New Bronchodilators. 3. Imidazo[4,5-c][1,8]naphthyridin-4(5H)-ones

Fumio Suzuki,* Takeshi Kuroda, Takashi Kawakita, Haruhiko Manabe, Shigeto Kitamura, Kenji Ohmori, Michio Ichimura, Hiroshi Kase, and Shunji Ichikawa

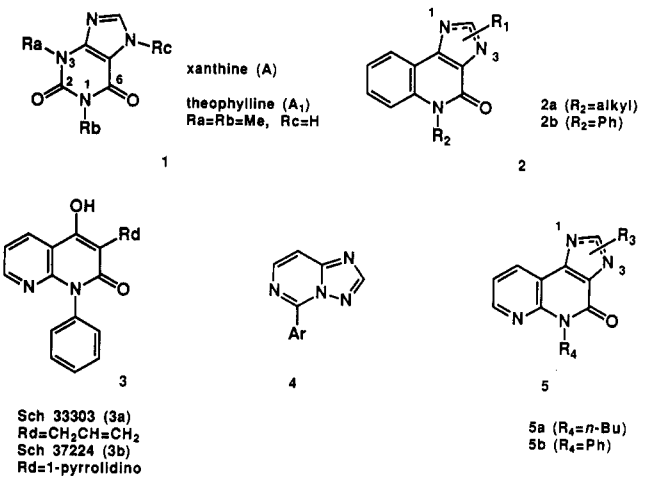
Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Shizuoka 411, Japan

Received June 15, 1992

In order to develop new oral bronchodilators, a series of novel imidazo[4,5-c][1,8]naphthyridin-4(5H)-ones **5** were designed and synthesized. Some of these new heterocycles exhibited more potent bronchodilator activity in vitro and in vivo than theophylline. With respect to modification at the 5-position, both phenyl and *n*-butyl substitution produced potent activity. Though bulk tolerance at N-3 is observed with short and small lipophilic groups, any substitution at the other positions and transformations of the parent skeleton eliminated activity. Thus 5-phenyl-1H-imidazo[4,5-c][1,8]naphthyridin-4(5H)-one (**23**) (KF17625), which satisfied these conditions, was selected for further studies (antigen inhalation-induced bronchospasm model; minimum effective dose (MED) = 1 mg/kg, po; antigen-induced contraction of trachea (the Schultz–Dale reaction), IC_{50} = 2.2 μM). Compound **23** inhibited carbachol-, histamine-, or leukotriene D_4-induced contraction and relaxed spontaneous tone in guinea pig isolated tracheal preparations with, 4- to 16-fold greater potency than aminophylline. Thus it appeared to relax directly the airway smooth muscle. **23** did not have any influence on adenosine binding at 10 μM, but inhibited canine tracheal phosphodiesterase (PDE) IV (IC_{50} = 12 μM) and concanavalin-A-induced histamine release from rat mast cells (44% inhibition at 10 μM). Although the detailed mechanisms of these compounds remain to be elucidated, this series of novel tricyclic heterocycles represents a new class of bronchodilator.

Introduction

Xanthines **1**, represented by theophylline (**1a**), have long been used in asthma therapy[1] despite a very low margin of safety due to their multiple pharmacological activities.[2] Since the appearance of theophylline, many new xanthine analogues, with more potent bronchodilator activities, have been reported.[3] However, none of these compounds have replaced theophylline as a useful therapeutic drug for asthma. Thus, this situation prompted us to explore nonxanthine compounds with potent bronchodilator activity and less side effects compared to theophylline. As a result, new tricyclic heterocycles, the imidazo[4,5-c]-quinolin-4(5H)-one derivatives **2**, were recently reported,[4] where many of these compounds exhibited more potent activity (in vitro) than theophylline, but showed poor oral bioavailability. Therefore, we have continued further research for new bronchodilators with better oral bioavailability.



During our research, Sch 33303 (**3a**) and 37224 (**3b**) bearing a 1,8-naphthyridin-2(1H)-one skeleton were reported by Sherlock et al.[5] and Kreutner et al.,[6] respectively, to show potent antiallergic activity, mainly due to their inhibition of the release of leukotrienes. Prevention of the release of mediators from mast cell and basophils

* To whom all correspondence should be addressed: Fumio Suzuki, Ph. D., Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411, Japan. Phone No. 81-559-89-2025. Fax No. 81-559-86-7430.

(1) Persson, C. G. A. Some Pharmacological Aspects on Xanthines in Asthma. *Eur. J. Resp. Dis.* **1980**, *61*, 7–16.

(2) Persson, C. G. A.; Andersson, K. E.; Kjellin, G. Minireview, Effects of Enprofylline and Theophylline, May Show the Role of Adenosine. *Life Sci.* **1986**, *38*, 1057–1072.

(3) (a) Stefanovich, V.; Porsche, E. On the Biochemical Mechanism of Action of 1-Propyl-3-methyl-7-(5-hydroxyhexyl)-xanthine (HWA 153), a New Bronchospasmolytically Active Methyl Xanthine Derivative. *Arzneim.-Forsch.* **1979**, *29*, 917–920. (b) Pascal, J. C.; Beranger, S.; Pinhas, H.; Poizot, A.; Desiles, J. P. New Antihistaminic Theophylline or Theobromine Derivatives. *J. Med. Chem.* **1985**, *28*, 647–652. (c) Regnier, G. L.; Guillonneau, C. G.; Duhault, J. L.; Tisserand, F. P.; Saint-Romas, G.; Holstorp, S. M. New Xanthine Derivatives with Potent and Long Lasting Anti-Bronchoconstrictive Activity. *Eur. J. Med. Chem.* **1987**, *22*, 243–250. (d) Berti, F.; Magni, F.; Rossoni, G.; Schiantarelli, P.; Bongrani, S.; Pasargiklian, R. New Pharmacological Data on the Bronchospamolytic Activity of Bamifylline. *Arzneim.-Forsch.* **1988**, *38*, 40–44. (e) Grosa, G.; Caputo, O.; Ceruti, M.; Biglino, G.; Franzone, J. S.; Cirillo, R. Synthesis and Antibronchospastic Activity of Theophylline Thioacetal Derivatives. *Eur. J. Med. Chem.* **1989**, *24*, 635–638.

(4) (a) Suzuki, F.; Kuroda, T.; Nakasato, Y.; Ohmori, K.; Manabe, H. U.S. Patent 4 994 468, 1991; *Chem. Abstr.* **1991**, *114*, 185499h. (b) Suzuki, F.; Kuroda, T.; Nakasato, Y.; Ohmori, K.; Manabe, H. Eur. Patent 4 113 194, 1991; *Chem. Abstr.* **1991**, *114*, 247276u. (c) Suzuki, F.; Kuroda, T.; Nakasato, Y.; Manabe, H.; Ohmori, K.; Kitamura, S.; Ichikawa, S.; Ohno, T. New Bronchodilators. 1. 1,5-Substituted 1H-imidazo[4,5-c]-quinolin-4(5H)-ones. *J. Med. Chem.* **1992**, *35*, 4045–4053. (d) Suzuki, F.; Kuroda, T.; Hayashi, H.; Nakasato, Y.; Manabe, H.; Ohmori, K. New Bronchodilators 2. 3H-Imidazo[4,5-c]quinolin-4(5H)-ones. *Chem. Pharm. Bull.* **1992**, *40*, 3245–3252.

(5) Sherlock, M. H.; Kaminski, J. J.; Tom, W. C.; Lee, J. F.; Wong, S. C.; Kreutner, W.; Bryant, R. W.; McPhail, A. T. Antiallergy Agents. 1. Substituted 1,8-Naphthyridin-2(1H)-ones as Inhibitors of SRS-A Release. *J. Med. Chem.* **1988**, *31*, 2108–2121.

(6) Kreutner, W.; Sherwood, J.; Sehring, S.; Rizzo, C.; Chapman, R. W.; Siegel, M. I.; Egan, R. W. Antiallergy Activity of Sch 37224, a New Inhibitor of Leukotriene Formation. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 997–1003.

0022-2623/92/1835-4866$03.00/0 © 1992 American Chemical Society

is considered to be useful as one approach in the treatment of asthma. Medwid et al. have tried to develop triazolo-[1,5-c]pyrimidines 4 as potential antiasthmatic agents from this concept.[7] We have been interested in both their pharmacological profiles and the parent structure (1,8-naphthyridin-2(1*H*)-one), which is known to be a bioisostere of the 1*H*-2-quinolone ring.

