Perspective

5-Lipoxygenase: Properties, Pharmacology, and the Quinolinyl(bridged)aryl Class of Inhibitors

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Introduction

Feldberg, Kellaway, and Trethewie[1,2] a half century ago first recognized that stimulated lung tissue can produce a material, SRS-A (slow reacting substance of anaphylaxis), which slowly induces sustained contractions of guinea pig smooth muscle; however, it was not until the chemical characterization of SRS-A[3] by Samuelsson[4-6] and Corey[7,8] as several discrete chemical entities that rapid progress was made in the elucidation of the 5-lipoxygenase (5-LO) pathway. Products of the 5-LO pathway include the peptide and non-peptide leukotrienes (LTs), the hydroperoxy- and hydroxyeicosatetraenoic acids (HPETEs and HETEs)[9] and the lipoxins, most of which are involved in a broad variety of functions and disease processes. Inhibitors of 5-LO could represent a major breakthrough in asthma therapy and could have therapeutic utility in a number of other inflammatory disease states including rheumatoid arthritis, inflammatory bowel disease, psoriasis, and glomerulonephritis. In this review, we describe the properties and pharmacology of 5-LO and its products, then place the 2-quinolinyl(bridged)aryl class of 5-LO inhibitors into perspective, and finally make a prognosis for clinical efficacy. Special emphasis is placed on the discovery of 2-quinolinyl(bridged)aryl system as modulators AA cascade because we thought that the history of this particular class would be informative, especially when related to all the other classes of 5-LO inhibitors. In addition, there are frequent references to cyclooxygenase (CO), the other major enzyme in the AA cascade, since inhibition of the CO pathway is thought to explain the therapeutic effects of nonsteroidal antiinflammatory agents (NSAIDs) in rheumatic diseases.[10] Since a Perspective entitled "Peptide Leukotrienes: Current Status of Research" was published in *J. Med. Chem.* which summarized the state-of-the-art of peptide LT antagonists,[11]

we will not concentrate on this aspect of modulation of the 5-LO pathway, although many of the 2-quinolinyl-(bridged)aryl compounds have LTD$_4$ antagonist properties. For a comprehensive monograph on LTs, their involvement in disease, and compounds that modulate their ef-

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(2) Kellaway, C. H.; Trethewie, E. R. The Liberation of Slow Reacting Smooth Muscle-stimulating Substance in Anaphylaxis. *Q. J. Exp. Physiol.* 1940, *38*, 121–145.

(3) Actually, Brocklehurst in 1960 first used the term "slow reacting substance of anaphylaxis" (SRS-A) to describe material produced by human lungs on immunological challenge by antigens. See: Brocklehurst, W. E. The Release of Histamine and the Formation of a Slow-reacting Substance (SRS-A) during Anaphylactic Shock. *J. Physiol.* 1960, *151*, 416–435.

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(9) For a recent review of HPETEs, see: Pace-Asciak, C. R.; Asotra, S. Biosynthesis, Catabolism, and Biological Properties of HPETEs, Hydroperoxide Derivatives of Arachidonic Acid. *Free Radical Biol. Med.* 1989, *7*, 409–433.

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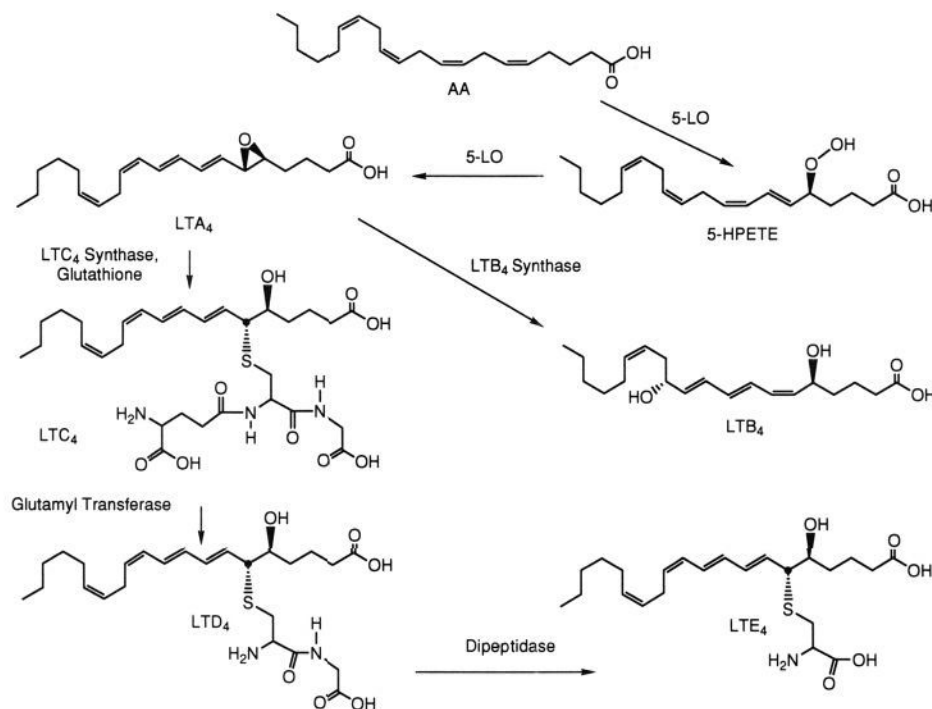† Current address: Glycomed, Inc., 860 Atlantic Ave., Alameda, CA 94501.
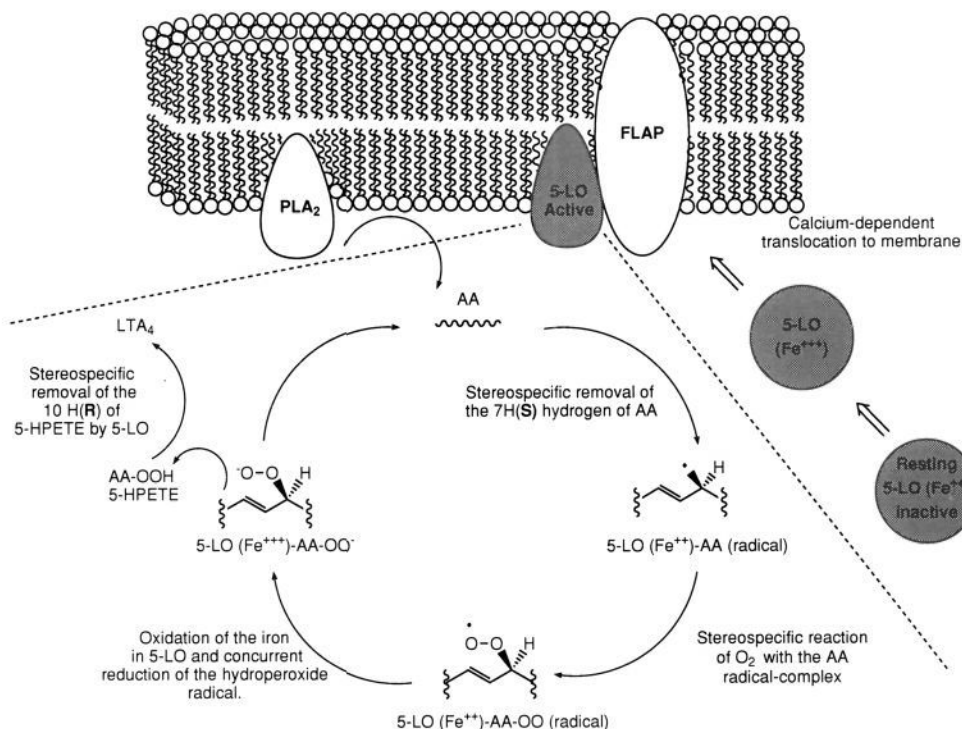
Figure 1. Arachidonic acid cascade.

Figure 2. A mechanism of the reaction of 5-LO with AA.

fects, see *Leukotrienes and Lipoxygenases* edited by J. Rokach.[12]

I. Properties and Sources of the 5-LO Enzyme

The 5-LO enzyme, which is found primarily in cells of myeloid origin such as polymorphonuclear leukocytes (PMNs) and eosinophils, catalyzes the first step of the biochemical pathway (Figure 1) in which arachidonic acid (AA) is converted into the LTs. Recently, reviews on the

properties of 5-LO as a class in general[13] and in mammals in particular[14] were published. We will introduce what is known about the mechanism of action for the 5-LO enzyme, then discuss the sources of the enzyme, and end the section with an assessment of the impact recombinant

(12) *Leukotrienes and Lipoxygenases*; Rokach, J., Ed.; Elsevier: New York, 1989.

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deoxyribonucleic acid (DNA) technology is having on the study of 5-LO structure and function.

5-LO Mechanism of Action. Excellent discussions of LO mechanisms were recently published[15,16] which can provide additional references beyond the scope of our Perspective. We have summarized a large amount of data on 5-LO and present it as a plausible mechanism of action which is shown in Figure 2. However, we caution the reader not to take this representation for the 5-LO mechanism of action too literally for reasons which will become apparent from our subsequent discussions on the oxidation states of iron, radical intermediates, and peroxides as cofactors.

The 5-LO enzyme is postulated to be normally in the dormant ferrous state (Fe^{2+}). It is found in the cytosol which is spatially removed from AA, the substrate found in the cell membrane. Upon activation by hydroperoxides, adenosine triphosphate (ATP) and calcium (Ca^{2+}), the 5-LO enzyme is converted to the active ferric form (Fe^{3+}) and this form translocates, possibly due to a change from a hydrophilic conformation into a hydrophobic conformation, to the cell membrane where it docks with a transmembrane protein FLAP (five lipoxygenase activating protein). Once docked to the cell membrane, it acts on the substrate AA which is then oxidized in a stereoselective, free radical process. First, there is a removal of the 7H(S) hydrogen of AA which is then followed by a reaction of oxygen (O_2) with the AA radical complex. All that remains is the oxidation of the iron in 5-LO and concurrent reduction of the hydroperoxide radical to complete the cycle and give 5-HPETE. In addition, the 5-LO enzyme serves another purpose, the catalytic conversion of 5-HPETE to LTA$_4$. What follows are some of the facts and controversies concerning the 5-LO mechanism of action.

Although it is generally accepted that 5-LO contains a non-heme iron,[17,18] the exact nature of the iron is controversial. Either Fe^{3+} or Fe^{2+} forms are claimed as the resting state of the iron in 5-LO. Most work investigating the nature of the iron in LO was done on soybean 15-LO due to its greater stability and availability. Incorporation of ^{57}Fe into 15-LO has unequivocally established that the native enzyme contains high spin Fe^{2+}.[19] Mossbauer spectral analysis of 15-LO enriched in ^{57}Fe revealed that the iron appears to cycle between Fe^{2+} and Fe^{3+}.[20] Recent work on 15-LO suggests that higher iron species such as ferryl are not involved.[21] The Fe^{2+} form was assumed to be involved in oxygen binding. This may not be a valid assumption since it was found that native 15-LO does not

bind oxygen.[22] Thus, it is assumed that resting 5-LO is in the inactive Fe^{2+} form and that the 5-LO enzyme functions largely as an activator of AA through interaction with Fe^{3+} iron. If the analogy to lipid peroxidation can be used, the iron would involve species that are not readily reducible.[23] The reduction potential for the iron in 15-LO was estimated to be +0.6V.[24] The Fe^{2+} iron in 15-LO appears to be surrounded by six oxygen and/or nitrogen ligands, four of which are imidazoles.[25] One water or hydroxide molecule appears to be coordinated to the active site Fe^{3+} iron of 15-LO.[26] Recently it was proposed that the iron in 5-LO may involve an "outer sphere" rather than "inner sphere" type process.[27]

There are considerable data which favor a radical mechanism for LO catalysis.[28–31] For example, dimers of fatty acids are formed in the absence of oxygen. Also, there is now evidence for the formation of a peroxy radical that is bound to soybean LO.[32] Further support for the radical mechanism with soybean LO was shown with radical trapping experiments.[33] Porcine leukocyte 5-LO was found to possess a pseudoperoxidase activity.[34] The steps in the radical mechanism involve a stereospecific hydrogen removal and O_2 addition which are reported to take place from opposite sides of the pentadiene system.[35,36] In the

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porcine leukocyte 5-LO system, hydroxamic acid- and hydroxyurea-containing 5-LO inhibitors are oxidized during the catalysis to nitroxide radicals which is indicative of a radical mechanism and this also suggests that the 5-LO enzyme can oxidize other substrates besides polyunsaturated fatty acids and may be regulated by reduction of the iron species.

The first step in the 5-LO reaction can be thought of as an enzymatically assisted lipid peroxidation. Both lipid peroxidation and the 5-LO reaction involve an oxidation of a fatty acid or ester in a radical process. From an evolutionary perspective, the lipid peroxidation pathway probably preceded the 5-LO enzyme and some insight into the mechanism of the 5-LO enzyme may be gained by considering its older nonenzymatic prototype. Iron is important in both 5-LO and lipid peroxidation and both reactions have free radical chain reaction character.[37] Tyrosine radical was postulated as the hydrogen abstracting species in the CO reaction.[38] It is tempting to hypothesize that a similar species may be involved in the 5-LO reaction.[39] Site-directed mutagenesis could partly answer this question concerning the need for a tyrosine residue in the active site of 5-LO.

