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Molecular Basis of Binding Interactions of NSAIDs and Computer-Aided Drug Design Approaches in the Pursuit of the Development of Cyclooxygenase-2 (COX-2) Selective Inhibitors

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Abstract

The nonsteroidal anti-inflammatory drugs (NSAIDs) are important class of therapeutic agents used for the treatment of pain, inflammation and fever. Nonselective inhibition of cyclooxygenase (COX-1 and COX-2) isoenzymes by classical NSAIDs is associated with undesirable side effects such as gastrointestinal (GI) and renal toxicities due to COX-1 inhibition. To circumvent this problem, several COX-2 selective inhibitors were developed with superior GI safety profile. However, the voluntary market withdrawal of potent COX-2 selective inhibitors (rofecoxib and valdecoxib) due to their severe cardiovascular toxicity which is also found to be associated with some of the traditional NSAIDs suggesting the need to relook into the entire class of NSAIDs rather than exclusively victimizing the COX-2 selective inhibitors. Furthermore, the recent evidences for the involvement of COX-2 selective inhibitors in the aetiology of many diseases, such as Alzheimer's disease, Parkinson's disease, diabetes, various cancers and so on, have gained much attention for researchers to design and develop novel COX-2 selective inhibitors with improved pharmacodynamics and pharmacokinetic profile. This chapter is focused on the detailed analysis of molecular basis of binding interactions of various NSAIDs by highlighting the role of crucial amino acid residues at the binding site of cyclooxygenase enzymes (COXs) to be considered for selective inhibition of COX-2 enzyme while emphasising the impact of significant CADD strategies employed for designing new potent COX-2 inhibitors with tuned selectivity.

Keywords: molecular binding interactions of NSAIDs, computer-aided drug design of COX-2 selective inhibitors, development of COX-2 selective inhibitors

1. Introduction

The nonsteroidal anti-inflammatory drugs (NSAIDs) are important therapeutic agents used for the treatment of pain, inflammation and fever [1, 2]. NSAIDs act by reducing the production of pro-inflammatory prostaglandins (PGs) at the sites of injury through the obstruction of cyclooxygenase enzyme (COX) binding site by sterically preventing the binding of the endogenous arachidonic acid (AA) [1–3]. There are two different isoforms of COX isoenzymes, a constitutive form (COX-1) and an inducible form (COX-2), respectively [4]. The constitutive COX-1 isozyme plays an important role in many physiological functions, such as cytoprotection of gastric mucosa, renal blood flow regulation and platelet aggregation. The expression of COX-2 isozyme is mainly induced by several stimuli such as hormones, growth factors, mitogens, oncogenes and disorders of water-electrolyte homeostasis resulting in its involvement to pathological processes such as inflammation and various types of cancer [5]. The classical NSAIDs (aspirin, ibuprofen, flurbiprofen, naproxen, indomethacin, diclofenac, mefenamic acid, piroxicam, etc. (**Figure 1**)) are associated with side effects such as gastrointestinal (GI) ulcer and renal toxicity due to their nonselective inhibition of COX-1 pathway [6, 7]. As a result, a number of COX-2 selective inhibitors such as rofecoxib, celecoxib, valdecoxib and etoricoxib (**Figure 2**) were introduced into the market as safer NSAIDs which were devoid of GI toxicity. The voluntary market withdrawal of rofecoxib (Vioxx) by Merck in September 2004 based on APROVe (Adenomatous Polyp Prevention on Vioxx) study followed by valdecoxib (Bextra) in 2005 (Pfizer) due to their association with increased cardiovascular risk imposed a big question

Figure 1. Representative structures of classical NSAIDs (nonselective COX inhibitors).

Figure 2. Structures of COX-2 selective inhibitors.

on the safety profile of this class of COX-2 selective inhibitors [8]. Interestingly, no such increased cardiovascular risk was observed from the Celecoxib Long-term Arthritis Safety Study (CLASS) trial. Celecoxib (Celebrex) is the only COX-2 selective inhibitor currently available in the US market with cautions of cardiac risk. Moreover, some of the nonselective NSAIDs such as high dosage of diclofenac and ibuprofen are also found to be associated with similar incidences of cardiovascular toxicity like COX-2 selective inhibitors [9]. Several research findings suggested that the adverse cardiovascular effects of COX-2 selective inhibitors might be dependent on the dose as well as duration of action [10–12]. However, the most potent COX-2 selective inhibitor, lumiracoxib (with different structures from other coxibs (**Figure 2**)), did not exhibit considerable cardiovascular adverse effects in Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET) but found to be associated with serious hepatic toxicity which resulted in its withdrawal both from the Australian and European market [13, 14]. Furthermore, recent studies revealed the association of COX-2 with various other pathophysiological processes such as Alzheimer's disease, Parkinson disease, schizophrenia, epilepsy, depression, diabetic peripheral neuropathy and various cancers. [15, 16]. The recognition of new roles for COX-2 selective inhibitors imposed a great challenge to the researchers to design and explore alternative scaffolds to develop COX-2 selective inhibitors with improved potency and efficacy in order to circumvent various side effects associated with the currently available NSAIDs. Thus, a detailed analysis of the characteristic structural differences between the two isoenzymes along with the binding interactions of different NSAIDs is essential to construct a novel structure-based pharmacophore model for designing potent inhibitors with augmented COX-2 affinity as well as selectivity. This chapter is focused on the molecular basis of binding interactions of various NSAIDs by highlighting the role of crucial amino acid residues at the binding site of cyclooxygenase enzymes to be considered for selective inhibition of

COX-2 enzyme while emphasising the impact of various significant computer-aided drug design (CADD) approaches employed for designing new potent COX-2 inhibitors with tuned selectivity.

