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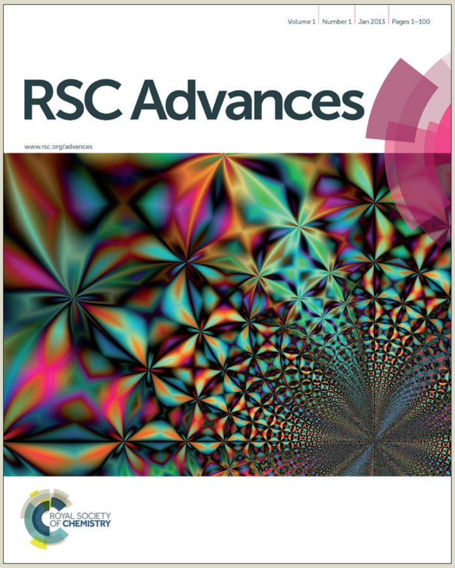

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PAPER

Fluorescence staining of salicylaldehyde azine and applications in the determination of potassium *tert*-butoxide

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Salicylaldehyde azine (**1**) with an aggregation-induced emission (AIE) function was synthesized from salicylaldehyde and characterized by Fourier transform infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), high resolution mass spectrometry (HRMS), and X-ray single crystal diffraction. Compound **1** exhibited high thermal stability and good light-emitting behavior in the solid state. It could be preferably used to dye cellulose and KBr. No obvious changes in fluorescence intensity occurred when compound **1** was dissolved in different pH buffer solutions. The addition of TFA led to slight fluorescence quenching, and the fluorescence intensity gradually increased with increasing potassium *tert*-butoxide solution concentrations. The fluorescence intensity of compound **1** showed a good linear relationship with the concentration of potassium *tert*-butoxide (0.5-4.0×10^{-4} M), y=99.193x+93.599, R^2=0.9902; the limit of detection (LOD) was 1.07×10^{-7} M. It could measure the content of potassium *tert*-butoxide with a relative standard deviation (RSD) value of 1.5%.

1. Introduction

In recent years, new organic fluorescent probes[1-9] and solid light-emitting materials[10-16] have been greatly described due to their good utility in fluorescence. Many fluorescent compounds with a fluorescence enhancement function in a solution state exhibit fluorescence quenching in the solid state. This has limited the development of fluorescence dyes.[17] Some fluorescent compounds have been successfully synthesized to overcome this disadvantage such as polythiophene and its derivataives,[18,19] as well as tetraphenylethene derivataives,[20] 1-methyl-1,2,3,4,5-pentaphenylsilole,[21] and *p*-sexiphenyl.[22]

Concurrently, some studies on salicylaldehyde azines have been reported including pharmacological proprieties[23,24] and optical properties.[25,26] The AIE characteristic of salicylaldehyde azine was first fully studied by Tang.[27] In addition, two-photon absorption and two-photon excitation fluorescence of salicylaldehyde azines were investigated.[28] This was also used as a simple fluorescent probe for Zn(II).[29] Next, some derivatives of salicylaldehyde azine were synthesized for optical application. A novel fluorescence turn-on detection method in human serum albumin (HSA) and bovine serum albumin (BSA) in aqueous solution was investigated with 2,4-dihydroxyl-3-iodo salicylaldehyde azine.[30] Meanwhile, chloro-salicylaldehyde azine and bromo-salicylaldehyde azine exhibited AIEE properties and iodo-salicylaldehyde azine could be applied as a turn-on fluorescence probe for egg albumin detection.[31]

Anti-counterfeiting technologies in paper products (banknote) are very important.[32,33] Fluorescent materials can identify the source information after being irradiated, and fluorescence plays a very important role in the field of anti-counterfeiting.[34-36] Because of good light-emitting behavior, favorable permeability and adhesive force with cellulose, compound **1** could be used an anti-counterfeiting material and dye.

