Synthesis of Symmetrical Acetophenone Azine Derivatives as Colorimetric and Fluorescent Cyanide Chemosensors

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Received: March 1, 2021
Accepted: October 17, 2021

DOI: 10.22146/ijc.64428

Abstract: *Cyanide is a highly toxic anion and poison to the environment. Therefore, fast, effective, and efficient analysis methods to detect cyanide are needed. Herein, symmetrical chemosensor of 2'-hydroxy acetophenone azine (**1**) and 2',4'-dihydroxy acetophenone azine (**2**) has been synthesized tested as colorimetric and fluorescent cyanide chemosensor. The azines were produced from the condensation of acetophenone derivatives with hydrazine hydrate in ethanol under reflux or ultrasonic irradiation methods. Colorimetric and fluorescent chemosensor tests showed selectivity to acetate and cyanide anions in DMSO. The limit of detection (LOD) for colorimetric measurement of cyanide anion was 9.68×10^{-4} M for compound (**1**) and 9.63×10^{-5} M for compound (**2**), while the fluorescent method showed 15.90×10^{-4} M for compound (**1**) and 8.95×10^{-5} M for compound (**2**), respectively. In addition, test paper-strips containing sensor **2** indicated noticeable results for 'naked eye' detection of cyanide in an aqueous medium.*

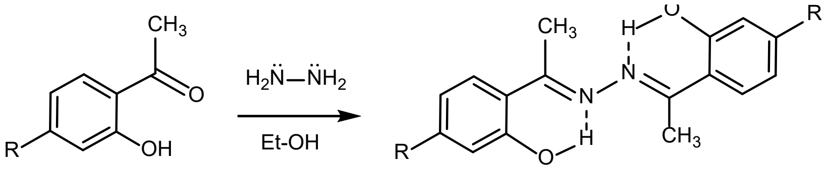
Keywords: *acetophenone; azine; colorimetry; fluorescents; cyanide*

INTRODUCTION

Cyanide is a toxic anion that can affect the function of organ systems such as the vascular system, the sense of sight, central nervous system, heart, endocrine, and metabolic systems [1]. When cyanide ion enters the bloodstream, it disrupts the electron transport chain leading to cytotoxic hypoxia and death [2]. A small dose of cyanide ion around 0.5–3.5 mg of each kg body weight can cause death [3]. According to WHO, the cyanide detection limit in drinking waters is 0.07 mg per liter or about 2.69×10^{-6} M [4].

Cyanide is widely used in industries such as gold mining, electroplating, metallurgy, and polymer productions. These industries produced 140,000 tons of waste per year [5]. Cyanide also occurs naturally as cyanogen glycosides in at least 2,000 types of plants, including apple, mango seeds, and several essential crops such as almonds, cassava, cherries, corn, cotton, and potatoes. Cyanide is also found in cigarette smoke, incomplete combustion of nitrogen-containing compounds from a vehicle, reaction of coke, coal, and limestone [6]. Therefore, detection of cyanide is essential. Some techniques such as chromatography [7], atomic absorption spectrometry [8], and electrochemical [9] have been used to detect cyanide anion. Nowadays, colorimetric and fluorescence chemosensors are attracting much attention because of low cost, high selectivity, simplicity, sensitivity, and fast recognition in real-time circumstances [10].

Some organic molecules containing amide, pyrrole, thiourea, and phenol groups can be used as cyanide chemosensors. Detections involve the formation of hydrogen bonding or deprotonation between cyanide and chemosensors. For example, Azine derivatives bearing hydroxyl group have been used as colorimetric and fluorescent chemosensors [1,5,11-13]. Most azine chemosensors were produced from aryl benzaldehydes and hydrazine hydrates as starting materials under reflux condition [14-16]. This paper reports the synthesis of symmetrical azine **1** and **2** from acetophenone derivatives and hydrazine hydrate (Scheme 1). The azines were then applied as colorimetric and fluorescent chemosensors for cyanide anion.



1. R=H **2.** R=OH

Scheme 1. Synthesis targeted compounds **1** and **2**

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were hydrazine hydrate 80%, 2'-hydroxy acetophenone, 2',4'-dihydroxy acetophenone, sodium fluoride, sodium chloride, sodium bromide, sodium iodide, sodium cyanide, sodium acetate, sodium hydroxide and hydrochloric acid 37%, acetone, acetonitrile, dimethyl sulfoxide, ethanol, and distilled water. All chemicals used in this study were purchased from E. Merck with p.a. quality, while distilled water is obtained from the Organic Chemistry Laboratory of FMIPA-UGM.

Instrumentation

The equipment used in the experiment were laboratory glassware, hot plate, magnetic stirrer, thermometer 100 °C, Buchner filter, analytical scales (Libror EB-330 Shimadzu), and ultrasonic irradiation (Ultrasonic Branson 43 KHz, 43 Watts). The structure elucidation used FT-IR (Shimadzu Prestige-21), GC-MS (Shimadzu QP2010S), and ^{1}H-NMR and ^{13}C-NMR (JEOL JNM ECA-500, 500 MHz). The colorimetric study used UV-Vis Spectrophotometer (Shimadzu UV-1800), and the fluorescents study used Spectrofluorometer (Shimadzu RF-6000). The melting point was measured using uncorrected Electrothermal-9100.

