

Fluorescent Sensing of both Fe(III) and pH Based on 4-Phenyl-2-(2-Pyridyl)Thiazole and Construction of OR Logic Function

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Abstract In the presented paper we investigated a 2-pyridylthiazole derivative, 4-phenyl-2-(2-pyridyl)thiazole (2-PTP), as the molecular fluorescent switches. It was firstly found that 2-PTP could perform a “turn-on” fluorescent sensing for Fe(III) with selectivity and reversibility. A 2:1 stoichiometry between 2-PTP and Fe(III) was determined according to the molar ratio method. The binding constant was evaluated as $(1.90 \pm 0.05) \times 10^5$ (L/mol)². The detection limit was found as 2.2×10^{-7} M ($S/N = 3$). Secondly, 2-PTP also exhibited a pH-dependent dual-emission. The $pK_a(2\text{-PTP-H}^+/2\text{-PTP})$ value was then estimated as 2.0. To explain the identical emission at 479 nm of both the Fe(III) coordinated form and the protonated form of the ligand, we proposed a “locked” conformation. Finally, combining the two external stimuli as inputs, an OR logic gate was constructed using the fluorescent emission at 479 nm as the output channel.

Keywords Fluorescence · Fe(III) · pH · 2-pyridylthiazole · OR logic gate

Introduction

Molecular fluorescent switches have been developing rapidly in recent because of their wide applications in chemosensors for various species of biological and/or environmental significance and in construction of logic gates in molecular level [1–4]. Great efforts have been devoted to the development of different fluorescent sensors based on many fluorophores [5, 6]. For example, being the most abundant transition metal species, iron plays important roles in various enzyme catalysis, cellular metabolism, and DNA, RNA synthesis [7–11]. Imbalance of iron could lead to cell damage and death [12, 13]. Detection of Fe(III) is thus of great importance and has been intensively pursued based on the fluorescent sensors with high selectivity [14]. Several rhodamine or BODIPY derivatives have been reported as chemosensors for Fe(III) based on the switch-on fluorescence [15–20], which is more appreciated due to the improved sensitivity compared to the quenching mechanism. However, the reported probes were generally involved in relatively complicated structures and time-consuming synthesis. In our opinion, it remains as a challenge to perform the turn-on sensing of Fe(III) with selectivity and reversibility on the platform based on a fluorophore with structural simplicity.

Pyridylthiazole unit (PT) has been applied in the development of various fluorescent probes [21–24]. Here we hope to report a PT derivative with structural simplicity, 4-phenyl-2-(2-pyridyl)thiazole (2-PTP), as a “turn-on” fluorescent sensor for Fe(III) with high selectivity and reversibility (Scheme 1). We also investigated the protonation effects on both absorption and emission of the titled compound. An OR logic gate was then constructed based on the fluorescent responses of 2-PTP to the two external stimuli, Fe(III) and protons.

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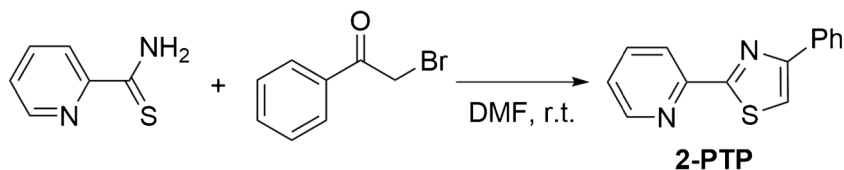
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Scheme 1 Synthesis of 4-phenyl-2-(2-pyridyl)-5-(2-thiophenyl)thiazole



Experimental

Materials and Methods

All chemicals were purchased from Acros and used as received without further purification. All reactions were performed under argon atmosphere using purified solvents by standard methods. For all spectrometric measurements, HPLC grade solvents were used. Flash chromatography was performed with 100–200 mesh silica gel (Qingdao, China) and thin-layer chromatography (TLC) was carried out on silica coated glass sheets (Qingdao silica gel 60 F-254).

UV-vis absorption spectra were measured with a Hitachi UV-3010 spectrophotometer and the fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. All spectra measurements were recorded at 20 °C. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and uncorrected. ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra were recorded with a Burkert AV300 (300 MHz) instrument using tetramethylsilane as the internal standard. IR spectra were recorded on a Perkin-Elmer 1200 FT-IR spectrometer. Elemental analysis were performed at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, China.

