Nicotinamide Derivatives as a New Class of Gastric H^+/K^+-ATPase Inhibitors. 1. Synthesis and Structure−Activity Relationships of *N*-Substituted 2-(Benzhydryl- and benzylsulfinyl)nicotinamides

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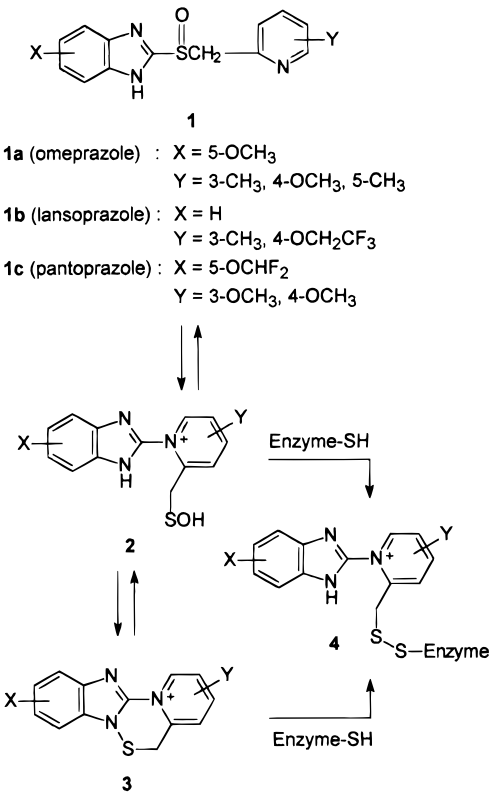
A new series of *N*-Substituted 2-(benzhydryl- and benzylsulfinyl)nicotinamides **7** and **8** were synthesized. Upon acid activation in the acidic environment of the parietal cell, these compounds are converted into their active forms, 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridines **5**, which inhibit gastric H^+/K^+-ATPase. Inhibitory activities against [^{14}C]aminopyrine accumulation stimulated by dibutyryl cAMP in isolated rabbit parietal cells in vitro and histamine-induced gastric acid secretion in pylorus-ligated rats by intraduodenal administration in vivo were evaluated, and the structure−activity relationships were examined. Among the compounds synthesized, 2-[(2,4-dimethoxybenzyl)sulfinyl]-*N*-(4-pyridyl)nicotinamide (**8b**) showed potent inhibitory activities in vitro and in vivo equivalent to those of omeprazole, a typical H^+/K^+-ATPase inhibitor. Moreover, **8b** was much more stable at neutral and weakly acidic pH than omeprazole, lansoprazole, and pantoprazole. Compound **8b** is considered to be a promising agent for treating acid-related gastrointestinal disorders.

Peptic ulcer has been generally thought to result from an imbalance between the aggressive factors of acid and pepsin and defensive forces of resistance. Consequently, antiulcer therapy has been directed toward these factors. Since the discovery of the histamine H_2 receptor antagonist cimetidine and its clinical success, the inhibition of gastric acid secretion has been the major focus of antiulcer therapy. Subsequently, gastric H^+/K^+-ATPase, which is located in the apical membrane of the parietal cell and plays a major role in acid secretion, has become the next target for numerous investigations. Among studies on H^+/K^+-ATPase inhibitors, 2-[(2-pyridylmethyl)sulfinyl]benzimidazoles (PSBs) such as omeprazole (**1a**),[1] lansoprazole (**1b**),[2] and pantoprazole (**1c**)[3] (Scheme 1) have been found to have superior properties responsible for complete suppression of gastric acid secretion, and **1a**−**c** have recently been introduced as clinically useful agents. The PSBs act as prodrugs, being chemically transformed into the highly thiophilic sulfenic acid **2** and the cyclic sulfenamide **3** in an acidic environment such as the apical membrane of the parietal cell (Scheme 1). The thiophilic sulfenic acid **2** and the sulfenamide **3** react readily with thiol groups on the enzyme to form an enzyme−inhibitor complex (**4**) with a tight S−S bond.[4] However, there is another possibility of this successive reaction taking place not only within the acid compartment of the parietal cells but also at weakly acidic pH conditions outside the parietal cells.[3] All known irreversible H^+/K^+-ATPase inhibitors do not exhibit activity by the mechanism described above, but they are structurally similar to the PSBs[5,6] and their analogues.[7−14] On the basis of the mechanism of pH-dependent inhibition as observed in the PSBs, we aimed to find out potent and more selective inhibitors of H^+/K^+-ATPase in vivo, which have a different structure from PSBs.

On the basis of our random screening, we have found that *N*-substituted 2,3-dihydro-3-oxoisothiazolo[5,4-*b*]-pyridines **5a**,**b** inhibited the H^+/K^+-ATPase in vitro

Scheme 1



(Table 1); however, they did not exhibit inhibitory activity against gastric acid secretion in vivo by intraduodenal (id) administration. The potent in vitro activities of the isothiazolopyridines **5** are due to their high-thiophilic property; compound **5a** reacted rapidly with *N*-acetyl-L-cysteine to give **6** in high yield due to its thiophilic property as shown in Scheme 2. So, we speculated that the compounds reacted with thiol groups on other proteins before they reached the target enzyme, gastric H^+/K^+-ATPase. To get good in vivo efficacy, it appeared necessary to find prodrugs which are con-

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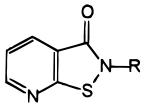
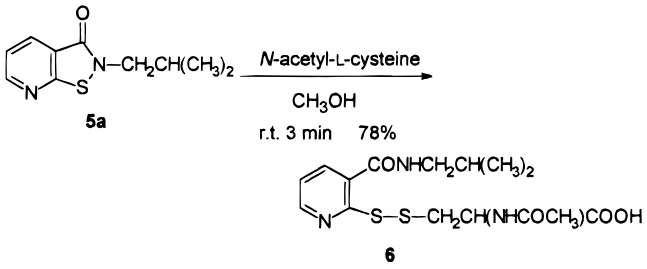
Table 1. Inhibitory Activity against Gastric H^+/K^+-ATPase of *N*-Substituted 2,3-Dihydro-3-oxoisothiazolo[5,4-*b*]pyridines **5**



compd	R	inhibition of porcine gastric H^+/K^+-ATPase, IC_{50} (μM)[a,b]
5a	isobutyl	0.53
5b	phenyl	0.19
5c	4-pyridyl	0.22

[a] See the Experimental Section. [b] IC_{50} values were calculated from the regression lines.

Scheme 2



verted into the active isothiazolopyridines **5** only within the acid compartment of the parietal cells.

Recently, we reported a convenient method for preparing **5** from 2-(benzhydrylsulfinyl)nicotinamides **7** or 2-(benzylsulfinyl)nicotinamides **8** substituted with alkoxy groups at the ortho and para positions of the benzyl group at room temperature in a diluted hydrochloric acid−methanol solution in high yields.[15] We considered that the mechanism for the conversion of **7** and **8** might involve the sulfonium salt **9** as an intermediate followed by elimination of the leaving group R^1, as shown in Scheme 2, and that the conversion rate might depend on the stability of the carbonium ion of the leaving group R^1. This finding suggested the possibility of seeking compounds acting as prodrugs with a desirable chemical profile of being converted into the isothiazolopyridines **5** at low pH but being more stable at both neutral and weakly acidic pH than the PSBs to avoid interaction with thiol groups on proteins except H^+/K^+-ATPase in the body.

The present study was focused on finding a new class of potent gastric H^+/K^+-ATPase inhibitors which possess in vivo gastric antisecretory activity by id administration and are more stable at both neutral and weakly acidic pH than the PSBs. The present paper deals with syntheses, chemical stability, and antisecretory activities of *N*-substituted 2-(benzhydryl- and benzylsulfinyl)-nicotinamides; the structure−activity relationships (SARs) of these compounds are also discussed.

