Structural Approach for COX-2 Inhibition

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Abstract: The design of selective COX-2 inhibitors is a new approach to obtain potent, anti-inflammatory drugs but with less side effects. Several families of such inhibitors were reported in literature. In this review, the drug design processes used to understand their binding mode and the origin of selectivity of these compounds are described.

Keywords: COX-2 inhibitor, selectivity, molecular modelling, X-ray, drug design, COX binding site, molecular features

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics for the treatment of pain and inflammation [1]. Aspirin, derived from the acetylation of salicylic acid, was the first one introduced in 1897 (Fig. **1**) [2]. Since the 60s, several other NSAIDs were marketed, for example, flurbiprofen (Cebucid®), indomethacin (Indocid®), naproxen (Apranax®),…(Fig.**1**). They share a carboxylic acid in their structures.

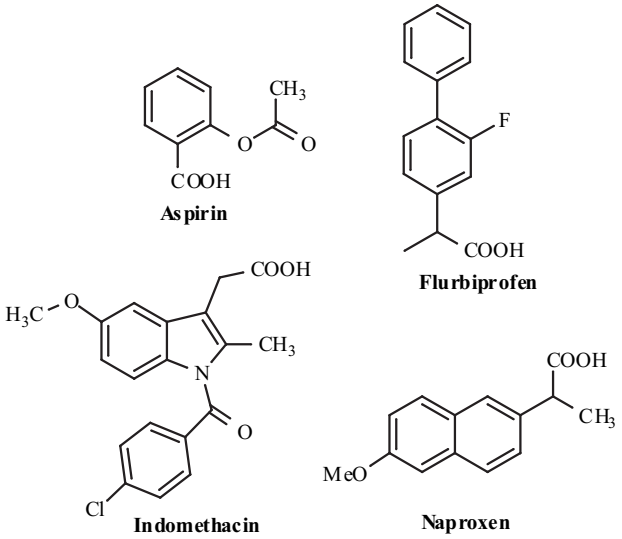

Fig. (1). Some classical NSAIDs.

In the 70s, Vane and his colleagues showed that these drugs prevent the biosynthesis of prostaglandins (PG) by blocking cyclooxygenase (COX) [3]. This enzyme has two catalytical activities: the cyclooxygenase and the hydroperoxidase ones. The arachidonic acid (AA), its natural substrate, is cyclised to PGG_2 by the COX activity and reduced to PGH_2 by the hydroperoxidase one. Then PGH_2 is transformed by isomerases and synthases in PGs (PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2) and thromboxane A_2 (TXA_2) that display different physiological functions [4]. Among the PGs, the most important are PGE_2 and PGI_2, which are potent vaso- and bronchodilators, unlike TXA_2. They regulate the renal blood flow and allow stomach protection. TXA_2 induces platelet aggregation although PGI_2 inhibits it [5].

NSAIDs are efficient in the treatment of inflammatory diseases and especially in the antirheumatic therapy [6,7]. However these drugs show side effects such as GI irritations and renal disorders [8,9].

In the 90s, Needleman *et al.* postulated the existence of two isoenzymes of COX: a constitutive one, called COX-1, responsible for a basic level of PGs; and an inducible one, COX-2, activated by inflammatory stimuli [10,11].

This discovery helped in understanding the side effects associated with classical NSAIDs. Indeed the complications arise from the inhibition of COX-1, which is constitutively expressed in many cells. In contrast, inhibition of COX-2 would be responsible for the anti-inflammatory effect [12].

As a result, the development of selective COX-2 inhibitors could provide anti-inflammatory drugs with fewer risks. Several research programmes were performed in order to find such new drugs. Until now, two compounds are on the market: celecoxib (Celebrex®) and rofecoxib (Vioxx®) (Fig. **2**) [13,14]. Research is going on to enhance selectivity and to elucidate the role of selective COX-2 inhibitors in various cancers and neurological disorders like Alzheimer's and Parkinson's diseases [15-18].

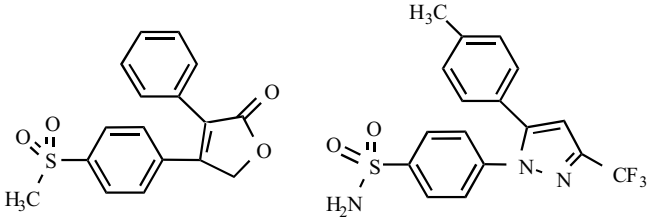

Fig. (2). Selective COX-2 inhibitors on the market.

In this review, we describe the structural differences between the two isoenzymes and especially, the small differences between the two active sites that make the research of new selective inhibitors so complicated. The main families of inhibitors and the drug design processes used to understand their binding mode will also be depicted.

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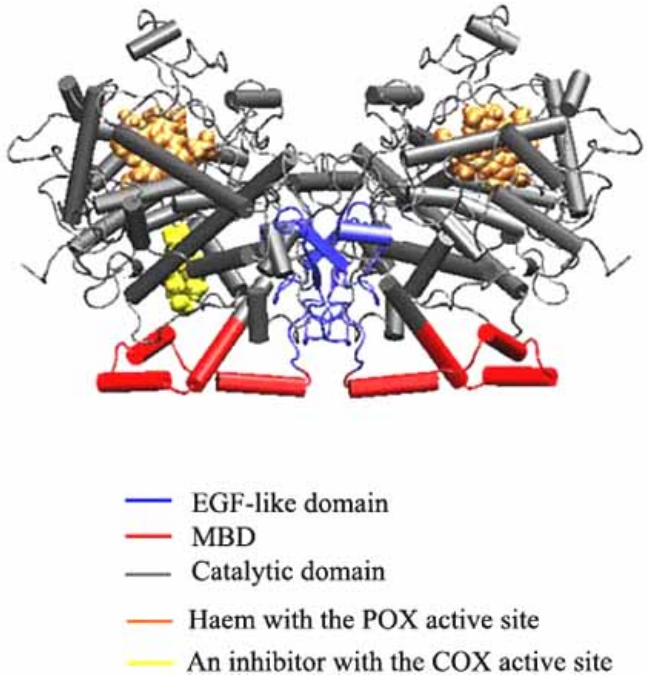

Fig. (3). 3D structure of the COX with its three domains.
(This image was made with VMD by the Theoretical and Computational Biophysics Group, an NIH Resource for Macromolecular Modeling and Bioinformatics, at the Beckham Institute, University of Illinois at Urbana-Champaign).

2. STRUCTURE OF THE CYCLOOXYGENASES [19,20]

The COXs are monotopic, membrane-bound, heam-dependent enzymes located in the endoplasmic reticulum and the nuclear envelope. The three dimensional structure of the ovine COX-1 was first reported in 1994 and those of human and murine COX-2 followed [21-23]. These are homodimers with similar structure, consistent with a high sequence identity (60%). Their tertiary structures are characterised by three domains: an N-terminal epidermal growth factor-like (EGF) domain, a membrane-binding domain (MBD) and a globular catalytic domain with the COX and hydroperoxidase (POX) active sites (Fig. **3**).

