Research paper

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PII: S0020-1693(18)30148-8
DOI: https://doi.org/10.1016/j.ica.2018.03.033
Reference: ICA 18178

To appear in: *Inorganica Chimica Acta*

Received Date: 27 January 2018
Revised Date: 20 March 2018
Accepted Date: 21 March 2018

Please cite this article as: W-D. Wang, Y. Hu, Q. Li, S-L. Hu, A carbazole-based turn-on fluorescent probe for the detection of hydrazine in aqueous solution, *Inorganica Chimica Acta* (2018), doi: https://doi.org/10.1016/j.ica.2018.03.033

A carbazole-based turn-on fluorescent probe for the detection of hydrazine in aqueous solution

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Abstract

A carbazole-based fluorescent probe, 2-(9-ethyl-9H-carbazol-3-yl) isoindoline-1,3-dione, with a low detection limit (2.673× 10^{-6} M) for the detection of hydrazine is designed and synthesized based on Gabriel reaction. The probe responds selectively to hydrazine over other amino compounds with marked fluorescence enhancement. Moreover, test paper experiments indicated its great potential in the environment monitoring of hydrazine in aqueous solution.

Key words

Carbazole, Fluorometric, Hydrazine, Gabriel reaction.

1. Introdution

As a type of highly reactive base and reduction agent [1], hydrazine has also played a vital role in pharmaceuticals, pesticides, dyes and emulsifiers [2-4]. However, hydrazine is highly poisonous and the exposure of hydrazine in elevated levels could

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cause serious damage to the liver, lungs, kidneys, especially for the human nervous system [5-7]. As a result, it is necessary to develop the highly selective and sensitive analytical methods for the detection of trace hydrazine in both environmental and biological science.

Recently, the development of fluorescent probes toward metal ions, anions and biomolecules has attracted great attention because of its simplicity, low cost, good selectivity, and high sensitivity [8-12]. Of particular interest is the design of "turn-on" fluorescent probes for selective detection because fluorescence enhancement are more sensitive and more easily monitored than fluorescence quenching [13-15]. A variety of fluorescent probes based on coumarin[16, 17] , naphthalimide [18-20], pyrazoline[21,22], benzothiazole [23], BODIPY[24], fluorescein[25, 26] , pyrene and anthracene [27] for hydrazine have been reported. Carbazoles are known for their good chemical stability and high fluorescence quantum yield [28, 29]. Many carbazole-based compounds as fluorescent probe for detection of anion, metal ions and biomolecules have also been reported [30-36]. However, carbazole-based fluorescent chemosensors for hydrazine is still rare [37].

Herein, we designed and synthesized a carbazole-based fluorescence probe, 2-(9-ethyl-9H-carbazol-6-yl) isoindoline-1,3-dione (**1**, Scheme 1). This probe is constructed via two functional moieties: 9-ethyl-9*H*-carbazole acts as a fluorophore for its excellent photophysical property, and isoindoline-1,3-dione linked to 9-ethyl-9*H*- carbazole provides the recognition and binding site for hydrazine. Probe **1** displays high selectivity for hydrazine among other analyst examined and exhibits

turn-on fluorescence upon binding of hydrazine in aqueous solution.

2. Experimental

2.1. Chemicals and apparatus

9-ethyl-9H- carbazol-3-amine (**4**) was synthesized according to the reported method [38]. NMR spectra were measured on a Varian Mercury 300 spectrometer operating. ESI-MS spectra were obtained on a Finnigan Trace MS spectrometer. Absorption spectra were determined on UV-2501 PC spectrophotometer. Fluorescence spectra measurements were performed on a Fluoro-Max-P spectrofluorimeter. The X-ray crystal structure determinations of **1** were obtained on a Bruker SMART APEX CCD system.

*2.2. Synthesis of probe **1***

The synthetic route of compound **1** is shown in Scheme 1.

To a 25 mL flask, 9-ethyl-9H-carbazol-3-amine (**4**) (1.0 mmol, 0.21 g) and *o*-phthalic anhydride (1.0 mmol, 0.15 g) were added to acetic acid (7ml) and refluxed for 6 h. Then acetic acid was removed and the afforded solid was purified by column chromatography on silica gel to obtain **1** as a white solid (0.24 g, 71%). M.p.: 212-213°C (lit.[39], 212-213°C). ^{1}H NMR($CDCl_3$, 300 MHz): δ1.43-1.48 (t, 3H), 4.36-4.44 (q, 2H), 7.72-8.11 (m, 11H); ^{13}C NMR ($CDCl_3$, 75MHz): 13.8, 37.6, 108.6, 10 8.8, 119.1, 119.3, 120.7, 122.61, 122.65, 123.2, 123.6, 124.5, 125.6, 126.1, 131.9, 134.2, 135.9, 139.3, 140.4, 168.1. ESI-MS: *m/z* 702.83 $[2M+Na]^+$. Crystal data for $C_{22}H_{16}N_2O_2$: Crystal size: 0.32×0.17×0.12 mm, monoclinic, space group $P2_1/c$. *a* =

10.069(3) Å, b = 10.968(4) Å, c = 15.670(5) Å, V = 1701.9(10) Å^3, Z = 4, T = 298(2) K, θ_{max} = 30.00°, 14031 reflections measured, 3230 unique (R_{int} = 0.0244). Final R indices ($I > 2\sigma(I)$): R_1 = 0.0418, wR_2 = 0.1212 and GOF =1.056. CCDC number: 1561225.

