Synthesis and Structure–Activity Relationships of *N*-Substituted 2-[(2-Imidazolylsulfinyl)methyl]anilines as a New Class of Gastric H^+/K^+-ATPase Inhibitors

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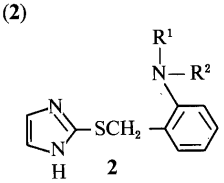
A series of *N*-substituted 2-[(2-imidazolylsulfinyl)methyl]anilines (3) was synthesized and evaluated for its biological activity against gastric H^+/K^+-ATPase prepared from rabbit stomach and gastric acid secretions in Heidenhain pouch dogs. Monoalkyl substituents on the nitrogen atom of the aniline moiety markedly inhibited the enzyme activity to the same degree as omeprazole, a representative H^+/K^+-ATPase inhibitor. Most of these compounds, administered at 3 mg/kg i.v. inhibited histamine-stimulated gastric acid secretion. The inhibitory activity of these derivatives on the enzymes at pH 6.0 was more potent than that at pH 7.4, and was distinctly correlated to stability in aqueous solution at pH 5.0.

Keywords 2-[(2-imidazolylsulfinyl)methyl]aniline; H^+/K^+-ATPase inhibitor; proton pump inhibitor; antisecretory effect; stability; structure–activity relationship

In recent years there has been considerable interest in gastric H^+/K^+-ATPase as the proton pump in parietal cells. This enzyme is responsible for the secretion of acid into the gastric lumen, and has thus been widely regarded as an important target for peptic ulcer therapy. Since the discovery of the enzyme inhibitors 2-[(2-benzimidazolylsulfinyl)-

TABLE I. *N*-Substituted 2-[(2-Imidazolylthio)methyl]anilines (**2**)



2

Compd. No.	R^1	R^2	Yield[a] (%)	Appearance	mp (°C)	HR-Mass Found	(Calcd)	^{1}H-NMR δ ($CDCl_3$), (J, Hz)
2a	H	H	81	Pale yellow powder	130—133	205.0677	(205.0674)	4.11 (2H, s), 6.4—7.1 (4H, m), 7.00 (2H, s)
2b	Me	H	66	White powder	173.5—175	219.0830	(219.0831)	2.84 (3H, s), 4.10 (2H, s), 6.3—7.2 (4H, m), 7.0 (2H, s)
2c	Me	Me	84	White powder	64—65	233.0986	(233.0987)	2.60 (6H, s), 4.22 (2H, s), 6.7—7.3 (6H, m)
2d	Et	H	100	Yellow powder	99.5—105	233.0989	(233.0987)	1.25 (3H, t, 7), 3.13 (2H, q, 7), 4.17 (2H, s), 6.3—7.3 (4H, m), 7.03 (2H, s)
2e	Me_2CH	H	59	Pale brown powder	125—126	247.1144	(247.1144)	1.19 (6H, d, 6), 3.4—3.8 (1H, m), 4.17 (2H, s), 6.3—7.2 (4H, m), 7.02 (2H, s)
2f	$Me(CH_2)_3$	H	71	White powder	95.5—97	261.1299	(261.1301)	0.7—1.8 (7H, m), 3.11 (2H, t, 7), 4.18 (2H, s), 6.3—7.2 (4H, m), 7.03 (2H, s)
2g	Me_2CHCH_2	H	94	White powder	106.5—107	261.1299	(261.1301)	0.88 (6H, d, 7), 1.84 (1H, m), 2.84 (2H, d, 7), 4.12 (2H, s), 6.2—7.1 (6H, m)
2h	$Me(CH_2)_4$	H	48	White powder	87	275.1457	(275.1457)	0.7—1.9 (9H, m), 3.11 (2H, t, 7), 4.19 (2H, s), 6.3—7.2 (4H, m), 7.03 (2H, s)
2i	$Me_2CHCH_2CH_2$	H	56	White powder	103—104	275.1455	(275.1457)	0.94 (6H, d, 6), 1.3—1.9 (3H, m), 3.13 (2H, t, 7), 4.19 (2H, s), 6.3—7.2 (4H, m), 7.03 (2H, s)
2j	Me_3CCH_2	H	43	White powder	127—128	275.1458	(275.1457)	1.00 (9H, s), 2.98 (2H, s), 4.20 (2H, s), 6.3—7.2 (4H, m), 7.02 (2H, s)
2k	$Me(CH_2)_5$	H	59	White powder	101	289.1617	(289.1614)	0.7—1.8 (11H, m), 3.10 (2H, t, 7), 4.18 (2H, s), 6.3—7.2 (4H, m), 7.03 (2H, s)
2l		H	61	White powder	93—97	259.1146	(259.1144)	0.1—0.4 (2H, m), 0.4—0.7 (2H, m), 0.8—1.3 (1H, m), 3.97 (2H, d, 7), 4.20 (2H, s), 6.4—7.3 (4H, m), 7.03 (2H, s)
2m		H	44	White powder	130—131.5	301.1616	(301.1614)	0.7—2.0 (11H, m), 2.97 (2H, d, 7), 4.20 (2H, s), 6.3—7.3 (4H, m), 7.03 (2H, s)
2n	$MeOCH_2CH_2$	H	88	Colorless prisms	73—74.5	263.1093	(263.1093)	3.44 (3H, s), 3.2—3.5 (2H, m), 3.6—3.8 (2H, m), 4.13 (2H, s), 6.4—7.2 (4H, m), 7.00 (2H, s)
2o	$EtOCH_2CH_2$	H	43	Pale yellow prisms	—[b]	—[b]	(277.1250)	1.22 (3H, t, 7), 3.37 (2H, t, 5), 3.61 (2H, q, 7), 3.78 (2H, t, 5), 4.13 (2H, s), 6.4—7.3 (4H, m), 6.99 (2H, s)
2p	$MeO(CH_2)_3$	H	66	Pale yellow oil		277.1252	(277.1250)	1.92 (2H, m), 3.23 (2H, t, 6), 3.32 (3H, s), 3.54 (2H, t, 6), 4.14 (2H, s), 6.3—7.2 (4H, m), 7.02 (2H, s)
2q	$Me_2CHOCH_2CH_2$	H	37	White powder	89.5—90.5	291.1406	(291.1406)	1.20 (6H, t, 6), 3.34 (2H, t, 5), 3.4—3.9 (3H, m), 4.12 (2H, s), 6.3—7.3 (4H, m), 6.98 (2H, s)
2r	$H_2C=CHCH_2$	H	79	White powder	103.5—105.5	245.0987	(245.0987)	3.76 (2H, dt, 2, 5), 4.19 (2H, s), 5.0—5.4 (2H, m), 5.6—6.1 (1H, m), 6.4—7.2 (4H, m), 7.04 (2H, s)
2s	$HC\equiv CCH_2$	H	71	Pale yellow powder	122—124	243.0831	(243.0831)	2.30 (1H, t, 2), 3.99 (2H, d, 2), 4.13 (2H, s), 6.5—7.3 (4H, m), 7.01 (2H, s)[c]
2t	$PhCH_2$	H	61	White powder	113—115	295.1141	(295.1144)	4.23 (2H, s), 4.36 (2H, s), 6.4—7.6 (11H, m)
2u	Thenyl	H	69	White powder	121—122	301.0707	(301.0708)	4.20 (2H, s), 4.55 (2H, s), 6.4—7.3 (7H, m), 6.95 (2H, s)
2v	Ph	H	68	Yellow prisms	126—128	281.0982	(281.0987)	4.23 (2H, s), 6.6—7.3 (9H, m), 7.03 (2H, s)

