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Review

5-Lipoxygenase Antagonist therapy: a new approach towards targeted cancer chemotherapy

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Leukotrienes are the bioactive group of fatty acids and major constituents of arachidonic acid metabolism molded by the catalytic activity of 5-lipoxygenase (5-LOX). Evidence is accumulating in support of the direct involvement of 5-LOX in the progression of different types of cancer including prostate, lung, colon, and colorectal cancers. Several independent studies now support the correlation between the 5-LOX expression and cancer cell viability, proliferation, cell migration, invasion through extracellular matrix destruction, metastasis, and activation of anti-apoptotic signaling cascades. The involvement of epidermal growth factor receptor and 5-oxo-ETE receptor (OXER1) is the major talking point in the downstream of the 5-LOX pathway, which relates the cancer cells to the proliferative pathways. Antisense technology approaches and use of different kinds of blocker targeted to 5-LOX, FLAP (5-LOX-activating protein), and OXER1 have shown a greater efficiency in combating different cancer cell types. Lastly, suppression of 5-LOX activity that reduces the cell proliferation activity also induces intrinsic mitochondrial apoptotic pathway in either p53-dependent or independent manner. Pharmacological agents that specifically inhibit the LOX-mediated signaling pathways have been used during last few years to treat inflammatory diseases such as asthma and arthritis. Studies of these well-characterized agents are therefore warranted for their use as possible candidates for chemotherapeutic studies against the killer disease cancer.

Keywords leukotriene; 5-LOX; receptor; proliferation; apoptosis

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Introduction

Cancer is a dreadful disease primarily caused by abnormal and uncontrolled cell proliferation. The disease is triggered by various extrinsic and intrinsic factors. Some agents are considered initiators and some as promoters of cancer. While some of the dietary components can act as antagonists of cancer [1], some are known to contribute to the cause of cancer progression [2]. One such example is that of high-fat diet that has been linked to the progression of cancer.

Diet is then a critical determinant of cancer risk. The risk has been attributed both to dietary chemical constituents and to overall energy consumption. As much as 14%–20% of cancer deaths have been attributed to overweight and obesity. Overweight and obesity, as defined by the ratio of weight to height known as body mass index [3], are on the rise in the developing countries. Traditionally overweight and obesity have been associated with elevated risk of cancers of the colon, breast, endometrium, kidney, prostate, lung, and esophagus [1]. The metabolites of fatty acid intend to form different bioactive molecules like eicosanoids. These derived substances implicated in the pathogenesis of variety of human diseases, including cancer, are now believed to play greater role in tumor progression, metastasis, angiogenesis, etc. [4]. Two main enzymes namely cyclooxygenase (COX) and lipoxygenase (LOX) are responsible for production of eicosanoids while metabolizing fats [5]. Inhibition of these two enzymes delays tumorigenesis in animals and humans by many non-steroidal anti-inflammatory drugs, which in turn can obstruct tumor progression in various tissues [6]. In the last decade, agents that specifically inhibit the LOX metabolic pathway have been developed to treat inflammatory diseases, such as asthma and arthritis [7]. In recent days these compounds, showing inhibitory action on LOX pathway, are also showing promising block against cell proliferation. This profound activity of LOX as proliferation-blocker may play a significant role as a key factor in the treatment of the killer disease cancer to a certain extent.

Thus treating cancer as a disease of cells brings up the basic question: what is the cause of abnormal proliferation of cancer cells? Inappropriate number of chromosomes with their incorrect structure or the faulty metabolism which produces mitogen like substances has often been reported when studying cancer cells.

5-LOX: Key Enzyme in Leukotriene Biosynthesis

Human 5-LOX is a non-heme iron containing dioxygenase [8]. Its gene spans >82 kb and consists of 14 exons [9]. This enzyme is also known as arachidonate:oxygen 5-oxido-reductase that catalyzes the formation of leukotriene (LT) or eicosatetraenoic acid from arachidonic acid [10]. Arachidonic acid (5,8,11,14-eicosatetraenoic acid), a common member of the omega-6 poly-unsaturated fatty acids in high-fat product, is a strong stimulating agent for different types of cancer [11]. The progression of carcinogenicity is associated with the formation of bioactive arachidonic acid metabolites like eicosanoids, which act as mitogen [12].

5-LOX catalyzes the first two steps in LT formation [4], and the reaction starts with the intracellular release of arachidonic acid. 5-LOX in the presence of FLAP (5-LOX-activating protein) catalyzes the oxidation of arachidonic acid into 5(*S*)-hydroxy-6-*trans*-8,11,14-cis-eicosatetraenoic acid (5-HETE) [10], followed by a second reaction in which 5-HETE is dehydrated to form the epoxide LTA_4 [13]. Once formed, LTA_4 is further metabolized to either LTB_4 via stereo-selective hydration by LTA_4 hydrolase or to LTC_4 through glutathione conjugation catalyzed by LTC_4 synthase [14]. Sequential metabolic reactions, catalyzed by γ-glutamyltransferase and a specific membrane-bound dipeptidase, convert LTC_4 into LTD_4 and LTE_4, respectively [15]. Now in resting cells, 5-LOX resides in either the nucleus or the cytosol, in several tissues and cells, including epithelial cells, and vascular smooth muscle cells [16]. Upon activation, 5-LOX translocates to the nuclear membrane, where the FLAP is thought to facilitate the transfer of phospholipid-derived arachidonic acid to 5-LOX and to enhance the efficiency of conversion of 5-HETE to LTA_4, thereby triggering 5-LOX product formation [4]. The oxidized forms of 5-HETE, LTs, increased cell proliferation, and viability of cancer cells [17] by binding to the G-protein-coupled transmembrane receptor OXER1 [18] (**Fig. 1**).

Involvement of 5-LOX Metabolites in Tumor Cell Proliferation

Several metabolites of arachidonic acid synthesized by the 5-LOX-mediated pathway are known to have the potential to promote the cell proliferation, increase the cellular viability, and protect the cells from different chemo-preventive measures [19], but the proper molecular mechanism of action of these metabolites still remains in the dark though these metabolites play a critical role in the tissue repair and lipid homeostasis [20]. Tumor cells, like growing tissues or embryonic cells, emit signals that initiate the formation of new blood vessels. This adoptive process, termed angiogenesis, is a general feature of every tissue, mainly activated during wound repair process, and is a pre-requisite for tumor expansion beyond a limiting size [21]. Direct proliferative and anti-apoptotic stimuli are an enhanced tumor angiogenesis which can contribute to the tumor metastasis in the later stages. Recent studies demonstrated the involvement of growth factors, such as epidermal growth factor (EGF) and neurotensin in the 5-LOX-mediated tumor progression in prostate cancer [22,23]. Recent studies with 5-LOX siRNA [10] and specific blocker of 5-LOX [24] revealed the relation of this gene with the tumor cell proliferation.

