Bronchodilator Activity of Xanthine Derivatives Substituted with Functional Groups at the 1- or 7-Position

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Xanthine derivatives with several functional groups at the 1- or 7-position were synthesized, and their pharmacological activities in guinea pigs were studied. In general, the *in vitro* tracheal relaxant action and positive chronotropic action of 3-propylxanthines were increased by substitutions with nonpolar functional groups at the 1-position, but decreased by any substitution at the 7-position. On the other hand, because positive chronotropic actions of substituents with allyl, aminoalkyl, alkoxyalkyl, and normal alkyl groups were much less than tracheal muscle relaxant action, these compounds had high tracheal selectivity. The selectivity for tracheal muscle became very high with substitutions of 3′-butenyl, (dimethylamino)ethyl, 2′-ethoxyethyl, 3′-methoxypropyl, and *n*-propyl groups at the 1-position and of 2′-ethoxyethyl, 2′-oxopropyl, and *n*-propyl groups at the 7-position, compared with theophylline and the corresponding unsubstituted xanthines, 3-propylxanthine and 1-methyl-3-propylxanthine. When compounds were intraduodenally administered to the guina pig, 1-(2′-ethoxyethyl)-, 1-(3′-methoxypropyl)-, 1-(3′-butenyl)-, and 1-[(dimethylamino)-ethyl]-3-propylxanthines, 1-methyl-7-(2′-oxopropyl)-3-propylxanthine, and denbufylline (1,3-di-*n*-butyl-7-(2′-oxopropyl)xanthine) effectively inhibited the acetylcholine-induced bronchospasm without heart stimulation or central nervous system-stimulation at the effective dosage range. Particularly, the bronchodilatory effect of 1-(2′-ethoxyethyl)-3-propylxanthine was much stronger and more continuous than those of theophylline and pentoxifylline. On the other hand, there were certain relationships among the *in vitro* tracheal relaxant activities of these compounds, their affinities for adenosine (A_1) receptors in the brain membrane, and their inhibition of cyclic AMP-phosphodiesterase (PDE) in the tracheal muscle. The affinity for A_2 receptors of these compounds was very low or negligible. This suggests that both the action on A_1 receptors or interaction with adenosine and the cyclic AMP-PDE inhibitory activity contribute to the bronchodilator action of 1- and 7-substituted xanthines. This study indicates that the substitutions with none or low polar functional groups at the 1-position could improve the selectivity and duration of the bronchodilator effects of xanthines.

Introduction

Because theophylline used for asthma has some adverse reactions such as tachycardia and central nervous system stimulation,[1,2] we have tried to develop selective bronchodiltors from xanthine derivatives.[3–7] In our previous paper we described the structure–activity relationships of a series of 1,3,7-trialkylxanthines. We indicated that while the 3-substitution increased both tracheal smooth muscle relaxant action and positive chronotropic action, substitution at the 1- and 7-positions was important for tracheal relaxation and decline of heart stimulation, respectively, and both increased bronchoselectivity.[7] On the other hand, pentoxifylline and propentofylline were developed for peripheral and central circulatory disturbances with low cardioexcitatory and central nervous system-stimulatory actions.[8–10] Although these xanthines caused only weak tracheal relaxation, 1-(5′-oxohexyl)-3-*n*-propylxanthine was the strongest tracheal relaxant, and further prolongation of the alkyl chain length at the 3-position and alkylation at the 7-position decreased heart stimulation more than tracheal relaxation, resulting in selectivity for the trachea.[6] However, the 5′-oxohexyl group of these compounds is assumed to be rapidly degraded by reductive and oxidative metabolism *in vivo*, as reported for pentoxifylline.[11,12] Then, in this study, to obtain selective, long-acting bronchodilators we synthesized a series of 3-*n*-propylxanthines substituted by several functional groups at the 1- and 7-position and evaluated their bronchodilator effects in guinea pigs.

Results and Discussion

Because substitution by the normal propyl group at the 3-position of the xanthine skeleton induced the strongest relaxation of the tracheal muscle in our studies on the structure–activity relationships of alkylxanthines[3–5,7] and 1-(5′-oxohexyl)xanthine derivatives,[6] we synthesized a series of 3-*n*-propylxanthines with several functional groups at the 1- or 7-position by the route outlined in Scheme I. According to our previously described method for introduction of the alkyl groups,[7] xanthine derivatives (**4–20**) with the functional groups at the 1-position were synthesized by acid deprotection of 7-(*p*-methoxybenzyl) derivatives (II) and/or hydrolysis of ethyl ester III. Intermediate II was obtained from enprofylline (1) by treatment with *p*-methoxybenzyl chloride/K_2CO_3, followed by alkylation with various alkyl halides containing a functional group (route A). Xanthine derivatives (**25–40**) with the functional groups at the 7-position were prepared by direct alkylation for the 1,3-disubstituted compound

