

HETEROCYCLES, Vol. 94, No. 9, 2017, pp. 1719 - 1727. © 2017 The Japan Institute of Heterocyclic Chemistry
Received, 6th May, 2015, Accepted, 30th June, 2017, Published online, 14th July, 2017
DOI: 10.3987/COM-15-13242

A NEW FLUORESCENCE CHEMOSENSOR FOR Hg²⁺ BASED ON CARBAZOLE AND THIOUREA

Fang-fang Yin, Wei-ju Zhu, Min Fang, Zhen-yu Wu, Ying Xu, Hai-lun Wang, Yao Wang, and Cun Li*

School of Chemistry and Chemical Engineering & Anhui Province Key Laboratory of Environment-friendly Polymer Materials, Anhui University, Hefei 230601, China. Email: cun_li@126.com

Abstract – A new thiourea-based Schiff-base (**L**) as a fluorescent chemosensor has been designed and synthesized, which exhibits selectivity toward Hg²⁺ by forming a 1:2 compound based on the method of Job's plot. The detection limit was calculated to be 6.5×10⁻⁶ mol/L. The prominent recognizable changes of **L** could be observed by the naked eyes and fluorescence sensing conditions. The color of the **L** solution changed instantly from colorless to yellow after adding Hg²⁺ and returned to colorless by adding I⁻. The coordination reaction was reversible.

Along with the rapid development of the economy, hazardous contaminants have earned closed attention due to their harmful effects on human health and the environment.¹ In order to detect different kinds of ions, many kinds of chemical chemosensors have been reported,² such as Zn²⁺,³ Cu²⁺,⁴ Fe³⁺,⁵ Cd²⁺,⁶ Al³⁺,⁷ Cr³⁺,⁸ Hg²⁺,⁹ Pb²⁺¹⁰ and Ag⁺.¹¹

Mercury as one of hazardous contaminants has attracted extensive attention. Due to their special chemical, physical properties, they are widely used in chemical and petroleum chemical industry, pharmaceutical, pulp papermaking, electrical and electronic instrumentation, and other industrial sectors. However, it is harmful to our health. Mercury can gradually accumulate in our brain tissue, and it will damage the brain when reaching a certain amount.¹² And various techniques are available for the determination of Hg²⁺ such as atomic spectroscopy, inductively coupled plasma mass spectroscopy and so forth.¹³

However, most of these methods are restricted to complex instruments and procedures, high cost, long reaction time, low sensitivity and selectivity. For these reasons, colorimetric methods, in particular, are extremely attractive because they allow naked-eye detection of the color change without using any spectroscopic instruments.

Carbazole group with heterocyclic structure containing a nitrogen atom, a condensed ring with a special rigid structure is a good fluorescence group. It contains the larger conjugated system and strong intramolecular electron transfer. It also has a strong electron-donating ability and hole transmission capacity.¹⁴ Thiourea derivatives contain strong complexing ability of nitrogen, sulfur and other coordinating atoms. Coordination compounds may be formed through sulfur atom and nitrogen atom. Thiourea derivatives not only have an electron donor, they also have a group that can form hydrogen bonds by imine.¹⁵ Thiourea derivatives were widely used in the field of molecular recognition, such as Hg^{2+} ,¹⁶ Ni^{2+} ,¹⁷ and so on.¹⁸ Here we synthesized a kind of Schiff base (**L**), which consisted of carbazole and thiourea. The fluorescent chemosensor **L** has high selectivity and sensitivity for detecting Hg^{2+} ions. It differs from the fluorogenic chemodosimeter for Hg^{2+} based on the dimerization of desulfurized product,¹⁹ or fluorogenic chemodosimeter directly desulfurized and generated a ring.²⁰ The complexing of **L**- Hg^{2+} formed two stable rings of 5-membered ring.

