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5-Lipoxygenase-Activating Protein (FLAP) Inhibitors. Part 4: Development of 3-[3-*tert*-Butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (AM803), a Potent, Oral, Once Daily FLAP Inhibitor

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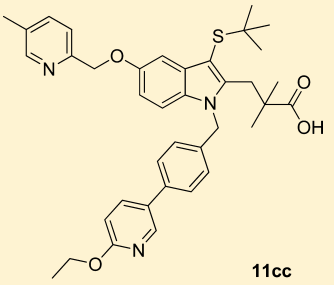
Supporting Information

ABSTRACT: The potent 5-lipoxygenase-activating protein (FLAP) inhibitor 3-[3-*tert*-butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid **11cc** is described (AM803, now GSK2190915). Building upon AM103 (**1**) (Hutchinson et al. *J. Med Chem.* **2009**, *52*, 5803−5815; Stock et al. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 213−217; Stock et al. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4598−4601), SAR studies centering around the pyridine moiety led to the discovery of compounds that exhibit significantly increased potency in a human whole blood assay measuring LTB_4 inhibition with longer drug preincubation times (15 min vs 5 h). Further studies identified **11cc** with a potency of 2.9 nM in FLAP binding, an IC_{50} of 76 nM for inhibition of LTB_4 in human blood (5 h incubation) and excellent preclinical toxicology and pharmacokinetics in rat and dog. **11cc** also demonstrated an extended pharmacodynamic effect in a rodent bronchoalveolar lavage (BAL) model. This compound has successfully completed phase 1 clinical studies in healthy volunteers and is currently undergoing phase 2 trials in asthmatic patients.



FLAP binding IC_{50} = 2.9 nM

Human whole blood IC_{50} = 76 nM

INTRODUCTION

The leukotrienes (LTs) are a family of eicosanoid bioactive lipids implicated in a variety of pathophysiological states such as asthma, allergic rhinitis, and atherosclerosis. Biosynthesis of leukotrienes is initiated through an immune, allergic, or inflammatory stimulus.[2] They are derived from arachidonic acid (AA) through the concerted action of the enzyme 5-lipoxygenase (5-LO) with 5-lipoxygenase activating protein (FLAP), initially converting AA to (5*S*,6*E*,8*Z*,11*Z*,14*Z*)-5-hydroperoxy-6,8,11,14-eicosatetraenoic acid (5-HpETE, Figure 1). Subsequent dehydration of 5-HpETE yields leukotriene A_4 (LTA_4), an unstable epoxide that is transformed to either leukotriene B_4 (LTB_4) by the enzyme LTA_4 hydrolase or leukotriene C_4 (LTC_4) via conjugation of glutathione by LTC_4 synthase. 5-HpETE can alternatively be converted to 5-oxo-ETE, a potent human chemoattractant for eosinophils, immune cells that play important roles in a variety of diseases including asthma and cardiovascular disease (CVD).[3] LTC_4 is further metabolized to leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) through sequential amide bond cleavages, and collectively these three molecules are known as the cysteinyl leukotrienes (CysLTs) or historically as the slow-reacting substance of anaphylaxis (SRS-A). The biological effects of the LTs arise through binding to their respective G-protein-coupled receptors (GPCRs).[4] LTB_4 binds to two distinct GPCRs, namely, BLT_1 and BLT_2, resulting in neutrophil and eosinophil chemotaxis.[5] The CysLTs activate at least two well-defined GPCRs, namely, $CysLT_1$,[6] and $CysLT_2$,[7] the activation of the former leading to contraction of smooth muscle (bronchoconstriction), edema, eosinophil migration, and mucus secretion. The precise role of the $CysLT_2$ receptor remains unclear, but mice deficient in this receptor indicate a role for $CysLT_2$ in vascular permeability and inflammation[8] and its expression in myocytes, fibroblasts, and vascular smooth muscle cells may point to a function within the cardiovascular system.[9] Recent evidence also points to the existence of other receptors that may preferentially bind CysLTs, namely, GPR17,[10] $P2Y_{12}$,[11] and a putative $CysLT_E$ receptor.[12]

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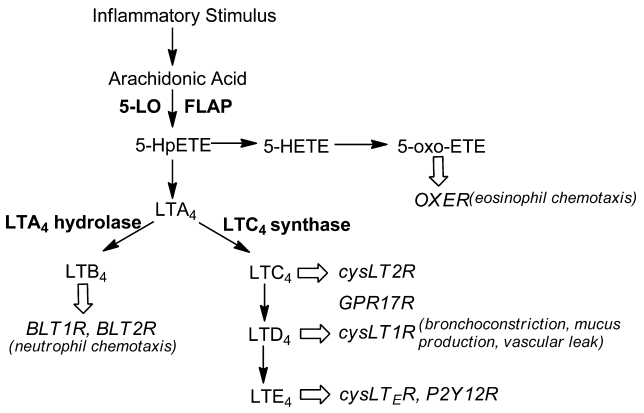

Figure 1. Leukotriene biosynthesis pathway and associated GPCRs (in italics).

Chemical interference in the LT pathway is not a novel concept, as both a 5-LO inhibitor (zileuton, **3**) and $CysLT_1$ receptor antagonists (e.g., montelukast, **2**) (Figure 2) are currently marketed for the treatment of asthma. Direct 5-LO inhibition has been an active research area for several decades, and only the iron chelator (redox inhibitor) **3** is used clinically, with no nonredox inhibitors currently on the market. However, **3** is not widely prescribed for asthma because of a high dose regimen (600 mg q.i.d. or 1200 mg b.i.d.), low but significant hepatotoxicity, and subsequent need for liver function testing.[13] **2**, a $CysLT_1$ receptor antagonist, is widely prescribed to asthmatics because of its efficacy, excellent safety profile, and low dosing requirements (10 mg q.d.). $CysLT_1$ antagonism obviously only blocks the action of this receptor and does not perturb the biosynthesis or pathophysiological action of the other LTs. Several clinical trials have evaluated FLAP inhibitors (DG-031/BAY X1005 **4**,[14] MK-886 **5**,[15] MK-591 **6**[16]) in human and shown them to be effective. For various reasons, none of these compounds have reached the market. Development of compounds **4** and **5** was likely halted because of incomplete LT synthesis inhibition at clinically tolerable doses, and compound **6** was halted after phase 2 trials in favor of the LTD_4 receptor antagonist **2**.[17]

Significant evidence also points to the potential for LT inhibitors to have a positive impact on CVD. Genetic data implicate polymorphic variants in the ALOX5AP (FLAP) gene that predispose these individuals to an increased risk of myocardial infarction and stroke (deCODE study).[18] This genetic correlation has now been confirmed in several other populations[19] and more recently associated with an increased risk for coronary arterial disease in a European−American population.[20] A murine 5LO-knockout model of CVD also supports the rationale for LT inhibition in this disease setting, as these mice were protected against the development of atherosclerotic lesions when fed a high fat, high cholesterol diet.[21] Inhibition of the FLAP protein by **5** has also been shown to reduce atherosclerosis in an apoE/LDLR-double knockout mouse model.[22] Of more significance is the observation that in humans, the 5-LO inhibitor **7** (VIA-2291, formerly known as ABT-761) reduces biomarkers of CVD and shows statistically significant reduction in the rate of plaque volume growth in patients with acute coronary syndrome.[23] The FLAP inhibitor **4** has also been shown to reduce biomarkers in a CVD setting in a phase 2b study.[24]

The significant animal and human data implicating leukotrienes in various disease states prompted us to reassess the potential of FLAP inhibitors as an anti-inflammatory treatment paradigm. Safe and potent inhibitors of FLAP would prevent the biosynthesis of all LTs and may provide an improved therapy for respiratory diseases and potentially CVD.

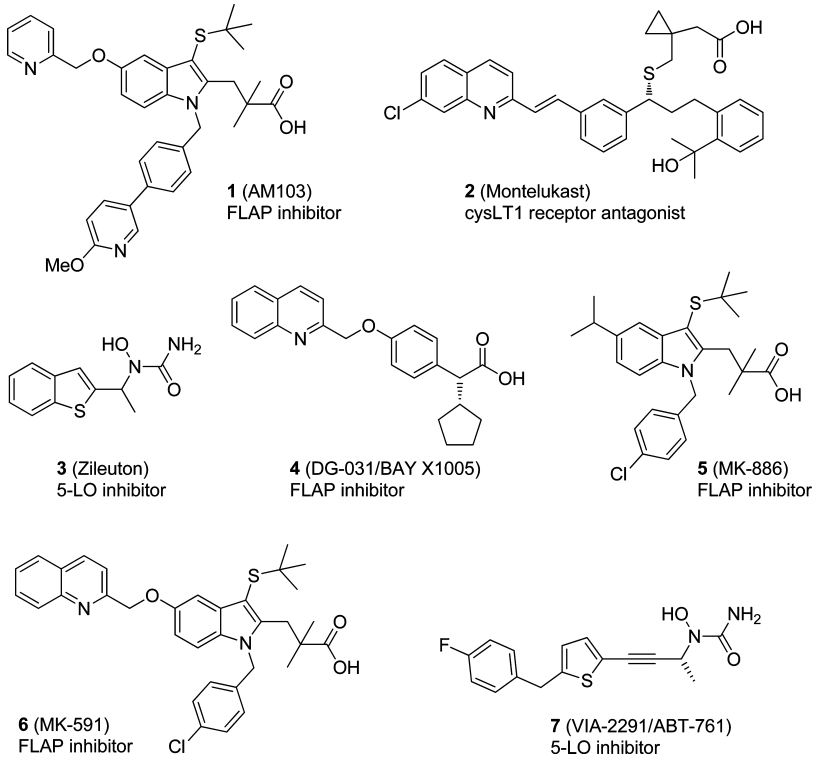

Figure 2. Structures of leukotriene pathway inhibitors.

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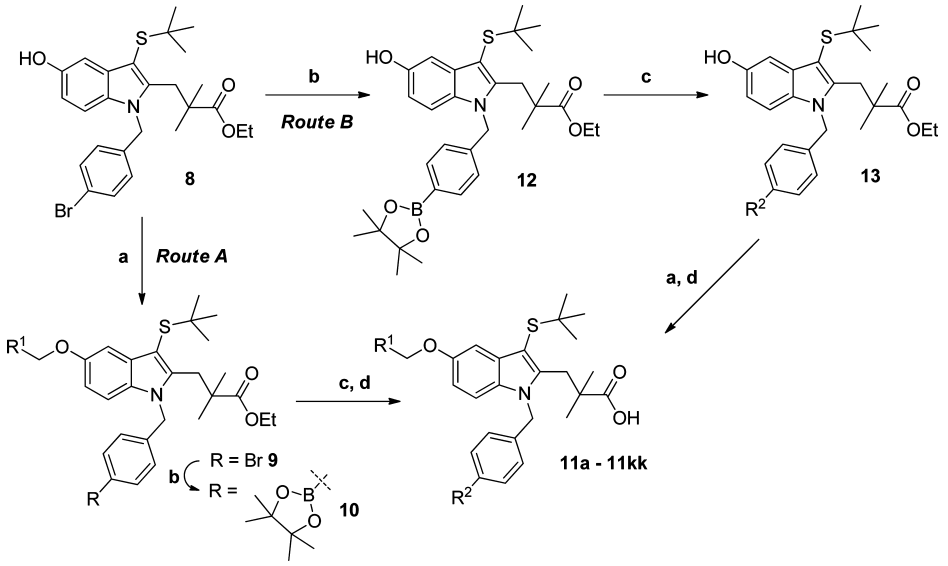
Scheme 1[a]



[a](a) R^1CH_2Br (see Scheme 2), Cs_2CO_3, MeCN or DMF, room temp to 60 °C; (b) $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (cat.), bis(pinacolato)diboron, KOAc, *p*-dioxane, 85 °C; (c) heteroaryl-X, $Pd(PPh_3)_4$ (cat.), DME/H_2O, K_2CO_3, 80−85 °C; (d) LiOH·H_2O, THF, H_2O, MeOH, 60 °C.

In this paper we detail the design of 3-[3-*tert*-butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid **11cc** (AM803, now GSK2190915), a potent derivative of our recently disclosed FLAP inhibitor AM103 **1**.[1] A key part in the discovery of **11cc** was the finding that certain compounds exhibit a heretofore unknown time-dependent increase in potency when assayed for LTB_4 inhibition in a human blood assay (hWB). We herein discuss our findings, the SAR around this time-dependent phenomenon, and the ultimate identification of **11cc** as a once a day, orally bioavailable FLAP inhibitor that has successfully finished phase 1 clinical trials and is currently in phase 2 trials in asthmatic patients.

