Inhibition of 5-Lipoxygenase Product Synthesis by Natural Compounds of Plant Origin

Oliver Werz

Abstract

The biosynthesis of leukotrienes (LTs) is initiated by the transformation of free arachidonic acid to LTA_4 by 5-lipoxygenase (5-LO). Subsequent enzymatic conversion of LTA_4 yields LTB_4 and the cysteinyl-LTs C_4, D_4 and E_4. LTs have prominent functions in pathophysiology and are connected to numerous disorders including bronchial asthma, allergic rhinitis, inflammatory bowel and skin diseases, rheumatoid arthritis, cancer, osteoporosis and cardiovascular diseases. Pharmacological and genetic interruption of the 5-LO pathway or blockade of LT receptors, serving as means for intervention with LTs, may be of therapeutic value for certain related disorders. Natural or plant-derived substances were among the first 5-LO inhibitors identified in the early 1980 s. To date, a huge number of diverse plant-derived compounds have been reported to interfere with 5-LO product synthesis. However, many investigations have addressed the efficacy of a given compound solely in cellular test systems and analysis of direct interference with 5-LO has been neglected. In the first part of this review, the biology and molecular pharmacology of the 5-LO pathway is summarized in order to understand its overall regulation and complexity as well as to comprehend the possible points of attack of compounds that eventually lead to inhibition of 5-LO product formation in intact cells. In the second part, natural compounds that interfere with 5-LO product formation are compiled and grouped into structural classes, and the underlying molecular mechanisms and structure-activity relationships are discussed.

Key words

5-Lipoxygenase · arachidonic acid · leukotrienes · leukocytes · inflammation · plants · natural compounds · inhibitors

Abbreviations

AA:	arachidonic acid
CLP:	coactosine-like potein
COX:	cyclooxygenase
GPCR:	G protein-coupled receptor
GPX:	glutathione peroxidase
GSH:	glutathione
FLAP:	5-lipoxygenase-activating protein
H(P)ETE:	hydro(pero)xyeicosatetraenoic acid
iNOS:	inducible nitric oxide synthase
LO:	lipoxygenase
LOOH:	lipid hydroperoxide
LT:	leukotriene
MAPEG:	membrane-associated proteins in eicosanoid and glutathione metabolism
MAPK:	mitogen-activated protein kinase
NDGA:	nordihydroguaiaretic acid
NFκB:	nuclear factor κB
PAF:	platelet-activating factor
fMLP:	*N*-formyl-methionyl-leucyl-phenylalanine
PC:	phosphatidylcholine
PL:	phospholipase
PK:	protein kinase
PMNL:	polymorphonuclear leukocytes
PT:	pentacylic triterpene
SAR:	structure-activity relationships

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The Leukotriene Synthetic Pathway

Leukotrienes (LTs) are bioactive lipid mediators mainly produced and released from activated leukocytes [1]. The initial step in LT biosynthesis is the dioxygenation of free arachidonic acid (AA) by 5-lipopxygenase (5-LO), yielding 5(S)-hydroperoxyeicosatetraenoic acid (5-HPETE) that is further metabolized by 5-LO to the instable epoxide LTA_4 (Fig. **1**, for review see [2]). In neutrophils and monocytes, LTA_4 can be converted to LTB_4 by LTA_4 hydrolase, whereas in mast cells and eosinophils, LTC_4 synthase or membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEGs) can conjugate LTA_4 with glutathione (GSH), yielding the cysteinyl-LT (Cys-LT)C_4 that can be cleaved in the extracellular environment yielding LTD_4 and then LTE_4 (Fig. **1**). Alternatively, *de novo* synthesized LTA_4 can be transferred to neighbouring cells that are unable to produce LTA_4 itself but express LTA_4 hydrolase or LTC_4 synthase/MAPEGs (e.g., parenchymal cells or erythrocytes), allowing subsequent generation of LTB_4 or LTC_4, respectively [3]. Finally, when 5-LO and 12-LO or 15-LO act together, lipoxins (LXs) might be formed, that are bioactive trihydroxytetraene-containing lipid mediators functioning as stop signals for inflammatory responses [4].

Biological Effects of Leukotrienes

LTB_4 is a potent chemoattractant for leukocytes causing adherence of phagocytes to vessel walls, neutrophil degranulation and release of superoxide anions, whereas cysLTs are potent bronchoconstrictors increasing vascular permeability and stimulating mucus secretion from airways [5]. These biological actions of LTs are mediated by specific G protein-coupled receptors (GPCRs) on the surface of their target cells. The CysLTs bind to CysLT1 and CysLT2 receptors that signal via Gq proteins (for review, see [6]). For the CysLT1 receptor the agonist potency is $LTD_4 >> LTC_4 > LTE_4$, whereas LTC_4 and LTD_4 exhibit similar potency at the CysLT2 receptor and LTE_4 is a weak agonist [6]. The CysLT1 receptor is expressed in eosinophils, monocytes, macrophages, and bronchial smooth muscle cells whereas the CysLT2 receptor is expressed more ubiquitously. CysLT1 receptor mediates vascular leakage, bronchoconstriction, dendritic cell maturation and migration, but both receptors contribute to macrophage activation, smooth muscle proliferation and fibrosis. The CysLTR antagonists pranlukast, montelukast, zafirlukast, acting primarily on CysLT1, have been shown to be clinically efficacious in chronic asthma and have been successfully introduced into market [7]. The CysLT2 receptor is insensitive to these antagonists and the therapeutic potential of this receptor is unclear.

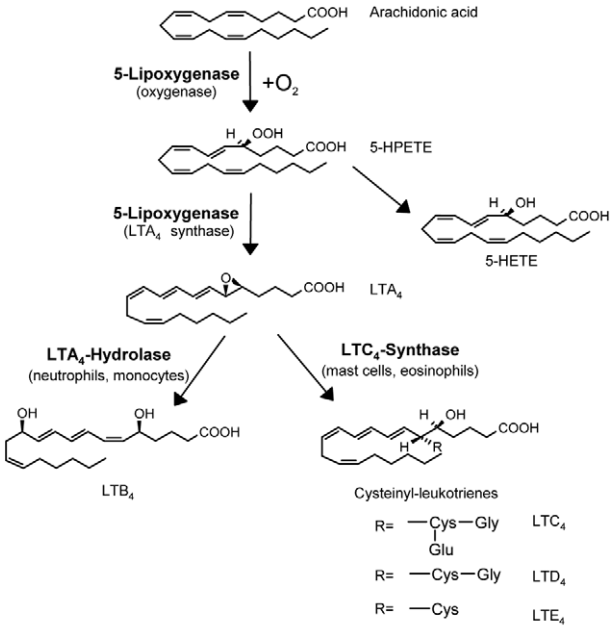


Fig. **1** Conversion of arachidonic acid by 5-lipoxygenase. LT = leukotriene; 5-H(P)ETE = 5-hydro(pero)xyeicosatetraenoic acid.

LTB_4 binds BLT1 and BLT2 receptors, which show high homology (36–45%), but quite distinct tissue distribution and distinct pharmacological properties (for review, see [6]). The BLT_1 receptor is prominently expressed in leukocytes, possesses high affinity (K_d = 0.39 nM) and specificity for LTB_4, and is coupled to Gi and Gq proteins [8]. BLT1 mediates typical effects of LTB_4 including chemotaxis, formation of superoxide, release of lysosomal enzymes and leukocyte adhesion to endothelial cells. The BLT1 seems to be related to atherogenesis, bronchial asthma, glomerulonephritis, arthritis and chronic inflammatory bowel diseases (for review see [9]). The more widely expressed BLT2 binds LTB_4 with a 20-fold lower affinity as BLT1 and the physiological and pathophysiological roles of the BLT2 are hardly understood.

Finally, a Gi-coupled GPCR specific for 5-oxo-ETE (formed by oxidation of 5-HETE) may exist in eosinophils, neutrophils and monocytes [10]. 5-Oxo-ETE is a chemoattractant for leukocytes and induces actin polymerization, Ca^{2+} mobilization, integrin expression and degranulation, stimulates the proliferation of prostate cancer cells [11], and may play a role in allergic asthma [12].

5-LO and Diseases

Initially, LTs have been identified as the chemical nature of the slow-reacting substance of anaphylaxis (SRSA) that induced a pronounced contraction of smooth muscles in a slow mode, and it soon became clear that LTs play important roles in inflammatory and allergic disorders. Therefore, bronchial asthma, allergic rhinitis, inflammatory skin diseases, rheumatoid arthritis, inflammatory bowel diseases, have long been accounted as 5-LO-mediated or -associated (for review see [13]). Today, it is generally agreed that bronchial asthma is the major 5-LO-associated disease and, in fact, the available anti-LT therapy is approved for treatment of asthma only. On the other hand, LTs play a minor role in the pathophysiology of inflammatory bowel diseases and rheumatoid arthritis in humans. Similarly, the rather disappointing results of anti-LT drugs in psoriasis led to the conclusion that the 5-LO pathway is negligible in this disease.

Novel technologies in molecular biology and cell biology, mainly attributable to the elucidation of the receptors of LTs, genotyping approaches, and cumulating data from 5-LO knock-out mice led to deeper insights into the pathophysiology of 5-LO and its products in recent years. Accordingly, there are indications for novel disorders related to 5-LO products including osteoporosis [14], [15], cancer (i.e., prostate, pancreas and breast) [16], [17], [18],

and cardiovascular diseases including atherosclerosis, heart attack and stroke [19], [20]. Clinical trials will reveal if anti-LT therapy is valuable for the treatment of these disorders.

Expression and Structure of 5-LO

Since LTs display strong biological effects it is not surprising that LT biosynthesis is tightly regulated. This regulation occurs on the transcriptional level (5-LO expression) as well as on the level of postranslational regulation and modulation by cofactors (5-LO enzyme activation) [21]. 5-LO is mainly expressed in mature leukocytes or dentritic cells, and the capability of cells to express 5-LO is acquired during cell maturation [22]. Although the gene and promoter of 5-LO are well characterized and important insights into the molecular mechanism of 5-LO expression have been obtained during the past years [21], pharmacological inhibition of expression 5-LO is a minor strategy for intervention with LT formation. Nevertheless, when compounds are analyzed for inhibition of 5-LO using cellular test systems, an effect on the 5-LO protein level must be considered, in particular when long incubation periods (hours) are applied. Thus, plant extracts or plant-derived compounds able to induce apoptosis may reduce 5-LO protein levels. In fact, functionally 5-LO protein can be cleaved by caspase-6 [23] that is activated in leukocytes in response to apoptosis-inducing agents.

Particular efforts have been made in order to establish pharmacological possibilities to block LT synthesis by interfering with 5-LO enzyme activity. Along these lines, elucidation of the structure of the 5-LO protein and the understanding of the regulation of 5-LO activation and catalysis is of importance [24]. Thus far, the 3D structure of 5-LO has not been resolved, however, practicable computational models of 5-LO based on the structure of 15-LO from rabbit have been created [23], [25]. These models indicate that 5-LO consists of an N-terminal C2-like β-barrel domain (AA 1 – 121), with distinct functions and a C-terminal catalytic domain (AA 121 – 673) [26], [27].

The C-terminal catalytic domain contains a non-heme iron in the active site, coordinated by His-367, His-372, His-550, Asn-554 and the C-terminal Ile. This iron acts as an electron acceptor or donor during catalysis (for review see [28]). In the inactive form, the iron is in the ferrous state (Fe^{2+}) whereas catalytically active 5-LO requires conversion to the ferric (Fe^{3+}) iron, accomplished by oxidation by lipid hydroperoxides (LOOH). Note that most 5-LO inhibitors, synthetic ones as well as those from natural sources (e.g., polyphenols, coumarins, quinones) act at the catalytic domain by reducing or chelating the active-site iron, by reduction of activating LOOH, or simply by scavenging electrons participating in the redox cycle of the iron [2], [24]. On the other hand, no data are available that demonstrate pharmacological inhibition of 5-LO by interfering with the C2-like domain.

The C2-like domain has a regulatory function, visualized by its ability to bind various lipids [phosphatidylcholine (PC), glycerides or lipid membranes] [29], [30], Ca^{2+} or Mg^{2+} ions [25], and coactosin-like protein (CLP) [31]. These interactions stimulate 5-LO catalysis in cell-free assays. In the cell, the C2-like domain functions as an anchor for association with the nuclear membrane, mediated by tryptophan residues (Trp-13, -75, and -102) in conjunction with Ca^{2+} [30], [32], and this process seemingly is essential for cellular 5-LO product synthesis. It is conceivable that (preferrably lipophilic) compounds may interrupt such 5-LO membrane association (5-LO translocation inhibitors), thereby reducing 5-LO product synthesis in the cell. In addition, also the catalytic domain is subject for external regulation, in particular by phosphorylation of serine residues (see below). In view of the complexity of the 5-LO regulation, compounds that suppress cellular 5-LO product synthesis may not necessarily confer their inhibitory effect by direct interference with 5-LO catalysis. Instead, inhibition of proteins that act in conjunction with 5-LO (e.g., FLAP, CLP), prevention of stimulatory effects by certain regulatory co-factors, or blockade of signaling molecules that transduce external cell stimulation to 5-LO activation may be potential points of attack (see Table **1**). Accordingly, the complex regulation of 5-LO activity in the cell often makes it difficult to distinguish if a certain compound that suppresses LT formation acts on 5-LO directly or alternatively interferes with concomitant or regulatory processes. The following chapters will focus on these regulatory elements and mechanisms that might be subject of interference with external compounds culminating in reduced 5-LO product synthesis.

