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Laboratory note

4-Substituted 1-(2-methylphenyl)thieno[2,3-*c*]-1,5-naphthyridines as possible reversible inhibitors of gastric H^+,K^+-ATPase

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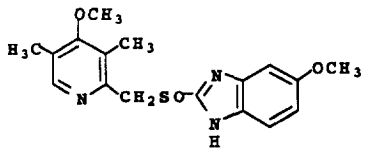
Dedicated to Professor Richard Neidlein on the occasion of his 65th birthday

Summary — Seven 1-(2-methylphenyl)thieno[2,3-*c*]-1,5-naphthyridines substituted in the 4-position with different amino-containing groups have been prepared. The reaction route to these compounds consisted of a palladium(0)-catalyzed cross-coupling between 1-bromothieno[2,3-*c*]-1,5-naphthyridine and 2-methyl-1-trimethylstannylbenzene, then oxidation of the 5-nitrogen, followed by treatment with thionyl chloride to give the 4-chloro derivative. The compounds obtained after nucleophilic substitution were tested with regard to their effects on H^+,K^+-ATPase activity and on acid formation in gastric glands. However, the inhibitory potency in vitro of the substituted naphthyridines was not high enough to be of interest from a pharmacological point of view.

gastric H^+,K^+-ATPase / cross-coupling / heterocycle / chlorination / oxidation

Introduction

The final step of acid secretion in the stomach is mediated by gastric H^+,K^+-ATPase. Accordingly, this protein has been identified as a pharmacological target for antisecretory drugs. Inhibition of acid secretion by substituted benzimidazoles such as omeprazole **1**, which after acid-catalyzed conversion binds to H^+,K^+-ATPase, has been shown to be long-lasting in nature [1]. In order to find compounds with a shorter duration of action, a variety of reversible inhibitors have been prepared and tested [2–5]. It has previously been found that some 4-substituted 1-aryl-pyrrolo[3,2-*c*]-quinolines such as **2** [3] are effective as reversible inhibitors of gastric H^+, K^+-ATPase. In this work the pyrrolo[3,2-*c*]quinoline system has been substituted for the thieno[3,2-*c*]-1,5-naphthyridine system as the parent heterocycle. The 1-position has been substituted with 2-methylphenyl and the 4-position has been substituted with a variety of amines in order to find a potent compound.



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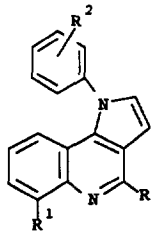

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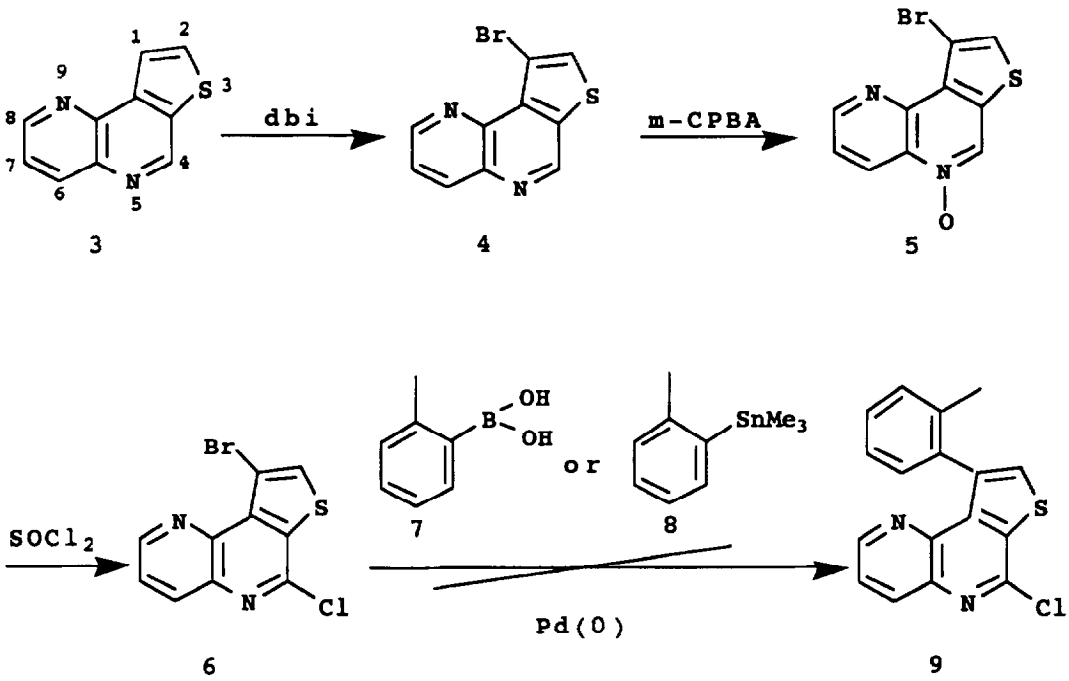
Chemistry

