(2,3-dihydro-5-methoxy-1*H*-inden-1-amine (9.9 g, 61 mmol) in absolute methanol (25 mL) was added to a hot solution of (*R*)-*N*-acetyl-(3,4-dimethoxyphenyl)alanine (18.1 g, 68 mmol) in absolute methanol (150 mL). The solution was kept at room temperature for 18 h. The crystalline solid obtained was filtered and dried, affording 6.0 g of salt A. The filtrate was concentrated to a total volume of 100 mL and, upon standing, gave 6.25 g of salt B. The filtrate was further concentrated to a volume of 50 mL and, upon standing, 6.5 g of salt C was obtained. Evaporation of solvent from the filtrate afforded 7.4 g of salt D.

Salt A (1.8 g, 42 mmol) was dissolved in 10% NaOH solution (15 mL) and extracted with chloroform (3 × 50 mL). The chloroform extract was washed with water, dried over $MgSO_4$, filtered, and evaporated to yield liquid amine. It was dissolved in 25 mL of ethanol and to this solution, 6-chloropurine ribonucleoside (0.9 g, 32 mmol) and triethylamine (0.48 g, 48 mmol) were added, and the reaction mixture was refluxed for 20 h. Evaporation of ethanol gave solid material which was treated with cold water. The solid was filtered, dried, and taken into methanol (20 mL). The solid material thus obtained was filtered and dried to afford 0.75 g of diastereomer **5** having a melting point of 152–155 °C; $[\alpha]_D$ = −140.2° (*c* = 1.18% DMF). Anal. C, H, N.

Similarly, diastereomer **6** [mp 170–173 °C; $[\alpha]_D$ = −6.73° (*c* = 1.01% DMF)] was obtained from the salt D. Anal. C, H, N.

Preparation of Pure Diastereomers (16 and 17) of *N*-(5-Butoxy-2,3-dihydro-1*H*-inden-1-yl)adenosine. Resolution of 5-butoxy-1-indanylamine. 5-Butoxy-2,3-dihydro-1*H*-inden-1-amine was resolved with commercially available dibenzoyl-L-tartaric acid monohydrate. To a solution of dibenzoyl-L-tartaric acid (9.5 g, 25 mmol) in methanol (100 mL) was added a solution of 5-butoxy-2,3-dihydro-1*H*-inden-1-amine (5.2 g, 25 mmol) on a steam bath. The solution upon cooling gave 7.2 g of sale E. This salt was further recrystallized twice from methanol–water to afford 2.2 g of salt G. The filtrate from the first crystallization was concentrated gradually (five times) to a volume of 50 mL and at each concentration, the salt that crystallized was filtered off. Following final concentration, the filtrate was evaporated to dryness to afford 6.8 g of salt H. In this experiment, the salts were analyzed by HPLC for their diastereomeric purity. Both salts G and H were >97% pure as determined by HPLC.

Salt G was dissolved in 10% NaOH and extracted with chloroform (3 × 75 mL). The extract was dried over $MgSO_4$, filtered, and evaporated, yielding the liquid amine (0.65 g, 31 mmol). It was dissolved in ethanol (20 mL) and to this solution, 6-chloropurine ribonucleoside (0.81 g, 28 mmol) and triethylamine (0.31 g, 31 mmol) were added. The reaction was refluxed for 20 h. The volatiles were removed under reduced pressure, and the residue was treated with cold water. The solid obtained was filtered, dried, and purified by flash column chromatography on silica gel using 5% methanol–chloroform as an eluent. Evaporation of solvent from the pure fractions gave solid material which was crystallized from chloroform–hexane to afford 0.89 g of the diastereomer **16** having a melting point of 126–128 °C: $[\alpha]_D$ = −120.6° (*c* = 1.12% DMF). Anal. C, H, N.

Similarly, diastereomer **17** [mp 148–151 °C, $[\alpha]_D$ = +11.7° (*c* = 1.1% DMF)] was obtained from salt H. Anal. C, H, N.

Pharmacological Method. Compounds were evaluated for effects on blood pressure and heart rate in conscious male, spontaneously hypertensive rats (20–24 weeks old, Charles River Laboratories). Indwelling aortic catheters were implanted for continuous direct measurement of aortic blood pressure and heart rate in free-moving unanesthetized rat as previously described.[17] Rats were housed in individual cages and allowed to recover from surgery for 24–48 h before dosing. Compounds were suspended in 4% gum acacia and administered by gavage in a 2 mL/kg volume. All doses are expressed as the free base.

Blood pressure and heart rate were measured continuously for up to 10 h postdose with a computer-based data-acquisition system. Predrug control values were obtained by averaging the two 30-min intervals obtained before dosing.

Acknowledgment. We thank Mr. J. Fergus and Ms. G. Lu for receptor binding data and Ms. B. Olszewski for pharmacological results.

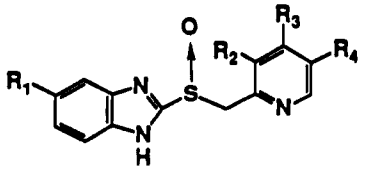
Studies on (H^+-K^+)-ATPase Inhibitors of Gastric Acid Secretion. Prodrugs of 2-[(2-Pyridinylmethyl)sulfinyl]benzimidazole Proton-Pump Inhibitors

John C. Sih,* Wha Bin Im, André Robert, David R. Graber, and David P. Blakeman

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001. *Received February 22, 1990*

The synthesis of N-substituted benzimidazole (H^+-K^+)-ATPase or proton-pump inhibitors is described. These compounds were prepared to function as prodrugs of the parent N–H compound and evaluated for their ability to inhibit gastric (H^+-K^+)-ATPase and gastric acid secretion. The prodrugs reported rely on either in vivo esterase hydrolysis for liberation of the parent compound (type I and type II) or require an acid environment for release of the active drug (type III and type IV). The *N*-(acyloxy)alkyl-substituted benzimidazoles **9**, 11, and **24** showed improved chemical stability in the solid state and in aqueous solutions when compared to their parent N–H compounds. When given orally, **24** was found to be twice as potent as omeprazole in both the Shay rat and inactivation of gastric (H^+-K^+)-ATPase in the rat. The *N*-ethoxy-1-ethyl-substituted benzimidazoles **48–50** were found equally as effective as the N–H compound for inhibition of rat (H^+-K^+)-ATPase activity. In the Shay rat **48** at 10 mg/kg was approximately twice as active as parent timoprazole.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole (1, timoprazole) is a substituted benzimidazole analogue and the prototype of a new class of antisecretory agents which



1, Timoprazole (R_1=R_2=R_3=R_4=H)

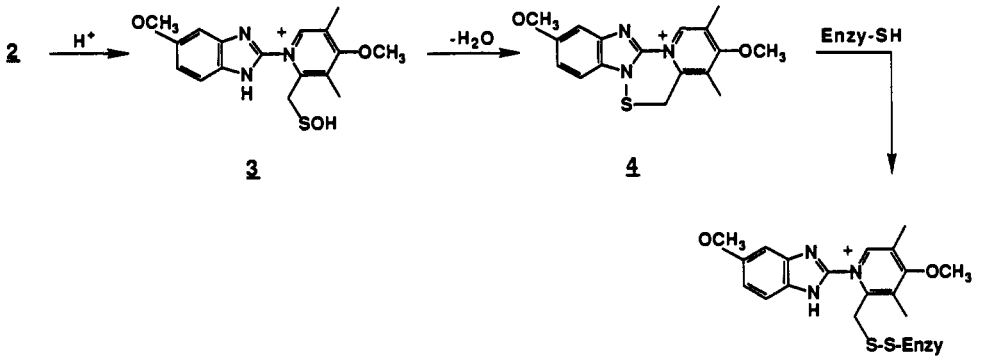
2, Omeprazole (R_1=R_3=OCH_3; R_2=R_4=CH_3)

inhibit gastric ATPase.[1] The proton pump, located in the apical membrane of the parietal cell, is responsible for the secretion of acid in the stomach when stimulated by the enzyme adenosine triphosphatase (ATPase). The substituted benzimidazoles are thought to reduce acid secretion in vivo by selectively blocking the hydrogen/potassium-adenosine triphosphatase enzyme.[2] Omeprazole

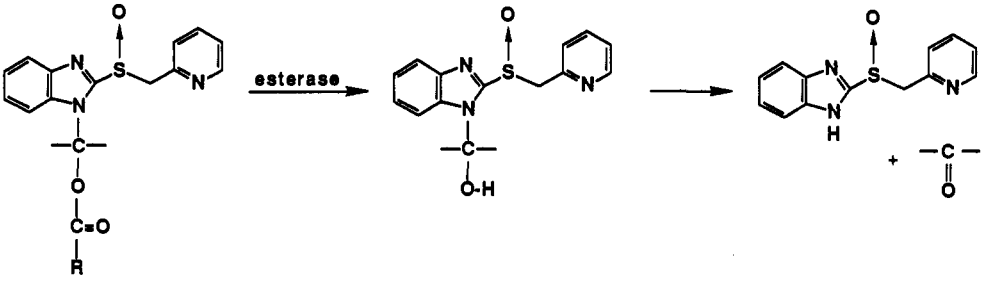
(1) (a) Olbe, L.; Berglindh, T.; Elander, B.; Helander, H.; Fellenius, E.; Sjöstrand, S. E.; Sundell, G.; Wallmark, B. *Scand. J. Gastroenterol.* **1979**, *Suppl. 14* (*55*), 131. (b) Fellenius, E.; Berglindh, T.; Sachs, G.; Olbe, L.; Elander, B.; Sjöstrand, S. E.; Wallmark, B. *Nature (London)* **1981**, *290*, 159. (c) Brändström, A.; Lindberg, P.; Junggren, U. *J. Gastroenterol.* **1985**, *Suppl. 20* (*108*), 15. (d) Brändström, A.; Lindberg, P.; Junggren, U.; Wallmark, B. *Scand. J. Gastroenterol.* **1986**, *Suppl. 21* (*118*), 54.

0022-2623/91/1834-1049$02.50/0

Scheme I



Scheme II



(2), a more highly substituted analogue of 1, has attracted considerable attention as a potential therapeutic drug for the treatment of peptic ulcers and is presently under extensive clinical investigation.[3] Omeprazole itself is not the active inhibitor of the ATPase but is transformed within the acid compartments of the parietal cell into the active inhibitor.[4a,b] The active inhibitor proposed is a cyclic sulfenamide 4. It is believed to arise from dehydration of a highly reactive sulfenic acid intermediate 3 generated from omeprazole via an acid-promoted rearrangement. The inhibitor is believed to react with essential sulfhydryl groups of the ATPase and inactivate the enzyme by formation of a covalent disulfide bond[4a,c] (Scheme I).

The generation and isolation of sulfenic acid 3 and sulfenamide 4 have received much attention and have been previously reported.[4a-c,5] The acid-activation mechanism makes omeprazole an ideal and selective inhibitor of gastric acid secretion in vivo; however, its instability in aqueous media, especially in an acidic environment, poses several problems. The great propensity of this class of compounds to undergo decomposition is evident by the rapid purple coloration of freshly prepared aqueous solutions. At room temperature in the solid state, these compounds have a limited shelf life and are prone to colorize during storage. Omeprazole is effective by the oral route in man only when formulated in enteric coated capsules;[6] otherwise most of the drug is destroyed in the acidic environment of the stomach. The chemical instability and biological activity of omeprazole appear to be associated with the behavior of the N-H substituent of the benzimidazole ring and its transformation via a complex series of reversible acid-catalyzed reactions to sulfenamide 4. We thought derivatization at this position would render omeprazole more chemically stable for storage, handling, and formulation for oral and parenteral delivery and make omeprazole more bioavailable.

This paper describes the synthesis of derivatives of timoprazole where we have converted the N-H group of the benzimidazole to biolabile N-substituted derivatives (prodrugs). The prodrugs described in this report can be classified into two categories. Those in the first class rely on in vivo esterase hydrolysis for liberation of the parent N-H compound (type I and type II); those in the second class require an acid environment for releasing the active drug (type III and type IV). Also reported are in vitro and in vivo antisecretory activities, inhibition of hog (H^+-K^+)-ATPase activities, and comparisons of the activities of the prodrugs to those of the parent N-H compounds.

Prodrug Approach

Type I. In recent years acyloxyalkylation has become a commonly used approach to obtain prodrugs of various amides, imides, hydantoins, uracils, tertiary or N-heterocyclic amines, and other N-H acidic compounds.[7] The usefulness of this approach stems from the fact that by varying the acyl portion of the derivatives, it is possible to control the rate of generation of the parent drug and to obtain prodrugs with varying physicochemical properties such as water solubility or lipophilicity. Whereas the derivatives show good stability in aqueous solution similar to other esters, they are in general rapidly cleaved by virtue of enzyme-mediated hydrolysis. The regeneration of the parent N-H acidic drug takes place via a two-step reaction.

(2) (a) Lorentzon, P.; Eklundh, B.; Brändström, A.; Wallmark, B. *Biochim. Biophys. Acta* **1985**, *25*, 817. (b) Im, W. B.; Blakeman, D. P.; Davis, J. P. *Biochem. Biophys. Res. Commun.* **1985**, *78*, 126.

(3) (a) Gustavsson, S.; Lööf, L.; Adami, H. O.; Nyberg, A.; Nyren, O. *Lancet* **1983**, *2*, 124. (b) Lauritsen, K.; et al. *N. Engl. J. Med.* **1985**, *312*, 958.

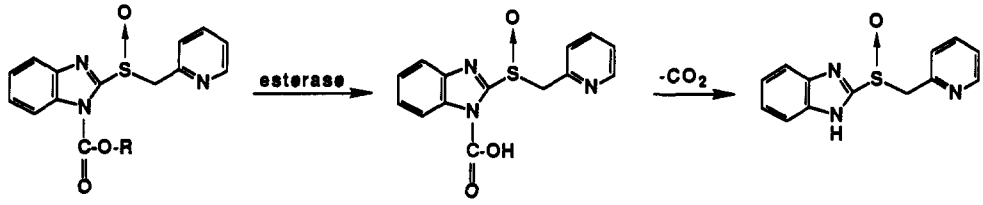
(4) (a) Lindberg, P.; Nordberg, P.; Alminger, T.; Brändström, A.; Wallmark, B. *J. Med. Chem.* **1986**, *29*, 1329. (b) Senn-Bilfinger, J.; et al. *J. Org. Chem.* **1987**, *29*, 4582. (c) Sturm, E.; et al. *J. Org. Chem.* **1987**, *52*, 4573.

