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Short communication

Relationship between quantum-chemical descriptors of proton dissociation and experimental acidity constants of various hydroxylated coumarins. Identification of the biologically active species for xanthine oxidase inhibition

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Abstract

Quantum-chemical descriptors related to proton dissociation constants of a set of coumarins hydroxylated in various positions have been computed and related to the experimental pK_a values. An excellent correlation was found between the computed deprotonation energies of hydroxycoumarins in water and their experimental pK_a values, and the results were used to predict the pK_a of other hydroxycoumarins. Then, predicted and experimental pK_a values were used as a basis for interpreting and discussing the variation of xanthine oxidase inhibitory activities within a subset of coumarins, with the aim of identifying the molecular species most relevant for enzyme inhibition.

Keywords: Quantum-chemical descriptors; pK_a; Coumarin; Xanthine oxidase inhibitors

1. Introduction

Xanthine oxidase (XO) is an important enzyme of the purine metabolism that catalyzes the oxidation of xanthine and hypoxanthine to uric acid [1]. Inhibiting XO is desirable for two principal reasons: first, it decreases the excess uric acid developed under hyperuricemic conditions that ultimately cause gout [2,3]; second, it prevents the formation of superoxide radicals, thereby protecting against post-ischemic reperfusion injury [4].

In a previous paper we proposed a model of the interaction of substrates and inhibitors with XO [5]. The model, based on quantum-chemical calculations with the inclusion of solvent effects, molecular superimpositions, and molecular electrostatic potential calculations, was able to rationalize a number of experimental evidences relating to the mechanism and structure–activity relationships of XO. Taking into account inhibitors, the main binding features of inhibitors belonging to different classes, including flavones (2-phenyl-benzopyran-4-one derivatives), were investigated together with substrates to build a single model that rationalized structure–activity relationships [5]. Flavones have already been optimized as XO inhibitor in the past [6,7]. In particular, they were proposed to inhibit XO in their anionic form, originated by dissociation of the 7-hydroxyl group [6,7].

Coumarin derivatives, whose structure is similar to that of benzopyran-4-one, proved to be active as XO inhibitors [8]. Indeed, the hydroxylation pattern of coumarins turned out to be important for XO inhibition, as previously observed also for hydroxylated flavones [6,7] and anthocyanidins [9]. However, a qualitative relationship between hydroxylation pattern, dissociation constants, and inhibitory activity of hydroxycoumarins has not been investigated, and the molecular species responsible for XO inhibition has not been identified.

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In the present communication, quantum-chemical descriptors related to proton dissociation constants of coumarins hydroxylated in various positions have been computed and related to the experimental pK_a values, and the results have been used to infer the biologically active species involved in XO inhibition.

2. Results and discussion

Quantum-chemical descriptors, being able to account for geometric, electronic and energetic properties of molecular species in equilibrium, are useful descriptors for proton dissociation. In this context, computed deprotonation energies, corresponding to the difference between the calculated heat of formation of the anionic (dissociated) and the neutral form of a molecule, can be related to the experimental dissociation constant because they represent the relative stability of the two forms in the dissociation equilibrium.

Table 1 reports the deprotonation energies (Kcal/mol) of a series of various hydroxylated coumarins, calculated with the semiempirical Hamiltonian PM3. The deprotonation energies have been calculated both in vacuo ($\Delta\Delta H_f^0$(vacuo)) and in water ($\Delta\Delta H_f^0$(water)), using the SM3 method for modeling water solvent. The table also reports the experimental pK_a values of 17 hydroxylated coumarins [10–14] spanning 4.5 pK_a units, and the available XO inhibitory activities (IC_{50}) [8].