Chemistry

The requisite nicotinic acids **11**−**27** (Table 2) were prepared by condensation of 2-mercaptonicotinic acid (**10**) with the corresponding benzyl or benzhydryl chloride (procedure A) or with the corresponding benzyl alcohols under acidic condition (procedure B) according to the method reported previously[15] (Scheme 3). 2-[[4-Methoxy-2-[2-(methoxymethoxy)ethoxy]benzyl]thio]-nicotinic acid (**28**) (Table 2) was prepared from 2-[[4-methoxy-2-(2-hydroxyethoxy)benzyl]thio]nicotinic acid (**25**) via alkylation with chloromethyl methyl ether (procedure C) (Scheme 3).

The nicotinamides **7a**−**d**,**f**−**t** and **8a**−**1**,**n**−**p** listed in Tables 3 and 4 were prepared from the corresponding nicotinic acids **11**−**24** and **26**−**28** with the appropriate amines by (i) the 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride method and (ii) the acid chloride method followed by oxidation with *m*-chloroperbenzoic acid (*m*CPBA) (procedures D and E, respectively), as reported previously[15] (Scheme 3). The nicotinamide **7e** was prepared starting from **11**, which was converted to 2-(benzhydrylthio)-*N*-(4-nitrophenyl)-nicotinamide (**29**) by condensation with 4-nitroaniline by the use of oxalyl chloride, followed by catalytic reduction and subsequent oxidation with *m*CPBA (procedure F). The nicotinamide **8m** was prepared by condensation of 4-aminopyridine with 2-[[4-methoxy-2-(2-acetoxyethoxy)benzyl]thio]nicotinic acid derived from the nicotinic acid **25** by acetylation followed by cleavage of the acetyl group with K_2CO_3 and subsequent oxidation with *m*CPBA (procedure G). In the case of **7d**,**g**, oxidation of the respective 2-(benzhydrylthio)nicotinamides with *m*CPBA was performed in the presence of hydrochloric acid to avoid formation of the *N*-oxide derivatives.

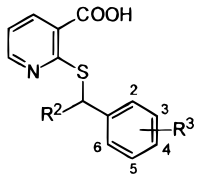
Pharmacological Results and Discussion

Compounds **7a**−**t** and **8a**−**p** were first evaluated for their ability to inhibit [^{14}C]aminopyrine (AP) accumulation stimulated by dibutyryl cyclic AMP (dbcAMP) in isolated rabbit parietal cells. The compounds which significantly inhibited the AP accumulation were further evaluated for the ability to inhibit histamine-induced gastric acid secretion in pylorus-ligated rats by id administration. The results are summarized in Tables 5 and 6.

We focused our initial effort on the SARs associated with substitution on the nitrogen atom of the carbamoyl moiety of the 2-(benzhydrylsulfinyl)nicotinamides **7** (Table 5). Most compounds **7** were supposed to be readily converted into the isothiazolopyridines **5** at room temperature at low pH as described in our previous report:[15] Half-lives at pH 1 of the compounds **7j**,**k** were less than 0.02 h. The *N*-(1-benzyl-4-piperidyl) (**7d**) and the *N*-phenyl derivatives bearing an amino (**7e**), dimethylamino (**7f**), or 2-(dimethylamino)ethyl (**7g**) group at the para position exhibited inhibitory activity against the AP accumulation, whereas the *N*-isobutyl (**7a**), the *N*-phenyl (**7b**), and the *N*-naphthyl (**7c**) derivatives did not inhibit the accumulation. It was deduced from this finding that the basic part of a substituent on the carbamoyl moiety was necessary for inhibitory activity against the AP accumulation.

The effect of substitution of heteroaromatic rings having a basic nitrogen atom on the nitrogen atom of the carbamoyl moiety was examined (Table 5). The 4-pyridyl (**7j**,**k**), 5-quinolyl (**7m**), 6-quinolyl (**7n**), and 2-naphthyridinyl (**7p**) derivatives exhibited inhibitory activity against the AP accumulation; particularly, compounds **7j**,**k** bearing the 4-pyridyl group showed the greatest activity in this series. The 4-pyridyl group was therefore selected as the optimal substituent on the nitrogen atom of the carbamoyl moiety. Compounds such as **7j**,**k** which inhibited the AP accumulation, however, did not show significant inhibitory activity

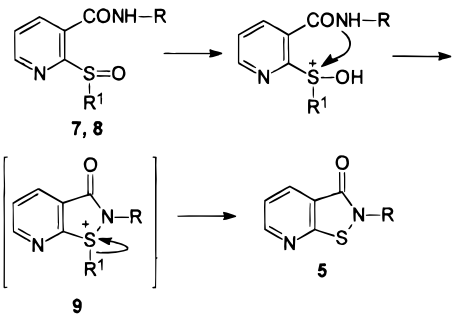
Table 2. Nicotinic Acids **11**−**28**



compd	R^2	R^3	procedure[a]	mp, °C	recrystn solvent[b]	yield, %	formula[c]
11[d]	phenyl	H	A	209−211	A	60	
12[d]	4-methylphenyl	4-CH_3	A	211−214	A	92	
13	H	4-OCH_3	A	215−217	A	81	$C_{14}H_{13}NO_3S$
14[d]	H	2-OCH_3, 4-OCH_3	B	189−192	B	96	
15	H	2-OCH_3, 6-OCH_3	B	224−225	C−D	92	$C_{15}H_{15}NO_4S$
16	H	2-OCH_3, 3-OCH_3, 4-OCH_3	B	185−187	A−C−D	85	$C_{16}H_{17}NO_5S$
17	H	2-OCH_3, 4-OCH_3, 5-OCH_3	B	181−183	A−B−C	98	$C_{16}H_{17}NO_5S$
18[d]	H	2-OCH_3, 4-OCH_3, 6-OCH_3	B	189−192	B−D	99	
19	H	3-OCH_3, 4-OCH_3, 5-OCH_3	A	214−215	F	85	$C_{16}H_{17}NO_5S$
20	H	2-OCH_3, 3-CH_3, 4-OCH_3	B	214−216	A−B−C	98	$C_{16}H_{17}NO_5S$
21	H	2-OCH_3, 4-OCH_3, 6-CH_3	B	215−218	B−D	94	$C_{16}H_{17}NO_5S$
22	H	2-OCH_3, 4-OCH_3, 5-Br	B	209−211	B	97	$C_{15}H_{14}BrNO_4S$
23	H	2-OC_2H_5, 4-OCH_3	B	160−162	A	85	$C_{16}H_{17}NO_4S{\cdot}0.35H_2O$
24	H	2-OC_2H_5, 4-OC_2H_5	A	140−142	A	95	$C_{17}H_{19}NO_4S$
25	H	2-OCH_2CH_2OH, 4-OCH_3	B	124−129	B	97	*f*
26	H	2-OCH_2CH_2F, 4-OCH_3	B	152−154	A	91	*e*
27	H	2-OCH_2OCH_3, 4-OCH_3	B	149−162	A	77	$C_{16}H_{17}NO_5S{\cdot}0.25H_2O$
28	H	2-$OCH_2CH_2OCH_2OCH_3$, 4-OCH_3	C	98−100	B	93	*g*

[a] Capital letters refer to the procedures in the Experimental Section. [b] A = CH_3CN, B = CH_3OH, C = acetone, D = H_2O, E = $CHCl_3$, F = toluene, G = $(C_2H_5)_2O$, H = C_2H_5OH, I = *n*-hexane, J = $(iC_3H_7)_2O$. [c] All compounds were analyzed for C, H, N, S, and halogen; analytical results were within ±0.4% of the theoretical values. [d] These compounds were previously prepared.[15] [e] MS (SIMS): *m*/*z* 338 (MH^+). [f] MS (APCI): *m*/*z* 336 (MH^+). [g] SIMS: *m*/*z* 380 (MH^+).

Scheme 3



against the histamine-induced gastric acid secretion in pylorus-ligated rats by id administration. On the other hand, intravenous administration led **7k** to exhibit the inhibitory activity significantly: Its inhibition percents at doses of 3 and 10 mg/kg were 46.1% and 73.5%, respectively. So, we assumed that the weak inhibitory activities of these compounds in vivo could be attributed to the highly hydrophobic benzhydryl groups.

