Eur. J. Med. Chem. 34 (1999) 671–685

Invited review

Inhibitors of 5-lipoxygenase: a therapeutic potential yet to be fully realized?

Robert N. Young*

Department of Medicinal Chemistry, Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire - Dorval, Québec H9R 4P8, Canada

(Received 21 June 1999; accepted 21 June 1999)

Abstract – Inhibition of leukotriene biosynthesis has been extensively studied as a potential for the development of novel therapies for inflammation and respiratory diseases and, in particular, for asthma. Many compounds have been identified which inhibit the key enzyme, 5-lipoxygenase. Four distinct classes of compounds have been identified, namely, (1) redox inhibitors (alternative substrates), (2) iron chelating inhibitors, (3) competitive reversible inhibitors, and (4) inhibitors of the 5-lipoxygenase activating protein. Experience over the past two decades with redox inhibitors has been disappointing and although a number of potent compounds have been identified, they have often been associated with ancillary toxicity and non-specificity due to their redox activity. Iron chelating inhibitors have been more successful and one compound, Zileuton®, has reached the market. However, more potent analogues have often encountered toxicity problems. Competitive inhibitors have been identified by a number of research groups but, as yet, none has been successful. Inhibitors of the 5-lipoxygenase activating protein (FLAP) have been identified and compounds such as MK-0591 and BaY-X-1005 have shown efficacy in asthma trials. To date, however, no clear advantage for inhibitors of lipoxygenase has been demonstrated relative to the leukotriene D_4 receptor antagonists such as Singulair® and Accolate®. © 1999 Éditions scientifiques et médicales Elsevier SAS

5-lipoxygenase / inhibitor / 5-lipoxygenase activating protein / asthma / inflammation

1. Introduction

After the initial characterization of the slow-reacting substance of anaphylaxis in 1940 [1], nearly four decades passed before the characterization by Professor Bengt Samuelsson of the Karolinska Institute [2, 3] of these potent contractile substances as novel peptido-lipid hybrids, which became known as leukotrienes C_4, D_4 and E_4. These substances and a companion lipid which became known as leukotriene B_4 were proposed by Samuelsson to be derived from a common epoxide intermediate which he named leukotriene A_4. Samuelsson's biosynthetic detective work, which led to the proposal of the leukotriene biosynthetic pathway *(figure 1)* was a scientific triumph. Considering the potent pro-inflammatory properties of leukotriene B_4 [4] and the multiple activities of the peptido-lipid leukotrienes LTC_4, D_4 and E_4, it became apparent that modulation of this pathway could have important implications in the development of novel therapeutics for diseases such as asthma, allergy and a host of other inflammatory diseases. It was recognized in the course of this work that a key enzyme in the process, 5-lipoxygenase, could transform arachidonic acid (AA) in a two-step process to first, 5-hydroperoxyeicosatetraenoic acid (5-HPETE), and thence through a dehydration step to leukotriene A_4. This common unstable intermediate is then taken on to leukotriene B_4 via leukotriene B_4 synthase in certain cells, such as neutrophils, or could be converted via a specific glutathione-transferase enzyme (leukotriene C_4 synthase) to provide the peptido-lipid leukotrienes C_4, D_4 and E_4 in cells such as eosinophils [5].

In the early 1980s, a number of pharmaceutical research laboratories throughout the world recognized that novel therapeutics could be derived from the development of inhibitors of 5-lipoxygenase (which would potentially modulate the entire pathway) or from receptor antagonists at the specific leukotriene receptors. At the onset, it was not clear which of these alternative approaches would prove most successful, although, as of this date, it would appear that the latter approach, namely

*Correspondence and reprints

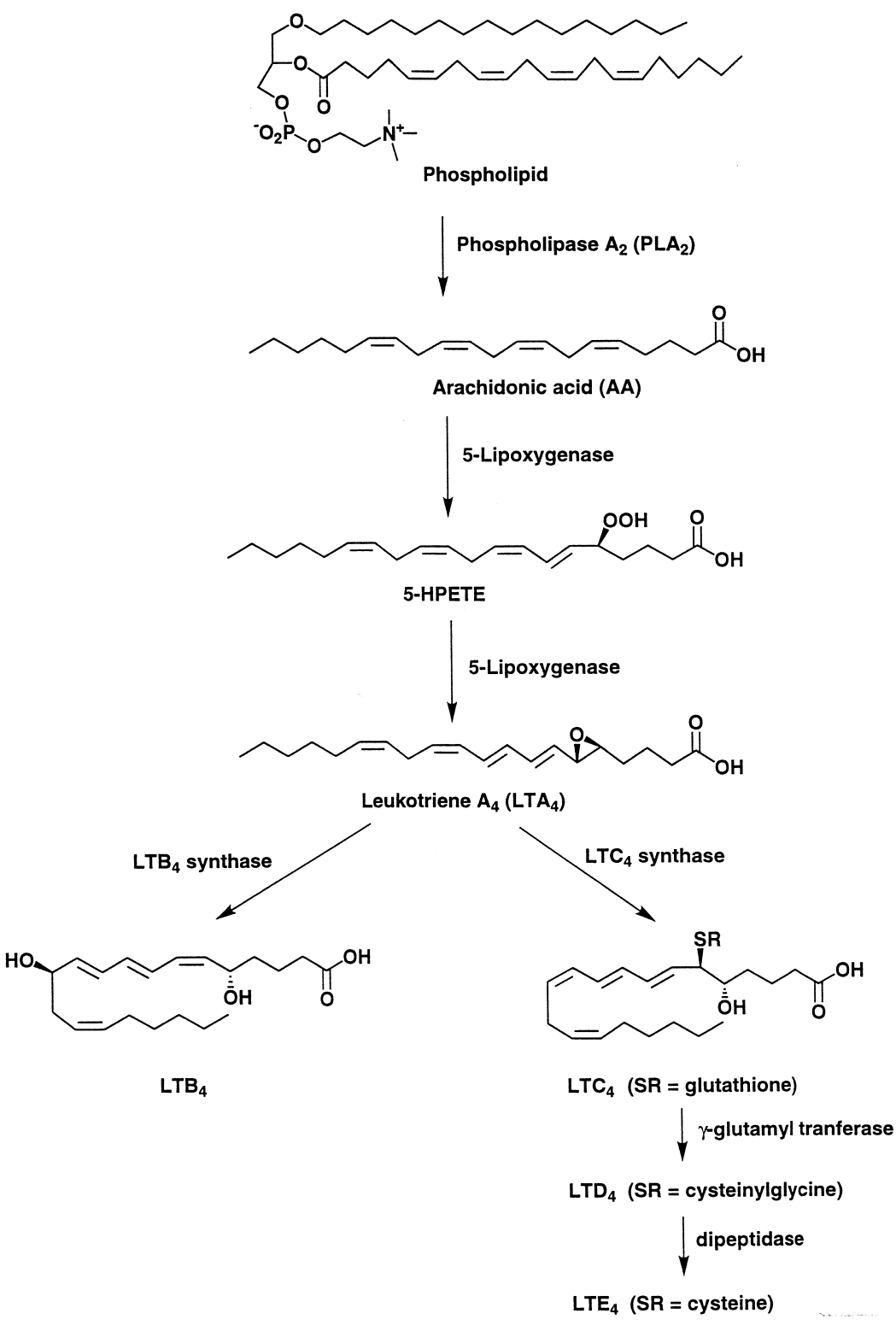


Figure 1. Leukotriene biosynthetic pathway.

the development of leukotriene D_4 receptor antagonists, has been most successful. Nonetheless, the efforts that have gone into the development of specific inhibitors of the 5-lipoxygenase enzyme have been extensive and have yielded at least one novel therapeutic agent, namely Zileuton®. A number of reviews on the development of 5-lipoxygenase inhibitors have been published in the past [6–8]. This review will attempt to provide an overview of what has been learned in the course of these efforts and, hopefully, provide a useful perspective for future drug development.

2. 5-Lipoxygenase mechanism

A detailed review on the 5-lipoxygenase mechanism has been published [7] and doesn't bear detailed repeating here. However, a number of key factors are pertinent in understanding the efforts to discover a novel and safe inhibitor of 5-lipoxygenase. 5-lipoxygenase was found to be a cytosolic enzyme which contained a non-haem iron atom. For maximum activity, it is necessary that the enzyme be converted from an inactive reduced state (Fe(II) state (E_r)) to the active Fe(III) state (E_o) through interaction with an oxidizing agent such as fatty acid hydroperoxide, AOOH.

