Theoretical proton affinities of α1 adrenoceptor ligands

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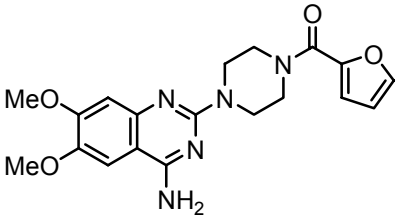
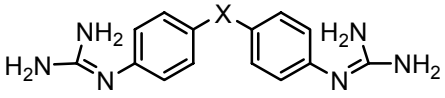
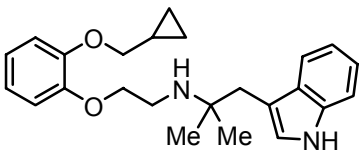
Graphical Abstract:



Prazosin



5 (X=NH), **6** (X=CO),
7 (X=SO_2), **8** (X=CH_2)



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The figure illustrates some of the α1-adrenoceptor ligands object of this study. Their Proton Affinity has been theoretically computed at B3LYP/6-31G* level.

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Abstract:

A systematic study has been performed of the proton affinity of a large family of agonists and antagonists of the α1-adrenoceptor at the B3LYP/6-31G* level of theory. After a conformational search, all the N atoms were considered as protonation sites and protonation energy values were determined. The inclusion of solvation by means of the Onsager model yielded stabilization in the proton affinity values obtained. In addition, a good correlation was found between the proton affinity values corresponding to the first protonation in gas phase of some of the compounds and their corresponding experimental affinity constants K_i for the α1A adrenergic receptor.

Keywords: Adrenoceptor, α1, Density Functional Theory, Proton affinity.

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Introduction

The medical condition known as Benign Prostatic Hyperplasia (BPH) is characterised by a nodular enlargement of prostatic tissue resulting in obstruction of the urethra. The percentage incidence of BPH approximately equals a mature man's age (*i.e.* 60% of men over 60 years of age),[1] and leads to a variety of uncomfortable urological symptoms. Current research has presented significant evidence that α1 adrenoceptors (α1-AR), in particular the α1A subtype (α1A-AR), can be targeted for the treatment of BPH.[2]

Adrenaline (AD) and noradrenaline (NA, see Figure 1) are the natural agonists of all α-adrenoceptors and a number of subtype specific antagonists have also been developed. The first generation of α1-AR antagonists used in treating BPH (*i.e.*, prazosin, terazosin, doxazosin, and alfuzosin, Figure 1), were originally developed as antihypertensives and their ameliorative effects in the treatment of BPH were not observed until their introduction into clinical practice.[3] Furthermore, we found two families of compounds with guanidinium or 2-iminoimidazolidinium cations at both ends of a chain formed by diphenyl derivatives that exhibit α1A-AR antagonist activity (**1**-**8**, see Figure 1).[4] Recently, we performed pharmacological studies on slices of human prostate with BPH, which showed that guanidinium derivatives were able to inhibit between 90 and 95 % of the contractions induced by NA. These results are comparable to the percentage of inhibition observed for doxazosin (95 %), a compound being used for the treatment of BPH.[5]

Other compounds have been developed and studied as antagonists of the α1A-AR in the treatment of BPH, such as the piperazine pyriminedione derivatives 5-methylurapidyl (**9**)[6] and RS-100,975 (**10**),[7] the later being an antagonist of the α1A-AR and α1D-AR. Screening the R. W. Johnson PRI library compounds, Kuo *et al*. found that the piperazine oxazoline derivative RWJ-37914 (**11**)[8] showed very good selectivity towards the α1A-AR. Furthermore, a number of benzodioxanes were prepared and tested by Barbaro *et al.*[9] and, in particular

compound **12**, showed affinity towards the α1A-AR, and was determined to fit the pharmacophore model previously developed by their group.[10] Chern *et al.*[11] prepared α1-AR antagonists for the treatment of BPH, which included tricyclic fused quinazolines with compound **13** exhibiting the largest selectivity for α1A-AR binding sites over the α1B-AR. In addition, the benzodioxan derivatives studied by Quaglia *et al.*[12] were related to WB 4101 (**14**) a known α1-AR antagonist. Inclusion of a phenyl ring led to phendioxan (**15**) with a marked drop in affinity towards the α1D-AR and α1B-AR subtypes while not affecting the affinity for α1A-AR. Further work, resulted in the development of compound **16** as a potent and selective antagonist for α1A-AR. Additionally, phenoxybenzamine (**17**), a known α-AR antagonist and compound RS-100,329 (**18**),[13] a α1A-AR selective antagonist with 126- and 50-fold selectivity over human α1B- and α1D-AR respectively shall be considered.

