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Synthesis and Determination of Acid Dissociation Constants of Bis-Acyl Thiourea Derivatives

Cagla Efeoglu[1], Sit Tiken[1], Hayati Sari[2], Yahya Nural[1]*

[1]Mersin University, Faculty of Pharmacy, Department of Analytical Chemistry, 33169, Mersin, Turkey
[2]Department of Chemistry, Faculty of Science and Arts, Tokat Gaziosmanpasa University, Tokat 60250, Turkey

Abstract: N,N'-((dodecane-1,12-diylbis(azanediyl))bis(carbonothioyl))bis(4-nitrobenzamide) 5 and N,N'-((dodecane-1,12-diylbis(azanediyl))bis(carbonothioyl))bis(3-nitrobenzamide) 6 as bis acyl thiourea derivatives were synthesized and their molecular structures were characterized using 1 H NMR, 13 C NMR, COSY, DEPT, HMQC, FT-IR, and HRMS techniques. The acid dissociation constants (p K a) of the bis-acyl thiourea derivatives 5, 6 were determined potentiometrically and spectrophotometrically. The pK a values of products 5, 6 were determined in 50% (v/v) dimethyl sulfoxide–water hydro-organic solvent in the presence of 0.1 mol□L □1 ionic strength of NaCl and in the acidic medium at 25±0.1 °C, and two p K a values were calculated for each compound with the HYPERQUAD computer program using the data obtained from the potentiometric titrations performed. In addition, three pK a values for each compound were determined in the calculations using the HypSpec program from the data obtained from the spectrophotometric titrations performed under the conditions where the potentiometric titrations were performed. For compounds 5 and 6, spectrophotometrically, pK a1 was 3.56±0.08 and 3.87±0.01, respectively, pK a2 was 7.11±0.08 and 7.05±0.01, respectively, and pK a3 was 12.30±0.08 and 11.82±0.02, respectively. It can be said that pK a1 , pK a2 , and pK a3 values may belong to enol, enthiol, and NH, respectively. Moreover, for compounds 5 and 6, potentiometrically, pK a2 was 7.06±0.13 and 6.94±0.11, respectively, and pK a3 was 12.11±0.06 and 11.17±0.06, respectively, and it can be said that pK a2 and pK a3 values may belong to enthiol and NH, respectively. It is seen that the pK a values determined spectrophotometrically and potentiometrically are compatible with each other.

Keywords: Acid dissociation constant, Bis-Acyl thiourea, Potentiometric titration, Spectrophotometric titration.

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***Corresponding author.** E-mail: ynural1805@yahoo.com, yahyanural@mersin.edu.tr

1. INTRODUCTION

Acyl thiourea derivatives are widely studied in analytical chemistry because they show chemosensory properties in the determination of various anions, cations, and neutral compounds (1–5) and can be used in heavy metal removal due to their very good chelating properties with metal ions (6,7). In addition, acyl thiourea derivatives have an important place in pharmaceutical chemistry (8,9) because they can be used as an intermediate product in the synthesis of many bioactive molecules (10,11) and show a wide range of bioactivity such as anti(mico)bacterial (12–14), antifungal (15,16), antioxidant (17), and anticancer (18) as well as enzyme inhibitory (19–23) properties. Since acyl thiourea compounds can easily form chelate complexes on carbonyl and thiocarbonyl groups, their Pt(II), Ni(II), Zn(II), Co(II), and other many metal complexes have been synthesized (24–30).

It is also known that various bis-acyl thiourea derivatives have been synthesized (31,32) to examine their bioactivity and that these compounds show antimicrobial (33) and antifungal (34,35), anticancer (36–38), antioxidant (39) as well as anti-diabetic (40) and tissue-nonspecific alkaline phosphatase inhibitor properties (41). It has also been stated that some bis-acyl thiourea derivatives may be potential drug candidates for Alzheimer's disease by showing acetylcholinesterase enzyme inhibition activity (42). Pt(II), Ni(II), Cu(II), and Zn(II) complexes of various bis-acyl thiourea derivatives are also synthesized (43–45). It has been stated that the Ni(II) and Cu(II) complexes of bis-acyl thiourea derivatives show anticancer properties as well as DNA and protein binding properties (46). The chemosensory properties of bis-acyl thiourea derivatives were also investigated and it was determined that some compounds showed chemosensory properties against Cu(II) ions (47,48). Moreover, hexamethylene-1,6-bis[(*N*'-benzoyl)thiourea], a bis-acyl thiourea derivative, showed high palladium extraction properties in hydrochloric acid solutions (49).

