Studies on Proton Pump Inhibitors. I. Synthesis of 8-[(2-Benzimidazolyl)sulfinyl]-5,6,7,8-tetrahydroquinolines and Related Compounds

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Many 8-[(2-benzimidazolyl)sulfinyl]-5,6,7,8-tetrahydroquinolines were synthesized and examined for their ($H^+ + K^+$) adenosine triphosphatase ATPase-inhibitory and antisecretory activities. These sulfinyl compounds could be considered to be rigid analogues of the 2-[(2-pyridyl)methylsulfinyl]benzimidazole class of antisecretory agents. All the compounds tested were potent inhibitors of ($H^+ + K^+$)ATPase. Most of the compounds also inhibited histamine-induced gastric acid secretion in rats. Among them, 8-[(5-fluoro-2-benzimidazolyl)sulfinyl]-3-methyl-5,6,7,8-tetrahydroquinoline (XIVm) was found to have the most potent activity. The structure–activity relationships are discussed.

Keywords proton pump inhibitor; ($H^+ + K^+$)ATPase-inhibitory activity; 2-[(2-pyridyl)methylsulfinyl]benzimidazole; antisecretory activity; 8-[(5-fluoro-2-benzimidazolyl)sulfinyl]-3-methyl-5,6,7,8-tetrahydroquinoline; antiulcer activity; cytoprotective activity; structure–activity relationship

Introduction

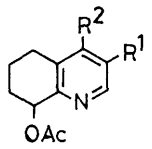
Peptic ulcer has been generally thought to result from an imbalance between the aggressive forces of acid and pepsin and the defensive forces of resistance. Consequently, antiulcer therapy has been directed toward these two factors.

Since the discovery of the histamine H_2 receptor antagonist cimetidine[1] and its clinical success, the inhibition of gastric acid secretion has been the major focus of antiulcer therapy. Recently, the benzimidazole derivative omeprazole, one of a series of compounds which act by inhibiting the parietal cell ($H^+ + K^+$) adenosine triphosphatase (ATPase), was discovered by Fellenius *et al.*[2] Clinical studies have shown that omeprazole affords a complete healing of peptic ulcers. Proton pump inhibitors are promising drugs for the treatment of peptic ulcer diseases. Therefore, we were interested in synthesizing analogues of substituted benzimidazole derivatives.

5,6,7,8-Tetrahydroquinolines are well known as rigid analogues of 2-methylpyridine, which are components of antiulcer agents[3] and antitumor agents.[4] We describe here the synthesis and biological activity of some 2-sulfinylbenzimidazole derivatives having a substituted 5,6,7,8-tetrahydroquinoline moiety.

Synthesis 8-Bromo-5,6,7,8-tetrahydroquinoline derivatives (Va—f), which are versatile key intermediates in the synthesis of the sulfides (XIIIa—v), were synthesized as shown in Chart 1. Tetrahydroquinoline *N*-oxides (IIa, b, VIIa—d) were rearranged with acetic anhydride to give the

TABLE I. 8-Acetoxy-5,6,7,8-tetrahydroquinolines



Compd. No.[a]	R^1	R^2	Yield (%)	^{1}H-NMR δ ($CDCl_3$) (J, Hz)
IIIb	CH_3	H	81	1.60—2.00 (2H, m), 2.00—2.30 (2H, m), 2.09 (3H, s), 2.30 (3H, s), 2.60—2.90 (2H, m), 5.92 (1H, t, 7.5), 7.25 (1H, s), 8.33 (1H, s)
IIIc	H	OCH_3	23	1.60—3.00 (6H, m), 2.08 (3H, s), 3.86 (3H, s), 5.92 (1H, t, 4.5), 6.67 (1H, d, 6), 8.38 (1H, d, 6)
IIId	CH_3	OCH_3	75	1.70—2.90 (6H, m), 2.08 (3H, s), 2.25 (3H, s), 3.78 (3H, s), 5.92 (1H, t, 4.5), 8.30 (1H, s)
IIIe	CH_3	$OCH_2CH=CH_2$	57	1.70—3.10 (6H, m), 2.09 (3H, s), 2.25 (3H, s), 4.30—4.50 (2H, m), 5.20—5.50 (2H, m), 5.91 (1H, t, 4.5), 5.90—6.30 (1H, m), 8.30 (1H, s)
IIIf	CH_3	$OCH_2C\equiv CH$	44	1.70—2.00 (4H, m), 2.11 (3H, s), 2.29 (3H, s), 2.54 (1H, t, 2.5), 2.80—3.10 (2H, m), 4.61 (2H, d, 2.5), 5.94 (1H, t, 4.5), 8.33 (1H, s)

a) All compounds are oils and were purified by column chromatography.

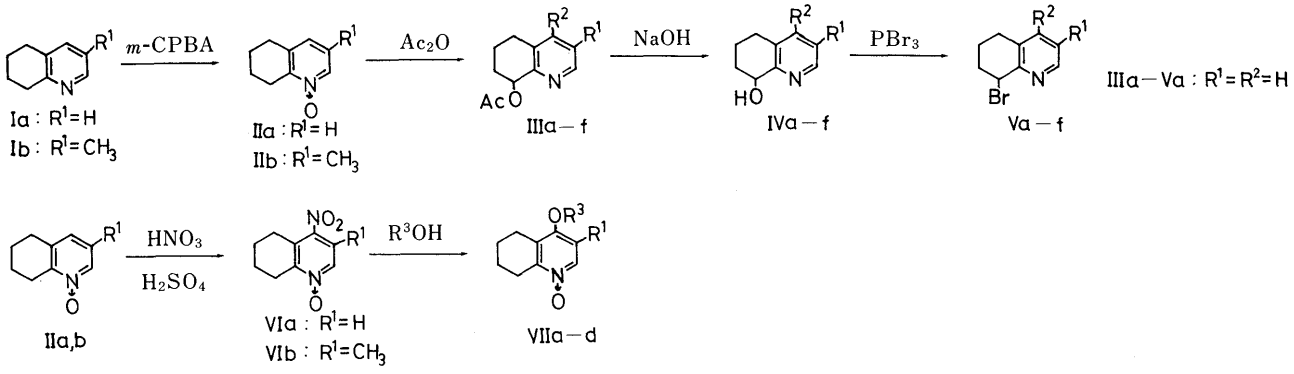
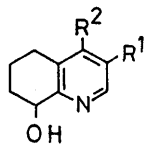


Chart 1

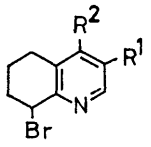
TABLE II. 8-Hydroxy-5,6,7,8-tetrahydroquinolines



