Polycyclic *N*-Heterocyclic Compounds. **50** [1]. Synthesis and Pharmacological Evaluation of 2,3,6,7-Tetrahydrothieno[2,3-*h*]-imidazo[2,1-*f*][1,6]naphthyridines, 3,4,7,8-Tetrahydro-2*H*-thieno-[2,3-*h*]pyrimido[2,1-*f*][1,6]naphthyridines and their Precursor

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Some novel 5-hydroxyalkylamino-1,2-dihydrothieno[2,3-*h*][1,6]naphthyridines were prepared by the reaction of 5-chloro-1,2-dihydrothieno[2,3-*h*][1,6]naphthyridine derivatives with some aminoalcohols in the presence of base. These derivatives were cyclized to the corresponding imidazo or pyrimido derivatives. The bronchodilatory activity of these compounds was evaluated on the basis of their relaxation activity to tracheal contraction induced by carbamylcholine chloride as a primary *in vitro* assays. Effect of some naphthyridines on carbamylcholine chloride-induced contractions of trachea in the presence or absence of milrinone or 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one, which is inhibitor of phosphodiesterase III or IV, were also evaluated.

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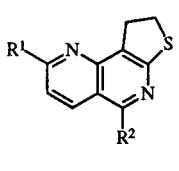
Recently we prepared new tricyclic heterocycles, 5-amino-1,2-dihydrothieno[2,3-*h*][1,6]naphthyridine **1a** and its 8-methyl analogue **1b** [2,3], and found that compound **1a** had the potent relaxation activity on isolated guinea pig tracheal muscle contraction induced by carbamylcholine chloride *in vitro* [4]. During our research, Sherlock *et al.* [5] and Kreutner *et al.* [6] respectively reported that some derivatives bearing a 1,8-naphthyridin-2(1*H*)-one skeleton showed potent antiallergic activity, mainly due to their inhibition of the release of leukotrienes. Prevention of the release of mediators from mast cell and basophils is considered to be useful as one approach in the treatment of asthma.



1a,	$R^1 = H$,	$R^2 = NH_2$
b,	$R^1 = CH_3$,	$R^2 = NH_2$
2a,	$R^1 = H$,	$R^2 = OH$
b,	$R^1 = CH_3$,	$R^2 = OH$
3a,	$R^1 = H$,	$R^2 = Cl$
b,	$R^1 = CH_3$,	$R^2 = Cl$

Figure

On the other hand, Suzuki *et al.* [7] reported the synthesis and structure-activity relationships of derivatives of imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones as new bronchodilators. We have been interested in both their pharmacological profiles and the parent structure, that is, 1,8-naphthyridin-2(1*H*)-one. Because their structure is a bioisostere of 1,6-naphthyridine-7(8*H*)-thione which is regarded as a partial moiety of **1a**. This fact inspired us to perform molecular modifications of **1a** and **1b** and correlate structure *vs* activity in the aim of developing new potential drug candidates for asthmatic diseases. Furthermore, as described above, imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones were reported to exhibit potent bronchodilator activity *in vitro* and *in vivo* [7]. Medwid *et al.* also have developed triazolo[1,5-*c*]pyrimidines as potential antiasthmatic agents [8]. On these basis, hybridization of the 1,6-naphthyridine-7(8*H*)-thione moiety onto imidazo[4,5-*c*]-[1,8]naphthyridin-4(5*H*)-one or triazolo[1,5-*c*]pyridine skeleton resulted in novel tetracyclic compounds, thieno[2,3-*h*]imidazo(or pyrimido)[2,1-*f*][1,6]naphthyridines **5** (or **7**) as further synthetic targets.

This paper describes the synthesis of additional analogs of **1a** and **1b** and their cyclized products as well as the tracheal muscle relaxation activity of the new compounds and those reported previously [2,3].