Procedure

Synthesis of acetophenone azine

Acetophenone azines were synthesized based on the previous report with some modifications [16]. First, acetophenone derivative (10 mmol) was dissolved in 10 mL of absolute ethanol, then 0.25 mL (5 mmol) of hydrazine hydrate (80%) was added dropwise under stirring conditions. The solution was then heated under reflux for 2 h until a yellow precipitate formed. The stirring was continued at room temperature overnight. The residue was filtered and washed with cold ethanol and then dried. The solid was then determined its melting point, and the structures were elucidated by FTIR, GC-MS, and ^{1}H- and ^{13}C-NMR. A similar procedure was also carried out through ultrasonic irradiation methods at room temperature for 1 h.

Compound of 2'-hydroxy acetophenone azine (**1**) was obtained as a yellow solid in 73.13% yield for reflux method and 88.80% yield for ultrasonic irradiation methods (m.p. 197–198 °C, literature 197–198 °C [17]). IR (KBr cm^{-1}): 3448 (O–H), 1604 (C=N), 1496 and 1442 (C=C aromatic), 1356 ($-CH_3$), and 840 (Ar–**C**–CH_3); ^{1}H-NMR ($CDCl_3$): δ 2.56 (s, 6H), 6.93 (t.d, *J* = 8.4, 1.3 Hz, 2H), 7.02 (d.d, *J* = 8.3, 1.2 Hz, 2H), 7.36 (t.d, *J* = 8.6, 1.6 Hz, 2H), 7.63 (d.d, *J* = 8.1, 1.6 Hz), and 13.26 (s, 2H); ^{13}C-NMR ($CDCl_3$): δ 14.96, 118.01, 119.09, 119.14, 129.06, 133.00, 160.69, and 168.19. Mass spectra (m/z): 268 (M^+).

Compound of 2',4'-dihydroxy acetophenone azine (**2**) was obtained as a pale-yellow solid in 68% yields from reflux method, and 82.67% yields from ultrasonic irradiation methods (m.p. 271–272 °C, literature 269–270 [18]). IR (KBr cm^{-1}): 3417 (O–H), 1604 (C=N), 1504 and 1442 (C=C aromatic), 1357 ($-CH_3$), and 833 (Ar–**C**–CH_3); ^{1}H-NMR (DMSO): δ 2.41 (s, 6H), 6.27 (d, *J* = 2.5 Hz, 2H), 6.35 (d.d, *J* = 8.8, 2.5 Hz, 2H), 7.56 (d, *J* = 8.9, 2H), 10.12 (s, 2H), and 13.55 (s, 2H); ^{13}C-NMR (DMSO): δ 14.48, 103.42, 108.15, 111.84, 131.69, 162.16, 162.63, and 167.54. Mass spectra (m/z): 300 (M^+).

Solvatochromic effect on sensor compounds

Compounds **1** and **2** were dissolved in acetone, acetonitrile, DMSO, and ethanol, respectively, in a concentration of 10^{-3} M. Color of the solutions was observed and then measured with a UV-Vis spectrophotometer at a concentration of 5×10^{-5} M for **1** and 2×10^{-5} M for **2**.

Acid-base effect on sensor compounds

Color changes of solution (**1** and **2**) were observed after dropping 1 mL of HCl or NaOH 10% (w/v) into each sensor solution (5×10^{-5} M for **1** and 2×10^{-5} M for **2**) in DMSO for a total volume of 10 mL. The color of the solution was observed and then measured with a UV-Vis spectrophotometer. Distilled water (1 mL) was used as a reference solution.

Ionic effect on sensor compounds

Sodium salt solutions of NaF, NaCl, NaBr, NaI, NaCN, and CH_3COONa were prepared with a concentration of 1 M in distilled water. The 1 mL of sodium salt solution was added into solution **1** (5×10^{-5} M) and solution **2** (2×10^{-5} M) to a total volume of 10 mL. The color of the solution was observed and then measured with a UV-Vis spectrophotometer and spectrofluorometer.

Limit of detection for cyanide anion

The limit of detection was carried out by adding 1 mL of various concentrations of NaCN (10^{-4}–10^{-1} M) into solution **1** (5×10^{-5} M) and **2** (2×10^{-5} M) to a total volume of 10 mL. The spectra were measured using a UV-Vis spectrophotometer and spectrofluorometer. Then, a calibration curve was plotted cyanide concentration vs. absorbance correlation. The limit of detection (LOD) values were determined by the $3\sigma/m$ equation, where σ is the standard deviation and m is the slope of the calibration curve [19].

Interaction study between sensor compounds and cyanide

The interaction between sensor compounds and cyanide anion was determined by measuring Job's plot curve [20]. The concentration of sensor solution was 5×10^{-5} M for compound **1** and 2×10^{-5} M for compound **2** in DMSO, and a standard solution of NaCN 1 M was used to make a fraction of 0.1–0.9 into sensor solutions. Then the absorbance was measured using a UV-Vis spectrophotometer. The curve was plotted as the mole fraction of cyanide vs. the absorbance. Finally, the ^{1}H-NMR titration was conducted from a mixture of **2** and cyanide solutions in DMSO-d6.

Paper-strip sensor

Paper-strip sensor was carried out by immobilizing compounds **1** and **2** (1×10^{-3} M in DMSO) to filter paper (Whatman no. 42 - 1×3 cm) for 2 h and then dried using an oven. The test was conducted by dropping 2–3 drops of cyanide anion solution to the paper-strip sensor. The color change of paper-strip was observed directly and under a 365 nm UV lamp. As a negative control, distilled water was dropped on a paper-strip sensor.

■ RESULTS AND DISCUSSION

Solvatochromic Effect on Compounds 1 and 2

The solvatochromic test was carried out by dissolving compounds **1** and **2** in several solvents of acetone, acetonitrile, ethanol, and DMSO. The optical color change and UV-Vis spectra are shown in Fig. 1.