(Scheme 1)

(Fig. 1)

2.3. *UV–Vis absorption and fluorescence spectroscopy analysis*

The stock solution of probe **1** in CH_3CN was diluted and pH of the solution was adjusted to about 7.0 using HEPES solution to deliver the final concentration of the probe (10 μM, pH=7.0) in CH_3CN-HEPES (1:1, v/v) solution. Hydrazine, metal ions, anion, and primary amines stock solutions with concentration of (1mM) were prepared in water, respectively. Fluorescence spectra were recorded using a fluorescence spectrophotometer (λ_{ex} = 371 nm, λ_{em} = 439 nm, slit widths: 5 nm/5 nm) after 60 min with the addition of determinand at 25°C. The relative fluorescence quantum yields, Φ, were determined by standard methods with respect to rhodamine B (Φ = 0.69 in ethanol) as a reference [40].

3. Results and discussion

3.1. *Synthesis and characteristics of probe1*

The probe **1** was obtained in 71% yield by interacting o-phthalic anhydride and 9-ethyl-9H-carbazol-3-amine (**4**) in refluxing acetic acid for 6 h. The structure of **1** was identified by NMR, MS (Fig. S1-3) and X-ray diffraction analysis. The crystal structure of **1** is shown in Fig. 1. In the molecule, the carbazole ring displays a

co-planar conformation. However, **t**he dihedral angle (124.3°) between *o*-phthalic anhydride and carbazole ring indicated that probe **1** had a very big distortion.

3.2. *Spectral response of probe **1** to hydrazine.*

The absorption and emission spectra of probe **1** was first investigated in HEPES buffer (10 mM, pH 7.0, 50% CH_3CN, 25^oC). As shown in Fig. 2, the free probe **1** showed absorption bands centered at 290, 331 and 346 nm. Upon addition of hydrazine (50 equiv.), this absorption bands disappeared and two new bands at 364 and 299 appeared. The presence of three well-defined isosbestic points at 296, 311 and 351 nm indicates the formation of the hydrazone product. As shown in Fig 3. The free probe **1** displayed a very weak emission peak at 439 nm (λ_{ex}= 371 nm). The significant increase of the fluorescence intensity at 439 nm was evidenced after addition of hydrazine and the fluorescent color changed from colorless to bright blue.

(Fig. 2)

(Fig. 3)

To further evaluate its sensing properties, the dynamics of the reaction between probe **1** (10 μM) in CH_3CN–HEPES (1:1, v/v) solution (pH=7.0) and hydrazine were studied by monitoring time-dependent fluorescence spectra. As shown in Fig. 4, when hydrazine (50 equiv.) was added to the solution of the probe, the emission intensity at 439 nm increased gradually over time and peaked within 60 min. Thus, the reaction time required to produce stable fluorescence intensity was 60 min.

(Fig. 4)

To conduct a quantitative analysis of probe **1**, we then examined the sensitivity of probe **1** for different concentrations of hydrazine by the fluorescence spectra. As shown in Fig. 5, free probe **1** (10 μM) exhibited an extremely weak fluorescence intensity, its fluorescence quantum yield (Φ) was calculated to be 0.0078. However, the fluorescence intensity at 439 nm gradually increased with the addition of hydrazine (0-55 equiv.). The fluorescence quantum yield of **1** in the present of 50 equiv. hydrazine was calculated to be 0.36. Moreover, an excellent linear relationship of emission intensity versus hydrazine concentration (10–40 equiv.) was observed (R_2 = 0.9906, y = 9.719x - 66.522) (Fig. 6). The limit of detection (LOD) of probe **1** is 2.673×10^{-6} M based on the definition of IUPAC (C_{DL}=3Sb/m) [41]. Compared with some reported hydrazine probes (Table 1), probe **1 s**till showed a good LOD for hydrazine in aqueous solution. These results demonstrate that the probe **1** could be used to detect N_2H_4 quantitatively using the fluorescence spectroscopy method.

(**Fig. 5**)

(**Fig. 6**)

(Table 1)

3.3. *Selectivity of probe **1** toward various analytes*

To evaluate the selectivity of the proposed probe, we examined the fluorescence response of **1** to various metal ions, anions and several common amine-containing

species, including Fe^{3+}, Cd^{2+}, K^{+}, Na^{+}, Mg^{2+}, Al^{3+}, Mn^{2+}, Cu^{2+}, Cr^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, NO_3^{-}, HSO_4^{-}, ClO_4^{-}, $H_2PO_4^{-}$, Cl^{-}, Br^{-}, I^{-}, F^{-}, ClO^{-}, ethylenediamine, diethylamine, triethylamine, ammonia, thiamine, urea, glutathione (GSH) and cysteine (Cys). As demonstrated in Fig. 7, these interferences did not lead to any significant fluorescence enhancement of **1** at 439 nm. The competition experiments (Fig. 8) also indicated that there was hardly any interference to monitor hydrazine in the presence of various interfering analytes. These results showed that probe **1** could provide high specifically for hydrazine detection.