The oxidized enzyme then interacts with the fatty acid substrate (AH) to yield the Fe(II) enzyme with a bound pentadienyl radical ($A^{\bullet}$), which then reacts with molecular oxygen to yield a hydropyroxy radical ($A00^{\bullet}$), which then picks up a hydrogen atom to yield the fatty acid hydroperoxide and to regenerate the oxidized enzyme. The exact mechanism whereby the 5-HPETE is then converted to leukotriene A_4 is not fully understood but is catalysed in a second rapid step by the same enzyme *(figure 2)*. The enzyme itself is subject to turnover inactivation, presumably through the generation of reactive radical biproducts. The arachidonic acid substrate is generally limiting in the system and is generated near the cell wall surface by the enzyme phospholipase A_2. Processes are in place to rapidly reacylate liberated arachidonic acid, such that free levels of arachidonic acid are normally very low in the cells. Thus, in order to have optimal activity, the 5-lipoxygenase enzyme translocates under mediation of a variety of factors, such as calcium levels in the cell, to interact with the cell membrane. Thus, a variety of approaches can be considered for the development of inhibitors of 5-lipoxygenase. Considering the redox cycling nature of the enzyme, it should be possible to inhibit the enzyme by competition with an alternative substrate which would itself be oxidized through radical transfer, and thus, divert the enzyme from its normal task. Such a substrate could itself produce reactive intermediates which could facilitate turnover inactivation of the enzyme. The inhibitor radicals thus produced, if stable, could be cycled in their own right or go on to decompose. Alternatively, inhibitors which are not substrates for the enzyme could interact with either the reduced state or the oxidized state of the enzyme (or both) and form reversible dead-end inhibitor-enzyme complexes. Finally, it could be possible to inhibit the translocation of 5-lipoxygenase to the membrane where it obtains substrate or to inhibit the transfer of the substrate to the enzyme. Although the details of these possibilities were not known as research began in the early 1980s to find inhibitors of 5-lipoxygenase, inhibitors in all three of these classes have been discovered and, in some cases, shown to be useful drugs.

3. Redox inhibitors of 5-lipoxygenase *(figure 3)*

Many small organic compounds such as phenols, quinones, dihydroquinones, etc., can interact in redox mechanisms. Early screening to find lipoxygenase inhibitors employed cell models such as human or rat polymorphonuclear cells (PMNs) stimulated with calcium ionophores, measuring the production of leukotriene B_4. These initial efforts were rewarded by the discovery of a wide variety of inhibitors, some of which showed quite potent and apparently selective inhibition (selectivity was generally determined relative to other oxygenase enzymes such as cyclooxygenase or other lipoxygenases such as 12-lipoxygenase). Considering the lipophilic nature of the substrate, these inhibitors were generally small lipophilic molecules such as mono- and polycyclic aromatics.

In our own laboratories, we were initially elated to discover the tricyclic benzothiazinone, L-615,919, which showed nanomolar potency for inhibition of leukotriene synthesis in PMN cells stimulated with calcium ionophore. The compound also showed bioavailability via the oral route and biochemical activity ex vivo. Our initial elation, however, was rapidly quenched when it was found that the compound promoted methaemoglobin information in the blood of dogs treated with the drug. It was apparent that the drug not only interfered with the redox cycling of 5-lipoxygenase but also could serve to convert iron in haemoglobin to the oxidized Fe(III) state producing profound toxicity. This was the first indication of problems of non-specificity and ancillary activity due to redox cycling that were to plague the development of redox inhibitors of 5-lipoxygenase for many years to come. Our initial feelings were that L-615,919 was too redox-active and the product of its reduction (a dihydroaminoquinone) would be too powerful an oxidizing

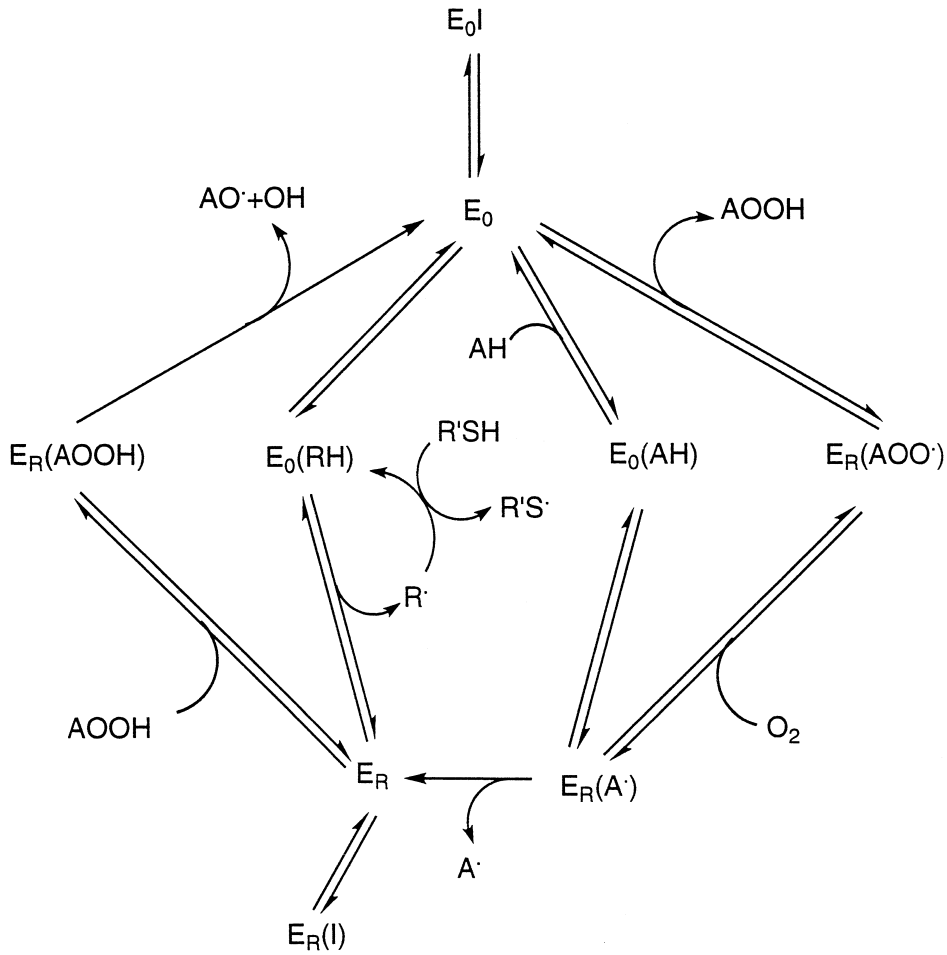


Figure 2. A kinetic mechanism of 5-lipoxygenase: oxygenase activity (right hand side), results from the reaction of the Fe(II) enzyme (E_R) with the arachidonic acid substrate (AH) to yield the Fe(II) enzyme (E_R) with a bound arachidonyl radical ($A^{\bullet}$), followed by binding of O_2 and reaction to yield hydroperoxyl radical ($AOO^{\bullet}$) bound to E_R and then regeneration of E_O and release of 5-hydroperoxyeicosatetraenoic acid (AOOH). Free E_R can result from dissociation of $A^{\bullet}$ from $E_R(A^{\bullet})$ or from reduction of E_O by various reducing agents (RH). The product of one electron oxidation of RH ($R^{\bullet}$) can be reduced by thiols. Reduced enzyme (E_R) can be reoxidized by fatty acid hydroperoxide (AOOH) to yield a hydroxide ion and an alkoxy radical ($AO^{\bullet}$). Non-redox reversible dead end inhibitors (I) can bind to E_R, E_O and possibly other states of the enzyme as well.

agent, and thus shows the observed toxicity. Examination of the literature indicated a number of redox-active substances such as menadione [9] which were known to cause methaemoglobinaemia and also to produce superoxide anions which led to other manifestations of toxicity such as Heinz body formation and haemolysis of blood cells. Our efforts were then directed to produce compounds with less potent redox activity hoping that these types of toxicities could be differentiated from the inhibition of the lipoxygenase enzyme itself. Modulation of redox potential via substitution led to the discovery of L-651,392 [10] which did not show methaemoglobin formation in dog blood. The compound was, however, a potent 5-lipoxygenase inhibitor (IC_{50} = 60 nM (rat PMN)). Unfortunately, the compound was only poorly soluble and poorly absorbed. It also showed a variety of toxicities including genotoxicity and therefore the compound was abandoned. Further studies on phenothiazinone analogues including the naphthyl analogue, such as L-649,927, provided compounds which were free of the tendency to form methaemoglobin, although, again, poorly absorbed. A prodrug carbonate, L-654,623, with better solubility and bioavailability which showed good in vitro activity was identified (Y. Girard, unpublished

