Journal Pre-proof

UV/vis spectrophotometric determination of slow equilibrated N(1)-H missing deprotonation constant of a pyrimidine and thiopyrimidine: The final situation of the four pKa values

H. Kılıç

PII: S1386-1425(19)31257-0

DOI: https://doi.org/10.1016/j.saa.2019.117867

Reference: SAA 117867

To appear in: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*

Received date: 23 August 2019

Revised date: 26 November 2019

Accepted date: 26 November 2019

Please cite this article as: H. Kılıç, UV/vis spectrophotometric determination of slow equilibrated N(1)-H missing deprotonation constant of a pyrimidine and thiopyrimidine: The final situation of the four pKa values, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*(2019), https://doi.org/10.1016/j.saa.2019.117867

UV/vis spectrophotometric determination of slow equilibrated N(1)-H missing deprotonation constant of a pyrimidine and thiopyrimidine: The final situation of the four pK_a values

H. Kılıç

Department of Physical Chemistry, Faculty of Arts and Sciences, Marmara University, Ziverbey 34722 Istanbul, Turkey

ABSTRACT

The missing acidity constant of recently synthesized 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-one (**I**) and 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-thione (**II**) are investigated as a function of time at pH values from 1.0 to 3.20 for **I** and from 1.0 to 4.06 for **II** using UV/vis spectroscopic analysis of their aged solutions, at a temperature of 25 ± 0.1 °C. In this study, a novel time-dependent isosbestic point method was developed to confirm the presence of any weak acid – base equilibrium and the detection of related equilibration time. The missing acidity constants for each of **I** and **II** were determined in 5.0% v/v aqueous methanol. Plausible acid base equilibrium mechanism for the missing acidity constant was presented for each of the compounds based on the extensive experimental UV/vis data. In this context, all the acid base equilibria for each of the regarding compounds were completely revised through different pH values.

Keywords: acidity constants; pyrimidines; thiopyrimidines; semicarbazones; thiosemicarbazones; uv/vis. spectroscopy.

---

* Tel: (+90) 216 - 346 4553; fax: (+90) 216 - 347 8783.

E-mail address: hkilic@marmara.edu.tr

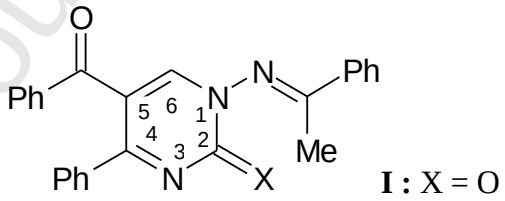
1. Introduction

The pyrimidine ring and its heterocyclic derivatives are well known bioactive compounds [1] that contains heteroatoms. Therefore it has a wide range of uses in various fields of industry [2,3] including biological systems in our daily life [4,5]. Since heterocyclic pyrimidine compounds have remarkable pharmacological activities such as antiviral [7], analgesic [8], anti-microbial [8] and anticancer activities [9], and etc., there are many novel applications of sulfur-containing pyrimidines in drug-delivery systems [6]. Moreover, accurate determination of acidity constants, pK_a values of these compounds are important for drug analysis.

Every weak acid and/or base has one or more protonable groups. Thus in each case one or more definite acidity constants are expected to be obtained. Acidity constants correspond to an equilibrium level in which one hydrogen ion, i.e. a proton (H^+) is received or released. The term of acidity constant or protonation constant is not only use for weak acid(s) but also is used for weak base(s). Both acidic and basic groups in any molecule undergo ionization by protonation or deprotonation to different extents depending on acidity or basicity of the aqueous solution. As the majority of drugs are weak organic acids and/or bases, knowledge of the protonation ↔ deprotonation constant or vice versa helps in understanding the ionic form of the drug molecule that will take across a range of pH values. Therefore, an acidity constant info is useful as a physicochemical indicator that showing the extent of dissociation of ionizable groups in the medium with respect to pH, by which the changes in concentrations of the components can be calculated. By using the pK_a value of an acid or base the extent of the dissociation can be determined at a particular pH using the Henderson-Hasselbalch equation via UV/vis spectroscopy [10-13] potentiometric titration [14] and conductometry [15].. Molecules containing proton-binding or proton-releasing groups control the polarity of the

medium and, consequently, the physicochemical properties of the respective aqueous solution, thereby enabling interactions with target molecules. For this reason, acid dissociation constant, pK_a value of an organic compound is one of the important physicochemical parameter that influence the pharmaceutical characteristics in choosing appropriate acidic or basic reagents in drug discovery and development of new medicines. In this sense, pK_a value of a drug molecule influences its solubility, hydrophilicity, lipophilicity, protein binding and permeability. Additionally, pKa value of an organic compound also directly affects kinetic (pharmacokinetic) and thermodynamic (pharmacodynamic) properties of the related drug compound in terms of absorption, distribution and metabolism. In the case of all aforementioned importance of the calculation of acidity constant, computational programs have been developed to screen and predict pK_a values with a high degree of accuracy [16].

Considering the aforementioned significance of the recently synthesized pyrimidine derivatives 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-one (**I**) and 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-thione (**II**), are expected to present novel biological, pharmaceutical and industrial features. The structures of the compounds are given in Figure 1.



I : X = O
II : X = S

Fig. 1. Pyrimidine compounds investigated in this study

As shown in Fig. 1, the pyrimidine compounds are analogs of each other containing both semi- and thiosemicarbazone moiety at the same time. The numbered benzene ring containing two nitrogen atoms shown in Fig. 1 is a pyrimidine ring. Thus, pyrimidine ring and all derivatives of it are basic in character due to presence of nitrogen atoms. Therefore, the title compounds undergo protonation in acidic solutions. So, pyrimidine compounds exist in their

cationic forms, i.e., protonated amine cations in acidic solutions. Using fresh solutions of these two compounds, acid-base properties were investigated by Kılıç and three pKa values were found for each compound [13]. However, by looking at the structure of the two title compounds four protonable nitrogen atoms are seen, indicating four pK_a values are expected to be obtained for each compound. This difference clarifies that one acidity constant was missing for each compound in the recent study using fresh solutions. The reason for this result is slow balance of the equilibrium. Thus, the aim of this current study is to determine the missing pK_a value of each compounds using their aged solutions against varying pH and time with the related acid base equilibrium mechanism using UV/vis absorption spectroscopy. Since UV/vis spectrophotometric method provides high precision and accuracy in order to establish the dissociation constants of organic compounds, aged solutions of the compounds were used in this current study. However, fresh solutions of the same compounds were used in the recent study [13].

2. Material and methods

2.1. Synthesis of Pyrimidine Compounds

Compounds **I** and **II** were synthesized in our laboratory according to procedures described elsewhere [17,18]. Briefly, for the synthesis of **I**, 0.28 g furan-2,3-dione and 0.18 g methylphenyl semicarbazone (molar ratio 1:1) are refluxed in toluene for 45 minutes, yielding 0.14 g (38%) of **I**. For the synthesis of **II**, 5 g furan-2,3-dione and 3.5 g methylphenyl thiosemicarbazone are refluxed in 20 mL of toluene in a 100-mL round-bottom flask for 1 h, affording 2.4 g (33%) of **II**. The compounds are filtered from the reaction mixtures and purified by recrystallization from acetic acid. Their characteristic physical and chemical properties including IR data have been reported previously [17,18]. Their purity is assessed by thin-layer chromatography (TLC) using methanol/chloroform (1:9 *v/v*) and methanol/benzene (1:9 *v/v*) as given in [19]. All other chemicals are obtained from Fluka as reagent-grade materials. Triple-

distilled water is used for all aqueous solutions. Preparation of triple-distilled water is as described recently [14]. Methanol is distilled before use. Stock solutions of the compounds are prepared in an appropriate volume of distilled methanol.

