Malaysian Journal of Analytical Sciences, Vol 21 No 6 (2017): xxx - xxx
DOI: https://doi.org/10.17576/mjas-2017-2106-zz

MALAYSIAN JOURNAL OF ANALYTICAL SCIENCES
Published by The Malaysian Analytical Sciences Society

ISSN
1394 - 2506

SYNTHESIS OF THIOUREA DERIVATIVES AND BINDING BEHAVIOR TOWARDS THE MERCURY ION

(Sintesis Sebatian Terbitan Tiourea dan Sifat Pengikatan Terhadap Ion Merkuri)

Fatimatul Akma Awang Ngah, Emma Izzati Zakariah, Nurul Izzaty Hassan, Bohari Yamin, Suhaila Sapari,
Siti Aishah Hasbullah*

School of Chemical Sciences and Food Technology,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

**Corresponding author: aishah80@ukm.edu.my*

Received: 19 July 2017 Accepted: 30 September 2017

Abstract

Four new thiourea derivatives namely 1-(2-aminophenyl)-3-(naphthlene-1-yl)thiourea, **3a**, *N*-(2-aminophenyl)-*N*-(1-naphthalenyl)thiourea, **3a'**, 1-(3-aminophenyl)-3-(naphthalene-1-yl)thiourea, **3b**, and 1,4-phenylene-bis[3-(α-naphthyl)thiourea], **3c** have been synthesized by the reaction of 1-naphthyl isothiocyanate, **1a** with 1,2-phenylenediamine, **2a**, 1,3-phenylenediamine, **2b,** 1,4-phenylenediamine, **2c**. The binding behavior of **3b** for mercury ion was evaluated by 'naked- eye', ^{1}H Nucleus Magnetic Resonance, Fourier Transformation Infrared, and UV-Vis titration methods. Stoichiometry of the host-guest complexation of **3b** and mercury ion was found to be 1:1 by plotting molar-ratio curves.

Keywords*:* synthesis, thiourea, sensing, mercury

Abstrak

Empat sebatian baru terbitan tiourea iaitu 1-(2-aminofenil)-3-(naftil-1-il)tiourea, **3a**, *N*-(2- aminofenil)-*N*-(1-naftilin-il)tiourea, **3a'**, 1-(3- aminofenil)-3-(naftilin-1-il)tiourea, **3b**, and 1,4-fenilin-bis[3-(α-naftil)tiourea], **3c** telah berjaya disintesis melalui tindak balas di antara 1-naftil isotiosianat, **1a** dengan 1,2-fenilenadiamina, **2a**, dan 1,3-fenilenadiamina, **2b** dan 1,4-fenilenadiamina, **2c**. Sifat kelakuan pengikatan sebatian **3b** terhadap ion merkuri dikaji dengan menggunakan kaedah mata kasar, ^{1}H Resonan Magnetik Nukleus, Infra Merah Transformasi Fourier, dan juga pengtitratan Ultralembayung-Cahaya Nampak (UV-Vis). Stoikiometri kompleks 3b dan ion merkri ditemui pada nisbah 1:1 melalui lengkung nisbah molar yang telah diplot.

Kata kunci: sintesis, tiourea, penderia, merkuri

Introduction

Thiourea, $SC(NH_2)_2$ is a compound that is analogous to urea ($CO(NH_2)_2$), where by the oxygen atom has been replaced with a sulfur atom. It has different properties compared to urea due to the difference in electronegativity between sulfur and oxygen [1]. Thiourea is one of the most important compounds used in relation to metallic electrodes [2]. It can absorb to a metal surface and in so doing block active sites [2]. It is also one of the most important industrial chemical products [3]. The presence of heavy metal ions in the environment is a large problem due to their acute toxicity. They are not biodegradable and harmful to human organs [4]. Among the variety of metal ions, mercury is hugely harmful because its compounds are widely spread in air, soil, and water also can subsequently bio-accumulate through the food chain [5].

Thioureas are also selective analytical reagents, especially for the determination of metals in the presence of other metal ions [6]. Thiourea derivatives with conjugated oxygen or nitrogen donor sites represent some of the most important chelating ligands in coordination chemistry and their metal complexes are useful for homogenous catalysis, chemical vapor deposition, supramolecular chemistry, redox sensing, magnetic materials and so on [7]. Copper is one of the important trace metals and has been used in complexes to disinfect human solids, liquids and also human tissues. The high concentrations of the metals in the environment are harmful and toxic to living organisms [8]. Mercury is very hazardous to the environment and living organisms. It can exist as either an inorganic or organic species in the environment. It can pass through biological membranes and can cause many dangerous conditions like cancer, brain damage and also motion disorder [9]. The thiourea compound can bind with metal ions to make a stable complex due to the sulfur atom's excellent donor properties [10]. Moreover, it is also extremely flexible as a ligand, either neutral, mono-anion or dianions that able to bind to metal center [11]. Metal complexes of thiourea are commonly semi organic materials, in which the organic and inorganic parts of complex confer the advantageous properties of both elements [12].

