concentration, nonspecific binding), or assay buffer (control); (b) 50 μL of radioligand in assay buffer with 0.05% CHAPS; (c) 200 μL of assay buffer; (d) 200 μL of membranes in assay buffer. After 30 min of incubation at 37 °C, 1 mL of ice-cold buffer was added followed by rapid filtration under reduced pressure over Whatman glass fiber GF/B filters, presoaked with buffer, by means of a Millipore sampling manifold. Filters were washed with 1 mL of buffer used to wash the incubation tubes and three times with 2 mL of ice-cold buffer. Filters were dried at 75 °C for 45 min. The radioactivity trapped on the filters was counted in 3.5 mL of Packard Emulsifier Safe after at least a 2 h extraction time.

Data Analysis. Specific binding was defined as the difference between total binding and nonspecific binding in presence of 10 μM lorglumide. IC_{50} values were determined from pseudo-Hill plots of the displacement curves. Inhibitor dissociation constants (K_i) were calculated from the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + [L^*]/K_d)$. L^* denotes the concentration and K_d the equilibrium dissociation constant of the radioligand. A K_d value of 0.21 nM was determined for ^{3}H-(±)-L364,718 binding to rat pancreas membranes.

Acknowledgment. Use of the services of the Dutch CAOS/CAMM Center, under grant numbers SON-11-20-700 and STW-NCH-44.0703, is gratefully acknowledged.

Registry No. 1, 103420-77-5; 2, 118919-27-0; 3, 138354-37-7; 4, 138354-38-8; 5, 138354-39-9; 6, 138354-40-2; 7, 138354-41-3; 8, 138354-42-4; 9, 138354-43-5; 10, 138384-12-0; 11, 138354-44-6; 12, 138354-46-8; 13, 138354-46-8; 14, 138354-47-9; 15, 138354-48-0; 16, 138354-49-1; 17, 138354-50-4; 18, 138354-51-5; 19, 138354-52-6; 20, 138354-53-7; 21, 138354-54-8; 22, 138384-13-1; 23, 138384-14-2; II, 3531-24-6; III, 5666-18-2; IV, 5785-66-0; V, 6078-95-1; VI·HCl, 93007-57-9; VII, 122-39-4; VIII, 42393-65-7; IX, 1140-29-0; ClC-H_2-COOH, 79-11-8; diethyl (cyanomethyl)phosphonate, 2537-48-6; benzophenone, 119-61-9; indole-2-carboxylic acid, 1477-50-5; 4-chlorophenyl isocyanate, 104-12-1; potassium phthalimide, 1074-82-4; 4,4-diphenyl-3-butenyl-1-phthalimide, 95958-02-4.

(H^+,K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. 4.[1] A Novel Series of Dimethoxypyridyl-Substituted Inhibitors with Enhanced Selectivity. The Selection of Pantoprazole as a Clinical Candidate

Bernhard Kohl,* Ernst Sturm, Jörg Senn-Bilfinger, W. Alexander Simon, Uwe Krüger, Hartmann Schaefer, Georg Rainer, Volker Figala, and Kurt Klemm

Byk Gulden Pharmaceuticals, D-7750 Konstanz, Germany

Robert J. Ife and Colin A. Leach

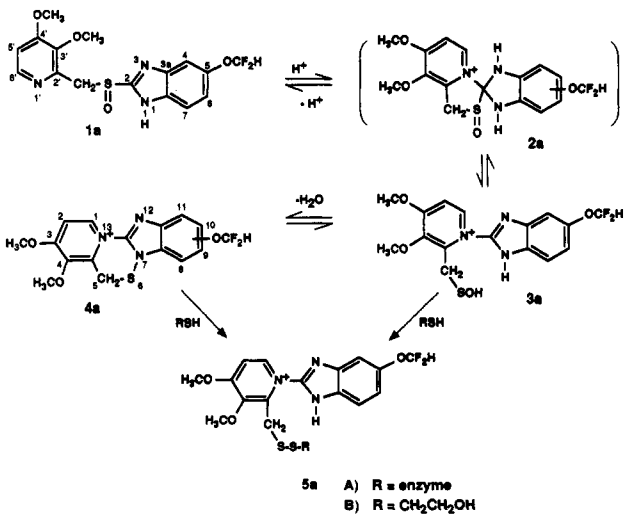
SmithKline Beecham Pharmaceuticals Ltd., The Frythe, Welwyn, Hertfordshire, England. *Received July 15, 1991*

[(Pyridylmethyl)sulfinyl]benzimidazoles 1 (PSBs) are a class of highly potent antisecretory (H^+,K^+)-ATPase inhibitors which need to be activated by acid to form their active principle, the cyclic sulfenamide 4. Selective inhibitors of the (H^+,K^+)-ATPase in vivo give rise to the nonselective thiophile 4 solely at low pH, thus avoiding interaction with other thiol groups in the body. The propensity to undergo the acid-catalyzed transformation is dependent on the nucleophilic/electrophilic properties of the functional groups involved in the formation of 2 since this step is both rate-determining and pH-dependent. The aim of this study was to identify compounds with high (H^+,K^+)-ATPase inhibitory activity in stimulated gastric glands possessing acidic pH, but low reactivity (high chemical stability) at neutral pH as reflected by in vitro (Na^+,K^+)-ATPase inhibitory activity. The critical influence of substituents flanking the pyridine 4-methoxy substituent present in all derivatives was carefully studied. The introduction of a 3-methoxy group gave inhibitors possessing a combination of high potency, similar to omeprazole and lansoprazole, but increased stability. As a result of these studies, compound 1a (INN pantoprazole) was selected as a candidate drug and is currently undergoing phase III clinical studies.

Introduction

Control of gastric pH by means of antisecretory drugs has proven to be a valuable principle in the treatment of peptic ulcers.[2] The identification of the gastric (H^+,K^+)-ATPase,[3,4] located in the apical membrane of parietal cells, as the gastric proton pump has stimulated considerable interest in the inhibitors of this enzyme as antiulcer therapeutics. Its unique environment[5] in the

Scheme I



(1) Krüger, U.; Senn-Bilfinger, J.; Sturm, E.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J. $(H^+$-$K^+)$-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. Part 3. Evidence for the Involvement of a Sulfenic Acid in Their Reactions. *J. Org. Chem.* **1990**, *55*, 4163–4168.

(2) Prous, J. R. The year's new drugs. *Drug News Perspect* **1988**, *1* (1), 38.

(3) Olbe, L.; Berglindh, T.; Elander, B.; Helander, H.; Fellenius, E.; Sjöstrand, S. E.; Sundell, G.; Wallmark, B. Properties of a New Class of Gastric Acid Inhibitors. *Scand. J. Gastroenterol.* **1979**, *14* (Suppl. 55), 131–133.

(4) Fellenius, E.; Berglindh, T.; Sachs, G.; Olbe, L.; Elander, B.; Sjöstrand, S. E.; Wallmark, B. Substituted Benzimidazoles Inhibit Gastric Acid Secretion by Blocking (H^+,K^+)-ATPase. *Nature* **1981**, *290*, 159–161.

0022-2623/92/1835-1049$03.00/0

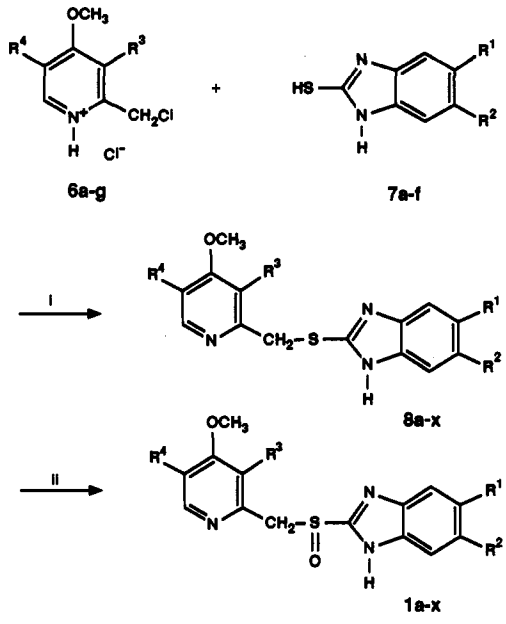
apical membrane separating the neutral cytosol from the acidic lumen is distinct from other H^+-translocating ATPases. Furthermore, it is structurally and mechanistically quite distinct, providing an excellent conceptual basis for the design of selective drugs.

[(Pyridylmethyl)sulfinyl]benzimidazoles (PSBs) 1 have proved to be highly active inhibitors of the gastric (H^+,K^+)-ATPase both in vitro and in vivo, with high and long lasting antisecretory activity.[6,7] In particular, omeprazole has been developed clinically and was successfully introduced to the market in 1989.[8] The enzyme-blocking and antisecretory activity of the PSBs is believed to be exerted by covalent modification of one or more thiol groups on the acidic lumenal side of the enzyme.[9]

Recently, we[10] and others[11] established the chemical transformations that take place under conditions resembling those of the extracytosolic part of the parietal cell (e.g. 0.1 N HCl). These involve unique reactions leading to the highly thiophilic cyclic sulfenamide 4, which is able to react rapidly with thiol groups on the enzyme to form the tightly bound enzyme-inhibitor complex 5 (Scheme I).

