

## Cyclooxygenase-2 – An attractive target for fruitful drug design

G. Felcy Fabiola, Lakshminarasimhan Damodharan, Vasantha Pattabhi and Kuppuswamy Nagarajan\*

*Cyclooxygenase, an enzyme involved in the conversion of C-20 acids to prostaglandins, exists in two isoforms. A third isoform has been recently encountered. COX-1 is constitutively expressed and has a gastroprotective function. COX-2, induced at the site of injury, is responsible for the expression of pro-inflammatory prostaglandins. Despite overall similarities, COX-1 and COX-2 show subtle differences in amino acid composition at the active sites. COX-2 has valine at positions 89 and 523, while COX-1 has isoleucine, resulting in larger space availability in the former. Further, the presence of valine at position 434 in COX-2 as against isoleucine in COX-1 allows a gate mechanism to operate in favour of the former. Molecular modelling studies explain the preferential COX-2 inhibitory activity of some nonsteroidal anti-inflammatory agents like celecoxib (3), rofecoxib (4), nimesulide (5), meloxicam (6), nabumetone (10) and etodolac (13) in terms of binding, destabilizing and intermolecular energies. A few modified meloxicam derivatives like 19 and 20 are likely to have superior COX-2 selectivity.*

THE revolution in biology over the past two decades has resulted in radically new approaches and opportunities for drug discovery. There has been an incredibly rapid

increase in the rate of determination of three-dimensional structures of biomolecules. Many of these macromolecules are important drug targets and it is now possible to use the knowledge of the three-dimensional structures as a good basis for drug design. We propose to illustrate this in the case of cyclooxygenase-2, an enzyme responsible for inflammation<sup>1</sup>. This area has attracted immense attention in the last few years and a large number of original

G. Felcy Fabiola, Lakshminarasimhan Damodharan and Vasantha Pattabhi are in Department of Crystallography and Biophysics, University of Madras, Chennai 600 025, India. Kuppuswamy Nagarajan is in the Recon R&D Centre, Bannerghatta Road, Bangalore 560 076, India.

\*For correspondence. (e-mail: n\_kuppuswamy@hotmail.com)

research articles and a good number of scientific and popular review articles have been published<sup>1-6</sup>.

Aspirin or acetylsalicylic acid, the prototype of non-steroidal anti-inflammatory agents (NSAIDs) was first produced and marketed by Bayer in March 1899. The German chemist, Felix Hoffman employed by Bayer, who was responsible for the production of this drug was motivated by his concern for his father's severe rheumatism<sup>1</sup>. NSAIDs are even today among the most widely used therapeutic agents with a total annual sale in excess of US \$ 10 billion. They are used for the treatment of a broad spectrum of pathophysiological conditions such as headaches, discomfort associated with minor injuries and alleviation of severe pain caused by inflammatory and degenerative joint diseases such as osteo and rheumatoid arthritis<sup>1</sup>.

### Mode of action of anti-inflammatory agents

Corticosteroids inhibit the activity of phospholipase A<sub>2</sub> and hence reduce the release of arachidonic acid and ultimately inhibit the formation of proinflammatory prostaglandins. Vane<sup>7</sup> made the seminal proposal in 1971 that in contrast to steroids, NSAIDs exerted their activity by inhibiting cyclooxygenase (COX), a dual function enzyme. Prostaglandins are formed by the oxidative cyclization of the central 5 carbons within 20 carbon polyunsaturated fatty acids. The key regulatory enzyme of this pathway is COX, also known as PGH synthase, which catalyses the conversion of C-20 acids with varying degrees of unsaturation to prostaglandins PGG<sub>2</sub> and PGH<sub>2</sub>. The latter is subsequently transformed to a variety of eicosanoids such as PGE<sub>2</sub> and thrombaxane TXA<sub>2</sub>. Apart from the activity to bring about cyclization, COX has also peroxidase activity which leads to the hydroxylation of cyclopentenes through endo-peroxidation. All NSAIDs in clinical use have been shown to inhibit COX, leading to a marked reduction in PG synthesis<sup>8</sup>. The inhibition by aspirin is due to irreversible acetylation of the cyclooxygenase component of COX, leaving the peroxidase activity unaffected<sup>9</sup>. In contrast, NSAIDs like indometacin or ibuprofen inhibit COX reversibly by competing with the substrate, arachidonic acid, for the active site of the enzyme<sup>10</sup>.

All the activities of NSAIDs such as prevention of pathological overproduction of proinflammatory prostaglandins and the physiological formation of prostanoids are explained well by the postulate of inhibition of prostaglandin synthesis. The unwelcome ulcerogenic and renal side effects of NSAIDs such as aspirin and ibuprofen have been related to the inhibition of production of prostacyclin, which has a cytoprotective effect on the gastric mucosa and regulation of kidney function. It thus appeared that the ulcerative effect of classical NSAIDs was an inevitable price to be paid for the desired anti-inflammatory activity, until the discovery that COX ex-

isted in two isoforms, COX-1 and COX-2. More recently, the presence of a new isoform COX-3 has been speculated upon<sup>11</sup>.