Thioureas show high biological activity where they act as corrosion inhibitors and antioxidants and are also the component of polymers. In 2006, researchers reported acyl thiourea derivatives are for a wide range of biological activities like bactericidal, fungicidal, herbicidal and insecticidal also possess regulating activity for plant growth [13]. In continuation of our research theme, herein we wish to report the synthesis and characterization of four thioureas based 1,2-, 1,3- and 1,4-phenylenediamine. The binding behavior of **3b** toward mercury ion was investigated by 'naked eyes', ^{1}H NMR, FTIR and UV–vis titration method.

Materials and Methods

Chemicals

This research was using some chemicals such as 1,2-phenylenediamine (Sigma Aldrich), 1,3-phenylenediamine (Sigma Aldrich), 1,4-phenylenediamine (Merck), 1-naphthyl isothiocyanate (Sigma Aldrich), and solvent such as dimethyl sulfoxide (DMSO) and dichloromethane (DCM) from Fisher company. All the chemicals and solvents were used directly without any purification.

Physical measurement

All reactions were performed under reflux condition. Chemicals and solvents were purchased from Sigma Aldrich and Acros and were used directly without further purification. The compound was analyzed using FTIR Perkin Elmer Model Spectrum GX in the range 400-4000cm^{-1} using KBr method, ^{1}H and ^{1}C NMR spectrometer model Bruker Advance 400 MHz, Liquid Chromatography Mass Spectroscopy model Dionex/Bruker MicroToF Q and Ultraviolet visible (UV-vis) CARY Agilent 100.

Synthesis of 1-(2-aminophenyl)-3-(naphthlene-1-yl)thiourea, 3a

A solution of 1-naphthyl isothiocyanate (0.185 g, 1.00 mmol) and 1, 2-phenylenediamine (0.054 g, 0.50 mmol) in 10 mL dichloromethane (DCM) was refluxed for 23 hours. The white precipitate was filtered out and the pure product was isolated (63 %, 0.092 g).

Synthesis of N-(2-aminophenyl)-N-(1-naphthalenyl)thiourea, 3a'

A solution of 1-naphthyl isothiocyanate (0.093 g, 0.025 mmol) and 1-(2-aminophenyl)-3-(naphthlene-1-yl)thiourea (**3a**) (0.073 g, 0.025 mmol) in 20 mL DCM was refluxed for 24 hours. The white precipitate was filtered, and the pure product was isolated (85 %, 0.010 g).

Synthesis of 1-(3-aminophenyl)-3-(naphthalene-1-yl)thiourea, 3b

A solution of 1-naphthyl isothiocyanate (0.705 g, 2.00 mmol) and 1, 3-phenylenediamine (0.109 g, 1.00 mmol) in 20 mL dichloromethane (DCM) was refluxed for 24 hours. The white precipitate was filtered out and the pure product was isolated (82 %, 0.241 g).

Synthesis of 1,4-phenylene-bis[3-(α-naphthyl)thiourea], 3c

A solution of 1-naphthyl isothiocyanate (0.927 g, 5.00 mmol) and 1, 4-phenylenediamine (0.270 g, 2.50 mmol) in 20 mL dichloromethane (DCM) was refluxed for 28 hours. The white precipitate was filtered out and the pure product was isolated (95 %, 1.136 g).

Binding behaviors for compound 3b towards selected metal salts

The binding experiments were carried out using naked-eye method, FTIR, UV-Vis titration and ^{1}H NMR titration. For naked-eye detection, stock solutions (1 x 10^{-3} M) of Fe^{2+}, Pb^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Sn^{2+} ions and compound **3b** in dimethyl sulfoxide (DMSO) were prepared. 1.0 ml vial containing Fe^{2+} and 1.0 ml of **3b** was mixed together in vials and shaken to ensure homogeneity. The color changes were observed and the data were collected. The experiment was repeated using Pb^{2+}, Hg^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Sn^{2+} ions.

