Recent Advances in Inducible Cyclooxygenase (COX-2) Inhibition

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Abstract: Cyclooxygenase is the key enzyme in the biosynthesis of prostanoids, biologically active substances that are involved in several physiological processes but also in pathological conditions such as inflammation. Since ten years now, it is well known that this enzyme exists under two forms: a constitutive (COX-1) and an inducible form (COX-2). Both enzymes are sensitive to inhibition by conventional nonsteroidal anti-inflammatory drugs (NSAIDs). Observations that COX-1, involved in several homeostatic processes, played a housekeeping role while COX-2 expression was associated with inflammation and other pathologies such as cancer proliferation have led to the development of COX-2 selective inhibitors in order to reduce the classical side-effects, of which gastric irritation is the most common, associated with the use of conventional NSAIDs.

1. Introduction

Cyclooxygenase (prostaglandin endoperoxide synthase or COX) catalyses the transformation of arachidonic acid into prostaglandin H₂ (PGH₂) as the first step in the biosynthesis of prostanoids (prostaglandins, prostacyclin and thromboxane). Until recently, one COX was thought to be responsible of both the physiological production of prostaglandins (PGs) and their increased production when inflammation occurs. In 1991 however, an inducible COX was identified as a different isofom from the constitutive enzyme [1] and called COX-2 in opposition to the constitutive form : COX-1. Following this discovery, it was important to determine the involvement of prostanoïds deriving from the constitutive and the inducible forms of cyclooxygenases in both physiological and pathological processes. High levels of COX-1 are expressed in platelets [2], vascular endothelial cells [3], stomach [4,5,6] and kidneys collecting tubules [4,7,8,9]. Prostanoids deriving from COX-1 play an important role in thrombogenesis [10] and in the homeostasis of the gastrointestinal tract and kidneys [10,11,12,13]. In contrast, COX-2 is almost undetectable in physiological conditions but its expression is induced by a wide range of stimuli such as growth factors [14,15], phorbol esters [16], interleukin-1 (IL-1) [17,18,19] or lipopolysaccharide (LPS) [20,21,22]. High levels of COX-2 are detected in exudates and in the spinal cord in different animal models of inflammation [23]. Moreover, specific COX-2 inhibitors show anti-inflammatory, antipyretic and analgesic properties in several animal and human models. All those points clearly demonstrate the implication of COX-2 in inflammation processes. Prostaglandins also seem to be involved in other pathological conditions. Indeed, because they affect mitogenesis [24], cellular adhesion [25] and apoptosis [26], prostaglandins appear to play a major role in the pathogenesis of several types of cancers: overproduction of PGs has yet been well established in cancer of the head and the neck [27], the breast [28], the lung [29,30], the colon [31,32] and the pancreas [33] where an overexpression of COX-2 has been demonstrated, suggesting the involvement of COX-2 in cancerogenesis and, subsequently, the interest of selective COX-2 inhibitors in cancer prevention. This approach is supported by several epidemiological studies of regular use of NSAIDs indicating their effectiveness in chemoprevention of colorectal cancers [34,35]. Finally, long term treatment by NSAIDs has also been shown to decrease the incidence of Alzheimer’s disease. Although the contribution of each COX isoform in this pathology is not well known, a trend for higher COX-2 mRNA message abundance in Alzheimer’s disease neocortex has been noted [36]. Following those findings, it seems now evident that

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COX-2 is expressed in pathological conditions such as inflammation or cancer proliferation whereas COX-1 is mainly expressed as a constitutive enzyme playing key-roles in homeostasis.

Acting as non selective COX inhibitors, classical NSAIDs have been widely used in the treatment of several ailments for a long time. Possessing anti-inflammatory, analgesic and antipyretic properties, this class of agents is chiefly used to treat chronic inflammation states. However, all those agents cause untoward side-effects related to COX-1 inhibition of which gastrointestinal irritation [37,38], sometimes leading to haemorrhage and ulceration, is the most common. So, the evidence of therapeutic benefit deriving from selective COX-2 inhibition led to the development of COX-2 selective inhibitors.

The aim of this review was to give the reader a global view of the recent evolutions of this therapeutic class of agents.

2. Biochemistry of Prostanoids

Cyclooxygenase (prostaglandin endoperoxide synthase) catalyses the conversion of arachidonic acid into prostaglandins and related metabolites (Fig. 1).

![Fig. (1). Biosynthesis of eicosanoids.](image1)

![Fig. (2). Transformation catalyzed by cyclooxygenase](image2)
COX enzymes possess two distinct catalytic activities: a cyclooxygenase activity which catalyses the transformation of arachidonic acid into PGG₂ by reaction with two molecules of oxygen, and a peroxidase activity which reduces the 15S-hydroperoxide PGG₂ to 15S-alcohol PGH₂ [39,40,41] (Fig. 2). Those two enzymatic activities require heme of which there is one per isozyme subunit. In a second step, several isomerases catalyse the transformation of PGH₂ into different other prostaglandins (PGD₂, PGE₂, PGF₂α), prostacyclin synthase catalyses the transformation of PGH₂ into prostacyclin (PGI₂), and thromboxane synthase carries out the transformation of PGH₂ into thromboxane A₂ (TXA₂) (Fig. 3). Moreover, arachidonic acid is also the substrate of other oxidative enzymes such as 5-, 12- and 15-lipoxygenases which also generate biologically active lipids such as leukotrienes.