CHEMISTRY

The compounds prepared in this study were synthesized predominantly through two synthetic routes, A and B, as depicted in Scheme 1, and the resulting product structures are given in Tables 1−4 along with the route employed. Route A involves alkylation of the phenol of the previously reported indole **8**[1] with the appropriate electrophile, using Cs_2CO_3 as base, yielding the indoles **9**. Conversion of these aryl bromides to the pinacol boronate esters **10** was achieved using standard conditions ($Pd(dppf)Cl_2 \cdot CH_2Cl_2$, bis(pinacolato)diboron, *p*-dioxane, heat). This boronate served as a robust handle to cross-couple a variety of heteroaryl halides under Suzuki coupling conditions ($Pd(PPh_3)_4$, K_2CO_3, DME/H_2O). Hydrolysis of the hindered geminal dimethyl ester using LiOH in MeOH/THF/H_2O yielded the desired carboxylic acids **11**. Route B reverses the order of steps by converting the indole **8** to the boronate derivative **12** and cross-coupling with the appropriate heteroaryl halide to generate compounds **13**. Alkylation of these phenols using Cs_2CO_3 as base and the requisite electrophiles and subsequent ester hydrolysis afforded the target compounds **11**. The commercially unavailable alkylating reagents were prepared as follows (Scheme 2). The pyrazinyl and quinoxalinyl chlorides **14** and **15** were synthesized using standard radical chlorination conditions (NCS with 2,5-dimethylpyrazine or trichloroisocyanuric acid with 2-methylquinoxaline, respectively).[25] The symmetrical 2,5-dimethylpyrazine gave a mixture of monochlorides and bischlorides that were readily separable by column chromatography. The 2-chloromethyl alkyl substituted pyridines (**16a−d,f**) and 2-chloromethyl-6-methylquinoline **17** hydrochlorides were prepared through synthesis of the *N*-oxide (*m*-CPBA), rearrangement of the *N*-oxide under refluxing acetic anhydride conditions to furnish the pyridine methylacetate, hydrolysis with concentrated hydrochloric acid, and reaction of the resulting alcohols with thionyl chloride to give the desired chloromethyl pyridines/quinoline as the HCl salt. Pyridine **16d** was obtained from reaction of the commercially available 6-hydroxymethyl-2-methylpyridine with thionyl chloride. The pyridine *N*-oxide **16g** was synthesized through *m*-CPBA oxidation of the pyridine **16c**. 5-Methoxy-2-methylpyridine, prepared from the corresponding phenol by reaction with $TMSN_2$, was brominated using NBS and benzoyl peroxide in refluxing benzene to afford bromide **18**. Similar bromination conditions were used to provide the 5-cyano-2-bromomethylpyridine **19** and the 6- and 7-fluoroquinoline derivatives **20** and **21**. Heterocycles **22−25** were prepared from the commercially available alcohols using refluxing thionyl chloride. 5,6-Dimethyl-2-chloromethylpyridine hydrochloride **26** was prepared from 2,3-lutidine through conversion to the *N*-oxide (*m*-CPBA) and then rearrangement to 2-cyano-5,6-dimethylpyridine (TMSCN, diethylcarboamoyl chloride). Acidic methanolysis of the resulting nitrile to the methyl ester, reduction of the ester with DIBAl-H under standard conditions, and treatment of the alcohol with thionyl chloride afforded **26**. 5-Chloro-2-chloromethylpyridine **27** was derived from borane reduction of 5-chloropyridine-2-carboxylic acid and conversion of the resulting alcohol to the chloride using thionyl chloride. Finally, the Boc-protected indole **28** was synthesized from 1*H*-indole-2-carboxylic acid ethyl ester via reaction with Boc anhydride under standard conditions (DMAP, MeCN), reduction of the ester with DIBAl-H, and conversion of the resulting alcohol to the corresponding halide using a mixture of LiBr and MsCl in dichloromethane.[26] The mixture of products from this halide displacement was used without further

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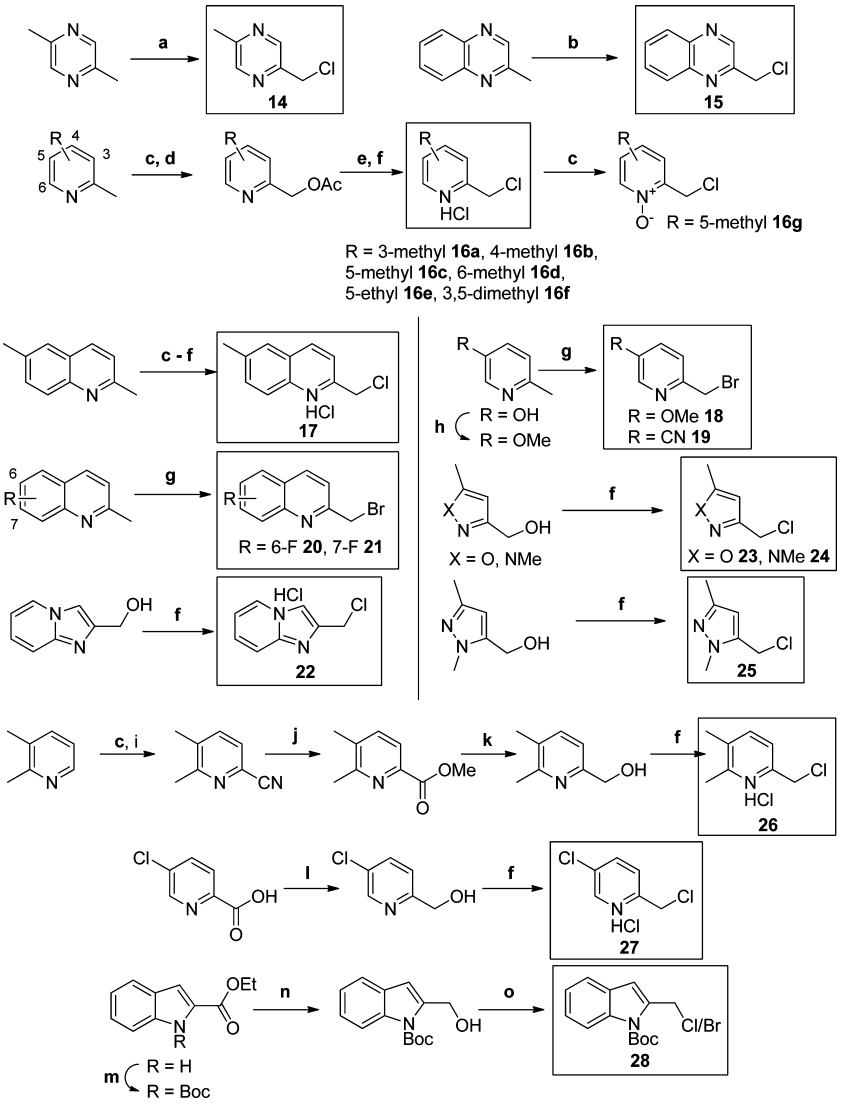
Scheme 2. Preparation of Alkylating Agents[a]



[a](a) *N*-Chlorosuccinimide, C_6H_6, $(PhCO)_2$, reflux; (b) trichloroisocyanuric acid, $CHCl_3$, reflux; (c) *m*-CPBA, $CHCl_3$; (d) Ac_2O, 100 °C to reflux; (e) HCl (conc), reflux; (f) $SOCl_2$, reflux; (g) *N*-bromosuccinimide, C_6H_6, $(PhCO)_2$, reflux; (h) $TMSCH_2N_2$, toluene, MeOH; (i) TMSCN, $Et_2NC(O)Cl$, CH_2Cl_2; (j) HCl (g), MeOH; (k) DIBAl-H, THF, −78 °C; (l) BH_3·THF, THF, 0 °C; (m) Boc_2O, DMAP, MeCN; (n) DIBAl-H, toluene, −40 °C; (o) LiBr, CH_3SO_2Cl, Et_3N, CH_2Cl_2.

purification in the subsequent alkylation of the indole phenol **8**. The Boc-protecting group was also removed during hydrolysis of the ester (route A) to give the indole compound **11g**. As shown in Scheme 3, the racemic sulfoxide **29** was prepared via oxidation of **11cc** with 1 equiv of *m*-CPBA. The acyl derivatives **31a**,**b** were prepared through removal of the *tert*-butylthio group with $AlCl_3$ and water[27] to give the 3*H* indole **30** and subsequent Friedel−Crafts like acylation with $AlCl_3$ and the relevant acid chloride in 1,2-dichloroethane and then ester hydrolysis. The pyridone **11jj** was isolated after prolonged reaction of the sodium salt of **11cc** in ethylene glycol with excess lithium hydroxide at 165 °C. The amide compound **11q** was the result of concomitant hydrolysis of the precursor nitrile (compound **19** used in the alkylation of the phenol **3**, Scheme 1) upon synthesis of the final carboxylic acid. Finally, the methoxypyrimidine derivative **11kk** was obtained from an S_NAr reaction. After cross-coupling of 2-chloro-5-fluoropyrimidine (Scheme 1, route B) and alkylation of the phenol with 2-chloromethyl-5-methylpyridine, methanol displaced the aromatic fluoride under the hydrolysis reaction conditions to afford the methoxypyrimidine **11kk**.

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.[28] The ligand used was tritiated 3-[5-(pyrid-2-ylmethoxy)-3-*tert*-butylthio-1-benzylindol-2-yl]-2,2-dimethylpropionic acid (American Radiolabeled Chemicals, St. Louis, MO). Compounds were also screened in a cell-based human leukocyte assay (hLA) to measure the inhibition of LTB_4 production following calcium ionophore (A23187) challenge. To investigate the effect of blood protein binding on potency, compounds were tested in a human whole blood (hWB) or rat whole blood (rWB) assay to measure the inhibition of LTB_4 production following A23187 challenge (15 min or 5 h of compound preincubation).[17a] Selected compounds were

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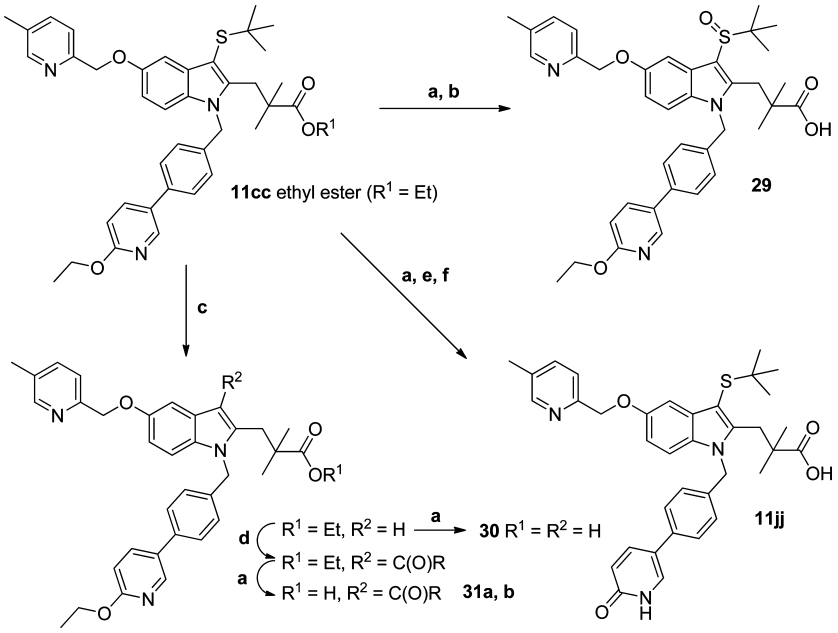
Scheme 3. SAR at 3-Position of the Indole and Preparation of Pyridinone 11jj[a]



[a](a) $LiOH·H_2O$, MeOH, THF, H_2O, 60 °C; (b) *m*-CPBA (1 equiv), CH_2Cl_2, −20 to 0 °C; (c) $AlCl_3$, H_2O, CH_2Cl_2 0 °C to room temp; (d) RC(O)Cl, $AlCl_3$, DCE, 65 °C; (e) 1 N NaOH, EtOH; (f) $LiOH·H_2O$, ethylene glycol, 165 °C.

screened for inhibition of thromboxane B_2 synthesis in a human whole blood cyclooxygenase-1 assay (COX-1, 15 min of preincubation; see Experimental Section).

RESULTS AND DISCUSSION

During our research to identify a backup compound to the recently disclosed FLAP inhibitor **1**, we observed that in a rat bronchoalveolar lavage (BAL) LT inhibition assay compound **6** showed complete inhibition of LTB_4 and CysLTs in vivo 16 h after oral dosing. The measured plasma concentration (10 nM) at 16 h was significantly below its rat whole blood assay (rWB) potency against LTB_4 inhibition (IC_{50} = 117 nM, 15 min assay preincubation). The apparent discrepancy between the measured LT inhibition and the in vitro potency of **6** in the rWB assay led us to repeat this assay with an extended compound preincubation time (4 h). Interestingly, when compound **6** was repeated in the rWB assay with this 4 h preincubation, the IC_{50} had significantly decreased to 10 nM, a value more closely aligned with the in vivo potency observed in the rat BAL model. This indicated that the previous 15 min preincubation protocol did not permit the full potency of this class of FLAP inhibitors to be observed in blood. We had also previously screened and optimized compound selection using the hWB assay at 15 min preincubation times to ascertain their potency in the presence of human blood proteins. Therefore, we reanalyzed compounds in human blood with an extended compound preincubation time and identified certain compounds that gave significantly higher inhibitory potencies after these prolonged assay conditions. A time course analysis of both compounds **5** and **6** (15 min, 5 h, and 11 h) in the hWB assay indicated that near maximal inhibition occurred after 5 h of preincubation in human blood (hWB data for **5**, 15 min IC_{50} = 1.4 μM, 5 h IC_{50} = 1.1 μM, 11 h IC_{50} = 1.0 μM; hWB data for **6**, 15 min IC_{50} = 536 nM, 5 h IC_{50} = 95 nM, 11 h IC_{50} = 80 nM). For screening purposes the 5 h time point (5 h hWB) was chosen as the routine extended preincubation time. This time-dependent increase in potency represents a previously unrecognized property that has important implications for the design and selection of FLAP inhibitors suitable for clinical development. Furthermore, this phenomenon appears selective for this particular series of FLAP inhibitors, as compound **3** showed no such improvement in LT synthesis inhibition in hWB after prolonged incubation (15 min hWB IC_{50} = 2.2 ± 1.2 μM (n = 3), 5 h hWB IC_{50} = 4.2 ± 2.4 μM (n = 3).

As discussed in our previous paper,[1] both **1** (15 min hWB IC_{50} = 349 nM) and **6** (15 min hWB IC_{50} = 536 nM) are potent FLAP inhibitors, the latter of which has demonstrated efficacy in human trials for asthma.[16] Replacement of the chlorobenzyl substituent of **6** with a 2-methoxy-5-pyridyl moiety was part of the strategy in the identification of **1** to address some of the perceived liabilities of **6**. Keeping the methoxypyridine component constant (R^2 = 5-methoxypyridin-2-yl, Scheme 1, compound **11**), we prepared a small, focused series of compounds, varying R^1 with aromatic groups including quinoline or similar aromatic bicycles (Table 1). All compounds were assayed in a human FLAP membrane binding assay to ascertain their intrinsic potency and in a cell-based LTB_4 inhibition assay (hLA). Compounds were further profiled in a 15 min LTB_4 inhibition assay (hWB) to measure the inhibition of FLAP in the presence of human blood. Inhibitors that showed good activity in the hWB assay (15 min hWB IC_{50} < 1 μM) were further profiled using a 5 h compound preincubation period to determine if the blood LT inhibition potency increases over time. As can be seen from Table 1, we identified several quinoline replacement groups that showed good potency in all three assays. Of particular note is the observation that compounds **6**, **11a**, and **11c** show an increase in hWB potency upon extended preincubation in this assay (**6**, 15 min IC_{50} = 554 nM, 5 h IC_{50} = 108 nM; **11a**, 15 min IC_{50} = 502 nM, 5 h IC_{50} = 194 nM). The quinoxinyl derivative **11b** possesses a similar in vitro profile, but the presence of a nitrogen at the 4-position apparently prevents this time

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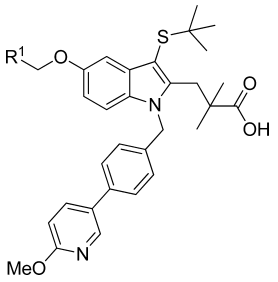
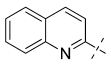
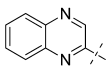
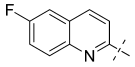
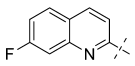
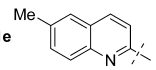
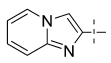
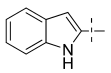
Table 1. IC_{50} Results of FLAP Inhibitors[a]