UV–Vis absorption spectral studies

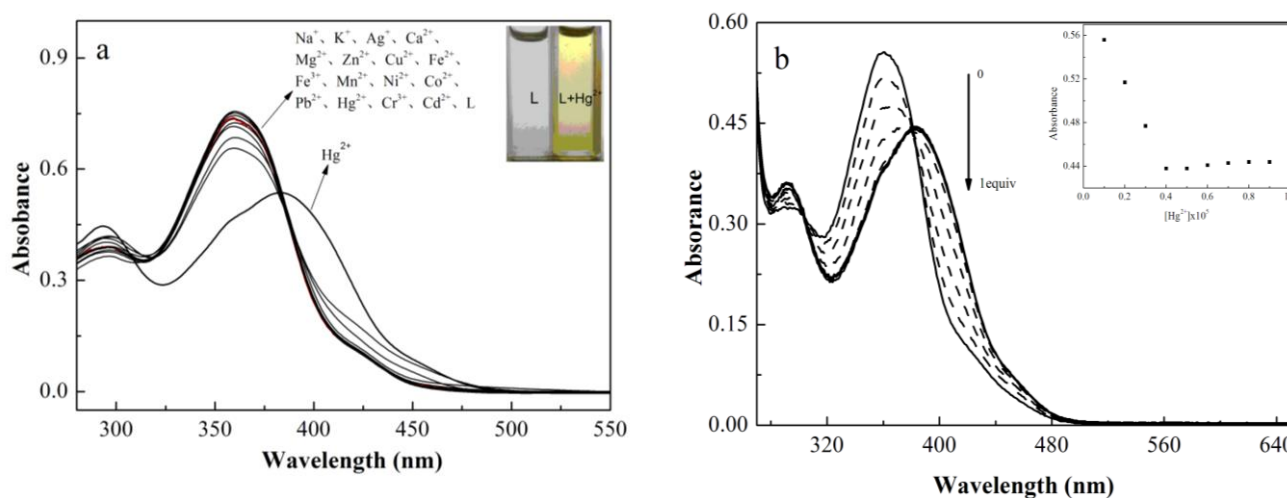


Figure 1. (a) UV–Vis absorption spectra of **L** (10.0 μM) upon addition of various metal ions (10.0 μM) in DMF solution, and the changes of absorbance peak (inset: photograph of **L** and **L**+ Hg^{2+}), (b) UV–Vis absorption spectra of **L** (10 μM) with gradual addition of Hg^{2+} at 359 nm (inset: changes of absorbance upon addition of Hg^{2+} at 359 nm)

The interaction between **L** and cations was investigated by measuring UV–Vis absorption spectroscopy with different metal ions in DMF. Different metal ions were added to the solution of **L** (1×10^{-5} M). As shown in **Figure 1(a)**, the addition of 1 equiv. Hg^{2+} resulted in an obvious change, where the peak of compound **L** at 290 nm increased and the peak at 359 nm decreased with a red shift of about 26 nm (from 359 nm to 385 nm). The result caused the change of solution color from colorless to yellow (inset of **Figure 1(a)**). And under the same condition, other metal ions (Na^+ , Ag^+ , Ca^{2+} , Mg^{2+} , Zn^{2+} , Cu^{2+} , Fe^{2+} ,

Fe^{3+} , K^+ , Cd^{2+} , Cr^{3+} , Co^{2+} , Mn^{2+} , Pb^{2+} and Ni^{2+}) had no obvious response. The peak at 290 nm reflected a π - π^* transition of the C=N group while the peak at 359 nm reflected an intermolecular charge transfer (ICT) band of the entire conjugated molecule. The phenomenon of a red shift suggested that the complex of L-Hg^{2+} was formed in DMF solution.²¹

The change of absorbance in the UV-Vis spectra of **L** as a function of the concentration of Hg^{2+} was shown in **Figure 1(b)**. Upon addition of Hg^{2+} ions to the solution of **L**, the absorption peaks at 359 nm gradually decreased and bathochromic shifted to 384 nm. This indicates the formation of a L-Hg^{2+} complex. When the proportion of $\text{C}(\text{Hg}^{2+})$ and $\text{C}(\text{L})$ was greater than 0.5, the fluorescence intensity was almost a constant, suggesting a complex of $\text{Hg}^{2+}\text{-L}(1:2)$ was formed. These results indicate that **L** could be used in potential candidate of colorimetric chemosensor for Hg^{2+} with good selectivities.