**Biological Properties of Prostanoids**

Prostanoids are divided into three distinct groups: prostaglandins, prostacyclin and thromboxane. This classification is based upon the nature and the substituents of their central ring. Biological actions of prostanoids on the cardiovascular system can be summarized by the observation that prostaglandins (PGs) and prostacyclin (PGI₂) are potent vasodilatators [42,43,44] while thromboxane (TXA₂) constricts. TXA₂ induces platelet aggregation while PGI₂ exhibits anti-aggregatory properties. PGs and TXA₂ provoke vascular permeability which can lead to oedema.

Actions on other tissues are also observed. The longitudinal smooth muscle of the gastrointestinal tract appears to be contracted by prostaglandins while the circular muscle appears to be relaxed [45]. Uterine smooth muscle is also strongly contracted by prostaglandins [46]. Finally, prostaglandins appear to play a key role in the kidney by increasing renal blood flow and stimulating diuresis [47,48].

**3. Differences Between COX-1 and COX-2**

**A. Gene Expression**

The comparison of COX genes reveals several differences. First, human COX genes are assigned to different chromosomes, with the COX-1 gene on chromosome 9 and the COX-2 gene on chromosome 1 [49,50]. Human COX-1 gene contains 11 exons in 22 Kb instead of 10 exons in 8.3 Kb for COX-2 gene...
The relatively small genomic size of COX-2 fits one of the characteristics of early-immediate genes [53]. Furthermore, multiple 5’ transcriptional elements including NFkB, AP-2 and NF-IL-6 exist in COX-2 and are not present in COX-1 gene [54]. Although gene size favors COX-1, mRNA size favors COX-2 (4.1 Kb for COX-2 versus 2.8 Kb for COX-1) [55]. This size difference is mainly due to the presence of a larger 3’ untranslated region on COX-2 mRNA. This untranslated region of more than 2000 nucleotides contains 22 copies of AUUUA repeats that are known to mediate rapid and selective mRNA degradation [55,56,57]. Such motifs are not found on COX-1 message.

An additional important difference distinguishing COX-1 from COX-2 is the inducibility of the gene expression by a wide range of stimuli (Fig. 4). Indeed, COX-2 expression can be induced in many cell types by bacterial endotoxin [20,21,22], cytokines such as IL-1 [17,18,19], IL-17 [58] or TNFα [59,60], growth factors such as EGF [14,15], or mitogens such as phorbol esters [16]. It should be noted that COX-1 levels are also increased in several cell types by IL-1 and phorbol ester, although the magnitude of the response to those two stimuli is very weak [54]. Finally, it was observed that COX-2 induction can also be triggered by hypoxia [61], synaptic excitation [62], peroxisome proliferators [63] or laminar shear stress [64]. In contrast, the induction of COX-2 is strongly repressed by glucocorticoids which have been demonstrated to act by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA [65,66]. This double action lead to an inhibition of the induction of mRNA for COX-2 as well as the production of proteins.

B. Enzymes Structures

COX-1 and COX-2 from human, sheep and mouse show approximately 60% identity at the amino acid level [67]. Between species, there is about 90% homology in the amino acid sequence. The COX isozymes are integral membrane enzymes found in the endoplasmic reticulum (E.R.) [68,69], although COX-2 enzymes appear to be also present in the nuclear envelope [70]. After posttranslational processing in the E.R. membrane (modifications at 3 of the 4 potential glycosylation sites with 3 high mannose oligosaccharides), cleavage of the signal peptide and insertion to the E.R. membrane, the mature glycosylated COX-1 and COX-2 proteins have an apparent molecular weight of 67,000 and 72,000, respectively [71]. The major isozymes differences in the primary structure are a truncated signal peptide and a 18 amino acid insert in COX-2. This unique sequence has been used to raise specific COX-2 antibodies [72]. It has also been suggested that this sequence could contain regulatory factors for protein turnover and E.R. retention [68]. Cyclooxygenasic and peroxidasic mechanisms are essentially identical between the isozymes [68] although minor differences in fatty acid substrate preferences led to the suggestion that the COX-2 active site was larger than the COX-1 active site [68,73]. Tertiary and quaternary structures of the COX enzymes are virtually identical [74] consisting in 3 folding domains: a N-terminal epithelial growth factor (EGF)-like module [75], a membrane binding domain containing a spiral of 4 amphipathic helices and finally the globular catalytic site. X-ray crystal structures of mouse [77], sheep and human [74] COX isozymes have been determined. The structure of human COX-2 active site seems to be very similar to that of sheep COX-1 with a substantially larger binding site for NSAIDs. This structure modification is mainly due to a difference in amino acid at position 523: the replacement of isoleucine 523 in COX-1 by valine in COX-2 creates a substantial increase in the accessible volume with the catalytic site [78,79,80]. Serine 516 in the ovine enzyme (Vs serine 530 in the human enzyme) was confirmed as the acetylation site of aspirin [81].

**Fig. (4).** Arachidonic acid metabolism pathways.
C. Tissue Distribution

In general terms, COX-1 enzymes are found ubiquitously while COX-2 is only found in inflammation sites or in certain pathological conditions. Nevertheless, several observations demonstrated that a more complex relationship between COX-1 and COX-2 may exist. COX-2 mRNA and enzymes were found in normal tissues such as brain, stomach, kidneys and vascular endothelium [82]. In the rat stomach, it was demonstrated that COX-1 and COX-2 were present. Of particular interest is the cellular distribution of COX enzymes: COX-1 was found in the mucous neck cells while COX-2 was found in the surface mucous cell. This observation led to the suggestion that COX-2 may also have positive effects in the gastric cytoprotection [83]. COX-2 mRNA was found in kidneys and its expression was demonstrated to be increased upon sodium restriction, suggesting that COX-2 may play an homeostatic role in kidneys [84]. Finally, increased COX-2 levels are detected in uterine tissue upon onset of labor suggesting that COX-2 mediates the overproduction of prostanoids associated with labor [85]. All these observations contribute to the hypothesis that, despite their well established pathological role, prostanoids deriving from COX-2 may act in several physiological processes.

