Interactions of some PGHS-2 Selective Inhibitors with the PGHS-1: an Automated Docking Study by BioDock*

by **Alessandro Pedretti**, **Anna Maria Villa***, **Luigi Villa**, **Giulio Vistoli**

Istituto di Chimica Farmaceutica , Università di Milano, viale Abruzzi, 42, I-20131 Milano

Running title

An Automated Docking Study of some PGHS-2 Inhibitors with the PGHS-1.

Keywords

PGHS-1 inhibitors, PGHS-2 inhibitors, BioDock, automated docking, cyclooxygenase

---

Summary

The automated stochastic docking procedure BioDock has been applied to a series of inhibitors of PGH synthase, the key enzyme in the synthesis of eicosanoids from arachidonic acid.

Some PGHS-2 selective inhibitors have been docked to the structure of the ovine PGHS-1 enzyme, as recently obtained by means of X-ray crystallographic analysis, in order to highlight possible structural bases for selectivity.

Riassunto

La procedura stocastica di docking automatico BioDock è stata applicata ad una serie di inibitori della PGH sintasi, enzima chiave nella sintesi di eicosanoidi a partire da acido arachidonico.

E' stata simulata l' interazione di alcuni inibitori selettivi per la PGHS-2 con la struttura sperimentale della PGHS-1 ovina, recentemente ottenuta analisi cristallografica, allo scopo di evidenziare possibili basi strutturali per la selettività.

Introduction

Prostaglandin endoperoxide synthase (PGHS) is the key enzyme of the biosynthetic pathway leading to the formation of prostaglandins. This enzyme has been recently found[1] to exist in two isoforms, which apparently perform the same biochemical function. The two isozyme forms are about 60% identical in amino acid composition, and the regions which are thought to be important for catalysis

* Registration of the trade mark in progress

are widely conserved [2]. Also their affinity towards arachidonic acid, the natural substrate, appears to be quite similar. However, their synthesis is encoded by separate genes, and their pattern of expression appears to be quite different. While PGHS-1 is constitutively present in a large number of tissues, the PGHS-2 is induced by cytokines, mitogens and endotoxins in inflammatory cells[3].

Although the reason for these physiological features are not known, the most likely hypothesis is that while PGHS-1 has a "house-keeping" role under physiological conditions, i.e. the production of prostaglandins important to the maintenance of gastrointestinal, kidney and vascular functions, PGHS-2 is responsible for the elevated production of prostaglandins during inflammation[2].

These findings, however, need to be supported by the availability of selective inhibitors of the two isozymes. In addition, it could build the basis for a quite important therapeutic application. Aspirin and many other nonsteroidal anti inflammatory drugs have been recognized as inhibitors of the PGHS isozymes for a long time. However, the therapeutic benefit of these drugs is often accompanied by severe side effects, mainly related to their ulcerogenic properties towards the gastrointestinal tract. Therefore, the availability of agents which are specifically directed to the inhibition of the induced enzyme, without affecting the homeostatic one, is one of the main goals of a large research effort in this area[2].

Recently, the three-dimensional structure of ovine PGHS-1, also cocrystallized with some NSAIDs has been determined at 3.5 Å resolution by X-ray crystallography[4,5,6]. Its structure suggests that the enzyme is a monotopic membrane protein with two well distinct active sites. The cyclooxygenase active site appears to be a long hydrophobic channel.

Both the structural information and the results of site-directed mutagenesis experiments[7,8] supported the hypothesis of a binding between the NSAIDS carboxylate and the Arg_{120} positively charged group. The latter has also been shown to be involved in a salt bridge with Glu_{524}, and a steric interaction with Tyr_{355} may explain the stereoselectivity of flurbiprofen binding.

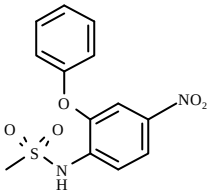
More recently, also the other isozyme has been crystallized[9]. However, the experimental coordinates are not yet available in the Brookhaven protein data bank[10].