On this basis, hybridization of the 1,8-naphthyridin-2(1*H*)-one moiety onto the imidazo[4,5-*c*]quinolin-4(5*H*)-one skeleton resulted in novel tricyclic heterocycles, imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives **5** as our next synthetic targets. It is known from previous studies that antigen-induced contractions of trachea isolated from guinea pigs are mediated primarily by endogenously released histamine and 5-lipoxygenase products.[8] Such a contraction of airway smooth muscle (in vitro) may reflect airway constriction and subsequent increase in airway resistance normally associated with allergic asthma.[9] Thus the Schultz–Dale (SD) reaction using trachea isolated from passively sensitized guinea pigs was employed as a primary screen. This assay can select not only nonselective bronchodilators but also inhibitors of the mediator release.

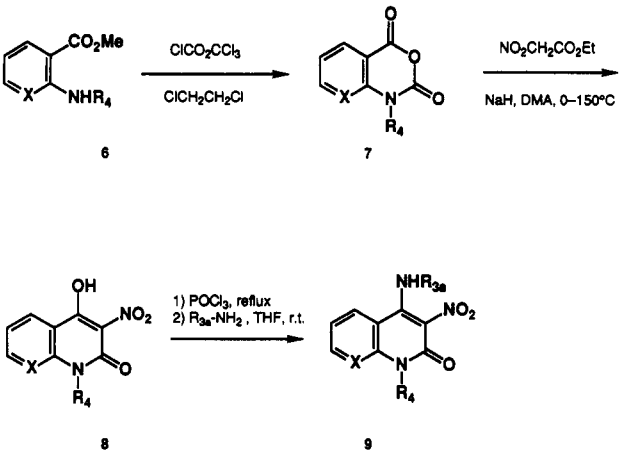
Now we describe the synthesis and structure–activity relationships of 5-substituted imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives which show greater in vitro and in vivo activity than theophylline. The mechanisms of action of this series are speculated to be the inhibition of phosphodiesterase and histamine release instead of antagonism of adenosine.

Chemistry

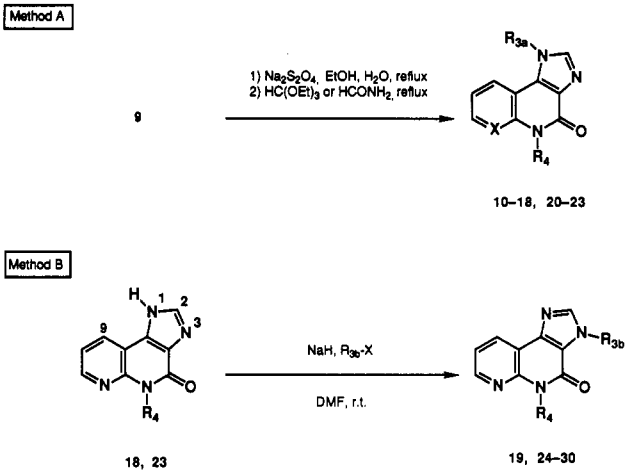
In this paper, three kinds of compounds based on 5-butylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (**5a**), 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (**5b**), and 5-phenylimidazo[4,5-*c*]quinolin-4(5*H*)-one **2b** derivatives were prepared. Compounds **5a** and **2b** were prepared from the corresponding methyl ester **6**, by using a procedure similar to that for the preparation of **5b**[10] (Schemes I and II). Starting materials such as methyl 2-(alkylamino)- or 2-anilinonicotinate[5] and methyl 2-(phenylamino)benzoate[11] (**6**) were prepared according to the reported procedures.

Recently we found that treatment of methyl 2-anilinonicotinate with trichloromethyl chloroformate provided *N*-phenyl-3-azaisatoic anhydride in a 87% yield (Scheme I).[10] This reaction was used to obtain the oxazinedione derivatives **7** (Table I), which were converted to **8** by reaction with ethyl nitroacetate anion (Table II). Compound **8** was chlorinated by phosphorus oxychloride, followed by amination using aqueous ammonia to afford amines **9** (Table III). After reduction of the nitro group of **9** with sodium hydrosulfite, the imidazole moiety was

Scheme I



Scheme II



constructed by heating with triethyl orthoformate to afford compounds **20–23**. In the preparation of **10–18**, formamide was used instead of triethyl orthoformate in order to avoid side reactions (Scheme II, Method A, Table IV). Compounds **21–23** were prepared as described previously.[10]

Compounds **18** and **23** were reacted with appropriate electrophiles to furnish the corresponding 3-substituted products **19** and **24–30**, regioselectively (Scheme II, Method B, Table V).[10] In this reaction, no 1-substituted products were observed. Steric interaction between the 1-substituent and 9-H and the linear conjugation of the imidazole double bonds with the carbonyl group presumably favor 3-substitution of **18** or **23** under alkylation conditions. The 3-acetic acid derivative **31** was prepared by the treatment of **23** with *tert*-butyl bromoacetate, followed by the hydrolysis using trifluoroacetic acid (Scheme III, Method C). Compound **23** was reacted with 1-bromo-3-chloropropane, to give **32** which was transformed into the iodide derivative with sodium iodide, followed by amination with diethylamine or morpholine to afford **33** or **34** (Scheme III, Method D). Formation of the triazole ring was achieved by the treatment of the reduced product of **35** with sodium nitrite[12] to afford **36** (Scheme IV, Method E). The reduction of **35**, followed by acylation with acetyl chloride or benzoyl chloride, and cyclization under basic conditions gave 2-methyl or 2-phenyl derivative **37** or **38**, respectively (Scheme IV, Method F).

(7) Medwid, J. B.; Paul, R.; Baker, J. S.; Brockman, J. A.; Du, M. T.; Hallett, W. A.; Hanifin, J. W.; Hardy, R. A.; Tarrant, J. M. E.; Torley, L. W.; Wrenn, S. Preparation of Triazolo[1,5-*c*]pyrimidines as Potential Antiasthma Agents. *J. Med. Chem.* **1990**, *33*, 1230–1241.

(8) Jones, T. R.; Charette, L.; Denis, D. Antigen-Induced Contraction of Guinea-Pig Isolated Trachea: Studies with Novel Inhibitors and Antagonists of Arachidonic Acid Metabolites. *Br. J. Pharmacol.* **1988**, *95*, 309–321.

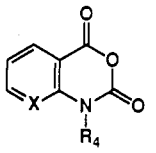
(9) Chand, N.; Eyre, P. The Schultz–Dale Reaction: A Review. *Agents Actions* **1978**, *8*, 171–184.

(10) Kuroda, T.; Suzuki, F. A Facile and Novel Synthesis of 5-Phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones. *J. Heterocycl. Chem.* **1991**, *28*, 2029–2034.

(11) Craig, D. The Nitro and Amino Derivatives of *t*-Butylbenzene. *J. Am. Chem. Soc.* **1935**, *57*, 195–198.

(12) Blicke, F. F.; Godt, H. C. J. Reactions of 1,3-Dimethyl-5,6-diaminouracil. *J. Am. Chem. Soc.* **1954**, *76*, 2798–2800.

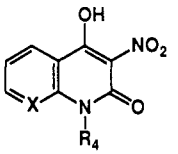
Table I



compd	R_4	X	yield, %	mp, °C	recrystn solvent	formula[a]
7a	4-$CH_3OC_6H_4$	N	92	241–243	$ClCH_2CH_2Cl$	$C_{14}H_{10}N_2O_4$
7b	3-$CH_3OC_6H_4$	N	72	224	$CHCl_3$–*i*-Pr_2O	$C_{14}H_{10}N_2O_4$
7c	4-$CH_3C_6H_4$	N	93	238–241	$ClCH_2CH_2Cl$	$C_{14}H_{10}N_2O_3$
7d	3-$CH_3C_6H_4$	N	79	142–145	$CHCl_3$–*i*-Pr_2O	$C_{14}H_{10}N_2O_3$
7e	3-ClC_6H_4	N	72		crude[b]	$C_{13}H_7N_2O_3Cl$
7f	4-$C_2H_5O_2CC_6H_4$	N	90		crude[b]	$C_{16}H_{12}N_2O_5$
7g	3-$C_2H_5O_2CC_6H_4$	N	86		crude[b]	$C_{16}H_{12}N_2O_5$
7h	*n*-C_4H_9	N	90	122–124	*i*-Pr_2O	$C_{11}H_{12}N_2O_3 \cdot {}^1/_{10}H_2O$
7i[c]	C_6H_5	CH	72		crude[b]	$C_{14}H_9N_2O_3$

[a] Compounds except for crude products were analyzed for C, H, and N. Their results agreed to ± 0.4% of theoretical values. [b] Materials used in the next step without further purification. [c] See ref 11.

