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ACID IONIZATION CONSTANT OF POTENTIAL BIOACTIVE FUNCTIONALIZED *N* BENZOYLTHIOUREA/ 2-THIOHYDANTOIN-PYRROLIDINE DERIVATIVES BY POTENTIOMETRIC TITRATION IN ACETONITRILE-WATER

Samet POYRAZ,[a,*] H. Ali DÖNDAŞ,[b,c] Samet BELVEREN[a] and Hayati SARI[d]

[a]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Mersin University, Yenişehir 33169, Mersin, Türkiye
[b]Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Çukurova University, Balcalı 01330, Adana, Türkiye
[c]Department of Biotechnology, Institute of Natural and Applied Sciences, Çukurova University, Balcalı 01330, Adana, Türkiye
[d]Department of Chemistry, Faculty of Science and Arts, Gaziosmanpasa University, 60250 Tokat, Türkiye

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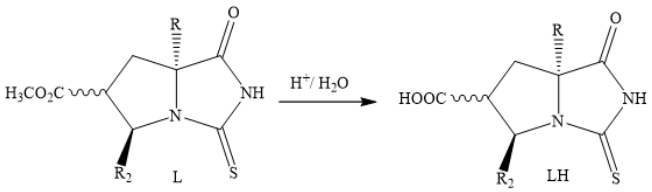
The acid ionization constants (pK_a) of the *N*-benzoylthiourea / 2-thiohydantoin-pyrrolidines were determined by the potentiometric titration method. Titrations were performed by adding sodium chloride, which gives ionic strength, and hydrochloric acid, which is required for the protonation of the ionizable groups, to $2x10^{-4}$ M solutions of the synthesized compounds prepared in an acetonitrile-water (20:80, v / v) solvent system at 25 ± 0.1 °C and sodium hydroxide was used as a titrant. The HYPERQUAD computer program was utilized to determine acid ionization constants based on the data obtained from the Molspin Titration System. Analyzing the obtained results, it is possible to propose three pK_a (pK_{a1}, pK_{a2}, and pK_{a3}) values that correspond to the enol, thiol, and NH species for *N*-benzoylthiourea derivatives in the range of 3.02–10.91 and carboxyl, enol and enthiol species for thiohydantoin derivatives in the range of 2.13–10.96.



INTRODUCTION

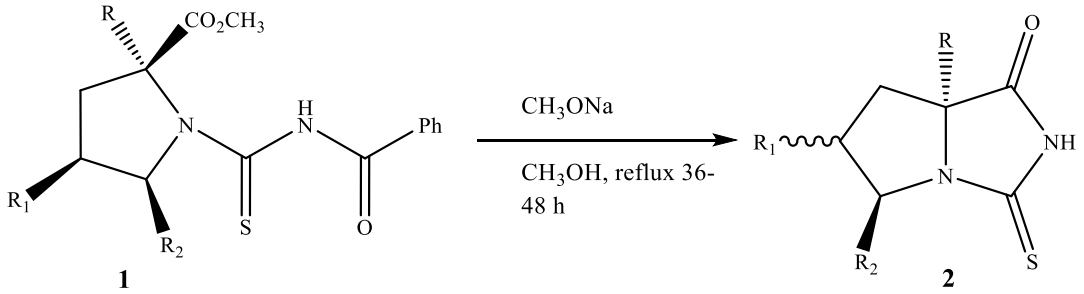
The acid ionization constant (pK_a), which is one of the significant parameters for drug molecules, is crucial as it affects drug absorption, distribution, metabolism, and elimination in the body.[1] Acid ionization constants have been reported to provide critical information while designing novel drug candidates.[2] Various methods have been proposed to determine ionization constants such as spectroscopic, chromatographic, electrophoretic, and potentiometric.[3] Potentiometric titrations give more reasonable results than those obtained from traditional titrations using chemical indicators and can be applied in particular to colored or turbid solutions.[4] In addition, species that are unknown in the sample solution could be observed thanks to potentiometric titrations.[5] Potentiometry is an extensively used method because of its high accuracy and repeatability in a short period.[6,7]

The pyrrolidine ring serves as the fundamental structure in numerous natural products and plays a

* Corresponding author: s.poyraz88@gmail.com

crucial role as a structural component in some alkaloids and physiologically significant compounds.[8–12] Furthermore, thiohydantoins and several of its derivatives have been shown to have a variety of biological activities[13], including antiviral[14], antibacterial[15], anti-inflammatory[16], anticancer[17], antiandrogenic[18], and agronomic uses.[19] Thiohydantoin and its derivatives are appealing to obtain more potent derivatives because of their valuable biological and pharmacological characteristics. Furthermore, because *N*-benzoyl thiourea derivatives exhibit diverse biological and pharmacological characteristics, including urease inhibitory, antioxidant, anti-HIV, anti-nociceptive, anticancer, antibacterial, antifungal and antimycobacterial activity, they have been studied extensively.[20]

As a continuation of our previous work[21] in this study, we report the investigations of ionization constant (pK_a) for some newly prepared potential bioactive heterocycles by the potentiometric titration method.



