Dual Inhibition of Cyclooxygenase-2 (COX-2) and 5-Lipoxygenase (5-LOX) as a New Strategy to Provide Safer Non-Steroidal Antiinflammatory Drugs

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Invited review

Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs

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Abstract

Dual COX/5-LOX (cyclooxygenase/5-lipoxygenase) inhibitors constitute a valuable alternative to classical non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors for the treatment of inflammatory diseases. Indeed, these latter present diverse side effects, which are reduced or absent in dual-acting agents. In this review, COX and 5-LOX pathways are first described in order to highlight the therapeutic interest of designing such compounds. Various structural families of dual inhibitors are illustrated.

Keywords: Cyclooxygenase; 5-Lipoxygenase; Dual; Inhibitor; NSAIDs; Inflammation

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), which include Aspirin®, are among the most widely prescribed drugs worldwide [1]. Through their anti-inflammatory, anti-pyretic and analgesic activities, they represent a choice treatment in various inflammatory diseases such as arthritis, rheumatisms as well as to relieve the aches and pain of everyday life [2]. However, their use is often limited by the side effects they produce, particularly in the gastrointestinal (GI) tract and the kidney [3]. Therefore, several new strategies have been considered, notably the dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) [4]. Both are key enzymes involved in the arachidonic acid (AA) cascade, leading to important bioactive fatty acids known as eicosanoids [5].

The objective of this review is to attempt to put in perspective the rationale for dual inhibition with the progress to date in the design of new compounds.

2. The cyclooxygenase pathway

AA is the most abundant polyunsaturated fatty acid found in the phospholipid cell membranes. Activation of the phospholipase A_2 (PLA_2), in response to various stimuli, releases AA, which can be further metabolised by two major enzymatic pathways: cyclooxygenase and 5-LOX, leading to pro-inflammatory mediators, prostanoids and leukotrienes (LTs), respectively.

2.1. Biochemistry of prostanoids

Prostaglandin endoperoxide H synthase, colloquially known as cyclooxygenase or COX has two distinct catalytic activities (Fig. 1): (1) cyclooxygenase which oxides AA to the hydroperoxy-endoperoxide PGG_2 and (2) peroxidase which subsequently reduces PGG_2 to the hydroxy-endoperoxide PGH_2 [6]. Afterwards, the unstable PGH_2 is transformed by diverse tissue-specific synthases and isomerases into prostanoids. This term includes the prostaglandins (PGD_2, PGE_2 and $PGF_{2\alpha}$), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) [7].

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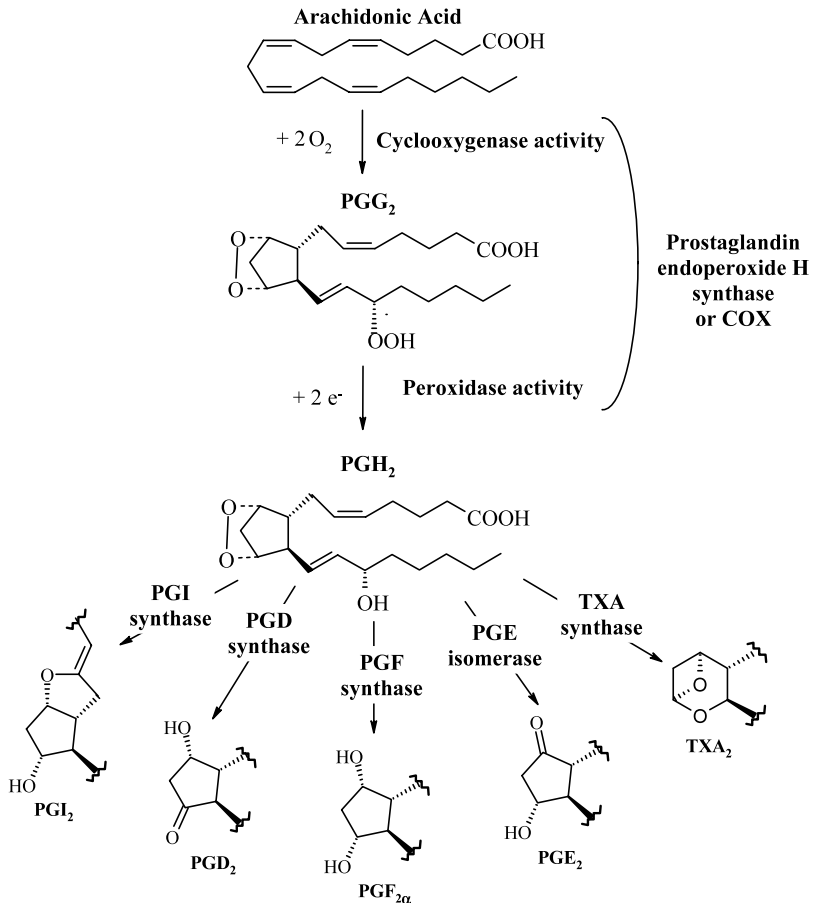


Fig. 1. The cyclooxygenase pathway.

2.2. Biological effects of prostanoids

As they have both autocrine and paracrine functions, these bioactive lipids can be thought of as local hormones. Indeed, once a prostanoid is formed, it exits the cell and then interacts with G protein-coupled receptors, either on the parent cell or on closely neighboring cells to modulate second messenger levels [8].

Although their tissue distribution depends on the cellular enzymatic material, prostanoids are involved in a very broad range of physiological and pathophysiological responses [9].

In the cardiovascular system, PGD_2 and PGE_2 as well as PGI_2 are potent vasodilators whereas TXA_2 displays vasoconstrictor properties. TXA_2 also plays a major role in the induction of platelet aggregation while PGI_2 presents anticoagulant properties. In the airways, $PGF_{2\alpha}$ and TXA_2 are bronchoconstrictors whereas PGI_2 and PGE_2 act as bronchodilators. In the GI tract, PGE_2 and $PGF_{2\alpha}$ as well as PGI_2 ensure the protection of the gastric mucosa by lowering acid secretions, enhancing mucosal blood flow and stimulating mucus formation and bicarbonate secretion. TXA_2 induces increased vascular permeability, leading to edema. In the compromised kidney, PGE_2 and PGI_2, unlike TXA_2, stimulate renal blood flow and diuresis. PGE_2 and $PGF_{2\alpha}$, in contrast to PGI_2, strongly contract the uterine smooth muscle [10].

Prostanoids also mediate the body's responses to tissue injury or inflammation. PGE_2 and PGI_2 are potent vasodilators acting in synergy with other autacoids (i.e., compounds involved in inflammation) such as histamine or bradykinin. Their combined action on capillaries contributes to the redness and increased blood flow in acute inflammatory regions. They enhance vascular permeability leading to the characteristic swelling of tissues. They also produce hyperalgesia by a sensitising action on the peripheral terminals of sensory fibers. Moreover, PGE_2 acts on neurons and contributes to the systemic responses to inflammation such as fever, fatigue and pain hypersensitivity [11].

2.3. Two isoforms of COX

Despite the wide use of NSAIDs over the last century, their mechanism of action was not fully appreciated until 1971 when Vane [12] identified their molecular target, the COX enzyme. In the early 1990s, a second isoform (COX-2) was discovered, distinct from the first

one, then renamed COX-1 [13,14]. While both enzymes carry out essentially the same catalytic reaction, they differ in expression [15], function [16] and structure [17].