The pK_a value, which provides information about the acidity/basicity of the compounds, their solubility in the solvent and pH environments they are in, and their ability to interact with the receptors in the environments where they will exert pharmacological effects, is a very useful parameter. Since drug molecules generally show activity in ionized form, it is important to determine the relevant pK_a values, which give information about the ionization state of each functional group in the molecule. The pK_a values are also an important parameter in knowing which ionized forms of the molecule will be effective in various pH environments. Since pK_a values provide critical information about molecules, they are also very important parameters in the development of molecular docking programs, which have a very important place in drug design (50–55). pK_a values carry critical information about the complexes that ligands to be used in heavy metal removal will form with metal ions and their adsorption properties (56). While potentiometric (57) and spectrophotometric (58,59) titrations are widely used in determining pK_a values of compounds, voltammetric (60), chromatographic (61,62), and nuclear magnetic resonance (NMR) spectroscopy (63) methods are also known to be used.

Herein, we report the synthesis of *N*,*N*'-((dodecane-1,12-diylbis(azanediyl))bis(carbonothioyl)) bis(4-nitrobenzamide) and *N*,*N*'-((dodecane-1,12-diylbis(azanediyl))bis(carbonothioyl))bis(3-nitrobenzamide) as bis acyl thiourea derivatives and the determination of their pK_a values potentiometrically and spectrophotometrically in 50:50 v/v DMSO: water hydro-organic medium.

2. MATERIAL AND METHODS

2.1. Materials and Instrumentation

All used chemicals were of reagent grade. They were purchased from Merck or Aldrich and used without further purification. Fourier transform infrared (FTIR) and NMR spectra were recorded using the Varian Scimitar Series 1000 FTIR spectrophotometer and a Bruker Ultrashield Plus Biospin GmbHt at 400 MHz, respectively. Melting points were determined on a Mettler Toledo MP90 apparatus and were uncorrected. High-resolution mass spectra were recorded by electrospray ionization technique in Waters SYNAPT G1 MS (ESI-TOF-MS). Potentiometric titrations were performed using a Titroline 7000 automated titrator with SI-Analytics combined with a glass pH electrode and having an automatic micro-burette that could be controlled by a computer. Spectrophotometric titrations were performed using a Shimadzu 1800 240V model UV-Vis spectrophotometer.

2.2. Synthesis

Potassium thiocyanate (10.3 mmol) was added to a stirred solution of 4-nitrobenzoyl chloride or 3-nitrobenzoyl chloride in acetone (75 mL) and then the mixture was stirred and heated to reflux for 4 h. After the reaction was complete, the mixture was filtered through filter paper. The filtrate was added dropwise to the stirred flask containing 1,12-diaminododecane (1g, 5 mmol) dissolved by heating in chloroform (100 mL) and heated to reflux temperature for 36 h (Scheme 1). After completion of the reaction monitored using TLC, the solvent was removed using a rotary evaporator and the crude product was purified using column chromatography (ethyl acetate:hexane, 1:4 v/v). The molecular structures of the desired pure products were characterized using NMR, FT-IR, and HRMS techniques.

2.2.1. *N*,*N*'-((dodecane-1,12-diylbis(azanediyl))bis(carbonothioyl))bis(4-nitrobenzamide) (**5**). Brown powder. Yield, 0,52 g, 84%. m.p.: 107-109 °C. FT-IR (cm^{-1}): υ_{max} 3399, 3237, 3111, 3081, 2919, 2850, 1668, 1604, 1552, 1515, 1489, 1435, 1345, 1303, 1250, 1202, 1158, 1042. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.67 (s, 2H, 2 x C(O)NH), 10.74 (t, 2H, J = 5.1