1,2a. R = $PhCH_2$ R_1 = CO_2CH_3 R_2 = 2,4-Dimethoxyphenyl
1,2b. R = $PhCH_2$ R_1 = CO_2CH_3 R_2= 2,4-Dichlorophenyl
1,2c. R = Ph R_1 = Phenylsulphonyl R_2 = 2,4-Dimethoxyphenyl
1,2d. R = Ph R_1 = Phenylsulphonyl R_2 = 2,4-Dichlorophenyl

Scheme 1 – Synthetic route of 2-thiohydantoins from *N*-benzolylthioureas.

RESULTS AND DISCUSSION

The ionization constants determination of the synthesized compounds **1a–d,** and **2a–d** were carried out by potentiometric titration method in a 20:80 % acetonitrile: water hidroorganic solvent system at 25.0 ± 0.1 °C and calculated by HYPERQUAD program (Tables 1, 2). Three deprotonated species formulated as LH_3, LH_2, and LH were observed during titration. The hydrolysis of the ligands in acidic media (Fig. 1, 4), titration, and distribution curves of **1a–d**, **2a–d** are given in Figs. 3, 6. The deprotonation equilibrium (1) is as seen in the following equations (charges are omitted for simplicity):

$$\mathbf{LH}_n = \mathbf{LH}_{n-1} + \mathbf{H}$$

and deprotonation constants (K_n) (2) are given as[22]

$$\mathbf{K}_n = \frac{[\mathbf{LH}_{n-1}][\mathbf{H}]}{[\mathbf{LH}_n]}$$

The HYPERQUAD uses the Gauss-Newton-Marquardt algorithm to minimize the sum of squares of the residuals of pH values, which are the difference between the measured and computed values. The approach, initially suggested by Bjerrum, demonstrates great accuracy and applies to a wide variety of metal ions and ligands. Specifically, a titration curve was created by plotting the pH versus the volume of NaOH solution that was added to the mixture. A preliminary refinement was performed with all of the data provided. If the refinement process did not reach a convergent state, which was observed in certain intricate systems, data that exhibited higher levels of inaccuracy or provided limited information regarding pK_a values were omitted from the refinement. This exclusion encompassed data from the initial, final, and inflection areas. The program produces the values of log(β_n) together with their corresponding standard uncertainties. These uncertainties are determined using the covariance matrix of the residuals from the fitted values. Additionally, the program takes into account the user's input on the uncertainties of the volumetric equipment and the purities of the reagents. Since any pK_a value may be represented as the discrepancy between successive log(β_n) values, the estimated standard uncertainty for the given pK_a values can be obtained by assuming that the log(β_n) values are not correlated.[23–25]

Although the number of pK_a values that can be seen in titration curves is directly proportional to the number of inflection points, software such as

HYPERQUAD makes it possible to calculate more than one pK_a value. Processing the titration data in the HYPERQUAD program allows for the calculation of additional pK_a values, but it is not possible to observe the inflection points that should be observed for values close to each other in the titration curves formed with a large amount of data collected by potentiometric titration.

The pK_a values of tripeptides comprising glutamic acid, glycine, and histidine were computed by Khoury[25] *et al*. utilizing the HYPERQUAD program and the data obtained from potentiometric titrations. The titration curve indicates that five pK_a values were determined for tripeptide 1, but only two pK_a were expected. Similarly, four pK_a values were computed for tripeptide 2, despite the expectation of three pK_a values.

Meloun[26] *et al*. used the HYPERQUAD program to compute four pK_a ranging from 3.29 to 11.29 for bosutinib, a cytostatic and anticancer drug, using data from the potentiometric titration method. Since three inflection points were observed, three separate pK_a values would need to be computed when the pK_a value was determined by examining the titration curve without the HYPERQUAD algorithm.