2.3.1. Expression and biological functions of COX-1 and -2

The isozyme COX-1 is constitutively expressed in most tissues and is involved in the regulation of physiological "housekeeping" functions such as platelet aggregation and homeostasis of the GI tract and the kidney. The second isoform, COX-2, in contrast, is inducible. Indeed, almost undetectable in healthy man, its expression is rapidly induced in inflammatory cells in response to pro-inflammatory stimuli such as cytokines, growth factors, tumor-promoting agents, bacterial endotoxin, ... The prostaglandins (PGs) produced by COX-2 play a major role in inflammatory reactions and are responsible for the characteristic inflammatory symptoms (redness, pain, edema, fever and loss of function) [18]. The inducible isozyme has also been implicated in pathological processes such as various cancer types (colorectal [19,20], breast [21]), and Alzheimer and Parkinson's diseases [22].

However, recent studies have shown that the relation between the two isoforms is not so straightforward. Indeed, COX-1 may contribute to inflammation processes whereas COX-2 is constitutively expressed in several tissues and organs (see Section 2.5).

2.3.2. Enzymatic structure

The primary structures of COX-1 and COX-2 from numerous species are known. Mature mammalian COX-1 and COX-2 contain 576 and 587 amino acids, respectively [23]. They share a high degree of sequence identity (about 60–65%) [24].

COXs are heme-containing integral membrane proteins [2], located on the lumenal surface of the endoplasmic reticulum and also, for COX-2 mainly, on the nuclear envelope [25].

The three-dimensional (3D) structure of these two enzymes was determined by X-ray diffraction [26,27]. They both exist as homodimers, each monomer comprised of three folding units (Fig. 2): (1) a N-terminal epidermal growth factor (EGF)-like module; (2) an α-helical membrane-binding domain, which anchors the protein to one leaflet of the lipid bilayer (*monotopic* membrane attachment); and (3) a large C-terminal globular catalytic domain with the COX active site which accommodates the substrate or the inhibitors and the peroxidase one which contains the heme cofactor. These sites are distinct but functionally and structurally interconnected [28].

The COX active site, quite similar in both isozymes [27], consists of a long narrow hydrophobic channel extending from the membrane-binding domain (the lobby) to the core of the catalytic one (Fig. 3) [26,29]. The arachidonate-binding site is located in the upper half of the canal, from Arg-120 to near Tyr-385. Ser-530, positioned in the middle of the channel is the site of acetylation by aspirin [30].

Despite their similarity, the COX-2 active site is about 20% larger and has a slightly different form than that of COX-1 (Fig. 3). These size and shape differences are caused mainly by two changes in the amino acid sequence [17]. Ile-**523** in COX-1 is replaced by a valine in COX-2. This difference opens up a small hydrophilic side pocket off the main channel, appreciably increasing the volume of the COX-2 active site. Access to this nook is sterically denied in COX-1 by the longer side chain of Ile-523. In addition, the exchange of Ile-**434** for a valine in COX-2 allows a neighboring residue Phe-518 to swing out of the way, increasing further access to the side cavity.

Another essential amino acid difference between the two isoforms exists, which does not alter the shape of the drug-binding site but rather changes its chemical environment. Within the side pocket of COX-2 is an arginine in place of His-**513** in COX-1, which can interact with polar moieties [31].

These differences between the COX active sites have major implications for the selectivity profile of inhibitors [29].

2.4. Non-steroidal anti-inflammatory drugs

NSAIDs target COX enzymes. They compete with AA for binding to the COX active site [8]. These drugs can be subdivided into two classes: (a) classical, "isozyme non-specific", NSAIDs (discovered before 1995) and (b) selective COX-2 inhibitors.

In 1899, Aspirin® (acetylsalicylic acid, Fig. 4) was introduced as the first potent drug to treat rheumatic diseases [32]. Between the 1960s and 1980s, numerous anti-inflammatory agents were developed and reached the market, e.g. ibuprofen, indomethacin, diclofenac, naproxen (Fig. 4). Despite an extensive chemical diversity, they all possess a carboxylate function that, like one of the AA, forms an ion pair with Arg-120 at the bottom of the COX active site [33]. They share the same therapeutic properties but are also responsible for GI lesions and renal toxicity, leading at high doses to erosions, ulcerations, bleedings, and even to death [3]. Indeed, because of their non-specific inhibition of both COX isoforms, classical NSAIDs reduce the production of pro-inflammatory PGs at sites of injury (via COX-2 inhibition) but also the formation of physiological PGs in the stomach and the kidney (via COX-1 inhibition).

These observations provided a rationale for the development of COX-2 selective inhibitors that should retain the potent anti-inflammatory and analgesic effects of classical NSAIDs with less GI adverse effects [34].

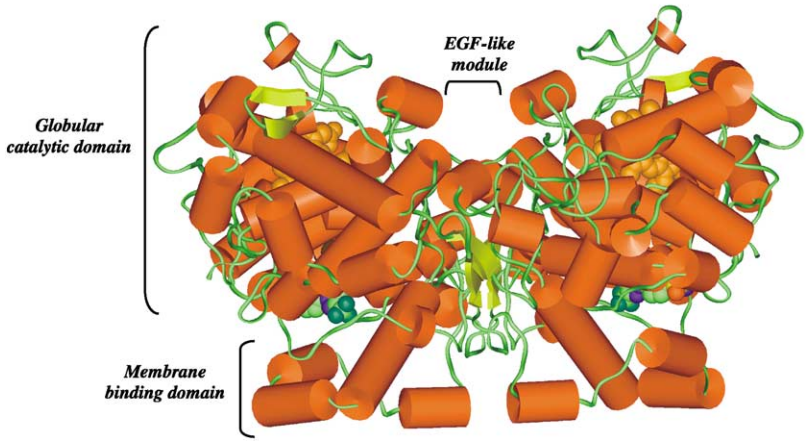


Fig. 2. Structure of mouse COX-2 homodimer (PDB entry 6cox), with the selective COX-2 inhibitor SC-558 bound (colored by atom type) within the COX active site. The heme (orange) marks the peroxidase active site (figure composed using Insight II [134]).

The first generation of these compounds came from animal models, before the notion of an inducible isoform was introduced. Nimesulide, meloxicam and etodolac (Fig. 5) were the first NSAIDs possessing an enhanced safety profile. Later, they were shown to preferentially inhibit the inducible isoform. Currently, more than 500 COX-2-specific inhibitors have been designed. The main structural features of these compounds are the absence of the carboxylate group, characteristic of classical NSAIDs, and generally, the presence of a sulfone ($-SO_2-$) or sulfonamide ($-SO_2NH_2$) moiety, which can interact with Arg-513 in the hydrophilic side pocket of the COX-2 active site [10]. Although the majority of these compounds were discovered before the structure of COX-2 was solved, crystallographic data can now be used to rationally design selective inhibitors [35]. So far, two compounds, celecoxib and rofecoxib (Fig. 5), have been launched for the treatment of inflammatory processes [36]. Several others are undergoing clinical development, such as valdecoxib and etoricoxib (Fig. 5) [37].

The experimental and clinical studies available indicate that selective COX-2 inhibitors are effective anti-inflammatory agents accompanied by a reduced risk of GI toxicity compared with classical NSAIDs [38].

These compounds also open new therapeutic insights in the treatment of several diseases where COX-2 implication has been shown, notably in various cancer types [39] and in Alzheimer's disease [40,41].

