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Structural Basis for Selective Inhibition of COX-2 by Nimesulide

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Abstract—Nimesulide **1** is a novel nonsteroidal antiinflammatory drug which inhibits the enzyme cyclooxygenase 2 (COX-2) more selectively than cyclooxygenase 1 (COX-1). Molecular modelling studies have been carried out on complexes of **1** with COX-1 and with mutants of COX-1 simulating COX-2. These indicate that the mutations I523V and S516A largely contribute to the selectivity. A comparative study with SC-558 **2** has also been performed.

Introduction

Cyclooxygenase (COX) also known as prostaglandin synthase (PGH) is a potent mediator of inflammation.[1] Nonsteroidal anti-inflammatory drugs (NSAIDs) bind to cyclooxygenase thereby inhibiting the production of prostaglandins. COX-1 and COX-2 are two isoforms of cyclooxygenase known to date.[2] COX-1, a constitutive enzyme produces gastroprotective prostaglandins[3] and COX-2 is induced by cytokines, mitogens and endotoxins in inflammatory cells which are responsible for the production of inflammatory prostaglandins.[4] Complexes of COX-1 with NSAIDs[5–7] and COX-2 with flurbiprofen, indomethacin and SC-558, **2**[8] have been reported. Most of the NSAIDs bind at the active sites of both COX-1 and COX-2 with little specificity and lead to side effects like gastric lesions and renal toxicity. Inhibitors that selectively bind to COX-2 show anti-inflammatory action in vivo with minimal gastric side effects.[9] Hence there has been considerable global effort to discover newer selective COX-2 inhibitors.[10] The compound **2** is one of such selective COX-2 inhibitors belonging to the diarylpyrazole group of compounds out of which SC 586315 (celecoxib) is in phase III clinical trials for rheumatoid- and osteo-arthritis.[11] Selective inhibition of **2** is mainly attributed to the presence of the sulphonamide group.[8] Crystallographic studies on human COX-2 and complexes with some NSAIDs have appeared recently.[12]

Total number of amino acids in the polypeptide chain of COX-1 and COX-2 differs slightly from each other with one insertion of proline after Ile 106 in COX-2. Comparison of the sequences of COX-1 and COX-2 shows 60% sequence identity, but the overall structures of COX-1 and COX-2 are highly conserved.[8] As the overall structures of COX-1 and COX-2 are similar, the binding sites in both the isoforms are similar as expected.[8]

Nimesulide **1** which is a drug in clinical usage[13] for the last 4 years[14] has the property of selective inhibition of COX-2[15] similar to SC-558. It also has a methyl sulphonamide group and the overall conformation shows some relation to structure and function of SC-558 (Fig. 1). With the knowledge of the three dimensional structures of COX-1, COX-2 and their inhibitors, molecular modeling in conjunction with molecular energetics using molecular mechanical approach was initiated to understand the mechanism and structural aspects of inhibition of cyclooxygenases by **1**. Where crystallographic data are unavailable, the molecular basis of selectivity is generally probed by mutating the crucial residues and then evaluating the ligand binding activity of the enzyme.[16]

Key words: NSAIDS; selective inhibition of COX-2; computer modelling; nimesulide.
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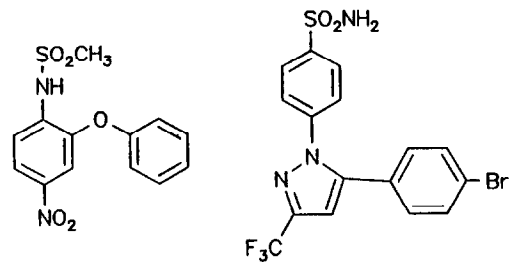
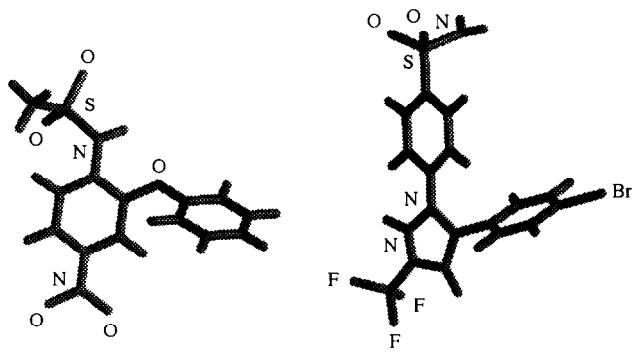