### COX-1 and COX-2: Two isoforms of cyclooxygenase

An early clue to the existence of COX-2 came from a study of cell-growth signalling pathways, which pointed to a unique inducible gene product related to the known COX (i.e. COX-1)<sup>12</sup>. Meanwhile investigators looking at PG production in response to cytokines and other inflammatory factors observed increases in COX activity that could only arise by increased expression of another cyclooxygenase<sup>13</sup>. Immunoprecipitation techniques allowed the isolation of the COX-2 protein and the identification of the two distinct isoforms. Subsequent research established that the COX-1 and COX-2 proteins are derived from distinct genes that diverged well before birds and mammals<sup>14</sup>.

#### *Properties of COX-1 and COX-2*

COX-1 is constitutively expressed in most tissues and performs a housekeeping function to synthesize PGs with normal cell regulatory activity. It is a membrane-bound haemo and glycoprotein with a molecular weight of 71 kDa. It cyclizes arachidonic acid and adds the 15 hydroperoxy group to PGG<sub>2</sub> and then reduces the molecule to PGH<sub>2</sub>, both through its own peroxidase activity.

COX-2 with a molecular weight of 70 kDa has similar functions. However, it is not found to any appreciable extent in resting cells. On the other hand, it is expressed considerably after exposure to fibroblasts, cytokines, etc. Levels of COX-2 protein increase in parallel with overproduction of prostaglandins in many cells and tissues in chronic inflammation. Both COX-1 and COX-2 are homodimeric proteins.

With the availability of purified COX-1 and COX-2 enzymes of different origins and by various methods, including recombinant technology, the classical NSAIDs could be readily tested and were shown to inhibit both COX-1 and COX-2, some of them in fact inhibiting the first more than the second.

A logical outcome of the recognition of the role of the two COX forms appeared to be that a selective COX-2 inhibiting NSAID should not have the principal side effects associated with use of the earlier drugs, such as gastrointestinal inhibition and ulcers as well as renal perturbations<sup>2,3</sup>.

Additionally, since COX-2 overexpression is observed in diseases like Alzheimer's and colorectal cancer, selective COX-2 inhibitors may have useful therapeutic benefits in such conditions<sup>1</sup>. COX-2 selective NSAID treatment may be an important advance in the prevention

of preterm delivery<sup>15</sup>. The design of a selective COX-2 inhibitor would be desirable and possible if structural differences could be identified between the two isoforms and exploited, although doubts have been raised to temper this optimism, since among other reasons, COX-2 has also useful physiological functions which may suffer in the process of inhibition<sup>16</sup>. The situation is confounded further by the preliminary data on COX-3 which is expressed in the 'resolution' phase of the inflammatory process. Since this has anti-inflammatory activity, the administration of selective COX-2 inhibitors at this stage may actually retard the healing phase<sup>11</sup>. Nevertheless the scientific, therapeutic and monetary rewards that successful design of selective COX-2 inhibiting NSAIDs would confer have been so beguiling that both the pharmaceutical industry and academia have jumped into the arena and at least two new COX-2 selective inhibitors have been introduced into the market, while some of the earlier NSAIDs have also been found to possess this virtue (*vide infra*). This article will review briefly the literature and discuss in a little greater detail our approach to the study and design of such agents.

### Structure of COX-1 and COX-2

X-ray crystallography of the 3-D structures of COX-1 and COX-2 as well as complexes with NSAIDs has thrown light on the mechanism of action<sup>17-19</sup>. COX-1 and COX-2 are very similar enzymes consisting of a long narrow channel with a hairpin bend at the end. Both isoforms are membrane associated. Arachidonic acid released from damaged membranes adjacent to the opening of the enzyme channel, mostly hydrophobic, is sucked in, twisted around the hairpin bend and subjected to chemical reactions, resulting in the formation of the cyclopenta ring of PGs. Experiments have revealed the site of catalysis at about half-way down the channel and mechanism of action of NSAIDs at that site<sup>20</sup>. Subtle differences existing at the active site in COX-1 and COX-2 can be expected to regulate specificity as has been convincingly shown by the elegant study of complexes of the classical, nonspecific NSAIDs, flurbiprofen and indometacin and the recently developed SC-558 (**1**) with selectivity for COX-2 (ref. 19). It was postulated that L-valine at 523 in the active site of COX-2 as against the bulkier isoleucine in COX-1 gave better access to the inhibitor in the case of former. This has been convincingly demonstrated with SC-58125 (**2**), an analogue of SC-558, which selectively inhibited COX-2. Targetted single amino acid substitution of valine in COX-2 at position 509 (active site) gave a mutant with a COX-1 profile, which was poorly inhibited by **2** (ref. 21).

We now proceed to discuss in somewhat greater detail the differences between the binding sites of COX-1 and COX-2.

### Differences between binding sites of COX-1 and COX-2

COX-1 and COX-2 are 63% identical and 77% similar at the amino acid level. The catalytic domain is highly conserved, with the major residues known to be involved in catalysis, Arg 120, His 206, Tyr 385, His 386 and His 388, all conserved along with the residue Ser 530. Differences that could be responsible for the selectivity are most likely to be found in the cyclooxygenase active site, due to the fact that the known selective inhibitors inhibit the cyclooxygenase activity. The active site is preponderantly hydrophobic in nature with two internal hydrophilic pockets I and II (Figure 1 *a* and *b*), both of which have a valine in COX-2 and an isoleucine in COX-1 (positions 523 and 89) at the opening of the pocket, leading to the constriction of this pocket in COX-1. One was reported in 1996 (ref. 19), while we have encountered the other recently<sup>22</sup>. Figure 2 gives a stereoview of the environments at the binding centres. The accessibility of these pockets is reported to be controlled by a valine in COX-2 as against isoleucine in COX-1, at position 434. The side chain of Ile residue at 434 packs against Phe 518 which forms a molecular gate that extends to the hydrophilic pocket I. In COX-1, this gate is closed because of the bulkier Ile side-chain, whereas in COX-2, with the less voluminous Val at 434, the gate has room to swing open, allowing the entry of the inhibitor<sup>19</sup>. The substitution of Val 523 in COX-2 by Ile in COX-1 has consequences for the size and shape of this hydrophobic region. This difference has been implicated in the selectivity of some