Cofactors associated with 5-LO include various peroxides, ATP, and calcium. Contrary to earlier reports,[40,41] not only organic hydroperoxides but also hydrogen peroxide can activate 5-LO;[42,43] however, other recent studies with high levels (100 μM) of *tert*-butyl hydroperoxide failed to show an increase in 5-LO products.[44,45] Superoxide generation in PMNs from asthmatics is significantly increased after antigen challenge.[46] Therefore, we speculate there is a link between NADPH oxidase, the superoxide producing enzyme,[47] and activation of 5-LO, since dismutation of superoxide generates hydrogen peroxide. Conversely, 5-LO inhibitors attenuate hydrogen peroxide-induced lung injury[48] and high concentrations can inhibit the formation of 5-LO products by depletion of the essential cofactor ATP.[49] An activated 5-LO, therefore, appears to require the presence of hydroperoxides or "hydroperoxide tone" to remain active. A kinetic scheme of 5-LO from human PMN shows that calcium plays a major regulatory role.[50] It is not clear, however, whether calcium is a cofactor for 5-LO. Finally, a pyrroloquinoline quinone was reported as a cofactor for 5-LO, but this work has not been reproduced.[51,52]

Additional physical characteristics of the 5-LO enzyme recently determined include the energy of activation, stability, and kinetics. The determination of the activation energy of the steps in the reactions of 5-LO with AA from human PMN as a function of temperature[53] indicates that the activation energy for the first step in the 5-LO reaction is 5.2 kcal/mol (essentially diffusion controlled); however, the second step, the conversion of 5-HPETE to LTA$_4$ has a considerably higher activation energy (16 kcal/mol). Therefore, the first is consistent with a radical mechanism. The 5-LO enzyme is fairly unstable, possessing a half-life of 45 min at 37 °C.[54-56] Although the enzyme has complex kinetics, the K_m and V_{max} for human PMNs was recently reported as 11.9 μM and 8.5 mmol/min per mg of protein, respectively.[57] These values should be accepted with caution since the manner of substrate preparation may influence enzyme kinetics due to poor water solubility of fatty acid substrates and the tendency of AA to form micelles.[58]

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As stated above, 5-HPETE is converted to LTA_4 by 5-LO. It is known that the synthesis of LTA_4 proceeds from 5-HPETE through stereospecific removal of the *pro-R* C-10 hydrogen followed by radical migration and epoxide formation.[13] However, one of the anomalies associated with the LTA_4 synthase component of 5-LO is the relatively inefficient manner in which 5-HPETE is converted to LTA_4 compared to the rate of AA conversion. This could be a reflection of its higher activation energy.[52] Only 20% of the 5-HPETE produced from AA is converted to LTA_4 in an isolated 5-LO enzyme preparation, indicating a loose coupling of the two activities. This is in contrast to whole cell studies, where as much as 60–80% of the AA is converted to LTA_4. Further studies have demonstrated that the steady-state concentration of 5-HPETE has no effect on the rate of LTA_4 production from AA or on the fraction of 5-HPETE produced from AA that is converted to LTA_4, even though exogenous 5-HPETE is capable of inhibiting the oxidation of AA which suggests the presence of a single catalytic site for the two activities. The fact that exogenous 5-HPETE does not effectively compete with 5-HPETE produced from AA for LTA_4 production implies that equilibration between free and enzyme-bound 5-HPETE does not occur prior to dissociation from the enzyme.[59]

A final cautionary note involves the possibility for the existence of 5-LO isozymes. Although not much is known about 5-LO isozymes, it is likely that the various subtypes will have different physical properties and will be located in different cell types. Certainly, isozymes of 15-LO are known.

The Cellular Presence of 5-LO. There is considerable controversy in the literature concerning cellular presence of 5-LO and the sources of LTs. This is due to the fact that few cells actually possess the 5-LO enzyme whereas virtually all cells possess LTA_4 hydrolase.[13,60] Consequently, if a cell is provided with an external source of LTA_4, it will make LT products even though it does not possess the 5-LO enzyme. Indeed, human tonsil B lymphocytes and peripheral B and T cells are devoid of 5-LO activity but can synthesize LTB_4 in the presence of activated monocytes that provide them with LTA_4.[61,62] Immunohistochemical studies of the distribution of 5-LO can definitively answer the question of whether certain cell types possess the 5-LO enzyme. A recent report on porcine leukocytes revealed that neutrophils and eosinophils are positively stained but lymphocytes are not.[63] In summary, 5-LO is largely found in cells of myeloid origin, for instance mast cells, basophils, and macrophages.[60]

The profiles of 5-LO products are similar in PMNs from various species under given conditions of stimulation, but vary drastically according to incubation conditions.[64,65] Nonphysiological stimuli such as A23187 tend to overwhelm the enzymatic capacity of LTA_4 hydrolase leading to nonenzymatic hydrolysis. In human whole blood, LTs are synthesized predominantly by PMNs;[66] however, care must be taken in assigning 5-LO to certain cell types. For example, it was thought that infiltrating PMNs were the source of 5-LO products in reperfusion injury, but it was latter found that canine myocytes also possess the 5-LO enzyme.[67]

In contrast to PMNs, human eosinophils from asthmatics produce significantly more LTC_4 than eosinophils from healthy donors.[68–70] Human eosinophils produce approximately 10 times more LTC_4 than PAF and the amount of LTC_4 that remains cell-associated depends on the activating stimulus.[71]

Hypereosinophilia may play a proinflammatory role in bronchial asthma, and the recent characterization of LTB_4 receptors on guinea pig eosinophils suggests that LTB_4 may play a role in the recruitment of eosinophils.[72] It was shown, however, that guinea pig PMNs do not participate in the late phase reaction.[73] Although there appears to be no causal link between eosinophil infiltration and the development of increased airway reactivity,[74] it may be

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necessary for the cells to be activated.[75]

Recombinant DNA Technology and the 5-LO Enzyme. Many aspects of 5-LO research are impacted by the development of molecular biological techniques for the detection and sequence analysis of genes that encode for the enzymes, as well as for the cloning, site-specific mutagenesis and expression of their cDNAs in cells. For example, human 5-LO, recently cloned, sequenced, and expressed, appears to contain 674 amino acids, has a hydrophobic nature, and lacks signal sequences. This sequence is homologous to lipases, suggesting a mechanism for peripheral association of 5-LO with the plasma membrane.[76–79] The human 5-LO gene spans more than 82 kilobases, consists of 14 exons, and has been expressed in a baculovirus/insect system.[80] The human 5-LO promoter was characterized[81] and antibodies to human 5-LO were prepared.[82] Human 5-LO was recently expressed in yeast.[83] Indeed, recombinant 5-LO work has recently not only made this scarce enzyme readily available[84] but has also provided both an alternate source for antibody generation and, through site-directed mutagenesis, new knowledge of which residues are important for catalytic iron binding.[13,60,85,86] Polymerase chain reaction technology has recently been applied to human eicosanoid-forming enzyme RNA.[87] The FLAP gene was characterized[88] and

thus it is anticipated that a source of this protein produced in significant quantities will soon be available for study in drug design.

Site-directed mutagenesis of human 5-LO was performed to determine if several histidines, which are known to coordinate iron, are involved as ligands of iron.[89] Three groups independently located essential histidines at positions 367 and 372 of the human 5-LO enzyme. Recombinant 5-LO studies have also confirmed that the 5-LO enzyme contains both 5-LO and LTA_4 synthase activities.[13] The recombinant biology performed on the 5-LO enzymes has thus provided the tools for significant progress to be made in understanding this enzyme on a molecular level and should facilitate future studies on inhibitors of 5-LO.[13]

II. Pathophysiology of 5-LO Products

The elucidation of the 5-LO biosynthetic pathway and the chemical synthesis of LTs has provided the theoretical bases and the tools to gain insight into many aspects of asthma pathophysiology. Smooth muscle contraction, inflammatory cell influx, mucus production, and edema are the principal pathophysiological characteristics of asthma. In fact, the marked ability of the peptide LTs (LTC_4, LTD_4, and LTE_4) to promote bronchoconstriction and mucus hypersecretion is well documented in animals and man.[90] In addition, LTD_4 produces airway hyperreactivity, a hallmark of asthma, in normal humans,[91] and in a related study, LTC_4, LTD_4, and LTE_4 in asthmatics cause a long-lasting hyperreactivity which was not observed in normal subjects.[92] Another metabolite of the 5-LO cascade is the nonpeptide LT, LTB_4.[93] This LT is a potent chemokinetic, chemotactic, and aggregating agent for a variety of leukocytes, and in vivo, it stimulates cell accumulation and effects vascular smooth muscle, which may partially account for the edema component of asthma.[94]

Supplies of well-characterized and pure LTs made available by synthetic organic chemistry[95,96] have allowed

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for extensive pharmacological studies to be conducted on the effects of LTs. Because of the ready availability of 5-LO metabolites and analogs it was soon discovered that LTs have far-ranging pharmacological activities that go well beyond the range of the bronchopulmonary system.[60] Indeed, an enormous amount of evidence has accumulated that describes the deleterious actions of LTs in human allergic diseases[97] and inflammation.[98] In this section, we first establish that the production of LTs is harmful in asthma and diseases of the lung, and then proceed to discuss the effects these endogenously generated agents have on other systems including the immune, cardiovascular, and central nervous systems.

5-LO Products in Asthma and Other Diseases of the Lung. The clinical evidence for the adverse effects of the products of the 5-LO cascade in asthma is extensive.[99] Both the peptide and nonpeptide LTs are implicated in the etiology of asthma and other diseases of the lung including adult respiratory distress syndrome (ARDS), bronchopulmonary dysplasia, and cystic fibrosis. Higher plasma levels of LTC_4 are found in asthmatics compared with control subjects, and the levels of LTC_4 are significantly higher during asthmatic attack than during remission. In atopic asthmatics, levels of LTC_4 in bronchoalveolar lavage fluid are significantly elevated after antigen challenge.[100] In asthmatics, LTC_4 causes narrowing not only of central airways but also of peripheral airways,[101] with higher levels of LTC_4 correlated with the severity of the disease.[102] Bronchial lavage of asthmatics reveals significant levels of LTE_4 in contrast to healthy subjects who have no LTs.[103] The amount of LTE_4 in the blood of asthmatics in remission is comparable to controls and is significantly lower than that in blood of asthmatics undergoing a wheezing attack.[104] LTs may also be involved in aspirin-induced asthma since elevated levels of LTC_4 are found in the nasal fluid of aspirin-sensitive asthmatics.[105] This is corroborated by related studies

measuring LTE_4 levels in the urine of subjects with this disease.[106] Indeed, peptide LT levels in urine are elevated in asthmatics.[107] Urinary levels of LTE_4 in asthmatics are elevated after antigen challenge.[108] Exercise-induced asthma also appears to involve LTs. For example, levels of LTE_4 in urine are significantly increased in severe asthmatics who had just exercised.[109] Thus, LTC_4, LTE_4, and LTE_4 are clearly present in humans and correlate with the severity of asthma.

The severity of ARDS, bronchopulmonary dysplasia, and cystic fibrosis also correlates to synthesis and excretion of levels of peptide LTs.[110] The involvement of LTs in ARDS was the subject of a recent review.[111] By measuring urinary LTE_4 levels, it was shown that there is a persistent generation of peptide LTs in ARDS.[112] Both LTD_4 and 5-HETE produce significant mucous thickening.[113] The amount of LTE_4 in the urine of patients with multiple trauma with and without ARDS is elevated compared to controls.[114] In infants with bronchopulmonary dysplasia, both alveolar macrophage numbers and peptide LT levels are elevated relative to normal infants.[115] Finally, LT levels in both sputum and urine are elevated in children with cystic fibrosis.[116] The sum of the above evidence clearly shows that 5-LO products play a fundamental role as pathophysiological mediators of asthma and other pulmonary diseases.

Inflammation and Other Diseases Which Involve

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the 5-LO Pathway. In addition to asthma, other inflammatory diseases known to involve products of the 5-LO pathway include psoriasis, ulcerative colitis, and rheumatoid arthritis.[117,118] LTs induce inflammatory changes in human skin following local administration.[119] Both 5-LO and 12-LO products are important for cell recruitment,[120] whereas both LTC$_4$ and LTD$_4$ are growth factors for human melanocytes.[121] Continuous antigen challenge in atopics resulted in persistent release of histamine and LTB$_4$.[122] LTC$_4$ levels are significantly elevated after antigen challenge of human skin.[123] LTB$_4$ levels are elevated in patients with atopic eczema.[124] In rheumatoid arthritis, LTB$_4$ levels in both peripheral blood and synovial fluid are significantly elevated in patients.[125,126] LTB$_4$ is also elevated in synovial fluid obtained from patients with osteoarthritis.[127] In patients with rheumatoid arthritis, serum levels of LTB$_4$ are not only elevated relative to normal subjects but also correlate with concentrations of LTB$_4$ in the synovium.[128]

With respect to the gastrointestinal effects, evidence is equivocal for involvement of LTs in NSAID-induced lesion formation.[129] It appears that LTs are not the exclusive or primary mediators in ethanol-induced gastric mucosal damage and that the protection observed with some 5-LO inhibitors is due to effects other than inhibition of LT synthesis.[130] However, 5-LO products do appear to be involved in inflammatory bowel disease.[131]

Peptide LTs are also implicated in the pathophysiology of several cardiovascular disorders,[132] including coronary vasospasm and myocardial ischemia.[133,134] Peptide LTs are reported to be among the most potent coronary vasoconstrictors and appear to act as important mediators of shock and reperfusion injury.[135] A 5-LO product, 5-HETE, inhibits angiotensin II-mediated aldosterone secretion.[136] 5-LO products are also modulators of cardiac potassium channels.[137] LTs appear to mediate arteriolar oxygen reactivity which can be blocked by 5-LO inhibitors.[138] LTB$_4$ can promote leukocyte endothelium adhesion and this may be a significant event in ischemia reperfusion injury. It was thought that activated human monocytes oxidize low-density lipoproteins by a LO-dependent pathway; however, this effect is not blocked by 5-LO inhibitors.[139] Nevertheless, LTD$_4$ is a potent coronary constrictor in the perfused cat coronary artery.[140] Although 5-LO inhibitors are not being developed for coronary vasospasm and myocardial ischemia indications, these agents may be discovered to have beneficial side effects on the cardiovascular system in clinical studies (for example, see FLM 5011 below which has entered clinical trials for a myocarditis indication).