2.5. Controversy concerning selective COX-2 inhibitors

Recent pharmacological studies have raised questions about the two principal tenets underlying the design of specific COX-2 inhibitors, namely that PGs responsible for the gastric mucosal integrity and renal function are

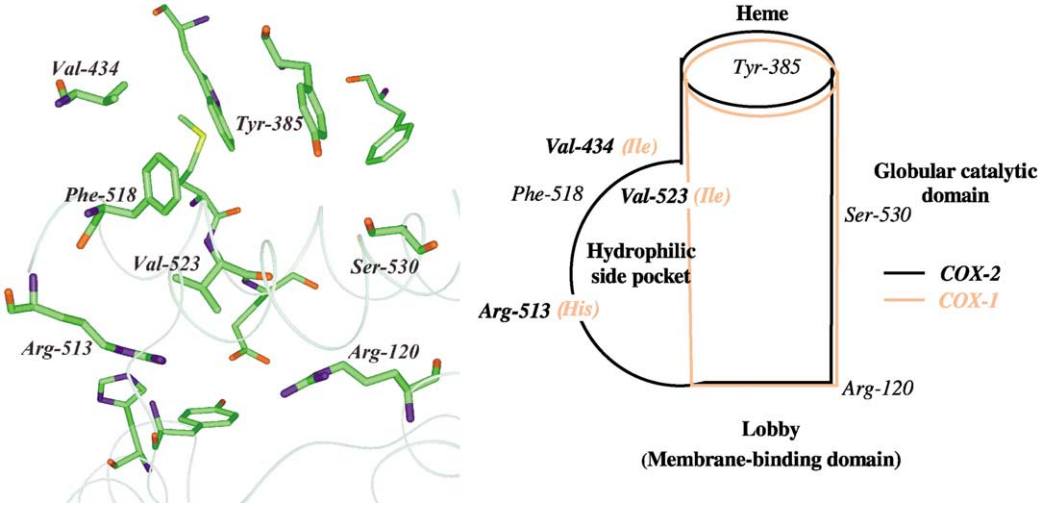


Fig. 3. The COX-2 active site and its schematic representation (figure composed using Insight II [134]).

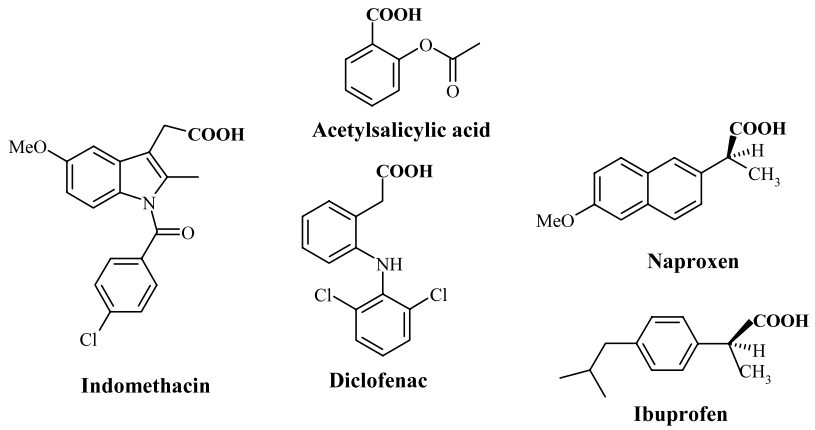


Fig. 4. Classical NSAIDs.

produced solely via COX-1 whereas PGs that mediate inflammatory responses are produced solely via COX-2 [42].

Firstly, COX-2 is constitutively expressed in the kidney and the reproductive tract.

In addition to its implication in the kidney development, this isoform plays an important part in the regulation of renal function (perfusion, water handling, renin release) in both normal and paraphysiological conditions (i.e., in patients with liver cirrhosis, renal insufficiency or congestive heart failure). These patients are, therefore, at risk of renal ischemia when NSAIDs and/or selective COX-2 inhibitors reduce vasodilatory PG synthesis [4,43]. Moreover, cyclic hormonal induction of COX-2 plays an important role in ovulation. This enzyme is also expressed in the uterine epithelium at different stages of pregnancy: in the beginning, it contributes to the ovum implementation and to angiogenesis essential for placenta establishment, while, at the end, it is important for the onset of the labor (parturition). As a result, like for classical NSAIDs, the use of selective COX-2 inhibitors should be avoided in the

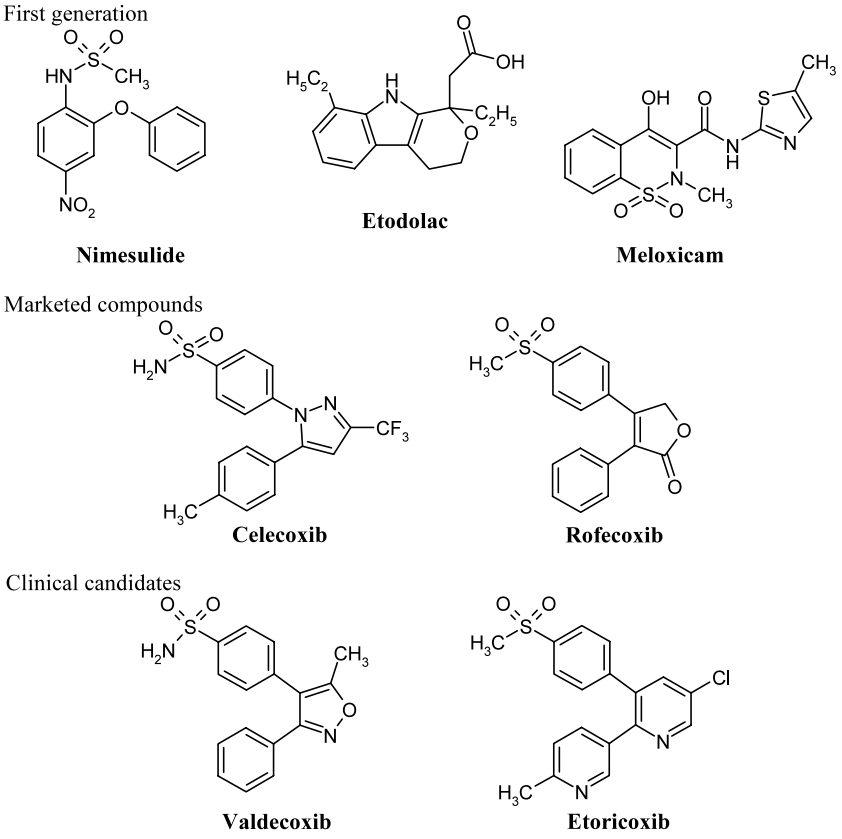


Fig. 5. Selective COX-2 inhibitors.

early stages of pregnancy whereas they should be useful in delaying premature delivery [10].

Secondly, COX-2 may be involved in the "adaptative cytoprotection" response in GI mucosa. When the latter is inflamed or ulcerated, COX-2 is rapidly induced at sites of injury where it produces large amounts of PGs involved in the healing process. So, selective COX-2 inhibitors should be avoided in patients with gastric susceptibility [44].

Thirdly, the incidence of the use of these compounds on cardiovascular diseases still requires vigilance. Indeed, COX-2 has been shown to generate PGI_2 in endothelial cells [45]. Therefore, by decreasing vasodilatory and antiaggregatory PGI_2 production, selective COX-2 inhibitors may tip the natural balance PGI_2–TXA_2 in favor of prothrombotic TXA_2 and may lead to increased cardiovascular thrombotic events [46].

Finally, PGs produced by COX-1 have also been shown to contribute to inflammatory responses and hyperalgesia. In these cases, the anti-inflammatory efficacy of selective COX-2 inhibitors was only observed at doses that inhibited COX-1 [47].

In conclusion, it appears that selective COX-2 inhibitors do not fully satisfy the search for new safer anti-inflammatory agents.

In addition, the 5-LOX pathway, which generates products particularly important in inflammation (LTs), is up-regulated during COX blockade and is thus potentially responsible for undesirable adverse effects, such as asthma. Dual inhibition of COX-2 and 5-LOX is, therefore, an interesting alternative to provide safer NSAIDs [1].