Analysis of Inhibition of 5-LO Product Synthesis: Possible Points of Attack for External Compounds

Test systems for evaluation of 5-LO inhibitors

When reviewing the overwhelming amount of studies reporting about the identification of plant-derived compounds as "5-LO inhibitors" it became obvious that most studies applied cell-based test systems for assessing inhibition of 5-LO product synthesis, but only few studies have actually addressed a direct interaction of the test compound with 5-LO itself (by means of cell-free assays). Certainly, novel compounds that can be structurally and functionally grouped into the well-recognized classes of 5-LO inhibitors (see below) may be assumed to act directly on 5-LO. However, unless direct inhibition of 5-LO activity has not be demonstrated, the designation "5-LO inhibitor" is actually not justified. Moreover, the potency of a given compound may depend on the experimental settings and assay conditions that are different in each and every study.

Numerous different screening assays have been applied for the identification of inhibitors of 5-LO product synthesis that can be basically divided into (1) cellular test systems (e.g., whole blood, isolated primary leukocytes, 5-LO expressing cell lines) or (2) cell-free assays (e.g., leukocyte homogenates or cytosol, purified 5-LO enzyme). For conclusive analysis, both types of test systems should be applied. As discussed in the section below and summarized in Table **1**, for cellular assays many possibilities aside from direct interference with 5-LO exist, eventually suppressing 5-LO product synthesis. On the other hand, the fact that a test compound inhibits 5-LO in cell-free assays does not unequivocally mean that this applies also for 5-LO in the cell.

For the evaluation of test compounds utilizing cellular test systems, the correct choice of the 5-LO metabolite(s) to be analyzed is essential. For example, many studies investigating plant-de-

Table **1** Co-factors and mechanisms involved in 5-LO product formation and respective pharmacological interference by plant-derived compounds

Factor/mechanism	*Proposed function leading to INDUCTION of 5-LO product synthesis*	*Interference leading to INHIBITION of 5-LO product synthesis*	*(Plant-derived) compound*
cell viability/integrity	prerequisite for cellular response	acute cytotoxicity causes loss of cellular functionality	saponins [204]
agonist or respective signaling molecules	(receptor-coupled) cell stimulation and signal transduction of 5-LO	interruption of agonist-induced signal (e.g. receptor blockade) and signal transduction	gingkolide BN52021 [43]
$cPLA_2$	liberation and supply of AA	lack of substrate for 5-LO product formation	manoalide [37], ochnaflavone **24** [102]
FLAP	facilitation of AA transfer to 5-LO at nuclear membrane	failure of 5-LO to access AA as substrate	knipholone **54** [46]
CLP	binds and stimulates 5-LO at the C2-like domain	interruption of stimulatory CLP – 5-LO interaction	unknown
phosphorylation of 5-LO by a) MK2 or ERKs b) by PKA	a) 5-LO activation in the cell b) inhibition of 5-LO catalysis and nuclear trafficking	a) blockade of MK2/ERK pathway b) elevation of cAMP/stimulation of PKA	a) quercetin **11** [58], [59] b) ginkgetin **25** [40], [60]
Ca^{2+}	stimulates 5-LO by decreasing K_m for AA, facilitates membrane binding, protects against GPx	suppression of Ca^{2+} mobilization; Ca^{2+} chelation	deoxypodophyllotoxin **55** [50]
PC or membranes	stimulation of 5-LO catalysis	interruption of interaction with PC/membranes	unknown
(diacyl)glycerides	stimulation of 5-LO catalysis, protects against GPx	suppression of glyceride generation or interaction	1-butanol [205]
ATP	binds and stimulates 5-LO catalysis	ATP depletion or blockade of interaction with 5-LO	2-deoxyglucose [206]
redox tone	LOOH facilitate to enter the redox cycle of 5-LO catalysis	suppression of LOOH formation; reduction of LOOH	vitamin C and E, carotenoids [207]
nuclear membrane trafficking	enables 5-LO to access FLAP and AA	blockade of 5-LO translocation	genisteine [47]
LTA_4 hydrolase	conversion of LTA_4 to LTB_4	selective suppression of LTB_4 levels	bestatin [208]
LTC4 synthase/MAPEGs	conversion of LTA_4 to LTC_4	selective suppression of LTC_4, D_4, and E_4 levels	thymoquinone **77** [63], helenalin **93** [62]

rived compounds as "5-LO inhibitors" used ELISA techniques or HPLC methods in order to measure LTB_4 or cysLTs (i.e., LTC_4, D_4 or E_4). These techniques are indeed sensitive and (often) selective. However, if used as sole method, caution should be taken when interpreting the results. Thus, a given "active compound" could affect the enzymatic activities of LTA_4 hydrolase or LTC_4 synthases. Therefore, analysis of direct 5-LO derived products [i.e., 5-H(P)ETE and the *trans*- and *epi-trans* isomers of LTB_4] is important in order to exclude such interrelations. In the subsequent section, the regulation of 5-LO enzyme and 5-LO product synthesis is described in order to understand possible points of attack that all may lead to suppression of 5-LO product synthesis.

Release of arachidonic acid, redistribution/binding of 5-LO to the nuclear membrane, and the role of FLAP

Based on the complex interplay of various proteins and the manifold mechanisms involved in cellular 5-LO product formation, a number of points of attack for a given compound are conceivable. First of all, acute cytotoxicity coupled to a loss of cell functionality by disruption of cell integrity must be taken into account as a factor leading to reduced LT synthesis, in particular when lipophilic extracts using organic solvents or high concentrations of detergent-like compounds [e.g., triterpenes (-sapononinns), fatty acid derivatives] are applied.

Upon cell stimulation by an adequate agonist, 5-LO together with cytosolic phospholipase A_2 ($cPLA_2$) redistributes from a soluble cellular compartment to the nuclear membrane, where $cPLA_2$ liberates AA from phospholipids, which is then transferred to 5-LO by the membrane-bound 5-LO-activating protein (FLAP) [2], [33] (Fig. **2**). Stimuli able to induce LT formation [e.g., *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), platelet-activating factor (PAF), LTB_4, C5a, interleukin-8, phagocytic particles like zymosan, and ionophores] cause activation of 5-LO and $cPLA_2$[2]. Thus, a given test compound may simply interfere with the stimulus used to evoke 5-LO product formation and/or with the distal signaling, or with AA release by inhibiting PLA_2 enzymes. Interestingly, 5-LO and $cPLA_2$ share structural (C2 domain) and regulatory properties [activated by Ca^{2+} and by phosphorylation by members of the mitogen-activated protein kinase (MAPK) family] [31], [34]. Indeed, many studies revealed that natural compounds (e.g., flavonoids and other polyphenols) leading to suppression of LT formation also act as PLA_2 inhibitors preventing AA release [35], [36], [37], [38], [39], [40]. The anti-inflammatory sesterterpenoid manoalide, for instance, is an irreversible inhibitor of PLA_2 enzymes by covalently modifying lysine residues [41]. Initially, manoalide was proposed as a selective 5-LO inhibitor based on its ability to potently suppress the formation of LTs in human PMNL and in rat basophilic leukemia (RBL)-1 cells (IC_{50} = 0.3 μM) [42]. Later, more detailed investigations confirmed suppression of LT biosynthesis by manoalide, but the authors showed that the compound does not directly inhibit 5-LO activity by analysis of the compound under cell-free assay conditions, suggesting that the inhibitory action is eventually

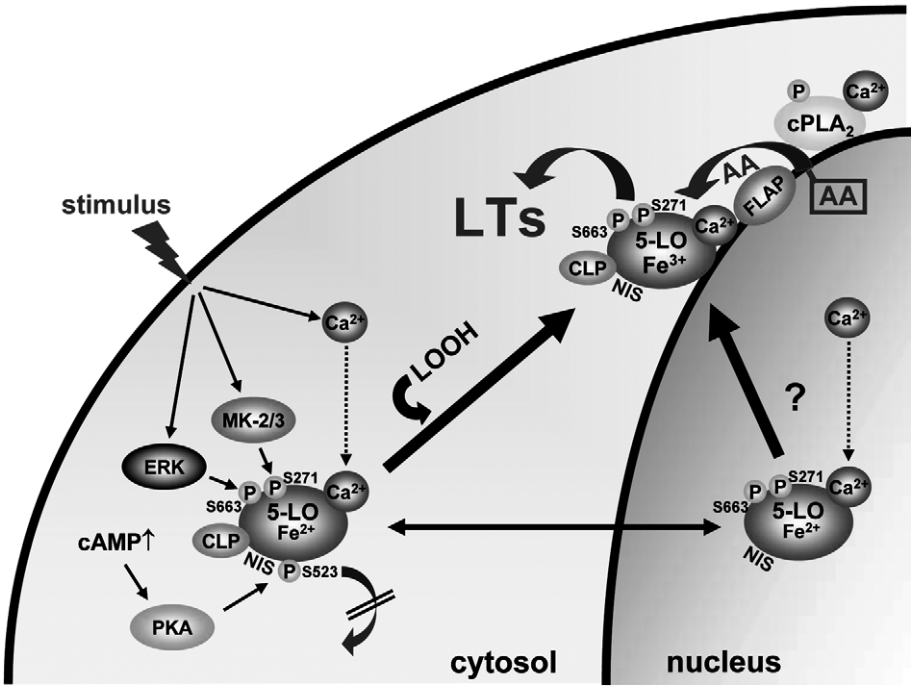


Fig. **2** Activation and signal transduction of 5-lipoxygenase in leukocytes. Upon cell stimulation, mobilisation of Ca^{2+} and activation of MK-2/3 and ERKs stimulate 5-LO in the cytosol. Moderate cell stimulation may lead to nuclear import of 5-LO, depending on the overall 5-LO phosphorylation state. Subsequent challenge may induce nuclear membrane association of intranuclear 5-LO. Robust stimulation causes direct translocation of cytosolic 5-LO to the nuclear membrane where 5-LO binds in close proximity to the 5-lipoxygenase-activating protein (FLAP). In parallel, lipid hydroperoxides (LOOH) confer the conversion of the active site iron from the ferrous to the ferric state. AA is released at the nuclear membrane (by $cPLA_2$) and transferred via FLAP towards activated 5-LO for metabolism to LTA_4 and 5-HPETE. Coactosin-like protein (CLP) is bound to 5-LO and stimulates catalysis.

due to a blockade of PLA_2 [37]. Also, (receptor) antagonists directed against the applied stimuli were found to suppress 5-LO product synthesis. For example, the gingkolide BN52021, a well-recognized PAF receptor antagonist inhibited LT formation in primed neutrophils, seemingly by antagonizing the actions of PAF [43].

The nuclear membrane-bound FLAP facilitates the access of AA to 5-LO, and in cells that lack FLAP or where FLAP is pharmacologically inhibited, transformation of endogenous AA by 5-LO is efficiently blocked [44]. It is clear that not only the supply of AA but also the access towards 5-LO is a prerequisite for LT biosynthesis [44], [45], and one important class of synthetic inhibitors of LT biosynthesis are so-called FLAP inhibitors (e.g., MK-886, Bay-X-1005). Among plant-derived compounds, the phenylanthraquinone knipholone **54** (Table **3**), from the roots of *Kniphofia foliosa*, was suggested to suppress cellular 5-LO product synthesis by inhibition of FLAP [46].

Conversion of endogenously provided AA to LTA_4 occurs at the nuclear membrane, implying that 5-LO has to be present at this locale. In resting cells, 5-LO resides in the cytosol and/or in a nuclear soluble compartment, depending on the cell type [2]. For those cells with intranuclear 5-LO, an import process from the cytosol is needed, mediated by Arg- and Lys-rich nuclear import sequences (NIS), and putative NIS are present in the N-terminal part of 5-LO, and close to the C-terminus [33]. Regardless of the localization in resting cells, upon agonist challenge 5-LO translocates to the nuclear membrane, a process that requires elevation of $[Ca^{2+}]_i$ and/or phosphorylations (Fig. **2**). Conclusively compounds that interfere with cellular 5-LO trafficking will cause a suppression of LT formation. Among natural compounds, the isoflavone genisteine from soybean suppressed 5-LO product synthesis in ionophore-stimulated HL-60 cells due to inhibiting 5-LO translocation [47].

