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Theoretical prediction of relative and absolute pK_a values of aminopyridines

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Abstract

This work presents a study aimed at the theoretical prediction of pK_a values of aminopyridines, as a factor responsible for the activity of these compounds as blockers of the voltage-dependent K^+ channels. To cover a large range of pK_a values, a total of seven substituted pyridines is considered as a calibration set: pyridine, 2-aminopyridine, 3-aminopyridine, 4-aminopyridine, 2-chloropyridine, 3-chloropyridine, and 4-methylpirydine. Using ab initio G1, G2 and G3 extrapolation methods, and the CPCM variant of the Polarizable Continuum Model for solvation, we calculate gas phase and solvation free energies. pK_a values are obtained from these data using a thermodynamic cycle for describing protonation in aqueous and gas phases. The results show that the relatively inexpensive G1 level of theory is the most accurate at predicting pK_a values in aminopyridines. The highest standard deviation with respect to the experimental data is 0.69 pK_a units for absolute values calculations. The difference increases slightly to 0.74 pK_a units when the pK_a is computed relative to the pyridine molecule. Considering only compounds at least as basic as pyridine (the values of interest for bioactive aminopyridines) the error falls to 0.10 and 0.12 pK_a units for the absolute and relative computations, respectively. The technique can be used to predict the effect of electronegative substituents in the pK_a of 4-AP, the most active aminopyridine considered in this work. Thus, 2-chloro and 3-chloro-4-aminopyridine are taken into account. The results show a decrease of the pK_a, suggesting that these compounds are less active than 4-AP at blocking the K^+ channel.

Keywords: K^+ channel blocking; Aminopyridines; pK_a prediction; Activity factors

1. Introduction

Aminopyridines are bioactive N-heterocyclic tertiary amines, which increase the strength of the nerve signal by blocking of the voltage-dependent K^+ channel. In this form, the efflux of intracellular K^+ is suppressed and the presynaptic action potential is maintained. Thus, the calcium influx is enhanced, leading to an increase in the release of neurotransmitter and, therefore, to an increase of the nerve signal [1–4]. Due to this capacity, aminopyridines have been proposed as drugs for the treatment of spinal cord injuries [5], botulism [6], multiple sclerosis [7], and myasthenia gravis [8]. Also they have been tested as putative agents for the symptomatic treatment of Alzheimer's disease [9]. In particular, 4-aminopyridine, under the name fampridine, is being tested as an agent for compensating the loss of the myelin cover in nerves [10]. Recently, an experimental work has been presented, which uses 4-aminopyridine to identify novel compounds restoring conduction in the injured spinal cord [11]. The results show that derivatives of 4-aminopyridine, like the methyl and ethyl carbamates, maintain the ability to restore nerve function in injured nerves.

Aminopyridines are weak bases with pK_a values between 6 and 9. Therefore, they can exist in neutral and protonated form at physiological pH. The experimental [4,12–16] and theoretical evidences [17,18] support that the protonated form is the active species, being responsible for blocking the K^+ channel through an intracellular and reversible way of action. These works also identified the aminopyridines pharmacophore, which is constituted by the positive charge on the protonated nitrogen and one or more amine groups suitable for hydrogen bonding. Nevertheless, the action mechanisms to

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inactivate the K^+ channel, and the physical nature of the receptor, are still unknown. On this respect, a theoretical work [19] has proposed two possible receptor sites. The first is defined by four threonine–threonine–valine (Thr–Thr–Val) chains in C_4 symmetry with a bed of four oxygens pointing from the narrow exit channel of the pore to the large central cavity in the channel. The second corresponds to four threonine (Thr) residues in the pore, with their four OH groups arranged in a square. On the other hand, a functional model of activity for the K^+ channel blocking by aminopyridines has been developed [20]. This model shows that the algorithm of the experimental activity index (concentration of neutral form that produces a given result) depends linearly on the minus pK_a and on the Gibbs energy variation (ΔG) for interaction with the receptor. The pK_a value determines the amount of neutral and protonated form present in the extracellular medium. Only the neutral form enters the lipidic cell membrane to protonate in the cytoplasmic side and to bind to the receptor site. The pK_a of the most common aminopyridines is known, but predicting the pK_a value of novel compounds could be extremely useful in the search for new active molecules.

