hexane–ethyl acetate 2:1) to give 560 mg (85%) of the product as white crystals: mp 61–62 °C (lit.[1b] mp 61–62 °C); R_f 0.41 (hexane–ethyl acetate 2:1); $[\alpha]^{25}_D$ −13.56° (c 1.0, chloroform–methanol 1:1); IR (CCl_4) 2919, 2849, 1749, 1367, 1224, 1118, 1090, 1038, 911 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.24 (dd (apparent t), J = 9.42 Hz, J = 9.39 Hz, 1 H, glucosyl-$H_{3'}$), 5.12 (dd (apparent t), J = 9.76 Hz, J = 9.43 Hz, 1 H, glucosyl-$H_{4'}$), 5.01 (dd, $J_{H2'-H3'}$ = 9.42 Hz, $J_{H1'-H2'}$ = 7.98 Hz, 1 H, glucosyl-$H_{2'}$), 4.59 (d, J_{H1-H2} = 7.91 Hz, 1 H, glucosyl-$H_{1'}$), 4.31 (dd, $J_{H6'-H6'b}$ = 12.6 Hz, $J_{H6'a-H5'}$ = 4.75 Hz, 1 H, glucosyl-$H_{6'a}$), 4.17 (dd, $J_{H6'a-H6'b}$ = 12.6 Hz, $J_{H6'b-H5'}$ = 2.30 Hz, 1 H, glucosyl-$H_{6'b}$), 3.93 (dd, $J_{H1'-H3a}$ = 3.62 Hz, $J_{H3a-H3b}$ = 9.65 Hz, 1 H, C_3-H_a), 3.74 (m, 1 H, glucosyl-$H_{5'}$), 3.68 (dd, $J_{H1'-H3b}$ = 4.41 Hz, $J_{H3a-H3b}$ = ~9.6 Hz, 1 H, C_3-H_b), 3.41–3.58 (m, 8 H, with a singlet at δ 3.46, $CH_2OCH_2C_{15}H_{31}$, CH_3OCH), 2.09 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.56 (br t, 2 H, $OCH_2CH_2C_{14}H_{29}$), 1.26 (br s, 26 H, $(CH_2)_{13}$), 0.88 (br t, J = 7.8 Hz, 3 H).

3-*O*-Hexadecyl-2-*O*-methyl-*sn*-glycero-1-*O*-β-D-glucopyranoside 2,3,4,6-Tetraacetate (12′, X = O, R = Ac). This compound was prepared in 87% yield by the same procedure as described above: mp 56–58 °C [lit.[1b] mp of racemate at C-2 52–54 °C]; R_f 0.41 (hexane–ethyl acetate 2:1); $[\alpha]^{25}_D$ −6.33° (c 1.0, chloroform–methanol 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) same as its C-2 enantiomer in the region δ 4.17–5.24; different at δ 3.98 (dd, $J_{H1'-H1b}$ = 3.65 Hz, $J_{H1a-H1b}$ = 9.71 Hz, 1 H, C_1-H_a), 3.74 (m, 1 H, glucosyl-$H_{5'}$), 3.61 (dd, $J_{H1'-H1b}$ = 4.45 Hz, J_{H1a-Hb} = 9.7 Hz, C_1-H_b).

1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (1, X = O, R = H). The hydrolysis procedure of Weber and Benning[1b] was used without modification: yield, 100%; mp 200 °C dec; $[\alpha]^{25}_D$ −11.9° (c 1.0, chloroform–methanol 1:1); lit.[1b] $[\alpha]^{20}_D$ −11° (c 1.0, chloroform–methanol 1:1); IR (KBr) 3359, 2919, 2849, 1112, 1090, 1037, 913 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (d, 1 H, J = 5.31 Hz), 3.41–3.8 (m with a singlet at δ 3.44, 17 H), 1.55 (br t, 2 H), 1.26 (br s, 26 H, $(CH_2)_{14}CH_3$), 0.89 (br t, 3 H, ω-CH_3).

3-*O*-Hexadecyl-2-*O*-methyl-1-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (1′, X = O, R = H). This compound was prepared as described above: $[\alpha]^{25}_D$ −7.30° (c 1.0, chloroform–methanol 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) essentially identical with that of 1, except singlet at δ 3.46.

Acknowledgment. We thank Dr. John Schrader of the Biomedical Research Centre, University of British Columbia, for providing the cell lines used in this study. We thank Dr. William F. Berkowitz of Queens College of CUNY for advice in interpreting the 2D-NMR spectra. This research was supported in part by the British Columbia Health Care Research Foundation.

Synthesis and in Vitro LTD_4 Antagonist Activity of Bicyclic and Monocyclic Cyclopentylurethane and Cyclopentylacetamide *N*-Arylsulfonyl Amides

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The dissociation constants (K_B) at the LTD_4 receptor on guinea pig trachea of a series of monocyclic and bicyclic cyclopentylurethane and cyclopentylacetamide *N*-arylsulfonyl amides have been measured. The K_B was found to be remarkably tolerant of changes in the electronic constitution and lipophilicity of the bicyclic ring system (template). Thus, *N*-[4[[6-[[(cyclopentyloxy)carbonyl]amino]benzimidazol-1-yl]methyl]-3-methoxybenzoyl]benzenesulfonamide (**11a**) and *N*-[4-[[5-[[(cyclopentyloxy)carbonyl]amino]benzo[*b*]thien-3-yl]methyl]-3-methoxybenzoyl]benzenesulfonamide (**25a**) had closely similar affinities (pK_B, 9.20 and 9.31, respectively; LTE_4 as agonist). It has been shown that the hetero-ring of the template need not be aromatic in order to achieve high affinity, since indoline **31** and 2,3-dihydrobenz-1,4-oxazines **37a–c** had pK_Bs > 9. Further, it has been shown that an *o*-aminophenone (see **42** and Figure 3) can function as a template; the template in **42** [see iii] is bicyclic by virtue of the presence of an intramolecular hydrogen bond. In contrast, when the template is a phenyl ring (**48**), receptor affinity is markedly reduced. These findings support the notion that central bicyclic ring system in this family of peptidoleukotriene antagonists is a molecular feature which helps to preorganize the acylamino and acidic chains and thereby facilitate the molecular recognition event.

Previous papers from these laboratories have described the discovery and some aspects of structure/activity relationships of a novel family of leukotriene (LT) D_4 antagonists.[1] Two series of indoles and two series of indazoles were highlighted in those reports (Figure 1): cyclopentylurethane and cyclopentylacetamide indole arylsulfonyl amides (**1**[1a,b] and **3**[1c]), and the corresponding indazole arylsulfonyl amides (**2**[1a,b] and **4**[1c]), where the group R in **3** and **4** was frequently a methyl group.

The series of compounds **1–4** were shown to contain selective LTD_4 antagonists of unusually high affinity, dissociation constants for the most part being in the range 10^{-9}–10^{-11} M against LTE_4 on isolated guinea pig trachea. In addition, many of the compounds were found to be orally effective in blocking leukotriene-induced dyspnea in guinea pigs. *N*-Methylindole ICI 204,219[1c,2] (a compound belonging to series **3**) was a product of those investigations, and is currently under clinical evaluation for asthma.

The previous studies demonstrated that the bicyclic ring system (the template) in this family of molecules could be modified in specific ways which were compatible with effective receptor recognition. In a considerable extension of that work, the present paper describes modification of

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

(1) (a) Brown, F. J.; Yee, Y. K.; Cronk, L. A.; Hebbel, K. C.; Snyder, D. W.; Krell, R. D. *J. Med. Chem.* **1990**, *33*, 1771. (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) Matassa, V. G.; Maduskuie, T. P., Jr.; Shapiro, H. S.; Hesp, B.; Snyder, D. W.; Aharony, D.; Krell, R. D.; Keith, R. A. *J. Med. Chem.* **1990**, *33*, 1781.

(2) 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. Respir. Dis.* **1990**, *141*, 978.

0022-2623/90/1833-2621$02.50/0

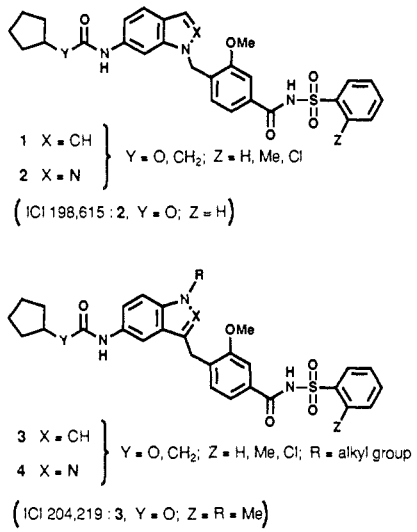
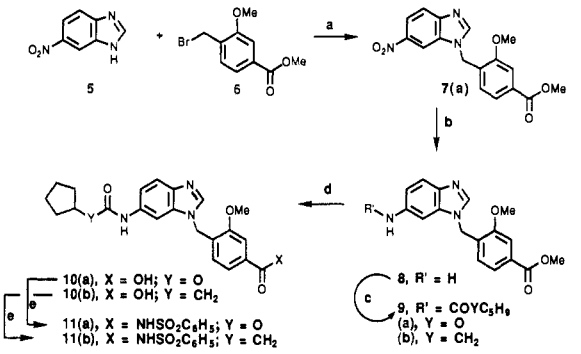


Figure 1. Peptidoleukotriene antagonists: indoles and indazoles.

