Journal of Medicinal Chemistry Article

J. Med. Chem. **2009,** *52,* 5803–5815 **5803**
DOI: 10.1021/jm900945d

5-Lipoxygenase-Activating Protein Inhibitors: Development of 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (AM103)

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Received June 26, 2009

The potent and selective 5-lipoxygenase-activating protein leukotriene synthesis inhibitor 3-[3-*tert*-butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid (**11j**) is described. Lead optimization was designed to afford compounds with superior *in vitro* and *in vivo* inhibition of leukotriene synthesis in addition to having excellent pharmacokinetics and safety in rats and dogs. The key structural features of these new compounds are incorporation of heterocycles on the indole N-benzyl substituent and replacement of the quinoline group resulting in compounds with excellent *in vitro* and *in vivo* activities, superior pharmacokinetics, and improved physical properties. The methoxypyridine derivative **11j** has an IC_{50} of 4.2 nM in a 5-lipoxygenase-activating protein (FLAP) binding assay, an IC_{50} of 349 nM in the human blood LTB_4 inhibition assay, and is efficacious in a murine ovalbumin model of allergen-induced asthma. Compound **11j** was selected for clinical development and has successfully completed phase 1 trials in healthy volunteers.

Introduction

Leukotrienes (LTs[a]) are pro-inflammatory lipid mediators derived from arachidonic acid that play important roles in a number of biological processes.[1] Arachidonic acid is converted to leukotriene A_4 (LTA_4) in a two-step process mediated by the enzyme 5-lipoxygenase (5-LO) (Figure 1). The initial step is the oxygenation of arachidonic acid to form 5(*S*)-hydroperoxy-6,8,11,14(*E*,*Z*,*Z*,*Z*)-eicosatetraenoic acid (5-HPETE) followed by dehydration to produce the unstable epoxide LTA_4. LTA_4 is converted to either LTB_4 by LTA_4 hydrolase or to LTC_4 through conjugation with glutathione mediated by LTC_4 synthase. Subsequent amide bond cleavage by γ-glutamyl transferase converts LTC_4 to LTD_4, and then dipeptidase action removes glycine to form LTE_4. Collectively, LTC_4, LTD_4, and LTE_4 are known as the cysteinyl leukotrienes (cysLTs) or, previously, as the slow reacting substance of anaphylaxis (SRS-A). In cells, the initial oxidation/dehydration step to produce LTA_4 is a process that requires the intimate involvement of both 5-LO and the membrane bound 5-lipoxygenase-activating protein (FLAP).[2] Thus, inhibition of either FLAP or 5-LO results in the inhibition of all LT production. LTB_4 is a high affinity ligand for the G protein-coupled receptors (GPCRs) BLT_1 and BLT_2. Stimulation of these receptors results in a number of biological activities including neutrophil and eosinophil chemotaxis and activation of inflammatory responses. The cysLTs activate at least two GPCRs, namely, $CysLT_1$ and $CysLT_2$, and this activation results in bronchoconstriction, airway edema, and hypersecretion of mucus. The $CysLT_1$ receptor is expressed on airway smooth muscle cells and on immune inflammatory cells, such as eosinophils, monocytes, and pregranulocytic CD34+ cells.[3] The second CysLT receptor ($CysLT_2$) is expressed in the human heart and on immune inflammatory cells, such as eosinophils, monocytes, and macrophages.[4] Recently, GPR17 has been reported to bind LTC_4 and LTD_4 at a site distinct from the uracil nucleotide binding site. GPR17 is highly expressed in the brain, heart, and kidney.[5] There is also strong evidence for the existence of additional receptors that preferentially bind LTE_4, the most abundant cysLT in biological fluid. The human $P2Y_{12}$ receptor has been shown to bind LTE_4 as an agonist with an EC_{50} of 1.3 nM.[6] In addition, using $CysLT_1R$/$CysLT_2R$ double knockout mice, Austen et al. have shown that intradermal injection of LTE_4 elicits a vascular leak response that exceeds the response derived through the intradermal injection of either LTC_4 or LTD_4.[7] This response is independent of the $P2Y_{12}$ receptor and, presumably, is through an as yet unidentified receptor termed the $CysLT_E$ receptor.

Antagonism of the $CysLT_1$ receptor or inhibition of 5-LO has been shown to be effective methods for the treatment of asthma.[8] Montelukast (**1**, a $CysLT_1$ antagonist; Figure 2) dominates the antileukotriene market because of an excellent

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[a] Abreviations: LT, leukotriene; 5-LO, 5-lipoxygenase; FLAP, five-lipoxygenase activating protein; 5-HPETE, 5(*S*)-hydroperoxy-6,8,11,14(*E*,*Z*,*Z*,*Z*)-eicosatetraenoic acid; cysLT, cysteinyl leukotriene; CVD, cardiovascular disease; MI, myocardial infarction; TAO, troleandomycin; hLA, human leukocyte assay; hWBA, human whole blood assay; OVA, ovalbumin; BAL, bronchoalveolar lavage; EPO, eosinophil peroxidase; GSH, glutathione; TDI, time-dependent inhibition.

Published on Web 09/09/2009

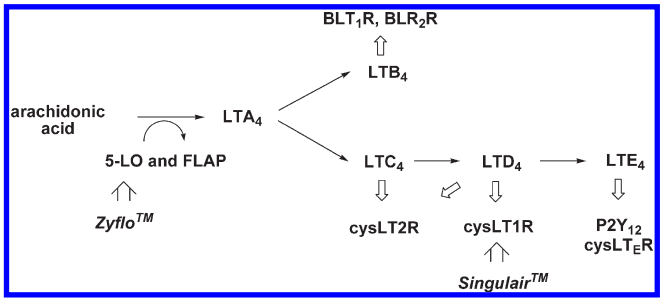


Figure 1. Leukotriene pathway.

safety profile, low therapeutic dose, and once-a-day dosing regimen.[9] While zileuton (**2**, a 5-LO inhibitor) inhibits both cysLTs and LTB_4, it has a low but significant incidence of hepatotoxicity and requires liver function testing. In addition, the high dose and poor pharmacokinetic profile (dosed at 600 mg qid or 1200 mg BID) has resulted in this compound not being widely prescribed. In several clinical trials, it has been shown that FLAP inhibitors are also efficacious in the allergen challenge and asthma trials; however, no FLAP compounds have made it to market.[10] The most potent FLAP inhibitor, **3** (MK-591), showed clinical efficacy and oral activity in phase 2 trials in asthmatics, but further development was halted in favor of the development of **1**.[11,9]

The deCODE group, studying Icelandic disease gene links, announced a strong genetic linkage of both the FLAP and LTA_4 hydrolase genes to the incidence of myocardial infarction (MI) and stroke.[12,13] The linkage of FLAP with cardiovascular disease (CVD) and stroke has also been confirmed by a number of groups using different populations.[14] These genetic data validate earlier work in animal models demonstrating that LTs play an important role in CVD. For example, Folco et al. demonstrated the protective effects of **4** (formerly BAY X1005, now DG031; a FLAP inhibitor) in a rabbit model of acute MI.[15] Further evidence of the link of 5-LO and CVD is provided by 5-LO knockout mouse studies that showed that 5-LO KO mice fed on a high-fat, high cholesterol diet are protected from the development of atherosclerosis and the formation of aortic aneurysms.[16,17] The small molecule FLAP inhibitor **5** (MK-886) has been shown to decrease atherosclerosis in apoE/LDLR-double knockout mice.[18] Clinical evidence derived from the analysis of human atherosclerotic plaque demonstrates that the expression of LT pathway proteins is highly correlated with the extent of disease.[19] These findings have prompted the investigation of **4** for human CVD, and a phase 2b trial successfully demonstrated that a FLAP inhibitor can reduce biomarkers implicated in the progression of CVD.[20] Similarly, patients taking the 5-LO inhibitor **6** (VIA2291; formerly known as ABT761) have been shown to have reduce biomarkers of CVD, and they show statistically significant lower plaque volumes when compared to those of patients taking placebo in a phase 2 trial in acute coronary syndrome.[21]

The clinical and commercial success of **1** and the reemergence of the importance of LTs in vascular disease have reawakened interest in the development of novel molecules to intervene in the LT pathway. Thus, compounds that inhibit FLAP and/or 5-LO hold the promise of improved therapies for the treatment of respiratory diseases as well as novel anti-inflammatory therapies for CVD. In this article, we describe the design of a new series of potent FLAP inhibitors leading to the identification of 3-[3-*tert*-butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid (**11j**, **AM103**) as a development candidate.

Chemistry. Compounds prepared in this study (compounds **11b**−**v**) were synthesized using five general routes A to E, and the structures of the compounds prepared and their route of synthesis are given in Tables 1 and 4. As shown in Scheme 1, benzylation of hydrazine **7** followed by a Fisher-indole synthesis reaction using the known ketone ethyl 5-*tert*-butylsulfanyl-4-oxo-2,2-dimethylpentanoate and subsequent demethylation afforded indole **8** in 38% yield for three steps.[22] The phenol was alkylated with 2-picolylchloride using standard conditions (59%; Route A) and the benzyl halide then quantitatively transformed into the corresponding pinacol-boronate derivative **9** using bis(pinocolato)-diborane and $PdCl_2$(dppf) as catalyst. Cross-coupling methodologies ($Pd(Ph_3P)_4$, K_2CO_3 in DME−water) using a variety of arylhalides provided compounds of structure **10** in generally high yields (70−95%.) Alternatively, compound **10** can be prepared from **8** following Route B. Thus, alkylation of the phenol as before followed by coupling of the benzylhalide with a range of commercially available heteroarylboronic acids (or heteroaryl-pinacolboronates) using the conditions described above yielded compound **10**. Standard ester hydrolysis conditions then afforded **11**.

Route C involved direct N-alkylation of the NH indole **14** as shown in Scheme 2. Phenol **12** was converted to the known hydrazine **13** in 3 steps (53% overall yield), and this was converted in 61% yield to indole **14** using the standard Fisher-indole synthesis described for Scheme 1.[23] Alkylation of indole **14** using NaH in DMF and the appropriate benzylbromide (or mesylate) followed by hydrolysis of the ester provided the target compounds **11**. Installation of a 3-$(OH)_2$B-benzyl group followed by coupling with 2-methoxy-5-bromopyridine allowed for the preparation of the meta-substituted analogue **11l**. The sulfoxide and sulfone derivatives were prepared using Route D as shown in Scheme 3. The bromobenzyl intermediate **8** was converted to the pinacol-boronate and then cross-coupled using the conditions described above to yield **15a**. Oxidation using 1 equivalent of *m*-CPBA in CH_2Cl_2 provided sulfoxide **15b** and oxidation with 2 equivalents of *m*-CPBA provided the sulfone **15c**. Alkylation of the phenol and hydrolysis then gave the target sulfoxide **11m** or target sulfone **11n**.

