New Bronchodilators. 1. 1,5-Substituted 1*H*-Imidazo[4,5-*c*]quinolin-4(5*H*)-ones

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A series of novel xanthine-based tricyclic heterocycles in 1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones was designed, synthesized, and tested as potential active bronchodilators. Inhibition of the Schulz–Dale (SD) reaction-induced contraction in trachea and inhibition of antigen inhalation-induced bronchospasm in passively sensitized guinea pigs served as primary in vitro and in vivo assays, respectively. Simultaneous measurement of acute lethal toxicity (minimum lethal dose; MLD, po) in mice allowed determination of a safety margin. The bronchodilatory activity of these heterocycles was considerably varied with the nature of substituents at the 5-position. The most active substituents at the 2- and 5-positions and on the aromatic ring were found to be hydrogen, *n*-butyl, and hydrogen, respectively. There was a bulk tolerance for lipophilic substituents at the 1-position. 5-Butyl-substituted compounds appeared to be less toxic than theophylline on the basis of MLD data. Thus 5-butyl-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (**10**) (IC_{50} value of the SD assay = 0.25 μM, MLD > 300 mg/kg) was selected for further studies. Compound **10** (KF15570) reduced bronchoconstriction produced by antigen (Konzett–Rössler preparation in anesthetized guinea pigs, ED_{50} = 0.42 mg/kg, iv) more effectively than aminophylline (ethylenediamine salt of theophylline, ED_{50} = 7.8 mg/kg, iv) but had fewer side effects on the heart and CNS than theophylline. Compound **10** and its derivatives showed weak adenosine antagonism and phosphodiesterase (PDE) inhibition which could not account for their potent bronchodilation. Although their precise mechanism of action remains unclear, this series of novel tricyclic heterocycles represents a new class of bronchodilator.

Introduction

Bronchial asthma is a chronic debilitating disease which in its severe forms can threaten life. At present, four classes of drugs have been employed to combat the symptoms of this disease: β-sympathomimetic agents, bronchodilators, antiallergic agents, and corticosteroids. Traditionally, theophylline (**1a**), as a representative of bronchodilators, is extensively used in the treatment of asthma.[1] However, theophylline possesses cardiotonic, central nervous system (CNS) stimulatory, diuretic, and other pharmacological activities in addition to the bronchodilatory activity.[2] Side effects and toxicity are often noted at blood levels considered to be within the therapeutic range.[3] Therefore, theophylline has a narrow therapeutic index. Much effort has been invested to develop new xanthine derivatives which relax the smooth bronchial muscle in a fashion similar to that of theophylline but without its CNS and cardiovascular side effects. Most studies have modified substituents at the 1-, 3-, 7-, and 8-positions of the xanthine skeleton.[4,5] As a result, enprofylline (**1b**)[2] and doxofylline (**1c**)[6] were found, but these compounds provided little benefit over theophylline.[7] At present, none of xanthine derivatives have proved to be clinically more useful than theophylline. Therefore, our approach to new bronchodilators focused on the synthesis of new heterocycles.

Design

We postulated that diverse pharmacological actions of theophylline might be based on multiple biochemical mechanisms such as adenosine antagonism, phosphodiesterase (PDE) inhibition, and release of catecholamine.[2] Previous studies suggested that an increase in lipophilicity of the xanthine moiety enhances one of these biochemical activities to some degree. For example, 6-thiotheophylline (**2**) was reported to increase the activity of PDE inhibition, while its activity as an adenosine receptor antagonist remained unchanged.[8] Zaprinast (**3**), with a phenyl ring at the 2-position of the 8-azaxanthine skeleton, showed enhanced inhibition of cyclic guanosine monophosphate (cGMP) PDE and exhibited antiallergic activity.[9] En-

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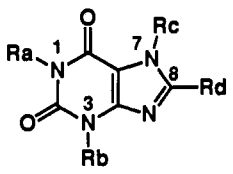
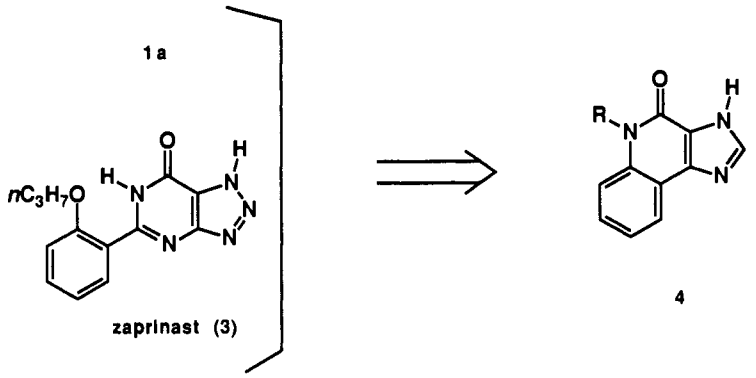
Scheme I



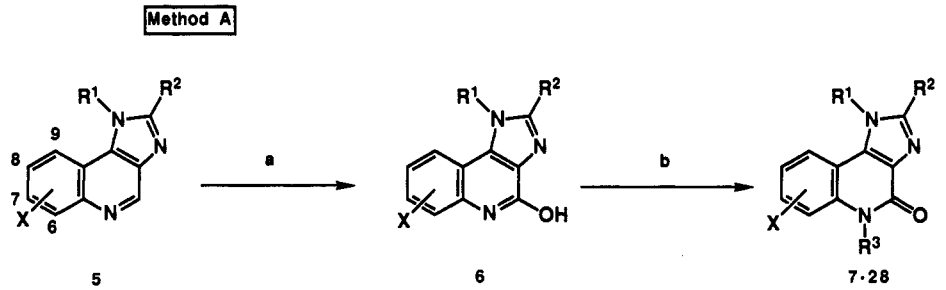
Xanthines (1)

theophylline (1a)
Ra=Rb=CH_3, Rc=Rd=H
enprofylline (1b)
Ra=Rc=Rd=H, Rb=nC_3H_7
doxofylline (1c)
Ra=Rb=CH_3, Rd=H
Rc=1,3-dioxolane-2-ylmethyl

6-thiotheophylline (2)



Scheme II [a]



[a] (a) (i) AcOH, H_2O_2, 80 °C; (ii) Ac_2O, Δ; (b) $(CH_3)_2NCOH$, R^3Y, NaH.

profylline (3-propylxanthine) is a weak adenosine receptor antagonist and a moderate PDE inhibitor, but is a more potent bronchodilator than theophylline.[2,10] On this basis, non-xanthine but xanthine-based compounds with different structural and functional features became the initial target for our studies. The design of these targets involved "combining" the methyl group at the 3-position of theophylline (**1a**) with the pyrimidine ring to form a tricyclic ring system **4**. Thus, 1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one derivatives **4** became a synthetic target as shown in Scheme I. Critical to the early success of this design is (1) evidence of bronchodilatory activity comparable to that of theophylline (**1a**) and (2) demonstration of fewer side effects. We report here the synthesis and structure–activity relationships of novel xanthine-based tricyclic heterocycles, 1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one derivatives, which exhibited potent bronchodilatory activities.[11]

