Studies on Antiulcer Drugs. 7.[1] 2-Guanidino-4-pyridylthiazoles as Histamine H_2-Receptor Antagonists with Potent Gastroprotective Effects against Nonsteroidal Antiinflammatory Drug-Induced Injury

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A series of 2-guanidino-4-pyridylthiazole derivatives were synthesized and evaluated for anti-aspirin-ulcer, gastric antisecretory, and histamine-H_2-receptor-antagonist activities. Several compounds showed superior anti-aspirin-ulcer activity to that of clinically used H_2-antagonists in the rat. Among them, 4-[6-(acetamidomethyl)pyridin-2-yl]-2-guanidinothiazole (**8**) demonstrated potent inhibitory activities against gastric lesions caused by two kinds of nonsteroidal antiinflammatory drugs, aspirin and indomethacin, respectively, in addition to strong antisecretory activity. Compound **8** possessed a preventable ability for the aspirin-induced reduction of the gastric mucosal blood flow at an intragastric administration of 32 mg/kg in the rat. On the other hand, famotidine (32 mg/kg) exhibited no significant effect and ranitidine (100 mg/kg) aggravated the blood flow in this system.

Nonsteroidal antiinflammatory drugs (NSAID) have been used for a variety of inflammatory diseases, among which rheumatoid arthritis is a representative. Although these drugs are generally well tolerated in patients with arthritic conditions, a high incidence of gastrointestinal side effects such as hemorrhage and ulceration has been a serious problem in medication.[2] A recent review[3] demonstrated that peptic ulcer symptoms occur in 25% of NSAID users. Furthermore, it is indicated that the ulcerations induced by NSAID are more severe than those not associated with NSAID.[4] Therefore, the use of these drugs is often limited, and 2–10% of patients with rheumatic disease eventually discontinue the NSAID treatment.[5]

Histamine H_2-receptor antagonists (H_2-antagonists) have been most widely used in the treatment of peptic ulcer disease as one of the safest classes of drugs.[6] However, despite their high healing rates of idiopathic ulcer symptoms, the therapeutic effect on NSAID-induced gastropathy is not satisfactory.[2,7] On the other hand, misoprostol, a synthetic analogue of prostaglandin which was recently marketed, is expected to be cytoprotective for the gastrointestinal mucosa.[8] Although misoprostol is a therapeutically useful drug for the treatment of NSAID-induced ulcers, this drug causes undesirable side effects, such as diarrhea and abdominal pain, in a significant ratio.[2,6,8,9] Antiulcer drugs in the other categories, omeprazole, a proton pump inhibitor, and sucralfate, a gastroprotective drug, are clinically available, but the usefulness of these drugs has been obscure in treating ulcerations caused by NSAID.[2b,c,3]

In a continuing search for novel H_2-antagonists, these clinical requirements prompted us to probe for an agent with anti-NSAID gastropathy as a pharmacological option. In a previous paper,[1] we reported the synthesis and antiulcer activities of a series of 4-furyl-2-guanidinothiazoles, which were structurally characterized as rigid analogues of conventional H_2-antagonists (**1**–**4**) (Figure 1).[10-13] Among them, 4-[5-[2-amino-2-[(aminosulfonyl)-imino]ethyl]furan-2-yl]-2-guanidinothiazole (**5**) demonstrated a superior cytoprotective activity to that of commercially available H_2-antagonists in additional to potent gastric antisecretory activity. We focused on the pharmacological properties of compound **5** and decided to prepare a new series of conformationally constrained H_2-antagonists. This paper describes the synthesis and the pharmacological evaluation of a series of 2-guanidino-4-pyridylthiazoles.

Chemistry

The target compounds listed in Table 1 were synthesized by the routes outlined in Schemes 1–6. The 6-(substituted methyl)-2-thiazolylpyridines (**7**–**34**) were obtained as follows. 2-Carbamoyl-6-(hydroxymethyl)pyridine (**39**)[14] was treated with phosphorus oxychloride in the presence of *N*,*N*-dimethylformamide (DMF) to generate 6-(chloromethyl)-2-cyanopyridine (**40**), which led to the phthalimide derivative **41**. Reaction of **41** with hydrazine hydrate followed by treatment with acid anhydrides yielded the 2-[(acylamino)methyl]-6-cyanopyridines **43a** and **43b**, which on treatment with methyl magnesium bromide gave the ketone derivatives **44a** and **44b**. Bromination of these ketones and subsequent cyclization with amidinothiourea afforded the desired compounds **8** and **9** (method A, Scheme 1). Acid hydrolysis of **8** gave a key intermediate, **46**. Treatment of **46** with several reagents, *e.g.*, (i) reactive carboxylic acid derivatives (method B_1–B_4), (ii) sulfonyl chloride (method C), (iii) potassium cyanate (method D_1) or alkyl isocyanates (method D_2), (iv) dimethyl dithioimidocarbonates or 1,1-bis(methylthio)-2-nitroethene followed by methylamine (method E), and (v) imidate (method F), resulted in the corresponding products, *i.e.*, (i) amides **7** and **10**–**24**, (ii) sulfonamide **25**, (iii) urea **26**–**29**, (iv) guanidines **30** and **31** or nitroethene **32**, and (v) amidine **33**, respectively (Scheme 2). Acetylation of **39** followed by dehydration yielded the nitrile derivative **47**, which was successively subjected to Grignard reaction, chlorination, and then cyanation to give 2-acetyl-6-(cyanomethyl)pyridine (**49**). Bromination of **49** and subsequent cyclization with amidinothiourea provided the thiazolylpyridine derivative **50**. Compound **50** was con-

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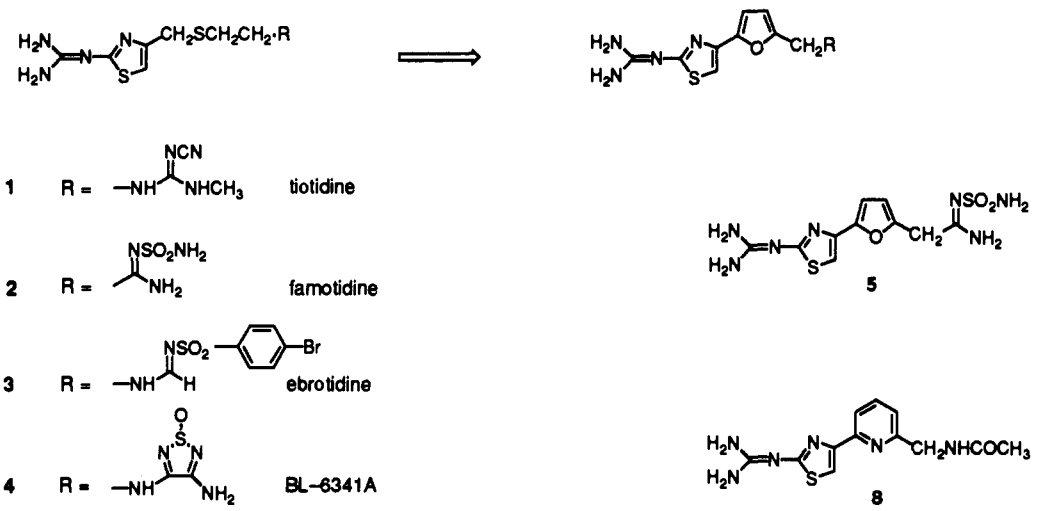
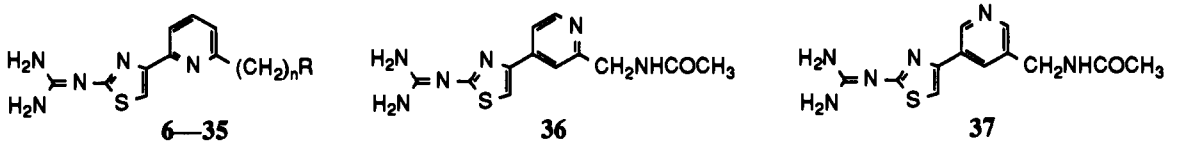


Figure 1.

