Synthesis of 4-(Phenylamino)quinoline-3-carboxamides as a Novel Class of Gastric H^+/K^+-ATPase Inhibitors

Minoru UCHIDA,* Kenji OTSUBO, Jun MATSUBARA, Tadaaki OHTANI, Seiji MORITA, and Katsuya YAMASAKI

Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Kagasuno 463–10, Kawauchi-cho, Tokushima 771–01, Japan. Received October 3, 1994; accepted November 24, 1994

In a search for inhibitors of gastric H^+/K^+-ATPase, 4-(phenylamino)quinoline-3-carboxamides were synthesized and evaluated for antisecretory activity against histamine-induced gastric acid secretion in rats. These compounds were synthesized by condensation of aniline derivatives with *N*-substituted 4-chloroquinoline-3-carboxamides, which were obtained from treatment of 4(1*H*)-quinolinone-3-carboxylic acid with thionyl chloride. Most of the compounds inhibited histamine-induced gastric acid secretion in rats. Among them, *N*-allyl-4-(2-ethylphenylamino)quinoline-3-carboxamide (4h) was the most potent inhibitor and had the best profile as a candidate antiulcer agent. This compound showed reversible, K^+-competitive gastric H^+/K^+-ATPase inhibitory activity.

Key words 4-(phenylamino)quinoline-3-carboxamide; antisecretory activity; H^+/K^+-ATPase inhibitor; antiulcer activity; cytoprotective activity; reversible inhibitor

The inhibition of gastric acid secretion has proved to be effective in the clinic for the treatment of peptic ulcer diseases. Since the discovery of the proton pump inhibitor omeprazole, this 2-[(2-pyridyl)methylsulfinyl]benzimidazole derivative has been the focus of intensive synthetic studies. The proton pump inhibitors omeprazole and lansoprazole produce faster healing of peptic ulcers than the H_2-antagonists ranitidine and famotidine, and overall healing rates tend to be superior with the proton pump inhibitors. However, a long duration of action appears to cause undesirable side effects.[1] Further, the instability of these compounds under various conditions, especially in aqueous media, poses problems. Therefore, it is difficult to use this class of compounds as injections. In earlier papers,[2] we reported that analogues of the benzimidazole class showed potent antisecretory and antiulcer activities. As a continuation of our search for much more active and stable compounds, we were interested in reversible inhibitors of the gastric H^+/K^+-ATPase, such as 4-(phenylamino)quinoline-3-carboxylic acid esters.[3] Here we wish to report the synthesis and biological activity of 4-(phenylamino)quinoline-3-carboxamides. The synthesis and biological activity of a similar series of compounds was disclosed by the Smith Kline Beecham group,[4] after we applied for a patent.[5]

Synthesis

4-(Phenylamino)quinoline-3-carboxamides were synthesized by the pathway shown in Chart 1. The *o*-substituted anilines (**1a—d**) were condensed with diethyl ethoxymethylenemalonate (EMME) at 100—110 °C and then cyclized in diphenyl ether at 200 °C. The crude esters were hydrolyzed with sodium hydroxide (NaOH) in ethanol (EtOH)–water (H_2O) to give 4(1*H*)-quinolinone-3-carboxylic acids (**2f—h** and **2l**). The 8-(acetoxymethyl)-4(1*H*)-quinolinone derivative (**2i**) was synthesized by acetylation of the hydroxymethyl compound (**2h**). Treatment of 4(1*H*)-quinolinone-3-carboxylic acids (**2a—l**)[6] with thionyl

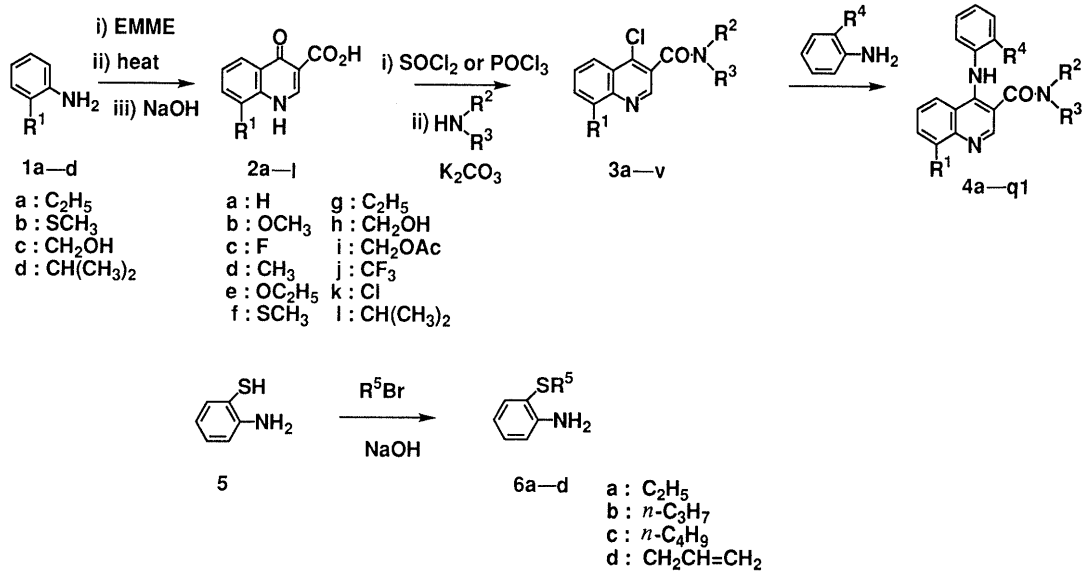
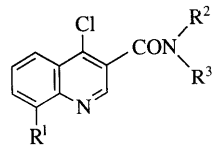


Chart 1

* To whom correspondence should be addressed.

