Computational study of the proton affinity and basicity of structurally diverse α₁-adrenoceptor ligands

C. Matijssen[a], G. K. Kinsella[b], G. W. Watson[a] and I. Rozas[a]*

The α₁-adrenoceptor is a target for the treatment of several conditions from hypertension to benign prostatic hyperplasia. In this paper, we describe a new analysis approach to explore the conformational space of several ligands of the α₁-adrenoceptor and we also present the calculation of their proton affinity and basicity. For each compound a conformational search followed by a semi-empirical optimisation was performed and a selection of conformations for each ligand was subjected to further optimisation using density functional theory methods. Different positions were explored to determine the favoured site of protonation, and then, the proton affinity (in the gas phase) and basicity (using the polarisable continuum model for the aqueous solution) were calculated for each of them. In addition, an alternative method using one explicit water molecule in combination with the polarisable continuum model for aqueous solvent was explored. Moreover, the acid dissociation constant (pK_a) in water of these 26 compounds was calculated because this is an important parameter for a ligand when binding to its receptor. The experimental pK_a values of six of these ligands and those of two compounds with a very low and a very large pK_a were used to validate the theoretical methodology. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: acid dissociation constant; α₁-adrenoceptor; basicity; polarisable continuum model; proton affinity

INTRODUCTION

Drugs interact with their receptor in an aqueous environment and at physiological pH. Therefore, their protonation state plays an important role in the interaction between the ligand and the receptor. In particular, looking at drugs that interact with α₁-adrenoceptors (α₁-ARs), which are G protein coupled receptors (GPCRs) involved in several diseases and conditions,[1] it has been found that a protonated N atom plays an important role in the binding of the ligands to a particular α₁-AR subtype (α₁A-AR). This protonated N has become a common feature in several pharmacophore models;[2–5] and thus, in docking studies, it has been shown that an aspartic acid (conserved amongst most of the GPCRs) can interact with this protonated functional group.[6]

The preference for each N atom to be protonated can be determined by calculating the corresponding basicity (i.e. proton affinity in the gas phase –PA-). Techniques such as electrospray ionization, matrix-assisted laser desorption or the extended kinetic method allow for the experimental determination of PA;[7,8] however, basicities can also be determined using computational methods. To obtain accurate PA values in the gas phase, or basicities (B) in solvation, the bioactive or, when this is not known, some of the most stable conformers for each compound should be considered. Hence, a conformational search can be performed followed by a thorough optimisation of a large number of possible conformations. This step is time consuming and often simplified by limiting the number of conformations that are optimised. However, the correct minimum energy conformation should contribute to a better description of molecular properties participating in the ligand–receptor interactions and, for that reason, we have chosen an approach that selects a number of low energy conformations based on ligand

structural flexibility and allows further refinement of each conformation. From the collection of optimised conformers, the minimum energy conformation can be selected. This minimum energy structure can then be used for PA/B determination searching for the most favourable N atom to be protonated.

Using density functional theory (DFT) methods, Alkorta *et al.* have previously studied the basicity of a number of hydrogen bonded complexes between halogen acids and different bases,[9] and that of different triple bonded molecules formed by C, N, Si, P, B and Al.[10] Also, Alkorta and colleagues have calculated the PA of different series of hydrocarbons,[11] triazoles,[12] and small molecules.[13] Moreover, Dmitrenko *et al.* obtained PAs within 3–5 kcal mol⁻¹ of the experimental values in a series of small molecules (enolate intermediates and phosphine complexes).[14,15] To mimic the solvent, different approaches can be used but we have previously found that the use of the polarisable continuum model (PCM) produces good results.[16,17]

Thus, using the energy of the minimum energy conformation, the PA (enthalpies in the gas phase) or the basicity (free energies in the solvated phase, PCM) for each of the N atoms can be determined using formula (1) or (2) respectively

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$$(\text{gas phase})PA = E_{protonated} - E_{unprotonated} \quad (1)$$

$$(PCM)B = E_{protonated} - E_{unprotonated} - E(H^+) \quad (2)$$

where $E_{protonated}/E_{unprotonated}$ are the energies of the protonated and unprotonated species (enthalpies in the gas phase or free energies in the PCM), $E(H^+)$ would be null in gas phase, but in the PCM is the solvation free energy of the proton $[\Delta G^0_{solv}(H^+)]$, which has been experimentally determined in water as -265.9 kcal mol^{-1}.[18,19]

As mentioned before, the basicity and hence the pK_a plays an important role in the pharmacokinetic profile of a potential drug. The experimental evaluation of the pK_a of a compound is not always possible and can be tedious. However, the pK_a in water can also be determined using a computational approach based on the thermodynamic cycle shown in Fig. 1. [7,20–22]

After calculation of the minimum energy of the molecule in the different protonation states, the pK_a in water can then be derived from this thermodynamic cycle using formula (3)

$$pK_a = \frac{\Delta G^0_{aq}}{2.303 \times RT} \quad (3)$$

where ΔG^0_{aq} is the aqueous solvation free energy, R is the universal gas constant and T is the temperature (298 K). ΔG^0_{aq} can be calculated using formula (4)

$$\Delta G^0_{aq} = \Delta G^0_{gas} - \Delta G^0_{solv}(AH^+) + \Delta G^0_{solv}(A) + \Delta G^0_{solv}(H^+) \quad (4)$$