Compd. No.[a]	R^1	R^2	Yield (%)	^{1}H-NMR δ ($CDCl_3$), (J, Hz)
IVb	CH_3	H	46	1.60—2.50 (4H, m), 2.73 (2H, t, 5), 4.65 (1H, t, 5), 7.23 (1H, s), 8.25 (1H, s)
IVc	H	OCH_3	98	1.50—2.40 (4H, m), 2.50—2.90 (2H, m), 3.83 (3H, s), 4.50—4.80 (1H, m), 6.62 (1H, d, 6), 8.30 (1H, d, 6)
IVd	CH_3	OCH_3	95	1.50—2.40 (4H, m), 2.22 (3H, s), 2.73 (2H, t, 6), 3.76 (3H, s), 4.66 (1H, t, 6), 8.20 (1H, s)
IVe	CH_3	$OCH_2CH=CH_2$	91	1.50—2.40 (4H, m), 2.22 (3H, s), 2.73 (2H, t, 6), 4.30—4.50 (2H, m), 4.67 (1H, t, 6), 5.20—5.50 (2H, m), 5.80—6.30 (1H, m), 8.21 (1H, s)
IVf	CH_3	$OCH_2C\equiv CH$	80	1.53—2.93 (4H, m), 2.27 (3H, s), 2.50 (1H, t, 2.5), 4.58 (1H, d, 2.5), 4.65 (1H, t, 7), 8.24 (1H, s)

a) All compounds are oils and were purified by column chromatography.

TABLE III. 8-Bromo-5,6,7,8-tetrahydroquinolines

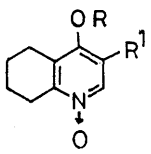


Compd. No.	R^1	R^2	Yield (%)	^{1}H-NMR δ ($CDCl_3$), (J, Hz)
Va[a]	H	H	31	1.70—2.60 (4H, m), 2.70—3.10 (2H, m), 5.40—5.60 (1H, m), 7.07 (1H, dd, 7, 4), 7.40 (1H, dd, 7, 1.5), 8.43 (1H, dd, 7, 1.5)
Vb[a]	CH_3	H	31	1.80—2.60 (4H, m), 2.28 (3H, s), 2.70—3.00 (2H, m), 5.53 (1H, brs), 7.23 (1H, d, 2), 8.30 (1H, d, 2)
Vc[a]	H	OCH_3	74	1.70—3.20 (6H, m), 3.83 (3H, s), 5.45 (1H, t, 3), 6.60 (1H, d, 6), 8.33 (1H, d, 6)
Vd[b]	CH_3	OCH_3	34	1.70—3.20 (6H, m), 2.23 (3H, s), 3.78 (3H, s), 5.49 (1H, brs), 8.27 (1H, s)
Ve[a]	CH_3	$OCH_2CH=CH_2$	29	1.60—2.40 (4H, m), 2.08 (3H, s), 2.60—3.10 (2H, m), 4.30—4.50 (2H, m), 5.20—5.50 (2H, m), 5.91 (1H, t, 4.5), 5.80—6.40 (1H, m), 8.30 (1H, s)
Vf[a]	CH_3	$OCH_2C\equiv CH$	90	1.80—3.20 (4H, m), 2.38 (3H, s), 2.54 (1H, t, 2.5), 4.61 (2H, d, 2.5), 5.54 (1H, brs), 8.30 (1H, s)

a) Oily compounds were purified by column chromatography. b) Colorless needles (from hexane), mp 82—83 °C. *Anal.* Calcd for $C_{11}H_{14}BrNO$: C, 51.58; H, 5.51; N, 5.47. Found: C, 51.51; H, 5.49; N, 5.07.

acetates (IIIa—f), which were converted to the hydroxy compounds (IVa—f) by hydrolysis with sodium hydroxide, followed by treatment with phosphorus tribromide to give the bromides (Va—f). The 4-alkoxy derivatives (VIIa—d)

TABLE IV. 4-Alkoxy-5,6,7,8-tetrahydroquinoline *N*-Oxides



Compd. No.[a]	R^1	R	Yield (%)	^{1}H-NMR δ ($CDCl_3$), (J, Hz)
VIIa	H	CH_3	47	1.50—2.10 (4H, m), 2.50—3.10 (4H, m), 3.86 (3H, s), 6.58 (1H, d, 7.5), 8.12 (1H, d, 7.5)
VIIb	CH_3	CH_3	71	1.50—2.00 (4H, m), 2.23 (3H, s), 2.72 (2H, t, 6), 2.90 (2H, t, 6), 3.76 (3H, s), 8.02 (1H, s)
VIIc	CH_3	$CH_2CH=CH_2$	68	1.50—2.10 (4H, m), 2.18 (3H, s), 2.70 (2H, t, 6), 2.88 (2H, t, 6), 4.00—4.20 (1H, m), 4.20—4.40 (1H, m), 4.90—5.00 (2H, m), 5.80—6.30 (1H, m), 8.00 (1H, s)
VIId	CH_3	$CH_2C\equiv CH$	78	1.60—1.90 (4H, m), 2.23 (3H, s), 2.53 (1H, t, 2.5), 2.76 (2H, t, 6), 2.91 (2H, t, 6), 4.60 (2H, d, 2.5), 8.03 (1H, s)

a) All compounds are oils and were purified by column chromatography.

were then synthesized as follows. Nitration of the *N*-oxide compounds (IIa, b) with nitric acid–sulfuric acid gave the 4-nitrotetrahydroquinolines (VIa, b), which were converted to the 4-alkoxy derivatives by treatment with alcohol (ROH) in the presence of sodium hydroxide (Chart 1, Tables I, II, III and IV).

On the other hand, the 2-mercaptobenzimidazole compounds (XII) were easily prepared from the phenylenediamines (XI) and potassium ethyl xanthate in the usual manner.[5] Some of the phenylenediamine compounds (XIa—c) were synthesized as shown in Chart 2. Nitration of 3′,4′-difluoroacetanilide (VIII) with nitric acid–sulfuric acid gave IX, which was hydrolyzed with hydrochloric acid to give the amino compound (Xa), followed by reduction with stannous chloride–hydrochloric acid to give XI. The alkoxy compounds (Xb, c) were synthesized from Xa and sodium alkoxide (Chart 2, Table V).

Condensation of the 8-bromotetrahydroquinolines (Va—f) with 2-mercaptobenzimidazoles (XII) in the presence of sodium hydroxide in methanol–water afforded the corresponding sulfides (XIIIa—v). Various sulfoxides (XIVa—v) were synthesized by oxidation of XIIIa—v with *m*-chloroperbenzoic acid (*m*-CPBA) (Chart 3, Tables VI and VII).

Structure–Activity Relationships The $(H^+ + K^+)$ATPase-inhibitory activity[6] and the antisecretory activity[7] against histamine-stimulated gastric acid secretion are summarized in Table VII. All compounds tested showed good activity in the *in vitro* $(H^+ + K^+)$ ATPase inhibition assay. Most compounds inhibited *in vivo* histamine-induced gastric acid secretion in rats. Since a non-substituted compound (XIVa) was first found to possess potent $(H^+ + K^+)$ATPase-inhibitory activity, various compounds having substituents in the benzimidazole ring and 5,6,7,8-tetrahydroquinoline ring were prepared.