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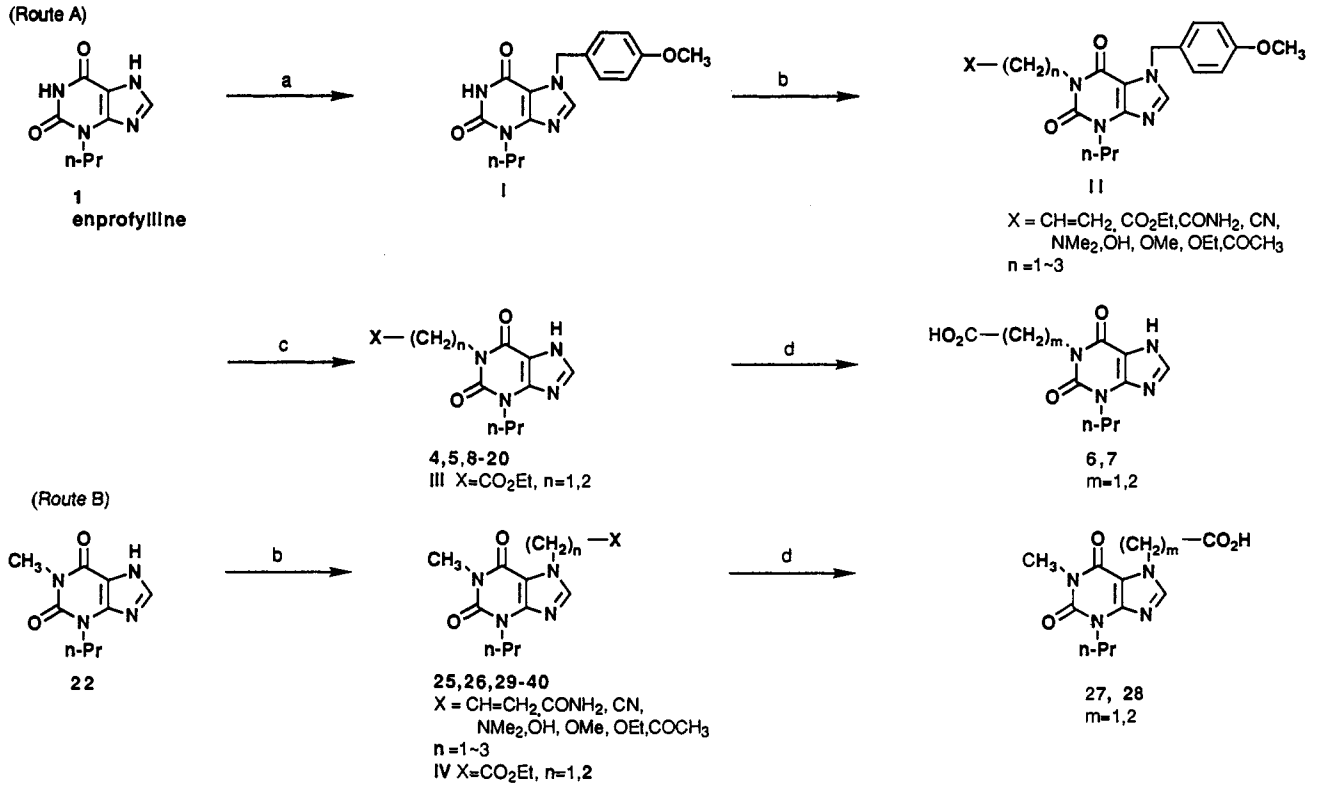
Scheme I [a]



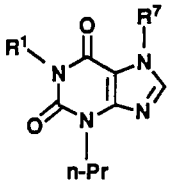
[a] (a) *p*-Methoxybenzyl chloride, K_2CO_3/DMF; (b) X-$(CH_2)_n$-Cl (or Br), K_2CO_3/DMF; (c) TFA, concentrated H_2SO_4/anisole; (d) 2 N NaOH/EtOH.

22 with alkyl halides containing a functional group and/or hydrolysis of ester (IV) (route B). The generalized chemical structures and compound numbers are shown in Table I.

