Thiopyranol[2,3,4-*c*,*d*]indoles as Inhibitors of 5-Lipoxygenase, 5-Lipoxygenase-Activating Protein, and Leukotriene C_4 Synthase

John H. Hutchinson,*,† Stella Charleson,§ Jilly F. Evans,§ Jean-Pierre Falgueyret,§ Karst Hoogsteen,⊥ Tom R. Jones,‡ Stacia Kargman,§ Dwight Macdonald,† Cyril S. McFarlane,‡ Donald W. Nicholson,§ Hanna Piechuta,‡ Denis Riendeau,§ John Scheigetz,† Michel Thérien,† and Yves Girard†

Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe-Claire, Dorval, Quebec, Canada H9R 4P8

Received August 16, 1995®

The attachment of an arylacetic or benzoic acid moiety to the thiopyrano[2,3,4-*c*,*d*]indole nucleus results in compounds which are highly potent and selective 5-lipoxygenase (5-LO) inhibitors. These compounds are structurally simpler than previous compounds of similar potency in that they contain a single chiral center. From the data presented, 2-[[1-(3-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]methoxy]-phenylacetic acid, **14b**, was shown to inhibit 5-hydroperoxyeicosatetraenoic acid (5-HPETE) production by human 5-LO (IC_{50} of 18 nM). The acid **14b** is highly selective as an inhibitor of 5-LO activity when compared to the inhibition of ram seminal vesicle cyclooxygenase (IC_{50} > 5 μM) or human leukocyte leukotriene A_4 (LTA_4) hydrolase (IC_{50} > 20 μM). In addition, **14b** was inactive in a 5-lipoxygenase-activating protein (FLAP) binding assay at 10 μM. *In vivo* studies showed that **14b** is bioavailable in rat and functionally active in the hyperreactive rat model of antigen-induced dyspnea (74% inhibition at 0.5 mk/kg po; 2 h pretreatment). In the conscious squirrel monkey model of asthma, **14b** showed excellent functional activity at 0.1 mg/kg against antigen-induced bronchoconstriction (94% inhibition of the increase in R_L and 100% inhibition in the decrease in C_{dyn}; $n = 4$). Resolution of this compound gave (−)-**14b**, the most potent enantiomer (IC_{50} = 10 nM in the human 5-LO assay), which was shown to possess the *S* configuration at the chiral center by X-ray crystallographic analysis of an intermediate. Subsequent studies on the aryl thiopyrano[2,3,4-*c*,*d*]indole series of inhibitors led to the discovery of potent *dual* inhibitors of both FLAP and 5-LO, the most potent of which is 2-[[1-(4-chlorobenzyl)-4-methyl-6-(quinolin-2-ylmethoxy)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]methoxy]phenylacetic acid, **19**. Acid **19** has an IC_{50} of 100 nM for the inhibition of 5-HPETE production by human 5-LO and is active in a FLAP binding assay with an IC_{50} of 32 nM. Furthermore, thiopyrano[2,3,4-*c*,*d*]indoles such as **1** and **14b** are capable of inhibiting the LTC_4 synthase reaction in a dose dependent manner (IC_{50}s of 11 and 16 μM, respectively, compared to that of LTC_2 at 1.2 μM) in contrast to other, structurally distinct 5-LO inhibitors. It has also been observed that the thiopyrano[2,3,4-*c*,*d*]indole class of compounds strongly promotes the translocation of 5-LO from the cytosol to a membrane fraction in the presence or absence of the ionophore A23187. The membrane-bound 5-LO retains its activity, and this association with the membrane is not reversed with the FLAP inhibitor MK-886. These observations, in part, support the hypothesis that these compounds act by binding at the arachidonic acid-binding site on FLAP, 5-LO, and LTC_4 synthase and that the thiopyrano[2,3,4-*c*,*d*]indole is capable of mimicking an active conformation of arachidonic acid.

Introduction

The biotransformation of arachidonic acid by 5-lipoxygenase (5-LO)[1] occurs by oxygenation to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE) followed by the conversion of 5-HPETE (by the same enzyme) into the unstable epoxide leukotriene A_4 (LTA_4). This epoxide can then be transformed into the proinflammatory mediator LTB_4. Alternatively, LTA_4 can be conjugated with glutathione to yield LTC_4 from which LTD_4 and LTE_4 are generated. A crucial factor in the production of LTA_4 is the involvement of a membrane-bound protein, 5-lipoxygenase-activating protein (FLAP), which has been shown to be required for leukotriene biosynthesis.[2] The leukotrienes thus produced have been implicated in a variety of disorders[3] which include asthma, rheumatoid arthritis, and inflammatory bowel disease. It has recently been shown that inhibitors of LT biosynthesis (either by FLAP inhibitors or by 5-LO inhibitors)[4] or LTD_4 receptor antagonists[5] have clinically significant effects in the treatment of asthma.

We have recently described a novel class of 5-LO inhibitors based on the thiopyrano[2,3,4-*c*,*d*]indole ring system.[6] These compounds have been shown to inhibit 5-LO in a manner which is consistent with the formation of a reversible dead-end complex.[7] From this class, 2-[2-[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)-methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl]ethoxy]butanoic acid (**1**, L-699,333) has been shown to be a selective and potent inhibitor of 5-LO which has oral activity in a number of animal models of asthma and inflammation.

* To whom correspondence should be addressed. Present address: Merck and Co. Inc., P.O. Box 4, WP44-J100, West Point, PA 19486.
† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Department of Biochemistry.
⊥ Department of Biophysical Chemistry, Rahway, NJ.
® Abstract published in *Advance ACS Abstracts*, October 15, 1995.

0022-2623/95/1838-4538$09.00/0 © 1995 American Chemical Society

1 (L-699,333)

In this paper we will describe the preparation and biological evaluation of a second generation of compounds which are structurally simpler than **1** in that they lack the chiral center α to the acid. The compounds detailed herein contain arylacetic or aryl carboxylic acids on the C-2 side chain and are potent, selective inhibitors of 5-LO which display oral efficacy in animal models.

Also, with knowledge from our previous work on FLAP inhibitors, we have been able to combine the salient features from each class to generate the first potent *dual* inhibitors of both FLAP and 5-LO. The results of these studies and the implication with regard to the interplay of arachidonic acid with FLAP and 5-LO will be discussed. In addition, some further observations on the interaction of the thiopyrano[2,3,4-*c*,*d*]-indole compounds with 5-LO and LTC_4 synthase will be presented.

Chemistry

Compounds containing a 5-phenyl-2-pyridylmethoxy substituent at C-6 of the thiopyrano[2,3,4-*c*,*d*]indole ring were prepared from the known alcohols **2** and **3**[6,8] using the synthetic routes shown in Schemes 1 and 2. For the carba analog (Scheme 1), the alcohol **2a** was oxidized to the aldehyde **4** using MnO_2, and this was then condensed with the Wittig reagent generated from **5**. The resulting alkene **6** (mixture of double-bond isomers) was reduced using Et_3SiH and $BF_3 \cdot OEt_2$ in CH_2Cl_2, and subsequent hydrolysis of the nitriles under forcing conditions then provided the desired acid **7**.

Scheme 2 describes the preparation of compounds containing an O or S linkage in the acid-bearing side chain. The direct introduction of a thiobenzoic acid moiety onto **2** (to give **8**) was achieved by stirring **2** with 2-thiosalicylic acid and $BF_3 \cdot OEt_2$ in 1,2-dichloroethane. For the homologous compound, acid **9**, the starting alcohol **3** was transformed into the corresponding bromide which was then displaced with the anion derived from 2-thiosalicylic acid using NaH in DMF. The ether **10** was synthesized by coupling **2** with 2-(bromomethyl)benzonitrile followed by hydrolysis of the nitrile to the acid. The remaining ether compounds (**11**–**15**) were assembled from **2** or **3** and the appropriate phenolic ester using a Mitsunobu reaction followed by saponification.