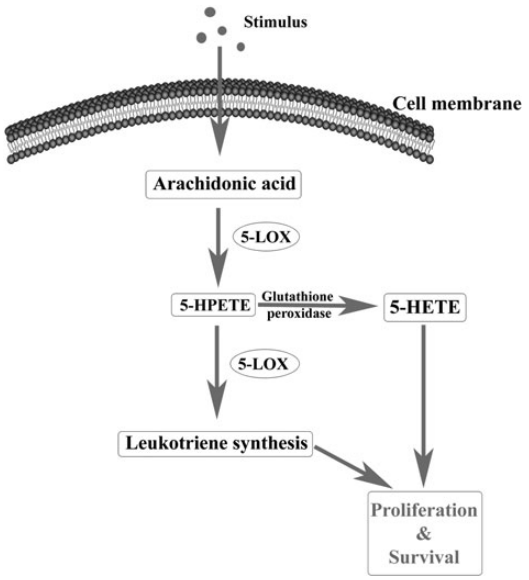
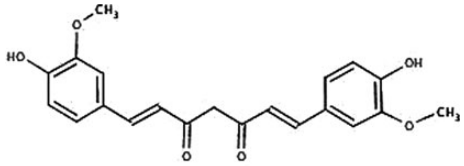
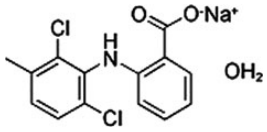
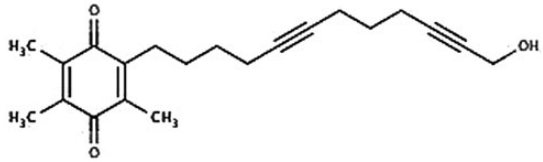
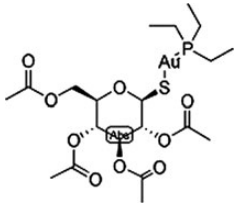
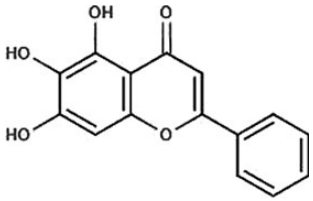


Figure 1 Schematic of LT biosynthesis

Involvement of 5-LOX Metabolites in Angiogenesis

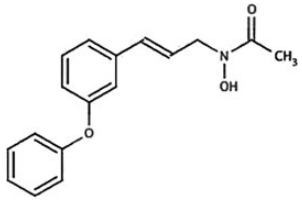
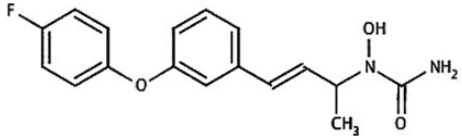
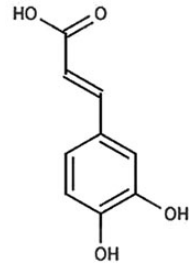
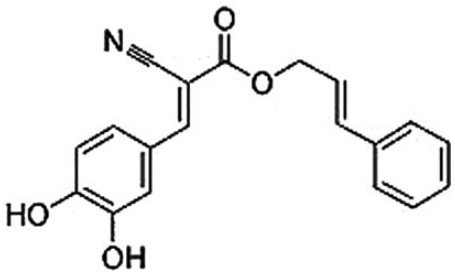
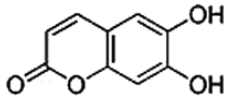
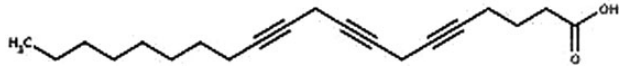
Growth factors are the key regulators of angiogenic genes. Requirements of nutrients and oxygen in the growing tissue make them emit signals that initiate the formation of new blood vessels. Formation of new blood vessels is a general feature in the wound healing process and is a pre-requisite for tumor expansion beyond a limiting size of 2–3 mm^3. Vascular endothelial growth factor (VEGF) is the most potent tumor angiogenic factor identified and a critical initiator for vessel formation. Recently, 5-HETE, a metabolite of 5-LOX, was found to stimulate angiogenesis by inducing the expression of VEGF in case of colon cancer [25–27]. The involvement of AKT (also known as Protein Kinase B) and ERK (extracellular-signal-regulated kinases) in the downstream of 5-LOX activation also could play a crucial role in the formation of vasculogenesis or angiogenesis in the tumor cells [28].

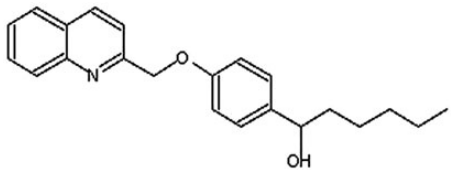
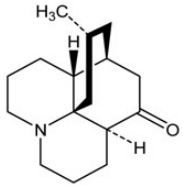
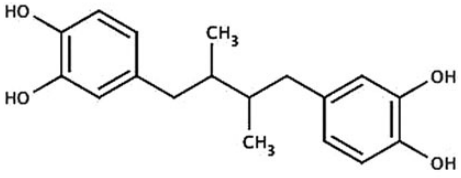
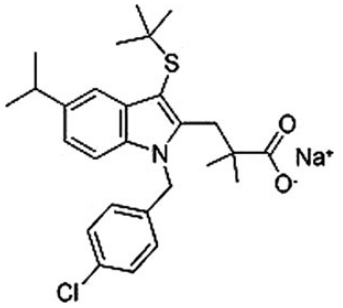
Table 1 Names and structures of 5-LOX inhibitors, their targets of action, and types of cancer tested along with respective references

Name and structure	Target	Status[a]	Types of cancer tested and IC_{50}/EC_{50} values	References
Curcumin 	5-LOX	Pancreatic neoplasms: phase II	Inhibit 5-LOX activity in RAW264.7 cells (IC_{50} = 0.3 μM), inhibits 5-LOX (IC_{50} = 8 μM), Cox-2 (IC_{50} = 52 μM) in colorectal cancer	[36]
Meclofenamate sodium 	5-LOX and COX		Inhibit human recombinant hCox-1 (IC_{50} = 1.5 μM) and hCox-2 (IC_{50} = 9.7 μM)	[37–39]
AA-861 	5-LOX	Discontinued phase II (asthma, allergy) Phase III (rheumatoid arthritis)	Human leukemia cells (IC_{50} = 6–20 mM), lung cancer cells (IC_{50} = 5–10 mM), guinea pig peritoneal PMNL 5-LOX (IC_{50} = 0.8 mM), mouse epidermal 12-LO (IC_{50} = 1.9 mM), murine bladder cancer cell line MBT-2 (IC_{50} = 8.2 μM), Capan-2 (IC_{50} = 57 μM), Panc-1 (IC_{50} = 27 μM), THP-1 (IC_{50} = 40 μM), U937 (IC_{50} = 12 μM), Capan-Z (IC_{50} = 25 μM)	[36,40–45]
Auranofin 	5-LOX/LTA synthase and disrupt MMP	Phase II (chronic lymphocytic leukemia, stage IV non-small cell lung cancer)	Human lung macrophages, human neutrophils stimulated with either fMLP (IC_{50} = 10 μM), human neutrophils (IC_{50} = 17.4 μM), MCF-7 human breast cancer cells (IC_{50} = 11.4 μM)	[46–50]
Baicalein 	5- and 12-LOX		Gastric cancer cell lines, murine bladder cancer cell line MBT-2 (IC_{50} = 0.43 μM), rat heart endothelial cells (IC_{50} = 20 μM), rat platelet (IC_{50} = 0.12 μM), rat PMN (IC_{50} = 9.5 μM)	[51–57]