Accordingly, the design of selective inhibitors of the (H^+,K^+)-ATPase in vivo requires PSBs to form the non-

Scheme II. Method A

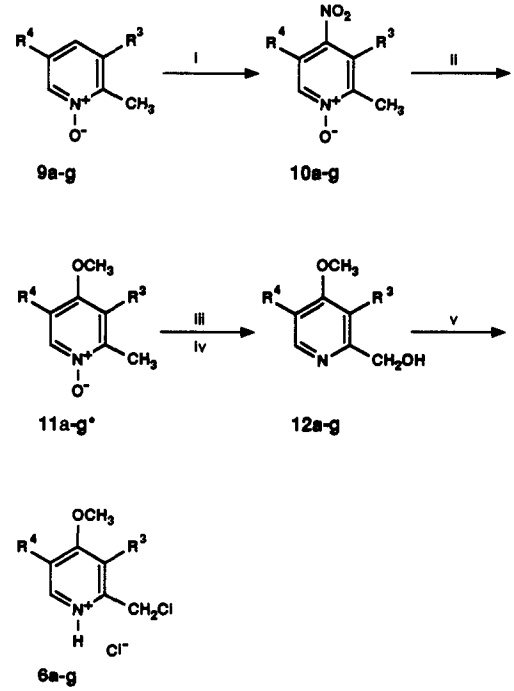


7: a: R^1 = OCF_2H, R^2 = H; b: R^1 = OCH_2CF_3, R^2 = H; c: R^1 = OCF_2CF_2H, R = H; d: R^1 + R^2 = O-CF_2-O; e: R^1 = OCF_2H, R^2 = OCH_3; f: R^1 = OCF_2H, R^2 = F

I) NaOH/EtOH/50°C

II) Method A1: mCPBA/-30°C or Method A2: NaOH/1 eq. NaOCl

Scheme III. Method B



a: R^3 = OCH_3, R^4 = H; b: R^3 = H, R^4 = OCH_3; c: R^3 = CH_3, R^4 = OCH_3; d: R^3, R^4 = H; e: R^3, R^4 = CH_3; f: R^3 = CH_3, R^4 = H; g: R^3 = H, R^4 = CH_3

(I) HNO_3/CH_2Cl_2/RT, (II) $NaOCH_3/\Delta$, (III) Ac_2O/100°C, (IV) NaOH, (V) $SOCl_2/CH_2Cl_2$

* 11c was synthesised according to Scheme 4 (Method C)

selective thiophile 4 solely at low pH, thus avoiding interaction with other thiol groups in the body. This can

(5) Sachs, G.; Carlsson, E.; Lindberg, P.; Wallmark, B. Gastric (H^+,K^+)-ATPase as Therapeutic Target. *Ann. Rev. Pharmacol. Toxicol.* **1988**, *28*, 269–284.

(6) Wallmark, B.; Lorentzon, O.; Larsson, H. The Mechanism of Action of Omeprazole—a Survey of Its Inhibitory Actions in vitro. *Scand. J. Gastroenterol.* **1985**, *20* (Suppl. 108), 37–51.

(7) Larsson, H.; Carlsson, E.; Junggren, U.; Olbe, L.; Sjöstrand, S. E.; Skanberg, I.; Sundell, G. Inhibition of Gastric Acid Secretion by Omeprazole in the Dog and Rat. *Gastroenterology* **1983**, *85*, 900–907.

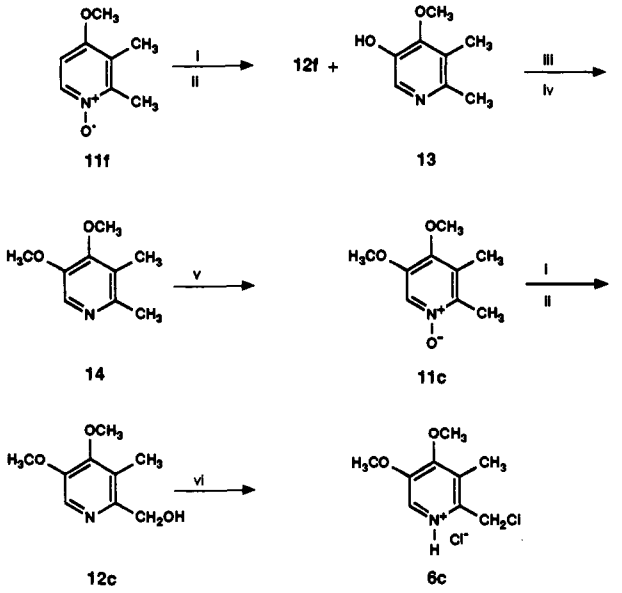
(8) Lindberg, P.; Brändström, A.; Wallmark, B.; Mattson, H.; Rikner, L.; Hoffmann, K.-J. Omeprazole: The First Proton Pump Inhibitor. *Med. Res. Rev.* **1990**, *10*, 1–54.

(9) Wallmark, B.; Brändström, A.; Larson, H. Evidence for Acid-induced Transformation of Omeprazole into an Active Inhibitor of (H^+,K^+)-ATPase within the Parietal Cell. *Biochim. Biophys. Acta* **1984**, *778*, 549–558.

(10) (a) Figala, V.; Klemm, K.; Kohl, B.; Krüger, U.; Rainer, G.; Schaefer, H.; Senn-Bilfinger, J.; Sturm, E. Acid Activation of (H^+,K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulphinyl]-benzimidazoles: Isolation and Characterization of the Thiophilic 'Active Principle' and Its Reactions. *J. Chem. Soc., Chem. Commun.* **1986**, 125–127. (b) Sturm, E.; Krüger, U.; Senn-Bilfinger, J.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. (H^+,K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. 1. Their Reaction with Thiols under Acidic Conditions. Disulfide Containing 2-Pyridiniobenzimidazolides as Mimics for the Inhibited Enzyme. *J. Org. Chem.* **1987**, *52*, 4573–4581. (c) Senn-Bilfinger, J.; Krüger, U.; Sturm, E.; Figala, V.; Klemm, K.; Kohl, B.; Rainer, G.; Schaefer, H.; Blake, T. J.; Darkin, D. W.; Ife, R. J.; Leach, C. A.; Mitchell, R. C.; Pepper, E. S.; Salter, C. J.; Viney, N. J.; Huttner, G.; Zsolnai, L. (H^+,K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. 2. The Reaction Cascade Induced by Treatment with Acids. Formation of 5*H*-Pyrido[1′,2′:4,5][1,2,4]thiadiazino[2.3-*a*]benzimidazol-13-ium Salts and Their Reactions with Thiols. *J. Org. Chem.* **1987**, *52*, 4582–4592.

(11) (a) Lindberg, P.; Nordberg, P.; Alminger, T.; Brändström, A.; Wallmark, B. The Mechanism of Action of the Gastric Acid Secretion Inhibitor Omeprazole. *J. Med. Chem.* **1986**, *29*, 1327–1329. (b) Brändström, A.; Lindberg, P.; Bergman, N.-A.; Alminger, T.; Ankner, K.; Junggren, U.; Lamm, B.; Nordberg, P.; Erickson, M.; Grundevik, I.; Hagin, I.; Hoffmann, K.-J.; Johansson, S.; Larsson, S.; Löfberg, I.; Ohlson, K.; Persson, B.; Skanberg, I.; Tekenbergs-Hjelte, L. Chemical Reactions of Omeprazole and Omeprazole Analogues. I. A Survey of the Chemical Transformations of Omeprazole and Its Analogues. *Acta Chem. Scand.* **1989**, *43*, 536–548.

Scheme IV. Method C

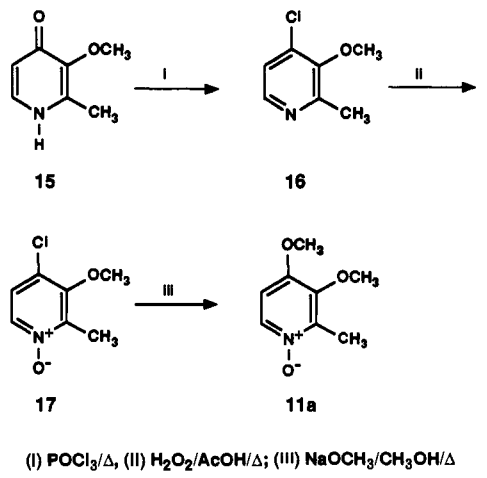


(I) $Ac_2O/100°C$, (II) NaOH, (III) KOH/DMSO, (Iv) DMS, (v) mCPBA, (vI) $SOCl_2/CH_2Cl_2$

be achieved by fine tuning the nucleophilic/electrophilic properties of the functional groups involved in the formation of **2**, since this step is both rate-determining and pH-dependent. The influence of substituents in the aromatic rings of PSBs, in particular the dominating electronic effect in the pyridine ring, has been discussed previously.[12,13]

We have studied the influence of substituents in both the pyridine and the benzimidazole moiety in a new series of dimethoxypyridyl-substituted PSBs compared with their monomethoxy congeners.[14] The purpose was to identify compounds with high (H^+,K^+)-ATPase inhibitory activity in stimulated gastric glands possessing acidic pH, but low reactivity (high chemical stability) at neutral pH, reflected by in vitro (Na^+,K^+)-ATPase inhibitory activity. As a result of these studies, compound **1a** (INN pantoprazole)[15] was selected as a clinical candidate, and is currently undergoing phase III clinical studies. The biochemical characteristics[16] and the in vivo activity profile

Scheme V. Method D



(I) $POCl_3/\Delta$, (II) $H_2O_2/AcOH/\Delta$; (III) $NaOCH_3/CH_3OH/\Delta$

have been published elsewhere.[17]

Chemistry

All compounds **1a–x** in Table I were synthesized according to method A (Scheme II) by condensation of the appropriately substituted 2-(chloromethyl)pyridines **6a–g** with the substituted 2-mercaptobenzimidazoles **7a–f**, followed by oxidation of the sulfides **8a–x** either with *m*-chloroperbenzoic acid at low temperature (method A1) or with sodium hypochlorite in alkaline aqueous solutions (method A2).[18,19]

The substituted 2-(chloromethyl)pyridine·HCl **6a–g**[20] were known or were prepared according to known methods as outlined in Scheme III (method B). Controlled nitration of the pyridine *N*-oxides **9a–g** with concentrated nitric acid gave the 4-nitro derivatives **10a–g**[20] which were converted to the 4-methoxy *N*-oxides **11a–g** by treatment with sodium methoxide in refluxing methanol. The rearrangement of **11a–g** was performed in acetic anhydride at 100 °C. Hydrolysis with NaOH readily afforded the 2-(hydroxymethyl)pyridines **12a–g** which were converted to the corresponding 2-(chloromethyl)pyridine·HCl **6a–g** by treatment with thionyl chloride in CH_2Cl_2.

