as measured with a Malvern 2600C droplet and particle sizer. The guinea pigs were pretreated with indomethacin (10 mg/kg, ip) and propranolol (5 mg/kg ip) and then positioned in the chamber for a 30-min acclimation period prior to the aerosol challenge.

The challenge consisted of an aerosolized solution of LTD_4 (60 μM) delivered for a maximum time of 5 min, during which time changes in the breathing patterns of the guinea pigs were visually monitored. The end point was defined as a consistent, slow, deep, deliberate respiratory pattern with marked involvement of the abdominal muscles. Time, in seconds, to reach the end point was determined for each guinea pig and percent protection was calculated using the following equation:

$$\% \text{ protection} = [(\text{drug time} - \text{mean control time})/(\text{maximal aerosoltime} - \text{mean control time})] \times 100$$

Mean control time was the time to dyspnea for all vehicle-treated animals run concomitantly with a given compound. The animals in each run were pretreated with compound or vehicle at the indicated times prior to LTD_4 challenge. At least two vehicle-treated animals were contained in each test run and the experimenter was blind as to treatment groups. Differences in means between the drug group and vehicle group were compared by using Student's unpaired t test with $p < 0.05$ considered significant.

Evolution of a Series of Peptidoleukotriene Antagonists: Synthesis and Structure/Activity Relationships of 1,3,5-Substituted Indoles and Indazoles

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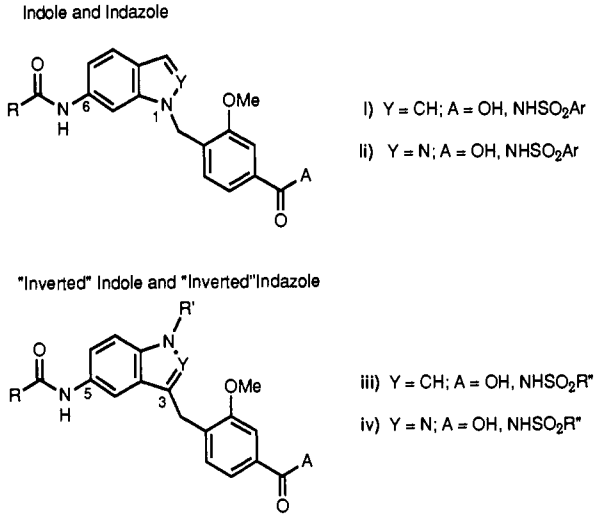
1,3,5-Substituted indoles and indazoles have been studied as receptor antagonists of the peptidoleukotrienes. The best of these compounds generally had a methyl group at the N1 position, a [(cyclopentyloxy)carbonyl]amino or 2-cyclopentylacetamido or *N'*-cyclopentylureido group at the C-5 position, and an arylsulfonyl amide group as part of the acidic chain at the C-3 position of the ring. Such compounds had in vitro dissociation constants (K_B) in the range 10^{-9}–10^{-11} M on guinea pig trachea against LTE_4 as agonist and inhibition constants (K_i) $\leq 10^{-9}$ M on guinea pig parenchymal membranes against [^{3}H]LTD_4. A number of compounds were orally effective at doses $\leq$1 mg/kg in blocking LTD_4-induced "dyspnea" in guinea pigs. Compound **45** [N-[4-[[5-[[(cyclopentyloxy)carbonyl]-amino]-1-methylindol-3-yl]methyl]-3-methoxybenzoyl]-2-methylbenzenesulfonamide, ICI 204,219; $pK_B = 9.67 \pm 0.13$, $K_i = 0.3 \pm 0.03$ nM, po $ED_{50} = 0.3$ mg/kg] is currently under clinical investigation for asthma. In the indole series, certain *alkyl*sulfonyl amides possessing a 3-cyanobenzyl substituent at the N-1 position (**60**, **61**) were produced that had $K_B \leq 10^{-9}$ M on guinea pig trachea.

The discovery of potent and selective peptidoleukotriene (LTC_4, LTD_4, and LTE_4) antagonists for the treatment of asthma, and other allergic conditions, remains a major focus for pharmaceutical research. The likelihood of success in this endeavor for asthma has been strengthened by the recent suggestion that, unlike on guinea pig trachea where there are distinct receptors for LTC_4 and LTD_4/LTE_4,[1a] human intralobar airways have a single receptor for the peptidoleukotrienes.[1b-e] The Fisons' hydroxyacetophenone FPL 55712 and the structures of the peptidoleukotrienes have been widely used as points of departure in the search for such antagonists. Over the past few years, these efforts have produced pharmacologically interesting molecules, some of which have been important enough to warrant clinical evaluation. Thus, the Lilly (LY 171,883)[2] and Merck (L-648,051[3] and L-649,923[4]) hydroxyacetophenones and the SK&F leukotriene-derived hydroxy acid (SKF 104,353)[5] have shown varying degrees of efficacy as LTD_4 antagonists in man, the last of these being especially interesting, although not orally active.

Recently, other classes of LTD_4 antagonists have been reported.[6-8] These new molecules bear little structural resemblance to hydroxyacetophenones or leukotrienes, and some[6,7] are significantly more potent than the earlier compounds. An accompanying report from these laboratories[9a] and previous work[6a,b,9b] have described the evolution of a new family of indole (i) and indazole (ii) benzoic acids and their *N*-arylsulfonyl amide derivatives (Chart I) which were potent and selective LTD_4 antagonists.

The goal of the present study was a better understanding of the geometric and electronic requirements of the central

Chart I. Peptidoleukotriene Antagonists: Indoles and Indazoles



bicyclic ring system for receptor binding of this class of compounds. This paper describes the ("inverted") indole

† Present address: Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

(1) (a) Hogaboom, G. K.; Mong, S.; Wu, H.-L.; Crooke, S. T. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 1136. (b) Buckner, C. K.; Saban, R.; Castleman, W. L.; Will, J. A. *Ann. N. Y. Acad. Sci.* **1988**, *524*, 181. (c) Buckner, C.; Fedyna, J.; Krell, R.; Robertson, J.; Keith, R.; Matassa, V.; Brown, F.; Bernstein, P.; Yee, Y.; Will, J.; Fishleder, R.; Saban, R.; Hesp, B.; Giles, R. *FASEB J.* **1988**, 2:A1264 (Abstr). (d) Jones, T. R.; Charette, L.; Denia, D. *Can. J. Physiol. Pharmacol.* **1988**, *66*, 762. (e) Hay, D. W. P.; Muccitelli, R. M.; Tucker, S. S.; Vickery-Clark, L. M.; Wilson, K. A.; Gleason, J. G.; Hall, R. F.; Wasserman, M. A.; Torphy, T. J. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 474.

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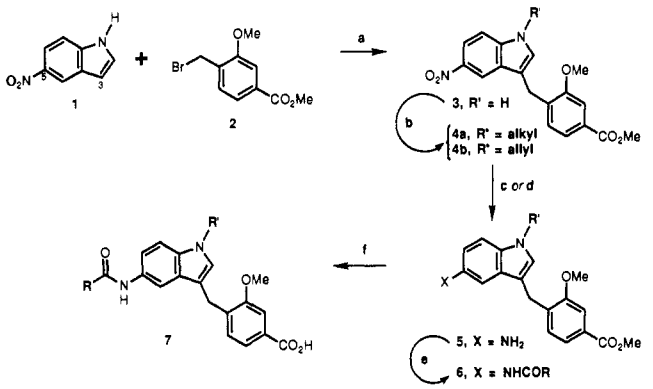
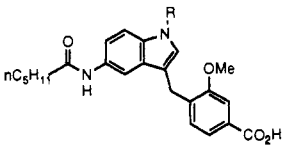
Scheme I[a]



[a] (a) Ag_2O, dioxane, 60 °C; (b) NaH, R'X, DMF; (c) H_2, 10% Pd/C, THF; (d) $SnCl_2 \cdot 2H_2O$, ethanol, Δ; (e) RCOCl, *N*-methylmorpholine, CH_2Cl_2 (method A); RCO_2H, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 4-(dimethylamino)pyridine, CH_2Cl_2 (method B); (i) trichloromethyl chloroformate, dioxane, (ii) RNH_2, 70 °C (method C); (f) LiOH, H_2O, THF, methanol.

(iii), and ("inverted") indazole (iv) series of compounds (Chart I) and structure/activity relationships of their appended acylamino and acidic chains, and N1 substituents. Structures iii and iv were chosen for study since they were anticipated to have, qualitatively speaking, an overall similar geometry to the earlier indole i and indazole ii but different electronic properties (e.g., direction of dipole moment, molecular electrostatic potential). A compound for clinical trials, ICI 204,219 (**45**), resulted from this investigation.

Table I. Indole Hexanamide Carboxylic Acids: N1 Modification



compd	R	% inhibition/ concentration,[a] μM
18	H	64/3.3
19	Me	77/1
20	allyl	43/1
21	benzyl	38/1
22	CH_2CO_2H	NS/3.3[b]

[a] Percent inhibition of the LTE_4-induced contraction of guinea pig tracheal spirals by the antagonist, at the indicated concentration, as compared to control tissues. [b] NS = not statistically significant ($p > 0.05$).

Chemistry

The general routes of synthesis of the indole carboxylic acid series of compounds are summarized in Scheme I.