Meloun[27] *et al*. also computed the pK_a values of the hematological disease treatment Eltrombopag by inputting the data acquired *via* potentiometric titration into the ESAB program, which operates on a similar logical framework as the HYPERQUAD program. Despite eltrombopag having six ionizable sites that can potentially undergo protonation, only one pK_a value should be examined when analyzing the number of inflection points in the titration curve. However, using the ESAB program, they obtained four pK_a values ranging from 6.59 to 9.29.

N-benzoylthiourea pyrrolidines **1a–d** have five possible ionizable groups that are exposed to protonation in the acidic media (Fig. 2). As a result of the calculations from the data obtained by titration, three ionization constants were found for the compounds (**1a–b**) having a methoxy group at the R_1 position and two constants for the compounds (**1c–d**) having phenylsulfonyl group at the same position (Table 1).

These three ionization constants were identified as thiol, enol, and NH groups for compound **1a** and as carboxyl, enol, and NH groups for **1b.** It could be said that the ionization constant 3.02 may be attributed to **1b** which contains the methyl ester group at the R_1 position which is exposed to hydrolysis in acidic media and converted to the carboxyl group. However, the reason for not determining the ionization constant which is thought to belong to the carboxyl group resulting from the hydrolysis of the methyl ester group in the structure of the compound **1a** is that the expected value is out of the detection limit of the glass electrode.

The pK_{a1}, pK_{a2}, and pK_{a3} values calculated for the *N*-benzoylthiourea compounds ranged from 3.02–7.32, 6.75–8.72, and 9.80–10.91 respectively. Comparing compound **1a** having the 2,4-dimethoxyphenyl substituent with **1b** containing the 2,4-dichlorophenyl substituent in terms of acidity character; it is expected that compound **1b** is more acidic than compound **1a** since the fact that the methoxy groups are electron donors led to decrease in acidity and the chlorine atoms are electron-withdrawing groups increase the acidity. Comparing compound **1a** containing the 2,4-dimethoxyphenyl substituent with compound **1b** containing the 2,4-dichlorophenyl substituent in terms of their acidity, compound **1b** is expected to be more acidic than compound **1a**, because methoxy groups are electron donors that reduce acidity, while chlorine atoms are electron-withdrawing groups that increase acidity. The results indicate that compound **1b** is more acidic than compound **1a** as expected. The acidity constants for compounds **1a** and **1b**, respectively; pK_{a3} values thought to belong to the amide NH proton are 10.76 and 9.80, and pK_{a2} values thought to belong to the enthiol groups are 8.12 and 6.75. 7.32 for compound **1a** is thought to belong enol group, and 3.02 is thought to belong carboxyl group which results from the hydrolysis of the methyl ester in the structure for **1b**. Compound **1d**, which bears the phenylsulfonyl group as opposed to the chlorophenyl substituent, is anticipated to have a more acidic nature when compared to compound **1c**. By evaluation of the obtained values, the pK_a of compound **1c** is attributed to the thiol group which is more acidic than that of the **1d** compound, whereas the pK_a value of the NH moiety for **1d** was found to be more acidic. The results show that there was no significant difference between pK_a values of **1c** and **1d** compounds. These results are in line with those of previous studies.[21] The ionization constants of the compounds were compared concerning their acidity, it could be said that compound **1b** which contains a methyl ester group together with chlorophenyl substituent has the most acidic character among them.

Compounds **1c** would require one pK_a calculation, and compounds **1a** and **1b** would require two pK_a if the pK_a calculation had been done by examining the inflection points in the titration curves. Calculating three pK_a values for compounds **1a–b** and two pK_a values for

compounds **1c**–**d** using the HYPERQUAD program is consistent with the literature because compounds could have two or three ionizable groups depending on the substituents they include.

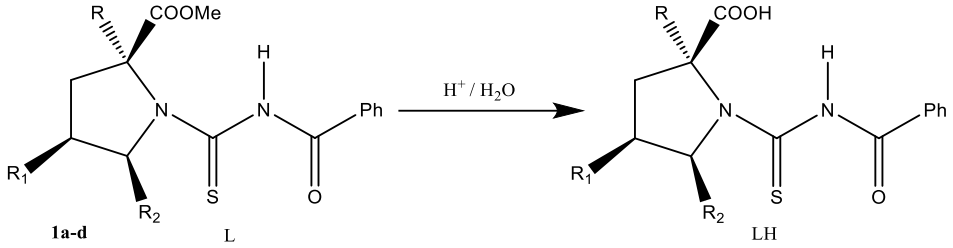


Fig. 1 – Hydrolysis of *N*-benzoylthiourea derivatives in acidic environment.