Potassium *tert*-butoxide is an important catalyst and can also be employed in organic synthesis for condensation, rearrangement, ring-opening, etc.[37-40] However, the main method to detect the content of potassium *tert*-butoxide is titration.[41] Thus, it would be useful to detect the content of potassium *tert*-butoxide. In this paper, compound **1** was used to fluorescently detect potassium *tert*-butoxide.

2. Experimental

2.1. Materials and instruments

All the raw materials and solvents were purchased from commercial suppliers and used without further purification. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 500 MHz FT-NMR spectrometer in DMSO with TMS as internal standard, respectively. Fourier-transform infrared (FT-IR) spectra were recorded on a Nicolet 380 FT-IR spectrophotometer. Melting points were measured using an X-6 microscopic melting point apparatus. UV-vis absorption spectra were measured on a UV-2450 spectrophotometer (SHIMADZU) using a quartz cuvette with a 1-cm path length. Fluorescence emission spectra were determined on a Perkin Elmer LS 55 fluorescence spectrophotometer, excitation wavelength at 385 nm, scan from 450 nm to 750 nm with a 100 nm/min of scan speed. Fluorescence microscopic images were photographed with a fluorescence microscopic imaging system (BX51 Olympus). X-ray data were collected on a Bruker D8 Venture area diffractometer.

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Confocal fluorescence microscopic images were read on a LSM710 confocal laser scanning microscopy.

2.2. Synthesis of compound 1

A 250 mL dried three-necked flask equipped with a thermometer, stirrer and condenser was charged with salicylaldehyde (2.44 g, 20 mmol), and hydrazine hydrate (0.63 g, 10 mmol) in 100 mL of ethanol. Then the resulting mixture was refluxed for 2 h with a large amount of precipitation appeared (**Scheme 1**).[33] The precipitation was also refluxed with 1 ml hydrochloric acid for 1 h in 100 mL of ethanol to offer the reaction products, which was purified twice by recrystallization with 100 mL ethanol. Acicular yellow crystal; yield of 90%; mp: 227.2-227.4 ^{o}C; FT-IR (KBr) ν (cm^{-1}): 3436, 1623, 1575, 1486, 1402, 752; ^{1}H NMR (DMSO, 300 MHz): 6.89-6.94 (t, 4H), 7.32-7.37 (m, 2H), 7.63-7.65 (d, 2H), 8.95 (s, 2H), 11.07 (s, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz), δ (ppm): 116.48, 118.15, 119.54, 130.82, 133.16, 158.61, 162.75; HRMS (m/z): $[M+H]^+$ calcd for $C_{14}H_{12}N_2O_2+H^+$, 241.0971; found, 241.0959.

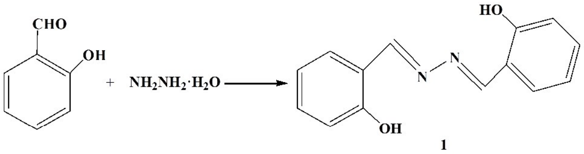
3. Results and discussion

3.1. Synthesis

Compound **1** was first synthesized by a reaction of salicylaldehyde and aqueous hydrazine. The compound was characterized by HRMS, IR, ^{1}H-NMR, ^{13}C-NMR, and X-ray single crystal diffraction (**Table S1**, Supplementary data and **Fig. 1)**. These analyses confirmed compound **1** to be a salicylaldehyde azine.

3.2. Thermal stability

Good thermal stability is required for fluorescence dyes, and we assessed this for compound **1** using TGA under a nitrogen stream (**Fig. S1**, Supplementary data). Compound **1** was thermally stable up to ca. 232 ^{o}C corresponding to a 5% of the material weight loss. Thus, compound **1** had good thermal stability.



Scheme 1. Synthesis of compound **1**

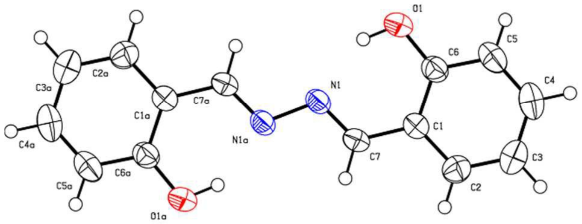


Fig. 1 X-Ray crystal structure of compound **1**.