Table 1. *N*-Substituted 4-Chloroquinoline-3-carboxamides



3a—v

No.	R^1	R^2 R^3	Yield (%)	mp (°C)	Appearance (Recryst. solv.)	Formula	Analysis (%) Calcd (Found) C	H	N
3a	OCH_3	H H	77	238.5—239 (dec.)	White powder (E–A)	$C_{11}H_9ClN_2O_2$	55.83 (55.64)	3.83 3.88	11.84 11.77)
3b	OCH_3	H CH_3	70	175—176	Colorless prisms (E–A–H)	$C_{12}H_{11}ClN_2O_2 \cdot 1/4H_2O$	56.48 (56.63	4.54 4.40	10.98 10.79)
3c	OCH_3	H C_2H_5	42	155—156	Colorless needles (A–H)	$C_{13}H_{13}ClN_2O_2$	58.99 (58.79	4.95 4.90	10.58 10.57)
3d	C_2H_5	H C_2H_5	83	127—129	Colorless needles (A–H)	$C_{14}H_{15}ClN_2O$	64.00 (63.87	5.75 5.66	10.66 10.67)
3e	OCH_3	H n-C_3H_7	82	145—147	Colorless needles (A)	$C_{14}H_{15}ClN_2O_2$	60.33 (60.15	5.42 5.12	10.05 9.93)
3f	C_2H_5	H n-C_3H_7	71	133—134.5	Colorless needles (A–H)	$C_{15}H_{17}ClN_2O$	65.10 (64.98	6.19 5.85	10.12 10.22)
3g	OCH_3	H $CH_2CH=CH_2$	79	114—116	Brown prisms (A–H)	$C_{14}H_{13}ClN_2O_2$	60.77 (60.57	4.74 4.74	10.12 9.82)
3h	OCH_3	$CH_2CH=CH_2$ $CH_2CH=CH_2$	42	146.5—147.5	Colorless needles (A–H)	$C_{17}H_{17}ClN_2O_2$	64.46 (64.29	5.41 5.11	8.84 8.73)
3i	OCH_3	H $CH_2C\equiv CH$	58	189.5—190	Colorless needles (A)	$C_{14}H_{11}ClN_2O_2$	61.21 (61.17	4.04 3.80	10.20 10.23)
3j	OCH_3	H cyclo-C_3H_7	63	192—193	Colorless needles (A)	$C_{14}H_{13}ClN_2O_2$	60.77 (60.45	4.74 4.40	10.12 9.79)
3k	OCH_3	H $CH_2C(CH_3)=CH_2$	83	115—116	Colorless prisms (A–H)	$C_{15}H_{15}ClN_2O_2 \cdot 1/2H_2O$	60.10 (60.27	5.38 5.11	9.35 9.45)
3l	OCH_3	H CH_2CF_3	69	204—206	Pale yellow powder (A)	$C_{13}H_{10}ClF_3N_2O_2$	49.00 (48.77	3.16 2.93	8.79 8.76)
3m	H	H $CH_2CH=CH_2$	62	179.5—180.5	Colorless flakes (E–A–H)	$C_{13}H_{11}ClN_2O \cdot H_2O$	58.99 (59.23	4.95 5.20	10.58 10.28)
3n	F	H $CH_2CH=CH_2$	79	133—134.5	Colorless needles (E–A–H)	$C_{13}H_{10}ClFN_2O$	58.99 (58.87	3.81 3.52	10.58 10.58)
3o	OC_2H_5	H $CH_2CH=CH_2$	50	151.5—152.5	Colorless needles (A)	$C_{15}H_{15}ClN_2O_2$	61.97 (62.11	5.20 5.02	9.64 9.67)
3p	SCH_3	H $CH_2CH=CH_2$	92	138—139.5	Pale brown powder (A–H)	$C_{14}H_{13}ClN_2OS$	57.43 (57.14	4.48 4.10	9.57 9.41)
3q	CH_2OAc	H $CH_2CH=CH_2$	82	146—147	Pale brown powder (A)	$C_{16}H_{15}ClN_2O_3$	60.29 (60.18	4.74 4.89	8.79 8.79)
3r	C_2H_5	H $CH_2CH=CH_2$	79	133.5—134.5	Brown prisms (A–H)	$C_{15}H_{15}ClN_2O$	65.57 (65.44	5.50 5.37	10.20 10.11)
3s	CH_3	H $CH_2CH=CH_2$	63	120—121	Colorless needles (A–H)	$C_{14}H_{13}ClN_2O$	64.50 (64.41	5.03 4.99	10.74 10.80)
3t	$CH(CH_3)_2$	H $CH_2CH=CH_2$	73	99—100	White powder (A–H)	$C_{16}H_{17}ClN_2O$	66.55 (66.66	5.93 5.78	9.70 9.58)
3u	Cl	H $CH_2CH=CH_2$	69	140—141	Colorless needles (A–H)	$C_{13}H_{10}Cl_2N_2O$	55.54 (55.69	3.59 3.31	9.96 10.04)
3v	CF_3	H $CH_2CH=CH_2$	76	153—155	White powder (A–H)	$C_{14}H_{10}ClF_3N_2O$	53.43 (53.38	3.20 2.95	8.90 8.96)

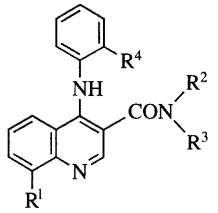
Recrystallization solvent: E, EtOH; A, AcOEt; H, hexane.

chloride or phosphorus oxychloride followed by Shotten–Baumann reaction with amines in the presence of potassium carbonate gave *N*-substituted 4-chloroquinoline-3-carboxamides (**3a—v**). The desired 4-(phenylamino)quinoline-3-carboxamides (**4a—q1**) were prepared by condensation of the 4-chloroquinoline derivatives (**3a—v**) with anilines. The 8-(hydroxymethyl)quinoline derivative (**4n1**) was obtained by hydrolysis of the corresponding acetoxy derivative (**4m1**) with NaOH. Several starting alkylthioanilines (**6a—d**) were synthesized by the reaction of 2-aminothiophenol (**5**) with alkyl halides in the presence of NaOH.

Biological Results

The antisecretory activity against histamine-induced gastric acid secretion of the synthesized compounds is summarized in Table 2. Compounds **4d, e, h, t, u, h1 i1** and **4j1** inhibited histamine-induced gastric acid secretion.