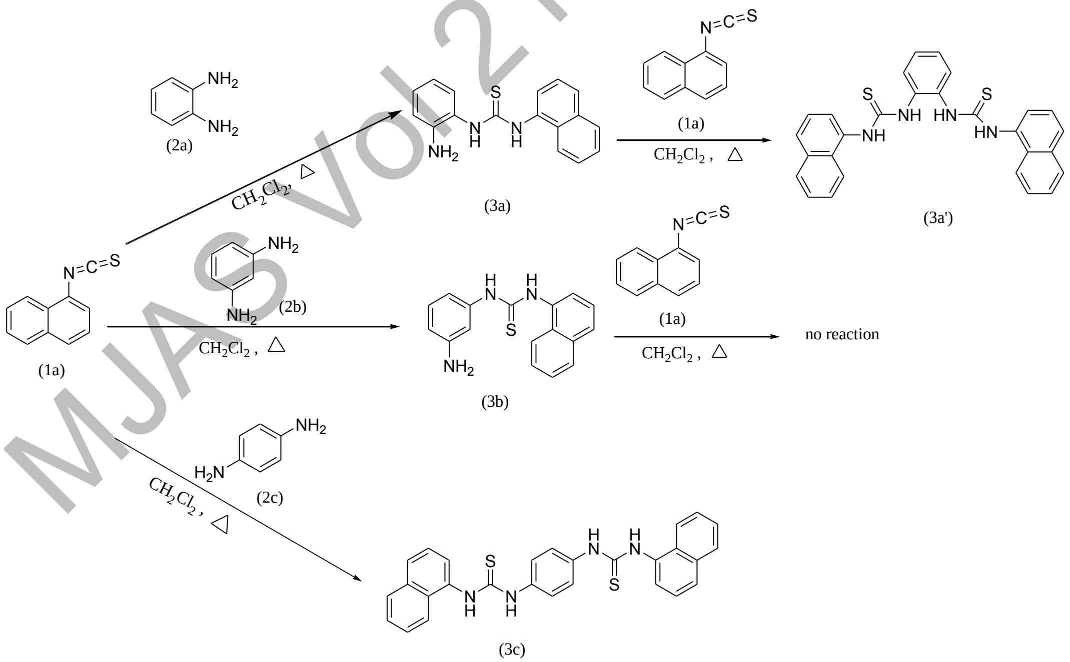
For UV-Vis titrations, 10 ml stock solutions (1 x 10^{-3} M) of **3b** and selected metal ions (1 x 10^{-3} M) were prepared in DMSO as solvent. The screening of these solutions was conducted on a UV-Vis spectrophotometer. 200 µL of **3b** and 0-600 µL of the mercury ion solution were titrated and directly analyzed using UV-Vis spectrophotometer [5].

For the ^{1}H NMR titrations, 300 µL of a stock solution of Hg^{2+} (0 – 0.06 M) was added into 560 µL of stock solution of **3b** (0.03 M) and then was placed in an NMR tube. Both of solutions were in DMSO-*d*$_6$. The titration solutions were analyzed using ^{1}H NMR spectroscopy [14].

For analysis using FTIR spectroscopy, the sample was prepared by using 0.03 M of compound **3b** and 0.03 M of $Hg(OAc)_2$, both in DMSO as solvent.

Results and Discussion

Compound **3a, 3a', 3b** and **3c** were synthesized by the reaction of ethyl isothiocyanate **1a** with diamines **2a, 2b** and **2c** (Scheme 1).



Scheme 1. Reaction schemes for synthesis of compounds **3a**, **3a'**, **3b** and **3c**

1-(2-aminophenyl)-3-(naphthlene-1-yl)thiourea, 3a
Mp: 164.0 – 168.1 ºC. IR (KBr pellet) υ(cm^{-1}): 3467, 3358, 3313, 3145, 3135, 2970, 1595, 1508, 1284, 747. ^{1}H RMN (DMSO-d_6, 400 MHz) δ (ppm): 9.62 (s, 1H, N*H*), 7.21 (s, 1H, N*H*), 8.01 (dd, 2H, J= 7.6 Hz and 7.2 Hz, Ar-*H*), 7.84 (d, 1H, J=8.0 Hz, Ar-*H*), 7.53 (m, 4H, Ar-*H*), 7.11 (d, 1H, J= 7.2 Hz, Ar-*H*), 6.99 (t, 1H, J= 8.0 Hz, Ar-*H*), 6.77 (d, 1H, J= 7.6 Hz, Ar-*H*), 6.60 (t, 1H, J= 7.2 Hz, Ar-*H*), 4.93 (s, 2H, N*H*$_2$). ^{13}C RMN (DMSO-d_6, 100 MHz) δ (ppm): 182.1 (*C*=S), 144.6 (Ar-*C*), 135.6 (Ar-*C*), 134.3 (Ar-*C*), 127.9 (Ar-*C*), 124.6 (Ar-*C*), 130.5 (Ar-*C*H), 128.9 (Ar-*C*H), 128.5 (Ar-*C*H), 127.2 (Ar-*C*H), 126.6 (Ar-*C*H), 126.5 (Ar-*C*H), 126.1 (2 × Ar-*C*H), 123.5 (Ar-*C*H), 117.0 (Ar-*C*H), 116.4 (Ar-*C*H). LC-MS (ESI) m/z: calculated for $C_{17}H_{15}N_3S$ (293.39), found (M-H$^+$) (292.08) [15].