In order to prepare optically pure **14b**, the intermediate phenol **16** was separated by HPLC using a Chiralcel AS column to give the pure enantiomers (+)- and (−)-**16** (Scheme 3). These were then alkylated with 2-(chloromethyl)-5-phenylpyridine using Cs_2CO_3 in DMF/CH_3-CN to give (+)- and (−)-**2b**, respectively, which were transformed, as before, into (+)- and (−)-**14b**.

The compounds incorporating various nitrogen heterocycles at C-6 were prepared from the known phenol **17** as shown in Scheme 4. Coupling of **17** with the appropriate alkyl halide using Cs_2CO_3 in DMF afforded **18** which was then transformed into the target acids **19** (cf. Scheme 2, step c) and **20a**–**d** (cf. Scheme 2, step a).

Results and Discussion

Selective 5-LO Inhibitors. The potent 5-LO inhibitor **1** possesses two asymmetric centers—one at the carbon bearing the methyl group on the thiopyran ring and the second α to the acid group. This, then, is a mixture of four possible diastereomers. We sought to reduce the degree of complexity present in this molecule by removal of a chiral center. Since we have shown in a related series that a methyl-substituted thiopyran ring is optimum for activity,[9] attention was focused on removal of asymmetry on the acid-bearing chain. One avenue that was explored was the incorporation of an aromatic ring wherein the phenyl ring is able to occupy the same lipophilic site as the ethyl side chain of **1**. The results for these interphenylated compounds are given in Table 1. The carba analog **7** was significantly less potent than **1** at inhibiting the human 5-LO reaction.

Scheme 1[a]

2a —(a)→ **4**

5 —(b)→ **6** —(c), (d)→ **7**

[a] Reagents: (a) MnO_2, THF; (b) **5**, KHMDS, THF, then **4**; (c) Et_3SiH, $BF_3 \cdot OEt_2$, CH_2Cl_2; (d) 8 N KOH, 2-(ethoxyethoxy)ethanol, 180 °C.

Scheme 2[a]

[a] TPI = 1-(halobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c*,*d*]indol-2-yl. Reagents: (a) thiosalicylic acid, $BF_3 \cdot OEt_2$, CH_2ClCH_2Cl; (b) Ph_3P, CBr_4, THF, then NaH, thiosalicylic acid, THF; (c) NaH, 2-$BrCH_2C_6H_4CN$, THF, then 8 N KOH, 2-(ethoxyethoxy)ethanol, 180 °C; (d) Ph_3P, DEAD, 2-$HOC_6H_3YCO_2Me$, THF, then aqueous LiOH, THF, MeOH; (e) Ph_3P, DEAD, 2-$HOC_6H_4CH_2CO_2Me$, THF, then aqueous LiOH, THF, MeOH.

Scheme 3

Gratifyingly, introduction of a heteroatom (which in a previous series was shown to be beneficial)[6] improved potency in the case of both a sulfur atom (compounds **8** and **9**) as well as an oxygen atom (compounds **10**–**15**).

The position of substitution of the acid was investigated by comparing the ortho acid **11** to the meta analog **13**, and it was found that the meta analog is about 2-fold less active (60 versus 130 nM). However, there is little difference in activity between benzoic or phenylacetic acid derivatives (cf. **15a** and **11**, both 60 nM). Similarly, the position of the heteroatom in the chain makes little difference (compare **10** with **11**, both 60 nM). In contrast, substitution of the phenyl ring in compound **11** with chlorine **12** resulted in a diminution of activity by ca. 2–3-fold.

The data presented show that variations in the chain length of the indole C-2 substituent are well tolerated. In addition, it has been shown previously that the carboxylate group is required for inhibition of 5-LO activity.[9,10] Presumably, the carboxylate of the thiopyrano[2,3,4-*c*,*d*]indoles as well as that of arachidonic acid is capable of interacting with a basic amino acid residue located at or near the active site. Indeed, recently the 3-dimensional structure of soybean lipoxygenase-1 has been determined,[11] and it shows that the arachidonic acid-binding domain contains an arginine and a histidine as well as a lysine (located near the surface of the protein). Given that there is considerable homology between soybean lipoxygenase-1 and mamalian lipoxygenases, it is reasonable to speculate that similar basic residues are located in the arachidonic acid-binding region of human 5-LO. The observation that inhibitors of different chain length can have similar potencies may be a result of the carboxylate group interacting with the different resonance forms present in the flexible basic residues of arginine or histidine.

Replacement of the *N*-4-chlorobenzyl substituent with other halobenzyl groups was investigated. Thus, when the chlorine of **15a** was replaced with a fluorine (**15c**) or when the chlorine was moved to the 3-position (**15b**), little change in activity was observed. However, the 3-fluoro derivative **15d**, with an IC_{50} of 23 nM, is ca. 2 times as potent as **15a**. Similarly, comparison of the 4-chloro compound **14a** (26 nM) with the 3-chloro analog **14b** (18 nM) shows that this modification is marginally beneficial in terms of inhibitor potency.

Since most of the analogs prepared exhibit excellent *in vitro* activity, many of them were also screened *in vivo* to determine the bioavailability in rat and their efficacy in a rat model of asthma.[12] Both of the sulfur analogs **8** and **9** showed poor bioavailability in rat (<10%) and so were not examined further. The oxygen-containing analogs exhibited somewhat better bioavailability (up to 44% in the case of **15d**). However, the

Scheme 4[a]

[a] Reagents: (a) RCH_2X, Cs_2CO_3, DMF; (b) $2\text{-}HOC_6C_4CH_2CO_2Me$, DEAD, Ph_3P, THF, then LiOH, MeOH, THF, H_2O; (c) 2-thiosalicylic acid, $BF_3{\cdot}OEt_2$, CH_2ClCH_2Cl.

Table 1. Structure–Activity Relationship of C-2 Side Chain

compd	W	X	Y	Z	human 5-LO (IC_{50}, nM)[a]	F (rat)[b]	hyperreactive rat (%)[c]
1					22	88	50
7	4-Cl	CH_2CH_2	H	$2\text{-}CO_2H$	360	–[d]	–
8	4-Cl	CH_2S	H	$2\text{-}CO_2H$	30	<5	–
9	4-Cl	CH_2CH_2S	H	$2\text{-}CO_2H$	30	10	–
10	4-Cl	CH_2OCH_2	H	$2\text{-}CO_2H$	62*	–	–
11	4-Cl	CH_2CH_2O	H	$2\text{-}CO_2H$	60	37	42
12	4-Cl	CH_2CH_2O	Cl	$2\text{-}CO_2H$	130*	–	–
13	4-Cl	CH_2CH_2O	H	$3\text{-}CO_2H$	170*	–	–
14a	4-Cl	CH_2O	H	$2\text{-}CH_2CO_2H$	26	35	27
14b	3-Cl	CH_2O	H	$2\text{-}CH_2CO_2H$	18	11	74
(+)-14b	3-Cl	CH_2O	H	$2\text{-}CH_2CO_2H$	95	–	–
(−)-14b	3-Cl	CH_2O	H	$2\text{-}CH_2CO_2H$	10	32	58
15a	4-Cl	CH_2CH_2O	H	$2\text{-}CH_2CO_2H$	60*	15	47
15b	3-Cl	CH_2CH_2O	H	$2\text{-}CH_2CO_2H$	40*	–	–
15c	4-F	CH_2CH_2O	H	$2\text{-}CH_2CO_2H$	60*	4	36
15d	3-F	CH_2CH_2O	H	$2\text{-}CH_2CO_2H$	23	44	59

[a] Each IC_{50} value corresponds to an average of at least two independent determinations except those identified with an asterisk which are the result of a single titration. [b] 20 mg/kg po, 5 mg/kg iv (n = 2). Bioavailability (0–6 h) determined by $AUC_{po}/4 \times AUC_{iv}$. [c] Percent inhibition of dyspnea in hyperreactive rats dosed at 5 mg/kg po; n = 6. [d] Value not determined denoted by a dash.