The pyridine *N*-oxide **11c** leading to the 4,5-dimethoxy-3-methyl series of **1** (compounds **1c,h,l,r**) was synthesized according to method C (Scheme IV). Isomerization of **11f** with acetic anhydride gave, after hydrolysis, **12f** and as a side product the 5-hydroxypyridine **13**. Methylation of **13** with dimethyl sulfate in the presence of KOH in DMSO afforded the 4,5-dimethoxypyridine **14**, which was oxidized with mCPBA to give the *N*-oxide **11c**. This was further treated according to method B to give **6c** via **12c**.

(12) (a) Brändström, A.; Lindberg, P.; Junggren, U. Structure Activity Relationships of Substituted Benzimidazoles. *Scand. J. Gastroenterol.* **1985**, *20* (Suppl. 108), 15–23. (b) Brändström, A.; Lindberg, P.; Junggren, U.; Wallmark, B. Structure-Activity Relationships of Substituted Benzimidazoles. *Scand. J. Gastroenterol.* **1986**, *21* (Suppl. 118), 54–56. (c) Brändström, A.; Bergman, N.-A.; Lindberg, P.; Grundevik, I.; Johansson, S.; Tekenbergs-Hjelte, L.; Ohlson, K. Chemical Reactions of Omeprazole and Omeprazole Analogues. II. Kinetics of the Reaction of Omeprazole in the Presence of 2-Mercaptoethanol. *Acta Chem. Scand.* **1989**, *43*, 549–568.

(13) Ife, R. J.; Dyke, C. A.; Keeling, D. J.; Meenan, E.; Meeson, M. L.; Parsons, M. E.; Price, C. A.; Theobald, C. J.; Underwood, A. H. 2-[[(4-Amino-2-pyridyl)methyl]sulfinyl]benzimidazole (H^+,K^+)-ATPase Inhibitors. The Relationship between Pyridine Basicity, Stability, and Activity. *J. Med. Chem.* **1989**, *32*, 1970–1977.

(14) Parts of this paper have been presented at the 198th ACS Meeting 1989, Miami. (Kohl, B.; Sturm, E.; Simon, W. A.; Schaefer, H.; Keeling, D. J. Selection of BY1023/SK&F 96022 from a New Series of (H^+,K^+)-ATPase Inhibitors).

(15) Pantoprazole Sodium. *Drugs Fut.* **1990**, *15* (8), 801–804.

(16) Simon, W. A.; Keeling, D. J.; Laing, S. M.; Fallowfield, C.; Taylor, A. G. BY1023/SK&F 96022: Biochemistry of a Novel (H^+,K^+)-ATPase *Inhibitor Biochem. Pharm.* **1990**, *39*, 1799–1806.

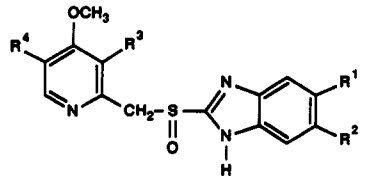
(17) (a) Kromer, W.; Postius, S.; Riedel, R.; Simon, W. A.; Hanauer, G.; Brand, U.; Gönne, S.; Parsons, M. E. BY 1023/SK&F 96022 INN Pantoprazole, a Novel Gastric Proton Pump Inhibitor, Potently Inhibits Acid Secretion But Lacks Relevant Cytochrome P450 Interactions. *J. Pharmacol. Exp. Therap.* **1990**, *254*, 129–135. (b) Hanauer, G.; Graf, U.; Meissner, T. In Vivo Cytochrome P 450 Interactions of the Newly Developed (H^+,K^+)-ATPase Inhibitor Pantoprazole (BY 1023/SK&F 96022) Compared to Other Antiulcer Drugs. *Meth. Find. Exp. Clin. Pharmacol.* **1991**, *13* (1), 63–68.

(18) Kohl, B.; Klemm, K.; Riedel, R.; Figala, V.; Rainer, G.; Schaefer, H.; Senn-Bilfinger, J. Eur. Pat. 166 287, 1985. US Pat. 4, 758, 579, 1988.

(19) Rainer, G.; Riedel, R.; Senn-Bilfinger, J.; Klemm, K.; Schaefer, H.; Figala, V. Eur. Pat. 134 400, 1984.

(20) Avoid contact with skin; handle under a well ventilated hood.

Table I. Synthesis of 2-{[(4-Methoxy-2-pyridyl)methyl]sulfinyl}benzimidazoles



1

compd[a]	R^1	R^2	R^3	R^4	method of oxidation	yield,[b] %	crystn solvent[c]	mp, °C[d]	formula	MW
1a	OCF_2H	H	OCH_3	H	A1	85	DCM/DIPE	139–40	$C_{16}H_{15}F_2N_3O_4S$	383.4
b	OCF_2H	H	H	OCH_3	A2	65	DIPE	159–60	$C_{16}H_{15}F_2N_3O_4S$	383.4
c	OCF_2H	H	CH_3	OCH_3	A1	96	DIPE	133–35	$C_{17}H_{17}F_2N_3O_4S$	397.4
d	OCF_2H	H	H	H	A1	74	EtOAc	159–61	$C_{15}H_{13}F_2N_3O_3S$	353.3
e	OCF_2H	H	CH_3	CH_3	A1	83	DCM/DIPE	145–46	$C_{17}H_{17}F_2N_3O_3S$	381.4
f	OCF_2H	H	CH_3	H	A1	77	EtOAc	166–68	$C_{16}H_{15}F_2N_3O_3S$	367.4
g	OCH_2CF_3	H	OCH_3	H	A1	87	DCM/DIPE	113–15	$C_{16}H_{17}F_3N_3O_4S$	415.4
h	OCH_2CF_3	H	CH_3	OCH_3	A1	72	DCM/DIPE	154–56	$C_{16}H_{16}F_3N_3O_4S$	429.4
i	OCH_2CF_3	H	CH_3	H	A1	81	CH_3CN	172–73	$C_{17}H_{16}F_3N_3O_3S$	399.4
j	OCF_2CF_2H	H	OCH_3	H	A1	66	DCM/DIPE	117–19	$C_{17}H_{15}F_4N_3O_4S$	433.4
k	OCF_2CF_2H	H	H	OCH_3	A1	82	DCM/DIPE	161–62	$C_{17}H_{15}F_4N_3O_4S$	433.4
l	OCF_2CF_2H	H	CH_3	OCH_3	A1	51	DCM/DIPE	125–26	$C_{18}H_{17}F_4N_3O_4S$	447.4
m	OCF_2CF_2H	H	H	H	A2	94	EtOAc/DIPE	132–34	$C_{16}H_{13}F_4N_3O_3S$	403.4
n	OCF_2CF_2H	H	CH_3	H	A1	77	DIPE	135–36	$C_{17}H_{15}F_4N_3O_3S$	417.4
o	OCF_2CF_2H	H	H	CH_3	A1	93	DIPE	163–64	$C_{17}H_{15}F_4N_3O_3S$	417.4
p	$O-CF_2-O$		OCH_3	H	A1	85	DCM/DIPE	177–78	$C_{16}H_{13}F_2N_3O_5S$	397.4
q	$O-CF_2-O$		H	OCH_3	A2	83	EtOAc	211–13	$C_{16}H_{13}F_2N_3O_5S$	397.4
r	$O-CF_2-O$		CH_3	OCH_3	A1	75	DIPE	189–90	$C_{17}H_{15}F_2N_3O_5S$	411.4
s	$O-CF_2-O$		H	H	A2	83	EtOAc	184–85	$C_{15}H_{11}F_2N_3O_4S$	367.3
t	$O-CF_2-O$		CH_3	H	A2	88	EtOAc	201–02	$C_{16}H_{13}F_2N_3O_4S$	381.3
u	OCF_2H	OCH_3	OCH_3	H	A1	83	DCM/DIPE	170–71	$C_{17}H_{17}F_2N_3O_5S$	413.4
v	OCF_2H	OCH_3	CH_3	H	A1	89	EtOAc	169–70	$C_{17}H_{17}F_2N_3O_4S$	397.4
w	OCF_2H	F	OCH_3	H	A1	73	DCM/DIPE	155–57	$C_{16}H_{14}F_3N_3O_4S$	401.4
x	OCF_2H	F	CH_3	H	A1	96	DIPE	163–64	$C_{16}H_{14}F_3N_3O_3S$	385.4

[a] All products exhibited IR and ^1H NMR spectra consistent with the assigned structures and gave satisfactory C, H, and N combustion analyses within ±0.4% of calculated values. [b] Yields are given for the oxidation step and have not been optimized. [c] DCM, dichloromethane; DIPE, diisopropyl ether. [d] Compounds decompose on melting; clearly defined melting points are not always obtainable.

An alternative approach for the 3,4-dimethoxypyridine *N*-oxide **11a**, a key intermediate for the 3,4-dimethoxy series of **1**, (compounds **1a,g,j,p,u,w**) is outlined in Scheme V (method D). Chlorination of the methoxypyridone[21] **15** with $POCl_3$ afforded the 4-chloropyridine **16**, which was oxidized to the corresponding *N*-oxide **17** by treatment with H_2O_2 in hot acetic acid, followed by exchange of the chloro substituent for methoxy by means of sodium methoxide to yield **11a**.

The mercaptobenzimidazoles **7** were prepared according to the reported methods.[19]

Results and Discussion

Stability Properties. The pH-dependent rate of activation of PSBs is a key parameter for the understanding of their biological effects. The variation of this rate with substituents on the pyridine and the benzimidazole rings is related to changes in the pK_a values (Table II) describing the equilibria for the protonation on the pyridine nitrogen and for the release of a proton from the benzimidazole ring. The activation of PSBs is reflected by their stability in solution (Table II), since the decomposition proceeds exclusively via the activated form represented by the spiro intermediate **2**.[10c]

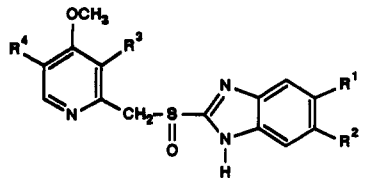
Biological Effects. In order to study in vitro potency the PSBs were evaluated for their ability to inhibit the (H^+,K^+)-ATPase (at pH < 3) in the model of fundic glands isolated from the rabbit stomach. The in vitro inhibitory activity on the (Na^+,K^+)-ATPase, representative of other SH-containing peptides or proteins, was studied to assess unwanted effects arising from nonselective inhibition at neutral pH. These data are summarized in Table II.