Since its discovery from rabbit neutrophils in 1976 [48], 5-LOX has aroused considerable interest [49].

3. The 5-LOX pathway

3.1. Diversity of the LOX family

5-LOX belongs to a family of lipid peroxidising enzymes, which are expressed in both the vegetal and animal kingdoms [50,51].

These dioxygenases catalyse the oxygenation of polyunsaturated fatty acids containing a 1,4-*cis,cis*-pentadiene moiety to produce hydroperoxy derivatives. They require one non-heme iron atom per molecule, which oscillates between Fe^{2+} (inactive enzyme) and Fe^{3+} (active form) during the catalytic cycle [52]. Although the detailed mechanism of the LOX reaction still remains controversial, its radical nature is commonly agreed upon. It consists of three consecutive steps[1]: (1) stereo-selective hydrogen abstraction from a doubly allelic methylene group, (2) radical rearrangement, and (3) stereo-specific insertion of molecular oxygen and reduction of hydroperoxy-radical intermediate to the corresponding anion [53].

Until now, three major isozymes have been observed in human beings, classified according to their positional specificity of AA oxygenation: the 5-, 12- and 15-LOX insert O_2 at the C-5, -12 and -15 positions of AA, respectively and produce the 5-, 12- and 15-HPETE (hydroperoxy-eicosatetraenoic acid) [53].

The knowledge about the biological roles of 12- and 15-LOX is limited [59–61] and needs to be investigated further. 5-LOX, in contrast, has been widely studied and it might be biologically the most important LOX. It is involved in the biosynthesis of potent inflammatory mediators, the LTs, the second major metabolic pathway of AA.

3.2. Biosynthesis of LTs

The term leukotriene reflects the cells of origin of these compounds, the leukocytes, and their characteristic structure of conjugated trienes [62].

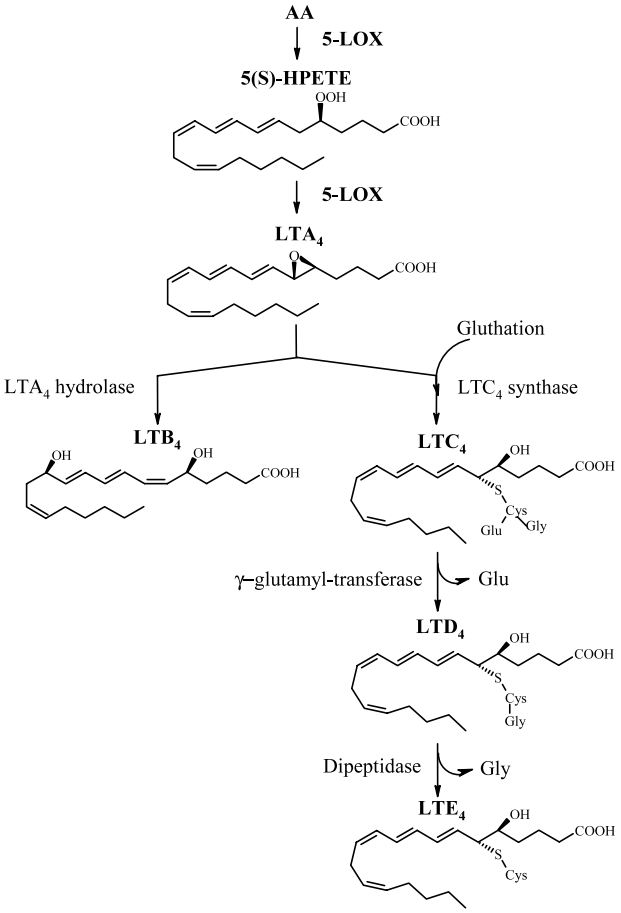


Fig. 6. The 5-LOX pathway.

[1] For further details concerning the mechanism and positional specificity of the LOX reaction, see [53–58].

5-LOX catalyses the first two steps in their biosynthesis (Fig. 6): first, the oxidation of AA at C-5 to yield 5-HPETE followed by the subsequent dehydration of this hydroperoxide leading to the key intermediate LTA_4 [63,64].

Depending upon the available enzymes, this highly unstable epoxide may either undergo enzymatic hydrolysis into the dihydroxy-acid LTB_4 by LTA_4 hydrolase, or be conjugated with glutathione to form LTC_4 by LTC_4 synthase. This last compound can be metabolised into LTD_4 and LTE_4 by successive elimination of glutamic acid and glycine, through the action of a γ-glutamyl-transferase and a specific dipeptidase, respectively. These compounds (LTC_4, D_4 and E_4) are collectively referred to as cysteinyl- or peptido-LTs.

Unlike prostanoids, LTs are almost exclusively produced by inflammatory cells. However, while 5-LOX is specifically expressed in cells of myeloid lineage, LTA_4 hydrolase and LTC_4 synthase are more widely distributed throughout the body: LTA_4 hydrolase is particularly abundant in the intestine, spleen, lung, kidney and erythrocytes [65]; LTC_4 synthase is expressed in mast cells, basophiles, eosinophils [66], endothelial cells and platelets [67].

The broad distribution of these two enzymes allows transcellular metabolisms to occur [68]. Indeed, once available in the extracellular space, LTA_4 can be exported to another cell, which contains enzymes able to metabolise it further. For instance, LTA_4 produced by polymorphonuclear leukocytes can be converted either into LTB_4 in erythrocytes, or to LTC_4 in endothelial cells. These enzymatic interactions between different cell types increase the biosynthesis of LTs and, therefore, their pathophysiological impact.

3.3. Biological effects of LTs

LTs are paracrine hormones and they exhibit a broad spectrum of biological activities, mediated by specific G protein-coupled receptors [69].

LTB_4 is a potent chemotactic agent for inflammatory cells such as neutrophils, macrophages and eosinophils. It is involved in leukocyte migration towards inflammatory sites. By activating neutrophils, it causes their degranulation associated with enzyme release as well as superoxide generation. It also increases their adhesion to vascular endothelium and promotes their tissue infiltration. Finally, it plays an important role in immune reactions by enhancing the release of pro-inflammatory cytokines by macrophages and lymphocytes [70].

Cysteinyl-LTs (LTC_4, D_4 and E_4) constitute the biologic mixture previously known as "slow-reacting substance of anaphylaxis" [62]. These compounds play a pathophysiological role in immediate hypersensitivity reactions. They are potent constrictors of smooth muscles (100–1000 times as potent as histamine), particularly active in the airways leading to important bronchoconstriction. They also stimulate mucus secretion and play an important role in bronchial smooth muscle cells proliferation. In the micro-vascular system, by contracting endothelial cells, they increase the vascular permeability and lead to edema, due to plasma extravasations. Furthermore, they present chemoattractive properties for eosinophils. Finally, it has been shown in guinea pigs that cysteinyl-LTs can interact with sensory fibers, leading to changes in their excitability and enhanced release of tachykinins [71].

In regard to these potent biological activities, LTs have been recognised to be important mediators of numerous inflammatory diseases and allergic disorders such as rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, asthma, psoriasis and allergic rhinitis [68].

3.4. Molecular biology and regulation of 5-LOX

5-LOX has been characterised from various mammalian species. It is a monomeric protein of 75–80 kDa, containing about 673 amino acids [72,73].

While its 3D structure has not been determined yet, several structures of LOXs (two isoforms from soybeans, LOX-1 and LOX-3 [74,75] and one from rabbit, 15-LOX [76]) have been reported. Based on these crystallographic data, the different LOX enzymes are known to share an overall folding pattern (Fig. 7) comprised of two distinct units: a small N-terminal, β-barrel domain, suggested to interact with lipids according to its similarity with the C-terminal domain of human pancreatic lipase [76], and a larger C-terminal catalytic domain, mainly composed of α-helices and containing the active site with the catalytic non-heme iron atom [77].