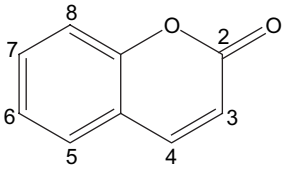
The values of $\Delta\Delta H_f^0$(vacuo) and $\Delta\Delta H_f^0$(water) in Table 1 have been correlated with the available experimental pK_a values of the hydroxylated coumarins and the results are shown in Fig. 1. Interestingly, while deprotonation energies calculated in vacuo ($\Delta\Delta H_f^0$(vacuo)) are linearly correlated with experimental pK_a values with a squared regression coefficient of 0.54 (Fig. 1A), inclusion of solvent effects ($\Delta\Delta H_f^0$(water)) significantly improves the correlation, giving a squared regression coefficient of 0.96 (Fig. 1B). The statistical parameters of the two regression equations at a 95% confidence interval are $pK_a = 0.1178(0.0580–0.1777)\Delta\Delta H_f^0(\text{vacuo}) + 12.8010(9.9940–15.6088)$, $R^2 = 0.54$, $s = 0.88$, $F = 17.6$ for the regression in vacuo, and $pK_a = 0.3085(0.2722–0.3448)\Delta\Delta H_f^0(\text{water}) + 35.3691(32.0681–38.6700)$, $R^2 = 0.96$, $s = 0.27$, $F = 328$ for the regression in water. The excellent correlation obtained using deprotonation energies in water further highlights the importance of including solvent effects, especially when charged species are being calculated. Interestingly, coumarins **2** and **9** are clear outliers in the plot of Fig. 1A but not of Fig. 1B, indicating that a proper description of solvent effects is required to reliably predict pK_a values. Importantly, the correlation holds for molecular species arising from dissociation of hydroxyl groups in different positions of the coumarin ring. Therefore, the theoretical approach is sensible and can serve our scope of identifying the biologically active species among a number of different anionic species. Using the regression equation $pK_a = 0.3085\Delta\Delta H_f^0(\text{water}) + 35.3691$ to recalculate the pK_a values of the 17 analogs, we found that the average difference between the calculated and the experimental pK_a values is significantly low (0.19 ± 0.16). To further validate the regression, the ''leave-one-out'' method, which consists of recomputing the equation by excluding from the data set one molecule at a time, was performed. This validation served to check if the regression was tightly dependent on one particular compound and to verify if the pK_a value of each excluded molecule is predicted to be in agreement with experiment. In each case, the squared regression coefficient was never lower than 0.94, the average difference between the calculated and experimental pK_a values was 0.22 ± 0.18, and the predicted pK_a value of each excluded molecule never differed by more than 0.1 units from the prediction performed on the entire data set.

Then, qualitative arguments based on predicted pK_a values and XO inhibitory activities were put forward to help identifying the molecular species most relevant for XO inhibition.

Among mono-hydroxylated compounds **1**–**5**, 4-hydroxycoumarin is the most acidic coumarin in the series ($pK_a = 5.1$) and is completely dissociated at physiological pH. 3-Hydroxy and 7-hydroxy coumarins, which are less acidic ($pK_a = 7.6$ and 8.3, respectively) and are only partially dissociated at physiological pH, have inhibitory activities comparable to 4-hydroxycoumarin. Therefore, these data alone do not support a direct link between the availability of a general dissociated form in solution and its inhibitory activity, at least for the three analyzed mono-hydroxylated compounds. Unfortunately, the activities of 6-hydroxy- and 8-hydroxycoumarin are not available.

6,7-Dihydroxycoumarin **18** is, by far, the more active derivative in the series (IC_{50} 8.2 μM). The pK_a prediction of the dissociation constants of each of the two hydroxyls in 6,7-dihydroxycoumarin, performed using the regression equation of Fig. 1B, gave values of 8.4 for the 6-hydroxyl and 6.9 for the 7-hydroxyl; compared to the computed pK_a values of the corresponding mono-hydroxylated coumarins (9.3 and 7.9), the simultaneous presence of both the 6- and 7-hydroxyls is predicted to lower the pK_a value of both hydroxyls by one pK_a unit. Overall, the finding that the pK_a value of the 6,7-dihydroxycoumarin gets closer to the physiological pH, thereby increasing the amount of dissociated form, combined with the higher inhibitory activity of this molecule, gives a first indication that dissociation of the 6- or 7-hydroxyls could be important for activity. Other indications supporting this evidence come from the effects of selective methoxylation of the two hydroxyl groups. While 6-hydroxy-7-methoxycoumarin **20** retains activity, though weaker than 6,7-dihydroxycoumarin, 6-methoxy-7-hydroxycoumarin **21** is completely inactive. Therefore, dissociation of the 6-hydroxyl seems to be more relevant than dissociation of the 7-hydroxyl, because methoxylation necessarily implies that proton dissociation is prevented. The pK_a predictions of these two methoxylated derivatives show that the 7-methoxyl increases the pK_a value of the 6-hydroxyl by 0.3 units, while the 6-methoxyl lowers the pK_a value of the 7-hydroxyl by 0.3 units, compared to the correspondent mono-hydroxycoumarins. The finding that 6-methoxy-7-hydroxycoumarin is completely inactive despite having a slightly more acidic 7-hydroxyl, combined with the evidence that 6-hydroxy-7-methoxycoumarin retains activity despite having a less acidic 6-hydroxyl, suggests that dissociation of the 6-hydroxyl is more important for inhibitory activity. In