In the central nervous system, 12-LO and 9-LO pathways seem more important.[141-143] LTs were the major eicosanoids found in cerebrospinal fluid of preterm infants with posthemorrhagic hydrocephalus.[144] A recent report implicated a LO metabolite as a mediator of somatosta-

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tin-induced increase of neuronal M-current and this effect could be blocked by 5-LO inhibitors.[145]

The 5-Lipoxygenase Cascade and the Immune System. The effects of products of the 5-LO cascade on the immune system are of increasing interest[146,147] since these effects are manifested in cancer and autoimmune diseases. For example, in brain tumors there is a correlation between increasing malignancy and increased LT production.[148] Also, the action of tumor promoters can be blocked by 5-LO inhibitors.[149] 5-LO inhibitors enhance the proliferation of human B cells[150] and at the same time block the proliferation of murine mastocytoma cells[151] and human glioma cells.[152] 5-HETE stimulated malignant and normal cell growth which can be blocked by a 5-LO inhibitor.[153] Finally, LTD$_4$ and LTB$_4$ stimulated proliferation of leukemia cells can be blocked by 5-LO inhibitors.[154] It is important to note these effects that LTs have on cell proliferation since it would implicate the use of 5-LO inhibitors in the treatment of cancer.

However, in contrast to the above detrimental effects of LTs and the potential of 5-LO inhibitors in the treatment of cancer, it is reported that LTs can stimulate macrophages to destroy tumor cells and this effect can be blocked by 5-LO inhibitors.[155] In addition, peritoneal macrophages significantly enhance NK cell activity by production of 5-HPETE and LTB$_4$.[156] If considered in terms of warding off cancer or infection, this finding is significant since there is very little evidence for the beneficial effects of LTs.

Interestingly, LTs do protect against radiation and this has implications for radiation therapy of tumors.[157] Possibly, a 5-LO inhibitor or LT antagonist could prevent this undesired radioprotection within tumors.

5-LO inhibitors may have a positive effect with respect to mutagenesis induced by xenobiotics. For example, benzopyrene is metabolized to protein-binding species in the presence of 5-LO and this is blocked by 5-LO inhibitors.[158]

A 5-LO inhibitor structurally related to LTA$_4$ suppresses antigen-specific immunoglobulin E· (IgE) responses in lymphocytes.[159] The 5-LO enzyme expression is apparently enhanced in virally infected rats.[160] Patients that have undergone bone marrow transplants frequently suffer from capillary leak syndrome which is associated with elevated LT levels.[161] It would be interesting to see if a 5-LO inhibitor could block this leakage.

The question of whether lymphocytes can actually synthesize LTs has been controversial.[162,163] Recent evidence implicates 5-LO products in transduction of mitogenic signals in T lymphocytes[164] and human B cells.[165] In fact, human B cells possess 5-LO activity.[166]

There are reports of LT effects on renal and hepatic function and renal transplantation. Synthesis of LTs is associated with glomerulonephritis.[167] Peptide LTs produce a decrease in renal blood flow and glomerular filtration rate.[168] Infiltration of monocytes and PMNs is found in experimental models of liver injury, which can

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be inhibited by a 5-LO inhibitor.[169] Production of LTs is stimulated during acute renal allograft rejection.[170] There have been recent reports that 5-LO inhibitors can prevent renal allograft rejection.[171]

The lipoxins (LXs) were recently reviewed,[172] and although the pharmacology of these compounds is still being defined, they appear to counter some of the untoward effects of the LTs. For example, LXA_4 antagonizes LTD_4 at the receptor which provides evidence of endogenous LT receptor antagonists.[173] LXA_4 also inhibits the microvascular effects of LTB_4 in a hamster cheek pouch model of inflammation.[174] Production of LTB_4 in human PMNs is inhibited by LXA_4 and LXB_4.[175] LXA_4 is found in bronchoaveolar fluids of patients with various pulmonary diseases and is absent in BAL (bronchial alveolar lavage) from normal subjects.[176]

Thus, with the exception of the lipoxins and data concerning whether LTs can stimulate macrophages, there is clear and ample reason to inhibit the synthesis of both the peptide and nonpeptide LTs by 5-LO inhibitors.

Since modification of cell–cell adhesion has generated considerable interest of late, it is interesting to note that LTB_4 induces hyperadhesiveness in human endothelial cells which leads to increased binding of PMNs.[177] In fact, MK-886 was shown to prevent oxidized LDL (low-density lipoprotein) induced adhesion of leukocytes to the endothelium.[178] In addition, LTB_4 increases IgE binding, IgE-dependent adherence, and cytotoxicity of human eosinophils.[179]
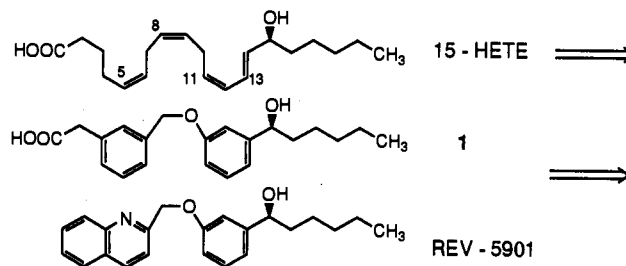

Figure 3. Diagram denoting the stepwise structural evolution of REV-5901.
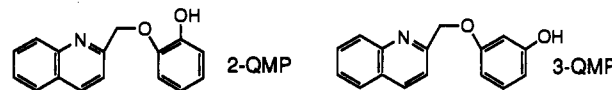

Figure 4. Dewhirst's compound, 2-QMP, and Wyeth-Ayerst's 3-QMP.

III. Quinolinyl(bridged)aryl Class of Inhibitors

Since there are extensive tabulations of 5-LO inhibitors in the literature,[180-182] no attempt is made in this Perspective to comprehensively review all these compounds; only the more significant examples from each class will be discussed. First, the discovery of the 2-quinolinyl-(bridged)aryl system as modulators of the AA cascade is placed in perspective with respect to other classes of 5-LO inhibitors. We then discuss many compounds that have entered Phase 1 clinical trials and some of the newer, more interesting preclinical compounds, e.g. ICI-216,800.[183] We also add half inhibitory concentration (IC_{50}) values routinely after the first mention of a 5-LO inhibitor.

Since the elucidation of the 5-LO biosynthetic pathway there has been a debate as to whether it is better with respect to drug discovery to inhibit the 5-LO enzyme or to antagonize peptide or nonpeptide LTs. Recent evidence suggests that 5-LO enzyme inhibitors may be superior to LT receptor antagonists. For example, 5-LO inhibitors block the action of the full spectrum of 5-LO products,[184,185] whereas LT antagonists by definition can effectively block the effect of only one LT. In addition, LT antagonists appear to prolong the half-lives of LTs by hindering metabolism.[186] Finally, 5-LO inhibitors also block some of the effects of PAF.[187-189]

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One approach to generating new leads as modulators of the AA cascade was based on work by Vanderhoek who reported that 15-HETE is an inhibitor of 5-LO.[190] It was speculated that HETEs may be acting through feedback inhibition to attenuate 5-LO activity. Therefore in 1980, we prepared analogs of 15-HETE, where the aliphatic double bonds were replaced by aromatic rings.[191] When comparing computer-generated, space-filling models of 15-HETE with aromatic ring-stabilized analogs, it was apparent that the 11,13-cis, trans double bonds could satisfactorily be substituted with a phenyl ring. Less straightforward, however, was finding a surrogate ring system for the remaining 5 or 8 double bond while retaining a good isosteric fit with 15-HETE. A number of systems were tried but the solution consisted of bridging C-3 to C-7 and substituting a methyleneoxy group for the C-8–C-9 double bond(Figure 3). Target structure 1 was prepared along with a number (>30) of analogs including ortho, meta, and para isomers of the tethered carboxylic acid group. Compound 1 possesses 5-LO inhibitory activity versus rat PMNs (IC_{50} = 2.5 μM); however, versus ovalbumin (OA) induced bronchoconstriction in the guinea pig, compound 1 and its direct analogs are devoid of activity. Since all of the compounds in the series are very hydrophobic, we considered inserting a basic nitrogen into the terminal ring, with the goal of obtaining a compound capable of forming a water-soluble salt. Several pyridyl and quinolyl derivatives were prepared[192] and one, REV-5901 (IC_{50} = 0.16 μM, rat PMN), is active in vivo.[193]

REV-5901 significantly inhibits the release of LTs from human lung tissue challenged with either antigen or calcium ionophore at 1 and 10 μM. As an antagonist of peptide LTs, it has a K_i value of 0.7 μM versus [^{3}H]-LTD$_4$ binding to membranes from guinea pig lung. REV-5901 does not appear to have either significant antihistamine or anticholinergic activity. In guinea pig bronchoconstriction studies, REV-5901 (id, 30 mg/kg) inhibits the response to LTD$_4$ by 74%. Upon oral administration in
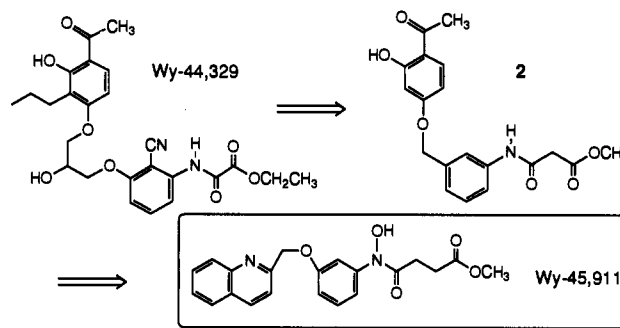

Figure 5. Parallel evolution of Wy-45,911.

man, it is rapidly absorbed, undergoes facile and extensive metabolism, and the metabolites are highly protein bound. No limiting toxicity was observed in animals and, in man, no significant adverse toxicity was observed up to doses of 1000 mg. Recently, REV-5901 was acquired by Perdue Frederick as PF-5901 and is under development as a treatment of inflammatory bowel disease.[194]

Although the 2-quinolinyl(bridged)aryl structure of REV-5901 has served as a template for most of the compounds in this class of modulators of the AA cascade, the first report of a 2-quinolinyl(bridged)aryl system as a modulator of eicosanoid synthesis was by Dewhirst and the Forsyth Dental Infirmary for Children. He claimed in the patent literature several bridged bis-aryl systems including 2-(2-quinolinylmethoxy)phenol (2-QMP) (Figure 4) as inhibitors of sheep vascular gland CO with utility as antiinflammatory agents.[195] Thus, we prepared a number of agents mentioned in Dewhirst's patent including 2-QMP and related isomers.[196] Using rat PMNs, 2-QMP has CO inhibitory activity [IC_{50} = 5.82 μM vs prostaglandin E$_2$ (PGE)$_2$] but is only marginally active as a 5-LO inhibitor (IC_{50} = 53.0 μM vs 5-HETE). In contrast, the meta hydroxyl isomer, 3-QMP, possesses selective 5-LO inhibitory activity (IC_{50} = 6.07 μM vs 5-HETE and >100 μM vs PGE$_2$), also using rat PMNs. To our surprise, in vivo, 3-QMP has significant LTD$_4$ antagonist activity (50 mg/kg, 82% inhibition of LTD$_4$-induced bronchoconstriction in the guinea pig). By contrast, the ortho hydroxyl isomer, 2-QMP, is only marginally active (50 mg/kg, 28% inhibition) as an antagonist of peptide LTs.

A Strategy for the Eighties. The observation that there are agents that both inhibit 5-LO and antagonize peptide LTs was critical to our future design strategy. Data to support the dual nature of some modulators of the AA cascade came from our laboratories and others. For example, the hydroxyacetophenone peptide LT antagonists Wy-44,329 (IC_{50} = 32 μM, rat PMN)[197] and FPL-55,712 (IC_{50} = 21 μM, rat RBL-1)[198] were reported as 5-LO inhibitors, and conversely, the 2-(2-quinolinylmethoxy)-phenol 5-LO inhibitor, REV-5901, was reported as a weak peptide LT antagonist.[193] In addition, several 5-LO inhibitors reported in the literature have a bis-aryl system,[199]

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(198) Casey, F. B.; Appleby, B. J.; Buck, D. C. Selective Inhibition of the Lipoxygenase Metabolic Pathway of Arachidonic Acid by the SRS-A Antagonist, FPL-55,712. *Prostaglandins* 1983, 25, 1–11.

which is also found in many CO inhibitors.[200] Thus, *we adopted as a design strategy, the synthesis of agents that had the potential to not only inhibit the AA cascade but had as an additional feature the potential to antagonize the effects of LTs.*

Hydroxyacetophenones. Our next design focused on the LTD$_4$ antagonist component of dual modulation by employing the hydroxyacetophenone moiety of FPL-55,712.[201] FPL-55,712 has been extensively used as a pharmacological tool to define the role that peptide LTs play in immediate hypersensitivity reactions in man and animals.[202] Although many research groups have synthesized LT antagonists by substantial modification of the right-hand portion of FPL-55,712 while retaining the left-hand hydroxyacetophenone moiety,[203] our approach was different in that we wanted to explore the 5-LO inhibitory activities of the hydroxyacetophenone moiety.