The **EGF-like domain** would be involved in the integration of matured COX into the lipid bilayer but its role remains unclear. The **MBD** contains four amphipathic alpha helices and allows access of the arachidonic acid, the substrate of COXs, through the interior of the bilayer to the active site. The **COX active site** is a narrow hydrophobic channel extending from the MBD to the core of the catalytic domain. The heam-dependent **POX active site** is on the opposite side of the MBD and is exposed to the solvent.

3. THE COX BINDING SITE OF COX-1 AND -2 AND STRUCTURAL BASIS OF THE COX-2 SELECTIVITY [19]

The COX binding site is a hydrophobic channel. The main amino acids of the active site of COX-1 and -2 are illustrated in Figure **4**. The charged residues in the two COXs are Glu524 and Arg120. X-ray data show that Arg120, located midway down the COX channel, interacts with the carboxylic group of the AA and classical NSAIDs in COX-1. The fact that all selective COX-2 inhibitors reported to date do not contain a carboxylic moiety raised the question about the role of such residue in COX-2.

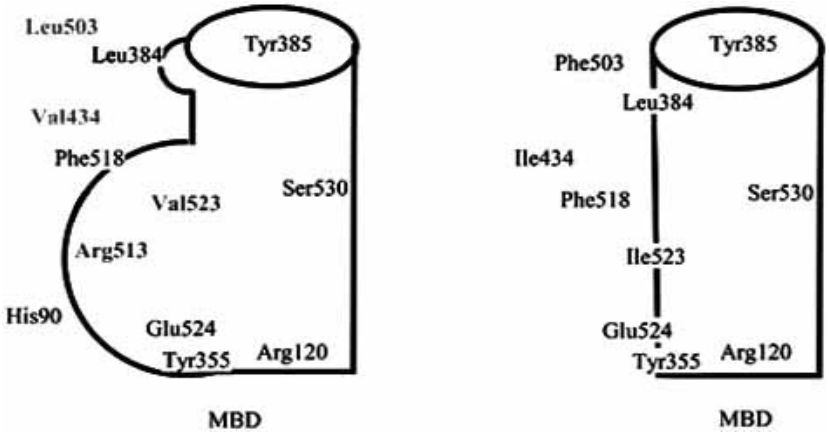

Fig. (4). Active site of COX-2 and COX-1.

Ser530, which is acetylated by aspirin, is located in the upper portion of the channel. Tyr385, at the top of the channel, between the COX active site and the POX one, plays a part in the hydroperoxidase activity.

Several differences between the COX active site of COX-1 and -2 can explain the selectivity of some COX-2 inhibitors.

Firstly, the substitution of Ile523 in COX-1 with the less bulky Val523 in COX-2 allows access to an additional polar side pocket and increases the volume of the COX active site. Such enhancement is also due to the substitution of Ile434 in COX-1 with Val in COX-2 that allows the Phe518 to move back [22]. The existence of this side pocket in COX-2 allows additional interactions with amino acids such as Arg513, replaced by a His in COX-1. This change would be another contribution to COX-2 specificity [23]. Indeed, site directed mutagenesis studies suggest that Arg513 is a key residue for the activity of the diaryl heterocycle inhibitors (see section 5.1) [24].

Secondly, the conserved Leu384, situated at the top of the channel, is oriented differently in the COX-1 and -2 because of the effects of a residue at position 503. In COX-1, the presence of a phenylalanine forces the Leu384 side chain to lie into the active site. In COX-2, a smaller leucine at this position allows the Leu384 side chain to move away from the active site and generates an accessible space in the apex of the COX-2 binding site [25]. This "extra space" in COX-2 was revealed by the resolution of the X-ray structure of human COX-2 complexed with L-758048, an analogue of indomethacine, and RS-104897 and RS57067, zomepirac analogues [22].

All these slight differences in the sequence of two enzymes lead to structural differences between COX-1 and COX-2 active site which could be exploited to design COX-2 selective inhibitors.

4. ENZYME KINETICS OF THE COX-2 INHIBITORS

Inhibition of COX activity is complex and for some compounds corresponds to a multi-step model of interaction. In addition to the irreversible inhibition mode of aspirin, some NSAIDs exhibit a time-independent inhibition, in which the ligand competes reversibly with the natural substrate to form an enzyme-inhibitor complex (EI). Others display a time-dependent inhibition including an initial reversible binding mode which progresses to a tight irreversible one to form a new enzyme-inhibitor complex (EI*). In many cases, selective COX-2 inhibition is correlated with a time-dependent COX-2 inhibition and a time-independent COX-1 inhibition [26-29].

Currently, the structure of the EI complex and the transition status from EI to EI* are unknown. Analysis of the crystal structures and several modelling studies were performed in order to understand this mechanism. On the one hand, examination of the membrane domain (MBD) reveals a first hydrophobic cavity of the size of the COX binding site and with similar structural features. Docking and molecular dynamics studies suggested that inhibitors bind first to this cavity, forming the EI complex and explaining the time-independent inhibition.

On the other hand, a network of hydrogen bonds involving Arg120, Tyr355, Arg513 (His513 in COX-1) and Glu524 acting as a gate for ligand entrance to the COX active site was revealed. Moreover, molecular dynamics studies suggest that this gate doesn't open easily and that Glu524 fluctuates to interact with either Arg120 or Arg513 [30-33]. If the inhibitors are able to perturb this H bond network, they can reach the COX active site and form the EI* complex. This is the second step of the time-dependent inhibition [34]. Such multi-step sequence was described for celecoxib by Salter *et al.* [35].

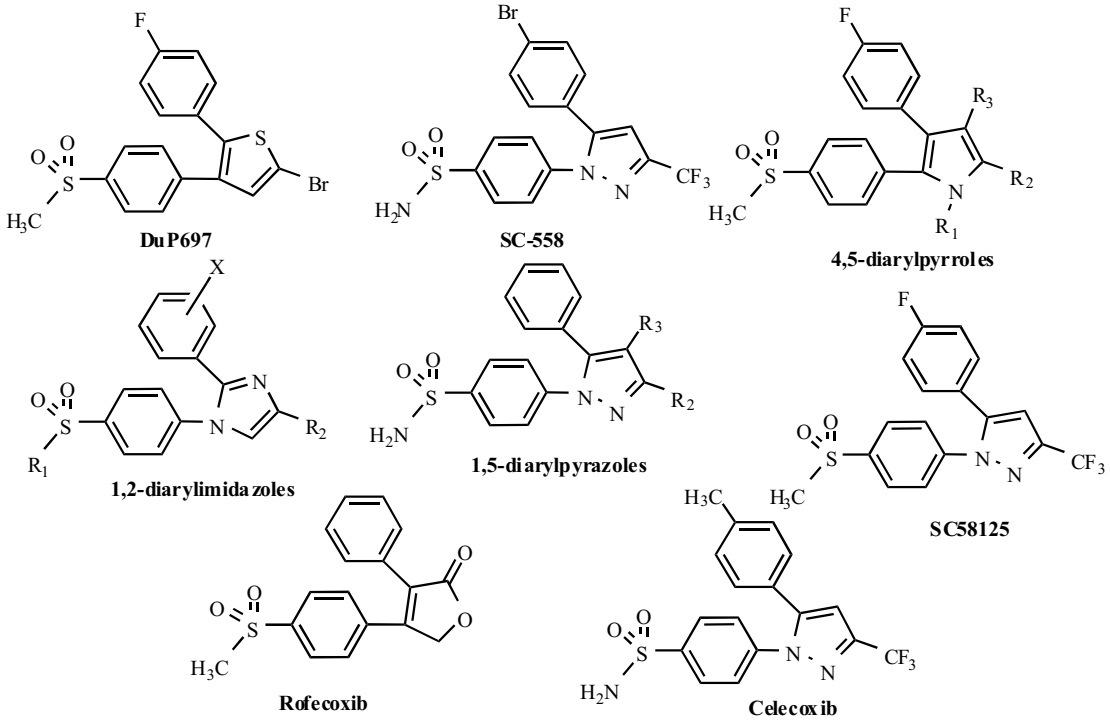

Fig. (5). Diaryl heterocycle family.