In order to design new potential drugs, we would like to model the interaction of these antagonists with the α1A-AR, a model of which we have recently developed.[14] Drugs interact with their receptor in an aqueous environment and at physiological pH. Thus, the protonation state of the ligand, in such conditions, will play an essential role in ligand-receptor interactions. Hence, a thorough conformational analysis of the drug or ligand and the determination of its protonation state should be an essential step in the study of ligand-receptor interactions and in the design of new drugs. For this reason, we have studied the conformational space and calculated the proton affinity (PA) of α1-AR agonists and antagonists to determine which atoms would be protonated when interacting with the α1A-AR under physiological conditions.

Techniques such as electrospray ionization, matrix-assisted laser desorption or the extended kinetic method, allow for the experimental determination of PAs and examples of the application of these methods can be found in the recent literature.[15] However, computational approaches, and more particularly Density Functional Theory (DFT)

calculations, are valuable tools in determining PAs,[16] not only in gas phase but also including solvation effects. Furthermore, DFT studies using the B3LYP functionals with 6-31G* and 6-31+G** basis sets have been shown to produce PA values within 3-5 kcal mol^{-1} of experimental results.[17,18] For this reason, we have chosen the B3LYP hybrid DFT method,[19] coupled with the Onsager[20] solvation model for our present study. The molecules chosen are the two α1-AR natural agonists (noradrenaline and adrenaline) and a selection of α1- and α1A-AR antagonists. Namely, the four compounds already used in clinical practice, prazosin, terazosin, doxazosin and alfuzosin (Figure 1), the eight bis-2-iminoimidazolidinium and bis-guanidinium derivatives developed previously by us (**1-8**), and the α1-AR and α1A-AR antagonists previously mentioned, **9** to **18** (Figure 2). To our knowledge, no experimental proton affinities are available for these compounds.

Computational Methods

The minimum energy conformation of all the ligands studied was determined by means of conformational analyses using the Random Search tool implemented in Sybyl (versions 6.81 and 6.9).[21] The following conditions were established in the conformational analyses performed: in compounds **1-8**, only the C(arom.)-X bonds [X= N(2-iminoimidazolidinium, guanidinium), N(H), $C(H_2)$, C(O), $S(O_2)$] were rotated; in prazosin, terazosin, doxazosin, alfuzosin and compounds **9** to **18** all single bonds were rotated. All the atomic charges were evaluated with the Gasteiger-Hückel method. Each generated conformer was minimized over 300 cycles using the Conjugate Gradient method and the maximum number of cycles in the search was set to 6000 with an energy cut-off of 10 kcal mol^{-1}. For each compound, the lowest energy conformer was chosen and the proton was placed in turn on each of the N atoms present in each molecule. The labeling of all the N present are indicated in the schemes of Tables 1, 2 and 3.

The geometries of all the protonated structures were fully optimised with the program Gaussian-98[22] using the hybrid method B3LYP with a 6-31G* basis set. Due to the size of the compounds studied a larger basis set could not be used. The inter- and intramolecular interactions can cause substantial changes in the geometry and electronic structure of compounds in solution in comparison with the isolated gas phase. Therefore, the aqueous phase calculations should be preferred in theoretical approaches and for this reason the Onsager solvation method was included in the calculations. The radii for the cavities used in this approach were chosen after a volume calculation of each molecule, and the dielectric constant of water was used. Additionally, vibrational frequency calculations were performed, at the B3LYP/6-31G* level, to characterise the stationary points as minima. The variation in zero-point vibration energies (ZPE) and thermal corrections from zero degrees to 298 K have been considered in the calculations. No scaling factor for the ZPE values has been taken into account.