The thieno[2,3-*c*]-1,5-naphthyridine ring system **3** has previously been prepared at this department, in our work on the syntheses and properties of the isomeric 24 thieno[*b*]- and [*c*]-fused naphthyridines [6–9]. Thieno[2,3-*c*]-1,5-naphthyridine **3** was prepared through a one-pot approach developed by us, in which 2-bromo-3-aminopyridine and 2-formyl-3-thiopheneboronic acid were reacted through a modified Suzuki reaction followed by spontaneous ring-closure. This system was chosen as the parent compound because it is fairly easily synthesized and extensive work has already been done at this department on the functionalization of this compound [10–13].



Our first synthetic approach was to prepare 1-bromo-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **6** according to Malm et al [13]. The bromine would supply the handle for attaching the important 2-methylphenyl group, through a palladium(0)-catalyzed cross-coupling reaction. Furthermore the handle for substituting the 4-position with various amines would be available through the chlorine. We tried to attach the 2-methylphenyl group in the 1-position, by using the marked difference in reactivity between the bromine and the chlorine in the palladium(0)-catalyzed cross-coupling reactions. An attempt to react 1-bromo-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **6** with 2-methylphenylboronic acid **7** under standard cross-coupling conditions did not give the desired product, even when using Suzuki's new cross-coupling conditions [14] for sterically-hindered arylboronic acids. We also tried to use 2-methyl-1-trimethylstannylbenzene **8** as the organometallic coupling partner. This approach was also unsuccessful, even when copper(II) oxide was used as co-reagent [15] to promote the reaction.

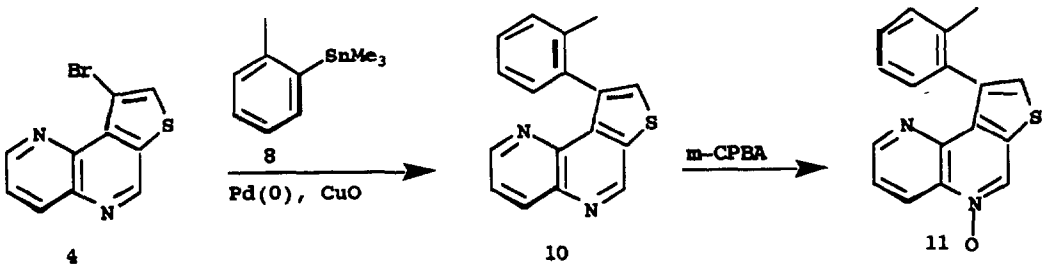
In another more successful approach we reacted 1-bromothieno[2,3-*c*]-1,5-naphthyridine **4** with 2-methyl-1-trimethylstannylbenzene **8** using dichloro-(diphenylphosphinebutane)palladium(II), $PdCl_2$(dppb) [16], as catalyst and copper(II)oxide as co-reagent [15], which gave the desired 1-(2-methylphenyl)thieno[2,3-*c*]-1,5-naphthyridine **10** in 42% yield. Once again we found that tetrakis(triphenylphosphine)palladium(0) is not the most effective catalyst in this type of reactions [8, 15].

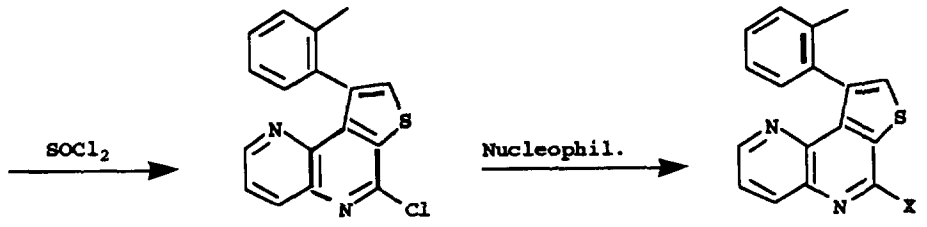
Oxidation of **10** with *m*-chloroperbenzoic acid according to Malm et al [10] gave 1-(2-methylphenyl)thieno[2,3-*c*]-1,5-naphthyridine-5-oxide **11**. The selectivity for the 5-nitrogen was as high as 70%, which was expected from the work of Malm et al where the same high regioselectivity was found in the *N*-oxidation of the parent compound thieno[2,3-*c*]-1,5-naphthyridine **3** [10], and in the bromo derivative **4** [12].