4. Evaluation Methods of COX-2 Inhibitors

Several methods have been reported for the evaluation of the COX-1 and COX-2 inhibitory potency and selectivity of new drugs. In vitro assays can be classified in two distinct categories: assays using enzymes or cells. Enzymatic methods use purified [86] or recombinant enzymes [87]. Methods using microsomial preparation of cell line U937 can be joined to this category of tests [88]. Because selective COX-2 inhibitors are typically time-dependent rapidly reversible competitive inhibitors of COX-1 [89], differences in incubation times or in arachidonic acid concentrations can affect the apparent selectivity and potency against COX-2. Concerning the cellular model, several methods using human whole blood [90,91], insect cells [92] or various mammalian cells and platelets [93] were described. It is important to note that several COX-2 inhibitors were found to be more potent and more selective on cellular prostaglandin production than on COX-2 enzymatic assay. In fact, the major difficulty in comparative studies of selective COX-2 inhibitors is the large variations found in COX-2 potency and selectivity depending on the use of different methods. In order to have a precise idea of the new COX-2 inhibitors profile, studies should be led using at least an enzymatic and a cellular assay and reference drugs should be incorporated. In vivo assays have also been employed to evaluate COX-2 inhibitors. Carrageenan-induced paw oedema assay and carrageenan-induced algesia models in rats [94] are currently used to respectively evaluate the anti-inflammatory and analgesic properties of new drugs. In order to measure the anti-inflammatory potency in chronic inflammation, the adjuvant-induced arthritis model [95] is frequently used. Finally, the antipyretic potency of new drugs is commonly determined against the endotoxin-induced pyretic response in rats [96].

5. Medicinal Chemistry

A. Methanesulfonanilide Inhibitors

This important class of COX-2 inhibitors derives from two chemically close compounds: nimesulide or 4-nitro-2-phenoxymethanesulfonanilide (1) and NS-398 (2). Since these two agents were found to possess anti-inflammatory activity [97,98] with an interesting degree of COX-2 selectivity [22,98,99,100,101], a renewed interest was focused on this class of anti-inflammatory agents leading to the synthesis of a great number of analogues. Structurally, these COX-2 inhibitors are characterized as alkylsulfonanilides. The alkyl portion (R) (Fig. 5) of the sulfonyl group is typically a methyl substituent, although halogenated substituents, such as trifluoromethyl substituent, have also been reported [102]. The 2-position is frequently substituted with an arylxy or aroylsulfanyl group but cycloalkyloxy or cycloalkylsulfanyl groups are commonly described. The

![Fig. (5). General structure of sulfonanilide COX-2 inhibitors.](image-url)
4-position invariably bears an electron withdrawing group, sometimes incorporated as part of an adjacent ring (3). Thus, all the members of the sulfonanilide class of COX-2 inhibitors have three common structural features summarized in Fig. 5.

Investigations on the anti-inflammatory activity of alkylsulfonanilide began during the 1960s with nimesulide and diflumidone (4) (Fig. 6) [103]. A large body of informations related to the mechanism of action [104], the pharmacological profile [99,105] and the clinical evaluation of nimesulide [106,107,108,109] have been published, clearly demonstrating that this compound was a promising anti-inflammatory agent. From this point, a large variety of nimesulide derivatives with different electron withdrawing groups at the 4-position including carboxy, ethoxycarbonyl, aminocarbonyl, cyano, aminosulfonyl or trifluoromethyl groups have been synthesized by different groups [110,111]. Following this strategy, the Schering group obtained CGP-28238, also called flosulide (5), whom the structural feature was exploited by the Merck Frosst group for the elaboration of L-745,337 (6), which is the thioether analogue of flosulide [103,112,113]. A large number of in vitro and in vivo trials were performed on these two compounds, demonstrating that the thioether analogue had a greater in vivo anti-inflammatory potency, a better oral bioavailability and gastrointestinal safety profile, although in vitro COX-2 inhibitory potency appeared quite identical for the two drugs [88]. Compared to L-745,337, the ethylthiazolyl analogues (7) and (8) showed superior COX-2 inhibition profile in cell culture. Furthermore, the particular high potency of the lactone analogue (8) in the carrageenan-induced foot pad oedema (ED$_{50}$ = 0.9 mg/kg p.o.) [114] designates this product as the most potent methanesulfonanilide NSAID prepared to date.

B. Diaryl-substituted Cycles as COX-2 Inhibitors

In contrast to the sulfonanilide class, the origins of diaryl-substituted heterocycles as COX-2 inhibitors are not well established. However, two diaryl-substituted heterocycles synthesized in the 1960s, indoxole (9) and oxaprozin (10), were identified as potent anti-inflammatory agents [115,116,117]. It was also suggested that oxaprozin could derive from phenylbutazone (11) [118], leading to the suggestion that this well known NSAID was the first representative member of the diaryl-substituted heterocycles COX-2 inhibitors class (Fig. 7). Of particular interest is the absence of well characterized acidic function in indoxole designating this agent as one of the first non-acidic NSAID described. During the 70s and 80s, several groups of medicinal chemists became interested in this class of agents and a wide range of analogues were synthesised and evaluated for their COX-2 inhibitory potency. The major difference between compounds of this class is the nature of the central ring. Thiazole, oxazole, furan, pyrrole, pyrazole, imidazole, isoxazole, thiophene, cyclopentene structures and many others were proposed. The summary of the literature devoted to the biological evaluation of this class leads to the general statement that 4-methoxy- or 4-halo-substituted diaryl-substituted heterocycles most frequently possess enhanced anti-

![Fig. (6). Examples of COX-2 inhibitors of the sulfonanilide class.](image-url)
inflammatory potency compared to their unsubstituted analogues.