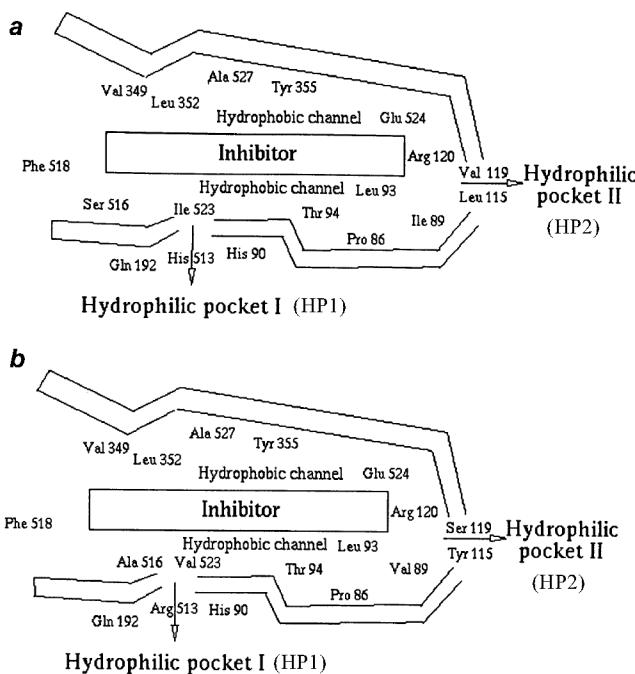


Figure 1. Schematic representation of active sites of (a) COX-1 and (b) COX-2.

inhibitors<sup>19</sup>. Access to the hydrophilic pocket II situated at one end of the hydrophobic channel is facilitated in COX-2 because of Val at 89 instead of Ile in COX-1 at that position. The combined effect of the amino acid differences at 89 and 523 contributes to the larger space of this site in COX-2. NSAIDs, occupying both the pockets in COX-2, may be expected to have greater specificity compared to drugs which may bind at only one of them. In addition to the above-mentioned differences between COX-1 and COX-2, significant changes occur at position 115, where a nonpolar leucine is replaced by an uncharged polar tyrosine; at position 119, where a nonpolar valine is replaced by an uncharged polar serine, and at position 357, where a nonpolar leucine is replaced by the much larger nonpolar phenylalanine. The substrate channel is oriented from top to bottom with heme at the top and amino acid residues 112, 115 and 119 at the bottom. Leucine 357 is situated slightly below the active site/NSAID binding pocket.

### Modelling studies as a tool to probe reasons for COX-2 vs COX-1 selectivity

There are clear differences among NSAIDs regarding their relative inhibition of COX-1 and COX-2. The selectivity of a NSAID is based on four factors – the ease of entry of the drug into the enzyme channel, binding energy, destabilizing energy and intermolecular interaction energy. Modelling studies cannot gauge the ease of entry of the drug which is controlled by the molecular gate mechanism<sup>19</sup>, as the drug is positioned at the active site to start with. However, once the drug has passed through the gate, binding, destabilizing and intermolecular energies can clearly explain the binding efficiency and selectivity of COX inhibitors.

We have recently looked at several NSAIDs which have been evaluated for their ability to selectively inhibit

COX-2 and carried out modelling studies to delineate features which may usher in COX-2 selectivity<sup>22,23</sup>. We shall briefly review the results, extend the observation to a few more known drugs and going a step further, propose the design of some highly selective COX-2 inhibitors. For the modelling exercise, we have used available crystal structure data for COX-1, COX-2 and known NSAIDs. For other known NSAIDs and newly proposed molecules, structures have been built using the Builder module of Biosym software<sup>24</sup>. The enzyme-inhibitor complex was modelled with Insight II of the same software. The inhibitor was moved in the active site to maximize the intermolecular interactions and minimize steric hindrances. Molecular mechanics calculations were done using Discover and CFF 91 force-field.

### Results of modelling studies with known COX-2 selective and nonselective inhibitors

Our studies cover SC-558 (1), celecoxib (3), rofecoxib (4), nimesulide (5), meloxicam (6), piroxicam (7), nabumetone (10), naproxen (12) and etodolac (13). Stick diagrams of 3, 5 and 9 are displayed in Figure 3. It has been reported that compounds having the structural feature of an aryl methyl sulphone or aryl sulphonamide may display a propensity for COX-2 selectivity<sup>25</sup>. 1 and 3 (ref. 26) are aryl sulphonamides of which the latter has been introduced recently as a COX-2 selective anti-inflammatory drug with negligible side effects. 4 an aryl methyl sulphone, has been also accepted for similar claims<sup>27</sup>. Nimesulide (5) having an acyclic sulphonamide<sup>22,23</sup> and meloxicam (6)<sup>22</sup>, a cyclic sulphonamide have lesser selectivity for COX-2 than 1–4 in enzyme inhibition tests, but have been found to have high gastro-intestinal tolerability, while piroxicam (9), the 2-pyridyl analogue of 6 is non-selective. 5 and 6 have been called preferential COX-2