In the ^{1}H NMR spectrum of **11** the normal shielding of the proton in the 4-position and the deshielding of the proton in the 6-position could be observed [10, 12]. Chlorination of **11** with thionyl chloride [13] gave 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** in a yield of 49%. This compound was treated with a variety of amino nucleophiles. The reactions were run either in a sealed tube or, if the amine had a high enough boiling point, in a round-bottomed flask. All these nucleophilic substitutions went fairly well, producing compounds **12–18** with yields ranging from 47–88%.

Biochemistry

Compounds **9** and **12–18** were tested for their ability to inhibit H^+,K^+-ATPase activity and, in the most interesting cases, also for their effect on acid formation in gastric glands detected by [^{14}C]-aminopyrine accumulation. Results obtained from these two assays are given in table I. As a reference, in vitro activities of SR & F 96067, a substituted 4-aminoquinoline developed by Smith Kline, Beecham Pharmaceuticals,





12 X = $NHCH_3$

13 X = $N(CH_3)_2$

14 X = $NHCH_2CH_3$

15 X = $NH(CH_2)_2OH$

16 X = $NH(CH_2)_3OH$

17 X = $NH(CH_2)_4OH$

18 X = $N(CH_2)_2(CH_2)_2NMe$

are included in table I. SK & F 96067 has been tested for antisecretory effects in man and shown to have greater efficacy than ranitidine, an H_2-receptor antagonist [17].

The choice of substituents in the 4-position was of those which had been found to be most effective in the 1-aryl pyrrolo[3,2-*c*]quinoline series [3]. However, these compounds were not active enough to be of pharmacalogical interest with regard to both their ability to inhibit H^+,K^+-ATPase activity and acid formation in gastric glands. Interestingly enough, **18** with the *N*-methylpiperazinyl substituent, a substituent not included in the the 1-arylpyrrolo[3,2-*c*]quinoline series, was found to be the most active compound in both preparations.

Experimental protocols

Chemistry

^1H NMR spectra were recorded on a Varian XL-300 Spectrometer. Mass spectra and high resolution mass spectra were

Table I. Effects on H^+,K^+-ATPase activity and acid formation in gastric glands.

Compound	*H^+,K^+-ATPase IC_{50} (μM) or % inhibition at 100 μM*	*Gastric glands IC_{50} (μM) or % inhihbition at 100 μM*
SK & F96067	3.6	2.7
9	27%	0%
12	101	ND
13	48.3	69.6
14	52.8	ND
15	44%	ND
16	36%	ND
17	94.6	24.8
18	31.6	11.4

ND = not determined.

recorded on a JEOL JMS-SX 102 spectrometer. All melting points are uncorrected. Deuteriochloroform was used as the solvent for all ^{1}H NMR samples. *m*-Chloroperbenzoic acid (*m*-CPBA) and ethylamine (70% in water) were purchased from Merck. 2-Bromotoluene, methylamine (40% in water), 2-amino-1-ethanol and 3-amino-1-propanol were purchased from Janssen. 4-Amino-1-butanol was purchased from Aldrich. Thionyl chloride, dimethylamine (40% in water) and 1-methylpiperazine were purchased from Riedel de Haën.

Chloroform was distilled over phosphorous pentoxide, diethyl ether was distilled over sodium and dimethylformamide was distilled and stored over molecular sieves. All other solvents were purchased from the manufacturer in analytical grade and used without further purification. Dichloro(diphenylphosphinebutane)palladium(II) [16], 2-methyl-1-trimethylstannyl benzene **8** [18], 1-bromothieno[2,3-*c*]-1,5-naphthyridine **4** [12] and 1-bromo-4-chloro-thieno[2,3-*c*]-1,5-naphthyridine **9** [13] were prepared by published procedures.

