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Perspective

Peptide Leukotrienes: Current Status of Research

A. Shaw* and R. D. Krell

Biomedical Research Department, ICI Pharmaceuticals Group, A Business Unit of ICI Americas Incorporated, Wilmington, Delaware 19897. *Received August 13, 1990*

Background

It is now almost 10 years since the publication here of the Perspective entitled *Leukotrienes: A Major Step in Understanding Immediate Hypersensitivity Reactions* by Borgeat and Sirois,[1] a review that nicely caught the excitement of that period when "the question of the chemical nature of an important mediator (had) recently been answered" and a new biosynthetic sequence from arachidonic acid had been identified. In this review we will attempt to overview some developments in the area since then, particularly in the area of new drug discovery. The pace of research in the linear pathway of arachidonic acid metabolism has not slackened during the eighties, nor has the pace of writing of reviews; we bring to the interested reader's attention numerous excellent recent articles that expand and complete our own.[2-6] This review will concentrate on the peptide leukotrienes (pLTs), their receptors, and their antagonists. The medicinal chemistry of the 5-lipoxygenase inhibitors will not be discussed. Consistent with the general emphasis in the pharmaceutical industry, we will deal predominantly with pLTs in the context of pulmonary pathophysiology.

The now-familiar linear pathway of arachidonic acid metabolism via 5-lipoxygenase (5-LO) to LTA_4 and the subsequent bifurcation to LTB_4 and the peptide leukotrienes LTC_4, LTD_4, and LTE_4 is shown in Figure 1.[7] LTB_4 is a potent chemoattractant for inflammatory cells and may play a role in the late phase of inflammation commonly seen in asthmatics,[8] whereas LTC_4, LTD_4, and LTE_4, collectively identified with the classical slow-reacting substance of anaphylaxis, SRS-A, have potent, acute pharmacological effects such as smooth muscle contraction, stimulation of bronchial mucus secretion, and provocation of increases in vascular permeability which make them potential mediators of allergic symptoms. While many cell types[9] are capable of producing linear products upon activation, the response of IgE-bearing cells (basophils, mast cells) to antigen challenge by brisk production of leukotrienes supports the continued emphasis on allergic conditions as a probable end point for drugs operating via effects on this pathway.[10] For reasons discussed later, research on blockade of the pLTs has come to focus predominantly on antagonism of the effects of LTD_4.

Discovery of pLT Antagonists

The 1980s are distinguished in retrospect as an era when high capacity ligand binding assays, creative use of substructure searching and molecular modeling, chemical intuition, synthetic firepower, and good fortune allowed some remarkable achievements in the structured discovery of small ("drug-like") but highly potent and selective antagonists of large and complex natural agonists, such as AII,[11] CCK,[12] and PAF.[13] The compounds listed in Table I,

(1) Borgeat, P.; Sirois, P. *J. Med. Chem.* **1981**, *24* (2), 121.
(2) Perchonok, C. D.; Torphy, T. J.; Mong, S. *Drugs Future* **1987**, *12* (9), 871.
(3) Musser, J. H. *Drug News Perspect.* **1989**, *2* (4), 202.
(4) Cashman, J. R.; Oliva, S. L. *Drugs Today* **1988**, *24* (10), 723.
(5) Bisgaard, H. *Danish Med. Bull.* **1989**, *36* (2), 142.
(6) Snyder, D. W.; Fleisch, J. H. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 123.
(7) Samuelson, B. *Science* **1983**, *220*, 568.
(8) Ford-Hutchinson, A. W. *Crit. Rev. Immunol.* **1990**, *10* (1), 1.
(9) Maycock, A. L.; Pong, S-S.; Evans, J. F.; Miller, D. K. In *Leukotrienes and Lipoxygenases: Chemical, Biological and Clinical Aspects*; Rokach, J., Ed.; Elsevier: Amsterdam, 1989; p 143.
(10) MacGlashan, D. W.; Schleimer, R. P.; Peters, S. P.; Schulman, E. S.; Adams, G. K.; Newball, H. H.; Lichtenstein, L. M. *J. Clin. Invest.* **1982**, *70*, 747.
(11) Duncia, J. V.; Chiu, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. M. *J. Med. Chem.* **1990**, *33*, 1312.
(12) Evans, B. E.; Bock, M. G.; Rittle, K. E.; DiPardo, R. M.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Friedinger, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4918.

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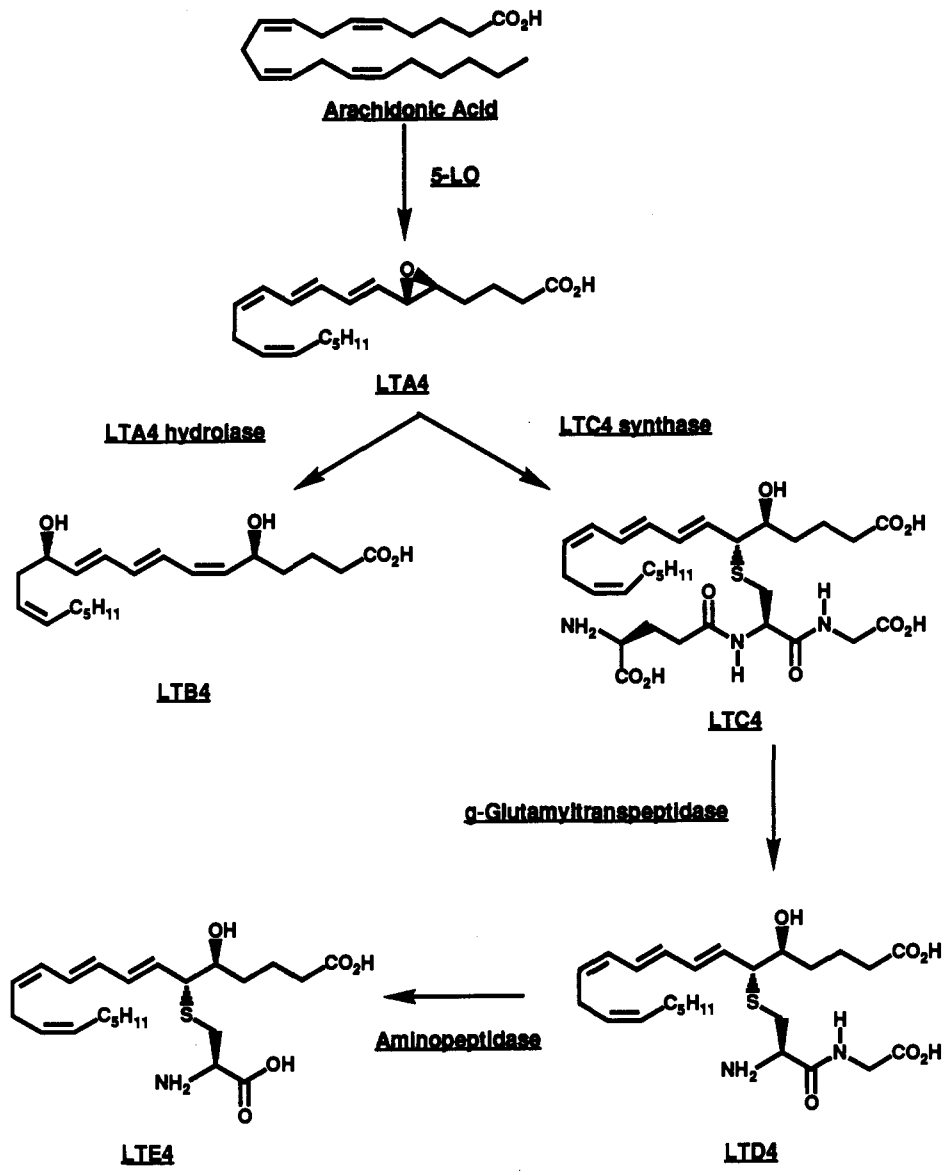

Figure 1. Biosynthesis of the leukotrienes.