Table 2. 4-(Phenylamino)quinoline-3-carboxamides



4a—q1

No.	R^1	R^2 R^3	R^4	Yield (%)	mp (°C)	Appearance (Recryst. solv.)[a]	Formula	Analysis Calcd (Found) C	H	N	Activity (ED_{50})[b]
4a	OCH_3	H H	CH_3	52	258—260 (dec.)	Pale yellow needles (E–H)	$C_{18}H_{17}N_3O_2\cdot$ HCl	62.88 (62.97	5.28 5.33	12.22 12.29)	35.1
4b	OCH_3	H CH_3	C_2H_5	67	252—252.5 (dec.)	Pale yellow powder (E–A–H)	$C_{20}H_{21}N_3O_2\cdot$ HCl	64.60 (64.54	5.96 6.05	11.30 11.35)	5.3
4c	OCH_3	H C_2H_5	CH_3	71	254.5—255.5 (dec.)	Pale yellow powder (E–A–H)	$C_{20}H_{21}N_3O_2\cdot$ HCl	64.60 (64.52	5.96 6.01	11.30 11.09)	4.4
4d	OCH_3	H C_2H_5	C_2H_5	34	234—235 (dec.)	Pale yellow powder (E–A–H)	$C_{21}H_{23}N_3O_2\cdot$ HCl	65.36 (65.27	6.27 6.13	10.89 10.73)	2.4
4e	C_2H_5	H C_2H_5	CH_3	57	251—253	Pale brown powder (E–A–H)	$C_{21}H_{23}N_3O\cdot HCl$	68.19 (68.10	6.54 6.62	11.36 11.16)	2.2
4f	OCH_3	H n-C_3H_7	C_2H_5	66	91—93	Yellow powder (E–A–H)	$C_{22}H_{25}N_3O_2\cdot$ $HCl\cdot 2H_2O$	60.61 (60.29	6.94 6.96	9.64 9.35)	3.2
4g	OCH_3	H $CH_2CH=CH_2$	CH_3	44	231—232 (dec.)	Yellow powder (E–A–H)	$C_{21}H_{21}N_3O_2\cdot$ HCl	65.71 (65.68	5.78 5.79	10.95 10.84)	6.7
4h	OCH_3	H $CH_2CH=CH_2$	C_2H_5	47	222—223 (dec.)	Yellow powder (E–H)	$C_{22}H_{23}N_3O_2\cdot$ HCl	66.41 (66.33	6.08 6.07	10.56 10.49)	1.0
4i	OCH_2	$CH_2CH=CH_2$ $CH_2CH=CH_2$	CH_3	34	182—183 (dec.)	Yellow powder (E–A–H)	$C_{24}H_{25}N_3O_2\cdot$ $HCl\cdot H_2O$	65.22 (65.08	6.39 6.34	9.51 9.44)	—
4j	OCH_3	H $CH_2C\equiv CH$	CH_3	56	237—238 (dec.)	Yellow powder (E–A–H)	$C_{21}H_{19}N_3O_2\cdot$ $HCl\cdot 1/2H_2O$	64.53 (64.65	5.42 5.53	10.75 10.57)	—
4k	OCH_3	H cyclo-C_3H_7	CH_3	31	272—273 (dec.)	Pale yellow powder (E–A)	$C_{21}H_{21}N_3O_2\cdot$ HCl	65.71 (65.57	5.78 5.84	10.95 10.79)	—
4l	OCH_3	H $CH_2C(CH_3)=CH_2$	C_2H_5	79	235—236 (dec.)	Yellowish brown powder (E–A–H)	$C_{23}H_{22}N_3O_2\cdot$ $HCl\cdot 1/2H_2O$	66.10 (66.13	5.79 6.51	10.05 10.04)	6.4
4m	OCH_3	H CH_2CF_3	C_2H_5	68	214—215 (dec.)	Yellow powder (E–A–H)	$C_{21}H_{20}F_3N_3O_2\cdot$ $HCl\cdot 1/2H_2O$	56.19 (56.05	4.94 4.85	9.36 9.24)	4.9
4n	OCH_3	H $CH_2CH=CH_2$	F	29	232—233 (dec.)	Yellow powder (E–A–H)	$C_{20}H_{18}FN_3O_2\cdot$ HCl	61.94 (61.90	4.94 4.87	10.83 10.88)	—
4o	OCH_3	H $CH_2CH=CH_2$	SCH_3	33	242—243 (dec.)	Yellow powder (E–A–H)	$C_{21}H_{21}N_3O_2S\cdot$ HCl	60.64 (60.18	5.33 5.28	10.10 9.90)	8.4
4p	OCH_3	H $CH_2CH=CH_2$	SC_2H_5	19	246.5—247.5 (dec.)	Pale yellow powder (E–A–H)	$C_{22}H_{23}N_3O_2S\cdot$ $HCl\cdot 1/4H_2O$	60.82 (60.96	5.68 5.66	9.67 9.52)	7.1
4q	OCH_3	H $CH_2CH=CH_2$	SC_3H_7-n	50	206—207 (dec.)	Pale yellow powder (E–A–H)	$C_{23}H_{25}N_3O_2S\cdot$ HCl	62.22 (61.86	5.90 5.86	9.46 9.41)	2.8
4r	OCH_3	H $CH_2CH=CH_2$	SC_4H_9-n	40	175—176	Pale yellow flakes (A–H)	$C_{24}H_{27}N_3O_2S$	68.38 (68.25	6.46 6.43	9.97 9.83)	—
4s	OCH_3	H $CH_2CH=CH_2$	$SCH_2CH=CH_2$	31	205.5—207 (dec.)	Pale yellow granules (E–A–H)	$C_{23}H_{23}N_3O_2S\cdot$ HCl	62.50 (62.25	5.47 5.56	9.51 9.50)	7.2
4t	OCH_3	H $CH_2CH=CH_2$	$CH(CH_3)_2$	31	228—229 (dec.)	Pale yellow powder (E–A)	$C_{23}H_{25}N_3O_2\cdot$ HCl	67.06 (67.06	6.36 6.31	10.20 10.27)	1.7
4u	OCH_3	H $CH_2CH=CH_2$	n-C_3H_7	49	232.5—233.5 (dec.)	Pale yellow powder (E–A–H)	$C_{23}H_{25}N_3O_2\cdot$ $HCl\cdot 1/3H_2O$	66.10 (66.13	6.43 6.30	10.05 9.97)	1.4
4v	OCH_3	H $CH_2CH=CH_2$	$C(CH_3)=CH_2$	55	230—231 (dec.)	Pale yellow powder (E–A–H)	$C_{23}H_{23}N_3O_2\cdot$ HCl	67.39 (67.39	5.90 5.92	10.25 10.26)	7.0
4w	OCH_3	H $CH_2CH=CH_2$	n-C_4H_9	33	208—210 (dec.)	Yellow powder (E–A–H)	$C_{24}H_{27}N_3O_2\cdot$ HCl	67.67 (67.30	6.63 6.67	9.87 9.70)	15.2
4x	OCH_3	H $CH_2CH=CH_2$	OCH_3	69	257.5—258.5 (dec.)	Yellow powder (E–A–H)	$C_{21}H_{21}N_3O_3\cdot$ $HCl\cdot 1/4H_2O$	62.37 (62.58	5.61 5.53	10.39 10.48)	—
4y	OCH_3	H $CH_2CH=CH_2$	$COCH_3$	27	204—206 (dec.)	Pale yellow powder (E–A–H)	$C_{26}H_{23}N_3O_2\cdot$ HCl	70.03 (69.65	5.42 5.44	9.42 9.27)	16.3
4z	OCH_3	H $CH_2CH=CH_2$	CN	30	245—246 (dec.)	Pale yellow powder (E–A)	$C_{21}H_{18}N_4O_2\cdot$ $HCl\cdot 1/3H_2O$	62.92 (62.97	4.95 5.06	13.98 13.65)	—
4a1	OCH_3	H $CH_2CH=CH_2$	Cl	34	238.5—239.5 (dec.)	Pale yellow powder (E–A–H)	$C_{20}H_{18}ClN_3O_2\cdot$ HCl	59.42 (59.27	4.74 4.78	10.39 10.40)	25.1
4b1	H	H $CH_2CH=CH_2$	CH_3	52	283—285	Pale yellow powder (E–A)	$C_{20}H_{19}N_3O\cdot HCl$	67.89 (67.89	5.70 5.73	11.88 11.82)	—
4c1	F	H $CH_2CH=CH_2$	CH_3	51	236.5—237.5	Brown needles (E–A–H)	$C_{20}H_{18}FN_3O$	71.63 (71.60	5.41 5.43	12.53 12.49)	—
4d1	OC_2H_5	H $CH_2CH=CH_2$	CH_3	65	177—178	Pale brown needles (A–H)	$C_{22}H_{23}N_3O_2$	73.11 (73.07	6.41 6.42	11.63 11.44)	—
4e1	SCH_3	H $CH_2CH=CH_2$	CH_3	49	263.5—265 (dec.)	Yellow powder (E)	$C_{21}H_{21}N_3OS\cdot$ HCl	63.07 (62.98	5.54 5.54	10.51 10.32)	—