(Fig. 7)

(Fig. 8)

*3.4. Mechanism of probe **1** in sensing hydrazine*

To explore the sensing mechanism of probe **1** for hydrazine, the reaction products of probe**1** and hydrazine were separated and characterized. The fluorescence product was characterized to be 9-ethyl-9H-carbazol-3-amine (**4)** by ^{1}H NMR, ^{13}C NMR and ESI-MS (Figs. 4-6), which is in agreement to the previous Gabriel-type hydrazinolysis reaction mechanism [42]. Therefore, a reasonable binding mechanism based on the Gabriel reaction was proposed in Scheme 2.

(Scheme 2)

The probe **1** showed no obvious fluorescence in solution because of its distorted

molecular structure, which can be observed from its X-ray structure (Fig. 1). However, After Gabriel-type hydrazinolysis of probe **1** in the present of hydrazine, the fluorescence product, 9-ethyl-9H-carbazol-3-amine (**4)** was released into the solution and then the ‘‘turn-on” fluorescence change was observed.

3.5. *Practical applications*

Because hydrazine has been widely used in a variety of industrial processes, hydrazine detection in aqueous samples is of interest. We explored opportunities for probe**1** to analyze hydrazine in aqueous solution for practical applications. Firstly, test papers were prepared by immersing filter papers into a CH_3CN solution of probe **1** (5 μm) and dried under room temperature. Then, the filter papers coated with probe **1** were immersed into a water sample of different amine-containing species for 5.0 min, respectively. As shown in Fig. 9, in the case of hydrazine, distinctive changes were observed in the fluorescence (from blue to blue-green) of the test paper. While in the present of other amines, a visual change in the fluorescent is negligible. Moreover, the fluorescence intensity was dependent on the hydrazine concentration in aqueous solution and was easy to observe visually (Fig. 10). Therefore, these observations indicated that the as-prepared test paper can be used for hydrazine detection in a water sample.

(Fig. 9)

(Fig. 10)

4. Conclusion

In summary, a carbazole-based fluorescent probe was synthesized for hydrazine detection. The probe displayed distinct changes in the intensity of emission spectra upon addition of hydrazine and the remarkable fluorescence color changes can be observed visually. The detection limit was 2.673×10^{-6} M. Test paper experiments indicated its great potential in the environment monitoring of hydrazine.

Acknowledgments

This study was supported by 2015 open foundation of Hubei Key Laboratory of Pollutant Analysis & Reuse Technology (No. PA150209) .

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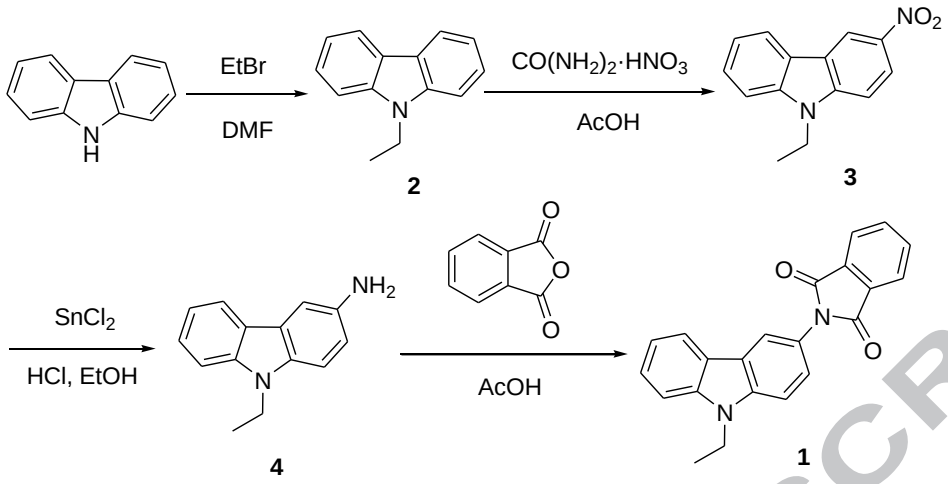


Scheme 1

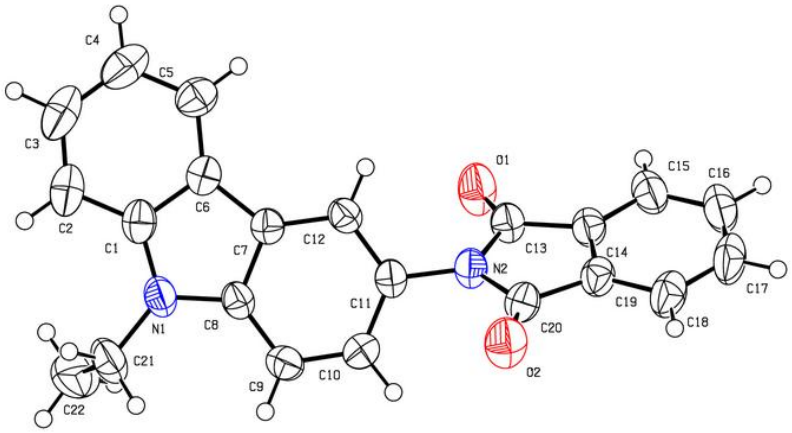


Fig. 1.