2. Progress in the pursuit of the development of cyclooxygenase-2 selective inhibitors

The discovery of the specific role of COX-2 enzyme in inflammation resulted in the development of several COX-2 selective inhibitors to overcome the GI side effects of classical NSAIDs [15, 16]. Interestingly, before the confirmation of existence of COX-2 enzyme, the DuPont company discovered a compound Dup-697 (**Figure 2**) as a potent anti-inflammatory agent without having the ulcerogenic effects of NSAIDs [17]. After the discovery of COX-2 enzyme, Dup-697 became the lead molecule for the development of COX-2 selective inhibitors (coxibs); as a result celecoxib and rofecoxib became the pioneer COX-2 selective inhibitors to reach the market [18, 19]. Dup-697 is a diaryl heterocyclic compound with cis-stilbene moiety. It has been observed from the structure-activity relationship (SAR) studies that the diaryl heterocyclic compounds possessing cis-stilbene moiety with variation in the para-position of one of the aryl rings play an important role in inducing selectivity for COX-2 as compared to COX-1 enzyme [20]. Celecoxib, valdecoxib and parecoxib (prodrug of valdecoxib) possess sulphonamide (SO_2NH_2) group, whereas etoricoxib and rofecoxib have a methyl sulphone (SO_2CH_3) group at the para-position of one of the aryl rings (**Figure 2**). Several attempts were made to extensively manipulate the ring system that is fused with the cis-stilbene system to include every possible heterocyclic ring of varying sizes as well as by altering the scaffolds of classical NSAIDs to convert them into COX-2 selective inhibitors, but none could successfully reach the market. Recently, a series of thiazole derivatives [21], cycloalkyl/aryl-3,4,5-trimethylgallates [22], thienopyrimidine derivatives [23], 3-alkoxy-4-methanesulfonamido acetophenone derivatives [24] and 8/10-trifluoromethyl-substituted-imidazo[1,2-*c*]quinazolines [25], have been designed, synthesised and reported from our research group in search of compounds with novel scaffold as potent anti-inflammatory agents.

Computer-aided drug design (CADD) strategies have been emerged as a potential tool for the discovery of new drugs. In the pursuit of the discovery and development of novel NSAIDs with selective inhibition of the COX-2 enzyme, various ligand-based 3D-QSAR and pharmacophore models were reported [15]. But these 3D-QSAR models are developed mainly based on particular classes of compounds which may not be applicable for the prediction of structurally diverse compounds. In contrast, the structure-based drug design approaches such as molecular docking and molecular dynamics (MD) simulation studies are based on detailed analysis of the binding site of target protein for designing novel drugs with improved potency. The availability of various 3D X-ray crystal structures of COX-1 and COX-2 isoenzymes co-crystallised with diverse selective and nonselective inhibitors provides an opportunity to gain insight into various physicochemical requirements for effective binding of a ligand with selective inhibition of COX-2.

3. Structural and functional insights of cyclooxygenase enzymes

The COX-1 enzyme is constitutively expressed in most tissues where it is encoded by PTGS-1 gene (codes for a relatively stable 2.8 kb mRNA). On the other hand, COX-2 is encoded by PTGS-2 gene (codes for a relatively less stable 4 kb mRNA), which is activated by several inflammatory and proliferative stimuli [3]. The difference in gene expression account for the existence of two COX isoforms, signifying that COX-1 provides PGs essential for maintaining homeostasis including gastric cytoprotection, whereas COX-2 plays an important role in producing PGs during various pathological conditions such as inflammation and tumourigenesis [26]. These observations became the driving force for the rapid development of COX-2 selective inhibitors having anti-inflammatory activity while avoiding GI side effects associated with traditional nonselective NSAIDs.

Human COX-1 and COX-2 enzymes exist as homodimers of 576 and 581 amino acids, respectively, and each monomer having a molecular mass of about 70 kDa [3, 26]. Both enzymes are almost identical in their general tertiary structure with 60% sequence similarity. The signal peptide of COX-1 is longer (with seven amino acid residues) than COX-2, and the N-terminus of COX-1 has an insertion of eight residues, while the C-terminus of COX-2 has an insertion of eighteen residues. Each subunit of COX-1 and COX-2 dimers consists of three structural domains: the N-terminal epidermal growth factor (EGF) domain (amino acid residues 34–72), the α -helical membrane-binding domain (amino acid residues 73–116) and the C-terminal catalytic domain, which comprises the bulk of the protein. The catalytic domain contains the cyclooxygenase and peroxidase active sites on either side of the heme prosthetic group [26–28]. Recent studies revealed that only one monomer of the COX homodimer is active at a given time [29]. It has been postulated that these monomers can act additionally through an allosteric/catalytic couple, with AA oxygenation being controlled in the ‘catalytic’ monomer (E_{cat}) through the binding of non-substrate fatty acids and nonselective NSAIDs to the opposite monomer, the ‘allosteric’ monomer (E_{allo}) [30, 31]. Moreover, the major differences between COX-1 and COX-2 are the substitutions of the bulkier amino acid residues Ile434, His513 and Ile523 in COX-1 by comparatively smaller residues Val434, Arg513 and Val523, respectively, in COX-2 at the main channel of cyclooxygenase binding site (**Figure 3**). These substitutions produce a 25% increase in the volume of the active site at COX-2 along with

Figure 3. Structures of amino acid residues playing crucial role at the active site of COX enzymes.

the creation of a side pocket off the main channel with Arg513 located at its base [26]. The sulphonamide or methyl sulphone moieties of diaryl heterocycle-based coxibs were mainly designed to bind within this side pocket to provide selective inhibition of COX-2.

4. Molecular basis of inhibition of cyclooxygenase enzymes and computer-aided drug design (CADD) approaches employed for the design and discovery of COX-2 selective inhibitors

The experimental methodologies such as site-directed mutagenesis, X-ray crystallographic analysis along with various CADD approaches such as structure-based molecular docking studies (employing Amber, Flexi dock, Fast dock, Glide), MD simulation, metadynamics simulation studies and ligand-based 3D-QSAR (by using COMFA, COMSiA) and pharmacophore modelling were extensively used to understand the molecular basis of interactions of NSAIDs with COX enzymes as well as to design novel potent COX-2 selective inhibitors. The site-directed mutagenesis and X-ray crystallographic structures of COX-1 and COX-2 isoenzymes indicate that selective and nonselective inhibitors generally bind in two different patterns which provides an impetus for the rational modulation of existing binders to improve selectivity and potency. It has been observed that the selectivity pocket of COX-1 is comparatively smaller due to the presence of bulky amino acid residue Ile523, whereas in COX-2 the presence of smaller amino acid residue Val523 enlarged the selectivity pocket providing a more stable binding opportunity for selective inhibitors [32, 33]. Further, the kinetics of selective and nonselective inhibitors were found to be different, and it has been postulated that the association of COX-2 selective inhibitor SC-299 with COX-1 and COX-2 occurs at similar rate, while the dissociation of SC-299 from COX-2 is 100-fold slower than COX-1 indicating the correlation between the relative rate of dissociation and the selective inhibition of COX-2 isoenzyme [34]. This correlation was also confirmed by Walker et al. in additional experiments on other COX-2 selective inhibitors [35]. The stable binding mode of selective inhibitors to COX-2 isoform is also attributed to the presence of a different amino acid residue Val434 instead of Ile434 as found in the binding site of COX-1 enzyme.