In principle, it is possible to use different theoretical approaches for the prediction of pK_a values. The most direct is a discrete approach, where water molecules are represented explicitly, and the Gibbs energy variations are determined from ab initio calculations. This approach has been recently applied to the study of the protonation mechanism of pyridine [21]. This job shows that four water molecules are needed for protonation to occur in a spontaneous way. Otherwise, five water molecules are needed to get a pK_a value of the same order of magnitude than the one obtained for pyridine in water solution. However, this discrete approach fails to reproduce pK_a values, since a large number of water molecules would be needed, which turns unfeasible the electronic structure calculations. Another approach is based on a continuous model for the solvent and a thermodynamic cycle for the protonation process. In this form, pK_a values have been accurately reproduced for carboxylic acids [22,23] to within half a pK_a unit. These works combine ab initio extrapolation methods, such as the Complete Basis Set (CBS) [24] or the Gaussian-*n* (Gn) [25–28] approaches, with continuum solvation models, such as the polarizable conductor model (CPCM), [29].

In this work, we consider the prediction of the pK_a of aminopyridines, which is one key factor defining their biological activity. Thus, we will apply a thermodynamic cycle for protonation, and high level (extrapolation) ab initio methods for determining Gibbs and solvation free energies. These extrapolation methods include an empirical factor derived from a reference set of molecules. No substituted pyridines are included in the set. Thus, to calibrate the different extrapolation methods, we use a set of substituted pyridines, including, but not limited to, aminopyridines. The goal is to determine the most appropriate procedure for predicting pK_a values in novel compounds. The results are analyzed in relative and absolute terms applying regression techniques. The application to new molecular systems is also presented.

2. Theory

To determine the most suitable strategy for prediction of pK_a values in aminopyridines, and more generally in substituted pyridines, a total of seven substituted pyridines, for which pK_a values are available, is considered in this work. These are pyridine (Pyr), 2-aminopyridine (2-AP), 3-aminopyridine (3-AP), 4-aminopyridine (4-AP), 2-chloropyridine (2-ClP), 3-chloropyridine (3-ClP), and 4-methylpirydine (4-MeP) (see Fig. 1). The pyridine is used as a reference for the relative determination of pK_a values, as describe below. The 2-, 3- and 4-aminopyridines are K^+ channel blockers with pK_a in the interval 6–9. The 2- and 3-chloropyridine are used to extend the range of pK_a to low values (0–3). Finally, 4-MeP is included to test pyridines with aliphatic substituents.

The thermodynamic cycle represented in Fig. 2 will be used. In this cycle, the deprotonation reaction in both gas and aqueous phase is taken into account. In Fig. 2, P represents a generic substituted pyridine, HP^+ the protonated form, and the "g" and "a" subscripts mean gas and aqueous phases, respectively. In addition, the "s" subscript identifies solvation free energies. Clearly,

$$pK_a(P) = 0.43429\Delta G_a(HP^+)/RT \quad (1)$$

On the other hand, we have

$$\Delta G_a(HP^+) = \Delta G_g(HP^+) + \Delta G_s(P) + \Delta G_s(H^+) - \Delta G_s(HP^+) \quad (2)$$

where

$$\Delta G_g(HP^+) = G_g(P) + G_g(H^+) - G_g(HP^+) \quad (3)$$

Combining Eqs. (1)–(3), we can compute absolute pK_a values assuming that the gas phase Gibbs energies are determined at a temperature of 298.15 K and at a concentration of 1 M. Electronic structure calculations, and some gas phase data, provide values in a reference state of 298.15 K and 1 atm. Conversion of Gibbs energies from the 1 atm to the 1 M reference state is done as follows,

$$G(1\ M) = G(1\ atm) + RT\ \ln[P(1\ M)/P(1\ atm)] = G(1\ atm) + RT\ \ln(22.46) \quad (4)$$

In addition, $G_g(H^+)$, and $\Delta G_s(H^+)$ are needed. In this work, we take the Liptak et al. values [23] of −6.28 kcal/mol and

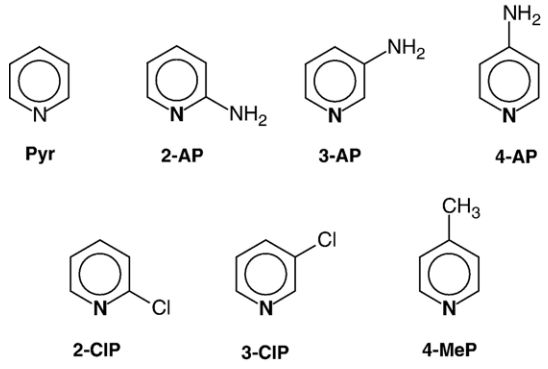

Fig. 1. Molecular structures of the substituted pyridines considered in this work.