3.3. Solid state fluorescence properties

3.3.1. Fluorescence enhancement properties

Stable light-emitting behavior in the solid state is the foundation of fluorescence. **Figs. 2a** and **2b** show that compound **1** has good fluorescence and emits Kelly light. To study pH effects, three KBr pellets were prepared for fluorescence analysis after evenly grinding three different mixtures (a mixture of 1.0 g KBr and 1.0×10^{-6} mol compound **1**; a mixture of 1.0 g KBr, 1.0×10^{-6} mol compound **1** and 1.0×10^{-6} mol TFA; a mixture of 1.0 g KBr, 1.0×10^{-6} mol compound **1** and 1.0×10^{-6} mol potassium *tert*-butoxide). The addition of TFA or potassium *tert*-butoxide enhanced the fluorescence (**Fig. 2b)**. TFA had the most pronounced effects. Thus, the addition of an organic acid or alkali could not lead to fluorescence quenching in the solid state.

3.3.2. Application of compound 1

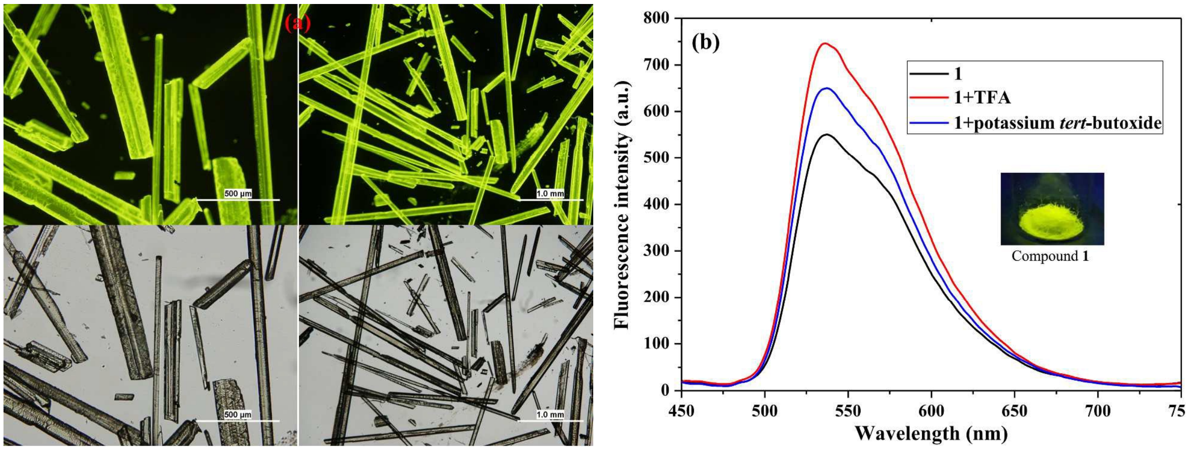


Fig. 2 (**a**) Fluorescence microscopic images (upper) and microscopic images (lower) of compound **1**. (**b**) The fluorescence intensity of compound **1** as a KBr pellet was affected by TFA and potassium *tert*-butoxide ($\lambda_{ex385\ nm}$). Inset is a photo of compound **1** under a 365 nm light.

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Compound **1** solution (1.0×10^{-3} M) was used to dye filter paper, pledget, cotton and KBr. The images were obtained under sunlight and 365 nm UV light (**Fig. 3**). Under sunlight, the color of the dyed materials changed only a little, but they emitted strong Kelly light under 365 nm irradiation and could be a fluorescence dye. Confocal fluorescence microscopy images showed the microstructures of these dyed materials (**Fig. S2**). Compound **1** had good permeability with cellulose and was well-mixed with KBr. Then, an anti-counterfeiting icon was prepared with compound **1** and filter paper (**Fig. S3**). The icon was not seen under the sunlight, but it was very clear under a 365 nm irradiation. Compound **1** could also be used to indicate devices at night (**Fig. S4**).

