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3D-QSAR Studies on 4-Hydroxyphenylpyruvate Dioxygenase Inhibitors by Comparative Molecular Field Analysis (CoMFA)

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Abstract—A comparative molecular field analysis (CoMFA) of alkanoic acid 3-oxo-cyclohex-1-enyl ester and 2-acylcyclohexane-1,3-dione derivatives of 4-hydroxyphenylpyruvate dioxygenase inhibitors has been performed to determine the factors required for the activity of these compounds. The substrate's conformation abstracted from dynamic modeling of the enzyme–substrate complex was used to build the initial structures of the inhibitors. Satisfactory results were obtained after an all-space searching procedure, performing a leave-one out (LOO) cross-validation study with cross-validation q^2 and conventional r^2 values of 0.779 and 0.989, respectively. The results provide the tools for predicting the affinity of related compounds, and for guiding the design and synthesis of new HPPD ligands with predetermined affinities.

Introduction

4-Hydroxyphenylpyruvate dioxygenase (HPPD)[1,2] is a non-heme Fe(II)-dependent enzyme involved in the metabolism of tyrosine in most organisms and in the biosynthesis of tocopherols in plants. It catalyzes the conversion of 4-hydroxphenylpyruvate (HPP) and molecular oxygen to homogentisate through the oxidative decarboxylation and subsequent hydroxylation of the aromatic ring as shown in Scheme 1.

The inhibition study of HPPD has recently become the focus of considerable research interest because potent HPPD inhibitors can provide an alternative treatment for the life-threatening tyrosinaemia type I disease[3] and also has the potential to serve as a new class of bleaching herbicide for weed control. Previous studies have demonstrated that 2-benzoylcyclohexane-1,3-dione derivatives, referred to as triketones,[4,5] are potent HPPD inhibitors with IC_{50} values as low as 40 nM. Recently, we have also described a new family of compounds, alkanoic acid 3-oxo-cyclohex-1-enyl esters,[6] which appear to be non-triketone type HPPD inhibitors. In the present study, we have performed the quantitative activity relationship studies (QSAR) of two classes of HPPD inhibitors, that is, alkanoic acid 3-oxo-cyclohex-1-enyl esters (class 1) and cyclohexane-1,3-diones (class 2), by the comparative molecular field analysis method (CoMFA).[7] CoMFA is one of the well known 3D-QSAR descriptors which has been used regularly to produce the three dimensional models to indicate the regions that affect biological activity with a change in the chemical substitution. It operates on a set of compounds superimposed to reflect their anticipated common bonding orientation. CoMFA models can describe the relative change in magnitude of the steric and electrostatic fields as a function of the sampled compound chosen from the data set, and can account for the variance in measured biological activity. Thus, the resulting CoMFA study will not only illustrate the conformation or spatial orientation of HPPD inhibitors but also provide indicators useful for further design of new drug candidates for tyrosinaemia type I disease.

Data sets

A data set of 32 compounds has been taken from the published results.[8,9] The structures of two classes of HPPD inhibitors used in the training set for the CoMFA study are given in Figure 1 and their biological activities are listed in Table 1.

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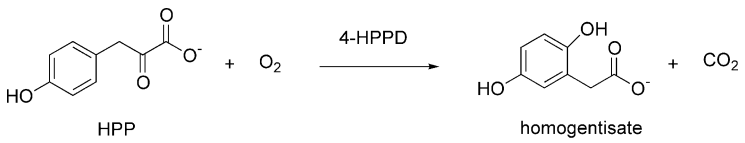

Scheme 1. Reaction catalyzed by HPPD.

