MEM supplemented with 5% FCS was added into each dish, and then 0.1 mL of test compound solution at given concentrations was added and incubated at 37 °C. The final concentration of DMSO in media was 0.15%, and those of test compounds ranged from 0.97 to 3030 μM. Each test was run in duplicate. The media were collected at 18 h postinfection and frozen for plaque assays of virus present in the media. The virion samples were thawed at room temperature and serially diluted into five different concentrations, with phosphate buffered saline (pH 7.0; PBS) solution. These serially diluted virus samples (0.2 mL) were then used to inoculate DBT cells separately. After virus absorption for 1 h at room temperature, 4 mL of 0.75% agarose in MEM solution was added to each culture and allowed to solidify at room temperature. The cultures were incubated for 48 h at 37 °C and 3 mL of neutral red was added to stain uninfected cells while the virus-infected cells appear as unstained plaques. Each plaque represents one virus particle. The number of virus particles was calculated by the following equation:

$$\text{no. of virus particles/mL} = \text{no. of virus particles in duplicated plates} / 2 \times 5 \times \text{dilution factor}$$

The percent inhibition of virus growth was calculated as follows:

$$\% \text{ inhibition} = (1 - T/C) \times 100\%$$

where T = number of virion particles/mL in treated samples and C = number of virion particles/mL in control group. The dose required for 50% inhibition of virus growth in the tissue culture ($TCID_{50}$) for each compound was calculated by using probit analysis.[36,37]

Acknowledgment. This paper was taken in part from the Ph.D. dissertation of P.H.W., University of Southern California, 1989.

Registry No. 1, 123541-05-9; 1 (free base), 123541-04-8; 2, 123541-07-1; 2 (free base), 123541-06-0; 3, 123541-09-3; 3 (free base), 123541-28-6; 4, 123541-11-7; 4 (free base), 123541-10-6; 5, 123541-13-9; 5 (free base), 123541-12-8; 6, 123541-15-1; 6 (free base), 123541-14-0; 7, 123541-17-3; 7 (free base), 123541-16-2; 8, 123541-19-5; 8 (free base), 123541-18-4; 9, 123541-21-9; 9 (free base), 123541-20-8; 10, 123541-23-1; 10 (free base), 123541-22-0; 11, 123541-25-3; 11 (free base), 123541-24-2; 12, 123541-27-5; 12 (free base), 123541-26-4; 13, 96826-63-0; 13 (free base), 96826-62-9; 14, 96826-58-3; 14 (free base), 96826-57-2; 15, 96826-69-6; 15 (free base), 96826-68-5; 10-chloro-9-anthracenecarboxaldehyde, 10527-16-9; 2-hydroxy-3-(2-propenyl)benzaldehyde, 24019-66-7; 6-chloro-1,3-benzdioxole-5-carboxaldehyde, 15952-61-1; 2,3-dihydro-1,4-benzodioxin-6-carboxaldehyde, 29668-44-8; 3-hydroxy-2-methyl-5-[(phosphonooxy)methyl]-4-pyridinecarboxaldehyde, 54-47-7; 2-hydroxy-3,5-dinitrobenzaldehyde, 2460-59-5; 3,5-dichloro-2-hydroxybenzaldehyde, 90-60-8; 3,4,5-trihydroxybenzaldehyde, 13677-79-7; 2,3,4-trihydroxybenzaldehyde, 2144-08-3; 2,4,6-trihydroxybenzaldehyde, 487-70-7; 2-hydroxybenzaldehyde, 90-02-8; 3-hydroxy-2-pyridinecarboxaldehyde, 1849-55-4; *n*-hydroxy-*n*-aminoguanidine tosylate, 36826-58-1; 3-hydroxy-2-(hydroxymethyl)pyridine, 14047-53-1.

(36) Finney, D. J. *Probit Analysis*; Cambridge University Press: Cambridge, UK, 1947; p 20.
(37) Miller, L. C.; Tainter, M. C. *Proc. Soc. Exp. Biol. Med.* **1944**, *57*, 261.

Potential Antisecretory Antidiarrheals. 2. α_2-Adrenergic 2-[(Aryloxy)alkyl]imidazolines†

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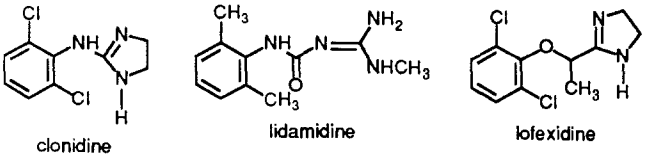
Preclinical Research, G. D. Searle and Company, 4901 Searle Parkway, Skokie, Illinois 60077. *Received October 9, 1987*

Lofexidine, an α_2-agonist, has central hypotensive activity and peripheral intestinal antisecretory activity. Analogues were synthesized with increased polarity in an attempt to prevent penetration of the blood–brain barrier. The compounds were evaluated in the cholera toxin treated ligated jejunum of the rat and in the Ussing chamber with a rabbit ileum preparation. Active compounds were determined to be α_2-adrenergic agonists by yohimbine reversals of their Ussing chamber activities. The 2,6-dimethyl derivative of lofexidine, **4a**, was as active as lofexidine in vivo, but derivatives with 2,6-substituents larger than ethyl were inactive. (Aryloxy)alkyl derivatives which have an imidazoline and a methyl or larger group as part of the alkyl exhibited the best antisecretory activity. Compounds with substituents in the para position of the phenyl ring were generally inactive. 3-Amino-2,6-dimethyl derivative **21** was twice as active as **4a**. A 2-methyl substituent is required in the 3-amino series to retain good activity. 2-Methyl derivative **12a** had activity comparable to that of **4a**, while 6-methyl derivative **12f** was inactive. Substituents on the 3-amino group did not affect the activity, but substituting a hydroxyl for the amino group produced an inactive compound. Replacing the phenyl moiety with a 4-indole resulted in retention of activity, but other heterocycles were inactive. Compound **12a** was resolved and *d* isomer **32** was five times more potent than *l* isomer **33**. The more active compounds in the rat cholera toxin assay (RCTA), when evaluated in the dog, exhibited antisecretory activity but also exhibited central nervous system (CNS) effects, sedation and ataxia, at 10 mg/kg, and in spontaneously hypertensive rats at 50 mg/kg. A measure of polarity, log *P*, was calculated for the (aryloxy)alkyl groups. Regression analysis showed no correlation of antisecretory ED_{50} to the calculated log *P*. The active compounds did not show a separation of the central CNS effects from the peripheral antisecretory activity by increasing the polarity.

We have recently described a series of compounds which offer an alternate approach to antidiarrheal therapy.[1] Instead of focusing on the motility component of diarrhea, we investigated a new therapeutic strategy of using α_2-adrenergic agonists to block both secretion and motility.

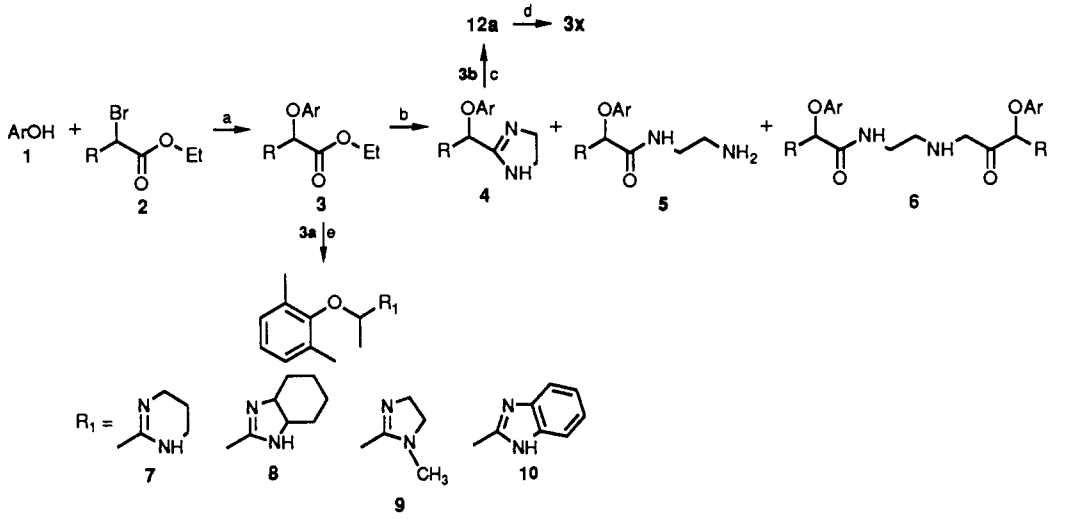
Chart I



† This paper has been presented in part; see: Moormann, A. E.; Pitzele, B. S.; Jones, P. H.; Gullikson, G. W.; Bianchi, R. G.; Monroy, M.; Rubin, B.; Casler, J.; Grebner, M.; Yu, S. at the 190th National Meeting of the American Chemical Society, Chicago, IL, September 8–13, 1985; paper MEDI 72. Derivatives of Lofexidine as Intestinal α_2 Adrenergic Antisecretory Agents.

0022-2623/90/1833-0614$02.50/0

Scheme I[a]



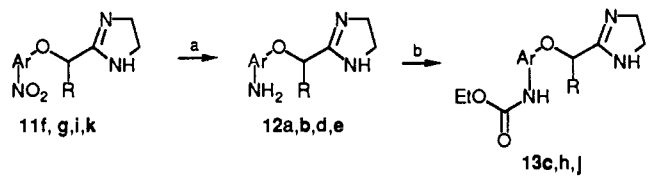
Ar	R	Ar	R
a: 2,6-dimethylphenyl	Me	n: 1-naphthyl	Me
b: 2-methyl-3-nitrophenyl	Me	o: 2-naphthyl	Me
c: 5-isoquinolyphenyl	Me	p: 2-methyl-1-naphthyl	Me
d: 4-indyl	Me	q: 2,6-dimethylphenyl	H
e: 2-methyl-3-pyridyl	Me	r: 2,6-dimethylphenyl	Et
f: 4-bromo-2,6-dimethylphenyl	Me	s: 2,6-dimethylphenyl	Me_2
g: 2,6-diethylphenyl	Me	t: 2,6-dimethyl-4-(*tert*-butoxycarbonyl)phenyl	Me
h: 2,6-di-*n*-propylphenyl	Me	u: 2,6-dimethyl-4-carboxylphenyl	Me
i: 2,6-diallylphenyl	Me	v: 2,6-dimethyl-4-aminophenyl	Me
j: 2,6-dimethyoxyphenyl	Me	w: 2,6-dimethyl-4-nitrophenyl	Me
k: 2,4,6-trimethylphenyl	Me	x: 3-(*N,N*-dimethyamino)-2-methylphenyl	Me
l: 2,4-dimethylphenyl	Me	y: 3-methoxy-2-methylphenyl	Me
m: 2-ethylphenyl	Me	z: 2-methyl-3-nitrophenyl	Et

[a] (a) K_2CO_3/DMF; (b) $AlMe_3$/EDA; (c) Raney Ni/H_2; (d) $NaCNBH_3$/formaldehyde/HOAc/CH_3CN; (e) $AlMe_3$/1,3-diaminopropane (**7**), 1,2-diaminocyclohexane (**8**), *N*-methylethylenediamine (**9**), 1,2-phenylenediamine (**10**).

Clonidine, lofexidine, and lidamidine (Chart I), all α_2-adrenergic agonists, suppress intestinal hypersecretion and reduce motility.[2-5] Since lidamidine, unlike clonidine and lofexidine, has poor penetration into the central nervous system (CNS), it does not produce hypotension at therapeutically active antisecretory doses, as do the other two drugs.