In a former work, the docking of several inhibitors like flurbiprofen, indometacin, meclofenamic acid and piroxicam into the experimental structure of PGHS-1 has been simulated. In addition, a preliminary simulation on two PGHS-2 selective inhibitors (SC-57666 and nimesulidee) had been attempted[11]

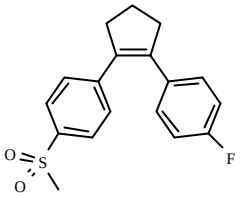
In the present study, an automated docking experiment between PGHS-1 and three PGHS-2 selective inhibitors (nimesulidee (**1**), SC-57666 (**2**) and CGP-28238 (**3**) see *Chart I*) has been

performed, in order to gain an insight into possible differences in the interactive behaviour with respect to the non selective inhibitors like flurbiprofen.

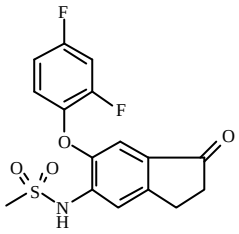
Chart I



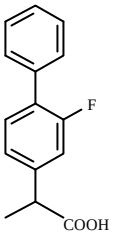
1 *nimesulide*



2 *SC-57666*



3 *CGP-28328*



4 *flurbiprofen*

Computational Methods

The crystallographic structure of the PGHS-1 enzyme[4,5,6], as reported in the Brookhaven PDB file, had already been used as a basis for the modelling of the interactions of some inhibitors in the previous work [10]. The experimental coordinates of the protein were kept fixed as far as the backbone structure is concerned. A suitable optimization of the amino acid side chains with the CHARMm[12] force field gave rise to the model structure which was used in the present study. The ligand structures were built from standard fragments and optimized with the MOPAC[13] method (AM1 algorithm). After the optimization, the structures of the ligand and the enzyme were joined and the complex was subjected to a BioDock[14] simulation. BioDock is a docking software recently developped in our laboratory, which is able to produce evaluate and classify a high number of complexes between two interaction partners in a rapid and efficient way. In the present version, both the ligand and the protein are kept fixed. For each compound, 10000 complexes have been screened. The most stable were chosen both according to energetical and functional criteria and subjected to a second BioDock simulation with the optimization option. This means that the rototranslational intervals into which the

ligand is allowed to move have been restricted to a spheric region of 8 Å around a favourable frame and optimized by means of a suitable convergence algorithm.

For some reasons which will be explained in the discussion below, in all the minima located by BioDock the ligand is not able to penetrate into the channel leading to the active site of PGHS-1, but arrests itself at the mouth of the same channel, in proximity to two arginine residues protruding into the channel itself (Arg_{79} and Arg_{83}).

Therefore, starting from the BioDock minima, a molecular dynamics for each compound was performed by means of the CHARMm force field. Only a limited region around the ligand and active site has been allowed to move. After 100 ps the complex has been optimized again. The results are illustrated below.

All calculations have been performed with a Silicon Graphics Indigo^2 workstation with a Solid Impact graphics card.

Results and Discussion

The figures 1,2 and 3 show the most stable frame identified by the BioDock screening and optimization procedure for the compounds **1**, **2** and **3**, respectively (see Chart I). For a comparison, also the corresponding frame obtained by the same procedure for flurbiprofen (compound **4**, Chart I) in the former work is shown in Fig. 4. For clarity, the protein backbone is depicted as a ribbon.

