JOTCS Journal of the Turkish Chemical Society Section: A Chemistry
TURKISH CHEMICAL SOCIETY

Synthesis and Determination of Acid Dissociation Constants in Dimethyl Sulfoxide–Water Hydro-organic Solvent of 5,5-Diphenylpyrrolidine *N*-aroylthiourea Derivatives

Yahya Nural[1]*

[1]Mersin University, Faculty of Pharmacy, Department of Analytical Chemistry, 33169, Mersin, Turkey

Abstract: Novel 5,5-diphenylpyrrolidine *N*-aroylthioureas, containing 4-methylbenzoyl, 2-chlorobenzoyl, 2,4-dichlorobenzoyl, and 2-naphthoyl were synthesized, and their structural analyses were performed using ^{1}H Nuclear Magnetic Resonance (NMR), ^{13}C NMR, Fourier transform infrared spectroscopy (FT-IR), mass spectrometry (MS), and high-resolution MS (HRMS) techniques. The acid dissociation constants of the 5,5-diphenylpyrrolidine *N*-aroylthiourea derivative compounds were determined using Hyperquad computer program for data obtained using potentiometric titration method in 25% (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, using sodium hydroxide base as a titrant. Two acid dissociation constants were obtained for **3b** and **3d**, and it was suggested that they were related to N-H and enol groups. Furthermore, three acid dissociation constants obtained for **3a** indicated that they were related to N-H, enthiol, and enol groups, and four acid dissociation constants obtained for **3c** suggested that they were related to N-H, enthiol, enol, and carboxyl groups.

Keywords: Acid dissociation constant, Potentiometric titration, 5,5-Diphenylpyrrolidine, Aroylthiourea.

Submitted: July 31, 2017. **Accepted:** August 10, 2017.

Cite this: Nural Y. Synthesis and Determination of Acid Dissociation Constants in Dimethyl Sulfoxide–Water Hydro-organic Solvent of 5,5-Diphenylpyrrolidine N-aroylthiourea Derivatives. JOTCSA. 2017;4(3):841–54.

DOI: To be assigned.

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

INTRODUCTION

The compounds that contain one or more nitrogen, oxygen, and sulfur atoms as heteroatom in their structures represent a significant part of the drug molecules (1). The *N*-aroyl(acyl)thiourea derivatives, represented by the general formula $R_1C(O)NHC(S)NR_2R_3$, contain at least two nitrogen, one oxygen, and one sulfur atoms. *N*-Aroyl(acyl)thioureas have been extensively studied in the fields of pharmaceutical chemistry, coordination chemistry, and analytical chemistry (2-4). It has been known that the compounds containing the *N*-aroyl(acyl)thioureas exhibit various pharmacological properties such as antimicrobial (5-7), anticancer (8, 9), cholinesterase (10), and urease inhibitors (11). In addition, Ni(II) and Pt(II) complexes of the *N*-aroyl(acyl)thioureas exhibit antimicrobial (6, 12, 13) and antitumor (14, 15) activities and Co(III) complexes of the *N*-aroyl(acyl)thioureas exhibit anticancer and antileishmanial activities (16). Furthermore, the *N*-aroyl(acyl)thioureas are also used as chemosensors (17) and in the extraction of some heavy metals (18, 19). The pyrrolidine ring, a five-membered heterocyclic moiety containing nitrogen, is another important pharmacophore extensively used in drug research due to the presence of the structure of many natural and synthetic bioactive compounds (20-22). It has been known that many compounds containing the pyrrolidine ring exhibit antimicrobial (23, 24), antitumor (24-26), and antiviral (27, 28) activities.

Acid dissociation constant (pK_a) is considered to be an important physicochemical parameter that can be used to understand the nature of molecules because it can provide valuable information about many properties, such as acidity, solubility, and hydrogen-bonding capacity, of the compounds (29-31). Acid dissociation constants provide relevant information for studies in the field of pharmaceutical chemistry because most of the drug molecules gain and/or lose a proton in aqueous media (30, 32-34). Acid dissociation constant is one of resorted data for the studies on molecular modeling and drug design and those determining the optimal conditions of analysis methods (35-37). Many studies in the literature focused on determining the acidity of the constants of the compounds (29-40).

As a continuation of our previous studies (5, 41), this study reports the synthesis and determination of acid dissociation constants of 5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives in dimethyl sulfoxide–water hydro-organic solvent.

MATERIALS AND METHODS

Materials and Instrumentation

All used chemicals were of reagent grade. They were purchased from Merck or Aldrich and used without further purification. FT-IR spectra were recorded in the range of 4000–600 cm^{-1} with a Varian Scimitar Series 1000 Fourier transform infrared (FT-IR) spectrometer using horizontal attenuated total reflectance. Nuclear magnetic resonance (NMR) spectra were determined at 400 MHz on a Bruker Ultrashield Plus Biospin GmbH. Melting points were determined on Stuart SMP3 hot stage apparatus and were uncorrected. Mass spectra were recorded using an Agilent 6460 Triple Quad LC/MS/MS mass spectrometer. High-resolution mass spectra were recorded by the time of flight liquid chromatography–mass spectrometry electro-spray ionization technique. Potentiometric titrations were performed using a Titroline 7000 automated titrator with SI-Analytics combined with a glass pH electrode, which could be controlled by a computer and had an automatic micro-burette.