The active site of COX-2 is mainly hydrophobic where most of the protein-ligand interactions are stabilised by van der Waals forces. According to the X-ray crystal structure of COX-2 bound with the SC-558 (PDB: 1CX2; 2.5 Å resolution) [33], the ligand binds in the cyclooxygenase active site where the bromophenyl ring occupies a hydrophobic pocket formed by Tyr348, Phe381, Leu384, Tyr385, Trp387, Gly526, Ala527 and Ser530, respectively (**Figure 4**). The trifluoromethyl group attached to the pyrazole ring is surrounded by a close hydrophobic cavity formed by Met113, Val116, Val349, Tyr355, Leu359 and Leu531 where only Arg120 (located near to CF₃ group) introduces a strong electrostatic field. This cavity is referred to as common pocket (**Figure 4A**) as it is also found to be occupied by the aromatic ring bearing the carboxylate functional group of many nonselective COX inhibitors such as ibuprofen and flurbiprofen, respectively [38]. Furthermore, these two features of SC-558 binding are almost equivalent to the binding mode of flurbiprofen and indomethacin [33]. The phenylsulphonamide moiety of

Figure 4. (A) 2D X-ray crystallographic pose of SC-558 in COX-2 (unpublished pose taken with the help maestro academic visualiser 10.4 [36] by using the crystal structure of COX-2, PDB ID: 1CX2). (B) UBEXTRACT plots (electrostatic, van der Waals and total energy) for the interaction of SC-558 with the different residues of the protein (values shown correspond to the average of 1.5 ns of MD simulation) (reprinted with permission from Robert et al. [37], copyright © 2003 American Chemical Society).

SC-558 is found to be anchored within a selectivity pocket formed by His90, Asn192, Leu352, Ser353, Arg513, Ala516, Ile517, Phe518 and Val523 assuming a conformation in which one of the oxygen atoms forms H-bond with Arg513 and is close enough to interact with His90, while the amide hydrogens are able to interact with the backbone of Phe518 through two water bridges. The phenylsulphonamide moiety of SC-558 is mainly responsible for inducing selectivity for COX-2, where it is easily accessible to the selectivity pocket due to the presence of smaller residue Val523 that is more restricted in COX-1 because of the substitution of valine to isoleucine at the same position-523 [33, 38].

Robert et al. demonstrated the binding mechanism of different NSAIDs (mainly celecoxib and rofecoxib analogues) to the cyclooxygenase active site of COX-2 based on molecular dynamics (MD) simulation and free energy calculation studies [37]. The MD simulation study carried out by using the homology model of human COX-2 also revealed similar binding mode of interaction of SC-558 as observed from crystallographic pose [33]. To investigate the key residues involved in the interaction of NSAIDs with COX-2, the MD trajectories were analysed by using UBEXTRACT programme. For example, the UBEXTRACT analysis [37] indicated that mainly four residues (Arg120, Asn192, Leu352 and Arg513) made significant electrostatic interactions, whereas thirteen residues involved in van der Waals interaction with the SC-558 (**Figure 4B**). The inspection of experimental data of valdecoxib suggested that the methyl group attached to the central isoxazole ring is favourable for binding by making hydrophobic interaction. The MD simulation study of rofecoxib [37] showed that the carbonyl group of furanone ring formed an H-bond with Ser530. It is interesting to note that to achieve this contact the side chain of Ser530 adopted a 'down orientation', whereas it favours 'up conformation' while interacting with celecoxib because of water-mediated H-bond between Ser530 and Tyr385 residues (**Figure 5A and B**).

Vittorio et al. used an advanced metadynamics-based computational technique to simulate the full dissociation process of a highly potent and selective inhibitor SC-558 in both COX-1 and COX-2 isoenzymes [38]. The metadynamics study of SC-558 dissociation process in COX-2 was able to reproduce the X-ray crystallographic pose and also revealed the possibility of an alternative binding mode (**Figure 6A and B**). In this alternative binding mode (**Figure 6B**), the bromophenyl moiety is found to be anchored within a highly hydrophobic cavity formed by Ile345, Val349, Leu359, Leu531 and Met535, while the trifluoromethylpyrazole moiety undergone 180° rotation which resulted in improved interactions with neighbouring residues such as Leu352, Phe518, Val523, Gly526 and Ala527, respectively. Finally, the sulphonamide group engages in the formation of H-bond with Tyr355 and Arg120 (**Figure 6B**). Similar observation is also evident from another study where the mutation Tyr355Phe disfavoured the binding of many ligands to COX [40]. Further support to this alternative binding mode of SC-558 comes from the very similar binding mode of some of the experimentally observed nonselective COX inhibitors, in particular the binding mode of ibuprofen to COX-1 (PDB ID: 1EQG) where the main interactions with the protein are well conserved [41]. The carboxylate group of ibuprofen forms polar interactions with Tyr355 and Arg120 similarly to the sulphonamide moiety of SC-558 (**Figure 6C**). In either case, the common pocket is occupied by the phenyl ring in ibuprofen and the pyrazole moiety in SC-558. The similarity is even greater in case of flurbiprofen or diclofenac, where a halogen atom is substituted in the phenyl ring enforcing the hydrophobic interactions with Leu352, Phe518 and Val523 similar to trifluoromethyl group of SC-558 (in alternative pose).

Figure 5. (A) Comparison of the SC-558 and rofecoxib binding modes after superposition of both protein binding sites. (B) Details of the rofecoxib binding site when Ser530 is in the 'up' (magenta) and in the 'down' (green) conformation. The conformation found in the crystal structure (brown) is also displayed for comparison (reprinted with permission from Robert et al. [37], copyright © 2003 American Chemical Society).