Activation pathways of 5-LO in the cell

Signaling pathways and molecules that initialize the recruitment of resting 5-LO in the cell to become a catalytically active enzyme capable of converting AA essentially include Ca^{2+} ions and certain protein kinases (PKs). Ca^{2+} binds to the regulatory C2-like domain, mediates binding of 5-LO to PC-containing membranes or lipid vesicles, lowers the K_m value for AA as substrate, and decreases the requirement of 5-LO for activating LOOH, which altogether potently enhances 5-LO catalysis *in vitro* [21], [24], [31]. In the cell, Ca^{2+} facilitates 5-LO association with the nuclear membrane and protects 5-LO activity against GPx activity [48]. Hence, it is reasonable to assume that natural compounds that chelate Ca^{2+} or interfere with Ca^{2+} mobilization (i.e., by blocking channels, or interaction with signaling molecules such as PLC, IP3 receptors) prevent activation of 5-LO without inhibiting the enzyme directly. For instance, the lignan deoxypodophyllotoxin **55** from *Hernandia nymphaeifolia* potently impairs the production of LTC_4 (IC_{50} = 0.37 μM) in intact cells [49], but a direct effect on 5-LO was not demonstrated. Of interest, it inhibited the increase in $[Ca^{2+}]_i$ induced by PAF, LTB_4, and thapsigargin [50] that is required for activation of 5-LO. Also, the coumarin derivative osthol **70** from *Angelica pubescens* exhibits Ca^{2+}-channel blocking properties [51] and inhibits 5-LO in intact cells [52], [53], but direct inhibition of 5-LO has not been shown.

Recently, 5-LO kinases, namely the p38 MAPK-regulated MAPKAPK-2/3, the ERK1/2, CaMKII and PKA [54], [55], [56], [57] that phosphorylate 5-LO *in vitro* were identified. Phosphorylations by MAPKAPKs and ERKs at Ser-271 and Ser-663, respectively, activate 5-LO in the cell, whereas phosphorylation by PKA at Ser-523 suppresses 5-LO translocation and 5-LO catalysis. Interestingly, most stimuli that activate 5-LO in the cell are able to raise $[Ca^{2+}]_i$ and/or lead to activation of ERK1/2 and MAPKAPK-2 [24]. Since certain natural compounds possess the ability to block PK activities, those interfering with ERK1/2 and/or MAPKAPK-2 or

the respective upstream signaling pathways could block cellular 5-LO product formation by such routes as well. In fact, the flavonoid quercetin **11** that suppresses 5-LO product synthesis also blocks the ERK1/2 and p38 MAPK pathway [58], [59], and plant-derived tyrosine kinase inhibitors such as genistein reduced 5-LO activation in HL60 cells [47]. Moreover, agents that cause elevation of cAMP (e.g., ginkgetin **25** from *Ginkgo biloba*, a potent inhibitor of cellular 5-LO product synthesis [40], [60]) and thus stimulation of PKA may suppress 5-LO activity and translocation by such mechanisms.

In order to enter the catalytic cycle, 5-LO requires conversion of the ferrous iron to the active ferric state, conferred by certain LOOHs. Accordingly, the capacity of the cell to form LT is strongly linked to the peroxide tone. Agents that promote lipid peroxidation upregulate 5-LO product synthesis, whereas on the other hand, withdrawal of peroxides suppresses 5-LO product formation (for review, see [2]). It should be observed that many plant-derived natural compounds (i.e., flavonoids, coumarins, chinones, polyphenols) have reducing properties which may cause reduction of 5-LO product formation also by decreasing the peroxide tone in addition to acting on 5-LO directly.

As outlined above, in addition to these regulatory mechanisms acting on or upstream of 5-LO product synthesis, the subsequent transformation of LTA_4 to LTB_4 by LTA_4 hydrolase or to LTC_4 by LTC_4 synthase/MAPEGs provide additional points of attack. This should be taken into account, in particular when only LTB_4 or cysLTs are measured as 5-LO derived products. Well-recognized inhibitors of LTA_4 hydrolase are bestatin, captopril, and kelatorphan that reduce formation of LTB_4 without affecting 5-LO [61]. The sesquiterpene lactone helenalin **93**, present in species of the Asteraceae family, and thymoquinone **77** from *Nigella sativa* directly inhibit LTC_4 synthase besides inhibition of 5-LO [62], [63].

Molecular Pharmacology of 5-Lipoxygenase Inhibitors

Based on the pathophysiological implications of 5-LO products and the potential benefit of an anti-LT therapy in a variety of diseases, great efforts have been made within the past 25 years in order to develop selective and potent pharmacological agents that intervene with LTs. Basically, two different strategies have been pursued to reach this aim: (I) inhibition of the biosynthesis of LTs and (II) inhibition of the action of LTs.

Synthetic derivatives of LTs or prostaglandins were first described as inhibitors of LT biosynthesis [64], [65]. To achieve reduction of LT formation, reasonable targets include PLA_2 enzymes, 5-LO, FLAP, LTA_4 hydrolase and LTC_4 synthase/MAPEGs, with 5-LO being the preferred target. Although experiments in $cPLA_2$-deficient mice support the concept that inhibition of PLA_2 enzymes prevents the formation of basically all eicosanoids [66], clinical studies using glucocorticoids that suppress $cPLA_2$ enzymes proved to be ineffective in reducing the levels of LTs [5].

Most of the compounds that interfere with LT synthesis are direct 5-LO inhibitors, that advantageously block the formation of both LTB_4 and cysLTs as well as the synthesis of 5-H(P)ETE. These direct 5-LO inhibitors are classified according to their molecular mode of action as (1) redox-active 5-LO inhibitors, (2) iron-ligand inhibitors, (3) non-redox-type 5-LO inhibitors, and finally (4) compounds that act on 5-LO by so far unrecognized mechanisms (for review see [2]).

Redox-active 5-LO inhibitors comprise lipophilic reducing agents, and among those there are many prominent plant-derived classes like flavonoids, coumarins, quinones, coumarins, lignans and other polyphenols. Also the first synthetic 5-LO inhibitors belong to the class of redox-type 5-LO inhibitors, represented by AA-861 **1**, L-656,224 **2**, phenidone, or BW755C [67], [68] (Fig. **3**). These drugs act by keeping the active site iron in the ferrous state, thereby uncoupling the catalytic cycle of the enzyme and are highly efficient inhibitors of 5-LO product formation *in vitro* and partially also *in vivo*. However, most of them lack suitable oral bioavailability, possess only poor selectivity for 5-LO and thus, exert severe side-effects (e.g., methemoglobin formation) due to interference with other biological redox systems or by the production of reactive radical species [69]. These detrimental features hampered the substances to enter the market.

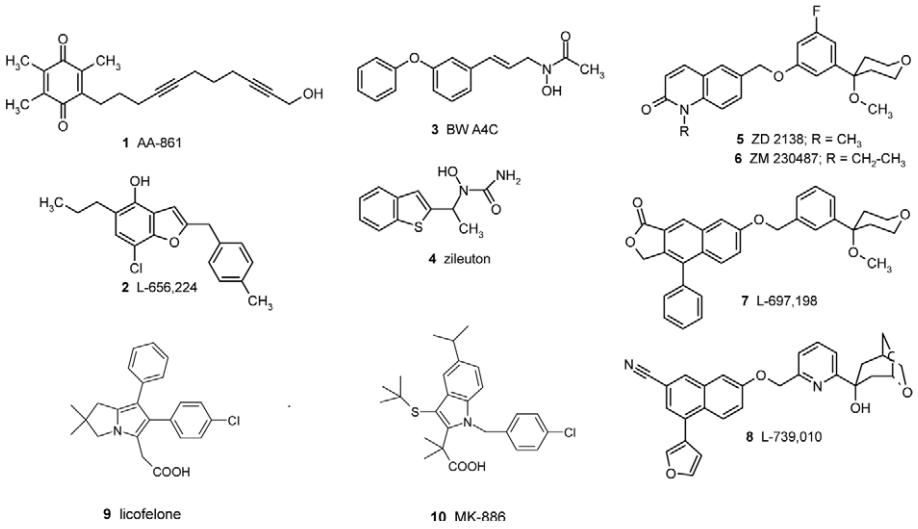


Fig. **3** Chemical structures of different types of synthetic inhibitors of 5-LO product synthesis. AA-861 **1** and L-656,224 **2** are redox-type 5-LO inhibitors, BWA4C **3** and zileuton **4** are iron-ligand-type 5-LO inhibitors, ZD 2138 **5**, ZM 230487 **6**, L-697,198 **7** and L-739,010 **8** are non-redox-type 5-LO inhibitors, MK-886 **10** is a FLAP inhibitor, and licofelone **9** is a dual inhibitor of COX and 5-LO product synthesis.

Iron ligand inhibitors chelate the active site iron via a hydroxamic acid or an *N*-hydroxyurea moiety and also exert weak reducing properties. BWA4C **3**, a hydroxamic acid and zileuton **4**, a hydrolytic stable *N*-hydroxyurea derivative belong to this class of potent and orally-active 5-LO inhibitors [70], [71]. Zileuton (Zyflo®, IC_{50} = 0.5 – 1 μM in stimulated leukocytes) has been available in the USA for the treatment of asthma, but was discontinued in June, 2003 and is currently being re-evaluated for clinical efficacy. Zileuton improved acute and chronic airway functions, associated with a decreased need for beta-agonists or glucocorticoids [72]. However, the therapeutic potential in allergic rhinitis, rheumatoid arthritis, and inflammatory bowel disease was low [73].

The non-redox-type 5-LO inhibitors compete with AA or LOOH for binding to 5-LO without redox properties and encompass structurally diverse molecules. It is still unclear if the binding-site of these compounds is in fact the AA substrate-binding cleft in the active site. Thus, experimental data from molecular and biochemical studies suggest an allosteric mode of action [74]. Nonetheless, representatives out of this class such as the orally active compounds ZD 2138 **5** or its ethyl analogue ZM 230487 **6** or L-739.010 **8** are highly potent and selective for 5-LO in cellular assays [24]. We found that elevated peroxide levels and/or phosphorylation of 5-LO by MAPKAPK-2 and/or ERKs strongly impaired the potency of non-redox-type 5-LO inhibitors in activated PMNL [74], [75], [76].

Recent developments in the field of anti-inflammatory drugs yielded compounds that act not solely on 5-LO, but also on other relevant targets including COX enzymes, the PAF or the H1 receptor (so-called dual inhibitors) [77]. A prominent representative is licofelone **9** (ML-3000, currently undergoing phase III trials) that potently inhibits 5-LO and COX product formation in the submicromolar range [78]. In fact, many plant-derived compounds have been described to suppress the activities of both 5-LO and COX enzymes (see below). Incorporation of an *N*-hydroxyurea functionality onto well-characterized PAF receptor antagonists consisting of 2,5-diaryltetrahydrofurans (CMI-392) resulted in a potent dual function compound (5-LO and PAF) [79] which may provide therapeutic advantages over agents with only single activity [77]. Similarly, hybrid molecules containing *N*-hydroxyureas or *N*-hydroxycarbamates (5-LO pharmacophore) connected to benzhydryl piperazines (H_1 receptor antagonistic moiety) are currently developed for the treatment of asthma [80], [81].

An indirect pharmacological strategy to reduce the formation of LTs is the inhibition of FLAP. Cells expressing 5-LO but lacking FLAP produced no LTs, although 5-LO was active in the corresponding homogenates [82]. No LT synthesis is detectable in FLAP-deficient macrophages from knock-out mice [83]. The indole MK-886 **10** was the first synthetic FLAP inhibitor that potently inhibits 5-LO product formation in intact isolated leukocytes (IC_{50} = 2.5 nM) [84]. However, in whole blood MK-886 **10** is much less efficient (IC_{50}, 1.1 μM), and LTB_4 biosynthesis *ex vivo* in whole blood was only partially blocked [85]. In addition to indole structures, quinolines and hybrid structures of indoles and quinolines were found to bind FLAP and to inhibit LT biosynthesis in intact cells. Obviously, these FLAP inhibitors are potent blockers of LT synthesis in isolated PMNL, whereas in whole blood assays the drugs are 50- to 200-fold less active [86], [87], possibly due to high plasma protein binding of the drugs and/or competition with AA and other fatty acids for binding to FLAP [88].