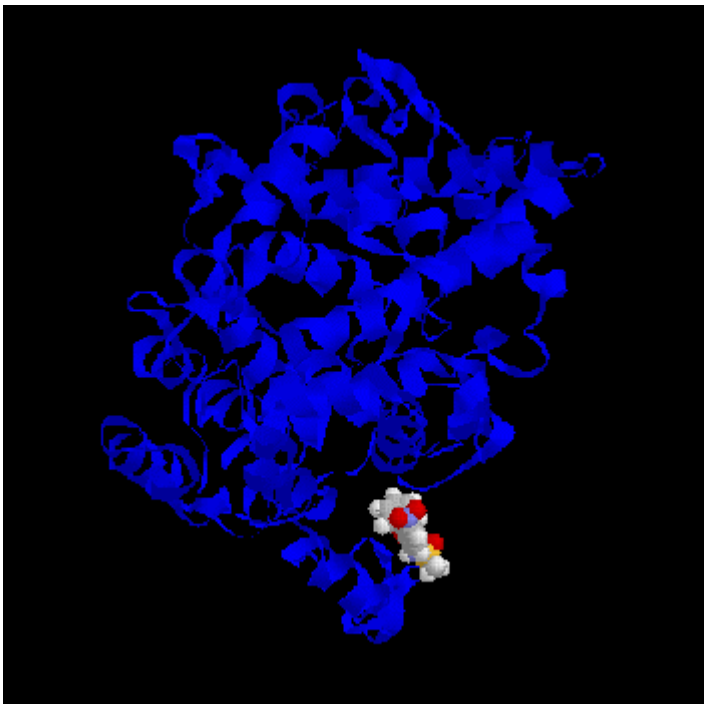


Fig. 1 - Complex between nimesulide and PGHS-1 as obtained by BioDock (not optimized). The protein backbone is illustrated as a ribbon.

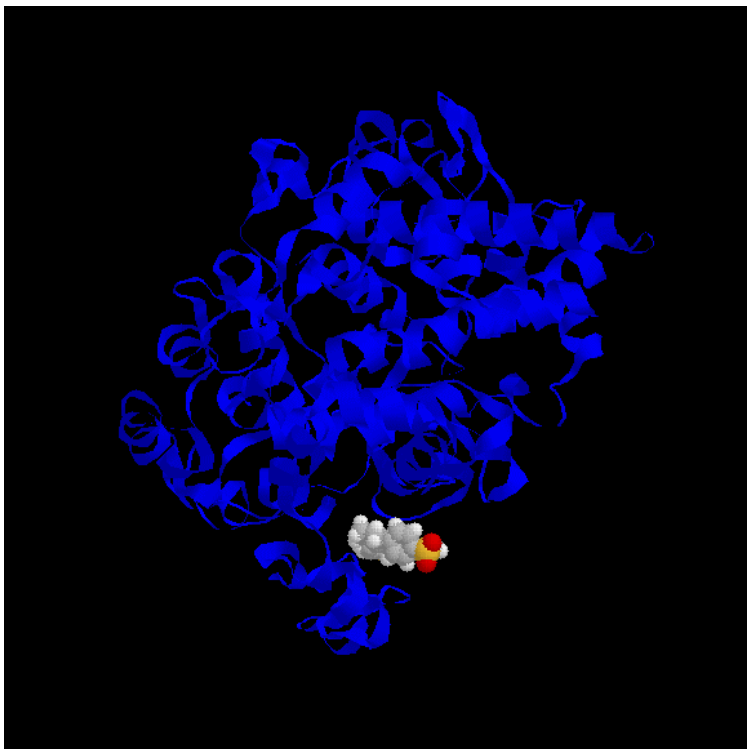


Fig. 2 - Complex between SC-57666 and PGHS-1 as obtained by BioDock (not optimized). The protein backbone is illustrated as a ribbon.

