REVIEW

Leukotriene Biosynthesis Inhibitors

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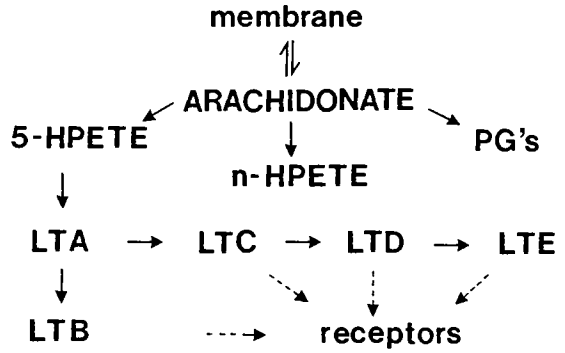
Abstract: This review describes the design and current development of leukotriene biosynthesis inhibitors as potential antiinflammatory agents. Knowledge of the enzymatic mechanism of 5-lipoxygenase led to specific inhibitors of this enzyme which catalyzes a key step in the leukotriene pathway. Competitive inhibitors include iron chelators, redox agents and alternate substrates. Further, the potential of product inhibitors and mechanism based inactivators of 5-lipoxygenase is discussed.

The release and metabolism of polyunsaturated fatty acids to biologically active metabolites constitutes a new and fascinating area of bioorganic chemical research (1–3). Biotransformation of arachidonic acid (AA) gives rise to a family of chemical mediators many of which possess potent and complex physiological, pathological, and pharmacological actions (4, 5). Some of the most potent agents in this series are the leukotrienes LTC_4, LTD_4, and LTE_4, which for many years were termed the "slow reacting substance of anaphylaxis" SRS-A (6). Selective inhibition of leukotriene (LT) biosynthesis could provide at least two significant advances. Leukotriene biosynthesis inhibitors could be useful as 1) pharmacological probes for *in vitro* molecular investigations and 2) as potential agents useful in the design of therapeutic entities useful for the treatment of disease states.

There are some problems associated with employing LT biosynthesis inhibitors (5, 7). First, metabolism of AA is very complex. New metabolic reactions of polyunsaturated fatty acids are routinely being described. As a consequence, the number of biologically active metabolites of AA is large, and the enzymes capable of metabolizing those metabolites and producing other mediators involved in disease states is correspondingly large. As a result, a large number of anabolic and catabolic processes may be triggered by the branching metabolic cascades stemming from AA (8). Secondly, the disease states that metabolites of AA are involved with are exceedingly complex and currently poorly understood (5, 9). This presumably arises because AA is involved in complex interactions at many levels, as well as with many other cellular mediators (some of which remain undiscovered) that may be produced in response to AA metabolites.

Metabolism of Arachidonic Acid

The oxidative products of AA metabolism (i.e., prostaglandins, leukotrienes, thromboxanes, and epoxides etc.) can be thought of as members of a family of chemical substances produced in the body as part of the immunochemical response. The agents produced by AA constitute a method for protection by which viruses, bacteria, and also certain cells such as tumor or damaged cells are recognized and dealt with.



Upon release from cellular phospholipids (10), AA is oxidatively metabolized by cyclooxygenase or lipoxygenase(s) to produce a variety of physiologically important compounds (11, 12). Oxidative metabolism of AA via the cyclooxygenase pathway sequentially generates two cyclic prostaglandin (PG) endoperoxides, PGG_2 and the corresponding cyclic endoperoxide alcohol, PGH_2 (13). The biological effects of prostaglandin endoperoxides include smooth muscle contraction (14) (i.e. prostaglandin H_2 is a potent contractor of the isolated human umbilical artery), complex cardiovascular effects (6) (i.e., the blood pressure response showed a complex triphasic pattern including a transient fall consistently followed by a short-lasting rise and then a sustained reduction), and unique effects on platelets (15) (i.e., PGG_2, and PGH_2 induce rapid and irreversible aggregation of human platelets). Depending on the cell type, PGH_2 may be rapidly converted to $PGF_{2\alpha}$, PGE_2, PGD_2, prostacyclin (PGI_2), or thromboxane A_2 (TXA_2) (3, 16). Thus, many of the effects of PG endoperoxides are not attributable to stable prostaglandin metabolites but rather unstable intermediates. This is a feature seemingly common to the AA metabolic cascade; minute amounts of biologically active and chemically unstable metabolites are biosynthesized to perform a biochemical task and then are dispensed metabolically or chemically. However, in cases where rapid metabolism or chemical decomposition of prostaglandin-endoperoxides to stable materials occurs, the range of biological activity may be due to these stable metabolites (17). The thromboxane class of arachidonate metabolite offers an extreme example of a biological activity-chemical reactivity relationship. Thromboxane A_2 (TXA_2) has been postulated to consist of a highly unstable bicyclic acetal compound (half life 30 seconds) with potent vasoconstricting and platelet aggregatory activity (18). TXA_2 is rapidly hydrolyzed to the relatively stable thromboxane B_2 that possesses considerably less biological activity (19). The main conclusions drawn from the early work concerning prostaglandin endoperoxides and thromb-