Continued

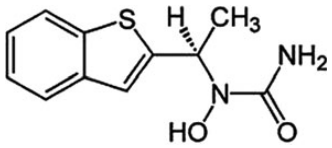
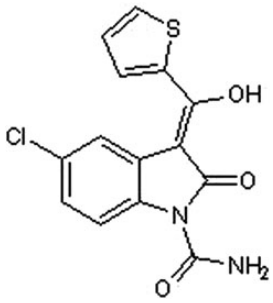
Table 1 *Continued*

Name and structure	Target	Status[a]	Types of cancer tested and IC_{50}/EC_{50} values	References
BW A4C 	5-LOX		Murine adenocarcinomas: MAC16 (IC_{50} = 5 μM), MAC13 (IC_{50} = 2 μM), MAC26 (IC_{50} = 4 μM), U937 (IC_{50} = 29 μM), Capan-Z (IC_{50} = 13 μM)	[15,58–62]
BW B70C 	Arachidonic acid, 5-LOX		Murine adenocarcinomas: MAC16 (IC_{50} = 5 μM), MAC13 (IC_{50} = 2 μM), MAC26 (IC_{50} = 4 μM)	[61]
Caffeic acid 	5-LOX	Discontinued phase III (common cold)	HepG2 (IC_{50} = 100 μg/ml), RAW 264.7 macrophages, DPE inhibited platelet (IC_{50} = 4.2 μM), DPE inhibited PMNL (IC_{50} = 13 μM), intact platelets (IC_{50} = 50 μM), intact PMNL (IC_{50} = 26 μM), colorectal cancer cells (IC_{50} = 0.8 μM), human lung macrophages, human neutrophils stimulated with either fMLP (IC_{50} = 10 μM), human neutrophils (IC_{50} = 17.4 μM), MCF-7 human breast cancer cells (IC_{50} = 11.4 μM)	[63–67]
CDC 	12-LOX inhibitor also inhibits 5-LOX and 15-LOX	Phase I (adenocarcinoma) Phase II (renal cell cancer, kidney cancer)	Inhibit 5-LOX at 1.89 μM, growth inhibition at 10 μM, induce apoptosis at 24.2 μM in epithelial cancer cell lines, prostate cancer cells PC3, breast cancer cells MCF-7, Bladder cancer cells (BOY, T24, HT1376, SCaBeR, RT4) $IC_{50} \geq 50$ μM, lung pulmonary arteries (IC_{50} = 10 μM)	[68–71]
Esculetin 	12-LOX and LT synthesis		Inhibits 5-LOX at 0.1–1.0 μM, mastocytoma 12-LOX (IC_{50} = 2.5 μM)	[72,73]
ETI 	5-LOX and biosynthesis of LT C4	Phase I (healthy volunteers)	Cox (IC_{50} = 14 μM), human 12-LO (IC_{50} = 0.46 μM), 5-LO (IC_{50} = 25 μM), effective against mammary cancer	[74,75]

L-655,238 	FLAP		Platelets (IC_{50} = 10 μM), cerebellar granule neurons (IC_{50} = 25 μM), 5(*S*)-HETE production in RBL-2H3 cells was inhibited by L-655,238 with an IC_{50} of 135.2 ± 11.5 nM, bronchial epithelial cells (IC_{50} = 1 μM)	[45,75–77]
Lycopodine 	5-LOX		Inhibit prostate cancer PC3 (IC_{50} = 121 μM), LnCap (IC_{50} = 101 μM)	[78]
NDGA 	5-LOX	Discontinued phase I (prostate cancer), completed phase II (prostate cancer, brain tumor, CNS tumors, cervical intraepithelial neoplasia)	Murine bladder cancer cell line MBT-2 (IC_{50} = 5.8 μM)	[79,80]
MK-886 sodium salt 	FLAP	Discontinued phase I (asthma)	Human and rat neutrophil LT biosynthesis (IC_{50} = 3–5 nM), human PMNs (IC_{50} = 2.5 nM), DNA synthesis inhibition in acute myelogenousleukemia cells at 100 nM, growth inhibition of chronic myelogenous leukemia (10–20 mM), lung cancer cells (5–10 mM), Capan-1 (IC_{50} = 37 μM), THP-1 (IC_{50} = 32 μM), U937 (IC_{50} = 10 μM), Capan-2 (IC_{50} = 15 μM), Capan-Z (IC_{50} = 24 μM)	[76,81,82]

Continued

Table 1 *Continued*

Name and structure	Target	Status[a]	Types of cancer tested and IC_{50}/EC_{50} values	References
Zileuton 	5-LOX, LTB4 synthesis	Phase II (lung cancer, head and neck cancer) Completed phase II (head and neck cancer) FDA approved 12/96, launched in the USA January 1997 (asthma)	FDA approved 12/96, launched in the USA January 1997 (asthma), RBL-1 (IC_{50} = 0.5 mM) and rat PMNLa (IC_{50} = 0.3 mM) 5-HETE synthesis, human whole blood (IC_{50} = 0.9 mM) and PMNL (IC_{50} = 0.4 mM) LTB4, murine colon adenocarcinoma cells (IC_{50} = 43–58 mM), murine adenocarcinomas: MAC16 (IC_{50} = 58 μM), MAC13 (IC_{50} = 43 μM), MAC26 (IC_{50} = 58 μM)	[82,83]
Tenidap 	5-LOX, COX-1		Plasma-free leucocyte suspensions (IC_{50} = 10 μM), inhibition of IL-1 production (IC_{50} = 3 μM) *in vitro*	[84–86]

5-LOX inhibitors can block the metabolism of arachidonic acid, which in turn produces LTs at the downstream of this pathway. These LTs directly help the cancer cells to proliferate via up-regulating the EGFR.

[a]Data were obtained from the site: ClinicalTrials.gov (A service of the US National Institutes of Health).

DPE, 2-(3,4-dihydroxyphenyl)ethanol; FDA, Food And Drug Administration; IL-1, interleukin-1; fMLP, formylmethionyleucylphenylalanine; PMNL, endometrial epithelial cell line.