measured bioavailability did not generally correspond well with the inhibition of dyspnea observed in the hyperreactive rat. Using the hyperrreactive rat model, compounds **15d** and **14b** stand out as having good *in vivo* efficacy at the screening dose of 0.5 mg/kg with a 59% and 74% inhibition of dyspnea, respectively. It is interesting to note that the subtle change from a 4-chloro substituent (**14a**) to a 3-chloro substituent (**14b**) results in a dramatic increase in *in vivo* potency (27% versus 74% inhibition of dyspnea). Inhibitor **14b** (L-705,302) was further examined in a monkey model of asthma[13] where it showed excellent functional activity at 0.1 mg/kg against antigen-induced bronchoconstriction in the conscious squirrel monkey (94% inhibition of the increase in R_L and 100% inhibition in the decrease in C_{dyn}; $n = 4$). For comparison, **1** showed 89% inhibition of the increase in R_L and 68% inhibition in the decrease in C_{dyn} when dosed at 0.1 mg/kg, $n = 3$. Both of these compounds (**14b** and **1**) are superior in efficacy in this model to MK-0591 when given at a 3-fold lower dose (44% inhibition of the increase in R_L and 46% inhibition in the decrease in C_{dyn}; 0.3 mg/kg, $n = 5$).[14]

Figure 1. Ortep diagram of intermediate (−)-**2b**.

MK-0591 is a FLAP inhibitor which has shown efficacy against antigen-induced asthma in clinical trials.[4e]

The acid **14b** is highly selective as an inhibitor of 5-LO activity when compared to the inhibition of ram seminal vesicle cyclooxygenase (IC_{50} > 5 μM) or human leukocyte LTA_4 hydrolase (IC_{50} > 20 μM). In addition, **14b** was inactive in a FLAP binding assay at 10 μM.

Due to the excellent *in vitro* and *in vivo* activity of **14b**, it was of interest to assess the activity of the individual enantiomers and, if possible, determine the absolute stereochemistry of the most active isomer. Toward this end, the enantiomers (+)- and (−)-**14b** were synthesized. They show an 8-fold difference in activity in the human 5-LO assay with (−)-**14b**, at 10 nM, being equal in activity to the most active diastereomer derived from **1**. The enantiomer (−)-**14b** is also functionally active in the hyperreactive rat model with a 58% inhibition of dyspnea observed at a dose of 0.5 mg/kg.

In order to determine the absolute configuration of the chiral center present in (−)-**14b**, an X-ray crystal structure of the intermediate (−)-**2b** was solved.[15] The anomalous dispersion effect established, with statistically acceptable significance, that the C-4 atom (this corresponds to the atom numbered C-11 on the Ortep diagram, Figure 1) has the *S* configuration.

Thus we have demonstrated that replacement of the ethyl substituent and elimination of the chiral center α to the acid in **1** by incorporation of an aryl group results in potent, selective inhibitors of 5-LO. These highly optimized compounds are structurally simpler than the predecessors of comparable potency in that they contain only a single chiral center. In addition, several of these compounds are orally bioavailable and show *in vivo* activity in animal models. In particular, the oxyphenylacetic acid derivative **14b** has excellent oral activity in the rat and monkey models of asthma, comparable to that of **1** and superior to that of MK-0591. The latter compound is currently undergoing clinical trials and has shown efficacy in patients with mild to moderate asthma. Therefore, this approach may lead to the identification of a novel chemical entity for the treatment of leukotriene-mediated diseases.

Preparation of Dual 5-LO/FLAP Inhibitors. Upon activation, 5-LO is known to translocate from the cytosol to a sedimentable fraction.[16] Through the use of immunoelectronmicroscopic labeling of activated leukocytes, it has been determined that the site to which translocation occurs is the nuclear envelope, and in activated cells *both* 5-LO and FLAP are localized here.[17] FLAP is a membrane-bound protein containing three putative transmembrane-spanning domains.[2] Recently, it has been determined that FLAP binds arachidonic acid and may stimulate 5-LO by selectively transferring arachidonic acid to 5-LO.[18] In addition, FLAP inhibitors have been developed by Merck Frosst (e.g., MK-886[19] and MK-0591) and others (e.g., BAY X1005[20] and WAY-50,295[21]) which bind at the arachidonic acid-binding site on FLAP. Site-specific mutagenesis work carried out at these laboratories[22] has demonstrated that an aspartic acid residue located at the putative interface of the cytosol and the membrane is crucial for the binding of the FLAP inhibitor MK-886, and presumably, this is where the arachidonic acid-binding site is located. The binding of these inhibitors or fatty acids[23] is not dependent on the carboxylic acid but is influenced by changes to the lipophilic polyene chain. In contrast, the direct 5-LO inhibitors in the thiopyrano[2,3,4-*c*,*d*]indole series are competitive for arachidonic acid binding to the enzyme 5-LO, and the activity is *critically* dependent on the presence of a carboxylic acid or acid equivalent. Thus, it appears that the binding of arachidonic acid to FLAP and 5-LO is a result of binding to different regions of the same substrate. It therefore seems logical to assume that mixed inhibitors of 5-LO and FLAP are possible especially in light of the structural similarity of MK-886/MK-0591 and the thiopyrano[2,3,4-*c*,*d*]indoles. Indeed, the thiopyrano[2,3,4-*c*,*d*]indole series evolved from work done in our FLAP inhibitor program, which suggests a degree of commonality in the active site of both FLAP and 5-LO. Moreover, critical to the development of the 5-LO inhibitors was the identification of the weak mixed inhibitors **21** (9300 nM FLAP, 700 nM rat 5-LO) and **22** (19 nM FLAP, 600 nM rat 5-LO). Subsequently, the Bayer group also proposed that mixed inhibitors of this system should be possible,[24] although they appear not to be aware of these earlier results.

21

22

We were intrigued by the possibility of being able to prepare *potent* dual inhibitors of both FLAP and 5-LO by the judicious combination of structural features which are responsible for the separate activities. Ex-

amination of both series reveals that the thiopyran ring is essential for 5-LO inhibitory activity and is also a potency-enhancing feature for FLAP inhibitory activity. In addition, the *N*-benzyl substituent is required for FLAP inhibition but plays a lesser role in determining 5-LO inhibition. As stated previously, the acid chain is not a required structural feature in a FLAP inhibitor, whereas it is essential for inhibitory activity against the enzyme. Therefore an indole substituted with a thiopyran ring, an *N*-benzyl group, and an optimized acid-bearing side chain (such as is described above) should be capable of binding to both FLAP and 5-LO. The remaining substituent at the C-5 position (indole numbering) is either a quinolin-2-ylmethoxy (for MK-0591) or a (5-phenylpyridin-2-yl)methoxy (for 5-LO inhibitors). The quinoline group is an important pharmacophore for FLAP inhibition,[21] although it is not an essential requirement since it can be replaced with, among other things, a pyridin-2-ylmethoxy substituent.[6] In contrast, the (5-phenylpyridin-2-yl)methoxy group has been shown to be optimum for 5-LO inhibition in the case of compounds containing a 2,2-dimethyl-3-propanoic acid side chain.[1] In this series, replacement with a quinolin-2-ylmethoxy group (to give **22**) leads to a mixed inhibitor with a significant loss of potency against 5-LO activity. The consequence of replacing the (5-phenylpyridin-2-yl)methoxy group with other basic heterocycles on a molecule containing a C-2 side chain optimized for 5-LO inhibition, such as the arylbenzoic and arylacetic acids (described above), was not known. We hypothesized that, by using such an optimized compound, the 5-phenylpyridine moiety could be replaced with other groups that would maintain the 5-LO potency and perhaps also endow the molecule with significant FLAP activity. Several basic heterocyclic groups were selected, and the hybrid compounds synthesized are given in Table 2.

