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Amino acid amides of 2-[(2-aminobenzyl)sulfinyl]benzimidazole as acid-stable prodrugs of potential inhibitors of H^+/K^+ ATPase

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Summary — A series of amino acid amides of 2-[(2-aminobenzyl)sulfinyl]benzimidazole were prepared and found to possess gastric antisecretory activity on oral administration. (Glycylaminobenzyl)sulfinyl compound **23a**, stable in artificial gastric juice (pH 1.2), was given orally to dogs. It was absorbed efficiently and converted into aniline derivative **7a** which showed a very high plasma concentration. Compound **23a** was hydrolyzed by the action of aminopeptidase present in plasma or the brush border fraction of the small intestine to release the terminal glycine. *o*-Aniline derivatives showed good activity in *in vitro* H^+/K^+-ATPase inhibition as well as in the inhibition of histamine stimulated acid secretion in isolated bullfrog gastric mucosa. Although these *o*-aniline derivatives showed no or weak gastric antisecretory activity in rat by id administration, they were active when administered ip. Therefore, these amino acid amides were considered to be acid stable prodrugs of proton pump inhibiting *o*-aniline derivatives. The mechanism of H^+/K^+-ATPase inhibition of **7a** was also examined.

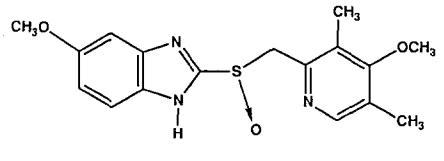
Résumé — Amides d'aminoacides du 2-[(2-aminobenzyl)sulfinyl]benzimidazole, prodrogues stables en milieu acide d'inhibiteurs potentiels de la H^+/K^+-ATPase. *Préparation d'une série d'aminoamides du 2-[(2-aminobenzyl)sulfinyl]benzimidazole présentant une activité antisécrétoire gastrique par administration orale. Le composé (glycylaminobenzyl) sulfinyl* ***23a*** *stable en milieu gastrique artificiel (pH 1.2) a été administré oralement à des chiens. Il était absorbé efficacement et converti en dérivé de l'aniline* ***7a*** *qui révélait une très forte concentration plasmatique. Ce composé* ***23a*** *était hydrolysé sous l'action de l'aminopeptidase présente dans le plasma ou la fraction de bordure en brosse de l'intestin grêle pour libérer la glycine terminale. Les dérivés de l'*o*-aniline ont montré une bonne activité dans l'inhibition* in vitro *de la H^+/K^+-ATPase ainsi que dans l'inhibition de la sécrétion acide stimulée par l'histamine dans la muqueuse gastrique isolée de grenouille. Bien que ces dérivés de l'*o*-aniline n'aient révélé soit aucune activité, soit une faible activité antisécrétoire gastrique chez le Rat par administration id, ils se sont montré actifs par administration intrapéritonéale. En conséquence, ces aminoamides peuvent être considérés comme les prodrogues stables en milieu acide de dérivés de l'*o*-aniline inhibant la pompe à protons. Le mécanisme de l'inhibition H^+/K^+-ATPase du composé* ***7a*** *a été aussi examinée.*

H^+/K^+-ATPase inhibitor / antisecretory / *o*-aniline derivatives / proton pump / amino acid amides / aminopeptidase

Introduction

The new gastric acid secretion inhibitor omeprazole (**1**) has been shown to be a highly effective agent for curing both peptic diseases [1], and controlling severe hypersecretion, such as that encountered in the Zollinger-Ellison syndrome [2]. Its effective inhibition of gastric acid secretion has been ascribed to the inhibition of H^+/K^+-ATPase, the proton pump enzyme present in the secretory membranes of parietal cells of the gastric mucosa [3–5]. Recent investigations have revealed that **1** inhibits acid secretion by binding the acid-induced transformed form of 1 with H^+/K^+-ATPase [6–8].

In the model reactions, omeprazole is converted *via* acid-catalyzed Smiles rearrangement into sulfenamide, the active principle, and subsequently reacts with thiol to form asymmetric disulfide. In this reac-



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tion, the mercapto group of the H^+/K^+-ATPase reacts with the active principle of omeprazole to cause modification of the enzyme leading to inhibition of acid secretion [9–12].

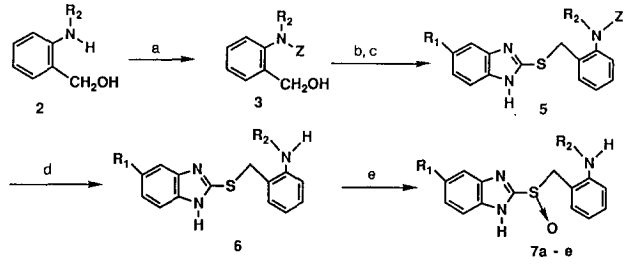
The weak basic properties of omeprazole enable the compound to accumulate in the acidic canalicular lumen, and the pKa values of analogues demonstrated the great dependence of the biological effect on substituents in the pyridine ring [13]. We synthesized a variety of benzimidazole derivatives in which the pyridine ring of omeprazole was replaced with substituted anilines. Of these compounds, 2-aminobenzyl derivatives showed distinct H^+/K^+-ATPase-inhibiting activity and suppressed histamine-stimulated acid secretion in isolated bullfrog gastric mucosa. Intraperitoneal administration also strongly inhibited histamine-stimulated acid secretion in the rat (Schild rat). 2-Aminobenzylsulfoxides were considered to be analogues in which the nitrogen atom of the pyridine ring in omeprazole was situated outside the benzene ring. Independent of our work, a similar series of aniline derivatives were also described in [14–17].

2-Aminobenzylsulfoxide **7a** and the related compounds have strong inhibitory activity on acid secretion in the Schild rat by ip administration, but the inhibition diminishes on id administration. This suggests that the bioavailability of **7a** is poor.

As omeprazole and its analogues are very sensitive to gastric acid, they need to be developed as stable oral formulations, for example, with enteric coating. We have synthesized a variety of amino acid amides of proton pump-inhibiting aniline derivatives as acid-stable and highly bioavailable prodrugs.

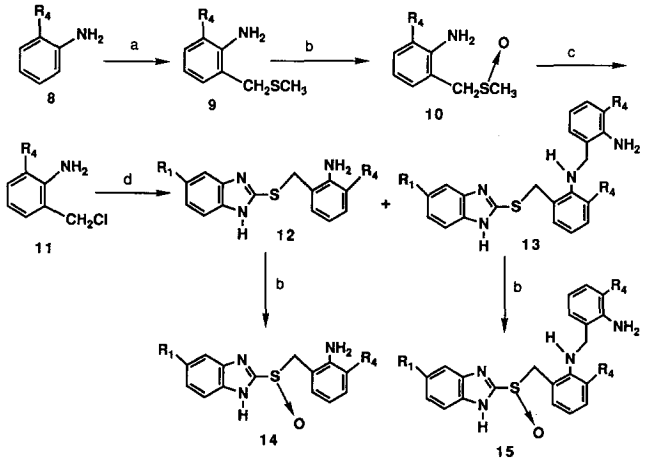
Chemistry

2-Aminobenzyl alcohols **2** were made to react with benzyloxycarbonyl chloride (ZCl) to obtain the *Z*-amino compounds **3** which were then chlorinated with thionyl chloride ($SOCl_2$) and made to react with the 2-mercaptobenzimidazoles **4** giving the sulfides **5**. After removing the Z group of **5** by treatment with HBr-HOAc, the resulting sulfides **6** were oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) affording the sulfoxides **7a–e** (scheme 1). 2-[(Methylthio)methyl]anilines **9** obtained from the substituted anilines **8** were oxidized to give the sulfoxides **10** which were treated with HCl, affording the substituted 2-aminobenzyl chlorides **11** [18]. Alkylation of 2-mercaptobenzimidazole with **11** gave the sulfides **12** and **13**. Oxidation of **12** and **13** with *m*-CPBA afforded the corresponding sulfoxides **14** and **15**, respectively (scheme 2). 2-(3-Aminobenzylsulfinyl)benzimidazole (**19**) was obtained from the *Z*-aminosulfide **18** by deblocking with HBr-HOAc and subsequent oxidation with *m*-CPBA. Compound **18** was derived from 3-



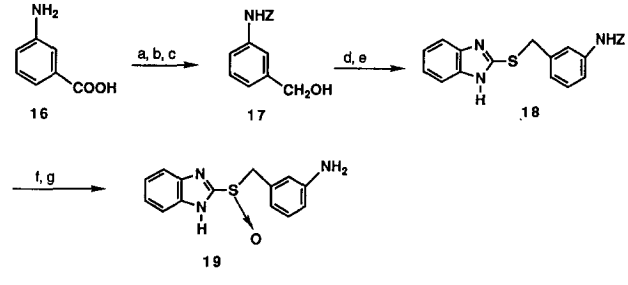
(a) Z-Cl, K_2CO_3; (b) $SOCl_2$; (c) 2-mercaptobenzimidazole (**4**) / NaOH; (d) HBr-HOAc; (e) m-CPBA.

Scheme 1.



(a) Me_2S, NCS, Et_3N; (b) m-CPBA; (c) HCl; (d) **4**, NaOH.

Scheme 2.

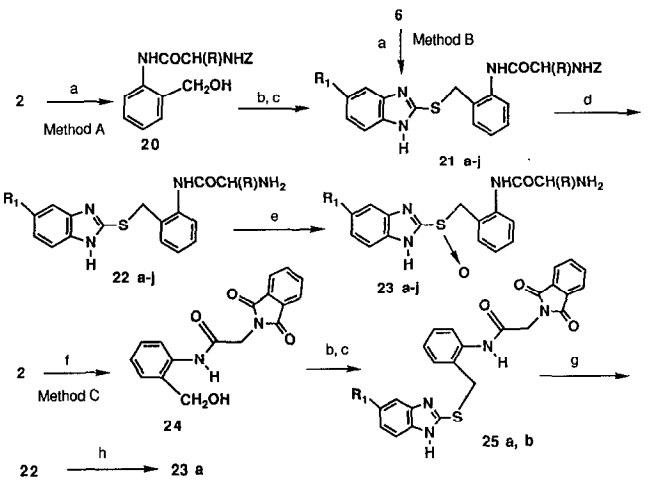


(a) Z-Cl, K_2CO_3; (b) ClCOOEt - Et_3N ; (c) $NaBH_4$; (d) $SOCl_2$; (e) **4**, NaOH ; (f) HBr-HOAc ; (g) m-CPBA.

Scheme 3.

aminobenzoic acid (**16**) by blocking the amino group with the *Z* group and then reacting with ClCOOEt-Et_3N and $NaBH_4$ (scheme 3).

The amino acid amides of 2-[(2-aminobenzyl) sulfinyl]benzimidazole (**23a–j**) were prepared as shown in scheme 4. 2-Aminobenzyl alcohol (**2**) (R_2 =



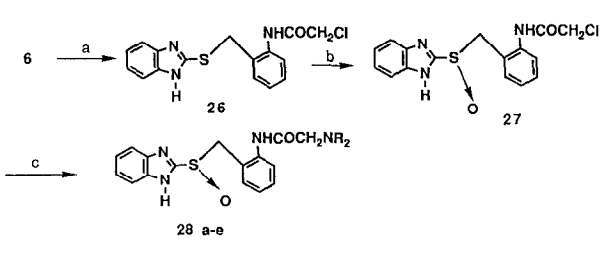
(a) ZNHCH(R)COOH, SOCl2 - HMPA ; (b) SOCl2 ; (c) 4 / NaOH ; (d) HBr - HOAc; (e) m-CPBA ; (f) Pht - Gly-Cl ; (g) MeNH2 ; (h) AcOOH.

Scheme 4.

H) was coupled with the appropriate activated *Z*-amino acids. The coupling products **20** were chlorinated with $SOCl_2$ and then made to react with 2-mercaptobenzimidazoles. The resulting sulfides **21** were treated with HBr-HOAc to remove the *Z* group and then oxidized with *m*-CPBA giving the desired sulfoxides **23** (method A). Sulfide **21** was also obtained from the 2-aminobenzylsulfide **6** (R_2 = H) by coupling with activated *Z*-amino acids (method B). 2-[(2-Glycylaminobenzyl)sulfinyl]benzimidazole (**23a**) was conveniently prepared from 2-[(2-(phthalylglycyl)amino benzyl)thio]benzimidazole (**25**) by removing the phthalyl group with methylamine followed by oxidation with peracetic acid (method C) (scheme 4).