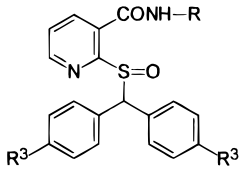
Accordingly, it appeared that replacement of the benzhydryl group by less hydrophobic benzyl groups was required to improve the bioavailability. Since 2-(benzylsulfinyl)-*N*-(4-pyridyl)nicotinamide is not readily converted into its active form, *N*-(4-pyridyl)-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine (**5c**) (Table 1), at room temperature under acidic conditions as reported previously,[15] it was necessary to introduce an electron-donating alkoxy group into the ortho and/or para position(s) of the phenyl ring of the benzyl group to raise the conversion rate. The inhibitory activities against the AP accumulation in vitro and the histamine-induced gastric acid secretion in vivo as well as half-lives ($t_{1/2}$) of conversion at pH 5.0, 3.0, and 1.0 for **8a**−**p** are summarized in Table 6. Concerning the effect of substitution of methoxy groups on the phenyl ring of **8a**−**j**, decreasing order of $t_{1/2}$ at pH 1 was 3,4,5-trimethoxy (**8g**) ≫ 4-methoxy (**8a**) ≫ 2,6-dimethoxy (**8c**) > 2,3,4-trimethoxy (**8d**) > 2,4-dimethoxy-5-methyl (**8h**) > 2,4-dimethoxy-5-bromo (**8j**) > 2,4-dimethoxy (**8b**), 2,4,5-trimethoxy (**8e**), 2,4,6-trimethoxy (**8f**), and 2,4-dimethoxy-6-methyl (**8i**). Compounds **8a**,**c**−**e**,**g**,**h** showed weak inhibitory activity against the AP accumulation, whereas **8b**,**f**,**i**,**j** inhibited the accumulation potently. The result suggested that the ready conversion into **5c** at pH 1 tended to enhance the potency in inhibition of the AP accumulation. Compounds **8b**,**f**,**i** which potently inhibited the AP accumulation exhibited potent inhibitory activity against the histamine-induced gastric acid secretion in vivo with the exception of **8j**; particularly, the activity of **8b** (ED_{50} = 4.2 mg/kg id) was comparable to that of omeprazole. In addition, **8b** was much more stable at weakly acidic pH (pH 5.0) than **8f**,**i**.

The effect of replacement of the methoxy groups of **8b** by other alkoxy groups was examined (Table 6). Replacement of the methoxy groups by an ethoxy group (**8k**,**l**) had little effect on inhibition of the AP accumulation but resulted in a considerable decrease in inhibitory activity against the histamine-induced gastric acid secretion in vivo. Replacement by other alkoxy groups (**8m**−**p**) caused a decrease in inhibitory activities against both the AP accumulation in vitro and the histamine-induced secretion in vivo. As shown in Table 6, there was not a correlation between the AP accumulation assay and in vivo in every case. This may be because there was a disparity in the ability of the compounds to reach the acidic compartments of the parietal cell.

Structure−activity relationship studies on *N*-(4-pyridyl)nicotinamides **7** and **8** revealed that decreasing the hydrophobicity tended to enhance the inhibitory activity against the histamine-induced gastric acid secretion in pylorus-ligated rats.

To clarify whether the compounds **8** are mainly converted into the isothiazolopyridine **5c** in various

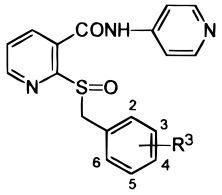
Table 3. *N*-Substituted 2-(Benzhydrylsulfinyl)nicotinamides **7**



compd	R	R^3	procedure[a]	mp, °C	recrystn solvent[b]	yield,[c] %	formula[d]
7a	isobutyl	H	E	138–140	J	77	$C_{23}H_{24}N_2O_2S$
7b	phenyl	H	D	179–183	E	51	$C_{25}H_{20}N_2O_2S$
7c	1-naphthyl	H	D	152–154	B	22	$C_{29}H_{22}N_2O_2S$
7d	1-benzyl-4-piperidyl	H	E	168–170	B	27	$C_{31}H_{31}N_3O_2S$
7e	4-aminophenyl	H	F	104–106	A–E–I	27	$C_{25}H_{21}N_3O_2S{\cdot}0.6CH_3CN$
7f	4-(dimethylamino)phenyl	H	E	155–157	A	33	$C_{27}H_{25}N_3O_2S$
7g	4-[2-(dimethylamino)ethyl]phenyl	H	E	119–121	G	22	$C_{29}H_{29}N_3O_2S{\cdot}0.25H_2O$
7h	2-pyridyl	H	E	160–163	A	16	$C_{24}H_{19}N_3O_2S$
7i	3-pyridyl	H	E	165–166	B	52	$C_{24}H_{19}N_3O_2S$
7j[e]	4-pyridyl	H	E	175–178	B	23	
7k[e]	4-pyridyl	CH_3	E	159–163	A	23	
7l	2-methoxy-3-pyridyl	H	E	177–173	B	40	$C_{25}H_{21}N_3O_3S$
7m	5-quinolyl	CH_3	E	157–159	A	59	$C_{30}H_{25}N_3O_2S$
7n	6-quinolyl	H	E	165–168	H	40	$C_{28}H_{21}N_3O_2S$
7o	2-methyl-8-quinolyl	H	E	145–150	F	53	$C_{29}H_{23}N_3O_2S$
7p	5,7-dimethyl-2-naphthyridinyl	H	E	141–145	A	51	$C_{29}H_{24}N_4O_2S$
7q	9-acridinyl	H	E	260–265	A	9	$C_{32}H_{23}N_3O_2S$
7r	pyrimidinyl	H	E	153–156	A	18	$C_{23}H_{18}N_4O_2S$
7s	pyrazinyl	H	E	164–165	B–E	49	$C_{23}H_{18}N_4O_2S$
7t	2-thiazolyl	H	E	172–175	A	32	$C_{22}H_{17}N_3O_2S_2$

[a] See footnote a in Table 2. [b] See footnote b in Table 2. [c] Total yields (%) of the *N*-substituted 2-(benzhydrylsulfinyl)nicotinamides were based on the corresponding nicotinic acids. [d] See footnote c in Table 2. [e] See footnote d in Table 2.

