Cyclooxygenase inhibitors—current status and future prospects. Eur J Med Chem

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · MARCH 2001

Impact Factor: 3.45 · DOI: 10.1016/S0223-5234(01)01197-7 · Source: PubMed

2 AUTHORS:

Gerd Dannhardt
Johannes Gutenberg-Universität Mainz
194 PUBLICATIONS **2,221** CITATIONS
SEE PROFILE

Werner Kiefer
Johannes Gutenberg-Universität Mainz
31 PUBLICATIONS **888** CITATIONS
SEE PROFILE

Eur. J. Med. Chem. 36 (2001) 109–126

PII: S0223-5234(01)01197-7/REV

Review

Cyclooxygenase inhibitors – current status and future prospects

Gerd Dannhardt*, Werner Kiefer

Johannes Gutenberg-University of Mainz, Institute of Pharmacy, Staudingerweg 5, D-55099 Mainz, Germany

Received 8 November 2000

Abstract – Prostaglandins are formed from arachidonic acid by the action of cyclooxygenase and subsequent downstream synthetases. Two closely related forms of the cyclooxygenase have been identified which are now known as COX-1 and COX-2. Both isoenzymes transform arachidonic acid to prostaglandins, but differ in their distribution and their physiological roles. Meanwhile, the responsible genes and their regulation have been clarified. COX-1, the pre-dominantly constitutive form of the enzyme, is expressed throughout the body and performs a number of homeostatic functions such as maintaining normal gastric mucosa and influencing renal blood flow and platelet aggregation. In contrast, the inducible form is expressed in response to inflammatory and other physiological stimuli and growth factors, and is involved in the production of the prostaglandins that mediate pain and support the inflammatory process. All the classic NSAIDs inhibit both COX-1 and COX-2 at standard anti-inflammatory doses. The beneficial anti-inflammatory and analgesic effects are based on the inhibition of COX-2, but the gastrointestinal toxicity and the mild bleeding diathesis are a result of the concurrent inhibition of COX-1. Agents that inhibit COX-2 while sparing COX-1 represent a new attractive therapeutic development and could represent a major advance in the treatment of rheumatoid arthritis and osteoarthritis. Apart from its involvement in inflammatory processes, COX-2 seems to play a role in angiogenesis, colon cancer and Alzheimer's disease, based on the fact that it is expressed during these diseases. The benefits of specific and selective COX-2 inhibitors are currently under discussion and offer a new perspective for a further use of COX-2 inhibitors. © 2001 Éditions scientifiques et médicales Elsevier SAS

cyclooxygenase (COX-1, COX-2) / classic NSAIDs / selective COX-2 inhibitors / inflammation / angiogenesis / colon cancer / Alzheimer's disease

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation, especially arthritis. From a historical viewpoint, the first NSAID with therapeutic benefits was aspirin, which has now been used for more than 100 years as an NSAID. The overall worldwide production of about 50 000 tons a year reflects the importance of this substance even today [1].

In the 1970s, a scientific breakthrough occurred with the elucidation of the molecular mechanism of aspirin and other NSAIDs. Vane, Samuelson and Bergstrom succeeded in showing that these anti-inflammatory substances block the biosynthesis of prostaglandins (PGs) which contribute to a variety of physiological and pathophysiological functions.

Figure 1 summarizes the biosynthesis of PGs: the initial step in the biosynthesis of prostanoids is the liberation of arachidonic acid (AA) from the phospholipids of the cell membrane catalyzed by phospholipase A_2. The following decisive step is the biotransformation of AA by cyclooxygenase. In a bifunctional action, this first generates the unstable PGG_2, the cyclooxygenase reaction itself, which is then immediately converted into PGH_2 by the same enzyme in a peroxidase reaction. As shown in *figure 1*, the final products of the AA metabolism are PGs, thromboxanes and prostacyclin [2–5].

PGs are produced by most cells and are also present in tissues, which explains their broad spec-

* Correspondence and reprints.
E-mail address: dannhrdt@mail.uni-mainz.de (G. Dannhardt).

trum of biological responses. PGs mediate a number of characteristic features of the body's response to tissue injury or inflammation. The outstanding effects of the PGs include their cytoprotective properties in the gastrointestinal (GI) tract and control of renal functions in the kidney.

PGE_2 is the most important PG which mediates the typical symptoms of inflammation: rubor, calor, tumor, dolor and functio laesa. Dilatation of small blood vessels initiates the development of redness and heat; the increase in vascular permeability causes the characteristic swelling of tissues. Moreover, PGs sensitize peripheral nerve endings and nociceptors to transmit pain signals to the brain and the spinal cord.

In addition to the well-accepted proinflammatory role of PGs, there is also evidence of anti-inflammatory activity in certain COX-2-derived PGs in vivo, an experiment recently reported by Gilroy et al. [6].

Like aspirin, all other NSAIDs such as ibuprofen, ketoprofen and naproxen develop their mode of action by blocking cyclooxygenase. Therefore, administration of NSAIDs, for example to treat inflammatory diseases such as osteoarthritis or rheumatoid arthritis, unavoidably leads to a lack of the prostaglandins required for the physiological functions mentioned above. Therapeutic effects and side-effects of this class of anti-inflammatory drugs are closely related to their biochemical mechanism of action.

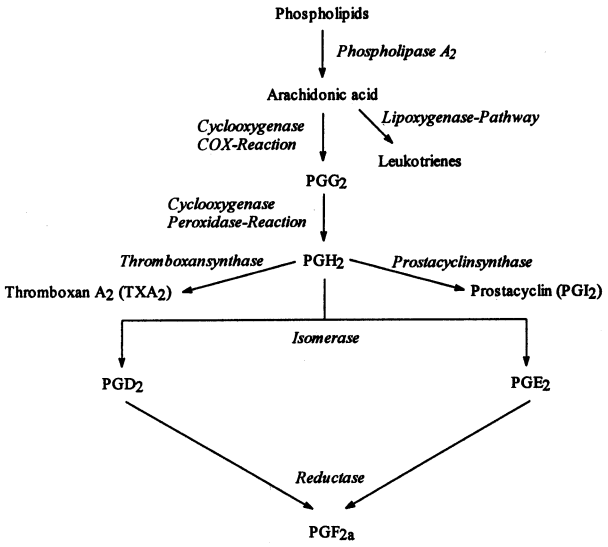

Figure 1. Arachidonic acid cascade.

As a consequence, long-term NSAID users suffer from a high incidence of GI irritation or, in the worst case, from the development of life threatening GI ulcers and bleeding. These lesions can lead to increased morbidity in patients [7–9]. Administration of NSAIDs may also lead to renal disorders and have hypertensive effects. Due to a reduced production of PGs, such as PGI_2, PGE_2 and PDG_2, in the regulation of renal blood circulation, the rate of glomeruleric filtration is reduced. Especially in patients with reduced renal function, this leads to retention of water, hypertension and, in some cases, to renal failure [10–12].

The inhibition of cyclooxygenase in thrombocytes results in decreased production of thromboxane A_2. This phenomenon prolongs bleeding time and leads to inhibition of platelet aggregation. A severe side-effect of NSAIDs is bronchoconstriction with resultant asthmatic events. The reduced amount of bronchodilatating PGE_2 on the one hand and a shift in the metabolic pathway from the cyclooxygenase pathway to the 5-lipoxygenase pathway on the other hand, seems to be responsible for the bronchoconstricting effect of NSAIDs [13]. The latter pathway metabolizes 'overflow' AA, which cannot be transformed by the blocked cyclooxygenase pathway. The resulting leukotrienes act as bronchoconstrictors [14].

Because of these problems, a major target of drug research is the development of NSAIDs with anti-inflammatory and analgesic activity but without side-effects.

2. The discovery of COX-2 and its distribution

Until the latter part of the 1980s, it was common belief that the formation of PGs was only limited by the amount of AA available, the substrate of cyclooxygenase. However, some observations made in 1988 by Needleman suggested that this point of view was incorrect. It was found that the amount of COX protein in inflamed tissues was significantly higher than in normal tissues. The same effect was shown in human fibroblasts stimulated with interleukin (IL)-1 [15].

Moreover, it was evident that the endotoxin lipopolysaccharide (LPS) can stimulate the activity of cyclooxygenase and consequently the generation of PGs in monocytes in the same manner [16]. Further experiments showed that LPS-induced PGH synthesis

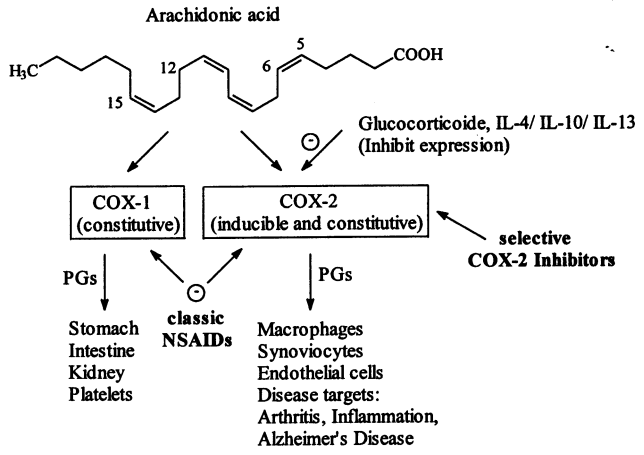

Figure 2. Roles of COX-1 and COX-2.

can be suppressed by dexamethasone or anti-inflammatory cytokines (IL-4, IL-11, IL-13), which inhibit the induction of COX-expression [17] (*figure 2*).

However, it became clear that there is also basic PG production which is not inducible by LPS and which is not inhibited by dexamethasone [18]. Needleman et al. consequently postulated that two isoenzymes of cyclooxygenase are responsible for these effects: a so-called 'house keeping' enzyme (COX-1) which is responsible for a basic level of PGs and an inducible enzyme (COX-2) which is activated by different stimuli mediating inflammatory reactions. In 1991, the inducible form of the enzyme COX-2 was cloned and hence its existence confirmed [19, 20]. Meanwhile, it is known that this isoform is also constitutively expressed (see section 5.2.6).

It is now known that under basal conditions the constitutive enzyme COX-1 is expressed in nearly all tissues including the colon, kidney, spleen, stomach, liver, lung, heart and brain. In both the kidney and the stomach, for example, prostanoids synthesized by COX-1 act as vasodilatators. In the kidney these prostanoids help to maintain renal plasma flow and glomerular filtration during periods of systemic vasoconstriction. Similarly, in the gastric antrum, local vasodilatation appears to be critical in maintaining mucosal defenses. COX-1 in platelets on the other hand generates thromboxane which plays a key role in mediating platelet aggregation [21].