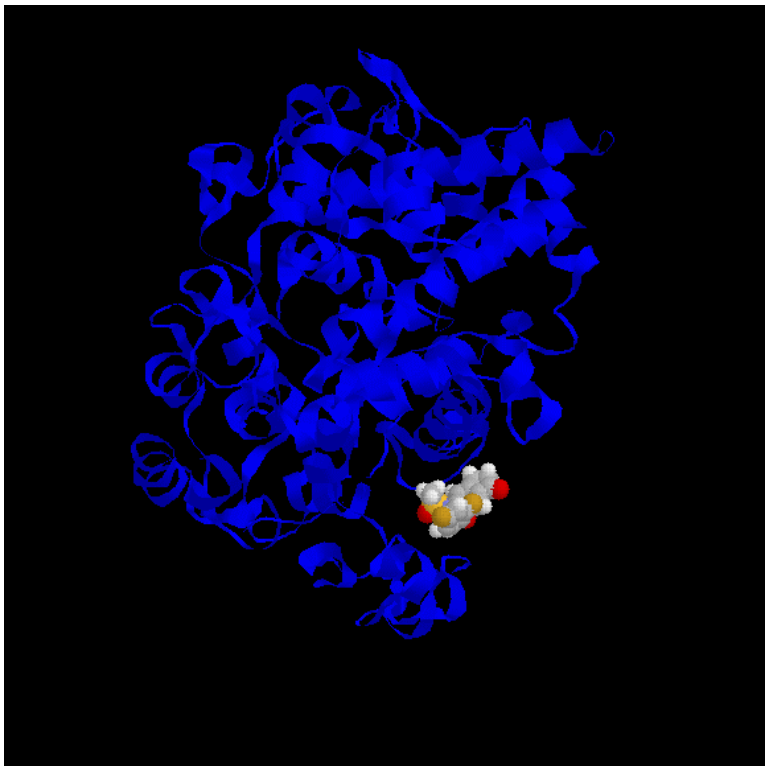


Fig. 3 - Complex between CGP-28328 and PGHS-1 as obtained by BioDock (not optimized). The protein backbone is illustrated as a ribbon.

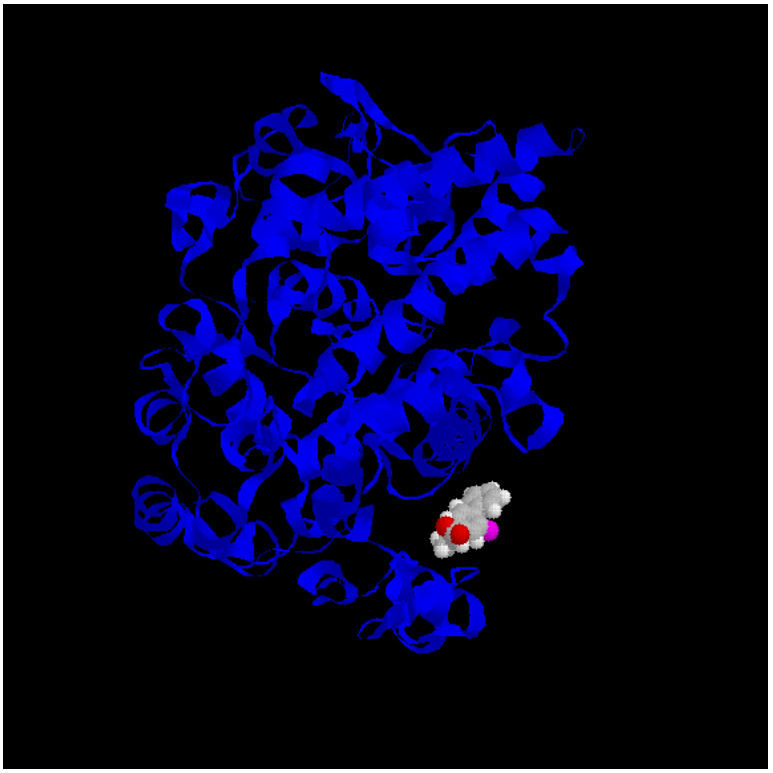


Fig. 4 - Complex between flurbiprofen and PGHS-1 as obtained by BioDock (not optimized). The protein backbone is illustrated as a ribbon.