(5) For a detailed review of the chemical reactions of omeprazole and omeprazole analogues, See: Brändström, A. et al. *Acta Chem. Scand.* **1989**, *43*, 536 and references to accompany papers cited therein.

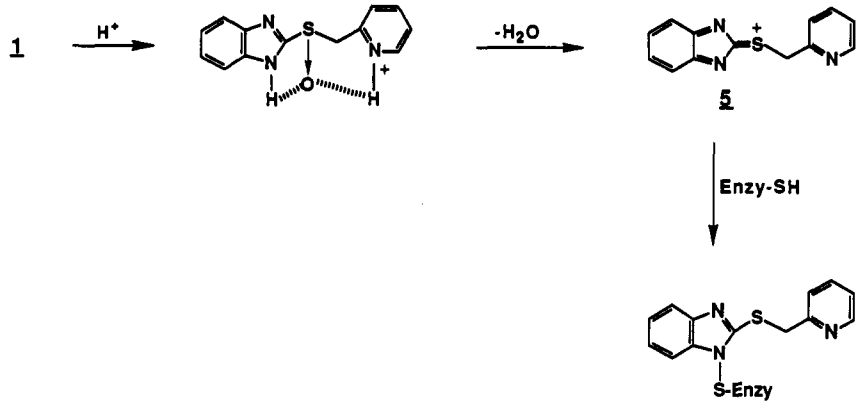
(6) Howden, C. W.; Forrest, J. A. H.; Reid, J. L. *Gut* **1984**, *25*, 707.

(7) (a) Pitman, I. H. *Med. Res. Rev.* **1981**, *1*, 189. (b) Bodor, N. *Drugs Future* **1981**, *6*, 165. (c) Bundgaard, H. In *Optimization of Drug Delivery*; Bundgaard, H.; Hansen, A. B.; Kofod, H., Eds.; Munksgaard: Copenhagen, 1982; p 178. (d) Bundgaard, H. *Adv. Drug Delivery Rev.* **1989**, *3*, 39.

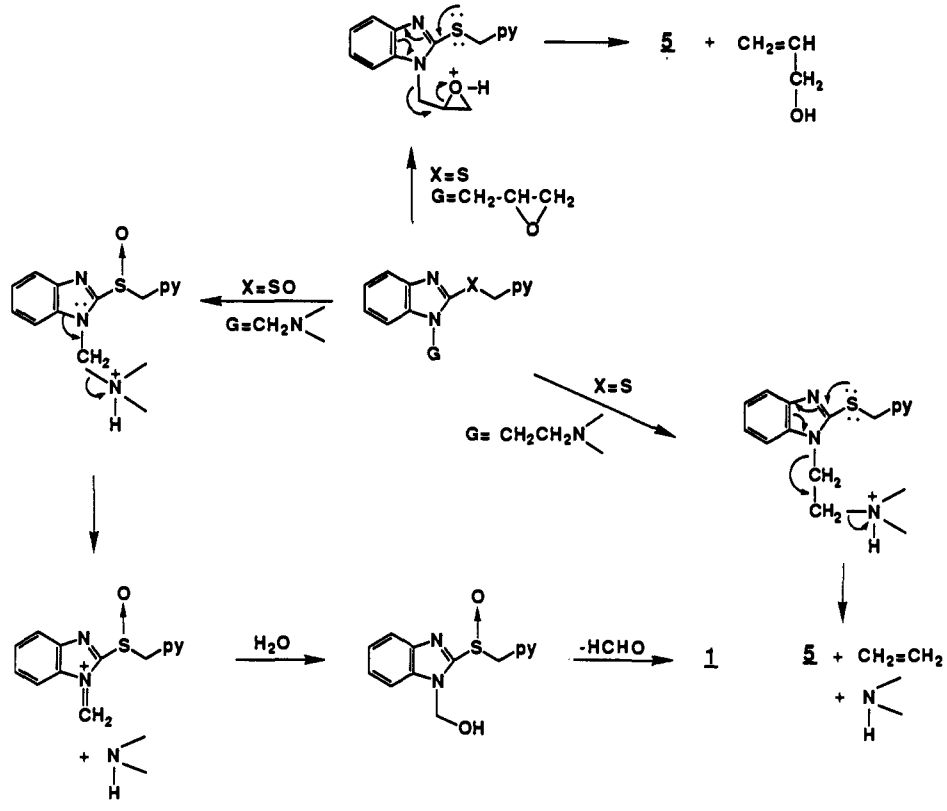
Scheme III



Scheme IV



Scheme V

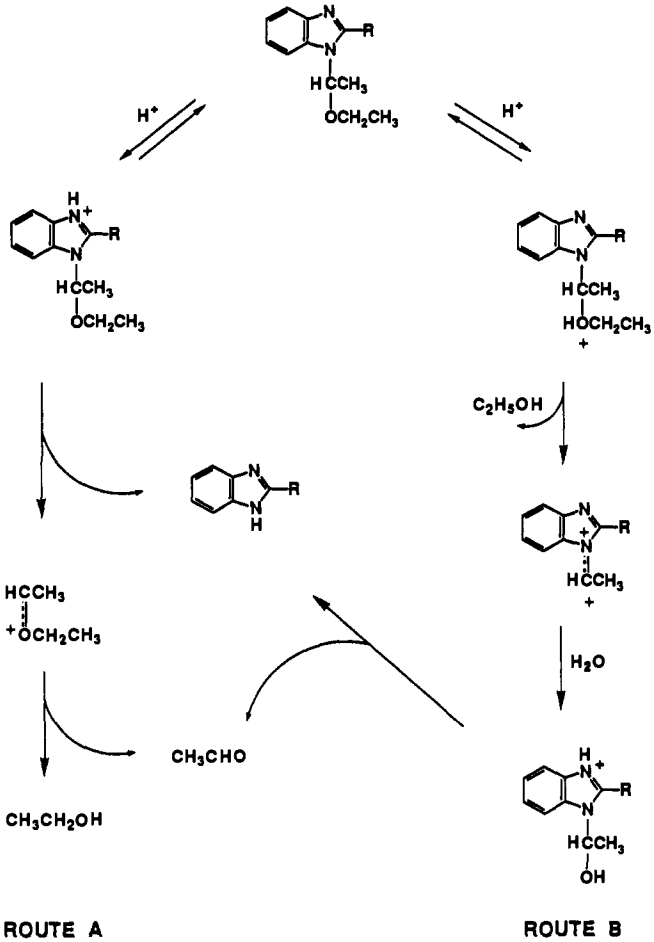


Enzymatic cleavage of the ester grouping results in the formation of an *N*-hydroxyalkyl derivative which subsequently is assumed to decompose rapidly into the corresponding carbonyl compound and the N–H acidic drug (Scheme II). Thus the rate of drug formation is dependent on the rate of the initial ester cleavage, which can be controlled by steric and electronic factors. The *N*-(acyloxy)alkyl derivatives of theophylline[8] and allopurinol[9] have proven to be stable prodrugs with improved aqueous solubility and enhanced transport properties.

Type II. Carbamate prodrugs have been used primarily as transport groups for drugs exhibiting restricted distribution in the body. Although carbamate hydrolysis enzymes per se probably do not exist in vivo, nonspecific amidases and esterases are probably responsible for hydrolysis.[10] Various carbamate derivatives have been assessed as prodrugs for normeperidine, amphetamine, ephedrine, and phenethylamine.[10,11] It was our hope that

(8) Bodor, N.; Sloan, K. B. U.S. Patent 4,061,753, Dec 6, 1977.
(9) Bundgaard, H.; Falch, E. *Int. J. Pharm.* **1985**, *25*, 27.
(10) Verbiscar, A. J.; Abood, L. G. *J. Med. Chem.* **1970**, *13*, 1176.
(11) (a) Kupchan, S. M.; Isenberg, A. C. *J. Med. Chem.* **1967**, *10*, 960. (b) Baker, G. B.; Coutts, R. T.; Nazarali, A. J.; Danielson, T. J.; Rubens, M. *Proc. West. Pharmacol. Soc.* **1984**, *27*, 523.

Scheme VI



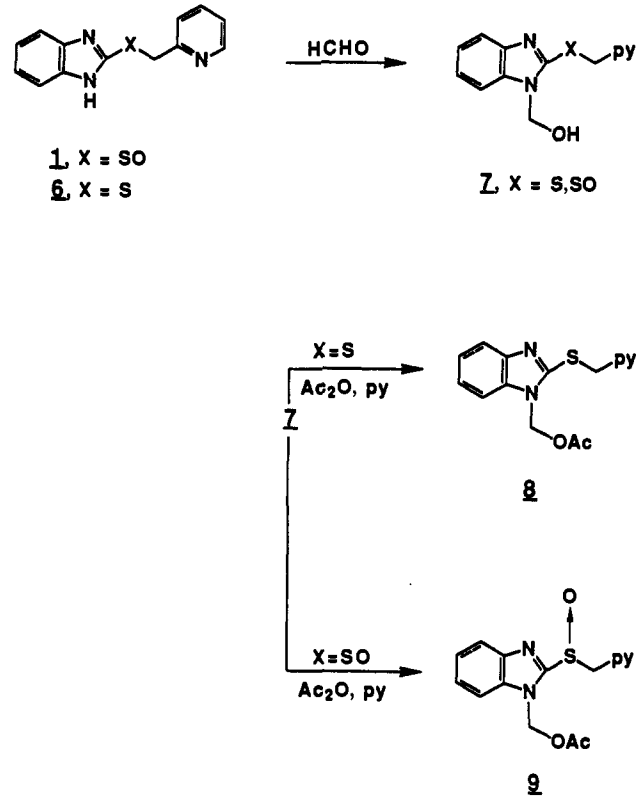
the *N*-aryloxy, *N*-araalkyloxy- and *N*-alkoxycarbonyl derivatives of timoprazole would be susceptible to undergo cleavage by plasma and hepatic enzymes and afford the parent drug (Scheme III).

Type III. When this work was in progress, we considered the isobenzimidazole sulfonium species **5** as a hypothetical unstable intermediate for the active inhibitor produced in acid.[12] The proposed formation of **5** and its mechanism of inhibition is shown in Scheme IV. To generate **5** in vivo from N-substituted derivatives, we considered the three classes of N-substituted derivatives shown in Scheme V, i.e., *N*-epoxymethyl, *N*-aminoethyl, and *N*-aminomethyl. The rationale we conceived for the synthesis of **5** is based on acid activation at a remote nucleophilic site (G) followed by a "push–pull" mechanism involving the sulfur and nitrogen atoms. The driving force behind this process to produce **5** is the loss of a small stable molecule, i.e., allyl alcohol (G = epoxymethyl) or ethylene (G = aminoethyl). In the case when G = aminomethyl, we envisioned a slightly different series of events. Protonation at nitrogen would lead to an ammonium cation, which, after loss of a neutral amine fragment, would afford an iminium ion. In an aqueous milieu, we hoped the iminium ion would rapidly react and give an *N*-hydroxymethyl species, an intermediate previously discussed, which could briskly eliminate formaldehyde to give the parent N–H compound.

Type IV. The 1-(1-alkoxyalkyl)- and 1-[(1-alkoxyalkyl)thio]benzimidazole ether derivatives in this category release the parent N–H compound in an acid environment.

(12) Im, W. B.; Sih, J. C.; Blakeman, D. P.; McGrath, J. P. *J. Biol. Chem.* **1985**, *260*, 4591.

Scheme VII



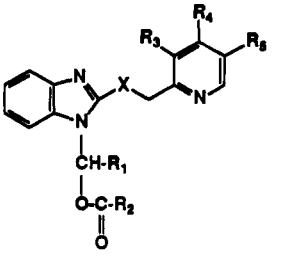
To illustrate the acid-promoted mechanism, Scheme VI shows two pathways formulated for the acid-catalyzed cleavage of the ethoxyethyl ether moiety.[13] In pathway A protonation occurs on the benzimidazole nitrogen. Bond breakage yields the benzimidazole drug and an oxocarbenium ion. Alternatively, in pathway B protonation of the ether oxygen is followed by the loss of ethanol to give a resonance-stabilized carbonium ion, which in turn is further degraded to the benzimidazole drug and acetaldehyde via an amino alcohol intermediate.