Nimesulide (1) SC-558 (2)



Nimesulide (1) SC-558 (2)

Figure 1. Structures of nimesulide (1) and SC-558 (2).

Materials and methods

Silicon graphics Iris Crimson was used to perform all energy calculations and modeling studies. Modelling of the enzyme–inhibitor complex was carried out using INSIGHT II of Biosym software. Crystallographic data on COX-2 are not available. However comparison of the published amino acid sequence of COX-1 with COX-2 gives a sequence identity of 67% with 87% identity and strict sequence conservation at the cyclooxygenase active site.

Crucial sequence dissimilarity at the active site[8] are:

Residue	COX-1	COX-2
513	His	Arg
516	Ser	Ala
523	Ile	Val

and in the neighbourhood, presence of Ile at position 434 in COX-1 versus Val in COX-2.

Therefore, mutants of COX-1 were constructed to mimic COX-2 around the active site and to evaluate the contribution of these residues to the specificity.

COX-1 coordinates at 3.5 Å resolution available in PDB data base were used as the initial coordinates.[5] Hydrogens were fixed and the energy was minimised. Crystallographically determined coordinates[17] of **1** were used. Docking of **1** with COX-1 was performed using DOCKING module of Biosym software[18] where the intermolecular energies are displayed interactively. The inhibitor was moved in the active site to maximise the intermolecular interactions and positioned such that the interactions were reasonably good with least steric hindrances. This model was taken as the starting one for further energy calculations. Molecular mechanics calculations were done using DISCOVER with CFF91 forcefield.

Results and discussion

Component energies of complexes of inhibitor **1** with native COX-1 and **1** with mutant COX-1 are given in Table 1. Binding energy, the energy released due to the complex formation, is less for the native–nimesulide complex when compared with those of **1** with COX-1 having mutants I523V, S516A and the four mutants, I434V, H513R, S516A and I5213V. The drug molecule

Table 1. Component energies of complexes of nimesulide with COX-1 and its mutants

Name	Binding energy (kcal/mol)	Destabilisation energy (kcal/mol)
Native	−5	44
S516A mutant	−25	22
I523V mutant	−35	12
I434V, S516A, I523V, H513K mutant	−38	10

positioned into the binding site has a few steric interactions (Fig. 2). When the model was energy minimised to get rid of these steric contacts, e.g. those arising from the presence of extra methyl group in Ile 523, the side chains reorient, which destabilize the protein conformation. As a result, the native complex has more destabilization energy and less binding energy than the mutant complex.

