

Review

Inflammation, Cancer and Oxidative Lipoxygenase Activity are Intimately Linked

Rosalina Wisastra and Frank J. Dekker *

Pharmaceutical Gene Modulation, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands; E-Mail: r.wisastra@gmail.com

* Author to whom correspondence should be addressed; E-Mail: f.j.dekker@rug.nl;
Tel.: +31-5-3638030; Fax: +31-5-3637953.

Received: 16 April 2014; in revised form: 27 June 2014 / Accepted: 2 July 2014 /

Published: 17 July 2014

Abstract: Cancer and inflammation are intimately linked due to specific oxidative processes in the tumor microenvironment. Lipoxygenases are a versatile class of oxidative enzymes involved in arachidonic acid metabolism. An increasing number of arachidonic acid metabolites is being discovered and apart from their classically recognized pro-inflammatory effects, anti-inflammatory effects are also being described in recent years. Interestingly, these lipid mediators are involved in activation of pro-inflammatory signal transduction pathways such as the nuclear factor κ B (NF- κ B) pathway, which illustrates the intimate link between lipid signaling and transcription factor activation. The identification of the role of arachidonic acid metabolites in several inflammatory diseases led to a significant drug discovery effort around arachidonic acid metabolizing enzymes. However, to date success in this area has been limited. This might be attributed to the lack of selectivity of the developed inhibitors and to a lack of detailed understanding of the functional roles of arachidonic acid metabolites in inflammatory responses and cancer. This calls for a more detailed investigation of the activity of arachidonic acid metabolizing enzymes and development of more selective inhibitors.

Keywords: inflammation; cancer; oxidative stress; lipoxygenases; nuclear factor κ B

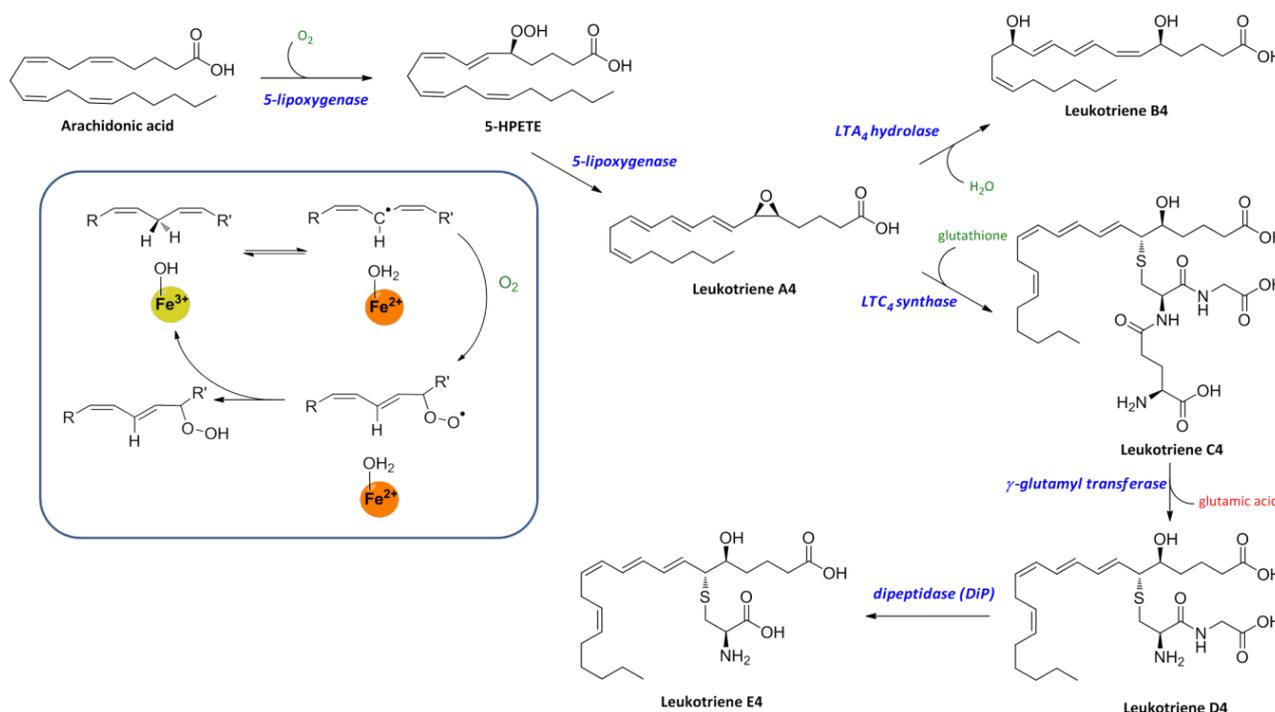
1. Introduction

Inflammation and cancer are closely linked by specific oxidative processes in the tumor microenvironment [1]. Therefore, oxidative enzymes that are known to play a key role in inflammation are increasingly investigated in connection to cancer. The immune response on the cellular levels is carefully orchestrated by signal transduction pathways such as the nuclear factor κ B (NF- κ B) pathway. In this review we will discuss the lipid mediators that are produced by lipoxygenases, their role in the regulation of inflammatory responses among others via the NF- κ B pathway, their connection with inflammatory diseases and cancer as well as small molecule lipoxygenase inhibitors.

2. Lipid Mediators Produced by Lipoxygenases

Lipoxygenases are a group of oxidative enzymes with a non-heme iron atom in their active site, which are involved in the regulation of inflammatory responses by generation of pro-inflammatory mediators known as leukotrienes or anti-inflammatory mediators known as lipoxins. These enzymes catalyze the insertion of oxygen (O_2) into poly-unsaturated fatty acids (PUFAs) such as arachidonic acid and linoleic acid. It has been described that the catalytic reaction of lipoxygenases involves a single electron oxidation by the active site iron atom which switches between Fe^{2+} and Fe^{3+} redox states [2]. In the catalytic reaction, Fe^{3+} is reduced to Fe^{2+} with concomitant oxidation of the lipid substrate by hydrogen abstraction from a bis-allylic methylene to give a pentadienyl radical, which is re-arranged to provide a 1-cis,3-trans-conjugated diene moiety. Subsequently, a stereo-specific insertion of oxygen at the pentadienyl radical takes place to form an oxygen centered fatty acid hydroperoxide radical. The intermediate hydroperoxide radical is reduced to the corresponding anion with concomitant re-oxidation of iron to Fe^{3+} (Scheme 1) [3].

Scheme 1. Oxidation reactions of lipoxygenases in the leukotriene (LT) biosynthesis pathways.



Lipoxygenases catalyze the formation of hydroperoxy eicosatetraenoic acids (HPETEs) from arachidonic acid. These HPETEs are subsequently reduced and transformed to form so called eicosanoids, which are signaling molecules that play an important regulatory role in the immune responses and other physiological processes. In general, lipoxygenases are classified as 5-, 8-, 12-, and 15-lipoxygenases according to their selectivity to oxygenate fatty acids in a specific position [4]. The importance of fatty acids oxygenation by lipoxygenase enzymes has been described for many physiological processes (Table 1).

Lipoxygenases are commonly found in the plant and animal kingdoms. Although the overall architecture of plant lipoxygenases such as soybean lipoxygenase is similar to mammalian lipoxygenases, they share little sequence similarity (about 25%) [5]. In contrast, there are sequence similarities of about 60% among human 5-, 12- and 15-lipoxygenases [6]. Even though these enzymes show a high sequence similarity, the regulatory mechanism of 5-lipoxygenase (5-LOX) is more complex compared to the other human lipoxygenases. In general, lipoxygenases are comprised of two domains; N-terminal and C-terminal domains. The N-terminal domain is a regulatory domain and consists mostly of β -barrels, while the C-terminal domain is a catalytic domain and consists mostly of α -helices [6]. The non-heme iron atom is located in the catalytic C-terminal domain, whereas the function of the N-terminal domain is not unambiguously characterized. For 5-LOX, it is clear that the N-terminal domain is essential for translocation to the nuclear membrane whereas for the other LOXs, this is still under debate [6,7].

Table 1. Human lipoxygenases and their most important substrates, products, and functions.

Lipoxygenase	Substrate	Product	Physiological function	Ref.
5-lipoxygenase (5-LOX)	arachidonic acid	5(S)-HPETE, Leukotriene A4	Pro-inflammatory mediator	[8]
	γ -linoleic acid	Dihomo- γ -linoleic acid (DGLA)	Inhibition of arachidonic acid conversion	[9]
	Eicosapentaenoic acid (EPA)	Leukotriene A5	Anti-inflammatory mediator/inhibitor LTA4 hydrolase	[10]
Platelet 12-lipoxygenase (p12-LOX)	arachidonic acid	12(S)-HPETE	Modulation of platelet aggregation	[11–13]
	Dihomo- γ -linoleic acid (DGLA)	12(S)-HPETrE		
	Eicosapentaenoic acid (EPA)	12(S)-HPEPE		
12R-lipoxygenase (12R-LOX)	α -linoleic acid	12(S)-HPOTrE	Epidermal barrier acquisition	[14]
	arachidonic acid	12(R)-HPETE		
epidermis LOX3 (eLOX3)	Linoleyl- ω -hydroxy ceramide	9(R)-hydroperoxylinoleoyl- ω -hydroxy ceramide		
15-lipoxygenase-1 (15-LOX1)	9(R)-hydroperoxylinoleoyl- ω -hydroxy ceramide	9(R)-10(R)-trans-epoxy-11E-13(R)-hydroxylinoleoyl- ω -hydroxy ceramide	modulation of MAP kinase signaling pathways	[15–17]
	linoleic acid	13(S)-HPODE	modulation of leukotriene B4, pro-inflammatory mediators	
15-lipoxygenase-2 (15-LOX2)	arachidonic acid	15(S)-HPETE	negative cell cycle regulator and tumor suppressor	[18,19]

Human 5-LOX activity is influenced by the presence of Ca^{2+} , which reversibly binds to the enzyme with maximum binding of two Ca^{2+} ions per 5-LOX. Ca^{2+} binding causes an increase in hydrophobicity, which promotes membrane association of 5-LOX [20]. Furthermore, the presence of adenosine tri-phosphate (ATP) appears to be important for optimal 5-LOX activity. It has been reported that 5-LOX has an ATP binding site, in which both the adenine-base and the phosphate moieties of ATP are essential for the activation. However, the stoichiometry, the affinity and the location of ATP binding on 5-LOX remain elusive [21]. In addition, the cellular 5-LOX activity is essentially dependent on a small membrane protein called five-lipoxygenase-activating protein (FLAP). Although, FLAP shares about 50% sequence similarity with human LTC₄ synthase, its activity is not glutathione dependent. The influence of FLAP on 5-LOX activity is exerted via an allosteric mechanism. FLAP plays a role in arachidonic acid recruitment to 5-LOX and its conversion to 5-HPETE and LTA₄ [22].