The fluorescence spectral studies

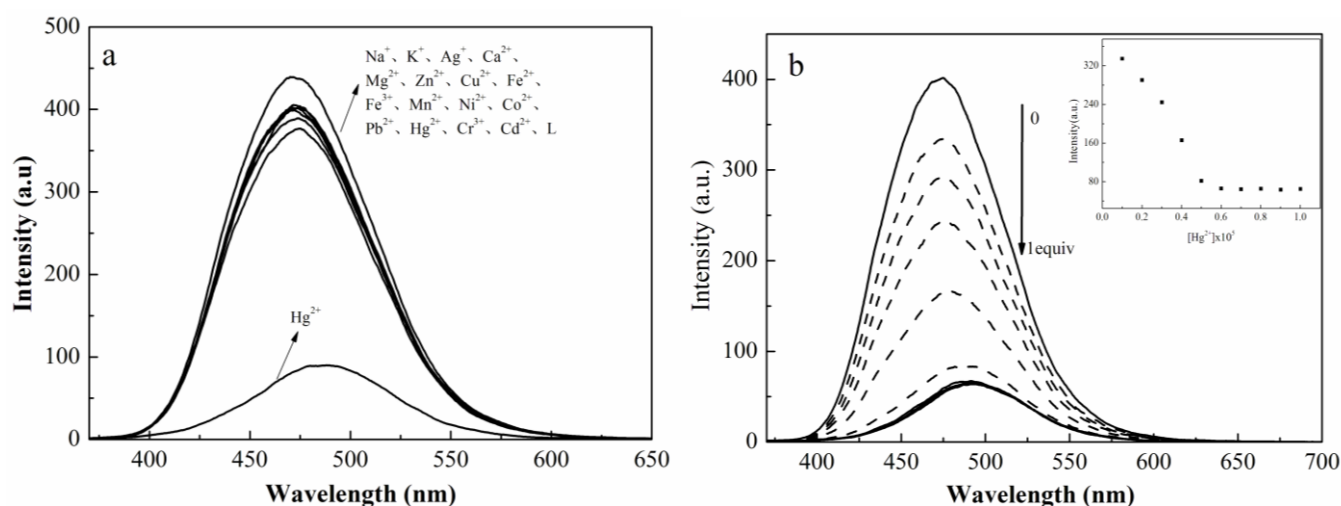


Figure 2. (a) Fluorescence responses of compound **L** (10.0 μM) upon addition of various metal ions (10.0 μM) in DMF solution ($\lambda_{\text{ex}} = 365$ nm), (b) Fluorescence responses of compound **L** (10 μM) with gradual addition of Hg^{2+} (inset: changes of fluorescence upon addition of Hg^{2+} at 480 nm) ($\lambda_{\text{ex}} = 365$ nm)

To explore the fluorescence behavior of **L**, we investigated it with various metal ions in DMF. As **Figure 2(a)** shows, with the addition of other ions into the solution, the fluorescence intensity has no obvious increase or decrease. But only the addition of Hg^{2+} , the emission peak at 480 nm decreased significantly with red shift from 480 nm to 490 nm.²² This phenomenon might be caused by the complexation of Hg^{2+} . Excitation by light ($\lambda = 365$ nm), there was an ICT from the electron-rich group (carbazole) to the electron-withdrawing group (thiourea) before addition of Hg^{2+} , resulting in a strong fluorescence. After addition of Hg^{2+} , the above-mentioned ICT was suppressed.²³ To get more information on binding mechanism of compound **L** toward Hg^{2+} , spectrometric titration experiment was performed in the

presence of Hg^{2+} . The titration results of fluorescence spectrum of **L** and Hg^{2+} were shown in **Figure 2(b)**. With the addition of Hg^{2+} , gradually decrease of the fluorescence intensity could be distinguished visually at 480 nm. However, when the proportion of $\text{C}(\text{Hg}^{2+})$ and $\text{C}(\text{L})$ was greater than 0.5, the fluorescence intensity basically remained unchanged, suggesting a complex of Hg^{2+} and **L** (1:2) was formed. The association constant of Hg^{2+} ions with **L** which determined by the Benesi-Hildebrand plot (**Figure S1**) came as a result of 2.8×10^4 .²⁴

To get more information on binding mechanism of compound **L** and Hg^{2+} , the sensitivity was assessed by a Stern–Volmer plot (**Figure S2**). A Stern–Volmer plot exhibits linear relationship ($R^2 = 0.98$), suggesting the limited detection is 6.5×10^{-6} mol/L. To further determinate the stoichiometry between compound **L** and Hg^{2+} , Job's plot (**Figure S3**) analyses were used. The plot of fluorescence intensity vs molar fraction of **L** shows a turning point at 0.7, which was indicative of a 2:1 stoichiometry complexation between **L** and Hg^{2+} .