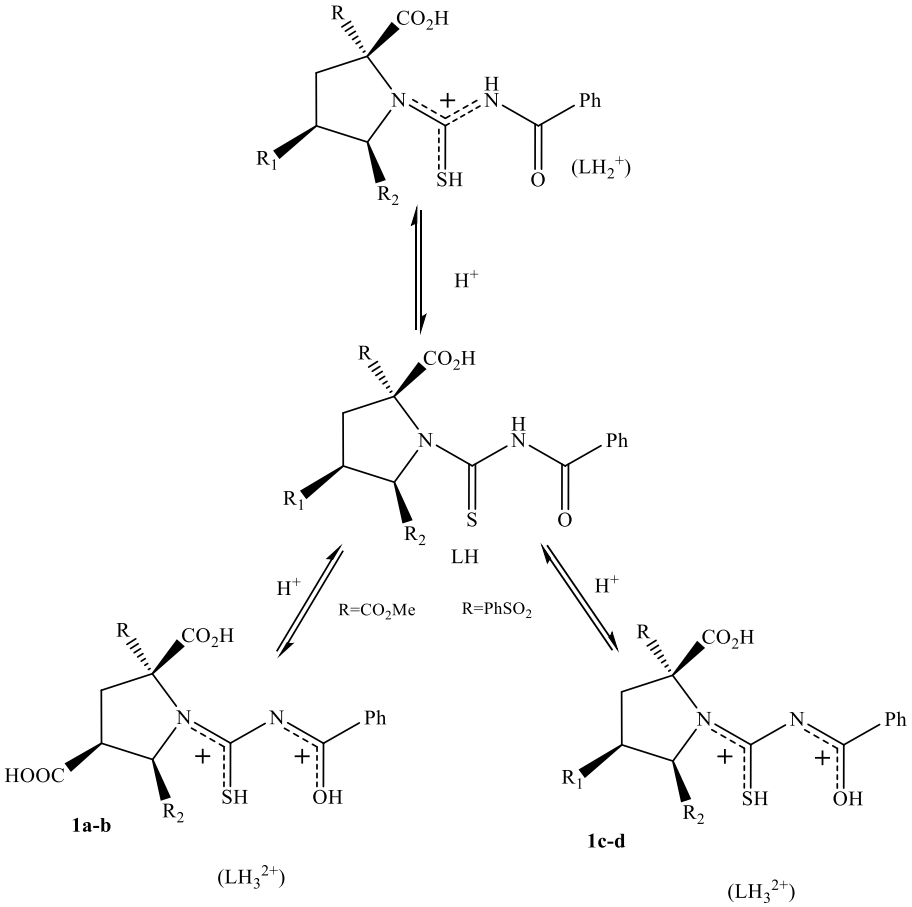


Fig. 2 – Proposed mechanism for protonation of *N*-benzoylthiourea derivatives **1a–d**.

Table 1

Ionization constants of *N*-benzoylthiourea (298 K, I:0.1 mol dm^{-3} NaCl, 20% acetonitrile (v/v))
($\log_{10}(\beta)$ is cumulative acid dissociation constants)

Compound	Species	$\log_{10}(\beta)$	pK_a values		σ
1a	LH_3	26.20±0.09	pK_{a1}	7.32 ± 0.09	12.46
	LH_2	18.88±0.09	pK_{a2}	8.12 ± 0.09	
	LH_1	10.76±0.07	pK_{a3}	10.76 ± 0.07	
1b	LH_3	19.57±0.13	pK_{a1}	3.02 ± 0.13	13.16
	LH_2	16.55±0.03	pK_{a2}	6.75 ± 0.03	
	LH_1	9.80±0.02	pK_{a3}	9.80 ± 0.02	
1c	LH_2	19.01±0.04	pK_{a2}	8.10 ± 0.04	12.17
	LH_1	10.91±0.07	pK_{a3}	10.91 ± 0.07	
1d	LH_2	18.89±0.09	pK_{a2}	8.17 ± 0.09	12.38
	LH_1	10.72±0.08	pK_{a3}	10.72 ± 0.08	

