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Novel styrylbenzimidazolium-based fluorescent probe for reactive sulfur species: Selectively distinguishing between bisulfite and thiol amino acids

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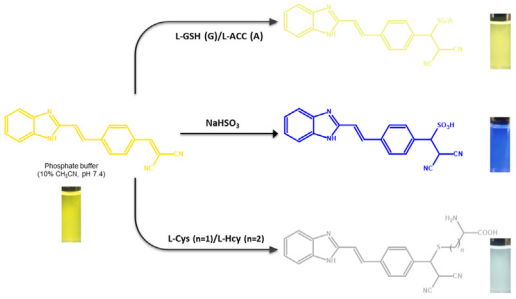
HIGHLIGHTS

- New styrylbenzimidazolium derivative with dicyanovinyl moiety exhibit sensitivity and selectivity for HSO^{3-} and discriminative fluorescence sensing of Cys/Hcy over GSH.
- The detection limits of HSO^{3-} was estimated to be 0.25 μM.
- The styrylbenzimidazolium derivative was used to detect bisulfite in real samples.
- The cytotoxicity of styrylbenzimidazolium derivative was examined against the HeLa cells.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, a new fluorescent probe containing dicyanovinyl moiety has been designed and synthesized. Fluorescent probe based on styrylbenzimidazolium derivative was reported for the effective detection of bisulfite and distinguish it from biothiols by exploiting dicyanovinyl group as the recognition site. The photophysical properties of the novel styrylbenzimidazolium derivative were assessed by determination of absorption and fluorescence spectra, fluorescence quantum yield, and fluorescence lifetime. Its spectroscopic behavior towards various analytes has been evaluated in aqueous media at a pH of 7.4. The highest increase in fluorescence intensity of compound **5** in the presence of different analytes was observed for sodium bisulfite and the limit of detection was estimated to be 0.25 μM. The styrylbenzimidazolium dye was applied to detect bisulfite in various wine sample using fluorimetry. Finally, the ability of this novel probe to detect HSO_3^- in red wine samples was evaluated.

1. Introduction

Sulfites are naturally present in many foods, such as eggs, some fish, asparagus, lettuce, garlic, onions, soybeans, and tomatoes. Also, they are very often found in sugar, wine, and generally in fermented products and fruit/vegetable preserves. For several reason sulfites are added during vinification. They eliminate harmful microorganisms and wild yeasts that can cause incorrect fermentation. Sulfites also prevent wine from oxidation because they are stabilizers and have preservative properties [1,2]. Moreover, in white wine, they act as bleaching agents that maintain the desired color of the wine [3]. Simultaneously, sulfites are popular food products additives. They are also components of acid rain or smog inhaled by residents of large urban agglomerations [4]. Except that,

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sulfites are also used as the additives of some pharmaceuticals and cosmetics during production and storage [5]. Due to the widespread using of sulfates, World Health Organization has established a safe daily limit of sulfur dioxide consumption at 0.7 mg per kilogram of body weight [6]. The certain concentration level of sulfur compounds are especially dangerous for asthmatics because they can cause very strong allergic reactions, e.g. breathing problems. According to the epidemiological research, an excess sulfur dioxide in consumed products can reduce blood pressure and cause bronchitis [7]. The sulfur compounds also destroy the vitamins providing with food, especially A, B_1 and B_{12}. Undoubtedly, sulfites are present in many areas of life, so it is important to detect and control their quantity. Hence, many different analytical methods have been used to determine sulfur derivatives including electrochemistry, conductivity, chromatography, flow injection analysis [1,8–12]. Fluorescent probes have become very useful and efficient tools for the detection and the identification of various analytes in food and beverages. In the last five years, many fluorescent probes have been reported for the detection of bisulfite [13]. They are mainly fluorescent probes based on through different chemical reaction mechanisms, such as Michael addition [14–16], nucleophilic reaction to aldehyde moiety [17–19], and other reaction [20–22]. All of these mechanisms exploit the strong nucleophilicity and reducing properties of sulfites to achieve selectivity over other reactive sulfur species. The presence of strongly electron-withdrawing group such as dicyanovinyl group in the fluorescent probe structure reduces the electron density of the carbon–carbon double bond and, therefore, it may undergo nucleophilic attack. Various types of nucleophiles, including thiol compounds [23–25], reactive sulfur species [26–30], and cyanide anions [31,32] react with this kind of compounds.

Recently, we have synthesized and characterized in detail styrylbenzimidazolium dye containing a maleimide moiety [33]. Here, we report the synthesis and characterization of a novel styrylbenzimidazolium-based derivative with dicyanovinyl group at the site of the maleimide moiety.

Then, we characterized and validated this compound as probe for the direct detection of bisulfite over other sulfur species. Compared with the previously reported probe for compounds containing a sulfhydryl group, probe **5** is able to distinguish different reactive sulfur species.