Synthesis

The 5,5-diphenylpyrrolidine *N*-aroylthiourea derivative novel compounds **3a–d** were synthesized by modifying the literature procedures [5, 42]. They were synthesized by the reaction of the dimethyl 5,5-diphenylpyrrolidine-2,4-dicarboxylate **1** [5] and the substituted isothiocyanate **2** [42] in acetone at reflux temperature for 36 h. Upon the reaction completion monitoring by TLC, the solvent was removed using a rotary evaporator, and the mixture was quenched with saturated aqueous NaCl and extracted with dichloromethane. The crude product was purified using column chromatography (ethyl acetate:hexane, 1:4). Structural characterization was performed using ^{1}H NMR, ^{13}C NMR, FT-IR, MS, and HRMS.

Dimethyl (*2S, 4S*)-1-((4-methylbenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**3a**). Yellow crystals. Yield, 0,45 g, 87%. m.p.: 143-145 °C. FT-IR (cm^{-1}): υ_{max} 3340, 3075, 2982, 1733, 1701, 1481, 1349, 1266, 1207. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (brs, 1H, NH), 7.93 (d, 2H, *J*= 7.2 Hz, Ar-H), 7.57-7.27 (m, 8H, Ar-H), 6.93 (d, 2H, *J*= 7.8 Hz, Ar-H), 6.76 (d, 2H, *J*= 7.9 Hz, Ar-H), 5.39 (dd, 1H, *J*= 8.3 Hz, 8.3 Hz, 2-H), 4.03 (dd, 1H, *J*= 8.1 Hz, 8.1 Hz, 4-H), 3.88 (s, 3H, OC*H*$_3$), 3.39 (s, 3H, OC*H*$_3$), 2.46-2.41 (m, 2H, 3-H, 3-H'), 3.28 (s, 3H, C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.0 (C=S), 170.7 (C=O), 169.3 (C=O), 163.5 (C=O), 143.0, 140.1, 135.8, 130.5, 129.4 (3 x C), 129.0 (4 x C), 128.9 (2 x C), 128.6, 127.8 (2 x C), 127.1 (2 x C), 79.7, 64.9, 61.1, 52.6, 52.0, 28.7, 21.4. MS (ESI, M-H^+): m/z 515.4 (M-H^+, 100). HRMS (ESI-TOF-MS): calcd. for $C_{29}H_{28}N_2O_6S$ $[MH]^+$ 517.1797; found 517.1786.

Dimethyl (*2S, 4S*)-1-((2-chlorobenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**3b**). Yellow crystals. Yield, 0,46 g, 89%. m.p.: 191-193 °C. FT-IR (cm^{-1}): υ_{max} 3336, 3064, 2981, 1736, 1692, 1470, 1331, 1254, 1209. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (brs,

1H, NH), 7.93 (d, 2H, *J*= 7.4 Hz, Ar-H), 7.47-7.02 (m, 10H, Ar-H), 6.50 (d, 2H, *J*= 7.6 Hz, Ar-H), 5.26 (dd, 1H, *J*= 7.6 Hz, 9.4 Hz, 2-H), 4.03 (dd, 1H, *J*= 6.5 Hz, 10.4 Hz, 4-H), 3.85 (s, 3H, OC*H*$_3$), 3.40 (s, 3H, OC*H*$_3$), 2.46-2.32 (m, 2H, 3-H, 3-H'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2 (C=S), 170.4 (C=O), 169.1 (C=O), 164.5 (C=O), 140.3, 135.0, 134.1, 131.3, 130.9, 129.9, 129.3, 129.2, 129.1 (4 x C), 128.9 (2 x C), 128.7, 128.0 (2 x C), 126.3, 79.7, 65.0, 60.8, 52.6, 52.0, 28.6. MS (ESI, M-H$^+$): m/z 535.2 (M-H$^+$, 100), 537.2 (M-H$^+$, 35). HRMS (ESI-TOF-MS): calcd. for $C_{28}H_{25}ClN_2O_5S$ $[MH]^+$ 537.1251; found 537.1274.

Dimethyl (*2S, 4S*)-1-((2,4-dichlorobenzoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**3c**). Yellow crystals. Yield, 0,51 g, 89%. m.p.: 152-154 °C. FT-IR (cm^{-1}): υ_{max} 3334, 3082, 2954, 1732, 1710, 1469, 1326, 1261, 1209. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 1H, NH), 7.82 (s, 2H, Ar-H), 7.51-7.36 (m, 8H, Ar-H), 7.20 (d, 1H, *J*= 1.6 Hz, Ar-H), 7.00 (dd, 1H, *J*= 1.6 Hz, 8.3 Hz, Ar-H), 6.31 (d, 1H, *J*= 8.3 Hz, Ar-H), 5.23 (dd, 1H, *J*= 7.3 Hz, 9.8 Hz, 2-H), 4.06 (dd, 1H, *J*= 6.3 Hz, 10.8 Hz, 4-H), 3.84 (s, 3H, OC*H*$_3$), 3.41 (s, 3H, OC*H*$_3$), 2.47-2.31 (m, 2H, 3-H, 3-H'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2 (C=S), 170.3 (C=O), 168.9 (C=O), 164.2 (C=O), 140.4, 136.7, 134.8, 132.6, 132.1, 130.1, 129.8, 129.5, 129.2 (2 x C), 129.1 (2 x C), 128.9 (2 x C), 128.8, 128.1 (2 x C), 126.6, 79.7, 65.1, 60.7, 52.6, 52.1, 28.7. MS (ESI, M-H$^+$): m/z 569.2 (M-H$^+$, 100), 570.2 (M-H$^+$, 30), 571.2 (M-H$^+$, 75). HRMS (ESI-TOF-MS): calcd. for $C_{28}H_{24}Cl_2N_2O_5S$ $[MH]^+$ 571.0861; found 571.0864.