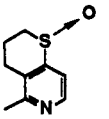
Chemistry

Type I. *N*-[(Acyloxy)methyl]- and *N*-[(Acyloxy)ethyl]benzimidazole Derivatives (Table I). The *N*-[(acyloxy)methyl]benzimidazole derivatives were prepared by treatment of thioether **6** or sulfoxide **1** with aqueous formaldehyde to give an *N*-hydroxymethyl intermediate, **7** (Scheme VII). The *N*-hydroxymethyl thioethers were stable when X = S, but less stable and not isolable when X = SO. The *N*-hydroxymethyl sulfoxide **7** (X = SO) could not be isolated without extensive reconversion to **1**, and as a result was used without isolation in situ for the preparation of ester **9**. In sharp contrast, the *N*-hydroxymethyl thioethers, like **7** (X = S), were, in general, stable crystalline solids. These intermediates were smoothly converted to the final products by first treatment with an acylating agent, e.g., acetic anhydride, and then oxidation of the resulting thioether to the sulfoxide with *m*-chloroperbenzoic acid. However, in some cases we could not use sulfide **6** for the starting material and were forced to employ the parent sulfoxide in the synthesis. For example, oxidation of **10** with *m*-chloroperbenzoic acid afforded exclusively undesired product **12** (Scheme VIII). Similarly, oxidation of sulfide **13** gave **14**. In both these

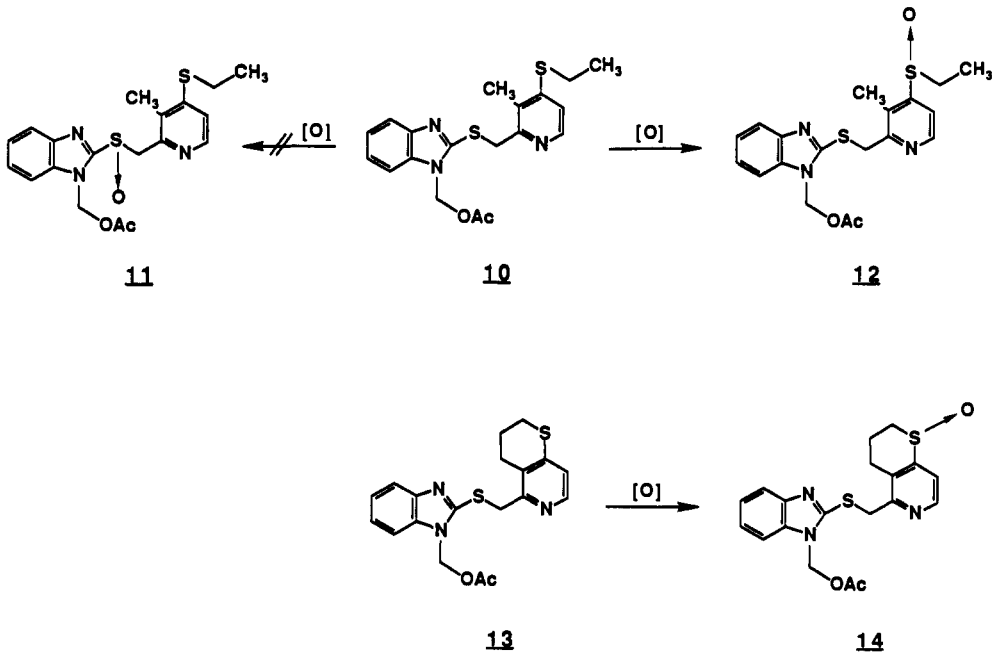
(13) The type IV prodrugs are based on the acid hydrolysis studies of 2-substituted 1-(1-ethoxyethyl)benzimidazoles reported by Lönnberg and Käppi. See: Lönnberg, H.; Käppi, R. *Tetrahedron* **1980**, *36*, 913.

Table I



compd no.	X	R_1	R_2	R_3	R_4	R_5	antisecretory activity, isolated rabbit glands: ID_{50}, M	inhibn of isolated hog (H^+-K^+)-ATPase: IC_{50}, M	inhibn of rat (H^+-K^+)-ATPase: ED_{50}, mg/kg so (os)
9	SO	H	CH_3	H	H	H	1×10^{-4}	8×10^{-6}	5 (5)
11	SO	H	CH_3	CH_3	SC_2H_5	H	3×10^{-7}	4×10^{-4}	2 (15) same duration as omeprazole
14	S	H	CH_3					–	inactive at 10
18a (less polar)	SO	CH_3	CH_3	H	H	H	no effect at 1×10^{-4}	10	
18b (more polar)	SO	CH_3	CH_3	H	H	H	no effect at 1×10^{-4}	10	
19a (less polar)	SO	CH_3	CH_3	CH_3	SC_2H_5	H			2.0
19b (more polar)	SO	CH_3	CH_3	CH_3	SC_2H_5	H			2.0
20	SO	H	$(CH_2)_2CO_2CH_3$	H	H	H	2×10^{-5}		7.0
21	SO	H	$CH_3(CH_2)_6$	H	H	H	2×10^{-5}		9.0
22	SO	H	$(CH_3)_2CHCH_2O$	H	H	H	2×10^{-5}		6.0
23	SO	H	$(CH_3)_2CH$	H	H	H	3×10^{-5}		4.0
24	SO	H	CH_3	CH_3	OCH_3	CH_3	2×10^{-7}	5×10^{-4}	1.0 (5.0)
25	SO	CH_3	CH_3	CH_3	OCH_3	CH_3	4×10^{-7}	1.5×10^{-3}	<1
27	SO	H	Ph	CH_3	SC_2H_5	H	1.5×10^{-6}	no effect at 5×10^{-4}	30% at 10
28	SO	H	*t*-Bu	CH_3	SC_2H_5	H	5×10^{-6}	30% at 5×10^{-4}	5.0
29a (less polar)	SO	CH_3	Ph	H	H	H	4×10^{-5}	4×10^{-4}	27% at 10
29b (more polar)	SO	CH_3	Ph	H	H	H	no effect; 1×10^{-4}		35% at 10
30a (less polar)	SO	CH_3	*t*-Bu	H	H	H	no effect; 1×10^{-4}		32% at 10
30b (more polar)	SO	CH_3	*t*-Bu	H	H	H	2×10^{-5}		40% at 10

Scheme VIII

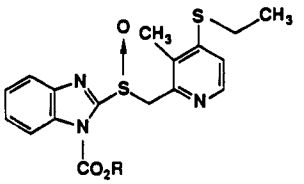


11 **10** **12**

13 **14**

reactions oxidation occurred at the sterically less hindered sulfur. Thus, 11 had to be prepared directly from sulfoxide **30** (disuprazole), albeit in poor yield. The prodrug acyl esters prepared were chemically more stable than their corresponding parent N–H compounds as evidenced by the lack of coloration in solutions of these prodrugs when kept in protic solvents. In most instances, the prodrugs were highly crystalline, sharp-melting solids. As previously discussed, the *N*-acetoxymethyl derivatives are converted to their parent compounds in vivo by esterase hydrolysis followed by loss of formaldehyde. To avoid the liberation of formaldehyde in the body, we synthesized some *N*-(1-acetoxyethyl)benzimidazole derivatives. With this class of compounds, in vivo esterase hydrolysis would yield a labile *N*-(1-hydroxyethyl)benzimidazole intermediate. Loss of acetaldehyde, a more innocuous molecule than form-

Table II

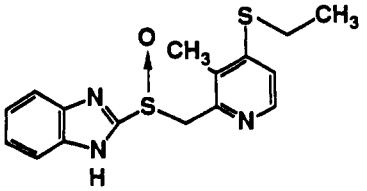


compd no.	R	antisecretory activity, isolated rabbit glands; ID_{50}, M	inhibn of isolated hog (H^+-K^+)-ATPase: IC_{50}, M	inhibn of rat (H^+-K^+)-ATPase, ED_{50}, mg/kg sc
31	C_2H_5	2×10^{-6}	2×10^{-5}	61% at 10
32	$CH_2CH(CH_3)_2$	4×10^{-6}	5×10^{-6}	5
33	CH_2Ph	1.5×10^{-6}	2×10^{-5}	40% at 10
34	Ph	1.5×10^{-6}	2×10^{-5}	5
35	$CH_2C_6H_4$-p-NO_2	1.5×10^{-6}	2×10^{-5}	44% at 10
36	$CH{=}CH_2$	1.5×10^{-6}	3×10^{-5}	10
37	$CH(CH_3)_2$	1.5×10^{-5}		10
38	$(CH_2)_7CH_3$	1×10^{-5}		25% at 10
39	CH_2CH_2Cl	2×10^{-6}	1×10^{-5}	40% at 10
40	$C(Me)_2CCl_3$	5×10^{-5}	1×10^{-5}	no effect at 10

aldehyde, would again lead to the parent N–H compound.

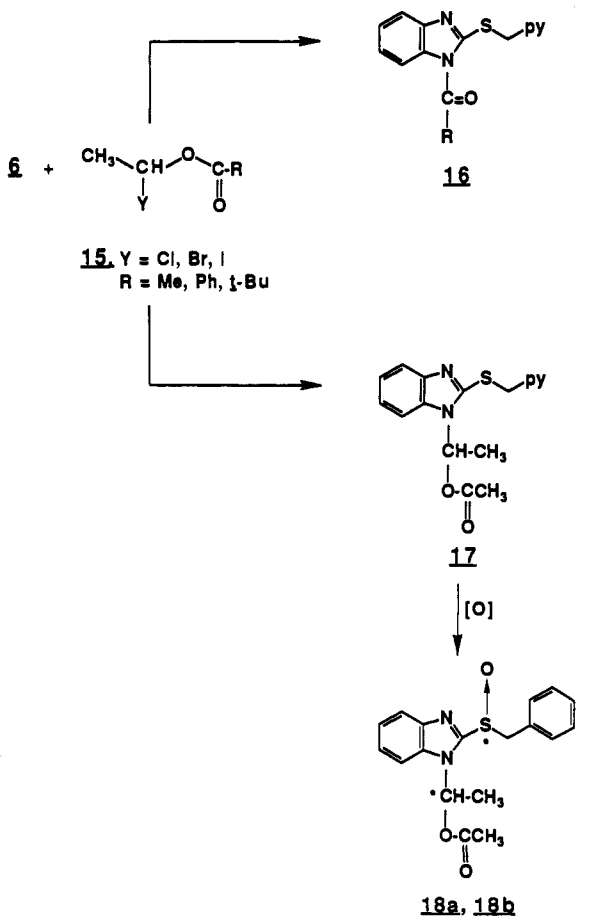
The preparation of the *N*-(1-hydroxyethyl) derivatives required the synthesis of α-haloalkyl esters **15**[14] for use as alkylating agents in the reaction with benzimidazole thioether **6** (Scheme IX). The low yields obtained in these reactions can be attributed in part to a side reaction, i.e., nucleophilic attack of the benzimidazole nitrogen at the acyl carbon of **15** to yield the N-acylated compound **16**. This undesired product became more prevalent with the poorer X = Cl leaving group than with X = Br. However, we were not able in every instance to prepare the more reactive, but less stable, bromo reagent due to difficulties in handling and storage. A partial solution to this problem was to employ the choro reagent and add sodium iodide to generate the iodo reagent in situ via exchange of chloride for iodide. While this method worked satisfactorily in some cases, it was not always reproducible. The best condition found for optimizing the yield of **17** was to simply warm **6** and **15** (X = Br, R = Me) in acetonitrile at 60 °C for 24 h. This method provided **17** in 41% yield. The oxidation of benzimidazole thioether **17** to the sulfoxide final products were again carried out with *m*-chloroperbenzoic acid. Due to the creation of a new chiral sulfoxide functionality, the final products were obtained as a mixture of diastereomers. The diastereomers were readily separated by silica gel chromatography, and we assigned **18a** to the "less polar" and **18b** to the "more polar" isomer.

Oxidation of **10** occurred exclusively at the undesired sulfur attached to the pyridine ring. In light of this result, we anticipated the same problem for securing **19** by oxidation of its corresponding thioether. A solution to this problem was realized when we discovered that treatment of disuprazole (**26**) directly with α-bromoethyl acetate and



26, DISUPRAZOLE

Scheme IX

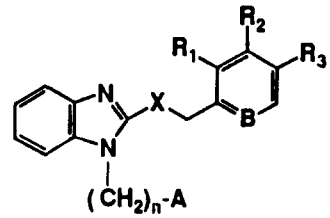


potassium carbonate as the base in acetonitrile provided in 29% yield a mixture of chromatographically separable diastereomers, **19a** (less polar isomer) and **19b** (more polar isomer).

Type II. *N*-(Carbalkoxy)-, *N*-(Carbaryloxy)-, and *N*-(Carbobenzyloxy)benzimidazole Derivatives (Table II). The synthesis of the carbamate esters was accomplished in a straightforward manner. At the time this work was in progress, disuprazole (**26**) was a lead candidate selected for further development as an antiulcer drug. With the hope of improving its chemical and metabolic stability, disuprazole was selected as the model compound

(14) Ulich, L. H.; Adams, R. *J. Am. Chem. Soc.* **1921**, *43*, 660. French, H. E.; Adams, R. *J. Am. Chem. Soc.* **1921**, *43*, 651.

Table III



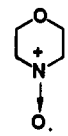
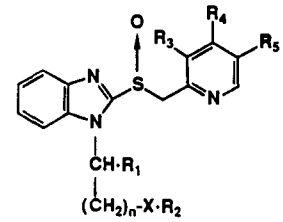
compd no.	n	A	X	B	R_1	R_2	R_3	antisecretory activity, isolated rabbits glands: ID_{50}, M	inhibn of isolated hog (H^+-K^+)-ATPase: IC_{50}, M	inhibn of rat (H^+-K^+)-ATPase: ED_{50}, mg/kg sc
41	2		S	N	H	H	H	inactive 1×10^{-4}		inactive at 10
42	1		S	N	H	H	H	inactive 1×10^{-4}		inactive at 10
43	1		S	N	Me	SEt	H	8×10^{-5}		inactive at 10
44	1		S	N	H	H	H	inactive 1×10^{-4}		inactive at 10
45	2		SO	N	Me	OMe	Me	7×10^{-5}	1×10^{-3}	inactive at 10
46	2		SO	N	Me	SEt	H	2×10^{-5}	3×10^{-5}	inactive at 10
47	2		SO	N	H	H	H	inactive 1×10^{-4}		

Table IV



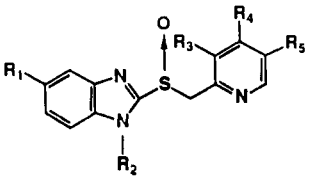
compd no.	n	X	R_1	R_2	R_3	R_4	R_5	antisecretory activity, isolated rabbit glands: ID_{50}, M	inhibn of isolated hog (H^+-K^+)-ATPase: IC_{50}, M	inhibn of rat (H^+-K^+)-ATPase: ED_{50} mg/kg sc (os)
48	0	O	Me	C_2H_5	H	H	H	inactive	1 mM	5
49	0	O	Me	C_2H_5	Me	OMe	Me	1×10^{-6}	1.5×10^{-3}	1 (5)
50	0	O	Me	C_2H_5	Me	SEt	H	5×10^{-7}	2×10^{-4}	1.5 (10)
51	0	S	H	Me	Me	SEt	H	9×10^{-6}	no effect	inactive at 10
52	0	S	H	Ph	Me	SEt	H	8×10^{-6}	$>1 \times 10^{-3}$	inactive to 10
53	1	O	H	C_2H_5	Me	SEt	H	2×10^{-6}	1×10^{-3}	inactive at 10
54	0	O	H	Me	Me	SEt	H	no effect		inactive at 10
55	0	O	H	$(CH_2)_2OMe$	Me	SEt	H	1×10^{-5}		inactive at 10

for evaluating carbamate esters as prodrugs. The parent N–H compound was treated in dimethylformamide (DMF) with sodium hydride, and then reacted with appropriate and commercially available chloroformate reagents. This method afforded the desired esters in good yield (75–90%).