Synthesis of 4-Phenyl-2-(2-Pyridyl)Thiazole (2-PTP) [25]

To 25 mL N,N-dimethyl formamide (DMF) containing α -bromo acetophenone (1.1 g, 5.5 mmol), 2-pyridinecarbothioamide (760 mg, 5.5 mmol) was added. After kept stirring for 24 h, the reaction mixture was diluted

with 200 mL EtOAc followed by washing with brine. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Separation through flash column chromatography using a mixture of *n*-hexane and EtOAc as eluents provided a white solid as the product.

Yield: 81.0 %. Mp: 72–73 °C. IR (film, cm^{-1}): 3111, 3057, 1576, 1499, 1462, 1461, 1273, 1240, 1065, 993, 781, 740, 683. ^1H NMR (CDCl_3 , 300 MHz, ppm) δ = 7.37–7.39 (m, 2H), 7.46–7.51 (m, 2H), 7.63 (s, 1H), 7.83–7.88 (m, 1H), 8.03 (d, 2H, J = 7.0 Hz), 8.36 (d, 1H, J = 7.8 Hz), 8.58 (d, 1H, J = 4.6 Hz). ^{13}C NMR (CDCl_3 , 75 MHz, ppm) δ = 115.3, 119.9, 124.5, 126.4, 128.2, 128.8, 134.5, 137.1, 149.5, 151.5, 156.7, 168.8. Anal. Calcd. (%) for $\text{C}_{14}\text{H}_{10}\text{N}_2\text{S}$: C 70.56, H 4.23, N 11.76. Found: C 70.48, H 4.31, N 12.01.

Results and Discussion

As shown in Scheme 1, preparation of 2-PTP was very simple and the starting materials could be obtained commercially with cheapness. Although the titled compound had been reported about 60 years ago [26], the application in chemosensor remains unknown. Considering that the PT unit is a structural analogous to 2,2'-bipyridyl, its possible chelation to metal ions could be expected. We then examined experimentally the effects of various metal ions (Li(I), Na(I), K(I), Ag(I), Mg(II), Ca(II), Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), Pb(II), and Fe(III)) on both absorbance and emission of the ligand.

Figure 1 depicted the changes of UV-Vis absorption of 2-PTP after addition of 5 eq. metal ions, which could be divided

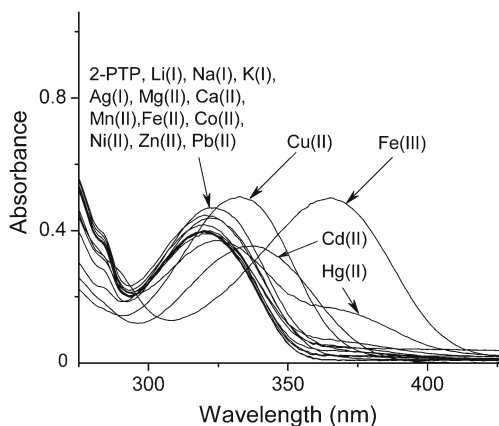


Fig. 1 UV-Vis absorption of 2-PTP (10 μM in MeCN) with the presence of various screened metal ions (10 eq.) at 25 °C

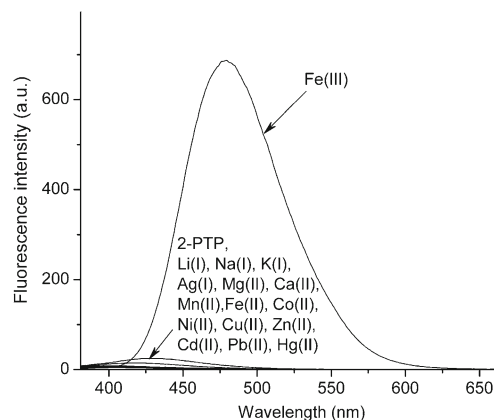


Fig. 2 Fluorescence spectra of 2-PTP (1 μM in MeCN, excited at 365 nm) with the presence of various screened metal ions (10 eq.) at 25 °C