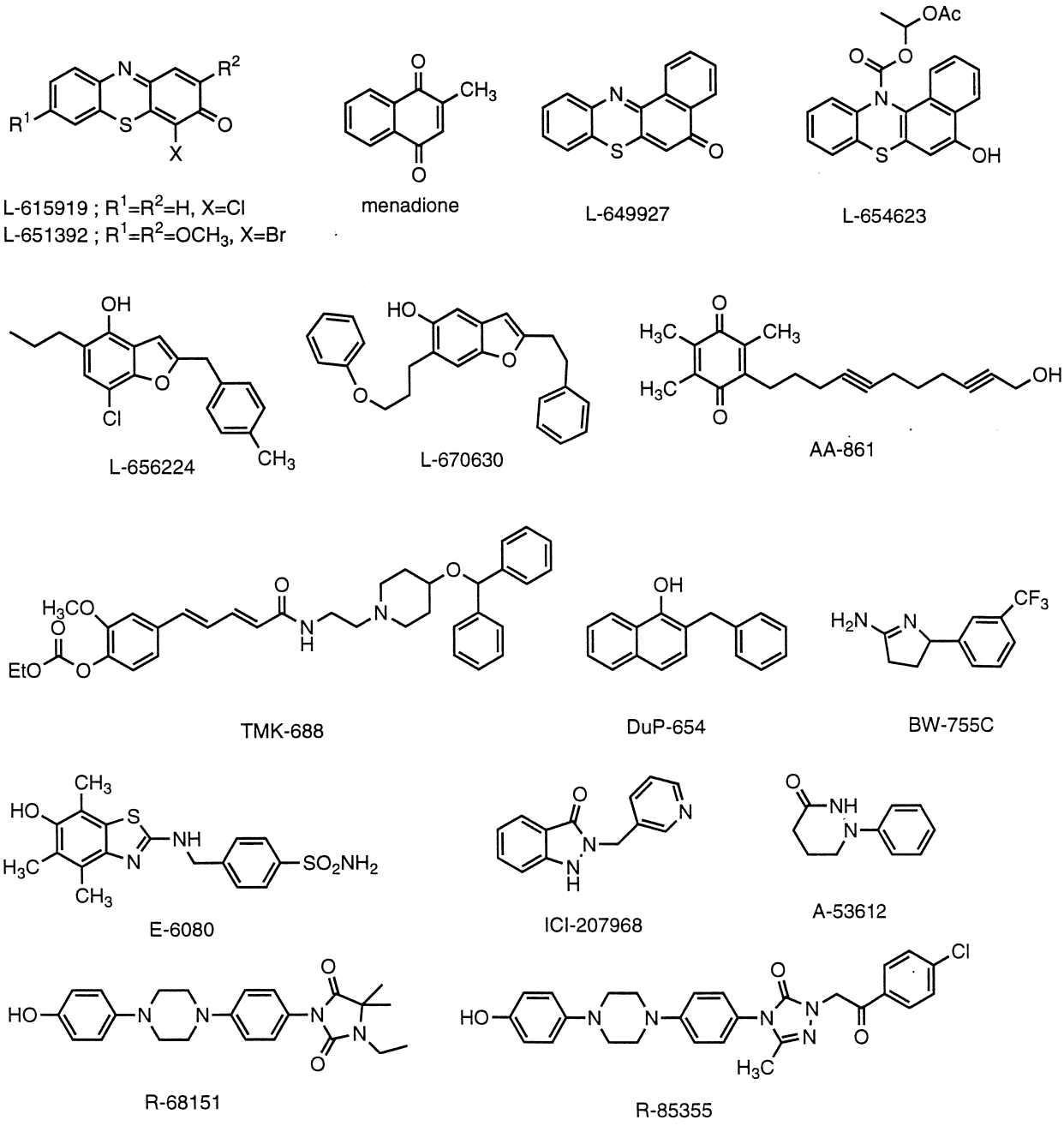


Figure 3. Redox inhibitors of 5-lipoxygenase.

results). Unfortunately, again, problems were encountered in toxicological evaluation and L-654,623 was shown to cause Heinz body formation in blood cells in dogs. As this kind of toxicity could be attributed to the redox activity of this series, further work on phenothiazinones was stopped. In later work, a number of hydroxybenzofurans were identified through screening. Further studies and optimization led to the identification of L-656,224 [11, 12]. Although the compound showed good in vitro and in vivo activity, toxicological evaluation

revealed indications of hypersensitivity reactions, apparently due to metabolic conversion to quinone-type metabolites [13], and development was suspended. Further efforts at Merck Frosst derived a second dihydrobenzofuranol, L-670,630 [14]. Unfortunately, again, signs of toxicity similar to that observed for L-656,224 were observed and investigation of redox inhibitors was abandoned at Merck Frosst in preference for a search for competitive non-redox inhibitors.

Other companies have investigated a variety of redox inhibitors over the years, although none to date have been brought to market. The quinone AA-861 has been reported to be in clinical development but little recent information is available [15, 16]. Other phenolic 5-lipoxygenase inhibitors have entered development and clinical trials, such as, TMK-688 (linazolast) [17], DuP-654 [18], R-68151 [19] and E-6080 [20]. None, however, have proceeded to market to this date and presumably have encountered a variety of problems. Many redox-active heterocyclic compounds such as BW-755C [21], ICI-207968 [22] and A-53612 [23] have been reported as 5-lipoxygenase inhibitors over the years. However, studies have shown that compounds of this type are prone to one electron oxidation [24] and can cause methaemoglobin formation in blood [22] and, thus, they apparently have not been developed further.

Recently, a study on a series of tetrahydro-1,2,4-triazien-3-ols has been published by workers at Abbott [25] which are reported to be free of methaemoglobinaemia in rats. This suggests that it may be possible to derive such inhibitors free of the toxicity problems observed in related phenothiazinone and phenol series of 5-lipoxygenase inhibitors. The heterocyclic phenol from Jansen, R-68151, has been reported to be in clinical trials for psoriasis by the topical route and has apparently shown mild to moderate therapeutic effect [26]. A more potent analogue, R-85355, was also investigated for psoriasis but, unfortunately, was found not to show significant clinical activity [27].

4. Iron chelator inhibitors *(figure 4)*

The most successful efforts to derive non-toxic redox type inhibitors of 5-lipoxygenase have been in the area of hydroxamic acids and related N-hydroxy ureas. These compounds were designed basically with the expectation that the functional groups might chelate iron and therefore inhibit the enzyme. Studies in this area have been pursued by researchers from Glaxo/Burroughs Wellcome, Abbott Laboratories, SmithKline Beecham, Wyeth Ayerst, Ciba Geigy and many others.

Early studies of N-acylhydroxylamine compounds led to the discovery of BW-A4C [28] and A-63162 [29] as potent and selective 5-lipoxygenase inhibitors. However, BW-A4C was found to be rapidly metabolized in humans [30] and shown to be oxidized by 5-lipoxygenase to form nitroxide radicals [31]. It was also found that O-glucuronidization was a problem in this series, as well as the hydrolysis of the hydroxamic acid which impaired in vivo activity [30]. Researchers at Abbott continued extensive studies in this area concentrating on an analogous series of N-hydroxyureas which were more hydrolytically stable, had reduced glucuronidization and therefore superior in vivo properties. These efforts ultimately yielded A-64077 (Zileuton) which has undergone extensive clinical evaluation [32]. Zileuton has shown efficacy in chronic asthma where it provided some degree of bronchodilation and anti-inflammatory and steroid sparing effects [33]. The compound was brought to market in 1996 as the first of the new class of anti-leukotriene drugs as a 600 mg q.i.d. dose. The compound, however, has shown a variety of adverse effects including elevated liver enzymes and other hepatic toxicities as well as significant drug interactions [32]. Zileuton has been shown to induce a variety of liver enzymes, including P450-2B and P450-4A, and induces hepatomegaly on chronic treatment in rats [34]. Recent reports have indicated that Zileuton showed efficacy in a rat model of ulcerative colitis [35]. However, a subsequent clinical trial at a dose of 600 mg q.i.d. for six months failed to show activity significantly better than placebo, and showed less efficacy when compared with mesalazine [36]. Efforts by Abbott researchers have derived a number of more potent analogues of Zileuton with better pharmacokinetics which promise the potential for a q.d. drug with doses lower than required for Zileuton. Studies derived an optimized compound, A78773, and its more potent R(+) enantiomer, A79175 [37] which was reported to have entered clinical trials [38, 39]. A phenol analogue, A-76745 (fenleuton) has been evaluated as potential treatment for allergic and inflammatory disorders in horses [40]. The compound has been reported to have some efficacy in a horse model of chronic obstructive pulmonary disease [41]. A thiophene analogue (ABT-761) (atreleuton) has apparently proceeded to Phase III trials as a second generation hydroxyurea 5-lipoxygenase inhibitor and is reported to show potent biochemical efficacy for nine hours after a single oral dose of 200 mg. The compound showed efficacy in blocking bronchoconstriction in asthmatics following a challenge by exercise [42]. ABT-761 has a long elimination half-life of about 15 hours consistent with once-a-day dosing [43]. However, the compound has been reported to show some