Two different types of 12-LOX have been identified based on the differences in tissue distribution, which are respectively named as platelet 12-LOX (p12-LOX) and 12R-LOX. Platelet 12-LOX is mostly found in platelets as a platelet aggregation modulator, whereas 12R-LOX is mostly found in skin cells in which it plays a role in epidermal barrier properties [13,14]. There are also two subtypes of 15-LOX, named as 15-LOX-1 and 15-LOX-2. 15-LOX-1 is highly expressed in leukocytes and airway endothelial cells [23,24] while, in contrast, 15-LOX-2 is expressed in prostate, lung, cornea, and many tissues such as liver, colon, kidney, spleen, ovary, and brain, but not in leukocytes [25]. Moreover, cells induced by interleukin (IL)-4 and IL-13 show selective increase of 15-LOX-1 expression and not 15-LOX-2 [26]. A lack of similarity between 15-LOX-1 and 15-LOX-2 at the primary sequence level contributes to their distinct biological roles [27].

3. Biosynthesis of Leukotrienes: Initiation of Inflammatory Responses

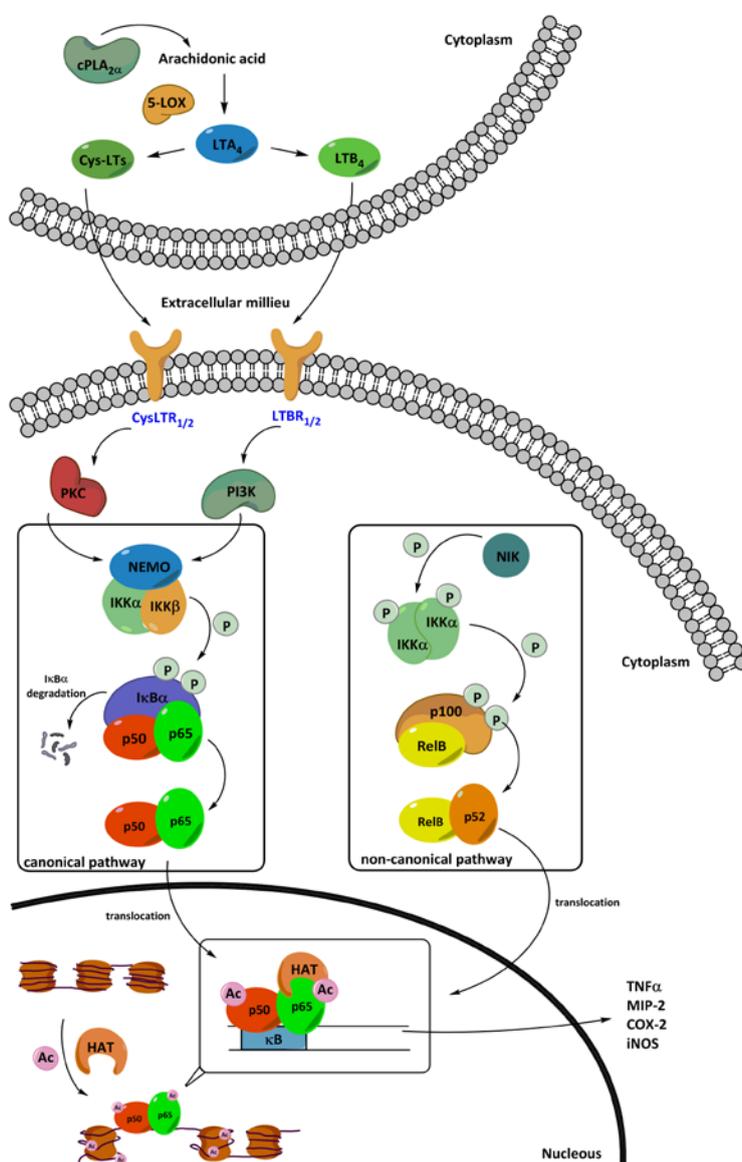
Leukotrienes (LTs) received their name because they were found in various types of leukocytes, such as granulocytes, monocytes and mast cells. The pro-inflammatory leukotrienes are divided into two classes, dihydroxy acid leukotriene LTB₄ and the cysteinyl leukotrienes LTC₄, LTD₄, and LTE₄ [28].

Biosynthesis of leukotrienes is regulated by the activity of 5-lipoxygenase. Upon inflammatory stimulation, cytosolic phospholipase A₂- α (cPLA₂ α) releases arachidonic acid from membrane lipids to start the leukotrienes biosynthesis. 5-lipoxygenase catalyzes the oxidation of arachidonic acid to 5-HPETE, which is subsequently converted into Leukotriene A₄ (LTA₄). LTA₄, which is a LT precursor, is hydrolyzed by LTA₄ hydrolase to form dihydroxy acid leukotriene LTB₄. Another route is the conversion of LTA₄ to cysteinyl leukotriene LTC₄ by addition of a glutathione group by LTC₄ synthase. Conversion of LTC₄ by γ -glutamyl transferase results in LTD₄ and glutamic acid release. Furthermore, dipeptidase (DiP) breaks the amide bond in LTD₄ to give LTE₄ (Scheme 1).

LTB₄ has an important function as chemo-attractant and is also involved in the formation of reactive oxygen species. Binding of LTB₄ to the Leukotriene B₄ receptor 1 or 2 (LTBR1/2) activates the phosphatidylinositol 3-kinase (PI3K) pathways [29]. In this way LTB₄ is involved in the NF- κ B pathway by stimulating the phosphorylation of I κ B α , which results in activation of the NF- κ B pathway. The cysteinyl leukotrienes LTC₄, LTD₄, and LTE₄ activate two cysteinyl leukotriene receptors (CysLTR) 1 and 2, which also play a role in the regulation of NF- κ B pathway [30]. LTC₄ induces the phosphorylation of NF- κ B p65 and activates the NF- κ B complex p50-p65. It also has been

proposed that the LTC₄ binding to the CycLT2 receptor will induce the phosphorylation of IκBα by involving protein kinase C (PKC) family enzymes (Figure 1) [31].

Figure 1. The roles of leukotrienes and acetylation in the expression of pro-inflammatory mediators through the NF-κB pathway. The activated cPLA₂α produces arachidonic acid, which is further converted to LTA₄ by the 5-LOX. LTA₄ is then converted to LTB₄ and cys-LTs and their binding to the leukotriene receptors activate the NF-κB pathway in leukocytes during inflammation. cPLA₂α—cytosolic phospholipase A₂-α; 5-LOX—5-lipoxygenase; LTA₄—leukotriene A₄; LTB₄—leukotriene B₄; Cys-LTs—cysteinyl leukotrienes; LTBR1/2—leukotriene B receptors 1 or 2; CysLTR1/2—cysteinyl leukotriene receptors 1 or 2; PI3K—phosphoinositide 3-kinase; PKC—protein kinase C; NEMO—NF-κB essential modulator; IκBα—inhibitor NF-κB; IKK—IκB kinase; NIK—NF-κB activation of inducing kinase; HAT—histone acetyltransferase. TNFα—tumor necrosis factor α; MIP-2—macrophage inflammatory protein-2; COX-2—cyclooxygenas-2; iNOS—inducible nitric oxide synthase.



4. Nuclear Factor κ B (NF- κ B) in Inflammation

Among all the lipoxygenase products, leukotrienes have exceptional biological functions. A particular function is their action as pro-inflammatory mediators in the activation of the NF- κ B pathway [32]. The nuclear factor κ B (NF- κ B) is an inducible transcription factor comprised of homo- and hetero-dimers of the NF- κ B and Rel protein family [33]. The NF- κ B sub-family is comprised of two precursor proteins, p105 and p100, while the Rel sub-family is comprised of RelA/p65, RelB and c-Rel. p105 and p100 respectively are precursors of p50 and p52, which are transcription factors in the NF- κ B pathways. The transcription factors of NF- κ B are normally present in the cytoplasm in their inactive state in a complex with the inhibitory proteins of I κ B family [33]. The production of pro- and anti-inflammatory mediators is highly correlated with gene expression through the NF- κ B pathway [34]. There are two major pathways for NF- κ B activation, the canonical pathway and the non-canonical pathway. In addition, an atypical pathway has also been identified. The heterodimer of RelA/p65 and p50 is involved in the canonical pathway, whereas the heterodimer of RelB and p52 is involved in the non-canonical pathway [35,36]. The activated NF- κ B pathway is involved in the pathogenesis of inflammatory diseases such as asthma, arthritis, inflammatory bowel diseases (IBD) and chronic obstructive pulmonary diseases (COPD) [37–39]. During inflammatory responses, both pro- and anti-inflammatory mediators are produced. The regulation of inflammatory responses relies on the careful orchestration of the expression of mediators that activate or suppress the immune response.