5-Nitroindole (**1**; Aldrich) was alkylated at C3 with bromo ester **2**, under catalysis by silver oxide, to give ester **3**. Alkylation of the indole nitrogen was carried out using NaH in anhydrous DMF, in the presence of the requisite alkyl bromide or iodide, to give **4a,b**. Reduction of the nitro group was effected either catalytically with H_2/Pd–C or, in the case of *N*-allyl derivative **4b**, with stannous chloride to give primary amines **5**. Acylation of **5** with an acid chloride (method A) or a carboxylic acid (method B) gave urethane and amide esters **6**. Treatment of **5** with diphosgene[9a] in dioxane gave the corresponding isocyanate, which, without isolation, was converted to the desired urea (**6**, R = NHR″) by condensation with an amine at 70 °C (method C). The urethane, amide, and urea methyl esters **6** were hydrolyzed at room temperature with lithium hydroxide to give the corresponding carboxylic acids **7**.

The general routes of synthesis of the indazole carboxylic acid series of compounds are summarized in Scheme II. The method features a novel indole to indazole conversion, which could be carried out on cyclopentylurethane substrate **6a** (to give indazole **11a**) or on benzylurethane **6b** (to give **11b**). In the latter case, reductive cleavage of the benzyl group allowed exploration of structure/activity

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(3) (a) Jones, T. R.; Guindon, Y.; Young, R. N.; Champion, E.; Charette, L.; Denis, D.; Ethier, D.; Hamel, R.; Ford-Hutchinson, A. W.; Fortin, R.; Letts, G.; Masson, P.; McFarlane, C.; Piechuta, H.; Rokach, J.; Yoakim, C.; DeHaven, R. N.; Maycock, A.; Pong, S. S. *Can. J. Physiol. Pharmacol.* **1986**, *64*, 1535. (b) Barnes, N. C.; Evans, J.; Zakrzewski, J. T.; Piper, P. J.; Costello, F. *2nd International Congress on Pharmacology*, Aug 23–28, 1987, Sydney, Australia.

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(7) Young, R. N. *Third Chemical Congress of North America*, June 5–10, 1988, Toronto, Canada, Medicinal Abstract 98.

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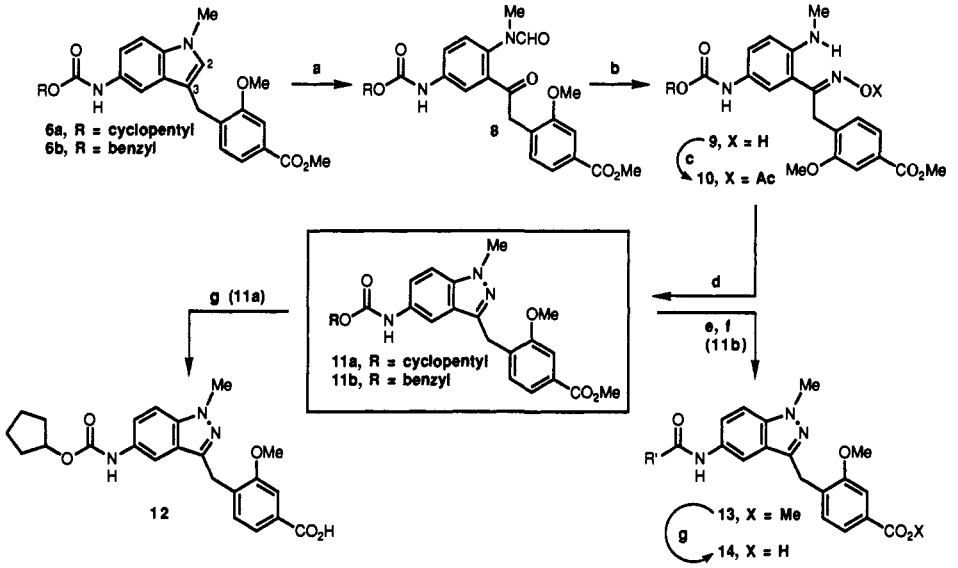
(9) (a) Brown, F. J.; Yee, Y. K.; Cronk, L. A.; Hebbel, K. C.; Snyder, D. W.; Krell, R. D. *J. Med. Chem.*, preceeding paper in this issue. (b) Yee, Y. K.; Brown, F. J.; Hebbel, K. C.; Cronk, L. A.; Snyder, D. W.; Krell, R. D. *Ann. N. Y. Acad. Sci.* **1988**, *524*, 458. (c) LTD_4 and LTE_4 share common receptors in guinea pig lung membranes and trachea. A strong correlation exists between radioligand binding sites and contractile activity; for a review, see: Crooke, S. T.; Mong, S.; Clark, M.; Hogaboom, G. K.; Lewis, M.; Gleason, J. *Biochem. Actions Horm.* **1987**, *14*, 81; see also: Aharony, D.; Falcone, R. C.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 921.

Scheme II[a]



[a] (a) $^1O_2$, methanol, Rose Bengal; (b) $NH_2OH{\cdot}HCl$, pyridine, Δ; (c) Ac_2O, pyridine, CH_2Cl_2; (d) melt, 170 °C; (e) H_2, 10% Pd–C, ethyl acetate; (f) methods A–C—see Scheme I; (g) LiOH, H_2O, THF, methanol.

relationships for the acylamino chain.

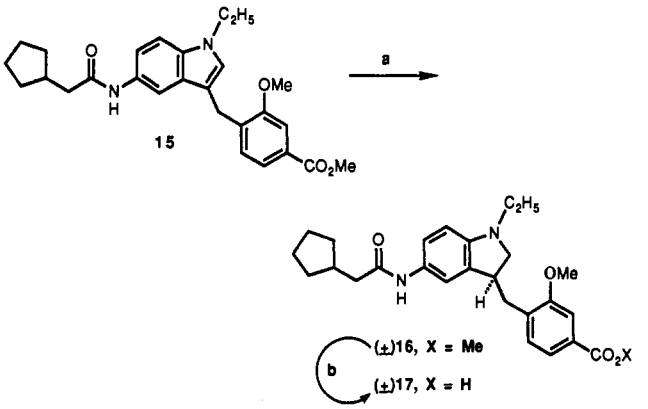
Cleavage of the (nucleophilic) C2–C3 double bond of the indole (**6a,b**, prepared as described in Scheme I) with singlet oxygen in methanol[10] (Rose Bengal as sensitizer) gave keto amide **8** in virtually quantitative yield. Treatment of **8** with hydroxylamine in hot pyridine simultaneously removed the formyl group and converted the ketone to its (*E*)-oxime **9**. On the basis of literature precedent,[12a] the *E* geometry of the oxime was indicated by the low-field resonance (δ 7.45, DMSO-d_6) of the amino NH proton in the ^1H NMR of **9**. The (*E*)-oxime is presumably the thermodynamic product, since it can take advantage of a strong,[11] mutually reinforcing intramolecular hydrogen bond between the amino NH and the imino nitrogen lone pair. Acetylation with acetic anhydride in the presence of pyridine in methylene chloride gave *E*-oxime acetate **10**. Heating the crystalline oxime acetate **10** in the melt at 170 °C for 10 min (under vacuum) effected quantitative closure to the indazole ring system[12] (**11a, 11b**). Subsequent hydrolysis of the cyclopentylurethane methyl ester **11a** gave indazole acid **12**.

Reductive cleavage of the benzylurethane in **11b** gave the primary amine, which was acylated with an acid chloride (method A) or a carboxylic acid (Method B) to give urethane and amide derivatives, or was converted to urea derivatives via the isocyanate (method C) to give esters **13**. Acids **14** were obtained, as before, by base-catalyzed hydrolysis of **13**.

Indoline **17** was prepared as shown in Scheme III, by reduction of indole **15** to indoline **16** using catalytic hydrogen transer,[24] followed by hydrolysis of the methyl ester under the usual conditions.

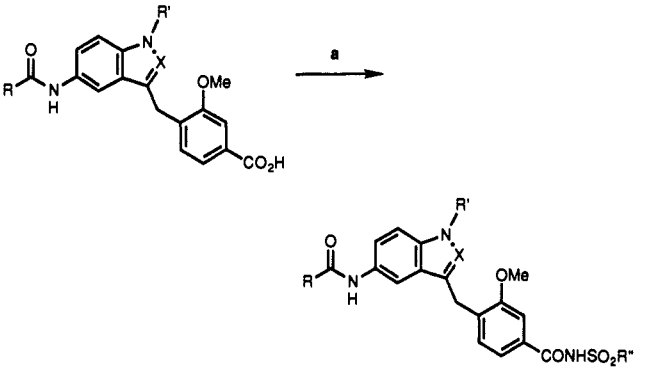
In all cases, the carboxylic acid derivatives were cleanly converted to the required aliphatic or aromatic *N*-sulfonyl amide by coupling the acid with the requisite sulfonamide in the presence of a (water-soluble) carbodiimide in methylene chloride (Scheme IV).

Scheme III[a]



[a] Formic acid, 10% Pd/C, Δ; (b) LiOH, H_2O, THF, methanol.

Scheme IV[a]



[a] (a) H_2NSO_2R'', 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, 4-(dimethylamino)pyridine, CH_2Cl_2.

Results and Discussion

Structure/Activity Relationships for Indole Acids. The "inverted" indole acids were examined for percent inhibition of LTE_4-induced contraction of guinea pig tra-

(10) H. H. Wassermann; B. H. Lipshutz In *Singlet Oxygen*, H. H. Wassermann, R. W. Murray, Eds.; *Organic Chemistry, A Series of Monographs*; Academic: New York 1979; Vol. 40, Chapter 9.

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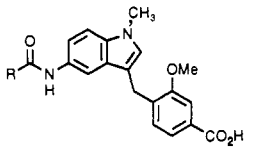
(12) (a) For a related photochemical ring closure, see: Pfoertner, K-H.; Foricher, J. *Helv. Chim. Acta* **1982**, *65*, 798. (b) For a related thermal closure to produce a benzisoxazole, see: Blatt, A. H.; Russell, L. H. *J. Am. Chem. Soc.* **1936**, *58*, 1903.

