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Computational approach to the basicity of a series of α1-adrenoceptor ligands in aqueous solution

Gemma K. Kinsella,[a,†] Fernando Rodriguez,[a,b] Graeme W. Watson[a] and Isabel Rozas[a,b,*]

[a]*School of Chemistry, University of Dublin, Trinity College, Dublin 2, Ireland*
[b]*Centre for Synthesis and Chemical Biology, School of Chemistry, University of Dublin, Trinity College, Dublin 2, Ireland*

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Abstract—In order to design any new potential drug, it is crucial to know their corresponding pK_a since their protonation state will be critical in the ligand–receptor interaction and it will play an essential role in their pharmacokinetic profile. Several authors have developed approaches for the computational determination of pK_a which involve the use of a thermodynamic cycle relating pK_a to the gas-phase proton basicity via the solvation energies of the products and the reactants. Such methods are very dependent on the solvation model used and the nature of the system. The theoretical pK_a of a number of agonists and antagonists of the α1A-adrenoceptor has been computed and the performance of this approach has been tested through comparison with the available and/or measured experimental pK_a values.
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1. Introduction

Considering that drugs and endogenous ligands interact with their corresponding receptor in an aqueous environment at physiological pH, the basicity and, hence, the pK_a of these compounds is critical in the ligand–receptor interaction and plays an essential role in the pharmacokinetic profile of any potential drug. Pharmaceutical companies nowadays need to evaluate the pK_a of any potential lead before proceeding to its optimization. However, the experimental evaluation of the pK_a of potential drugs is not always possible and can be tedious. Therefore, an accurate computational approach is needed.

Several authors have developed approaches for the computational determination of pK_a which involve the use of a thermodynamic cycle relating pK_a to the gas-phase proton basicity via the solvation energies (ΔG^0_{solv}) of the products and the reactants (Fig. 1).[1] 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 are essential for pK_a determination and, for this reason both the Onsager[2] and polarisable continuum model (PCM)[3,4] solvation methods have been included in the present calculations.

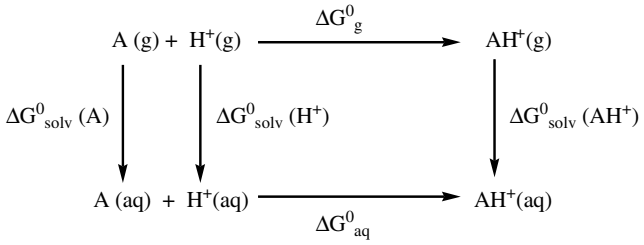

Figure 1. Thermodynamic cycle illustrating the calculation of theoretical pK_a values. For details on the different terms, see Section 4.

Calculation of the pK_a of a molecule, A, involves quantum mechanical calculations to characterise the gas-phase system (ΔG^0_g) for both the associated acid, AH^+(g), and the dissociated species, H^+(g) and A(g), and to characterise the solvated system (ΔG^0_{aq}) for the associated [AH^+(aq)] and dissociated [H^+(aq) and A(aq)] species. Thus, the pK_a of A(aq) is given by

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* Corresponding author. Tel.: +353 1 896 3731; fax: +353 1 671 2826; e-mail: rozasi@tcd.ie
† Present address: Dublin Software Lab., IBM Software Group, IBM Technology Campus, Damastown Industrial Park, Mulhuddart, Dublin 15, Ireland.

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$$pK_a = \Delta G^0_{aq}/2.303RT \quad (1)$$

where

$$\begin{aligned}\Delta G^0_{aq} &= \Delta G^0_g + \Delta G^0_{solv}(AH^+) - \Delta G^0_{solv}(H^+) \\ &\quad - \Delta G^0_{solv}(A) \\ &= \Delta G^0(AH^+(g)) - \Delta G^0(H^+(g)) - \Delta G^0(A(g)) \\ &\quad + \Delta G^0_{solv}(AH^+) - \Delta G^0_{solv}(H^+) - \Delta G^0_{solv}(A)\end{aligned}$$

Most of the terms in this equation will be taken from our computations, but there are two terms that need a careful consideration, the free energy of a proton in the gaseous phase [$\Delta G^0(H^+(g))$] and its solvation free energy [$\Delta G^0_{solv}(H^+)$]. In the case of the free energy of a proton in the gaseous phase, it can be easily calculated by the Sackur-Tetrode equation,[5]

$$\Delta G^0(H^+(g)) = 2.5RT - T\Delta S = -6.28 \text{ kcal mol}^{-1} \quad (2)$$

However, the reference state of the solvation energies is very important and, thus, a correction should be included taking into account a change of reference state (from atm to mol L^{-1}).[6] In this way, a corrected value for $\Delta G^0(H^+(g))$ of -4.40 kcal mol^{-1} is obtained and it will be the one used in the present study.