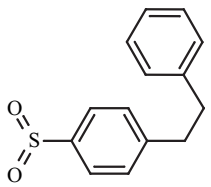

Fig. (6). General structure of the diaryl heterocycle family.

5. MOLECULAR AND STRUCTURAL APPROACH FOR SELECTIVE COX-2 INHIBITION

Since the discovery of COX-2, research efforts were focused on the development of selective COX-2 inhibitors [36-40]. Several chemical classes were developed and analysed. Here, rational approaches to understand the binding mode and the origin of selectivity of five families have been described: the diaryl heterocycles, the acidic sulfonamides, the modified NSAIDs, the zomepirac analogues and the enolcarboxamides. Such analyses can help to design new compounds.

5.1 Diaryl Heterocycles

This family of compounds is the most developed in the primary and patent literature. Its lead compound is DuP697, the first non-ulcerogenic, anti-inflammatory, diarylheterocycle derivative described (Fig. **5**) [41]. The common structural feature of this family is depicted in figure **6**.

X-ray structure of SC-558 (with a 1,900-fold selectivity for COX-2 over COX-1) in complex with COX-2 highlighted the binding mode of this class of compounds [23] (Figs. **5** and **7**). The bromophenyl ring is surrounded by several hydrophobic residues such as Phe381, Leu384, Tyr385, Trp387, Phe518 and by Ser530. The trifluoromethyl group fills the bottom of the channel formed by Met113, Val116, Val349, Tyr355, Leu359 and Leu531. The phenyl sulfonamide moiety binds in the additional pocket which is made accessible by the substitution I523V and the sulfonamide moiety interacts with His90, Gln192 and Arg513. Site directed mutagenesis indicates that the presence of valine instead of isoleucine is sufficient to confer selectivity to this family [42,43]. His90 and Gln192 are conserved in the two isoforms but Arg513 is replaced by histidine in COX-1. X-ray data show that an imidazole ring at this position would not be sufficient to interact with the sulfonamide group.

Several molecular modelling studies were performed on this family in order to clarify the structural basis for the selectivity and the binding affinity of such inhibitors and to propose new selective COX-2 inhibitors.

5.1.1 Rationalisation of the Selectivity and the Binding Mode

Rodriguez *et al.* developed a **3D-pharmacophore model** searching the most stable conformers of the diaryl heterocycle (derived from SC-558) and sulfonamide families (see section 5.2) [44]. Three points were obtained for the diaryl heterocycles: the first is the O and N atoms of the

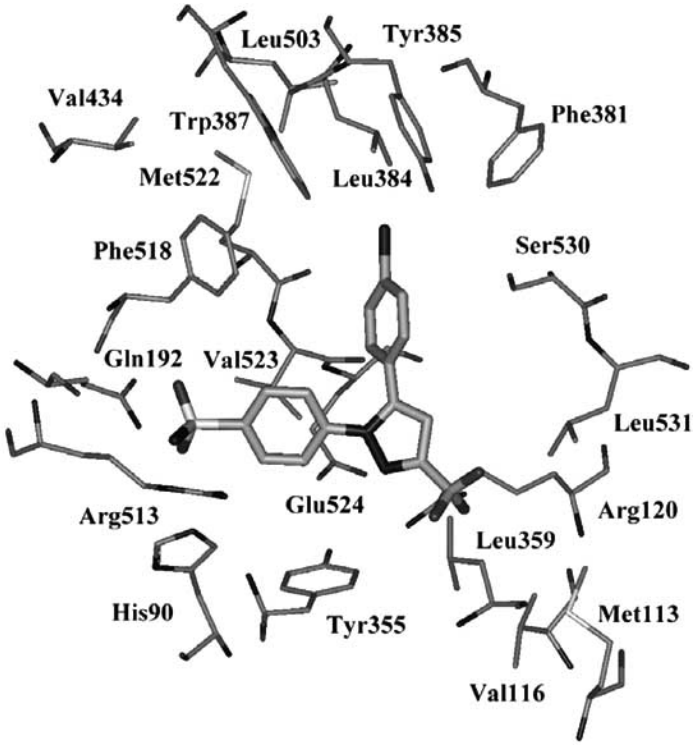

Fig. (7). SC-558 complexed with COX-2 (figure composed using InsightII [76]).

sulfonamide group (P1); the second, the N atom of the pyrazole ring (P2); and the third, the halogen or H atoms at position 4 of the non-sulfonamide phenyl ring (P3) (see section 5.2 for the sulfonamide family) (Fig. **8**).

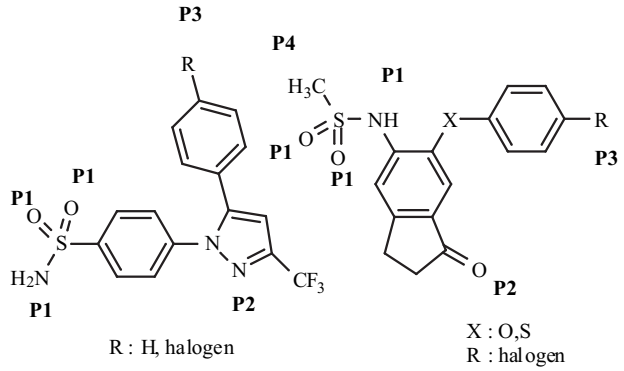

Fig. (8). Pharmacophore model of the diaryl heterocycle and sulfonamide families.

3D-QSAR studies which applied comparative molecular field analysis (CoMFA) and/or comparative molecular similarity indices analysis (CoMSIA) including the steric, electrostatic and lipophilic fields were performed to derive the predictive models. Contour maps in relation to the enzyme site structure were also described. Four studies were reported and the results generally agree [45-48].

It is shown that favourable steric region is close to the tensor between the two aromatic rings and the no sulfonamide-containing phenyl ring (Fig. **9**). Only medium-sized and electropositive substituents at the para, meta and ortho positions of ring A are allowed. The lipophilic nature of the para position near Tyr385 is also important.

Moreover the presence of a hydrophilic area close to the sulfonamide moiety,which may be explained by the possibility of such group to interact with His90 and Arg513, was observed. A hydrogen bond donor at this position would also enhance the inhibitory activity.

The existence of an electropositive zone on the ring B itself and an electronegative one at the 4-position of this cycle indicates that an electron-withdrawing group near the Arg120 may be ideal. Such a substituent would not only make the ring electron deficient but also leads to an accumulation of charge at this position. Moreover this substituent should be lipophilic to suit the best for activity.

