CoMFA/CoMSIA/HQSAR and Docking Study of the Binding Mode of Selective Cyclooxygenase (COX-2) Inhibitors

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Full Paper

The intermolecular interaction between four types of anti-inflammatory inhibitors (oxazoles, pyrazoles, pyrroles and imidazoles) and COX-2 receptor was studied. The results of docking suggest that they have similar interaction mechanism. The most active compounds of these four types of inhibitors could both form several hydrogen bonds with residues His90, Arg513, Leu352 and Arg120, and develop hydrophobic interaction with residues Phe518, Leu352 and Leu359. This is consistent with the investigation reported by R. G. Kurumbail et al. (Nature. 1996, 384, 644-648). A common 3D-QSAR model could be constructed with these four categories of COX-2 inhibitors using the method of docking- guided conformer selection. The cross-validated q² values are found as 0.741 and 0.632 for CoMFA and CoMSIA respectively. And the non-cross-validated r² values are 0.887 and 0.885. 54 inhibitors constitute the test set used to validate the model. The results show that this model possesses good predictive ability for diverse COX-2 inhibitors. Furthermore, a HQSAR model was used to evaluate the influence of substituents on anti-inflammatory activity. Compared with the results of previous works, our model possesses significantly better prediction ability. It could help us to well understand the interaction mechanism between inhibitors and COX-2 receptor, and to make quantitative prediction of their inhibitory activities.

1 Introduction

In human body inflammation appears when immune system is attacked by virus (for example coronavirus, influenza ...). It is necessary to investigate the anti-inflammatory mechanism for the goal of designing new drugs. COX-1 and COX-2 are two cyclooxygenase (COX) isoforms [1]. Their principal pharmacological effect is inhibiting prostaglandin synthesis. This discovery led to the hypothesis that side effects such as ulcers and renal failure associated with the clinically used nonsteroidal anti-inflammatory drugs (NSAIDs) are caused by the inhibition of COX-1. Whereas COX-2 is an inducible enzyme, it is mainly produced during inflammation processes [2]. Then the study of selective inhibition of COX-2 led to a new class of anti-inflammatory, analgesic and antipyretic drugs with significantly reduced side effects. Recent works suggest that inhibiting COX-2 could also be an important anti-cancer strategy [3] and could be used to delay or slow down the clinical expression of Alzheimer’s disease [4]. But extended use of NSAIDs, such as ibuprofen, may increase the risk of developing Alzheimer disease up to 80% [5]. Therefore we need to develop more specific and efficient COX-2 inhibitors with improved safety profile [6].

A large number of research studies aimed at finding selective COX-2 inhibitors have been reported [7–10]. Many studies have been carried out using computer simulations to develop protocols and methods for designing new COX-2 inhibitors such as oxazoles, pyrazoles, pyrroles and imidazoles [11–15]. But these investigations did not clearly explain how substituents in these inhibitors influence the anti-inflammatory activity. In this paper, we report the binding mode between four types of selective inhibitors mentioned above and the COX-2 receptor using automated molecular docking methodology. The study will show that a unique docking model can be built up on a population of 227 diarylhetereocyclic derivatives, suggesting a possible common inhibitory mechanism for the four types of above mentioned compounds. This allows us for attempting to construct 3D-QSAR models by using the conformers obtained from molecular docking.
investigation. These 3D-QSAR models can help us to better understand the interaction mechanism between inhibitors and COX-2 receptor, and make quantitative prediction of their inhibitory activities.

2 Computational Methods and Materials

2.1 Data sets

The structures and activities IC$_{50}$ (IC$_{50}$ is the concentration in µM for 50% inhibition of COX-2 enzyme) for four types of COX-2 inhibitors (oxazoles, pyrazoles, pyrroles and imidazoles), extracted from literature [16-20], are gathered in tables 1 to 7. The structures marked with ™*∫ symbol constitutes the test set, and the others the training set.

2.2 Molecular Docking

The complex of cyclooxygenase (COX-2) receptor and SC558 was extracted from Brookhaven Protein Databank (PDB code: 1CX2). The structure of ligand SC558 is shown in figure 1. The most active structures for each type (A9, B2, C13 and D59) were optimized using Gaussian98 (at HF/6-31G level) [21]. Other structures were built based on these geometries, and then optimized using Tripos force field [22] with SYBYL 6.9 [23]. All calculations were performed on a SGI Octane2 workstation.

Because the four types of COX-2 inhibitors investigated have a skeleton similar to that of SC558 (Figure 1), we only need to align their five-membered heterocycle with that of SC558. The active site was determined based on the co-crystallized SC558-protein complex. Firstly, the ligand SC558 was extracted from the cyclooxygenase complex, and then the remaining COX-2 receptor structure was completed by adding the missing polar hydrogen atoms and residues. This structure was optimized with constrained

![Figure 1](image)

**Figure 1.** Structure of SC558 extracted from COX-2 receptor complex.

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**Table 1.** IC$_{50}$ activities of oxazole derivatives (subset I)

| No. | X     | Substituent | Z     | Log(1/IC$_{50}$) |
|-----|-------|-------------|-------|------------------|
| A1  | H     | Me          | NH$_2$| 4.65             |
| A2  | 4-Cl  | Me          | NH$_2$| 8.22             |
| A3  | 4-Br  | Me          | NH$_2$| 8.00             |
| A4  | 3-F-4-OMe | Me   | NH$_2$| 7.27             |
| A5  | 4-Cl  | CH$_2$OMe   | NH$_2$| 7.40             |
| A6  | 4-Br  | CH$_2$OMe   | NH$_2$| 7.82             |
| A7  | H     | CH$_2$OH    | NH$_2$| 6.62             |
| A8  | H     | CF$_3$H     | NH$_2$| 7.70             |
| A9  | 4-Cl  | CF$_3$      | NH$_2$| 9.00             |
| A10 | 3,4-Cl$_2$ | CF$_3$ | NH$_2$| 8.70             |
| A11 | 3,4-F$_2$ | CF$_3$ | NH$_2$| 7.40             |
| A12 | 3-Cl-4-OMe | CF$_3$ | NH$_2$| 7.02             |
| A13 | 3-F-4-OMe | CF$_3$ | NH$_2$| 8.52             |
| A14 | 4-F   | Me          | Me    | 6.85             |
| A15 | H     | CH$_2$OH    | Me    | 5.71             |
| A16 | 4-F   | C$_6$H$_5$  | Me    | 7.40             |
| A17 | 4-F   | CH$_2$C$_6$H$_5$ | Me | 7.50             |
| A18 | H     | CH$_2$OCH$_2$C$_6$H$_5$ | Me | 7.01             |
| A19 | 3-F-4-OMe | CF$_3$     | Me    | 8.52             |
molecular dynamics. Partial atom charges of COX-2 receptor were calculated with Kollman-all-atom approximation [24]. Gasteiger-Hückel charges were calculated for all inhibitor molecules, as recommended in the AutoDock 3.0 package which was used for performing automated docking of inhibitors to COX-2 receptor. The development and the principle of this software have been described elsewhere [25–26]. During the docking process, a series of docking parameters were set. The number of generations, energy evaluations, and docking runs were set to 370000, 1500000, and 50, respectively.

### 2.3 Docking Guided Conformation Selection

CoMFA results may be extremely sensitive to a number of factors, such as conformers, alignment rules, overall orientation of aligned compounds, lattice shifting, step size and the probe atom type [27]. Because the investigated inhibitors are flexible and have a set of possible conformers, selecting probable conformations is important for CoMFA study.