Table I

compound no.	compound name	^{3}H-LTD$_4$ binding: K_i	LTD$_4$ contraction guinea pig trachea: pK_B	comments
1	FPL-55712	0.8 μM (15)	6.7[a] (16)	
2	CGP-35949		7.4[a] (17)	preclinical
3	L-648,051		7.3[a] (18)	aerosol only, weak clinical activity
4	L-649,923	0.4 μM (19)	7.2[a] (19)	weakly active po against LTD$_4$ challenge in man
5	LY163443		7.5 (20)	
6	LY171883	0.6 μM (15)	6.9 (21)	withdrawn from clinical studies; see text
7	LY203647		6.4 (22)	selected for clinical trial
8	Ro 23-3544	4 μM[b] (23)	6.6[a] (23)	in Phase II studies, aerosol only
9	SC-39070		8.2[c] (24)	preclinical
10	YM-16638		0.16 μM[b] (25)	in Phase II studies
11	MK-571	0.2 nM (26)	9.3 (26)	in Phase II; see text for clinical results
12	RG 12525	3 nM (27)	8.4[a,d] (27)	selected for clinical study
13	SR2640	23 nM[b] (28)	8.7 (28)	in Phase II studies
14	Wy-48,252	35 nM (29)	7.8 (30)	in Phase II studies
15	Ro 24-5913		9.3[e] (31)	preclinical
16	LY170680		8.1[a] (32)	in Phase I as an aerosol
17	SKF 106203	60 nM (33)	7.6 (34)	orally active congener of SKF 104353; in trial
18	SKF 104353	5 nM (35)	8.6[a] (35)	in Phase II; see text for clinical results; aerosol only
19	ICI 198615	0.3 nM (36)	10.1[a] (36)	
20	IC 204219	0.3 nM (37)	9.5 (37)	in Phase II; see text for clinical results
21	ONO-RS-411		7.5 (38)	active po against LTD$_4$ and antigen challenge in man

[a] pA_2. [b] IC$_{50}$. [c] GP ileum. [d] GP lung parenchymal strips. [e] Human bronchus.

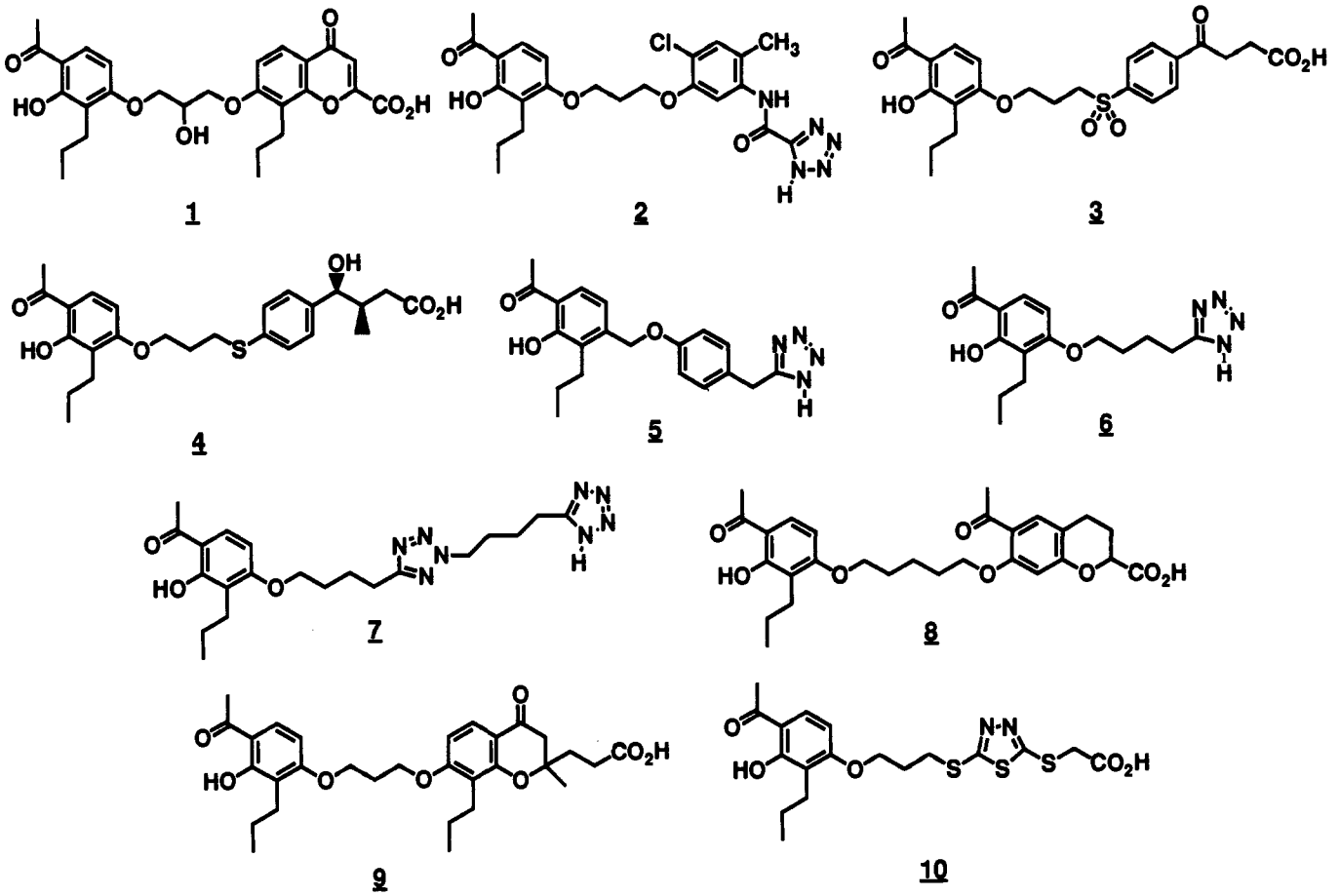

Figure 2. Hydroxyacetophenone (HAP) pLT antagonists.