Involvement of 5-LOX in the Regulation of Migration and Invasion

There are also evidences for the role of 5-LOX in cell migration and invasion through extracellular matrix (ECM) destruction. Matrix metalloproteinases (MMPs) are involved in the degradation of matrix components and they play an important role in the invasion of tumor cells through basement membrane barriers [27]. Distinct changes in ECM homeostasis, which in some respects imitate those that occur in fibrotic diseases, play a crucial role in tumor development. They occur due to destruction of the balance between ECM synthesis and secretion, and owing to alterations in the normal levels of matrix-remodeling enzymes such as LOX [28] and MMPs. Elevated LOX expression is ominously connected with metastasis and is known to reduce survival in cancer patients and mouse models of cancer [29]. LOX has been validated as a predictive marker in patients with head and neck cancer [30,31]. Augmented LOX activity results in increased ECM stiffness [32], and has been shown to increase the invasiveness of many cancer cell types [33,34]. Thus, in addition to proliferation and angiogenesis, 5-LOX and MMP are now linked to the tumor migration and invasion. So, 5-LOX can be believed as a potent cancer-causing agent, which helps in tumor progression, angiogenesis, migration, and lastly in invasion.

5-LOX Inhibitors in Combating Cancer

Barbey *et al*. [35] used 3-[[3-fluoro-5-(tetrahydro-4-methoxy-2H-pyran-4-yl)phenoxy]methyl]-1-[4-(methylsulfonyl)phenyl]-5-phenyl-1H-pyrazole to inhibit growth of prostate cancer cell lines, and surprisingly it brought a marked reduction in the growth of PC3 and LnCaP cell lines, showing a greater efficiency on the androgen-dependent cell lines. The growth was reduced to 50% at 83 μM of the drug used (see **Table 1** for other details). Meclofenamate sodium (MS) is known for its anti-inflammatory activity, and apart from this, Boctor *et al.* [37] reported that it caused reduction in the formation of 5-HETE in human leucocytes when used. MS can thus be considered a dual inhibitor of 5-LOX and COX pathways of arachidonic acid cascade. Further investigation with this substance revealed that it could interfere with the LT receptors in the lung carcinoma [38]. In a recent study, a group of scientists have shown the effect of MS on prostate cancer cells both *in vitro* and *in vivo* [39], and their result suggests a profound reduction in the tumor growth and cancer metastasis.

Studies have shown that 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861) can inhibit 5-LOX of guinea pig peritoneal polymorphonuclear leucocytes [40] and also can suppress the formation of 5-HETE and LTD_4 [41]. Zou *et al.* [42] reported that AA-861 reduced gastric cancer metastasis and induced apoptosis, and could act as a potent, selective, and orally active 5-LOX inhibitor. Inhibition of 5-LOX has shown to accelerate and enhance fracture healing and increase Ca^{2+} in MDCK cells (also known as NBL-2, Canis familiaris kidney disease) by releasing Ca^{2+} from multiple internal stores. Hoque *et al.* [36] studied the role of AA-861 on the esophageal cancer, and found that increased expression of 5-LOX prevented the cells from apoptosis and by using this inhibitor, a reduced growth of cancer cell lines was noted. Hayashi *et al.* [43] studied the effect of AA-861 on bladder cancer cells and suggested that the inhibition of 5-LOX pathway suppressed the growth of bladder cancer cells. Goto *et al.* [44] showed that AA-861 could bring apoptosis on estrogen-responsive mouse Leydig cell tumor cells by inhibiting LT production. Uz *et al.* [45] also showed that AA-861 could reduce proliferation in immature cerebellar granule neurons *in vitro*.

Auranofin, a gold(I)-phosphine thiolate small molecule, is reported to completely block all LOX products through inhibition of 5-LOX and to induce mitochondrial permeability transition [46–49]. It can bring up apoptosis on cisplatin-resistant human ovarian cancer cells [50].

Baicalein is a cell-permeable flavone, originally isolated from the roots of *Scutellaria baicalensis*. Baicalein has been shown to inhibit platelet 5-LOX and 12-LOX. Baicalein is a potent anti-inflammatory and anti-tumor agent. Hsu *et al.* [51] reported about this active flavone and also reported it to have anti-proliferative effect on several cell types; it could bring apoptosis to them by arresting the cell cycle mechanism [52]. This compound has an anti-inflammatory activity on leucocytes [53,54]. For the first time, Deschamps *et al.* [55] stated about the LOX inhibitory nature of this active principle. Baicalein can induce apoptosis in human gastric cancer cells and also on murine bladder cancer cell line by inhibiting LOX pathway [56,57].

BW A4C is a selective 5-LOX inhibitor and can induce apoptosis on lymphoma [58,59]. This compound shows its inhibitory effect on MAC26 and MAC16 tumors and at dose levels in between 5 and 25 mg/kg [60]. Similar inhibition occurs in murine colon adenocarcinoma cell lines MAC16, MAC13, and MAC26 treated with BWA4C at micro-molar concentrations and it has also been reported to have inhibitory growth effect on colorectal cancer by modulating 5-LOX pathway [61]. BWA4C is the most effective inhibitor, significantly decreasing both growth rate and tumor volume after 8–13 days of treatment [15]. Fischer *et al.* [62] showed the effect of BW A4C on various kinds of tumor cell including pancreatic cancer cell line (Capan-2).

Caffeic acid is an endogenous phenolic phytochemical compound that exists in plants and many foods [63]. A major metabolite product upon hydrolization of chlorogenic acid, caffeic acid inhibits a number of LOXs such as 5-LOX in a

non-competitive manner [64] in various kinds of cancer like colorectal cancer [65], liver cancer [66], and gastric cancer [67].

Esculetin, a phenolic compound, acts as a 5-LOX inhibitor. Additionally, esculetin also inhibits the activity of 12-LOX, and decreases LT biosynthesis during 5-LOX inhibition [72]. This compound acts as a potent therapeutic agent for leukemia, and induces apoptosis through Bcl-2-mediated pathway [73].

Other compounds that can also inhibit 5-LOX and 12-LOX selectively are also used as an anti-cancer agent. ETI, 5,8,11-eicosatriynoic acid, demonstrates the ability to inhibit 5-LOX activity and halt biosynthesis of LT C4 by mastocytoma cells [71,74]. L-655,238 is a potent and selective inhibitor of FLAP (5-LO-activating protein) [45,75–77]. NDGA (nordihydroguaiaretic acid) [79,80], MK-886 sodium salt [76,81,82], zileuton [82,83], tenidap [84–86], CDC (cinamyl-3,4-dihydroxy-a-cyanocinnamat, ametabolite of ciprofloxacin) [68–71], BW B70C [61], curcumin [36], and lycopodine [78] can alter LOX metabolism by inhibiting 5-LOX, 12-LOX, and 15-LOX; otherwise they can also inhibit FLAP receptor and other proteins of related pathways. These effects, on the other hand, induce apoptosis of different kinds of cancer *in vitro* and *in vivo*.