Table 2. (continued)

No	R^1	R^2 R^3	R^4	Yield (%)	mp (°C)	Appearance (Recryst. solv.)[a]	Formula	Analysis Calcd (Found) C	H	N	Activity (ED_{50})[b]
4f1	CH_3	H $CH_2CH=CH_2$	CH_3	14	234—235	Pale yellow powder (E–A–H)	$C_{21}H_{21}N_3O \cdot HCl$	68.56 (68.45)	6.03 6.16	11.42 11.38)	6.7
4g1	CH_3	H $CH_2CH=CH_2$	C_2H_5	30	149—150	Colorless needles (A–H)	$C_{22}H_{23}N_3O$	76.49 (76.56	6.71 6.91	12.16 12.10)	3.5
4h1	CH_3	H $CH_2CH=CH_2$	$CH(CH_3)_2$	33	252.5—254.5	Pale yellow powder (E–A–H)	$C_{23}H_{25}N_3O \cdot HCl$	69.77 (69.76	6.62 6.56	10.61 10.57)	2.1
4i1	CH_3	H $CH_2CH=CH_2$	n-C_3H_7	2	131.5—132.5	Pale yellow needles (A–H)	$C_{23}H_{25}N_3O$	76.85 (76.87	7.01 7.13	11.69 11.52)	1.9
4j1	C_2H_5	H $CH_2CH=CH_2$	CH_3	48	231.5—232.5	Pale yellow powder (E–A–H)	$C_{22}H_{23}N_3O \cdot HCl$	69.19 (69.27	6.33 6.44	11.00 10.98)	1.6
4k1	C_2H_5	H $CH_2CH=CH_2$	C_2H_5	5	117—118	Yellow needles (A–H)	$C_{23}H_{25}N_3O$	76.85 (76.62	7.01 7.17	11.69 11.58)	—
4l1	C_2H_5	H $CH_2CH=CH_2$	H	29	176—178	Pale yellow powder (A)	$C_{24}H_{27}N_3O \cdot HCl$	70.31 (70.27	6.88 6.90	10.25 10.25)	4.2
4m1	CH_2OAc	H $CH_2CH=CH_2$	C_2H_5	16	114—115	Pale yellow powder (A–H)	$C_{24}H_{25}N_3O_3$	71.44 (71.31	6.25 6.25	10.41 10.41)	6.7
4n1	CH_2OH	H $CH_2CH=CH_2$	C_2H_5	39	151—152	Pale yellow powder (A–H)	$C_{22}H_{23}N_3O_2$	73.11 (73.16	6.41 6.39	11.63 11.55)	8.7
401	$CH(CH_3)_2$	H $CH_2CH=CH_2$	CH_3	49	228—230	Yellow granules (A—H)	$C_{23}H_{25}N_3O \cdot HCl \cdot 1/2H_2O$	68.22 (68.48	6.72 6.86	10.38 10.37)	12.4
4p1	Cl	H $CH_2CH=CH_2$	C_2H_5	33	243—245	Yellow powder (E–A–H)	$C_{21}H_{20}ClN_3O \cdot HCl$	62.69 (62.45	5.26 5.17	10.44 10.28)	5.6
4q1	CF_3	H $CH_2CH=CH_2$	C_2H_5	11	155—156	Pale yellow needles (A–H)	$C_{22}H_{20}F_3N_3O$	66.16 (66.19	5.05 4.95	10.52 10.43)	—

a) Recrystallization solvent: E, EtOH; A, AcOEt; H, hexane. *b*) ED_{50} in mg/kg i.v. For comparison: omeprazol, ED_{50} 0.45 μmol/kg i.v.[7]; ethyl 4-(2-methylphenylamino)quinoline-3-carboxylate, ED_{50} 1.03 mg/kg i.v.