**Diaryl-substituted Cycles with a Central 4-Membered Ring**

Some cyclobutene derivatives such as (12), (13) and (14) are representative members of this class (Fig. 8) [119]. In this series, introduction of a geminal dimethyl substituent (13) in the cyclobutene ring of (12) remarkably increased the COX-2 inhibitory potency ((12): COX-1 IC$_{50}$ not determined; COX-2 IC$_{50}$ > 5 µM and (13): COX-1 IC$_{50}$ = 0.12 µM; COX-2 IC$_{50}$ = 0.002 µM). Incorporation of a ketone functionality (14) also appeared favourable ((14): COX-2 IC$_{50}$ > 5µM, COX-2 IC$_{50}$ = 0.11 µM). Combination of the incorporation of a ketone moiety and of a geminal dimethyl substituent (15) gave an enhanced COX-2 selectivity without affecting the inhibitory potency. Surprisingly, the regioisomeric (16) geminal dimethyl ketone analogue of (15) was almost 30 times less potent on cellular culture although results obtained in carrageenan-induced foot oedema were quite similar for both regioisomers.

**Diaryl-substituted Cycles with a Central 5-Membered Carbocyclic Ring**

Cyclopentene derivatives were one of the first series of diaryl-substituted cycles successfully
investigated as COX-2 inhibitors [120, 121, 122, 123]. For example, SC-57,666 (17) (Fig. 9) possesses a high degree of COX-2 selectivity (COX-1 IC$_{50}$ > 100 µM, COX-2 IC$_{50}$ = 0.026 µM). Replacement of the fluorine atom of SC-57,666 with an hydrogen atom, methyl, methoxy, trifluoromethyl or cyano groups had minor effects on the inhibition of COX-1 enzymes. On the other hand, replacement of the fluorine atom of (17) with a chlorine atom (18) remarkably enhanced the potency against COX-2 (COX-1 IC$_{50}$ > 100 µM; COX-2 IC$_{50}$ = 0.003 µM). Although compound (18) is one order of magnitude more potent than SC-57,666 in COX-2 enzyme inhibition, it is almost two times less potent than (17) in the adjuvant-induced arthritis model in rats (ED$_{50}$ = 3.2 and 1.7 mg/kg p.o., respectively). The superior in vivo performances of SC-57,666 seems to be due to a combination of better absorption, longer half-life and lower first-pass clearance.

Furthermore, no gastric lesions were observed in mice after five hours when this compound was administered intragastrically at the dose of 600 mg/kg. The oral bioavailability and half-life time of SC-57,666 is enhanced by replacing the methylsulfone group with a sulfonamide functionality (19) but this structural modification also leads to a loss of selectivity (COX-1 IC$_{50}$ = 5.1 µM; COX-2 IC$_{50}$ = 0.01 µM). Several structural modifications on the cyclopentene ring have been reported, leading for example to compound (20) which showed a similar potency against COX-2 than SC-57,666 although it was less selective (COX-1 IC$_{50}$ = 18.3 µM; COX-2 IC$_{50}$ = 0.015 µM). Incorporation of 4,4-substituents into a spirocyclic ring [124] led to derivatives (21), (22), (23) and (24) whom in vitro performances are described in Fig. 10. Increasing the size of the spirocyclic ring to cyclobutane appears to be required to obtain a similar COX-2 selectivity than SC-
57,666 although large rings (n > 3, see Fig. 10) dramatically alter the potency against COX-2. Unfortunately, the in vivo potency of spirocyclic derivatives appears clearly lower compared to SC-57,666. Substitution of the 3-position of the cyclopentene template with a ketone moiety \[125,126,127\] as in (25) and (26) (Fig. 11) has also been reported and both of these regioisomers possessed the same COX-2 inhibitory potency. Diarylcyclopentadienes such as (27) were also described \[128,129,130\]. Compared to its cyclopentene analogue, (27) appeared less selective in vitro while its in vivo potency was slightly enhanced. Fusion of a benzene ring to cyclopentene gave diarylindene derivatives like (28) and (29). In this series, the methylsulfonyl derivative (28) is a very potent and selective COX-2 inhibitor while its sulfonamide analogue (29) appears very potent but non-selective (COX-1 IC\(_{50}\) ≥ 100 µM; COX-2 IC\(_{50}\) = 0.011 µM and COX-1 IC\(_{50}\) = 0.007 µM; COX-2 IC\(_{50}\) = 0.005 µM, respectively) \[131\].