2.2. Instrumentation

IR spectra are recorded from KBr disks using a Shimadzu 8300 FT-IR spectrometer in the range 4000–400 cm^{-1}. UV/vis spectroscopy studies are carried out using a Shimadzu 1601 PC UV/vis spectrophotometer with quartz cells (1.0-cm path length). The pH of the aqueous and methanol solutions are measured using a digital pH meter (Hanna Instruments 8314, Italy; ± 0.01 pH unit) and a combined pH electrode that is calibrated using standard aqueous buffers (pH 4.00, 7.00, and 9.00) as described in previous report [20]. The measured pH values are not corrected, and the symbol pH (defined as -log $[H^+]$) is used in all cases. Glass electrodes in solvents containing up to 95% alcohol show responses very similar to the theoretical response in water. Of the organic solvents, methanol is the most similar to water in structure and properties. Therefore, acidic dissociation in methanol takes place in a fashion analogous to that of in water [21]. An Eppendorf micro-pipette is used for precise volumetric measurement of the solutions. The temperature of the solutions is kept constant at 25 ± 0.1 °C using a thermostat (WiseCircu, Daihan Scientific Co., Ltd. Korea). A Sartorius A120 S analytical balance (sensitivity of ± 0.0001 g) is used for weighing the chemicals.

2.3. Measurements

Stock solutions of the compounds are prepared in methanol and stored in the dark to avoid decomposition. Aqueous Britton-Robinson buffer (BRB) solutions [22] are prepared with the desired pH values for the experiments. Working solutions with concentrations of 1.641×10^{-5} M for **I** and 2.044×10^{-5} M for **II** are prepared daily in 5.0% v/v aqueous methanol by adding 1 mL of the stock solution of **I** or **II** to a flat-bottomed volumetric flask (20 mL) containing some BRB solution at 25 ± 0.1 °C and diluting it to 20 mL rapidly with the same solution.

The experiments are conducted in BRB media over a pH range of 3.0 to 5.0 in increments of 1.0 pH unit at regular time intervals. For more acidic solutions, 0.1 M HCl, 0.01 M HCl are used. The ionic strength of the solution is constant with 0.10 M LiCl as the supporting electrolyte.

Typically, experiments are initiated as soon as 1.0 mL of the stock solution of **I** or **II** is mixed with the required buffer solution in a 20 mL volumetric flask at 25 ± 0.1 °C. After preparation of the aqueous methanol working solution, the pH is first adjusted to the desired pH by dotting with a relatively concentrated KOH or concentrated HCl on a thin glass rod and then an appropriate volume of this solution is transferred immediately to the 1.0-cm quartz cells and the corresponding spectrum is recorded. Experiments are initiated as soon as 1.0 mL of the stock solution of **I** or **II** is mixed with the required buffer solution in a 20-mL volumetric flask at 25 ± 0.1 °C. The absorption maxima are monitored by recording the UV/vis spectra at regular time intervals until the maximum absorbance (A_∞) is reached. The absorbance is determined after baseline correction. The reference beam contained a blank of the buffer containing the same amount of aqueous methanol as the solvent. The UV/vis spectra are recorded from 600 to 200 nm for each compound at a medium scan rate (ca. 100 nm/minute) between pH 1.0 and 13.0.

2.4. Determination of missing acidity constants

Since the compounds **I** and **II** are basic in character, they undergo protonation completely in a strong acidic solution. Therefore, the both compounds undergo deprotonation on increasing pH of the medium. So, the missing acidity constants for the protonated form of the both compounds in their aged solutions were determined from their spectral behavior in buffer solutions against varying pHs and time, at selected wavelengths, by using the Henderson-Hasselbalch equation as described by Albert et al. [11]. For this purpose, the pH media of

from 1.0 to 3.06 for **I** and of from 1.0 to 4.06 for **II** were scanned in BRB solutions for each compound and the approximate position of each pK_a value was determined by monitoring the spectral changes over the pH range. The pK_a values were calculated after the baseline correction of the spectra.

3. Results and discussion

The acid-base properties of the two compounds were investigated in their fresh solutions from the pH-dependence of the absorbance values at selected wavelengths in aqueous methanol [13]. From the obtained spectra the pH-dependence of the absorbance values of keto and enol maxima at selected wavelengths were evaluated. By means of these evaluations three acidity constants were obtained for each compound. Although the fourth one is expected to occur for each compound it was not obtained in the recent study [13]. In other words, using the pH dependence of the absorbance values of the keto and enol forms of both compounds obtained in fresh solutions at different pHs, the fourth acidity constant cannot yet be determined so far. During our previous study [23] in which we investigated hydration behavior of the title compounds using their aged solutions, a clue about the loss acidity constant for each compound was observed. Therefore, in this current study the acid base behaviors of the title compounds are studied also as a function of time using their aged solutions. The use of the aged solutions of the compounds provides an advantage as follows; as the solution is kept at any pH level for a while (from 2 minutes to 360 minutes) hydration of the related molecule occurs depending on pH and the passage of time. Thus, in addition to the keto and its enolic form maxima obtained using fresh solutions in the recent study [13] a new maximum, i.e. a hydration maximum is obtained in this current study. The examination of the changes in the absorptions and their wavelengths of this new maximum with the other keto and enol maxima have created a new hope in the determination of the lost acidity constant of the compounds. The results related to the missing acidity constant obtained from

the aged solutions of each compound in the pH range from 1.0 to 3.20 for **I** and from 1.0 to 4.06 for **II**, are presented below separately.

The UV/Vis spectra of each compound are obtained in the aqueous methanol. The results obtained from the aged solutions of compounds **I** and **II** are summarized below.

*3.1. Effect of pH and time on hydration property of **I** in aqueous solution*

In the recent study [13], a keto maximum and its enolic form were obtained. However, in addition to the keto and its enolic form in the recent study [13], a new well-separated hydration maximum is obtained in this current study as function of time for **I** at pH values from 1.0 to 3.20, above which the new maximum is not observed. As the aging time increased from 1 minute to 20 minutes, the absorbance of the new maximum increases in pH 1.0 solution. Moreover, the absorbance of the new maximum increases and simultaneously the keto maximum decreases by showing a time dependent isosbestic point depending on varying aging time in the UV/vis spectra obtained at pHs from 1.0 to 3.20. A time dependent isosbestic point is reported for the first time in this study. Increasing of the new maximum and decreasing of the keto maximum indicates the presence of two absorbing species which are interconvertible from carbonyl to enol for **I** in that pH range. It is just similar to the isosbestic point which is seen in absorption *vs.* wavelength plots in UV/vis absorption spectroscopy. The observed time dependent isosbestic point agrees well with the literatures [24,25] and thus it confirms the presence of the missing acid-base equilibrium for **I**.