Scheme I[a]



[a] (a) Methyl ethyl ketone, K_2CO_3; (b) $SnCl_2 \cdot 2H_2O$, ethanol; (c) cyclopentyl chloroformate, 2,6-lutidine, CH_2Cl_2 (method A) or cyclopentylacetic acid, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide·HCl (carbodiimide*), 4-(dimethylamino)pyridine (DMAP), CH_2Cl_2 (method B); (d) LiOH, H_2O, methanol, THF; (e) benzenesulfonamide, carbodiimide*, DMAP, CH_2Cl_2.

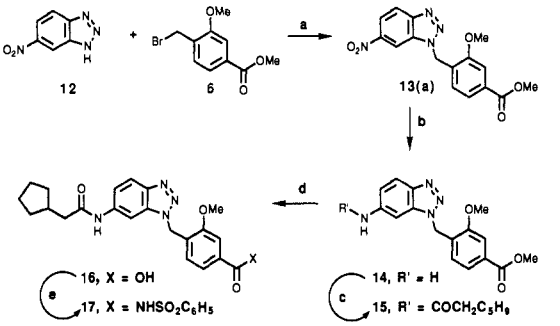
the electronic and geometric features of the template, and presents pharmacologic data which shows that the LTD_4 receptor is remarkably tolerant of changes in the nature of the template in these antagonist molecules. The ring systems which are described include one in which part of the template is a six-membered and nonaromatic ring, and another ring system which is bicyclic by virtue of the existence of an intramolecular hydrogen bond. Evidence is presented to support the notion that the presence of a bicyclic template is a necessary prerequisite for achieving high affinity at the LTD_4 receptor, in this family of antagonists.

Discussion is limited to the in vitro structure/activity relationships of those compounds which have either a cyclopentylurethane or a cyclopentylacetamide as the acylamino chain, and which have an *N*-arylsulfonyl amide as the acidic group; these groups were previously shown to be generally preferred.[1]

Chemistry

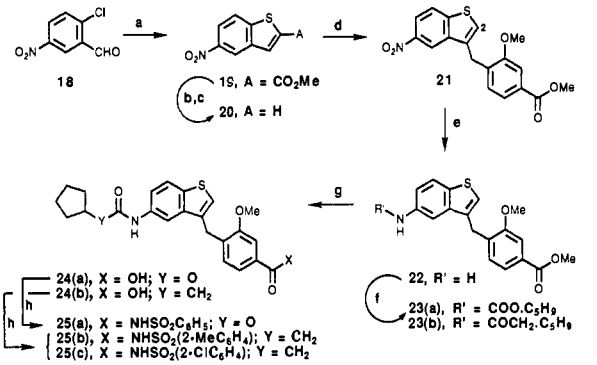
The synthesis of the benzimidazole series is summarized in Scheme I. 5-Nitrobenzimidazole (**5**) was alkylated with bromo ester **6**,[1a] to give a mixture of the required nitro ester **7a**, and its N3 isomer **7b**. The mixture was separable by chromatography on silica gel, and the desired isomer was identified through ^1H NMR experiments by a strong nuclear Overhauser enhancement (NOE) of H-7 of the benzimidazole ring upon irradiation of the benzylic methylene protons (see Table I). The nitro group in **7a** was reduced to the primary amine **8** by using stannous chloride. Acylation of **8** with an acid chloride (method A), or a carboxylic acid in the presence of a water-soluble carbodiimide (method B), gave esters **9**. Hydrolysis of **9** with aqueous lithium hydroxide gave the corresponding carboxylic acids **10**, which could be condensed with benzenesulfonamide in the presence of a water-soluble carbodiimide[1c] to furnish the desired *N*-phenylsulfonyl amides **11**.

Scheme II[a]



[a] (a) Methyl ethyl ketone, K_2CO_3; (b) H_2, 10% Pd/C, ethyl acetate; (c) method B, Scheme I; (d, e) steps d, e, Scheme I.

Scheme III[a]



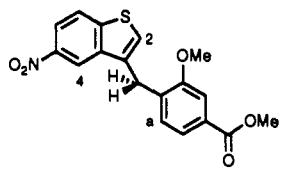
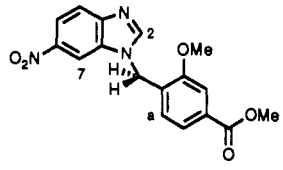
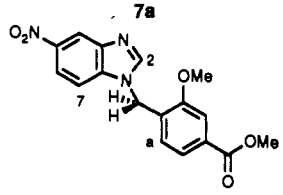
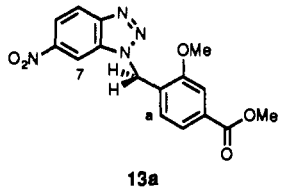
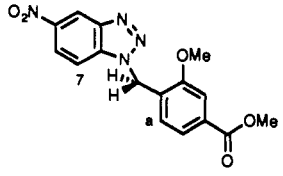
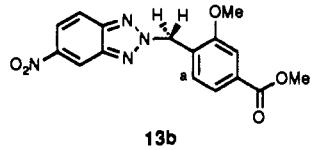
[a] (a) Methyl thioglycolate, NaH, DMF; (b) LiOH, H_2O, CH_3OH; (c) Cu^0, quinoline, 170–180 °C; (d) $SnCl_4$, **6**, CH_2Cl_2; (e) $SnCl_2 \cdot 2H_2O$, C_2H_5OH; (f) method A, Scheme I; (g) step d, Scheme I; (h) arylsulfonamide, carbodiimide*, DMAP, CH_2Cl_2.

The synthesis of the benzotriazole compound **17** followed a similar route to that for the benzimidazole above; however, in this case, reduction of the nitro group in **13a** was accomplished by catalytic reduction. The route is summarized in Scheme II. The formation of **13a** was accompanied by **13b** and **13c**, the N2 and N3 alkylation products, respectively. The desired isomer was identified through ^1H NMR experiments by a strong NOE of H-7 of the benzotriazole ring upon irradiation of the benzylic methylene protons (see Table I).

The synthesis of the benzothiophene series is summarized in Scheme III. 5-Nitrobenzothiophene (**20**) was prepared by a modification of a literature procedure:[3] condensation of 2-chloro-5-nitrobenzaldehyde (**18**) with methyl thioglycolate under basic conditions in hot DMF gave directly 2-carbomethoxy-5-nitrobenzothiophene (**19**). Hydrolysis and decarboxylation gave 5-nitrobenzothiophene (**20**).[3b] Condensation of **20** with bromo ester **6** under catalysis by stannic chloride, gave nitro ester **21**, together with the C2-alkylated isomer. The major product

(3) (a) Fries, K.; Heering, H.; Hemmecke, E.; Siebert, G. *Ann.* **1936**, *527*, 83. (b) Fieser, L. F.; Kennelly, R. G. *J. Am. Chem. Soc.* **1935**, *57*, 1611.

Table I. Assignment of Regiochemistry: NOE Measurements

	proton[a]	δ
 **21**	H-2	7.22[b]
	H-4	8.73[b]
	H-a	7.16[b]
 **7a**	H-2	8.57[c]
	H-7	8.57[c]
	H-a	7.44[c]
 **7b**	H-2	8.50[c]
	H-7	7.77[c]
	H-a	7.39[c]
 **13a**	H-7	8.59[b]
	H-a	7.31[b]
 **13c**	H-7	7.59[b]
	H-a	7.21[b]
 **13b**	H-a	7.20[b]

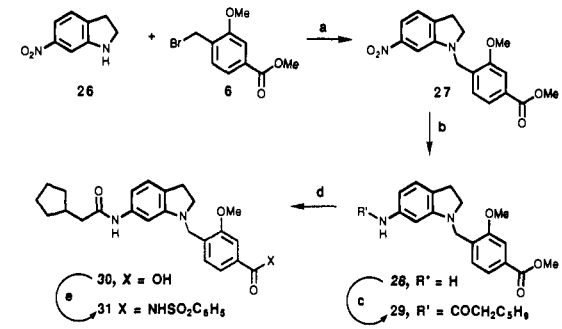
[a] Aromatic protons experiencing NOE when benzylic methylene irradiated. [b] $CDCl_3$ as solvent. [c] Acetone-d_6 as solvent

was separable by chromatography on silica gel and was identified as the desired isomer through ^1H NMR experiments by a strong NOE of H-4 of the benzothiophene ring upon irradiation of the benzylic methylene protons (see Table I). Reduction of the nitro group in **21** with stannous chloride, followed by acylation of the resulting primary amine **22** with an acid chloride (method A), gave esters **23**. Hydrolysis of **23** to give the carboxylic acids **24**, and subsequent coupling with a sulfonamide furnished *N*-arylsulfonyl amides **25**.