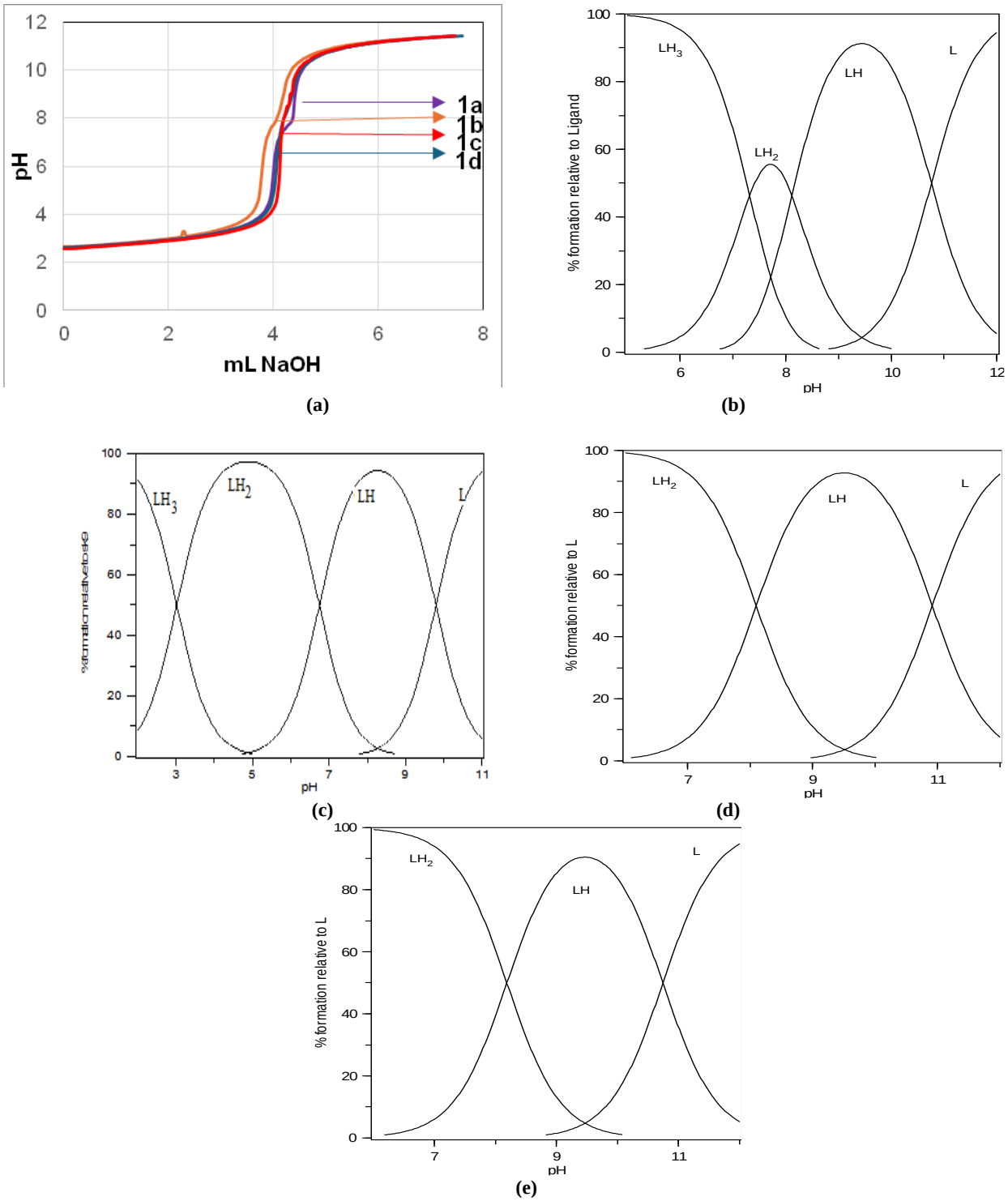


Fig. 3 – Potentiometric titration curves (a) and distribution curves of the ligands [(b) **1a**, (c) **1b**, (d) **1c**, (e) **1d** in 20% (v/v) acetonitrile-water mixture].

The ionization constants for the novel 2-thiohydantoin derivatives were determined by the same procedure that was applied to *N*-benzoyl thiourea compounds (**1a–d**). Four ionizable groups can be protonated in acidic media for thiohydantoin compounds (Fig. 5). Three ionization constants were calculated for compounds with methyl ester groups at position R_1 and two ionization constants were calculated for those bearing the phenylsulfonyl group at the same position (Table 2). The ionization constants of the novel 2-thiohydantoin derivatives were evaluated

with respect to containing substituents. The acidity of compounds **2a** and **2c** containing methoxy groups is expected to be lower than that of compounds **2b** and **2d** because chlorine atoms are electron-withdrawing groups and raise the acidity. When the calculated acid ionization constants for these structures are evaluated, compounds **2a** and **2b**, respectively; pK_{a1} values resulting from hydrolysis of the methyl ester in aqueous solution are 2.80 and 2.13, pK_{a2} values of enol groups are 8.90 and 8.16, and pK_{a3} values of the enthiol groups are 9.69 and 9.01. In this case, compound **2b** with lower pK_a values is more acidic than expected. For compounds **2c** and **2d**, respectively; pK_{a1} values of enol groups were found to be 8.29 and 7.85, and pK_{a2} values of amide NH proton were 10.96 and 10.68. These results indicate that as expected, compound **2d** is more acidic.