The different colors of sensor solution in various solvents were observed. Compound **1** showed colorlessness, while compound **2** showed a brighter yellow color in ethanol and DMSO (Fig. 1(a, b)). From UV-Vis spectra (Fig. 1(c)), compound **1** gave two absorption peaks at λ_{max} of 294, 290, 296, and 292 nm and the second peak at λ_{max} of 362, 354, 360, and 360 nm for acetone, acetonitrile, DMSO, and ethanol solvent respectively. Thus, the absorption peak of compound **1** solutions was at adjacent λ_{max}. These data indicated that the effect of solvent polarity is small for the color changes. From Fig. 1(d), compound **2** exhibits one λ_{max} at 378 nm in DMSO. Two absorption peaks were observed from acetone, acetonitrile, and ethanol solvents, respectively. One low peak was around λ_{max} at 300 nm and a peak at λ_{max} at 370, 368, 374 nm for acetone, acetonitrile, and ethanol solvent, respectively. These data also indicated that the effect of solvent polarity is small for the color changes. However, compound **2** in DMSO and ethanol still provided shoulder absorption at λ_{max} of the visible area.

Effect of Acid-Base Addition on Sensor Compounds

Strong acid and base addition were applied into sensor solutions by adding HCl 10% or NaOH 10%. As shown in Fig. 2, the color of solutions **1** and **2** was unchanged after adding HCl 10%. However, the addition of NaOH 10% caused the color change from colorless to a very light yellow for compound **1** and yellow for compound **2**.

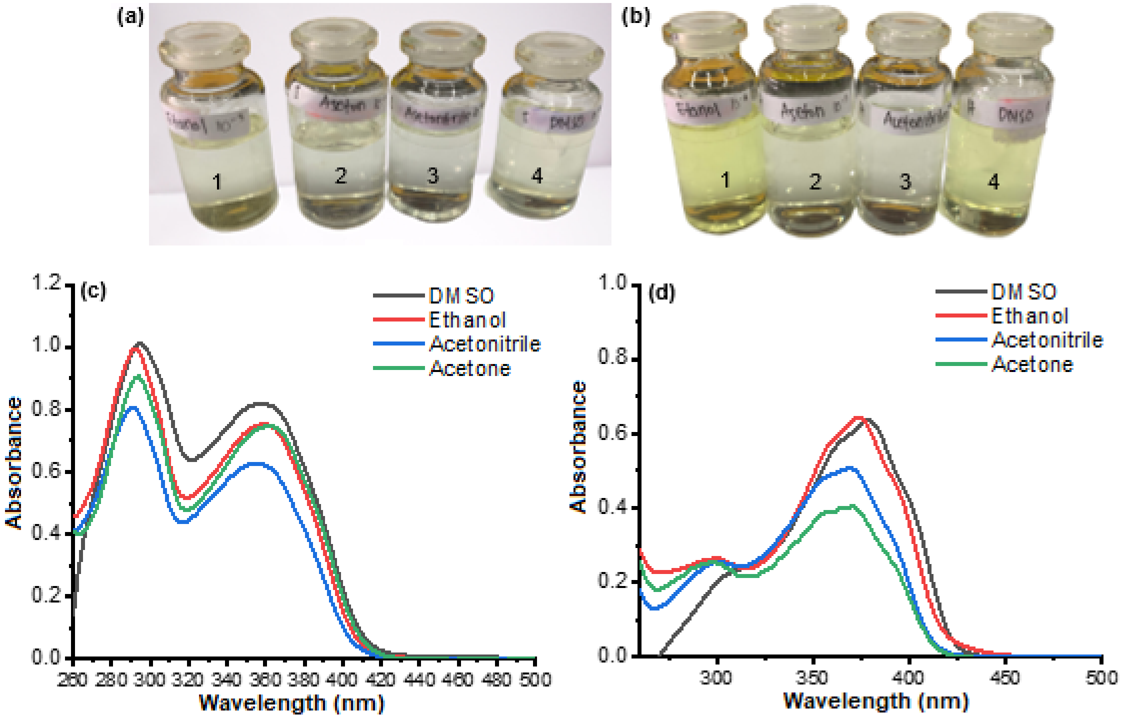


Fig 1. Optical color of (a) compound **1** and (b) **2** with a concentration of 10^{-3} M changes in (1) ethanol, (2) acetone, (3) acetonitrile, and (4) DMSO and UV-Vis absorption spectra of (c) compound **1** (5×10^{-5} M) and (d) **2** (2×10^{-5} M) in various solvents

The yellow color was obtained because the addition of bases can deprotonate the pH-sensitive groups [21]. Deprotonation of the H atom on hydroxyl group of compound **2** produced phenoxide ion and followed delocalization of electrons. Compound **2** has a more probable resonance structure than compound **1**. More resonance structures will create a distinct color change in the solution [22]. Therefore, compound **2** has stronger yellow color than compound **1**. The light-yellow color of compound **1** is produced from compound **1** with cyanide anion via hydrogen bonding formation, not deprotonation.

The color change of compounds **1** and **2** on the acid-base addition supported UV-vis spectra shown in Fig. 3. The addition of HCl to compound **1** causes a shift of two absorption peaks at λ_{max} of 296 and 360 nm into a peak at λ_{max} of 324 nm. The addition of NaOH produced a shift of λ_{max} from 296 and 360 nm to 266 and 354 nm. Both additions of acid and base did not create a bathochromic shift, so no color change was observed.