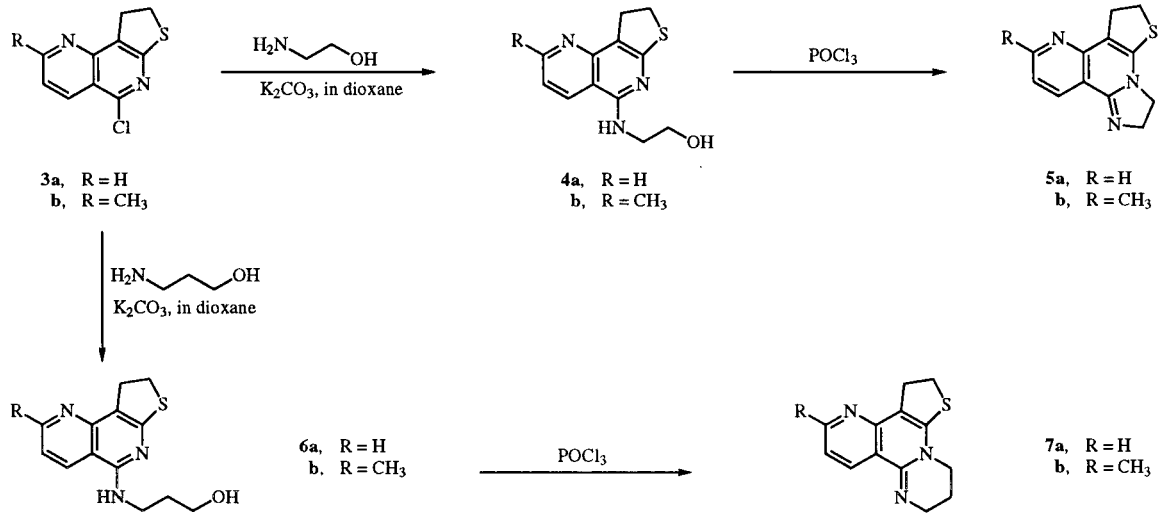
Chemistry.

A preparation of 5-oxo-1,2,4,5-tetrahydrothieno[2,3-*h*]-[1,6]naphthyridine (keto form of **2a**), 5-chloro-1,2-dihydrothieno[2,3-*h*][1,6]naphthyridine **3a**, and their 8-methyl analogues **2b** and **3b** starting from 2-(3-cyanopropylthio)-pyridine-3-carbonitrile (or its 6-methyl derivative) *via* **1a** (or **1b**) has been described in the previous paper [3].

The chloro group of **3a,b** is an useful replaceable group for substitution with a functional moiety. Therefore, introducing a hydroxyalkylamino group onto 5 position of thieno-[2,3-*h*][1,6]naphthyridine ring was employed as the first stage of synthetic strategy for the purposes of biological evaluation.

As shown in Scheme, 5-(2-hydroxyethylamino) derivatives **4a,b** were prepared from **3a,b** by the reaction with 2-aminoethanol in the presence of potassium carbonate in dioxane. Cyclization of the hydroxylalkylamino derivatives **4a,b** to the corresponding imidazo derivatives **5a,b** was attempted using phosphorus oxychloride which was previously employed in our laboratory for the cyclization of this type [9]. 5-(3-Hydroxypropylamino) derivatives **6a,b** and their cyclized products **7a,b** were also prepared by the similar manner. The cyclized products **5** and **7**, both and **6a** on carbamylcholine chloride-induced contraction of guinea pig trachea in the presence or absence of phosphodiesterase inhibitor were studied, and milrinone, 1-methyl-3-(2-methylpropyl)-3,7-dihydro-1*H*-purine-2,6-dione, rolipram, and 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one were also used as a reference. To assess the selectivity of compounds on phosphodiesterase isozymes, milrinone (3 μg/ml) for the inhibition of phosphodiesterase III or 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one (3 μg/ml) for phosphodiesterase IV were

Scheme



of which have a novel ring system, were characterized by the disappearance of hydroxyl group in the ir spectrum, observation of the parent peak (or the fragment peak by elimination of hydrogen chloride from the parent peak in the case of hydrochloride of the free base **5a** and **7a**) in the FAB-ms, ^{1}H-nmr data, and elemental analysis.

Pharmacological Results and Discussion.