Regarding the free energy of solvation of the proton, $\Delta G^0_{solv}(H^+)$, it has been the subject of some uncertainty and controversy, since this energy is difficult to measure or predict accurately.[7] To date, experimental determination of the free energy of a proton in water,[8] has provided values in a range between -254.0 and -264.0 kcal mol^{-1}. Additionally, theoretical studies have reported a considerable range of values between -244.9 and -264.0 kcal mol^{-1}.[9] These values differ depending on both the computational method and the system used in each case. However, very recently, Camaioni et al.[10] have clarified the topic stressing the importance of the reference standard states. Hence, to convert from 1-atm gas phase/1-m solution standard state to the 1-M gas/1-M solution standard state they subtract 1.9 kcal mol^{-1} from the most reliable reported experimental value for hydration of a proton[8d,e] of -264 kcal mol^{-1} resulting in a corrected $\Delta G^0_{solv}(H^+)$ value of -265.9 kcal mol^{-1}. This will be the value used in the present study.

We are interested in the development of new specific antagonists of α-adrenoceptors (α-AR) and their subtypes, and, with that in mind we have recently developed a model of the α1A-AR subtype[11] and modelling of the other two subtype receptors is on-going.[12] Adrenaline and noradrenaline (AD and NA, Fig. 2) are the natural agonists of α-AR, several subtype selective antagonists have been developed with different medical applications,[13] and in our group, we have previously prepared a series of compounds (**1**–**6**)[14] that exhibit α1-AR antagonist activity (Fig. 2).

In this article and following the outlined procedure, we have attempted to evaluate the theoretical pK_a of all these compounds and we have examined the performance of this method through comparison of the computational results obtained to the available experimental pK_a values. In some cases, these experimental values were accessible from the literature or databases; however, in the case of those compounds already prepared by us (**1**–**6**), we have experimentally measured their pK_a.

2. Results and discussion

In aqueous solution, the more acidic proton will be lost first when increasing the pH, so that the experimental pK_a value corresponds to the preferred ionization site. Predictions of pK_a depend on accurate solvated PA values. Considering our previous study[15] on the computational calculation of the proton affinity of a number of agonists and antagonists of α1A-adrenoceptors, we have used those values in the present study.

2.1. Natural α1A-AR agonists' basicity

Both endogenous ligands that bind to the adrenoceptors contain an amino function that has a pK_a greater than seven so that the ligand will be partly or fully positively charged at neutral pH.[16] The experimental pK_a values for adrenaline and noradrenaline are very similar, 8.59[17] and 8.58,[18] respectively. Using these experimental values we tried to calculate the corresponding $\Delta G^0_{solv}(H^+)$ to compare with the value established by Camaioni. In this manner and with both solvation models, we did not obtain any value near to -265.9. Then, applying the thermodynamic cycle and using the value already established for the solvation of the proton, we obtained the pK_a values presented in Table 1.

At the B3LYP/6-31G* level of theory, the theoretical pK_a values obtained are very far from those experimentally obtained. At least, by using the PCM solvation technique we obtained positive values that follow the same trend as the experimental ones.

2.2. Basicity of antagonists used in clinical practice

To further study the theoretical determination of pK_a values, we have examined the experimental data for some of the antagonists already used in clinical practice (Fig. 2). In all these compounds there are a number of possible protonation centres. Following the results obtained in a previous article,[15] the N atoms labelled as N*b* in the Scheme of Table 2 were chosen as the first protonation centre and consequently the experimental pK_a values were associated to the protonation of N*b*. The experimental pK_a values of terazosin,[19] prazosin,[20] doxazosin[16] and alfuzosin[21] were provided by different pharmaceutical companies (Table 2).