Two inflection points were found in the titration curves of **2a**–**b** compounds, hence two pK_a values should have been determined. Thanks to the HYPERQUAD program, the computation of three pK_a values for compounds **2a**–**b**, which include three ionizable groups, is consistent with previous research.

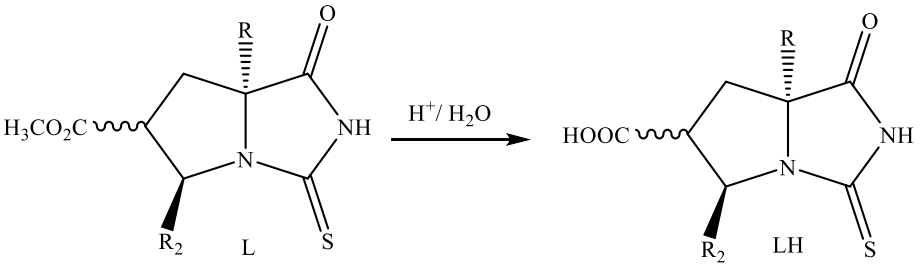


Fig. 4 – Hydrolysis of 2-thiohydantoin derivatives in the acidic environment.

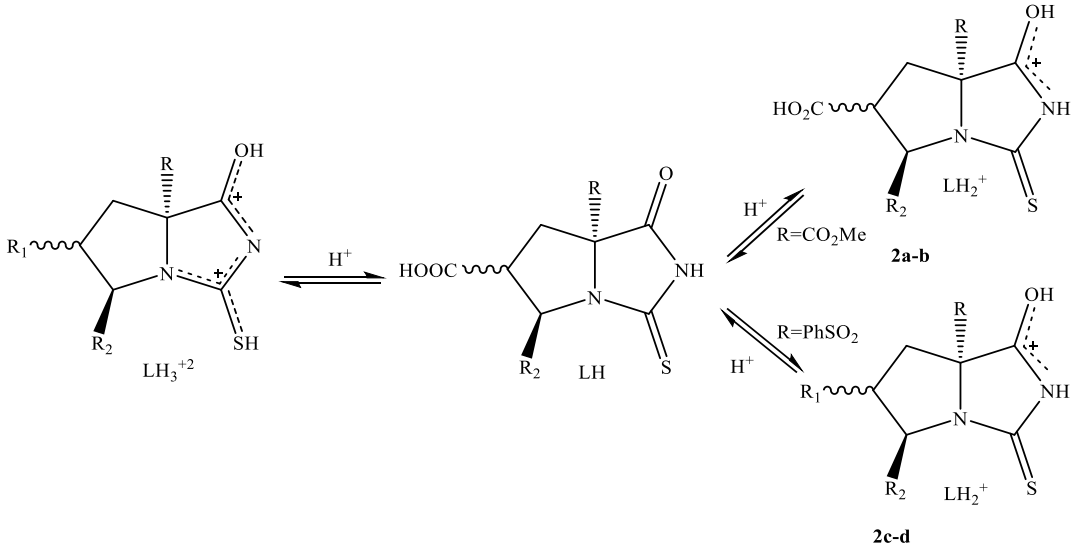


Fig. 5 – Proposed mechanism for protonation of thiohydantoin derivatives **2a–d**.

Table 2

Ionization constants of 2-thiohydantoin derivatives (298 K, I:0.1 mol dm^{-3} NaCl, 20% acetonitrile (v/v))
($\log_{10}(\beta)$ is cumulative acid dissociation constants)

Compound	Species	$\log_{10}(\beta)$	pK_a values		σ
2a	LH_3	21.39±0.13	pK_{a1}	2.80 ± 0.13	5.61
	LH_2	18.59±0.02	pK_{a2}	8.90 ± 0.02	
	LH_1	9.69±0.02	pK_{a3}	9.69 ± 0.02	
2b	LH_3	19.30±0.17	pK_{a1}	2.13 ± 0.17	4.93
	LH_2	17.17±0.01	pK_{a2}	8.16 ± 0.01	
	LH_1	9.01±0.01	pK_{a3}	9.01 ± 0.01	
2c	LH_2	19.25±0.06	pK_{a2}	8.29 ± 0.06	10.21
	LH_1	10.96±0.05	pK_{a3}	10.96 ± 0.05	
2d	LH_2	18.53±0.03	pK_{a2}	7.85 ± 0.03	5.77
	LH_1	10.68±0.03	pK_{a3}	10.68 ± 0.03	