In order to test bronchodilatory activity of the above compounds, the inhibition of carbamylcholine chloride-induced contraction in trachea isolated from guinea pigs was employed. Compounds which produced more than 30% relaxation at 10 μg/ml, which was calculated from the percent of maximum relaxation by papaverine, were regarded as active, their IC_{30} value were obtained by a cumulative method, and aminophylline, milrinone, and 1-methyl-3-(2-methylpropyl)-3,7-dihydro-1*H*-purine-2,6-dione were employed as a reference compound. Subsequently, effects of some active and non-active compounds **4a**, **4b** added to the buffer before the addition of carbamylcholine chloride. Their IC_{30} values shows the concentration of each compound which gives 30% relaxation to tracheal contraction by 1 μ*M* of carbamylcholine chloride. It was calculated from the percent of maximum relaxation by papaverine. The pharmacological activity of these compounds is summarized in Table I and II. Half of the tested compounds in this experiment caused relaxation of trachea isolated from guinea pigs. The introduction of methyl group at the 8-position apparently reduced the activity. Compounds **4a** and **6a** bearing secondary alkylamine at the 5-position possessed higher activity than the compound bearing primary amine **1a**. Tetracyclic compounds except for **7a**, possessed no activity. The effect of compound **7a** was the most potent among the tested compounds, however, its activity was almost the same as **6a** which is the precursor of the cyclized product **7a**. At least, the changing from tricyclic compounds to tetracycles had no advances for possessing the activity.

Table I

IC_{30} Values of Thieno[2,3-*h*][1,6]naphthyridine Derivatives on Carbamylcholine Chloride-Induced Tracheal Response *in vitro*

Compound	IC_{30} Value μg/ml	μ*M*
1a	6.76	28.2
1b	>10	—
2a	5.01	24.5
2b	5.75	26.3
3a	5.01	22.5
3b	>10	—
4a	4.90	19.8
4b	>10	—
5a	>10	—
5b	>10	—
6a	4.90	18.7
6b	>10	—
7a	5.13	18.3
7b	>10	—
aminophylline	36.7	87.3
milrinone	5.42	25.7
1-methyl-3-(2-methylpropyl)-3,7-dihydro-1*H*-purine-2,6-dione	1.42	6.52

EXPERIMENTAL

All melting points were determined on a Yanagimoto micro-melting point apparatus, and are uncorrected. Elemental analyses were performed on a Yanagimoto MT-5 CHN Corder elemental analyzer. The EI-, FAB-, and hrms spectra were recorded on a VG 70-SE mass spectrometer, using glycerol or *m*-nitrobenzyl alcohol as a matrix agent. The ir spectra were recorded on a Japan Spectroscopic IRA-102 diffraction grating infrared spectrophotometer. Unless otherwise stated, they were measured as potassium bromide pellets and frequencies are expressed in cm^{-1}. The nmr spectra were recorded on a Varian VXR-200 instrument (200 MHz) in the solvent indicated with tetramethylsilane as the internal standard. Chemical shifts are reported in ppm (δ) and J values in Hz, and the signals are designated as follows; s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; quin, quintet; br, broad. Unless otherwise stated extracted solutions were dried over anhydrous sodium sulfate. Evaporation refer to the removal of volatile materials under reduced pressure at 40-60° on a rotary vacuum evaporator.

5-(2-Hydroxyethylamino)-1,2-dihydrothieno[2,3-*h*][1,6]naphthyridine (**4a**).

To a stirred suspension of **3a** (900 mg, 4.05 mmoles) in dioxane (8 ml) were gradually added 2-aminoethanol (3.08 g, 40.5 mmoles) and potassium carbonate (1.12 g, 8.12 mmoles). The resulting mixture was refluxed for 2 hours. Excess reagent was removed and then ice-water (20 ml) was poured onto the residue. The resulting mixture was acidified with acetic acid (*p*H 4-5) and precipitated yellowish crystalline solid was collected. The filtrate was extracted with ethyl acetate (50 ml) and the organic layer was washed with brine, dried, evaporated, and combined with the above solid obtained by suction. This combined solid was recrystallized from a mixture of benzene and ethanol to afford **4a** (920 mg, 92%) as yellow prisms, mp 149-152°; ir: cm^{-1}

Table II

Effects of Naphthyridines and Phosphodiesterase Inhibitors on Carbamylcholine Chloride-Induced Contraction in the Presence or Absence of Milrinone or 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one *in vitro*