Fig 2. The color of solutions (a) compound **1** (5×10^{-5} M) and (b) compound **2** (2×10^{-5} M) in DMSO changes after addition (1) HCl 10%, (2) H_2O, and (3) NaOH 10%

For compound **2**, the addition of HCl causes a shift of λ_{max} of 378 nm to two absorption peaks at λ_{max} of 280 and 316 nm. The addition of NaOH produced a change of λ_{max} on 378 nm to 272 nm and appearance shoulder peak at λ_{max} > 400 nm. The shoulder peak at λ_{max} > 400 nm supported a yellow color formation after the addition of NaOH.

Ionic Effect on Sensor Compounds

The ionic effect was carried out by anion addition into sensor solutions. The color change after addition 1 mL of 1 M sodium salt from NaF, NaCl, NaBr, NaI,

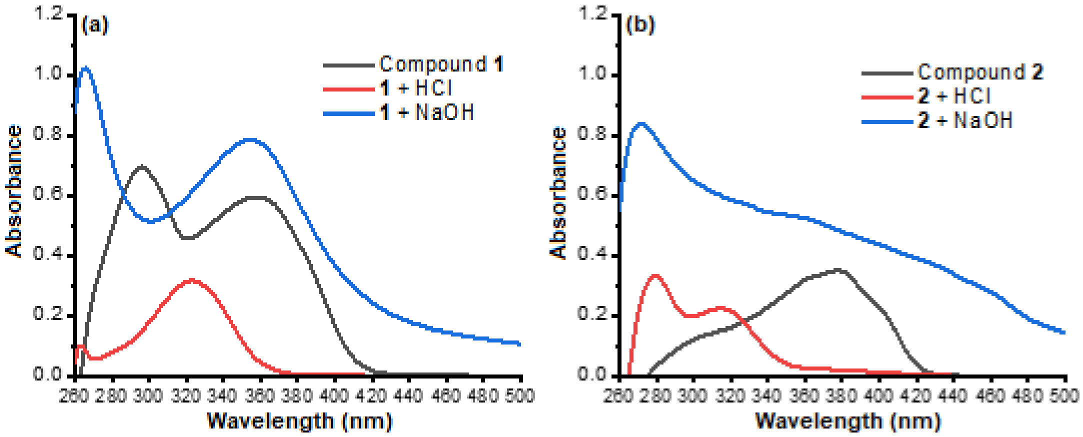


Fig 3. UV-Vis absorption spectra of (a) compound **1** (5×10^{-5} M) and (b) compound **2** (2×10^{-5} M) in DMSO after addition of HCl 10% and NaOH 10%

NaCN, NaOAc, and a mixture of all salts is shown in Fig. 4. Adding Cl^-, Br^- and I^- anion into compounds **1** and **2** did not produce a color change. The color change to yellow and cloudy was observed after addition F^- into compound **2**, while compound **1** was only cloudy with no color change. A significant color change was observed when CN^- anion was added to a solution of compounds **1** and **2**. The addition of AcO^- gave a significant intense yellow color change for compound **2**. The addition of a mixture of all anions into compounds **1** and **2** caused the color change to a slightly yellowish color for compound **1** and a significant yellow color change for compound **2**. These color changes indicated that there is no anion interference from those anions.

The color changes after the addition of anions were supported by the UV-Vis spectra shown in Fig. 5. The addition of Cl^-, Br^-, I^-, AcO^- to **1** did not give new absorption peaks in the visible light region. CN^- and the mixture of all anions increased absorbance at λ 420–440 nm. The addition of Cl^-, Br^- and I^- into the solution of **2** did not produce a change in λ_{max}. The λ_{max} shift toward visible light (about 430 nm) occurred when AcO^-, CN^- and the mixture of all anions was added into a solution of **2**.

The addition of F^- into compounds **1** and **2** caused the color of solutions to become cloudy, making good UV-VIS spectra impossible. The formation of cloudiness is due to a strong hydrogen bond formed

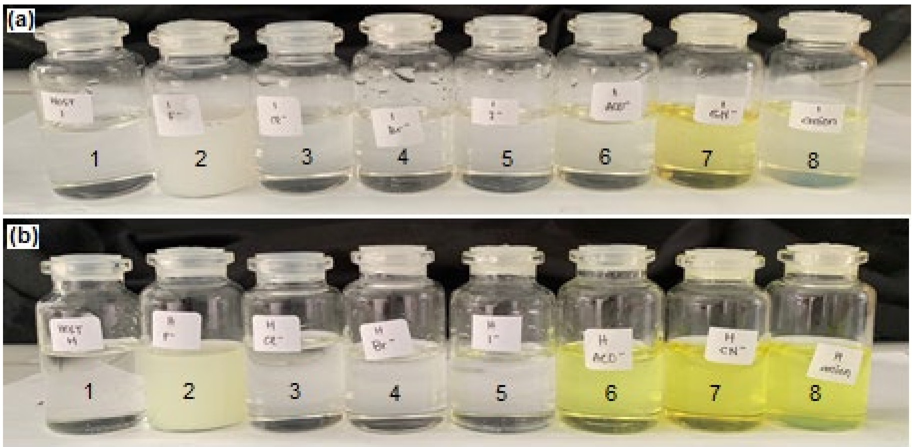


Fig 4. Color of solutions (a) compound **1** (5×10^{-5} M) and (b) compound **2** (2×10^{-5} M) in DMSO changes after addition (1) H_2O, 2) F^-, (3) Cl^-, (4) Br^-, (5) I^-, (6) AcO^-, (7) CN^-, and (8) mixture of all anions