First, the enzyme-inhibitory activity of monosubstituted compounds in the benzimidazole ring was similar to that of

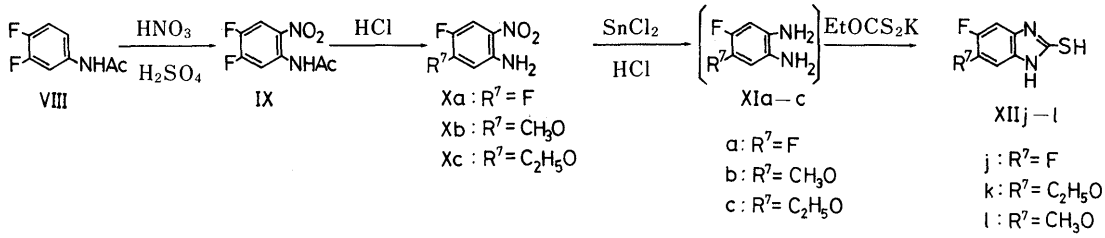


Chart 2

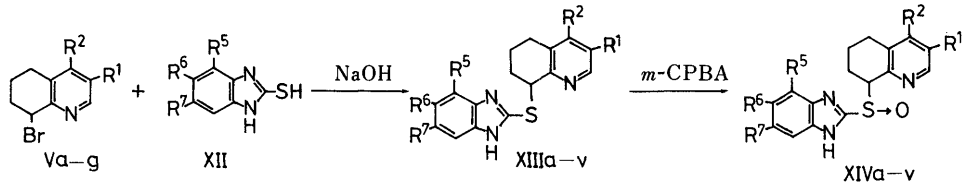
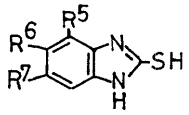


Chart 3

TABLE V. 2-Mercaptobenzimidazoles



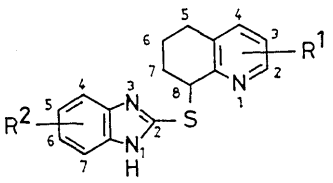
Compd. No.	R^5	R^6	R^7	Yield (%)	Appearance (Recrystn. solv.)	mp (°C)	Formula	Analysis (%) Calcd (Found)		
								C	H	N
XIIa	H	OCH_3	H	91	Brown powder (EtOH)	263—264	$C_8H_8N_2OS$	53.51 (53.39)	4.47 4.42	15.54 15.48)
XIIb	H	CH_3	H	85	White powder (EtOH)	293—295	$C_8H_8N_2S$	58.51 (58.52)	4.91 4.68	17.06 17.10)
XIIc	H	Cl	H	85	White powder (EtOH)	301—303	$C_7H_5ClN_2S$	45.53 (45.09)	2.73 2.53	15.17 14.82)
XIId	H	F	H	78	Brown powder (iso-PrOH)	289—292	$C_7H_5FN_2S$	49.99 (49.92)	3.00 3.08	16.66 16.81)
XIIe	H	NO_2	H	86	Yellow powder (EtOH–H_2O)	273—276 (dec.)	$C_7H_5N_3O_2S$	43.07 (43.32)	2.58 2.72	21.53 21.20)
XIIf	H	OCH_3	OCH_3	47	Colorless flakes (EtOH)	289—290	$C_9H_{10}N_2O_2S$	51.41 (51.57)	4.79 4.72	13.32 13.16)
XIIg	H	CH_3	CH_3	92	Pale brown needles (EtOH)	>300	$C_9H_{10}N_2S$	60.64 (60.68)	5.65 5.52	15.72 15.52)
XIIh	CH_3	H	H	54	Brown powder (EtOH)	>300	$C_8H_8N_2S$	58.51 (58.80)	4.91 4.88	17.06 17.37)
XIIi	H	CF_3	H	72	Pale yellow powder (iso-PrOH)	280—282 (dec.)	$C_8H_5F_3N_2S$	44.04 (43.93)	2.31 2.36	12.84 12.83)
XIIj	H	F	F	57	Brown powder (EtOH–H_2O)	297—299 (dec.)	$C_7H_4F_2N_2S$	45.16 (45.27)	2.17 2.06	15.05 15.57)
XIIk	H	OC_2H_5	F	80	Brown powder (EtOH)	297—300 (dec.)	$C_9H_9FN_2OS$	50.93 (50.84)	4.27 4.57	13.20 12.72)
XIIl	H	F	OCH_3	84	Pale brown powder (EtOH)	291—292	$C_8H_7FN_2OS$	48.48 (48.48)	3.56 3.63	14.13 14.14)

the non-substituted compound (XIVa). The disubstituted benzimidazole derivatives (XIVh, i) were more active than XIVa in *in vitro* assay. But the antisecretory activities of fluoro-substituted compounds (XIVf, q) in the benzimidazole ring were more potent than those of non-substituted benzimidazoles (XIVa, p). So, many fluoro-substituted benzimidazole derivatives were synthesized.

Next, the substitution effect on the tetrahydroquinoline ring was examined; it was found that methoxy substitution (XIVn, o) at the 4-position resulted in potent enzyme inhibitory activity. Disubstitution on the tetrahydroquinoline ring by methyl and methoxy groups (XIVp, q) maintained high activity, whereas methyl- and allyloxy-substituted derivatives (XIVt, u) were a little less active than XIVp. The enzyme-inhibitory activities of compounds (XIVp, q) which had the same substitution pattern as omeprazole were about 10 times higher than that of omeprazole.

The antisecretory activity of the tetrahydroquinolines against histamine-stimulated gastric acid secretion in rats was reduced compared with that of omeprazole. The lack of *in vivo* antisecretory activity suggests that the tetrahydroquinoline derivatives may either be unable to permeate