Table II



compd	R_4	X	yield, %	mp, °C	recrystn solvent	formula[a]
8a	4-$CH_3OC_6H_4$	N	55	220–222	DMF–H_2O	$C_{15}H_{11}N_3O_5$
8b	3-$CH_3OC_6H_4$	N	66	216–217	DMF–H_2O	$C_{15}H_{11}N_3O_5$
8c	4-$CH_3C_6H_4$	N	78	225–226	DMF–H_2O	$C_{15}H_{11}N_3O_4$
8d	3-$CH_3C_6H_4$	N	69	205–206	DMF–H_2O	$C_{15}H_{11}N_3O_4$
8e	3-ClC_6H_4	N	74	189–191	DMF–H_2O	$C_{14}H_8N_3O_4Cl$
8f	4-$C_2H_5O_2CC_6H_4$	N	38	158–161	EtOH–H_2O	$C_{17}H_{13}N_3O_6$[b]
8g	3-$C_2H_5O_2CC_6H_4$	N	41	143–145	EtOH–H_2O	$C_{17}H_{13}N_3O_6$
8h	*n*-C_4H_9	N	44	106–109	*i*-PrOH–H_2O	$C_{12}H_{13}N_3O_4 \cdot {}^9/_{10}H_2O$
8i	C_6H_5	CH	89	175	DMF–H_2O	$C_{15}H_{10}N_2O_4$

[a] All compounds were analyzed for C, H, and N and their results agreed to ±0.4% of theoretical values except for **8f**. [b] N: calcd, 11.83; found, 11.39.

Table III

compd	R_4	X	R_{3a}	yield, %	mp, °C	recrystn solvent	formula[a]
9a	4-$CH_3OC_6H_4$	N	H	78	>300	EtOH–H_2O	$C_{15}H_{12}N_4O_4$
9b	3-$CH_3OC_6H_4$	N	H	85	>300	EtOH–H_2O	$C_{15}H_{12}N_4O_4$
9c	4-$CH_3C_6H_4$	N	H	84	>300	EtOH–H_2O	$C_{15}H_{12}N_4O_3 \cdot {}^3/_5H_2O$[b]
9d	3-$CH_3C_6H_4$	N	H	85	>300	crude[c]	$C_{15}H_{12}N_4O_3$
9e	3-ClC_6H_4	N	H	70	>300	crude[c]	$C_{14}H_9N_4O_3Cl$
9f	4-$C_2H_5O_2CC_6H_4$	N	H	91	>300	EtOH–H_2O	$C_{17}H_{14}N_4O_5$
9g	3-$C_2H_5O_2CC_6H_4$	N	H	87	>300	EtOH–H_2O	$C_{17}H_{14}N_4O_5$
9h	*n*-C_4H_9	N	H	40	221–225	EtOH–H_2O	$C_{12}H_{14}N_4O_3$
9i	C_6H_5	CH	H	60	>300	DMF–H_2O	$C_{15}H_{11}N_3O_3$[c]
9j	*n*-C_4H_9	N	CH_3	40	233–235	EtOH–H_2O	$C_{13}H_{16}N_4O_3$

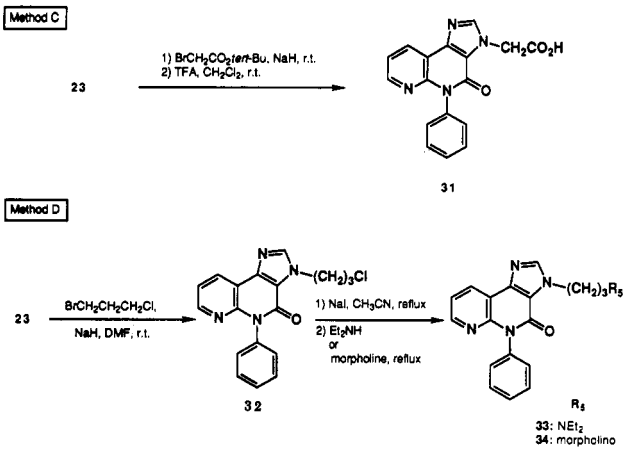
[a] Compounds except for crude products were analyzed for C, H, and N. [b] C: calcd, 58.67; found, 58.24. [c] See footnote *b* in Table I. [c] C: calcd, 64.05; found, 63.19.

Pharmacological Results and Discussion

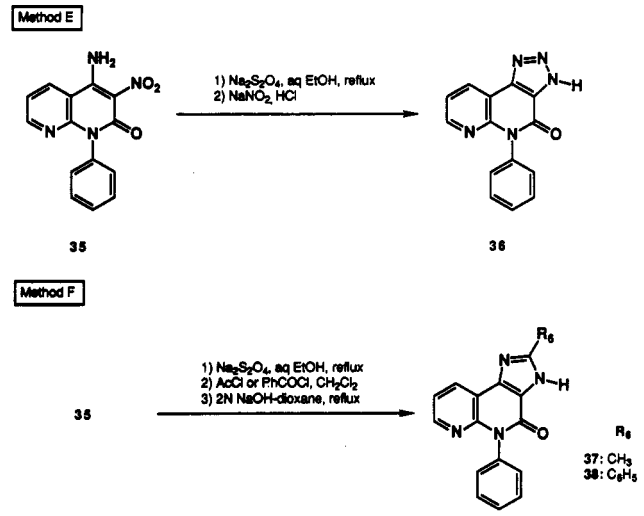
In order to test for bronchodilator activity of this series of compounds, the inhibition of antigen-induced contraction in trachea, isolated from passively sensitized guinea pigs (Schultz–Dale (SD) reaction), was employed. Compounds which produced more than 50% relaxation at 30 μM were regarded as active, and their IC_{50} values were obtained by a cumulative method. Subsequently, active compounds were evaluated orally against antigen-induced bronchospasm in passively sensitized guinea pigs. The time (s) of onset of the asphyxic convulsion was defined as the collapse time. Compounds which prolong the collapse time in this model were regarded as active. Acute lethal toxicity of these compounds was examined in mice as an index of side effects. The pharmacology data of our tested compounds are summarized in Table VI, in comparison with that of theophylline.

In the 5-butylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives **17–19** (R_4 = *n*Bu), introduction of the methyl group at the 1- or 3-position produced bronchod-

Scheme III



Scheme IV



ilation (17, 19), while the lack of substitution at either, as in 18, abolished activity. The 3-methyl derivative 19 was more active in vitro than the 1-methyl derivative 17 or theophylline. However, 17, 19, and theophylline exhibited no significant oral activity against antigen-induced bronchospasm at a dose of 10 mg/kg and showed lethal acute toxicity at a dose of 200 or 300 mg/kg (po).

On the other hand, in the 5-phenylimidazo[4,5-*c*][1,8]-naphthyridin-4(5*H*)-one (R_4 = Ph), the effects of the substituents (R_3) on bronchodilator activity were largely different from those in the 5-butyl-substituted derivatives. Thus, 1-substitution with methyl or isopropyl groups, eliminated the activity (21 and 22), whereas no substitution or 3-substitution resulted in potent activity. The unsubstituted compound 23 exhibited 3-fold more potent in vitro relaxant activity and less acute lethal toxicity than theophylline. Substitution of short alkyl chains such as methyl or ethyl at the 3-position enhanced both activity and lethal acute toxicity (24 and 25). However, it is worth noting that 24 exhibited 30-fold more potent activity than theophylline. On the other hand, introduction of longer substituents such as *n*-butyl and *n*-hexyl diminished bronchodilator effects (28, 30). With increasing bulk and lipophilicity of R_{3b}, activity and acute lethal toxicity tended to decrease (25–30). Furthermore, compounds 23, 24, 26, and 29 significantly inhibited bronchoconstriction induced by antigen at a dose of 10 mg/kg. Acidic or basic moieties at the 3-position, eliminated activity (31, 33, 34). Among the compounds described above, 23 was regarded as the most interesting compound because of its potent bronchodilator activity and reduced acute lethal toxicity. Thus, minor modifications of 23 were then assessed. Surprisingly, substitution at the 2-position and the 5-phenyl ring of 23 eliminated activity regardless of the nature and positions of the substituents (10–16, 37, and 38). Furthermore, replacement of the pyridine moiety by benzene or of imidazole by triazole also abolished activity (20 and 36).

Structure–activity relationships of our tested compounds are as follows. With respect to modification at the 5-position, both phenyl and *n*-butyl substitution produced potent activity. Though the bulk tolerance of R_{3b} for activity is observed with short and small lipophilic groups, any substitution at the other positions and transformations of the parent skeleton eliminated bronchodilator activity. Thus 23 was selected for further evaluation.