Off-target Effect of 5-LOX Inhibitors

The increasing numbers of publications that report a crucial role of 5-LOX, and its products in tumorogenesis have been accompanied by additional studies that question the correlation between 5-LOX and cancer. There is little disagreement that 5-LOX inhibitors exert strong cytotoxic activities against 5-LOX over-expressing tumor types and cultured tumor cells, which represents significant basis for concluding that 5-LOX products directly stimulate tumor cell proliferation. These metabolites might induce cancer progression, but not carry sole responsiveness. Other factors related to fat deposition also play a crucial role in inducing neoplasia. LOX pathway also carries out a metabolism related to normal physiological growth. Blocking of this pathway on the other hand hinders normal physiological conditions related to metabolism. So, it is most important to carry out study onside/off-target effects of these blocker substances. Cytotoxic natures of these substances generally harm the normal body cells.

However, it was recently demonstrated that the common 5-LO inhibitors could reduce the viability of pancreatic cancer cells, cervix carcinoma cells, and leukemic cells independently of the suppression of 5-LO product formation [62]. The hypothesis of 5-LO-independent cytotoxicity and anti-proliferation was substantiated using several experimental approaches. While the commonly used inhibitors produced strong cytotoxicity, notably, zileuton, the only commercialized 5-LOX inhibitor, failed to induce an anti-proliferative or cytotoxic response in all other types of tumor cells where 5-LOX was in inactive state (e.g. HeLa cells); however, where 5-LOX was in active state, zileuton could effectively inhibit progression, as in case of prostate cancer.

In fine, the cytotoxic and chemo-preventive effects of 5-LOX inhibitors in cell culture assays and in animal tumor models may derive from molecular mechanisms other than suppression of LT biosynthesis and warrant re-assessment in some cases.

Apoptosis Induction

The blocking of 5-LOX enzyme can induce apoptosis in several cancer cells. Initiation of cells' programmed-killing process is generally triggered by depolarization of mitochondrial membrane potential. Recently in our lab, we have shown the activation of mitochondrial-mediated apoptosis without the involvement of p53 gene. Lycopodine, a plant-derived active compound, could activate caspase 3 protein in PC3 and LnCaP prostate cancer cells [78]. In another study, Rev-5901, a 5-LOX inhibitor, showed its efficiency to reduce cancer growth in lung tissue, and activated cytochrome c-mediated caspase-dependent pathway to induce apoptosis [87]. Again a group of researchers have shown licofelone, a dual COX/5-LOX inhibitor, could depolarize mitochondrial membrane potential to induce apoptosis; on the other hand, it also affected the arachidonic pathway by inhibiting 5-LOX enzyme activity in HCA-7 colon cancer cells [88]. MK-591 in prostate cancer cells could induce protein kinase C-epsilon at the downstream of 5-LOX inhibition [89]. From all these cumulative data, it is clear that, the LOX inhibitor acts upon mitochondria to depolarize its potential and releases out the cytochrome c to activate caspase cascade. The involvement of proteins like p53 [78] and AKT [89] is always not needed for caspase cascade initiation. Proteins like p21 and PKC could play here a greater role in the initiation of apoptosis through mitochondrial membrane potential imbalance (**Fig. 2**).

Implications and Future Directions

This review aims at presenting a conceptual framework for integrative signaling and proposes 5-LOX as a cancer-

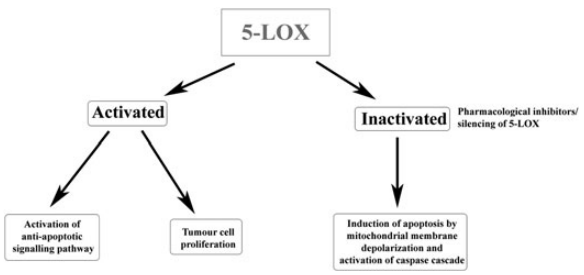


Figure 2 Effect of 5-LOX inhibition

causing element. A casual connection between arachidonic acid cascade and cell proliferation and cancer has been proposed for many years and many events on COX pathway have been well documented in recent years, but the implication of LOX pathway for the metabolism of arachidonic acid and its relation with the cancer growth is not yet well understood. As 5-HETE and LTs synthesis have been associated with several malignancies involving epithelial cells, these arachidonic acid metabolites might be the missing link between arachidonic acid metabolites and cancer progression.

The well-recognized over-expression of 5-LOX in various types of malignant cells, the reduction of tumor cell viability by 5-LOX gene silencing approaches, as well as experiments involving 5-LOX knockout mice, together constitute a substantial rationale for the relation between 5-LOX gene and malignancy. However, considering that the cytotoxic activity of 5-LOX inhibitors is substance-specific and may, in many cases, have not derived from inhibition of 5-LOX activity, the traditional hypothesis that 5-LOX products are the exclusive players in 5-LOX-triggered tumorigenesis may warrant re-consideration. Experiments on the role of 5-LOX product formation in proliferation of cultured tumor cells using high concentrations (>1 μM) of certain 5-LOX inhibitors may be misleading and the use of these agents as pharmacological tools should be critically considered. Also, possible indirect tumorigenic effects of 5-LOX products (e.g. promotion of angiogenesis) with relevance towards the observed situation *in vivo* but not for cell culture assays should be taken into account. Notably, non-enzymatic functions, including an interaction with cytoskeleton proteins, or with the EGF receptor (EGFR) molecule, or with mitogen-activated protein kinases (MAPKs) have been reported for 5-LOX. Because of the crucial role of oncogenic EGFR and MAPK signaling in cancer progression, a disrupted growth factor signaling may contribute to the reduction in tumor cell viability by 5-LOX gene silencing approaches. Taken together, a broad body of evidence from the literature suggests a crucial, albeit poorly defined, role of 5-LOX in tumorigenesis of several cancer types. Elucidation of the molecular mechanisms underlying these effects may include direct proliferative actions of 5-LOX products on tumor cells as well as indirect and so far neglected effects of 5-LOX, and thereby tempt one to draw novel connections between pathways that are currently regarded as unrelated.