The terminal substituted glycylaminobenzyl derivatives **28a–e** were obtained from the aminobenzylsulfide **6** by reacting with chloro acetyl chloride followed by oxidation with *m*-CPBA and then reaction with a variety of amines (scheme 5).

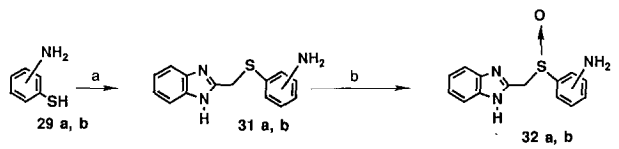
2-[2-/4-(Aminophenylsulfinyl)methyl]benzimidazoles (**32a, b**) were prepared by alkylation of 2-/4-



(a) $ClCOCH_2Cl$; (b) m-CPBA ; (c) R_2NH.

Scheme 5.

aminothiophenols (**29a, b**) with 2-chloromethylbenzimidazole (**30**) followed by oxidation with *m*-CPBA (scheme 6).



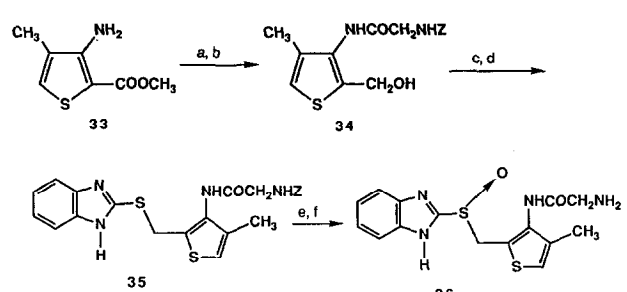
(a) 2-chloromethylbenzimidazole, Et_3N ; (b) m - CPBA.

Scheme 6.

2-[((3-Glycylamino-4-methylthiophen-2-yl)methyl)sulfinyl]benzimidazole (**36**) was prepared as shown in scheme 7. Methyl 3-amino-4-methylthiophene-2-carboxylate (**33**) [19] was coupled with activated Z-glycine followed by reduction with $LiBH_4$, giving the alcohol **34**. Compound **34** was chlorinated with $SOCl_2$ and reacted with 2-mercaptobenzimidazole followed by oxidation with *m*-CPBA after removal of the Z group by HBr-AcOH, and gave the desired **36**.

2[((3-Phenyl-4-glycylaminoisothiazol-5-yl)methyl)sulfinyl]benzimidazole (**40**) was obtained as shown in scheme 8. Ethyl 3-phenyl-4-aminoisothiazole-2-carboxylate (**37**) [20] was coupled with activated Z-glycine followed by reduction with $LiBH_4$, giving the alcohol **38**. Reaction of **38** with benzimidazole-2-disulfide in the presence of n-Bu_3P-pyridine gave the sulfide **39**. After removal of the *Z* group of **39** using HBr-AcOH, oxidation with *m*-CPBA afforded the desired **40**.

2-(2-Aminophenylsulfinyl)benzimidazole (**43**) was prepared by oxidation of the sulfides **42** with m-CPBA. Compound **42** was obtained by the reaction of 2-aminothiophenol with 2-chlorobenzimidazole (**41**) or 2-methylsulfinylbenzimidazole (**44**). Compound **42** was coupled with activated *Z*-glycine and the resulting



(a) Z-Gly, $SOCl_2$ - HMPA ; (b) $LiBH_4$; (c) $SOCl_2$; (d) 4 / NaOH ; (e) HBr - AcOH ; (f) m-CPBA.

Scheme 7.

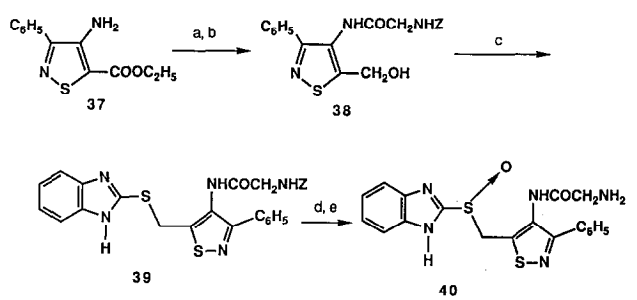
amide was oxidized with *m*-CPBA after deblocking by HBr-HOAc, giving the desired sulfoxide **47** (scheme 9).

Biological test methods

The compounds were evaluated for inhibitory activities upon H^+, K^+-ATPase and gastric acid secretion. The inhibition of H^+, K^+-ATPase activity (assay 1) [21] was used for the primary screening. In this test, the compounds were added to the incubation medium at 100 μM.

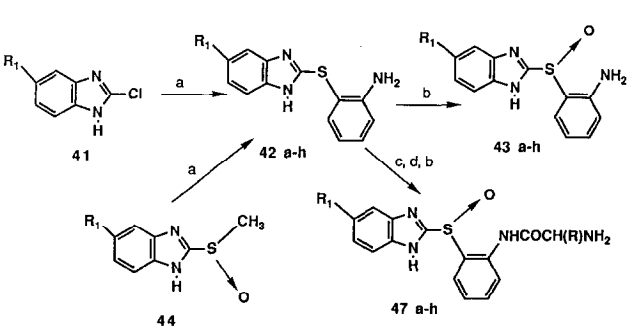
The secondary screening was performed by assessing the inhibition of histamine-stimulated acid secretion in isolated bullfrog gastric mucosa (assay 2) [22]. The mucosal surface was superfused to measure the acid secretion and the compounds were added to the serosal solution. The rate of inhibition of acid secretion was determined 60 min after addition of 100 μM of compound.

The *in vivo* antisecretory effect of the test compounds was assayed in the perfused stomach of histamine-stimulated rat [23]. The compounds were



(a) Z-Gly, $SOCl_2$ - HMPA ; (b) $LiBH_4$; (c) benzimidazole-2-disulfide, n-Bu_3P - Py ; (d) HBr - AcOH ; (e) m-CPBA.

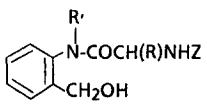
Scheme 8.



(a) 2-aminothiophenole ; (b) m- CPBA ; (c) Z-NHCH(R)COOH, $SOCl_2$ - HMPA ; (d) HBr - HOAc.

Scheme 9.

Table I. Structures and chemical data of hydroxymethylaniline derivatives.



No	R	R'	Mp, °C	% yield	Formula	Analyses
20a	H	H	120-122	67.6	$C_{17}H_{18}NO_4$	C, H, N
20b	CH_3 (L)	H	152-153	88.4	$C_{18}H_{20}N_2O_4$	C, H, N
20c	$(CH_3)_2CH$ (L)	H	192-194	67.4	$C_{20}H_{24}N_2O_4$	C, H, N
20d	$(CH_3)_2CHCH_2$ (L)	H	109-111	86.5	$C_{21}H_{26}N_2O_4$	C, H, N
20e	$C_6H_5CH_2$ (L)	H	180-182	79.2	$C_{24}H_{24}N_2O_4$	C, H, N
20f	H	CH_3	oil			

administered intraperitoneally (assay 3) or intraduodenally (assay 4). The inhibition rate of acid secretion was determined 90 min after administration of the compound.

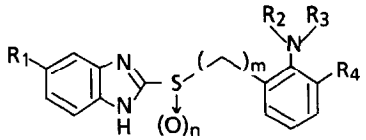
The antisecretory effect of the finally selected compounds was examined in histamine-stimulated Heidenhain pouch dogs. The compound was administered orally and the effect on acid secretion was observed for 3.5 h.

Results and discussion

As shown in table III, many of the *o*-aniline derivatives showed good activity in the *in vitro* H^+/K^+-ATPase inhibition assay (assay 1) as well as in the inhibition assay for histamine-stimulated acid secretion in isolated bullfrog gastric mucosa (assay 2). The *m*-aminobenzyl derivative **19** (scheme 2) however, did not show inhibitory activity in these tests. Some of the *o*-aniline derivatives also showed inhibitory activity in the histamine stimulated acid secretion in rat by ip administration (assay 3). Although compounds **7** showed more than 60% inhibition at the dose of 3 mg/kg in assay 3, they showed less than 60% inhibition at 10 mg/kg by id administration (assay 4). Compound **32a**, in which the methylene and sulfinyl groups in **7a** are exchanged, was inactive in assay 2 and 3. *p*-Aniline derivative **32b** was also almost inactive in assay 2.

Since the weak activity of **7a** in id administration seemed to be due to the lack of absorption from the duodenal mucosa, an attempt was made to increase the bioavailability of **7a** by converting it into amino acid amides as prodrugs. This concept was derived from our recent observations that peptidoaminobenzophenones and related compounds, a novel class of ring-opened derivatives of 1,4-benzodiazepines [25–29], are useful as minor tranquilizers or hypnotics (*eg*, lorzafone: INN [30–32] and rilmazafone hydrochloride: INN [33–36]). These compounds serve as

Table II. Structures and chemical data of benzimidazole derivatives.



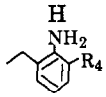
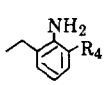
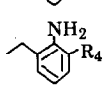
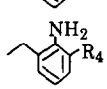
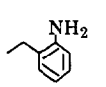
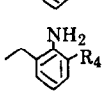
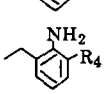
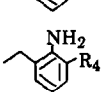
No	R_1	R_2	R_3 [a]	R_4	n	m	Mp, °C	% yield	formula	analyses
5a	H	H	Z	H	0	1	155-156	91.8	$C_{22}H_{19}N_3SO_2$	C, H, N, S
5b	CH_3O	H	Z	H	0	1	172-174	56.5	$C_{23}H_{21}N_3SO_3$	C, H, N, S
5c	CF_3	H	Z	H	0	1	207-208	84.3	$C_{23}H_{18}N_3SO_2F_3$	C, H, N, S, F
5d	F	H	Z	H	0	1	195-196	57.7	$C_{22}H_{18}N_3SO_2F$	C, H, N, S, F
6a	H	H	H	H	0	1	149-151	84.4	$C_{14}H_{13}N_3S$	C, H, N, S
6b	CH_3O	H	H	H	0	1	122-124	63.1	$C_{15}H_{15}N_3SO$	C, H, N, S
6c	CF_3	H	H	H	0	1	oil			
6d	F	H	H	H	0	1	oil			
7a	H	H	H	H	1	1	182-183	97.4	$C_{14}H_{13}N_3SO$	C, H, N, S
7b	CH_3O	H	H	H	1	1	167-168	80.4	$C_{15}H_{15}N_3SO_2$	C, H, N, S
7c	CF_3	H	H	H	1	1	159-161	73.6	$C_{15}H_{12}N_3SOF_3$	C, H, N, S
7d	F	H	H	H	1	1	168-169	87.1	$C_{14}H_{12}N_3SOF$	C, H, N, S, F
7e	H	CH_3	H	H	1	1	151-152	30.0	$C_{15}H_{15}N_3SO$	C, H, N, S
12a	H	H	H	CH_3	0	1	135-137	20.0	$C_{15}H_{15}N_3S$	C, H, N, S
12b	H	H	H	CF_3	0	1	143-145	65.0	$C_{15}H_{12}N_3SF_3$	C, H, N, S
12c	H	H	H	CH_3O	0	1	161-163	13.8	$C_{15}H_{15}N_3SO$	C, H, N, S
12d	H	H	H	CH_3	0	1	135-137	20.0	$C_{15}H_{11}N_3S$	C, H, N, S
12e	H	H	H	C_2H_5	0	1	124-126	20.2	$C_{16}H_{17}N_3S \cdot 1/6C_6H_{14}$	C, H, N, S
13a	H	H		CH_3	0	1	173-175	5.2	$C_{23}H_{24}N_4S$	C, H, N, S
13c	H	H		CH_3O	0	1	165-167 (decomp)	3.9	$C_{23}H_{24}N_4SO_2 \cdot 1/4C_2H_5OH$	C, H, N, S
13d	H	H		CH_3	0	1	173-175	5.2	$C_{23}H_{24}N_4S$	C, H, N, S
13e	H	H		C_2H_5	0	1	184-186	6.5	$C_{25}H_{28}N_4S$	C, H, N, S
14a	H	H	H	CH_3O	1	1	155-156	90.0	$C_{15}H_{15}N_3SO_2 \cdot 3/4H_2O$	C, H, N, S
14b	H	H	H	CF_3	1	1	192-194	94.1	$C_{15}H_{12}N_3SOF_3$	C, H, N, S, F
14c	H	H	H	MeOCO	1	1	158-160	59.0	$C_{17}H_{17}N_3SO_3 \cdot H_2O$	C, H, N, S
14d	H	H	H	CH_3	1	1	157-159	66.2	$C_{15}H_{15}N_3SO \cdot 1/4C_2H_5OH$	C, H, N, S
14e	H	H	H	C_2H_5	1	1	157-159	94.9	$C_{16}H_{17}N_3SO$	C, H, N, S
15a	H	H		H	1	1	150-152	58.5	$C_{21}H_{20}N_4SO$	C, H, N, S
15b	H	H		CH_3O	1	1	135-137	50.0	$C_{23}H_{24}N_4SO_3 \cdot H_2O \cdot 1/2C_2H_5OH$	C, H, N, S
15c	H	H		CH_3	1	1	141-143	45.0	$C_{23}H_{24}N_4SO_3$	C, H, N, S
15d	H	H		C_2H_5	1	1	133-134	69.5	$C_{25}H_{28}N_4SO$	C, H, N, S