Dimethyl (*2S, 4S*)-1-((2-naphthoyl)carbamothioyl)-5,5-diphenylpyrrolidine-2,4-dicarboxylate (**3d**). Yellow crystals. Yield, 0,52 g, 94%. m.p.: 197-199 °C. FT-IR (cm^{-1}): υ_{max} 3333, 3055, 2981, 1732, 1694, 1485, 1347, 1260, 1213. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (brs, 1H, NH), 7.97 (d, 2H, *J*= 3.8 Hz, Ar-H), 7.98-7.15 (m, 15H, Ar-H), 5.40 (dd, 1H, *J*= 8.4 Hz, 8.4 Hz, 2-H), 4.07 (dd, 1H, *J*= 8.2 Hz, 8.2 Hz, 4-H), 3.90 (s, 3H, OC*H*$_3$), 3.40 (s, 3H, OC*H*$_3$), 2.49-2.45 (m, 2H, 3-H, 3-H'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9 (C=S), 170.6 (C=O), 169.3 (C=O), 163.6 (C=O), 140.2, 135.7, 135.0, 130.7, 129.5, 129.4 (2 x C), 129.2 (2 x C), 129.0 (2 x C), 128.9, 128.7, 128.3, 128.2, 127.8 (2 x C), 127.7 (2 x C), 127.3, 126.7, 123.7, 79.7, 64.9, 61.0, 52.6, 52.1, 28.8. MS (ESI, M-H$^+$): m/z 551.3 (M-H$^+$, 100). HRMS (ESI-TOF-MS): calcd. for $C_{32}H_{28}N_2O_5S$ $[MH]^+$ 553.1797; found 553.1793.

Determination of Acid Dissociation Constants

The pK_a values of the ligands were determined using the titrator, controlled with a computer, with an ultra-combination pH electrode. The electrode system was calibrated with potassium hydrogen phthalate and sodium tetraborate (43-45). Stock solutions of the ligands were prepared with a concentration of 1×10^{-3} mol·L^{-1} in dimethyl sulfoxide (DMSO) and stock solutions of NaOH, HCl, and NaCl were prepared with concentrations of 0.025, 0.1, and 1.0 mol·L^{-1} in deionized water, respectively. Nitrogen gas (99.9%) was passed through the titration cell during the titration process. The pK_a values were calculated with Hyperquad, which was one of the most

important computer programs in this area (46), using the data obtained from potentiometric titration.

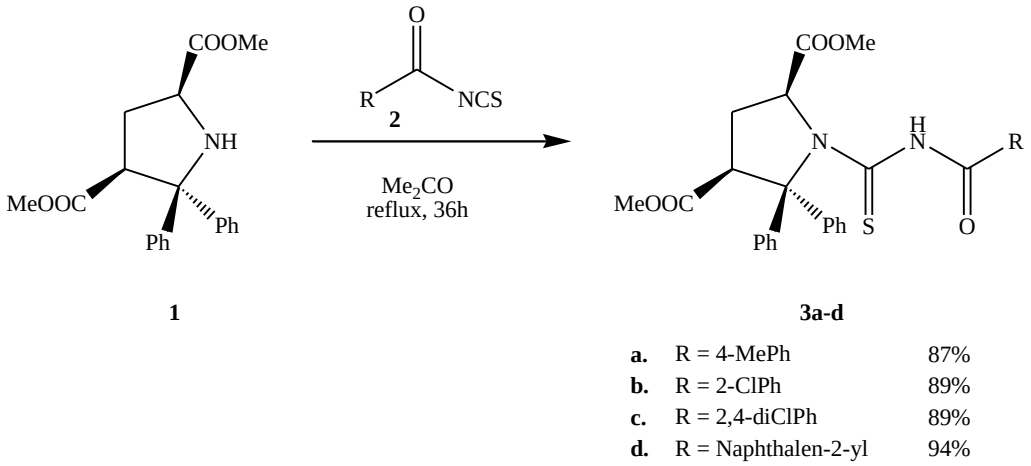
Potentiometric titrations were performed in a double-walled glass titration cell. The temperature was kept constant at 25.0±0.1 °C using a thermostat. The solution in the titration cell was stirred at a constant rate throughout the titration using a magnetic stirrer. The titration cell was washed and dried before and after each titration. The titration cell was kept closed during titration. Air bubbles were removed before each titration. The syringe was washed several times with deionized water and then the base solution. Then, 8 mL of the stock ligand solution and 2 mL of DMSO solution were added to the titration cell. After that, 1 mL of the stock HCl solution and 4 mL of the NaCl solution were added to the titration cell, and the cell was filled to 40 mL with deionized water. Titration process with the addition of 0.04 mL of the stock NaOH solution was carried out using an automatic titrator and computer program. Titration process for each ligand was repeated three times.

RESULTS AND DISCUSSION

Synthesis

The (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives **3a–d** were synthesized by the reaction of the dimethyl 5,5-diphenylpyrrolidine-2,4-dicarboxylate **1** [5], which was obtained by the cycloaddition reaction of methyl 2-(diphenylmethyleneamino)acetate and methyl acrylate, and the substituted isothiocyanates **2,** which were prepared with potassium thiocyanate and acyl chloride (41) in excellent yield (87-94%) (**Scheme 1**). Structural characterization of the compounds was carried out using ^{1}H NMR, ^{13}C NMR, FT-IR, MS and HRMS techniques.

In the FT-IR spectra, NH stretching vibrations of **3a–d** were observed in the range of 3333–3340 cm^{-1}. The FT-IR bands of **3a–d**, observed in the range of 1732–1736 cm^{-1} and 1254–1266 cm^{-1}, were assigned to the ester ν(C=O) and ν(C–O), respectively. In addition, the amide C=O vibration band of **3a–d** was observed in the range of 1692–1710 cm^{-1} due to conjugation with the aromatic moiety. Furthermore, vibration bands of the ν(C=S) and ν (C–S) of **3a–d** were assigned in the range of 1326–1349 cm^{-1} and 1207–1213 cm^{-1}, respectively.



