ORIGINAL ARTICLE

AB INITIO AND DENSITY FUNCTIONAL THEORY STUDY ON IONIZATION OF BETAHISTINE AND CIMETIDINE NANO DRUG IN AQUEOUS SOLUTION

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Abstract

In this paper, pK_a values of the drugs betahistine and cimetidine were determined in aqueous solution. We used *ab initio* and density functional theory (DFT) methods with the B3LYP 6-31+G(d) functional and basis sets and polarizable continuum solvation model (PCM) to include the effects of aqueous solvation. Also, we have calculated the free energies for determining the pK_a values, and intermolecular hydrogen bonds (IHBs) in aqueous solution by Tomasi's model. In the present work, there is comparable agreement between the experimentally determined pK_a values for the acid-base reactions selected by potentiometric and spectrophotometric methods and those reported in the literature, demonstrating the theoretically calculated pK_a values.

Rezumat

În acest studiu, cele două valori ale pK_a pentru betahistidină și cimetidină au fost determinate pentru soluții apoase. A fost folosită teoria densității funcționale cu seturi bazice de B3LYP 6-31+G(d) și modelul de solvatare polarizabil continuu (MSC) pentru a include efectele solvatării apoase. A fost calculată energia liberă pentru determinarea valorilor pK_a și energia legăturilor de hidrogen intermoleculare în soluție apoasă, folosind model Tomasi. În prezentul studiu, există o corelație între valorile experimentale ale pK_a-ului stabilite pentru reactanții reacțiilor acido-bazice, selectate prin metodele spectrofotometrice și potențiometrice și cele teoretice, din literatură.

Keywords: betahistine, cimetidine, acid dissociation constant, intermolecular hydrogen bonding

Introduction

The ability to deliver therapeutic agents to a sick person in a pulsatile or staggered release profile has been a major target in drug delivery research over the last two decades. A key issue in drug delivery system is to maximize the drug access to specific sites, and to be able to control the release of drugs, in order to maintain a desired drug concentration level for long periods of time without reaching a toxic level or dropping below the minimum effective level [1]. Betahistine is an antivertigo drug. The chemical formula of betahistine is $C_8H_{12}N_2$, it has the molar mass (g/mol) 136.194 grams per mole, the half-life of 3 to 4 hours, and the protein band is very low. It is effective for treating abnormal noise hearing in the ears and Meniere's disease [2]. Cimetidine is the most used drug for stomach pain disease. Cimetidine is administered as gastric acid secretion inhibitor, for the prevention and treatment of active benign gastric and duodenal ulcers and reflux of gastric contents into the oesophagus, and in other cases is for reducing stomach acidity. At the time of the drug administration, it is best to avoid driving until full recovery [3].

The important drug properties to be taken into consideration are the molecular weight, chemical functionality, and especially the physico-chemical properties. These properties have the greatest effect on the drug delivery. pK_a is an important physico-chemical parameter in drug absorption. The gastric or intestinal pH values and the blood pH are indicative for the driving force of pH gradient absorption.

Many drug compounds include at least one acid and/or basic functionality, and the ionization state of these groups play an important role in determining the compound physio-chemical properties. The pK_a for a compound creates a means of specifying the extent of ionization of the compound at any solution pH. Information of a compound's pK_a value plays a major role in the expansion of drug delivery formulations [4-6].

For the determination of acid constants; capillary electrophoresis [7], calorimetric adsorption method [8], potentiometric titration method [9], chromatographic methods [10] and IR, NMR or UV-visible spectrometric [11-13] determinations in water or in solvents mixtures are used. In addition to experimental methods, quantitative chemical methods have been developed for calculating the pK_a values based on

chemical structures. The pK_a can be calculated from the energy of the following reaction:

$$HA \Leftrightarrow H^+ + A^- \quad (1)$$

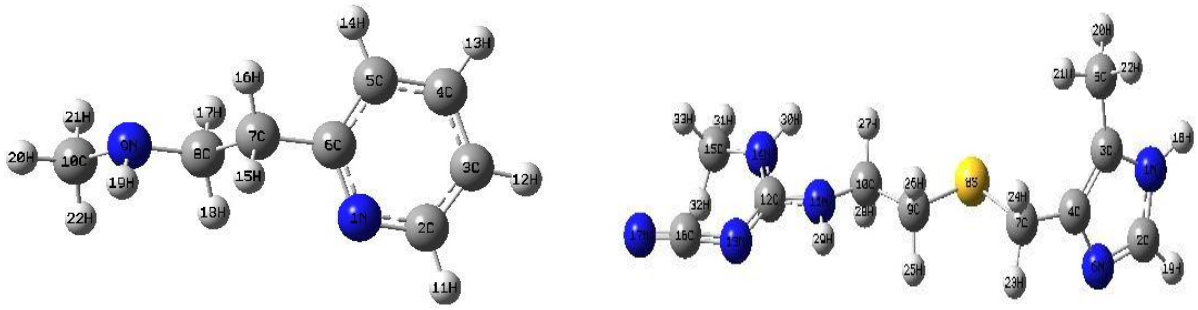
Using the relation:

$$pK_a = \frac{\Delta G}{2.303RT} \quad (2)$$

In the main reaction, ΔG is the difference between the free energy of products and reactant [14-18].
Ab initio Hartree-Fock and density functional geometry optimizations were performed with the Gaussian 09 program. The *ab initio* geometries were employed for calculating the free energies solvation using the B3LYP/6-31+G(d) [19]. For considering the interactions in aqueous solution, we added one, two, three, and four water molecules to the model using the polarizable continuum model (PCM) [20]. This article refers to the factors influence, such as the Self-Consistent Reaction Field (SCRF) model applied, choice of a particular thermodynamic equation, atomic radii used to build a cavity in the solvent (water), optimization of geometry in water, electron inclusion of correlation, and the dimension of the basis set on the solvation free energies and on the calculated pK_a values. In this study, betahistine and cimetidine pK_a values were specified in aqueous solution by an *ab initio* method at a temperature of 25°C. To explain the acid dissociation constants obtained, we investigated the molecular conformations and solute-solvent interactions of the cation, anion, and neutral species of betahistine and cimetidine, using *ab initio* and density functional theory (DFT) methods.

Materials and Methods

All the theoretical computations were performed using HyperChem version 7.0 and Gaussian 09 program package [21]. The initial geometry and different conformers of betahistine and cimetidine (Figure 1) were built by the semi empirical parameterized model number 3 (PM3) method. All the initial geometries and solvated species (in water) were optimized with the Gaussian 09 program packages using the B3LYP/6-31+G(d) methods and the default convergence criteria [22]. The polarized continuum model (PCM), which is the ideal conceptual frame-work to describe solvent effects on all species involved in the selected ionization reaction, was used [23]. Furthermore, to focus on the experimental pK_a values of these drugs in water, all possible conformers were tested using the Excel program. Among them, all calculated pK_a values that were not compatible with experimental values were rejected. We then focused on reactions having pK_a near to experimental values. Finally, we selected the solvation of the species by means of intermolecular hydrogen bonds (IHBs) that involve one molecule of the mentioned species and some molecules of water.



betahistine cimetidine

Figure 1.
The structures of the (a) betahistine and (b) cimetidine for carrying out the calculations

Results and Discussion

The total energies of the single and solvated betahistine and cimetidine (cationic, zwitterion natural, and anionic) species were calculated in water at the B3LYP/6-31+G(d) level of the theory, using Tomasi's model. Table I summarizes the variations of the total energy (kJ/mol) of the species per water molecule as a function of the total number of solvation water molecules. Figure 2 and Table I show the marked increase of the total energies of cations, when the number of water molecules increases in the solvation indicating the endothermic nature of the reaction.

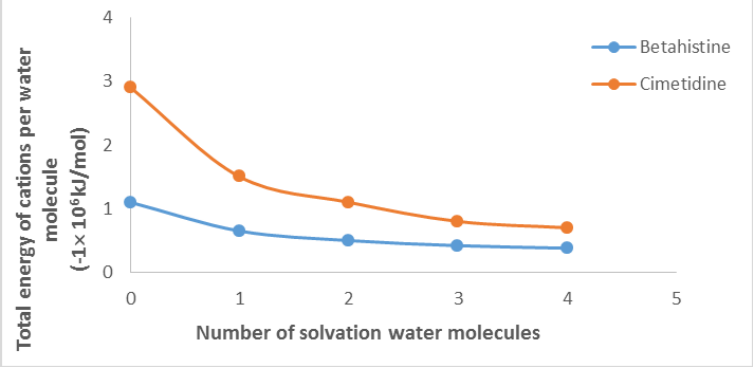


Figure 2.
Plot of the total energy (kJ/mol) of solvated betahistine and cimetidine cations per water molecule against the total number of solvation water molecules