Through browsing the docking results, we found that hydrogen atoms were not included in the docking result files except the polar ones. We could not select the conformers directly from docking results to construct CoMFA models. Thus the “docking-guided” selection method has been

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### Table 2. IC₅₀ activities of oxazole derivatives (subset II)

| No. | X     | Y     | Z     | Log(1/IC₅₀) |
|-----|-------|-------|-------|-------------|
| A20 | 4-F   | Me    | Me    | 6.85        |
| A21 | H     | CH₂OH | Me    | 6.70        |
| A22 | 4-F   | CMe₃  | Me    | 7.40        |
| A23 | 4-F   | CH₂CH₃| Me    | 7.50        |
| A24 | 4-F   | CH₂CH₂CH₃| Me | 7.07        |
| A25 | H     | CH₂OCH₂CH₂CH₃| Me | 7.07        |
| A26 | 4-Me  | CF₃   | Me    | 8.70        |
| A27 | 3-F-4-OMe | CF₃| Me    | 8.52        |
| A28 | H     | Me    | NH₂   | 7.57        |
| A29 | 4-F   | Me    | NH₂   | 8.05        |
| A30 | 4-Cl  | Me    | NH₂   | 8.52        |
| A31 | 4-Br  | Me    | NH₂   | 8.52        |
| A32 | 3-F   | Me    | NH₂   | 7.21        |
| A33 | H     | H     | NH₂   | 4.10        |
| A34 | H     | CF₃   | NH₂   | 7.92        |
| A35 | H     | CF₂-H | NH₂   | 8.30        |
| A36 | H     | Cl    | NH₂   | 8.40        |
| A37 | H     | CH₂OH | NH₂   | 7.46        |
| A38 | H     | CH₂OMe| NH₂   | 7.77        |
| A39 | H     | CH₂COOH| NH₂| 6.54        |
| A40 | H     | SH    | NH₂   | 6.34        |
| A41 | H     | SMe   | NH₂   | 8.40        |
| A42 | H     | SOMe  | NH₂   | 6.00        |
| A43 | H     | SO₂Me | NH₂   | 6.43        |
| A44 | H     | OH    | NH₂   | 6.31        |
| A45 | H     | OMe   | NH₂   | 7.77        |
| A46 | H     | NH₂   | NH₂   | 4.68        |
| A47 | H     | NMe₂  | NH₂   | 7.20        |
| A48 | H     | CH=CH₂| NH₂   | 8.40        |
| A49 | H     | CCH   | NH₂   | 8.22        |
| A50 | H     | CCMc  | NH₂   | 7.70        |
| A51 | H     | CONH₂ | NH₂   | 5.65        |
applied. For each of the four most active compounds (A9, B2, C13 and D59) the conformer with the lowest binding free energy was extracted from the corresponding docking result file and used as a template for alignment. In another hand, for the other studied compounds, 50 conformers were computed by using the Multisearch method implemented in CoMFA/CoMSIA/HQSAR and Docking Study of the Binding Mode of Selective Cyclooxygenase (COX-2) ...
SYBYL 6.9. Then these conformers were aligned with the obtained template. The measure of the fit of two conformers is the root mean square distance between pairs of atoms (RMSD):

\[
\text{RMSD} = \sqrt{\frac{\sum_{i=1}^{S_{\text{atom}}} d_i^2}{S_{\text{atom}}}}
\]

Where \(S_{\text{atom}}\) is the total number of atoms over which the RMSD is measured and \(d_i\) the distance between the coordinates of atom \(i\) in the two conformers which are overlaid. The conformation with minimum RMSD value is selected in CoMFA analysis.

2.4 CoMFA Models

After consistently aligning the molecules within a lattice of 2 Å mesh which extend 4 Å units beyond the aligned molecules in all directions; a sp\(^3\) carbon atom with +1 net charge was employed as probe. The steric and electrostatic interactions between the probe and the atoms of the molecule were calculated. Electrostatic interactions are modeled using a Coulomb potential and Van der Waals interactions by Lennard-Jones potential. The regression analysis was carried out using the partial least-squares (PLS) [28] method. The final model was developed with the optimum number of components (that yielding the highest \(q^2\)). The total set of inhibitors was divided into two groups in an approximate ratio 3:1 (for example, 173 in the training set and 54 in the test set). The selection of test set and training set compounds was done manually such that low, moderate and high activity compounds are present in roughly equal proportions in both sets.

2.5 CoMSIA Models

CoMSIA similarity indices [29–30] were derived within the same lattice box of the CoMFA calculations. Five physicochemical properties related to steric, electrostatic, hydrophobic, hydrogen bond donor, and hydrogen bond acceptor fields were evaluated using the probe atom. A Gaussian-type distance dependence was employed to describe the attenuation of the fields with distance, using the default value (0.3) as attenuation factor. This leads to a much smoother sampling of the fields around the molecules compared with CoMFA.

2.6 Optimization of \(q^2\) by All-Orientation and All-Placement Searches

Cross-validated \(q^2\) usually serves as a quantitative measurement of the predictive power of CoMFA and CoMSIA. However, Cho et al. reported that \(q^2\) value was sensitive to the orientation of aligned molecules on the computer terminal and might vary with the orientation by as much as 0.5 \(q^2\) units [27]. So all molecules under investigation were geometrically aligned, then the molecular aggregate was rotated systemati-
every 30° for X, Y, Z axis and translated every 0.2 Å. At every place, the cross-validated q^2 of PLS was evaluated, until the maximum q^2 value was found [31].

2.7 HQSAR Models

Molecular fingerprinting (hologram) is a method that represents a compound as a unique string of numbers or “bins” [32]. The bins represent all of the unique fragments included within a particular molecule and are assigned by a cyclic redundancy check (CRC) algorithm [33]. A number of holograms with differing bin lengths are created for each molecule. The hologram length that leads to the best PLS analysis is used to construct HQSAR model. A set of factors such as fragment size, number of fragments, atom types, bond types, atom hybridization, hydrogen bond donor and acceptor, were modified to search the best cross-validation r^2 of the model.

Table 6. IC50 activities of imidazole derivatives (subset I)

| No. | X     | Y   | Z   | Log(1/IC50) |
|-----|-------|-----|-----|-------------|
| D1  | 4-Cl  | Me  | CF3 | 6.96        |
| D2  | 4-F   | Me  | CF3 | 7.00        |
| D3  | H     | Me  | CF3 | 6.92        |
| D4  | 4-Me  | Me  | CF3 | 6.80        |
| D5  | 4-OMe | Me  | CF3 | 6.24        |
| D6  | 4-NHMe| Me  | CF3 | 5.83        |
| D7  | 4-NMe2| Me  | CF3 | 6.16        |
| D8  | 4-SMe | Me  | CF3 | 6.80        |
| D9  | 4-SO2Me| Me | CF3 | 5.24        |
| D10 | 4-CI  | NH2 | CF3 | 8.00        |
| D11 | 4-F   | NH2 | CF3 | 8.00        |
| D12 | H     | NH2 | CF3 | 7.40        |
| D13 | 4-Me  | NH2 | CF3 | 7.40        |
| D14 | 3-CI  | Me  | CF3 | 7.22        |
| D15 | 3-F   | Me  | CF3 | 6.92        |
| D16 | 3-Br  | Me  | CF3 | 7.10        |
| D17 | 3-Me  | Me  | CF3 | 7.22        |
| D18 | 3-CF3 | Me  | CF3 | 6.68        |
| D19 | 3-OMe | Me  | CF3 | 6.46        |
| D20 | 3-SMe | Me  | CF3 | 6.46        |
| D21 | 3-CH2OMe| Me | CF3 | 4.17        |
| D22 | 3-NMe2| Me  | CF3 | 5.50        |
| D23 | 3-NHMe| Me  | CF3 | 6.04        |
| D24 | 3-NH2 | Me  | CF3 | 5.23        |
| D25 | 3-NO2 | Me  | CF3 | 6.24        |
| D26 | 3-Cl  | NH2 | CF3 | 8.10        |
| D27 | 3-F   | NH2 | CF3 | 7.52        |
| D28 | 3-Br  | NH2 | CF3 | 8.16        |
| D29 | 3-Me  | NH2 | CF3 | 7.52        |
| D30 | 3-CI  | Me  | CF3 | 6.05        |
| D31 | 2-F   | Me  | CF3 | 6.40        |
| D32 | 2-Me  | Me  | CF3 | 6.10        |
| D33 | 2-OMe | Me  | CF3 | 4.00        |
| D34 | 2-CF3 | NH2 | CF3 | 7.00        |
| D35 | 2-Me  | NH2 | CF3 | 6.70        |
| D36 | 3-F-4-OMe| Me | CF3 | 6.82        |
| D37 | 3-CI-4-OMe | Me | CF3 | 6.89        |
| D38 | 3-CI-4-SMe| Me | CF3 | 7.40        |
| D39 | 3-CI-4-NMe2| Me | CF3 | 6.50        |