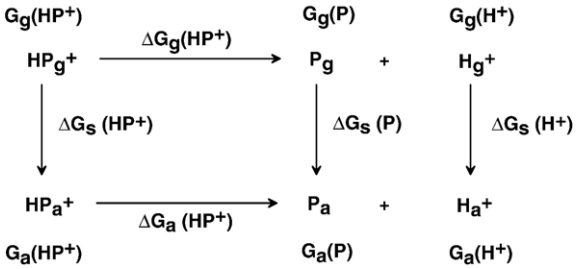

Fig. 2. Schematic representation of the thermodynamic cycle considered in this work. The diagram shows the deprotonation reaction in gas and aqueous phase. In the figure, P represents a generic substituted pyridine, HP^+ is the protonated form and the "g" and "a" subscripts represent gas and aqueous phases, respectively. Solvation free energies are denoted by an "s" subscript.

−264.61 kcal/mol, respectively. The first value was obtained theoretically at a pressure of 1 atm [22,30,31]. This value is within 0.19 kcal/mol of the value obtained from the gas-phase deprotonation data available from NIST [32]. The second value is in the 1 M standard state and is obtained experimentally from the acetic acid system [22,31]. With these considerations, and at a temperature of 298.15 K,

$$\mathrm{p}K_a(\mathrm{P}) = 0.73299\cdot[G_g(\mathrm{P}) + \Delta G_s(\mathrm{P}) - G_g(\mathrm{HP}^+) + \Delta G_s(\mathrm{HP}^+) - 269.00] \quad (5)$$

Eq. (5) provides $\mathrm{p}K_a$ values in the 1 M reference state, using Gibbs energies in kcal/mol and computed at a pressure of 1 atm.

Relative $\mathrm{p}K_a$ values can be obtained using the cycle in Fig. 2, and applying the previous treatment to a generic pyridine and to a reference molecule, in our case, pyridine (Pyr). By making the difference, we obtain

$$\mathrm{p}K_a(\mathrm{P}) = \mathrm{p}K_a(\mathrm{Pyr}) + 0.73299\cdot\{[G_g(\mathrm{P}) - G_g(\mathrm{Pyr})] - [G_g(\mathrm{HP}^+) - G_g(\mathrm{HPyr}^+)] + [\Delta G_s(\mathrm{P}) - \Delta G_s(\mathrm{Pyr})] - [\Delta G_s(\mathrm{HP}^+) - \Delta G_s(\mathrm{HPyr}^+)]\} \quad (6)$$

Using Eq. (6), the values of the $G_g(H^+)$, and $\Delta G_s(H^+)$ cancel out.

The comparison of the results obtained with the different methods can be done by defining an average of absolute differences index, AD, as

$$\mathrm{AD} = \frac{\sum_{i=1}^{n} |\mathrm{p}K_{a\ \mathrm{calc}} - \mathrm{p}K_{a\ \mathrm{exp}}|}{n} \quad (7)$$

where n is the number of molecules used, $\mathrm{p}K_{a\ \mathrm{calc}}$ is the calculated value and $\mathrm{p}K_{a\ \mathrm{exp}}$ the experimental datum. In addition, another index, related with the least squares procedure, will be used: the sum of squares of differences, SSD,

$$\mathrm{SSD} = \sum_{i=1}^{n} [\mathrm{p}K_{a\ \mathrm{calc}} - \mathrm{p}K_{a\ \mathrm{exp}}]^2 \quad (8)$$