The $\Delta G^0_{solv}(A)$ and $\Delta G^0_{solv}(AH^+)$ terms can be obtained from our calculations by subtracting the gas-phase free energy from the PCM free energy. The $\Delta G^0_{solv}(H^+)$ term, as mentioned before, is a known experimental value.[13,14] The ΔG^0_{gas} term can be obtained using formula (5)

$$\Delta G^0_{gas} = G^0_{gas}(A) + G^0_{gas}(H^+) - G^0_{gas}(AH^+) \quad (5)$$

where $G^0_{gas}(AH^+)$ and $G^0_{gas}(A)$ are the free energy of the protonated and unprotonated states respectively. $G^0_{gas}(H^+)$ is the free energy of the proton and can be calculated using the Sackur–Tetrode equation resulting in -6.28 kcal mol^{-1}.[23] Hence, knowing the free energy of all the solvated species (A and AH$^+$) calculated with PCM we can calculate ΔG^0_{aq} and determine the pK_a of the compounds.

Therefore, the aim of this study is to explore the conformational space of several α_1-AR's ligands and to theoretically calculate their PA, basicity and pK_a to determine their optimal protonated state for future molecular modelling and docking studies.

METHODS

The agonists adrenaline and noradrenaline and a selection of α_1-AR antagonists taken from the literature[2] resulted in a set of 26 compounds (Fig. 2) covering a wide range of binding affinities for the different α_1-ARs subtypes. Considering that the experimental pK_a value of adrenaline (**1**),[24] noradrenaline (**2**),[25] prazosin (**3**),[26] alfuzosin (**4**),[27] doxazosin (**5**)[28] and terazosin (**6**)[29] are already known, these compounds will serve as a validation set for the determination of the computational pK_a values presented here.

These compounds were subdivided into six different groups based on common scaffolds (Fig. 3) and thus, this common substructure will be used to derive the site of protonation for similar ligands.

To explore the conformational space of these molecules, a random search using the Monte Carlo method was performed with SYBYL (version 7.2, Tripos International, St. Louis, MO, USA)[30] applying the conjugate gradient minimisation method, a maximum number of iterations of 1000, a gradient of 0.0005, the Tripos force-field and Gasteiger–Hückel charges. This conformational search produced 200–1500 different conformations for each compound. Each conformer was further optimised using GAUSSIAN03 (Gaussian, Inc., Wallingford, CT, USA)[31] with the AM1 semi-empirical method, and the energy and various dihedral angles were used to select different conformations.

Taking into account the number of dihedral angles that could account for molecule flexibility, two different approaches were used to select the most representative conformations for further optimisation: (i) in the case of molecules with ≤5 rotatable bonds, the corresponding dihedrals of each possible pair of rotatable bonds were plotted against each other. Considering that each rotatable bond of the AM1-optimised structures produced two, four, six or eight possible dihedral angles, different clusters were formed. From each cluster the lowest energy conformation was selected; (ii) in the case of molecules with >5 rotatable bonds, an alternative approach was used. Thus, the dihedral angle for each rotatable bond was plotted against the conformer's energy and in that way several clusters of two to eight angles were formed. From each cluster the conformation with the lowest energy was selected.

Using either of these methods for the selection of low energy conformations produced a smaller set of 10–25 conformations per compound. Each of these AM1 conformations was further optimised with GAUSSIAN03 using the Becke 3-Parameter (Exchange), Lee, Yang and Parr (B3LYP) hybrid method with the 6-31 G* basis set. From the different optimised conformers, that with the lowest electronic energy was selected.

To determine the PA, each of the N atoms of each structure was individually protonated. Optimisation of the neutral and the different protonated conformations was performed using B3LYP/6-31 G* level of theory in the gas phase and in the PCM solvent model. Additionally, vibrational frequency calculations were performed at the B3LYP/6-31 G* level, to characterise the stationary points as minima. The variation in zero-point energies (ZPE) and thermal corrections from zero degree to 298 K have been considered in the calculations. No scaling factor for the ZPE values was taken into account.

An alternative strategy to account for the solvation effects was pursued by adding one explicit water molecule[32,33] interacting through hydrogen bonds (HBs) with the structures considered (Fig. 4). We considered that an explicit water molecule would have a different effect on the compounds than a continuous solvation model such as PCM. Thus, we explored if different minimum energy conformations were found and, perhaps, more accurate pK_a values. For the unprotonated state of each molecule, the H atom of the water molecule was orientated towards each of the
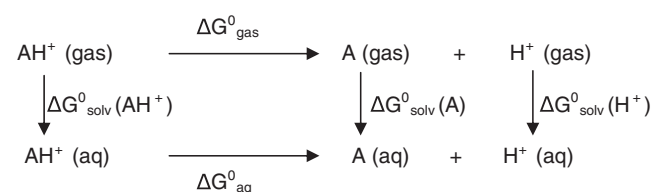
Figure 1. Thermodynamic cycle of a compound in gas and solvent states describing the energy terms which arise from the change from one state to another.