Results and Discussion

Proton Affinities of the natural agonists and current clinical antagonists of the α1 adrenoceptor

In the case of the natural agonists, adrenaline and noradrenaline, only one N atom is available for protonation (Figure 1). When performing the structural optimisations in the gas phase, the protonation energy determined for adrenaline was 243.4 kcal mol^{-1} and for noradrenaline was 240.2 kcal mol^{-1}. These values were thermally corrected to 233.9 and 231.0 kcal mol^{-1}, respectively (Table 1). Furthermore, when solvation effects where included a stabilization of the protonated state due to solvation, was observed at 0 K and 298 K (Table 1).

For the α1-AR antagonist prazosin, the gas phase calculations indicate that the N atom labelled as N*b* in the scheme of Table 1 was the most likely to be protonated in agreement with other authors.[23] As NH_2 groups are electron donating, the N*c* group is *ortho* / *para* directing. A study by de Benedetti *et al.*[24] highlighted that the N*a* of prazosin, which is in *ortho* position with respect to the NH_2 group, is unlikely to be protonated. This corroborates with our results since the energy difference for protonating at N*a* with respect to N*b* is approximately of 10 kcal mol^{-1} (Table 1).

For terazosin and doxazosin, with structures very similar to prazosin, the results follow a similar trend; thus, the N*b* nitrogen has the highest proton affinity both at 0 K and 298 K and the second N atom to be protonated is the other pyrimidinic nitrogen, N*a*, showing a difference in energy of around 10 kcal mol^{-1}. In the case of alfuzosin, with a related structure to the previous antagonists, N*b* again seems the most likely to be first protonated in gas phase, followed by N*a* and N*e* with a difference of 10 kcal mol^{-1} (Table 1).

When solvation effects are included, stabilization due to the solvent is observed in all cases and larger PAs are obtained (Table 1). For prazosin, terazosin and doxazosin the same PA order is observed, N*b* having the highest PA followed by N*a* (ΔPA= 10 kcal mol^{-1}). However, the change in the PA values is not homogeneous. The largest changes are observed in the thermally corrected values of prazosin with stabilizations of 17 for N*d*, 19 for N*a* and N*b*, 29 for Nc and 34 kcal mol^{-1} for N*e*. In the case of terazosin the largest solvent stabilization occurs for the protonations of N*e* and N*c* (14 and 11 kcal mol^{-1}) and in the case of doxazosin the largest ΔPA corresponds to the solvated protonation of N*c* (22 kcal mol^{-1}). Surprisingly, the inclusion of solvent effects for alfuzosin produces a change in its order of protonation. Thus, according to these calculations, the N atom most likely to be protonated in an aqueous environment is N*e* followed by N*b* (ΔPA of approximately 8 kcal mol^{-1}). Due to these unexpected results we utilised another more computationally expensive solvation

model, the PCM (Polarisable Continuum Model) approach.[25] Using this solvation model we obtained PA values of 297.2 and 289.3 kcal mol^{-1} (without and with thermal correction) for N*b* and 292.9 and 284.0 kcal mol^{-1} for N*e*, in agreement with the expected order of protonation.

Proton Affinities of the bisphenyl antagonists ***1-8***

For the symmetric bisphenyl derivatives previously developed by us,[4] there are two unique N atoms which can be protonated in the guanidinium moieties. They have been labelled N*a* to N*b* as shown in the scheme of Table 2. In addition when the bridge group (X) is the NH group an additional protonation site is available, N*c*.

The gas phase protonation energies, of the N*a* atoms range from 246 to 263 kcal mol^{-1}, in a 17 kcal mol^{-1} interval (Table 2). These differences are due to the various minimum energy conformations considered. In the gas phase, compound **1** shows, overall, the greatest protonation energy for the N*a* position, and, generally, the PA values obtained for those atoms are in a similar range to those obtained in the previous section. The thermally corrected PA values for the N*a* atom falls in the range between 238 and 254 kcal mol^{-1}. Regarding the outer N*b* position, the PAs obtained are in intervals of 19 and 18 kcal mol^{-1} (without and with thermal correction).

Generally, in these compounds, when solvation is considered, a stabilization of the PA for the protonations of N*a* and N*b* are observed and the range of protonation energies slightly increases (Table 2). However, solvation does not seem to affect the protonation of the bridge N*c* atoms possibly because these atoms are less exposed to the effects of solvent than those in the guanidinium or 2-iminoimidazolidinium groups. In summary, the N*a* position will be the first one to be protonated whereas N*b* will be the last one. In the case of compounds **1** and **5** the bridge N*c* will be the second position to be protonated.