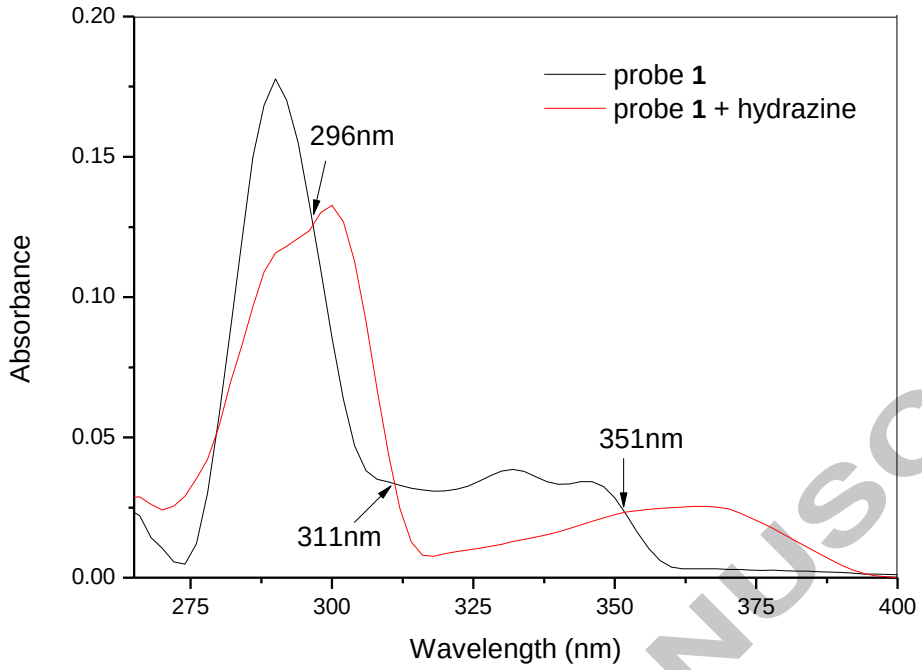


Fig. 2

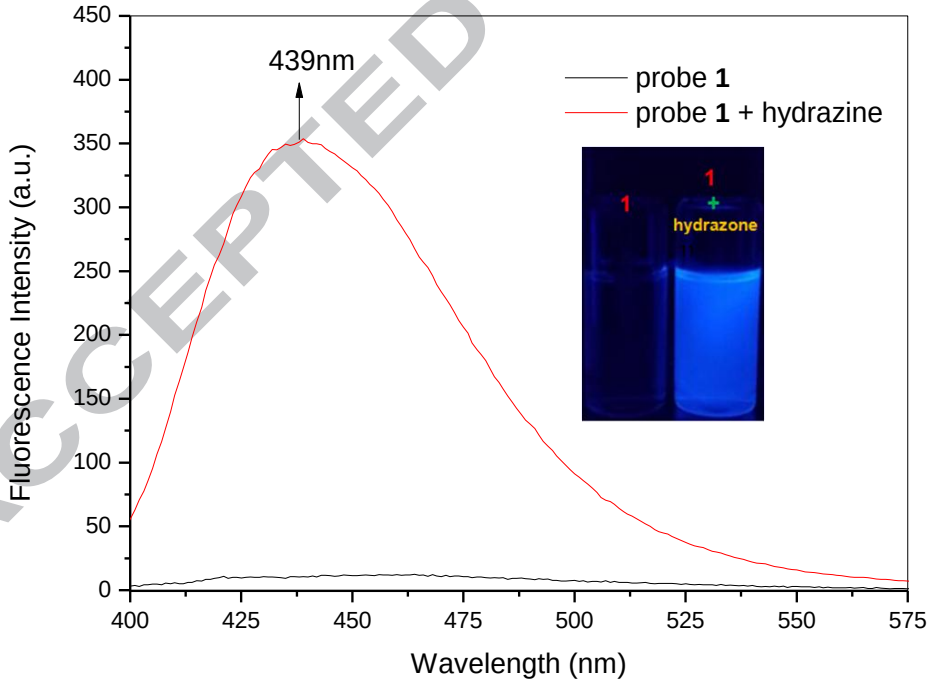


Fig. 3

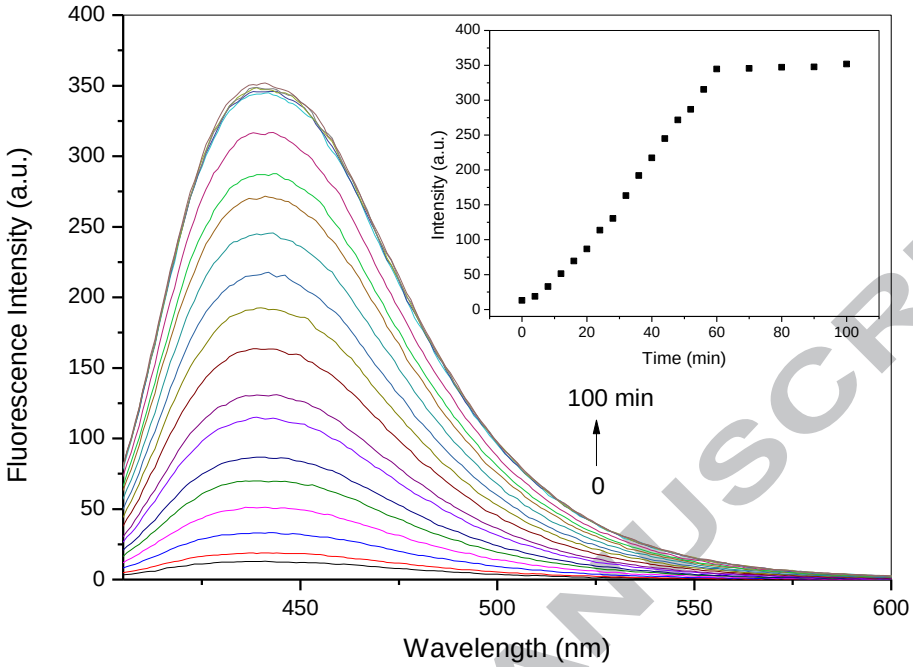


Fig. 4

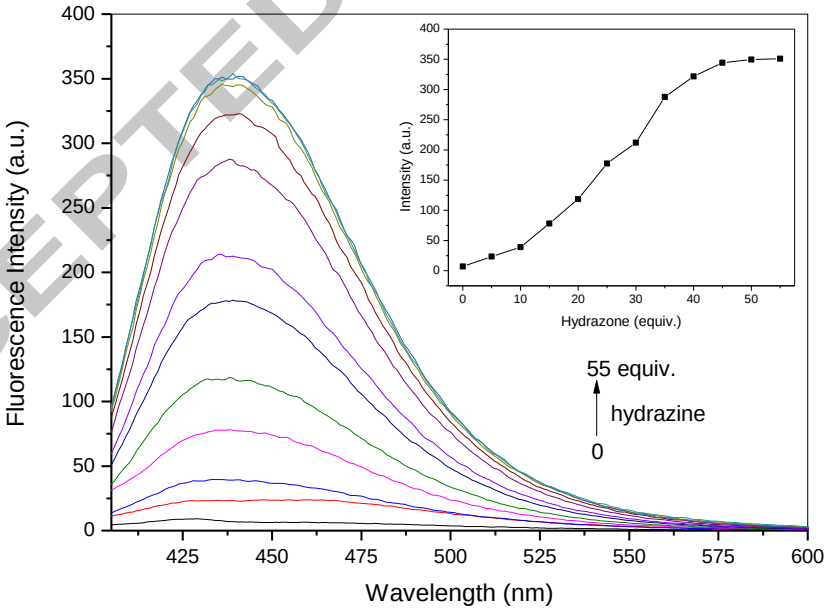


Fig. 5

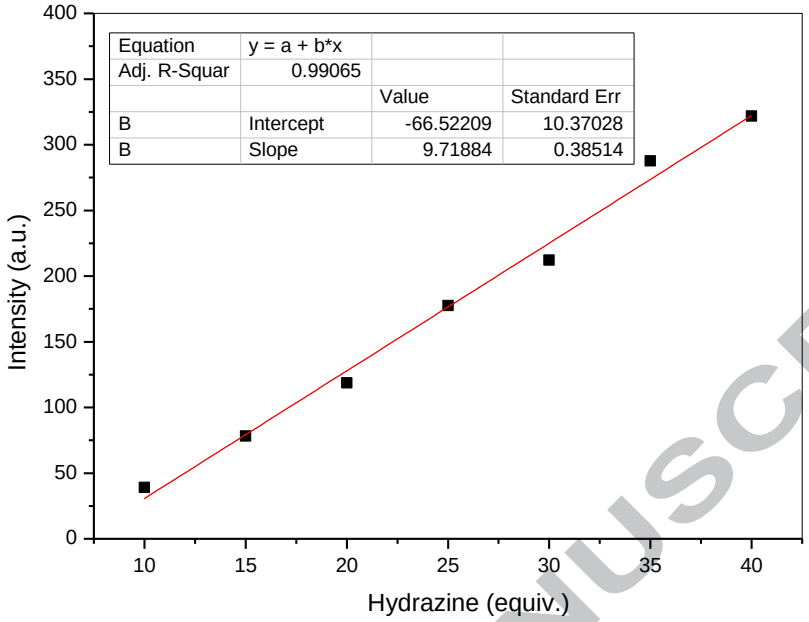


Fig. 6

Ref.	Probes	Solution (v/v)	LOD (mol/L)
[16]	Coumarin	DMSO/acetate (1: 9)	2.0×10^{-5}
[17]	Coumarin	CH_3CN	3.38×10^{-6}
[19]	Naphthalimide	CH_3CN/H_2O (9:1)	1.0×10^{-7}
[20]	Naphthalimide	DMSO/HEPES (4:6)	1.4×10^{-7}
[21]	Pyrazoline	CH_3CN/acetate (1:1)	6.16×10^{-6}
[22]	Pyrazoline	CH_3CN/PBS (3:7)	6.22×10^{-8}
[23]	Benzothiazole	EtOH/PBS((1:99)	1.4×10^{-7}
[24]	BODIPY	DMSO/acetate (1: 9)	1.6×10^{-6}
[25]	Fluorescein	CH_3OH/H_2O(1: 1)	3.88×10^{-8}
[26]	Fluorescein	Tris	3.1×10^{-8}
[37]	Carbazole	CH_3CN/HEPES (8:2)	1.02×10^{-6}
This work	Carbazole	CH_3CN/HEPES (1:1)	2.67×10^{-6}