4.1. The Canonical NF- κ B Activation Pathway

Under normal conditions, the activity of the transcription factor complex RelA/p65-p50 is inhibited by its natural inhibitors, I κ B proteins. Upon stimulation by pro-inflammatory cytokines such as TNF α and IL-1, I κ B kinase (IKK) complex phosphorylates I κ B proteins that cause the release of the RelA/p65-p50 dimer, which can subsequently translocate to the nucleus. The IKKs consist of the subunits IKK α , IKK β and IKK γ , which is also known as the NF- κ B essential modulator (NEMO) protein. In addition, the functions of RelA/p65 are also regulated by two group of enzymes; phosphoinositide 3-kinase (PI3K) and protein kinase B (PKB)/Akt kinases [40]. Kinases in the PI3K and PKB/Akt pathways induce the activation of I κ B kinase to phosphorylate the I κ B and stimulate the activation of transcription factors [41,42]. Furthermore, the phosphorylated I κ B α protein is ubiquitinated and subsequently degraded [43]. Degradation of I κ B leads to the translocation of the free p65-p50 dimer to the nucleus, in which p65-p50 then bind to the κ B promoter regions and activates gene expression (Figure 1) [35,43].

4.2. The Non-Canonical NF- κ B Activation Pathway

RelB in complex with p100 is present in the cytoplasm as inactive form of the transcription factor RelB-p52. The activation of the NF- κ B via the non-canonical pathway is mediated by the IKK complex, which comprises two IKK α sub-units. The activation of the homodimer of IKK α is involving NF- κ B activation of inducing kinase (NIK) and tumor necrosis factor receptor-associated factor (TRAF) [5,40]. Upon stimulation, the IKK complex is activated by NIK through a phosphorylation process, then the activated IKK α phosphorylates the inactive form of p100 subunit. Phosphorylation of

p100 then leads to another post-translational modification; ubiquitination, which induces the proteolytic processing of p100 to form the active transcription factor p52. The formed heterodimer RelB-p52 is recruited to the nucleus to initiate the gene transcription (Figure 1) [44].

5. Role of Leukotrienes in Inflammatory Diseases

Over-expression of lipoxygenases and their pro-inflammatory products, leukotrienes, has been implicated in many human acute and chronic inflammatory diseases such as asthma, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, dermatitis, and cancer. In some cases a connection between lipoxygenase activity and activation of the NF- κ B pathway has been described (Table 2).

Table 2. Connection between lipoxygenase activity and NF- κ B activity in specific diseases.

Disease	Observations	Ref.
Asthma	Ectopic expression of 15-LOX induces NF- κ B mediated reporter gene expression in epithelial cells.	[45]
Cardiovascular diseases	Increased levels of 5-LOX metabolites in patients with atherosclerosis.	[46]
	The 15-LOX metabolite 15-HETE activates the NF- κ B pathway and stimulates 15-LOX expression in a positive feedback loop.	[47]
Rheumatoid Arthritis	The 15-LOX metabolite 15-HETE increases I κ B α degradation and activation of the NF- κ B pathway.	[48]
Cancer	The 5-LOX metabolite LTB4 is capable of activating the transcription factor NF- κ B in cancer cells	[49]

5.1. Asthma

Highly increased levels of LTC₄, LTD₄, and LTE₄, which are 5-LOX metabolites, have been observed in lung tissues that were challenged with allergens. Up-regulation of these mediators is considered as the main cause of asthma since leukotrienes are potent regulators for smooth muscle contraction in bronchoconstriction. In addition, cysteinyl leukotrienes can cause plasma leakage from post-capillary venules in respiratory tissues, which can lead to inflammatory edema [50]. In addition, it has been shown that the expression of 15-LOX in lung epithelial cells activates the NF- κ B pathway [45], which demonstrates a connection between LOX activity and NF- κ B activation. These findings indicate that the modulation of the production of pro-inflammatory leukotrienes using small molecule inhibitors has potential for treatment of asthma.

5.2. Cardiovascular Diseases

Lipoxygenase activity has been implicated in the pathogenesis of cardiovascular diseases such as atherosclerosis. Lipoxygenases, as oxidative enzymes, are believed to have an important role in the oxidation of low density lipoproteins (LDLs) in macrophages to form foam cells [46]. The formed foam cells will develop plaques of atheroma and their accumulation in the arteries leads to atherosclerosis. In addition, an increase of the 5-LOX metabolites cysteinyl LTE₄ levels in urine and LTB₄ in the atheroma were observed in patients with atherosclerosis. In addition, it has been shown that the 15-LOX-1 and 15-LOX-2 metabolite 15-hydroxyeicosatetraenoic acid (15-HETE) promotes

pulmonary artery inflammation via activation of the NF- κ B pathway, which leads to increased expression of the 15-LOX enzymes in a positive feedback loop [47]. This demonstrates that inhibition of lipoxygenase activity can provide a treatment strategy for this cardiovascular disease.

5.3. Rheumatoid Arthritis

Since 5-lipoxygenase is the main catalyst for the formation of LTB₄, its role in the development of rheumatoid arthritis becomes apparent with the identification of high LTB₄ levels in the synovial fluid of arthritis patient [51]. This leukotriene is produced mainly by neutrophils, which are the most abundant leukocytes in rheumatoid joints [52]. A crucial role of LTB₄ in arthritis induction and severity has been revealed in a mouse serum transfer model of inflammatory arthritis [53]. Importantly, the inflammatory responses are reduced in mice with 5-LOX and leukotriene A₄ hydrolase enzyme deficiency [54]. In addition, another lipoxygenase type, namely 15-lipoxygenase, is also involved in the pathogenesis of rheumatoid arthritis via the NF- κ B pathway. It has been described that the 15-lipoxygenase metabolite, 15-(*S*)-HETE increases the I κ B α degradation and the nuclear translocation of NF- κ B subunit [48]. It has been observed that the NF- κ B pathway is activated in the early stage of joint inflammation and NF- κ B DNA binding activity is increased in rheumatoid arthritis patients [55]. These results indicate NF- κ B activity and LOX activity are also closely linked in rheumatoid arthritis and that inhibition of lipoxygenases could also find a therapeutic application in this field.

5.4. Inflammatory Bowel Disease

The role of leukotrienes in inflammatory bowel disease (IBD) has been explored. A colonic biopsy test from patients with IBD showed 3-7 fold enhancement of 5-lipoxygenase, FLAP and LTA₄ hydrolase expression in the colonic mucosa and the rectal dialysates, which form the cellular basis for LTB₄ synthesis [56]. More recently, Cys-leukotiene E₄ (LTE₄) was considered as a biomarker for IBD since the urinary excretion of LTE₄ was significantly increased in patients with IBD [57]. All these data together suggest that inhibition of lipoxygenase activity and leukotriene bio-synthesis can be a valuable approach for treatment of such inflammatory diseases.

5.5. Lipoxygenase in Cancer

Lipoxygenases and their catalysis products are associated with carcinogenic processes such as tumor cell proliferation, differentiation, and apoptosis [58]. Several lines of evidence have proven the crucial role of lipoxygenases in cancer. In human prostate cancer cells, the overexpression of platelet 12-lipoxygenase (p12-LOX) has been observed, which is a trigger for angiogenesis and tumor growth [59]. The increased expression of the 5-LOX enzyme and the LTB₄ receptors were observed in pancreatic cancer. In addition, 5-LOX expression levels were suggested as indicator for early neoplastic lesions [60]. Leukotriene LTB₄ is a potential stimulator for cancer cell growth and also plays a role in the formation of ROS in response to hypoxia [60,61]. It has also been shown that the 5-LOX metabolite LTB₄ is capable of activating the transcription factor NF- κ B in cancer cells, which suggest a tumor promoting role via this route [49]. The roles of 15-LOX-1 metabolites are reported in

the development of breast cancer by promoting the invasion of tumor cells into the lymphatic vessels and the formation of lymph node metastasis [62]. In colon cancer cells it has been shown that 15-LOX-1 expression suppresses the metastatic phenotype of these cells [63] and this enzyme is linked to increased NF- κ B transcriptional activity [46]. Contrary to a tumor promoter role of 15-LOX-1 a tumor suppressor role of 15-LOX-2 has been described in prostate cancer [18,64]. For 15-LOX-2, however, no connection with NF- κ B signaling has been described so far. These studies indicate that the lipoxygenase expression is associated with the development of cancer. For 5-LOX and 15-LOX-1 the activity is linked to NF- κ B activity, whereas such a connection has not been described for the other lipoxygenases. Taking all this evidence together, lipoxygenases are an emerging group of cancer targets.