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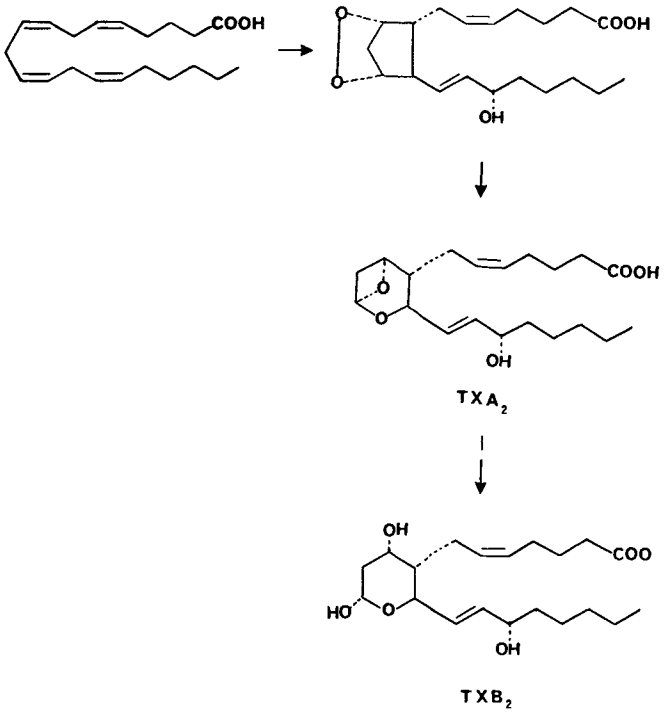
oxane A_2 are as follows: 1) once liberated, AA is converted very efficiently to exceedingly active biological agents, 2) the amount of material involved (i.e., 10–100 ng per 10^6 purified cells) is quite small, and 3) the extreme chemical or biochemical reactivity of unstable endoperoxide or thromboxane metabolites requires a short half life for biological activity.

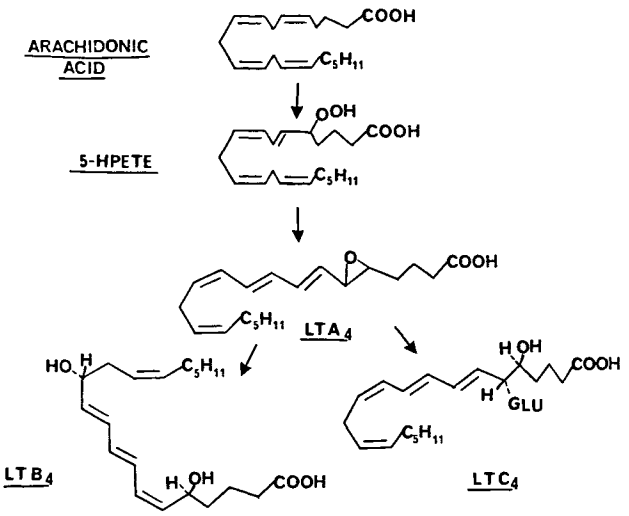


Recently, an entirely new class of arachidonic acid metabolites has been discovered. Unconjugated monoepoxide metabolites of AA have been shown to arise via a cytochrome P-450 mediated oxidation (20). Unconjugated epoxides are relatively chemically stable but are efficiently hydrated by cytosolic epoxide hydrolase to produce diols. There is an interesting regiochemistry associated with diol formation in that the most biologically potent epoxides are not substrates for cytosolic epoxide hydrase (21). In contrast, conjugated epoxides of the leukotriene or lipoxin class of AA metabolite formed via lipoxygenases are chemically unstable to hydrolysis, as well as metabolically unstable to biotransformation (22).

The observations concerning unstable prostaglandin and epoxide intermediates is recognizable in another branch of the arachidonate pathway, namely, the lipoxygenase cascade which leads to leukotrienes (2, 23, 24).

Leukotriene Biosynthesis

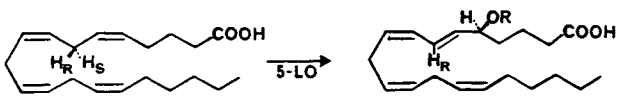
The biological effects of LTs can be divided into the important chemotactic effects of leukotriene B_4 (LTB_4) on the circulating neutrophil (25) and the effects of leukotriene C_4 (LTC_4) and LTD_4 as a bronchoconstrictor and pulmonary vasoconstrictor both *in vivo* and *in vitro* (26). LTC_4, LTD_4, and LTE_4 comprise the compounds that were once referred to as the "slow reacting substance of anaphylaxis" (SRS) introduced by Feldberg and Kellaway and characterized as producing a slow and prolonged contraction of a test smooth muscle strip maintained under tension in a suitable physiological bath (27). Later, Brocklehurst demonstrated the presence of SRS in the lung perfusate of an animal undergoing anaphylaxis (28). He named this material the "slow reacting substance of anaphylaxis" (SRS-A). Austen showed the involvement of SRS in asthma and other types of immediate hypersensitivity reactions (29). However, it was not until the structure determination studies confirmed by total synthesis of leukotrienes by Corey (30) that allowed Corey and Samuelsson to provide a structure for leukotriene C_4 (24, 31). A knowledge of the structure of leukotrienes and their precursors allowed a proposal for a biosynthetic mechanism (2). LTC_4 is biosynthesized by the following sequence (32–34): arachidonate is lipoxygenated to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is converted by the action of leukotriene A_4 (LTA_4) synthetase to the unstable allylic epoxide, LTA_4. LTA_4 may undergo two diverse biochemical reactions. Enzymatic hydrolysis produces LTB_4 by the action of LTA_4 epoxide hydrolase (6, 35). Alternatively, LTA_4 may be conjugated with glutathione by glutathione transferase to produce LTC_4 (23, 33). For the metabolism of arachidonate to LTC_4 it is generally accepted that the first and critical rate limiting step in the overall biosynthetic pathway is the 5-lipoxygenation catalyzed by the 5-lipoxygenase. Knowledge of the detailed enzymatic mechanism for this enzyme could be extremely helpful in the design of specific 5-lipoxygenase inhibitors.