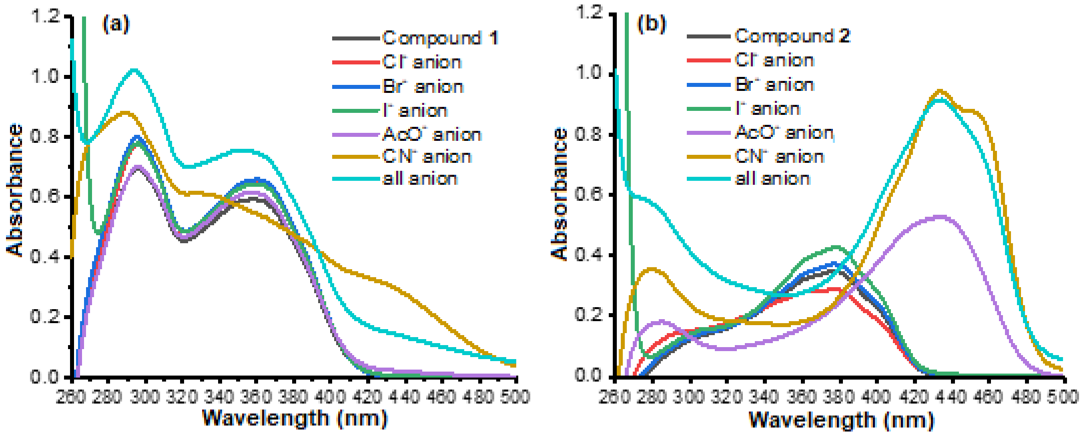


Fig 5. UV-Vis absorption spectra of (a) compound **1** (5×10^{-5} M) and (b) compound **2** (2×10^{-5} M) in DMSO after addition of anions

between F⁻ and water and may decrease anion solubility in DMSO.

Fluorescence properties were observed with a 365 nm UV light (Fig. 6). Both compounds **1** and **2** did not exhibit fluorescence. The addition of all tested anions showed the fluorescence intensities of compound **1**, while compound **2** did not give fluorescence with the addition of iodide ion only (Fig.6(a, b)).

The fluorescents changes were supported by emission spectra shown in Fig. 6(c) and d. In addition, CN⁻ anion gives a high-intensity emission for both compounds **1** and **2**. Excitation of compound **1** occurs at λ 335 nm with the highest emissions at λ_{max} around 400–450 nm, while compound **2** excites at λ_{max} 415 nm with the highest emission at 500–530 nm. The increasing fluorescents intensity of sensor solutions after addition

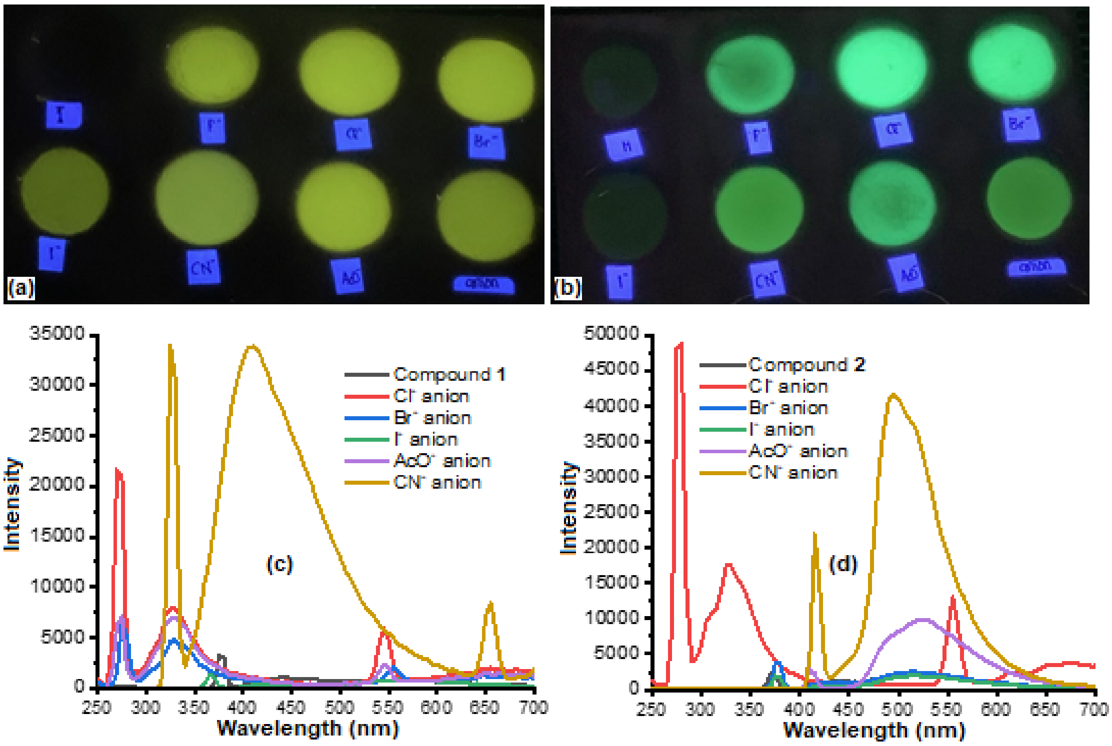


Fig 6. Fluorescents of solution (a) compound **1** (5×10^{-5} M) and (b) compound **2** (2×10^{-5} M) under a UV lamp 365 nm; and emission spectra of (c) compound **1** and (d) compound **2** in DMSO after addition of Cl⁻, Br⁻, I⁻, AcO⁻, and CN⁻

of CN^- indicates an OFF-ON system due to interaction between sensor compound and anion [23].

Limit of Detection Value for Cyanide on Sensor Compounds

The color changes of sensor solutions after addition various CN^- concentrations are shown in Fig. 7. The solution color change into yellow was observed when the CN^- concentrations were 4×10^{-2} M for **1** and 1×10^{-3} M for **2**.

