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Benzo[*d*]imidazo[2,1-*b*]thiazole-based fluorescent sensor for Zn^{2+} ion detection

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ABSTRACT

In this study, we have synthesized and evaluated three benzo[*d*]imidazo[2,1-*b*]thiazole-based sensors (**BIT-1**, **BIT-2** and **BIT-3**). Among them, **BIT-3** namely 2-(benzo[*d*]imidazo[2,1-*b*]thiazol-2-yl)-5-methoxyphenol was introduced as a selective fluorescence chemosensor for Zn^{2+} ion detection. The presence of zinc ions enhanced fluorescence property of **BIT-3** at 404 nm due to formation of a 1:1 complex between the chemosensor and the Zn^{2+} ion. Control experiments showed that the analogous ions do not have fluorescence enhancement effect and some of them even quench the fluorescence dramatically. The data obtained from UV–vis absorption analysis, fluorescence measurements and ^{1}H NMR spectroscopy approved the interaction between **BIT-3** and Zn^{2+} ion. The sensor probe **BIT-3** exhibits a selective fluorescence enhancing property via a chelation-enhanced fluorescence (CHEF) upon addition of Zn^{2+}. Thus, a selective fluorescent chemosensor **BIT-3** establishes an important sensing platform for real-time monitoring of Zn^{2+} ion in aqueous environment.

1. Introduction

Zinc has been recently recognized as an essential element in organisms [1]. It has attracted a considerable attention due to its existential role in many biological processes [2]. Appropriate levels of zinc ions prevent teeth caries and osteoporosis [3]. The lack of zinc in human body have deleterious effect on health including growth retardation, diarrhea, malfunctioning of wound healing and dermatitis [4], while high concentration of zinc can be toxic. To that end, the elevated levels of zinc compromises cell survival and is a risk factor in stroke [5] and Alzheimer's disease [6]. Blood and urine Zn^{2+} ion concentration depends on a wide range of parameters such as biological, habitual, and environmental conditions [7]. Thus, quantification of zinc ions concentration in real water and biological samples could provide a unique diagnostic tool in the clinical, environmental and industrial areas [8].

Currently, many methods are available for determination of zinc ion, including atomic absorption spectrometry [9], inductively coupled plasma [10], electrochemical techniques [11], UV–Vis methods based on surface plasmon resonance [12] and fluorescence assays [13]. In particular, fluorescent chemosensors are at the center of attentions due to their high response speed, good selectivity, high sensitivity and easy operation character [13–20]. In the recent years, intensive investigations have focused on the design, development and discovery of fluorescent sensors for Zn^{2+} ions [13,21–24]. However, only a few sensors surmount the drawbacks including weak selectivity, low sensitivity and detection in aqueous medium. Also, the prominent obstacle for most of the sensors is the requirement of toxic organic solvent for their operation and single ion detection. Therefore, developing chemosensors capable to overcome these limitations is troublesome [25,26].

In this work, we designed and synthesized a fluorescence probe **BIT-3** derived from benzo[*d*]imidazo[2,1-*b*]thiazole (**BIT**) for Zn^{2+} ion detection (Fig. 1). Fluorescent sensor **BIT-3** was very selective and sensitive for Zn^{2+} in comparison with other cations. We also copiously studied the optical properties and recognition behaviors of **BIT-3** and related compounds in detail using fluorescence and UV–vis spectroscopy. Furthermore, we have also investigated the usefulness of designed sensor for the detection of the Zn^{2+} ions in real water and biological samples.

2. Experimental

2.1. Reagents and materials

All the starting materials including 2-aminobenzothiazole, 2-

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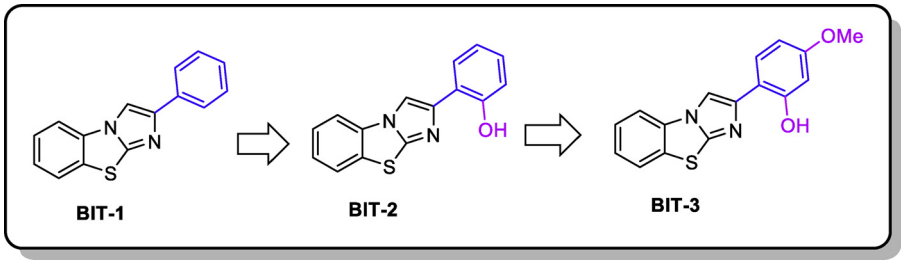


Fig. 1. Chemical structures of designed fluorescent sensors.

bromoacetophenone, 1-(2-hydroxyphenyl)ethanone and 1-(2-hydroxy-4-methoxyphenyl)ethanone were purchased from Sigma-Aldrich and used for synthesis of sensors without further purification unless otherwise mentioned. All solvents used in spectroscopic tests were spectroscopic grade without fluorescent impurity. The solutions of Na^+, K^+, Cs^+, Ag^+, Cd^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Pd^{2+}, Fe^{3+} and Al^{3+} ions were prepared from their sulfate salts (Sigma-Aldrich).

2.2. Apparatus

General information about instruments used in this study can be found in Supplementary Material.

2.3. Synthesis of chemosensors

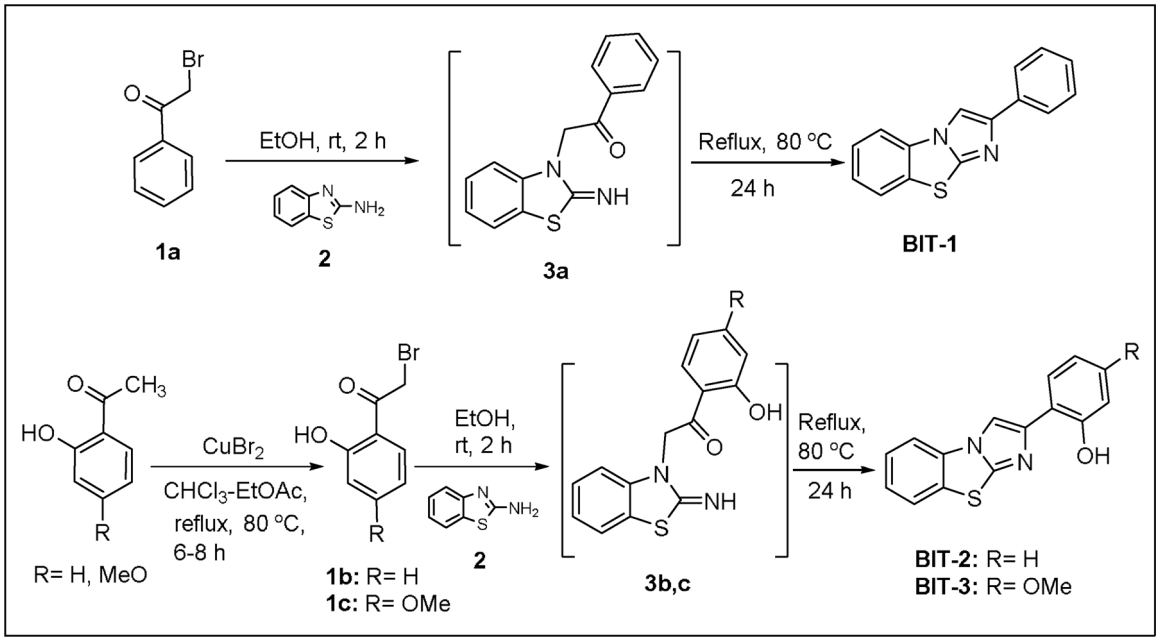
Scheme 1 presents the synthetic routes to **BIT-1**, **BIT-2** and **BIT-3**. The bromo- intermediates **1b** and **1c** were prepared according to the reported methods [27,28]. The synthetic procedures for preparation of other compounds are described here in details.