1 **3a-d**

a.	R = 4-MePh	87%
b.	R = 2-ClPh	89%
c.	R = 2,4-diClPh	89%
d.	R = Naphthalen-2-yl	94%

Scheme 1: Synthesis of the (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthioureas.

The (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthiourea derivatives **3a–d** were characterized using ^{1}H and ^{13}C NMR spectra. The C(O)NHC(S) proton signals for **3a–d** were observed as a singlet in the range of 7.83–8.33 ppm. Shift values of proton signals of the pyrrolidine ring were observed as expected. In the ^{13}C NMR spectra of **3a–d**, the shift value of the carbon signal of the thiocarbonyl group, which was obtained by the reaction of 5,5-diphenylpyrrolidine with 4-substituted-benzoylisothiocyanate, was observed in the range of 177.24–178.0 ppm and shift values of carbon signals of three carbonyl groups were observed in the range of 163.5–170.7 ppm.

Acid dissociation constants

The pK_a values of (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthioureas **3a–d** were determined potentiometrically in a 25% (v/v) DMSO–water hydro-organic solvent system at 25.0±0.1 °C (**Table 1**). Titration process was performed in the acidic medium, and ionic strength was kept constant at 0.1. As a result of the calculations, three different pK_a values 5.87±0.06, 8.87±0.05, and 11.62±0.03 were determined for **3a**. Two different pK_a values were determined for **3b** and **3d**, and pK_{a1} was 5.85±0.08 and 6.06±0.06, and pK_{a2} was 10.65±0.03 and 11.04±0.03, respectively. In addition, four different pK_a values 2.53±0.08, 5.70±0.06, 8.37±0.05, and 10.11±0.03 were determined for **3c**. Schröder *et al.* [47] determined, potentiometrically, one pK_a value in the range of 9.82–10.19 for each of the benzoylthiourea derivatives in 75% dioxane–water mixture. Binzet *et al.* [48] reported a pK_a value in the range of 8.79 (±0.02)–9.62 (±0.05) for each *N*-aroylthiourea compound in a 50% (v/v) dioxane–water mixture. The previous study [41] reported three potentiometrically different pK_a values for each (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthiourea compound in the 30% (v/v) acetonitrile–water hydro-organic solvent system. The present study suggested that the pK_{a1} values in the range of 3.49±0.07 to 5.19±0.04 were related to carboxyl, the pK_{a2} values in the range of 6.96±0.03 to

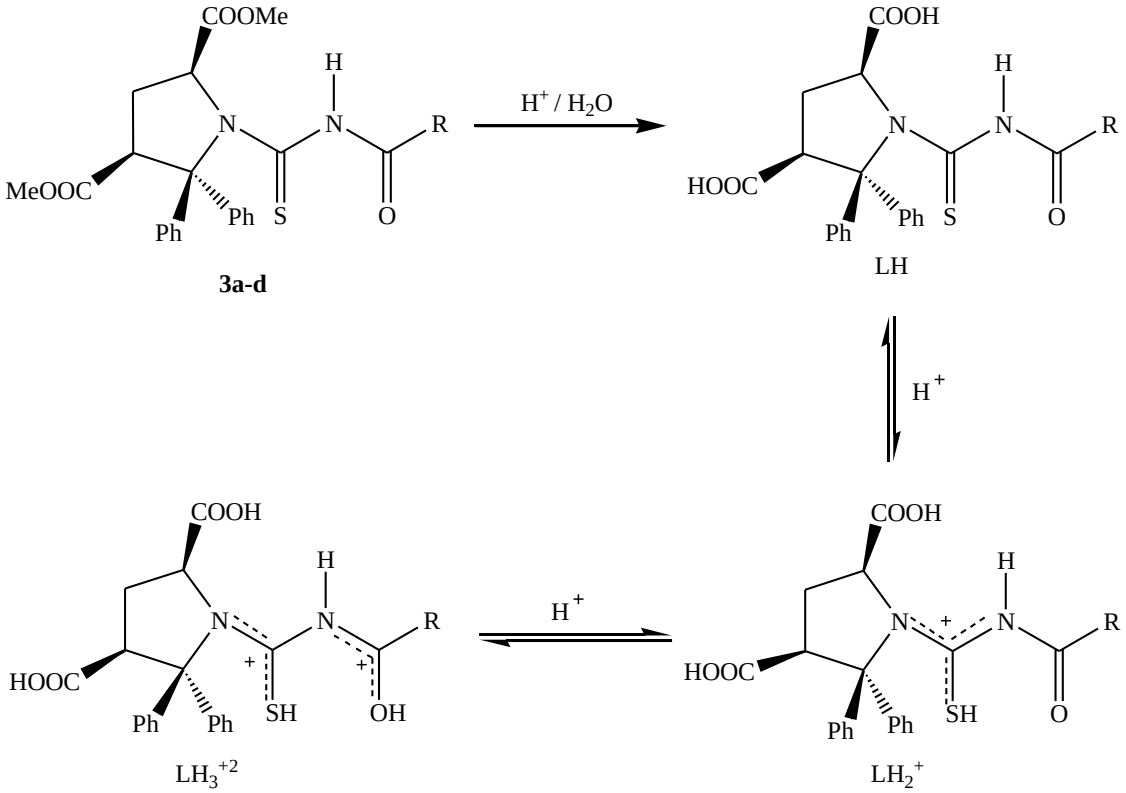
7.84±0.04 were related to enol, and the pK_{a3} values in the range of 8.29±0.02 to 9.94±0.08 were related to enthiol groups.