Table 1. 2-Guanidino-4-pyridylthiazoles



no.	n	R	mp, °C	recrystn solvent[a]	yield, %	formula[b]	method
6	0	$NHCOCH_3$	>300	E-W	66	$C_{11}H_{12}N_6OS\cdot 2HCl\cdot H_2O$	A
7	1	NHCHO	192–193	M-W	27	$C_{11}H_{12}N_6OS$	B_1
8	1	$NHCOCH_3$	228–229	M-I	21	$C_{12}H_{14}N_6OS\cdot 2HCl\cdot H_2O$	A
9	1	$NHCOC_2H_5$	216–217	E-DX-1	64	$C_{13}H_{16}N_6OS$	A
10	1	NHCO-*n*-C_3H_7	233–234	D-I	36	$C_{14}H_{18}N_6OS$	B_4
11	1	NHCO-*i*-C_3H_7	228–229	E-I	39	$C_{14}H_{18}N_6OS$	B_3
12	1	NHCO-*t*-C_4H_9	245–246	M-I	48	$C_{15}H_{20}N_6OS$	B_3
13	1	NHCO-c-C_3H_5	219	M-DX-I	29	$C_{14}H_{16}N_6OS$	B_2
14	1	NHCO-c-C_4H_7	242	D-EA	62	$C_{15}H_{16}N_6OS$	B_2
15	1	NHCO-c-C_5H_9	239	D-EA	78	$C_{16}H_{20}N_6OS$	B_2
16	1	NHCO-c-C_6H_{11}	243	D-EA	76	$C_{17}H_{22}N_6OS$	B_2
17	1	NHCO-c-C_7H_{13}	240	D-EA	61	$C_{16}H_{24}N_6OS$	B_2
18	1	$NHCOCF_3$	271	M-DX-I	26	$C_{12}H_{11}F_3N_6OS$	B_4
19	1	$NHCOCH_2SCH_3$	202	M-DX-I	24	$C_{13}H_{16}N_6OS_2$	B_2
20	1	$NHCOCH_2SCH_2$-(2-furyl)	182	M-DX-I	43	$C_{17}H_{16}N_6O_2S_2$	B_2
21	1	NHCO-(2-furyl)	229	D-EA	44	$C_{15}H_{16}N_6O_2S$	B_2
22	1	NHCO-(3-furyl)	227	D-EA	48	$C_{15}H_{16}N_6O_2S$	B_2
23	1	NHCO-(4-tetrahydropyranyl)	232	D-EA	64	$C_{17}H_{20}N_6O_2S$	B_2
24	1	NHCO-(2-tetrahydrofuryl)	157–158	W-IA	48	$C_{15}H_{14}N_6O_2S\cdot 2HCl\cdot 1.5H_2O$	B_3
25	1	$NHSO_2CH_3$	196	M-W	24	$C_{11}H_{14}N_6O_2S_2$	C
26	1	$NHCONH_2$	210–211	M-W	48	$C_{11}H_{13}N_7OS\cdot 2HCl\cdot 0.5H_2O$	D_1
27	1	$NHCONHCH_3$	238–239	M-I	55	$C_{12}H_{15}N_7OS\cdot 2HCl$	D_2
28	1	$NHCONHC_2H_5$	229	M-I	56	$C_{13}H_{17}N_7OS\cdot 2HCl$	D_2
29	1	NHCONH-*n*-C_3H_7	228–229	M-I	82	$C_{14}H_{19}N_7OS\cdot 2HCl$	D_2
30	1	$NHC(=NCN)NHCH_3$	245–246	D-W	64	$C_{13}H_{16}N_8S\cdot 0.2H_2O$	E
31	1	$NHC(=NSO_2CH_3)NHCH_3$	197	E-W	26	$C_{13}H_{18}N_8O_2S_2\cdot HCl\cdot 0.5H_2O$	E
32	1	$NHC(=CHNO_2)NHCH_3$	184–185	E-W	36	$C_{13}H_{16}N_8O_2S\cdot HCl\cdot H_2O$	E
33	1	$NHCH=NSO_2C_2H_5$	201	M-DX-I	29	$C_{13}H_{17}N_7O_2S_2$	F
34	1	$C(=NSO_2NH_2)NH_2$	207–208	D-EA	27	$C_{11}H_{14}N_8O_2S_2$	G
35	2	$NHCOCH_3$	272–273	IA-W	16	$C_{13}H_{16}N_6OS\cdot 2HCl$	A
36			246–248	A-E-W	44	$C_{12}H_{14}N_6OS\cdot 2HCl\cdot H_2O$	B_4
37			291–221	M-I	23	$C_{12}H_{14}N_6OS$	B_4

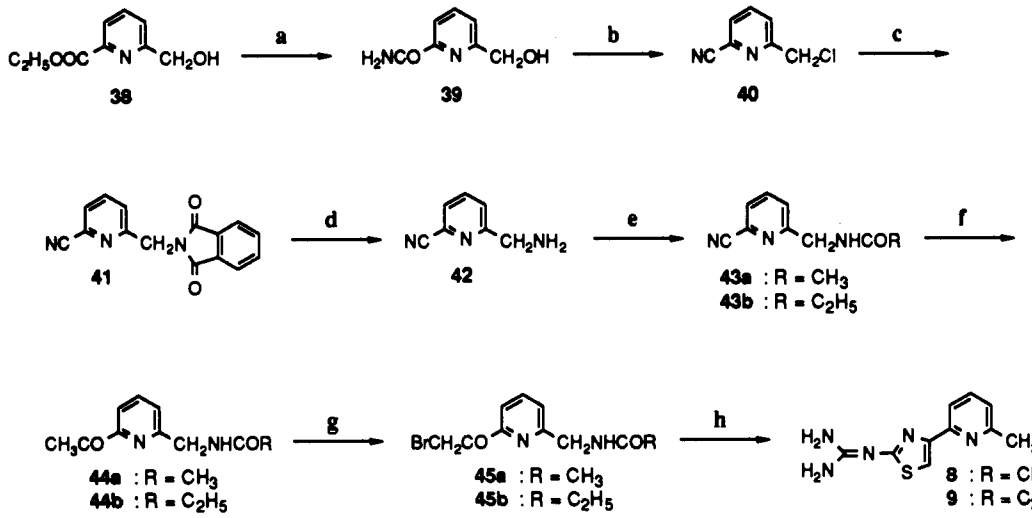
[a] A = Me_2O, D = *N,N*-dimethylformamide (DMF), DX = dioxane, E = EtOH, EA = EtOAc, I = $(i\text{-Pr})_2O$, IA = *i*-PrOH, M = MeOH, and W = H_2O. [b] Analyses for C, H, and N are within ±0.4% of the theoretical values.

verted to the imidate which was reacted with sulfamide to afford the final product **34** (method G, Scheme 3).

Modifications in the side chain of **8** were carried out as shown in Scheme 4. Dehydration on the carbamoyl group of **53**[15] or cyanation onto the α-position of **55** gave the nitrile derivatives **56a** and **56b**, which were converted to the final products, a shorter side-chain (**6**) and a longer side-chain (**35**) analogue of **8**, in a similar manner to the formation of **8**.

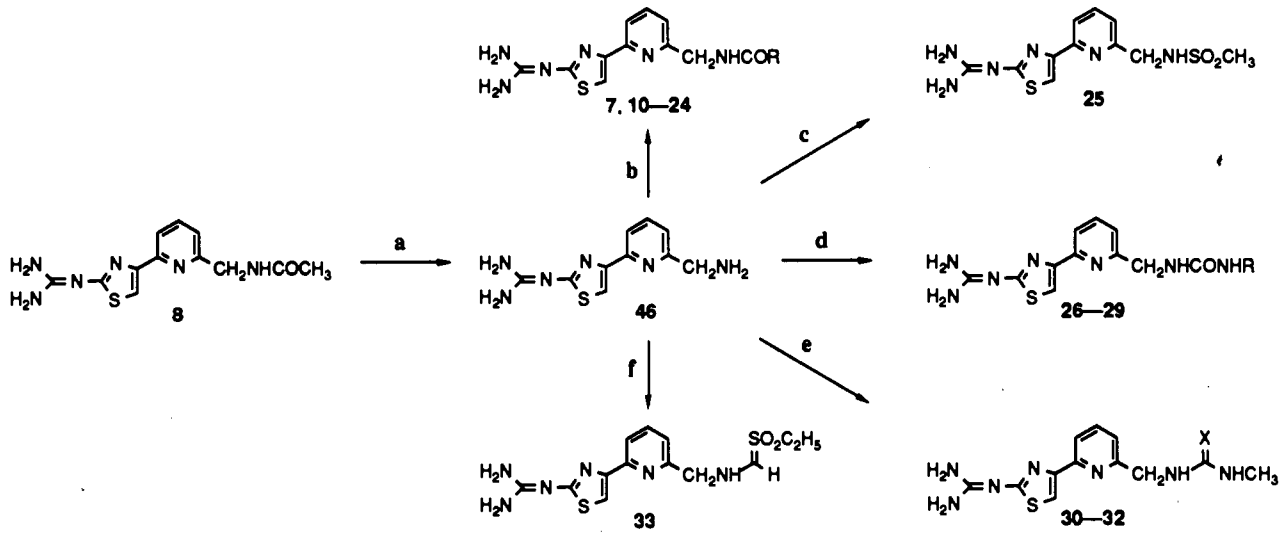
The positional isomers of **8** were prepared by the routes

Scheme 1[a]



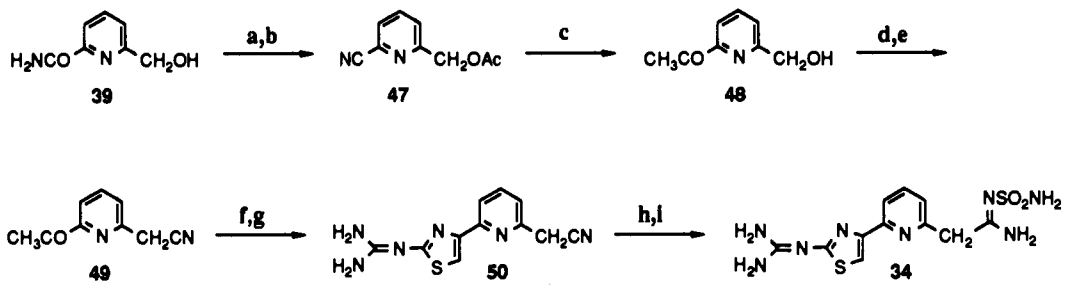
[a] Reagents: (a) c-NH_4OH; (b) $POCl_3$/DMF; (c) potassium phthalimide; (d) $H_2NNH_2 \cdot H_2O$; (e) $(RCO)_2O$; (f) MeMgBr; (g) Br_2; (h) $H_2NC(=NH)NHC(=S)NH_2$ (method A).