Compounds containing 3-acylindoles or 3-alkylindoles were prepared using Route E according to the procedures described in Scheme 4. Removal of the *t*-butylthio group from the **11b** ethyl ester was achieved using moist $AlCl_3$ in CH_2Cl_2 to give **16a.**[24] A Friedel−Crafts acylation installed the 3-acyl substituent (**16b**), and this acyl group was then reduced to the saturated analogue (**16c**) using $NaBH_4$ and TFA in CH_2Cl_2. Hydrolysis of **16a**, **b**, or **c** then provided the target compounds **11o**−**r**.

Biology. All compounds were assayed in a FLAP binding assay using membranes prepared from human polymorphonuclear cells following a previously described protocol with minor modifications.[25] The ligand used was tritiated 3-[5-(pyrid-2-ylmethoxy)-3-*tert*-butylthio-1-benzyl-indol-2-yl]-2,2-dimethylpropionic acid (**11**, R^1 = H; American Radiolabeled Chemicals, St Louis, MO). Compounds were also tested in a cell based assay (hLA) using human leukocytes to measure the inhibition of LTB_4 production following a calcium ionophore (A23817) challenge. To investigate the

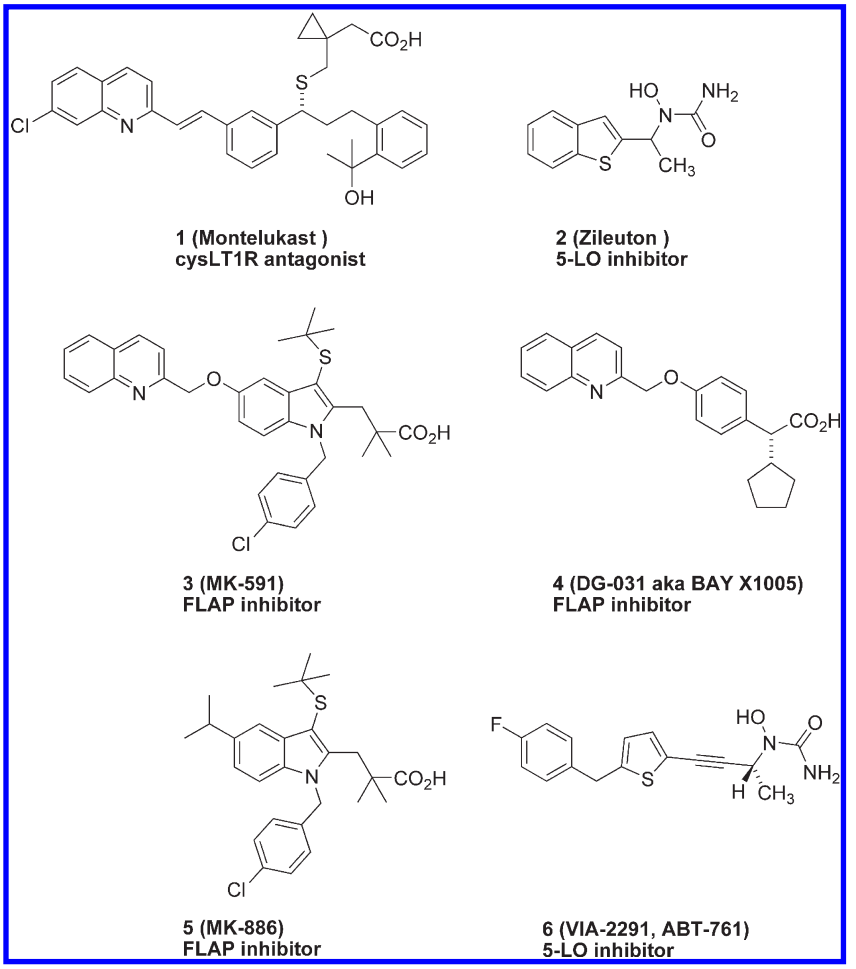


Figure 2. Structures of leukotriene pathway modulators.

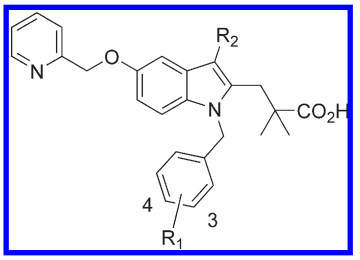
effect of blood protein binding on potency, compounds were tested in a human whole blood assay to measure the inhibition of LTB_4 production following A23817 challenge.[11]

Results and Discussion

We decided to prepare a FLAP inhibitor that addressed the potential weaknesses of **3**, a compound that has proven efficacy in human clinical trials for asthma. Compound **3** was efficacious in phase 2 asthma trials as a 125 mg twice-a-day compound, but it has been reported to cause a low incidence of a mild reversible maculopapular rash in humans.[26] Although **3** binds FLAP with high affinity (see Table 1), it suffers from a large protein shift in biological fluids as shown by the significantly weaker potency of inhibiting LTB_4 production in human whole blood (**3** IC_{50} = 0.550 μM). Additionally, it has been reported that the formulation of **3** is difficult as the crystalline form of the sodium salt is not bioavailable, and during the course of our studies, we found that it was a modest inhibitor of CYP3A4 and a potent inhibitor of CYP2C9 (5.2 μM and 0.5 μM, respectively; Table 2), the two most common CYP-isoforms.[27] Thus, a more soluble FLAP inhibitor devoid of CYP inhibition issues with improved potency and pharmacokinetics suitable for once-a-day dosing in humans could be of major clinical benefit for the treatment of respiratory and cardiovascular diseases.

Attempts to establish an *in vivo* rat model to screen for the potential of FLAP inhibitors to cause an idiosyncratic drug-induced rash were unsuccessful.[28] Therefore, we decided to focus on specific pharmacophores that we hypothesized may be responsible for this side effect. Quinoline-containing compounds can undergo CYP-mediated formation of an unstable arene oxide that can either be opened by epoxide-hydrolase to give the 5,6-diol or, alternatively, be opened by a nucleophile such as GSH.[29] Thus, bioactivation of the quinoline group followed by *in vivo* labeling of protein is one potential cause of the rash. Compound **3** differs from **5** in that it possesses a quinoline group, and no rashes were observed for **5**, although the number of subjects exposed to the drug was limited. However, in contrast, **4** is a FLAP inhibitor that contains a similarly substituted quinoline group, and no clinical experience of rash has been reported for this compound. To eliminate this possibility, we chose to focus on the pyrid-2-ylmethyl group as a quinoline replacement. The pyridyl analogue **11a** is equipotent to **3** in FLAP binding (Table 1) and is slightly more potent in the blood assay (512 nM). In order to design compounds with improved inhibition of LTB_4 in whole blood potency and an improved CYP profile and solubility, we decided to investigate the effects of introducing heterocyclic groups into the molecule. We hypothesized that substitution on the N-benzyl group may be an appropriate place in which to place heterocyclic substituents; therefore, we chose to focus part of our SAR efforts on this. In addition, we investigated the effect of combining these N-benzyl modifications with a variety of 3-indole substituents. Compounds were assayed in a human FLAP binding assay to establish the intrinsic affinity of compounds. We used the human leukocyte assay to provide the potency of the

Table 1. SAR of FLAP Inhibitors[a]



compd	route	R_1	pos	R_2	FLAP[b] IC_{50} (nM)	hLA[c] IC_{50} (nM)	hWBA[d] IC_{50} (nM)
3					2.0 ± 0.3	0.5 ± 0.5	540 ± 270
11a		Cl	4	*S*-*tert*-butyl	1.7 ± 0.5	0.8 ± 0.3	512 (551, 472)
11b	A	thiazol-2-yl	4	*S*-*tert*-butyl	4.8 ± 5.0	1 ± 0.2	273 ± 58
11c	A	pyramid-2-yl	4	*S*-*tert*-butyl	6.9 ± 2.8	6.4 ± 3.4	1210 (1311, 1109)
11d	A	pyramid-5-yl	4	*S*-*tert*-butyl	7.7 ± 2.7	3.3 ± 1.8	1043 (890, 1196)
11e	A	pyrazin-2-yl	4	*S*-*tert*-butyl	28 ± 33	12 ± 6.5	1650 (1584, 1716)
11f	B	pyrid-3-yl	4	*S*-*tert*-butyl	9 ± 2.2	3.4 ± 2.3	370 ± 228
11g	A	6-methyl pyridazin-2-yl	4	*S*-*tert*-butyl	27 ± 10	17 ± 12	4370 (3191, 5549)
11h	A	6-methoxy pyridazin-2-yl	4	*S*-*tert*-butyl	4 ± 2.4	3.6 ± 1.8	1577 ± 874
11i	B	6-methyl pyridine-3-yl	4	*S*-*tert*-butyl	6.5 ± 2.4	1.3 ± 0.6	577 (666, 487)
11j	C	6-methoxy pyridine-3-yl	4	*S*-*tert*-butyl	4.2 ± 2.4	0.5 ± 0.7	349 ± 86
11k	A	5-methoxy pyridine-2-yl	4	*S*-*tert*-butyl	6.8 ± 3.2	4.1 ± 4.6	277 (355, 199)
11l	C	6-methoxy pyridine-3-yl	3	*S*-*tert*-butyl	5.5 ± 1.5	1.2 ± 0.4	934 (773, 1094)
11m	D	6-methoxy pyridine-3-yl	4	*S*(*O*)-*tert*-butyl	793 (128,1457)	2005 ± 59	nd
11n	D	6-methoxy pyridine-3-yl	4	$S(O)_2$-*tert*-butyl	205 ± 70	59 ± 43	3420
11o	E	thiazol-2-yl	4	H	299 (99, 500)	19 ± 4.4	5517
11p	E	thiazol-2-yl	4	C(O)-c-butyl	444 ± 111	130 ± 111	33500 (29535, 37407)
11q	E	thiazol-2-yl	4	CH_2-c-butyl	6.6 ± 3.0	1.1 ± 0.4	391 (344, 437)
11r	E	thiazol-2-yl	4	$CH_2CH_2C(CH_3)_3$	229 ± 70	5.3 ± 3.6	4860 (3806, 5909)

[a] nd, not determined; values shown are ±standard deviation. Where two values were obtained, the individual values are shown in parentheses. A single value is the result of a single assay, which is the average of results using two donors. See Experimental Section for details. [b] FLAP inhibition of ^{3}H-ligand binding to FLAP membranes. [c] hLA: inhibition of LTB_4 synthesis following ionohore challenge in human leukocytes. [d] hWB: inhibition of LTB_4 synthesis following ionophore challenge in human blood.