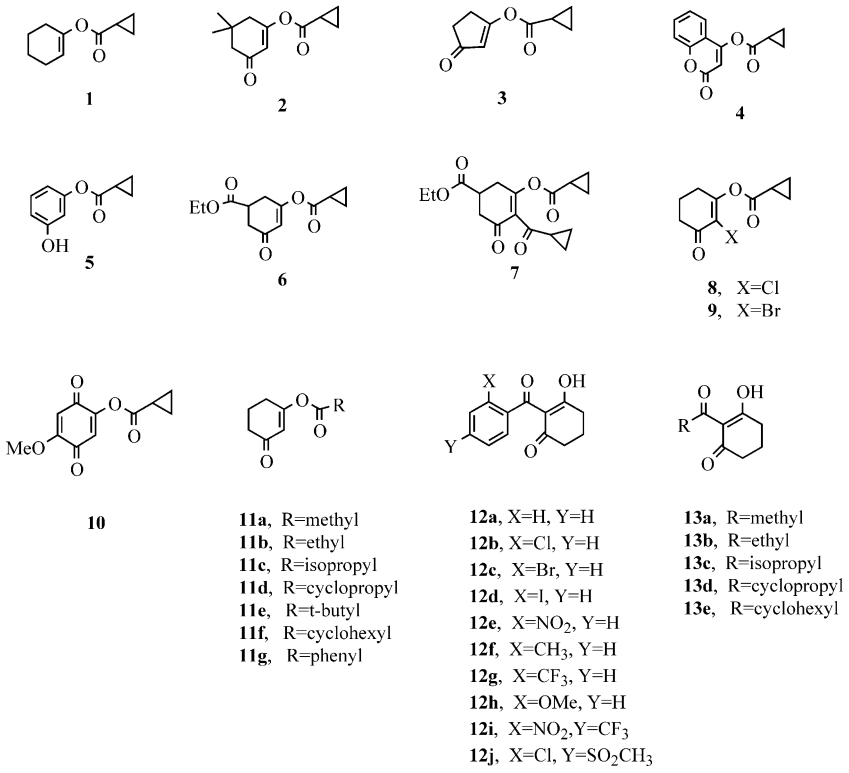

Figure 1. Structures of the CoMFA training set.

Table 1. Experimental and calculated pIC_{50}[a] values for HPPD inhibitors in the data set

No.	Class 1				No.	Class 2			
	pIC_{50} (μm)	pIC_{50} (obsd)	pIC_{50} (calcd)	Residue		pIC_{50} (μm)	pIC_{50} (obsd)	pIC_{50} (calcd)	Residue
1	0.70	6.15	6.15	0.01	**12a**	11.20	4.95	4.91	0.04
2	0.33	6.48	6.56	−0.08	**12b**	0.50	6.30	6.18	0.13
3	0.07	7.15	7.01	0.14	**12c**	0.56	6.25	6.26	−0.01
4	0.11	6.96	7.20	−0.24	**12d**	0.76	6.12	5.99	0.13
5	0.25	6.60	6.73	−0.12	**12e**	0.16	6.80	6.96	−0.17
6	0.09	7.05	7.14	−0.09	**12f**	3.75	5.43	5.57	−0.14
7	0.04	7.40	7.51	−0.11	**12g**	0.25	6.60	6.64	−0.04
8	0.02	7.70	7.57	0.12	**12h**	11.70	4.93	4.88	0.05
9	0.03	7.52	7.42	0.11	**12i**	0.04	7.40	7.33	0.07
10	30.00	4.52	4.37	0.15	**12j**	0.05	7.35	7.42	−0.07
11a	3.62	5.44	5.41	0.03	**13a**	11.20	4.95	5.04	−0.09
11b	0.11	6.96	6.88	0.08	**13b**	17.80	4.75	4.76	−0.01
11c	4.16	5.38	5.50	−0.12	**13c**	93.30	4.03	3.99	0.04
11d	0.03	7.52	7.19	0.33	**13d**	6.00	5.22	5.24	−0.02
11e	79.60	4.10	4.28	−0.18	**13e**	364.50	3.44	3.37	0.07
11f	3.70	5.43	5.55	−0.11					
11g	1.58	5.80	5.68	0.12					

[a]The potency was defined as log (1/C) (pIC_{50}), while C is the effective inhibitory concentration of compound required to achieve 50% (IC_{50}) inhibition against HPPD.

CoMFA study

The CoMFA studies described here were performed on a Silicon Graphics workstation using the SYBYL molecular modeling software from Tripos Inc., St. Louis, MO, USA. The compounds were built from fragments in the Sybyl database. The molecular geometry of each compound was then minimized using a standard Tripos molecular mechanics force field with a 0.005 kcal/mol energy gradient convergence criterion. Charges were calculated by the Gasteiger–Hückel method at the beginning. All of the 32 compounds were superimposed onto a template using an atom-by-atom least-square fit as implemented in the SYBYL FIT option, and the most active compound **8** was used as the reference molecule. The aligned molecules were put into a 3D grid with a spacing of 1.5 Å, using the five selected atoms on both classes of the compounds designated in Figure 2 as the fitting centers. The steric and electrostatic fields were then calculated using a sp^3 C-atom with +1 charge, and the default cutoff energy was 30 kcal/mol. Regression analysis of the resulting field matrix was performed by Partial Least Squares (PLS) for the compounds.