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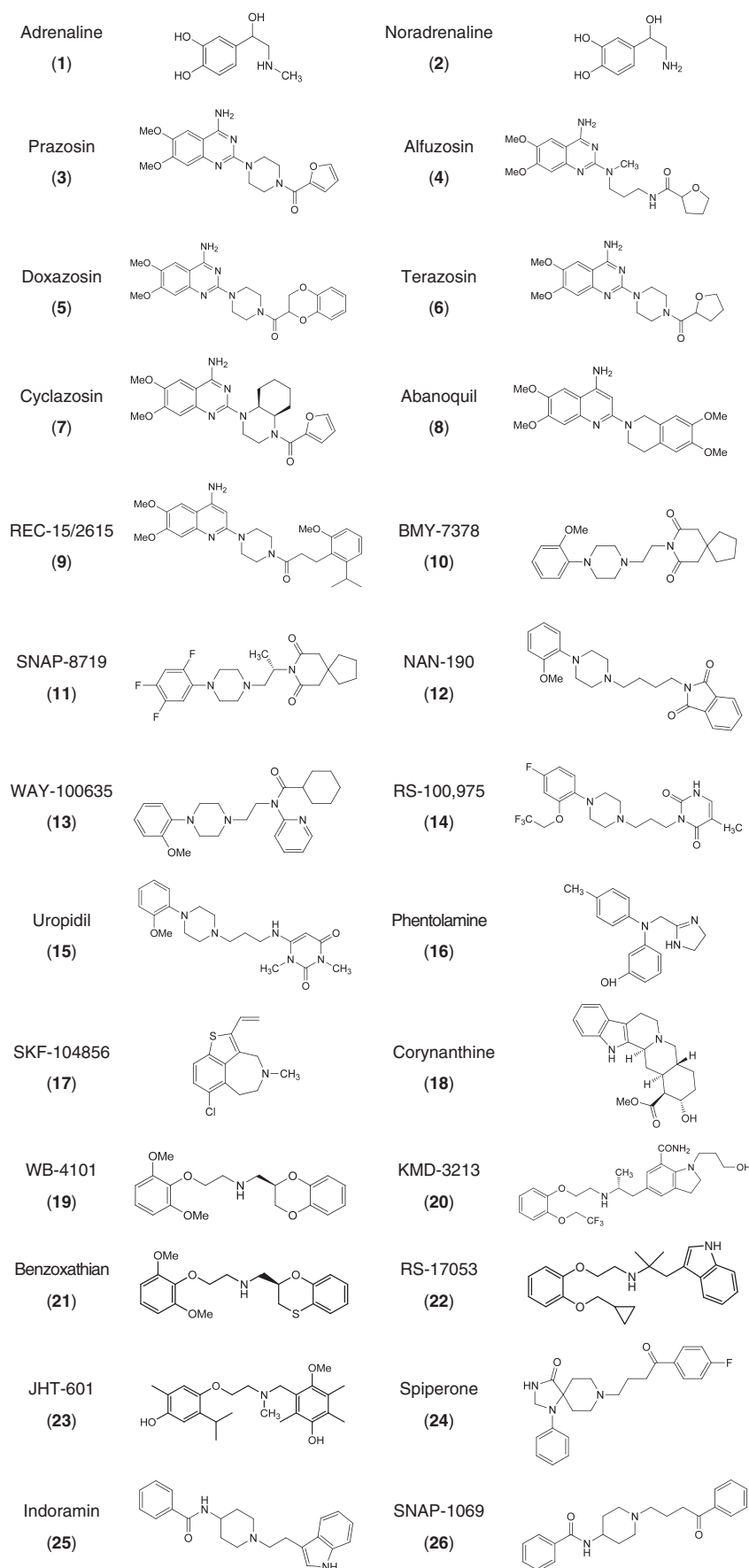
Figure 2. Structures of the 26 compounds studied in this work.

Group 1

Group 2

Group 3

Group 4

Group 5

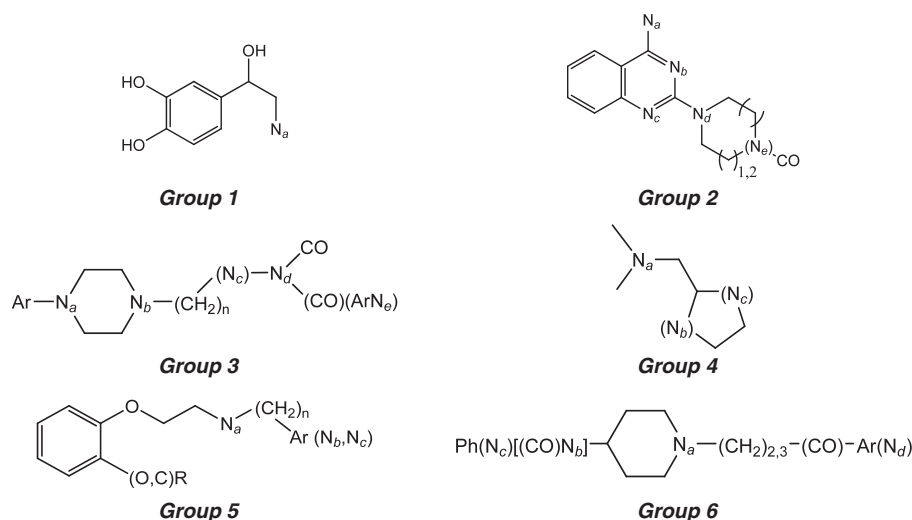*Group 6*

Figure 3. Different scaffolds considered: *Group 1* (compounds **1** and **2**), *Group 2* (compounds **3** to **9**), *Group 3* (compounds **10** to **15**), *Group 4* (compounds **16** to **18**), *Group 5* (compounds **19** to **23**) and *Group 6* (compounds **24** to **26**). The N atoms to be protonated are indicated with subindex letters for each group.
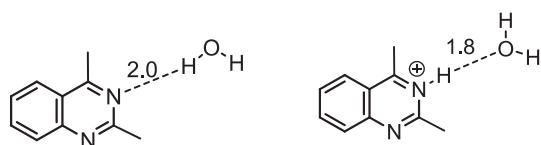