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References

1 Divisi D, Tommaso SD, Salvemini S, Garramone M and Crisci R. Diet and cancer. Acta Biomed 2006, 77: 118–123.
2 Willett WC. Diet and cancer. Oncologist 2000, 5: 393–404.
3 Guo SS, Wu W, Chumlea CW and Roche AF. Predicting overweight and obesity in adulthood from body mass index values in childhood and adolescence. Am J Clin Nutr 2002, 76: 653–658.
4 Steinhilber D, Fischer AS, Metzner J, Steinbrink SD, Roos J, Ruthardt M and Maier TJ. 5-lipoxygenase: underappreciated role of a pro-inflammatory enzyme in tumorigenesis. Front Pharmacol 2010, 1: 143.
5 Brash AR. Arachidonic acid as a bioactive molecule. J Clin Invest 2001, 107: 1339–1345.
6 August EM, Nguyen T, Malinowski NM and Cysyk RL. Non-steroidal anti-inflammatory drugs and tumor progression: inhibition of fibroblast hyaluronic acid production by indomethacin and mefenamic acid. Cancer Lett 1994, 82: 49–54.
7 Martel-Pelletier J, Lajeunesse D, Reboul P and Pelletier J-P. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann Rheum Dis 2003, 62: 501–509.
8 Needleman P, Turk J, Jakschik BA, Morrison AR and Lefkowith JB. Arachidonic acid metabolism. Annu Rev Biochem 1986, 55: 69–102.
9 Funk CD, Hoshiko S, Matsumoto T, Rdmark O and Samuelsson B. Characterization of the human 5-lipoxygenase gene. Proc Natl Acad Sci USA 1989, 86: 2587–2591.
10 Sundaram S and Ghosh J. Expression of 5-oxoETE receptor in prostate cancer cells: critical role in survival. Biochem Biophys Res Commun 2006, 339: 93–98.
11 Cianchi F, Cortesini C, Magnelli L, Fanti E, Papucci L, Schiavone N and Messerini L, *et al*. Inhibition of 5-lipoxygenase by MK886 augments the antitumor activity of celecoxib in human colon cancer cells. Mol Cancer Ther 2006, 5: 2716–2726.
12 Anderson KM, Seed T, Vos M, Mulshine J, Meng J, Alrefai W and Ou D, *et al*. 5-Lipoxygenase inhibitors reduce PC3 cell proliferation and initiate nonnecrotic cell death. Prostate 1998, 37: 161–173.
13 Peters-Golden M. Cell biology of the 5-lipoxygenase pathway. Am J Respir Crit Care Med 1998, 157: 227–232.
14 Avis I, Hong SH, Martinez A, Moody T, Choi YH, Trepel J and Das R, *et al*. Five-lipoxygenase inhibitors can mediate apoptosis in human breast cancer cell lines through complex eicosanoid interactions. FASEB J 2001, 15: 2007–2009.
15 Steele VE, Holmes CA, Hawk ET, Kopelovich L, Lubet RA, Crowell JA and Sigman CC, *et al*. Lipoxygenase inhibitors as potential cancer chemopreventives. Cancer Epidemiol Biomarkers Prev 1999, 8: 467–483.
16 Abramovitz M, Wong E, Cox ME, Richardson CD, Li C and Vickers PJ. 5-lipoxygenase-activating protein stimulates the utilization of arachidonic acid by 5-lipoxygenase. Eur J Biochem 1993, 215: 105–111.
17 Nieves D and Moreno JJ. Hydroxeicosatetraenoic acids released through the cytochrome P-450 pathway regulate 3T6 fibroblast growth. J Lipid Res 2006, 47: 2681–2689.
18 Ghosh J and Myers CE. Arachidonic acid stimulates prostate cancer cell growth: critical role of 5-lipoxygenase. Biochem Biophys Res Commun 1997, 235: 418–423.
19 Rioux N and Castonguay A. Inhibitors of lipoxygenase: a new class of cancer chemopreventive agents. Carcinogenesis 1998, 19: 1393–1400.
20 Manigrasso MB and O'Connor JP. Accelerated fracture healing in mice lacking the 5-lipoxygenase gene. Acta Orthop 2010, 81: 748–755.
21 Moreno JJ. New aspects of the role of hydroxyeicosatetraenoic acids in cell growth and cancer development. Biochem Pharmacol 2009, 77: 1–10.
22 Hassan S and Carraway RE. Involvement of arachidonic acid metabolism and EGF receptor in neurotensin-involved prostate cancer PC3 cell growth. Regul Pept 2006, 133: 105–114.

23 Karlage KL, Mogalian E, Jensen A and Myrdal PB. Inhalation of an ethanol-based zileuton formulation provides a reduction of pulmonary adenomas in the A/J mouse model. AAPS Pharm Sci Technol 2010, 11: 168–173.

24 Cuendet M and Pezzuto JM. The role of cyclooxygenase and lipoxygenase in cancer chemoprevention. Drug Metabol Drug Interact 2000, 17: 109–157.

25 Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M and DuBois RN. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell 1998, 93: 705–716.

26 Romano M, Catalano A, Nutini M, D'Urbano E, Crescenzi C, Claria J and Libner R, *et al*. 5-lipoxygenase regulates malignant mesothelial cell survival: involvement of vascular endothelial growth factor. FASEB J 2001, 15: 2326–2336.

27 Ye YN, Wu WK, Shin VY, Bruce IC, Wong BC and Cho CH. Dual inhibition of 5-LOX and COX-2 suppress colon cancer formation promoted by cigarette smoke. Carcinogenesis 2005, 26: 827–834.

28 Payne SL, Hendrix MJ and Kirschmann DA. Paradoxical roles for lysyl oxidases in cancer-a prospect. J Cell Biochem 2007, 101: 1338–1354.

29 Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT and Chi JT, *et al*. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 2006, 440: 1222–1226.

30 Le QT, Kong C, Lavori PW, O'Byrne K, Erler JT, Huang X and Chen Y, *et al*. Expression and prognostic significance of a panel of tissue hypoxia markers in head-and-neck squamous cell carcinomas. Int J Radiat Oncol Biol Phys 2007, 69: 167–175.

31 Le QT, Harris J, Magliocco AM, Kong CS, Diaz R, Shin B and Cao H, *et al*. Validation of lysyl oxidase as a prognostic marker for metastasis and survival in head and neck squamous cell carcinoma: radiation therapy oncology group trial 90–03. J Clin Oncol 2009, 27: 4281–4286.

32 Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT and Fong SF, *et al*. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 2009, 139: 891–906.

33 Kirschmann DA, Seftor EA, Fong SF, Nieva DR, Sullivan CM, Edwards EM and Sommer P, *et al*. A molecular role for lysyl oxidase in breast cancer invasion. Cancer Res 2002, 62: 4478–4483.

34 Erler JT, Bennewith KL, Cox TR, Lang G, Bird D, Koong A and Le QT, *et al*. Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. Cancer Cell 2009, 15: 35–44.

35 Barbey S, Goossens L, Taverne T, Cornet J, Choesmel V, Rouaud C and Gimeno G, *et al*. Synthesis and activity of a new methoxytetrahydropyran derivative as dual cyclooxygenase-2/5-lipoxygenase inhibitor. Bioorg Med Chem Lett 2001, 12: 779–782.

36 Hoque A, Lippman SM, Wu TT, Xu Y, Liang ZD, Swisher S and Zhang H, *et al*. Increased 5-lipoxygenase expression and induction of apoptosis by its inhibitors in esophageal cancer: a potential target for prevention. Carcinogenesis 2005, 26: 785–791.