Alignment

Two alignment rules were used for the superimposition of all compounds. In alignment 1, the conformational space of the most active compound **8** was sampled using the SYBYL/SYSTEMATIC SEARCH procedure. All of the rotatable bonds were driven from 0 to 360° in 30° increments. The lowest-energy conformation was subjected to full minimization and was chosen as a starting point for modeling the remaining compounds in the training set. The aggregate 1 was set up using compound **8** as the alignment template. In alignment 2, dynamic molecular modeling of the enzyme–substrate complex was conducted as described in the literature.[10] The substrate's conformation was abstracted from the complex and was used for modeling the compounds in the data set. Minimization was then performed using the molecular mechanics method with key torsion constrained. Again, compound **8** was chosen as the alignment template and aggregate **2** was obtained. A stereoview of the superimposed complexes based on both alignments is shown in Figure 3.

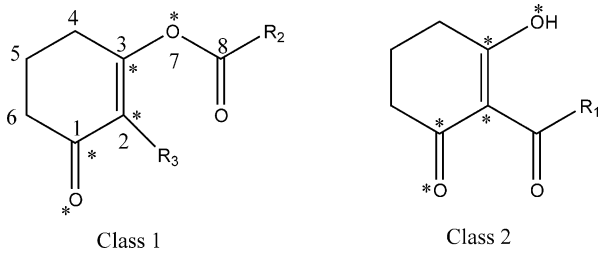

Figure 2. General structures and numbering system of two types of HPPD inhibitors. Stars indicate the atoms selected as the fitting centers.

Since 3D-QSAR models are highly sensitive to the different space orientations of the molecular aggregate with respect to the lattice, all-space searching (ASS) has also been conducted in order to obtain the best model with the most suitable molecular orientation. In the present work, the translation and rotation procedures were performed using SYBYL Programming Language (SPL) script written by Hou.[11] For each orientation of the molecular aggregate, the CoMFA analyses were carried out to generate the q^2 value of the model.

Results and Discussion

The CoMFA models were developed using both aggregates 1 and 2 to determine the factors required for the HPPD inhibition of these compounds. The HPPD enzyme inhibition activity (pIC_{50}) as related to the independent variables (steric and electrostatic fields) by the PLS methodology (see Table 2). Analyzing the conformations of compound **8** in both aggregates 1 and 2, we found that the torsional angles caused by the circumrotating of the C–O bond (τC2–C3–O7–C8, shown in Fig. 2) are 165.2 and −50.5°, respectively. Although the former has lower energy (105.114 kcal/mol) than the latter (110.332 kcal/mol), the better 3D-QSAR derived from aggregate 2 indicated that it is the reasonable one to model the initial structure of the inhibitors from the dynamic-modeled complex. This result suggested that the pharmaceutical conformation adopted by the inhibitors when interacting with the enzyme is a local minimal conformation instead of the lowest-energy one. We have also derived CoMFA models with different charge calculation using MOPAC/AM1, MOPAC/PM3 and

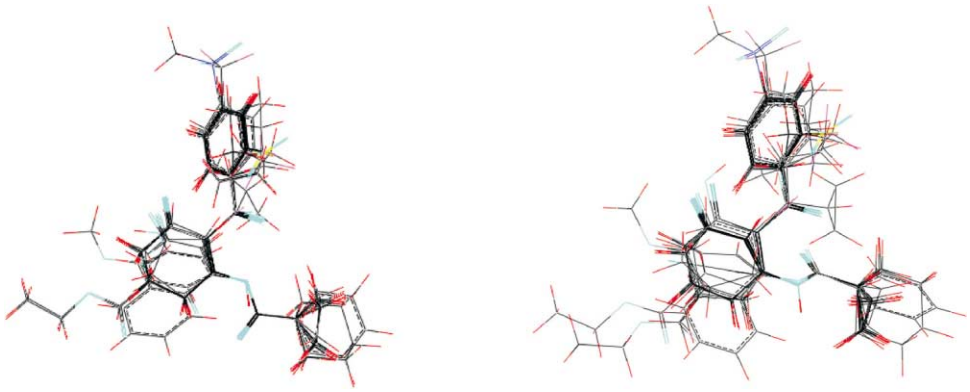

Figure 3. Stereoview of the superimposed complexes based on alignment 1 (aggregate 1) and alignment 2 (aggregate 2).