Figure 4. Examples of water molecule orientation and initial distance used (in Å) in the complexes with the neutral (left) and protonated states (right).

N atoms at an initial distance of 2.0 Å. For the protonated form, the O atom of the water molecule was orientated towards the H atom protonating each N atom with a distance of 1.8 Å between both atoms as the starting point for the optimisation.

The computation of the ligands with an explicit water molecule in the gas phase and PCM will help to decide if such 'supermolecule' (ligand + H_2O) facilitates the computation of PA and basicity or, on the contrary, will not provide a better description only adding more computational time.

RESULTS AND DISCUSSION

Determination of proton affinities and basicities

The PA for each compound was calculated as the difference in enthalpy between the neutral and protonated species in the gas phase Eqn (1) whereas when using aqueous salvation, the basicities were calculated using the free energies including the solvation energy of the proton Eqn (2). The natural agonists adrenaline (**1**) and noradrenaline (**2**) only have one N atom that can be protonated; therefore, only one PA/B for each of the structures was calculated (Table 1). Comparing the 298 K thermally corrected structures with the uncorrected ones, both in the gas phase and the PCM solvent optimised structures, a decrease of the PAs (~9 kcal mol^{-1}) is observed. The difference between the gas phase optimisations and PCM optimisations is ~213 kcal mol^{-1} for adrenaline and ~209 kcal mol^{-1} for noradrenaline. These differences account for the solvation of the proton (-265.9 kcal mol^{-1}) and other interactions that the molecule and
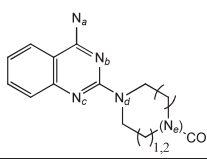
the proton will establish with the environment in the aqueous solution model.

The results for *Group 2* (compounds **3** to **9**) are shown in Table 2. A similar PA/B pattern is observed for all the compounds within this set and the previous one, with the N_c atom being favoured for protonation. Nitrogen N_e, in compounds **3–7** and **9**, belongs to an amide group and, therefore, would not be likely protonated; while N_a is an 'aniline' type of amine and it would require very acidic conditions to be protonated. Accordingly, the PA/B values obtained for those N atoms were very low and are omitted from the table. Again the basicities (PCM results in aqueous solution) are smaller than the gas phase PA. In articular, the B thermally corrected values obtained for N_d are so small (even negative in the case of compound **4**) that it seems to indicate that this N would not be protonated at physiological conditions. Interestingly, N_d is not part of a six-membered ring (piperazine or piperidine) only in compound **4**.

All compounds in *Group 3* (**10** to **15**), which are favourably protonated at N_b in the gas phase (Table 3), have a N atom as part of an amide group (N_d) and because this type of N atom is rarely protonated the PA/Bs obtained were very low and they

Table 1. Calculated proton affinities and basicities (kcal mol^{-1}) of *Group 1* compounds

| | | Gas phase | | PCM (water) | |
	Nitrogen	PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
Adrenaline (**1**)	N_a	241.5	232.5	28.2	19.5
Noradrenaline (**2**)	N_a	236.4	227.5	27.6	18.6

Table 2. Calculated proton affinities and basicities (kcal mol^{-1}) of *Group 2* compounds

	Nitrogen	Gas phase		PCM (water)	
		PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
3	N_b	243.7	236.6	20.0	13.4
	N_c	255.6	247.3	26.5	18.3
	N_d	242.7	234.6	14.1	4.8
4	N_b	259.6	250.4	19.9	10.0
	N_c	264.7	256.0	27.0	17.6
	N_d	253.2	244.9	10.6	-0.1
5	N_b	241.9	235.1	19.7	10.9
	N_c	253.2	245.9	26.2	17.0
	N_d	240.1	232.4	13.4	3.7
6	N_b	244.9	237.5	20.1	9.6
	N_c	256.0	248.0	26.6	17.4
	N_d	243.5	234.9	14.4	2.6
7	N_b	248.1	241.0	21.2	12.7
	N_c	256.5	249.1	27.5	18.5
	N_d	247.4	239.8	15.2	4.3
8	N_c	261.1	253.6	30.1	22.4
	N_d	249.0	241.3	18.8	8.4
9	N_c	257.6	250.6	29.9	22.9
	N_d	240.3	233.0	15.0	5.6

are not shown in the table. The N_b position corresponds to a piperazine N atom attached to an aliphatic chain. Compound **11**, which is the only derivative with a methyl branch in the aliphatic chain, seems to have a different PA/B pattern with slightly lower values than the rest of the compounds in the set. Compound **15** has another amino group, N_c, which is an aniline type of nitrogen, which could explain the negative thermally corrected B value obtained (see Table 3).