In our search for a peripherally active α_2-agonist, polar derivatives of lofexidine were synthesized in an attempt to decrease their ability to cross the blood–brain barrier. Replacing the chloro groups of clonidine with methyls increases its basicity and at physiological conditions the 2,6-dimethyl derivative is not as active centrally.[6] The substitution of methyl for chloro in lofexidine will not increase its basicity because the imidazoline ring is insulated from the aromatic ring by two atoms but will avoid potential toxicity problems associated with phenoxy halides.[7] The dimethyl derivative of lofexidine (**4a**) was synthesized and found to be as active as lofexidine in inhibiting intestinal hypersecretion in the rat cholera toxin assay (RCTA). Polar groups such as hydroxy and amino were added to the phenyl ring in an attempt to decrease partitioning into the CNS. Specifically, a series of 2-[(aryloxy)alkyl]- and 2-[(heteroaryloxy)alkyl]imidazolines were synthesized. Changes in the imidazoline ring and the

Scheme II[a]



Ar	R
a: 3-amino-2-methylphenyl	Me
b: 3-amino-4,6-dimethylphenyl	Me
c: 3-acetamido-2-methylphenyl	Me
d: 3-amino-2-methylphenyl	CH_2CH_2OH
e: 3-aminophenyl	Me
f: 6-methyl-3-nitrophenyl	Me
g: 3-amino-2-methylphenyl	Et
h: 3-carbethoxy-2,6-dimethylphenyl	Me
i: 4-bromo-3-nitro-2,6-dimethylphenyl	Me
j: 3-carbethoxy-2-methylphenyl	Me
k: 3-nitrophenyl	Me

[a] (a) 10% Pd/C, H_2; (b) HCl, 12% phosgene/toluene, CH_3CN, EtOH.

(1) Pitzele, B. S.; Moormann, A. E.; Gullikson, G. W.; Albin, D.; Bianchi, R. G.; Palicharla, P.; Sanguinetti, E. L.; Walters, D. E. *J. Med. Chem.* **1988**, *31*, 138.

(2) (a) Pictrusko, R. G. *Am. J. Hosp. Pharm.* **1979**, *36*, 757. (b) Powell, D. W.; Field, M. *Secretory Diarrhea*; Field, M., Fordtran, J. S., Schultz, S. G. Eds.; American Physiology Society: Bethesda, Maryland, 1980.

(3) (a) Mir, G. N.; Alioto, R. L.; Sperow, J. W.; Eash, J. R.; Krebs, J. B.; Yelnosky, J. *Arzneim-Forsch/Drug Res.* **1978**, *28II*, 1448. (b) Beattie, D. E.; Dover, G. M.; Ward, T. J. *J. Med. Chem.* **1985**, *28*, 1617.

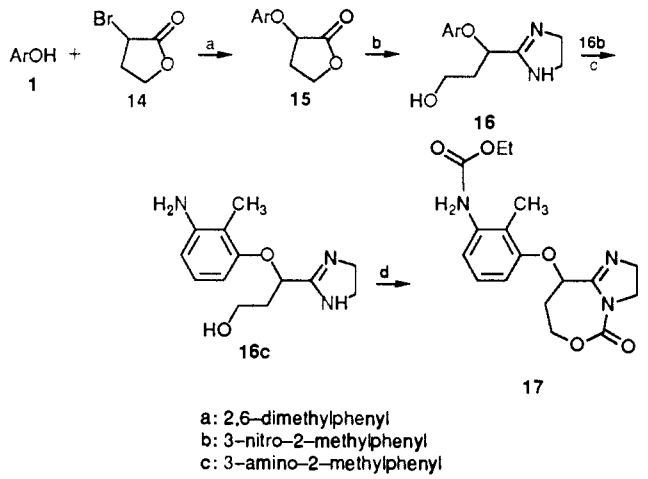
(4) Lal, H.; Shearman, G. T.; Ursillo, R. C. *J. Clin. Pharmacol.* **1981**, *21*, 16.

(5) Lal, H.; Shearman, G. T. *Drug Dev. Res.* **1981**, *1*, 37.

(6) Timmermans, P. B. M. W. M.; Hoefke, W.; Stahle, H.; Van Zwieten, P. A. Structure–Relationships in Clonidine-Like Imidazolidines and Related Compounds. In *Progress in Pharmacology*; G. F. Verlag: Stuttgart, New York, 1980; Vol. 3, No. 1.

(7) Jenner, P.; Testa, B. Concepts in Drug Metabolism Part A. In *Drugs and the Pharmaceutical Sciences*; Marcel Dekker: New York and Basel, 1980; Vol. 10.

Scheme III[a]



[a] (a) K_2CO_3/DMF; (b) $AlMe_3$/EDA; (c) 10% Pd/C, H_2; (d) HCl, 12% phosgene/toluene, CH_3CN, EtOH.

connecting unit were also investigated.

Chemistry

General Synthesis. As shown in Scheme I, a phenol (1) was alkylated with an α-bromo ester (2) in DMF with K_2CO_3. Imidazoline 4 was formed from phenoxy ester 3 in the presence of trimethylaluminum/ethylenediamine ($AlMe_3$/EDA) complex. The conversion to 4 was generally excellent, with small amounts of amide dimer 6 and uncyclized amide 5 being the only other products. These impurities were removed by crystallization. Other diamino substrates were used to synthesize compounds 7–10.

General Synthesis of Amino Derivatives. Nitro derivative 11 was synthesized from the nitrophenol as described in Scheme I. Compound 11 was then reduced to amino derivative 12, as shown in Scheme II, through catalytic hydrogenation over Pd/C. Compound 12 was converted to urethane 13 by selectively functionalizing the aniline nitrogen of the dihydrochloride salt with phosgene in acetonitrile, then quenching with the appropriate alcohol.

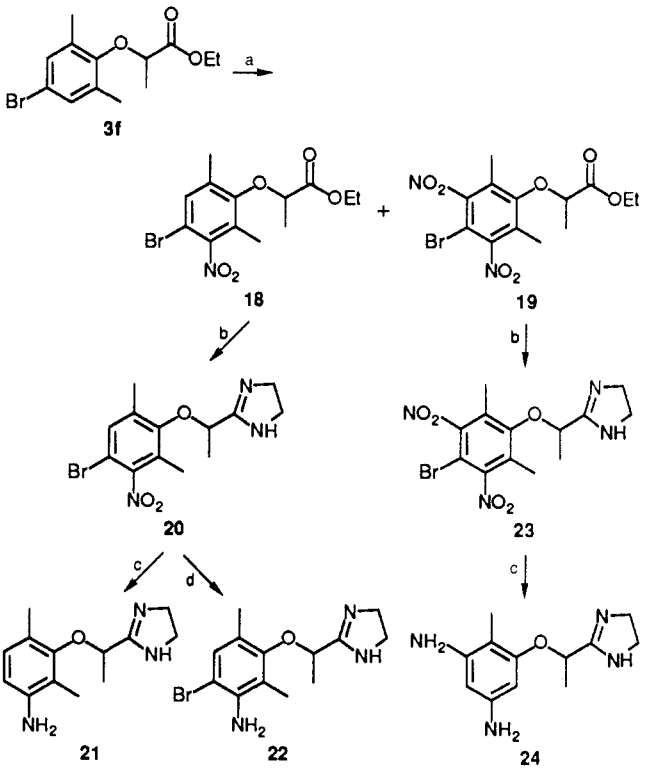
Hydroxyethyl Side Chain. As shown in Scheme III, phenol 1 was alkylated with α-bromobutyrolactone (14), giving α-butyrolactone 15. The yields with 14 were not as high as with the other alkylating agents and an excess of 14 was needed to consume the phenol.

The reaction of lactone 15 with $AlMe_3$/EDA yielded hydroxyethyl derivatives 16. The dihydrochloride of 16c was reacted with 2 equiv of phosgene, and solvolyzed with ethanol to yield 17.

2,6-Dimethylamino Derivatives. As shown in Scheme IV, nitration of 3f, in which reaction at the 4-position is blocked by the bromo substituent, in concentrated H_2SO_4 with 70% HNO_3, produced a mixture of the mononitro (18) (major component) and dinitro (19) compounds. After chromatographic separation, each was cyclized with $AlMe_3$/EDA complex to produce imidazolines 20 and 23. Hydrogenation of 20 and 23 over Pd/C reduced the nitro function to the amine and removed the bromine to provide 21 and 24, respectively. When 20 was hydrogenated over Raney nickel, only the nitro function was reduced to produce 22.

Synthesis of *d*- and *l*-12a (32 and 33). As shown in Scheme V, *d*-mandelic acid was used to resolve the *d* and *l* enantiomers of nitro derivative 4b. *d*-Mandelic acid was esterified with $SOCl_2$/methanol, and the resulting hydroxy ester 26 was converted to 27 with phenyl chloroformate. Nitro derivative 4b was heated with 27 at 150 °C (neat)

Scheme IV[a]



[a] (a) HNO_3/H_2SO_4; (b) $AlMe_3$/EDA; (c) 10% Pd/C, H_2; (d) Raney Ni/H_2.

to form the diastereometric pair 29. Reverse-phase chromatography and two cycles on a Waters Prep 500 Porosil column separated the two diastereomers 30 and 31. If normal-phase chromatography was performed on 29 before the reverse-phase chromatography, the sample degraded, but 29 was stable to normal-phase conditions after the reverse-phase treatment. Compounds 30 and 31 were hydrogenated separately over Pd/C, removing the benzyloxy group and reducing the nitro to the aniline, to obtain 32 ($[\alpha]_D$ +39°) and 33 ($[\alpha]_D$ -34°). The optical purity of 32 and 33 was also checked by NMR. One equivalent of a chiral shift reagent[8] was capable of separating the chemical shift of the aliphatic methyl groups of 12a (a mixture of 32 and 33) by 11 Hz. The separated isomers did not show cross-contamination to the detection level of NMR. The chiral shift reagent was also capable of separating the aliphatic methyl groups of 4a by 7 Hz.

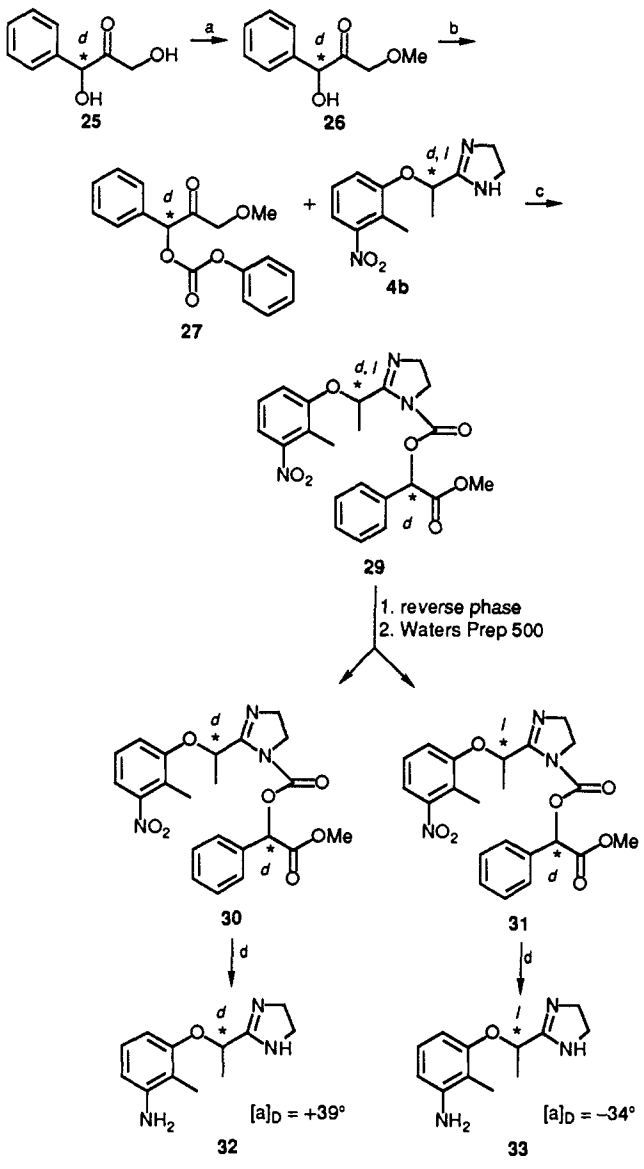
Heterocyclic Derivatives. 3-Hydroxy-2-methylpyridine,[9] 5-hydroxyisoquinoline, and 4-hydroxyindole[10] were converted to their imidazoline derivatives (Scheme I).