SAR Discussion. Structure–activity relationships of PSBs have previously been reported.[12,13] For a given substituent on the benzimidazole ring, the rate of activation as well as the antisecretory effect in gastric gland preparations were found to correlate with the pyridine pK_a (py-pK_a). The modulation of this key parameter in a series of PSBs bearing 4-amino substituents on the pyridine ring by variation of 3-halo substituents has been demonstrated, and even lowering of the pyridine pK_a down to values of around 3 was shown to be acceptable.[13] With regard to the specific accumulation of PSBs from the neutral pH cytosolic part of the parietal cell as an important factor contributing to the activity in vivo, we believed a pyridine pK_a just below 4 to be optimal. The effect of benzimidazole substituents has also been discussed.[12]

The PSBs presented in Table II were studied with the aim of optimizing biological activity and neutral pH stability. A methoxy group in the 4-position of the pyridine ring, which promotes the acid-catalyzed activation, is common to all the derivatives **1a–x**. Compounds bearing only this substituent on the pyridine ring (**1d,m,s**) exhibit a py-pK_a of 4.3. They are potent (H^+,K^+)-ATPase inhibitors but they proved to be insufficiently stable at neutral pH and showed considerable activity in the (Na^+,K^+)-ATPase test system under these conditions. An additional methyl group in the 5-position of the pyridine ring does not improve the enzyme-inhibiting properties, but reduces the neutral pH stability, probably because of the increased

(21) Hwang, D. R.; Proctor, G. R.; Driscoll, J. S. Pyridones as Potential Antitumor Agents II: 4-Pyridones and Bioisosteres of 3-Acetoxy-2-pyridone. *J. Pharm. Sci.* **1980**, *69*, 1074–1076.

Table II. Biological Activity and Stability of 2-[[(4-Methoxy-2-pyridyl)methyl]sulfinyl]benzimidazoles



1

compd	R^1	R^2	R^3	R^4	py-pK_a	imidazole pK_a	stability: $t_{1/2}$ pH 3, min	pH 5, h	pH 7.4, h	H^+/K^+-ATPase of gastric glands: -log IC_{50},[a] mol/L	Na^+/K^+-ATPase: -log IC_{50},[a] mol/L
1a	OCF_2H	H	OCH_3	H	3.92	8.19	9	3.0	130	6.0	3.7
b	OCF_2H	H	H	OCH_3	3.75	8.03	17	2.1	>200	4.5	<3.5
c	OCF_2H	H	CH_3	OCH_3	3.73	8.35	12	3.7	60	5.6	4.6
d	OCF_2H	H	H	H			7	0.8	51	5.8	4.3
e	OCF_2H	H	CH_3	CH_3	4.16	8.23	12	1.9	15	6.3	4.4
f	OCF_2H	H	CH_3	H			5	0.17	3	6.2	5.2
g	OCH_2CF_3	H	OCH_3	H	3.90	8.52	11	7.6	>200	6.0	3.5
h	OCH_2CF_3	H	CH_3	OCH_3	3.72	8.61	10	3.3	130	5.6	4.3
i	OCH_2CF_3	H	CH_3	H	4.97	8.51	5	0.15	5	6.1	5.4
j	OCF_2CF_2H	H	OCH_3	H	3.86	8.08	18	3.6	160	5.3	3.8
k	OCF_2CF_2H	H	H	OCH_3			19	2.9	140	4.7	<3.0
l	OCF_2CF_2H	H	CH_3	OCH_3			20	6.8	43	5.1	4.2
m	OCF_2CF_2H	H	H	H	4.25	7.91	10	0.8	63	5.9	4.4
n	OCF_2CF_2H	H	CH_3	H			5	0.3	insol	6.4	5.5
o	OCF_2CF_2H	H	H	CH_3	4.95	8.00	6	0.2	18	5.9	5.5
p	O-CF_2-O		OCH_3	H	3.84	7.97	38	6	>200	5.6	3.6
q	O-CF_2-O		H	OCH_3			insol	insol	insol	4.2	<3.0
r	O-CF_2-O		CH_3	OCH_3			insol	insol	insol	4.8	<3.0
s	O-CF_2-O		H	H	4.29	7.92	20	1.4	insol	5.6	<3.5
t	O-CF_2-O		CH_3	H	4.73	7.93	10	0.5	insol	6.0	4.6
u	OCF_2H	OCH_3	OCH_3	H			15	2.1	800	5.4	3.6
v	OCF_2H	OCH_3	CH_3	H	4.96	8.31	6	0.2	11	5.8	5.0
w	OCF_2H	F	OCH_3	H	3.76	7.76	26	8.3	150	5.1	3.4
x	OCF_2H	F	CH_3	H			8	0.5	4	5.5	4.7
omeprazole[b]	OCH_3	H	CH_3	CH_3	4.13	8.98	4	1.0	80	6.3	4.5
lansoprazole[c]	H	H	CH_3	H	4.01	8.78	4	1.1	40	6.4	4.4

[a] $n \geq 3$; SD $\leq \pm 0.2$. [b] Omeprazole (Hässle). [c] Lansoprazole (Takeda) (the substituent in 4-position of the pyridine is OCH_2CF_3 instead of OCH_3, as with all other compounds).

py-pK_a (compare **1m** and **1o**). Although the py-pK_a's of the 3-methyl and the 5-methyl isomers are almost identical, the (H^+,K^+)-ATPase inhibitory activity is generally enhanced by the 3-methyl group; up to -log IC_{50} values of 6.4.

Unfortunately, these highly active 4-methoxy-3-methyl compounds are among the least stable derivatives at neutral pH, and due to their significant (Na^+,K^+)-ATPase inhibition (compare pairs **1d/1f**, **1m/1n**, **1s/1t**), they are not of interest. The unfavorable contribution of the 3-methyl substituents has been explained by steric assistance of ring closure generating **2**.[12c] The most striking effect in the series of monomethoxy PSBs was found when two methyl groups were present flanking the 4-methoxy substituent (compound **1e**). In this sterically demanding arrangement the electronic effects of the pyridine substituents are not additive,[11c] and the py-pK_a is in the same range as for the derivatives with only the 4-methoxy group on the pyridine ring. Despite this, neutral stability as well as (Na^+,K^+)-ATPase inhibition of **1e** was not satisfactory.

Further variation of pyridine substituents was aimed at improving stability at neutral and weakly acidic pH, while simultaneously retaining a high level of (H^+,K^+)-ATPase inhibition. By introduction of a second methoxy group in the 3- or 5-position of the 4-methoxypyridine derivatives, py-pK_a values between 3.7 and 4.0 were obtained. Although both the 3,4- and 4,5-dimethoxy isomers display similar py-pK_a's and high stabilities under neutral conditions, as well as low activity in the (Na^+,K^+)-ATPase assay, they are far from equivalent with regard to their (H^+,K^+)-ATPase potency (compare pairs **1a/1b**, **1j/1k**, **1p/1q**). An additional methyl group in the 3-position of the pyridine of the 4,5-dimethoxy series leaves the py-pK_a almost unchanged, but enhances the reactivity at neutral pH as well as the inhibitory activity in both enzyme systems and thus does not result in further improvement. Again there seems to be a particular effect of a methyl group in the 3-position adversely affecting the pH-dependent reactivity.

Only in the series of 3,4-dimethoxypyridine substituted derivatives did we observe a high level of (H^+,K^+)-ATPase inhibition combined with low activity in the (Na^+,K^+)-ATPase assay (-log $IC_{50} < 4$). These derivatives not only display good stability at neutral pH but sustain this higher level of stability down to pH 5. This stability profile has the potential advantage of minimizing the risk of activation at low pH values found outside the parietal cell, for example, in lysosomes.[22] The activation of these PSBs to the cyclic sulfenamide is therefore optimally restricted to the parietal cell canaliculus. Depending on the benzimidazole substituent, the 3,4-dimethoxypyridine derivatives cover a range of -log IC_{50} values for the (H^+,K^+)-ATPase inhibition from 5.1 to 6.0. Incomplete stability data for the (difluoromethylene)dioxy-substituted deriv-

(22) Silver, M. S.; Haskell, J. H. Acid Sensitive Inhibitors of Proteolytic Enzymes: Synthesis and Characterization. *J. Med. Chem.* **1989**, *32*, 1253–1259 and references cited therein.

atives 1p–t is due to their low solubility at pH values near to neutral and excluded compound 1p from further evaluation. The two compounds 1a and 1g both displayed promising characteristics with regard to pH-dependent stability and enzyme inhibition. They act in vitro as (H^+,K^+)-ATPase inhibitors with -log IC_{50} values of 6.0. This is slightly less than that observed for omeprazole and lansoprazole,[23] but the differentiation between (H^+,K^+)-ATPase and (Na^+,K^+)-ATPase inhibition (Δ log $IC_{50} > 2.3$) is more pronounced (Δ log IC_{50} omeprazole 1.8, lansoprazole 2.0). Compound 1a was selected for further evaluation over 1g because of its greater water solubility (c_s, pH 10, 25 °C: 1a, 1.8×10^{-2} mL^{-1}; 1g, 2.3×10^{-3} mL^{-1}). With a second methoxy substituent in the pyridine ring of 1a, lipophilicity is minimized (log P = 2.05, octanol/aqueous buffer pH 7.4), and low global lipophilicity is believed to be of benefit with respect to potential cytochrome P450 system interactions.[16,17b]

Implications of the Mechanism of Action on Sulfoxide Stereochemistry. In selecting pantoprazole 1a for further evaluation we also studied the chemical transformations leading to its "active principle", the sulfenamide 4a, in more detail (see Scheme I). We were able to prepare analytically pure 4a very easily according to our previously described procedure by treating a methanolic solution of 1a with HPF_6 (see Experimental Section). The achiral regioisomeric 4a which is formed from racemic pantoprazole shows the characteristic long wavelength absorption in its UV spectrum (λ_{max} 334 nm). The rapid reaction of 4a with thiols such as 2-mercaptoethanol was also found to be in line with our previous results, yielding the disulfides 5. In the preceding papers[10] we discussed this reaction with respect to the covalent inhibition of the gastric (H^+,K^+)-ATPase. Due to their unique mechanism of action, therefore, the in vitro inhibitory activity of the enantiomers of 1a is anticipated to be identical as has been shown for omeprazole[24] and Ro 185364.[25]