some of which show a higher affinity for the LTD_4 receptor than LTD_4 itself, adequately demonstrate another striking success for modern methods against an agonist of challenging complexity. However, chemists recruited to the search for pLT antagonists by the excitement of the structure determination of SRS-A in the late 1970s found that the new structure was not the only starting point, as Fison's chemiists had already made the empirical observation of the modest anti-SRS-A activity of FPL-55712 (Table I, Figure 2, 1) several years earlier.[14] It is apparent from Figure 2 that this structure influenced much medicinal chemistry in the area and led to many of the earliest clinical candidates, some of which are discussed in a later section. While distinct advances on the pharmacological profile of FPL-55712 have been achieved, it is clear, especially in comparison to some of the later structures (Figure 3), that the hydroxyacetophenone series are generally of modest in vitro potency as LTD_4 antagonists. Nevertheless, new clinical candidates are still coming from this area, and Ro 23-3544 (8) appears particularly effective upon aerosol dosing in animal models.[23] As the FPL-55712-derived series are generally profiled by functional and binding studies as competitive ligands to pLT receptors, it is reasonable to seek some structural overlap with the pLTs themselves. It has become customary to analyze the structural elements of pLT binding in terms of the agonist structure–activity relationship (SAR) of LTD_4 analogues[39] and to recognize three physicochemically

(13) Weber, K. H.; Heuer, H. O. *Med. Res. Rev.* **1989**, *9*, 181.
(14) Augstein, J.; Farmer, J. B.; Lee, T. B.; Sheard, P.; Tattersall, M. L. *Nat. New Biol.* **1973**, *245*, 215.
(15) Aharony, D.; Falcone, R. C.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 921.
(16) Chand, N. *Agents Actions* **1979**, *9*, 133.
(17) VanSprecher, A.; Ernest, I.; Main, A.; Beck, A.; Brietenstein, W.; Marki, F.; Bray, M. A. *Adv. Prostaglandin Thromboxane Leukotriene Res.* **1987**, *17*, 519.
(18) Jones, T. R.; Guindon, Y.; Champion, E.; Charette, L.; Young, R.; DeHaven, R. N.; Denis, D.; Ethier, D.; Ford-Hutchinson, A. W.; Fortin, R.; Letts, G.; Masson, P.; Hamel, R.; Maycock, A.; McFarlane, C.; Piechuta, H.; Pong, S. S.; Rokach, J.; Yoakim, C. *Can. J. Physiol.* **1986**, *64*, 1535.
(19) Jones, T. R.; Young, R.; Champion, E.; Charette, L.; Denis, D.; Ford-Hutchinson, A. W.; Frenette, R.; Gauthier, J-Y.; Guindon, Y.; Kakushima, M.; Masson, P.; McFarlane, C.; Piechuta, H.; Rokach, J.; Zamboni, R.; DeHaven, R. N.; Maycock, A.; Pong, S. S. *Can. J. Physiol.* **1986**, *64*, 1068.
(20) Rinkema, L. E.; Haisch, K. D.; McCullough, D.; Carr, F. P.; Dillard, R. D.; Fleisch, J. M. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1985**, *44* (3), No. 472.
(21) Gapinski, D. M.; Roman, C. R.; Richardson, L. E.; Fleisch, J. M. *J. Med. Chem.* **1988**, *31*, 172.
(22) Whitesitt, C. A.; Sigmund, S. K.; Lifer, S. L.; Hahn, R. A.; Roman, C. R.; Rinkema, L. E.; Fleisch, J. M.; Marshall, W. S. *Pharmacologist* **1988**, *30* (3), A90.
(23) Cohen, N.; Weber, G.; Banner, B. L.; Lopresti, R. J.; Schaer, B.; Focella, A.; Zenchoff, G. B.; Chiu, A-M.; Todaro, L.; O'Donnell, M.; Welton, A. F.; Brown, D.; Garippa, R.; Crowley, H.; Morgan, D. W. *J. Med. Chem.* **1989**, *32*, 1842.
(24) Carnathan, G. W.; Sanner, J. H.; Thompson, J. M.; Prusa, C. M.; Miyano, M. *Agents Actions* **1987**, *20*, 124.
(25) Tomioka, K.; Yamada, T.; Mase, T.; Hara, H.; Murase, K. *Arzneim.-Forsch.* **1988**, *38*, 682.
(26) Jones, T. R.; Zamboni, R.; Belley, M.; Champion, E.; Charette, L.; Ford-Hutchinson, A. W.; Frenette, R.; Gauthier, J. Y.; Leger, S.; Masson, P.; McFarlane, C. S.; Piechuta, H.; Rokach, J.; Williams, H.; Young, R. N.; DeHaven, R. N.; Pong, S-S. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 17.
(27) Van Inwegen, R. G.; Nuss, G. W.; Carnathan, G. W. *Life Sci.* **1989**, *44*, 799.
(28) Ahnfelt-Ronne, I.; Kirstein, D.; Kaergaard-Nielsen, C. *Agents Actions* **1989**, *26*, 82.
(29) O'Sullivan, B. P.; Mong, S. *Mol. Pharmacol.* **1989**, *35*, 795.