The benzimidazolyl derivative was synthesized as shown in Scheme VI. Compound 3a was nitrated to give dinitro derivative 34, which was hydrogenated to diamino compound 35. Refluxing 35 with formic acid produced benzimidazole 36 which was converted to compound 37 as described before.

Triazolopyridine 43 was synthesized as shownin Scheme VII. Compound 38 was reacted with *N,N*-dimethylformamide dimethyl acetal and then hydroxylamine-*O*-sulfonic acid to form 40. Reductive removal of the benzyl group over Pd/C formed 41, which was alkylated to ester 42 and

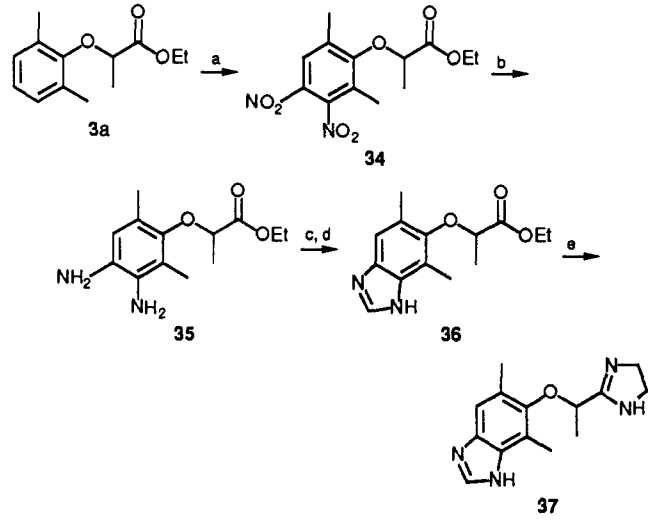
(8) (*S*)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol purchased from Aldrich #21,134-6.
(9) Chien, P.; Cheng, C. *J. Med. Chem.* **1970**, *13*(5), 867.
(10) Purchased from Aldrich.

Scheme V[a]



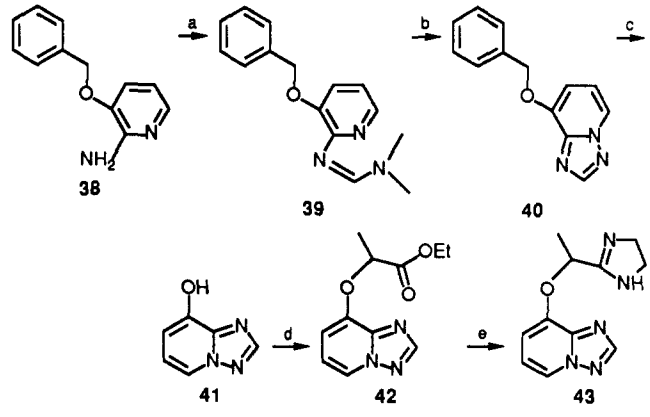
[a] (a) $SOCl_2$/MeOH, reflux; (b) phenyl chloroformate, pyridine/CH_2Cl_2; (c) 152 °C, 0.25 h; (d) 10% Pd/C, H_2.

Scheme VI[a]



[a] (a) HNO_3/H_2SO_4; (b) Raney Ni/H_2; (c) Formic acid, reflux; (d) $SOCl_2$/EtOH, reflux; (e) $AlMe_3$/EDA.

Scheme VII[a]



[a] (a) *N,N*-dimethylformamide dimethyl acetal, EtOH; (b) hydroxylamine-*O*-sulfonic acid, MeOH; (c) 5% Pd/C, H_2, THF/EtOH; (d) ethyl 2-bromopropionate, K_2CO_3/DMF; (e) $AlMe_3$/EDA.

then converted to **43** as described before.

5-Methylindole **51** was synthesized as shown in Scheme VIII. 2-Methyl-5-nitrophenol (**44**) was alkylated with allyl bromide to give allyl ether **45**. The key step was the Claisen rearrangement of nitro allyl ether **45** to nitroallylphenol **46**, which was alkylated as before to produce ester **47**. The allyl group of **47** was oxidized and cleaved with $OsO_4/NaIO_4$ to nitro aldehyde **48**, which when hydrogenated spontaneously cyclized to indole **50**. Compound **50** was converted to compound **51** as described before.

Sulfur, Nitrogen, and Carbon Replacements for Oxygen. The alkylation of thiophenol **52a** and aniline **52b** to form **54a** and **54b**, respectively, proceeded as before as shown in Scheme IX.

Carbon derivative **58** was synthesized as shown in Scheme X. The Heck reaction of 2,6-dimethylbromobenzene (**55**) and the appropriate olefin **56** formed compound **57**. The ester was converted with $AlMe_3$/EDA complex and the double bond in the resulting imidazoline **57a** was hydrogenated over Pd/C to form **58**.

General Synthesis of Phenoxy Derivatives. 3-Hydroxy derivative **64** and 3-methoxy derivative **63a** were synthesized as shown in Scheme XI. 2-Methylresorcinol (**59**) was dibenzylated and then partially hydrogenated with termination at 1 mol of hydrogen uptake. Monophenol **61** was chromatographically separated from a mixture with **60** and **59**. Compound **61** was alkylated with ethyl 2-bromopropionate to **62** and cyclized with $AlMe_3$/EDA complex to **63**. Reductive removal of the benzyl group over Pd/C formed the phenolic derivative **64**.

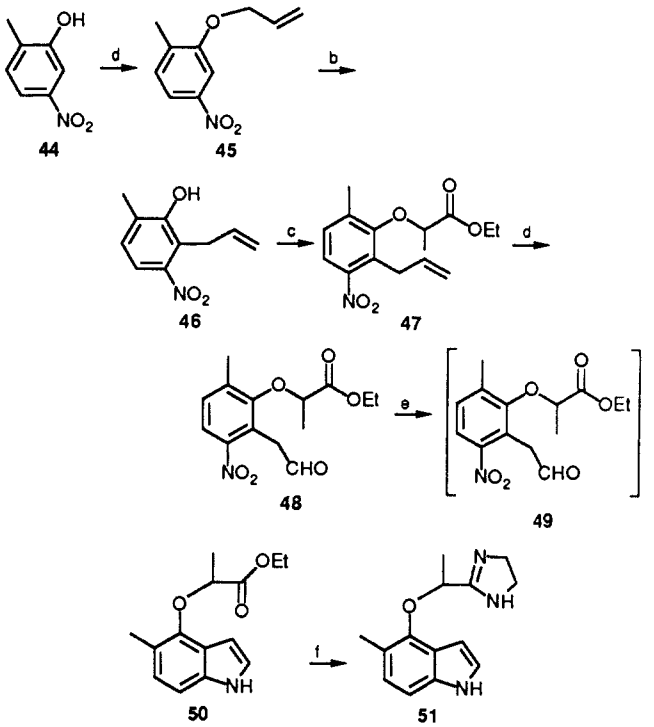
Monophenol **61** was also methylated with dimethyl sulfate to **60a** and the benzyl group was removed by hydrogenating **60a** over Pd/C to form **61a**. Compound **61a** was alkylated with ethyl 2-bromopropionate to **62a**, then cyclized with $AlMe_3$/EDA to **63a**.

Synthesis of the Thiazoline Derivative. As shown in Scheme XII, ester **3a** was hydrolyzed to the acid with NaOH. The acid was converted to the acid chloride with thionyl chloride and then quenched with NH_3 to give amide **65**. This amide was dehydrated with phosgene and triethylamine to the nitrile **66**. The thiazoline ring was formed by fusing the nitrile with cysteamine hydrochloride, and **67** was isolated through column chromatography.

Biological Results and Discussion

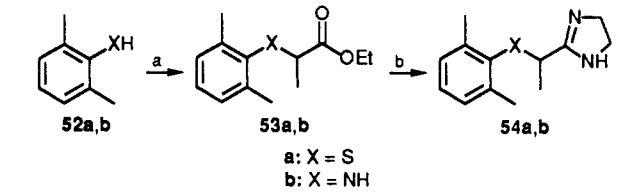
In order to determine the effect of the test compounds on intestinal fluid movements in an intact animal, the rat

Scheme VIII[a]



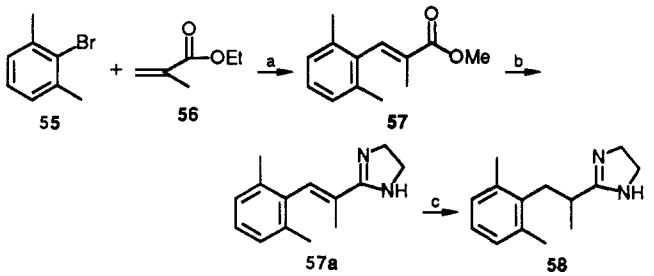
[a] (a) Allyl bromide, K_2CO_3/DMF; (b) cumene, reflux 4 h; (c) ethyl 2-bromopropionate, K_2CO_3/DMF; (d) $NaIO_4$, 1% OsO_4, dioxane/H_2O; (e) Raney Ni/H_2; (f) $AlMe_3$/EDA.

Scheme IX[a]



[a] (a) Ethyl 2-bromopropionate, NaH/THF; (b) $AlMe_3$/EDA.