Compounds in *Group 4* (**16–18**) show a consistent preference of protonation at nitrogen N_a in both the gas phase and aqueous PCM (Table 4). Compound **16** has other N atoms but differences larger than 20 kcal mol^{-1} are observed in favour of nitrogen N_a, which seems logical considering that N_b and N_c are involved in an imidazoline ring. Compound **17** has only one N atom that can be protonated. In compound **18**, the N_c belongs to an indole system and therefore is too acidic to be likely protonated (data not included in Table 4). Despite the limited number of compounds in this group and only two of them having multiple N atoms to be protonated, the large difference in PA/B observed indicates that nitrogen N_a is the one that will be protonated.

Compounds in *Group 5* (**19–23**) show consistent results both in gas phase and PCM, all of them showing a preference for protonation at nitrogen Na (Table 5). Compounds **19**, **21** and **23** have only one N atom that can be protonated. Compound **22** has two N atoms but N_b belongs to an indole system, hence,
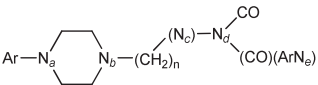
it is too acidic to be protonated. In the case of compound **20**, a large difference favouring protonation on N_a over N_b (with a certain indole-like nature) is observed and N_c (amide) is too acidic to be likely protonated, for that reason the corresponding results are not included in the table.

The structures in *Group 6* (**24–26**) show consistent results in both gas phase and PCM with a preference for protonation on the N_a atom (Table 6). In compound **24**, there is a large difference in PA/B between nitrogen N_a and other N atoms (amide and aniline type); hence, nitrogen N_a is the preferred site for protonation. Nitrogen atom N_b belongs to an amide group (in **24** and **25**) and N_d belongs to an indole ring (in **26**), hence they are too acidic to be protonated.
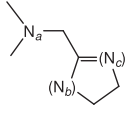
The values of the PA/Bs obtained in each group of ligands have allowed us to identify which N atom is most likely to be protonated. Our results show that, in structurally similar compounds, as expected, the same N atom is preferred for protonation, which is useful when modelling the receptor–molecule interaction in series of similar compounds.

Modelling the solvent with the addition of an explicit molecule of water

An alternative approach for the calculation of basicities in solvated molecules was taken by introducing a properly orientated water

Table 3. Calculated proton affinities and basicities (kcal mol^{-1}) of *Group 3* compounds

		Gas phase		PCM (water)	
	Nitrogen	PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
10	N$_a$	250.0	240.6	22.8	12.8
	N$_b$	252.4	244.5	23.6	14.9
11	N$_a$	236.6	238.2	14.7	5.3
	N$_b$	246.7	238.2	19.3	12.0
12	N$_a$	248.9	239.4	22.9	15.6
	N$_b$	251.1	242.5	27.4	16.6
13	N$_a$	252.0	242.1	22.6	11.6
	N$_b$	252.9	243.6	25.4	15.5
	N$_e$	236.6	228.2	14.9	5.3
14	N$_a$	246.8	236.5	17.2	8.5
	N$_b$	247.9	237.9	27.2	17.2
15	N$_a$	245.6	236.1	22.3	11.9
	N$_b$	250.1	240.4	23.2	13.0
	N$_c$	244.2	233.0	2.3	-7.2

Table 4. Calculated proton affinities and basicities (kcal mol^{-1}) of *Group 4* compounds

		Gas phase		PCM (water)	
	Nitrogen	PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
16	N$_a$	251.4	243.8	11.5	2.4
	N$_b$	233.0	224.5	11.4	1.4
	N$_c$	226.9	218.4	9.4	0.6
17	N$_a$	241.6	231.9	24.4	15.6
18	N$_a$	249.1	240.0	28.4	19.4

molecule interacting with a selection of compounds from our set, and performing calculations at the B3LYP/6-31 G* level in gas phase and with the PCM aqueous solvation. Considering that water molecules can form HBs with any of the N or NH groups present in these molecules, this study was performed to investigate if explicit water molecules can provide a more realistic picture for the solvation influencing the calculated PA/B values. The molecules chosen for this study were: (i) both agonists (**1** and **2**); (ii) a representative of each *Group* (*2* to *6*) (compounds **6**, **13**, **16**, **22** and **24**); (iii) a conformationally restricted compound (**17**); and (iv) a compound with a unique site for protonation (**23**).

The results presented in Table 7 show similar results with and without an explicit water molecule, and in all cases the same N

atom (usually belonging to a piperidine/piperazine ring or within an aliphatic chain) is preferably protonated.