In contrast, COX-2 expression is largely undetectable unless induced by inflammatory stimuli in cells such as synoviocytes, macrophages and endothelial cells. Such stimuli are proinflammatory cytokines (IL-1β, TNFα), lipopolysaccharides such as LPS, mitogenes and oncogenes (phorbolic esters), growth factors (fibroblast growth factor, FGF; platelet derived growth factor, PDGF; epidermal growth factor, EGF), hormones (luteinizing hormone, LH) and disorders of water-electrolyte hemostasis [15, 22, 23].

Indeed, the functions of COX-1 and COX-2 are more complex. Findings, largely from animal studies, have suggested a broader spectrum of biological activity of COX-2. Apart from its induction in inflammatory cells, COX-2 is known to be induced in the kidney in response to sodium depletion or in hyperfiltration states, in postsynaptic excitatory neurons in the brain after electroconvulsive stimulation and in colon adenoma and carcinoma cells; this opens a new spectrum for therapy with COX-2 inhibitors [24].

The development of selective COX-2 inhibitors could be a big step ahead in the therapeutic treatment of inflammatory diseases with fewer risks and side-effects.

3. COX-1/COX-2

3.1. From the gene to the protein

COX-1 and COX-2 are isoenzymes. Since isoenzymes are genetically independent proteins, the genes in humans for the two enzymes are located on different chromosomes and show different properties. The COX-1 gene is located on chromosome 9, COX-2 is encoded by a gene on chromosome 1 [25, 26]. Both genes also differ in size; the human COX-1 gene with 22 Kb contains 11 exons, whereas the human COX-2 gene containing only 10 exons has a relatively small genomic size of 8.3 Kb [27, 28]. This smaller size is characteristic for the so-called early-immediate genes [29]. Looking at the transcriptional elements of both genes a further difference can be seen: the role of a number of transcriptional factors and signaling pathways was investigated to clarify the transcriptional activation of COX-2 gene in endotoxin-treated macrophages [30].

The investigation showed that in contrast to the COX-1 gene, induction of a murine COX-2 reporter gene by LPS requires a cyclic AMP response element (CRE)-site, a nuclear factor-IL-6 (NF-IL-6) site of the COX-2 promoter and probably not the transcriptional element NFκB [31]. Additionally, kinases such as MAPK/ERK kinase (MEKK1) and c-Jun *N*-terminal

kinase (JNK) take part in the induction with a recently discovered adapter protein which is involved in this process, the evolutionary conserved signaling intermediate in toll pathways (ECSIT) [30].

The COX isoenzymes are membrane-bound enzymes in the endoplasmic reticulum (ER). Post-translational modification, cleavage of the signal peptide and insertion into the ER membrane produces the mature glycosylated COX-1 and COX-2 proteins with a molecular weight of 67 000 and 72 000 Da, respectively [32]. A specificity of COX-2 in the primary structure consists of a truncated signal peptide, and insertion of an additional 18 amino acid sequence acts as an epitope for specific COX-2 antibodies [33].

Tertiary and quarternary structures of the COX-enzymes are characterized by three distinct domains: an *N*-terminal EGF domain), a membrane-binding motif and the catalytic site which contains the cyclooxygenase and peroxidase active sites. Consistent with a high sequence identity (ca. 60%) the overall structure of COX-1 and COX-2 are highly conserved. The significant difference between the two enzymes seems to be the much larger binding site in COX-2 for NSAIDs [34].

3.2. The binding site of COX-1 and COX-2

What is the reason for the different behavior of anti-inflammatory agents towards COX-1 and COX-2? The IC_{50} values measured in human COX-1 and human COX-2 and the resulting COX-2/COX-1 ratios for flurbiprofen, indomethacin and the pyrazole derivative SC-588 reflect the differences in potency and the striking increase in enzyme selectivity of SC-588 (*figure 3*) [34].

Lipophilicity has definitely been proved to be an important physico–chemical parameter for the efficacy of NSAIDs. The different binding sites of the two isoenzymes, however, and the interaction of drugs with

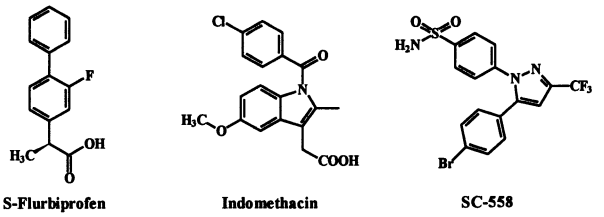

Figure 3. Chemical structures and IC_{50}-values of COX inhibitors.

the protein structure of COX-1 and COX-2 contribute towards clarifying and understanding the pharmacological action and drug specificity. The 3D structure of COX-1 is known from the first X-ray crystal structure described by Picot et al. of the isoenzyme complexed with flurbiprofen, a nonselective NSAID [35]. The structure of the human COX-2 enzyme was obtained by X-ray diffraction [36] or by homology modeling [37].

The structure of bovine COX-1 complexed with several NSAIDs as well as the structure of unliganded murine COX-2 and the corresponding complexes with flurbiprofen, indomethacin and the selective COX-2 inhibitor SC-558 have been published [34].

The cyclooxygenase active site is created by a long hydrophobic channel that is the site of non-steroidal anti-inflammatory drug binding. The channel contains areas with high electron density interacting with the aromatic system of flurbiprofen. The carboxylic group of flurbiprofen is directed towards the mouth of the channel and lies in a favorable position for interacting with the guanidinium group of arginine 120 (ARG 120). Glutaminic acid 524 (GLU 524) is located near ARG 120, and these two residues may form a salt bridge thus blocking the mouth of the enzyme. ARG 120 and GLU 524 are the only polar residues in the otherwise hydrophobic channel. It can be argued that flurbiprofen inhibits the cyclooxygenase reaction by preventing AA from invading the channel. This may also be the reason why flurbiprofen is a slow-binding time-dependent inhibitor of both COX-1 and COX-2 [34, 38].

Indomethacin, a classic nonselective cyclooxygenase inhibitor, causes slow, time-dependent inhibition of COX-1 and COX-2. Indomethacin seems to penetrate very deeply into the channel without a strong effect on COX-2. Its benzoyl group occupies an environment similar to that of the distal phenyl ring of flurbiprofen and is stabilized by hydrophobic interactions. The time-dependence may also result from the formation of a salt bridge between the acidic function of indomethacin and ARG 120 of the enzyme within the long hydrophobic cyclooxygenase channel followed by conformational changes.

The differences between COX-1 and COX-2 are genetically determined and cause an amino acid exchange in the enzyme proteins. Evidently the exchange of a valine at position 523 in COX-2 for a relatively bulky isoleucine residue in COX-1 at the same position of the active site of the enzyme causes a structural modification. This modification in the COX-2 enzyme allows access to an additional side pocket, which is a pre-requi-

Table I. Relative potency of NSAIDs according to COX-2/COX-1 selectivity in different test systems.

Inhibitor	Reference								
	[40]	[41]	[42]	[43]	[44]	[45]	[46]	[47]	[48]
ASS							166		>60
Indomethacin	2.9	30	9	0.05	>74	22.3	60		13.1
Diclofenac	0.35	2.2	2.5	0.0004	7.6		0.7		0.7
Ibuprofen	>6.3		11.4		3.1	0.67[a]	15		6.1
Ketoprofen	54		1	0.5				1.6[a]; 2.8[b]	15
Naproxen	9.5		32.7	0.3	5.9		0.6		
Flurbiprofen	14.6	317	4	0.4	6.4	5.7			12.7
Ketorolac	2.6				1.9				
Tolmetin	5.7								
Tenoxicam	6.2	15							
Piroxicam	11.8	33	>7.7	0.004	>28	9.5			2.6
Meloxicam		0.33							
Nimesulid				0.0004					0.19
NS-398	0.097		>0.001	0.0025				0.006	0.03

[a] S-Enantiomer.
[b] R-Enantiomer.

site for COX-2 drug selectivity. Access to this side pocket is restricted in the case of COX-1.

A further exchange of valine/isoleucine at position 434 is responsible for the formation of a 'gate'. In COX-2 the less bulky valine is able to 'swing' like a gate because of less steric hindrance and offers enough space for the entry of compounds with room-filling substituents.

Such a highly selective compound is SC-588, a diaryl heterocyclic inhibitor with a 1 900 fold selectivity of COX-2 over COX-1. In COX-2 the channel that leads from the membrane to the active site forks at the SC-558 binding site. One branch forms a cavity that accepts the bromophenyl residue of the compound whereas the entire phenylsulfonamide moiety stretches into the COX-2 selective side pocket. Additionally the sulfonamide group extends into a region near the surface of COX-2 that is relatively polar and probably interacts with histidine 90 (HIS 90), glutamine 192 (GLN 192) and arginine 513 (ARG 513).

These studies of the static 3D structure of the enzyme have been very helpful in the process of understanding the enzyme inhibition mechanisms and in the design of selective compounds. However, they neglect to take into consideration that the enzyme is not static but dynamic. Hence, dynamic factors such as enzyme flexibility and rearrangement of the hydrogen-bonding network at the entrance of the active site have to be considered as well. Investigations of the selectivity of ketoprofen and structural analogues reflect that water present in the ligand-binding site of a protein plays a major role in ligand–protein interactions. Molecular models have been constructed by considering such implicit water molecules that participate in the dynamic hydrogen-bonding network at the polar active site entrance with residues 355, 524, 120 and 513. These models suggest that active site hydration is essential for understanding inhibitor selectivity [39].

4. Methods of evaluating COX inhibitors

Since NSAIDs have been shown to exert their effects through the inhibition of PG synthesis by interaction with cyclooxygenase, a great number of in vitro and in vivo test models have been developed to study drug interactions with AA metabolism and to screen anti-inflammatory compounds.

With the detection of the two isoenzymes, the aspect of enzyme selectivity had to be taken into consideration as well. Meanwhile, the quotient of the IC_{50}-values of COX-2/COX-1 inhibition is an admitted and widely used parameter for the determination of enzyme selectivity. The value of these quotients, however, is restricted because it strongly depends on the test system, cell types, stimulating agents and culture conditions used (*table I*) [40–48].