N-(2-aminophenyl)-N-(1-naphthalenyl)thiourea, 3a’
Mp: 165.0 – 169.8 ºC. IR (KBr pellet) υ(cm^{-1}): 3457, 3307, 3129, 2958, 1525, 1500, 1251, 752. ^{1}H NMR (DMSO-d$_6$, 400MHz) δ (ppm): 10.14 (s, 1H, N*H*), 9.27 (s, 1H, N*H*), 8.01 (m, 3H, Ar-*H*), 7.88 (d, 1H, J=8.0 Hz, Ar-*H*), 7.56 (t, 3H, J= 4.0 Hz, Ar-*H*), 7.48 (d, 2H, J= 3.6 Hz, Ar-*H*). ^{13}C NMR (DMSO-d$_6$, 100MHz) δ (ppm): 182.1 (*C*=S), 144.6, 134.3, 130.5, 123.5 (4 Ar-*C*), 128.9, 128.5, 127.9, 127.2, 126.6, 126.5, 126.1, 117.0, 116.4 (9 Ar-*C*H). LC-MS (ESI) m/z: calculated for $C_{28}H_{22}N_4S_2$ (478.63), found (477.10).

1-(3-aminophenyl)-3-(naphthalene-1-yl)thiourea, 3b
Mp: 117.4 – 121.8 ºC. IR (KBr pellet) υ(cm^{-1}): 3433, 3345, 3322, 3207, 3043, 1595, 1519, 1225, and 775. ^{1}H RMN (DMSO-d_6, 400 MHz) δ (ppm): 9.62 (d, 2H, J= 5.2 Hz, N*H*), 7.94 (s, 1H, Ar-*H*), 7.84 (d, 1H, J= 8 Hz, Ar-*H*), 7.52 (m, 4H, J= 8, 4 Hz, Ar-*H*), 6.99 (t, 1H, J= 8 Hz, Ar-*H*), 6.65 (d, 1H, J= 8 Hz, Ar-*H*), 6.78 (s, 1H, Ar-*H*), 6.39 (d, 1H, J= 8 Hz, Ar-*H*), 5.12 (s, 2H, N*H*$_2$). ^{13}C RMN (DMSO-d_6, 100 MHz) δ (ppm): 181.3 (*C*=S), 149.4 (Ar-*C*), 140.2 (Ar-*C*), 135.7 (Ar-*C*), 123.5 (Ar-*C*), 111.4 (Ar-*C*), 134.3 (Ar-*C*H), 130.5 (Ar-*C*H), 129.4 (Ar-*C*H), 128.5 (Ar-*C*H), 127.1 (Ar-*C*H), 126.6 (Ar-*C*H), 126.5 (Ar-*C*H), 126.0 (Ar-*C*H), 126.0 (Ar-*C*H), 112.3 (Ar-*C*H), 110.1 (Ar-*C*H). LC-MS (ESI) m/z: calculated for $C_{17}H_{15}N_3S$ (M+H$^+$) (293.39), found (294.10) [16].

1,4-phenylene-bis[3-(α-naphthyl)thiourea], 3c
Mp: 214.3 – 219.1 ºC. IR (KBr pellet) *v*(cm^{-1}): 3325, 3186, 3000, 1542, 1499, 1221, 767. ^{1}H RMN (DMSO-d$_6$, 400 MHz) δ (ppm): 9.87 (s, 1H, N*H*), 9.77 (s, 1H, N*H*), 7.98 (t, 2H, J= 8.0 Hz, Ar-*H*), 7.86 (d, 1H, J= 6.8 Hz, Ar-*H*), 7.55 (m, 6H, Ar-*H*). ^{13}C RMN (DMSO-d$_6$, 100 MHz) δ (ppm): 181.6 (*C*=S), 136.5 (Ar-*C*), 135.5 (Ar-*C*), 134.3 (Ar-*C*), 124.7 (Ar-*C*), 130.4 (Ar-*C*H), 128.6 (Ar-*C*H), 127.2 (Ar-*C*H), 126.6 (Ar-*C*H), 126.5 (Ar-*C*H), 126.1 (Ar-*C*H), 125.8 (Ar-*C*H), 124.6 (Ar-*C*H), 123.5 (Ar-*C*H). LC-MS (ESI) m/z: calculate for $C_{28}H_{22}N_4S_2$ (M+H$^+$) (478.63), found (479.1) [17].

Binding behaviors for 3b towards some selected metal ions
For the visible observations, the most remarkable color change was observed in the presence of Hg^{2+}, Cu^{2+}, Fe^{2+}, and Ag^{+} ions. The color changes before and after adding the metal salts into a solution of **3b** are seen in Figure 1 and Table 1. Based on the observation, the colorless solution became dark brown upon the addition of Hg^{2+} (with acetate as counter ion). Next, the screening of **3b** with some selected metals in 2.0 ml solvent was carried out using UV-Vis spectroscopy (Figure 2). Based on the screening, Hg^{2+} was found to show the highest absorption at 260 nm, together with new bands appearing at 305 nm and 370 nm which indicated strong binding behavior of **3b** towards the Hg^{2+} ion. Due to this high affinity, the Hg^{2+} ion was chosen for further titration studies using UV-vis and ^{1}H NMR titrations.