Scheme X[a]

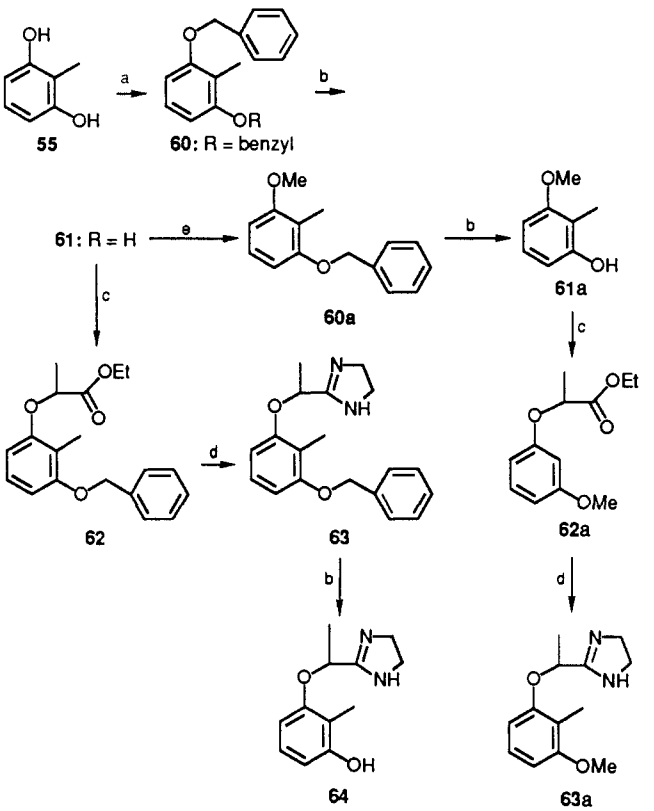


[a] (a) $Pd(OAc)_2$, Et_3N; (b) $AlMe_3$/EDA; (c) 5% Pd/C, H_2.

cholera toxin secretion assay (RCTA) was used.[11] The ID_{50} of the compound with statistical limits was calculated from data on at least two doses and from at least two different experiments, by the method of maximum likelihood.[12]

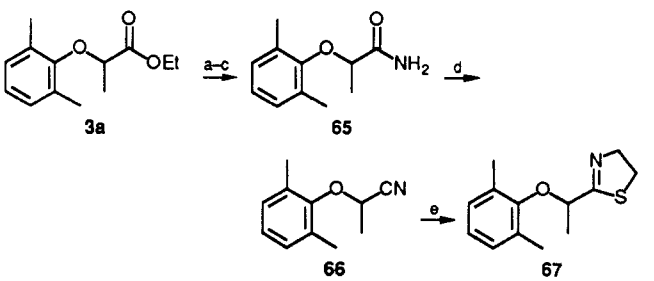
Intestinal antisecretory drugs which act by an α_2-adrenergic mechanism have been demonstrated to inhibit the in vitro increases in intestinal potential difference (PD) caused by diarrhea-inducing secretogogues.[13] Experiments

Scheme XI[a]



[a] (a) Benzyl chloride, K_2CO_3/DMF; (b) 10% Pd/C, H_2; (c) ethyl 2-bromopropionate, K_2CO_3/DMF; (d) $AlMe_3$/EDA; (e) Me_2SO_4, NaOH, EtOH.

Scheme XII[a]



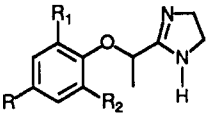
[a] (a) NaOH, MeOH/H_2O; (b) $SOCl_2$; (c) NH_3/Et_2O; (d) Et_3N, 12% phosgene/toluene; (e) 2-aminoethanethiol hydrochloride, fused.

to determine actions of intestinal antisecretory compounds on potential difference in the Ussing chamber were done as described by Field.[14] Determination of drug effect was made on at least two tissues.[15]

A compound was considered active if it returned the aminophylline-augmented PD to control values. Moderate activity was defined as a reduction of PD only at 10^{-5} M. Inactive compounds did not change PD. Yohimbine, a specific competitive antagonist of α_2-receptors, was always incubated after activity was seen. Reversal of PD changes was taken as evidence of α_2-receptor activation.

(11) Jacoby, H. I.; Marshall, C. H. *Nature (London)* **1972**, *235*, 163.
(12) Fisher, R. A. Principles of Statistical Estimation. In *Statistical Methods for Research Workers*, 14th ed.; Hafner: New York, 1970.
(13) (a) Gullikson, G. W.; Albin, D.; Yang, P. C.; Bauer, R. *Fed. Am. Soc. Exp. Biol. 67th Annu. Meet. Abstr.* #6300, Chicago, IL, April, 1983. (b) Chang, E. B.; Field, M.; Miller, R. J. *Am. J. Physiol.* **1982**, *242*, G237–G242.
(14) Field, M.; Fromm, D.; McColl, I. *Am. J. Physiol.* **1971**, *220*, 1388.
(15) An ID_{50} > 10 mg/kg in the RCTA was used as a cutoff for further evaluation. These compound usually exhibited very weak or no activity in the dog antisecretory assay.

Table I. Alkyl Derivatives of Lofexidine



R	R_1	R_2	compound	log P^a	Ussing[b] chamber	rat cholera toxin assay:[c] ID_{50} (sc), mg/kg
			clonidine			0.3 (0.2–0.35)
H	Cl	Cl	lofexidine	4.13		<2.0[e]
H	Me	Me	**4a**	4.39	++	1.7 (1.2–2.5)
Br	Me	Me	**4f**			>100
H	Et	Et	**4g**	5.41	++	2.3 (1.5–3.4)
H	*n*-Pr	*n*-Pr	**4h**		+	>100
H	allyl	allyl	**4i**			>100
H	OMe	OMe	**4j**			>100
Me	Me	Me	**4k**	5.00	0	11.1 (7.0–19.8)
Me	Me	H	**4l**	4.35		24.1 (13.3–95.2)
H	H	Et	**4m**	4.23	+[d]	22.9 (12.4–98.0)
t-BuOCO	Me	Me	**4t**			>100
CO_2H	Me	Me	**4u**		0	>100
NH_2	Me	Me	**4v**		0	>100
NO_2	Me	Me	**4w**		0	>100

[a] log *P* calculated by using CLOGP3 from the MedChem software release 3.42 by C. Hansch and A. Leo, Medicinal Chemistry Project, Pomona College, Claremont, CA. The imidazoline fragment was excluded from the calculation (refer to the text). [b] legend: ++ = active, final concentration 10^{-6} M; + = moderate activity, final concentration 10^{-5} M; 0 = inactive, final concentration $>10^{-5}$ M. [c] 95% confidence limits of the mean ID_{50} are shown in parentheses. [d] At 10^{-5} M the compound was found to be a partial agonist/antogonist. [e] ID_{50} limits could not be calculated because the doses tested (10 and 20 mg/kg) produced a >95% inhibition of fluid secretion.

As shown in Table I, the 2,6-dimethyl derivative of lofexidine **4a** was found to have antisecretory activity comparable to that of lofexidine. To determine the structural features needed to retain activity while altering the polarity of **4a**, three portions of the molecule were studied: (1) the imidazoline (heterocyclic) ring, (2) the connecting chain, and (3) the aromatic ring.

As shown in Table II, the 2,6-dimethylphenyl group in **4a** was held constant and changes in the imidazoline and oxyalkyl connecting chain were investigated to establish the best combination. The benzimidazolyl (**10**), tetrahydropyrimidyl (**7**), and thiazolinyl (**67**) compounds were inactive. The *N*-methylimidazolinyl (**9**) and hexahydrobenzimidazolyl (**8**) derivatives were weakly active. When sulfur (**54a**), nitrogen (**54b**), and carbon (**58**) were substituted for oxygen in the oxyalkyl chain in **4a**, all activity was lost as shown in both the Ussing chamber and the RCTA. Addition of a second methyl (**4s**) or replacement with ethyl (**4r**) or hydroxyethyl (**16a**) moieties on the carbon of the connecting chain of **4a** showed comparable activity, whereas desmethyl compound **4q** was inactive. Thus, the unsubstituted imidazoline ring retaining the N–H and a connecting chain with an oxygen and bulk on the carbon are required for optimal activity.

The 4-position of the aromatic ring was very sensitive to changes. As shown in Table I, the addition of a 4-methyl moiety to **4a** (**4k**) resulted in a decrease in activity, while all other 4-substitutions, 4-bromo (**4f**), 4-nitro (**4w**), 4-amino (**4v**), 4-carbo-*tert*-butoxy (**4t**), and 4-carboxylic acid (**4u**), were inactive. This may be explained by a filled region within the receptor which prevents a 4-substituent larger than hydrogen from binding to the receptor. This is consistent with the structure–activity relationships (SAR) of clonidine at the central α_2-receptor, where a 4-substituent decreases the hypotensive activity markedly (exception: 4-aminoclonidine).[6]

The addition of a 3-amino group to **4a** produces compound **21**, which has a slightly increased potency (Table III). Removal of the 6-methyl group (**12a**) reduced the activity when compared to that of **21**. Compounds in which both methyls (**12e**) or the 2-methyl (**12f**) were removed were inactive. This demonstrates the importance of the 2-substituent for good activity. In addition, the importance of the nature of the 2,6-disubstitution was shown: The compounds with a 2,6-dimethyl (**4a**) and 2,6-diethyl (**4g**) were active, those with 2,4-dimethyl (**4l**) and 2-ethyl (**4m**) were weakly active, and the 2,6-di-*n*-propyl (**4h**), 2,6-diallyl (**4i**), and 2,6-dimethoxy (**4j**) analogues were inactive (Table I). These results demonstrate very specific electronic (**4g** compared to **4j**) and steric requirements within the receptor. The crowding around the oxygen atom forces the remainder of the connecting chain and imidazoline ring out of the plane of the aromatic ring into a hairpin configuration. Figures 1 and 2 show this with the lowest energy conformations for **4a** and **12a**. These conformations appear to be important for good binding.

Substituents on the 3-amino group such as an *N*-acetyl (**13c**), *N*-ethoxycarbonyl (**13a** and **13h**), and *N,N*-dimethyl **4x** cause little diminution in activity (Table III). In this series, an ethyl (**12g**) or hydroxyethyl (**16c**) on the carbon of the connecting chain maintained or improved the activity of the parent compound **12a**.

Replacement of the 3-amino group with a hydroxyl (**64**) reduced activity (Table III), while formation of the methyl (**63a**) or benzyl (**63**) ethers produced inactive compounds.

As shown in Table IV, indoles **4d** and **51** showed activity comparable to that of the parent compound **4a**. Heterocycles such as the 2-methyl-3-pyridyl (**4e**) and 5-(4,6-dimethyl)benzimidazolyl (**37**) derivatives were inactive and other heterocycles such as the 5-isoquinolyl (**4c**) and triazoimidazopyridyl (**43**) derivatives showed very weak activity. 1-Substituted naphthalene **4n** showed weak activity. 2-Substituted naphthalene **4o** and 2-methyl-1-substituted-naphthalene **4p** were inactive.

d isomer **32** was about 5 times as active as *l* isomer **33** and the average activity of the two equaled the activity of **12a** (Table III).

As shown in the tables, there was good correlation of the activity of the RCTA to that of the Ussing chamber. A compound with an $ID_{50} \leq 10$ mg/kg ig in the RCTA model was generally active in the Ussing chamber (e.g. **4a**, **4d**, **4g**, **12a**, and **21**). An $ID_{50} \geq 10$ mg/kg sc in the RCTA model paralleled moderate activity or inactivity in the

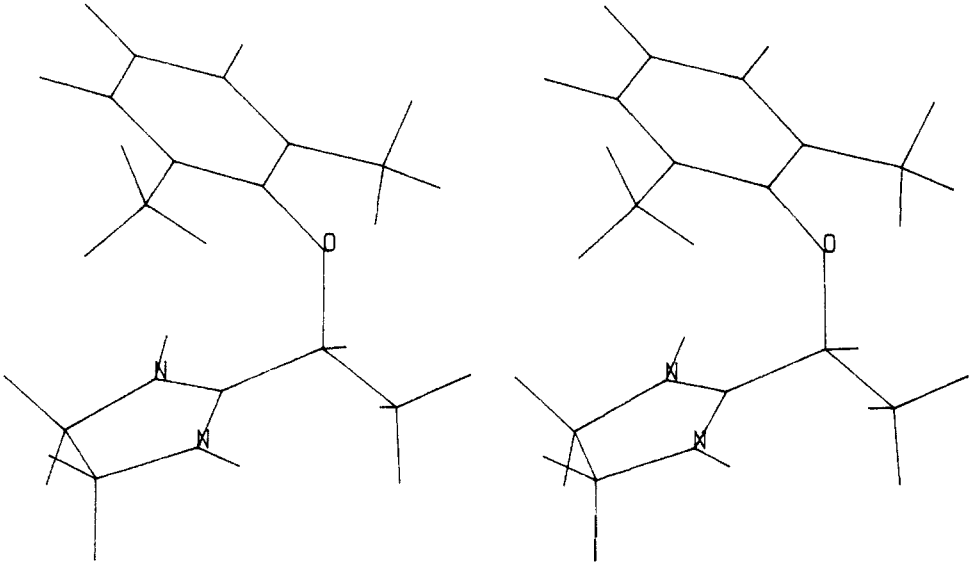


Figure 1. Lowest energy conformation for compound **4a**.