Mitchell et al., for example, measured COX-1 and COX-2 activity in purified enzyme systems, microso-

mal membranes and cultured intact cells [46]. Meade et al. investigated COX inhibition on microsomal membranes from cos-1 cells transfected with murine COX-1 or COX-2 in purified enzyme systems [45]. Patrignani et al. used a human whole blood (HWB) assay [47] and Laufer et al. developed an in vitro test system which uses isolated monocytes from whole blood in a one-step centrifugation procedure [49]. Dannhardt et al. established a test system for the in vitro evaluation of 5-LO and COX inhibitors using bovine neutrophils and platelets [50].

Hence, it is evident that values for selectivity can only be compared if the same test assays are used. To underline and to confirm the variations of COX-2/COX-1 ratios depending on the test model, indomethacin is a striking example with an astonishingly wide range of selectivity ratios (in this case COX-1/COX-2 ratio, i.e. Patrignani = 1.94 [51], Futaki et al. = 7.63 [52] and Grossmann et al. = 14.2 [53]. It is therefore important to compare compounds against known inhibitors to make a meaningful interpretation of the data from different laboratories. An aspect not regarded in all cellular test systems is a possible drug effect on COX-1/COX-2-expression at the mRNA and protein level. An in vitro cellular test system recently published by Tordjman et al. using mouse resident peritoneal macrophages allows us to differentiate if a reduction of PGE_2 production results from an exclusive enzymatic inhibition (aspirin, NS-398) or an enzymatic inhibition associated with a slight decrease of COX-2 protein level (indomethacin). In contrast, in the case of paracetamol and salicylic acid, two weak inhibitors of COX enzymatic activity, reduction of PGE_2 synthesis seems to be related to a reduced level of COX-2 protein [54].

Different in vivo systems have been established to evaluate COX-2 inhibitors. The carrageenan-induced paw edema assay in the rat is a test system often used for the testing of NSAIDs and COX-2 inhibitors in acute inflammatory processes [55]. In this test the increased paw volume after injection of carrageenan is used as the parameter for inflammation. The Hargreaves model is used to assess the analgesic potency of anti-inflammatory compounds [56]. The effect of anti-inflammatory drugs in chronic inflammation is evaluated in the adjuvant-induced arthritis assay [57, 58]. The ulcerogenicity of drugs can be determined in an animal model using fasted rats; the rat is examined for lesions 72 h after application of the anti-inflammatory drug [59].

Table I confirms these results and shows some commonly used NSAIDs and their COX-2/COX-1 ratio under different conditions. It also becomes evident that none of the presently used classic NSAIDs, except aspirin, can be classified as a selective inhibitor either for COX-1 or for COX-2. Only meloxicam shows an increased ability to inhibit COX-2 and therefore it is often classified as a 'preferential' COX-2 inhibitor. This allows a differentiation of commonly used NSAIDs:

1. Selective COX-1 inhibitors (low dose aspirin).
2. Nonselective COX inhibitors (high dose aspirin, i.e. indomethacin).
3. Preferential COX-2 inhibitors (i.e. meloxicam).
4. Highly selective COX-2 inhibitors (i.e. celecoxib).

The classification in current use was established by Kurumbail. Inhibitors are distinguished according to their interaction with the enzyme protein [34]:

1. **Irreversible inhibitors of COX-1 or COX-2.** Aspirin or *o*-(acetoxyphenyl)hept-2-inyl-sulfide (APHS) acetylates the amino acid serine so that endogenic AA is prevented from reaching the catalytic center.
2. **Reversible, competitive inhibitors of COX-1 and COX-2.** Inhibitors such as ibuprofen, piroxicam or mefenaminic acid compete against AA to bind at the catalytic center
3. **Slow time-dependent, reversible inhibitors of COX-1 and COX-2.** Indomethacin and flurbiprofen seem to act by ionic interactions between their carboxylic function and the arginine residue of the enzyme. This effect seems to influence the helix D of the protein followed by a remarkable loss of flexibility of the enzyme protein.
4. **Slow, time-dependent irreversible inhibitors of COX-2.** Representatives of this group are selective COX-2 inhibitors such as celecoxib, rofecoxib and others. They are weak competitive inhibitors of COX-1, but inhibit COX-2 in a slow time-dependent process.

5. Therapeutics

5.1. Classic NSAIDs

Commonly used NSAIDs play an enormous role in the therapy of inflammatory diseases. Over the years the pool of therapeutic substances has grown rapidly. *Figure 4* shows the presently most important COX inhibitors for antirheumatic therapy. These substances are subdi-

vided into the class of fenac-, profen- and enol-derivatives according to their chemical structure.

Substances such as indomethacin, diclofenac, ibuprofen or piroxicam are still the preferred therapeutics. The formerly used anthranilic acid derivatives and pyrazolon derivatives are no longer mentioned here. For a long time the presence of an acidic moiety was thought to be a pre-requisite for the classic NSAIDs. Today we know that this structural feature accounts for the formation of a salt bridge between the carboxylic group of the anti-inflammatory drug and ARG 120 at the bottom of the cyclooxygenase enzyme thus generating COX-1 inhibiting activity.

As mentioned before, depending on their chemical structure, NSAIDs inhibit both COX-1 and COX-2 to different extents. This accounts for their anti-inflammatory and analgesic activities and also their unwanted GI side-effects [60].

Table II summarizes the results for COX-1 and COX-2 inhibition of classic NSAIDs and experimental compounds. In this case the IC_{50}-values based on a HWB cyclooxygenase assay were measured as biochemical index for COX-2 and COX-1 activity. This means that in this study PGE_2 levels in LPS-stimulated human blood on the one hand and TXB_2 levels following blood coagulation on the other hand were determined [40].

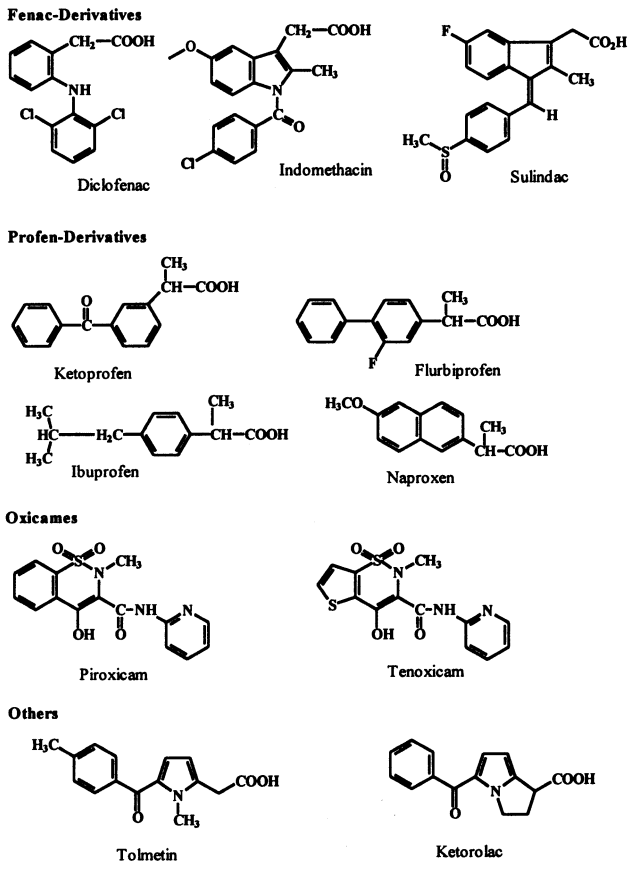

Figure 4. Classic NSAIDs.

As shown, all currently marketed NSAIDs are inhibitors of both COX-1 and COX-2 (*table II*, section A). The aspect of enzyme selectivity of NSAIDs becomes important particularly under the point of view of low-risk NSAIDs with reduced side-effects. Therefore, the classic NSAIDs are being pushed increasingly into the background, whereas selective COX-2 inhibitors with an attractive pharmacological profile and reduced side-effects are being favored (*table II*, section B).

5.2. COX-2 inhibitors

Prior to the identification of the COX-2 enzyme, researchers identified a potent anti-inflammatory compound, DuP-697, which was a relatively weak inhibitor of bovine seminal vesicle PG synthesis, but potent in a variety of anti-inflammatory assays [61]. At first these results could not be explained, but after identification of COX-2 it became evident that this compound possessed a selective inhibitory activity against COX-2. This was the beginning of the search for new anti-inflammatory compounds focussing on COX-2 as the target enzyme.

The large number of newly developed COX-2 inhibitors demonstrates how promising this field of anti-inflammatory agents is expected to be [62]. More than 500 COX-2 inhibitors have been described over the past few years. Until now two compounds, celecoxib and rofecoxib, have been launched for the treatment of inflammatory processes.

The chemical structures of COX-2 inhibitors are heterogenic so that a further classification of this group will be made in the following chapter. Contrary to the classic NSAIDs, this new class of enzyme inhibitors is lacking a carboxylic group, thus effecting COX-2 affinity by a different orientation within the enzyme without formation of a salt bridge in the hydrophobic channel of the enzyme.

Selective COX-2 inhibitors belong to different structural classes (*figure 5*):

1. Diaryl- or aryl-heteroaryl-ethers (sulfonanilide inhibitors): nimesulide, NS-398, flosulide, L-745337.
2. Vicinal diaryl heterocycles: celecoxib, rofecoxib, SC-57666, DuP-697.

Table II. In vitro human whole blood (HWB) assays for inhibition of COX-1 and COX-2 (endotoxin-induced PGE_2 production) [40].

Inhibitor	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-2/COX-1
A. Classic NSAIDs			
Diclofenac	0.14 ± 0.03	0.05 ± 0.01	0.35
Indomethacin	0.16 ± 0.01	0.46 ± 0.06	2.9
Ketorolac	0.33 ± 0.05	0.86 ± 0.14	2.6
Ketoprofen	0.02 ± 0.003	1.08 ± 0.25	54
Tolmetin	1.23 ± 0.13	7.09 ± 1.59	5.7
Flurbiprofen	0.44 ± 0.07	6.42 ± 0.16	14.6
Piroxicam	0.76 ± 0.05	8.99 ± 1.36	11.8
Sulindac sulfide (active metabolite of sulindac)	1.02 ± 0.33	10.43 ± 1.23	10.2
Tenoxicam	2.30 ± 0.83	14.22 ± 2.53	6.2
Naproxen	7.76 ± 0.83	73.74 ± 3.12	9.5
Ibuprofen	4.75 ± 0.50	>30	>6.3
B. Experimental compounds			
Dup-697	1.18 ± 0.36	0.06 ± 0.01	0.051
NS-398	4.81 ± 1.24	0.47 ± 0.07	0.097
SC-58125	>30	2.25 ± 0.43	–
L-745337	>30	9.67 ± 2.02	–

3. Modified, known NSAIDs to improve COX-2 selectivity: L-748780, L-761066, meloxicam, etodolac
4. Antioxidative compounds.
5. 1,2-Diarylethylene derivatives (*cis*-stilbenes).