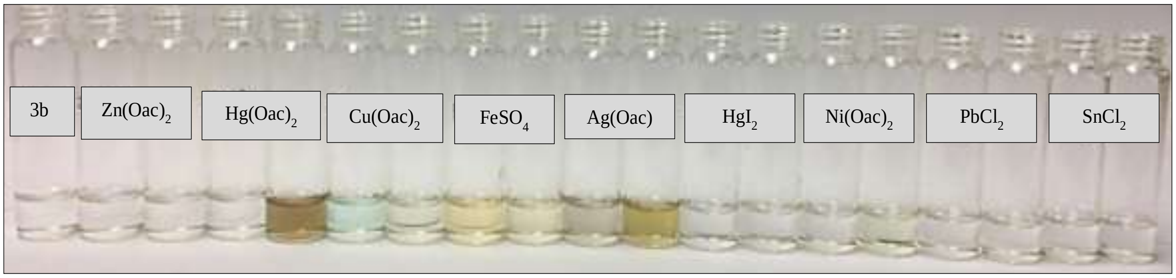


Figure 1. Color changes for naked-eyes sensor of 1-(3-aminophenyl)-3-(naphthalene-1-yl)thiourea, **3b** (1 x 10^{-3} M) towards selected metals (1 x 10^{-3} M)

Table 1. Color changes before and after adding the metal salts solution into the 1-(3-aminophenyl)-3-(naphthalene-1-yl)thiourea, **3b** solution

Metal salts	**1-(3-aminophenyl)-3-(naphthalene-1-yl)thiourea, 3b (colorless)**
$Zn(OAc)_2$ (colorless)	Unchanged
$Hg(OAc)_2$ (colorless)	Dark brown
$Cu(OAc)_2$ (blue)	Light yellow
$FeSO_4$ (yellowish)	Light yellow
Ag(OAc) (light grey)	Brown
HgI_2 (colorless)	Unchanged
$Ni(OAc)_2$ (colorless)	Very light yellow
$PbCl_2$ (colorless)	Unchanged
$SnCl_2$ (colorless)	Unchanged

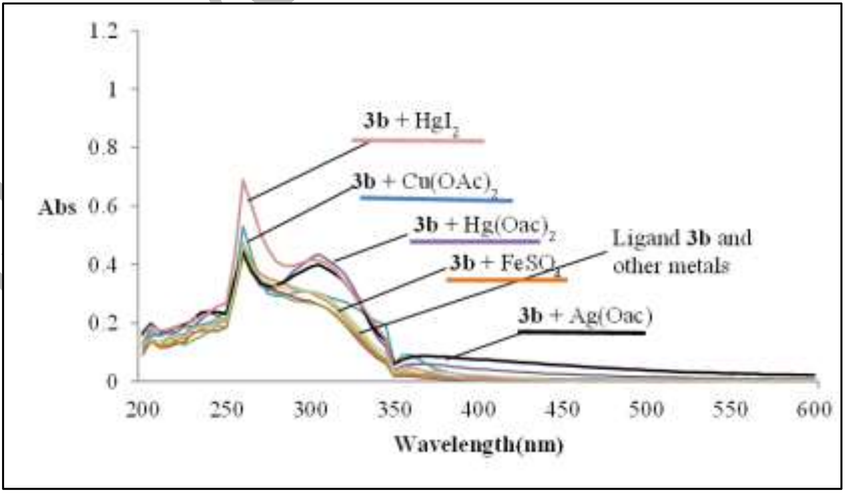


Figure 2. UV-Vis spectra of **3b** (1 x 10^{-3} M) with all metal ions (1 x 10^{-3} M) in DMSO as solvent

The titration studies of **3b** toward Hg^{2+} ions were performed in DMSO. Figure 3 shows that upon the gradual addition of 25 µL equivalent of Hg^{2+} (1x10^{-3} M) to the 200 µL ligand solution (1x10^{-3} M), new absorption bands at 305 nm and 370 nm appeared with increasing intensity. The absorbance maxima increased linearly with the concentration of Hg^{2+} in a range of 0 – 1.0 M with a correlation coefficient of 0.99306 (Figure 4) after 10 readings. There was an isosbestic point at 280 nm which indicated that there were only two species present at equilibrium [18]. In addition, a new shoulder band appeared at 345 nm, something which relates to the intramolecular charge transfer (ICT) on the thiourea moiety of compound **3b**. The band at 370 nm shows a hyperchromic effect with an increasing amount of Hg^{2+}. Based on the UV-Vis titration, we were able to calculate the stoichiometry of the binding interaction between ligand **3b** and Hg^{2+} through the molar-ratio curve method (Figure 4) [19]. The binding ratio of **3b**- Hg^{2+} was 1:1 and the binding constant (K$_d$) was calculated using the nonlinear regression equation using Sigma Plot 12.0 software. The K$_d$ value of **3b**-Hg^{2+} was 1.659 M.