Table 6. (cont.)

| No. | X     | Y   | Z   | Log(1/IC50) |
|-----|-------|-----|-----|-------------|
| D40 | 3-F-4-NMe2| Me | CF3 | 6.48        |
| D41 | 3-Cl-4-NHMe| Me | CF3 | 6.18        |
| D42 | 3-Cl-4-Me  | Me  | CF3 | 7.52        |
| D43 | 3-F-4-Me   | Me  | CF3 | 6.96        |
| D44 | 3-Me-4-F   | Me  | CF3 | 6.77        |
| D45 | 3-Me-4-Cl  | Me  | CF3 | 7.05        |
| D46 | 3-OMe-4-Cl | Me  | CF3 | 6.60        |
| D47 | 3-NMe2-4-Cl| Me | CF3 | 5.98        |
| D48 | 3.4-OCH3O | Me  | CF3 | 6.77        |
| D49 | 3.4-F1    | Me  | CF3 | 6.92        |
| D50 | 3.4-Me2   | Me  | CF3 | 6.48        |
| D51 | 3-Me-5-Cl  | Me  | CF3 | 7.10        |
| D52 | 3-Cl-5-Me  | Me  | CF3 | 6.96        |
| D53 | 3-OMe-5-Cl | Me  | CF3 | 6.02        |
| D54 | 3.5-CI2   | Me  | CF3 | 6.77        |
| D55 | 3-Cl-4-OMe | NH2 | CF3 | 7.52        |
| D56 | 3-Cl-4-OMe | NH2 | CF3 | 7.70        |
| D57 | 3-Br-4-OMe | NH2 | CF3 | 7.52        |
| D58 | 3-Cl-4-SMe | NH2 | CF3 | 8.00        |
| D59 | 3.4-CI4-Me | NH2 | CF3 | 8.52        |
| D60 | 3-OMe-4-Cl | NH2 | CF3 | 7.70        |
| D61 | 3.4-F1    | NH2 | CF3 | 7.52        |
| D62 | 3-Cl-5-Me  | NH2 | CF3 | 7.40        |
| D63 | 3-OMe-5-F  | NH2 | CF3 | 7.52        |
| D64 | 3-OMe-5-F  | NH2 | CF3 | 6.34        |
| D65 | 3.5-F-4-OMe| Me  | CF3 | 6.77        |
| D66 | 3.5-CI-4-OMe| Me | CF3 | 6.85        |
| D67 | 3-Br-5-4-OMe| Me | CF3 | 7.05        |
| D68 | 3.5-Me-4-OMe| Me | CF3 | 6.14        |
| D69 | 2.5-Me-4-OMe| Me | CF3 | 4.91        |
| D70 | 3.5-CI-4-NMe2| Me | CF3 | 6.85        |
| D71 | 3.5-F1-4-OMe| NH2 | CF3 | 7.52        |
| D72 | 4-CI     | Me  | CF3 | 6.62        |
| D73 | 4-CI     | Me  | CHF2 | 6.22        |
| D74 | 4-CI     | Me  | CH2F | 6.39        |
| D75 | 4-CI     | Me  | CHO  | 5.80        |
| D76 | 4-CI     | Me  | CN   | 6.64        |
| D77 | 4-CI     | Me  | COOC6H5| 5.24        |
| D78 | 4-CI     | Me  | C6H5 | 6.62        |
| D79 | 4-CI     | Me  | CHOC6H5-4-Cl | 7.52 |
| D80 | 4-CI     | Me  | CH2SCH3 | 7.30 |
| D81 | 4-CI     | Me  | CH2SC6H4-4-Cl | 5.43 |
| D82 | 4-CI     | Me  | CH2OMe | 6.50        |
| D83 | 4-CI     | Me  | CH2OH  | 5.08        |
| D84 | 4-CI     | Me  | CH2CN  | 5.81        |
3 Results and Discussion

3.1 Action Mechanism of COX-2 Inhibitors

For each molecule, 50 conformers were selected to dock with COX-2 receptor. The most active molecules for four types of inhibitors (oxazoles, pyrazoles, pyrroles and imidazoles) were selected to study the binding mode with COX-2 receptor. The complexes formed by A9 of oxazoles, B2 of pyrazoles, C13 of pyrroles and D59 of imidazoles with COX-2 receptor were then investigated, as shown in Figure 2.

For all complexes, two oxygen atoms of sulphonamide are linked by hydrogen bond to residues His90 and Arg513. This is consistent with the investigation reported by R. G. Kurumbail et al. [6]. Beside these hydrogen bond interactions, there are also hydrophobic effects around sulphonamide group bonded to phenyl with the residues Phe518, Leu352, Leu359, Trp387 and Met522. This hydrophobic interaction seems to be favourable to the activity. The other residues such as Tyr355, Tyr385 and Ser530 are polar. The electrostatic interaction between these residues and phenyl ring could stabilise the formed complex. Except the complex of C13, there is one hydrogen bond between the nitrogen atom of sulphonamide and carbonyl oxygen of Leu352. This may explain that the sulphonamide containing compounds are more active than those containing sulphomethyl. In the complexes of A9 and B2, there is also electrostatic interaction between halogen atom and residue Arg120. It is perhaps why the anti-inflammatory activity of A9 and B2 is higher than that of D59. For B2-COX complex, there is a hydrogen bond between nitrogen atom of pyrazole ring and −OH of residue Tyr355 with 127.8° for N–H–O angle and 2.85 Å for N–H distance. These values (angle < 140° and distance > 2.0 Å) suggest that this hydrogen bond should be very weak and does not significantly contribute to stabilise the complex. By contrast, for A9 complex, electrostatic interaction between the chlorine atom located in phenyl para position and the residue Trp387 could increase the interaction between ligand and receptor. This leads to the higher activity for A9 compared with B2. The docking study is in agreement with experiments. It seems that the anti-inflammatory activity increases with the number of hydrogen bonds for these four compounds, as shown in the following sequences:

| Number of H-bonds: A9 (5) B2 (6) D59 (4) C13 (3) Activity: 9.00 8.77 8.52 7.70 |

The docking results suggest that the oxazoles, pyrazoles, pyrroles and imidazoles derivatives here investigated, might have similar interaction mechanism with COX-2. There are at least three hydrogen bonds, favourable hydrophobic interactions between sulphone bonded to phenyl and residues Phe518, Leu352, Leu359, Trp387 and Met522, and electrostatic interactions with residues Tyr355, Tyr385 and Ser530. Figure 3 illustrates the superposition results of A9, B2, C13, and D59 with ligand SC558. Their RMSD values with SC558 are 1.38, 1.32, 1.36 and 1.39 Å, respectively. Their benzenesulphonyl and five-membered heterocycle could be aligned together. We could then attempt to construct a common 3D-QSAR model for these four types of COX-2 inhibitors.

At the same time, we investigated these compounds using the method of hierarchical clustering. 34 descriptors were calculated with default methods implemented in SYBYL 6.9, including 27 Charged Partial Surface Area (CPSA) descriptors [34], 4 polarity, volume, surface and hydrophobicity descriptors. The aim of this analysis was to examine whether the 227 compounds can be gathered into a unique model or must be split into different subpopulations according to their structural features. As shown in Figure 4, cluster analysis indicates that the 227 compounds were classified into two categories. As an example, the black dots illustrate one branch of the cluster, including compounds B1, B32, B35, D18, D61, D97, A9, A10 and A11. This mixture of different types of compounds (A, B and D) suggests that these four categories of molecules cannot be really separated according to their structural features. Therefore all these compounds could be treated together in a common 3D-QSAR model.