As already mentioned, COX activity originates from two distinct and independently regulated isoenzymes. COX-1 is a constitutive enzyme, whereas COX-2 is inducible, short-lived and also constitutive (see section 5.2.6). The differential tissue distribution of the COX-2 isoenzyme has provided a rationale for the development of COX-2 selective inhibitors as non-ulcerogenic, anti-inflammatory and analgesic agents.

5.2.1. Diaryl- and aryl-heteroaryl ether

A selective COX-2 inhibitor at the beginning of this era was the compound NS-398 with a completely different structure from classic NSAIDs. The compound showed inhibition of PG synthesis in inflammatory cells and was largely free of unwanted GI effects in animal models. Moreover, NS-398 did not affect PG production in the stomach or kidney.

On recognizing that NS-398 was a preferential more or less selective inhibitor of COX-2, new interest in this class of anti-inflammatory agents evolved. Structurally, closely related to NS-398 there are two other compounds, nimesulide and flosulide, a diaryl ether and thioether structure, respectively, which bear a methansulfonanilide moiety [51, 63, 64]. The sulfonamide structure with its NH-acidity in all these compounds seems to be obligatory. It appears that nimesulide was the first member of this class of drugs. Its mechanism of action, pharmacology and clinical results in rheumatic diseases, osteoarthritis and acute inflammation demonstrated that nimesulide possesses novel anti-inflammatory qualities.

Flosulide is similar to nimesulide [65]. The main difference between them is the incorporation of the electron-withdrawing substituent into the five-membered carbocyclic ring. L-745337, the thioether analogue of flosulide, is reported to have higher COX-2 specificity, better bioavailability, improved in vivo potency and a greater GI safety profile than flosulide [66].

A patent application describes a series of highly active COX-2 inhibitors which bear an isobenzofurane system additionally (*figures 5* and *6*).

It seems to be highly potent in cell culture with COX-1 $IC_{50}>100$ μmol and COX-2 $IC_{50} = 0.005$ μmol).

In the carrageenan paw edema assay, this compound had an ED_{50} of 0.9 mg kg^{-1} p.o., thus representing the most potent anti-inflammatory methanesulfonamide prepared to date [67].

Hence, a general structure of a COX-2 inhibitor with diarylether and sulfonamide moiety as COX-2 inhibitor can be developed (*figure 7*).

5.2.2. Vicinal diaryl heterocycles

By far the greatest amount of research in the COX-2 area has been performed in the preparation and evaluation of this class of compounds. The compounds are characterized by a central carbocyclic or heterocyclic ring system bearing two vicinal aryl moieties. These compounds represent the most important group of COX-2 inhibitors. During the last few years a large

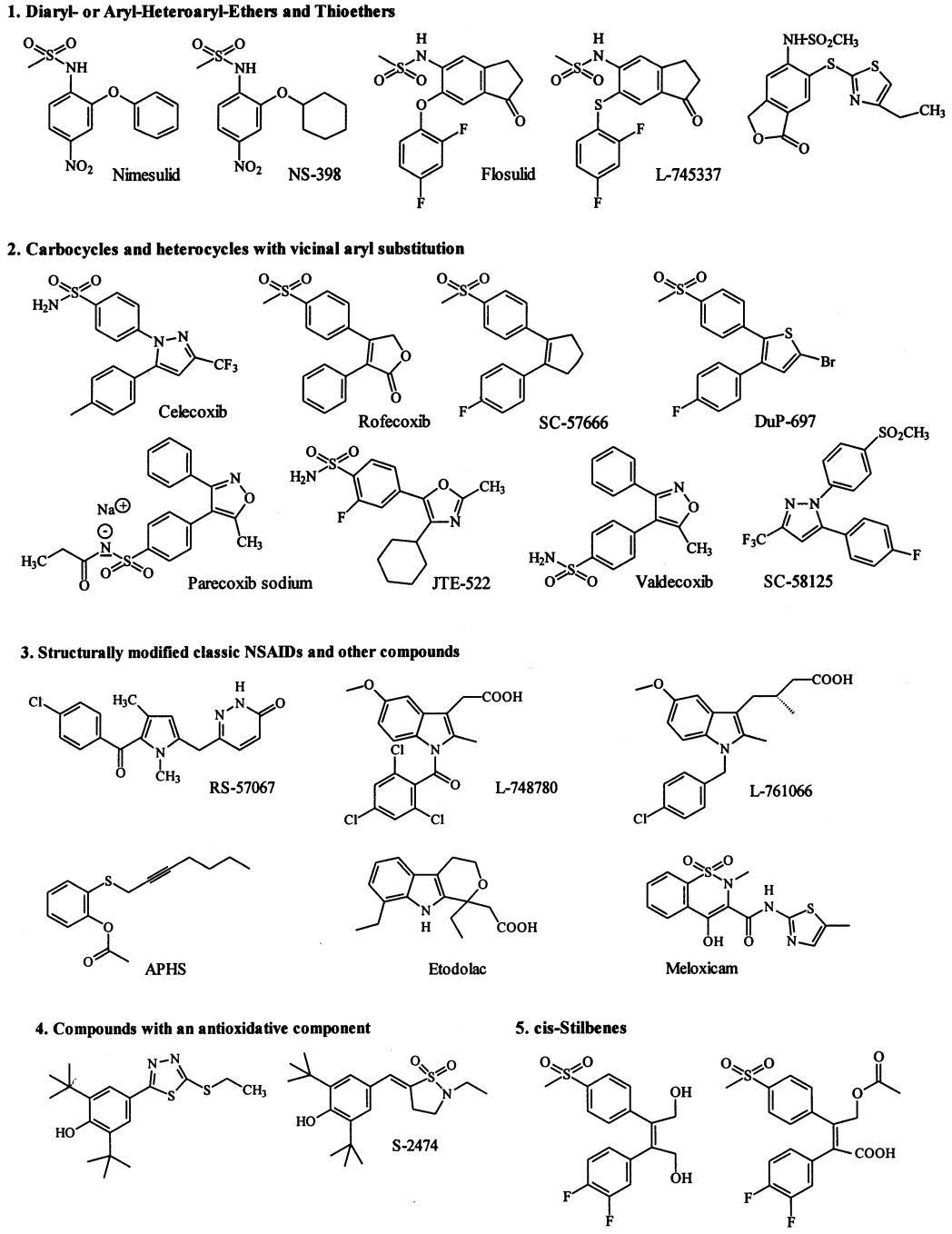

Figure 5. COX-2 inhibitors.

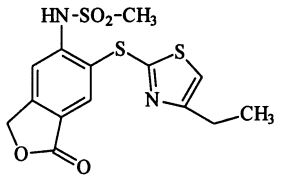

Figure 6. Isobenzofurane derivative as COX-2 inhibitor.

number of compounds has been developed as potential candidates. Occasionally this structure is defined in the literature as a 'tricyclic compound' which is not really appropriate according to chemical nomenclature.

It can be assumed that the heterocycle is responsible for the appropriate orientation to the aromatic rings in space and finally for the binding to the enzyme. A wide variety of heterocycles can serve as a template for COX-2 inhibitors, i.e. pyrrole, thiazole, oxazole, furane, imidazole, isoxazole, pyrimidine and thiophene, but at the moment pyrazole and cyclopentenone seem to be the most appropriate tools for COX-2 specificity. For optimal activity, one aromatic ring must be substituted with a methylsulfonyl or a sulfonamide substituent in para position [61, 62].

Substitution at position 4 of one of the aromatic systems with a sulfonamide or a methylsulfonyl group is essential for COX inhibition. Replacement of the methylsulfonyl group by a sulfonamide group reduces COX-2 selectivity but improves oral bioavailability [68].

These structural pre-requisites are obligatory for enhanced activity towards COX-2 and lead to unsymmetrically substituted analogues. Compound DuP-697 with a bromo-substituted thiophene ring is a typical representative which fulfils these pre-requisites. However, clinical data of DuP-697 showed a very long plasma half-life of

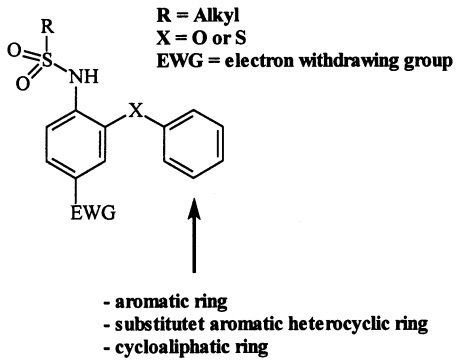

Figure 7. General structure of a sulfonanilide COX-2 inhibitor.

242 h in humans because of its enterohepatic recirculation and rendered it unacceptable for further evaluation. Today this compound serves as a pharmacological tool [69].

Two heterocycles with the substitution pattern mentioned above were of special interest: the 1,5 diarylpyrazole derivative celecoxib and 3,4-diarylfuranone derivative rofecoxib.

Celecoxib (Celebrex®) was approved by the FDA for the treatment of osteoarthritis and chronical polyarthritis in 1998. Celecoxib has an optimal in vitro and in vivo profile. The substance shows potent and selective in vitro activity (COX-1 $IC_{50} = 13$ μmol, COX-2 $IC_{50} = 0.04$ μmol) and marked anti-inflammatory activity in the rat adjuvant-induced arthritis assay ($ED_{50} = 0.4$ mg kg^{-1}) [70, 71]. Studies showed that the analgesic and antiphlogistic efficacy of 400 mg celecoxib daily is comparable to a daily dosage of 1 000 mg naproxen or 150 mg diclofenac. Celecoxib is contraindicated during pregnancy and interestingly in patients with GI ulcers. At this point the role of COX-2 for wound healing becomes evident, once an ulcerative injury is present, COX-2 expression is elevated in response to this disease and the COX-2 enzyme seems to be essential for wound healing in the stomach by enhancing gastric blood flow, reducing gastric acid secretion and allowing epithelial cell proliferation and granulation tissue contraction. Consequently, highly selective COX-2 inhibitors such as celecoxib lead to delayed wound healing and can aggravate the injury [72, 73].

Rofecoxib (Vioxx®) has been launched in the USA for the treatment of osteoarthritis, acute pain and primary dysmenorrhea and is meanwhile also available in Germany, and approved within the EU for the treatment of osteoarthritis [74].