2. Materials and methods

2.1. General

All chemicals were purchased from commercial suppliers (Sigma-Aldrich, Poland) and used directly without further refinement. Melting points were determined on the Boeöthius melting point apparatus and uncorrected. The reaction progress and the purity of the obtained compounds were routinely checked by thin layer chromatography (TLC) on silica gel 60 F254 plates (Merck, Germany). Plates were visualized with UV light (λ = 254 nm or 365 nm). Crude materials were purified by column chromatography on silica gel 60 (0.063–0.200 mm, Merck, Germany). ^{1}H NMR and ^{13}C NMR spectra were obtained with tetramethylsilane (TMS) as the internal standard and dimethylsulfoxide (DMSO d_6) as the solvent on a Bruker Avance DPX 250 (Germany) spectrometer. The chemical shifts (δ values) and coupling constants (*J* values) are expressed in parts per million (ppm) and in hertz (Hz), respectively. NMR peak multiplicities are described as follows: s (singlet), d (doublet), m (multiplet), brs (broad singlet). High-resolution mass spectrometry (HRMS) experiments were performed with a mass spectrometer equipped with an electrospray ionization source operated in the positive ion mode and quadrupole-time-of-flight mass analyser (Synapt G2-Si mass spectrometer, Waters). UV–vis absorption spectra and fluorescence spectra were recorded using a Jasco V-670 UV–vis/NIR spectrophotometer (Jasco, Japan) and a FLS-920 spectrofluorometer (Edinburgh Instruments, UK), respectively. In each case quartz cuvettes (1 cm) were used. The excitation wavelength for fluorescence measurements was 395 nm (slit width: ex/em = 1.5 nm). The pH values were determined with a CPI-551 microcomputer pH/ion meter (Elmetron, Poland)

2.2. Preparation of compound **5**

2.2.1. Synthesis of trans-2-(4-formylstyryl)-[1H]-benzimidazole (3) [34,35]

A mixture of 2-methyl-[1*H*]-benzimidazole **1** (1.32 g, 0.01 mol), terephthalaldehyde **2** (1.34 g, 0.01 mol), acetic acid (1.5 ml), and acetic anhydride (3 ml) was stirred at 120 °C for 20 h. The reaction progress was monitored by TLC (eluent: toluene/ethanol 3:1 (v/v), R_f = 0.73). Then, the solution was cooled to room temperature and 30% hydrochloric acid (15 ml) was added and after that the solution was filtered. The filtrate was neutralized with 30% sodium hydroxide solution (30 ml) and resulted a yellowish precipitate that was filtered and dried. The crude product **3** was recrystallized from ethanol to yield 1.10 g (44%); mp 178–180 °C.

2.2.2. Synthesis of 2-(4-dicyanovinylstyryl)-[1H]-benzimidazole (**5**)

Malononitrile **4** (0.03 g, 0.5 mmol) was added to a solution of *trans*-2-(4-formylstyryl)-[1*H*]-benzimidazole **3** (0.124 g, 0.5 mmol) in absolute ethanol (15 ml), and then three drops of piperidine were added to the mixture. The reaction mixture was stirred under reflux until the disappearance of starting compounds (15 h), which was checked by TLC using toluene/ethanol (3:1, v/v) as eluent (R_f = 0.58). Then the solution was cooled to room temperature and the resulting precipitate was filtered and dried. The crude product **5** was recrystallized from ethanol to give an orange solid (0.04 g, 28%); mp greater than 360 °C without decomposition.

^{1}H NMR (DMSO d_6, 250 MHz) δ 7.24–7.17 (m, 2H), 7.64–7.50 (m, 2H), 7.47 (d, *J* = 16.5 Hz, 1H), 7.78 (d, *J* = 16.5 Hz, 1H), 7.91 (d, *J* = 8.5 Hz, 2H), 8.02 (d, *J* = 8.5 Hz, 2H), 8.53 (s, 1H), 13.00 (brs, 1H).

^{13}C NMR (DMSO d_6, 62 MHz) δ 80.7, 87.4, 113.6, 114.5, 121.3, 121.6, 122.5, 127.9, 130.2, 131.2, 131.4, 132.8, 135.4, 141.7, 150.4, 160.5. HRMS (MS ESI) *m*/*z*: $[M + H]^+$ calcd. for $C_{19}H_{12}N_4$ 297.1140, found 297.1141.

2.3. Fluorescence quantum yield and fluorescence lifetime

For the determination of fluorescence quantum yields, fluorescein in 0.1 M NaOH (Φ_{st} = 0.85) [36] was used as the standard. Fluorescence quantum yields (Φ) of tested compound **5** were determined using following Eq. (1):

$$\phi = \phi_{st}\left(\frac{Grad_{sensor}}{Grad_{st}}\right) \times \left(\frac{\eta^2_{sensor}}{\eta^2_{st}}\right) \quad (1)$$

where the subscripts 'st' and 'sensor' denote the standard fluorescein and the tested compound, respectively. Φ is the quantum yield of fluorescence, Grad is the gradient obtained from the plot of the integrated fluorescence intensity as a function of the absorbance, at their excitation wavelengths, and η is the solvent refractive index.

Fluorescence lifetime measurements were made using a time–correlated single photon counting system (TCSPC) with a pulsed picosecond diode (EPLED-380) as the excitation source. The instrument response function was measured by collecting scattered light from a Ludox silica suspension. Fluorescence lifetimes were calcu-

lated from intensity decay analyses. Fluorescence decay was acquired at a level of 1×10^4 counts at the peak and was fitted to a sum of exponentials (2):

$$I(t) = \sum_{i=1}^{n} \alpha_i \exp(-t/\tau_i) \quad (2)$$

with amplitudes, αi, and decay lifetimes τ_i. Average lifetimes (τ) for multiexponential fluorescence decay were calculated from decay times and pre-exponential factors using (3):

