Determination and evaluation of acid dissociation constants of some novel benzothiazole Schiff bases and their reduced analogs by spectroscopy

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Abstract
The acid dissociation constants (K_a) of eight novel biologically active benzothiazole Schiff bases were determined by UV-vis spectroscopic technique at 25 ± 0.1 °C. The calculated acidity constants, pK_a values, were evaluated in structure elucidation and protonation – deprotonation mechanisms. The prototropic tautomerism was also studied.

Keywords: Benzothiazole Schiff bases, reduced analogs, acidity constant, tautomerism

Introduction

There has been a considerable interest in Schiff bases derived from salicylaldehyde because of their thermochromic and photochromic properties in the solid state.[1-2] The biological activities of benzothiazole derivatives are well known.[3-4] Much research has been devoted to study the metal ligand interaction and biological behavior of such derivatives which possess the azomethine (CH=N) linkage.[5-6] The biological activity of these compounds may be connected to their ability to form complexes with certain metal ions which may lead to a ‘‘locked geometry’’ via the coordination mechanism so that only certain substances are able to become attached to the framework of this interaction.[7-10] Many anticancer and antibacterial drugs are known to behave as versatile ligands, some of which exhibit increased anticancer activity when administered in the complex form with metal ions.[11-13] It has been suggested that certain types of cancers are caused by viruses. The interaction between the metal ion and their ligands with cancer-associated viruses might represent an important route in designing, a new anticancer therapies for tumors that become resistant to the conventional drugs.[14] A recent methodology to design novel antiviral therapies is achieved by coordinating a metal ion from an important bio-molecule, such as a zinc finger protein, with the antiviral agent, usually containing sulfur functionalities with good complex forming behavior.[15]

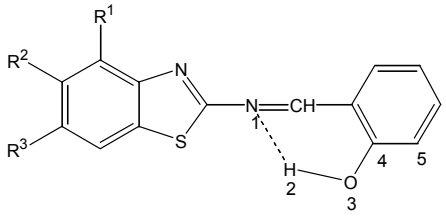
The acidity concept have been used in various areas of research, such as stereo-chemical and conformational structure determinations, the directions of nucleophilic and electrophilic attack, the stabilities of intermediates, the size of activation energies in organic reactions and the determination of the active sites of enzymes in biochemistry.[16-19] Following our work on the synthesis of some novel benzothiazole Schiff bases we now are reporting on the acid dissociation constants of them to elucidate structure-reactivity relation-ships of these novel compounds.[20]

Results and Discussions

There are two series of molecules in this work. The first series consists of four compounds **1 – 4** and they are all of them are novel Schiff bases. The second series contains the reduced forms of these novel Schiff bases and they are compound **5 – 8** (Table 1).

The first four compounds exhibit proton tautomerism. Therefore, we have concentrated on the proton tautomerism and intramolecular H-bond forming of compounds **1-4**. These two phenomena can have pronounced effect on acid-base behaviours as well as structural properties of organic molecules.

Tautomerism and H-bonding



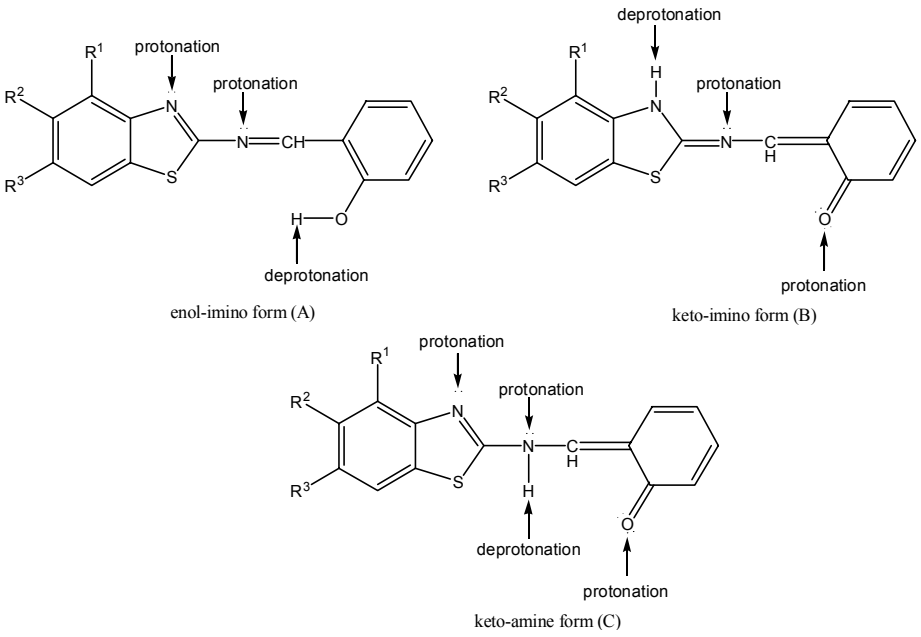
Scheme 1. H-bonding possibility in compounds **1 – 4**.