2-Methylphenylboronic acid ***7***

To a solution of 115.5 g (0.675 mol) 2-bromotoluene in 450 mL anhydrous ether, 372 mL of 2.10 M butyllithium in cyclohexane was added at –70° C under nitrogen. After stirring for 30 min, 189 g (0.825 mol) butyl borate in 750 mL anhydrous ether was added all at once. The reaction mixture was stirred at –70 °C for an additional 4 h, and then allowed to warm to 0° C. With ice-cooling, 900 mL of 2 M hydrochloric acid was added. The phases were separated and the aqueous phase was extracted with ether. The combined ether phases were extracted with 1050 mL of cooled 2 M sodium hydroxide solution in three portions. Upon acidification with 2 M hydrochloric acid and with ice-cooling, 39 g (36%) of the boronic acid was isolated. Its physical properties were identical with those given in the literature [19].

1-(2-Methylphenyl)thieno[2,3-c]-1,5-naphthyridine ***10***

A mixture of 530 mg (2.00 mmol) 1-bromothieno-[2,3-*c*]-1,5-naphthyridine **4**, 60 mg (0.10 mmol) dichloro(diphenylphosphinebutane)palladium(II) and 160 mg (2.00 mmol) copper-(II)oxide in 8.0 mL *N,N*-dimethylformamide was stirred at 100° C under nitrogen. After 5 min, 763 mg (3.00 mmol) 2-methyl-1-trimethylstannylbenzene **8** in 2.0 mL *N,N*-dimethylformamide was added all at once to the reaction mixture. When the starting materials were consumed (10 h), the reaction mixture was allowed to reach room temperature. The precipitate was filtered off and the filtrate was evaporated. The residue was subjected to column chromatography on silica, using chloroform/methanol 99:1 as eluent, whereupon it was purified by HPLC on a silica column using chloroform/2-propanol (99.5:0.5) as eluent. The title compound was obtained as white crystals in a yield of 234 mg (42%), mp 98–100 °C. ^{1}H NMR: δ 9.39 (s, 1H, H4), 8.67 (dd, 1H, H8, *J* = 4.2, 1.7 Hz), 8.46 (dd, 1H, H6, *J* = 8.4, 1.7 Hz), 7.65 (s, 1H, H2), 7.53 (dd, 1H, H7, *J* = 8.4, 4.2 Hz), 7.25–7.40 (m, 4H, Ph), 2.04 (s, 3H, CH_3). MS: *m/z* 276 (M, 38), 261 (M-CH_3, 100), 242 (10), 130 (10); peak matching on M$^+$. Calc for $C_{17}H_{12}N_2S$: 276.0721; found 276.0723.

1-(2-Methylphenyl)thieno[2,3-c]-1,5-naphthyridine-5-oxide ***11***

To a stirred mixture of 138 mg (0.50 mmol) 1-(2-methylphenylthieno[2,3-*c*]-1,5-naphthyridine **10** in 5.0 mL chloroform, 130 mg (0.75 mmol) *m*-CPBA was added in small portions over a period of 15 min at room temperature. After 4 h the reaction mixture was diluted with 100 mL chloroform, washed with 2 × 25 mL of 2.0 M sodium hydroxide and 25 mL water, and finally dried over magnesium sulfate. After evaporation the product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. The title compound was obtained as yellow crystals, in a yield of 102 mg (70%), mp 81–83 °C. ^{1}H NMR: δ 9.11(dd, 1H, H6, *J* = 8.7, 1.7 Hz), 9.05 (s, 1H, H4), 8.73 (dd, 1H, H8, *J* = 4.3, 1.7 Hz), 7.59 (dd, 1H, H7, *J* = 8.7, 4.3 Hz), 7.56 (s, 1H, H2), 7.25–7.40 (m, 4H, Ph), 2.04 (s, 3H, CH_3). MS: *m/z* 292 (M, 60), 277 (M-CH_3, 100), 261 (M-O, 55), 248 (10), 237 (10); peak matching on M$^+$. Calc for $C_{17}H_{12}N_2OS$: 292.0670; found 292.0668.