The indoline **31** was prepared from 6-nitroindoline (**26**) by the method shown in Scheme IV. The route of synthesis is similar to that in Scheme I.

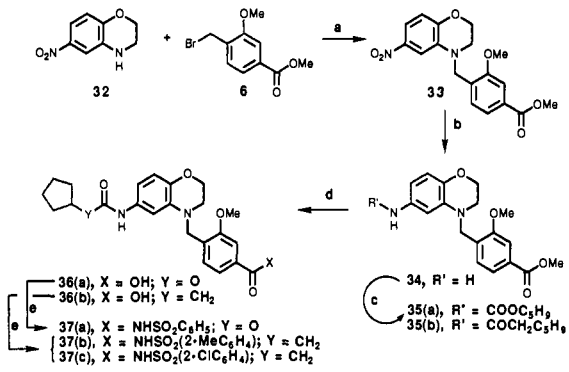
The synthesis of the 2,3-dihydrobenz-1,4-oxazine series is summarized in Scheme V. 6-Nitro-2,3-dihydrobenz-1,4-oxazine[4] (**32**) was alkylated with bromo ester **6** to give nitro ester **33**. Catalytic reduction of **33** to the primary amine **34** and acylation (method A), followed by hydrolysis of the esters and coupling of the carboxylic acids **36** with a sulfonamide, gave the *N*-sulfonyl amides **37**.

Scheme IV[a]



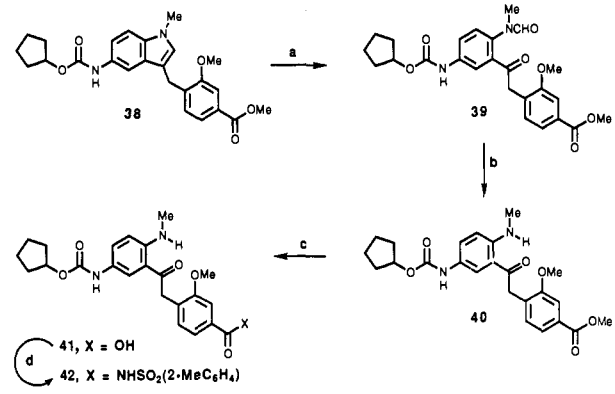
[a] (a, b) Steps a, b, Scheme I; (c) method B, scheme I; (d, e) steps d, e, Scheme I.

Scheme V[a]



[a] (a) K_2CO_3, acetone, NaI; (b) H_2, 10% Pd/C, ethyl acetate; (c) method A, Scheme I; (d,e) steps d,e, Scheme I.

Scheme VI[a]



[a] (a) See ref 1c; (b) HCl, CH_3OH; (c) step d, Scheme I; (d) *o*-toluenesulfonamide, carbodiimide*, DMAP, CH_2Cl_2.

The synthesis of the amino ketone **42** is summarized in Scheme VI. Oxidative cleavage of the indole **38** with singlet oxygen,[1c] followed by deformylation of the resulting keto amide **39** under acidic conditions, gave the amino ketone ester **40**. The presence of the intramolecular hydrogen bond in **40** was indicated[5] by the low-field resonance (δ 8.31, DMSO-d_6) of the NH proton, which appeared as a broadened quartet in the NMR spectrum. The ester **40** was converted to the *N*-phenylsulfonyl amide **42** in the usual way, via the carboxylic acid **41**.

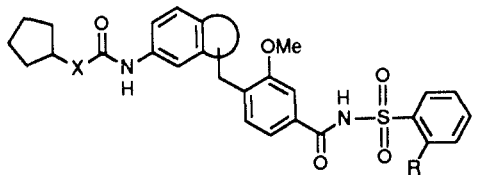
The synthesis of the ether **48** is summarized in Scheme VII. 3-Aminophenol (**43**) was bis-acylated with cyclopentyl chloroformate to give urethane ester **44**, which was subsequently hydrolyzed with potassium carbonate to give

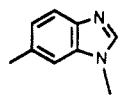
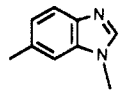
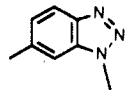
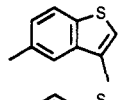
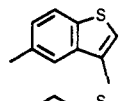
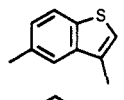
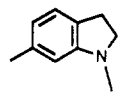
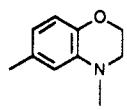
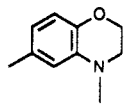
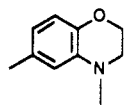
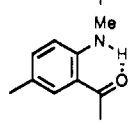
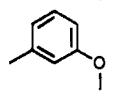
(4) Bugaut, A.; Estradier, F.; U.S. Patent 3,690,810; *Chem. Abstr.* **1970**, *73*, 36576p.

(5) Pfoertner, K.-H.; Foricher, J. *Helv. Chim. Acta* **1982**, *65*, 798.

(6) Pomona89 Database, Daylight Chemical Information Systems, 2 Corporate Park, Suite 204, Irvine, Ca 92714.

Table II. Modification of Template: Dissociation Constants on Guinea Pig Trachea



compd	X		R	pK_B[a] (n)[b]
11a	O		H	9.20 (8)
11b	CH_2		H	9.22 (5)
17	CH_2		H	9.86 (8)
25a	O		H	9.31 (4)
25b	CH_2		Me	9.25 (8)
25c	CH_2		Cl	9.60 (8)
31	CH_2		H	9.11 (8)
37a	O		H	9.02 (8)
37b	CH_2		H	9.10 (4)
37c	CH_2		Me	9.68 (4)
42	O		Me	9.07 (8)
48	O		H	7.14 (6)

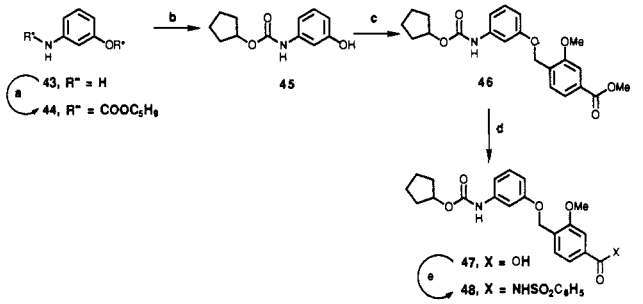
[a] –log molar dissociation constant on guinea pig trachea, LTE_4 as agonist; standard error of the mean <3% in all cases. [b] Number of determinations.

phenol **45**. O-Alkylation with bromo ester **6** gave urethane ester **46** which was converted, via the carboxylic acid **47**, to *N*-phenylsulfonyl amide **48** in the usual way.

Assignment of Regiochemistry by ^1H NMR

The preceding section indicated that alkylation of nitro heterocycles **5**, **12**, and **20** with bromide **6** gave mixtures of isomeric products. In the former two cases, the isomers were separated and the regiochemistry assigned by ^1H NMR spectroscopy with use of nuclear Overhauser enhancement techniques. The desired isomer from alkylation of **20** was identified in a similar manner, except that the unwanted (minor) C-2 isomer could not be obtained in pure form, and was therefore not subjected to the NOE analysis. The relevant findings are summarized in Table I.

Scheme VII[a]



[a] (a) Cyclopentyl chloroformate, 2,6-lutidine, THF, –10 °C; (b) NaOH, H_2O, CH_3OH, THF; (c) K_2CO_3, **6**, acetone; (d, e) steps d, e, Scheme I.

Table III. log *P* Values for Bicyclic Ring Systems

ring system	log *p*[a]
benzimidazole	1.20
benzotriazole	1.34
indazole	1.82
indole	2.14
N-methylindole	2.72
benzothiophene	3.09

[a] Octanol–water (see ref 6).

Table IV. Physical Data for Compounds of Table II

compd	scheme	yield,[a] %	mp, °C	formula	anal.[b]
11a	I	7	242–243	$C_{28}H_{28}N_4O_6S$	C,H,N
11b	I	61	220–222	$C_{29}H_{30}N_4O_5S\cdot0.5H_2O$	C,H,N
17	II	95	211–213	$C_{28}H_{29}N_5O_5S\cdot0.5H_2O$	C,H,N
25a	III	51	253–254	$C_{29}H_{28}N_2O_6S_2\cdot0.5H_2O$	C,H,N
25b	III	70	227–229	$C_{31}H_{32}N_2O_5S_2$	C,H,N
25c	III	32	223–236[c]	$C_{30}H_{29}ClN_2O_5S_2$	C,H,N
31	IV	7	136–137	$C_{30}H_{33}N_3O_5S\cdot0.2H_2O$	C,H,N
37a	V	54	186–187	$C_{29}H_{31}N_3O_7S$[d]	C,H,N
37b	V	61	190–192	$C_{30}H_{33}N_3O_6S$	C,H,N
37c	V	45	207–208	$C_{31}H_{35}N_3O_6S\cdot H_2O$	C,H,N
42	VI	48	179–181	$C_{30}H_{33}N_3O_7S\cdot0.5H_2O$	C,H,N
48	VII	67	179–181	$C_{27}H_{28}N_2O_7S$	C,H,N

[a] Yield for formation of *N*-arylsulfonyl amide. [b] Analyses were within ±0.4% of theoretical values, unless otherwise indicated. [c] Compound has a broad melting range. [d] H: calcd, 6.26; found, 5.83.