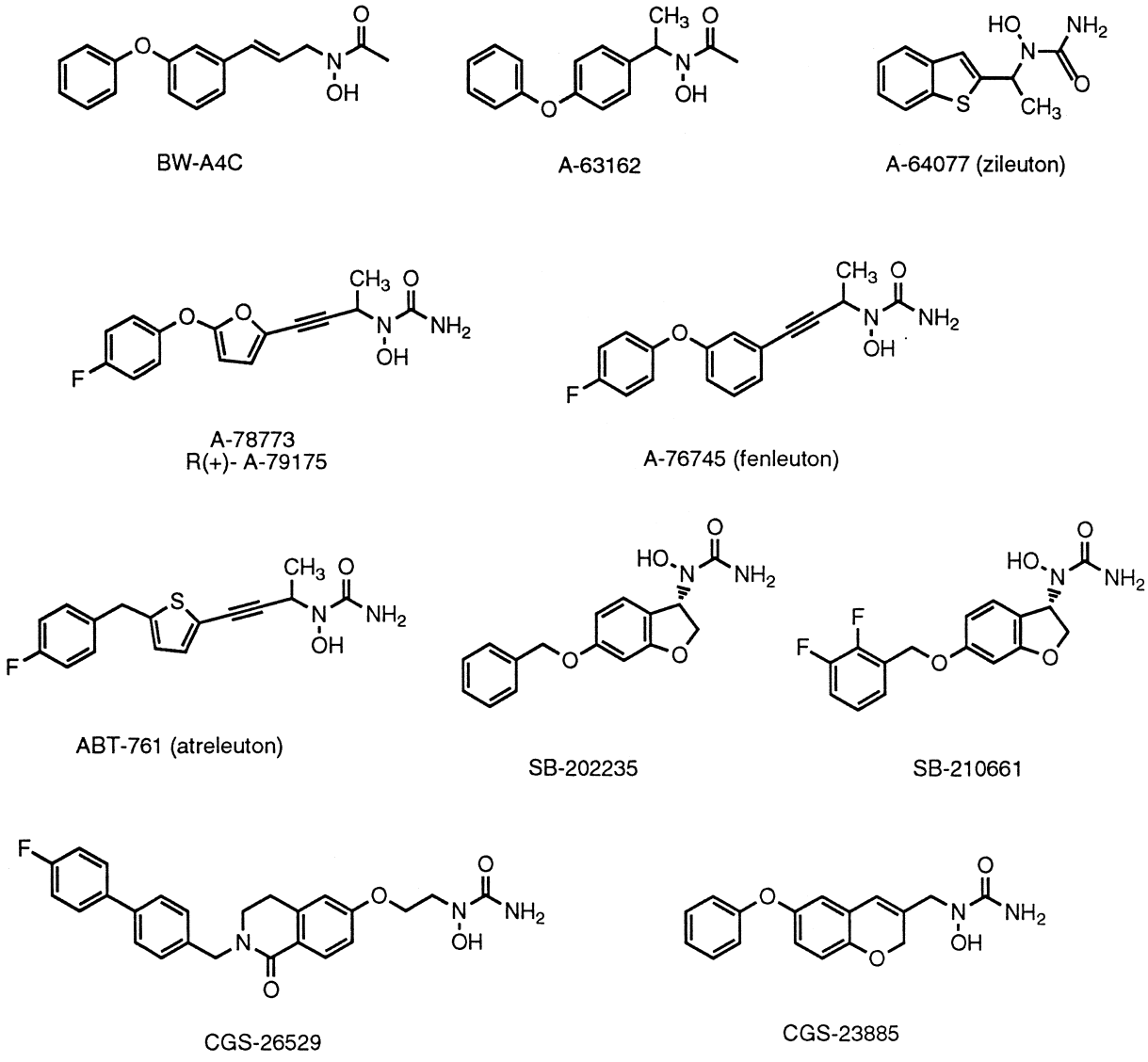


Figure 4. Iron chelator inhibitors of 5-lipoxygenase.

drug interaction with oral contraceptive steroids [44]. It remains to be seen whether atreleuton will reach the market. Structure activity studies on the research which derived atreleuton have been published [45].

SmithKline Beecham have studied a series of dihydrobenzofuran-N-hydroxy ureas (which have derived SB-210661 and SB-202235) [46, 47]. These compounds have not yet been reported to have reached clinical trials. Ciba-Geigy/Novartis have reported on a series of heterocyclic hydroxy ureas including CGS-26529 [48] and CGS 23885 [49, 50], the latter compound has been reported to have been in Phase I clinical trials. Wyeth Ayerst researchers have reported structure-activity studies on a series of azophenoxyhydroxy ureas [51], however, the developmental stages of this series is unknown. Although the N-hydroxy ureas have been designed as iron chelators, it has been shown that these compounds can be turned over by the 5-lipoxygenase enzyme to derive radical by-products. In particular, Zileuton has been shown to be metabolized to form nitroxides and, as such, serves as a reducing substrate for 5-lipoxygenase [52]. If the degree of turnover by the enzyme

correlates with potency of these compounds, it is possible that potent analogues of Zileuton will continue to be plagued by toxicities derived from production of radicals and radical by-products.

5. 5-Lipoxygenase inhibitors with dual activities *(figure 5)*

Many companies have reported over the last decades on compounds that inhibit both 5-lipoxygenase and other enzymes involved in inflammation as approaches to treat a variety of inflammatory diseases. BW-B70C has been reported to have dual 5- and 15-lipoxygenase inhibitor activity [53]. The compound showed some efficacy in allergic models in guinea-pigs. The phenolic benzothiazole analogue, E-3040, from Eisai, was reported to have potent dual 5-lipoxygenase and thromboxane A2 synthase inhibitory activity. The compound has been evaluated in models of experimental ulcerative colitis [54] and has been reported to be in Phase II clinical trials. Another quinonoid dual inhibitor of 5-lipoxygenase and thromboxane A2 synthase (CV-6504) has been reported to show anti-tumour activity in murine models of adenocarcinoma [55].

Most efforts, however, have been directed towards development of dual 5-lipoxygenase/cyclooxygenase inhibitors. The compound tepoxaline, or RWJ-20485, has received extensive biochemical and clinical evaluation by

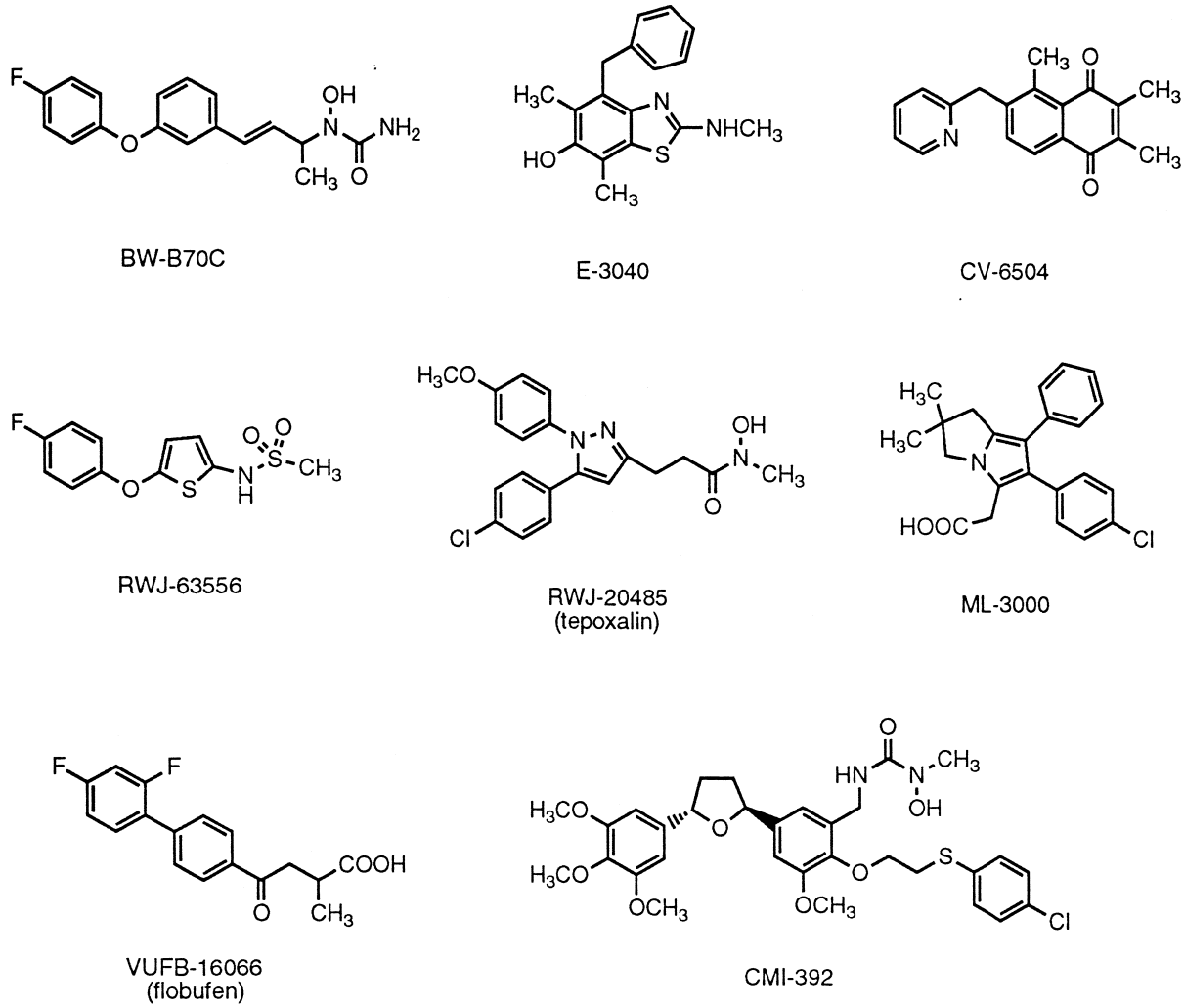


Figure 5. Inhibitors of 5-lipoxygenase with dual activities.