Scheme 2[a]



[a] Reagents: (a) HCl; (b) HCOOH–Ac_2O (method B_1), RCOOH/$Me_2N(CH_2)_3N{=}C{=}NEt$ (method B_2), RCOCl/Et_3N (method B_3), or $(RCO)_2O$ (method B_4); (c) $MeSO_2Cl/Et_3N$ (method C); (d) KOCN (method D_1) or RNCO (method D_2); (e) (1) $(MeS)_2C{=}X$, (2) $MeNH_2$ (method E); (f) $EtOCH{=}SO_2Et$ (method F).

Scheme 3[a]



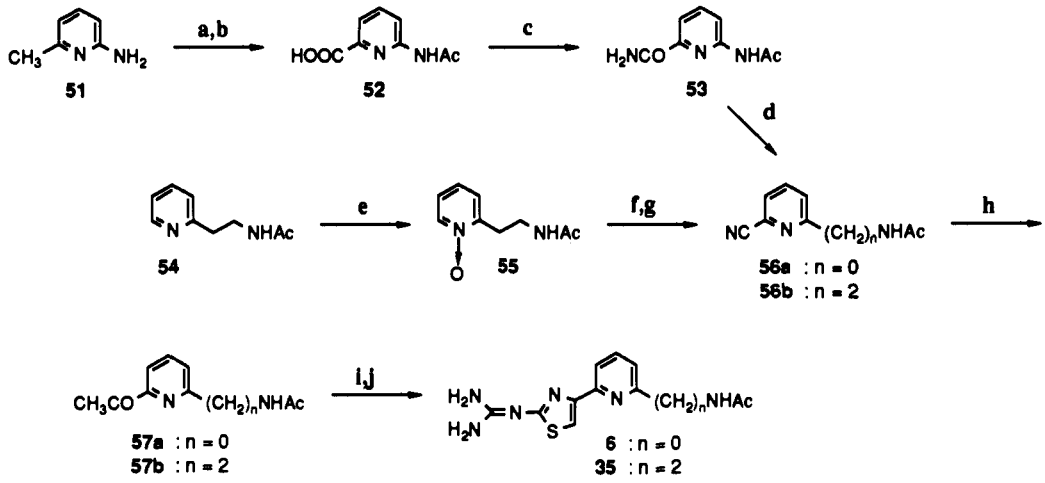
[a] Reagents: (a) Ac_2O; (b) $POCl_3$/DMF; (c) MeMgBr; (d) $POCl_3$/DMF; (e) KCN; (f) Br_2; (g) $H_2NC(=NH)NHC(=S)NH_2$; (h) HCl gas/MeOH; (i) $H_2NSO_2NH_2$ (method G).

shown in Scheme 5 and 6. The key intermediate (**59**) for the synthesis of 4-thiazolyl-2-(acetamidomethyl)pyridine **36** was obtained from 4-acetylpyridine (**58**) by homolytic ethoxycarbonylation (Minisci reaction).[16] Bromination of **59** and subsequent cyclization yielded the 2-(ethoxycarbonyl)-4-thiazole derivative **60**, which on reduction with sodium borohydride gave **61**. The hydroxymethyl moiety of **61** was converted to an acetamidomethyl group *via* chlorination followed by Gabriel amino synthesis and then acetylation to afford the desired compound **36** (Scheme 5). The synthesis of the 3,5-substituted pyridine derivative **37** was carried out in a manner similar to that of **36** by using 3-acetyl-5-(methoxycarbonyl)pyridine (**62**)[17] as the starting material (Scheme 6).

Results and Discussion

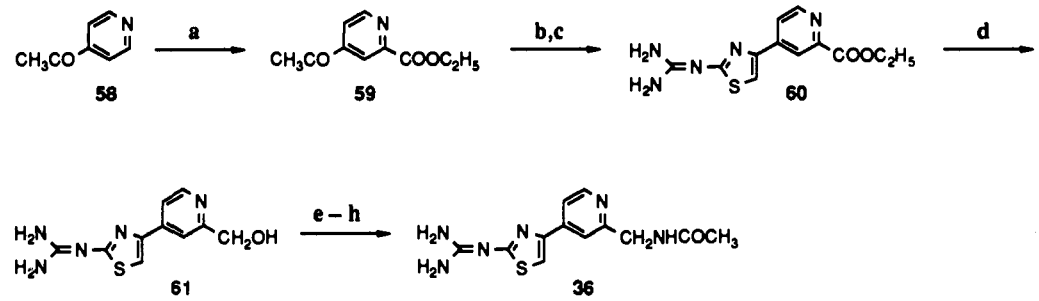
The compounds obtained were evaluated for antiulcer activity against aspirin-induced gastric lesions (aspirin

Scheme 4[a]



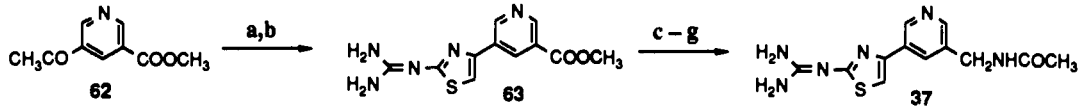
[a] Reagents: (a) Ac_2O; (b) $KMnO_4$; (c) DCC/c-NH_4OH; (d) $POCl_3$/DMF; (e) AcOH/H_2O_2; (f) Me_2SO_4; (g) KCN/DMSO; (h) MeMgBr; (i) Br_2; (j) $H_2NC(=NH)NHC(=S)NH_2$.

Scheme 5[a]



[a] Reagents: (a) MeCOCOOEt/H_2O_2/$FeSO_4$; (b) Br_2; (c) $H_2NC(=NH)NHC(=S)NH_2$; (d) $NaBH_4$; (e) $SOCl_2$; (f) potassium phthalimide; (g) $H_2NNH_2 \cdot H_2O$; (h) Ac_2O.

Scheme 6[a]



[a] Reagents: (a) Br_2; (b) $H_2NC(=NH)NHC(=S)NH_2$; (c) $NaBH_4$; (d) $SOCl_2$; (e) potassium phthalimide; (f) $H_2NNH_2 \cdot H_2O$; (g) Ac_2O.

ulcer) in rats, antisecretory activity on histamine-stimulated gastric acid secretion in lumen-perfused anaesthetized rats, and H_2-antagonist activity using the histamine-stimulated chronotropic response of the isolated guinea pig right atrium. Several derivatives with good profiles in those tests were also assessed for antisecretory activity on tetragastrin-stimulated gastric acid secretion in conscious Heidenhain-pouch dogs.

As shown in Table 2, in the 2,6-substituted pyridine series, several derivatives having an amidomethyl substituent showed potent anti-aspirin-ulcer activity. Among them, compounds with a cyclic acyl substituent (**14**, **15**, and **17**) possessed excellent potency. Incorporation of an oxygen (**21** and **22**) into the cyclic alkyl ring of **15** resulted in diminished activity. Similarly, compounds with an introduced heteroatom (**18**, **20**, **23**, and **24**) in the acyl group conferred reduced activity except for the case of **19**. The ureido derivatives showed marginal activity. However, compounds to which were introduced guanidines **30** and **31**, nitroethene **32**, and amidines **33** and **34**, which are all representative substituents of those existing in known H_2-antagonists, were found to exhibit, at best, only weak activity. The clinically used H_2-antagonists, cimetidine, ranitidine, roxatidine acetate, and famotidine, also revealed no significant effect at the dose levels tested.

Concerning antisecretory activity, some compounds having an amido (**7–10**) or an ureido (**26–29**) substituent exhibited good activity. Introduction of the cyclic alkylamido groups (**14–17**), except for the case of **13**, or the amido groups with a heteroatom (**18–24**) tended to cause a decrease in activity. Contrary to our expectation, compounds **30**, **32**, and **34** having the substituents which exist in cimetidine, ranitidine, and famotidine, respectively, also revealed only slight activity.