Table 1: pK_a values of the ligands (25% (v / v) DMSO-water mixture, 25.0 ± 0.1 °C, I = 0.1 mol·L^{-1} by NaCl) ($\log_{10}\beta$ is cumulative acid dissociation constants).

Ligand	Species	$\log_{10}\beta$	pK_a values
	LH_3	26.36±0.06	5.87±0.06
3a	LH_2	20.49±0.05	8.87±0.05
	LH	11.62±0.03	11.62±0.03
	LH_2	16.50±0.08	5.85±0.08
3b	LH	10.65±0.03	10.65±0.03
	LH_4	26.71±0.08	2.53±0.08
	LH_3	24.18±0.06	5.70±0.06
3c	LH_2	18.48±0.05	8.37±0.05
	LH	10.11±0.03	10.11±0.03
	LH_2	17.10±0.06	6.06±0.06
3d	LH	11.04±0.03	11.04±0.03

When the hydrolysis of methyl esters of the compounds in acidic media and the proposed protonation mechanisms were examined, five ionizable groups were identified in **Scheme 2**. In this study, the pK_{a1} value was found as 11.63±0.03 when the pK_a values determined in the 25% (v/v) DMSO–water hydro-organic solvent system was examined for the compound **3a** containing 4-MePh as R group, and it could be said that the constant was related to the N-H group. The pK_{a2} and pK_{a3} values were found to be 8.87±0.05 and 5.87±0.06, respectively, and it could be said that these constants were related to the enthiol and enol groups, respectively. For the compound **3b** containing 2-ClPh as R group, two different pK_a values were determined as 10.65±0.03 and 5.85±0.08; it was suggested that these constants were related to N-H and enol groups, respectively. For **3c** containing 2,4-diClPh as R group, four different pK_a values were obtained as 10.11±0.03, 8.37±0.05, 5.70±0.06, and 2.53±0.08; it was suggested that these constants were related to N-H, enthiol, enol, and carboxyl groups, respectively. Two different pK_a values were determined as 11.04±0.03 and 6.06±0.06, for **3d** containing 2-naphthoyl as R group, and it was suggested that these constants were related to N-H and enol groups, respectively. When the acid dissociation constants were compared concerning the R groups bound to the compounds, the N-H group of **3a** having a methyl group on the phenyl ring was more basic than the N-H groups of other compounds as expected. Similarly, the N-H group of **3c** containing 2,4-dichlorophenyl group as R group was less basic than the N-H groups of other compounds as expected. Additionally, it could be said that **3c** having chlorine atoms at the ortho and para positions of the phenyl ring instead of the methyl group at the para position of the phenyl ring had a less basic character than **3a**. On comparing the pK_a values of the enol groups determined for all compounds, the pK_a value of **3c** was found to be lower than those of the others as expected. The pK_a value 2.53±0.08, which was determined for only the compound **3c**,

it was related to the carboxyl group, indicating that the methyl ester of (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthiourea derivative compounds was hydrolyzed in the acidic medium. The acid dissociation constants of the carboxyl groups of the compounds **3a**, **3b,** and **3d** could not be determined because their pK_a values were not in the range of the limit of detection of the glass electrode.



Scheme 2: Hydrolysis of methyl esters of (*2S, 4S*)-5,5-diphenylpyrrolidine *N*-aroylthioureas **3a–d** in acidic medium and proposed mechanism for their protonation.

As a result of the calculation performed using the Hyperquad program, four deprotonated species formulated as LH4, LH3, LH2, and LH were determined. The deprotonation equilibrium for the ligands is shown in Eq. (1) (charges are omitted for simplicity), and the deprotonation constants (K_n) are shown in Eq. (2) [49].

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

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

While increasing the pH value of the medium, the protonated ligands lost its protons and converted into another species of the ligand (see **Figure 1**). The titration curves of **3a–d** and the distribution curves of species H in a 25% (v/v) DMSO–water mixture are given in **Figure1**.