Inhibitors of 5-LO Product Synthesis from Plant Origin

Starting from the early 1980 s until today, several hundred reports have described plant extracts and/or specific ingredients thereof capable of suppressing the biosynthesis of 5-LO products. In most of these studies, plant-derived compounds were tested for their ability to block LT synthesis in isolated cells from rat, mice or human sources. In 1981, Bokoch and Reed published the polyphenol nordihydroguaiaretic acid **41** (NDGA) from the Mexican dessert plant *Larrea divaricata* as the first plant-derived 5-LO inhibitor [65]. Later, Koshihara et al. reported about natural compounds isolated from the Chinese plant *Artemisia rubris*, which were caffeic acid **36**, eupatilin and 4-demethyleupatilin that inhibited 5-LO activity in a cell free-assay as well as the formation of LTC_4 and D_4 in ionophore-stimulated mastocytoma cells [89]. Like NDGA **41**, caffeic acid **36** is a lipophilic phenolic compound, and eupatilin and 4-demethyleupatilin are flavones, and all of them possess reducing properties, thus, acting as antioxidants. In parallel, esculetin **65**, an *ortho*-dihydroxycoumarin derivative present in many plants, was identified as a 5-LO inhibitor [90]. Note that these different "5-LO active" compounds mentioned (and many more, see below) have a cathechol partial structure in common, and it is assumed that the combined iron-chelating and antioxidant feature of this moiety is eventually responsible for uncoupling of the 5-LO catalytic cycle. Since the plant kingdom is a rich source for various polyphenols, flavonoids and coumarins, subsequent investigations addressed various representatives with phenolic or coumarin structure from different plants [91], [92], [93]. A second class of plant-derived 5-LO inhibitors constitutes compounds lacking reducing properties that may mimic fatty (arachidonic) acid structures including polyacetylenes, and triterpenes and thus, inhibit 5-LO activity by binding and/or competing at the substrate-binding site or at a hypothetical fatty acid-binding cleft of 5-LO.

In the following section it is attempted to compile and group plant-derived compounds that have been reported to interfere with 5-LO product synthesis primarily according to structural aspects but also with respect to their mode of action. The respective test systems, differentiated into cell-based (intact cells) or cell-free assays have been considered and specified. Moreover, focus is placed on the effects of compounds on 5-LO, whereas effects on related enzymes including PLA_2, 12/15-LO, COX and NOS (that are in fact quite frequent) as well as *in vivo* efficacy in animal models or human studies have been mainly neglected. This list is far away from being complete and only compounds are considered that efficiently (IC_{50} < 50 μM) block 5-LO product formation. It is worthwhile to mention that most of these compounds have been evaluated in only one type of assay, preferably a cellular test system, which makes it exceedingly difficult to deduce concrete structure-activity relationships (SARs) due to multiple possible targets. As discussed above, from results obtained from only a cell-based assay, it is critical to designate an active compound as a "direct 5-LO inhibitor".

Flavonoids and other polyphenols

Polyphenols, basically grouped into flavonoids, phenolic acids (mainly caffeic acid derivatives), stilbenes and lignans are the most abundant antioxidants in consumed food together with other dietary reducing agents (e.g., carotenoids, vitamin C and E). They protect cells and tissues against oxidative stress and against associated pathologies such as cancers, cardiovascular disease and inflammation [94]. Epidemiological studies indicate that populations consuming specific polyphenol-rich food have a lower incidence of chronic inflammatory disorders and there is evidence that the health benefits associated with fruits, vegetables and red wine are linked to the antioxidant properties of polyphenols [95]. Nevertheless, most beneficial effects of polyphenols have been obtained from *in vitro* studies, and the bioavailability and bioefficacy is affected by various metabolic transformations during digestion and absorption *in vivo*. Potential molecular mechanisms of polyphenols underlying the anti-inflammatory activities include differential interference with AA cascade-dependent (PLA_2, COX, LOs) and AA cascade-independent enzymes or receptors [e.g., NOS, NFκB, peroxisome proliferator-activated receptors (PPARs) and NAG-1], depending on the overall structure of each individual polyphenol [94], [96]. Seemingly, a combination of iron-chelating and iron-reducing properties (frequently as catechol structure) mediates inhibition of 5-LO activity.

Flavonoids are widely distributed in the plant kingdom and more than 4000 derivatives have been identified [97]. Besides anticancer, antiviral, antimicrobial, immunmodulatory and antithrombotic activities, the anti-inflammatory properties, observed *in vitro* and *in vivo*, are most recognized. Thus, flavonoids have been shown to inhibit acute or chronic inflammation in various animal models such as rat Freund's adjuvant arthritis [98], [99] and carrageenan-induced mouse paw edema, when given orally [99], [100]. In particular, however, flavonoids showed beneficial effects in AA-induced ear edema, when applied topically (for review see [97]). The reduction of reactive oxygen species and the suppression of eicosanoid biosynthesis due to interference with PLA_2, COX and LO enzymes may be the major underlying molecular mechanisms. However, flavonoids can also act on the transcriptional level, interfering with the expression of iNOS, COX-2 and cytokines.

Together with other polyphenols (see below), flavonoids constitute the most prominent class of plant-derived inhibitors of 5-LO product synthesis. Since many (potent) flavonoids have been analyzed for inhibition of 5-LO product synthesis only in intact cells, it is not always clear if reduced formation of 5-LO products is caused by direct inhibition of 5-LO or by inhibition of PLA_2 enzymes (reduced AA supply). In fact, certain biflavonoids like ginkgetin **25** [101] and ochnaflavone **24** [102] or papyriflavonol **20** [103] and quercetin **11** [104] that all suppress 5-LO product synthesis, efficiently inhibit PLA_2 enzymes.

As shown in Table **2**, 5-*O*-demethylnobiletin **16**, cirsiliol **17**, luteolin **19**, papyriflavonol **20**, ginkgetin **25**, and rhamnetin **14** potently suppress 5-LO product formation in intact cells with IC_{50} values below 1 μM. In cell-free assays, quercetin **11**, 5-*O*-demethylnobiletin **16**, cirsiliol **17**, artonin E **22**, sophoraflavanone G **30**, and kenunsanone A **31** are potent 5-LO inhibitors (IC_{50} < 1 μM) with sophoraflavanone G **30**, a prenylated flavanol from *Sophora flavescens*, being most efficient (IC_{50} = 0.09 – 0.25 μM) [105]. Solely for quercetin **11**, 5-*O*-demethylnobiletin **16**, cirsiliol **17** and papyriflavonol **20** was the efficacy in both cellular and cell-free assays determined (Table **2**), where the effectiveness appeared generally somewhat higher under cell-free conditions. An exception is papyriflavonol **20** which is more potent in intact cells, presumably due to higher lipophilicity (two prenyl residues) and thus, intracellular accumulation and/or additional intracellular effects. SARs imply that compounds with vicinal phenolic hydroxy groups, that is hydroxylation in positions 3′ and 4′ of ring C, seem superior over unsubstituted (i.e., baicalein **18**), mono-hydroxylated (xanthomicrol **15**) or tri-hydroxylated (myricetin **12**) derivatives (IC_{50} = 7 – 29 μM, Table **2**). Nevertheless, an *ortho*-dihydroxy structure is not essential since sophoraflavanone G **30** and kenusanone A **31** lacking this moiety are most potent. It is interesting that also flavonoids where the hydroxy groups are replaced by methoxy functions are highly effective. For example, the polymethoxyflavone 5-*O*-demethylnobiletin **16** from *Sideritis tragoriganum* carries only one free OH group but is one of the most potent representatives (IC_{50} = 0.25 and 0.35 μM) [106]. On the other hand no clear SARs are obvious with respect to variations of the nature and number of substitutents at ring A. Also, the C-2,3-double bond as well as the C-3-OH in ring B seem not absolutely required, since kenusanone A **31** lacking the double bond [105], and epicatechin **32**, a flavanol [107], are significantly active. Regarding the C-3-OH in ring B, luteolin **19** (lacking this moiety) is even more potent than its direct counterpart quercetin **11**, a flavon-3-ol derivative [108]. Of interest, the prenylated flavonoids including payriflavonol **20**, morusin **21**, artonin E **22**, sanggenon D **26**, sophoraflavanone G **30** and kenusanone A **31** are highly efficient direct 5-LO inhibitors with IC_{50} values 0.09 to 7 μM in cell-free assays [105]. Unfortunately, among those, only for papyriflavonol **20** has the efficacy in intact cells been demonstrated [103]. Although for inhibition of cellular 5-LO, the flavonoid aglycones are generally superior over the corresponding glycosides, the myricetin 3-*O*-β-D-glucuronide **13** is more efficient than the corresponding aglycone myricetin **12** under comparable assay conditions [99], [109], probably related to more effective cellular uptake of the glycoside by specific transporters. Finally, one should observe that different studies evaluating essentially the same compound may come up with completely different efficacies, as in the case of quercetin **11** (IC_{50} = 0.3 or 25 μM [110], [111]) or morusin **21** (IC_{50} = 2.9 or >100 μM [105], [112]) analyzed in cell-free assays, again implying that the results obtained in each and every study strongly depend on the respective experimental settings of the test system.

Since conventional flavonoids show only weak efficacy after oral administration, presumably due to poor bioavailability (low absorption) and/or rapid metabolism and elimination, the more lipophilic prenylated derivatives may possess the advantage of facilitated membrane (and skin) penetration in addition to their higher potency as 5-LO inhibitors, suggesting a potential for topical treatment of inflammatory skin diseases [103], [105], [113]. Nevertheless, oral intake of flavonoid-rich nutrition (i.e., chocolate) led to significant decreases in the plasma concentrations of cys-LTs in human subjects [114] supporting the beneficial effects of daily dietary intake of flavonoids.

Table **2** Flavonoids that inhibit 5-LO product synthesis (n. d. = not determined)

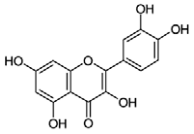
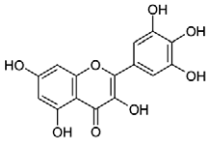
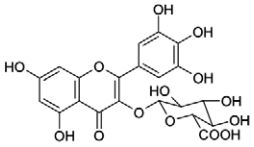
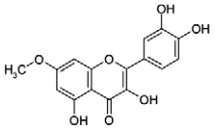
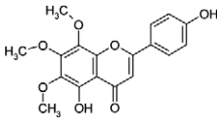
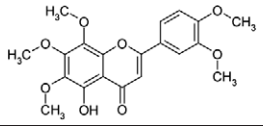
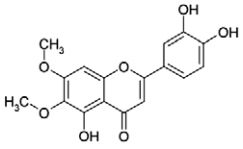
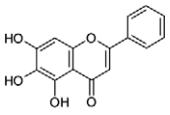
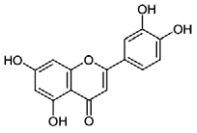
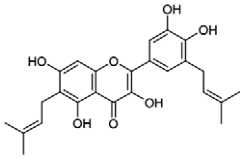
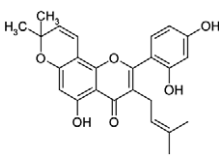
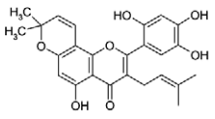
No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
11	quercetin		3.2 μM	0.3 μM, 25 μM	*Lonicera japonica*, ubiquitous	[209], [110], [111]
12	myricetin		13 μM	n. d.	ubiquitous	[109]
13	myricetin-3-*O*-β-D-glucuronide		0.1 μM (rat), 2.2 μM (human)	n. d.	*Epilobium angustifolium*	[99]
14	rhamnetin		0.7 μM	n. d.	*Guiera senegalensis*	[210]
15	xanthomicrol		29.2 μM	n. d.	*Stachys chrysantha*	[211]
16	5-*O*-demethylnobiletin		0.35 μM	≈ 0.25 μM	*Sideritis tragoriganum*	[106]
17	cirsiliol		0.4 μM	0.1 μM	*Salvia officinalis*	[91]
18	baicalein		7.13 μM 9.5 μM	n. d.	*Scutellaria baicalensis*	[212], [98]
19	luteolin		0.1 μM	n.d	ubiquitous	[108]
20	papyriflavonol		0.64 μM	7 μM	*Broussnetia papyriferra*	[105], [103]
21	morusin		n. d.	2.9 μM, > 100 μM	*Morus alba*	[112], [105]
22	artonin E		n. d.	0.36 μM	*Artocarpus communis*	[112]

Table **2** cont.