This indicates that the native COX-1-inhibitor complex is less favoured and this also confers the selectivity of **1**. Complex of **1** with S516A mutant of COX-1 is energetically more favourable than native COX-1 complex but less than 1523V mutant complex. These results show that position Val 523 is the first contributor and Ala 516 is the second contributor to the selectivity of nimesulide. Model with four mutants is energetically better only by a small value (3 kcal/mole) and so the contribution of Val 523 and Ala 516 are considered appreciable for the selectivity. With the increase in binding energy there is a decrease in destabilisation energy and this inverse correlation between the binding and destabilisation energy can be seen from Table 1. Visually examining the superposition of the backbone of the crystal structure of COX-1 and the energy refined model of complex of nimesulide with 1523V mutant COX-1 shows that the binding site of COX-1 and COX-2 are quite similar,[8] (r.m.s deviation = 0.415 Å) but the bulkier side chain of isoleucine at 523 in COX-1 restricts the methyl sulphonamide group from a favourable interaction. The distance between the nearest heavy atom of nimesulide and the beta carbon atom of Ile 523 in COX-1 is 2.47 Å whereas the shortest distance between the drug and Val 523 is 3.51 Å in the COX-1 mutant (Fig. 3). This shows that the bulkier side chain of Ile of COX-1 offers steric hindrance to the drug. Since there is no room for the methyl sulphonamide group in the active site pocket of COX-1, the enzyme is expected to be destabilised from its native structure and hence the destabilisation energy is large (44 kcal/mol) in the refined COX-1–nimesulide complex. Comparison of the destabilisation energies of the native and the 1523V mutant indicates that Val at position 523 is crucial for the binding of the nimesulide to COX-2 as in the case of **2**. Another residue that contributes to the specificity is Ser 516. When Ser 516 in COX-1 is replaced by Ala, the binding and the destabilising energies are in favour of the binding of nimesulide with this mutant COX-1. A partial COX-2 environment in the active site of COX-1 was realised by making all the four mutants 1523V, S516A, H513R, and

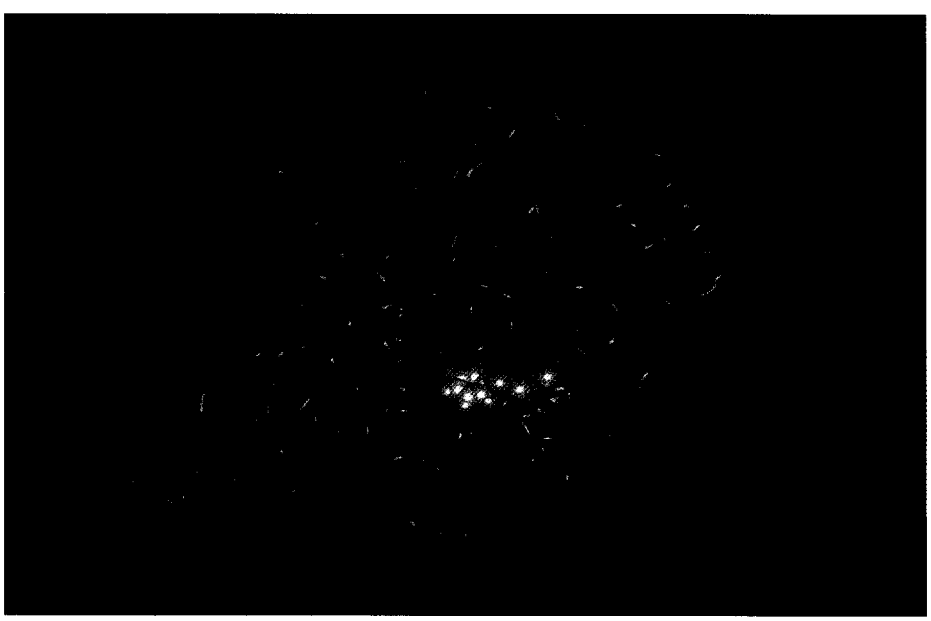


Figure 2. Colour photograph of four mutant COX-1 (COX-2 environment) with **1** bound in the active site.