*Proton Affinities of the α1A adrenoceptor antagonists **9** to **18***

Compounds **9** to **13** can be represented by a generic scheme in which three moieties are identified. At one end of the molecule there is a heterocyclic moiety containing a N*a*-CO-N*b* group (Table 3), and on the other end a piperazine ring (labelled N*c* and N*d* in the scheme of Table 3) with a heterogeneous bridge including a N atom (labelled N*e*). In compounds **9**, **10** and **13**, the N atoms with the largest PA in the gas phase are those of the piperazine rings (N*c* and N*d*, see Table 3), and they will be the first ones to be protonated. When present, N*a* and N*b* have the lowest PA values in this set of compounds (~200 kcal mol^{-1}). In both compounds **11** and **12**, there is a C=N*e* group and similar PA values were obtained (see Table 3).

For compounds **14** to **17** there is only one N atom to be protonated and in the case of compound **18** there are two N atoms, one in an alkyl chain and the other in the indole ring (labelled N*e* and N*f* respectively in the schemes of Table 3). The gas phase PA values of the N atom of the linker of these compounds are approximately 240 kcal mol^{-1} and 230 kcal mol^{-1} without and with thermal corrections. In general, the PA in gas phase of the N in the alkyl linkers of all these compounds (N and N*e* in compounds **9**, **12**, **14**, **15**, **16**, **17** and **18** in Table 3) are analogous, which seems logical due to the similarity in their structures. An exception is compound **15** with PA values that are larger that those of the other mono amino derivatives.

When solvation is included a general increase in the PA values is observed (Table 3) indicating stabilization of the protonated state. Similar patterns are detected between the PA of solvated molecules and those in the gas phase. Thus, the piperazine N*c* and N*d* atoms in compounds **9**, **10** and **13** will be the first ones to be protonated, while those of the heterocyclic ring (N*a* and N*b*) show the lowest PA values both at 0 K and 298 K. However, compounds **11** and **12**, show larger PA values at the N*e* atoms than at the piperazine N atoms (see Table 3). It should be pointed out that the N*c* atoms of the piperazine ring should be more likely to be first

protonated than the N*d* atoms since these last ones are directly connected to an aromatic ring. However, our solvation results indicate the contrary for compounds **9** and **10**. This, again, could be probably due to the limitations of the Onsager model in reproducing solvation conditions.

The mono amine derivatives (**14** to **17**) follow the same trend as in the gas phase studies with compound **15** having the largest PA. This could possibly be explained by the presence of a near phenyl ring and the dioxane O atoms. Compound **16**, which has a PA slightly smaller than that of **15**, has a similarly positioned phenyl ring but a piranone system instead of the dioxane ring. In the case of compound **18**, both in the gas phase and solvation environments N*e* is the first Nitrogen being protonated.

Overall, the PA values obtained for the first protonation of all these compounds are in similar ranges to those obtained for the first protonation of previous compounds.

Correlations between PA and experimental biological affinity towards α1A adrenoceptors

Correlations were examined between the determined PA values and the experimental affinities when available. A good correlation has been found between the experimental affinities (K_i, nM) for α_{1A}-ARs antagonists, prazosin, alfuzosin, doxazosin, terazosin, **9** (5-methylurapidil), **10** (RS-100,975), **14** (WB4101) and **18** (RS-17053)[24c] and the PA at 298 K without solvent effects (Figure 3). The good logarithmic relation with the unsolvated PA values ($PA= 5.57 \ln(K_i) + 237.85$; $R^2= 0.96$; $n= 8$) may indicate that the actual interaction between the ligand and the active site of the α_{1A}-AR occurs in the absence of water molecules and could possibly be better modelled by the gas phase protonation results.

Further correlations between the PA values and the activity data for the compounds previously prepared by us (**1** to **8**) were also pursued. No clear correlation was found perhaps due to the nature of the biological data obtained. The antagonistic activities of compounds **1**

to **8** were measured in isolated aortic tissue of rat and rabbit. No direct K_i over the α1A-AR was measured and, therefore, different effects from the tissue could be influencing the final K_i values.

