Thiopyrano[2,3,4-*cd*]indoles as 5-Lipoxygenase Inhibitors: Synthesis, Biological Profile, and Resolution of 2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethoxy]butanoic Acid

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Leukotriene biosynthesis inhibitors have potential as new therapies for asthma and inflammatory diseases. The recently disclosed thiopyrano[2,3,4-*cd*]indole class of 5-lipoxygenase (5-LO) inhibitors has been investigated with particular emphasis on the side chain bearing the acidic functionality. The SAR studies have shown that the inclusion of a heteroatom (O or S) in conjunction with an α-ethyl substituted acid leads to inhibitors of improved potency. The most potent inhibitor prepared contains a 2-ethoxybutanoic acid side chain. This compound, **14d** (2-[2-[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethoxy]-butanoic acid, L-699,333), inhibits 5-HPETE production by human 5-LO and LTB_4 biosynthesis by human PMN leukocytes and human whole blood (IC_{50}s of 22 nM, 7 nM and 3.8 μM, respectively). The racemic acid **14d** has been shown to be functionally active in a rat pleurisy model (inhibition of LTB_4, ED_{50} = 0.65 mg/kg, 6 h pretreatment) and in the hyperreactive rat model of antigen-induced dyspnea (50% inhibition at 2 and 4 h pretreatment; 0.5 mg/kg po). In addition, **14d** shows excellent functional activity against antigen-induced bronchoconstriction in the conscious squirrel monkey [89% inhibition of the increase in R_L and 68% inhibition in the decrease in C_{dyn} (0.1 mg/kg, n = 3)] and in the conscious sheep models of asthma (iv infusion at 2.5 μg/kg/min). Acid **14d** is highly selective as an inhibitor of 5-LO activity when compared to the inhibition of human 15-LO, porcine 12-LO and ram seminal vesicle cyclooxygenase (IC_{50} > 5 μM) or competition in a FLAP binding assay (IC_{50} > 10 μM). Resolution of **14d** affords **14g**, the most potent diastereomer, which inhibits the 5-HPETE production of human 5-LO and LTB_4 biosynthesis of human PMN leukocytes and human whole blood with IC_{50}s of 8 nM, 4 nM, and 1 μM respectively. The *in vitro* and *in vivo* profile of **14d** is comparable to that of MK-0591, which has showed biochemical efficacy in inhibiting *ex vivo* LTB_4 biosynthesis and urinary LTE_4 excretion in clinical trials.

Introduction

The leukotrienes (LTs) are a family of lipophilic eicosanoic acid compounds which are biosynthesized from arachidonic acid.[1] The first enzyme of the LT cascade is 5-lipoxygenase (5-LO), a cytosolic enzyme which contains a non-heme iron at the active site.[2] Through the action of 5-LO, arachidonic acid is initially oxygenated to give 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which is then transformed by the same enzyme to LTA_4 (Figure 1). An essential component for both steps is the presence of an 18-kDa membrane bound protein, 5-lipoxygenase activating protein (FLAP).[3] Upon cellular activation, 5-LO has been shown to translocate to the membrane[4] where it presumably can interact with FLAP and perhaps also phospholipase A_2, in an as yet to be elucidated fashion, to produce LTs.[5] LTA_4 is converted by the action of LTA_4 hydrolase to LTB_4, a potent chemotactic agent.[6] Alternatively, LTA_4 may be coupled with glutathione to produce LTC_4 which is further metabolized to LTD_4 and then LTE_4. These peptidoleukotrienes are potent constrictors of airway smooth muscle and collectively they account for the properties of the slow reacting substance of anaphylaxis (SRS-A).[7]

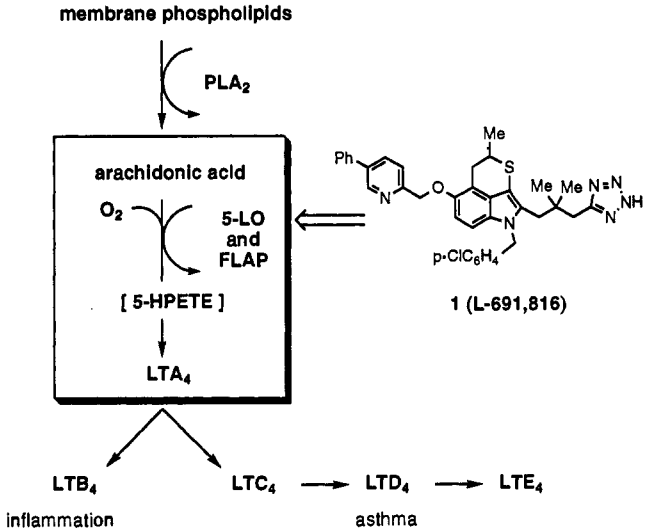

Figure 1. Biosynthesis of leukotrienes from arachidonic acid and structure of 5-LO inhibitor 1.

During the past 5 years it has become clear that it is possible to reduce the clinical symptoms of asthma through the use of chemical entities that block the action of LTs *in vivo*.[8] Such evidence has been obtained from clinical trials involving LTD_4 antagonists,[9,10] 5-LO inhibitors,[11,12]

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and FLAP inhibitors.[13] The latter two approaches involve inhibiting the production of LTA_4 from arachidonic acid, and this may also provide therapies for diseases in which LTB_4 has been implicated,[14] for example inflammatory bowel disease.

We have recently described a new class of direct 5-LO inhibitors based on the thiopyrano[2,3,4-*cd*]indole skeleton[15] which is exemplified by 1 (5-[3-[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-2,2-dimethylpropyl]-1*H*-tetrazole, L-691,816).[16] The tetrazole 1 has been shown to be a potent and highly selective 5-LO inhibitor which has excellent oral activity in a number of animal models of asthma and inflammation. Also, in contrast to several other classes of 5-LO inhibitors which can function as reducing agents for the non-heme iron of the enzyme, 1 has been shown to inhibit the enzyme reaction by a nonredox mechanism, most likely by the formation of a reversible dead-end complex with 5-LO.

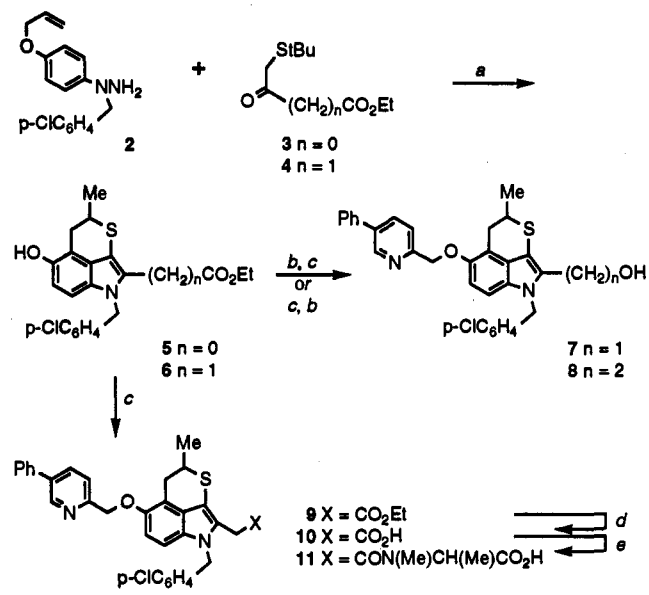
This paper describes the continuation of our SAR investigations into this new series with particular emphasis on modifications to the chain bearing the acidic functionality. As a result of this work, we have been able to identify **14d** (designated L-699,333), which has improved *in vitro* and *in vivo* potency over 1. The profile of **14d** will be presented as well as its resolution to provide **14g**, the most active diastereoisomer.

Chemistry

Previous SAR studies on the thiopyrano[2,3,4-*cd*]indole series have shown that the phenylpyridine group and the thiopyran ring as well as an acidic group on the C-2 side chain are all required for activity as an inhibitor of 5-LO.[16] In addition it has been shown that the enzyme can discriminate between compounds that are enantiomeric at the C-4 methyl of the thiopyran ring. The (-)-isomer is approximately 3–5 times more potent than the (+)-isomer.[15] The most significant modifications in terms of increasing *in vitro* potency were those made to the C-2 side chain. Therefore, other C-2 side chain derivatives were prepared with particular emphasis on chains containing O, N, and S heteroatoms. In conjunction with this, the effect of alkyl substituents on the side chain was investigated.

The key intermediates for all the compounds described (with the exception of the amide acid **11**) are the racemic alcohols **7** and **8**. They were synthesized as depicted in Scheme 1 using previously described materials and methodologies.[16,18] The thiopyrano[2,3,4-*cd*]indole skeleton was constructed in rapid fashion by a Fischer condensation between the allyl hydrazine **2** and a tert-butyl thioketone (**3** or **4**) followed by a Claisen rearrangement and an intramolecular cyclization to give the tricyclic ring systems **5** or **6**. The phenol **5** was alkylated with 2-(chloromethyl)-5-phenylpyridine and the ester reduced with $LiAlH_4$ in THF to give **7**. Although this sequence can be used to prepare **8**, the alkylation of **6** (to give **9**) is complicated by alkylation α to the ester, and the yields are somewhat reduced. This byproduct can be avoided by reversing the sequence of reactions (reduction to the diol then chemoselective alkylation of the phenol) to afford **8** in high yield (79% for two steps). Nevertheless, ester **9** can be obtained and hydrolyzed to give the acid **10**. The amide acid **11** was prepared from **10** using 1,1′-carbonyldiimidazole and *N*-methyl-D,L-alanine methyl ester hydrochloride in DMF/CH_3CN and subsequent hydrolysis (43% for both steps).