Table 4. 2-(Benzylsulfinyl)-*N*-(4-pyridyl)nicotinamides **8**



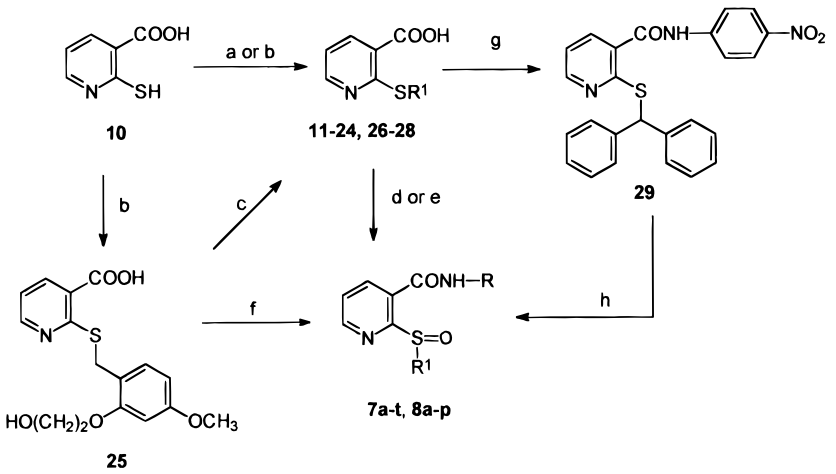
compd	R^3	procedure[a]	mp, °C	recrystn solvent[b]	yield,[c] %	formula[d]
8a	4-OCH_3	D	210–212	A	68	$C_{19}H_{17}NO_3S{\cdot}0.25H_2O$
8b[e]	2-OCH_3, 4-OCH_3	D	190–192	A	33	
8c	2-OCH_3, 6-OCH_3	D	243–246	A	23	$C_{20}H_{19}N_3O_4S$
8d	2-OCH_3, 3-OCH_3, 4-OCH_3	D	164–166	A	26	$C_{21}H_{21}N_3O_5S$
8e	2-OCH_3, 4-OCH_3, 5-OCH_3	D	103–106	A	61	$C_{21}H_{21}N_3O_5S$
8f[e]	2-OCH_3, 4-OCH_3, 6-OCH_3	D	182–185	A	35	
8g	3-OCH_3, 4-OCH_3, 5-OCH_3	D	207–210	A	36	$C_{21}H_{21}N_3O_5S$
8h	2-OCH_3, 3-CH_3, 4-OCH_3	D	105–108	A	41	$C_{21}H_{21}N_3O_4S$
8i	2-OCH_3, 4-OCH_3, 6-CH_3	D	195–198	A	39	$C_{21}H_{21}N_3O_4S$
8j	2-OCH_3, 4-OCH_3, 5-Br	D	209–211	A	42	$C_{20}H_{18}BrN_3O_4S$
8k	2-OC_2H_5, 4-OCH_3	E	200–202	A	8	$C_{21}H_{21}N_3O_4S$
8l	2-OC_2H_5, 4-OC_2H_5	D	165–166	A	10	$C_{22}H_{23}N_3O_4S$
8m	2-OCH_2CH_2OH, 4-OCH_3	G	182–184	C	16	$C_{21}H_{21}N_3O_5S$
8n	2-OCH_2CH_2F, 4-OCH_3	E	163–164	A	55	$C_{21}H_{20}FN_3O_4S$
8o	2-OCH_2OCH_3, 4-OCH_3	D	189–191	A	18	$C_{21}H_{21}N_3O_5S$
8p	2-$OCH_2CH_2OCH_2OCH_3$, 4-OCH_3	E	177–180	A–E	64	$C_{23}H_{25}N_3O_6S$

[a] See footnote a in Table 2. [b] See footnote b in Table 2. [c] See footnote c in Table 3. [d] See footnote c in Table 2. [e] See footnote d in Table 2.

acidic conditions and whether their conversion rates depend on pH, the concentrations of the representative **8b** and the formed **5c** were determined at pH 2.0, 3.0, and 4.0 in the conversion. As shown in Figure 1, **8b** was efficiently converted into **5c** depending on pH; **8b** was immediately converted into **5c** at pH 1 ($t_{1/2}$ < 0.02 h). Moreover, **8** prepared in the present study with the exception of **8f** did not inhibit the H^+/K^+-ATPase in vitro for themselves; the activity of **8f** seems to be due to a little **5c** formed during the assay because of its ready conversion. We deduced from these findings that the nicotinamides **8** were converted into the isothiazolopyridine **5c** (IC_{50} = 0.22 μM) in the acidic environment of the parietal cell and then the formed **5c** inhibited the H^+/K^+-ATPase.

The stability of **8b** at pH 5.0 and 7.0 was compared with those of the reference compounds omeprazole (**1a**), lansoprazole (**1b**), and pantoprazole (**1c**) (Table 7). Compound **8b** was much more stable than the reference compounds **1a–c** at both neutral and weakly acidic pH (pH 5.0). This stability profile has the potential advantage of minimizing a risk of activation at weakly acidic pH found outside the parietal cells, for example, lysosomes.[16]

Scheme 4[a]



[a] (a) R^1Cl, $N(C_2H_5)_3$; (b) R^1OH, concentrated HCl; (c) chloromethyl methyl ether, diisopropylethylamine; (d) (1) RNH_2, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, (2) *m*CPBA; (e) (1) RNH_2, $(COCl)_2$, (2) *m*CPBA; (f) (1) $(CH_3CO)_2O$, (2) 4-aminopyridine, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide, (3) K_2CO_3, (4) *m*CPBA; (g) 4-nitroaniline, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide; (h) (1) H_2—Pd/C, (2) *m*CPBA.

Table 5. Antisecretory Activities of *N*-Substituted 2-(Benzhydrylsulfinyl)nicotinamides **7**

compd	[^{14}C]AP accumulation,[a,b] % inhibtn at 10 μM	inhibtn of gastric acid secretion in pylorus-ligated rats,[b] % inhibtn at 100 mg/kg id
7a	1.3	
7b	5.0	
7c	NE[c]	
7d	84.3[d]	NE
7e	57.4[d]	
7f	58.3[d]	
7g	93.5[d]	
	7.4 (at 1 μM)	
7h	8.0	
7i	43.7[d]	NE
7j	55.0 (at 1 μM)[d]	30.2
7k	IC_{50} = 1.5 μM[e]	48.1
7l	NE	
7m	74.1[d]	30.4
7n	61.8[d]	21.7
7o	24.4[d]	
7p	68.6[d]	NE
7q	47.6[d]	
7r	NE	
7s	NE	
7t	14.7	
omeprazole	IC_{50} = 0.37 μM[e]	

[a] Inhibition of [^{14}C]aminopyrine (AP) accumulation determined in isolated rabbit parietal cells after dbcAMP stimulation. [b] See the Experimental Section. [c] NE: not effective. [d] $p < 0.01$. [e] See footnote b in Table 1.

As a result of the present study, compound **8b** was found to be a selective inhibitor of H^+/K^+-ATPase; its inhibitory activities against the [^{14}C]aminopyrine accumulation stimulated by dbcAMP in isolated rabbit parietal cells and against the histamine-induced gastric acid secretion in pylorus-ligated rats by id administration were comparable to those of omeprazole. In addition, **8b** was readily converted into the active form **5c** at low pH and was much more stable at both neutral and weakly acidic pH than pantoprazole. Therefore, this compound, having a different structure from the PSBs, seems to be a promising agent for treating acid-related gastrointestinal disorders.

Experimental Section

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Shimazu FTIR-8200PC spectrophotometer. ^{1}H NMR spectra were taken at 200 MHz with a Varian Gemini-200 spectrometer in $(CH_3)_2SO$-d_6. Chemical shifts are expressed in δ (ppm) values with tetramethylsilane as the internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Mass spectra were obtained on a JOEL JMS D-300 mass spectrometer for electron impact-mass spectra (EIMS), Hitachi M-80-B mass spectrometer for secondary-ion mass spectra (SIMS) and fast atom bombardment mass spectra (FABMS), or Hitachi M-1000 LC API mass spectrometer for atmospheric pressure chemical ionization mass spectra (APCIMS). The HPLC was carried out on Shimazu LC-10A system. Organic extracts were dried over anhydrous $MgSO_4$. 2-(Methoxymethoxy)-4-methoxybenzyl alcohol was prepared according to the cited literature.[17]

2-(Acetylamino)-3-[3-(isobutylcarbamoyl)-2-pyridyl]-dithiopropanoic Acid (6). To a stirred solution of the *N*-isobutyl-2,3-dihydro-3-oxoisothiazolo[5,4-*b*]pyridine[15] (6.0 g, 0.029 mol) in CH_3OH (200 mL) was added 4.7 g (0.029 mol) of *N*-acetyl-L-cysteine at room temperature. The resulting mixture was stirred at the same temperature for 3 min and concentrated to dryness in vacuo. The residue was recrystallized from C_2H_5OH to give 7.6 g (71%) of **6**: mp 175—177 °C; ^{1}H NMR δ 0.90 (6H, d, J = 6.2 Hz), 1.86 (3H, s), 4.01 (1H, m), 7.33 (1H, dd, J = 5.1, 7.9 Hz), 7.94 (1H, dd, J = 2.0, 7.9 Hz), 8.63 (1H, dd, J = 2.0, 5.1 Hz), 12.80 (1H, s); SIMS *m*/*z* 372 (MH^+); Anal. ($C_{15}H_{21}N_3O_4S_2$) C, H, N, S.