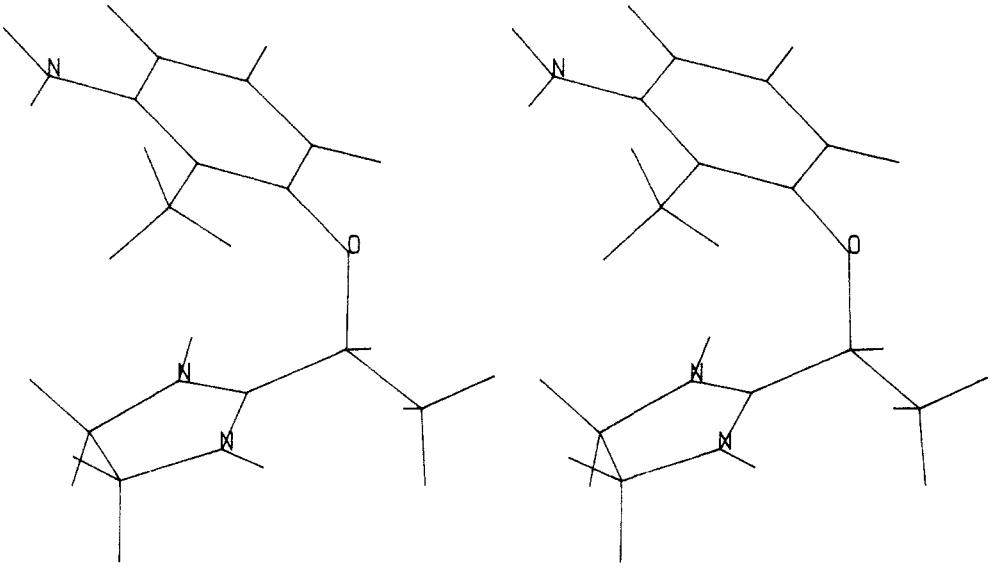


Figure 2. Lowest energy conformation for compound **12a**.

Ussing chamber (e.g. **64**). An $ID_{50} > 100$ in the RCTA model was rated inactive and such compounds were inactive in the Ussing chamber (e.g. **4u**, **4v**, **12e**, and **58**).

Four compounds (**4r**, **4x**, **12b**, and **13a**) were very active in the RCTA but were inactive in the Ussing chamber. Compound **4r** has an ethyl side chain which could be hydroxylated, and **4x** and **13a** have substituted 3-amino groups which could be metabolized to the free amino group in vivo. Compound **13h** was moderately active and also an antagonist.

Another group of compounds, **4m** (Table I), **4n** (moderately active), **4o**, **4p**, and **37** (Table IV), were inactive in the RCTA and Ussing chamber assays but were determined to be antagonists through further testing in the Ussing chamber.

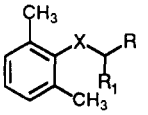
The more active compounds in the RCTA ($ID_{50} \leq 10$ mg/kg) also exhibited antisecretory activity in the dog. When evaluated in dogs at 10 mg/kg for gross symptomatology, these compounds exhibited central effects, notably sedation and ataxia.[16]

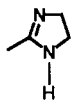
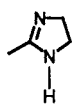
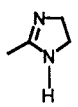
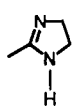
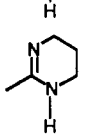
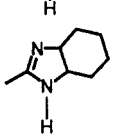
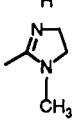
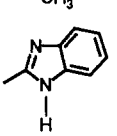
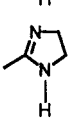
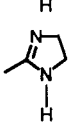
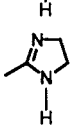
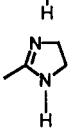
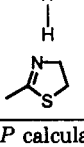
The hypotensive effect of centrally active α_2-adrenergic agents such as clonidine in the spontaneously hypertensive rat (SHR) is one measure of penetration into the CNS.[6] Several of the compounds were tested at 50 mg/kg ig in the SHR assay. As seen in Table V, there was no correlation between the RCTA ID_{50} and activity in the SHR assay. Compounds **4g**, **4k**, and **16a** exhibited good antisecretory activity, but were inactive in the SHR assay. Though compound **16a** was inactive as a hypotensive agent, it exhibited exophthalmic and lethargic side effects in the rat, both side effects attributable to centrally active α_2-adrenergic agents. Compounds **4d**, **12a**, **13a**, **13h**, **17**, **20**, **32**, **33**, and **51** exhibited good antisecretory and hypotensive activity. log P was calculated as a measure of polarity, for the more active compounds, to investigate any correlations between the RCTA ED_{50} and the compound's ability to penetrate into the CNS (SHR assay). To sim-

(16) For many of these compounds the rats exhibited CNS effects at the 20 mg/kg dose of the RCTA.

(17) Heating **45** above 160 °C caused extensive decomposition, yet no reaction occurred below 150 °C. The best results were achieved by heating **45** neat at 152 °C or refluxing in cumene for several hours and then separating **46** from **45** and recycling **45**.

Table II. Evaluation of the Nonaromatic Fragment



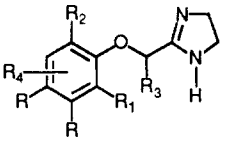
R	R_1	X	compd	log P^a	Ussing[b] chamber	rat cholera toxin assay:[c] ID_{50} (sc), mg/kg
	Me	O	**4a**	4.39	++	1.7 (1.2–2.5)
	H	O	**4q**		+	>100
	Et	O	**4r**	4.88	0[d]	5.2 (3.5–8.0)
	$(CH_3)_2$	O	**4s**	4.66	++	3.7 (2.5–5.5)
	Me	O	**7**		0	>100
	Me	O	**8**			24.1 (13.2–104.6)
	Me	O	**9**			32.4 (10.8–9999)
	Me	O	**10**			>100
	CH_2CH_2OH	O	**16a**	2.89	+	6.9 (4.6–10.8)
	Me	S	**54a**		0	>100
	Me	NH	**54b**		0	>100
	Me	CH_2	**58**		0	>100
	Me	O	**67**		0	>100

[a] log P calculated by using CLOGP3 from the MedChem software release 3.42 by C. Hansch and A. Leo, Medicinal Chemistry Project, Pomona College, Claremont, CA. The imidazoline fragment was excluded from the calculation. [b] Legend: ++ = active, final concentration 10^{-6} M; + = moderate activity, final concentration 10^{-5} M; 0 = inactive, final concentration $>10^{-5}$ M. [c] 95% confidence limits of the mean ID_{50} are shown in parentheses. [d] Antagonist (blocked the action of clonidine).

plify the calculations of log P, the common imidazoline fragment, because it is insulated from the aromatic center and should give a constant value to the log P, was factored out (Tables I–V). These simplified values should not affect any trends. Regression analysis showed no correlation of log P to ID_{50}. In addition, the compounds having higher log P values were inactive in the SHR assay, whereas compounds containing amino and hydroxyl groups having lower log P values exhibited CNS effects.

Thus separation of the central activity from the peripheral antisecretory effects on the basis of increasing polarity was not successful. The increase of basicity, which

Table III. Amino and Hydroxy Derivatives



R	R_1	R_2	R_3	R_4	compd	log P^a	Ussing[b] chamber	rat cholera toxin assay[c] ID_{50} (sc), mg/kg	rat cholera toxin assay[c] ID_{50} (ig) mg/kg
H	Me	Me	Me	H	**4a**	4.39	++	1.7 (1.2–2.5)	2.7 (1.3–4.7)
$N(Me)_2$	Me	H	Me	H	**4x**	4.00	0	4.0 (2.7–6.0)	
NH_2	Me	H	Me	H	**12a**	2.00	++	5.6 (3.7–8.7)	1.3 (0.9–2.1)
NH_2	H	Me	Me	4-Me	**12b**	3.01	0	7.8 (12.7–23.5)	
NH_2	H	H	Me	H	**12e**		0	>100	
NH_2	H	Me	Me	H	**12f**		0	>100	
NH_2	Me	H	Et	H	**12g**	3.19		4.5 (3.1–68)	5.2 (3.3–8.8)
EtOCONH	Me	H	Me	H	**13a**	3.27	0	4.6 (3.1–6.9)	9.5 (5.7–18.3)
AcNH	Me	H	Me	H	**13c**	2.43	0	7.0 (4.6–11.2)	
EtOCONH	Me	Me	Me	H	**13h**	4.40	+[d]	3.0 (2.0–4.4)	
NH_2	Me	H	CH_2CH_2OH	H	**16c**	1.20		1.2 (0.8–1.7)	1.3 (0.9–2.2)
NH_2	Me	Me	Me	H	**21**	3.31	++	1.0 (0.7–1.5)	
NH_2	Me	Me	Me	4-Br	**22**			>100	
NH_2	Me	Me	Me	5-NH_2	**24**			32.0 (0–9999)	
NH_2	Me	H	Me (*d*)	H	**32**	2.00		1.4 (1.0–2.0)	
NH_2	Me	H	Me (*l*)	H	**33**	2.00		7.7 (5.1–12.5)	
OBnz	Me	H	Me	H	**63**		+	>100	
OMe	Me	H	Me	H	**63a**		0[e]	>100	
OH	Me	H	Me	H	**64**	3.21	+	17.2 (9.7–46.1)	

[a] log *P* calculated by using CLOGP3 from the MedChem software release 3.42 by C. Hansch and A. Leo, Medicinal Chemistry Project, Pomona College, Claremont, CA. The imidazoline fragment was excluded from the calculation. [b] Legend: ++ = active, final concentration 10^{-6} M; + = moderate activity, final concentration 10^{-5} M; 0 = inactive, final concentrations $>10^{-5}$ M. [c] 95% confidence limits of the mean ID_{50} are shown in parentheses. [d] At 10^{-5} M the compound was found to be a partial agonist/antagonist. [e] Antagonist (blocked the action of clonidine).

is achieved in the clonidine series but not in the lofexidine series by substituting methyl for chloro, appears to be more important than increasing the polarity in preventing penetration across the blood–brain barrier. Although good intestinal antisecretory potency was achieved, the CNS side effect associated with all the compounds, though mild or nonexistent at the therapeutic antisecretory doses for several of the compounds, precluded this series from further development.

Experimental Section

General Procedures. Products were crystallized from an appropriate solvent. Extracts were concentrated, after washing with water and drying over $MgSO_4$, on a rotary evaporator at reduced pressure, the temperature being maintained below 40 °C. Column chromatography was performed on a medium-pressure glass column using silica gel 60 (Merck) or on a Waters Prep 500 using Porisil. All reactions were performed under an atmosphere of nitrogen. ^1H NMR spectra were measured with TMS (0 ppm) as an internal standard on a Varian A-60 or FT-80 spectrometer; IR spectra were measured on Perkin-Elmer 283b and 681 instruments. Microanalyses were performed for the stated elements and were within ±0.4% of the theoretical values for the stated empirical formula. Melting points are corrected and measured on a Thomas-Hoover capillary melting point apparatus.