Scheme 1[a]



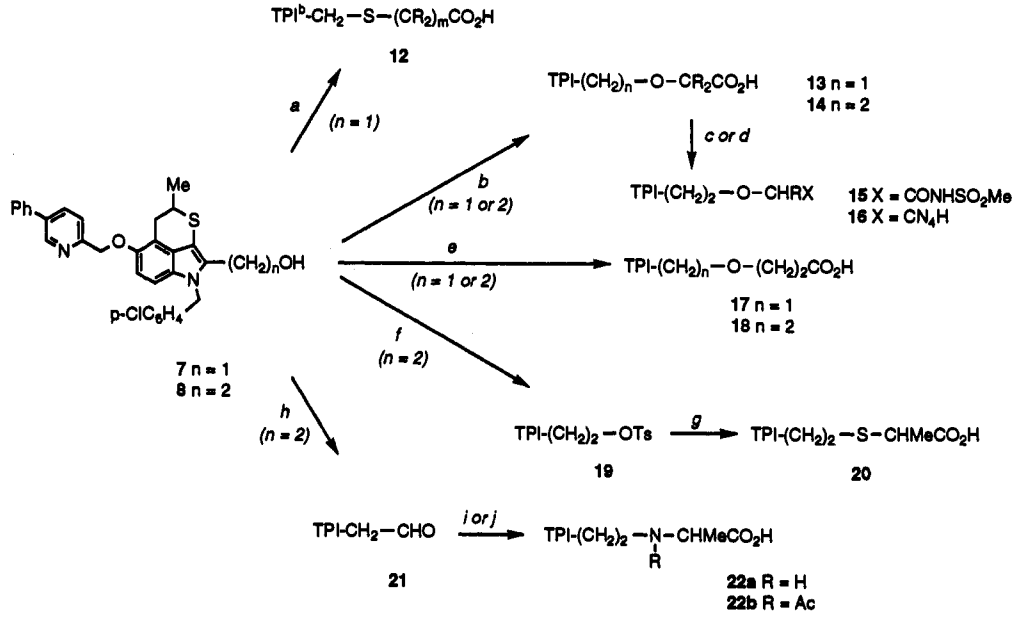
[a] Reagents: (a) toluene, HOAc, NaOAc; 1,2-$Cl_2C_6H_4$, 200 °C then p-TSA, 150 °C; (b) $LiAlH_4$, THF; (c) RCH_2Cl, Cs_2CO_3, CH_3CN, DMF; (d) aq LiOH, THF, MeOH; (e) MeHNCH(Me)CO_2Me·HCl, 1,1′-carbonyldiimidazole, Et_3N, DMF.

Alcohols **7** and **8** thus prepared were converted to the final products as outlined in Scheme 2. Alcohol **7** was smoothly transformed into a thioether upon treatment with BF_3·OEt_2 and the appropriate thiol in 1,2-dichloroethane.[19] The thioether acid **12** is obtained directly in the case where the thiol contains an acid, or after basic hydrolysis when a thiol ester is used. This methodology allows for the convenient introduction of substituted and unsubstituted thioether acid chains (compounds **12a–12g**, Table 1). To prepare the corresponding homologated thiol acid **20**, the alcohol **8** was first converted to a tosylate **19** and the tosylate then displaced using thiolactic acid and NaH in DMF.

For compounds containing an oxygen atom α to the acid, the sodium alkoxides of both **7** and **8** may be used to displace an α-bromo ester or an α-bromo acid to provide (after hydrolysis in the case where an ester is used) the acids **13** and **14**. Acid **14** can then be converted to the *N*-sulfonyl amide **15** via the acid chloride. For the tetrazole **16**, the ester precursor of acid **14** was reacted with dimethylaluminum amide[20] in THF and the resulting amide produced was dehydrated to the nitrile using TFAA in pyridine[21] (90% yield for two steps). It is possible to convert the ester into the nitrile directly using dimethylaluminum amide in xylene,[22] but the yield is low (20%), so the two-step procedure was employed. Reaction of the nitrile with *n*-Bu_3SnN_3[23,24] in 1,2-dichlorobenzene then afforded the tetrazole **16**. The 3-substituted propionic acid derivatives **17** and **18** were obtained by a Michael addition of either **7** or **8** to methyl acrylate in THF with Triton B followed by hydrolysis of the ester.

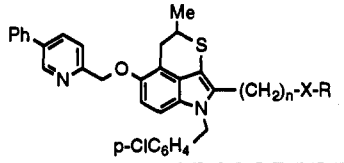
Lastly, a nitrogen link was introduced by a reductive amination sequence using the aldehyde **21** (prepared by the oxidation of **8** with SO_3–pyridine complex and Et_3N in DMSO and CH_2Cl_2) and D,L-alanine methyl ester hydrochloride in the presence of $NaBH_3CN$. The product was then hydrolyzed to yield compound **22a** or acetylated and then hydrolyzed to give **22b**.

Scheme 2[a]



[a] Generic groups are defined in Table 1. [b]TPI = 1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl. Reagents: (a) thiol, BF_3·OEt_2, CH_2ClCH_2Cl (then aq LiOH, THF, MeOH on ester); (b) NaH, THF $BrCR_2CO_2R'$; aq LiOH, THF, MeOH on ester; (c) NaH, pyridine, $(COCl)_2$, Et_3N, $MeSO_2NH_2$, DMAP; (d) Me_2AlNH_2, *m*-xylene; pyridine, TFAA, THF; *n*-Bu_3SnN_3, 1,2-$Cl_2C_6H_4$, Δ; (e) Triton B, CH_2=$CHCO_2Me$, THF; aq LiOH, THF, MeOH; (f) TsCl, pyridine, (g) NaH, $HSCHMeCO_2H$, DMF; (h) SO_3·pyridine, DMSO, Et_3N, CH_2Cl_2; (i) D,L-$H_2NCHMeCO_2Me$·HCl, $NaBH_3CN$, THF, MeOH; aq LiOH, THF, MeOH; (j) D,L-$H_2NCHMeCO_2Me$·HCl, $NaBH_3CN$, THF, MeOH; AcCl, THF, Et_3N; aq LiOH, THF, MeOH.

Table 1. SAR of C-2 Side Chain



compd	*n*	X-R	IC_{50}, nM[a] human 5-LO	IC_{50}, nM[a] HPMN
1		$CH_2CMe_2CH_2CN_4H$	75	8
13	1	OCH_2CO_2H	–[b]	17
17	1	$OCH_2CH_2CO_2H$	250*	65
12a	1	SCH_2CO_2H	–	15
12b	1	$SCH_2CH_2CO_2H$	55	10
12c	1	$SCH_2CHMeCO_2H$	–	20
12d	1	$SCHMeCH_2CO_2H$	70*	50
12e	1	$SCH_2CHEtCO_2H$ (2*R*,*S*)	–	6
12f	1	$SCH_2CHEtCO_2H$ (2*R*)	40	7
12g	1	$SCH_2CHEtCO_2H$ (2*S*)	120	12
14a	2	OCH_2CO_2H	–	31
18	2	$OCH_2CH_2CO_2H$	105	11
20	2	$SCHMeCO_2H$	–	17
22a	2	$NHCHMeCO_2H$	4000*	1100*
22b	2	$NAcCHMeCO_2H$	900*	113
11	1[c]	$C(O)NMeCHMeCO_2H$	300	200
14b	2	$OCHMeCO_2H$	20	5
14c	2	$OCMe_2CO_2H$	50	7
14d	2	$OCHEtCO_2H$	22	7
15a	2	$OCHMeCONHSO_2Me$	22	4.5
15b	2	$OCHEtCONHSO_2Me$	20	6.5
16	2	$OCHMeCN_4H$	60	10

[a] Each IC_{50} value corresponds to an average of at least two independant determinations, except those identified with an asterisk, which are the result of a single titration. [b] Values not determined denoted by a dash. [c] There are two carbon atoms between the thiopyranoindole and the nitrogen atom.

In Vitro Studies

All of the compounds prepared were evaluated as inhibitors of LTB_4 biosynthesis using Ca^{2+}-ionophore activated human polymorphonuclear (PMN) leukocytes.[25] The potency of each compound as an inhibitor of 5-LO activity was also determined using a spectrophotometric assay monitoring 5-H(P)ETE production by the 100000*g* supernatant fraction (S100) from insect cells overexpressing human 5-LO.[26] The drug-dependent inhibition of LTB_4 biosynthesis in human whole blood activated with the ionophore A23187 was assessed using previously described methodology.[25] The results are listed in Tables 1 and 4.

In this study, two series of compounds which differ in the number of carbon atoms between the thiopyranoindole ring and the heteroatom were prepared and evaluated. The first series contains only one carbon between the ring and the heteroatom while the second has two.

Compounds Containing One Carbon between the Thiopyranoindole Ring and the Heteroatom (Table 1, *n* = 1). Introduction of an oxyacetic acid chain (**13**) results in a compound of similar potency to the tetrazole **1** in the human PMN leukocyte assay while the longer β-oxypropanoic acid derivative **17** is about 4 times less potent. In contrast, the sulfur-containing analogues **12a** and **12b** are both equipotent with **1**. It is therefore apparent that, in this series, the chain length alone is not enough to explain the differences in potency. Presumably the conformation of the side chain is also important. The effect of a substituent α or β to the acid group of **12b** was then investigated (compounds **12c–f**). A β-methyl substituent (**12d**; mixture of four diastereomers) leads to a slight reduction in activity whereas an α-methyl group (**12c**) does not appear to confer any advantage over the unsubstituted parent chain. In contrast, an ethyl group α to the acid, **12e**, is marginally more potent than **12c** and similar to **1**. Since **12e** is a mixture of the four possible diastereomers, the effect of the stereochemistry of the ethyl group on inhibitor potency was then examined. Both the *R* and the *S* thiol acids were prepared and coupled with racemic alcohol **7** to give compounds **12f** and **12g**, respectively (both compounds are diastereomeric mixtures

at the methyl group on the thiopyran ring) to see if there is a stereochemical preference at this center. The 2*R* isomer **12f** is preferred, as it is 3–4 times more potent in the human 5-LO assay than the 2*S* analogue **12g** (40 nM versus 120 nM). The ethyl group may be holding the acid-bearing side chain in the most favorable orientation, or alternatively, it may be involved in binding in a lipophilic pocket. Despite these modifications, **12f** represents only a modest improvement in potency in the human 5-LO assay as compared to **1**. This then prompted us to examine those compounds in which the heteroatom is two carbon atoms removed from the thiopyranoindole ring (Table 1, $n = 2$).