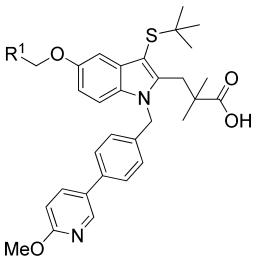


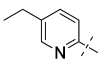
No.	R^1	FLAP binding (nM)[b]	hLA (nM)[c]	hWB (nM)[d] 15 min	hWB (nM)[d] 5 hr	Route
6	NA	1.9±0.3	0.5±0.5	536±271	108±40	NA
11a		5.3±1.6	0.8±0.5	502±216	194±97	**B**
11b		11.4±10.7	0.7±0.1	542±157	456±42	**B**
11c		7.6±7.0	0.7±0.2	747±229	289±58	**A**
11d		327±48	2.1±1.3	3220±2420	ND	**A**
11e		148±76	1.7±0.6	3880±2130	ND	**A**
11f		8.9±8.1	1.4±0.7	980±503	552±36	**A**
11g		1250±214	11.1±7.1	11800±885	ND	**B**

[a]NA, not applicable. ND, not determined. Values shown are ±standard deviation. [b]FLAP inhibition of ^{3}H-ligand binding to FLAP membranes. [c]hLA: inhibition of LTB_4 synthesis following ionophore (A23187) challenge in human leukocytes. [d]hWB: inhibition of LTB_4 synthesis following ionophore challenge (A23187) in human blood after either 15 min or 5 h of preincubation of target compound.

dependent potency enhancement (15 min IC_{50} = 542 nM, 5 h IC_{50} = 486 nM), despite excellent cell-based hLA LTB_4 inhibition and FLAP binding IC_{50} (0.7 and 11.4 nM, respectively). The 6-fluoro derivative **11c** shows good hLA activity, FLAP binding, and 15 min hWB LTB_4 inhibition and improves in potency by approximately 2-fold after extended preincubation in hWB. The 7-fluoroquinoline **11d** exhibits an unexpected dramatic loss of affinity in FLAP binding, presumably due to an unfavorable electronic effect. The 6-methylquinoline derivative **11e** also shows marked decrease in binding affinity for the FLAP protein and concomitant reduction in hWB potency. Subsequent to the completion of this work, X-ray crystal data of the FLAP protein were published indicating a restricted binding pocket for the quinoline moiety of **1**.[29] This correlates well with our findings that the steric nature of the quinoline group alters activity against FLAP (e.g., compounds **11d** and **11e**). Replacement of the quinoline with the more basic imidazopyridine **11f** retained potency in both FLAP binding and leukocyte LTB_4 inhibition assays, but the extended preincubation hWB potency proved modest (15 min IC_{50} = 980 nM, 5 h IC_{50} = 552 nM). Replacement with an indole, compound **11g**, led to a significant decrease in potency in all three assays. With the knowledge that a pyridine replacement for the quinoline maintained high affinity for FLAP (compound **1**) we prepared a series of substituted pyridine derivatives in an attempt to probe the SAR around this group and find compounds that exhibited the same time dependent increase in potency in the hWB assay as demonstrated by **6**, **11a**, and **11c** (Table 2). A scan of methyl

Table 2. IC_{50} Results of FLAP Inhibitors: Pyridine Replacement SAR[a]



No.	R^1	FLAP binding (nM)[b]	hLA (nM)[c]	hWB (nM)[d] 15 min	hWB (nM)[d] 5 hr	Route
1		4.2±2.4	0.5±0.7	349±86	149±44	NA
11h		17.4±4.0	1.1±0.1	1290±643	ND	**A**
11i		21.8±12.4	1.8±0.9	3300±2020	ND	**A**
11j		1.7±0.4	0.5±0.3	328±138	87±28	**B**
11k		3.1±2.2	0.5±0.1	535±227	359±46	**B**
11l		10.1±3.6	0.6±0.3	1200±565	1390±178	**A**
11m		59.3±5.7	1.2±0.3	1790±714	ND	**A**
11n		3.4±2.0	0.6±2.7	570±245	225±52	**B**
11o		26.5±11.9	0.7±0.1	1160±50	ND	**B**
11p		8.0±3.6	0.7±0.2	688±106	652±141	**B**
11q		3270±2020	229±70	39000±34700	ND	**B**
11r		5.4±2.2	3.3±4.4	208±106	281±28	**B**
11s		114±48	2.4±1.6	2424±21	ND	**A**
11t		1600±279	26.8±4.5	26300±1800	ND	**A**
11u		2470±1308	19.7±10	16600±12300	ND	**A**

[a]NA, not applicable. ND, not determined. Values shown are ±standard deviation. [b]FLAP inhibition of ^{3}H-ligand binding to FLAP membranes. [c]hLA: inhibition of LTB_4 synthesis following ionophore (A23187) challenge in human leukocytes. [d]hWB: inhibition of LTB_4 synthesis following ionophore challenge (A23187) in human blood after either 15 min or 5 h of preincubation of target compound.

groups around the pyridine moiety (**11h**, **11i**, **11j**, and **11k**) showed that the 5- and 6-methyl derivatives (**11j** and **11k**) kept intrinsic potency comparable to that of **1**, but only the 5-methyl derivative **11j** showed a significant increase in potency in the 5 h hWB assay (15 min IC_{50} = 328 nM, 5 h IC_{50} = 87 nM for **11j** vs 15 min IC_{50} = 349 nM to 5 h IC_{50} = 149 nM for **1**).

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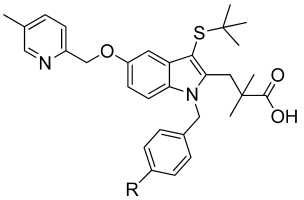
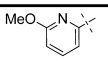
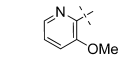
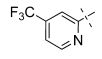
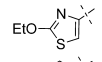
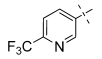
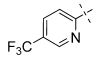
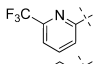
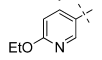
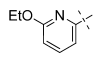
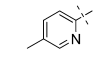
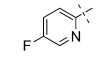
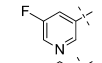
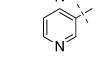
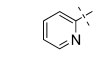
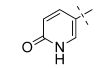
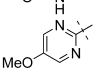
Surprisingly, the 5-ethyl derivative **11l** did not show any increase in potency in blood, presumably because of the steric difference between methyl and ethyl groups, albeit small, highlighting that a subtle difference could have a significant impact on the extended preincubation enhancement in hWB. Combining the 5-methyl group with another methyl substituent as in compounds **11m** and **11n** showed no advantage, the 3,5-dimethyl analogue **11m** possessing intrinsically poorer potency in all assays and the 5,6-dimethyl compound **11n** exhibiting a weaker 5 h hWB IC_{50} (225 nm) compared to **11j**. On the basis of the 5-methylpyridine imparting a favorable impact on hWB potency, several more 5-substituted analogues were examined. The 5-chloropyridine **11o** loses some affinity for FLAP and hence shows less inhibition of LTB_4 in the hWB assay, and the carboxamide **11q** suffers a dramatic loss in activity. However, the 5-methoxy derivative **11p** retained intrinsic hLA and FLAP binding activity but did not demonstrate any time-dependent increase in the 5 h hWB assay, a result similar to that observed with the 5-ethylpyridyl compound **11l**. The pyrazinyl derivative **11r**, like the quinoxinyl compound **11c**, again exemplified that although heterocycles incorporating a nitrogen at the 4-position are well tolerated in hLA and FLAP binding, they also show no significant change in potency with extended preincubation in the hWB assay. Attempts to mimic the 5-methylpyridine with other small heterocycles yielded significantly less potent compounds (**11s**, **11t**, and **11u**).

After identification of the 5-methylpyridine as a moiety that conferred significant potency in the 5 h hWB assay, continued SAR studies, primarily through modification of the indole *N*-benzyl substituent, addressed off-target activities and pharmacokinetic properties (Table 3). Apart from 5-LO and FLAP, several other enzyme systems utilize AA as their substrate, including the cyclooxygenase (COX) enzymes. Therefore, we counterscreened compounds of interest against COX-1 in a human blood thromboxane inhibition assay. We were surprised to discover that heteroaryl rings on the biaryl possessing a meta-substituent (**11v**, **11x**, **11bb**, **11dd**, **11gg**) or pseudo-meta in the case of the ethoxythiazole **11y** showed significant affinity for the COX-1 enzyme, with one compound, **11dd**, possessing considerable potency (IC_{50} = 615 nM) in the COX-1 whole blood assay. The unsubstituted pyridines **11hh** and **11ii** also conferred some affinity for the COX-1 enzyme. Compounds bearing a para-substitutent on the terminal biphenyl ring (**11cc**, **11ee**, **11ff**, **11kk**) show low affinity for COX-1. The pyridinone derivative **11jj** is an example of a very polar biaryl functionality appended to the indole. This proved detrimental to inhibition of LTB_4 in the leukocyte assay but retained affinity for FLAP (binding IC_{50} = 8.3 nM) and also shows the most significant improvement with extended preincubation in the hWB assay (15 min IC_{50} = 10028 nM to 5 h IC_{50} = 1279 nM ∼ 8-fold increase). However, this compound was not potent enough to warrant further investigation. To check for a potential time-dependent increase in potency against the COX-1 enzyme, we assayed compound **11j**, a compound that shows an increase in LT synthesis inhibition over time in hWB, in the COX-1 assay with both a 15 min and 5 h preincubation. Importantly, no increase in potency against COX-1 was measured over time (hWB COX-1, 15 min IC_{50} = 12.3 μM, 5 h IC_{50} = 17.3 μM). Manipulation of the 3-position of the indole core via removal of the *tert*-butylthio group of **11cc** significantly attenuates the binding to FLAP and potency in blood (compound **29**). Oxidation to the racemic sulfoxides **30** and the pyridine *N*-oxide (**11ll**) also significantly reduces affinity for the FLAP protein (Table 4). Appreciable activity could be returned to the scaffold through introduction of the *tert*-butylacetyl group at C3 of the indole, compound **31a**, and this was the most potent of these derivatives prepared. However, despite the presence of the 5-methylpyridine, no significant increase in hWB potency was observed in the 5 h preincubation assay for **31a**.

Table 3. IC_{50} Results of FLAP Inhibitors: SAR of Benzylic Biphenyl and COX-1 Counterscreen[a]



No.	R	FLAP binding (nM)[b]	hLA (nM)[c]	hWB (nM)[d] 15 min	hWB (nM)[d] 5 hr	COX-1 hWB[e]	Route
11v		1.3±1.1	0.3±0.2	233±115	ND	81%	**A**
11w		4.6±2.4	0.8±1.4	1460±459	ND	ND	**B**
11x		2.1±1.5	0.4±0.1	452±22	ND	18μM	**B**
11y		1.4±1.3	0.4±0.1	419±32	ND	92%	**A**
11z		3.2±1.1	0.4±0.1	401±31	180±14	ND	**A**
11aa		2.7±0.5	0.5±0.1	435±9	202±40	ND	**A**
11bb		1.8±1.2	0.5±0.1	504±41	ND	74%	**B**
11cc		2.9±1.0	0.7±0.3	506±109	76±15	58μM	**B**
11dd		1.4±0.6	0.6±0.1	556±12	ND	0.62μM	**B**
11ee		2.2±1.0	0.5±0.1	283±78	130±1	33%	**A**
11ff		1.5±0.5	0.4±0.1	194±100	75±15	>30μM	**B**
11gg		2.1±0.9	0.6±0.3	225±14	ND	3.2μM	**B**
11hh		2.6±0.5	0.5±0.1	268±33	ND	97%	**B**
11ii		1.3±0.5	0.6±0.3	285±25	ND	9μM	**B**
11jj		8.3±7.5	94±42	10000±6400	1280±989	ND	scheme 3
11kk		2.0±0.2	0.6±0.2	161±58	81±26	25μM	**B**

[a]ND, not determined. Values shown are ±standard deviation. [b]FLAP inhibition of ^{3}H-ligand binding to FLAP membranes. [c]hLA: inhibition of LTB_4 synthesis following ionophore (A23187) challenge in human leukocytes. [d]hWB: inhibition of LTB_4 synthesis following ionophore challenge (A23187) in human blood after either 15 min or 5 h of preincubation of target compound. [e]Inhibition of TXB2 in human whole blood presented as either percent inhibition at 10 μM or IC_{50}.

Investigation of Time Dependent Increase in Potency of FLAP Inhibitors. Having identified the structural motifs responsible for imparting improved 5 h potency in the hWB assay, we attempted to determine the physical basis for this phenomenon. Our initial hypothesis was that compounds exhibiting a potency shift between the 15 min and 5 h hWB time points may reflect an intrinsic difference in the on-rate or off-rate of the compound to the FLAP protein itself. However,

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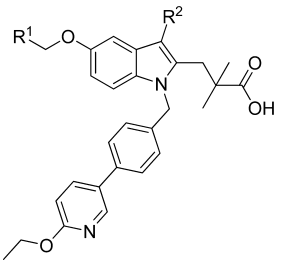
Table 4. IC_{50} Results of FLAP Inhibitors: Modification of 3-Indole Position[a]



No.	R^1	R^2	FLAP binding (nM)[b]	hLA (nM)[c]	hWB (nM)[d] 15 min	hWB (nM)[d] 5 hr	Route
11ll			89.5	4.6±3.4	873±862	(1863, 1394)	**B**
29		H	219±89	22.6±1.3	22300±9270	ND	scheme 3
30			559±6	253±171	33200±9720	ND	scheme 3
31a			29.3±20.4	1.5±0.2	1580±169	2000±1020	scheme 3
31b			22500±7630	64±62	32600±18100	ND	scheme 3

[a]ND, not determined. Values shown are ±standard deviation. Values in parentheses are the results of one experiment with two different donors. [b]FLAP inhibition of ^{3}H-ligand binding to FLAP membranes. [c]hLA: inhibition of LTB_4 synthesis following ionophore (A23187) challenge in human leukocytes. [d]hWB: inhibition of LTB_4 synthesis following ionophore challenge (A23187) in human blood after either 15 min or 5 h of preincubation of target compound.

after preparing radiolabeled (^{3}H) versions of compounds **1** and **11a**, we determined that both compounds display fast on- and off-rates with no significant difference in on- or off-rates between compounds that demonstrate improved potency over time and those that do not (Figure 3). By employment of the