Type III. *N*-(Epoxymethyl)-, *N*-(Aminoethyl)-, and *N*-(Aminomethyl)benzimidazole Derivatives (Table III). The morpholinylmethyl compound was prepared according to the literature procedure described by Collino and Volpe.[15] The synthesis of the morpholinylethyl and piperidinylethyl compounds was accomplished by direct alkylation by first generating the anion of benzimidazole with sodium hydride in DMF and then reaction with (β-chloroethyl)morpholine or (β-chloroethyl)piperidine, respectively. The corresponding sulfoxides were prepared

(15) Collino, F.; Volpe, S. *Boll. Chim. Farm.* **1982**, *121* (*4*), 167.

Table V



compound	antisecretory activity, isolated rabbit glands: ID_{50}, M	inhibn of isolated hog (H^+-K^+)-ATPase: IC_{50}, M	inhibn of rat (H^+-K^+)-ATPase: ED_{50}, mg/kg sc (os)	inhibn of rat acid secretion: ED_{50}, mg/kg sc (os)
timoprazole (1) $R_1 = R_2 = R_3 = R_4 = R_5 = H$	2×10^{-5}	27% at 3×10^{-3}	4.0 (15)	2.5 (5.0)
N-methyltimoprazole $R_2 = Me$; $R_1 = R_3 = R_4 = R_5 = H$	1×10^{-3}	18% at 3×10^{-3}	inactive 10.0	not available
omeprazole (2) $R_1 = R_4 = OMe$; $R_2 = H$; $R_3 = R_5 = Me$	2×10^{-7}	5×10^{-5}	1.0 (10)	<1.0 (2.5)
5-desmethoxyomeprazole $R_1 = R_2 = H$; $R_3 = R_5 = Me$; $R_4 = OMe$	2×10^{-7}	2×10^{-5}	not available	not available
disuprazole (26) $R_1 = R_2 = R_5 = H$; $R_3 = Me$; $R_4 = SEt$	1×10^{-7}	2×10^{-5}	1.0 (15)	<1.0 (2.0–5.0)

in the same manner except the alkylation was carried out directly on the sulfoxide starting material. Epoxide **43** was prepared in a similar manner employing epibromohydrin as the alkylating agent.

Type IV. *N*-(Alkoxyalkyl)- and *N*-[(Alkoxyalkyl)thio]benzimidazole Derivatives (Table IV). The prodrugs in this group were prepared in a straightforward manner. The benzimidazole sulfoxide was treated in DMF with sodium hydride and the appropriate alkylating agent. In other instances the benzimidazide thioether was used as the starting material. Alkylation followed by oxidation afforded the desired product.

Biological Activities

The compounds reported in this study (Tables I–IV) were evaluated for their antisecretory effect in isolated rabbit gastric glands as measured by (^{14}C)aminopyrine uptake,[16] examined for their inhibitory effect in vitro on hog gastric (H^+-K^+)-ATPase,[17] and examined for inhibition of gastric (H^+-K^+)-ATPase activity in the rat. The in vivo antisecretory activity of selected compounds was determined in the pylorous ligated rat by the method of Shay.[18] For the reader's convenience and discussion purposes, the activities of the parent N–H benzimidazole compounds, timoprazole (1), omeprazole (2), disuprazole (26), *N*-methyltimoprazole,[19] and 5-desmethoxyomeprazole[19] are shown in Table V.

Prodrugs Type I (Table I). The *N*-hydroxymethyl and *N*-hydroxyethyl ester derivatives prepared in this study were chemically more stable in the solid and liquid (oil) state and in solution than their corresponding N–H compounds. In order to compare the chemical stability of omeprazole and **11**, we followed by UV the decomposition of the drugs (10 μM) in 0.1 N HCl. We have previously reported[12] that omeprazole is unstable in an acidic environment and interacts with H^+ ions to produce a species[21] with an absorption maxima at 357 nm. Omeprazole was converted about 6 times faster than **11**, i.e., the initial rate of absorbance increase at 357 nm was 0.260/min for omeprazole and 0.045/min for **11**. Unlike the parent N–H benzimidazoles, aqueous ethanolic solutions of **9**, **11**, and **24** could be kept for several weeks at room temperature without any coloration. Improvement in aqueous stability with the prodrug may be potentially useful in formation of the drug for parenteral delivery. In most instances the prodrugs with *N*-acetoxymethyl and other acyl substituents, when administered subcutaneously (sc) in the rat, inhibited gastric (H^+-K^+)-ATPase with comparable potency to their parent compounds. For example, **9**, **11**, and **24** had ED_{50} values of 5.0, 2.0, and 1.0 mg/kg in the rat, which were almost identical with that of timoprazole, disuprazole, and omeprazole, respectively. With the in vitro assay using hog gastric (H^+-K^+)-ATPase, however, **11** and **24** were about 10–20 times less potent than their parent drugs, i.e., the IC_{50} values were 4–5 $\times$ 10^{-4} M for the prodrugs and 5×10^{-5} and 2×10^{-5} M for omeprazole and disuprazole, respectively. The antisecretory activities of **9**, **11**, and **24** in isolated rabbit gastric glands were similar to that of their parent compounds. These observations suggest that the isolated glands were able to convert these prodrugs to their parent N–H compounds. Conversion of these labile N-substituted derivatives in the glands to the N–H compounds was also consistent with the observation that replacement of the benzimidazole hydrogen of timoprazole with a stable methyl substituent to give the *N*-methyltimoprazole derivative resulted in loss of antisecretory activity.

When **9** was compared to timoprazole in a dose–response manner for their ability to inhibit (H^+-K^+)-ATPase in the rat, **9** was found to be equally potent whether given orally or subcutaneously at 5.0, 10.0 and 15.0 mg/kg. On the other hand, oral administration of timoprazole at low doses (less than 10 mg/kg) appeared not to be as effective as the corresponding subcutaneous injection of the compound. These data suggest that a certain portion of orally administered timoprazole was inactivated during its passage through the acidic environments of the stomach.

The *N*-acyl prodrugs **9** and **25** were tested orally for antisecretory activity in the Shay rat model and were found to be approximately equal in potency to omeprazole. In-

(16) Berglindh, T.; Obrink, K. J. *Acta Physiol. Scand.* **1976**, *96*, 150.
(17) Tsai, C. M.; Chen, K. Y.; Canellakis, E. S. *Biochim. Biophys. Acta* **1975**, *401*, 196.
(18) Shay, H.; Sun, D. C. H.; Gruenstein, M. *Gastroenterology* **1954**, *26*, 906.
(19) These data have not been published yet.
(20) For preparation of compound see Sih, J. C., U.S. Patents 4,619,997 and 4,565,554.
(21) (a) Rackur, G.; et al. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 477. (b) Brändström, A.; et al. *Acta Chem. Scand.* **1989**, *43*, 536.

terestingly, **24** when given orally was found to be twice as potent as omeprazole in both the Shay rat and inactivation of gastric (H^+-K^+)-ATPase in the rat. This again suggests that the prodrug was able to resist better the acid milieu of the stomach.

As was noted earlier in the discussion, **9**, **11**, and **24**, *N*-acetoxymethyl derivatives, are converted to their parent compounds in vivo by esterase hydrolysis followed by the loss of formaldehyde. We were concerned that the liberation of formaldehyde from these derivatives would make these compounds potential mutagens. When **9**, **11**, and **24** were tested in the Ames assay using TA 98 and TA 100 and both strains, the compounds were found to be weakly positive with metabolic activation. We were pleased, therefore that **18b**, the N-acetoxy ethan-2-ol derivative, which in vivo is hydrolyzed and liberates acetaldehyde, showed no mutagenicity in the Ames assay.

Prodrugs Type II (Table II). The *N*-carbalkoxy, *N*-carbaryloxy, and *N*-carbobenzyloxy ester prodrugs of disuprazole shown in Table II were high-melting crystalline solids which possessed greater chemical stability in the solid state and in solution than the parent compound. The *N*-substituted esters were generally active in vivo for inhibition of rat (H^+-K^+)-ATPase (ED_{50} 5–10 mg/kg sc), but 5–10 times less potent than disuprazole. The activity of these prodrugs was not greatly affected by changing the R group of the ester (CO_2R) from alkyl (**31** and **32**) to aryl (**34**). On the other hand, the more lypophilic esters **38** and **40** were less active than the shorter chain esters **31**, **32**, and **37**.

Prodrugs Type III (Table III). The derivatives prepared in Table III had no antisecretory activity in isolated rabbit gastric glands and were found to be inactive when tested subcutaneously at 10 mg/kg for inhibition of (H^+-K^+)-ATPase in the rat. During the course of the work, we discovered that the *N*-morpholinylethyl derivative **41**, the *N*-morpholinylmethyl derivative **42**, and epoxide **43** in solution were stable when exposed in the laboratory to 0.1 N HCl for several hours and could be recovered unchanged. Therefore, the acid-catalyzed process we proposed (Scheme V) for the breakdown of these derivatives to generate our suspected active metabolite **5** was not observed.

Prodrugs Type IV (Table IV). *N*-Ethoxy-1-ethyl prodrugs **48–50** were equally as effective as timoprazole, omeprazole, and disuprazole for inhibition of (H^+-K^+)-ATPase activity in the rat. It is noteworthy that **54**, the *N*-methoxymethyl derivative, was inactive compared to **50**. On the basis of mechanistic considerations, protonation and cleavages of **54** to the parent N–H compound would be less favored than with **50**, since the carbonium ion formed will be less stable than that produced from **50**. As would be expected of prodrugs, **48–50** in vitro were found to be 10–20 times lower in potency than their parent compounds to inhibit hog (H^+-K^+)-ATPase activity. In the Shay rat, **49** and **50** when tested orally at 10.0, 17.5, and 25.0 mg/kg were found to be as active as omeprazole and disuprazole. On the other hand, **48** at 10 mg/kg was approximately twice as active as timoprazole.

In summary, we have prepared some N-substituted benzimidazole (H^+-K^+)-ATPase or proton-pump inhibitors which were equipotent and sometimes slightly better than the parent N–H compounds for inhibition of acid secretion and inhibition of rat (H^+-K^+)-ATPase activity. As would be expected of prodrugs, the potency of these compounds to inhibit (H^+-K^+)-ATPase in vitro was 10–20 times lower than that of the unsubstituted N–H compounds. The N-substituted benzimidazole derivatives showed improved chemical stability in the solid state and in aqueous solutions when compared to their parent N–H compounds. In terms of ease in handling, storage, and formulation for oral and parenteral delivery, N-substituted derivatives may offer practical advantages in the future drug development of benzimidazole proton-pump inhibitors. Significant improvement of oral efficacy with the prodrugs in humans cannot be easily inferred from the data. However, the extent of decomposition in the lumen should be reduced with the N-substituted derivatives.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were recorded at 90 Hz with a Varian Associates EM-390 and at 60 Hz on a Varian A-60D spectrometer. Chemical shifts are reported in δ (parts per million) relative to internal tetramethylsilane. Unless otherwise indicated, all samples were prepared in deuteriochloroform ($CDCl_3$). High-resolution mass spectra were obtained with a Varian MAT-CH5 spectrometer using the fast atom bombardment (FABMS) technique. In the FAB mode mass spectral data is reported as the positive-ion parent [$(M + H)^+$]. Infrared spectra and combustion analyses were obtained by the Physical and Analytical Chemistry Department of the Upjohn Co.

Chromatography refers to column chromatography using E. Merck silica gel 60 (70–230 mesh). LPLC refers to low-pressure liquid chromatography employing either E. Merck prepacked silica gel Lobar columns or Michel-Miller columns (Ace Glass, Inc.) packed with silica gel 60 (40–62 mesh). The solvents were driven by a Milton-Roy D pump. All solvents for chromatography were Burdick and Jackson reagent grade distilled in glass. Thin-layer chromatography (TLC) was conducted with Analtech 0.25-mm plates precoated with silica gel GF.

Workup in the usual manner refers to dilution of the reaction with water, extraction of the aqueous phase with chloroform or ether, washing of the organic layer with water, aqueous sodium bicarbonate solution, water, saturated brine, drying of the chloroform or ether extract with anhydrous sodium sulfate, and removal of the solvent under vacuum in a 40–45 °C water bath.

The thioethers reported in this study were oxidized to the corresponding sulfinyl compounds (sulfoxides) with 80% *m*-chloroperbenzoic acid (Aldrich Chemical Co.). Sodium hydride 60% oil dispersion was purchased from Alfa Chemical Co. Dimethylformamide (DMF) and tetrahydrofuran (THF) solvents used in the reactions were Burdick and Jackson reagent grade and stored over molecular sieves. The free base of (β-chloroethyl)morpholine and (β-chloroethyl)piperidine hydrochloride salts was obtained by washing the salts from methylene chloride with aqueous base and the solvent removed in vacuo.

For the preparation of **1**, **6**, **26**, and other compounds not included in the Experimental Section, procedures can be found in ref 20.