5-Lipoxygenase Enzyme Mechanism

The lipoxygenase enzyme catalyzes the reaction involving the oxygenation of a 1,4-*cis,cis*-pentadiene to a 1-hydroperoxy-2,4-*trans,cis*-pentadiene subunit (36). The stereochemical aspects of the reaction have been investigated using linoleic (37) and arachidonic acid (38). In all cases studied thus far, the following absolute stereochemistry is followed. For arachidonic acid, in two different enzyme systems, the 5-lipoxygenase derived from potato and rat basophil leukemic (RBL-1) cells gave the absolute stereochemistry shown below. It was shown that 7(*R*)-deuterioarachidonic acid (compound **1**, Figure 4) is converted to 5(*S*)hydroperoxyeicosa-*trans*-6-*cis*-8,11,14-tetraenoic acid (5-HPETE) with essentially 100% of the deuterium retained. When the 7(*S*)-deuterioarachidonic acid was subjected to the same conditions, a large isotope effect on product formation was observed. Thus, in both the vegetable and mammalian lipoxygenase reactions studied, the 7-H_s hydrogen is removed in the conversion of arachidonate to 5(*S*)-HPETE (see Figure 4). The biosynthesis of leukotrienes from stereospecifically labeled arachidonic (39) and

eicosapentaenoic (40) acid with isotopic substitution at C-10 has also been investigated. Removal of hydrogen from C-10 is stereospecific in the biosynthetic conversion of HPETEs to leukotrienes. Thus, fatty acid oxygenation at C-5 by lipoxygenase is stereospecific and occurs before C-10 hydrogen elimination. Once formed, HPETEs undergo stereospecific elimination to form leukotriene A.



1 H_R = D

2 R=OH

3 R=H

It is also apparent from this work that molecular oxygen enters the diene system from the opposite side of the plane of the carbon system from that which the proton is abstracted (36). The double bond that moves always ends up *trans*. The involvement of singlet oxygen in the production of the *trans*-hydroperoxide is extremely unlikely since the latter species requires both proton abstraction and oxygen addition to take place on the same side of the plane of the allylic system (41). Knowledge that the leukotriene biosynthetic pathway involves an initial requirement for an enzymatic step of stereochemical preference discussed above provides important information to guide the rational design of 5-lipoxygenase inhibitors.

In general, the design of metabolic pathway inhibitors has focused on the inhibition of the critical or rate limiting step in the overall cascade. For the biosynthesis of leukotrienes, the rate determining enzyme is arguably the 5-lipoxygenase. There has been some suggestion that phospholipase A_2 is rate limiting but because of its important physiological role in cellular homeostasis, phospholipase A_2 has not been a major target of leukotriene inhibitor design. Lipoxygenase activity has been located in various tissues. Human platelets (18), guinea pigs lung (42), spleen homogenates (43), rabbit polymorphonuclear leukocytes (PMNL) (44), human neutrophil (45), rat basophilic leukemia (RBL-1) cells (33), rabbit reticulocytes (46), guinea pig peritoneal PMNL (47), as well as a great many other tissues have lipoxygenase activity. 5-Lipoxygenase activity has also been demonstrated from vegetable sources (48, 49). The choice of an enzyme source depends on an investigator's requirements for product regiospecificity and purity, and enzyme preparation convenience. However, it is not sufficient to demonstrate the production of HETEs from tissues in order to demonstrate lipoxygenase activity, since the ubiquitous enzyme cytochrome P-450 has been shown to form hydroxyeicosatetraenoic acid (HETE) metabolites of arachidonic acid in relatively high yield (50, 51).

Generally, subcellular localization of 5-lipoxygenase activity resides in the cytosolic fractions from post microsomal supernatants. However, there have been some reports of other 5-lipoxygenase subcellular distribution (47); LTC_4 synthetase activity apparently is also present in greatest concentration in the 105 000 × g pellet of the post microsomal fraction (53).

Although there are many lipoxygenase isozymes from various mammalian and plant sources, our discussion will primarily focus on the 5-lipoxygenase from RBL-1 cells. The molecular weight of the dilute enzyme is approximately 73 000 (54). In the presence of Ca^{2+}, the apparent molecular weight of the lipoxygenase increases two-fold. Unpurified 5-lipoxygenase activity is generally stimulated by the presence of Ca^{2+} (55), nucleotides (i.e., ATP and to a lesser degree GTP) (47), and divalent cations ionophore A23187 (56). 12-HPETE has been reported to specifically stimulate its own production and stimulate leukotriene biosynthesis in leukocytes by increasing lipoxygenase activity (57).