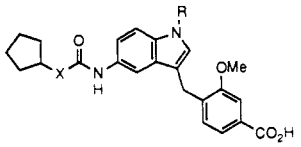
Table II. *N*-Methylindole Carboxylic Acids: Acylamino Chain Variations



compd	R	% inhibition/ concentration,[a] nM	K_i,[b] nM
19	*n*-pentyl	NS/100	1900
23	$CH_3(CH_2)_2CH(Et)$	70/330	1830
24	cyclopentyl-O	75/100	157
25	cyclopentyl-CH_2	94/100	80
26	cyclopentyl-NH	70/1000	1119

[a] Defined in Table I, footnote *a*. [b] Inhibition constant for displacement of [^{3}H]LTD_4 on guinea pig lung parenchymal membranes; K_i values are the mean of two experiments conducted in duplicate with separate batches.

Table III. Indole Cyclopentylurethanes and Cyclopentylacetamides: N1 Modification



compd	X	R	K_i,[a] nM	pK_B[b] (*n*)[c]
24	O	Me	157	7.84 (4)
27	O	H	236	7.32 (6)
28	O	*n*-propyl	58	7.72 (4)
29	O	$(CH_2)_2OMe$	41	
30	O		57	
31	O		53	7.67 (8)
25	CH_2	Me	80	
32	CH_2	ethyl	71	
33	CH_2	*n*-propyl	84	
34	CH_2	benzyl	70	
35	CH_2		10	8.55 (8)

[a] Defined in Table II, footnote *b*. [b] -log molar dissociation constant on guinea pig tracheal strips, LTE_4 as agonist; standard error of the mean <3% in all cases. [c] Number of determinations.

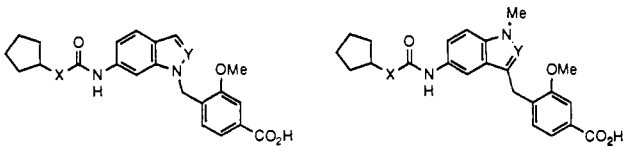
cheal strips, beginning with a hexanamide[9a] as acylamino chain (Table I). Initially, a selection of simple indole N1 substituents was evaluated. Compounds **19–21**, which had lipophilic groups at N1, were active at 1 μM in the functional assay on guinea pig trachea against LTE_4, and the unsubstituted derivative **18** was active at 3.3 μM. Compound **22**, which had the polar acetic acid group at N1, was significantly less potent than **18**. On the basis of these results, a methyl group at the indole N1 position was retained during investigation of the acylamino chain.

Table II summarizes the in vitro activity of N1-methylindoles when the acylamino chain was varied. Consonant with previous work from these laboratories,[9a] the cyclopentylurethane and cyclopentylacetamide groups conferred good in vitro activity on the series. The compounds in question, **24** and **25**, had K_i's against [^{3}H]LTD_4 of 157 and 80 nM, respectively, and were active at 100 nM in the functional assay against LTE_4.[9c] Cyclopentylurea **26** was about 1 order of magnitude less potent.

Further investigation of the indole N1 substituent was undertaken with the cyclopentylurethane present as the acylamino chain. Removing the methyl group in **24** gave (considerably more polar) NH derivative **27**, which possessed similar K_i and K_B (Table III). A variety of lipophilic substituents at N1 of the indole—for example propyl (**28**), 2-methoxyethyl (**29**), cyclopropylmethyl (**30**), and cyclopentyl (**31**)—were accommodated at the receptor and gave compounds of similar potency (K_i) to *N*-methyl derivative **24**. The potency of *N*-cyclopentyl compound **31** suggested that the indole N1 substituent was occupying a relatively spacious (lipophilic?) site at the LTD_4 receptor. However, the intrinsic binding potential[13] of the cyclopentyl ring (ca. 3 kcal/mol) was not being utilized, since **31** was no more potent than methyl derivative **24**. The opportunity therefore existed for optimization of the receptor binding characteristics of the indole N1 substituent, but subsequent developments (vide infra) with sulfonyl amide analogues of these compounds made further investigation of lipophilic N1 substituents unattractive in the urethane series.

Lipophilic substituents were also well-tolerated when the acylamino chain was cyclopentylacetamide (lower half of Table III), the *m*-cyanobenzyl group (**35**) being the only one of these which provided a noticeable improvement (ca. 10-fold) in in vitro activity over that of methyl analogue **25**. The exceptionally potent carboxylic acid **35** has been investigated further in the form of *N*-alkylsulfonyl amide derivatives (vide infra).

Table IV. Cyclopentylurethane and Cyclopentylacetamide Indoles and Indazoles: Comparison of Templates



compd	X	Y	pK_B[a]	K_i,[b] nM	compd	X	Y	pK_B[a]	K_i,[b] nM
c	O	CH	7.80	160	24	O	CH	7.84	157
c	CH_2	CH	7.68	200	25	CH_2	CH		80
c	O	N	7.50	80	12	O	N		160
c	CH_2	N	7.79	50	36	CH_2	N		132

[a] Defined in Table III, footnote *b*. [b] Defined in Table II, footnote *b*. [c] Reference 9a.

Structure/Activity Relationships for Indazole Acids

In similar fashion to the N1-methylindole series above, cyclopentylurethane **12** and cyclopentylacetamide **36** derivatives in the N1-methylindazole series were potent and approximately equipotent (Table IV). Table IV shows a comparison of the activity of a cyclopentylurethane indole and indazole, and a cyclopentylacetamide indole and indazole, all from the present study, with the corresponding indoles and indazoles from previous work.[9a]

The similar activities of the eight compounds in Table IV led to the conclusion that the bicyclic ring system in this family of LTD_4 receptor antagonists functions as a template, which presents the acylamino and acidic chains in a certain ("correct") orientation at the receptor. The template may therefore impart a degree of preorganization[14] to the antagonist molecules and thereby facilitate the molecular recognition event. The electronic properties of the individual templates appear to be less important, a finding which could not have been anticipated at the outset.

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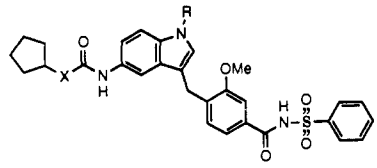
(14) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039.

Table V. Indole Phenylsulfonyl Amides: In Vitro and in Vivo Vivo Activities



compd	X	R	pK_B[a] (n)[b]	K_i,[c] nM	po ED_{50},[d] mg/kg
37	O	H	9.84 (8)	1.4	
38	O	Me	9.69 (8)	0.8	2.5
39	O	$(CH_2)_2OMe$	9.39 (6)	0.2	1.1
40	O	*n*-propyl	8.44 (10)		1.0
41	O	cyclopentyl	7.84 (4)	67	
42	CH_2	Me	9.80 (6)	0.3	1.0
43	NH	Me	9.83 (5)	0.8	~35
44	NH	*n*-propyl	9.20 (6)	0.5	

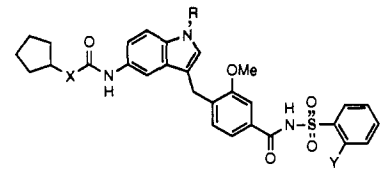
[a,b] Defined in Table III, footnotes *b,c*. [c] Defined in Table II, footnote *b*. [d] Guinea pig "dyspnea" model, 180-min pretreatment time (see the Experimental Section).

There are restrictions on the relative geometry of the substituents on the template, however. The importance of the planarity of the template was suggested by the reduced receptor affinity of (racemic) indoline **17** (K_i = 1.3 μM) compared to indole **32** (K_i = 0.07 μM). Three important molecular changes, one geometrical and two electronic, are introduced when an indole is reduced to an indoline: first, C2 and C3 of the indole rehybridize from trigonal to tetrahedral geometry; second, the ring nitrogen atom becomes more basic; and third, the pyrrole ring loses its capacity for Π-stacking donor/acceptor[26] (or related[27]) interactions. The last of these features may be ruled out as an explanation of the reduced receptor affinity of indoline **17**, since related templates in which the right-hand ring is nonaromatic have been shown to be capable of producing high-affinity antagonists.[15] The pK_a of an indoline[16a] is too low to result in protonation at physiological pH, and the drop in activity (ca. 20-fold[16b]) was therefore attributed to the change in geometry at C3, which produces a marked change in the position of the benzoic acid group.

Structure/Activity Relationships for Indole *N*-Phenylsulfonyl Amides

In Vitro. Aliphatic and aromatic *N*-sulfonyl amides are known as acid mimics,[17] the pK_a of the acidic NH proton being similar to that of a carboxylic acid.[17] Previous reports from these laboratories showed that replacement of the carboxyl group by an *N*-arylsulfonyl amide in related LT antagonists produced a substantial increase in receptor affinity.[9b] In the present indole urethane series, the observed benefits were found to be strongly dependent upon the nature of the indole N1 substituent (Table V). For example, whereas about a 100-fold improvement in dissociation constant was observed between N1-methylindole acid **24** and its phenylsulfonyl amide **38**, there was *no change* when the same modification was made to N1-cyclopentyl acid **31** (compare to **41**). Similarly, only a 5-fold difference in in vitro activity was observed between the N1-propyl acid **28** and its phenylsulfonyl amide **40**.

Table VI. Indole Arylsulfonyl Amides: In Vitro and in Vivo Vivo Activities



compd	X	R	Y	pK_B[a] (n)[b]	K_i,[c] nM	po ED_{50},[d] mg/kg
45	O	Me	Me	9.67 (8)	0.3	0.3
46	CH_2	H	Me	9.69 (6)	1.0	1.3
47	CH_2	Me	Me	9.64 (4)	0.5	0.2
48	CH_2	Me	Cl	9.85 (6)	0.3	
49	CH_2	Me	Br	10.11 (8)	0.5	
50	NH	Me	Me	9.31 (21)	1.4	0.9
51	NH	Me	Cl	10.17 (4)	0.7	

[a,b] Defined in Table III, footnotes *b,c*. [c] Defined in Table II, footnote *b*. [d] Defined in Table VI, footnote *d*.