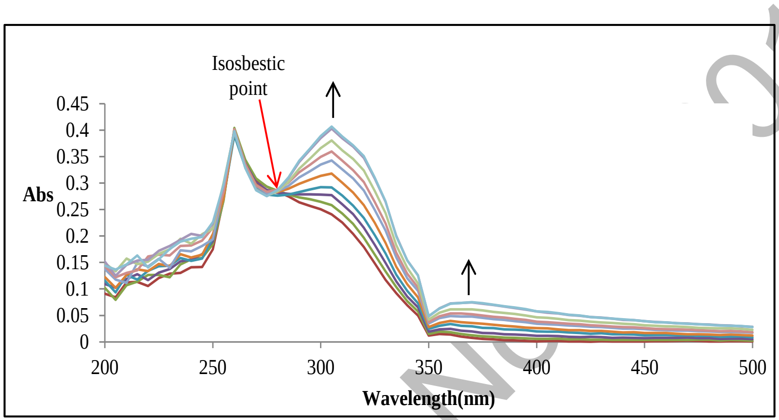


Figure 3. Changes in absorption spectra of **3b** (1 x 10^{-3} M) with increasing amount of Hg^{2+} ions (Hg(OAc)$_2$) (1 x 10^{-3} M)

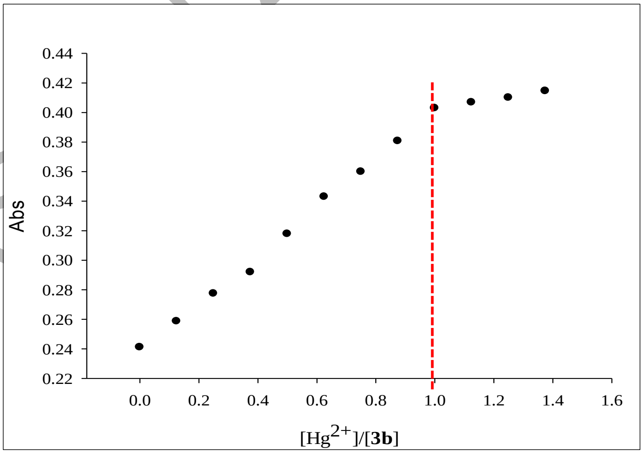


Figure 4. Variation of absorbance at 305 nm versus [Hg^{2+}]/[**3b**]

To further study the binding behavior, ^{1}H NMR titrations were performed in DMSO-d$_6$ (Figure 5). Protons for the two NH of compound **3b** were observed at 9.61 ppm and 9.59 ppm respectively. The integral of the peaks were both 2.00. After gradually adding the Hg^{2+} solution (0 – 0.06 M), the size of the signals decreased and their integration value became less than 1. This was due to the strong interaction between **3b** and Hg^{2+}. In addition, the protons for the NH_2 groups in the original spectra for compound **3b** were singlet, but in combination with Hg^{2+} solution, the signal became broader and less intense (integration less than 1.00).

Next, the FTIR spectrum showed that the complex of **3b** and Hg^{2+} showed a decreased intensity for both the CN and C=S groups, whilst a new peak appeared at 998 cm^{-1} (Figure 6) [20]. This peak was due to the complexation of the Hg^{2+} ion and the thiourea moiety. The peaks responsible for the NH_2 region disappeared due to the intramolecular charge transfer formed with the thiourea moiety. The proposed reaction mechanism of the **3b**-Hg interaction is shown in Figure 7.

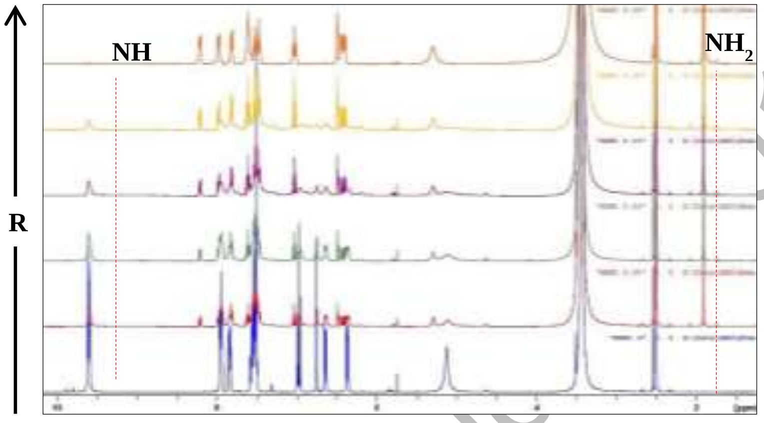


Figure 5. ^1H NMR titration of **3b** showing changes in the NH and NH_2 chemical shifts of the receptor **3b** (0.03M) with increasing amount of Hg^{2+} (**R**) (0 - 0.06 M) in DMSO-*d_6*