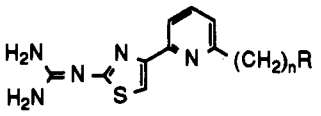
H_2-Antagonist activity of the amido and ureido derivatives also tended to have higher potency compared to that of the guanidine or amidine derivatives.

In the effects of the side-chain length, both shortening (**6**) and lengthening (**35**) the side chain of the derivatives of **8** showed a decrease in the activities for all assays.

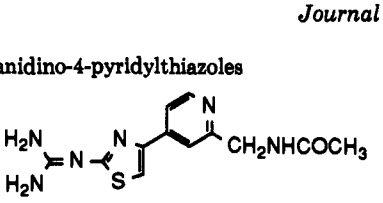
With regard to the positional isomers of **8**, on the anti-aspirin-ulcer assay, the 2-(acetamidomethyl)-4-thiazolyl derivative **36** and the 3-(acetamidomethyl)-5-thiazolyl derivative **37** showed marginal activity, whereas no significant antisecretory and H_2-antagonist activities of these derivatives were observed.

In the structure–activity relationships, it is interesting that the H_2-antagonist and antisecretory activities of the famotidine analogue **34** were very low in contrast to the high activities of the furan homologue **5**. On the other

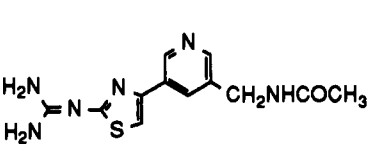
Table 2. Pharmacological Activities of 2-Guanidino-4-pyridylthiazoles



6—35



36



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no.	n	R	inhibition, % aspirin ulcer,[a] 32 mg/kg po	gastric secretion rat,[b] 1 mg/kg iv	gastric secretion dog,[c] 0.32 mg/kg po	H_2-antagonism,[d] 1×10^{-6} g/mL
5			49**	99	92	84
6	0	$NHCOCH_3$	43**	16		-49
7	1	NHCHO	57**	83		46
8	1	$NHCOCH_3$	68**	82	95	66
9	1	$NHCOC_2H_5$	70**	81	50	72
10	1	NHCO-*n*-C_3H_7	56**	71		25
11	1	NHCO-*i*-C_3H_7	65**	21		62
12	1	NHCO-*t*-C_4H_9	58**	22		-13
13	1	NHCO-c-C_3H_5	62**	91	48	60
14	1	NHCO-c-C_4H_7	82**	61		2
15	1	NHCO-c-C_5H_9	92**	67	22	32
16	1	NHCO-c-C_6H_{11}	62**	23		23
17	1	NHCO-c-C_7H_{13}	93**	43	67	13
18	1	$NHCOCF_3$	43**	43		72
19	1	$NHCOCH_2SCH_3$	80**	4		37
20	1	$NHCOCH_2SCH_2$-(2-furyl)	49**	16		76
21	1	NHCO-(2-furyl)	66**	6		7
22	1	NHCO-(3-furyl)	42**	51		32
23	1	NHCO-(4-pyridyl)	62**	37		2
24	1	NHCO-(3-pyridyl)	69**	34		13
25	1	$NHSO_2CH_3$	60**	46		50
26	1	$NHCONH_2$	34*	98	78	100
27	1	$NHCONHCH_3$	57**	79		86
28	1	$NHCONHC_2H_5$	34	91		52
29	1	NHCONH-*n*-C_3H_7	42*	90		82
30	1	$NHC(=NCN)NHCH_3$	37	15		43
31	1	$NHC(=NSO_2CH_3)NHCH_3$	10	24		29
32	1	$NHC(=CHNO_2)NHCH_3$	-27	18		-14
33	1	$NHCH=NSO_2C_2H_5$	1	-18		4
34	1	$C(=NSO_2NH_2)NH_2$	16	42		-32
35	2	$NHCOCH_3$	31	58		3
36			42*	40		-33
37			42*	10		22
		cimetidine	33	53	22[f]	43
		ranitidine	3	72	41	44
		roxatidine acetate	31[e]	84	58	76
		famotidine	22	96	96	87

[a] Inhibitory effect on gastric lesions induced by HCl (0.2 N)–aspirin (200 mg/kg) in rats ($n = 7$); double asterisk, $p < 0.01$ and asterisk, $P < 0.05$: statistical significant from control (Student's t test). [b] Inhibition of histamine-stimulated gastric acid secretion in the lumen-perfused stomachs of the anaesthetized rats ($n = 2$). [c] Inhibitory effect on gastric acid secretion induced by gastrin in the conscious Heidenhain-pouch dogs ($n = 2$). [d] Inhibition of the histamine-stimulated chronotropic response in the isolated guinea pig right atrium. [e] 100 mg/kg. [f] 1 mg/kg.

hand, in the acetamidomethyl derivatives, such activities of the pyridyl derivative 8 were superior to those of the furan isostere.[1] Thus, no simple isosteric replacement between the furan ring and the pyridine ring was accomplished in our arylthiazole series.

Among the compounds tested, 8 demonstrated a good pharmacological profile, and therefore, further evaluation of this compound was carried out (Table 3). In additional high antisecretory activity based on potent H_2-antagonism, compound 8 indicated significant inhibitory activity against gastric lesions induced by two kinds of NSAIDs, aspirin and indomethacin, and the ED_{50} values are 13.3 and 6.5 mg/kg po, respectively. These activities are clearly superior to those of the referenced H_2-antagonists. In particular, famotidine having a more potent antisecretory effect than 8 revealed weak activities (47% inhibition on aspirin ulcers and 33% inhibition on indomethacin ulcers at 100 mg/kg po, respectively). This indicates that the gastroprotective effect of 8 against these NSAID-induced injuries is not completely dependent on the ability of gastric antisecretion. Furthermore, the result that roxatidine acetate, reported as a cytoprotective H_2-antagonist,[18] also displayed only weak activity on the aspirin ulcer at 100 mg/kg (Table 2) seems to indicate that 8 has a different quality of cytoprotection from such a classical H_2-antagonist.

Next, we examined an effect on gastric mucosal blood flow which is known as an important function to maintain the integrity of the gastric mucosa. The occurrence of gastric injury by NSAID intake is closely associated with the reduction of mucosal blood flow.[19] Compound 8 antagonized the aspirin-induced decrease in the gastric

Table 3. Further Evaluation of 8 and the Reference Compounds

compd	aspirin ulcer[a] ED_{50}, mg/kg po[f]	indomethacin ulcer[b] ED_{50}, mg/kg po[f]	gastric secretion: pylorus-ligated rat[c] ED_{50}, mg/kg id[f]	gastric secretion: dog[d] ED_{50}, mg/kg po	H_2-antagonism[e] IC_{50}, μM
8	13.3 (4.8–37.5)[g]	6.5 (2.5–16.5)	12.6 (3.3–48.3)	0.063 (0.016–0.25)	1.3 (0.29–5.8)
ranitidine	>100[h]	nt[j]	30.5 (3.9–239)	0.30 (0.10–1.14)	3.4 (0.74–14.5)
famotidine	>100[i]	>100[k]	3.5 (0.79–15.2)	0.042 (0.0087–0.18)	0.30 (0.042–2.2)

[a] See footnote *a* in Table 2. [b] Inhibitory effect on gastric lesions induced by HCl (0.35 N)–indomethacin (5 mg/kg) in rats. [c] Inhibition of basal gastric acid secretion on pylorus-ligated rats. [d] See footnote c in Table 2. ED_{50} values were estimated from three or four doses. Three or four animals were used per dose. [e] See footnote *d* in Table 2. IC_{50} values were obtained from three doses. Three atria were used per dose. [f] ED_{50} values were estimated from three doses. Ten animals were used per dose. [g] 95% confidence limits are in parentheses. [h] 1% inhibition at 100 mg/kg. [i] 47% inhibition at 100 mg/kg. [j] Not tested. [k] 33% inhibition at 100 mg/kg.