**Diaryl-substituted Cycles with a Central 5-Membered Heterocyclic Group**

**Thiophenes**

Investigations performed by a team of researchers at DuPont group on the diaryl heterocyclic class of anti-inflammatory agents led to the discovery of DuP 697 (30) (Fig. 12). This thiophenic compound has been
shown to possess a great selectivity and potency against COX-2 (COX-1 IC\textsubscript{50} = 1 µM; COX-2 IC\textsubscript{50} = 0.01 µM) [132]. Further investigations demonstrated that compound (30) was orally active and very potent in the adjuvant arthritis assay with an ED\textsubscript{50} of 0.18 mg/kg p.o. [133]. Clinical evaluations performed on this compound revealed that it was not metabolized and possessed a plasmatic half-life of about 292 hours in human [134] attributed to an enterohepatic recirculation. From this point, different groups became interested in this compound and several analogues of DuP 697 were prepared with the aim to obtain compounds showing a similar COX-2 inhibitory profile and a lower plasmatic half-life. Replacement of the bromine atom with different substituents [134,135] such as hydrogen atom, alkyl, aminosulfonyl or methoxycarbonyl groups were found to be acceptable substitutions. Investigations on the replacement of the methylsulfonyl group of (30) with an aminosulfonyl moiety usually led to compounds with increased potency against COX-1 and COX-2 [136,137]. For example, sulfone (31) exhibits a COX-1 IC\textsubscript{50} > 100 µM and a COX-2 IC\textsubscript{50} = 0.25 µM while the sulfonylamide analogue (32) shows a COX-1 IC\textsubscript{50} = 1.3 µM and a COX-2 IC\textsubscript{50} = 0.03 µM. Moreover, replacement of the methylsulfonyl group of (31) with an acetyl group [136] led to compound (33) which was demonstrated to be a COX-1 selective inhibitor. This inversion of selectivity has also been observed with compounds of which the sulfonyl group was replaced with other functions. Regioisomeric 3,4-diarylthiophenes [134,137,138] such as (34), 2,4-diarylthiophenes such as (35) and 2,5-diarylthiophenes such as (36) have also been investigated (Fig. 13). While compound (32) and (34) showed a similar in vitro activity (COX-1 IC\textsubscript{50} ≥ 100 µM; COX-2 IC\textsubscript{50} = 0.08 µM for compound (34)), compounds (35) and (36) showed a significantly lower activity whatever the in vitro test performed. These results clearly demonstrate that the potency against COX-2 is only observed when the aromatic groups are on adjacent position in the thiophene ring. Substitution on the thiophene ring of (34) with a bromine atom led to isomers (37) and (38) (Fig. 14). Analogue (37), where the bromine atom is located in the adjacent position to the aromatic ring bearing the methylsulfone was weakly active while isomer (38) appeared very potent against COX-2 (COX-1 IC\textsubscript{50} ≥ 100 µM; COX-2 IC\textsubscript{50} > 100 µM and COX-1 IC\textsubscript{50} ≥ 100 µM; COX-2 IC\textsubscript{50} > 0.08 µM, respectively). Thus, benefits of the substitution of the thiophene ring with a bromine atom are only observed when the bromine atom is located in distal position to the methylsulfonylphenyl ring. In the 3,4-diarylthiophene series, it was observed that 4-methylsulfonyl or 4-aminosulfonyl groups were required to obtain selective COX-2 inhibitors. In addition, it was observed that the substituents on the other phenyl ring could seriously affect both the selectivity and potency against COX-2. For example, replacement of the fluoride atom of (34) with a methoxy group (39) led to an increased potency against COX-1 (COX-1 IC\textsubscript{50} = 0.9 µM; COX-2 IC\textsubscript{50} = 0.05 µM). However, the COX-2 specificity can be reobtained by introduction of a fluoride atom in adjacent position to the methoxy group, like in (40) (COX-1 IC\textsubscript{50} ≥ 100 µM; COX-2 IC\textsubscript{50} = 0.03 µM). A wide range of analogues bearing different substituents on the aromatic ring that did not bear the methylsulfonyl function were evaluated and most of those compounds appeared to be specific and potent against COX-2.
**Pyrroles**

A series of 1,2-diaryl/pyrroles has been synthesized and were found to contain potent and selective COX-2 inhibitors [139]. For example, compound (41) (Fig. 15) shows a great COX-2 inhibitory potency (COX-1 \( IC_{50} > 100 \mu M \); COX-2 \( IC_{50} = 0.06 \mu M \)) and appears to be potent in the carrageenan-induced oedema in rats (25% inhibition in the paw oedema assay at the dose of 10 mg per kg p.o.). In order to evaluate the importance of the 1,2-diaryl arrangement in (41), the N-aryl ring was replaced with hydrogen atom, benzyl, alkyl or cycloalkyl substituents. Neither of these new compounds showed inhibition of the COX enzymes up to 100 \( \mu M \). Only the cyclohexyl compound (42) gave interesting results (COX-1 \( IC_{50} > 100 \mu M \); COX-2 \( IC_{50} = 0.52 \mu M \)) although it was eight times less potent than (41), suggesting that the 1,2-diaryl arrangement was crucial for the selectivity and potency against COX-2 in this series. Replacement of the fluoro substituent in (41) with an hydrogen, a trifluoromethyl or a methyl group gave compounds showing a comparable profile. In contrast, replacement of the fluoro substituent of (41) with an acetyl group led to a considerably less active compound, suggesting that minor modifications of the size and/or the polarity on the N-aromatic ring can seriously alter the activity against COX-2. Regioisomer of (41), represented by compound (43), showed a good potency against COX-2 (COX-1 \( IC_{50} > 100 \mu M \); COX-2 \( IC_{50} = 0.05 \mu M \)) and appeared to be more potent than (41) in the carrageenan-induced paw oedema in rats (42% inhibition at the dose of 10 mg/kg). This result suggests that the in vivo potency is enhanced when the 4-fluorophenyl group is in position 2 of the pyrrole ring. The isosteric sulfonamide derivative (44) of (43) was less selective but more potent than (43) against COX-2 (COX-1 \( IC_{50} = 10.5 \mu M \); COX-2 \( IC_{50} = 0.014 \mu M \)). Placement of substituents at the 4-position of the pyrrole ring led to interesting tetrasubstituted pyrroles such as compound (45), which appeared remarkably selective and potent against COX-2 (COX-1 \( IC_{50} > 100 \mu M \); COX-2 \( IC_{50} = 0.03 \mu M \)). The 2,3-diarylpyrrole series [140,141,142] was also extensively studied. A series of 2-(4-methylsulphonylphenyl) 3-(4-fluorophenyl)pyrroles bearing different substituents in the 5-position of the pyrrole ring (46) has been prepared and evaluated by the DuPont Merck group. In this series, best in vitro results were obtained by placing electron withdrawing groups like chlorine (46) or bromine (47) in the 5-position. In the adjuvant arthritis assay, (46) and (47) had \( ED_{50} \) of 0.5 and 1.05 mg/kg respectively.