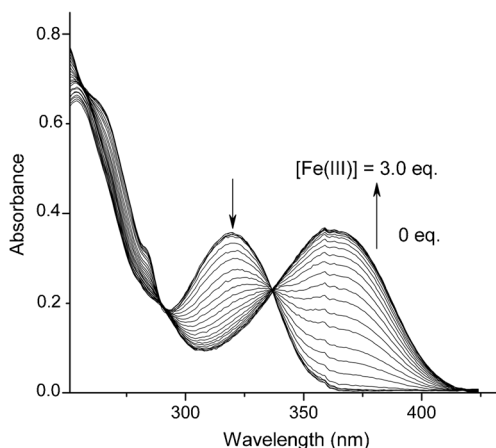


Fig. 3 UV-Vis absorption of 2-PTP (10 μ M in MeCN) upon continuous addition of 0–3 eq. of Fe(III) at 25 $^{\circ}$ C

to three classes. Only Fe(III) could result in a significant red-shift of the absorption maximum from 320 nm to 365 nm while the three ions (Cu(II), Cd(II), and Hg(II)) led to the bathochromic shift to some extent, respectively. In the other hand, the other metal ions had little effects on the absorption of 2-PTP. We further investigated the fluorescent responses of the ligand to the metal ions in screen. In the experimental conditions ([2-PTP] = 1 μ M in MeCN, excited at 365 nm), the free ligand exhibited a weak emission. Only addition of Fe(III) could significantly enhance the emission intensity at 479 nm whereas other metal ions had little effects on the emission (Fig. 2). According to the observed selectivity and the change of fluorescence, 2-PTP should be suggested as a “turn-on” fluorescent sensor for Fe(III) with selectivity.

We described the enhanced fluorescent emission at 479 nm to the coordinative complex of 2-PTP to Fe(III). Formation of the stable Fe(III) complex could be judged from UV-Vis titration experiment (Fig. 3), where it could be found that a gradual decrease of absorbance at 320 nm of 2-PTP with addition of Fe(III), accompanied with a gradual increase at 365 nm. After

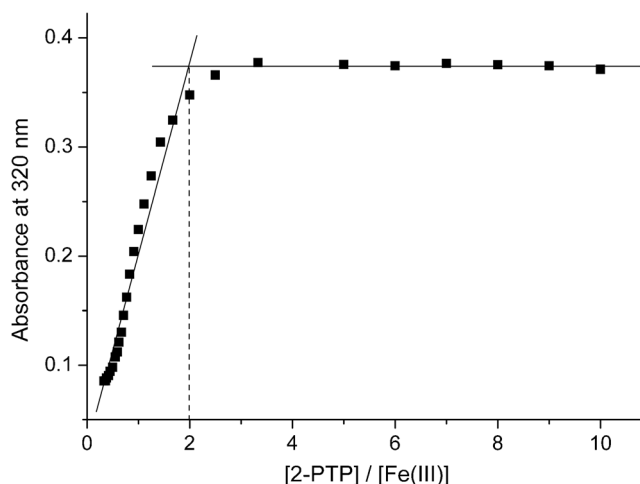


Fig. 4 Mole ratio method for determination of the binding stoichiometry of 2-PTP with Fe(III) obtained from variations in absorbance at 320 nm

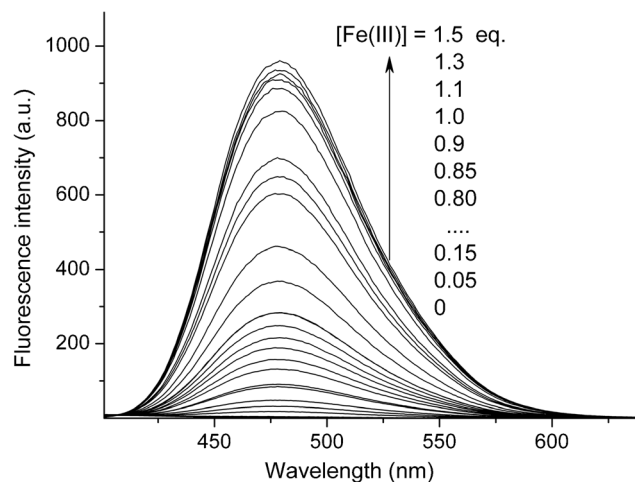


Fig. 5 Fluorescence spectra of 2-PTP (1 μ M) in the presence of different concentrations of Fe(III) in MeCN at 25 $^{\circ}$ C

addition of 1.5 eq. Fe(III), the spectral difference could be neglected. The clear and fixed isobestic point at 343 nm in the evolution of UV-Vis absorption indicated the presence of a coordination equilibrium between the ligand and Fe(III). The stoichiometry between Fe(III) and 2-PTP was then determined as 1:2 with mole ratio method (Fig. 4).