2-(2-Hydroxyethoxy)-4-methoxybenzyl Alcohol. A mixture of 2-hydroxy-4-methoxybenzaldehyde (20 g, 0.13 mol), acetic acid 2-chloroethyl ester (17 mL, 0.16 mol), K_2CO_3 (27 g, 0.20 mol), and dimethylformamide (DMF) (200 mL) was stirred at 150 °C for 3 days and concentrated to dryness in vacuo. The residue was taken up in 50 mL of water, and the aqueous mixture was extracted with two 200-mL portions of $(C_2H_5)_2O$. The combined extracts were dried and concentrated to dryness in vacuo to give 31 g (99%) of crude 2-(2-acetoxyethoxy)-4-methoxybenzaldehyde: APCIMS *m*/*z* 239 (MH^+).

To a stirred solution of the crude 2-(2-acetoxyethoxy)-4-methoxybenzaldehyde (31 g, 0.13 mol) in CH_3OH (500 mL) was added slowly 4.5 g (0.12 mol) of $NaBH_4$. The resulting mixture was stirred at room temperature for 30 min and concentrated to dryness in vacuo. The residue was taken up in 50 mL of water, and the aqueous mixture was extracted with two 200-mL portions of $CHCl_3$. The combined extracts were washed with brine, dried, and concentrated to dryness in vacuo to give 25 g (97%) of crude 2-(2-hydroxyethoxy)-4-methoxybenzyl alcohol: ^{1}H NMR δ 3.65—3.75 (2H, m), 3.73 (3H, s), 3.98 (2H, t, J = 5.2 Hz), 4.43 (2H, d, J = 5.7 Hz), 4.78 (1H, t, J = 5.2 Hz), 4.85 (1H, t, J = 5.4 Hz), 6.44—6.53 (2H, m), 7.22 (1H, m).

Table 6. Antisecretory Activities and Half-Lives of Conversion of 2-(Benzylsulfinyl)-*N*-(4-pyridyl)nicotinamides **8**

compd	[^{14}C]AP accumulation,[a,b] IC_{50}[c] or % inhibtn (μM)	inhibtn of gastric acid secretion in pylorus-ligated rats,[b] ED_{50}[d] or % inhibtn (id dose mg/kg)[e]	$t_{1/2}$ of conversion (h)[b] pH 1.0	pH 3.0	pH 5.0	inhibtn of H^+/K^+-ATPase,[b] IC_{50} (μM)
8a	33.1% (10)[f]	44.4% (100)[f]	2.14			
8b	0.59	4.2 [1.4−12.2]	<0.02	0.84	38.51	>100
8c	26	60.7% (100)[f]	0.66	38.51		>100
		12.7% (10)				
8d	19.6% (10)		0.45	115.52		
8e	32.5% (10)[f]	21.7% (100)	<0.02	0.14		>100
8f	1.2	6.8 [2.0−22.7]	<0.02	<0.03	0.29	7.3
8g	11.3% (10)		7.70			
8h	16.6	38.5% (10)[g]	0.12	11.55		>100
8i	0.46	5.7 [1.0−31.2]	<0.02	0.09	4.28	>100
8j	1.9	34.8% (100)	0.07	28.88		
8k	0.45	19 [7.3−49.4]	<0.02	0.71		>100
8l	0.40	11.2 [2.9−42.7]	<0.02	0.49		>100
8m	59	41.6% (10)[g]	<0.02	1.78		
8n	1.7	8.1 [1.1−59.1]	<0.02	3.30		>100
8o	2.2	37.7% (100)	<0.02	8.25		
8p	2.0	76.6% (10)[f]	<0.02	2.75		
		14.4% (3)				
omeprazole	0.37	4.1 [1.5−10.9]				

[a] See footnote a in Table 5. [b] See the Experimental Section. [c] See footnote b in Table 1. [d] ED_{50} values were calculated from the regression lines; 95% confidence limits in brackets. [e] $n = 4-7$ at each dose. [f] $p < 0.01$. [g] $0.01 < p < 0.05$.

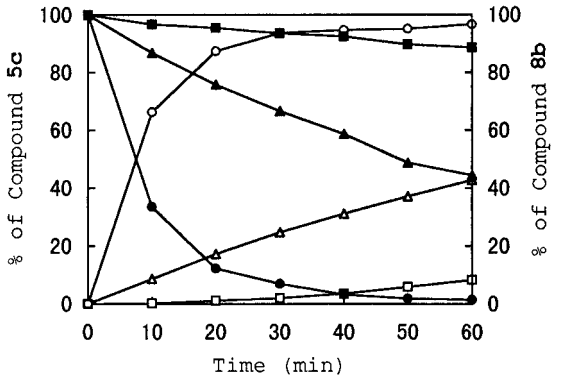


Figure 1. Kinetics of conversions of compound **8b** in aqueous solution (pH 2.0, 3.0, 4.0) at 37 °C. Compound **8b** was incubated at each pH; the concentrations of **8b** (pH 2.0, ●; pH 3.0, ▲; pH 4.0, ■) and compound **5c** (pH 2.0, ○; pH 3.0, △; pH 4.0, □) were determined at the time points indicated using HPLC.

Table 7. pH-Dependent Chemical Stability of Compound **8b** and Reference Compounds

compd	$t_{1/2}$[a] (h) pH 5	pH 7
8b	38.51	>100
omeprazole	0.32	10.50
lansoprazole	0.28	6.08
pantoprazole	1.17	23.11

[a] See the Experimental Section.

2,4-Diethoxybenzyl alcohol was prepared starting from 2,4-dihydroxybenzaldehyde in a manner similar to that described above; the overall yield was 81%. Crude 5-bromo-2,4-dimethoxybenzyl alcohol and 2-ethoxy-4-methoxybenzyl alcohol were prepared from 5-bromo-2,4-dimethoxybenzaldehyde and 2-ethoxy-4-methoxybenzaldehyde, respectively, by reduction with $NaBH_4$ in a manner similar to that described above; the yields were 97% and 85%. The crude benzyl alcohols, without further purification, were used for the preparation of the corresponding nicotinic acids.

2-(2-Fluoroethoxy)-4-methoxybenzyl Alcohol. A mixture of methyl 2-hydroxy-4-methoxybenzoate (10 g, 0.055 mol), 1-chloro-2-fluoroethane (4.7 mL, 0.066 mol), K_2CO_3 (11.4 g, 0.079 mol), and DMF (100 mL) was stirred at 90 °C for 2 days and concentrated to dryness in vacuo. The residue was taken up in 50 mL of water, and the aqueous mixture was extracted with two 50-mL portions of $(C_2H_5)_2O$. The combined extracts were dried and concentrated to dryness in vacuo to give 12 g (96%) of crude methyl 2-(2-fluoroethoxy)-4-methoxybenzoate: SIMS *m/z* 229 (MH^+).

To a stirred solution of the crude benzoate (12 g, 0.053 mol) in $(C_2H_5)_2O$ (150 mL) was added slowly 1.9 g (0.049 mol) of $LiAlH_4$ at 0 °C. After the resulting mixture was stirred at the same temperature for 30 min, the remaining $LiAlH_4$ was allowed to decompose by the cautious addition of 10 mL of CH_3OH and 5 mL of water. The organic solvent was removed by distillation in vacuo, and the resulting aqueous mixture was extracted with two 100-mL portions of $CHCl_3$. The combined extracts were washed with brine, dried, and concentrated to dryness in vacuo to give 10 g (92%) of crude 2-(2-fluoroethoxy)-4-methoxybenzyl alcohol: ^{1}H NMR δ 3.74 (3H, s), 4.13−4.87 (5H, m), 4.44 (2H, d, $J = 5.6$ Hz), 6.52−6.58 (2H, m), 7.26 (1H, m).

Crude 2,4-dimethoxy-6-methylbenzyl alcohol was prepared from ethyl 2,4-dihydroxy-6-methylbenzoate in a manner similar to that described above; the overall yield was 97%. The crude benzyl alcohols, without further purification, were used for the preparation of the corresponding nicotinic acids.