$$(\tau) = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} \quad (3)$$

2.4. General procedure for preparation of probe test solutions

L-cysteine (L-Cys), L-homocysteine (L-Hcy), L-glutathione (L-GSH), *N*-acetyl-L-cysteine (L-ACC), Glycine (Gly), L-glutamic acid (L-Glu), L-lysine (L-Lys), 2-mercaptoethanol (ME), thioglycolic acid (TGA), sodium bisulfite ($NaHSO_3$), sodium hydrosulfide (NaSH), sodium sulfide (Na_2S), hydrogen peroxide (H_2O_2), and *N*-ethylmaleimide (NEM, a trapping reagent for thiol species [37] stock solutions were prepared in distilled water (30 mM). The stock solutions were diluted with distilled water to the desired concentration and used immediately after preparation. For all experiments a stock solution of **5** was prepared by dissolving accurately weighted the solid compound in acetonitrile (CH_3CN, 1 mM **5**). The stock solution of probe was diluted with CH_3CN to acquired 0.1 mM dye solution. All the detection experiments were performed in phosphate buffer (0.1 M, pH 7.4) at room temperature. Because of the relatively poor solubility of the **5** probe in water, CH_3CN was used as a cosolvent (10%; v/v). Additionally, in the pH studies, an acetate buffer (0.1 M, pH 3.6–5.6), and carbonate-bicarbonate buffer (0.1 M, pH 9.5–10.5) were also used.

In a typical experiment, the concentration of the probe **5** was 10 μM. The procedure was as follows: 0.3 ml of appropriate dye solution (**5**) was added to 2.4 ml phosphate buffer (0.1 M, pH 7.4) and 0.3 ml of analyte solution was added. For the control experiments, a solution of L-Cys (30 mM was pretreated with a solution of NEM (30 mM) and incubated for 2 h at ambient temperature (20–23 °C), and then the reaction mixture was added to the test solution containing compound **5**. The probe was incubated with an appropriate amount of each analyte in a phosphate buffer (0.1 M, pH 7.4) for 5 min. Then, absorption and fluorescence spectra were recorded.

All experiments were carried out at least in triplicate and the results are presented as means ± standard deviation.

2.5. Determination of the detection limit

The limit of detection for styrylbenzimidazolium derivative was calculated according to the definition by IUPAC (3σ/m, where σ is the standard deviation of the blank measurement of compound **5** and m is the slope of a plot of fluorescence intensity against HSO_3^- concentration). In the absence of analytes, the fluorescence emission spectrum of probe **5** was measured five times and the standard deviation of blank measurement was achieved.

2.6. Cell viability

The human cell line HeLa was obtained from CBMM PAN Lodz and was cultured in RPMI (Roswell Park Memorial Institute 1640) medium with 10% fetal bovine serum (FBS, Invitrogen) supplemented with penicillin and streptomycin antibiotic at 37 °C in 5% CO_2 air atmosphere/95% air atmosphere. The cells (1×10^4 cells per 100 μL) were plated into each well of 96-well microtitre plates and treated with the indicated concentrations (0, 6.25, 12.5, 18.75 μm) of compound **5** for 24 h. Cells viability was quantified with PrestoBlue reagent from Life Technologies (Van Allen Way, CA, USA) according to the procedure described in literature [38]. The cytotoxicity of the compound **5** was determined by measuring the fluorescent signal at λ_{ex} = 530 nm and λ_{em} = 590 nm using a Synergy 2 Microplate Reader (Bio-Rad, CA, USA). The obtained fluorescence values were used to calculate cell viability expressed as the percentage of viability of the untreated control.

2.7. Preparation of wine samples

The three types of wine were selected: rose wine 1 (semi-dry type), red wine 2 (semi-dry type), red wine 3 (sweet type) for the practical study. The wine sample (1 ml) was mixed (1:100) with distilled water. Then 0.3 ml of the prepared solution was added to the solution of compound of **5** in phosphate buffer (10% CH_3CN, pH 7.4). For the control experiment, 10 μM of HSO_3^- was added to each test solution and, the fluorescence intensity of all wine samples at 410 nm were recorded.

3. Results and discussion

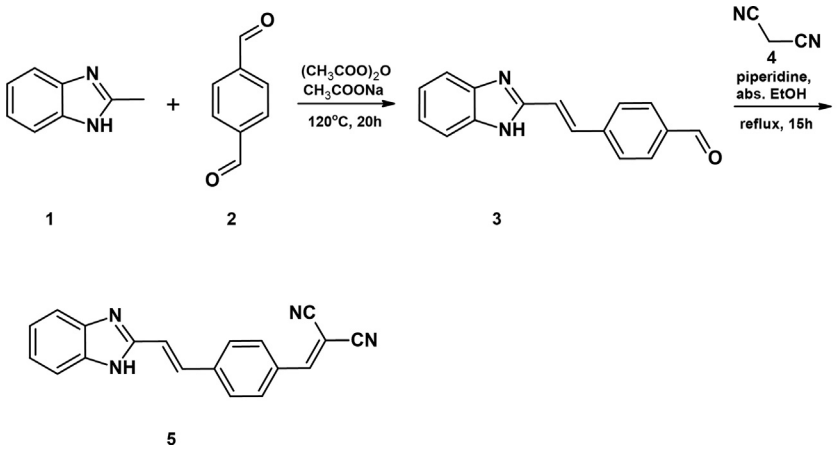
3.1. Synthesis and spectroscopic characterization of *5*

2-(4-dicyanovinylstyryl)-[1*H*]-benzimidazole **5** was prepared by a simple two-step synthetic route as shown in Scheme 1. In the first stage, the reaction between 2-methyl-[1*H*]-benzimidazole **1** with terephthalaldehyde **2** was carried out by refluxing in acetic anhydride – acetic acid mixture [34,35]. The compound **3** was obtained in good yield (44%). Finally, compound **5** was synthesized by condensation of *trans*-2-(4-formylstyryl)-[1*H*]-benzimidazole **3** with malononitrile **4** in absolute ethanol in the presence of catalytic amounts of piperidine. The crude compound **5** was purified by column chromatography and recrystallization from ethanol until a TLC purity was obtained. ^{1}H NMR, ^{13}C NMR spectroscopy and ESI MS spectrometry studies evidenced the chemical structure of probe **5** (Fig. S1).