TABLE VI. 8-[(2-Benzimidazolyl)thio]-5,6,7,8-tetrahydroquinoline Derivatives



Compd. No.	Substituents R^1	Substituents R^2	Yield (%)	^{1}H-NMR δ ($CDCl_3$), (J, Hz)
XIIIa	H	H	92	1.70—2.20 (2H, m), 2.30—2.50 (2H, m), 3.87 (2H, t, 6), 4.80 (1H, t, 4.5), 7.00—7.30 (3H, m), 7.30—7.70 (3H, m), 8.47 (1H, dd, 4.5, 1.5)
XIIIb	H	5-CH_3O	68	1.70—2.00 (2H, m), 2.10—2.40 (2H, m), 2.73 (2H, t, 6), 3.77 (3H, s), 4.83 (1H, t, 4.5), 6.70—7.50 (5H, m), 8.42 (1H, dd, 4.5, 1.5)
XIIIc	H	5-CH_3	89	1.70—2.10 (2H, m), 2.20—2.50 (2H, m), 2.43 (3H, s), 2.83 (2H, t, 6), 4.77 (1H, t, 4.5), 6.90—7.60 (5H, m), 8.48 (1H, dd, 4.5, 1.5)
XIIId	H	4-CH_3	54	1.70—2.10 (2H, m), 2.20—2.40 (2H, m), 2.62 (3H, s), 2.87 (2H, t, 6), 4.77 (1H, t, 4.5), 6.80—7.60 (5H, m), 8.48 (1H, dd, 4.5, 1.5)
XIIIe	H	5-Cl	87	1.70—2.10 (2H, m), 2.20—2.50 (2H, m), 2.87 (2H, t, 6), 4.80 (1H, t, 4.5), 7.00—7.60 (5H, m), 8.46 (1H, dd, 4.5, 1.5)
XIIIf	H	5-F	50	1.70—2.10 (2H, m), 2.20—2.50 (2H, m), 2.85 (2H, t, 6), 4.82 (1H, t, 4.5), 6.70—7.50 (5H, m), 8.48 (1H, dd, 4.5, 1.5)
XIIIg[a]	H	5-NO_2	33	1.80—2.10 (2H, m), 2.20—2.50 (2H, m), 2.83 (2H, t, 6), 5.38 (1H, t, 4.5), 7.23 (1H, dd, 9, 4.5), 7.50—7.70 (2H, m), 8.08 (1H, dd, 9.0, 1.5), 8.30—8.50 (2H, m)[b]
XIIIh[a]	H	5,6-$(CH_3O)_2$	94	1.80—2.10 (2H, m), 2.20—2.50 (2H, m), 2.85 (2H, t, 6), 3.91 (6H, s), 4.73 (1H, t, 4.5), 7.00—7.60 (4H, m), 8.52 (1H, dd, 4.5, 1.5)
XIIIi[a]	H	5,6-$(CH_3)_2$	48	1.70—2.20 (2H, m), 2.20—2.50 (2H, m), 2.34 (3H, s), 2.85 (2H, t, 6), 4.74 (1H, t, 4.5), 7.10—7.60 (4H, m), 8.48 (1H, dd, 4.5, 1.5)
XIIIj	H	5-C_2H_5O 6-F	31	1.50 (3H, t, 7), 1.80—2.00 (2H, m), 2.20—2.40 (1H, m), 2.50—2.70 (1H, m), 2.74 (2H, t, 6), 4.13 (2H, q, 7), 4.88 (1H, t, 4.5), 7.00—7.20 (2H, m), 7.20—7.40 (2H, m), 8.33 (1H, dd, 4.5, 1.5)
XIIIk	H	5,6-diF	29	1.70—2.10 (2H, m), 2.10—2.50 (2H, m), 2.81 (2H, t, 6), 4.92 (1H, t, 4.5), 7.00—7.60 (4H, m), 8.42 (1H, dd, 4.5, 1.5)
XIIIl	3-CH_3	5-CH_3O	68	1.60—2.00 (2H, m), 2.00—2.40 (2H, m), 2.27 (3H, s), 2.72 (2H, t, 7), 3.78 (3H, s), 4.78 (1H, t, 4.5), 6.77 (1H, dd, 9, 2), 7.00 (1H, d, 2), 7.22 (1H, dd, 2, 1), 7.40 (1H, t, 9), 8.23 (1H, dd, 2, 1)
XIIIm	3-CH_3	5-F	55	1.70—2.10 (2H, m), 2.10—2.60 (2H, m), 2.27 (3H, s), 2.75 (2H, t, 7), 4.82 (1H, t, 4.5), 6.70—7.50 (3H, m), 7.24 (1H, d, 2), 8.25 (1H, d, 2)
XIIIn	4-CH_3O	5-CF_3	88	1.70—2.10 (2H, m), 2.10—2.40 (2H, m), 2.40—2.80 (2H, m), 3.87 (3H, s), 4.87 (1H, t, 4.5), 6.37 (1H, d, 6), 7.30—7.70 (2H, m), 7.77 (1H, s), 8.30 (1H, d, 6)
XIIIo	4-CH_3O	5-F	69	1.70—2.10 (2H, m), 2.20—2.50 (2H, m), 2.50—2.90 (2H, m), 3.90 (3H, s), 4.72 (1H, t, 4.5), 6.72 (1H, d, 6), 6.70—7.60 (3H, m), 8.39 (1H, d, 6)
XIIIp	3-CH_3 4-CH_3O	H	78	1.70—2.00 (2H, m), 2.16 (3H, s), 2.10—2.50 (2H, m), 2.62 (2H, t, 6.0), 3.60 (3H, s), 4.85 (1H, t, 4.5), 7.00—7.40 (2H, m), 7.50—7.70 (2H, m), 8.17 (1H, s)
XIIIq	3-CH_3 4-CH_3O	5-F	83	1.70—2.10 (2H, m), 2.10—2.40 (2H, m), 2.24 (3H, s), 2.60—2.90 (2H, m), 3.78 (3H, s), 4.80 (1H, t, 4.5), 6.70—7.50 (3H, m), 8.25 (1H, s)
XIIIr	3-CH_3 4-CH_3O	5-F 6-CH_3O	48	1.70—2.10 (2H, m), 2.10—2.50 (2H, m), 2.27 (3H, s), 2.60—2.90 (2H, m), 3.80 (3H, s), 3.87 (3H, s), 4.78 (1H, t, 4.5), 7.08 (1H, d, 7.5), 7.24 (1H, d, 10.5), 8.28 (1H, s)
XIIIs	3-CH_3 4-CH_3O	5-C_2H_5O 6-F	47	1.43 (3H, t, 7.5), 1.70—2.10 (2H, m), 2.10—2.30 (2H, m), 2.27 (3H, s), 2.60—2.90 (2H, m), 3.80 (3H, s), 4.08 (2H, q, 7.5), 4.76 (1H, t, 4.5), 7.08 (1H, d, 9), 7.23 (1H, d, 12), 8.27 (1H, s)
XIIIt	3-CH_3 4-CH_2=$CHCH_2O$	H	93	1.60—2.10 (2H, m), 2.10—2.40 (2H, m), 2.22 (3H, s), 2.50—3.00 (2H, m), 4.20—4.40 (2H, m), 4.80 (1H, t, 4.5), 5.10—5.50 (2H, m), 5.80—6.30 (1H, m), 7.00—7.70 (4H, m), 8.25 (1H, s)
XIIIu	3-CH_3 4-CH_2=$CHCH_2O$	5-F 6-CH_3O	71	1.70—2.10 (2H, m), 2.10—2.50 (2H, m), 2.30 (3H, s), 2.60—3.00 (2H, m), 3.92 (3H, s), 4.30—4.50 (2H, m), 4.70 (1H, t, 4.5), 5.20—5.60 (2H, m), 5.80—6.40 (1H, m), 7.00—7.40 (2H, m), 8.33 (1H, s)
XIIIv	3-CH_3 4-CH≡CCH_2O	5-F	60	1.80—3.10 (6H, m), 2.31 (3H, s), 2.56 (1H, t, 2.5), 4.65 (2H, d, 2.5), 4.81 (1H, t, 5), 6.91 (1H, dt, 2.5, 10), 7.22 (1H, d, 9), 7.43 (1H, dd, 6, 10), 8.32 (1H, s)