2.3.1. Preparation of 2-phenylbenzo[d]imidazo[2,1-b]thiazole (*BIT-1*)

A solution of 2-bromoacetophenone (**1a**, 199 mg, 1.0 mmol) in ethanol (2 mL) was added to a solution of 2-aminobenzothiazole (**2**, 150 mg, 1.0 mmol) in ethanol (3 mL). After stirring at room temperature (2 h), the reaction mixture was refluxed for 24 h. A white precipitated solid was formed after cooling. The solid was separated by filtration, then washed with cold ethanol and dried to give **BIT-1**. Yield 78%; mp 107–108 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.89 (s, 1 H), 8.40 (d, J = 8.0 Hz, 1 H), 8.04 (d, J = 8.0 Hz, 1 H), 7.97 (d, J = 7.2 Hz, 2 H), 7.58 (t, J = 8.0 Hz, 1 H), 7.31–7.51 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.94 (1C), 147.41 (1C), 133.63 (1C), 131.95 (1C), 130.06 (1C), 128.64 (2C), 127.43 (1C), 126.06 (1C), 125.06 (2C), 124.77 (1C), 124.19 (1C), 112.53 (1C), 106.80 (1C). MS (*m/z*, %): 250 (M^+, 100), 223 (5), 125 (9), 116 (8), 103 (5), 52 (7), 63 (4). Anal. Calcd for $C_{15}H_{10}N_2S$: C, 71.97; H, 4.03; N, 11.19. Found: C, 72.09; H, 3.96; N, 11.31.

2.3.2. Preparation of 2-(benzo[d]imidazo[2,1-b]thiazol-2-yl)phenol (*BIT-2*)

A solution of 2-bromo-1-(2-hydroxyphenyl)ethanone (**1b**, 215 mg, 1.0 mmol) in ethanol (2 mL) was added to a solution of 2-aminobenzothiazole (**2**, 150 mg, 1.0 mmol) in ethanol (3 mL). After stirring at room temperature (2 h), the reaction mixture was refluxed for 24 h. The precipitated solid was filtered, washed with cold ethanol and dried to give **BIT-2**. Yield 56%; mp 190–193 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.99 (s, 1 H), 8.27 (d, J = 7.6 Hz, 1 H), 8.18 (d, J = 8.0 Hz, 1 H), 7.83 (d, J = 7.6 Hz, 1 H), 7.67 (t, J = 8.0 Hz, 1 H), 7.56 (t, J = 7.6 Hz, 1 H), 7.25 (t, J = 8.0 Hz, 1 H), 7.04 (d, J = 8.0 Hz, 1 H), 6.97 (t, J = 7.6 Hz, 1 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 154.84 (1C), 146.53 (1C), 138.08 (1C), 131.98 (1C), 130.45 (1C), 130.03 (1C), 127.86 (1C), 127.05 (1C), 125.87 (1C), 120.13 (1C), 117.00 (1C), 115.61 (1C), 115.10 (1C), 111.79 (1C). MS (*m/z*, %): 266 (M^+, 100), 237 (24), 211 (6), 135 (14), 108 (9), 82 (10). Anal. Calcd for $C_{15}H_{10}N_2OS$: C, 67.65; H, 3.78; N, 10.52. Found: C, 67.82; H, 3.86; N, 10.44.



Scheme 1. Synthesis of sensors **BIT-1**, **BIT-2** and **BIT-3**.

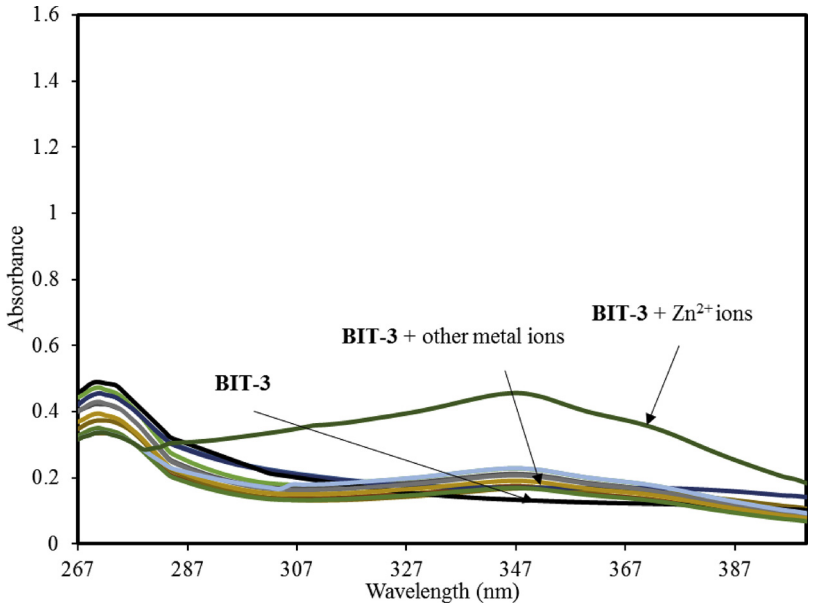


Fig. 2. Absorption spectral changes of **BIT-3** (100 μM) in combination with 1.0 equiv of ions in DMSO/H_2O (95:5, v/v) solution.

*2.3.3. Preparation of 2-(benzo[d]imidazo[2,1-b]thiazol-2-yl)-5-methoxyphenol (**BIT-3**)*

A solution of 2-bromo-1-(2-hydroxy-4-methoxyphenyl)ethanone (**1c**, 245 mg, 1.0 mmol) in ethanol (2 mL) was added to a solution of 2-aminobenzothiazole (**2**, 150 mg, 1.0 mmol) in ethanol (3 mL). After stirring at room temperature (2 h), the reaction mixture was refluxed for 24 h. The precipitated product (brown solid) was filtered and washed with cold ethanol. Further purification was carried out by column chromatography (silica gel, eluting with $CHCl_3$:MeOH = 98:2, v/v) to afford compound **BIT-3**. Yield 48%; mp 173–175 °C ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.85 (s, 1 H), 8.59 (s, 1 H), 8.06 (d, J = 7.6 Hz, 1 H), 8.03 (d, J = 8.4 Hz, 1 H), 7.80 (d, J = 6.4 Hz, 1 H), 7.55 (t, J = 7.6 Hz, 1 H), 7.42 (t, J = 7.6 Hz, 1 H), 6.45–6.55 (m, 2 H), 3.75 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 159.99 (1C), 156.31 (1C), 145.84 (1C), 144.47 (1C), 132.27 (1C), 129.56 (1C), 127.62 (1C), 127.11 (1C), 125.49 (1C), 125.38 (1C), 113.93 (1C), 112.41 (1C), 109.42 (1C), 105.99 (1C), 101.94 (1C), 55.47 (1C). MS (*m/z*, %): 296 (M^+, 100), 253 (44), 225 (11), 199 (5), 134 (10), 108 (4), 63 (4). Anal. Calcd for $C_{16}H_{12}N_2O_2S$: C, 64.85; H, 4.08; N, 9.45. Found: C, 64.98; H, 3.97; N, 9.44.