In all cases, the behaviour is quite similar. The ligand is located at the distal part of the channel which starts from the membrane binding domain. This is mainly due to the presence of the two positively charged arginine residues 83 and 79 near the entrance of the channel leading to the active site. The electrostatic attraction towards partial or unitary negative charges in the ligand and the steric hindrance of the two side chains cannot be easily overcome because of the rigidity of the two interaction partners in the present version of BioDock. Some work is presently in progress[15] in order to allow the conformational freedom at least of the ligand in the simulation.

The situation is quite different when the relaxation of the geometry is allowed. Indeed, the following results have been obtained after a 100 ps constant energy dynamics simulation and optimization. In Fig. 5, the details of the interactions of flurbiprofen with the surrounding residues, and particularly those which have been shown to be crucial for inhibition are shown. The presence of the carboxylate function clearly disrupts the hydrogen bond reticulum which is present in the native enzyme between Arg_{120}, Tyr_{355} and Glu_{524} by competition for the interaction with the Arginine residue. The flurbiprofen molecule is able to reach the cavity containing Arg_{120}, Glu_{524} and Tyr_{355}, in agreement with the site-directed mutagenesis observations[3,4].

The minimum shows an interaction between the carboxylate oxygen of the ligand and one of the guanidinium protons of the Arginine residue; the second carboxylate oxygen forms an H-bond with the Arg_{120} amidic NH hydrogen; a proton on the second guanidinium nitrogen is still free to interact with the Glu_{524} carboxylate.

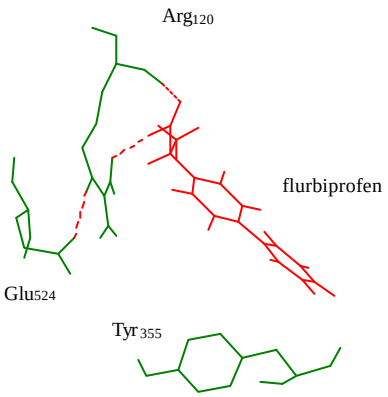


Fig. 5 - Interactions of flurbiprofen with the cyclooxygenase active site. For clarity, only polar hydrogens of the aminoacidic residues are illustrated.

By contrast, in our model the non-charged PGHS-2 selective inhibitors are not able to overcome the strength of the intra residue interactions. In fig. 6,7 and 8 the pictures of the active site after the dynamics and optimization procedure for compounds **1**, **2** and **3**, respectively, are shown.

This is clearly shown by the case of nimesulide , the most well known PGHS-2 inhibitor. In the present model the optimized complex shows a double hydrogen bonding patterns between the protons of Arg_{120} and the Tyr_{355} oxygen on one side and the Glu_{524} carboxylate on the other side, whereas the nimesulide molecule is located in a cavity facing the three residues of the active site. No specific interactions can be shown (see Fig. 6), although the presence of the inhibitor somehow modifies the conformation of the residues in the region.

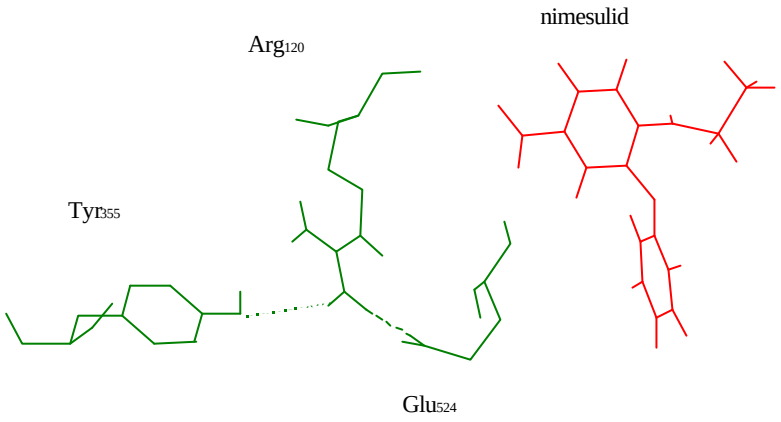


Fig. 6 - Interactions of nimesulide with the cyclooxygenase active site. For clarity, only polar hydrogens of the aminoacidic residues are illustrated.