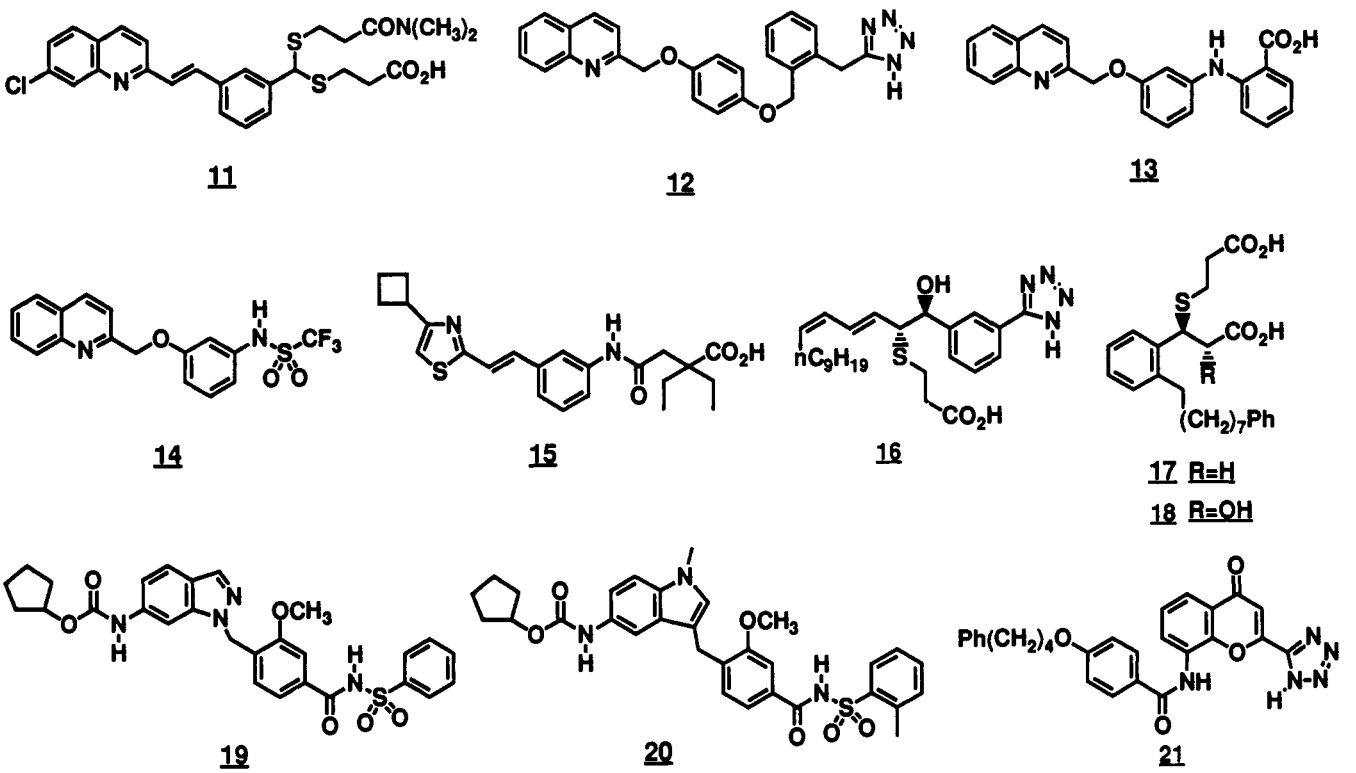

Figure 3. Non-HAP pLT antagonists.

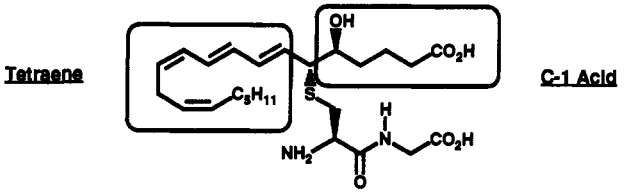

Figure 4. Binding domains of LTD_4.

distinct arms to the ligand, as shown in Figure 4. There is postulated to be a hydrophobic pocket for the tetraene chain, a hydrophilic, anion-binding site binding the peptidic unit and a polar, but not necessarily anion-binding site associated with the C-1 acid. This analysis allowed Young[40] to speculate that the chromone acid unit of FPL-55712 was occupying the peptide-binding pocket of the pLT receptor, and consistent with this analysis, the chromone (22; Figure 5) was found to displace ^{3}H-LTD_4 from guinea pig lung membranes with an IC_{50} of 100 nM.

A second group of pLT antagonists is now customarily recognized, comprising those compounds (e.g. 11–14, Table I and Figure 3) containing a quinoline nucleus. (Ro 24-5913 (15) is included here as a possible new structural variant on this theme.) The origins of the methoxyquinline motif is not entirely clear; certainly, in the mid 1980s a number of compounds appeared, such as REV-5,901 (23),[41] OT3473 (24),[42] and Wy-45,911 (25),[43] which showed both 5-LO inhibition and pLT antagonism, and evidently rapid optimization of the pLT antagonism component was achieved. On the other hand, the styrylquinoline of MK-571 (11) has been described as resulting from the finding in random screening of significant pLT antagonism in the simple quinoline (26).[40] The contention that the quinoline unit is a mimic of the pLT tetraene chain appears particularly acceptable in the case of MK-571, where all three arms of the pLT binding model are then present, and it is an intriguing extrapolation that a quinoline moiety can perform the same polyene mimic function in the binding of drug molecules to 5-LO. Overall, the quinoline-based compounds are an impressive group of clinical candidates, with Wy-48,252 (14) probably representing the chemically most simple pLT antagonist undergoing advanced study.

(30) Musser, J. H.; Kreft, A. F.; Bender, R. H. W.; Kubrak, D. M.; Carlson, R. P.; Chang, J.; Hand, J. M. *J. Med. Chem.* **1989**, *32*, 1176.
(31) Pharmaprojects, 1990, PJB Publications Ltd., Richmond, Surrey, UK.
(32) Boot, J. R.; Bond, A.; Gooderham, R.; O'Brien, A.; Parsons, M.; Thomas, K. H. *Br. J. Pharmacol.* **1989**, *98*, 259.
(33) Gleason, J. G.; Hall, R. F.; Perchonock, C. D.; Erhard, K. F.; Frazee, J. S.; Ku, T. W.; Kondrad, K.; McCarthy, M. E.; Mong, S.; Crooke, S. T.; Chi-Rosso, G.; Wasserman, M. A.; Torphy, T. J.; Muccitelli, R. M.; Hay, D. W.; Tucker, S. S.; Vickery-Clark, L. *J. Med. Chem.* **1987**, *30*, 959.
(34) Hay, D. W. P.; Muccitelli, R. M.; Vickery-Clark, L. M.; Wilson, K. A.; Bailey, L.; Tucker, S. S.; Yodis, L. P.; Eckardt, R. D.; Newton, J. F.; Smallheer, J. M.; McCarthy, M. E.; Gleason, J. G.; Wasserman, M. A.; Torphy, T. J. *Pharmacologist* **1988**, *30* (3), A96.
(35) May, D. W. P.; Newton, J. F.; Torphy, T. J.; Gleason, J. G. *Drugs Future* **1990**, *15*, 240.
(36) Snyder, D. W.; Giles, R. E.; Keith, R. A.; Yee, Y. K.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 548.
(37) Krell, R. D.; Aharony, D.; Buckner, C. K.; Keith, R. A.; Kusner, E. J.; Snyder, D. W.; Bernstein, P. R.; Matassa, V. G.; Yee, Y. K.; Brown, F. J.; Hesp, B.; Giles, R. E. *Am. Rev. Respir. Dis.* **1990**, *141*, 978.
(38) Nakai, H.; Konno, M.; Kosuge, S.; Sakuyama, S.; Toda, M.; Arai, Y.; Obata, T.; Katsube, N.; Miyamoto, T.; Okegawa, T.; Kawasaki, A. *J. Med. Chem.* **1988**, *31*, 84.
(39) Krell, R. D.; Brown, F. J.; Willard, A. K.; Giles, R. E. In *The Leukotrienes, Chemistry and Biology*; Chakrin, L. W.; Bailey, D. M., Eds.; Academic Press: New York, 1984; Chapter 11.
(40) Young, R. N. *Adv. Prostagland. Thrombox. Leukotriene Res.* **1989**, *19*, 643.
(41) Musser, J. H.; Chakraborty, U. R.; Sciortino, S.; Gordon, R. J.; Khandwala, A.; Neiss, E. S.; Pruss, T. P.; Van Inwegen, R.; Weinryb, I.; Coutts, S. M. *J. Med. Chem.* **1987**, *30*, 96.
(42) Ahnfelt-Ronne, I.; Kaergaard-Nielsen, C.; Kirstein, D. *6th Int. Conf. Prostaglandins Rel. Comp. (Florence)* **1986**, 360.
(43) Musser, J. H.; Kubrak, D. M.; Chang, J.; Lewis, J. *J. Med. Chem.* **1986**, *29*, 1429.