Table II. *Continued.*

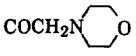
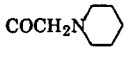
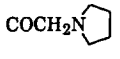
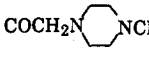
no.	R_1	R_2	R_3[a]	R_4	n	m	mp, °C	% yield	formula	analyses
21 a	H	H	Z-Gly-	H	0	1	173-175	74.8	$C_{24}H_{22}N_4SO_3$	C, H, N, S
21 b	CH_3O	H	Z-Gly-	H	0	1	187-189	58.7[b]	$C_{25}H_{24}N_4SO_4$	C, H, N, S
21 c	H	H	Z-L-Ala-	H	0	1	166-168	66.6[b]	$C_{25}H_{24}N4SO_3$	C, H, N, S
21 d	H	H	Z-L-Val-	H	0	1	174-175	77.2[b]	$C_{27}H_{28}N_4SO_3 \cdot 1/2H_2O$	C, H, N, S
21 e	H	H	Z-L-Leu-	H	0	1	oil[b]			
21 f	H	H	Z-D-Leu-	H	0	1	oil[c]			
21 g	H	H	Z-L-Phe-	H	0	1	90>	73.2[b]	$C_{31}H_{28}N_4SO_3$	C, H, N, S
21 h	H	H	Z-L-Lys-	H	0	1	oil[c]			
21 i	H	CH_3	Z-Gly-	H	0	1	164-166	69.9[b]	$C_{25}H_{24}N_4SO_3$	C, H, N, S
22 a	H	H	Gly-	H	0	1	209-213	82.4	$C_{16}H_{16}N_4SO$	C, H, N, S
22 b	CH_3O	H	Gly-	H	0	1	165-167	82.4[b]	$C_{17}H_{18}N_4SO_2 \cdot 1/4H_2O$	C, H, N, S
22 c	H	H	L-Ala-	H	0	1	195-197	87.9[b]	$C_{17}H_{18}N_4SO$	C, H, N, S
22 d	H	H	L-Val-	H	0	1	146-148	81.3[b]	$C_{19}H_{22}N_4SO$	C, H, N, S
22 e	H	H	L-Leu-	H	0	1	144-146	87.3[b]	$C_{20}H_{24}N_4SO$	C, H, N, S
22 f	H	H	D-Leu-	H	0	1	145-146	67.1[b]	$C_{20}H_{24}N_4SO$	C, H, N, S
22 g	H	H	L-Phe-	H	0	1	162>	92.3[b]	$C_{23}H_{22}N_4SO \cdot 1/2H_2O$	C, H, N, S
22 h	H	H	L-Lys-	H	0	1	oil[b]			
22 i	H	CH_3	Gly-	H	0	1	oil[b]			
22 j	F	H	Gly-	H	0	1	197-198	76.9[c]	$C_{16}H_{15}N_4SOF \cdot 1/2H_2O$	C, H, N, S, F
23 a	H	H	Gly-	H	1	1	190-192	63.9	$C_{16}H_{16}N_4SO_2$	C, H, N, S
23 b	CH_3O	H	Gly-	H	1	1	169-171	46.5	$C_{17}H_{18}N_4SO_3 \cdot 1.3C_7H_5O_2Cl \cdot H_2O$	C, H, N, S
23 c	H	H	L-Ala-	H	1	1	128>	13.4	$C_{17}H_{18}N_4SO_2 \cdot 1/4H_2O$	C, H, N, S
23 d	H	H	L-Val-	H	1	1	204-206	93.2	$C_{19}H_{22}N_4SO_2 \cdot 1/2H_2O$	C, H, N, S
23 e	H	H	L-Leu-	H	1	1	159-161	93.2	$C_{20}H_{24}N_4SO_2$	C, H, N, S
23 f	H	H	D-Leu-	H	1	1	167-168	59.4	$C_{20}H_{24}N_4SO_2$	C, H, N, S
23 g	H	H	L-Phe-	H	1	1	154-157	38.8	$C_{23}H_{22}N_4SO_2 \cdot 1/4H_2O$	C, H, N, S
23 h	H	H	L-Lys-	H	1	1	229-231		$C_{20}H_{25}N_5SO_2 \cdot H_2O$	C, H, N, S
23 i	H	CH_3	Gly-	H	1	1	105>	42.2	$C_{17}H_{18}N_4SO_2 \cdot 1/2H_2O$	C, H, N, S
23 j	F	H	Gly-	H	1	1	170-172	46.2	$C_{16}H_{15}N_4SO_2F \cdot C_2H_5OH$	C, H, N, S, F
28 a	H	H		H	1	1	oil	95.8	$C_{20}H_{22}N_4SO_3$	C, H, N, S
28 b	H	H		H	1	1	204-206	96.6	$C_{21}H_{24}N_4SO_2$	C, H, N, S
28 c	H	H		H	1	1	201-203	89.1	$C_{20}H_{22}N_4SO_2$	C, H, N, S
28 d	H	H		H	1	1	192-194	90.9	$C_{21}H_{25}N_5SO_2 \cdot 1/2H_2O$	C, H, N, S
28 e	H	H	$COCH_2N(CH_3)_2$	H	1	1	167-169	98.1	$C_{18}H_{20}N_4SO_2$	C, H, N, S

Table II. *Continued.*

no.	R_1	R_2	R_3[a]	R_4	n	m	mp, °C	% yield	formula	analyses
42a	H	H	H	H	0	0	210-222	52.0	$C_{13}H_{11}N_3S$	C, H, N, S
42b	CF_3	H	H	H	0	0	186-188	98.2[c]	$C_{14}H_{10}N_3SF_3$	C, H, N, S, F
42c	CH_3O	H	H	H	0	0	152-154	91.0[c]	$C_{14}H_{13}N_3SO$	C, H, N, S
42d	CH_3OCO	H	H	H	0	0	160>	87.9[c]	$C_{15}H_{13}N_3SO_2 \cdot 1/4CH_3CO_2C_2H_5$	C, H, N, S
42e	CH_3	H	H	H	0	0	135-137	85.9[c]	$C_{14}H_{13}N_3S$	C, H, N, S
42f	F	H	H	H	0	0	190-192	91.2[c]	$C_{13}H_{10}N_3SF$	C, H, N, S, F
42g	H	CH_3	CH_3	H	0	0	166-168	76.2[c]	$C_{15}H_{15}N_3S$	C, H, N, S
42h	H	CH_3	H	H	0	0	182-185	48.1[b]	$C_{14}H_{13}N_3S$	C, H, N, S
43a	H	H	H	H	1	0	174-176	63.2	$C_{14}H_{11}N_3SO$	C, H, N, S,
43b	CF_3	H	H	H	1	0	181-183	38.9	$C_{14}H_{10}N_3SF_3O$	C, H, N, S, F
43c	CH_3O	H	H	H	1	0	152-154	64.6	$C_{14}H_{13}N_3SO_2$	C, H, N, S
43d	CH_3OCO	H	H	H	1	0	188-191	63.2	$C_{15}H_{13}N_3SO_3$	C, H, N, S
43e	CH_3	H	H	H	1	0	191-193	69.1	$C_{14}H_{13}N_3SO$	C, H, N, S
43f	F	H	H	H	1	0	193-195	63.7	$C_{13}H_{10}N_3SFO$	C, H, N, S, F
43g	H	CH_3	CH_3	H	1	0	116>	88.6	$C_{15}H_{15}N_3SO$	C, H, N, S
43h	H	CH_3	H	H	1	0	171-173	15.1	$C_{14}H_{13}N_3SO$	C, H, N, S
45a	H	H	Z-Gly-	H	0	0	142-144	88.4	$C_{23}H_{20}N_4O_3$	C, H, N, S
45b	CH_3	H	Z-Gly-	H	0	0	oil			
45c	H	H	Z-L-Phe-	H	0	0	133-135	91.8	$C_{30}H_{26}N_4SO_3$	C, H, N, S
45d	CH_3	H	Z-L-Phe-	H	0	0	oil			
45e	CH_3O	H	Z-L-Phe-	H	0	0	oil			
45f	H	H	Z-L-Leu-	H	0	0	102-104	85.7	$C_{27}H_{28}N_4SO_3 \cdot 1/4H_2O$	C, H, N, S
45g	CH_3	H	Z-L-Leu-	H	0	0	oil			
45h	CH_3O	H	Z-L-Leu-	H	0	0	oil			
46a	H	H	Gly-	H	0	0	165-168	75.0	$C_{15}H_{14}N_4SO$	C, H, N, S
45b	CH_3	H	Z-Gly-	H	0	0	oil			
45c	H	H	Z-L-Phe-	H	0	0	133-135	91.8	$C_{30}H_{26}N_4SO_3$	C, H, N, S
45d	CH_3	H	Z-L-Phe-	H	0	0	oil			
45e	CH_3O	H	Z-L-Phe-	H	0	0	oil			
45f	H	H	Z-L-Leu-	H	0	0	102-104	85.7	$C_{27}H_{28}N_4SO_3 \cdot 1/4H_2O$	C, H, N, S
45g	CH_3	H	Z-L-Leu-	H	0	0	oil			
45h	CH_3O	H	Z-L-Leu-	H	0	0	oil			
46b	CH_3	H	Gly-	H	0	0	167-168	86.4	$C_{16}H_{16}N_4SO$	C, H, N, S
46c	H	H	L-Phe-	H	0	0	167-168	87.7	$C_{22}H_{20}N_4SO$	C, H, N, S
46d	CH_3	H	L-Phe-	H	0	0	oil			
46e	CH_3O	H	L-Phe-	H	0	0	130-132	95.4	$C_{23}H_{22}N_4SO_2$	C, H, N, S
46f	H	H	L-Leu-	H	0	0	168-169	89.3	$C_{19}H_{22}N_4SO$	C, H, N, S
46g	CH_3	H	L-Leu-	H	0	0	151-153	94.7	$C_{20}H_{24}N_4SO$	C, H, N, S
47a	H	H	Gly-	H	1	0	100>	4.7	$C_{15}H_{14}N_4SO_2 \cdot 1/4H_2O$	C, H, N, S
47b	CH_3	H	Gly-	H	1	0	140-142	42.6	$C_{16}H_{16}N_4SO_2$	C, H, N, S
47d	CH_3	H	L-Phe-	H	1	0	179-181	45.6	$C_{23}H_{22}N_4SO_2$	C, H, N, S
47e	CH_3O	H	L-Phe-	H	1	0	167-169	63.2	$C_{23}H_{22}N_4SO_3$	C, H, N, S
47f	H	H	L-Leu-	H	1	0	77>	51.3	$C_{19}H_{22}N_4SO_2$	C, H, N, S
47g	CH_3	H	L-Leu-	H	1	0	78>	77.5	$C_{20}H_{24}N_4SO_2 \cdot 1/6C_6H_{14}$	C, H, N, S
47h	CH_3O	H	L-Leu-	H	1	0	80>	72.0	$C_{20}H_{24}N_4SO_3$	C, H, N, S

[a]Ala: alanyl, Leu: leucyl, Lys: lysyl, Phe: phenylalanyl, Val: valyl, Z: benzyloxycarbonyl. [b]Method A. [c]Method.

Table III. Biological activities of compounds used in this study.