Compound	IC_{30} (μg/ml) - [a]	+ milrinone [b]	+ 4-(3-butoxy-4-methoxyphenyl)-imidazolidin-2-one [c]
4a	4.90	3.72	>10
4b	>10	2.15	>10
6a	4.90	2.65	>10
milrinone	5.42	4.62	0.433
1-methyl-3-(2-methylpropyl)-3,7-dihydro-1*H*-purine-2,6-dione	1.45	0.668	1.32
rolipram	>10	<0.025	>10
4-(3-butoxy-4-methoxyphenyl)-imidazolidin-2-one	>10	0.0343	>10

[a] Absence of milrinone and 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one; [b] Presence of milrinone; [c] Presence of 4-(3-butoxy-4-methoxyphenyl)-imidazolidin-2-one.

The effects of naphthyridines tested here on tracheal relaxation were not so potentiated by the pre-treatment with milrinone or 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one. With regard to the selectivity of naphthyridine derivatives on phosphodiesterase isozymes, all of them showed a slightly stronger selectivity on phosphodiesterase IV than III. However, the selectivity of these compounds is less than that of milrinone and rolipram.

3340 (OH), 3220 (NH); ms: (FAB) m/z 248 (MH^+); ^1H nmr (dueteriochloroform): δ 3.56 (m, 4H, H1 and 2), 3.77 (td, J_t = 5, J_d = 3, 2H, CH_2N), 3.92 (t, J = 5, 2H, OCH_2), 5.84 (br t, J = 3, 1H, NH), 7.18 (dd, $J_{7,6}$ = 8.4, $J_{7,8}$ = 4.4, 1H, H7), 8.01 (dd, $J_{6,7}$ = 8.4, $J_{6,8}$ = 1.6, 1H, H6), 8.85 (dd, $J_{8,7}$ = 4.4, $J_{8,6}$ = 1.6, 1H, H8).

Anal. Calcd. for $C_{12}H_{13}N_3OS$: C, 58.28; H, 5.30; N, 16.99. Found: C, 58.14; H, 5.37; N, 16.99.

5-(2-Hydroxyethylamino)-8-methyl-1,2-dihydrothieno[2,3-*h*]-[1,6]naphthyridine (**4b**).

To a stirred suspension of **3b** (200 mg, 0.85 mmole) in dioxane (2 ml) were added 2-aminoethanol (516 mg, 8.47 mmoles) and potassium carbonate (233 mg, 1.69 mmoles), the resulting mixture was refluxed for 16 hours. The post-treatment was performed in a manner similar to that of **4a**, and **4b** (190 mg, 86%) was recrystallized from ethanol as yellowish prisms, mp 199-201°; ir: cm^{-1} 3370 (OH), 3220 (NH); hrms: (FAB, MH^+) m/z Calcd. for $C_{13}H_{16}N_3OS$: 262.1014. Found: 262.0978; ^1H nmr (dueteriochloroform): δ 2.67 (s, 3H, CH_3), 3.43-3.66 (m, 4H, H1 and 2), 3.76 (q, J = 5, 2H, NCH_2), 3.91 (t, J = 5, 2H, CH_2O), 5.71 (br, deuterium oxides exchangeable, 1H, NH), 7.07 (d, $J_{7,6}$ = 8.5, 1H, H7), 7.88 (d, $J_{6,7}$ = 8.5, 1H, H6).

Anal. Calcd. for $C_{13}H_{15}N_3OS \cdot 0.5H_2O$: C, 57.76; H, 5.97; N, 15.54. Found: C, 57.97; H, 5.76; N, 15.69.

2,3,6,7-Tetrahydrothieno[2,3-*h*]imidazo[2,1-*f*][1,6]naphthyridine (**5a**) as the Hydrochloride.