Valdecoxib, parecoxib sodium and JTE-522 are the latest developments of COX-2 inhibitors and belong to the same chemical group.

Valdecoxib is a diarylisoxazole derivative which is currently in clinical evaluation for the management of pain and inflammation with high selectivity. Valdecoxib showed weak inhibitory activity towards COX-1 ($IC_{50} = 140 +/- 19$ μmol), whereas COX-2 was inhibited with an $IC_{50} = 0.005$ μmol $+/- 0.001$ μmol), screened on human recombinant enzymes. These in vitro data were confirmed in in vivo models, i.e. rat carrageenan foot edema for acute inflammation assay, the rat adjuvant arthritis model for chronic anti-inflammatory activity and the rat carrageenan air pouch model for blocking PG synthesis at the inflammatory site [75].

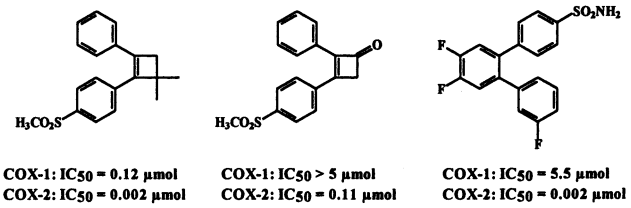

Figure 8. Four- and six-membered carbocycles as COX inhibitors.

Interestingly, valdecoxib is metabolized in rodents and dogs to a hydroxymethyl derivative with additional anti-inflammatory activity. Substances such as celecoxib or rofecoxib possess a modest aqueous solubility which prevents their use for parenteral administration. The development of parecoxib sodium, which is a prodrug of valdecoxib, as an injectable COX-2 inhibitor broadens the spectrum for the treatment of severe acute pain and particularly postoperative pain [76].

JTE-522 is currently undergoing phase II clinical trials for the treatment of rheumatoid arthritis and osteoarthritis. This compound seems to be one of the most specific COX-2 inhibitors described to date which inhibits both sheep and human recombinant COX-2 with IC_{50} values of 0.64 and 0.085 μmol, respectively, without inhibiting either sheep or human platelet COX-1 at concentrations up to 100 μmol. JTE-522 does not produce gastric ulcers at therapeutic doses, because it does not affect COX-1-mediated PGE_2 production in the gastric mucosa [77, 78].

Variations of the central five-membered ring are tolerated as well. Hence, ring contraction to smaller carbocycles such as cyclobutenes also leads to potent COX-2 inhibitors as well as insertion of a six-membered carbocyclic or heterocyclic group (*figure 8*). Compounds with a cyclobutene central ring show IC_{50} values for COX-1 of 0.12 and >5 μmol, for COX-2 = 0.002 and 0.11 μmol [79]. A central six-membered ring with a vicinal substitution pattern seems to be accepted as well (IC_{50} value for COX-1 = 5.5 μmol, for COX-2 = 0.002 μmol) [80]. Recently MK-0663 was developed, a compound that belongs to a novel series of bis-pyridinyl(4-methyl-sulfonyl-phenyl) compounds. The compound was tested for selectivity in the HWB assay for COX-1 and COX-2 before being evaluated for oral absorption and efficacy in a number of animal models of inflammation, pyrexia and pain. The compound produced IC_{50} values of 0.08 μmol (COX-2) and 12 μmol (COX-1), ratio COX-2/COX-1 = 0.006 in cells assays and 1.1 and 116 μmol in the HWB COX-2 and COX-1 assay, respectively (ratio = 0.009), indicating that it is one of the most selective COX-2 inhibitors developed to date [81, 82].

5.2.3. Modification of known NSAIDs and compounds without common structural features

Modifying well known NSAIDs into selective COX-2 inhibitors represents an interesting strategy [83]. Indomethacin, zomepirac, aspirin and flurbiprofen have been successfully elaborated into selective COX-2 inhibitors (*figures 9* and *10*). However, the methodology utilized in NSAID modification does not follow a general scheme. Classic NSAIDs such as indomethacin possess both COX-1 and COX-2 inhibiting activity. Various attempts have been made to shift the enzyme selectivity of indomethacin from COX-1 to COX-2 while keeping the potency on the same level and reducing the unwanted side-effects at the same time. In principle, the strategy consisted of introducing larger substituents to fit into the active site volume of COX-2 (L-748780).

Introducing a larger trichlorobenzoyl analogue instead of the chlorobenzoyl analogue optimized COX-2 selectivity, altering the side chain by a beta-branched butyric acid and replacing the benzoyl group of indomethacin by a 4-bromo benzyl-substituent finally pro-

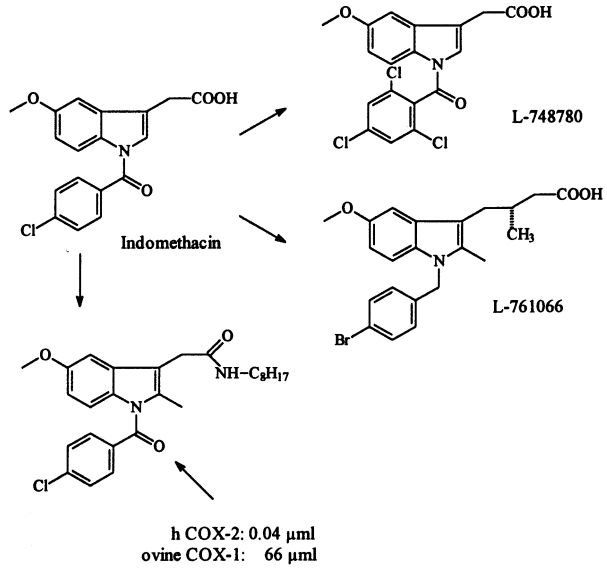

Figure 9. Conversion of indomethacin to selective COX-2 inhibitors.

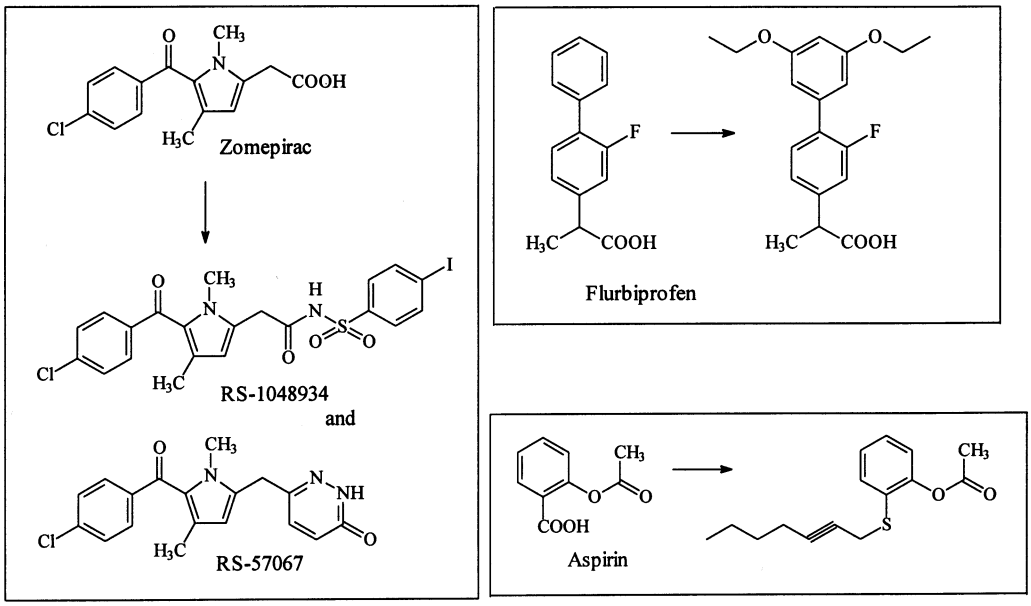

Figure 10. Conversion of nonselective COX inhibitors to COX-2-selective inhibitors.

duced compound L-761066 with a high potency and a remarkable selectivity (COX-1: $IC_{50}>10$ μmol, COX-2: $IC_{50}=0.06$ μmol) [84, 85]. Transformation of the aryl acetic acid moiety of indomethacin to esters or amides furnishes molecules capable of binding tightly to COX-2 but not COX-1 [86]. The amide synthesis by combinatorial methods makes it easy to obtain a highly diverse library appropriate for a broad screening in order to optimize COX-2 inhibitory potency (*figure 9*).

The indomethacin esters or amides inhibited purified human COX-2 with IC_{50} values in the low nanomolar range whereas their effect on ovine COX-1 was relatively weak. Further modifications such as replacement of the 4-chlorobenzoyl group of indomethacin esters or amides with the 4-bromobenzyl moiety afforded inactive compounds. Moreover, it was shown that the 2-methyl group at the indole ring is essential for the potency [86]. As for kinetic aspects it was shown that indomethacin amides behave as slow, tight-binding inhibitors of COX-2 and it becomes evident that selectivity is a function of the time-dependent step. *Figure 9* shows some of the most potent and selective indomethacin derivatives.

A similar strategy was used for the modification of zomepirac, basically a COX-1 selective drug [87]. The desired COX-2 selectivity was achieved by replacing the acetic acid group by other moieties such as the pyridazinone ring or an *N*-acyl aminosulfonyl phenyl group to yield RS-57067 and RS-1048934, respectively (*figure 10*). Occasionally this class of compounds is designated as aryl-heteroaryl ketones [88].

Aspirin is the only known NSAID that covalently binds to serine and more significantly inhibits COX-1 than COX-2. A lot of systematic structural modifications have been made resulting in the development of APHS characterized by a 60 times higher and 100 times more selective activity towards COX-2 than aspirin. Inhibition of COX-2 also occurs by acetylation of the same serine residue that aspirin acetylates, indicating that APHS is the first selective covalent inhibitor of COX-2. In other words, the binding site of APHS is not identical with those of other selective COX-2 inhibitors [89].

A class of compounds which have attracted attention over the past few years are the enol-carboxamides. One representative of this class, meloxicam, was reported to have COX-2 selectivity but meanwhile it is accepted as a so-called preferential COX-2 inhibitor with a certain COX-2 specificity, and a lower incidence of gastric and renal side-effects than associated with classic NSAIDs [90]. IC_{50} values for meloxicam tested in guinea-pig macrophages underline these characteristics: 0.58 μmol for COX-1 and 0.19 μmol for COX-2, which is a COX-2/COX-1 ratio of 0.32 [91].