We next studied the pH effects of compound **1** (1×10^{-4} M) with solutions of buffered 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 20 mM, 90% (v/v) C_2H_5OH) (**Fig. S5**). The fluorescence intensity had no significant change in different pH values; there was no fluorescence quenching. This showed that compound **1** was stable, and its fluorescence intensity was pH-stable. The adhesive force and influence of light on compound **1** and the substrate were other important factors influencing the applications. Thus, a study on adhesive force and influence by light was finished (**Figs. S6**, **S7**, **S8**). No significant fluorescence quenching occurred after the dyed pledget and filter paper were washed with water for 5 min and treated with sunlight for 120 h. There was no fluorescence when they were washed at the same time with ethanol due to the dye's solubility in ethanol. The results showed that compound **1** had good adhesive force because it could not be easily washed off by water. The light stability test also proved that compound **1** was stable on the substrate.

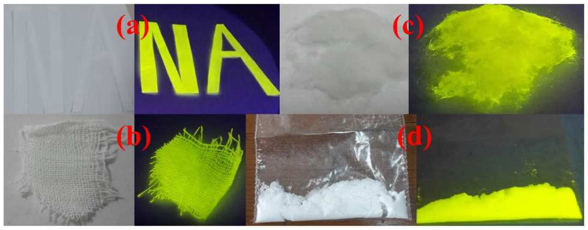

Fig. 3 Photographs of filter paper (**a**), pledget (**b**), cotton (**c**) dyed by compound **1** (1.0×10^{-3} M) and a mixture of compound **1** and KBr powder (1.0×10^{-6} mol/g, **d**) under sunlight (left) and 365 nm UV light (right).

3.4. Optical properties in solution

Compound **1** was readily soluble in methanol, ethanol, isopropanol, ethyl acetate, trichloromethane, dichloromethane, and cyclohexane. UV-vis absorption and fluorescence spectra of compound **1** in these solvents (1×10^{-4} M) were studied (**Fig. S9**, Supplementary data). Compound **1** had two absorption maximum peaks at 294 nm and 357 nm, and there were no significant changes in different solvents (**Fig. S9a**). In contrast, the fluorescence intensity was markedly affected by solvents (**Fig. S9b**). A methanol solution of compound **1** had a peak intensity at 540 nm, but other solutions red-shifted from 540 nm to 550 nm. These solutions of compound **1** had good fluorescence after dissolution in cyclohexane,