Chemistry

Methods A and B have been developed, respectively, in order to introduce a variety of substituents at the 5- and 1-positions of 1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones (Scheme II–IV). 1*H*-Imidazo[4,5-*c*]quinolines **5**[12] were prepared from the corresponding anthranilic acids. Introduction of the hydroxy group into the 4-position of imidazoquinorines was carried out by the oxidation of **5** (Table I) with hydrogen peroxide, followed by rearrangement of *N*-oxide in refluxing acetic anhydride (Table II). Compounds **7–28** were obtained by the treatment of the sodium anion of **6** with appropriate electrophiles (Scheme II, method A, Table III). Compound **29** was prepared from **6a** by the reaction with *tert*-butyl bromoacetate in the presence of sodium hydride and was hydrolyzed with trifluoroacetic acid (TFA) in methylene chloride to afford the acetic acid derivative **30** (Scheme III). 4-Chloro-3-nitro-quinolin-2(1*H*)-ones were prepared from isatoic anhydride.[13–15] 1-Butylisatoic anhydride (**31**)[15] was reacted with the anion of ethyl nitroacetate to give 1-butyl-

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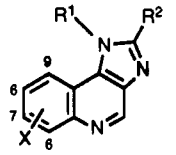
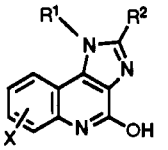
Table I. 1*H*-Imidazo[4,5-*c*]quinolines



compd	R^1	R^2	X	mp, °C	recryst solvent	formula[a]
5a[b]	CH_3	H				
5b	CH_3	H	7-Cl	176–178	*i*PrOH–H_2O	$C_{11}H_8N_3Cl$
5c[b]	CH_3	H	8-CH_3			
5d	CH_3	H	8-Cl	218–220	*i*PrOH–H_2O	$C_{11}H_8N_3Cl \cdot {}^4/_5H_2O$
5e	CH_3	H	9-CH_3	70	iPr_2O	$C_{12}H_{11}N_3$
5f	CH_3	H	7,8-$(CH_3O)_2$	139–142	*i*PrOH–H_2O	$C_{16}H_{13}N_3O_2$
5g	CH_3	CH_3	H	84–85	EtOAc–hexane	$C_{12}H_{11}N_3$
5h	CH_3	furyl	H	150–153	EtOH–H_2O	$C_{16}H_{11}N_3O \cdot {}^3/_8H_2O$
5i	CH_3	C_6H_5	H	131–135	EtOH–H_2O	$C_{17}H_{13}N_3 \cdot {}^2/_5H_2O$
5j	CH_3	4-$CH_3OC_6H_4$	H	166–167	EtOH–H_2O	$C_{16}H_{15}N_4O \cdot {}^1/_2H_2O$
5k	CH_3	3,4-$Cl_2C_6H_4$	H	208–211	MeOH–H_2O	$C_{17}H_{11}N_3Cl_2$
5l[b]	$C_6H_5CH_2$	H				

[a] All compounds were analyzed for C, H, and N and results agreed to ±0.4% of theoretical values. [b] See ref 13.

Table II. 4-Hydroxy-1*H*-imidazo[4,5-*c*]quinolines



compd	R^1	R^2	X	yield, %	mp, °C	recryst solvent	starting material	formula[a]
6a	CH_3	H	H	38	>300	DMF–MeOH	**5a**	$C_{11}H_8N_3O \cdot {}^1/_2H_2O$
6b	CH_3	H	7-Cl	48	>300	DMSO–H_2O	**5b**	$C_{11}H_8N_3OCl$
6c	CH_3	H	8-CH_3	40	>300	DMSO–H_2O	**5c**	$C_{12}H_{11}N_3O$
6d	CH_3	H	8-Cl	26	>300	DMSO–H_2O	**5d**	$C_{11}H_8N_3OCl$
6e	CH_3	H	9-CH_3	40	>300	DMSO–H_2O	**5e**	$C_{12}H_{11}N_3O \cdot {}^1/_8H_2O$
6f	CH_3	H	7,8-$(CH_3O)_2$	51	>300	DMSO–H_2O	**5f**	$C_{13}H_{13}N_3O_3 \cdot {}^1/_{10}H_2O$
6h	CH_3	CH_3	H	52	>300	DMSO–H_2O	**5g**	$C_{12}H_{11}N_3O \cdot {}^3/_2H_2O$
6h	CH_3	furyl	H	41	>300	DMSO–H_2O	**5h**	$C_{16}H_{11}N_3O_2 \cdot {}^1/_{10}H_2O$
6i	CH_3	C_6H_5	H	41	>300	DMSO–H_2O	**5i**	$C_{17}H_{13}N_3O \cdot {}^1/_5H_2O$
6j	CH_3	4-$CH_3OC_6H_4$	H	54	>300	DMSO–H_2O	**5j**	$C_{16}H_{15}N_3O_2$
6k	CH_3	3,4-$Cl_2C_6H_4$	H			crude	**5k**	
6l[b]	$C_6H_5CH_2$	H	H	60			**5l**	

[a] See footnote *a* in Table I. [b] See ref 13.

4-hydroxy-3-nitroquinolin-2(1*H*)-one (Scheme IV). Without purification, the hydroxy group was chlorinated with phosphorus oxychloride under reflux to give **32**. The chlorine in **32** was readily replaced[14] by primary amines in tetrahydrofuran (THF) at room temperature to provide **33** (Table IV). Hydrogenation of the nitro group in **33**, followed by cyclization in refluxing triethyl orthformate, afforded the target compounds **10** and **34–38** (Scheme IV, method B, Table V). Methylamino derivative **33a** was used for introduction of a substituent into the 2-position of the imidazole moiety. Hydrogenation of **33a** gave the crude diamino derivative (3-amino-1-butyl-4-(methylamino)quinolin-2(1*H*)-one) which was treated with sodium nitrite under acidic condition to afford triazole **39**. 2-Hydroxy derivative **40** was obtained by the treatment of the diamino derivative with 1,1′-carbonyldiimidazole (CDI) in refluxing THF. 2-Thiol **41** was prepared in the same manner as the preparation of **40** except that 1,1′-thiocarbonyldiimidazole (thioCDI) was used instead of CDI (Scheme V). Hydrogenation of **10** over platinum oxide (PtO_2) at 60 psi in TFA[16] afforded a tetrahydro derivative **42** in a moderate yield (Scheme VI).