Etodolac is another preferential COX-2 inhibitor available in some European countries and in the USA for the treatment of rheumatoid arthritis and osteoarthritis [92].

Modern medicinal chemistry offers a wide range of different strategies for finding selective COX-2 inhibitors with a novel structure. Computational and combinatorial chemistry methodology helped to create a highly selective phenothiazine derivative (*figure 11*) which can serve as a novel lead compound for further developments in the field of COX-2 inhibitors. It processes a >50 fold enzyme selectivity and a low micromolar potency [93].

5.2.4. Compounds with antioxidative moieties

These compounds, which are under investigation, develop their mode of action by an antioxidative mechanism. Since COX enzyme catalysis involves radical intermediates, a radical scavenging moiety such as a di-*tert*-butylphenol interferes with the cyclooxygenase reaction [94]. Linkage of phenolic substructure with a thiazolone, oxazolone, thiadiazole or oxadiazole derivative produces non-ulcerogenic, orally active anti-inflammatory agents as a novel class of COX-2 inhibitors.

The most potent and COX-2 selective compound of this class was the thiadiazole derivative (*figure 5*) with IC_{50} COX-1 >100 μmol and COX-2 $IC_{50} = 0.14$ μmol) on purified enzymes [94].

A new class of compounds is reported to be dual inhibitors. They have a γ-sultam skeleton and show potent inhibitory effects on both COX-2 and 5-lipoxygenase as well as production of IL-1 in in vitro assays. It has also proved to be effective in several animal arthritic models without any ulcerogenic activity. Among these compounds (E)-(5)-(3,5-di-*tert*-butyl-4-hydroxy-benzylidene)-2-ethyl-1,2-isothiazolidine-1,1-dioxide (S-2474) was selected as an anti-arthritic drug candidate and is now under clinical investigation [95].

5.2.5. 1,2 Diarylethylene derivatives (cis-stilbene-derivatives)

Reduction of the furanone ring led to active inhibitors with a ring open diol structure. Ring opening and elimination of the heteroatom led to *cis*-stilbene derivatives which still contain the pre-requisites for COX-2 inhibition: vicinal orientation of two aromatic rings, substitution pattern at the aryl moiety as seen in potent COX-2 inhibitors, i.e. methylsulfonyl moiety in combination with a halogen [96]. This group of compounds is presently undergoing biological testing.

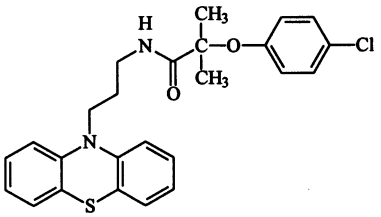

Figure 11. Phenothiazine derivative as COX-2 inhibitor.

5.2.6. Side-effects of selective COX-2 inhibitors

The clinical results for selective COX-2 inhibitors such as celecoxib and rofecoxib are promising. However, the tendency to search for more specific inhibitors has also provoked critical reactions. Certainly selective COX-2 inhibitors reduce the risk of GI side-effects, but COX-2 is not only a proinflammatory inducible enzyme, it also has a number of physiological functions which means that it is constitutively expressed to a high extent in the human body [97].

COX-2 seems to be involved into the regulation of the renin–angiotensin system [98, 99], and to possess vasoactive and anti-atherogenic properties [100]. Moreover, the hormonal induction is important for ovulation and, at the end of pregnancy, high uterine levels of COX-2 are necessary for the onset of labor [98, 101]. The role of elevated COX-2 enzyme levels in ulcerative tissues for wound healing has already been mentioned. Therefore, the following side-effects have been taken into account under therapy with selective COX-2 inhibitors:

Influence on the renin–angiotensin-system in the kidneys induces hypertension and renal failure; deleterious effects on ovulation and parturition; delayed wound healing. However, at the recommended therapeutic doses these drugs retain selectivity and do not affect platelet aggregation or bleeding time [102]. Hence drug safety should be discussed at the same time as the benefits of selective COX-2 inhibitors. As an alternative, balanced COX-1/COX-2 inhibitors should be further investigated. Furthermore additional compounds being developed as GI-sparing anti-inflammatory drugs might be of interest: nitric oxide-releasing NSAIDs (NO-NSAIDs) which show markedly reduced renal toxicity and dual inhibitors of both cyclooxygenase and 5-lipoxygenase, a further enzyme of the AA metabolism responsible for leukotriene biosynthesis such as ML 3000. This compound is presently undergoing clinical testing and shows a promising pharmacological profile with low GI risks [103, 104].

6. COX-2 and new therapeutic targets

6.1. Angiogenesis – colon cancer – Alzheimer's disease (AD)

Apart from its role in inflammatory sites, COX-2 is involved in numerous physiological and pathophysiological functions. COX-2 is constitutively expressed in the developing kidney and brain, playing a role in their maturation and function. Additionally, COX-2 expression may be upregulated at certain sites: in the kidney during sodium restriction, in the microglia of cognitive centers within the hippocampus and cortex in AD and in intestinal adenomas and colon tumors. Some details are discussed in the following sections [105].

The formation of new blood vessels by angiogenesis to provide a blood supply is a major requirement for the growth of many tumors [106].

While mature blood vessels express COX-1, new angiogenic cells express inducible COX-2 [107]. Based on these observations, Masferrer et al. hypothesized that tumor-derived growth factor promotes angiogenesis by inducing the production of COX-2-derived PGE_2. This is supported by the fact that PGs are known to be pro-angiogenic molecules and contribute to tumor growth by inducing the newly formed blood vessels (neoangiogenesis) that sustain tumor cell viability and growth.

COX-2 is expressed within human tumor neovascularization as well as in neoplastic cells present in human colon, breast, prostate and lung tissues. Celecoxib demonstrated a potent anti-angiogenetic activity in a rat model of angiogenesis, in which corneal blood vessel formation is suppressed by celecoxib (corneal micropocket model) [108]. It has been suggested that angiogenesis is inhibited through direct effects on endothelial cells by lowering the expression of angiogenic factors such as vascular endothelial growth factor (VEGF).

Hence, the gate to a new field of research has opened. As monotherapeutics, anti-angiogenics are expected to be safer and better tolerated than classic chemotherapeutic drugs and therefore an ideal candidate for the prevention of cancer. A combination of anti-angiogenic drugs and cytostatic agents could have a synergistic antitumor activity via an impact on two independent mechanisms such as neovascularization and neoplastic cell proliferation.

Closely related to these observations is the fact that COX-2 plays a role in the development of colon cancer. Normally COX-2 expression is strictly regulated; however, a constitutive overexpression of COX-2 seems to be important in colon carcinogenesis. In cultured human colonic fibroblasts it was shown that growth factors such as hepatocyte growth factor are involved in the progression of tumors. COX-2 inhibitors are now assumed to inhibit COX-2-mediated PG synthesis which is responsible for hepatocyte growth factor expression [109–113].

In addition to the well known peripheral role of COX-2 in inflammation, recent results indicate an important role in the central nervous system (CNS). COX-2 is expressed constitutively in some excitatory neurons in the CNS. Moreover, expression of this isoform is markedly induced in CNS neurons by excitatory stimuli such as ischemia and seizures so that a role of COX-2-derived PGs in certain forms of neurodegeneration can be assumed. It has also been shown that celecoxib maximally inhibits COX-2 in the CNS at anti-inflammatory doses. Interestingly, application of COX-2 inhibitors to the spinal cord of rats, where COX-2 is also expressed, prevents peripheral inflammation and hyperalgesia. Evidently, COX-2 plays an important role in the CNS in inflammation and pain and it can be deduced that therapeutic effects of COX inhibitors might be caused not only by peripheral enzyme inhibition but also at least partly by central inhibition.

COX-2 in CNS may have an ambivalent functionality in the brain since the basal production of PGs through COX-2 may participate in neuronal homeostasis, whereas the expression of COX-2 is associated with brain development [114]. COX-2 is constitutively expressed in neurons and is upregulated in degenerative brain regions in AD such as the microglia of the cognitive centers within the hippocampus and cortex. Enhanced COX-2 expression in the brain may be associated with beta-amyloid protein deposition in the neuritic plaques of AD. This protein and its peptide precursors are thought to be elaborated as part of an inflammatory cascade in which microglia, a rich source of prostanoids, probably participate. The role of activated microglia, which express COX-2 in cerebral inflammatory processes, was recently demonstrated in the rat [115].

The fact that COX-2 mRNA is elevated in areas related to memory (hippocampus, cortex) and that the amount of COX-2 correlates with the deposition of beta-amyloid protein represents a possible therapeutic benefit and a hopeful new strategy in the prevention or treatment of AD [116].

Despite this interesting and optimistic outlook for future uses of COX-2 inhibitors, most of the findings are based on in vitro and in vivo assays and must urgently undergo investigation in man.

7. COX-1 and COX-2 – the end of the story?

So far it is generally agreed that the constitutively expressed COX-1 enzyme is the predominant form in the GI tract, kidney and platelets, providing a rich source of physiologically important PGs. It is also evident that COX-2 is expressed at inflammatory sites by leukocytes as well as by activated mesenchymal cells.

The conceptual framework and the properties of selective COX-2 inhibitors have been demonstrated as effective NSAIDs in blocking the signs and symptoms of inflammation.

Although this beneficial effect of specific COX-2 inhibitors is evident, some data suggest that in certain models PGs may be unexpectedly beneficial in the resolution of inflammation or tissue injury. Gilroy and Willoughby described the effect of selective COX-2 inhibitors on carrageenan pleurisy in the rat over a time course ranging from 0–48 h after injection of the irritant. This investigation produced surprising results and showed that efficacy by COX-2 inhibitors strongly depends on the time course of the inflammatory process: onset of inflammation, peak inflammation and resolution [117].

COX-2 inhibitors showed anti-inflammatory activity early on during the onset of the inflammatory response coincident with the expression of COX-2 protein. After 6 h, the COX-2 inhibitors were without effect whereas dual inhibitors still showed efficacy [118]. As shown by western blotting, at this point the COX-2 protein was no longer present. After 48 h, the time of nearly complete resolution of inflammation in this model, there was a second surprising increase in COX-2 protein expression. This newly formed COX-2 protein, however, did not produce the proinflammatory PGE_2 but anti-inflammatory PGs [69]. Apart from PGD_2 and $PGF_{2\alpha}$, one of the proposed anti-inflammatory prostanoids assumed to be important in the resolution of carrageenan pleurisy is 15-deoxy$\Delta^{12-14}PGJ_2$, a member of the cyclopentenone family.