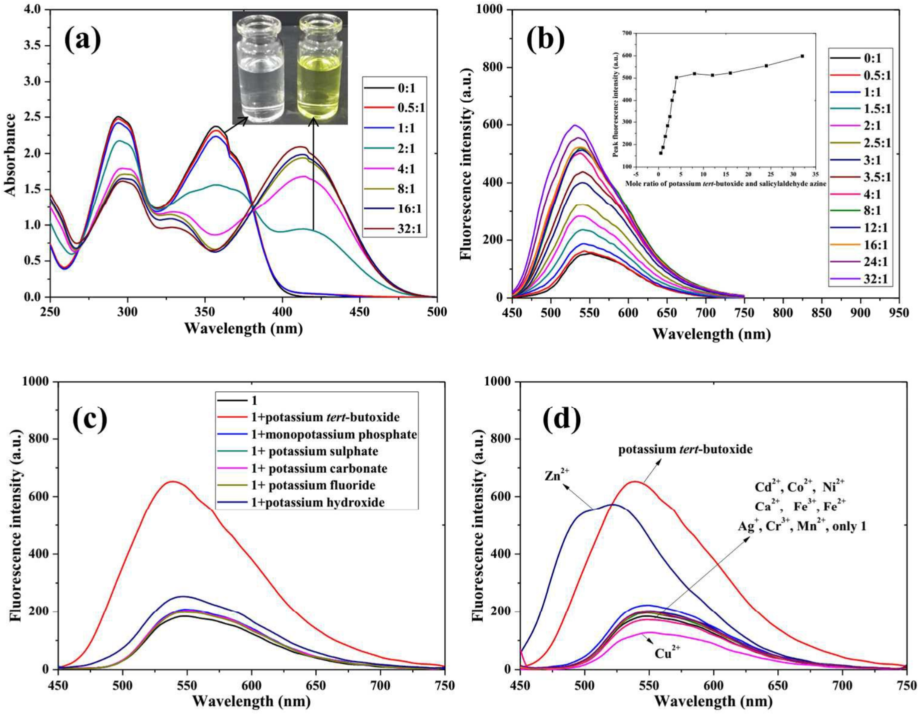

Fig. 4 UV-vis absorption spectral change (**a**) and fluorescence spectral change (**b**) of compound **1** in ethanol (1.0×10^{-4} M) with different molar ratios of potassium *tert*-butoxide and compound **1**. The fluorescence intensity of compound **1** in ethanol (1×10^{-4} M) with equal K^+ (**c**) and other metal ions (**d**), $\lambda_{ex385\ nm}$.

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trichloromethane, dichloromethane, or isopropanol.

To further study the stabilities of compound **1** affected by TFA or potassium *tert*-butoxide in solution, the UV-vis absorption and fluorescence spectra of compound **1** in ethanol (1.0×10^{-4} M) with different equivalents of TFA or potassium *tert*-butoxide were shown in **Fig. S10** and **Fig. 4**.

Fig. S10a shows that the absorbance peak was stable with increasing TFA, but the fluorescence intensity was affected by increased TFA. This lead to a slight decrease without minimal changes (**Fig. S10b**). Compound **1** has two nitrogen atoms and two sites to bind protons.[42] The two nitrogen atoms were unaffected by H^+, and the UV-vis absorption and fluorescence spectra were stable. Thus, the structure of compound **1** was stable.

Versus **Fig. S10a**, the absorbance of compound **1** was markedly affected by potassium *tert*-butoxide (**Fig. 4a**). The color turned from colorless to yellow when the solution of compound **1** had more than 2 equivalents of potassium *tert*-butoxide. The absorbance of the two peaks at 294 nm and 356 nm decreased gradually with the addition of an increasing potassium *tert*-butoxide. A new absorption peak appeared at 411 nm when the molar ratio of potassium *tert*-butoxide and compound **1** was 1:1. An obvious absorption peak emerged after more than 2 equivalents of potassium *tert*-butoxide were added to the solution. That is, the color change indicated a response.

The fluorescence spectra of compound **1** in ethanol (1.0×10^{-4} M) with different equivalents of potassium *tert*-butoxide are also provided (**Fig. 4b**). Fluorescence enhancement occurred, and the intensity increased as a function of potassium *tert*-butoxide concentration. The peak fluorescence intensity increased rapidly with potassium *tert*-butoxide concentration until 4 equivalents were added at which point it plateaued.

To study the mechanism, the impact of K^+ and OH^- was analyzed (**Fig. 4c**). Except potassium *tert*-butoxide, the addition of monopotassium phosphate, potassium sulphate, potassium carbonate, potassium fluoride, or potassium hydroxide, led to no significant fluorescence enhancement. Potassium hydroxide had OH^-, which could only lead to a slight fluorescence enhancement. The –OH of compound **1** might lead to deprotonation with OH^-,[42] but this was not the main reason for fluorescence enhancement. To further understand the mechanism, the fluorescence intensity of compound **1** (1×10^{-4} M) and equal metal ion concentrations in ethanol solution were discussed (**Fig. 4d**). The system with Zn^{2+} also had fluorescence enhancement, which agreed with the simple fluorescent probe data.[33] No significant fluorescence change occurred when other metal ions were added to the solution. The solution with compound **1** and Cu^{2+} had quenching.