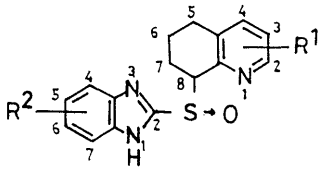
a) XIIIg: Yellow needles (from AcOEt–hexane), mp 150—152 °C, *Anal.* Calcd for $C_{16}H_{14}N_4O_2S \cdot 1/2\,H_2O$: C, 57.30; H, 4.51; N, 16.71. Found: C, 57.05; H, 4.43; N, 16.86. XIIIh: Pale brown powder (from AcOEt), mp 133—137 °C, *Anal.* Calcd for $C_{18}H_{19}N_3O_2S \cdot 1/3\,H_2O$: C, 62.23; H, 5.71; N, 12.09. Found: C, 62.28; H, 5.40; N, 11.87. XIIIi: White powder (from AcOEt), mp 200.5—201.5 °C, *Anal.* Calcd for $C_{18}H_{19}N_3S$: C, 69.87; H, 6.19; N, 13.58. Found: C, 70.10; H, 6.20; N, 13.23. *b*) In dimethyl sulfoxide-d_6 (DMSO-d_6).

through the membrane or have poor stability.

Four compounds (XIVd, m, o, q) were selected for further study. The antiulcer activity against aspirin-induced gastric ulcer[8] was determined in the rat (Table VIII). Three compounds (XIVd, m, q) showed good antiulcer activity. The compound (XIVo) with the best antisecretory activity had poor antiulcer activity. The cytoprotective activity[9] of these compounds was also tested. All the compounds tested showed a cytoprotective effect against gastric necrosis in rats induced by 0.6 N hydrochloric acid (Table VIII).

Among these compounds, 8-[(5-fluoro-2-benzimidazolyl)-

TABLE VII. 8-[(2-Benzimidazolyl)sulfinyl]-5,6,7,8-tetrahydroquinoline Derivatives



Compd. No.	Substituent R^1	Substituent R^2	Yield (%)	H^+/K^+ ATPase IC_{50}, M[a]	Histamine-stimulated rat % inhibn. (at i.v. dose, mg/kg)[b]	Appearance (Recrystn. solv.)	mp (°C)[c]	Formula	Analysis (%) Calcd (Found) C	H	N
XIVa	H	H	22	1.2×10^{-6}	85.5 (30)	Colorless prisms (CH_2Cl_2–Et_2O)	119—120.5	$C_{16}H_{15}N_3OS$	64.62 (64.41)	5.08 (4.91)	14.13 (13.64)
XIVb	H	5-CH_3O	48	2.7×10^{-6}	95.7 (30)	White powder (AcOEt)	114.5—115.5	$C_{17}H_{17}N_3O_2S\cdot H_2O$	59.11 (59.07)	5.54 (5.47)	12.17 (12.08)
XIVc	H	5-CH_3	22	1.9×10^{-6}	37.0 (10)	White powder (CH_2Cl_2–Et_2O)	122—123	$C_{17}H_{17}N_3OS$	65.57 (65.59)	5.50 (5.35)	13.49 (13.65)
XIVd	H	4-CH_3	30	2.6×10^{-6}	45.0 (10)	White powder (Et_2O)	113—114	$C_{17}H_{17}N_3OS$	65.57 (65.62)	5.50 (5.50)	13.49 (13.52)
XIVe	H	5-Cl	29	1.0×10^{-6}	91.3 (30)	Pale brown powder (Et_2O)	115—116	$C_{16}H_{13}ClN_3OS$	57.92 (58.28)	4.25 (4.06)	12.66 (12.74)
XIVf	H	5-F	14	1.7×10^{-6}	55.8 (10)	White powder (Et_2O)	118—119	$C_{16}H_{14}FN_3OS$	60.97 (61.22)	4.47 (4.48)	13.32 (13.40)
XIVg	H	5-NO_2	15	3.0×10^{-6}	ND	Pale brown powder (AcOEt–EtOH–hexane)	152—154	$C_{16}H_{14}N_4O_3S\cdot H_2O$	53.32 (53.56)	4.48 (3.83)	15.55 (15.77)
XIVh	H	5,6-$(CH_3O)_2$	36	1.8×10^{-7}	ND	White powder (AcOEt)	125—127	$C_{18}H_{19}N_3O_3S$	60.49 (60.35)	5.36 (5.21)	11.76 (11.31)
XIVi	H	5,6-$(CH_3)_2$	38	9.3×10^{-7}	89.3 (30)	White powder (CH_2Cl_2–Et_2O)	141—142	$C_{18}H_{19}N_3OS$	66.43 (66.33)	5.89 (5.72)	12.91 (12.71)
XIVj	H	5-C_2H_5O 6-F	39	3.4×10^{-6}	69.4 (30)	White powder (CH_2Cl_2–Et_2O)	128—129.5	$C_{18}H_{18}FN_3O_2S$	60.15 (60.20)	5.05 (4.73)	11.69 (11.48)
XIVk	H	5,6-diF	24	5.0×10^{-6}	35.9 (10)	White powder (CH_2Cl_2–Et_2O–hexane)	131—132	$C_{16}H_{13}F_2N_3OS$	57.65 (57.69)	3.93 (4.01)	12.61 (12.46)
XIVl	3-CH_3	5-CH_3O	51	3.7×10^{-6}	101.3 (30)	White powder (CH_2Cl_2–Et_2O)	114—114.5	$C_{17}H_{16}FN_3OS$	63.32 (63.36)	5.61 (5.52)	12.31 (12.45)
XIVm	3-CH_3	5-F	36	1.9×10^{-6}	40.3 (3)	White powder (CH_2Cl_2–Et_2O)	122.5—114.5	$C_{17}H_{16}FN_3OS$	61.99 (61.54)	4.90 (4.92)	12.76 (12.26)
XIVn	4-CH_3O	5-CF_3	38	2.3×10^{-7}	ND	Pale yellow powder (CH_2Cl_2–Et_2O)	140—142	$C_{18}H_{16}F_3N_3O_2S\cdot 1/2H_2O$	53.46 (53.44)	4.24 (3.88)	10.39 (10.26)
XIVo	4-CH_3O	5-F	43	9.9×10^{-8}	76.9 (3)	White powder (CH_2Cl_2–Et_2O)	138—139	$C_{17}H_{16}FN_3O_2S$	59.12 (58.86)	4.67 (4.72)	12.17 (12.13)
XIVp	3-CH_3 4-CH_3O	H	39	2.5×10^{-7}	96.7 (30)	Pale brown powder (Et_2O)	120.5—104	$C_{18}H_{19}N_3O_2S$	63.32 (63.19)	5.61 (5.48)	12.31 (12.10)
XIVq	3-CH_3 4-CH_3O	5-F	33	2.2×10^{-7}	25.9 (3)	Pale brown powder (Et_2O)	117—118	$C_{18}H_{18}FN_3O_2S$	60.15 (60.03)	5.05 (4.92)	11.59 (11.23)
XIVr	3-CH_3 4-CH_3O	5-F 6-CH_3O	14	4.6×10^{-6}	40.0 (30)	White powder (CH_2Cl_2–Et_2O–hexane)	142—143	$C_{19}H_{20}FN_3O_3S$	58.60 (58.37)	5.18 (5.07)	10.79 (10.48)
XIVs	3-CH_3 4-CH_3O	5-C_2H_5O 6-F	35	2.4×10^{-7}	114.8 (30)	White powder (CH_2Cl_2–Et_2O–hexane)	129—130	$C_{20}H_{22}FN_3O_3S$	59.54 (59.36)	5.50 (5.51)	10.41 (9.82)
XIVt	3-CH_3 4-CH_2=$CHCH_2O$	H	55	7.4×10^{-7}	23.9 (10)	White powder (Et_2O)	100—102	$C_{20}H_{21}N_3O_2S$	65.37 (65.19)	5.76 (5.63)	11.44 (11.07)
XIVu	3-CH_3 4-CH_2=$CHCH_2O$	5-F 6-CH_3O	56	7.4×10^{-7}	69.0 (30)	Pale brown powder (Et_2O)	110—112	$C_{21}H_{22}FN_3O_3S$	60.71 (60.28)	5.34 (5.38)	10.11 (10.04)
XIVv	3-CH_3 4-$CH\equiv CCH_2O$	5-F	26	1.1×10^{-6}	48.1 (10)	White powder (Et_2O)	100—101	$C_{20}H_{18}FN_3O_2S$	62.65 (62.54)	4.73 (4.66)	10.96 (10.89)