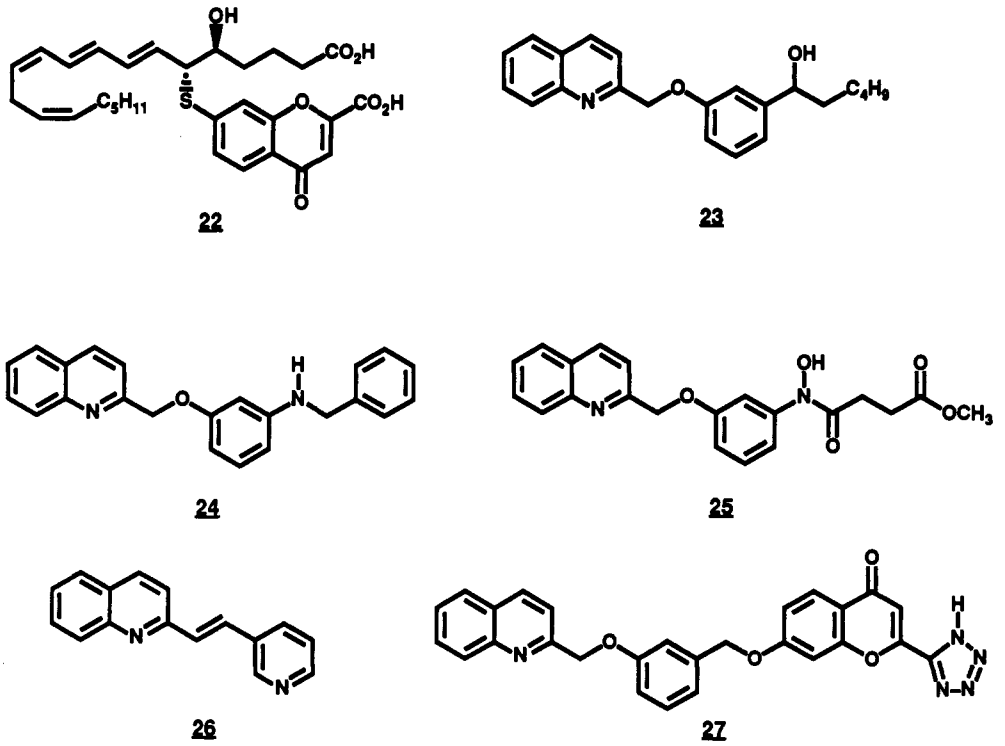

Figure 5.

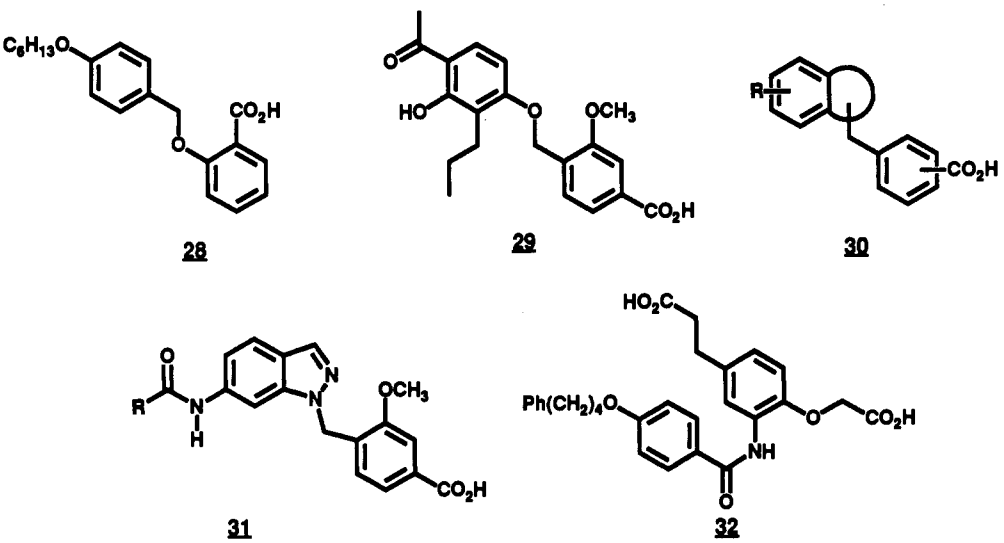

Figure 6.

MK-571 (11) is discussed in further detail in the section on clinical studies. Intriguingly, a very recent further reworking of design concepts in this area has led to very potent quinoline–chromone acid hybrids such as (27).[44]

The influence of the structure of LTD_4 on the drug discovery process is most clearly seen with compounds 16–18, particularly in 16 and 18, where the same relative and absolute stereochemistry of the chiral hydroxyl and thioether centers of the natural ligand are retained. It is thus probable that these antagonists are bound in a mode substantially resembling the agonist binding mode; while none of these compounds are indeed agonistic, agonism is found in congeners of 16. While the loss of the hydroxyl group of 18 leads to distinct loss of binding affinity in 17, a useful gain in oral bioavailability is observed.

The ICI series of pLT antagonists, exemplified by ICI 198615 (19) and ICI 204219 (20), is at first sight difficult to relate structurally to either the agonists or the other known antagonists. In fact, its origins are imbedded in studies of the SAR of both FPL-55712 and the pLTs themselves. A program of dissection of the LTD_4 structure had led, via a spectrum of weak agonists and partial agonists, to structures such as (28; Figure 6), which, while having lost 3 orders of magnitude of affinity compared to LTD_4, displayed reasonably selective antagonism in isolated tissues ($IC_{50} \sim 5\ \mu M$). Separately from this work, attempts to modify the structure of FPL-55712 had led to a second series of benzoic acids (e.g. 29) showing micromolar LTD_4 antagonism.[45] One of the responses to this

(44) Galemmo, R. A.; Learn, K. S.; Poli, G. B.; Morrissette, M. M.; Johnson, W. H.; Dankulich, W. P.; Huang, F-C.; Campbell, H. F.; Van Inwegen, R. G. *Abstracts of Papers*, 200th National Meeting of the American Chemical Society, 1990; American Chemical Society: Washington, DC, 1990; MEDI99.