Conclusions

A systematic computational study of the PA of a large family of agonists and antagonists of the α1-ARs has been performed. After an initial conformational analysis process the minimum energy conformation of each compound was chosen for the PA study. All the possible N protonations were considered for all the molecules and similar trends were observed in the protonation energy values obtained. The PA values obtained for the first protonation of all the compounds studied are in the same range (~250 kcal mol^{-1}). The inclusion of solvation by means of the Onsager model yielded stabilization in the PA values obtained, indicating that protonation of these compounds in a solvated medium will be energetically favoured. However, it has to be mentioned that some of the protonation sites indicated by the results using this solvation method do not agree with the expected sites according to chemical intuition. This could indicate that the simple Onsager method would not describe properly solvation for all types of ligands, and that results obtained with this method should be carefully analysed.

Furthermore, a good correlation was found between the PA values corresponding to the first protonation in gas phase of some of the compounds studied and their corresponding affinity constants K_i which measure the affinity of the compounds for the α1A-AR receptor.

Knowledge gained from this study will be useful for the design and future synthesis of new and more selective α1A-AR antagonists that could find application for the treatment of BPH. Furthermore, this work will allow for further studies into the interactions of these ligands with our previously developed model of an α1A-AR.

Acknowledgements

This research was supported by an HEA PRTLI grant (Cycle III), an Enterprise Ireland postgraduate research scholarship (G.K.), and a Trinity College Dublin postgraduate scholarship (G.K.).

Figures:

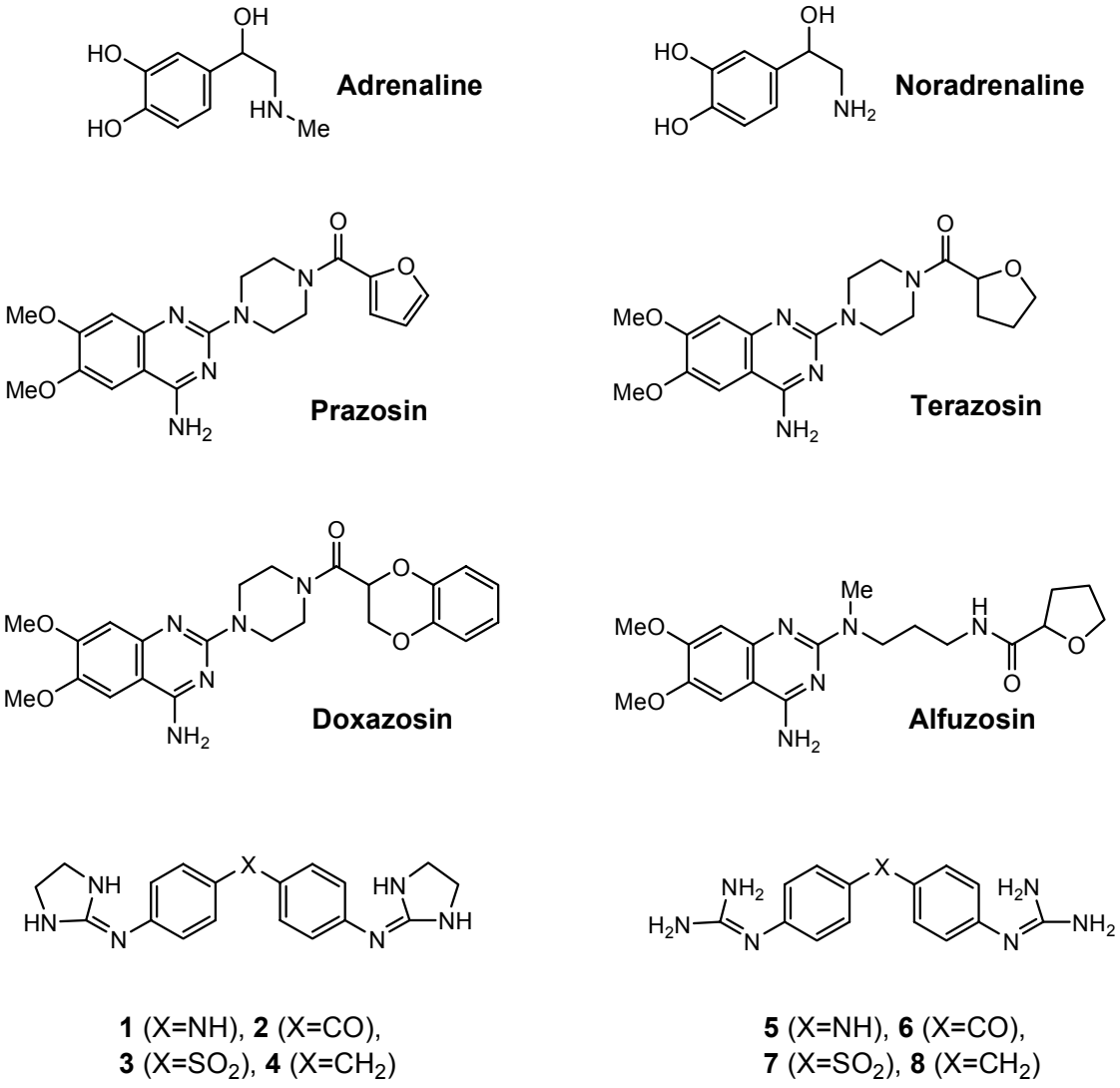


Figure 1.- Agonists of α1-AR (adrenaline and noradrenaline), antagonists used in clinical practice (prazosin, terazosin, doxazosin and alfuzosin) and α1A-AR antagonists prepared previously by our group (**1** to **8**).