2-[(2-Pyridinylmethyl)thio]-1*H*-benzimidazole-1-methanol (7, X = S). To a solution of 10.00 g (41.45 mmol) of **6**[13] in 220 mL of acetonitrile was added 5.00 mL of 37% formaldehyde solution dissolved in 10 mL of acetonitrile. After addition, the mixture was heated in an oil bath maintained at 70 °C for 15 min. At the end of this time, the major portion of the solvent was removed in vacuo, the residue diluted with chloroform, and the reaction worked up in the usual manner. Removal of the solvent in vacuo gave the crude product which was recrystallized from ethyl acetate–ether–Skellysolve B to give 9.68 g of **7** (X = S): mp 80–83 °C from ethyl acetate–ether; ^1H NMR 8.50, 7.80–7.10, 5.70, 4.40. Anal. ($C_{14}H_{13}N_3OS$) C, H, N, S.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]-1*H*-benzimidazole-1-methanol (7a). Following the same procedure described above, 1.63 g (5.45 mmol) of 2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]benzimidazole[13] afforded 1.44 g of **7a**: mp 162–165 °C from ethyl acetate–Skellysolve B; ^1H NMR 8.00, 7.80–7.20, 5.80, 4.50, 3.80, 2.35, 2.20. Anal. ($C_{17}H_{19}N_3O_2S$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]thio]-1*H*-benzimidazole-1-methanol (7b). Following the same procedure described above, 2.00 g (6.35 mmol) of 2-[[[4-(ethylthio)-3-

methyl-2-pyridinyl]methyl]thio]benzimidazole[20] afforded 1.86 g of a tan solid, which by ^{1}H NMR indicated a 80:20 mixture of **7b** and starting material. This mixture was not separable by silica gel chromatography and therefore was used in subsequent reactions without further purification: ^{1}H NMR 8.10 (d, 1 H, J = 5 Hz), 7.70–6.90 (m, 5 H), 5.70 (s, 2 H), 4.52 (s, 2 H), 2.85 (q, 2 H, J = 7 Hz), 2.25 (s, 3 H), 1.35 (t, 3 H, J = 7 Hz).

2-[[(3,4-Dihydro-2*H*-thieno[2,3-*c*]pyridinyl)methyl]thio]-1*H*-benzimidazole-1-methanol (7c). Following the same procedure described above, 0.300 g (0.96 mmol) of 2-[[(3,4-dihydro-2*H*-thieno[2,3-*c*]pyridinyl)methyl]thio]benzimidazole[13] afforded 0.322 g of **7c** as a light pink solid, which was used in subsequent reactions without further purification. ^{1}H NMR indicated the crude product contained 20% starting material: ^{1}H NMR 7.95, 7.85–6.90, 5.80, 4.50, 2.90, 2.10.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-methanol Acetate (9). To a magnetically stirred solution of **7** (X = S, 5.00 g, 18.45 mmol) in 20 mL of pyridine was added 7 mL of acetic anhydride and 100 mg of 4-(*N*,*N*-dimethylamino)-pyridine. Stirring was continued at room temperature for 1.5 h. At the end of this period, the solution was poured onto crushed ice and water and worked up in the usual manner to yield 5.30 g of acetate ester **8** as a viscous oil: FABMS calcd for $C_{16}H_{16}N_3O_2S$ 314.0963, found 314.0940; ^{1}H NMR 8.60, 7.80–7.13, 6.10, 4.80, 2.03. The crude ester was dissolved in 100 mL of chloroform, cooled in a 0–5 °C bath, and treated in several portions with 3.98 g (18.45 mmol) of *m*-chloroperbenzoic acid. Stirring was continued at 0–5 °C for 15 min. At the end of this period, the reaction was diluted with 300 mL of additional chloroform and worked up in the usual way. Concentration of the solvent in vacuo afforded a viscous brown oil. The crude product was chromatographed on 400 g of silica gel and eluted with ethyl acetate to afford 5.34 g of **9**: mp 99–100 °C from ethyl acetate–ether; ^{1}H NMR 8.70 (m, 1 H), 7.90–7.20 (m, 7 H), 6.45 (s, 2 H), 4.95 (s, 2 H), 2.07 (s, 3 H). Anal. ($C_{16}H_{15}N_3O_3S$) C, H, N, S.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole-1-methanol Acetate (24). Following the acetylation conditions previously described, 1.30 g (3.95 mmol) of **7a** afforded 1.29 g of the crude acetate as a white solid: FABMS calcd for $C_{19}H_{22}N_3O_3S$ 372.1382, found 372.1379; ^{1}H NMR 8.27, 7.90–7.20, 6.15, 4.85, 3.80, 3.32, 2.20, 2.02. The crude thioether product (1.22 g, 3.29 mmol) was oxidized with *m*-chloroperbenzoic acid in the same manner as previously described to give, after chromatography (100 g of silica gel, acetone–Skellysolve B 4:1) 0.637 g of **24**, mp 131–133 °C from ethyl acetate–ether; ^{1}H NMR 8.20 (s, 1 H), 7.90–7.30 (m, 4 H), 6.54 (s, 2 H), 5.00 (s, 2 H), 3.70 (s, 3 H), 2.30 (s, 3 H), 2.20 (s, 3 H), 2.10 (s, 3 H). Anal. ($C_{19}H_{21}N_3O_4S$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]thio]-1*H*-benzimidazole-1-methanol Acetate (10). To a magnetically stirred solution of **7b** (1.86 g, containing approximately 15–20% of the N–H compound) in 12 mL of pyridine was added 4 mL of acetic anhydride and 20 mg of 4-(*N*,*N*-dimethylamino)pyridine. Stirring was continued at room temperature for 24 h. At the end of this period, the reaction contents were poured onto water–crushed ice and the resulting solid which appeared was filtered, washed with ether–Skellysolve B, and air-dried to give 1.55 g of **10** as a fluffy white crystalline solid: mp <50 °C; ^{1}H NMR 8.32 (d, 1 H, J = 5 Hz), 7.95–7.20 (m, 4 H), 7.05 (d, 1 H, J = 5 Hz), 6.10 (s, 2 H), 4.80 (s, 2 H), 2.95 (q, 2 H, J = 7 Hz), 2.30 (s, 3 H), 2.03 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{18}H_{19}N_3O_2S_2$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-methanol Acetate (11). To a magnetically stirred suspension of disuprazole (**26**; 1.00 g, 3.02 mmol) in 55 mL of acetonitrile was added in one portion 1.13 mL of 37% formaldehyde solution and the mixture heated in a 65 °C oil bath for 10 min. At the end of this time the acetonitrile was removed in vacuo and the residue treated with 25 mL of pyridine, 8 mL of acetic anhydride and 150 mg of 4-(*N*,*N*-dimethylamino)pyridine. Stirring was continued at room temperature for 1.5 h. Workup in the usual manner and chromatography of the crude product (200 g of silica gel, ethyl acetate) afforded 0.768 g of **11**: mp 132–133 °C from ethyl acetate–ether; ^{1}H NMR 8.30 (d, 1 H, J = 4 Hz), 8.00–7.30 (m, 4 H), 7.05 (d, 1 H, J = 4 Hz), 6.55 (s, 2 H), 5.05 (s, 2 H), 3.00 (q, 2 H, J = 7 Hz), 2.30 (s, 3 H), 2.10 (s, 3 H), 1.40 (t, 3 H), J = 7 Hz). Anal. ($C_{19}H_{21}N_3O_3S_2$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-methanol 4-Methoxy-4-oxobutanoate (20). To a magnetically stirred solution of **7** (X = S, 1.08 g, 4.00 mmol) in 12 mL of pyridine was added 0.600 g (6.00 mmol) of succinic anhydride and 10–15 mg of 4-(*N*,*N*-dimethylamino)pyridine. Stirring was continued at room temperature for 17 h. At the end of this time, the reaction was worked up with chloroform in the usual manner. The residual oil was dissolved in methanol–ether (5:1) and treated with ethereal diazomethane. The solvents were then removed in vacuo, and the crude product was chromatographed (100 g of silica gel, ethyl acetate) to afford 1.42 g of the succinyl methyl ester as a colorless viscous oil: ^{1}H NMR 8.55, 7.75–7.20, 6.08, 4.85, 3.55, 2.55. The ester was oxidized as previously described with *m*-chloroperbenzoic acid to afford, after chromatography (100 g of silica gel, ethyl acetate), 0.465 g of **20**: mp 114–115 °C from Skellysolve B; FABMS calcd for $C_{19}H_{19}N_3O_5S$ 402.1124, found 402.1130; ^{1}H NMR 8.65 (m, 1 H), 8.10–7.20 (m, 7 H), 6.50 (s, 2 H), 5.00 (s, 2 H), 3.55 (s, 3 H), 2.50 (br s, 4 H). Anal. ($C_{19}H_{19}N_3O_5S$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-methanol Octanoate (21). To a magnetically stirred solution of octanoic acid (0.864 g, 6.00 mmol) in 50 mL of acetone was added triethylamine (1.25 mL, 9.00 mmol) and isobutyl chloroformate (1.16 mL, 9.00 mmol). The contents were stirred at room temperature for 15 min. At the end of this time, **7** (X = S, 1.084 g, 4.00 mmol), dissolved in 10 mL of pyridine, was added and the reaction stirred at room temperature for 1.5 h. The reaction was then diluted with 300 mL of chloroform and worked up in the usual way to yield the crude product, which was chromatographed (100 g of silica gel, ethyl acetate–Skellysolve B, 1:1) to afford 0.838 g of the octyl ester as a viscous oil. The product was oxidized with *m*-chloroperbenzoic acid in the usual manner to afford, after chromatography (100 g of silica gel, ethyl acetate), 0.598 g of **21**: mp 50–51 °C from Skellysolve B; ^{1}H NMR 8.60 (m, 1 H), 8.10–7.15 (m, 7 H), 6.46 (s, 2 H), 4.97 (s, 2 H), 2.20 (m, 2 H), 1.70–1.00 (m, 10 H), 0.82 (m, 3 H). Anal. ($C_{22}H_{27}N_3O_3S$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-methanol 2-Methylpropyl Carbonate (22). To a magnetically stirred solution of **7** (X = S, 0.750 g, 2.76 mmol) in 20 mL of methylene chloride was added 0.40 mL (2.80 mmol) of triethylamine and 0.39 mL (3.00 mmol) of isobutyl chloroformate. Stirring was continued at room temperature for 1.5 h, the reaction diluted with 300 mL of chloroform and worked up in the usual way. Removal of the solvent in vacuo gave 1.107 g of an orange semisolid. Without further purification, the crude thioether product was oxidized with *m*-chloroperbenzoic acid in the usual manner to afford, after chromatography (100 g of silica gel, ethyl acetate), 0.594 g of **22**: mp 79–81 °C Skellysolve B; ^{1}H NMR 8.60 (m, 1 H), 8.10–7.10 (m, 7 H), 6.50 (s, 2 H), 4.97 (s, 2 H), 3.90 (d, 2 H, J = 6 Hz), 1.90 (m, 1 H), 0.90 (d, 6 H, J = 6 Hz). Anal. ($C_{19}H_{21}N_3O_4S$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-methanol 2-Methylpropanoate (23). Following the same procedure as described for the preparation of **21**, 0.750 g (2.76 mmol) of **7** (X = S), 0.264 g (3.00 mmol) of isobutyric acid, 0.42 mL (3.00 mmol) of triethylamine, and 0.39 mL (3.00 mmol) of isobutylchloroformate provided, after chromatography (100 g of silica gel, ethyl acetate–Skellysolve B 1:1), 0.297 g of the ester as a viscous oil: ^{1}H NMR ($CDCl_3$) 8.60, 7.80–7.05, 6.05, 4.80, 2.50, 1.10. The thioether ester was oxidized in the usual manner with *m*-chloroperbenzoic acid to give, after chromatographed (100 g of silica gel, ethyl acetate–Skellysolve B 3:1), 0.281 g of **23**: mp 69–71 °C from Skellysolve B; ^{1}H NMR 8.60 (m, 1 H), 8.10–7.10 (m, 7 H), 6.48 (s, 2 H), 4.97 (s, 2 H), 2.50 (m, 1 H), 1.05 (d, 6 H, J = 6 Hz). Anal. ($C_{18}H_{19}N_3O_3S$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-methanol Benzoate (27). To a magnetically stirredd suspension of **26** (0.500 g, 1.51 mmol) in 20 mL of acetonitrile was added 0.58 mL of 37% formaldehyde solution. The contents were warmed in a 65 °C oil bath for 15 min and cooled to room temperature and the acetonitrile removed in vacuo to give a dark oil. The oil was dissolved in 80 mL of methylene chloride and treated with 0.42 mL (3.02 mmol) of triethylamine and 0.314 mL (2.72 mmol) of benzoyl chloride. After

stirring at room temperature, the reaction was worked up in the usual manner to afford, after chromatography (150 g of silica gel, Skellysolve B–acetone 2:1), 0.150 g of **31**: mp 138–140 °C from ethyl acetate–ether; ^{1}H NMR 8.30–7.00 (m, 11 H), 6.80 (s, 2 H), 5.10 (s, 2 H), 3.00 (d, 2 H, J = 7 Hz), 2.30 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{24}H_{23}N_3O_3S_2$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-methanol *tert*-Butyl Ester (28). Following the same procedure described for the preparation of **27**, 0.500 g (1.51 mmol) of **26** and 0.24 mL (1.96 mmol) of trimethylacetyl chloride gave 0.358 g of **28**: mp 138–139 °C from ethyl acetate–ether; ^{1}H NMR 8.30 (d, 1 H, J = 4 Hz), 8.00–7.30 (m, 4 H), 7.05 (d, 1 H, J = 4 Hz), 6.48 (s, 2 H), 5.05 (s, 2 H), 3.00 (q, 2 H, J = 7 Hz), 2.30 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz), 1.20 (s, 12 H). Anal. ($C_{22}H_{27}N_3O_3S_2$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Acetates (18a and 18b). To a magnetically stirred partial solution of **6** (0.241 g, 1.00 mmol) in 10 mL of acetonitrile was added 0.200 g (1.20 mmol) of α-bromoethyl acetate. After the reaction was stirred overnight at room temperature, an additional 0.400 g of α-bromo ester was added and the reaction placed in a 60 °C oil bath for 1 h. The reaction was diluted with ether and worked up in the usual manner. The crude product was chromatographed (100 g of silica gel, methylene chloride–acetone, 4:1) to give 0.134 g of ester: FABMS calcd for $C_{17}H_{18}N_3O_2S$ 328.1120, found 328.1147; ^{1}H NMR 8.60 (m, 1 H), 7.80–6.90 (m, 7 H), 4.80 (s, 2 H), 2.00 (s, 3 H), 1.75 (d, 3 H, J = 6 Hz). The thioether ester (0.214 g, 0.65 mmol) was oxidized in the same manner as previously described with *m*-chloroperbenzoic acid (0.148 g, 0.68 mmol) to give, after chromatography (75 g of silica gel, methylene chloride–acetone, 4:1), 0.115 g of **18a** (less polar isomer) and 0.037 g of **18b** (more polar isomer): TLC (methylene chloride–acetone 4:1) R_f **18a** 0.18; **18b** 0.14; FABMS calcd for $C_{17}H_{18}N_3O_3S$ 344.1069, found (**18a**) 344.1070; (**18b**) 344.1064; ^{1}H NMR (**18a**) 2.05 (s, 3 H), 1.90 (d, 3 H, J = 6 Hz); **18b** 8.65 (m, 1 H), 8.00–7.15 (m, 7 H), 5.05 (q, 1 H, J = 12 Hz), 2.10 (s, 3 H), 1.90 (d, 3 H, J = 6 Hz).