1-(2-Methylphenyl)-4-chlorothieno[2,3-c]-1,5-naphthyridine ***9***

In 0.5 mL thionyl chloride, 50 mg (0.17 mmol) 1-(2-methylphenyl)thieno[2,3-*c*]-1,5-naphthyridine-5-oxide **11** was quickly dissolved. The reaction mixture was stirred at room temperature for 6 h. The thionyl chloride was evaporated, the residue was treated with 40 mL saturated sodium bicarbonate solution and then extracted with 3 × 10 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The crude product was purified by HPLC on a silica column using chloroform/2-propanol (99:1) as eluent. After purification 26 mg (49%) of the title compound was isolated as white crystals, mp 170–172 °C. ^{1}H-NMR δ 8.66 (dd, 1H, H8, *J* = 4.3, 1.7 Hz), 8.38 (dd, 1H, H6, *J* = 8.4, 1.7 Hz), 7.68 (s, 1H, H2), 7.54 (dd, 1H, H7, *J* = 8.4, 4.3 Hz), 7.28–7.43 (m, 4H, Ph), 2.04 (s, 3H, CH_3). MS: *m/z* 310 (M, 35), 295 (M-CH_3, 100), 241 (80), 239 (75), 160 (30), 116 (35); peak matching on M$^+$. Calc for $C_{17}H_{11}ClN_2S$: 310.0331; found 310.0331.

1-(2-Methylphenyl)-4-(methylamino)thieno[2,3-c]-1,5-naphthyridine ***12***

A mixture consisting of 31 mg (0.10 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 3.0 mL methylamine (40% in water) was heated to 180 °C in a pressure vessel for 18 h. The reaction mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. The title compound was obtained as white crystals in a yield of 15 mg (49%), mp 215–220 °C. ^{1}H NMR: δ 8.31 (dd, 1H, H8, *J* = 4.3, 1.7 Hz), 8.10 (dd, 1H, H6, *J* = 8.4, 1.7 Hz), 7.41 (s, 1H, H2), 7.33 (dd, 1H, H7, *J* = 8.4, 4.3 Hz), 7.23–7.37 (m, 4H, Ph), 4.87 (br m, 1H, NH), 4.83 (d, 3H, N-CH_3, *J* = 4.8 Hz), 2.03 (s, 3H, CH_3). MS: *m/z* 305 (M, 40), 290 (M-CH_3, 100), 274 (M-$(CH_3)_2$, 15), 261 (10), 152 (10); peak matching on M$^+$. Calc for $C_{18}H_{15}N_3S$: 305.0987; found 305.0986.

1-(2-Methylphenyl)-4-(dimethylamino)thieno[2,3-c]-1,5-naphthyridine ***13***

A mixture consisting of 31 mg (0.10 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 3 mL dimethylamine (40% in water) was heated to 180 °C in a pressure vessel for 18 h. The mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. The title compound was obtained as a slightly yellow oil in a yield of 15 mg (47%). ^{1}H NMR: δ 8.28 (dd, 1H, H8, *J* = 4.2, 1.7 Hz), 8.04 (dd, 1H, H6, *J* = 8.4, 1.7 Hz), 7.46 (s, 1H, H2), 7,31 (dd, 1H, H7, *J* = 8.4, 4.2 Hz), 7.25–7.36 (m, 4H, Ph), 3.48 (s, 6H, N$(CH_3)_2$), 2.01 (s, 3H, CH_3). MS: *m/z* 319 (M, 40), 304 (M-CH_3, 100), 275 (M-N$(CH_3)_2$, 30), 261 (M-CH_3-N$(CH_3)_2$, 20), 45 (N$(CH_3)_2$, 50); peak matching on M$^+$. Calc for $C_{19}H_{17}N_3S$: 319.1143; found 319.1141.

1-(2-Methylphenyl)-4-(ethylamino)thieno[2,3-c]-1,5-naphthyridine ***14***

A mixture consisting of 31 mg (0.10 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 2 mL ethylamine (70% in water) was heated to 180 °C in a pressure vessel for 18 h. The mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. The title compound was obtained as white crystals in a yield of 28 mg (88%), mp 93–95 °C. ^{1}H NMR: δ 8.31 (dd, 1H, H8, J = 4.3, 1.7 Hz), 8.07 (dd, 1H, H6, J = 8.3, 1.7 Hz), 7.40 (s, 1H, H2), 7.32 (dd, 1H, H7, J = 8.4, 4.3 Hz), 7.25–7.36 (m, 4H, Ph), 4.78 (t, 1H, N-H, J = 4.6 Hz), 3.82 (m, 2H, CH_2), 2.03 (s, 3H, CH_3), 1.42 (t, 3H, CH_3, J = 7.2 Hz). MS: *m/z* 319 (M, 45), 304 (M-CH_3, 100), 276 (M-CH_3-CH_2CH_3, 15), 261 (M-CH_3-$NHCH_2CH_3$); peak matching on M$^+$. Calc for $C_{19}H_{17}N_3S$: 319.1143; found 319.1147.