Let us assume the experimental $\mathrm{p}K_a$ to be an undefined function of the calculated value, $\mathrm{p}K_{a\ \mathrm{exp}} = \mathrm{p}K_{a\ \mathrm{calc}}$. This function will correct for the deficiencies in the theoretical models employed. In this case, expanding in a Taylor series around an arbitrary value $\mathrm{p}K_{a\ 0}$ we will have

$$\mathrm{p}K_{a\ \mathrm{exp}} = f(\mathrm{p}K_{a\ \mathrm{calc}}) = f_0(\mathrm{p}K_{a\ \mathrm{calc}}) + \left.\frac{\partial f(\mathrm{p}K_{a\ \mathrm{calc}})}{\partial(\mathrm{p}K_{a\ \mathrm{calc}})}\right|_0 (\mathrm{p}K_{a\ \mathrm{calc}} - \mathrm{p}K_{a\ 0}) + \frac{1}{2}\left.\frac{\partial^2 f(\mathrm{p}K_{a\ \mathrm{calc}})}{\partial(\mathrm{p}K_{a\ \mathrm{calc}})^2}\right|_0 (\mathrm{p}K_{a\ \mathrm{calc}} - \mathrm{p}K_{a\ 0})^2 + \cdots \quad (9)$$

or formally,

$$\mathrm{p}K_{a\ \mathrm{exp}} = a + b\cdot\mathrm{p}K_{a\ \mathrm{calc}} + c\cdot\mathrm{p}K_{a\ \mathrm{calc}}^2 + \cdots \quad (10)$$

The coefficients a, b, c, … can be obtained by a regression analysis. The regression analysis will permit to quantify statistically the reliability of the calculated $\mathrm{p}K_a$ values.

3. Methods

The values of Gibbs energies in vacuum (gas phase), ΔG_g, will be obtained by high level ab initio methods. Extrapolation methods will be used, based on the good results obtained for the $\mathrm{p}K_a$ of carboxylic acids by Liptak et al. [22,23]. In particular, the Gaussian-1 (G1), Gaussian-2 (G2) and Gaussian-3 (G3) approaches will be considered, which belongs to a series called Gaussian-*n* theories [25–28]. These procedures are designed to give an estimation of the "infinite correlation, infinite basis" results. The goal of these methods is to produce energy differences accurate to about 1 kcal/mol compared with the experimental data. After an initial geometry optimization at the MP2(full)/6-31G(d) theory level, the methods perform a series of different single point calculation with different basis sets and electron correlation methods. From these results the extrapolation to the infinite limit is done, [25–28]. The methods have been calibrated on a reference set of molecules, using atomization energies, ionization potentials, and electron and proton affinities.

The solvation free energies for the protonated and neutral forms, ($\Delta G_s(HP^+)$ and $\Delta G_s(P)$), will be theoretically obtained using a continuum solvation method. In particular, we will apply the Polarizable Continuum Model, PCM [33], using the CPCM polarizable conductor variant [29]. The molecular geometries employed are those optimized at the MP2(full)/6-31G(d) level, which are the structures used by the G1, G2, and G3 theories.

The regression analysis has been performed with the SPSS statistical package [34]. The "best" regression equation is selected by using the stepwise regression procedure. This procedure uses *F*-tests to determine the inclusion or removal of terms in the regression equation. The *F* probabilities of the null hypothesis used for inclusion or removal of terms are 0.05 and 0.10, respectively.

All electronic structure calculations, in vacuum and solution, have been carried out with the Gaussian-03 package [35], running on a computational Grid formed by three clusters: Hermes and Tales (Ciudad Real, Spain), and Popocatepetl

(Puebla, Mexico). The Grid uses GridWay [36] as metascheduler, with Globus 2.4 [37] as the basic Middleware. The three clusters run under the cluster configuration and management system Rocks [38].

4. Results and discussion

Table 1 collects the experimental and absolute pK_a values computed using Eq. (5) on the molecular set represented in Fig. 1. The AD and SSD indexes show that the difference with the experimental data follows the order G1<G2<G3. To quantify the accuracy of the results, we have performed a regression to a quadratic form of the experimental versus the calculated data for the G1 results, see Eq. (10). The most significant regression model found by the stepwise method is lineal,

$$pK_{a\,exp} = 0.840 + 0.884 \cdot pK_{a\,calc} \quad \text{with}$$
$$R = 0.974;\ \sigma = 0.69 \qquad (11)$$

The σ value shows that we can expect an error of the estimate of 0.69 pK_a units.

On the other hand, Table 2 collects the relative pK_a values obtained from Eq. (6), using the calculated pK_a of pyridine as a reference. The AD and SSD indexes show that the difference with the experimental data follows the same trend than in the previous case: G1<G2<G3. The values of these indexes are slightly smaller than for absolute pK_a values. This can be attributed to partial cancellation of systematic errors in the theoretical models. Again, a linear regression analysis of the G1 results to a quadratic form, Eq. (10), produces a linear model as the most significant,

$$pK_{a\,exp} = 0.800 + 0.885 \cdot pK_{a\,calc} \quad \text{with}$$
$$R = 0.975;\ \sigma = 0.74 \qquad (12)$$

Now the σ value shows an expected error of the estimate of 0.74 pK_a units, greater than for absolute computations, see Eq. (11).