Most evidence points to essential ferric iron and sulfur containing amino acids at the active site of lipoxygenases (58). The mechanistic details of non heme, non acid labile sulfide iron (to be discussed below) have eluded most investigators, primarily because attempts to purify and stabilize mammalian lipoxygenase have met with little success. Recent reports outlining new enzyme purification procedures indicate that this situation may be changing, however (49, 59).

The best unsaturated fatty acid substrates for the 5-lipoxygenase are: arachidonic (20:4); 5,8,11,14,17-eicosapentaenoic (20:5); 5,8,11-eicosatrienoic (20:3); 5,8,11,14-octadecatetraenoic (18:4); 5,8,11,14-nonadecatetraenoic (19:4), which were readily converted to HETEs (60, 61). The primary structural requirement for lipoxygenase activity is $\Delta^{5,8,11}$ unsaturation in a polyenoic fatty acid. Fatty acids with initial unsaturation at Δ^4, Δ^6, Δ^7, or Δ^8 are poor substrates for the RBL 5-lipoxygenase. Apparently, the most critical structural feature for substrate interaction with the 5-lipoxygenase is a 1,4-*cis,cis* pentadiene moiety (or something resembling it) five carbons removed from a carboxylate (or chemical isostere) which should be capable of hydrogen bonding. Surprisingly, it was discovered that an arachidonate carboxylate functionality is not an absolute requirement for lipoxygenase substrate activity (62). Other arachidonate (C_1) substituents are also tolerated for substrate as well as inhibitor activity (63). Chain length does not appear to play a dominant role for substrate activity but as discussed below, many chain shortened materials are active inhibitors and serve as interesting lipoxygenase active site probes.

Mechanistic Information Useful in Inhibitor Design

In the following discussions we will consider how the mechanistic details of lipoxygenase action (discussed above) have contributed to the design of potent tight binding reversible as well as mechanism-based inhibitors of 5-lipoxygenase. For optimal competitive enzyme inhibitory efficiency, an inhibitor I should form tight reversible complexes with the enzyme E. Tight reversible binding (i.e., small K_1) will increase specificity and result in selective occupation of the enzyme (i.e. see equation [1])

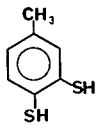
$$E + I \overset{K_1}{\rightleftharpoons} E \cdot I \overset{K_2}{\rightleftharpoons} E \sim I \qquad \text{eq. [1]}$$

The structural features of the active site (i.e., a ferric non heme-iron, a hydrophobic domain, and a hydrogen-bond donor five carbons removed from the peroxidized olefin) are the features available for inhibitor design. As a result, at least four classes of inhibitors have been designed and evaluated including: 1) iron chelating inhibitors, 2) substrate-related analogs; 3) antioxidants, and 4) structurally unrelated (discovered) inhibitors.

Competitive Inhibitors

Iron Chelators

It has been observed that iron chelators are effective inhibitors of mammalian lipoxygenases. The dithiophenol **4**, the thioureido compound **5**, and 6-mercaptopurine **6** are effective inhibitors of platelet lipoxygenase (64). A variety of catechol



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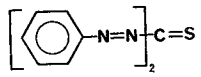

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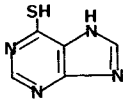

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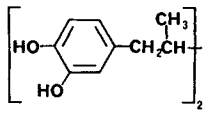
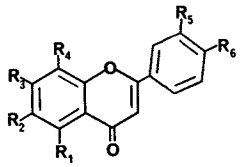
structures are also effective lipoxygenase inhibitors. Caffeic acid (and caffeic acid methyl ester) (65), esculetin (66), baicalein (67), anthralin (68), cirsiliol and other flavonoids (69), guaiacol, nordihydroquararetic acid (NDGA, see Figure 6) as well as other compounds possess catechol or related functionality and demonstrate efficient lipoxygenase inhibition activity (70). The compounds are shown in Figure 6. It

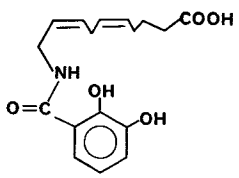


NDGA



	R_1	R_2	R_3	R_4	R_5	R_6
Cirsiliol	OH	OCH_3	OCH_3	H	OH	OH
Quercetin	OH	H	OH	H	OH	OH
Baicalein	OH	OH	OH	H	H	H

remains unclarified whether the catechol containing inhibitors function to inhibit 5-lipoxygenase activity because of chelation of prosthetic iron or by some other mechanism. In fact, data suggest that some catechol inhibitors may serve as antioxidants, since for some inhibitors non-competitive inhibitor kinetics are observed. Recently, some catechol derivatives of fatty acids have been prepared and tested as inhibitors of RBL 5-lipoxygenase (63). The catechol **7**, a potential metal coordinator, was designed to be placed distal to the carboxylic function and on the side of the C(5)-C(9)-1,4-diene subunit, proximate to the region that might be occupied by catalytic metal ion. The 2,3-dihydroxybenzoyl group is known to possess high affinity for Fe(III), and catechol **7** was found to inhibit RBL 5-lipoxygenase with an EC_{50} of 10 μM. Other similar truncated arachidonate derivatives were synthesized and tested but were significantly less active as inhibitors of RBL 5-lipoxygenase (i.e. compounds **8** and **9** have EC_{50} values of 50 and 100 μM, respectively). Modification of the carbonyl