2-(Benzylthio)nicotinic Acids 11−28 (Table 2). Procedure A (1). 2-[(4-Methoxybenzyl)thio]nicotinic Acid (13). This compound was prepared from 2-mercaptonicotinic acid (**10**) and 4-methoxybenzyl chloride in a manner similar to that described previously:[15] ^{1}H NMR δ 3.72 (3H, s), 4.30 (2H, s), 6.85 (2H, m), 7.23 (1H, dd, $J = 5, 7$ Hz), 7.31 (2H, m), 8.20 (1H, dd, $J = 2, 7$ Hz), 8.65 (1H, dd, $J = 2, 5$ Hz); EIMS *m/z* 275 (M^+); IR (KBr) 1681 cm^{-1} (C=O).

Procedure A (2). 2-[(2,4-Diethoxybenzyl)thio]nicotinic Acid (24). To a stirred solution of the crude 2,4-diethoxybenzyl alcohol (20 g, 0.11 mol) in toluene (400 mL) was added 60 mL of concentrated HCl at room temperature. The resulting mixture was stirred at the same temperature for 30 min, washed successively with water and brine, dried, and concentrated to dryness in vacuo. The residue was dissolved in 500 mL of CH_2Cl_2, and then a suspension of 2-mercaptonicotinic acid (14 g, 0.089 mol) in CH_2Cl_2 (500 ml) was added. To the stirred resulting mixture was added slowly 14 g (0.14 mol) of triethylamine at 0 °C. The mixture was stirred at room temperature for 6 h, taken up in 100 ml of water, and extracted with two 100-mL portions of $CHCl_3$. The combined extracts were dried and concentrated to dryness in vacuo to give 28 g (95%) of **24**: ^{1}H NMR δ 1.29 (3H, t, $J = 6.8$ Hz), 1.30 (3H, t, $J = 6.8$ Hz), 3.99 (2H, q, $J = 6.8$ Hz), 4.04 (2H, q, $J = 6.8$ Hz), 4.27 (1H, s), 6.42 (1H, m), 6.52 (1H, m), 7.17−7.26 (2H, m), 8.17 (1H, dd, $J = 1.9, 7.6$ Hz), 8.63 (1H, dd, $J = 1.9, 4.9$ Hz); SIMS *m/z* 333 (M^+); IR (KBr) 1695 cm^{-1} (C=O).

Procedure B. 2-[(2,4-Dimethoxy-6-methylbenzyl)thio]-nicotinic Acid (21). Compound **21** was derived from 2-mercaptonicotinic acid (**10**) that was condensed with 2,4-dimethoxy-6-methylbenzyl alcohol in the presence of concentrated HCl in a manner similar to that described previously:[15] ^{1}H NMR δ 2.28 (3H, s), 3.74 (3H, s), 3.76 (3H, s), 4.24 (2H, s), 6.42 (2H, m), 7.18 (1H, dd, J = 4.8, 7.9 Hz), 8.15 (1H, dd, J = 2.0, 7.9 Hz), 8.59 (1H, dd, J = 2.0, 4.8 Hz); EIMS m/z 319 (M^+); IR (KBr) 1772 cm^{-1} (C=O).

Procedure C. 2-[[4-Methoxy-2-[2-(methoxymethoxy)-ethoxy]benzyl]thio]nicotinic Acid (28). A mixture of **25** (7.0 g, 0.021 mol), chloromethyl methyl ether (4.8 mL, 0.063 mol), diisopropylethylamine (18 mL, 0.10 mol), and CH_2Cl_2 (200 mL) was stirred at room temperature for 16 h and concentrated to dryness in vacuo. The residue was taken up in 100 mL of water, and the aqueous mixture was extracted with two 200-mL portions of $CH_3CO_2C_2H_5$. The combined extracts were dried and concentrated to dryness in vacuo. The residue was dissolved in 400 mL of CH_3OH, and then 60 mL of 1 N NaOH was added. The resulting mixture was stirred at reflux temperture for 1 h. The reaction mixture was neutralized with 1 N HCl and concentrated to dryness in vacuo. The residue was taken up in 50 mL of water, and the aqueous mixture was extracted with two 200-mL portions of $CHCl_3$. The combined extracts were washed with brine, dried, and concentrated to dryness in vacuo to give 7.4 g (93%) of **28**: ^{1}H NMR δ 3.18 (3H, s), 3.73 (3H, s), 3.77 (2H, m), 4.15 (2H, m), 4.28 (2H, s), 4.59 (2H, s), 6.48 (1H, dd, J = 2.2, 8.7 Hz), 6.58 (1H, d, J = 2.2 Hz), 8.19 (1H, dd, J = 1.9, 7.6 Hz), 8.65 (1H, J = 1.9, 4.8 Hz); IR (KBr) 1682 cm^{-1} (C=O).

2-(Benzhydrylsulfinyl)nicotinamides 7a−t and 2-(Benzylsulfinyl)nicotinamides 8a−p (Tables 3 and 4). Procedure D. 2-(Benzhydrylsulfinyl)-*N*-phenylnicotinamide (7b). 2-(Benzhydrylthio)-*N*-phenylnicotinamide was derived from 2-(benzhydrylthio)nicotinic acid (**11**) in 82% yield by condensation with aniline by the use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride in a manner similar to that described previously:[15] mp 156−159 °C (CH_3OH); ^{1}H NMR δ 6.58 (1H, s), 7.87 (1H, dd, J = 1.9, 7.9 Hz), 8.00 (1H, s), 8.46 (1H, dd, J = 1.9, 5.1 Hz); EIMS m/z 396 (M^+); IR (KBr) 1645 cm^{-1} (C=O).

Compound **7b** was derived from 2-(benzhydrylthio)-*N*-phenylnicotinamide in 62% yield by oxidation with *m*CPBA in a manner similar to that described previously:[15] ^{1}H NMR δ 5.87 (1H, s), 7.59 (1H, dd, J = 4.8, 5.2 Hz), 8.11 (1H, dd, J = 1.7, 8.2 Hz), 8.66 (1H, dd, J = 1.7, 4.8 Hz); SIMS m/z 413 (MH^+); IR (KBr) 1043 (S=O), 1668 (C=O) cm^{-1}.

Procedure E (1). 2-[[2-(2-Fluoroethoxy)-4-methoxybenzyl]sulfinyl]-*N*-(4-pyridyl)nicotinamide (8n). 2-[[2-(2-Fluoroethoxy)-4-methoxybenzyl]thio]-*N*-(4-pyridyl)nicotinamide was prepared starting from 2-[[2-(2-fluoroethoxy)-4-methoxybenzyl]thio]nicotinic acid (**26**), which was chlorinated with oxalyl chloride followed by condensation with 4-aminopyridine in a manner similar to that described previously.[15] This compound was obtained as an oily product. The overall yield was 61%: ^{1}H NMR δ 3.73 (3H, s), 4.16−4.82 (4H, m), 4.36 (2H, s), 6.47 (1H, dd, J = 2.4, 8.6 Hz), 6.58 (1H, d, J = 2.4 Hz), 7.65 (2H, m), 7.96 (1H, dd, J = 1.9, 7.6 Hz), 8.47 (2H, m), 8.64 (1H, dd, J = 1.9, 5.2 Hz), 10.79 (1H, s); APCIMS m/z 414 (MH^+); IR (KBr) 1684 cm^{-1} (C=O).

Compound **8n** was derived from 2-[[2-(2-fluoroethoxy)-4-methoxybenzyl]thio]-*N*-(4-pyridyl)nicotinamide in 90% yield by oxidation with *m*CPBA in a manner similar to that described previously:[15] ^{1}H NMR δ 3.73 (3H, s), 4.19 (1H, d, J = 12.0 Hz), 4.42 (1H, d, J = 12.0 Hz), 6.47 (1H, dd, J = 2.2, 8.3 Hz), 6.56 (1H, d, J = 2.2 Hz), 7.02 (1H, d, J = 8.3 Hz), 7.65 (2H, m), 7.71 (1H, dd, J = 4.6, 8.0 Hz), 8.22 (1H, dd, J = 1.9, 8.0 Hz), 8.85 (1H, dd, J = 1.9, 4.6 Hz), 10.95 (1H, s); APCIMS m/z 430 (MH^+); IR (KBr) 1037 (S=O), 1672 (C=O) cm^{-1}.