No.	Inhibition of H^+, K^+-ATPase (Assay 1) (% inhib at 10-4 M)	Inhibition of Acid Secretion in Isolated Mucosa (Assay 2) (% inhib at 10-4 M)	Suppression of Acid Secretion in Rat (Assay 3, 4) (% inhib) ip dose, mg/kg		id dose, mg/kg	
			10	3	10	3
7a	44	80	NT[a]	68	57	NT
7b	16	0	NT	80	53	NT
7c	30	0	NT	70	58	NT
7d	NT	NT	46	NT	NT	NT
7e	100	69	NT	86	NT	NT
14a	91	0	55	NT	NT	NT
14b	5	6	58	NT	NT	NT
14c	19	7	44	NT	NT	NT
14d	94	80	67	NT	NT	NT
14e	94	39	60	NT	NT	NT
15a	79	44	61	NT	29	NT
15b	75	52	59	NT	NT	NT
15c	98	10	42	NT	NT	NT
15d	63	42	67	NT	NT	NT
19	0	0	2	NT	NT	NT
23a	22	14	NT	67	NT	71
23b	55	6	71	NT	NT	NT
23c	8	0	78	NT	42	NT
23d	1	0	22	NT	NT	NT
23e	40	6	89	NT	78	NT
23f	NT	NT	38	NT	NT	NT
23g	21	5	74	NT	64	NT
23h	4	NT	14	NT	NT	NT
23i	0	NT	45	NT	34	NT
23j	7	NT	70	NT	NT	NT
32a	18	0	34	NT	NT	NT
32b	75	10	NT	NT	NT	NT
36	NT	NT	56	NT	NT	NT
40	NT	NT	48	NT	NT	NT
43a	79	44	61	NT	NT	NT
43b	NT	NT	27	NT	NT	NT
43c	NT	NT	50	NT	NT	NT
43d	NT	NT	23	NT	NT	NT
43e	NT	NT	51	NT	NT	NT
43f	NT	NT	38	NT	30	NT
43g	38	NT	49	NT	NT	NT
43h	NT	NT	46	NT	NT	NT
47a	97	NT	81	NT	NT	NT
47b	100	NT	47	NT	57	NT
47c	NT	NT	44	NT	53	NT
47d	NT	NT	37	NT	NT	NT
47e	89	NT	50	NT	NT	NT
47g	NT	NT	28	NT	NT	NT
47h	NT	NT	26	NT	NT	NT
Omepr azole	77	92	NT	95	NT	95

[a]NT: not tested.

double prodrugs which are hydrolyzed enzymatically to release the terminal amino acids and then cyclize chemically to become transformed into 1,4-benzodiazepines in the body [25–29]. Amino acid amides **23a–j** showed no activity *in vitro* (assay 1 and 2). However, glycyl-, L-alanyl-, L-leucyl-, and L-phenylalanyl-amides **23a, c, e, g** displayed strong activity *in vivo* (assay 3). Compounds **23a, e, g** also showed strong acid secretory inhibition in Schild rat by id administration (assay 4). Therefore, glycine and L-amino acid amides of **7** may be efficiently absorbed from the duodenum and cleaved enzymatically, being converted into active aniline derivatives which display the activity. This is supported by the absence of activity for the D-amino acid amide **23f** and the terminal substituted glycylamide derivatives **28a–e**, which could not be enzymatically hydrolyzed.

Compound **43a**, in which the methylene group in **7a** is lacking, also showed inhibitory activity in assay 1, 3 and 4. Amino acid amides of **47**, however, did not show improved activity.

Compound **23a** at 3 mg/kg, po, decreased the gastric acid secretion stimulated by histamine in Heidenhain pouch dogs. It was not effective at 1 mg/kg, but showed 45% inhibition at 3 mg/kg and 95% inhibition at 10 mg/kg at 2 h post-dose. Its suppressive effect lasted during the observation period (3.5 h) (fig 1). Compound **23a** also inhibited Shay ulcers, water-immersion stress-induced erosions, and erosions induced by HCl-ethanol with ED_{50} 14.6 mg/kg (id), 2.8 mg/kg (po) and 66.8 mg/kg (po), respectively.

Compound **23a** is of particular interest because of its stability in acid and because it greatly improved the bioavailability of aniline derivatives that inhibit acid secretion. No degradation of **23a** was observed in the artificial gastric juice (pH 1.2) [37] after it had been left standing for 6 h at 37°C, although omeprazole is degraded at below pH 4 with a half-life of less than 10 min [38]. When **23a** was given to dog by oral administration at a dose of 500 mg/kg, its metabolite **7a** increased rapidly in plasma according to HPLC measurement and reached a maximum at 2 h after administration, as shown in fig 2. This indicates that the orally given glycinamide **23a** is absorbed

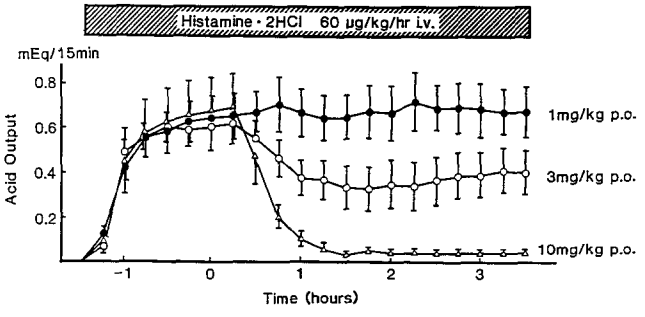


Fig 1. Effect of **23a** on histamine-stimulated acid secretion in Heidenhain pouch dogs. Acid secretion was stimulated by an intravenous infusion of histamine-2HCl (60 μg/kg per h). After 90 min of histamine infusion, **23a** was given orally and the effect on acid secretion was monitored for the next 3.5 h. $N = 4$. Values represent means ± standard errors.

efficiently and converted into aniline **7a**, as demonstrated by the very high plasma concentration of **7a**. Compound **23a** was converted into **7a** when incubated with plasma or the brush border fraction of rat small intestine homogenate although the conversion appeared to be slow (figs 3 and 4). This suggests that **23a** is hydrolyzed by the action of amino-peptidase present in plasma or in the brush border of the small intestine to release the terminal glycine and that the liver might also be an important site of conversion.

As a model for studying the mechanism of H^+, K^+-ATPase inhibition by **7a**, the chemical behavior of **7a** in acidic media with/without thiol was examined. In aqueous acidic medium (*eg* 0.1 N HCl), which resembles the conditions present at the site of action in the parietal cell, compound **7a** degraded rapidly at room temperature, first giving the sulfide **6**, which in turn degraded to afford 2-mercaptobenzimidazole **4** and 2-aminobenzyl alcohol **2** (fig 5). Treatment of the sulfide **6a** with 0.1 N HCl at room temperature also gave **4** and **2**. When **7a** was treated with 0.1 N HCl in the presence of 2-mercaptoethanol at 37°C, **4** and 2-amino(2-hydroxyethyl)benzylsulfide (**48**) were obtained in good yields (scheme 10). No change was found for the *m*-aminobenzyl sulfoxide **19**, benzylsulfinylbenzimidazole and benzylthiobenzimidazole in a 0.1 N HCl solution, which accounts for their lack of activity.

The present study suggests that orally administered **23a** remains intact in gastric acid and is absorbed from the intestine to be converted into **7a**. Under acidic conditions, such as in the target cell, **7a** is attacked by the SH group of H^+, K^+-ATPase at its benzyl position to irreversibly form the enzyme-inhibitor complex **49** as exemplified by compound **48**

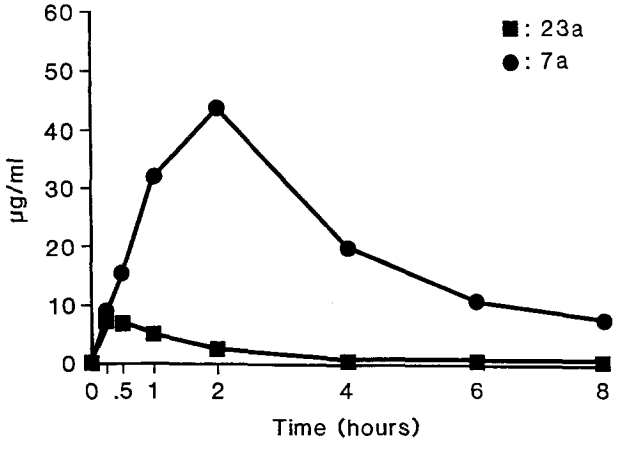


Fig 2. Plasma concentrations of **7a** and **23a** when **23a** was given to Heidenhain pouch dogs by oral administration at the dose of 500 mg/kg. $N = 1$.

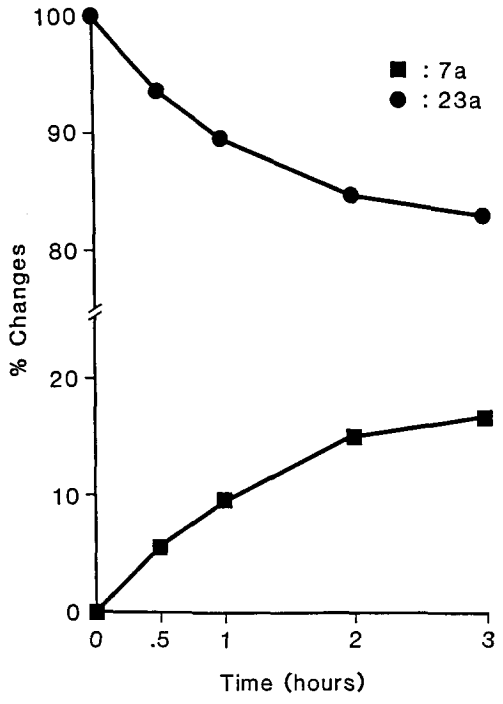


Fig 3. Changes of **7a** and **23a** when **23a** was incubated with rat small intestine brush borders. $N = 1$.

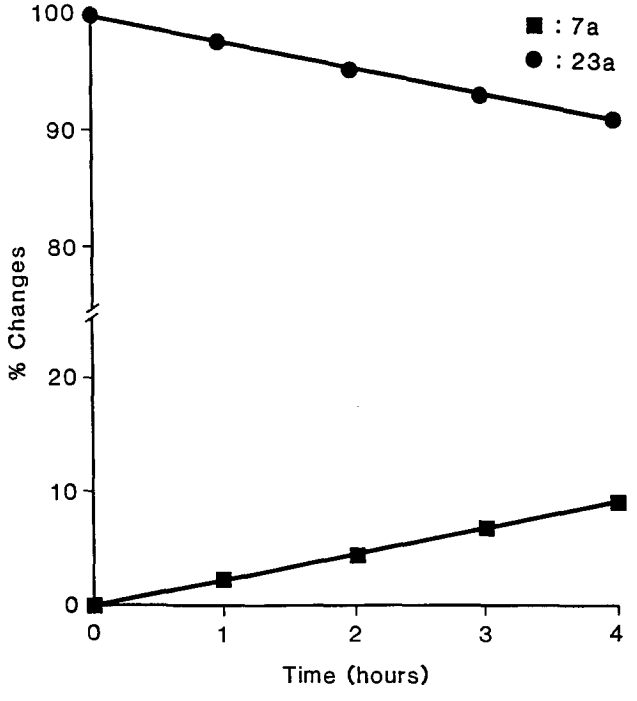


Fig 4. Changes of **7a** and **23a** when **23a** was incubated with rat plasma. $N = 1$.

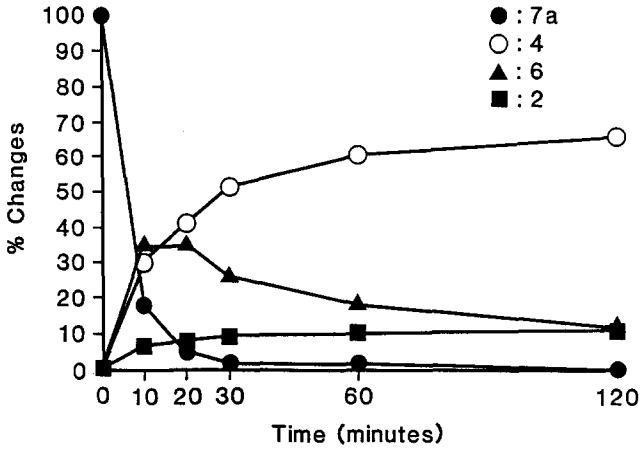
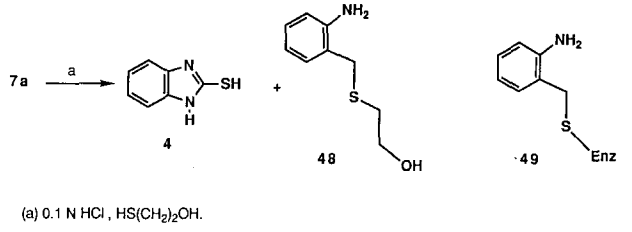


Fig 5. Degradation pattern of **7a** in 0.1 N HCl. $N = 1$.