(45) Brown, F. J.; Bernstein, P. R.; Cronk, L. A.; Dosset, D. A.; Hebbel, K. C.; Maduskie, T. P.; Shapiro, H. S.; Vacek, E. P.; Yee, Y. K.; Willard, A. K.; Krell, R. D.; Snyder, D. W. *J. Med. Chem.* **1989**, *32*, 807.

apparent converging evolution of inhibitor structures was to perform a methodical search over the generic structure **30**, where a new geometrical constraint was added as a potential source of enhanced affinity.[46] The tactics of the search strategy led to the recognition of the indazole **31** and the related indole as novel, selective, and yet still only moderately potent LTD_4 antagonists and LTD_4 receptor ligands. Further intensive efforts on these lead structures, optimizing the acyl chain,[46] central heterocyclic template,[47] and the benzoic acid moiety[48] gave a series of dramatic increases in functional and binding potency, leading ultimately to the orally active clinical compound ICI 204219 (**20**).[37] The indazole ICI 198615 (**19**) is now available in tritiated form as a metabolically stable and highly potent ligand for further investigation of pLT receptors. Other members of these and related series have subsequently been identified with even higher affinity.

Further refinement and unification of binding postulates will probably come from a consideration of the structures of YM-17690 (**32**) and ONO-RS-411 (Table I, **21**). Compound **32** has been pharmacologically profiled as a potent, full agonist at LTD_4 receptors on guinea pig trachea (EC_{50} 2×10^{-8} M, $LTD_4 = 1.9 \times 10^{-8}$ M) and to displace ^{3}H-LTD_4 from guinea pig lung membranes with a pK_i of 9.28 (LTD_4 = 9.79).[49]

Heterogeneity of pLT Receptors

The earliest studies carried out with the newly available synthetic pLTs were designed to determine if they exerted their potent biological activities by an interaction with membrane receptors. Two pieces of evidence suggested that receptor activation was indeed involved: Firstly, biological responses to the pLTs demonstrated a strong chiral preference for the naturally occurring 5*S*,6*R* configuration,[50,51] and secondly, in most systems, FPL-55712 showed a competitive antagonism of their effects. Subsequently, ligand binding studies directly demonstrated the existence of membrane receptors for LTD_4 and LTE_4.[52,53] The unequivocal demonstration of a receptor for LTC_4 by ligand binding techniques has yet to be described, although in guinea pig trachea, at least, functional studies support the presence of such a receptor.[54] A particular problem in this area is the ability of LTC_4 to bind to the ubiquitous glutathione transferase.[55]

Some of the first quantitative pharmacological studies carried out with FPL-55712 suggested pLT receptor heterogeneity. Fleisch et al. postulated on the basis of differences in the apparent affinity constant of FPL-55712 in antagonizing LTD_4 that pLT receptors in guinea pig ileum were not identical with those found in guinea pig trachea and lung parenchymal strips.[56] Similar discrepancies were observed with LY171883 (**6**).[57] Schild analysis of FPL-55712 antagonism of LTD_4 contractions in guinea pig tracheal strips consistently failed to demonstrate competitive antagonism. This observation led Krell et al. to provide circumstantial evidence that LTD_4 receptors on this tissue might be of two subtypes,[58] to which FPL-55712 bound with a half log unit difference in affinity. LTE_4 appeared selective for the higher affinity FPL-55712 site. Recently it was suggested that in guinea pig lung parenchymal membranes LTE_4 might bind to subset of LTD_4 receptors,[59] although this proposal is not consistent with studies finding LTD_4 and LTE_4 to interact with identical sites of equal density.[52]

FPL-55712, in addition to antagonizing LTD4 and LTE_4, also antagonized LTC_4 contractions in most tissues. In guinea pig trachea, antagonism of LTC_4 contractions by FPL-55712 was not independent of the concentration of the antagonist, as expected of competitive antagonism. This problem was solved when it was determined that guinea pig trachea and parenchymal smooth muscle were capable of metabolizing LTC_4, first to LTD_4 by the action of γ-glutamyltranspeptidase and then to the less potent (10-fold) LTE_4.[54] In the presence of L-serine borate, a γ-glutamyltranspeptidase inhibitor, FPL-55712 did not block the effects of LTC_4. These findings led to the postulate that LTC_4 interacted in guinea pig trachea with a receptor distinct from the two receptors (high and low FPL-55712 affinity) with which LTD_4/LTE_4 interact. These functional pharmacologic findings have been confirmed[60,61] and extended to parenchyma by ligand binding studies.[62] In summary, guinea pig trachea contains three pLT receptors: two with which LTD_4/E_4 interact and the third, an LTC_4 receptor.

The main question arising from these studies for those involved in drug discovery was—what are the pharmacologic characteristics of pLT receptors in human airway smooth muscle? To answer this question, an extensive series of experiments were carried out with human intralobar airway smooth muscle.[63] The affinities of both FPL-55712 (**1**) and LY171883 (**6**) did not change when assayed against LTC_4 or LTD_4, with or without added metabolic inhibitors. The apparent dissociation constant (K_B) for FPL-55712 was approximately 1 half log unit lower in human tissue compared to guinea pig smooth muscle. Similar results were obtained when the more potent and selective antagonists ICI 204219 (**20**) and ICI 198615 (**19**) were investigated.[64,65] ICI 204,219 demon-

(46) Brown, F. J.; Yee, Y. K.; Cronk, L. A.; Hebbel, K. C.; Krell, R. D.; Snyder, D. W. *J. Med. Chem.* **1990**, *33*, 1771.
(47) Matassa, V. G.; Maduskie, T. P.; Shapiro, H. S.; Hesp, B.; Snyder, D. W.; Aharony, D.; Krell, R. D.; Keith, R. A. *J. Med. Chem.* **1990**, *33*, 1781.
(48) Yee, Y. K.; Bernstein, P. R.; Adams, E. J.; Brown, F. J.; Cronk, L. A.; Hebbel, K. C.; Vacek, E. P.; Krell, R. D.; Snyder, D. W. *J. Med. Chem.* **1990**, *33*, 2437.
(49) Tomioka, K.; Yamada, T.; Teramura, K.; Terai, M.; Hidaka, K.; Mase, T.; Hara, H.; Murase, K. *J. Pharm. Pharmacol.* **1987**, *39*, 819.
(50) Baker, S. R.; Boot, J. R.; Jamieson, W. B.; Osborne, D. J.; Sweatman, W. J. F. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 1258.
(51) Tsai, B.; Bernstein, P. R.; Macia, R. A.; Conaty, J.; Krell, R. D. *Prostaglandins* **1982**, *23*, 489.
(52) Mong, S.; Scott, M. O.; Lewis, M. A.; Wu, H-L.; Hogaboom, G. K.; Clark, M. A.; Crooke, S. T. *Eur. J. Pharmacol.* **1985**, *109*, 183.
(53) Pong, S. S.; DeHaven, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 7415.
(54) Snyder, D. W.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 616.
(55) Sun, F. F.; Chau, L. Y.; Austen, K. F. *Fed. Proc.* **1987**, *46*, 204.
(56) Fleisch, J. H.; Rinkema, L. E.; Baker, S. R. *Life Sci.* **1982**, *31*, 577.
(57) Fleisch, J. H.; Rinkema, L. E.; Haisch, K. D.; Swanson Bean, D.; Goodson, T.; Ho, P. K. P.; Marshall, W. S. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 148.
(58) Krell, R. D.; Tsai, B. S.; Berdoulay, A.; Barone, M.; Giles, R. E. *Prostaglandins* **1983**, *25*, 171.
(59) Aharony, D.; Catanese, C. A.; Falcone, R. C. *J. Pharmacol. Exp. Ther.* **1989**, *248*, 581.
(60) Hand, J. M.; Schwalm, S. F. *Prostaglandins* **1987**, *33*, 709.
(61) Hand, J. M.; Schwalm, S. F.; Englebach, I. M.; Auen, M. A.; Musser, J. H.; Kreft, A. F. *Prostaglandins* **1989**, *37*, 181.
(62) Hogaboom, G. K.; Mong, S.; Wu, H-L.; Crooke, S. T. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 1136.
(63) Buckner, C. K.; Krell, R. D.; Laravuso, R. B.; Coursin, D. B.; Bernstein, P. R.; Will, J. A. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 558.
(64) Krell, R. D.; Giles, R. E.; Yee, Y. K.; Snyder, D. W. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 557.