The competitive binding studies

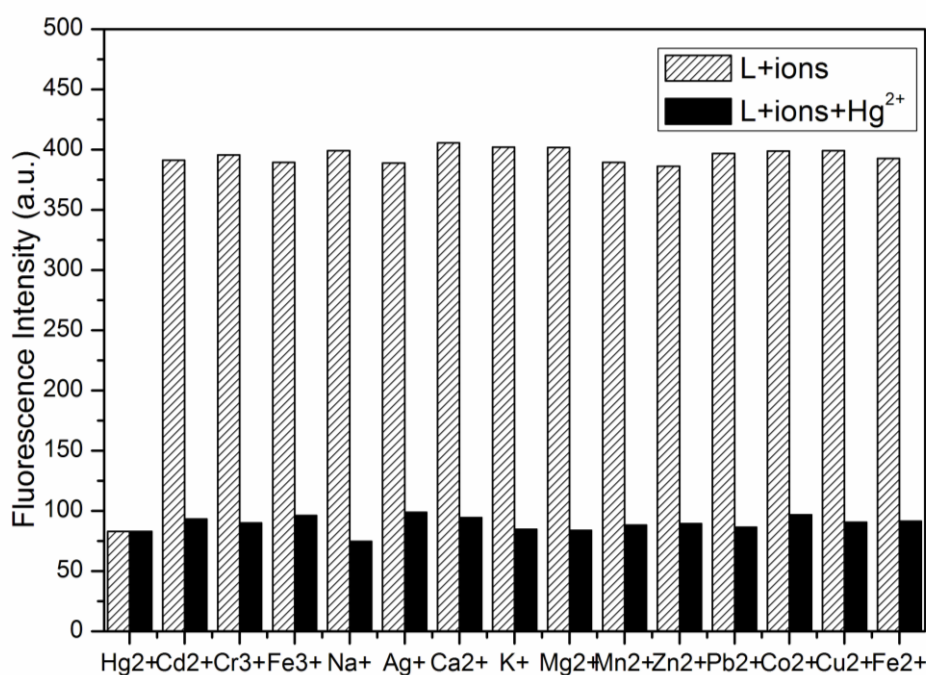


Figure 3. Bar graph shows the relative emission intensity of **L** at 359 nm upon treatment with various metal ions ($\lambda_{\text{ex}} = 365$ nm)

Whether the coexisting ions will influence the recognition of Hg^{2+} by the fluorescence probe is one of the important properties. As shown in **Figure 3**, the high fluorescence intensity was observed in the presence of other metal ions, while weak fluorescence was observed in the presence of Hg^{2+} . The miscellaneous competition of cations did not induce significant fluorescence intensity changes of compound **L** in the

absence of Hg^{2+} . This phenomena indicates that fluorescence quenching effect has less influence with the presence of coexisting ions, and the recognition of Hg^{2+} ions has strong anti-interference abilities.

The binding mode of L-Hg^{2+}

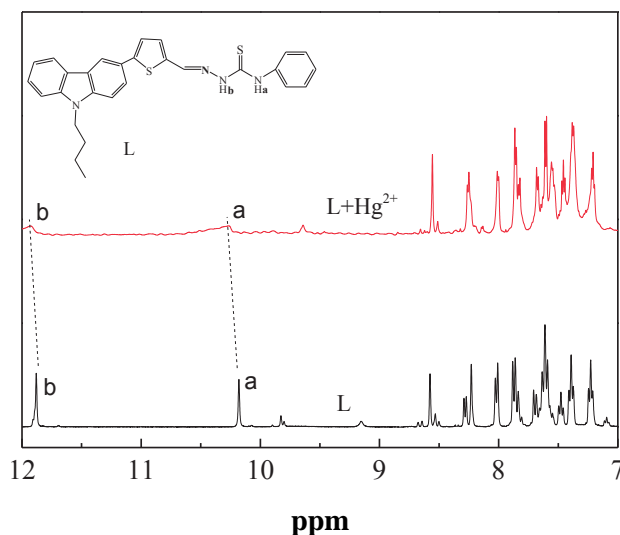


Figure 4. ^1H NMR spectra of **L** (black) with $\text{Hg}(\text{NO}_3)_2$ (red) in $\text{DMSO-}d_6$