Inclusion of an explicit water molecule results, as expected, in larger basicities, with an increment of ~7 kcal mol^{-1} in the gas phase and ~5 kcal mol^{-1} in the PCM water solvation. In general, the effect of the addition of the water molecule seems to be independent on the compound studied. Our results indicate that the influence of an explicit water molecule confirms the findings obtained with the PCM solvation model alone and, clearly, this added water molecule provides extra stabilization to the protonated system helping provide a better description of the protonation state of these α1-AR ligands. However, taking into account computational time issues, we can conclude that the

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Table 5. Calculated proton affinities and basicities (kcal mol⁻¹) of *Group 5* compound

	Nitrogen	Gas phase		PCM (water)	
		PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
19	N_a	250.3	240.3	25.2	14.8
20	N_a	252.1	241.3	26.0	19.7
	N_b	234.7	223.8	17.3	8.6
21	N_a	247.3	237.5	24.1	14.8
22	N_a	258.4	247.0	30.4	20.0
23	N_a	249.0	239.9	22.7	11.1

Table 6. Calculated proton affinities and basicities (kcal mol⁻¹) of *Group 6* compounds

	Nitrogen	Gas phase		PCM (water)	
		PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
24	N_a	256.0	245.4	30.1	19.7
	N_c	237.0	226.8	16.4	5.9
25	N_a	246.3	238.0	27.5	16.8
26	N_a	259.3	250.0	31.5	21.2

Table 7. Calculated proton affinities and basicities (kcal mol⁻¹) using an explicit water molecule

	Nitrogen	Gas phase + H₂O		PCM (water) + H₂O	
		PA (0 K)	PA (298 K)	B (0 K)	B (298 K)
1	N_a	248.1	241.9	28.9	23.1
2	N_a	244.4	237.8	28.5	22.3
6	N_b	252.8	245.0	24.3	14.8
	N_c	260.0	252.9	29.1	22.3
	N_d	253.4	245.8	19.7	9.2
13	N_a	259.6	251.2	25.3	16.2
	N_b	268.8	255.5	27.6	18.2
	N_e	245.6	238.0	20.5	11.2
16	N_a	259.5	253.4	33.8	28.4
	N_b	248.6	236.9	19.2	8.7
	N_c	227.8	222.1	13.3	5.3
17	N_a	251.3	243.0	27.5	20.0
22	N_a	262.2	253.7	31.7	19.8
23	N_a	256.2	247.7	27.6	19.4
24	N_a	261.2	250.7	30.6	19.6
	N_c	247.2	239.0	20.7	12.2

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use of the PCM alone is enough to achieve a good description of such protonated species.

Determination of the pK_a of the compounds studied

The basicity of the ligands and their pK_a are critical when analyzing ligand–receptor interactions because basicity determines the interaction with the receptor and the pharmacokinetic properties of the ligand. Considering that the overall proportion of nonionizable/ionizable compounds for drug-like substances is not well known, Manallack reviewed the literature with regard to both the proportion of ionizable substances and pK_a distributions.[34] However, the experimental determination of the pK_a is not always straightforward and there are computational alternatives to predict this parameter. Several commercial packages include an option to evaluate pK_a; however, these packages work as a black box and the methodology used is not always clearly explained. Our approach involves applying the thermodynamic cycle presented in Fig. 1. To validate this theoretical approach, we have computed, first, the pK_a values of a number of compounds for which an experimental pK_a had been previously determined (Table 8). These compounds include adrenaline (**1**), noradrenaline (**2**) and compounds **3** to **6**, which are currently commercially available α_1-adrenoceptor antagonists and, hence, their pK_a values are in agreement with the pharmacokinetic properties required for an actual drug. However, because these six values fall within a narrow range (see Table 8), we have also calculated the pK_a value of two compounds, debrisoquin and phenothiazine (Fig. 5), where experimental pK_a values are very large (11.9 [35]) and very small (2.9[35]), respectively.

Table 8. Experimental and computational pK_a values obtained for compounds **1** to **6** and for reference compounds debrisoquin and phenothiazine

	Experimental pK_a	Computational pK_a
1	8.6[24]	9.7
2	8.6[25]	9.0
3	7.0[26]	8.8
4	8.1[27]	8.3
5	6.9[28]	7.9
6	7.1[29]	8.1
Debrisoquin	11.9[35]	20.6
Phenothiazine	2.5[35]	−5.9

Debrisoquin is an antihypertensive drug and phenothiazine is present in different neuroleptic and antihistaminic drugs, such as chlorpromazine and promethazine, respectively. These compounds, even though not related to our data set in terms of biological activity, can expand the scope of the computational method because of the extreme pK_a values that they exhibit.

In the case of debrisoquin, the results (Table 8) are in agreement with the experimental pK_a showing that the guanidine moiety [NH_2–C(=NH)–N–] is a very strong base. This is well known, because guanidine itself is a very strong base in water (exp. pK_a =13.6).[36] Regarding phenothiazine, the small calculated pK_a (Table 8) indicates that the amino group in this compound is weakly basic. Therefore, even though a very large value was calculated for debrisoquin and a very low one was computed for phenothiazine, large deviations from the experimental pK_a were obtained. However, for those values in the middle range, in general, the computed pK_a were slightly larger than the experimental ones but they are more or less within the experimental range (see graph in Fig. 5).

Considering that using this theoretical method, acceptable pK_a values were obtained for the compounds with values in the middle range, the pK_a value of all the compounds in the dataset was determined in a similar manner. The results (Table 9) indicate that the compounds will be mostly protonated at physiological pH, with most compounds showing pK_a values larger than 7.

In *Group 2*, the pK_a of most of the compounds are within a 7.9 to 9.0 range, except for compounds **8** and **9**, which pK_a are >10 (Table 9). This can be explained because these two compounds contain a quinoline core instead of a quinazoline core, which is present in the other compounds of the group (**3–7**). The pK_a of quinoline (4.85[37]) is known to be larger than that of quinazoline (3.31[29]). Moreover, the pK_a of pyridine, which is larger than that of pyrimidine (5.14 and 1.10, respectively[29]), becomes even larger with a NH_2 group in *para* (9.17 for 4-aminopyridine[38] vs 5.71 for 4-aminopyrimidine[39]), and all the compounds in this *Group 2* have an amino functionality in position 4 of the quinoline/quinazoline cores.