Johnson & Johnson as an anti-inflammatory agent. However, clinical trials indicated potent cyclooxygenase inhibition at doses from 25–800 mg p.o., whereas only weak inhibition of leukotriene synthesis was observed at the maximum 800 mg dose [56]. Development of the compound has been reported to be discontinued (company communication, March 1995). More recently, Johnson & Johnson have reported a cyclooxygenase 2/5-lipoxygenase dual inhibitor, RWJ-63556 [57]. The company Merckle has reported on a series of perazolene analogues as dual cyclooxygenase/5-lipoxygenase inhibitors from which they derived the clinical candidate ML-3000. The compound has shown some oral activity in rat models of inflammation [58] and is reported to work by a non-redox mechanism [59] and to show activity in a sheep model of asthma [60] by aerosol administration. The compound is reported to be in Phase II clinical trials. Another dual cyclooxygenase/5-lipoxygenase inhibitor, VUFB-16066 (flobufen), has reportedly been evaluated as inflammatory in clinical trials for rheumatoid arthritis [61]. Researchers from Cytomed have recently reported on the hybridizaton of N-hydroxyurea functionality to diaryltetrahydrofuran to derive a novel series of compounds showing dual 5-lipoxygenase and platelet activating factor receptor (PAF) antagonism. An optimized compound CMI-392 was reported [62].

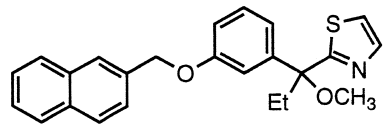
Although many companies continue to try and develop dual inhibitors, it is clear that the difficulty to balance the inhibitory activity toward two different targets in vivo has made it difficult to optimize compounds in this area.

6. Competitive (non-redox) inhibitors of 5-lipoxygenase *(figure 6)*

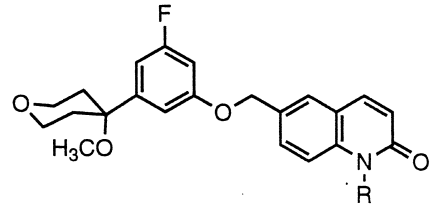
The multiple toxicities and difficulties encountered in developing redox inhibitors of 5-lipoxygenase has led many research groups to strive to find competitive non-redox inhibitors of the enzyme. Only as a better understanding of the mechanism of the enzyme became available, has it become possible to develop reliable criteria for the evaluation and identification of non-redox inhibitors [52]. The observation by ICI (Zeneca) that a series of methoxyalkylthiazoles [63] and methoxytetrahydropyrans [64] were potent inhibitors of 5-lipoxygenase which, in some cases, showed enantioselective activity [63, 65] suggested they might act by stereospecific binding at the active site of 5-LO. Structural features suggested they were unlikely to enter into redox reactions. Subsequent studies showed that compounds in this series, such as ZM-211965, did not act as reducing substrates for 5-lipoxygenase, while they did inhibit turnover-dependent inactivation of the enzyme and therefore could be considered as true non-redox inhibitors [52]. Optimization of these compounds led to the discovery of ZD-2138 [65, 66]. This compound was evaluated extensively by Zeneca in clinical trials for arthritis and asthma, and in normal volunteers. For example, a 350 mg p.o. dose was shown to maximally inhibit leukotriene synthesis for at least 24 hours [67]. Unfortunately, in spite of this potent activity, Zeneca has recently reported to have discontinued development of this compound due to mixed and unconvincing results as an anti-arthritis agent [68]. It may still be in development for asthma. An analogue, ZM-230487, has also been evaluated preclinically by Zeneca [69, 70] and more recently structure-activity relationships on a series of thiene analogues have been published [71].

Recent studies have suggested that compounds such as ZM-230487 are potent inhibitors of 5-lipoxygenase under conditions of low peroxide tone and are less efficient under conditions of oxidative stress [72]. This could explain the disappointing activity observed for these compounds as anti-inflammatory agents.

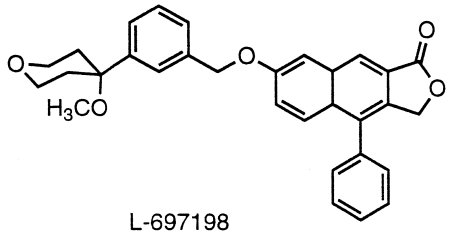
A related series of non-redox inhibitors of 5-LO has been discovered by Merck Frosst scientists. Screening of the Merck sample collection identified a class of lignans derived from a natural product, Justicidin E, as a moderately potent non-redox inhibitor 5-lipoxygenase [73]. Based on similarities between these compounds and ZD-2138, hybrid molecules such as L-697,198 were prepared which were not only markedly more potent than the initial lignans, but also by virtue of the possibility of dosing as a prodrug dihydroxyacid form, showed excellent bioavailability and oral activity [74]. However, extensive metabolism of the pyran ring in these compounds [75] complicated their development. Structure-activity studies were carried out with the intention of deriving a metabolically stable analogue. These efforts resulted in the identification of L-708,780 [76], L-739,010 [77] and L-746,530 [78]. These compounds showed only traces of metabolism and had excellent bioavailability and duration in a number of animal species. Unfortunately, it was noted that recovery of drug under in vitro metabolic conditions was poor for these compounds and subsequent studies showed that both the bicyclo moiety and the furan moiety were metabolized to derive reactive metabolites which labelled plasma and hepatic proteins [79, 80]. Toxicological evaluation of L-739,010 showed a variety of toxicities [81] and, thus, development of these compounds was discontinued. Other studies in the Merck Frosst Laboratories have identified a series of thiopyranylindoles [82] structurally related to series which had been shown to indirectly inhibit lipoxygenase activity in cells (vide infra). These



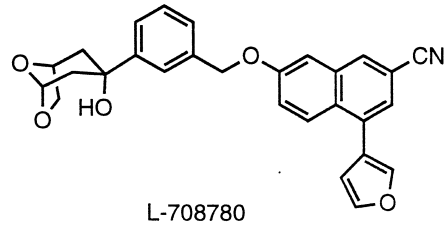
ZM-211965



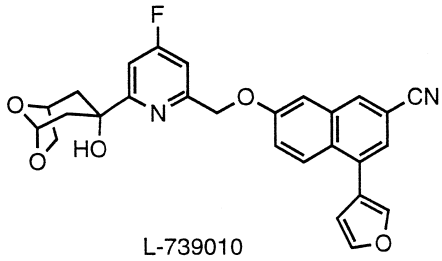
R = CH_3 ; ZD-2138
R = C_2H_5 ; ZM-230487



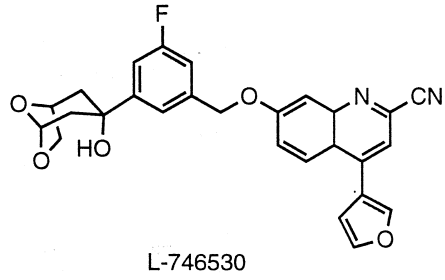
L-697198



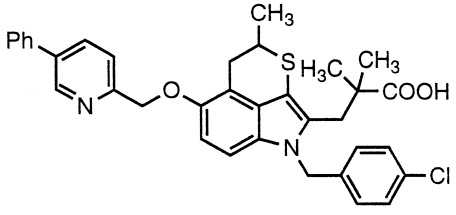
L-708780



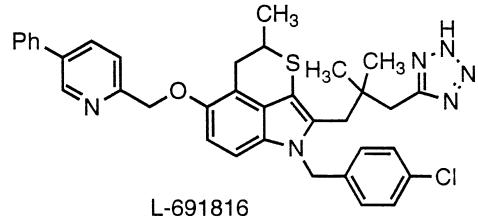
L-739010



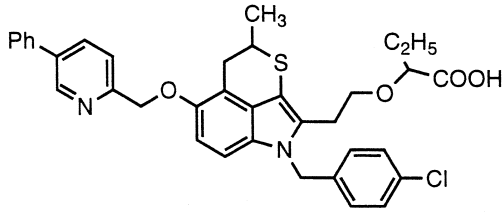
L-746530



L-689065



L-691816



L-699333

Figure 6. Competitive (non-redox) inhibitors of 5-lipoxygenase.

compounds, exemplified by L-689,065 [83], showed potent in vitro activity against human 5-lipoxygenase and optimized compounds L-691,816 [84] and L-699,333 [85] were subsequently identified with excellent in vivo activity in a variety of models of asthma when dosed by the oral route. Development of this series was, however, inhibited by the modulation of ex vivo activity through binding to plasma proteins in blood and by competing development of a series of indirect inhibitors in the same laboratory (see following discussion).