Table I
Calculated total energy using the Tomasi method at the B3LYP/6-31+G(d) level of theory for cationic, neutral, and anionic species of betahistine and cimetidine, at 298.15 K

N [a]	solvated species	G^0_{sol} (Hartree)	$G^0_{sol/molecule}$ (Kj.mol^{-1})
	betahistine		
0	HL:UZ	-422.054468	-1108103.899
1	HL:UZ	-498.49119	-654394.2469
2	HL:UZ	-574.937239	-503165.8587
3	HL:UZ	-651.38316	-427551.5806
4	HL:UZ	-727.826292	-382181.5492
0	HL:Z	-421.599903	-1106910.439
1	HL:Z	-498.042731	-653805.5324
2	HL:Z	-574.485192	-502770.2423
3	HL:Z	-650.926641	-427251.933
4	HL:Z	-727.368698	-381941.2667
0	H_2L^+	-421.597388	-1106903.836
1	H_2L^+	-498.036583	-653797.4616
2	H_2L^+	-574.487431	-502772.2018
3	H_2L^+	-650.920888	-427248.1569
4	H_2L^+	-727.373315	-381943.691
0	L^-	-421.061022	-1105495.607
1	L^-	-497.515477	-653113.3797
2	L^-	-573.993907	-502340.2861
3	L^-	-650.407726	-426911.3302
4	L^-	-726.887053	-381688.3549
	cimetidine		
0	H_2L^+	-1117.865042	-2934954.386
1	H_2L^+	-1194.306783	-1567826.079
2	H_2L^+	-1270.764512	-1112130.635
3	H_2L^+	-1347.19977	-884268.1642
4	H_2L^+	-1423.644751	-747555.787
0	HL	-1117.449684	-2933863.864
1	HL	-1193.890075	-1567279.046
2	HL	-1270.328636	-1111749.171
3	HL	-1346.777371	-883990.912
4	HL	-1423.218238	-747331.825
	Water		
0	H_2O	-76.434	-200677.4477
0	OH^-	-75.952	-199411.9569
2	$(H_2O)_2$	-152.87	-133786.7155
0	$2H_2O$	-152.868	-401354.8955
0	$3H_2O$	-229.302	-602032.3432
1	$OH^-(H_2O)$	-152.4	-200063.0808

N [a]: total number of solvation water molecules; G_{sol}^o, total free energy in solution; G_{sol}^o/molecule, total energy of solvated species per water molecule; H_2L^+, cation species; HL, neutral; L^-, anion species.

Fully protonated betahistine loses proton in two stages and cimetidine in one stage. For betahistine, the proton is lost from NH both in R- group and ring to give different ionized species. The ring has the electron-donor effect on nitrogen atom. Therefore, the nitrogen atom in R- group has more positive charge than the one in the ring. For this reason, the R- group loses proton easier than the ring. In Table II, it can be seen that the value of pK_{a2} is greater than pK_{a1}. For cimetidine, the proton is lost from NH_2^+ in the R- group (Figure 3).

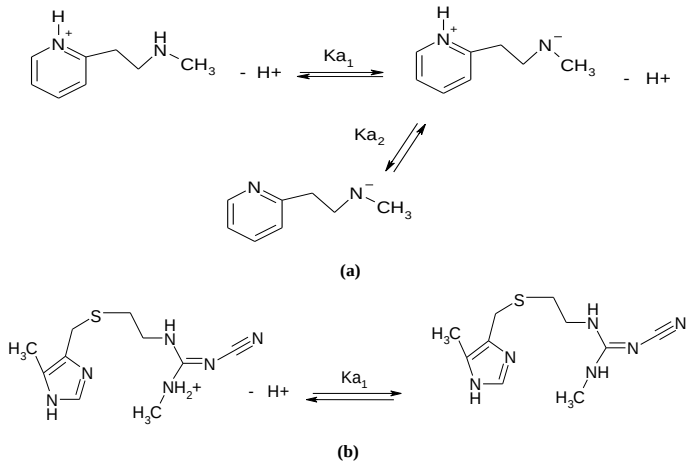


Figure 3.
Suggested protonation scheme of (a) betahistine and (b) cimetidine

Table II

Values of pK_a for the protonation of betahistine and cimetidine obtained using the Tomasi method at the B3LYP/6-31+G(d) level of theory, at 298.15 K