The formation of B and C in acidic or basic media seems to be very unlikely due to loss of aromatic property of the phenol ring (Scheme 2).

Acidity constants

Considering the structures of the Schiff bases **1–4** we can deduce that they have two protonation sites the first one is the aza-nitrogen atom of the benzothiazole ring and imino- nitrogen atom which is substituted at 2C position of the benzothiazole ring. These compounds, however, possess one deprotonation site (i.e. OH group of the phenol ring) and they are in enol-imino form (Scheme 2). On the other hand when they are in keto-imino tautomeric form (B) they have two protonation sites (i.e. imino nitrogen atom and ketonic oxygen atom of the phenol ring) and one deprotonation site (i.e. –NH- group of the benzothiazole ring). However, when they are in keto-amine form (C), they have three protonation sites (i.e. aza nitrogen atom =NH of benzothiazole

ring, amine group –NH- which is substituted at 2C position of benzothiazole ring and ketonic oxygen atom =O of the phenol ring) and they possess one deprotonation site (i.e. –NH-group which is located at 2C position of the benzothiazole ring) (Scheme 2).



Scheme 2. Possible tautomeric forms of Schiff bases **1-4** via proton migration.

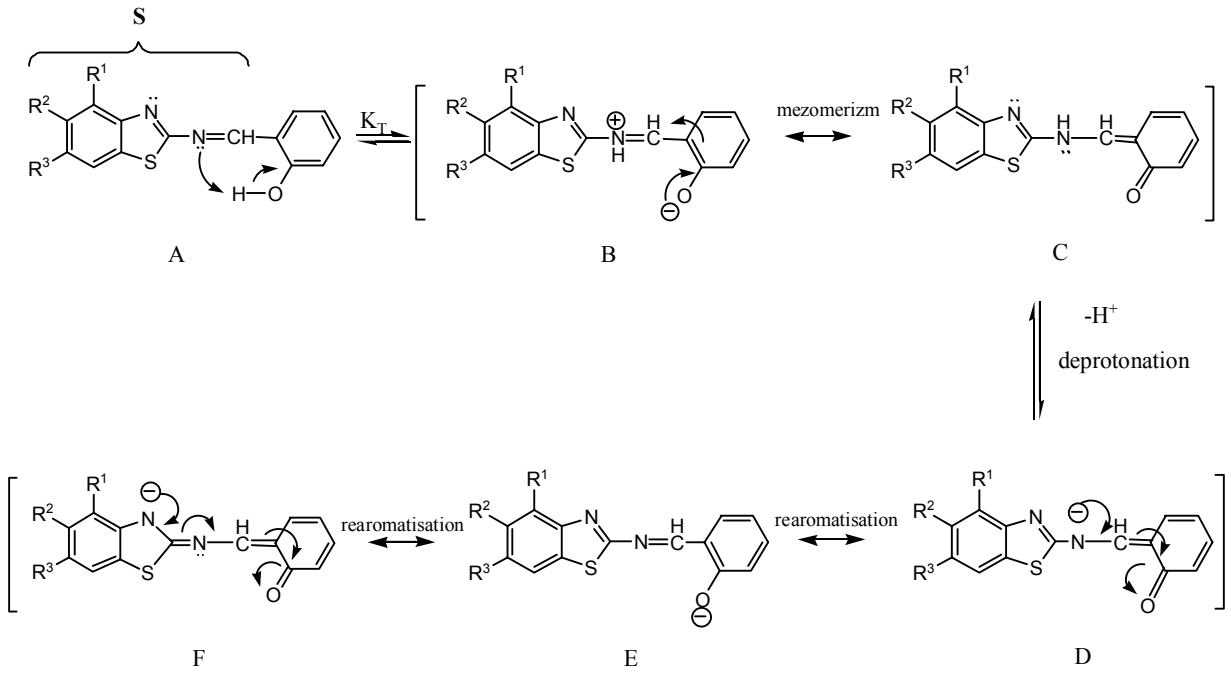
Deprotonation (pK_{a1} values*)*

When we arrange the studied Schiff bases and their reduced derivatives in an increasing acidity order for deprotonation process we get the following sequence;