7. Inhibitors of the 5-lipoxygenase activating protein (FLAP) *(figure 7)*

Early screening studies in PMN cell assays at Merck Frosst looking for novel inhibitors of leukotriene biosynthesis identified a new class of indole inhibitors which showed potent inhibitory activity in intact PMN cells. Optimization of this series of indoles led to the discovery of L-663,536 (or MK-886) [86]. This was a specific inhibitor of leukotriene biosynthesis in a variety of intact

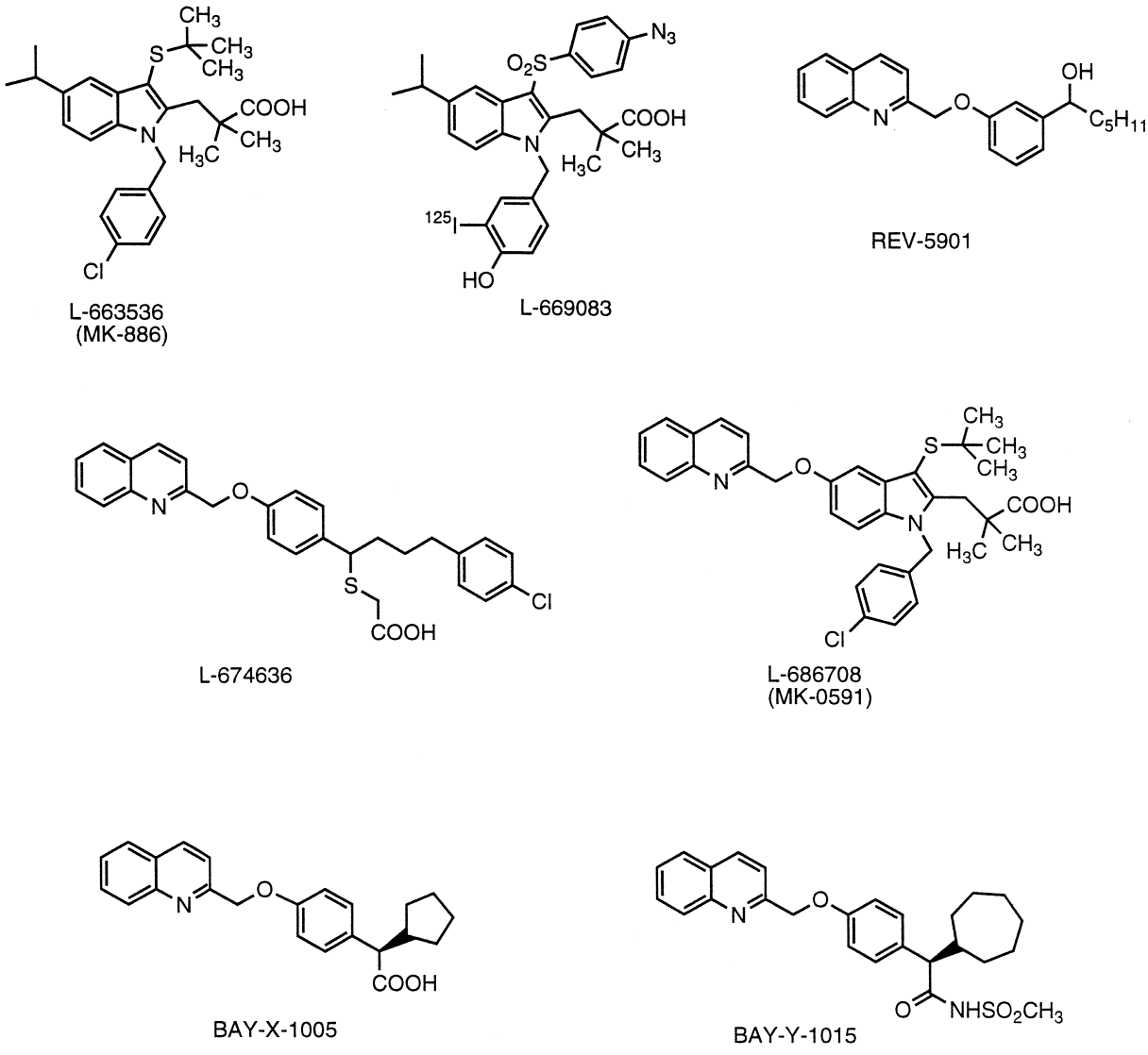


Figure 7. Inhibitors of the 5-lipoxygenase activating protein (FLAP).

cell preparations but had no effect on either cyclooxygenase or 15- or 12-lipoxygenase derived products. In broken cell preparations or on purified 5-lipoxygenase, the compound had no activity. In addition, the compound had no activity on phospholipase A_2 and did not inhibit arachidonic acid release. The compound also showed excellent activity in a variety of animal models of asthma. Attempts to find the molecular target for MK-886 led to the preparation of a photoaffinity label ^{125}I-L-669,083 which was shown to label an 18 Kd protein in cell membrane preparations [87]. In addition, MK-886 and a variety of analogues inhibited the binding of the photo probe to this protein in a dose-dependent and potency-dependent manner. An analogue of MK-886 was used to prepare an affinity column from which it was possible to isolate this 18 Kd protein and based on sequence information attained from the rat protein, the cDNA for both the rat and human protein were isolated, cloned and expressed [87, 88]. Subsequent studies indicated that this novel protein (termed 5-lipoxygenase activating protein or FLAP) is a necessary factor which must be present in cells to facilitate the transfer of arachidonic acid to 5-lipoxygenase [89, 90]. Cells which contain 5-lipoxygenase and not FLAP are unable to biosynthesize leukotriene products unless provided with a large excess of exogenous arachidonic acid substrate. It was thus proposed that MK-886 inhibited the biosynthesis of leukotrienes in PMNs and other cells by blocking the FLAP-mediated transfer of the arachidonic acid substrate to the enzyme. Other photo-affinity studies showed that arachidonic acid binds to FLAP and this binding is competed by MK-886 and related analogues [91]. The discovery of this protein led to the possibility of setting up a binding assay whereby inhibitors of arachidonic acid binding to FLAP could be evaluated directly [92]. MK-886 itself entered clinical trials for asthma and while it showed some efficacy, the results were somewhat disappointing [93]. At maximally tolerated doses, however, inhibition of leukotriene biosynthesis was not complete [94] and it was felt that a more potent compound would be necessary.

After the discovery of MK-886, a survey of the literature identified a weak inhibitor, REV-5901 [95], a quinoline structure, which appeared to have similar activity to MK-886 in that it was active in whole cells but not in broken cells. Evolution of structures related to REV-5901 identified a series of quinoline inhibitors characterized by L-674,636 [96]. These compounds, though relatively potent and orally absorbed, were plagued by significant modulation of their activity through binding to plasma proteins. Observation of similarities between the quinoline series and the indole series of MK-886 led to the investigation of hybrid structures [97]. The utilization of the FLAP binding assay allowed optimization of analogues of MK-886 and the discovery of a significantly more potent FLAP inhibitor, L-686,708 (MK-0591) [98–100]. This compound inhibited FLAP binding with a potency at least 10-fold greater than MK-886, and showed potent inhibition of LT biosynthesis in stimulated whole blood and also showed good oral absorption and pharmacokinetics and was less attenuated by plasma proteins. MK-0591 has been extensively evaluated in clinical trials where it showed efficacy both for biochemical inhibition of leukotriene biosynthesis in vivo, as measured by excretion of LTE_4 in urine in asthmatic patients, and in ex vivo assays as measured by inhibition of leukotriene B_4 biosynthesis in stimulated whole blood [101]. Subsequent trials in chronic asthma showed efficacy by a variety of parameters including increase of FEV_1, decrease in β-agonist usage and symptom scores, although a small incidence of rash was observed as well [102]. The compound was administered under a b.i.d. regimen at 125 mg dose. Although MK-0591 showed clinical efficacy and oral activity, its development was suspended by Merck in favour of the leukotriene D_4 receptor antagonist MK-0476 (montelukast), which had superior activity and could be given by a once-a-day regimen. MK-0591 has also been evaluated in clinical trials in ulcerative colitis. Again, MK-0591 showed excellent biochemical efficacy [103, 104]. However, similar to trials previously described for Zileuton, clinical efficacy in the disease was marginal and not statistically significantly different than placebo [104].

Other laboratories have pursued the quinoline series of leukotriene biosynthesis inhibitors and, in particular, Bayer have derived compounds which have been evaluated in clinical trials. BAY-X-1005 has been extensively evaluated in allergen and cold air challenged asthmatics in clinical trials where it has shown efficacy [105, 106]. In spite of this activity, the compound has been reported to show rather limited anti-inflammatory potential [107]. The compound has been reported to be in Phase II clinical trials and with Phase III trials planned, no recent information has been available as to its status. A related compound from Bayer, BaY-Y-1015, has been investigated in animal models of IBD where it has shown efficacy better than olsalazine [108] but, to date, no reports of human clinical evaluation have been published.