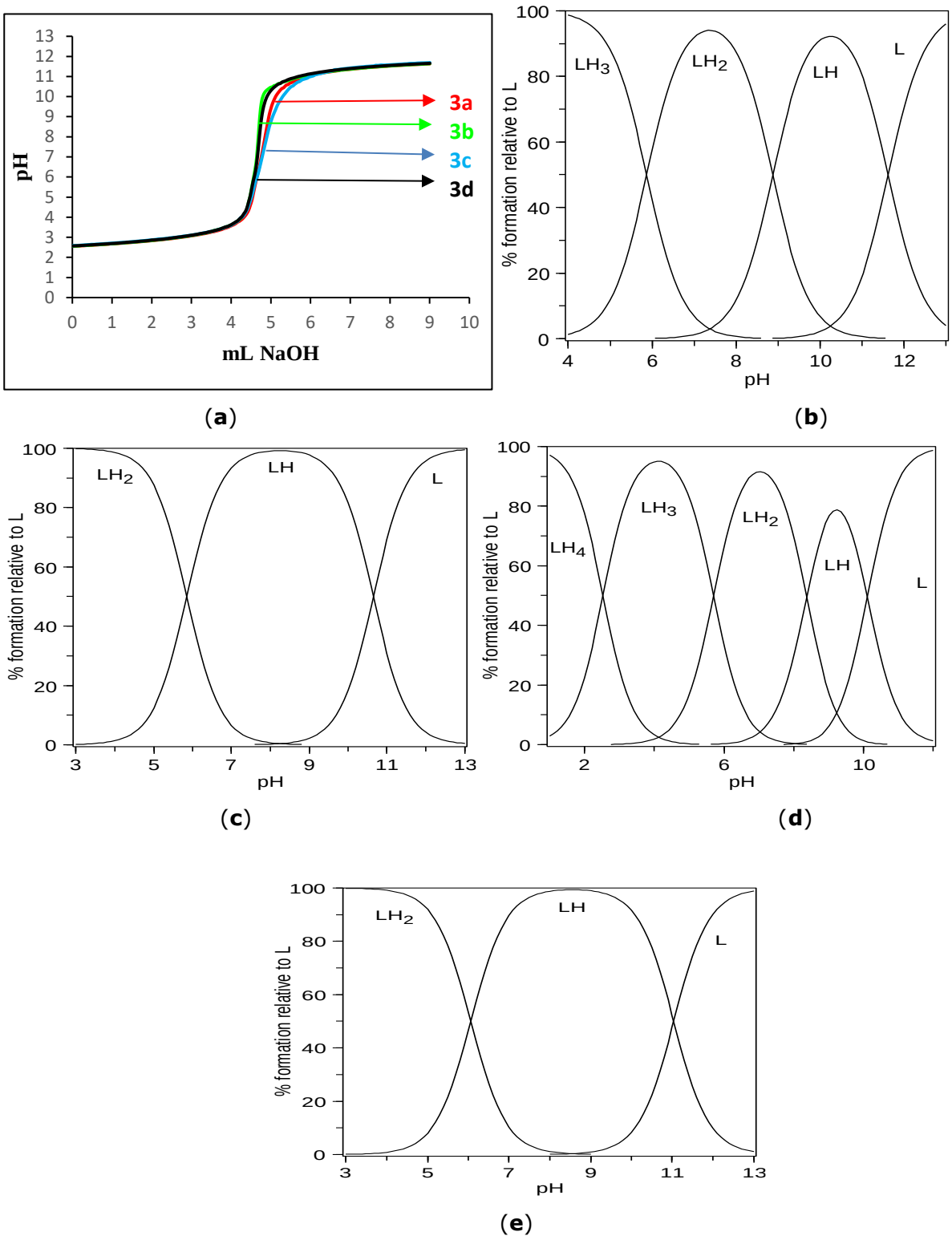


Figure 1: Potentiometric titration curves (a) and distribution curves of the ligands [(b) **3a**, (c) **3b**, (d) **3c**, and (e) **3d** in a 25% (v/v) DMSO–water mixture].

ACKNOWLEDGMENTS

The author thanks the Scientific and Technological Research Council of Turkey (TÜBİTAK, project grant 214Z289) for financial support and Mersin University Advanced Technology Education, Research, and Application Center (MEITAM). The author would like to give special thanks to Prof.

Dr. Hayati Sarı (Gaziosmanpasa University, Faculty of Science and Arts, Department of Chemistry) for his help in calculating acid dissociation constants.

REFERENCES

1. Ilardi EA, Vitaku E, Njardarson JT. Data-Mining for Sulfur and Fluorine: An Evaluation of Pharmaceuticals To Reveal Opportunities for Drug Design and Discovery: Miniperspective. Journal of Medicinal Chemistry. 2014 Apr 10;57(7):2832–42.

2. Saeed A, Qamar R, Fattah TA, Flörke U, Erben MF. Recent developments in chemistry, coordination, structure and biological aspects of 1-(acyl/aroyl)-3-(substituted) thioureas. Research on Chemical Intermediates. 2017 May;43(5):3053–93.

3. Saeed A, Flörke U, Erben MF. A review on the chemistry, coordination, structure and biological properties of 1-(acyl/aroyl)-3-(substituted) thioureas. Journal of Sulfur Chemistry. 2014 May 4;35(3):318–55.

4. Koch KR. New chemistry with old ligands: N-alkyl- and N,N-dialkyl-N'-acyl(aroyl)thioureas in co-ordination, analytical and process chemistry of the platinum group metals. Coordination Chemistry Reviews. 2001 Jun;216–217:473–88.

5. Erşen D, Ülger M, Mangelinckx S, Gemili M, Şahin E, Nural Y. Synthesis and anti(myco)bacterial activity of novel 5,5-diphenylpyrrolidine N-aroylthiourea derivatives and a functionalized hexahydro-1H-pyrrolo[1,2-c]imidazole. Medicinal Chemistry Research. 2017 Sep;26(9):2152–60.

6. Gemili M, Sari H, Ulger M, Sahin E, Nural Y. Pt(II) and Ni(II) complexes of octahydropyrrolo[3,4-c]pyrrole N -benzoylthiourea derivatives: Synthesis, characterization, physical parameters and biological activity. Inorganica Chimica Acta. 2017 Jul;463:88–96.

7. Saeed A, Zaib S, Pervez A, Mumtaz A, Shahid M, Iqbal J. Synthesis, molecular docking studies, and in vitro screening of sulfanilamide-thiourea hybrids as antimicrobial and urease inhibitors. Medicinal Chemistry Research. 2013 Aug;22(8):3653–62.

8. Koca İ, Özgür A, Er M, Gümüş M, Açikalin Coşkun K, Tutar Y. Design and synthesis of pyrimidinyl acyl thioureas as novel Hsp90 inhibitors in invasive ductal breast cancer and its bone metastasis. European Journal of Medicinal Chemistry. 2016 Oct;122:280–90.

9. Saeed S, Rashid N, Jones PG, Ali M, Hussain R. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. European Journal of Medicinal Chemistry. 2010 Apr;45(4):1323–31.