37 Boctor AM, Eickholt M and Pugsley TA. Meclofenamate sodium is an inhibitor of both the 5-lipoxygenase and cyclooxygenase pathways of the arachidonic acid cascade *in vitro*. Prostaglandins Leukot Med 1986, 23: 229–238.

38 Stadler I, Kapui Z and Ambrus JL. Study of the mechanisms of action of sodium meclofenamic acid (Meclomen) a 'double inhibitor' of the arachidonic acid cascade. J Med 1994, 25: 371–382.

39 Soriano-Hernández AD, Galvan-Salazar HR, Montes-Galindo DA, Rodriguez-Hernandez A, Martinez-Martinez R, Guzman-Esquivel J and Valdez-Velazquez LL, *et al*. Antitumor effect of meclofenamic acid on human androgen-independent prostate cancer: a preclinical evaluation. Int Urol Nephrol 2012, 44: 471–477.

40 Yoshimoto T, Yokoyama C, Ochi K, Yamamoto S, Maki Y, Ashida Y and Terao S, *et al*. 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. Biochim Biophys Acta 1982, 713: 470–473.

41 Ashida Y, Saijo T, Kuriki H, Makino H, Terao S and Maki Y. Pharmacological profile of AA-861, a 5-lipoxygenase inhibitor. Prostaglandins 1983, 26: 955–972.

42 Zou LY, Li JY, Chen FL, Chen ZX and Wang XZ. Tumor 5-Lipoxygenase expression correlates with gastric cancer metastasis and its selective inhibitor induces cancer cell apoptosis. J Cancer Mol 2006, 2: 227–233.

43 Hayashi T, Nishiyama K and Shirahama T. Inhibition of 5-lipoxygenase pathway suppresses the growth of bladder cancer cells. Int J Urol 2006, 13: 1086–1091.

44 Goto HG, Nishizawa Y, Katayama H, Murashima T, Yamasaki M, Tanigaki Y and Kimura S, *et al*. Induction of apoptosis in an estrogen-responsive mouse Leydig tumor cell by leukotriene. Oncol Rep 2007, 17: 225–232.

45 Uz T, Manev R and Manev H. 5-Lipoxygenase is required for proliferation of immature cerebellar granule neurons *in vitro*. Eur J Pharmacol 2001, 418: 15–22.

46 Betts WH, Hurst NP, Murphy GA and Cleland LG. Auranofin stimulates LTA hydrolase and inhibits 5-lipoxygenase/LTA synthase activity of isolated human neutrophils. Biochem Pharmacol 1990, 39: 1233–1237.

47 Rigobello MP, Scutari G, Boscolo R and Bindoli A. Induction of mitochondrial permeability transition by auranofin, a gold(I)-phosphine derivative. Br J Pharmacol 2002, 136: 1162–1168.

48 Elmgreen J, Ahnfelt-Rønne I and Nielsen OH. Inhibition of human neutrophils by auranofin: chemotaxis and metabolism of arachidonate via the 5-lipoxygenase pathway. Ann Rheum Dis 1989, 48: 134–138.

49 Liu C, Liu Z, Li M, Li X, Wong YS, Ngai SM and Zheng W, *et al*. Enhancement of auranofin-induced apoptosis in MCF-7 human breast cells by selenocystine, a synergistic inhibitor of thioredoxinreductase. PLoS ONE 2013, 8: e53945.

50 Marzano C, Gandin V, Folda A, Scutari G, Bindoli A and Rigobello MP. Inhibition of thioredoxinreductase by auranofin induces apoptosis in cisplatin-resistant human ovarian cancer cells. Free Radic Biol Med 2007, 42: 872–881.

51 Hsu SL, Hsieh YC, Hsieh WC and Chou CJ. Baicalein induces a dual growth arrest by modulating multiple cell cycle regulatory molecules. Eur J Pharmacol 2001, 425: 165–171.

52 Lee HZ, Leung HW, Lai MY and Wu CH. Baicalein induced cell cycle arrest and apoptosis in human lung squamous carcinoma CH27 cells. Anticancer Res 2005, 25: 959–964.

53 Shen YC, Chiou WF, Chou YC and Chen CF. Mechanisms in mediating the anti-inflammatory effects of baicalin and baicalein in human leukocytes. Eur J Pharmacol 2003, 465: 171–181.

54 Lin HY, Shen SC, Lin CW, Yang LY and Chen YC. Baicalein inhibition of hydrogen peroxide-induced apoptosis via ROS-dependent hemeoxygenase 1 gene expression. Biochim Biophys Acta 2007, 1773: 1073–1086.

55 Deschamps JD, Kenyon VA and Holman TR. Baicalein is a potent *in vitro* inhibitor against both reticulocyte 15-human and platelet 12-human lipoxygenases. Bioorg Med Chem 2006, 14: 4295–4301.

56 Ikemoto S, Sugimura K, Kuratukuri K and Nakatani T. Antitumor effects of lipoxygenase inhibitors on murine bladder cancer cell line (MBT-2). Anticancer Res 2004, 24: 733–736.

57 Hsieh YC, Hsieh SJ, Chang YS, Hsueh CM and Hsu SL. The lipoxygenase inhibitor, baicalein, modulates cell adhesion and migration by up-regulation of integrins and vinculin in rat heart endothelial cells. Br J Pharmacol 2007, 151: 1235–1245.

58 Tateson JE, Randall RW, Reynolds CH, Jackson WP, Bhattacherjee P, Salmon JA and Garland LG. Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids: biochemical assessment *in vitro* and ex vivo. Br J Pharmacol 1988, 94: 528–539.

59 Thornber K, Colomba A, Ceccato L, Delsol G, Payrastre B and Gaits-Iacovoni F. Reactive oxygen species and lipoxygenases regulate the