The analysis the chemical shifts, multiplicities, and integration of the relevant groups of protons are in accordance with the structure of the molecule. As expected, in the structure of **5** a styryl scaffold have a trans configuration, as evidenced by the value of coupling constants for vinyl protons (J = 16.5 Hz). In addition, the characteristic broad singlet about 13 ppm derived from the proton of NH group is observed.

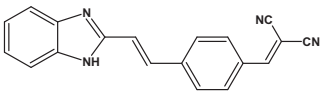
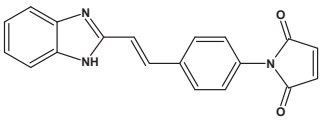
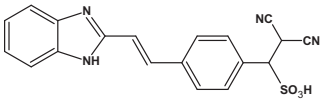
The spectroscopic properties of compound **5** in pure acetonitrile and in aqueous solution (10% CH_3CN, pH 7.4) were studied by means UV–vis absorption and fluorescence spectroscopy. The data obtained are summarized in Table 1 and compared with styrylbenzimidazolium derivative possessing maleimide group [33]. The normalized absorption and fluorescence spectra of compound **5** were provided in Fig. S2.

Dye **5** exhibits an absorption band with a maximum at 390 nm at pH 7.4. The electron-withdrawing group in the structure of compound **5** affect the absorption and fluorescence properties. The replacement of a maleimide group by dicyanovinyl moiety cause a bathochromic shift of λ_{abs} and λ_{em}. The value of Stokes shift of compound **5** is greater than 100 nm suggest that the geometries of two states (ground and excited) are different. It should be emphasized that probes with a large Stokes shift (greater than80 nm) are essential for biological applications due to minimal self-quenching and scattered light during fluorescence imaging. Moreover, the compound with dicyanovinyl group possess higher fluorescence quantum yield (Φ) and longer lifetime of singlet excited state (τ) compared with previously described probe with



Scheme 1. The synthesis of compound **5**.

Table 1
Spectroscopic characterization of compounds with dicyanovinyl and maleimide group in $CH_3CN^{(a)}$ and in phosphate buffer (0.1 M, pH 7.4) containing 10% $CH_3CN^{(b)}$.

	λ_{abs} [nm]	ε [$M^{-1}*cm^{-1}$]	λ_{em} [nm]	Φ	τ [ns]	*SS* [nm]
	395[a] 390[b]	14300[a] 13300[b]	601[a] 550[b]	0.58[a] 0.05[b]	2.27[a] 0.55[b]	206[a] 160[b]
	339[a] 339[b]	25300[a] 24400[b]	409[a] 410[b]	0.20[a] 0.07[b]	1.36[a] -	70[a] 71[b]
	340[b]	14100[b]	410[b]	0.44[b]	1.29[b]	70[b]

maleimide moiety. The fluorescence decay profile of **5** was described by single-exponential fit (Fig. S3).

3.2. Studies of the fluorescent response to bisulfite

Compounds possessing dicyanovinyl moiety are susceptible to nucleophilic attack by several nucleophiles [23–32]. As is well known, the presence of dicyanovinyl moiety in the molecule is used as recognition of bisulfite [26–29]. Thus, UV–vis absorption spectra and fluorescence emission spectra of compound **5** upon the addition of $NaHSO_3$ in phosphate buffer (10% CH_3CN, pH 7.4) were measured and are shown in Fig. 1. It was observed that the addition of bisulfite to solutions of compound **5** causes significant changes to the UV–vis absorption spectrum. The visible absorption band located *ca.* 400 nm was gradually disappeared and the appearance of a new peak *ca.* 346 nm, with an isosbestic point at 365 nm, which indicates the formation of a new reaction product between **5** and bisulfite, was observed. The hipsochromic effect in the fluorescence emission spectra after the addition of bisulfite to the solution of compound **5** was also observed. More importantly, the fluorescence intensity of the product formed in the reaction between the probe **5** and bisulfite at 410 nm is over 68-fold higher in comparison with the fluorescence intensity of **5** at the same wavelength.

In order to determine the limit of detection of compound **5** against HSO_3^- in phosphate buffer (10% CH_3CN, pH 7.4), the absorp-

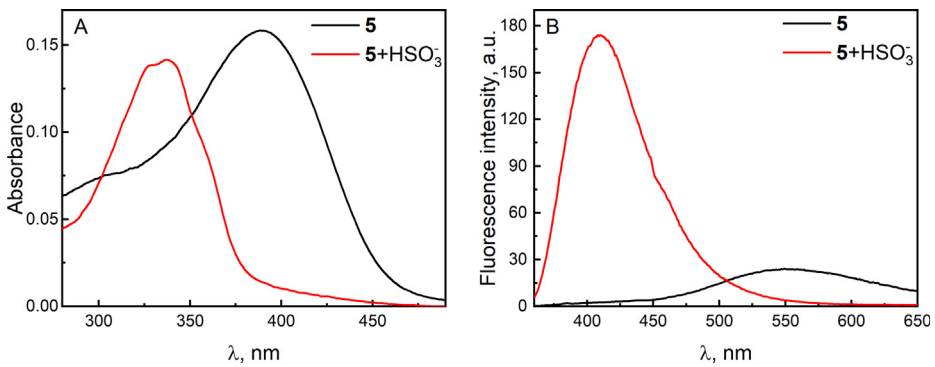


Fig. 1. (A) Absorption (B) fluorescence spectra changes of **5** (10 μM) in phosphate buffer (10% CH_3CN, pH 7.4) after addition of bisulfite (3 mM).

tion and emission spectra in the presence of various concentrations bisulfite (in the range of 0–40 μM) were registered (Fig. 2). After the addition of HSO_3^- the absorption band located in the visible range of the spectrum gradually undergoes a hipsochromic shift and an increase in fluorescence intensity is observed.

