A theoretical study of the inhibition of human 4-hydroxyphenylpyruvate dioxygenase by a series of pyrazalone-quinazolone hybrids

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ABSTRACT

A Density Functional Theory study was carried out to find relationships between the electronic/molecular structure of a group of pyrazalone-quinazolone hybrids and their inhibition of human 4-hydroxyphenylpyruvate dioxygenase (HPPD). The geometries were fully optimized at the B3LYP/6-31G(d,p) level. A statistically significant equation was obtained. The equivalent 2D pharmacophore was built and some atom-site interactions were suggested. The analysis of the equation and the pharmacophore should provide new information about possible substitution sites for an enhancing of the inhibitory activity.

Keywords: HPPD, 4-hydroxyphenylpyruvate dioxygenase, QSAR, DFT calculations, reactivity indices, inhibition constant, electronic structure.

INTRODUCTION

In aerobic metabolism, the conversion of 4-hydroxyphenylpyruvate into 2,5 dihydroxyphenylacetate (homogentisate) is catalyzed by the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD). In animals this pathway is required to control blood tyrosine levels. In Homo sapiens, the abnormal metabolism in the tyrosine catabolism pathway gives rise to various diseases, such as Type I tyrosinemia, Type II tyrosinemia, Type III tyrosinemia, hawkinsinuria and alkaptonuria (alcaptonuria, black urine disease or black bone disease). HPPD is linked directly to alkaptonuria (a deficiency in active homogentisate 1,2-dioxygenase), Type III tyrosinemia (a deficiency of active HPPD) and hawkinsuremia (a result of uncoupled turnover of HPPD). The specific inhibition of HPPD can relieve the symptoms of alkaptonuria, Type I tyrosinemia and perhaps hawkinsinuria, by ending the flux of metabolites through four of the five steps of tyrosine catabolism. Also, naturally occurring allelopathic diketone and triketone alkaloids inhibit HPPD in a specific way which prevents the creation of homogentisate and consequently the synthesis of tocopherols and plastoquinones, the latter of which is vital for photosynthesis. Therefore, molecules that specifically inhibit HPPD have potential use as herbicides. Several groups of molecules have been synthesized and tested for HPPD inhibition [1-14]. Recently, Yang et al. synthesized a series of pyrazolone–quinazolone hybrids that are novel potent human HPPD inhibitors [3]. As a contribution for a better understanding the action mechanism of HPPD inhibitors, we present here the results of a study relating the electronic/molecular structure of the abovementioned molecules with their inhibition constants against recombinant human HPPD.
MATERIALS AND METHODS

The method
Considering that the formal model employed here has been presented in several papers [15-23], we shall present here only its main lines of development and discuss below only the results obtained here. Starting from the statistical-mechanical definition of the equilibrium constant, an expression relating this experimental value with several local atomic reactivity indices and orientational parameters was developed. This model is the sole member of the class of formal models. Its application to several different molecules and receptors gave very good results (see [23-38] and references therein). Its extension to all kinds of biological activities was very fruitful (see [38-52] and references therein).

Selection of biological activity
The molecules were selected from Ref. [3]. The chosen experimental values are the inhibition constants (Kᵢ) against recombinant human HPPD. Both are shown in Fig. 1 and Table 1.

Calculations
As usual, we worked within the common skeleton hypothesis asserting that there is a set of atoms, common to all the molecules analyzed, that explains practically all the biological activities. The effect of the substituents is to change...
the electronic structure of the common skeleton and/or influencing the correct placement of the inhibitor molecule. The common skeleton is shown in Fig. 2, together with the atom numbering employed in the resultant statistical equations.

![Common skeleton numbering](image)

Molecular geometries were fully optimized at the B3LYP/6-31G(d,p) level of the theory with the Gaussian 03 suite of programs [53]. From the corrected Mulliken Population Analysis results [54], the numerical values for all electronic local atomic reactivity indices were obtained. The D-Cent-QSAR software was used [55]. The orientational parameters for the R₂-R₄ substituents were calculated with the Steric software [56]. As the system of linear equations cannot be solved because the number of molecules is smaller than the number of unknown coefficients, a linear multiple regression analysis (LMRA) was carried out. The Statistica software was used [57].