Treating this newly formed COX with selective COX-2 inhibitors 24–48 h after application of the irritant showed an unexpected effect on the inflammatory process, inflammation was not inhibited and still continued. It might therefore be postulated that this newly formed enzyme is not COX-2. But what is it? Is it a catalytic variant of COX-2 or can we postulate a third isoenzyme, a COX-3? If this hypothesis is true, expression of this third inducible isoform of COX could result in the typical periods of remission often seen in many clinical cases of chronic inflammatory diseases. Application of COX-2 inhibitors during the phase of naturally occurring remission of the inflammation could hence delay healing. Indeed, it is known that COX-2 inhibitors given over a long period lead to delayed wound healing.

If this hypothesis is really true, a marker for disease activity is urgently needed in order to determine the appropriate time for the use of selective COX-2 inhibitors and, if a COX-3 enzyme really exists, this fact will lead to a generation of new anti-inflammatory drugs with a new therapeutic target.

References

[1] Kuhnert N., Pharmazie in unserer Zeit 1 (2000) 32–39.

[2] J.R. Vane, R.M. Botting, in: Aspirin and Other Salicylates, Chapman & Hall Medical, London, 1992, pp. 3–16.

[3] Vane J.R., Nature 231 (1971) 232–235.

[4] Smith W.L., Willis A.L., Nature 231 (1971) 235–239.

[5] Frölich J.C., Arzneim.-Forsch./Drug Res. 47 (II) (1997) 1289–1296.

[6] Gilroy D.W., Colville-Nash P.R., Willis D., Chivers J., Paul-Clark M.J., Willoughby D.A., Nature Med. 5 (1999) 698–701.

[7] Allison M.C., Howatson A.G., Torrance C.J., Lee F.D., Russell R.I.G., N. Engl. J. Med. 327 (1992) 749–754.

[8] MacDonald T.M., Morant S.V., Robinson G.C., Shield M.J., McGilchrist M.M., Murray F.E., McDevitt D.G., Br. Med. J. 315 (1997) 1333–1337.

[9] Pilotto A., Franceschi M., Leandro G., Dimario F., Valerio G., Eur. J. Gastroenterol. Hepatol. 9 (1997) 951–956.

[10] Clive D.M., Stoff J.S., N. Engl. J. Med. 310 (1984) 563–572.

[11] Pirson Y., van Ypersele D.E., Strihou C., Am. J. Kidney Dis. 8 (1986) 337–344.

[12] Venturini C.M., Isakson P., Needleman P., Curr. Opin. Nephrol. Hypertension 7 (1998) 79–82.

[13] Kuel F.A., Daugherty H.W., Ham E.A., Biochem. Pharmacol. 33 (1984) 1–5.

[14] Jäger L., Kroegel C., Drug Res. 48 (1998) 205–211.

[15] Needleman P., Isakson P.C., J. Rheumatol. 1 (1997) 129–131.

[16] Fu J.Y., Masferrer J.L., Seibert K., Raz A., Needleman P., J. Biol. Chem. 265 (1990) 16737–16740.

[17] Ristimäki A., Narko K., Hla T., Biochem. J. 318 (1996) 325–331.

[18] Masferrer J.L., Seibert K., Zweifel B., Needleman P., Proc. Natl. Acad. Sci. USA 89 (1992) 3917–3921.

[19] Xie W., Chipman J.G., Robertson D.L., Erikson R.L., Simmons D.L., Proc. Natl. Acad. Sci. USA 88 (1991) 2692–2696.

[20] Kujubu D.A., Fletcher B.S., Varnum B.C., Lim R.W., Herschman H.R., J. Biol. Chem. 266 (1991) 12866–12872.

[21] Crofford L., J. Rheumatol. 24 (1997) 15–19.

[22] Bakhle Y.S., Botting R.M., Mediators Inflamm. 5 (1996) 305–323.

[23] Ristimäki A., Garfinkel S., Wessendorf J., Maciag T., Hla T., J. Biol. Chem. 269 (1994) 11769–11775.

[24] Lipsky P.E., J. Rheumatol. 26 (1999) 25–30.

[25] Tazawa R., Xa X.M., Wu K.K., Wang L.H., Biochem. Biophys. Res. Commun. 203 (1994) 190–199.

[26] Kosaka T., Miyata A., Ihara H., Hara S., Sugimoto T., Takeda O., Eur. J. Biochem. 221 (1994) 889–897.

[27] Yokoyama C., Tanabe T., Biochem. Biophys. Res. Commun. 165 (1989) 888–894.

[28] Kraemer S.A., Meade E.A., DeWitt D.L., Arch Biochem. Biophys. 293 (1992) 391–400.

[29] Herschman H.R., Annu. Rev. Biochem. 60 (1991) 281–319.

[30] Wadleigh D.J., Srinivasa T.R., Kopp E., Ghosh S., Herschman H.R., J. Biol. Chem. 275 (2000) 6259–6266.

[31] Herschman H.R., Cancer Metastasis Rev. 13 (1994) 241–256.

[32] Garavito R.M., Nature Struct. Biol. 3 (1996) 897–901.

[33] DeWitt D.L., Meade E.A., Arch. Biochem. Biophys. 1306 (1993) 94–102.

[34] Kurumbail R.G., Stevens A.M., Gierse J.K., McDonald J.J., Stegeman R.A., Pak J.Y., Gildehaus D., Miyashiro J.M., Penning T.D., Seibert K., Isakson P., Stallings W.C., Nature 384 (1996) 644–648.

[35] Picot D., Loll P.J., Garavito M., Nature 367 (1994) 243–249.

[36] Luong C., Miller A., Barnett J., Chow J., Ramesha C., Browner M.F., Nature Struct. Biol. 3 (1996) 927–933.

[37] Filizda M., Pérez J.J., Palomer A., Mauléon D., J. Mol. Graph. 15 (1997) 290–300.

[38] Llorens O., Pérez J.J., Palomer A., Mauléon D., Bioorg. Med. Chem. Lett. 9 (1999) 2779–2784.

[39] Palomer A., Pérez J.J., Navea S., Llorens O., Jaume P., Garcia L., Mauléon D., J. Med. Chem. 43 (2000) 2280–2284.

[40] Brideau C., Kargman S., Liu S., Dallob A.L., Ehrich E.W., Rodger I.W., Chan C.C., Inflamm. Res. 45 (1996) 68–74.

[41] Engelhardt G., Bögel R., Schnitzer C., Utzmann R., Biochem. Pharmacol. 51 (1996) 21–28.

[42] Gierse J.K., Hauser S.D., Creely D.P., Koboldt C., Rangwala S.H., Isakson P.C., Seibert K., Biochem. J. 305 (1995) 479–484.

[43] Grossman C.J., Wiseman J., Lucas F.S., Trevethick M.A., Birch O.J., Inflamm. Res. 44 (1995) 253–257.

[44] Laneuville O., Breuer D.K., DeWitt D.L., Hla T., Funk C.D., Smith W.L., J. Pharmacol. Exp. Ther. 271 (1994) 927–934.

[45] Meade E.A., Smith W.L., DeWitt D.L., J. Biol. Chem. 268 (1993) 6610–6614.

[46] Mitchell J.A., Akarasereenont P., Thiemermann C., Flower R.J., Vane J.R., Proc. Natl. Acad. Sci. USA 90 (1993) 11693–11697.

[47] Panara M.R., Greco A., Santini G., Sciulli M.G., Rotondo M.T., Padovano R., di Giamberardino M., Cipollone F., Cuccurullo F., Patrono C., Patrignani P., Br. J. Pharmacol. 116 (1995) 2429–2434.

[48] Young J.M., Panah S., Satchawatcharaphong C., Cheung P.S., Inflamm. Res. 45 (1996) 246–253.

[49] Laufer S., Neher K., Bayer B., Homann J., Reutter E., Tries S., Pharm. Pharmacol. Lett. 4 (1995) 166–169.

[50] Dannhardt G., Lehr M., J. Pharm. Pharmacol. 44 (1992) 419–424.

[51] Patrignani P., Panara M.R., Greco A., Fusco O., Natoli C., Jacobelli S., Cipollone F., Ganci A., Creminon C., Maclouf J., J. Pharmacol. Exp. Ther. 271 (1994) 1705–1712.

[52] Futaki N., Takahashi S., Yokoyama M., Arai J., Higuchi S., Otomo S., Prostaglandins 47 (1994) 55–59.

[53] Grossman C.J., Wiseman J., Lucas F.S., Trevethick M.A., Birch P.J., Inflamm. Res. 44 (1995) 253–257.

[54] Tordjman C., Coge F., Andre N., Rique H., Spedding M., Bonnet J., Biochim. Biophys. Acta 1256 (1995) 249–256.

[55] Winter C.A., Risley E.A., Nuss G.W., Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.

[56] Hargreaves K., Dubner R., Brown F., Flores C., Joris J.A., Pain 32 (1998) 77–88.

[57] Anderson G.D., Hauser S.D., McGarity K.L., Bremer M.E., Isakson P.C., Gregory S.A., J. Clin. Invest. 97 (1996) 2672–2679.

[58] Jaffee B.D., Kerr J.S., Jones E.A., Ginnaras J.V., McGowan M., Ackerman N.R., Agents Actions 27 (1989) 344–346.

[59] Seibert K., Zhang Y., Leahy K., Hauser S., Masferrer J., Perkins W., Lee L., Isakson P., Proc. Natl. Acad. Sci. USA 91 (1994) 12013–12017.

[60] Carabaza A., Cabré F., Rotllan E., Gómez M., Gutiérrez M., García L., Mauléon D., J. Clin. Pharmacol. 36 (1996) 505–512.

[61] Gaus K.R., Galbraith W., Roman R.J., Haber S.B., Kerr J.S., Schmidt W.K., Smith C., Hewew W.E., Ackerman N.R., J. Pharmacol. Exp. Ther. 254 (1990) 180–187.

[62] Talley J.J., Prog. Med. Chem. 36 (1999) 201–234.

[63] Huff R., Collins P., Kramer S., Seibert K., Koboldt C., Gregory S., Isakson P., Inflamm. Res. 44 Suppl. 2 (1995) 145–146.

[64] Davis R., Brogden R.N., Drugs 48 (1994) 431–454.

[65] C.S. Li, C. Soucy-Breau, N. Ouimet, Synthesis (1995) 1355–1356.

