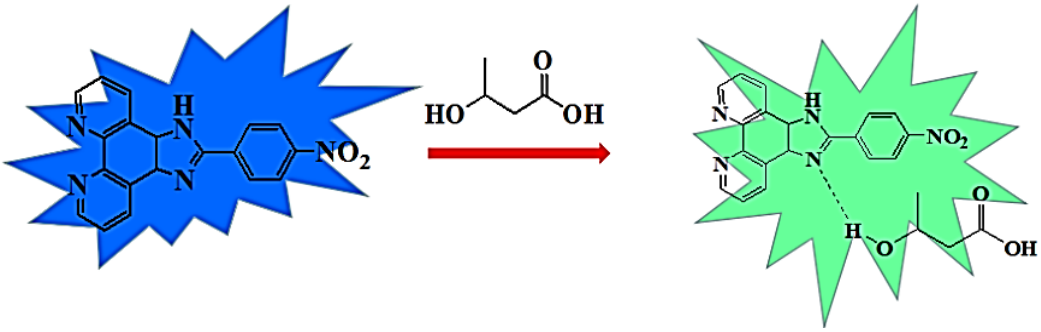


FIG. 5. **Reaction time profile of probe 1 (1.0 µM) and D-3-HB (4.0 µM) at 506 nm.**

Proposed mechanism

To better understand the complexation behavior of probe 1 with D-3-HB, Mass spectrometry analysis of a product obtained from the reaction of the probe 1 with D-3-HB in CH_3OH shows the binding between probe 1 and D-3-HB (FIG. 5). A peak at m/z=470.1278 (cal: m/z=470.14), corresponding to $[1+D\text{-}3\text{-}HB+Na]^+$, is clearly observed, which is consistent with a 1:1 stoichiometry between probe 1 and D-3-HB. It is similar with our early report [20]. Therefore, we proposed a possible mechanism, as shown in SCHEME 2.



SCHEME. 2. **Proposed binding mode of probe 1 with D-3-HB.**

Conclusions

A new probe 4-(1H-imidazo [4,5-f] [1,10] phenanthrolin-2-yl) nitrobenzene probe 1) was synthesized and characterized by ^{1}H-NMR, ^{13}C-NMR , EAs , FT-IR, ESI-MS. Probe 1 showed a remarkable colorimetric selectivity to D-3-HB over common

anions and metal ions, and could form stable 1:1 complex with D-3-HB and generated color change from colorless to yellow in DMSO/H_2O (v:v=1:1). It could be serve as an effective probe for colorimetric detection of D-3-HB with a detection limit as low as 0.25 μM using the UV-Vis spectra and the visual color changes by the naked eye respectively. So, we trust probe 1 has an ability to serve as a practical sensor for D-3-HB detection.

Acknowledgements

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