Compounds Containing Two Carbons between the Thiopyranoindole Ring and the Heteroatom (Table 1, *n* = 2). The results for those compounds containing an oxygen atom in the side chain such as the unsubstituted oxyacetic (**14a**) and β-oxypropanoic acid (**18**) side chains are unremarkable; both compounds are similar in potency to **1**. Inclusion of a thiolacetic acid moiety to give **20** yielded a similar result. When the heteroatom is nitrogen as in compound **22a** (derived from D,L alanine), there is a significant reduction in potency with the IC_{50} in human 5-LO being approximately 4 μM. Some of the potency can be recovered when the basic amine of **22a** is transformed into the branched amide **22b**. Nevertheless, **22b** is still 10-fold less potent than **1**. The amide functionality can be included as part of the side chain as shown by compound **11**, but the potency in the human 5-LO assay is not improved (IC_{50} = 300 nM). Several other analogues of **11** incorporating different amino acids (e.g. phenylalanine, valine, and leucine) were prepared and evaluated, but the results were equally disappointing.

From the results shown above, a substituent α to the acid can lead to compounds with improved potency, albeit modest (cf. thio acid **12f**), and so a similar strategy was tried in this series. The α-methyl derivative **14b** was prepared and proved to be significantly more potent than the unsubstituted analogue **14a**. Indeed, **14b** is some 3-fold more active than **1**. The *gem*-dimethyl derivative **14c** is not as potent as **14b**, but the ethyl analogue **14d** is comparable. Previous work has shown that other functional groups which contain an acidic proton are well tolerated, and so compounds containing the *N*-sulfonyl amide and the tetrazole groups were prepared. The *N*-sulfonyl amides **15a** and **15b** are equipotent to the carboxylic acid derivatives **14b** and **14d**, respectively. This is not the case for the tetrazole **16**, which is 3 times less active *in vitro* than **14b**.

Thus significant improvements to the *in vitro* potency of tetrazole **1** can be made by the inclusion of oxygen or sulfur heteroatoms in the acidic side chain. The most potent inhibitors prepared (e.g. **14b**, **14d**, **15a**, and **15b**) are those containing a 2-ethoxyacetic acid (or the corresponding *N*-sulfonyl amide) unit with an alkyl substituent α to the acid group.

In Vivo Studies

The most promising compounds from the preceeding *in vitro* SAR studies (**14b**, **14d**, and **15b**; **15a** was not assessed) were evaluated in the rat to compare their *in vivo* properties. The plasma profile of each compound was obtained and their effectiveness in a hyper-reactive rat model of dyspnea was determined (Table 2). Plasma levels were obtained after oral (20 mg/kg in 1% methocel) and iv (5 mg/kg in PEG 200) dosing of the respective sodium salts. Bioavailabilities were then calculated from the $AUC_{po}/4 \times AUC_{iv}$. For the hyperreactive rat model,[27] inbred rats were pretreated with methysergide (3 mg/kg, iv) and the drugs administered po in 1 or 0.5% methocel 2 h prior to antigen (ovalbumin) challenge. The effect was measured as the percent inhibition of dyspnea duration compared to litter-mate-matched vehicle-treated controls.

Table 2. *In Vivo* Data of Selected Compounds in the Rat

	rat plasma levels[a]			% inhibn in hyperreactive rat[c]	
compd	% bioavailability[b]	T_{max} (h)	C_{max} (μM)	2-h pretreatment	4-h pretreatment
14b	35	2	3.8	53 ($p < 0.001$)	2[d]
14d	88	6	5.5	55 ($p < 0.01$)	51 ($p < 0.001$)
15b	72	4	4		

[a] 20 mg/kg po in 1% methocel; 5 mg/kg iv in PEG 200. [b] $AUC_{po}/4 \times AUC_{iv}$. [c] Inhibition of dyspnea (0.5 mg/kg po using 1% methocel (**14a**) and 0.5% methocel (**14d**), n = 6). [d] Not significant.

The α-methyl acid **14b** has the poorest plasma profile (Table 2; T_{max} = 2 h and C_{max} = 3.8 μM) and the lowest bioavailability (35%) of the three compounds studied. The ethyl analogue **14d** is very well absorbed, the peak plasma concentration is 5.5 μM at 6 h and the bioavailability is 88%. When the *N*-sulfonyl amide analogue **15b** was examined, it was also found to be well-absorbed, with the bioavailability being over 70%. Unfortunately, examination of the rat plasma by HPLC showed, in addition to **15b**, a second peak with the same retention time as that of **14d**. After 6 h the HPLC peak corresponding to **14d** was about 60% of the area of the parent drug peak. Clearly the *N*-sulfonyl amide functionality is being metabolized to the acid. This was somewhat disappointing, as we had previously observed that the *N*-sulfonyl amide of **23** is stable under the same conditions. Presumably the presence of the ether α to the carbonyl makes this functionality more labile due to the inductive effect of the oxygen. Since **15b** is metabolized to give **14d**, further studies of **15b** were not conducted.

The remaining two compounds showed similar activity in the rat functional model with a 2-h pretreatment time (53% and 55% inhibition of dyspnea for **14b** and **14d**, respectively, Table 2). However when the pretreatment time was increased to 4 h only **14d** showed significant activity. These results are consistent with the measured rat plasma levels: at 4 h postdose the plasma concentration of **14b** is significantly less than that of **14d**.

From the *in vitro* and *in vivo* data presented, compound **14d** (L-699,333) stands out as having the best overall profile. Selectivity studies show that this compound is at least 200-fold more potent in inhibiting 5-LO activity than human 15-LO, porcine 12-LO, and cyclooxygenase from ram seminal vesicle microsomes ($IC_{50} > 5$ μM). The acid **14d** was shown to be inactive in a FLAP binding assay[28] ($IC_{50} > 10$ μM). As previously reported for other inhibitors of the thiopyrano[2,3,4-*cd*]indole class,[17] **14d** did not function as a reducing substrate for 5-LO in a pseudoperoxidase assay (10 μM) indicating that it inhibits enzyme activity by a nonredox mechanism.

Due to the excellent *in vitro* profile, **14d** was then further evaluated in two other animal models of asthma (conscious squirrel monkey and conscious sheep) as well as an inflammation model (rat pleurisy model). In addition, since **14d** is a mixture of four diastereomers, it was resolved to give the most active isomer.

Table 3. Inhibition of *Ascaris* Antigen-Induced Bronchoconstriction in the Conscious Squirrel Monkey by **14d** and MK-0591[a]

	changes in airway resistance (R_L)		changes in dynamic compliance (C_{dyn})	
	14d	MK-0591	**14d**	MK-0591
		0.3 mg/kg Dose		
control	55.1	60.5 ± 6.3	-29.4	-33.0 ± 1.9
drug	-1.6	34.0 ± 11.5	16.8	-17.9 ± 6.8
% inhibition	100 (n = 2)	44 (p < 0.05)	100 (n = 2)	46[b]
		0.1 mg/kg Dose		
control	62.8 ± 2.4		-33.2 ± 2.0	
drug	6.9 ± 7.7	not tested	-10.7 ± 8.2	not tested
% inhibition	89 (p < 0.05)		68[b]	

[a] Results are shown as means ± SEM, n = 3 for **14d** (at 0.1 mg/kg) and n = 5 for MK-0591. [b] Not significant.

The effect of the acid **14d** on antigen-induced bronchoconstriction in allergic conscious squirrel monkeys[29] was measured after a 4-h pretreatment with the drug followed by a challenge with an aerosol of *Ascaris* antigen (1:25 dilution). A dose-dependent inhibition of the induced bronchoconstriction was observed, and the results are shown in Table 3 along with results for MK-0591 (shown for comparison). At the higher dose of 0.3 mg/kg, complete inhibition of the increase in resistance (R_L) and the decrease in dynamic compliance (C_{dyn}) was observed in the two monkeys used for the experiment. Reducing the dose to 0.1 mg/kg (n = 3), an 89% inhibition of the increase in R_L and a 68% inhibition in the decrease in C_{dyn} was measured. These results are significantly better than the values obtained in the same assay under identical conditions for MK-0591, a leukotriene biosynthesis (FLAP) inhibitor (see Table 3). MK-0591 is currently undergoing clinical trials and has been shown to be effective at inhibiting both *ex-vivo* LTB_4 biosynthesis and urinary LTE_4 production in humans.[13c]

In the allergic sheep model,[30] **14d**, when administered as a bolus dose of 0.2 mg/kg followed by a constant infusion of 2.5 μg/kg/min (n = 3), was effective at inhibiting both the early- and late-phase bronchoconstriction in conscious sheep exposed to *Ascaris* antigen (Figure 2a). MK-0591 was also effective at this dose whereas **1** was not. However both **14d** and MK-0591 showed no activity at a lower dose of 0.8 μg/kg/min. Urinary LTE_4 production was also measured throughout the experiment (Figure 2b) and **14d** was shown to be marginally effective although the numbers are not significant.