The method described below, alkylation of a phenol with the appropriate bromo ester and cyclization to the imidazoline ring with EDA and Me_3Al, was used to synthesize 2-[(aryloxy)alkyl]- and 2-[(heteroaryloxy)alkyl]imidazolines in all cases. Whenever possible the nitro derivative was reduced to the amino derivative after imidazoline ring formation. The experimental results of similar reactions are collected in Table VI. ^1H NMR and IR are collected in Table VII of the supplementary material.

Synthesis of 2-[(Aryloxy)alkyl]- and 2-[(Heteroaryloxy)alkyl]imidazolines. Method A. Ethyl 2-[(2-Methyl-3-pyridyl)oxy]propionate (3e). A mixture of 16.7 g (0.15 mol) of 3-hydroxy-2-methylpyridine,[9] 19.8 mL (0.15 mol) of ethyl 2-bromopropionate, and 21 g (0.15 mol) of K_2CO_3 in 150 mL of DMF was stirred for 18 h. The mixture was poured into water (500 mL), then extracted with ethyl ether. The organic layer was washed with water, dried, and concentrated to an oil, **3e** (14.2 g, 45%).

3-[1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethoxy]-2-methylpyridine (4e). $AlMe_3$/toluene (2 M, 35 mL, 0.07 mol) and 50 mL of toluene were cooled to −10 °C. Ethylenediamine (4.7 mL, 0.07 mol) was added dropwise to keep the temperature below 5 °C ($AlMe_3$/EDA complex). The heterogeneous mixture was stirred for 15 min before adding 14.2 g (0.067 mol) of **3e** dissolved in 20 mL of toluene. The mixture was allowed to warm to room temperature and then refluxed for 3 h. After cooling to 25 °C, 5.0 mL of H_2O was carefully added to the reaction mixture (exothermic reaction!). CH_2Cl_2 and then MeOH (50 mL) were added to the reaction mixture, the slurry was filtered, and the filter cake was washed with 50 mL of MeOH. The filtrate was slowly evaporated to about $^1/_4$ the volume, and the crystalline solid was collected and washed well with ethyl ether to yield 5.9 g (43%) of **4e**.

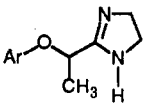
Method B (Amino Derivatives). Ethyl 2-(4-Bromo-2,6-dimethyl-3-nitrophenoxy)propionate (18). Concentrated H_2SO_4 (320 mL) was cooled to −20 °C with an acetone/dry ice bath (H_2SO_4 just starts to freeze!). Ester **3f** (92.8 g, 0.307 mol) was added with vigorous stirring. The mixture was warmed to −10 °C, and 22.4 mL (0.352 mol) of 70% HNO_3 was added dropwise to keep the temperature at or below −10 °C. After stirring for 15 min, the reaction mixture was poured onto ice and extracted with ethyl ether (2 × 500 mL). The combined extracts were washed with 5% $NaHCO_3$ and then H_2O, dried, and concentrated to an oil (96.2 g). The oil was chromatographed by eluting with EtOAc/hexane. The first component was discarded. The next component was 4-bromo-3-nitro derivative **18** (31.1 g, 28%).

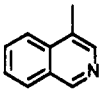
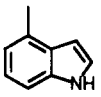
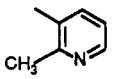
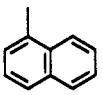
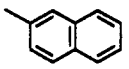
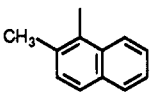
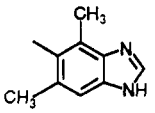
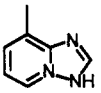
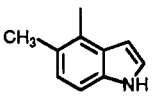
The last fraction was rechromatographed by eluting with toluene/hexane to give the 4-nitro derivative and 3,5-dinitro derivative **19**.

Nitro ester **18** (31.1 g, 0.0866 mol) was converted to 24.8 g (87%) of a crystalline solid (**20**) as described in method A. Anal. ($C_{13}H_{16}BrN_3O_3$) C, H, N.

4,5-Dihydro-2-[1-(3-amino-2,6-dimethylphenoxy)ethyl]-1*H*-imidazole (21). Compound **20** (14.8 g, 0.043 mol) was hydrogenated in MeOH over 5% Pd/C. The filtrate was concentrated and the oil crystallized from ethyl ether, yielding 11.2 g

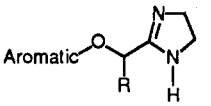
Table IV. Heterocyclic and Polycyclic Derivatives



Ar	compd	log P^a	Ussing[b] chamber	rat cholera toxin assay[c] ID_{50} (sc), mg/kg	rat cholera toxin assay[c] ID_{50} (ig), mg/kg
	**4c**			21.7 (11.3–79.7)	
	**4d**	3.12	++	5.7 (3.8–8.1)	6.6 (3.8–15.1)
	**4e**			>100	
	**4n**	4.35	0[d]	17.9 (10.4–44.4)	16.1 (10.8–9999)
	**4o**		0[d]	>100	
	**4p**		0[d]	>100	
	**37**		0[d]	>100	
	**43**			26.5 (14.2–207.9)	
	**51**	3.77		1.6 (1.1–2.4)	1.2 (0.8–1.9)

[a] log *P* calculated by using CLOGP3 from the MedChem software release 3.42 by C. Hansch and A. Leo, Medicinal Chemistry Project, Pomona College, Claremont, CA. The imidazoline fragment was excluded from the calculation. [b] Legend: ++ = active, final concentration 10^{-6} M; + = moderate activity, final concentration 10^{-5} M; 0 = inactive, final concentration $>10^{-5}$ M. [c] 95% confidence limits of the mean ID_{50} are shown in parentheses. [d] Antagonist (blocked the action of clonidine).

Table V. Comparison of the Activity in the Spontaneously Hypertensive Rat (SHR) and the RCTA



aromatic	R	compd	ID_{50} RCTA	log *P*	SHR assay[a]
4-indyl	Me	**4d**	5.7	3.12	active(−50)[b,c]
2,6-diethylphenyl	Me	**4g**	2.3	5.41	inactive
2,4,6-trimethylphenyl	Me	**4k**	11.1	5.00	inactive
1-naphthyl	Me	**4n**	17.9	4.35	inactive
3-amino-2-methylphenyl	Et	**12g**	4.5	3.19	active(−49)[b]
3-carbethoxy-2-methylphenyl	Me	**13a**	4.6	3.27	active(−75)
3-carbethoxy-2,6-dimethylphenyl	Me	**13h**	3.0	4.40	active(−20)[b]
2,6-dimethylphenyl	CH_2CH_2OH	**16a**	6.9	2.89	inactive[b,c]
3-amino-2-methylphenyl	CH_2CH_2OH	**16c**	1.2	1.20	died[b]
3-amino-2,6-dimethylphenyl	Me	**21**	1.0	3.31	active(−20)[b]
2,6-dimethylphenyl	Me (*d*)	**32**	1.4	2.00	active(−43)[b,d]
2,6-dimethylphenyl	Me (*l*)	**33**	7.7	2.00	active(−45)
5-methyl-4-indyl	Me	**51**	1.6	3.77	died[d]
3-hydroxyl-2-methylphenyl	Me	**64**	17.2	3.21	inactive

[a] Spontaneously hypertensive rats (SHR) were dosed with 50 mg/kg of compound ig. The mean arterial blood pressure was measured directly via a previously implanted catheter immediately before administration of the test compound. Blood pressure readings were repeated at 4 h after treatment. The compound was rated active, if the posttreatment blood pressure change (from pretreatment base line) was −20 mmHg or greater in a single animal. [b] Exophthalmic side effects. [c] Lethargic side effects. [d] Convulsive side effects.

Table VI. Experimental Results

compd	% yield	method	formula	anal.	mp, °C
3b	80	B	$C_{12}H_{15}NO_5$	C,H,N	
3c	45	A	$C_{14}H_{15}NO_3$	C,H,N	oil
3d	93	A	$C_{14}H_{15}N_3O \cdot {}^1/_4H_2O$	C,H,N	oil
3z	91	B	$C_{13}H_{17}NO_5$	C,H,N	
4a	61	A	$C_{13}H_{18}N_2O$	C,H,N	waxy solid
4c	35	A	$C_{14}H_{15}N_3O \cdot {}^1/_4H_2O$	C,H,N	60–118
4d	52	A	$C_{13}H_{15}N_3 \cdot {}^1/_2H_2O$	C,H,N	
4g	58	A	$C_{15}H_{22}N_2O$	C,H,N	waxy solid
4l	62	A	$C_{13}H_{18}N_2O$	C,H,N	72–95
4m	55	A	$C_{13}H_{18}N_2O$	C,H,N	oil
4r	60	A	$C_{13}H_{18}N_2O \cdot {}^1/_8H_2O$	C,H,N	108–115
4s	45	A	$C_{14}H_{20}N_2O$	C,H,N	67–83
4x	34	A	$C_{14}H_{21}N_3O$	C,H,N	
4z	30	B	$C_{13}H_{17}N_3O_3$	C,H,N	
7	43	A	$C_{14}H_{20}N_2O \cdot {}^1/_8H_2O$	C,H,N	
8	13	A	$C_{17}H_{24}N_2O \cdot {}^1/_8H_2O$	C,H,N	waxy solid
9	32	A	$C_{14}H_{20}N_2O$	C,H,N	oil
10	19	A	$C_{17}H_{18}N_2O$	C,H,N	
11k	21	B	$C_{12}H_{15}N_3O_3 \cdot {}^1/_2H_2O$	C,H,N	
12a	80	B	$C_{13}H_{17}N_3O \cdot 2HCl \cdot H_2O$	C,H,N	waxy solid
13j	68	B	$C_{15}H_{21}N_3O$	C,H,N	~79 dec
15b	58	B	$C_{11}H_{11}NO_5$	C,H,N	
16a	35	B	$C_{14}H_{20}N_2O_2 \cdot {}^3/_8H_2O$	C,H,N	111–135
16b	15	B	$C_{11}H_{11}NO_5$	C,H,N	
16c	86	B	$C_{13}H_{19}N_3O_2 \cdot 2HCl \cdot {}^1/_4H_2O$	C,H,N	
17	24	C	$C_{17}H_{21}N_3O_3$	C,H,N	
42	59	A	$C_{11}H_{13}N_3O_3$	C,H,N	
43	31	A	$C_{11}H_{13}N_5O \cdot {}^1/_4MeOH$	C,H,N	
45	56	A	$C_{10}H_{11}NO_3$	C,H,N	57–118
51	53	A	$C_{14}H_{17}N_3O \cdot {}^1/_4CH_2Cl_2$	C,H,N	
54a	48	A	$C_{13}H_{18}N_2S$	C,H,N,S	
54b	5	A	$C_{13}H_{19}N_3 \cdot {}^1/_4H_2O$	C,H,N	
57	32	A	$C_{13}H_{16}O_2$		
57a	30	A	$C_{14}H_{18}N_2 \cdot {}^1/_4H_2O$	C,H,N	
58	24	A	$C_{14}H_{20}N_2 \cdot {}^1/_4H_2O$	C,H,N	
62	93	A	$C_{19}H_{22}O_4$		
62a	94	A	$C_{13}H_{18}O_4$		
63	61	A	$C_{19}H_{22}N_2O_2$	C,H,N	
63a	41	A	$C_{13}H_{18}N_2O_2$	C,H,N	
64	45	A	$C_{12}H_{18}N_2O_2 \cdot HCl \cdot {}^1/_8H_2O$	C,H,N,Cl	173.5–176.5

(86%), of **21**, mp 189–194 °C. Anal. ($C_{13}H_{19}N_3O \cdot HBr \cdot {}^1/_4H_2O$) C, H, N, Br.