The UV-Vis spectra after the addition of various cyanide anion concentrations are shown in Fig. 8. For example, the addition of cyanide from a range concentration of 10^{-4}–10^{-2} M into compound **1** caused a decrease in absorbance at λ ~ 290 and 360 nm and increased a new peak at λ ~ 450 nm region (Fig. 8(a)). For compound **2**, increasing CN^- concentration from 10^{-5}–10^{-3} M caused a decrease in absorbance at λ_{max} 380 nm and a new peak at λ ~440 nm (Fig. 8(b)).

A ratiometric calibration curve was plotted from UV-Vis spectra to determine the limit of detection (LOD) cyanide anion of compounds **1** and **2**. The ratiometric calibration curve against various CN^- concentrations had a regression value (R^2) of 0.9916 with a LOD value of 9.6×10^{-4} M for compound **1** and 0.9917 with a LOD value of 9.63×10^{-5} M for compound **2**.

Fig 7. Optical color of (a) compound **1** and (b) compound **2** changes after addition of various concentrations of cyanide anion (10^{-4}–10^{-2} M for compound **1** and 10^{-5}–10^{-2} M for compound **2**)

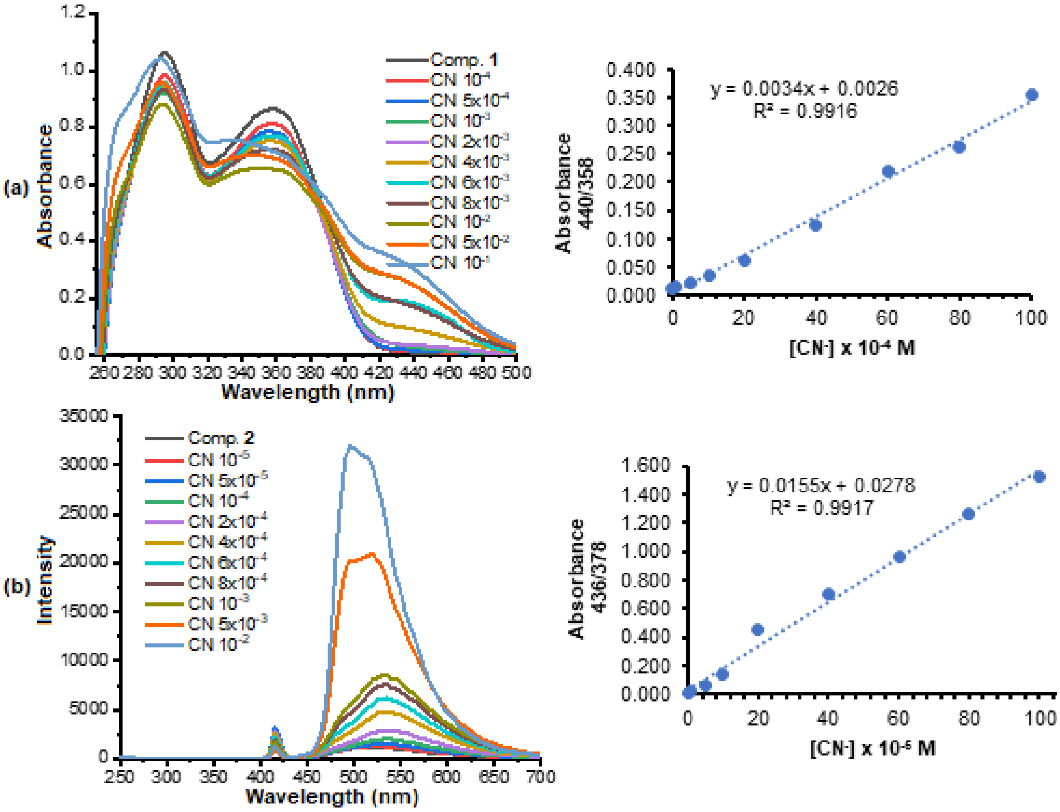


Fig 8. UV-Vis absorption spectra with the ratiometric curve of (a) compound **1** and (b) compound **2** after addition various concentrations of CN^-

The LOD value for CN$^-$ anion was also performed using a spectrofluorometer. The fluorescence emission spectra and calibration curve are shown in Fig. 9. The emission spectra of compounds **1** and **2** increased with an increase of CN$^-$ concentration. As a result, the calibration curve of the emission spectra of compounds **1** and **2** had an R^2 value of 0.9776 and 0.9928, and the LOD value was 15.90×10^{-4} M and 8.95×10^{-5} M.

Interaction Study between Sensor Compounds and Cyanide

The interaction study between sensor compounds and cyanide anions was performed through Job's plot [20]. The curves of absorbances against the cyanide fraction are shown in Fig. 10. The maximum absorbance was obtained at addition a cyanide fraction of 0.5. This value indicates that interaction between compounds **1** and **2** with the cyanide anion has a mole ratio of 1:1. The 1:1 ratio means that only one hydroxyl from the symmetrical azine interacts with one mole of cyanide anion.