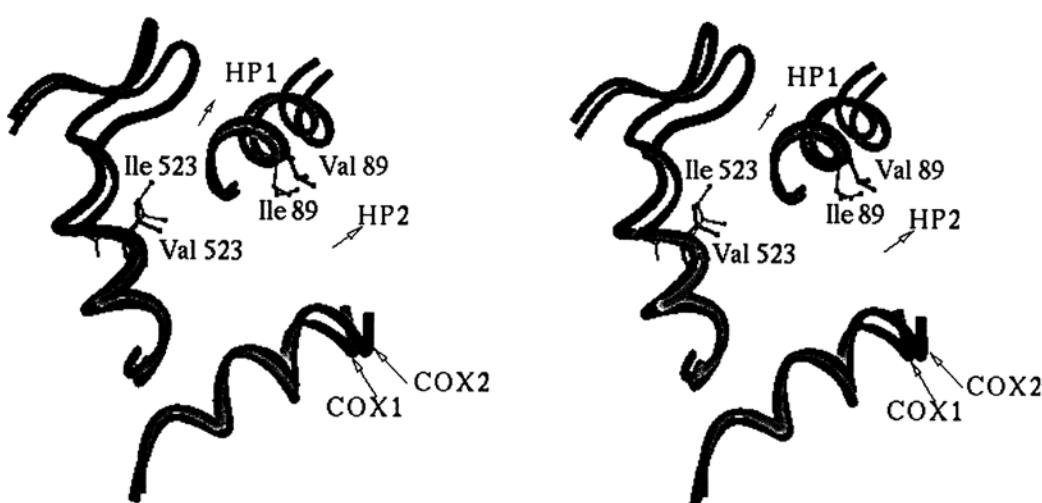
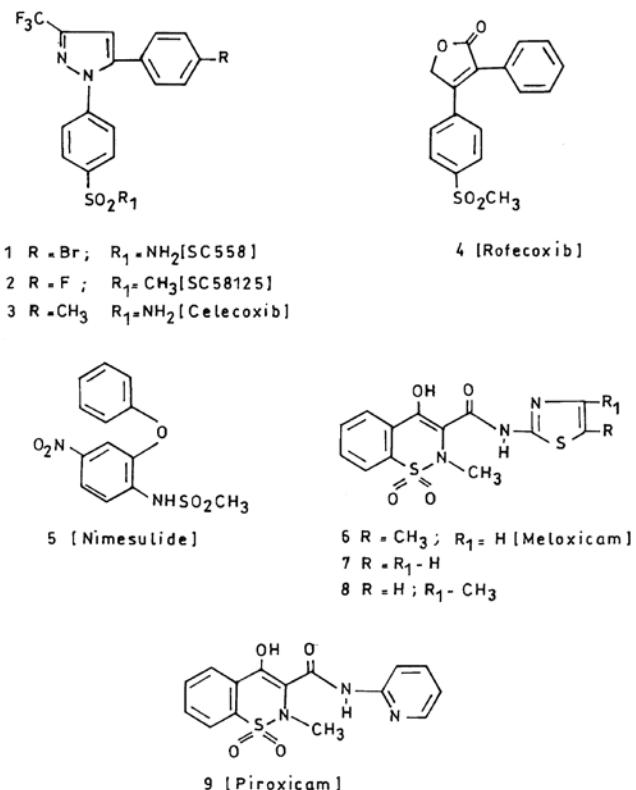


Figure 2. Stereoview of the environment of binding centres of COX-1 and COX-2.



Schemes 1–9.

inhibitors, while 3 and 4 have been designated as selective ones<sup>2</sup>. Nabumetone (10) and naproxen (12) are naphthalene derivatives<sup>22</sup>. The former is a butanone and is reported to have selectivity for COX-2, reflected in increased tolerability, while the latter is a propionic acid and non-selective. Etodolac (13) is a heteroaryl propionic acid with claims for COX-2 selectivity and clinical reports of good safety<sup>28</sup>.

Table 1 gives the binding and destabilizing energies for complexes of different drugs and some analogues with slight variations, with COX-1 and COX-2. Table 2 gives the intermolecular energies between the inhibitor and COX. These data have allowed a correlation with severity of inhibition of COX-1 and COX-2.

Binding energy is the energy released due to the formation of a complex between inhibitor and enzyme and is calculated as  $\Delta E = E_{\text{complex}} - (E_{\text{enzyme}} + E_{\text{drug}})$ . A more negative energy indicates better complexation<sup>29</sup>. Destabilization energy relates to the constraint imposed upon the native COX by deviating from the preferred conformation to accommodate the substrate and is given by<sup>30</sup>

$$\text{DE} = \text{Energy of the protein in the complex} - \text{Energy of the protein in the native state.}$$

A favourable complex is one which does not disturb the native protein to a less stable form. A more negative potential (intermolecular) energy signifies that the