Species	Selected equations	pK_a (calculated)	pK_a (experimental)	Ref
betahistine	$H_2L^+(H_2O)_2 + H_2O \rightleftarrows HL(H_2O)_2 + H_3O^+$	3.78	3.900 (I^a = 0)	28
	$HL + 5H_2O \rightleftarrows L^-(H_2O)_4 + H_3O^+$	10.33	10.020 (I^a = 0)	28
cimetidine	$H_2L^+(H_2O)_3 + 2H_2O \rightleftarrows HL(H_2O)_4 + H_3O^+$	6.88	6.900 (I^a = 0)	28

I^a: Ionic strength

First ionization constant of betahistine

It was selected that in alkaline solution betahistine suffers a reaction of partial neutralization as follows:

$$H_2L^+(H_2O)_2 + OH^- \overset{K_{C1}}{\rightleftarrows} HL(H_2O)_2(Z) + H_2O \quad (3)$$

It is well-known that all aqueous solutions contain hydrogen (H^+) and hydroxyl (OH^-) ions. In pure water these ions are derived completely from the ionization of the water molecules. Considering that the H^+ ion is hydrated, appearing predominantly as H_3O^+, the auto-protolysis of two molecules of water is better represented by the following reaction:

$$2H_2O \Leftrightarrow H_3O^+ + OH^- \quad K_w \quad (4)$$

At T = 298.15K, Kw = 1.008×10^{-14} shows that only a few of the water molecules are ionized [24].

In the reaction (3), $H_2L^+(H_2O)_2$ is the betahistine cation solvated with two water molecules, and $HL(H_2O)_2$:Z represents the betahistine zwitterion natural solvated with two water molecules. The reaction (5) is characterized by equilibrium constants (K_{al}) which was theoretically obtained by incorporating equations (4) and (3). The equation (5) defines the first ionization constant of betahistine:

$$H_2L^+(H_2O)_2 + H_2O \overset{K_{a1}}{\rightleftarrows} HL(H_2O)_2(Z) + H_3O^+ \quad (5)$$

It is obvious that:

$$K_{al} = K_{C1} \times K_w \quad (pK_{a1} = -\log K_{a1}) \quad (6)$$

The equation (6) was applied for determining the values of the first ionization constant of betahistine in water. Table III summarizes the optimized values of molecular properties of the $H_2L^+(H_2O)_2$ cation (Figure 4A), and the $HL(H_2O)_2$:Z natural (Figure 4B) obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at 298.15 K. The formation of the betahistine zwitterion implies that the electronic density of the N_1 atom decreases notably (in absolute value) with respect to the N_1 atom of the betahistine cation. It can be observed that the pK_{a1} value theoretically calculated (pK_{a1} = 3.78) is relatively comparable with the experimentally determined pK_a(pK_{a1} = 3.9) [25].

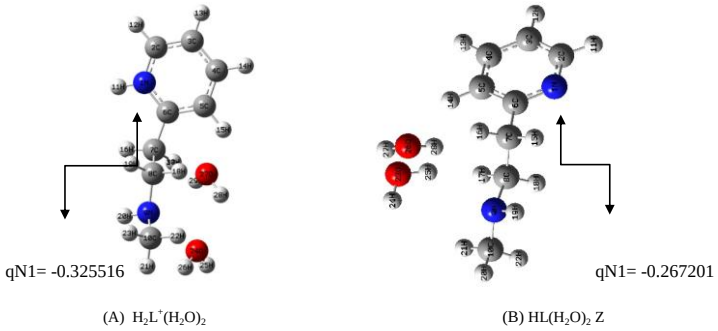


Figure 4.
Calculated structure for the betahistine cation (A) and neutral (B) betahistine zwitterion, at the B3LYP/6-31+G(d) level of theory and using Tomasi's method in water at 298.15 K

Table III
Calculated structural magnitudes using Tomasi's method at the B3LYP/6-31+G(d) level of theory for the cations, anion and neutral molecules of betahistine at 298.15 K