2.4. UV–vis and fluorescence spectroscopic studies

The titrations for recording absorption and fluorescence spectra were performed in DMSO/H_2O (95:5, v/v) solution. Inorganic salts solutions were prepared by using distilled water to acquire 2.0 mM aqueous solution. Due to the high lipophilicity of ligands, the stock solutions of ligands (2.0 mM) were prepared in DMSO. Aliquot of stock solution of each ligand was diluted to 2 mL DMSO/H_2O (95:5, v/v) solution in order to provide the final concentration of 20 μM. The resulting solutions contained 20 μM of ligand and cation. The UV–vis spectra were provided at 200–600 nm using a 1 cm quartz cell. The fluorescence spectra of ligand (20 μM) in the presence of various competitive species under the same conditions were recorded at 404 nm (excitation was provided at 343 nm) in DMSO/H_2O (95:5). Both the excitation and emission slits were set at 5 nm. The selectivity of the ligand toward Zn^{2+} was confirmed by adding excess amount (1 equiv.) of competitive metal ions in the mentioned experimental medium and the fluorescence emissions were recorded in the absence and presence of ions.

2.5. Determination of figures of merit

To obtain the detection limit of **BIT-3**, fluorescence titration curve was obtained by addition of Zn^{2+} (0.1–20 μM). Fluorescence intensity of **BIT-3** was measured five times and the standard deviation of blank control was calculated. The following equation was used for determining detection limit:

$$\text{Detection limit} = \frac{3\sigma_i}{k} \quad (1)$$

Where σ_i stands for the standard deviation of the blank, and k is the slope between the fluorescence intensity versus Zn^{2+} concentration.

2.6. Quantum yield measurements

The fluorescence quantum yield was measured by using anthracene as a standard fluorophore (Φ_s = 0.27, in ethanol) and the following equation:

$$\Phi_x = \frac{n_x^2 \cdot F_x \cdot A_s}{n_s^2 \cdot F_s \cdot A_x} \cdot \Phi_s \quad (2)$$

where, subscripts s and x refer to the reference and the sample, respectively. Letter A stands for absorbance at the excitation wavelength, n is the solution refractive index, and F represents emission integrated area. **BIT-3** and Zn^{2+} were dissolved in DMSO/H_2O to make a 10 μM solution. Fluorescence spectra of anthracene (10 μM) were taken in ethanol.

2.7. Binding constant

Emission titration studies of **BIT-3** with a Zn^{2+} solution (effective concentration between 0–60 μM) were carried out at its effective concentration (20 μM). The spectrophotometric titration data were applied to calculate the apparent binding constants by Benesi–Hildebrand (B–H) plot.

$$\frac{1}{F - F_0} = \frac{1}{K(F_{max} - F_0)[Zn^{+2}]} + \frac{1}{F_{max} - F_0} \quad (3)$$

F_0 and F are symbols for the **BIT-3** fluorescence intensity at maximum and particular wavelength in the presence of a certain concentration of the Zn^{2+}, respectively. F_{max} is the maximum emission intensity observed in the presence of Zn^{2+} at 404 nm during titration with varying

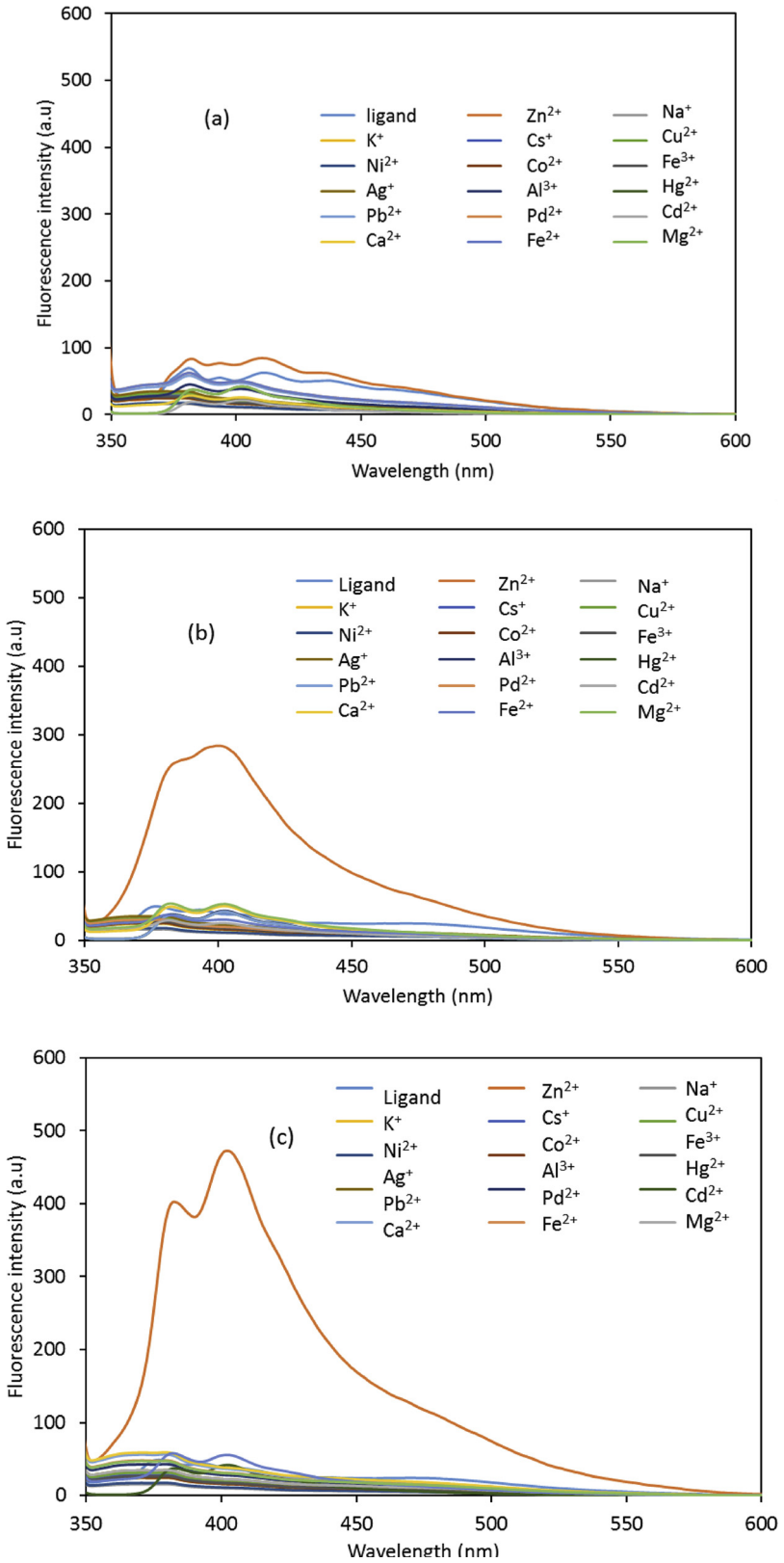


Fig. 3. Fluorescence emission spectra of (a) **BIT-1**, (b) **BIT-2** and (c) **BIT-3** (20 μM) in absence and presence of different metal ions in DMSO/H_2O (95:5, v/v) solution (1.0 equiv., λ_{ex} = 343 nm).