Discussion of Structure/Activity Relationships

This section describes the dissociation constants of compounds at the LTD_4 receptor on guinea pig trachea, when LTE_4 is used as agonist[15] (Table II). The affinities of the benzimidazole cyclopentylurethane **11a** and cyclopentylacetamide **11b** *N*-phenylsulfonyl amides (pK_B 9.20 and 9.22, respectively) at the LTD_4 receptor were comparable to those of their indole analogues **1**.[1b] Similarly high activity was observed for the analogous benzotriazole cyclopentylacetamide **17**, although this compound was somewhat more potent than its benzimidazole counterpart **11b** (ΔpK_B ca. 0.6). The benzimidazole and benzotriazole ring systems are isosteric with the previously reported indoles and indazoles, but are more hydrophilic (Table III).

The question of whether a nitrogen atom was an obligatory component of the template in this family of LTD_4 antagonists was addressed by preparing benzothiophene analogues of existing compounds. The benzothiophene ring is a relatively close structural analogue of the nitrogen-containing ring systems examined thus far, although

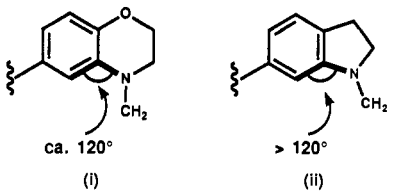


Figure 2. A comparison of the dihydrobenzoxazine and indoline templates.

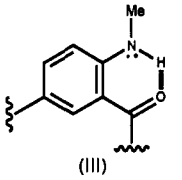


Figure 3. The *o*-aminophenone template.

markedly more lipophilic (Table III). Despite this change, benzothiophene **25a** was a high affinity antagonist (pK_B 9.31), equipotent with the substantially more hydrophilic benzimidazole **11a**. As expected,[1b] other members of this series which had a methyl or chloro substituent in the ortho position of the sulfonamide ring, **25b** and **25c**, respectively, were of similarly high affinity (pK_B 9.25–9.6). The relative insensitivity of the in vitro dissociation constant to changes in the hydrophilicity and atomic constitution of the template is a remarkable feature of this family of leukotriene antagonists and suggests that it is the geometrical features of the template which are of paramount importance. However, a certain latitude even in geometry has been shown to be permissible (vide infra).

Until this point, discussions of structure/activity relationships have been restricted to templates which comprised a five-membered (and frequently nitrogen-containing) aromatic ring fused to a phenyl ring. In a departure from this structural regularity, the indoline and the 2,3-dihydrobenz-1,4-oxazine templates were examined. The hetero-ring of these bicyclic systems are nonaromatic, and the dihydrobenzoxazine has a six-membered (although still electron rich) ring. The endocyclic "anilinoid" nitrogen atom in each case is expected to be a predominantly planar center. As can be seen from Table II, the indoline **31** and the dihydrobenzoxazines **37a** and **37b** were potent and were of comparable potency. From these results, it may be concluded that the right-hand ring of the template is not involved in π-stacking donor/acceptor[7] (or related[8]) interactions at the LTD_4 receptor. Tolyl sulfonamide **37c** was about 3-fold more potent than its phenyl counterpart **37b**.

The virtually identical dissociation constants of the indoline **31** and dihydrobenzoxazine **37b** at the LTD_4 receptor suggest that the (small) inherent difference in the geometries of these templates—a difference which affects the benzyl group (see i and ii, Figure 2) and, therefore, the positioning of the acidic *N*-sulfonyl amide group—is unimportant for overall effective recognition by the receptor. In contrast, a previous study[1c] showed that changing the hybridization (sp^2 to sp^3) of the template atom to which the benzylic carbon was attached, resulted in a sharp drop in affinity.

In trying to extend the concept of a template still further, the amino ketone iii (Figure 3) was considered for study. On the basis of previous work[1c] with N1-methylated indoles and indazoles, it was felt that the presence of the methyl group in iii would not be intrusive at the LTD_4 receptor. Although iii is, in a constitutional sense, monocyclic, the ortho juxtaposition of the amino and ketone functionalities would be expected to ensure that a strong,[9] mutually reinforcing,[10] and entropically favorable intramolecular hydrogen bond would exist, in effect "locking" the system in a ring (as indicated). That the proposed hydrogen bond in **42** does exist, even in a polar solvent, is evident[5] from the low-field resonance (δ 8.3, DMSO-d_6) of the MeN*H* proton in the ^1H NMR spectrum. The vinylogously amidic nature of the arrangement suggested that this template would be relatively planar. Further, the atom bearing the benzylic group was sp^2-hybridized, a seeming requirement for effective receptor antagonism.[1c] Indeed, urethane **42** did exhibit high affinity for the LTD_4 receptor. The existence of the intramolecular hydrogen bond in **42** at the receptor remains a supposition, but the result to be discussed below with a (truly) monocyclic template provides indirect evidence for its importance.

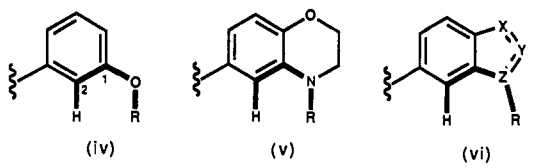


Figure 4. A comparison of the ether iv with the bicyclic templates.

The question of whether a bicyclic template is necessary at all in these molecules, can be addressed with the ether **48**, a compound possessing a phenyl ring as the (monocyclic) template. On the basis of the known conformational preference of *O*-aryl ethers,[14] the arrangement indicated in iv (i.e. O–R bond eclipsed with C^2-H bond, Figure 4) is expected to be a low-energy conformer. The local conformation around the highlighted bonds in iv is similar to that which is enforced by the bicyclic templates [see highlighted bonds in v and vi, N–R/Z–R eclipsed with C–H, Figure 4]. **48** was 100-fold less potent than, for example, the dihydrobenzoxazine **37a** or the benzothiophene **25a** and was some 1000-fold less potent than the indazole analogues reported previously (ICI 198,615, pA_2 = 10.1;[1b] **4**, Y = O, Z = H, R = CH_3, pK_B = 10.30[1c]). The greater conformational freedom of **48** compared to the bicyclic analogues presumably accounts for at least part of its reduced receptor affinity.[11]

Summary and Conclusions

Controlled modifications to the polarity and electronic constitution of the bicyclic ring system in this family of peptidoleukotriene antagonists, and certain minor geometric modifications, have been shown to be compatible with high affinity binding (K_B ca. 10^{-9}–10^{-10} M) at the LTD_4 receptor. Replacing the bicyclic ring by a phenyl ring markedly reduced affinity. These results provide further support for the notion that the bicyclic ring system functions as a template for presenting the acylamino chain (i.e. cyclopentylurethane and cyclopentylacetamide) and acidic chain (i.e. arylsulfonyl amide) in a favorable orien-

(7) Bender, C. J. *Chem. Soc. Rev.* **1986**, *15*, 475.
(8) (a) Singh, J.; Thornton, J. M. *FEBS Lett.* **1985**, *191*, 1. (b) Burley, S. K.; Petsko, G. A. *Science* **1985**, *229*, 23. (c) Pawliszyn, J.; Szczesniak, M. M.; Scheiner, S. *J. Phys. Chem.* **1984**, *88*, 1726.
(9) Yonometo, T.; Reynolds, W. F.; Hutton, H. M.; Schaefer, T. *Can. J. Chem.* **1965**, *43*, 2668.
(10) For a recent theoretical and structural discourse on "Resonance-Assisted Hydrogen Bonding", see: Gilli, G.; Bellucci, F.; Ferretti, V.; Bertolasi, V. *J. Am. Chem. Soc.* **1989**, *111*, 1023.
(11) (a) Cram, D. J. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 1039. (b) Breslow, R. *Isr. J. Chem.* **1979**, *18*, 187.

tation at the receptor. To paraphrase Breslow,[11b] flexibility would appear to be an enemy in this family of molecules. The bicyclic ring may therefore be considered as a structural feature which helps to preorganize[11a] the antagonist molecule, thereby facilitating the formation of the receptor/antagonist supramolecular[12] complex. The practical importance of a bicyclic ring system in this family of molecules was forseen in the early stages of the design process.[1a] It is tempting to suggest that the (conformationally restricted) bicyclic templates may mimic the (conformationally well-defined)[16] planar triene fragment of the agonist molecule at its receptor.