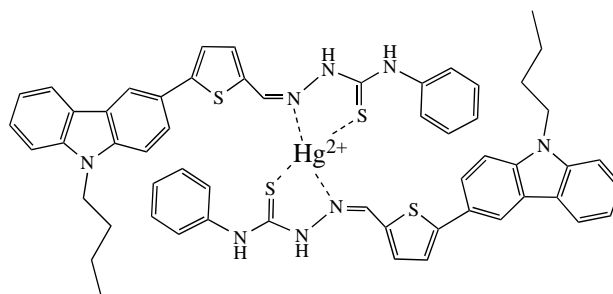


Figure 5. Propose the structure of a 1:2 complex of Hg^{2+} and **L**

To examine the binding mode of L-Hg^{2+} , the ^1H NMR experiments of **L** with Hg^{2+} ion were studied in $\text{DMSO-}d_6$ (**Figure 4**). Upon the addition of 1 equiv Hg^{2+} , the intensity of the protons of $-\text{NH}_2$ in thiourea group H_a (s, 1H), and H_b (s, 1H) became weaker and the chemical shift increased by 0.06 and 0.15, respectively. And the chemical shifts of other hydrogen atoms did not change. It can be seen, N and S were the main ion binding sites, and other places did not attend the complexing, estimating the **L** and Hg^{2+} may in the following way form two stable rings of 5-membered ring as **Figure 5** shown.²⁵

The reversibility experiment of **L** was tested by addition of I^- to the mercury-sensor complex.²⁶ The I^- was well known to bind strongly to Hg^{2+} ions. As shown in **Figure 6**, **L** was colorless, turning yellow after the addition of Hg^{2+} , then yellow returned to colorless following by addition 10 equiv I^- . And the fluorescence of **L** recovered after addition 10 equiv I^- . It showed that the **L** with Hg^{2+} was reversible. As

a result, a fluorescence quenching was observed. The whole process was shown in **Scheme 1**.

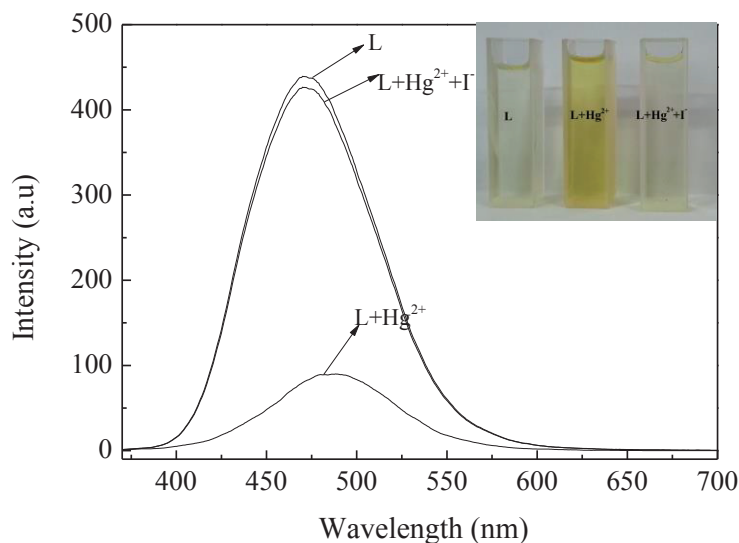
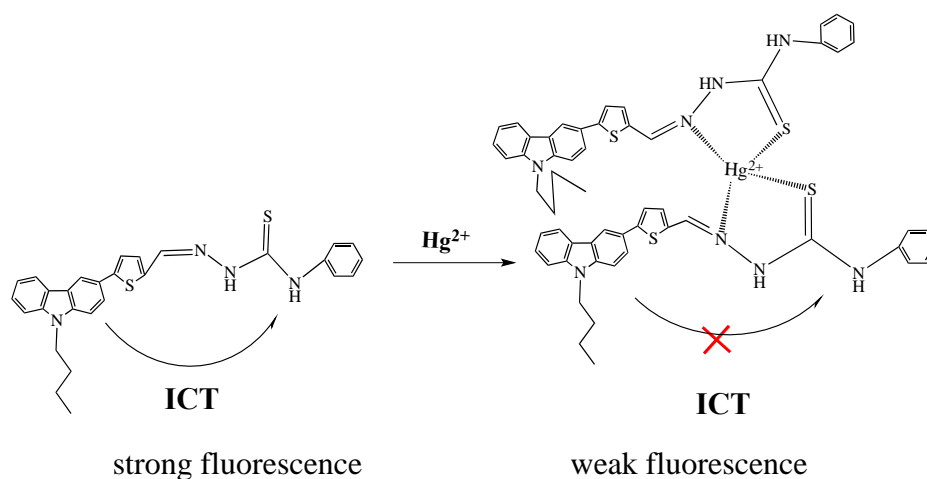