Molecule:	8	5	6	7	phenol	1	3	2	4
Half protonation ($H^{1/2}$)	12.45	12.43	12.26	11.36	10.00	9.78	9.44	8.40	8.37

⟶ increasing acidity

If we take the ionization constant of phenol (i.e. pK_a=10) as reference, we can say that the big groups (i.e. **S** and **R**) act as *ortho* substituent on the ionization of Schiff bases and their reduced derivatives (Scheme 4 and 7) respectively. It seems that in molecules **1 – 4** both the benzothiazole ring and phenol ring are on the same plane and the through conjugation lead to proton transfer from OH group to N atom of imino group, forming the zwitter ion iminium form (B), followed by a subsequent rearrangement into the keto-amine form (C). Under these conditions, the deprotonation may occur from NH group and rearrangement of anion (D) leads to anion (E). This means that deprotonation mechanism of these four molecule **1 – 4** is different from the other molecules **5 – 8** (Scheme 3 and Table 2).



Scheme 3. Possible deprotonation patterns for molecules 1 **– 4**.

In molecule **4**, the two methyl groups at **5** and **6** position of benzothiazole ring make **S** group more electron-donating than the other molecules of **1-3** and keeping the proton more firmly make the molecule **4** more acidic (less basic) among the others. In molecule **1,** however, there is no methyl group on benzothiazole ring and the **S** group becomes less electron-donating and consequently molecule **1** becomes less acidic (more basic).

In molecules **5 – 8,** there is no possibility of thorough conjugation. Therefore formation of keto forms are not feasible and presumably the deprotonation occurs at phenolic OH group. In here also dimethyl groups in molecule **8** were effective by making the **R** group more electron withdrawing but in opposite direction of molecule **4** as expected. Since there is no thorough conjunction, the only effect of group **R** is inductive electron donation and making the whole molecule more basic or less acidic. If we think the position of methyl group in molecule **7,** the unusual effect of the **R** group can be expected (Scheme 3).

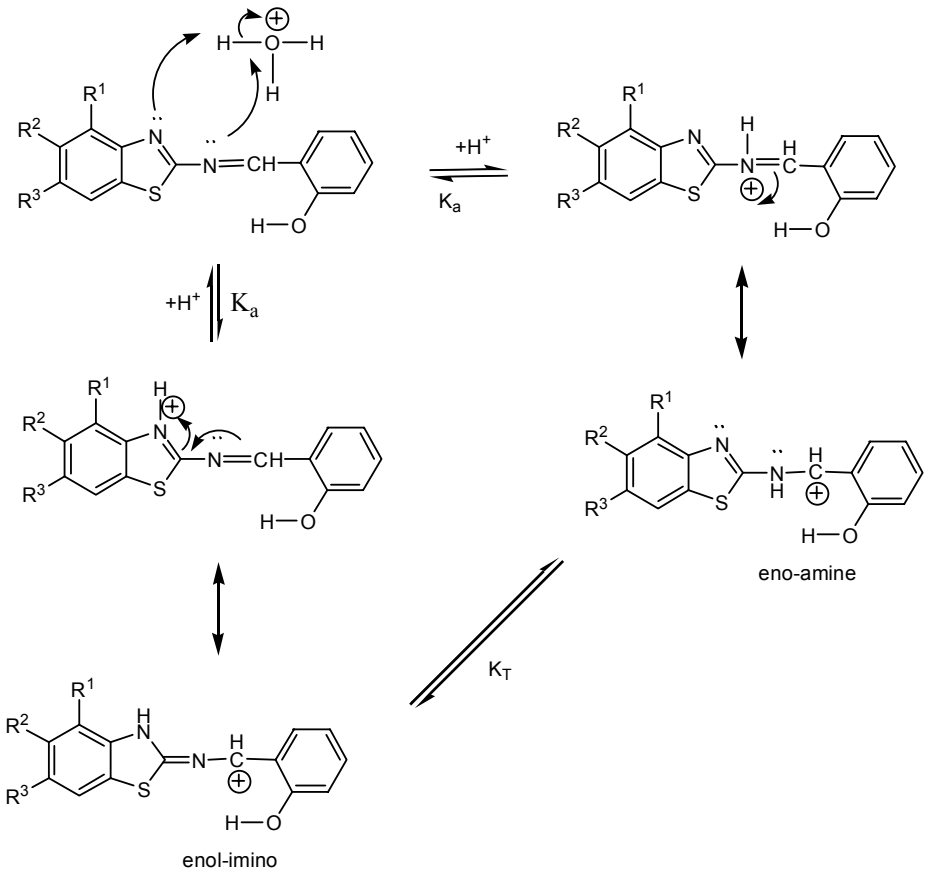
First protonation (pK_{a2} values)