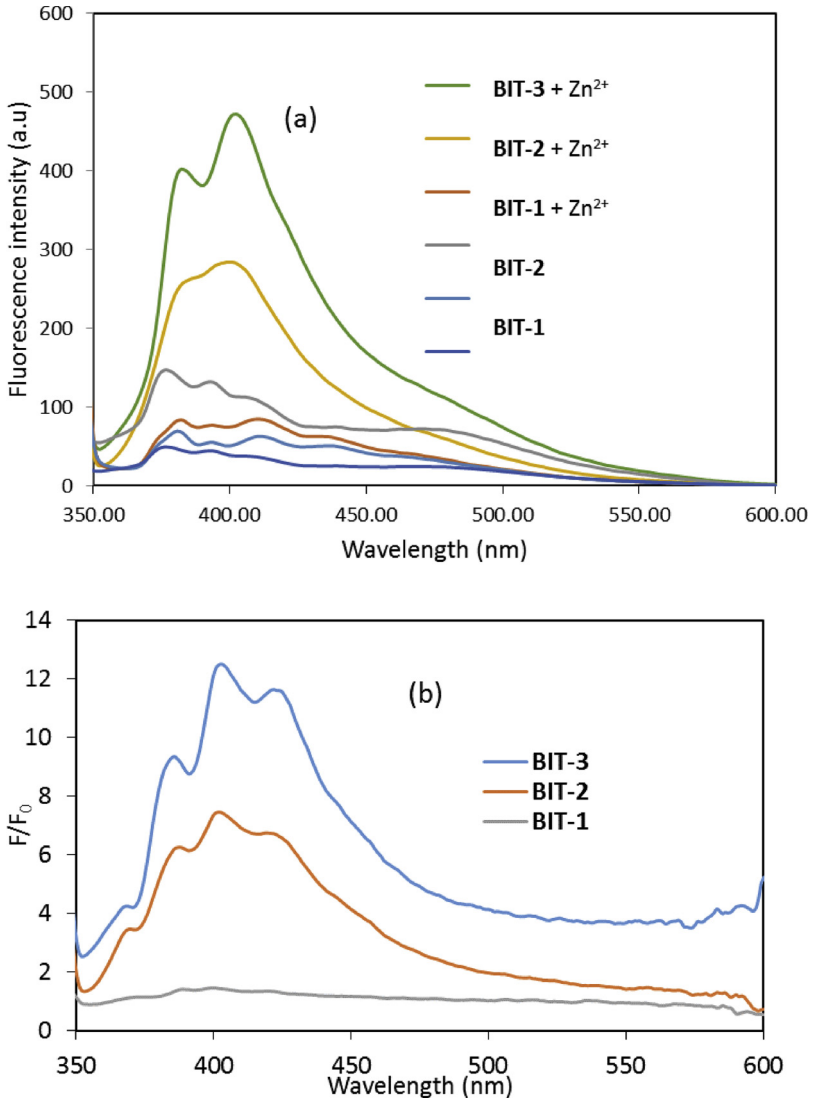


Fig. 4. (a) Fluorescence emission intensity changes and (b) F/F_0 values of **BITs** (20 μM) in the presence of Zn^{2+} ions in a DMSO/H_2O (95:5, v/v) mixture (1.0 equiv., λ_{ex} = 343 nm).

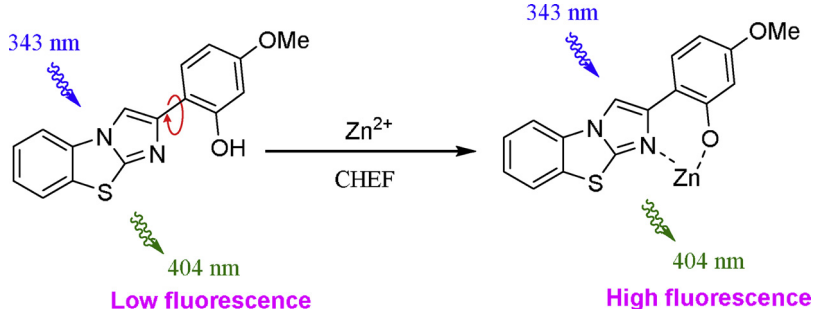


Fig. 5. Fluorescence enhancement mechanism and probable binding mode of **BIT-3**-Zn^{2+} complex.

analytes concentration, and K (M^{-1}) is the slope of the linear plot, which is equal to apparent binding constant.

2.8. ^1H NMR titration of *BIT-3* with Zn^{2+}

The **BIT-3** stock solution (1.0 mM) was prepared in DMSO-d_6 while Zn^{2+} ions solution (1.0 mM) was separately prepared in D_2O. After addition of Zn^{2+} (1 equiv.) to **BIT-3** solution, the ^1H NMR spectrums were recorded immediately.

2.9. Preparation of real water and bio-fluid samples for Zn^{2+} determination

The potential applicability of **BIT-3** to detect Zn^{2+} ion in real water and bio-fluid samples was studied using spikes and recovery method. Tap water sample was attained at our laboratory water pipe and the river water samples were acquired from Tajan River (Sari, Mazandaran, Iran). Human blood serum samples were collected from the Tooba clinical laboratory (Sari, Mazandaran, Iran). The stock solution of blood serum sample was prepared by diluting 1 mL of each blood serum

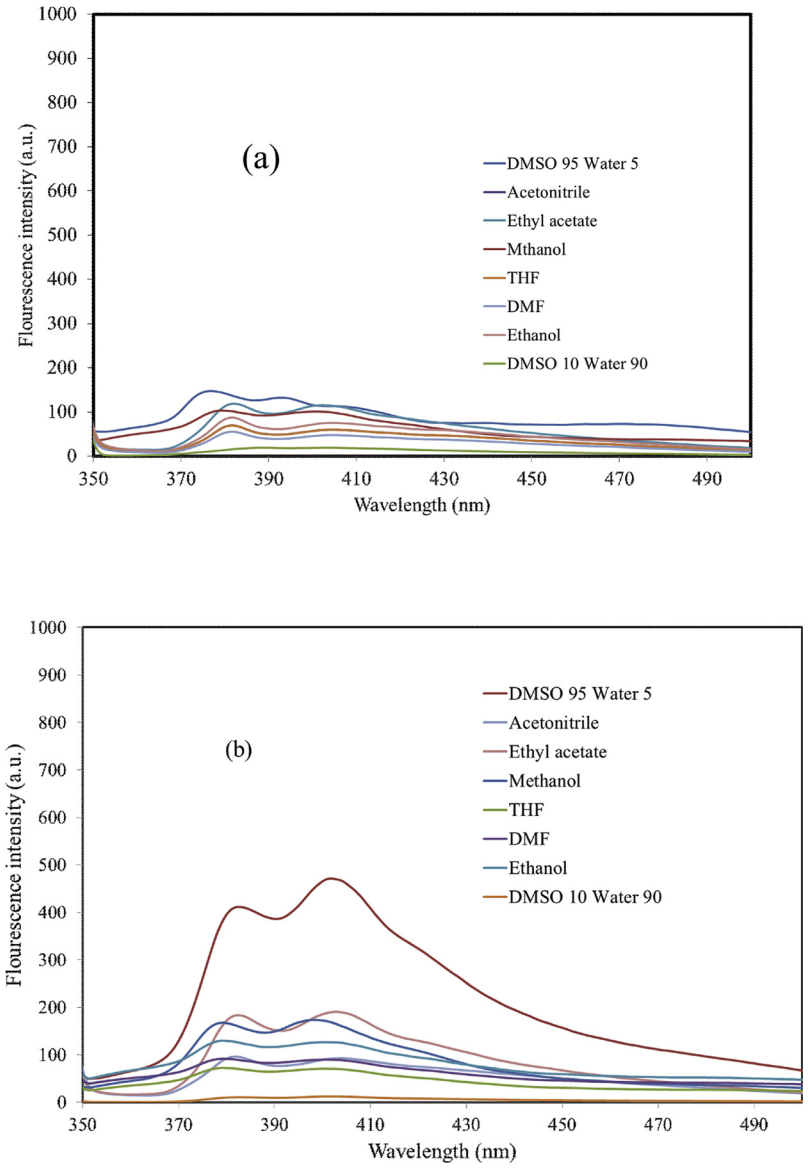


Fig. 6. (a) Fluorescence emission intensity change of **BIT-3** (20 μM) alone and (b) **BIT-3** (20 μM) in the presence of Zn^{2+} ions in different solvent systems (1.0 equiv., λ_{ex} = 343 nm).