Table 1
Computed deprotonation energies, experimental and calculated pK_a values, and xanthine oxidase inhibitory activities of selected hydroxycoumarins



No.	Substituents	Anionic species	pK_a^{expt}	$\Delta\Delta H_f^0$(vacuo)[f]	$\Delta\Delta H_f^0$(water)[g]	pK_a^{calch}	IC_{50}(μM)[i]
1	3-Hydroxy	$3O^-$	7.6[a]	−40.89	−90.52	7.4	131
2	4-Hydroxy	$4O^-$	5.1[a]	−45.41	−96.79	5.5	195
3	6-Hydroxy	$6O^-$	9.4[a]	−34.42	−84.38	9.3	
4	7-Hydroxy	$7O^-$	8.3[a]	−42.96	−89.18	7.9	236
5	8-Hydroxy	$8O^-$	8.5[a]	−33.19	−86.85	8.6	
6	4-Methyl-5-hydroxy	$5O^-$	8.26[b]	−44.90	−89.80	7.7	
7	4-Methyl-6-hydroxy	$6O^-$	9.14[b]	−34.05	−84.42	9.3	
8	4-Methyl-7-hydroxy	$7O^-$	7.8[b]	−41.78	−89.05	7.9	
9	4-Methyl-6,8-difluoro-7-hydroxy	$7O^-$	4.9[c]	−51.53	−98.78	4.9	
10	3-Oxo-acetaldehyde-7-hydroxy	$7O^-$	6.7[d]	−56.50	−93.70	6.5	
11	3-(2-Furyl)-7-hydroxy	$7O^-$	7.41[e]	−47.22	−90.09	7.6	
12	3-(2-Thienyl)-7-hydroxy	$7O^-$	7.71[e]	−48.16	−88.39	8.1	
13	3-Phenyl-7-hydroxy	$7O^-$	7.8[e]	−45.44	−89.23	7.8	
14	3-(2-Benzoxazolyl)-7-hydroxy	$7O^-$	6.84[e]	−52.98	−92.27	6.9	
15	3-(2-Benzothiazolyl)-7-hydroxy	$7O^-$	7.02[e]	−52.33	−91.32	7.2	
16	3-(2-Benzoxazolyl)-4-cyano-7-hydroxy	$7O^-$	6.07[e]	−57.82	−95.66	5.9	
17	3-(2-Benzothiazolyl)-4-cyano-7-hydroxy	$7O^-$	6.38[e]	−57.00	−93.83	6.4	
18	6,7-Dihydroxy	$6O^-$		−39.23	−87.37	8.4	8.2
		$7O^-$		−47.48	−92.13	6.9	
19	7,8-Dihydroxy	$7O^-$		−43.08	−90.56	7.4	390
		$8O^-$		−36.25	−89.32	7.8	
20	6-Hydroxy-7-methoxy	$6O^-$		−34.99	−83.50	9.6	138
21	6-Methoxy-7-hydroxy	$7O^-$		−44.67	−89.90	7.6	Inactive
22	4-Methyl-6,7-dihydroxy	$6O^-$		−39.01	−87.63	8.3	246
		$7O^-$		−46.82	−92.49	6.8	

[a] Ref. [10].
[b] Ref. [11].
[c] Ref. [12].
[d] Ref. [13].
[e] Ref. [14].
[f] Deprotonation energies (Kcal/mol) corresponding to the difference between the calculated heat of formation of the anionic (dissociated) and the neutral form of each molecule in vacuo.
[g] Deprotonation energies (Kcal/mol) in water.
[h] Recalculated and predicted pK_a values using the regression equation $pK_a = 0.3085\Delta\Delta H_f^0(\text{water}) + 35.3691$.
[i] Inhibitory activity toward XO.

support of this, it can be noted that while both hydroxyls in 7,8-dihydroxycoumarin **19** are predicted to be more acidic than the correspondent mono-hydroxyl derivatives (with pK_a values 0.5 and 0.8 units lower for the 7-OH and 8-OH, respectively), 7,8-dihydroxycoumarin is significantly less active than 6,7-dihydroxycoumarin (Table 1). Therefore, dissociation of the 7-hydroxyl does not seem to be very important for activity. Taken all together, these results indicate that the anionic form originating from dissociation of the 6-hydroxyl is likely to be the biologically relevant species.