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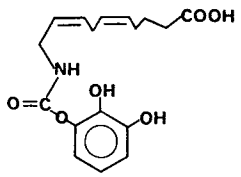

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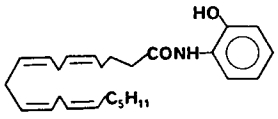

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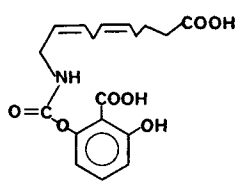

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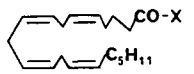
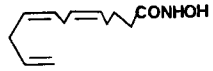
terminus of arachidonic acid with a good hydrogen atom donor group produced a compound with reasonable 5-lipoxygenase inhibitory activity (63). The phenolic arachidonamide **10** inhibits 5-HPETE formation with a K_i value of 5.5 μM. Recently, workers at the Ono Pharmaceutical Company have extended this type of 5-lipoxygenase inhibitor studies. They showed that 4-arylthio-2-aminophenol was much more potent *in vitro* and *in vivo* than *ortho*-aminophenol (IC_{50} value is 2.5 μM) at inhibiting RBL 5-lipoxygenase (71).

In view of the effective degree of inhibition by aminophenol derivatives of arachidonic acid, other amide derivatives of arachidonic acid in which strong chelation to iron was possible were synthesized. *N*-Hydroxyarachidonamide is capable of mimicking arachidonic acid as well as coordination to a metal ion (*N*-hydroxyamides are excellent ligands for Fe(III) with $K_{assoc} = 10^{12}$). Hydroxamates **11**, **12** and **13** are potent inhibitors of the 5-lipoxygenase from RBL cells (62). In addition, chain shortened hydroxamate analogs of arachidonic acid are also effective inhibitors. For instance, the synthetic hydroxamates **14** and **15** had EC_{50} values of 1.9 and 15 μM, respectively, for RBL 5-lipoxygenase inhibition. The thiohydroxamate of arachidonic acid was synthesized and also proved to be effective at inhibiting the 5-lipoxygenase (i.e. arachidonate thiohydroxamate has an EC_{50} value of 2.0 μM) (72).





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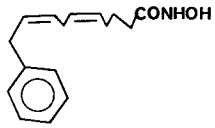
	substituent	K_i (μM)
11	X = NHOH	0.17
12	X = $N(CH_3)OH$	0.04
13	X = N(t-Bu)OH	0.11



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Redox Agents as Inhibitors

The conversion of arachidonic acid to 5-HPETE can be modulated by a variety of redox agents (73). There are at least two possible mechanisms for antioxidant inhibition. First, antioxidants can reduce hydroperoxides and depress 5-lipoxygenase activity since there is some evidence that hydroperoxides initiate the enzymatic reaction. Antioxidants could also serve to reduce other important enzyme active site functionalities and impair function. The workers at Merck Sharp and Dohme Research Laboratories have demonstrated both of these mechanisms (74). Incubation of RBL 5-lipoxygenase with glutathione peroxidase and glutathione decreased 5-HETE formation in a concentration dependent fashion. They also showed that a series of substituted diphenyl disulfides were reasonably effective at inhibiting the 5-lipoxygenase (74). A number of agents have been shown to be effective chemical antioxidants and also effective antiinflammatory agents. Inhibition of lipoxygenase activity has been implicated in the antiinflammatory mechanism of action. Antioxidants nordihydroquairetic acid (75), quercetin (70), 3-*t*-butyl-4-hydroxyanisole (BHA) (76), α-tocopherol (vitamin E) (77) as well as others (78) have demonstrated some degree of lipoxygenase inhibitory activity. In addition, some diynyl substituted 1,4-benzoquinones are effective 5-LO inhibitors (79).

Alternate Substrates as Inhibitors

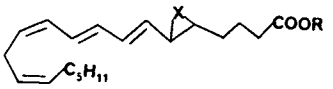
As mentioned above, the primary structural requirement for RBL 5-lipoxygenase substrate activity is a $\Delta^{5,8,11}$ center of unsaturation in a polyenoic fatty acid (60). Fatty acids with the initial site of unsaturation at Δ^4, Δ^6, Δ^7 or Δ^8 are poor substrates for the 5-lipoxygenase. Although not expressly tested, presumably these poor alternative substrates should also be competitive inhibitors. This has been borne out in a few studies. For example, docosahexaenoic acid is a $\Delta^{4,7,10,13,16,19}$ polyunsaturated fatty acid that is converted by RBL 5-lipoxygenase to HETEs to an extent of about 3 % that of arachidonic acid (80). Further docosahexaenoic acid was a poor 5-lipoxygenase competitive inhibitor (i.e. $K_i \sim 390$ μM). Interestingly, the hydroxamate of docosahexaenoic acid, although a less potent inhibitor of 5-lipoxygenase than arachidonate hydroxamate was nevertheless a competitive inhibitor (i.e. $K_i \approx 0.43$ μM) (62). The physiological significance of dietary fatty acids such as docosahexaenoic acid to serve as lipoxygenase inhibitors is doubtful since sufficient quantities of free docosahexaenoic acid to inhibit the 5-lipoxygenase may not exist (81). However, docosahexaenoic acid is a powerful competitive inhibitor of cyclooxygenase (i.e. K_i = 360 nM), and the cardiovascular protective effects of certain populations to dietary intake of fish lipid may be due in part to this feature of docosahexaenoic acid (80). 5,8,11,14,17-Eicosapentaenoic acid has been reported to be a poorer substrate for prostaglandin biosynthesis than arachidonate (82, 84). This is consistent with observations of long bleeding times and decreased platelet aggregation of some groups of people on a high fish diet (83). Eicosapentaenoic acid has also been shown to be a slightly better substrate than arachidonate for PMNL 5-lipoxygenase. In addition, eicosapentaenoic acid is a competitive inhibitor of 5-HPETE formation (i.e. K_i = 25 μM) as well as an inhibitor of thromboxane A_2 synthesis (84).