6. Biosynthesis of Lipoxins: Termination of Inflammatory Responses

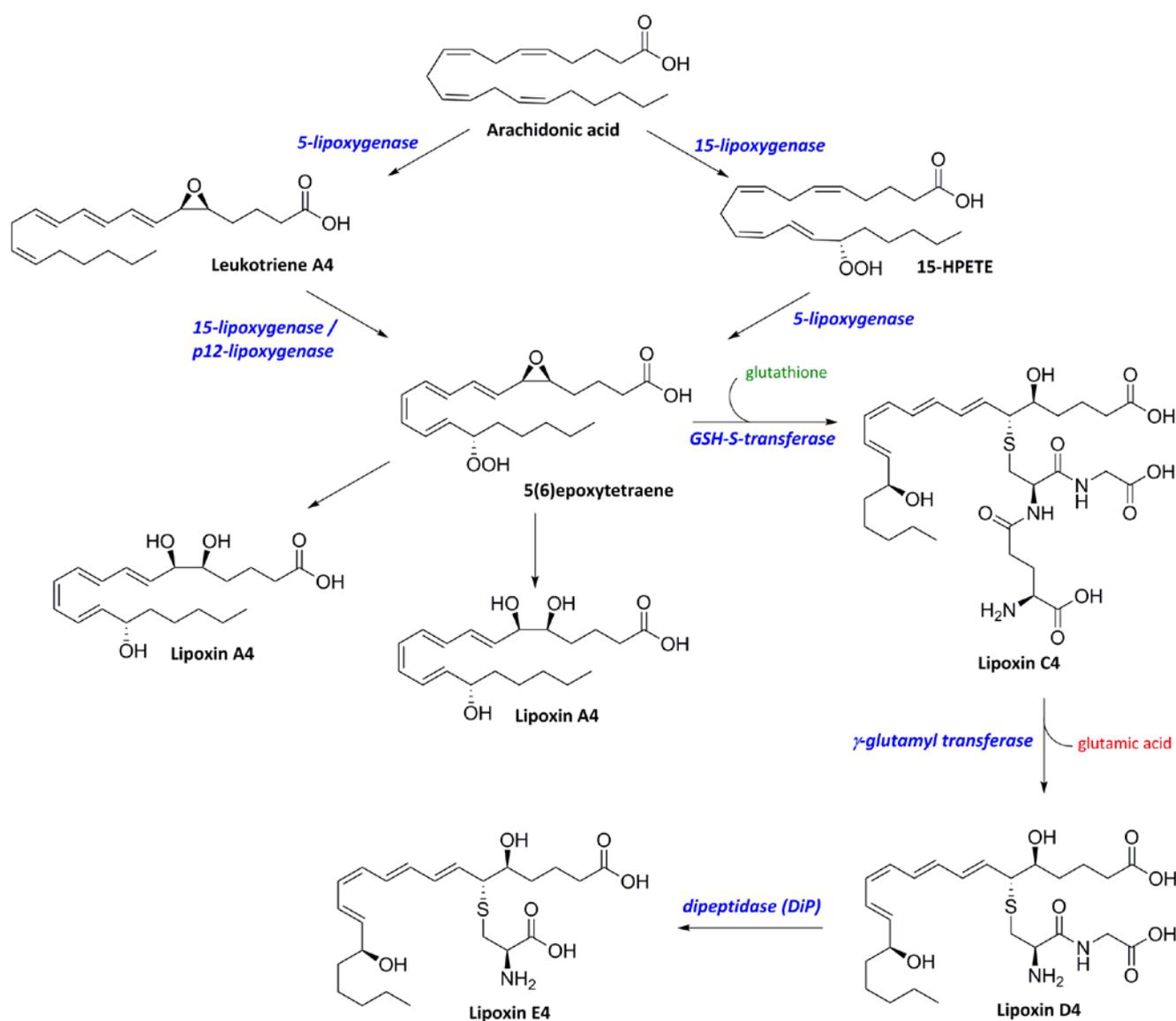
Within the eicosanoid cascade, lipoxins that are formed by lipoxygenases have potential as counter-regulator to resolve inflammation and to restore cellular homeostasis. Lipoxins (LXs) are generated from arachidonic acid through two lipoxygenase-based synthesis routes. The first route involves the formation of LTA₄ by 5-LOX and the conversion of LTA₄ to the intermediate 5(6)-epoxytetraene, which is subsequently converted into LXA₄ and LXB₄. The second route for LXs formation is initiated by 15-LOX activity to oxidize arachidonic acid to 15-HPETE then followed by 5-LOX activity, which convert 15-HPETE to 5(6)-epoxytetraene [65]. Both routes, which are involving 5-LOX activity in the lipoxin production, show that 5-LOX activity is important, not only in the formation of pro-inflammatory mediators, but also in the formation of anti-inflammatory mediators. Moreover, like the leukotrienes, an addition of glutathione (GSH) by GSH-S-transferase activity generates cysteinyl lipoxin LXC₄. LXD₄ and LXE₄ are generated in a similar manner as in the leukotriene biosynthesis pathways (Scheme 2).

Only a few explorations on LXC₄, LXD₄, and LXE₄ have been done and their biological roles have not been investigated in detail. However, it has been reported that LXC₄, LXD₄, and LXE₄ are selectively generated by eosinophils and not by neutrophils and platelets [65]. LXA₄ and LXB₄, with LXA₄ being the most studied, are emerging as mediators to stop the inflammatory responses and to switch the cells to normal homeostasis [66]. LXA₄ and LXB₄ actions in cells and tissues are mediated through their interactions with lipoxin receptors. The lipoxin A receptor (ALXR) transmits stop signals to reduce the pro-inflammatory signals to terminate neutrophil migration. Furthermore, it stimulates the activation of monocytes and macrophages, and inhibits the leukotriene B₄ formation [67]. In addition, LXA₄ can also act as a partial agonist for the LTD₄ receptor by blocking the LTD₄ binding [65]. LXA₄ stimulated-ALXR is able to block the NF- κ B-mediated gene expression and inhibits the degradation of I κ B α [66,68].

Lipoxins production, which is related to the activity of 5-, p12-, and 15-LOXs, has been proven to be important and the alteration of the enzyme activity determines the levels of lipoxin [69,70]. Up-regulation of arachidonate 15-lipoxygenase genes has been reported in the gene profiling of glucocorticoid-treated nasal polyps [71], which is also an indication of 15-HPETE production during the termination of inflammatory process. Another study on the blood polymorphonuclear cells (PMN) from asthmatic patients shows an increase of lipoxin production together with the activation of 5-lipoxygenase [72]. In addition, aspirin, a non-steroidal anti-inflammatory drug which inhibits the

activity of pro-inflammatory eicosanoids produced by cyclooxygenase (COX), triggers the biosynthesis of LXA4 and the 15-epimer of LXA4 accompanied by the increase of brain 5-LOX activity in rat infused with lipopolysaccharide (LPS) [73]. Taking all these findings together, these studies indicate that the increase of 5-LOX activity does not solely contribute to the production of leukotrienes but also to the increase of lipoxin levels. Since the 5-LOX activity is important for both initiation and termination of inflammation, the modulation of this enzyme is crucial for inflammation therapy. Furthermore, its dual functions in the inflammatory processes make 5-LOX an interesting enzyme for further investigation of both inhibitors and activators.

Scheme 2. Two lipoxygenase-based synthesis routes of lipoxins (LXs).

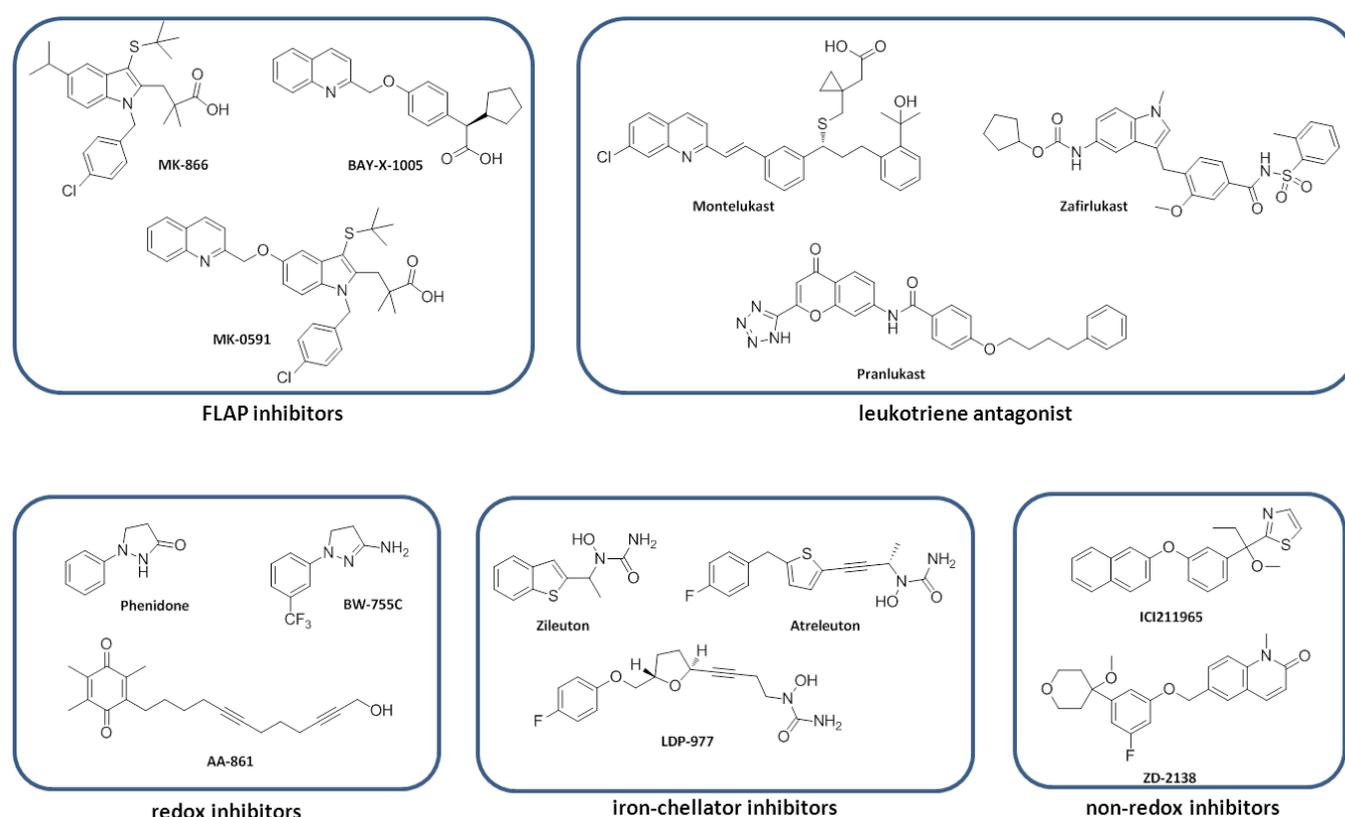


7. Lipoxygenase Inhibitors

Considering the potent pro-inflammatory properties of lipoxygenases and their products, the modulation of the lipoxygenase pathways using small molecule inhibitors should provide new therapeutic approaches for numerous inflammatory diseases and cancer. Various approaches have been

developed to inhibit lipoxygenases. Several synthetic small molecules as well as isolated natural compounds have been tested for the inhibition of lipoxygenases (Figure 2). Recently, it was reported that Δ^9 -tetrahydrocannabinol (Δ^9 -THC), which is an active component extracted from cannabis, shows an inhibition of 15-lipoxygenase with an IC_{50} of 2.42 μ M [74]. Nordihydroguaiaretic acid (NDGA), which is a well-known antioxidant, inhibits platelet 12-lipoxygenase and 15-lipoxygenase [75]. Another compound with iron binding properties; 4-(2-oxopentadeca-4-yne)phenyl propanoic acid (OPP) (Figure 2), shows a mixed type inhibition towards leukocyte 12-lipoxygenase with K_i and K_i' values respectively are 0.2 μ M and 4.5 μ M [76]. The natural product curcumin, which is found in turmeric, is a modulator of arachidonic acid metabolism through the 5-LOX pathway [77].