(a) 0.1 N HCl, $HS(CH_2)_2OH$.

Scheme 10.

shown in a study model. This mechanism is different from that proposed by Adelstein [17], who suggested a mechanistic pathway analogous to that of omeprazole. Omeprazole reacts with acid in the presence of mercaptans giving omeprazole sulfide through formation of the reactive sulfenamide and reaction with the enzyme-SH group to show deactivation of the target enzyme H^+, K^+-ATPase [11, 12]. However, no formation of sulfide **6a** and bis(2-hydroxyethyl)disulfide were observed in our study model, which suggests a different type of deactivation mechanism by 2-[(2-aminobenzylsulfinyl)benzimidazole on the target enzyme H^+, K^+-ATPase.

In preliminary subacute toxicity tests in dogs, edema and hemorrhage of the bladder were observed after oral administration of **23a** for 4 weeks. Thus, the further development of this compound was precluded.

Experimental protocols

Chemistry

Melting points were determined in a Yamato capillary melting point apparatus and are uncorrected. Microanalyses were performed by the Analytical Department of Shionogi Research Laboratories, and analytical values were within 0.4% of the theoretical values unless otherwise noted. NMR and/or mass spectra were obtained for all compounds and were consistent with the structures and assignments.

2-(Benzyloxycarbonyl)aminobenzyl alcohol 3
To a suspension of **2** (7.6 g, 61.7 mmol) and K_2CO_3 (8.65 g, 62.6 mmol) in 150 ml of $CHCl_3$, benzyloxycarbonyl chloride (8.8 ml, 61.6 mmol) was added. The mixture was stirred for 4 h and allowed to stand overnight at room temperature. H_2O was added to the reaction mixture and the separated $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography with 1/1 $CHCl_3$/AcOEt as eluent and 3.1 g (82.4%) of 3 was obtained. Recrystallization from Et_2O gave colorless crystals, mp = 87-89°C. NMR ($CDCl_3$ + d_6-DMSO) δ 2.30 (t, 1H, J = 5 Hz), 4.61 (d, 2H, J = 5 Hz), 5.15 (s, 2H), 6.80-8.07 (m, 4H), 7.33 (s, 5H). Anal $C_{15}H_{15}NO_3$ (C, H, N).

2-[2-((Benzyloxycarbonyl)aminobenzyl)thio]1-H-benzimidazole 5a
A solution of **3** (9.5 g, 36.9 mmol) and $SOCl_2$ (6 ml, 82.3 mmol) in 200 ml of benzene was refluxed for 1 h. The reaction mixture was concentrated with a rotary evaporator. The residue was combined with a solution of 1.55 g of NaOH, **4** (5.55 g, 60 mmol), 5 ml of H_2O, and 50 ml of EtOH. The mixture was stirred for 2 h at room temperature and the crystals that formed were collected, washed with H_2O, and dried, giving 13.2 g (91.8%) of **5a**. Recrystallization from AcOEt gave colorless crystals, mp = 155-156°C. NMR ($CDCl_3$ + d_6-DMSO) δ 4.47 (s, 2H), 5.27 (s, 2H), 6.90-7.83 (m, 13H), 11.47 (bs, 1H). Anal $C_{22}H_{19}N_3SO_2$ (C, H, N).

Compounds **5b–d** were prepared in a similar manner.

2-[(2-Aminobenzyl)thio]-1H-benzimidazole 6a
A solution of **5a** (1.95 g, 5.0 mmol) in 4 ml of 30% HBr-AcOH was stirred for 3 h at room temperature and Et_2O was added. The crystals that formed were collected, and the filtrate was neutralized with aqueous $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The crude product was purified by silica gel column chromatography with 5/1 $CHCl_3$/MeOH as the eluent to obtain 1.08 g (84.4%) of **6a**. Recrystallization from EtOH gave Colorless crystals, mp = 149-151°C. NMR ($CDCl_3$ + d_6-DMSO) δ 3.27 (br, 2H), 4.52 (s, 2H), 6.43-7.60 (m, 8H). Anal $C_{14}H_{13}N_3S$ (C, H, N, S).

Compounds **6b–d** were prepared in a similar manner.

2-[(2-Aminobenzyl)sulfinyl]-1H-benzimidazole 7a
To a solution of **6a** (1.79 g, 7.0 mmol) in 150 ml of $CHCl_3$ and 5 ml of MeOH *m*-CPBA (80% purity) (1.66 g, 7.7 mmol) was added at – 5°C followed by stirring for 30 min at – 5°C. An aqueous $NaHCO_3$ solution was added to the reaction mixture, and the crystals that formed were collected, washed with H_2O, dried over $MgSO_4$, and evaporated to dryness to afford 1.85 g (97.4%) of **7a**. Recrystallization from EtOH gave colorless crystals of mp 182-183°C decomp. NMR ($CDCl_3$ + d_6-DMSO) δ 4.40, 4.61 (ABq, 2H, J = 8 Hz), 6.07 (b, 2H), 6.33-7.73 (m, 8H). Anal $C_{14}H_{13}N_3SO$ (C, H, N, S).

Compounds **7b–e**, **14a–e**, **15a–d** were prepared in a similar manner.

2-[(2-Amino-3-methylbenzyl)thio]-1H-benzimidazole 12a and 2-[(2-((2-amino-3-methylbenzyl)amino)-3-methylbenzyl)thio]-1H-benzimidazole 13a
A mixture of **4** (2.25 g, 15.0 mmol), NaOH (1.2 g, 30.0 mmol), 4 ml of H_2O, and 40 ml of EtOH was stirred for 15 min at room

temperature. To this solution 2-amino-3-methylbenzyl chloride hydrochloride (2.95 g, 15.0 mmol) was added. The mixture was stirred for 2 h at room temperature then evaporated to dryness and the residue was extracted with $CHCl_3$, washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. Purification of the crude product by silica gel column chromatography with 5/1 $CHCl_3$/AcOEt as the eluent gave 0.8 g (20.0%) of **12a**, mp = 135-137°C (from Et_2O), NMR ($CDCl_3$ + d_6-DMSO) δ 2.13 (s, 3H), 4.57 (s, 2H), 6.47-7.70 (m, 7H), Anal ($C_{15}H_{15}N_3S$) C, H, N, S; and 0.3 g (5.2%) of **13a**, mp = 173-175°C (from EtOH). NMR ($CDCl_3$ + d_6-DMSO) δ 2.15 (s, 3H), 2.37 (s, 3H), 4.10 (s, 2H), 4.62 (s, 2H), 6.47-7.40 (m, 10H). Anal $C_{23}H_{24}N_4S$ (C, H, N, S).

Compounds **12b–e** and **13c–e** were prepared in a similar manner.

3-(Benzyloxycarbonyl)aminobenzyl alcohol ***17***

A mixture of **16** (5.42 g, 20 mmol), $ClCO_2Me$ (1.89 g, 20 mmol), Et_3N (2.02 g, 20 mmol), and 30 ml of THF was stirred for 20 min at 0°C to room temperature. $NaBH_4$ (1.89 g, 50 mmol) was then added, followed by stirring for 4 h at room temperature. The reaction mixture was concentrated with a rotary evaporator, and the residue was extracted with AcOEt, washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography with 5/1 CH_2Cl_2/AcOEt as the eluent and gave 4.75 g (92.3%) of **17**, mp = 70-73°C. NMR ($CDCl_3$) δ 4.52 (s, 2H), 5.06 (s, 2H), 6.90-7.40 (m, 9H).

2-[(3-(Benzyloxycarbonyl)aminobenzyl)thio]-1H-benzimidazole ***18***

To a solution of **17** (2.74 g, 10.6 mmol) in 20 ml of CH_2Cl_2 $SOCl_2$ (0.93 ml, 12.8 mmol) was added dropwise at – 50°C. After the mixture was stirred at room temperature for 3 h, the reaction mixture was evaporated to dryness to give 2.46 g (84.2%) of 3-(benzyloxycarbonyl)aminobenzyl chloride as an oil. A mixture of **4** (0.84 g, 5.6 mmol), KOH (0.32 g, 5.6 mmol), and 15 ml of EtOH was stirred for 15 min at room temperature. To this solution 3-(benzyloxycarbonyl)aminobenzyl chloride (1.54 g, 5.6 mmol) was added followed by stirring for 2 h at room temperature. The reaction mixture was concentrated with a rotary evaporator, and the residue was extracted with $CHCl_3$, washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*, giving crude **18** as an oil (2.1 g, 96.1%). NMR ($CDCl_3$) δ 4.28 (s, 2H), 5.05 (s, 2H), 6.80-7.60 (m, 8H).

2-[(3-Aminobenzyl)sulfinyl)]-1H-benzimidazole ***19***

A solution of **18** (2.18 g, 5.6 mmol) in 12 ml of 30% HBr-AcOH was stirred for 4 h at room temperature, then Et_2O was added. The separated crystals were collected, and the filtrate was neutralized with aqueous $NAHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness to afford 1.43 g (74.8%) of 2-[3-aminobenzyl)thio]-1H-benzimidazole (**19'**), mp = 190-195°C. Anal $C_{14}H_{13}N_3S \cdot 0.8\ HBr \cdot 0.2\ CH_3COOC_2H_5 \cdot 0.2\ H_2O$ (C, H, N, S).

To a solution of 2-[(3-aminobenzyl)thio]-1H-benzimidazole (0.9 g, 3.5 mmol) in 20 ml of $CHCl_3$ was added *m*-CPBA (80% purity) (0.76 g, 3.5 mmol) at – 20°C followed by stirring for 45 min at – 15°C. To the reaction mixture an aqueous $NaHCO_3$ solution was added, and the $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by silica gel column chromatography with AcOEt as the eluent to obtain 0.41 g (47.4%) of **19**, mp = 190-193°C. NMR ($CDCl_3$) δ 4.18, 4.45 (ABq, 2H, J = 12 Hz), 6.20-7.60 (m, 8H). Anal $C_{14}H_{13}N_3OS \cdot 0.1\ CH_3CO_2C_2H_5$ (C, H, N, S).

2-[(Benzyloxycarbonyl)glycyl]aminobenzyl alcohol ***20a***

To a solution of (benzyloxycarbonyl)glycine (2.3 g, 11.0 mmol) in 15 ml of HMPA and 1.5 ml of CH_3CN $SOCl_2$ (0.77 ml, 10.6 mmol) was added dropwise at – 5-4°C. After the mixture had been stirred at 0°C for 15 min, **2** (1.23 g, 10.0 mmol) was added. The reaction mixture was stirred at room temperature for 4 h, then left standing overnight at room temperature. The reaction mixture was mixed with aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The extract was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The crude product was purified by silica gel column chromatography with 1/1 $CHCl_3$/AcOEt as the eluent to obtain 2.12 g (67.6%) of **20a**. Recrystallization from AcOEt gave colorless crystals, mp = 120-122°C. NMR ($CDCl_3$ + d_6-DMSO) δ 3.87 (d, 2H, J = 6 Hz), 4.56 (d, 2H, J = 7 Hz), 5.08 (s, 2H), 6.97-8.07 (m, 4H), 7.33 (s, 5H), 9.67 (bs, 1H). Anal $C_{17}H_{18}NO_4$ (C, H, N).

Compounds **20b–f** were prepared in a similar manner.

2[(2-((Benzyloxycarbonyl)glycyl)aminobenzyl)thio]-1H-benzimidazole ***21a***

Method A. A solution of **20a** (1.79 g, 5.7 mmol) and $SOCl_2$ (0.46 ml, 6.3 mmol) in 50 ml of benzene was refluxed for 20 min. The reaction mixture was concentrated *in vacuo* and the residue was mixed with a solution of NaOH (0.23 g, 5.8 mmol), **4** (0.86 g, 5.7 mmol), 1 ml of H_2O and 10 ml of EtOH. The mixture was stirred for 2 h at room temperature and concentrated *in vacuo*. The residue was purified by silica gel column chromatography with 1/1 $CHCl_3$/AcOEt as the eluent to obtain 1.9 g (74.8%) of **21a**. Recrystallization from AcOEt gave colorless crystals, mp = 173-175°C. NMR ($CDCl_3$ + d_6-DMSO) δ 4.10 (d, 2H, J = 6 Hz), 4.48 (s, 2H), 4.93 (s, 2H), 6.93-7.83 (m, 8H), 7.18 (s, 5H). Anal $C_{24}H_{22}N_4SO_3$ (C, H, N, S).