The phenylpyridine moiety of compounds **8** and **14a** is clearly responsible for selectivity for the 5-LO enzyme as neither compound shows any inhibitory activity in the FLAP binding assay at 10 μM. In contrast, replacement of the phenylpyridine with a quinoline (to give **19** and **20a**) has minimal effect on the potency versus 5-LO activity but boosts the FLAP inhibition and yields compounds which are potent dual inhibitors. The IC_{50} of the quinoline compound **19** is 100 nM in the 5-LO assay and 32 nM in the FLAP assay, while that of **20a** is 70 nM in 5-LO and 72 nM in FLAP assays. These compounds are similar in potency to the most potent 5-LO inhibitors (e.g., **1** and **14b**; Table 1) as well as to the most potent FLAP inhibitors (e.g., MK-886 at 23 nM and MK-0591 at 2 nM). Other nitrogen-containing heterocycles were examined (examples **20a–c**; Table 2). The pyridine analog **20b** and the 3-methoxypyridine analog **20c** were both able to maintain 5-LO activity at about 50 nM, but their ability to bind to FLAP is compromised. These analogs were 5–10-fold less active as FLAP inhibitors than the corresponding quinoline **20a**. The isoquinoline analog **20d** was considerably weaker at inhibiting both the 5-LO reaction and FLAP binding than the other heterocycles studied.

Thus it is apparent that thiopyrano[2,3,4-*c,d*]indoles which incorporate an acid side chain optimized for inhibition of 5-LO activity will tolerate modifications to the phenylpyridine portion of the molecule. This is in contrast to previous findings in an earlier, nonoptimized series in which such modifications resulted in a dramatic reduction in potency. Not surprisingly, the incorporation of a quinoline group engenders these molecules with significant FLAP inhibitory properties (many FLAP inhibitors contain the quinoline pharmacophore). The unique aspect of this series of compounds is that they are potent dual inhibitors capable of preventing leukotriene biosynthesis by limiting the access of 5-LO to its substrate, arachidonic acid, either by binding to 5-LO or by binding to FLAP, or both.

Table 2. Effect of *N*-Heterocycle on Selectivity

compd	R	Y	n	Human 5-LO (IC_{50}, nM)[a]	FLAP (IC_{50}, nM)[a]
8	Ph, N, CH_2	S	0	32	>10,000*
14a	Ph, N, CH_2	O	1	26	>10,000*
19	N, CH_2	O	1	100*	32
20a	N, CH_2	S	0	70	72
20b	N, CH_2	S	0	55	760
20c	MeO, N, CH_2	S	0	52	370
20d	CH_2, N	S	0	175*	2900
MK-886				>10,000	23
MK-0591				>10,000	1.6

[a] Each IC_{50} value corresponds to an average of at least two independent determinations except those identified with an sterisk which are the result of a single titration.

Further *in Vitro* Observations. The complete mechanism by which 5-LO and FLAP cooperate in order to produce LTA_4 from arachidonic acid is not known, although recent studies are beginning to unravel the mystery. As mentioned previously, it is known that, upon cellular stimulation, cytosolic 5-LO becomes activated and translocates from the cytosol to the nuclear membrane.[16] We have found that, in addition to the known cellular activating factors (such as antigen stimulation and the calcium ionophore A23187) which induce translocation, the thiopyrano[2,3,4-*c,d*]indole class of 5-LO inhibitors strongly promote this translocation in the presence or absence of A23187. Also, the subsequent addition of a FLAP inhibitor (MK-886) does not reverse this process. Presumably, upon activation, 5-LO undergoes a conformational change, rendering the enzyme surface hydrophobic, whereupon it then translocates to the lipophilic membrane. In this active conformation, 5-LO is readily able to accept the sub-

Figure 2. LTC_4 synthase activity versus drug concentration: LTC_2 (●), 1 (■), **14b** (□), MK-886 (▲), MK-0591 (△), and **24** (○).

strate arachidonic acid as transferred to it by FLAP. Perhaps the thiopyrano[2,3,4-*c,d*]indole inhibitors mimic arachidonic acid and, upon binding to 5-LO, induce and stabilize the active hydrophobic conformation thus enhancing the enzyme association with the membrane and/or stabilizing it at the membrane. It is also interesting to note that, after translocation of 5-LO in the presence of the thiopyrano[2,3,4-*c,d*]indole inhibitor **23** (L-689,065), this membrane-bound enzyme retains its activity after dilution of the inhibitor (data not shown). Ordinarily, translocation to the membrane

23 (L-689,065)

results in the formation of inactive 5-LO. These results complement findings by Hill et al.[25] in which the direct 5-LO inhibitor zileuton was also able to protect 5-LO from inactivation following cellular activation. The mechanism for protection of membrane-associated 5-LO by zileuton may be different from the thiopyrano[2,3,4-*c,d*]indole inhibitor stabilization since, in the case of the zileuton experiments, MK-886 was able to release 5-LO from the membrane. However, the protective properties of the thiopyrano[2,3,4-*c,d*]indole series of inhibitors are further evidence that they are able to bind at and protect the active site in a reversible manner.

Interestingly, it has also been recently reported that LTC_4 synthase (which catalyzes the conversion of LTA_4 to LTC_4) has significant structural homology to FLAP and, indeed, is inhibitable by the FLAP inhibitor MK-886.[26] In an independent study to identify inhibitors of LTC_4, we hypothesized that, since the molecule produced by the 5-LO reaction (LTA_4) is the substrate for LTC_4 synthase, we may be able to identify inhibitors of LTC_4 synthase by screening 5-LO inhibitors. Following this reasoning, several compounds were selected and tested as potential LTC_4 synthase inhibitors using a previously described protocol.[27] The results are shown in Figure 2 and are shown in comparison with LTC_2, a metabolically stable, competitive end product inhibitor of LTC_4 synthase, used as a standard. Both thiopyrano[2,3,4-*c,d*]indoles **1** and **14b** proved to inhibit LTC_4 synthase activity in a dose-related manner with IC_{50}s of 11 and 16 μM, respectively (compared to LTC_2 with an IC_{50} of 1.2 μM). For comparison, both MK-886 and MK-0591 were shown to have approximately the same potency at 85 μM. Interestingly, the potent 5-LO inhibitor **24** (L-702,539)[28] which is a member of the lignan class of compounds did not show any inhibition up to 100 μM. The lignan inhibitors (such as **24**) have

MK-886 **MK-0591**

24

been shown to inhibit the 5-LO reaction in a manner consistent with the compounds binding competitively at the arachidonic acid-binding site. However, there is little structural homology between this series and the thiopyrano[2,3,4-*c,d*]indole series, and all attempts to prepare hybrid analogs containing the key structural features of each series have failed. Thus, it seems that compounds from the lignan series do not appear to be structural mimics for the bound conformation of arachidonic acid, and hence, perhaps it is not suprising that **24** shows no inhibitory activity against LTC_4 synthase. The results obtained in this study are consistent with the hypothesis that compounds which mimic arachidonic acid in their binding to 5-LO (or FLAP) may be capable of inhibiting LTC_4 synthase. Since the inhibition of the function of LTC_4 synthase is a potential therapeutic target for the regulation of the production of peptidoleukotrienes, the thiopyrano[2,3,4-*c,d*]indoles **1** and **14b** represent attractive lead compounds for the development of more potent inhibitors of LTC_4.