When we arrange the molecules with increasing acidity power for the first protonation, we get the following order:

Molecule:	**1**	**4**	**5**	**3**	**2**	**8**	**7**	**6**
Half protonation ($H^{1/2}$)	4.57	4.45	3.94	3.84	3.80	3.65	3.50	3.02

→ increasing acidity

If we compare the obtained half protonation values of the studied molecules (Table 3) with the literature pK_a values of 1.84 and 4.51 for the aza nitrogen atom protonation of benzothiazole ring[22,23] and 2-aminobenzothiazole[24] molecules respectively , due to similarity of the obtained pK_a values of this study we can conclude that the studied molecules were protonated with the same mechanism of these two molecules and that is the aza nitrogen atom protonation of the benzothiazole ring. The reduced derivatives of the corresponding Schiff bases are less basic (i.e. $pK_{a\,(5,6,7,8)} < pK_{a\,(1,2,3,4)}$ 5<1; 6<2; 7<3 and 8<4). This is an expected result because there are a through conjugation between the two rings namely benzothiazole and phenol ring in Schiff bases **1 – 4** which don't exist in their reduced derivatives **5 – 8** (Scheme 4).



Scheme 4. Possible first protonation patterns for molecules **1 – 4**.

Second protonation (pK_{a3} values)

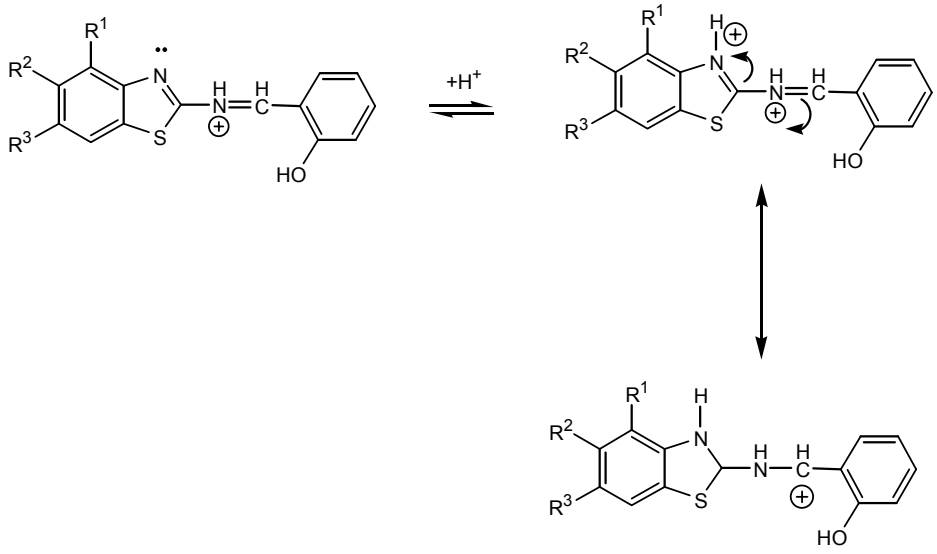
When we are arrange the studied molecules with increasing acidity power for the second protonation, we get the following order:

Molecule:	**4**	**2**	**3**	**1**
Half protonation ($H^{1/2}$)	3.10	2.18	1.86	1.30

⟶ increasing acidity

The half protonation values of 1.86 and 1.30 for compounds **3** and **1** are comparable with benzothiazole aza nitrogen atom protonation (i.e.1.84) . So obviously the protonation had taken place at aza nitrogen atom of benzothiazole ring of these two compounds. Obviously strong electron – withdrawing effect of protonated imine group makes the ring nitrogen electron deficient. This means that molecules become more acidic. Following the second protonation, a subsequent mezomerization seems to occur to form a more stable carbonium ion (Scheme 5).

By considering the pK_a value of 3.10 for molecule **4** which is quite different from the other three molecules **1 – 3** which contains one methyl or no methyl group, we can predict that this bigger pK_a value of this compound is expected because of inductively electron donating effect of dimethyl groups on benzothiazole ring.



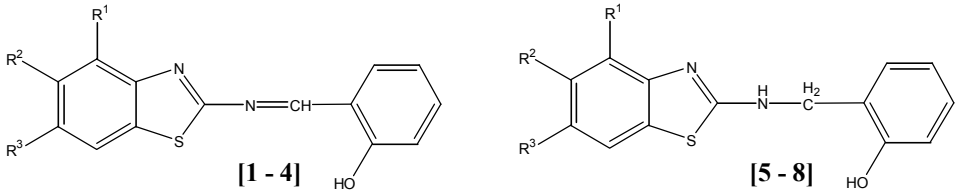
Scheme 5. Possible second protonation patterns for molecules **1** and **3**.