Hz, 2 x NH), 8.31 (d, 4H, *J* = 8.7 Hz, Ar-H), 8.11 (d, 4H, *J* = 8.7 Hz, Ar-H), 3.62-3.58 (m, 4H, $C1H_2$, $C12H_2$), 1.66-1.59 (m, 4H, $C2H_2$, $C11H_2$), 1.34-1.26 (m, 16H, $C3H_2$, $C4H_2$, $C5H_2$, $C6H_2$, $C7H_2$, $C8H_2$, $C9H_2$, $C10H_2$). ^{13}C NMR (100 MHz, DMSO-d_6): □ 179.6 (2 x C=S), 166.5 (2 x C=O), 149.7 (2 x C4'), 138.1 (2 x C1'), 130.1 (2 x C2', 2 x C6'), 123.2 (2 x C3', 2 x C5'), 44.8 (C1, C12), 28.92 (C2, C11), 28.89 (C5, C8), 28.6 (C6, C7), 27.5 (C4, C9), 26.4 (C3, C10). HRMS (ESI-TOF-MS): calcd. for $C_{28}H_{37}N_6O_6S_2$ $[MH]^+$ 617.2211; found 617.2216.

2.2.2. *N*,*N*'-((dodecane-1,12-diylbis(azanediyl)) bis(carbonothioyl))bis(3-nitrobenzamide) (**6**). White powder. Yield, 0,48 g, 78%. m.p.: 142-144 °C. FT-IR (cm^{-1}): υ_{max} 3376, 3236, 3158, 3049, 2924, 2849, 1668, 1615, 1557, 1519, 1470, 1346, 1307, 1260, 1189, 1149, 1073. ^{1}H NMR (400 MHz, DMSO-d_6): *δ* 11.73 (s, 2H, 2 x C(O)NH), 10.74 (t, 2H, *J* = 5.2 Hz, 2 x NH), 8.73-8.72 (m, 2H, Ar-H), 8.46-8.44 (m, 2H, Ar-H), 8.33-8.30 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.82-7.78 (m, 2H, Ar-H), 3.63-5.57 (m, 4H, $C1H_2$, $C12H_2$), 1.65-1.59 (m, 4H, $C2H_2$, $C11H_2$), 1.33-1.23 (m, 16H, $C3H_2$, $C4H_2$, $C5H_2$, $C6H_2$, $C7H_2$, $C8H_2$, $C9H_2$, $C10H_2$). ^{13}C NMR (100 MHz, DMSO-d_6): δ 179.7 (2 x C=S), 166.1 (2 x C=O), 147.4 (2 x C3'), 134.9 (2 x C1'), 134.0 (2 x C6'), 130.1 (2 x C5'), 127.1 (2 x C4'), 123.5 (2 x C2'), 44.8 (C1, C12), 28.91 (C2, C11), 28.88 (C5, C8), 28.6 (C6, C7), 27.5 (C4, C9), 26.3 (C3, C10). HRMS (ESI-TOF-MS): calcd. for $C_{28}H_{37}N_6O_6S_2$ $[MH]^+$ 617.2211; found 617.2216.

2.3. Determination of Acid Dissociation Constants

Potentiometric determination: Potentiometric determination of the pK_a values of the products was performed using an automatic titrator which is controlled with a computer, with an ultra-combination pH electrode in 50% (v/v) DMSO–water hydro-organic solvent in the presence of 0.1 mol·L^{-1} ionic strength of NaCl and in the acidic medium at 25±0.1 °C. Stock solutions of the bis-acyl thiourea derivatives **5**, **6** were prepared with a concentration of 1×10^{-3} mol·L^{-1} in DMSO. NaOH, HCl, and NaCl stock solutions were prepared with concentrations of 0.025, 0.1, and 1.0 mol·L^{-1} in deionized water, respectively. Potentiometric titrations were performed in a double-walled glass titration cell, the temperature was kept constant at 25.0±0.1 °C using a thermostat. The titration cell was washed and dried before and after each titration, and the syringe was washed several times with deionized water and then the base solution. 5 mL of the stock solution of **5** or **6**, 20 mL of DMSO solution, 1 mL of the stock HCl solution, 5 mL of the NaCl solution, and 19 mL of deionized water were added to the titration cell. The titration cell was kept closed during titration, the solution in the titration cell was stirred at a constant rate throughout the titration using a magnetic stirrer, and nitrogen gas (99.9%) was continuously passed through the titration cell throughout the titration process. After nitrogen has passed through the titration cell for 5 minutes titration process was started, and the titration process with the additions of 0.04 mL of the stock NaOH solution was carried out using an automatic titrator and computer program. Using the data obtained from potentiometric titration, pK_a values were calculated with HYPERQUAD, one of the most widely used computer programs in this field (64).