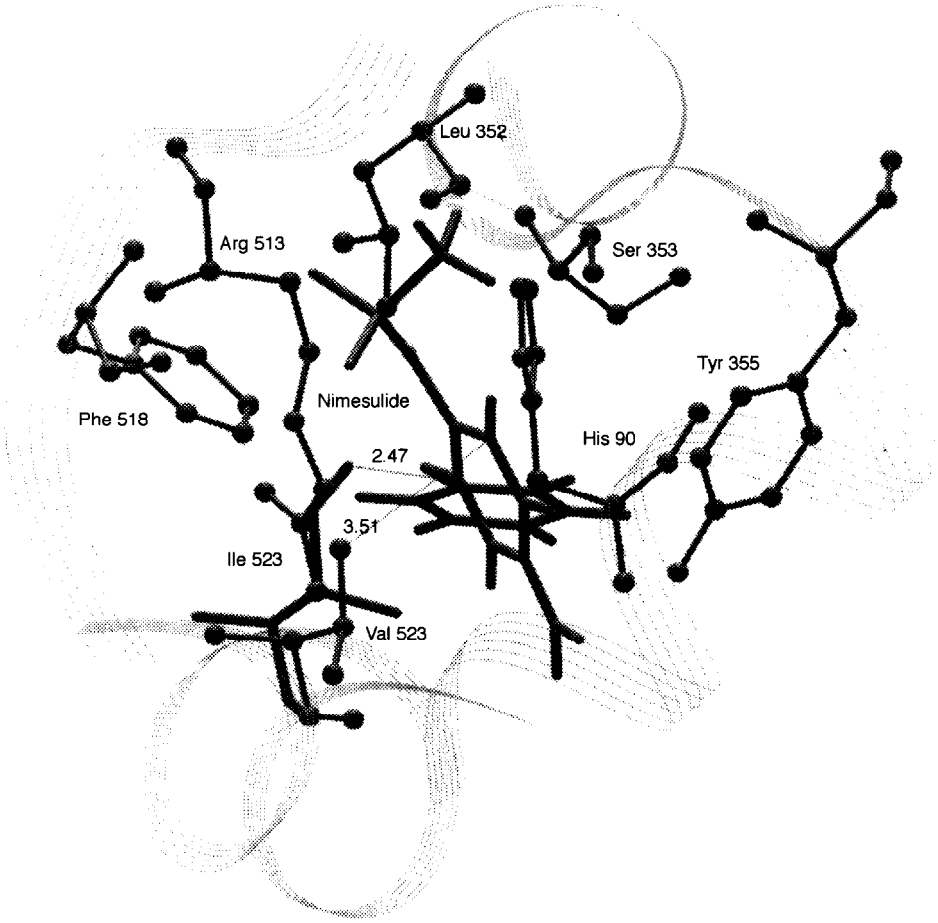


Figure 3. View showing the side chain of Ile 523 (stick) in COX-1 hindering the binding of inhibitor **1**.

I434V in the native structure. The complex of **1** with four mutants shows a further decrease in the destabilisation energy and also a more favourable binding energy. From the above energy results, when COX-1 is mutated at the four sites mentioned earlier, creating a COX-2 binding site environment, COX-2 specific inhibitors may show greater prospects for binding. I434V does not show any significant role in the binding site excepting the fact that it may participate in the gate mechanism as seen in Figure 4 which has been proposed already for **2**.[8]

Drug interaction

Nimesulide **1** belonging to a new class of COX-2 inhibitors, consists of a phenyl and a nitrophenyl ring that are bridged by an oxygen atom making an angle of 74.69° in the crystal structure. In comparison with **2**, a strong COX-2 selective inhibitor with a sulphonamide group, nimesulide has a methyl sulphonamide group. The binding feature of the methyl sulphonamide group and the nitrophenyl ring of **1** has good equivalence with the binding feature of the sulphonamide and the phenyl ring of **2**. Binding of the phenyl ring in **1** is quite different from that of other COX-2 inhibitors. Intermolecular interaction energies of **1** with COX-1 and COX-1 mutants are recorded in Table 3. From the table, it is very clear that the intermolecular interaction energy is more favourable (by 2.5 kcal/Mol) in the complex of **1** with COX-1 mutant than with native COX-1. Most of the difference arises in the intermolecular energy between the methyl sulphonamide group of the inhibitor and COX-1 mutant. Though the energy differences are small, in a relative sense they still help us to appreciate the importance of the methyl sulphonamide group for the specificity of nimesulide towards COX-2.