Species	Calculated magnitudes			
betahistine	$H_2L^+(H_2O)_2$	$HL(H_2O)_2$	HL	$L^-(H_2O)_4$
K^a_{C1}	6.02936E+17	-	-	-
K^a_{C2}	-	-	3.34223E+29	-
K^a_{al}	6077.59093	-	-	-
K^a_{a2}	-	-	2.144E+10	-
a_0	4.84	4.83	4.38	4.94
qN_1	-0.325516	-0.267201	-0.291449	-0.278065
qC_2	-0.114362	-0.166267	-0.197335	-0.200069
qN_9	-0.741437	-0.756594	-0.572144	-0.830403
qH_{12}	0.280085	0.223397	0.223844	0.224065
qH_{17}	0.269507	0.224549	0.206586	0.201861
qO_{23}	-	-1.142653	-	-
qO_{24}	-1.127553	-	-	-
qO_{26}	-	-1.121146	-	-
qO_{27}	-1.125145	-	-	-
qH_{22}	0.226103	0.199510	-	-
qO_{22}	-	-	-	-1.342936
qO_{25}	-	-	-	-1.209226
qO_{28}	-	-	-	-1.199325
$dH_{26}N_9$	1.87620	-	-	-
$dH_{28}O_{24}$	2.49797	-	-	-
$dH_{29}O_{24}$	2.56010	-	-	-
dN_9H_{21}	-	-	2.019597	-
$dH_{24}N_9$	-	-	-	1.74586
$dH_{17}H_{25}$	-	1.74662	-	-
$dH_{25}N_9$	-	1.81100	-	-
$dO_{23}H_{27}$	-	2.49633	-	-
$dH_{34}O_{28}$	-	-	-	1.72090
dN_9H_{30}	-	-	-	1.91971
A $O_{24}H_{26}N_9$	162.21245	-	-	-
$AN_9H_{25}O_{23}$	-	175.03024	-	-
$AO_{23}H_{25}H_{22}$	-	53.53124	-	-
$AN_9H_{19}O_{23}$	-	23.52878	-	-
A $H_{19}N_9H_{21}$	-	-	138.68177	-
$AO_{28}H_{30}N_9$	-	-	-	167.09575
$AO_{31}H_{32}N_9$	-	-	-	128.30898
D-$C_7C_6N_1C_2$	-178.990194	179.772057-	-179.173993	-178.017024
D-$H_{20}C_{10}N_9C_8$	-	177.171022	176.572701	-65.377886
D-$H_{21}C_{10}N_9C_8$	177.171022	-64.203571	-65.660557	55.605016
D-$H_{29}O_{27}C_7C_6$	120.9078132	-	-	-
D-$H_{13}C_4C_3C_2$	-	179.938810	179.982580	-179.765909

[a]K_{C1} and K_{C2}, equilibrium constants of equations; K_{a1} and K_{a2}, first and second acid dissociation constants of species in water; D, dihedral angle between the indicated atoms (Å); a_0, bohr radius (Å); q, total atomic charge (Muliken) (au); d, bond lengths between the indicated atoms; A, angles (°).

Second ionization constant of betahistine

It is selected that the HL:Z suffers a total neutralization process as follows:

$$HL(Z) + OH^-(H_2O)_3 \xrightleftharpoons{K_{C2}} L^-(H_2O)_4 \quad (7)$$

In the above reaction, $L^-(H_2O)_4$ and HL represent the solvated anion with four water molecules and zwitterion natural of betahistine, respectively. The autoprotolysis of five molecules of water is better represented by the following reaction [26, 27]:

$$5H_2O \xrightleftharpoons{K_{N3}} H_3O^+ + OH^-(H_2O)_3 \quad (8)$$

$$K_{N3} = \frac{K_W}{[H_2O]^3} = 6.4149\times10^{-20} \quad (9)$$

The described reaction in equation (7) is characterized by another equilibrium constant, K_{C2}, which was also theoretically determined. By combining equations (7) and (8), the second ionization reaction of betahistine was obtained:

$$HL(Z) + 5H_2O \xrightleftharpoons{K_{a2}} L^-(H_2O)_4 + H_3O^+ \quad (10)$$

The second ionization constant (K_{a2}) that characterizes the above reaction is associated with constants K_{C2} and K_{N2} by equation (7):