QSAR and Classification (k-nearest neighbour) models have been developed by Kauffman *et al.* for a set of specific COX-2 inhibitors using only topological descriptors [49]. While the calculated descriptors used in their models provide limited insight into the major structural contributions to potent COX-2 inhibition, this work demonstrated that they could be used as a screening tool for larger libraries of potential target compounds which share a similar core scaffold. Wilkerson showed by QSAR studies the influence of lipophilicity, size and electronic effects on substituents of the pyrrole moiety for a series of 4,5-diarylpyrroles (Fig. **5**) [50]. It was suggested that the electronic environment of the pyrrole proton would appear to be more important to the inhibition of COX-2 than of COX-1. Clearly the pyrroles where R1=R3=H and R2 is inductively electron withdrawing produced the best activity. Moreover selectivity could be influenced by the size of R1 and R2 and the molecular lipophilicity.

In parallel to the ligand study, an analysis of the protein and the protein-ligand interaction is here described.

A **chemometric procedure** was applied to identify the most suitable regions of the isoenzymes for the design of selective ligands [31]. GRID multivariate characterisation of the enzymes and subsequent Principal Component Analysis (PCA) of the descriptor variables allowed the identification of chemical groups that could be added to a core template structure to increase its selectivity. In these studies, the importance of the side pocket in COX-2 was highlighted, as probes with a hydrophobic core and a polar head were able to interact selectively with it. The PCA results showed the well-established role of Arg513 residue. It was also shown that a charged group might increase the affinity for both target proteins, to the detriment of selectivity.

The interactions between several diaryl heterocycles such as SC-558, its derivatives, SC58125, celecoxib, 1,5-diarylpyrazole or 1,2-diarylimidazole, rofecoxib and the two isoenzymes were investigated by **docking studies** in order to understand their binding mode and predict their binding free energy (Fig. **5**) [30,32,35,47,48,51]. Different methods were used: on the one hand, automated molecular docking (FlexiDock, Affinity, Autodock) and on the other hand, manually docking guided by the crystal structures and site directed mutagenesis.

All the studied compounds show a similar orientation to that of SC-558 into COX-2. Hydrogen-bond interactions with His90, Arg513 and Phe518 are observed. The substituted phenyl group at the top of the channel interacts with the side chains of residues through not only hydrophobic interaction, but also electrostatic interaction, to

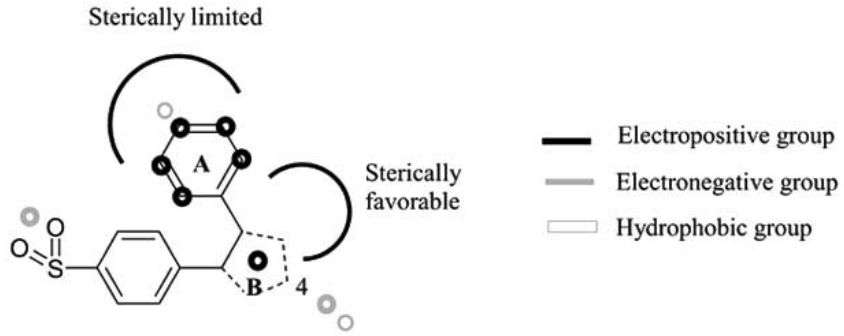

Fig. (9). Results of 3D-QSAR studies of the diaryl heterocycle family.

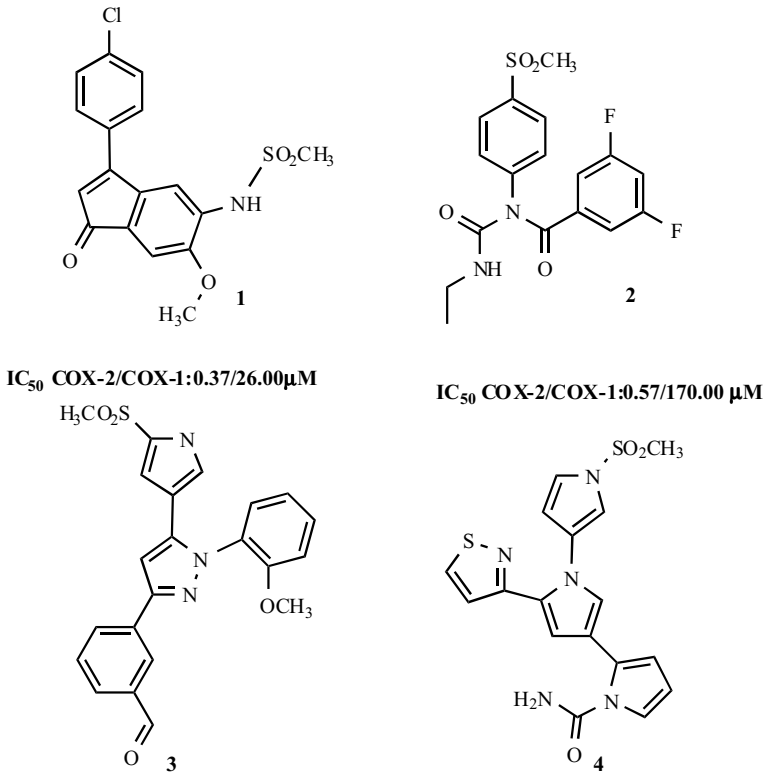

Fig. (10). New leads, selective COX-2 inhibitors, designed from 3D-QSAR models and from *de novo* structure-based drug design.

some extent. This side of the binding site bordered (limited) by Tyr385 is quite sterically restricted. In general, small substituents lead to a better binding in agreement with the 3D-QSAR study.

In some 4,5-diarylpyrroles and 1,5-diarylpyrazoles, the R3 group, not present in SC-558, interacts with the side chain of Ala527 through a hydrophobic interaction (Fig. **5**). The smaller substituents bind generally the best. Unlike SC-558, rofecoxib has a furanone moiety that can be responsible for extra interactions with, for example, Ser530.

A novel docking method, based on a combined Tabu and Monte Carlo (MC) protocol, was used to determine starting conformations for MC simulations [52;53]. From these conformations, relative changes in binding free energies were computed for ten celecoxib analogues with the MC free energy perturbation (FEP) method. This one yielded to relative binding free energies in excellent agreement with the experimental data. The docked complexes obtained help to explain experimentally observed trends.

Another method was used by Wesolowski *et al.* to predict the binding affinities of celecoxib analogues for the COX-2 enzyme: the Monte Carlo-Extended Linear Response approach. Such method can successfully accommodate both small and large structural variations for inhibitor series [54].

The binding mode of the diaryl heterocycles into COX-1 was also studied. These inhibitors seem to interact with Ser530, forcing them to adopt another position, being pushed to the top of the binding pocket [30]. These ligands have no electrostatic and H bond interactions with Arg120 whereas mutagenesis experiment indicated the importance of this residue in the binding with COX-1. As a result, withdrawing this interaction within COX-1 would be another way to increase COX-2 selectivity.