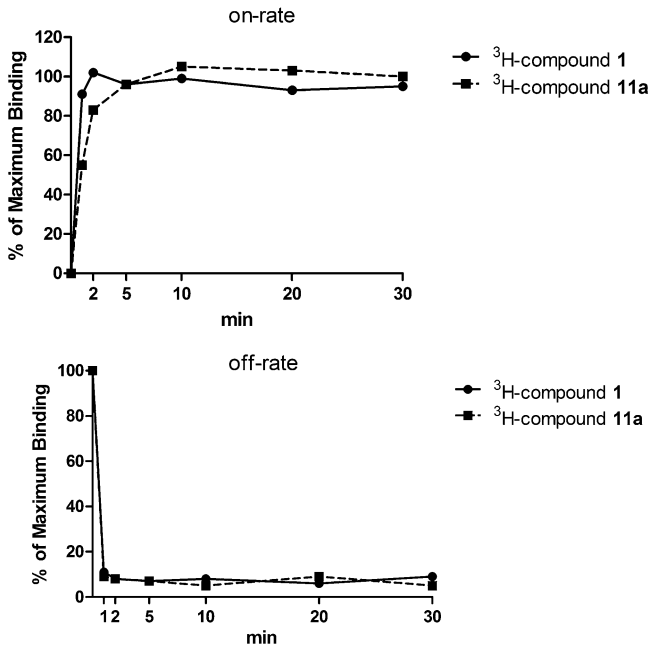


Figure 3. On-rates and off-rates for radiolabeled compounds **1** and **11a**.

hLA assay, in the absence of added human serum albumin (hSA) to the assay buffer, no potency increase was apparent between a 5 min and 1 h preincubation time for any of the compounds tested (Table 5). However, addition of 0.5% hSA

Table 5. Human Leukocyte Assay (hLA) IC_{50} after Varying Preincubation Time in the Presence or Absence of 0.5% hSA[a]

	no hSA (nM)		0.5% hSA (nM)	
compd	5 min	1 h	10 min	3 h
1	1.2	1.1	10	14
5	2.2	5.7	19	27
6	1.0	1.0	66	12
11cc	1.0	1.0	26	4

[a]hLA: inhibition of LTB_4 synthesis following ionophore (A23187) challenge in human leukocytes resuspended in assay buffer either lacking (no hSA) or containing 0.5% hSA. hSA = human serum albumin.

to the assay buffer resulted in a measurable shift in potency between a 10 min and 3 h preincubation time. These data indicate an equilibrium shift between compounds binding hSA and the target, FLAP. All of the compounds measured in these studies are highly lipophilic carboxylic acids and as such possess a very large degree of protein binding (≥99.5%, individual data not shown). Consequently, the time dependent increase in potency of this class of FLAP inhibitors in hWB might reflect subtle differences in the degree of protein binding that cannot be distinguished using conventional protein binding assays. Additionally, the time-dependent inhibition may also reflect differences in the off-rate from serum albumin. However, this does not fully explain the observed 5 h hWB differences between compounds such as **11z**, **11aa**, and **11cc**, for example. All three possess almost identical in vitro potencies in FLAP binding, hLA, and 15 min hWB, but **11cc** is twice as potent after 5 h of incubation in hWB. By analogy to the reported X-ray crystal structure for FLAP with compound **6** bound,[29] the differences between these three compounds were not anticipated to impart much variation in potency because of the biaryl portion of the molecule being directed away from the target protein and into the membrane space. The fact that **11cc** is the significantly more potent of the three compounds in 5 h hWB may be due to other, as yet undetermined factors or that, for certain compounds (e.g., **11z** and **11aa**), a 5 h preincubation in the hWB assay may still not be a fully equilibrated situation. We recently reported on a similar potency enhancement in our research on prostaglandin D_2 receptor (DP_2) antagonists using an eosinophil shape change assay in human blood with extended compound preincubation.[30]

As part of our drug discovery effort, compounds are screened against the common human cytochrome P450 (hCYP) enzymes 3A4, 2C9, and 2D6 to minimize the possibility of drug−drug interactions or drug concentration variations upon long-term dosing. Table 6 shows the hCYP inhibition data for the four most potent compounds in the 5 h hWB assay: **11cc**, **11ee**, **11ff**, **11kk**. In general they show modest inhibition toward the hCYP isoforms tested, the fluoropyridine **11ff** showing the most activity against 2C9 with an IC_{50} of 1.5 μM. Of particular note was compound **11cc**, which was essentially inactive against all three of the enzymes listed at the highest concentration tested (50 μM).

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Table 6. CYP Inhibition Parameters for Four Most Potent FLAP Inhibitors (5 h hWB)[a]

	CYP inhibition IC_{50} (nM)		
compd	3A4	2C9	2D6
11cc	>50	>50	>50
11ee	8.5	12	>50
11ff	5.5	1.5	>50
11kk	8.8	4.5	>50

[a]Inhibition assays were performed in a manner similar to those published.[32] All positive control inhibitors were within their published IC_{50} values.

Further Profiling of 11cc (AM803). Compound **11cc**, 3-[3-*tert*-butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid, was selected for further profiling because of its excellent in vitro potency against LTB_4 inhibition in the 5 h hWB assay, inactivity against the three major human CYP isoforms, and no COX-1 cross-reactivity. Additionally, it showed no induction or time-dependent inhibition of the CYP3A4 enzyme (measured at 10 μM; 0.01 min^{-1} vs 0.054 min^{-1} for troleandomycin control) and no inhibition of the CYP2C19, 2D6, and 1A2 enzymes (IC_{50} > 40 μM). Furthermore, **11cc** showed very little activity toward other enzymes in the leukotriene pathway (LTA_4 hydrolase IC_{50} > 100 μM, LTC_4 synthase IC_{50} = 36 μM, 5-LO IC_{50} = 20 μM) and no activity against the COX-2 enzyme (IC_{50} > 100 μM in a human blood assay measuring PGE_2 inhibition). Pharmacokinetic parameters were assessed in Sprague–Dawley rats (Table 7), and upon oral administration (10 mg/kg sodium carboxylate salt), **11cc** showed good bioavailability (76%) and a C_{max} of 10 μg/mL. Intravenous administration of **11cc** (2 mg/kg) showed a low systemic clearance of 5.2 mL min^{-1} kg^{-1} and a low volume of distribution (0.77 L/kg) with a 2–3 h terminal half-life. When dosed in dogs, **11cc** demonstrated good bioavailability (51%), very low clearance (0.6 mL min^{-1} kg^{-1}), low volume of distribution (0.2 L/kg), and a long iv half-life of 13 h. The low volume of distribution observed in dog is primarily attributable to **11cc** possessing a very high degree of plasma protein binding (rat, 99.8%; dog, 99.7%; human, 99.5%). To ascertain the ability of **11cc** to inhibit leukotriene synthesis in vivo, we employed a rodent BAL model.[31] After a 3 mg/kg po dose 4 h prior to A23187 ionophore challenge, LTB_4 and CysLT concentrations in the rat BAL fluid were significantly reduced (100% and 80%, respectively, n = 5, average measured plasma concentration of **11cc** at 4 h of 1 μM). Of note is the observation that after a 3 mg/kg po dose of **11cc** 16 h prior to ionophore challenge in the rat lung, inhibition of LTB_4 (86%) and CysLT (41%) was maintained despite the measured plasma concentration (average of 3 nM, n = 5) being below the rWB IC_{50} (15 min preincubation, 239 nM), potentially indicating an extended pharmacodynamic effect. However, the potency of **11cc** increased substantially with an extended 4 h preincubation period in rWB, yielding an IC_{50} of 16 nM. The discrepancy between the measured plasma concentration in this 16 h pretreatment BAL experiment and the 4 h rWB potency (3 nM vs 16 nM) may reflect a concentration difference between lung tissue and plasma in the rat.

CONCLUSIONS

We have discovered that a certain subset of FLAP inhibitors in this indole series require longer incubation times in blood to reach their equilibrium potency for LTB_4 inhibition. From the SAR presented, this "time-dependent" effect appears limited to compounds bearing certain functional groups appended to the hydroxymethyl portion of the indole nucleus, the most potent of which are quinolines (for example, compounds **6**, **11a**, and **11c**) or 5-methylpyridines (e.g., **11cc**, **11ff**, **11kk**). Comparing the 5-methylpyridine derivative **11j** to **1** clearly shows that addition of the methyl group increases affinity in the FLAP binding assay and potency in the 5 h preincubation hWB LTB_4 assay. Utilizing this extended assay paradigm in hWB, we identified compound **11cc** (3-[3-*tert*-butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid), which is a very effective inhibitor of LTB_4 production in human blood, does not inhibit any of the major human CYP isoforms or the COX enzymes, and demonstrates excellent pharmacokinetic parameters in rat and dog. Furthermore **11cc** demonstrated excellent in vivo inhibition of leukotrienes in a rodent BAL ionophore challenge model. **11cc** has subsequently completed phase 1 clinical trials and is currently in phase 2 studies in patients, the results of which will be reported in a separate publication.

EXPERIMENTAL SECTION

FLAP Binding Assay. Packed human polymorphonuclear cell pellets (1.8 × 10^9 cells) (Biological Specialty Corporation) were resuspended and lysed, and 75000*g* pelleted membranes were prepared as described.[28] The 75000*g* pelleted membranes were resuspended in a Tris buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM DTT, 30% glycerol) to yield a protein concentration of ~4 mg/mL. An amount of 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, 0.05% Tween-20) and ~30000 cpm of [^{3}H]-3-[5-(pyrid-2-ylmethoxy)-3-*tert*-butylthio-1-benzylindol-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 mixtures 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 and the bottoms sealed. An amount of 100 μL of scintillant was added. The plates were incubated for 1 h before reading on a Perkin-Elmer TopCount. Specific binding was defined as total radioactive binding minus nonspecific binding in the presence of 10 μM MK886. IC_{50}

Table 7. Measured Pharmacokinetic Parameters for Four Most Potent FLAP Inhibitors (5 h hWB)[a]

compd	$T_{1/2}$ iv (h)	C_{max} (μM)	F (%)	AUC_{po} (h·μg/mL)	Cl (mL min^{-1} kg^{-1})	Vd_{ss} (L/kg)
11cc	2.0	12.2	100	26	8.0	0.70
11ee	1.0	14.6	40	26	3.0	0.16
11ff	1.9	23.3	100	77	4.0	0.38
11kk	1.7	30.6	100	63	3.1	0.18

[a]Compounds were dosed as the corresponding sodium carboxylate salt, 10 mg/kg po (0.5% methylcellulose) and 2 mg/kg iv (water) in Sprague–Dawley rats (male).

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values were determined using Graphpad Prism analysis of drug titration curves. Compounds **1** and **11a** were tritiated at American Radiolabeled Chemicals, St. Louis, MO. Analysis of on-rate was performed by incubating membranes with 10 μM cold competitor (for nonspecific binding) or vehicle (DMSO) for 5 min before addition of radioligand and further incubation with the radioligand for 1−60 min before harvesting by filtration. Analysis of the off-rate was performed by incubating membranes with radioligand for 30 min before addition of 10 μM cold competitor and further incubation with the cold competitor for 1−60 min before harvesting and filtration.

Human Leukocyte Inhibition Assay (hLA). 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 were sedimented at 1200 rpm. The pellet was resuspended at 1.25×10^5 cells/mL in assay buffer (Hanks balanced salt solution containing 15 mM Hepes) 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 the mixture incubated for 5 min at 37 °C. Samples were centrifuged at 1200 rpm (~300*g*) for 10 min at 4 °C. The supernatant was removed and a 1:4 dilution assayed for LTB_4 concentration using ELISA (Assay Designs). Drug concentrations to achieve 50% inhibition (IC_{50}) of vehicle LTB_4 were determined by nonlinear regression analysis (Graphpad Prism) of % inhibition versus logarithmic drug concentration. Modifications to the assay for analysis of time-dependent inhibition included resuspending the leukocytes in assay buffer plus or minus 0.5% human serum albumin and incubating leukocytes with test compound for 5 min to 3 h before stimulation with calcium ionophore.

Human Blood LTB_4 Inhibition Assay (hWB). Blood was drawn from consenting human volunteers into heparinized tubes, and 150 μL aliquots were added to wells containing 1.5 μL of DMSO (vehicle) or 1.5 μL of test compound in DMSO. Samples were incubated for either 15 min or 5 h at 37 °C. An amount of 2 μL of calcium ionophore A23187 (freshly diluted from a 50 mM DMSO stock to 1.5 mM in Hanks balanced salt solution) was added. The solution was mixed and incubated for 30 min at 37 °C. Samples were centrifuged at 1500 rpm (~300*g*) for 10 min at 4 °C. Plasma was removed and a 1:100 dilution assayed for LTB_4 concentration using ELISA (Assay Designs). Drug concentrations to achieve 50% inhibition (IC_{50}) of vehicle LTB_4 were determined by nonlinear regression analysis (Graphpad Prism) of percent inhibition versus logarithm of drug concentration. Each assay was performed using two different donors; drug concentrations are singletons.

COX-1 Human Whole Blood Assay. Human blood was obtained by venipuncture from consenting adult volunteers. Blood was anticoagulated by drawing into heparinized tubes and used within 2 h of draw. Aliquots of fresh blood (150 μL) were incubated for 15 min at 37 °C with 1 μL of test compound diluted in DMSO or with DMSO alone as a vehicle control. A concentration of 10 μM for routine screening was used, or for the generation of IC_{50} values the final concentrations of the test compound were 100, 30, 10, 3, 1, 0.3, and 0.1 μM. Calcium ionophore A23187 (25 μM final concentration) was added from a 1.5 mM stock in HBSS (prepared by dilution from a 50 mM stock in DMSO). Incubations were continued for 30 min at 37 °C. Incubation mixtures were centrifuged at 1500 rpm for 10 min at 4 °C, and aliquots of the plasma were diluted 1:100 with assay buffer and analyzed for TXB2 by a competitive enzyme immunoassay according to the manufacturer's instructions (Assay Designs, Ann Harbor, MI, catalog no. 900-002).

Pharmacokinetic Studies. Compounds were converted from the acid form to the sodium salt by treatment of the acid in ethanol/water with 1 equiv of 1 N NaOH and then removal of the solvent in vacuo. Male Sprague−Dawley rats surgically cannulated in their jugular vein (approximate weight of 300 g for male) were purchased from Charles River (Wilmington, MA). Compounds were administered intravenously (iv) (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 (po) (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 μL) 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 5000 ng/mL. Plasma samples were precipitated using MeCN (1:5, v/v) containing the internal standard (IS) buspirone. An amount of 10 μL of the analyte mixture was injected using a Leap PAL autosampler. The calibration curves were constructed by plotting the peak areas 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 mm × 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% MeCN/50% MeOH 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 then next 0.5 min; B was held constant for 1 min at 95%; 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 Pre-Clinical Research International, Inc., Laval, Quebec, Canada, using female beagle dogs (n = 3 per group). Compound **11cc** 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 rat.

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 either using glass columns packed with silica gel, eluting with the solvents reported, or using prepacked silica gel cartridges on a CombiFlash Rf 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 a Waters Symmetry C18 3.5 μm, 4.6 mm × 150 mm. Final products had a purity of ≥95%. LCMS 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.