Figure 6. Fluorescence emission spectra of **L**, **L+Hg²⁺**, **L+Hg²⁺+I⁻** in DMF solution ($\lambda_{\text{ex}} = 365 \text{ nm}$) (inset: photographs of **L+Hg²⁺**, **L+Hg²⁺+I⁻**)



Scheme 1. The proposed binding mode of **L** with **Hg²⁺** and sensing mechanism

In conclusion, a carbazole-thiourea Schiff's base was synthesized, and it had better recognition effect to **Hg²⁺**. After addition of **Hg²⁺**, the UV-vis absorption spectroscopy had a red-shift from 358 to 385 nm. Also, the fluorescence intensity can be found from 414 to 454. The change of solution color was from colorless to yellow. While the rest of ions had no such phenomena. The carbazole-thiourea Schiff's-base can be used to develop fluorescent chemosensors for **Hg²⁺**.

EXPERIMENTAL

Materials

4-Phenylthiosemicarbazide was purchased as reagent-grade from Sinopharm Chemical Reagent Co.

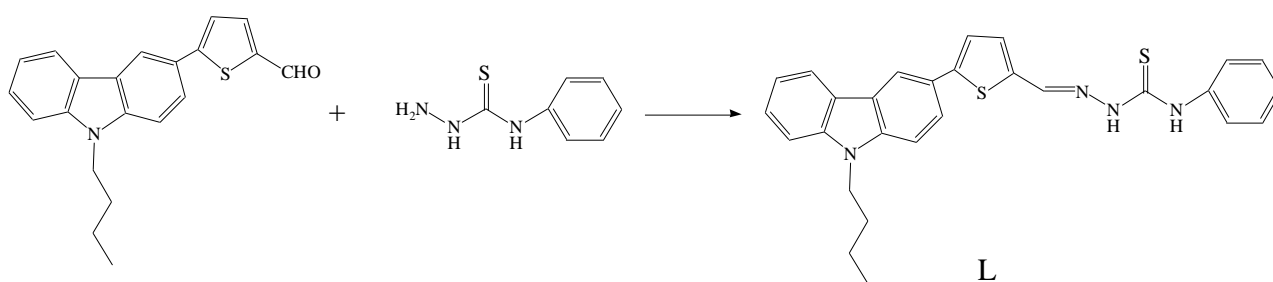
Ltd. (Shanghai, China) and used without further purification. 3-(2-Formyl)thienyl-9*H*-butylcarbazole was synthesized and characterized.²⁷ The stock solutions for compound **L** (1 mM) were prepared in HPLC grade DMF. DMF was HPLC grade without impurities. The solutions of metal ions were prepared from NO₃⁻ salts except for FeSO₄ and CdCl₂. Stock solutions of metal salts were prepared in distilled water. Double distilled water was used for spectral detection.

Characterization

The FT-IR spectra were recorded with KBr pellets on a 170 sx spectrometer (Nicolet, USA). ¹H NMR spectra were obtained on an AV-400 spectrometer (Bruker, Switzerland). Chemical shifts (δ) were given in ppm relative to DMSO-*d*₆. All spectral characterizations were carried out in HPLC-grade solvents at 20 °C within a 10 mm quartz cell. UV-Visible absorption spectra were recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). Fluorescence emission spectra were recorded on a LS-55 FL spectrophotometer (Perkin Elmer, USA). Elemental analysis was determined on a Vario EL III spectrometer (Elementar, Germany). MALDI-TOF MS was determined on Voyager DE-STR MALDI-TOF (Applied Biosystems, USA).

Synthesis of the Schiff base (**L**)

As shown in **Scheme 2**, the mixture of 4-phenylthiosemicarbazide (0.17 g, 1 mmol) and 3-(2-formyl)-thienyl-9*H*-butylcarbazole (0.34 g, 1.2 mmol) in EtOH (2 mL) were stirred at room temperature for 2 h during which a pale yellow solid was obtained. The solid compound was filtered, washed, and recrystallized from EtOH. The yield was 85.46%.