**Furans**

COX-2 selective inhibitors were also found in the 3,4-diaryl-substituted furan series (48) [143,144] (Fig. 16) but, in general, these drugs were found to be less potent in vitro compared to the corresponding thiophenes. More interesting was the finding that the furanone (49) displayed a 3000 fold selectivity. Further investigations conducted on the 5H-furanone-based inhibitors revealed that the selectivity for COX-2 was enhanced by substituting the 5,5'-positions with dialkyl (50) or alkoxyalkyl groups. For example, compound (50) possesses a very high selectivity for COX-2. With 50% inhibition in the carrageenan-induced paw oedema in rats at the dose of 0.1 mg/kg, compound

![Fig. (15). Examples of pyrrole-based selective COX-2 inhibitors.](image-url)
Fig. (16). Examples of 3,4-diaryl-substituted furans.

(50) is also one of the most potent NSAID COX-2 inhibitors ever synthesized. Moreover, it was described that certain substituents at the 5,5′-positions of the furanone ring could provide additional metabolic stability [145,146]. Finally, MK-0966 (51), with a COX-2 IC$_{50}$ = 0.77 µM and a COX-1 IC$_{50}$ not determined because inhibition was not seen at single doses up to 1000 mg in the human whole blood assay [147,148], was selected for clinical evaluation. Excellent results were obtained with this compound also called Rofecoxib in clinical trials and it has been recently commercialized in several countries.

**Pyrazoles**

Interest for 1,5-diarylpyrazole-based COX-2 selective inhibitors began in the early 90’s [149,150]. For example, compound (52) (Fig. 17) was found to possess a good in vitro potency (COX-1 IC$_{50}$ ≥ 100 µM; COX-2 IC$_{50}$ = 0.24 µM) and to cause 81% inhibition in

Fig. (17). Examples of 1,5-diarylpyrazole-based COX-2 inhibitors.
the adjuvant-arthritis model in rats at the dose of 3.2 mg/kg per os. Compound (53) or SC-58125, one of the first well documented pyrazole-based COX-2 inhibitor [9], showed a promising profile but suffered from possessing a long terminal half-life in rats (>200 hours). Subsequently, this compound was found to be unacceptable for clinical evaluation. Replacement of the 4-fluoro group with a metabolically more labile methyl group and changing the methylsulfonyl for an aminosulfonyl moiety led to SC-58635 (54). This compound appeared selective and potent against COX-2 in vitro (COX-1 IC_{50} = 13 µM; COX-2 IC_{50} = 0.04 µM) and gave good results in the carrageenan-induced foot oedema and adjuvant-induced arthritis models in rats with ED_{50} of 0.4 and 7 mg/kg, respectively. Furthermore, SC-58635 was demonstrated to possess a better oral bioavailability and a decreased half-life (10 hours) compared to its sulfone congener [151]. Subsequently, this compound, Celecoxib, was clinically evaluated and the successful results obtained allowed its commercialisation. Other interesting examples of pyrazole-based inhibitors are given by compound (55) and (56) which possess an additional ring that provide them a restricted conformational freedom. Observations that these two compounds are potent COX-2 inhibitors (COX-1 IC_{50} = 11 µM; COX-2 IC_{50} = 0.04 µM, respectively) suggest that they possess the required conformation for inhibiting COX-2 [152,153].

**Oxazoles and Isoxazoles**

A large number of oxazole-based inhibitors have been investigated by different laboratories. It was clearly demonstrated that regioisomeric oxazoles showed very different in vitro activities [154]. For example, compound (57) (Fig. 18) showed a COX-1 IC_{50} ≥ 100 µM and a COX-2 IC_{50} = 10 µM whereas regioisomer (58) had a COX-1 IC_{50} ≥ 100 µM and a COX-2 IC_{50} = 0.14 µM. In general, sulfonamide analogues showed an enhanced potency against both COX-1 and COX-2. This finding is illustrated by the profile of compound (59) which shows a COX-1 IC_{50} = 25 µM and a COX-2 IC_{50} = 0.02 µM and causes 50% inhibition at 0.2 mg/kg per os in the carrageenan-induced paw oedema assay in rats [155]. Replacement of the phenyl ring of (59) with a cyclohexyl ring and introduction of a fluorine atom adjacent to the sulfonamide moiety led to JTE 522 (60) which was found, in comparison to compound (59), to be more selective and more potent against COX-2 [156,157]. Investigations performed in the isoxazole series also gave interesting results. For example, hydroxymethylisoxazole (61) exhibited a selectivity ratio superior than 5000-fold and caused 50% inhibition at the dose of 1.1 mg/kg p. o. in the carrageenan-induced foot oedema model in rats [158].