The inhibitory effects of compound 23 on the contraction of guinea pig tracheal strips induced by the spasmogens carbachol, histamine, or leukotriene (LT) D_4, which are known to be important mediators in asthma, were examined. The results together with the effect on the SD reaction are outlined in Table VII, compared with those of aminophylline, the ethylenediamine salt of theophylline. Relaxant activity of 23 against the SD reaction-, carbachol-, histamine-, and LTD_4-induced contraction (in vitro) were approximately 7.7-, 3.7-, 5.8-, and 7.8-fold greater than that of aminophylline, respectively. No receptor ligands binding of dopamine (D_1, D_2), histamine (H_1, H_2), acetylcholine (M_1), serotonin ($5HT_{1A}$, $5HT_2$), or catecholamines (α_1, α_2, β) was significantly antagonized by 23 at 100 μM. Furthermore, 23 inhibited spontaneous tone in guinea pig isolated tracheas (IC_{50} = 1.99 ± 0.04 μM, n = 4) more potently than aminophylline (IC_{50} = 32.3 ± 12.1 μM, n = 4).[13] Therefore, 23 directly relaxes airway smooth muscle.

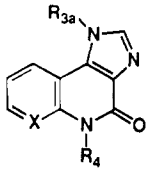
Subsequently, a further detailed oral comparison of 23 with theophylline was performed against anitgen-induced bronchospasm in the guinea pig (Table VIII). Compound 23 exhibited significant antispasmogenic activity at doses of 0.5 to 25 mg/kg, but theophylline showed no statistically significant activity even at 25 mg/kg. These results indicate that oral bioavailability can be improved as a result of the conversion of imidazo[4,5-*c*]quinolin-4(5*H*)-one derivatives[4] into 5-phenylimidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one derivatives.[14] Although the pharmacological effects of theophylline have been studied extensively,[1,2] the molecular mechanism responsible for its activity in asthma remain ill-defined. Of its many activities, adenosine antagonism and phosphodiesterase inhibition may be the most important.[15] Thus the effects of our tricyclic heterocycles on adenosine receptor binding and PDE were examined. With respect to adenosine A_1 and A_2 receptor binding, values of percent inhibition of compound 23 were 66 and 36% at 100 μM, respectively,

(13) Buckle, D. R.; Arch, J. R. S.; Fenwichk, A. E.; Houge-Frydrych, C. S. V.; Pinto, I. L.; Smith, D. G.; Taylor, S. G.; Tedder, J. M. Relaxant Activity of 4-Amido-3,4-dihydro-2*H*-1-benzopyran-3-ols and 4-Amido-2*H*-1-benzopyrans on Guinea Pig Isolated Trachealis. *J. Med. Chem.* **1990**, *33*, 3028–3034.

(14) Suzuki, F.; Kuroda, T.; Kitamura, S.; Ohmori, K. Eur. Patent 459505, 1991; *Chem. Abstr.*, in press.

(15) (a) Fredholm, B. B. Are Methylxanthine Effects due to Antagonism of Endogenous Adenosine? *Trends Pharmacol. Sci.* **1980**, *1*, 129–133. (b) Torphy, T. J.; Undem, B. J. Phosphodiesterase Inhibitors; New Opportunities for the Treatment of Asthma. *Thorax* **1991**, *46*, 512–523.

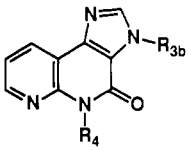
Table IV



compd	R_4	X	R_{3a}	yield, %	mp, °C	recrystn solvent	formula[a]
10	4-$CH_3OC_6H_4$	N	H	54	>300	DMF–H_2O	$C_{16}H_{12}N_4O_2$
11	3-$CH_3OC_6H_4$	N	H	50	>300	DMF–H_2O	$C_{16}H_{12}N_4O_2$
12	4-$CH_3C_6H_4$	N	H	52	>300	DMF–H_2O	$C_{16}H_{12}N_4O$
13	3-$CH_3C_6H_4$	N	H	37	>300	DMF–H_2O	$C_{16}H_{12}N_4O$
14	3-ClC_6H_4	N	H	47	>300	DMF–H_2O	$C_{15}H_9N_4OCl$
15	4-$C_2H_5O_2CC_6H_4$	N	H	60	>300	DMF–H_2O	$C_{18}H_{14}N_4O_3$[b]
16	3-$C_2H_5O_2CC_6H_4$	N	H	48	>300	DMF–H_2O	$C_{18}H_{14}N_4O_3$
17	n-C_4H_9	N	CH_3	85	199–202	i-PrOH–i-Pr_2O	$C_{14}H_{16}N_4O$
18	n-C_4H_9	N	H	45	>300	DMF–H_2O	$C_{13}H_{14}N_4O$
20	C_6H_5	CH	H	66	>300	DMF–H_2O	$C_{16}H_{11}N_3O$

[a] All compounds were analyzed for C, H, and N. [b] Calcd: H, 4.22. Found: H, 3.80.

Table V



compd	R_4	R_{3b}	yield, %	mp, °C	recrystn solvent	formula[a]
19	n-C_4H_9	CH_3	72	173–174	i-Pr_2O	$C_{14}H_{16}N_4O$
26	C_6H_5	n-C_3H_7	71	194–204	EtOAc–hexane	$C_{18}H_{16}N_4O$
27	C_6H_5	i-C_3H_7	41	192–193	i-PrOH–H_2O	$C_{18}H_{16}N_4O$
28	C_6H_5	n-C_4H_9	70	192–194	EtOAc–i-Pr_2O	$C_{19}H_{18}N_4O$
30	C_6H_5	n-C_6H_{13}	83	166–168	$CHCl_3$–i-Pr_2O	$C_{21}H_{22}N_4O$

[a] See footnote *a* in Table II.

and below 20% at 10 μM. Those of theophylline at 100 μM were 77 and 67%, respectively. Compound **24**, which exhibited the most potent in vitro relaxant activity among our tested compounds, inhibited adenosine binding at both receptor subtypes below 50% (A_1, 36%; A_2, 43%) at 100 μM. Furthermore, recently reported selective and potent adenosine antagonists such as 8-cyclopentyl-1,3-dipropylxanthine (CPX),[16] 1,3-dipropyl-8-(3-noradamantyl)-xanthine (KW3902)[17] for the A_1 receptors, and (*E*)-8-(3,4-dimethoxystyryl)-7-methyl-1,3-dipropylxanthine (KF17837)[18] and 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine (CGS15943)[19] for the A_2 receptors did not inhibit the SD reaction at 30 μM (<20% inhibition). Thus adenosine receptors do not play an important role in antigen-induced contractions of the guinea pig isolated trachea or in the relaxant activity of **23** and **24**.

On the other hand, a papaverine-derived bronchodilator AH 21-132 was recently reported to selectively inhibit PDE IV.[20] Thus inhibitory effects of **19**, **23**–**27**, and **29** on PDE IV derived from canine tracheal smooth muscle[21] were evaluated to compare them with those of aminophylline and rolipram (Table VIII). Rolipram is a typical PDE IV inhibitor and an antidepressant.[20] Some of these imidazo-[4,5-*c*]-naphthyridine-2-one derivatives potently inhibited PDE IV. The 3-isobutyl derivative was proved to be the most potent inhibitor among them (IC_{50} = 0.15 μM). However, the bronchodilator effects of these compounds do not correlate well with their PDE inhibitory activities. Possible explanations might be differences in their membrane permeabilities and/or in their effects on other isozymes. Compound **23** inhibited PDE I (IC_{50} = 6.2 μM) but did not inhibit PDE II, III, and V (IC_{50} > 20, > 100, > 100 μM, respectively). Detailed studies are in progress and will be included in a future publication.

We also examined the effects of **23** and aminophylline on concanavalin-A-induced histamine release from rat mast cells.[22] Though aminophylline showed no significant inhibition at a concentration of 100 μM (% inhibition: 21.1 ± 3.7%), compound **23** significantly inhibited histamine release even at 10 μM (% inhibition: 43.6 ± 3.5%).

(16) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. Binding of the A_1-Selective Adenosine Antagonist 8-Cyclopentyl-1,3-dipropylxanthine to Rat Brain Membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 59–63.

(17) (a) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A. 8-Polycycloalkyl-1,3-dipropylxanthines as Potent and Selective Antagonists for A_1-Adenosine Receptors. *J. Med. Chem.* **1992**, *35*, 924–930. (b) Suzuki, F.; Shimada, J.; Mizumoto, H.; Karasawa, A.; Kubo, K.; Nonaka, H.; Ishii, A.; Kawakita, T. Adenosine A_1 Antagonists 2. Structure–Activity Relationships on Diuretic Activities and Protective Effects against Acute Renal Failure. *J. Med. Chem.* **1992**, *35*, 3066–3075.