The optimization of the SC-57666 derivative complex (see *Fig. 7*) shows little tendency for this compound to escape the local minimum near the two Arg residues 79 and 83. An interaction between Arg_{79} and the fluorine atom is maintained also in the completely optimized complex. Some interactions between the two aryl groups of the ligand and the hydrophobic aminoacids (e.g. Ile_{89} and Trp_{100}) surrounding the binding site stabilize the complex, although they are not so easily described because of their aspecific nature.

This behaviour could be mainly ascribed to the lack of a unitary charge in the molecule, and to the bulkiness and relative rigidity of the two phenyl substituents at the cyclopentene system, who tend to

interact with the otherwise hydrophobic surroundings of the distal part of the channel. In the postulated binding site, all intra-residue interactions are conserved: a double interaction (salt bridge) between the Glu_{524} carboxylate oxygens and the Arg_{120} guanidinium group hydrogens and a H-bond between the Tyr_{355} phenolic oxygen and another guanidinium proton are present (see *Fig. 7*). The active site region is even less perturbed than in the case of nimesulide.

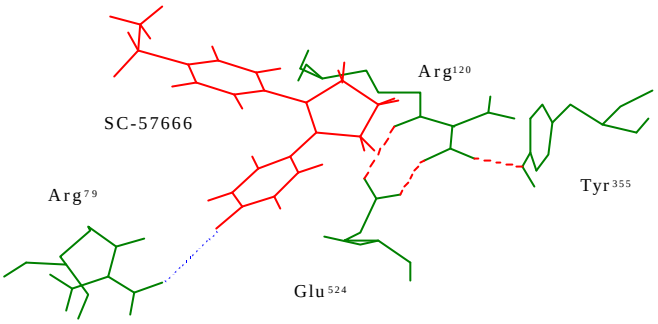


Fig. 7 - Interactions of SC-57666 with the cyclooxygenase active site. For clarity, only polar hydrogens of the aminoacidic residues are illustrated.

The last derivative examined in the present work is the CGP-28238, which has a relatively bulky indanone derivative. In this case, like in the complex with nimesulide, the hydrogen bonding patterns in the active site is perturbed through the conformational changes induced by the bicyclic indanone system. No direct interaction can be visualized between the CGP-28238 molecule and the active site residues.

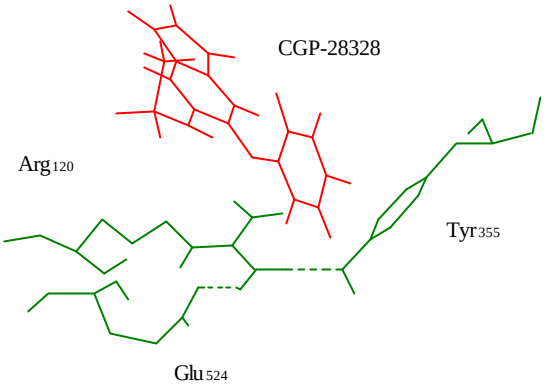


Fig. 8 - Interactions of CGP-28328 with the cyclooxygenase active site. For clarity, only polar hydrogens of the aminoacidic residues are illustrated.

Conclusions

The computational procedure used in the present work, which included a BioDock screening simulation, a BioDock optimization and a subsequent molecular dynamics and optimization yielded a model for the interaction of some PGHS-2 selective inhibitors with the cyclooxygenase site of the ovine PGHS-1 experimental solid state structure. In all cases which were examined, the presence of the non-charged inhibitor was not able to modify in a substantial way the H-bonding patterns between Arg_{120}, Tyr_{355} and Glu_{524}, which includes a double interaction between the two Glu carboxylate oxygens and two Arg protons and between another Arg protons and the Tyr oxygen in the native enzyme. Upon binding, nimesulide and CGP-28328 cause a conformational rearragement of the region with the effect of taking apart one of the Arg protons and one of the carboxylate oxygens. SC-57666 doesn't perturb the original disposition of the three residues.