a) Yields from alcohol (**1**) to sulfide (**2**) have not been optimized. *b*) Melting point and HR-Mass for this compound was not determined. *c*) $CDCl_3 : CD_3OD = 3 : 1$.

TABLE II. *N*-Substituted 2-[(2-Imidazolylsulfinyl)methyl]anilines (**3**): Synthesis

Compd. No.	Method[a]	Yield[b] (%)	Appearance	Recrystn.[c] solv.	mp[d] (°C)	IR ν cm^{-1} (S→O)	Formula	Analysis (%) Found (Calcd) C	H	N	^{1}H-NMR δ (J, Hz)	Solvent[e]
3a	A	13	Pale brown powder	a	170—172	1035	$C_{10}H_{11}N_3OS$	54.45 (54.28)	4.84 5.01	18.88 18.99)	4.44 (2H, s), 5.16 (2H, br), 6.2—7.1 (4H, m), 7.26 (2H, s)	B
3b	A	66	Pale yellow prisms	a	168	1040	$C_{11}H_{13}N_3OS$	56.33 (56.15	5.47 5.57	17.71 17.86)	2.67 (3H, s), 4.37 (1H, d, 14), 4.52 (1H, d, 14), 5.60 (1H, br), 6.2—7.6 (6H, m)	C
3c	A	44	Colorless prisms	a	115—117	1005	$C_{12}H_{15}N_3OS$	57.78 (57.81	6.22 6.06	16.59 16.85)	2.66 (6H, s), 4.50 (1H, d, 12), 4.73 (1H, d, 12), 6.8—7.4 (4H, m), 7.22 (2H, s)	A
3d	B	82	Yellow prisms	b	145—146.5	995	$C_{12}H_{15}N_3OS$	57.65 (57.81	5.91 6.06	16.86 16.85)	1.27 (3H, t, 7), 3.11 (2H, q, 7), 4.32 (1H, d, 14), 4.52 (1H, d, 14), 6.4—7.3 (4H, m), 7.23 (2H, s)	B
3e	B	61	Pale brown powder	c	130—132	1040	$C_{13}H_{17}N_3OS$	59.22 (59.29	6.54 6.51	15.86 15.96)	1.21 (6H, d, 6), 3.60 (1H, m), 4.24 (1H, d, 14), 4.51 (1H, d, 14), 6.4—7.3 (4H, m), 7.21 (2H, s)	B
3f	A	46	Pale brown powder	a	136—139	1040	$C_{14}H_{19}N_3OS$	60.32 (60.62	6.98 6.90	14.97 15.15)	0.98 (3H, t, 6), 1.2—1.9 (4H, m), 3.07 (2H, t, 6), 4.29 (1H, d, 14), 4.52 (1H, d, 14), 6.4—7.3 (4H, m), 7.23 (2H, s)	B
3g	A	57	Colorless prisms	a	132—133	1020	$C_{14}H_{19}N_3OS$	60.47 (60.62	6.87 6.90	15.25 15.15)	1.02 (6H, d, 7), 1.94 (1H, m), 2.90 (2H, d, 7), 4.32 (1H, d, 13), 4.54 (1H, d, 13), 6.4—7.3 (4H, m), 7.24 (2H, s)	B
3h	B	63	White powder	a	137—138	1040	$C_{15}H_{21}N_3OS$	61.76 (61.82	7.22 7.26	14.52 14.42)	0.8—1.9 (9H, m), 3.06 (2H, t, 7), 4.31 (1H, d, 14), 4.52 (1H, d, 14), 6.4—7.3 (4H, m), 7.24 (2H, s)	B
3i	A	55	Pale brown powder	a	150—151	995	$C_{15}H_{21}N_3OS$	61.92 (61.82	7.25 7.26	14.17 14.42)	0.98 (6H, d, 6), 1.4—2.0 (3H, m), 3.08 (2H, t, 7), 4.31 (1H, d, 14), 4.53 (1H, d, 14), 6.4—7.3 (4H, m), 7.24 (2H, s)	B
3j	A	41	Pale yellow powder	d	133	1005	$C_{15}H_{21}N_3OS$	61.90 (61.82	7.35 7.26	14.28 14.42)	0.99 (9H,s), 2.79 (2H, d, 6), 4.25 (1H, d, 14), 4.62 (1H, d, 14), 6.4—7.3 (6H, m)	A
3k	B	69	White powder	c	151—154	1035	$C_{16}H_{23}N_3OS$	63.10 (62.92	7.52 7.59	13.55 13.76)	0.7—1.9 (11H, m), 3.06 (2H, t, 7), 4.30 (1H, d, 14), 4.53 (1H, d, 14), 6.4—7.3 (4H, m), 7.23 (2H, s)	B
3l	A	58	Pale yellow powder	c	138—138.5	1020	$C_{14}H_{17}N_3OS$	61.11 (61.06	6.27 6.22	15.13 15.26)	0.1—0.7 (4H, m), 0.9—1.2 (1H, m), 2.92 (2H, d, 7), 4.32 (1H, d, 14), 4.56 (1H, d, 14), 6.4—7.3 (4H, m), 7.22 (2H, s)	A
3m	B	46	White powder	c	145—146	1010	$C_{17}H_{23}N_3OS$	64.34 (64.32	7.47 7.30	13.06 13.24)	0.8—2.0 (11H, m), 2.91 (2H, d, 6), 4.25 (1H, d, 14), 4.53 (1H, d, 14), 6.4—7.3 (4H, m), 7.22 (2H, s)	B
3n	B	50	Colorless prisms	c	126—128	1000	$C_{13}H_{17}N_3O_2S$	55.93 (55.89	6.10 6.13	14.88 15.04)	3.0—3.4 (2H, m), 3.34 (3H, s), 3.44—3.70 (2H, m), 4.26 (1H, d, 14), 4.56 (1H, d, 14), 6.4—7.3 (6H, m)	B