Scheme 2. The synthetic route of **L**

Compound **L**

FT-IR (KBr, cm⁻¹) (**Figure S4**): 3448 (-NH), 2954 (-C-H), 1595 (-C=N), 1546 (phenyl), 1267 (-Me), 868 (Ar-H). ¹H-NMR (DMSO-*d*₆, 400 Hz) (**Figure S5**): δ_H 11.88 (s, 1H), 10.18 (s, 1H), 8.58 (s, 1H), 8.23 (s, 1H), 8.02 (d, *J* = 8.04 Hz, 1H), 7.86 (m, 2H), 7.68 (d, *J* = 8.80 Hz, 1H), 7.64~7.57 (m, 4H), 7.48 (t, *J* = 8.04 Hz, 1H), 7.39 (d, *J* = 8.00 Hz, 2H), 7.23 (t, *J* = 7.60 Hz, 2H), 4.30 (t, *J* = 7.20 Hz, 2H), 1.92~1.85 (m, 2H), 1.41~1.38 (m, 2H), 0.87 (t, *J* = 7.30 Hz, 3H). Anal. Calcd for C₂₈H₂₆N₄S₂: C 69.68, H 5.43, N 11.61,

S 13.29. Found: C 69.59, H 5.51, N 11.47, S 13.23. MS (**Figure S6**), m/z : calculated for $[M + H]^+$ 482.6, found 481.0.

ACKNOWLEDGEMENTS

This research was financially supported by the Anhui Province University Natural Science Research Project (No. KJ2013A013), the Dr. Research Project Fund of Anhui University (No. 33190105, 33190184 and 33190213), Youth Scientific Research Found of Anhui University, the Excellent Youth Fund in University of Anhui Province (No. 2012SQRL025).

REFERENCES

1. P. Vrhovnik, J. P. Arrebola, T. Serafimovski, T. Dolenc, and N. R. Smuc, M. Dolenc, *E. Mutch. Environ. Pollut.*, 2013, **80**, 92.
2. K. P. Carter, A. M. Young, and A. E. Palmer, *Chem. Rev.*, 2014, **114**, 4564; X. Q. Chen, T. H. Pradhan, F. Wang, J. S. Kim, and J. Y. Yoon, *Chem. Rev.*, 2012, **112**, 1910.
3. R. Azadbakht and H. Keypour, *Spectrochim. Acta, Part A*, 2012, **85**, 293; Y. Zhou, Z. X. Li, S. Q. Zang, Y. Y. Zhu, H. Y. Zhang, H. W. Hou, and T. C. W. Mak, *Org. Lett.*, 2012, **14**, 1214; S. C. Yin, J. Zhang, H. K. Feng, Z. J. Zhao, L. W. Xu, H. Y. Xu, and B. Z. Tang, *Dyes Pigm.*, 2012, **95**, 174.
4. L. N. Wang, J. X. Yan, W. W. Qin, W. S. Liu, and R. Wang, *Dyes Pigm.*, 2012, **92**, 1083; H. Shoaee, M. Roshdi, and N. Khanlarzadeh, *Spectrochim. Acta, Part A*, 2012, **98**, 70; H. P. Zhou, J. Q. Wang, Y. X. Chen, W. G. Xi, Z. Zheng, D. L. Xu, Y. L. Cao, G. Liu, W. J. Zhu, J. Y. Wu, and Y. P. Tian, *Dyes Pigm.*, 2013, **98**, 1.
5. Z. Q. Li, Y. Zhou, K. Yin, Z. Yu, Y. Li, and J. Ren, *Dyes Pigm.*, 2014, **105**, 7; A. Y. Yuan, C. L. Zheng, Z. Y. Zhang, L. Yang, C. Liu, and H. B. Wang, *J. Fluoresc.*, 2014, **24**, 557.
6. Y. L. Cai, X. M. Meng, S. X. Wang, M. Z. Zhu, Z. W. Pan, and Q. X. Guo, *Tetrahedron Lett.*, 2013, **54**, 1125.
7. Z. Köstereli and K. Severin, *Org. Biomol. Chem.*, 2015, **13**, 252.
8. H. Y. Liu, X. J. Wan, T. Q. Liu, Y. Q. Li, and Y. W. Yao, *Sens. Actuators, B*, 2014, **200**, 191.
9. X. Zhou, W. Yan, T. Zhao, Z. X. Tian, and X. Wu, *Tetrahedron*, 2013, **69**, 9535; N. Vasimalai, G. Sheeba, and S. A. John, *J. Hazard. Mater.*, 2012, **213**, 193; J. Hatai, S. Pal, G. P. Jose, and S. Bandyopadhyay, *Inorg. Chem.*, 2012, **51**, 10129; H. Lee, H.-S. Lee, J. H. Reibenspies, and R. D. Hancock, *Inorg. Chem.*, 2012, **51**, 10904; C. Arivazhagan, R. Borthakur, and S. Ghosh, *Organometallics*, 2015, **34**, 1147; Y. Zhang, Y. Y. Yan, S. Y. Chen, Z. N. Gao, and H. Xu, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 5373.
10. F. Y. Wu, S. W. Bae, and J. I. Hong, *Tetrahedron Lett.*, 2006, **47**, 8851; G. Pina-Luis, M.