Regarding *Group 3*, most of the pK_a values obtained (Table 9) are consistent with this type of *N,N'*-arylalkylpiperazines. The pK_a value of 1-phenylpiperazine is 6.30 and the average pK_a values for a number of 1-arylpiperazines such as ofloxacin, enoxacin, norfloxacin, ciprofloxacin or pipemidic acid is around 5.6.[40] To explain the low pK_a values of **10**, **11** and **15**, it should be considered that, when protonated in the piperazine *N*-alkyl
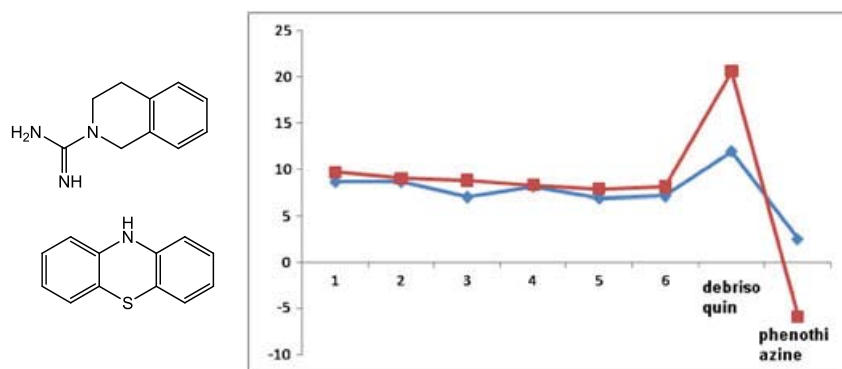
Figure 5. Structure of debrisoquin (left, top) and phenothiazine (left, down) and comparison between experimental (blue) and theoretical (red) pK_a values.

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Table 9. Calculated pK_a values from the thermally corrected B values of all the compounds studied here

Compound	pK_a	Compound	pK_a
Group 1		*Group 2*	
1	9.7	**3**	8.8
2	9.0	**4**	8.3
Group 3		**5**	7.9
10	6.3	**6**	8.1
11	4.2	**7**	9.0
12	7.6	**8**	11.8
13	6.7	**9**	12.2
14	8.0	*Group 4*	
15	5.0	**16**	−3.0
Group 5		**17**	6.8
19	6.2	**18**	9.7
20	9.9	*Group 6*	
21	6.3	**24**	9.9
22	10.0	**25**	7.7
23	3.6	**26**	10.9

position, they are able to form intramolecular HBs with the O atom of an amide group connected by an ethylene linker (**10** in Fig. 6, and **11**) or with the π system of a double bond[41] (**15** in Fig. 6). This intramolecular HB could stabilise the protonated species. Compounds **12**, **13** and **14** (pK_a ~7 or more) in their protonated N–H$^+$ form cannot establish such an HB interaction (i.e. compound **12** in Fig. 5) and therefore their protonated species are less stabilised.

Compounds **17** and **18**, in *Group 4*, show pK_a values around 7 or more (Table 9) in agreement with aliphatic tertiary amines.[29] In the case of the *N*-substituted diphenylamine **16**, the pK_a obtained is negative in agreement with the low

experimental values reported for diphenylamine itself (0.78), *N*-methyldiphenylamine (1.71), 4-carboxydiphenylamine (−1.24) or 4-nitrodiphenylamine (−2.78).[42]

The pK_a obtained for compounds in *Group 5*, can be discussed in three sets even though they all contain an aliphatic amine (secondary or tertiary). Derivatives **19** and **21** both have two OCH$_3$ groups attached to the phenyl ring at the *ortho* positions of the *O*-alkyl chain containing the amine to be protonated (see Fig. 2). In both cases, an HB seems to be formed between the NH group in the aliphatic chain and one of these OCH$_3$ in the neutral forms (d[O...H] for **19**: 2.18 Å and for **21**: 2.27 Å). When the amino group is protonated, this HB becomes shorter (d[O...H] for **19**: 1.81 Å and for **21**: 1.85 Å), stabilising the cationic species and thus explaining the low pK_a values obtained (see Table 9). However, this HB is not formed in compounds **20** and **22** because of the large size of the substituents at the *ortho* position of the aromatic ring (OCH$_2$CF$_3$ and OCH$_2$–cyclopropane). In the case of **23**, the *O*-alkyl chain does not have OCH$_3$ groups in *ortho* positions in the phenyl ring; however, at the end of the *O*-alkyl chain there is another aromatic ring containing an *ortho* OCH$_3$ group. The N atom to be protonated is a tertiary amine and thus in the neutral form no HB can be form. When the tertiary amine is protonated, though, an HB is formed between this NH+ group and the OCH$_3$ of the nearest aromatic ring (d[O...H]: 1.91 Å) forming a pseudo six-membered ring, stabilizing this protonated species and justifying the very low pK_a value computed.