| No. | X       | Y       | Z       | Log(1/IC<sub>50</sub>) |
|-----|---------|---------|---------|------------------------|
| D85 | 2-Me    | Me      | CF<sub>3</sub> | 5.02                   |
| D86 | 6-Me    | Me      | CF<sub>3</sub> | 5.75                   |
| D87*| 5-Me    | Me      | CF<sub>3</sub> | 5.75                   |
| D88 | 4-Me    | Me      | CF<sub>3</sub> | 4.27                   |
| D89 | 6-OMe   | Me      | CF<sub>3</sub> | 5.92                   |
| D90 | 5-OMe   | Me      | CF<sub>3</sub> | 4.43                   |
| D91 | 5-Br    | Me      | CF<sub>3</sub> | 6.02                   |
| D92*| H       | NH<sub>2</sub> | CF<sub>3</sub> | 6.36                   |
| D93 | 2-Me    | NH<sub>2</sub> | CF<sub>3</sub> | 5.55                   |
| D94 | 6-Me    | NH<sub>2</sub> | CF<sub>3</sub> | 6.54                   |
| D95 | 5-Me    | NH<sub>2</sub> | CF<sub>3</sub> | 6.29                   |
| D96*| 4-Me    | NH<sub>2</sub> | CF<sub>3</sub> | 4.29                   |
| D97 | 5-Br    | NH<sub>2</sub> | CF<sub>3</sub> | 6.47                   |
| D98 | H       | Me      | CF<sub>3</sub> | 5.77                   |
| D99 | H       | Me      | CHF<sub>2</sub> | 4.68                   |
| D100| H       | Me      | CN      | 4.61                   |
| D101| H       | Me      | Me      | 4.10                   |
| D102*| H     | Me      | CH<sub>2</sub>OH | 3.03                   |
According to the minimum RMSD of the training set, the most probable conformer of every compound was selected. The alignment diagram of the 173 compounds of the training set within the active site of COX-2 is shown in Figure 5. For each type of COX-2 inhibitors, the structures were divided into training and test sets. Then, the structures of these training sets were put together for building a new model. Based on this model, two other models were constructed by the method of all-orientation and all-placement searches and random orientation. For all-orientation and all-placement searches, training set was rotated systematically every 30° for X, Y, Z axis and translated every 0.2 Å, then cross-validated q^2 of PLS was evaluated. Because the number of training set of pyrrole is not enough to build a 3D-QSAR model, only six models were constructed. Model 1 is for oxazole, model 2 for pyrazole, model 3 for imidazole, model 4 corresponds to the mixture of all training sets described above. Model 5 results from all-placement searching based on model 4. Model 6 is constructed by random orientation for 173 compounds. The parameters of these models are given in table 8. The predicted anti-inflammatory activities (PA) are shown in table 9 and 10.

3.2 3D-QSAR Models

The interactions between ligands and the surrounding residues of COX-2 within 6 Å. The central skeleton is the ligand, the other ones are residues of COX-2. The dotted lines represent hydrogen bonds between ligands and the residues of COX-2.

Figure 2. The interactions between ligands and the surrounding residues of COX-2 within 6 Å. The central skeleton is the ligand, the other ones are residues of COX-2. The dotted lines represent hydrogen bonds between ligands and the residues of COX-2.
3.2.1 Evaluation of 3D-QSAR Model 1, 2 and 3

We examine now the three correlations between experimental and predicted anti-inflammatory activities for the three classes of inhibitors (oxazoles, pyrazoles and imidazoles).

For model 1 (oxazoles), the cross-validated $q^2$ values of CoMFA and CoMSIA of training set are 0.810 and 0.669, respectively, with six and three principal components. The conventional $r^2$ values are 0.975 and 0.880, with standard errors (SE) equal to 0.183 and 0.382 for an overall variation of 9.00 log units. The corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models for test set are 0.907 and 0.848, with standard errors (SE) 0.417 and 0.562, respectively. The correlations between experimental (EA) and predicted (PA) activities are shown in figure 6. We remark that model 1 is good enough. For model 2 (pyrazoles), the cross-validated $q^2$ values of CoMFA and CoMSIA of training set are 0.650 and 0.426, respectively, with six and three principal components.

We also report the conventional $r^2$ values, which are 0.965 and 0.846, respectively, with 0.259 and 0.529 standard errors (SE). The corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models for test set are 0.893 and 0.883, with standard errors (SE) 0.442 and 0.413, respectively. The correlations between experimental (EA) and predicted (PA) activities are shown in figure 6. For model 3 (imidazoles), the cross-validated $q^2$ values of CoMFA and CoMSIA of training set are 0.666 and 0.719, respectively, with six and three principal components.

We also report the conventional $r^2$ values, which are 0.937 and 0.885, respectively, with 0.308 and 0.265 standard errors (SE). The corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models for test set are 0.870 and 0.876, with standard errors (SE) 0.442 and 0.413, respectively. The correlations between experimental (EA) and predicted (PA) activities are shown in figure 6. We remark that model 1 is good enough. For model 2 (pyrazoles), the cross-validated $q^2$ values of CoMFA and CoMSIA models of training set are 0.650 and 0.426,
| No.  | Log(1/IC₅₀) | Model 1 | Model 2 | Model 3 | Model 4 | Previous Work[^15] |
|------|-------------|---------|---------|---------|---------|-------------------|
|      | CoMFA       | CoMSIA  | CoMFA   | CoMSIA  | CoMFA   | CoMSIA           |
| A1   | 4.65        | 4.57    | 5.06    | -       | -       | 4.72             |
| A2   | 8.22        | 8.13    | 7.96    | -       | -       | 8.15             |
| A4   | 7.27        | 7.09    | 7.71    | -       | -       | 7.40             |
| A5   | 7.40        | 7.56    | 7.59    | -       | -       | 7.48             |
| A6   | 7.82        | 7.61    | 7.67    | -       | -       | 7.45             |
| A7   | 6.62        | 6.84    | 7.14    | -       | -       | 7.25             |
| A9   | 9.00        | 8.79    | 8.76    | -       | -       | 8.45             |
| A10  | 8.70        | 8.73    | 8.90    | -       | -       | 8.45             |
| A11  | 7.40        | 7.33    | 7.66    | -       | -       | 7.58             |
| A13  | 8.52        | 8.66    | 8.69    | -       | -       | 8.75             |
| A14  | 6.85        | 6.99    | 7.41    | -       | -       | 7.27             |
| A16  | 7.40        | 7.28    | 7.68    | -       | -       | 7.53             |
| A17  | 7.50        | 7.41    | 7.71    | -       | -       | 7.56             |
| A18  | 7.01        | 6.98    | 7.12    | -       | -       | 8.02             |
| A19  | 8.52        | 8.42    | 7.70    | -       | -       | 8.31             |
| A20  | 6.85        | 7.30    | 7.17    | -       | -       | 7.03             |
| A22  | 6.70        | 6.55    | 7.54    | -       | -       | 6.86             |
| A23  | 7.40        | 7.49    | 7.45    | -       | -       | 7.13             |
| A24  | 7.50        | 7.64    | 7.37    | -       | -       | 7.27             |
| A26  | 8.70        | 8.73    | 7.94    | -       | -       | 7.98             |
| A27  | 8.52        | 8.46    | 8.26    | -       | -       | 8.62             |
| A29  | 8.05        | 7.73    | 7.93    | -       | -       | 7.71             |
| A30  | 8.52        | 8.33    | 8.02    | -       | -       | 8.02             |
| A31  | 8.52        | 8.37    | 8.10    | -       | -       | 7.98             |
| A32  | 7.21        | 7.38    | 7.00    | -       | -       | 7.85             |
| A34  | 7.92        | 8.15    | 7.92    | -       | -       | 8.58             |
| A35  | 8.40        | 8.41    | 8.41    | -       | -       | 8.51             |
| A36  | 7.46        | 7.44    | 7.21    | -       | -       | 7.52             |
| A38  | 6.54        | 6.17    | 6.59    | -       | -       | 6.24             |
| A39  | 6.34        | 6.37    | 6.66    | -       | -       | 6.51             |
| A41  | 6.00        | 6.02    | 6.03    | -       | -       | 6.27             |
| A42  | 6.43        | 6.53    | 6.61    | -       | -       | 6.17             |
| A43  | 6.31        | 6.25    | 6.51    | -       | -       | 6.65             |
| A45  | 4.68        | 4.81    | 4.10    | -       | -       | 4.64             |
| A46  | 7.20        | 7.34    | 7.39    | -       | -       | 6.70             |
| A48  | 8.22        | 8.29    | 8.17    | -       | -       | 7.83             |
| A49  | 7.70        | 7.94    | 7.29    | -       | -       | 7.71             |
| A50  | 5.65        | 5.62    | 5.27    | -       | -       | 5.56             |
| B2   | 8.77        | -       | -       | 8.50    | 8.33    | 8.36             |
| B3   | 7.66        | -       | -       | 7.74    | 7.53    | 7.72             |
| B5   | 7.10        | -       | -       | 7.33    | 7.63    | 7.18             |
| B6   | 5.45        | -       | -       | 5.20    | 5.44    | 5.90             |
| B7   | 7.31        | -       | -       | 7.02    | 7.13    | 7.35             |
| B8   | 7.51        | -       | -       | 7.14    | 6.94    | 7.22             |
| B10  | 4.33        | -       | -       | 4.36    | 4.25    | 4.83             |
| B11  | 7.12        | -       | -       | 7.37    | 6.94    | 7.47             |
| B12  | 6.54        | -       | -       | 6.66    | 5.92    | 6.61             |
| B13  | 4.70        | -       | -       | 4.84    | 5.54    | 5.23             |
| B15  | 7.55        | -       | -       | 7.37    | 7.94    | 8.07             |
| B16  | 6.47        | -       | -       | 6.60    | 6.81    | 6.58             |
| B17  | 8.00        | -       | -       | 8.19    | 7.22    | 7.51             |
| B19  | 5.96        | -       | -       | 6.32    | 5.86    | 6.02             |
| B20  | 7.50        | -       | -       | 7.29    | 7.41    | 7.29             |
| B21  | 7.24        | -       | -       | 7.45    | 7.40    | 7.38             |
| B23  | 7.39        | -       | -       | 7.24    | 7.32    | 7.12             |
| B24  | 7.25        | -       | -       | 7.28    | 7.42    | 7.16             |
| B25  | 8.00        | -       | -       | 7.79    | 7.05    | 7.44             |
| B26  | 8.00        | -       | -       | 8.04    | 8.00    | 7.89             |
| B28  | 6.96        | -       | -       | 6.65    | 7.26    | 7.04             |
Table 9. (cont.)