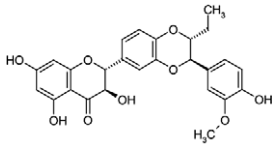
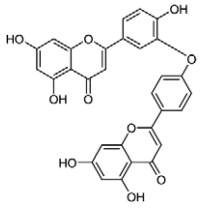
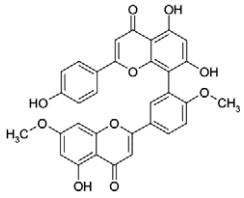
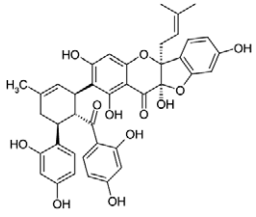
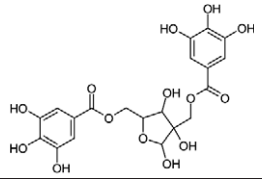
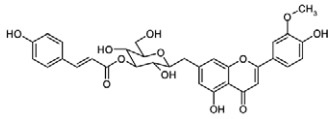
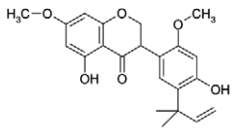
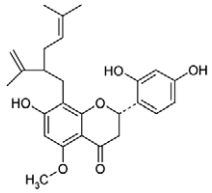
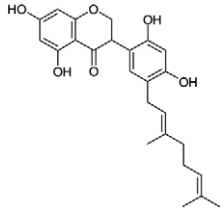
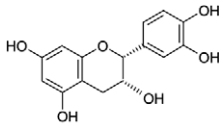
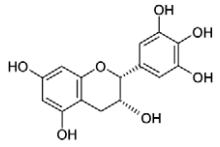
No.	Compound	Structure	IC_{50}, intact	IC_{50}, cell-free	Plant	Ref.
23	silibinin		15 μM	n. d.	*Silybum marianum*	[213]
24	ochnaflavone		6.56 μM	n. d.	*Lonicera japonica*	[214]
25	ginkgetin		0.33 μM	n. d.	*Ginkgo biloba*	[40]
26	sanggenon D		n. d.	4 μM	*Morus mongolica*	[105]
27	hamamelitannin		n. d.	1 μM	*Hamamelis virginiana*	[215]
28	chrysoeriol 7-*O*-β-(3-*E*-*p*-coumaroyl)-glucopyranoside		11.1 μM	n. d.	*Stachys chrysantha*	[211]
29	echinoisosophoranone		n. d.	19 μM	*Echinosophora koreensis*	[105]
30	sophoraflavanone G		n. d.	0.09 – 0.25 μM	*Sophora flavescens*	[105]
31	kenusanone A		n. d.	0.5 – 0.9 μM	*Echinosophora koreensis*	[105]

Table **2** cont.

No.	Compound	Structure	IC_{50}, intact	IC_{50}, cell-free	Plant	Ref.
32	epicatechin		n. d.	22 μM	*Erythroxylum coca*	[107]
33	epigallocatechin		n. d.	< 30 μg/mL	*Camellia sinensis*	[216]

In addition to flavonoids, **other groups of polyphenols** constitute rich sources of inhibitors of 5-LO product synthesis. These compounds greatly vary in their structure and consequently in their efficacy. Mechanistically, polyphenols (like flavonoids) may act as antioxidants thereby keeping the active site iron of 5-LO in the inactive ferrous state, and the *para*-hydroxy group might mediate the iron-chelating properties. In Table **3**, a number of structurally different polyphenols are compiled. Just as for the flavonoids, most of the 5-LO inhibition experiments were exclusively performed using cell-based assays, whereas only few studies have addressed inhibition of cell-free 5-LO and even less data are available from evaluations in both assay systems.

In intact cells, gingerols **53** [115], [116], 6-hydroxy-2-(2-hydroxy-4-methoxyphenyl)benzofuran **51** [117], 2-[(2′*E*)-3′,7′-dimethyl-2′,6′-octadienyl]-4-methoxy-6-methylphenol **40** [118], hyperforin **48** [119], NDGA **41** [65], 3,4,2′,4′-tetrahydroxy-2-geranyldihydrochalcone **60** [120], deoxypodophyllotoxin **55** [49], and the related lignan derivative diphyllin acetylapioside **56** [121] are highly efficient inhibitors of 5-LO with IC_{50} values of 0.004 to 3 μM. From these data, clear SARs are not immediately apparent, although it seems that increased lipophilicity (due to extended alkyl or alkylene chains) and increasing numbers of phenolic hydroxy groups govern the potency. This can be visualized by comparison of hydroxytyrosol **34**, composed of a catechol structure and a hydroxyethyl residue (IC_{50} = 10 – 26 μM) [122], [123], and urushiol **43**, possessing an extended alkyl chain (IC_{50} = 2 μM) [124].

For some compounds, the potency for 5-LO inhibition correlates to the ability to scavenge reactive oxygen species or to unspecifically suppress lipid peroxidation [109], but on the other hand polyphenols without free phenolic OH groups (i. e., deoxypodophyllotoxin **55**) or derivatives lacking strong antioxidant properties (hyperforin **48**, myrtucommulone **49**) are highly efficient 5-LO inhibitors [119], [125], [126]. Also, many polyphenols that efficiently block 5-LO fail to inhibit the related 12- and 15-LOs that also exert a sensitive iron redox cycling. It is obvious that most of the active polyphenols resemble fatty acid-like structures, either possessing a (vinylogue) carboxylic acid moiety (e. g., caffeic acid **36**, hyperforin **48**, myrtucommulone **49**, lobaric acid **57**, and rosmarinic acid **42**) or an acidic phenol core (e. g., NDGA **41**, curcumin **52**). For caffeic acid phenethyl ester (IC_{50} for purified 5-LO < 10 μM) exhibiting antioxidant properties, an uncompetitive binding to the 5-LO-substrate complex, but not to the free 5-LO enzyme, was demonstrated [127]. A recent study [128] applied surface plasmon resonance biosensor technology to investigate the binding features of typical 5-LO inhibitors, among them NDGA **41**, caffeic acid **36** and the 6,7-dihydroxylated coumarin esculetin **65** (see below) that are all assumed to act as antioxidants at the active site of 5-LO. The equilibrium dissociation constants (K_D) values showed a good correlation to the reported IC_{50} values implying that in addition to the antioxidant capacity the binding of these compounds parallels 5-LO interference [128]. Collectively, multiple structural and chemical features determine the 5-LO inhibitory action of polyphenols and definite SARs are hard to be deduced.

Remarkably potent polyphenols that inhibit 5-LO in cellular as well as in cell-free assays in the submicromolar range are the benzofuran derivatives medicarpin **50** and 6-hydroxy-2-(2-hydroxy-4-methoxyphenyl)benzofuran **51** from the Chinese plant *Dalbergia odorifera* (Jiangxiang) (IC_{50} = 50 – 500 nM) [117], 3,4,2′,4′-tetrahydroxy-2-geranyldihydrochalcone **60** from *Artocarpus communis* (IC_{50} = 0.05 – 1 μM) [120] and the acylphloroglucinols hyperforin **48** (IC_{50} = 0.09 – 1.2 μM) from St. John's wort (*Hypericum perforatum* L.) [119], and myrtucommulone **49** (IC_{50} = 1.8 – 5 μM) from myrtle (*Myrtus communis* L.) [126]. These compounds may be regarded as potent and direct 5-LO inhibitors. Similarly, the well-recognized curcumin **52** from *Curcuma longa* L. [39], [129] and the structurally related gingerols **53** from *Zingiber officnialis* Roscoe [115], [116] proved to inhibit 5-LO in cell-based and cell-free assays with IC_{50} < 1 μM. Interestingly, compared to the prenylated hyperforin **48**, the structurally related polyphenols such as erychristagallin **46** [130], or kuraridin **47** [105] possessing lipophilic prenyl residues (that seemingly enhance the potency of flavonoids) are significantly less effective with IC_{50} values > 20 μM. Among the most recognized bioactive polyphenols, also the stilbene resveratrol **37** has been reported to inhibit 5-LO product synthesis in PMNL with IC_{50} values in the low micromolar range [93], [131]. Taken together, polyphenols (including flavonoids) are a rich source of plant-derived inhibitors of 5-LO product synthesis. Given their inhibitory action also on PLA_2 and related diooxygenases within the AA cascade (such as 12-LO and COX-

Table **3** Polyphenols that inhibit 5-LO product synthesis

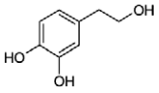
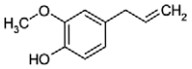
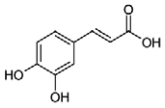
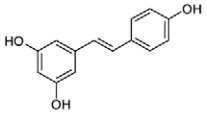
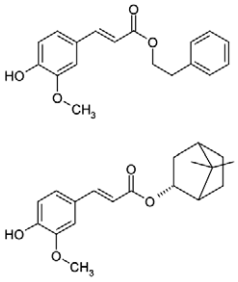
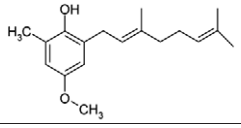
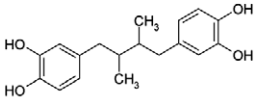
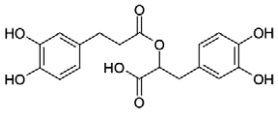
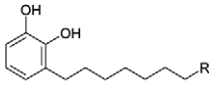
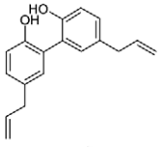
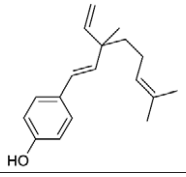
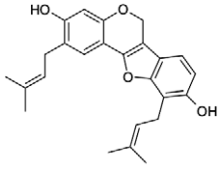
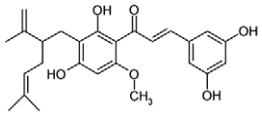
No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
34	hydroxytyrosol		10 to 26 μM	13 μM	*Olea europaea*	[123], [122], [217]
35	eugenol		20 μM	26 μM	*Syzygium aromaticum*	[218], [111]
36	caffeic acid		n. d.	3.7 μM	*Artemisia rubripes*	[89]
37	resveratrol		1.37 – 8.9 μM	n. d.	*Polygonum multiflorum*	[93] [131]
38; 39	phenylethyl ferulate; bornyl ferulate		5.75 μM; 10.4 μM	n. d.; n. d.	*Notopterygium incisum*	[219]
40	2-[(2′*E*)-3′,7′-dimethyl-2′, 6′-octadienyl]-4-methoxy-6-methylphenol		0.1 μM	n. d.	*Atractylodes lancea*	[118]
41	nordihydroguaiaretic acid (NDGA)		0.8 μM	28 μM	*Larrea divaricata*	[65], [109], [111]
42	rosmarinic acid		< 10 μM	n. d.	*Rosmarinus officinalis*	[220]
43	urushiol		2 μM	n. d.	*Toxicodendron radicans*	[124]
44	magnolol		2 – 10 μM	15 – 25 μM	*Magnolia obovata*	[35], [221], [222], [223]
45	bakuchiol		23.5 μM	n. d.	*Psoralea glandulosa*	[36]
46	erycristagallin		23.4 μM	n. d.	*Erythrina mildbraedii*	[130]
47	kuraridin		n. d.	22 μM	*Sophora flavescense*	[105]

Table **3** cont.

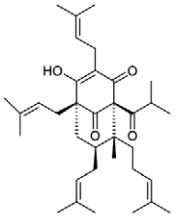
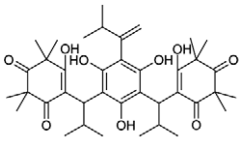
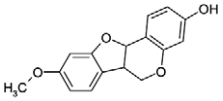
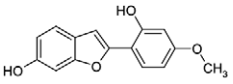
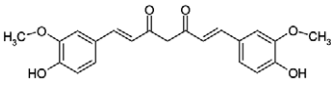
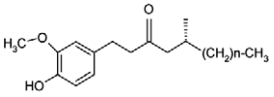
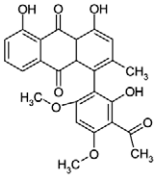
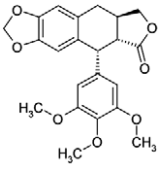
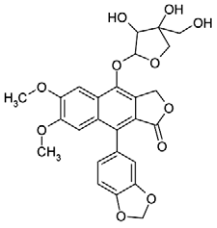
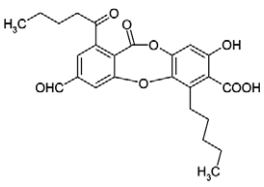
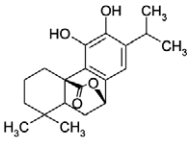
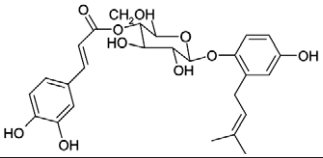
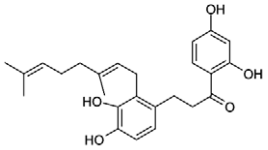
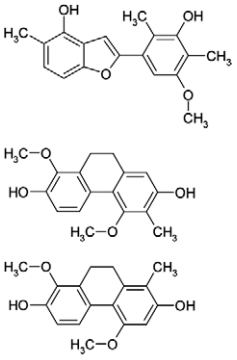
No.	Compound	Structure	IC_{50}, intact	IC_{50}, cell-free	Plant	Ref.
48	hyperforin		1.2 μM	0.09 μM	*Hypericum perforatum*	[119]
49	myrtucommulone		1.8 μM	5 μM	*Myrtus communis*	[126]
50	medicarpin		0.5 μM	n. d.	*Dalbergia odorifera*	[117]
51	6-hydroxy-2-(2-hydroxy-4-methoxyphenyl)-benzofuran		0.05 μM	0.08 μM	*Dalbergia odorifera*	[117]
52	curcumin		2.7 μM	0.7 – 30 μM	*Curcuma longa*	[39], [129], [111]
53	gingerols		0.004 – 3 μM	n. d.	*Zingiber officinalis*	[116], [115]
54	knipholone		4.2 μM	n. d.	*Kniphofia foliosa*	[46]
55	deoxypodophylotoxin		0.37 μM	n. d.	*Anthriscus sylvestris*	[49]
56	diphyllin acetylapioside		0.5 μM	≈ 10 μM	*Haplophyllum hispanicum*	[121]
57	lobaric acid		5.5 μM	n. d.	*Stereocaulon alpinum*	[224]
58	carnosol		2 μM	n. d.	*Rosmarinus offcinalis*	[109]

Table **3** cont.