According to the titration plot, the lowest concentration of sodium bisulfite that can be determined using the presented compound **5** was estimated to be 0.25 μM and it is comparable with the values for other probes described in literature so far [19,39–41].

3.3. Determination of the reaction responsible for bisulfite recognition

To verify the proposed sensing mechanism shown in Fig. 3A, the product of the reaction of HSO_3^- with compound of **5** was confirmed by hydrogen nuclear magnetic resonance (^{1}H NMR) spectroscopy. As shown in Fig. 3B, the signal at 8.02 ppm (Ha), corresponding to the double bond in dicyanovinyl moiety of compound **5**, disappeared and two new signals at 4.38 and 4.33 ppm (Hb, Hc) appeared. Moreover, the adduct of **5-HSO$_3^-$** was further confirmed by HRMS analysis (*m*/*z*: $[M + Na]^+$ calcd. for $C_{19}H_{14}N_4O_3$-NaS 401.0684, found 401.0679) (Fig. S4).

3.4. Studies of the fluorescent response to various amino acids and reactive sulfur species

The chromophore system attached to a dicyanovinyl group can also be used as fluorescent probe to amino acids containing sulfhydryl group [23–25]. Therefore, to check the selectivity, absorption spectra and fluorescence emission spectra of compound **5** upon the addition of various amino acids, namely of thiol amino acids, L-cysteine (L-Cys), L-homocysteine (L-Hcy), L-glutathione (L-GSH), *N*-acetyl-L-cysteine (L-ACC), in phosphate buffer (10% CH_3-CN, pH 7.4) were measured and are shown in Fig. 4. Additionally, in Fig. 5 compound **5** towards representative anions, including reactive sulfur species (NaSH, Na_2S) and reactive oxygen species (H_2O_2) at the same conditions are shown. In the presence of amino acid and other compound tested, some changes in the absorption spectra were observed. These changes were observed after about 30 min upon the addition the thiol amino acids (Fig. 6A-C). In the case of sodium bisulfite the change was observed immediately (Fig. 6D). It is worth that, almost complete disappearance of the band *ca.* 400 nm and the highest absorbance value of the band formed *ca.* 346 nm was observed for the bisulfite.

Fig. 4B and Fig. 5B illustrates the fluorescence spectra of **5** in the presence of different amino acids and reactive sulfur species, respectively. Upon the addition of thiol amino acids and reactive sulfur species the formation a new maximum emission band located at approximately 410 nm, but with a lower fluorescence intensity was also observed. However, the addition sodium bisulfite to solution of **5** results in the highest increase of fluorescence intensity only at 410 nm. The changes in color of fluorescence of compound **5** in phosphate buffer (10% CH_3CN, pH 7.4) after the addition of amino acids containing sulfhydryl group and bisulfite under UV lamp are shown in Fig. S5.

Furthermore, similar results were observed for aliphatic thiol compounds, such as 2-mercaptoethanol (ME) and thioglycolic acid (TGA) (Fig. S6).

Moreover, in order to confirm that the optical response toward thiol amino acid is the results of the reaction of the probe with sulfhydryl group present in the structure of amino acid, the emission spectra of compound **5** in the presence of L-Cys which was previously treated with thiol masking reagent (NEM) were recorded. As shown in Fig. S7, the styrylbenzimidazolium derivative in the presence L-Cys pretreated with NEM did not enhance fluorescence intensity at 410 nm.

The results indicate the probe **5** can be used to detect of bisulfite and is able to distinguish HSO_3^- from other reactive sulfur species.

3.5. Selectivity and interference for the detection of bisulfite

The addition of bisulfite to compound **5** induces the highest fluorescence intensity increase. To further assess the selectivity of compound **5** for HSO_3^-, the competition tests were conducted. The changes of fluorescence intensity ratios (I/I_0) in the presence of bisulfite after the addition of various amino acids (L-Cys, L-Hcy, L-GSH, L-ACC, Gly, L-Glu, L-Lys), reactive sulfur species (SH^-, S_2^-) and H_2O_2 were monitored (Fig. 7). As shown, the addition of other competitive analytes does not affect the fluorescence response of compound **5** to bisulfite. It can therefore be concluded that the probe **5** can be successfully used for the detection of sodium bisulfite over other amino acids.