[66] Chan C.C., Black C., Boyce S., Brideau C., Ford-Hutchinson A.W., Gordon R., Guay D., Hill R., Li C.S., Mancini J., Penneton M., Prasit P., Rasori R., Riendeau D., Roy P., Tagari P., Vickers P., Wong E., Rodger W., J. Pharmacol. Exp. Ther. 274 (1995) 1531–1537.

[67] Otterness I.G., Moore P.F., Methods Enzymol. 162 (1988) 320–327.

[68] Li J.J., Anderson G.D., Burton E.G., Cogburn J.N., Collins J.T., Garland D.J., Gregory S.A., Huang H.C., Isakson P.C., Koboldt C.M., Logusch E.W., Norton M.B., Perkins W.E., Reinhard E.J., Seibert K., Veenhuizen A.W., Zhang Y., Reitz D.B., J. Med. Chem. 38 (1995) 4570–4578.

[69] Pinto D.J.P., Copeland R.A., Covington M.B., Pitts W.J., Batt D.G., Orwat M.J., Lam G.N., Joshi A., Chan Y.C., Wang S., Tzraskos J.M., Magolda R.L., Kornhauser D.M., Bioorg. Med. Chem. Lett. 6 (1996) 2907–2912.

[70] Penning T.D., Talley J.J., Bertenshaw S.R., Carter J.S., Collins P.W., Docter S., Graneto M.J., Lee F.J., Malecha J.W., Miyashiro J.M., Rogers R.S., Rogier D.J., Yu S.S., Anderson G.A., Burton F.G., Cogburn J.N., Gregory S.A., Koboldt C.M., Perkins W.E., Seibert K., Veenhuizen A.W., Zhang Y.Y., Isakson P.C., J. Med. Chem. 40 (1997) 1347–1365.

[71] Simon L.S., Lanza F.L., Lipsky P.E., Hubbard R.C., Tolwalker S., Schwarz B.D., Isakson P.C., Geis G.S., Arthritis Rheum. 41 (1998) 1591–1602.

[72] Schlassman A., Perkar B.M., Stettler C., Netzer P., Stroff T., Flogerzi B., Halter F., Br. J. Pharmacol. 123 (1998) 795–804.

[73] Konturek P.C., Brzozowski T., Konturek S., Taut A., Sekiwowski Z., Stachura J., Hahn E.G., Eur. J. Pharmacol 342 (1998) 55–65.

[74] Ehrlich E.W., Dallob A., De Lepeleive I., Van Hecken A., Riendeau D., Yuan W., Porras A., Wittreich J., Seibold J., De Schepper P., Mehlisch D.R., Gertz B.J., Clin. Pharmacol. Ther. 65 (1999) 336–347.

[75] Talley J.J., Brown D.L., Carter J.S., Graneto M.J., Koboldt C.M., Masferrer J.L., Perkins W.E., Rogers R.S., Shaffer A.F., Zhang Y.Y., Zweifel B.S., Seibert K., J. Med. Chem. 43 (2000) 775–777.

[76] Talley J.J., Bertenshaw S.R., Brown D.L., Carter J.S., Graneto M.J., Kellogg M.S., Koboldt C.M., Yuan J., Zhang Y.Y., Seibert K., J. Med. Chem. 43 (2000) 1661–1663.

[77] JTE-522, Drugs of the Future 23 (6) (1998) 598–601.

[78] Wakitani K., Tazaki H., Matsushita M., Iwamura H., Inflamm. Res. 49 (2000) 117–122.

[79] Friesen R.W., Dubé D., Fortin R., Frenette R., Prescott S., Cromlish W., Greig G.M., Kargman S., Wong E., Chan C.C., Gordon R., Xu L., Riendeau D., Bioorg. Med. Chem. Lett. 6 (1996) 2677.

[80] Li J.J., Norton M.B., Reinhard E.J., Anderson G.D., Gregory S.A., Isakson P.C., Koboldt C.M., Masferrer J.L., Perkins W.E., Seibert K., Zhang Y., Zweifel B.S., Reitz D.B., J. Med. Chem. 39 (1996) 1846–1856.

[81] D. Dube, R. Fortin, R.W. Friesen, Z. Wang, J.Y. Gauthier, US Patent 5, 861, 419.

[82] Warner T.D., Giuliano F., Vojnovic J., Bukasa A., Mitchel J.A., Vane J.R., Proc. Natl. Acad. Sci. USA 96 (1999) 7563–7568.

[83] Dannhardt G., Laufer S., Curr. Med. Chem. 7 (2000) 1101–1112.

[84] Black W.C., Bayly C., Belley M., Chan C.C., Charleson S., Denis D., Gauthier J.Y., Gordon R., Guay D., Kargman S., Lan C.K., Leblanc Y., Mancini J., Quellet M., Percival D., Roy P., Skorey K., Tagari P., Vickers P., Wong E., Xu L., Prasit P., Bioorg. Med. Chem. Lett. 6 (1996) 725–730.

[85] Leblance Y., Black W.C., Chan C.C., Charleson S., Delorme D., Denis D., Gauthier J.Y., Grimm E.L., Gordon R., Guay D., Hamel P., Kargman S., Lan C.K., Mancini J., Quellet M., Percival D., Roy P., Skorey K., Tagari P., Vickers P., Wang E., Xu L., Prasit P., Bioorg. Med. Chem. Lett. 6 (1996) 731–736.

[86] Kalgutkar A.S., Marnett A.B., Crews B.C., Remmel R.P., Marnett L.J., J. Med. Chem. 43 (2000) 2860–2870.

[87] J.W. Barnett, J.P. Dunn, D.J. Kertesz, A.B. Morgans, C.S. Ramesha, C.E. Sigal, E.B. Sjogren, D.B. Smith, D.B. Talamas, Eur. Pat. Appl. EP 714895; Chem. Abstr. 125, 142546.

[88] Carson J.R., Wong S., J. Med. Chem. 16 (1973) 172–174.

[89] Kalgutkar A.S., Crews B.C., Rowlinson S.W., Garner C., Seibert K., Marnett L.J., Science 280 (1998) 1268–1270.

[90] Distel M., Mueller C., Bluhmki E., Fries J., Br. J. Rheumatol. 35 (1996) 68–77.

[91] Engelhardt C., Bögel R., Schnitzer C., Utzmann R., Biochem. Pharmacol. 51 (1996) 21–28.

[92] Lanza F., Rack M.F., Lynn M., Wolf J., Sanda M., J. Rheumatol. 14 (1987) 338–341.

[93] Stewart K.D., Loren S., Frey L., Otis E., Klinghofer V., Hulkower K.I., Bioorg. Med. Chem. Lett. 8 (1998) 529–534.

[94] Song Y., Connor D.T., Doubleday R., Sorensen R.J., Sercel A.D., Unangst P.C., Roth B.D., Gilbertsen R.B., Chan K., Schrier D.J., Guglietta A., Bornemeier D.A., Dyer R.D., J. Med. Chem. 42 (1999) 1151–1160.

[95] Inagaki M., Tsuri T., Jyoyama H., Ono T., Yamada K., Kobayashi M., Hori Y., Arimura A., Yasui K., Ohno K., Kakudo S., Koizami K., Suzuki R., Kato M., Kawai S., Matsumoto S., J. Med. Chem. 43 (2000) 2040–2048.

[96] Black C., Girard M., Guay D., Wang Z., Chem. Abstr. 127 (1997) 220465.

[97] Katori M., Majima M., Inflamm. Res. 49 (2000) 367–392.

[98] Morham S.G., Langenbach R., Loftin C.D., Tiano H.F., Vouloumanos N., Jennette J.C., Mahler J.F., Kluckman K.D., Ledford A., Lee C.A., Cell 83 (1995) 473–482.

[99] Harris R.C., McKanna J.A., Akai Y., Jacobson H.R., Dubois R.N., Breyer M.D., J. Clin. Invest. 93 (1994) 2504–2510.

[100] Mitchel J.A., Evans T.W., Inflamm. Res. 47 Suppl. 2 (1998) 88–92.

[101] Chakraborty I., Das S.K., Wang J., Dey S.K., J. Mol. Endocrinol. 16 (1996) 107–122.

[102] Pairet M., Netter P., Thérapie 54 (1999) 433–445.

[103] Laufer S., Striegel H.G., Neher K., Zechmeister P., Donat C., Stolingwa K., Baur S., Tries S., Kammermeier T., Dannhardt G., Kiefer W., Arch. Pharm. Pharm. Med. Chem. 330 (1999) 307–312.

[104] Augustin J., Dannhardt G., Kiefer W., Laufer S., J. Med. Chem. 37 (1994) 1894–1897.

[105] Lipsky P.E., Am. J. Med. 5B (1999) 515–575.

[106] Masferer J.L., Koki A., Seibert K., Ann. NY Acad. Sci. 889 (1999) 84–86.

[107] Hanahan D., Folkman J., Cell 86 (1996) 353–364.

[108] Form D.M., Auerbach R., Proc. Soc. Exp. Biol. Med. 172 (1983) 214–218.

[109] Ota S., Tanaka Y., Bamba H., Kato A., Matsuzaki F., Eur. J. Pharm. 367 (1) (1999) 131–138.

[110] Jones M.K., Wang H., Peskar B.M., Levin E., Itani R.M., Sarfeh I.J., Tarnawski A.S., Nat. Med. 5 (1999) 1418–1423.

[111] Tomozawa S., Nagawa H., Tsuno N., Hatano K., Osada T., Kitayama J., Sunami E., Nita M.E., Ishihara S., Yano H., Tsuruo T., Shibata Y., Muto T., Br. J. Cancer 81 (1999) 1274–1279.

[112] Reddy B.S., Hirose Y., Lubet R., Steele V., Kelloff G., Paulson S., Seibert K., Rao C.K., Cancer Res. 60 (2000) 293–297.

[113] Elder J.E.D., Halton D.E., Crew T.E., Paraskeva C., Int. J. Cancer 86 (2000) 553–560.
[114] Halliday G., Robinson S.R., Shepherd C., Kril J., Clin. Exp. Pharmacol. Physiol. 27 (2000) 1–8.
[115] Bauer M.K.A., Lieb K., Schulze-Osthoff K., Eur. J. Biochem. 243 (1997) 726–731.
[116] Tocco G., Freire-Moar J., Schreiber S.S., Exp. Neurol. 144 (1997) 339–349.
[117] Willoughby D.A., Moore A.R., Colville-Nash P.R., Lancet 355 (2000) 646–648.
[118] Gilroy D.W., Tomlinson A., Willoughby D.A., Eur. J. Pharmacol. 355 (1998) 211–217.