10. Saeed A, Shakil Shah M, Ali Larik F, Ullah Khan S, Ali Channar P, Flörke U, et al. Synthesis, computational studies and biological evaluation of new 1-acetyl-3-aryl thiourea derivatives as potent cholinesterase inhibitors. Medicinal Chemistry Research. 2017 Aug;26(8):1635–46.

11. Saeed A, Khan MS, Rafique H, Shahid M, Iqbal J. Design, synthesis, molecular docking studies and in vitro screening of ethyl 4-(3-benzoylthioureido) benzoates as urease inhibitors. Bioorganic Chemistry. 2014 Feb;52:1–7.

12. Nural Y, Kilincarslan R, Dondas HA, Cetinkaya B, Serin MS, Grigg R, et al. Synthesis of Ni(II), Pd(II) and Cu(II) metal complexes of novel highly functionalized aroylaminocarbo-N-thioyl pyrrolidines and their activity against fungi and yeast. Polyhedron. 2009 Sep;28(14):2847–54.

13. Binzet G, Arslan H, Flörke U, Külcü N, Duran N. Synthesis, characterization and antimicrobial activities of transition metal complexes of N,N -dialkyl- N′ -(2-chlorobenzoyl)thiourea derivatives. Journal of Coordination Chemistry. 2006 Aug 5;59(12):1395–406.

14. Yeşilkaynak T, Muslu H, Özpınar C, Emen FM, Demirdöğen RE, Külcü N. Novel thiourea derivative and its complexes: Synthesis, characterization, DFT computations, thermal and electrochemical behavior, antioxidant and antitumor activities. Journal of Molecular Structure. 2017 Aug;1142:185–93.

15. Sacht C, Datt M. Synthesis and characterisation of mixed-ligand platinum(II)–sulfoxide complexes, [PtCl(DMSO)(L)], for potential use as chemotherapeutic agents (HL=N,N-dialkyl-N′-(3-R-benzoyl)thiourea). Polyhedron. 2000 Jun;19(11):1347–54.

16. Yaseen S, Rauf MK, Zaib S, Badshah A, Tahir MN, Ali MI, et al. Synthesis, characterization and urease inhibition, in vitro anticancer and antileishmanial studies of Co(III) complexes with N,N,N′-trisubstituted acylthioureas. Inorganica Chimica Acta. 2016 Mar;443:69–77.

17. Li S, Cao X, Chen C, Ke S. Novel salicylic acid-oriented thiourea-type receptors as colorimetric chemosensor: Synthesis, characterizations and selective naked-eye recognition properties. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2012 Oct;96:18–23.

18. Alkherraz AM, Lusta ZI, Zubi AE. Synthesis and use of thiourea derivative (1-phenyl-3-benzoyl-2-thiourea) for extraction of cadmium ion. Int J Chem Mol Nucl Mater Metall Eng. 2014;8:108–110.

19. Luckay RC, Sheng X, Strasser CE, Raubenheimer HG, Safin DA, Babashkina MG, et al. Competitive bulk liquid membrane transport and solvent extraction of some metal ions using RC(S)NHP(X)(OiPr)2 (X = O, S) as ionophores. Formation of the polynuclear complex of [Ag(N□C–NP(S)(OiPr)2)]n. Dalton Transactions. 2009;(39):8227.

20. Li X, Li J. Recent Advances in the Development of MMPIs and APNIs Based on the Pyrrolidine Platforms. Mini-Reviews in Medicinal Chemistry. 2010 Aug 1;10(9):794–805.

21. Pandey G, Banerjee P, Gadre SR. Construction of Enantiopure Pyrrolidine Ring System via Asymmetric [3+2]-Cycloaddition of Azomethine Ylides. Chemical Reviews. 2006 Nov;106(11):4484–517.

22. Felpin F-X, Lebreton J. Recent Advances in the Total Synthesis of Piperidine and Pyrrolidine Natural Alkaloids with Ring-Closing Metathesis as a Key Step. European Journal of Organic Chemistry. 2003 Oct;2003(19):3693–712.

23. Hensler ME, Bernstein G, Nizet V, Nefzi A. Pyrrolidine bis-cyclic guanidines with antimicrobial activity against drug-resistant Gram-positive pathogens identified from a mixture-based combinatorial library. Bioorganic & Medicinal Chemistry Letters. 2006 Oct;16(19):5073–9.

24. Arun Y, Bhaskar G, Balachandran C, Ignacimuthu S, Perumal PT. Facile one-pot synthesis of novel dispirooxindole-pyrrolidine derivatives and their antimicrobial and anticancer activity against A549 human lung adenocarcinoma cancer cell line. Bioorganic & Medicinal Chemistry Letters. 2013 Mar;23(6):1839–45.

25. Arun Y, Saranraj K, Balachandran C, Perumal PT. Novel spirooxindole–pyrrolidine compounds: Synthesis, anticancer and molecular docking studies. European Journal of Medicinal Chemistry. 2014 Mar;74:50–64.

26. Mokale SN, Dube PN, Bhavale SA, Sayed I, Begum A, Nevase MC, et al. Synthesis, in-vitro screening, and docking analysis of novel pyrrolidine and piperidine-substituted ethoxy chalcone as anticancer agents. Medicinal Chemistry Research. 2015 May;24(5):1842–56.

27. Nájera C, Sansano JM. 1,3-Dipolar cycloadditions: applications to the synthesis of antiviral agents. Organic & Biomolecular Chemistry. 2009;7(22):4567.

28. Martín-Rodríguez M, Nájera C, Sansano JM, de Cózar A, Cossío FP. Chiral gold(I) vs chiral silver complexes as catalysts for the enantioselective synthesis of the second generation GSK-hepatitis C virus inhibitor. Beilstein Journal of Organic Chemistry. 2011 Jul 19;7:988–96.