Experimental Section

Biology. All of the compounds prepared were evaluated as inhibitors of 5-LO activity using a spectrophotometric assay monitoring 5-HPETE production by the 100000*g* supernatant fraction (S100) from insect cells overexpressing human 5-LO.[29] The FLAP binding assay was carried out using human leukocyte membrane preparations and measuring the binding of drug in the presence of [^{125}I]L-691,831.[30]

Selected compounds (dosed as the sodium salts) were also evaluated in the rat to compare their *in vivo* properties. The plasma profile of each compound was obtained, and their effectiveness in a hyperreactive rat model of dyspnea was determined. Plasma levels were obtained after oral (20 mg/kg in 1% methocel) and iv (5 mg/kg in PEG 200) dosing of the respective sodium salts. Bioavailabilities were then calculated from the $AUC_{po}/4 \times AUC_{iv}$. For the hyperreactive rat model,[12] inbred rats ($n = 6$) were pretreated with methysergide (3 μg/kg iv) and the drugs administered po at 0.5 mg/kg dissolved in 1% or 0.5% methocel 2 h prior to antigen (ovalbumin) challenge. The effect was measured as the percent inhibition

Table 3. Physical Data for Compounds

compd	R	W	X	Y	Z	formula	anal.[a]	mp (°C)
7	R1	4-Cl	CH_2CH_2	H	2-CO_2H	$C_{39}H_{33}N_2O_3ClS$	C,H,N	209–211
8	R1	4-Cl	CH_2S	H	2-CO_2H	$C_{38}H_{31}N_2O_3ClS_2$	C,H,N,S	246–247
9	R1	4-Cl	CH_2CH_2S	H	2-CO_2H	$C_{39}H_{33}N_2O_3ClS_2\cdot 1.4H_2O$	C,H,N	164–167
10	R1	4-Cl	CH_2OCH_2	H	2-CO_2H	$C_{39}H_{33}N_2O_4ClS$	*b*	218.5–219
11	R1	4-Cl	CH_2CH_2O	H	2-CO_2H	$C_{39}H_{33}N_2O_4ClS\cdot 0.3H_2O$	C,H,N	195–198
12	R1	4-Cl	CH_2CH_2O	Cl	2-CO_2H	$C_{39}H_{32}N_2O_4Cl_2S$	C,H,N	170–173
13	R1	4-Cl	CH_2CH_2O	H	3-CO_2H	$C_{39}H_{33}N_2O_4ClS$	C,H,N	210–213
14a	R1	4-Cl	CH_2O	H	2-CH_2CO_2H	$C_{39}H_{33}N_2O_4ClS\cdot 0.3H_2O$	C,H,N	176–177
14b	R1	3-Cl	CH_2O	H	2-CH_2CO_2H	$C_{39}H_{33}N_2O_4ClS$	C,H,N	153–154
(+)-**14b**	R1	3-Cl	CH_2O	H	2-CH_2CO_2H	$C_{39}H_{32}N_2O_4ClSNa\cdot 1.5H_2O$	C,H,N	153.5–154
(−)-**14b**	R1	3-Cl	CH_2O	H	2-CH_2CO_2H	$C_{39}H_{33}N_2O_4ClS\cdot 1.0H_2O$	C,H,N	153–155
15a	R1	4-Cl	CH_2CH_2O	H	2-CH_2CO_2H	$C_{40}H_{35}N_2O_4ClS\cdot 0.3H_2O$	C,H,N	201–202.5
15b	R1	3-Cl	CH_2CH_2O	H	2-CH_2CO_2H	$C_{40}H_{35}N_2O_4FS\cdot 0.2H_2O$	C,H,N	196–198
15c	R1	4-F	CH_2CH_2O	H	2-CH_2CO_2H	$C_{40}H_{35}N_2O_4ClS$	C,H,N	118–125
15d	R1	3-F	CH_2CH_2O	H	2-CH_2CO_2H	$C_{40}H_{35}N_2O_4FS\cdot 0.5H_2O$	C,H,N	171.5–173.5
19	R2	4-Cl	CH_2O	H	2-CH_2CO_2H	$C_{37}H_{31}N_2O_4ClS$	C,H,N	128–130
20a	R2	4-Cl	CH_2S	H	2-CO_2H	$C_{36}H_{29}N_2O_3ClS_2$	C,H,N	218.5–220
20b	R3	4-Cl	CH_2S	H	2-CO_2H	$C_{32}H_{27}N_2O_3ClS_2\cdot 0.8H_2O$	C,H,N	137
20c	R4	4-Cl	CH_2S	H	2-CO_2H	$C_{33}H_{29}N_2O_4ClS_2\cdot 0.8H_2O$	C,H,N	206–207
20d	R5	4-Cl	CH_2S	H	2-CO_2H	$C_{36}H_{29}N_2O_3ClS_2\cdot 1.2H_2O$	C,H,N	220

[a] All elemental analyses gave results ±0.4% of the theoretical value. [b] Exact mass obtained.

of dyspnea duration compared to litter mate-matched vehicle-treated controls.

The 5-LO translocation assays were performed essentially as described by Rouzer and Kargman.[16] Briefly, human leukocytes were preincubated with compounds, diluted in DMSO, for 10 min prior to calcium ionophore A23187 challenge. Leukocytes were lysed by sonication and cytosolic and 100000*g* sedimentable membrane fractions prepared for 5-LO activity and immunoblot protein assays.

LTC_4 synthase activity was measured essentially as described previously[27] by the formation of LTC_4 in incubation mixtures containing reduced glutathione and the free acid of LTA_4. Partially purified human LTC_4 synthase (anion-exchange-active fraction, specific activity = 16.1 nmol of LTC_4 formed min^{-1} mg^{-1} [31]) was used as the enzyme source. Drugs were introduced from 100-fold concentrated stocks in DMSO, and data were calculated as LTC_4 synthase enzymatic activity expressed as a percentage of vehicle-only controls to which no drug was added.

Chemistry. Proton nuclear magnetic resonance spectra were obtained on a Bruker AM250 or AM300 spectrometer, and proton chemical shifts are relative to tetramethylsilane (TMS) as internal standard. Melting points were measured using either a Buchi 535 or an Electrothermal IA9100 melting apparatus in open capillary tubes and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN, or by Oneida Research Services, Whitesboro, NY. Where elemental analyses are reported only by symbols of the elements, results are within 0.4% of the theoretical values. The high-resolution mass spectral analyses were provided by the mass spectroscopy department of Merck, West Point, PA. All reactions were carried out under a nitrogen atmosphere, and all worked-up reaction solutions were dried using $MgSO_4$.

Compounds used for *in vivo* studies were converted to the corresponding sodium salt by treatment of a solution of the acid in water/THF with 1 equiv of 1 N NaOH. Excess THF was removed *in vacuo*, and the resulting solution was frozen and lyophilized to afford the desired sodium salt as an amorphous powder. The physical data for the final compounds prepared are give in Table 3.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]ethyl]benzoic Acid, 7. Aldehyde 4. A solution of 1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*c,d*]indole-2-methanol (1.25 g, 2.37 mmol) in 100 mL of THF was treated with 4 × 1 g (23.7 mmol total) portions of MnO_2. After 2.5 h, the mixture was filtered through Celite and the solvent removed to give **4** as a yellow solid which was used as such.

Nitrile 6. A mixture of 2-cyanobenzyl bromide (2.0 g, 10.2 mmol) and Ph_3P (3.2 g, 12.2 mmol) in CH_3CN was stirred at room temperature for 3 days under nitrogen. The mixture was concentrated to 15 mL, Et_2O added, and the precipitate collected to afford (2-cyanobenzyl)triphenylphosphonium bromide (**5**) as a white solid (4.3 g, 92%). To a suspension of **5** (916 mg, 2.0 mmol) in 20 mL of THF at −78 °C was added 4 mL of a 0.5 M solution of KHMDS in toluene. After 45 min, the aldehyde **4** (500 mg, 0.95 mmol) was added and the solution warmed to room temperature. Stirring was continued for 16 h; then the solution was poured into 1 N HCl and extracted with EtOAc (3×). The organic layer was washed with brine, dried, and evaporated and the residue chromatographed (silica gel; hexane:EtOAc, 3:2) to give **6** as a yellow solid (532 mg, 92%; 1:1 mixture of cis:trans isomers).