Table VII. Comparison of In Vitro Activity of 45 (ICI 204,219) with That of Other Prominent LTD_4 Antagonists

compd	pK_B[a]	K_i,[b] nM
45 (ICI 204,219)	9.67 ± 0.13[c]	0.34 ± 0.03[c]
ICI 198,615	10.26 ± 0.05[d]	0.28 ± 0.03[d]
SKF 104,353	>8.9[e]	5 ± 2[f]
LY 171,883	7.21 ± 0.04[g]	2818[h]

[a] -Log molar dissociation constant on guinea pig tracheal strips, LTE_4 as agonist. [b] Inhibition constant for displacement of [^{3}H]-LTD_4 on guinea pig lung parenchymal membranes. [c] Present manuscript. [d] Reference 6c. [e] Reference 1e. [f] Reference 5b. [g] Reference 2d. [h] Aharony, D.; et al *J Pharm Exp. Ther.* **1989**, *248*, 581.

This unexpected trend cannot be interpreted as the result of an unfavorable steric interaction of the indole N1 substituent with the receptor, since *N*-(2-methoxyethyl) derivative **39** showed excellent affinity. A possible explanation is that the propyl and cyclopentyl derivatives were too lipophilic. Support for this notion was provided by urea **44**, a high-affinity antagonist despite the presence of a propyl group at N1 of the indole. Urea **44** is a more hydrophilic molecule than urethane **40**, and it is reaonable to assume that the urea chain per se does not provide improved receptor binding, since the cyclopentylurethane **38** and cyclopentylurea **43** had closely similar dissociation constants (Table V). Compound **44** was somewhat less potent than **43**, however, on trachea.

The fact that urethane (**38**), amide (**42**), and urea (**43**) *N*-phenylsulfonyl amides were equipotent is noteworthy, since the urea was 10-fold less potent than the others in the carboxylic acid series (Table II). This means that introduction of the phenylsulfonyl amide led to a 10^3-fold increase (4.2 kcal/mol) in receptor affinity, for this particular urea (compare **43** with **26**).

In Vivo. When given by oral administration in the guinea pig, **38**, **39**, **42**, and **40** were effective in blocking (aerosolized) LTD_4-induced dyspnea,[18] the last of these

(15) Matassa, V. G.; Brown, F. J.; Bernstein, P. R.; Shapiro, H. S.; Maduskuie, Jr., T. P.; Cronk, L. A.; Vacek, E. P.; Yee, Y. K.; Snyder, D. W.; Krell, R. D., unpublished observations.

(16) (a) Clark, J.; Perrin, D. D. *Q. Rev. Chem. Soc.* **1964**, *18*, 295. (b) The drop in activity of **17** compared to that of **32** may only be 10-fold, if it is assumed that one enantiomer is inactive.

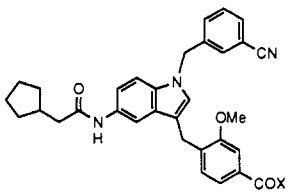
(17) Schaaf, T. K.; Hess, H-J. *J. Med. Chem.* **1979**, *22*, 1340.

(18) Snyder, D. W.; Liberati, N. J.; McCarthy, M. M. *J. Pharmacol. Methods* **1988**, *19*, 219. LTD_4 is normally used as agonist in the in vivo studies since it is the most potent of the peptidoleukotrienes in this model.

(19) (a) A. Fersht *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1985; pp 296–299. (b) Fersht, A. R.; Shi, J.-P.; Knill-Jones, J.; Lowe, D. M.; Wilkinson, A. J.; Blow, D. M.; Brick, P.; Carter, P.; Waye, M. M. Y.; Winter, G. *Nature* **1985**, *314*, 235. (c) Vedani, A.; Dunitz, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7653.

(20) Thomas, K. A.; Smith, G. M.; Thomas, T. B.; Feldman, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4843.

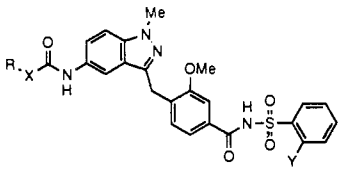
Table VIII. Indole Alkylsulfonyl Amides



compd	X	pK_B[a] (n)[b]	K_i,[c] nM
35	OH	8.55 (8)	10
60	$NHSO_2Me$	9.00 (8)	5.6
61	$NHSO_2$-*i*-Pr	9.28 (8)	6.8

[a,b] Defined in Table III, footnotes *b*,*c*. [c] Defined in Table II, footnote *b*.

Table IX. *N*-Methylindazole Arylsulfonyl Amides: In Vitro and in Vivo Activities



compd	R	X	Y	pK_B[a] (n)[b]	K_i,[c] nM	po ED_{50},[d] mg/kg
52	⬠	O	H	10.30 (6)	0.25	
53	⬠	O	Me	10.95 (11)	0.3	0.5
54	⬠	O	Cl	10.43 (11)	0.3	1.0
55	⬠	O	Br	10.73 (6)	0.3	1.1
56	⬠	CH_2	Me	10.75 (14)	0.3	47%/0.6 mg/kg[e] 100%/1.8 mg/kg[e]
57	⬠	CH_2	Cl	10.80 (13)	0.3	48%/0.6 mg/kg[e]
58	⬡	CH_2	Cl	10.75 (5)	0.25	NS/0.6 mg/kg[e,f] 71%/1.8 mg/kg[e]
59	□	NH	Cl	10.85 (6)	0.1	1.0

[a,b] Defined in Table III, footnotes *b*,*c*. [c] Defined in Table II, footnote *b*. [d] See Table VI, footnote *d*. [e] Percent protection of animals against aerosolized LTD_4-induced "dyspnea" at given antagonist concentration. [f] NS = not statistically significant ($p > 0.05$).

surprisingly so in view of its modest in vitro activity (Table V). In contrast, the cyclopentylurea **43** was much less potent in vivo.

Structure/Activity Relationships for Indole *N*-Arylsulfonyl Amides

Introduction of a methyl, chlorine, or bromine substituent into the ortho position of the sulfonamide ring (Table VI) had little effect on receptor binding. As expected from previous work,[9b] however, oral activity was improved in these derivatives in comparison to their phenyl analogues. For example, (2-methylphenyl)sulfonyl amide **45** (ICI 204,219) was some 10-fold more potent following oral administration than its phenyl analogue **38**. This may reflect better absorption of **45**, since the in vitro dissociation constants were the same for both compounds. Compound **45** had a long duration of action following oral administration in the conscious guinea pig ($t_{1/2} > 14$ h at a dose of 0.5 μmol/kg),[22a] and was also active by the aerosol route.[18,22a] The in vitro activity of ICI 204,219 is compared to that of ICI 198,615, SKF 104,353 and LY 171,883 in Table VII.

Table X. Physical Data for Compounds of Tables I–IX

compd	% yield[a]	mp, °C	formula	analysis[b]
12	98	216–217	$C_{23}H_{25}N_3O_5$	C, H, N
17	84	183–186	$C_{28}H_{32}N_2O_4$	C, H, N
18	77	205–210	$C_{23}H_{26}N_2O_4 \cdot 0.25H_2O$	C, H, N
19	66	227–228	$C_{24}H_{28}N_2O_4$	C, H, N
20	39	203–204	$C_{26}H_{30}N_2O_4$	C, H, N
21	67	210–211	$C_{30}H_{32}N_2O_4$	C, H, N
22	59	235–240	$C_{25}H_{28}N_2O_6$	C, H, N
23	66	204–208	$C_{26}H_{32}N_2O_4$	C, H, N
24	88	157–158	$C_{24}H_{26}N_2O_5$	C, H, N
25	84	238–241	$C_{25}H_{28}N_2O_4$	C, H, N
26	70	203–206	$C_{24}H_{27}N_3O_4$	C, H, N
27	80	184–186	$C_{23}H_{24}N_2O_5$	C, H, N
28	89	157–158	$C_{28}H_{30}N_2O_5$	C, H, N
29	65	158–159	$C_{26}H_{30}N_2O_6$	C, H, N
30	72	177–179	$C_{27}H_{30}N_2O_5$	C, H, N
31	75	189–191	$C_{28}H_{32}N_2O_5$	C, H, N
32	83	219–220	$C_{26}H_{30}N_2O_4$	C, H, N
33	82	196–197	$C_{27}H_{32}N_2O_4$	C, H, N
34	85	239–241	$C_{31}H_{32}N_2O_4$	C, H, N
35	89	130–132	$C_{32}H_{31}N_3O_4 \cdot 0.6H_2O$	C, H, N
36	98	241–242	$C_{24}H_{27}N_3O_4 \cdot 0.5H_2O$	C, H, N
37	30	244–245	$C_{29}H_{29}N_3O_6S$	C, H, N
38	98	132–138	$C_{30}H_{31}N_3O_6S$	C, H, N
39	65	107–117	$C_{32}H_{35}N_3O_7S$	C, H, N
40	84	109–115	$C_{32}H_{35}N_3O_6S$	C, H, N
41	89	179–181	$C_{34}H_{37}N_3O_6S$	C, H, N
42	86	228–232	$C_{31}H_{33}N_3O_5S$	C, H, N
43	89	197–200	$C_{30}H_{32}N_4O_5S$	C, H, N
44	66	207–208	$C_{32}H_{36}N_4O_5S$	C, H, N
45	69	138–140	$C_{31}H_{33}N_3O_6S$	C, H, N
46	56	248–250	$C_{31}H_{33}N_3O_5S$	C, H, N
47	81	194–196	$C_{32}H_{35}N_3O_5S \cdot 0.2H_2O$	C, H, N
48	47	197–199	$C_{31}H_{32}ClN_3O_5S \cdot 0.5H_2O$	C, H, N
49	67	202–204	$C_{31}H_{32}BrN_3O_5S$	C, H, N
50	58	212–215	$C_{31}H_{34}N_4O_5S \cdot H_2O$	C, H, N
51	88	158–160	$C_{30}H_{31}ClN_4O_5S \cdot 0.3H_2O$	C, H, N
52	63	145	$C_{29}H_{30}N_4O_6S \cdot 0.5H_2O$	C, H, N
53	91	179–181	$C_{30}H_{32}N_4O_6S$	C, H, N
54	92	179–181	$C_{29}H_{29}ClN_4O_6S$	C, H, N
55	93	181.5–183	$C_{29}H_{29}BrN_4O_6S$	C, H, N
56	57	140–145	$C_{31}H_{34}N_4O_5S \cdot 0.65H_2O$	C, H, N
57	74	144–155	$C_{30}H_{31}ClN_4O_5S \cdot 0.25H_2O$	C, H, N
58	90	148–150	$C_{31}H_{33}ClN_4O_5S \cdot 0.25H_2O$	C, H, N
59	79	221–222.5	$C_{28}H_{28}ClN_5O_5S$	C, H, N
60	45	182–185	$C_{33}H_{34}N_4O_5S \cdot 0.4H_2O$	C, H, N
61	76	148–150	$C_{35}H_{38}N_4O_5S$	C, H, N