Finally, the values obtained for the three compounds in *Group 6*, which are all *N*-alkylpiperidines, are in the range of experimental pK_a of related compounds such as 1-propylpiperidine (10.48[29]).

In general, and considering the results found for debrisoquin and phenothiazine (compounds with extreme pK_a values), the method seems to work properly only in the middle range (from 6 to 9 pK_a units) and, thus, those computed values obtained outside the middle range (compounds **8**, **9**, **11**, **15**, **16**, **22**, **23**, **26**) should be considered only in qualitative terms.
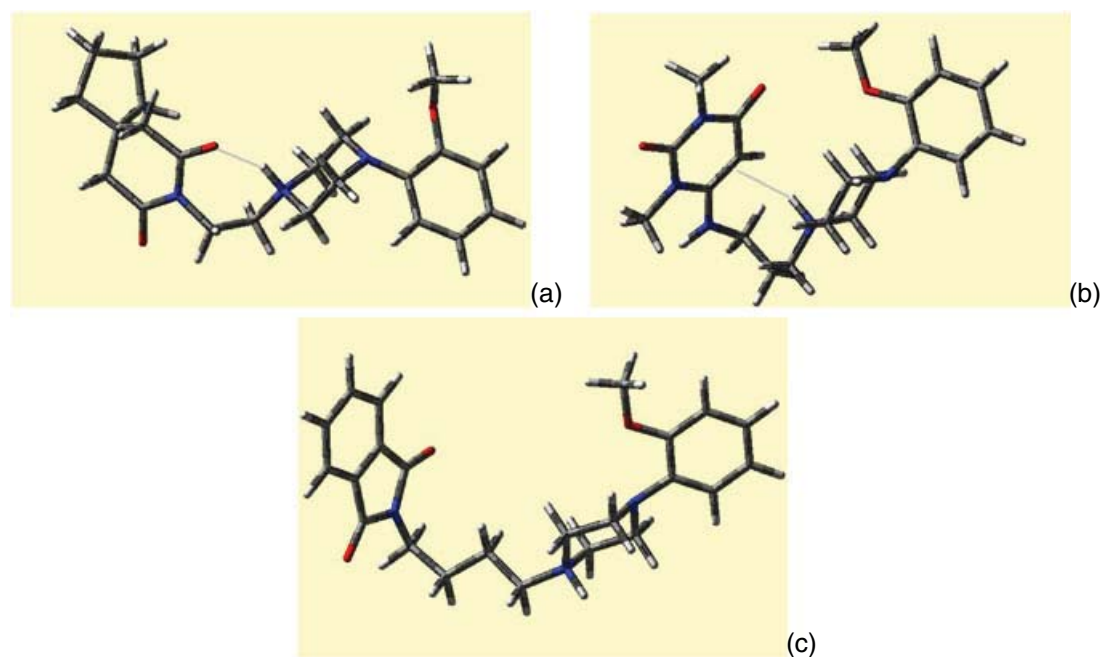

(a)

(b)

(c)

Figure 6. Optimised structures of the protonated forms of compounds (a) **10**, (b) **15** and (c) **12**, calculated at B3LYP/6-31 G* level with the PCM solvation model approach, showing the possible HB formation (grey line) in the case of compounds **10** (between the N–H$^+$ and a C=O of a ring) and **15** (between the N–H$^+$ and a C=C of a ring), and the lack of such a HB in **12**.

CONCLUSIONS

We have performed an extensive conformational search and optimisation to find the optimal minimum energy conformation of a series of 26 α_1-AR ligands (two agonists and 24 antagonists). For each compound a conformational search followed by a semi-empirical optimisation was carried out and then, for each ligand, some of these conformations were subjected to further optimisation using DFT methods.

The determination of the proton affinities and basicities of these compounds shows in very few cases different results between optimisation in gas phase or using the PCM solvent model. Because of the fact that compounds interacting with a receptor are normally in an aqueous environment, the basicity values obtained (using the PCM) are of more biological interest than the PAs.

Consistent results are obtained regarding the preferred N atom for protonation according to the PA/Bs within each group of compounds. This suggests that when structurally similar compounds are studied, their site for protonation can be determined by exploring only a small number of compounds within the set.

To improve the solvation model for our molecules, we have studied the effect that an explicit water molecule would have in the solvation effects. We have found that the addition of an explicit water molecule to our models results in similar results for PA and basicity. The same N atom was preferred for protonation with or without the explicit water molecule. Therefore, this approach, which is longer in computational time, does not provide any improvement to the determination of PA and/or B.

The interaction between a drug and its receptor at physiological pH is determined by the pK_a of the drug. Thus, computational tools that allow for the theoretical determination of the pK_a have been developed and, by using this type of approximation, we have computationally evaluated the pK_a values of our set of molecules and those of two reference drugs (debrisoquin and phenothiazine) with extreme pK_a values (very large and very small pK_a, respectively). The results in this evaluation produced a very large pK_a for debrisoquin and a very small one for phenothiazine but with very large deviations from the experimental values. However, better agreement was found for those compounds with experimental pK_a values in the middle range (compounds **1** to **6**). Hence, for those compounds with no experimental pK_a values available, the results obtained could be explained based on similar derivatives reported in the literature. Even though the approach seems to work better for pK_a values in the middle range, it can be used in a qualitative manner outside this range.

Acknowledgement

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