Among the plethora of bicyclic templates which can now be utilized to produce antagonist molecules with sub-nanomolar dissociation constants at the LTD_4 receptor, only the previously reported indazole ring systems (series **2**[1b] and **4**[1c]) have provided antagonists with dissociation constants approaching 10^{-11} M. It would appear, therefore, that the indazoles (fortuitously, in retrospect) embody a particularly refined blend of geometric and electronic characteristics for antagonist preorganization,[11a] desolvation, and recognition.[13]

The present paper has dealt only with in vitro receptor affinity, and the question of how modification of the template affects in vivo activity in the different series is a separate issue; as a generalization, however, the compounds reported in this paper are less potent than ICI 204,219[1c] in blocking LTD_4-induced bronchoconstriction in the guinea pig, following oral administration.[17]

Experimental Section

General. ^1H NMR spectra were recorded on a Brucker WM 250 (250 MHz) or an IBM NR-80 (80 MHz) instrument with the indicated solvents, 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 uncorrected. Combustion analyses were performed on a Perkin-Elmer 241 instrument, by ICI Americas Analytical Department, and were within ±0.4% of theoretical values. Chromatography was performed according to the method of Still,[18] using the indicated solvent ratios (v/v) on Kieselgel 60 (230–400 mesh) supplied by E. Merck. Tetrahydrofuran (THF) was 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 with anhydrous $MgSO_4$ and removing the solvent by evaporation under reduced pressure.

Methyl 3-Methoxy-4-[(6-nitrobenzimidazol-1-yl)methyl]benzoate (7a). Potassium carbonate (1.9 g, 13.75 mmol) was added to a solution of 6-nitrobenzimidazole (**5**) (2.0 g, 12.3 mmol) and methyl 4-(bromomethyl)-3-methoxybenzoate (**6**) (3.5 g, 13.5 mmol) in methyl ethyl ketone (61 mL), and the mixture was heated under reflux for 24 h. The solvent was evaporated, the residue was extracted with ethyl acetate, and the inorganic material was removed by filtration. The solvent was evaporated and the product isolated by chromatography (6 × 30 cm column), eluting with 1:1 ethyl acetate/hexanes (100 mL fractions), to give **methyl 3-methoxy-4-[(5-nitrobenzimidazol-1-yl)methyl]benzoate 7b** (fractions 42–45) (1.97 g, 47%) as a white solid: ^1H NMR (250 MHz, acetone-d_6) δ 3.87 (s, 3 H, OCH_3), 4.0 (s, 3 H, OCH_3), 5.66 (s, 2 H, NCH_2), 7.39 (d, 1 H), 7.59 (d, 2 H), 7.78 (d, 1 H, H^7 benzimidazole), 8.19 (dd, 1 H, H^6 benzimidazole), 8.52 (s, 1 H, H^2 benzimidazole), 8.57 (dd, 1 H, H^4 benzimidazole), and **7a** (fractions 49–55) (1.07 g, 26%) as a white solid: ^1H NMR (250 MHz, acetone-d_6) δ 3.9 (s, 3 H, OCH_3), 4.0 (s, 3 H, OCH_3), 5.7 (s, 2 H, NCH_2), 7.4 (d, 1 H), 7.8 (d, 1 H, H^4 benzimidazole), 8.1 (dd, 1 H, H^5 benzimidazole), 8.6 (br s, 2 H, H^2 and H^7 benzimidazole).

Methyl 4-[(6-Aminobenzimidazol-1-yl)methyl]-3-methoxybenzoate (8). Stannous chloride dihydrate (2.6 g, 11.3 mmol) was added to a stirred solution of **7** (0.77 g, 2.2 mmol) in anhydrous ethanol (23 mL). The mixture was heated at 80 °C for 18 h. The cooled mixture was washed with saturated sodium bicarbonate solution, water, and brine, then dried, and evaporated to give **8** (0.63 g, 90%) as tan foam: partial ^1H NMR (80 MHz, $CDCl_3$) δ 3.4 (br s, 2 H, NH_2), 5.2 (s, 2 H, NCH_2).

Method A. General procedure for acylation of an amine with an acid chloride:

Methyl 4-[[6-[[(Cyclopentyloxy)carbonyl]amino]benzimidazol-1-yl]methyl]-3-methoxybenzoate (9a). Cyclopentyl chloroformate (0.33 g, 2.2 mmol) was added to a stirred solution of **8** (0.63 g, 2.0 mmol) and 2,6-lutidine (0.36 mL, 3.0 mmol) in CH_2Cl_2 (10 mL). After 24 h, CH_2Cl_2 was added, and the mixture was washed with 20% w/v sodium hydroxide solution, water, and brine, then dried, and evaporated. The product was isolated by chromatography (6 × 20 cm column), eluting with 3:1 ethyl acetate/hexanes, to give **9a** (0.57 g, 93%) as a white solid: ^1H NMR (80 MHz, $CDCl_3$) δ 1.7 [m, 8 H, $(CH_2)_4$], 3.8 (s, 3 H, OCH_3), 3.9 (s, 3 H, OCH_3), 5.2 (m, 1 H, CHO), 6.17–7.0 (m, 3 H), 7.5–7.8 (m, 4 H).

General procedure for the hydrolysis of a methyl ester to a carboxylic acid:

4-[[6-[[(Cyclopentyloxy)carbonyl]amino]benzimidazol-1-yl]methyl]-3-methoxybenzoic Acid (10a). A solution of lithium hydroxide monohydrate (0.3 g, 8.1 mmol) in water (1.4 mL) was added to a stirred solution of **9a** (0.57 g, 1.3 mmol) in 1:1 THF/methanol (7 mL). After 4 h, the solvent was evaporated, water was added, and the solution was acidified with 10% v/v hydrochloric acid. The precipitate was collected by filtration to give **10a** (0.43 g, 78%). Recrystallization from aqueous ethanol gave a white powder: mp 241–242 °C. Anal. ($C_{22}H_{23}N_3O_5 \cdot 0.6\ H_2O$) C, H, N; C: calcd, 64.54; found, 62.88.

Method B. General procedure for the acylation of an amine by coupling with a carboxylic acid:

Methyl 4-[[6-(2-Cyclopentylacetamido)benzimidazol-1-yl]methyl]-3-methoxybenzoate (9b). Cyclopentylacetic acid (0.26 g, 2.0 mmol) was added to a stirred solution of **8** (0.56 g, 1.8 mmol), 4-(dimethylamino)pyridine (0.25 g, 2.0 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.39 g, 2.0 mmol) in CH_2Cl_2 (9 mL), under nitrogen. After 52 h, the mixture was diluted with CH_2Cl_2, washed successively with 10% v/v hydrochloric acid, water, 20% w/v sodium hydroxide, water, and brine, and dried. The solvent was evaporated to give **9b** (0.64 g, 84%) as a pink solid: partial ^1H NMR (80 MHz, DMSO-d_6) δ 1.0–1.6 [br m, 8 H, $(CH_2)_4$], 2.26 (br s, 3 H, $CHCH_2N$), 9.81 (s, 1 H, NH).

(12) Lehn, J.-M. *Science* **1985**, *227* (4689), 849.
(13) Some of the ways in which the indazole rings (series **2** and **4**) could contribute to improved receptor recognition have been discussed in reference **1c**.
(14) Anderson G. M., III; Kollman, P. A.; Domelsmith, L. N.; Houk, K. N. *J. Am. Chem. Soc.* **1979**, *101*, 2344. For a recent indepth analysis of the conformation of methoxy-substituted benzenes using crystallographic data, see: Hummel, W.; Huml, K.; Buergi, H.-B. *Helv. Chim. Acta* **1988**, *71*, 1291.
(15) LTE_4 was used as agonist for the determination of dissociation constants since (a) LTD_4 and LTE_4 are believed to share a common receptor on guinea pig lung (Hogaboom, G. K.; Mong, S.; Wu, H.-L.; Crooke, S. T. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 1136), and since (b) LTD_4 is known to be metabolized to LTE_4 in the presence of isolated guinea pig tracheal strip preparations (Snyder, D. W.; Aharony, D.; Dobson, P.; Tsai, B. S.; Krell, R. D. *J. Pharmacol. Exp. Ther.* **1984**, *231*, 224). (c) ICI 204,219 has similar dissociation constants on isolated guinea pig tracheal strips against LTE_4 and LTD_4, in the latter case either in the presence or absence of 3 mM L-cysteine (see ref 2).
(16) The conformation of LTD_4 has been studied (i) in aqueous solution: Sugiura, M.; Beierbeck, H.; Kotovych, G.; Belanger, P. C. *Can. J. Chem.* **1984**, *62*, 1640; (ii) in methanol: Loftus, P.; Bernstein, P. R. *J. Org. Chem.* **1983**, *48*, 43. See also earlier work on protected LTD_4 derivatives: Rackham, D. M.; Morgan, S. E.; Paschal, J. W.; Elzey, T. E. *Spectrosc. Lett.* **1981**, *14*, 589.
(17) Snyder, D. W.; Krell, R. D., unpublished results.
(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

4-[[6-(2-Cyclopentylacetamido)benzimidazol-1-yl]methyl]-3-methoxybenzoic Acid (10b). By using the general hydrolysis procedure described above, **10b** was obtained in 62% yield from **9b** as white needles: mp 280–281 °C. Anal. ($C_{23}H_{25}N_3O_4$) C, H, N.