A bathochromic shift by 1.6 nm in the wavelength of the new hydration maximum of **I** is observed on increasing the pH from 1.0 to 3.20 above which the new hydration maximum is not observed. A hypsochromic shift by 16.1 nm in the wavelength of the keto maximum of **I** is also observed in the same pH range. The absorbance of the new hydration maximum decreased significantly, and the absorbance of the keto maximum increased steadily. From both shifts, a fine isosbestic point at 249.7 nm (A = 0.795) is obtained, covering the pH range

of from 1.0 to 3.20. Such decrease in the absorbance of the hydration maximum with simultaneous increase in the keto form with pH clearly indicates that the two maxima are in equilibrium [24,25] which established after 30 minutes of equilibration period. Thus, the obtained single isosbestic point determined for **I** also confirms the presence of one dissociation step, i.e. one equilibrium [24,25]. The related spectra in due to course of 30 minutes are given in Fig. 2 below. The related data are given in Table 1 below.

Figure 2 may be placed here

Table 1 may be placed here

*3.2. Effect of pH and time on hydration property of **II** in aqueous solution*

Very fast new hydration maximum of the thioenol form of **II** is observed in 2 minutes after start the experiment. Like **I**, in addition to this thioenol maximum a new well-separated hydration maximum is obtained for also **II** in this current study. The new hydration maximum is obtained as function of time at pH values 4.06 and below, above which the new maximum is not observed. The absorbance of the new maximum and the thioenol maximum changed continually depending on varying aging time in the UV/vis spectra obtained, in the range of pH from 1.0 to 5.07. The related spectra for duration of 120 minutes are given in Fig. 3 below. The related spectral characteristics are given also in Table 1 above.

Figure 3 may be placed here

A bathochromic shift by 15.6 nm in the wavelength of the new hydration maximum of **II** is observed on increasing the pH from 1.0 to 4.06 above which the new hydration maximum is not observed. A bathochromic shift by 2.8 nm in the wavelength of the thioenol maximum of **II** is observed too in the same pH range. The absorbance values of both the new hydration and of the thioenol maxima are decreased depending on varying aging time. Increasing one of them and simultaneously decreasing the other one is never observed. Therefore, there is no clear time dependent isosbestic point was obtained for **II**, covering the pH range from 1.0 to

5.07, despite 240 minutes waiting period. The reason for this can be explained as follows: On increasing pH, the thioenol group, –SH of **II** undergoes deprotonation by producing sulfur ions, $-S^-$ which re-reacts with proton of the solvent water immediately to reform –SH. Because, H^+ ions accelerate the thioenolization (≡C–SH) of **II**, which in turn accelerates its hydration. Thus, the continuous refreshing of the –SH form prevents its concentration from falling. For this reason, the thioketo form of **II** is not seen in the spectra obtained, despite using 240 minutes aged solutions at all pHs investigated. Although a clear time dependent isosbestic point is not seen for **II**, this does not mean that there is no acid base equilibrium between the new and thioenol maxima of **II**. As long as a time dependent isosbestic point is observed for **I**, it is definitely expected to observe also for **II** by an analogical approach. Fortunately, presence of the missing acid-base equilibrium for **II** is also proved from the pH dependency of absorbance values obtained using aged solutions of 40 minutes as shown in Fig. 4 in the section *3.4* below. As shown in Fig. 4, the obtained absorbance *vs.* pH curve for **II** is an S-shaped indicating clearly to the presence of an acid-base equilibrium [11-13].

*3.3. Comparative interpretations of the findings obtained for **I** and **II***

The protonated cationic forms of **I** and **II** molecules are capable of binding water molecules that is known as hydration. Thus, the hydration is too fast in pH 1.0 solution so that each of **I** and **II** molecules has four types of binding.

I undergoes protonation and thus of hydration in its aged solutions at pHs below 3.20. Similarly, **II** undergoes also protonation and thus of hydration in its aged solutions at pH values 4.06 and below. Protonation, thus of hydration behavior, of each compound in pH 1.0 aqueous solution is displayed in Scheme 1 below.

Scheme 1 may be placed here

As shown by Ia→Ia' and IIa→IIa' in Scheme 1, both the keto and enol forms of **I**, and only the thioenol form of **II** exist in their protonated forms in pH 1.0 aged solutions. Although **I**

exists in comparable amounts of the keto and the enol forms in pH 1.0 aged solutions, **II** exists wholly in the thioenol form (IIa→IIa') in the same medium. As displayed in Scheme 1, each of Ia and IIa molecules has four protonated amine cations in pH 1.0 solutions. These protonated forms can capable of forming hydrogen bonding as shown in red color in Scheme 1 with adjacent solvent or solute molecules. Thus, **I** and **II** undergo very fast hydration in pH 1.0 solutions. It should be also noted that each protonated form corresponds to a private pK_a value. Thus, there should be four acidity constants in total for each compound in pH 1.0 solutions. As clearly predictable, the number of protonated amine cations will decrease as the pH increased and this will cause the observed hydrogen bonding formation to slow down. For this reason, no significant hydration is observed at pH 3.20 and above for **I** and, above 4.06 for **II**. Consequently, the pH values of 3.20 for **I** and of 4.06 for **II** can be considered roughly as limit upper pH values for hydration to be observed in the acidic pH region investigated.

Thus, the reason of stopping of the hydration at pHs 3.20 and below for **I** and at pHs 4.06 and below for **II** are investigated at a protonation - deprotonation mechanism level. The possible equilibrium processes for **I** and **II** are schematically presented by the deprotonation mechanism given in Scheme 2 below.

Scheme 2 may be placed here

As shown in Scheme 2 above, the reason for the observed stop is found to be the deprotonation of the previously protonated nitrogen $(\text{-NH=})^+$ substituted to the ring nitrogen at position 1 of both **I** (Ib') and **II** (IIb'). The aforementioned isosbestic point obtained for **I** confirms this finding. Thus, this deprotonation causes to stop the observed hydrations of **I** and **II** in the acidic solutions by forming a new intramolecular hydrogen bonding between the deprotonated nitrogen −N= substituted to the ring nitrogen, =N< at position 1, and the carbonyl oxygen at position 2 of **I** and **II.** The new hydrogen bonds are >C=O-H·····:N≡ by

Ib→Ib' for **I** and >C=S-H·····:N≡, by IIb→IIb' for **II**. Thus, the deprotonation corresponds just to one that we are looking for, i.e. to the missing pK_a value.

As it is seen in Scheme 2, it is plausible to show the stopping of the hydration, and thus of protonation - deprotonation equilibrium of the related local amino form for **I** and **II** in acidic medium. In this respect, Scheme 2 is faultless and very useful to understand the reason of the stops occurring at pH values below 3.20 for **I**, and 4.06 and below for **II**. The related missing pK_a value for the related deprotonation of each compound is investigated as given below.

*3.4. Determination of the missing acidity constant of the each compound of **I** and **II***

The compounds, **I** and **II** exist in their cationic protonated amine forms, mostly in aged acidic solutions. Therefore, the obtained pK_a values are explained in this current study on the basis of the relative contribution of acidic character of respective species. For each of the compounds, the experiments are repeated twice in aged aqueous methanol solutions.

As shown in Scheme 1 and 2 above, the dissociation mechanisms are the same for the both compounds. Every protonation – deprotonation equilibrium has its own acidity constant. For this reason, the absorbance vs. pH curves in the related pH ranges are examined carefully, and thus the value of the related missing pK_a value of each compound is investigated at a selected wavelength by using the Henderson-Haselbalch equation as described by Albert et al [11]. Using the preliminary findings of this examination, a preliminary edition is presented in [26] in which the missing acidity constant for the each compound could not be determined at the time of congress event.