a) Omeprazole, 2.0×10^{-6} M. *b*) Omeprazole, 78.6% (1 mg/kg). *c*) All compounds decomposed. ND = not determined.

sulfinyl]-3-methyl-5,6,7,8-tetrahydroquinoline (OPC-22321, XIVm) was found to have the most potent activity.

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IRA-2 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on Varian EM-390 and Brucker AC-200 NMR spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Varian MAT-312 instrument.

Compounds Ia, b, IIa, b, IIIa and IVa were prepared according to the reported methods.[4,10,11]

Preparation of IIIb—f. 8-Acetoxy-3-methyl-5,6,7,8-tetrahydroquinoline (IIIb) Acetic anhydride (40 ml) was added to IIb (7.0 g, 43 mmol) with stirring. After being stirred at 90 °C for 4 h, the reaction mixture was concentrated, made alkaline with dilute NaOH, and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue

TABLE VIII. Antiulcer and Cytoprotective Activities of 8-[(2-Benzimidazolyl)sulfinyl]-5,6,7,8-tetrahydroquinolines

Compd. No.	Antiulcer actvity ED_{50} (mg/kg) *p.o.*	Cytoprotective activity ED_{50} (mg/kg) *p.o.*
XIVd	7.1	3.6
XIVm	1.1	2.7
XIVo	30>	15.8
XIVq	11.4	13.8
Omeprazole	8.3	30.2

was purified by column chromatography (silica gel; eluent, CH_2Cl_2) and gave IIIb as an oily material (7.8 g, 81%). IR ν (neat): 2930, 1730, 1460, 1370, 1230 cm^{-1}. MS *m/z*: 43 (16%), 107 (12), 144 (15), 146 (29), 162 (100), 163 (16), 206 ($M^+ + 1$, 15). NMR data are given in Table I.

Compounds IIIc—f were obtained by the same procedure as described for IIIb; the yields and NMR data are given in Table I.

Preparation of IVb—f. 8-Hydroxy-3-methyl-5,6,7,8-tetrahydroquinoline (IVb) A mixture of IIIb (4.1 g, 20 mmol), 30% NaOH (15 ml) and MeOH (15 ml) was refluxed for 3 h. After removal of MeOH, the residue was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 5 : 1) and gave IVb as an oily material (1.5 g, 46%). IR ν (neat): 3200, 2930, 1460, 1070, 750 cm^{-1}. MS *m/z*: 77 (11%), 106 (12), 107 (100), 134 (18), 135 (18), 162 (M^+, 6). NMR data are given in Table II.

Compounds IVc—f were obtained by the same procedure as described for IVb; the yields and NMR data are given in Table II.

Preparation of Va—f. 8-Bromo-5,6,7,8-tetrahydroquinoline (Va) Phosphorus tribromide (1.9 g, 7 mmol) was added dropwise to a stirred solution of IVa (3.0 g, 20 mmol) in benzene (15 ml) at 0—10 °C. The reaction mixture was stirred at room temperature over night. The cooled mixture was treated with water and the solution was adjusted to pH 9 (NaOH) and extracted with CH_2Cl_2. The combined extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, CH_2Cl_2: MeOH = 100 : 1) to give Va as an oily material (1.3 g, 31%). IR ν (neat): 2950, 1570, 1440, 1190, 790, 590 cm^{-1}. MS *m/z*: 117 (39%), 130 (23), 132 (100), 133 (12), 212 (M^+, 5), 214 (5). NMR data are given in Table III.

Compounds Vb—f were obtained by the same procedure as described for Va; the yields and NMR data are given in Table III.

Preparation of VIa and VIb. 4-Nitro-5,6,7,8-tetrahydroquinoline *N*-Oxide (VIa) Va (10.9 g, 73 mmol) was added to a stirred and ice-cooled solution of fuming HNO_3 (20 ml) and H_2SO_4 (20 ml). After being stirred at 70 °C for 2 h, the reaction mixture was poured into ice-water. The solution was made alkaline with dilute NaOH solution and extracted with $CHCl_3$. The combined extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, CH_2Cl_2 : MeOH = 50 : 1). Recrystallization from AcOEt–hexane gave VIa (3.9 g, 27%) as pale yellow needles, mp 114—115 °C. NMR δ: 1.75—1.85 (2H, m), 1.90—2.00 (2H, m), 2.94 (2H, t, J = 6 Hz), 3.10 (2H, t, J = 6 Hz), 7.81 (1H, d, J = 7 Hz), 8.19 (1H, d, J = 7 Hz). IR ν (KBr): 1570, 1515, 1335, 1275 cm^{-1}. *Anal.* Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.53; H, 5.17; N, 14.32.