Acid 7. A solution of the nitrile **6** (1.28 g, 2.1 mmol) and triethylsilane (5 mL) in 40 mL of CH_2Cl_2 at room temperature was treated with 2.5 mL of $BF_3\cdot OEt_2$. The reaction mixture was stirred for 15 min and then poured into 1 N HCl, extracted three times with EtOAc, washed with brine, dried, and evaporated. The product was swished with Et_2O and filtered to give the saturated nitrile as a solid (933 mg, 73%). This nitrile (335 mg), 8 N KOH (6 mL), and 2-(ethoxyethoxy)ethanol (20 mL) were heated at 180 °C for 16 h. The solution was cooled, poured into 1 N HCl, extracted (3×) with EtOAc, washed with brine, dried, and concentrated to give a solid which was swished with EtOAc and then filtered to give **7** (205 mg, 59%).

General Procedure for Alkylation of Phenols. (+)-1-(3-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-*1H*-thiopyrano[2,3,4-*c,d*]indole-2-methanol, (+)-2b. A solution of the phenol (+)-**16** (200 mg, 0.56 mmol), 2-(chloromethyl)-5-phenylpyridine (124 mg, 0.61 mg), Cs_2CO_3 (218 mg, 0.67 mmol) in DMF (6 mL), and CH_3CN (6 mL) was stirred at 60 °C under argon for 16 h. The solution was poured into water and extracted with THF/EtOAc and then twice more with EtOAc. The combined organic layers were washed with water and then brine, dried, and evaporated to give a solid. This material was swished with ether/hexane and filtered to yield (+)-**2b** as a solid: mp 177–178 °C; $[\alpha]_D$ = +7.1° (*c* 0.82, $CHCl_3$).

Using this procedure, compounds (−)-**2b** and **18a–d** were prepared.

(−)-1-(3-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-*1H*-thiopyrano[2,3,4-*c,d*]indole-2-methanol, (−)-2b: prepared as above to give (−)-**2b** as a solid; mp 178.5–180 °C; $[\alpha]_D$ = −8.0° (*c* 0.62, $CHCl_3$).

General Procedure for the Introduction of Thiosalicylic Acid Group. 2-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-*1H*-thiopyrano-[2,3,4-*c,d*]indol-2-yl]methyl]thio]benzoic Acid, 8. A suspension of the alcohol **2a** (339 mg, 0.64 mmol) and thiosalicylic acid (151 mg, 0.98 mmol) in 1,2-dichloroethane (12 mL) was treated with $BF_3 \cdot OEt_2$ (0.12 mL, 0.98 mmol). After 10 min the heterogeneous mixture was poured into 1 N HCl and THF. The solution was extracted with EtOAc (2×), washed with brine, dried, and evaporated to give a solid which was swished with EtOAc. Chromatography of the residue (hexane:EtOAc, 1:1; loaded in DMF) afforded the acid **8** as a solid (190 mg, 44%).

Using this procedure, compounds **20a–d** were prepared.

General Procedure for the Mitsunobu Coupling and Hydrolysis. 2-[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-*1H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]methoxy]phenylacetic Acid, 14a. To a suspension of the alcohol **2a** (250 mg, 0.47 mmol), methyl 2-hydroxyphenylacetate (118 mg, 0.71 mmol), and Ph_3P (137 mg, 0.52 mmol) in THF (7 mL) was added DEAD (83 μL, 0.52 mmol), and the solution was then stirred for 30 min. The solvent was removed and the product purified on silica gel (hexane:EtOAc, 2:1) to give the corresponding ester (110 mg, 34%).

A solution of this ester (100 mg, 0.14 mmol), 0.5 mL 1 N LiOH, THF (2 mL), and MeOH (2 mL) was stirred at 50 °C for 1.5 h. The mixture was poured into 1 N HCl, extracted with EtOAc (3×), washed with water and then brine, dried, and evaporated. After a swish with ether/EtOAc, the solid was obtained by filtration to give **14a** (80 mg, 82%).

Using this procedure, compounds **11–13**, **14b**, (+)-**14b**, (−)-**14b**, **15a–d**, and **19** were prepared.

2-[[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-*1H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]ethyl]thio]benzoic Acid, 9. To a solution of **3a** (400 mg, 0.76 mmol) in CH_2Cl_2 (12 mL) were added Ph_3P (408 mg, 1.6 mmol) and CBr_4 (491 mg, 1.5 mmol). The mixture was stirred for 20 min at room temperature and then concentrated and chromatographed (5% Et_2O in CH_2Cl_2) to give the corresponding bromide (341 mg, 76%).

To a solution of thiosalicylic acid (38 mg, 0.25 mmol) in DMF (2 mL) at 0 °C was added NaH (12 mg, 0.52 mmol). After 10 min, the bromide (100 mg, 0.17 mmol) was added; the mixture was stirred for 2 h and then poured into 1 N HCl. This was extracted with EtOAc (3×), washed twice with brine, dried, and evaporated. The residue was purified by silica gel chromatography (hexane:EtOAc, 1:1; then 5% HOAc added) to give **9** (71 mg, 64%).

2-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-*1H*-thiopyrano[2,3,4-*c,d*]indol-2-yl]methoxy]methyl]benzoic Acid, 10. A suspension of **2a** (425 mg, 0.81 mmol) in DMF (15 mL) was treated sequentially with NaH (24 mg, 1.0 mmol) and then 2-cyanobenzyl bromide (198 mg, 1.0 mmol) at room temperature. After 2 h, the mixture was poured into 1 N HCl, extracted with EtOAc (3×), washed twice with brine, dried, and evaporated. The residue was triturated with Et_2O to provide the coupled product as a solid (460 mg, 89%).

This nitrile (230 mg, 0.37 mmol) was heated with 8 N KOH (1 mL), ethylene glycol (7.5 mL), and 2-(ethoxyethoxy)ethanol (2.5 mL) at 150 °C for 8 h. The mixture was cooled and EtOAc added followed by 1 N HCl. A precipitate formed which was collected by filtration and then chromatographed (hexane: EtOAc, 1:2, and then EtOAc:HOAc, 10:1) to give **10** as a solid (10 mg, 5%): FAB MS found M^+ = 660.1840, $C_{39}H_{33}N_2O_4ClS$ requires 660.1850.

X-ray Crystallography. Compound (−)-**2b**: $C_{31}H_{27}N_2O_2SCl$, M = 527.090; orthorhombic, $P2_12_12$; a = 20.863(4), b = 21.272(2), and c = 11.825(3) Å; V = 5248(3) Å^3, Z = 8, D_x = 1.334 g cm^{-3}; monochromatized radiation λ(Cu K$_\alpha$) = 1.541 838 Å, μ = 2.27 mm^{-1}, $F(000)$ = 2208, T = 294 K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 72.5° which yielded 5774 unique reflections. There are 2587 unique, observed reflections (with $I \geq 3\sigma(I)$ as the criterion for being observed) out of the total measured. The structure was solved by direct methods (SHELXS-86)[32] and refined using full-matrix least-squares on F (SDP-PLUS, Structure Determination Package version 3, Enraf-Nonius, Delft, The Netherlands, 1985). The final model was refined using 667 parameters and the observed data. All non-hydrogen atoms were refined with anisotropic thermal displacements. The hydrogen atoms were included at their calculated positions and constrained via a riding model. The final agreement statistics are R = 0.053, R_w = 0.050, and S = 1.54 with $(\Delta/\sigma)_{max}$ = 0.96. The least-squares weights were defined using $1/\sigma^2(F)$. The maximum peak height in a final difference Fourier map is 0.24(5) eÅ^{-3}.

Acknowledgment. The authors gratefully acknowledge the following for their contributions to this work: D. Denis, M. McAuliffe, W. Grzywacz, J. F. Kaysen, and R. G. Ball.

Supporting Information Available: NMR data and analytical data for final compounds (10 pages). Ordering information can be found on any current masthead page.