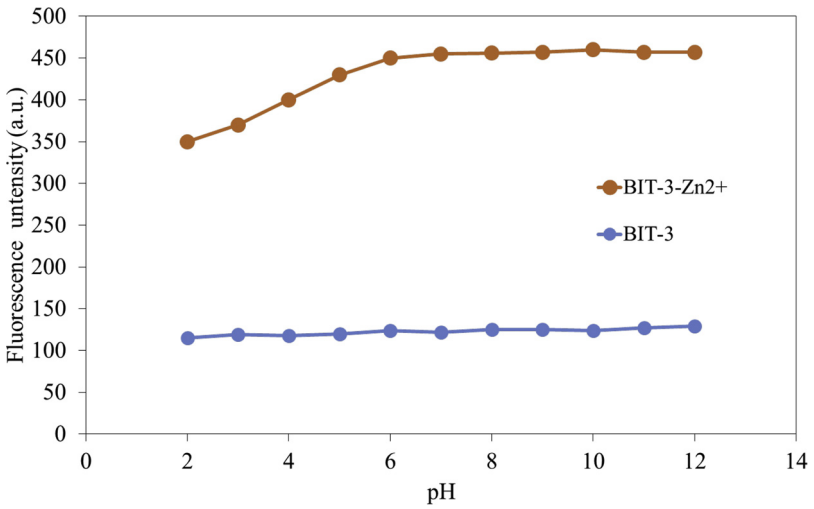


Fig. 7. Effect of pH on the fluorescence intensity of **BIT-3** (20 μM) in DMSO/H_2O mixture (95:5, v/v) in the absence and presence of Zn^{2+} ions (20 μM).

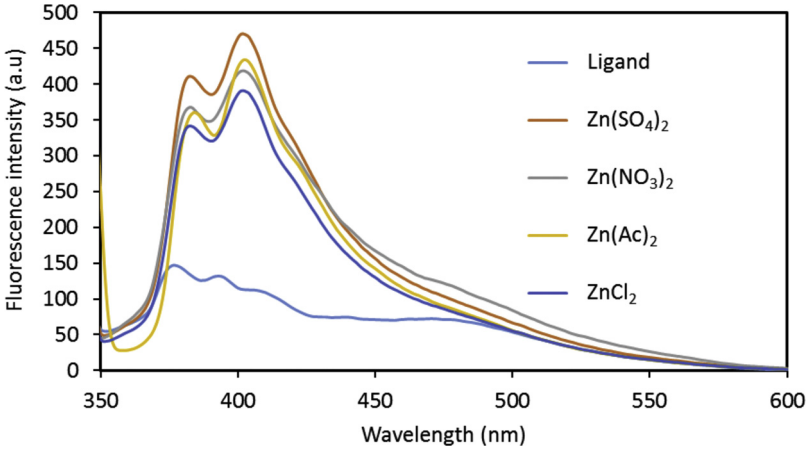


Fig. 8. Fluorescence emission intensity change of **BIT-3** (20 μM) in the presence of Zn^{2+} ion salts (1.0 equiv., λ_{ex} = 343 nm).

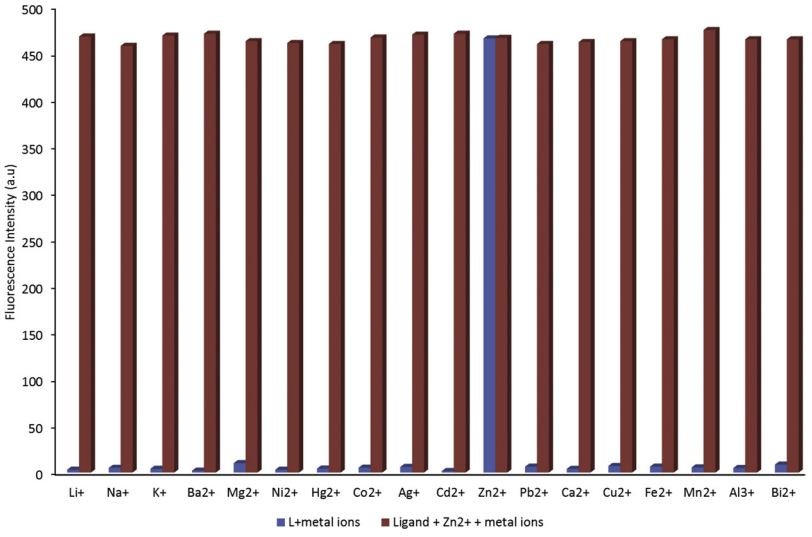


Fig. 9. Competitive selectivity of **BIT-3** (20 μM) toward Zn^{2+} (20 μM) in the presence of other metal ions (1.0 equiv.) with the excitation of 343 nm in a DMSO/H_2O (95:5, v/v) mixture.

Table 1
The obtained tolerance limits of different interfering ions in the determination of Zn^{2+} (20 μM).

Interfering ions	Tolerance limit (x/Zn^{2+})
Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Ba^{2+}, Ni^{2+},Cu^{2+}, Ag^+, Pb^{2+}, Hg^{2+}, and Al^{3+}	500
Co^{2+}, Cd^{2+}, Mn^{2+} and Fe^{2+}	300

samples into 50 mL of double distillated water. The human urine samples were collected from 2 male volunteers. Prior to experiment ultrapure concentrated HNO_3 (about 2.0 mL) was added to the urine samples and the acidified samples were stored at −4 °C. In order to assure of homogeneity of subsamples, all samples were shaken vigorously for 1 min before each experiment. The quantification was conducted by standard spiked sample addition and recovery.

Fluorescence spectra measurement of real water samples containing Zn^{2+} were carried by adding 100 μL of 400 μM stock solution of **BIT-3** (final concentration 20 μM) to 1.90 mL sample solutions. After well mixing, the solutions were allowed to stand at 25 °C for 1 min before test.

3. Results and discussion

3.1. Spectroscopic studies of *BIT-3*

3.1.1. UV–vis absorption studies

The UV–vis sensing properties of **BIT-3** (100 μM) in DMSO/H_2O (95:5, v/v) were investigated with the addition of metal ions Na^+, K^+, Cs^+, Ag^+, Cd^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Pd^{2+}, Fe^{3+} and Al^{3+}. As shown in Fig. 2, UV–vis spectra of free **BIT-3** exhibited two maximal absorptions at 272 and 343 nm (broad band) before titrations. When Zn^{2+} ions (1.0 equiv) were added, the absorbance band at 272 nm gradually decreased in intensity, while the intensity of absorbance band at 343 nm was gradually increased. The isosbestic point at 286 nm might be attributable to the coordination between **BIT-3** and Zn^{2+}. Whereas, the other tested metal ions displayed an obvious decrease in absorption bands at 272 nm with a week bond at 343 nm. So, a good distinction between Zn^{2+} and other ions

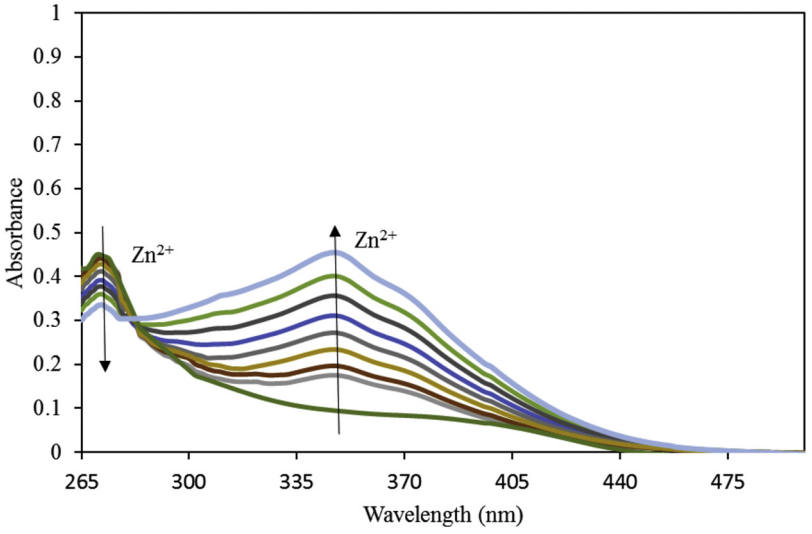


Fig. 10. UV–vis spectra for gradual addition of Zn^{2+} (0 equivalence to 2.0 equivalence) to **BIT-3** (100 μM in DMSO/H_2O (95:5, v/v)).