| No. | Log(1/IC₅₀) | Model 1 | Model 2 | Model 3 | Model 4 | Previous Work[^1] |
|-----|-------------|---------|---------|---------|---------|------------------|
|     |             | CoMFA   | CoMSIA  | CoMFA   | CoMSIA  | CoMFA           |
|     |             |         |         |         |         | CoMFA           |
|     |             |         |         |         |         | CoMSIA          |
|     |             |         |         |         |         | CoMSIA          |
| B29 | 7.40        | –       | –       | 7.21    | 7.14    | 7.37            |
| B30 | 7.89        | –       | –       | 7.67    | 7.19    | 7.55            |
| B31 | 6.07        | –       | –       | 5.79    | 5.57    | 5.80            |
| B32 | 5.09        | –       | –       | 5.26    | 4.99    | 4.89            |
| B34 | 4.33        | –       | –       | 4.32    | 4.42    | 4.16            |
| B35 | 5.58        | –       | –       | 5.09    | 5.79    | 5.29            |
| B36 | 6.54        | –       | –       | 6.43    | 6.79    | 7.01            |
| B37 | 8.10        | –       | –       | 7.81    | 7.54    | 7.66            |
| B39 | 6.19        | –       | –       | 6.57    | 5.91    | 5.94            |
| B41 | 6.47        | –       | –       | 6.98    | 7.28    | 7.53            |
| B42 | 4.85        | –       | –       | 4.65    | 6.23    | 4.94            |
| B44 | 8.33        | –       | –       | 8.39    | 8.59    | 8.01            |
| B45 | 4.03        | –       | –       | 3.85    | 3.88    | 4.05            |
| B46 | 4.95        | –       | –       | 5.40    | 6.13    | 5.52            |
| B47 | 7.00        | –       | –       | 7.01    | 7.52    | 7.12            |
| B49 | 8.17        | –       | –       | 8.45    | 7.78    | 7.59            |
| B50 | 5.32        | –       | –       | 5.37    | 4.60    | 5.58            |
| B51 | 6.13        | –       | –       | 6.22    | 6.18    | 5.96            |
| B52 | 6.13        | –       | –       | 6.24    | 6.36    | 6.62            |
| B53 | 4.13        | –       | –       | 4.40    | 4.32    | 4.45            |
| C1  | 7.22        | –       | –       | –       | –       | 6.65            |
| C3  | 7.10        | –       | –       | –       | –       | 7.08            |
| C4  | 7.40        | –       | –       | –       | –       | 7.50            |
| C5  | 6.60        | –       | –       | –       | –       | 6.24            |
| C6  | 4.99        | –       | –       | –       | –       | 5.10            |
| C7  | 6.92        | –       | –       | –       | –       | 6.35            |
| C9  | 5.99        | –       | –       | –       | –       | 6.07            |
| C10 | 7.22        | –       | –       | –       | –       | 7.52            |
| C12 | 6.13        | –       | –       | –       | –       | 6.45            |
| C13 | 7.70        | –       | –       | –       | –       | 8.14            |
| C14 | 7.30        | –       | –       | –       | –       | 6.31            |
| C15 | 4.00        | –       | –       | –       | –       | 3.83            |
| C17 | 5.41        | –       | –       | –       | –       | 5.90            |
| C18 | 7.52        | –       | –       | –       | –       | 7.23            |
| C19 | 7.10        | –       | –       | –       | –       | 7.32            |
| C20 | 5.84        | –       | –       | –       | –       | 6.42            |
| D1  | 6.96        | –       | –       | 6.99    | 7.02    | 6.69            |
| D3  | 6.92        | –       | –       | 6.61    | 6.34    | 6.36            |
| D4  | 6.80        | –       | –       | 7.01    | 6.68    | 7.00            |
| D5  | 6.24        | –       | –       | 6.62    | 6.23    | 7.33            |
| D7  | 6.16        | –       | –       | 5.98    | 6.41    | 6.17            |
| D8  | 6.80        | –       | –       | 6.56    | 6.62    | 6.92            |
| D9  | 5.24        | –       | –       | 5.70    | 5.42    | 5.48            |
| D10 | 8.00        | –       | –       | 7.86    | 8.076   | 7.55            |
| D12 | 7.40        | –       | –       | 7.44    | 7.20    | 7.28            |
| D13 | 7.40        | –       | –       | 7.90    | 7.55    | 7.93            |
| D14 | 7.22        | –       | –       | 6.91    | 6.93    | 6.90            |
| D16 | 7.10        | –       | –       | 6.85    | 7.17    | 6.90            |
| D17 | 7.22        | –       | –       | 6.78    | 6.46    | 6.75            |
| D18 | 6.68        | –       | –       | 6.80    | 7.32    | 7.45            |
| D19 | 6.46        | –       | –       | 6.47    | 6.47    | 6.57            |
| D21 | 4.17        | –       | –       | 4.25    | 3.94    | 4.34            |
| D22 | 5.50        | –       | –       | 5.68    | 5.62    | 6.14            |
| D24 | 5.23        | –       | –       | 5.93    | 5.32    | 5.60            |
| D25 | 6.24        | –       | –       | 6.23    | 6.34    | 6.18            |
| D26 | 8.10        | –       | –       | 7.83    | 7.76    | 7.80            |
| D28 | 8.16        | –       | –       | 7.72    | 8.01    | 7.80            |
| D29 | 7.52        | –       | –       | 7.59    | 7.30    | 7.65            |
| D30 | 6.05        | –       | –       | 6.01    | 5.96    | 5.87            |
Table 9. (cont.)