oncogenicity of NPM-ALK-positive anaplastic large cell lymphomas. Oncogene 2009, 28: 2690–2696.
60 Hussey HJ and Tisdale MJ. Inhibition of tumour growth by lipoxygenase inhibitors. Br J Cancer 1996, 74: 683–687.
61 Rao CV, Janakiram NB and Mohammed A. Lipoxygenase and cyclooxygenase pathways and colorectal cancer prevention. Curr Colorectal Cancer Rep 2012, 8: 316–324.
62 Fischer AS, Metzner J, Steinbrink SD, Ulrich S, Angioni C, Geisslinger G and Steinhilber D, *et al*. 5-Lipoxygenase inhibitors induce potent antiproliferative and cytotoxic effects in human tumour cells independently of suppression of 5-lipoxygenase activity. Br J Pharmacol 2010, 161: 936–949.
63 Koshihara Y, Neichi T, Murota S, Lao A, Fujimoto Y and Tatsuno T. Caffeic acid is a selective inhibitor for leukotriene biosynthesis. Biochim Biophys Acta 1984, 792: 92–97.
64 Kohyama N, Nagata T, Fujimoto S and Sekiya K. Inhibition of arachidonate-lipoxygenase activities by 2-(3,4-dihydroxyphenyl)ethanol, a phenolic compound from olives. Biosci Biotechnol Biochem 1997, 61: 347–350.
65 Shureiqi I, Chen D, Lee JJ, Yang P, Newman RA, Brenner DE and Lotan R, *et al*. 15-LOX-1: a novel molecular target of nonsteroidal anti-inflammatory drug-induced apoptosis in colorectal cancer cells. J Natl Cancer Inst 2000, 92: 1136–1142.
66 Chung TW, Moon SK, Chang YC, Ko JH, Lee YC, Cho G and Kim SH, *et al*. Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J 2004, 18: 1670–1681.
67 Wu J, Xia HH, Tu SP, Fan DM, Lin MC, Kung HF and Lam SK, *et al*. 15-Lipoxygenase-1 mediates cyclooxygenase-2 inhibitor-induced apoptosis in gastric cancer. Carcinogenesis 2003, 24: 243–247.
68 Cho H, Ueda M, Tamaoka M, Hamaguchi M, Aisaka K, Kiso Y and Inoue T, *et al*. Novel caffeic acid derivatives: extremely potent inhibitors of 12-lipoxygenase. J Med Chem 1991, 34: 1503–1505.
69 Nie D, Nemeth J, Qiao Y, Zacharek A, Li L, Hanna K and Tang K, *et al*. Increased metastatic potential in human prostate carcinoma cells by overexpression of arachidonate 12-lipoxygenase. Clin Exp Metastasis 2003, 20: 657–663.
70 Nunez-Anita RE, Cajero-Juarez M and Aceves C. Peroxisome proliferator-activated receptors: role of isoform gamma in the antineoplastic effect of iodine in mammary cancer. Curr Cancer Drug Targets 2011, 11: 775–786.
71 Berg C, Hammarström S, Herbertsson H, Lindström E, Svensson AC, Söderström M and Tengvall P, *et al*. Platelet-induced growth of human fibroblasts is associated with an increased expression of 5-lipoxygenase. Thromb Haemost 2006, 96: 652–659.
72 Neichi T, Koshihara Y and Murota S. Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. Biochim Biophys Acta 1983, 753: 130–132.
73 Park C, Jin CY, Kwon HJ, Hwang HJ, Kim GY, Choi IW and Kwon TK, *et al*. Induction of apoptosis by esculetin in human leukemia U937 cells: roles of Bcl-2 and extracellular-regulated kinase signaling. Toxicol In Vitro 2010, 24: 486–494.
74 Orning L and Hammarström S. Inhibition of leukotriene C and leukotriene D biosynthesis. J Biol Chem 1980, 255: 8023–8026.
75 Evans JF, Lévillé C, Mancini JA, Prasit P, Thérien M, Zamboni R and Gauthier JY, *et al*. 5-Lipoxygenase-activating protein is the target of a quinoline class of leukotriene synthesis inhibitors. Mol Pharmacol 1991, 40: 22–27.
76 Ford-Hutchinson AW. FLAP: a novel drug target for inhibiting the synthesis of leukotrienes. Trends Pharmacol Sci 1991, 12: 68–70.
77 Ozeki Y, Nagamura Y, Ito H, Unemi F, Kimura Y, Igawa T and Kambayashi Ji, *et al*. An anti-platelet agent, OPC-29030, inhibits translocation of 12-lipoxygenase and 12-hydroxyeicosatetraenoic acid production in human platelets. Br J Pharmacol 1999, 128: 1699–1704.
78 Bishayee K, Chakraborty D, Ghosh S, Boujedaini N and Khuda-Bukhsh AR. Lycopodine triggers apoptosis by modulating 5-lipoxygenase, and depolarizing mitochondrial membrane potential in androgen sensitive and refractory prostate cancer cells without modulating p53 activity: signaling cascade and drug-DNA interaction. Eur J Pharmacol 2012, 698: 110–121.
79 Meyers RO, Lambert JD, Hajicek N, Pourpak A, Kalaitzis JA and Dorr RT. Synthesis, characterization, and anti-melanoma activity of tetra-O-substituted analogs of nordihydroguaiaretic acid. Bioorg Med Chem Lett 2009, 19: 4752–4755.
80 Coffey MJ, Jarvis GE, Gibbins JM, Coles B, Barrett NE, Wylie OR and O'Donnell VB. Platelet 12-lipoxygenase activation via glycoprotein VI: involvement of multiple signaling pathways in agonist control of H(P)ETE synthesis. Circ Res 2004, 94: 1598–1605.
81 Gillard J, Ford-Hutchinson AW, Chan C, Charleson S, Denis D, Foster A and Fortin R, *et al*. L-663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-t-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. Can J Physiol Pharmacol 1989, 67: 456–464.
82 Aoki Y, Qiu D, Zhao GH and Kao PN. Leukotriene B4 mediates histamine induction of NF-kappaB and IL-8 in human bronchial epithelial cells. Am J Physiol 1998, 274: L1030–LL1039.
83 McMillan RM, Spruce KE, Crawley GC, Walker ER and Foster SJ. Pre-clinical pharmacology of ICI D2138, a potent orally-active non-redox inhibitor of 5-lipoxygenase. Br J Pharmacol 1992, 107: 1042–1047.
84 Kirchner T, Argentieri DC, Barbone AG, Singer M, Steber M, Ansell J and Beers SA, *et al*. Evaluation of the anti-inflammatory activity of a dual cyclooxygenase-2 selective/5-lipoxygenase inhibitor, RWJ 63556, in a canine model of inflammation. J Pharmacol Exp Ther 1997, 282: 1094–1101.
85 Proudman KE and McMillan RM. Are tolfenamic acid and tenidap dual inhibitors of 5-lipoxygenase and cyclo-oxygenase? Agents Actions 1991, 34: 121–124.
86 Otterness IG, Bliven ML, Downs JT, Natoli EJ and Hanson DC. Inhibition of interleukin 1 synthesis by tenidap: a new drug for arthritis. Cytokine 1991, 3: 277–283.
87 Tong WG, Ding XZ, Witt RC and Adrian TE. Lipoxygenase inhibitors attenuate growth of human pancreatic cancer xenografts and induce apoptosis through the mitochondrial pathway. Mol Cancer Ther 2002, 1: 929–935.
88 Tavolari S, Bonafè M, Marini M, Ferreri C, Bartolini G, Brighenti E and Manara S, *et al*. Licofelone, a dual COX/5-LOX inhibitor, induces apoptosis in HCA-7 colon cancer cells through the mitochondrial pathway independently from its ability to affect the arachidonic acid cascade. Carcinogenesis 2008, 29: 371–380.
89 Sarveswaran S, Thamilselvan V, Brodie C and Ghosh J. Inhibition of 5-lipoxygenase triggers apoptosis in prostate cancer cells via down-regulation of protein kinase C-epsilon. Biochim Biophys Acta 2011, 1813: 2108–2117.