3-Methyl-4-nitro-5,6,7,8-tetrahydroquinoline *N*-Oxide (VIb) Compound VIb was obtained by the same procedure as described for VIa. Yield 64%, pale yellow powder (from AcOEt–hexane), mp 140—142 °C NMR δ: 1.60—2.10 (4H, m), 2.26 (3H, s), 2.72 (2H, t, J = 6 Hz), 2.90 (2H, t, J = 6 Hz), 8.08 (1H, s). IR ν (KBr): 1515, 1335, 1300 cm^{-1}. *Anal.* Calcd for $C_{10}H_{12}N_2O_3$: C, 57.69; H, 5.81; N, 13.45. Found: C, 57.48; H, 5.84; N, 13.58.

Preparation of VIIa—d. 4-Methoxy-5,6,7,8-tetrahydroquinoline *N*-Oxide (VIIa) VIa (2.8 g, 14 mmol) was added to a stirred solution of sodium metal (0.7 g, 30 mmol) in MeOH (30 ml). The reaction mixture was refluxed for 30 min and concentrated *in vacuo*. The residue was poured into water and extracted with $CHCl_3$. The extracts were combined and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (silica gel; eluent, CH_2Cl_2:MeOH = 100 : 1) to give VIIa as an oily material (1.2 g, 47%). IR ν (neat): 2930, 1575, 1290 cm^{-1}. MS *m/z*: 77 (20%), 84 (23), 86 (15), 146 (38), 147 (36), 160 (17), 162 (100), 179 (M^+, 47), 180 (20). NMR data are given in Table IV.

Compounds VIIb—d were obtained by the same procedure as described for VIIa; the yields and NMR data are given in Table IV.

Preparation of 4',5'-Difluoro-2'-nitroacetanilide (IX) HNO_3 (d = 1.42, 9.2 ml) was added dropwise to a stirred solution of 3',4'-difluoroacetanilide[12] (VIII, 3.0 g, 18 mmol) in H_2SO_4 (10 ml) at 5—10 °C. After being stirred at the same temperature for 1 h, the reaction mixture was poured into ice-water. The precipitates were filtered off and recrystallized from EtOH to give IX (2.9 g, 82%) as pale yellow needles, mp 104—105 °C. NMR δ: 2.31 (3H, s), 8.10 and 8.15 (1H, d, J = 7.5 Hz), 8.81 and 8.87 (1H, d, J = 7.5 Hz), 10.44 (1H, br s). IR ν (KBr): 1700, 1600, 1515, 1270 cm^{-1}. *Anal.* Calcd for $C_8H_6F_2N_2O_3$: C, 44.46; H, 2.80; N, 12.96. Found: C, 44.44; H, 2.97; N, 12.83.

Preparation of 4,5-Difluoro-2-nitroaniline (Xa) A suspension of IX (2.0 g, 10 mmol) in HCl (5 ml) and EtOH (20 ml) was refluxed for 2 h, then the reaction mixture was poured into water. The precipitates were collected by filtration. Recrystallization from EtOH–H_2O gave Xa (1.2 g, 70%) as yellow needles, mp 107.5—109.5 °C. NMR δ: 5.70—6.30 (2H, br s), 6.52 and 6.65 (1H, d, J = 7.5 Hz), 7.92 and 8.12 (1H, d, J = 7.5 Hz). IR ν (KBr): 3475, 3355, 1525, 1255 cm^{-1}. *Anal.* Calcd for $C_6H_4F_2N_2O_2$: C, 41.39; H, 2.32; N, 16.09. Found: C, 41.36; H, 2.47; N, 16.29.

Preparation of Xb, c. 4-Fluoro-5-methoxy-2-nitroaniline (Xb) Xa (0.9 g, 5 mmol) was added to a solution of sodium metal (0.14 g, 6 mmol) in MeOH (10 ml). The reaction mixture was refluxed for 3 h and concentrated *in vacuo*. The residue was poured into water and the precipitates were collected by filtration. Recrystallization from MeOH gave Xb (0.53 g, 57%) as yellow needles, mp 165.5—166.5 °C. NMR δ: 3.91 (3H, s), 6.47 (1H, d, J = 7.5 Hz), 7.02 (2H, br s), 7.76 (1H, d, J = 12 Hz). IR ν (KBr): 3490, 3385, 1520, 1260 cm^{-1}. *Anal.* Calcd for $C_7H_7FN_2O_3$: C, 45.17; H, 3.79; N, 15.05. Found: C, 44.98; H, 3.50; N, 15.12.

5-Ethoxy-4-fluoro-2-nitroaniline (Xc) Compound Xc was obtained by the same procedure as described for Xb. Yield 50%, yellow needles (from EtOH), mp 119—121 °C. NMR δ: 1.37 (3H, t, J = 7 Hz), 3.24 (2H, br s), 4.00 (2H, q, J = 7 Hz), 6.39 (1H, d, J = 8 Hz), 6.49 (1H, q, J = 12 Hz). IR ν (KBr): 3480, 3370, 1590, 1510, 1240, 1210, 840 cm^{-1}. *Anal.* Calcd for $C_8H_9FN_2O_3$: C, 48.00; H, 4.53; N, 14.00. Found: C, 47.99; H, 4.53; N, 14.09.

Preparation of XIa—c. 4-Fluoro-5-methoxy-1,2-phenylenediamine (XIb) Xb (53 g, 0.28 mol) was added to $SnCl_2 \cdot 2H_2O$ (250 g, 1.1 mol) in HCl (250 ml) and stirred at 50—60 °C for 2 h. The reaction mixture was poured into ice-water, made alkaline with dilute NaOH and extracted with $CHCl_3$. The extracts were combined and dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from AcOEt–hexane to give XIb (41.5 g, 93%) as a white powder, mp 89—90 °C (dec.). NMR δ: 3.23 (4H, br s), 3.80 (3H, s), 6.39 (1H, d, J = 8 Hz), 6.51 (1H, d, J = 12 Hz). IR ν (KBr): 3380, 3300, 3200, 1520, 1500, 1230, 1200, 860 cm^{-1}.

Compounds XIa and XIc were obtained by the same procedure as described for XIf; these samples were immediately used in the next step since they were unstable.

4,5-Difluoro-1,2-phenylenediamine (XIa) Yield 42%, colorless prisms (from AcOEt–hexane), mp 130—131 °C. NMR δ: 3.30 (4H, br s), 6.51 (2H, t, J = 9.5 Hz). IR ν (KBr): 3410, 3390, 3320, 3270, 3210, 1520, 1220, 1180, 860 cm^{-1}.

4-Ethoxy-5-fluoro-1,2-phenylenediamine (XIc) Yield 93%, a brown oil. NMR δ: 3.23 (4H, br s), 3.80 (3H, s), 6.39 (1H, d, J = 8 Hz), 6.51 (1H, d, J = 12 Hz). IR ν (neat): 3400, 3340, 2980, 1520, 1230, 1200, 1120, 860 cm^{-1}

Preparation of XIIa—l , These compounds were prepared according to the reported methods.[5,13]

5-Fluoro-6-methoxy-2-mercaptobenzimidazole (XIII) A mixture of XIb (41 g, 0.26 mol), potassium ethyl xanthate (50.4 g, 0.32 mol), 95% EtOH (400 ml) and H_2O (100 ml) was refluxed for 8 h, then allowed to cool. Acetic acid (50 ml) and H_2O (300 ml) were added and the precipitates were filtered off and recrystallized from EtOH to give XIII (43.6 g, 84%) as a pale brown powder, mp 291—292 °C. NMR δ (DMSO-d_6): 3.82 (3H, s), 6.86 (1H, d, J = 7.5 Hz), 7.03 (1H, d, J = 10.5 Hz), 12.44 (2H, br s). IR ν (KBr): 3090, 1485, 1350, 1165 cm^{-1}. MS *m/z*: 111 (15%), 155 (43), 183 (88), 198 (M^+, 100). The elemental analysis data are given in Table V.