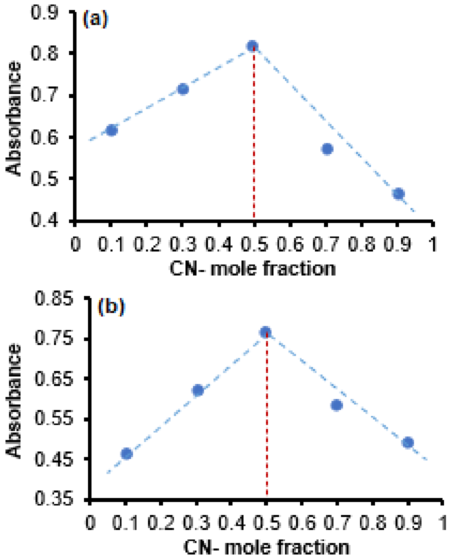


Fig 10. Job's Plot curve interaction between (a) compound **1** and (b) compound **2** with a fraction of cyanide addition

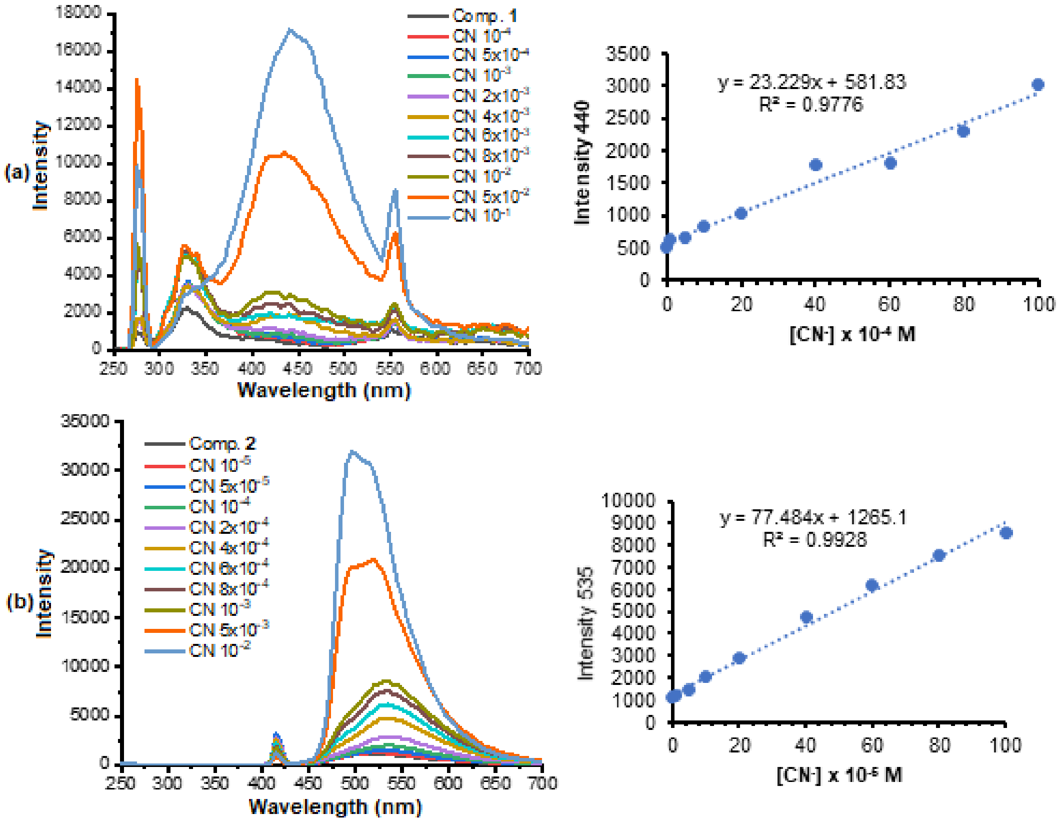


Fig 9. Emission spectra with the calibration curve of (a) compound **1** and (b) compound **2** at the excitation of 415 nm after addition various concentrations of CN$^-$

The ^{1}H-NMR titration supported 1:1 ratio interaction for compound 2 (Fig. 11). The addition of 1 equivalent mole ratio of cyanide anion caused a signal at 13.55 ppm to disappear and followed a new signal at 8.51 ppm. The addition of 5 and 10 moles equivalents of cyanide anion, a signal at δ 8.51 ppm shifted towards upfield at 8.48 and 8.44 ppm accompanied by an increase in the absorption intensity. The proton of benzene peaks gradually showed a shift to the upfield area. This data indicated that deprotonation of the hydroxyl group has occurred in one hydroxyl group. These results are proposed to the mechanism of interactions in Scheme 2.

The proposed mechanism supported color change after the addition of cyanide. Interaction of compound **1** with cyanide indicated the formation of H-bond with cyanide anion in one hydroxyl group. This interaction did not produce delocalization of the benzene ring, so the color change was not intense. Interaction of compound **2** with cyanide indicated the deprotonation of one hydroxyl group followed with delocalization of the phenol anion producing color change.

Paper-Strip Sensor

A paper-strip sensor was done by dropping various concentrations of cyanide anion solution (0–1 M) to a Whatman filter immobilized with compounds **1** and **2**.