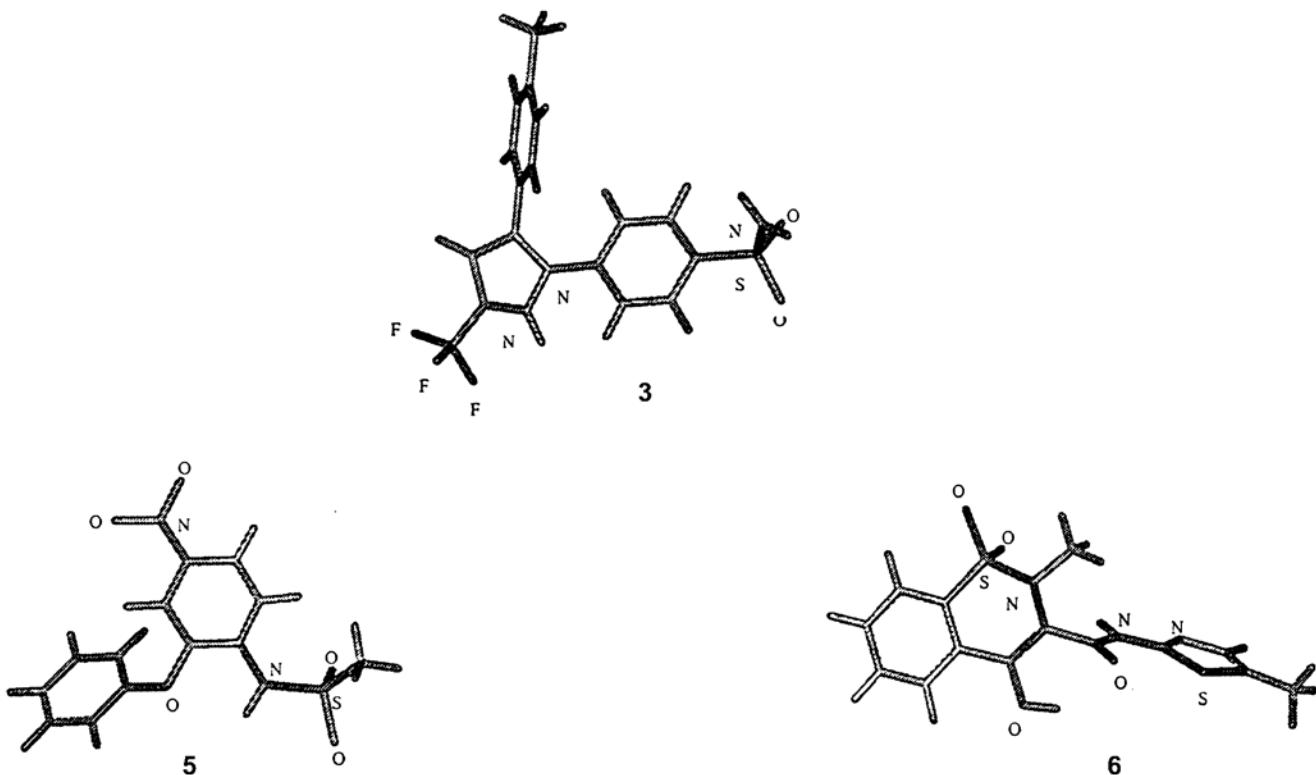


Figure 3. Stick diagrams of 3, 5 and 6.

attractive force is more than the repulsive force and the molecule is in a minimum energy conformation.

It is seen from Table 1 that **3**, **4** and **5** can exhibit selectivity to COX-2 in terms of favourable binding energies. Regarding intermolecular energies, **4** cannot be expected to have partiality for COX-2 over COX-1, while **3** has a definite edge over **5**. It is possible that the operation of the gate mechanism<sup>19,23</sup> accounts for the experimentally observed higher COX-2 selectivity of **3** and **4**. As far as destabilizing energies are concerned, **3**, **4** and **5** prefer COX-2 over COX-1, although **5** seems to be better than the other two.

In comparison to **6**, **9** has less favourable margins for COX-2 over COX-1 in terms of binding and destabilizing energies. The disparity is more glaring for intermolecular energy and favours COX-1 complexation. Desmethyl meloxicam (**7**) and the 4-methyl isomer (**8**) would not be expected to show preferential inhibition of COX-2 and have been found to be so. Among the naphthalenes, data for nabumetone (**10**) in Tables 1 and 2 support its COX-2 selectivity. It is somewhat disconcerting to note that the data for naproxen (**12**) require it to be COX-2 selective, whereas literature reports that the ratio of inhibitory

power of COX-2 vs COX-1 is close to 1. Nevertheless, this is considerably superior to piroxicam or ibuprofen which inhibits COX-1 preferentially<sup>3</sup>. In an interesting excursion, we looked at an isomer of nabumetone with structure **11** in our modelling studies and concluded that it should also be COX-2 selective in terms of binding and intermolecular energies, provided it had anti-inflammatory activity *per se*. This caveat is added since the activity of **10** has been attributed to its active metabolite, 6-methoxynaphthalene-2-acetic acid which we have shown has favourable data for the parameters of Tables 1 and 2 (ref. 22). We are also gratified to find that data for etodolac (**13**) are in line with its selectivity.

In our earlier publications, we have presented detailed information on the binding features of various inhibitors referred to above, giving group-wise splitting of intermolecular energies and hydrogen-bonding schemes<sup>22,23</sup>. We had also shown that **1**, **3** and **5** bind into hydrophilic pocket I through the sulphonamide residue. More interestingly, we observed for **6**, a reinforcing binding with hydrophilic pocket II through the thiazole ring. Figure 4 overlaps of **3**, **5** and **6** at the binding site of COX-2. Figure 5 shows the binding of **6** with the two cyclooxygenases.