**Other 5-Membered Ring Derivatives**

Several thiazole, isothiazole, thiazadiazole, imidazole, and oxadiazole derivatives were described as potent and selective COX-2 inhibitors (Fig. 19). For example, thiazole (62) is a potent and selective inhibitor of COX-2 [159] and its isothiazole (63) and thiazadiazole (64) analogues are both potent COX-2 inhibitors [138]. In vivo studies performed on compound (64) demonstrated that it was very potent in the
carrageenan-induced foot oedema model with an ED$_{50}$ lower than 1 mg/kg. The study of the imidazole series clearly demonstrated that 1,2-diarylimidazole derivatives were more selective and potent against COX-2 than their 4,5-diarylimidazole isomers [160,161]. Compound (65), which is one of the more potent COX-2 inhibitor belonging to the 1,2-diarylimidazole class, showed an COX-1 IC$_{50}$ $\geq 1000$ μM and a COX-2 IC$_{50}$ = 0.12 μM. In the 1,2-diarylimidazole series, substitution of the sulfone moiety with a sulfonamide function leads, in general, to compounds showing an enhanced potency against both COX-1 and COX-2. Finally, 3,4-diaryloxazolone derivative (66) showed a great potency against COX-2 but appeared poorly selective (COX-1 IC$_{50}$ = 3.5 μM; COX-2 IC$_{50}$ = 0.06 μM). In vivo tests performed on this compound revealed that (66) caused 67% inhibition in the adjuvant arthritis model at the dose of 1 mg/kg [162].

**Derivatives with a Central 6-Membered Carbocyclic or Heterocyclic Group**

Several research groups performed investigations on ortho-terphenyl (67) and ortho-terpyridine (68)

Fig. (19). Examples of COX-2 inhibitors bearing different 5-membered central rings.

Fig. (20). Examples of COX-2 inhibitors bearing a central 6-membered ring.
analogs as COX-2 inhibitors [163,164,165] (Fig. 20). In both series, it was clearly established that substitution of the central ring provided a potency enhancement. Ortho-terphenyl sulfonamide (67) displayed a selectivity ratio of 2700 fold (COX-1 IC$_{50}$ = 5.5 µM; COX-2 IC$_{50}$ = 0.002 µM) and revealed a high potency in the adjuvant arthritis model in rats with an ED$_{50}$ of 0.05 mg/kg. Insertion of an heteroatom like an oxygen atom (69) between the central ring and the ring that does not carry the sulfonamide or the methylsulfone function was found to be an acceptable structural modification. Derivatives of this series were found to possess a good in vivo activity (inhibitory effects in the range of 45-62% at the dose of 1 mg/kg in the adjuvant arthritis model) [166]. In the ortho-terpyridine series, 2,3-diaryl [167,168] and 3,4-diarylpyridine [169] derivatives have been described but no important variations concerning the selectivity and potency against COX-2 were demonstrated. In vivo evaluation of compound (70), which belongs to the 2,3-diarylpyridine class, revealed a great potency with an ED$_{50}$ of 0.6 mg/kg in the carrageenan-induced paw oedema assay.

**Derivatives with a Central Bicyclic Ring**

Several compounds bearing a fused ring system as central template and still possessing potency against COX-2 have been reported (Fig. 21). The imidazopyridine (71) has been described as a COX-2 selective inhibitor (COX-1 IC$_{50}$ > 100 µM; COX-2 IC$_{50}$ = 0.14 µM) but has not yet been evaluated in vivo [170]. The imidazothiazole (72) was found to be a highly potent COX-2 inhibitor in chinese hamster ovary cells transfected with human COX (COX-1 IC$_{50}$ > 50 µM; COX-2 IC$_{50}$ = 0.016 µM) and this compound also showed a good potency in the carrageenan-induced paw oedema (ED$_{50}$ = 2 mg/kg). The thiazolotriazole (73) was also reported to be selective and potent against COX-2 (COX-1 IC$_{50}$ = 43 µM; COX-2 IC$_{50}$ = 0.01 µM). No chromium leakage in the gastro-intestinal integrity test was observed for compounds (72) and (73) after administration for 5 days at the dose of 100 mg/kg twice daily in rats [171,172,173]. Fusion of a lactone to a central thiophene ring provided the selective inhibitors (74) and (75) (COX-1 IC$_{50}$ = 32 µM; COX-2 IC$_{50}$ = 0.051 µM and COX-2 IC$_{50}$ = 0.03 µM, respectively) [174], which can exist in the hydroxy carboxylate form at high pH and in the lactone form at lower pH. So, these two compounds have a greater solubility at high pH than their thiophene analogues and, subsequently, show a greater oral bioavailability [175].

**1,2-Diarylethylene Derivatives**

First examples of COX-2 inhibitors without central ring are given by (76) and (77) [176,177] (Fig. 22). Compound (76) was obtained by reduction of the furanone analogue to the corresponding diol. Results obtained with (76) and (77) demonstrated that both compounds were selective COX-2 inhibitors and potent in the classical inflammation models.