General procedure for the conversion of a carboxylic acid to a *N*-sulfonyl amide:

***N*-[4-[[6-[[(Cyclopentyloxy)carbonyl]amino]benzimidazol-1-yl]methyl]-3-methoxybenzoyl]benzenesulfonamide (11a).** A solution of **10a** (0.43 g, 1.05 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.21 g, 1.10 mmol), 4-(dimethylamino)pyridine (0.14 g, 1.10 mmol), and benzenesulfonamide (0.17 g, 1.10 mmol) in CH_2Cl_2 (5 mL) was stirred under nitrogen for 48 h. The mixture was diluted with CH_2Cl_2, washed sequentially with 10% v/v hydrochloric acid, water, and brine, and dried. The solvent was evaporated to give a white solid, and the product was isolated by chromatography (447 mL silica gel), eluting with 3:97 methanol/CH_2Cl_2, to give a solid which was crystallized from methanol/water to give **11a** (0.043 g, 7.4%) as a white powder: mp 242–243 °C. Anal. ($C_{28}H_{28}N_4O_6S$) C, H, N.

Methyl 3-Methoxy-4-[(6-nitrobenzotriazol-1-yl)methyl]benzoate (13a). Potassium carbonate (2.84 g, 20.5 mmol) and **6** (5.22 g, 20.2 mmol) were added to a stirred suspension of 5-nitrobenzotriazole (3.0 g, 18.3 mmol) in anhydrous methyl ethyl ketone (55 mL) and THF (10 mL). After 3.5 h, the mixture was filtered and the solvent evaporated. The residue was purified by chromatography on silica gel (200 g), eluting with CH_2Cl_2 (500 mL), 2:98 diethyl ether/CH_2Cl_2 (900 mL), and 5:95 diethyl ether/CH_2Cl_2 (600 mL), to give **methyl 3-methoxy-4-[(6-nitrobenzotriazol-2-yl)methyl]benzoate (13b)** (1.14 g; R_f = 0.75, 5:95 diethyl ether/CH_2Cl_2), and a mixture of **13a** and **methyl 3-methoxy-4-[(6-nitrobenzotriazol-3-yl)methyl]benzoate (13c).** The mixture of **13a** and **13c** was separated by HPLC (4:96 diethyl ether/CH_2Cl_2, Waters Prep 500 instrument) in a double recycle, to give **13c** (1.63 g, 26%) as a solid (mp 188–189 °C; R_f = 0.67 (5:95 diethyl ether/CH_2Cl_2)) and **13a** (1.67 g, 27%) as a solid (mp 165–166.5 °C; R_f = 0.61 (5:95 diethyl ether/CH_2Cl_2)): ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.91 (s, 3 H, OCH_3), 4.00 (s, 3 H, OCH_3), 5.97 (s, 2 H, ArCH_2), 7.29 (d, 1 H), 7.61 (m, 2 H), 8.22 (m, 2 H), 8.60 (m, 1 H); MS *m/z* 343 (M^+ + 1).

Methyl 4-[[6-(2-Cyclopentylacetamido)benzotriazol-1-yl]methyl]-3-methoxybenzoate (15). Palladium on carbon (10% w/w, 0.07 g) was added to a solution of **13a** (0.7 g) in ethyl acetate (140 mL) in a hydrogenation bottle, and the mixture was hydrogenated for 4.5 h (17 lb H_2) and for a further 1 h (20 lb H_2). The catalyst was removed by filtration through diatomaceous earth, the filter pad washed with ethyl acetate, and the combined filtrate evaporated to give **14** which was used without further purification. By using method B, **15** was obtained from **14** (58% overall yield from **13a**) as a white powder: mp 206–208 °C; ^{1}H NMR (80 MHz, DMSO-d_6) δ 1.0–2.0 [complex m, 8 H, $(CH_2)_4$], 2.1–2.5 (m, 3 H, $CHCH_2CON$), 3.84 (s, 3 H, OCH_3), 3.92 (s, 3 H, OCH_3), 5.85 (s, ArCH_2), 7.05–7.56 (4 H), 7.95 (d, 1 H), 8.32 (d, 1 H), 10.16 (br s, 1 H, NH); MS *m/z* 423 (M^+ + 1).

4-[[6-(2-Cyclopentylacetamido)benzotriazol-1-yl]methyl]-3-methoxybenzoic Acid (16). By using the general hydrolysis procedure described above, **16** as a white powder was obtained in 98% yield from **15** as a partial hydrochloride salt: mp 235–238 °C. Anal. ($C_{22}H_{24}N_4O_4 \cdot 0.25HCl \cdot 1.0H_2O$) C, H, N, Cl.

Methyl 5-Nitrobenzo[*b*]thiophene-2-carboxylate (19). Methyl thioglycolate (1.14 g, 0.97 mL, 10.77 mmol) was added dropwise to a stirred suspension of oil-free sodium hydride (0.31 g, 12 mmol) in anhydrous DMF (20 mL), under a nitrogen atmosphere (CAUTION: H_2 evolution). After 10 min, a solution of 2-chloro-5-nitrobenzaldehyde (**18**) (2.0 g, 10.77 mmol) in anhydrous DMF (5 mL) was added, giving a red color. After 1 h at ambient temperature, the mixture was heated at 100 °C for 5 h. The cooled mixture was poured into 1 M hydrochloric acid. The precipitate was collected by filtration, washed with water, and recrystallized from a methanol/ethyl acetate mixture to give **19** (2.0 g, 78%) as a yellow solid: mp 213–215 °C.

Methyl 3-Methoxy-4-[(5-nitrobenzo[*b*]thien-3-yl)methyl]benzoate (21a). Stannic chloride (0.41 g, 0.18 mL, 1.56 mmol) was added to a stirred solution of 5-nitrobenzo[*b*]thiophene (**20**) (0.28 g, 1.56 mmol) and **6** (0.61 g, 2.34 mmol) in CH_2Cl_2 (5 mL), under a nitrogen atmosphere. The mixture was heated under reflux for 18 h. The cooled mixture was poured into water (10 mL) and extracted with CH_2Cl_2 (2 × 20 mL). The combined extracts were dried and evaporated, and the product was isolated by chromatography (100 mL silica gel), eluting with 1:9 ethyl acetate/hexanes, to give a solid which was crystallized from a toluene/hexane mixture to give **21a** (0.16 g, 30%) as a powder: mp 156–159 °C; IR (Nujol) 1720 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.92 (s, 3 H, OCH_3), 3.98 (s, 3 H, OCH_3), 4.28 (s, 2 H, ArCH_2), 7.15 (d, 1 H), 7.22 (s, 1 H), 7.56 (m, 2 H), 7.93 (d, 1 H), 8.19 (dd, 1 H), 8.73 (d, 1 H); MS *m/z* 357 (M^+).

Methyl 4-[(5-Aminobenzo[*b*]thien-3-yl)methyl]-3-methoxybenzoate (22). Stannous chloride dihydrate (0.38 g, 1.68 mmol) was added to a stirred solution of **21a** in ethanol (5 mL), under a nitrogen atmosphere. The mixture was heated under reflux for 2 h, and then the cooled mixture was basified with saturated sodium bicarbonate solution (15 mL). The mixture was extracted with ethyl acetate (2 × 25 mL), the extracts were dried and evaporated, and the product was isolated by chromatography (50 mL silica gel), eluting with 3:7 ethyl acetate/hexanes, to give **22** (0.06 g, 54%) as an oil: ^{1}H NMR (80 MHz, DMSO-d_6) δ 3.84 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 4.03 (s, 2 H, ArCH_2), 5.0 (br s, 2 H, NH_2), 6.80 (m, 2 H), 7.14 (m, 2 H), 7.52 (m, 3 H).

Methyl 4-[[5-[[(Cyclopentyloxy)carbonyl]amino]benzo[*b*]thien-3-yl]methyl]-3-methoxybenzoate (23a). By using method A, **23a** was obtained in 78% yield from **22** as a white powder: mp 163–164 °C.

Methyl 4-[[5-(2-Cyclopentylacetamido)benzo[*b*]thien-3-yl]methyl]-3-methoxybenzoate (23b). By using method B, **23b** was obtained in 60% yield from **22** as a white solid: mp 185–187 °C.

4-[[5-[[(Cyclopentyloxy)carbonyl]amino]benzo[*b*]thien-3-yl]methyl]-3-methoxybenzoic Acid (24a). By using the general hydrolysis procedure described above, **24a** was obtained in 95% yield from **23a** as a white powder: mp 259–261 °C. Anal. ($C_{23}H_{23}NO_5S$) C, H, N.

4-[[5-(2-Cyclopentylacetamido)benzo[*b*]thien-3-yl]methyl]-3-methoxybenzoic Acid (24b). By using the general hydrolysis procedure described above, **24b** was obtained in 96% yield from **23b** as a white powder: mp 247–249 °C. Anal. ($C_{24}H_{25}NO_4S$) C, H, N.