Pharmacological Results and Discussion

In order to test bronchodilatory activity of this series of compounds, the Schultz–Dale (SD) reaction-induced contraction in trachea, isolated from passively sensitized guinea pigs, was employed. Compounds which produced more than 50% relaxation at 30 μM were regarded as active and their IC_{50} values were obtained by the cumulative method. Subsequently, active compounds were evaluated by the oral administration in the antigen inhalation-induced bronchospasm model in passively sensitized guinea pigs. The time (seconds) of onset of the asphyxial convulsion was defined as the collapse time. Compounds which elongated 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 pharmacological data of 1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one derivatives are listed in Table VI. Some of these novel xanthine-based tricyclic heterocycles exhibited

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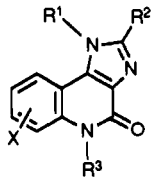
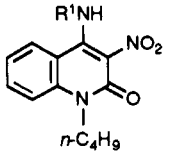
Table III. Substituted 1*H*-Imidazo[4,5-*c*]quinolin-4(5*H*)-ones



compd	R^1	R^2	R^3	X	Y	yield, %	mp, °C	recryst solvent	starting material	formula[a]
7	CH_3	H	CH_3	H	I	46	296–298	MeOH–iPr_2O	6a	$C_{12}H_{11}N_3O\cdot{}^1/_6H_2O$
8	CH_3	H	C_2H_5	H	I	71	216–218	$iPrOH$–iPr_2O	6a	$C_{13}H_{13}N_3O\cdot{}^1/_{10}H_2O$
9	CH_3	H	nC_3H_7	H	I	73	233–235	$iPrOH$–iPr_2O	6a	$C_{14}H_{15}N_3O$
10	CH_3	H	nC_4H_9	H	I	65	208–209	$iPrOH$–iPr_2O	6a	$C_{15}H_{17}N_3O$
11	CH_3	H	iC_4H_9	H	I	50	245–248	chromat–HCl salt[b]	6a	$C_{15}H_{17}N_3O\cdot HCl$[c]
12	CH_3	H	nC_6H_{11}	H	I	73	237–239	chromat–HCl salt	6a	$C_{16}H_{19}N_3O\cdot HCl$
13	CH_3	H	$CH_2C_6H_5$	H	Br	48	238–240	chromat–HCl salt	6a	$C_{16}H_{15}N_3O\cdot HCl\cdot{}^1/_5H_2O$
14	CH_3	H	CH_2-furyl	H	Cl	50	>300	DMF–iPr_2O	6a	$C_{16}H_{13}N_3O_2$
15	CH_3	H	$CH_2CH_2OCH_3$	H	Cl	50	207–210	$iPrOH$–iPr_2O	6a	$C_{14}H_{15}N_3O_2$
16	CH_3	H	cyclo-Pen	H	Br	65	155–158	iPr_2O	6a	$C_{16}H_{17}N_3O$
17	CH_3	H	$CH_2CH_2CH{=}CH_2$	H	Br	55	208–211	Toluene	6a	$C_{15}H_{15}N_3O$
18	CH_3	C_6H_5	nC_4H_9	H	I	74	153–155	chromat–HCl salt	6i	$C_{21}H_{21}N_3O\cdot{}^1/_2HCl$
19	CH_3	CH_3	nC_4H_9	H	I	72	214–215	chromat–HCl salt	6g	$C_{16}H_{19}N_3O\cdot HCl$
20	CH_3	2-furyl	nC_4H_9	H	I	74	180–183	chromat–HCl salt	6h	$C_{19}H_{19}N_3O_2\cdot HCl$
21	CH_3	3,4-$Cl_2C_6H_4$	nC_4H_9	H	I	3[d]	174–176	chromat–HCl salt	6k	$C_{21}H_{19}N_3OCl_2\cdot HCl$
22	CH_3	4-$CH_3OC_6H_4$	nC_4H_9	H	I	24	157–161	chromat–HCl salt	6j	$C_{22}H_{23}N_3O_2\cdot HCl$
23	CH_3	H	nC_4H_9	7,8-$(CH_3O)_2$	I	67	179–181	chromat–HCl salt	6f	$C_{17}H_{21}N_3O_3\cdot HCl$[e]
24	CH_3	H	nC_4H_9	7-Cl	I	63	218–222	chromat–HCl salt	6b	$C_{15}H_{16}N_3OCl\cdot{}^3/_4HCl$
25	CH_3	H	nC_4H_9	8-Cl	I	56	224–227	chromat[f]	6d	$C_{15}H_{16}N_3OCl$
26	CH_3	H	nC_4H_9	8-CH_3	I	69	186–189	chromat	6c	$C_{16}H_{19}N_3O$
27	CH_3	H	nC_4H_9	9-CH_3	I	68	160–164	chromat	6e	$C_{16}H_{19}N_3O$
28	$C_6H_5CH_2$	H	nC_4H_9	H	I	76	230–233	EtOH–H_2O	6l	$C_{21}H_{21}N_3O$

[a] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values except for C of 7 and 19. [b] Purification of the compound was conducted by isolation as hydrochloric salts after chromatography. [c] C: calcd, 61.75; found, 61.28. [d] A yield was based on from **5k**. [e] C: calcd, 58.04; found, 57.44. [f] Purification of the compound was conducted by tritilation with isopropyl ether after chromatography.

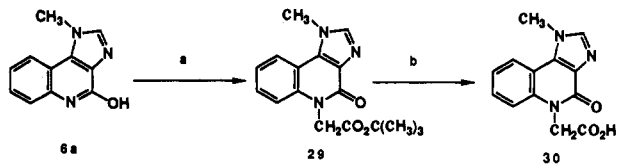
Table IV. 4-(Substituted amino)-1-butyl-3-nitroquinolin-2(1*H*)-ones



compd	R^1	yield, %	mp, °C	recryst solvent	formula[a]
33a	CH_3	85	175–177	$iPrOH$–H_2O	$C_{14}H_{17}N_3O_3$
33b	C_2H_5	97	137–138	$iPrOH$–H_2O	$C_{16}H_{19}N_3O_3$
33c	nC_3H_7	95	124–126	$iPrOH$–H_2O	$C_{16}H_{21}N_3O_3$
33d	iC_3H_7	90	150–152	iPr_2O–hexane	$C_{16}H_{21}N_3O_3$
33e	nC_4H_9	95	124–125	$iPrOH$	$C_{17}H_{23}H_3O_3$
33f	C_6H_5	75	155–157	$iPrOH$–H_2O	$C_{19}H_{19}N_3O_3$

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

Scheme III [a]

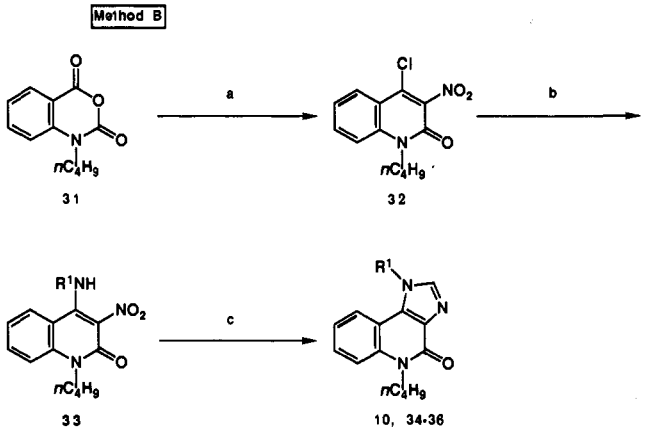


[a] (a) $(CH_3)_2NCOH$, $BrCH_2CO_2C(CH_3)_3$, NaH; (b) CH_2Cl_2, TFA.

potent bronchodilatory activities as expected from our drug design.