A stirred suspension of **4a** (1.05 g, 4.25 mmoles) in phosphorus oxychloride (4.31 ml, 42.5 mmoles) was refluxed for 1 hour. The reaction mixture was evaporated and ice-water (20 ml) was poured onto the residue. Thus obtained mixture was basified with saturated sodium hydrogen carbonate solution (*p*H 8-9) to precipitate yellowish solid, which was collected by suction. The filtrate was extracted with ethyl acetate (100 ml), and the organic layer was washed with brine, dried and evaporated to give a yellowish residue. This residue was combined with the above solid obtained by suction, and it was dissolved in ethanol to convert to corresponding hydrochloride by the treatment with hydrogen chloride gas. This salt was recrystallized from a mixture of ethanol and water to afford **5a** (1.0 g, 89%) as yellow needles, mp 260° dec; ir: cm^{-1} 3440 (NH); hrms: (FAB, MH^+ - HCl) m/z Calcd. for $C_{12}H_{12}N_3S$: 230.0751. Found: 230.0752; ^1H nmr (dimethyl-d_6 sulfoxide): δ 3.55 and 3.80 (each t, J = 8, each 2H, H6 and 7), 4.17 and 4.58 (each dd, J_1 = 11, J_2 = 9, each 2H, H2 and 3), 7.61 (dd, $J_{10,11}$= 8.4, $J_{10,9}$ = 4.6, 1H, H10), 8.81 (dd, $J_{11,10}$ = 8.4, $J_{11,9}$ = 1.7, 1H, H11), 9.09 (dd, $J_{9,10}$ = 4.6, $J_{9,11}$ = 1.7, 1H, H9), 11.31 (br s, deuterium oxide exchangeable, 1H, NH).

Anal. Calcd. for $C_{12}H_{12}ClN_3S \cdot 2.3H_2O$: C, 46.92; H, 5.45; N, 13.68. Found: C, 47.11; H, 5.52; N, 13.56.

9-Methyl-2,3,6,7-tetrahydrothieno[2,3-*h*]imidazo[2,1-*f*][1,6]-naphthyridine (**5b**).

To a suspension of **4b** (190 mg, 0.73 mmole) in chloroform (2 ml) was added phosphorus oxychloride (0.13 ml, 1.46 mmoles), and the reaction mixture was stirred at room temperature for 3 hours. After removal of the excess solvent ice-water (20 ml) was poured onto the residue. The resulting aqueous mixture was basified with saturated sodium hydrogen carbonate solution (*p*H 8-9), extracted with ethyl acetate (40 ml), and the organic layer was washed with brine, dried, and evaporated. Thus obtained residue was subjected to column chromatography on ODS gel with acetonitrile-methanol (90:10-50:50, v/v) as an eluent. After evaporation of the solvent the residue was recrystallized from a mixture of benzene and ethanol to afford **5b** (60 mg, 34%) as pale yellow plates, mp 151-154°; hrms: (FAB, MH^+) m/z Calcd. for $C_{13}H_{14}N_3S$: 244.0908. Found: 244.0867; ^1H nmr (deuteriochloroform): δ 2.56 (s, 3H, CH_3), 3.33-3.56 (m, 4H, H6 and 7), 4.08 (m, 4H, H2 and 3), 6.94 (d, $J_{10,11}$ = 8, 1H, H10), 8.18 (d, $J_{11,10}$ = 8, 1H, H11).

Anal. Calcd. for $C_{13}H_{13}N_3S \cdot 1.5H_2O$: C, 57.76; H, 5.97; N, 15.54. Found: C, 57.88; H, 6.10; N, 15.47.

5-(3-Hydroxypropylamino)-1,2-dihydrothieno[2,3-*h*][1,6]naphthyridine (**6a**).

To a suspension of **3a** (650 mg, 2.93 mmoles) in dioxane (6 ml) were added portionwise 3-amino-1-propanol (2.2 g, 29.3 mmoles) and potassium carbonate (808 mg, 5.86 mmoles), and the resulting mixture was refluxed for 4 hours. The post-treatment was performed in a manner similar to that of **4a**, and **6a** (650 mg, 85%) was recrystallized from ethanol as yellow prisms, mp 108-110°; ir: cm^{-1} 3210 (NH), 3150 (OH); hrms: (FAB, MH^+) m/z Calcd. for $C_{13}H_{16}N_3OS$: 262.1014. Found: 262.1015; ^1H nmr (dimethyl-d_6 sulfoxide): δ 1.78 (quin, J = 7, 2H, CH_2CH_2O), 3.30-3.90 (m, 8H, H1 and 2 and NCH_2 and CH_2O), 7.33 (dd, $J_{7,6}$ = 8.3, $J_{7,8}$ = 4.4, 1H, H7), 7.78 (br, deuterium oxide exchangeable, 1H, NH), 8.01 (dd, $J_{6,7}$ = 8.3, $J_{6,8}$ = 1.5, 1H, H6), 8.61 (dd, $J_{8,7}$ = 4.4, $J_{8,6}$ = 1.5, 1H, H8).