No.	Compound	Structure	IC_{50}, intact	IC_{50}, cell-free	Plant	Ref.
59	monocaffeoylprenylhydroquinone glucoside		33 μM	n. d.	*Phagnalon rupestre*	[225]
60	3,4,2′,4′-tetrahydroxy-2-geranyldihydrochalcone (AC-5 – 1)		1 μM	0.05 μM	*Artocarpus communis*	[120]
62; 63; 64	stemofuran G; stemanthrenes A and D		3.7 μM; 8.5 μM; 4.8 μM	n. d.	*Stemona* species	[226]

1/2), intervention with the biosynthesis of eicosanoids may be one important mode of action underlying the anti-inflammatory properties of these compounds.

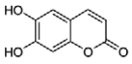
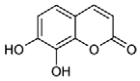
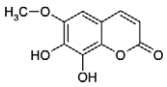
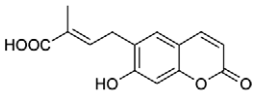
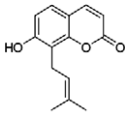
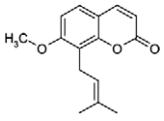
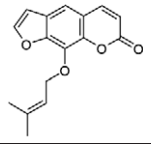
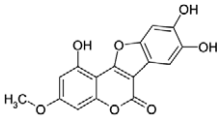
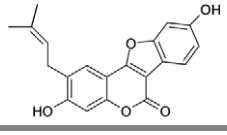
Coumarins

The almost two thousand coumarins identified from plants, fungi and bacteria comprise a class of phenolic compounds made of fused benzene and α-pyrone rings (see Table **4**). The biological effects of coumarin (1,2-benzopyrone) itself and 7-hydroxycoumarin (umbeliferrone) have been well studied [132]. The manifold possible substitutions and conjugations at the basic coumarin structure offer a great variety of distinct derivatives that occur naturally, and these modifications eventually determine the pharmacological and biochemical properties of the respective coumarin [133], [134]. Although coumarin itself and 7-hydroxycoumarin do not inhibit 5-LO, the 6,7-dihydroxycoumarin esculetin **65** has long been recognized as a 5-LO inhibitor [90]. Coumarin was suggested to act as prodrug, since metabolism in man leads to hydroxylation at C-7, and obviously, 5-LO-active coumarins possess a 7-hydroxy moiety. In general, coumarins with an *ortho*-dihydroxy moiety such as esculetin **65** [90], [135], 4-methylesculetin, daphnetin **66** and fraxetin **67** [135] suppress 5-LO product formation with IC_{50} values of 1.46 to 10 μM (compare Table **4**), more or less accompanied by efficient inhibition of lipid peroxidation and scavenging of superoxide and aqueous alkylperoxyl radicals [135], [136]. It was suggested that, like polyphenols, the high potency of dihydroxylated coumarins is related to the combined effect of the compounds to chelate the active-site iron and to interrupt the iron redox cycle by donating electrons [134], [136]. Similarly, the dihydroxylated coumestan derivative wedelolactone **72** from *Ecliptica alba* potently inhibits 5-LO in porcine leukocytes (IC_{50} = 2.5 μM), seemingly by an oxygen radical scavenger mechanism [137]. However, the 6-(3-carboxybut-2-enyl)-7-hydroxycoumarin **68** from *Peucedanum ostruthium,* characterized by only one OH moiety, a prenyl residue and a carboxylic group, is the most potent coumarin derivative (IC_{50} = 0.25 μM in a cell-based assay) [138]. This compound showed marked effectiveness in carrageenan-induced rat paw edema after oral administration (ED_{50} = 0.03 mg/kg) [138]. Also other monohydroxylated (osthenol **69** and psoralidin **73** [52], [53], [105]) or O-alkyl derivatives (osthol **70** and imperatorin **71**, that lack free phenolic hydroxy groups at all [52], [53], [139]) inhibit 5-LO product synthesis, albeit less efficiently. The latter compounds contain prenyl residues that apparently govern interference with 5-LO product formation. As for polyphenols, substantial data are available for the efficacy of coumarin derivatives on 5-LO in intact cell assays, but only sparsely have results been reported about their direct inhibitory action on 5-LO (Table **4**). In contrast to many flavonoids and other polyphenols, coumarins are relatively selective for 5-LO and hardly inhibit other enzymes within the AA cascade such as 12/15-LOs, PLA_2 and COX enzymes.

Quinones

Compounds possessing an 1,4-benzoquinone moiety exemplified by the synthetic substance 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA-861; **1**), have long been reported as potent 5-LO inhibitors [67]. In the cell, the quinone moiety is reduced to hydroquinone which is able to reduce the active site iron in 5-LO, suggesting that the reducing character confers 5-LO enzyme inhibition [140]. However, as in the case of flavonoids, polyphenols and coumarins the potency of quinones

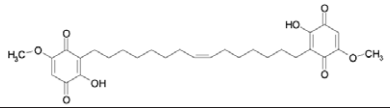
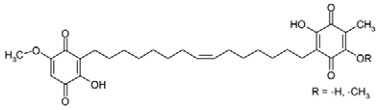
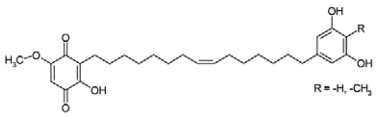
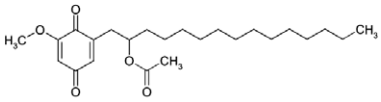
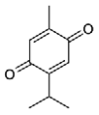
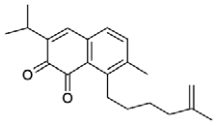
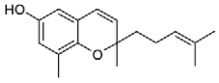
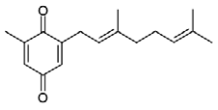
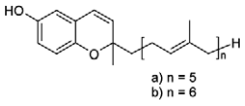
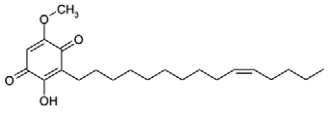
Table **4** Coumarins that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
65	esculetin		1.46 μM, 4 μM	n. d.	ubiquitous	[135], [90]
66	daphnetin		6.9 μM	n. d.	*Daphne giraldii*	[135]
67	fraxetin		2.57 μM	n. d.	*Aesculus hippocastanum*	[135]
68	6-(3-carboxybut-2-enyl)-7-hydroxycoumarin		0.25 μM	n. d.	*Peucedanum ostruthium*	[138]
69	osthenol		43.1 μM	n. d.	*Angelica pubescence*	[52], [53]
70	osthol		36.2 μM	n.d	*Atractylodes lancea*	[52], [53]
71	imperatorin		< 15 μM	n. d.	*Cachrys trifida*	[139]
72	wedelolactone		2.5 μM	n. d.	*Eclipta alba*	[137]
73	psoralidin		n. d.	3.6 – 8.8 μM	*Psoralea corylifolia*	[105]

does not solely depend on the reducing properties but also parallels their lipophilicity. For example, inhibition of 5-LO by AA-861 **1** was found to be competitive with regard to AA [67]. Also, a mechanistic analysis of the interaction of α-tocopherol (vitamin E, also a 1,4-benzoquinone derivative) with purified 5-LO showed that the potent 5-LO inhibition (IC_{50} = 5 μM) is unrelated to the antioxidant function, but instead is accounted by a selective and tight binding of α-tocopherol to a single 5-LO peptide [141].

In 1993, Fukuyama first reported about naturally occurring 5-LO inhibitors with a quinone structure derived from the rhizome of *Ardisia japonica* (Blume). This [142] and subsequent studies [143], [144], [145], [146] identified ardisianone A **76**, ardisiaquinones A **74**, B **75a**, D **75b**, E **75c**, and F **75d**, as well as maesanin **83** (Table **5**) as potent inhibitors of 5-LO in cell-free assays with IC_{50} values = 0.2 – 1 μM. In intact cells, ardisiaquinone A **74**, the most potent analogue in cell-free assays was less efficient (IC_{50} = 5.56 μM) [146]. Structurally related compounds with extended alkyl, alkylene or isoprenyl residues such as chromenols **82** from *Ircina spinosula* [147] or atracylochromene **80** and 2-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-6-methyl-2,5-cyclohexadiene-1,4-dione **81** from *Atractylodes lancea* (Thunb.) [53], [118] might be superior, at least in intact cells (IC_{50} = 0.2 – 7.5 μM, see Table **5**). Thymoquinone **77** and its polymer nigellone **78** from *Nigella sativa* (L.) that lack pronounced lipophilic residues are quite efficient as well [63], [148], [149]. Direct inhibition of 5-LO in cell-free assays was demonstrated for the *ortho*-quinone aethiopinone **79** from *Salvia aethiopsis* (L.) [150] and thymoquinone **77**[63] with IC_{50} values of 0.11 and 3 μM, respectively, and both compounds suppressed 5-LO in activated leukocytes with comparable efficacies (Table **5**). More detailed analysis of thymoquinone's **77** action revealed that, in addition to 5-LO (IC_{50} = 3 μM), it also blocks LTC_4 synthase activity, albeit less potently (IC_{50} = 10 μM) [63], and aethiopinone **79** inhibited synovial PLA_2 (IC_{50} = 10 μM), but had no effects on COX enzymes [150]. Some of the investigated quinones {e. g., thymoquinone **77**, atracylochromene **80** and 2-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-6-methyl-2,5-cyclohexadiene-1,4-dione **81**} also inhibited COX-1 at about 10- or more-fold higher concentrations [53], [118], [148]. Recently, it was shown that *i. p.* administration of ardisiaquinone A **74** to rats prevented the ischemia-induced increase in LTB_4 in the liver with an ID_{50} = 0.645 mg/kg,

Table **5** Quinones that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
74	ardisiaquinone A		5.56 μM	≈ 0.2 μM	*Ardisia sieboldii*	[146], [145]
75a; 75b; 75c; 75d	ardisia-quinones B, D, E, and F	 	n. d.	0.3 – 1 μM	*Ardisia sieboldii*	[144]
76	ardisianones		n. d.	> 80 % inhibition at 10 μM	*Ardisia japonica*	[142]
77; 78	a) thymoquinone b) nigellone = carbonyl polymer of thymoquinone		a) 0.26 μg/mL; 2.3 μM; b) 11.9 μg/mL	a) 3 μM; b) n. d.	*Nigella sativa*	[149], [148], [63]
79	aethiopinone		0.2 μM	0.11 μM	*Salvia aethiopsis*	[150], [227]
80	atractylochromene		0.6 μM	n. d.	*Atractylodes lancea*	[53]
81	2-[(2*E*)-3,7-dimethyl-2,6-octadienyl]-6-methyl-2,5-cyclohexadiene-1,4-dione		0.2 μM	n. d.	*Atractylodes lancea*	[53]
82	chromenols		a) 1.9 μM; b) 7.5 μM	n. d.	*Ircina spinosula*	[147]
83	maesanin		0.7 μM	n. d.	*Maesa lanceolata*	[143]

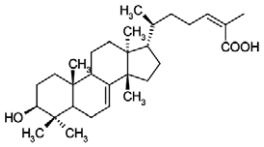
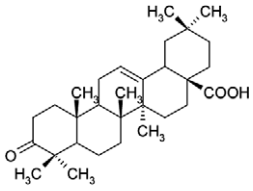
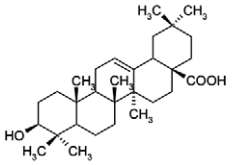
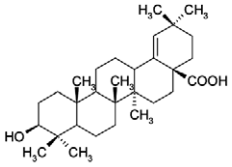
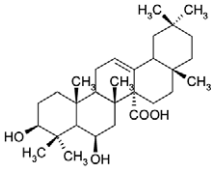
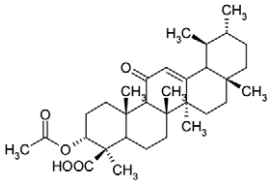
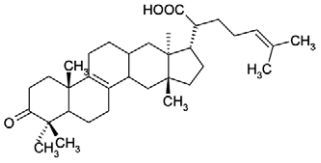
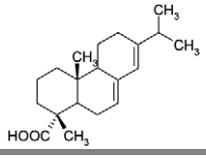
being slightly superior over AA-861 **1** (ID_{50} = 0.728 mg/kg) [151].