To evaluate the binding constant K_{as} , $[(F - F_{min}) / (F_{max} - F_{min})]$ was plotted against $\log[\text{Fe(III)}]$ according to the fluorescence titration experiment (Fig. 5) [24], where F_{min} and F_{max} are the fluorescence intensity at 479 nm in absence of Fe(III) and in presence of the saturated concentration of Fe(III) (1.5 eq.), respectively. Here the fraction, $(F - F_{min}) / (F_{max} - F_{min})$, is named after α , which could be regarded as the representative of the formed Fe(III) complex in the system. The binding constant K_{as} could be rewritten as below (Eq. 1).

$$K_{as} = [\text{Fe(III)} - \text{L}_2] / [\text{L}]^2 [\text{Fe(III)}] = \alpha / (1 - \alpha/2)^2 [\text{Fe(III)}] \quad (1)$$

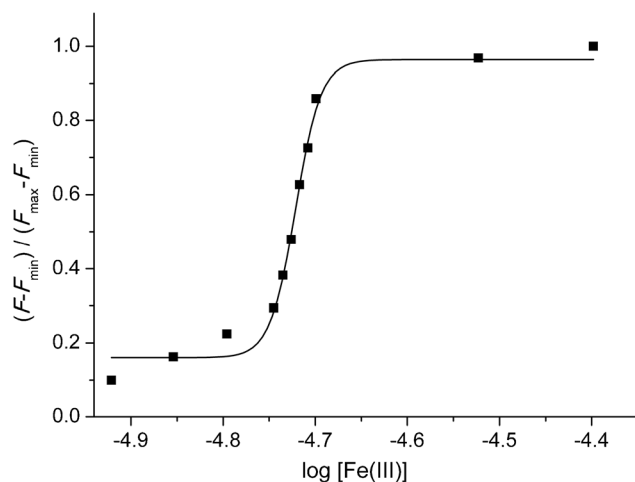


Fig. 6 Relative fluorescence ratio, α , as a function of $\log[\text{Fe(III)}]$ (M) in MeCN. The curve fitting for experimental data was calculated as mentioned in text (■, experimentally observed data points)

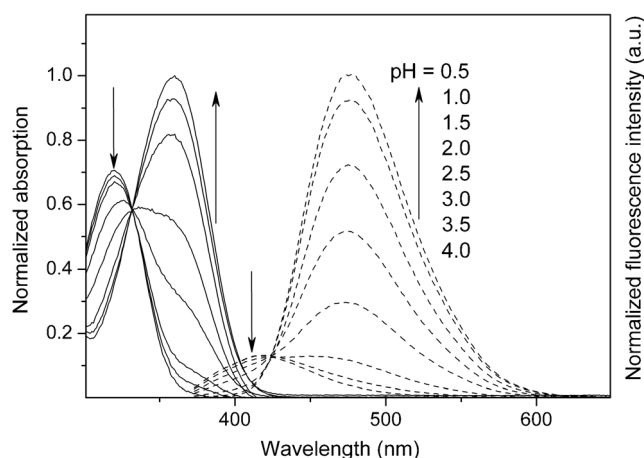


Fig. 7 Evolution of absorption and fluorescence of 2-PTP excited at 350 nm with pH in MeCN:H₂O = 1:9 at 25 °C. Solid line: absorption; dashed line: fluorescence

According to the 1:2 stoichiometry and Eq. 1, K_{as} was fitted as $(1.90 \pm 0.05) \times 10^5$ (L/mol)² with $R^2 = 0.988$ (Fig. 6) [27, 28]. Accordingly, the detection limit was found as 2.2×10^{-7} M ($S/N = 3$) [29]. We also noticed that the Fe(III) complex could be destroyed by addition of excess of EDTA, and the emission at 479 nm was thus quenched as before used.

We also investigated the effects of protonation on both absorption and emission of 2-PTP. When pH was higher than 4.0, both spectra have little changes compared to the free ligand. With decrease of pH from 4.0 to 0.5, the absorption maxima was red-shifted from 320 nm to 355 nm while a new emission at 479 nm appeared (Fig. 7). A clear and fixed isosbestic point as observed in pH-dependent evolution of both spectra indicated the presence of protonation equilibrium of 2-PTP. The red-shifted spectra were then reasonably ascribed to the protonated form of 2-PTP (2-PTP-H⁺). The $pK_a(2\text{-PTP-H}^+/2\text{-PTP})$ value was then estimated as 2.0 from the UV-Vis absorption titration experiment (Fig. 7).