(18) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A.; Ichikawa, S. (*E*)-1,3-Dialkyl-7-methyl-8-(3,4,5-trimethoxystyryl)xanthines: Potent and Selective Adenosine A_2 Antagonists. *J. Med. Chem.* **1992**, *35*, 2342–2345.

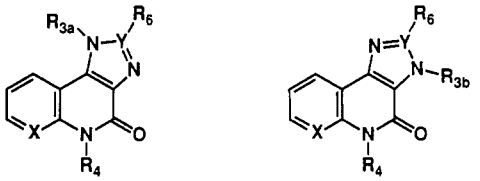
(19) Williams, M.; Francis, J.; Ghai, G.; Brannwalder, A.; Psychos, S.; Stone, G.; Cash, W. D. Biochemical Characterization of the Triazoloquinazoline, CGS15943, a Novel Non-Xanthine Adenosine Antagonist. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 415–420.

(20) Giembycz, M. A.; Barnes, P. J. Selective Inhibition of a High Affinity Type IV Cyclic AMP Phosphodiesterase in Bovine Trachealis by AH 21-132. *Biochem. Pharmacol.* **1991**, *42*, 663–677.

(21) (a) Torphy, T. J.; Cieslinski, L. B. Characterization and Selective Inhibition of Cyclic Nucleotide Phosphodiesterase Isozymes in Canine Tracheal Smooth Muscle. *Mol. Pharmacol.* **1990**, *37*, 206–214. (b) Silver, P. J.; Hamel, L. T.; Perrone, M. H.; Bentley, R. G.; Bushover, C. T.; Evans, D. B. Differential Pharmacologic Sensitivity of Cyclic Nucleotide Phosphodiesterase Isozymes Isolated from Cardiac Muscle, Arterial and Airway Smooth Muscle. *Eur. J. Pharmacol.* **1988**, *150*, 85–94.

(22) Suzuki, T.; Mori, K.; Uchida, M. Inhibition of Calcium Antagonists of Histamine Release and Calcium Influx of Rat Mast Cells: Difference between Induction of Histamine Release by Concanavalin A and Compound 48/80 *Eur. J. Pharmacol.* **1982**, *85*, 155–161.

Table VI. Effects of 1*H*-Imidazo[4,5-*c*]quinolin-4(5*H*)-ones on Schultz–Dale (SD) Reaction in Tracheal Strip Isolated from Passively Sensitized Guinea Pig, Collapse Time in Antigen Inhalation-Induced Bronchospasm Model (passively sensitized Guinea Pigs), and Acute Lethal Toxicity in Mice



compd	R_{3a}	R_{3b}	R_4	R_6	X	Y	SD IC_{50} ± SEM (μM)	collapse time MCT ● SEM[a] at 10 mg/kg (s)	acute lethal toxicity, MLD[f] (mg/kg po)
10	H		4-$CH_3OC_6H_4$	H	N	C	>30		
11	H		3-$CH_3OC_6H_4$	H	N	C	>30		
12	H		4-$CH_3C_6H_4$	H	N	C	>30		
13	H		3-$CH_3C_6H_4$	H	N	C	>30		
14	H		3-ClC_6H_4	H	N	C	>30		
15	H		4-$C_2H_5O_2CC_6H_4$	H	N	C	>30		
16	H		3-$C_2H_5O_2CC_6H_4$	H	N	C	>30		
17	CH_3		*n*-C_4H_9	H	N	C	13.0 ± 1.40	335 ± 74[b]	300
18	H		*n*-C_4H_9	H	N	C	>30		
19		CH_3	*n*-C_4H_9	H	N	C	3.57 ± 1.26	215 ± 35	200
20	H		C_6H_5	H	C	C	>30		
21	*i*-C_3H_7		C_6H_5	H	N	C	>30		
22	CH_3		C_6H_5	H	N	C	>30		
23	H		C_6H_5	H	N	C	2.18 ± 0.54	457 ± 58[c]	>300
24		CH_3	C_6H_5	H	N	C	0.22 ± 0.08	543 ± 35[c]	100
25		C_2H_5	C_6H_5	H	N	C	0.47 ± 0.11		100
26		*n*-C_3H_7	C_6H_5	H	N	C	8.30 ± 2.15	505 ± 95[c]	200
27		*i*-C_3H_7	C_6H_5	H	N	C	4.98 ± 1.93		200
28		*n*-C_4H_9	C_6H_5	H	N	C	>30		
29		*i*-C_4H_9	C_6H_5	H	N	C	6.78 ± 2.49	551 ± 49[c]	300
30		*n*-C_6H_{13}	C_6H_5	H	N	C	>30		
31		CH_2CO_2H	C_6H_5	H	N	C	>30		
33		(diethylamino)propyl	C_6H_5	H	N	C	>30		
34		morpholinopropyl	C_6H_5	H	N	C	>30		
36	H		C_6H_5	H	N	N	>30		
37	H		C_6H_5	CH_3	N	C	>30		
38	H		C_6H_5	C_6H_5	N	C	>30		
theophylline[d]							6.52 ± 1.26	343 ± 76	300

[a] MCT indicated mean collapse time of treated animals. The mean collapse time for untreated animals was 254 ± 18. [b] Values of MCT ± SEM at 50 mg/kg po. [c] Significant differences from the control at $P < 0.01$ (Scheffe's multiple range test). [d] MCT at 50 mg/kg po was 414 ± 48. [e] Significant differences from the control at $P < 0.01$. [f] MLD indicated minimum lethal dose (see Experimental Section).

Table VII. Effects of Compound **23** and Aminophylline on Spasmogen-Contracted Guinea Pig Trachea

	IC_{50} ± SEM (μM)[a]			
compd	SD	carbachol	histamine	LTD_4
23	2.18 ± 0.54	10.2 ± 4.65	5.72 ± 2.48	3.04 ± 0.85
aminophylline	16.7 ± 2.23	37.7 ± 8.22	33.2 ± 5.97	23.6 ± 4.69

[a] Concentration inhibition curves were carried out in triplicate with four or five concentrations of test agents, and IC_{50} values were calculated from computerization of logit-log curve.

These results suggest that compound **23** might exhibit activity against the SD reaction partly via inhibition of histamine release. Frossard reported that cyclic AMP was not predominantly involved in an inhibition of histamine release from rat mast cell.[23a] Consequently inhibition of histamine release could not be mediated via inhibition of PDE IV. Furthermore zaprinast,[23a,23c,23d] which is an inhibitor of PDE V (IC_{50} = 0.22 μM) and an old mast cell stabilizer, did not effectively inhibit the SD reaction (inhibition % at 10 μM = 33%). Thus compound **23** might be a unique antiasthmatic agent which can relax trachea directly (bronchodilator) and inhibit histamine release from mast cells (antiallergic agent).[23]

Further elucidation of the mechanism of action and toxicological investigation of **23** (KF17625) are currently under way together with evaluation of the pharmacological activity using other models, which will be reported in near future.

In conclusion, some of new nonxanthine heterocycles, imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-ones **5**, exhibited more potent antibronchospastic activity in vitro and in vivo than theophylline and will be a new class of bronchodilator.

Experimental Section

Melting points were determined on a Yanagimoto hot plate micro melting point apparatus and are uncorrected. Infrared (IR) spectra were measured on a JASCO IR-810 spectrometer. Proton nuclear magnetic resonance (^1H NMR) spectra were measured on a JEOL JNM GX-270 spectrometer or a Hitachi R-90H spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra (MS) were determined on a JEOL JMS-D300 instrument at an ionization potential of 70 eV. Elemental analyses were performed with a Perkin-Elmer 2400CHN. For column chromatography, Silica gel 60 (E. Merck, 0.063–0.200

(23) (a) Frossard, N.; Landry, Y.; Pauli, G.; Ruckstuhl. Effects of Cyclic AMP- and Cyclic GMP-Phosphodiesterase Inhibitors of Immunological Release of Histamine and Lung Contraction. *Br. J. Pharmacol.* **1981**, *73*, 933–938. (b) Barnes, P. J.; Chung, K. F.; Page, C. P. Inflammatory Mediators and Asthma. *Pharmacol. Rev.* **1988**, *40*, 49–84. (c) Torphy, T. J. Action of Mediators of Airway Smooth Muscle: Functional Antagonism as a Mechanism for Bronchodilator Drugs. *Agents Actions Suppl.* **1988**, *23*, 37–53. (d) Broughton, B. J.; Chalplen, P.; Knowles, P.; Lunt, E.; Pain, D. L.; Wooldrigde, K. R. H.; Ford, R.; Marchall, S.; Walker, J. L.; Maywell, D. R. New Inhibitor of Reagin-Mediated Anaphylaxis. *Nature* **1974**, *251*, 650–652.