1-(2-Methylphenyl)-4-[(2-hydroxyethyl)amino]thieno[2,3-c]-15-naphthyridine ***15***

A mixture consisting of 31 mg (0.10 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 1.0 mL 2-amino-1-ethanol was heated to 150 °C in a sealed round-bottomed flask for 3 h. The mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. The title compound was obtained as a slightly yellow oil in a yield of 20 mg (60%). ^{1}H NMR: δ 8.34 (dd, 1H, H8, J = 4.3, 1.7 Hz), 8.03 (dd, 1H, H6, J = 8.4, 1.7 Hz), 7.46 (s, 1H, H2), 7.34 (dd, 1H, H7, J = 8.4, 4.3 Hz), 7.26–7.40 (m, 4H, Ph), 5.49 (br s, 1H, OH), 5.42 (m, 1H, NH) 3.88–4.02 (m, 4H, CH_2), 2.03 (s, 3H, CH_3). MS: *m/z* 335 (M, 60), 320 (M-CH_3, 100), 304 (M-CH_2-OH, 30), 276 (M-$NHCH_2CH_2OH$, 50), 260 (M-$NHCH_2CH_2OH$ and CH_3, 20); peak matching on M$^+$. Calc for $C_{19}H_{17}N_3OS$; 335.1092; found 335.1090.

(2-Methylphenyl)-4-[(3-hydroxypropyl)amino]thieno-[2,3-c]-1,5-naphthyridine ***16***

A mixture consisting of 31 mg (0.10 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 1.0 mL 3-amino-1-propanol was heated to 150 °C in a sealed round-bottomed flask for 3 h. The mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. The title compound was obtained as a slightly yellow oil in a yield of 30 mg (86%). ^{1}H NMR: δ 8.32 (dd, 1H, H8, J = 4.3, 1.6 Hz), 8.02 (dd, 1H, H6, J = 8.4, 1.6 Hz), 7.44 (s, 1H, H2), 7.35 (dd, 1H, H7, J = 8.4, 4.3 Hz), 7.23–7.40 (m, 4H, Ph), 5.66 (s br, 1H, OH), 5.22 (t, 1H, N-H, J = 6.5 Hz), 3.96 (m, 2H, CH_2), 3.70 (m, 2H, CH_2), 2.04 (s, 3H, CH_3), 1.91 Hz (m, 2H, CH_2). MS: *m/z* 349 (M, 75), 334 (M-CH_3, 100), 318 (30), 290 (35), 276 (40), 260 (15), 151 (15); peak matching on M$^+$. Calc for $C_{20}H_{19}N_3OS$: 349.1249; found 349.1257.

(2-Methylphenyl)-4-[(4-hydroxybutyl)amino-thieno[2,3-c]-1,5-naphthyridine (17)

A mixture consisting of 31 mg (0.10 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 1.0 mL 4-amino-1-butanol was heated to 150 °C in a sealed round-bottomed flask for 3 h. The mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The product was purified by HPLC on a silica column using chloroform/2-propanol (96:4) as eluent. 31 mg (85%) of a transparent oil was obtained, ^{1}H NMR: δ 8.30 (dd, 1H, H8, J = 4.3, 1.7 Hz), 8.10 (dd, 1H, H6, J = 8.4, 1.7 Hz), 7.41 (s, 1H, H2), 7.32 (dd, 1H, H7, J = 8.4, 4.3 Hz), 7.20–7.39 (m, 4H, Ph), 5.16 (t, 1H, NH, 5.6), 3.80–3.88 (m, 4H, $(CH_3)_2$), 3.18 (s br, 1H, OH), 2.03 (s, 3H, CH_3), 1.88–1.98 (m, 2H, CH_2), 1.71–1.82 (m, 2H, CH_2). MS: *m/z* 363 (M, 65), 348 (M-CH_3, 100), 318 (25), 304 (30), 276 (55), 260 (20), 151 (20); peak matching on M$^+$. Calc for $C_{21}H_{21}N_3OS$: 363.1405; found 363.1405.