Figure 6. (A) The X-ray crystallographic pose of SC-558 in COX-2 (PDB ID code 1CX2) reproduced during the metadynamics simulations. (B) The alternative binding pose of SC-558 in COX-2 found during metadynamics simulations. (C) The X-ray binding conformation of ibuprofen in complex with COX-1 (PDB ID code 1EQG). The ligands are represented as yellow, whereas the protein is represented as green cartoon with the α -helices forming the gate coloured in orange and the hydrogens are not displayed for clarity (reprinted with permission from Vittorio et al. [38]).

The experimentally observed time-dependent slow tight-binding inhibition of other diaryl heterocyclic compounds similar to SC-558 is interpreted due to the presence of an additional binding step, where a vital role might be played by the rearrangement of the hydrogen-bonding network formed by Arg120, Tyr355 and Glu524, respectively, which are assumed to be critical for the transition from the relaxed to the tightened state of the enzyme. The involvement of these key residues (Arg120 and Tyr355) in the newly observed alternative binding mode of SC-558 suggests that the time-dependent inhibition kinetics of SC-558 results from the ability of the ligand to bind in two distinct but equally strong ways [38].

The metadynamics simulation study of SC-558 dissociation process in COX-1 reveals that the conformation of SC-558 in COX-1 is very similar to the crystallographic pose of SC-558 in COX-2, where the ligand is more weakly bound because of the partial insertion of the sulphonamide group into the selectivity pocket of COX-1 due to the presence of bulkier Ile523 in contrast to Val523 in COX-2 [38]. Moreover, the presence of the bulkier residue Ile523 in COX-1

also nullifies the occurrence of alternative binding mode that is observed in case of COX-2 binding interaction. This hypothesis of selective inhibitors with their high residence time in COX-2 due to the existence of additional binding step/mode is also evident from another study on a series of COX-2 selective inhibitors [34, 35]. The binding conformation of SC-558 in COX-1 determined through metadynamics simulation study is very similar to the crystallographic pose found for celecoxib in COX-1 [39] with a low RMSD of 1.46 Å for the ligand heavy atoms.

The analysis of X-ray crystallographic binding mode of Vioxx (rofecoxib) with huCOX-2 (PDB ID: 5KIR) [42] shows that the inhibitor makes a total of 42 contacts with amino acid residues (mainly hydrophobic in nature), while the methyl sulphone moiety occupies the side pocket of the cyclooxygenase channel and the phenyl ring extended up towards the side chain of Tyr385 (**Figure 7A–C**). The oxygen (O) atoms of the methyl sulphone moiety of the inhibitor

Figure 7. Comparison of celecoxib and Vioxx (rofecoxib) bound within the cyclooxygenase channel of COX-2. (A and B) Chemical structures of (A) celecoxib and (B) Vioxx. Although both inhibitors share a common diaryl heterocycle scaffold, celecoxib contains a pyrazole heterocycle and a sulphonamide moiety, whereas Vioxx contains a furanone heterocycle and a methyl sulphone moiety. (C) Stereoview showing an overlay of Vioxx (yellow) and celecoxib (magenta) from PDB entry 3LN1 bound within the cyclooxygenase channel of COX-2 (C^{α} r.m.s.d. of 0.31 Å for 542 pairs). The binding mode of the two coxibs is conserved, with the sulphone/sulphonamide moieties penetrating into the COX-2-specific side pocket. C-atoms of residues lining the channel are coloured green for huCOX-2 and salmon for muCOX-2, whereas N- and O-atoms are coloured blue and red, respectively (reprinted with permission from Orlando and Malkowski [42]).

made the only significant hydrophilic interactions with the side-chain N-atoms of His90 and Arg513, respectively, positioned at the base of the side pocket. It is surprising to note that Vioxx is approximately 60-fold more COX-2 selective than celecoxib [43], but it binds in the same general conformation as evident from celecoxib binding within the cyclooxygenase active site (**Figure 7C**) [44]. The reason for the difference in COX-2 selectivity between these two inhibitors presumed to be because of their differences in binding kinetics rather than in a particular enzyme-inhibitor interaction. Hence, the binding and dissociation kinetic analysis of both Vioxx and celecoxib might provide a clear rationale for their different degrees of isoform selectivity.

Further, the X-ray crystallographic pose of celecoxib/ovCOX-1 [39] shows that the Ile523 propagates a shift of side-chain residues such as His513, Pro514 and Asn515 in the β -turn loop of the 'side pocket' (**Figure 8**). The trifluoromethyl (CF_3) group attached to the pyrazole ring of celecoxib does not interact with Arg120 typically observed with substrates and other carboxylic acid-containing inhibitors. Instead, the trifluoromethyl group adjoins Tyr355 where the phenyl ring of Tyr355 makes edge-to-face contact with the aromatic ring of the benzene-sulphonamide group of celecoxib (**Figure 8**). It has been also observed that both inhibitors, SC-558 and celecoxib, bind in a similar manner with two exceptions. The sulphonamide group forms a hydrogen bond with His513 in ovCOX-1 which is Arg513 in muCOX-2. In ovCOX-1, the rigid histidine residue does not form a hydrogen bond with celecoxib. The amide nitrogen of sulphonamide moiety of celecoxib forms short N—H—O H-bonds with the side chain of Gln192 and main chain of Leu352 (**Figure 8**). Moreover, Ile434 (Val434 in COX-2) is proposed to act as a gate to prevent Phe518 from moving away when bound to COX-2 selective inhibitors. As a result, Phe518 forms hydrophobic contacts with the benzene ring of the inhibitor in COX-1.

The most commonly used nonselective NSAID, aspirin, covalently modifies COX-1 and COX-2 in a time-dependent manner via the acetylation of the hydroxyl group of Ser-530 [45].

Figure 8. Celecoxib binding to ovCOX-1 as determined by X-ray crystallography [39] (reprinted with permission from Gilad et al. [39]).