4,5-Dihydro-2-[1-(3-amino-4-bromo-2,6-dimethylphenoxy)ethyl]-1*H*-imidazole (22). Bromonitroimidazoline **20** (3.0 g, 0.0087 mol) was hydrogenated in MeOH over Raney Ni: TLC indicated two components. The mixture was chromatographed by eluting with ($MeOH/CH_2Cl_2$/0.25% NH_4OH). The second component was **22**, yielding 580 mg (21%). Anal. ($C_{13}H_{18}BrN_3O$) C, H, N, Br.

4,5-Dihydro-2-[1-(3,5-diamino-2,6-dimethylphenoxy)ethyl]-1*H*-imidazole (24). Compound **23** (2.1 g, 0.0054 mol) was hydrogenated over 10% Pd/C in EtOH. The mixture was concentrated and crystallized from MeOH to yield 1.2 g (63%) of **24**, mp 231–240 °C. Anal. ($C_{13}H_{20}N_4O \cdot HBr \cdot {}^1/_2MeOH$) C, H, N, Br.

Method C. Ethyl *N*-[3-[[1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethyl]oxy]-2,6-dimethylphenyl]carbamate (13h). Compound **21** (5.0 g, 0.0159 mol) was suspended in 130 mL of acetonitrile, and 13.8 mL (0.016 mol) of a 12% phosgene/toluene solution was added to the suspension and the mixture was stirred for 18 h. Absolute ethanol (25 mL) was added and the mixture stirred for 2 h. The reaction mixture was concentrated, the residue was dissolved in H_2O, and the pH was adjusted to 10 with 5% NaOH. The solution was decanted from the resulting solid. Ethyl ether was added to the residue, and the organic solution was decanted from the insoluble material. The organic solution was dried and concentrated and the product crystallized from ethyl ether, yielding 2.2 g (43%) of **13h**. Anal. ($C_{16}H_{23}N_3O_3 \cdot H_2O$) C, H, N.

Method D. 4,5-Dihydro-2-[1-(2,6-dimethylphenoxy)-3-hydroxypropyl]-1*H*-imidazole (16a). α-bromobutyrolactone (8.24 mL, 0.1 mol) was added to a solution of 12.2 g (0.1 mol) of 2,6-dimethylphenol and 13.8 g (0.1 mol) of K_2CO_3 in 150 mL of DMF, and the mixture stirred was for 18 h. The reaction mixture was poured into H_2O and then partitioned into Et_2O. The organic layer was washed with H_2O, dried, and concentrated to an oil (14 g), which was chromatographed by eluting with EtOAc/hexane, to obtain 3.6 g (17%) of an oil, **15a**.

Compound **15a** (3.6 g, 0.017 mol) was converted to the imidazoline with $AlMe_3$/EDA as before (exothermic!).[18] This yielded 4.4 g of an oil which was chromatographed by eluting with ($MeOH/CH_2Cl_2$/0.25% NH_4OH), yielding 2.9 g (69%) of **16a**. Anal. ($C_{14}H_{20}N_2O_2 \cdot {}^3/_8H_2O$) C, H, N.

Ethyl 2-[3-(Dimethylamino)-2-methylphenoxy]propanoate (3x). Compound **12a** (7.7 g, 0.034 mol), 2.1 mL of acetic acid, and 30 mL of 37% formaldehyde were stirred in 50 mL of acetonitrile. $NaCNBH_3$ (2.3 g, 0.037 mol) was added to the reaction mixture and the progress of the reaction was monitored by TLC (5% $MeOH/CH_2Cl_2$). NaOH was added to the reaction mixture after the starting material was consumed and the mixture was then extracted with Et_2O. The combined organic extracts were washed with H_2O and then dried over $MgSO_4$, and the mixture was concentrated to an oil, which was chromatographed on silica, eluting with EtOAc/hexane, to yield 4.2 g (49%) of **3x**.

Synthesis of *d*- and *l*-12a (32 and 33). *d*-Mandelic acid (**25**, 15.2 g, 0.1 mol) was refluxed in 100 mL of MeOH with 1.0 mL of $SOCl_2$ for 1 h, cooled, and then poured into H_2O and extracted with Et_2O. The organic layer was washed with H_2O, dried, and concentrated to an oil (**26**), which crystallized, giving 12.8 g (77%): $[\alpha]_D$ +659.5°; IR ($CHCl_2$) 1741 cm^{-1}.

Methyl ester **26** (12.8 g, 0.077 mol) and 6.2 mL (0.077 mol) of pyridine were dissolved in 300 mL of CH_2Cl_2 and cooled to −10 °C. Phenyl chloroformate (9.6 mL, 0.077 mol) was added drop-

(18) The reaction of lactone **15** with $AlMe_3$/EDA complex was very exothermic when compared to that of ester **3**, and it produced a fair yield of hydroxy ethyl derivative **16**.

wise, and the mixture was stirred for 15 min. The reaction mixture was washed with saturated citric acid and then H_2O, dried, and concentrated to an oil (**27**): $[\alpha]_D$ +403.5; IR (KBr) 1760 cm^{-1}.

Compound **4b** (5.0 g, 0.02 mol) and 8.6 g (0.03 mol) of **27** were combined and placed in an oil bath at 150 °C for 15 min. The residue was placed on a C_{18} reverse-phase column and eluted with THF/H_2O. This yielded 8.3 g (97.5%) of an oil (**29**) which was then chromatographed on a Waters Prep 500 using two cartridges and eluting with 45% EtOAc/hexane. The sample was recycled two times with collection of the forerun and tail each time to yield 1.185 g (14%) of **30** and 1.435 g (17%) of **31**. Compound **30** (*d,d*): $[\alpha]^{589}_D$ +44.7°; Calcd for $C_{22}H_{23}N_3O_7$ 425.43. Compound **31** (*l,d*): $[\alpha]^{589}_D$ +2.8°; Calcd for $C_{22}H_{23}N_3O_7$ 425.43.

Each isomer (**30** and **31**) was hydrogenated separately over 10% Pd/C. This reduced the nitro group and cleaved the benzyloxy group. Each was isolated as the HCl salt.

Compound **32**: $[\alpha]^{365}_D$ +39.8°. Anal. $C_{12}H_{17}N_3O{\cdot}2HCl{\cdot}{}^1/_2MeOH$ (308.22) C, H, N, Cl.

Compound **33**: $[\alpha]^{365}_D$ −33.8°. Anal. $C_{12}H_{17}N_3O{\cdot}2HCl{\cdot}{}^1/_8MeOH$ (296.20) C, H, N, Cl.

5,7-Dimethyl-6-[[1-(4,5-dihydro-1*H*-imidazol-2-yl)ethyl]oxy]-1*H*-benzimidazole (37). Concentrated H_2SO_4 (300 mL) was cooled to 0 °C with a MeOH/ice bath and 14.7 mL (0.22 mol) of 70% HNO_3 was added to it, and the mixture was recooled to 0 °C. Compound **3a** (22.2 g, 0.1 mol) was added and the temperature was kept below 10 °C. The reaction mixture was stirred at 0 °C for 1 h and then poured on to ice and extracted with ethyl ether. The organic layer was washed with dilute $NaHCO_3$ and then with H_2O and then dried and concentrated to an oil. The sample was chromatographed by eluting with EtOAc/hexane. The first component was discarded. The second was collected and concentrated, yielding 20.6 g (66%) of **34**.

Compound **34** (20.6 g, 0.065 mol) was hydrogenated over Pd/C in EtOH and then concentrated to yield 16.7 g of **35**.

Compound **35** (20 g, 0.08 mol) was refluxed with 8 mL of formic acid in 200 mL of 4 N HCl for 40 min. The reaction mixture was concentrated, the residue was dissolved in 500 mL of EtOH, pretreated with 2.0 mL of $SOCl_2$, and the mixture was refluxed for 0.5 h. The mixture was concentrated and then partitioned between dilute NH_4OH and ethyl ether. The organic layer was washed with H_2O, dried, concentrated, and then crystallized from Et_2O/hexane to yield 14.6 g (57%) of **36**.

Compound **36** (14.6 g, 0.0556 mol) was converted to the imidazoline derivative as above. Column chromatography, eluting with (EtOH/CH_2Cl_2/0.25% NH_4OH) and collecting the second component, yielded 2.2 g (13%) of **37**. Anal. $(C_{14}H_{18}N_4O{\cdot}{}^3/_4EtOH)$ C, H, N.

8-[[1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethyl]oxy][1,2,4]triazolo[1,5-*a*]pyridine (43). A mixture of 25.0 g (0.125 mol) of 2-amino-3-(benzyloxy)pyridine (**38**),[10] and 16.6 mL (0.125 mol) of *N,N*-dimethylformamide dimethyl acetal in 25 mL of EtOH was refluxed for 20 h. The mixture was concentrated to a crude oil of **39** (31.9 g).

Crude **39** was dissolved in 180 mL of methanol and 20 mL of pyridine and was cooled to 0 °C. Hydroxylamine-*O*-sulfonic acid (15.4 g, 0.130 mol) was added to the reaction mixture. The mixture was allowed to warm to room temperature; crystallization started after 2 h. After 18 h the crystals were filtered and washed with methanol and then ethyl ether to yield 16.8 g (60%) of **40**. Anal. ($C_{13}H_{11}N_3O$) C, H, N.

Compound **40** (16.4 g, 0.0769 mol) was hydrogenated in THF/EtOH over 5% Pd/C to yield 9.0 g (86%) of **41** (C, H, N), which was converted to compound **43** following method A.

4,5-Dihydro-2-[1-(5-methyl-4-indoloxy)ethyl]-1*H*-imidazole (51). 2-Methyl-5-nitrophenol (40.0 g, 0.26 mol) was alkylated with 45.6 mL (0.52 mol) of allyl bromide as in method A. The residue was crystallized from hexane to yield 29.6 g (59%) of **45**. Anal. ($C_{10}H_{11}NO_3$) C, H, N.

Compound **45** (29.6 g, 0.15 mol) was refluxed in cumene for 4 h.[17] The mixture was concentrated and chromatographed by eluting with EtOAc/hexane. The starting material was recycled and yielded 7.3 g (25%) of phenol **46** and was converted to **47** by using method A.

Compound **47** (9.0 g, 0.03 mol) and 13.4 g (0.063 mol) of $NaIO_4$ and 1.0 mL of 1% OsO_4 in butanol were stirred in 150 mL of dioxane and 35 mL of H_2O. The solid was filtered and washed with ethyl ether. The filtrate was washed with H_2O, dried, and concentrated to an oil. This oil was chromatographed by eluting with EtOAc/hexane, yielding 3.9 g, 44% of nitroaldehyde **48**.

Nitroaldehyde **48** (1.0 g, 0.0033 mol) was hydrogenated over Raney Ni in EtOH to yield 896.6 mg of indole **50**. Following method A **50** was converted to compound **51**.

4,5-Dihydro-2-[1-[(2,6-dimethylphenyl)thio]ethyl]-1*H*-imidazole (54a). 2,6-Dimethylthiophenol (5.5 g, 0.04 mol) was dissolved in 100 mL of THF. This solution was added to a suspension of 1.98 g (0.04 mol) of 50% NaH/mineral oil (washed with hexane to remove the mineral oil) and 50 mL of THF, and the mixture was stirred for 0.5 h after addition was complete. Ethyl 2-bromopropionate (7.2 g, 0.04 mol) dissolved in 20 mL of THF was slowly added to the mixture (exothermic). The mixture was concentrated and the residue was partitioned between Et_2O/H_2O. The organic layer was washed with H_2O, dried, and concentrated. The residue was chromatographed on silica eluting with EtOAc/hexane to give 3.5 g of an oil (**53a**).