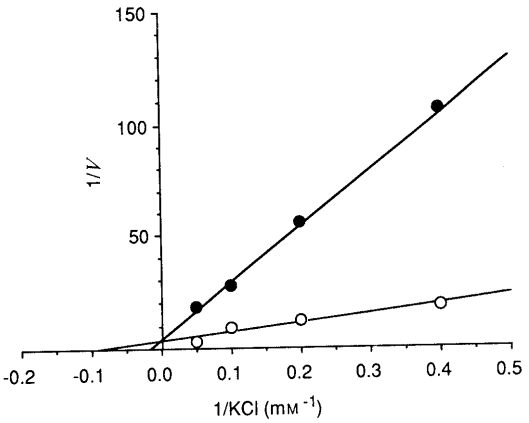


Fig. 1. Lineweaver–Burk Plot for Inhibition of H^+/K^+-ATPase by Compound **4h**

The concentration of K^+ was varied from 2.5 to 20 mM and MgATP was kept constant at 2 mM. The cuvettes contained 15 mg protein of enzyme, 100 mM Tris–HCl buffer (pH 7.4), 2 mM $MgCl_2$. Compound **4h** was present at 0 (○) and 3 μM (●). Lines show the best fit to a competitive pattern of inhibition. Values shown are the mean of duplicate determinations.

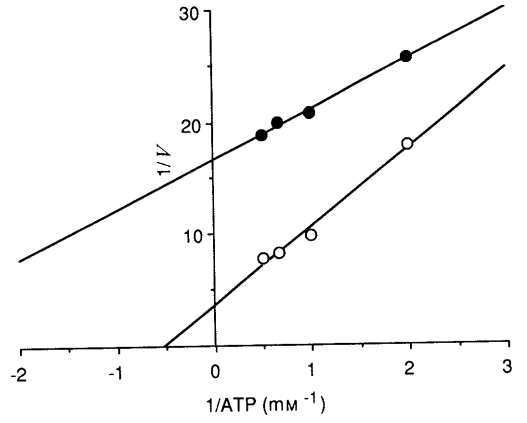


Fig. 2. Lineweaver–Burk Plot for Inhibition of H^+/K^+-ATPase by Compound **4h**

The concentration of MgATP was varied from 0.5 to 2 mM and KCl was kept constant at 20 mM. The cuvette contained 15 mg protein of enzyme, 100 mM Tris–HCl buffer (pH 7.4), 2 mM $MgCl_2$. Compound **4h** was present at 0 (○) and 3 μM (●). Lines show the best fit to an uncompetitive pattern of inhibition. Values shown are the mean of duplicate determinations.

Among them, compounds **4e**, **h**, **t**, **u**, **h1** and **4j1** showed remarkable antiulcer activity (Table 3) and, in particular, compound **4h** showed the best profile as a potential antiulcer agent. Compound **4h** inhibited the H^+/K^+-ATPase activity in a concentration-dependent manner with an IC_{50} value of 2.2 μM at pH 7.4 in the presence of 10 mM KCl. The inhibition was reversed by dialysis; we observed 7.1% of control enzyme activity on treatment with 10 μM compound **4h**, and the activity recovered to 82.0% of the control (mean of duplicate determinations) after dialysis. This result suggested that inhibition of the enzyme is reversible. Steady-state enzyme kinetics showed compound **4h** to be competitive with respect to K^+ and to be uncompetitive with respect to ATP (Figs. 1 and 2). This compound also showed cytoprotective activity (Table 3) and was more stable than 2-[(2-pyridyl)methylsulfinyl]-benzimidazole class compounds in aqueous media.[8]

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IRA-2 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard.

Compounds **2a**—**e**, **j** and **2k** were prepared according to the reported methods.[6]

8-Ethyl-4(1*H*)-quinolinone-3-carboxylic Acid (2g). A Typical Procedure

Table 3. Antiulcer and H^+/K^+-ATPase Inhibitory Activities of 4-(Phenylamino)quinoline-3-carboxamides

Compd. No.	Aspirin-induced ulcer ED_{50} (mg/kg) *p.o.*	Stress ulcer ED_{50} (mg/kg) *p.o.*	Cytoprotective activity ED_{50} (mg/kg) *p.o.*	H^+/K^+-ATPase IC_{50} (μM)
4d	NE	ND	NE	ND
4e	3.3	ND	NE	ND
4h	0.48	12.5	1.5	2.2
4t	0.42	8.7	3.9	4.9
4u	2.6	>100	NE	1.6
4h1	2.7	ND	NE	ND
4i1	NE	ND	NE	ND
4j1	2.7	ND	NE	ND
Patented compd.	9.1	53	4.1	0.87
Omeprazol	8.3	9.8	30.2	2.0

NE = no effect. ND = not determined. Patented compound = ethyl 4-(2-methylphenylamino)quinoline-3-carboxylate.

A mixture of *o*-ethylaniline (**1a**) (24.2 g, 0.2 mol) and EMME (43.2 g, 0.2 mol) was heated at 100—110 °C for 2 h. Diphenyl ether (200 ml) was added to the reaction mixture and the mixture was heated at 200 °C for 5 h. After cooling of the reaction mixture, hexane was added and the resulting precipitates were collected by filtration. A mixture of the crude ester and NaOH (10 g) in EtOH (100 ml) and H_2O (50 ml) was refluxed for 2 h. After removal of EtOH under reduced pressure, the residue was poured into water and the solution was acidified with concentrated HCl. The resulting precipitates were collected by filtration. Recrystallization from EtOH–$CHCl_3$ gave **2g** (2.9 g, 7%) as a white powder, mp 278.5—279.5 °C (dec.). NMR (DMSO-d_6) δ: 1.26 (3H, t, J=7.4 Hz), 2.97 (2H, q, J=7.4 Hz), 7.53 (1H, dd, J=7.4, 8.0 Hz), 7.76 (1H, dd, J=1.6, 7.4 Hz), 8.16 (1H, dd, J=1.6, 8.0 Hz), 8.61 (1H, s), 12.63 (1H, br s), 14.78 (1H, br s). IR (KBr): 3050, 2970, 2880, 1700, 1620, 1580, 1550, 1510, 1430, 1200, 1010, 790 cm^{-1}. *Anal.* Calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45. Found: C, 66.45; H, 5.09; N, 6.37.