The binding mode of flurbiprofen is found to be identical in both COX-1 and COX-2 (PDB ID: 3PGH) isoenzymes [33]. Moreover, it also shows similarity with the binding mode of two features of SC-558, where the distal phenyl ring and the fluorophenyl ring of flurbiprofen superimpose with the bromophenyl ring and pyrazole ring of SC-558, respectively. In contrast, the distal phenyl ring of flurbiprofen forms a π -stacking interaction with Tyr385 while interacting with Ser530, the residue that is selectively acetylated by aspirin, whereas the carboxylate group forms a salt bridge with the guanidine moiety of Arg120 and a hydrogen bond with Tyr355, respectively (**Figure 9B**). Furthermore, the fluorine atom of fluorophenyl ring interacts with the side chain of Ile523 in COX-1, which is absent in COX-2 because of the presence of a smaller residue valine at the corresponding position-523.

Similarly, the X-ray crystallographic binding mode of another nonselective COX inhibitor, indomethacin (PDB ID: 4COX) [33], reveals that it anchors well within the cyclooxygenase active site of COX-2 (**Figure 9A**) by forming a salt bridge between the carboxylate group and guanidine moiety of Arg120 (similar to flurbiprofen), while the indole ring mainly interacts with Val349 and Ser353 and forms additional contact with Tyr355, Val523 and Ala527, respectively. The six-member ring of indole forms close interaction with Leu352 and Ser353, whereas the *o*-methoxy group protrudes slightly into a relatively large cavity adjacent to Ser353, Tyr355 and Val523 in COX-2. The benzoyl group occupies a position very similar to that of the distal phenyl ring of flurbiprofen and forms stable hydrophobic interactions with Phe381, Leu384, Tyr385 and Trp387, respectively, while the benzoyl oxygen forms H-bonding interactions with side-chain hydroxyl group of Ser530 and with Val349, and the chlorine atom interacts with Leu384. It has to be noted that the benzoyl ring can adopt either *cis* or *trans* conformation with respect to indole ring and possibility of *cis* conformation is claimed to be preferable based on the conformation of indomethacin complex with COX-1. It has also been anticipated that the benzoyl oxygen plays a vital role in enhancing the affinity for COX-1.

Duggan et al. in their study based on site-directed mutagenesis and X-ray crystallography, postulated that the binding mode of naproxen (**Figure 9C**) is similar to other members of the 2-arylpropionic acid family of NSAIDs [46]. It is important to note that the majority of 2-arylpropionic acid family of NSAIDs are marketed as racemic mixtures except naproxen which is exclusively sold as the (*S*)-enantiomer. The (*S*)- α -methyl group of naproxen plays a crucial role by forming a critical interaction with the COX enzymes and occupies into a hydrophobic cleft below Val-349, whereas the removal or substitution of methyl group with a range of substituents of varying size and stereochemistry at the α -position results in a dramatic loss of potency. The carboxylate group makes hydrogen-bonding interactions with Arg-120 and Tyr-355 at the base of the active site. The *p*-methoxy group of naproxen forms van der Waals interactions with Tyr-385 and Trp-387, while the naphthyl moiety of naproxen makes hydrophobic interactions with Ala-527, Gly-526 and Leu-352, respectively (**Figure 9C**). It has been observed that the side chain of Leu-352 adopts an alternate conformation as compared to that observed in case of binding interactions of co-crystal structures of flurbiprofen, indomethacin and diclofenac bound to mCOX-2 (**Figure 9A–D**) [46].

Comparative analysis of X-ray crystal structures of other 2-arylpropionic acids and the diaryl heterocyclic compounds bound to the COX enzymes shows that the α -methyl or

Figure 9. (A) Binding mode of the crystal structure of indomethacin (PDB ID: 4COX) at the COX-2 active site showing H-bonding with constriction residues Arg-120 and Tyr-355 at the base of the active site. (B) Binding mode of the crystal structure of flurbiprofen (PDB ID: 3PGH) at the COX-2 active site showing H-bonding with constriction residues Arg-120 and Tyr-355. (C) Binding mode of the crystal structure of naproxen (PDB ID: 3NT1) at the COX-2 active site showing H-bonding with constriction residues Arg-120 and Tyr-355 (similar to indomethacin and flurbiprofen). The inhibitor does not enter the side pocket into which the phenyl sulphonamide or phenyl sulphone moieties of diaryl heterocyclic compounds protrude. (D) Binding mode of the crystal structure of diclofenac (PDB ID: 1PXX) bound at the COX-2 active site showing the opposing binding mode where the position of acidic groups coordinated to the catalytic Tyr-385 as well as Ser-530 at the top of the pocket. The inhibitor carbon atoms are coloured green. All the poses were taken with the help of maestro academic visualiser 10.4, Schrodinger [36].

4-trifluoromethyl (SC-558) group makes interaction in a similar manner to the naproxen [33, 41]. Interestingly, COX inhibitors belonging to carboxylate-containing family without having a methyl group in the α -position exploit different interactions to reinforce binding within the active site of COX enzymes. For example, diclofenac binds with an inverted

orientation (**Figure 9D**) where the carboxylate group makes H-bonds with Ser-530 and Tyr-385 and a chlorine atom attached to the lower aniline ring occupies into a hydrophobic pocket above Val-349.

Juan et al. carried out docking studies by using the AMBER programme to provide proper insights of the differential binding mode of diverse families of COX inhibitors, including selective and nonselective ligands: rofecoxib, ketoprofen, suprofen, carprofen, zomepirac, indomethacin, diclofenac and meclofenamic, respectively [47]. Their study concluded the importance of several structural features that should be attached to a scaffold required for efficient COX inhibition such as (i) a carboxylate moiety essential for interaction with the Arg120 side chain, (ii) a carbonyl moiety important for making hydrogen bond with the side chain of Ser530 and (iii) a distal aromatic ring crucial for fitting into a hydrophobic pocket underneath the Tyr385 side chain. Desiraju et al. reported 3D-QSAR models with high predictive abilities from a set of 114 substituted 1,2-diarylimidazole analogues for computer-aided design of COX-2 selective inhibitors based on 3D-QSAR studies employing comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA) approaches, by including hydrophobic (lipophilic), electrostatic, steric and hydrogen bond donor and acceptor fields as well as docking studies with FlexiDock (using the X-ray crystal structures of COX-1 and COX-2 with PDB ID: 1PGG and 6COX, respectively) [48]. Adinarayana et al. studied the interactions of COX-2 active site residues with selective inhibitors (rofecoxib, etoricoxib, valdecoxib, celecoxib and its analogues) and assessed the importance of scoring functions based on docking studies employing X-score scoring function and FastDock programme. The study concluded that the main interactions of COX-2 inhibitors within the binding pocket enzymes are hydrogen bonding and hydrophobic interactions indicating that sulphonamide and substituted pyrazole groups act as potential pharmacophore features for the design of highly potent and COX-2 selective inhibitors [49]. Sundar et al. developed a virtual library of drug-like novel molecules by employing structure-based de novo drug designing and 2D-fingerprinting approaches followed by molecular docking and MD simulation studies and reported two compounds as promising highly COX-2 selective inhibitors [50]. Chakraborti et al. in their extensive review [15] highlighted various molecular modelling-based approaches in search of novel potential COX-2 selective inhibitors overcoming various side effects posed by the drugs in clinical use.