Therefore, a series of compounds combining a hydroxyacetophenone moiety with the (phenylamino)oxocarboxylic acid group found in Wy-44,329 was prepared (Figure 5).[201] We wanted to determine the minimum requirements of dual activity for Wy-44,329; this resulted in removing much of the bridging element. We also wanted to insert a methylene spacer of various lengths in the oxamate moiety because an analogous system was previously reported in the patent literature as having LTD$_4$ antagonist activity.[204] We were able to generate 5-LO inhibitors in this series. Indeed, for 5-LO inhibitory activity the overall structure can be significantly varied since many analogs were active; however, against LTD$_4$-induced bronchospasm in the guinea pig only compound **2** has any significant inhibitory activity. Although we were successful in preparing a 5-LO inhibitor with LTD$_4$ antagonist activity, the oral in vivo potency for this hydroxyacetophenone bridged (phenylamino)-4-oxoalkanoic acid is not sufficient to warrant further development.

Hydroxamic Acids Reported As 5-LO Inhibitors. We continued to be attracted to the (phenylamino)-4-oxobutanoic acid substructure on theoretical grounds because it partially approximates the C-1 through 10 hydrocarbon backbone of AA and 5-HETE, and the 5-hydroxy-6-mercapto-6-vinylhexanoic acid portion of LTD$_4$. It was considered that the amide pK_a is similar to that of the C-5 carbinol of both LTD$_4$ and 5-HETE and that the amide/acid functional distance approximates the carbinol/acid functional distance in 5-HETE and LTD$_4$.

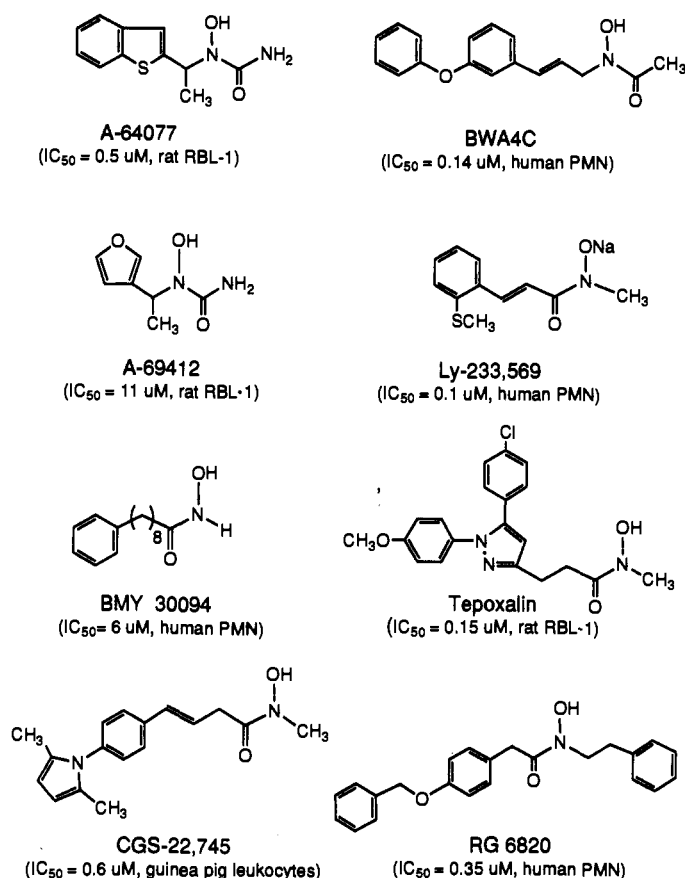In order to increase in vivo potency, we decided to substitute the 2-oxymethylquinoline group found in 3-

A-64077
(IC$_{50}$ = 0.5 uM, rat RBL-1)

BWA4C
(IC$_{50}$ = 0.14 uM, human PMN)

A-69412
(IC$_{50}$ = 11 uM, rat RBL-1)

Ly-233,569
(IC$_{50}$ = 0.1 uM, human PMN)

BMY 30094
(IC$_{50}$= 6 uM, human PMN)

Tepoxalin
(IC$_{50}$ = 0.15 uM, rat RBL-1)

CGS-22,745
(IC$_{50}$ = 0.6 uM, guinea pig leukocytes)

RG 6820
(IC$_{50}$ = 0.35 uM, human PMN)

Figure 6. Hydroxamic acids and hydroxyureas subsequently reported as 5-LO inhibitors.

QMP for the hydroxyacetophenone of **2**.[205] It was also thought that a 2-oxymethylquinoline group could either simulate the fatty acid backbone of 5-HETE or LTD$_4$, or the cysteinylglycine of LTD$_4$. In sharp contrast to the hydroxyacetophenone series, many of the initial compounds inhibit LTD$_4$-induced bronchoconstriction in the guinea pig while only a few are 5-LO inhibitors. We then decided to synthesize a hydroxamic acid analog.

A hydroxamic acid function would more closely approximate the C-5 carbinol of 5-HETE and LTD$_4$ and it would also have the potential to chelate iron in the active sites of 5-LO/CO.[206] Therefore, Wy-45,911 (Figure 5) was prepared and it both inhibits the 5-LO enzyme (IC$_{50}$ = 1.4 μM, rat PMN) and antagonizes LTD$_4$ receptors.[207] Note that Wy-45,911 is the first hydroxamic acid-containing 5-LO inhibitor described in the literature that was investigated in vivo.[208] Actually, iron was shown to be an essential component of 15-LO and is postulated to be an essential part of the active site of 5-LO.[209] Corey was the

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(201) Kees, K. L.; Musser, J. H.; Chang, J.; Skowronek, M.; Lewis, A. J. Synthesis and Antiallergic Activity of a Novel Series of 5-Lipoxygenase Inhibitors. *J. Med. Chem.* **1986**, *29*, 2329–2334.

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(204) Kadin, S. B. Carboxyacylamino)phenylalkenamides and Esters Thereof as SRS-A Antagonists. U.S. Patent 4,296,129, 1981.

(205) Musser, J. H.; Kubrak, D. M.; Chang, J.; Lewis, A. J. Synthesis of [[(Naphthalenylmethoxy)- and [[(Quinolinylmethoxy)phenyl]amino]oxoalkanoic Acid Esters. A Novel Series of Leukotriene D$_4$ Antagonists and 5-Lipoxygenase Inhibitors. *J. Med. Chem.* **1986**, *29*, 1429–1435.

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(208) Corey was the first to publish (1984) that hydroxamic acids are 5-LO inhibitors but only in vitro results were reported; see: Corey, E. J.; Cashman, J. R.; Kantner, S. S.; Corey, D. R. Rationally Designed, Potent Competitive Inhibitors of Leukotriene Biosynthesis. *J. Am. Chem. Soc.* **1984**, *106*, 1503–1504.

first to demonstrate that certain iron chelating hydroxamic acids can inhibit 5-LO.[208] More recent hydroxamic acids and hydroxyureas reported as 5-LO inhibitors include A-64077 (IC_{50} = 0.5 μM, rat RBL-1),[210] BWA4C (IC_{50} = 0.14 μM, human PMN),[211] A-69412 (IC_{50} = 11 μM, rat RBL-1),[212] Ly-233,569 (IC_{50} = 0.1 μM, human PMN),[213] BMY 30094 (IC_{50} = 6 μM, human PMN),[214] tepoxalin (IC_{50} = 0.15 μM, rat RBL-1),[215] CGS-22,745 (IC_{50} = 0.6 μM, guinea pig PMN),[216] and RG-6820 (IC_{50} = 0.35 μM, human PMN)[217] (Figure 6). An interesting series of hydroxamic acid-containing 5-LO inhibitors based on CO inhibitors was recently reported.[218] Definitive proof that hydroxamic acids and hydroxyureas inhibit 5-LO by iron chelation is still lacking and it is possible that they also inhibit the enzyme by radical scavenging (vide infra).

Not only did hydroxamic acid Wy-45,911 markedly inhibit the formation of 5-LO products but also it inhibited CO products to a lesser extent in rat PMNs.[219] Similarly, in mouse peritoneal macrophages the IC_{50} value against LTC_4 is 0.45 μM and maximal inhibition is obtained with 5 μM. Wy-45,911 also reduces thromboxane B_2 (TxB_2) synthesis; however, the activity is significantly weaker (IC_{50} = 4.52 μM). PGE_2 synthesis is also reduced by Wy-45,911
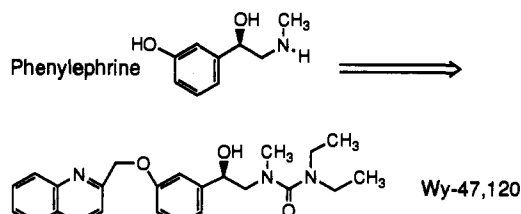

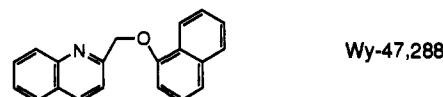**Figure 7.** Dual 5-LO inhibitors/LTD_4 antagonists derived from phenylephrine.

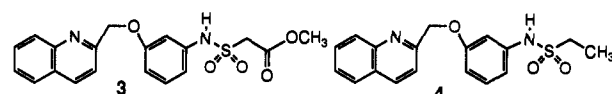**Figure 8.** A 2-oxymethylquinoline system as a topical CO/LO inhibitor.

Figure 9. Sulfonamide LTD_4 antagonist series that have 5-LO/CO inhibitory activity.

(IC_{50} = 7.20 μM). We were gratified with respect to our dual-modulation strategy to find out that in addition to its effect on AA metabolism, Wy-45,911 inhibits competitively LTD_4-induced contractions of the isolated guinea pig trachea but not those of LTC_4 in the presence or absence of glutathione (GSH), a γ-glutamyltranspeptidase inhibitor. Tracheal contractions induced by histamine or pilocarpine are not significantly altered by Wy-45,911, suggesting a LT-specific effect. The drug inhibits the tracheal contractions induced by antigen, even in the presence of GSH. This latter effect could be the result of 5-LO inhibition since the conversion of the progenitor, LTC_4, to LTD_4 is inhibited by GSH. The drug inhibits both LTD_4-induced and antigen-induced bronchoconstriction when administered either intraduodenally or intragastrically into intact guinea pigs though it is more potent against LTD_4-induced bronchoconstriction.

However, Wy-45,911 was positive in the Ames assay for mutagenicity and consequently no further work was carried out with this series. The (phenylamino)-4-oxobutanoic acid group was used in the preparation of an Ames negative compounds but these agents are metabolically unstable; they undergo cyclodehydration to yield a pyrolidine-2,5-dione moiety.[220] To avoid molecules capable of metabolic activation resulting in electrophile formation, consideration of a 5-hydroxyhexanoate group was abandoned.

We wanted to continue to explore additional surrogates of the 5-hydroxyhexanoate group. Therefore, it was decided to replace the hydroxamic acid of Wy-45,911 with a carbinol moiety and the C-1 carboxylate with a function incapable of intramolecular reaction with the C-5 hydroxyl moiety. With these requirements in mind, the use of phenylephrine for the synthesis of potential LT antagonists of novel structure was considered.

Phenylephrine was an attractive starting material for the following reasons: (a) it is meta-substituted which is important for activity as demonstrated in the Wy-45,911 series, (b) it has an acidic alcohol function which can be selectively alkylated, (c) the *R* and *S* enantiomers of phenylephrine are commercially available, and (d) it has

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a basic nitrogen which can be reacted with various acylating agents to give amides and the resulting amides are unlikely to react intramolecularly to form a ring.

After completing a thorough structure–activity relationship (SAR) study of phenylephrine derivatives as 5-LO inhibitors and LTD$_4$ antagonists, Wy-47,120 (IC$_{50}$ = 2 μM, rat PMN) was designated the lead (Figure 7).[221] Wy-47,120 is Ames negative and metabolically stable; however, it has an unacceptable ancillary pharmacological profile. An intravenous (iv) bolus injection of Wy-47,120 causes convulsions in guinea pigs. So we sought to prepare other systems containing the 2-oxymethylquinoline substructure that are nontoxic.