Table 4. Preventive Effect of 8 and the Reference Drugs against the Decrease of Gastric Mucosal Blood Flow Caused by HCl–Aspirin

compd	dose, mg/kg ig	gastric mucosal blood flow, mL/min/100 g,[a] (ratio:% of initial): basal[b]	drug[c]	30 min[d]	60 min[e]
control		65.7 ± 8.0 (100)	65.4 ± 7.5 (99.9 ± 0.8)	54.9 ± 10.9 (81.4 ± 9.0)	47.7 ± 8.0 (71.4 ± 4.9)
8	10	70.4 ± 7.1 (100)	70.2 ± 9.4 (98.9 ± 5.4)	67.9 ± 10.2 (97.3 ± 10.6)	61.0 ± 9.7 (87.6 ± 11.8)
	32	58.9 ± 8.9 (100)	65.3 ± 11.9* (109.4 ± 2.6)	63.1 ± 12.7** (104.6 ± 4.7)	60.4 ± 9.5** (102.3 ± 2.3)
famotidine	10	68.6 ± 5.7 (100)	73.0 ± 10.3 (105.3 ± 8.8)	62.5 ± 7.9 (89.6 ± 5.7)	50.5 ± 5.4 (73.4 ± 3.7)
	32	58.4 ± 3.6 (100)	60.5 ± 6.2 (103.8 ± 9.7)	46.9 ± 7.0 (80.1 ± 11.0)	43.4 ± 5.7 (75.2 ± 10.7)
ranitidine	100	85.3 ± 3.5 (100)	82.3 ± 3.8 (96.7 ± 4.2)	53.4 ± 7.1 (62.9 ± 8.3)	55.4 ± 5.7 (64.8 ± 5.9)

[a] The values are means ± SEM from five determinations; double asterisk, $P < 0.01$, and asterisk, $P < 0.05$: statistical significant from control (Student's *t* test). [b] Basal value; 15 min before dosing of the drugs. [c] Immediately after dosing of the drugs; 15 min before administration of HCl (0.2 N)–aspirin (200 mg/kg ig). [d] 30 min after administration of HCl–aspirin. [e] 60 min after administration of HCl–aspirin.

mucosal blood flow at 10 mg/kg ig, though the degree was not significant, and completely prevented it at 32 mg/kg ig. On the other hand, famotidine showed no preventive effect at 32 mg/kg ig, and ranitidine aggravated the blood flow at 100 mg/kg ig (Table 4).

Compound 8 displayed a competitive H_2-antagonism in the assay using the isolated rabbit gastric gland (pA_2 = 6.81; slope of Schild plot = 1.0), and the ability was greater than that of cimetidine (pA_2 = 5.95). With respect to the specificity for the H_2-receptor, this compound did not antagonize the contractile responses to histamine and acetylcholine in isolated guinea pig ileum (histamine H_1 and muscarinic receptor) at a concentration of 200 μM and, furthermore, demonstrated no effect on the cardiovascular system, blood pressure, and heart rate in rats at 320 mg/kg po.

Conclusions

We investigated a series of 2-guanidino-4-pyridylthiazoles as novel H_2-antagonists. Of the compounds obtained, 8 demonstrated potent antisecretory activities in rats and dogs. Furthermore, this compound displayed antiulcer activities against lesions induced by aspirin or indomethacin. In contrast with this result, the therapeutic H_2-antagonists expressed no significant inhibitory activities on such antiulcer assays even at a high dose. Therefore, from the clinical point of view, compound 8 may be a useful antisecretory drug having a new therapeutic option, *i.e.*, a prophylactic effect for NSAID-induced gastropathy. Although the mechanism of compound 8 on these antiulcer activities has not yet been clarified, maintenance of gastric mucosal blood flow under NSAID-irritated circumstances may be one of the possible causes.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Infrared (IR) spectra were taken in Nujol using a Hitachi 260-10 spectrometer. Proton nuclear magnetic resonance (^1H NMR) spectra were recorded in dimethylsulfoxide-d_6 (DMSO) with tetramethylsilane as an internal standard on a Bruker AC-200P spectrometer. Mass spectral measurements (MS) were made on a JEOL JMS D-300 mass spectrometer. Analytical results are within ±0.4% of the theoretical values unless otherwise indicated. All extracted solutions were dried over magnesium sulfate and concentrated to dryness on a rotary evaporator under reduced pressure.

6-(Chloromethyl)-2-cyanopyridine (40). Phosphorus oxychloride (220 mL, 2.4 mol) was added dropwise to a solution of 2-carbamoyl-6-(hydroxymethyl)pyridine (**39**)[14] (120 g, 0.8 mol) in *N*,*N*-dimethylformamide (DMF) (1.2 L) at 0–5 °C with stirring, and the mixture was stirred for 6 h at the same temperature. After removal of the solvent, the residue was added to cold water (2 L). The mixture was basified to pH 10 with 20% aqueous K_2CO_3 and extracted with EtOAc. The extract was dried and concentrated to give **40** (106 g, 88%) as an irritant powder. An analytical sample was obtained by recrystallization from EtOAc/hexane, mp 64–65 °C. IR: 2240 cm^{-1}. ^1H NMR: δ 4.87 (2H, s), 7.90 (1H, dd, J = 1.2, 7.7 Hz), 8.03 (1H, dd, J = 1.2, 7.7 Hz), 8.14 (1H, t, J = 7.7 Hz). MS: *m/z* 152 (M^+). Anal. ($C_7H_5ClN_2$) C, H, N.

2-Cyano-6-(phthalimidomethyl)pyridine (41). A solution of **40** (100 g, 0.66 mol) and potassium phthalimide (120 g, 0.66 mol) in DMF (750 mL) was stirred for 4 h at room temperature. After removal of the solvent, the residue was added to water (1 L). The resulting precipitate was collected by filtration and washed with water to give **41** (130 g, 77%). An analytical sample was obtained by recrystallization from aqueous DMF, mp 200–201 °C. IR: 2250, 1775, 1715 cm^{-1}. ^1H NMR: δ 4.99 (2H, s), 7.81 (1H, dd, J = 1.0, 7.7 Hz), 7.85–7.98 (5H, m), 8.06 (1H, t, J = 7.7 Hz). Anal. ($C_{15}H_9N_3O_2$) C, H, N.

2-(Aminomethyl)-6-cyanopyridine Hydrochloride (42). A solution of hydrazine hydrate (25 g, 0.5 mol) in MeOH (150 mL)

was added dropwise to a suspension of 41 (120 g, 0.46 mol) in MeOH (500 mL)/tetrahydrofuran (THF) (500 mL) at room temperature. After the mixture was stirred for 2 h, 2 N HCl (250 mL, 0.5 mol) was added and the resulting mixture was stirred for an additional 3 h. The solvent was evaporated, and the residue was mixed with water (600 mL). The insoluble material was removed by filtration, and the filtrate was concentrated to dryness. The residue was recrystallized from MeOH/diisopropyl ether (IPE) to give **42** (74 g, 95%), mp >300 °C. IR: 2240 cm^{-1}. ^1H NMR: δ 4.27 (2H, s), 7.94 (1H, dd, J = 1.2, 7.7 Hz), 8.08 (1H, dd, J = 1.2, 7.7 Hz), 8.16 (1H, t, J = 7.7 Hz), 8.83 (3H, br s). Anal. ($C_7H_7N_3$·HCl) C, H, N.

2-(Acetamidomethyl)-6-cyanopyridine (43a). Acetic anhydride (42 mL, 0.44 mol) was added dropwise to a mixture of **42** (69 g, 0.4 mol) and $NaHCO_3$ (34 g, 0.4 mol) in water (700 mL). After being stirred for 4 h at room temperature, the mixture was basified to pH 10 with K_2CO_3 and extracted with EtOAc. The extract was dried and concentrated to give **43a** (45 g, 62%). An analytical sample was obtained by recrystallization from EtOAc/hexane, mp 92–93 °C. IR: 3260, 2230, 1650 cm^{-1}. ^1H NMR: δ 1.92 (3H, s), 4.39 (2H, d, J = 6.0 Hz), 7.62 (1H, dd, J = 1.0, 7.7 Hz), 7.97 (1H, dd, J = 1.0, 7.7 Hz), 8.03 (1H, t, J = 7.7 Hz), 8.56 (1H, t, J = 6.0 Hz). Anal. ($C_9H_9N_3O$) C, H, N.

2-(Acetamidomethyl)-6-acetylpyridine (44a). An etheral solution of methyl magnesium bromide (3 M) (200 mL, 0.6 mol) was added dropwise to a solution of **43a** (35 g, 0.2 mol) in THF (525 mL) at 0–5 °C with stirring. After being stirred for 2 h at the same temperature, the reaction mixture was poured into ice-cold water (500 mL) and extracted four times with EtOAc/THF (1/1). The extracts were combined, dried, and concentrated to give **44a** (35 g, 92%). An analytical sample was obtained by recrystallization from EtOAc/hexane, mp 90–91 °C. IR: 3300, 1690, 1650 cm^{-1}. ^1H NMR: δ 1.94 (3H, s), 2.64 (3H, s), 4.43 (2H, d, J = 6.0 Hz), 7.54 (1H, dd, J = 1.0, 7.7 Hz), 7.82 (1H, dd, J = 1.0, 7.7 Hz), 8.00 (1H, t, J = 7.7 Hz), 8.53 (1H, t, J = 6.0 Hz). Anal. ($C_{10}H_{12}N_2O_2$) C, H, N.