3o	B	52	White powder	c	119—121	1040	$C_{14}H_{19}N_3O_2S$	57.19 (57.31	6.59 6.53	14.16 14.32)	1.24 (3H, t, 7), 3.28 (2H, t, 5), 3.59 (2H, q, 7), 3.69 (2H, t, 5), 4.31 (1H, d, 13), 4.53 (1H, d, 13), 6.4—7.3 (6H, m)	B
3p	A	31	Yellow prisms	d	100—107	1000	$C_{14}H_{19}N_3O_2S$	57.25 (57.31	6.44 6.53	14.54 14.32)	1.7—2.0 (2H, m), 2.9—3.2 (2H, m), 3.33 (3H, s), 3.49 (2H, t, 6), 4.24 (1H, d, 14), 4.54 (1H, d, 14), 6.4—7.3 (6H, m)	A
3q	B	76	White powder	c	140—142	1035	$C_{15}H_{21}N_3O_2S$	58.85 (58.61	6.94 6.89	13.33 13.67)	1.21 (6H, d, 6), 3.26 (2H, t, 5), 3.5—3.8 (3H, m), 4.31 (1H, d, 14), 4.54 (1H, d, 14), 6.4—7.3 (4H, m), 7.21 (2H, s)	B
3r	A	46	Colorless prisms	e	139	1000	$C_{13}H_{15}N_3OS$	59.90 (59.75	5.63 5.79	16.03 16.08)	3.72 (2H, d, 6), 4.31 (1H, d, 14), 4.53 (1H, d, 14), 4.9—5.4 (2H, m), 5.7—6.2 (1H, m), 6.4—7.4 (4H, m), 7.21 (2H, s)	B
3s	A	37	Pale yellow powder	c	158—159	1025	$C_{13}H_{13}N_3OS$	60.16 (60.21	4.91 5.05	15.99 16.20)	2.32 (1H, t, 3), 3.99 (2H, d, 3), 4.29 (1H, d, 13), 4.53 (2H, d, 13), 6.5—7.3 (4H, m), 7.22 (2H, s)	B
3t	A	43	Pale brown powder	a	135—138	1030	$C_{17}H_{17}N_3OS$	65.77 (65.57	5.48 5.50	13.63 13.49)	4.32 (2H, d, 6), 4.57 (2H, s), 6.2—7.5 (11H, m)	C
3u	A	72	White powder	f	137—139	1020	$C_{15}H_{15}N_3OS_2$	56.67 (56.76	4.47 4.76	13.00 13.24)	4.35 (1H, d, 14), 4.56 (1H, d, 14), 4.51 (2H, s), 6.4—7.3 (9H,m)	B
3v	A	42	Pale yellow powder	c	133—135	1025	$C_{16}H_{15}N_3OS$	63.87 (64.62	5.25 5.08	14.07 14.13)	4.33 (1H, d, 14), 4.62 (1H, d, 14), 6.6—7.4 (11H, m)	A

a) Method A: *m*-CPBA oxidation, method B: H_2O_2/NH_4VO_3 oxidation. *b*) Yields from sulfide have not been optimized. *c*) a, this compound was crystallized with addition of aqueous NH_4Cl solution to the NaOH solution of this compound; b, dichloromethane–ethanol; c, chloroform–ether; d, acetonitrile–ether; e, chloroform–acetonitrile; f, chloroform–hexane. *d*) Compounds decompose on melting; clearly defined melting points are not always obtainable. *e*) As the solvent of ^{1}H-NMR: A: $CDCl_3$, B: $CDCl_3/CD_3OD$, C: DMSO-d_6.

methyl]pyridines, represented by omeprazole,[1] there have been reports of many analogues with benzimidazole and pyridine moieties.[2]

In the replacement of the pyridine ring with other heterocycles, we previously reported the biochemical and pharmacological activities of *N*,*N*-dimethyl-2-[(2-benzimidazolylsulfinyl)methyl]anilines (NC-1300)[3] and *N*,*N*-dimethyl-2-[[2-(5-methoxy)benzimidazolylsulfinyl]-methyl]anilines (NC-1300-B)[4]; and Adelstein also reported those of *N*-unsubstituted 2-[[2-(5-methoxy)benzimidazolylsulfinyl]methyl]anilines.[5]

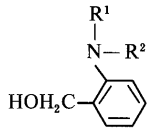
To obtain more potent compounds, we replaced the benzimidazole ring of NC-1300 with many other heterocycles. The fact that aniline (pK_a 4.60) is less basic than pyridine (pK_a 5.22) drew our attention to the imidazole ring (pK_a 6.95), which is a more basic heterocycle than benzimidazole (pK_a 5.48) coupled with the aniline ring.