Substrate analogs of arachidonic acid have also been synthesized and tested as inhibitors for RBL 5-lipoxygenase. Arachidonamide is a weak competitive inhibitor of RBL 5-lipoxygenase (i.e. K_i = 64 μM) as well as a very poor substrate (62).

Recently, several structurally modified arachidonate analogs have been evaluated as inhibitors of leukotriene biosynthesis. As discussed above, the rate determining enzyme catalyzed reaction for leukotriene biosynthesis is C-7 proton abstraction. Consequently, 7,7-disubstituted arachidonate analogs were synthesized and investigated as potential leukotriene biosynthesis inhibitors. Such inhibitors could interfere at two points in leukotriene biosynthesis: 1) compounds might block the formation of 5-HPETE or 2) might inhibit the conversion of 5-HPETE to LTA_4. However, 7,7-dimethyl as well as 10,10-dimethylarachidonate actually stimulated 5-HPETE formation from human PMNs and were only mild inhibitors of RBL 5-lipoxygenase (85–89). 7,7'-Ethanoarachidonic acid as well as 5,6-benzoarachidonic acid have been shown to be weak competitive inhibitors (88, 89). Substitution of the C-7 methylene bridge of arachidonic acid with sulfur has resulted in the discovery of an entirely new class of 5-lipoxygenase inhibitors (discussed below). Interestingly, the C-7 *S*-oxide derivatives of thiaarachidonic acid and truncated analogs (Figure 11) are strictly competitive inhibitors. As discussed below, studies of thiaarachidonic and S-oxide derivatives thereof provide interesting mechanistic insight into 5-lipoxygenase activity (90).

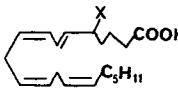
Product Inhibitors

It has been reported that the products of lipoxygenase reactions (i. e. HPETEs and HETEs) can serve to modulate the 5-lipoxygenase from various sources. 12- and 15-HPETE have been shown to be irreversible inhibitors and 12- and 15-HETE reversible inhibitors of 5-lipoxygenase (91–93). In addition, some analogs of a 5-lipoxygenase product, leukotriene A_4, have shown considerable 5-lipoxygenase inhibitory activity. Replacement of the allylic epoxide oxygen of LTA_4 with a methylene group afforded effective 5-lipoxygenase inhibitory activity. Interestingly, 5,6-methano LTA_4 methyl ester (Figure 9, compound **16**) is a more effective inhibitor (i. e. ED_{50} = 18 μM) than the corresponding free acid (94). The sulfur-containing analog of LTA_4 (i. e. thiirane **17**) was synthesized but all attempts to hydrolyze the methyl ester met with failure (95, 88). The methyl ester itself was tested as an inhibitor but was found to be only weakly active against the 5-lipoxygenase. The aziridine analog of LTA_4 has been synthesized but biological results have not been reported, as yet (97).



16 X = CH_2
17 X = S
18 X = NH

Various stable analogs of 5-HPETE have been synthesized and tested as potential 5-lipoxygenase inhibitors. As discussed above, 5-HETE and 5-HPETE (i. e. compounds **3** and **2,** respectively) demonstrate competitive and irreversible 5-lipoxygenase inhibitory activity, respectively. The carba analog of 5-HPETE (i. e. compound **19**) has an IC_{50} = 100 μM when tested against the RBL 5-lipoxygenase (98). The *cis* and *trans* oximes of 5-keto-6,7-*trans*-8,11,14-*cis*-eicosatetraenoic acid are poor competitive inhibitors (i. e. compounds **20** and **21** have IC_{50} values in the range 300 to 500 μM, respectively) (99). From the relatively poor inhibitor activity of product-like compounds it is apparent that product-like inhibitors are probably inappropriate goals for inhibitor design.



3 X = OH
2 X = OOH
19 X = CH_2OH
20,21 X = =N–OH

Mechanism Based Inactivators

Although extremely tight binding high affinity competitive inhibitors are desirable, there are certain advantages to mechanism based irreversible inactivators. First, much mechanistic information about enzyme binding and catalysis can be obtained (100). Second, mechanism based inactivators are potentially more selective. For optimal efficacy, mechanism based inactivators should form tight, reversible complexes with the enzyme. Tight reversible binding will enhance specificity and, in most cases, decrease the concentration of inhibitor required to obtain maximal inactivation.