4-[6-(Acetamidomethyl)pyridin-2-yl]-2-guanidinothiazole Dihydrochloride (8). Method A. A solution of bromine (6.7 mL, 0.13 mol) in AcOH (50 mL) was added dropwise to a solution of **44a** (25 g, 0.13 mol) and a 30% HBr/AcOH solution (55.7 mL, 0.26 mol) in AcOH (500 mL)/MeOH (125 mL) at room temperature with stirring. After being stirred at 60 °C for 2 h, the solution was concentrated to dryness. The residue was basified to pH 9 with 20% aqueous K_2CO_3 and extracted twice with EtOAc/THF (1/1). The extracts were combined, dried, and concentrated to give an oil (29.4 g). A solution of the oil obtained and amidinothiourea (8.85 g, 75 mmol) in Me_2CO (210 mL) was refluxed for 1 h with stirring. After removal of the solvent, the residue was dissolved in water (60 mL). The solution was basified to pH 10 with 20% aqueous K_2CO_3 and extracted with EtOAc/THF (1/1). The extract was dried and concentrated to give a residue, which was chromatographed on alumina eluting with $CHCl_3$/MeOH (9/1). The free base obtained was converted to the dihydrochloride in the usual manner, and the salt was recrystallized from MeOH/IPE to afford **8** (5.6 g, 21%). IR: 3340, 3160, 1705, 1650 cm^{-1}. ^1H NMR: δ 1.96 (3H, s), 4.56 (2H, d, J = 6.0 Hz), 7.45 (1H, d, J = 7.5 Hz), 8.10 (1H, t, J = 7.5 Hz), 8.24 (1H, d, J = 7.5 Hz), 8.25 (1H, s), 8.46 (4H, br s), 8.74 (1H, t, J = 6.0 Hz).

4-[6-(Aminomethyl)pyridin-2-yl]-2-guanidinothiazole Trihydrochloride (46). A solution of **8** (12.0 g, 41 mmol) and concentrated HCl (34 mL, 410 mmol) in EtOH (120 mL) was refluxed for 9 h. After cooling in an ice bath, the resulting precipitate was collected by filtration and recrystallized from aqueous EtOH to give **46** (13.0 g, 88%), mp 288–289 °C. IR: 3375, 3275, 3175, 1685 cm^{-1}. ^1H NMR: δ 4.25 (2H, q, J = 5.8 Hz), 6.12 (3H, br s), 7.46 (1H, d, J = 7.7 Hz), 7.95 (1H, t, J = 7.7 Hz), 8.18 (1H, d, J = 7.7 Hz), 8.33 (1H, s), 8.41 (4H, s), 8.62 (2H, br s). Anal. ($C_{10}H_{15}Cl_3N_6S$). The trihydrochloride was treated with 20% aqueous K_2CO_3, and the product obtained was recrystallized from aqueous MeOH to afford the free base of **46**, mp 228–229 °C. IR: 3350, 3150 cm^{-1}. ^1H NMR: δ 3.85 (2H, s), 6.96 (4H, br s), 7.31 (1H, dd, J = 2.5, 6.1 Hz), 7.41 (1H, s), 7.74 (1H, d, J = 2.5 Hz), 7.76 (1H, d, J = 6.1 Hz). Anal. ($C_{19}H_{12}N_6S$).

4-[6-(Formamidomethyl)pyridin-2-yl]-2-guanidinothiazole (7). Method B_1. A mixture of formic acid (0.47 mL, 13 mmol) and acetic anhydride (0.87 mL, 9 mmol) was stirred for 30 min at 40–50 °C. After cooling in an ice bath, the mixture was added to a solution of **46** (3.0 g, 8 mmol) and triethylamine (3.5 mL, 25 mmol) in DMF (45 mL) and the resulting mixture was stirred for 5 h at room temperature. The reaction solution was mixed with water and extracted with EtOAc/THF. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on alumina eluting with $CHCl_3$/MeOH (9/1) and recrystallized from aqueous MeOH to give **7** (0.62 g, 27%). IR: 3420, 3350, 1685 cm^{-1}. ^1H NMR: δ 4.43 (2H, d, J = 5.9 Hz), 6.93 (4H, s), 7.19 (1H, t, J = 4.5 Hz), 7.47 (1H, s), 7.82 (2H, d, J = 4.5 Hz), 8.21 (1H, s), 8.63 (1H, t, J = 5.9 Hz).

4-[6-[[(Cycloheptylcarbonyl)amino]methyl]pyridin-2-yl]-2-guanidinothiazole (17). Method B_2. A solution of **46** (0.8 g, 2.2 mmol), cycloheptanecarboxylic acid (0.4 mL, 2.9 mmol), 1-hydroxybenzotriazole monohydrate (0.4 g, 2.9 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (0.56 g, 2.9 mmol), and triethylamine (0.9 mL, 6.6 mmol) in DMF (10 mL) was stirred for 2 h at room temperature. After the reaction solution was poured into water, the mixture was basified to pH 10 with 20% aqueous K_2CO_3 and extracted with EtOAc. The extract was dried and concentrated to give a residue, which was recrystallized from EtOAc/DMF to afford **17** (0.51 g, 61%). IR: 3410, 3320, 1650 cm^{-1}. ^1H NMR: δ 1.30–1.95 (12H, m), 2.40 (1H, quint, J = 7.8 Hz), 4.35 (2H, d, J = 5.9 Hz), 6.93 (4H, s), 7.11 (1H, dd, J = 3.4, 4.5 Hz), 7.38 (1H, s), 7.75 (1H, d, J = 4.5 Hz), 7.77 (1H, d, J = 3.4 Hz), 8.31 (1H, t, J = 5.9 Hz).

4-[6-[(2-Furoylamino)methyl]pyridin-2-yl]-2-guanidinothiazole Dihydrochloride (24). Method B_3. 2-Furoyl chloride (0.66 mL, 6.7 mmol) was added to a solution of **46** (2.0 g, 5.6 mmol) and triethylamine (3.4 mL, 25 mmol) in DMF (40 mL) at 0–5 °C. After being stirred for 6 h at the same temperature, the mixture was poured into water and extracted with EtOAc. The extract was dried and concentrated to give a residue, which was converted to the dihydrochloride in the usual manner, and the salt was recrystallized from aqueous isopropyl alcohol to afford **24** (1.6 g, 48%). IR: 3380, 1690 cm^{-1}. ^1H NMR: δ 4.73 (2H, d, J = 5.9 Hz), 6.67 (1H, dd, J = 1.8, 3.5 Hz), 7.26 (1H, d, J = 3.5 Hz), 7.48 (1H, d, J = 7.8 Hz), 7.91 (1H, d, J = 1.8 Hz), 8.09 (1H, t, J = 7.8 Hz), 8.22 (1H, s), 8.25 (1H, d, J = 7.8 Hz), 8.48 (4H, s), 9.24 (1H, t, J = 5.9 Hz).

2-Guanidino-4-[6-[(trifluoroacetamido)methyl]pyridin-2-yl]thiazole (18). Method B. Trifluoroacetic anhydride (2.4 mL, 17 mmol) was added dropwise to a solution of **46** (3.0 g, 8.4 mmol) and triethylamine (4.7 mL, 34 mmol) in DMF (60 mL) at 0–5 °C. After being stirred for 20 h at room temperature, the reaction mixture was poured into water, basified to pH 9.5 with 20% aqueous K_2CO_3, and extracted with EtOAc/THF. The extract was dried and concentrated to give a residue, which was recrystallized from MeOH/dioxane/IPE to afford **18** (0.74 g, 26%). IR: 3430, 3320, 1705, 1650 cm^{-1}. ^1H NMR: δ 4.54 (2H, d, J = 5.8 Hz), 6.94 (4H, s), 7.18 (1H, t, J = 4.3 Hz), 7.35 (1H, s), 7.83 (2H, d, J = 4.3 Hz), 10.05 (1H, t, J = 5.8 Hz).

2-Guanidino-4-[6-[[(methylsulfonyl)amino]methyl]pyridin-2-yl]thiazole (25). Method C. Methylsulfonyl chloride (0.58 mL, 7.6 mmol) was added dropwise to a solution of the free base of **46** (1.8 g, 5 mmol) and pyridine (0.81 mL, 10 mmol) in CH_2Cl_2 (40 mL) at 0–5 °C. After being stirred for 23 h at room temperature, the reaction mixture was poured into water, basified to pH 9.5 with 20% aqueous K_2CO_3, and extracted with EtOAc/THF. The extract was dried and concentrated to give a residue, which was recrystallized from aqueous MeOH to afford **25** (0.40 g, 24%). IR: 3475, 3380, 1640, 1300, 1145 cm^{-1}. ^1H NMR: δ 2.96 (3H, s), 4.31 (2H, s), 6.93 (4H, s), 7.35 (1H, dd, J = 3.1, 4.6 Hz), 7.46 (1H, s), 7.66 (1H, br s), 7.81 (1H, d, J = 4.6 Hz), 7.83 (1H, d, J = 3.1 Hz).

2-Guanidno-4-[6-(ureidomethyl)pyridin-2-yl]thiazole Dihydrochloride (26). Method D_1. A solution of the free base of **46** (0.64 g, 2.6 mmol), potassium cyanate (0.21 g, 2.6 mmol), and 1 N HCl (5.2 mL) in water (6.4 mL) was stirred for 19 h at room temperature. The mixture was basified to pH 10 with 20% aqueous K_2CO_3, and the resulting precipitate was collected by filtration. The basic material obtained was converted to the dihydrochloride in the usual manner, and the salt was recrystallized from aqueous MeOH to give **26** (0.45 g, 48%). IR: 1705,

1650 cm^{-1}. ^{1}H NMR: δ 4.57 (2H, s), 7.64 (1H, d, J = 7.5 Hz), 8.30 (1H, t, J = 7.5 Hz), 8.39 (1H, d, J = 7.5 Hz), 8.51 (10H, s).