Table 1

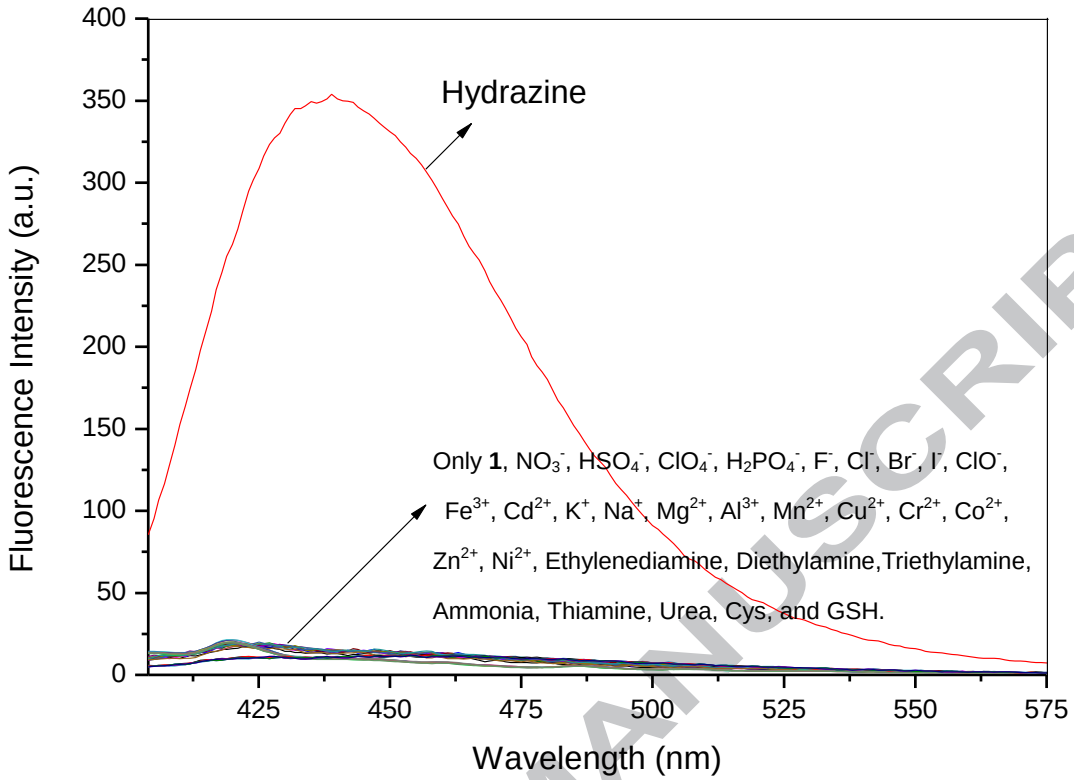


Fig. 7

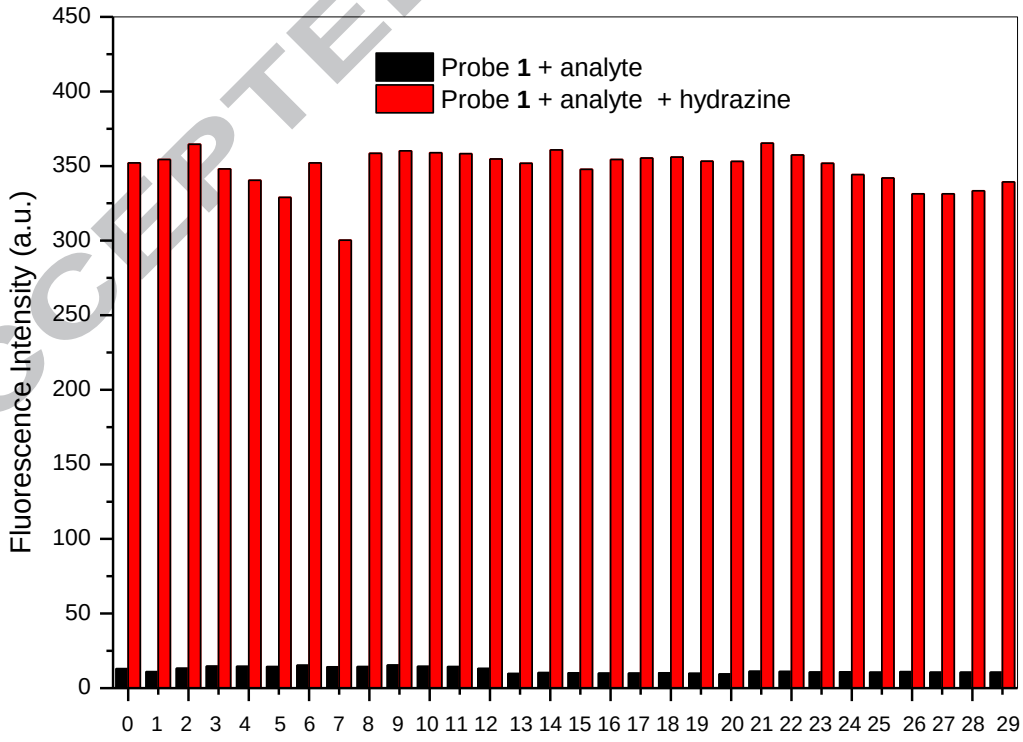
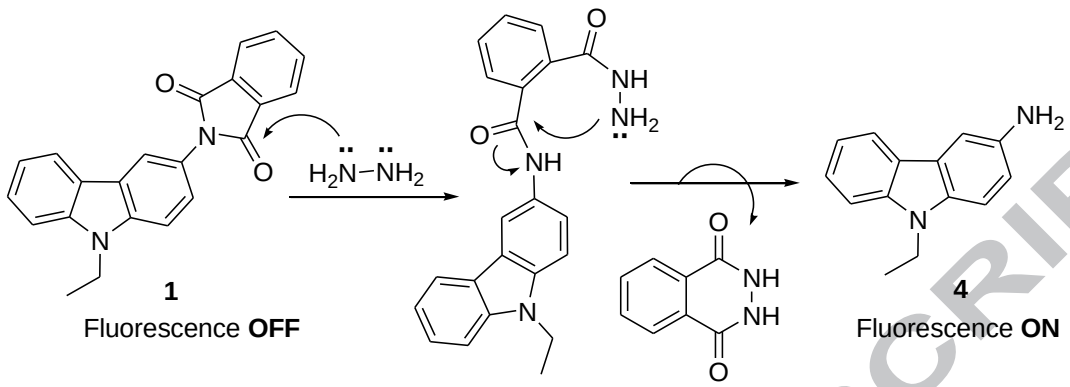


Fig. 8



Scheme 2

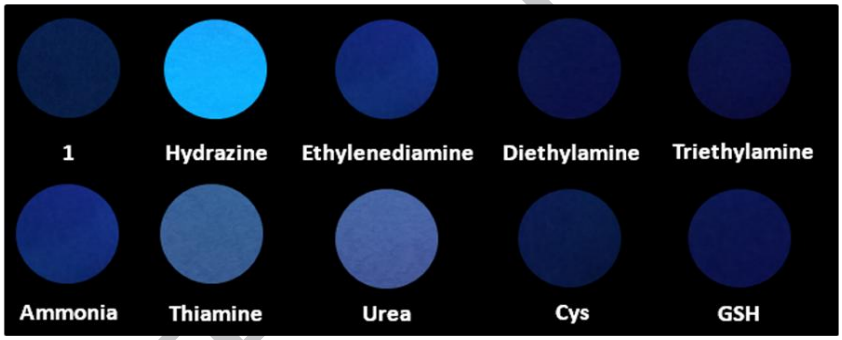


Fig. 9

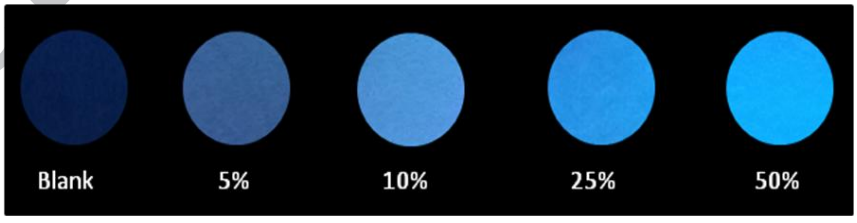


Fig. 10

Captions:

Scheme 1 Synthesis of the probe, **1**.

Fig. 1. The molecular structure of **1**.

Fig. 2 Absorption spectra of probe **1** (10 μM) recorded without and with 50 equiv. hydrazine in a CH_3CN–HEPES (1:1, v/v) solution (pH =7.0).