Experimental Section

General Procedures. The studied compounds (Table 1) were synthesized and the procedures of synthesis are described elsewhere.[20] Methanol, ethanol, glycine, KOH, H_2SO_4, HCl, CH_3COOH, CH_3COONa, NaOH, KH_2PO_4, Na_2CO_3, $NaHCO_3$, phenolphthalein indicator, and standard buffer solutions were from Aldrich and were not purified further.

Apparatus. pH measurements were performed using a glass electrode. pH values of the standard buffer solutions were 1, 7, and 14 they were used in the calibration of the Orion pH/ion analyzer. The Ohaus Advanturer balance; a Scimadzu UV2450 PC UV-vis scanning spectrometer was used for measurements.

Table 1. Nomenclature and formulae of the studied molecules [**1-8**]



Molecule	IUPAC Name	R^1	R^2	R^3
1	2-(2-aza-2-benzothiazol-2-ylvinyl)phenol	H	H	H
2	2-[2-aza-2-(4-methylbenzothiazol-2-yl)vinyl]phenol	CH_3	H	H
3	2-[2-aza-2-(6-methylbenzothiazol-2-yl)vinyl]phenol	H	H	CH_3
4	2-[2-aza-2-(5,6-dimethylbenzothiazol-2-yl)vinyl]phenol	H	CH_3	CH_3
5	2-[(benzothiazol-2-ylamino)methyl]phenol	H	H	H
6	2-{[(4-methylbenzothiazol-2-yl)amino]methyl}phenol	CH_3	H	H
7	2-{[(6-methylbenzothiazol-2-yl)amino]methyl}phenol	H	H	CH_3
8	2-{[(5,6-dimethylbenzothiazol-2-yl)amino]methyl}phenol	H	CH_3	CH_3

Procedure. Acid solutions were prepared with H_2SO_4 (w/w) (0.0049 to 96 % H_2SO_4) in water.[21] The CO_2-free NaOH solutions were prepared with NaOH pellets (1 to 16.4 mol.dm^{-3}) in water.[25] Buffer solutions were prepared with procedures well described by using Perrin's desciription.[26] Spectrometry is an ideal method[27] when a substance is not soluble enough for potentiometry or when its pK_a value is particularly low or high (e.g., less than 2 or more than 11).

The protonation of a weak base can be defined as follows:[21]

$$HX + SH \rightleftharpoons X^- + SH_2^+ \quad (1)$$

where SH is the solvent, then the equilibrium constant might be expressed in terms of concentration and activity coefficient:

$$K_a = \frac{a_{X^-} a_{SH_2^+}}{a_{HX}}$$

where a= cγ; a= activity constant; γ= activity coefficient; c= concentration:

$$K_a = \frac{[X^-]}{HX} \frac{\gamma_X}{\gamma_{HX}} a_{SH_2^+} = H_x \frac{[X^-]}{[HX]} \quad (2)$$

Therefore, the Eq. 2 can be rearranged as follows:

$$H_x = -\log h_x = pK_a - \log \frac{[HX]}{[H^+]} \quad (3)$$

where H_x is an acidity function. The H_o scale is defined such that, for the uncharged primary aniline indicators used, the plot of log *I* (i.e., log([BH^+]/[B])) against H_0 has unit slope. It was observed from work on bases other than the Hammett type that the slopes of the plots of log *I* against H_o, donated by *m,* were not always unity. Thus, series of structurally similar bases, like triarylmethanols[28], primary amides[29,30] and tertiary aromatic amines[31] defined individual acidity functions *(*H_R, H_A, and H_), which have a linear relationship to H_o with *m* values of 2.0, 0.6, and 1.3, respectively. Yates proposed that any acidity function H_x would be proportional to H_o over the entire acidity range, that is, $H_{x=}$ mH_o, with a common point H_o=0.[32]

As explained in the previous paragraph plot of log *I* against H_o does not yield the pK_a at log *I*=0, unless it is an Hammett base, but yields the H_o at half-protonation value *(*$H_o^{1/2}$). The general Eq. 3 may therefore be applied and Eq. 4 can be obtained.

$$\log I = m(H_o^{1/2} - H_o) \quad (4)$$

By rearranging Eq.4 we obtain the following expression;

$$\log I = mH_o^{1/2} - mH_o$$

$$\log I = -mH_o^{1/2} + pK_a \text{ and for } \log I = 0$$

rearranging the last expression we will produce Eq. 5:

$$pK_a = mH_o^{1/2} \quad (5)$$

Where $H^{1/2}$ describes the half protonation value.