Compounds **21b–e, g, j** were prepared in a similar manner.

2-[(2-((Benzyloxycarbonyl)-D-leucyl)aminobenzyl)thio]-1H-benzimidazole ***21f***

Method B. To a solution of benzyloxycarbonyl-D-leucine (1.17 g, 4.4 mmol) in 10 ml of HMPA and 1 ml of CH_3CN $SOCl_2$ (0.31 ml, 4.2 mmol) was added dropwise at – 8 to – 2°C. After the mixture had been stirred for 15 min at – 5°C, **6a** (1.01 g, 4.0 mmol) was added, followed by stirring for 3 h at room temperature. The reaction mixture was mixed with aqueous $NaHCO_3$ solution and extracted with Et_2O. The Et_2O extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by silica gel column chromatography with 4/1 $CHCl_3$/AcOEt as the eluent to obtain 2.0 g (100% yield) of **21f**. NMR ($CDCl_3$) δ 0.92 (d, 6H, J = 4 Hz), 1.60-2.00 (m, 3H), 4.35, 4.14 (ABq. 2H, J = 10 Hz), 4.7 (b, 1H), 5.08 (s, 2H), 5.73 (d, 1H, J = 6 Hz), 6.90-7.83 (m, 8H), 7.25 (s, 5H).

2-[(2-Glycylaminobenzyl)thio]-1H-benzimidazole ***22a***

Method A. A solution of **21a** (1.7 g, 3.8 mmol) in 5 ml of 30% of HBr-AcOH was stirred for 2.5 h at room temperature and mixed with Et_2O. The separated crystals were collected, and the filtrate was neutralized with aqueous $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness to obtain 0.98 g (82.4%) of **22a**. Recrystallization from EtOH gave colorless crystals, mp = 209-213°C. NMR ($CDCl_3$ + d_6-

DMSO) δ 3.45 (s, 2H), 4.55 (s, 2H), 6.93-7.80 (m, 8H). Anal $C_{16}H_{16}N_4SO$ (C, H, N, S).

Compounds **22b–j** were prepared in a similar manner.

Method C. To a suspension of **25a** (9.6 g, 21.7 mmol) in 96 ml of EtOH 30% $MeNH_2$ (9.0 g, 86.9 mmol) in EtOH was added, followed by stirring for 15 h at room temperature. The reaction mixture was refluxed for 10 min, the crystals that formed were collected, the filtrate was washed with EtOH, and dried to obtain crude **22a**. The crude **22a** was dissolved in 120 ml of 3% HCl, then aqueous $NaHCO_3$ solution was added to neutralize the mixture. The crystals that formed were collected and 6.55 g (96.6%) of **22a** was obtained.

Compound **22j** was prepared in a similar manner.

2-[(2-Glycylaminobenzyl)sulfinyl]-1H-benzimidazole ***23a***

Method A. To a solution of **22a** (1.25 g, 4.0 mmol) in 50 ml of CH_2Cl_2 and 12 ml of MeOH *m*-CPBA (80% purity) (0.86 g, 4.0 mmol) was added at – 10°C, followed by stirring for 30 min at – 5°C. 40 ml of aqueous $NaHCO_3$ and 1 ml of Et_3N were then added to the reaction mixture, and crystals that formed were collected, washed with H_2O, and dried to obtain 0.84 g (63.9%) of **23a**. Recrystallization from EtOH gave colorless crystals, mp = 190-192°C. NMR (d_6-DMSO) δ 3.38 (s, 2H), 4.55, 4.84 (ABq, 2H, J = 13 Hz), 7.00-7.65 (m, 8H).

Compounds **23b–j** were prepared in a similar manner.

Method C. To a solution of **22a** (11.08 g, 35.3 mmol) in 88 ml of $CHCl_3$, 88 ml of MeOH and 4.6 ml of AcOH a mixture of 40% peracetic acid (6.97 g, 35.3 mmol) and 10 ml of $CHCl_3$ was added dropwise for 5 min at – 30 to – 25°C. The reaction mixture was stirred for 4 h at – 25°C, quenched with aqueous Na_2SO_3 solution and neutralized with saturated aqueous $NaHCO_3$ solution. The separated crystals were collected, washed with H_2O, and recrystallized from EtOH to obtain 7.25 g (62.5%) of **23a**, mp = 190-192°C. Anal $C_{16}H_{16}N_4SO_2$ (C, H, N, S).

2-(Phthaloylglycyl)aminobenzyl alcohol ***24***

To a mixture of 2-aminobenzylalcohol (**2**) (6.16 g, 50 mmol) and K_2CO_3 (13.8 g, 100 mmol) in 110 ml of CH_2Cl_2 and 5 ml of HMPA Pht-Gly-Cl (11.2 g, 50 mmol) was added, followed by stirring for 2 h at room temperature. H_2O was added and the crystals that formed were collected, washed with CH_2Cl_2, and dried to obtain 11.7 g (75.5%) of **24**. Recrystallization from MeOH gave colorless crystals, mp = 87-89°C (decomp). NMR ($CDCl_3$ + d_6-DMSO) δ 4.48 (s, 2H), 4.54 (d, 2H, J = 4 Hz), 5.23 (b, 1H), 7.00-7.93 (m, 8H), 9.67 (bs, 1H). Anal $C_{17}H_{14}N_2O_4 \cdot CH_3OH$ (C, H, N).

2-[((Phthaloylglycyl)aminobenzyl)thio]-1H-benzimidazole ***25a***

A suspension of **24** (11.7 g, 34.2 mmol) and $SOCl_2$ (6.8 ml, 35.0 mmol) in 117 ml of ethylene dichloride was refluxed for 1 h. The reaction mixture was concentrated *in vacuo*, the residue was mixed with **4** (5.7 g, 37.9 mmol) and K_2CO_3 (15.6 g, 112.9 mmol) in 30 ml of DMF, and stirred for 3 h at room temperature. The mixture was then concentrated *in vacuo*, the residue washed with H_2O and then CH_2Cl_2, and dried to obtain 12.6 g (75.4%) of **25**. Recrystallization from MeOH gave colorless crystals, mp = 208-210°C. NMR ($CDCl_3$ + d_6-DMSO) δ 4.57 (s, 2H), 4.70 (s, 2H), 7.00-7.92 (m, 12H). Anal $C_{24}H_{18}N_4SO_3 \cdot 1/2\ CH_3OH$ (C, H, N, S).

Compound **25b** (R_1 = F) was prepared in a similar manner, 63.0% yield, mp = 190-194°C (decomp). Anal $C_{24}H_{17}N_4SFO_3$ (C, H, N, S, F).

2-[(2-Chloroacetaminobenzyl)-thio]-1H-benzimidazole ***26***

To a suspension g of 2-[(2-aminobenzyl)thio]-1H-benzimidazole (**6**) (1.28 g, 5.0 mmol) and anhydrous K_2CO_3 (0.7 g, 5.0 mmol) in 8 ml of DMF chloroacetyl chloride (0.565 g, 5.0 mmol) was added, followed by stirring for 2 h at room temperature. The reaction mixture was evaporated *in vacuo* and the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over $MgSO_4$, and evaporated to dryness. The crude product was purified by silica gel column chromatography with 5/1 $CHCl_3$/AcOEt as the eluent to obtain 0.7 g (42.4%) of **26**. Recrystallization from EtOH gave colorless crystals, mp = 182-184°C. NMR ($CDCl_3$ + d_6-DMSO) δ 4.40 (s, 2H), 4.48 (s, 2H), 7.03-7.73 (m, 8H). Anal $C_{16}H_{14}N_3SClO$ (C, H, N, S, Cl).

2-[(2-Chloroacetaminobenzyl)sulfinyl]-1H-benzimidazole ***27***

To a solution of **26** (0.332 g, 1.0 mmol) in 15 ml of $CHCl_3$ and 1.5 ml of MeOH *m*-CPBA (0.237 g, 1.1 mmol) was added at – 10°C, followed by stirring for 40 min at 0°C. The reaction mixture was washed with H_2O and the organic layer was separated. The $CHCl_3$ layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by silica gel column chromatography with 1/1 $CHCl_3$/AcOEt as the eluent to obtain 0.31 g (88.5%) of **27**. Recrystallization from EtOH gave colorless crystals, mp = 188-189°C (decomp). NMR ($CDCl_3$ + d_6-DMSO) δ 4.23 (s, 2H), 4.84, 4.42 (ABq, 2H, J = 9 Hz), 6.97-7.70 (m, 8H), 9.90 (b, 1H).

2-[(2-Morpholinoacetaminobenzyl)sulfinyl]-1H-benzimidazole ***28a***

To a solution of **27** (0.209 g, 0.6 mmol) in 10 ml of $CHCl_3$ and 2 ml of MeOH morpholine (0.153 g, 1.8 mmol) and KI (0.1 g, 0.6 mmol) was added, followed by stirring for 6 h at room temperature. H_2O was added to the reaction mixture, and the $CHCl_3$ layer was separated, dried over $MgSO_4$, and evaporated to dryness to obtain 0.23 g (95.8%) of **28a**. NMR ($CDCl_3$) δ 2.70-2.53 (m, 4H), 3.13 (s, 2H), 3.67-3.83 (m, 4H), 4.68, 4.33 (ABq, 2H, J = 9 Hz), 6.73-7.80 (m, 8H), 9.80 (s, 1H). Anal $C_{20}H_{22}N_4SO_3$ (C, H, N, S).

Compounds **28b–e** were prepared in a similar manner.

2-[((2-Aminophenyl)thio)methyl]-1H-benzimidazole ***31a***

To a solution of 2-aminothiophenol (**29a**) (0.376 g, 2.9 mmol) and Et_3N (1.5 ml, 10.8 mmol) in 15 ml of $CHCl_3$ 2-chloromethylbenzimidazole (**30**) (0.5 g, 3.0 mmol) was added, followed by stirring for 2 h at room temperature. The reaction mixture was mixed with aqueous $NaHCO_3$, and the separated $CHCl_3$ layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography with AcOEt as the eluent to obtain 0.61 g (79.5%) of **31a**. NMR ($CDCl_3$ + d_6-DMSO) δ 4.13 (s, 2H), 6.40-7.63 (m, 8H).

2-[((4-Aminophenyl)thio)methyl]-1H-benzimidazole (**31b**) was prepared in a similar manner from 4-aminothiophenol (**29b**). 63.9% yield. NMR ($CDCl_3$ + d_6-DMSO) δ 4.13 (s, 2H), 6.40-7.60 (m, 8H).

2-[((2-Aminophenyl)sulfinyl)methyl]-1H-benzimidazole (**32a**). To a solution of **31a** (0.383 g, 1.5 mmol) in 15 ml of $CHCl_3$ and 1 ml of MeOH *m*-CPBA (80% purity) (0.324 g, 1.5 mmol) was added at – 10°C, followed by stirring for 30 min at – 5°C. The reaction mixture was mixed with aqueous $NaHCO_3$ and concentrated *in vacuo*, and the residue was purified by silica gel column chromatography with AcOEt as the eluent, giving 0.29 g (69.0%) of **32a**. Recrystallization from EtOH gave colorless crystals, mp = 181-183°C (decomp). NMR ($CDCl_3$ + d_6-DMSO) δ 4.43, 4.68 (ABq, 2H, J = 9 Hz), 5.78 (b, 2H), 6.53-7.67 (m, 8H). Anal $C_{14}H_{13}N_3SO \cdot 1/2\ H_2O$ (C, H, N, S).

2-[((4-Aminophenyl)sulfinyl)methyl]-1H-benzimidazole **32b** was prepared in a similar manner as **31b**. 46.7% yield, mp = 187-189°C (EtOH). NMR ($CDCl_3$ + d6-DMSO) δ 4.17, 4.34 (ABq, 2H, J = 8 Hz), 5.27 (b, 2H), 6.57-7.60 (m, 8H). Anal $C_{14}H_{13}N_3SO \cdot 1/2H_2O$ (C, H, N, S).

