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

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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.

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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.

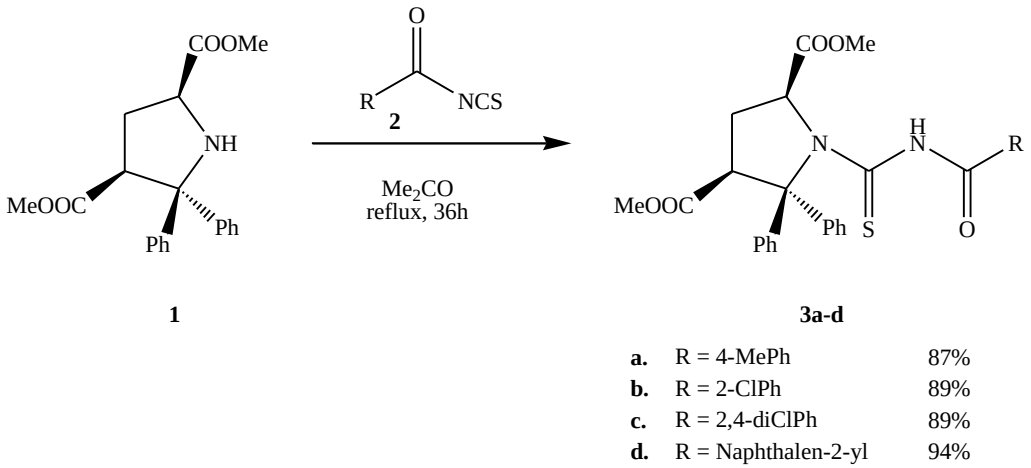
Potentiometric titrations were performed in a double-walled glass titration cell. The temperature was kept constant at 25.0±0.1 °C using a thermostat. The solution in the titration cell was stirred at a constant rate throughout the titration using a magnetic stirrer. The titration cell was washed and 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

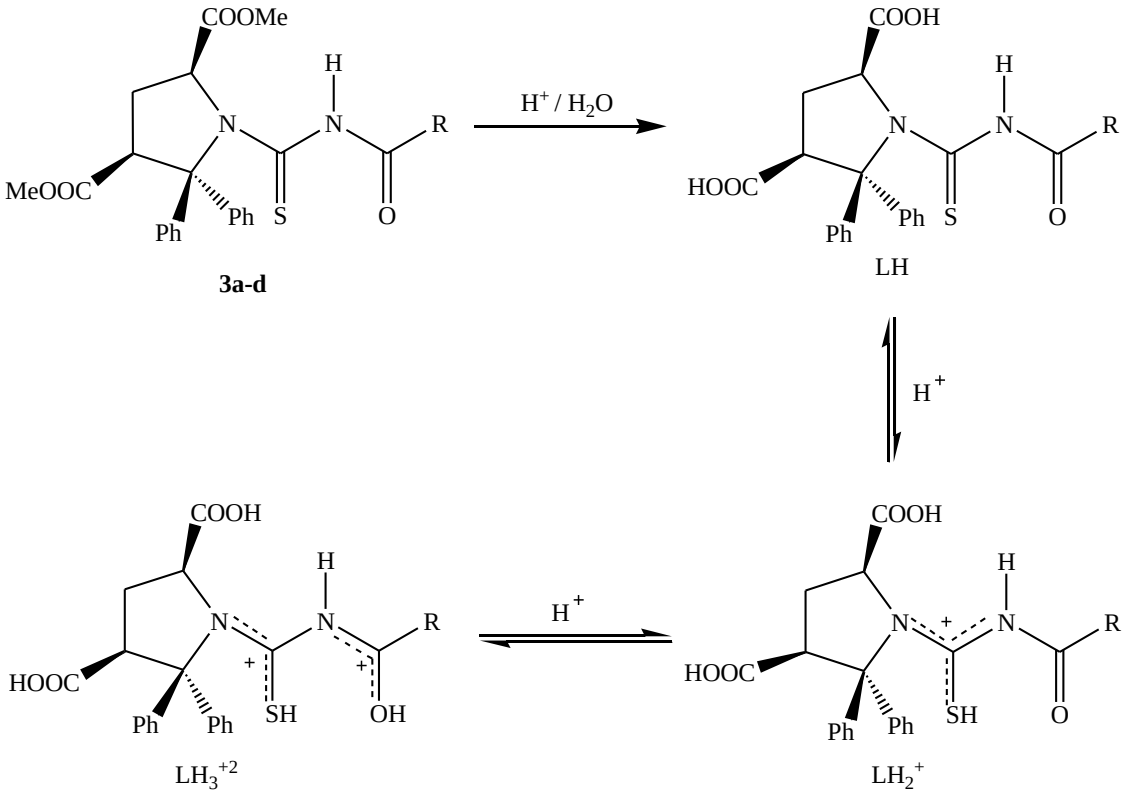
7.84±0.04 were related to enol, and the pK_{a3} values in the range of 8.29±0.02 to 9.94±0.08 were related to enthiol groups.