Conclusion

The sulfoxides 1 are potent inhibitors of gastric acid secretion and elicit their activity by reaction of the corresponding sulfenamide 4 ("active principle") with thiol groups on the gastric (H^+,K^+)-ATPase in the acidic compartment of the parietal cell. The design of selective inhibitors was guided by modulating the pyridine basicity by substituents in the 3- and 5-positions. The introduction of an additional methoxy group in the 3-position gave compounds with very high (H^+,K^+)-ATPase inhibitory activity combined with high stability at neutral pH (1a and 1g). 1a (pantoprazole) was selected for further development over 1g on the basis of its higher solubility. Pantoprazole, as its sodium salt, is currently in phase III clinical trials,[26] and data available so far fit the preclinical profile.[17]

Experimental Section

Melting points are uncorrected and were determined with a Büchi 510 apparatus. ^1H and ^{13}C NMR spectra were recorded at 200.13 and 50.32 MHz, respectively, on a Bruker AC 200 spectrometer, and chemical shifts are reported in parts per million (δ) downfield from the internal standard Me_4Si. A Kratos MS 25 RFA mass spectrometer was used to obtain electron impact MS spectra. Elemental analyses (C, H, N, S) were performed by Dr. W. Rozdzinski, Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität, Pfaffenwaldring 55, D-7000 Stuttgart 80, West Germany. Analytical figures were all within ±0.4% of theoretical unless otherwise indicated. IR spectra were recorded with a Perkin-Elmer 257 grating spectrometer.

Synthesis of Benzimidazole Sulfoxides.[18,19] General Procedures. Synthesis of Sulfoxides by Oxidation with *m*-Chloroperbenzoic Acid (Method A1). 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (1a) (INN Pantoprazole). A solution of *m*-chloroperbenzoic acid (0.4 M, 20 mL) in dichloromethane was added over 15 min to a stirred solution of 8a (2.92 g, 8.0 mmol) in dichloromethane (40 mL) while maintaining the temperature at -30 °C. After 30 min, 2 mL of triethylamine were added, and the cold reaction mixture was poured into a stirred solution of sodium thiosulfate and sodium carbonate (each 10 mL of 5% solution). The organic layer was separated and two further extractions with dichloromethane were performed. The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to low volume in vacuo. Addition of diisopropyl ether afforded 1a as an off-white solid (2.61 g, 85%): mp 139–140 °C dec; H NMR ($CDCl_3$) δ 3.82 (3 H, s, OCH_3), 3.84 (3 H, s, OCH_3), 4.82 (2 H, AB, J = 13.1 Hz, $\Delta\nu$ = 14.1 Hz, CH_2SO), 6.53 (1 H, t, J = 74.2 Hz, OCF_2H), 6.78 (1 H, d, $J_{5',6'}$ = 5.5 Hz, H-5'), 7.08 (1 H, dd, $J_{6,7}$ = 8.8 Hz, $J_{6,4}$ = 2.1 Hz, H-6), 7.3 (1 H, br, H-4), 7.6 (1 H, br, H-7), 8.15 (1 H, d, H-6'), 12.71 (1 H, s, NH); EI (70 eV) MS m/z (%) 383 (11, M^+), 335 (11), 152 (53), 138 (14), 122 (23), 92 (100). Anal. ($C_{16}H_{15}F_2N_3O_4S$) C, H, N, S.

Preparation of the Sodium Salt of 1a. [Sodium 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazolide (Pantoprazole Sodium Sesquihydrate)]. NaOH (6 N, 0.43 mL, 2.6 mmol) was added dropwise to a stirred solution of 1a (1.0 g, 2.6 mmol) in a mixture of ethanol (30 mL) and dichloromethane (5 mL) at 20 °C. After 10 min diisopropyl ether (10 mL) was added slowly until the mixture became turbid, and stirring was continued for 2 h. The precipitate formed was collected by filtration, washed with diisopropyl ether, and dried in vacuo at 40 °C to yield the sodium salt of 1a as a white to off-white solid (1.0 g, 89%): decomposition

(23) Kubo, K.; Oda, K.; Kaneko, T.; Satoh, H.; Nohara, A. Synthesis of 2-[[(4-Fluoroalkoxy-2-pyridyl)methyl]sulfinyl]-1*H*-benzimidazoles as Antiulcer Agents. *Chem. Pharm. Bull. Jpn.* **1990**, *38*, 2853–2858.

(24) Erlandson, P.; Isaksson, R.; Lorentzon, P.; Lindberg, P. Resolution of the Enantiomers of Omeprazole and Some of Its Analogues by Liquid Chromatography on a Trisphenylcarbamoylcellulose-based Stationary Phase. The Effect of the Enantiomers of Omeprazole on Gastric Glands. *J. Chromatogr. Biomed. Appl.* **1990**, *532*, 305–319.

(25) Sigrist-Nelson, K.; Krasso, A.; Müller, R. K. M.; Fischli, A. E. Ro 18-5364, a potent new inhibitor of the gastric (H^+,K^+)-ATPase. *Eur. J. Biochem.* **1987**, *166*, 453–459.

(26) (a) Simon, B.; Müller, P.; Hartmann, M.; Bliesath, H.; Lühmann, R.; Huber, R.; Bohnenkamp, W.; Wurst, W. Pentagastrin-stimulated Gastric Acid Secretion and Pharmacokinetics Following Single and Repeated Intravenous Administration of the Gastric (H^+,K^+)-ATPase Inhibitor Pantoprazole (BY1023/SK&F 96022) in Healthy Volunteers. *German J. Gastroenterol.* **1990**, *28*, 443–447. (b) Simon, B.; Müller, P.; Bliesath, H.; Lühmann, R.; Hartmann, M.; Huber, R.; Wurst, W. Single Intravenous Administration of the (H^+,K^+)-ATPase Inhibitor BY1023/SK&F 96022—Inhibition of Pentagastrin—Stimulated Acid Secretion and Pharmacokinetics in Man. *Aliment. Pharmacol. Therap.* **1990**, *4*, 239–245. (c) Acton, G.; Howland, K.; Pue, M.; Broom, C. Antisecretory Activity and Preliminary Pharmacokinetics of Single Oral Doses of SK&F 96022 (BY1023). Poster PP 1023 presented at the 9th Congress of Gastroenterology, Sydney, Australia, 1990. (d) Simon, B.; Müller, P.; Marinis, E.; Bliesath, H.; Huber, R.; Hartmann, M.; Lühmann, R.; Wurst, W. Inhibition of Pentagastrin-stimulated Gastric Secretion and Pharmacokinetics in Man Following Single and Repeated Oral Doses of the (H^+,K^+)-ATPase Inhibitor BY1023/SK&F96022. Poster PP 1003 presented at the 9th Congress of Gastroenterology, Sydney, Australia, 1990. (e) Lloyd-Davies, K. A.; Acton, G.; Wareham, K.; Broom, C. Effect of Intravenous SK&F 96022 (BY 1023) on Intragastric pH in Healthy Subjects. Poster PP 1020 presented at the 9th Congress of Gastroenterology, Sydney, Australia, 1990.

above 130 °C; IR (KBr) 1576, 1304, 1166, 1118, 1068, 1035 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 289 (1.64 × 10^4); H NMR (DMSO-d_6) δ 3.77 (3 H, s, 3'-OCH_3), 3.89 (3 H, s, 4'-OCH_3), 4.50 (2 H, AB, J = 13.0 Hz, $\Delta\nu$ = 54 Hz, CH_2SO), 6.74 (1 H, dd, $J_{6,7}$ = 8.6 Hz, $J_{6,4}$ = 2.4 Hz, H-6), 7.04 (1 H, t, $J_{H,F}$ = 75.8 Hz, OCF_2H), 7.09 (1 H, d, $J_{5',6'}$ = 5.6 Hz, H-5'), 7.26 (1 H, d, $J_{4,6}$ = 2.5 Hz, H-4), 7.46 (1 H, d, H-7), 8.23 (1 H, d, H-6'); ^{13}C NMR (DMSO-d_6; 100 mg of **1a** per milliliter) δ 56.09 (q, J = 146.0 Hz, 4'-OCH_3), 57.11 (t, J = 141.9 Hz, CH_2SO), 61.10 (q, J = 145.0 Hz, 3'-OCH_3), 107.63 (d, J = 163.8 Hz, C-4), 108.09 (d, J = 161.7 Hz, C-5'), 111.21 (d, J = 158.8 Hz, C-6), 117.69 (d, J = 227.8 Hz, t, J_{CF} = 255.6 Hz, 5-$OCHF_2$), 117.69 (d, J = 158.6 Hz, C-7), 144.36 (t, $^3J_{C,F}$ = 2.9 Hz C-5), 144.58 (s, C-3'), 144.71 (s, C-7a), 146.02 (d, J = 179.2 Hz, C-6'), 146.80 (s, C-3a), 147.13 (s, C-2'), 158.51 (s, C-4'), 164.34 (s, C-2). Anal. $C_{16}H_{14}F_2N_3NaO_4S$ (water content 6.5%) C, H, N, S.

The following examples represent prototype cases of the H-NMR spectra of the title compounds 1 (Table I), prepared in analogy to **1a** (method A1) from appropriately substituted chloromethylpyridine HCl **6** and benzimidazoles **7** via the corresponding sulfides **8**.

5-(Difluoromethoxy)-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (1e): H NMR ($CDCl_3$) δ 2.16 (3 H, s, CH_3), 2.22 (3 H, s, CH_3), 3.63 (3 H, s, OCH_3), 4.75 (2 H, AB, J = 13.8 Hz, $\Delta\nu$ = 9.2 Hz, CH_2SO), 6.53 (1 H, t, J = 74 Hz, OCF_2H), 7.10–7.70 (3 H, m, benzimidazole), 8.19 (1 H, s, H-6').