Structure–Activity Relationships. (A) R^3 Substituents (7–17 and 30). The bronchodilatory activity was considerably varied with the nature of the R^3 groups in 1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones. Compound **7** or **8** bearing methyl or ethyl was inactive. Introduction of an *n*-propyl or cyclopentyl group showed

Scheme IV [a]

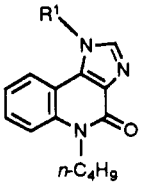


[a] (a) (i) $(CH_3)_2NCOCH_3$, $NO_2CH_2CO_2Et$, NaH, 0–120 °C; (ii) $POCl_3$, Δ; (b) THF, R^1NH_2; (c) (i) EtOH, H_2, 10% Pd–C; (ii) $HC(OEt)_3$, Δ.

moderate activity in the SD assay (**9** and **16**). Though *n*-butyl-substituted compound **10** exhibited potent bronchodilatory activity in vitro and in vivo, isobutyl or *n*-pentyl substitution abolished the activity (**11** and **12**). Furthermore, introduction of lipophilic aralkyl groups such as benzyl and furfuryl groups (**13** and **14**) and a hydrophilic part such as a acetic acid moiety (**30**) eliminated activity. 3-Butenyl-substituted compound **17**, which is a dehydrogenated product of **10**, showed moderate inhibition against the SD reaction, but significant activity was not observed in vivo. Compound **10** was 26-fold more active than theophylline in vitro and was the most potent among **7–17**.

(B) R^1 Substituents (34–38). The effects of R^1 on the activity were examined with 5-butyl-1*H*-imidazo[4,5-*c*]-

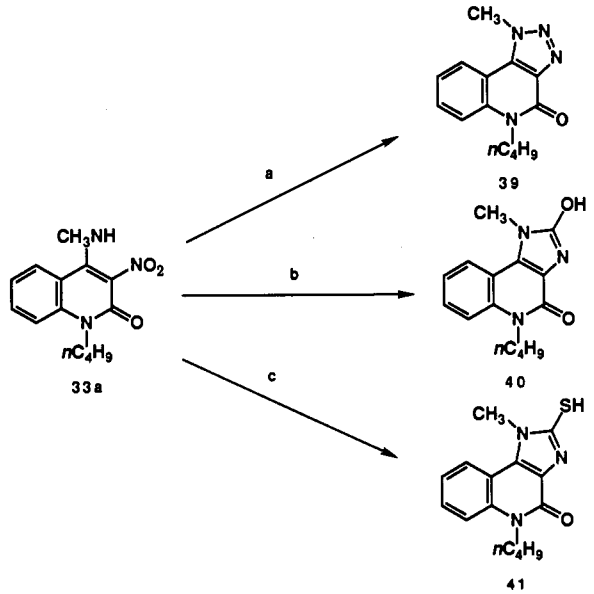
Table V. 1-Substituted 5-Butyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-ones



compd	R_1	yield, %	mp, °C	recryst solvent	starting material	formula[a]
10	CH_3	82	208–209	*i*PrOH–*i*Pr_2O	33a	$C_{16}H_{17}N_3O$
34	C_2H_5	86	192–194	HCl salt[b]	33b	$C_{16}H_{18}N_3O\cdot HCl\cdot H_2O$
35	*n*C_3H_7	81	180–183	HCl salt	33c	$C_{17}H_{21}N_3O\cdot HCl$
36	*i*C_3H_7	82	163–165	HCl salt	33d	$C_{17}H_{21}N_3O\cdot HCl$
37	*n*C_4H_9	82	199–200	HCl salt	33e	$C_{18}H_{23}H_3O\cdot HCl$
38	C_6H_5	50	160–164	DMF–H_2O	33f	$C_{20}H_{19}N_3O$

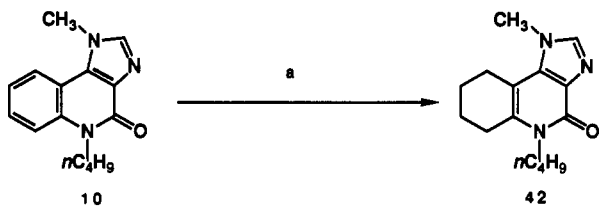
[a] See footnote *a* in Table I. [b] See Experimental Section.

Scheme V [a]



[a] (a) (i) EtOH, H_2, 10% Pd–C; (ii) EtOH, H_2O, concentrated HCl, $NaNO_2$; (b) (i) EtOH, H_2, 10% Pd–C; (ii) THF, CDI, Δ; (c) (i) EtOH, H_2, 10% Pd–C; (ii) THF, thioCDI, Δ.

Scheme VI [a]



[a] (a) TFA, H_2 (60 psi), PtO_2.

quinolin-4(5*H*)-one. When R^1 substituents were the low alkyl groups such as ethyl, *n*-propyl, isopropyl, and *n*-butyl, potent activities were observed in vitro and in vivo (**34–37**). However, at the low dose (25 mg/kg), **34–37** displayed diminished in vivo activities. Substitution by phenyl or benzyl eliminated the activity (**28** and **38**).

(C) R^2 Substituents and Y Atoms (18–22 and 39–41). Triazole **39** (Y = N) decreased in vivo activity compared to that of **10**. Introduction of hydroxy, alkyl, and aryl groups into the 2-position of imidazole (R^2) abolished the activity (**18–22**) in the SD assay. Compound **41** (R^2 = thiol) showed bronchodilatory activity in vitro, but no activity in vivo.

(D) X Substituents (23–27). The effects of substituents in the benzene ring of **10** on the activities were examined. This modification surprisingly diminished the activity. Among these compounds, only **24** (7-Cl) and **26** (8-Me) showed moderate activity in the SD assay but did not show significant in vivo activity.

(E) Mother Skeleton (42). Tetrahydro compound **42** prepared from **10** by hydrogenation abolished the activity.

Summary of these SAR are as follows: (a) SAR concerning R^2, R^3, X, and Y groups were very narrow, and the most effective substituents on the activity were found to be hydrogen, *n*-butyl, hydrogen, and carbon, respectively; (b) there was a bulk tolerance of R^1 substituents to show bronchodilatory activity as long as R^1 was a low alkyl group; (c) the imidazo[4,5-*c*]quinoline skeleton was essential for activity. With respect to acute lethal toxicity in mice, compounds **10**, **24**, **34**, **35**, **36**, **37**, **39**, and **41** did not cause death at a dose of 300 mg/kg, but compounds **9**, **16**, **17**, and theophylline did. Consideration of the above-mentioned SAR and the result of acute lethal toxicity led to the choice of compound **10** for further studies.

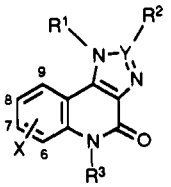
Pharmacological Activities of Compound 10

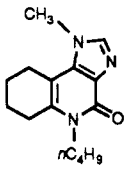
The inhibitory effects of compound **10** and aminophylline (the ethylenediamine salt of theophylline) on contraction of guinea pig tracheal strips induced by several smasmogens were examined (Table VII). Aminophylline moderately inhibited the contraction elicited by antigen, carbachol, histamine, and leukotriene $(LT)D_4$ at almost equal concentrations. On the other hand, **10** showed a profile different from that of aminophylline. It inhibited the SD reaction-induced contraction 108-fold, 8-fold, and 37-fold more potently than the carbachol-, histamine-, and LTD_4-induced contractions, respectively, and furthermore antagonized these contractions more effectively than aminophylline. The difference in the bronchodilatory profiles of **10** and aminophylline suggests that they exhibit different mechanisms of action.