Figure 2. Lipoxygenase inhibitors.



The role of 5-lipoxygenase in inflammation has been intensively investigated. The fact that the mechanism of 5-LOX activation is more complex compared to the others lipoxygenases, opens opportunities for alternative strategies of inhibition. Inhibitors for leukotriene biosynthesis via 5-lipoxygenase can be divided into FLAP inhibitors, redox inhibitors, non-redox inhibitors, and iron-chelator inhibitors (Figure 2).

7.1. FLAP Inhibitors

Compound MK-866, which was introduced by Gillard *et al.*, is a potent leukotriene biosynthesis inhibitor [78]. This compound selectively inhibits FLAP without affecting 5-LOX, or phospholipase in the leukotriene biosynthesis pathway [78,79]. MK-866 was shown to be safe for consumption and has an effect on the early and late stages of asthmatic responses to allergens [80]. The other compounds

that belong to the FLAP inhibitor class are MK-0591 and Bay-X-1005 (Figure 2). These compounds show a potent leukotriene inhibition in the nanomolar range [81,82]. However, the presence of arachidonic acid and other *cis*-unsaturated fatty acids in blood can compete with those inhibitors for FLAP binding, causing a low inhibitors efficacy in whole blood assay [83]. This results in a 50–200 fold reduction in potency in whole blood assays in comparison with assays in isolated leukocytes [81,82]. This reduced efficacy for FLAP inhibition in excess of arachidonic acid indicates that inhibition of FLAP in the leukotriene biosynthesis pathway might be less effective [84].

7.2. Redox Inhibitors

Redox inhibitors basically act as antioxidants for the oxidation reaction performed by lipoxygenases. The redox inhibitors phenidone, BW755C, and AA-861 are well known as reducing agents (Figure 2) [85,86]. Structure activity relationships for this class of inhibitors are relatively difficult to describe. Nevertheless, it has been recognized that, apart from the redox potency [87], lipophilicity is also important [86]. Recently, a new redox inhibitor for 5-LOX has been reported, which is a trimer or tetramer of caffeoyl clusters (Figure 2), with IC₅₀ values of 0.79 μM and 0.66 μM, respectively [88]. Furthermore, redox inhibitors have a low selectivity for 5-LOX inhibition compared to COXs inhibition [85]. Although they display a high potency to inhibit leukotriene biosynthesis, an interference with other biological redox processes has been reported. The formation of methaemoglobin is one of the problems that were reported upon application of redox inhibitors [89].

7.3. Iron-Chelator Inhibitors

In general a non-heme iron atom in lipoxygenases coordinates with amino acid residues and a water molecule forming an octahedral complex [90]. The coordinated water molecule in the active site is stabilized by a hydrogen bond with the carboxylate of an Ile residue. The iron atom in the 12-lipoxygenase active site is more ordered in comparison to 5- or 15-lipoxygenase. The water molecule in 5-lipoxygenase still coordinates with the iron atom but is slightly off the position to form an octahedral complex, while in contrast no water molecule is coordinated with the iron atom in the 15-lipoxygenase active site. Besides coordinating with a water molecule, in 5-lipoxygenase the iron atom coordinates with three His residues, and one Asn, whereas in 12- and 15-lipoxygenases four His residues with one Ile are coordinated with the iron [91]. The crystal structure of the enzymes with their iron complex provides an understanding about the regio- and stereoselectivity of the catalytic reaction, which is important for the development of inhibitors of the iron-chelator class.

Inhibition of 5-LOX can be achieved by replacing one of the iron ligands with a small molecule ligand to create a complex. Molecules with iron-chelating functionalities such as hydroxamic acid or N-hydroxyurea are potent inhibitors for 5-LOX (Figure 2) [92]. Zileuton is one of the 5-LOX iron-chelator inhibitors that is already on the market for the treatment of asthma. In a number of clinical trials, zileuton has been shown to improve airway function and reduce the asthmatic symptoms as well as the inflammation in the respiratory system. Despite its effectiveness, zileuton is not the first choice therapy due to its side effect such as nausea and idiosyncratic effects on the liver [93]. Further development of this class of inhibitors led to the identification of atreleuton, which inhibits LTB₄ and cys-LTE₄ production and has a potency that is about 5-fold enhanced in comparison to zileuton [94].

Atreleuton, which has entered clinical trials for atherosclerosis and cardiovascular diseases, is one of the leading 5-LO inhibitors in clinical development [95]. Another N-hydroxyurea derivative, CMI-977 (LDP-977) [96] showed potency as a new drug for asthma by suppressing 5-LOX activity in blood and also by inhibition of anti-IgE-induced contractions of the airway tissue [97,98]. These studies suggest that the development of iron-chelator inhibitors for lipoxygenases could be an interesting concept for further exploration.

7.4. Non-Redox Inhibitors

Non-redox inhibitors do not interfere with the oxidation reaction of lipoxygenases or have apparent iron-binding properties. Inhibition of the enzyme activity can take effect by competitive binding to the active site or by binding to an allosteric binding site that regulates the activity of the enzyme. The (methoxyalkyl)thiazole (ICI211965) (Figure 2) selectively inhibits 5-LOX activity, which reduces LTC₄ and LTB₄ synthesis in animal and human blood samples [99]. Unfortunately, steady-state kinetic analyses of this compound for 5-LOX have not been successfully performed and therefore it has not been possible to determine whether the inhibition is competitive with the substrate arachidonic acid or not [100]. Although, ICI211965 is a highly potent 5-LOX inhibitor from a novel structural class, it has been reported to have a low oral potency. The methoxytetrahydropyran compound ZD-2138 (Figure 2) shows an improvement of the oral potency compared to ICI211965 for the treatments of arthritis and asthma [101]. Furthermore, ZD-2138 inhibits antigen-induced leukotriene release at the micromolar concentration range [102]. However, the results from a clinical trial for its application as an anti-arthritis agent were disappointing and therefore research on this molecule was discontinued [103]. Interestingly, recently a compound class containing a salicylate core structure has been identified to inhibit or activate lipoxygenases presumably via an allosteric mechanism [104–106].

7.5. Leukotriene Antagonist

Recently, leukotriene receptor antagonists have appeared as a class of compounds that have superior properties for suppression of leukotriene biosynthesis. Pranlukast, zafirlukast and montelukast (Figure 2), three of the leukotriene receptor antagonists, have also shown good efficacy in the treatment of asthma [107,108]. These drugs block the binding of leukotriene D₄ and also LTC₄ and LTE₄ to the CysLTR1 in the lungs and bronchial tubes, which resulted in the reduction of airway constriction, and mucus accumulation in the lungs and airways. Interestingly, it has also been reported that montelukast possess secondary anti-inflammatory properties to inhibit the activity of 5-LOX and HATs [109]. Montelukast suppresses the leukotriene biosynthesis by selective inhibition of 5-LOX and gives no effect on the other enzymes involved in the leukotrienes biosynthesis pathway such as LTA₄ hydrolase and LTC₄ synthase [110]. Moreover, montelukast alters the activity of the NF- κ B transcription factor p65-associated HAT activity and reduces the TNF- α -stimulated IL-8 expression [111]. However, it has been reported that the usage of this leukotriene antagonist produces neuropsychiatric side effects which is a major concern for its safety.

8. Conclusions

Lipoxygenases are an intensively studied class of enzymes and an increasing number of functions in various diseases are being found for their lipid metabolites. Although lipoxygenases have been recognized classically as drug targets for treatment of inflammation more recently anti-inflammatory effects have been discovered for the lipoxins, which are also lipoxygenase metabolites. Interestingly, also connections between lipoxygenases and diseases such as cancer and atherosclerosis have been identified. There is a limited amount of data on the connections between lipoxygenase metabolites and signal transduction pathways such as the NF- κ B pathway. Taken this all together, literature demonstrates a key regulatory role for lipoxygenases and their metabolites in many physiological processes, which positions them at the center of many disease models. Nevertheless, their versatile roles and their connection to signaling cascades indicates that it can be difficult to redirect specific physiological processes using small molecule inhibitors of lipoxygenases.

A variety of compounds have been introduced to modulate lipoxygenase enzyme activity and ultimately to provide new drugs for inflammation. Despite of their high potency to inhibit leukotriene production, their limitation in efficacy in specific disease models are still a concern that needs to be resolved. In view of that the development of lipoxygenase modulator with an improved potency and selectivity for specific therapeutic applications as well as novel methods to study the functional consequences of these oxidative enzymes remains an important challenge.

Acknowledgments

We acknowledge the COST action “Biomimetic radical chemistry” CM1201. This work was supported by a VIDI grant (016.122.302) for FJD from the Netherlands Organization for Scientific Research (NWO).

Author Contributions

Rosalina Wisastra and Frank J. Dekker wrote the text.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Newshean, K.; Aziz, T.B.; Kryston, N.F.; Ferguson, N.F.; Georgakilas, A. The interplay between inflammation and oxidative stress and carcinogenesis. *Curr. Mol. Med.* **2012**, *12*, 672–680.
2. Haining, J.L.; Axelrod, B. Induction period in the lipoxygenase-catalyzed oxidation of linoleic acid and its abolition by substrate peroxide. *J. Biol. Chem.* **1958**, *232*, 193–202.
3. Solomon, E.I.; Zhou, J.; Neese, F.; Pavel, E.G. New insights from spectroscopy into the structure/function relationships of lipoxygenases. *Chem. Biol.* **1997**, *4*, 795–808.
4. Brash, A.R. Lipoxygenases: Occurrence, Functions, Catalysis, and Acquisition of Substrate. *J. Biol. Chem.* **1999**, *274*, 23679–23682.

5. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410.
6. Sigal, E. The molecular biology of mammalian arachidonic acid metabolism. *Am. J. Physiol.* **1991**, *260*, L13–L28.
7. Chen, X.; Funk, C.D. The N-terminal “ β -Barrel” Domain of 5-Lipoxygenase is Essential for Nuclear Membrane Translocation. *J. Biol. Chem.* **2001**, *276*, 811–818.
8. Samuelsson, B. Leukotrienes: Mediators of immediate hypersensitivity reactions and inflammation. *Science* **1983**, *220*, 568–575.
9. Iversen, L.; Fogh, K.; Bojesen, G.; Kragballe, K. Linoleic acid and dihomogammalinolenic acid inhibit leukotriene B₄ formation and stimulate the formation of their 15-lipoxygenase products by human neutrophils *in vitro*. Evidence of formation of antiinflammatory compounds. *Agents Actions* **1991**, *33*, 286–291.
10. Nathaniel, D.J.; Evans, J.F.; Leblanc, Y.; Léveillé, C.; Fitzsimmons, B.J.; Ford-Hutchinson, A.W. Leukotriene A₅ is a substrate and an inhibitor of rat and human neutrophil LTA₄ hydrolase. *Biochem. Biophys. Res. Commun.* **1985**, *131*, 827–835.
11. Brüne, B.; Ullrich, V. 12-hydroperoxyeicosatetraenoic acid inhibits main platelet functions by activation of soluble guanylate cyclase. *Mol. Pharmacol.* **1991**, *39*, 671–678.
12. Yeung, J.; Holinstat, M. 12-Lipoxygenase: A Potential Target for Novel Anti-Platelet Therapeutics. *Cardiovasc. Hematol. Agents Med. Chem.* **2011**, *9*, 154–164.
13. Ikei, K.N.; Yeung, J.; Apopa, P.L.; Ceja, J.; Vesci, J.; Holman, T.R.; Holinstat, M. Investigations of human platelet-type 12-lipoxygenase: Role of lipoxygenase products in platelet activation. *J. Lipid Res.* **2012**, *53*, 2546–2559.
14. Epp, N.; Fürstenberger, G.; Müller, K.; de Juanes, S.; Leitges, M.; Hausser, I.; Thieme, F.; Liebisch, G.; Schmitz, G.; Krieg, P. 12R-lipoxygenase deficiency disrupts epidermal barrier function. *J. Cell Biol.* **2007**, *177*, 173–182.
15. Profita, M.; Sala, A.; Riccobono, L.; Pace, E.; Paternò, A.; Zarini, S.; Siena, L.; Mirabella, A.; Bonsignore, G.; Vignola, A.M. 15(S)-HETE modulates LTB₄ production and neutrophil chemotaxis in chronic bronchitis. *Am. J. Physiol. Cell Physiol.* **2000**, *279*, C1249–C1258.
16. Sordillo, L.M.; Weaver, J.A.; Cao, Y.; Corl, C.; Sylte, M.J.; Mullarky, I.K. Enhanced 15-HPETE production during oxidant stress induces apoptosis of endothelial cells. *Prostaglandins Other Lipid Mediat.* **2005**, *76*, 19–34.
17. Hsi, L.C.; Wilson, L.C.; Eling, T.E. Opposing Effects of 15-Lipoxygenase-1 and -2 Metabolites on MAPK Signaling in Prostate: Alteration in peroxisome proliferator-activated receptor γ . *J. Biol. Chem.* **2002**, *277*, 40549–40556.
18. Bhatia, B.; Maldonado, C.J.; Tang, S.; Chandra, D.; Klein, R.D.; Chopra, D.; Shappell, S.B.; Yang, P.; Newman, R.A.; Tang, D.G. Subcellular localization and tumor-suppressive functions of 15-lipoxygenase 2 (15-LOX2) and its splice variants. *J. Biol. Chem.* **2003**, *278*, 25091–25100.
19. Tang, S.; Bhatia, B.; Maldonado, C.J.; Yang, P.; Newman, R.A.; Liu, J.; Chandra, D.; Traag, J.; Klein, R.D.; Fischer, S.M.; *et al.* Evidence That Arachidonate 15-Lipoxygenase 2 Is a Negative Cell Cycle Regulator in Normal Prostate Epithelial Cells. *J. Biol. Chem.* **2002**, *277*, 16189–16201.

20. Rådmark, O. The Molecular Biology and Regulation of 5-Lipoxygenase. *Am. J. Respir. Crit. Care Med.* **2000**, *161*, S11–S15.
21. Noguchi, M.; Miyano, M.; Matsumoto, T. Physicochemical characterization of ATP binding to human 5-lipoxygenase. *Lipids* **1996**, *31*, 367–371.
22. Peters-Golden, M.; Brock, T.G. 5-Lipoxygenase and FLAP. *Prostaglandins Leukot. Essent. Fat. Acids* **2003**, *69*, 99–109.
23. Hunter, J.A.; Finkbeiner, W.E.; Nadel, J.A.; Goetzl, E.J.; Holtzman, M.J. Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 4633–4637.
24. Nadel, J.A.; Conrad, D.J.; Ueki, I.F.; Schuster, A.; Sigal, E. Immunocytochemical localization of arachidonate 15-lipoxygenase in erythrocytes, leukocytes, and airway cells. *J. Clin. Investig.* **1991**, *87*, 1139–1145.
25. Brash, A.R.; Boeglin, W.E.; Chang, M.S. Discovery of a second 15S-lipoxygenase in humans. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6148–6152.
26. Brown, C.D.; Kilty, I.; Yeadon, M.; Jenkinson, S. Regulation of 15-lipoxygenase isozymes and mucin secretion by cytokines in cultured normal human bronchial epithelial cells. *Inflamm. Res.* **2001**, *50*, 321–326.
27. Chanez, P.; Bonnans, C.; Chavis, C.; Vachier, I. 15-Lipoxygenase. *Am. J. Respir. Cell Mol. Biol.* **2002**, *27*, 655–658.
28. Haeggstrom, J.Z.; Wetterholm, A. Enzymes and receptors in the leukotriene cascade. *Cell Mol. Life Sci.* **2002**, *59*, 742–753.
29. Okamoto, F.; Saeki, K.; Sumimoto, H.; Yamasaki, S.; Yokomizo, T. Leukotriene B4 Augments and Restores FcγRs-dependent Phagocytosis in Macrophages. *J. Biol. Chem.* **2010**, *285*, 41113–41121.
30. Lee, K.S.; Kim, S.R.; Park, H.S.; Park, S.J.; Min, K.H.; Lee, K.Y.; Jin, S.M.; Lee, Y.C. Cysteinyl leukotriene upregulates IL-11 expression in allergic airway disease of mice. *J. Allergy Clin. Immunol.* **2007**, *119*, 141–149.
31. Thompson, C.; Cloutier, A.; Bossé, Y.; Poisson, C.; Larivée, P.; McDonald, P.P.; Stankova, J.; Rola-Pleszczynski, M. Signaling by the Cysteinyl-Leukotriene Receptor 2: Involvement in chemokine gene transcription. *J. Biol. Chem.* **2008**, *283*, 1974–1984.
32. Kawano, T.; Matsuse, H.; Kondo, Y.; Machida, I.; Saeki, S.; Tomari, S.; Mitsuta, K.; Obase, Y.; Fukushima, C.; Shimoda, T.; *et al.* Cysteinyl leukotrienes induce nuclear factor kb activation and rantes production in a murine model of asthma. *J. Allergy Clin. Immunol.* **2003**, *112*, 369–374.
33. Hoffmann, A.; Natoli, G.; Ghosh, G. Transcriptional regulation via the NF-kappaB signaling module. *Oncogene* **2006**, *25*, 6706–6716.
34. Lawrence, T. The Nuclear Factor NF-κB Pathway in Inflammation. *Cold Spring Harb. Perspect. Biol.* **2009**, doi:10.1101/cshperspect.a001651.
35. Zheng, C.; Yin, Q.; Wu, H. Structural studies of NF-kappaB signaling. *Cell Res.* **2011**, *21*, 183–195.
36. Karin, M.; Ben-Neriah, Y. Phosphorylation Meets Ubiquitination: The Control of NF-κB Activity. *Annu. Rev. Immunol.* **2000**, *18*, 621–663.