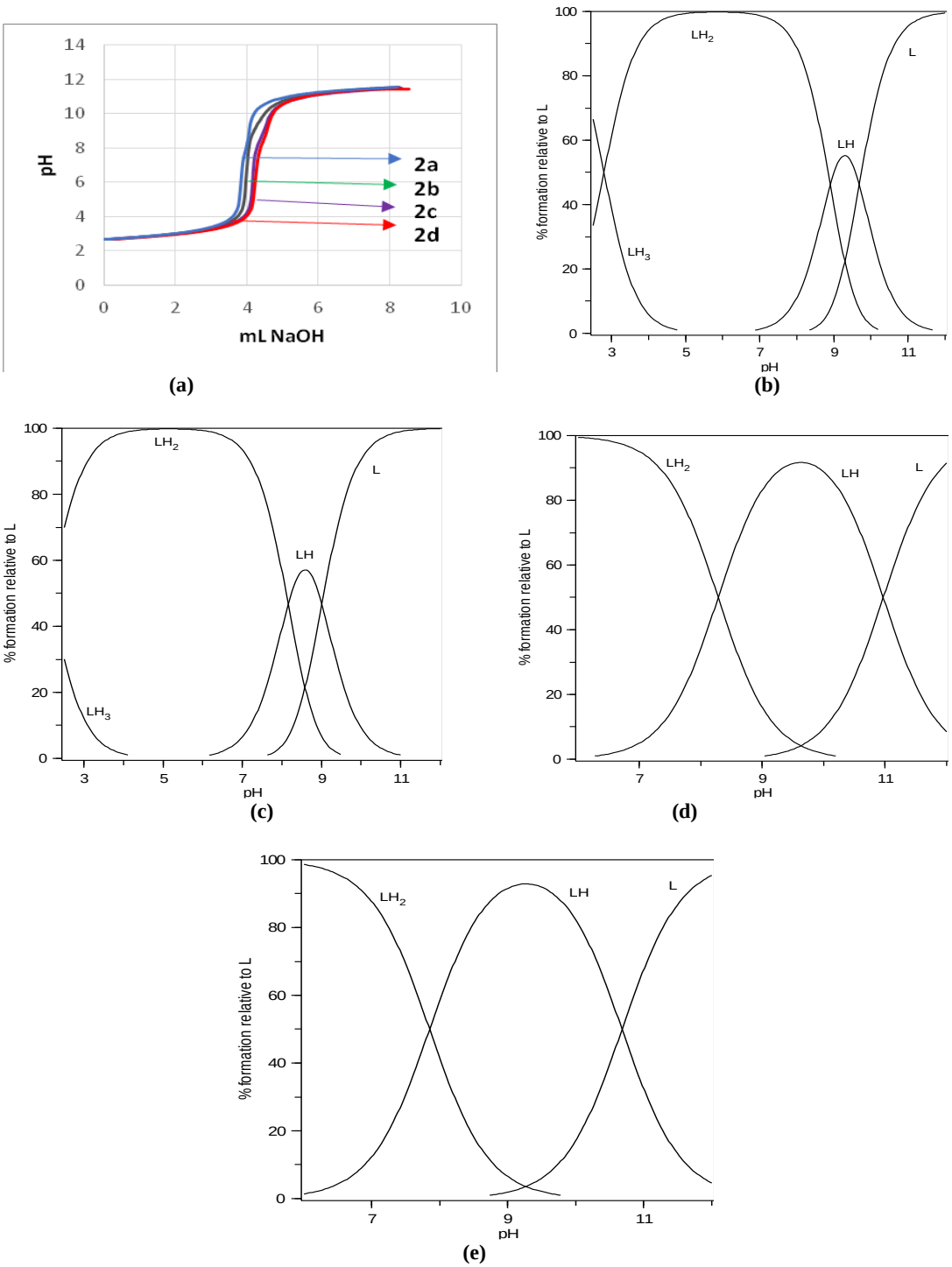


Fig. 6 – Potentiometric titration curves (a) and distribution curves of the ligands [(b) **2a**, (c) **2b**, (d) **2c**, (e) **2d** in 20% (v/v) acetonitrile-water mixture].