Table 2. Inhibition of CYP Isoforms by Selected Compounds[a]

compd	CYP3A4 IC_{50} (μM)	CYP2C9 IC_{50} (μM)	CYP2D6 IC_{50} (μM)	CYP2C19 IC_{50} (μM)	CYP1A2 IC_{50} (μM)
3	5.8	0.5	11.0	nd	nd
11a	11.5	1.0	> 30	nd	nd
11b	1.4	9.1	4.0	nd	nd
11c	> 10	> 10	> 10	nd	nd
11f	4.7	15.5	2.6	nd	nd
11h	15.1	26.9	> 30	nd	nd
11i	64%@10	10	> 10	nd	nd
11j	> 50	> 50	> 30	> 50	> 50
11k	61%@10	> 10	> 10	nd	nd

[a] nd: assay was not done.

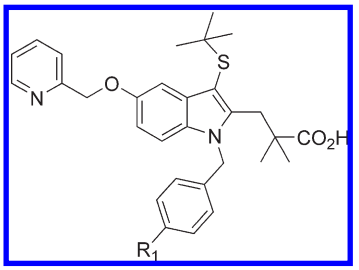
Table 3. Pharmacokinetic Parameters in Rats[a]

compd	%F	$T_{1/2}$ i.v. (h)	C_{max} (μg/mL)	AUC_{po} (h·μg/mL)	Cl (mL/min/kg)	V_{Dss} (L/kg)
11b	50%	6.3	4.3	9.54	8.6	0.71
11f	8%	7.6[b]	0.92	1.88	7	0.38
11h	42%	2.1	7.9	19.7	8.1	0.47
11j	100%	2.2	9.0	26	5.4	0.30
11k		(1.9)[c]	21.8	61.6	(2.8)	(0.51)

[a] Compounds were dosed at 2 mg/kg i.v. (water) and 10 mg/kg p.o. (0.5% methylcellulose). [b] Half-life calculated from 6 to 24 h. [c] Data extrapolated from oral dosing.

compounds in a cellular system and the human blood assay as a measure of the shift in LT synthesis inhibitor potency due to the presence of blood proteins. As can be seen from Table 1, replacement of the 4-chloro group with unsubstituted thiazol-2-yl (**11b**), pyrimidin-2-yl (**11c**), pyrimidin-5-yl (**11d**), or pyridine-3-yl (**11f**) resulted in compounds that are potent in FLAP binding (IC_{50} < 10 nM) and the leukocyte assay (IC_{50} < 10 nM). The pyrazin-2-yl analogue **11e** has a slightly lower affinity in FLAP binding with an IC_{50} of 28 nM. There are significant differences in potencies in the blood LTB_4-inhibition assay, suggesting that the presence of the heterocyclic group has a significant effect on protein binding. For example, the thiazole (**11b**) and the pyridyl (**11f**) are the most potent of these derivatives with blood LTB_4 IC_{50} values of 273 and 370 nM, respectively. Both of the pyrimidine analogues (**11c** and **11d**) as well as the pyrazine (**11e**) have IC_{50} values > 1 μM in the blood LTB_4-inhibition assay. Substituents on the heterocyclic ring also have a significant effect on potency as shown by the difference between **11g** and **11h**. The 6-methyl pyridazin-3-yl **11g** (IC_{50} of 27 nM in FLAP binding) has about 10-times lower affinity for FLAP than the 6-methoxypyridazin-3-yl analogue (**11h**; IC_{50} of 4 nM in FLAP binding). This difference is not apparent with pyridine derivatives; both the 6-methyl and 6-methoxy-pyridin-3-yl analogues (**11i** and **11j**) are equipotent in both the FLAP binding and leukocyte assays. The 6-methoxypyridin-3-yl derivative has an IC_{50} of 349 nM in the blood assay which is approximately two times more potent than **3** and **11a**. The isomer of **11j**, the 5-methoxypyridin-2-yl analogue **11k**, is even more potent in the blood assay (IC_{50} of 277 nM). The position of attachment was investigated as shown by the comparison of the 3-substituted (**11l**) and 4-substituted (**11j**) analogues. Both compounds are equipotent in the binding and leukocyte assays,

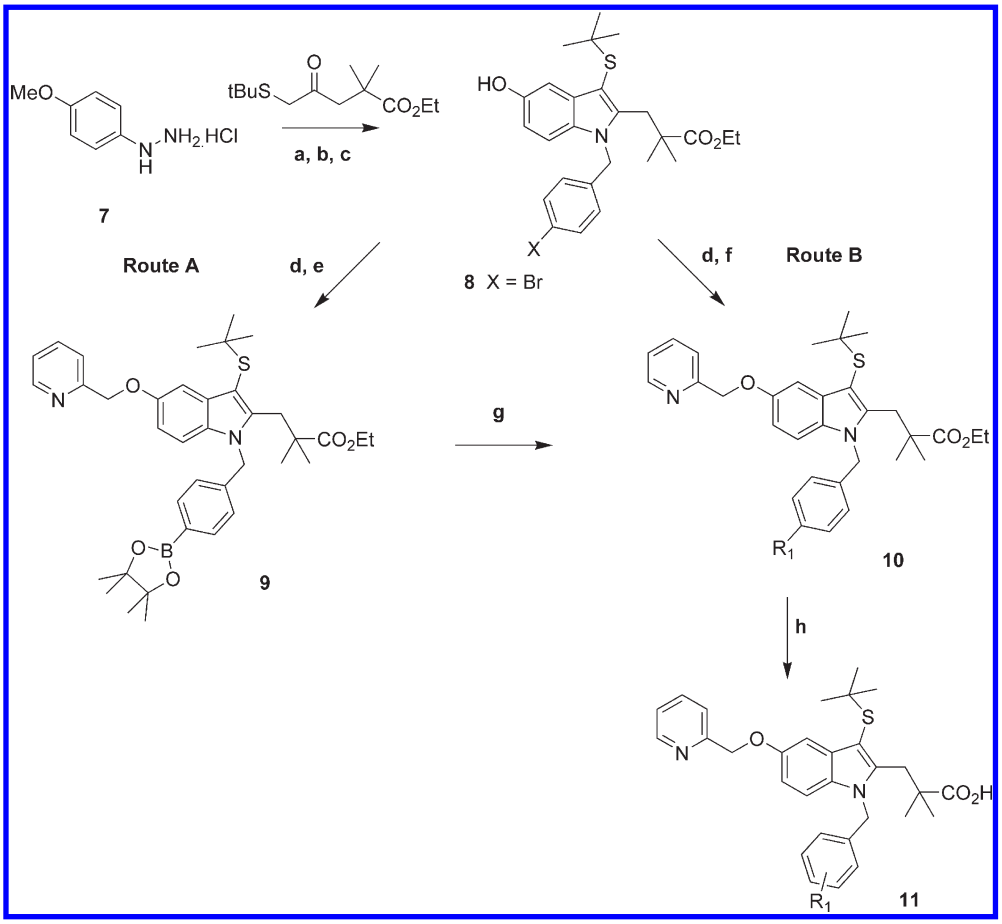
Table 4. SAR and Time-Dependent Inhibition of CYP3A4[a]



compd	Rte	R1	FLAP[b] IC_{50} (nM)	hWBA[c] IC_{50} (nM)	CYP3A4 TDI (min^{-1})
TAO[d]			nd	nd	0.039 ± 0.013
3			2.0 ± 0.3	540 ± 270	0.009
11j			4.2 ± 2.4	349 ± 86	0.015 (0.019, 0.01)
11b	A	thiazol-2-yl	4.8 ± 5.0	273 ± 58	0.033 (0.041, 0.025)
11s	C	5-methyl thiazol-2-yl	4.8 ± 1.0	527 (399,654)	0.014
11t	C	5-fluoro thiazol-2-yl	2.1 ± 0.8	150 (177, 124)	0.017
11u	A	4-methyl thiazol-2-yl	7.3 ± 0.4	1153 ± 630	0.006
11v	A	2-methoxy thiazol-4-yl	3.3 ± 0.9	255 (296, 213)	0.018 (0.021, 0.015)

[a] nd, not determined; values shown are ±standard deviation. Where two values were obtained, the individual values are shown in parentheses. For the hWB assay, a single value is the result of a single assay, which is the average of results using two donors. See Experimental Section for details. [b] FLAP inhibition of ^{3}H-ligand binding to FLAP membranes. [c] hWB: inhibition of LTB_4 synthesis following ionophore challenge in human blood. [d] TAO: troleandomycin positive control.

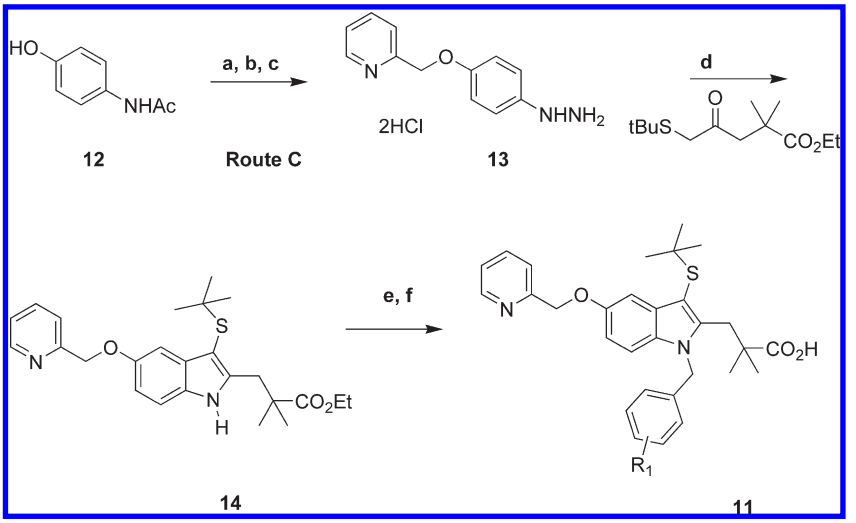
Scheme 1[a]



[a](a) 4-bromobenzylbromide, Et_3N, toluene; (b) ketone NaOAc, HOAc, toluene; (c) tBuSH, $AlCl_3$, CH_2Cl_2; (d) 2-picolylchloride.HCl, Cs_2CO_3, DMF; (e) bis(pinacolato)diborane, $PdCl_2$(dppf), KOAc, dioxane;(f) R1B-pinacol or $R^1B(OH)_2$, $Pd(Ph_3P)_4$, K_2CO_3, DME, H_2O; (g) R^1I or R^1Br, $Pd(Ph_3P)_4$, K_2CO_3, DME, H_2O; (h) LiOH, H2O, THF, MeOH.