Anal. Calcd. for $C_{13}H_{15}N_3OS \cdot 0.7H_2O$: C, 57.00; H, 6.03; N, 15.34. Found: C, 56.82; H, 6.17; N, 15.48.

5-(3-Hydroxypropylamino)-8-methyl-1,2-dihydrothieno[2,3-*h*]-[1,6]naphthyridine (**6b**).

To a stirred suspension of **3b** (400 mg, 1.69 mmoles) in dioxane (5 ml) were gradually added 3-amino-1-propanol (760 mg, 10.2 mmoles) and potassium carbonate (280 mg, 2.03 mmoles), and the resulting mixture was refluxed for 10.5 hours. The post-treatment was performed in a manner similar to that of **4a**, and **6b** (350 mg, 75%) was recrystallized from chloroform as yellow, mp 150-152°; ir: cm^{-1} 3310 (OH), 3190 (NH); ms: (FAB) m/z 276 (MH^+); ^1H nmr (dueteriochloroform): δ 1.84 (quin, J = 5.7, 2H, CH_2CH_2O), 2.67 (s, 3H, CH_3), 3.43-3.66 (m, 4H, H1 and 2), 3.68 and 3.78 (each t, J = 5.7, each 2H, CH_2N and OCH_2), 5.72 (br, deuterium oxide exchangeable, 1H, NH), 7.06 (d, $J_{7,6}$ = 8.5, 1H, H7), 7.87 (d, $J_{6,7}$ = 8.5, 1H, H6).

Anal. Calcd. for $C_{14}H_{17}N_3OS$: C, 61.07; H, 6.22; N, 15.26. Found: C, 60.87; H, 6.19; N, 15.07.

3,4,7,8-Tetrahydro-2*H*-thieno[2,3-*h*]pyrimido[2,1-*f*][1,6]naphthyridine (**7a**) as the Hydrochloride.

A suspension of **6a** (250 mg, 0.96 mmole) in phosphorus oxychloride (0.97 ml, 9.58 mmoles) was refluxed for 1 hour. The post-treatment was performed in a manner similar to that of **5a**, and **7a** (220 mg, 82%) was recrystallized from ethanol as yellowish prisms, mp >300°; ir: cm^{-1} 3480 (NH); hrms: (FAB, MH^+ - HCl) Calcd. for $C_{13}H_{14}N_3S$: 244.0908. Found: 244.0897; ^1H nmr (dimethyl-d_6 sulfoxide): δ 2.21 (quin, J = 7, 2H, H3), 3.54-3.66 (m, 6H, H2, 7 and 8), 4.24 (t, J = 5.8, 2H, H4), 7.63 (dd, $J_{11,12}$ = 8.5, $J_{11,10}$ = 4.5, 1H, H11), 9.01 (dd, $J_{12,11}$ = 8.5, $J_{12,10}$ = 1.4, 1H, H12), 9.06 (dd, $J_{10,11}$ = 4.5, $J_{10,12}$ = 1.4, 1H, H10).

Anal. Calcd. for $C_{13}H_{14}ClN_3S \cdot 1.7H_2O$: C, 50.30; H, 5.65; N, 13.54. Found: C, 50.49; H, 5.69; N, 13.79.

10-Methyl-3,4,7,8-tetrahydro-2*H*-thieno[2,3-*h*]pyrimido[2,1-*f*]-[1,6]naphthyridine (**7b**).