In this paper, we report the synthesis and the pharmacological activities of *N*-substituted 2-[(2-imidazolylsulfinyl)methyl]anilines (**3**) as a new class of potent inhibitors of gastric H^+/K^+-ATPase. We also discuss the relationships between H^+/K^+-ATPase inhibitory activity (pH 6.0 and 7.4), antisecretory effects and stability in aqueous solutions (pH 3.0, 5.0, 7.0).

Synthesis *N*-Substituted 2-chloromethylaniline hydrochloride derivatives prepared by the chlorination of corresponding benzyl alcohols (**1**) with $SOCl_2$ in methylenechloride or gaseous HCl in ethanol were unstable and used without isolation. Condensation of 2-mercaptoimidazole with *N*-substituted 2-chloromethylanilines under neutral or acidic conditions gave the corresponding sulfides (**2**). The condensation reaction was carried out in ethanol and the sulfides (**2**) were isolated as the free bases by neutralization of the resultant hydrochlorides with aqueous Na_2CO_3 followed by extraction with chloroform or methylenechloride, as summarized in Table I.

Oxidation of the sulfides (**2**) with *m*-chloroperbenzoic acid (*m*-CPBA) in chloroform or methylenechloride at 0 °C gave sulfoxides (**3**) (method A). As an alternative method, sulfide was oxidized using H_2O_2 catalyzed by NH_4VO_3 in a mixed solvent (CH_2Cl_2–MeOH–AcOH) (method B). In the latter

TABLE III. *N*-Substituted 2-Aminobenzyl Alcohol (**1**)



Compd. No.	R^1	R^2	Method	Yield	
				Step 1	Step 2
1b	Me	H	—	—[a]	100
1c	Me	Me	—	—[a]	100
1d	Et	H	D	100	80
1e	Me_2CH	H	D	66	100
1f	$Me(CH_2)_3$	H	C	95	100
1g	Me_2CHCH_2	H	D	77	100
1h	$Me(CH_2)_4$	H	C	82	59
1i	$Me_2CHCH_2CH_2$	H	C	100	66
1j	Me_3CCH_2	H	C	98	95
1k	$Me(CH_2)_5$	H	C	97	97
1l		H	C	62	41
1m		H	C	55	97
1n	$MeOCH_2CH_2$	H	C	81	41
1o	$EtOCH_2CH_2$	H	C	100	90
1p	$MeO(CH_2)_3$	H	C	37	94
1q	$Me_2CHOCH_2CH_2$	H	C	68	100
1t	$PhCH_2$	H	C	91	99
1u	Thenyl	H	C	56	29

a) Starting compound is commercially available.

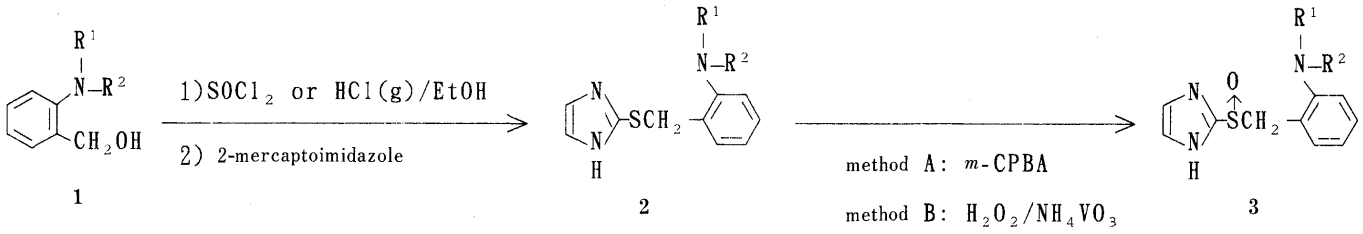


Chart 1

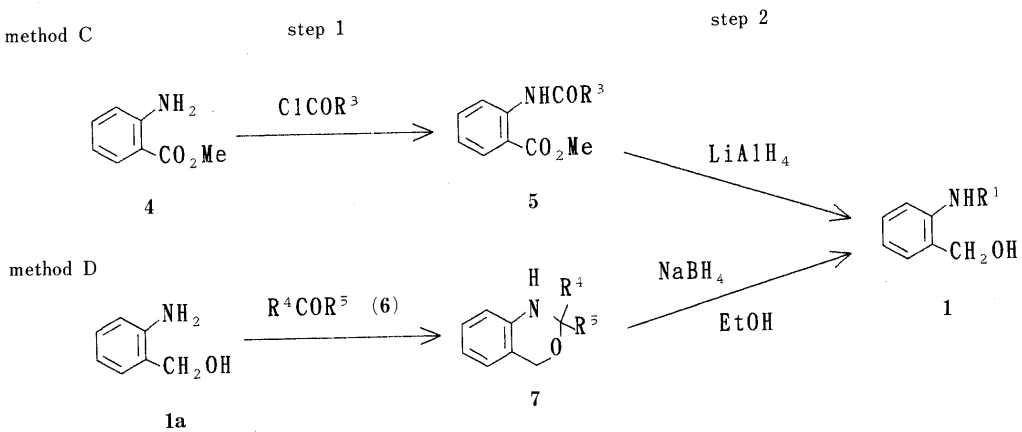
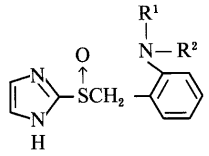


Chart 2

4 8 1r (19%) 1s (41%)

Chart 3

TABLE IV. *N*-Substituted 2-[(2-Imidazolylsulfinyl)methyl]anilines (**3**): Activity and Stability