8-Methylthio-4(1*H*)-quinolinone-3-carboxylic Acid (2f) Yield 12%, mp 270.5—271.5 °C (dec.), white powder (from EtOH). NMR (DMSO-d_6) δ: 2.60 (3H, s), 7.59 (1H, dd, J=7.6, 8.1 Hz), 8.00 (1H, dd, J=1.4, 7.6 Hz), 8.20 (1H, dd, J=1.4, 8.1 Hz), 8.65 (1H, s), 12.58 (1H, br s), 14.95 (1H, br s). IR (KBr): 3050, 1720, 1610, 1550, 1480, 1440, 1420, 1380, 1330, 1200, 790 cm^{-1}. *Anal.* Calcd for $C_{11}H_9NO_3S$: C, 56.16; H, 3.86; N, 5.95. Found: C, 56.26; H, 3.78; N, 5.81.

8-Isopropyl-4(1*H*)-quinolinone-3-carboxylic Acid (2l) Yield 41%, mp 277—277.5 °C (dec.), white powder (from EtOH). NMR (DMSO-d_6) δ: 1.31 (6H, d, J=6.6 Hz), 3.54 (1H, m), 7.58 (1H, dd, J=7.8, 8.1 Hz), 7.84 (1H, dd, J=1.6, 7.8 Hz), 8.18 (1H, dd, J=1.6, 8.1 Hz), 8.62 (1H, s), 12.64 (1H, br s), 14.75 (1H, br s). IR (KBr): 2970, 1700, 1610, 1580, 1550, 1500, 1430, 1370, 1200, 920, 770 cm^{-1}. *Anal.* Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.62; H, 5.46; N, 5.95.

8-Hydroxymethyl-4(1*H*)-quinolinone-3-carboxylic Acid (2h) Yield 19%, mp 267—268 °C (dec.), brown powder (from EtOH). NMR (DMSO-d_6) δ: 4.89 (2H, s), 5.75 (1H, br s), 7.57 (1H, dd, J=7.3, 8.1 Hz), 7.86 (1 H, dd, J=1.4, 7.3 Hz), 8.24 (1H, dd, J=1.4, 8.1 Hz), 8.71 (1H, s), 12.56 (1H, br s), 14.70 (1H, br s). IR (KBr): 3250, 3070, 2950, 2900, 1700, 1630, 1590, 1520, 1360, 1010, 770 cm^{-1}. *Anal.* Calcd for $C_{11}H_9NO_4$: C, 60.28; H, 4.14; N, 6.39. Found: C, 60.35; H, 4.29; N, 6.00.

8-Acetoxymethyl-4(1*H*)-quinolinone-3-carboxylic Acid (2i) Sulfuric acid (10 drops) was added to a mixture of **2h** (1.1 g, 5 mmol) in acetic anhydride (20 ml) and then the reaction mixture was heated at 70—80 °C for 3 h. It was poured into ice-water and the resulting precipitates were collected by filtration. Recrystallization from EtOH gave **2i** (0.8 g, 62%) as a brown powder, mp 244—245.5 °C (dec.). NMR (DMSO-d_6) δ: 2.07 (3H, s), 5.38 (2H, s), 7.61 (1H, dd, J=7.3, 8.1 Hz), 7.93 (1H, dd, J=1.4, 7.3 Hz), 8.34 (1H, dd, J=1.4, 8.1 Hz), 8.65 (1H, s), 12.78 (1H, br s), 14.80 (1H, br s). IR (KBr): 2950, 2900, 1750, 1690, 1630, 1580, 1550, 1440, 1230, 1030, 770 cm^{-1}. *Anal.* Calcd for $C_{13}H_{10}NO_5$: C, 60.00; H, 3.87; N, 5.38. Found: C, 59.97; H, 4.08; N, 4.92.

***N*-Substituted 4-Chloroquinoline-3-carboxamides (3a—v). A Typical Procedure** Thionyl chloride (10 ml) was added to 8-methoxy-4(1*H*)-quinolinone-3-carboxylic acid (1.5 g, 6.8 mmol). The reaction mixture was refluxed for 1 h and concentrated *in vacuo*. The residue was added to a stirred and ice-cooled solution of allylamine (0.47 g, 8.2 mmol) and K_2CO_3 (0.94 g, 6.8 mmol) in acetone (50 ml) and water (20 ml), and the mixture was stirred at 0—10 °C for 1 h. After removal of acetone, the residue was poured into water. The resulting precipitates were collected by filtration. Recrystallization from AcOEt–hexane gave **3g** (1.5 g, 79%) as brown prisms, mp 114—116 °C. NMR ($CDCl_3$) δ: 4.09 (3H, s), 4.10—4.30 (2H, m), 5.20—5.40 (2H, m), 5.80—6.10 (1H, m), 6.58 (1H, br s), 7.15 (1H, dd, J=1.1, 7.8 Hz), 7.59 (1H, dd, J=7.8, 8.5 Hz), 7.81 (1H, dd, J=1.1, 8.5 Hz), 8.89 (1H, s). IR (KBr): 3420, 3040, 1640, 1550, 1490, 1370, 1280, 810, 780 cm^{-1}. The elemental analysis data are given in Table 1.

Compounds **3a—f** and **3h—v** were obtained by a similar procedure to that described for **3g**; the yields, melting points and elemental analyses data are listed in Table 1.