3-((Benzyloxycarbonyl)glycyl)amino-2-hydroxymethyl-4-methylthiophene ***34***

To a solution of benzyloxycarbonylglycine (1.506 g, 7.2 mmol) in 8.4 ml of HMPA and 0.84 ml of CH_3CN $SOCl_2$ (0.785 g, 6.6 mmol) was added dropwise at – 5°C. After the reaction mixture was stirred at – 5°C for 10 min. **33** (1.027 g, 6.0 mmol) was added. The reaction mixture was stirred at room temperature then left standing overnight at room temperature. To the reaction mixture 50 ml of saturated aqueous $NaHCO_3$ solution and 25 ml of Et_2O was added slowly. The crystals that formed were collected by filtration. The filtrate was extracted with AcOEt, the organic layer combined with the crystals and purified by silica gel column chromatography with 4/1 CH_2Cl_2/AcOEt as the eluent to obtain 2.16 g (99.3%) of 3-((benzyloxycarbonyl)glycyl)amino-2-methoxycarbonyl-4-methylthiophene **34'**. To a solution of **34'** (1.087 g, 3.0 mmol) in 10 ml of THF $LiBH_4$ (0.196 g, 9.0 mmol) was added and the mixture was stirred for 4 d at room temperature. The reaction mixture was concentrated *in vacuo* and the residue mixed with 20 ml of iced water, acidified (pH 1) with 1 N HCl, then neutralized (pH 7) with 1 N NaOH, and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4, evaporated *in vacuo*, and purified by silica gel column chromatography with AcOEt as the eluent to obtain 0.816 g (81.3%) of **34**. NMR ($CDCl_3$) δ 2.00 (s, 3H), 3.72 (t, 1H, J = 4 Hz), 3.99 (d, 2H, 4 Hz), 4.49 (d, 2H, 4 Hz), 5.12 (s, 2H), 5.56 (b, 1H), 6.87 (s, 1H), 7.35 (s, 5H), 7.73 (b, 1H).

2-[((3-(Benzyloxycarbonyl)glycyl)amino-4-methylthiophen-2-yl)methyl)thio]-1H-benzimidazole ***35***

To a solution of **34** (0.687 g, 2.05 mmol) in 10 ml of benzene, $SOCl_2$ (0.293 g, 2.46 mmol) was added. After heating at 80°C, the mixture was cooled to room temperature and evaporated *in vacuo*. The residue was mixed with **4** (0.323 g, 2.15 mmol), K_2CO_3 (1.417 g, 10.25 mmol), and 6 ml of DMF at room temperature. After the mixture had been stirred for 17 h at room temperature, DMF was removed *in vacuo*, 30 ml of H_2O was added to the residue, and the resultant mixture was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with aqueous 10% K_2CO_3 solution, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified by silica gel column chromatography with 1/1 CH_2Cl_2/AcOEt as the eluent to obtain 0.767 g (80.2%) of **35** as a colorless amorphous powder. NMR ($CDCl_3$) δ 2.02 (s, 3H), 4.21 (d, 2H, J = 11 Hz), 4.30 (s, 2H), 5.08 (s, 2H), 5.73 (b, 1H), 6.73 (s, 1H), 7.06-7.73 (m, 4H), 7.28 (s, 5H).

2-[((3-Glycylamino-4-methylthiophen-2-yl)methyl)sulfinyl]-1H-benzimidazole ***36***

A mixture of **35** (0.757 g, 1.62 mmol) and 1.5 ml of 25% HBr-AcOH was stirred for 1 h at room temperature, the resulting product was trituated with Et_2O, and collected by filtration. The crystalline product was suspended in 30 ml of aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 and evaporated, and the residue was purified by silica gel column chromatography with 4/1 $CHCl_3$/MeOH as the eluent to obtain 0.484 g (87.4%) of 2-[(3-glycylamino-4-methylthiophen-2-yl)methyl)thio]-1H-benzimidazole **35'**, mp = 157-159°C (decomp). NMR (d_6-DMSO) δ 1.97 (s, 3H), 3.37 (s, 2H), 4.60 (s, 2H), 6.98 (s, 1H), 6.97-7.60 (m, 4H). Anal $C_{15}H_{16}N_4OS_2 \cdot 1/2\ H_2O$ (C, H, N, S).

To a solution of **35'** (0.332 g, 1.0 mmol) in 27 ml of $CHCl_3$ and 3 ml of MeOH *m*-CPBA (80% purity) (0.216 g, 1.0 mmol) was added at – 10°C, followed by stirring for 1 h at – 10°C.To the reaction mixture 3 ml of aqueous $NaHCO_3$ solution and 0.75 ml of aqueous 10% Na_2SO_3 solution was added. The organic layer was separated, washed with aqueous $NaHCO_3$ solution, dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography with 4/1–3/2 $CHCl_3$/MeOH as the eluent to obtain 0.159 g (44.9%) of **36** as colorless amorphous powder. NMR ($CDCl_3$) δ 1.98 (s, 3H), 3.40 (s, 2H), 4.53, 4.75 (ABq, 2H, J = 10 Hz), 6.77 (s, 1H), 7.27-7.77 (m, 4H). Anal $C_{15}H_{16}N_4O_2S_2 \cdot 1/3\ H_2O$ (C, H, N. S).

4-((Benzyloxycarbonyl)glycyl)amino-5-hydroxymethyl-3-phenylisothiazole ***38***

To a solution of (benzyloxycarbonyl)glycine (1.8 g, 8.6 mmol) in 11 ml of HMPA and 1.1 ml of CH_3CN, $SOCl_2$ (0.55 ml, 7.5 mmol) was added dropwise at – 2 to – 8°C. After the reaction mixture had been stirred for 15 min at – 5°C, **37** (1.87 g, 7.5 mmol) was added, followed by stirring for 4 h at room temperature. The reaction mixture was mixed with aqueous $NaHCO_3$ and Et_2O. The crystals that formed were collected, washed with H_2O, and dried, giving 2.85 g (86.4%) of 4-[(benzyloxycarbonyl)glycyl]amino-5-ethoxycarbonyl-3-phenylisothiazole (**38'**). Recrystallization from AcOEt gave colorless crystals, mp = 143-145°C. NMR ($CDCl_3$) δ 1.35 (t, 3H, J = 5 Hz), 3.92 (d, 2H, J = 4 Hz), 4.33 (q, 2H, J = 5 Hz), 5.10 (s, 2H), 5.52 (t, 1H, J = 4 Hz), 7.28-7.73 (m, 5H), 7.32 (s, 5H), 8.68 (s, 1H). Anal $C_{22}H_{21}N_3SO_5$ (C, H, N, S).

To a solution of $LiBH_4$ (0.13 g, 6.0 mmol) in 10 ml of THF **38'** (0.88 g, 2.0 mmol) was added at 0°C followed by stirring for 3 h at room temperature. The reaction mixture was concentrated *in vacuo*, and the residue was dissolved in aqueous HCl, neutralized with aqueous $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography with AcOEt as the eluent to obtain 0.50 g (62.9%) of **38**. Recrystallization from AcOEt gave colorless crystals, mp = 156-158°C. NMR ($CDCl_3$ + d_6-DMSO) δ 3.94 (d, 2H, J = 4 Hz), 4.73 (s, 2H), 5.10 (s, 2H), 7.03 (b, 1H), 7.30-7.83 (m, 5H), 7.35 (s, 5H), 9.45 (s, 1H). Anal $C_{20}H_{19}N_3SO_4$ (C, H, N, S).

2-[((4-(Benzyloxycarbonyl)glycyl)amino-3-phenylisothiazol-5-yl)methyl)thio]-1H-benzimidazole ***39***

A mixture of **38** (1.19 g, 3.0 mol), benzimdiazole-2-disulfide (1.34 g, 4.5 mmol), *n*-Bu_3P (1.12 ml, 4.5 mmol), and pyridine (1.08 ml, 13.3 mmol) was stirred for 3 h at room temperature and allowed to stand overnight at room temperature. $CHCl_3$ was added to the reaction mixture, the crystals that formed were collected, and the $CHCl_3$ layer was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography with 1/1 $CHCl_3$/AcOEt as the eluent to obtain 1.2 g (75.5%) of **39**. NMR ($CDCl_3$) δ 4.02 (d, 2H, J = 4 Hz), 4.35 (s, 2H), 4.98 (s, 2H), 5.80 (b, 1H), 7.07-7.63 (m, 9H), 7.23 (s, 5H), 9.90 (s, 1H).

2[((4-Glycylamino-3-phenylisothiazol-5-yl)methyl)sulfinyl]-1H-benzimidazole ***40***

A solution of **39** (1.2 g, 2.3 mmol) in 6.0 ml of 25% HBr-AcOH was stirred for 1.5 h at room temperature and Et_2O was added to the reaction mixture. The separated crystals were

collected and the filtrate was neutralized with aqueous $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by silica gel column chromatography with 5/1 $CHCl_3$/MeOH as the eluent to obtain 0.89 g (98.9%) of 2-[((4-glycylamino-3-phenylisothiazol-5-yl)methyl)thio]-1H-benzimidazole **39'**. Recrystallization from EtOH gave colorless crystals, mp = 165-167°C. NMR ($CDCl_3$ + d_6-DMSO) δ 3.57 (s, 2H), 4.70 (s, 2H), 5.30 (b, 2H), 7.08-7.80 (m, 9H). Anal $C_{19}H_{17}N_5S_2O$ (C, H, N, S).

To a solution of **39'** (0.99 g, 2.5 mmol) in 40 ml of $CHCl_3$ *m*-CPBA (80% purity) (0.54 g, 2.5 mmol) was added at − 10°C, followed by stirring for 0.5 h at − 5°C. Aqueous $NaHCO_3$ solution was added to the reaction mixture, and the organic layer was separated and dried over Na_2SO_4. After removal of the solvent, the crude product was purified by silica gel column chromatography with 3/1 $CHCl_3$/MeOH as the eluent to obtain 0.35 g (33.0%) of **40**. Recrystallization from EtOH gave colorless crystals, mp = 127°C (decomp). NMR ($CDCl_3$ + d_6-DMSO) δ 3.33 (s, 2H), 4.79, 5.01 (ABq, 2H, J = 10 Hz), 7.20-7.73 (m, 9H). Anal $C_{19}H_{17}N_5S_2O_2 \cdot 0.75\ H_2O$ (C, H, N, S).

2-[(2-Aminophenyl)thio]-1H-benzimidazole 42a

Method A. A mixture of 2-aminothiophenol (1.17 g, 9.3 mmol), 1.16 g of 2-chlorobenzimidazole (**41**) (1.16 g, 8.4 mmol), Et_3N (1.8 ml, 12.9 mmol), 10 ml of DMF, and 40 ml of $CHCl_3$ was refluxed for 2 h. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo* to obtain 1.06 g (52.0%) of **42a**. Recrystallization from EtOH gave colorless crystals, mp = 210-212°C. NMR ($CDCl_3$ + d_6-DMSO) δ 5.20 (b, 2H), 6.53-7.57 (m, 8H). Anal $C_{13}H_{11}N_3S$ (C, H, N, S).

Compound **42h** was prepared in a similar manner.

Method B. A solution of 2-aminothiophenol (0.5 g, 4.0 mmol) and 2-(methylsulfinyl)benzimidazole (0.36 g, 2.0 mmol) in 5 ml of EtOH was refluxed for 1 h. The crystals that formed were collected to obtain 0.46 g (95.1%) of **42a**.

Compounds **42b–h** were prepared in a similar manner.

*2-[(2-Aminophenyl)sulfinyl]-1H-benzimidazole **43a***

To a suspension of **42a** (0.99 g, 4.1 mmol) and $NaHCO_3$ (1.0 g, 11.9 mmol) in 40 ml of $CHCl_3$ and 15 ml of MeOH *m*-CPBA (80% purity) (0.88 g, 4.1 mmol) was added at − 10°C and stirred for 30 min at − 5°C. The reaction mixture was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness. The crude product was washed with Et_2O to obtain 9.67 g (63.2%) of **43a**. Recrystallization from EtOH gave colorless crystals, mp = 174-176°C (decomp). NMR ($CDCl_3$ + d_6-DMSO) δ 4.35 (s, 2H), 6.80-7.67 (m, 8H). Anal $C_{13}H_{11}N_3SO$ (C, H, N, S).