2-Chloromethyl-5-methylpyrazine (14). To a solution of 2,5-dimethylpyrazine (20.0 mL, 0.187 mol) in benzene (450 mL) were added *N*-chlorosuccinimide (25.0 g, 0.187 mol) and benzoyl peroxide (400 mg). The mixture was heated under reflux for 18 h. An additional 5.0 g of NCS was added, and heating continued for a further 6 h. TLC analysis indicated almost complete consumption of starting material and the presence of two less polar products. The reaction mixture was evaporated to dryness and purified on silica gel (ISCO, 0−15% EtOAc

in hexanes), and the desired fractions repurified (ISCO: 25−100% hexanes in CH_2Cl_2) to afford the title compound as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H), 4.67 (s, 2H), 8.44 (s, 1H), 8.61 (s, 1H). R_f = 0.4 (20% EtOAc in hexanes).

2-Chloromethylquinoxaline (15).[25] To a refluxing solution of 2-methylquinoxaline (10.0 g, 69.3 mmol) in $CHCl_3$ (100 mL) was added trichloroisocyanuric acid (7.10 g, 30.5 mmol) in ∼100 mg portions over the period of 1 h. The mixture was then heated at reflux for a further 30 min, allowed to cool, and poured into an ice−water mixture. Aliquots of a 50% aqueous NaOH solution were then added to adjust the pH to ∼9. The resulting solution was extracted with $CHCl_3$, dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified on silica gel (ISCO, 0−15% EtOAc in hexanes) to afford the title compound as a purple solid. ^{1}H NMR ($CDCl_3$) δ 4.89 (s, 2H), 7.81 (m, 2H), 8.11 (m, 2H), 9.04 (s, 1H).

2-Chloromethyl-5-methylpyridine Hydrochloride (16c). To an ice cooled solution of 2,5-lutidine (46.8 g, 0.436 mol) in $CHCl_3$ (750 mL) was added *m*-CPBA (129 g, 0.52 mol) in small portions over a period of 30 min. The reaction mixture was maintained at room temperature for 48 h, and then calcium hydroxide (81 g) was added with vigorous stirring leading to the formation of a thick precipitate. After being stirred for 4 h, the slurry was filtered through Celite, washing the residue with $CHCl_3$. Evaporation of the filtrate afforded 46 g (86%) of 2,5-dimethylpyridine 1-oxide as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3H), 2.49 (s, 3H), 7.00 (d, 1H), 7.14 (d, 1H), 8.13 (s, 1H).

To acetic anhydride (150 mL) heated to 100 °C was very carefully added 2,5-dimethylpyridine 1-oxide (46 g, 0.373 mol) over a period of 30 min (*caution*: significant exotherm can occur after an induction period). After complete addition, an additional 50 mL of Ac_2O was added and the reaction mixture heated to reflux for 1 h. Upon completion of heating, the reaction mixture was allowed to cool to ambient temperature and slowly quenched with the addition of EtOH (200 mL) and stirred for 12 h. Evaporation of the reaction mixture afforded a yellow oil which was purified using silica gel chromatography (0−40% EtOAc in hexanes), yielding acetic acid 5-methylpyridin-2-ylmethyl ester (52 g, 84%). ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H), 2.15 (s, 3H), 2.35 (s, 3H), 7.27 (d, 1H), 7.53 (d, 1H), 8.45 (s, 1H).

To acetic acid 5-methyl-pyridin-2-ylmethyl ester (52 g, 0.314 mol) was added concentrated HCl (200 mL), and the mixture was heated to reflux for 1 h. Evaporation of the mixture under vacuum afforded (5-methylpyridin-2-yl)methanol hydrochloride as an orange solid which was used without further purification.

To (5-methylpyridin-2-yl)methanol hydrochloride (40 g, 0.250 mol) was added $SOCl_2$ (140 mL) slowly with vigorous stirring under a reflux condenser (*caution*: significant gas evolution). After complete addition the reaction mixture was heated to reflux for 30 min, allowed to cool, and thoroughly evaporated to dryness to afford the title compound as a pale brown solid used without further purification.

Compounds **16a**, **16b**, **16e**, **16f** were prepared in an identical manner to the procedure given for compound **16b** from the commercially available alkylpyridines. Compound **16d** was prepared from the commercially available 6-methylpyridine-2-methanol through reaction with thionyl chloride as described for the final step of **16c**. Quinoline **17** was also prepared in an identical manner from commercially available 2,6-dimethylquinoline.

2-Chloromethyl-5-methylpyridine 1-Oxide (16g). To a mixture of 2-chloromethyl-5-methylpyridine hydrochloride (**16c**, 500 mg, 2.81 mmol) and *m*-CPBA (760 mg, 3.09 mmol) in CH_2Cl_2 (15 mL) was added $NaHCO_3$ (300 mg, 3.57 mmol). The solution was stirred at ambient temperature for 24 h. The mixture was diluted with CH_2Cl_2, washed with water and the organic phase dried ($MgSO_4$), filtered, and evaporated in vacuo. Purification of the residue on silica gel (ISCO: 0−100% EtOAc in hexanes) afforded 222 mg (50%) of the title compound as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H), 4.82 (s, 2H), 7.13 (d, 1H), 7.48 (d, 1H), 8.13 (s, 1H).

2-Bromomethyl-5-methoxypyridine (18). To a solution of 5-hydroxy-2-methylpyridine (1.0 g, 9.16 mmol) in a mixture of toluene (45 mL) and MeOH (45 mL) was added a solution of (trimethylsilyl)diazomethane (2 M in diethyl ether, 9.16 mL, 18.32 mmol). After the mixture was stirred at room temperature for 30 min, another 2 equiv of (trimethylsilyl)diazomethane was added and the solution maintained at ambient temperature for 12 h. Evaporation of the mixture in vacuo afforded 5-methoxy-2-methylpyridine, which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 3H), 3.83 (s, 3H), 7.08 (d, 1H), 7.13 (dd, 1H), 8.20 (d, 1H). To a solution of the previously isolated 5-methoxy-2-methylpyridine (400 mg, 3.25 mmol) in benzene (16 mL) was added *N*-bromosuccinimide (636 mg, 3.57 mmol) and benzoyl peroxide (30 mg). The reaction mixture was refluxed for 16 h, evaporated to dryness and the residue purified using silica gel chromatography (ISCO, 15% EtOAc in hexanes) to afford 320 mg (44%) of the title compound.

2-Bromomethyl-6-fluoroquinoline (20). To a solution of 6-fluoro-2-methylquinoline (5.00 g, 31.0 mmol) in benzene (160 mL) was added *N*-bromosuccinimide (6.07 g, 34.1 mmol) and benzoyl peroxide (370 mg), and the mixture was heated at reflux for 16 h. After the mixture was cooled, the solvent was removed in vacuo and the residue purified by column chromatography on silica gel (ISCO, 0−20% EtOAc in hexanes) to afford 3.19 g (43%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 4.70 (s, 2H), 7.50 (m, 2H), 7.58 (d, 1H), 8.06 (dd, 1H), 8.12 (d, 1H).

6-Bromomethylnicotinonitrile (19) and 2-Bromomethyl-7-fluoroquinoline (21). **19** and **21** were prepared in a similar manner to that described for **20**.

2-Chloromethylimidazo[1,2-*a*]pyridine Hydrochloride (22). Imidazo[1,2-*a*]pyridine-2-ylmethanol (500 mg, 3.37 mmol) and $SOCl_2$ (5 mL) were heated at reflux for 20 min and evaporated to dryness to afford the title compound used without further purification.

3-Chloromethyl-5-methylisoxazole (23), 3-Chloromethyl-1,5-dimethyl-1*H*-pyrazole (24), and 5-Chloromethyl-1,3-dimethyl-1*H*-pyrazole (25). **23**−**25** were prepared from their corresponding alcohols in a manner identical to that described for **22**.

6-Chloromethyl-2,3-dimethylpyridine Hydrochloride (26). 2,3-Lutidine (30.0 g, 0.28 mol) was dissolved in $CHCl_3$ (500 mL) and cooled to 0 °C, after which *m*-CPBA (76.0 g, 0.31 mol) was added in small portions over a period of 5 min. The solution was allowed to warm to ambient temperature, and after 12 h solid $Ca(OH)_2$ (52 g) was added with vigorous stirring. A thick precipitate forms, and after a further 4 h of being stirred, the slurry was filtered through Celite, washing with $CHCl_3$. Evaporation of the filtrate afforded a solid which was triturated with Et_2O (200 mL) to afford 20 g (58%) of 2,3-dimethylpyridine 1-oxide as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.51 (s, 3H), 7.04 (m, 2H), 8.16 (m, 1H).

To a solution of the previously isolated *N*-oxide (17.6 g, 0.143 mol) in CH_2Cl_2 was added trimethylsilyl cyanide (19.8 mL, 0.148 mol). After 30 min, *N*,*N*-diethylcarbamoyl chloride (18.6 mL, 0.148 mol) was added slowly and the resulting reaction mixture maintained at ambient temperature for 60 h. A 10% aqueous solution of K_2CO_3 was carefully added (100 mL) and the resulting two-phase mixture stirred rapidly for 30 min. The organic phase was separated, the aqueous layer extracted with CH_2Cl_2, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified using silica gel chromatography (ISCO, 0−15% EtOAc in hexanes) to yield 10.1 g (53%) of 5,6-dimethylpyridine-2-carbonitrile as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H), 2.55 (s, 3H), 7.45 (d, 1H), 7.53 (d, 1H).

5,6-Dimethylpyridine-2-carbonitrile (5.00 g, 37.8 mmol) in MeOH (500 mL) was cooled to −10 °C and dry HCl gas bubbled through the solution for 15 min. The vessel was sealed and allowed to stand at ambient temperature for 72 h. Water (12 mL) was added and the solution evaporated to dryness in vacuo. The residue was diluted with EtOAc, and saturated $NaHCO_3$ solution was added with caution (gas evolution) and vigorously stirred for 30 min. The EtOAc phase was separated, the aqueous layer was extracted (EtOAc), and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo to afford 5.86 g (94%) of 5,6-dimethylpyridine-2-carboxylic acid methyl ester as a light tan solid.

To the previously isolated methyl ester (5.86 g, 35.5 mmol) in dry THF (60 mL) cooled to −78 °C was slowly added DIBAl-H (1 M

solution in THF, 100 mL, 100 mmol) over a period of 5 min. The reaction mixture was allowed to warm to 0 °C, after which saturated aqueous sodium potassium tartrate solution was carefully added to quench the reduction. After the mixture was stirred vigorously for 30 min, solid citric acid was added to adjust the pH to ~8, the aqueous phase was extracted with EtOAc (×3), and the combined organics were dried ($MgSO_4$), filtered, and evaporated. The residue was purified using silica gel chromatography (ISCO, 100% EtOAc) to afford 4.43 g (91%) of (5,6-dimethylpyridin-2-yl)methanol as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 2.49 (s, 3H), 4.21 (br s, 1H), 4.68 (s, 2H), 6.98 (d, 1H), 7.39 (d, 1H).

Thionyl chloride (5 mL) and the previously isolated alcohol (1.00 g, 0.73 mmol) were heated to reflux for 15 min, allowed to cool, and thoroughly evaporated to dryness in vacuo to afford the title compound as a pale brown solid used without further purification.

5-Chloro-2-chloromethylpyridine (27). 5-Chloropyridine-2-carboxylic acid (0.50 g, 3.17 mmol) in THF (15 mL) was cooled to 0 °C, and BH_3·THF (1 M solution in THF, 19 mL, 19 mmol) was added dropwise. The reaction mixture was then heated to 50 °C for 12 h. After the mixture was cooled to ambient temperature, water was slowly added to quench excess reagent, the reaction mixture was partitioned between EtOAc and water, the organic phase was separated, the aqueous layer was extracted (EtOAc), and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. To the isolated alcohol (237 mg, 1.65 mmol) in CH_2Cl_2 (3 mL) was added $SOCl_2$ (0.6 mL, 8.25 mmol), and the mixture was stirred at ambient temperature for 30 min. Evaporation of the solvent in vacuo afforded the title compound which was used without further purification.

2-Chloromethylindole-1-carboxylic Acid *tert*-Butyl Ester (28). To a solution of 1*H*-indole-2-carboxylic acid ethyl ester (5.00 g, 26.0 mmol) in MeCN (85 mL) were added Boc_2O (6.92 g, 31.2 mmol) and DMAP (323 mg, 2.60 mmol). After 12 h at ambient temperature, 2-amino-5-diethylaminopentane (0.5 mL) was added and the reaction mixture evaporated in vacuo. Purification of the residue using silica gel chromatography (ISCO, 0−10% EtOAc in hexanes) afforded 7.5 g (99%) of indole-1,2-dicarboxylic acid 1-*tert*-butyl ester 2-ethyl ester. ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H), 1.63 (s, 9H), 4.38 (q, 2H), 7.10 (s, 1H), 7.25 (m, 1H), 7.40 (m, 1H), 7.60 (d, 1H), 8.08 (d, 1H).

To a solution of the previously isolated *tert*-butyl ester (7.50 g, 26.0 mmol) in toluene (50 mL) cooled to −40 °C was slowly added DIBAl-H (1 M soln in hexanes, 64.8 mL, 64.8 mmol) over a period of 20 min. After a further 20 min the reaction was carefully quenched upon addition of MeOH (20 mL) and then H_2O (20 mL), and the mixture was stirred vigorously at ambient temperature for 10 min. The mixture was filtered and the filtrate washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo. Purification of the residue using silica gel chromatography (ISCO, 0−25% EtOAc in hexanes) afforded 5.3 g (83%) of 2-hydroxymethylindole-1-carboxylic acid *tert*-butyl ester. ^{1}H NMR ($CDCl_3$) δ 1.71 (s, 9H), 3.77 (t, 1H), 4.80 (d, 2H), 6.57 (s, 1H), 7.16−7.26 (m, 2H), 7.50 (d, 1H), 7.98 (d, 1H).

2-Hydroxymethylindole-1-carboxylic acid *tert*-butyl ester isolated above (1.60 g, 6.47 mmol) was dissolved in CH_2Cl_2, and LiBr (5.45 g, 65 mmol) was added. Triethylamine (1.46 mL, 10.4 mmol) was added followed by methanesulfonyl chloride (816 μL, 10.4 mmol) and the solution maintained at ambient temperature for 24 h. The reaction mixture was diluted with CH_2Cl_2, washed with aqueous saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered, and evaporated in vacuo to afford the title compound which was used immediately without further purification.