$$K_{a2} = K_{C2} \times K_{N3} \quad (pK_{a2} = -\log K_{a2}) \quad (11)$$

The above equation was used to determine theoretically the value of the second ionization constant of betahistine in water. Table III summarizes the optimized values of molecular properties of the HL:Z zwitterion (Figure 5A), and $L^-(H_2O)_4$ anion molecule (Figure 5B) obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at 298.15 K. Obviously, the formation of the betahistine anion $L^-(H_2O)_4$ implies that the electronic density of the N_9 atom increases notably (in absolute value), with respect to the N_9 atom of the betahistine zwitterion HL. It can be observed that the pK_{a2} theoretically calculated value (pK_{a2} = 10.33) is relatively comparable with the experimentally determined pK_{a2} (pK_a = 10.02) [24].

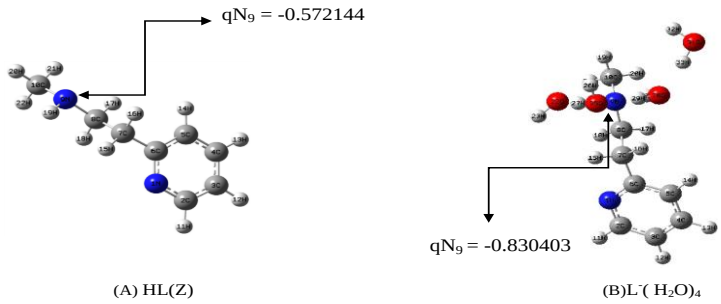


Figure 5.
Calculated structure for the betahistine zwitterion natural (A) and anion (B), at the B3LYP/6-31+G(d) level of theory and using Tomasi's method in water at 298.15 K

Ionization constant of cimetidine

It was chosen that the $H_2L^+(H_2O)_3$cation suffers a total neutralization as follows:

$$H_2L^+(H_2O)_3 + OH^-(H_2O)_2 \Leftrightarrow HL(H_2O)_4 + 2H_2O \quad K_C \quad (12)$$

The autoprotolysis of four molecules of water is better represented by the following reaction [27]:

$$4H_2O \xrightleftharpoons{K_{N2}} H_3O^+ + OH^-(H_2O)_2 \quad (13)$$

$$K_{N2} = \frac{K_W}{[H_2O]^2} = 3.326\times10^{-18} \quad (14)$$

In the reaction (12), $HL(H_2O)_4$ is the neutral cimetidine solvated with four water molecules. Also, $H_2L^+(H_2O)_3$ represents the cimetidine cation solvated with three water molecules. The reaction of equation (15) was obtained by incorporating equations (12) and (13):

$$H_2L^+(H_2O)_3 + 2H_2O \xrightleftharpoons{K_{a1}} HL(H_2O)_4 + H_3O^+ \quad (15)$$

The equilibrium constant K_a that characterizes the above reactions, is:

$$K_a = K_C \times K_{N2} \quad (pK_a = -\log K_a) \quad (16)$$

These equations were used to theoretically determine the value of the ionization constant of cimetidine in water. Table IV summarizes the optimized values of molecular properties of the $H_2L^+(H_2O)_3$ cation (Figure 6A), and $HL(H_2O)_4$ neutral molecule (Figure 6B) for cimetidine obtained at the B3LYP/6-31+G(d) level of theory with Tomasi's method in water at 298.15 K. Evidently, the formation of the neutral cimetidine implies that the electronic density of the N_{13} atom decreases notably (in absolute value) with respect to the N_{14} atom of the cimetidine cation, as shown in Table IV. It can be observed that the pK_a value theoretically calculated (pK_a = 6.88) is relatively comparable with the experimentally determined pK_a (pK_a = 6.9) [24].