2-[[(4,5-Dimethoxy-3-methyl-2-pyridinyl)methyl]-sulfinyl]-5-(2,2,2-trifluoroethoxy)-1*H*-benzimidazole (1h): H NMR ($CDCl_3$) δ 2.23 (3 H, s CH_3), 3.83 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 4.38 (2 H, q, J = 8.1 Hz, OCH_2CF_3), 4.73 (2 H, AB, J = 13.9 Hz, $\Delta\nu$ = 6.8 Hz, CH_2SO), 6.90–7.70 (3 H, m, benzimidazole), 8.02 (1 H, s, H-6'), 12.43 (1 H, br s, NH).

2-[[(4,5-Dimethoxy-2-pyridinyl)methyl]sulfinyl]-5-(1,1,2,2-tetrafluoroethoxy)-1*H*-benzimidazole (1k): H NMR (DMSO-d_6) δ 3.57 (3 H, s, OCH_3), 3.81 (3 H, s, OCH_3), 4.73 (2 H, AB, J = 13.0 Hz, $\Delta\nu$ = 22.8 Hz, CH_2SO), 6.77 (1 H, s, H-3'), 6.84 (1 H, t of t, J_1 = 52 Hz, J_2 = 3.1 Hz, OCF_2CF_2H), 7.20–7.70 (3 H, m, benzimidazole), 8.08 (1 H, s, H6').

2-[[(4-Methoxy-5-methyl-2-pyridinyl)methyl]sulfinyl]-5-(1,1,2,2-tetrafluoroethoxy)-1*H*-benzimidazole (1o): H NMR ($CDCl_3$) δ 2.06 (3 H, s, CH_3), 3.47 (3 H, s, OCH_3), 4.63 (2 H, AB, J = 13.1 Hz, $\Delta\nu$ = 37.5 Hz, CH_2SO), 5.96 (1 H, t of t, J_1 = 53 Hz, J_2 = 2.8 Hz, OCF_2CF_2H), 6.40 (1 H, s, H-3'), 7.15–7.65 (3 H, m, benzimidazole), 8.08 (1 H, s, H-6').

5-(Difluoromethoxy)-6-methoxy-2-[[(4-methoxy-3-methyl-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (1v): H NMR ($CDCl_3$) δ 2.12 (3 H, s, CH_3), 3.83 (3 H, s, OCH_3), 3.89 (3 H, s, OCH_3), 4.79 (2 H, AB, J = 13.8 Hz, $\Delta\nu$ = 8.0 Hz, CH_2SO), 6.54 (1 H, t, J = 75 Hz, OCF_2H), 6.80 (1 H, d, $J_{5',6'}$ = 5.7 Hz, H-5'), 7.20 (1 H, s, H-7), 7.48 (1 H, s, H-4), 8.25 (1 H, d, H-6').

5-(Difluoromethoxy)-6-fluoro-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1*H*-benzimidazole (1w): H NMR ($CDCl_3$) δ 3.83 (3 H, s, OCH_3), 3.87 (3 H, s, OCH_3), 4.79 (2 H, AB, J = 13.1 Hz, $\Delta\nu$ = 23.5 Hz, CH_2SO), 6.58 (1 H, t, J = 73.6 Hz, OCF_2H), 6.81 (1 H, d, $J_{5',6'}$ = 5.5 Hz, H-5'), 7.20–7.56 (2 H, br, benzimidazole), 8.17 (1 H, d, H-6'), 12.66 (1 H, br s, NH).

Synthesis of Sulfoxides by Oxidation with Aqueous Alkaline Sodium Hypochlorite Solution (Method A2). 2,2-Difluoro-6-[[(4-methoxy-2-pyridinyl)methyl]sulfinyl]-5*H*-[1,3]dioxolo[4,5-*f*]benzimidazole (1s). A mixture of sodium hypochlorite (10% strength, 0.24 mol) and 6 N NaOH (40 mL) was added to a well-stirred solution of **8s** (80 g, 0.228 mol) in ethyl acetate (800 mL) and 6 N NaOH (45 mL) during 1 h at 0 °C. After 30 min sodium thiosulfate (5 mL, 5% solution) was added. The organic layer was separated, washed with water, treated with active charcoal (2 g), and, after filtration, adjusted to pH 7.2 by addition of sodium dihydrogenphosphate solution to precipitate a solid, which was recrystallized from ethyl acetate to give **1s** (69.5 g, 83%): mp 184–85 °C. H NMR (DMSO-d_6) δ 3.75 (3 H, s, OCH_3), 4.68 (2 H, AB, J = 12.9 Hz, $\Delta\nu$ = 15.2 Hz, CH_2SO), 6.90 (1 H, s, H-3'), 6.91 (1 H, d, J = 4.7 Hz, H-5'), 7.66 (2 H, s, H-4 + H-7), 8.32 (1 H, d, J = 4.7 Hz, H-6'). Anal. ($C_{15}H_{11}F_2N_3O_4S$) C, H, N.

Isomeric Mixture (1:1) of 9(10)-(Difluoromethoxy)-3,4-dimethoxy-5*H*-pyrido[1',2':4,5][1,2,4]thiadiazino[2,3-*a*]benzimidazol-13-ium Hexafluorophosphate ($4a{\cdot}PF_6^-$). Aqueous hexafluorophosphoric acid (50%, 1.7 mL, 12.9 mmol) was added in one portion to a stirred suspension of **1a** (3.3 g, 8.6 mmol) in methanol (8 mL) at 0 °C. After 1.5 h at 0–5 °C the precipitated solid was filtered off, washed with little ice-cold methanol and diisopropyl ether, and dried in vacuo at 30 °C to give **4a** as a yellow solid (3.34 g, 76%): mp 146–147 °C dec; H NMR (CD_3OD) [partially separated signals of two isomers A (9-difluoromethoxy isomer) and B (10-difluoromethoxy isomer) with the ratio 1/1] 4.12 (3 H, s, OCH_3 of both isomers), 4.39 (3 H, s, OCH_3 of both isomers), 5.06 (2 H, s, CH_2S of both isomers), 6.88 and 6.93 (0.5 H, t and 0.5 H, t, J = 74 Hz, OCF_2H of both isomers), 7.27 (0.5 H, dd, J = 9 Hz, J = 2 Hz, H-10 of isomer A or H-9 of isomer B), 7.33 (0.5 H, dd, J = 9 Hz, J = 2 Hz, H-9 of isomer B or H-10 of isomer A), 7.39 (0.5 H, d, J = 2 Hz, H-11 of isomer B or H-8 of isomer A), 7.61 (0.5 H, s(br), H-8 of isomer A or H-11 of isomer B), 7.64 (0.5 H, d, J = 8.9 Hz, H-8 of isomer B or H-11 of isomer A), 7.84 (0.5 H, d, J = 8.9 Hz, H-11 of isomer A or H-8 of isomer B), 7.95 (1 H, d, $J_{2,1}$ = 7.5 Hz, H-2 of both isomers), 9.47 and 9.48 (0.5 H, d, and 0.5 H, d, H-1 of both isomers); UV (MeOH containing 0.4 vol% of 1 N HCl) λ_{max} (ϵ) 334 (1.39 × 10^4), 258 (7.4 × 10^3). Anal. ($C_{16}H_{14}F_8N_3O_3PS$) C, H, N.

In Situ Preparation of 9(10)-(Difluoromethoxy)-3,4-dimethoxy-5*H*-pyrido[1',2':4,5][1,2,4]thiadiazino[2,3-*a*]benzimidazol-13-ium Chloride ($4a{\cdot}Cl^-$) from 1a in the NMR Tube. Compound **1a** (10 mg, 3.5 × 10^{-5} M) was dissolved in CD_3OD (0.5 mL) and DCl (20% in D_2O, 20 μL) was added. The sulfenamide **4** salt produced reached a maximum concentration after 10–30 min but was transformed into further products (thiosulfinate and dimeric disulfide) analogous to those described in ref 1. The presence of Cl^- causes rapid isomerization of the 9- and 10-difluoromethoxy regioisomers,[10b] leading to the partial coalescence of the signals separated in the spectrum of the $4{\cdot}PF_6^-$ salt. $4a{\cdot}Cl^-$: H NMR δ 4.13 (3 H, s, OCH_3), 4.41 (3 H, s, OCH_3), 5.11 (2 H, s, SCH_2), 6.92 (1 H, t, $J_{H,F}$ = 73.9 Hz), 7.3 (1 H, s(br), H-9 (H-10)), 7.5 and 7.7 (1 H, s(br) and 1 H, d, J = 9 Hz, H-8 and H-11), 7.99 (1 H, d, $J_{2,1}$ = 7.5 Hz, H-2), 9.48 (1 H, d, H-1).

Formation of 5a (R = CH_2CH_2OH) from $4a{\cdot}PF_6^-$ and Excess 2-Mercaptoethanol in the NMR Tube. Compound $4a{\cdot}PF_6^-$ (5 mg, 9.8 × 10^{-6} M) was dissolved in CD_3OD (0.5 mL) and DCl (20% in D_2O, 5 μL), and 2-mercaptoethanol (50 μL, 7.2 × 10^{-4} M) was added. The spectrum indicated a complete reaction immediately after the addition and was found to be unchanged for at least 17 h: H NMR δ 4.15 (3 H, s, OCH_3), 4.38 (3 H, s, OCH_3), 4.60 (2 H, s, 2'-CH_2), 6.90 (1 H, t, $J_{H,F}$ = 74.1 Hz, CF_2H), 7.29 (1 H, dd, $J_{6,7}$ = 8.9 Hz, $J_{6,4}$ = 2.0 Hz, H-6), 7.53 (1 H, s(br), H-4), 7.77 (1 H, d, H-7), 7.91 (1 H, d, $J_{5',6'}$ = 7.3 Hz, H-5'), 9.01 (1 H, d, H-6'). The signals of the —SCH_2CH_2O— side chain [triplets at δ 2.6 (SCH_2) and 3.6 (OCH_2)] were superimposed by the signals of 2-mercaptoethanol starting material.