Procedure E (2). 2-(Benzhydrylsulfinyl)-*N*-(2-pyrimidinyl)nicotinamide (7r). Oxalyl chloride (10 g, 0.079 mol) was added to a stirred suspension of **11** (5.0 g, 0.016 mol) in dioxane (200 mL) at room temperature. The resulting mixture was heated at 80 °C for 2 h with stirring. The mixture was concentrated to dryness in vacuo. The residue was dissolved in 300 mL of pyridine, and then 2-aminopyrimidine (1.5 g, 0.016 mol) was added. The reaction mixture was stirred at room temperature overnight and concentrated to dryness in vacuo. The residue was taken up in 100 mL of water, and the aqueous mixture was extracted with two 300-mL portions of $CHCl_3$. The combined extracts were dried and concentrated to dryness in vacuo. The residue was chromatographed on silica gel, eluted with $CHCl_3-CH_3OH$ (50:1), and crystallized from CH_3CN to give 3.5 g (56%) of 2-(benzhydrylthio)-*N*-(2-pyrimidinyl)nicotinamide: mp 155−159 °C; ^{1}H NMR δ 6.41 (1H, s), 7.89 (1H, dd, J = 1.9, 8.1 Hz), 8.46 (1H, dd, J = 1.9, 5.1 Hz), 8.67 (2H, d, J = 4.1 Hz); FABMS m/z 399 (MH^+); IR (KBr) 1666 cm^{-1} (C=O).

To a stirred solution of the 2-(benzhydrylthio)-*N*-(2-pyrimidinyl)nicotinamide (2.0 g, 5.0 mmol) in CH_2Cl_2 (50 mL) was added dropwise a solution of 80% *m*CPBA (1.3 g, 6.0 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The resulting mixture was stirred at the same temperature for 3 min, washed with saturated aqueous $NaHCO_3$ (30 mL), and dried. The solvent was removed by distillation in vacuo. The residue was chromatographed on silica gel with $CHCl_3-CH_3OH$ (30:1) as the eluent and recrystallized from CH_3CN to give 0.66 g (32%) of **7r**: ^{1}H NMR δ 5.81 (1H, s), 7.52 (1H, dd, J = 5.0, 7.8 Hz), 8.03 (1H, dd, J = 1.9, 7.8 Hz), 8.61 (1H, dd, J = 4.9, 5.0 Hz), 8.66 (2H, d, J = 4.9 Hz), 11.30 (1H, s); SIMS m/z 415 (MH^+); IR (KBr) 1045 (S=O), 1683 (C=O) cm^{-1}.

Compound **7s** was prepared in a manner similar to that described above.

Procedure F. *N*-(4-Aminophenyl)-2-(benzhydrylsulfinyl)nicotinamide (7e). 2-(Benzhydrylthio)-*N*-(4-nitrophenyl)nicotinamide (**29**) was prepared from 2-(benzhydrylthio)-nicotinic acid (**10**), which was chlorinated with oxalyl chloride followed by condensation with 4-nitroaniline in a manner similar to that described previously.[15] The overall yield was 55%: mp 166−167 °C; ^{1}H NMR δ 6.44 (1H, s), 7.16−7.48 (10H, m), 8.28 (2H, m), 8.52 (1H, dd, J = 1.8, 4.8 Hz), 11.09 (1H, s); SIMS m/z 441 (M^+); IR (KBr) 1658 cm^{-1} (C=O). Anal. ($C_{25}H_{19}N_3O_3S$) C, H, N, S.

Compound **29** (1.0 g, 2.3 mmol) was hydrogenated in 100 mL of $CH_3CO_2C_2H_5$ containing 0.1 g of 10% Pd/C at 40 °C under atmospheric pressure. After removal of the catalyst by filtration, the solvent was removed in vacuo to give 0.90 g (97%) of crude 2-(benzhydrylthio)-*N*-(4-nitrophenyl)-nicotinamide: SIMS m/z 411 (M^+).

The crude nicotinamide was dissolved in 50 mL of $CH_2Cl_2-CH_3OH$ (2:1), and then a solution of 80% *m*CPBA (0.40 g, 1.9 mmol) in CH_2Cl_2 (50 mL) was added dropwise at 0 °C. The resulting mixture was stirred at the same temperature for 3 min, washed with saturated aqueous $NaHCO_3$ (20 mL), and dried. The solvent was removed by distillation in vacuo. The residue was chromatographed on silica gel with $CHCl_3-CH_3OH$ (20:1) as the eluent and recrystallized from *n*-hexane−$CHCl_3$ to give 0.37 g (51%) of **7e**: ^{1}H NMR δ 5.01 (2H, br), 5.83 (1H, s), 6.57 (2H, m), 7.55 (1H, dd, J = 4.5, 8.1 Hz), 8.07 (1H, dd, J = 1.8, 8.1 Hz), 8.62 (1H, J = 1.8, 4.5 Hz), 10.16 (1H, s); SIMS m/z 427 (M^+); IR (KBr) 1036 (S=O), 1661 (C=O) cm^{-1}.

Procedure G. 2-[[2-(2-Hydroxyethoxy)-4-methoxybenzyl]sulfinyl]-*N*-(4-pyridyl)nicotinamide (8m). To a stirred solution of **25** (1.0 g, 3.0 mmol) in pyridine (8 mL) was added 1 mL of $(CH_3CO)_2O$ at room temperature. The resulting mixture was stirred at same temperature for 30 min and at reflux temperature for 1 h. The resulting mixture was concentrated to dryness in vacuo and taken up in 100 mL of water, and the aqueous mixture was extracted with two 200-mL portions of $CHCl_3$. The combined extracts were washed with brine, dried, and concentrated to dryness in vacuo to give 1.1 g (98%) of crude 2-[[2-(2-acetoxyethoxy)-4-methoxybenzyl]-thio]nicotinic acid.

2-[[2-(2-Acetoxyethoxy)-4-methoxybenzyl]thio]-*N*-(4-pyridyl)-nicotinamide was derived from the crude 2-[[2-(2-acetoxyethoxy)-4-methoxybenzyl]thio]nicotinic acid in 57% yield by condensation with 4-aminopyridine by the use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride in a manner similar to that described previously.[15] This compound was obtained as an oily product: ^{1}H NMR δ 1.94 (3H, s), 3.72 (3H, s), 4.19 (2H, m), 4.30 (2H, m), 4.34 (2H, s), 6.45 (1H, dd, J =

2.6, 8.6 Hz), 6.57 (1H, d, J = 2.6 Hz), 7.65 (2H, m), 7.95 (1H, dd, J = 1.9, 7.6 Hz), 8.47 (2H, m), 8.64 (1H, dd, J = 1.9, 4.9 Hz); APCIMS m/z 454 (M^+); IR (KBr) 1684 cm^{-1} (C═O).

To a stirred solution of the crude 2-[[2-(2-acetoxyethoxy)-4-methoxybenzyl]thio]-*N*-(4-pyridyl)nicotinamide (0.77 g, 1.7 mmol) in CH_3OH (30 mL) was added 30 mL of 0.1 N K_2CO_3 at room temperature. The resulting mixture was stirred at the same temperature for 30 min, neutralized with 1 N HCl at 0 °C, and concentrated to about 30 mL. The aqueous mixture was extracted with two 30-mL portions of $CHCl_3$. The combined extracts were washed with two 10-mL portions of brine, dried, and concentrated to dryness in vacuo to give crude 2-[[2-(2-hydroxyethoxy)-4-methoxybenzyl]thio]-*N*-(4-pyridyl)-nicotinamide (0.63 g, 96%): APCIMS m/z 412 (M^+).