Recently, Pfizer Global Research and Development, USA [51], reported their structure-based drug design efforts and discovery of two novel orally available benzopyran class of COX-2 selective inhibitors (SD-8381 and SC-75416, structurally different from the diaryl heterocycle class of coxibs (**Figure 10**)) under clinical trials with superior potency and efficacy as an anti-inflammatory and analgesic agent as compared to other NSAIDs and COX-2 selective inhibitors. In particular, the compound SC-75416 exhibited a human half-life of 34 h, which is suitable for once daily dosing and also demonstrated superior analgesic efficacy in a phase II clinical trial of postsurgical dental pain. However, the entire membrane-binding helix cluster and the side chain of Tyr355 observed to move ~0.7 and 1.6 Å away from the active site to accommodate the bulky 7-*t*-butyl substituent. The binding orientation of both the compounds is very similar to each other and conserves many similar contacts between the

Figure 10. Chemical structures and biological data of novel benzopyran derivatives SD-8381 and SC-75416 [51].

enzyme and inhibitor, while the carboxylate group makes H-bond interactions with Tyr385 and Ser530, respectively.

5. Conclusion

Traditional NSAIDs act by nonselective inhibition of cyclooxygenase isoenzymes and are found to be associated with various undesirable side effects such as gastrointestinal (GI) and renal toxicity due to COX-1 inhibition. As a result, a number of COX-2 selective inhibitors (celecoxib, rofecoxib, valdecoxib, etc.) were developed as safer NSAIDs with improved GI safety profile. But the voluntary market withdrawal of rofecoxib and valdecoxib due to their strong association with cardiovascular toxicity imposed a big question on the safety profile of COX-2 selective inhibitors. However, similar cardiovascular toxicity was also found to be associated with some of the traditional NSAIDs suggesting the need to relook into the entire class of NSAIDs rather than exclusively victimising the COX-2 selective inhibitors. Furthermore, the recent evidences for the involvement of COX-2 selective inhibitors in the aetiology of many diseases, such as Alzheimer's disease, Parkinson's disease, diabetes, various cancers, etc. have gained much attention of researchers to design and develop novel COX-2 selective inhibitors with improved pharmacodynamic and pharmacokinetic profile. The availability of 3D X-ray crystal structures of cyclooxygenase enzymes (COX-1 and COX-2) co-crystallised with diverse selective as well as nonselective inhibitors provides an opportunity to gain insight into various physicochemical requirements of ligands for effective binding with selective inhibition of COX-2 enzyme. The main differences between COX-1 and COX-2 are found to be the substitutions of the bulkier amino acid residues Ile434, His513 and Ile523 in COX-1 by comparatively smaller residues Val434, Arg513 and Val523, respectively, in COX-2 at the main channel of cyclooxygenase binding site which resulted in the 25% increase in the volume of the active site at COX-2. The advancement of CADD approaches made a great impact in the discovery process by facilitating the proper understanding of the differential molecular interactions of various inhibitors with cyclooxygenase enzymes. The detailed analysis of molecular basis of binding interactions of NSAIDs and various insightful CADD approaches discussed in this chapter will be useful to build new strategies to develop novel potential COX-2 selective inhibitors to circumvent the limitations associated with the NSAIDs in clinical use.

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References

- [1] Vane JR, Botting RM. Mechanism of action of anti-inflammatory drugs. *Scandinavian Journal of Rheumatology*. 1996;**102**:9-21. DOI: 10.3109/03009749609097226
- [2] Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. *Inflammation Research*. 1998;**47**:S78-S87. DOI: 10.1007/s000110050284
- [3] Smith RM, DeWitt DL, Garavito RM. CYCLOOXYGENASES: Structural, cellular, and molecular biology. *Annual Review of Biochemistry*. 2000;**69**:145-182. DOI: 10.1146/annurev.biochem.69.1.145
- [4] Griswold DE, Adams JL. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): Rationale for selective inhibition and progress to date. *Medicinal Research Reviews*. 1996;**16**:181-206. DOI: 10.1002/(SICI)1098-1128(199603)16:2<181::AID-MED3>3.0.CO;2-X
- [5] Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L, Isakson P. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proceedings National Academy of Sciences of the United States of America*. 1994;**91**:12013-12017
- [6] Allison MC, Howatson AG, Torrance CJ, Lee FD, Russell RI. Gastrointestinal damage associated with the use of nonsteroidal anti-inflammatory drugs. *The New England Journal of Medicine*. 1992;**327**:749-754. DOI: 10.1056/NEJM199209103271101
- [7] Clive DM, Stoff JS. Renal syndromes associated with nonsteroidal anti-inflammatory drugs. *The New England Journal of Medicine*. 1984;**310**:563-572. DOI: 10.1056/NEJM198403013100905
- [8] FitzGerald GA. Coxibs and cardiovascular disease. *The New England Journal of Medicine*. 2004;**351**:1709-1711. DOI: 10.1056/NEJMp048288
- [9] Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclooxygenase-2 inhibitors and traditional nonsteroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. *BMJ*. 2006;**332**:1302-1308. DOI: 10.1136/bmj.332.7553.1302