However, when using these experimental pK_a values, the thermodynamic cycle and the calculations performed using the PCM solvation technique, which is considered a more realistic solvation model than Onsager, $\Delta G^0_{solv}(H^+)$ values of -292.2 (alfuzosin), -281.9 (doxazosin), -287.3 (prazosin) and -288.7 kcal mol^{-1} (terazosin) were obtained. In all cases, these values were

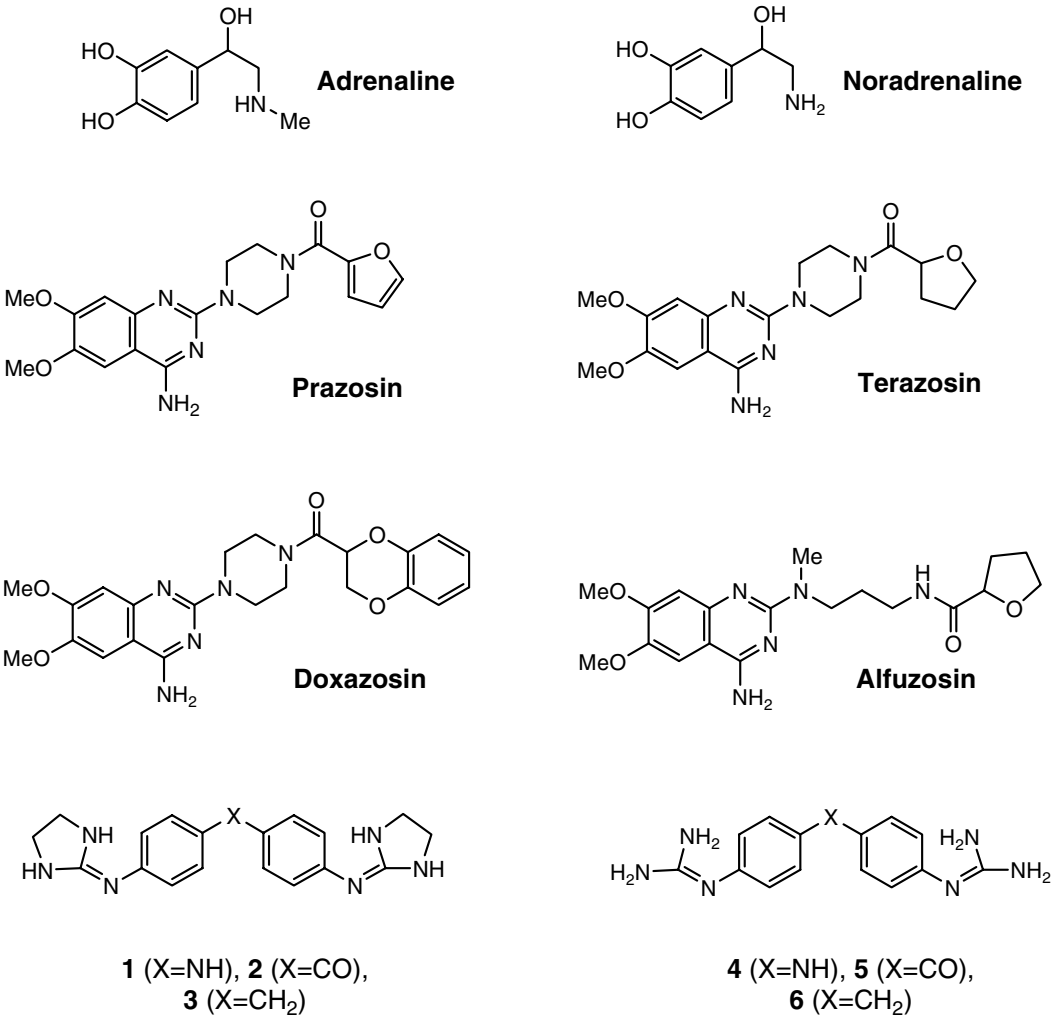
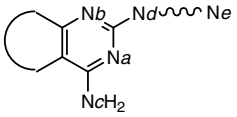