Pentacyclic triterpenes

In general, triterpenes, consisting of six isoprene units, represent the basic structure of a large number of biological active compounds including steroid hormones, vitamin D, heart-active "cardiac glycosides", steroid alkaloids, bile acids, and saponins. The pentacyclic triterpenes (PTs) are widely distributed among plants and, due to the complex biosynthesis including cyclization of squalene, only higher plants able to carry out this catalysis contain PTs. Many plants containing PTs, in particular boswellic acids (BAs) from *Boswellia serrata*, have been used as anti-inflammatory remedies in folk medicine (for review see [152], [153]).

In contrast to many other natural compounds that block 5-LO activity by chelating and redox actions, the PTs are assumed to act by a distinct mode, apparently by interference with a (regulatory) fatty acid-binding site of 5-LO [154], [155]. The most-recognized PTs that act on 5-LO are BAs, and many studies addressed the respective molecular interactions (for detailed review see [153], [156]). BAs with an 11-keto moiety, preferably 3-*O*-acetyl-11-keto-β-BA (AKBA **89**, Table **6**) are of particular interest, and AKBA **89** is the most efficient derivative with IC_{50} values 1.5 – 15 μM in intact cells [74], [154], [157], [158], [159]. Studies using isolated 5-LO or other types of cell-free assays showed that AKBA **89** is a direct, non-redox-type 5-LO inhibitor (IC_{50} values 8 – 50 μM) [74], [154], [159]. The discrepancies in the potency of AKBA **89** for 5-LO inhibition (1.5 – 50 μM) reported from different studies may be due to different experimental settings such as cell

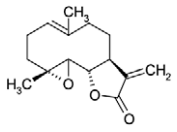
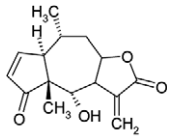
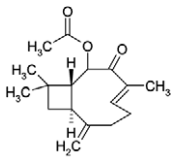
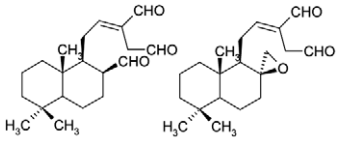
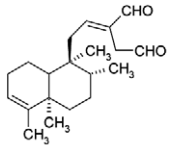
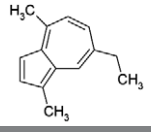
Table **6** Triterpenes and abietic acid that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
84	masticadienolic acid		16.6 μM	n. d.	*Pistacia terebinthus*	[162]
85; 86	oleanonic acid; oleanolic acid	 	17.3 μM; 16.79	n. d.; n. d.	*Pistacia terebinthus;* *Phillyrea latifolia*	[163], [164]
87	morolic acid		15.2 μM	n. d.	*Pistacia terebinthus*	[162]
88	astilbic acid		2.1 μM	n. d.	*Astilbe chinensis*	[161]
89	3-*O*-acetyl-11-keto-β-boswellic acid		1.5 – 15 μM	8 – 50 μM	*Boswellia serrata*	[154], [158], [74]
90	3-oxo-tirucallic acid		≈ 15 μM	3 μM	*Boswellia serrata*	[160]
91	abietic acid		n. d.	29.5 μM (S-LOX!)	*Abies normanniana* ssp. *equitrojani*	[165]

type, species and enzyme source (purified 5-LO, crude homogenates). It is obvious that AKBA **89** is more efficient in cell-based than in cell-free assays, suggesting additional actions in the intact cell, namely pro-oxidant activity that may irreversibly inactivate 5-LO [157]. Interestingly, the PT 3-oxotirucallic acid (3-oxo-TA **90**), also present in *B. serrata*, is more efficient on cell-free 5-LO (IC_{50} = 3 μM), whereas in intact cells 5-LO product synthesis is even elevated at low concentrations, and a significant higher IC_{50} value (15 μM) is evident [160].

Besides PTs from *Boswellia* species, the structurally related astilbic acid **88** (lacking the 11-keto group; Table **6**) from *Astilbe chinensis* blocks 5-LO-dependent LTC_4 generation in bone marrow-derived mast cells with IC_{50} = 2.1 μM [161], being equipotent to AKBA. Unfortunately, experiments addressing the effectiveness of astilbic acid **88** as a direct 5-LO inhibitor have not been performed yet. Similarly, the 11-keto-free PTs masticadienolic acid **84**, oleanonic acid **85** and morolic acid **87** from *Pistacia terebinthus* (L.) [162], [163] or oleanolic acid **86** from

Table **7** Sesquiterpenes that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
92	parthenolide		12 μM	n. d.	*Tanacetum parthenium*	[176]
93	helenalin		9 μM	n. d.	*Arnica montana*	[62]
94	buddledin		50.4 μM	n. d.	*Buddleja spec.*	[177]
95; 96	miogatrial; miogadial		n. d.; n. d.	7.5 μM; 4 μM	*Zingiber mioga*	[174]
97	*E*-isolinaridial		0.42 μM	0.2 μM	*Linaria saxatilis* var. *glutinosa*	[38]
98	chamazulene		15 μM	10 μM	*Chamomilla recutita*	[173]

Phillyrea latifolia (L.) [164] inhibit cellular 5-LO product synthesis with IC_{50} values 15.2 – 17.3 (Table **6**), but data regarding 5-LO inhibition in cell-free assays are not available. Finally, the tricyclic diterpenoid abietic acid **91** was found to inhibit soybean LO (IC_{50} = 29.5 μM) but the effectiveness on mammalian 5-LO and cellular LT biosynthesis remains to be determined [165].

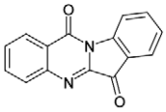
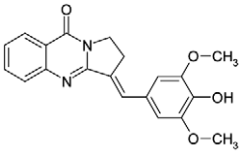
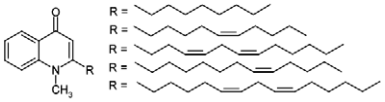
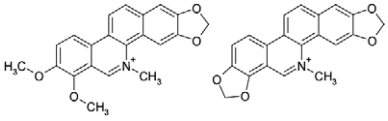
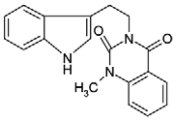
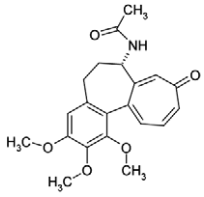
It was suggested that AKBA **89** acts at a selective binding site of 5-LO for various PTs that is different from the AA-binding cleft [155], where certain functional groups (i. e., the 11-keto and C-4-carboxylic moiety) are essential for 5-LO inhibitory activity [166]. Although previous reports demonstrated a selectivity of BAs for 5-LO, we recently showed that AKBA **89** potently suppresses 12-LO product formation, with even higher potency in cell-free assays (IC_{50} = 15 μM) as compared to 5-LO (IC_{50} = 50 μM) [167]. The direct interaction of AKBA **89** with platelet 12-LO was visualized by a protein fishing approach using immobilized KBA as bait and platelet lysates as protein source, where 12-LO was specifically precipitated [167].

Taken together, PTs carrying a carbocylic group at varying positions may be effective in suppressing cellular 5-LO product formation. Although 5-LO seemingly possesses a specific PT-binding site, and BAs (i. e., AKBA **89**) as well as 3-oxo-TA **90** directly inhibit 5-LO catalysis, it should be noted that PTs affect many signal transduction pathways (e. g., MAPK, Akt, Ca^{2+}) and effectors (i. e., reactive oxygen species) [157], [168], [169]. Hence, besides direct interference with 5-LO, these multiple (intra-)cellular targets and routes may be affected by PT that eventually governs inhibition of 5-LO product synthesis.

Sesquiterpenes

More than 3000 sesquiterpenes that all consist essentially of 3 isoprene units are known and most of them occur as polycycles, frequently with lactone, aldehyde or ketone functionality. Sesquiterpenes have long been recognized as anti-inflammatory agents being active *in vitro* and *in vivo*, and it is proposed that inhibition of NF-κB and MAPK are molecular modes of actions (see [170], [171] and references therein). Chamazulene **98** (Table **7**), an anti-inflammatory sesquiterpene from *Chamomilla recutita* (L.), is used to treat inflammatory skin and bowel diseases and was shown to scavenge hydroxyl radicals and to inhibit lipid peroxidation *in vitro* (IC_{50} = 2 – 18 μM) [172], [173]. At similar concentrations, chamazulene **98** inhibited LTB_4 formation in intact cells and in corresponding cell-free supernatants with IC_{50} = 15 and 10 μM, respectively [173], whereas the structurally related sesquiterpene lactone matricin was not effective up to 200 μM. The most potent sesquiterpene reported thus far is E-isolinaridial **97** (Table **7**), a sesquiterpene dialdehyde from *Linaria saxatilis var. glutinosa* that directly acts on 5-LO in cell-free as-

Table **8** Alkaloids that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
99	tryptanthrin		0.15 μM	n. d.	*Isatis tinctoria*	[184]
100	isaindigotone		< 10 μM	0.04 μM	*Isatis indigotica*	[185]
101	quinolone alkaloids		10 – 14.6 μM	n. d.	*Evodia rutaecarpa*	[183]
102; 103	chelerythrine; sanguinarine		0.8 μM; 0.4 μM	n. d.	*Chelidonium majus*	[181]
104	goshuyuamide-II		6.6 μM	n. d.	*Evodia rutaecarpa*	[182]
105	colchicine		< 10 μM	no inhibition	*Colchicum autumnale*	[179], [180]

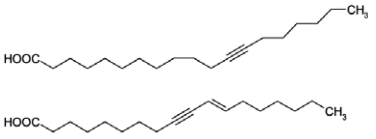
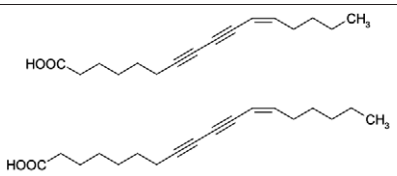
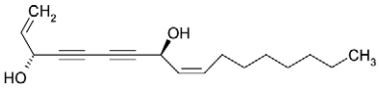
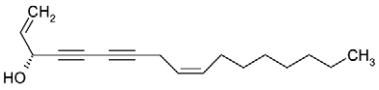
says (IC_{50} = 0.2 μM) as well as in intact neutrophils (IC_{50} = 0.42 μM) [38]. Although E-isolinaridial **97** was equipotent in suppression of synovial $sPLA_2$, no effects on COX enzymes, NOS or superoxide generation were evident. Similarly, the related sesquiterpene di- and trialdehydes miogadial **96** and miogatrial **95** from *Zingiber mioga* (Roscoe) inhibited 5-LO in cell-free assays [174], though less potently (IC_{50} = 4 and 7.5 μM); unfortunately, the effectiveness in intact cells was not addressed. Possibly, the aldehyde groups may form adducts with susceptible thiol or primary amino moieties that are essential for 5-LO catalysis. Along these lines, we recently found that the synthetic thiol-reactive compound U-73 122 (an aminosteroid with an electrophilic maleimide group, recognized as PLC inhibitor) potently inhibits 5-LO (IC_{50} = 30 nM) [175], apparently by covalent interaction with susceptible cysteine residues (unpublished data).

Among the sesquiterpene lactones, helenalin **93** from *Arnica Montana* [62] and parthenolide **92** from *Tanacetum parthenium* [176] interfere with 5-LO product synthesis. These agents usually act by forming covalent bonds via an α-methylene-γ-lactone group with free thiol groups of cysteine residues in their target enzyme. Whereas for helenalin **93** detailed mechanistic analysis was performed that indicates a time-dependent inhibitory effect on 5-LO (IC_{50} values in homogenates < 30 μM, in intact cells 9 μM), for parthenolide **92** the effectiveness was analyzed solely in intact cells (IC_{50} = 12 μM). The sesquiterpene ketone buddledin **94**, also possessing an exocyclic methylene group, was described as moderate blocker of 5-LO activity (IC_{50} = 50.4 μM) in rat peritoneal leukocytes [177]. Taken together, sesquiterpenes that inhibit 5-LO product formation encompass highly reactive compounds (α-methylene-γ-lactones, polyaldehydes) with antioxidant properties, but also with the ability to covalently modify susceptible moieties in their target protein, implying direct attack of 5-LO. Since most sesquiterpenes also interfere with other targets relevant for inflammation including NF-κB, MAPK and in particular PLA_2 and COX enzymes the extrapolation of 5-LO inhibition *in vitro* towards the anti-inflammatory activities *in vivo* needs more detailed analysis.