The pH-dependence of the absorbance curves due to 30 minutes for **I** and 40 minutes for **II** found at selected wavelengths in aqueous methanol are shown in Fig. 4. As shown in Fig. 4, the absorbance *vs.* pH curves obtained both for **I** and **II** are an S-shaped indicating clearly the existence of one dissociation step [11-13,27]. The isosbestic point reported in the section *3.1* for **I** above support the acid base equilibrium in the corresponding stage of the hydration

[24,25]. The mean missing pK_a values calculated both for **I** and **II** are given in Table 1 above. As shown in Table 1, newly calculated pK_a values of 2.881 for **I** and 3.684 for **II** in this study can be considered roughly as a limit upper value for the hydration of the respective compounds in the acidic media investigated. Because there is no hydration at pH values 3.06 and above for **I** and, above 4.06 for **II** in the acidic region. Thus, all the explanations and the data given in Table 1 are in good agreement with Scheme 1 and 2.

Figure 4 may be placed here

The wavelengths selected are 245.2 nm for compound **I** and 247.4 nm for compound **II**. The obtained inflection given by **I** in Fig. 4 is the missing acidity constant for which a pK_a value of 2.881 (R^2=0.9993) is obtained at λ = 245.2 nm. On the other hand, the obtained inflection given by **II** in Fig. 4 is the missing acidity constant for which a pK_a value of 3.684 (R^2=0.9981) was obtained at λ = 247.4 nm. The determined missed pK_a values with the related protonated amino form of **I** and **II** are displayed in Scheme 3 below.

Scheme 3 may be placed here

3.5. Final situation of the four acidity constants for each compound

The preferred four acid dissociation mechanisms are fully revised based on the data obtained in this current study, and the latest visual four acid – base equilibria of each compound against varying pH are given in Scheme 4, below. The latest situations of all acidity constants for both compounds are also given in Table 2 below.

Scheme 4 may be placed here

Table 2 may be placed here

As shown in Scheme 4, each of **I** and **II** must have theoretically four acidity constants. In the recent study [13], the pK_a values related to ring nitrogen at position 1 of **I** and **II** were not determined. However, the other three of them were determined in the recent study [13] and, the pK_{a1} values were found to be 5.121 (λ = 267.8 nm) for **I** and 4.684 (λ = 256.6 nm) for **II** both of

which were misattributed as the dissociation of one proton from the protonated nitrogen form (–NH=)$^+$ substituted to the ring nitrogen, (>N=)$^+$ at position 1 of **I** and **II**, respectively. Because, the pK_a value for the deprotonation mentioned earlier was obtained as 2.881 for **I** and 3.684 for **II** in this current investigation. The determination of this misattribution is a great accomplishment of this current study. Thus, the required correction and reinterpretation were done as given in below.

Before going into reinterpretation of those, it should be reminded that there were no problems with the obtained pK_{a2} and pK_{a3} values presented in the recent study [13]. The pK_{a2} value was attributed in the recent study [13] to the dissociation of one proton from the protonated ring nitrogen (–NH=)$^+$ at position 3 of **I** and **II**. The pK_{a3} value in the recent study [13] was attributed to the dissociation of the one proton from both the enol form of **I** and the thioenol form of **II**. No experimental data was given for the fourth dissociation constant, relating it to ring nitrogen at position 1 of both **I** and **II** in the recent study [13].

As for the reinterpretation, the missed acidity constant is fixed in this current study, in the case of using aged solutions for the each compound. This current study showed that the pK_{a1} value obtained in the recent study [13] actually belongs to the unavailable fourth dissociation constant in the recent study as relating it to ring nitrogen at position 1 of both **I** and **II**. Unfortunately, it was misattributed as the dissociation of one proton from the protonated nitrogen form (–NH=)$^+$ substituted to the ring nitrogen, (>N=)$^+$ at position 1 of **I** and **II**, respectively. Finding this misattribution is the innovation of this current study. Therefore, it can be seen clearly now from this current study that one of the four acidity constants are masked in the recent study [13] for which fresh solutions of **I** and **II** were used. In the light of this current study, it can thus be resulted that the inability in the determination of one of the four acidity constants led to a misattribution and so on a misinterpretation of the acidity constants determined for **I** and **II** in the recent study [13].

4. Conclusions

In this study, time-dependent isosbestic point method was improved for the first time to confirm the presence of any weak acid – base equilibrium and the detection of the related equilibration time. This is a great accomplishment of this study and will be a model for further studies on weak acids and bases. Besides, the finding of the missing acidity constant is the innovation of this study.

The pK_{a1} value of 5.121 (λ = 267.8 nm) for **I** and 4.684 (λ = 256.6 nm) for **II** obtained in the recent study is actually not related to the deprotonation of the protonated nitrogen $(\text{-NH=})^+$ substituted to the ring nitrogen at position 1. Rather, it is found in this study that it is to be related to the deprotonation of the previously protonated form of the ring nitrogen $(\text{>N=})^+$ at position 1 of the each compound. Thus, the required correction is done in this study and the most accurate four acidic dissociation mechanisms for **I** and **II** are identified. The final situation of the four pK_a values is displayed for each compound.

Acknowledgements

This work was supported by the Scientific Research Project Program of Marmara University (Project No: FEN-A-250608-0185). The author is grateful to Marmara University for their financial support and the provision of laboratory facilities.

References

[1] M. Radi, S. Schenone, M. Botta, Recent highlights in the synthesis of highly functionalized pyrimidines Org. Biomol. Chem. 7 (2009) 2841-2847.

[2] G. Hughes, C.S. Wang, A.S. Batsanov, S.S. Fern Mati, S. Chall, S. Konar, S. Rakchit, S.C. Bhattacharya, Pyrimidine-based fluorescent zinc sensor: photo-physical characteristics, quantum chemical interpretation and application in real samples, Sens Actuators B 201 (2014) 204-212.

[3] E.V. Verbitskiy, E.M. Cheprakova, J.O. Subbotina, A.V. Schepochkin, P.A. Slepukhin, G.L. Rusinov, et al, Synthesis, spectral and electrochemical properties of pyrimidine-containing dyes as photosensitizers for dye sensitized solar cells, Dyes Pigment 100 (2014) 201-214.

[4] M. Asif, A Mini Review: Biological Significances of Nitrogen Hetero Atom Containing Heterocyclic Compounds, International Journal of Bioorganic Chemistry, 2(3) (2017) 146-152.

[5] J. Toohey, A. Cooper, Thiosulfoxide (Sulfane) Sulfur: New Chemistry and New Regulatory Roles in Biology, Molecules 19 (2014) 12789–12813.

[6] Giulia Vignaroli, Pierpaolo Calandro1, Claudio Zamperini, Federica Coniglio, Giulia Iovenitti, Matteo Tavanti, David Colecchia, Elena Dreassi, Massimo Valoti, Silvia Schenone, Mario Chiariello, Maurizio Botta, Improvement of pyrazolo[3,4-*d*]pyrimidines pharmacokinetic properties: nanosystem approaches for drug delivery, Sci. Rep. **6**, 21509; doi: 10.1038/srep21509 (2016).