References

(1) Ford-Hutchinson, A. W.; Gresser, M.; Young, R. N. 5-Lipoxygenase. *Annu. Rev. Biochem.* **1994**, *63*, 383–417.

(2) (a) Miller, D. K.; Gillard, J. W.; Vickers, P. J.; Sadowski, S.; Léveillé, C.; Mancini, J. A.; Charleson, P.; Dixon, R. A. F.; Ford-Hutchinson, A. W.; Fortin, R; Gauthier, J.-Y.; Rodkey, J.; Rosen, R.; Rouzer, C. A.; Sigal, I. S.; Strader, C.; Evans, J. F. Identification and isolation of a membrane protein necessary for leukotriene synthesis. *Nature (London)* **1990**, *343*, 278–281. (b) Dixon, R. A. F.; Diehl, R. E.; Opas, E.; Rands, E.; Vickers, P. J.; Evans, J. F.; Gillard, J. W.; Miller, D. K. Requirement of a 5-lipoxygenase activating protein for leukotriene synthesis. *Nature (London)* **1990**, *343*, 282–284.

(3) Mayatepek, E.; Hoffmann, G. F. Leukotrienes: biosynthesis, metabolism, and pathophysiologic significance. *Pediatr. Res.* **1994**, *37*, 1–9.

(4) FLAP or 5-LO inhibitors, recent clinical results: (a) Hui, K. P.; Taylor, I. K.; Taylor, G. W.; Rubin, P.; Kesterson, J.; Barnes, N. C.; Barnes, P. J. Effect of a 5-lipoxygenase inhibitor on leukotriene generation and airway responses after allergen challenge in asthmatic patients. *Thorax* **1991**, *46*, 184–189. (b) Israel, E.; Drazen, J.; Pearlman, H.; Cohn, J.; Rubin, P. A double blind multicenter study of zileuton, a potent 5-lipoxygenase inhibitor versus placebo in the treatment of spontaneous asthma in adults. *J. Allergy Clin. Immunol.* **1992**, *89*, 236–238. (c) Collawn, C.; Rubin, P.; Perez, H.; Bobadilla, J.; Cabrera, G.; Reyes, E.; Borovoy, J.; Kershenobich, D. Phase II study of the safety and efficacy of a 5-lipoxygenase inhibitor in patients with ulcerative colitis. *Am. J. Gastroenterol.* **1992**, *323*, 342–346. FLAP inhibitors, recent clinical results: (d) Friedman, B. S.; Bel, E. H.; Buntinx, A.; Tanaka, W.; Han, Y. H.; Shingo, S.; Spector, R.; Sterk, P. Oral leukotriene inhibitor (MK-886) blocks allergen-induced airway responses. *Am. Rev. Respir. Dis.* **1993**, *147*, 839–844. (e) Diamant, Z.; Timmers, M. C.; van der Veen, H.; Friedman, B. S.; De Smet, M.; Depré, M.; Hilliard, D.; Bel, E. H.; Sterk, P. J. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. *J. Allergy Clin. Immunol.* **1995**, *95*, 42–51.

(5) LTD_4 antagonists, recent clinical results: (a) Margolskee, D.; Bodman, S.; Dockhorn, R.; Israel, E.; Kemp, J.; Mansmann, H.; Minotti, D. A.; Spector, R.; Stricker, W.; Tinkelman, D.; Townly, R.; Winder, J.; Williams, V. C. The therapeutic effects of MK-

571, a potent and selective leukotriene D_4 receptor antagonist in patients with chronic asthma. *J. Allergy Clin. Immunol.* **1992**, *87*, 309 (Abstract 677). (b) Impens, N.; Reiss, T. F.; Teahan, J. A.; Desmet, M.; Rossing, T. H.; Shingo, S.; Zhang, J.; Schandevyl, W.; Verbesselt, R.; Dupont, A. G. Acute bronchodilation with an intravenously administered leukotriene D_4 antagonist, MK-679. *Am. Rev. Respir. Dis.* **1993**, *147*, 1442–1446. (c) Taylor, I. K.; O'Shaughnessy, K. M.; Fuller, R. W.; Dollery, C. T. Effect of cysteinyl-leukotriene receptor antagonist ICI 204,219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* **1991**, *337*, 690–694. (d) Finnerty, J. P.; Wood-Baker, R.; Thomson, H.; Holgate, S. T. Role of leukotrienes in exercise-induced asthma. Inhibitory effect of ICI 204219, a potent leukotriene D_4 receptor antagonist. *Am. Rev. Respir. Dis.* **1992**, *145*, 746–749. (e) O'Shaughnessy, K. M.; Taylor, I. K.; O'Connor, B.; O'Connell, F.; Thomson, H.; Dollery, C. T. Potent leukotriene D_4 receptor antagonist ICI 204,-219 given by the inhaled route inhibits the early but not the late phase of allergen-induced bronchoconstriction. *Am. Rev. Respir. Dis.* **1993**, *147*, 1431–1435. (f) Makker, H. K.; Lau, L. C.; Thompson, H. W.; Binks, S. M.; Holgate, S. T. The protective effect of inhaled leukotriene D_4 receptor antagonist ICI 204,219 against exercise-induced asthma. *Am. Rev. Respir. Dis.* **1993**, *147*, 1413–1418. (g) Fujimura, M.; Sakamoto, S.; Kamio, Y.; Matsuda, T. Effect of a leukotriene antagonist, ONO-1078, on bronchial hyperresponsiveness in patients with asthma. *Respir. Med.* **1993**, *87*, 133–138.

(6) Hutchinson, J. H.; Riendeau, D.; Brideau, C.; Chan, C.; Falgueyret, J.-P.; Guay, J.; Jones, T. R.; Lépine, C.; Macdonald, D.; McFarlane, C. S.; Piechuta, H.; Scheigetz, J.; Tagari, P.; Thérien, M.; Girard, Y. Thiopyrano[2,3,4-c,d]indoles as 5-lipoxygenase inhibitors: synthesis, biological profile, and resolution of 2-[2-[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1H-thiopyrano[2,3,4-c,d]indol-2-yl]ethoxy]butanoic acid. *J. Med. Chem.* **1994**, *37*, 1153–1164.

(7) Falgueyret, J.-F.; Hutchinson, J. H.; Riendeau, D. Criteria for the identification of non-redox inhibitors of 5-lipoxygenase. *Biochem. Pharmacol.* **1993**, *45*, 978–981.

(8) Girard, Y.; Hutchinson, J. H.; Thérien, M.; Delorme, D. U.S. Patent 5,314,900, 1994.

(9) Hutchinson, J. H.; Riendeau, D.; Brideau, C.; Chan, C.; Delorme, D.; Denis, D.; Falgueyret, J.-P.; Fortin, R.; Guay, J.; Hamel, P.; Jones, T.; Macdonald, D.; McFarlane, C. S.; Piechuta, H.; Scheigetz, J.; Tagari, P.; Thérien, M.; Girard, Y. Substituted thiopyrano[2,3,4-c,d]indoles as potent, selective and orally active inhibitors of 5-lipoxygenase. Synthesis and biological evaluation of L-691,816. Synthesis and biological evaluation of L-691,816. *J. Med. Chem.* **1993**, *36*, 2771–2787.

(10) Hutchinson, J. H.; Prasit, P.; Choo, L. Y.; Riendeau, D.; Charleson, S.; Evans, J. F.; Piechuta, H.; Ball, R. G. Development of L-689,065 – the prototype of a new class of potent 5-lipoxygenase inhibitors. *BioMed. Chem. Lett.* **1992**, *2*, 1699–1702.

(11) Boyington, J. C.; Gaffney, B. J.; Amzel, L. M. The three-dimensional structure of an arachidonic acid 15-lipoxygenase. *Science* **1993**, *260*, 1482–1486.