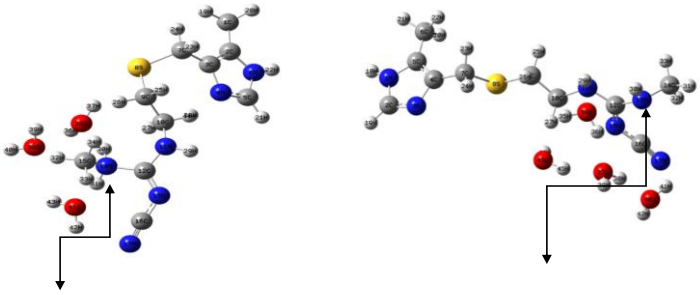


Figure 6.
Calculated structure for the cimetidine cation (A) and neutral (B), at the B3LYP/6-31+G(d) level of theory and using Tomasi's method in water at 298.15 K

Table IV
Calculated structural magnitudes using Tomasi's method at the B3LYP/6-31+G(d) level of theory for the cation and neutral molecules of cimetidine at 298.15 K[a]

Species	Calculated magnitudes	
cimetidine	$H_2L^+(H_2O)_3$	$HL(H_2O)_4$
K_{C1}	2.30873E+24	-
K_{a1}	7648840.838	-
a_0	5.11	5.41
qC_1	-1.085626	-
qC_2	0.592757	0.308655
qC_5	0.266249	0.922961
qS_8	0.165783	0.199486
qN_{11}	-0.495351	-0.573830
qN_{13}	-	-0.384547
qN_{14}	-0.987501	-
qN_{17}	-0.618701	-0.742527
qO_{34}	-	-1.131008
qO_{35}	-1.099028	-
qO_{37}	-	-1.154765
qO_{38}	-1.099028	-
qO_{40}	-	-1.119080
qH_{18}	0.238907	0.485744
qH_{26}	0.277584	0.257281
qH_{33}	0.281070	0.248261
$dO_{41}H_{31}$	1.77606	-
$dO_{38}H_{32}$	2.74581	-
$dO_{35}H_{30}$	1.82383	-
$dH_{32}H_{39}$	2.61321	-
$dO_{34}H_{30}$	-	2.75001
$dN_{17}H_{41}$	-	1.83638
$dO_{37}H_{36}$	-	1.80962
$dO_{40}H_{38}$	-	1.78686
$dO_{34}H_{29}$	-	2.49998
$AN_{14}H_{31}O_{41}$	164.70310	-
$AN_{17}H_{41}O_{40}$	-	171.07138
$AN_{14}H_{30}O_{35}$	148.32505	-
$AN_{14}H_{30}O_{38}$	108.64328	-
$AN_{14}H_{41}O_{40}$	-	124.09166
$D\text{-}N_4C_3C_2C_1$	-179.895876	-
$D\text{-}C_5N_4C_3C_2$	0.164437	0.382486
$D\text{-}C_{16}N_{13}C_{12}N_{11}$	170.808570	-
$D\text{-}H_{18}C_1C_2C_3$	-123.600739	-179.923216
$D\text{-}C_{12}N_{11}C_{10}C_9$	88.804696	-87.777321
$D\text{-}H_{22}N_6C_5N_4$	178.827607	1.544872
$D\text{-}C_{14}N_{13}C_{12}N_{11}$	-	-161.004070
$D\text{-}N_{17}C_{16}N_{15}C_{12}$	-	144.457480

[a]K_C, equilibrium constants of equations; K_a, acid dissociation constant of species in water; D, dihedral angle between the indicated atoms (Å); a_0, bohr radius (Å); q, total atomic charge (Muliken) (au); d, bond lengths between the indicated atoms; A, angles (°).

The molecule of water originated from the acid-base reaction, together with the hydration water molecule of the betahistine and cimetidine, and these are the molecules of water that interact with the betahistine and cimetidine molecules by means of IHBs. According to ref. [28], the properties of the weak, moderate and strong hydrogen bonds have classified. For species of this study, the distances and angles of intermolecular hydrogen bounds (IHBs) are shown in Tables III and IV. These values show that the IHB of cation and the neutral molecules of cimetidine and also, cation, anion and neutral molecules of betahistine are very close to class of the moderate IHBs. Finally, it must be noted that hydrogen bonds are at the minimum distance between molecules. Therefore, we can design nano drugs with lower volume, which are more effective at lower dosage that can be very useful in the treatment of different diseases. [4, 28-31].

Conclusions

In this study, we have theoretically determined the pK_a of betahistine and cimetidine in water at 298.15 K with the *ab initio* and DFT methods. There is a good agreement between the theoretically determined acid dissociation constants and the experimentally determined ones. This agreement along with the other data (the electronic density, q, structural properties, and IHBs) helps us to predict nano drug modelling of betahistine and cimetidine.

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