Route A. 3-{-3-*tert*-Butylsulfanyl-5-(6-fluoroquinolin-2-ylmethoxy)-1-[4-(6-methoxypyridine-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11c). Step 1: 3-[1-(4-Bromobenzyl)-3-*tert*-butylsulfanyl-5-(6-fluoroquinolin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid Ethyl Ester. To a solution of 3-[1-(4-bromobenzyl)-3-*tert*-butylsulfanyl-5-hydroxy-1*H*-indol-2-yl]-2,2-dimethylpropionic acid ethyl ester[1] (2.00 g, 3.86 mmol) in MeCN (25 mL) was added cesium carbonate (2.50 g, 7.67 mmol) followed by 2-bromomethyl-6-fluoroquinoline **20** (1.02 g, 4.25 mmol), and the suspension was maintained at ambient temperature with vigorous stirring for 16 h. The reaction mixture was partitioned between EtOAc and water. The aqueous phase was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. The product residue was triturated using a mixture of EtOAc and hexanes and the solid collected by filtration to afford 1.43 g of the title compound. The mother liquor was purified by silica gel column chromatography (ISCO, 0−15% EtOAc in hexane gradient) to isolate a further 1.00 g of the title compound. Combined yield obtained was 2.43 g (93%). ^{1}H NMR ($CDCl_3$) δ 1.13 (s, 9H), 1.18 (t, 3H), 1.20 (s, 6H), 3.24 (s, 2H), 4.03 (q, 2H), 5.32 (s, 2H), 5.24 (s, 2H), 6.65 (d, 2H), 6.90 (d, 1H), 7.02 (d, 1H), 7.36 (d, 2H), 7.41 (m, 1H), 7.47 (m, 1H), 7.75 (d, 1H), 8.10 (m, 2H). $[M + H]^+$: m/z calculated 679; observed 679.

Step 2: 3-{3-*tert*-Butylsulfanyl-5-(6-fluoroquinolin-2-ylmethoxy)-1-[4-(4,4,5,5-tetramethyl[1,3,2]dioxaborolan-2-yl)-benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid Ethyl Ester. To a solution of 3-[1-(4-bromobenzyl)-3-*tert*-butylsulfanyl-5-(6-fluoroquinolin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid ethyl ester (from step 1, 1.02 g, 1.51 mmol) in *p*-dioxane (15 mL) were added potassium acetate (443 mg, 4.52 mmol), bis(pinacolato)-diboron (1.08 g, 4.25 mmol), and Pd(dppf)Cl_2·CH_2Cl_2 (125 mg, 0.15 mmol) in a sealable vessel. The mixture was sparged with nitrogen gas for 5 min, sealed, and heated to 90 °C for 12 h. After cooling, the reaction mixture was partitioned between EtOAc and water, the aqueous layer was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified on silica gel (ISCO, 0−20% EtOAc in hexanes) to afford the title compound as off-white foam (910 mg, 84%) used without further analysis.

Step 3: 3-{3-*tert*-Butylsulfanyl-5-(6-fluoroquinolin-2-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid Ethyl Ester. A solution of the product of step 2 (250 mg, 0.345 mmol), K_2CO_3 (145 mg, 0.105 mmol), 5-bromo-2-methoxypyridine (91 mg, 0.484 mmol), and Pd(PPh_3)$_4$ (55 mg, 0.048 mmol) in DME/H_2O 2:1 (6 mL) was degassed via sparging with nitrogen and heated at 85 °C for 4 h. After cooling, the mixture was poured into water, extracted with EtOAc (×2) and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified using silica gel chromatography (ISCO, 0−15% EtOAc in hexanes) to afford 206 mg (85%) of the title ester. ^{1}H NMR ($CDCl_3$) δ 1.14 (s, 9H), 1.16 (t, 3H), 1.22 (s, 3H), 3.29 (s, 2H), 3.96 (s, 3H), 4.05 (q, 2H), 5.42 (s, 4H), 6.78 (d, 1H), 6.87 (d, 2H), 6.91 (d, 1H), 7.10 (d, 1H), 7.37−7.53 (m, 5H), 7.70 (d, 1H), 7.75 (d, 1H), 8.12 (m, 2H), 8.31 (d, 1H).

Step 4: 3-{-3-*tert*-Butylsulfanyl-5-(6-fluoroquinolin-2-ylmethoxy)-1-[4-(6-methoxypyridine-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11c). To a solution of the ethyl ester from step 3 (206 mg, 0.304 mmol) in a mixture of THF (3 mL), MeOH (1.5 mL), and H_2O (1.5 mL) was added LiOH·H_2O (75 mg, 1.79 mmol), and the reaction mixture was heated to 60 °C for 4 h. After cooling, the reaction mixture was diluted with EtOAc and H_2O, and solid citric acid was added to achieve an aqueous pH of ~3. The organic phase was separated and the aqueous layer extracted with EtOAc. The combined organic phases were washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo to afford the title compound as a colorless solid in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 9H), 1.26 (s, 6H), 3.33 (s, 2H), 3.95 (s, 3H), 5.40 (s, 2H), 5.43 (s, 2H), 6.76 (d, 1H), 6.85 (d, 2H), 6.92 (d, 1H), 7.07 (d, 1H), 7.35 (d, 2H), 7.44 (m, 1H), 7.49 (m, 1H), 7.69 (d, 1H), 7.73 (d, 1H), 8.12 (m, 2H), 8.30 (d, 1H). $[M + H]^+$: m/z calculated 678; observed 678.

The following compounds were prepared using route A.

3-{-3-*tert*-Butylsulfanyl-5-(7-fluoroquinolin-2-ylmethoxy)-1-[4-(6-methoxypyridine-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11d). For step 1, **21** was used as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 9H), 1.26 (s, 3H), 3.34 (s, 2H), 3.95 (s, 3H), 5.41 (s, 2H), 5.54 (s, 2H), 6.76 (d, 1H), 6.85 (d, 2H), 6.91 (d, 1H), 7.07 (d, 1H), 7.36 (m, 4H), 7.67−7.83 (m, 4H), 8.16 (d, 1H), 8.30 (d, 1H). $[M + H]^+$: m/z calculated 678; observed 678.

3-{-3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridine-3-yl)-benzyl]-5-(6-methylquinolin-2-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11e). For step 1, the alkylation was performed at 45 °C in MeCN using **17** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 9H), 1.26 (s, 6H), 2.54 (s, 3H), 3.33 (s, 2H), 3.95 (s, 3H), 5.40 (s, 2H), 5.43 (s, 2H), 6.76 (d, 1H), 6.87 (d, 2H), 6.91 (d, 1H), 7.04 (d, 1H), 7.37 (m, 3H), 7.55 (m, 2H), 7.68 (m, 2H), 8.03 (d, 1H), 8.08 (d, 1H), 8.30 (d, 1H). $[M + H]^+$: *m*/*z* calculated 674; observed 674.

3-{3-*tert*-Butylsulfanyl-5-(imidazo[1,2-*a*]pyridine-2-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11f). For step 1, the alkylation was performed at 55 °C in a 15:1 mixture of MeCN/DMF for 48 h using **22** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 9H), 1.28 (s, 6H), 3.34 (s, 2H), 3.95 (s, 3H), 5.29 (s, 2H), 5.43 (s, 2H), 6.75−6.83 (m, 5H), 6.94 (d, 1H), 7.20 (m, 1H), 7.30 (d, 2H), 7.38 (s, 1H), 7.64−7.73 (m, 3H), 8.07 (d, 1H), 8.28 (d, 1H). $[M + H]^+$: *m*/*z* calculated 649; observed 649.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(3-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11h). For step 1, the alkylation was performed at ambient temperature using DMF as solvent with the addition of catalytic TBAI and **16a** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 15H), 2.44 (s, 3H), 3.35 (s, 2H), 3.94 (s, 3H), 5.24 (s, 2H), 5.42 (s, 2H), 6.76 (d, 1H), 6.83 (m, 3H), 6.96 (s, 1H), 7.22 (dd, 1H), 7.32 (d, 2H), 7.40 (d, 1H), 7.55 (d, 1H), 7.68 (dd, 1H), 8.30 (d, 1H), 8.50 (d, 1H). $[M + H]^+$: *m*/*z* calculated 624; observed 624.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(4-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11i). For step 1, the alkylation was performed at ambient temperature using DMF as solvent with the addition of catalytic TBAI using **16b** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 1.27 (s, 6H), 2.37 (s, 3H), 3.34 (s, 2H), 3.95 (s, 3H), 5.20 (s, 2H), 5.43 (s, 2H), 6.76 (d, 1H), 6.86 (m, 3H), 7.03 (d, 1H), 7.06 (d, 1H), 7.34 (m, 3H), 7.43 (s, 1H), 7.68 (dd, 1H), 8.29 (d, 1H), 8.48 (d, 1H). $[M + H]^+$: *m*/*z* calculated 624; observed 624.

3-[3-*tert*-Butylsulfanyl-5-(5-ethylpyridin-2ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11l). For step 1, the alkylation was performed at 45 °C in MeCN with catalytic TBAI for 72 h with **16e** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 1.26 (m, 9H), 2.65 (q, 2H), 3.34 (s, 2H), 3.95 (s, 3H), 5.20 (s, 2H), 5.43 (s, 2H), 6.76 (d, 1H), 6.83 (m, 3H), 7.01 (d, 1H), 7.33 (m, 3H), 7.54 (m, 2H), 7.68 (dd, 1H), 8.30 (d, 1H), 8.48 (d, 1H). $[M + H]^+$: *m*/*z* calculated 638; observed 638.

3-[3-*tert*-Butylsulfanyl-5-(3,5-dimethylpyridin-2ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11m). For step 1, the alkylation reaction was performed at ambient temperature using DMF as solvent with catalytic TBAI and **16f** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 6H), 1.25 (s, 9H), 2.31 (s, 3H), 2.40 (s, 3H), 3.35 (s, 2H), 3.95 (s, 3H), 5.20 (s, 2H), 5.41 (s, 2H), 6.74−6.93 (m, 4H), 6.91 (d, 1H), 7.31 (d, 2H), 7.39 (m, 2H), 7.67 (dd, 1H), 8.28 (d, 1H), 8.30 (s, 1H). $[M + H]^+$: *m*/*z* calculated 638; observed 638.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(5-methylisoxazol-3-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11s). For step 1, the alkylation reaction was carried out at 45 °C in MeCN for 12 h using **23** as alkylating reagent. ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 15H), 2.41 (s, 3H), 3.35 (s, 2H), 3.95 (s, 3H), 5.15 (s, 2H), 5.45 (s, 2H), 6.13 (s, 1H), 6.77 (d, 1H), 6.76−6.87 (m, 3H), 7.08 (d, 1H), 7.34 (m, 3H), 7.68 (dd, 1H), 8.31 (d, 1H). $[M + H]^+$: *m*/*z* calculated 614; observed 614.

3-[3-*tert*-Butylsulfanyl-5-(1,5-dimethyl-1*H*-pyrazol-3-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11t). For step 1, the alkylation reaction was performed using MeCN as solvent with catalytic TBAI and **24** as alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 15H), 2.24 (s, 3H), 3.36 (s, 2H), 3.85 (s, 3H), 3.95 (s, 3H), 5.03 (s, 2H), 5.46 (s, 2H), 6.11 (s, 1H), 6.77 (d, 1H), 6.80 (dd, 1H), 6.84 (d, 2H), 7.07 (d, 1H), 7.36 (m, 3H), 7.71 (dd, 1H), 8.32 (d, 1H). $[M + H]^+$: *m*/*z* calculated 627; observed 627.

3-[3-*tert*-Butylsulfanyl-5-(2,5-dimethyl-2*H*-pyrazol-3-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11u). For step 1, the alkylation reaction was performed using MeCN as solvent with catalytic TBAI and **25** as alkylating reagent. ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 15H), 2.24 (s, 3H), 3.85 (s, 3H), 3.95 (s, 3H), 5.03 (s, 2H), 5.46 (s, 2H), 6.11 (s, 1H), 6.77 (d, 1H), 6.80 (dd, 1H), 6.84 (d, 2H), 7.07 (d, 1H), 7.36 (m, 3H), 7.71 (dd, 1H), 8.32 (d, 1H). $[M + H]^+$: *m*/*z* calculated 627; observed 627.

For the following compounds, step 1 was performed using **16c** as alkylating agent and the coupling partner in step 3 was chosen accordingly.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11v). For step 3, 6-bromo-2-methoxypyridine was used as the cross-coupling partner. ^{1}H NMR (DMSO-d_6) δ 1.11 (s, 15H), 2.28 (s, 3H), 3.91 (s, 3H), 5.15 (s, 2H), 5.53 (s, 2H), 6.74 (d, 1H), 6.83 (dd, 1H), 6.93 (d, 2H), 7.10 (d, 1H), 7.35 (m, 2H), 7.49 (d, 1H), 7.60 (d, 1H), 7.74 (m, 1H), 7.97 (d, 2H), 8.41 (s, 1H). $[M + H]^+$: *m*/*z* calculated 624; observed 624.

3-[3-*tert*-Butylsulfanyl-1-[4-(2-ethoxythiazol-4-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11y). For step 3, 4-bromo-2-ethoxythiazole was used as the cross-coupling partner. ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H), 1.25 (s, 6H), 1.43 (t, 3H), 2.34 (s, 3H), 3.32 (s, 2H), 4.48 (q, 2H), 5.20 (s, 2H), 5.39 (s, 2H), 6.74 (s, 1H), 6.80 (m, 3H), 7.00 (d, 1H), 7.27 (d, 1H), 7.47 (d, 1H), 7.50 (d, 1H), 7.63 (d, 2H), 8.45 (s, 1H). $[M + H]^+$: *m*/*z* calculated 644; observed 644.

3-[3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-[4-(6-trifluoromethylpyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11z). For step 3, 5-bromo-2-trifluoromethylpyridine was used as the coupling partner. ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H), 1.23 (s, 6H), 2.35 (s, 3H), 3.34 (s, 2H), 5.17 (s, 2H), 5.46 (s, 2H), 6.81 (dd, 1H), 6.90 (d, 2H), 6.95 (d, 1H), 7.32 (d, 1H), 7.41 (d, 2H), 7.47 (d, 1H), 7.54 (dd, 1H), 7.69 (d, 1H), 7.92 (dd, 1H), 8.47 (s, 1H), 8.83 (d, 1H). $[M + H]^+$: *m*/*z* calculated 662; observed 662.

3-[3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-[4-(5-trifluoromethylpyridin-2-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11aa). For step 3, 2-chloro-5-trifluoromethylpyridine was used as the coupling partner. ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 9H), 1.23 (s, 6H), 2.34 (s, 3H), 3.34 (s, 2H), 5.20 (s, 2H), 5.46 (s, 2H), 6.81 (dd, 1H), 6.88 (d, 2H), 6.98 (d, 1H), 7.31 (d, 1H), 7.46 (d, 1H), 7.53 (d, 1H), 7.72 (d, 1H), 7.84 (d, 2H), 7.93 (dd. 1H), 8.45 (s, 1H), 8.90 (s, 1H). $[M + H]^+$: *m*/*z* calculated 662; observed 662.