In resting cells, 5-LOX is localised in the cytosol and in some cell types, in the nucleus [78,79].

Unlike other isoforms, 5-LOX is activated by calcium. Indeed, it binds, through its N-terminal domain, two calcium ions in a reversible manner [80]. This enzyme is also stimulated by ATP in the presence of Ca^{2+} (co-stimulatory effect), by lipid hydroperoxides and phosphatidylcholine [49,81,82].

Once the cell is activated and the intracellular rate of Ca^{2+} is enhanced, 5-LOX, regardless of its cellular localisation, translocates to the nuclear envelope [83]. Then, it interacts with a small membrane protein of 18 kDa designated 5-LOX activating protein or FLAP [84,85]. Although the mechanism of action still remains obscure, it seems that this protein transfers the substrate AA to 5-LOX, this interaction between the enzyme and FLAP being crucial for cellular LT biosynthesis [86].

The LTs produced inside the nucleus might be important for the regulation of gene expression. For

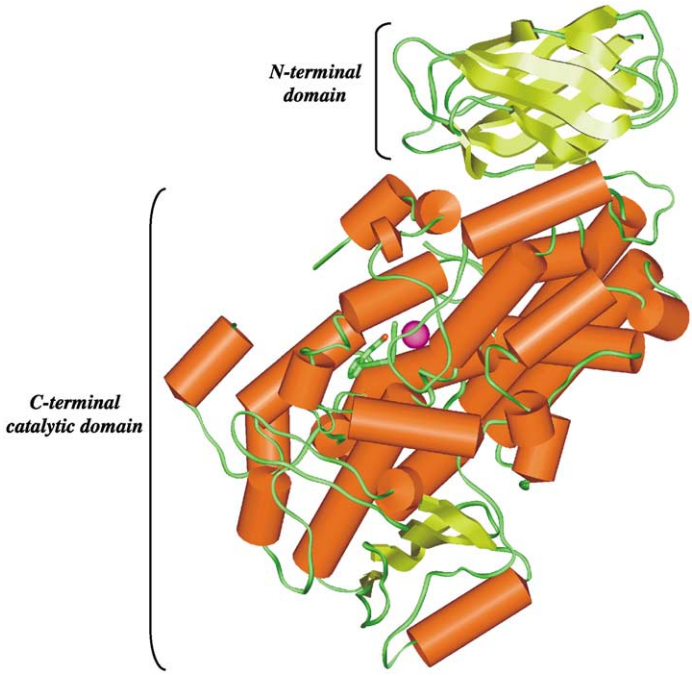


Fig. 7. Structure of rabbit 15-LOX (PDB entry LOX1) with a bound inhibitor (colored by atom type). The non-heme iron atom is shown in magenta (figure composed using Insight II [134]).

instance, LTB_4 binds to the nuclear receptor $PPAR_\alpha$ and activates the transcription of some target genes involved in lipid homeostasis [5].

3.5. Inhibition of LT biosynthesis

Considering the potent pro-inflammatory properties of LTB_4 and the multiple activities of cysteinyl-LTs, the modulation of this pathway should be interesting in the treatment of numerous diseases such as asthma [87,88], allergic and inflammatory disorders [89].

In regard to its characteristics and mechanism of action, different strategies have been developed to inhibit the 5-LOX pathway [90–92]. Direct approaches, on the one hand, involve (i) redox inhibitors or antioxidants, which interfere with the redox cycle of 5-LOX, (ii) iron-chelator agents, and (iii) non-redox competitive inhibitors, which compete with AA to bind the enzyme active site. On the other hand, indirect inhibitors, namely FLAP inhibitors, can prevent 5-LOX from interacting with FLAP and, therefore, inhibit the LT biosynthesis.

Antioxidants are generally small lipophilic aromatic molecules, such as phenols and quinones [91]. The prototypes of this class are the pyrazoline derivatives, phenidone and BW-755C (Fig. 8) [90]. Despite diffuse structure-activity relationships (SAR), lipophilicity is an essential feature for the activity of these compounds [93]. Whereas most are potent inhibitors, they reveal many side effects due to their poor selectivity against 5-LOX. They readily interfere with other biological redox systems, yielding mainly methaemoglobin formation and genotoxicity [73]. Therefore, this class of drug has not been developed further, except some compounds

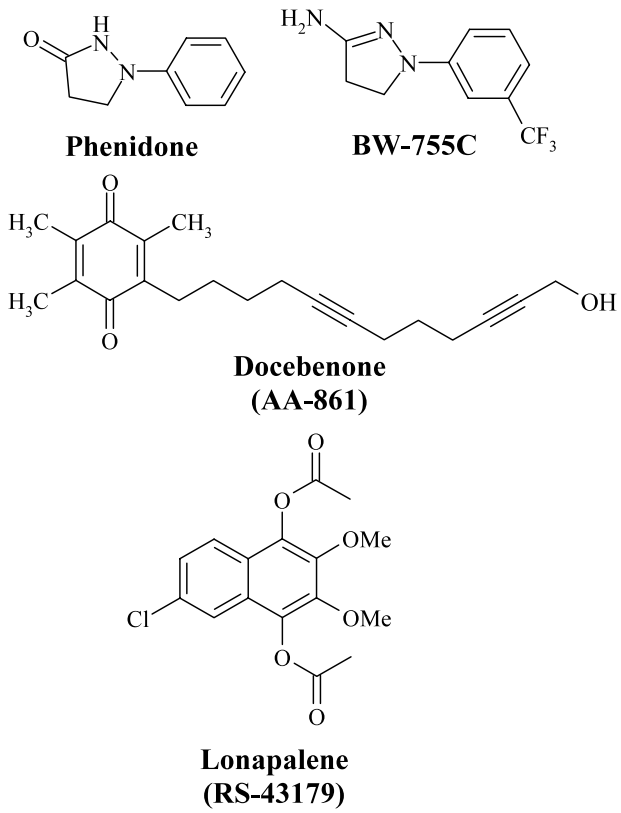


Fig. 8. Redox compounds as 5-LOX inhibitors.

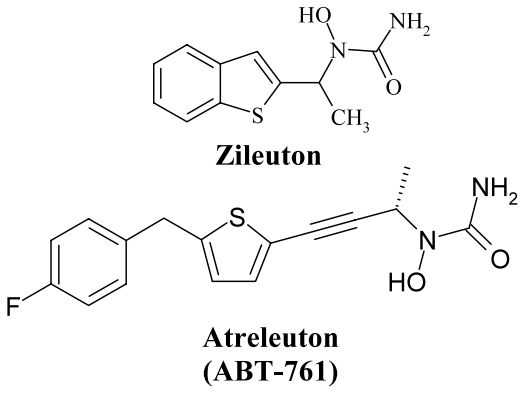


Fig. 9. Iron-chelators as 5-LOX inhibitors.

such as lonapalene and docebenone (Fig. 8), which display clinical efficacy for psoriasis and arthritis by the topical route [91].

Rational design of iron-chelator inhibitors, widely described by hydroxamic acids, one of the most powerful metal ligand groups [94] and *N*-hydroxyurea derivatives, has been more fruitful. Zileuton (Fig. 9) was the first 5-LOX inhibitor [95] brought to market for the treatment of bronchial asthma [96]. This compound, however, has shown a variety of adverse effects, including hepatic toxicities and drug interactions. Further studies yielded potent analogues with better pharmacokinetics. Atreleuton (Fig. 9), for instance, has entered phase III clinical trials [97].