In the binding site of COX-2, the nitrophenyl ring of **1** interacts with the hydrophobic residues Pro 86, Ile 89, Leu 93, Leu 352, Phe 518, Val 523 and Ala 527 (Fig. 5). This also strongly interacts with some of the polar

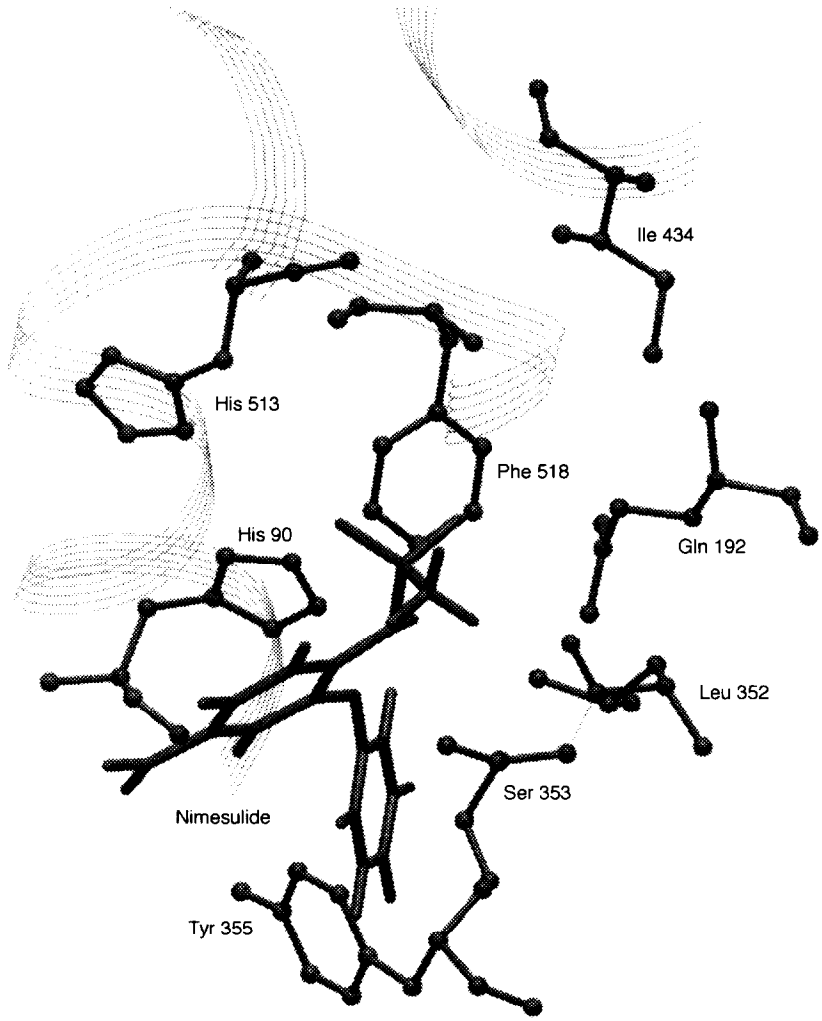


Figure 4. Figure showing Ile 434 as a molecular gate far from the binding site.

residues His 90, Arg 120, Tyr 355, Arg 513 and with the main chain of Ser 353. The presence of the nitro group facilitates its interaction with Pro 86, Ile 89, Leu 93 and Arg 120 which are not found in the complex of **2** with COX-2.[8] The nitrogen and one of the oxygen atoms of the nitro group form O...H–N and O...H–O hydrogen bonds with the hydroxyl group of Tyr 355 (Fig. 6). Data for hydrogen bonds are given in Table 4.