29. Mazák K, Noszál B. Advances in microspeciation of drugs and biomolecules: Species-specific concentrations, acid-base properties and related parameters. Journal of Pharmaceutical and Biomedical Analysis. 2016 Oct;130:390–403.

30. Babić S, Horvat AJM, Mutavdžić Pavlović D, Kaštelan-Macan M. Determination of pKa values of active pharmaceutical ingredients. TrAC Trends in Analytical Chemistry. 2007 Dec;26(11):1043–61.

31. Roda G, Dallanoce C, Grazioso G, Liberti V, De Amici M. Determination of acid dissociation constants of compounds active at neuronal nicotinic acetylcholine receptors by means of electrophoretic and potentiometric techniques. Analytical Sciences. 2010;26(1):51–54.

32. Aslan N, Erden PE, Doğan A, Canel E, Kılıç E. Protonation Constants of Some Alanyl Dipeptides in Mixed Aqueous Organic Solvents. Journal of Solution Chemistry. 2016 Feb;45(2):299–312.

33. Dohoda D, Tsinman K, Tsinman O, Wang H, Tam KY. Spectrophotometric pKa determination of ionizable pharmaceuticals: Resolution of molecules with weak pH-dependent spectral shift. Journal of Pharmaceutical and Biomedical Analysis. 2015 Oct;114:88–96.

34. Shelley JC, Cholleti A, Frye LL, Greenwood JR, Timlin MR, Uchimaya M. Epik: a software program for pK a prediction and protonation state generation for drug-like molecules. Journal of Computer-Aided Molecular Design. 2007 Dec;21(12):681–91.

35. Grover M, Singh B, Bakshi M, Singh S. Quantitative structure–property relationships in pharmaceutical research – Part 1. Pharmaceutical Science & Technology Today. 2000 Jan;3(1):28–35.

36. Onufriev A, Case DA, Ullmann GM. A Novel View of pH Titration in Biomolecules †. Biochemistry. 2001 Mar;40(12):3413–9.

37. Şanli S, Altun Y, Şanli N, Alsancak G, Beltran JL. Solvent Effects on p K a values of Some Substituted Sulfonamides in Acetonitrile−Water Binary Mixtures by the UV-Spectroscopy Method. Journal of Chemical & Engineering Data. 2009 Nov 12;54(11):3014–21.

38. Nural Y, Döndaş HA, Sarı H, Atabey H, Belveren S, Gemili M. Determination of Acid Dissociation Constants (p K a) of Bicyclic Thiohydantoin-Pyrrolidine Compounds in 20% Ethanol-Water Hydro-organic Solvent. International Journal of Analytical Chemistry. 2014;2014:1–6.

39. Atabey H, Sari H. The effects of ionic strength and temperature on the dissociation constants of adefovir and cidofovir used as antiviral drugs. TURKISH JOURNAL OF CHEMISTRY. 2014;38:806–14.

40. Mumcu A, Küçükbay H. Determination of p K a values of some novel benzimidazole salts by using a new approach with 1 H NMR spectroscopy: p K a determination using new approches in NMR. Magnetic Resonance in Chemistry. 2015 Dec;53(12):1024–30.

41. Ersen D, Gemili M, Sarı H, Nural Y. Acid Dissociation Constants of 5, 5-Diphenylpyrrolidine N-Aroylthioureas and Stability Constants of their Pt (II) and Ni (II) Complexes in Acetonitrile-Water Hydro-organic Solvent. 2017;

42. Arslan H, Külcü N, Flörke U. Synthesis and characterization of copper (II), nickel (II) and cobalt (II) complexes with novel thiourea derivatives. Transition Metal Chemistry. 2003;28(7):816–819.

43. Pettit L. Academic Software. Sourby Farm, Timble, Otley, LS21 2PW, UK; 1992.

44. Mussini PR, Mussini T, Rondinini S. Reference value standards and primary standards for pH measurements in D2O and aqueousorganic solvent mixtures: New accessions and assessments (Technical Report). Pure and Applied Chemistry [Internet]. 1997 Jan 1 [cited 2017 Aug 1];69(5). Available from: https://www.degruyter.com/view/j/pac.1997.69.issue-5/pac199769051007/pac199769051007.xml

45. Rondinini S, Mussini PR, Mussini T. Reference value standards and primary standards for pH measurements in organic solvents and water + organic solvent mixtures of moderate to high permittivities. Pure and Applied Chemistry [Internet]. 1987 Jan 1 [cited 2017 Aug 1];59(11). Available from: https://www.degruyter.com/view/j/pac.1987.59.issue-11/pac198759111549/pac198759111549.xml

46. Gans P, Sabatini A, Vacca A. Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs. Talanta. 1996 Oct;43(10):1739–53.

47. Schröder B, Schröder U, Dietze F, Beyer L. Protonation, complexation and thermochemical behaviour of N-benzoylthiocarbamic-O-alkylester anions in solution. Inorganic Chemistry Communications. 2001 Aug;4(8):398–401.

48. Binzet G, Zeybek B, Kılıç E, Külcü N, Arslan H. Determination of the Ionization Constants of Some Benzoyl Thiourea Derivatives in Dioxane-Water Mixture. Journal of Chemistry. 2013;2013:1–7.

49. Atabey H, Sari H, Al-Obaidi FN. Protonation Equilibria of Carminic Acid and Stability Constants of Its Complexes with Some Divalent Metal Ions in Aqueous Solution. Journal of Solution Chemistry. 2012 Jun;41(5):793–803.