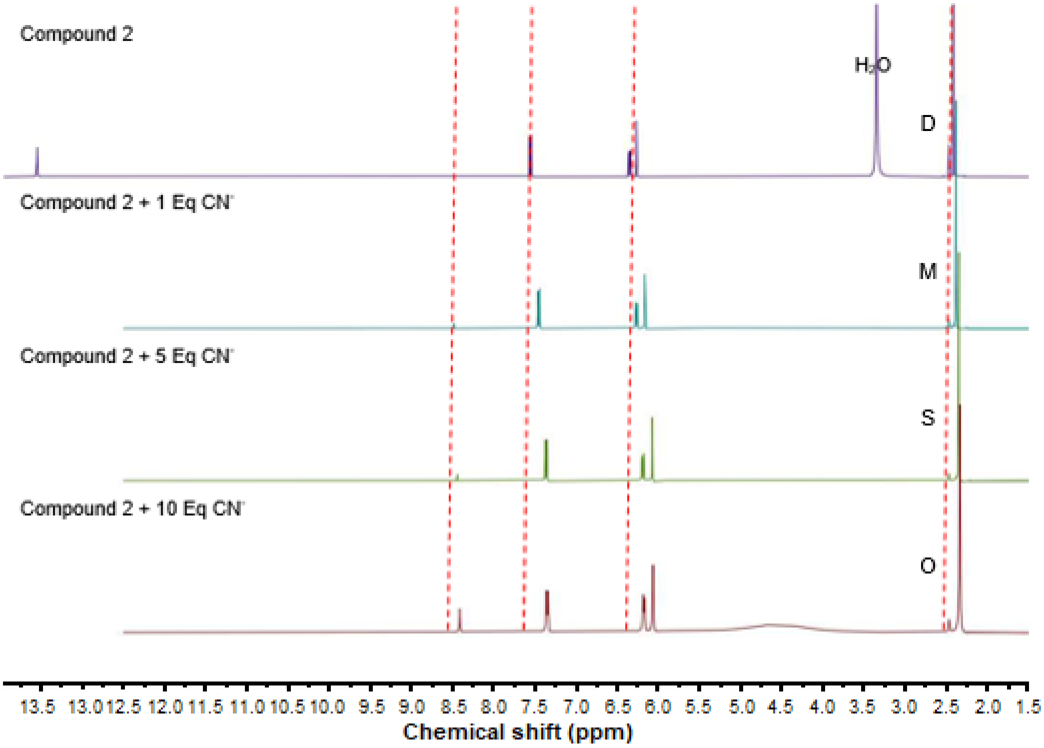
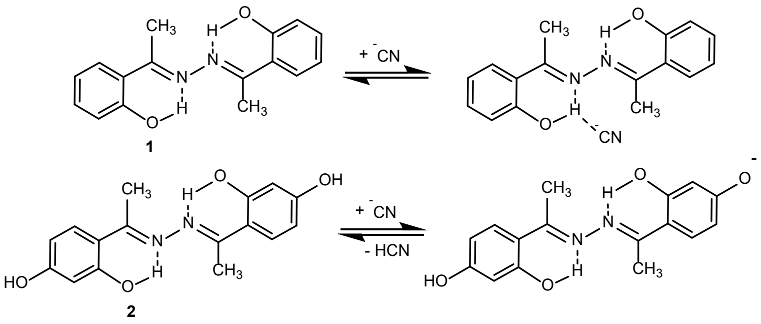


Fig 11. ^{1}H-NMR titration on compound **2** with the addition of cyanide ion



Scheme 2. Proposed mechanism of interaction **1** and **2** with cyanide anion

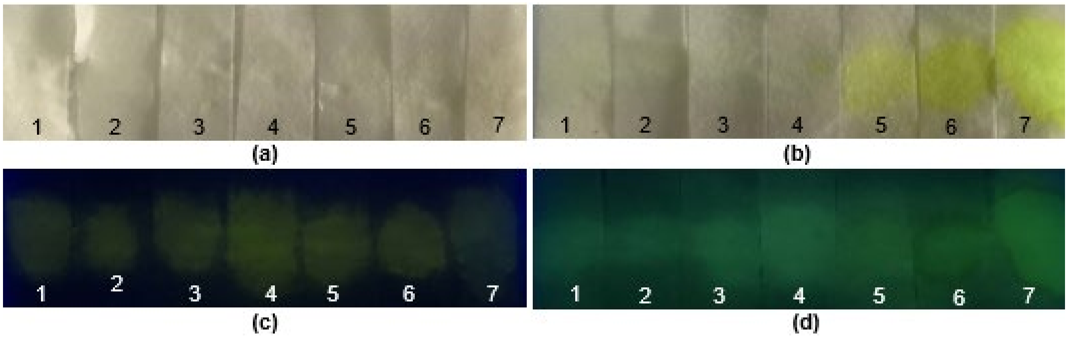


Fig 12. The paper-strip color of (a) sensor **1** and (b) sensor **2** changes after addition of various CN^- concentration (1) 0 M; (2) 10^{-5} M; (3) 10^{-4} M; (4) 10^{-3} M; (5) 10^{-2} M; (6) 10^{-1} M; and (7) 1 M, (c) sensor **1** and (d) sensor **2** under a 365 nm UV lamp

The results are shown in Fig. 12. The paper-strip sensor **1** produced no 'naked-eye' color change in addition to various cyanide anion concentrations. In contrast, paper-strip sensor 2 could detect the presence of cyanide anions at a concentration of 10–2 M with a concentration of a pale-yellow color. Observing the paper-strips under a 365 nm UV lamp, paper-strip sensors **1** and **2** glow when the cyanide is dripped with distilled water. Paper-strip **1** brought out a yellow glow, while paper-strip **2** gave a greenish-yellow glow. However, based on these results, it can be said that compound **1** and **2** is not sensitive to detect cyanide anions under UV light irradiation at a 365 nm UV lamp.

■ CONCLUSION

Acetophenone azine derivatives **1** and **2** have been synthesized in one-step condensation of acetophenone derivatives with hydrazine hydrate. Compounds **1** and **2** can act as dual-mode chemosensors (colorimetry and fluorometry) to detect cyanide anion. Compound **2** has more sensitivity than compound **1**. The LOD values for cyanide anion by colorimetry was 9.68×10^{-4} M for compound **1** and 9.63×10^{-5} M for compound **2**, while LOD values by fluorescent were 15.90×10^{-4} M for compound **1** and 8.95×10^{-5} M for compound **2**.

■ ACKNOWLEDGMENTS

We gratefully acknowledge to Indonesian Government through the LPDP scholarship to Nur Masyittah Irmi.

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