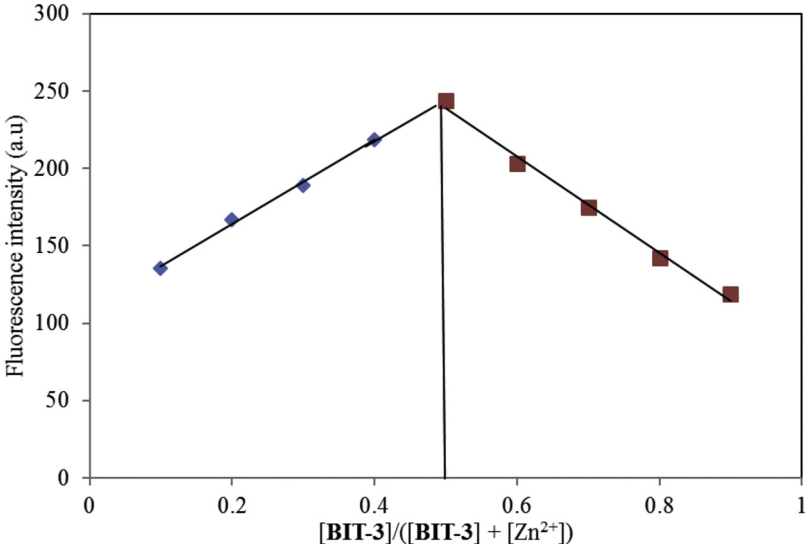


Fig. 11. Job's plot of **BIT-3**-Zn^{2+} complex resulted from the measurement of fluorescence emission at 404 nm (λ_{ex} = 343 nm).

was observed at 343 nm. Based on these results, 343 nm was selected as excitation wavelength in fluorescence emission studies.

3.1.2. Fluorescence emission studies

3.1.2.1. Selectivity. The selectivity of **BITs** ligands as chemosensors was investigated by fluorescence titration (Fig. 3) using a wide range of metal ions (Na^+, K^+, Cs^+, Ag^+, Cd^{2+}, Ba^{2+}, Pb^{2+}, Zn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Pd^{2+}, Fe^{3+} and Al^{3+}). Addition of 1.0 equiv of all ions except Zn^{2+} caused a complete quenching or decreasing of fluorescence at 404 nm. Certainly, 1.0 equiv Zn^{2+} resulted in a significant fluorescence increment at 404 nm, which was completely distinguishable from other metal ions spectrum.

3.1.2.2. Effect of chemical structure. The presence of hydroxy group in *ortho* position and methoxy group in *para* position of phenyl moiety in **BITs** chemosensors affect their fluorescence sensitivity and intensity for Zn^{2+} ions (Fig. 4a). To that end, **BITs** relative fluorescence intensity (Fig. 4b) was calculated and compared. As seen in Fig. 4b, the 2-hydroxy-4-methoxy derivative **BIT-3** showed a higher F/F_0 value in comparison to 2-hydroxy analog **BIT-2** and unsubstituted compound **BIT-1**. These findings demonstrate that the 2-hydroxy and 4-methoxy substituents as strong electron-donating fluorophores have important role in the fluorescence properties of **BIT-3**.

As a parent ligand, **BIT-1** didn't show any selectivity toward the aforementioned cations with lower fluorescence signal enhancement (Fig. 4). The 2-hydroxyphenyl derivative **BIT-2** showed better selectivity and fluorescence enhancement in comparison with **BIT-1**. The introduction of an electron donating group such as the methoxy on the phenyl moiety of **BIT-2** resulted in **BIT-3** with fluorescence enhancing property and better selectivity towards Zn^{2+} ions. Accordingly, chelation enhanced fluorescence (CHEF) could be a main mechanism for **BIT-3** (Fig. 5). Chemosensors with CHEF mechanism generally have separated conjugated units in their semi-rigid structure and would be integrated into a rigid coplanar conjugation system via metal coordination. When the Zn(II) ion is coordinated with nitrogen and oxygen atoms of **BIT-3**, the free rotation around single bond between aryl ring and tricyclic benzo[*d*]imidazo[2,1-*b*]thiazole system is restricted and eventually π-conjugation between them is increased, resulting in CHEF phenomena. The electron donating characteristic of the methoxy group on the phenyl moiety of **BIT-3** facilitates the 2-phenoxy group for more strongly coordination with Zn ion, and preferring CHEF to be as main mechanism.

3.1.2.3. The effect of solvents. The fluorescence emission of a solution

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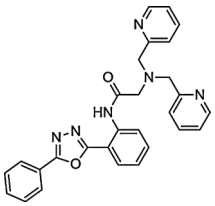
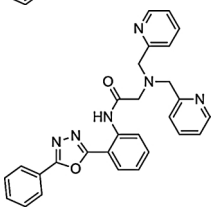
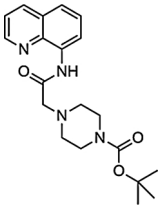
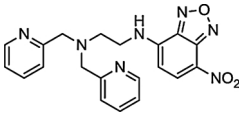
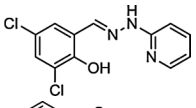
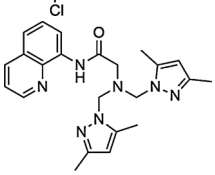
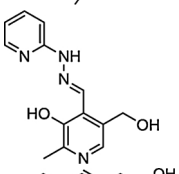
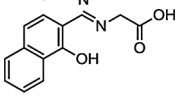
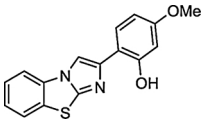
Table 2
Comparison of organic fluorescence chemosensors for the detection of Zn^{2+}.

Sensors	Detection limit (μM)	Binding constant (M^{-1})	Reference
	2.5	1.5×10^4	[31]
	0.86	2.82×10^8	[32]
	0.52	3.4×10^3	[33]
	0.06	No data	[34]
	0.03	5×10^4	[25]
	0.03	1.1×10^7	[35]
	0.07	2.7×10^6	[26]
	0.018	1.25×10^4	[36]
	0.03	6.8×10^9	[This work]

composed of **BIT-3** alone or **BIT-3** with 1.0 equiv. of Zn^{2+} was studied in different solvents including THF, DMF, ethanol, methanol, acetonitrile, ethyl acetate, DMSO/H_2O (10:90, v/v) and DMSO/H_2O (95:5, v:v), and the obtained data are presented in Fig. 6 (a and b, respectively). **BIT-3** in different solvents showed weak and same fluorescence peak in whole studied domain (350–500 nm). As presented in Fig. 6 b, the **BIT-3**-Zn^{2+} complex showed good fluorescence emission intensity in the DMSO/H_2O (95:5, v/v) solution compared with other solvents; therefore this solvent system was used for further studies.

Table 3
Determination of Zn(II) in real water and biological samples (n = 2).