The general procedure applied as follows: a stock solution of the compound under investigation was prepared by dissolving the compound (about 10 to 20 mg) in water or sulfuric acid of known strength (25 mL) in a volumetric flask. Aliquots (1 mL) of this solution were transferred into 10 mL volumetric flask and diluted to the mark with sulfuric acid solutions of various strengths or buffers of various pH. The total mass of solution in each flask was measured, and the mass percent of sulfuric acid in each solution was than calculated knowing the mass of sulfuric acid added and the total mass of the final solution. In the case of buffer solutions, the pH was measured before and after addition of the new solution. The optical density of each solution was then measured in 1 cm cells, against solvent blanks, using a constant temperature cell-holder Scimadzu UV2450 PC UV-Vis. A scanning spectrometer was thermostated at 25 °C (to within ± 0.1 °C). The wavelengths were chosen such that the fully protonated form of the substrate had a much greater or a much smaller extinction coefficient than the neutral form. The analytical wavelengths, the half-protonation values, and the UV absorption maxima for each substrate were depicted in Tables 2 and 4.

Calculations of half-protonation values were carried out as follows; the sigmoid curve of optical density or extinction coefficients at the analytical wavelength (OD,λ) was first obtained (Figure 1). The optical density of the fully protonated molecule (OD_{ca}, optical density of conjugated acid) and the pure free base (OD_{fb}, optical density of free base) at an acidity were then calculated by linear extrapolation of the arms of the curve. Eq. 6 provides the ionization ratio where the OD_{obs} (the observed optical density) was in turn converted into molar extinction $\in_{obs}$ using Beers law of OD=$\in bc$ *(b*=cell width, cm; *c*= concentration, mol.dm^{-3}):

$$I = \frac{[BH^+]}{[B]} = \frac{(OD_{obs} - OD_{fb})}{(OD_{ca} - OD_{obs})} = \frac{(\in_{obs} - \in_{fb})}{(\in_{ca} - \in_{obs})} \quad (6)$$

The linear plot of log I against Hx or pH, using the values -1.0<log I<1.0, had slope m, yielding half-protonation value as $H_x^{1/2}$ or $pH^{1/2}$ at log I= 0 The pK$_a$ values were calculated by using Eq. 7 (Figure 2).

$$pK_a = mH_x^{1/2} \text{ (or } pH^{1/2}) \quad (7)$$

Table 2. The UV Spectral data and the acidity constants, pK$_{a1}$ values, of compounds 1-8 for deprotonation process

compound	spectral maximum λ/nm		acidity measurements		
	neutral[a] species (log ∈$_{max}$)	anion[b] (log ∈$_{max}$)	λ[c]nm	H$^{1/2d}$	corr.[e]
1	264 (3.21)	262 (3.35)	258	9.78	0.99
2	259 (3.33)	265 (3.12)	258	8.40	0.99
3	262 (3.37)	264 (3.34)	262	9.44	0.99
4	258 (3.82)	265 (4.00)	265	8.37	0.99
5	269 (3.74)	271 (3.80)	243	12.43	0.99
6	271 (3.71)	275 (3.76)	292	12.26	0.99
7	271 (3.74)	272 (3.70)	294	11.36	0.99
8	271 (3.65)	273 (3.79)	294	12.45	0.99

[a] Measured in pH= 7 buffer solution. [b] Measured in pH=13 buffer solution. [c] The analitical wavelength for pK$_a$ determination. [d] Half-protonation values. [e] Correlations for log I as a function of pH graph.

Table 3. The UV Spectral data and the acidity constants, pK$_{a2}$ values, of compounds 1-8 for the first protonation process

compound	spectral maximum λ/nm		acidity measurements		
	neutral[a] species (log ∈$_{max}$)	monocation[b] (log ∈$_{max}$)	λ[c]nm	H$^{1/2d}$	corr.[e]
1	264 (3.21)	255 (3.45)	286	4.57	0.99
2	259 (3.33)	257 (3.28)	258	3.80	0.99
3	262 (3.37)	259 (3.41)	290	3.84	0.99
4	258 (3.82)	257 (4.02)	293	4.45	0.99
5	269 (3.74)	263 (3.68)	243	3.94	0.99
6	271 (3.71)	267 (3.67)	292	3.02	0.99
7	271 (3.74)	266 (3.61)	294	3.50	0.99
8	271 (3.65)	272 (3.59)	294	3.65	0.99

[a] Measured in pH=7 buffer solution. [b] Measured in pH=1 buffer solution. [c] The analytical wavelength for pK_a determination. [d] Half-protonation value. [e] Correlation for log I as a function of pH graph.