**RESULTS**

The best equation obtained with LMRA is:

\[
\log(K_i) = 1.47 - 0.69 F_{19}(HOMO - 2)^* + 0.001 \phi_{R_4} + 6.92 F_{17}(HOMO - 2)^* + 6.48 F_{19}(LUMO + 2)^* - 0.21 S_{N}^V(LUMO + 1)^* + 1.18 F_{18}(HOMO - 2)^* - 2.50 F_{14}(HOMO - 1)^*
\]

with n=25, R= 0.96, R²= 0.92, adj. R²= 0.89, F(7,17)=28.62 (p<0.000001) and a standard error of estimate of 0.11. No outliers were detected and no residuals fall outside the ±2σ limits. Here, \(F_i(HOMO - 2)^*\) is the Fukui index of the third highest occupied MO localized on atom 5, \(\phi_{R_4}\) is the orientational parameter of the R₄ substituent, \(F_{17}(HOMO - 2)^*\) is the Fukui index of the third highest occupied MO localized on atom 17, \(F_{19}(LUMO + 2)^*\) is the Fukui index of the third vacant MO localized on atom 19, \(S_{N}^V(LUMO + 1)^*\) the nucleophilic superdelocalizability of the second vacant MO localized on atom 5, \(F_{18}(HOMO - 2)^*\) is the Fukui index of the third highest occupied MO localized on atom 18 and \(F_{14}(HOMO - 1)^*\) is the Fukui index of the second highest occupied MO localized on atom 14 (see Fig. 2). Table 2 shows the beta coefficients and the results of the t-test for significance of coefficients of Eq. 1. Table 3 displays the squared correlation coefficients for the variables appearing in Eq. 1. With the exception of two reactivity indices belonging atoms 5 and 19 (Fig. 2) showing a 32% correlation, there are no important internal correlations. Note that these two atoms are separated by a long distance. Fig. 3 displays the plot of observed vs. calculated log (Kᵢ) values. The associated statistical parameters of Eq. 1 indicate that this equation is statistically significant and that the variation of the numerical value of a set of seven local atomic reactivity indices of atoms belonging to the common skeleton explains about 89% of the variation of the HPPD inhibition constant.
Table 2. Beta coefficients and t-test for significance of the coefficients in Eq. 1

<table>
<thead>
<tr>
<th>Beta ID VAR</th>
<th>Beta (t(17))</th>
<th>p-level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Var5</td>
<td>-0.38</td>
<td>F_1(HOMO - 2)*</td>
</tr>
<tr>
<td>Var564</td>
<td>0.53</td>
<td>F_R4</td>
</tr>
<tr>
<td>Var325</td>
<td>0.41</td>
<td>F_R4</td>
</tr>
<tr>
<td>Var370</td>
<td>0.58</td>
<td>F_19(LUMO + 2)*</td>
</tr>
<tr>
<td>Var95</td>
<td>-0.46</td>
<td>S_5(LUMO + 1)*</td>
</tr>
<tr>
<td>Var345</td>
<td>0.27</td>
<td>F_18(HOMO - 2)*</td>
</tr>
<tr>
<td>Var266</td>
<td>-0.19</td>
<td>F_14(HOMO - 1)*</td>
</tr>
</tbody>
</table>

Table 3. Squared correlation coefficients for the variables appearing in Eq. 1

<table>
<thead>
<tr>
<th></th>
<th>F_1(HOMO - 2)*</th>
<th>F_R4</th>
<th>F_19(HOMO - 2)*</th>
<th>F_19(LUMO + 2)*</th>
<th>S_5(LUMO + 1)*</th>
<th>F_14(HOMO - 2)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_R4</td>
<td>0.01</td>
<td>1.00</td>
<td>0.06</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
</tr>
<tr>
<td>F_19(HOMO - 2)*</td>
<td>0.12</td>
<td>0.005</td>
<td>0.03</td>
<td>1.00</td>
<td>0.0004</td>
<td>0.004</td>
</tr>
<tr>
<td>F_19(LUMO + 2)*</td>
<td>0.002</td>
<td>0.004</td>
<td>0.10</td>
<td>0.32</td>
<td>1.00</td>
<td>0.002</td>
</tr>
<tr>
<td>S_5(LUMO + 1)*</td>
<td>0.0004</td>
<td>0.05</td>
<td>0.06</td>
<td>0.07</td>
<td>0.004</td>
<td>1.00</td>
</tr>
<tr>
<td>F_18(HOMO - 2)*</td>
<td>0.03</td>
<td>0.002</td>
<td>0.05</td>
<td>0.01</td>
<td>0.02</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Figure 3. Observed versus calculated values (Eq. 1) of log (K_i). Dashed lines denote the 95% confidence interval.