3

Group	Compd. No.	R^1	R^2	H^+/K^+ ATPase IC_{50} (μM)		Heidenhain pouch dog inhibn. at i.v.	$T_{1/2}$ (h)		
				pH 6.0	pH 7.4	% (3 mg/kg)	pH 3.0	pH 5.0	pH 7.0
I	**3a**	H	H	>100	>100	NE	—	—	—
	3b	Me	H	2.8	44	82	0.04	0.93	35.1
	3c	Me	Me	29	>100	NE	—	—	—
II	**3d**	Et	H	14	100	80	0.06	1.07	41.2
	3e	Me_2CH	H	10	>100	66	0.07	0.88	52.0
	3f	$Me(CH_2)_3$	H	5.2	23	92	0.04	0.76	29.0
	3g	Me_2CHCH_2	H	12	100	81	0.03	0.93	63.0
	3h	$Me(CH_2)_4$	H	19	96	86	0.04	0.93	60.3
	3i	$Me_2CHCH_2CH_2$	H	4.4	19	93	0.04	0.94	62.5
	3j	Me_3CCH_2	H	6.8	63	95	0.02	0.81	51.6
	3k	$Me(CH_2)_5$	H	8.4	>100	NE	0.04	0.99	55.3
	3l		H	6.2	18	52	0.07	1.34	61.8
	3m		H	8.6	98	77	0.02	0.83	45.0
III	**3n**	$MeOCH_2CH_2$	H	43	>100	89	0.15	4.60	175.0
	3o	$EtOCH_2CH_2$	H	22	53	98	0.16	4.00	65.4
	3p	$MeO(CH_2)_3$	H	13	60	92	0.07	1.77	50.9
	3q	$Me_2CHOCH_2CH_2$	H	46	>100	97	0.11	4.58	179.5
IV	**3r**	$H_2C=CHCH_2$	H	18	40	94	0.08	2.88	106.3
	3s	$HC\equiv CCH_2$	H	>100	140	70	1.03	79.1	155.9
	3t	$PhCH_2$	H	100	>100	57	0.14	8.16	180.6
	3u	Thenyl	H	>100	80	49	0.28	27.7	94.4
	3v	Ph	H	>100	>100	NE	7.53	54.1	46.6
	Omeprazole			3.8	54	95	0.05	0.34	27.1
	NC-1300			6.6	20	22	0.06	0.26	3.8

NE, not effective.

oxidation method, the formation of by-products was minor or not observed except for the corresponding sulfone. The sulfones were removed by washing the reaction mixture with a dilute aqueous alkaline solution. Sulfoxides were purified by extraction with 1 N NaOH followed by the addition of aqueous NH_4Cl solution. This purification method is critical as these sulfoxides are unstable under acidic solutions. The yield and chemical data of sulfoxides (**3**) are summarized in Table II.

2-Mercaptoimidazole was synthesized by a previously reported method.[6] *N*-Substituted 2-aminobenzyl alcohols (**1**) were also synthesized by accepted or modified methods. Alkyl groups were introduced into the aniline nitrogen atom by the acylation of methyl anthranilate (**4**), followed by the reduction of the resultant amide with $LiAlH_4$ (method C) or the cyclization of 2-aminobenzyl alcohol (**1a**) with aldehydes or ketones (**6**)[7] and subsequent reduction of the resultant benzoxazine derivatives (**7**) with $NaBH_4$ in ethanol (method D) as shown in Chart 2. The yield of the synthesis of **1** is summarized in Table III.

N-Allyl and *N*-propargyl derivatives (**1r** and **1s**) were synthesized by the pathway shown in Chart 3. Reduction with $LiAlH_4$ of *N*-propargyl derivative (**8**) prepared by the condensation of methyl anthranilate (**4**) and propargyl bromide gave the mixture of **1r** (19%) and **1s** (41%).

Results and Discussion

Three 2-[(2-imidazolylsulfinyl)methyl]anilines in which the nitrogen atoms of aniline moiety were unsubstituted (**3a**) and substituted with either a monomethyl (**3b**) or a dimethyl group (**3c**) were initially synthesized and evaluated for inhibitory activity against gastric H^+/K^+-ATPase prepared from rabbit stomach. A significant difference was found in the inhibitory activities between the three compounds shown as group I in Table IV. The *N*-methyl compound (**3b**) had potent inhibitory activity similar to

that of omeprazole or NC-1300, while the activity of the *N,N*-dimethyl derivative (**3c**) was one tenth that of **3b**, whereas the *N*-unsubstituted compound (**3a**) showed only a very weak inhibitory effect.

The inhibitory effects of these compounds against histamine-stimulated gastric acid secretion were also evaluated *in vivo* with Heidenhain pouch dogs by intravenous administration (3 mg/kg). The *N*-methyl compound (**3b**) showed a potent effect but the other two compounds were inactive.

On the basis of these results, a variety of *N*-monosubstituted derivatives were then synthesized and evaluated for H^+/K^+-ATPase inhibitory activity. Compounds investigated were classified into groups depending upon the type of substituent at the nitrogen atom of the aniline moiety: alkyl substituent group (II), alkoxyalkyl substituent group (III), and the π-electron-possessing substituent group (IV), as shown in Table IV.

Group II and III compounds all inhibited H^+/K^+-ATPase at pH 6.0, while group IV compounds had little or no inhibitory activity, with the exception of the *N*-allyl derivative (**3r**).

The pH dependent stability of omeprazole analogous in an aqueous solution serves as a model for the rate of activation essential for H^+/K^+-ATPase inhibition at low pH and for undesired reactions with other enzymes, such as Na^+/K^+-ATPase, under neutral conditions.[8] Accordingly, the stability of these new compounds in aqueous solution was measured at pH 7.0, 5.0 and 3.0. Half-lives of the compounds in the respective solutions are also listed in Table IV. All compounds synthesized were very stable in an aqueous solution at pH 7.0 but unstable at pH 3.0. The stability at pH 5.0 differed markedly depending on the compound.

The compounds with potent inhibitory activity against H^+/K^+-ATPase had half-lives of less than 5 h in an aqueous solution at pH 5.0. In contrast, compounds with little or no activity on the enzyme were stable in aqueous solution at pH 5.0 and the half-lives were more than 8 h. The *N*-allyl derivative (**3r**) had a half-life of 2.88 h, while other group IV compounds were very stable. The half-lives of group II or III compounds in aqueous solution at pH 5.0 were correlated with the potency of *in vitro* H^+/K^+-ATPase inhibitory activity at pH 6.0 as shown in Fig. 1 ($r=0.89$, $p<0.001$).