[a] Yield for final step. [b] Analyses were within ±0.4% of theoretical values.

Structure/Activity Relationships for Indole *N*-Alkylsulfonyl Amides

As discussed above, investigation of indole N1 substituents in the carboxylic acid series revealed that a *m*-cyanobenzyl group was particularly interesting, cyclopentylacetamide **35** having a pK_B of 8.55 on trachea and K_i of 10 nM in the radioligand binding assay (Table VIII).

In an attempt to improve the activity of **35**, without having to resort to an *N*-arylsulfonyl amide, a selection of *aliphatic* sulfonyl amide derivatives of **35** was investigated. As shown in Table VIII, the methylsulfonyl (**60**) and isopropylsulfonyl (**61**) amides were somewhat more potent than acid **35**, and had pK_B's ≥ 9. These results demonstrate that molecules possessing subnanomolar dissociation constants can be engineered in the absence of the arylsulfonyl group. Despite their high in vitro activities, **61** and **60** were disappointing in vivo.

Structure/Activity Relationships for Indazole *N*-Arylsulfonyl Amides

In Vitro. This series of (N1-methyl) indazoles consistently produced antagonists with exceptionally high affinity for the LTD_4 receptor (Table IX). For example, cyclopentylurethanes **53–55** had dissociation constants in

the 10^{-10}–10^{-11} M range on guinea pig trachea. There was a trend for these ortho-substituted sulfonamides[9b] to be significantly more potent on trachea than the phenyl analogue **52**, in contrast to the observation for indoles (vide supra). Cyclopentylacetamides **56** and **57** were of comparable potency to the urethanes **53–55**. In addition to the low dissociation constants on guinea pig trachea against LTE_4, the indazoles had K_i values $<10^{-9}$ M against [^{3}H]-LTD_4 on guinea pig parenchyma (Table IX); the discrepancy between the K_B and K_i values in this series is probably due to the inherent insensitivity of (tritium-based) radioligand binding assays at low ligand concentrations (10^{-9}–10^{-11} M).[21]

It was interesting to discover that indazoles were more potent than indoles, on guinea pig trachea, when an arylsulfonyl amide was present in the antagonist molecule, since this contrasted with the situation for carboxylic acids discussed above. Remarkably, a similar relationship had been found between the earlier indole and indazole series.[6b]

The indazoles are as much as 1 order of magnitude more potent on trachea than the indoles (e.g., compare **53** with **45**). The reason for this is unclear, but one speculation is that the indazole N2 nitrogen atom sp^2 lone pair (which has an identical spatial orientation in both types of indazoles) could engage in an energetically favorable interaction with a suitable site at the LTD_4 receptor. Since the observed energy difference is relatively small (ca. 1–1.5 kcal/mol), the interaction could take the form of a hydrogen bond between uncharged centers,[19a,b,13] or an electrostatic interaction with the edge of a protein-bound aryl ring.[20] The indoles would not, of course, be able to enjoy these kinds of stabilization.

Just why the indole- and indazolecarboxylic acids were closely similar in activity (Table IV) and yet their *N*-arylsulfonyl amides were significantly different from each other is also unclear. However, a minor difference in binding mode for acids and sulfonyl amides could easily account for this observation, since noncovalent interactions of the kind suggested above are generally sensitive to small changes in the interatomic separation and (particularly for hydrogen bonds) in the directional properties of the donor and acceptor centers.[19a,c,20]

As a consequence of the apparently superior receptor binding properties of the indazole ring system, acylamino chains other than the (previously optimal) cyclopentylurethane, cyclopentylacetamide, or cyclopentylurea could be incorporated to produce high-affinity antagonists. Thus, cyclo*hexyl*acetamide **58** and cyclo*butyl*urea **59** retained exceptional affinity in both the radioligand binding and functional assays (Table IX).

In Vivo. The indazoles were generally active at doses ≤1 mg/kg, following oral administration in the guinea pig. Cyclopentylurethane **53** was one of the most potent compounds in the series (po ED_{50} = 0.5 mg/kg, Table IX). This compares well with the corresponding urethane from the indole series (**45**; po ED_{50} = 0.3 mg/kg). However, since the in vitro dissociation constant (Table IX) and intravenous ED_{50} of **53** (po ED_{50}:iv ED_{50} ca. 80) were about 10-fold better than those of **45** (po ED_{50}:iv ED_{50} ca. 10),[22a] absorption of **53** may have been lower. Other indazoles from the present study also had high po ED_{50}:iv ED_{50} ratios, consistent with the observations for ICI 198,615.[6d] As a generalization, the indazole templates ii and iv seem to predispose the antagonist molecules to low bioavailability.

Selectivity. The in vitro selectivities of **38** and **45** have been examined.[22a] At a concentration of 10 μM, neither compound had any effect on α_1 (rat aorta, phenylephrine as agonist), α_2 (rat vas deferens/clonidine), β_1 (guinea pig right atria/norepinephrine), β_2 (guinea pig trachea/salbutamol), H_1 (rabbit aorta/histamine), H_2 (guinea pig right atria/histamine), 5-HT_2 (rabbit aorta/serotonin), muscarinic (guinea pig trachea/carbachol), or PGE_2 (guinea pig trachea/PGE_2) receptors. On guinea pig trachea, **38** had a nonsignificant effect (pK_B = 5.1 ± 0.3), and **45** had a barely significant effect (pK_B = 5.60 ± 0.3) against U46619 at the TxA_2 receptor. The compounds therefore show extremely high selectivity for the LTD_4 receptor.

Summary

A study of the bicyclic ring system (template) of a newly reported family of high-affinity LTD_4 antagonists has been undertaken. The study has shown that the LTD_4 receptor is surprisingly tolerant of changes in the electronic constitution of the template, providing that the rings remain planar. Novel series of indoles and indazoles have thereby been discovered and have been shown to be equipotent in vitro with the original indoles and indazoles. In accord with previous work, the best of these compounds generally have a cyclopentyl ring as part of the acylamino chain, and have an *N*-arylsulfonyl amide as the acidic group, although some exceptions have been discussed. In vitro dissociation constants were generally in the range 10^{-9}–10^{-11} M, and a number of compounds were orally effective in the guinea pig at doses <1 mg/kg.

The lipophilicity of the indole N1 substituent in the cyclopentylurethane series was found to play an important role in modulating the in vitro activity of the *N*-arylsulfonyl amide derivatives but had an insignificant role in the case of the carboxylic acids. The affinities of the (more polar) cyclopentylurea *N*-arylsulfonyl amides had a lesser, but still significant, dependence upon the indole substituent. By controlled manipulation of the indole N1 substituent in the cyclopentylacetamide series, certain N-*alkyl*sulfonyl amides were produced which had dissociation constants $\leq 10^{-9}$ M on guinea pig trachea.

N1-Methylindole **45** (ICI 204,219) emerged from this study as the clinical candidate of choice. In addition to its excellent in vitro and in vivo profile in guinea pig and its in vitro selectivity, the compound has high bioavailability in rat (68%)[28] and dog (67%)[28] and is a potent antagonist of the peptidoleukotrienes on isolated human bronchus (pK_B ca. 8.5).[1c,22b]

Experimental Section

General Procedures. ^{1}H NMR spectra were recorded on a Brucker WM 250 (250 MHz) or a Brucker AM 300 (300 MHz) instrument using DMSO-d_6 as solvent, with tetramethylsilane as internal standard. Infrared spectra were recorded on a Perkin-Elmer 781 spectrophotometer. Mass spectra were recorded on a Kratos MS-80 instrument. Melting points were measured on a Thomas-hoover capillary melting point apparatus and are un-

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(22) (a) Krell, R. D.; Aharony, D.; Buckner, C. K.; Keith, R. A.; Kusner, E. J.; Snyder, D. W.; Matassa, V. G.; Yee, Y. K.; Brown, F. J.; Bernstein, P. R.; Hesp, B.; Giles, R. E. *Am. Rev. Res. Dis.*, in press. (b) Buckner, C. K.; Fedyna, J. S.; Robertson, J. L.; Will, J. A.; England, D. M.; Krell, R. D.; Saban, R. *J. Pharmacol. Exp. Ther.*, in press.
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corrected. Combustion analyses were performed on a Perkin-Elmer 241 instrument, by ICI Americas Analytical Department. Chromatography was performed according to the method of Still,[23] using the indicated solvent ratios (v/v) on Kieselgel 60 (230–400 mesh) supplied by E. Merck. Tetrahydrofuran (THF) and dioxane were distilled from sodium benzophenone ketyl. Pyridine was distilled from calcium hydride. Aromatic sulfonamides were generally recrystallized from ethanol prior to use. All other reagents were used as received. Unless otherwise indicated, reaction workups culminated in drying the solution with anhydrous $MgSO_4$ and removing the solvent by evaporation under reduced pressure.