As part of the SAR on 3-QMP, the phenol was replaced by naphthalene to give 2-oxymethylquinoline Wy-47,288 (Figure 8). It was tested in several cell-free and cellular models of AA metabolism.[222] In both rat PMNs and mouse macrophages, it is markedly more potent against 5-LO-catalyzed reactions (IC$_{50}$ = 0.4, 0.2 μM, respectively) than CO-catalyzed reactions (IC$_{50}$ = 6.3, 44.0 μM, respectively). Several animal models of skin inflammation were employed to characterize Wy-47,288. When applied to mouse ear surfaces, it inhibits AA (ED$_{50}$ = 0.3 mg/ear) and tetradecanoylphorbol acetate (TPA) induced inflammation (40% at 1 mg/ear). Similarly, ear edema and epidermal proliferation are reduced by 50% upon administration of Wy-47,288 (1 mg/ear) at 30 min and 5 h after TPA. It also inhibits oxazoline-induced contact hypersensitivity in mouse ears (ED$_{50}$ = 0.4 mg/ear) and UVB-induced guinea pig skin erythema (ED$_{50}$ = 0.25 mg/spot). These antiinflammatory effects are attributed to inhibition of 5-LO and CO since Wy-47,288 demonstrated no appreciable inhibition of 12-LO (rabbit platelet), 15-LO (soybean), or phospholipase A$_2$ (human platelet). Furthermore, no systemic adverse effects are observed after topical, parenteral, or oral administration of Wy-47,288, suggesting that Wy-47,288 is a safe, topically active 5-LO/CO inhibitor for treating skin inflammation. However, topical activity was not sufficient for development since our design strategy now included the synthesis of agents that not only inhibit the AA cascade and have LTD$_4$ antagonist effects but also have activity via the oral route.

We then sought an alternative, achiral isostere corresponding to the C-5 hydroxyl of 5-HETE to append to our 2-oxymethylquinoline substructure. The sulfonamide moiety is recognized as a bioisostere of an alcohol.[223] By incorporating a sulfonamide into Wy-45,911 we arrived at target structure 3 (Figure 9).[224] As a first approximation of target 3, we decided to prepare the simple ethanesulfonyl derivative 4 and test it as a 5-LO inhibitor and inhibitor of both LTD$_4$- and OA-induced bronchoconstriction in the guinea pig. Many sulfonamides in the series clearly demonstrate the ability to inhibit 5-LO and LTD$_4$-
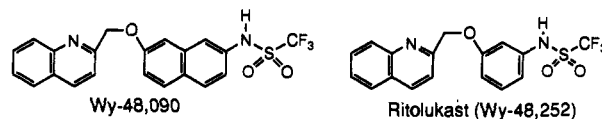

Figure 10. Ritolukast—a LTD$_4$ antagonist with 5-LO/CO inhibitory activity.

and OA-induced bronchoconstriction in the guinea pig; however, inhibition is highly variable. Since the pK_a for the hydroxamic acid of Wy-45,911 is lower than the leads in subsequent series, we considered making analogs of 4 in which the sulfonamide NH is more acidic.

Ritolukast—A Leukotriene D$_4$ Antagonist with 5-Lipoxygenase Inhibitory Activity. Several sulfonamide analogs were prepared of which ritolukast (Wy-48,252) was shown to be chemically stable and Ames negative, and was our most potent intragastrically active LTD$_4$ antagonist (Figure 10). Ritolukast contains a trifluoromethanesulfonamide group. Apparently, replacement of alkyl with trifluoromethyl not only lowers the pK_a of the NH but also provides a potent LTD$_4$ antagonist with 5-LO inhibitory activity that is consistently active by intragastric administration.

Ritolukast was assessed for its ability to modulate AA metabolism in several inflammatory cells.[225] In rat PMNs, it effectively inhibits the conversion of exogenous AA to 5-HETE and thromboxane B$_2$ (TxB$_2$). Synthesis of immunoreactive LTB$_4$ (IC$_{50}$ = 4.6 μM) and TxB$_2$ (IC$_{50}$ = 3.3 μM) from endogenous substrate by these cells in the absence of exogenous AA is similarly inhibited. Ritolukast also inhibits LTC$_4$ and PGE$_2$ synthesis by zymosan-activated mouse peritoneal macrophages (IC$_{50}$ = 4.4 and 4.3 μM, respectively). 5-LO-catalyzed reactions in human PMNs, lung mast cells, and basophils activated by various stimuli are dose-dependently inhibited by ritolukast, while PGD$_2$ synthesis by lung mast cells is inhibited at 100 μM. By contrast, 12-LO, phosphodiesterase activity, and histamine release from mast cells and basophils are unaffected by ritolukast. These data suggest that ritolukast inhibits the synthesis of AA metabolites, a feature that may contribute to its pharmacological actions in vivo.

In a guinea pig lung LTD$_4$ receptor binding assay, ritolukast's affinity for the receptor is 28 times that of LY-171,883, a LTD$_4$ receptor antagonist with demonstrated clinical efficacy.[226] This affinity is corroborated by a related study with a soluble LTD$_4$ receptor preparation (K_i = 60 nM).[227] In another study, ritolukast is twice as effective as LY-171,883 in displacing LTD$_4$ from guinea pig lung membranes.[228] The binding affinity of ritolukast translated into potent in vitro antagonism of both LTD$_4$ and antigen-induced contractions of guinea pig tissue.

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Ritolukast is approximately 1 order of magnitude more potent than LY-171,883 in blocking LTD$_4$-induced contractions of guinea pig trachea (pA_2 = 7.62 and 6.68, respectively).[229] In the presence of indomethacin and chlorpheniramine, ritolukast inhibits antigen-induced contractions of guinea pig trachea in a dose-dependent manner.[230] Ritolukast also inhibits LTD$_4$-induced calcium mobilization in differentiated U-937 cells.[231]

In several in vivo animal models of asthma, ritolukast showed impressive activity relative to first generation compounds, substantiating the LTD$_4$ binding studies and in vitro testing results. In allergic sheep, ritolukast (10 mg/kg, ig) effectively reduces both the early and late responses to inhaled antigen.[232] The potency of ritolukast in this model is comparable to YM-16,638, a LTD$_4$ receptor antagonist with demonstrated clinical efficacy.[233] In the same study, ritolukast also significantly reduces the increase in specific lung resistance induced by LTD$_4$. In guinea pigs, intraduodenally administered ritolukast is approximately 1 order of magnitude more potent than LY-171,883 in preventing LTD$_4$-induced changes in dynamic compliance and airway conductance.[234] In contrast to pure 5-LO inhibitors, ritolukast is able to reverse an ongoing bronchoconstriction induced by LTD$_4$. This ability to reverse an ongoing contraction induced by LTD$_4$ is consistent with previous studies[235] of other LTD$_4$ antagonists. Ritolukast is also effective in preventing antigen-induced bronchoconstriction in guinea pigs (ID$_{50}$ = 600 μg/kg, po).

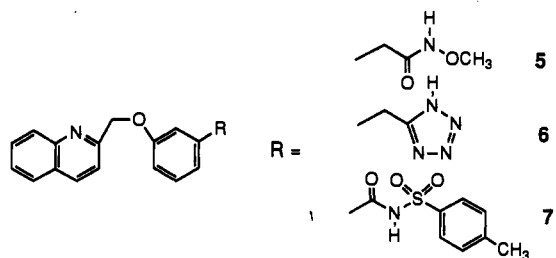In several animal species, ritolukast has good oral

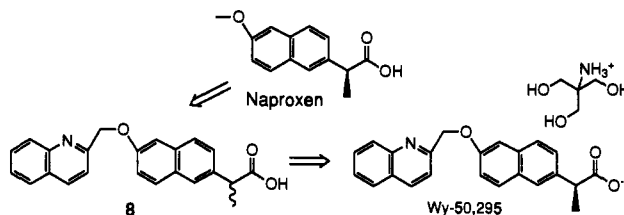**Figure 11.** Other LTD$_4$ antagonists with 5-LO and/or CO inhibitory activity.

Figure 12. 5-LO inhibitor with LTD$_4$ antagonist activity.

bioavailability (49–102%) and acceptable plasma half-life (0.5–17.7 h).[236] Due to the excellent preclinical pharmacological activity in LT-mediated disease models and favorable pharmacokinetic profile, ritolukast was selected for clinical development; however, it was withdrawn because of a constellation of minor undesirable side effects. None of the side effects, such as reversible bladder hyperplasia, precluded development but taken together made the safety margin for ritolukast, a chronically administered drug, unacceptable.

The search for additional sulfonyl moieties to bond with the aniline of ritolukast brought us back to the initial target structure 3. Both ester 3 and the corresponding carboxylic acid shows LTD$_4$ antagonist and 5-LO inhibitory activity (intraduodenal administration), but both are less potent than ritolukast.

We also examined ritolukast in inflammatory models. In the rat PMN assay, ritolukast inhibits both 5-LO and CO, but demonstrates only modest activity in rat carrageenan paw edema assay (20% inhibition at 100 mg/kg, ig) and is inactive in the mouse ear edema assay (ig).

The structural feature common to many of our 5-LO inhibitors and LTD$_4$ antagonists and to 5-HETE and LTD$_4$ is an acidic moiety. Although at the time we were not aware of the structure of the more recent LTD$_4$ antagonists, such as, SKF-104,353,[237] ICI-204,219[238] and RS-411,[239] we did know that the pK_a of the hydroxamic

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(234) Hand, J. M.; Auen, M. A.; Chang, J.; Englebach, I. M. Prevention and Reversal of Aerosol LTD$_4$-induced Changes in Guinea Pig Pulmonary Mechanics by Wy-48,252, an Orally Active LTD$_4$/E$_4$ Receptor Antagonist. *Int. Arch. Allergy Appl. Immunol.* 1989, *89*, 78–82.

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acid in Wy-45,911, and the trifluoromethanesulfonamide in Wy-48,252 is low. Therefore, we started to design and synthesize novel [(quinolinylmethoxy)phenyl] analogs containing acidic moieties. We also wanted to identify a compound to follow ritolukast into development and to determine whether the addition of acidic moieties to our [(quinolinylmethoxy)phenyl] system would provide potent orally active inhibitors of 5-LO and LTD$_4$-induced bronchoconstriction.

Three series of [(quinolinylmethoxy)phenyl] compounds were then prepared as 5-LO inhibitors and LTD$_4$ antagonists.[240] The hydroxamate 5, the tetrazole 6, and the sulfonylcarboxamide 7 (Figure 11) inhibit LTD$_4$-induced bronchoconstriction in guinea pigs with oral ED$_{50}$s of 7.9, 0.4, and 3.0 mg/kg, respectively. Compounds 5, 6, and 7 are also active as 5-LO inhibitors in the rat PMN assay with IC$_{50}$s values of 14.2, 6.2, and 3.9 μM, respectively. Only 6 shows a dose-related inhibition of CO (IC$_{50}$ = 13.4 μM). In contrast to ritolukast, 5–7 in the rat carrageenan paw edema assay show good oral activity. The antiinflammatory activity of 5–7 may be due not only to LTD$_4$ antagonism but also to their ability to inhibit 5-LO and in the case of 6 to inhibit CO. Although we were not able to develop more potent LTD$_4$ antagonists relative to ritolukast, we were able to discover LTD$_4$ antagonists with a dual action which may have potential therapeutic use against both the early and late phases of asthma.

In order to convert Wy-47,288 into an orally active compound, hybrids of it and naproxen, an orally active CO inhibitor, were prepared. Of these, 8 (Figure 12) generated the most interest.[241]

Compound 8 is a 5-LO inhibitor both in vitro (IC$_{50}$ = 0.13 μM vs LTB$_4$, rat PMN) and in vivo (ED$_{50}$ = 11.1 mg/kg vs LTC$_4$, mouse zymosan peritonitis) but is marginally active (30% at 100 μM) as a CO inhibitor (rat PMN). The selectivity of 8 is further demonstrated by the fact that it has no affect on soybean 15-LO or rabbit platelet 12-LO. Furthermore, 8 has neither a low redox potential (peak potential = 1.41 V) nor free radical scavenging activity. Finally, 8 does not interfere with substrate availability since human platelet phospholipase A$_2$ hydrolysis is not affected. Compound 8 does possess antiinflammatory activity (ED$_{50}$ = 75 mg/kg po, rat carrageenan edema) without gastrointestinal toxicity (doses up to 300 mg/kg, po).

Although 8 appeared promising, two further requirements needed to be met for further development. First, a single enantiomer was needed since 8 is a racemic mixture, and second, greater in vivo potency was required. Both the R and S enantiomers were prepared and both are equally active as 5-LO inhibitors in vitro and in vivo. We decided to go with the S enantiomer, Wy-50,295, because of the considerable literature on NSAIDs which indicates that the S configuration of arylpropanoic acids is metabolically favored. The second obstacle was surmounted by the preparation of the tromethamine salt.[242] Appar-

ently, the increased water solubility afforded by tromethamine salt formation greatly improves the oral absorption which results in increased in vivo potency.

Wy-50,295—A 5-Lipoxygenase Inhibitor with Leukotriene D$_4$ Antagonist Activity. Wy-50,295 tromethamine is a potent, selective 5-LO inhibitor in several in vitro 5-LO enzyme systems (rat PMN, IC$_{50}$ = 0.1 μM; human PMN, IC$_{50}$ = 0.8 μM).[243] Consistent with previously reported 5-LO inhibitors, the enantiomers are equipotent.[244] This contrasts with a recently reported 5-LO inhibitor ICI-216,800.[245] The inhibitory activity appears to be both competitive and reversible, and does not involve antioxidant mechanisms. Selectivity of Wy-50,295 tromethamine for the 5-LO enzyme was demonstrated by a lack of effect against 12-LO, 15-LO, CO, and phospholipase A$_2$ enzymes. In vivo inhibition of 5-LO by orally administered Wy-50,295 tromethamine was demonstrated in the rat passive Arthus model (ED$_{50}$ = 1.7 mg/kg).[246] In this model, the potency of Wy-50,295 tromethamine at 3 h is superior to A-64,077 and L-663,536, two 5-LO inhibitors with demonstrated clinical efficacy. The in vivo activity of Wy-50,295 tromethamine was corroborated by demonstration of activity in an ex vivo model of 5-LO inhibition, the rat blood leukocyte assay (ED$_{50}$ = 23 mg/kg, po).[247] Wy-50,295 is also an LTD$_4$-receptor antagonist with a binding affinity in guinea pig lung membranes comparable to LY-171,883 (K_i = 2.8 μM)[246] and a pA_2 value of 6.06 against LTD$_4$-induced contractions of isolated guinea pig trachea.[249] In a model of allergic asthma where bronchoconstriction in sensitized guinea pigs was induced by antigen, prior oral administration of Wy-50,295 tromethamine inhibits the bronchoconstriction response (ED$_{50}$ = 7.3 mg/kg).[250] These results

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are corroborated by in vitro data where Wy-50,295 tromethamine inhibits antigen-induced contractions of sensitized guinea pig tracheal rings with greater efficacy than either A-64077 or L-663,536.[251] Wy-50,295 also displays LTD$_4$-receptor antagonist activity in vivo, giving an ED$_{50}$ of 6.6 mg/kg po against LTD$_4$-induced bronchoconstriction in anesthetized guinea pigs.