Methyl 3-Methoxy-4-[(6-nitro-2,3-dihydroindol-1-yl)methyl]benzoate (27). By using a similar procedure to that described for the preparation of **7**, **27** was obtained in quantitative yield from **26** as an oil: ^{1}H NMR (80 MHz, $CDCl_3$) δ 3.10 (m, 2 H, NCH_2CH_2), 3.70 (m, 2 H, NCH_2CH_2), 4.40 (s, 2 H, ArCH_2), 4.85 (s, 3 H, OCH_3), 4.90 (s, 3 H, OCH_3), 7.00–7.64 (m, 6 H, aromatic).

Methyl 4-[(6-Amino-2,3-dihydroindol-1-yl)methyl]-3-methoxybenzoate (28). By using a similar procedure to that described for the preparation of **8**, **28** was obtained in quantitative yield from **27** as a foam: ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.87 (m, 2 H, NCH_2CH_2), 3.22–4.54 (m, 4 H, NCH_2CH_2 and NH_2), 3.85 (s, 3 H, OCH_3), 3.90 (s, 3 H, OCH_3), 4.22 (s, 2 H, ArCH_2), 5.74 (d, 1 H), 6.00 (dd, 1 H), 7.30–7.68 (m, 3 H).

Methyl 4-[[6-(Cyclopentylacetamido)-2,3-dihydroindol-1-yl]methyl]-3-methoxybenzoate (29). By using method B, **29** was obtained in 26% yield from **28** as an oil: ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.24–1.76 (m, 8 H, cyclopentyl ring), 2.24 (m, 3 H, $CHCH_2CO$), 2.93 (m, 2 H, NCH_2CH_2), 3.41 (m, 2 H, NCH_2CH_2), 3.86 (s, 6 H, 2 × OCH_3), 4.27 (s, 2 H, ArCH_2), 6.55–7.72 (m, 7 H, aromatic and NH).

4-[[6-(Cylopentylacetamido)-2,3-dihydroindol-1-yl]methyl]-3-methoxybenzoic Acid (30). By using the general hydrolysis procedure described above, **30** was obtained in 14% yield from **29** as a white solid: mp 212–213 °C. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

Methyl 3-Methoxy-4-[(6-nitro-2,3-dihydrobenz-1,4-oxazin-4-yl)methyl]benzoate (33). A mixture of 6-nitro-2,3-dihydrobenz-1,4-oxazine (**32**) (0.45 g, 2.5 mmol), **6** (0.65 g, 2.5 mmol), anhydrous potassium carbonate (0.35 g, 2.5 mmol), and sodium iodide (0.38 g, 2.5 mmol) in acetone (25 mL) was stirred and heated under reflux for 48 h, under nitrogen. The cooled mixture was filtered, and the filtrate was washed with acetone. The combined filtrate was evaporated, and the product was isolated by chro-

matography (3-cm-diameter column), eluting with 1:10 ethyl acetate/toluene, to give **33** (0.75 g, 84%) as a solid: mp 130–132 °C; IR ($CHCl_3$) 1720 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.48 (t, 2 H, CH_2N), 3.91 (s, 3 H, OCH_3), 3.95 (s, 3 H, OCH_3), 4.33 (t, 2 H, OCH_2), 4.51 (s, 2 H, $ArCH_2$), 6.74–6.88 (m, 1 H), 7.48–7.65 (m, 5 H); MS *m/z* 358 (M^+).

Methyl 4-[(6-Amino-2,3-dihydrobenz-1,4-oxazin-4-yl)-methyl]-3-methoxybenzoate (34). Palladium on carbon (10% w/w, 0.2 g) was added to a solution of **33** (0.69 g) in ethyl acetate (50 mL) in a hydrogenation bottle, and the mixture was hydrogenated at a pressure of 3.17 bars. When uptake of hydrogen had ceased, the catalyst was removed by filtration through diatomaceous earth, the filter pad was washed with ethyl acetate, and the combined filtrate was evaporated to give **34** (0.625 g, 91%) as a foam: IR ($CHCl_3$) 3440, 3370 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.23 (br s, 2 H, NH_2), 3.40 (t, 2 H, NCH_2), 3.91 (s, 3 H, OCH_3), 4.21 (t, 2 H, OCH_2), 4.41 (s, 2 H, $ArCH_2$), 5.88–5.99 (m, 2 H), 6.60 (d, 1 H), 7.24 (d, 1 H), 7.53–7.65 (m, 2 H); MS *m/z* 329 (M^+ + 1).

Methyl 4-[[6-[[(Cyclopentyloxy)carbonyl]amino]-2,3-dihydrobenz-1,4-oxazin-4-yl]methyl]-3-methoxybenzoate (35a). By using method A, **35a** was obtained in 52% yield from **34** as an oil: IR ($CHCl_3$) 3440, 1720 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.63–1.70 [m, 8 H, $(CH_2)_4$], 3.39 (t, 2 H, CH_2N), 3.90 (s, 3 H, OCH_3), 3.91 (s, 3 H, OCH_3), 4.23 (t, 2 H, OCH_2), 4.45 (s, 2 H, $ArCH_2$), 5.10 (m, 1 H, CHO), 6.29 (br s, 1 H, NH), 6.59–7.62 (complex m, 6 H).

4-[[6-[[(Cyclopentyloxy)carbonyl]amino]-2,3-dihydrobenz-1,4-oxazin-4-yl]methyl]-3-methoxybenzoic Acid (36a). By using the general hydrolysis procedure described above, **36a** was obtained in 67% yield from **35a** as a solid: mp 221–222 °C. Anal. ($C_{23}H_{26}N_2O_6$) C, H, N.

Methyl 4-[[6-(2-Cyclopentylacetamido)-2,3-dihydrobenz-1,4-oxazin-4-yl]methyl]-3-methoxybenzoate (35b). By using method B, **35b** was obtained in 80% yield from **34** as a white powder: mp 152–153 °C.

4-[[6-(2-Cyclopentylacetamido)-2,3-dihydrobenz-1,4-oxazin-4-yl]methyl]-3-methoxybenzoic Acid (36b). By using the general hydrolysis procedure described above, **36b** was obtained in 97% yield from **35b** as a powder: mp 224–225 °C. Anal. ($C_{24}H_{28}N_2O_5$) C, H, N.

Methyl 4-[2-[5-[[(Cyclopentyloxy)carbonyl]amino]-2-(*N*-formyl-*N*-methylamino)phenyl]-2-oxoethyl]-3-methoxybenzoate (39). **39** was prepared from methyl 4-[[5-[[(cyclopentyloxy)carbonyl]amino]-1-methylindol-3-yl]methyl]-3-methoxybenzoate (**38**) as described previously.[1c]

Methyl 4-[2-[5-[[(Cyclopentyloxy)carbonyl]amino]-2-(methylamino)phenyl]-2-oxoethyl]-3-methoxybenzoate (40). Hydrochloric acid (6N; 0.25 mL) was added to a solution of **39** (2.78 g, 5.93 mmol) in methanol (100 mL), under nitrogen. The mixture was stirred and heated under reflux for 80 h and then cooled, and the yellow solid **40** (1.25 g) which had formed was isolated by filtration, washed with a little methanol, and dried. The filtrate was evaporated and the residue crystallized from methanol to give a further quantity of **40** (0.43 g, 64% combined yield) as a yellow powder: ^1H NMR (250 MHz, DMSO-d_6) δ 1.57–1.90 [m, 8 H, $(CH_2)_4$], 2.79 (m, 3 H, NCH_3), 3.79 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 4.29 (s, 2 H, $ArCH_2$), 5.07 (m, 1 H, CHO), 6.69 (d, 1 H), 7.29 (d, 1 H), 7.40–7.60 (m, 3 H), 8.12 (br s, 1 H), 8.31 (br q, ca. 1 H, $NHCH_3$), 9.24 (br s, 1 H, NHCO); IR ($CHCl_3$) 3440, 3340, 1715, 1645 cm^{-1}; MS *m/z* 441 (M^+ + 1), 440 (M^+).

4-[2-[5-[[(Cyclopentyloxy)carbonyl]amino]-2-(methylamino)phenyl]-2-oxoethyl]-3-methoxybenzoic Acid (41). By using the general hydrolysis procedure described above, **41** was obtained in 62% yield from **40** as a yellow solid: mp 224–226 °C.