5.1.2 Design of New Inhibitors

Molecular modelling studies were used to design new selective inhibitors. For example **3D-QSAR models** were used by Chavatte *et al.* to select five compounds to synthesise according to their predicted COX-2 inhibitory potency and their chemical accessibility. These molecules, displaying some selectivity for COX-2, were considered as new leads for chemical optimisation (Fig. **10**: compounds **1** and **2**) [55].

A new method of ***de novo*** **structure-based drug design**, Dycoblock, was applied to design new selective COX-2 inhibitors. A multiple-copy stochastic molecular dynamics was developed to dynamically assemble molecular building blocks into binding sites [56]. Compounds with five or six building blocks were found but not yet tested. Two of these compounds, **3** and **4**, are depicted in figure **10**.

An original method to find new selective COX-2 inhibitors consists in transforming a non-selective inhibitor to a selective one. Palomer *et al.* identified three **pharmacophoric points** from the 3D structure of four diaryl heterocycles with the knowledge of the X-ray crystal structure of SC-558: two aromatic rings and a sulfonyl moiety [57,58]. From the superposition of indomethacin, a non-selective inhibitor to this pharmacophore, it was observed that despite the absence of the sulfonyl group, this inhibitor reasonably maps the model. As a result, Palomer proposed analogues of indomethacin with the sulfonyl feature and some of these are potent and selective COX-2 inhibitors. The same work was performed with the

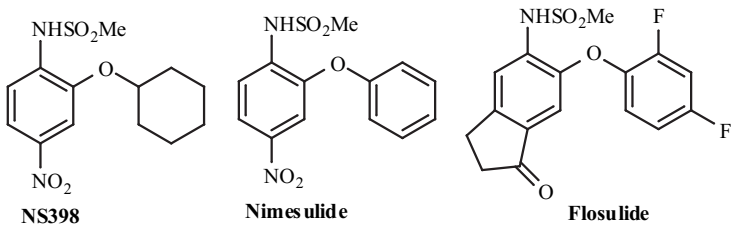

Fig. (11). Compounds of the acidic sulfonamide family.

arylalkanoic acid ketoprofen to transform it into a selective COX-2 inhibitor. All these compounds have been described in section 5.3.

5.2 Acidic Sulfonamides

No crystal structure of compounds of this family in complex with the COXs has been published yet. NS398 was the first compound of this family and subsequently, flosulide and nimesulide were also characterised as COX-2 specific inhibitors (Fig. **11**) [59-61]. The general structure of this family is shown in figure **12**. Some modelling studies were performed in order to understand the binding mode of these compounds.

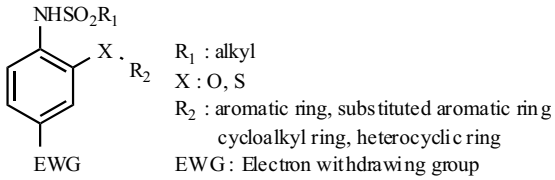

Fig. (12). General structure of the acidic sulfonamide family.

First Fabiola showed, by **docking studies** and molecular mechanics calculations, that the residues at positions 523 and 516 largely contribute to the selectivity of nimesulide [62]. Its methyl sulfonamide group would interact with His90 and Arg513 in the side pocket, like the sulfonamide of SC-558 (Fig. **13**). The nitro group points towards Arg120 and forms hydrogen bonds with Tyr355. Such interaction was not found in the SC-558 complex. This study also suggested that nimesulide may have a higher selectivity towards COX-2 than SC-558 due to its bulkier methyl sulfonamide group that helps to have strong interactions with the enzyme.

Then Garcia-Nieto *et al.* showed, using automated docking, like Autodock and DOCK software, and molecular dynamics simulations, two binding modes for nimesulide within COX-2 making use of the adjacent pocket [33]. An orientation A, similar to that described above, and an other orientation B, where the methyl sulfonamide is placed near the Arg120 and the nitro group in the vicinity of Arg513, were found (Fig. **13**). In both cases, the phenoxy ring binds in a position comparable to that of the bromophenyl ring of SC-558. The overall intermolecular interaction energy is very similar for the two complexes given that a better electrostatic term in orientation A is compensated by an improved van der Waals contribution in complex B. Like for SC-558, the Ser530 residue is not important for the binding of nimesulide. The sulfonamide NH of nimesulide does not appear to make any direct contacts with the enzyme. Its major role appears to be in decreasing the conformational flexibility of the phenoxy moiety and in enforcing the co-planarity of the sulfonamide group with respect to the nitrophenyl ring.

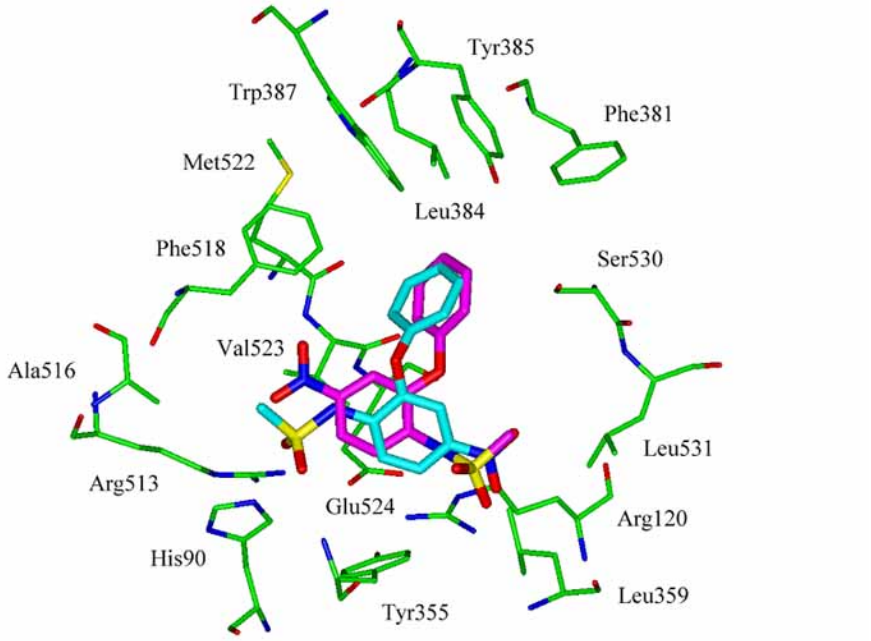

Fig.**13** : Orientation A and B of nimesulide complexed with the COX-2 (figure composed using InsightII [76]).

Docking simulations of NS398 were also performed to study interaction of such drug with COX-2 and to analyse perturbative changes after its complexation [32,63]. The orientation of NS398 described was similar to the orientation B of nimesulide. It was also shown that through its binding with the cyclooxygenase cavity, this drug affects the dynamic flexibility in the membrane binding domain and in a small catalytic lobe close to haem binding site.

Interactions of flosulide and analogues with the COX-2 were described by Rodriguez *et al.* [44]. The sulfonamide oxygen atom of flosulide was shown to interact with the NH_2 group of Arg120. A hydrogen bond was observed between the oxygen atom of the indanone moiety and the -OH group of Ser530. Moreover the para fluorophenyl substituent interacts with Tyr385.

3D-QSAR models of selective COX-2 inhibitors such as DuP697, NS398, flosulide and their analogues superimposed the sulfonamide group of DuP697 with the nitro or indanone groups of NS398 and flosulide [46]. Moreover the bromo atom in DuP697 and the methylsulfonamide of the other family are in the same region. Such alignment is in agreement with the orientation B of nimesulide.