Conformational changes occur in the side chain of Arg 120 and the mutant residue Val 523 to accommodate the nitrophenyl ring in the hydrophobic pocket (Table 5). The resultant side chain conformation of the Ile 523 (native complex) after energy refinement does not show significant deviation from the crystal structure; however Chi 2 torsion of Val 523 shows significant deviation from the crystal structure. Chi 2 of Val 523 could take gauche+ whereas Ile at that position could only take trans because of the, extra methyl group. Visual examination of the side chain conformation clearly shows that gauche+ for Chi 2 torsion is advantageous since this conformation opens up the gate of the hydrophilic pocket. As a result of the conformational changes in Arg 120, the salt bridge between Arg 120 and Gln 192 is lost but a salt bridge is formed with the oxygen of the nitro group of the inhibitor in nimesulide–COX-1 (four mutant) complex.

The phenyl ring of **1** is bound in a hydrophobic cavity formed by Val 349. Leu 352, Tyr 355, Phe 518, Val 523, Gly 526, Ala 527 and Leu 531. In contrast, Val 349, Tyr 355 and Leu 531 show interactions with the trifluromethyl group and Gly 526 and Ala 527 interact with the bromophenyl ring of **2** in its reported complex

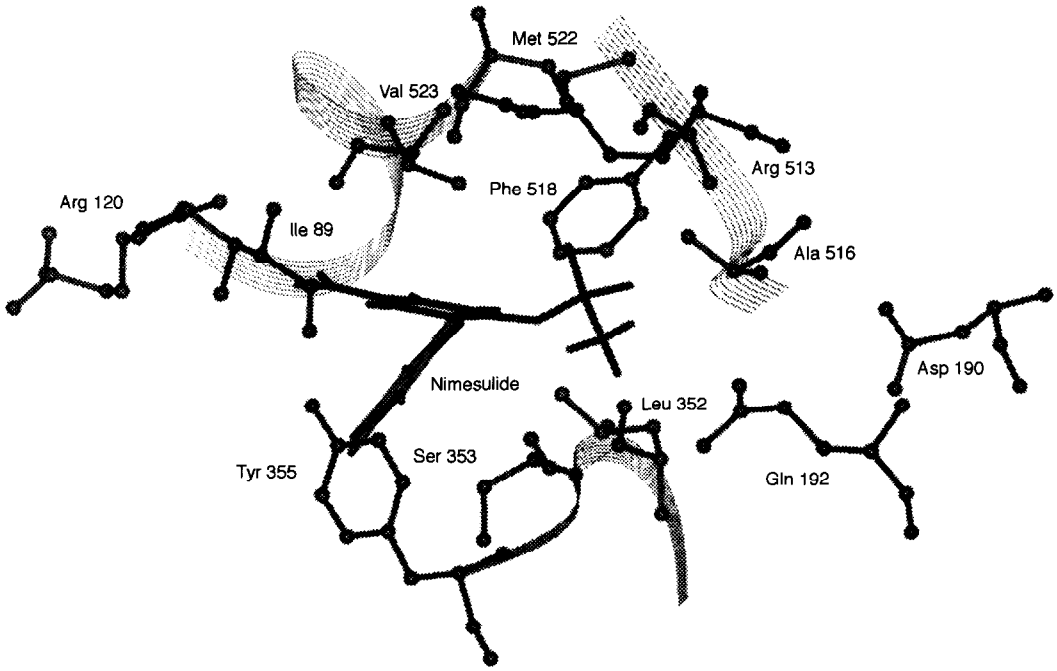


Figure 5. View of the binding of **1** at the active site.