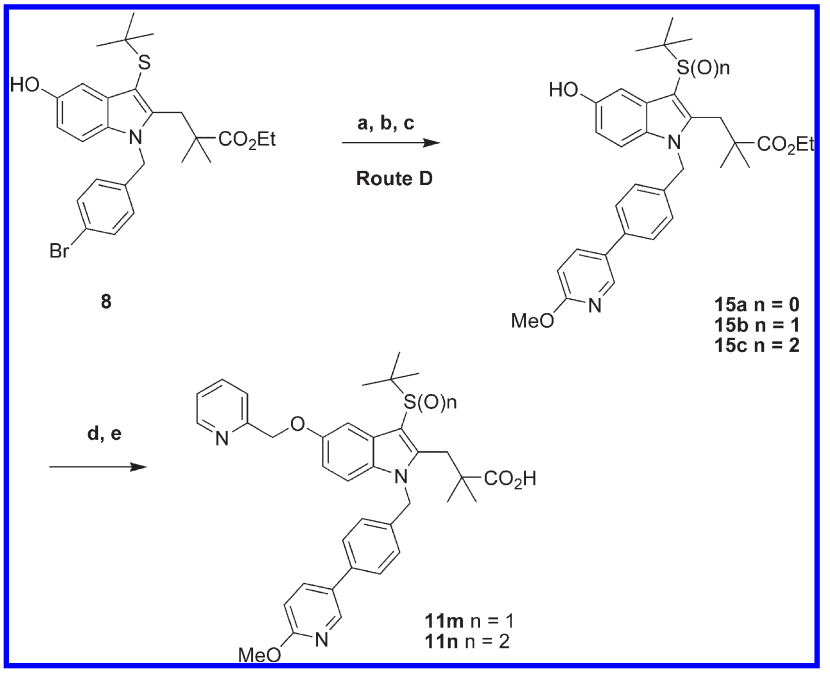
although **11l** is about 3-fold less active in the blood assay with an IC_{50} of 934 nM. 3-Substituted benzyl analogues were not pursued as they showed significant inhibition of COX-1 activity (**11l** has an IC_{50} of 420 nM in a blood COX-1 assay).[30] This cross-reactivity was not seen for the 4-substituted benzyl analogues.

Scheme 2[a]



[a](a) 2-picolylchloride·HCl, Cs_2CO_3, DMF; (b) KOH, H_2O, EtOH; (c) $NaNO_2$, H_2O, $Na_2S_2O_4$, diethyl ether, H_2O; (d) NaOAc, HOAc, toluene; (e) NaH, R^1-$C_6H_4CH_2X$, DMF; (f) LiOH, H_2O, THF, MeOH.

Scheme 3[a]

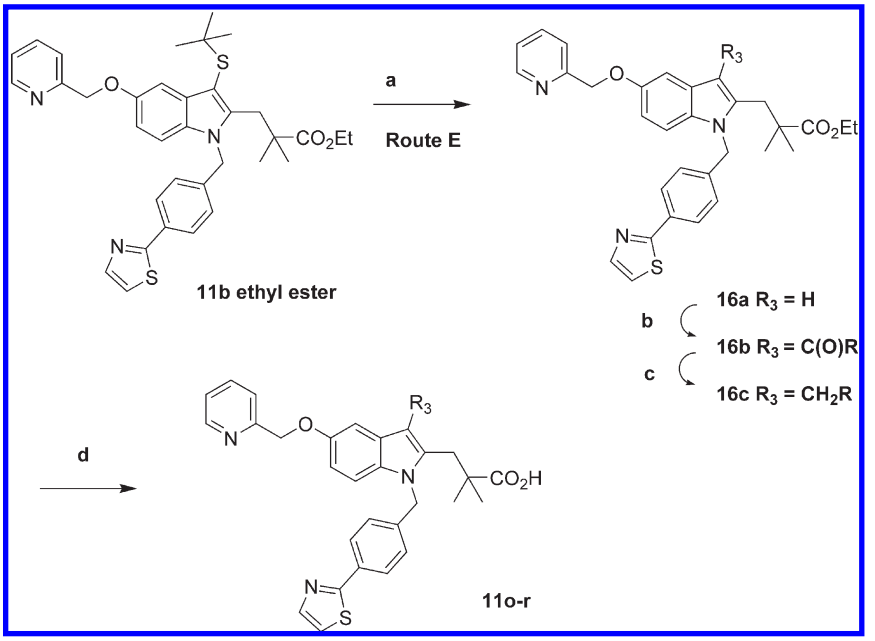


[a](a) bis(pinacolato)diborane, $PdCl_2$(dppf), KOAc, dioxane; (b) 2-methoxy-5-bromopyridine, $Pd(Ph_3P)_4$, K_2CO_3, DME, H_2O; (c) mCPBA, CH_2Cl_2; (d) 2-picolylchloride·HCl, Cs_2CO_3, DMF; (e) LiOH, H_2O, THF, MeOH.

Our studies show that compounds containing both 3-substituted and 4-substituted benzyl derivatives are potent FLAP inhibitors, and this indicates that the FLAP protein must be able to accommodate these bulky groups. After completion of this work, the X-ray crystal structure of the FLAP protein cocrystallized with **3** was published, and examination of the data confirms these observations.[31] From the structure, it is apparent that the 4-chlorobenzyl group is orientated away from the FLAP protein into the membrane space. The X-ray structure also shows that the acid group is similarly pointing into the membrane, and this is not unexpected as FLAP was originally isolated by affinity chromatography using an immobilized indole inhibitor attached to a Sephadex bead via the acid moiety.[2]

Compounds **11m**−**11r** show the results obtained from varying the 3-substituent of the indole ring. The sulfoxide **11m** is not well tolerated leading to a decrease in intrinsic potency of 300−500-fold when compared to the parent compound **11j.** Further oxidation to give the sulfone **11n** partially restores the affinity for the FLAP protein (IC_{50} = 205 nM in FLAP binding), and the whole blood potency is improved (IC_{50} of 3420 nM). Further C-3 modifications were all made on compounds containing the thiazol-2-yl benzyl N-substituents. Removal of the *S-tert*-butyl group from **11b** afforded **11o**, which was significantly less potent in the leukocyte assay (IC_{50} of 19 nM versus 1 nM for the parent compound **11b**). This loss of activity is also apparent in the blood assay. Acyl substituents were not well tolerated. For example, the cyclobutylacyl analogue **11p** has an IC_{50} of 444 nM in the FLAP binding assay and 33 μM in the blood assay. Reduction of the acyl carbonyl to the saturated

Scheme 4[a]



[a](a) $AlCl_3$, CH_2Cl_2; (b) $AlCl_3$, RC(O)Cl, CH_2Cl_2; (c) $NaBH_4$, TFA, CH_2Cl_2; (d) LiOH, H_2O, THF, MeOH.

derivative **11q** restored the activity (IC_{50} values of 6.6 nM in FLAP binding and 391 nM in blood).

On the basis of the SAR studies, compounds with potent LT synthesis inhibition were selected for further profiling. Compounds were counter-screened for their inhibitory potential against the 3 most common CYP-isoforms, and a subset of these were dosed in rats to establish their pharmacokinetic parameters. The results are shown in Tables 2 and 3. In our assay, **3** is a potent inhibitor of CYP2C9 with an IC_{50} of 0.5 μM and is also a modest inhibitor of CYP3A4 (IC_{50} 5.2 μM; Table 2). Replacement of the quinoline of **3** with a pyridine (**11a**) did not significantly affect the *in vitro* CYP profile. However, in general, the replacement of the 4-chloro group of **3** or **11a** with heterocyclic ring systems had a beneficial effect in the CYP inhibition assays. All compounds tested are significantly less potent at inhibiting the CYP2C9 isoform. For CYP3A4, the thiazol-2-yl derivative **11b** was an exception, and it is approximately 4-fold more active than **3** (IC_{50} 1.4 μM), while the remaining compounds in Table 2 are all of similar potency or are significantly less potent than **3**. The most notable compound is the methoxypyridyl **11j**, which has an excellent CYP profile against the 5 most common CYP isoforms with IC_{50} values greater than 30 μM for CYP2D6 and >50 μM for CYPs 3A4, 2C9 2C19, and 1A2.