[7] S. Massari, G. Nannetti, J. Desantis, G. Muratore, S. Sabatini, G. Manfroni, B. Mercorelli, V. Cecchetti, G. Palù, G. Cruciani, A. Loregian, L. Goracci, O. Tabarrini, A broad anti-influenza hybrid small molecule that potently disrupts the interaction of polymerase acidic protein-basic protein 1 (PA-PB1) subunits, J. Med. Chem. 58 (2015) 3830-3842.

[8] S. Kumar, B. Narasimhan, Therapeutic potential of heterocyclic pyrimidine scaffolds, Chem Cent J. 12(38) (2018) 1-29.

[9] A.M. Gamal-Eldeen, N.A. Hamdy, H.A. Abdel-Aziz, E.A. El-Hussieny, I.M.I. Fakhr, Induction of intrinsic apoptosis pathway in colon cancer HCT-116 cells by novel 2-substituted-5, 6, 7, 8-tetrahydronaphthalene derivatives, Eur. J. Med. Chem. 77 (2014) 323-333.

[10] H. Kılıç, Ultraviolet–visible study on acid–base equilibria for some 7,8-ethylenedioxy coumarins, Journal of Molecular Liquids 187 (2013) 314–319.

[11] A. Albert, E.P. Serjeant, The Determination of Ionization Constants, A Laboratory Manual, 3rd ed., Chapman and Hall, London, 1984.

[12] H. Kılıç, Electronic absorption study on acid–base equilibria for some keto and thioketo pyrimidine derivatives Experimental and theoretical evidence of enolization and solute–solvent interactions, Spectrochimica Acta Part A 67 (2007) 1210–1219.

[13] H. Kılıç, Electronic absorption study on acid–base equilibria for some pyrimidine derivatives containing semi- and thiosemicarbazone moiety, Spectrochimica Acta Part A 75 (2010) 728–733.

[14] H. Kılıç, B. Er, Potentiometric investigation of acid-base equilibria of two new pyrimidine derivatives in various methanol–water media, J. Serb. Chem. Soc. 71(1) 43–54 (2006).

[15] Masashi Hojo, Tadaharu Ueda, Hideaki Hamada, Zhidong Chen, Shigeo Umetani, Condutometric studies on higher ion-aggregation from lithium fluoroalkanoates in propylene carbonate and N,N-dimethylformamide, Journal of Molecular Liquids 145 (2009) 24–32.

[16] S. Zhang, J. Baker, P. Pulay, A reliable and efficient first principles-based method for predicting pK_a values. 2. Organic acids, J. Phys. Chem. A 114 (2010) 432–442.

[17] Y. Akçamur, B. Altural, E. Saripinar, G. Kollenz, O. Kappe, A convenient synthesis of functionalized 1 H-pyrimidine-2-thiones, J. Heterocycl. Chem. 25 (1988) 1419-1422.

[18] B. Altural, Y. Akçamur, E. Saripinar, I. Yildirim, G. Kollenz, Reactions of cyclic oxalyl compounds, Part 29: a simple synthesis of functionalized 1 H-pyrimidines, Monatsh. Chem. 120 (1989) 1015-1020.

[19] H. Kılıç, Ultraviolet–visible study of tautomeric behavior of some carbonyl and thiocarbonyl pyrimidine derivatives: experimental evidence of enolization and thioketonization, Spectrochim. Acta Part A 71 (2008) 175-185.

[20] H. Kılıç, M.L. Berkem, Electrochemical behavior of some new pyrimidine derivatives, J. Serb. Chem. Soc. 69 (2004) 689-703.

[21] F. Rived, M. Rosés, E. Bosch, Dissociation constants of neutral and charged acids in methyl alcohol. The acid strength resolution. Anal. Chim. Acta. 374 (1998) 309-324.

[22] J. Heyrovský, P. Zuman, Practical Polarography, An Introduction for Chemistry Students, Academic Press, London, 1968.

[23] H. Kılıç, Experiment-based physicochemical aspects for the coulombic hydration kinetics and thermodynamics of a pyrimidine and thiopyrimidine, Journal of Molecular Liquids 266 (2018) 443–452.

[24] R.C. Denney, R. Sinclair, D. Mowthorpe, Visble and Ultraviolet Spectroscopy, Analytical Chemistry by Open Learning, John Wiley & Sons, Chichester, 1987 p.87.

[25] E.J. King, The International Encyclopedia of Physical Chemistry and Chemical Physics, Topic 15, first ed., vol. 4, Pergamon Press, Oxford, 1965, p.97.

[26] 5th International Congress on Fundamental and Applied Sciences 2018 (ICFAS2018) Proceeding Book.

[27] G.W. Ewing, Instrumental Methods of Chemical Analysis, 5th ed., McGraw-Hill Book Co., New York, 1987.

Captions to figures

Table 1

The related UV/vis spectroscopic data and the mean missing pK_a values calculated both for **I** and **II** in 95% *v/v* aqueous medium at 25 °C and constant ionic strength (0.10 M LiCl).

Table 2

The latest situation of all acidity constants and the related UV/vis spectroscopic data obtained for **I** and **II** in 95% *v/v* aqueous medium at 25 °C and constant ionic strength (0.10 M LiCl).

a Obtained from the recent study [13].

Fig. 1. Pyrimidine compounds investigated in this study

Fig. 2. In due to course of 30 minutes time dependent UV-Vis absorption spectra of 1.641×10^{-5} M aged solutions of **I** obtained at different pHs in 95% *v/v* aqueous solutions. Isosbestic point at 249.7 nm, A = 0.795.

Fig. 3. In due to course of 40 minutes time dependent UV-Vis absorption spectra of 2.044×10^{-5} M aged solutions of **II** obtained at different pHs in 95% *v/v* aqueous solutions.

Fig. 4. The pH-dependence of the absorbance curves due to 30 minutes for **I** and 40 minutes for **II** found at selected wavelengths in 95% *v/v* aqueous solutions.

Scheme 1: The suggested all protonation mechanisms for **I** and **II** in pH 1.0 aqueous aged solutions.

Scheme 2: The suggested deprotonation mechanisms of **I** and **II** causing to stop the hydrations observed at pHs below 3.20 for **I** and, 4.06 and below for **II**.

Scheme 3: The determined missed pK_a values with the related protonated amino form of **I** and **II**.

Scheme 4: A fully revised the latest four acid – base equilibria of each compound against varying pH.

Table 1

pH Range	Compound	Isosbestic Point		Inflection	Selected wavelength (nm)	The missing pK_a	R^2
		Wavelength (nm)	Absorbans (A)				
1.0 to 3.20	**I**	249.7	0.795	**I**	245.2	2.881	0.9993
1.0 to 4.06	**II**	-	-	**II**	247.4	3.684	0.9981

I: 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-one

II: 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-thione

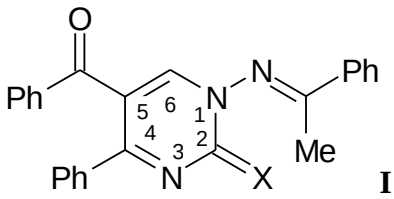
Table 2

Medium	Compound	Selected wavelength (nm)	Isosbestic Points (nm)	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
Aqueous methanol (v/v, 5% methanol)	**I**	**245.2**	**249.7**	**2.881**	-	-	-
		267.8	346.2	-	5.121[a]	-	-
		337.4	251.5	-	-	7.929[a]	-
		270.0	322.5	-	-	-	11.130[a]
	II	**247.4**	-	**3.684**	-	-	-
		256.6	315.1	-	4.684[a]	-	-
		301.2	265.0	-	-	7.245[a]	-
		299.6	395.8	-	-	-	10.630[a]

I: 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-one.