Figure 2. α1-AR agonists (adrenaline and noradrenaline), first generation antagonists (prazosin, terazosin, doxazosin and alfuzosin) and α1-AR antagonists prepared previously by our group (**1**–**6**).[14]

Table 1. Theoretical pK_a obtained using the Onsager and PCM solvation techniques and the experimental pK_a values found for adrenaline and noradrenaline

	Expt. pK_a	pK_a [Onsager]	pK_a [PCM]
Adrenaline	8.59	−23.6	24.76
Noradrenaline	8.58	−19.2	21.38

Table 2. Experimental and calculated pK_a values obtained for the clinical antagonists



	Expt. pK_a	pK_a [Onsager]	pK_a [PCM]
Prazosin	7.0	2.50	22.88
Terazosin	7.1	2.69	23.89
Doxazosin	6.9	1.27	18.69
Alfuzosin	8.1	7.41	27.42

far from Camaioni's corrected value of −265.9 kcal mol^{-1}.

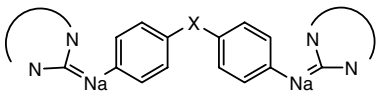
Thus, the theoretical pK_a values for the N*b* of all these clinical antagonists were computed using Camaioni's $\Delta G^0_{solv}(H^+)$ value and the results are presented in Table 2. The experimental pK_a values of this set of compounds follow the relative order: alfuzosin > terazosin > prazosin > doxazosin, and at Onsager and PCM levels, the computed pK_a values follow the same order.

2.3. Basicity of imidazolidinium/guanidinium antagonists

In the case of the compounds previously prepared by us (**1**–**6**, see Fig. 2) we performed the experimental determination of their pK_a with a method based on the Henderson-Hasselbach equation (pH = pK_a + log [A^-]/[AH]). According to this equation, when the concentration of the protonated acid and its conjugated base is the same the logarithmic term is cancelled and then the pK_a will be equal to the experimentally measured pH.

Using these experimental pK_a values (Table 3), and for the sake of comparison, we try to determine the

Table 3. Experimentally determined and theoretically calculated pK_a values for the antagonists **1**–**6**, with Onsager and PCM solvation method



Compound		Expt. pK_a	pK_a [Onsager]	pK_a [PCM]
1	N*a*	9.9	24.78	29.08
	N(–*X*–)	2.8	−11.51	12.35
2	N*a*	8.6	15.26	24.58
3	N*a*	9.8	11.32	27.78
4	N*a*	10.8	0.81	30.86
	N(–*X*–)	2.9	−12.38	16.13
5	N*a*	9.4	19.35	25.94
6	N*a*	10.5	16.30	27.78

corresponding $\Delta G^0_{solv}(H^+)$ values using both solvation approaches but all the values obtained were outside the experimental range and far from Camaioni's corrected figure.

Once again we used this $\Delta G^0_{solv}(H^+)$ corrected value to compute the theoretical pK_a values for these family of compounds using both solvation approximations. The results obtained are shown in Table 3. It is clear from these results that the Onsager method is not appropriate for the evaluation of the solvated species in the thermodynamic cycle and therefore cannot be used for an appropriated determination of theoretical pK_a values. This approach locates the molecule in a spherical cavity within the solvent and, unless the molecule is more or less spherical, the solvent effects on the structure studied are not homogeneous and the final description of the solvated molecule is wrong. On the contrary, results obtained using the PCM solvation approach show a certain agreement with the experimental data.

A linear correlation was found between all pK_a values experimentally measured (see Tables 1–3) and those computed with the PCM method.

$$pK_a[\mathrm{PCM}] = 1.939 pK_a[\exp] + 8.451,$$
$$R^2 = 0.86,\ n = 14 \quad (3)$$

Much better results are obtained when correlating experimental pK_a values to those calculated from the PCM solvent simulations for compounds **1**–**6.**

$$pK_a[\mathrm{PCM}] = 1.950 pK_a[\exp] + 8.543,$$
$$R^2 = 0.96,\ n = 8 \quad (4)$$

It seems that compounds belonging to the same chemical family provide better correlations between theoretical and experimental pK_as. Thus, Eq. (4) could be used to correct the theoretical values obtained in future calculations on this type of bis-aryl derivatives. Thus, by inputing a calculated pK_a[PCM] value in equation Eq. (4) it could be possible to predict relative pK_a values.