- [10] Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *The New England Journal of Medicine*. 2005;**352**:1092-1102. DOI: 10.1056/NEJMoa050493
- [11] Solomon SD, McMurray JJV, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E, Bertagnolli M. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. *The New England Journal of Medicine*. 2005;**352**:1071-1080. DOI: 10.1056/NEJMoa050405
- [12] Ritter JM, Harding I, Warren JB. Precaution, cyclooxygenase inhibition and cardiovascular risk. *Trends in Pharmacological Sciences*. 2009;**30**(10):503-508. DOI: 10.1016/j.tips.2009.07.007
- [13] Zarraga IGE, Schwarz ER. Coxibs and heart disease. *Journal of the American College of Cardiology*. 2007;**49**:1-14. DOI: 10.1016/j.jacc.2006.10.003
- [14] Hinz B, Renner B, Cheremina O, Besz D, Zolk O, Brune K. Lumiracoxib inhibits cyclooxygenase 2 completely at the 50 mg dose: Is liver toxicity avoidable by adequate dosing? *Annals of the Rheumatic Diseases*. 2009;**68**:289-291. DOI: 10.1136/ard.2008.092536
- [15] Chakraborti AK, Sanjeev KG, Raj K, Hasim FM, Pradeep SJ. Progress in COX-2 Inhibitors: A journey so far. *Current Medicinal Chemistry*. 2010;**17**:1563-1593. DOI: 10.2174/092986710790979980
- [16] Singh P, Mittal A. Current status of COX-2 inhibitors. *Mini Reviews in Medicinal Chemistry*. 2008;**8**:73-90. DOI: 10.2174/138955708783331577
- [17] Gans KR, Galbraith W, Roman RJ, Haber SB, Kerr JS, Schmidt WK, Smith C, Hewes WE, Ackerman NR. Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor. *Journal of Pharmacology and Experimental Therapeutics*. 1990;**254**:180-187
- [18] Prasit P, Wang Z, Briedeau C, Chan CC, Charleson S, Cromlish W, Ethier D, Evans JF, Ford-Hutchinson AW, Gauthier JY, Gordon R, Guay J, Gresser M, Kargman S, Kennedy B, Leblanc Y, Legar S, Mancini J, O'Neill GP, Ouellet M, Percival MD, Perrier H, Riendeau D, Rodger I, Tagari P, Therien M, Vickers P, Wong E, Xu LJ, Young RN, Zamboni R. The discovery of rofecoxib, [MK966, VIOXX, 4-(4'-methylsulfonylphenyl)-3-phenyl-2(5H)-furanone], an orally active cyclooxygenase-2 inhibitor. *Bioorganic & Medicinal Chemistry Letters*. 1999;**9**:1773-1778. DOI: 10.1016/S0960-894X(99)00288-7
- [19] Flower RJ. The development of COX-2 inhibitors. *Nature Reviews Drug Discovery*. 2003;**2**(3):179-191. DOI: 10.1038/nrd1034
- [20] Marnett LJ, Kalgutkar AS. Cyclooxygenase 2 inhibitors: Discovery, selectivity and the future. *Trends in Pharmacological Sciences*. 1999;**20**(11):465-469. DOI: 10.1016/S0165-6147(99)01385-1

- [21] Pran Kishore D, Rajwinder K, Balakumar C, Madhu B, Dilshad G, Venkat Rao K, Raghuram Rao A, Raghuprasad M. Synthesis, anti-inflammatory evaluation, and docking studies of some new thiazole derivatives. *Medicinal Chemistry Research*. 2014;**23**:2780-2792. DOI: 10.1007/s00044-013-0861-4
- [22] Mamta SD, Pran Kishore D, Renu C, Tejvir S, Maninder K. Synthesis, evaluation and molecular docking studies of cycloalkyl/aryl-3,4,5-trimethylgallates as potent non-ulcerogenic and gastroprotective anti-inflammatory agents. *Medicinal Chemistry Research*. 2014;**23**:87-106. DOI: 10.1007/s00044-013-0620-6
- [23] Pran Kishore D, Raghuprasad Maillabaram A, Raghuram RA, Mallikarjuna RP. Anti-inflammatory evaluation and docking studies of some new thienopyrimidines. *Asian Journal of Chemistry*. 2013;**25**(18):10583-10587. DOI: 10.14233/ajchem.2013.16184
- [24] Alka B, Ruchika O, Pran Kishore D. Synthesis, evaluation and docking studies on 3-alkoxy-4-methanesulfonamido acetophenone derivatives as non-ulcerogenic anti-inflammatory agents. *European Journal of Medicinal Chemistry*. 2012;**49**:397-405. DOI: 10.1016/j.ejmech.2012.01.018
- [25] Balakumar C, Lamba P, Pran Kishore D, Lakshminarayana B, Venkat Rao K, Rajwinder K, Raghuram Rao A, Shireesha B, Narsaiah B. Design, synthesis, biological evaluation and docking studies of some fluorinated fused-quinazolines as antiinflammatory agents. *European Journal of Medicinal Chemistry*. 2010;**45**:4904-4913. DOI: 10.1016/j.ejmech.2010.07.063
- [26] Garavito RM, Malkowski MG, DeWitt DL. The structures of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins & Other Lipid Mediators*. 2002;**68-69**:129-152. DOI: 10.1016/S0090-6980(02)00026-6
- [27] Mbonye UR, Yuan C, Harris CE, Sidhu RS, Song I, Arakawa T, Smith WL. Two distinct pathways for cyclooxygenase-2 protein degradation. *Journal of Biological Chemistry*. 2008;**283**:8611-8623. DOI: 10.1074/jbc.M710137200
- [28] Carol AR, Lawrence JM. Cyclooxygenases: Structural and functional insights. *Journal of Lipid Research*. 2009;**50**:S29-S34. DOI: 10.1194/jlr.R800042-JLR200
- [29] Yuan C, Rieke CJ, Rimon G, Wingerd BA, Smith WL. Partnering between monomers of cyclooxygenase-2 homodimers. *Proceedings of National Academy of Sciences of United States of America*. 2006;**103**(16):6142-6147. DOI: 10.1073/pnas.0601805103
- [30] Yuan C, Sidhu RS, Kuklev DV, Kado Y, Wada M, Song I, Smith WL. Cyclooxygenase Allosterism, fatty acid-mediated cross-talk between monomers of cyclooxygenase homodimers. *The Journal of Biological Chemistry*. 2009;**284**:10046-10055. DOI: 10.1074/jbc.M808634200
- [31] Mitchener MM, Hermanson DJ, Shockley EM, Brown HA, Lindsley CW, Reese J, Rouzer CA, Lopez CF, Marnett LJ. Competition and allostery govern substrate selectivity of