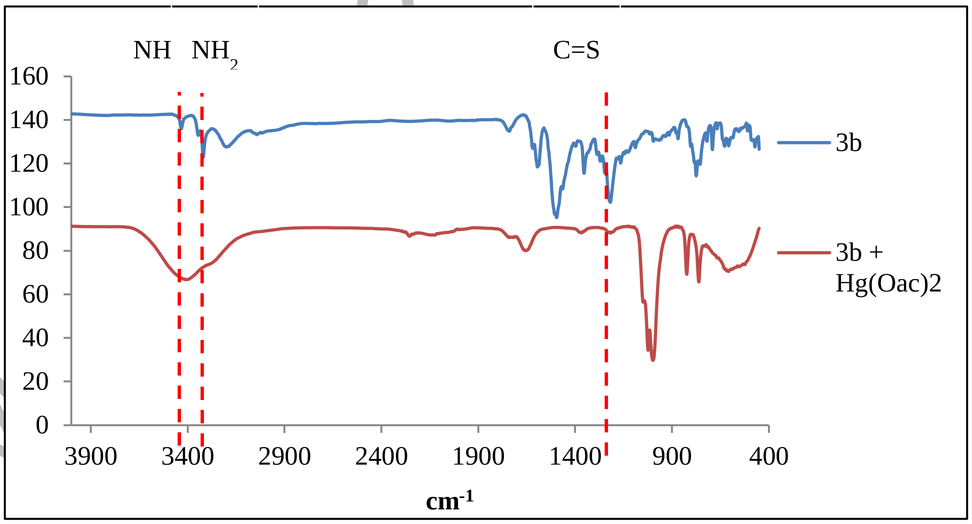


Figure 6. FTIR spectrum of compound **3b** with $Hg(OAc)_2$ solution.

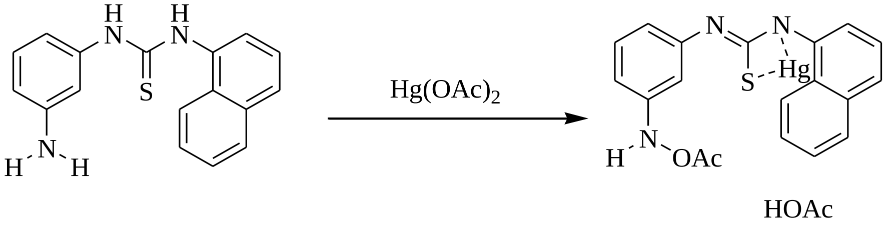


Figure 7. The proposed reaction mechanism of the **3b**-Hg interactions

Conclusion

In conclusion, we have reported the synthesis of thiourea derivatives, 1-(2-aminophenyl)-3-(naphthlene-1-yl) thiourea (**3a**), N-(2-aminophenyl)-N-(1-naphthalenyl)thiourea (**3a’**), 1-(3-aminophenyl)-3-(naphthalene-1-yl) thiourea (**3b**), and 1,4-phenylene-bis[3-(α-naphthyl)thiourea] (**3c**). Using observation by naked eye, UV-vis titration, FTIR, and ^{1}H NMR titration methods showed that the binding behavior of compound **3b** towards Hg^{2+} afforded a complex with a 1:1 binding ratio. Future work will be aimed at using compound **3b** for sensor based applications whereby the fate of Hg^{2+} can be tracked in the environment.

Acknowledgement

The authors thank the Ministry of Higher Education Malaysia for My Master Scholarship, School of Chemical Sciences and Food Technology, National University of Malaysia, Center for Research and Instrumentation (CRIM), Research Grant FRGS/1/2015/ST01/UKM/02/2 and PRGS/2/2015/SG01/UKM/02/1 and everyone that involved in this research.