Alkaloids

Although there is substantial evidence for an anti-inflammatory effectiveness of alkaloids *in vivo*, only a few studies demonstrated suppression of 5-LO product synthesis by alkaloids. Colchicine **105** (Table **8**) from *Colchicum autumnale* inhibits microtubule polymerization by binding to tubulin and has long been used for the treatment of gout and rheumatoid arthritis [178]. Reibmann et al. first showed that colchicine **105** (as well as vinblastine) inhibits ionophore-induced formation of LTB_4 in neutrophils, linked to decrements in microtubule numbers [179]. Others confirmed that suppression of 5-LO product synthesis by colchicine **105** relies on microtubular disruption, but also on abrogation of agonist-induced increase in $[Ca^{2+}]_i$, connected to re-

Table **9** Polyacetylenes as arachidonic acid mimetics that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
106; 107	crepenynic acid; ximenynic acid		85 μM; 60 μM	n. d.	*Ixiolaena brevicompta*	[193]
108; 109	*cis*-hexadec-11-ene-7,9-diynoic acid; *cis*-octadec-12-ene-7,9-diynoic acid		≈ 5 μM	n. d.	*Heisteria accumniata*	[195]
110	falcarindol		9.4 μM	7 μM	*Saposhnikovia divaricata*	[196], [52]
111	panaxynol		n. d.	2 μM	*Saposhnikovia divaricata*	[196]

duced AA release [180]. Importantly, the authors excluded that colchicine acts directly on 5-LO. Using intact bovine PMNL, the benzophenanthridine alkaloids sanguinarine **103** and chelerythrine **102** of *Chelidonium majus* (L.) were shown to inhibit 5-LO (IC_{50} = 0.4 and 0.8 μM), apparently acting in a nonredox fashion [181]. However, only cell-based experiments were performed but no data from cell-free assays are available, and the related soybean LO-1 was hardly inhibited (IC_{50} > 75 μM) by these compounds. Therefore, and in view of the manifold actions of these alkaloids on central signaling molecules (that is, PKC) associated with 5-LO activation, a direct interference with 5-LO is uncertain. Similarly, different alkaloids isolated from *Evodia rutaecarpa* (Benth.) namely goshuyuamide-II **104** and five alkyl-/alkylenequinolone alkaloids reduced 5-LO activity in RBL-1 cells (IC_{50} goshuyuamide-II = 6.6 μM) [182] and in neutrophils (quinolone alkaloids) [183], but direct inhibition of 5-LO in cell-free assays was not addressed. Two structurally related alkaloids from *Isatis* species with a quinazolinone core, tryptanthrin **99** from *I. tinctoria* [184] and isaindigotone **100** from *I. indigotica* [185] have been shown to potently suppress 5-LO in intact neutrophils (IC_{50} = 0.15 and < 10 μM, respectively). Isaindigotone **100** scavenges superoxide released by PMNL and inhibits cell-free 5-LO with an IC_{50} = 0.04 μM. Both alkaloids also inhibited the formation of COX products and NOS-1, and tryptanthrin **99** has proven effective in certain animal models of inflammation [186], [187].

Polyacetylenes as arachidonic acid mimetics

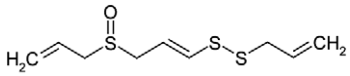
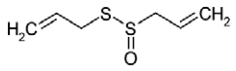
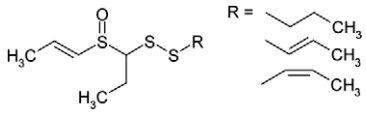
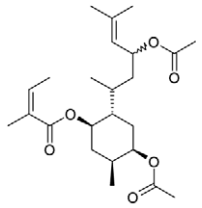
Short after the discovery of the 5-LO enzyme, various AA derivatives, in particular those with acetylene moieties such as 5,8,11,14-eicosatetraynoic acid (ETYA) [65] and 5,8,11-eicosatriynoic acid [188], but also 15-hydro(pero)xy-5,8,11,13-eicosatetraenoic acid (15-H(P)ETE) [189,190], 5,12-diHETE, or LT-analogues like 5,6-methano-LTA_4 [191], [192] were found to inhibit 5-LO activity. Moreover, AA-861 **1** [2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone, see above] contains two acetylene moieties in addition to the redox-active 1,4-benzoquinone structure [67].

A number of linear (poly)acetylenes consisting of C-16 to C-18 chains have been identified from various plants that inhibit 5-LO with rather moderate efficacy. Crepenynic acid **106** (*cis*-octadec-9-en-12-ynoic acid; Table **9**) and ximenynic acid **107** (*trans*-octadec-11-en-9-ynoic acid) from *Ixiolaena brevicompta* containing only one acetylene moiety inhibited 5-LO in intact leukocytes with IC_{50} values of 85 and 60 μM, respectively [193]. Derivatives containing more than one acetylene group, such as heptadeca-2*E*,8*E*,10*E*,16-tetraene-4,6-diyne, heptadeca-2*E*,8*Z*,10*E*,16-tetraene-4,6-diyne, heptadeca-2*E*,8*E*,16-triene-4,6-diyne-10-ol, and safynol from *Bidens campylotheca* Schultz Bip. ssp *campylotheca* (Compositae) [194], or 11(*S*),16(*R*)-dihydroxy-octadeca-9*Z*,17-diene-12,14-diyn-1-yl acetate from *Angelica pubescens f. biserrata* [52] are more efficient. Potent derivatives are *cis*-hexadec-11-ene-7,9-diynoic acid **108** and *cis*-octadec-12-ene-7,9-diynoic acid **109** [195], falcarindol **110** and panaxynol **111** from *Saposhnikoviae* [52], [196] that block 5-LO product synthesis with IC_{50} values of 2 – 10 μM. All these compounds lack substantial antioxidant activities, and it is assumed that polyacetylenes bind to the active site of 5-LO, thereby competing with AA as substrate. Hence, it is not surprising that these compounds interfere with other AA-binding/metabolizing enzymes (i. e. COX and other LOs) as well.

Diverse compounds suppressing 5-LO product synthesis

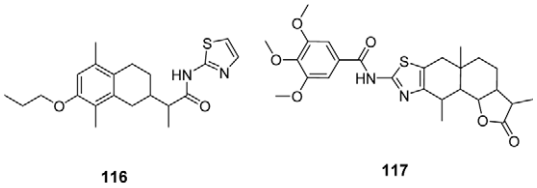
Sulfur-containing molecules, e. g., certain cepaenes **114**, ajoenes **112** and the thiosulfinate allicin **113** (Table **10**) from *Allium* species (i. e., onions and garlic) were found to inhibit 5-LO activity in cell-based assays (IC_{50} = 0.5 – 1, 1.5 and 25 μM, respectively), as well as COX product (TXB_2 and PGE_2) synthesis, and histamine release [197], [198]. No or only weak antioxidant properties have been reported for these compounds [199]. Unfortunately, no data are available demonstrating a direct inhibitory effect on 5-LO,

Table **10** Diverse compounds that inhibit 5-LO product synthesis

No.	*Compound*	*Structure*	*IC_{50}, intact*	*IC_{50}, cell-free*	*Plant*	*Ref.*
112	ajoene		1.5 μM	n. d.	*Allium sativum*	[198]
113	allicin		25 μM	n. d.	*Allium sativum*	[198]
114	cepaenes		0.5 – 1 μM	n. d.	*Allium cepa*	[197]
115	polyacetylenes		7.7 – 11.4 μM	n. d.	*Leontopodium alpinum*	[201]

and analysis of a related allyl sulfide from onion showed only poor efficacy in inhibiting purified 5-LO (IC_{50} = 83 μM) [111]. Thus, the molecular mechanisms underlying suppression of 5-LO product formation remain to be examined. Extracts of onions and garlic suppress inflammatory and allergic reactions *in vivo* (in animals as well as in human patients), correlating to the content of S-containing compounds [198], [200]. Recently, constituents (one lignan, one kaurenoate and three bisabolane derivatives) from the root of *Leontopodium alpinum* (Cass.) were shown to inhibit LTB_4 synthesis in ionophore-activated granulocytes with IC_{50} values 7.7 – 11.4 μM [201]. No significant effect on COX activity was observed. Especially the bisabolane derivatives are remarkable, since the compounds comprise a novel type of structure that may interfere with 5-LO, however, cell-free assays were not applied in this study.

Nowadays, the efficient discovery of new lead structures as 5-LO inhibitors from the plant kingdom applies different strategies including ligand-based virtual filtering experiments using pharmacophore models, docking studies and neural networks [202]. For example, by virtual screening of a natural product collection and natural-product-derived combinatorial libraries (430 substances) for potential 5-LO inhibitors grounded on the "similarity principle", we identified 18 compounds which had mutual pharmacophore features with at least one of 43 known 5-LO inhibitors (that served as query structures) [203]. Two new chemotypes (**116** and **117** in Fig. **4**) strongly inhibited 5-LO in a cell-based and cell-free assay with IC_{50} values around 1 and 0.1 μM, respectively [203], demonstrating the potential of such natural-product-derived screening libraries for hit and lead structure identification.



116 **117**

Fig. **4** Chemical structures of natural compounds that inhibit 5-LO product synthesis. The compounds **116** and **117** were identified by virtual screening of natural-product-derived combinatorial libraries (430 substances) grounded on the "similarity principle".

Conclusions

Based on the pivotal pathophysiological role of 5-LO products in the development and maintenance of common and severe disorders including bronchial asthma, rheumatoid arthritis, cancer, and cardiovascular diseases, the pharmacological intervention with 5-LO product formation is an important task, and accordingly, there is a strong need for efficient and selective 5-LO inhibitors. Although LT receptor antagonists (e. g., montelukast, pranlukast, zafirlukast) are nowadays frequently used in the therapy of bronchial asthma, and the synthetic 5-LO inhibitor zileuton has been approved for the treatment of asthma some years ago, at the moment, no 5-LO inhibitor is currently available on the market. The failure of many synthetic 5-LO inhibitors to enter the market have been attributed to the occurrence of severe side effects and the lack of efficacy. The latter point might be related to the complex regulation of 5-LO product synthesis in the cell and the influence of 5-LO activation by many co-factors such as phosphorylation, the redox tone, Ca^{2+} etc.

An enormous number of different plant-derived compounds from various species have been reported to interfere with 5-LO product formation. Whereas most studies have addressed the efficacy of a given test compound in a cell-based assay, considerably less reports have taken into account the molecular mechanisms by investigating a direct interference with 5-LO in cell-free assays, or even both test systems. Notably, besides direct inhibition of 5-LO, reduced 5-LO product synthesis in intact cells may result from many actions such as general cytotoxicity, inhi-

bition of LTA_4 hydrolase or LTC_4 synthase (if applicable), inhibition of PLA_2 and thus AA release, blockade of FLAP, interference with 5-LO kinases, Ca^{2+}-mobilization, lipid interactions, and 5-LO translocation/trafficking, as well as altering the cellular redox-tone. In general, it appears that lipophilic, often fatty acid-like compounds with (i.e., phenols) or without (i.e., triterpenes, polyacetylenes) reducing properties interfere with 5-LO, and the majority are phenolic structures including flavonoids, quinones (that become bio-activated to hydroquinones) hydroxylated coumarins, and many other polyphenols. Apparently, the combination of iron-reducing and iron-chelating features of these phenolic compounds (particularly *ortho*-dihydroxy moieties) are responsible for 5-LO inhibition, but still structural aspects play a role, in particular, prenyl residues or extended alkyl chains seem to govern the efficacy. Many phenolic compounds (and plant extracts containing them) are recognized and used as anti-inflammatory remedies and inhibition of 5-LO product synthesis might be one underlying mode of action. However, the poor bioavailability and the rapid metabolism and elimination of polyphenols after oral intake raises doubts regarding their effectiveness as 5-LO inhibitors *in vivo*. Also, the low selectivity of phenolic substances (for example, many inhibit COX, 12-LO and PLA_2) makes it difficult to correlate the 5-LO inhibitory effects *in vitro* to an anti-inflammatory action *in vivo*. Among the non-reducing compounds, the boswellic acids have been intensively studied, but still, important questions regarding the molecular mode of action and the contribution of 5-LO inhibition for the *in vivo* pharmacology remain open. Similarly, for alkaloids and sesquiterpenes that may inhibit 5-LO activity in a non-redox fashion, the molecular interactions with 5-LO catalysis are unclear. Finally, many of the plant-derived compounds exhibit only a moderate potency and respective plasma or tissue concentrations reached in patients taking such medication (if known at all) are often significantly lower.

Altogether, a huge number of studies demonstrating suppression of 5-LO product synthesis by plant-derived compounds have been conducted and published, but considerably less investigations have been performed that provide deeper insights into mechanistic interactions of the compounds with 5-LO. Most available data do not allow final conclusions about the *in vivo* relevance of 5-LO inhibition with respect to intervention with allergic or inflammatory diseases. Nevertheless, carefully performed contributions led to the identification of plant-derived compounds that potently interfere with 5-LO activity in intact cells as well as in cell-free assays. These discoveries encourage the future search of plant-derived compounds as 5-LO inhibitors and provide a suitable basis for pharmaceutical chemists for novel developments.

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