II: 5-benzoyl-1-(methylphenylmethyleneamino)-4-phenyl-1H-pyrimidine-2-thione.

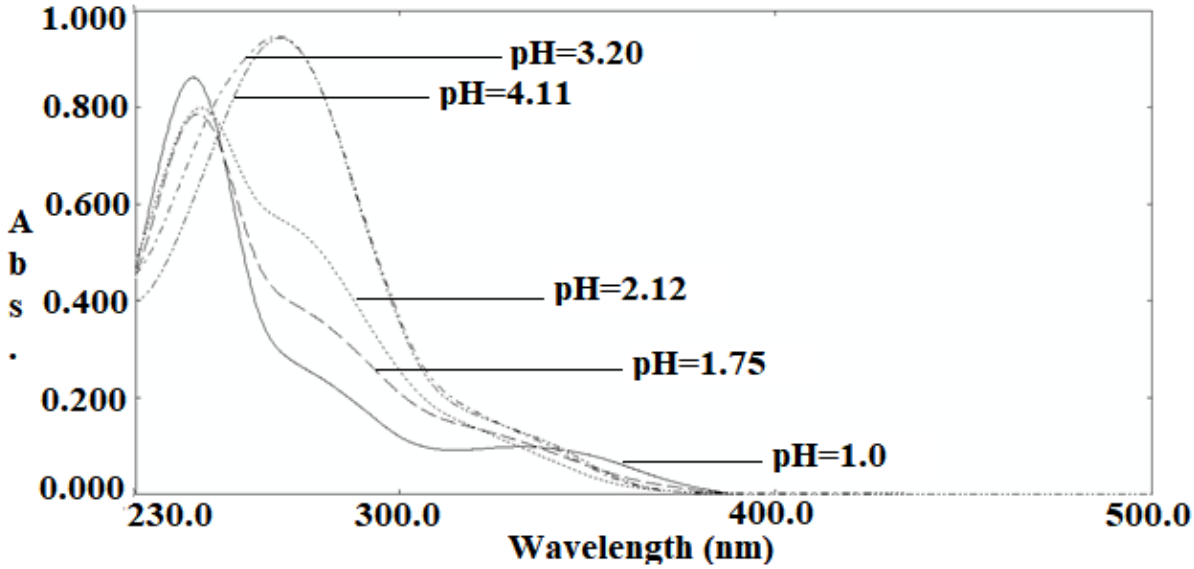
Fig. 1



I : X = O

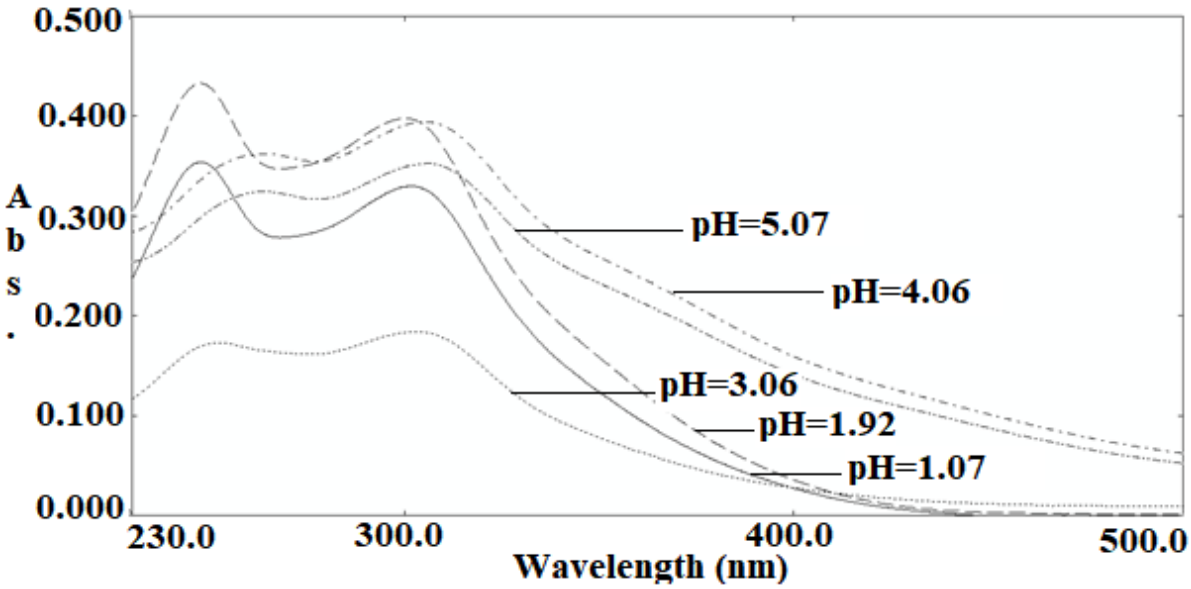
II : X = S

Revised Fig. 2



The old Figure 2 shown below is deleted

Revised Fig. 3



The old Figure 3 shown below is deleted