Intravenous or oral administration of drugs after or before antigenic challenge of passively sensitized guinea pigs (Konzett–Rössler preparation) produced dose-related increases in air overflow, and ED_{50} values are shown in Table VIII. The activity of **10** in the intravenous administration was about 19-fold more potent than that of aminophylline. However, surprisingly, the oral activity of **10** was comparable to that of aminophylline. This difference might be caused by the slow oral absorption or metabolism in the gastrointestinal region.

Compound **10** was then evaluated in cardiovascular and CNS pharmacology models in order to further define its therapeutic potential. After intravenous administration of **10** at a dose of 3.0 mg/kg, left ventricular dP/dt_{max} (LV

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



compd	R^1	R^2	R^3	X	Y	SD: $IC_{50} \pm SEM$[a] (μM)	collapse time: MCT ± SEM,[b] 50 mg/kg po (s)	acute lethal toxicity: MLD[c] (mg/kg)
7	CH_3	H	CH_3	H	C	>30		
8	CH_3	H	C_2H_5	H	C	>30		
9	CH_3	H	nC_3H_7	H	C	10.7 ± 3.00		300
10	CH_3	H	nC_4H_9	H	C	0.25 ± 0.13	517 ± 37[d] 342 ± 33[e]	>300
11	CH_3	H	iC_4H_9	H	C	>30		
12	CH_3	H	nC_6H_{11}	H	C	>30		
13	CH_3	H	$CH_2C_6H_5$	H	C	>30		
14	CH_3	H	CH_2-furyl	H	C	>30		
15	CH_3	H	$CH_2CH_2OCH_3$	H	C	>30		
16	CH_3	H	cyclo-Pen	H	C	6.58 ± 2.96		300
17	CH_3	H	$CH_2CH_2CH{=}CH_2$	H	C	5.62 ± 0.65	342 ± 91	200
18	CH_3	C_6H_5	nC_4H_9	H	C	>30		
19	CH_3	CH_3	nC_4H_9	H	C	>30		
20	CH_3	2-furyl	nC_4H_9	H	C	>30		
21	CH_3	3,4-$Cl_2C_6H_4$	nC_4H_9	H	C	>30		
22	CH_3	4-$CH_3OC_6H_4$	nC_4H_9	H	C	>30		
23	CH_3	H	nC_4H_9	7,8-$(CH_3O)_2$	C	>30		
24	CH_3	H	nC_4H_9	7-Cl	C	1.52 ± 0.82	289 ± 38	>300
25	CH_3	H	nC_4H_9	8-Cl	C	>30		
26	CH_3	H	nC_4H_9	8-CH_3	C	7.97 ± 2.74	356 ± 62	>300
27	CH_3	H	nC_4H_9	9-CH_3	C	>30		
28	$C_6H_5CH_2$	H	nC_4H_9	H	C	>30		
30	CH_3	H	CH_2CO_2H	H	C	>30		
34	C_2H_5	H	nC_4H_9	H	C	4.90 ± 1.53	422 ± 43[f]	>300
35	nC_3H_7	H	nC_4H_9	H	C	3.99 ± 1.66	484 ± 31[f]	>300
36	iC_3H_7	H	nC_4H_9	H	C	2.60 ± 1.11	425 ± 50[e]	>300
37	nC_4H_9	H	nC_4H_9	H	C	5.47 ± 1.02	504 ± 44[f]	>300
38	C_6H_5	H	nC_4H_9	H	C	>30		
39	CH_3		nC_4H_9	H	N	1.58 ± 0.78	296 ± 33	>300
40	CH_3	OH	nC_4H_9	H	C	>30		
41	CH_3	SH	nC_4H_9	H	C	8.20 ± 2.42	284 ± 48	>300
42						>30		
theophylline (1a)						6.52 ± 1.26	414 ± 48[d] 389 ± 46[e]	>300

[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. [b] MCT indicated mean collapse time of treated animals. The mean collapse time for untreated animals was 254 ± 18. [c] See Experimental Section. [d] Significant differences from the control at $P < 0.01$ (Scheffe's multiple range test). [e] Values of MCT ± SEM at 25 mg/kg po. [f] Significant differences from the control at $P < 0.05$ (Scheffe's multiple range test).

Table VII. Effects of Compound 10 and Aminophylline on Spasmogen Contracted Guinea Pig Trachea

compd	IC_{50}[a] ± SEM (μM)			
	SD	carbachol	histamine	LTD_4
10	0.25 ± 0.12	27.2 ± 13.3	2.12 ± 1.31	9.19 ± 2.59
aminophylline	16.7 ± 2.23	37.7 ± 8.22	33.2 ± 5.97	23.6 ± 4.69

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

Table VIII. Effects of Compound 10 and Aminophylline on Anaphylactic Bronchoconstriction in Passively Sensitized Guinea Pigs

compound	ED_{50}, mg/kg (95% confidence limits)[a]	
	po	iv
10	44.9 (32.1–62.7)	0.42 (0.19–0.95)
aminophylline	51.7 (23.6–113.3)	7.8 (1.6–36.8)

[a] ED_{50} as determined by linear-regression analysis.

dP/dt_{max}) and heart rate in anesthetized guinea pigs transiently decreased and immediately recovered to the pretreatment value as shown in Table IX. On the contrary, aminophylline significantly increased LV dP/dt_{max} and heart rate. Compound 10 seems to be devoid of cardiostimulant effects associated with theophylline at this dose. Furthermore, theophylline increased spontaneous locomotor activity of mice 2 h after oral administration at doses of 25 and 50 mg/kg (mean ± SEM = 13 209 ± 1110 and 13 058 ± 978 counts ($n = 4$), respectively; significant differences from the value of the placebo at $P < 0.01$ as determined by the Student's *t*-test ($n = 4$); the placebo, 4343 ± 499 counts ($n = 4$)), while compound 10 did not

Table IX. Effects of Compound 10 and Aminophylline on Left Ventricular dP/dt_{max} (LV dP/dt_{max}) and Heart Rate in Anesthetized Guinea Pigs after Their Intravenous Administration (3 mg/kg)

compd	time after administration (min)	percentages of pretreatment value[a] LV dP/dt_{max}	heart rate
	0	100	100
10	1	91.6 ± 3.9[b]	98.5 ± 1.7
	3	98.9 ± 4.6	101.0 ± 1.9
	5	99.4 ± 5.7	101.0 ± 0.6
aminophylline	0	100	100
	1	125 ± 6.7[b]	108 ± 1.9[b]
	3	117 ± 4.9[b]	105 ± 1.7[b]
	5	116 ± 6.3[b]	105 ± 1.9[b]

[a] Each percentage represents the mean ± SEM in groups of guinea pigs ($n = 5$). [b] Significant differences from the pretreatment value at 0 min at $P < 0.05$ determined by the Student's t-test ($n = 5$).

produce any meaningful changes at the same doses (4605 ± 1008 and 4325 ± 1220 counts ($n = 4$), respectively; the placebo, 5943 ± 1676 counts).[17a] These results suggest that compound 10 is superior to theophylline in animal models.