Preparation of 2-(Chloromethyl)pyridine Hydrochlorides (6) (Method B).[20] General Procedure. Compounds **6d,e,f,g**[27,28] were prepared according to the literature methods; **6c** was prepared according to method C.

2-(Chloromethyl)-3,4-dimethoxypyridine Hydrochloride (6a).[29] Thionyl chloride (2 mL, 27.5 mmol) in dry dichloromethane (10 mL) was added dropwise to a cooled (0–5 °C) stirred solution of **12a** (3.38 g, 20 mmol) in dichloromethane (30 mL). The mixture was allowed to warm up to 20 °C and, after 2 h, concentrated to low volume in vacuo. Addition of toluene afforded **6a** as a colorless solid (4.2 g, 93%): mp 158–59 °C dec (lit.[29] mp 158–59 °C); H NMR ($CDCl_3$) δ 4.09 (3 H, s, OCH_3), 4.23 (3 H, s, OCH_3), 5.06 (2 H, s, CH_2Cl), 7.56 (1 H, d, J = 6.6 Hz, H-5), 8.56 (1 H, d, H-6). Anal. ($C_8H_{10}ClNO_2{\cdot}HCl$) C, H, N, Cl.

2-(Chloromethyl)-4,5-dimethoxypyridine hydrochloride (6b): from **12b**;[30] yield 81%; mp 160–61 °C dec; H NMR ($CDCl_3$)

(27) Brändström, A.; Carlson, S.; Källson, B.; Lindberg, P. Ger. Offen. DE 3404610, 1984.
(28) Senn-Bilfinger, J.; Schaefer, H.; Figala, V.; Klemm, K.; Rainer, G.; Riedel, R.; Schudt, Ch.; Simon, W. Eur. Pat. 080602, 1981.
(29) (a) Brown, T. H. Eur. Pat. 0004793, 1979. (b) Nohara, A.; Maki, Y. Eur. Pat. 0208452, 1986.
(30) Besso, H.; Imafuku, K.; Matsamura, H. Tautomerism of 4-Pyridones. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 710–712.

δ 4.03 (3 H, s, OCH_3), 4.19 (3 H, s, OCH_3), 5.11 (2 H, s, CH_2Cl), 7.42 (1 H, s, H-3), 8.13 (1 H, s, H-6). Anal. ($C_8H_{10}ClNO_2 \cdot HCl$) C, H, N.

2-(Chloromethyl)-4,5-dimethoxy-3-methylpyridine hydrochloride (6c): from **12c**; yield 99%; mp 125–6 °C dec; H NMR ($CDCl_3$ + DMSO-d_6) δ 2.37 (3 H, s, CH_3), 4.04 (3 H, s, OCH_3), 4.27 (3 H, s, OCH_3), 5.07 (2 H, s, CH_2Cl), 8.24 (1 H, s, H-6). Anal. ($C_9H_{12}ClNO_2 \cdot HCl$) C, H, N.

Preparation of Sulfides 8. General Procedures. 5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1*H*-benzimidazole (8a). Compound **6a** (2.24 g, 0.01 mol) was added over 10 min with stirring to a heated (50 °C) solution of 5-(difluoromethoxy)-2-mercapto-1*H*-benzimidazole (**7a**) (2.16 g, 0.01 mol) in ethanol (20 mL) and 2 N NaOH (11 mL, 0.022 mol). After 2 h at the stated temperature, ethanol was removed on a rotary evaporator and the residual suspension was extracted three times with dichloromethane. The combined organic layers were washed with 0.1 N NaOH, dried ($MgSO_4$), and evaporated in vacuo to give crude 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]thio]-1*H*-benzimidazole (**8a**) (3.38 g, 92%) as an amorphous residue which was used in the next step without further purification.[31] Crude **8a** can be recrystallized from dichloromethane/diisopropyl ether to give **8a** as a white solid: mp 118–9 °C; H NMR ($CDCl_3$) δ 3.92 (3 H, s, OCH_3), 3.94 (3 H, s, OCH_3), 4.40 (2 H, s, CH_2S), 6.51 (1 H, t, J = 74.6 Hz, OCF_2H), 6.86 (1 H, d, $J_{5',6'}$ = 5.7 Hz, H-5'), 6.98 (1 H, dd, $J_{6,7}$ = 2.2 Hz, H-6), 7.32 (1 H, d, H-4), 7.63 (1 H, d, H-7), 8.26 (1 H, d, H-6'), 12.5 (1 H, br, NH). Anal. ($C_{16}H_{15}F_2N_3O_3S$) C, H, N.

2,2-Difluoro-6-[[(4-methoxy-2-pyridinyl)methyl]thio]-5*H*-[1,3]dioxolo[4,5-*f*]benzimidazole (8s). A solution of **6d** (19.4 g, 0.1 mol) in 50 mL of water was added over 15 min to a stirred mixture of **7d** (24.4 g, 0.105 mol), 4 N NaOH (55 mL, 0.22 mol), and ethanol (100 mL) at 60 °C. After 4 h, the mixture was diluted with water (100 mL) and allowed to cool to 20 °C. The solid was collected, washed subsequently with NaOH (0.01 N), water, and a 1:1 mixture of ethanol/water and dried to afford a solid which was recrystallized from toluene to give **8s** (28.45 g, 81%): mp 150–51 °C. H NMR ($CDCl_3$) δ 3.89 (3 H, s, OCH_3), 4.29 (2 H, s, CH_2S), 6.82 (1 H, dd, $J_{5',6'}$ = 5.8 Hz, $J_{3',5'}$ = 2.4 Hz, H-5'), 6.88 (1 H, d, H-3'), 7.22 (2 H, s, H-4 + H-6), 8.46 (1 H, d, H-6'). Anal. ($C_{15}H_{11}F_2N_3O_3S$) C, H, N.

3-Methoxy-2-methyl-4-nitropyridine *N*-Oxide (10a).[20] Nitric acid (98%, 40 mL) was added to a stirred, heated (80 °C) solution of 3-methoxy-2-methylpyridine *N*-oxide (18.0 g, 0.13 mol) in glacial acetic acid (40 mL) over a period of 6 h. After 18 h a further 40 mL of nitric acid was added over a period of 6 h. After a further 15 h, the mixture was cooled, poured onto ice (50 g), and adjusted to pH 6, with cooling, using 40% sodium hydroxide. The precipitate (3-methoxy-2-methyl-6-nitropyridine, mp 144 °C) was filtered off and the filtrate extracted with dichloromethane (4 × 50 mL). The combined organic layers were dried ($MgSO_4$), concentrated to low volume, and triturated with petroleum ether (50/70) to give **10a** (13.7 g, 58%): mp 103–04 °C. H NMR ($CDCl_3$) δ 2.52 (3 H, s, CH_3), 3.99 (3 H, s, OCH_3), 7.77 (1 H, d, $J_{5,6}$ = 7.3 Hz, H-5), 8.13 (1 H, d, H-6). Anal. ($C_7H_8N_2O_4$) C, H, N.

3,4-Dimethoxy-2-methylpyridine *N*-Oxide (11a).[32] A mixture of **10a**, (4.5 g, 25 mmol) and sodium methoxide (2.7 g, 50 mmol) in dry methanol (75 mL) was stirred at 40 °C for 16 h. After cooling, the solution was adjusted to pH 7 by addition of concentrated sulfuric acid. The mixture was evaporated in vacuo and the residue extracted with toluene (50 mL). After filtration, to remove insoluble inorganic salts, the filtrate was evaporated in vacuo to a yellow oil. Chromatography (silica gel, CH_2Cl_2/MeOH) followed by trituration with petroleum ether (50/70) at 40 °C afforded **11a** (5.2 g, 88%): mp 111–13 °C; H NMR ($CDCl_3$) δ 2.50 (3 H, s, CH_3), 3.85 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 6.71 (1 H, d, $J_{5,6}$ = 7.3 Hz, H-5), 8.10 (1 H, d, H-6). Anal. ($C_8H_{11}NO_3$) C, H, N.

2-(Hydroxymethyl)-3,4-dimethoxypyridine (12a). A solution of **11a** (4.8 g, 28.4 mmol) in acetic anhydride (25 mL) was heated at 90 °C for 2 h. After evaporation in vacuo, the dark oily residue was agitated with 2 N NaOH (20 mL) for 2 h at 80 °C. After cooling the product was extracted into dichloromethane, dried (K_2CO_3), and concentrated in vacuo to low volume. Addition of petroleum ether (50/70) afforded **12a** as a colorless solid (3.60 g, 76%): mp 93–95 °C; H NMR ($CDCl_3$) δ 3.85 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 4.76 (2 H, s, CH_2O), 6.82 (1 H, d, $J_{5,6}$ = 5.6 Hz, H-5), 8.22 (1 H, d, H-6). Anal. ($C_8H_{11}NO_3$) C, H, N.

Preparation of 12c (Method C). 5-Hydroxy-4-methoxy-2,3-dimethylpyridine (13). Compound **11f**[18] (1000 g, 6.53 mol) was added over 7 h in portions with stirring to heated (100 °C) acetic anhydride (3 L). After 3 h, excess acetic anhydride was removed in vacuo and the residue was distilled under reduced pressure. The fraction with a boiling range from 95–145 °C (1 Pa) was collected and added over 30 min to vigorously stirred 2 N NaOH (3.5 L) at 50 °C. The temperature was raised to 80 °C and, after 3 h, the solution was allowed to cool to room temperature and extracted three times with dichloromethane (3 L). The combined organic layers were back-extracted with 1 N NaOH (2 × 0.5 L). The combined aqueous layers were adjusted to pH 7.5 with 2 N HCl under vigorous stirring to precipitate an off-white solid which was rinsed with water and dried to afford **13** (130 g, 13%): mp 274–76 °C; H NMR (DMSO-d_6) δ 2.08 (3 H, s, CH_3), 2.28 (3 H, s, CH_3), 3.79 (3 H, s, OCH_3), 7.85 (1 H, s, H-6), 9.44 (1 H, s, OH). Anal. ($C_8H_{11}NO_2$) C, H, N.