In general, compounds in the indole propionic acid series have good pharmacokinetics in rats when dosed as the sodium salts. Compounds **11b**, **11h**, **11j**, and **11k** are all well absorbed following oral dosing at 10 mg/kg ($AUC_{po} > 10$ h·μg/mL and C_{max} ranging from 4.3 to 21.8 μM). These compounds have a low clearance rate and low volume of distribution (V_{DSS}). The pyridyl analogue **11f** is an outlier in that it performs poorly in rats following oral dosing. The bioavailability is 8%, and the C_{max} is 0.92 μM following an oral dose of 10 mg/kg. Presumably, this is due to poor absorption rather than first-pass metabolism as the i.v. parameters for **11f** are consistent with those for **11b**, **11h**, and **11j** (compound **11k** was not dosed intravenously). Compounds **11f** and **11j** are structurally similar with the only difference being the presence of a 6-methoxy group on **11j**. The methoxy group significantly alters the basicity of the pyridine, reducing it by approximately 2 log units (pK_a for pyridine is ~5.2 and for 2-methoxypyridine is 3.2).[32] The reduced basicity may be responsible for the improved oral absorption for **11j** compared to that of **11f**. A similar observation has been reported previously.[33]

Time-Dependent Inhibition of CYP3A4. During the process of profiling the most potent and bioavailable analogues, we discovered that the thiazol-2-yl analogue **11b** was a time dependent inhibitor of CYP3A4 (using midazolam as the substrate; see Table 4). Time-dependent inhibition is also referred to as mechanism-based inhibition, which usually results from irreversible binding of the test compound or one of its metabolites to the active site of a CYP enzyme during biotransformation.[34] Compound **11b** has an inhibition rate of 0.033 min^{-1}, and for comparison, the positive control in this assay is troleandomycin (TAO) with an inhibition rate of 0.039 min^{-1}. Compounds **3** and **11j** are both considered inactive in this assay with inhibition rates of <0.02 min^{-1}. It is known that thiazole groups can be metabolized to generate reactive species that may be capable of inactivating CYP3A4 or covalently labeling proteins.[35] Oxidation of **11b** by CYP3A4 followed by coupling of the reactive intermediate at the active site of CYP3A4 could be the mechanism for this time-dependent inhibition. To explore this possibility, we prepared a number of substituted thiazole derivatives. Introduction of a fluorine atom onto the thiazole ring has been shown to be a successful strategy for reducing ring oxidation metabolism.[36] When this analogue (**11t**) was prepared, it was found to have a 2- to 3-fold increase in potency in both the FLAP binding assay and the blood assay when compared to that of the parent **11b**. The fluorine did indeed slow down the rate of inactivation of CYP3A4, as the inhibition rate is 0.01 min^{-1}. Introduction of a metabolic soft spot intended to shift the position of oxidation away from the thiazole ring also proved successful. Thus, the 5-methylthiazol-2-yl derivative **11s** resulted in a compound with potency comparable to that of **11b**, and this compound was not a time-dependent inhibitor (rate = 0.014 min^{-1}). Similarly, **11u** (the 4-methylthiazol-2-yl analogue) was also shown not to be a

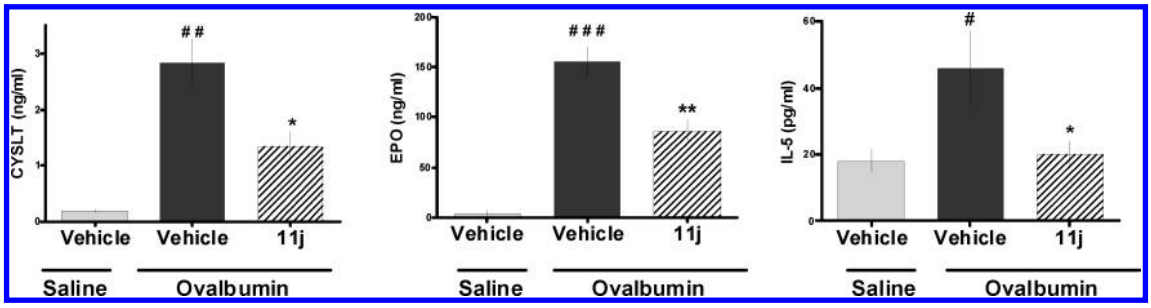


Figure 3. Intranasal ovalbumin (OVA) to OVA sensitized BALB/c mice caused an increase in BAL CysLT, eosinophil peroxidase (EPO), and IL-5. Compound **11j** (10 mg/kg q.i.d.) significantly reduced these three end points. ###$P < 0.001$, #$P < 0.05$ significant increase vs vehicle-saline; **$P < 0.01$, *$P < 0.05$ significant inhibition vs vehicle-OVA; Student−Newman−Keul's post hoc comparisons following ANOVA.

time-dependent inhibitor of CYP3A4 (rate = 0.006 min^{-1}). The 2-methoxythiazol-4-yl analogue **11v** is also not a CYP3A4 time-dependent inhibitor. In this case, the methoxy group may act as a metabolic soft spot directing the oxidation away from the thiazole ring.

Further Profiling of 11j (AM103). Compound **11j** (3-[3-*tert*-butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid) was selected for further *in vivo* testing based on the excellent *in vitro* potency in all three screening assays as well as the excellent CYP selectivity profile and rat pharmacokinetics (PK). When **11j** was dosed in dogs, it was found to have high bioavailability (64%), low clearance (2.9 mL/min/kg), low volume of distribution (0.41 L/kg), and a long i.v. half-life (5.2 h). *In vivo* efficacy for **11j** was established in a murine bronchoalveolar lavage (BAL) model. It has been shown that LTs play an important role in airway inflammation in ovalbumin (OVA)-sensitized mice, and both 5-LO inhibitors (e.g., **4**) and FLAP inhibitors (e.g., **5**) are effective in blocking airway mucus production and infiltration of eosinophils.[37] Female BALB/c mice were sensitized with OVA given by i.p. injection prior to intranasal OVA challenge. Drug treated animals were given 4 oral doses of **11j** (10 mg/kg each dose) every 6 h commencing 2 h prior to challenge. As shown in Figure 3, intranasal ovalbumin (OVA) caused an increase in BAL fluid concentrations of CysLT, eosinophil peroxidase (EPO), and IL-5. CysLTs increased from 0.18 ± 0.03 ng/mL to 2.82 ± 0.43 ng/mL. EPO increased from 4.1 ± 3.6 ng/mL to 156 ± 15 ng/mL, and IL-5 increased from 18.1 ± 3.43 pg/mL to 46 ± 11 pg/mL. Treating mice with **11j** (10 mg/kg q.i.d.) inhibited the increase in CysLTs and EPO by approximately 60%, and IL-5 levels were reduced to the concentrations obtained following saline treatment alone. Plasma drug concentrations measured at 6 h post oral dose at 10 mg/kg from a separate cohort of animals ($n = 6$) showed the average concentration of **11j** to be 406 ± 87 nM. Thus, compound **11j** is effective *in vivo* following oral administration in a murine model of allergen-induced airway inflammation.

Conclusions. The design of a series of FLAP inhibitors engineered to address the deficiencies present in compound **3** has led to the identification of (3-[3-*tert*-butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid (**11j**). Compound **11j** is a potent inhibitor of LTB_4 production in human blood, does not inhibit the five major CYP isoforms, and has excellent PK in both rats and dogs. Compound **11j** has been shown to have efficacy in a murine model of allergen-induced airway inflammation using OVA-primed and -challenged BALB/c mice. The OVA-induced increase in CysLTs, eosinophil peroxidase, and IL-5 levels measured in bronchoalveolar lavage fluid were significantly reduced by **11j**. On the basis of the overall profile, **11j** was selected for clinical development. From GLP safety assessment studies, **11j** was shown to have a significant therapeutic window based on the anticipated human therapeutic dose, and this compound has successfully completed phase 1 human clinical trials, the results of which will be reported in a separate paper.

Experimental Section

FLAP Binding Assay. Packed human polymorphonuclear cell pellets (1.8×10^9 cells) (Biological Specialty Corporation) were resuspended, lysed, and 75,000*g* membranes prepared as described.[25] The 75,000*g* pelleted membranes were resuspended in a Tris buffer (50 mM Tris HCl, pH 7.4, 1 mM EDTA, 1 mM DTT, and 30% glycerol) to yield a protein concentration of ~4 mg/mL. Then, 2.5 μg of membrane protein per well was added to 96-well deep well plates containing Tris-Tween buffer (100 mM Tris HCl, pH 7.4, 100 mM NaCl, 1 mM EDTA, 0.5 mM DTT, 5% glycerol, and 0.05% Tween-20) and ~30,000 cpm of [^3H]-3-[5-(pyrid-2-ylmethoxy)-3-*tert*-butylthio-1-benzyl-indol-2-yl]-2,2-dimethylpropionic acid and test compound in a total volume of 100 μL and incubated for 60 min at room temperature. The reactions were then harvested onto GF/B filter plates using a Brandel 96-tip harvester and washed 3× with 1 mL of ice-cold Tris-Tween buffer. The filter plates were dried, the bottoms sealed, and 100 μL of scintillant added. The plates were incubated for 1 h before reading on Perkin-Elmer TopCount. Specific binding was defined as total radioactive binding minus nonspecific binding in the presence of 10 μM MK886. IC_{50} values were determined using Graphpad prism analysis of drug titration curves.

Human Leukocyte Inhibition Assay. Blood was drawn from consenting human volunteers into heparinized tubes and 1/3 volume of 3% dextran was added. After sedimentation of red blood cells, a hypotonic lysis of remaining red blood cells was performed and leukocytes sedimented at 1200 rpm. The pellet was resuspended at 1.25×10^5cells/mL and 250 μL aliquoted into wells containing 1 μL of DMSO (vehicle) or 1 μL of drug in DMSO. Samples were incubated for 5 min at 37 °C, and 5 μL of calcium ionophore A23817 (freshly diluted from a 50 mM DMSO stock diluted to 0.5 mM in Hanks balanced salt solution) was added, mixed, and incubated for 5 min at 37 °C. Samples were centrifuged at 1,200 rpm (~300*g*) for 10 min at 4 °C, supernatant removed, and a 1:4 dilution assayed for LTB_4 concentration using ELISA (Assay Designs). Drug concentrations to achieve 50% inhibition (IC_{50} values) of vehicle LTB_4 were determined by nonlinear regression analysis (Graphpad Prism) of % inhibition versus log drug concentration.

Human Blood LTB_4 Inhibition Assay. Blood was drawn from consenting human volunteers into heparinized tubes and 150 μL aliquots added to wells containing 1.5 μL of DMSO (vehicle) or

1.5 μL of test compound in DMSO. Samples were incubated for 15 min at 37 °C. Two microliters of calcium ionophore A23817 (freshly diluted from a 50 mM DMSO stock to 1.5 mM in Hanks balanced salt solution) was added, solutions mixed, and incubated for 30 min at 37 °C. Samples were centrifuged at 1,500 rpm (~300*g*) for 10 min at 4 °C, plasma removed, and a 1:100 dilution assayed for LTB_4 concentration using ELISA (Assay Designs). Drug concentrations to achieve 50% inhibition (IC_{50} values) of vehicle LTB_4 were determined by nonlinear regression analysis (Graphpad Prism) of % inhibition versus log drug concentration. Each assay is done using two different donors; drug concentrations are singletons.

Pharmacokinetic Studies. Compounds were converted from the acid form to the sodium salt by treatment of the acid in THF/water with 1 equivalent of 1 N NaOH and then removal of the solvent *in vacuo*. Male Sprague−Dawley rats surgically cannulated in their jugular vein (approximate weight 300 g for male) were purchased from Charles River (Wilmington, MA).