Surprisingly, 34, 35, and 36 did not inhibit PDE isolated from canine tracheal smooth muscle at 10 μM (percent inhibition; below 30%). IC_{50} values of 10 and theophylline were 220 and 110 μM, respectively.[17a]

No receptor binding of a variety of ligands including adenosine (percent inhibition: A_1, 28%; A_2, 48%), histamine, muscarine, and catecholamines was significantly antagonized by 10, 34, 35, and 36 at 100 μM. Though theophylline showed no antagonistic effects on histamine, muscarine, and catecholamines at 100 μM, it moderately inhibited adenosine receptors binding at the same concentration (percent inhibition: A_1, 77%; A_2, 67%). K_i values for the A_1 and A_2 receptors were 23 and 16 μM, respectively.[24] It seems difficult to account for potent bronchodilatory activities of these compounds only by their weak PDE inhibition and adenosine antagonism. Although the precise mechanism of action of these compounds remains to be elucidated, this series of novel tricyclic heterocycles represents a new class of bronchodilator.

During our research, a similar concept was reported by Davey et al.[18] They reported a new series of inodilators without CNS activity, imidazo[1,2-*a*]quinoxalinones and imidazo[1,5-*a*]quinoxalinones and their aza analogs, which were designed from 3-isobutyl-1-methylxanthine (IBMX).

In conclusion, we have designed, synthesized, and evaluated a new series of non-xanthine bronchodilators, 1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one derivatives. Studies of structure–activity relationships led to the selection of 5-butyl-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (10) (KF 15570), which exhibited more potent bronchodilation and possessed fewer side effects than theophylline or aminophylline.

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 (^{1}H 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 mm) was used. The reactions were usually carried out under nitrogen. Organic extracts were dried over anhydrous sodium sulfate and concentrated by a rotary evaporator.

Method A. 5-Butyl-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (10). (a) 4-Hydroxy-1-methyl-1*H*-imidazo[4,5-*c*]quinoline (6a). A solution of 6.0 g (0.033 mol) of 5a in 7.4 mL (0.066 mol) of 30% hydrogen peroxide and 90 mL of acetic acid was stirred at 80 °C for 12 h. After cooling, the solvent was evaporated under reduced pressure, and the residue was neutralized with saturated aqueous sodium hydrogen carbonate. The resulting precipitate was filtered and dried. The suspension of the crude mixture in 80 mL of acetic anhydride was heated under reflux for 1 h. After cooling, the solvent was evaporated under reduced pressure and 40 mL of methyl alcohol was added to the residue. Twenty-eight percent solution of sodium methoxide in methyl alcohol was added dropwise to the suspension until pH 10. The resulting precipitate was filtered, washed with methyl alcohol, and dried. Recrystallization from DMF–methyl alcohol gave 2.5 g (38%) of 6a as brown crystals: mp >300 °C; IR 1655 cm^{-1}; NMR (DMSO-d_6) δ 4.18 (s, 3 H), 7.20–7.32 (m, 1 H), 7.40–7.55 (m, 2 H), 8.13 (br d, 1 H, $J = 8$ Hz), 8.15 (s, 1 H), 11.58 (s, 1 H). Anal. ($C_{11}H_9N_3O \cdot {}^1/_2H_2O$) C, H, N.

(b) 5-Butyl-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (10). To a suspension of 3.0 g (0.015 mol) of 6a in 50 mL of DMF was added 0.80 g (0.020 mol) of 60 wt % sodium hydride at 0 °C in portions, followed by stirring at 50 °C for 0.5 h. Then the mixture was again ice-cooled, and 2.6 mL (0.023 mol) of butyl iodide was added dropwise. After stirring at 50 °C for 2 h, 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 brine, dried, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel using $CHCl_3$–methyl alcohol 17:1 and recrystallized from isopropyl alcohol–isopropyl ether to give 2.5 g (65%) of 10 as colorless crystals: mp 208–209 °C; IR (KBr) 1645, 1004 cm^{-1}; NMR (DMSO-d_6) δ 0.94 (t, 3 H, $J = 7$ Hz), 1.37–1.45 (m, 2 H), 1.55–1.64 (m, 2 H), 4.17 (s, 3 H), 4.34 (t, 2 H, $J = 7$ Hz), 7.34 (br t, 1 H, $J = 8$ Hz), 7.55–7.65 (m, 2 H), 8.10 (s, 1 H), 8.20 (br d, 1 H, $J = 8$ Hz). Anal. ($C_{15}H_{17}N_3O$) C, H, N.

5-(Carboxymethyl)-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (30). (a) 5-[(*tert*-Butoxycarbonyl)methyl]-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (29). To a suspension of 3.0 g (0.015 mol) of 6a in 50 mL of DMF was added 0.90 g (0.023 mol) of 60 wt % sodium hydride at 0 °C in portions, followed by stirring at 50 °C for 0.5 h. Then the mixture was

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again ice-cooled, and 4.9 mL (0.030 mol) of *tert*-butyl bromoacetate was added dropwise. After stirring at 50 °C for 0.5 h, 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 brine, dried, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel using $CHCl_3$–methyl alcohol 15:1 to give 2.7 g (57%) of **29** as colorless crystals: mp 237–239 °C; IR (KBr) 1735, 1661 cm^{-1}; MS *m/z* (M^+); NMR ($CDCl_3$) δ 1.43 (s, 9 H), 4.12 (s, 3 H), 5.12 (s, 2 H), 7.05–7.47 (m, 3 H), 7.69 (s, 1 H), 7.93 (br t, 1 H, *J* = 8 Hz).

(b) 5-(Carboxymethyl)-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (30). To a solution of 2.3 g (7.4 mmol) of **29** in 50 mL of CH_2Cl_2 was added 50 mL of trifluoroacetic acid with ice cooling. The solution was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure, and ethyl ether was added to the residue. The resulting precipitate was filtered and dried. Recrystallization from DMF–isopropyl ether gave 1.5 g (77%) of **30** as colorless crystals: mp 215–218 °C; IR (KBr) 3700–2200, 1651 cm^{-1}; NMR (DMSO-d_6) δ 4.19 (s, 3 H), 5.13 (s, 2 H), 7.36 (br t, 1 H, *J* = 8 Hz), 7.46 (br d, 1 H, *J* = 8 Hz), 7.55 (br t, 1 H, *J* = 8 Hz), 8.13 (s, 1 H), 8.23 (br d, 1 H, *J* = 8 Hz), 12.85–13.15 (m, 1 H); HRMS (M^+) calcd for $C_{13}H_{11}N_3O_3$ 257.0801, found 257.0812.

Method B. 5-Butyl-1-ethyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one Hydrochloride (34). (a) 1-Butyl-4-chloro-3-nitroquinolin-2(1*H*)-one (32). To a solution of 2.4 mL (0.026 mol) of ethyl nitroacetate in 30 mL of dry dimethylacetamide (DMA) was added 1.0 g (0.026 mol) of 60 wt % sodium hydride at 0 °C in portions. When the evolution of hydrogen ceased, 5.2 g (0.024 mol) of **31** was added. The temperature was raised slowly to 120 °C and kept there for 5 h (carbon dioxide evolution occurred). After the solvent was evaporated under reduced pressure, 15 mL of water and 15 mL of CH_2Cl_2 were added to the residue. The resulting precipitate was filtered. The aqueous layer of the filtrate was made acidic with concentrated HCl, the precipitate was recollected by filtration and dried together with previously recovered solid.