The effect of **14d** on *in vivo* LTB_4 biosynthesis was assessed in a rat pleurisy model[31]. Male Sprague–Dawley rats were injected intrapleurally with 0.4 mL of 0.5% carrageenan in saline 16 h prior to drug pretreatment. The drugs were then administered (doses ranging from 3 to 0.3 mg/kg) and 6 h later the animals were challenged with calcium ionophore by intrapleural injection. After 1 h the LTB_4 levels in the pleural exudate were measured. Using a 6-h pretreatment time, **14d** was effective at inhibiting LTB_4 biosynthesis with an ED_{50} of 0.65 mg/kg (n = 6/group). Using the same pretreatment time, MK-0591 has an ED_{50} value of 0.16 mg/kg[25] whereas **1** with an 8-h pretreatment has an ED_{50} of 1.9 mg/kg.[16]

Further SAR

The acid **14d** contains two chiral centers and is therefore a mixture of four diastereomers. We were interested in separating the different diastereomers to determine which

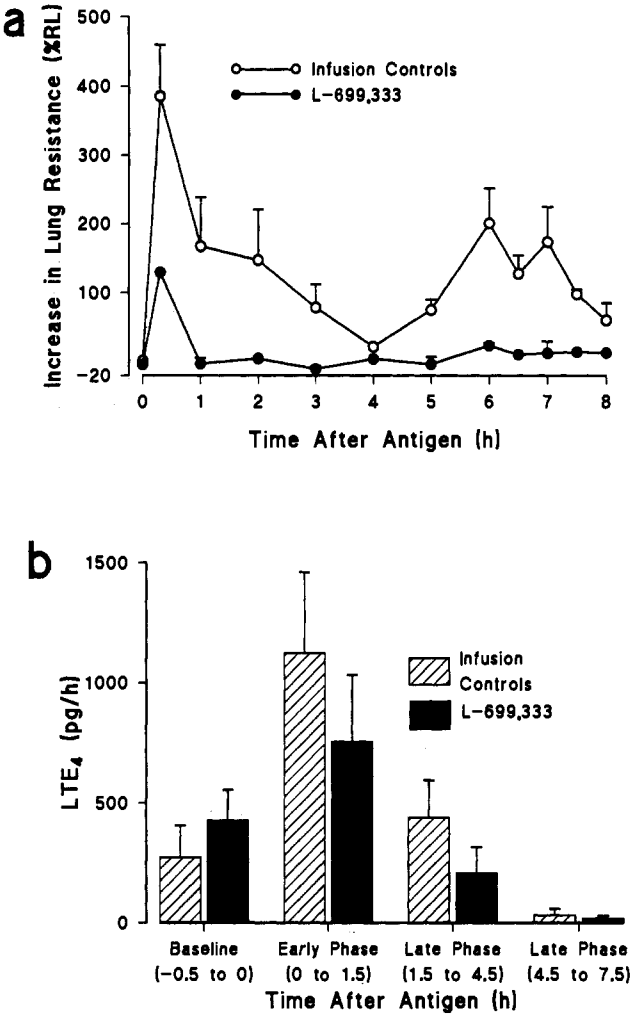
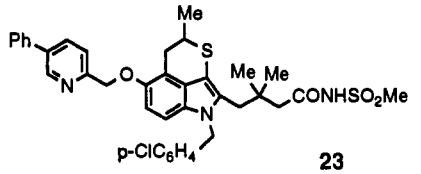


Figure 2. Intravenous infusion of **14d** at 2.5 μg/kg/min in allergic sheep (n = 3): (a) effect on postantigen bronchoconstriction and (b) effect on postantigen urinary LTE_4 excretion.



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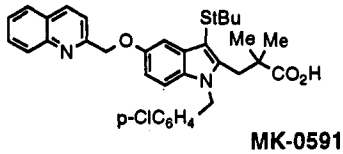
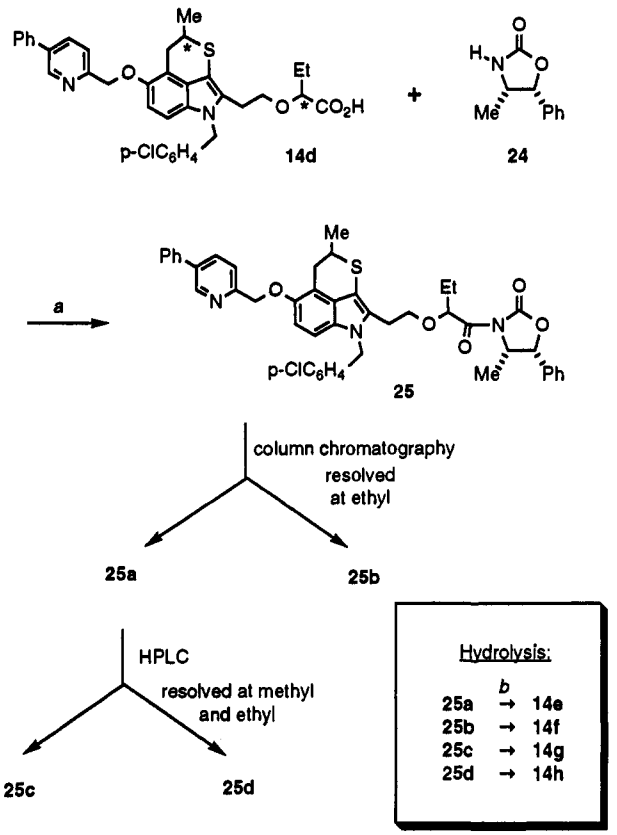


MK-0591

is the most potent. The SAR work described above indicates that the center α to the acid may play a large role in determining the inhibitor potency (compare compounds **12f** and **12g**; Table 1). The initial attempts to prepare compounds in which the stereochemistry of the ethyl group is controlled involved alkylation of the alcohol **8** with both (+)- and (-)-2-bromobutanoic acid.[32] Unfortunately this did not yield a stereochemically controlled product since approximately 15% of the unwanted stereoisomer was formed (determined by formation of the amide with (*S*)-(−)-α-methylbenzylamine and HPLC analysis).[33] As a result, it was decided to try to separate **14d** into individual diastereomers by using a chiral auxilary. The chiral auxilary chosen was (4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinone (**24**)[34] using methodology developed by Evans *et*

Scheme 3[a]



[a] Reagents: (a) 1,1'-carbonyldiimidazole, CH_3CN, Et_3N; (b) aq LiOH, THF.

al.[35] Thus **14d** was coupled with the oxazolidinone (using 1,1'-carbonyldiimidazole and Et_3N in CH_3CN) to give **25** (Scheme 3). Thin-layer chromatography showed two well-resolved spots of R_f 0.41 and 0.34, respectively (HOAc–EtOAc–hexane 5:25:70). Compound **25** was then separated into two diastereomeric mixtures by column chromatography to yield 33% of **25a** and 35% of **25b**. Presumably it is the center α to the carbonyl which has been resolved since the methyl center on the thiopyran ring is too remote from the chiral auxilary to be so strongly affected by it. Removal of the oxazolidinone group was achieved by hydrolysis with aqueous LiOH in THF at 0 °C[36] to regenerate the acids **14e** and **14f**. The stereochemical integrity of each diastereomeric pair was confirmed by HPLC analysis of the corresponding (*S*)-(−)-α-methylbenzylamine derivative, and they were both shown to contain < 3% of the other diastereomers.

When the oxazolidinone adduct **25a** was analyzed by HPLC it was found to separate into two peaks on a Porasil column eluting with 40% EtOAc in hexane. This then provided a method to obtain the individual diastereomers **14g** and **14h** by repeated HPLC chromatography of **25a** followed by careful hydrolysis.

The compounds prepared were then tested in the human 5-LO assay and the human PMN leukocyte assay as well as for inhibitory activity in the human whole blood assay. The results are given in Table 4. Resolving the 2-ethoxybutanoic acid chain gives rise to **14e**, which is about 10 times more potent in the human 5-LO assay than **14f** (IC_{50} = 13 nM compared to 150 nM). This result is consistent with the hypothesis that the conformation induced by the side chain is an important factor for inhibitor binding. Since **14e** is the most potent of the two diastereomeric pairs only this one was seperated further to give **14g** and **14h**. Most of the observed activity of **14e** resides with the single diastereomer **14g**, which is approximately 5 times more potent than **14h**. Compound **14g** represents the most potent compound prepared in the thiopyranoindole class of inhibitors to date. It has an IC_{50} of 4 nM in the human PMN leukocyte assay and 8 nM in the human 5-LO assay. All of the isomers were tested for their ability to inhibit LTB_4 production in whole blood and the rank order of potency obtained is the same as previously indicated (Table 4). The racemate **14d** has an IC_{50} in whole blood of 3.8 μM and the most active diastereomer **14g** is about 4 times more potent. The IC_{50} value of 1.0 μM for **14g** as an inhibitor of LTB_4 production in human whole blood is comparable to the value of 0.5 μM obtained for MK-0591.

Table 4. Effect of Selected Compounds on 5-LO Activity and on the Production of LTB_4 by Human Leukocyte and Whole Blood[a]

		IC_{50}, nM[a]		
compd	comments	5-LO	PMN	whole blood
14d	L-699,333	22	7	3.8 ± 0.8
14e	two diastereomers	13	3.5	1.8 ± 0.8
14f	two diastereomers	150	10	16 ± 1.8
14g	single diastereomer	8	4	1.0 ± 0.03
14h	single diastereomer	80*	17	4.8 ± 0.6
MK-0591	FLAP inhibitor	–	3	0.5 ± 0.05
zileuton		3500*	1060	2.0 ± 0.3
ICI D2138		400	14	0.06 ± 0.03

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

Also shown in Table 4 are the results obtained for zileuton[37] and ICI D2138.[38] Compound **14g** is somewhat more potent than ICI D2138 in the human PMN leukocyte assay while zileuton, with an IC_{50} of 1.0 μM, is considerably less active. However, in the human 5-LO assay, ICI D2138 is significantly less potent than **14g** (400 nM versus 8 nM) in comparison to the 3-fold difference observed in the human PMN leukocyte assay. The discrepancy between these assays may reflect a different sensitivity of the inhibitors to variations in the levels of arachidonic acid and activating hydroperoxides of the two systems, or it may suggest additional inhibitory effects of ICI D2138 in the whole cell assay.