As described on point 5.1.1, Rodrigues developed a **pharmacophore** model with diarylheterocycle and acidic sulfonamide compounds [44]. Four pharmacophoric points

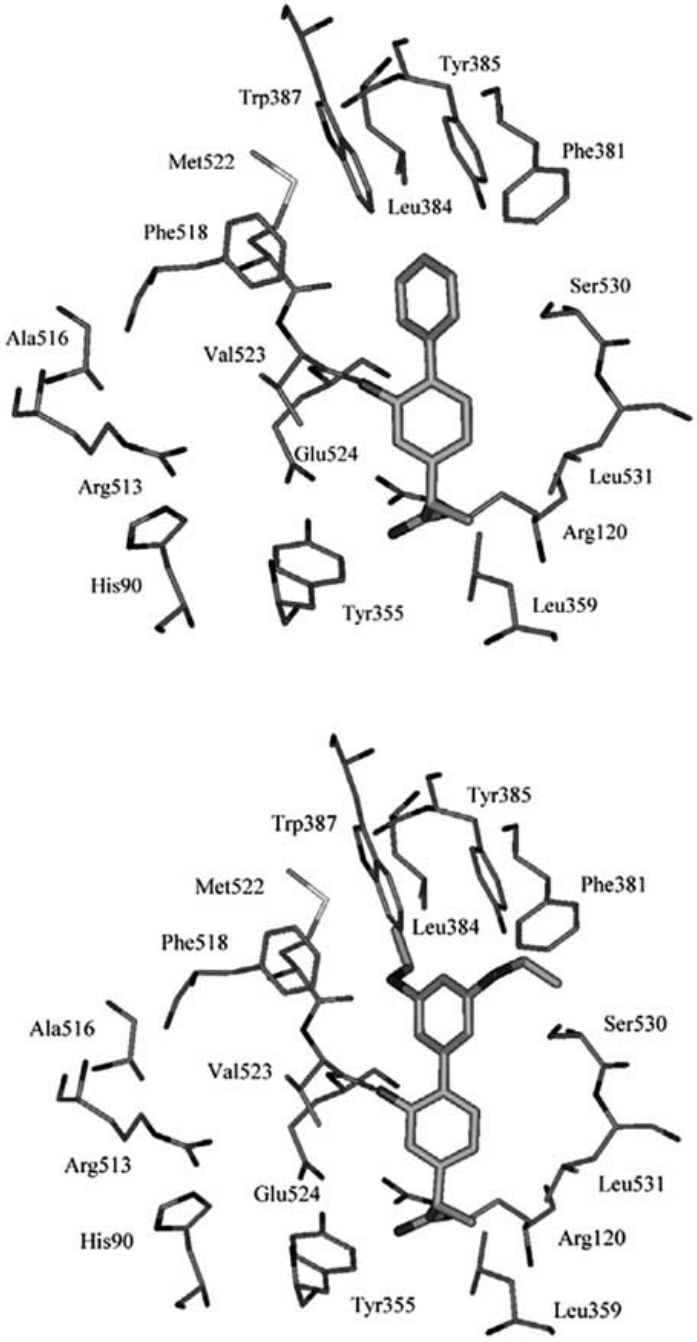

Fig. (14). Flurbiprofen and flurbiprofen analogue complexed with the COX-2 (figure composed using InsightII [76]).

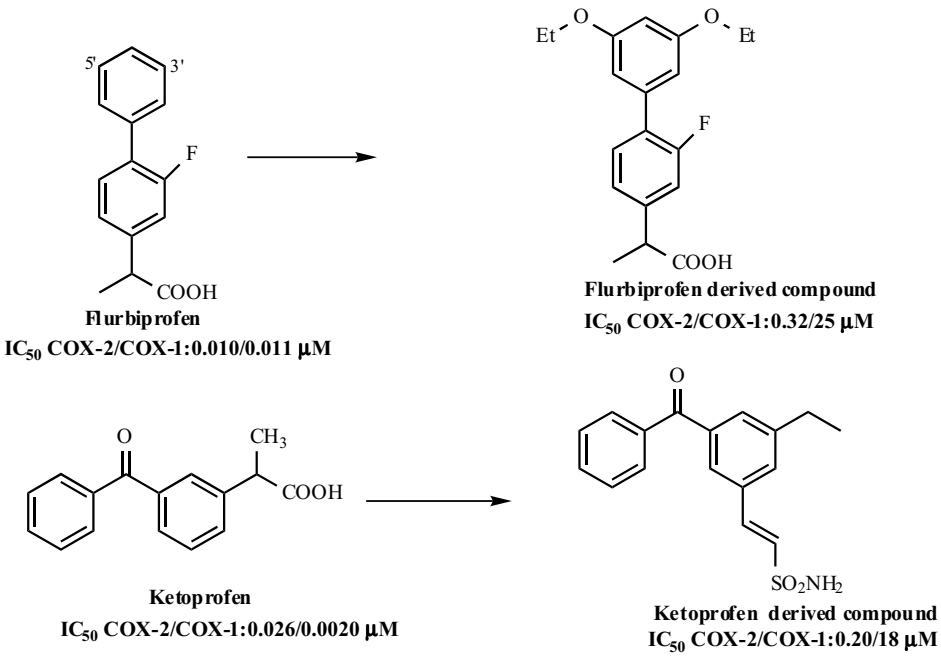

Fig. (15). Flurbiprofen and ketoprofen derived compounds as selective COX-2 inhibitors.

were obtained for the sulfonamide family: P1 is O and N atoms of the sulfonamide group; P2 is O atom of the indanone ring; P3 is halogen atom at position 4 of the phenyl ring; and P4 is C atom of the CH3 group of the sulfonamide (Fig. **8**). Such study shows that the acidic sulfonamide moieties would interact with the COX enzyme according to the orientation A.

From these studies, it is difficult to choose a preferential orientation for the acidic sulfonamide family but for the two ones, it seems that the selectivity is due partly to their access to the side pocket (Val523).

5.3 Modified NSAIDs

As explained above, one strategy used to obtain selective COX-2 inhibitors is to transform the structure of non-selective inhibitors such as arylpropionic acids (flurbiprofen, ketoprofen), indomethacin, zomepirac and aspirin.

5.3.1 Arylpropionic Acid Analogues

Crystal structure of flurbiprofen complexed with ovine COX-1 and murine COX-2 was resolved (Fig. **14**) [21,23]. The binding mode of this compound is similar in the two enzymes: the carboxylate moiety forms a salt bridge with Arg120 and an hydrogen bond with Tyr355. The distal phenyl ring forms van der Waals contacts with Val523, and the fluorophenyl ring interacts with Val349 and Ala527 and stacks with Tyr385. Flurbiprofen binds the hydrophobic channel without filling the hydrophilic pocket. This feature explains its non-selectivity.

In order to obtain a COX-2 specificity, 3',5'-bis-substituted flurbiprofen analogues were designed taking the lipophilic pocket at the top of the channel (with Leu384) into account [25]. Following this study, the substituent has to be small, lipophilic and coplanar with the phenyl ring of flurbiprofen. Moreover affinity is enhanced if the substituent can form hydrogen bond with Tyr385. The ethoxy substituents were found to be the best (Fig. **14**-**15**).