Table II. Proposed Provisional Classification of pLT Receptors

receptor	tissue	agonist(s)	antagonists
pLT_1	GP trachea	LTC_4	none
pLT_{2a}	GP trachea	LTD_4/E_4	high affinity for FPL-55712 (also LY171883, SKF 104353, MK571, IC 198615, ICI 204219)
pLT_{2b}	GP trachea	LTD_4/E_4	lower affinity for FPL-55712 than pLT_{2a}
pLT_{2c}	human bronchial smooth muscle	$LTC_4/D_4/E_4$	FPL-55712 SKF 104,353 ICI 204219 ICI 198615
pLT_{2d}	GP ileum	LTD_4/E_4	FPL-55712, LY171883

strates a pK_B of 8.5 against LTC_4, LTD_4, and LTE_4, a value approximately 1 log unit lower than that determined against LTD_4 and LTE_4 on guinea pig tissue. These results indicate that the three pLTs interact with the same receptor in human airway smooth muscle, which is similar to, but not identical with, guinea pig tracheal LTD_4/LTE_4 receptors. It is also noteworthy that LTC_4, LTD_4, and LTE_4 are essentially equipotent in isolated human tissue—thus metabolic conversion of LTC_4 through to LTE_4 does not result in decreased exposure of tissue to the contractile effects of pLT.

Proposal for a Provisional Classification of pLT Receptors

The above discussion serves to highlight some of the complexities of the pLT receptor family as we presently perceive them. Within the next few years we are likely to receive further insights into receptor heterogeneity as the powerful techniques of molecular biology are brought to bear on the problem. Nonetheless, it seems appropriate to present an attempt at receptor classification based on current knowledge as a framework for possible future work. A two-receptor system with multiple subtypes of one of the two is proposed. The pLT_1 receptor exists in guinea pig tracheal smooth muscle, interacts with LTC_4, and is not antagonized by any of the presently available antagonists. The second leukotriene receptor, pLT_2, appears to exist in a variety of subtypes (Table II). The agonists appear to be either LTD_4 or LTE_4, with one exception, viz. pLT_{2c}, where LTC_4 interacts as well, and all are antagonized by at least some of the currently available antagonists. The antagonists do not demonstrate similar affinity for these classes, thus their designation as subclasses of the pLT_2 receptor. Antagonists uniformly demonstrate highest affinity in guinea pig ileum, followed by guinea pig airway smooth muscle and human airway smooth muscle. It is recognized that several tissues that are responsive to pLTs have been omitted, perhaps most notably guinea pig lung parenchyma. In this instance, the complexity of the cell types giving rise to the contractile response makes appropriately quantitative pharmacologic analysis difficult and thus classification precarious indeed. For others that have been omitted (viz. pulmonary artery/vein, myocardium, segments of gastrointestinal smooth muscle, uterus, etc.) not enough information is presently available, particularly concerning the ability of the newer, more potent antagonists to inhibit responses to pLTs, to consider classification at this time. This proposed classification is based solely on functional studies. However, in many instances ligand binding studies do lend support to the proposed classification. The classification is undoubtedly oversimplified; however, it is hoped that it can be a useful framework for those studying the biochemistry and pharmacology of leukotrienes and their antagonists in the future.

Biochemistry of pLT Receptors

Physical isolation and characterization of the pLT receptors is a comparatively underdeveloped area, with most of the recent work emanating from the SKF laboratories. It is now considered likely that the LTD_4/LTE_4 (pLT_2?) receptor belongs to the G-protein-coupled receptor superfamily, and that IP_3 generation and subsequent intracellular Ca^{2+} mobilization are central components of the second messenger system activated by receptor occupancy.[66] To date, attempts to isolate LTD_4 receptors in soluble form have met with some success,[67] but little is known of the protein chemistry, and no receptor cloning experiments have been reported.

Clinical Results with pLT Antagonists

Most of the compounds in Table I are either in clinical trial or have been withdrawn from trial. Typically, candidates are being assessed initially for their ability to block the effects of exogenously administered LTD_4 on pulmonary function parameters, followed by an assessment of their ability to block the decline in lung function induced by exposure to antigen in atopic individuals. Finally, clinical efficacy in disease states such as asthma or rhinitis is sought.

LY171883 (6) at a total oral doses of up to 400 mg produced a 4.6- and 6.1-fold shift in the aerosol dose–response curve for LTD_4 on large and small airways, respectively,[68] whereas L-649923 (4) at a total oral dose of 1 g produced a 3.8-fold shift on both large and small airways.[69] Since neither compound altered base-line airway caliber, the effects are reasonably attributed to antagonism of LTD_4 rather than any nonspecific bronchodilator activity. L-649923 at 1000 mg po dose showed a modest effect in an antigen challenge study in mild asthmatics.[70] Given the comparatively small effects (<5-fold shifts) seen against exogenous LTD_4, it is perhaps reasonable not to expect a large impact on the antigen-induced effects, where it is possible to envision relatively large concentrations of pLTs being developed around their cellular source and close to their effector sites. Results of more chronic studies with LT antagonists have provided some further encouragement. LY171883 (6) after 2 weeks of treatment produced a decrease in symptom scores in mild asthmatics as well as a degree of antagonism of the bronchoconstrictor response to inhaled cold air[71] and, in a longer study in mild asthmatics, produced an improvement in lung function and reduced bronchodilator use.[72] The withdrawal of