Sample	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery rate (%)	Relative standard deviation (RSD)/ %
Rain water	0	N.D.[a]	–	–
	2	2.082	104.10	1.29
	10	10.051	100.51	0.62
River water (Tajan)	0	N.D.[a]	–	–
	2	2.018	100.90	1.76
	10	10.149	101.49	0.39
Tap water	0	N.D.[a]	–	–
	2	2.020	101.00	1.77
	10	9.950	99.50	0.28
Urine sample 1	0	N.D.[a]	–	–
	2	1.989	99.45	1.35
	10	9.918	99.18	0.90
Urine sample 2	0	N.D.[a]	–	–
	2	1.989	99.45	1.86
	10	9.885	98.85	0.47
Blood serum sample 1	0	N.D.[a]	–	–
	2	1.998	99.90	0.14
	10	10.007	100.07	2.16
Blood serum sample 2	0	N.D.[a]	–	–
	2	2.018	100.90	1.75
	10	9.918	99.18	1.01
Blood serum sample 3	0	N.D.[a]	–	–
	2	2.003	100.15	2.23
	10	9.971	99.71	0.61

[a] Not detected.

3.1.2.4. The effect of pH. A series of buffered solutions of **BIT-3** [20 μM, with various pH values (2–12)] with or without Zn^{2+} (20 μM) was prepared in HEPES buffer solution in DMSO/H_2O (95:5, v/v) mixture. The fluorescence intensity of the sensor **BIT-3** and **BIT-3**–Zn^{2+} over a wide range of pH (2.0 to 12.0) was studied as shown in Fig. 7. Almost no obvious change in the fluorescence intensity of **BIT-3** at 404 nm was observed in the pH range from 2.0 to 12.0, revealing that the free **BIT-3** is stable over the wide pH range.

As seen in Fig. 7, **BIT-3** exhibited a sensitive fluorescence enhancement response to Zn^{2+} in the pH range 6–12, but at pH below 5, **BIT-3** exhibited very weak fluorescence intensity. Thus, results of the pH study confirmed that **BIT-3** exhibited a sensitive response to Zn^{2+} in the 6–12 pH range, making it suitable for detecting Zn^{2+} at different pH levels.

*3.1.2.5. The effect of common anions on **BIT-3**-Zn^{2+} complex.* The impact of Zn^{2+} companion anion on fluorescence emission response of **BIT-3** was evaluated as well. The fluorescence emission of the solution containing **BIT-3** was investigated in the presence of common salts of zinc (1.0 equiv. of Zn^{2+}) including $ZnCl_2$, $Zn(NO_3)_2$, $Zn(SO_4)_2$ and $Zn(Ac)_2$ (Fig. 8). Fluorescence spectroscopic results showed that the type of anion negligibly affects fluorescence intensity of the **BIT-3**-Zn^{2+} complex.

3.1.2.6. Competition experiments. In order to evaluate the impact of other cations on fluorescence emission intensity, the fluorescence was obtained in the absence and presence of interfering ions in **BIT-3** or **BIT-3**-Zn^{2+} and the data were compared. Addition of 20 μM of aqueous solution to the solutions of Li^+, Na^+, K^+, Ag^+, Cd^{2+}, Ba^{2+}, Pb^{2+}, Mg^{2+}, Ca^{2+}, Fe^{2+}, Cu^{2+}, Ni^{2+}, Hg^{2+}, Co^{2+}, Mn^{2+}, Al^{3+} and Bi^{2+} ions, did not produce significant changes in fluorescence emission intensity of **BIT-3**-Zn^{2+} complex (Fig. 9).

Moreover, in order to find the maximum tolerable amounts of various interfering ions for determination of Zn^{2+} ion, 20 μM of Zn^{2+} ion alone and various concentrations of some diverse ions were added into the **BIT-3**–Zn^{2+} complex solution (20 μM of Zn^{2+} ion and **BIT-3**). Then, the inhibition effects of Zn^{2+} ion and the mixtures of Zn^{2+} + M^{n+} on

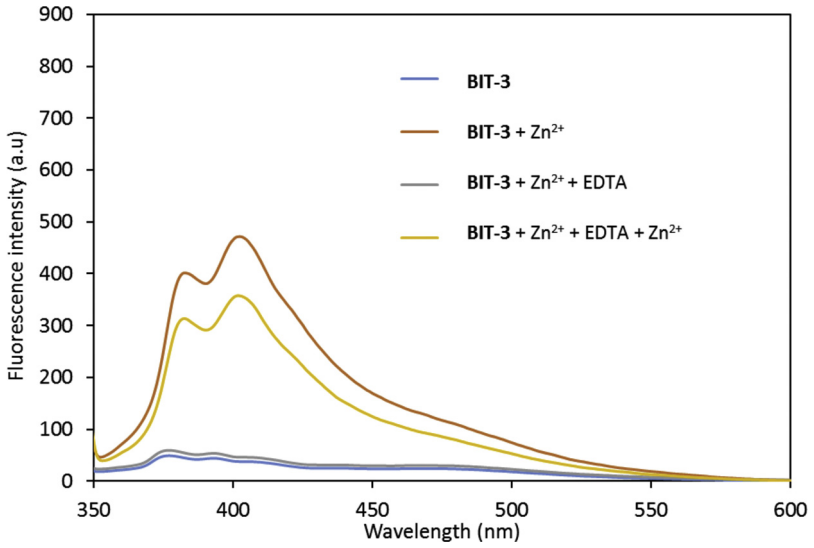


Fig. 12. Reversibility experiment of **BIT-3** (20 μM) in DMSO/H_2O (95:5, v/v) with EDTA and Zn^{2+} (10 equivalents amount of Zn^{2+} and EDTA was used).

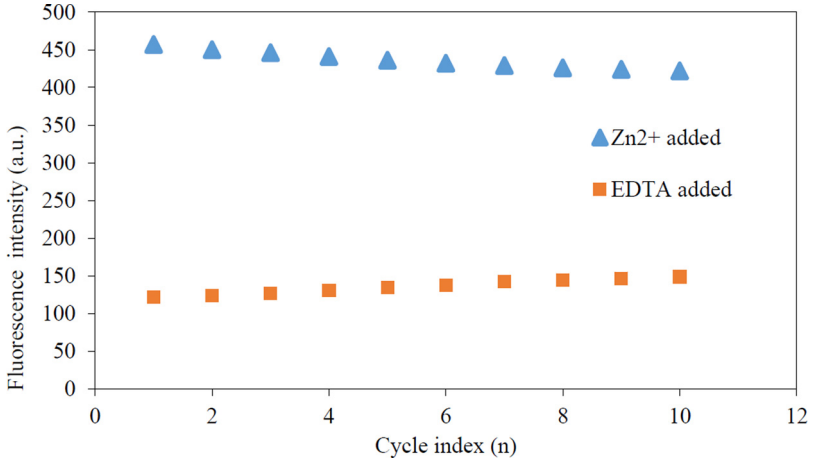


Fig. 13. The cycle index of **BIT-3**–Zn^{2+} (20.0 μM) reacting with Na_2EDTA (20.0 μM).