Methyl 3-Methoxy-4-[(5-nitroindol-3-yl)methyl]benzoate (3). Silver(I) oxide (7.15 g, 30.8 mmol) was added to a stirred solution of 5-nitroindole (5.0 g, 30.8 mmol) and methyl 4-(bromomethyl)-3-methoxybenzoate[9a] (7.99 g, 30.8 mmol) in dioxane (30 mL), under nitrogen. The mixture was heated at 60 °C for 20 h. The solvent was evaporated under reduced pressure, ethyl acetate (50 mL) was added, and the mixture was filtered through a pad of diatomaceous earth. The solvent was evaporated and the product was isolated by chromatography, eluting with 3:7 ethyl acetate/hexanes, to give a yellow oil, which was crystallized from a CH_2Cl_2/hexanes mixture to give **3** (4.6 g, 45%) as yellow needles: mp 153–155 °C; IR ($CHCl_3$) 3470, 1715 cm^{-1}; ^1H NMR δ 3.83 (s, 3 H, OCH_3), 3.93 (s, 3 H, OCH_3), 4.12 (s, 2 H, CH_2Ar), 7.25 (d, 1 H), 7.43 (d, 1 H), 7.49 (m, 3 H), 7.95 (dd, 1 H, indole H6), 8.47 (d, 1 H, indole H4), 11.65 (br s, 1 H, NH); MS m/z 340 (M^+).

General Procedure for Indole N1-Alkylation. Methyl 3-Methoxy-4-[(1-methyl-5-nitroindol-3-yl)methyl]benzoate (4a, R′ = CH_3). Nitro ester **3** (0.44 g, 1.29 mmol) was added to a stirred suspension of oil-free sodium hydride (0.031 g, 1.29 mmol) in dry THF (10 mL), under nitrogen. After 10 min, iodomethane (0.18 g, 1.29 mmol) was added to the dark-red solution. After 30 min, the mixture was poured into 1 M hydrochloric acid (30 mL) and extracted with ethyl acetate (2 × 50 mL), and the combined extracts were washed with brine, then dried, and evaporated. The product was isolated by chromatography, eluting with 50:45:5 hexanes/CH_2Cl_2/ethyl acetate, to give a yellow oil, which was crystallized from a mixture of CH_2Cl_2 and hexanes to give **4a** (R′ = CH_3) (0.33 g, 72%) as a yellow solid: mp 144–146 °C; IR ($CHCl_3$) 1715 cm^{-1}; ^1H NMR δ 3.81 (s, 3 H, NCH_3), 3.83 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 4.11 (s, 2 H, CH_2Ar), 7.27 (d, 1 H), 7.37 (s, 1 H, indole H2), 7.49 (m, 2 H), 7.60 (d, 1 H), 8.01 (dd, 1 H, indole H6), 8.50 (d, 1 H, indole H4); MS m/z 354 (M^+).

General Procedure for Catalytic Reduction of a Nitro Group. Methyl 4-[(5-Amino-1-methylindol-3-yl)methyl]-3-methoxybenzoate (5, R′ = CH_3). Palladium-on-carbon (10% w/w, 0.1 g) was added to a solution of **4a** (R′ = CH_3) (0.56 g, 1.57 mmol) in THF (30 mL) and the mixture was hydrogenated at 3.45 bars for 2 h. The mixture was filtered through a pad of diatomaceous earth, and the solvent was evaporated. The product was purified by chromatography, eluting with 1:1 ethyl acetate/hexanes, to give **5** (R′ = CH_3) (0.5 g, 98%), as an oil; ^1H NMR δ 3.60 (s, 3 H, NCH_3), 3.83 (s, 3 H, OCH_3), 3.9 (m, 5 H, OCH_3 and CH_2Ar), 4.45 (br, 2 H, NH_2), 6.54 (m, 2 H), 6.86 (s, 1 H), 7.04 (m, 2 H), 7.40 (m, 2 H).

Reduction of a Nitro Group Using Stannous Chloride. Methyl 4-[(1-Allyl-5-aminoindol-3-yl)methyl]-3-methoxybenzoate (5, R′ = Allyl). Stannous chloride dihydrate (1.80 g, 8.02 mmol) was added to a suspension of **4b** (0.61 g, 1.60 mmol, prepared from **3** by alkylation with allyl bromide) in ethanol (15 mL), under nitrogen. The mixture was heated at reflux for 18 h. The cooled mixture was evaporated and the residue was partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ solution (50 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined extracts were dried and evaporated, and the product was isolated by chromatography, eluting with 3:7 ethyl acetate/hexanes, to give **5** (R′ = allyl) (0.46 g, 82%) as an oil: ^1H NMR δ 3.83 (s, 3 H, OCH_3), 3.91 (s, 5 H, OCH_3 and CH_2Ar), 4.4–4.8 (br, 2 H, NH_2), 4.64 (d, 2 H, NCH_2), 4.9–5.25 (m, 2 H, $CHCH_2$), 5.8–6.05 (m, 1 H, $CHCH_2$).

General Procedure for Acylation of an Amine by an Acid Chloride (Method A). Methyl 4-[[5-[[(Cyclopentyloxy)carbonyl]amino]-1-methylindol-3-yl]methyl]-3-methoxybenzoate (6, R′ = CH_3, R = Cyclopentyloxy). Cyclopentyl chloroformate (0.11 g, 0.77 mmol) was added to a stirred solution of **5** (R′ = CH_3) (0.25 g, 0.77 mmol) and *N*-methylmorpholine (0.23 g, 0.77 mmol), in CH_2Cl_2 (3 mL), under nitrogen. The mixture was stirred for 2 h, then poured into 1 M hydrochloric acid (20 mL), and extracted with ethyl acetate (2 × 30 mL). The combined extracts were washed with saturated brine (20 mL), dried, and evaporated to give a viscous oil. The product was purified by chromatography, eluting with 3:7 ethyl acetate/hexanes, to give **6** (R′ = CH_3, R = cyclopentyloxy) (0.25 g, 74%), as a foam: IR ($CHCl_3$) 3440, 1710 cm^{-1}; ^1H NMR δ 1.62 [m, 8 H, $(CH_2)_4$], 3.68 (s, 3 H, NCH_3), 3.83 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 3.95 (s, 2 H, CH_2Ar), 5.05 (m, 1 H, CHO), 7.11 (m, 2 H), 7.26 (d, 1 H), 7.43 (m, 2 H), 7.59 (br s, 1 H), 9.18 (br s, 1 H, NH); MS m/z 437 (M^+).

General Procedure for Acylation of an Amine by a Carboxylic Acid (Method B). Methyl 4-[[5-(2-Cyclopentylacetamido)-1-methylindol-3-yl]methyl]-3-methoxybenzoate (6, R′ = CH_3, R = Cyclopentylmethyl). A mixture of **5** (R′ = CH_3) (0.57 g, 1.76 mmol), cyclopentylacetic acid (0.23 g, 1.79 mmol), 4-(dimethylamino)pyridine (0.22 g, 1.79 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.343 g, 1.79 mmol) was dissolved in CH_2Cl_2 (25 mL) under nitrogen. After 18 h, the mixture was poured into 1 M HCl (25 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The extracts were washed with water and brine, then dried, and evaporated. The residual oil was crystallized from ethyl acetate to give **6** (R′ = CH_3, R = cyclopentylmethyl) (0.56 g, 73%) as a white powder: mp 180–181 °C; IR ($CHCl_3$) 3440, 1715, 1670 cm^{-1}; ^1H NMR δ 1.17 (m, 2 H), 1.4–1.8 (m, 6 H), 2.25 (m, 3 H), 3.70 (s, 3 H, NCH_3), 3.82 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 3.97 (s, 2 H, CH_2Ar), 9.62 (br s, 1 H, NH); MS m/z 435 (M^+).

General Procedure for Conversion of an Amine to a Urea[9a] (Method C). Methyl 4-[[5-(*N*′-Cyclopentylureido)-1-methylindol-3-yl]methyl]-3-methoxybenzoate (6, R′ = CH_3, R = Cyclopentylamino). A solution of trichloromethyl chloroformate (0.66 g, 3.37 mmol) in dry dioxane (10 mL) was added over 10 min to a stirred solution of **5** (R′ = CH_3) (1.09 g, 3.37 mmol) in dry dioxane (15 mL). The vessel was continuously purged with nitrogen gas, and the effluent was bubbled through aqueous potassium hydroxide solution to destroy excess phosgene. The in situ formation of the isocyanate was followed by TLC. After 30 min, cyclopentylamine (0.574 g, 6.74 mmol) was added, the mixture was heated to 70 °C for 20 min and then cooled, and water (100 mL) was added. The precipitate was collected by filtration, dissolved in 95:5 CH_2Cl_2/methanol (100 mL), and the solution was washed with water and brine, then dried and evaporated. The solid was crystallized from acetonitrile to give **6** (R′ = CH_3, R = cyclopentylamino) (0.75 g, 56%) as a white solid: mp 210–212 °C; partial ^1H NMR δ 1.25–1.80 (3 m, 8 H, cyclopentyl ring), 3.68 (s, 3 H, NCH_3), 3.83 (s, 3 H, OCH_3), 3.90–4.00 (m, 6 H, OCH_3, CH_2Ar, $CHNH$), 5.93 (d, 1 H, $CHNH$), 7.99 (s, 1 H, $NHAr$); MS m/z 436 ($M^+ + 1$).