One last comment is worth for 4-methyl-6,7-dihydroxycoumarin **22**. The pK_a values predicted for the 6- and 7-hydroxyls are very close to those predicted for 6,7-dihydroxycoumarin. Therefore, the considerably lower inhibitory activity showed by 4-methyl-6,7-dihydroxycoumarin with respect to 6,7-dihydroxycoumarin cannot be attributed to dissociation effects but, rather, to unfavourable steric interaction of the 4-methyl with enzyme binding residues.

3. Conclusions

An excellent correlation between computed deprotonation energies and experimental pK_a values of a set of 17 various hydroxylated coumarins has been established. In particular, deprotonation energies turned out to be useful descriptors for identifying the molecular species more relevant for XO inhibition (the anionic, dissociated 6-hydroxyl form). One could use such information to predict the pK_a values of differently substituted coumarins before chemical synthesis. Having identified the active species, optimization of coumarins as XO inhibitors should consider maintaining the 6-hydroxyl, and introducing suitable substituents able to modulate its pK_a value

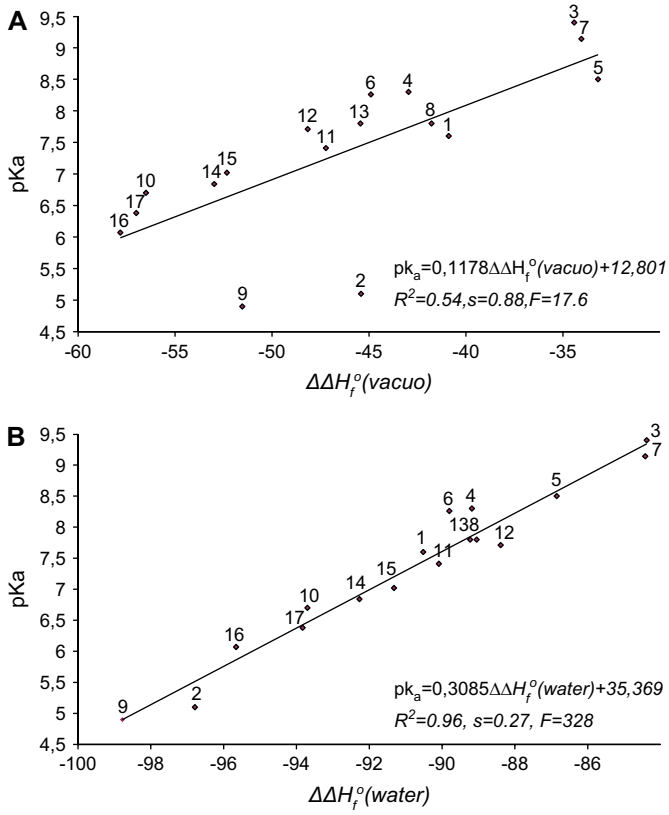


Fig. 1. Correlation between experimental pK_a values of various hydroxylated coumarins and computed deprotonation energies (Kcal/mol) in vacuo (A) and in water (B).

and/or other factors like hydrophobic interactions. Once a congeneric series of various substituted 6-hydroxycoumarins have been built, quantitative structure–activity relationships can be attempted.

4. Experimental

Quantum-chemical calculations were performed with the AMSOL 6.8 package [15] in the PM3 [16] parameterization. The geometry of each compound reported in Table 1 was fully optimized in both the neutral and the anionic form originated from proton dissociation of the hydroxyls. Solvation energies of each molecular species were calculated from single-point calculations on the geometry-optimized structures using the SM3 [17] method. Deprotonation energies in vacuo were defined as the difference between the heat of formation of the anionic (dissociated) and the neutral forms of each molecule in vacuo (PM3 calculations); deprotonation energies in water were obtained by correcting the deprotonation energies in vacuo with the relative solvation energies of the anionic and neutral forms (PM3/SM3 calculations).

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