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**.

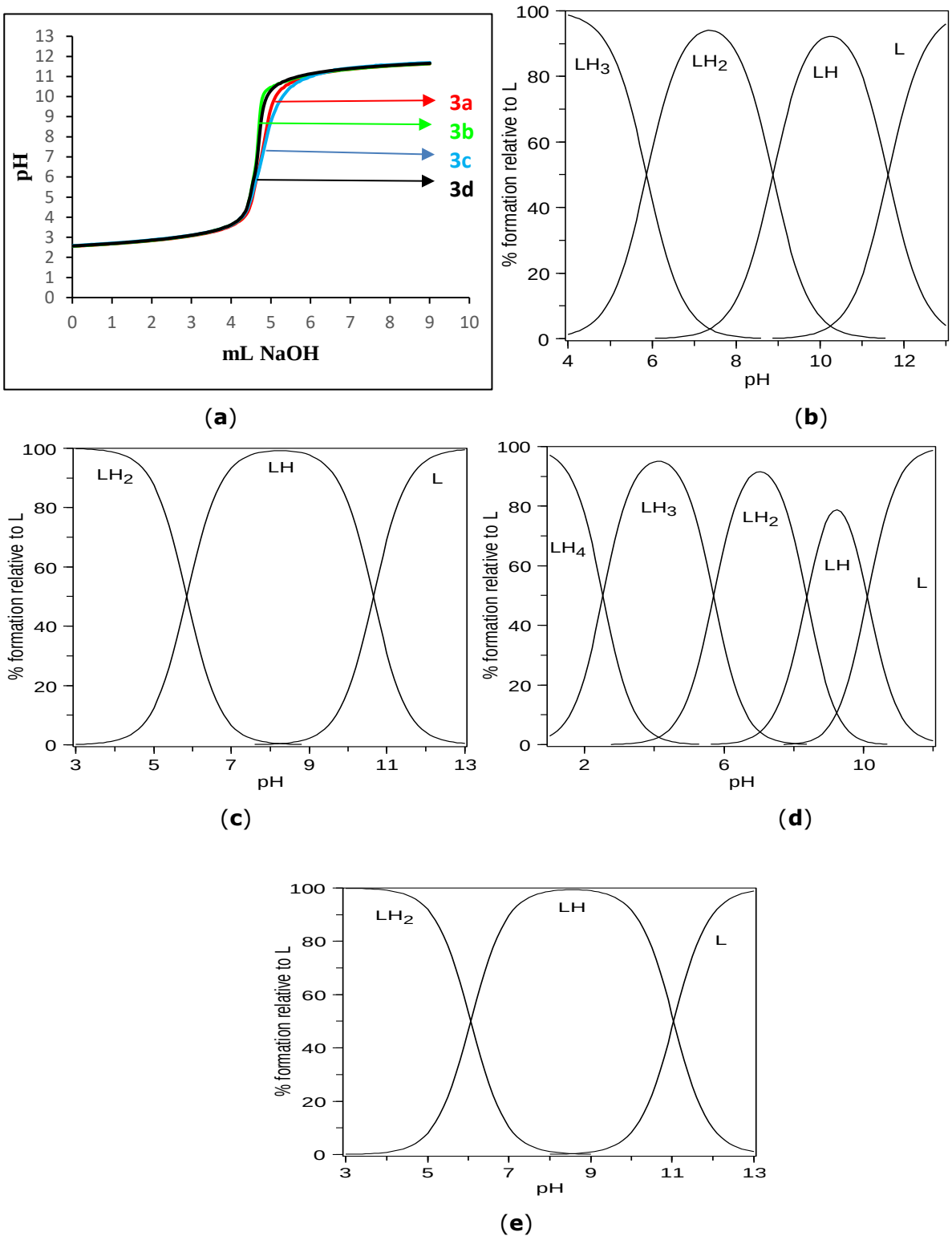


Figure 1: Potentiometric titration curves (a) and distribution curves of the ligands [(b) **3a**, (c) **3b**, (d) **3c**, and (e) **3d** in a 25% (v/v) DMSO–water mixture].

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