| No. | Log(1/IC₅₀) | Model 1 | Model 2 | Model 3 | Model 4 | Previous Work[15] |
|-----|-------------|---------|---------|---------|---------|------------------|
|     |             | CoMFA   | CoMSIA  | CoMFA   | CoMSIA  | CoMFA CoMSIA     |
| D32 | 6.10        | –       | –       | –       | –       | 6.09 6.06        |
| D33 | 4.00        | –       | –       | –       | –       | 3.91 4.46        |
| D34 | 7.00        | –       | –       | –       | –       | 6.76 6.93        |
| D36 | 6.82        | –       | –       | –       | –       | 6.56 6.47        |
| D37 | 6.89        | –       | –       | –       | –       | 6.75 6.79        |
| D38 | 7.40        | –       | –       | –       | –       | 6.99 7.06        |
| D40 | 6.48        | –       | –       | –       | –       | 6.29 6.67        |
| D41 | 6.18        | –       | –       | –       | –       | 6.84 6.10        |
| D42 | 7.52        | –       | –       | –       | –       | 7.23 7.27        |
| D43 | 6.96        | –       | –       | –       | –       | 7.19 6.94        |
| D45 | 7.05        | –       | –       | –       | –       | 6.76 7.14        |
| D46 | 6.60        | –       | –       | –       | –       | 7.02 6.52        |
| D47 | 5.98        | –       | –       | –       | –       | 5.93 6.21        |
| D48 | 6.77        | –       | –       | –       | –       | 6.42 6.56        |
| D50 | 6.48        | –       | –       | –       | –       | 6.71 6.73        |
| D51 | 7.10        | –       | –       | –       | –       | 6.71 6.68        |
| D52 | 6.96        | –       | –       | –       | –       | 6.51 6.66        |
| D53 | 6.02        | –       | –       | –       | –       | 5.63 6.06        |
| D55 | 7.52        | –       | –       | –       | –       | 7.50 7.259       |
| D56 | 7.70        | –       | –       | –       | –       | 7.65 7.65        |
| D57 | 7.52        | –       | –       | –       | –       | 7.55 7.89        |
| D58 | 8.00        | –       | –       | –       | –       | 7.90 7.92        |
| D60 | 7.70        | –       | –       | –       | –       | 7.56 7.73        |
| D61 | 7.52        | –       | –       | –       | –       | 7.75 7.87        |
| D62 | 7.40        | –       | –       | –       | –       | 7.86 7.57        |
| D63 | 7.52        | –       | –       | –       | –       | 7.70 7.54        |
| D65 | 6.77        | –       | –       | –       | –       | 6.90 6.71        |
| D66 | 6.85        | –       | –       | –       | –       | 6.99 7.05        |
| D67 | 7.05        | –       | –       | –       | –       | 6.79 7.33        |
| D69 | 4.91        | –       | –       | –       | –       | 5.07 5.37        |
| D71 | 7.52        | –       | –       | –       | –       | 7.75 7.48        |
| D72 | 6.62        | –       | –       | –       | –       | 6.05 6.49        |
| D73 | 6.22        | –       | –       | –       | –       | 6.10 6.61        |
| D74 | 6.39        | –       | –       | –       | –       | 6.00 6.62        |
| D76 | 6.64        | –       | –       | –       | –       | 6.55 6.46        |
| D77 | 5.24        | –       | –       | –       | –       | 5.30 5.33        |
| D78 | 6.62        | –       | –       | –       | –       | 6.23 6.93        |
| D79 | 7.52        | –       | –       | –       | –       | 7.59 7.72        |
| D81 | 5.43        | –       | –       | –       | –       | 5.77 5.51        |
| D82 | 5.08        | –       | –       | –       | –       | 5.85 4.97        |
| D83 | 6.50        | –       | –       | –       | –       | 6.41 6.58        |
| D85 | 5.02        | –       | –       | –       | –       | 4.98 4.89        |
| D86 | 5.75        | –       | –       | –       | –       | 5.99 5.78        |
| D88 | 4.27        | –       | –       | –       | –       | 4.40 4.36        |
| D89 | 5.92        | –       | –       | –       | –       | 6.10 5.53        |
| D90 | 4.43        | –       | –       | –       | –       | 4.16 4.13        |
| D91 | 6.02        | –       | –       | –       | –       | 5.82 5.98        |
| D93 | 5.55        | –       | –       | –       | –       | 5.87 5.82        |
| D94 | 6.54        | –       | –       | –       | –       | 6.59 6.22        |
| D95 | 6.29        | –       | –       | –       | –       | 6.59 6.18        |
| D97 | 6.47        | –       | –       | –       | –       | 6.70 6.90        |
| D98 | 5.77        | –       | –       | –       | –       | 5.91 5.87        |
| D99 | 4.68        | –       | –       | –       | –       | 4.16 4.69        |
| D100| 4.61        | –       | –       | –       | –       | 4.91 4.57        |
| D101| 4.10        | –       | –       | –       | –       | 4.54 4.62        |
Table 10. The experimental and predicted activities of test sets

| No. | Log(1/IC$_{50}$) | Model 1 | Model 2 | Model 3 | Model 4 | Previous Work [15] |
|-----|------------------|---------|---------|---------|---------|-------------------|
|     |                  | CoMFA   | CoMSIA  | CoMFA   | CoMSIA  | CoMFA  | CoMSIA  | CoMFA  | CoMSIA  |
| A3  | 8.00             | 8.18    | 8.04    |         |         | 8.11   | 7.94    |         |         |
| A8  | 7.70             | 8.18    | 7.35    |         |         | 8.03   | 7.81    |         |         |
| A12 | 7.02             | 7.42    | 7.02    |         |         | 6.89   | 7.40    |         |         |
| A15 | 5.71             | 5.50    | 6.15    |         |         | 6.11   | 5.56    |         |         |
| A21 | 6.25             | 6.31    | 6.32    |         |         | 6.06   | 6.15    |         |         |
| A25 | 7.07             | 7.54    | 7.00    |         |         | 7.41   | 6.73    |         |         |
| A28 | 7.57             | 7.68    | 7.80    |         |         | 7.80   | 7.76    |         |         |
| A33 | 4.10             | 5.12    | 5.05    |         |         | 5.23   | 5.38    |         |         |
| A35 | 8.30             | 8.17    | 7.51    |         |         | 8.53   | 8.77    |         |         |
| A38 | 7.77             | 7.51    | 7.29    |         |         | 8.02   | 8.30    |         |         |
| A41 | 8.40             | 8.01    | 7.32    |         |         | 8.12   | 8.72    |         |         |
| A45 | 7.77             | 7.54    | 6.87    |         |         | 7.10   | 7.24    |         |         |
| A48 | 8.40             | 8.13    | 7.51    |         |         | 8.21   | 8.70    |         |         |
| B1  | 8.28             |         |         | 8.43    | 8.00    |         |         | 8.78   | 8.43    |
| B4  | 7.55             |         |         | 7.45    | 7.49    |         |         | 7.04   | 7.79    |
| B9  | 5.33             |         |         | 4.74    | 5.67    |         |         | 5.48   | 6.35    |
| B14 | 4.53             |         |         | 4.42    | 3.75    |         |         | 4.97   | 3.76    |
| B18 | 6.80             |         |         | 6.87    | 6.57    |         |         | 6.56   | 6.81    |
| B22 | 5.11             |         |         | 4.48    | 4.54    |         |         | 4.49   | 5.28    |
| B27 | 7.16             |         |         | 7.74    | 7.31    |         |         | 7.31   | 7.37    |
| B33 | 4.53             |         |         | 3.74    | 4.56    |         |         | 3.51   | 5.14    |
| B35 | 7.82             |         |         | 7.48    | 7.75    |         |         | 7.95   | 8.40    |
| B40 | 8.05             |         |         | 8.49    | 8.29    |         |         | 8.47   | 8.57    |
| B43 | 7.80             |         |         | 7.54    | 7.74    |         |         | 7.75   | 7.44    |
| B48 | 8.00             |         |         | 7.19    | 7.19    |         |         | 7.67   | 8.82    |
| C2  | 7.22             |         |         |         |         |         |         | 8.07   | 7.46    |
| C8  | 5.79             |         |         |         |         |         |         | 5.31   | 5.77    |
| C11 | 5.49             |         |         |         |         |         |         | 5.36   | 5.80    |
| C16 | 6.33             |         |         |         |         |         |         | 6.41   | 6.29    |
| C21 | 6.85             |         |         |         |         |         |         | 6.26   | 6.76    |
| D2  | 7.00             |         |         | 6.57    | 6.76    |         |         | 6.43   | 6.58    |
| D6  | 5.83             |         |         | 5.79    | 5.74    |         |         | 5.62   | 5.83    |
| D11 | 8.00             |         |         | 7.46    | 7.82    |         |         | 7.27   | 7.62    |
| D15 | 6.92             |         |         | 6.94    | 6.58    |         |         | 6.87   | 6.59    |
| D20 | 6.46             |         |         | 5.73    | 6.00    |         |         | 6.21   | 6.33    |
| D23 | 6.04             |         |         | 7.02    | 5.10    |         |         | 6.59   | 6.65    |
| D27 | 7.52             |         |         | 7.84    | 7.42    |         |         | 7.82   | 7.65    |
| D31 | 6.40             |         |         | 6.62    | 6.35    |         |         | 6.71   | 6.76    |
| D35 | 6.70             |         |         | 6.25    | 6.36    |         |         | 6.15   | 6.97    |
| D39 | 6.50             |         |         | 5.97    | 6.98    |         |         | 6.73   | 7.08    |
| D44 | 6.77             |         |         | 6.63    | 6.87    |         |         | 6.65   | 6.63    |
| D49 | 6.92             |         |         | 6.77    | 6.98    |         |         | 6.92   | 6.74    |
| D54 | 6.77             |         |         | 7.14    | 7.19    |         |         | 7.16   | 7.25    |
| D59 | 8.52             |         |         | 8.07    | 8.10    |         |         | 8.38   | 8.18    |
| D64 | 6.34             |         |         | 7.00    | 6.09    |         |         | 6.23   | 7.22    |
| D68 | 6.14             |         |         | 6.41    | 6.29    |         |         | 6.77   | 6.34    |
| D70 | 6.85             |         |         | 6.72    | 7.24    |         |         | 7.50   | 7.48    |
| D75 | 5.80             |         |         | 6.49    | 5.92    |         |         | 5.53   | 5.54    |
| D90 | 7.30             |         |         | 7.31    | 6.49    |         |         | 7.44   | 7.31    |
| D84 | 5.81             |         |         | 5.96    | 6.51    |         |         | 5.80   | 6.13    |
| D87 | 5.75             |         |         | 5.53    | 5.26    |         |         | 5.54   | 5.14    |
| D92 | 6.36             |         |         | 6.38    | 6.16    |         |         | 6.02   | 6.02    |
| D96 | 4.29             |         |         | 6.10    | 6.80    |         |         | 4.21   | 4.51    |
| D102| 3.03             |         |         | 5.47    | 5.43    |         |         | 3.55   | 2.90    |