Then 16 mL (0.17 mol) of phosphorus oxychloride was added to the solid, and the suspension was refluxed for 1 h. After cooling, the solvent was evaporated under reduced pressure and water was added to the residue. The mixture was neutralized with 2 N NaOH solution and extracted with $CHCl_3$. The organic layer was washed with brine, dried, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel using $CHCl_3$ to give 1.1 g (16%) of **32** as yellow crystals. Hexane was used for recrystallization: mp 100–102 °C; IR (KBr) 1655, 1541 cm^{-1}; NMR ($CDCl_3$) δ 1.01 (t, 3 H, *J* = 7 Hz), 1.20–1.98 (m, 4 H), 4.34 (t, 2 H, *J* = 7 Hz), 7.12–7.60 (m, 2 H), 7.75 (br t, 1 H, *J* = 8 Hz), 8.11 (br d, 1 H, *J* = 8 Hz). Anal. ($C_{13}H_{13}N_2O_3Cl$) C, H, N.

(b) 1-Butyl-4-(ethylamino)-3-nitroquinolin-2(1*H*)-one (33b). To a solution of 3.5 g (0.013 mol) of **32** in 30 mL of tetrahydrofuran was added 8.0 mL (0.13 mol) of ethylamine with ice cooling. The solution was stirred at room temperature for 12 h. The solvent was evaporated under reduced pressure, and water was added to the residue. The resulting precipitate was filtered, washed with brine, and dried. Recrystallization from isopropyl alcohol–water gave 3.5 g (97%) of **33b** as yellow crystals: mp 137–138 °C; IR (KBr) 1612, 1549 cm^{-1}; NMR ($CDCl_3$) δ 0.98 (t, 3 H, *J* = 7 Hz), 1.37 (t, 3 H, *J* = 7 Hz), 1.26–2.00 (m, 4 H), 3.30–3.68 (m, 2 H), 4.24 (t, 2 H, *J* = 7 Hz), 6.15–6.42 (m, 1 H), 7.10–7.85 (m, 4 H). Anal. ($C_{15}H_{19}N_3O_3$) C, H, N.

(c) 5-Butyl-1-ethyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one Hydrochloride (34). To a suspension of 3.3 g (0.011 mol) of **33b** in 100 mL of ethyl alcohol was added 0.70 g of 10% palladium/carbon. The mixture was stirred under a hydrogen gas at room temperature for 4 h. Then the mixture was filtered and concentrated. Nineteen milliliters (0.11 mol) of ethyl orthoformate was added to the residue. The suspension was stirred at 110 °C for 1 h. After cooling, the solvent was evaporated under reduced pressure and the residue was dissolved in $CHCl_3$ and filtered. Ten milliliters of ethyl acetate saturated by hydrogen chloride gas was added to the residue. The resulting precipitate was filtered, washed with ethyl acetate, and dried to afford 3.0 g (86%) of **34** as a colorless solid: mp 190–194 °C; IR (KBr) 1678 cm^{-1}; NMR (DMSO-d_6) δ 0.95 (t, 3 H, *J* = 7 Hz), 1.55 (t, 3 H, *J* = 7 Hz), 1.36–1.68 (m, 4 H), 4.40 (t, 3 H, *J* = 7 Hz), 4.73 (q, 2 H, *J* = 7 Hz), 6.50–7.10 (m, 1 H), 7.48 (br t, 3 H, *J* = 8 Hz), 7.70–7.82 (m, 2 H), 8.20 (br d, 1 H, *J* = 8 Hz), 9.18 (s 1 H). Anal. ($C_{16}H_{19}N_3O_3 \cdot HCl \cdot H_2O$) C, H, N.

5-Butyl-1-methyl-1*H*-triazolo[4,5-*c*]quinolin-4(5*H*)-one (39). To a suspension of 1.7 g (6.1 mmol) of **33a** in 85 mL of ethyl alcohol was added 0.34 g of 10% palladium/carbon. The mixture was stirred under hydrogen gas at room temperature for 4 h. Then the mixture was filtered and concentrated, and 20 mL of ethyl alcohol was added to the residue. To the mixture was added 0.55 mL of concentrated HCl and 15 mL of water with stirring at 0 °C. Then a solution of 0.56 g (8.0 mmol) of sodium nitrite in 6.0 mL of water was added dropwise. After 30 min, the precipitate was filtered and recrystallized from DMF–water to give 1.1 g (70%) of **39** as yellow crystals: mp 144–145 °C; IR (KBr) 1670 cm^{-1}; NMR (DMSO-d_6) δ 0.95 (t, 3 H, *J* = 7 Hz), 1.34–1.50 (m, 2 H), 1.56–1.72 (m, 2 H), 4.35 (t, 2 H, *J* = 7 Hz), 4.56 (s, 3 H), 7.44 (br t, 1 H, *J* = 8 Hz), 7.65–7.76 (m, 2 H), 8.28 (br d, 1 H, *J* = 8 Hz); HRMS (M^+) calcd for $C_{14}H_{16}N_4O$ 256.1324, found 256.1351.

5-Butyl-2-hydroxy-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (40). To a suspension of 1.7 g (6.1 mmol) of **33a** in 85 mL of ethyl alcohol was added 0.34 g of 10% palladium/carbon. The mixture was stirred under hydrogen gas at room temperature for 4 h. Then the mixture was filtered and concentrated. Thirty milliliters of tetrahydrofuran and 1.5 g (9.3 mmol) of carbonyldiimidazole were added to the residue, and the mixture was refluxed for 4 h. After cooling, the precipitate was filtered, washed with isopropyl ether, and dried. Recrystallization from isopropyl alcohol–water gave 1.2 g (74%) of **40** as colorless crystals: mp 296–299 °C; IR (KBr) 1712, 1694, 1659 cm^{-1}; NMR (DMSO-d_6) δ 0.94 (t, 3 H, *J* = 7 Hz), 1.32–1.46 (m, 2 H), 1.54–1.69 (m, 2 H), 3.69 (s, 3 H), 4.34 (t, 2 H, *J* = 7 Hz), 7.33 (br t, 1 H, *J* = 8 Hz), 7.56 (br t, 1 H, *J* = 8 Hz), 7.65 (br d, 1 H, *J* = 8 Hz), 8.18 (br d, 1 H, *J* = 8 Hz), 11.47 (s, 1 H). Anal. ($C_{15}H_{17}N_3O_2$) C, H, N.