8. Summary

Intensive efforts to develop clinically useful drugs from inhibitors of the 5-lipoxygenase enzyme or of leukotriene biosynthesis have been carried out for almost

two decades now. As only one marketed drug, Zileuton, has emerged from this massive effort, this speaks strongly to the high risk and great difficulty of developing new therapies today. It is apparent from clinical results on MK-0591, BaY-X-1005 and ABT-761, that a variety of leukotriene biosynthesis inhibitors could provide useful therapy in asthma [109]. However, other issues of toxicity, pharmacokinetics or tolerability have frequently blocked bringing such compounds to the market. Of great importance has been the success of two leukotriene receptor antagonists, namely, zafraleukast and montelukast which have also shown good efficacy in the treatment of asthma [110–113].

To date, the leukotriene receptor antagonists appear to have superior properties of safety, pharmacokinetics and, to some degree, efficacy. It remains to be seen whether there are disease states or situations where the more complete blockade of leukotriene biosynthesis, including the elimination of leukotriene B_4, may prove to be an advantage.

Acknowledgements

The author wishes to acknowledge the efforts of Diane Sauvé and Mary Lynn Gaal for their help in preparing this manuscript.

References

[1] Kellaway C.H., Trethewie E.R., Q. J. Exp. Physiol. 30 (1940) 121–145.

[2] Murphy R.C., Hammarström S., Samuelsson B., Proc. Natl. Acad. Sci. USA 76 (1979) 4275–4279.

[3] Samuelsson B., Borgeat P., Hammarström S., Murphy R.C., Prostaglandins 17 (1979) 785–787.

[4] Ford-Hutchinson A.W., Bray M.A., Doig M.V., Shipley M.E., Smith M.J.H., Nature (London) 286 (1980) 264–265.

[5] Ford-Hutchinson A.W., in: Rokach J. (Ed.), Leukotrienes and Lipoxygenases, Elsevier, Amsterdam, 1989, pp. 405–425.

[6] Fitzsimmons B., Rokach J., in: Rokach J. (Ed.), Leukotrienes and Lipoxygenases, Elsevier, Amsterdam, 1989, pp. 427–502.

[7] Ford-Hutchinson A.W., Gresser M., Young R.N., Annu. Rev. Biochem. 63 (1994) 383–417.

[8] Bell R.L., Summers J.B., Harris R.R., Annu. Rep. Med. Chem. 32 (1997) 91–100.

[9] Goldberg B., Stern A., Biochim. Biophys. Acta 437 (1976) 628–632.

[10] Guindon Y., Girard Y., Maycock A., Ford-Hutchinson A.W., Atkinson J.G. et al., Adv. Prostaglandins Thromboxane Leuk. Res. 17A (1987) 554–557.

[11] Bélanger P., Maycock A., Guindon Y., Back T., Dollob A.L. et al., Can. J. Physiol. Pharmacol. 65 (1987) 2441–2448.

[12] Lau C.K., Bélanger P.C., Scheigetz J., Dufresne C., Williams H.W.R. et al., J. Med. Chem. 32 (1989) 1190–1197.

[13] Rouzer C.A., Riendeau D., Falgueyret J.-P., Lau C.K., Gresser M.J., Biochem. Pharmacol. 41 (1991) 1365–1373.

[14] Lau C.K., Bélanger P.C., Dufresne C., Scheigetz J., Thérien M. et al., J. Med. Chem. 35 (1992) 1299–1318.

[15] Ancill R.J., Takahashi Y., Kibune Y., Campbell R., Smith J.R., J. Int. Med. Res. 18 (1990) 75–88.

[16] Fujimura M., Sasaki F., Nakatsumi Y., Takahashi Y., Hifumi S. et al., Thorax 41 (1986) 955–959.

[17] Scrip No. 1306 (1988) p. 28.

[18] Harris R.R., Batt D.G., Galbraith W., Ackerman N.R., Agents Actions 27 (1989) 297–299.

[19] Marien K., Morren M., Degreef H., DeDoncker P., Rooman R.P., Cauwenbergh G., Arch. Dermatol. 128 (1992) 993–994.

[20] Beetens J.R., Somers Y., Goossens J., Vanwauwe J., De Clerck F., Arch. Int. Pharmacodyn. Ther. 290 (1987) 151.

[21] Fort F.L., Pratt M.C., Carter G.W., Lewkowski J.P., Heyman I.A. et al., Fundam. Appl. Toxicol. 4 (1984) 216–220.

[22] Bruneau P., Delvare C., Edwards M.P., McMillan R.M., J. Med. Chem. 34 (1991) 1028–1036.

[23] Albert D.H., Machinist J., Young P.R., Dyer R., Bouska J. et al., FASEB J. 3 (1989) A735.

[24] Duniec Z., Robak J., Gryglewski R., Biochem. Pharmacol. 32 (1983) 2283–2286.

[25] Bhatia P.A., Brooks C.D., Basha A., Ratajczyk J.D., Gunn B.P. et al., J. Med. Chem. 39 (1996) 3938–3950.

[26] Degreef H., Dockx P., DeDoncker P., De-Beule K., Cauwenbergh G., J. Am. Acad. Dermatol. 22 (1990) 751–755.

[27] van de Kerkhof P.C., van Pelt H., Lucker G.P., Steijlen P.M., Heremans A., Skin Pharmacol. 9 (1996) 307–311.

[28] Tateson J.E., Randall R.W., Reynolds C.H., Jackson W.P., Bhattacherjee P. et al., Br. J. Pharmacol. 94 (1988) 528–539.

[29] Summers J.B., Gunn B.P., Martin J.G., Mattin M.B., Mazdiyasni H. et al., J. Med. Chem. 31 (1988) 1960–1964.

[30] Payne A.N., Jackson W.P., Salmon J.A., Nicholls A., Yeadon M., Garland L.G., Agents Actions Suppl. 34 (1991) 189–199.

[31] Chamulitrat W., Mason R.P., Riendeau D., J. Biol. Chem. 267 (1992) 9574–9579.

[32] Wenzel S.E., Kamada A.K., Ann. Pharmacother. 30 (1996) 858–864.

[33] Liu M.C., Dube L.M., Lancaster J., J. Allergy Clin. Immunol. 98 (1996) 859–871.

[34] Rodrigues A.D., Machinist J.M., Toxicol. Appl. Pharmacol. 137 (1996) 193–201.

[35] Zarif A., Eiznhamer D., Callaghan C., Doria M.I., Broutman L., Keshavarzian A., Inflammation 20 (1996) 217–227.

[36] Hawkey C.J., Dube L.M., Rountree L.V., Linnen P.J., Lancaster J.F., Gastroenterology 112 (1997) 718–724.

[37] Bell R.L., Bouska J.B., Malo P.E., Lanni C., Harris R.R. et al., J. Pharmacol. Exp. Ther. 272 (1995) 724–731.

[38] Bouska J. et al., 6th Int. Conf. Inflamm. Res. Assoc., Sept. 20–24, White Haven (1992) Abst. P119.

[39] Brooks D.W., Stewart A.O., Basha A., Bhatia P., Ratajczyk J.D. et al., 206th ACS Nat. Meeting, Aug. 22–27, Chicago (1993) Abst. Medi 41.

[40] Annual Drug Data Report, 17 (No. 8) (1995) 711–712.

[41] Marr K.A., Lees P., Page C.P., Cunningham F.M., Inflamm. Res. 44 (Suppl. 1) Abst. W20/07, (1995) S289.

[42] Lehnigk B., Rabe K.F., Dent G., Herst R.S., Carpentier P.J., Magnussen H., Eur. Resp. J. 11 (1998) 617–623.

[43] Wong S.L., Drajesk J., Chang M.S., Witt G., Awni W.M., Biopharm. Drug. Disp. 19 (1998) 159–162.

[44] Wong S.L., O'Dea R., Dube L., Awni W.M., 99th Meeting Am. Soc. Clin. Pharm. Ther., New Orleans (1998) Abst. PI-106.

[45] Brooks C.D., Stewart A.O., Basha A., Bhatia P., Ratajczyk J.D. et al., J. Med. Chem. 38 (1995) 4768–4775.

[46] Adams J.L., Garigipati R.S., Sorenson M., Ross S., Schmidt S.J. et al., 204th ACS Nat. Meeting, Aug. 23–28, Washington (1992) Abst. Medi 121.

[47] Chabot-Fletcher M.C., Underwood D.C., Breton J.J., Adams J.L., Kagey-Sobotka A. et al., J. Pharmacol. Exp. Ther. 273 (1995) 1147–1155.

[48] Kimble E., Kowalski T., Peters P., Lee W., Koehler J. et al., Inflamm. Res. 44 (Suppl. 2) (1995) S147–S148.

[49] Satu Y., Stanton J.L., Hutchison A.J., Libby A.H., Kowalski T.J. et al., 205th ACS Nat. Meeting, March 28–April 2; Denver (1993) Abst. Medi 143.

[50] Raychaudhuri A., Chertock H., Chovan J., Jones L.S., Kimble E.F. et al., Naunyn-Schmiedeberg's Arch. Pharmacol. 355 (1997) 470–474.

[51] Malamas M.S., Carlson R.P., Grimes D., Howell R., Glaser K. et al., J. Med. Chem. 39 (1996) 237–245.

[52] Falgueyret J.-P., Hutchinson J.H., Riendeau D., Biochem. Pharmacol. 45 (1993) 978–981.