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respectively, with five and three principal components. The conventional $r^2$ values are 0.965 and 0.846, with standard errors (SE) 0.259 and 0.529, respectively. The corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models for test set are 0.941 and 0.942, with standard errors (SE) equal to 0.476 and 0.384, respectively. The correlations between EA and PA for training and test sets are shown in figure 7. For model 3 (imidazoles), the cross-validated $q^2$ values of CoMFA and CoMSIA model of training set are 0.666 and 0.719, respectively, with both six principal components. The non-cross-validated $r^2$ values are 0.914 and 0.937, with standard errors (SE) 0.308 and 0.265, respectively. The corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models of test set are 0.568 and 0.447, with standard errors (SE) equal to 0.757 and 0.828, respectively. The residues between predicted and experimental activity of compounds D96 and D102 are relatively large, presumably because of the bad orientation of their phenyl ring in the (active) conformer chosen, the ring is rotated about 60°. If these two compounds are excluded out of the test set, the corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models for test set are improved to 0.636 and 0.719, respectively.

This is why we need construct the common 3D-QSAR model. The correlations between EA and PA for training and test sets are shown in figure 8. It shows that the predictive ability of model 3 is unsatisfactory.

### 3.2.2 Evaluation of the common 3D-QSAR Model (Model 4)

This model was constructed with a training set of 173 molecules extracted from four types of COX-2 inhibitors. For the CoMFA and CoMSIA models (both with six principal components), the cross-validated $q^2$ values of the training set are 0.741 and 0.632, respectively. The non-cross-validated $r^2$ values are 0.887 and 0.885, with standard errors (SE) equal to 0.394 and 0.396, respectively. The corresponding correlation coefficient $r^2$ between EA and PA of CoMFA and CoMSIA models of test set are 0.884 and 0.889, with standard errors (SE) 0.428 and 0.422, respectively. The
The correlations between EA and PA are shown in Figure 9. This model could predict the pIC₅₀ values accurately for most compounds of test set, including D96 and D102, except compounds A33 and B33, which have relatively large error values (1.13 and 1.02 log units, respectively). Model 1 can not predict the pIC₅₀ value accurately for compound A33 (error = 1.02 log units), and model 2 is also incapable of accurately predicting the activity for molecule B33 (error = 0.79). Analyzing the conformers used in the models, it is found that the phenyl ring of these two compounds is rotated about 80° with respect to the five-membered ring, whereas for other compounds, the phenyl group and five-membered ring are located on a same plane. This may be the major cause of the failure of the models. Comparing these four models, the sequences of the corresponding correlation coefficients r² for test sets are 0.941 (model 2) > 0.907 (model 1) > 0.884 (model 4) > 0.568 (model 3) for CoMFA, and 0.942 (model 2) > 0.889 (model 4) > 0.848 (model 1) > 0.447 (model 3) for CoMSIA. Considering the results of CoMFA and CoMSIA, it seems that model 4 possesses great predictive ability for rather diverse compounds.

3.2.3 Model 4 versus models 5 and 6

Model 6 is the result of random orientation of the 173 compounds of the training set. Random orientation of training set was chosen by random method. The cross-validated q² values of CoMFA and CoMSIA models of training set are 0.693 and 0.651, respectively. Model 5 is obtained by the method of all-orientation and all-placement searching. The cross-validated q² values of CoMFA and CoMSIA models of training set for this model are 0.748 and 0.638, respectively. The parameter of CoMFA for model 5 is much higher than that of model 6. It suggests that CoMFA model is sensitive to the orientation of investigated training set, while the orientation of aligned compounds has little influence to CoMSIA model [30]. The parameters of model 5 for CoMFA and CoMSIA approaches are similar to those of model 4. This shows that the method of docking-guided conformer selection could eliminate the influence of orientation for CoMFA model. This docking-guided conformer selection seems to be a valuable method to search for the active conformers and build 3D-QSAR model.
position. The favorable negative charge (red contour) is near para substituent of benzene in position 5; green contour is near meta substituent in the same position. Because the para substituent of D2, D1 is F and Cl, respectively, their activity is larger than that of D3-D9 with para electro-positive substituents (−H, −Me, −OMe, −NHMe, −NMMe2, −SMe and −SO2Me).

3.3.2 Analysis of CoMSIA Model 4

The PLS analysis parameters and predicted log(1/IC50) are given in tables 8, 9 and 10. The steric, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor field contributions are 0.062, 0.304, 0.227, 0.259 and 0.149, respectively. We remark that the electrostatic, the hydrophobic and the hydrogen donor contributions are about 80%. This shows that these three fields are the main factors affecting anti-inflammatory activity in CoMSIA models.

Figure 11(A and B) are the contour plots of CoMSIA model. The meanings of the different contours for steric and electrostatic fields are the same as in CoMFA model. In figure 11-B, yellow contours indicate the regions where hydrophobic groups enhance the activity, and the white contours show the regions where hydrophilic groups increase the activity. In cyan regions hydrogen bond donor groups are favourable for activity, whereas purple contours delineate regions where they are unfavourable. Magenta contours indicate the zones where hydrogen bond acceptor group could increase the activity, and in red coloured regions, hydrogen bond acceptor groups would decrease the activity.