Compounds XIIa—k were obtained by the same procedure as described for XIII; the yields, melting points and elemental analysis data are listed in Table V.

Preparation of XIIIa—v. 8-[(2-Benzimidazolyl)thio]-5,6,7,8-tetrahydroquinoline (XIIIa) Va (0.81 g, 3.8 mmol) was added to a solution of NaOH (0.18 g, 4.2 mmol) and 2-mercaptobenzimidazole (0.57 g, 3.8 mmol) in H_2O (3 ml) and MeOH (30 ml), and the reaction mixture was refluxed for 1 h. After the removal of MeOH, the residue was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent CH_2Cl_2 : MeOH = 50 : 1). Recrystallization from CH_3CN gave XIIIa

(0.98 g, 92%) as colorless needles, mp 180—183 °C. IR ν (KBr): 2940, 1400, 750 cm^{-1}. *Anal.* Calcd for $C_{16}H_{15}N_3OS$: C, 64.62; H, 5.08; N, 14.13. Found: C, 64.41; H, 4.91; N, 13.64.

Compounds XIIIb—v were obtained by the same procedure as described for XIII and the oily compounds were purified by column chromatography; the yields and NMR data are given in Table VI.

Preparation of XIVa—v. 8-[(5-Fluoro-2-benzimidazolyl)sulfinyl]-3-methyl-5,6,7,8-tetrahydroquinoline (XIVm) A solution of 80% *m*-CPBA (0.52 g, 2.4 mmol) in CH_2Cl_2 (10 ml) was added dropwise to a stirred and cooled (−50—−40 °C) solution of XIIIm (0.8 g, 2.4 mmol) in CH_2Cl_2 (50 ml) and the reaction mixture was stirred at the same temperature for 20 min. The solution was washed with Na_2CO_3 aqueous solution and dried over $MgSO_4$. After removal of the solvent, the residue was triturated in Et_2O. The precipitates were collected by filtration. Recrystallization from CH_2Cl_2–Et_2O gave XIVm (0.3 g, 36%) as a white powder, mp 112.5—114.5 °C (dec.). NMR δ: 1.60—2.60 (4H, m), 2.18 (3H, s), 2.68 (2H, t, J=6 Hz), 4.83 (1H, t, J=4.5 Hz), 6.90—7.70 (4H, m), 8.12 (1H, d, J=1.5 Hz). IR ν (KBr): 3125, 1630, 1400, 1040 cm^{-1}. The elemental analysis data are given in Table VII.

Compounds XIVa—l and XIVn—v were obtained by the same procedure as described for XIVm; the yields, melting points and elemental analysis data are given in Table VII.

Biological Method. Gastric Antisecretory Activity Male Wistar rats, weighing 190 to 250 g, were used for gastric secretion studies. An *in vivo* system for stomach perfusion was prepared according to the method of Ghosh and Schild.[7] Rats fasted for 24 h were anesthetized with urethane (1.5 g/kg s.c.). The stomach was perfused continuously with warm saline containing 0.25 mM NaOH at the rate of 1 ml/min using a peristatic pump. The perfusate was passed over a glass electrode pH meter which recorded the pH on a chart recorder. Histamine (1 mg/kg/h) was infused intravenously at a constant rate. The first injection of test compound was made into the femoral vein 15 min after maximum acid production, followed by cumulative doses (1, 3, 10, 30 mg/kg). Percent inhibition by a test compound was calculated as follows: [(mean value of test pH − mean value of histamine pH)/(mean value of control pH − mean value of histamine pH)] × 100.

Aspirin-Induced Gastric Ulcer Male Wistar rats weighing between 200 and 250 g were used. The animals were housed in cages with wide mesh wire bottoms to prevent coprophagia. The rats were fasted for 24 h, but allowed access to water before the experiment. Test compound and the vehicle were given orally 30 min before the oral administration of 200 mg/kg of aspirin suspended in 0.5% carboxymethyl cellulose. Five hours after aspirin administration, the rats were killed. The stomachs were removed, instilled with 10 ml of 3% formalin for 10 min and opened along the greater curvature. The length of lesions in the glandular portion was measured. The ulcer index (mm) was given by the sum of the lengths of the lesions. Percent inhibition was calculated as follows: [(ulcer index of control-ulcer index of test compound)/ulcer index of control] × 100. The doses inhibiting aspirin−induced gastric ulcers by 50% (ED_{50}) were calculated by linear regression analysis.

Cytoprotective Activity[9] Male Wistar rats weighing between 200 and 250 g were fasted for 24 h, but allowed access to water *ad libitum* prior to the study. Test compound and the vehicle were given orally 30 min before the oral administration of 1 ml of 0.6 N HCl. The animals were killed 1 h after the irritant was given, and the stomachs were removed. After light fixation with formalin, the surface of the gastric mucosa was graded planimetrically. The total surface area damage in each animal was calculated and used as the lesion index. Percent inhibition was calculated as follows: [(lesion index of control − lesion index of test compound)/lesion index of control] × 100. The doses inhibiting 0.6 N HCl-induced lesions by 50% (ED_{50}) were calculated by linear regression analysis.

($H^+ + K^+$)ATPase Assay Hog gastric mucosal vesicles containing ($H^+ + K^+$)ATPase were prepared according to the method of Sachs *et al.*[14] Preincubation of the enzyme with a test compound was carried out at room temperature for 30 min in 2 mM Pipes–Tris buffer pH 6.1. ($H^+ + K^+$) ATPase was assayed at 37 °C for 30 min in a reaction mixture (2 ml) containing 100 mM Tris–HCl buffer pH 7.4, approximately 15 mg of membrane protein, 2 mM $MgCl_2$ and 2 mM ATP with or without 10 mM KCl. The reaction was stopped by adding 0.3 ml of 40% trichloroacetic acid. The enzyme activity was determined by subtracting the amount of inorganic phosphate released in K^+-free medium. Phosphate was measured by the method of Fiske and Subbarow.[15] Protein concentration was obtained by the Lowry method.[16] Percent inhibition was calculated as followed: [(mean value of control activity − mean value of test activity)/mean value of control activity] × 100. The doses inhibiting ($H^+ + K^+$)ATPase by 50% (IC_{50}) were calculated by linear regression analysis.

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