Spectrophotometric determination: Determination studies of pK_a values were carried out under conditions (50% (v/v) DMSO–water hydro-organic solvent in the presence of 0.1 mol·L^{-1} ionic strength of NaCl and in the acidic medium at 25±0.1 °C) where the pK_a values of bis-acyl thioureas **5**, **6** were determined potentiometrically, and were calculated using the HypSpec program using the data obtained as a result of titrations performed spectrophotometrically. 0.5 mL of the stock solution of **5** or **6**, 24.5 mL of DMSO solution, 1 mL of the stock HCl solution, 5 mL of the NaCl solution, and 19 mL of deionized water were added to the titration cell. The titration cell was kept closed during titration, the temperature was kept constant at 25.0±0.1 °C using a thermostat, the solution in the titration cell was stirred at a constant rate throughout the titration using a magnetic stirrer, and nitrogen gas (99.9%) was continuously passed through the titration cell throughout the titration process. After nitrogen has been passed through the titration cell for 5 minutes titration process was started. Blank measurement was made using only DMSO in the UV-Vis spectrophotometer. In the first measurement, the pH of the titration cell and then the absorbance of the solution was measured without the use of a titrant. For other measurements, 0.025 M NaOH titrant was added to the titration cell so that at least six absorbances were measured in each pH range between pH 3 and pH 12. At each addition of titrant, first, the pH and then immediately the absorbance were measured. Using the data obtained from spectrophotometric titration, pK_a values were calculated with the HypSpec computer program.