In whole blood, zileuton and **14g** are similar in their ability to inhibit LTB_4 biosynthesis (IC_{50}s of 2 and 1 μM, respectively) and both are inferior to ICI D2138 (IC_{50} = 0.06 μM). These results reflect the degree of protein binding observed with these compounds; **14g** is highly protein bound while ICI D2138 and zileuton are not. It should be noted that of the compounds for which clinical results have been reported [zileuton[12a] and MK-886[13a] (human whole blood $IC_{50} = 1.1 \pm 0.2$ μM)] both are effective for treating asthma although there is no correlation between *ex vivo* LTB_4 inhibition in whole blood and pulmonary changes. Finally, unlike **14d**, ICI D2138 was not active in the rat functional model of asthma [28% inhibition (n = 6, not significant) at 0.5 mg/kg, 2-h pretreatment], a result which is probably related to the pharmacokinetic properties of the compound. The thiopyranoindole **14d** is more soluble and better absorbed in the rat.

Conclusion

The C-2 side chain of the thiopyranoindole **1** has been modified by the inclusion of heteroatoms as well as alkyl substituents. Two series of compounds were studied which differ in the number of carbon atoms (either one or two)

between the thiopyranoindole ring and the heteroatom. Oxygen and sulfur heteroatoms present in the side chain of either series generally yield inhibitors similar to or more potent than 1. The exception is compound **13**, which has a methoxy-3-propanoic acid chain and is about 3-fold less active in the human 5-LO assay. When a nitrogen atom is included in the chain the potency of the inhibitor is drastically reduced. This is true for both amine and amide functionalities.

Although the length of the acid-bearing chain and the nature of the acidic group may vary considerably, the addition of an ethyl substituent α to the acidic functionality has a pronounced effect on the potency of the inhibitor. The 2-ethoxybutanoic acid chain yields a compound (**14d**) which has a superior *in vitro* and *in vivo* profile to all the inhibitors prepared. Resolution of this compound results in **14g**, the most potent diastereomer, which is approximately 10 times more potent than the parent compound 1 in the human 5-LO (IC_{50} = 8 nM versus 75 nM) and human whole blood (IC_{50} = 1 μM versus 10 μM) assays. The racemic acid **14d** shows enhanced *in vivo* potency over the tetrazole 1 in all the animal models of asthma and inflammation used (rat, monkey, and sheep) and the overall profile of this compound is similar to that of MK-0591.

Thus the continued investigation of the thiopyranoindole series has led to the identification of potent direct 5-LO inhibitors which have good aqueous solubility as the sodium salt and are both bioavailable and orally active in a variety of animal models. The profile of the best compound in the series, **14d**, is comparable to that of our current clinical candidate MK-0591. Further studies based on this series are in progress and may result in the identification of a new chemical entity for the treatment of asthma and other diseases in which LTs have been implicated.

Experimental Section

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

Preparation of Thiols. 2-(Mercaptomethyl)butanoic Acid. Step 1: Ethyl 2-[(Acetylthio)methyl]butanoate. Ethyl 2-ethyl-2-propenoate[39] (5 g, 39 mmol) was diluted with 5.6 mL (78 mmol) of thiolacetic acid and stirred at 65 °C for 36 h. The mixture was then diluted with Et_2O and washed with H_2O, and the organic phase was dried with Na_2SO_4. Evaporation of the solvent afforded the title material which was used as such.

Step 2: Ethyl 2-(Mercaptomethyl)butanoate. To a solution of the thioester from step 1 (5 g, 24.5 mmol) in MeOH (15 mL) at 0 °C, under nitrogen was added K_2CO_3 (9.67 g, 73.5 mmol). The mixture was stirred for 30 min, and then HOAc (8.82 g, 147 mmol) and 25% aqueous NH_4OAc were added. The title compound was extracted with EtOAc, dried, evaporated, and then distilled using a Kugelrohr apparatus (200 °C/760 mmHg).

Step 3: 2-(Mercaptomethyl)butanoic Acid. To a solution of the ester from step 2 (1 g, 6.2 mmol) in THF (10 mL) and MeOH (10 mL) was added 1 N LiOH (10 mL). The solution was stirred at room temperature for 3 days and partitioned between Et_2O and H_2O and the aqueous layer decanted. The aqueous layer was acidified with concentrated HCl and concentrated in vacuo to afford the crude product which was distilled under reduced pressure (short Vigreux column) to give the title compound as a colorless oil which solidified on cooling: bp 110–115 °C at ~2 mmHg.

2-(*S*)-(Mercaptomethyl)butanoic Acid. Step 1: 4(*S*)-(1-Methylethyl)-2-oxazolidinone. The title compound was prepared from (*S*)-(+)-2-amino-3-methyl-1-butanol and diethyl carbonate in the presence of K_2CO_3 using the procedure of Evans et al.[40]

Step 2: 3-(1-Oxobutyl)-4-(*S*)-(1-methylethyl)-2-oxazolidinone. A mechanically stirred, cooled (−78 °C) solution of the oxazolidinone from step 1 (32.3 g, 250 mmol) in THF (830 mL) was metalated with 163 mL (1.6 M in hexane, 261 mmol) of *n*-BuLi and treated with freshly distilled butanoyl chloride (28.1 mL, 271 mmol). The mixture was warmed to 0 °C and stirred for 30 min. Excess acid chloride was hydrolyzed by the addition of 165 mL of 1 M aqueous K_2CO_3 followed by stirring the resulting solution for 1 h at room temperature. Volatiles were removed in vacuo and the product was extracted into CH_2Cl_2 (3×). The combined organic extracts were successively washed with H_2O and brine, dried, and concentrated to give a yellow oil. Column chromatography on silica gel (hexane–EtOAc 4:1) gave a colorless liquid.

Step 3: 3-[1-Oxo-2(*S*)-[[(phenylmethyl)thio]methyl]butyl]-4(*S*)-(1-methylethyl)-2-oxazolidinone. A solution of the N-acylated product of step 2 (36.9 g, 185 mmol) in THF (70 mL) was added to a solution of LDA [prepared from 28.6 mL (204 mmol) of diisopropylamine and 127.5 mL (1.6 M in hexane, 204 mmol) of *n*-BuLi] in THF (240 mL) at −78 °C. After stirring for 30 min, the resulting lithium enolate was treated with benzyl bromomethyl sulfide (52.3 g, 241 mmol) for 2 h at −20 °C and the reaction then quenched by the addition of 200 mL aqueous NH_4Cl. The volatiles were removed in vacuo and the product was extracted into CH_2Cl_2 (3×). The organic extracts were washed with 1 M aqueous $NaHSO_3$ (2×), 1 M aqueous $KHCO_3$ (2×), brine, dried, and evaporated to give a yellow oil. Column chromatography (silica gel; hexane–EtOAc 99:1 then 95:5 then 90:10) provided the title compound.

Step 4: Benzyl 2(*S*)-[[(phenylmethyl)thio]methyl]butanote. To a cooled (−10 °C) solution of lithium benzyloxide in THF [prepared from benzyl alcohol (28.7 mL, 277 mmol) and 127.5 mL (1.6 M in hexane, 204 mmol] of *n*-BuLi, was added a solution of the product of step 3 (48.9 g, ~146 mmol) in 170 mL of THF over 30 min. After 15 min at −10 °C, the reaction mixture was warmed to 0 °C, stirred for 2 h, and then quenched by the addition of aqueous NH_4Cl (300 mL). Volatiles were removed in vacuo, and the product was extracted into CH_2Cl_2 (3×). The combined organic extracts were successively washed with H_2O (2×) and brine, dried, and concentrated to give 74 g of a pale yellow oil. This material was purified by chromatography (toluene) to afford the title compound as a colorless oil containing a small amount of an unidentified impurity. This was used as such in the next step.

Step 5: 2(*S*)-[[(Phenylmethyl)thio]methyl]butanoic Acid. Glacial HOAc (120 mL) was added to a suspension of the product from step 4 (32.4 g, ~103 mmol) in 210 mL of 30% anhydrous HBr in HOAc (~1.03 mol) to complete dissolution. The mixture was stirred for 6 h at 70 °C and at 50 °C overnight. After cooling, the solution was poured into H_2O and extracted with CH_2Cl_2 (3×). The solvent was removed in vacuo, the residue diluted with toluene (500 mL), and the solvent evaporated again. This was repeated five times to remove the excess HOAc. The residue was dissolved in 1 M aqueous KOH (750 mL), washed with CH_2Cl_2 (4×), acidified to pH 1 with concentrated HCl, and extracted with CH_2Cl_2 (6×). Evaporation of the dried solvent afforded a pale yellow liquid which was used as such.