The compounds with shorter half-lives had highly potent H^+/K^+-ATPase inhibitory activity at pH 6.0. Most compounds with an IC_{50} against H^+/K^+-ATPase inhibitory activity of less than 10 μM, had half-lives of less than 1 h at pH 5.0, except **31**, whose IC_{50} was 6.2 μM despite a half-life of 1.34 h. The data for the group II compounds are consistent with that of omeprazole and NC-1300.

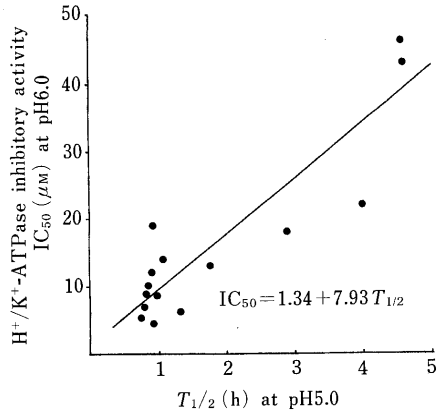


Fig. 1. Correlation of IC_{50} (pH 6.0) and $T_{1/2}$ (pH 5.0) for **3d**—**3r**
$r=0.89$, $p<0.001$.

Most group II and III compounds had good inhibitory effects against gastric acid secretion by intravenous administration (3 mg/kg), with the exception of the *n*-hexyl derivative (**3k**), although the potency differed somewhat depending on the compound. The difference in the potency of *in vivo* inhibitory activity against gastric acid secretion might be due to membrane permeability of the compounds or the ability of the compounds to reach the acidic compartments of the parietal cell.[9]

It is notable that compounds **3s**, **3t** and **3u** of group IV had moderate inhibitory effects against gastric acid secretion, whereas **3v** had no inhibitory effect. These compounds were very stable in aqueous solution at pH 5.0 and had little or no inhibitory effect in H^+/K^+-ATPase activity at pH 6.0.

Omeprazole is transformed under acidic conditions to give an active inhibitor, a cyclic sulfenamide.[10] An analogous mechanism of the inhibitory effect of H^+/K^+-ATPase activity for the new imidazole derivatives is suggested, based on the fact that the H^+/K^+-ATPase inhibitory activity of each compound at pH 6.0 was always more potent than that at pH 7.4 and that the lower stability in a solution at pH 5.0 was in proportion to higher inhibitory activity.

The *in vivo* inhibitory effect of **3s**, **3t** and **3u** against gastric acid secretion may be due to transformation into an active form under more acidic conditions in the parietal cell. In fact, **3s**, **3t** and **3u** were very unstable at pH 3.0 whereas **3v** was stable even at pH 3.0, as shown in Table IV.

These results show that *N*-alkyl or *N*-alkoxyalkyl 2-[(2-imidazolylsulfinyl)methyl]anilines in group II or III comprise a new class of H^+/K^+-ATPase inhibitors and that some of them might be candidates for antisecretory agents.

Experimental

Melting points determined with a Yamato MP-21 apparatus were uncorrected. Infrared (IR) spectra were recorded on a Hitachi 260-50 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL JNM-FX90Q NMR spectrometer with tetramethylsilane as the internal standard. High resolution mass spectra (MS) were obtained on a JEOL JMS-SX102 mass spectrometer. Elemental analyses (C, H, N) were performed on a Heraus C,H,N-Rapid instrument.

2-Aminobenzyl alcohol (**1a**) was commercially available. 2-Methylaminobenzyl alcohol (**1b**), 2-dimethylaminobenzyl alcohol (**1c**), and 2-phenylaminobenzyl alcohol (**1v**) were prepared by the reduction with $LiAlH_4$ of the corresponding benzoates which were commercially available.

Preparation of *N*-Substituted 2-Aminobenzyl Alcohol by Method C Step 1: Trimethylacetyl chloride (10.8 g, 89.6 mmol) and 12.3 g (89.6 mmol) of K_2CO_3 were added to a solution of 12.2 g (80.6 mmol) of methyl anthranilate (**4**) in 100 ml of benzene. The mixture was heated under reflux overnight, then cooled and poured into cold water (about 0 °C). The benzene layer was separated, washed with 6 N HCl and $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to yield 18.6 g (98%) of methyl *N*-trimethylacetylanthranilate (**5j**) as a pale brown oil. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (9H, s), 3.92 (3H, s), 7.04 (1H, dt, $J=1$, 8 Hz), 7.52 (2H, dt, $J=2$, 8 Hz), 8.02 (1H, dd, $J=2$, 8 Hz), 8.77 (1H, dd, $J=1$, 8 Hz). Exact MS *m/z* Calcd for $C_{13}H_{17}NO_3$: 235.1209. Found: 235.1207.

Step 2: A solution of 10.0 g (42.6 mmol) of **5j** in 20 ml of dry tetrahydrofuran (THF) (Al_2O_3) was added dropwise to a suspension of

3.2 g (85.2 mmol) of $LiAlH_4$ in 100 ml of dry THF (Al_2O_3) at 0 °C during a period of over 30 min. The mixture was then stirred for 30 min and heated under reflux for 1 h. The reaction mixture was cooled and saturated aqueous Na_2SO_4 was added dropwise at 0 °C. After Et_2O (100 ml) was added to the mixture, the precipitate was filtered and the filtrate was evaporated *in vacuo* to yield 7.8 g (95%) of 2-neopentylaminobenzyl alcohol (**1j**) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (9H, s), 2.90 (2H, s), 4.63 (2H, s), 6.5—7.3 (4H, m). Exact MS *m/z* Calcd for $C_{12}H_{19}NO$: 193.1468. Found: 193.1469.

Compounds **1f**, **h**, **i**, **k**—**q**, **t** and **u** were obtained by a similar procedure to that described for **1j**. The yields are listed in Table III.