2-Guanidino-4-[6-[(3-methylureido)methyl]pyridin-2-yl]-thiazole Dihydrochloride (27). Method D$_3$. A suspension of **46** (1.2 g, 3.4 mmol), methyl isocyanate (0.24 mL, 4 mmol), and triethylamine (1.4 mL, 10 mmol) in THF (18 mL)/MeOH (6 mL) was stirred for 1 h at room temperature. After the reaction solution was poured into water, the mixture was basified to pH 9.5 with 20% aqueous K_2CO_3 and extracted with EtOAc. The extract was dried and concentrated to give a residue, which was converted to the dihydrochloride in the usual manner, and the salt was recrystallized from MeOH/IPE to afford **27** (0.7 g, 55%). IR: 3310, 1680, 1660 cm^{-1}. ^{1}H NMR: δ 2.62 (3H, s), 4.53 (2H, s), 7.59 (1H, d, J = 7.0 Hz), 8.24 (1H, t, J = 7.0 Hz), 8.35 (1H, d, J = 7.0 Hz), 8.44 (1H, s), 8.50 (4H, s).

4-[6-[(2-Cyano-3-methylguanidino)methyl]pyridin-2-yl]-2-guanidinothiazole (30). Method E. A suspension of the free base of **46** (0.5 g, 2 mmol) and dimethyl N-cyanodithioiminocarbonate (0.29 g, 2 mmol) in EtOH (10 mL) was refluxed for 1 h with stirring. After removal of the solvent, 40% methanolic methylamine (1.6 mL) and DMF (10 mL) were added to the residue and the mixture was stirred for 5 h at 60 °C. The solvent was evaporated, and the residue was mixed with water. The resulting precipitate was collected by filtration and recrystallized from aqueous DMF to give **30** (0.42 g, 64%). IR: 3440, 3400, 3290, 2170, 1630 cm^{-1}. ^{1}H NMR: δ 2.76 (3H, d, J = 4.5 Hz), 4.46 (2H, d, J = 5.5 Hz), 6.93 (4H, s), 7.16 (1H, d, J = 7.9 Hz), 7.25 (1H, q, J = 4.5 Hz), 7.42 (1H, s), 7.57 (1H, t, J = 5.5 Hz), 7.80 (1H, d, J = 7.9 Hz), 7.85 (1H, d, J = 7.9 Hz).

4-[6-[[[[(Ethylsulfonyl)imino]methyl]amino]methyl]-pyridin-2-yl]-2-guanidinothiazole (33). Method F. A solution of **46** (3.0 g, 8.5 mmol), ethyl (ethylsulfonyl)formimidate (1.5 g, 9.2 mmol), and triethylamine (3.5 mL, 25 mmol) in MeOH (60 mL) was stirred for 6.5 h at room temperature. After removal of the solvent, the residue was mixed with water. The mixture was basified to pH 9.5 with 20% aqueous K_2CO_3 and extracted with EtOAc. The extract was dried and concentrated to give a residue, which was chromatographed on silica gel eluting with $CHCl_3$/MeOH (9/1) and recrystallized from MeOH/dioxane/IPE to give **33** (0.88 g, 29%). IR: 3390, 3270, 1640, 1310, 1125 cm^{-1}. ^{1}H NMR: δ 1.10 (3H, t, J = 7.3 Hz), 2.92 (2H, q, J = 7.3 Hz), 4.59 (2H, d, J = 5.4 Hz), 6.94 (4H, s), 7.25 (1H, t, J = 4.5 Hz), 7.53 (1H, s), 7.84 (2H, d, J = 4.5 Hz), 8.15 (1H, d, J = 4.9 Hz), 9.15–9.23 (1H, m).

4-[6-[2-Amino-2-[(aminosulfonyl)imino]ethyl]pyridin-2-yl]-2-guanidinothiazole (34). Method G. (1) A solution of 4-[(6-cyanomethyl)pyridin-2-yl]-2-guanidinothiazole (**50**) (2.0 g, 7.7 mmol) in $CHCl_3$ (10 mL)/MeOH (10 mL) was saturated with HCl gas at 0–5 °C with stirring. After the mixutre was stirred for 3 h at the same temperature, IPE (20 mL) was added to the mixture and the resulting precipitate was collected by filtration. The filtered cake was mixed with a cold solution of K_2CO_3 (5.3 g, 38 mmol) in water (50 mL) and extracted with EtOAc/THF. The extract was washed with water, dried, and concentrated to give an imidate derivative (1.75 g, 78%), mp 172–173 °C. IR: 3310, 1650 cm^{-1}. ^{1}H NMR: δ 3.60 (3H, s), 3.74 (2H, s), 6.92 (4H, s), 7.20 (1H, dd, J = 3.4, 5.3 Hz), 7.36 (1H, s), 7.76 (1H, d, J = 5.3 Hz), 7.80 (1H, d, J = 3.4 Hz), 8.13 (1H, s). (2) A solution of the imidate obtained (1.6 g, 5.5 mmol) and sulfamide (2.1 g, 22 mmol) in 2-methoxyethanol (8 mL) was stirred at 70 °C for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The extract was dried and concentrated to give a residue, which was recrystallized from EtOAc/DMF to afford **34** (0.7 g, 27%). IR: 3420, 3350, 3230, 1650, 1300, 1125 cm^{-1}. ^{1}H NMR: δ 3.71 (2H, s), 6.56 (2H, s), 6.92 (4H, s), 7.25 (1H, t, J = 4.7 Hz), 7.42 (1H, s), 7.48 (1H, s), 7.90 (2H, d, J = 4.7 Hz), 8.42 (1H, s).

2-Acetamido-6-cyanopyridine (56a). This compound was prepared from **53**[15] in a manner similar to that described for **40**, yield 95%, mp 191–193 °C. IR: 3230, 2240, 1665 cm^{-1}. ^{1}H NMR: δ 2.12 (3H, s), 7.72 (1H, dd, J = 0.7, 7.5 Hz), 8.01 (1H, t, J = 7.5 Hz), 8.37 (1H, dd, J = 0.7, 7.5 Hz), 10.95 (1H, s). MS: m/z 161 (M$^+$). Anal. ($C_8H_7N_3O$) C, H, N.

2-(2-Acetamidoethyl)pyridine N-Oxide (55). Aqueous hydrogen peroxide (30%, 80.3 mL, 0.79 mol) was added dropwise to a solution of 2-(2-acetamidoethyl)pyridine (**54**) (64.5 g, 0.39 mol) in AcOH (65 mL) at 70–75 °C. After being stirred at the same temperature for 8 h, the reaction mixture was cooled to room temperature and poured into a solution of Na_2SO_3 (56.9 g, 0.47 mol) in water (200 mL) under cooling in an ice bath. After removal of the solvent, THF and $MgSO_4$ were added to the residue and the mixture was stirred for 30 min. The insoluble material was removed by filtration, and the filtrate was concentrated to give **55** (70.8 g, 100%) as an oil. IR: 1640 cm^{-1}. ^{1}H NMR: δ 1.76 (3H, s), 2.93 (2H, t, J = 6.7 Hz), 3.84 (2H, q, J = 6.7 Hz), 7.25–7.40 (3H, m), 7.99 (1H, t, J = 6.7 Hz), 8.24–8.32 (1H, m).

2-(2-Acetamidoethyl)-6-cyanopyridine (56b). A mixture of **55** (70.8 g, 0.39 mol) and dimethyl sulfate (41 mL, 0.43 mol) was stirred for 1.5 h at room temperature. After a solution of potassium cyanide (25.6 g, 0.39 mol) in DMSO (420 mL) was added, the mixture was stirred for an additional 3 h. The reaction mixture was poured into water and extracted with $CHCl_3$. The extract was dried and concentrated to give **56b** (74.3 g, 100%) as an oil. IR: 3270, 2230, 1660 cm^{-1}. ^{1}H NMR: δ 1.76 (3H, s), 2.92 (2H, t, J = 7.0 Hz), 3.35–3.50 (2H, m), 7.61 (1H, dd, J = 1.3, 7.6 Hz), 7.86–8.02 (3H, m). MS: m/z 189(M$^+$).