2-[[(4-Methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Acetate (25). Following the same procedure and workup previously described above, 0.670 g (2.24 mmol) of 2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]thio]benzimidazole[20] and 0.486 g (2.91 mmol) of α-bromoethyl acetate afforded, after chromatography (150 g of silica gel, methylene chloride–acetone 6:1), 0.201 g of the ester as a colorless oil: FABMS calcd for $C_{20}H_{24}N_3O_3S$ 386.1538, found 386.1532; ^{1}H NMR 8.80 (s, 1 H), 7.70 (m, 2 H), 7.30 (m, 2 H), 7.10 (q, 1 H, J = 6 Hz), 4.85 (s, 2 H), 3.80 (s, 3 H), 2.40 (s, 3 H), 2.25 (s, 3 H), 2.10 (s, 3 H), 1.80 (d, 3 H, J = 6 Hz). The thioether ester (0.201 g, 0.52 mmol) was oxidized with *m*-chloroperbenzoic acid (0.118 g, 0.54 mmol) in the usual manner to give, after chromatography (LPLC, methylene chloride–acetone 6:1), 0.091 g of **25** as a mixture of diastereomers: mp 123–124 °C from ether–Skellysolve B; ^{1}H NMR 8.30 (s, 1 H), 7.85 (m, 2 H), 7.45 (m, 3 H), 5.05 (s, 2 H), 3.80 (s, 3 H), 2.40 (s, 3 H), 2.30 (s, 3 H), 2.10 (s, 3 H), 1.95 (d, 3 H, J = 6 Hz). Anal. ($C_{20}H_{23}N_3O_4S$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Benzoates (29a and 29b). To a magnetically stirred solution of **6** (1.50 g, 6.22 mmol) in 60 mL of acetonitrile was added 4.67 g (31.30 mmol) of powdered sodium iodide and 1.49 g (8.09 mmol) of α-chloroethyl benzoate.[14] The reaction mixture was stoppered and allowed to stir at room temperature for 20 **h**. The reaction was then diluted with ether and the ether successively washed with saturated sodium bicarbonate, water, 10% sodium sulfite solution, water, and saturated brine, and the ether solution dried over anhydrous sodium sulfate. Removal of the solvent in vacuo gave the crude product which was chromatographed on 250 g of silica gel. Elution with ethyl acetate–Skellysolve B (1:1) afforded 0.699 g of the benzoyl ester as a yellow oil: FABMS calcd for $C_{22}H_{20}N_3O_2S$ 390.1276, found 390.1286; ^{1}H NMR 8.65 (m, 1 H), 8.10 (m, 2 H), 7.90–7.10 (m, 11 H), 4.90 (s, 2 H), 1.98 (d, 3 H, J = 6 Hz). The thioether ester (0.699 g, 1.80 mmol) was oxidized with *m*-chloroperbenzoic acid (0.340 g, 1.98 mmol) in the usual manner to give, after chromatography (150 g of silica gel, methylene chloride–acetone 6:1), 0.460 g of **29a** (less polar isomer) and 0.070 g of **29b** (more polar isomer) both as oils: TLC (methylene chloride–acetone 4:1) R_f (**29a**) 0.23; (**29b**) 0.17; FABMS calcd for $C_{22}H_{20}N_3O_3S$ 406.1225, found (**29a**) 406.1211; (**29b**) 406.1219; ^{1}H NMR (**29a**) 8.70 (m, 1 H), 8.15–7.05 (13 H), 5.10 (q, 2 H, J = 12 Hz), 2.05 (d, 3 H, J = 6 Hz); (**29b**) 8.65 (m, 1 H), 8.25–7.10 (m, 13 H), 5.15 (q, 2 H, J = 12 Hz), 2.00 (d, 3 H, J = 6 Hz).

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol *tert*-Butyl Esters (30a and 30b). Following the same procedure and workup described above, 1.00 g (4.15 mmol) of **6** in 40 mL of acetonitrile, 1.87 g (12.45 mmol) of sodium iodide and 0.885 g (5.39 mmol) of α-chloroethyl pivaloate[14] gave, after chromatography (100 g of silica gel, ethyl acetate–Skellysolve B 1:1), 0.505 g of recovered **6** and 0.500 g of the *tert*-butyl ester: FABMS calcd for $C_{20}H_{24}N_3O_2S$ 370.1589, found 370.1610; ^{1}H NMR 8.60 (m, 1 H), 7.80–7.10 (m, 7 H), 7.00 (q, 1 H, J = 6 Hz), 4.80 (s, 2 H), 1.75 (d, 3 H, J = 6 Hz), 1.15 (s, 12 H). The thioether ester (0.936 g, 2.54 mmol) was oxidized with *m*-chloroperbenzoic acid (0.600 g, 2.79 mmol) in the usual manner to give, after chromatography (170 g of silica gel, methylene chloride–acetone 6:1), 0.519 g of **30a** (less polar isomer, mp 99–100 °C) and 0.088 g of **30b** (more polar isomer, as an oil): TLC (methylene chloride–acetone 4:1) R_f (**30a**) 0.30; (**30b**) 0.25; FABMS calcd for $C_{20}H_{24}N_3O_3S$ 386.1538, found (**30a**) 386.1514; (**30b**) 386.1514; ^{1}H NMR (**30a**) 8.70 (m, 1 H), 8.00–7.15 (m, 8 H), 5.05 (q, 2 H, J = 12 Hz), 1.90 (d, 3 H, J = 6 Hz), 1.15 (s, 12 H); (**30b**) 8.70 (m, 1 H), 7.90–7.10 (m, 8 H), 5.05 (q, 2 H, J = 12 Hz), 1.80 (d, 3 H, J = 6 Hz), 1.20 (s, 12 H).

Oxidation of 2-[[(3,4-Dihydro-2*H*-thieno[2,3-*c*]-pyridinyl)methyl]thio]-1*H*-benzimidazole-1-methanol Acetate (13). Oxidation of **13** (0.330 g, 0.857 mmol) in the usual manner with *m*-chloroperbenzoic acid (0.193 g, 0.900 mmol) gave, after chromatography (60 g of silica gel, acetone–methylene chloride 2:1), 0.201 g of **14**: mp 143–145 °C from ethyl acetate–ether; FABMS calcd for $C_{19}H_{20}N_3O_3S_2$ 402.0946, found 402.0934; ^{1}H NMR 8.65 (d, 1 H, J = 4 Hz), 7.80–7.20 (m, 5 H), 6.10 (s, 2 H), 4.80 (s, 2 H), 3.35–2.80 (m, 4 H), 2.60–1.90 (m, 2 H), 2.02 (s, 3 H). Anal. ($C_{19}H_{19}N_3O_3S_2$) C, H, N, S.

Oxidation of 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]-methyl]thio]-1*H*-benzimidazole-1-methanol Acetate (10). To a magnetically stirred solution of *m*-chloroperbenzoic acid (0.167 g, 0.78 mmol) was added at room temperature in several portions 0.300 g (0.78 mmol) of **10**. After stirring at room temperature for 15 min, the reaction was worked up in the usual manner. Chromatography (LPLC Michel–Miller 300 × 11 cm, 1% methanol in ethyl acetate) afforded 0.065 g of **12**: mp 101–104 °C; FABMS calcd for $C_{19}H_{21}N_3O_3S_2K$ 442.0661, found 442.0628; ^{1}H NMR 8.70 (d, 1 H, J = 4 Hz), 7.80–7.15 (m, 5 H), 6.10 (s, 2 H), 4.85 (s, 2 H), 3.10–2.45 (m, 2 H), 2.30 (s, 3 H), 2.00 (s, 3 H), 1.15 (m, 3 H). Further elution of the column provided 0.073 g of 2-[[[4-(ethylsulfinyl)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-methanol acetates, isolated as a mixture of diastereomers: FABMS calcd for $C_{19}H_{21}N_3O_4S_2K$ 458.0611, found 458.0618; ^{1}H NMR 8.65 (m, 1 H), 7.95–7.30 (m, 5 H), 6.60 (s, 1 H), 6.50 (s, 1 H), 5.10 (s, 2 H), 3.15–2.60 (m, 2 H), 2.40 (s, 1.5 H), 2.30 (s, 1.5 H), 2.10 (s, 3 H), 1.15 (m, 3 H).

2-[[4-(Ethylthio)-3-methyl-2-pyridinylmethyl]sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Acetates (19a and 19b). To a magnetically stirred suspension of **26** (1.00 g, 3.02 mmol) was added 1.67 g (12.08 mmol) of potassium carbonate and 0.53 mL (4.53 mmol) of α-bromoethyl acetate. The contents were stirred overnight at room temperature. The reaction was worked up in the usual manner and the crude product chromatographed on 130 g of silica gel. Elution with Skellysolve B–acetone (2:1) afforded 0.362 g of a mixture of esters as an oil. The oil was rechromatographed (LPLC, three Merck Lobar columns, acetone–chloroform–ethanol 5:93:2) to give 0.130 g of **19a** (less polar isomer, as a oil) and 0.194 g of **19b** (more polar isomer, mp 123–124.5 °C from ether): FABMS calcd for $C_{20}H_{24}N_3O_3S_2$: 418.1259, found (**19a**) 418.1249; (**19b**) 418.1257; ^{1}H NMR (**19a**) 8.20 (d, 1 H, J = 4 Hz), 7.90–7.20 (m, 4 H), 6.95 (d, 1 H, J = 4 Hz), 5.05 (s, 2 H), 2.95 (q, 2 H, J = 7 Hz), 2.40 (m, 1 H), 2.30 (s, 3 H), 2.00 (s, 3 H), 1.90 (d, 3 H, J = 6 Hz), 1.35 (t, 3 H, J = 6 Hz); (**19b**) 8.20 (d, 1 H, J = 4 Hz), 7.90–7.20 (m, 4 H), 6.95 (d, 1 H, J = 4 Hz), 5.10 (m, 1 H), 3.00 (q, 2 H, J = 7 Hz), 2.30 (s, 3 H), 2.20 (s, 3 H), 2.10 (s, 3 H), 1.95 (d, 3 H, J = 6 Hz), 1.35 (t, 3 H, J = 7 Hz).

Ethyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (31). To a mag-

netically stirred suspension of sodium hydride (0.067 g, 1.66 mmol) in 10 mL of dimethylformamide was added 0.500 g (1.51 mmol) of **26** and 0.196 g (1.81 mmol) of ethyl chloroformate. Stirring was continued at room temperature for 30 min. The reaction was then diluted with ether and worked up in the usual manner. The crude product was chromatographed on 75 g of silica gel and eluted with methylene chloride–acetone (4:1) to give 0.350 g of **31**: mp 135–136 °C dec from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.19–7.82 (m, 3 H), 7.60–7.31 (m, 2 H), 6.97 (d, 1 H, J = 6 Hz), 5.00–4.57 (m, 2 H), 4.64 (q, 2 H, J = 7 Hz), 2.98 (q, 2 H, J = 7 Hz), 2.38 (s, 3 H), 1.55 (t, 3 H, J = 7 Hz), 1.38 (t, 3 H, J = 7 Hz). Anal. ($C_{19}H_{21}N_3O_3S_2$) C, H, N, S.

Isobutyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (32). Following the same procedure and chromatographic conditions described above, 0.500 g (1.51 mmol) of **26** and 0.411 g (3.02 mmol) of isobutyl chloroformate gave 0.362 g of **32**: mp 85–91 °C from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.21–7.85 (m, 3 H), 7.64–7.31 (m, 3 H), 6.96 (d, 1 H, J = 5 Hz), 5.00–4.59 (m, 2 H), 4.38 (d, 2 H, J = 7 Hz), 2.96 (q, 2 H, J = 7 Hz), 2.55–2.01 (m, 1 H), 2.38 (s, 3 H), 1.36 (t, 3 H, J = 7 Hz), 1.10 (d, 6 H, J = 7 Hz). Anal. ($C_{21}H_{25}N_3O_3S_2$) C, H, N, S.

Benzyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (33). Following the same procedure and chromatographic conditions described previously, 0.500 g (1.51 mmol) of **26** and 0.310 g (1.81 mmol) of benzyl chloroformate afforded 0.444 g of **33**: mp 156–157 °C dec from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.22–7.80 (m, 3 H), 7.80–7.35 (m, 7 H), 7.00 (d, 1 H, J = 5 Hz), 5.65 (s, 2 H), 5.00–4.60 (m, 2 H), 2.99 (q, 2 H, J = 7 Hz), 2.40 (s, 3 H), 1.43 (t, 3 H, J = 7 Hz). Anal. ($C_{24}H_{23}N_3O_3S_2$) C, H, N, S.

Phenyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (34). Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.283 g (1.81 mmol) of phenyl chloroformate afforded 0.293 g of **34**: mp 136–137 °C dec from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.25–7.85 (m, 3 H), 7.71–7.30 (m, 7 H), 6.96 (d, 1 H, J = 5 Hz), 5.08–4.68 (m, 2 H), 2.93 (q, 2 H, J = 7 Hz), 2.38 (s, 3 H), 1.38 (t, 3 H, J = 7 Hz). Anal. ($C_{23}H_{21}N_3O_3S_2$) C, H, N, S.