3. Conclusions

A crucial step in the thermodynamic cycle to compute theoretical pK_a values is the accurate calculation of the energy difference between the neutral and protonated forms. Calculations of pK_a require reliable and highly accurate gas-phase protonation/deprotonation energy calculations as well as solvation energy calculations for both products and reactants. The combination of DFT and continuum solvation methods for the computation of pK_a has been shown to work well in some cases.[22] We have observed how the solvation model used for the computations plays a very important role in the final values obtained and the more developed methods, such as PCM, provide the most reasonable results.

Moreover, it has to be taken into account that there are a number of limitations to the current implementation of theoretical pK_a calculations. Camaioni's corrected value for $\Delta G_{solv}(H^+)$ could not be theoretically reproduced for all structural classes studied using the experimental pK_a values and the thermodynamic cycle. As well, higher accuracy in the prediction of proton affinity values will improve the theoretical calculation of pK_a. Therefore, it seems that this thermodynamic cyclic method cannot be used in a generalised way.

From our results we propose the following alternative within a chemically related family of compounds: to use the PCM method to model solvation, to utilise the corrected solvation energy for the proton reported by Camaioni and to correct the obtained values by using a linear correlation equation developed for a known experimental set of values, such as Eq. (4); thus, the input of the corresponding calculated pK_a[PCM] values in an equation similar to Eq. (4) will provide relative pK_a values.

4. Methods

4.1. Computational study

The minimum energy conformation of all the structures studied was determined by means of conformational analyses using the Random Search tool implemented in Sybyl (versions 6.81 and 6.9).[23] The following conditions were established in all the conformational analyses performed: in compounds **1**–**6**, only the C(arom)–X bonds [X = N(2-imino imidazolidinium, guanidinium), N(H), C(H_2), C(O)] were allowed to rotate; in

adrenaline, noradrenaline, prazosin, terazosin, doxazosin and alfuzosin all single bonds were allowed to rotate. 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}. The structures were optimized at the AM1 semiempirical level. For each compound the protonated species as determined in our previous study[15] were used and the lowest energy conformer was chosen.

The geometries of all the structures were fully optimized with the Gaussian-98[24] and Gaussian-03[25] programs using the hybrid B3LYP method with a 6-31G* basis set. Due to the size of the compounds studied no larger basis set could be 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 to 298 K have been considered in the calculations. A uniform overestimation of QM harmonic vibrational frequencies has been found and as a result generic frequency scaling factors are often applied. A scale factor of 0.9614 was recommended by Scott et al.[26] for the B3LYP/6-31G* level of theory and was utilised in this work.

Both the Onsager[2] and PCM (polarisable continuum model)[3,4] methods were used for the solvation calculations. All PCM calculations were performed using the Gaussian-03 set of programs. The radii for the cavities used in the Onsager approach were chosen after a volume calculation of each molecule, and the dielectric constant of water was used. In the PCM method a solute cavity is formed from a union of spheres centred on each atom, thus generating a more realistic cavity shape than that utilised in the Onsager technique. PCM also includes an accurate treatment of the electrostatic interaction with the surrounding medium. Typically, the spheres defining the cavity are taken to be 1.2 times the van der Waals radii.

4.2. Experimental determination of pK_a

The pK_a values of the first protonation of the hydrochlorides of compounds **1**–**6** were obtained by measuring the pH of solutions containing 0.08 mmol of each of the hydrochlorides and 4 mL of a 0.01 M NaOH standard solution. To determine the pK_a values of the second protonation, eight additional milliliters of the NaOH standard solution was added and the pH was measured afterwards.

In the cases of the hydrochlorides of compounds **1** and **4** the pK_a values of the third protonation were obtained measuring the pH of solutions containing 0.08 mmol of each compound and 4 mL of a 0.01 M HCl standard solution.

All the measurements were carried out in a Metrohm 632 pH-meter with a combined LL pH glass electrode (6.0232.100/0120 0336). A pH 7.00 standard solution with di-sodium hydrogen phosphate/potassium dihydrogen phosphate was used to calibrate the apparatus.

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