As described on 5.1.2, another study used the available information on the heterocycle diaryl family to modify ketoprofen [58]. Combination of a pharmacophore from the diaryl family and of computer 3D models of ketoprofen was performed. Several compounds were evaluated and the optimal activity was obtained combining the 3-sulfonylvinyl benzophenone frame with an additional non-polar substituent required for selectivity, for example meta-ethyl (Fig. **15**). From this study, we can conclude that the selectivity of such compounds results from their access to the side pocket (Val523) like the diaryl compounds.

5.3.2 Indomethacin Analogues

Indomethacin is one of the most potent NSAIDs. Crystal structure of iodo-indomethacin (the chlorine atom was replaced by iode) complexed within COX-1 and -2 was resolved (Fig. **16**) [64]. Indomethacin fills the entire hydrophobic channel and, as flurbiprofen, forms a salt bridge with Arg120. The iodophenyl group lies in a hydrophobic pocket at the top of the channel; the carbonyl moiety binds close to Ser530 and the indole ring is in contact with several aliphatic side chains, including Val349, Ala527, Leu352 and Val523. The O-methoxy interacts closely with the main-chain atoms of Tyr355 and Arg513.

Several modelling studies were performed in order to design analogues of indomethacin as COX-2 selective inhibitors.

Olgen *et al.* used the DOCK4.0 program to analyse potential differences in binding modes of N-substituted indole-2-carboxylic acid ester compounds, proposed to be selective towards COX-2, in the COX channel (Fig. **17**) [65]. This study indicates the COX-2 binding capability of some compounds. For compounds where R2 is a piperazine heterocycle, H bonds were found between the piperazine

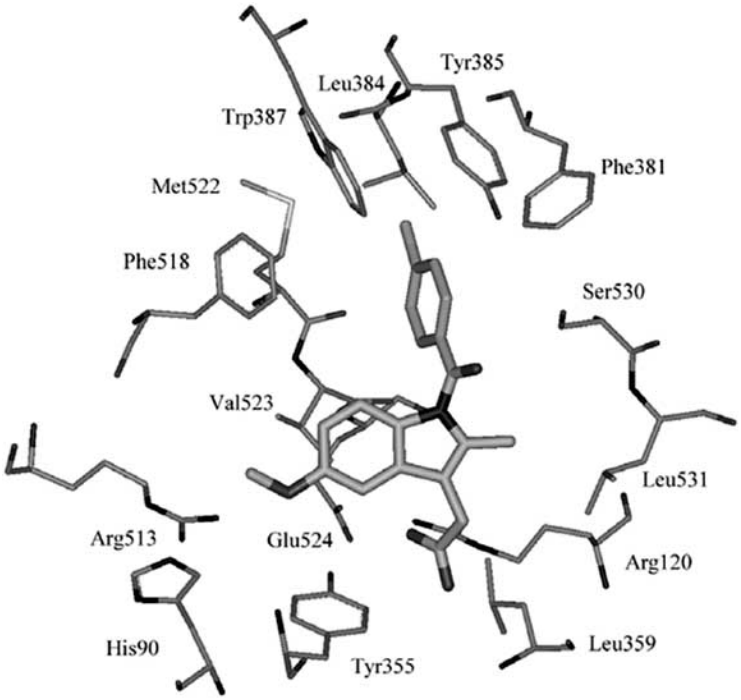

Fig. (16). Indomethacin complexed with COX-2 (figure composed using InsightII [76]).

hydrogen and the carbonyl oxygen of Phe518 and between the carbonyl and the amine hydrogen of Phe518.

As described above, Palomer *et al.* used, on the one hand, a pharmacophore developed from diarylheterocycle compounds and on the other hand, 3D crystal structure of COX-2 complexed with SC-558 and indomethacin to design indomethacin analogues having the basic N-benzyl- or N-benzoyl-5-sulfonylindole framework (Fig. **17**) [57]. Among these compounds one was confirmed as a promising selective COX-2 candidate. The substitution V523I is probably responsible for a part of the selectivity of this compound.

Moreover, replacement of the 4-chlorobenzoyl moiety of indomethacin with a 2,4,6-trichlorobenzoyl- or a 4-bromobenzyl group enhanced the COX-2 selectivity [66,67]. These larger groups are thought to bind in the "leucine tickle region" (Leu384). The carboxylate group was also replaced by ester and amide moieties that would interact with Tyr355 and Glu524 [68]. From these substitutions, selective COX-2 inhibitors were obtained. The reason of their selectivity is not clear.

5.3.3 Aspirin Analogues

Aspirin is the only NSAID that covalently modifies COX isoforms by acetylation of an active site serine residue [69,70]. In order to design COX-2 selective inhibitor from it, its carboxylate moiety was first replaced by a methyl sulfone group. There were two reasons for this substitution. First, the strong interaction between the carboxylate of

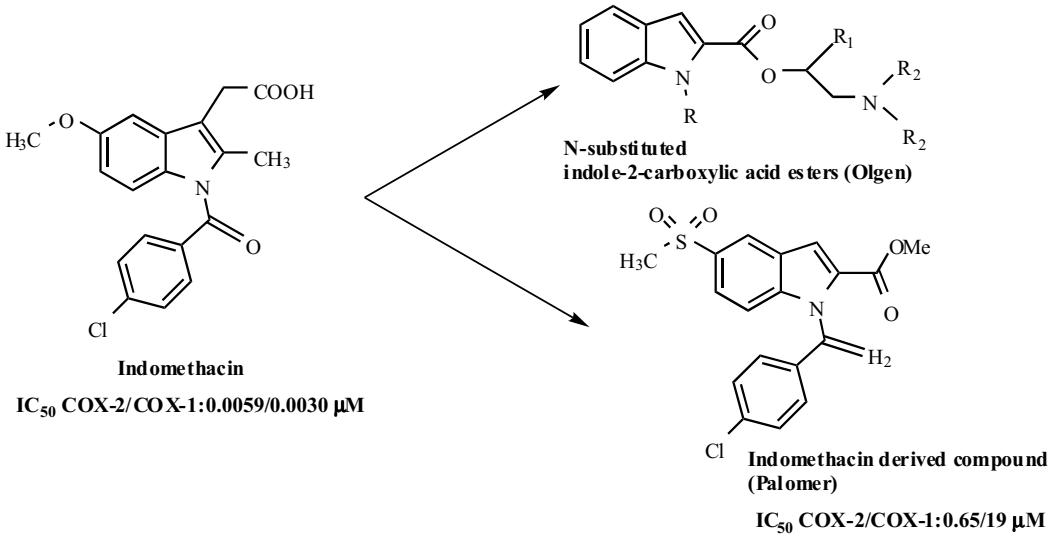

Fig. (17). Indomethacin derived compounds, as selective COX-2 inhibitors.