***p*-Nitrobenzyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (35).** Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.390 g (1.81 mmol) of *p*-nitrobenzyl chloroformate afforded 0.409 g of **35**: mp 152–153 °C dec from ethyl acetate–ether–Skellysolve B; ^{1}H NMR ($CDCl_3$ + MeOH-d_4) 8.51–8.22 (m, 2 H), 8.13–7.73 (m, 4 H), 7.65–7.33 (m, 3 H), 7.01 (d, 1 H, J = 5 Hz), 5.72 (s, 2 H), 5.03–4.57 (m, 2 H), 2.99 (q, 2 H, J = 7 Hz), 2.37 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{24}H_{22}N_4O_5S_2$) C, H, N, S.

Vinyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (36). Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.192 g (1.81 mmol) of vinyl chloroformate afforded 0.280 g of **36**, mp 120–122 °C dec from ethyl acetate–ether; ^{1}H NMR 8.21–7.85 (m, 3 H), 7.68–7.42 (m, 2 H), 7.35 (d, 1 H, J = 6 Hz), 6.97 (d, 1 H, J = 5 Hz), 5.45 (dd, 1 H, J = 13 Hz), 5.08–4.60 (m, 3 H), 2.95 (q, 2 H, J = 7 Hz), 2.38 (s, 3 H), 1.39 (t, 3 H, J = 7 Hz). Anal. ($C_{19}H_{19}N_3O_3S_2$) C, H, N, S.

Isopropyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (37). Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.221 g (1.81 mmol) of isopropyl chloroformate afforded 0.376 g of **37**: mp 121–123 °C dec from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.22–7.85 (m, 3 H), 7.65–7.32 (m, 2 H), 6.98 (d, 1 H, J = 5 Hz), 5.60–5.21 (m, 1 H), 5.00–4.60 (m, 2 H), 2.95 (q, 2 H, J = 7 Hz), 2.39 (s, 3 H), 1.57 (d, 3 H, J = 6 Hz), 1.54 (d, 3 H, J = 6 Hz), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{20}H_{23}N_3O_3S_2$) C, H, N, S.

Octyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (38). Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.350 g (1.81 mmol) of octyl chloroformate afforded 0.347 g of **38**: mp 61–63 °C from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.21–7.80 (m, 3 H), 7.65–7.32 (m, 2 H), 6.98 (d, 1 H, J = 5 Hz), 5.00–4.50 (m, 4 H), 2.98 (q, 2 H, J = 7 Hz), 2.40 (s, 3 H), 2.11–1.69 (m, 3 H), 1.69–1.12 (m, 12 H), 0.90 (t, 3 H, J = 7 Hz). Anal. ($C_{25}H_{33}N_3O_3S_2$) C, H, N, S.

2-Chloroethyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (39). Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.259 g (1.81 mmol) of 2-chloroethyl chloroformate afforded 0.382 g of **39**: mp 122–124 °C dec from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.21–7.82 (m, 3 H), 7.63–7.28 (m, 2 H), 6.95 (d, 1 H, J = 5 Hz), 4.98–4.64 (m, 4 H), 4.08–3.85 (m, 2 H), 2.91 (q, 2 H, J = 7 Hz), 2.35 (s, 3 H), 1.36 (t, 3 H, J = 7 Hz). Anal. ($C_{19}H_{20}ClN_3O_3S_2$) C, H, N, S.

2,2,2-Trichloro-1,1-dimethylethyl 2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-carboxylate (40). Following the same procedure and chromatographic conditions previously described, 0.500 g (1.51 mmol) of **26** and 0.471 g (1.81 mmol) of 2,2,2-trichloro-1,1-dimethylethyl chloroformate afforded 0.246 g of **40**: mp 162 °C dec from ethyl acetate–ether: ^{1}H NMR 8.36–7.82 (m, 3 H), 7.61–7.31 (m, 2 H), 6.92 (d, 1 H, J = 5 Hz), 5.00–4.63 (m, 2 H), 2.96 (q, 2 H, J = 7 Hz), 2.40 (s, 3 H), 2.18 (s, 6 H), 1.37 (t, 3 H, J = 7 Hz). Anal. ($C_{21}H_{22}Cl_3N_3O_3S_2$) C, H, N, S.

2-[(2-Pyridinylmethyl)thio]-1-(2-morpholinylethyl)benzimidazole (41). To a magnetically stirred solution of **6** (0.500 g, 2.07 mmol) in 7 mL of dimethylformamide was added 91 mg (2.28 mmol) of sodium hydride. The contents were stirred at 35–40 °C for 15 min and then (β-chloroethyl)morpholine, liberated from 0.766 g (4.14 mmol) of its HCl salt, was added in 3 mL of dimethylformamide. The reaction was heated in a 45 °C oil bath for 1 h and then at 85 °C for an additional 1 h. The reaction mixture was cooled to room temperature and worked up in the usual manner. Removal of the solvent in vacuo gave the crude product which was chromatographed on 70 g of silica gel. Elution with methylene chloride–acetone (4:1) gave 0.713 g of **41** as a viscous colorless oil: FABMS calcd for $C_{19}H_{22}N_4OS$ 355.1592, found 355.1601; ^{1}H NMR 8.65 (m, 1 H), 7.84–7.15 (m, 7 H), 4.80 (s, 2 H), 4.20 (t, 2 H, J = 6 Hz), 3.60 (m, 4 H), 2.55 (m, 6 H).

2-[(2-Pyridinylmethyl)thio]-1-(morpholinylmethyl)benzimidazole (42). To a magnetically stirred solution of **6** (2.41 g, 10 mmol) in 20 mL of methanol was added 0.87 mL (10 mmol) of morpholine and 1.10 mL of 37% formaldehyde solution. The solution was stirred at room temperature for 2 h, and then the solvents were removed in vacuo to give a colorless oil. The oil was chromatographed on a 300 × 11 cm Michel-Miller column and elution with Skellysolve B–acetone (2:1) gave 3.195 g of **42** as a viscous oil: ^{1}H NMR 8.60 (m, 1 H), 7.80–7.00 (m, 7 H), 4.80 (s, 2 H), 4.72 (s, 2 H), 4.70 (m, 4 H), 2.50 (m 4 H). Anal. ($C_{18}H_{20}N_4OS$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]thio]-1-(epoxymethyl)-1*H*-benzimidazole (43). To a magnetically stirred suspension of sodium hydride (70 mg, 1.75 mmol) in 10 mL of dimethylformamide was added 0.500 g (1.59 mmol) of **26** as a solid. Stirring was continued at room temperature for 10 min and then epibromohydrin (0.54 mL, 6.36 mmol) in 0.5 mL of dimethylformamide was added and the reaction placed in an oil bath at 85 °C for 15 min. The reaction was worked up in the usual manner. The crude product was chromatographed on 75 g of silica gel and elution with methylene chloride–acetone (4:1) gave 0.545 g of **43**: mp 111–113 °C from trituration with Skellysolve B; ^{1}H NMR 8.35 (m, 1 H), 7.90–7.00 (m, 5 H), 4.90 (s, 2 H), 4.60–4.10 (m, 2 H), 3.30–2.50 (m, 5 H), 2.33 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{19}H_{21}N_3OS_2$) C, H, N, S.

2-[(2-Pyridinylmethyl)thio]-1-(*N*-morpholinylmethyl)-1*H*-benzimidazole *N*-Oxide (44). To a magnetically stirred solution of **42** (0.500 g, 1.47 mmol) and sodium bicarbonate (0.185 g, 2.20 mmol) in 40 mL of chloroform, cooled in a 0–5 °C ice–water bath, was added a solution of *m*-chloroperbenzoic acid (0.332 g, 1.54 mmol) in 7 mL of chloroform over a 5-min period. Stirring was continued at 0–5 °C for 1 h. During this time period, two additional increments of peracid were added (0.052 g and 0.075 g, total 0.58 mmol). The reaction was then diluted with chloroform, washed with 10% sodium sulfite solution, saturated sodium bicarbonate, and saturated brine and the chloroform solution dried over anhydrous sodium sulfate. Removal of the solvent in vacuo

gave the crude product, which was chromatographed on 50 g of silica gel. Elution with Skellysolve B–acetone (2:1) afforded 0.221 g of an oil which slowly crystallized from Skellysolve B–ether to give 0.181 g of 1, identical by ^{1}H NMR and TLC to an authentic sample. Further elution of the column with chloroform–acetone (1:1) containing 10% methanol gave 0.129 g of **44** as a white solid: mp 169–170 °C from ethyl acetate methanol–ether; ^{1}H NMR 8.70 (m, 1 H), 7.90–7.10 (m, 7 H), 5.37 (s, 2 H), 4.90 (s, 2 H), 4.50 (m, 2 H), 4.00–2.90 (m, 6 H). Anal. ($C_{18}H_{21}N_4O_2S$) C, H, N, S.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1-(2-*N*-morpholinylethyl)-1*H*-benzimidazole (45). Following the same procedure and workup conditions described for the preparation of **41**, 0.500 g (1.59 mmol) of 2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]benzimidazole,[20] 0.070 g (1.75 mmol) of sodium hydride, and 0.441 g (2.38 mmol) of *N*-(β-chloroethyl)morpholine gave, after chromatography on 50 g of silica gel and elution with methylene chloride–acetone (2:1), 0.434 g of **45** as an oil. The oil crystallized from ethyl acetate–ether–Skellysolve B solvent mixtures to yield an hydroscopic solid: FABMS calcd for $C_{22}H_{29}N_4O_3S$ 429.1954, found 429.1960; ^{1}H NMR 8.20 (s, 1 H), 7.90 (m, 1 H), 7.40 (m, 3 H), 5.00 (s, 1 H), 4.55 (t, 2 H, J = 6 Hz), 3.70 (m, 7 H), 2.90–2.30 (m, 6 H), 2.25 (s, 3 H), 2.15 (s, 3 H).

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1-(2-*N*-piperidinylethyl)benzimidazole (46). Following the same procedure and workup described for the preparation of **41**, 0.500 g (1.59 mmol) of **26** and 0.585 g (3.18 mmol) of *N*-(β-chloroethyl)piperidine hydrochloride salt afforded, after chromatography on 100 g of silica gel and elution with methylene chloride–acetone (2:1), 0.591 g of **46**, mp 105–106.5 °C from ether–Skellysolve B; ^{1}H NMR 8.30 (d, 1 H, J = 5 Hz), 7.95 (m, 1 H), 7.70 (m, 3 H), 7.05 (d, 1 H, J = 5 Hz), 5.10 (m, 2 H), 4.60 (t, 2 H, J = 6 Hz), 3.15–2.30 (m, 8 H), 2.31 (s, 3 H), 1.80–1.20 (m, 9 H). Anal. ($C_{23}H_{30}N_4OS_2$) C, H, N, S.

2-[(2-Pyridinylmethyl)sulfinyl]-1-(2-*N*-morpholinylethyl)-1*H*-benzimidazole (47). Following the same procedure and workup conditions described for the preparation of **41**, 1.00 g (3.89 mmol) of **1** yielded, after chromatography on 75 g of silica gel and elution with 5% methanol in ethyl acetate, 0.897 g of **47**: mp 139–140 °C from ethyl acetate–ether; ^{H} NMR 8.70 (m, 1 H), 7.95–7.10 (m, 7 H), 4.98 (m, 2 H), 4.50 (m, 2 H), 3.50 (m, 4 H), 2.80–2.35 (m, 6 H). Anal. ($C_{19}H_{22}N_4O_2S$) C, H, N, S.

2-[(2-Pyridylmethyl)sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Ethyl Ether (48). In a 60-mL stainless steel tube was placed 1.20 g (5.0 mmol) of **6**, 0.700 g (6.45 mmol) of 1-chloroethyl ethyl ether, 2.00 mL (14.4 mmol) of triethylamine, and 15 mL of *m*-xylene. The contents were sealed and placed in a 160–165 °C oil bath for 18 h. At the end of this time, the reaction contents were cooled to room temperature, transferred with chloroform to a separatory funnel and worked up in the usual manner. Concentration of the solvent in vacuo gave a brown oil which was chromatographed on a 300 × 11 cm Michel-Miller column. Elution with ethyl acetate–Skellysolve B (8:1) gave 0.804 g of product as a viscous tan oil: ^{1}H NMR 8.70 (m, 1 H), 7.90–7.00 (m, 7 H), 5.80 (q, 2 H, J = 4 Hz), 4.85 (s, 2 H), 3.30 (m, 2 H), 1.70 (d, 3 H, J = 5 Hz), 1.11 (t, 3 H, J = 6 Hz). The crude product was placed in 18 mL of chloroform and 0.218 g (2.60 mmol) of sodium bicarbonate, cooled in a 0–5 °C ice–water bath, and treated with *m*-chloroperbenzoic acid (0.560 g, 2.60 mmol). After addition, stirring was continued at 0–5 °C for 10 min. The reaction was then diluted with chloroform and worked up in the usual manner. Removal of the solvent in vacuo gave an oil which was chromatographed on a Michel-Miller 300 × 11 cm column. Elution with ethyl acetate–Skellysolve B (8:1) gave 0.982 g of **48**, a colorless oil, as a mixture of diastereomers: ^{1}H NMR 8.65 (m, 1 H), 8.00–7.10 (m, 7 H), 6.35 (q, 1 H, J = 5 Hz), 5.10–4.85 (m, 2 H), 3.40 (m, 2 H), 1.79 (m, 3 H), 1.15 (m, 3 H). Anal. ($C_{17}H_{19}N_3O_2S$) C, H, N, S.