4-(Phenylamino)quinoline-3-carboxamides (4a—q1). A Typical Procedure A solution of **3g** (0.3 g, 1.1 mmol) and *o*-ethylaniline (0.27 g, 2.2 mmol) in dioxane (20 ml) was refluxed for 5 h. After removal of the solvent, the residue was recrystallized from EtOH–hexane to give **4h** (0.2 g, 47%) as a yellow powder, mp 222—223 °C (dec.). NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7.5 Hz), 2.68 (2H, q, J=7.5 Hz), 4.07 (3H, s), 4.10—4.20 (2H, m), 5.10—5.40 (2H, m), 5.90—6.20 (1H, m), 6.80—7.40 (7H, m), 9.63 (1H, s), 10.18 (1H, br s), 12.99 (1H, s), 14.20 (1H, br s). IR (KBr): 3120, 3000, 2960, 2920, 2870, 1640, 1620, 1580, 1550, 1520, 1470, 1310, 1290, 790, 750 cm^{-1}. The elemental analysis data are given in Table 2.

Compounds **4a—g**, **i—m1** and **4o1—q1** were obtained by a similar procedure to that described for **4h**; the yields, melting points and elemental analyses data are listed in Table 2.

***N*-Allyl 4-(2-Ethylphenylamino)-8-(hydroxymethyl)quinoline-3-carboxamide (4n1)** A solution of NaOH (0.1 g, 2.5 mmol) in H_2O (10 ml) was added to a solution of *N*-allyl 8-acetoxymethyl-4-(2-ethylphenylamino)-quinoline-3-carboxamide (**4m1**, 0.2 g, 0.5 mmol) in MeOH (10 ml) and the reaction mixture was stirred for 1 h at room temperature. After removal of MeOH, the residue was extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from AcOEt–hexane to give **4n1** (70 mg, 39%) as a pale yellow powder, mp 151—152 °C. NMR ($CDCl_3$) δ: 1.33 (3H, t, J=7.6 Hz), 2.82 (2H, q, J=7.6 Hz), 4.12 (2H, t, J=5.8 Hz), 5.09 (2H, s), 5.21—5.37 (2H, m), 5.53 (1H, br s), 5.89—6.05 (1H, m), 6.73—7.49 (7H, m), 8.83 (1H, s), 10.59 (1H, s). IR (KBr): 3300, 2960, 2920, 1630, 1610, 1580, 1550, 1490, 1430, 1280, 1200, 1020, 760 cm^{-1}. The elemental analysis data are given in Table 2.

2-Alkylthioaniline (6a—d). A Typical Procedure A solution of NaOH (0.48 g, 12 mmol) in H_2O (10 ml) was added to a solution of 2-aminothiophenol (1.25 g, 10 mmol) in MeOH (30 ml), then *n*-propyl bromide (1.5 g, 12 mmol) was added. The mixture was stirred at room temperature for 2 h. After removal of MeOH, the residue was poured into water and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and concentrated *in vacuo* to give oily **6b** (1.6 g, 95%). NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7.3 Hz), 1.50—1.65 (2H, m), 2.72 (2H, t, J=7.3 Hz), 4.34 (2H, br s), 6.60—6.80 (2 H, m), 7.11 (1 H, t, J=7.1 Hz), 7.37 (1H, d, J=7.7 Hz). IR (neat): 3450, 3350, 2960, 2930, 1610, 1480, 1450, 1300, 750 cm^{-1}.

2-Ethylthioaniline (6a) Yield 85%, a pale yellow oil. NMR ($CDCl_3$) δ: 1.23 (3H, t, J=7.3 Hz), 2.76 (2H, q, J=7.3 Hz), 4.33 (2H, br s), 6.65—6.75 (2H, m), 7.07—7.16 (1H, m), 7.37 (1H, dd, J=7.6, 1.5 Hz). IR (neat): 3450, 3350, 2970, 2920, 1600, 1480, 1450, 1300, 750 cm^{-1}.

2-Butylthioaniline (6c) Yield 88%, a pale yellow oil. NMR ($CDCl_3$) δ: 0.89 (3H, t, J=7.2 Hz), 1.31—1.62 (4H, m), 2.74 (2H, t, J=7.2 Hz), 6.64—6.74 (2H, m), 7.06—7.14 (1H, m), 7.37 (1H, dd, J=7.6, 1.4 Hz). IR (neat): 3450, 3350, 2950, 2930, 1600, 1480, 1450, 1300, 750 cm^{-1}.

2-Allylthioaniline (6d) Yield 80%, a pale yellow oil. NMR ($CDCl_3$) δ: 3.31 (2H, m), 4.34 (2H, br s), 4.90—5.00 (2H, m), 5.74—5.90 (1H, m), 6.62—6.75 (2H, m), 7.07—7.16 (1H, m), 7.35 (1H, dd, J=7.7, 1.6 Hz). IR (neat): 3450, 3350, 3060, 1600, 1500, 1480, 1450, 1310, 920, 750 cm^{-1}.

Biological Methods. Assay of Inhibitory Effect against Gastric Acid Secretion Male Wistar/ST rats, weighing 190 to 250 g, were used for gastric acid secretion studies. An *in vivo* system for stomach perfusion was prepared according to the method of Ghosh and Schild.[9] Rats fasted for 24 h were anesthetized with urethane (1.5 g/kg, s.c.). The stomach was perfused continuously with warm saline containing 0.25 mM NaOH at the rate of 1 ml/min using a peristaltic pump. The perfusate was passed over a glass electrode pH meter which recorded the pH on a chart recorder. Histamine (1 mg/kg/h) was infused intravenously at a constant rate (1 ml/h). The first injection of test compound was made

into the femoral vein 15 min after maximum acid production, followed by cumulative doses (1, 3, 10, 30 mg/kg). Percent inhibition by a test compound was calculated as follows: [(mean value of test pH − mean value of histamine pH)/(mean value of control pH − mean value of histamine pH)] × 100. The doses inhibiting gastric secretion by 50% (ED_{50}) were calculated by Probit analysis.