Table 4. The UV Spectral data and acidity constants, pK_{a3} values, of compounds 1-4 for the second protonation

	spectral maximum λ/nm		acidity measurements				
compound	mono cation[a] ($\log \in_{max}$)	dication[b] ($\log \in_{max}$)	λ[c]nm	$H^{1/2d}$	m[e]	pK_{a1}[f]	corr.[g]
1	255 (3.45)	256 (3.32)	284	1.30	0.57	0.74	0.90
2	257 (3.28)	258 (3.34)	265	2.18	1.02	2.18	0.98
3	259 (3.41)	258 (3.44)	267	1.86	0.17	0.31	0.93
4	257 (4.02)	258 (3.33)	293	3.10	0.20	0.62	0.99

[a] Measured in pH= 1 buffer solution. [b] Measured in %50 H_2SO_4. [c] The analytical wavelength for pK_a determination. [d] Half-protonation value. [e] Slopes for log I as a function of pH (or acidity function H_o) graph.[27] [f] Acidity constant value [g] Correlation for log I as a function of H_o graph.

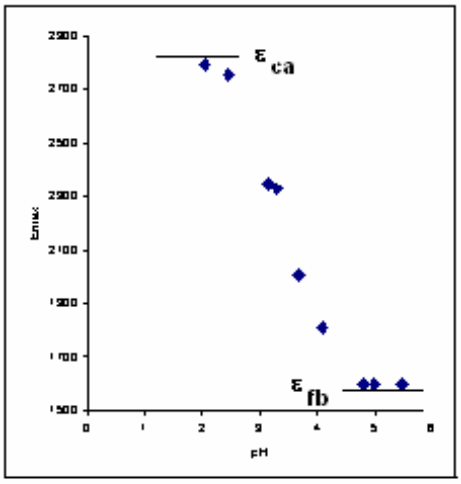


Figure 1. $\in_{max}$ as a function of pH (at 294 nm) for plot for the first protonation of 2-{[(6-methylbenzothiazol-2-yl)amino]methyl}phenol molecule **7.**