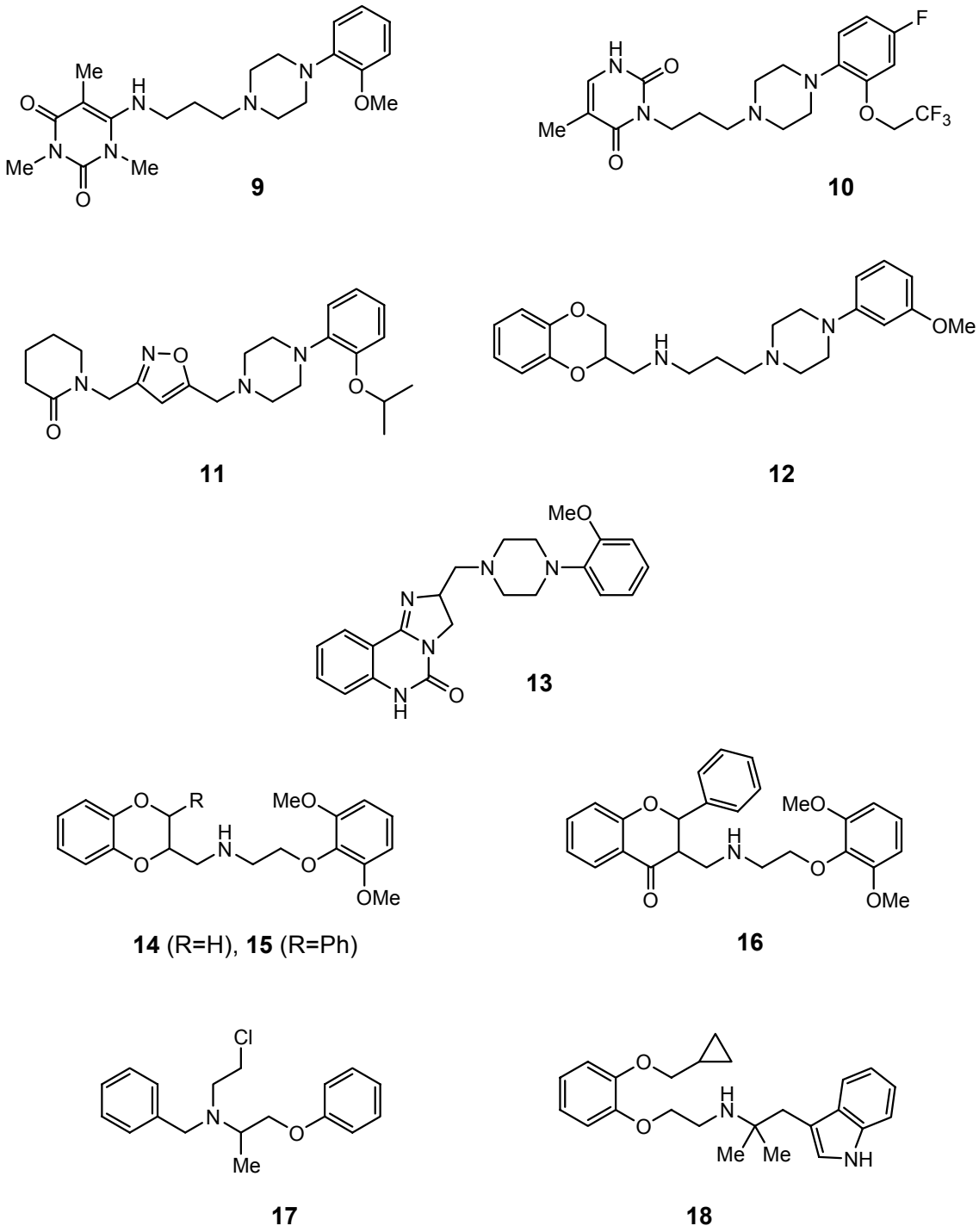


Figure 2.- Antagonists of the α1A adrenoceptor subtype developed by different authors (**9** to **18**).

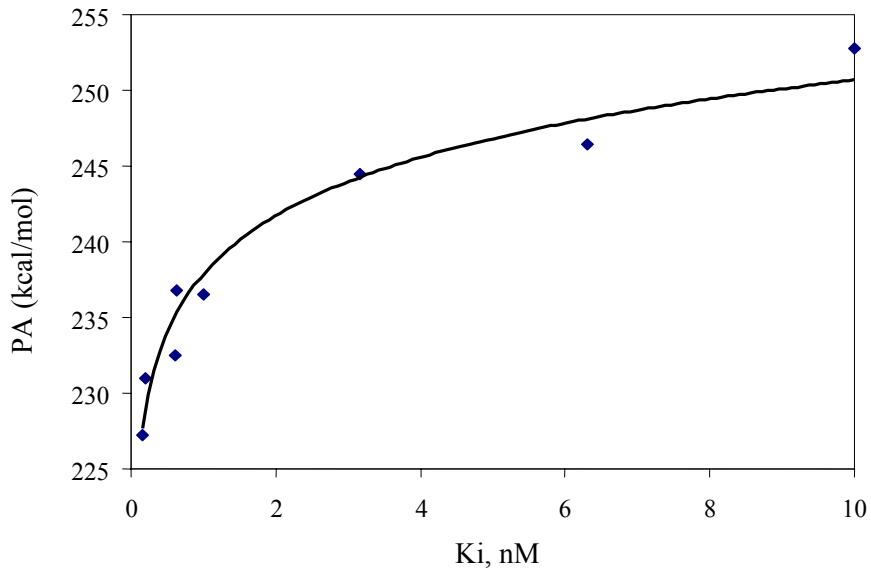
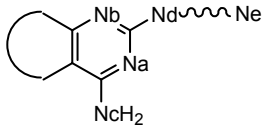


Figure 3.- Correlation of the (K_i, nM) for α_{1A}-ARs of prazosin, alfuzosin, doxazosin, terazosin, **9** (5-methylurapidil) **10** (RS-100,975)**, 14** (WB4101) and **18** (RS-17053) with the thermally corrected PA without solvent effects.

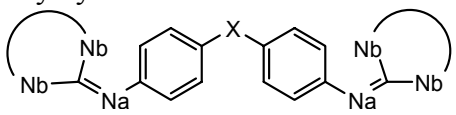
Tables:

Table 1.- Proton Affinity (PA, kcal mol^{-1}) in gas phase and including solvation effects without and with thermal correction at 298 K calculated at the B3LYP/6-31G* level for the α1-AR agonists noradrenaline and adrenaline and the antagonists prazosin, terazosin, doxazosin and alfuzosin.