3.6. pH-dependence in the detection of thiol amino acids and bisulfite

As is well known, pH is also an important factor, which significantly affects fluorescence measurements and the application of fluorescent probes. Therefore, the investigation of the influence of pH on the detection of bisulfite and thiol amino acid by probe **5** was performed. As shown in Fig. 8A, the fluorescence intensity of the compound **5** with sodium bisulfite and thiol amino acids at λ_{max} 410 nm increased with increasing pH, reaching the highest value at pH of 7.4. Above pH of 7.4, the decreased in fluorescence intensity was observed for different studied analytes. As can be seen in Fig. 8B, the fluorescence intensity of the compound **5** in the presence of HSO_3^- at 410 nm was decreased, whereas at 550 nm was enhanced with the increase of pH (above 10). The

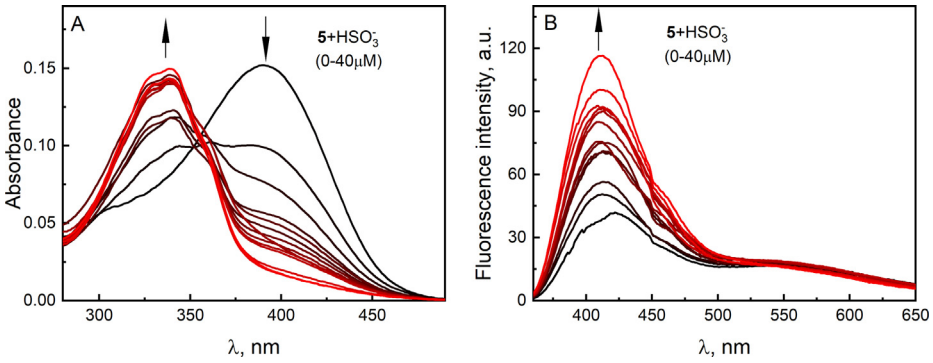


Fig. 2. (A) Absorption and (B) fluorescence spectra of compound **5** (10 μM) in the presence of different concentrations of HSO_3^- (0–40 μM) in phosphate buffer (10% CH_3CN, pH 7.4).

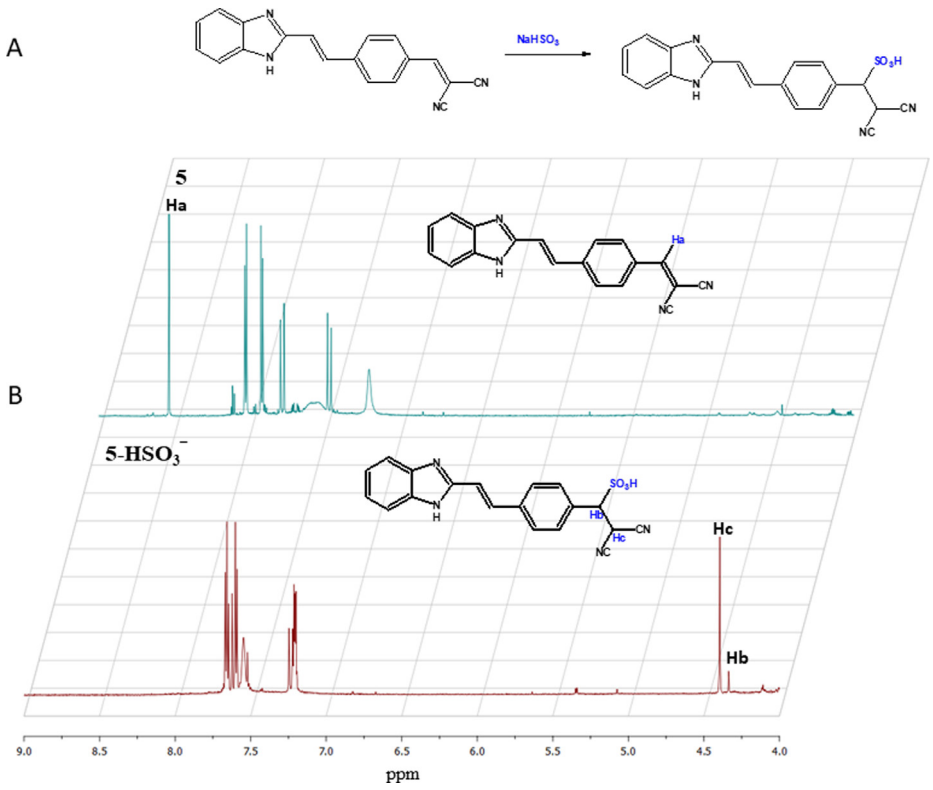


Fig. 3. (A) The proposed reaction of **5** for the determination of bisulfite anion. (B) Partial ^{1}H NMR spectral change of **5** in the absence and in the presence of excess HSO_3^- in dimethyl sulfoxide-d_6.

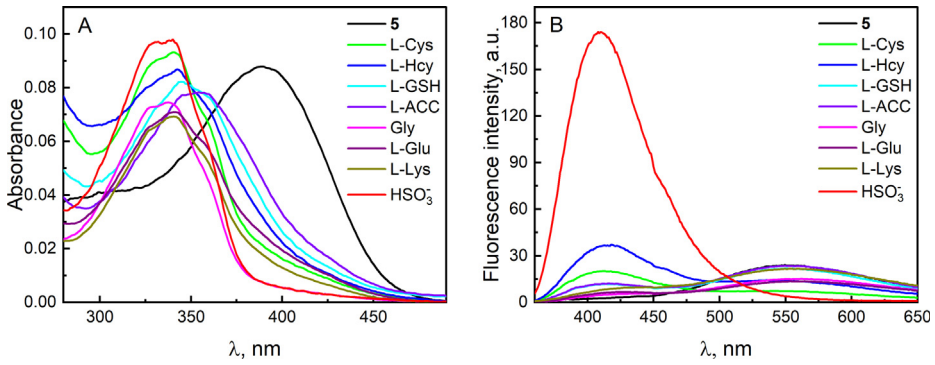


Fig. 4. (A) Absorption and (B) fluorescence spectra changes of **5** (10 μM) in phosphate buffer (10% CH_3CN, pH 7.4) after the addition of different amino acids (3 mM).