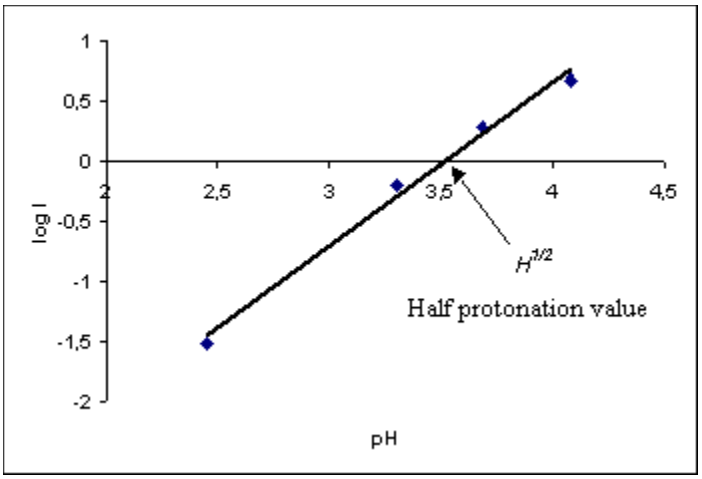
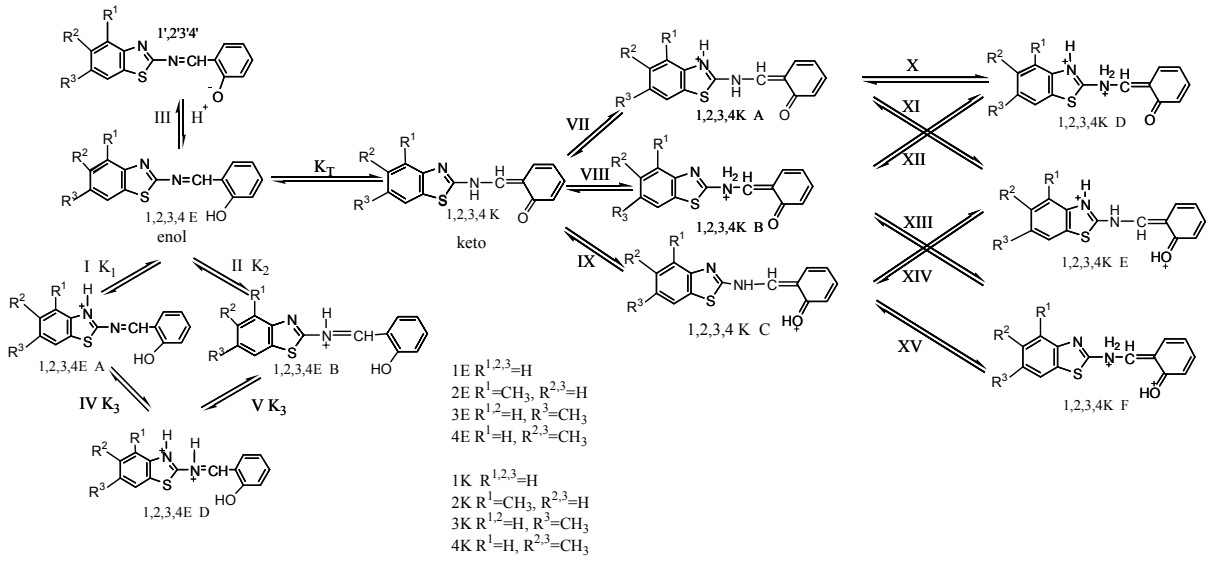
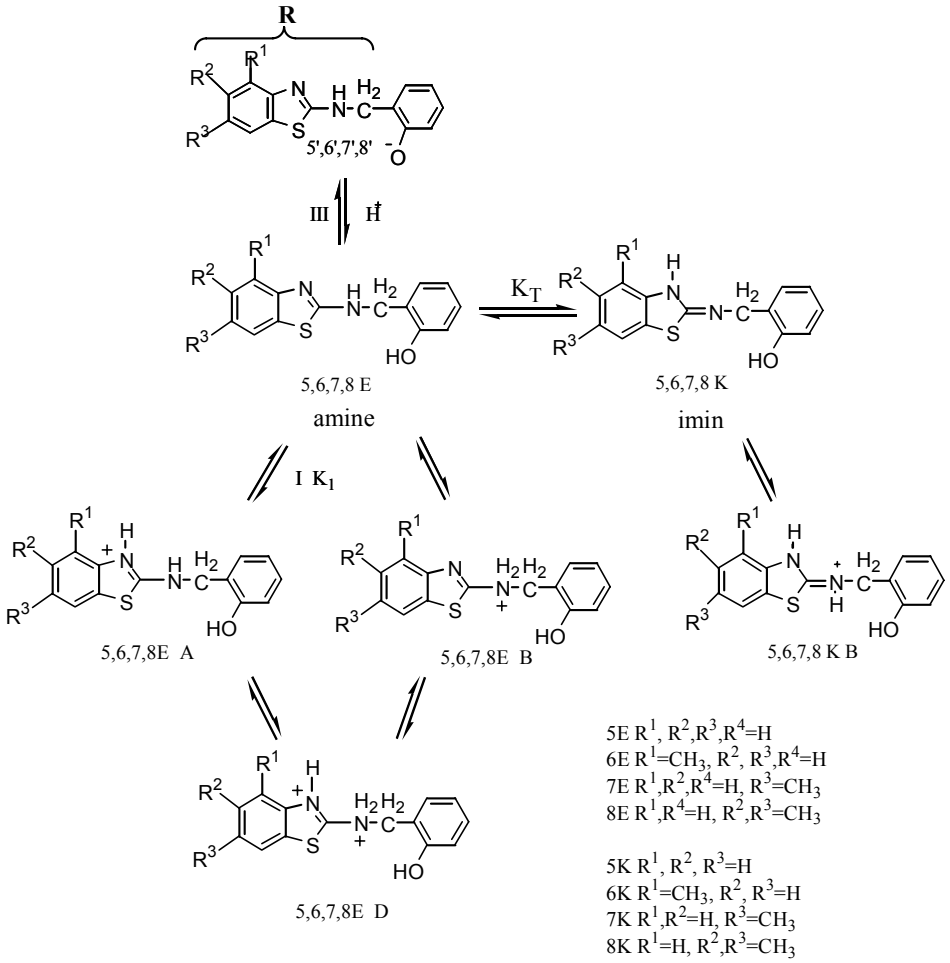


Figure 2. pH as a function of *log I* (at 294 nm) plot the first protonation of 2-{[(6- methylbenzothiazol-2- yl) amino]methyl}phenol molecule **7**.



Scheme 6. Possible deprotonation, first and the second protonation pathways for the enol and keto forms of studied benzothiazole Schiff bases [**1 – 4**].



Scheme 7. Possible deprotonation, the first protonation and the second protonation pathways for the amine and imin forms of the studied reduced benzothiazole Schiff bases [**5 – 8**].

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