- cyclooxygenase-2. *Proceedings of National Academy of Sciences of United States of America*. 2015;**112**(40):12366–12371. DOI: 10.1073/pnas.1507307112
- [32] Picot D, Loll PJ, Garavito RM. The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature*. 1994;**367**:243–249.
- [33] Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Dildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*. 1996;**384**:644–648.
- [34] Lanzo CA, Sutin J, Rowlinson S, Talley J, Marnett LJ. Fluorescence quenching analysis of the association and dissociation of a diarylheterocycle to cyclooxygenase-1 and cyclooxygenase-2: Dynamic basis of cyclooxygenase-2 selectivity. *Biochemistry*. 2000;**39**(20):6228–6234. DOI: 10.1021/bi992761o
- [35] Walker MC, Ravi GK, Kiefer JR, Morland KT, Koboldt CM, Isakson PC, Seibert K, Gierse JK. A three-step kinetic mechanism for selective inhibition of cyclo-oxygenase-2 by diarylheterocyclic inhibitors. *Biochemical Journal*. 2001;**357**(3):709–718. DOI: 10.1042/bj3570709
- [36] Maestro, version 10.4. New York, NY: Schrödinger, LLC; 2015.
- [37] Robert S, Carmen A, Susana GK, Javier LF, Modesto O. Theoretical studies on the inhibition mechanism of cyclooxygenase-2. Is there a unique recognition site? *Journal of Medicinal Chemistry*. 2003;**46**(8):1372–1382. DOI: 10.1021/jm0209376
- [38] Vittorio L, Massimiliano B, Luciana M, Francesco LG, Andrea C, Ettore N, Michele P. Molecular basis of cyclooxygenase enzymes (COXs) selective inhibition. *Proceedings of National Academy of Sciences of United States of America*. 2010;**107**(12):5411–5416. DOI: 10.1073/pnas.0913377107
- [39] Gilad R, Ranjinder SS, Adam LD, Jullia YL, Narayan PS, Chong Y, Ryan AF, Raymond CT, Benedict RL, William LS. Coxibs interfere with the action of aspirin by binding tightly to one monomer of cyclooxygenase-1. *Proceedings of National Academy of Sciences of United States of America*. 2010;**107**(1):28–33. DOI: 10.1073/pnas.0909765106
- [40] So OY, Scarafia LE, Mak AY, Callan OH, Swinney DC. The dynamics of prostaglandin H synthases—Studies with prostaglandin H synthase-2 Y355F unmask mechanisms of time-dependent inhibition and allosteric activation. *The Journal of Biological Chemistry*. 1998;**273**:5801–5807. DOI: 10.1074/jbc.273.10.5801
- [41] Selinsky BS, Gupta K, Sharkey CT, Loll PJ. Structural analysis of NSAID binding by prostaglandin H-2 synthase: Time-dependent and time-independent inhibitors elicit identical enzyme conformations. *Biochemistry*. 2001;**40**(17):5172–5180. DOI: 10.1021/bi010045s
- [42] Orlando BJ, Malkowski MG. Crystal structure of rofecoxib bound to human cyclooxygenase-2. *Acta Crystallographica Section F*. 2016;**F72**:772–776. DOI: 10.1107/S2053230X16014230

- [43] Marnett LJ. The COXIB experience: A look in the rearview mirror. *Annual Review of Pharmacology Toxicology* 2009;**49**:265-290. DOI: 10.1146/annurev.pharmtox.011008.145638
- [44] Wang JL, Carter J, Kiefer JR, Kurumbail RG, Pawlitz JL, Brown D, Hartmann SJ, Graneto MJ, Seibert K, Talley JJ. The novel benzopyran class of selective cyclooxygenase-2 inhibitors-part I: The first clinical candidate. *Bioorganic & Medicinal Chemistry Letters*. 2010;**20**(23):7155-7158. DOI: 10.1016/J.BMCL.2010.07.053
- [45] Lecomte M, Laneuville OJC, DeWitt DL, Smith WL. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *The Journal of Biological Chemistry*. 1994;**269**(18):13207-13215.
- [46] Duggan KC, Walters MJ, Musee J, Harp JM, Kiefer JR, Oates JA, Marnett LJ. Molecular basis for cyclooxygenase inhibition by the nonsteroidal anti-inflammatory drug naproxen. *Journal of Biological Chemistry*. 2010;**285**:34950-34959. DOI: 10.1074/jbc.M110.162982
- [47] Oriol L, Juan JP, Albert P, David M. Differential binding mode of diverse cyclooxygenase inhibitors. *Journal of Molecular Graphics and Modelling*. 2002;**20**(5):359-371. DOI: 10.1016/S1093-3263(01)00135-8
- [48] Desiraju GR, Gopalakrishnan B, Jetti RKR, Nagaraju A, Raveendra D, Sarma JARP, Sobhia ME, Thilagavathi R. Computer-aided design of selective cox-2 inhibitors: Comparative molecular field analysis, comparative molecular similarity indices analysis, and docking studies of some 1,2-diarylimidazole derivatives. *Journal of Medicinal Chemistry*. 2002;**45**(22):4847-4857. DOI: 10.1021/jm020198t
- [49] Adinarayana KPS, Reddy PA, Babu PA. Structural studies on docking selective COX-2 inhibitors. *Journal of Bioinformatics & Research*. 2012;**1**(1):21-26
- [50] Jaspreet KD, Anith KS, Khushboo B., Shashank PK, Sukriti G, Abhinav G, Sundar D. Computational structure-based de novo design of hypothetical inhibitors against the anti-inflammatory target COX-2. *Plos One*. 2015;**10**(8):1-18. DOI: 10.1371/JOURNAL.PONE.0134691
- [51] Wang JL, David L, Matthew JG, John S, Joseph RBH, Subo L, Jennifer LP, Ravi GK, Timothy M, John JT, James RK, Jeffery C. The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: The second clinical candidate having a shorter and favorable human half-life. *Bioorganic & Medicinal Chemistry Letters*. 2010;**20**(23):7159-7163. DOI: 10.1016/J.BMCL.2010.07.054