Preparation of *N*-Substituted 2-Aminobenzyl Alcohol by Method D Step 1: Isobutylaldehyde (19.8 g, 275 mmol) and 12.3 g of $CaCl_2$ were added to 24.6 g (200 mmol) of 2-aminobenzyl alcohol (**1a**) in 123 ml of CH_2Cl_2. The mixture was stirred for 1 h and the precipitate was filtered through celite. The filtrate was evaporated *in vacuo* and the residue was crystallized by the addition of 75 ml of hexane to yield 27.0 g (77%) of 1,2-dihydro-2-isopropyl-4*H*-3,1-benzoxazine (**7g**) as a pale brown solid. ^{1}H-NMR ($CDCl_3$) δ: 1.05 (6H, d, J=7 Hz), 1.7—2.1 (1H, m), 3.84 (1H, br), 4.2—4.4 (1H, m), 4.76 (1H, d, J=14 Hz), 4.95 (1H, d, J=14 Hz), 6.5—7.2 (4H, m). IR (KBr): 3290, 1580, 1480, 1250, 1080, 1040, 1020, 980, 885, 745 cm^{-1}. *Anal.* Calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53; N, 7.90%. Found: C, 74.36; H, 8.78; N, 8.05.

Step 2: Sodium borohydride (14.45 g, 380 mmol) was added in four portions over 4 h to a solution of 27.0 g (152 mmol) of 1,2-dihydro-2-isopropyl-4*H*-3,1-benzoxazine (**7g**) in 189 ml of EtOH and 81 ml of toluene. After the reaction was complete, the solvent was evaporated *in vacuo* and 20% aqueous NH_4Cl was added to the residue at 0 °C. A separated oil was extracted with ether and the ether layer was dried over anhydrous Na_2SO_4. Evaporation of the solvent yielded 27.2 g (100%) of 2-isobutylaminobenzyl alcohol (**1g**) as a pale brown oil. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (6H, d, J=7 Hz), 1.6—2.1 (1H, m), 2.90 (2H, d, J=7 Hz), 4.52 (2H, s), 6.4—7.3 (4H, m). Exact MS *m/z* Calcd for $C_{11}H_{17}NO$: 179.1311. Found: 179.1311.

Compounds **1d** and **1e** were obtained by a procedure similar to that described for **1g**. The yields are listed in Table III.

Methyl *N*-Propargylanthranilate (8) A mixture of 45.6 g (303 mmol) of methyl anthranilate (**4**) and 18 g (151 mmol) of propargyl bromide in 50 ml of MeOH was heated under reflux for 40 h. The solvent was evaporated and 500 ml of Et_2O was added to the residue. Insoluble solid was filtered and the filtrate was washed with 30 ml of 1 N HCl and with 30 ml of 1 N NaOH. The extract was dried over Na_2SO_4, and concentrated *in vacuo*. The residue was crystallized by the addition of hexane (150 ml), collected by filtration and washed with cold hexane to yield 20.4 g (35%) of methyl *N*-propargylanthranilate (**8**) as a yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 2.21 (1H, t, J=2 Hz), 3.85 (3H, s), 4.02 (2H, dd, J=2, 6 Hz), 6.5—8.0 (4H, m). *Anal.* Calcd for $C_{11}H_{11}NO_2$: C, 69.80; H, 5.86; N, 7.40%. Found; C, 69.73; H, 5.96; N, 7.70%.

2-Allylaminobenzyl Alcohol (1r) and 2-Propargylaminobenzyl Alcohol (1s) A solution of 5.0 g (26.4 mmol) of methyl *N*-propargylanthranilate (**8**) in 20 ml of dry THF (Al_2O_3) was added over 30 min under 0 °C to a suspension of 1.0 g of $LiAlH_4$ in 50 ml of dry THF (Al_2O_3) which was then cooled to −5 °C. After additional stirring for 30 min, a saturated aqueous Na_2SO_4 was added dropwise. The precipitate was filtered and the filtrate concentrated *in vacuo*. Column chromatography on silicagel (Et_2O : hexane = 5 : 2) of the residue yielded 0.8 g (19%) of 2-allylaminobenzyl alcohol (**1r**) as a colorless oil and 1.73 g (41%) of 2-propargylaminobenzyl alcohol (**1s**) as a white solid. ^{1}H-NMR ($CDCl_3$) for **1r**; δ: 1.7 (1H, br), 3.80 (2H, dt, J=2, 5 Hz), 4.64 (2H, s), 5.0—5.4 (2H, m), 5.7—6.2 (1H, m), 6.7—7.3 (4H, m). Exact MS *m/z* Calcd for $C_{10}H_{13}NO$: 163.0998. Found: 163.1002. ^{1}H-NMR ($CDCl_3$) for **1s**; δ: 1.84 (1H, br), 2.19 (1H, t, J=3 Hz), 3.93 (2H, d, J=3 Hz), 4.60 (2H, s), 5.0 (1H, br), 6.5—7.4 (4H, m). *Anal.* Calcd for $C_{10}H_{11}NO$: C, 74.51; H, 6.88; N, 8.69%. Found: C, 74.72; H, 6.85; N, 8.35%.

General Procedure for the Preparation of *N*-Substituted 2-[(2-imidazolylthio)methyl]anilines (2) A solution of 2.8 g (15.6 mmol) of 2-isobutylaminobenzyl alcohol (**1g**) in 23 ml of CH_2Cl_2 was cooled to −5 °C and 2.3 g of $SOCl_2$ was added dropwise. The mixture was stirred for 30 min, the solvent removed *in vacuo* under 40 °C and the residue was added to a suspension of 1.56 g (15.6 mmol) of 2-mercaptoimidazole in 15 ml of EtOH. The mixture was stirred for 1 h. The solvent was removed and $CHCl_3$ and water were added. The organic layer was separated, washed with 10% Na_2CO_3 solution, and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the addition of 6 ml of Et_2O–hexane (3 : 1) followed by filtration of the precipitate gave 4.1 g (94%) of *N*-isobutyl-2-[(2-imidazolylthio)methyl]aniline (**2g**) as a white powder. Most of the sulfides were obtained by a similar procedure to that described for **2g**. The yield, appearance, melting point, exact MS and ^{1}H-NMR data are given in Table I.