Compounds were administered intravenously (i.v.) (2 mg/kg) to two or three male rats (fasted overnight) as a solution in PEG400/ethanol/water (40/10/50, v/v/v) via a bolus injection into the jugular vein (2 mg/mL; 1 mL/kg) and orally (p.o.) (10 mg/kg) to two or three male rats as a suspension in 0.5% methylcellulose via an oral gavage to the stomach (3.33 mg/mL; 3 mL/kg). Blood samples (approximately 300 uL) were taken from each rat via the jugular vein cannula at time intervals up to 24 h postdose (8−9 samples per animal). After each sample, the cannula was flushed with an equivalent volume of heparinized saline (0.1 mL at 40 units/mL). Plasma samples, prepared by centrifugation of whole blood, were stored frozen (−80 °C) prior to analysis.

Sample Preparation and Calibration Curve. Known amounts of compound were added to thawed rat plasma to yield a concentration range from 5 to 5,000 ng/mL. Plasma samples were precipitated using acetonitrile (1:5, v/v) containing the internal standard (IS) buspirone. Ten microliters of the analyte mixture was injected using a Leap PAL autosampler. The calibration curves were constructed by plotting the peak-area of analyzed peaks against known concentrations. The data were subjected to linear regression analysis with $1/x$ weighting. The lower limits of quantitation (LLOQ) were 1−5 ng/mL.

LC/MS Analysis. Analyses were performed using an Agilent Zorbax SB-C8 column (2.1 × 50 mm; 5 μm) linked to a Shimadzu LC-10AD VP with SCL-10A VP system controller. Tandem mass spectrometric (MS/MS) detection was carried out on a PE Sciex API3200 in the positive ion mode (ESI) by multiple reaction monitoring (IS 386.2 → 122.2). The mobile phases contained 10 mM ammonium acetate in water with 0.05% formic acid (solvent A) and 10 mM ammonium acetate in 50% acetonitrile/50% methanol with 0.05% formic acid (solvent B). The flow rate was maintained at 0.7 mL/min, and the total run time was 3 min. Analytes were separated using a linear gradient as follows: mobile phase was held for 1 min at 5% B, B was increased from 5% to 95% over the next 0.5 min, B was held constant for 1 min at 95%, and B was returned to the initial gradient conditions.

Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated by a noncompartmental analysis using WinNonlin (Pharsight, Mountain View, CA). Maximum plasma concentrations (C_{max}) and their time of occurrence (T_{max}) were both obtained directly from the measured data. Half-life values were calculated from 4 to 8 h unless otherwise noted.

Dog pharmacokinetic studies were performed at LAB PreClinical Research International, Inc., Laval, Quebec using female beagle dogs (n = 3 per group). Compound **11j** was given intravenously at 2 mg/kg (2 mg/mL) in 75% PEG and 25% water and also orally at 5 mg/kg (1.67 mg/mL) dissolved in 0.5% methocel. Plasma samples were shipped to Amira Pharmaceuticals and analyzed as described for rats.

Mouse Ovalbumin-Induced Lung Inflammation Procedure. Mice (female BALB/c, 20−25 g) were immunized by intraperitoneal injections (i.p.) of ovalbumin (100 μg) complexed with alum (aluminum potassium sulfate, Sigma) in a volume of 0.2 mL/mouse on days 0 and 14. Seven days later (day 21), mice were challenged with intranasal ovalbumin (50 μg in 50 μL sterile saline; 25 μL/nostril) with the aid of isoflurane anesthesia. Two hours prior to intranasal OVA and then again at 6 h intervals, mice were given an oral dose of vehicle (0.5% methocel) or **11j** (10 mg/kg). Twenty-four hours following intranasal challenge (6 h following their final oral dose), mice were overdosed with sodium pentobarbital (100 mg/kg) and instilled via a tracheal catheter with 4 × 0.3 mL phosphate buffered saline solution. The resulting lavage fluid (~ 1 mL recovered) was centrifuged at 1,200 rpm for 10 min, the supernatant removed, and split into two aliquots; one aliquot (100 μL) was mixed with equal parts ice cold methanol for protein precipitation and supernatant analysis of CysLT concentrations and the remaining fluid analyzed for IL-5 and EPO, a marker for eosinophils. CysLTs and IL-5 concentrations were determined by EIA. EPO levels were determined using an eosinophil peroxidase assay. All animal handling procedures were approved by the local Institutional Animal Care and Use Committee.

Chemistry. All procedures were carried out under a nitrogen atmosphere using commercially available solvents that were used without further purification. Starting materials were obtained from commercial sources and were used without further purification. Column chromatography was carried out using either glass columns packed with silica gel eluting with the solvents reported or using prepacked silica gel cartridges on a CombiFlash R_f separation system by Teledyne ISCO. Yields refer to chromatographically pure compounds as determined by TLC (single spot) and/or HPLC. HPLC analysis was carried out using an Agilent 1100 binary pump system using a diode array detector. The column used was Waters Symmetry C18 3.5 μm, 4.6 × 150 mm. HPLC analysis were carried out either in-house or at IRIX Pharmaceuticals Inc., Florence, South Carolina. Final products had a purity ≥95%. LC/MS analyses were carried out using a Shimadzu LCMS-2010A system fitted with a SIL-HT autosampler, LC-10AD pumps, and a SPD-10AV UV−visible detector. NMR spectra were recorded using a Bruker Advance 300 MHz NMR spectrometer. Chemical shifts are reported in δ values relative to tetramethylsilane. Combustion analysis was performed by Atlantic Microlab, Inc. Norcross, Georgia, USA. Melting points were determined by differential scanning calorimetry using a TA Instrument Q1000 by Pharmorphix Ltd., Cambridge, U.K.

Route A. 1-(Thiazol-2-yl)benzyl-3-[3-*tert*-butylsulfanyl-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11b). Step 1: 1-(4-Methoxyphenyl)-1-bromobenzylhydrazine Dihydrochloride. 4-Methoxyphenylhydrazine hydrochloride (Aldrich; 100 g, 0.57 mol), 4-bromobenzylbromide (150 g, 0.6 mol), and triethylamine (167 mL, 1.2 mol) in toluene (1.6 L) were heated at 100 °C for 3 h. The solution was cooled, diluted with ether (800 mL), and the solids removed by filtration through Celite. After removal of the solvent, the residue was taken up in EtOAc (1 L) and treated with sat HCl/EtOAc solution. The precipitate was collected by filtration and dried *in vacuo* to give the title compound as a pale cream solid (167 g, 85%).

^{1}H NMR: (MeOD) δ 3.67 (s, 3H), 3.79 (s, 3H), 4.43 (s, 2H), 6.96 (d, 2H), 7.25 (d, 2H), 7.29 (d, 2H), 7.49 (d, 2H).

Step 2: 1-Bromobenzyl-3-[3-*tert*-butylsulfanyl-5-(methoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester. The hydrazine from step 1 (167 g, 0.48 mol), ethyl 5-(*tert*-butylsulfanyl)-2,2-dimethyl-4-oxo-pentanoate (130 g, 0.53 mol), NaOAc (40 g), toluene (1.6 L), and HOAc (800 mL) were combined and stirred at RT for 4 days.[22] The solution was washed with water (4×) then with brine, dried ($MgSO_4$), and concentrated to give a black−red oil. This oil was triturated with hexane/EtOAc 20:1 and the solid collected by filtration (105 g).

The mother liquors were purified by column chromatography (silica gel; eluted with hexanes then hexane/EtOAc 4:1) to give a further 16 g of solid. Combined total of the title compound: 121 g (55%).

^{1}H NMR: ($CDCl_3$) δ 1.15 (t, 3H), 1.19 (s, 6H), 1.25 (s, 9H), 3.24 (s, 2H), 3.85 (s, 3H), 4.02 (q, 2H), 5.31 (s, 2H), 6.63 (d, 2H), 6.75 (dd, 1H), 6.97 (d, 1H), 7.25 (s, 1H), 7.32 (d, 2H).

Step 3: 1-Bromobenzyl-3-[3-*tert*-butylsulfanyl-5-(hydroxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester (8). The methoxy indole from step 2 (121 g, 0.266 mol) and *t*-butylthiol (200 mL, 1.77 mol) were dissolved in CH_2Cl_2 (400 mL) and cooled to 0 °C. $AlCl_3$ (100 g, 0.75 mol) was added in portions over 5 min, the mixture stirred for 2 h, and then poured onto ice slowly (CARE). Then, 1 N HCl (500 mL) was added and the mixture extracted with CH_2Cl_2 (2×). The organic layers were washed with water (2×), dried ($MgSO_4$), and concentrated. The residue was taken up in hexane/EtOAc 5:1 and the title compound obtained as a crystalline solid (56 g). The mother liquors were filtered through a pad of silica gel and crystallized as before to give 35 g. The residue from the mother liquors was chromatographed (silica gel; eluted with hexane/EtOAc 20:1) to give a further 5 g of solid. Combined total of the title compound **8**: 96 g (82%).

^{1}H NMR: ($CDCl_3$) δ 1.17 (t, 3H), 1.22 (s, 6H), 1.25 (s, 9H), 3.24 (s, 2H), 4.03 (q, 2H), 4.76 (s, 1H), 5.31 (s, 2H), 6.65 (d, 2H), 6.68 (dd, 1H), 6.94 (d, 1H), 7.18 (d, 1H), 7.35 (d, 2H).

Step 4: 1-Bromobenzyl-3-[3-*tert*-butylsulfanyl-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester. To a solution of phenol **8** (1.5 g; 2.9 mmol) in DMF (60 mL) was added Cs_2CO_3 (8.2 g, 25 mmol) followed by 2-picolylchloride hydrochloride (0.92 g, 5.6 mmol) and TBAB (377 mg, 1 mmol). The mixture was stirred at RT overnight under N_2 then diluted with water and extracted with MTBE (3×). The MTBE solution was dried ($MgSO_4$) and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the title compound (1.22 g, 69%).

^{1}H NMR: ($CDCl_3$) δ 1.16 (t, 3H), 1.20 (s, 15H), 3.24 (s, 2H), 4.03 (q, 2H), 5.27 (s, 2H), 5.32 (s, 2H), 6.66 (d, 2H), 6.88 (dd, 1H), 7.02 (d, 1H), 7.20 (m, 1H), 7.35 (m, 3H), 7.57 (d, 1H), 7.72 (t, 1H), 8.61 (d, 1H).