Fig. 3 Fluorescence spectra of probe **1** (10 μM) recorded without and with hydrazine (50 equiv.) in a CH_3CN–HEPES (1:1, v/v) solution (pH =7.0). Insets: fluorescent color changes of **1** upon addition of 50 equiv. hydrazine with excitation at 365 nm.

Fig. 4. The fluorescence spectra of probe **1** (10 μM)) incubated with hydrazine (50 equiv.) in a CH_3CN–HEPES (1:1, v/v) solution (pH =7.0) at room temperature at different reaction times (0–100 min). Insert: Time-dependent fluorescence intensity (439 nm) changes of probe **1** (10 μM) upon addition of 50 equiv. of hydrazine in a CH_3CN–HEPES (1:1, v/v) solution (pH =7.0) at room temperature.

Fig. 5 Fluorescence spectra of probe **1** (10 μM) with the addition of increasing concentration of hydrazine (0-55 equiv.) in a CH_3CN–HEPES (1:1, v/v) solution (pH = 7.0). Insert: Plot of fluorescence intensity at 439 nm of probe **1** (10 μM) with varied concentrations of hydrazine (0–55 equiv.).

Fig. 6 The fluorescence intensities of probe **1**(10 μM) were linearly related to the concentration of hydrazine (10-40 equiv.), R = 0.9906 (λ_{em} = 439 nm).

Table 1 Comparison of the limit of detection (LOD) of probe **1** with some reported hydrazine fluorescent probes.

Fig. 7 Fluorescence spectra of probe **1** (10 μM) upon addition of hydrazine (50 equiv.)

and various anions, cations and amines (50 equiv.) in a CH_3CN–HEPES (1:1, v/v) solution (pH =7.0).

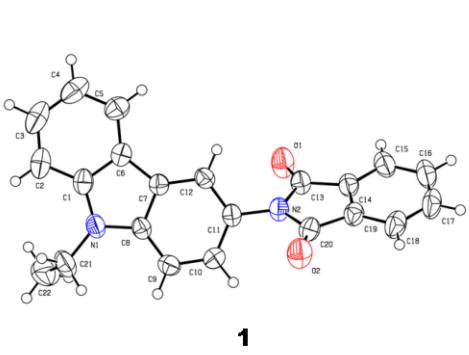
Fig. 8 Fluorescence intensity at 439 nm of probe **1**(10.0 μM) upon addition of various analytes (100 equiv.) and then addition of hydrazine (50 equiv.). Black bar: probe **1**+ various analytes. Red bar: probe **1**+ various analytes + hydrazine. The excitation wavelength was 378 nm (slit = 5 nm/5 nm). (0. Blank, **1**. Fe^{3+}, 2. Cd^{2+}, 3. K^+, 4. Na^+, 5. Mg^{2+}, 6. Al^{3+}, Mn^{2+}, 7. Cu^{2+}, 8. Cr^{2+}, 9. Co^{2+}, 10. Zn^{2+}, 11. Ni^{2+}, 12. NO_3^-, 13. HSO_4^-, 14. ClO_4^-, 15. $H_2PO_4^-$, 16. Cl^-, 17. Br^-, 18. I^-, 19. F^-, 20. ClO^-, 21. Hydrazine, 22. Ethylenediamine, 23. Diethylamine, 24.Triethylamine, 25. Ammonia, 26. Thiamine, 27. Urea, 28. Cys, 29. GSH).

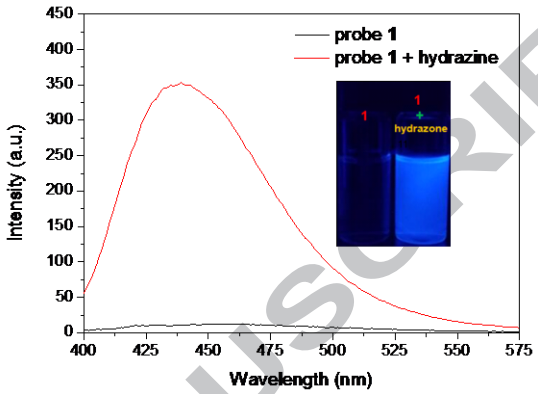
Scheme 2. Proposed sensing mechanism of probe **1** toward N_2H_4.

Fig. 9 Fluorescence color changes of the probe **1**-coated test papers treated with various common amine-containing species under 365 nm light.

Fig. 10 Fluorescence color changes of the probe **1**-coated test papers treated with different concentrations of hydrazine aqueous solution. Fluorescence color changes were observed using a hand-held UV lamp with excitation at 365 nm.

Graphical Abstract





Carbazole derivate (**1**) for selective detection of hydrazine with turn-on fluorescent changes by Gabriel-type hydrazinolysis reaction.

Highlights

- The structure of probe **1** was identified by X-ray diffraction analysis.
- The probe shows the turn-on fluorescent detection for hydrazine with high selectivity.
- Test paper experiments indicated its great potential in the environment monitoring of hydrazine in aqueous solution.