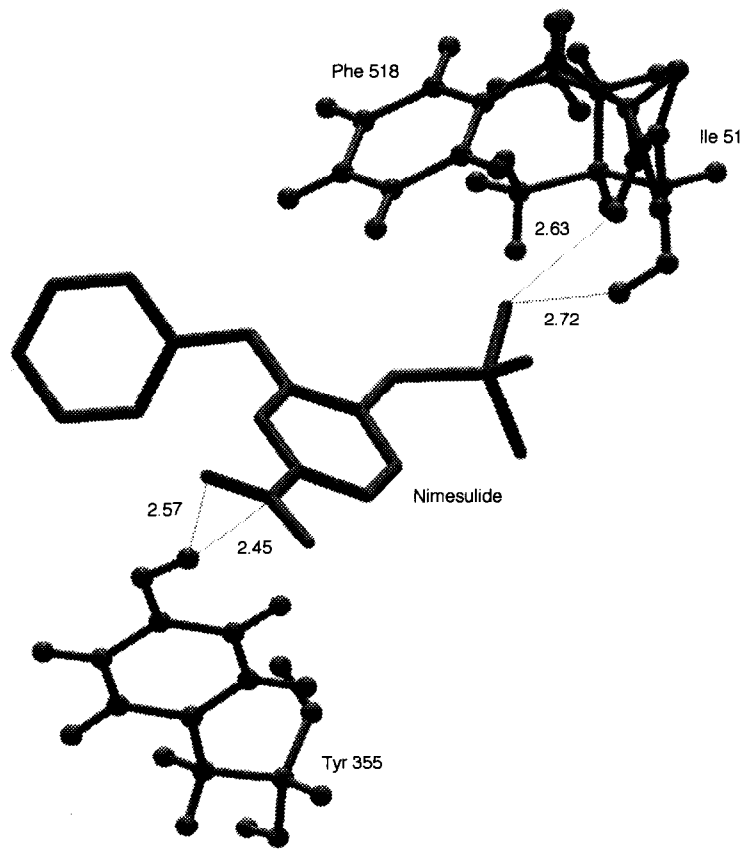


Figure 6. Hydrogen bonding scheme of **1**.

Table 2. Component energies of complexes of SC-558 with COX-1 and its mutants

Name	Binding energy (kcal/mol)	Destabilisation energy (kcal/mol)
Native	−39	10
I523V mutant	−40	10
I434V, S516A, I523V, H513K mutant	−42	10

with COX-2. This shows that the phenyl ring of nimesulide binds relatively in the middle of the binding sites of trifluro group and the bromophenyl ring. Phenyl ring of nimesulide also interacts with Ser 353, Ser 530 and Leu 352 which are new subsites in COX-2 binding.

Methyl sulphonamide is bulkier than the sulphonamide group and this facilitates it to have strong interactions with the enzyme. The sulphonamide group interacts with His 90, Gln 192. Leu 352, Ser 353, Tyr 355, Arg 513 Ala, 516, Ile 517, Phe 518, Met 522 and Val 523 and the methyl group extends deeply into the region which is polar and interacts with His 90, Asp 190, Gln 192, Leu 352, Ser 353, Gly 354 and Asn 515. Thus the methyl group attached to the sulphonamide residue in **1** occupies most of the hydrophilic pocket and hence the sulphonamide group is not deep inside the polar pocket as in the case of SC-558–COX-2 complex.[8]

Phe 518 shows good stacking interactions with the sulphonamide group and the intraction energy for the methyl sulphonamide group is maximum with Phe 518. Residues Leu 352, Ser 353, Tyr 355, Phe 518 and Val 523 interact well with all the three groups, viz methyl sulphonamide and nitrophenyl and the phenyl rings of the inhibitor and the energy, between the drug and these five residues alone is 20.58 kcal/mol.

Table 3. Groupwise split up of intermolecular energies of nimesulide with

	COX-1	COX-1 (mutants)
Methyl sulphonamide	−10.9	−12.5
Phenyl ring	−9.7	−10.5
Nitro phenyl ring	−14.5	−14.6
Total	−35.1	−37.6

Comparison of nimesulide 1 with SC 558 2

Among the diarylpyrazoles extensive structure-activity studies have shown that **2** in one of the most potent of the series.[8] Hence we considered that it would be interesting to compare the interaction and mode of binding of SC-558 and nimesulide. Towards this end we also performed modeling and energy calculations for complexes of the former with both cyclooxygenases. The SC-558–COX-1 model was built based on experimental observations from X-ray crystallography and retaining the hydrogen bonds. Table 2 gives the energy values for COX-1–SC 558 complex. From Table 2, it is clear that mutations at the active site do not considerably change the binding energy of SC-558–COX-1 complexes, but it still shows selectivity for COX-2.