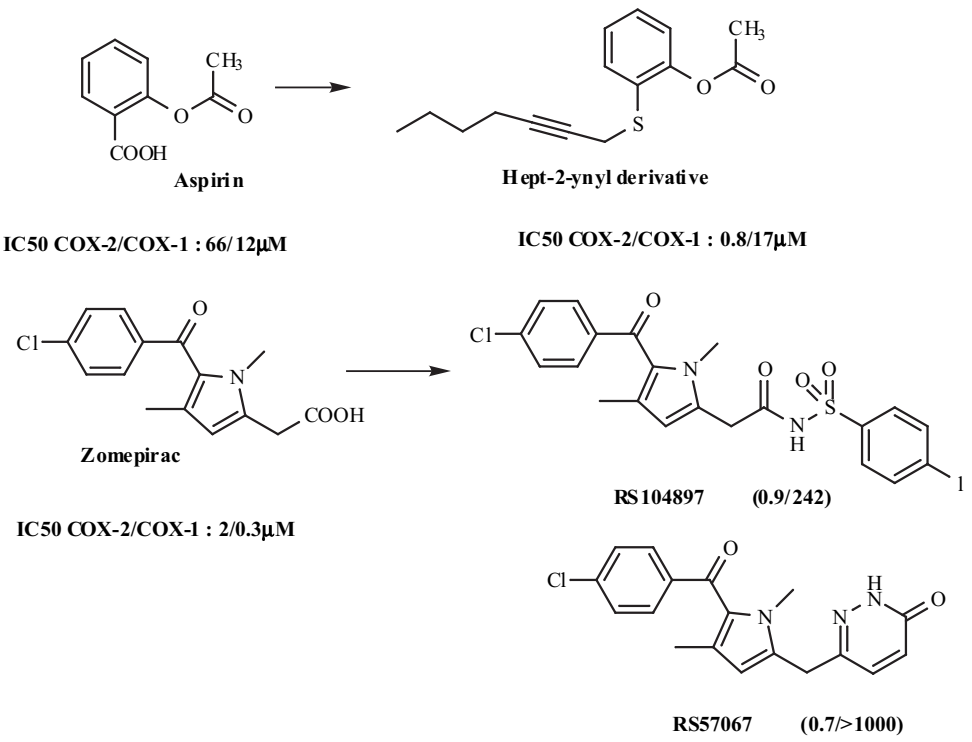

Fig. (18). Aspirin and zomepirac analogues as selective COX-2 inhibitors.

aspirin and Arg120 may overwhelm more subtle interaction necessary for the selectivity. Second, the methyl sulfone moiety is responsible for selective COX-2 inhibition in the diaryl heterocycle series.

Unfortunately this substitution was not efficient and was replaced by alkyl- or alkynylsulfide groups. This work led to an hept-2-ynyl derivative (APHS), a selective COX-2 inhibitor (Fig. **18**) [71]. No modelling study was performed on this family but site-directed mutagenesis was realised to understand the structural basis for COX-2 [72]. The results of those experiments reveal that the APHS selectively inhibits COX-2 by binding to previously uncharacterised regions in the COX-2 active site. The origin of the selectivity is so unclear yet.

5.4 Zomepirac Analogues

Zomepirac analogues, RS104897 and RS57067, were identified as COX-2 selective inhibitors by an in vitro screening assay and co-crystallised with the human COX-2 (data not available in the PDB) (Fig. **18**) [22]. In these derivatives, the common carboxylic acid has been replaced by other functional groups. These compounds are COX-2 selective whereas they only bind in the hydrophobic channel. The upper chlorophenyl ring of these compounds is positioned near Tyr385, which is very similar to the position of the upper phenyl ring of flurbiprofen in COX-1. H bond is observed between the carbonyl oxygen and Ser530. Arg120 and Tyr355 interact with the nitrogen and oxygens of the acyl-sulfonamide of RS104897, in the same way as the interaction with the acid of flurbiprofen. The iodophenyl ring lies into the membrane binding region.

Unlike RS104897 that binds in a closed conformation, RS57067 binds in an open conformation. In this latter complex, the pyridazinone ring induces a displacement of Arg120 causing a modification in the H bond network. The difference between these two complexes demonstrates the conformational flexibility at the juncture of the membrane binding domain.

As there are no direct interactions between these inhibitors and amino acids unique in COX-2, it is difficult to rationalise the selectivity of these inhibitors based on the crystal structure.

5.5 Enol Carboxamide Family

Meloxicam, an enolcarboxamide, was discovered as an anti-inflammatory agent with relatively low gastrointestinal side effects when compared with other NSAIDs (Fig. **19**) [73,74].

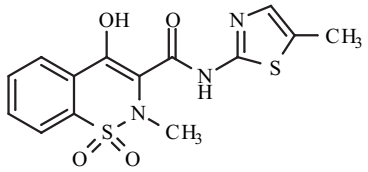

Fig. (19). Meloxicam.

A first modelling study showed for meloxicam a reinforced binding with an hydrophilic pocket at the bottom of the hydrophobic channel in COX-2 through the thiazole ring [75]. Access to this pocket is facilitated in COX-2 because of Val89 instead of Ile89 in COX-1. Complex of this compound with the two COXs clearly demonstrates the destabilising interferences of Ile523 and Ile89 of COX-1 for its binding. It was also observed that the SO_2 moiety of meloxicam has moved a little bit away from the hydrophilic pocket (Val523) compared to celecoxib and nimesulide.

Meloxicam does not fill the top of the hydrophobic pocket (Tyr385, Trp387).

A second study described a different binding mode for meloxicam where the 5-methyl group of the thiazole ring fills the flexible "extra space" at the top of the COX-2 channel [40]. The substitution of Ile434 in COX-1 by Val in COX-2 allows the side chain of Phe518 to open "extra space" which facilitates the binding of the compound to COX-2. Moreover it appears that the thiazine-sulfone can form hydrogen bonds with Arg120 and Tyr355.

As a result, the binding mode of meloxicam is yet unclear.

6. CONCLUSION

Since the discovery of a second COX isoform, several families of compounds were designed in order to identify selective COX-2 inhibitors. Some of them were cocrystallised with COX-1 and/or COX-2 and studied by molecular modelling in order to understand their binding mode and the molecular features essential for selectivity. Unlike the complexity of the problem, we can try to classify these compounds according to the origin of their selectivity.

Firstly, the COX-2 selectivity is sometimes related to the **V523I substitution** and to the access to the polar side pocket. It is the case of diaryl heterocycle family, acidic sulfonamide compounds and some flurbiprofen and indomethacin analogues.

Secondly, the origin of the specificity can come from the **L503F substitution**, allowing access to a lipophilic alcove at the top of the channel. The compounds matching to this criterion are other flurbiprofen and indomethacin derivatives.

Finally, there are series for which the cause of their selectivity is **unclear** or unknown. These are zomepirac and aspirin analogues.

As the binding mode of meloxicam is discussed, it is difficult to rationalise its selectivity.

In conclusion, this review shows that the mechanism of COX inhibition is quite complex and presents a challenge for the pharmaceutical research. Future researches should be interesting to elucidate completely the molecular requirements for the COX inhibition.

ACKNOWLEDGEMENTS

Catherine Michaux and Caroline Charlier are grateful to Prof. F. Durant for his expert advice. The authors thank the Belgian Foundation for Scientific Research (FNRS) and the French Community of Belgium – Concerted Research Action n°99/04-249 – for financial support.

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