To a stirred solution of the crude 2-[[2-(2-hydroxyethoxy)-4-methoxybenzyl]thio]-*N*-(4-pyridyl)nicotinamide (0.63 g, 1.5 mmol) in CH_2Cl_2 (50 mL) was added dropwise a solution of 80% *m*CPBA (0.36 g, 1.7 mmol) in CH_2Cl_2 (10 mL) at 0 °C. The resulting mixture was stirred at the same temperature for 3 min, washed with saturated aqueous $NaHCO_3$ (10 mL), and dried. The solvent was removed by distillation in vacuo. The residue was chromatographed on silica gel with $CHCl_3$−CH_3OH (10:1) as the eluent and recrystallized from acetone to give 0.20 g (31%) of **8m**: ^{1}H NMR δ 3.66 (2H, m), 3.73 (3H, s), 3.92 (2H, m), 4.24 (1H, d, J = 12.3 Hz), 4.43 (1H, d, J = 12.3 Hz), 4.75 (1H, t, J = 5.8 Hz), 6.44 (1H, dd, J = 2.3, 8.1 Hz), 6.53 (1H, d, J = 2.3 Hz), 6.99 (1H, d, J = 8.1 Hz), 7.66 (2H, m), 7.72 (1H, dd, J = 4.7, 7.8 Hz), 8.24 (1H, dd, J = 1.8, 7.8 Hz), 8.50 (2H, m), 8.86 (1H, dd, J = 1.8, 4.7 Hz), 10.97 (1H, s); APCIMS m/z 428 (MH^+); IR (KBr) 1032 (C═O), 1676 (S═O) cm^{-1}.

Oxidation with *m*CPBA in Acidic Conditions. 2-(Benzhydrylsulfinyl)-*N*-(1-benzyl-4-piperidyl)nicotinamide (7d). 2-(Benzhydrylthio)-*N*-(1-benzyl-4-piperidyl)nicotinamide was prepared from 2-(benzhydrylthio)nicotinic acid (**11**), which was chlorinated with oxalyl chloride followed by condensation with 4-amino-1-benzylpiperidine in a manner similar to that described previously.[15] The overall yield was 83%. This compound was obtained as an oily product: ^{1}H NMR δ 1.50−1.69 (2H, m), 1.97−2.04 (2H, m), 2.11−2.25 (2H, m), 2.79−2.88 (2H, m), 3.50 (2H, s), 3.92−4.09 (1H, m), 6.54 (1H, s), 6.99 (1H, dd, J = 5, 7 Hz), 7.76 (1H, dd, J = 1.9, 7.9 Hz), 8.40 (1H, J = 1.9, 4.9 Hz); EIMS m/z 493 (M^+); IR (KBr) 1649 cm^{-1} (C═O).

To a stirred solution of 2-(benzhydrylthio)-*N*-(1-benzyl-4-piperidyl)nicotinamide (2.8 g, 5.7 mmol) and 1.0 mL of 6 N HCl in CH_3OH (100 mL) was added dropwise a solution of 80% *m*CPBA (1.3 g, 6.0 mM) in CH_3OH (50 mL) at 0 °C. The resulting mixture was stirred at the same temperature for 1 min, taken up in 200 mL of saturated aqueous $NaHCO_3$, extracted with two 300-mL portions of $CHCl_3$, and dried. The solvent was removed by distillation in vacuo. The residue was chromatographed on silica gel with $CHCl_3$−CH_3OH (50:1) as the eluent and recrystallized from CH_3OH to give 1.0 g (35%) of **7d**: ^{1}H NMR δ 1.47−1.63 (2H, m), 1.77−1.89 (2H, m), 2.01−2.13 (2H, m), 2.79−2.87 (2H, m), 3.50 (2H, s), 3.70−3.85 (1H, m), 5.79 (1H, s), 7.51 (1H, dd, J = 5, 7 Hz), 7.96 (1H, dd, J = 2, 7 Hz), 8.60 (1H, dd, J = 2, 5 Hz); SIMS m/z 510 (MH^+); IR (KBr) 1043 (S═O), 1651 (C═O) cm^{-1}.

pH-Dependent Chemical Stability of Compounds 8a−p, Omeprazole, Lansoprazole, and Pantoprazole. The half-lives of each compound were determined in 100 mM HCl (pH 1), 10 mM HCl (pH 2), and McIlvaine buffer[18] (pH 3−7) which had been adjusted to the appropriate pH. Each compound was dissolved in $(CH_3)_2SO$ at a concentration of 10 μM. Then 20 μL of the prepared solution was added to 1.96 mL of the appropriate buffer and 0.02 mL of 1% Triton solution. The mixture was incubated at 37 °C for various periods of time, and 20 μL of this solution was injected into HPLC (Capcell Pack C_{18} SG120 4.6−250 mm, CH_3CN/10 mM phosphate buffer (pH 7.0), 1 mL/min, UV detector at 254 nm). On the basis of peak area, the amounts of the nicotinamides, the isothiazolopyridine, and the reference compounds were estimated, and the half-life for each compound in different media was determined from the liner regression of ln of the concentration vs time.

Reference Compounds. Omeprazole and lansoprazole were extracted from commercially available Omepral tablets (Fujisawa Pharmaceutical Co. Ltd.) and Takepron capsules (Takeda Chemical Industries Ltd.). Pantoprazole was prepared according to known procedures.[3]

Biology. For the in vitro study, the test compounds were dissolved in $(CH_3)_2SO$. All incubation mixtures contained less than 1% $(CH_3)_2SO$.

H^+/K^+-ATPase Activity in Porcine Gastric Microsomes. Porcine gastric microsomes containing H^+/K^+-ATPase were prepared as described by Nagaya.[19] The membrane protein (15 μg) was preincubated at 37 °C for 30 min in an assay medium containing 50 mM Tris-HCl buffer, pH 7.4, 4 mM $MgCl_2$, 5 μg/mL gramicidin, and test compound, with or without 20 mM KCl (total volume 1 mL). The enzyme reaction was started by adding ATP to a final concentration of 2 mM. After incubation for 20 min at 37 °C, the reaction was terminated by adding 1 mL of 16% trichloroacetic acid. Inorganic phosphate produced by hydrolysis of ATP was determined according to the method of Sanui.[20] The H^+/K^+-ATPase activity was calculated by subtracting the base rate (in the absent of K^+) from the rate of hydrolysis of ATP in the presence of K^+.

Acid Formation in Isolated Rabbit Parietal Cells. Rabbit parietal cells were isolated as described by Fryklund.[21] Acid formation in the parietal cells was assessed by accumulation of [^{14}C]AP.[22] The parietal cell-rich fraction ($1-2 \times 10^7$ cells/300 μL) was suspended in 1.5 mL of Earle's balanced salt solution containing 5.6 kBq of [^{14}C]AP, 25 mM HEPES-NaOH buffer, pH 7.4, 0.2% bovine serum albumin, and test compound. After dibutyryl cAMP (1 mM) was added, the reaction mixture was incubated at 37 °C for 30 min under an atmosphere of 95% O_2 and 5% CO_2. The cells were separated from medium by brief centrifugation and digested with a tissue solubilizer. After a liquid scintillator was added, radioactivity was counted using a liquid scintillation counter. The radioactivity accumulated by the cells in the presence of 0.1 mM dinitrophenol was subtracted from all data to correct for trapped [^{14}C]AP.

Histamine-Induced Gastric Acid Secretion in Pylorus-Ligated Rats.[2] Male stranded Wister rats weighing about 200 g were used. Rats were deprived of food but allowed free access to water for 24 h prior to experiments. Each experiment was performed using 4−7 rats/group. Under urethane anesthesia (1 g/kg), a midline laparotomy was performed and ligature tightly secured around the pylorus. Either control vehicle or drug was administered intraduodenally immediately just after ligating the pylorus, and the abdominal incision was closed. Thirty minutes later, histamine (30 mg/kg) was injected sc. Three hours later, the stomach was removed, and the gastric contents were collected. The volume of gastric juice was measured, and the acid concentration of 1.0-mL sample aliquots was determined by automatic titration to pH 7 with 0.01 N NaOH. The product of the gastric volume and acid concentration was used to calculate the total acid output. Total acid output during the 3-h period was compared with that obtained in control animals, and results are expressed as percent inhibition.

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