Table VIII. Effects of Compound 23 and Theophylline on Collapse Time in Antigen Inhalation-Induced Bronchospasm Model (Passively Sensitized Guinea Pigs)

	MCT ± SEM[a] (s) at dose (mg/kg po)						
compd	50	25	10	5	1	0.5	0.1
23		491 ± 68[b]	457 ± 58[b]	483 ± 31[b]	397 ± 50[b]	410 ± 37[b]	301 ± 42
theophylline	414 ± 48[c]	389 ± 46	343 ± 76				

[a] See footnote a in Table VI. [b] Significant differences from the control at $P < 0.01$ (Scheffe's multiple range test). [c] Significant differences from the control at $P < 0.05$ (Scheffe's multiple range test).

Table IX. Effects of Imidazonaphthyridine Derivatives on PDE IV Derived from Canine Tracheal Smooth Muscle and the SD Reaction-Induced Contraction

	IC_{50} (μM)	
compd	SD	PDE IV[a]
19	3.57 ± 1.26	3.6
23	2.18 ± 0.54	12
24	0.22 ± 0.08	6.6
25	0.47 ± 0.11	2.0
26	8.30 ± 2.15	0.52
27	4.98 ± 1.93	2.5
29	6.78 ± 2.49	0.15
rolipram	0.34 ± 0.33	1.6
aminophylline	16.7 ± 2.23	(34%)[b]

[a] Concentration inhibition curves were carried out in duplicate with four concentrations of test agents, and IC_{50} values were calculated from computerization of logit-log curve. The ranges for duplicate data were within ±10%. [b] Inhibition percent at 100 μM.

mm) was used. The reactions were usually carried out under nitrogen. Organic extracts were dried over anhydrous sodium sulfate and concentrated by rotary evaporation.

1-Butyl-2*H*-pyrido[2,3-*d*][1,3]oxazine-2,4(1*H*)-dione (*N*-Butyl-3-azaisatoic Anhydride) (7h). To a solution of 30 g (0.14 mol) of methyl 2-(butylamino)nicotinate in 300 mL of dry 1,2-dichloroethane was slowly dropped 35 mL (0.29 mol) of trichloromethyl chloroformate at 80 °C. The reaction mixture was stirred at this temperature for 3 h. After ice-cooling, 1.0 g of activated carbon was added and then the mixture was refluxed for 30 min. After ice-cooling, the mixture was filtered and the solvent was evaporated. The residue was recrystallized from isopropyl ether to afford 29 g (90%) of colorless crystals **7h**: mp 122–124 °C; NMR (DMSO-d_6) δ 0.92 (t, 3 H, J = 7 Hz), 1.25–1.45 (m, 2 H), 1.55–1.75 (m, 2 H), 4.14 (t, 2 H, J = 7 Hz), 7.33 (dd, 1 H, J = 8, 5 Hz), 8.38 (dd, 1 H, J = 8, 2 Hz), 8.77 (dd, 1 H, J = 5, 2 Hz); MS (EI) m/z 220 (M^+), 165, 133. Anal. ($C_{11}H_{12}N_2O_3 \cdot {}^1/_{10}H_2O$) C, H, N. Compounds **7a–d** were prepared in a manner similar to **7i**[11] and their physical data are listed in Table I.

1-Butyl-4-hydroxy-3-nitro-1,8-naphthyridin-2(1*H*)-one (8h). To a solution of 37 mL (0.39 mol) of ethyl nitroacetate in 600 mL of dry dimethylacetamide was added 16 g (0.40 mol) of 60% sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 72 g (0.33 mol) of **7h** was added. The temperature was raised slowly to 170 °C and kept there for 2 h (carbon dioxide evolved). After the mixture was cooled to room temperature, the solvent was evaporated under reduced pressure and water was added to the residue. The aqueous solution was washed with ethyl acetate and the aqueous phase was acidified with concentrated HCl. The resulting precipitate was filtered, washed with water, and recrystallized from isopropyl alcohol–water to afford 38 g (44%) of yellow crystals **8h**: mp 106–109 °C; NMR (DMSO-d_6) δ 0.92 (t, 3 H, J = 7 Hz), 1.1–1.8 (m, 4 H), 4.28 (t, 2 H, J = 7 Hz), 7.23 (dd, 1 H, J = 7, 3 Hz), 8.39 (brd, 1 H, J = 7 Hz), 8.60 (brd, 1 H, J = 3 Hz); MS (EI) m/z 245 ($M^+ - 18$). Anal. ($C_{12}H_{13}N_3O_4 \cdot {}^9/_{10}H_2O$) C, H, N. Compounds **8a–g** were prepared in a manner similar to **8i**[10] and their physical data are listed in Table II.

1-[4-(Ethoxycarbonyl)phenyl]-4-hydroxy-3-nitro-1,8-naphthyridin-2(1*H*)-one (8f): NMR (DMSO-d_6) δ 1.40 (t, 3 H, J = 7 Hz), 4.43 (q, 2 H, J = 7 Hz), 7.20–7.70 (m, 3 H), 7.80–8.20 (m, 2 H), 8.40–8.65 (m, 2 H); MS (EI) m/z 355 (M^+), 354.

4-Amino-1-butyl-3-nitro-1,8-naphthyridin-2(1*H*)-one (9h). A suspension of 46 g (0.18 mol) of **8h** in 230 mL (2.5 mol) of phosphorus oxychloride was refluxed for 30 min. After ice-cooling, the solvent was evaporated under reduced pressure and water was added to the residue. The mixture was neutralized with 2 N NaOH. The resulting precipitate was filtered, washed with water, and dried to afford 20 g of crude solid.

A mixture of 15 g of the solid and 150 mL (1.1 mol) of 28% aqueous ammonia solution in 35 mL of tetrahydrofuran was stirred at room temperature for 1 h. The solvent was evaporated and water was added to the residue. The resulting precipitate was filtered, washed with water, and recrystallized from ethyl alcohol–water to afford 14 g (40%) of yellow crystals **9h**: mp 221–225 °C; NMR (DMSO-d_6) δ 0.91 (t, 3 H, J = 7 Hz), 1.1–1.7 (m, 4 H), 4.27 (t, 2 H, J = 7 Hz), 7.31 (dd, 1 H, J = 8, 4 Hz), 8.1–8.4 (m, 2 H), 8.5–8.8 (m, 2 H). Anal. ($C_{12}H_{14}N_4O_3$) C, H, N. Compounds **9a–g** and **9i** were prepared in a manner similar to **9h** and their physical data are listed in Table III.

4-Amino-3-nitro-1-(*p*-tolyl)-1,8-naphthyridin-2(1*H*)-one (9c): NMR (DMSO-d_6) δ 2.84 (s, 3 H), 7.29 (d, 2 H, J = 8 Hz), 7.53 (d, 2 H, J = 8 Hz), 7.87 (dd, 1 H, J = 8, 5 Hz), 8.58 (dd, 1 H, J = 5, 2 Hz), 9.41 (dd, 1 H, J = 8, 2 Hz), 11.4 (brs, 2 H); MS (EI) m/z 296 (M^+).

4-Amino-3-nitro-1-phenylquinolin-2(1*H*)-one (9i): NMR (DMSO-d_6) δ 6.46 (brd, 1 H, J = 8 Hz), 7.10–7.70 (m, 7 H), 8.35 (brd, 1 H, J = 8 Hz); MS (EI) m/z 281 (M^+).

1-Butyl-4-(methylamino)-3-nitro-1,8-naphthyridin-2(1*H*)-one (9j). To 3.5 g of the solid (chloride) prepared in the synthesis of **9h** were added 21 mL (0.25 mol) of 40% aqueous methylamine solution and 35 mL of tetrahydrofuran. Then the mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure and water was added to the residue. The resulting precipitate was filtered, washed with water, and recrystallized from ethyl alcohol–water to afford 3.3 g (40% from **8h**) of yellow crystals **9j**: mp 233–235 °C; NMR (DMSO-d_6) δ 0.91 (t, 3 H, J = 7 Hz), 1.1–1.8 (m, 4 H), 3.30 (s, 3 H), 4.31 (t, 2 H, J = 7 Hz), 7.38 (dd, 1 H, J = 8, 4 Hz), 7.7–8.0 (m, 1 H), 8.58 (dd, 1 H, J = 8, 2 Hz), 8.71 (dd, 1 H, J = 4, 2 Hz). Anal. ($C_{13}H_{16}N_4O_3$) C, H, N.