General Procedure for Hydrolysis of a Methyl Ester to a Carboxylic Acid. 4-[[5-[[(Cyclopentyloxy)carbonyl]amino]-1-methylindol-3-yl]methyl]-3-methoxybenzoic Acid (24). A solution of lithium hydroxide monohydrate (0.12 g, 2.85 mmol) in water (2 mL) was added to a stirred solution of **6** (R′ = CH_3, R = cyclopentyloxy) (0.25 g, 0.57 mmol) in a mixture of methanol (5 mL) and THF (4 mL) under nitrogen. After 20 h, the mixture was concentrated in vacuo and acidified with 1 M hydrochloric acid (20 mL). The white precipitate which separated was collected by filtration, washed with a little water, and recrystallized from a toluene/hexanes mixture to give **24** (0.21 g, 88%), as a white powder: mp 157–158 °C. Anal. ($C_{24}H_{26}N_2O_5$) C, H, N.

Methyl 4-[2-[5-[[(Cyclopentyloxy)carbonyl]amino]-2-(formylmethylamino)phenyl]-2-oxoethyl]-3-methoxybenzoate (8, R = Cyclopentyl). Rose Bengal (25 mg) was added to a solution of ester **6a** (2.0 g, 4.6 mmol) in dry methanol (200 mL). The red solution was introduced into a quartz photolysis apparatus charged with a magnetic stirrer and fitted with a gas bubbler, drying-tube, and a water-cooled immersion tube housing a quartz tungsten–halogen lamp (type DVY, 650 W). Purified oxygen was bubbled through the stirred solution while it was irradiated. After 1.5 h (TLC monitoring), the solution was removed from the apparatus and concentrated. The residue was dissolved in a small volume of CH_2Cl_2 and filtered through a short

column of silica gel (eluting with 60:40 to 100:0 ethyl acetate/hexanes) to give keto amide **8** (R = cyclopentyl) (2.12 g, 98%) as a foam; ^{1}H NMR δ 1.50–2.0 (br m, 8 H, cyclopentyl ring), 3.04 and 3.24 (2 s, 3 H total, NCH_3)[29] 3.79, 3.82, and 3.86 (3 s, 6 H total, OCH_3 and $COOCH_3$),[29] 4.12 and 4.17 (2 s, 2 H total, CH_2Ar),[29] 5.13 (m, 1 H, CHOCO); MS m/z 469 ($M^+ + 1$).

Methyl 4-[2-[5-[[(Cyclopentyloxy)carbonyl]amino]-2-(methylamino)phenyl]-2-(hydroxyimino)ethyl]-3-methoxybenoate (9, R = Cyclopentyl). A solution of **8** (R = cyclopentyl) (1.0 g, 2.1 mmol) and hydroxylamine hydrochloride (0.84 g, 12.1 mmol) in freshly distilled pyridine (100 mL) was heated at reflux for 18 h, under nitrogen. The cooled solution was concentrated, the residue was dissolved in ethyl acetate (100 mL), washed with water and brine, dried, and evaporated. The residue was purified by flash chromatography, eluting with 1:1 ethyl acetate/hexanes, to give **9** (R = cyclopentyl) (0.57 g, 59%) as a waxy solid: IR (Nujol) 3430, 3300, 1680 cm^{-1}; ^{1}H NMR δ 1.5–2.0 (m, 8 H), 2.81 (d, NCH_3), 3.83 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 4.04 (s, 2 H, CH_2Ar), 4.98 (m, 1 H, CHOCO), 6.57 (d, 1 H), 6.92 (s, 1 H), 7.24 (m, 2 H), 7.46 (m, 5 H), 8.99 (br s, 1 H, NHCO); MS m/z 456 ($M^+ + 1$).

Methyl 4-[2-Acetoximino-2-[5-[[(cyclopentyloxy)carbonyl]amino]-2-(methylamino)phenyl]ethyl]-3-methoxybenzoate (10, R = Cyclopentyl). Acetic anhydride (0.27 mL, 2.85 mmol) was added to a stirred solution of **9** (R = cyclopentyl) (1.3 g, 2.85 mmol) and 4-(dimethylamino)pyridine (0.35 g, 2.9 mmol) in CH_2Cl_2 (120 mL) under nitrogen. After 18 h, the solution was concentrated, and the residue was crystallized from ethyl acetate/hexanes at −20 °C to give **10** (R = cyclopentyl) (1.36 g, 96%) as a pale-yellow solid: mp 124–126 °C; IR (Nujol) 3300, 1770, 1720, 1700 cm^{-1}; ^{1}H NMR δ 2.12 (s, 3 H, OAc), 2.82 (d, 3 H, NCH_3), 3.82 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 4.16 (s, 2 H, CH_2Ar), 5.00 (m, 1 H, CHO), 6.64 (d, 1 H), 7.02 (d, 1 H), 7.3–7.50 (m, 5 H), 9.10 (br s, 1 H), NHCO); MS m/z 497 (M^+), 437 (M − AcOH).

Methyl 4-[[5-[[(Cyclopentyloxy)carbonyl]amino]-1-methylindazol-3-yl]methyl]-3-methoxybenzoate (11a). Compound **10** (R = cyclopentyl) (1.3 g) was placed in a round-bottomed flask (100 mL volume) charged with a stirring bar, and the system was evacuated and maintained under vacuum. The solid was stirred and the flask was immersed in a preheated oil bath at 170 °C, until the solid melted and for 10 min thereafter. The product was purified by flash chromatography, eluting with 2:3 ethyl acetate/hexanes, to give **11a** (1.1 g, 96%) as a foam: IR ($CHCl_3$) 3450, 1725 cm^{-1}; ^{1}H NMR δ 1.5–2.0 (m, 8 H), 3.83 (s, 3 H, CH_3), 3.92 (s, 3 H, CH_3), 3.93 (s, 3 H, CH_3), 4.19 (s, 2 H, CH_2Ar), 5.07 (m, 1 H, CHO), 7.14 (d, 1 H), 7.33 (d, 1 H), 7.46 (m, 3 H), 7.78 (br s, 1 H), 9.46 (br s, 1 H, NHCO); MS m/z 438 ($M^+ + 1$).

4-[[5-[[(Cyclopentyloxy)carbonyl]amino]-1-methylindazol-3-yl]methyl]-3-methoxybenzoic Acid (12). By hydrolysis of **11a**, using the general procedure described above, **12** was obtained (98% yield) as a white powder: mp 216–217 °C. Anal. ($C_{23}H_{25}N_3O_5$) C, H, N.

Methyl 4-[[5-[[(benzyloxy)carbonyl]amino]-1-methylindazol-3-yl]methyl]-3-methoxybenzoate (11b): mp 132–133 °C; IR ($CHCl_3$) 3430, 1715 cm^{-1}; ^{1}H NMR δ 3.83 (s, 3 H, CH_3), 3.91 (s, 3 H, CH_3), 3.94 (s, 3 H, CH_3), 4.20 (s, 2 H, CH_2Ar), 5.14 (s, 2 H, OCH_2), 7.16 (d, 1 H), 7.3–7.55 (m, 9 H), 7.81 (br s, 1 H), 9.72 (br s, 1 H, NHCO); MS m/z 459 (M^+).

Methyl 4-[(5-Amino-1-methylindazol-3-yl)methyl]-3-methoxybenzoate. Palladium-on-carbon (10% w/w, 0.44 g) was added to a solution of **11b** (4.42 g, 9.6 mmol) in ethyl acetate (120 mL), and the mixture was hydrogenated at atmospheric pressure for 40 h. The mixture was filtered through a pad of diatomaceous earth, and the solvent was evaporated to give methyl 4-[(5-amino-1-methylindazol-3-yl)methyl]-3-methoxybenzoate (3.04 g, 97%) as an oil which was used directly in the acylation step (methods A–C).

General Procedure for Conversion of a Carboxylic Acid to an *N*-Sulfonyl Amide. *N*-[4-[[5-[[(Cyclopentyloxy)carbonyl]amino]-1-methylindol-3-yl]methyl]-3-methoxybenzoyl]benzenesulfonamide (38). A mixture of acid **24** (6.0 g, 14.2 mmol), benzenesulfonamide (2.34 g, 14.91 mmol), 4-(dimethylamino)pyridine (1.84 g, 14.91 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (2.86 g, 14.91 mmol) was dissolved in CH_2Cl_2 (250 mL), under nitrogen, and the mixture was stirred for 18 h. The mixture was poured into 1 M HCl (100 mL), the separated aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL), and the combined extracts were washed with water and brine, dried, and evaporated. The product was precipitated from hot methanol by water to give **38** (7.82 g, 98%) as a white powder: mp 132–138 °C. Anal. ($C_{30}H_{31}N_3O_6S$) C, H, N.