It was a interesting finding that both Fe(III) coordinated form and the protonated form of 2-PTP had the same emission. As proposed in our previous reports of 2-PT units as pH sensors [22], we believed that a possible hydrogen bond should result in the proton-locked conformation of 2-PTP-H⁺. Accordingly, a chelation of the ligand to Fe(III) should induce the similar form, which may be the reason that both forms had the identical emission properties (Fig. 8).

Combining the turn-on fluorescent responses of 2-PTP to the two external stimuli (Fe(III) and protons), a two-input OR

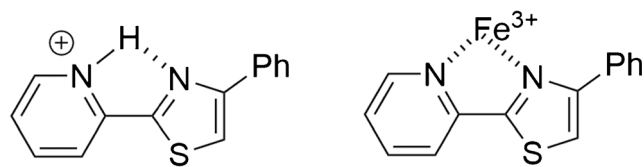
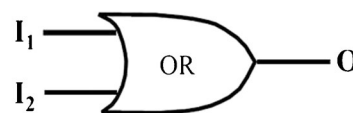


Fig. 8 Proposed locked conformations of 2-PTP after coordination to Fe(III) and protonation



I ₁ 2.0 eq Fe(III)	I ₂ 0.1 M TFA	O FL at 479 nm
0	0	0 (0.822)
1	0	1 (960.7)
0	1	1 (405.9)
1	1	1 (958.1)

Fig. 9 The two-input OR logic gate and its truth table. Addition of 2 eq. of Fe(III) and 0.1 M TFA was set as the two external inputs, respectively, while the emission at 479 nm of 2-PTP was monitored as the output channel. All operations were carried out in MeCN ([L] = 1 μM, excited at 365 nm) at 25 °C

logic gate was then constructed regarding the emission at 479 nm as the output. Either or both addition of the two species could trigger on the fluorescence. Missing either of the two inputs would make the output silent (Fig. 9). The changes induced by the input signals, 2.0 eq Fe(III) and 0.1 M trifluoroacetic acid (TFA), could be compensated by introduction of EDTA and triethylamine as the corresponding reset operations, respectively.

Conclusions

In summary, we investigated the possible applications of 2-PTP as fluorescent sensors in detail. Both introduction of Fe(III) and protons could red-shift the emission maxima to 479 nm. The turn-on fluorescent sensing for Fe(III) was firstly explored. According to the experimental, a stoichiometry of 1:2 was determined and the binding constant was fitted as $(1.90 \pm 0.05) \times 10^5$ (L/mol)² with $R^2 = 0.988$. The $pK_a(2\text{-PTP-H}^+/2\text{-PTP})$ value was estimated as 2.0. An OR logic function was then fulfilled based on the fluorescent responses at 479 nm to Fe(III) and protons. A possible mechanism was also suggested to explain the appearance of the similar bathochromic emission based on the locked conformation of the ligand.

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References

- de Silva AP, Gunaratne HQN, Gunnaugsson T, Huxley AJM, McCoy CP, Radmacher JT, Rice TE (1997) Signaling recognition events with fluorescent sensors and switches. *Chem Rev* 97:1515–1566