Method A. 5-Butyl-1-methyl-1*H*-imidazo[4,5-*c*][1,8]-naphthyridin-4(5*H*)-one (17). A mixture of 2.8 g (0.010 mol) of **9j** and 6.2 g (0.030 mol) of 85% sodium hydrosulfite in 30 mL of ethyl alcohol and 30 mL of water was stirred at 80 °C for 1 h. After ice-cooling, water was added to the mixture, and the resulting precipitate was filtered and dried. A suspension of the precipitate in 77 mL (0.46 mol) of triethyl orthoformate was stirred under reflux for 1 h. After ice-cooling, the resulting precipitate was filtered and recrystallized from isopropyl alcohol–isopropyl ether to afford 2.2 g (85%) of colorless crystals **17**: mp 199–202 °C; NMR (DMSO-d_6) δ 0.95 (t, 3 H, J = 7 Hz), 1.3–1.8 (m, 4 H), 4.16 (s, 3 H), 4.60 (t, 2 H, J = 7 Hz), 7.22 (dd, 1 H, J = 8, 4 Hz), 7.76 (s, 1 H), 8.26 (dd, 1 H, J = 8, 2 Hz), 8.58 (dd, 1 H, J = 4, 2 Hz); MS (EI) m/z 265 (M^+), 214, 200. Anal. ($C_{14}H_{16}N_4O$) C, H, N.

5-Butyl-1*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (18). A mixture of 13 g (0.050 mol) of **9h** and 30 g (0.15 mol) of 85% sodium hydrosulfite in 150 mL of ethyl alcohol and 30 mL of water was stirred at 80 °C for 1 h. After ice-cooling, water was added to the mixture, and the resulting precipitate was filtered and dried. A suspension of the precipitate in 60 mL (1.5 mol) of formamide was stirred at 150 °C for 2 h. After ice-cooling, the mixture was poured into water. The resulting precipitate was collected by filtration and recrystallized from DMF–water to afford 5.4 g (45%) of colorless crystals **18**: mp >300 °C; NMR (DMSO-d_6) δ 0.93 (t, 3 H, J = 7 Hz), 1.2–1.8 (m, 4 H), 4.52 (t, 2 H, J = 7 Hz), 7.39 (dd, 1 H, J = 8, 4 Hz), 8.30 (s, 1 H), 8.51 (dd, 1 H, J = 8, 2 Hz), 8.62 (dd, 1 H, J = 4, 2 Hz), 13.74 (brs, 1 H); MS (EI) m/z 242 (M^+), 200, 186. Anal. ($C_{13}H_{14}N_4O$) C, H, N.

Method B. 5-Butyl-3-methyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (19). To a solution of 1.3 g (5.4 mmol) of 18 in 30 mL of dry DMF was added 0.26 g (6.4 mmol) of 60 wt% sodium hydride at 0 °C in portions. After the evolution of hydrogen ceased, 0.67 mL (11 mmol) of methyl iodide was added. After stirring at room temperature for 2 h, aqueous saturated ammonium chloride solution was added under ice-cooling. The solvent was evaporated and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated. The residue was chromatographed on silica gel using $CHCl_3$ to afford 0.97 g (72%) of colorless crystals 19. An analytical sample was crystallized from isopropyl ether: mp 173–174 °C; NMR (DMSO-d_6) δ 0.93 (t, 3 H, J = 7 Hz), 1.3–1.5 (m, 2 H), 1.6–1.8 (m, 2 H), 4.09 (s, 3 H), 4.49 (t, 2 H, J = 7 Hz), 7.37 (dd, 1 H, J = 8, 4 Hz), 8.28 (s, 1 H), 8.47 (dd, 1 H, J = 8, 2 Hz), 8.50 (dd, 1 H, J = 4, 2 Hz); MS (EI) m/z 265 (M^+), 200, 186. Anal. ($C_{14}H_{16}N_4O$) C, H, N.

Method C. 3-(Carboxymethyl)-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (31). To a solution of 4.5 g (17 mmol) of 23 in 150 mL of dry DMF was added 1.0 g (26 mmol) of 60 wt% sodium hydride at 0 °C in portions. After the evolution of hydrogen ceased, 5.5 mL (34 mmol) of *tert*-butyl bromoacetate was added. After stirring at room temperature for 2 h, aqueous saturated ammonium chloride was added under cooling. The solvent was evaporated and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated. The residue was chromatographed on silica gel using $CHCl_3$ to afford 3.6 g (55%) of the *tert*-butyl acetate. Without analysis, to a solution of 3.6 g of the compound in 180 mL of methylene chloride was added 80 mL of trifluoroacetic acid under ice-cooling. The mixture was stirred at room temperature for 6 h, and the solvent was evaporated under reduced pressure. The residue was suspended in water, and 4 N NaOH was added to dissolve the residue. A 2 N HCl solution was added to the solution to give the precipitate, which was collected by filtration. Recrystallization from DMF–water gave 1.4 g (45%) of colorless crystals 31: mp >300 °C; NMR (DMSO-d_6) δ 5.27 (s, 2 H), 7.15–7.57 (m, 6 H), 8.30–8.40 (m, 2 H), 8.54 (dd, 1 H, J = 8, 2 Hz). MS (EI) m/z 320 (M^+), 319. Anal. ($C_{17}H_{12}N_4O_3$) C, H, N.

Method D. 3-[3-(Diethylamino)propyl]-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Hydrochloride (33·HCl). (a) 3-(3-Chloropropyl)-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (32). To a solution of 15 g (0.057 mol) of 23 in 400 mL of dry DMF was added 2.78 g (0.068 mol) of 60 wt% sodium hydride at 0 °C in portions. After the evolution of hydrogen ceased, 8.8 mL (0.086 mmol) of 3-bromo-1-chloropropane was added. After stirring at room temperature for 3 h, aqueous saturated ammonium chloride was added under cooling. The solvent was evaporated under reduced pressure, and water was added to the residue. The aqueous mixture was extracted with $CHCl_3$. The organic phase was washed with water, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel using $CHCl_3$ to afford 18 g (92%) of colorless crystals 32. An analytical sample was recrystallized from ethyl acetate–hexane: mp 186–190 °C; NMR (DMSO-d_6) δ 2.30–2.55 (m, 2 H), 3.53 (t, 2 H, J = 7 Hz), 4.67 (t, 2 H, J = 7 Hz), 7.11–7.62 (m, 6 H), 8.01 (s, 1 H), 8.41 (dd, 1 H, J = 4, 2 Hz), 8.62 (dd, 1 H, J = 8, 2 Hz). Anal. ($C_{18}H_{15}N_4OCl\cdot{}^1/_5H_2O$) C, H, N.

(b) 3-[3-(Diethylamino)propyl]-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Hydrochloride (33·HCl). A mixture of 18 g (0.052 mol) of 32 and 12 g (0.078 mol) of sodium iodide in 200 mL of acetonitrile was refluxed for 24 h. During reflux, 7.8 g (0.052 mol) of additional sodium iodide was added to the mixture. After cooling, the solvent was evaporated and water was added to the residue. The aqueous suspension was extracted with $CHCl_3$. The organic phase was washed with water, dried, and concentrated. A suspension of the residue in 50 mL of diethylamine was refluxed for 1 h. After cooling, the solvent was evaporated and water was added to the residue. The aqueous phase was extracted with $CHCl_3$. The organic phase was extracted with 2 N hydrochloric acid. The aqueous solution was adjusted to pH 11 with 8 N NaOH, followed by extraction with $CHCl_3$. The organic phase was washed with a brine and dried. To the solution was added ethyl acetate saturated with hydrogen chloride. The resulting precipitate was filtered and dried to afford 12 g (50%) of colorless crystals 33 as hydrochloride salts: mp 152–153 °C; NMR (DMSO-d_6) δ 1.18 (t, 6 H, J = 7 Hz), 2.20–2.35 (m, 2 H), 2.91–3.14 (m, 6 H), 4.60 (t, 2 H, J = 7 Hz), 7.22–7.60 (m, 6 H), 8.39 (dd, 1 H, J = 4, 2 Hz), 8.60 (dd, 1 H, J = 8, 2 Hz), 8.68 (s, 1 H), 10.57 (brs, 2 H); MS (EI) m/z 376 (M^+), 375. Anal. ($C_{22}H_{25}N_5O\cdot 2HCl\cdot{}^{13}/_{10}H_2O$) C, H, N.