Compound **53a** (3.0 g, 0.0125 mol) was reacted with 0.02 mol of EDA/$AlMe_3$ complex as previously described to yield 1.4 g (30%) of a solid (**54a**). Anal. ($C_{13}H_{18}N_2S$) C, H, N, S.

***N*-[1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethyl]-2,6-dimethylbenzenamine (54b).** Compound **53b** was prepared from 2,6-dimethylaniline in the same manner as **53a**.

Compound **53a** (4.7 g, 0.0226 mol) was reacted with 0.0373 mol of EDA/$AlMe_3$ complex as previously described to yield 250 mg of **54b**. Anal. $(C_{13}H_{19}N_3{\cdot}{}^1/_4H_2O)$ C, H, N.

4,5-Dihydro-2-[1-[(2,6-dimethylphenyl)methyl]ethyl]-1*H*-imidazole (58). 2-Bromo-*m*-xylene (**55**, 6.6 mL, 0.05 mol), 8.5 mL (0.08 mol) of methyl methacrylate (**56**), 112 mg (0.5 mmol) of $Pd(OAc)_2$, and 262 mg (1.0 mmol) of triphenylphosphine were dissolved in 22.3 mL (0.16 mol) of Et_3N and heated at 100 °C in a bomb for 8 h. The reaction mixture was dissolved in hexane/H_2O and filtered though Celite to remove the Pd^0. The organic layer was washed with cold, dilute HCl and cold H_2O, dried, and concentrated to 8.2 g of an oil, which was chromatographed on silica and eluted with EtOAc/hexane. The second component was **57** [3.3 g, 32%, $C_{13}H_{16}O_2$ (204.26)], and it was converted to **57a** following method A.

Compound **57a** (447 mg, 2.0 mmol) was hydrogenated in THF over 5% Pd/C. After concentrating the mixture the residue was chromatographed on silica by eluting with MeOH/CH_2Cl_2/0.25% NH_4OH to yield 105.7 mg, 23% of **58**. Anal. $(C_{14}H_{20}N_2{\cdot}{}^1/_4MeOH)$ C, H, N.

3-(Benzyloxy)-2-methylphenol (61). 2-methylresorcinol (50 g, 0.4 mol), 46.3 mL (0.4 mol) of benzyl chloride, and 55.5 g (0.4 mol) of K_2CO_3 in 0.5 L of DMF was heated at 95 °C for 7 h. An additional 10 mL of benzyl chloride was added and the mixture was heated for 18 h at 95 °C. The mixture was poured onto ice, acidified with concentrated HCl, and extracted with ethyl ether. The organic layer was washed with H_2O, dried, and concentrated to an oil (80.2 g). The oil was chromatographed by eluting with EtOAc/hexane; the first component was **60**, 20% (24.8 g). The second component was **61**, 20% (17.6 g).

Compound **60** (24.8 g, 0.081 mol) was hydrogenated over 10% Pd/C and the reaction was stopped after 1 equiv of H_2 was taken up. The mixture was concentrated and chromatographed, eluting with EtOAc/hexane, yielding 4.4 g (25%) of **61**.

1-Methoxy-2-methyl-3-(phenylmethoxy)benzene (60a). Compound **61** (4.4 g, 0.02 mol), 1.9 mL (0.02 mol) of Me_2SO_4 and 3.2 g (0.04 mol) of 50% NaOH in 40 mL of EtOH were stirred for 17 h. The mixture was diluted with H_2O and the pH was adjusted to 11. The product was extracted into ethyl ether, dried over $MgSO_4$, and concentrated to an oil. The yield of **60a** was 4.6 g. Calcd for $C_{15}H_{16}O_2$ 228.28.

3-Methoxy-2-methylphenol (61a). Compound **60a** (4.6 g, 0.02 mol) was hydrogenated in THF over Pd/C. The mixture was concentrated to yield 2.5 g of **61a**.

3-[[1-(4,5-Dihydro-1*H*-imidazol-2-yl)ethyl]oxy]-2-methylphenol (64). Compound **63** (12.0 g, 0.038 mol) was hydrogenated in THF over Pd/C. The product was concentrated and crystallized from ethyl ether to yield 8.3 g (84%) of **64**, which was isolated as the HCl salt. Anal. $(C_{12}H_{16}N_2O_2{\cdot}HCl{\cdot}{}^1/_8H_2O)$ C, H, N, Cl.

2-[1-(2,6-Dimethylphenoxy)ethyl]-4,5-dihydrothiazole (67). Compound **3a** (44.5 g, 0.2 mol) was dissolved in 150 mL of MeOH

and 10 mL of H_2O. To this mixture 40 mL of 50% NaOH was added dropwise, and the progress of the reaction was monitored by TLC. After the hydrolysis was complete, the reaction mixture was diluted to 700 mL with H_2O and washed with hexane. The aqueous solution was acidified with concentrated HCl and the product was extracted into Et_2O. The organic extracts were washed with H_2O and dried, and the solvent was evaporated to yield 38.2 g (98%, 0.196 mol) of the acid.

The acid was dissolved in 28 mL (0.39 mol) of $SOCl_2$ and refluxed for 1.5 h. The excess $SOCl_2$ was evaporated and the traces of $SOCl_2$ were removed by dissolving the residue in toluene and evaporating the mixture to dryness.

The residue was dissolved in 1.0 L of Et_2O and NH_3 gas was bubbled though the mixture until the solution was basic. Concentrated NH_4OH (50 mL) and then 400 mL of H_2O were added to the reaction mixture. The organic phase was washed with H_2O, dried, and evaporated to yield 32.8 g (50%) of amide **65**. Anal. ($C_{11}H_{15}NO_2$) C, H, N.

Compound **65** (18.9 g, 0.097 mol) and 37.8 mL (0.25 mol) of Et_3N were dissolved in 200 mL of toluene with 50 mL of CH_2Cl_2 and cooled with an ice bath. A solution of 12% phosgene in toluene (100.0 mL, 0.13 mol) was added dropwise to keep the temperature below 10 °C. The reaction mixture was warmed to room temperature and 50 mL of H_2O was added. The organic layer was washed with dilute HCl and H_2O, dried, and evaporated to yield 15.1 g of **66** as an oil.

Compound **66** (1.7 g, 0.01 mol) and 1.25 (0.011 mol) of 2-aminoethanethiol hydrochloride were heated with a Bunsen burner until the mixture was homogeneous. The residue was dissolved in H_2O and acidified with HCl, and the mixture was washed with Et_2O. The aqueous layer was made alkaline with NaOH. The product was extracted into Et_2O, washed with H_2O, dried, and then concentrated to an oil, which was chromatographed on silica, eluting with EtOAC/hexane to yield 229 mg of **67**. Anal. ($C_{13}H_{17}NOS \cdot {}^1/_8H_2O$) C, H, N.

Biological Methods. Cholera-Induced Intestinal Fluid Secretion. Excessive fluid secretion into the intestinal lumen is a major component of diarrhea.[19] In order to determine the effect of the test compounds on intestinal fluid movements, the rat cholera toxin secretion assay (RCTA) was used.[11] Female Charles River rats weighing 85–100 g with free access to water were fasted for 24 h prior to each experiment. Under ether anesthesia, a midline incision was made and a 20-cm ligated small intestinal segment was constructed starting 3.0 cm distal to the ligament of Treitz. Each segment was injected with equivalent secretory doses of either 1.0 mL of a 40 mg/mL saline solution of crude cholera toxin [Cholera Advisory Board, National Institutes of Allergy and Infectious Disease (Lot #001-Wyeth)] or 1.0 mL of saline containing 160 μg of crude cholera toxin (List Biologicals, Campbell, California).

Injections into the jejunum were made with a 27-gauge 0.5-in. needle. Animals were sacrificed 4 h later and the fluid content and exact length of the intestinal segments were measured. Fluid secretion was expressed in mL/cm of intestine.

Compounds were administered subcutaneously to groups of four rats at doses of 10 and 20 mg/kg at the time of cholera toxin injection into the intestinal segment. Rats given oral doses of drug were pretreated 30 min prior to cholera. The volumes obtained after compound treatment were compared to controls which did not receive drug.

(19) Binder, H. J. The Pathophysiologic Basis for Diarrhea. In *Mechanisms of Intestinal Secretion*; Alan R. Liss: New York, 1979; Chapter 1.

The ID_{50}'s of these compounds were estimated from data on at least two doses and from at least two different experiments, by the method for maximum likelihood.[12]

Ussing Chamber Transport Experiments. Intestinal antisecretory drugs such as clonidine which act by an α_2-adrenergic mechanism have been demonstrated to inhibit the increases in intestinal potential difference caused by diarrhea-inducing secretogogues.[13] Experiments to determine in vitro actions of this type of compound which might correlate with intestinal antisecretory actions in vivo in the rat were done as described by Field.[14] Male New Zealand white rabbits weighing between 2 and 3 kg were killed by cervical dislocation. A 40–50-cm segment of distal ileum was removed, washed thoroughly with cold saline, and placed in an ice-cold oxygenated modified Kreb's buffer (composition in mM: NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 1.1). The ileal segments were then stripped of serosa and muscularis by drawing the tissue over a 10-mL pipet and using a sharp scalpel for dissection. A 2.5-cm segment of mucosa was then mounted between two Plexiglas chambers providing an exposed mucosal area of 1.23 cm^2.

Ten milliliters of modified Krebs buffer was introduced into the chambers on both mucosal and serosal sides of the ileal mucosa. The buffer was oxygenated with 95% O_2/5% CO_2 and kept at 37 °C and pH 7.4.

Potential difference (PD) measurements on isolated ileal mucosa were made at 5-min intervals with a 742C dual voltage clamp (Bioengineering Dept., University of Iowa, Iowa City, IA). Tissue PD were monitored with matched reference electrodes (Fisher Scientific, cat. no. 13-639-79) connected to the chambers by salt–agar bridges containing Krebs solution and 2% agar.

Determination of drug effect was made on at least two tissues which had been exposed for 10 min to 5×10^{-3} M aminophylline to initiate net anion secretion into the lumenal compartment of the Ussing chamber. Ten minutes after the addition of aminophylline, the test compounds were added to both sides of the ileum at a final concentration of 10^{-6} M. A compound was considered active if it returned the aminophylline-augmented PD to control values. If the compound had no effect on PD, the concentration was raised to 10^{-5} M on both sides of the tissue. Moderate activity was defined as a reduction of PD only at 10^{-5} M. Inactive compounds did not change PD. Yohimbine, a competitive antagonist of α_2-receptors, was always incubated after activity was seen to determine whether α_2-receptor activation was responsible for the activity of the compounds.

If no activity was observed, the drug clonidine (10^{-6} M) was added to both sides of the bath to ascertain tissue viability. In some instances clonidine had a reduced or negligible effect. These agents were tested further against clonidine and were found to have α_2-adrenergic antagonism activity since they were able to produce a competitive antagonism of clonidine's action similar to yohimbine.

Antihypertensive Assay. Unaesthetized, 11–16 week old male spontaneously hypertensive rats were fitted with a caudal plethysmograph, and a blood pressure reading was made immediately before administration of compound (ig, 50 mg/kg).

Blood pressure readings were repeated 4 h after administration of the compound. A dose of test compound was rated active if the posttreatment blood pressures of the treated rats were significantly lower ($P \leq 0.05$) than the initial pressure reading. Statistical comparisons were made by using the nonpaired Student's t test.

Supplementary Material Available: Table VII listing spectroscopic data (NMR, IR) on target compounds (22 pages). Ordering information is given on any current masthead page.