Step 5: 1-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl-3-[3-*tert*-butylsulfanyl-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester (9). A solution of the indole from step 4 (1.22 g, 2.0 mmol), bis(pinacolato)diborane (0.76 g, 3.0 mmol), $PdCl_2$(dppf) (164 mg, 0.2 mmol), and KOAc (590 mg, 6.0 mmol) in dioxane (20 mL) was degassed with N_2 in a sealable tube. The vessel was sealed and heated to 110 °C for 6 h then cooled. The mixture was poured into water, extracted with EtOAc (2×), the organic layer dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the title compound (1.35 g, 100%).

^{1}H NMR: ($CDCl_3$) δ 1.16 (t, 3H), 1.20 (s, 18H), 1.30 (s, 9H), 3.25 (s, 2H), 4.04 (q, 2H), 5.27 (s, 2H), 5.39 (s, 2H), 6.77 (d, 2H), 6.85 (dd, 1H), 7.02 (d, 1H), 7.21 (m, 1H), 7.29 (m, 1H), 7.55 (d, 1H), 7.67 (m, 3H), 8.60 (d, 1H).

Step 6: 1-(Thiazol-2-yl)benzyl-3-[3-*tert*-butylsulfanyl-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11b). A solution of **9** (1.35 g, 2 mmol), 2-bromothiazole (370 mg, 2.2 mmol), $Pd(Ph_3P)_4$ (237 mg, 0.2 mmol), and K_2CO_3 (707 mg, 51 mmol) in DME/H_2O 2:1 (20 mL) was degassed with N_2, the vessel sealed, and heated to 90 °C overnight. The mixture was poured into water, extracted with EtOAc (2×), the organic layer dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the **11b ethyl ester** (1.30 g, 100%). A solution of the **11b ethyl ester** (1.3 g, 2.12 mmol), 1 N LiOH (10 mL), MeOH (10 mL), and THF (10 mL) was stirred at reflux for 4 h. After the addition of 1 N HCl, the mixture was poured into water, extracted with EtOAc (3×), the organic layer dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided **11b** (1.01 g, 81%).

^{1}H NMR ($CDCl_3$) δ: 1.20 (15H, m), 3.31 (2H, s) 5.24 (2H, s), 5.41 (2H, s), 6.80 (3H, m), 6.96 (1H, d), 7.26 (3H, m) 7.56 (1H, d), 7.68 (3H, m), 7.82 (1H, d), 8.61 (1H, d) $[M + H]^+$: *m*/*z* calculated 586; observed 586.

The following compounds were prepared using Route A: **11c**, **11d**, **11e**, **11 g**, **11h**, **11k**, **11u**, and **11v**.

Route B. 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methylpyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11i). A solution of 1-bromobenzyl-3-[3-*tert*-butylsulfanyl-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid ethyl ester (Route A, step 4; 50 mg, 0.076 mmol), 2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (20 mg, 0.092 mmol), $Pd(Ph_3P)_4$ (9 mg, 0.0076 mmol), and K_2CO_3 (30 mg, 0.22 mmol) in DME/H_2O 2:1 (2 mL) was degassed with N_2, the vessel sealed, and heated to 85 °C overnight. The mixture was poured into water, extracted with EtOAc (2×), the organic layer dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the **11i ethyl ester** (50 mg). Hydrolysis of the ester using the procedure described for Route A, step 7 provided **11i**.

^{1}H NMR ($CDCl_3$) δ: 1.21 (9H, s), 1.28 (6H, s), 2.58 (2H, s), 3.37 (2H, s), 5.29 (3H, s), 5.46 (2H, s), 6.85 (3H, m), 7.00 (1H, d), 7.24 (1H, m), 7.36 (3H, m), 7.49 (2H, m), 7.68 (2H), 8.61 (2H, m). $[M + H]^+$: *m*/*z* calculated 594; observed 594.

Compound **11f** was prepared using Route B.

Route C. 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11j). Step 1: *N*-[4-(Pyridin-2-ylmethoxy)-phenyl]-acetamide. A mixture of 4-acetamidophenol (**12**; Sigma-Aldrich; 73.6 g; 0.488 mol), 2-chloromethylpyridine hydrochloride (80 g; 0.488 mol), and cesium carbonate (320 g; 1.0 mol) in DMF (1 L) was stirred at 70 °C for 2 days. The mixture was cooled, poured into water (2 L), and extracted with EtOAc (6×). The organic layers were washed with brine, dried ($MgSO_4$), and filtered to give a tan solid (114 g; 96%), which was used as such in the next step.

^{1}H NMR: ($CDCl_3$) δ 2.12 (s, 3H), 5.16 (s, 2H), 6.90 (d, 2H), 7.22 (dt, 1H), 7.37 (d, 2H), 7.50 (d, 1H), 7.70 (dt, 1H), 7.78 (br s, 1H), 8.58 (dd, 1H).

Step 2: 4-(Pyridin-2-ylmethoxy)-phenylamine Hydrochloride. *N*-[4-(Pyridin-2-ylmethoxy)-phenyl]-acetamide (114 g; 0.47 mol) was dissolved in EtOH (1 L), and to this was added KOH (50 g; 0.89 mol) in water (200 mL). The solution was heated to 110 °C for 2 days, and KOH (20 g, 0.35 mol, in 100 mL water) was added and heating continued for a further 2 days. The solution was cooled, the EtOH was removed *in vacuo*, and the residue partitioned between EtOAc and water. After extraction of the water with EtOAc (3×), the organic layers were washed with brine, dried ($MgSO_4$), and filtered. To this solution was added saturated HCl in EtOAc, and a precipitate formed immediately. Collection of the solids by filtration followed by drying under vacuum provided the title compound (95 g; 74%) as a pink solid.

^{1}H NMR: ($CDCl_3$) δ 3.54 (br s, 2H), 5.13 (s, 2H), 6.63 (d, 2H), 6.81 (d, 2H), 7.20 (dt, 1H), 7.51 (d, 1H), 7.68 (dt, 1H), 8.58 (dd, 1H).

Step 3: [4-(Pyridin-2-ylmethoxy)-phenyl]-hydrazine Dihydrochloride (13). 4-(Pyridin-2-ylmethoxy)-phenylamine hydrochloride (95 g, 0.35 mol) was dissolved in water (1 L) at 0 °C, and to this was added $NaNO_2$ (26 g, 0.38 mol) in water (100 mL). The diazonium salt was allowed to form over 45 min, and then it was poured slowly over 15 min into a rapidly stirred mixture of $Na_2S_2O_4$ (350 g) in water (1 L) and ether (1 L) at 0 °C. Stirring continued for 40 min, then the mixture was made basic using conc. KOH. After extraction using EtOAc (2×), the organic layers were washed with water, then brine, dried ($MgSO_4$), and filtered. To this solution was added saturated HCl in EtOAc, and a precipitate formed immediately. Collection of the solids

by filtration followed by drying under vacuum provided **13** as a tan solid (75 g; 75%).

^{1}H NMR: (MeOD) δ 4.88 (br s, 5H), 5.17 (s, 2H), 7.02 (s, 4H), 7.41 (dt, 1H), 7.62 (d, 1H), 7.93 (dt, 1H), 8.54 (dd, 1H).

Step 4: 3-[3-*tert*-Butylsulfanyl-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester (14). Compound **13** (75 g 0.26 mol), ethyl 5-(*tert*-butylsulfanyl)-2,2-dimethyl-4-oxo-pentanoate (64 g, 0.26 mol), NaOAc (40 g; 0.57 mol) in toluene (800 mL), and HOAc (400 mL) were stirred at room temperature for 3 days. The mixture was poured into water and made basic with solid Na_2CO_3. The mixture was extracted with EtOAc (3×), then washed with water (2×) and brine, dried ($MgSO_4$), filtered, and concentrated to give a dark red−black oil. Column chromatography of the mother liquor (silica gel packed in hexanes; eluting with hexane, then hexane/EtOAc 9:1 rising to 4:1) afforded 68 g (61%) of **14** as a yellow solid.

^{1}H NMR: ($CDCl_3$) δ 1.20 (s, 15H), 1.24 (t, 3H), 3.20 (s, 2H), 4.18 (q, 2H), 5.28 (s, 2H), 6.92 (dd, 1H), 7.2 (m, 3H), 7.56 (d, 1H), 7.67 (dt, 1H), 8.59 (dd, 1H), 9.09 (s, 1H).

Step 5: 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester (11j Ethyl Ester). 3-[3-*tert*-Butylsulfanyl-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic acid ethyl ester (20.0 g, 45.4 mmol) was dissolved in DMF (150 mL) and cooled to −10 °C under N_2. Sodium hydride (60% dispersion in mineral oil; 2.0 g, 50.0 mmol) was added portionwise, and the reaction was stirred at −10 °C for 45 min until the foam had disappeared. To this dark brown-reddish solution was added dropwise 4-(6-methoxy-pyridin-3-yl)-benzyloxymethanesulfonate (16.0 g, 54.5 mmol) in DMF. The reaction was then stirred at −10 °C for 1 h and allowed to slowly warm to room temperature. After 16 h, LCMS confirmed the formation of the product. The reaction was quenched with saturated NH_4Cl and diluted with MTBE and water. The aqueous phase was extracted twice with MTBE. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated, and the crude product was purified by column chromatography (silica gel packed in hexanes; eluting with hexane, then hexane/EtOAc 9:1 rising to 4:1) to give the desired product **11j ethyl ester** (21.5 g, 74%).

^{1}H NMR: (d^6-DMSO) δ 1.1 (s, 15H), 1.24 (t, 3H), 3.25 (s, 2H), 3.87 (s, 3H), 4.04 (q, 2H), 5.20 (s, 2H), 5.50 (s, 2H), 6.84 (m, 4H), 7.10 (d, 1H), 7.3 (m, 2H), 7.49 (d, 1H), 7.55 (d, 2H), 7.77 (m, 1H), 7.96 (m, 1H), 8.40 (s, 1H), 8.58 (m, 1H).