Step 6: 2(*S*)-(Mercaptomethyl)butanoic Acid. A solution of the acid from step 5 (17.4 g, 77.6 mmol) in THF (30 mL) was added to ~200 mL of NH_3 at −78 °C. The temperature was raised to −50 °C and Na (5.2 g, 226 mmol) was added in portions over 30 min. After the mixture had remained dark blue for 30 min, the reaction was quenched by the addition of NH_4Cl (10 g), and then the ammonia was evaporated under a stream of nitrogen. The THF was removed in vacuo, the residue dissolved in 1 M aqueous KOH (400 mL) and the product was extracted into Et_2O

(4×). The organic extracts were dried and concentrated, and the residue was then distilled under reduced pressure to give the title compound as a colorless oil which solidified on standing: bp 102–104 °C at ~2 mmHg; $[\alpha]_D$ = -21.7° (c 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 0.98 (3H, t), 1.54 (1H, t), 1.64–1.82 (2H, m), 2.50–2.87 (3H, m).

2(*R*)-(Mercaptomethyl)butanoic Acid. Following the procedure described for 2(*S*)-(mercaptomethyl)butanoic acid but substituting (1*S*,2*R*)-(+)-norephenedrine for (*S*)-(+)-2-amino-3-methyl-1-butanol in step 1, the title compound was obtained: $[\alpha]_D$ = +20.0° (c 2.5, $CHCl_3$).

3-Mercapto-2-methylpropanoic Acid. Following the procedure described for 2-(mercaptomethyl)butanoic acid but using ethyl 2-methyl-2-propenoate as starting material, the title compound was obtained.

3-Mercaptobutanoic Acid. Step 1: Methyl 3-(Acetylthio)-butanoate. A mixture of methyl crotonate (50 mL, 0.47 mol) and thiolacetic acid (40.4 mL, 0.56 mol) were heated at 100 °C for 24 h. The solution was cooled and the product was obtained by distillation (67.7 g, 76%; bp 58–65 °C at 10 mmHg).

Step 2: 3-Mercaptobutanoic Acid. To a degassed suspension of K_2CO_3 (109 g, 0.79 mol) in MeOH (720 mL) at 0 °C was added $NaBH_4$ (700 mg) followed by the ester from step 2 (62.7 g, 0.36 mol). The mixture was stirred at room temperature for 1 h and then AcOH (140 mL) was added. Removal of the volatiles provided a solid which was dissolved in pH 7.2 phosphate buffer solution and extracted with CH_2Cl_2. After drying the organic layer and removal of the solvent the residue was distilled to provide the title compound: 32.9 g (68%); bp 40–50 °C at 100 mmHg.

Preparation of Alcohols. [1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]methanol (7). Step 1: Ethyl (*tert*-butylthio)pyruvate (3). To a solution of ethyl bromopyruvate (50 g, 0.256 mol) and *tert*-butyl thiol (30 mL, 0.266 mol) in THF (1 L) at 0 °C was added dropwise Et_3N (45 mL, 0.323 mol) over 30 min. After a further 1.5 h, hexane (400 mL) was added and the solution was then filtered through a bed of Celite. Removal of the solvent in vacuo afforded 41 g of the crude title product which was used without further purification in the next step.

Step 2: Ethyl [1-(4-Chlorobenzyl)-3-(*tert*-butylthio)-5-hydroxyindole-2-carboxylate. A mixture of 1-(4-chlorobenzyl)-1-[4-(allyloxy)phenyl]hydrazine hydrochloride[15] (30 g, 92.6 mmol), the crude ketone 3 from step 1 (41 g, ~200 mmol), NaOAc (15 g, 183 mmol), HOAc (150 mL), and toluene (300 mL) were stirred at 70 °C for 1.5 h under nitrogen. The solution was cooled, poured into H_2O, extracted with EtOAc (2×), washed with saturated $NaHCO_3$ and then brine, dried, and evaporated. Chromatography of the resulting dark red oil (62 g) on silica gel (hexane–EtOAc 10:1) gave 34 g (80%) of the title compound.

Step 3: Ethyl 1-(4-Chlorobenzyl)-4-methyl-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indole-2-carboxylate (5). A solution of the allyl ether from step 2 (34 g, 74.3 mmol) in 1,2-dichlorobenzene (250 mL) was heated to 200 °C under nitrogen for 7 h. The mixture was cooled to 150 °C and *p*-TSA (900 mg, 5.2 mmol) was added in portions. After 30 min, the reaction was complete (as monitored by TLC) and the solution was cooled to room temperature. The reaction mixture was applied directly to a silica gel column (eluting with hexane–EtOAc 19:1 then 2:1) to provide the title compound as a solid (22 g, 74%).

Step 4: Ethyl 1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indole-2-carboxylate. To a solution of the phenol 5 from step 3 (22 g, 54.8 mmol) and 2-(chloromethyl)-5-phenylpyridine[16] (12.2 g, 60.3 mmol) in CH_3CN (350 mL) and DMF (100 mL) was added Cs_2CO_3 (21.4 g, 65.7 mmol) and the mixture was stirred at room temperature overnight. The mixture was poured into 1 N HCl/brine and extracted with EtOAc–THF (4×). After washing of the combined organic layers with brine, the solvent was removed to give a solid which was swished in EtOAc–hexane and filtered to give the title compound as a solid (23.03 g, 74%).

Step 5: [1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]methanol (7). The ester from step 4 (13 g, 22.9 mmol) was dissolved in 400 mL of THF at 0 °C under nitrogen and $LiAlH_4$ (1.3 g, 34.2 mmol) was added in portions. After 30 min, the reaction was warmed to room temperature and stirred for a further 1 h. The solution was poured onto ice and acidified with 1 N HCl and the precipitate collected by filtration. The precipitate was dissolved in THF–EtOAc and washed twice with brine, dried, and evaporated to give the title compound 7 as a solid: ^1H NMR ($CDCl_3$): δ 1.52 (3H, d, J = 6.7 Hz), 1.93 (1H, t, J = 5.6 Hz, OH), 2.88 (1H, dd, J = 16.8 Hz, 9.6 Hz), 3.41 (1H, m), 3.53 (1H, dd, J = 16.8 Hz, 2.8 Hz), 4.64 (1H, dd, J = 15.2 Hz, 5.6 Hz), 4.72 (1H, dd, J = 15.6 Hz, 5.6 Hz), 5.18 (1H, d, J = 13.5 Hz), 5.26 (1H, d, J = 13.5 Hz), 5.37 (2H, s), 6.8–7.0 (4H, m), 7.2 (2H, m), 7.4–7.7 (6H, m), 7.93 (1H, dd, J = 7.3 Hz, 1.7 Hz), 8.82 (1H, d, J = 1.7 Hz).

2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethanol (8). Step 1: Ethyl [1-(4-chlorobenzyl)-4-methyl-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-acetate (6). Following the procedure described for alcohol 7 steps 1–3 but using ethyl 4-chloroacetoacetate as starting material, the title compound was obtained as a solid.

Step 2: 2-[1-(4-Chlorobenzyl)-4-methyl-6-hydroxy-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethanol. A solution of the ester 6 from step 1 (19.6 g, 47.2 mmol) in THF (800 mL) at 0 °C was treated with $LiAlH_4$ (4.4 g, 115 mmol) in portions over 30 min. After 1 h the mixture (a thick precipitate had formed) was added to ice/1 N HCl and extracted with EtOAc (3×). The organic layers were washed with H_2O and then brine, dried, and evaporated to give 19 g of the title compound, which was used as such in the next step.

Step 3: 2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethanol (8). The phenol from step 2 (19 g) was alkylated with 2-(chloromethyl)-5-phenylpyridine according to the procedure described for alcohol 7 step 4 to afford the title compound 8 (20.1 g, 79% for 2 steps): ^1H NMR ($CDCl_3$) δ 1.55 (3H, d, J = 6.7 Hz), 1.78 (1H, t, J = 6.2 Hz, OH), 2.93 (3H, m), 3.45 (1H, m), 3.56 (1H, dd, J = 16.9 Hz, 1.7 Hz), 3.72 (2H, m), 5.27 (4H, s), 6.8–7.0 (4H, m), 7.22 (2H, d, J = 7.3 Hz), 7.35–7.7 (6H, m), 7.92 (1H, dd, J = 7.3 Hz, 1.7 Hz), 8.85 (1H, d, J = 1.7 Hz).

Preparation of Final Products. [[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]methoxy]acetic Acid (13). The alcohol 7 (500 mg, 0.95 mmol) in 15 mL of DMSO was treated with NaH (10 mg) and then ethyl bromoacetate (0.1 mL) and the reaction stirred for 30 min. This sequence was repeated until 110 mg (4.75 mmol) of NaH and 1.1 mL (9.5 mmol) of ethyl bromoacetate had been added. After 6 h, the solution was poured into 1 N HCl and extracted (3×) with EtOAc. The organic layers were combined, washed with brine, and dried, and the solvent was evaporated. Purification of the residue by chromatography (hexanes–EtOAc 3:1) yielded the ethyl ester of title compound (200 mg, 34%).

The ester (200 mg, 0.33 mmol), 1 N LiOH (1.6 mL), THF (3 mL), and MeOH (4 mL) were stirred at room temperature overnight. The solution was acidified with 1 N HCl, filtered, washed with Et_2O, and dried in vacuo to give the title compound as a solid (140 mg, 72%): mp 158–159 °C; exact mass (FAB) $C_{33}H_{29}N_2O_4SCl + H^+$ calcd 585.1615, found 585.1618.

3-[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methoxy]propanoic Acid (17). The alcohol 7 (110 mg, 0.21 mmol) was dissolved in 10 mL of THF at room temperature then methyl acrylate (0.4 mL, 4.4 mmol) and Triton B (3 drops) were added, and the mixture was stirred for 2 h. The solution was poured into 1 N HCl, extracted with EtOAc/THF (3×), washed with brine, dried, and evaporated. Chromatography of the residue (hexane–EtOAc 2:1) afforded the methyl ester of the title compound (74 mg, 58%) as a solid.