Considering the toxicities and multiple difficulties encountered in the design of redox and iron-chelator inhibitors, the search for active-site directed 5-LOX inhibitors was considered as a new strategy [98]. Several compounds, which form enantio-specific interactions with the enzyme, were discovered, such as ZD-2138 (Fig. 10) [99]. Hybrid molecules of natural products and ZD-2138 led to compounds with improved bioavailability, such as L-697 198 (Fig. 10) [100,101]. Nevertheless, these series have not been further developed because of persistent pharmacokinetic problems and the easier design of another class of inhibitors, explicitly those of the FLAP [91].

MK-866 (Fig. 11) was first introduced as a potent inhibitor of LT biosynthesis in intact cells but had no activity on broken cells or purified 5-LOX. Further investigation led to the discovery of its molecular target, the FLAP protein [85]. Several compounds able to interact with FLAP, such as MK-0591 and Bay-X-1005 (Fig. 11), have been evaluated in clinical trials for asthma [102].

While intensive efforts to develop drugs from LT biosynthesis inhibitors have been rewarded by only one marketed compound, the design of LT receptor antagonists has been much more fruitful, leading to three marketed drugs (Fig. 12) [103].

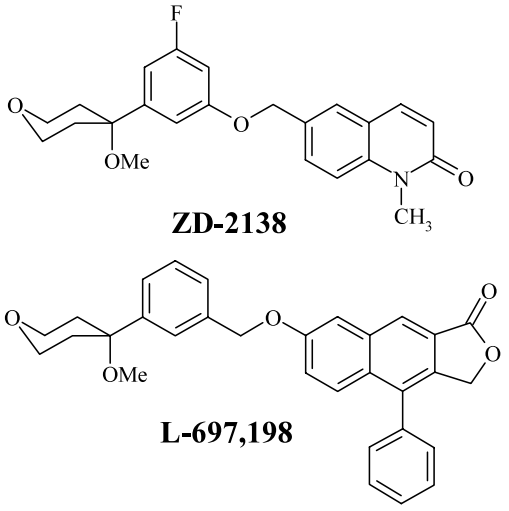


Fig. 10. Non-redox competitive inhibitors of 5-LOX.

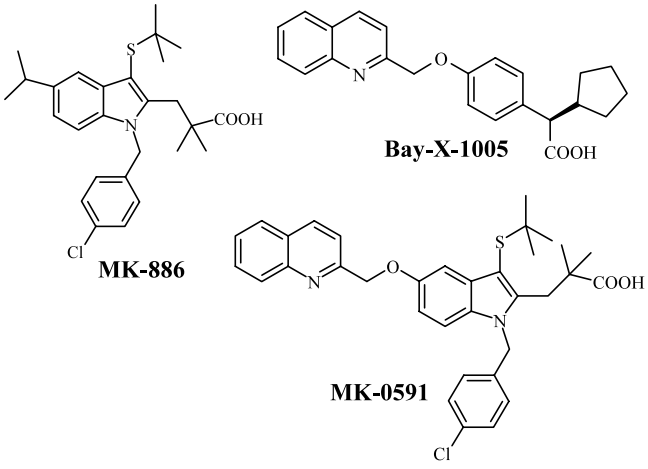


Fig. 11. FLAP inhibitors.

All these compounds, though effective in the treatment of asthma, appear to be an insufficient single therapeutic modality in other inflammatory diseases. Therefore, a promising approach consists of dual inhibition of both 5-LOX and COX-2 [92].

4. Dual COX/5-LOX inhibitors

4.1. Pharmacological and therapeutic benefits

Considering the pro-inflammatory properties of LTs and prostanoids, drugs able to block equally the synthesis of both eicosanoids (**dual inhibitors**), should not only present a superior anti-inflammatory profile but also fewer side effects than NSAIDs and selective COX-2 inhibitors [104].

Indeed, it has been shown that COX inhibition by NSAIDs, besides causing a reduction in the synthesis of vasodilatory and gastroprotective PGs, leads to an up-

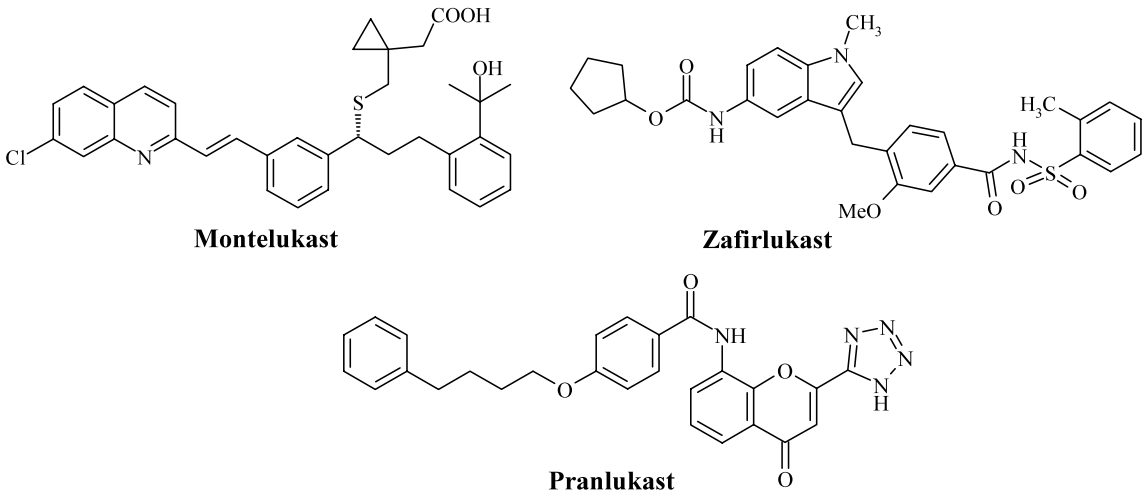


Fig. 12. Marketed cysteinyl-LT receptor antagonists.

regulation of AA metabolism by the 5-LOX pathway [105], increasing the formation of LTs and contributing to inflammation and NSAIDs-induced adverse effects.

On the one hand, LTs produced by 5-LOX, and especially LTB_4, contribute to developing and sustaining inflammation, notably by recruiting leukocytes at inflamed sites [1]. On the other hand, cysteinyl-LTs can generate allergic and hypersensitivity reactions, such as asthma, in sensitive patients [88]. Furthermore, LTs promote the development of GI damage, the most troublesome side effect of NSAIDs [106]. Indeed, LTC_4, by its vasoconstrictive action, lowers mucosal blood flow and consequently enhances the susceptibility of the gastric mucosa. LTB_4 increases damage by stimulating leukocyte infiltration through the mucosa. These latter release, among others, proteases and free radicals that can cause tissue necrosis [44].

Therefore, dual inhibitors can be expected to present an enhanced anti-inflammatory potency as well as not to cause GI injury and/or allergic adverse reactions.

In addition, both COX-2 and 5-LOX enzymes have been involved in the development and progression of numerous types of cancer such as pancreatic, lung, colorectal, prostate, ... [107–113]. So, the use of dual inhibitors opens up new perspectives in the prophylactic treatment of this dreadful disease.

While inhibitors of COXs and of 5-LOX in combination have been proved to be more effective than either class of drugs used alone [114], a single agent inhibiting both enzymes should avoid potential dosing and drug interaction complications of such polypharmacy [115].

4.2. Medicinal chemistry

Currently, various chemical families of dual COX/5-LOX inhibitors can be noticed throughout scientific literature [116]. Concerning COX inhibition, it should be pointed out that only a part of these compounds are selective of the COX-2 isoform. As seen previously, inhibition of both COX isoenzymes, in spite of producing a high anti-inflammatory efficacy, can lead to GI and renal toxicity through COX-1 inhibition. Inhibition of 5-LOX, in contrast, prevents pro-inflammatory and GI damaging effects of LTs.