General Procedure for the Preparation of *N*-Substituted 2-[(2-Imidazolylsulfinyl)methyl]anilines (3) by Method A *m*-Chloroperbenzoic acid (purity 80%, 774 mg, 3.61 mmol) was added portionwise to a solution of 1.0 g (3.61 mmol) of *N*-isobutyl-2-[(2-imidazolylthio)methyl]aniline (**2g**) in 40 ml of $CHCl_3$ and 10 ml of MeOH, at a constant temperature of 0—5 °C. The mixture was stirred for 1 h, then the solution was washed with saturated aqueous Na_2CO_3. The $CHCl_3$ extract was shaken successively with 10 ml of 0.1 N NaOH and extracted three times with 2 ml of 1 N NaOH to transfer the reaction product into aqueous fractions. The combined extract was made ammonia-alkaline by the addition of a 20% NH_4Cl solution. A precipitate deposited from the ammonia-alkaline solution was collected by filtration, washed with ether, and dried to give 600 mg (57%) of *N*-isobutyl-2-[(2-imidazolylsulfinyl)methyl]aniline (**3g**) as a white colorless prism.

Compounds **3a**—**c**, **f**, **i**, **j**, **l**, **p** and **r**—**v** were obtained by a similar procedure to that described for **3g**.

General Procedure for the Preparation of *N*-Substituted 2-[(2-Imidazolylsulfinyl)methyl]anilines (3) by Method B A mixture of 1.45 g (5.5 mmol) of *N*-(2-methoxyethyl)-2-[(2-imidazolylthio)methyl]aniline (**2n**), 13 ml of CH_2Cl_2, 13 ml of MeOH, and 1.3 ml of AcOH was stirred for 30 min at room temperature to dissolve **2n** in the solvent. The solution was cooled with ice water to below 5 °C and 2.6 ml of 35% H_2O_2 and 37 mg of NH_4VO_3 were added successively. The mixture was stirred for approx. 2.5 h at temperatures between −3 and 3 °C. Chloroform and saturated aqueous $NaHCO_3$ were added and the $CHCl_3$ layer was separated. The $CHCl_3$ extract was shaken successively twice with 10 ml of 1 N NaOH to transfer the reaction product into the aqueous fractions. The combined aqueous extract was rendered ammonia-alkaline by the addition of 20% aqueous NH_4Cl. A separated oil was extracted with $CHCl_3$, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. Addition of Et_2O followed by filtration of the precipitate yielded 760 mg (50%) of *N*-(2-methoxyethyl)-2-[(2-imidazolylsulfinyl)methyl]aniline (**3n**) as a colorless prism.

Compounds **3d**, **e**, **h**, **k**, **m**, **o** and **q** were obtained by a similar procedure to that described for **3n**. The yield, appearance, mp, IR (S O), elemental analyses and ^{1}H-NMR of all sulfoxides (**3**) are given in Table II.

Preparation of H^+/K^+-ATPase Enriched Rabbit Gastric Membrane Gastric H^+/K^+-ATPase was purified from the parietal cell-rich fraction of rabbit stomach as described by Saccoman *et al.*[11] with slight modification. The fundic mucosa of male Japanese white rabbits was homogenized in about 10 volumes of a cold solution containing 250 mM sucrose, 1 mM EGTA, and 20 mM Tris–HCl buffer at pH 7.4. The resulting homogenate was centrifuged at 9000 *g* for 10 min. The pellet was re-homogenized, and the combined supernatants were centrifuged at 77000 *g* for 60 min to yield a crude microsomal pellet. The 77000 *g* pellet was resuspended in the same solution, homogenized and centrifuged at 77000 *g* for 180 min in a discontinuous density gradient. Vesicles enriched in the gastric H^+/K^+-ATPase were collected at the interface of 250 mM sucrose, 7% Ficoll (w/w) +250 mM sucrose layers. The vesicle preparation was stored in 250 mM sucrose (unbuffered) at 4 °C. Protein concentration was determined by the Lowry method using bovine serum albumin as the standard.[12]

Assay Procedure of H^+/K^+-ATPase Inhibitory Activity The enzyme (*ca.* 10 μg protein) was preincubated in medium consisting of 5 mM imidazole buffer (pH 6.0 or 7.4) and a test compound in a final volume of 0.5 ml. These agents were dissolved in dimethyl sulfoxide (DMSO). All incubations contained less than 1% DMSO, which had no influence on the assay. The incubation time was 25 min at room temperature followed by 5 min at 37 °C, after which the enzyme reaction was started by the addition of 0.5 ml of a mixture containing 4 mM $MgCl_2$, 4 mM ATP, and 80 mM imidazole buffer (pH 7.4), with or without 20 mM KCl. After incubation for 15 min at 37 °C, the reaction was terminated by adding 1 ml of 24% trichloroacetic acid. Inorganic phosphate from adenosine triphosphate (ATP) was measured by the method of Taussky and Shorr.[13]

Assay Procedure of Inhibitory Effect against Gastric Acid Secretion Beagles of both sexes (12—14 kg) with Heidenhain pouch were fasted for 18 h before the experiments. Histamine dihydrochloride was continuously administered i.v. at a dose of 160 μg/kg/h. Gastric juice was collected at 15 min intervals and the volume was determined. Acid concentration was measured by titration with 0.1 N NaOH to pH 7.0. Test compounds were administered i.v. 75 min after the initiation of histamine

administration. The percent inhibition of acid output was determined 30 min after the administration of each compound by comparison with the control value.

Assay of Stability Stability at 37 °C was determined in Britten–Robinson buffer which had been adjusted to the appropriate pH. Compounds were dissolved in this buffer by supersonic waves, and the filtered solution was monitored by high performance liquid chromatography (HPLC) (Tosoh TSK-gel ODS-120T 4.6—250 mm, CH_3CN/10 mM phosphate buffer (pH 7.0), 1 ml/min, room temperature (25 °C), detector ultraviolet (UV) 240—250 nm). Half-lives ($T_{1/2}$) were determined from linear regression of ln of the concentration *vs.* time (h).

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