3-Morpholino-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one Hydrochloride (34·HCl). 34·HCl was obtained in a 55% yield from 32 according to the same procedure as the synthesis of 33 except that morpholine was used instead of diethylamine: mp 294–297 °C; NMR (DMSO-d_6) δ 2.28–2.58 (m, 2 H), 2.92–3.22 (m, 4 H), 3.32–3.49 (m, 2 H), 3.77–4.02 (m, 4 H), 4.45–4.80 (m, 2 H), 7.28–7.63 (m, 6 H), 8.40 (dd, 1 H, J = 4, 2 Hz), 8.67 (dd, 1 H, J = 8, 2 Hz), 8.87 (s, 1 H), 11.51 (brs, 2 H); MS (EI) m/z 390 (M^+), 389. Anal. ($C_{22}H_{23}N_5O_2\cdot 2HCl\cdot{}^7/_5H_2O$) C, H, N.

Method E. 5-Phenyl-3*H*-triazolo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (36). A suspension of 2.1 g (7.4 mmol) of 35 and 4.6 g (22 mmol) of 85% sodium hydrosulfite in a mixture of 25 mL of ethyl alcohol and 25 mL of water was refluxed for 10 min. After cooling, the solid was filtered and dried. To a suspension of the solid in a mixture of 20 mL of ethyl alcohol and 6.0 mL of water was added dropwise a solution of 0.54 g (7.7 mmol) of 98.5% sodium nitrite, 0.55 mL of concentrated hydrochloric acid, and 15 mL of water at 0 °C. The mixture was stirred at 0 °C for 2 h. The resulting precipitate was filtered, washed with water, and recrystallized from methyl alcohol to afford 0.87 g (44%) of yellow crystals 36: mp >300 °C; NMR (DMSO-d_6) δ 7.2–7.7 (m, 6 H), 8.45 (dd, 1 H, J = 5, 2 Hz), 8.58 (brd, 1 H, J = 8 Hz); MS (EI) m/z 263 (M^+), 206. Anal. ($C_{14}H_9N_5O$) C, H, N.

Method F. 2-Methyl-5-phenyl-3*H*-imidazo[4,5-*c*][1,8]naphthyridin-4(5*H*)-one (37). A suspension of 12 g (0.043 mol) of 35 and 35 g (0.17 mol) of 85% sodium hydrosulfite in a mixture of 10 mL of ethyl alcohol and 14 mL of water was refluxed for 10 min. After cooling, the solid was filtered and dried. To the suspension of the solid in 100 mL of methylene chloride were added 3.1 mL (0.022 mol) of triethylamine and 1.4 mL (0.019 mol) of acetyl chloride under ice-cooling. The mixture was stirred at room temperature for 1.5 h. Then methanol was added to the mixture and the solvent was evaporated. To the residue were added 10 mL of dioxane and 10 mL of 2 N NaOH, and the mixture was refluxed for 1.5 h. After the mixture was cooled with ice, concentrated hydrochloric acid was added to neutralize it. The resulting precipitate was collected by filtration and dried. Recrystallization from methyl alcohol afforded 2.0 g (40%) of colorless crystals 37: mp >300 °C; NMR (DMSO-d_6) δ 2.51 (s, 3 H), 7.18–7.61 (m, 6 H), 8.33 (dd, 1 H, J = 4, 2 Hz), 8.57 (dd, 1 H, J = 8, 2 Hz), 13.5 (brs, 1 H); MS (EI) m/z 276 (M^+), 275. Anal. ($C_{16}H_{12}N_4O\cdot{}^1/_2H_2O$) C, H, N.

2,5-Diphenyl-3*H*-imidazo[4,5][1,8]naphthyridin-4(5*H*)-one (38). 38 was prepared in a 73% yield from 35 according to the same procedure as the synthesis of 37 except that benzoyl chloride was used instead of acetyl chloride: mp >300 °C ($CHCl_3$); NMR (DMSO-d_6) δ 7.22–7.72 (m, 10 H), 8.29–8.41 (m, 3 H), 8.5–8.7 (brs, 1 H); MS (EI) m/z 338 (M^+), 337. Anal. ($C_{21}H_{14}N_4O$) C, H, N.

Antigen-Induced Contraction (Schultz–Dale Reaction) of Tracheal Strip Isolated from Passively Sensitized Guinea Pig.[24] Male Hartley strain guinea pigs weighing 350–450 g were passively sensitized by intraperitoneal injection of 1 mL/animal of rabbit anti-ovalbumin (OA) serum 16–18 h before use. The animals were killed by stunning and bleeding. Trachea were excised and cleaned of adhering adipose and connective tissues. Tracheal zigzag strips were prepared by the method of Emmerson and Mackay,[25] followed by equilibrating for 1 h in Krebs–Henseleit solution with 95% O_2–5% CO_2 at 37 °C. OA was administered at 10 μg/mL in a final bath concentration which was chosen because the resultant contraction had been shown to be approximately 80% of the maximum contraction obtained with OA (100 μg/mL). After the contraction of tracheal strips

(24) Koda, A.; Nagai, H.; Wada, H. Pharmacological Actions of Baicalin and Baicalein. I. *Nippon Yakurigaku Zasshi* **1970**, *66*, 194–213.

(25) Emmerson, J.; Mackay, D. The Zig-zag Tracheal Strip. *J. Pharm. Pharmacol.* **1979**, *31*, 798.

reached a plateau, test drugs were added cumulatively at 7-min intervals. Contractions were recorded isotonically using isotonic transducers (TD-112S: Nihon Kohden) connected to recorders (TYPE30066: Yokokawahokusin Electric). The inhibitory effects were calculated as a percentage of the relaxation induced by papaverine (10^{-4} M) added at the end of the experiment. The concentration of each drug required to produce 50% relaxation (IC_{50}) was determined from least-squares regression analysis.

Antigen Inhalation-Induced Bronchospasm Model in Passively Sensitized Guinea Pigs. Herxheimer's method[26] was modified as follows. Male Hartley guinea pigs (350–450 g) were passively sensitized by intraperitoneal injection of 1 mL/animal of rabbit anti-ovalbumin (OA) serum 16–24 h before use. Guinea pigs were placed individually in a clear plastic container (13 × 18 × 25 cm) and sprayed with 1.5% OA solution using a nebulizer (V type: Nihon Shoji) at a rate of about 0.83 L/min. The time (s) of onset of the asphyxial convulsion was defined as the collapse time. Animals not responding until 600 s were considered to be fully protected, and their collapse time was determined to be 600 s. Test drugs were orally administered 1 h before antigen exposure, and animals were preteated with diphenhydramine (20 mg/kg, ip) and propranolol (5 mg/kg, ip) 30 min before antigen exposure.

Carbachol-, Histamine-, or LTD_4-Induced Contraction of Tracheal Strips from Normal Guinea Pigs. Tracheal zigzag strips from normal guinea pigs were prepared by the method discribed before.[25] Contraction was induced by carbachol (3×10^{-6} M final concentration), histamine (3×10^{-5} M) or LTD_4 (50 μg/mL). These doses of spasmogens produced a contraction of tracheal strips approximately equivalent to 80% of maximum. In evaluation of inhibition of spontaneous tone, tension was allowed to develop spontaneously and was maintained at 0.5 g. Test drugs were administered cumulatively at 7-min intervals after the contraction of tracheal strips reached a plateau. The inhibitory effects were calculated as a percentage of the relaxation induced by papaverine (10^{-4} M) added at the end of the experiment. The concentration of each drug required to produce 50% relaxation (IC_{50}) was determined from least-squares regression analysis.

Adenosine Binding. Adenosine A_1 and A_2 binding were performed according to the same protocol as described before.[17]

Phosphodiesterase Activity. The cAMP-specific PDE (type IV) was isolated from canine tracheal smooth muscle basically according to Torphy's method.[21] The assay was done as described before (concentration of substrate ([^{3}H]cAMP) = 1 μM).[27]

Concanavalin-A-Induced Histamine Release from Rat Mast Cell. The assay was done as described before.[22]

Acute Lethal Toxicity. The compounds were orally administered to male ddY-mice weighing 20–25 g (n = 3) at three doses (100, 200, 300 mg/kg). Mice were monitored for 7 days after the administration, and minimum lethal doses (MLD) were determined by observing the death of at least one mouse.

Acknowledgment. We thank K. Takada, E. Tuchiya, and Y. Kato for their technical assistance, H. Nonaka and Y. Sasaki for biological assays, and K.T. for preparation of the manuscript. We are grateful to Dr. T. Hirata for encouragement.

(26) Herxheimer, H. Repeatable 'Microshocks' of Constant Strength in Guinea-Pig Anaphylaxis. *J. Physiol.* **1952**, *117*, 251–255.

(27) Kincaid, R. L.; Manganiello, V. C. Initiation and Termination of Cyclic Nucleotide Action. In *Methods in Enzymology*; Corbin, J. D., Johnson, R. A. Eds.; Academic Press Inc.: New York, 1988; Vol. 159, 457–470.