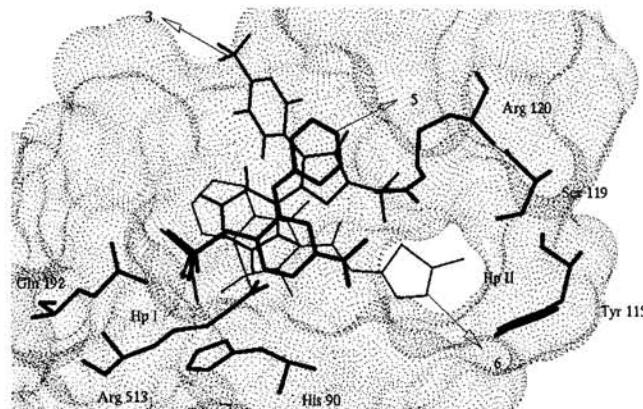
**Table 1.** Component energies (kcal/mol) of complexes of inhibitors with COX-1 and COX-2

Inhibitor	COX-1		COX-2	
	Binding energy	Destabilizing energy	Binding energy	Destabilizing energy
<b>1<sup>a</sup></b>	-45	17	-51	22
<b>1<sup>b</sup></b>	-39	10	-42	10
<b>3<sup>a</sup></b>	-59	12	-94	8
<b>4</b>	-39	10	-70	8
<b>5<sup>a</sup></b>	-5	44	-42	3
<b>5<sup>b</sup></b>	-5	44	-38	10
<b>6<sup>a</sup></b>	15	49	-40	10
<b>7</b>	-17	29	-38	10
<b>8</b>	-13	31	-32	12
<b>9<sup>a</sup></b>	-19	23	-27	13
<b>10<sup>a</sup></b>	-26	7	-37	6
<b>11<sup>a</sup></b>	-28	39	-33	11
<b>12<sup>a</sup></b>	-24	7	-38	6
<b>13</b>	12	42	-37	3

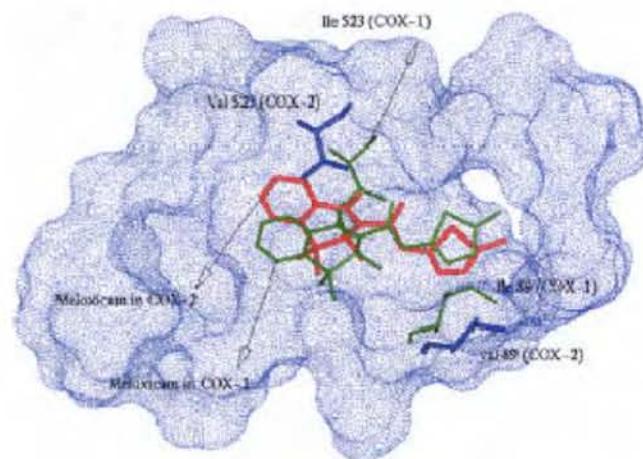
<sup>a</sup>, Ref. 22; <sup>b</sup>, Using I 434V, S 516A, I 523V, H 513K mutants of COX-1 (ref. 23) instead of COX-2 coordinates.

**Table 2.** Intermolecular energies (kcal/mol) between inhibitor and COX

Inhibitor	COX-1	COX-2
<b>1</b>	-53	-57
<b>3</b>	-50	-56
<b>4</b>	-47	-47
<b>5</b>	-35	-38
<b>6</b>	-40	-44
<b>9</b>	-46	-41
<b>10</b>	-34	-35
<b>11</b>	-32	-33
<b>12</b>	-29	-36



**Figure 4.** Overlapping of **3**, **5** and **6** in COX-2 binding site.



**Figure 5.** Binding of **6** with COX-1 and COX-2.

genases. This clearly demonstrates the destabilizing interferences of Ile 523 and Ile 89 of COX-1 for the binding of **6**. Note that Val 523 and Val 89 in COX-2 offer much less obstruction. It can also be seen from Figure 4 that the  $\text{SO}_2$  moiety of **6** has moved a little bit away from the hydrophilic pocket I compared to **3** and **5**.

*Design of analogues of **6** expected to have better COX-2 selectivity*

It is obvious from the above discussions that embellishing **6** with other suitable substituents on the thiazole and benzo rings may augment its selectivity by increasing hydrophobic attractions and more so by binding in the two hydrophilic pockets and further overall reinforcement of intermolecular energies. In these efforts we were also influenced by the published work on the nonselective NSAID, zomepirac (**14**) with an  $\text{IC}_{50}$  ( $\mu\text{M}$ ) COX-1 to COX-2 ratio of 0.15. Replacement of the  $\text{CO}_2\text{H}$  group in **14** by a pyridazinone afforded **15** with a selectivity ratio of more than 1500 in favour of COX-2 (ref. 18). Another input came from the work of Marnett's group. Aspirin, unlike other NSAIDs, binds irreversibly to COX-1 at low doses and to COX-2 at higher doses by acetylating serine at 530 and 516, respectively. The latter is responsible for its anti-inflammatory activity and the former for inhibition of platelet aggregation. Marnett's group investigated the possibility of modifying aspirin and designed the molecule APHS (**17**) with a 2-heptynol side-chain. **17** was found to bind COX-2 irreversibly with a selectivity ratio of 21 over COX-1 (ref. 31).