Table 2. Results of the CoMFA analyses of the training set

CoMFA model	Charge	Leave one out q^2	Conventional r^2	Standard error of estimate	F test values	No. of components
Model 1	Gasteiger–Hückel	0.524	0.971	0.220	141.595	6
Model 2	Gasteiger–Hückel	0.618	0.974	0.210	155.741	6
	AM1	0.628	0.978	0.192	226.004	5
	PM3	0.599	0.971	0.218	173.308	5
	MNDO	0.628	0.969	0.226	160.788	5
		ASS best orientation				
Rotational operation	AM1	0.719	0.941	0.310	83.062	5
Translational operation	AM1	0.779	0.989	0.138	369.223	6

MOPAC/MNDO. Among the three calculations, the CoMFA model with AM1 calculated charge had the best cross-validation value of 0.628 and conventional r^2 0.978 with five components. The modeling results were obviously affected by the method chosen to calculate the charge.

The molecular aggregate 2 was rotated around the x, y and z axes in 20° increments. A total of 162 orientations were explored. For each orientation, CoMFA fields were calculated and PLS analyses were performed. The best model after rotations was then selected and served as the initial orientation of the aggregate for the translations. Translations were performed along the x, y and z axes from 0 to 2.0 Å in steps of 0.5 Å. A total of 64 orientations were explored for the translations. Similarly, CoMFA fields were calculated and PLS was performed for each orientation. The calculated results of CoMFA and the best orientations after rotational and translational operations are given in Table 2. The leave-one-out (LOO) cross-validated PLS analysis of the best model from the ASS procedure had a q^2 value of 0.779 with standard error of 0.138. The high LOO cross-validated correlation coefficient reveals that the model is a useful tool for predicting HPPD affinities. The correlation coefficient between the calculated and experimental activities reached 0.994 with standard error 0.124. The conventional r^2 was 0.989 which means that the analyzed results have a 98.9% fitness compared to the biological in vitro test results. The respective relative contributions of steric and electrostatic fields were 51.4 and 48.6%, indicating that both fields contribute nearly equally. While the actual and calculated activities of all compounds by the best model are listed in Table 1, a plot of calculated biological activity versus experimental HPPD inhibition activity is illustrated in Figure 4.

Graphical representations of CoMFA results for HPPD inhibitors are shown in Figures 5 and 6, using compound **8** (cyan) and **12i** (white) as reference structures. The steric contour map shows a yellow region near the C-5 position of the six-membered ring, indicating the less bulky substituent is preferred in the position to produce higher inhibition activity. This fact is consistent with our previous results in which a decrease in activity was found for the C-5 dimethyl substituted compound.[9] The green region near the 2-position of the aryl ring of 2-benzoylcyclohexane-1,3-dione indicated that a bulky substituent is beneficial to the inhibition activity. Indeed, the bulky substituents like nitro and trifluoromethyl groups all exhibit potent inhibition activity as reported.[8] There is another green region near the C-2 position of the six-membered ring implies that a relatively bulky substituent is preferred for higher affinity. The observation of both yellow and green regions around the cyclopropane ring in the steric contour map suggests that the substitution effect of the alkyl group is complex. The most suitable substituent for this position should be the one that can specially match with the

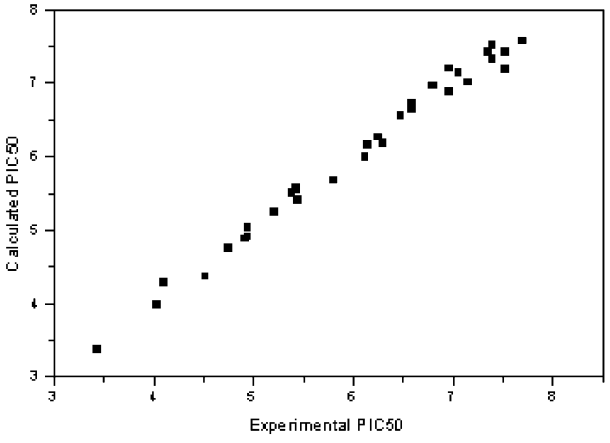

Figure 4. Calculated versus experimental pIC_{50} values for the training set derived from the CoMFA models.