The reason for the error trend observed in the three extrapolation methods, G3>G2>G1, can be explained by the inner working of these procedures. G2 theory improves G1 by including three factors [26]. First, the non-additivity caused by the assumption of separate basis set extensions for diffuse-sp functions. Second, a correction for the addition of a third d function to nonhydrogen atoms and a second p function to the hydrogens. Finally, G2 modifies the "higher level correction" introduced in the G1 theory. In G1, this correction is obtained to reproduce the electronic energy of the hydrogen atom and the H_2 molecule. In G2 the correction is introduced to give zero mean deviation from experiment of the calculated atomization energies of 55 molecules (no substituted pyridines are included in the set) [26]. The average absolute deviations of the four magnitudes used in G-*n* theories (atomization energy, ionization potential, electron affinity and proton affinity) is 1.43 and 1.18 kcal/mol for the G1 and G2 theories, respectively [26]. However, the average absolute deviation of proton affinities is 1.0 and 1.04 kcal/mol for G1 and G2, respectively [26]. Thus, G1 is slightly better at computing proton affinities. In turn, G3 theory is a variant of G2, which uses a different sequence of point calculations with different basis sets, a new formulation for the "higher level correction", spin-orbit correction for atoms and a correction for core correlation [28]. In particular, the "higher level correction" now distinguishes between molecules and atoms. The parameters used in the correction are derived by minimizing the average absolute deviation from experiments for the G2/97 test set [28]. This set contains 148 enthalpies of formation, 88 ionization potential, 58 electron affinities but only eight proton affinities. The average absolute deviations of the four magnitudes considered (atomization energy, ionization potential, electron affinity and proton affinity) decreases for the G2/97 set from 1.48 to 1.04 kcal/mol, when going from G2 to G3 theory. However, average deviation of proton affinities increases from 1.08 to 1.34 kcal/mol. Clearly, the introduction of more flexible empirical corrections for the "higher level correction" enhances the accuracy of the overall results in the order G3>G2>G1. However, the small relative weight given progressively to proton affinities in the corrections explains that the error of this magnitude is found in the order G3>G2>G1. Because of its direct relationship with protonation affinities, this variation is to be expected for the pK_a (as found in this job). Our

Table 1
Absolute pK_a values for the set of substituted pyridines considered in this work

	Exp.	G1	G2	G3
Pyr	5.25 [a]	4.89	5.74	5.88
2-AP	6.86 [b]	6.68	7.59	7.88
3-AP	5.98 [b]	5.75	6.77	6.96
4-AP	9.17 [b]	8.82	9.85	10.07
3-ClP	2.84 [a]	3.71	4.66	4.66
2-ClP	0.72 [c]	−0.86	0.06	0.17
4-MeP	6.02 [c]	6.01	6.89	7.02
SSD	0.00	3.59	7.44	7.82
AD	0.00	0.51	0.94	0.99

Values computed at the G1, G2 and G3 theory levels. All data obtained at a temperature of 298.15 K. The table includes the average differences, AD, and the sum of squares of differences, SSD, indexes, see text. Experimental pK_a values are included.
[a] From Ref. [40].
[b] From Molgó et al. [41].
[c] From Ref. [42].

Table 2
Relative pK_a values for the set of substituted pyridines considered in this work

	Exp.	G1	G2	G3
2-AP	6.86 [a]	6.72	7.54	7.79
3-AP	5.98 [a]	5.79	6.72	6.88
4-AP	9.17 [a]	8.86	9.8	9.98
3-ClP	2.84 [b]	3.68	4.54	4.57
2-ClP	0.72 [c]	−0.82	0.02	0.1
4-MeP	6.02 [c]	6.05	6.85	6.94
SSD	0.00	3.23	5.47	6.55
AD	0.00	0.51	0.88	0.98

Values computed at the G1, G2 and G3 theory levels. All data obtained at a temperature of 298.15 K. The table includes the average differences, AD, and the sum of squares of differences, SSD, indexes, see text. Experimental pK_a values are included.
[a] From Molgó et al. [41].
[b] From Ref. [40].
[c] From Ref. [42].

results show that the G1 theory can predict semiquantitatively pK_a values for substituted pyridines. This is an interesting result, since the computational expense of G1 is smaller than that of G2 or G3. Thus, larger molecules can be considered.