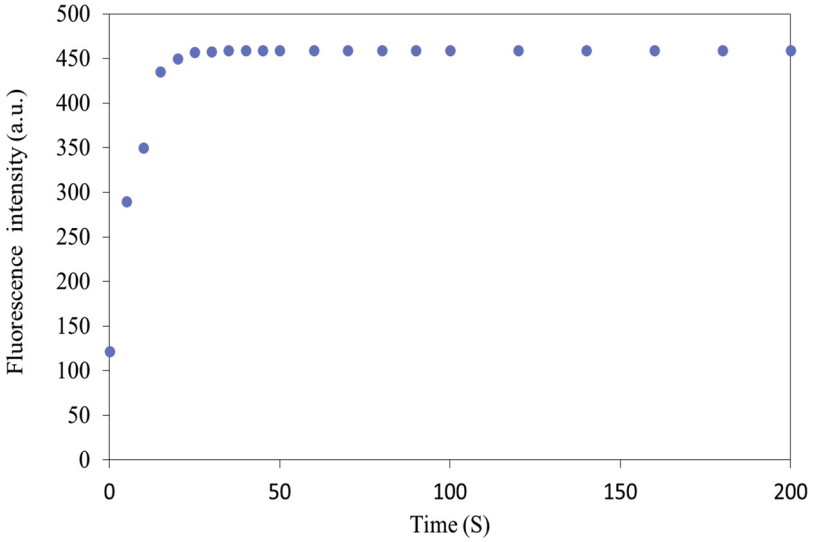


Fig. 14. Time-dependent fluorescence spectroscopy of **BIT-3** − Zn^{2+} complex (20 μM, 1 to 1 equiv.).

the **BIT-3**–Zn^{2+} complex were examined. As seen in Table 1, most of the tested ions do not have significant effect on the determination of Zn^{2+} (> 5.0% is considered tolerated).

3.1.2.7. Determination of quantum yield. The fluorescence quantum yield of **BIT-3** was calculated in comparison with anthracene (as the standard) in the absence and presence of Zn^{2+} ions in DMSO/H_2O (95:5, v/v). In the absence of Zn^{2+} ions, the quantum yield of **BIT-3** was 6.2%; which increased to 16.5% when 1 equiv. of Zn^{2+} ions was added. Therefore the presence of Zn^{2+} has a significant impact on quantum yield of **BIT-3**.

3.1.2.8. Determination of binding constant (K_a). By applying the obtained fluorescence emission spectroscopy data to Benesi-Hildebrand equation, the association constant (K_a) was calculated to be 6.8×10^9 M^{-1} for the **BIT-3**–Zn^{2+} complex [29].

3.1.3. Binding studies

In UV–vis titration study, different concentrations of Zn^{2+} ions (0.1–2 equiv.) were added to the **BIT-3** solution (100 μM) and the resulted data led to the calculation of **BIT-3** to Zn^{2+} ions ratio (Fig. 10). As seen, by increasing concentration of Zn^{2+} in solution, the absorption of **BIT-3** around 343 nm increased gradually. Conversely, the increasing amounts of Zn^{2+} resuled in decrease of absorption bond at 272 nm. The presence of isosbestic points at 282 nm revealed that a stable complex with a certain stoichiometric ratio is formed after addition of Zn^{2+}.

In addition to UV–vis titration experiment, to verify the ratio of **BIT-3** and Zn^{2+} ions, Job's plot experiment also was performed by using different concentration of ligand and the Zn^{2+} ion with a total concentration of 20 μM. The maximum point in Job's plot appears at the mole fraction of 0.5, indicating the 1:1 ratio of **BIT-3** and Zn^{2+} ion (Fig. 11).

Moreover, we examined the complexation of **BIT-3** with Zn^{2+} by ^1H NMR titration experiments. After addition of Zn^{2+} (1.0 equiv), the protons of −OH at 13.72 ppm disappeared completely. It is mainly due to the addition of D_2O and deuterium exchanges with hydrogen. Moreover, the protons of 6.52, 7.44, 7.55, 7.81 and 8.59 ppm shifted slightly downfield, which indicates Zn^{2+} ions can interact with **BIT-3** to form Zn^{2+}**–BIT-3** complex.

3.2. Analytical features

The validity of procedure was evaluated by determination of linear dynamic range, coefficient of determination (R^2) and limit of detection as quality parameters. Under optimum conditions, the calibration graph was linear over the range of 0.10–20.00 μM of Zn^{2+} with the determination coefficient of 0.9993 (n = 5), which means that **BIT-3** is suitable chemosensor for the detection of Zn^{2+} ions quantitatively. At the 0.2 μM concentration of Zn^{2+} (n = 5), the relative standard deviation was found to be 1.82%. According to the definition by IUPAC (CDL = 3Sb/m) the calculated detection limit was 0.03 μM, which is thousand times smaller than that of the value of WHO guideline for the detection of Zn^{2+} ions in the drinking water samples [30]. The performance of the developed chemosensor was compared with some of the figures of merit of the recently reported chemosensors for the determination of Zn^{2+} (Table 2). Obviously, the developed procedure with the newly designed compound **BIT-3** has a better or comparable detection limit and lower relative standard deviation in comparison with other reported compounds.

3.3. Real water and biological samples analysis

The **BIT-3** ligand as a chemosensor was applied to detect the content of Zn(II) in real samples such as different type of water (drinking, rain and tap water), urine and blood. The results are presented in Table 3. The data showed satisfactory recovery (in the range of 98.85–104.10%) and RSD values (lower than 2.23) for all samples.

3.4. Reversibility and response time for detection

The reversibility of the reaction between **BIT-3** and Zn^{2+} to form a complex during recognition process was checked by adding ethylenediamine tetraacetic acid disodium salt (EDTA) as the competing chelating agent, to the **BIT-3** and Zn^{2+} solution (10 equiv of **BIT-3**). As shown in Fig. 12, addition of EDTA to the Zn–**BIT-3** system immediately quenches the fluorescence peak at 404 nm. After addition of EDTA, introduction of Zn^{2+} to the solution recovers the main of primary fluorescence, which indicates that **BIT-3** and Zn^{2+} reaction in the sensing process is reversible to a certain degree. The probe reversibility can grantee its reusability in the presence of most interfering metal ions. This result indicates the high reversibility of **BIT-3** toward Zn^{2+} ion sensing and its potential for application in real medium monitoring.

To study the reversible cycle times of the **BIT-3** and Zn^{2+} ion complex formation and deformation, Zn^{2+} and EDTA periodically added to **BIT-3** (20 μM) in DMSO/H_2O mixture (95:5, v/v), and the fluorescent spectra (at 343 nm) after 10 cycles was scanned (Fig. 13). Obviously, the fluorescence intensity of recovered **BIT-3** appeared again by further adding Zn^{2+} ions, and restored steadily upon repeated addition of EDTA. As can be seen from Fig. 13, the **BIT-3** shows excellent stability with no significant fluorescence intensity decay for several successive cycles, which is important for the practical applications of the chemosensors.

Subsequently, the time-dependent changes of fluorescence intensity of **BIT-3** to Zn^{2+} were recorded and presented in Fig. 14. The fluorescence intensity of **BIT-3** at 404 nm was increased rapidly within 25 s upon addition of 20 μM Zn^{2+} ions to 20 μM **BIT-3** solutions, and even if the reaction time was extended to 180 s, the fluorescence intensity were substantially unchanged. It can conclude that **BIT-3** exhibits fast recognition to Zn^{2+} ions in aqueous solutions.

4. Conclusion

We have introduced a benzo[*d*]imidazo[2,1-*b*]thiazole-based fluorescent probe **BIT-3** as a novel chemosensor for the rapid detection of Zn^{2+} ions. The chemosensor **BIT-3** was synthesized from 2-aminobenzothiazole and an appropriate 2-bromoacetophenone. Job's plot disclosed a 1:1 stoichiometry between **BIT-3** and Zn^{2+}. The association constant was evaluated as 6.8×10^9 M^{-1}. **BIT-3** displayed high selectivity toward Zn^{2+} ions over other interfering ions with over 12-fold fluorescence enhancement, as well as high sensitivity with the detection limit of 0.03 μM. The recovery studies of the water and biological samples demonstrated the value of **BIT-3** in the practical application as a sensitive and selective probe for Zn^{2+} ion.

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.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jphotochem.2019.

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