The substituents at position 2 of the five-membered heterocycle near yellow-coloured, red-coloured and red-coloured (hydrogen bond acceptor field) regions, indicate that electronegative and small bulk groups are favourable to activity, while hydrogen bond acceptor field groups will decrease it. This could explain that A7, A15, A21, A37, A39, A40, A44, A46, A51, B16, B19, C11, C17, C20, D75, D82 and D102 with −CH3OH, −CH3COOH, −SH, −OH, −NH2, −CONH2, −CHO substituents at position 2 of the five-membered cycle are less active than molecules bearing −CF3 groups. In our docking investigation, we find that these former substituents with polar hydrogen at position 2 could form hydrogen bond with the residue Arg120 of COX-2, while the sum number of hydrogen bond between ligand and receptor is less than the compound with substituents −CF3. Therefore their binding free energies are lower than the latter one. Previous literature did not report why these compounds have low activity [11–12]. Electrostatic (red-colored) and hydrogen bond acceptor fields near the substituent of five-membered heterocycle at position 3 show that electronegative groups could increase the activity and hydrogen bond acceptor groups decrease it. Compounds of B1 (−Cl), B2 (−F) and B17 (−Cl) have higher activity than B6 (−OH) and B14 (−NH2). This agrees with the indication of CoMSIA model. There are red-colored,
cyan-colored and magenta-colored regions near the para substituent of the six-membered aromatic ring in position 4. It shows that polar, hydrogen bond donor and acceptor groups are favorable to the activity. This suggests that these molecules with substituents $-\text{SO}_3\text{NH}_2$ on position 4 are more active than those with $-\text{SO}_2\text{CH}_3$. The result is in agreement with our docking investigation discussed above.

Five-membered heterocycle is buried in a white-coloured contour, showing that hydrophobic interaction between residues and this ring is unfavourable to activity. The para substituent of phenyl at position 5 is near magenta-coloured, green-coloured and yellow-coloured (hydrophobic field) contours, suggesting that hydrogen bond acceptor, hydrophobic and bulky groups could increase anti-inflammatory activity. This could explain that compounds A2(4-Cl), A3(4-Br), A9(4-Cl), A10(3,4-Cl), A29(4-F), B40(4-SMe), B44(4-NMe$_2$), D10(4-Cl) and D11(4-F) are more active than A19, A23, A26, A28, B5-B15, molecules lacking substituent at the same position. There are blue-coloured, green-coloured and yellow-coloured (hydrophobic field), red-coloured (hydrogen bond acceptor field) areas near the meta substituent of phenyl at position 5. This shows that electropositive, bulky and hydrophobic groups are favourable to activity while groups with hydrogen bond acceptor are unfavourable. The CoMSIA model indicates that bulky substituents at ortho position of 5-phenyl are unfavourable to the anti-inflammatory effect. The activity of compounds have the order $21(2-F)<B25(4-F), B27(2-Me)<B29(4-Me), B36(2-OMe)<B37(4-OMe), B42(2-NMe$_2$)<B44(4-NMe$_2$), D30(2-Cl)<D14(3-Cl) and D33(2-OMe)<D19(3-OMe), in agreement with the bulk of the substituents.

### 3.4 HQSAR Model

For the 173 compounds of the training set, HQSAR model was constructed using fragment size varying from 3 to 8 and type Atom, Bond, Connectivity, Hydrogen donor or acceptor. Hologram length values were a set of prime numbers ranging from 53 to 401. The parameters of this HQSAR model are gathered in table 11.

Table 11. PLS analysis parameters for 173 training compounds of HQSAR model

| $q_{\text{cross}}^2$ | Components | $r^2$ | s | Best Length |
|---------------------|------------|-------|---|-------------|
| 0.409               | 6          | 0.685 | 0.656 | 353         |

Figure 11. CoMSIA contour plots; A shown steric and electrostatic fields, B shown hydrophobic, hydrogen bond donor and hydrogen bond acceptor fields. (ligand structure represented is A9) (Y represents yellow; B blue; G green; R red; C cyan; M magenta; P purple; W white)

Table 11. PLS analysis parameters for 173 training compounds of HQSAR model

Figure 12. Correlation between EA and PA for HQSAR model
approach gives no more than a very rough description of the relationship between structure and anti-inflammatory activity. The contribution of some fragments to activity is shown in figure 13. A9 is an example of active compound and A46 is one of less active ones. The sulphonamide fragment brings a positive contribution to anti-inflammatory activity. This is in consistent with the molecular docking investigation. A methylgroup at position 2 is favourable for activity. For A46, we could find that the fragment −NH2 gives a negative contribution to activity. This agrees with the CoMSIA model. Our HQSAR not only can testify the reliability of our 3D-QSAR but also complement it.

3.5 Comparison with Previously Reported Work

C. Hansch et al. [15] studied these four types of COX-2 inhibitors (table 1–7) with 2D-QSAR, and proposed a set of linear regressions between experimental activity and physicochemical parameters. In their regression equations, the anti-inflammatory activity is significantly correlated to hydrophobicity and polarization of the molecule. The contribution of hydrophobic and electrostatic fields in our CoMSIA model is 0.531, which shows that, among the five used parameters, hydrophobic and electrostatic fields are the main factors affecting activity. The calculated activities are listed in table 9 and 10. The correlation between EA and calculated activity (CA) is shown in Figure 14(A) for the all anti-inflammatory activity data. In this former work, the correlation coefficient r² between EA and CA was 0.695 and standard error 0.653, while the corresponding correlation coefficient r² of our CoMFA model, including training and test set, is 0.884, and the standard error 0.396. This shows that our model has better predictive ability.

A CoMFA study of similar compounds has been reported by Chavatte et al [11]. Three fields (steric, electrostatic and lipophilic) were used for constructing 3D-QSAR models. But the fields related to hydrogen bond (donor and receptor) were not taken into account. In the present work, docking study allowed us to understand the different interactions between ligand and the receptor.
of CoMSIA method provided more detailed information including the hydrogen bond effects and the contribution of different fields. In fact, the result of CoMSIA shows that the contribution of hydrogen bond donor plays also a significant role, as important as the electrostatic field. The predictive ability of our models seems to be better than the reported ones.

4 Conclusions

We applied molecular docking, CoMFA, CoMSIA and HQSAR to investigate the binding mode of four types of anti-inflammatory inhibitors (oxazole, pyrazole, pyrrole and imidazole) with COX-2 receptor. Docking inhibitors into COX-2 indicates that the most active compounds in each family form three hydrogen bonds between two oxygen atoms of sulphone and residues His90 and Arg513. This is consistent with the investigation reported by R. G. Kurumbail et al. The superposition of the docked conformations for the four types of compounds shows that these COX-2 inhibitors may have a similar interaction mechanism with COX-2 receptor, leading us to define a common 3D-QSAR model for these four types of COX-2 inhibitors. The most probable conformations were selected by a docking-guided conformer selection method. Then partial (one per family) and global (gathering the four families of compounds) 3D-QSAR models were built. For global 3D-QSAR model, the cross-validated $q^2$ values are 0.741 and 0.632; and conventional $r^2$ values 0.887 and 0.885 for CoMFA and CoMSIA. Comparing the four models, we can conclude that the global model possesses good predictive ability for diverse compounds.

For validating this result, we constructed other two models (“random orientation” and “all placement” search methods) with the same training set. The method of “all-orientation and all placement” searches could enhance the cross-validated $q^2$ for CoMFA model. The PLS parameters of the best orientation model are very similar to those of the global model. It shows that the method of docking-guided conformer selection could remove the influence of orientation for CoMFA model. This docking-guided selection is a valuable method for searching active conformers and building 3D-QSAR model.

For the 173 compounds constituting the training set, a HQSAR model was built. The result is consistent with those of molecular docking and 3D-QSAR model. Our HQSAR approach not only testifies the reliability of our 3D-QSAR but also complement it.

The global model gives a clue as to the influence of different substituents on anti-inflammatory activity. CoMSIA model gives reasonable evidence that hydrogen bond acceptor groups at position 2 are unfavorable to activity, in agreement with the results obtained from docking investigation. Literature did not report it. C. Hansch et al. [15] established 2D-QSAR models for each type of COX-2 inhibitors. For the same set of compounds, our 3D-QSAR model gives better results and has good robust prediction ability compared with their 2D-QSAR models.

Acknowledgements

The authors thank Professor Arthur J. Olson for his kindness in offering us the AutoDock 3.0.3 program. We gratefully acknowledge financial support from the Minister of Science and Technology of China (Grant No. 2002AA231011), the National Natural Science Foundation of China (Grants No. 20073058), Science and Technology Committee of Shanghai (02DJ14013), Chinese Academy of Sciences – National Center of Scientific Research in France Cooperation Program (CNRS/CAS No 14916) and Embassy of France in China.

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