3. RESULTS AND DISCUSSION

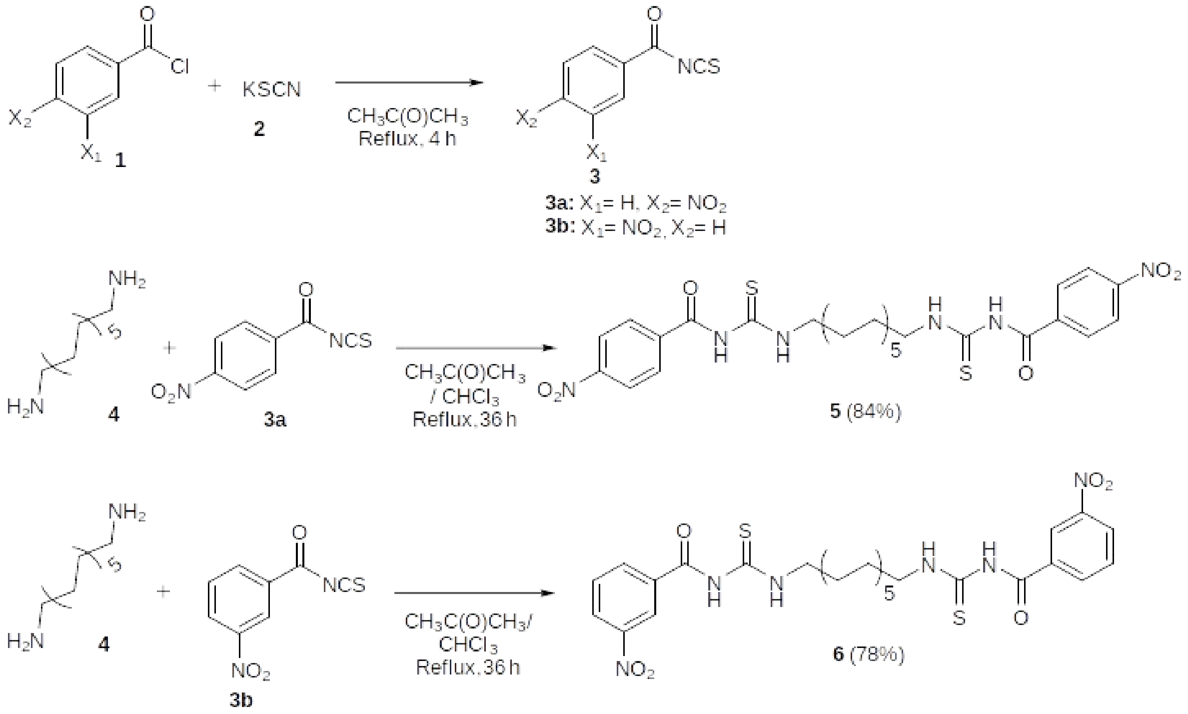
3.1. Synthesis

Bis-acyl thiourea derivatives **5**, **6** were synthesized by the reaction of 4-nitrobenzoyl isothiocyanate or 3-nitrobenzoyl isothiocyanate, which were prepared with the reaction of potassium thiocyanate and the corresponding acyl chloride, and 1,12-

diaminododecane in 84% and 78% yields, respectively (**Scheme 1**). Molecular structures of the bis-acyl thioureas **5**, **6** were characterized using ^{1}H NMR, ^{13}C NMR, COSY, DEPT, HMQC, FT-IR, and HRMS techniques (Figure S1-S12).

In the FT-IR spectra, NH stretching vibrations of **5** were observed at 3399 cm^{-1} and 3237 cm^{-1}, while NH stretching vibrations of **6** were observed at 3376 cm^{-1} and 3236 cm^{-1}. Aromatic C-H vibration band of **5** and **6** was observed at 3081 cm^{-1} and 3049 cm^{-1}, respectively, while vibration bands of the aliphatic C-H of **5** and **6** were assigned in the range of 2919–2850 cm^{-1} and 2924–2849 cm^{-1}, respectively. For **5** and **6**, the amide C=O vibration band was observed at 1668 cm^{-1}, while the C–O vibration band was observed at 1250 and 1260 cm^{-1}, respectively. In addition, vibration band of the C=S of compounds **5** and **6** was assigned at 1345 and 1346 cm^{-1}, respectively.

The bis-acyl thioureas **5**, **6** were characterized using ^{1}H and ^{13}C NMR, COSY, DEPT, and HMQC spectra (Figure S1-S12). The C(O)N*H*C(S) protons signal of **5** and **6** were observed as a singlet at 11.67 ppm and 11.73 ppm, respectively. Moreover, the other NH proton signal was observed as a triplet at 10.74 ppm for both **5** and **6**. Aromatic protons of **5** were observed in the range of 8.31–8.11 ppm, while aromatic protons of **6** were observed in the range of 8.73–7.78 ppm. In the ^{13}C NMR spectra of **5**, the thiocarbonyl and carbonyl carbon atoms peaks were observed at 179.6 ppm and 166.5 ppm, respectively, while the thiocarbonyl and carbonyl carbon atoms peaks of **6** were observed at 179.7 ppm and 166.1 ppm, respectively. All of the proton and carbon atoms peaks on the ^{1}H and ^{13}C NMR spectra, respectively, were assigned by using COSY, DEPT, and HMQC spectra.



Scheme 1: Synthesis of the bis-acyl thioureas **5**, **6**.

3.2. Acid Dissociation Constants

The pK_a values of bis-acyl thioureas **5** and **6** were determined potentiometrically and spectrophotometrically in a 50% (v/v) DMSO–water hydro-organic solvent system in the presence of 0.1 mol·L^{-1} ionic strength of NaCl and in the acidic medium at 25.0±0.1 °C (**Table 1**). As a result of the calculations of pK_a values with HYPERQUAD from the obtained potentiometric data, two different pK_a values 7.06±0.13, and 12.11±0.06 were determined for **5**, while 6.94±0.11 and 11.17±0.06 were determined for **6** (Table 1). Furthermore, as a result of the calculations of pK_a values with HypSpec from the obtained spectrophotometric data, three different pK_a values as 3.56±0.08, 7.11±0.08, and 12.30±0.08 were determined for **5**, while 3.87±0.01, 7.05±0.01, and 11.82±0.02 were determined for **6** (Table 1).