**Local Molecular Orbitals**

Tables 4 and 5 display the local molecular orbital structure of atoms appearing in Eq. 1 (Reading: molecule’s number (HOMO)/(HOMO-2)*, (HOMO-1)*, (HOMO)*, (LUMO)*, (LUMO+1)*, (LUMO+2)*).
DISCUSSION

Figure 4 shows the fully optimized structure of molecule 2. The most important fact to notice is that rings A, B-C and D are not coplanar. Therefore, the direct effect of the substituents of ring D on the electronic structure cannot propagate to the rest of the system.
Nevertheless, there is a second effect of the substituent that can alter the electronic structure. This effect is related to the appearance of new molecular orbitals that can alter the energy and/or location of the other MOs (a basis set effect). Table 2 shows that the importance of variables in Eq. 1 is $F_{19} (LUMO + 2) \ast > S_2^N (LUMO + 1) \ast > F_{17} (HOMO - 2) \ast > F_{14} (HOMO - 2) \ast > F_{18} (HOMO - 2) \ast > F_{14} (HOMO - 1) \ast$.

A high inhibitory activity is associated with a small orientational parameter value for $R_4$, a high values for $F_{14} (HOMO - 1) \ast$ and $F_{17} (HOMO - 2) \ast$, low values for $F_{17} (HOMO - 2) \ast$, $F_{19} (LUMO + 2) \ast$ and $F_{18} (HOMO - 2) \ast$. The case of $S_5^N (LUMO + 1) \ast$ will be discussed below. Atom 1 is a nitrogen in ring A (Fig. 2). The three highest occupied and the three lowest vacant local MOs have a $\pi$ nature (Table 4). A high value for $F_{17} (HOMO - 2) \ast$ suggests that atom 1 is interacting with an electron-deficient center through its three highest occupied MOs.

Atom 5 is the carbon of the methyl group attached to N1 in ring A (Fig. 2). All MOs have $\sigma$ nature (Table 4). If the numerical values of $S_5^N (LUMO + 1) \ast$ are positive, then a high inhibitory activity is associated with high values for this index. To obtain high values, the corresponding MO energy must be shifted downwards, making this MO more reactive. This suggests that atom 5 is interacting with a rich electron center through at least its first two lowest vacant MOs. It is probably that the center has occupied $\sigma$ MOs. Atom 17 is a carbon in ring C (Fig. 2). A high inhibitory activity is related to low numerical values of $F_{17} (HOMO - 2) \ast$. Table 5 shows that almost all (HOMO-2)$_{17}$ have a $\sigma$ nature, while all (HOMO-1)$_{17}$ and (HOMO)$_{17}$ have a $\pi$ nature. This suggests that this $\sigma$ MO can difficult the interaction of (HOMO-1)$_{17}$ and (HOMO)$_{17}$ with an electron-deficient center. Atom 19 is a carbon of the methyl group attached to C17 (Fig. 2). All MOs have a $\sigma$ nature (Table 5). A high inhibitory activity is associated with low numerical values for $F_{19} (LUMO + 2) \ast$. All the above suggestion are summarized in Fig. 5.
In summary, we obtained a statistically significant relationship between the variation of the HPPD inhibition constant and the variation of the numerical values of six local atoms reactivity indices and of the orientational parameter of one of the substituents in a series of pyrazalone-quinazolone hybrids. The associated pharmacophore should provide information to develop new molecules with enhanced inhibitory activity.

REFERENCES

[38] A Robles-Navarro, JS Gómez-Jeria, Der Pharma Chem., 2016, in press,