[53] Yeadon M., Price R.C., Payne A.N., Pulmon. Pharmacol. 8 (1995) 53–63.

[54] Oketani K., Murakami M., Inoue T., Yamanaka T., Yamatsu I., Jpn. J. Pharmacol. 64 (Suppl. I) Abst. (1994) P351.

[55] Hussey H.J., Bibby M.C., Tisdale M.J., Br. J. Cancer 73 (1996) 1187–1192.

[56] Depre M., VanHecken A., Verbesselt R., Verpooten G.A., Arnout J. et al., Int. J. Clin. Pharm. Res. 16 (1996) 1–8.

[57] Parnham M.J., Drug News Perspect. 9 (1996) 631–639.

[58] Laufer S.A., Augustin J., Dannhardt G., Kiefer W., J. Med. Chem. 37 (1994) 1894–1897.

[59] Laufer S.A., Tries S., Augustin J., 13th Int. Symp. Med. Chem., Sept. 19–23, Paris (1994) Abst. P181.

[60] Abraham W.M., Laufer S., Tries S., Pulmon. Pharmacol. Ther. 10 (1997) 167–173.

[61] Panajotova V., Anderova E., Jandera A., Kuchar M., Arzneim. -Forsch 47 (1997) 648–652.

[62] Cai X., Scannell R.T., Yaeger D., Hussoin M.S., Killian D.B. et al., J. Med. Chem. 41 (1998) 1970–1979.

[63] Bird T.G.C., Bruneau P., Crawley G.C., Edwards M.P., Foster S.J. et al., J. Med. Chem. 34 (1991) 2176–2186.

[64] Crawley G.C., Dowell R.I., Edwards P.N., Foster S.J., McMillan R.M. et al., J. Med. Chem. 35 (1992) 2600–2609.

[65] Lambert-van der Brempt C., Bruneau P., Lamorlette M.A., Forster S.J., J. Med. Chem. 37 (1994) 113–124.

[66] Bird T.G.C., 12th Int. Symp. Med. Chem., Sept. 13–17 Basel (1992) Abst. OC-05. 2.

[67] Walker E.R.H., 13th Int. Symp. Med. Chem., Paris, (1994) Abst. ML22.

[68] Scrip No. 2050 (1995) p. 7.

[69] Kusner E.J., Buckner C.K., Dea D.M., DeHaas C.J., Marks R.L. et al., Eur. J. Pharmacol. 257 (1994) 285–292.

[70] Teixeira M.M., Hellewell P.G., Brit. J. Pharmacol. 111 (1994) 1205–1211.

[71] Crawley G.C., Briggs M.T., J. Med. Chem. 38 (1995) 3951–3956.

[72] Werz O., Szellas D., Henseler M., Steinhilber D., Mol. Pharmacol. 54 (1998) 445–451.

[73] Thérien M., Fitzsimmons B.J., Sheigetz J., Macdonald D., Choo L.Y. et al., Bioorg. Med. Chem. Lett. 3 (1993) 2063–2066.

[74] Ducharme Y., Brideau C., Dubé D., Chan C.-C., Falgueyret J.-P. et al., J. Med. Chem. 37 (1994) 512–518.

[75] Chauret N., Li C., Ducharme Y., Trimble L.A., Yergey J.A. et al., Drug Metab. Disp. 23 (1995) 65–71.

[76] Delorme D., Ducharme Y., Brideau C., Chan C.C., Chauret N. et al., J. Med. Chem. 39 (1996) 3951–3970.

[77] Hamel P., Riendeau D., Brideau C., Chan C.C., Desmarais S. et al., J. Med. Chem. 40 (1997) 2866–2875.

[78] Dubé D., Blouin M., Brideau C., Chan C.C., Desmarais S. et al., Bioorg. Med. Chem. Lett. 8 (1998) 1255–1260.

[79] Zhang K.E., Naue J.A., Arison B., Vyas K.P., Chem. Res. Toxicol. 9 (1996) 547–554.

[80] Chauret N., Nicoll-Griffith D., Friesen R., Li C., Trimble L. et al., Drug Metab. Disp. 23 (1995) 1325–1334.

[81] Molon-Noblot S., Gillet J.P., Durand-Cavagna G., Huber A.C., Patrick D.H., Duprat P., Toxicol. Pathol. 24 (1996) 231–237.

[82] Hutchinson J.H., Charleson S., Evans J.F., Falgueyret J.P. et al., J. Med. Chem. 38 (1995) 4538–4547.

[83] Hutchinson J.H., Prasit P., Choo L.Y., Riendeau D., Charleson S. et al., BioMed. Chem. Lett. 45 (1993) 978–981.

[84] Hutchinson J.H., Riendeau D., Brideau C., Chan C., Delorme D. et al., J. Med. Chem. 36 (1993) 2771–2787.

[85] Hutchinson J.H., Riendeau D., Brideau C., Chan C., Falgueyret J.P. et al., J. Med. Chem. 37 (1994) 1153–1164.

[86] Gillard J., Ford-Hutchinson A.W., Chan C.C., Charleson S., Denis D. et al., Can. J. Physiol. Pharmacol. 67 (1989) 456–464.

[87] Miller D.K., Gillard J.W., Vickers P.J., Sadowski S., Léveillé C. et al., Nature 343 (1990) 278–281.

[88] Dixon R.A.F., Diehl R.E., Opas E., Rands E., Vickers P.J. et al., Nature 343 (1990) 282–284.

[89] Kargman S., Vickers P.J., Evans J.F., J. Cell. Biol. 119 (1992) 1701–1709.

[90] Kargman S., Rousseau P., Reid G.K., Rouzer C.A., Mancini J.A. et al., J. Lipid Mediators 7 (1993) 31–45.

[91] Mancini J.A., Abramovitz M., Cox M.E., Wong E., Charleson S. et al., FEBS Lett. 318 (1993) 277–281.

[92] Charleson S., Prasit P., Léger S., Gillard J.W., Vickers J.P. et al., Mol. Pharmacol. 41 (1992) 873–879.

[93] Friedman B.S., Bel E.H., Buntinx A., Tanaka W., Han Y.-H.R. et al., Am. Rev. Respir. Dis. 147 (1993) 839–844.

[94] Depre M., Friedman B., Tanaka W., VanHecken A., Buntinx A., DeSchepper P.J., Clin. Pharmacol. Ther. 53 (1993) 602–607.

[95] Musser J.H., Chakraborty U.R., Sciortino S., Gordon R.J., Khandwala A. et al., J. Med. Chem. 30 (1987) 96–104.

[96] Prasit P., Belley M., Evans J.F., Gauthier J.Y., Léveillé C. et al., Bioorg. Med. Chem. Lett. 1 (1991) 645–648.

[97] Young R.N., Gillard J.W., Hutchinson J.H., Léger S., Prasit P., J. Lipid Mediators 6 (1993) 233–238.

[98] Prasit P., Belley M., Blouin M., Brideau C., Chan C., Charleson S. et al., J. Lipid Mediators 6 (1993) 239–244.

[99] Mancini J.A., Prasit P., Coppolino M.G., Charleson S., Léger S. et al., Mol. Pharmacol. 41 (1992) 267–272.

[100] Brideau C., Chan C., Charleson S., Denis D., Evans J.F. et al., Can. J. Physiol. Pharmacol. 70 (1992) 799–807.

[101] Diamant Z., Timmers M.C., van der Veen H., Friedman B.S. et al., J. Allergy Clin. Immunol. 95 (1995) 42–51.

[102] Chapman K.R., Friedman B.S., Shingo S., Heyse J., Reiss T., Spector R., ALA/ATS Meeting, Boston, May 21–25 (1994) A215.

[103] Hillingso J., Kjeldsen J., Laursen L.S., Lauritsen K., von Spreckelsen S. et al., Clin. Pharmacol. Ther. 57 (1995) 335–341.

[104] Roberts W.G., Simon T.J., Berlin R.G., Haggitt R.C., Snyder E.S. et al., Gastroenterology 112 (1997) 725–732.

[105] Hamilton A.L., Watson R.M., Wyile G., O'Byrne P.M., Thorax 52 (1997) 348–354.

[106] Fischer A.R., Rosenberg M.A., Roth M., Loper M., Jungerwirth S., Isreal E., Thorax 52 (1997) 1074–1077.

[107] Muller-Peddinghaus R., J. Physiol. Pharmacol. 48 (1997) 529–536.

[108] Murthy S., Murthy N.S., Coppola D., Wood D.L., Inflamm. Res. 46 (1997) 224–233.

[109] Negro J.M., Miralles J.C., Ortiz J.L., Funes E., Garcia A., Allergol. Immunopathol. 25 (1997) 209–216.

[110] O'Byrne P.M., Chest III (2 Suppl.) (1997) 27S–34S.

[111] Larson J.S., Jackson S.K., Am. J. Health Syst. Pharm. 53 (1996) 2821–2830.

[112] Sorkness C.A., Pharmacotherapy 17 (1997) 50S–54S.

[113] Renzi P.M., Can. Med. Assoc. J. 160 (1999) 217–223.