Step 6: 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11j). The **11j ethyl ester** (21.5 g, 33.7 mmol) was dissolved in THF (100 mL) and MeOH (100 mL) and stirred until it became a clear solution. Then, 3 N LiOH aqueous solution (56 mL, 168.5 mmol) was added, and the reaction was refluxed at 80 °C for 2 h. The reaction was cooled to room temperature and partitioned between EtOAc and water. The pH of the aqueous solution was adjusted to pH 1 with 10% HCl, and the aqueous phase was extracted three times with EtOAc. The combined organic layers were washed with water, dried over $MgSO_4$, filtered, and concentrated to give the desired free acid **11j**. mp 206 °C.

^{1}H NMR: ($CDCl_3$) δ 1.21 (s, 9H), 1.27 (s, 6H), 3.34 (s, 2H), 3.95 (s, 3H), 5.24 (s, 2H), 5.43 (s, 2H), 6.76 (d, 1H), 6.78 (m, 3H), 7.03 (d, 1H), 7.22 (m, 1H), 7.33 (m, 3H), 7.59 (d, 1H), 7.70 (3H, m), 8.29 (d, 1H), 8.63 (d, 1H).

Step 6: 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Sodium Salt (11j-Na Salt). mp 290 °C. Analysis for $C_{36}H_{38}N_3O_4SNa \cdot 0.5H_2O$: calcd, C, 67.48; H, 6.13; N, 6.56. Found: C, 67.52; H, 6.06; N, 6.50.

^{1}H NMR (400 MHz; d^6-DMSO): δ 0.97 (6H, s), 1.12 (9H, s), 3.21 (2H, s), 3.87 (3H, s), 5.18 (2H, s), 5.68 (2H, br s), 6.78 (1H, dd), 6.85 (1H, d), 6.93 (2H, d), 7.08 (1H, d), 7.23 (1H, d), 7.31 (1H, m), 7.5 (3H, m), 7.78 (1H, dt), 7.94 (1H, dd), 8.41 (1H, d), 8.57 (1H, d).

The following compounds were prepared using Route C: **11l**, **11s**, and **11t**.

Route D. 3-[3-*tert*-Butylsulfoxide-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11m). Step 1: 1-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl-3-[3-*tert*-butylsulfanyl-5-(hydroxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester. The bromobenzyl indole **8** (1.0 g, 1.93 mmol), bis(pinacolato)diborane (0.54 g, 2.12 mmol), $PdCl_2$(dppf) (157 mg, 0.2 mmol), and KOAc (567 mg, 5.6 mmol) in dioxane (20 mL) were degassed with N_2 in a sealable tube. The vessel was sealed and heated to 110 °C for 6 h then cooled. The mixture was poured into water, extracted with EtOAc (2×), the organic layer dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the title compound (655 mg, 60%).

^{1}H NMR: ($CDCl_3$) δ 1.16 (t, 3H), 1.21 (s, 6H), 1.24 (s, 9H), 1.27 (s, 12H), 3.25 (s, 2H), 4.05 (q, 2H), 4.92 (s, 1H), 6.65 (dd, 1H), 6.776 (d, 2H), 6.95 (d, 1H), 7.18 (d, 1H), 7.67 (d, 2H).

Step 2: 1-(6-Methoxypyrid-3-yl)benzyl-3-[3-*tert*-butylsulfanyl-5-(hydroxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester. A solution of the indole from step 1 (655 mg, 1.16 mmol), 5-bromo-2-methoxypyridine (239 mg, 1.27 mmol), Pd-(Ph_3P)$_4$ (134 mg, 0.12 mmol), and K_2CO_3 (480 mg, 3.5 mmol) in DME/H_2O 2:1 (10 mL) was degassed with N_2, the vessel sealed, and heated to 90 °C overnight. The mixture was poured into water, extracted with EtOAc (2×), the organic layer dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the title compound.

^{1}H NMR: (d^6-DMSO) δ 1.14 (m, 9H), 1.22 (s, 9H), 3.25 (s, 2H), 3.87 (s, 3H), 4.04 (q, 2H), 5.46 (s, 2H), 6.58 (m, 1H), 6.9 (m, 3H), 7.01 (s, 1H), 7.16 (d, 1H), 7.55 (m, 2H), 7.95 (m, 1H), 8.43 (d, 1H), 8.87 (s, 1H).

Step 3: 1-(6-Methoxypyrid-3-yl)benzyl-3-[3-*tert*-butylsulfoxide-5-(hydroxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid Ethyl Ester. A solution of the ester from step 2 (75 mg, 0.14 mmol) and mCPBA (70%; 34 mg, 0.14 mmol) in CH_2Cl_2 (2 mL) was stirred at 0 °C for 15 min before being quenched with sat. $NaHCO_3$. The mixture was extracted with CH_2Cl_2 (3×), dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the title compound (60 mg).

Step 4: 3-[3-*tert*-Butylsulfoxide-1-[4-(6-methoxy-pyridin-3-yl)-benzyl]-5-(pyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11m). Starting with the product from step 3 above (60 mg) and following the procedure of Route A step 4, **11m ethyl ester** (50 mg) was prepared. The ester was hydrolyzed following the procedure of Route A step 7, to afford the title compound **11m**.

^{1}H NMR ($CDCl_3$) δ: 1.3 (15H, s), 3.08 (1H, d), 3.28 (1H, d), 3.98 (3H, s), 5.36 (3H, m), 5.50 (1H, d), 6.83 (3H, m), 6.92 (1H, dd), 7.06 (1H, d), 7.36 (2H, d), 7.64 (1H, m), 7.68 (1H, m), 7.78 (1H, dd), 7.87 (1H, d), 7.94 (1H, d), 8.20 (1H, t), 8.36 (1H, d), 8.79 (1H, d). $[M + H]^+$: m/z calculated 626; observed 626.16.

Compound prepared using Route D: **11n** (using 2 equivalents of mCPBA in step 3).

Route E. 1-(Thiazol-2-yl)benzyl-3-[5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11o). H_2O (67 μL) was added to a suspension of $AlCl_3$ (663 mg, 4.87 mmol) in CH_2Cl_2 (5 mL) at room temperature and stirred for 15 min.[24] A solution of **11b** ethyl ester (381 mg, 0.62 mmol) in CH_2Cl_2 (5 mL) was added and stirred for 48 h. LC-MS showed a 1:1 mixture of starting material and the desired product. The mixture was poured into aqueous Na−K tartrate solution and then extracted with CH_2Cl_2 (3×), dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the **11o** ethyl ester (200 mg). Hydroylsis of the ethyl

ester following the procedure of Route A, step 7 provided the title compound **11o** as a solid.

^{1}H NMR ($CDCl_3$) δ: 1.32 (6H, s), 2.98 (2H, s), 5.19 (2H, s), 5.35 (2H, s), 6.39 (1H, s), 6.90 (3H, m), 6.99 (1H, d), 7.08 (1H, d), 7.28 (3H, m), 7.60 (1H, d), 7.84 (4H, m), 8.60 (1H, d). $[M + H]^+$: *m*/*z* calculated 498; observed 498.

1-(Thiazol-2-yl)benzyl-3-[3-(cyclobutylcarbonyl)-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11p). A solution of **11o** ethyl ester (207 mg, 0.39 mmol) in CH_2Cl_2 (4 mL) was treated with $AlCl_3$ (210 mg, 12 mmol) and cyclobutylacylchloride (134 μL, 10 mmol), and heated to 80 °C for 2 h. The mixture was quenched with Na−K tartrate solution then extracted with EtOAc (3×), dried ($MgSO_4$), and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the **11p** ethyl ester (110 mg). Hydroylsis of the ethyl ester following the procedure of Route A, step 7 provided the title compound **11p** as a solid.

^{1}H NMR ($CDCl_3$) δ: 0.94 (2H, m), 1.26 (2H, t), 1.37 (6H, s), 1.87 (2H, m), 2.02 (4H, m), 3.71 (2H, s), 3.89 (1H, m), 5.22 (2H, s), 5.38, (2H, s), 6.43 (2H, m), 6.92 (1H, d), 7.30 (2H, m), 7.49 (1H, m), 7.55 (3H, m), 7.62 (1H, m), 8.65 (1H, d). $[M + H]^+$: *m*/*z* calculated 580; observed 580.

1-(Thiazol-2-yl)benzyl-3-[3-(cyclobutylmethyl)-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11q). A solution of **11p** ethyl ester (93 mg, 0.15 mmol) in CH_2Cl_2 (3 mL) was added slowly to a solution of TFA (3 mL) and $NaBH_4$ (58 mg, 1.5 mmol) at 0 °C. The mixture was warmed to ambient temperature overnight, diluted with water, and then 1 N NaOH until basic. After extraction with CH_2Cl_2, the organic layer was dried ($MgSO_4$) and concentrated. Purification of the residue (silica gel; ISCO hexane/EtOAc gradient) provided the **11q** ethyl ester (110 mg). Hydroylsis of the ethyl ester following the procedure of Route A, step 7 provided the title compound **11q** as a solid.

^{1}H NMR ($CDCl_3$) δ: 1.26 (6H, s), 1.7 (4H, m), 1.93 (2H, m), 2.67 (1H, m), 2.85 (2H, d), 3.06 (2H, s), 4.14 (1H, m) 5.20 (2H, s), 5.33 (2H, s), 6.70 (2H, d), 6.81 (1H, m), 6.96 (1H, m), 7.08 (1H, s), 7.64 (1H, d), 7.74 (4H, m), 7.84 (1H, m), 8.63 (1H, m). $[M + H]^+$: *m*/*z* calculated 566; observed 566.

1-(Thiazol-2-yl)benzyl-3-[3-(3,3-dimethylbut-1-yl)-5-(pyridine-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethyl-propionic Acid (11r). Following the procedure for **11q** but using 3,3-dimethylbutanoyl chloride, the title compound **11r** was obtained as a solid.

^{1}H NMR ($CDCl_3$) δ: 0.99 (9H, s), 1.27 (6H, s) 1.50 (2H, m), 2.69 (2H, m), 3.03 (2H, s), 5.23 (2H, s), 5.33 (2H, s), 6.80 (3H, m), 6.94 (1H, d), 7.07 (1H, s), 7.24 (2H, m), 7.63 (1H, m), 7.74 (3H, m), 7.84 (1H, d), 8.62 (1H, s). $[M + H]^+$: *m*/*z* calculated 582; observed 582.

Acknowledgment. We thank Dr. Brian Stearns for help with the preparation of the tritiated FLAP ligand.

Supporting Information Available: Information for the characterization of compounds **11b**−**v**. This material is available free of charge via the Internet at http://pubs.acs.org.

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