(65) Snyder, D. W.; Giles, R. E.; Keith, R. A.; Yee, Y. K.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 548.
(66) Mong, S.; Miller, J.; Wu, H. L.; Crooke, S. T. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 508.
(67) Mong, S.; Wu, H. L.; Stadel, J. M.; Clark, M. A.; Crooke, S. T. *Mol. Pharmacol.* **1986**, *29* (3), 235.
(68) Phillips, G. D.; Rafferty, P.; Robinson, C.; Holgate, S. T. *J. Pharmacol. Exp. Ther.* **1988**, *246*, 732.
(69) Barnes, M.; Piper, P. J.; Costello, J. *J. Allergy Clin. Immunol.* **1987**, *79*, 816.
(70) Britton, J. R.; Hanley, S. P.; Tattersfield, A. E. *J. Allergy Clin. Immunol.* **1987**, *79*, 811.
(71) Israel, E.; Juniper, E. F.; Callaghan, J. T.; Mathur, P. N.; Morris, M. M.; Dowell, A. R.; Enas, G. G.; Hargreave, F. E.; Drazen, J. M. *Am. Rev. Respir. Dis.* **1989**, *140*, 1348.
(72) Cloud, M. L.; Enas, G. G.; Kemp, J.; Platts-Mills, T.; Altman, L. C.; Townley, R.; Tinkelman, D.; King, T.; Middleton, E.; Shaffer, A. L.; McFadden, E. R.; Farlow, D. S. *Am. Rev. Respir. Dis.* **1988**, *140*, 1336.

LY171883 from trial left the question of the involvement of pLTs in asthma substantially unanswered by the first wave of clinical pLT antagonists, especially those of the hydroxyacetophenone type, and attention has shifted to the intrinsically more potent compounds of different structures.

SKF 104353 (18) is well advanced in clinical development. When dosed by aerosol, it has been shown to give potent blockade of aerosolized LTD_4 challenges in man, and to be selective for LTD_4 as opposed to histamine-induced bronchoconstriction.[73,74] Beneficial effects have also been shown in both allergen and exercise challenge protocols, and there is an intriguing suggestion that the late inflammatory response shown by some asthmatics upon allergen challenge is also blocked by SKF 104353. Such an effect has been seen with pLT antagonists in animal models[75] and is also seen clinically with ONO-RS-411 (21).[76]

ICI 204219 (20) is now established as a very effective LTD_4 antagonist in man. A 40-mg dose given orally 3 h prior to aerosol LTD_4 challenge produced a 2 log unit rightward shift in the LTD_4 dose–response curve, using FEV_1 as the measure of bronchoconstriction, and a significant degree of antagonism remained 24 h after dosing.[77] In a similar manner, the compound substantially shifts (13-fold) the antigen dose–response curve in allergic asthmatics challenged with cat dander.[78]

The published clinical data on MK-571 (11) is currently limited to iv studies and are consistent with the promising pre-clinical pharmacological profile of the compound. Normal and asthmatic subjects have tolerated iv doses up to 1500 mg,[79] and an iv infusion of 28 mg produced a >44-fold shift in the aerosol LTD_4 induced bronchoconstriction dose–response curve in asthmatics.[80] Evidence that the compound produces a significant, acute bronchodilating effect in asthmatic subjects has also been presented.[81]

Conclusion

The last 10 years of intense research effort by the pharmaceutical industry has brought forward a range of highly potent and selective antagonists that have the properties to define the role of peptide leukotrienes in human diseases and hopefully to become new therapeutic agents. While it is still early in their clinical evaluation, there has already been generated good evidence to support the contention, derived ultimately from the experiments of Kellaway[82] over 50 years ago, that pLTs play an important role in acute allergic bronchoconstriction in man, and consistent with one animal model at least,[75] there are preliminary indications that late-phase allergic bronchoconstriction is blocked by pLT antagonists also. Central issues now are to establish the impact of these pharmacological effects of pLT antagonism on a complex disease state such as asthma and to extend investigations to other allergically driven syndromes of the upper and lower airways, conjunctiva, skin and GI tract. The possibility that pLTs are involved in nonimmunologically triggered process such as urticaria will also be investigated.

The complexity of asthma as a disease is increasingly being recognized. It is highly likely that a mix of different receptor antagonists, e.g., histamine H_1 and pLT antagonists, will provide greater efficacy than any single therapeutic class. Moreover, 5-lipoxygenase inhibitors, capable of suppressing the synthesis of both pLT and the chemoattractant LTB_4, may prove more efficacious than pLT antagonists. In this regard, it should be recognized, however, that a variety of chemoattractants are released by antigen and inhibition of LTB_4 alone may not, in fact, provide any advantage for 5-LO inhibitors over pLT antagonists. However, with the arrival in the clinic of promising inhibitors of 5-lipoxygenase,[83,84] just behind the pLT antagonists, it is certain that the pathophysiological relevance of the pLTs and LTB_4 will be uncovered by the next few years of clinical research. It would be fitting indeed if the long series of high-quality scientific endeavors that have brought us to this position were to culminate in a major therapeutic advance.

(73) Evans, J. M.; Barnes, N. C.; Zakrzewski, J. T.; Glenny, H. P.; Piper, P. J.; Costello, J. F. *Br. J. Clin. Pharm.* **1988**, *26*, 677P.
(74) Joos, G. F.; Kips, J. C.; Puttemans, M.; Pauwels, R. A.; Van Der Straeten, M. E. *J. Allergy Clin. Immunol.* **1989**, *83*, 187.
(75) Abraham, W. *Ann. N.Y. Acad. Sci.* **1988**, *524*, 260.
(76) Yamai, T.; Watanabe, S.; Motojima, S.; Fukuda, T.; Makino, S. *Am. Rev. Respir. Dis.* **1989**, *139*, A462.
(77) Smith, L. J.; Geller, S.; Ebright, L.; Glass, M.; Thyrum, P. T. *Am. Rev. Respir. Dis.* **1990**, *141*, 988.
(78) Findlay, S. R.; Easley, C. B.; Glass, M.; Barden, J. W. *J. Allergy Clin. Immunol.* **1990**, *85* (1, part 2), No. 215.
(79) Van Hecken, A.; De Schepper, P.; Margolskee, D. J.; Hsieh, J. Y. K.; Robinette, R. S.; Buntix, A.; Rogers, J. D. *Eur. J. Clin. Pharmacol.*, in press.
(80) Kips, J.; Joos, G.; Pauwels, R.; Van Der Straeten, M. E.; De Lepeleire, I.; Williams, V.; Margolskee, D. *Am. Rev. Respir. Dis.* **1990**, *141* (4), A117.
(81) Gaddy, J.; Bush, R. K.; Margolskee, D.; Williams, V. C.; Busse, W. *J. Allergy Clin. Immunol.* **1990**, *85*, (1, part 2), No. 216.
(82) Kellaway, C. H.; Trethewie, E. R. *Q. J. Exp. Physiol.* **1940**, *30*, 121.
(83) Hui, K. P.; Taylor, I. K.; Barnes, N. C.; Barnes, P. J. *Am. Rev. Respir. Dis.* **1990**, *141* (4), A32.
(84) Tanaka, W.; Dallob, A.; Winchell, G.; Kline, W.; Spector, R.; Bjornsson, T.; DeSchepper, P. *Am. Rev. Respir. Dis.* **1990**, *141* (4), A32.