The overall dissociation constant for reversible binding (K_1) is part of the ratio (R) which includes the rate constant for covalent complex formation (eq. 2 and 3).

$$E + I \overset{K_1}{\rightleftharpoons} E \cdot I \overset{k_i}{\rightarrow} E \sim I \qquad \text{eq. [2]}$$

$$(E)\,(I)/(E \sim I) = K_1 k_i = R \qquad \text{eq. [3]}$$

Structural features of the inhibitor that increase K_1 will consequently enhance k_i. Observations about inhibitor binding

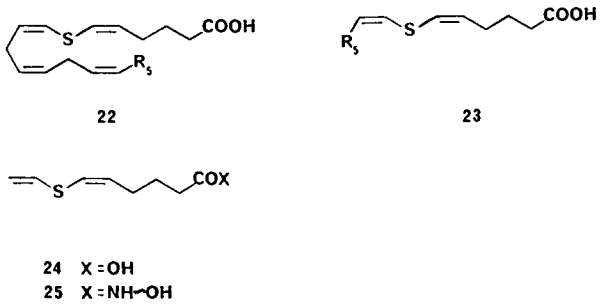
potentially could lead to the design of more potent irreversible inactivators, since an increase in K_1 should lead to an increase in R which describes the ratio of noncovalent to covalent enzyme complexes. Irreversible inhibitors have advantages over competitive inhibitors in that high intracellular concentrations of inhibitor are not required and consequently uptake, catabolism and disposition problems associated with lipoxygenase inhibitors may be obviated.

Quite early in the study of compounds effective as antiinflammatory agents the acetylenic agent eicosatetraynoic acid (ETYA) was observed to be effective *in vivo*. The mechanistic basis for this observation remained unclear until a number of groups reported that ETYA inhibits cyclooxygenase and lipoxygenase enzymes. Studies of the mechanism of ETYA were extended by Corey when he demonstrated that acetylene regioisomers inhibited various lipoxygenases in a regioselective fashion. For instance, 5,6-dehydroarachidonic (5,6-DHA) selectively inhibited RBL 5-lipoxygenase but not the arachidonate 15-lipoxygenase from soy bean, while 14, 15-dehydroarachidonic acid (14,15-DHA) demonstrated the converse regioselectivity (104, 105). However, 5,6-DHA behaves as a weak competitive inhibitor and substrate of cyclooxygenase and undergoes conversion to 5,6-dehydro-PGE_2 (106). A number of *in vitro* studies demonstrate that 5,6-DHA is a mechanism based inactivator of 5-lipoxygenase (107, 108). Inhibition of 5-lipoxygenase by 5,6-DHA requires O_2, Ca^{2+} and active enzyme and occurs in a time dependent irreversible manner. These observations support the hypothesis that 5-lipoxygenase inactivation is due to oxidation of 5,6-DHA to a vinylic hydroperoxide which rapidly decomposes to radicals capable of lethal damage to the catalytic site. This idea is further strengthened by the observation that when 5,6-DHA carries a deuterium (*7R*) at the site of hydrogen removal in the 5-lipoxygenase reaction of arachidonate, 5-lipoxygenase deactivation is subjected to a large primary kinetic isotope effect (63).

Rational chemical modification of the C-1 terminus of 5,6-DHA results in markedly improved 5,6-DHA inhibitory activity. Replacement of the carboxylate moiety with more polar and better hydrogen-bond acceptor groups results in a better C-1 substituted 5,6-DHA mechanism based inactivator (63). While the detailed mechanism of 5,6-DHA 5-lipoxygenase inactivation is still unclear, it is apparent that replacement of a *cis* HC=CH unit of arachidonic acid by a C≡C unit at the site of lipoxygenation leads to a dehydroarachidonic acid that effectively deactivates the enzyme.

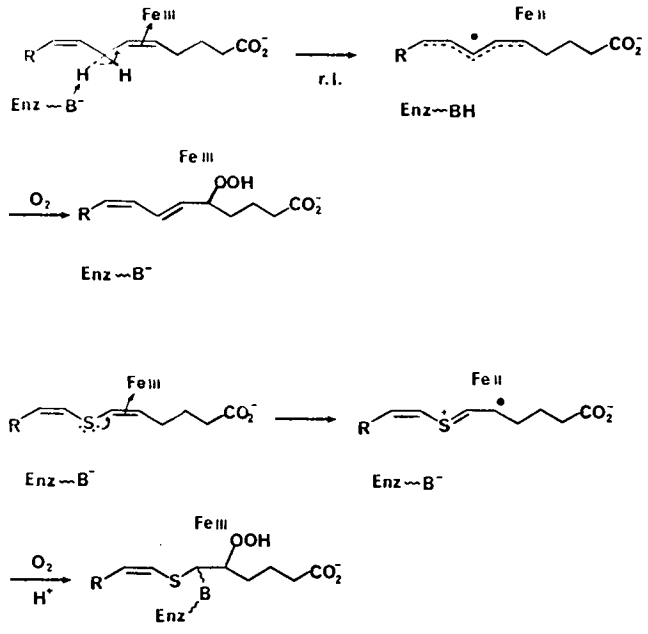
Further support for 5,6-DHA inactivation via allene hydroperoxide intermediate comes from studies of 4,5-dehydroarachidonic acid (4,5-DHA). Although considerably less stable to the incubation conditions than 5,6-DHA, the allene is significantly more potent than the acetylene as a mechanism based inactivator (109, 110). 4,5-DHA obeys all of the criteria set forth above for 5-lipoxygenase inactivation by 5,6-DHA. This is consistent with the hypothesis that the intermediacy of a vinyl hydroperoxide or other reactive rearrangment product is the critical intermediate that impairs 5-lipoxygenase function in an irreversible fashion for these acetylenic compounds. Other workers have noted that an essential methionine residue of reticulocyte lipoxygenase is *S*-oxidized by ETYA (107). Further work with the purified enzyme will be required to distinguish between these two mechanisms.