In studies on the determination of pK_a values of acyl thiourea derivative compounds, potentiometrically, one pK_a value in the range of 9.82–10.19 for each of the benzoylthiourea derivatives in 75:25 (v/v) dioxane–water was reported by Schröder et al. (65). In addition, for each 5,5-diphenylpyrrolidine *N*-aroylthiourea compound in 30:70 (v/v) acetonitrile–water, potentiometrically two pK_a values, which were in the range of 6.96±0.03 to 7.84±0.04 associated with enol and in the range of 8.29±0.02 to 9.94±0.08 associated with enthiol groups, were reported (66). In other study, for each pyrrolidine *N*-aroylthioureas in 25:75 (v/v) DMSO–water, potentiometrically two pK_a values, which were in the range of 5.85±0.08 to 6.06±0.06 associated with enol, in the range of 8.37±0.05 to 8.87±0.05 associated with enthiol groups, and in the range of 10.11±0.03 to 11.62±0.03 associated with NH, were reported (67). The synthesized bis-acyl thiourea derivative compounds potentially have eight different pK_a values, the pK_a values of the symmetrical same groups are expected to be very close to each other, and their experimental determination is difficult. The pK_a values of four NH groups, together with two enols and enthiols, can be expected due to protonation in the acidic medium. It can be said that the pK_{a2} value determined as potentiometrically 7.06±0.13 and 6.94±0.11 and spectrophotometrically 7.11±0.08 and 7.05±0.01, respectively for compounds **5** and **6** may be related to the enthiol group in the structure. Similarly, it can be said that the pK_{a3} value determined as potentiometrically 12.11±0.06 and 11.17±0.06 and spectrophotometrically 12.30±0.08 and 11.82±0.02, respectively for compounds **5** and **6** may be related to the NH. It can be said that the pK_{a1} value, which could not be determined potentiometrically for compounds **5** and **6**, but determined as 3.56±0.08 and 3.87±0.01 spectrophotometrically, respectively, may be related to the enol group in the structure. It can be said that the fact that NO_2, which is the electron-withdrawing group, is attached to the phenyl ring increases the acidity of the enol group significantly. Furthermore, when the NO_2 group is attached to the aromatic ring in the para position instead of the meta position, it is seen that the pK_{a1} value decreases, while the pK_{a2} and pK_{a3} values decrease.