**C. Di-tert-butylphenols**

Recently, substituted di-tert-butylphenols were described by the Parke-Davis group as a novel class of potent and selective COX-2 inhibitors [178,179]. In this class, a series of thiazole, oxazolone, 1,3,4-

![Fig. (21). Examples of derivatives with a central bicyclic ring as COX-2 inhibitors.](image-url)
thiadiazole and 1,3,4-oxadiazole derivatives were synthesized and pharmacologically evaluated (Fig. 23). For example, thiazolone di-tert-butylphenol (78) showed a good selectivity and potency against COX-2 in vitro with a COX-1 IC₅₀ = 9.2 µM and a COX-2 IC₅₀ = 0.17 µM. Compound (78), when orally administrated as its choline salt, appeared potent in the carrageenan-induced foot oedema in mice (ID₄₀ = 16 mg/kg p.o.) and showed analgesic properties in the acetic acid writhing test (ED₄₀ = 0.1 mg/kg p.o.). Surprisingly, compound (79), a weak and less selective COX-2 inhibitor, appeared more potent than (78) in vivo with an ID₄₀ of 8.1 mg/kg p.o. in the carrageenan-induced foot oedema assay in mice, suggesting that additional pharmacological properties of substituted di-tert-butylphenols may contribute to their efficacy in vivo. The isosteric replacement of the sulfur atom of compound (78) with an oxygen atom like in (80) resulted in a reversal of selectivity and this phenomena was observed in all the cases studied, clearly demonstrating that the selectivity and potency against COX-2 are extremely sensitive to minor changes in the chemical structure. 1,3,4-thiadiazole derivative (81) was identified as the most potent COX-2 inhibitor of the di-tert-butylphenol class (COX-1 IC₅₀ =100 µM and a COX-2 IC₅₀ = 0.14 µM on purified enzymes test) in all the in vitro and in vivo tests performed. Replacement of the sulfur atom of (81) with an oxygen atom gave compound (82) and this structural modification led to a loss of potency against COX-2. On the other hand, compound (83) appeared potent in cellular assays although it failed to inhibit either enzymes in isolated enzyme assays. Compound (81) and (83) were orally active and caused a minimal gastro-intestinal damage.

6. Clinical Trials with COX-2 Inhibitors

Recently, the literature began burgeoning of clinical findings with COX-2 inhibitors. From the phase I clinical trial data, Celecoxib (54) was shown to be rapidly absorbed with a Tmax of 3 hours and to be metabolised and eliminated with a half-life of 11.2 hours [180]. Celecoxib at the dose of 600 mg twice daily appeared to have no effect on platelet aggregation in human beings [181]. Celecoxib at the dose of 600 mg twice daily appeared to have no effect on platelet aggregation in human beings [181]. Single doses of celecoxib 100 or 400 mg were superior to placebo and as analgesic as aspirin in a post-surgical study of dental pain [182,183]. Dose
range of 100-400 mg daily of Celecoxib for osteoarthritis and 200-800 mg daily for rheumatoid arthritis [184] appeared similarly relevant as Naproxen 500 mg or Diclofenac 75 mg, both twice daily, in several studies performed over 12 or 24 weeks [185,186,187]. Levels of gastric mucosal injury with Celecoxib 100 or 200 mg twice daily were equivalent to those observed with placebo and significantly reduced compared with Naproxen 500 mg twice daily [188].

Rofecoxib (51) was shown to be rapidly absorbed in humans with a Tmax of 2 hours. Rofecoxib appeared to be at least as potent as Naproxen 550 mg for relief of post-surgical dental pain [189] and also showed antipyretic potency in human beings. In the treatment of osteoarthritis, Rofecoxib 12.5 or 25 mg once a day was as effective as ibuprofen 800 mg 3 times a day in studies performed over 6 weeks [191]. At the dose of 250 mg daily, Rofecoxib had no effects on bleeding time and caused levels of gastric mucosal injury that were equivalent to placebo [192].

Finally, Nimesulide was reported to be a COX-2 selective inhibitor. Long term tolerability of Nimesulide 100 mg twice daily was evaluated in patients with osteoarthritis over a 6 months period revealing a decreasing of the gastro-intestinal side effects of 10.9% compared with Diclofenac 50 mg thrice daily [99].

7. Conclusions

The promising clinical results obtained with COX-2 selective inhibitors such as Rofecoxib and Celecoxib offer the hope of relief from arthritic ailments, pain and fever without observing the classical side-effects attributed to classical NSAIDs. Furthermore, COX-2 selective inhibitors could rapidly hold an interesting place in the prevention, even the treatment, of several other diseases such as cancers associated with an overexpression of COX-2 or Alzheimer’s disease. Nevertheless, because COX-2 may be important for the regulation of several physiological processes, the use of COX-2 inhibitors could in theory be deleterious. First, observation that COX-2 is induced when gastric injury occurs led to the suggestion that COX-2 inhibitors should delay ulcer healing. Second, because COX-2 is constitutively expressed in kidneys and is also found in vascular endothelium, COX-2 selective inhibitors should induce renal failure and exacerbate hypertension. Third, hormonal induction of COX-2 is important for ovulation and at the end of pregnancy, uterine high levels of COX-2 are necessary for the onset of labor. Thus, selective COX-2 inhibitors should have deleterious effects on ovulation and parturition. Finally, COX-2 selectivity also lead to a restriction in the therapeutic applications: selective COX-2 inhibitors, because they did not inhibit COX-1, will never show the cardiovascular protective effects reported with aspirin and classical NSAIDs. So, despite their promising profile, several questions concerning the safety of selective COX-2 inhibitors remain without answer. Those interrogations will only find an answer in long term clinical studies that will exactly define the efficiency and the potential side-effects of COX-2 selective inhibitors.

8. Acknowledgements

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List of Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
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<tr>
<td>mRNA</td>
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<td>NSAIDs</td>
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PGI₂ = Prostacyclin
PGs = Prostaglandins
p.o. = Per os
TNFα = Tumor necrosis factor α
TXA₂ = Thromboxane A₂

References