4-Acetyl-2-(ethoxycarbonyl)pyridine (59). Aqueous hydrogen peroxide (30%, 130 mL, 1.2 mol) was added dropwise to ethyl pyruvate (216 g, 1.9 mol) at −5–5 °C with stirring. The resulting solution was added dropwise to a mixture of 4-acetylpyridine (**58**) (15.0 g, 0.12 mol), concentrated H_2SO_4 (12.4 g, 0.12 mol), and ferrous sulfate heptahydrate (345 g, 0.12 mol) in CH_2Cl_2 (1.5 L)/water (100 mL) over a 2-h period at room temperature. After the mixture was stirred for 30 min, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The organic layer obtained above and the extract were combined, washed with 5% aqueous Na_2SO_3 followed by water, dried, and concentrated. The residue was chromatographed on silica gel eluting with EtOAc/toluene (1/20) to give **59** (5.79 g, 24%). An analytical sample was obtained by recrystallization from EtOAc/hexane, mp 45–46 °C. IR: 1715, 1690 cm^{-1}. ^{1}H NMR: δ 1.36 (3H, t, J = 7.1 Hz), 2.69 (3H, s), 4.40 (2H, q, J = 7.1 Hz), 8.08 (1H, dd, J = 1.7, 4.9 Hz), 8.35 (1H, d, J = 1.7 Hz), 8.95 (1H, d, J = 4.9 Hz). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

4-[(2-Ethoxycarbonyl)pyridin-4-yl]-2-guanidinothiazole (60). This compound was prepared from **59** in a manner similar to that described for **8**, yield 62%, mp 234–236 °C (aqueous DMF). IR: 3375, 1715 cm^{-1}. ^{1}H NMR: δ 1.36 (3H, t, J = 7.1 Hz), 4.38 (2H, q, J = 7.1 Hz), 6.99 (4H, br s), 7.73 (1H, s), 8.04 (1H, dd, J = 1.7, 5.1 Hz), 8.38 (1H, d, J = 1.7 Hz), 8.69 (1H, d, J = 5.1 Hz). Anal. ($C_{12}H_{13}N_5O_2S$) C, H, N.

4-[2-(Hydroxymethyl)pyridin-4-yl]-2-guanidinothiazole (61). Sodium borohydride (1.6 g, 42 mmol) was added in portions to a solution of **60** (4.2 g, 14 mmol) in THF (80 mL)/MeOH (8 mL) at room temperature with stirring. After the solution was refluxed for 4h, the solvent was evaporated and the residue was mixed with water. The insoluble material was collected by filtration and recrystallized from aqueous DMSO to give **61** (3.3 g, 94%), mp 260–261 °C. IR: 3400, 3100, 1650 cm^{-1}. ^{1}H NMR: δ 4.58 (2H, d, J = 5.7 Hz), 5.43 (1H, t, J = 5.7 Hz), 6.67 (4H, br s), 7.51 (1H, s), 7.64 (1H, dd, J = 1.5, 5.2 Hz), 7.84 (1H, d, J = 1.5 Hz), 8.46 (1H, d, J = 5.2 Hz).

Histamine-H$_2$-Receptor-Antagonist Activity (atrium).[20] The atrial strip isolated from guinea pig was suspended under an initial tension of 0.3–0.6 g in an organ bath containing Thyrode solution at 30 °C and aerated by 95% O_2–5% CO_2 gas. The beating rate and the amplitude of contraction of the atrium were recorded by means of a transducer and a polygraph. Histamine hydrochloride (1×10^{-6} g/mL) was added to the beating fluid, and the increase in the beating rate after dosing was measured. The addition of the test compounds (1×10^{-6} g/mL) was done 30 min after washing out the histamine hydrochloride. The percent inhibitory effect of the test compound was calculated by comparing histamine-induced increases in the beating rate before and 30 min after dosing with the test compounds. The IC$_{50}$ values, the dose (μM) required for 50% inhibition of the chronotropic response, and 95% confidence limits were estimated by Probit analysis from three doses. Three atria were used per dose.

Histamine-H$_2$-Receptor-Antagonist Activity (gastric gland). The gastric glands were prepared following the procedure of Berglindh *et al.*,[21a] and aminopyrine accumulation was performed accoridng to the method of Sack and Spenney.[21b] The corpus mucosa from a rabbit was subjected to collagenase

digestion, and gastric glands were isolated. An assay medium containing the gland suspension, histamine, the test drug, and [^{14}C]aminopyrine was incubated at 37 °C for 30 min, and the radioactivity accumulated in the glands was then measured. In the control experiment, buffer was added instead of a test drug. The pA_2 value was determined by the method of Arunlakshana and Schild[22] from three doses. Three preparations were used per dose.

Gastric Antisecretory Activity in Lumen-Perfused Rats.[23] Male Sprague–Dawley (SD) rats, weighing about 250 g, were used. Rats were deprived of food for 24 h. The animals were anaesthetized with 1.25 g/kg urethane intraperitoneally. The abdomen was opened, and the gastric lumen was perfused with saline throughout the experiment. The perfusate was titrated by an autotitrator with 25 mM NaOH as a titrant. Gastric secretion was stimulated by intravenous infusion with histamine (3 mg/kg/h). After reaching a plateu, the test compound (1 mg/kg) was given intravenously. The effect of the drug was expressed as the maximal inhibition by acid output.

Gastric Antisecretory Activity in Pylorus-Ligated Rats.[24] Male SD rats, weighing about 250 g, were deprived of food for 24 h. The pylorus of the stomach was ligated under ether anaesthesia. The test compound was administered intraduodenally just after the pyloric ligation. Four hours later, the animals were sacrificed and gastric contents were collected. The volumes of the samples were measured, and the acidity was titrated with 0.1 N NaOH to pH 7.0 using an automatic titrater. The total gastric acid outputs in the treated animals were compared with those in the control animals, and the percentage inhibition for each dose was calculated. The ED_{50} values, the dose required for 50% inhibition of the gastric secretion, and 95% confidence limits were estimated by Probit analysis from three doses. Ten animals were used per dose.

Gastric Antisecretory Activity in Heidenhain-Pouch Dogs. Beagle dogs, weighing 8–13 kg, were used for the study on gastric acid secretion. The animals were surgically provided with a vagally denervated Heidenhain pouch. One month later, the dogs were fasted overnight. Gastric acid secretion was stimulated by an intravenous infusion of tetragastrin (10 μg/kg/h). Gastric samples were collected at 15-min intervals. After its volume was almost constant, the test compound suspended in 0.1% methylcellulose (MC) was administered orally. Acid concentration was determined by titrating an aliquot to pH 7 with 0.1 N NaOH using an automatic titrater. The total acid output was calculated by multiplying the total volume of the gastric samples by the acid concentration, and the percentage of change of the total acid output was calculated by comparison with the predosing value of the test compound. The ED_{50} values, the dose required for 50% inhibition of the gastric secretion, and 95% confidence limits were estimated by Probit analysis from three or four doses. Three or four animals were used per dose.

Gastric Ulcer Induced by HCl–Aspirin. Male SD rats, weighing about 200 g, were fasted for 24 h before the experiment. A suspension of the test compound in 0.1% MC was administered orally to the group of seven rats. After 30 min, a suspension of aspirin (200 mg/kg) in 0.2 N HCl/0.1% MC solution (10 mL/kg) was given orally, and the animals were sacrificed 1 h later. The stomachs were isolated and cut open along the greater curvature. The ulcer index was calculated as the sum of the length (mm) of each ulcer in the stomach. The inhibitory ratio (%) was obtained by comparing the ulcer index with that of the control group. The ED_{50} values, the dose required for 50% inhibition of the ulcer index, and 95% confidence limits were estimated by Probit analysis from three doses. Ten animals were used per dose.

Gastric Ulcer Induced by HCl–Indomethacin. Male SD rats, weighing about 200 g, were fasted for 24 h before the experiment. A suspension of the test compound in 0.1% MC was administered orally to the group of 10 rats. After 30 min, a suspension of indomethacin (5 mg/kg) in 0.1% MC was given orally and 1 h later, additional 0.35 N HCl (5 mL/kg) was dosed orally. After 1 h, the animals were sacrificed. The stomachs were isolated and cut open along the greater curvature. The ulcer index was calculated as the sum of the length (mm) of each ulcer in the stomach. The inhibitory ratio (%) was obtained by comparing the ulcer index with that of the control group. The ED_{50} values, the dose required for 50% inhibition of the ulcer index, and 95% confidence limits were estimated by Probit analysis from three doses. Ten animals were used per dose.

Measurement of Gastric Mucosal Blood Flow.[25] The gastric mucosal blood flow was measured by the electrolytically generated hydrogen gas clearance method. Male SD rats, weighing about 250 g, were fasted for 24 h before the experiment and anaesthetized with urethane (1.25 g/kg) intraperitoneally. The abdomen was incised along the midline, and the pylorus was ligated. A needle-type platinum electrode was inserted into the corpus mucosal layer through a small incision in the forestomach. A suspension of the test drug in 0.1% MC was administered intragastrically to the group of five rats. After 15 min, a suspension of aspirin (200 mg/kg) in 0.2 N HCl/0.1% MC solution (0.2 mL) was given intragastrically. The gastric mucosal blood flow was measured 30 and 60 min after aspirin dosing. The results obtained are presented as the mean ± the standard error and the percent change from the initial value (15 min before the drug administration). Statistical analysis was performed with the Student's t test.

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