The ester (74 mg, 0.12 mmol) was hydrolyzed according to the procedure described for compound 13 to afford the title compound 17 as a solid (50 mg, 69%): mp 179.5–180 °C. Anal. ($C_{34}H_{31}N_2O_4SCl$) C, H, N.

[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]acetic Acid (12a). The alcohol 7 (514 mg, 0.98 mmol) was suspended in 20 mL of 1,2-dichloroethane at room temperature under nitrogen. To the suspension was added

methyl thioglycolate (91 μL, 1.03 mmol) followed by $BF_3 \cdot OEt_2$ (0.18 mL, 1.47 mmol) and the reaction was stirred for 3 min. The solution was poured into 1 N HCl and extracted (2×) with EtOAc, and the organic layers were washed with brine. Removal of the dried solvent followed by chromatography (hexane–EtOAc 3:1) provided the methyl ester of the title compound as a solid (467 mg, 78%).

The ester (467 mg, 0.75 mmol) was hydrolyzed according to the procedure described for compound **13** to provide the title compound **12a** as a solid (330 mg, 72%): mp 170–172 °C. Anal. ($C_{33}H_{29}N_2O_3S_2Cl$) H, N, S; C: calcd 65.93, found 65.48.

The following compounds were prepared according to the procedure given for compound **12a** with the thiol used given in parenthesis.

3-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]propanoic acid (12b): (3-mercaptopropanoic acid) 61% yield; mp 182–183 °C. Anal. ($C_{34}H_{31}N_2O_3S_2Cl$) C, H, N.

3-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]-2-methylpropanoic acid (12c): (3-mercapto-2-methylpropanoic acid) 49% yield; mp 151–154 °C. Anal. ($C_{35}H_{33}N_2O_3S_2Cl$) C, H, N, S.

3-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]butanoic acid (12d): (methyl 3-mercaptobutanoate) 56% yield for two steps); mp 193–195 °C. Anal. ($C_{35}H_{33}N_2O_3S_2Cl$) C, H, N, S.

3-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]-2-ethylpropanoic acid (12e): (3-mercapto-2-ethylpropanoic acid) 25% yield; mp 135–137 °C. Anal. ($C_{36}H_{35}N_2O_3S_2Cl$) C, H, N.

3-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]-2(*R*)-ethylpropanoic acid (12f): (3-mercapto-2(*R*)-ethylpropanoic acid) 71% yield, mp 163–165 °C. Anal. ($C_{36}H_{35}N_2O_3S_2Cl$) C, H, N, S.

3-[[[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-methyl]thio]-2(*S*)-ethylpropanoic acid (12g): (3-mercapto-2(*S*)-ethylpropanoic acid) 72% yield, mp 161–162 °C. Anal. ($C_{36}H_{35}N_2O_3S_2Cl$) C, H, N, S.

[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]acetic Acid (14a). A solution of the alcohol 8 (1 g, 1.8 mmol) in DMF (20 mL) was cooled to 0 °C and NaH (216 mg, 9 mmol) added. After stirring for 20 min, *n*-Bu_4NI (666 mg, 1.8 mmol) was added, followed immediately by *tert*-butyl bromoacetate (1.7 mL, 10.8 mmol). The mixture was stirred for 18 h at room temperature and then poured into saturated NH_4Cl (50 mL) and extracted with Et_2O (2×). The organic layer was washed twice with brine, dried, and evaporated to give the crudes *tert*-butyl ester of the title compound as an oil which was used as such.

The ester was hydrolyzed to give the title compound **14a** as a solid (376 mg, 34% for two steps): mp 104 °C dec. Anal. ($C_{34}H_{31}N_2O_4SCl \cdot H_2O$) C, H, N.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]propanoic Acid (14b). Following the procedure for **14a** but using ethyl 2-bromopropanoate, the title compound **14b** was obtained (38% for two steps): mp 162–163 °C. Anal. ($C_{35}H_{33}N_2O_4SCl$) C, H, N.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]-2-methylpropanoic Acid (14c). Following the procedure for **14a** but using ethyl 2-bromo-2-methylpropanoate, the title compound **14c** was obtained (15% for two steps): mp 149–151 °C. Anal. ($C_{36}H_{35}N_2O_4SCl$) C, H, N.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]butanoic Acid (14d). To a solution of the alcohol 8 (3.0 g, 5.5 mmol) in THF (30 mL) at room temperature was added NaH (530 mg, 22 mmol). After 5 min, the mixture was cooled to 0 °C and (±)-2-bromobutanoic acid (1.84 g, 11 mmol) in THF (30 mL) was added slowly. The thick mixture was refluxed for 1.5 h and then cooled, poured into saturated NH_4Cl, and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried, and evaporated. Purification of the residue by chromatography (hexane–EtOAc 1:1 followed by addition of 5% HOAc) gave the title compound **14d** as a solid (1.97 g, 57%): mp 139–141 °C; ^1H NMR ($CDCl_3$) δ 0.88 (1.5H, t, J = 7.3 Hz), 0.89 (1.5H, t, J = 7.3 Hz), 1.51 (1.5H, d, J = 6.8 Hz), 1.53 (1.5H, d, J = 6.8 Hz), 1.78 (2H, m), 2.70–3.03 (3H, m), 3.36–3.72 (4H, m), 3.81 (1H, m), 5.23 (2H, s), 5.28 (2H, s), 6.88 (4H, m), 7.23 (2H, d, J = 7.9 Hz), 7.40–7.56 (3H, m), 7.56–7.73 (3H, m), 7.95 (1H, dd, J = 7.3 Hz, 2.8 Hz), 8.83 (1H, d, J = 2.8 Hz). Anal. ($C_{36}H_{35}N_2O_4SCl$) C, H, N, S.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]butanoic Acid (Diastereomers 14e). Step 1: (4*S*,5*R*) 3-[2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]-1-oxo-1-butyl]-4-methyl-5-phenyl-2-oxazolidinone (diastereomers 25a and 25b). A solution of the acid **14d** (4.80 g, 7.65 mmol) and 1,1′-carbonyldiimidazole (2.52 g, 15.3 mmol) in CH_3CN (140 mL) were heated to reflux under nitrogen for 45 min. After this time, (4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinone[34] (**24**) (2.71 g, 15.3 mmol) and Et_3N (2.1 mL, 15.31 mmol) were added and heating continued for 2 h. More oxazolidinone (1.4 g, 7.9 mmol) was added and heating was continued for a further 2 h. The solution was cooled to room temperature, poured into H_2O (100 mL), acidified with 6 N HCl (10 mL), and extracted with EtOAc (3×). The organic layers were washed with brine, dried, and concentrated to give crude **25** (9.5 g of an oily residue). Chromatography of this mixture on silica gel eluting with 30% EtOAc in hexane afforded 1.99 g (33%) of the less polar oxazolidinone derivative (diastereomers **25a**). Continued elution then yielded the more polar product (diastereomers **25b**; 2.1 g, 35%).

Step 2: 2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethoxy]butanoic Acid (Diastereomers 14e). The less polar oxazolidinone from step 1 (diastereomers **25a**; 1.94 g, 2.47 mmol) in 150 mL of THF and 50 mL of H_2O at 0 °C was treated with 1 N LiOH (10 mL, 10 mmol) for 1 h. The solution was then acidified using 1 N HCl (15 mL) followed by the addition of H_2O and extraction with EtOAc (3×). After washing with brine, the organic layer was dried and evaporated. Purification of the residue by chromatography on Biosil (30% EtOAc in hexane) followed by a swish in Et_2O gave the title compound **14e** as a solid (0.85 g, 55%): mp 144–147 °C. Anal. ($C_{36}H_{35}N_2O_4SCl$) C, H, N.

The acid (5 mg) was coupled with (*S*)-(−)-α-methylbenzylamine (isobutyl chloroformate, *N*-methylmorpholine in THF[32]) and the chiral amide produced was shown to contain <3% of diastereomers **14f** by HPLC (Porasil-A; 40% EtOAC in hexane).

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]butanoic Acid (Diastereomers 14f). Following the procedure described for diastereomers **14e**, the more polar diastereomers **25b** (from step 1; 2.0 g) was hydrolyzed to provide the title compound **14f** (0.933 g, 58%) as a solid: mp 145–149 °C. Anal. ($C_{36}H_{35}N_2O_4SCl$) C, H, N.

The acid (5 mg) was coupled with (*S*)-(−)-α-methylbenzylamine and the chiral amide produced was shown to contain <1.5% of diastereomers **14e** by HPLC (Porasil-A; 40% EtOAc in hexane).

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]butanoic Acid (Isomer 14g). The less polar oxazolidinone **25a** from compound **14f**, step 1, was chromatographed on a Delta prep HPLC machine using a Porasil column (125 Å; 10–20 μm; 50 × 300 mm) eluting with hexane–EtOAc 4:1 at 100 mL/min. The faster eluting diastereomer was collected and the solvent removed to give the pure oxazolidinone **25c** as a solid. In this manner 60 mg of material was obtained and hydrolyzed as before to provide the title compound **14g** as a solid: mp 120–122 °C.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]butanoic Acid (Isomer 14h). The slower eluting

diastereomer (from above) was collected and the solvent removed to give the pure oxazolidinone **25d** as a solid. Hydrolysis as before to provided the title compound **14h** as a solid: mp 160–161 °C.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]-*N*-(methylsulfonyl)propanamide (15a). A solution of the acid **14b** (400 mg, 0.64 mmol) in THF (8 mL) at room temperature was treated with NaH (23 mg, 0.96 mmol) followed 10 min later by 10 μL (0.12 mmol) of pyridine. After cooling to 0 °C over 30 min, oxalyl chloride (85 μL, 0.97 mmol) was added and the solution warmed back to room temperature over 20 min. Methanesulfonamide (321 mg, 3.38 mmol), Et_3N (0.48 mL, 3.45 mmol), and DMAP (16 mg, 0.14 mmol) were then added and the mixture stirred for 1.5 h. The solution was poured into saturated NH_4Cl, extracted with EtOAc (3×), washed twice with brine, dried, and concentrated. Chromatography of the residue (3% MeOH/CH_2Cl_2) afforded the title compound **15a** as a solid (328 mg, 73%): mp 154–157 °C. Anal. ($C_{36}H_{36}N_3O_5S_2Cl$) C, H, N.