37. Holgate, S.T. Cytokine and anti-cytokine therapy for the treatment of asthma and allergic disease. *Cytokine* **2004**, *28*, 152–157.
38. Williams, R.O.; Paleolog, E.; Feldmann, M. Cytokine inhibitors in rheumatoid arthritis and other autoimmune diseases. *Curr. Opin. Pharmacol.* **2007**, *7*, 412–417.
39. Chung, K.F. Cytokines as targets in chronic obstructive pulmonary disease. *Curr. Drug Targets* **2006**, *7*, 675–681.
40. Perkins, N.D.; Gilmore, T.D. Good cop, bad cop: The different faces of NF-kappaB. *Cell Death Differ.* **2006**, *13*, 759–772.
41. Haller, D.; Russo, M.P.; Sartor, R.B.; Jobin, C. IKK β and Phosphatidylinositol 3-Kinase/Akt Participate in Non-pathogenic Gram-negative Enteric Bacteria-induced RelA Phosphorylation and NF- κ B Activation in Both Primary and Intestinal Epithelial Cell Lines. *J. Biol. Chem.* **2002**, *277*, 38168–38178.
42. Madrid, L.V.; Mayo, M.W.; Reuther, J.Y.; Baldwin, A.S. Akt Stimulates the Transactivation Potential of the RelA/p65 Subunit of NF- κ B through Utilization of the I κ B Kinase and Activation of the Mitogen-activated Protein Kinase p38. *J. Biol. Chem.* **2001**, *276*, 18934–18940.
43. Perkins, N.D. Post-translational modifications regulating the activity and function of the nuclear factor kappa B pathway. *Oncogene* **2006**, *25*, 6717–6730.
44. Bonizzi, G.; Karin, M. The two NF- κ B activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* **2004**, *25*, 280–288.
45. Liu, C.; Xu, D.; Liu, L.; Schain, F.; Brunnström, A.; Björkholm, M.; Claesson, H.E.; Sjöberg, J. 15-Lipoxygenase-1 induces expression and release of chemokines in cultured human lung epithelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2009**, *297*, L196–L203.
46. Ylä-Herttuala, S.; Rosenfeld, M.E.; Parthasarathy, S.; Sigal, E.; Särkioja, T.; Witztum, J.L.; Steinberg, D. Gene expression in macrophage-rich human atherosclerotic lesions. 15-lipoxygenase and acetyl low density lipoprotein receptor messenger RNA colocalize with oxidation specific lipid-protein adducts. *J. Clin. Investig.* **1991**, *87*, 1146–1152.
47. Li, J.; Rao, J.; Liu, Y.; Cao, Y.; Zhang, Y.; Zhang, Q.; Zhu, D. 15-Lipoxygenase promotes chronic hypoxia-induced pulmonary artery inflammation via positive interaction with nuclear factor- κ B. *Arterioscler. Thromb. Vasc. Biol.* **2013**, *33*, 971–979.
48. Wu, M.; Lin, T.; Chiu, Y.; Liou, H.; Yang, R.; Fu, W. Involvement of 15-lipoxygenase in the inflammatory arthritis. *J. Cell. Biochem.* **2012**, *113*, 2279–2289.
49. Zhao, Y.; Wang, W.; Wang, Q.; Zhang, X.; Ye, L. Lipid metabolism enzyme 5-LOX and its metabolite LTB₄ are capable of activating transcription factor NF- κ B in hepatoma cells. *Biochem. Biophys. Res. Commun.* **2012**, *418*, 647–651.
50. Hui, A.Y.; McCarty, W.J.; Masuda, K.; Firestein, G.S.; Sah, R.L. A systems biology approach to synovial joint lubrication in health, injury, and disease. *Wiley Interdiscip. Rev. Syst. Biol. Med.* **2012**, *4*, 15–37.
51. Serhan, C.N. Novel Lipid Mediators and Resolution Mechanisms in Acute Inflammation: To Resolve or Not? *Am. J. Pathol.* **2010**, *177*, 1576–1591.
52. Dahlen, S.E.; Bjork, J.; Hedqvist, P.; Arfors, K.E.; Hammarstrom, S.; Lindgren, J.A.; Samuelsson, B. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary

- venules: *In vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3887–3891.
53. Chen, M.; Lam, B.K.; Kanaoka, Y.; Nigrovic, P.A.; Audoly, L.P.; Austen, K.F.; Lee, D.M. Neutrophil-derived leukotriene B₄ is required for inflammatory arthritis. *J. Exp. Med.* **2006**, *203*, 837–842.
54. Gheorghe, K.R.; Korotkova, M.; Catrina, A.I.; Backman, L.; Klint, E.; Claesson, H.; Radmark, O.; Jakobsson, P. Expression of 5-lipoxygenase and 15-lipoxygenase in rheumatoid arthritis synovium and effects of intraarticular glucocorticoids. *Arthritis Res. Ther.* **2009**, *11*, R83.
55. Asahara, H.; Asanuma, M.; Ogawa, N.; Nishibayashi, S.; Inoue, H. High DNA-binding activity of transcription factor NF-kappa B in synovial membranes of patients with rheumatoid arthritis. *Biochem. Mol. Biol. Int.* **1995**, *37*, 827–832.
56. Jupp, J.; Hillier, K.; Elliott, D.H.; Fine, D.R.; Bateman, A.C.; Johnson, P.A.; Cazaly, A.M.; Penrose, J.F.; Sampson, A.P. Colonic expression of leukotriene-pathway enzymes in inflammatory bowel diseases. *Inflamm. Bowel Dis.* **2007**, *13*, 537–546.
57. Stanke-Labesque, F.; Pofelski, J.; Moreau-Gaudry, A.; Bessard, G.; Bonaz, B. Urinary leukotriene E₄ excretion: A biomarker of inflammatory bowel disease activity. *Inflamm. Bowel Dis.* **2008**, *14*, 769–774.
58. Wang, D.; Dubois, R.N. Eicosanoids and cancer. *Nat. Rev. Cancer* **2010**, *10*, 181–193.
59. Pidgeon, G.P.; Tang, K.; Cai, Y.L.; Piasentin, E.; Honn, K.V. Overexpression of Platelet-type 12-Lipoxygenase Promotes Tumor Cell Survival by Enhancing $\alpha v \beta 3$ and $\alpha v \beta 5$ Integrin Expression. *Cancer Res.* **2003**, *63*, 4258–4267.
60. Hennig, R.; Grippo, P.; Ding, X.; Rao, S.M.; Buchler, M.W.; Friess, H.; Talamonti, M.S.; Bell, R.H.; Adrian, T.E. 5-Lipoxygenase, a Marker for Early Pancreatic Intraepithelial Neoplastic Lesions. *Cancer Res.* **2005**, *65*, 6011–6016.
61. Steiner, D.R.S.; Gonzalez, N.C.; Wood, J.G. Leukotriene B₄ promotes reactive oxidant generation and leukocyte adherence during acute hypoxia. *J. Appl. Physiol.* **2001**, *91*, 1160–1167.
62. Kerjaschki, D.; Bago-Horvath, Z.; Rudas, M.; Sexl, V.; Schneckeleithner, C.; Wolbank, S.; Bartel, G.; Krieger, S.; Kalt, R.; Hantusch, B.; *et al.* Lipoxygenase mediates invasion of intrametastatic lymphatic vessels and propagates lymph node metastasis of human mammary carcinoma xenografts in mouse. *J. Clin. Investig.* **2011**, *121*, 2000–2012.
63. Wu, Y.; Mao, F.; Zuo, X.; Moussalli, M.J.; Elias, E.; Xu, W.; Shureiqi, I. 15-LOX-1 suppression of hypoxia-induced metastatic phenotype and HIF-1 α expression in human colon cancer cells. *Cancer Med.* **2014**, *3*, 472–484.
64. Suraneni, M.V.; Schneider-Broussard, R.; Moore, J.R.; Davis, T.C.; Maldonado, C.J.; Li, H.; Newman, R.A.; Kusewitt, D.; Hu, J.; Yang, P.; *et al.* Transgenic expression of 15-lipoxygenase 2 (15-LOX2) in mouse prostate leads to hyperplasia and cell senescence. *Oncogene* **2010**, *29*, 4261–4275.

65. Serhan, C.N. Lipoxin biosynthesis and its impact in inflammatory and vascular events. *Biochim. Biophys. Acta* **1994**, *1212*, 1–25.
66. Chiang, N.; Arita, M.; Serhan, C.N. Anti-inflammatory circuitry: Lipoxin, aspirin-triggered lipoxins and their receptor ALX. *Prostaglandins Leukot. Essent. Fat. Acids* **2005**, *73*, 163–177.
67. Serhan, C.N. Resolving inflammation: Dual anti-inflammatory and pro-resolution lipid mediators. *Nat. Rev. Immunol.* **2008**, *8*, 349–361.
68. Fierro, I.M.; Colgan, S.P.; Bernasconi, G.; Petasis, N.A.; Clish, C.B.; Arita, M.; Serhan, C.N. Lipoxin A4 and aspirin-triggered 15-epi-lipoxin a4 inhibit human neutrophil migration: Comparisons between synthetic 15 epimers in chemotaxis and transmigration with microvessel endothelial cells and epithelial cells. *J. Immunol.* **2003**, *170*, 2688–2694.
69. Clària, J.; Titos, E.; Jiménez, W.; Ros, J.; Ginès, P.; Arroyo, V.; Rivera, F.; Rodés, J. Altered biosynthesis of leukotrienes and lipoxins and host defense disorders in patients with cirrhosis and ascites. *Gastroenterology* **1998**, *115*, 147–156.
70. Karp, C.L. Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. *Nat. Immunol.* **2004**, *5*, 388–392.
71. Benson, M.; Carlsson, L.; Adner, M.; Jernås, M.; Rudemo, M.; Sjögren, A.; Svensson, P.A.; Uddman, R.; Cardell, L.O. Gene profiling reveals increased expression of uteroglobin and other anti-inflammatory genes in glucocorticoid-treated nasal polyps. *J. Allergy Clin. Immunol.* **2004**, *113*, 1137–1143.
72. Chavis, C.; Vachier, I.; Chanez, P.; Bousquet, J.; Godard, P. 5(S),15(S)-dihydroxyeicosatetraenoic acid and lipoxin generation in human polymorphonuclear cells: Dual specificity of 5-lipoxygenase towards endogenous and exogenous precursors. *J. Exp. Med.* **1996**, *183*, 1633–1643.
73. Basselin, M. Anti-inflammatory effects of chronic aspirin on brain arachidonic acid metabolites. *Neurochem. Res.* **2011**, *36*, 139–145.
74. Takeda, S.; Jiang, R.; Aramaki, H.; Imoto, M.; Toda, A.; Eyanagi, R.; Amamoto, T.; Yamamoto, I.; Watanabe, K. 9-tetrahydrocannabinol and its major metabolite 9-tetrahydrocannabinol-11-oic acid as 15-lipoxygenase inhibitors. *J. Pharm. Sci.* **2011**, *100*, 1206–1211.
75. Whitman, S. Structure-activity relationship studies of nordihydroguaiaretic acid inhibitors toward soybean, 12-human, and 15-human lipoxygenase. *J. Med. Chem.* **2002**, *45*, 2659–2661.
76. Moody, J.S.; Marnett, L.J. Kinetics of Inhibition of Leukocyte 12-Lipoxygenase by the Isoform-Specific Inhibitor 4-(2-Oxapentadeca-4-yne)phenylpropanoic Acid. *Biochemistry* **2002**, *41*, 10297–10303.
77. Hong, J.; Bose, M.; Ju, J.; Ryu, J.; Chen, X.; Sang, S.; Lee, M.; Yang, C.S. Modulation of arachidonic acid metabolism by curcumin and related β -diketone derivatives: Effects on cytosolic phospholipase A2, cyclooxygenases and 5-lipoxygenase. *Carcinogenesis* **2004**, *25*, 1671–1679.
78. Gillard, J.; Ford-Hutchinson, A.W.; Chan, C.; Charleson, S.; Denis, D.; Foster, A.; Fortin, R.; Leger, S.; McFarlane, C.S.; Morton, H. L-663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-t-butylthio-5-isopropylindol-2-yl]-2,2—dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 456–464.