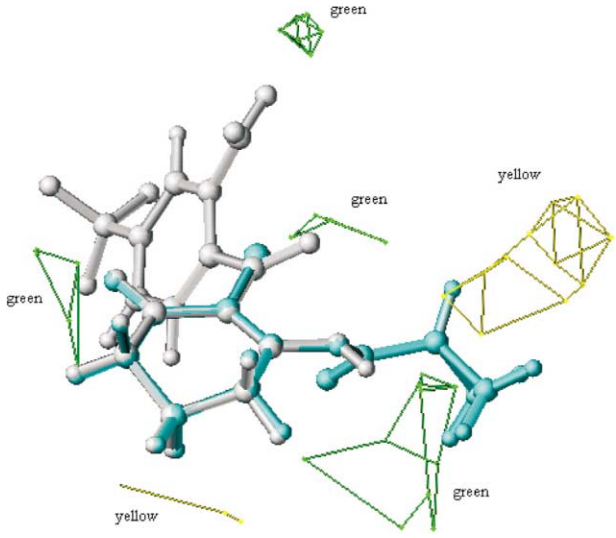

Figure 5. The CoMFA contour map for HPPD inhibitors: steric map in which green represents regions where steric bulk is predicted to increase activity, and yellow represents regions where an increase of steric bulk is predicted to decrease activity.

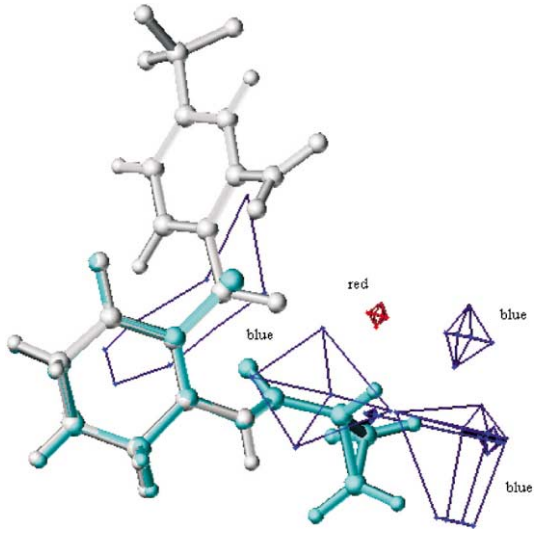

Figure 6. The CoMFA contour map for HPPD inhibitors: Electrostatic map indicating red contours represent regions where high electron density (negative charge) is expected to increase activity, and blue contours represent regions where low electron density (partial positive charge) is expected to increase activity.

receptor counterparts. The fact that all 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one analogues tested have higher inhibition activity against HPPD is presumably due to the particular steric effect of the cyclopropane ring, although further studies are needed to elucidate this point. In addition, the blue region near the right part of the common six-membered ring as well as the phenyl ring plane in class 2 in the electrostatic contour map suggested that the substituting which can increase the positive charge on the ring systems would result in a higher activity. Indeed, it was found that for 3-cyclopropanecarbonyloxy-2-cyclohexen-1-one inhibitors, an electron-withdrawing group like a chlorine or bromine atom at the 2-position of the ring system increases the inhibition activity. Finally, for 2-benzoylcyclohexane-1,3-dione derivatives, the most active analogues are those with the most electron-deficient aromatic rings. These results are in good agreement with previous SAR studies.[12]

Conclusions

In the current work, we successfully aligned two different structural classes of HPPD enzyme inhibitors for the CoMFA study by dynamic modeling of the complex of substrate and enzyme HPPD. A satisfactory model was obtained after the ASS procedure with LOO cross-validation q^2 and conventional r^2 values of 0.779 and 0.989, respectively. The effects of the electrostatic and steric fields around the aligned molecules on their activities are clarified by analyzing the CoMFA contour maps. The information obtained in this study provides the tools for predicting the affinity of related compounds, and for guiding further structural modifying and synthesizing new potent HPPD inhibitors.

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