2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]-*N*-(methylsulfonyl)butanamide (15b). Following the procedure described for **15a**, the acid **14d** (400 mg, 0.64 mmol) was converted to the title compound **15b** (328 mg, 73%): mp 153–155 °C. Anal. ($C_{37}H_{38}N_3O_5S_2Cl$) C, H, N.

5-[1-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethoxy]ethyl]-1*H*-tetrazole (16). Step 1: 2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-(5-phenylpyridin-2-ylmethoxy)-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]propionamide. To the ethyl ester of **14b** (500 mg, 0.78 mmol) in *m*-xylene (12.5 mL) was added 1.56 mL of 1 M dimethylaluminum amide (prepared by condensing NH_3 in 3 mL of CH_2Cl_2 at −78 °C and then adding 3 mL of 2 M trimethylaluminum in hexane; excess NH_3 is allowed to evaporate by warming to room temperature). The solution was heated at 75 °C for 2 h and then cooled to 25 °C and poured onto ice and 10 mL of 1 N HCl. The mixture was extracted with EtOAc (2×), washed twice with brine, dried, and evaporated to give the crude title compound as an oil. This material was used as such in the next step.

Step 2: 2-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethoxy]propionitrile. The amide from step 1 was dissolved in THF (20 mL) and to this solution was added pyridine (1 mL) and trifluoroacetic anhydride (1 mL). After 20 min, saturated NH_4Cl solution was added. The mixture was extracted with EtOAc (2×), washed twice with brine, dried, and evaporated. Trituration with Et_2O–hexane afforded the title compound, 418 mg (90% for two steps).

Step 3: 5-[1-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethoxy]ethyl]-1*H*-tetrazole (16). The nitrile from step 2 (400 mg, 0.67 mmol) was dissolved in 1,2-dichlorobenzene (5 mL) and *n*-Bu_3SnN_3 (1.12 g, 3.4 mmol) was added. The mixture was heated at 125 °C for 1 h and then cooled and HOAc (1 mL) added. After 20 min, the mixture was applied to a silica gel column (eluting with hexane–EtOAc 1:1, followed by the addition of 5% HOAc) and the title compound **16** was obtained as a solid (180 mg, 42%): mp 220 °C dec.

This material was converted into the sodium salt by dissolving in the minimum amount of ethanol followed by the addition of 1 equiv of 1 N NaOH and H_2O and subsequent lyophilization. Anal. ($C_{35}H_{33}N_6O_2SClNa\cdot4.5H_2O$) C, H, N.

3-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-ethoxy]propanoic Acid (18). To a solution of the alcohol **8** (300 mg, 0.55 mmol) in THF (8 mL) was added Triton B (2 drops) followed by methyl acrylate (0.2 mL, 2.2 mmol) and the mixture was stirred at room temperature overnight. After this time, 10 mL of saturated NH_4Cl was added and the solution extracted with EtOAc (2×). The organic layers were washed twice with brine and dried and the solvent removed in vacuo. Chromatography of the residue (hexane–EtOAc 70:30) afforded the methyl ester of the title compound (195 mg, 56%).

Following the procedure described for compound **13**, the ester (190 mg) was hydrolyzed to afford the title compound **18** as a solid (90 mg, 48%): mp 151–152 °C. Anal. ($C_{35}H_{33}N_2O_4SCl\cdot H_2O$) C, H, N.

2-[[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethyl]thio]propanoic Acid (20). Step 1: 2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethyl 4-Toluenesulfonate (19). To a solution of alcohol **8** (1 g, 1.8 mmol) in pyridine (20 mL) was added DMAP (1 crystal) and 4-toluenesulfonyl chloride (485 mg, 2.6 mmol). After 18 h at room temperature, the mixture was poured into 1 N HCl (40 mL) and extracted with EtOAc (2×). The organic layers were washed with H_2O and 20% citric acid (3×), dried, and concentrated. Chromatography on silica gel (hexane–EtOAc 3:2) afforded the title compound **19** (800 mg, 62%).

Step 2: 2-[[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethyl]thio]propanoic Acid (20). To a solution of thiolactic acid (48 μL, 0.54 mmol) in DMF (5 mL) at 0 °C was added NaH (26 mg, 1.08 mmol) and after 15 min the sulfonate **19** (250 mg, 0.36 mmol). After 18 h the mixture was cooled, 1 N HCl (4 mL) added, and the solution then extracted with EtOAc (2×). The organic layers were washed twice with brine, dried, and concentrated. Chromatography of the residue on silica gel (hexane–EtOAc 1:1, followed by addition of 5% HOAc) afforded the title compound **20** as a solid (85 mg, 37%): mp 159–160 °C. Anal. ($C_{35}H_{33}N_2O_3S_2Cl$) C, H, N.

***N*-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethyl]-D,L-alanine Hydrochloride (22a). Step 1: [1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]-acetaldehyde (21).** To a solution of the alcohol **8** (541 mg, 1 mmol) in DMSO (2 mL), CH_2Cl_2 (5 mL), and Et_3N (0.7 mL, 5 mmol) was added sulfur trioxide–pyridine complex (640 mg, 4 mmol). After 30 min, the mixture was purified by passing through a plug of silica gel eluting with hexane–EtOAc 1:1. Removal of the solvent afforded the title compound **21** as a solid (439 mg, 81%).

Step 2: Methyl *N*-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano-[2,3,4-*cd*]indol-2-yl]ethyl]-D,L-alanine. The aldehyde **21** (430 mg, 0.8 mmol) and D,L-alanine methyl ester hydrochloride (225 mg, 1.6 mmol) were stirred in THF (5 mL) and MeOH (5 mL) for 30 min. $NaBH_3CN$ (250 mg, 4 mmol) was added, and after 1 h, H_2O and EtOAc were added. The organic layer was washed with brine, dried, and evaporated. Chromatography of the residue (hexane–EtOAc 1:1 with 5% Et_3N) afforded the title compound as a solid (164 mg, 33%): mp 112–114 °C.

Step 3: *N*-[2-[1-(4-Chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethyl]-D,L-alanine Hydrochloride (22a). The ester from step 2 (164 mg, 0.26 mmol) was hydrolyzed using the conditions described for compound **13** (step 2) to afford the amino acid, (78 mg, 49%). The amino acid (63 mg) was converted to the corresponding hydrochloride salt by stirring in a mixture of THF (1 mL) and 3 N HCl (5 mL). After 10 min, the mixture was concentrated in vacuo to provide the title compound **22a** as a solid: 63 mg; mp 215–220 °C dec; exact mass (FAB) $C_{35}H_{34}N_3O_3SCl + H^+$ calcd 612.2088, found 612.2095.

***N*-Acetyl-*N*-[2-[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1*H*-thiopyrano[2,3,4-*cd*]indol-2-yl]ethyl]-D,L-alanine (22b).** A solution of the ester from compound **22a** (step 2, 157 mg, 0.25 mmol) in THF (10 mL) and Et_3N (0.5 mL) was treated with acetyl chloride (54 μL, 0.75 mmol) and the mixture was stirred for 1 h. After this time, 3 N HCl was added and the solution was then extracted with EtOAc. The organic layer was washed with brine, dried, and evaporated to give the methyl ester of the title compound as a foam (185 mg). The ester (180 mg) was hydrolyzed using the procedure described for compound **13** (step 2) to provide 135 mg of the acid. Purification by chromatography on silica gel (5% AcOH in EtOAc) afforded the title compound **22b** as a solid (100 mg, 61% for two steps): mp 206–209 °C. Anal. ($C_{37}H_{36}N_3O_4SCl\cdot0.5H_2O$) C, H, N.

N-Methyl-N-[[1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1H-thiopyrano[2,3,4-cd]indol-2-yl]acetyl]-D,L-alanine (11). To a solution of [1-(4-chlorobenzyl)-4-methyl-6-[(5-phenylpyridin-2-yl)methoxy]-4,5-dihydro-1H-thiopyrano[2,3,4-cd]indol-2-yl]acetic acid (10) (800 mg, 1.44 mmol) in CH_3CN (5 mL) and DMF (5 mL) was added 1, 1'-carbonyldiimidazole (256 mg, 1.58 mmol) and the mixture brought to reflux for 1 h. The solution was cooled, N-methyl-D,L-alanine methyl ester hydrochloride (243 mg, 1.58 mmol) and Et_3N (0.4 mL, 2.88 mmol) were added, and stirring was continued for 1 h at 60 °C. After cooling, the mixture was poured into water, extracted with EtOAc (3×), and washed with 1 N HCl (2×), saturated $NaHCO_3$ (2×), and then with brine. After drying, the solvent was removed and the residue chromatographed on silica gel (EtOAc) to afford the methyl ester of the title compound as a solid (690 mg, 73%).

The ester (300 mg) was hydrolyzed using the procedure described for compound 13 to yield (after trituration with EtOAc–hexane) the title compound 11 as a solid: 180 mg (61%); mp 192–194 °C. Anal. ($C_{36}H_{34}N_3O_4SCl$): C, H, N, S.

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Supplementary Material Available: NMR data for compounds 11, 12a–e, 13, 14a–c, 15a,b, 16–20, and 22a,b (3 pages). Ordering information is given on any current masthead page.

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