3-[3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-[4-(5-trifluoromethylpyridin-2-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11ee). For step 3, 2-chloro-5-methylpyridine was used as the coupling partner. The title compound was purified using preparative HPLC and isolated as the trifluoromethanesulfonic acid salt. ^{1}H NMR ($CDCl_3$) δ 1.20 (s, 6H), 1.23 (s, 9H), 2.52 (s, 3H), 2.55 (s, 3H), 3.35 (s, 2H), 5,46 (s, 2H), 5.52 (s, 2H), 6.84 (d, 1H), 6.99 (m, 3H), 7.29 (m, 1H), 7.67 (d, 2H), 7.85 (d, 1H), 7.92 (d, 1H), 8.13 (m, 2H), 8.70 (s, 1H), 8.76 (s, 1H). $[M + H]^+$: *m*/*z* calculated 592; observed 592.

Route B. 3-{-3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridine-3-yl)benzyl]-5-(quinoxalin-2-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11b). Step 1: 3-{3-*tert*-Butylsulfanyl-5-hydroxy-1-[4-(4,4,5,5-tetramethyl-1-[1,3,2]dioxaborolan-2-yl)-benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid Ethyl Ester (12). To a solution of 3-[1-(4-bromobenzyl)-3-*tert*-butylsulfanyl-5-hydroxyl-1*H*-indol-2-yl]-2,2-dimethylpropionic acid ethyl ester[1] (35.0 g, 67.5 mmol) in *p*-dioxane (350 mL) were added potassium acetate (19.9 g, 202.7 mmol), bis(pinacolato)diboron (25.0 g, 98.4 mmol), and [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane complex (2.50 g, 3.1 mmol), and the solution was sparged with nitrogen for 30 min. The resulting mixture was heated under nitrogen at 85 °C for 12 h, after which the solvent volume was reduced to ∼100 mL in vacuo, the residue taken up in EtOAc (1 L), washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo. Purification of the residue using silica gel chromatography (ISCO, 0−

15% EtOAc in hexanes) afforded 33.5 g (88%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3H), 1.21 (s, 6H), 1.25 (s, 9H), 1,31 (s, 12H), 3.25 (s, 2H), 4.04 (q, 2H), 4.69 (s, 1H), 5.37 (s, 2H), 6.65 (dd, 1H), 6.76 (d, 2H), 6.96 (d, 1H), 7.17 (d, 1H), 7.67 (d, 2H). $[M + H]^+$: m/z calculated 566; observed 566.

Step 2: 3-{3-*tert*-Butylsulfanyl-5-hydroxy-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid Ethyl Ester (13, R^2 = 6-Methoxypyridin-3-yl). To a solution of **12** (25.3 g, 44.8 mmol) in a mixture of 1,2-dimethoxyethane (300 mL) and water (150 mL) were added potassium carbonate (15.5 g, 112.1 mmol) and 5-bromo-2-methoxypyridine (10.9 g, 58.0 mmol). The mixture was degassed via sparging with nitrogen gas for 30 min and tetrakis(triphenylphosphine)palladium(0) (1.0 g, 0.86 mmol) and heated at 80 °C under nitrogen for 24 h. The reaction mixture was reduced in volume in vacuo and diluted in EtOAc and washed with water. The aqueous phase was extracted with EtOAc, and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified using silica gel chromatography (0−20% EtOAc in hexanes) to yield 23.7 g (96%) of the title compound as a colorless foam. ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H), 1.23 (s, 6H), 1.28 (s, 9H), 3.29 (s, 2H), 3.97 (s, 3H), 4.04 (q, 2H), 5.40 (s, 2H), 6.70 (dd, 1H), 6.79 (d, 1H), 6.85 (d, 2H), 7.20 (d, 1H), 7.37 (d, 2H), 7.72 (dd, 1H), 8.31 (d, 1H).

Step 3: 3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)-benzyl]-5-(quinoxalin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid Ethyl Ester. To the phenol isolated in step 2 (1.62 g, 2.96 mmol) in MeCN (45 mL) were added cesium carbonate (2.89 g, 8.87 mmol) and 2-chloromethylquinoxaline (**15**, 0.58 g, 3.25 mmol), and the suspension was stirred at ambient temperature for 12 h. The reaction mixture was partitioned between CH_2Cl_2 and water, and the organic phase was separated, washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified using silica gel chromatography (ISCO, 0−5% EtOAc in CH_2Cl_2) to afford 1.84 g (90%) of the title ester as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 9H), 1.17 (t, 3H), 1.23 (s, 6H), 3.29 (s, 2H), 3.96 (s, 3H), 4.05 (q, 2H), 5.43 (s, 2H), 5.50 (s, 2H), 6.78 (d, 1H), 6.85 (d, 2H), 6.94 (dd, 1H), 7.10 (d, 1H), 7.37 (m, 3H), 7.72 (dd, 1H), 7.78 (m, 2H), 8.11 (m, 2H), 8.31 (d, 1H), 9.14 (s, 1H).

Step 4: 3-{-3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridine-3-yl)benzyl]-5-(quinoxalin-2-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid. To the ester isolated in step 3 (150 mg, 0.22 mmol) in a mixture of THF (4 mL), MeOH (1.3 mL), and water (1.3 mL) was added $LiOH·H_2O$ (45 mg, 1.07 mmol), and the solution was heated at 60 °C for 4 h. Upon completion of the reaction the mixture was diluted with EtOAc and water, solid citric acid was added to attain an aqueous pH of ∼3, and the aqueous phase was extracted with EtOAc. The organic phase was washed with water, dried ($MgSO_4$), filtered, and evaporated in vacuo to yield 140 mg (97%) of the title compound. ^{1}H NMR ($CDCl_3$) δ 1.15 (s, 9H), 1.26 (s, 6H), 3.33 (s, 2H), 3.95 (s, 3H), 5.45 (s, 2H), 5.49 (s, 2H), 6.76 (d, 1H), 6.85 (d, 2H), 6.94 (dd, 1H), 7.10 (d, 1H), 7.38 (m, 3H), 7.70 (dd, 1H), 7.78 (m, 2H), 8.11 (m, 2H), 8.30 (d, 1H), 9.14 (s, 1H). $[M + H]^+$: m/z calculated 661; observed 661.

The following compounds were prepared according to route B using the intermediate prepared from step 2 (Scheme 1, R^2 = 6-methoxy-pyridin-3-yl).

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(quinolin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11a). For step 3, 2-chloromethylquinoline hydrochloride was used as the alkylating agent with catalytic TBAI and DMF as solvent. ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 15H), 3.32 (s, 2H), 3.95 (s, 3H), 5.43 (s, 4H), 6.77 (d, 1H), 6.84 (d, 2H), 6.90 (dd, 1H), 7.07 (d, 1H), 7.36 (m, 3H), 7.54 (m, 1H), 7.70 (dd, 1H), 7.74 (d, 2H), 7.81 (d, 1H), 8.11 (d, 1H), 8.17 (d, 1H), 8.30 (d, 1H). $[M + H]^+$: m/z calculated 660; observed 660.

3-{3-*tert*-Butylsulfanyl-5-(1*H*-indol-2-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11g). For step 3, 2-chloromethylindole-1-carboxylic acid *tert*-butyl ester (**28**) was used as the alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 1.26 (s, 6H), 3.34 (s, 2H), 3.95 (s, 3H), 5.27 (s, 2H), 5.44 (s, 2H), 6.53 (s, 1H), 6.76 (d, 1H), 6.85 (m, 3H), 7.07 (d, 1H), 7.08 (m, 1H), 7.13 (m, 1H), 7.32−7.40 (m, 4H), 7.58 (d, 1H), 7.69 (dd, 1H), 8.31 (d, 1H), 8.46 (s, 1H). $[M + H]^+$: m/z calculated 648; observed 648

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11j). For step 3, 2-chloromethyl-5-methylpyridine hydrochloride (**16c**) was used as the alkylating agent at 55 °C for 24 h. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.27 (s, 6H), 2.35 (s, 3H), 3.33 (s, 2H), 3.95 (s, 3H), 5.21 (s, 2H), 5.43 (s, 2H), 6.76 (d, 1H), 6.82 (m, 3H), 6.99 (d, 1H), 7.33 (m, 3H), 7.49 (d, 1H), 7.56 (dd, 1H), 7.68 (dd, 1H), 8.28 (d, 1H), 8.47 (s, 1H). $[M + H]^+$: m/z calculated 624; observed 624.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(6-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11k). For step 3, **16d** was used as the alkylating agent using catalytic TBAI in DMF as solvent at 50 °C. ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 15H), 2.81 (s, 3H), 3.34 (s, 2H), 3.95 (s, 3H), 5.45 (s, 2H), 5.54 (s, 2H), 6.78 (d, 1H), 6.88 (m, 3H), 7.07 (d, 1H), 7.35 (m, 4H), 7.71 (m, 2H), 7.95 (m, 1H), 8.32 (s, 1H). $[M + H]^+$: m/z calculated 624; observed 624.

3-{3-*tert*-Butylsulfanyl-5-(5,6-dimethylpyridin-2ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11n). For step 3, compound **26** was used as the alkylating agent at 35 °C for 12 h. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.26 (s, 6H), 2.28 (s, 3H), 2.53 (s, 3H), 3.33 (s, 2H), 3.95 (s, 3H), 5.18 (s, 2H), 5.43 (s, 2H), 6.76 (s, 1H), 6.85 (m, 3H), 7.03 (d, 1H), 7.32 (m, 4H), 7.42 (d, 1H), 7.69 (dd, 1H), 8.30 (d, 1H). $[M + H]^+$: m/z calculated 638; observed 638.

3-{3-*tert*-Butylsulfanyl-5-(5-chloropyridin-2-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11o). For step 3, compound **27** was used as the alkylating agent with catalytic TBAI in DMF as solvent for 12 h. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.27 (s, 6H), 3.35 (s, 2H), 3.96 (s, 3H), 5.24 (s, 2H), 5.45 (s, 2H), 6.78 (d, 1H), 6.87 (m, 3H), 7.08 (d, 1H), 7.30 (d, 1H), 7.37 (d, 2H), 7.54 (d, 1H), 7.69 (m, 2H), 8.30 (d, 1H), 8.57 (d, 1H). $[M + H]^+$: m/z calculated 644; observed 644.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(5-methoxypyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11p). For step 3, compound **18** was used as the alkylating agent with catalytic TBAI and DMF as solvent for 12 h. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.26 (s, 6H), 3.86 (s, 3H), 3.94 (s, 3H), 5.18 (s, 2H), 5.43 (s, 2H), 6.75 (d, 1H), 6.84 (m, 3H), 7.02 (d, 1H), 7.23 (dd, 1H), 7.32 (m, 3H), 7.49 (d, 1H), 7.68 (dd, 1H), 8.29 (d, 1H), 8.32 (d, 1H).). $[M + H]^+$: m/z calculated 641; observed 641.

3-{3-*tert*-Butylsulfanyl-5-(5-carbamoylpyridin-2-ylmethoxy)-1-[4-(6-methoxypyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11q). For step 3, compound **19** was used as the alkylating agent with catalytic TBAI and DMF as solvent for 12 h. ^{1}H NMR (DMSO-d_6) δ 1.14 (s, 15H), 3.26 (s, 2H), 3.89 (s, 3H), 5.24 (s, 2H), 5.56 (s, 2H), 6.92 (m, 4H), 7.11 (d, 1H), 7.38 (d, 1H), 7.58 (d, 2H), 7.66 (d, 1H), 7.99 (dd, 1H), 8.30 (dd, 1H), 8.46 (d, 1H), 9.10 (d, 1H). $[M + H]^+$: m/z calculated 654; observed 654.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-methoxypyridin-3-yl)benzyl]-5-(5-methylpyrazin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11r). For step 3, compound **14** was used as the alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.26 (s, 6H), 2.58 (s, 3H), 3.34 (s, 2H), 3.95 (s, 3H), 5.27 (s, 2H), 5.45 (s, 2H), 6.77 (d, 1H), 6.87 (m, 3H), 7.08 (d, 1H), 7.33 (m, 3H), 7.70 (dd, 1H), 8.30 (d, 1H), 8.46 (s, 1H), 8.71 (s, 1H). $[M + H]^+$: m/z calculated 625; observed 625.

The following compounds were prepared according to route B but varying the cross-coupling partner in step 2 as described and using compound **16c** as the alkylating agent in step 3.

3-[3-*tert*-Butylsulfanyl-1-[4-(3-methoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11w). For step 2, 2-bromo-3-methoxypyridine was used in the cross-coupling reaction. ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 6H), 1.05 (s, 9H), 2.29 (s, 3H), 3.20 (s, 2H), 3.61 (s, 3H), 5.15 (s, 2H), 5.34 (s, 2H), 6.74 (m, 3H), 6.89 (d, 1H), 7.08 (m, 2H), 7.22 (m, 1H), 7.41 (m, 2H), 7.59 (d, 2H), 8.11 (s, 1H), 8.39 (s, 1H). $[M + H]^+$: m/z calculated 624; observed 624.

3-{3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-[4-(4-trifluoromethylpyridin-2-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11x). For step 2, 2-chloro-4-trifluoromethylpyridine was used in the cross-coupling reaction. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.27 (s, 6H), 2.34 (s, 3H), 3.33 (s, 2H), 5.19 (s, 2H), 5.45 (s, 2H), 6.81 (dd, 1H), 6.84 (d, 2H), 6.96 (d, 1H), 7.31 (d, 1H), 7.41 (d, 1H), 7.47 (d, 1H), 7.52 (d, 1H), 7.82 (m, 3H), 8.46 (s, 1H), 8.32 (d, 1H). $[M + H]^+$: m/z calculated 662; observed 662.

3-{3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-[4-(6-trifluoromethylpyridin-2-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11bb). For step 2, 2-chloro-6-trifluoromethylpyridine was used as the coupling partner. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.26 (s, 6H), 2.33 (s, 3H), 3.34 (s, 2H), 5.19 (s, 2H), 5.44 (s, 2H), 6.81 (dd, 1H), 6.89 (d, 2H), 6.96 (d, 1H), 7.30 (d, 1H), 7.45 (d, 1H), 7.51 (dd, 1H), 7.56 (d, 1H), 7.77 (d, 1H), 7.84 (d, 1H), 7.87 (d, 2H), 8.44 (s, 1H). $[M + H]^+$: m/z calculated 662; observed 662.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (AM803, 11cc). For step 2, 5-bromo-2-ethoxypyridine was used in the cross-coupling reaction. ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H), 1.26 (s, 6H), 1.40 (t, 3H), 2.34 (s, 3H), 3.33 (s, 2H), 4.36 (q, 2H), 5.19 (s, 2H), 5.42 (s, 2H), 6.73 (d, 1H), 6.82 (m, 3H), 7.00 (d, 1H), 7.33 (m, 3H), 7.47 (d, 1H), 7.53 (dd, 1H), 7.67 (dd, 1H), 8.27 (d, 1H), 8.46 (s, 1H). $[M + H]^+$: m/z calculated 638; observed 638.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-ethoxypyridin-2-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11dd). For step 2, 2-chloro-6-ethoxypyridine was used in the cross-coupling reaction. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.26 (s, 6H), 1.40 (t, 3H), 2.33 (s, 3H), 3.34 (s, 2H), 4.42 (q, 2H), 5.20 (s, 2H), 5.43 (s, 2H), 6.62 (d, 1H), 6.82 (dd, 1H), 6.86 (d, 2H), 7.00 (d, 1H), 7.20 (d, 1H), 7.31 (d, 1H), 7.45–7.58 (m, 3H), 7.85 (d, 2H), 8.45 (s, 1H). $[M + H]^+$: m/z calculated 638; observed 638.