References

1. Alkherraz, A. M., Lusta, I. Z. and Zubi, E. A. (2014). Synthesis and use of thiourea derivative (1-phenyl-3-benzoyl-2-thiourea) for extraction of cadmium ion. *International Scholarly and Scientific Research & Innovation*, 8(2): 116 – 118.
2. Zhao, J. and Cui, G. (2011). Study on adsorption and complexation behavior of thiourea on copper surface. *International Journal of Electrochemical Science*, 6: 4048 – 4058.
3. Yahyazahed, A. and Ghasemi, Z. (2013). Synthesis of unsymmetrical thiourea derivatives. *European Chemical Bulletin*, 2(8): 573 – 575.
4. Zhang, Z., Lu, S., Sha, C. and Xu, D. (2015). A single thiourea-appended 1,8-naphthalimide chemosensor for three heavy metal ions: Fe^{3+}, Pb^{2+}, and Hg^{2+}. *Sensors and Actuators*, 208: 258 – 266.
5. Khansari, M. E., Wallace, K. D. and Hossain, M. A. (2013). Synthesis and anion recognition studies of dipodal thiourea-based sensor for anions. *Tetrahedron Letters*, 55: 438 – 440.
6. Singh, A., Bharty, M. K., Bharati, P., Bharti, A., Singh, S. and Singh, N. K. (2015). Synthesis, spectral, thermal and structural characterization of a hexanuclear copper(I) cluster and a cobalt(III) complex of 1-ethyl-3-phenyl-thiourea. *Polyhedron*, 85: 918 – 925.
7. Saad, F. A. (2014). Coordination chemistry of some first row transition metal complexes with multi-dentate ligand (1- benzoyl - 3-(4-methylpyridin -2-yl) thiourea), spectral, electrochemical and x – ray single crystal studies. *International Journal of Electrochemical Science*, 9: 4761 – 4775.
8. Rauf, M. K., Imtiaz-ud-Din, Badshah, A., Gielen, M., Ebihara, M., de Vos, D. and Ahmed, S. (2009). Synthesis, structural characterization and in vitro cytotoxicity and anti-bacterial activity of some copper(I) complexes with N,N'-disubstituted thioureas. *Journal of Inorganic Biochemistry*, 103: 1135 – 1144.
9. Lee, J. Y., Rao, B. A., Hwang, J. Y. and Son, Y. (2015). A novel sensing capabilities and structural modification from thiourea to urea derivative by $Hg(ClO_4)_2$: Selective dual chemodosimeter for Hg^{2+} and F^- ions. *Sensors and Actuators B*, 220: 1070 – 1085.
10. Arslan, H., Duran, N., Borekci, G., Koray, O. and Akbay, C. (2009). Antimicrobial activity of some thiourea derivatives and their nickel and copper complexes. *Molecules*, 14: 519 – 522.

11. Saeed, A., Abbas, N., Ashraf, Z. and Bolte, M. (2013). Synthesis, characterization and antibacterial activity of new 1,2- and 1,4-bis(n'-substituted thioureido)benzene derivatives. *South African Journal of Chemistry,* 66: 273 – 278.
12. Kumari, R. G., Ramakrishnan, V., Carolin, M. L., Kumar, J., Sarua, A. and Kuball, M. (2009). Raman spectral investigation of thiourea complexes. *Spectrochimica Acta Part A,* 73: 263 – 267.
13. Ke, S. Y. and Xue, S. J. (2006). Synthesis and herbicidal activity of n-(o-fluorophenoxyacetyl)thioureas derivatives and related fused heterocyclic compounds. *Arkivoc,* 10: 63 – 68.
14. Odago, M. O., Colabello, D. M. and Lees, A. J. (2010). A simple thiourea based colorimetric sensor for cyanide anion. *Tetrahedron Letters*, 66: 7465 – 7471.
15. Perry, C. J., Holding, K. and Tyrrell, E.. (2008). Simple, novel synthesis for 1-carbamoyl-1*H*-benzotriazole and some of its analogs. *Synthetic Communications,* 38: 3354 – 3365.
16. Awang Ngah, F. A., Lee, Y. H., Hasbullah, S. A. and Hassan, N. I. (2016). Synthesis and characterization of 1-(3-aminophenyl)-3-(naphthalen-1-yl)thiourea and preliminary study towards binding with metal ions. *AIP Conference Proceedings,* 1784 (1): 1 – 8.
17. Zhang, L. P., Shang, X. B., Wu, Q. F., Zhang, Y. and Li, J. P. (2012). Highly efficient method for the synthesis of 1,4-phenylenedithioureas under solvent- and catalyst- free condition promoted by microwave irradiation. *International Journal of Rapid Communication of Synthetic Organic Chemistry,* 42 (7): 1045 – 1052.
18. Kang, J., Lee, J. H., Kim, Y. H., Lee, S. K., Kim, E. Y., Lee, H. G. and Kim, C. (2011). Simple urea/thiourea sensors for the biologically important ions. *Journal of Inclusion Phenomena and Macrocyclic Chemistry,* 70: 29 – 35.
19. Fakhar, I., Yamin, B. and Hasbullah, S.A. (2017). A comparative study of the metal binding behavior of alanine based bis-thiourea isomers. *Chemistry Central Journal*, 11:76. 1 – 16.
20. Wiles, D. M., Gingras, B. A. and Suprunchuk, T. (1966). The C=S stretching vibration in the infrared spectra of some thiosemicarbazones. *Canadian Journal of Chemistry,* 45: 469 – 473.