On the basis of the 5-lipoxygenase inhibitory properties of C-7 substituted arachidonates a new class of 5-lipoxygenase inhibitors has emerged. Replacement of the C-7 methylene bridge with a sulfur provides a hydrophobic arachidonate analog which retains the correct geometry in a *cis,cis*-divinyl sulfide arrangement. 7-Thiaarachidonic acid **22** as well as a number of chain shortened *cis,cis*-divinyl sulfides **23, 24** demonstrated time dependent inactivation of RBL 5-lipoxygenase (Figure 11) (90). Enzyme inactivation requires oxygen,



Ca^{2+} and active enzyme, suggesting that mechanism based inactivation involves at least in part normal catalytic function. Modification of the carboxylate function of divinyl sulfide did not improve the potency of the inhibitor to the same extent as in the case of 5,6-DHA. Possibly, this is related to the fact that the divinyl sulfide is a more potent inactivator than 5,6-DHA. Interestingly, replacement of the carboxylate function of divinyl sulfide with a hydroxamate moiety abolished time dependent 5-lipoxygenase inactivation (i.e. hydroxamate **25** was a competitive inhibitor $K_i = 4.2\,\mu M$).

Divinyl sulfide hydroxamate presumably chelates prosthetic iron and prevents the normal catalytic function that converts divinyl sulfide into a mechanism based inactivator. This suggestion is in agreement with the mechanism proposed for 5-lipoxygenase action summarizing known mechanistic data as well as information about enzyme mechanism developed from inhibitor studies. As discussed above, 7-thiaarachidonic acid *S*-oxide and truncated homologues are not mechanism-based, but rather, simple competitive inhibitors. Taken together, the above studies (as well as many others) are consistent with the mechanism shown in Figure 12 as introduced by Corey for 5-lipoxygenase activity (90).



Summary

Oxidative metabolites of arachidonic acid produced by the 5-lipoxygenase pathway are potent mediators of hypersensitivity and inflammation (111, 112). Many pathophysiological conditions including asthma, chronic inflammation, smooth muscle spasm and psoriasis have been associated with high lipoxygenase activity. While experimental paradigms of inflammation for edema, pyrexia, hyperaemia, hyperalgesia and leukocyte migration are available, the rapid screening of antiasthmatic agents is not readily accomplished (113, 114). As a convenient model system for screening antiinflammatory agents, the *in vitro* inhibition of lipoxygenase and leukotriene products has become widely accepted. There are several problems associated with 5-lipoxygenase inhibitors that must be overcome before effective *in vivo* agents are established.

Biotransformation of 5-lipoxygenase inhibitors to metabolites that have no 5-lipoxygenase inhibitory activity or that possess other distinct biological activities can pose complications to *in vivo* evaluation of inhibitors. For instance, 5,6-DHA, a potent and selective 5-lipoxygenase inhibitor *in vivo* is metabolized to a dehydro prostaglandin *in vivo*. Metabolic *N*-demethylation of 3-amino-methyl-1-[3-(trifluoromethyl)-phenyl)]-2-pyrazoline (BW 540C) to BW755C markedly alters the specificity of inhibition for 5-lipoxygenase and produces an agent equipotent against cyclooxygenase (115). Dual inhibition of both 5-lipoxygenase and cyclooxygenase pathways could lead to enhanced beneficial antiinflammatory properties, and this has been suggested for nordihydroquairetic acid (116), BW 755C (117), timegadine (116), and 2-acetylthiophene-2-thiazolyhydrazone (CBS-1108) (118).

Fatty acid analogue inhibitors of 5-lipoxygenase may be taken up and incorporated into phospholipid *in vivo*. For this reason, agents that are designed to circumvent this problem may be potentially more effective *in vivo*. Another approach to producing high concentrations of antiinflammatory agents at secretory cells envisions selective drug uptake, internalization and accumulation. The antiallergic drug disodium cromoglycate (DSCG) binds to the outer membrane of mast cells and probably exerts its effect on Ca^{2+} gating (119). The effectiveness of agents such as DSCG is limited because the agent is reversibly associated and conjugation or other modification can occur (120). Specific uptake of a 5-lipoxygenase inhibitor prodrug or mediator release prodrug and subsequent enzymatic action (i.e. hydrolysis) could afford an active agent with high internal concentration of drug per cell. Such an agent (i.e. REV 2871) is currently being investigated by the Revlon Health Care Group. Although there are shortcomings of agents currently available as 5-lipoxygenase inhibitors, understanding of the mechanism and action *in vitro* of 5-lipoxygenase should provide important insight for the design of potent leukotriene biosynthesis inhibitors.

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