79. Evans, J.F.; Ferguson, A.D.; Mosley, R.T.; Hutchinson, J.H. What's all the FLAP about? 5-lipoxygenase-activating protein inhibitors for inflammatory diseases. *Trends Pharmacol. Sci.* **2008**, *29*, 72–78.
80. Friedman, B.S.; Bel, E.H.; Buntinx, A.; Tanaka, W.; Han, Y.R.; Shingo, S.; Spector, R.; Sterk, P. Oral Leukotriene Inhibitor (MK-886) Blocks Allergen-induced Airway Responses. *Am. Rev. Respir. Dis.* **1993**, *147*, 839–844.
81. Fruchtmann, R.; Mohrs, K.H.; Hatzelmann, A.; Raddatz, S.; Fugmann, B.; Junge, B.; Horstmann, H.; Muller-Peddinghaus, R. *In vitro* pharmacology of BAY X1005, a new inhibitor of leukotriene synthesis. *Agents Actions* **1993**, *38*, 188–195.
82. Mancini, J.A.; Prasit, P.; Coppolino, M.G.; Charleson, P.; Leger, S.; Evans, J.F.; Gillard, J.W.; Vickers, P.J. 5-Lipoxygenase-activating protein is the target of a novel hybrid of two classes of leukotriene biosynthesis inhibitors. *Mol. Pharmacol.* **1992**, *41*, 267–272.
83. Charleson, S.; Evans, J.F.; Leger, S.; Perrier, H.; Prasit, P.; Wang, Z.; Vickers, P.J. Structural requirements for the binding of fatty acids to 5-lipoxygenase-activating protein. *Eur. J. Pharmacol.* **1994**, *267*, 275–280.
84. Werz, O. 5-Lipoxygenase: Cellular Biology and Molecular Pharmacology. *Curr. Drug Targets Inflamm. Allergy* **2002**, *1*, 23–44.
85. McMillan, R.M.; Walker, E.R.H. Designing therapeutically effective 5-lipoxygenase inhibitors. *Trends Pharmacol. Sci.* **1992**, *13*, 323–330.
86. Batt, D.G.; Maynard, G.D.; Petraitis, J.J.; Shaw, J.E.; Galbraith, W.; Harris, R.R. 2-Substituted-1-Naphthols as Potent 5-Lipoxygenase Inhibitors with Topical Antiinflammatory Activity. *J. Med. Chem.* **1990**, *33*, 360–370.
87. Corey, E.J.; Wright, S.W.; Matsuda, S.P.T. Stereochemistry and mechanism of the biosynthesis of leukotriene A₄ from 5(S)-hydroperoxy-6(E),8,11,14(Z)-eicosatetraenoic acid. Evidence for an organoiron intermediate. *J. Am. Chem. Soc.* **1989**, *111*, 1452–1455.
88. Doiron, J.; Boudreau, L.H.; Picot, N.; Villebonet, B.; Surette, M.E.; Touaibia, M. Synthesis and 5-lipoxygenase inhibitory activity of new cinnamoyl and caffeoylclusters. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1118–1121.
89. Ford-Hutchinson, A.; Gresser, M.; Young, R.N. 5-Lipoxygenase. *Annu. Rev. Biochem.* **1994**, *63*, 383–417.
90. Gillmor, S.A.; Villasenor, A.; Fletterick, R.; Sigal, E.; Browner, M.F. The structure of mammalian 15-lipoxygenase reveals similarity to the lipases and the determinants of substrate specificity. *Nat. Struct. Biol.* **1997**, *4*, 1003–1009.
91. Xu, S.; Mueser, T.; Marnett, L.; Funk, M., Jr. Crystal structure of 12-lipoxygenase catalytic-domain-inhibitor complex identifies a substrate-binding channel for catalysis. *Structure* **2012**, *20*, 1490–1497.
92. Connolly, P.J.; Wetter, S.K.; Beers, K.N.; Hamel, S.C.; Chen, R.H.K.; Wachter, M.P.; Ansell, J.; Singer, M.M.; Steber, M.; Ritchie, D.M.; *et al.* N-Hydroxyurea and hydroxamic acid inhibitors of cyclooxygenase and 5-lipoxygenase. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 979–984.

93. Nelson, H.; Kemp, J.; Berger, W.; Corren, J.; Casale, T.; Dube, L.; Walton-Bowen, K.; LaVallee, N.; Stepanians, M. Efficacy of zileuton controlled-release tablets administered twice daily in the treatment of moderate persistent asthma: A 3-month randomized controlled study. *Ann. Allergy Asthma Immunol.* **2007**, *99*, 178–184.
94. Brooks, C.D.; Stewart, A.O.; Basha, A.; Bhatia, P.; Ratajczyk, J.D.; Martin, J.G.; Craig, R.A.; Kolasa, T.; Bouska, J.B.; Lanni, C. (*R*)-(+)-N-[3-[5-[(4-fluorophenyl)methyl]-2-thienyl]-1-methyl-2-propynyl]-N-hydroxyurea (ABT-761), a second-generation 5-lipoxygenase inhibitor. *J. Med. Chem.* **1995**, *38*, 4768–4775.
95. Back, M. Inhibitors of the 5-lipoxygenase pathway in atherosclerosis. *Curr. Pharm. Des.* **2009**, *15*, 3116–3132.
96. Gurjar, M.K.; Murugaiah, A.M.S.; Radhakrishna, P.; Ramana, C.V.; Chorghade, M.S. A novel and simple asymmetric synthesis of CMI-977 (LDP-977): A potent anti-asthmatic drug lead. *Tetrahedron Asymmetry* **2003**, *14*, 1363–1370.
97. Pergola, C.; Werz, O. 5-Lipoxygenase inhibitors: A review of recent developments and patents. *Expert Opin. Ther. Patents* **2010**, *20*, 355–375.
98. Werz, O. Pharmacological intervention with 5-lipoxygenase: New insights and novel compounds. *Expert Opin. Ther. Patents* **2005**, *15*, 505–519.
99. Bird, T.G.C.; Bruneau, P.; Crawley, G.C.; Edwards, M.P.; Foster, S.J.; Girodeau, J.M.; Kingston, J.F.; McMillan, R.M. (Methoxyalkyl)thiazoles: A new series of potent, selective, and orally active 5-lipoxygenase inhibitors displaying high enantioselectivity. *J. Med. Chem.* **1991**, *34*, 2176–2186.
100. Falgoutyret, J.; Hutchinson, J.H.; Riendeau, D. Criteria for the identification of non-redox inhibitors of 5-lipoxygenase. *Biochem. Pharmacol.* **1993**, *45*, 978–981.
101. Crawley, G.C.; Dowell, R.I.; Edwards, P.N.; Foster, S.J.; McMillan, R.M.; Walker, E.R.H.; Waterson, D.; Bird, T.G.; Bruneau, P.; Girodeau, J.M. Methoxytetrahydropyrans. A new series of selective and orally potent 5-lipoxygenase inhibitors. *J. Med. Chem.* **1992**, *35*, 2600–2609.
102. Kusner, E.J.; Buckner, C.K.; Dea, D.M.; DeHaas, C.J.; Marks, R.L.; Krell, R.D. The 5-lipoxygenase inhibitors ZD2138 and ZM230487 are potent and selective inhibitors of several antigen-induced guinea-pig pulmonary responses. *Eur. J. Pharmacol.* **1994**, *257*, 285–292.
103. Young, R.N. Inhibitors of 5-lipoxygenase: A therapeutic potential yet to be fully realized? *Eur. J. Med. Chem.* **1999**, *34*, 671–685.
104. Wisastra, R.; Ghizzoni, M.; Boltjes, A.; Haisma, H.J.; Dekker, F.J. Anacardic acid derived salicylates are inhibitors or activators of lipoxygenases. *Bioorg. Med. Chem.* **2012**, *20*, 5027–5032.
105. Baggelaar, M.P.; Huang, Y.; Feringa, B.L.; Dekker, F.J.; Minnaard, A.J. Catalytic asymmetric total synthesis of (*S*)-(-)-zearenone, a novel lipoxygenase inhibitor. *Bioorg. Med. Chem.* **2013**, *21*, 5271–5274.
106. Wisastra, R.; Kok, P.A.; Eleftheriadis, N.; Baumgartner, M.P.; Camacho, C.J.; Haisma, H.J.; Dekker, F.J. Discovery of a novel activator of 5-lipoxygenase from an anacardic acid derived compound collection. *Bioorg. Med. Chem.* **2013**, *21*, 7763–7778.
107. Sorkness, C.A. The use of 5-lipoxygenase inhibitors and leukotriene receptor antagonists in the treatment of chronic asthma. *Pharmacotherapy* **1997**, *17*, 50S–54S.
108. Renzi, P.M. Antileukotriene agents in asthma: The dart that kills the elephant? *CMAJ* **1999**, *160*, 217–223.

109. Tintinger, G.R.; Feldman, C.; Theron, A.J.; Anderson, R. Montelukast: More than a cysteinyl leukotriene receptor antagonist? *ScientificWorldJournal* **2010**, *10*, 2403–2413.
110. Ramires, R.; Caiaffa, M.F.; Tursi, A.; Haeggström, J.Z.; Macchia, L. Novel inhibitory effect on 5-lipoxygenase activity by the anti-asthma drug montelukast. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 815–821.
111. Tahan, F. Montelukast inhibits tumour necrosis factor- α -mediated interleukin-8 expression through inhibition of nuclear factor- κ B p65-associated histone acetyltransferase activity. *Clin. Exp. Allergy* **2008**, *38*, 805–811.

© 2014 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).