(12) Piechuta, H.; Ford-Hutchinson, A. W.; Letts, L. G. Inhibition of allergen-induced bronchoconstriction in hyperreactive rats as a model for testing 5-lipoxygenase inhibitors and leukotriene D_4 receptor antagonists. *Agents Actions* **1987**, *22*, 69–74.

(13) McFarlane, C. S.; Piechuta, H.; Hall, R. A.; Ford-Hutchinson, A. W. Effects of a contractile prostaglandin antagonist (L-640,-035) upon allergen-induced bronchoconstriction in hyperreactive rats and conscious squirrel monkeys. *Prostaglandins* **1984**, *28*, 173–182.

(14) Brideau, C.; Chan, C.; Charleson, S.; Denis, D.; Evans, J. F.; Ford-Hutchinson, A. W.; Fortin, R.; Gillard, J. W.; Guay, J.; Guévremont, D.; Hutchinson, J. H.; Jones, T. R.; Léger, S.; Mancini, J. A.; McFarlane, C. S.; Picket, C.; Piechuta, H.; Prasit, P.; Riendeau, D.; Rouzer, C. A.; Tagari, P.; Vickers, P. J.; Young, R. N.; Abraham, W. M. Pharmacology of MK-0591 (3-[1-(4-chlorobenzyl)-3-(t-butylthio)-5-(quinolin-2-ylmethoxy)-indol-2-yl]-2,2-dimethylpropanoic acid), a potent, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.* **1992**, *70*, 799–807.

(15) The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Rd., Cambridge CB2 1EZ, U.K. See also supporting information.

(16) Rouzer, C. A.; Kargman, S. Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A23187. *J. Biol. Chem.* **1988**, *263*, 10980–10988.

(17) Woods, J. W.; Evans, J. F.; Ethier, D.; Scott, S.; Vickers, P. J.; Hearn, L.; Heibein, J. A.; Charleson, S.; Singer, I. I. 5-Lipoxygenase and 5-lipoxygenase-activating protein are located in the nuclear envelope of activated human leukocytes. *J. Exp. Med.* **1993**, *178*, 1935–1946.

(18) Abramovitz, M.; Wong, E.; Cox, M.; Richardson, C. D.; Li, C.; Vickers, P. J. 5-Lipoxygenase-activating protein stimulates the utilization of arachidonic acid by 5-lipoxygenase. *Eur. J. Biochem.* **1993**, *215*, 105–111.

(19) Gillard, J. W.; Ford-Hutchinson, A. W.; Chan, C.; Charelson, S.; Denis, D.; Foster, A.; Fortin, R.; Leger, S.; McFarlane, C. S.; Morton, H.; Piechuta, H.; Riendeau, D.; Rouzer, C. A.; Rokach, J.; Young, R. N.; MacIntyre, D. E.; Peterson, L.; Bach, T.; Eirmann, G.; Hopple, S.; Humes, J.; Leull, S.; Metzger, J.; Meuer, R.; Miller, D. K.; Opas, E.; Pacholok, S. L-663,536 (MK-886) (3-[1-(4-chlorobenzyl)-3-t-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.* **1989**, *67*, 454–464.

(20) Gorenne, I.; Labat, C.; Gascard, J. P.; Norel, X.; Müller-Peddinghaus, R.; Mohrs, K.-H.; Taylor, W. A.; Gardiner, P. J.; Brink, C. (R)-2-[4-Quinolin-2-ylmethoxy)phenyl]-2-cyclopentyl] acetic acid (BAY X1005), a potent leukotriene synthesis inhibitor: effects on anti-IgE challenge in human airways. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 868–872.

(21) Kreft, A. F.; Marshall, L. A.; Wong, A. Structure-activity relationships in the quinoline-containing class of inhibitors of 5-lipoxygenase (5-LO) enzyme translocation and activation. *Drugs Furture* **1994**, *19*, 255–264.

(22) Vickers, P. J.; Adam, M.; Charleson, S.; Coppolino, M. G.; Evans, J. F.; Mancini, J. A. Identification of amino acid residues of 5-lipoxygenase-activating protein essential for the binding of leukotriene biosynthesis inhibitors. *Mol. Pharmacol.* **1992**, *42*, 94–102.

(23) Charleson, S.; Evans, J. F.; Léger, S.; Perrier, H.; Prasit, P.; Wang, Z.; Vickers, P. J. Structural requirements for the binding of fatty acids to 5-lipoxygenase-activating protein. *Eur. J. Pharmacol.* **1994**, *267*, 275–280.

(24) (a) Hatzelmann, A.; Fruchtmann, R.; Mohrs, K. H.; Matzke, M.; Pleiss, U.; Keldenich, J.; Müller-Peddinghaus, R. Mode of action of the leukotriene synthesis (FLAP) inhibitor BAY X1005: implications for biological regulation of 5-lipoxygenase. *Agents Actions* **1994**, *43*, 64–68. (b) Hatzelmann, A.; Goosens, J.; Fruchtmann, R.; Mohrs, K.-H.; Raddatz, S.; Müller-Peddinghaus, R. Inversley-correlated inhibition of human 5-lipoxygenase activity by BAY X1005 and other quinoline derivatives in intact cells and a cell-free system – implications for the function of 5-lipoxygenase activating protein. *Biochem. Pharmacol.* **1994**, *47*, 2259–2268. (c) Kast, R.; Fruchtmann, R.; Mohrs, K.-H.; Raddatz, S.; Müller-Peddinghaus, R.; Hatzelmann, A. Role of 5-lipoxygenase-activating protein in the regulation of 5-lipoxygenase activity in human neutrophils. *Agents Actions* **1994**, *41*, C166–C168.

(25) Hill, E.; Maclouf, J.; Murphy, R. C.; Henson, P. M. Reversible membrane association of neutrophil 5-lipoxygenase is accompanied by retention of activity and a change in substrate specificity. *J. Biol. Chem.* **1992**, *267*, 22048–22053.

(26) Lam, B. K.; Penrose, J. F.; Freeman, G. J.; Austen, K. F. Expression cloning of a cDNA for human leukotriene C_4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A_4. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7663–7667.

(27) Nicholson, D. W.; Klemba, M. W.; Rasper, D. M.; Metters, K. M.; Zamboni, R. J.; Ford-Hutchinson, A. W. Purification of human leukotriene C_4 synthase from dimethylsulfoxide-differentiated U937 cells. *Eur. J. Biochem.* **1992**, *208*, 725–734.

(28) Chauret, N.; Li, C.; Ducharme, Y.; Trimble, L. A.; Yergey, J. A.; Ramachandran, C.; Nicoll-Griffith, D. A. In vitro and in vivo biotransformations of the naphthalenic lignan lactone 5-lipoxygenase inhibitor, L-702,539. *Drug Metab. Depos.* **1995**, *23*, 65–71.

(29) Denis, D.; Falgueyret, J.-P.; Riendeau, D.; Abramovitz, M. Characterization of the activity of purified recombinant human 5-lipoxygenase in the absence and presence of leukocyte factors. *J. Biol. Chem.* **1991**, *266*, 5072–5079.

(30) Charleson, S.; Prasit, P.; Léger, S.; Gillard, J. W.; Vickers, P. J.; Mancini, J. A.; Charleson, P.; Guay, J.; Ford-Hutchinson, A. W.; Evans, J. F. Characterisation of a 5-lipoxygenase-activating protein binding assay: correlation of affinity for 5-lipoxygenase-activating protein with leukotriene synthesis inhibition. *Mol. Pharmacol.* **1992**, *41*, 873–879.

(31) Nicholson, D. W.; Ali, A.; Vaillancourt, J. P.; Calaycay, J. R.; Mumford, R. A.; Zamboni, R. J.; Ford-Hutchinson, A. W. Purification to homogeneity and N-terminal sequence of human leukotriene C_4 synthase: A homodimeric glutathione S-transferase composed of 18 kDa subunits. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2015–2019.

(32) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467–473.

JM9506130