5-Butyl-2-mercapto-1-methyl-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (41). To a suspension of 1.7 g (6.1 mmol) of **33a** in 85 mL of ethyl alcohol was added 0.34 g of 10% palladium/carbon. The mixture was stirred under a hydrogen gas at room temperature for 4 h. The mixture was filtered and concentrated. To this residue was added 50 mL of tetrahydrofuran and 1.8 g (9.2 mmol) of thiocarbonyldiimidazole, and the mixture was refluxed for 30 min. After cooling, the precipitate was filtered, washed with isopropyl ether, and dried. Recrystallization from isopropyl alcohol–water gave 1.5 g (85%) of **41** as colorless crystals: mp >300 °C; IR (KBr) 1663, 1616 cm^{-1}; NMR (DMSO-d_6) δ 0.94 (t, 3 H, *J* = 7 Hz), 1.33–1.49 (m, 2 H), 1.60–1.72 (m, 2 H), 4.10 (s, 3 H), 4.35 (t, 2 H, *J* = 7 Hz), 7.39 (br t, 1 H, *J* = 8 Hz), 7.59–7.70 (m, 2 H), 8.29 (br d, 1 H, *J* = 8 Hz), 13.55 (s, 1 H). Anal. ($C_{15}H_{17}N_3OS$) C, H, N.

5-Butyl-1-methyl-6,7,8,9-tetrahydro-1*H*-imidazo[4,5-*c*]quinolin-4(5*H*)-one (42). To a solution of 1.6 g (6.3 mmol) of **10** in 30 mL of trifluoroacetic acid was added 0.32 g of platinum oxide and stirred under hydrogen gas at 60 psi at room temperature for 5 d. After the mixture was filtered, the solvent was evaporated under reduced pressure and water was added to the residue. The mixture was neutralized with 4 N NaOH solution and extracted with $CHCl_3$. The organic layer was washed with brine, dried, and concentrated under reduced pressure. The residue was chromatographed on a column of silica gel using $CHCl_3$–methyl alcohol 50:1 to give 1.2 g (72%) of **42** as colorless crystals. Isopropyl ether–isopropyl alcohol was used for recrystallization: mp 225–227 °C; IR (KBr) 1652, 1568 cm^{-1}; NMR (DMSO-d_6) δ 0.92 (t, 3 H, *J* = 7 Hz), 1.26–1.57 (m, 4 H), 1.63–1.85 (m, 4 H), 2.72 (t, 2 H, *J* = 6 Hz), 2.94 (t, 2 H, *J* = 6 Hz), 3.35 (s, 3 H), 3.95 (t, 2 H, *J* = 7 Hz), 7.80 (s, 1 H). Anal. ($C_{15}H_{21}N_3O$) C, H, N.

Schultz-Dale Reaction in Tracheal Strips of Passively Sensitized Guinea Pigs.[19] Male Hartley strain guinea pigs weighing 350–450 g were passively sensitized by intraperitoneal injection of 1 mL/animal of rabbit antiovalubumin (Anti-OA) serum 16–18 h before use. The animals were killed by stunning and bleeding. Trachea were excised and adhering adipose and connective tissues cleaned. Tracheal zig-zag strips were prepared by the method of Emmerson and Mackay,[20] followed by equil-

ibration 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 final bath concentration. Test drugs were added cumulatively at intervals of 7 min after the contraction of tracheal strips reached a plateau. Contractions were recorded isotonically using isotonic transducers (TD-112S: Nihon Kohden) connected to recorders (TYPE 30066: Yokokawahokusin electric), and percent relaxation of each drug concentration was calculated by the maximum relaxation of papaverine at 10^{-4} mol. The concentration of each drug required to produce 50% relaxation (EC_{50}) was determined from the least-squares regression analysis.

Antigen Inhalation-Induced Bronchospasm Model in Passively Sensitized Guinea Pigs. The modified methods by Herxheimer[21] was used as follows. Male Hartley guinea pigs (350–450 g) were passively sensitized by intraperitoneal injection of 1 mL/animal of rabbit Anti-OA serum 16–24 h before use. Guinea pigs were placed individually in a clear plastic container (13 × 18 × 25 cm) and challenged with 1.5% OA using a nebulizer (V type: Nihon Shoji) at a rate of about 0.83 L/min. The time (seconds) 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 compounds were suspended in 0.3% Tween 80 and were orally administered 1 h before antigen exposure. Animals were pretreated with diphenhydramine (20 mg/kg, ip) and propranolol (5 mg/kg, ip) 30 min before antigen exposure.

Acute Toxicity. The compounds were orally administered to male dd mice weighing 20–25 g ($n = 3$). Minimum lethal doses (MLD) were determined for 7 days after the administration by observing the death of at least one mouse.

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 described above. Contraction was induced by carbachol (3×10^{-6} mol final concentration), histamine (3×10^{-5} mol), or LTD_4 (50 μg/mL). Test drugs were administered cumulatively at intervals of 7 min after the contraction of tracheal strips reached a plateau. The concentration of each drug required to produce 50% relaxation (IC_{50}) was determined from the least-squares regression analysis.

Anaphylactic Bronchoconstriction in Passively Sensitized Guinea Pigs. Male Hartley guinea pigs (350–450 g) were passively sensitized by intraperitoneal injection 1 mL/animal of rabbit anti-OA serum 16–24 h before use. Bronchoconstriction was measured by a modified technique of Konzet and Rössler method.[22] The guinea pigs were anesthetized with urethane (1.5 g/kg, ip). The right jugular vein was cannulated for the administration of test drugs and the left carotid artery, for measurement of blood pressure. The trachea was cannulated and animals were subsequently administered gallamine triethiodide (10 mg/kg, iv) for arrest of spontaneous respiration. Guinea pigs were then artificially ventilated with a rodent respirator (TB-101, Takashima) at 60–70 strokes/min. Bronchoconstriction was induced with OA challenge (5 mg/kg, iv) and measured as percentage of maximum contraction every minute. A side arm to the tracheal cannula was connected to a bronchospasm transducer (Ugo Basil) to obtain a continuous measure of air overflow, which was recorded on a polygraph (RM45: Nihon Kohden). Area under curve of bronchoconstriction was measured by a imaging analyzer (MCID system: Imaging Research). Test compounds were suspended in 0.3% Tween 80 and were administered orally 1 h before antigen challenge. In the intravenous administration, test compounds were suspended in polyethylene glycol 400 (2 mg/mL), diluted with saline, and injected 90 s after challenge. These vehicles were also used in other pharmacological experiments that followed. Data of area under curve of bronchoconstriction were then plotted versus the log of drug concentration, and the value of ED_{50} was estimated by linear-regression analysis.

Cardiovascular Effects in Anesthetized Guinea Pigs. Male guinea pigs were anesthetized with pentobarbital sodium (50 mg/kg, ip). The animal was ventilated via a tracheotomy with room air (60 respirations per min, stroke volume, 10 mL/kg). A cannula was advanced through an incision in the left common carotid artery into the left ventricle. LVP and LV dP/dt_{max} were measured with a pressure transducer (MPC-500, Millar Instruments) connected with the cannula. Heart rate was triggered by LVP. The left jugular vein was cannulated for intravenous drug injections. Changes in heart rate and LV dP/dt_{max} were expressed as percentage of pretreatment values.[23] Administration of vehicle did not make any changes in these parameters.

Locomotor Activity of Mice. Male dd mice (20–25 g) were monitored in the vertical activity in Automex-II (Columbus Instruments) for 120 min after the oral administration of test drugs.

Adenosine Binding. Adenosine A_1 and A_2 binding were performed according to the same protocol as described before.[24]

Phosphodiesterase Activity. The cAMP-specific PDE (type IV)[25] was isolated from canine tracheal smooth muscle according to Silver's method.[17c] The assay was done as described before.[17b]

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