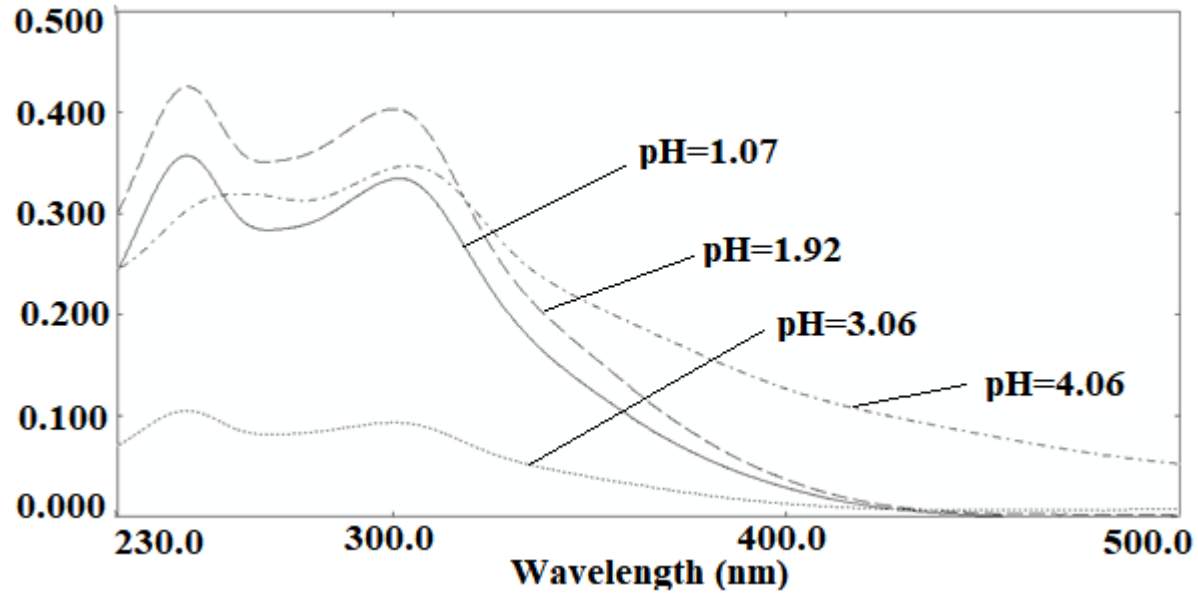
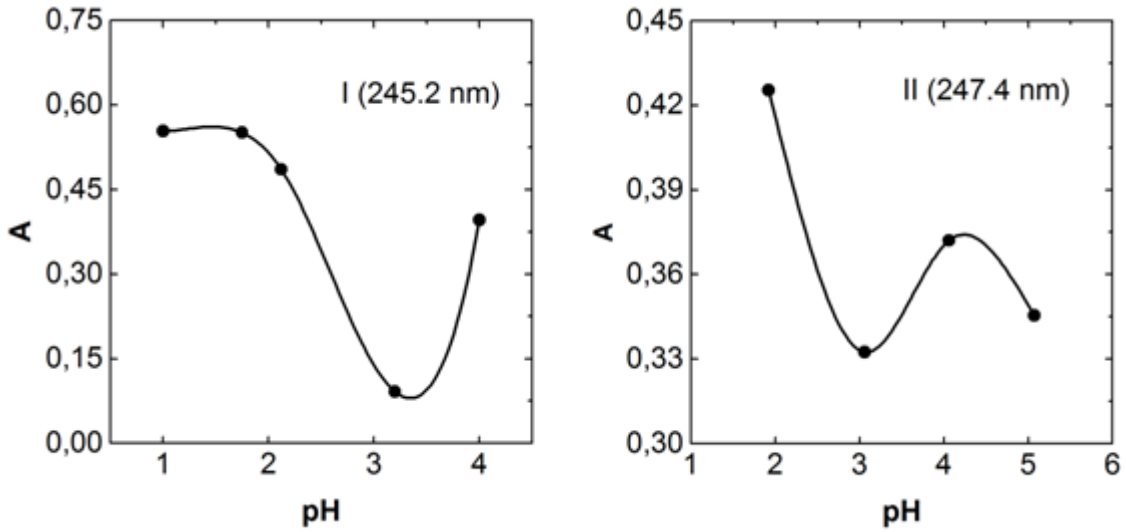
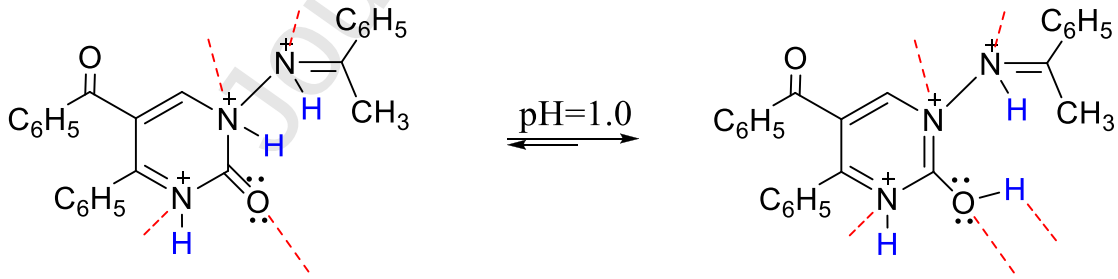


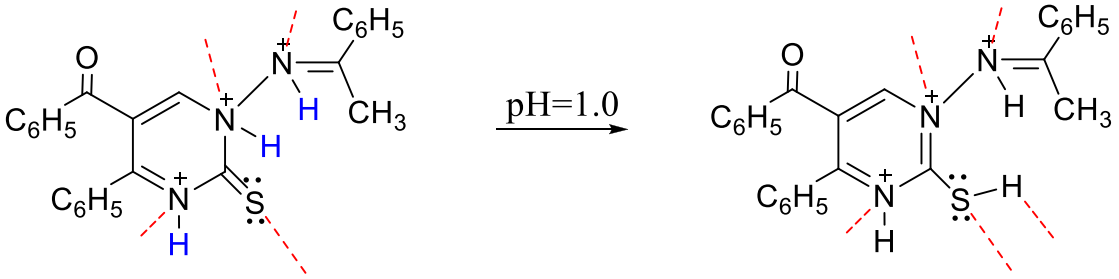
Fig. 4


0,75
0,60
0,45
0,30
0,15
0,00
A
I (245.2 nm)
1
2
3
4
pH
0,45
0,42
0,39
0,36
0,33
0,30
A
II (247.4 nm)
1
2
3
4
5
6
pH