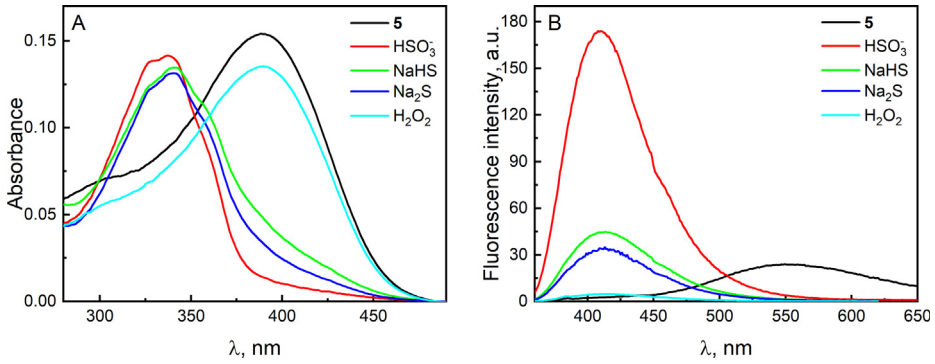


Fig. 5. (A) Absorption and (B) fluorescence spectra changes of **5** (10 μM) in phosphate buffer (10% CH_3CN, pH 7.4) after the addition of different analytes (3 mM).

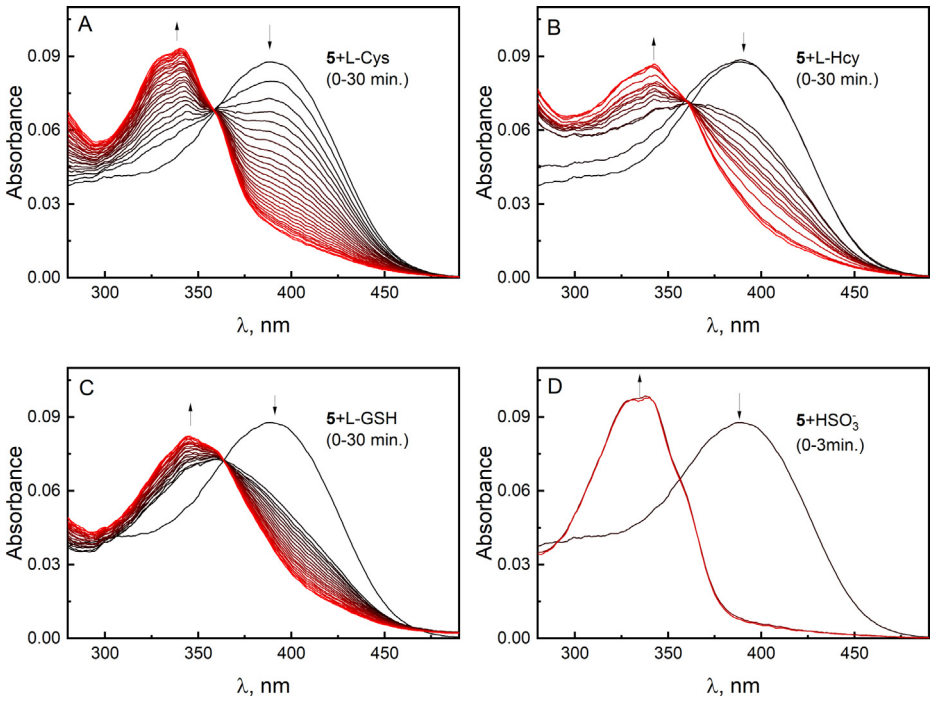


Fig. 6. Absorption spectra changes of **5** (10 μM) in phosphate buffer (10% CH_3CN, pH 7.4) in the presence of amino acids containing thiol and bisulfite (3 mM) in time (0–30 min.)

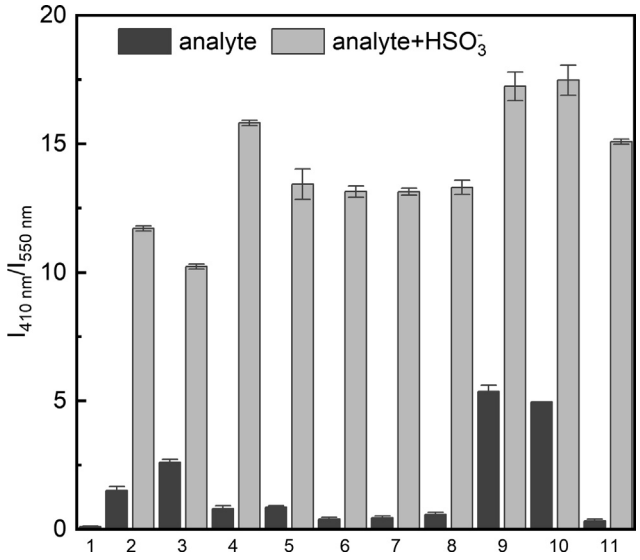


Fig. 7. The fluorescence intensity changes of compound **5** (10 μM) in the presence of bisulfite after the addition of different competitor (3 mM) in phosphate buffer (10% CH_3CN, pH 7.4) (1. blank, 2. L-Cys, 3. L-Hcy, 4. L-GSH, 5. L-ACC, 6. Gly, 7. L-Glu, 8. L-Lys, 9. NaHS, 10. Na_2S, 11. H_2O_2).

results indicate that the probe **5** can be used for the detection of HSO_3^- under physiological conditions.