2. Valuer B (2002) Molecular fluorescence: principles and applications. Wiley-VCH, Weinheim
3. Feringa BL (2001) Molecular switches. Wiley-VCH, Weinheim
4. Szacilowski K (2008) Digital information processing in molecular systems. *Chem Rev* 108:3481–3548
5. Gonçalves MST (2009) Fluorescent labeling of biomolecules with organic probes. *Chem Rev* 109:190–212
6. Terai T, Nagano T (2013) Small-molecule fluorophores and fluorescent probes for bioimaging. *Pflugers Arch - Eur J Physiol* 465: 347–359
7. Dai S, Schwendtmayer C, Schürmann P, Ramaswamy S, Eklund H (2000) Redox signaling in chloroplasts: cleavage of disulfides by an iron-sulfur cluster. *Science* 287:655–658
8. Beutler E (2004) “pumping” iron: the proteins. *Science* 306:2051–2053
9. Kaplan CD, Kaplan J (2009) Iron acquisition and transcriptional regulation. *Chem Rev* 109:4536–4552
10. Theil EC, Goss DJ (2009) Living with iron (and oxygen): questions and answers about iron homeostasis. *Chem Rev* 109:4568–4579
11. Atkinson A, Winge DR (2009) Metal acquisition and availability in the mitochondria. *Chem Rev* 109:4708–4721
12. Levi S, Rovida E (2009) The role of iron in mitochondrial function. *Biochim Biophys Acta* 1790:629–636
13. Cabantchik ZI (2014) Labile iron in cells and body fluids. Physiology, pathology and pharmacology. *Front Pharmacol* 5: 45
14. Sahoo SK, Sharma D, Bera RK, Crisponi G, Callan JF (2012) Iron(III) selective molecular and supramolecular fluorescent probes. *Chem Soc Rev* 41:7195–7227
15. Wang B, Hai J, Liu Z, Wang Q, Yang Z, Sun S (2010) Selective detection of iron(III) by rhodamine-modified Fe₃O₄ nanoparticles. *Angew Chem Int Ed* 49:4576–4579
16. Lee MH, Giap TV, Kim SH, Lee YH, Kang C, Kim JS (2010) A novel strategy to selectively detect Fe(III) in aqueous media driven by hydrolysis of a rhodamine 6G Schiff base. *Chem Commun* 46: 1407–1409
17. Yang Z, She M, Yin B, Cui J, Zhang Y, Sun W, Li J, Shi Z (2011) Three rhodamine-based “off-on” chemosensors with high selectivity and sensitivity for Fe³⁺ imaging in living cells. *J Organomet Chem* 77:1143–1147
18. Chen W-D, Gong W-T, Ye Z-Q, Lin Y, Ning G-L (2013) FRET-based ratiometric fluorescent probes for selective Fe³⁺ sensing and their applications in mitochondria. *Dalton Trans* 42:10093–10096
19. Sui B, Tang S, Liu T, Kim B, Belfield KD (2014) Novel BODIPY-based fluorescence turn-on sensor for Fe³⁺ and its bioimaging application in living cells. *ACS Appl Mater Interfaces* 6:18408–18412
20. Li G, Tang J, Ding P, Ye Y (2015) A rohdamine-benzimidazole based chemosensor for Fe³⁺ and its application in living cells. *J Fluoresc*. doi:10.1007/s10895-0151696-9
21. Zheng M-H, Jin J-Y, Sun W, Yan C-H (2006) A new series of fluorescent 5-methoxy-2-pyridylthiazoles with a pH-sensitive dual-emission. *New J Chem* 30:1196–1196
22. Zheng M-H, Zhang M-M, Li H-H, Jin J-Y (2012) Digital pH fluorescent sensing shown by small organic molecules. *J Fluoresc* 22: 1421–1424
23. Zheng M-H, Sun W, Jin J-Y, Yan C-H (2014) Molecular keypad locks based on gated photochromism and enhanced fluorescence by protonation effects. *J Fluoresc* 14:1169–1176
24. Zheng M-H, Hu X, Yang M-Y, Jin J-Y (2015) Ratiometrically fluorescent sensing of Zn(II) based on dual-emission of 2-pyridylthiazole derivatives. *J Fluoresc* 25:1831–1834
25. Khoroshilov GE, Yarotskii YV, Brovarets VS, Chernega AN (2010) Synthesis and structure of N-(aroylmethyl)-2-(4-aryl-2-thiazolyl)pyridinium bromides. *Zh Org Farm Khim* 8:57–60
26. Knott RF, Breckenridge JG (1954) Analogues of 2,2'-bipyridyl with isoquinoline and thiazole rings. Part I. *Can J Chem* 32:512–522
27. Yang R, Li K, Wang K, Zhao F, Li N, Liu F (2003) Porphyrin assembly on β -cyclodextrin for selective sensing and detection of a zinc ion based on the dual emission fluorescence ratio. *Anal Chem* 75:612–621
28. Du J, Fan J, Peng X, Li H, Sun S (2010) The quinoline derivative of ratiometric and sensitive fluorescent zinc probe based on deprotonation. *Sensors Actuators B* 144:337–341
29. Shortreed M, Kopelman R, Kuhn M, Hoyland B (1997) Fluorescent fiber-optic calcium sensor for physiological measurements. *Anal Chem* 68:1414–1418