In contrast, flurbiprofen efficiently competes for interaction with the Arg residue, due to the presence of the unitary negative charge. In addition, its presence in the cyclooxygenase region strongly alters the conformational features.

Furthermore, the consideration that the rigid docking experiment had to be integrated with a molecular dynamics procedure in order for the inhibitor to reach the cyclooxygenase active site, further supports the hypothesis that the interaction of the inhibitors with this enzyme is by no means a static process, but a dynamic one, which may require several steps before the complex is actually formed.

References

[1] J.R. Vane et al., "*Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammetion*" *Proc.Natl.Acad.Sci U.S.A., 91,* 2046-2050 (1994).

[2] D.E.Griswold, J.L.Adams, "*Constitutive cyclooxygenase and inducible cyclooxygenase: Rationale for selective inhibition and progress to date*" *Med.Res.Rev., 16,* 181-206 (1996) and refs. quoted herein.

[3] H.R. Herschman "*Prostaglandin synthase 2", Biochim.Biophys.Acta, 1299,* 125-140, (1996).

[4] D.Picot, P.J. Loll. R.M. Garavito, "*The X ray crystal structure of the integral membrane enzyme prostaglandin H2 synthase*" *Nature, 367,* 243-249, (1994).

[5] P.J.Loll, D.Picot, R.M. Garavito, "*The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase*" *Nat.Struct.Biol.,2,* 637-643 (1995).

[6] P.J.Loll, D.Picot, O.Ekabo, R.M. Garavito, "*Synthesis and use of iodinated nonsteroidal antiinflammatory drug analogs as crystallographic probes of prostanglandin H_2 synthase cyclooxygenase active site Biochemistry, 35,* 7730-7740 (1996).

[7] J.H.Mancini, D.Riendeau, J.P.Falgueyret, P.H. Vickers, G.P.O'Neill, "*Arginine 120 of protaglandin G/H synthase 1 is required for the inhibition by nonsteroidal anti-inflammatory drugs containing a carboxylic acid moiety" J.Biol.Chem., 270,* 29372-29377 (1995).

[8] D.H. Bhattacharyya, M.Lecomte, C.J.Rieke, M.Garavito, W.L. Smith,"*Involvement of arginine 120 glutamate 524 and tyrosine 355 in the binding of arachidonate and 2-fenilpropionic acid inhibitors to the cyclooxygenase active site of ovine prostaglanndin endoperoxide H-synthase 1" J.Biol.Chem., 271,* 2179-2184 (1996).

[9] R.G. Kurumbail et al., "*Structural basis for selective inhibition of cyclooxygenase-2 by antyinflammatory agents", Nature, 384,* 644-648 (1996).

[10] Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, NY,U.S.A.

[11] A. Pedretti, A.M.Villa, L.Villa, G.Vistoli,"*Modelling of the interactions of some inhibitors whith the PGHS1 by BioDock, a stochastic aproch to the automated docking of ligands to biomacromolecules*" in *Computer-Assisted Lead Finding and Optimization,* Helvetica Chimica Acta Verlag, Basel, in press (1996).

[12] MSI, Burlington, MA, USA.

[13] J. J. P. Stewart, "*MOPAC: a semiempirical molecular orbital program*" *J. Comp.-Aided Molecular Design, 4,*1-105, (1990).

[14]A.Pedretti, "*Nuovo metodo per il docking automatico di ligandi con macromolecole a struttura 3D nota*" degree thesis, Milan University 1995.

[15]A. Pedretti, work in progress.