EXPERIMENTAL

Apparatus and Materials

Prior to their utilization, analytical grade sodium hydroxide (Merck), potassium hydrogen phthalate (Fluka), and sodium tetraborate (Fluka) were dehydrated at 110 °C. Additionally, calibration solutions were prepared from 0.05 mol/kg potassium hydrogen phthalate and 0.01 mol/kg sodium tetraborate. Deionized water produced in the aqua MAXTM-Ultra water system was used for potentiometric titrations, Nitrogen gas (99.9% purity) was passed through the cell during titration to remove the effect of the carbon dioxide present in the medium. The ambient temperature

(25 ± 0.1 ºC) was controlled using the Digiterm 100 Selecta thermostat. 0.025 M sodium hydroxide standardized according to potassium hydrogen phthalate (4.005 at 25.0 ± 0.1 ºC) was used as the titrant. The glass electrode was calibrated at two points with potassium hydrogen phthalate (4.005 at 25.0 ± 0.1 ºC) and borax (9.180 at 25.0 ± 0.1 ºC).[28] Molspin pH-Meter and Orion 8102BNUWP ROSS ultra-combined pH glass electrodes were used to obtain titration data using the Molspin Titration System. pKa values were calculated using the HYPERQUAD computer program.[24]

Method

We have previously reported the synthetic procedure for *N*-benzoylthiourea -pyrrolidines (**1a–d**) and 2-thiohydantoin pyrrolidines (**2a–d**) **(**Scheme 1**)**.[29] The pK_a values of prepared (**1a–d**) and (**2a–d**) compounds were determined by the potentiometric titration method by following the literature method.[21] The most reasonable pH-mL graphs were obtained as a result of the titrations of thiohydantoin and thiourea derivatives at 2×10^{-4} M concentrations prepared according to the procedure in Table 3 against 0.025 M NaOH base. For experimental studies; 10 mL of 1×10^{-3} M **1a–d, 2a–d**, 0.5 mL of 0.1 M HCl solution, and 5 mL of 1 M NaCl solution were added respectively, and then the titration cell would contain 2×10^{-4} M (water: acetonitrile (80:20 v / v)) **1a–d**, **2a–d,** and the total cell volume was 50 mL Potentiometric titrations were performed with the addition of 0.03 mL of **1a–d**, **2a–d** solutions against 0.025 M NaOH base at 25 ± 0.1 ºC. Experimental studies were performed twice for each compound and acid ionization constants were determined by using HYPERQUAD computer program.

Table 3

1a-d and 2a-d titration procedure with 0,025 M NaOH (25 ˚C ± 0.1 ºC, 0.1 M HCl, 1 M NaCl acetonitrile-water (20:80 *v/v*))

1×10^{-3} M 1**a–d** and **2a–d** (mL)	0.1 M HCl (mL)	1 M NaCl (mL)
10	0.5	5

Potentiometric titrations were conducted using a Molspin pH meter equipped with an Orion 8102BNUWP ROSS ultra-combined electrode. The thermostat was used to keep the temperature of the titration constant at 25.0 ± 0.1 °C throughout the experiment (DIGITERM 100, SELECTA). The titration was performed in a double-walled glass tank with magnetic stirring to mix the titration solution. The double-walled glass titration tank was cleaned with distilled water before and after each titration, and then a piece of tissue was used to dry the inside of the tank. The titration vessel was capped by a lid containing three holes for the electrode, glass tubing for nitrogen purging, and plastic tubing for alkali from the burette. There were no air bubbles during the alkali solution drop. Before being filled with the alkali solution, the syringe was washed with deionized water and rinsed with the alkali solution at least three times. Three titrations were performed for each ligand to obtain the acid ionization constants by titrating 50.00 mL of ligands with a standard NaOH solution. The acid ionization constants were calculated using the HYPERQUAD computer program. The standard deviations mentioned are only for random errors. For every experiment, 130 titration data were collected. The NaOH solution increased in volume by 0.03 mL. The pK_w value for the aqueous system was determined to be 13.98 at the employed ionic strength.

CONCLUSION

The acid ionization constants were determined by analyzing the titration data using the HYPERQUAD program. As a result of the findings, it is possible to recommend three ionization constants for derivatives (**1**,**2a**–**b**) that contain methyl ester substituents in their structures, while only two constants are for derivatives (**1**,**2c**–**d**) that do not contain such substituents. The findings are consistent with our previous investigations. Compounds (**1b**, **2b**) with halogen substituents in their structures are considered to be more acidic than those without. The results may offer valuable guidance for the development of novel potential bioactive molecules to be discovered in further research.

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