Based upon these considerations, we are proposing that analogues of **6** represented by structures **18–22** are likely to have better COX-2 selectivity. In **18**, the  $\text{CH}_3$  group in **6** has been enlarged to an isopropyl residue with a view to increasing hydrophobic interactions. Nitro and isopropyl

groups at positions **5** and **6**, respectively on the benzene ring provide additional hydrophobic and hydrophilic interactions. Compound **19** has a pyridazinone ring connected to the thiazole through a  $\text{CH}_2$  spacer, to allow this part of the inhibitor to penetrate deep into the hydrophilic pocket II. Fusion of the pyridazinone to the thiazole in **6** leads to **20** and would be expected to have the same outcome. Addition of alkyl groups to the pyridazinone and benzo rings in **20** leads to **21**, which now has increased possibility of space-filling hydrophobic interactions. In compound **22**, the long heptynol moiety replaces the entire carboxamide residue of **6**.

Binding and destabilizing energies are given in Table 3 and intermolecular energies in Table 4. It is seen that the complexation efficiencies of all the compounds **18–22** are better for COX-2 than for COX-1 like meloxicam (**6**) in terms of both binding and destabilizing energies. Intermolecular energies also support COX-2 selectivity for the five compounds. It is also possible to speculate that all of them, particularly **19** and **20** may be superior to **6** taking into account all the data of Tables 3 and 4. It is interesting to note that **22** with an alkyl side chain in the place of the (more or less) obligatory hetero amide moiety of the oxicams (cf. **6** and **9**) seems to retain COX-2 preference. The group-wise split-up of intermolecular energies of **6** and **18–22** with COX-1 and COX-2 given in Table 5 reveals that differences arise mainly due to modifications to the thiazole ring of **6** (compounds **18** to **21**) and to the replacement of the entire amide appendage by the heptynol group (**22**).

**Table 3.** Component energies (kcal/mol) of complexes of **6** and **18–22** with COX-1 and COX-2

Inhibitor	COX-1		COX-2	
	Binding energy	Destabilizing energy	Binding energy	Destabilizing energy
<b>6</b>	15	49	–40	10
<b>18</b>	8	21	–42	2
<b>19</b>	7	49	–60	7
<b>20</b>	15	49	–50	7
<b>21</b>	1	35	–55	7
<b>22</b>	–4	35	–49	3

**Table 4.** Intermolecular energy (kcal/mol) between **6** and **18–22** with COX-1 and COX-2

Inhibitor	COX-1	COX-2
<b>6</b>	–39.7	–43.6
<b>18</b>	–48.0	–56.0
<b>19</b>	–46.0	–56.0
<b>20</b>	–40.6	–47.4
<b>21</b>	–45.8	–57.4
<b>22</b>	–42.1	–48.4

**Schemes 10–17.**

**Table 5.** Group-wise split-up of intermolecular energies (kcal/mol) of complexes of **6** and **18–22** with COX-1 and COX-2

Inhibitor	COX-1			COX-2		
	Benzothiazine ring	CONH	Thiazole/ equivalent	Benzothiazine ring	CONH	Thiazole/ equivalent
<b>6</b>	–26.6	–6.3	–6.7	–28.2	–7.0	–8.8
<b>18</b>	–29.7	–6.0	–5.2	–29.3	–8.1	–11.2
<b>19</b>	–21.3	–5.5	–13.7	–23.2	–7.4	–20.2
<b>20</b>	–21.3	–5.3	–8.7	–23.1	–7.9	–13.4
<b>21</b>	–25.3	–5.1	–7.5	–27.5	–6.1	–15.9
<b>22</b>	–27.3	–8.3	–	–26.7	–	–16.5