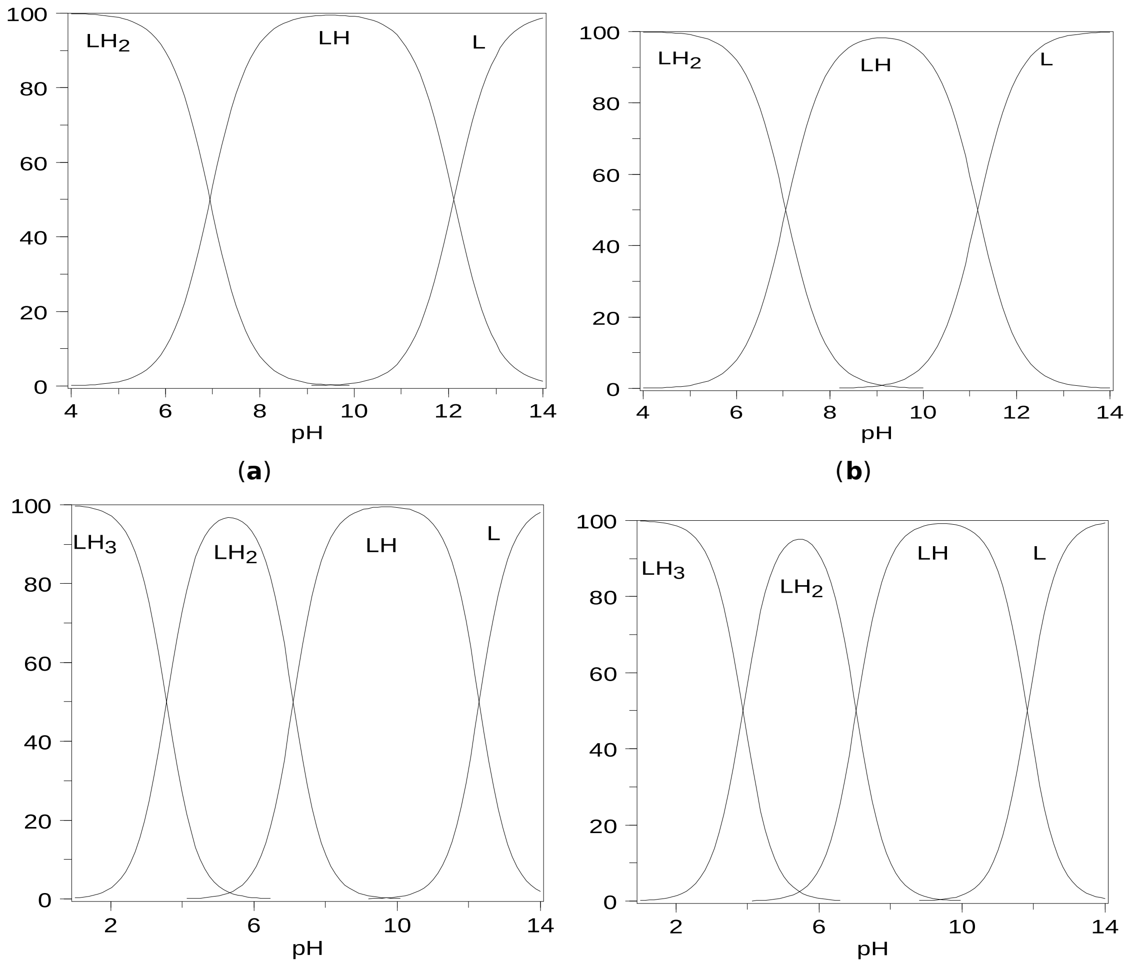
Table 1. The Potentiometric and spectrophotometric pK_a values of bis-acyl thioureas **5**, **6** (25.0 ± 0.1 °C, I = 0.1 mol·L^{-1} NaCl, 50% (v/v) DMSO-water)

Ligand	Spectrophotometric pK_a		Potentiometric pK_a
5	pK_{a1}	3.56±0.08	-
	pK_{a2}	7.11±0.08	7.06±0.13
	pK_{a3}	12.30±0.08	12.11±0.06
6	pK_{a1}	3.87±0.01	-
	pK_{a2}	7.05±0.01	6.94±0.11
	pK_{a3}	11.82±0.02	11.17±0.06

While three species formulated as LH_3 ($C_{28}H_{39}N_6O_6S_2^{3+}$), LH_2 ($C_{28}H_{38}N_6O_6S_2^{2+}$), and LH ($C_{28}H_{37}N_6O_6S_2^{+}$) were determined in the calculations performed using the HypSpec program from the spectrophotometric data, two species formulated as LH_2 and LH were determined in the calculations performed using the HYPERQUAD program in the potentiometric data. Deprotonation equilibrium for the ligands is shown in Eq. (1) and deprotonation constants in Eq. (2), omitting charges for simplicity (68). The potentiometric and spectrophotometric distribution curves of bis-acyl thioureas 5, 6 in a 50:50 (v/v) DMSO–water mixture are given in Figure 1.

$$LH_n \rightleftharpoons LH_{n-1}+H \qquad \text{(Eq. 1)}$$

$$K_n = [LH_{n-1}][H]/[LH_n] \qquad \text{(Eq. 2)}$$



(**a**) (**b**)

(**c**) (**d**)

Figure 1: Potentiometric distribution curves [(a) **5**, (b) **6**] and spectrophotometric distribution curves [(c) **5**, (d) **6**] of **5** and **6** (50% (v/v) DMSO-water, 25.0±0.1 °C, I = 0.1 mol·L^{-1} by NaCl).

4. CONCLUSION

The synthesis and characterization of bis-acyl thiourea derivatives **5**, **6**, which may have potential bioactivity and chemosensor properties as well as can be ligands for the coordination of metal ions, were demonstrated. In addition, pK_a values, which carry critical information for future studies on such compounds, were calculated with HYPERQUAD and HypSpec programs using potentiometric and spectrophotometric titration data, respectively.

5. ACKNOWLEDGMENTS

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