Analyzing the data in Tables 1 and 2, we observe that the two substituted pyridines with low pK_a values are affected by a large error. From the standpoint of the study of aminopyridines, we are specially interested in compounds with pK_a equal or higher than pyridine. Using the G1 results, after removing 2Cl-AP and 3-ClAP, we obtain AD values for the absolute and relative calculations of 0.23 and 0.17 pK_a units, respectively. If we repeat the regression analysis, we find the quadratic model as the most significant in both cases:

$$\mathrm{p}K_{\mathrm{a\ exp}} = 3.512 + 0.073 \cdot \mathrm{p}K^2_{\mathrm{a\ calc}} \quad \text{with}$$
$$R = 0.998;\ \sigma = 0.10 \tag{13}$$

for absolute values, and

$$\mathrm{p}K_{\mathrm{a\ exp}} = 3.498 + 0.072 \cdot \mathrm{p}K^2_{\mathrm{a\ calc}} \quad \text{with}$$
$$R = 0.998;\ \sigma = 0.12 \tag{14}$$

for the relative ones.

The results collected in Eqs. (13) and (14) show a better correlation, R coefficient, than the previous results of Eqs. (11) and (12). We observe that now the error of the estimate is about 0.10 pK_a units in both cases.

Having determined an appropriate theory method for pK_a predictions in our kind of compounds, new molecules for which no experimental data are available can be treated. As an example, we can consider the effect of electronegative substituents in the pK_a of 4-AP the most active aminopyridine considered in this work (for activity data, see reference [39]). Thus, 2-chloro-4-aminopyridine (2Cl-4AP), and 3-chloro-4-aminopyridine (3Cl-4AP) are considered. The absolute pK_a values obtained at the G1 level are 2.91 and 6.89 for 2Cl-4AP and 3Cl-4AP, respectively. Correcting these data with Eq. (11) we obtain predicted pK_a values of 3.41 and 6.90 for 2Cl-4AP and 3Cl-4AP, respectively. Comparing with the 8.64 pK_a value for 4-AP obtained with Eq. (11) using the absolute 8.82 G1 value, the new values represent an important decrease from the 4-AP pK_a. The decrease is specially high for 2Cl-4AP. This can be attributed to the electron withdrawal effect of chlorine, which is translated in a smaller affinity of the pyridinic nitrogen for the proton. This effect is specially strong in the 2 position due to the direct conjugation with the nitrogen. Without considering additional factors, these results suggest that 2Cl-4AP and 3Cl-4AP should present smaller activity against K^+ channel blocking than 4-AP.

5. Conclusions

This work deals with the computation of accurate pK_a values for aminopyridines. To consider a wide range of pK_a values, seven substituted pyridines have been used as a calibration set: pyridine, 2-aminopyridine, 3-aminopyridine, 4-aminopyridine, 2-chloropyridine, 3-chloropyridine, and 4-methylpyridine. These compounds expand a range of pK_a values form 0.72 to 9.17.

Applying a thermodynamic cycle, absolute and relative (to pyridine) pK_a values are calculated. The calculations use data obtained from ab initio results at the G1, G2 and G3 levels of theory and the CPCM solvation model. We find the relatively inexpensive G1 results to be the most accurate in absolute and relative terms. A regression analysis of the experimental versus the calculated pK_a, gives the linear regression equation as the most significant. The results establish an error of the estimate of 0.69 and 0.74 pK_a units for the absolute and relative results, respectively.

Since, from the activity models, high pK_a values favour the activity index, we focus in compounds at least as basic as pyridine. In these conditions, G1 gives still the best results. However, the regression analysis of experimental versus calculated pK_a finds a quadratic form as the most significant. The error of the estimate falls to 0.10 and 0.12 pK_a units for the absolute and relative cases, respectively.

Application of the present methodology permits to predict pK_a values of 3.41 and 6.90 for 2Cl-4AP and 3Cl-4AP, respectively. These results represent a significant decrease from the 4-AP pK_a. Despite other factors, the results suggest that 2Cl-4AP and 3Cl-4AP should exhibit smaller activity than 4-AP for the blocking of K^+ channels.

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