Future clinical data on dual inhibitors (COX-2/5-LOX as well as COX/5-LOX) should, therefore, inform us about the interest of a balanced inhibition of the two COX isoforms, associated with the blockade of the 5-LOX pathway.

4.2.1. Di-*tert*-butylphenols

The class of di-*tert*-butylphenol derivatives has been extensively investigated to identify new dual COX/5-LOX inhibitors. The most representative molecules are depicted in Fig. 13. Their general structure of 2,6-di-*tert*-butyl-1-hydroxy-benzene substituted in fourth position is optimum for dual activity [117]. The substituents are either five- or six-membered heterocycles or chains.

The phenol moiety gives them antioxidant properties, supposed to be responsible for their anti-inflammatory potency and their low ulcerogenic potential. Indeed, the therapeutic index of these compounds (ratio of anti-

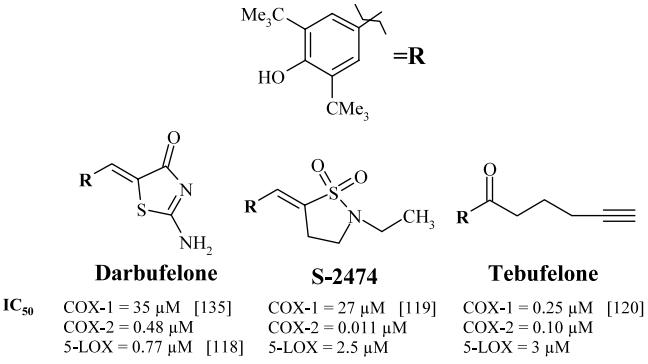


Fig. 13. Examples of dual inhibitors of the di-*tert*-butylphenol class.

inflammatory efficacy to GI safety profile) has uniformly been shown to be superior to that of classical NSAIDs [115].

As darbufelone [118,135], S-2474 is a selective COX-2/5-LOX inhibitor. In addition to its anti-inflammatory efficacy, this latter agent displays cytokine-modulating properties. It is currently being evaluated in clinical trials for arthritis [119].

Among the metabolites of tebufelone, the dihydrodimethyl-benzofuran (DHDMBF, Fig. 14) appears particularly interesting. Despite its lack of a phenol moiety with antioxidant properties, it presents an anti-inflammatory activity equal to tebufelone in the rat carrageenan paw-edema model. Additional studies have shown that DHDMBF, in contrast to tebufelone, selectively inhibits both 5-LOX and COX-2 enzymes. Structural modification of these metabolites resulted in a series of dihydrobenzofurans, exemplified by PGV-20229 (Fig. 14). This dual COX-2/5-LOX inhibitor has been reported to provide analgesic efficacy and excellent gastric safety in a variety of in vivo tests [120–122].

4.2.2. Thiophene derivatives

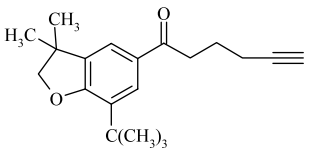
The lead compound of this family is RWJ-63556 [123], a potent orally active COX-2/5-LOX inhibitor (Fig. 15), structurally related to the selective COX-2 inhibitor nimesulide (Fig. 5). It produces significant anti-inflammatory activity in a canine model of carrageenan-induced inflammation [124].

4.2.3. Pyrazoline derivatives

The main compounds of these series are phenidone and BW-755C (Fig. 8). These antioxidant 5-LOX inhibitors appear to be rather non-selective, inhibiting also the COX isoforms [116].

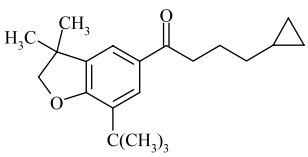
4.2.4. Modified NSAIDs

In an attempt to find potent balanced dual inhibitors, structural modifications were introduced to classical NSAIDs and selective COX-2 inhibitors. The new compounds, designed on the basis of this strategy, demonstrated a dual inhibitory activity against both 5-LOX and COX [115].



DHDMBF

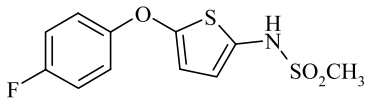
IC_{50} COX-1 = 0.06 μM
COX-2 = 0.015 μM
5-LOX = 6 μM



PGV-20229

IC_{50} COX-1 = 7 μM
COX-2 = 0.22 μM
5-LOX = 8 μM

Fig. 14. Derivatives of tebufelone as dual inhibitors [120].



IC_{50} COX-1 > 10 μM
COX-2 = 1.86 μM
5-LOX = 0.13 μM

Fig. 15. Structure of RWJ-63556 [124].

For instance, the carboxylic acid group of indomethacin, a classical NSAID, was exchanged for *N*-hydroxyurea, able to chelate the non-heme iron of 5-LOX. This led to derivatives, such as compound **1** (Fig. 16), inhibiting not only 5-LOX but also preferentially the inducible isoform COX-2 [125].

Analogues of other potent NSAIDs, fenamates, resulting from the bioisosteric replacement of the carboxylic acid moiety by a tetrazole, were first reported to inhibit COX and, to some extent, 5-LOX. Then, several fenamates, among which flufenamic acid, were converted to potent dual COX/5-LOX inhibitors, after substitution of their carboxylate moiety with other acidic heterocycles, namely 1,3,4-oxadiazole-2-thione (**2**) and 1,3,4-thiadiazole-2-thione (**3**) (Fig. 17). The thione function of these derivatives seems to play an important role in the 5-LOX inhibitory activity. Indeed, substitution of the carboxylate moiety with heterocycles having a carbonyl function (1,3,4-oxadiazole-2-one and 1,3,4-thiadiazole-2-one) led to inactive compounds [126].

The dual COX/5-LOX inhibitor tepoxaline (Fig. 18), which displays the tricyclic pattern of selective COX-2 inhibitors, is a pyrazole-containing hydroxamic acid, able to chelate the non-heme iron atom of 5-LOX [127]. It has undergone clinical evaluation for psoriasis and rheumatoid arthritis [1]. In order to enhance the metabolic stability of its hydroxamate function, structural features of the in vivo active 5-LOX inhibitor atreleuton (Fig. 9) have been incorporated to tepoxaline, leading to two chemical hybrid series (**4** and **5**) of potent dual inhibitors (Fig. 18). Unfortunately, neither improved on the duration of 5-LOX inhibitory activity [128].

The combination of COX-2 and 5-LOX structural characteristics, namely the classic tricyclic sulfonamide moiety (celecoxib-like) and the 4-methoxytetrahydropyran substituent of ZD-2138 (Fig. 10), led to a potent selective COX-2/5-LOX inhibitor (**6**), devoid of redox and iron ligand-binding properties (Fig. 19) [129].

4.2.5. Pyrrolizine derivatives

Several pyrrolizine derivatives possess a dual inhibitory activity. Unlike most of the described compounds, they are neither antioxidants nor iron chelators [130].