3-[3-*tert*-Butylsulfanyl-1-[4-(5-fluoropyridine-2-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11ff). For step 2, 2-bromo-5-fluoropyridine was used in the cross-coupling reaction. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.26 (s, 6H), 2.34 (s, 3H), 3.33 (s, 2H), 5.20 (s, 2H), 5.43 (s, 2H), 6.83 (m, 3H), 6.99 (d, 1H), 7.31 (d, 1H), 7.38–7.54 (m, 3H), 7.60 (dd, 1H), 7.72 (d, 2H), 8.45 (s, 1H), 8.50 (d, 1H). $[M + H]^+$: m/z calculated 612; observed 612.

3-[3-*tert*-Butylsulfanyl-1-[4-(5-fluoropyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11gg). For step 2, 3-bromo-5-fluoropyridine was used as the coupling partner. ^{1}H NMR ($CDCl_3$) δ 1.22 (s, 9H), 1.28 (s, 6H), 2.34 (s, 3H), 5.19 (s, 2H), 5.45 (s, 2H), 6.81 (dd, 1H), 6.88 (d, 2H), 6.96 (d, 1H), 7.32 (d, 1H), 7.36 (d, 2H), 7.49 (m, 3H), 8.40 (d, 1H), 8.47 (s, 1H), 8.53 (s, 1H). $[M + H]^+$: m/z calculated 612; observed 612.

3-[3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-(4-pyridin-3-yl-benzyl)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11hh). For step 2, 3-bromopyridine was used in the cross-coupling reaction. ^{1}H NMR (DMSO-d_6) δ 1.11 (s, 6H), 1.14 (s, 9H), 2.28 (s, 3H), 3.23 (s, 2H), 5.15 (s, 2H), 5.54 (s, 2H), 6.83 (dd, 1H), 6.93 (d, 2H), 7.11 (d, 1H), 7.33 (d, 1H), 7.38 (d, 1H), 7.42 (dd, 1H), 7.58 (d, 1H), 7.62 (d, 2H), 8.00 (d, 1H), 8.40 (s, 1H), 8.53 (d, 1H), 8.83 (d, 1H), 12.41 (br s, 1H). $[M + H]^+$: m/z calculated 594; observed 594.

3-[3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-(4-pyridin-2-yl-benzyl)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11ii). For step 2, 2-chloropyridine was used in the cross-coupling reaction. ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 15H), 2.28 (s, 3H), 3.22 (s, 2H), 5.15 (s, 2H), 5.54 (s, 2H), 6.83 (dd, 1H), 6.92 (d, 2H), 7.10 (d, 1H), 7.35 (m, 3H), 7.60 (dd, 1H), 7.80–7.98 (m, 4H), 8.41 (s, 1H), 8.61 (d, 1H), 12.42 (br s, 1H). $[M + H]^+$: m/z calculated 594; observed 594.

3-[3-*tert*-Butylsulfanyl-1-[4-(5-methoxypyrimidin-2-yl)-benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11kk). For step 2, 2-chloro-5-fluoropyrimidine was used in the cross-coupling step. In step 4 (ester hydrolysis), the reaction time was prolonged to 72 h at 65 °C to force complete displacement of the fluoride with methoxide. ^{1}H NMR ($CDCl_3$) δ 1.21 (s, 9H), 1.26 (s, 6H), 2.33 (s, 3H), 3.33 (s, 2H), 3.92 (s, 3H), 5.21 (s, 2H), 5.44 (s, 2H), 6.81 (m, 3H), 6.99 (d, 1H), 7.30 (d, 1H), 7.46 (d, 1H), 7.52 (d, 1H), 8.12 (s, 1H), 8.15 (s, 1H), 8.43 (s, 2H), 8.45 (s, 1H). $[M + H]^+$: m/z calculated 625; observed 625.

3-[3-*tert*-Butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methyl-1-oxypyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (11ll). For step 2, 5-bromo-2-ethoxypyridine was used as the coupling partner, and in step 3 compound **16g** was used as the alkylating agent. ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 9H), 1.25 (s, 6H), 1.40 (t, 3H), 2.33 (s, 3H), 3.34 (s, 2H), 4.36 (q, 2H), 5.39 (s, 2H), 5.44 (s, 2H), 6.74 (d, 1H), 6.84 (m, 3H), 7.04 (d, 1H), 7.22 (d, 1H), 7.35 (m, 3H), 7.60 (d, 1H), 7.69 (dd, 1H), 8.29 (s, 2H). $[M + H]^+$: m/z calculated 654; observed 654.

3-{3-*tert*-Butylsulfanyl-5-(5-methylpyridin-2-ylmethoxy)-1-[4-(6-oxo-1,6-dihydropyridin-3-yl)benzyl]-1*H*-indol-2-yl}-2,2-dimethylpropionic Acid (11jj). To the sodium salt of **11cc** (prepared through reaction of **11cc** with 1 equiv of NaOH in EtOH for 1 h and evaporation in vacuo; 700 mg, 1.06 mmol) in ethylene glycol (5 mL) was added $LiOH·H_2O$ (400 mg, 10 mmol) in a sealable vessel, and the mixture was heated to 165 °C for 1 week. LCMS analysis indicated incomplete reaction. The reaction mixture was allowed to cool and diluted with EtOAc. Water and solid citric acid were added until aqueous pH was ~4. The aqueous layer was extracted with EtOAc and the organic phase dried ($MgSO_4$), filtered, and evaporated in vacuo. The residue was purified using silica gel chromatography (0–7% MeOH in CH_2Cl_2) to afford the title compound as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.23 (s, 15H), 2.33 (s, 3H), 3.56 (s, 2H), 5.23 (s, 2H), 5.59 (br s, 2H), 6.54 (d, 1H), 6.66 (d, 1H), 6.75 (dd, 1H), 6.94 (d, 2H), 7.01 (d, 2H), 7.13 (s, 1H), 7.31 (s, 1H), 7.44 (d, 1H), 7.51 (d, 1H), 7.63 (d, 1H), 8.42 (s, 1H). $[M + H]^+$: m/z calculated 610; observed 610.

3-[1-[4-(6-Ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (29). To an ice cooled suspension of anhydrous $AlCl_3$ (1.38 g, 10.34 mmol) in CH_2Cl_2 (12 mL) was added water (138 μL, 7.66 mmol) dropwise, and the solution was allowed to attain ambient temperature. After 20 min the solution was recooled to 0 °C and a solution of the ethyl ester of **11cc** (isolated product from step 3 of route B; 1.70 g, 2.55 mmol) in CH_2Cl_2 (12 mL) was added over a period of 5 min. After 1 h, TLC analysis indicated a 60:40 product/SM mixture. The mixture was cooled to 0 °C, and anhydrous $AlCl_3$ (1.25 g, 9.37 mmol) and water (125 μL, 6.94 mmol) were added sequentially. The mixture was stirred at ambient temperature for 1 h and the reaction quenched upon addition of 0.5 N aqueous sodium potassium tartrate solution with vigorous stirring for 1 h. The mixture was extracted with CH_2Cl_2, and the organic phase was dried ($MgSO_4$), filtered, and evaporated in vacuo. Purification of the residue using silica gel chromatography (ISCO, 0–35% EtOAc in hexanes) afforded 1.07 g (73%) of the ethyl ester of **29**. To the isolated ester (100 mg, 0.173 mmol) in a mixture of THF (3 mL), MeOH (1 mL) and water (1 mL) were added $LiOH·H_2O$ (35 mg, 0.833 mmol). The mixture was heated at 60 °C for 4 h. The mixture was diluted with EtOAc and water, and solid citric acid was added to acidify the aqueous layer to a pH of ~3, after which the aqueous layer was extracted with EtOAc and the organic phase dried ($MgSO_4$), filtered, and evaporated in vacuo to yield the title compound as a colorless solid in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 1.32 (s, 6H), 1.40 (t, 3H), 2.33 (s, 3H), 3.00 (s, 2H), 4.36 (q, 2H), 5.13 (s, 2H), 5.34 (s, 2H), 6.38 (s, 1H), 6.74 (d, 1H), 6.81 (dd, 1H), 6.90 (d, 2H), 6.99 (d, 1H), 7.03 (d, 1H), 7.33 (d, 2H), 7.46 (d, 1H), 7.53 (dd, 1H), 7.67 (dd, 1H), 8.27 (d, 1H), 8.45 (s, 1H). $[M + H]^+$: m/z calculated 550; observed 550.

***rac*-3-[1-[4-(6-Ethoxypyridin-3-yl)benzyl]-3-(2-methylpropane-2-sulfinyl)-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (30).** 3-[3-*tert*-Butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic acid (**11cc**, 75.0 mg, 0.118 mmol) was dissolved in CH_2Cl_2 (1 mL) and cooled to −20 °C. *m*-CPBA (29.0 mg, 0.118 mmol) was added and the solution slowly warmed to 0 °C. Upon completion of the reaction as judged by TLC analysis, the reaction mixture was diluted with CH_2Cl_2, washed with water and the

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organic phase separated, dried ($MgSO_4$), filtered, and evaporated in vacuo. Purification of the residue using silica gel chromatography (ISCO, 0−5% MeOH in CH_2Cl_2) afforded 65 mg (85%) of the title compound as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 9H), 1.37 (s, 6H), 1.40 (t, 3H), 2.34 (s, 3H), 3.11 (d, 1H), 3.23 (d, 1H), 4.36 (q, 2H), 5.14 (app q, 2H), 5.29 (d, 1H), 5.47 (d, 1H), 6.74 (d, 1H), 6.80 (m, 3H), 7.00 (d, 1H), 7.38 (d, 2H), 7.43 (d, 1H), 7.53 (dd, 1H), 7.67 (dd, 1H), 7.78 (s, 1H), 8.26 (d, 1H), 8.45 (s, 1H). $[M + H]^+$: m/z calculated 654; observed 654.

3-[3-(3,3-Dimethylbutyryl)-1-[4-(6-ethoxypyridin-3-yl)-benzyl]-5-(5-methylpyridin-2-ylmethoxy)-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (31a). To a solution of the ethyl ester of **29** (150 mg, 0.26 mmol) in 1,2-dichloroethane were added anhydrous $AlCl_3$ (104 mg, 0.78 mmol) and *tert*-butylacetyl chloride (72 μL, 0.52 mmol), and the reaction mixture was heated to 65 °C under nitrogen for 1 h. The mixture was diluted with CH_2Cl_2 (25 mL) and 0.5 N aqueous sodium potassium tartrate solution added (25 mL). The mixture was vigorously stirred for 1 h. The aqueous phase was extracted with CH_2Cl_2 and the organic phase dried ($MgSO_4$), filtered, and evaporated in vacuo. Purification of the residue using silica gel chromatography (ISCO, 0−40% EtOAc in hexanes) afforded 114 mg (65%) of the ester of the title compound. The isolated ester (114 mg, 0.17 mmol) was dissolved in a mixture of THF (3 mL), MeOH (1 mL), and water (1 mL). LiOH·H_2O (40 mg, 0.95 mmol) was added, and the mixture was heated to 60 °C. Upon completion of the hydrolysis (∼2 h) the mixture was allowed to cool and diluted with EtOAc and water. The aqueous pH was adjusted to ∼3 with the addition of solid citric acid, the mixture extracted with EtOAc, and the organic phase dried ($MgSO_4$), filtered, and evaporated in vacuo to yield the title compound as a colorless solid in quantitative yield. ^{1}H NMR ($CDCl_3$) δ 1.09 (s, 9H), 1.32 (s, 6H), 1.40 (t, 3H), 2.36 (s, 3H), 2,90 (s, 2H), 3.64 (s, 2H), 4.36 (q, 2H), 5.17 (s, 2H), 5.40 (s, 2H), 6.74 (d, 1H), 6.81 (dd, 1H), 6.85 (d, 2H), 6.99 (d, 1H), 7.33 (d, 2H), 7.47 (s, 1H), 7.49 (d, 1H), 7.57 (dd, 1H), 7.67 (dd, 1H), 8.26 (d, 1H), 8.48 (s, 1H). $[M + H]^+$: m/z calculated 648; observed 648.

3-[1-[4-(6-Ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-ylmethoxy)-3-phenylacetyl-1*H*-indol-2-yl]-2,2-dimethylpropionic Acid (31b). **31b** was prepared in the same manner as **31a** using phenylacetyl chloride as electrophile. ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 6H), 1.40 (t, 3H), 2.35 (s, 3H), 3.66 (s, 2H), 4.33 (s, 2H), 4.36 (q, 2H), 5.08 (s, 2H), 5.36 (s, 2H), 6.74 (d, 1H), 6.79 (dd, 1H), 6.82 (d, 2H), 6.92 (d, 1H), 7.22−7.33 (m, 8H), 7.49 (s, 1H), 7.58 (dd, 1H), 7.65 (dd, 1H), 8.25 (d, 1H), 8.47 (s, 1H). $[M + H]^+$: m/z calculated 668; observed 668.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Information for the characterization of final compounds **11b**, **11d−11z**, **11bb−11ll** and **29**, **30**, **31a,b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AA, arachidonic acid; LT, leukotriene; 5-LO, 5-lipoxygenase; FLAP, five-lipoxygenase activating protein; 5-HpETE, (5*S*,6*E*,8*Z*,11*Z*,14*Z*)-5-hydroperoxy-6,8,11,14-eicosatetraenoic acid; 5-oxo-ETE, 5-oxo-(6*E*,8*Z*,11*Z*,14*Z*)-6,8,11,14-eicosatetraenoic acid; CysLT, cysteinyl leukotriene; CVD, cardiovascular disease; hLA, human leukocyte assay; hWB, human whole blood; BAL, bronchoalveolar lavage; GPCR, G-protein-coupled receptor; IC_{50}, concentration required to achieve 50% inhibition; hSA, human serum albumin

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