In inflammatory models, Wy-50,295 tromethamine displays a profile quite different from classical nonsteroidal antiinflammatory drugs.[252] Although structurally related to naproxen, a CO inhibitor with gastrointestinal side effects, Wy-50,295 does not cause gastrointestinal lesions at doses up to 300 mg/kg, po. In the AA-induced mouse ear edema model, Wy-50,295 tromethamine displays good activity when administered orally (ED$_{50}$ = 12.7 mg/kg). In cotton top tamarin monkeys with ulcerative colitis, Wy-50,295 free acid at 50 mg/kg b.i.d. not only reduces LTB$_4$ synthesis ex vivo but also prevents relapse of the active disease.[253] In the collagen-induced arthritis rat model, Wy-50,295 tromethamine reduces both inflammation and histopathology at day 42 and continues to retard the disease even at day 98, in contrast to indomethacin.[254]

Both Wy-50,295 and REV-5901 block the synthesis of PAF (platelet-activating factor), unlike other inhibitors of LT biosynthesis such as MK-886.[255] This newly described activity may contribute to the observed pharmacological profile of these compounds and may differentiate the quinoline-containing 5-LO inhibitors from the other types of 5-LO inhibitors. The exact mechanism of this effect remains to be clarified. Wy-50,295 tromethamine is 100% bioavailable with a long duration of action. Due to Wy-50,295s dual mechanism of action in modulating the effects of LTs and its excellent in vivo activity, this quinolinyl-(bridged)aryl compound was selected for development in LT-mediated diseases such as asthma and allergic rhinitis.

Other 2-Quinolinyl(bridged)aryl Compounds. The quinolinyl(bridged)aryls are now approaching the hydroxacetophenones as the key structural class of 5-LO inhibitors and LTD$_4$ antagonists.[256] Following our lead, 20 other pharmaceutical companies have synthesized 2-quinolinyl(bridged)aryl systems and listed below are representative examples.

SR-2,640 and ETH-615. SR-2,640 is a 2-quinolinyl-(bridged)aryl LTD$_4$ antagonist in clinical development by
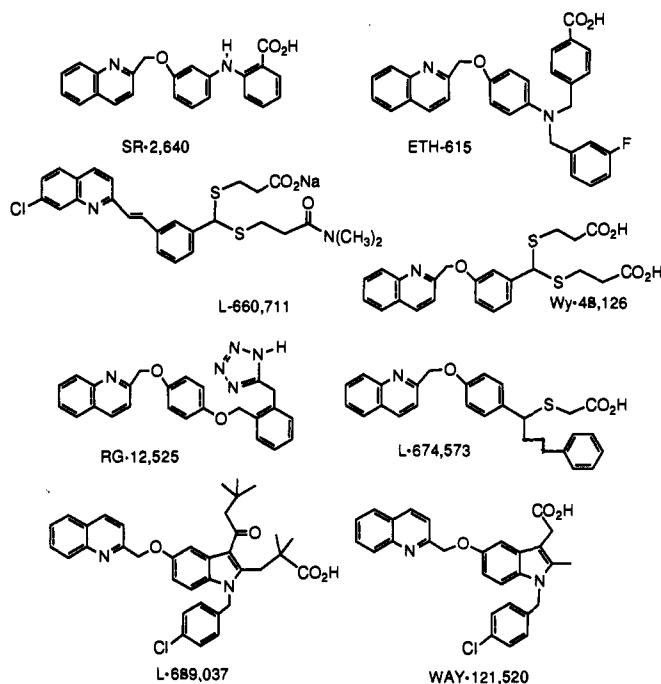

Figure 13. Other 2-quinolinyl(bridged)aryl 5-LO inhibitors and LTD$_4$ antagonists.

Leo of Denmark (Figure 13).[257] At relatively high concentrations in rat PMNs, SR-2,640 inhibits 5-LO (IC$_{50}$ = 10 μM) and CO (IC$_{50}$ = 2 μM), whereas with LTD$_4$-induced contraction of guinea pig ileum, inhibition is in the nanomolar range (IC$_{50}$ = 3 nM). In guinea pigs, SR-2,640 inhibits both LTD$_4$ and antigen-induced bronchoconstriction, and a single dose in human volunteers is well tolerated. The poor pharmacokinetic profile of SR-2,640 in guinea pigs does not translate to man, indicating the limited value of animal pharmacokinetic testing, at least for this series. Interestingly, a related quinoline-containing 5-LO inhibitor ETH-615 inhibits not only 5-LO (IC$_{50}$ = 0.02 μM, human PMN) but also IL-8 gene expression.[258]

RG-12,525. Not all analogs of REV-5901 retain 5-LO inhibition. RG-12,525 is Rorer's 2-quinolinyl(bridged)aryl peptide LT antagonist currently undergoing clinical evaluation which apparently lacks 5-LO inhibitory activity.[259] RG-12,525 has the tetrazole moiety that is also found in LY-171,883; the latter compound was withdrawn from clinical trials because of long-term toxicity. RG-12,525 competitively inhibits [^{3}H]-LTD$_4$ binding to lung membranes (K_i = 3.0 nM). It is also antagonizes the spasmogenic activity of LTC$_4$, LTD$_4$, and LTE$_4$ on lung strips with a K_B of 2.7 nM; however, the LTC$_4$ results may be an artifact since this peptide LT is rapidly metabolized to LTD$_4$. LTD$_4$-induced wheal formation is inhibited with

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orally dosed RG-12,525 (ED_{50} = 5 mg/kg). Likewise, anaphylactic death is also inhibited by oral RG-12,525 (ED_{50} = 2.2 mg/kg).

L-660,711. The quinoline L-660,711 (MK-571)[260] emerged from Zamboni's group at Merck Frosst as one of their most potent LTD_4 antagonists. Structurally, L-660,711 combines a 2-quinolinyl(bridged)aryl system with the dithioacetal moiety developed by SKF researchers. In contrast to most other quinoline-containing LT modulators, L-660,711 does not inhibit 5-LO. Apparently, the substitution of a trans double bond for an oxymethylene bridge eliminates the 5-LO inhibitory component. In the conscious monkey, L-660,711 causes total blockade of bronchoconstriction induced by LTD_4. In vitro, the (+)-enantiomer is significantly more active in binding to the receptor; however, in vivo not much difference between the enantiomers is seen. L-660,711 was in advanced clinical development; however, reports of hepatotoxicity lead its withdrawal. Like the Merck group, we also employed the 2-quinolinyl(bridged)aryl system in combination with SKF's dithioacetal moiety to design novel LT modulators, a representative example is Wy-48,126.[261]

Recently, others have joined us in speculating that the ideal compound modulating the effects of the LTs would possess both LT-receptor antagonism and 5-LO synthesis inhibitory activity.[262] These activities appear to be functionally additive[184] and, coupled with the reported ability of LTD_4 antagonists to reverse ongoing bronchoconstriction in asthma models, may extend the use of these compounds beyond solely prophylatic therapy.[263]

IV. Mechanisms of 5-LO Inhibition

At least four mechanisms can be considered for 5-LO inhibition: (1) the antioxidant and/or free radical scavenger mechanism, (2) the iron chelation mechanism, (3) the inhibition of 5-LO translocation mechanism, and (4) the substrate mimic mechanism. The study of the mechanisms of the various 5-LO inhibitors may afford more than mere academic knowledge. It may be possible that co-administration of two 5-LO inhibitors acting by dif-

ferent mechanisms may have a synergistic effect and thus would not compete with but rather complement each other. This is especially likely with a combination of a translocation inhibitor with a compound from one of the other three classes of inhibitors since the compounds will not be competing for the same site of action. Likewise, an agent which possesses more than one mechanism of action may have a net therapeutic effect which is greater than the sum of the individual component mechanisms.

With respect to our 2-quinolinyl(bridged)aryl system, we decided early not to design compounds with low oxidation potentials as possible 5-LO inhibitors because there are many physiologically important enzymes with a mechanism of action based on one-electron transfer and universal redox inhibition can translate into toxicity. Results for Wy-45,911 (peak potential = 1.19 V), Wy-47,288 (peak potential = 1.34 V), and ritolukast (peak potential = 1.20 V) indicate that the 5-LO/CO inhibitory activities for these agents are not likely due to an antioxidant mode of action. Note also that the 5-LO inhibitor Wy-50,295 has a peak potential of 1.41 V.

Considering that conversion of AA into LTs via 5-HPETE is a radical-based oxidation, it is not surprising that the largest class of 5-LO inhibitors is the antioxidant/free radical scavengers. Although lack of specificity can be a problem in this class due to the ubiquitous nature of oxidative processes in biological systems, several antioxidant 5-LO inhibitors have demonstrated clinical efficacy: lonapalene (psoriasis), docebenone (AA-861, allergic rhinitis), and R-68,151 (psoriasis).

Mechanism of Inhibition of 5-LO by Hydroxamic Acids. The mechanism of the inhibition of the 5-LO enzyme by hydroxamic acids and hydroxyureas is not well established and currently is somewhat controversial. Initially, it was assumed that the compounds inhibited 5-LO by virtue of chelation of the essential iron in the active site of the enzyme; however, recently several papers have provided evidence that this is not the whole story and that there also seems to be a antioxidant component to these compounds. Some of these hydroxamic acids and hydroxyureas are oxidized by the 5-LO enzyme. It was recently shown that hydroxamic acid-containing 5-LO inhibitors reduce the active-site iron of soybean LO and in the process are oxidized.[264] The prototypical hydroxyurea A-64,077 (zileuton) is oxidized by 5-LO to a nitroxide.[265] Related hydroxyurea derivatives are also able to reduce 5-LO.[34] Interestingly, nitroxides are capable of bearing free radical chains so that the nitroxides generated from hydroxamic acids and hydroxyureas may also inhibit the 5-LO enzyme but by radical scavenging. The hydroxamic acid BWA4C and the related hydroxyurea BW70C are not powerful redox compounds yet both inhibited lipid peroxidation. This property appears to be separate from their iron-chelating ability, thus suggesting a site-directed peroxyl radical scavenging ability.[266]

Low Redox Inhibitors, Toxicity and Specificity. In contrast to Wy-50,295 (peak potential = 1.41 V), BW-755C (IC_{50} = 3 μM, human PMN) has a low oxidation potential

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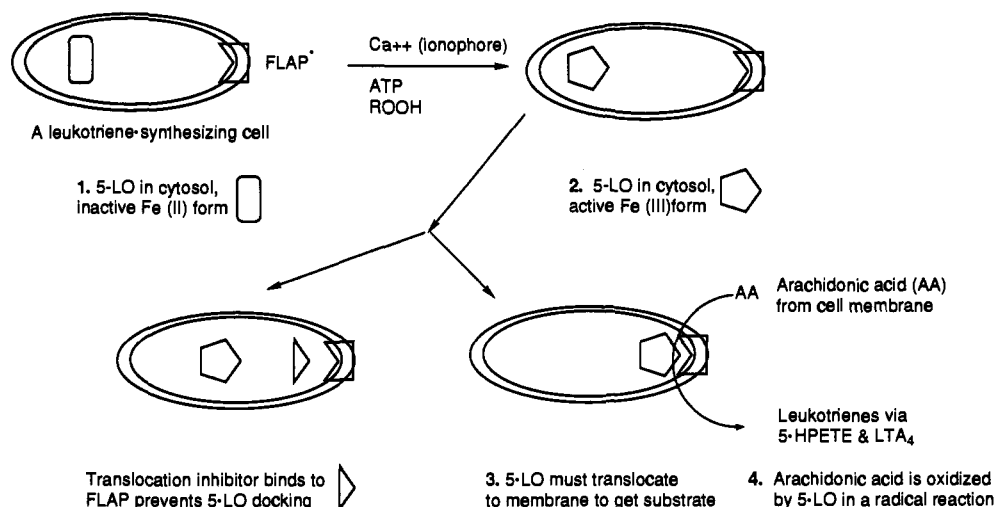
Figure 14. Translocation of the 5-LO enzyme. (*) FLAP is five lipoxygenase activating protein.