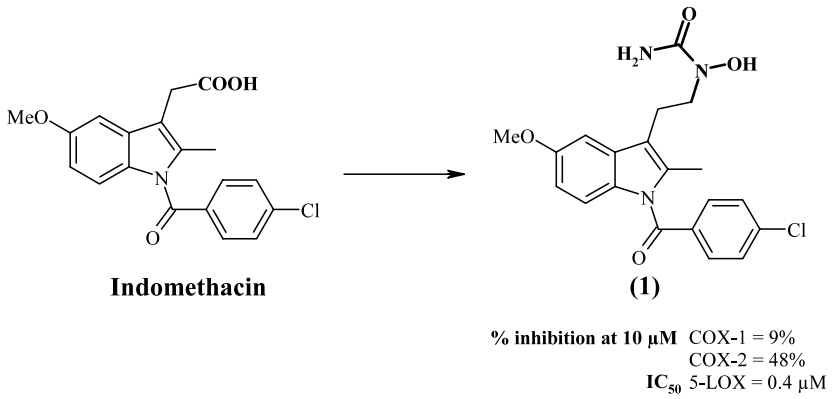


Fig. 16. An indomethacin derivative as dual COX-2/5-LOX inhibitor [125].

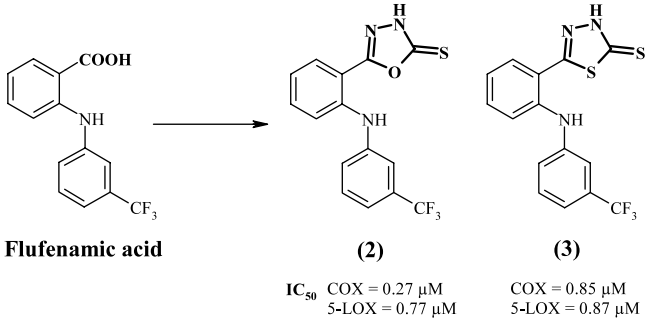


Fig. 17. Dual COX/5-LOX inhibitors derived from flufenamic acid [126].

Licofelone (Fig. 20) has entered phase III clinical trials for the treatment of osteoarthritis. In several animal models, this compound has shown anti-inflammatory, analgesic and anti-asthmatic effects [131]. Whereas it inhibits 5-LOX and preferentially COX-1 [116], it does not cause any GI damage [132]. The mechanism of this gastric-sparing action has not been fully clarified yet.

Other pyrrolizine derivatives, such as compound **7**, combine the structural requirements for COX-1/COX-2 and 5-LOX inhibition, with a higher selectivity towards COX-2 (Fig. 20) [133].

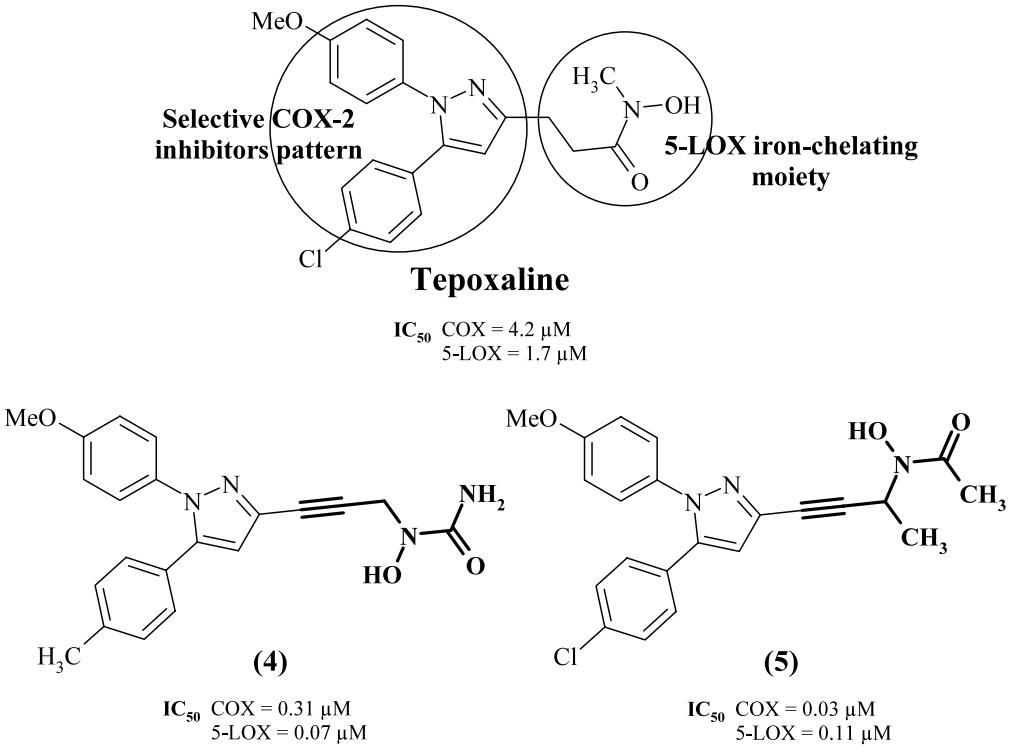
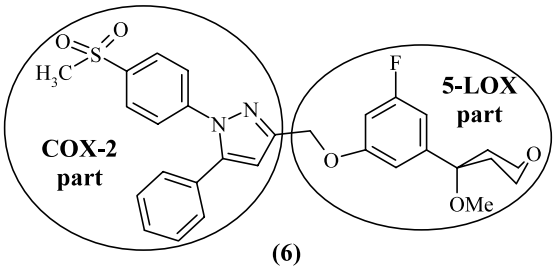


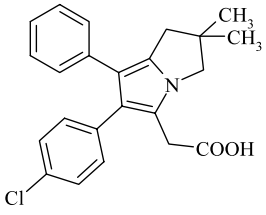
Fig. 18. Structure of tepoxaline and derivatives [128].



(6)

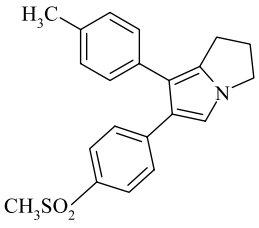
IC_{50} COX-1 >10 μM
COX-2 = 0.05 μM
5-LOX = 0.003 μM

Fig. 19. Association of two pharmacore groups leading to a potent COX-2/5-LOX inhibitor [129].



Licofelone (ML-3000)

IC_{50} COX-1 = 0.16 μM
COX-2 = 0.37 μM
5-LOX = 0.21 μM



(7)

IC_{50} COX-1 = 0.7 μM
COX-2 = 0.005 μM
5-LOX = 10 μM

Fig. 20. Structure of pyrrolizine derivatives [133].

5. Conclusion

NSAIDs are widely used for the treatment of inflammatory diseases, such as arthritis. However, their chronic use has often been impaired by the adverse effects they cause, especially in the GI tract and the kidney. Selective COX-2 inhibitors have been developed and marketed in order to reduce NSAIDs-induced side effects associated with COX-1 inhibition.

However, recent findings have highlighted an important role for COX-2 in several physiological processes, as well as a key role in inflammation and pain perception for COX-1, therefore, raising questions about the "selective COX-2 inhibitor" theory. Moreover, COX inhibition leads to an up-regulation of the 5-LOX pathway, yielding various adverse effects.

As a result, a new strategy has been considered: the dual inhibition of 5-LOX and COX enzymes. Various structural families of dual inhibitors have been designed and several compounds are currently undergoing preclinical or clinical development. By preventing the biosynthesis of both prostanoids and LTs, they are potent anti-inflammatory agents.

Though none of these compounds have reached the market yet, they might represent a valuable therapeutic alternative to classical NSAIDs and to some extent, to selective COX-2 inhibitors, notably because of their almost complete lack of GI toxicity.

All of these drugs do not present a selective COX-2 inhibition and the future clinical data of compounds, such as licofelone and S-2474, could point out the interest of a balanced inhibition of the two COX isoforms, associated with the blockade of the 5-LOX pathway.

Finally, as COX-2 and 5-LOX are up-regulated in various cancers, development of drugs targeting both enzymes would be a useful future direction for chemoprevention.

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