2-[[(4-Methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Ethyl Ether (49). To a magnetically stirred suspension of sodium hydride (0.074 g, 1.84 mmol) in 3 mL of dimethylformamide was added 0.500 g (1.67 mmol) of 2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl]thio]-1*H*-benzimidazole.[20] Stirring was continued at room temperature for 30 min. At the end of this time, 0.361 g (3.34 mmol) of 1-chloroethyl ethyl ether was added, and the contents were stirred at room temperature for 30 min. The reaction was diluted with chloroform and worked up in the usual manner. Removal of the solvent in vacuo gave the crude product which was chromatographed on 50 g of silica gel. Elution with Skellysolve B–acetone (3:1) gave 0.530 g of product: FABMS calcd for $C_{20}H_{26}N_3O_2S$ 372.1746, found 372.1751; ^{1}H NMR 8.32 (s, 1 H), 7.80 (m, 2 H), 7.30 (m, 2 H), 5.80 (q, 1 H, J = 6 Hz), 4.90 (s, 2 H), 3.85 (s, 3 H), 3.40 (m, 2 H), 2.40 (s, 3 H), 2.28 (s, 3 H), 1.70 (q, 3 H, J = 4 Hz), 1.20 (t, 3 H, J = 6 Hz). The product was oxidized with *m*-chloroperbenzoic acid (0.322 g, 1.50 mmol) and 0.180 g (2.14 mmol) of sodium bicarbonate in 25 mL of chloroform in the same manner as described for the preparation of **48** to give, after chromatography on 60 g of silica gel and elution with Skellysolve B–acetone (2:1), 0.248 g of **49** as a golden viscous oil: FABMS calcd for $C_{20}H_{26}N_3O_3S$ 388.1695, found 388.1697; ^{H} NMR 8.20 (s, 1 H), 7.90 (m, 2 H), 7.40 (m, 2 H), 6.50 (m, 1 H), 5.10 (m, 2 H), 3.78 (s, 3 H), 3.50 (s, 3 H), 2.30 (s, 3 H), 2.20 (s, 3 H), 1.85 (m, 3 H), 1.20 (m, 3 H).

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazole-1-ethan-2-ol Ethyl Ether (50). To a magnetically stirred suspension of sodium hydride (0.133 g, 3.32 mmol) in 25 mL of dimethylformamide was added under nitrogen at room temperature 1.00 g (3.02 mmol) of **26** as a solid. Stirring was continued at room temperature for 10 min. At the end of this time, 1-chloroethyl ethyl ether (0.392 g, 3.62 mmol) was added, and the reaction contents were stirred at room temperature for 20 min. The reaction was diluted with chloroform and worked up in the usual manner. Removal of the solvent in vacuo gave the crude product which was chromatographed on 80 g of silica gel. Elution with Skellysolve B–acetone (3:1) gave 0.913 g of **50**, a viscous oil, as a mixture of diastereomers. The oil slowly crystallized from ether–Skellysolve B solvent mixtures to yield 0.500 g of **50** (mp 114–115 °C, a 1:1 mixture of diastereomers) and 0.237 g of **50a** (mp 115–117 °C, 9:1 mixture in favor of more soluble isomer): ^{1}H NMR (**50a**) 8.25 (d, 1 H, J = 5 Hz), 7.90 (m, 2 H), 7.40 (m, 2 H), 7.05 (d, 1 H, J = 5 Hz), 6.45 (q, 1 H, J = 5 Hz), 5.20 (q, 2 H, J = 12 Hz), 3.60 (m, 2 H), 3.00 (q, 2 H, J = 6 Hz), 2.40 (s, 3 H), 1.80 (d, 3 H, J = 6 Hz), 1.40 (t, 3 H, J = 6 Hz), 1.20 (t, 3 H, J = 6 Hz). Anal. ($C_{20}H_{25}N_3O_2S_2$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazolemethan-1-ol Methyl Thioether (51). Following the same procedure and workup conditions described for the preparation of **50**, 0.500 g (1.51 mmol) of **26**, 0.067 g (1.66 mmol) of sodium hydride, and 0.189 mL (2.27 mmol) of chloromethyl methyl sulfide in dimethylformamide afforded, after chromatography on 70 g of silica gel and elution with methylene chloride–acetone (4:1), 0.400 g of **51**: mp 157–158 °C from ethyl acetate–methanol–ether: ^{1}H NMR 8.27 (d, 1 H, J = 5 Hz), 7.90 (m, 1 H), 7.50 (m, 3 H), 7.05 (d, 1 H, J = 5 Hz), 5.60 (m, 2 H), 4.90 (q, 2 H, J = 8 Hz), 3.00 (q, 2 H, J = 7 Hz), 2.35 (s, 3 H), 2.20 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{18}H_{22}N_3OS_3$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazolemethan-1-ol Phenyl Thioether (52). Following the same procedure and workup conditions described for the preparation of **50**, 0.500 g (1.51 mmol) of **26**, 0.067 g (1.66 mmol) of sodium hydride, 0.358 g (2.26 mmol) of phenyl chloromethyl sulfide, and 10 mL of dimethylformamide yielded, after chromatography on 75 g of silica gel and elution with methylene chloride–acetone (6:1), 0.335 g of **52**: mp 128–129.5 °C from ethyl acetate–ether–Skellysolve B; ^{1}H NMR 8.35 (d, 1 H, J = 5 Hz), 7.95 (m, 1 H), 7.35 (m, 8 H), 7.10 (d, 1 H, J = 5 Hz), 5.90 (s, 2 H), 4.90 (m, 2 H), 3.00 (q, 2 H, J = 6 Hz), 2.30 (s, 3 H), 1.35 (t, 3 H, J = 6 Hz). Aral. ($C_{23}H_{23}N_3OS_3$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazoleethan-1-ol Ethyl Ether (53). Following the same procedure and workup conditions described for the preparation of **50**, 0.500 g (1.51 mmol) of **26**, 0.067 g (1.67 mmol) of sodium hydride, 0.255 mL (2.26 mmol) of 2-bromoethyl ethyl ether, and 10 mL of dimethylformamide yielded, after chromatography on 90 g of silica gel and elution with methylene chloride–acetone (5:1), 0.294 g of **53** as a oil, which slowly crystallized from ethyl acetate–ether–Skellysolve B: mp 100–101 °C; ^{1}H NMR 8.25 (d, 1 H, J = 5 Hz), 7.90 (m, 1 H), 7.40 (m, 3 H), 7.00 (d, 1 H, J = 5 Hz), 5.10 (s, 2 H), 4.70 (t, 2 H, J = 4 Hz), 3.80 (t, 2 H, J = 4 Hz), 3.45 (q, 2 H, J = 7 Hz), 3.00 (q, 2 H, J = 7

Hz), 2.30 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz), 1.10 (t, 3 H, J = 7 Hz). Anal. ($C_{20}H_{25}N_3O_2S_2$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazolemethan-1-ol Methyl Ether (54). Following the same procedure and workup conditions described for the preparation of **50**, 0.500 g (1.51 mmol) of **26**, 0.067 g (1.67 mmol) of sodium hydride, 0.148 mL (1.82 mmol) of bromomethyl methyl ether, and 10 mL of dimethylformamide yielded, after chromatography on 50 g of silica gel and elution with methylene chloride–acetone (4:1), 0.309 g of **54** as a oil, which slowly crystallized from ethyl acetate–ether–Skellysolve B: mp 122–123 °C; ^{1}H NMR 8.20 (d, 1 H, J = 5 Hz), 7.90 (m, 1 H), 7.50 (m, 3 H), 7.00 (d, 1 H, J = 5 Hz), 5.80 (s, 2 H), 5.10 (q, 2 H, J = 10 Hz), 3.30 (s, 3 H), 3.00 (q, 2 H, J = 7 Hz), 2.30 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{18}H_{22}N_3O_2S_2$) C, H, N, S.

2-[[[4-(Ethylthio)-3-methyl-2-pyridinyl]methyl]sulfinyl]-1*H*-benzimidazolemethan-1-ol Methoxyethyl Ether (55). Following the same procedure and workup conditions described for the preparation of **50**, 0.500 g (1.51 mmol) of **26**, 0.067 g (1.67 mmol) of sodium hydride, 0.223 mL (1.96 mmol) of methoxyethyl chloromethyl ether (MEM chloride) and 10 mL of dimethylformamide yielded, after chromatography on 75 g of silica gel and elution with methylene chloride–acetone (3:1), 0.316 g of **55** as a oil, which slowly crystallized from ethyl acetate–ether–Skellysolve B: mp 80–81 °C; ^{1}H NMR 8.20 (d, 1 H, J = 5 Hz), 7.90 (m, 1 H), 7.65 (m, 1 H), 7.45 (m, 2 H), 7.00 (d, 1 H, J = 5 Hz), 5.95 (s, 2 H), 5.10 (q, 2 H, J = 10 Hz), 3.80–3.40 (m, 4 H), 3.30 (s, 3 H), 3.00 (q, 2 H, J = 7 Hz), 2.30 (s, 3 H), 1.40 (t, 3 H, J = 7 Hz). Anal. ($C_{20}H_{26}N_3O_3S_2$) C, H, N, S.

Antisecretory Activity in Isolated Rabbit Glands. Isolated rabbit gastric glands were prepared according to the method of Berglindh and Obrink.[16] The stomachs of male New Zealand albino rabbits weighing 1.5–2.0 kg were perfused through arterial gastric vessels under high pressure to produce edematous mucosa. The separated mucosa tissues were digested at 37 °C in a buffered solution, pH 7.4 containing 70 units collagenase/mL, 0.25 mg/mL soybean trypsin inhibitor, 2 mg/mL rabbit albumin, and 2 mg/mL glucose. The degree of acid secretion in these isolated glands was quantitated by measuring the distribution of ratio of (^{14}C)-aminopyrine, a weak base with a pK_a value of 5, between the intracellular and extracellular water spaces. Dibutyl cAMP (10^{-3} M) was added to stimulate gastric acid secretion in the glands. Incubation was at 37 °C for 45 min while shaking vigorously. The incubation was terminated by rapid centrifugation in an Eppendorf centrifuge (Model 5414). The dried pellets were solubilized in 0.1 mL 1 N NaOH per 1 mg of dry weight and added to 10 mL Dimilume. Each supernatant, (0.20 mL) was added to 10 mL of Insta-Gel. All samples were counted in a liquid scintillation counter. The ID_{50} values were obtained from a dose–response curve consisting of at least six doses (duplicate measurement at each dose). Standard errors between experiments were less than 20%.

Isolated Hog (H^+-K^+)-ATPase Assay. The inhibition of parietal cell (H^+-K^+)-ATPase was determined with microsomal preparations obtained from hog stomachs fundic mucosa that were homogenized in 40 mL of a solution containing 250 mM mannitol, 2 mM $MgCl_2$, and 2 mM Hepes/Tris, pH 7.4 (mannitol buffer). The ATPase enzyme activity was determined in a 1-mL incubation medium containing 40 mM Tris-acetate, pH 7.4, 2 mM ATP with or without KCl, and 7 mM NH_4Cl.[21] Usually the membrane vesicles (30 μg) were incubated in the above media without ATP for an indicated time (preincubation time) at 37 °C. Then the reaction was initiated by adding ATP to a final concentration of 2 mM. After incubation for 10 min at 37 °C, the reaction was terminated by adding 1 mL of ice-cold 10% trichloroacetic acid solution and 0.1 g of HCl-washed charcoal. The charcoal was removed by centrifugation and passage through glass-woolfilters. A 1-mL aliquot of the filtrate thus obtained was assayed for the inorganic phosphate released from ATP by the method of Tsai et al.[17] The charcoal treatment was designed to facilitate the colorimetric assay of inorganic phosphate by removing possibly interfering organic chemicals and protein precipitate. Under our experimental conditions, the formation of inorganic phosphate by the membrane ATPase was linear under the 10-min incubation time. The IC_{50} values were obtained from a typical dose–response curve and represent the mean of triplicate measurements. Standard errors were less than 5%.

Inhibition of (H^+-K^+)-ATPase Activity in the Rat. Male Sprague–Dawley rats weighing about 230 g were fasted for 16 h without food and water in a restraining cage to prevent coprophagy. An indicated dose of test compound in 1 mL of isotonic saline containing 25% ethanol was given subcutaneously (sc) or orally by gavage (po). The rats were sacrificed by cervical dislocation 3 h after drug treatment. The gastric mucosal tissues were obtained by thoroughly scraping the fundic region of the rat stomach. Typically, the scraped tissues from four rats (per treatment) were suspended in 20 mL of sucrose–EGTA buffer containing 250 mM sucrose, 2 mM $MgCl_2$, 1 mM EGTA and 2 mM Hepes/Tris, pH 7.4. The tissues were homogenized in a Sorvall Omni-Mixer for 3 min at the maximum speed. The homogenates were fractionated by differential centrifugation; the nuclei, the mitochondria-enriched fractions, and the microsomes were obtained by successive centrifugation at 1000×g for 10 min, 20000g, for 15 min, and 170000g for 35 min. The assay procedures for (H^+-K^+)-ATPase were the same as described above. The ED_{50} values were obtained from a typical dose–response curve consisting of at least five different doses (each triplicate measurement). Standard deviation between experiments was less than 20%.

Inhibition of Acid Secretion in the Pylorous-Ligated Rat. Gastric antisecretory activity was determined using the Shay rat preparation.[18] Female Upjohn rats or Charles River rats were fasted for 24 h. During the last 18 h, they were placed in individual semirestraining cages to prevent coprophagy and water was withheld. Weight ranges for rats before fasting were 185–200 g or 200–220 g. On the following morning the test compounds were given once either subcutaneously (sc) or orally (os). In all studies, the pylorus was ligated under ether anesthesia and 10 mL of saline was injected subcutaneously immediately after ligation to compensate for dehydration that may have occurred during fasting. To stimulate gastrin secretion, carbachol (50 μg/kg) was injected subcutaneously. Two hours after carbachol, the animals were killed with carbon dioxide. The esophagus was clamped, the stomach was dissected out and its contents emptied into a graduated test tube. The volume was read to the nearest 0.1 mL. Aliquots of gastric juice were taken for acid and pepsin determination. Acidity was determined by titration with 0.1 M NaOH either automatically (Copenhagen radiometer) or manually and was measured as output (mequiv/2 or 3 h). In most cases, 12 animals served as controls with five or six animals used for each dose of test compound.

Acknowledgment. We are grateful to the Physical and Analytical Chemistry of the Upjohn Co. for obtaining the mass spectra and microanalytical data, to Drs. M. J. Cho, P. S. Burton, and W. Morozowich from Drug Delivery Systems Research for helpful discussions and suggestions, and Mr. R. A. Morge for his expert technical assistance.