Compounds **43b–h** were prepared in a similar manner.

*2-[(2-((Benzyloxycarbonyl)glycylamino)phenyl)thio]-1H-benzimidazole **45a***

To a solution of benzyloxycarbonylglycine (1.15 g, 5.5 mmol) in 10 ml of HMPA and 1.5 ml of CH_3CN $SOCl_2$ (0.39 ml, 5.3 mmol) was added dropwise at − 8 to − 4°C. After the reaction mixture was stirred at − 5°C for 30 min, 1.2 g of **42a** was added. After the reaction mixture was stirred for 4 h at room temperature, aqueous $NaHCO_3$ solution was added, followed by extraction with Et_2O. The Et_2O extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The crude product was purified by silica gel column chromatography with AcOEt as the eluent, and 1.9 g (88.4%) of **45a** was obtained. Recrystallization from AcOEt gave colorless crystals, mp = 142-144°C. NMR ($CDCl_3$ + d_6-DMSO) δ, 3.94 (d, 2H, J = 4 Hz), 4.92 (s, 2H), 7.03-8.10 (m, 8H), 7.28 (s, 5H), 10.17 (s, 1H). Anal $C_{23}H_{20}N_4O_3$ (C, H, N, S).

Compounds **45b–h** were prepared in a similar manner.

*2-[(2-(Glycylamino)phenyl)thio]-1H-benzimidazole **46a***

A solution of **45a** (1.85 g, 4.3 mmol) in 6 ml of 30% HBr-AcOH was stirred for 2 h at room temperature and Et_2O was added to the reaction mixture. The crystals that formed were collected, and the filtrate was neutralized with aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4, and evaporated *in vacuo*. The crude product was purified by silica gel column chromatography with 1/1 $CHCl_3$/MeOH as the eluent to obtain 0.96 g (75.0%) of **46a**. Recrystallization from EtOH gave colorless crystals, mp = 165-168°C. Anal $C_{15}H_{14}N_4SO$ (C, H, N, S).

Compounds **46b–h** were prepared in a similar manner.

*2-[(2-(L-Phenylalanylamino)phenyl)sulfinyl]-1H-benzimidazole **47c***

To a solution of **46c** (1.36 g, 3.5 mmol) in 40 ml of $CHCl_3$ and 4 ml of MeOH m-CPBA (80% purity) (0.76 g, 3.5 mmol) was added at − 10°C, and the mixture was stirred for 30 min at − 5°C. To the reaction mixture aqueous $NaHCO_3$ solution was added and the organic layer was dried over Na_2SO_4, evaporated to dryness and the crude product purified by silica gel column chromatography with 1/1 $CHCl_3$/MeOH as the eluent to obtain 0.82 g (57.7%) of **47c**. Recrystallization from AcOEt gave colorless crystals, mp = 170-172°C (decomp). NMR ($CDCl_3$) δ, 2.55-4.10 (m, 3H), 7.10-8.23 (m, 8H), 7.28 (s, 5H). Anal $C_{22}H_{20}N_4SO_2$ (C, H, N).

Compounds **47a**, **b** and **d–h** were prepared in a similar manner.

*Determination of plasma concentration of **23a** and **7a** in dogs*

23a was given to Heidenhain pouch dogs by oral administration at a dose of 500 mg/kg and plasma samples were collected at different times. A 200 µl portion of plasma was mixed with 400 µl of MeOH by using a vibrator and was centrifuged at 0°C, 13 000 rpm, and 20 µl of supernatant was subjected to HPLC. The concentrations of **23a** and **7a** were calculated from the area of the peak on the HPLC chart by using the calibration curve previously prepared (fig 2). HPLC was performed with a LC-6A system (Shimadzu), using a nucleosil $5C_{18}$ (150 x 4.6 mm) column and was eluted with CH_3CN/PIC B-7 (26:74) at 1.0 ml/min. The spectrometric UV detector was set at 282 nm and the retention times of **23a** and **7a** were 6.45 and 9.23 min, respectively.

*Conversion of **23a** by rat intestine homogenate and plasma*

a) Rat small intestine brush border homogenate (1 ml) was added to a solution of **23a** (4.1 mg, 12 nmol) in 4 ml of 0.1 M phosphate buffer (pH 7.0). The mixture was incubated at 37°C and samples were collected at different times, quenched by adding 1 ml of acetone and analyzed by HPLC (fig 3). HPLC conditions were the same as in figure 2.

b) The procedure was the same as a) except that rat plasma (4 ml) was used instead of rat intestine homogenate (fig 4). HPLC conditions were the same as in figure 2.

*Reaction of **7a** in acid*

To a 30 ml solution of 0.1 N-HCl **7a** (200 mg, 609 nmol) was added at room temperature followed by stirring for 3 h. After the reaction mixture was neutralized with saturated $NaHCO_3$ solution, the product was extracted with $CHCl_3$ (x 3), and removal of the solvent gave 186 mg of resin. Unreacted crys-

talline **7a** (35 mg, 17.5%) was collected after the addition of 5 ml of EtOAc to the resin. The filtrate was concentrated *in vacuo*, and the residual products were separated by silica gel column chromatography, giving 2-mercaptobenzimidazole (56 mg, 50.6%), 2-aminobenzylalcohol (19 mg, 20.9%), and **7a** (19 mg, 9.5%).

Under stirring, **7a** (20 mg, 74 nmol) was added to 20 ml of 0.1 N HCl aqueous solution at 37°C . Samples were collected (0.5 ml) at different times, neutralized with phosphate buffer (pH 7, 1 ml), diluted with MeOH (1 ml), and analyzed by HPLC (fig 5). HPLC was performed with the same system as in figure 2, using Fine Pack SIL C_{18}-10 (ODS) (4.6 x 250 mm) column and the UV detection was run at 282 nm. The sample was eluted at 1.0 ml/min with 15% CH_3CN/85% 10 mM phosphate buffer (pH 7.5) for 10 min and then the ratio of CH_3CN was changed with a linear gradient from 15% to 35% for 10 min, and finally the ratio of CH_3CN was retained for 15 min. The retention times of 2-aminobenzyl alcohol, 2-mercaptobenzimidazole, **7a**, and **6** (R_1, R_2 = H) were 7.18, 8.48, 26.48 and 32.65 min, respectively.

*Reaction of **7a** with mercaptan in acid*

To a mixture of 0.1 N HCl and 5 ml of 2-mercaptoethanol **7a** (500 mg, 1.84 mmol) was added at 37°C. After stirring for 2 h at 37°C, crystalline 2-mercaptobenzimidazole (85 mg, 30.7%) was separated and the filtrate was neutralized with saturated $NaHCO_3$ solution. The solution was extracted with Et_2O and the aqueous layer was chromatographed with an HP-20 column. The combined products were separated by silica gel column chromatography and gave 2-mercaptobenzimidazole (112 mg, 40.5%) and 2-aminobenzyl-β-hydroxyethylsulfide (225 mg, 66.7%).

*Reaction of **6a** in acid*

To 100 ml of 0.1 N HCl **6a** (300 mg, 1.17 mmol) was added at 37°C, and the mixture was stirred for 3 h. After neutralizing with saturated aqueous $NaHCO_3$, 130 mg of crystals were removed, and the mother liquor was chromatographed (HP-20 column), giving 125 mg of products. Silica gel column chromatography of these products using 5/1 CH_2Cl_2/EtOAc 3/1 CH_2Cl_2/MeOH as the eluent gave 2-mercaptobenzimidazole (132 mg, 74.8%) and *o*-aminobenzylalcohol (52 mg, 20.7%).

Pharmacology

Assay of H^+, K^+-ATPase

Mucosal scraped from the fundic region of fresh porcine stomach were homogenized with homogenizing medium (250 mM sucrose and Tris 20 μM Tris/HCl pH 7.4) to prepare 20% homogenate. The homogenate was centrifuged at 2000 *g* for 30 min. The resulting supernatant was centrifuged again at 105 000 *g* for 60 min. The crude microsomal pellet was suspended in the sucrose buffer and layered over the sucrose buffer containing 7% (w/v) Ficol. After centrifugation for 90 min at 105 000 *g*, the membrane banding above the lower layer was collected, diluted with the sucrose buffer, recovered by centrifugation (105 000 *g*, 60 min) and stored at – 20°C. After freezing and thawing twice, the membrane fraction was used for assay of the enzyme activity. The assay medium (1 ml) consisted of 2 mM $MgCl_2$, 2 mM ATP, 40 mM Tris/HCl pH 7.4 with or without 2.5 mM KCl. Enzyme reaction was carried out for 10 min at 37°C and terminated by addition of 2 ml cold mixture of 4.5% (w/v) ammonium molybdate and 60% (v/v) $HClO_4$ (4/1). The phosphomolybdate that formed was extracted with *n*-butyl acetate and colored by reduction with $SnCl_2$ [39]. The absorbance was measured at 630 nm. H^+, K^+-ATPase activity was determined by subtracting the activity in the absence of KCl from that in its presence. The test compound was dissolved in dimethylsulfoxide and 10 μl of its solution was added to the assay medium for pre-incubation with the enzyme for 60 min at 37°C. Since this assay was used for the primary screen and omeprazole was sufficiently inhibitory at neutral pH, the test compounds were not pretreated with acid, although the inhibitory activity of omeprazole is potentiated in the acidic environment [40].

Acid secretion in isolated bullfrog gastric mucosa

Bullfrogs were killed by decapitation. The stomachs were removed and opened along the lesser curvature. The mucosa was stripped from muscle layer by dissection with fine scissors and stretched, with the mucosal surface inward, over a glass cylinder (1.54 cm^2). The tissue was immersed in serosal solution (87 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_4, 18 mM $NaHCO_3$ and 11 mM glucose) which was bubbled vigorously with 95% O_2-5% CO_2 at room temperature. The mucosal surface was superfused at a rate of 0.5 ml/min with an unbuffered mucosal solution (105 mM NaCl, 4 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM KH_2PO_4 and 11 mM glucose) bubbled with 100% O_2. Test compounds were dissolved in dimethylsulfoxide and added to the serosal solution after maximal stimulation of acid secretion by serosal addition of 1 μM histamine. The acid concentration of mucosal perfusate was determined every 15 min by titrating with 0.01 N NaOH to pH 5.0.

Acid secretion in rats

The trachea and jugular vein of male rats (Jcl Sprague-Dawley strain), 250-320 g of body weight, were cannulated. The abdomen was incised along the median line. Perfusion cannular were inserted and fixed in the antrum of the stomach and the esophagus. Warmed physiological saline (37°C) was perfused through the esophagus at the rate of 1 ml/min and the gastric effluent was collected every 15 min through the antrum cannula. The perfusate was titrated with 0.01 N NaOH to pH 7.0 to determine the acid secretion which was continuously stimulated by intravenous infusion of histamine dihydrochloride (3 mg/kg per h) through the jugular vein cannula. The test compounds suspended in 5% gum arabic solution were administered intraperitoneally or intraduodenally 90 min after the beginning of histamine infusion and the infusate was further collected for 90 min to determine the maximal suppression of acid secretion. The percent suppression was calculated from the acid secretion 90 min after the beginning of histamine infusion and the acid secretion at the maximal suppression.

Acid secretion in dogs

Heidenhain pouches were prepared in female dogs (Beagle strain), 10-12 kg of body weight, several months before experiments. Gastric juice was collected at 15 min intervals through a cannula and aliquots were titrated with 0.1 N NaOH to pH 7.0. The acid secretion was continuously stimulated by intravenous infusion of histamine dihydrochloride (60 μg/kg per h). The test compound was suspended in 5% gum arabica solution and administered orally 90 min after the beginning of histamine infusion.

Preparation of rat small intestinal brush borders

Brush borders were prepared from intestinal homogenates by the method of Wojnarcowska and Gray [41]. Mucosa scraped from the jejunum and ileum of rats (Jcl Sprague-Dawley strain) were homogenized with 300 mM sucrose/5 mM EDTA pH 7.4. The homogenate was filtered through cotton gauze and the filtrate was centrifuged at 1500 *g* for 10 min. The precipitate was purified to obtain brush borders by sequential 10 min exposure to increasingly hypotonic solutions of sucrose/5 mM EDTA, in which the sucrose was decreased from 300 mM in increments of 75 mM with a final washing with 37.5 mM sucrose/5 mM EDTA. The final precipitate was suspended in 0.05 mM Tris/HCl pH 7.5 and dialyzed against the same buffer.

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