Methyl 4-[[5-(Cyclopentylacetamido)-2,3-dihydro-1-ethylindol-3-yl]methyl]-3-methoxybenzoate (16). Palladium-on-carbon (10% w/w, 0.5 g) was added to a mixture of methyl 4-[[5-(cyclopentylacetamido)-1-ethylindol-3-yl]methyl]-3-methoxybenzoate (**15**) and formic acid (99%, 20 mL), under nitrogen. The mixture was vigorously stirred and heated at 80 °C for 1 h. The cooled mixture was filtered through a pad of diatomaceous earth, the filter cake washed with methanol, and the filtrate was evaporated. The product was isolated by chromatography, eluting with 2:3 ethyl acetate/hexanes, to give **16** (0.37 g, 80%), as a foam: IR ($CHCl_3$) 3440, 1715, 1650 cm^{-1}; ^{1}H NMR δ 1.04 (t, 3 H, CH_2CH_3), 1.15 (m, 2 H), 1.4–1.8 (m, 6 H), 2.20 (m, 3 H), 2.7 (dd, 1 H), 2.85–3.20 (m, 5 H), 3.45 (m, 1 H), 3.86 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 6.43 (d, 1 H), 7.02 (m, 2 H), 7.30 (d, 1 H), 7.50 (m, 2 H), 9.47 (s, 1 H, NH); MS m/z 451 ($M^+ + 1$).

4-[[5-(2-Cyclopentylacetamido)-1-ethyl-2,3-dihydroindol-3-yl]methyl]-3-methoxybenzoic Acid (17). By hydrolysis of **16**, using the general procedure described above, **17** was obtained (84% yield) as an off-white powder; mp 183–186 °C. Anal. ($C_{26}H_{32}N_2O_4$) C, H, N.

Biology. Functional Assay. In vitro evaluation of compounds on isolated guinea pig tracheal strips was carried out as described in the accompanying manuscript.[9a]

Radioligand Binding Assay. Male albino, Hartley strain guinea pigs (300–400 g) were decapitated, and the lungs were perfused in situ with modified Tyrode's buffer and then excised. Large blood vessels and all visible necrotic tissue were resected and the remainder were frozen at −70 °C. Pooled frozen lungs (50 g) were thawed, chopped with a McIlwain tissue chopper into small segments, and washed several times with ice-cold phosphate-buffered saline (0.1 M, pH 7.5). The lung tissue was suspended in Tris-HCl/sucrose buffer (10 mM, pH 7.5/0.25 M) containing several protease inhibitors, homogenized with a Brinkman PT-20 Polytron, and subjected to a differential centrifugation. The 32000*g* pellet was suspended carefully in buffer with a Teflon homogenizer and recentrifuged. The final pellet was resuspended in Tris-HCl/piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (10 mM/10 mM, pH 7.5) to a final concentration of 1.61 ± 0.17 mg of protein/mL (mean ± SEM, n = 6) and stored at −70 °C until used. (No deterioration of receptor binding was observed after storage for up to 18 months.) Incubations were carried out in 10 mM PIPES buffer (pH 7.5) containing 10 mM $CaCl_2$, 10 mM $MgCl_2$, 2 mM cysteine, and 2 mM glycine. In drug competition assays, incubation mixtures (0.31 mL) containing 1 nM [^{3}H]LTD_4, receptor protein (170 ± 30 μg/mL), and competing agents were incubated at 22 °C for 30 min with or without 2 μM LTD_4. Separation of receptor-bound from free [^{3}H]LTD_4 was achieved by dilution into ice-cold buffer (5 mL of 10 mM Tris-HCl/100 mM NaCl) and immediate filtration under vacuum (Whatman GF/C filters) and thorough washing (20 mL of the dilution buffer at 0 °C). The radioactivity retained on rinsed filters was determined by a liquid-scintillation counter (Beckman Spectrometer LS7500). Specific binding was defined as the difference in total [^{3}H]LTD_4 binding minus nonspecific binding determined in the presence of 2 μM LTD_4. Data from binding assays were plotted as log concentration versus percent inhibition, and the half-maximal inhibition (IC_{50}) was determined by computerized nonlinear least-squares analysis. The binding constant (K_i) was then calculated from the Cheng-Prusoff equation:[25]

$$K_i = IC_{50}/(1 + [L])/[K_D]$$

where [L] is the [^{3}H]LTD_4 concentration and K_D is the affinity constant of LTD_4, determined separately for each batch. K_i values are the mean of two experiments conducted in duplicate with separate batches.

(29) Doubling of resonances was due to amide *E* and *Z* isomers.

In Vivo Assay. Evaluation of compounds in conscious guinea pigs was carried out as described in the accompanying paper.[9a]

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Registry No. 1, 6146-52-7; 2, 70264-94-7; 3, 107786-36-7; 4a (R′ = CH_3), 107754-15-4; 4b, 126502-40-7; 5 (R′ = CH_3), 107754-14-3; 5 (R′ = CH_3, isocyanate), 126502-41-8; 6 (R′ = CH_3, R = cyclopentyloxy), 107754-19-8; 6 (R′ = CH_3, R = cyclopentylmethyl), 107786-49-2; 6 (R′ = CH_3, R = cyclopentylamino), 107753-65-1; 8 (R = cyclopentyloxy), 107786-92-5; 9 (R = cyclopentyloxy), 107786-93-6; 10 (R = cyclopentyloxy), 107786-91-4; 11a, 107786-90-3; 11b, 126644-52-8; 12, 126502-21-4; 13 (R′ = Me), 126502-22-5; 14, 126502-23-6; 15, 107786-85-6; 16, 126541-14-8; 17, 126541-14-8; 18, 107754-21-2; 19, 107786-37-8; 20, 107754-25-6; 21, 107754-26-7; 22, 107753-99-1; 23, 126541-15-9; 24, 107754-20-1; 25, 107786-57-2; 26, 107753-66-2; 27, 107754-23-4; 28, 107786-51-6; 29, 107803-03-2; 30, 107754-39-2; 31, 107786-56-1; 32, 107786-88-9; 33, 107786-58-3; 34, 126502-24-7; 35, 107753-76-4; 36, 126502-25-8; 37, 107786-63-0; 38, 107754-31-4; 39, 107786-65-2; 40, 107786-60-7; 41, 107786-67-4; 42, 107786-68-5; 43, 126502-26-9; 44, 126502-27-0; 45, 107753-78-6; 46, 126541-16-0; 47, 126502-28-1; 48, 126502-29-2; 49, 126502-30-5; 50, 107754-08-5; 51, 126502-31-6; 52, 107753-53-7; 53, 126502-32-7; 54, 126502-33-8; 55, 126502-34-9; 56, 126502-35-0; 57, 126541-17-1; 58, 126502-36-1; 59, 126502-37-2; 60, 126502-38-3; 61, 126502-39-4; LTE4, 75715-89-8; LTD4, 73836-78-9; trichloromethyl chloroformate, 503-38-8; cyclopentylamine, 1003-03-8; methyl 4-[(5-amino-1-methylindazol-3-yl)methyl]-3-methoxybenzoate, 126502-42-9; benzenesulfonamide, 98-10-2; cyclopentyl chloroformate, 50715-28-1; cyclopentylacetic acid, 1123-00-8.

Substituted Vitamin K Epoxide Analogues. New Competitive Inhibitors and Substrates of Vitamin K_1 Epoxide Reductase

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2- and 3-substituted vitamin K 2,3-epoxide analogues were synthesized and tested as inactivators, inhibitors, and substrates for beef liver microsomal vitamin K_1 epoxide reductase. 2-(X)-3-phytyl-1,4-naphthoquinone 2,3-epoxides, where X is hydroxymethyl, chloromethyl, fluoromethyl, difluoromethyl, and formyl were all competitive inhibitors, but none was an inactivator. Only the 2-hydroxymethyl analogue was reduced to a quinone that was stable enough under the conditions of the experiment to be detected. Vitamin K_1 epoxide analogues with modified phytyl chains (1′-hydroxy, 3′-fluoro with isomerized double bond, 1′-hydroxy and 1′-fluoro with saturated double bond, and the corresponding unsubstituted chains) were synthesized. All of the analogues were competitive inhibitors of vitamin K_1 epoxide reductase. The nonfluorinated analogues also were shown to be substrates, being reduced to the corresponding quinone without enzyme inactivation. At least one other enzyme besides vitamin K_1 epoxide reductase in beef liver microsomes also metabolizes all of these analogues.

The biosynthesis of plasma clotting factors II, VII, IX, X, and various other proteins is dependent on vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone).[1,2] The initial event in the utilization of vitamin K_1 is its conversion to the corresponding hydroquinone catalyzed by a dithiol-dependent reductase that is sensitive to the oral anticoagulant warfarin.[3,4] Reduced vitamin K_1 is involved in an oxygen and carbon dioxide dependent reaction that converts the cofactor to vitamin K_1 epoxide and concomitant γ-carboxylation of specific glutamyl residues of the clotting proteins.[5] These two apparently concomitant events, which result in the activation of the clotting proteins, appear to be catalyzed by the same enzyme.[6,7] Vitamin K_1 epoxide then is converted back to vitamin K_1 by a dithiol-dependent enzyme, vitamin K_1 epoxide reductase,[3,8] which is thought to be the principal site of action of oral anticoagulants such as warfarin.[9-11] Although the mechanism of inhibition of vitamin K_1 epoxide reductase by warfarin is unknown, we have found that warfarin is noncompetitive against vitamin K_1 epoxide for the beef liver microsomal enzyme, but is competitive with the reducing agent dithiothreitol.[12] This suggests that warfarin interacts with a site other than the substrate binding site.

Recently, we reported the synthesis of 2-(fluoromethyl)-3-phytyl-1,4-naphthoquinone 2,3-epoxide (1, R = CH_2F) and showed that it was a potent *competitive* inhibitor of vitamin K_1 epoxide reductase from beef liver.[13] It was designed, however, as a potential mechanism-based inactivator[14] (Scheme I) in order to gain support for a

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† Dr. Nandi only carried out the experiments to test the effects of warfarin and inhibitors of epoxide hydrase on the metabolism on the analogues.

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