3-[[(Cyclopentyloxy)carbonyl]amino]phenol (45). Lutidine (2.4 g, 2.6 mL, 22.3 mmol) was added to a solution of 3-aminophenol (1.0 g, 9.1 mmol) in dry THF (20 mL). The solution was cooled to −10 °C, and cyclopentyl chloroformate (3.0 g, 2.7 mL, 20.2 mmol) was added slowly. The mixture was allowed to warm to ambient temperature. After 3 h, ether was added, the mixture was filtered, and the solvent was evaporated to give a pink oil; the sequence of dilution with ether, filtration, and evaporation was repeated. The solid (2.8 g) obtained was dissolved in THF (5 mL) and methanol (2 mL), and 10% w/v sodium hydroxide (3.0 mL) was added. The solution was stirred at ambient temperature for 1 h. The solvents were evaporated, the residue was partitioned between ether and water, and the organic layer was washed with water (twice). The aqueous layer was acidified and extracted with ether (2 × 75 mL), and the ether extracts were dried over anhydrous sodium sulfate. Evaporation of solvent gave **45** (1.0 g, 50%) as a solid: mp 135–137 °C; ^1H NMR (80 MHz, $CDCl_3$ and DMSO-d_6) δ 1.4–2.0 [m, 8 H, $(CH_2)_4$], 5.15 (m, 1 H, CHO), 6.41–6.55 (m, 1 H), 6.78–7.18 (m, 3 H), 7.65 (br s, 1 H), 8.64, s, 1 H); MS *m/z* 222 (M^+ + 1).

Methyl 4-[[3-[[(Cyclopentyloxy)carbonyl]amino]phenoxy]methyl]-3-methoxybenzoate (46). Potassium carbonate (784 mg, 5.7 mmol) was added to a stirred solution of **45** (1.01 g, 4.6 mmol) and **6** (1.74 g, 6.7 mmol) in dry acetone (20 mL). After 18 h, ether was added, the mixture was filtered, and the filtrate was evaporated. The product was isolated by chromatography (75 g of silica gel), eluting with 1:10 hexane/$CHCl_3$, to give **46** (1.59 g, 82%) as a solid: partial ^1H NMR (80 MHz, $CDCl_3$) δ 3.90 (s, 6 H, 2 × OCH_3), 5.20 (m, 3 H, CHO and CH_2O); MS *m/z* 399 (M^+).

4-[[3-[[(Cyclopentyloxy)carbonyl]amino]phenoxy]-methyl]-3-methoxybenzoic Acid (47). By using the general hydrolysis procedure described above, **47** was obtained in 86% yield from **46** as a solid: mp 193–195 °C. Anal. ($C_{21}H_{23}NO_6$) C, H, N.

Biological Test Procedure. In vitro dissociation constants were obtained from cumulative dose–response curves on guinea pig tracheal strips. Guinea pigs were sacrificed by a sharp blow to the head, and the trachea were removed and cut into spirals. Each trachea was divided into two sections for paired experiments. Each section was placed in a jacketed, 10-mL tissue bath maintained at 37 °C, and bathed with modified Kreb's buffer which was bubbled with 95% O_2 and 5% CO_2. The Kreb's buffer consisted of the following composition (mM): NaCl (119), KCl (4.6), $CaCl_2$ (1.8), $MgCl_2$ (0.5), $NaHCO_3$ (24.9), NaH_2PO_4 (1.0), and glucose (11.1). The bath fluid also contained indomethacin (5 μM). Isometric tension was monitored via a Grass force–displacement transducer and was displayed on a Beckman Dynograph (Model R 612). Resting tension was set at 2 g, and the tissues were allowed to stabilize for 60 min, during which time the bath fluid was changed every 15 min.

LTE_4 concentration–response curves were obtained by addition of the agonist to the tissue bath to establish log increments of bath agonist concentration over a particular range according to the method of van Rossum.[19] Each successive concentration was added only after the plateau of the contraction due to the preceding agonist concentration was reached. Contractile responses were expressed as a percentage of the response abtainable to a maximally effective concentration of carbachol (30 μM), which was added to the bath after the 60-min stabilization period. Following the carbachol challenge, the tissues were washed and allowed 60 min to restabilize to base-line tension before the LTE_4 concentration–response curves were begun. EC_{50} values, the molar concentration of agonist required to produce a contraction equal to 50% of the maximal response, were derived by linear regression. The test compound was incubated for 30 min prior to starting the curves. Paired control tissues received vehicle. EC_{50} values were determined in the absence and presence of test compounds and significance ($p < 0.05$) was established with Student's paired *t* test. Dissociation constants for the receptor–antagonist complex were calculated by the method of Furchgott[20] by using the equation K_B = [antagonist]/(dose ratio − 1). The dose ratio (DR) represents the EC_{50} value in the presence of antagonist divided by the EC_{50} value in the absence of antagonist. Only one concentration–response curve was obtained from each tissue.

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Registry No. **5**, 94-52-0; **6**, 70264-94-7; **7a**, 107754-55-2; **7b**, 127973-84-6; **8**, 107754-54-1; **9a**, 107754-53-0; **9b**, 107786-75-4; **10a**, 107754-56-3; **10b**, 107786-76-5; **11a**, 107786-78-7; **11b**, 107786-77-6; **13a**, 107786-81-2; **13b**, 127973-85-7; **13c**, 127973-86-8; **14**, 107786-80-1; **15**, 107753-88-8; **16**, 107753-89-9; **17**, 107753-90-2; **18**, 6361-21-3; **19**, 20699-86-9; **20**, 4965-26-8; **21a**, 107754-50-7; **22**, 107754-49-4; **23a**, 107786-72-1; **23b**, 127973-87-9; **24a**, 107786-73-2; **24b**, 127973-88-0; **25a**, 107786-74-3; **25b**, 127973-89-1; **25c**, 127973-90-4; **26**, 19727-83-4; **27**, 127973-91-5; **28**, 104447-63-4; **29**, 104447-64-5; **30**, 104448-19-3; **31**, 104437-13-0; **32**, 28226-22-4; **33**, 107754-42-7; **34**, 107754-41-6; **35a**, 107754-44-9; **35b**, 107786-69-6; **36a**, 107754-45-0; **36b**, 107786-70-9; **37a**, 107754-48-3; **37b**, 107786-71-0; **37c**, 107786-89-0; **39**, 107786-92-5; **40**, 127973-92-6; **41**, 127973-93-7; **42**, 127973-94-8; **45**, 127973-95-9; **46**, 127973-96-0; **47**, 127973-97-1; **48**, 127973-98-2; cyclopentyl chloroformate, 50715-28-1; cyclopentylacetic acid, 1123-00-8; 5-nitrobenzotriazole, 2338-12-7; methyl thioglycolate, 2365-48-2; 3-aminophenol, 591-27-5.

Dihydropyrimidine Calcium Channel Blockers. 2.[1] 3-Substituted-4-aryl-1,4-dihydro-6-methyl-5-pyrimidinecarboxylic Acid Esters as Potent Mimics of Dihydropyridines

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To enhance the intrinsic potency of dihydropyrimidine calcium channel blockers, we have modified the structure of previously described 2-heteroalkyl-1,4-dihydropyrimidines **2** to 3-substituted 1,4-dihydropyrimidines **3**. Structure–activity studies using potassium-depolarized rabbit aorta show that ortho,meta-disubstituted aryl derivatives are more potent than either ortho- or meta-monosubstituted compounds. While vasorelaxant activity was critically dependent on the size of the C5 ester group, isopropyl ester being the best, a variety of substituents (carbamate, acyl, sulfonyl, alkyl) were tolerated at N3. Our results show dihydropyrimidines **3** are significantly more potent than corresponding 2-heteroalkyl-1,4-dihydropyrimidines **2** and only slightly less potent than similarly substituted 2-heteroalkyl-1,4-dihydropyridines **4** and **5**. Whereas dihydropyridine enantiomers usually show 10–15-fold difference in activity, the enantiomers of dihydropyrimidine **3j** show more than a 1000-fold difference in activity. These results strengthen the requirement of an enamino ester for binding to the dihydropyridine receptor and indicate a nonspecific role for the N3-substituent.

Introduction

Calcium channel blocking agents are widely used in the management of angina pectoris and hypertension.[2] Within this class of cardiovascular agents, the dihydropyridines (e.g., nitrendipine, **1**) have found widespread use in the clinic and have served as important tools for the study of calcium channel structure and function.[3-5] Although the effects on potency resulting from modifications of every substituent on the dihydropyridine ring have been reported,[6] efforts involving the modification of a dihydropyridine ring have been limited.[7] Metabolic oxidation to form the inactive pyridine derivative frequently results in short duration of action of these drugs.

We have reported that 2-heteroalkyl-1,4-dihydropyrimidines **2** mimic the biological effects of dihydropyridines.[1] Although some analogues of **2** show potent vasorelaxant activity in vitro, these compounds generally demonstrate lower affinity for the dihydropyridine receptor than similarly substituted 2-heteroalkyl-1,4-dihydropyridines **4** and **5**. To enhance the potency of 2-heteroalkyl-1,4-dihydropyrimidines **2**, we have further modified their structure to 3-substituted 1,4-dihydropyrimidines **3**. In this publication we demonstrate that this modification results in an increase in calcium channel blocking potency relative to 2-hetero-1,4-dihydropyrimidines **2**.

When the esters at C3/C5 and the alkyl groups at C2/C6 are equivalent, dihydropyridines are C_s symmetric. Nonidentical esters and/or C2/C6 substituents impart chirality to the molecule and, generally, result in 5–100-fold dif-

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