**Table 6.** Possible intermolecular hydrogen bonds in COX-2 inhibitor complexes

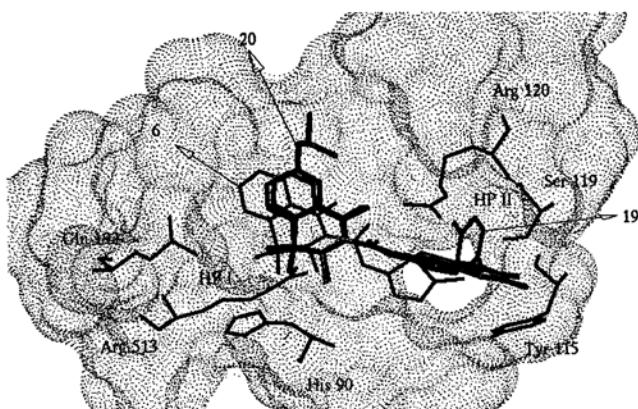
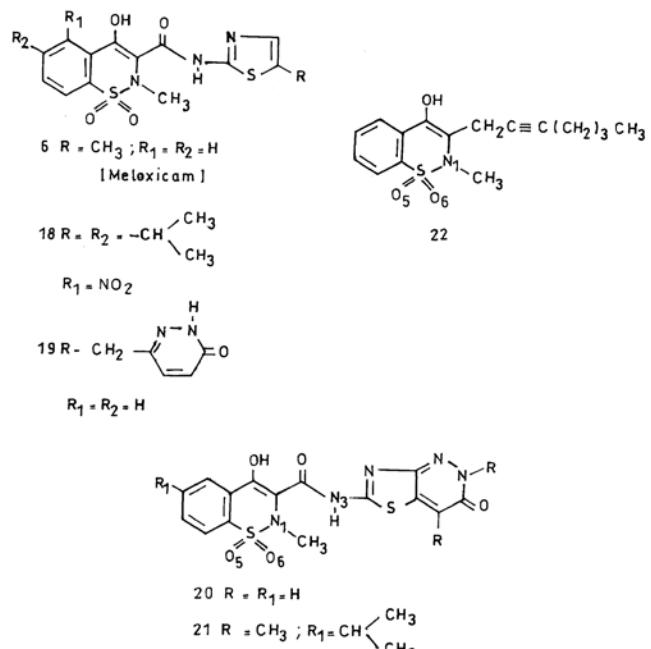
Donor (D)	Acceptor (A)	Distance (Å)		Angle (°)
		D...A	DH...A	
<b>18</b> (N3)	OH (Tyr 355)	3.04	2.08	174.1
<b>19</b> (N3)	OH (Tyr 355)	3.14	2.21	153.8
<b>20</b> (N3)	OH (Tyr 355)	2.75	2.21	131.2
<b>22</b> (N1 of Arg 513)	O6	3.04	2.08	174.1

### Binding features of the designer compounds **18–22**

The mode of binding of the five molecules does not differ from that of meloxicam (**6**)<sup>22</sup>. The binding features of the benzothiazine ring and CONH group of **18–21** have good equivalence with **6** and the substituents on the thiazole ring of **18–21** and the heptynyl group in **22** have additional hydrophobic and hydrophilic interactions around the region of the hydrophilic pocket II. Some specific interactions of these compounds have been described in detail elsewhere<sup>32</sup>. We shall illustrate this in the case of **19**.

The pyridazinone ring in **19** is attached to the thiazole ring through a CH<sub>2</sub> group, which leads to new subsets in its complex formation with COX-2. The benzo ring interacts with Arg 120 and Glu 524 which are not found in the complex of **6** with COX-2. The CONH group interacts strongly with Pro 86 and Val 89. It also forms a hydrogen bond with Tyr 355 which is not found in the complex with COX-1. Table 6 gives the hydrogen bonding scheme of **19** along with those of **18, 20** and **22**. It is interesting to note that the N atom involved in hydrogen bonding differs from one compound to another, illustrative of the larger phenomenon of neighbouring and distal atoms influencing various attractive forces in such enzyme–substrate complexes.

Figure 6 overlaps **6, 19** and **20** bound at the active site of COX-2 and portrays the increased penetration of the thiazolyl moiety into the hydrophilic pocket II, consequent to the proposed attachments.

**Figure 6.** Overlapping of **6, 19** and **20** in COX-2 binding site.**Schemes 18–22.**

Our modelling studies also indicated an alternate conformation for the COX-2 structure with the inhibitor bound at amino acid residues Lys 83, Thr 94, Val 116, Ser 119, Arg 120, Leu 352, Trip 387, Ile 517, Phe 518,

Met 522, Glu 524, Gly 526 and Ala 527. The possibility of such a conformational change has been noted previously in the work on zomepirac and led to the proposal of a second 'bottom-open' structure for COX-2. Comparison of the catalytic core for the open and closed COX-2 structures shows an rms deviation of 0.6 Å for backbone atoms. Hence such conformational changes do not affect the other regions of the binding site<sup>18</sup>. Conformational changes had been suggested even earlier based on the structural diversity of NSAIDs and the buried binding site<sup>33</sup>.

## Conclusion

Selective inhibition of COX-2 promises to provide NSAIDs with increased safety and has already become a purposeful approach. Detailed X-ray crystal structure studies of COX-1 and COX-2, and their complexes with classical and newer NSAIDs have revealed subtle structural differences at the active sites of COX-1 and COX-2 which can be exploited for the design of NSAIDs with improved tolerability. We have embarked upon modelling studies of NSAIDs and candidate drugs to evaluate and correlate complexation efficiencies with COX selectivity. These studies have explained the COX-2 selectivity observed for celecoxib (3), rofecoxib (4), nimesulide (5), meloxicam (6), nabumetone (10) and etodolac (13) and the nonselectivity of piroxicam (9). The molecular gate mechanism may additionally contribute to the increased COX-2 selectivity of 3 and 4 which are poised to become blockbuster NSAIDs. (A recent publication provides evidence suggesting that COX-2 inhibitors impair renal function and cause sodium retention in patients with mild pre-existing renal failure and presumably also in some elderly patients with volume depletion<sup>34</sup>.) Modelling studies confirm the loss of selectivity in 7 and 8, but predict that designer molecules 18–22 would yield meloxicam analogues with increased COX-2 selectivity. We do realize that as of now, our approach is qualitative and requires refinement to offer the quantitation needed to discriminate between 'preferential' and 'selective' COX-2 inhibitors<sup>2</sup>. More accurate modelling may require consideration of interactions with heme also. Finally, as is the case with any other rational design of drugs, the approach is necessarily related to an *in vitro* activity and will operate on the usual assumption and requirement that the designed molecule reaches the targeted enzyme in adequate concentration, unmodified or as an active metabolite.

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