There are many reasons why Zn^{2+} might enhance fluorescence.[43-45] The unfixed C=N structure is non-fluorescent due to the predominant decay process of C=N isomerization in the excited states. After complexing with metal ions, the fluorescence increases strongly because it restricts the rotation of the C=N bond and leads to the suppression of C=N isomerization. The reason for potassium *tert*-butoxide causing fluorescence enhancement might be a complexation reaction with compound **1,** which maintains the Zn^{2+} condition. Mass spectrometry (MS) of compound **1** without and with equivalent of potassium *tert*-butoxide in ethanol (1×10^{-4} M) were measured by LC-MS (**Fig. S11**). It was clear to see that the m/z =241.3 peak was for compound **1** and another m/z =375.1 peak was for the complex of compound **1** and potassium *tert*-butoxide (the stoichiometric ratio was 1:1).

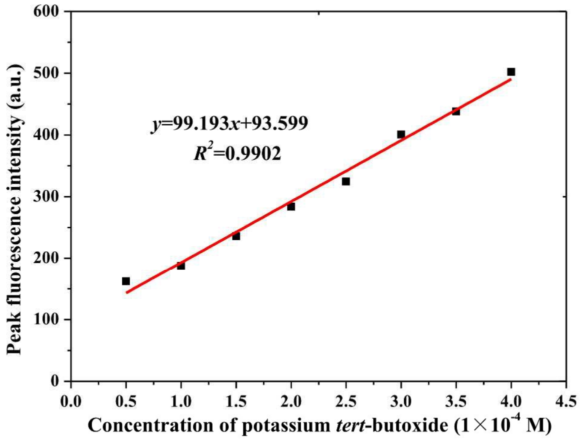

Fig. 5 The linear relationship of peak fluorescence intensity and the concentration of potassium *tert*-butoxide.

Fig. 5 depicts a good linear relationship of the peak fluorescence intensity and concentration of potassium *tert*-butoxide ($0.5\text{-}4.0\times10^{-4}$ M), $y=99.193x+93.599$, $R^2=0.9902$. The limit of detection (LOD) for determining potassium *tert*-butoxide was 1.07×10^{-7} M using $3\sigma_{bi}/m$,[46] where σ_{bi} is the standard deviation of six groups of blank signals; m is the slope of the linear calibration plot. To detect the content of potassium *tert*-butoxide purchased from the supplier, two samples of compound **1** (1×10^{-4} M) with potassium *tert*-butoxide were prepared (**Table S2**). The content was calculated after measuring the peak fluorescence intensity of the two samples. The two content were calculated to be 97.81% and 99.91%. The concentration of potassium *tert*-butoxide was successfully measured via this new method with a RSD of 1.5%. This highlights the utility of compound **1**.

4. Conclusion

Salicylaldehyde azine (**1**) was directly synthesized from salicylaldehyde. Compound **1** had good fluorescence, thermal stability, adhesive forces, and light stability in the solid state. The dye could be used to dye filter paper, pledget, cotton and KBr. It is a candidate material for anti-counterfeiting and is a fluorescence indicator. Compound **1** was stable at different pH values in the solution state and was not quenched by TFA. However, the fluorescence intensity of compound **1** gradually increased with increasing potassium *tert*-butoxide concentrations. A good linear relationship of the peak fluorescence intensity and concentration of potassium *tert*-butoxide ($0.5\text{-}4.0\times10^{-4}$ M) was also obtained ($y=99.193x+93.599$, $R^2=0.9902$); the LOD was 1.07×10^{-7} M. This is new tool to measure potassium *tert*-butoxide concentrations, and it increases the value of salicylaldehyde azine.

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Graphical Abstract:

Fluorescence staining of salicylaldehyde azine and applications in the determination of potassium *tert*-butoxide

Jinlai Yang,[a] Jian Rui,[a] Xu Xu,[ab] Yiqin Yang,[c] Jun Su,[a] Haijun Xu,[ab] Yunyun Wang,[a] Nan Sun,[a] Shifa Wang*[ab]