Scheme 1

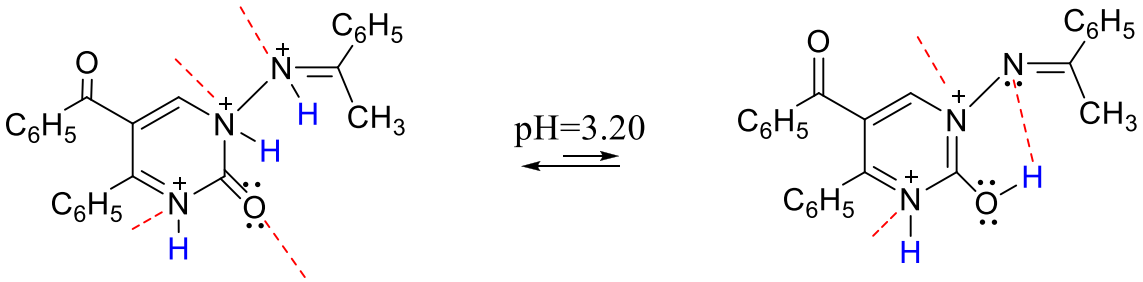


Ia **Ia'**

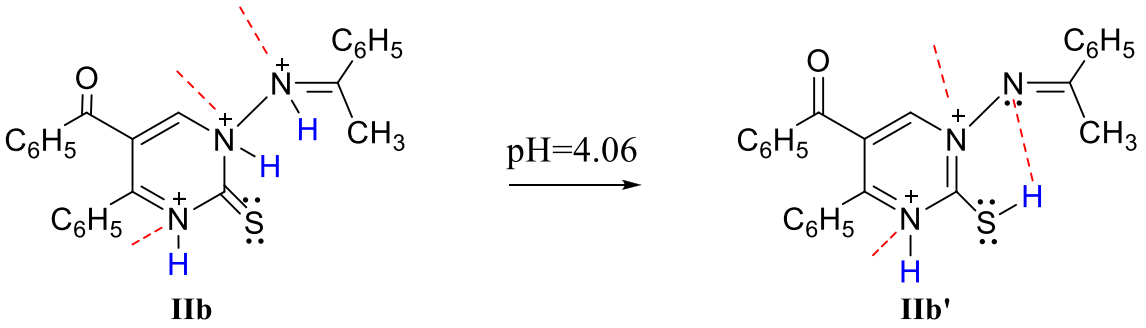


IIa **IIa'**

Scheme 2

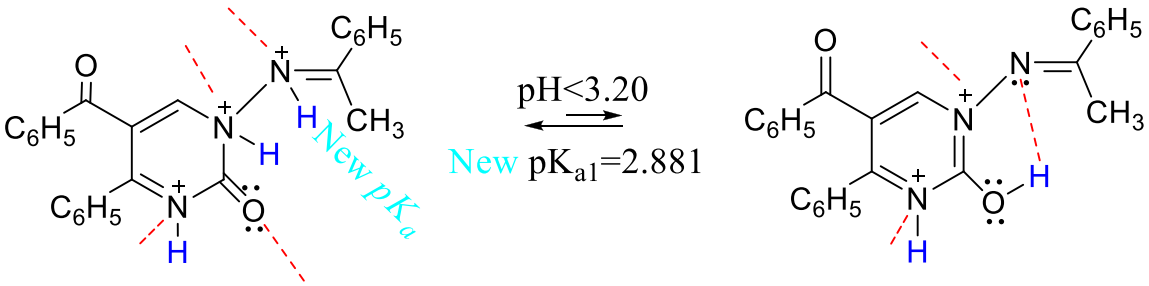


Ib **Ib'**

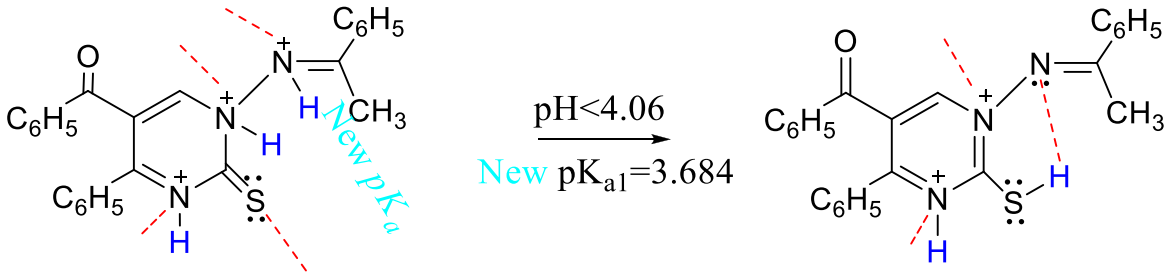


IIb **IIb'**

Scheme 3

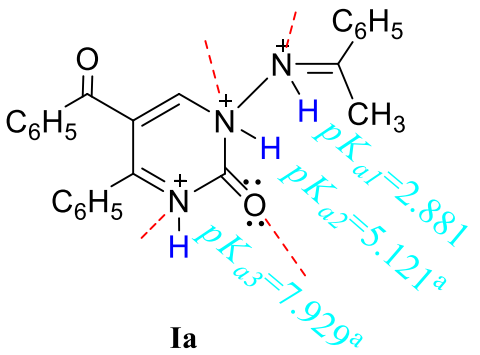


Ib **Ib'**

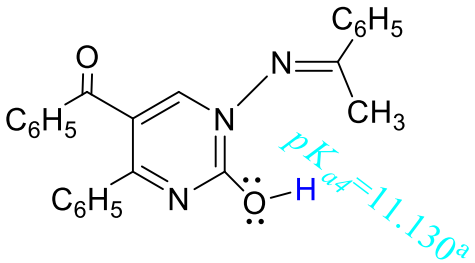


IIb **IIb'**

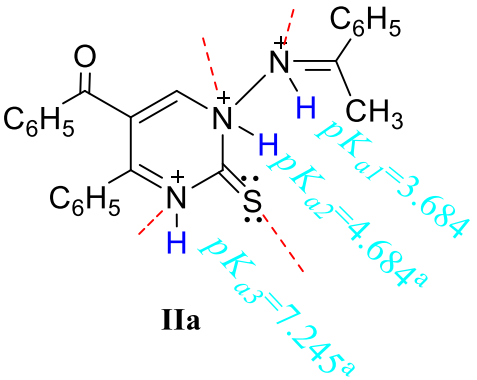
Scheme 4



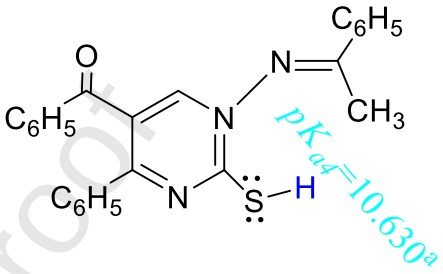
Ia



Ia'



IIa



IIa'

Declaration of interests

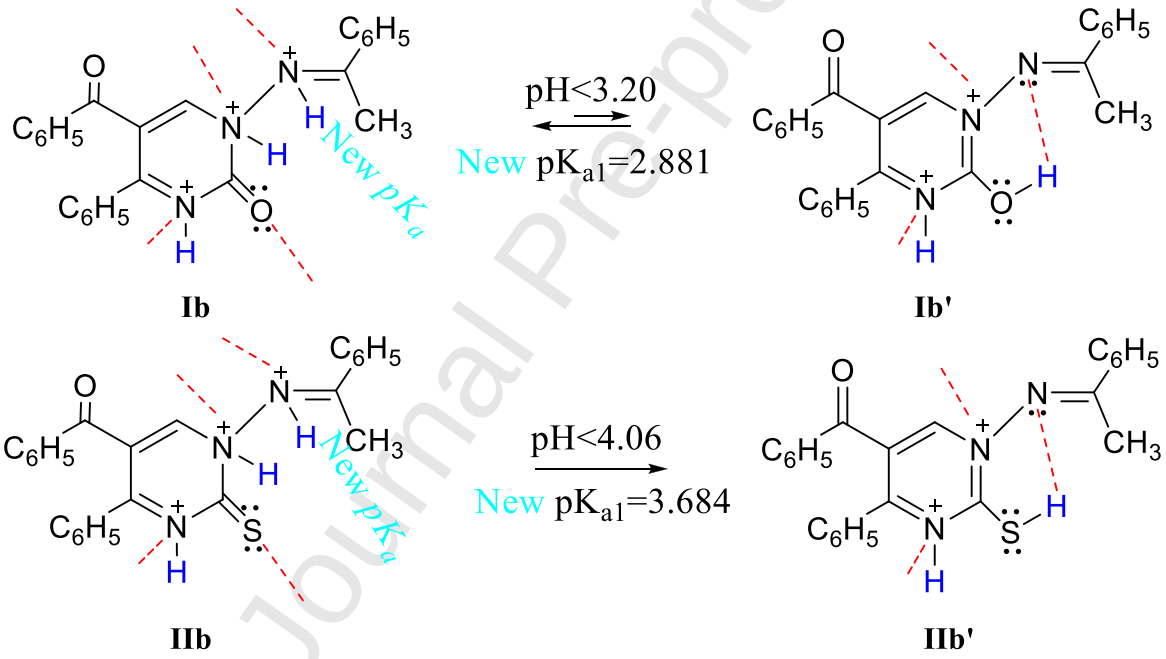
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Graphical abstract

UV/vis spectrophotometric determination of slow equilibrated N(1)-H missing deprotonation constant of a pyrimidine and thiopyrimidine: The final situation of the four pK_a values

H. Kılıç

Department of Physical Chemistry, Faculty of Arts and Sciences, Marmara University, Ziverbey 34722 Istanbul, Turkey



Highlights

► All the acid base equilibria for the each compound were identified firmly

► Missing acidity constants were determined using aged solutions

► The use of the aged solutions is provided an increased detection level

► Time-dependent isosbestic point method was improved to find weak acid–base equilibria

► The results are expected to contribute in the drug improvement of **I** and **II**