3.7. Cytotoxicity of styrylbenzimidazolium derivative

An important feature of new derivative that can be potentially used in biological systems, is the low toxicity. Therefore, cytotoxicity of the novel styrylbenzimidazolium derivative was performed using a Prestoblue-based assay. To this end, the human cells line (HeLa) were treated with various concentrations (0-18.75 μmol/L) of the compound **5**. More than 70% of HeLa cells survived after incubation with compound **5** (Fig. S8). That indicate the slight cytotoxicity of the tested styrylbenzimidazolium derivative toward the cell line tested.

3.8. Application in wine samples

It is important to control the amount of bisulfite due to its wide use in many products. The HSO_3^- anions are present in each wine, which affect the product quality. To test the feasibility detecting bisulfite in real samples using probe **5**, the three type of wine were selected. Based on the experimental results presented above, the fluorescence response of the compound **5** toward bisulfite in these samples was investigated. As shown in Table 2, the HSO_3^- levels in the three wine samples were range from 3.88 to 7.17 μM. The recovery values were in the range of 90.78%-99.67% in various wine samples. It indicates that the probe **5** can be potential used for the detection of bisulfite in real samples.

4. Conclusion

Here, the synthesis as well as detailed spectroscopic characterization of novel styrylbenzimidazolium-based fluorescent probe with dicyanovinyl group as the recognition site for the detection of bisulfite are described. Upon reaction with bisulfite a strong fluorescence signal at 410 nm was generated. The probe reacts with bisulfite *via* nucleophilic addition and it was confirmed by ^1H NMR measurements. The limit of detection for bisulfite was estimated to be 0.25 μM. Moreover, the probe **5** has been successfully implemented for the detection of bisulfite in various wine sample. Additionally, this compound have slight cytotoxicity for the human cells line.

Based on the observed reactivity of the probe **5**, we conclude that it can be used for the detection of bisulfite, but its ability to report thiols cannot be neglected. However, bisulfite reacts with the probe faster than other reactive sulfur species. The presented novel styrylbenzimidazolium-based fluorescent probe is favored by its high reactivity toward bisulfite with the formation of the product that exhibit strong fluorescence signal.

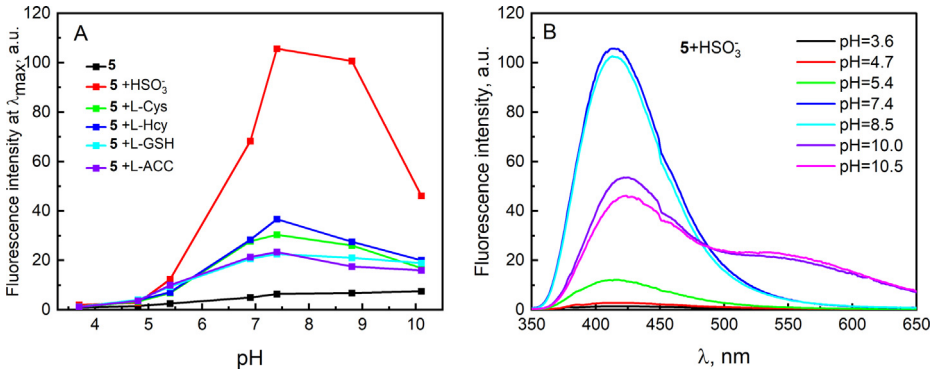


Fig. 8. (A) pH effect on the fluorescence intensity at λ_{max} of compound **5** (10 μM) with and without thiol amino acids and sodium bisulfite (3 mM) in phosphate buffer (10% CH_3CN, pH 7.4) (B) pH-dependent fluorescence spectra of compound **5** (10 μM) with HSO_3^- (3 mM) in phosphate buffer (10% CH_3CN, pH 7.4).

Table 2
Results for the determination of HSO_3^- in three wine samples.

Samples	HSO_3^- level (μM)	HSO_3^- added (μM)	HSO_3^- found (μM)	Recovery %
Rose wine 1	7.17	10	17.0	99.00
Red wine 2	5.35	10	15.3	99.67
Red wine 3	3.88	10	12.6	90.78

CRediT authorship contribution statement

Jolanta Kolińska: Conceptualization, Methodology, Investigation, Visualization, Writing - original draft. **Aleksandra Grzelakowska:** Methodology, Investigation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data (**Fig. S1.** ESI mass spectrum of **5**; **Fig. S2.** The normalized UV–vis absorption (black) and fluorescence (red) spectra of dye **5** (10 μM) in CH_3CN; **Fig. S3.** Fluorescence intensity decay of compound **5** in CH_3CN; **Fig. S4.** HRMS spectrum of **5-HSO_3^-**; **Fig. S5.** Changes in the fluorescence color of the compound **5** (10 μM) solution after addition of L-Cys, L-Hcy, L-GSH, L-ACC, HSO_3^- (3 mM) in phosphate buffer (10% CH_3CN, pH 7.4) under illumination 365 nm UV light; **Fig. S6.** (A) Absorption (B) fluorescence spectra changes of **5** (10 μM) in phosphate buffer (10% CH_3CN, pH 7.4) in the presence of aliphatic thiols (TGA, ME) (3 mM); **Fig. S7.** Fluorescence spectra changes of **5** (10 μM) in phosphate buffer (10% CH_3CN, pH 7.4) in the presence of L-cysteine, NEM (3 mM); **Fig. S8.** Percentage of viable HeLa cells with various concentrations of **5**) to this article can be found online at https://doi.org/10.1016/j.saa.2021.120151.

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