Aspirin-Induced Gastric Ulcers Male Wistar/ST rats weighing between 160 and 220 g were used. The animals were housed in cages with wide mesh wire bottoms to prevent coprophagia. The rats were starved for 24 h, but allowed access to water before the experiment. Test compound and the vehicle were given orally 30 min before the oral administration of 200 mg/kg of aspirin suspended in 0.5% carboxymethyl cellulose. Five hours after aspirin administration, the rats were killed. The stomachs were removed, instilled with 10 ml of 3% formalin for 10 min and opened along the greater curvature. The length of lesions in the glandular portion was measured. The ulcer index (mm) was given by the sum of the lengths of the lesions. Percent inhibition was calculated as follows: [(ulcer index of control − ulcer index of test compound)/ulcer index of control] × 100. The doses inhibiting aspirin-induced gastric ulcers by 50% (ED_{50}) were calculated by Probit analysis.

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

Stress-Induced Gastric Ulcer[10] Male Wistar/ST rats weighing 160—200 g were starved but allowed free access to water for 24 h before the experiment. The test compounds suspended in 0.5% carboxymethyl cellulose (CMC) and the vehicle were given orally 30 min before the water immersion. Each rat was placed in a stress cage and immersed at 23 °C. Seven hours later, the animals were killed and the stomachs were removed. The stomach was fixed by instilling 10 ml of 1% formalin solution and then immersing it in the formalin solution for 30 min, after which it was incised along the greater curvature. The length of each lesion in the glandular portion was measured under a dissecting microscope. The sum of the length (mm) of all lesions for each rat was used as an ulcer index. Percent inhibition was calculated as follows: [(ulcer index of control − ulcer index of test compound)/ulcer index of control] × 100. The doses inhibiting water-immersion stress ulcer by 50% (ED_{50}) were calculated by Probit analysis.

Assay of H^+/K^+-ATPase Inhibition Hog gastric mucosal vehicles containing H^+/K^+-ATPase were prepared according to the method of Sachs *et al.*[11] Preincubation of enzyme with a test compound was carried out at room temperature for 30 min in 2 mM Pipes–Tris buffer pH 6.1. H^+/K^+-ATPase was assayed at 37 °C for 30 min in a reaction mixture (2 ml) containing 100 mM Tris–HCl buffer pH 7.4, approximately 15 mg of membrane protein, 2 mM $MgCl_2$ and 2 mM ATP with or without 10 mM KCl. The reaction was stopped by adding 0.3 ml of 40% trichloroacetic acid. The enzyme activity was determined by subtracting the amount of inorganic phosphate released in K^+-free medium. Phosphate was measured by the method of Fiske and Subbarow.[12] H^+/K^+-ATPase activity was taken as the difference between with and without KCl. Protein concentration was obtained by the Lowry method.[13] Percent inhibition was calculated as followed: [(mean value of control activity − mean value of test activity)/mean value of control activity] × 100. The doses inhibiting H^+/K^+-ATPase by 50% (IC_{50}) were calculated by Probit analysis.

Stability Measurement Stability at room temperature (15—25 °C) was determined in water. A 0.25% solution of compound **4h** was left to stand for 20 d, and the decrease in concentration was monitored by HPLC (column, Cosmosil 5C18, 30—90% acetonitrile in 40 min 1 ml/min, detector UV 246 nm). The remaining concentration was determined to be from 98.9% to 98.1% of the initial value. For comparison, the half-life of omeprazole in acetonitrile–buffer (1 : 3) (pH 7.4) at ambient temperature (20 °C) was 456 h.[8]

References

1) Ekman L., Hasson E., Havu N., Carlsson E., Lundberg C., *Scand. J. Gastroenterol.*, **20**, suppl. 108, 53 (1985).
2) Uchida M., Morita S., Chihiro M., Kanbe T., Yamasaki K., Yabuuchi Y., Nakagawa K., *Chem. Pharm. Bull.*, **37**, 1517 (1989); Uchida M., Chihiro M., Morita S., Kanbe T., Yamashita H., Yamasaki K., Yabuuchi Y., Nakagawa K., *ibid*, **37**, 2109 (1989); *idem*, *ibid.*, **38**, 534 (1990); *idem*, *ibid.*, **38**, 1575 (1990).
3) Munson Jr. H. R., Alphin R. S., Patent U. S., 4343804 [*Chem. Abstr.*, **98**, 143284*e* (1983)].
4) Ife R. J., Brown T. H., Keeling D. J., Leach C. A., Meeson M. L., Parsons M. E., Reavil D. R., Theobald C. J., Wiggall K. J., *J. Med. Chem.*, **35**, 3413 (1992).
5) Uchida M., Morita S., Otsubo K., Yamasaki K., Shimizu T., Japan. Patent 081773 [*Chem. Abstr.*, **116**, 214368*p* (1992)].
6) Erickson E. T., Hainline C. F., Lenon L. S., Matson C. J., Rice T. K., Swingle K. F., Winkle M. Van., *J. Med. Chem.*, **22**, 816 (1979); Ozeki K., Ishizuka Y., Sawada M., Ichikawa T., Sato M., Yaginuma H., *Yakugaku Zasshi*, **107**, 123 (1987); Renault S., Renault J., Cavier R., *Eur. J. Med. Chem. Chim. Ther.*, **11**, 555 (1976); Abdul-Ahad P. G., Webb G. A., *ibid.*, **17**, 301 (1982).
7) Brown T. H., Ife R. J., Keeling D. J., Laing S. M., Leach C. A., Parsons M. E., Price C. A., Reavill D. R., Wiggall K. J., *J. Med. Chem.*, **33**, 527 (1990).
8) Ife R. J., Dyke C. A., Keeling D. J., Meenan E., Meeson M. L., Parsons M. E., Price C. A., Theobald C. J., Underwood A. H., *J. Med. Chem.*, **32**, 1970 (1989).
9) Ghosh M. N., Schild M. O., *Brit. J. Pharmacol.*, **13**, 54 (1958).
10) Takagi K., Okabe S., *Jpn. J. Pharmacol.*, **18**, 9 (1968).
11) Sachs G., Chang H. H., Rabon E., Schackman R., Lewin M., Saccomani G., *J. Biol. Chem.*, **251**, 7690 (1976).
12) Fiske C. H., Subbarow Y., *J. Biol. Chem.*, **66**, 375 (1925).
13) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 165 (1951).