(peak potential = 0.65 V), suggesting it inhibits 5-LO and CO by donating an electron during catalysis. Furthermore, a recent spectrophotometric study revealed that BW-755C is quickly oxidized by cytochrome oxidase in mitochondria, suggesting that the oxidative conversion is associated with its inhibitory action.[267] In support of this, it was shown that both BW-755C and phenidone must first be oxidized by the peroxidase activity of the 5-LO enzyme before inhibition can occur.[268] The inactivation of the 5-LO enzyme by these compounds appears to involve the formation of a covalent bond between the enzyme and either a phenidone or BW755C metabolite. BW-755C has not been developed as a drug presumably because of toxicity which may be attributed to promiscuous redox enzyme inhibition. The toxicity of both BW755C[269] and the structurally-related phenidone (IC_{50} = 12 μM, human PMN) is apparently due to methemoglobin formation associated with nonspecific redox properties of these compounds.[182] Furthermore, phenidone analogs also induce methemoglobinemia,[270] and NDGA, a commercial antioxidant-type 5-LO inhibitor, failed early toxicological evaluation presumably for the same reasons.[269] Some of the compounds in a new series of redox 5-LO inhibitors, the indazolinones, also cause methemoglobinemia.[271] Others have warned of the potential problems of utilizing an antioxidant approach for orally active compounds that will be systemically absorbed.[272,273]

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Table I. Translocation SAR in RBL2H3 Cells

compd	inh LTC_4 syn (nM)[a]	inh translocn (nM)	inh isolated 5-LO (nM)[b]
Wy-50,295	50 ± 1	53 ± 5	6400 ± 1600
Wy-47,288	450 ± 25	500 ± 10	>10000
Wy-45,911	780 ± 50	750 ± 250	1530 ± 370
MK-886	3 ± 0.5	35 ± 5	>20000
naproxen	>10000	>10000	>20000

[a] Measures inhibition of release of LTC_4 from whole cells stimulated with A-23187, n = 3–4. [b] Measures inhibition of LTB_4 synthesis on the isolated enzyme, n = 2–3.

However, we do not contend that all low redox 5-LO inhibitors are toxic. In fact, the orally active 5-LO inhibitor AA-861 and several topically active 5-LO inhibitors [e.g., lonapalene (RS-43179) and R-68,151] have shown clinical efficacy with no reports of side effects. As noted above, hydroxamic acids and hydroxyureas also have a radical scavenging component and it appears that the majority of 5-LO inhibitors may fall under this class. We only caution that compounds with a low redox potential may be predisposed with toxicological problems, such as methemoglobinemia.

Evidence for the specificity of low redox 5-LO inhibitors is mixed. For example, indazolinones, BW-755C, phenidone, and NDGA inhibit CO in addition to 5-LO.[274] NDGA also inhibits cytochrome P-450[275] and voltage-activated calcium currents, independently of 5-LO inhibition.[276] Work on antioxidant 5-LO inhibitors, the (arylethenyl)phenols, has produced more potent inhibitors of CO than 5-LO.[277] Finally, lonapalene and AA-861,

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(274) See Table III in Bruneau, P.; Delvare, C.; Edwards, M. P.; McMillan, R. M. Indazolinones, a New Series of Redox-Active 5-Lipoxygenase Inhibitors with Built-In Selectivity and Oral Activity. *J. Med. Chem.* **1991**, *34*, 1028–1036.

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which are claimed to be selective 5-LO inhibitors, were recently shown to inhibit platelet CO[278] and both PG production and histamine release in skin mast cells.[279]

Inhibition of Five Lipoxygenase Activating Protein (FLAP). In contrast to NSAIDs which largely inhibit the CO enzyme by either redox inhibition or substrate mimicry (important exceptions are aspirin and related compounds), 5-LO inhibitors can modulate 5-LO by different mechanisms acting at different sites of the biosynthetic pathway. The putative steps involved in the 5-LO biosynthetic pathway were discussed in the 5-LO Mechanism of Action section. Inhibition of 5-LO translocation is the most recently discovered mechanism of 5-LO inhibition, and possible sites of inhibition are shown in Figure 14. Both SmithKline Beecham[280] and Merck[281] recently have shown that the 5-LO enzyme translocates from the cytosol to the cell membrane upon activation by binding to a transmembrane protein designated FLAP.[282,283] This trans-
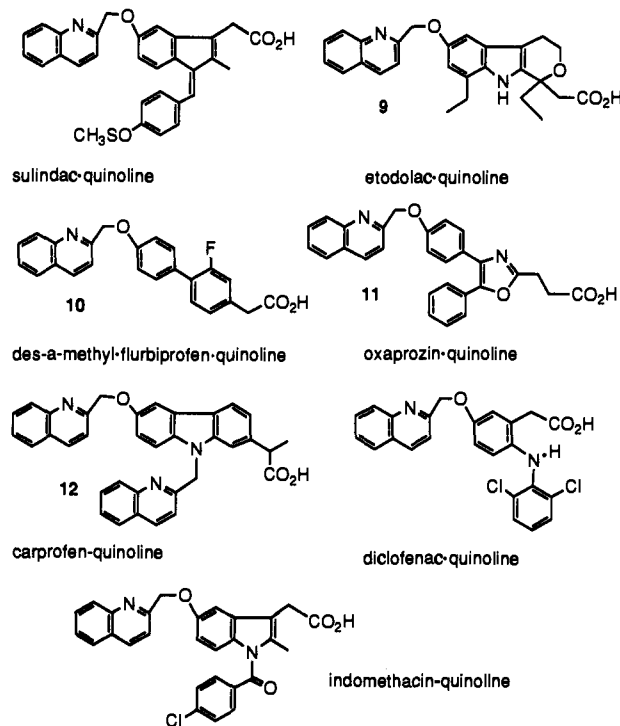

Figure 15. Examples of CO inhibitor to 5-LO inhibitor conversion: hybrid structures of NSAIDs and 2-oxymethylquinoline.

location step is necessary not only because the majority of the AA substrate is found in the cell membrane but also because the 5-LO enzyme is auto-inactivating and a continuous supply of fresh enzyme is required for an uninterrupted production of LTs. One very distinctive feature of pure 5-LO translocation inhibitors is that they are inactive against the isolated 5-LO enzyme or against broken cell preparations. The 5-LO translocation inhibitor MK-886 (IC_{50} = 2.5 nM, human PMN) appears to have modest clinical efficacy in allergic asthma.

A recent study clearly indicated that inhibition of 5-LO translocation is significant part of the mechanism of action of both Wy-50,295 and Wy-47,288 (Table I).[284] In both rat RBL-2H3 cells and human monocytes, the inhibition of A-23,187-induced LT synthesis by racemic Wy-50,295 (Wy-49,232) is mirrored by the inhibition of 5-LO translocation in a dose-related manner at similar concentrations. The effect is specific since a close structural analog (naproxen) which is inactive in inhibiting LT synthesis is also inactive against 5-LO translocation. The activity of a simpler analog of Wy-50,295 lacking the carboxylic acid side chain, Wy-47,288, in preventing 5-LO translocation argues that the quinolinylmethoxyaryl group may be a pharmacophore for the inhibition of 5-LO translocation. At low concentrations (<150 nM) of racemic Wy-50,295, the translocation mechanism is predominant with the substrate-mimic mechanism only becoming significant at higher concentrations. The potency of racemic Wy-50,295

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in inhibiting LT synthesis is 1 order of magnitude less than the known 5-LO translocation inhibitor MK-886 in RBL-2H3 cells (IC_{50} = 46.8 and 5 nM, respectively). In human monocytes racemic Wy-50,295 is comparable to MK-886 in preventing LT synthesis (IC_{50} = 188 and 50 nM, respectively); however, in human PMN's racemic Wy-50,295 is significantly less potent than MK-886 (IC_{50} = 2000 and 60 nM, respectively). The reason for these differences is not clear. The individual enantiomers of Wy-50,295 are of comparable potency to the racemic mixture in both inhibition of LT synthesis and 5-LO translocation in RBL-2H3 cells. Evidence that Wy-50,295 inhibits translocation by binding to FLAP is derived from competition studies with radiolabeled MK-886. Indeed, Wy-50,295 and its racemate Wy-49,232 can displace radiolabeled MK-886 from FLAP.[285] The results of our translocation studies are corroborated by other researchers who also claim that both racemic Wy-50,295 and REV-5901 are translocation inhibitors.[286]

The recognition that the indole class of 5-LO translocation inhibitors competes with the quinoline-class of 5-LO translocation inhibitors at the same binding site of FLAP has lead to the design of a new type of quinoline-containing 5-LO translocation inhibitor. This compound, L-689,037 (IC_{50} = 2 nM, human PMN), is termed a quindol since it is a hybrid of the indole and quinoline classes of translocation inhibitors. It binds to FLAP tighter than members of either class.[287] The structural similarity of the quinoline–indomethacin hybrid WAY-121,520 (IC_{50} = 4 nM, mouse macrophage) to the quindol L-689,037 should be noted. Interestingly, WAY-121,520 is also a PLA_2 inhibitor, although at much higher concentrations than those necessary for 5-LO inhibition.[288]

An interesting question is why is MK-886 a pure 5-LO translocation inhibitor whereas Wy-50,295 is not only a 5-LO translocation inhibitor but also an inhibitor of the isolated 5-LO enzyme. It is speculated that Wy-50,295 possesses direct inhibitory activity on the 5-LO enzyme because, unlike MK-866, it can mimic the shape of AA. In support of this argument, the inhibition of LT biosynthesis by Wy-50,295 is reversed by the addition of AA. In contrast, addition of AA in the presence of MK-886 has no
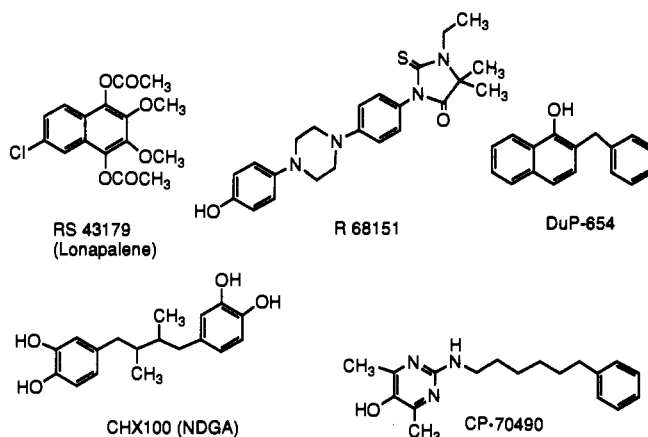

Figure 16. 5-LO inhibitors in the clinic for topical indications.

effect on the ability of MK-886 to block LT biosynthesis. What MK-886 and Wy-50,295 have in common is that both can mimic the shape of 5-HPETE which we speculate may be an endogenous ligand for FLAP. The 5-LO enzyme self-destructs after several turnovers and for the 5-LO reaction to continue there must be a signal continuously generated that promotes 5-LO enzyme translocation to replenish the destroyed enzyme. Since it is known that AA does not promote 5-LO translocation, this leaves 5-HPETE as the most likely candidate for an endogenous FLAP ligand. This hypothesis could be tested by examining 5-HPETE for FLAP binding.

Generality of Cyclooxygenase to 5-Lipoxygenase Inhibitor Conversion. As part of our design strategy to introduce oral activity into our quinoline-containing 5-LO inhibitors, we turned to other orally active CO inhibitors to determine if the naproxen example is an isolated case or if the conversion of CO inhibitors into 5-LO inhibitors is a general phenomenon. Replacement of either an existing substituent or a hydrogen in the CO inhibitors, sulindac, etodolac, carprofen, diclofenac, oxaprozin, indomethacin, and des-α-methylflurbiprofen (Figure 15), by a quinolinylmethoxy group afforded new hybrid structures which are 5-LO inhibitors. In contrast to Wy-50,295, which is a selective 5-LO inhibitor, some of these new hybrids are dual inhibitors of 5-LO and CO. For example, the quinoline/etodolac hybrid, 9, is a dual inhibitor of 5-LO and CO (91% and 47% inhibition, respectively, at 10 μM, rat PMN). In contrast, the quinoline/fluribprofen hybrid 10, the quinoline/oxaprozin hybrid, 11, and the quinoline/carprofen hybrid, 12, are pure 5-LO inhibitors (100%, 96%, and 92% inhibition of 5-LO at 10 μM, rat PMN, respectively). Apparently, replacement of an existing substituent or a hydrogen atom of a CO inhibitor by a quinolinylmethoxy group is a general route to novel 5-LO inhibitors. It is tempting to speculate that the mechanism of inhibition of 5-LO by these quinoline hybrids may involve the inhibition of 5-LO translocation as has been demonstrated for other members of this class of 5-LO inhibitors (Wy-50,295, Wy-47,288, L-674,573).[289]

V. 5-LO Inhibitors in the Clinic

There are two types of 5-LO inhibitors in clinical trials: topically- and orally-administered drugs. Several 5-LO

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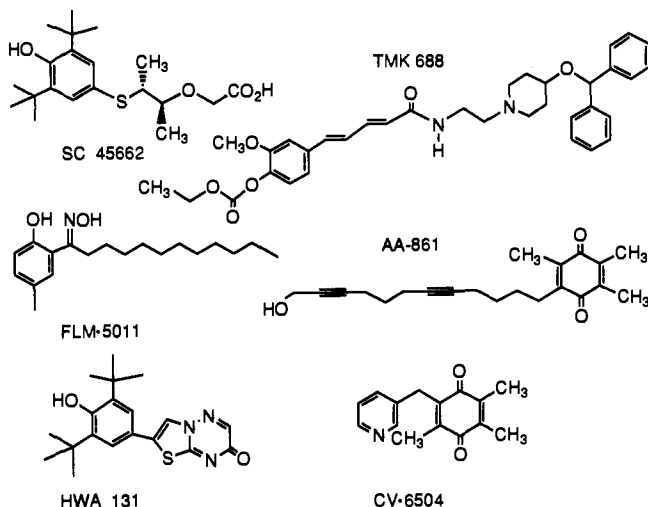
Figure 17. Orally active 5-LO inhibitors in the clinic.

inhibitors of the antioxidant type are undergoing clinical trials for the treatment of psoriasis. One of the more advanced compounds, lonapalene (IC_{50} = 0.5 μM, rat RBL-1) (Figure 16) has shown efficacy in psoriasis in several studies.[290] Other topically active antioxidant-type 5-LO inhibitors include R-68151 (IC_{50} = 0.5 μM, rat RBL-1) which has also shown efficacy in psoriasis,[291] DuP-654 (IC_{50} = 19 nM, rat RBL-1),[292] NDGA (IC_{50} = 5 μM, rat blood), and CP-70490 (IC_{50} = 0.3 μM, mouse).[293] NDGA lacks specificity in that it also inhibits cytochrome P-450,[275] whereas CP-70490 is also an IL-1 synthesis inhibitor.