4,5-Dimethoxy-2,3-dimethylpyridine (14). Compound **13** (9.18 g, 60 mmol) was dissolved in a solution of KOH (3.60 g, 64 mmol) in methanol (40 mL) and dimethyl sulfoxide (50 mL). The methanol was removed in vacuo, and methyl iodide (8.95 g, 63 mmol) was added dropwise at 20 °C with rapid stirring. After 15 h, the mixture was subjected to steam distillation. The distillate was adjusted to pH 13 with sodium hydroxide and extracted with dichloromethane in a continuous extractor. The combined extracts were dried (KOH) and evaporated to give **14** as a low melting solid (7.4 g, 74%): mp 36–38 °C; H NMR ($CDCl_3$) δ 2.17 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 3.88 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 7.99 (1 H, s, H-6). Anal. ($C_9H_{13}NO_2$) C, H, N.

2-(Hydroxymethyl)-4,5-dimethoxy-3-methylpyridine (12c). *m*-Chloroperbenzoic acid (9.7 g, 0.056 mol) in dichloromethane (50 mL) was added over 30 min to a stirred solution of **14** (6.3 g, 0.038 mol) in dichloromethane (50 mL) at 20–30 °C. After 2 h, the solution was heated at reflux for 4 h, cooled, and washed with a mixture of aqueous sodium carbonate and sodium thiosulfate (5% each). The water layer was back-extracted with dichloromethane, and the combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo. The residual crude 4,5-dimethoxy-2,3-dimethylpyridine *N*-oxide **11c** (4.6 g, 66%) was dissolved in acetic anhydride (20 mL), heated at 110 °C for 30 min, and evaporated in vacuo to a dark brown oil. NaOH (3 N, 30 mL) was added and the mixture heated at 80 °C for 2 h. After cooling the product was extracted into dichloromethane, dried (K_2CO_3), and evaporated in vacuo to a solid. Trituration with petroleum ether (50/70) afforded **12c** (overall yield 4.0 g, 57%): mp 91–2 °C; H NMR ($CDCl_3$) δ 2.10 (3 H, s, CH_3), 3.92 + 3.93 (2 × 3 H, 2 × s, 2 × OCH_3), 4.60 (2 H, s, CH_2O), 4.71 (1 H, brs, OH), 8.06 (1 H, s, H-6). Anal. ($C_9H_{13}NO_3$) C, H, N.

Alternative Synthesis of 11a from 15 (Method D). 4-Chloro-3-methoxy-2-methylpyridine (16). Phosphorus oxychloride (100 mL) and 3-methoxy-2-methyl-4(1*H*)-pyridone **15**[21] (13.9 g, 0.1 mol) were stirred at 90 °C for 18 h, concentrated in vacuo, and cooled to 20 °C. The residue was treated with ice–water and adjusted to pH 12 with 40% sodium hydroxide, and the product was extracted into dichloromethane. The residue obtained on evaporation of the combined extracts was distilled at reduced pressure to give **16** (15.1 g, 96%): bp 75 °C (10^3Pa); H NMR ($CDCl_3$) δ 2.55 (3 H, s, CH_3), 3.86 (3 H, s, OCH_3), 7.18 (1 H, d, $J_{5,6}$ = 5.1 Hz, H-5), 8.15 (1 H, d, H-6). Anal. (C_7H_8ClNO) C, H, N.

4-Chloro-3-methoxy-2-methylpyridine *N*-Oxide (17).[20] A mixture of hydrogen peroxide (67 mL of 30% solution, 0.68 mol), **16** (27 g, 0.17 mol), and acetic acid (0.5 L) was heated to 90 °C. After 24 h the solution was evaporated in vacuo to an oil. Purification by chromatography (silica gel, CH_2Cl_2/MeOH) gave **17** (26.5 g, 90%): mp 95–96 °C; H NMR ($CDCl_3$) δ 2.53 (3 H, s, CH_3), 3.89 (3 H, s, OCH_3), 7.16 (1 H, d, $J_{5,6}$ = 7.0 Hz, H-5), 8.08 (1 H, d, H-6). Anal. ($C_7H_8ClNO_2$) C, H, N.

(31) The sulfides were used in turn without further purification in the following oxidation step.

(32) An alternative synthesis of **11a** is described in method D.

3,4-Dimethoxy-2-methylpyridine *N*-Oxide (11a) from 17. Sodium methoxide (12.4 g, 0.23 mol) and 17 (20 g, 0.115 mol) in methanol (100 mL) were heated at reflux. After 18 h, the solvent was evaporated in vacuo, the residue was extracted with hot toluene (100 mL), and the insolubles were filtered off. Addition of diisopropyl ether to the filtrate afforded 11a as a colorless solid (17.7 g, 91%), mp 111–12 °C.

Stability and pK_a Measurements. Stability at ambient temperature (25 °C) was determined in methanol/phosphate buffer (5:95) which had been adjusted to the appropriate pH. The initial concentration of compounds was between 1×10^{-3} and 3×10^{-4} M, and the decrease in concentration was monitored by HPLC (μBondapak C_{18}, CH_3CN/KH_2PO_4, pH 7.4, 20–80% CH_3CN in 25 min, 1 mL min^{-1}, 40 °C, detector UV 280 nm). Half-lives ($t_{1/2}$) were determined from the linear regression of natural log of the concentration vs time (h). The pK_a's of the PSBs were measured spectrophotometrically at ambient temperature (25 °C).

In Vitro Biological Assays. Acid Secretion in Rabbit Isolated Gastric Glands.[33] Rabbit fundic glands (White New Zealanders, 2–3 kg body weight) were obtained by high-pressure perfusion of the circulation of the stomach and subsequent collagenase treatment of pieces of fundic mucosa. After the glands had been washed several times, they were placed in 20-mL vials with dibutyryl cyclic AMP (1 mmol/L) and the test compound (3×10^{-8} to 10^{-4} mol/L) in the presence of 0.125 μmol/L [^{14}C]-aminopyrine (^{14}C AP) and were incubated at 37 °C. The incubate was agitated (150 oscillations/min) for 30 min and the reaction stopped by centrifugation (10 s at 20000*g*).

The ability of the glands to maintain a pH gradient to the medium (pH 7.4) on stimulation with dibutyryl cyclic AMP is measured by means of the concentration ratio of [^{14}C]aminopyrine between glands and medium.[34]

(Na^+,K^+)-ATPase Activity Test. Dog kidney enzyme (20 μg, Sigma, München) was incubated in 50 mmol/L HEPES buffer pH 7.4 in presence of (mmol/L) 140 NaCl, 10 KCl, 3 ATP-Mg, 0.5 EDTA, and PSBs in the concentration range 0–300 μmol/L. At the end of the incubation the inorganic phosphate released from ATP was determined.[35]

Registry No. **1a**, 102625-70-7; **1a**·Na, 138786-67-1; **1b**, 102625-55-8; **1c**, 102625-68-3; **1d**, 97964-04-0; **1e**, 138786-68-2; **1f**, 97964-05-1; **1g**, 138786-69-3; **1h**, 138786-70-6; **1i**, 97964-02-8; **1j**, 102625-69-4; **1k**, 102625-57-0; 1l, 102625-61-6; 1m, 97963-96-7; **1n**, 97963-97-8; **1o**, 97963-98-9; **1p**, 102625-81-0; **1q**, 102625-80-9; **1r**, 102625-72-9; **1s**, 97966-85-3; **1t**, 97966-86-4; **1u**, 138786-71-7; **1v**, 97964-10-8; **1w**, 138786-72-8; **1x**, 138786-73-9; **4a**·PF_6^- (isomer 1), 138786-75-1; **4a**·PF_6^- (isomer 2), 138786-76-2; **4a**·Cl^- (isomer 1), 138786-77-3; **4a**·Cl^- (isomer 2), 138786-78-4; **5a** (R = CH_2CH_2OH), 138786-80-8; **6a**, 72830-09-2; **6b**, 102625-83-2; **6c**, 102625-85-4; **6d**, 62734-08-1; **6e**, 86604-75-3; **6f**, 86604-74-2; **6o**, 94452-59-2; **7a**, 97963-62-7; **7g**, 102625-84-3; **7j**, 97963-60-5; **7p**, 97967-01-6; **7u**, 97963-65-0; **7w**, 97963-64-9; **8a**, 102625-64-9; **8b**, 102625-52-5; **8c**, 102625-62-7; **8d**, 97964-23-3; **8e**, 138786-81-9; **8f**, 97964-24-4; **8g**, 138786-82-0; **8h**, 138786-83-1; **8i**, 97964-21-1; **8j**, 102625-63-8; **8k**, 102625-56-9; 8l, 102625-60-5; **8m**, 97964-15-3; **8n**, 97964-16-4; **8o**, 97964-17-5; **8p**, 102625-75-2; **8q**, 102625-79-6; **8r**, 102625-71-8; **8s**, 97966-99-9; **8t**, 97967-00-5; 8u, 102625-65-0; **8v**, 97963-58-1; **8w**, 138786-84-2; **8x**, 138786-85-3; **10a**, 15931-25-6; **11a**, 72830-07-0; **11c**, 102625-93-4; **11f**, 102625-96-7; **12a**, 72830-08-1; **12b**, 62885-49-8; **12c**, 102625-92-3; **13**, 102625-95-6; **14**, 102625-94-5; **15**, 76015-11-7; **16**, 107512-34-5; **17**, 122307-41-9; 3-methoxy-2-methylpyridine *N*-oxide, 35392-65-5; ATPase, 9000-83-3.

(33) Berglindh, T.; Öbrink, K. J. A Method for Preparing Isolated Glands from the Rabbit Gastric Mucosa. *Acta Physiol. Scand.* **1976**, *96*, 150–159.

(34) Berglindh, T.; Helander, H. F.; Öbring, K. J. Effects of Secretagogues on Oxygen Consumption, Aminopyrine Accumulation and Morphology in Isolated Gastric Glands. *Acta Physiol. Scand.* **1976**, *97*, 401–414.

(35) Yoda, A.; Hokin, L. E. On the Reversibility of Binding of Cardiac Steroids to a Partially Purified (Na^+,K^+)-activated ATPase From Beef Brain. *Biochem. Biophys. Res. Commun.* **1979**, *40*, 880.