1-(2-Methylphenyl)-4-(4-N-methylpiperazinyl)thieno[2,3-c]-1,5-naphthyridine ***18***

A mixture consisting of 31 mg (0.1 mmol) 1-(2-methylphenyl)-4-chlorothieno[2,3-*c*]-1,5-naphthyridine **9** and 1.0 mL 1-methylpiperazine was heated to 120 °C in a sealed round-bottomed flask for 24 h. The mixture was poured into 10 mL water, neutralized with 2 M hydrochloric acid, and extracted with 3 × 5 mL chloroform. The organic phase was dried over magnesium sulfate and evaporated. The title compound was obtained as a slightly yellow oil in a yield of 18 mg (48%). ^{1}H NMR: δ 8.37 (dd, 1H, H8, J = 4.3, 1.7 Hz), 8.12 (dd, 1H, H6, J = 8.4, 1.7 Hz), 7.46 (s, 1H, H2), 7.35 (dd, 1H, H7, J = 8.4, 4.3 Hz), 7.23–7.40 (m, 4H, Ph), 3.88 (t, 4H, Ar-N-$(CH_2)_2$, J = 4.9 Hz), 2.70 (t, 4H, $(CH_2)_2$, J = 4.9 Hz), 2.42 (s, 3H, N-CH_3), 2.01 (s, 1H, CH_3). MS: *m/z* 374 (M, 10), 304 (100), 292 (20), 275 (12), 261 (10), 83 (35, piperazine); peak matching on M$^+$. Calc for $C_{22}H_{22}N_4S$: 374.1565; found 374.1558.

Biochemistry

Reagents

All chemicals were of the highest grade available. Collagenase Type IA, was obtained from Sigma. [Dimethyl amine-C^{14}]aminopyrine was obtained from Amersham Life Science.

Preparation of gastric membrane vesicles

Gastric membrane vesicles containing H$^+$,K$^+$-ATPase were prepared from hog stomachs as previously described [20]. Briefly, tissue was homogenized and a microsomal fraction was obtained by differential centrifugation. The pelleted material was separated on a discontinuous density gradient and the fraction at the interface between the 0.25 M sucrose and the 0.25 sucrose plus 7.5% Ficoll layers, designated fraction GI, was collected.

Permeable vesicles

The GI fraction was diluted with 1 mM Pipes/Tris (pH 7.4), to obtain a 1% sucrose concentration, homogenized, and centrifuged at 100 000 *g* for 2 h. The resulting pellet was suspended in water and lyophilized twice.

Determination of H$^+$,K$^+$-ATPase activity

Permeable membrane vesicles (2.5–5.0 μg) were incubated for 15 min at 37 °C in 18 mM Pipes/Tris buffer pH 7.4 containing 2 mM magnesium chloride, 10 mM potassium chloride and 2 mM ATP. The ATPase activity was estimated as the release of inorganic phosphate from ATP, as described by LeBel et al [21]. The ATPase activity was approximately 5 μmol P_i/mg.h in the absence of potassium chloride and 160 μmol P_i/mg.h in the presence of 10 mM potassium chloride.

Protein determination
Protein was determined according to Bradford, using the Bio-Rad Protein Assay kit [22].

Preparation of gastric glands
Isolated gastric glands were prepared from rabbit, according to Berglindh et al [23]. The minced gastric corpus mucosa was digested with collagenase at 37 °C for approximately 60 min. After digestion the glands were rinsed three times and resuspended to a concentration of 80 mg wet weight per mL.

Determination of acid formation in gastric glands
The glands were resuspended in medium, pH 7.4 containing (in mM); sodium chloride, 132.4; potassium chloride, 5.4; magnesium sulphate, 1.2; calcium chloride, 1.0; sodium dihydrogen phosphate, 1.0; disodium hydrogen phosphate, 5.0; indomethacin, 0.01; glucose 2 mg/mL and albumin 2 mg/mL. Acid formation in the glands was monitored by the uptake of the weak base ^{14}C-aminopyrine [23]. Secretagogue activation was carried out by the addition of 1 mM dibutyryl cAMP.

Compounds
The compounds were dissolved in methanol. Aliquots were pipetted into the incubation media giving a final methanol concentation of 1%, which on its own had no effect on the enzyme or gland preparation.

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