- Martínez-Quiroz, A. Ochoa-Terán, H. Santacruz-Ortega, and E. Mendez-Valenzuela, *J. Lumines.*, **2013**, **134**, 729; F. Zapata, A. Caballero, A. Espinosa, A. Tarraga, and P. Molina, *J. Org. Chem.*, **2009**, **74**, 4787.
11. C. Y. Li, X. F. Kong, Y. F. Li, C. X. Zou, D. Liu, and W. G. Zhu, *Dyes Pigm.*, **2013**, **99**, 903; M. Mirzaeia and J. Saeed, *Spectrochim. Acta, Part A*, **2011**, **82**, 351; N. J. Wang, C. M. Sun, and W. S. Chung, *Sens. Actuators, B*, **2012**, **26**, 984.
 12. X. M. Liu, Q. J. Song, Y. Tang, W. L. Li, J. M. Xu, J. J. Wu, F. Wang, and P. C. Brookes, *Sci. Total Environ.*, **2013**, **463**, 530; C. Copat, M. Vinceti, M. G. D'Agati, G. Arena, V. Mauceri, A. Grasso, R. Fallico, S. Sciacca, and M. Ferrante, *Ecotoxicol. Environ. Safe.*, **2014**, **100**, 87.
 13. R. Dulare, M. K. Bharty, S. K. Kushawaha, S. Singh, and N. K. Singh, *Polyhedron*, **2011**, **30**, 1960.
 14. C. N. Chuang, H. J. Chuang, Y. X. Wang, S. H. Chen, J. J. Huang, M. Leung, and K. H. Hsieh, *Polymer*, **2012**, **53**, 4983; W. J. Zhu, Z. Y. Wu, J. M. Song, and C. Li, *Chin. Chem. Lett.*, **2011**, **22**, 1017.
 15. L. L. Wang, B. Li, L. M. Zhang, and Y. S. Luo, *Dalton Trans.*, **2013**, **42**, 459.
 16. M. Ghanei-Motlagh, M. Fayazi, and M. A. Taher, *Sens. Actuators, B*, **2014**, **199**, 133.
 17. P. Bharati, A. Bharti, M. K. Bharty, B. Maiti, R. J. Butcher, and N. K. Singh, *Polyhedron*, **2013**, **63**, 156.
 18. M. Saleem, R. Abdullah, A. Ali, B. J. Park, E. H. Choi, I. S. Hong, and K. H. Lee, *Bioorg. Med. Chem.*, **2014**, **22**, 2045; H. J. Zhang, X. Qin, K. Liu, D. D. Zhu, X. M. Wang, and H. L. Zhu, *Bioorg. Med. Chem.*, **2011**, **19**, 5708.
 19. P. Zhang, B. B. Shi, Y. M. Zhang, Q. Lin, H. Yao, X. M. You, and T. B. Wei, *Tetrahedron*, **2013**, **69**, 10292.
 20. J. L. Liu, C. Y. Li, and F. Y. Li, *J. Mater. Chem.*, **2011**, **21**, 7175.
 21. D. Maity and T. Govindaraju, *Chem. Eur. J.*, **2011**, **17**, 5479.
 22. B. Leng, L. Zou, J. Jiang, and H. Tian, *Sens. Actuators, B*, **2009**, **140**, 162.
 23. H. J. Dai, F. Liu, Q. Q. Gao, T. H. Fu, and X. M. Kou, *Luminescence*, **2011**, **26**, 523.
 24. S. Zhang, T. Yu, M. T. Sun, H. Yu, Z. P. Zhang, S. H. Wang, and H. Jiang, *Talanta*, **2009**, **126**, 185.
 25. S. P. Wu and S. R. Liu, *Sens. Actuators, B*, **2009**, **141**, 187.
 26. B. B. Shi, P. Zhang, T. B. Wei, H. Yao, Q. Lin, J. Liu, and Y. M. Zhang, *Tetrahedron*, **2013**, **69**, 7981.
 27. L. L. Yang, W. J. Zhu, M. Fang, Q. Zhang, and C. Li, *Spectrochim. Acta, Part A*, **2013**, **109**, 186.