Unlike SC 558-COX-1 mutant complex, binding energy of nimesulide-COX-1 mutant complex shows significant difference for each mutation. Difference in binding energies of the native and mutant SC-558–COX-1 complexes fill within a short range (3 kcal/mol). So it could be concluded that the selectivity of SC-558 to COX-2 is

Table 4. Possible intermolecular hydrogen bonds in COX-1 (mutant)–nimesulide complex

Donor (D)	Acceptor (A)	Distance (Å)		Angle (°)
		D...A	DH...A	
O (Tyr 355)	O4	3.36	2.45	158.7
O (Tyr 355)	N2	3.27	2.57	129.4
N (Ile 517)	O2	3.50	2.72	134.0
N (Phe 518)	O2	3.51	2.63	145.5

Table 5. Side chain conformations of Arg 120 and Val 523. (Ile in COX-1)

Arg 120:								
	Phi	Psi	Omega	Chi 1	Chi 2	Chi 3	Chi 4	Chi 5
Crystal structure of COX-1	−68.7	−45.5	−172.9	−58.4	−64.9	179.4	48.9	−119.0
COX-1–nimesulide model	−63.8	−48.2	−165.6	−61.1	−67.8	179.0	117.3	167.7
COX-1 (four mutant)–nimesulide model	−71.3	−45.4	−165.5	−62.8	−65.8	−179.2	116.0	167.1
Val 523:								
	Phi	Psi	Omega	Chi 1	Chi 2	Chi 2	Chi 2′	Chi 3
Crystal structure of COX-1	−63.8	−60.5	−174.0	−64.7	49.7	171.0	−179.4	175.4
COX-1–nimesulide model	−69.4	−55.4	−178.7	−71.9	52.9	1274.9	172.9	−177.2
COX-1 (four mutant)–nimesulide model	−73.9	−38.6	175.8	−67.7	64.9	54.9		

mainly due to the presence of Val 434 in the gate allowing free entry compared to Ile 434 in COX-1 which being bulkier causes obstruction. This demands further experimental study of I434V COX-1 mutant with SC-558 to prove the contribution of valine at position 434.

On the other hand, nimesulide shows large differences in the binding energies for different mutants. Whether or not Val 434 plays a role in the selectivity of **1** to COX-2, Val 523 and Ser 516 play an important role flor the favourable binding environment. The net binding energy for SC-558–COX-1 mutant is 4 kcal/mol lower than that of nimesulide–COX-1 mutant suggesting that the binding is more favourable in the case of the former. Although the importance of Val 523 is not clear from SC-558 mutant complex energy values, this has been demonstrated recently by elegant experiments.[16] The time dependent activity of **1** against cyclooxygenases[15] can be explained as being due to initial combination with the active site of the enzyme in competition with the substrate followed by inhibitor induced structural changes in the enzyme.[9]

Conclusion

Hence, the above modelling studies offer an explanation for the known selectivity of nimesulide towards COX-2 as against COX-1 and suggest that the methyl sulphonamide in **1** could be responsible for this property. They also indicate that nimesulide may have a higher selectivity towards COX-2 than SC-558 **2**. The applicability of similar studies to another COX-2 selective inhibitor, viz NS-398, which has the unsubstituted phenyl ring of **1** fully saturated[19] and a few other newer NSAIDs is under study and will be reported shortly. Such studies can pave the way for the rational design of newer NSAIDs with minimal gastric and renal disturbances.

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