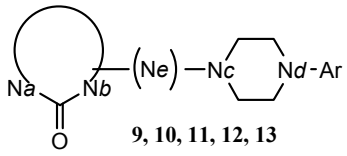
		Gas phase		Solvation	
		PA (0 K)	PA (298 K)	PA (0 K)	PA (298 K)
Adrenaline	N*a*	243.4	233.9	258.5	248.9
Noradrenaline	N*a*	240.2	231.0	246.2	237.1
Prazosin	N*a*	244.4	220.5	247.4	239.1
	N*b*	254.7	231.0	258.4	250.0
	N*c*	222.8	198.2	235.2	226.6
	N*d*	191.8	173.8	193.6	191.1
	N*e*	233.7	209.7	251.7	243.5
Terazosin	N*a*	244.1	235.8	248.7	240.4
	N*b*	254.9	246.4	259.7	251.1
	N*c*	222.8	214.5	235.1	226.3
	N*d*	239.4	231.1	241.6	232.5
	N*e*	231.4	223.4	244.9	237.0
Doxazosin	N*a*	242.1	233.9	255.5	247.3
	N*b*	253.0	244.5	264.6	256.3
	N*c*	221.1	212.3	242.2	233.6
	N*d*	242.4	233.5	241.6	232.5
	N*e*	214.7	206.6	218.4	210.4
Alfuzosin	N*a*	255.8	247.3	255.9	247.4
	N*b*	261.3	252.8	263.7	255.1
	N*c*	235.8	227.3	236.4	227.9
	N*d*	242.0	232.9	243.9	234.8
	Ne	257.0	248.3	271.7	263.0

Table 2.- Proton Affinity (PA) in gas phase and including solvation effects without and with thermal correction at 298 K calculated at the B3LYP/6-31G* level for the α1A-AR antagonists developed previously by us **1**-**8**.

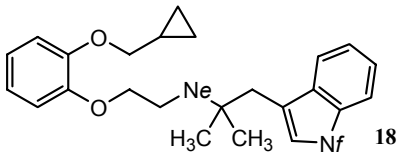


		Gas phase		Solvation	
		PA (0 K)	PA (298 K)	PA (0 K)	PA (298 K)
1	N*a*	262.5	253.8	292.5	284.5
	N*b*	230.6	221.7	274.3	266.4
	N*e*	246.2	236.9	244.0	234.8
2	N*a*	254.1	245.5	284.1	276.4
	N*b*	223.5	214.6	264.8	257.0
3	N*a*	251.0	242.5	274.0	266.0
	N*b*	220.0	211.5	252.1	244.5
4	N*a*	258.8	250.1	293.5	285.4
	N*b*	225.5	216.8	265.2	257.3
5	N*a*	262.6	252.2	289.7	281.3
	N*b*	225.3	217.3	243.5	234.6
	N*e*	242.2	233.6	241.6	233.0
6	N*a*	250.0	242.1	287.6	278.9
	N*b*	216.0	207.7	275.4	267.2
7	N*a*	246.0	238.3	283.5	275.1
	N*b*	212.0	204.0	261.6	268.2
8	N*a*	255.1	246.8	292.0	284.1
	N*b*	219.4	211.1	240.3	232.2

Table 3.- Proton Affinity (PA) in gas phase and including solvation effects without and with thermal correction at 298 K calculated at the B3LYP/6-31G* level for the α1A-AR antagonists **9-18**.



9, 10, 11, 12, 13



18

		Gas phase		Solvation	
		PA (0 K)	PA (298 K)	PA (0 K)	PA (298 K)
9	N*a*	207.6	199.4	222.2	214.6
	N*b*	207.4	199.6	220.1	212.7
	N*c*	246.3	236.8	251.0	241.6
	N*d*	246.0	236.3	276.8	267.8
	N*e*	219.5	211.1	228.5	220.3
10	N*a*	189.3	181.6	246.9	237.5
	N*b*	196.9	189.2	248.1	241.6
	N*c*	245.2	235.5	251.2	241.6
	N*d*	246.1	236.5	267.9	261.5
11	N*b*	220.1	211.1	244.1	218.6
	N*c*	239.9	231.9	241.3	233.3
	N*d*	246.9	236.9	245.9	236.3
	N*e*	263.8	236.8	262.4	253.3
12	N*c*	249.9	240.3	256.0	246.5
	N*d*	245.4	236.1	229.6	224.6
	N*e*	270.4	261.2	270.1	260.9
13	N*a*	204.9	196.7	241.5	233.8
	N*b*	206.7	198.8	231.1	223.3
	N*c*	256.9	248.2	265.9	257.4
	N*d*	245.8	235.9	246.0	236.1
	N*e*	253.8	243.8	258.9	249.2
14	N	236.6	227.2	245.4	236.1
15	N	264.7	254.7	265.1	255.1
16	N	246.4	237.1	246.3	243.5
17	N	239.2	229.7	242.3	232.6
18	N*e*	241.9	232.5	254.9	246.0
	N*f*	229.3	220.1	258.8	249.2

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