Many of the reported orally active 5-LO inhibitors being evaluated in the clinic have more than one physiological property. For example, SC-45662 (IC_{50} = 3.7 μM vs 5-HETE, rat RBL-1) (Figure 17) is one of a number of 2,6-dialkylphenol-containing antioxidant-type, orally active 5-LO inhibitors which have entered clinical trials;[294] it also is claimed to inhibit superoxide generation induced by either fMLP or C5a. From a series of 250 analogs of caffeic acid, TMK-688 (IC_{50} = 0.32 μM vs LTB$_4$, mouse mastocytoma) was selected for clinical development.[295] It is also

a prodrug of TMK-777, an antioxidant-type 5-LO inhibitor that results from hydrolysis of the carbonate found in TMK-688. Interestingly, these compounds also are antihistamines. FLM 5011 is a orally active 5-LO inhibitor, presumably of the iron chelator class, that has entered clinical trials for a myocarditis indication.[296]

AA-861 (docebenone) is an orally active, antoxidant-type 5-LO inhibitor (IC_{50} = 80 nM vs LTB$_4$, rat macrophages) that has shown clinical efficacy in a number of diseases;[297] however, the selectivity of AA-861 was questioned recently.[279]

HWA 131 is a orally active, antioxidant-type 5-LO inhibitor that has entered clinical trials for an antirheumatic indication. Although HWA 131 is only moderately potent as a 5-LO inhibitor (IC_{50} = 25 μM vs LTB$_4$, human PNMs), it also has immunomodulating properties.[298]

CV-6504 is a novel, orally active, antioxidant-type 5-LO inhibitor (IC_{50} = 62 nM, rat RBL-1) with added activity against thromboxane synthesis and lipid peroxidation which has entered clinical trials for a glomerular nephritis indication.[299]

Perhaps the most impressive clinical results have been shown by an iron chelating-type 5-LO inhibitor, A-64077 (zileuton), despite the fact that it has a relatively short half-life in man (2.5 h). In 11 patients with mild to moderate inflammatory bowel disease, 800 mg of A-64077 administered orally for 28 days results in significant improvement of symptoms in eight of the patients.[300] In a related double-blind, placebo-controlled study of 11 patients with inflammatory bowel disease, 800 mg of A-64077 given orally results in marked improvement in total sigmoidal score, total symptom score, biopsy score, and global evaluation.[301] In a double-blind, placebo-controlled study of eight patients suffering from allergic rhinitis, oral administration of 800 mg of A-64077 results not only in inhibition of LT synthesis in nasal lavage but also in a significant reduction in nasal congestion.[302] Preliminary double-blind, placebo-controlled studies of oral A-64077 (800 mg, b.i.d.) in rheumatoid arthritis shows a trend toward a greater decrease in the number of swollen and

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painful joints.[303] In a randomized, double-blind, crossover study of cold air-induced asthma, oral administration of 800 mg of A-64077 significantly ameliorated the asthmatic response.[304] In a multicenter, randomized, double-blind, placebo-controlled study of spontaneous asthma, oral administration of either 600 mg or 800 mg of A-64,077 for 4 weeks significantly improved airway function and symptomatology.[305] BWA4C is also an iron chelating-type 5-LO inhibitor that has entered clinical trials; however, its short half-life precluded evaluation of efficacy. The clinical efficacy shown by A-64077 in a wide range of inflammatory diseases has been the most convincing demonstration of the promise of 5-LO inhibitors.

Further back in clinical development are the newest class of inhibitors of LT synthesis, the translocation inhibitors. MK-886 is the prototype inhibitor of 5-LO translocation (Figure 18). In a double-blind, placebo-controlled, crossover clinical trial of asthma, oral administration of MK-886 resulted in significant reduction of the antigen-induced early-phase reaction and delayed the onset of the late reaction.[306] PF-5901 (formerly REV-5901) is a translocation inhibitor under clinical development for an inflammatory bowel disease indication. The related quinoline ETH-615 is in clinical development as a topical agent for the treatment of psoriasis and other skin diseases. Finally, Wy-50,295 tromethamine is a translocation inhibitor with a secondary direct 5-LO inhibitory component which has been given safely in up to 1000-mg doses to normal volunteers.

VI. Summary

In conclusion, an effective modulator of the AA cascade for the treatment of asthma and other inflammatory diseases may require 5-LO inhibitory activity as well as LTD_4 antagonism in order to limit the effects of LTB_4, LTD_4, and 5-HPETE. The unknown role of LTC_4 with respect to bronchoconstriction and mucus production could mask the efficacy of a pure LTD_4 antagonist in man, whereas the chemotactic property of LTB_4 for eosinophils can contribute to lung inflammation. Indeed, it is observed that the blood of patients with bronchial asthma has increased numbers of hypodense eosinophils.[307] In addition, the formation of lipid-derived peroxide radicals, such as 5-HPETE, are believed to be responsible for various types of cellular injuries associated with the inflammatory disease process. Because inhibition of the CO pathway is thought to explain the therapeutic effects of nonsteroidal antiinflammatory agents in rheumatic diseases, a 5-LO

inhibitor with CO inhibitory activity may also be desirable profile for an antiasthma agent.

The validation of the LT hypothesis of disease had to wait for the demonstration of a clinical effect by either a LTD_4 receptor antagonist or a LT synthesis inhibitor (5-LO inhibitor). Only very recently has this evidence become available and it is now apparent that compounds that antagonize LTD_4 receptors[308] or inhibit LT synthesis[309]

(308) Clinical efficacy has been shown for the following leukotriene receptor antagonists (the disease studied is in parentheses): (a) Ly-171,883 (allergic asthma): Cloud, M. L.; Enas, G. C.; Kemp, J.; PlattsMills, T.; Altman, L. C.; Townley, R.; Tinkelman, D.; King, T.; Middleton, E.; Sheffer, A. L.; Mcfadden, E. R.; Farlow, D. S. A Specific LTD_4/LTE_4-receptor Antagonist Improves Pulmonary Function in Patients with Mild, Chronic Asthma. *Am. Rev. Respir. Dis.* **1989**, *140*, 1336–1339. (b) Ly-171,883 (cold air-induced asthma): Israel, E.; Juniper, E.; Callaghan, J. T.; Mathur, P. N.; Morris, M. N.; Dowell, A. R.; Enas, G. G.; Hargrave, F. E.; Drazen, J. M. Effect of a Leukotriene Antagonist, Ly171883, on Cold Air-induced Bronchoconstriction in Asthmatics. *Am. Rev. Respir. Dis.* **1989**, *140*, 1348–1353. (c) Ym-16,638 (aspirin-induced asthma): Filep, J. Leukotrienes in Health and Disease. *Drugs Today* **1989**, *25*, 140. (d) MK-571 (exercise-induced bronchoconstriction): Manning, P. J.; Watson, R. M.; Margolskee, D. J.; Williams, V. C.; Schwartz, J. I.; O'Byrne, P. M. Inhibition of Exercise-induced Bronchoconstriction by MK-571, a Potent Leukotriene D_4-Receptor Antagonist. *N. Engl. J. Med.* **1990**, *323*, 1736–1739. (e) SKF-104,353 (aspirin-induced asthma): Smith, C.; Christie, P. E.; Lee, T. H. The Effect of the Leukotriene D4 (LTD4) Antagonist SKF 104353 on Aspirin-induced Asthma. *J. Allergy Clin. Immunol.* **1991**, *87*, 139. (f) ICI-204,219 (allergen-induced bronchoconstriction): Nathan, R. A.; Storms, W. W.; Bodman, S. F.; Minkwitz, M. C.; Glass, M. Inhibition by Aerosolized ICI 204,219, LTD_4 Receptor Antagonist, of Allergen-induced Bronchoconstriction. *J. Allergy. Clin. Immunol.* **1991**, *87*, 256. (g) ICI-204,219 (allergen-induced bronchoconstriction): Taylor, I. K.; O'Shaughnessy, K. M.; Fuller, R. W.; Dollery, C. T. Effect of Cyseinylleukotriene Receptor Antagonist ICI 204,219 on Allergen-induced Bronchoconstriction and Airway Hyperreactivity in Atopic Subjects. *Lancet* **1991**, *337*, 690–694.

(309) Clinical efficacy has been shown for the following 5-lipoxygenase inhibitors (the disease studied in in parentheses): (a) Lonapalene (psoriasis): Lassus, A.; Forsstrom, S. A Dimethoxynaphthalene Derivative (RS-43179 Gel) Compared with 0.025% Fluocinolone Acetonide Gel in the Treatment of Psoriasis. *Br. J. Dermatol.* **1985**, *113*, 103–106. (b) Lonapalene (psoriasis): Akers, W. A.; Tanenbaum, L.; Young, J.; Jones, G.; Venuti, M.; Lassus, A. RS-43179, a Nonsteroidal Topical Antipsoriatic Compound: Preclinical and Clinical Profile. *J. Invest. Dermatol.* **1985**, *84*, 354. (c) Docebenone (allergic rhinitis): Ancill, R.; Takahashi, Y.; Kibune, Y.; Campbell, R.; Smith, J. R. Randomized Double-blind, Placebo-controlled Clinical Trial of a Selective 5-Lipoxygenase Inhibitor (AA-861) for the Prevention of Seasonal Allergic Rhinitis. *J. Int. Med. Res.* **1990**, *18*, 75–88. (d) Lonapalene (psoriasis): Black, A. K.; Camp, R. D. R.; Mallet, A. I.; Cunningham, F. M.; Hofbrauer, M.; Greaves, M. W. Pharmacologic and Clinical Effects of Lonapalene (RS 43179), a 5-Lipoxygenase Inhibitor, in Psoriasis. *J. Invest. Dermatol.* **1990**, *95*, 50–54. (e) Docebenone (primary nephrotic syndrome): Shibashi, T.; Nakano, H.; Matsumoto, H.; Miyahara, T. Treatment of Primary Nephrotic Syndrome with a Lipoxygenase Inhibitor. *Curr. Ther. Res.* **1990**, *47*, 13–21. (f) quercetin (glomerulonephritis): Mune, M.; Goto, T.; Matoba, K.; Mori, Y.; Yamada, Y.; Yukawa, S.; Nomoto, H. Effects of a 5-Lipoxygenase Inhibitor on Primary Mesengial Proliferative Glomerulonephritis. 7th International Conference on Prostaglandins and Related Compounds. Florence, Italy, May 28–June 1, 1990; p 243 of abstract book. (g) Docebenone (bullous pemphigoid): Kawana, S.; Ueno, A.; Nishiyama, S. Increased Levels of Immunoreactive Leukotriene B_4 in Blister Fluids of Bullous Pemphigoid Patients and Effects of a Selective 5-Lipoxygenase Inhibitor on Experimental Skin Lesions. *Acta Derm. Venereol.* **1990**, *70*, 281–285.
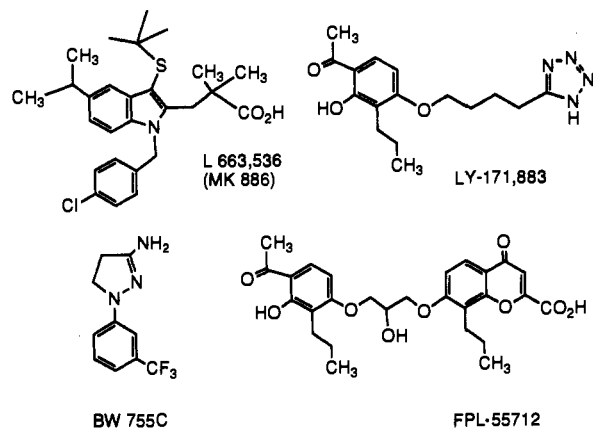
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Figure 18. Standard 5-LO inhibitors and LTD$_4$ antagonists.

have shown clinical efficacy in a wide range of diseases. Due to the breakthrough nature of this approach, certain of these compounds are being considered for expedited development.[310] The absence of side effects seen in the clinical trials of selective 5-LO inhibitors is gratifying and argues that LTs are not important in homeostasis. Only time will tell whether 5-LO inhibitors will take their place in the therapeutic armamentarium; however, the recent demonstration of clinical efficacy by a number of these compounds is a significant step in this direction.

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