To a mixture of **3d** (120 mg, 0.44 mmole) in chloroform (1 ml) was added phosphorus oxychloride (0.07 ml, 0.87 mmole) under gentle stirring, and the resulting mixture was stirred at room temperature for 3 hours. The post-treatment was performed in a manner similar to that of **5b**, and the column chromatography on ODS gel eluted with acetone-methanol (9:1, v/v) gave **7b** which was recrystallized from a mixture of benzene and ethanol to afford yellow plate (60 mg, 54%), mp 160-161°; hrms: (FAB, MH^+) m/z Calcd. for $C_{14}H_{16}N_3S$: 258.1064. Found 258.1048; ^1H nmr (dueteriochloroform): δ 2.12 (quin, J = 5.5, 2H, H3), 2.62 (s, 3H, CH_3), 3.54 (s, 4H, H7 and 8), 3.72 and 4.00 (each t, J = 5.5, each 2H, H2 and 4), 7.12 (d, $J_{11,12}$ = 8.4, 1H, H11), 8.87 (d, $J_{12,11}$ = 8.4, 1H, H12).

Anal. Calcd. for $C_{14}H_{15}N_3S \cdot 0.6H_2O$: C, 62.71; H, 6.09; N, 15.67. Found: C, 62.95; H, 6.02; N, 15.36.

Tracheal Relaxation *in vitro*.

Tracheal tubes were removed from male Hartley guinea pigs and cut into small pieces (0.5 cm). Each preparation was mounted in an organ bath with 10 ml of Tyrode's solution (sodium chloride 137.9 m*M*, potassium chloride 2.7 m*M*, calcium chloride 1.8 m*M*, magnesium chloride 0.5 m*M*, sodium dihydrogen phosphate 1.1 m*M*, sodium hydrogen carbonate 11.9 m*M*, glucose 5.6 m*M*) prewarmed at 37° and gassed with 95% oxygen-5% carbon dioxide under the initial resting tension of 1 g. The change in contractile force was isometrically measured by a force transducer coupled to an amplifier and recorded on a pen recorder. Tissues were equilibrated for 20 minutes and then carbamylcholine chloride (final 1 μ*M*) was added. After the contraction reached to the stable level, a test compound was cumulatively added. Finally, papaverine (final 0.1 m*M*) was added to obtain the maximal relaxation.

In experiments to assess activities on phosphodiesterase isozymes, either milrinone (3 μg/ml) for the inhibition of phosphodiesterase III or 4-(3-butoxy-4-methoxyphenyl)imidazolidin-2-one (3 μg/ml) for phosphodiesterase IV were added to the buffer 10 minutes before the addition of carbamylcholine chloride.

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REFERENCES AND NOTES

[1] Part **49**, T. Hirota, T. Matsushita, K. Sasaki and S. Kashino, *Heterocycles*, **41**, 2565 (1995).

[2] K. Sasaki, A. S. S. Rouf, S. Kashino and T. Hirota, *J. Chem. Soc., Chem. Commun.*, 1767 (1994).

[3] K. Sasaki, A. S. S. Rouf, S. Kashino and T. Hirota, *Heterocycles*, **41**, 1307 (1995).

[4] Unpublished biological data of naphthyridines.

[5] M. H. Sherlock, J. J. Kaminski, W. C. Tom, J. F. Lee, S. C. Wong, W. Kreutner, R. W. Bryant and A. McPhail, *J. Med. Chem.*, **31**, 2108 (1988).

[6] W. Kreutner, J. Sherwood, S. Sehring, C. Rizzo, R. W. Chapman, M. I. Siegel and R. W. Egan, *J. Pharmacol. Exp. Ther.*, **247**, 997 (1988).

[7] F. Suzuki, K. Kuroda, T. Kawakita, H. Manabe, S. Kitamura, K. Ohmori, M. Ichimura, H. Kase and S. Ichikawa, *J. Med. Chem.*, **35**, 4866 (1992), and related references are cited therein.

[8] J. B. Medwid, R. Paul, J. S. Baker, J. A. Brockman, M. T. Du, W. A. Hallett, J. W. Hanifin, R. A. Hardy, J. M. E. Tarrant, L. W. Torley and S. Wrenn, *J. Med. Chem.*, **33**, 1230 (1990).

[9] T. Hirota, M. Fukumoto, K. Sasaki, T. Namba and S. Hayakawa, *Heterocycles*, **24**, 143 (1986).