The *in vitro* pharmacological activities of 1,7-substituted 3-*n*-propylxanthine derivatives were examined and compared with those of their parent compounds and the existent xanthine compounds and are shown in Table II. The *in vitro* relaxant activity on the spontaneous tone of isolated tracheal ring chains and positive chronotropic action on the isolated right atrium were generally increased by substitutions with low or nonpolar functional groups at the 1-position but decreased by any substitutions at the 7-position. Regarding 1-substituents, normal alkyl, allyl, cyanoalkyl, hydroxyalkyl, and alkoxyalkyl groups (**2–5, 10, 11, 14–18**) potentiated, and the carboxyalkyl and carbamylalkyl groups (**6–9**) decreased the tracheal relaxant activity of the unsubstituted parent compound, enprofylline (**1**). On the other hand, allyl and cyanoalkyl groups (**4, 5, 10, 11**) increased, but the carboxyalkyl, carbamylalkyl, aminoalkyl, and carbonylalkyl groups (**6–9, 12, 13, 19, 20**) almost abolished the positive chronotropic action. Furthermore, the effects of carbon chain length on these pharmacological activities were different among the kinds of functional groups at the 1- or 7-position. In substituents with normal alkyl, allyl, and cyanoalkyl groups at the 1-position (**2–5, 10, 11**), the effect on the trachea became strong but the effect on the heart was diminished or barely changed by prolongation in one methylene length, and among alkoxyalkyl substituents, introduction of the 2′-ethoxyethyl group at the 1-position (**17**) markedly increased tracheal relaxation but decreased heart stimulation, while the 2′-methoxyethyl group (**16**) increased both activities. With other substitutions, the alkyl carbon chain prolongation changed both pharmacological activities to be strong or weak. From these results, the selectivity for tracheal muscle became very high upon substitutions of *n*-propyl (**3**), 3′-butenyl (**5**), (dimethylamino)ethyl (**12**), 2′-ethoxyethyl (**17**), and 3′-methoxypropyl (**18**) groups at the 1-position and of *n*-propyl (**24**), 2′-ethoxyethyl (**38**), and 2′-oxopropyl (**39**) groups at the 7-position, compared with theophylline and the corresponding unsubstituted xanthines, enprofylline (**1**) and 1-methyl-3-propylxanthine (**22**).

Then we evaluated the *in vivo* pharmacological effects of compounds that showed high bronchoselectivity *in vitro*, comparing them with unsubstituted parent compounds and existent drugs, theophylline, pentoxifylline, and denbufylline. When compounds were administered into the duodenum of guinea pigs, all compounds reduced the acetylcholine-induced bronchospasm and increased the heart rate, in a dose-dependent manner. Among them, the 50% inhibition dosages for the bronchospasm (ED_{50}) of 1-[(dimethylamino)ethyl]- (**12**), 1-(2′-ethoxyethyl)- (**17**), and 1-(3′-methoxypropyl)-3-propylxanthines (**18**), 1-methyl-7-(2′-oxopropyl)-3-propylxanthine (**39**), and denbufylline were over 10-fold lower than the dosages inducing 15% increase in the heart rate (ED_{15}) by them, and they had high bronchoselectivity (Table III). Other compounds and control drugs had low selectivity for bronchodilation, particularly, although 1,3-dipropylxanthine (**3**) and 1-(5′-oxohexyl)-3-*n*-propylxanthine (**21**) had very strong relaxant effect and high selectivity for the trachea *in vitro*, their *in vivo* bronchoselectivity was only about 5. This difference between the *in vitro* effects and the *in vivo* effects of these xanthine derivatives seems to be due to their pharmacokinetic behaviors in the animals. Figure 1 shows the course of inhibition of acetylcholine-induced bronchospasm after the intraduodenum administration of compounds at a dose inducing about 50% inhibition at

Table I. Physicochemical Data for 3-Propylxanthine Derivatives



compd no.	R^1	R^7	yield[a] (%)	mp (°C)	recryst solvent	formula[b]
1[7]	H	H		291–292	DMF–Et_2O	$C_8H_{10}N_4O_2$
2[7]	Et	H		180–181	EtOH	$C_{10}H_{14}N_4O_2$
3[7]	*n*-Pr	H		205–207	MeOH	$C_{11}H_{16}N_4O_2$
4	$CH_2CH{=}CH_2$	H	75	161–163	AcOEt	$C_{11}H_{14}N_4O_2$
5	$CH_2CH_2CH{=}CH_2$	H	52	201–203	AcOEt	$C_{12}H_{16}N_4O_2$
6	CH_2CO_2H	H	98	>300	DMF–EtOH	$C_{10}H_{12}N_4O_4$
7	$CH_2CH_2CO_2H$	H	86	233–234	DMF	$C_{11}H_{14}N_4O_4$
8	CH_2CONH_2	H	89	276.5–278	aqueous DMF	$C_{10}H_{13}N_5O_3$
9	$CH_2CH_2CONH_2$	H	94	257–259	MeOH	$C_{11}H_{15}N_5O_3$
10	CH_2CN	H	46	209–211	EtOH	$C_{10}H_{11}N_5O_2$
11	CH_2CH_2CN	H	24	183–185	i-PrOH	$C_{11}H_{13}N_5O_2$
12	$CH_2CH_2N(CH_3)_2$	H	42	235–240	EtOH	$C_{12}H_{19}N_5O_2{\cdot}HCl$
13	$CH_2CH_2CH_2N(CH_3)_2$	H	60	220–225	i-PrOH	$C_{13}H_{21}N_5O_2{\cdot}HCl$
14[22]	CH_2CH_2OH	H	90	204–206	EtOH	$C_{10}H_{14}N_4O_3$
15[22]	$CH_2CH_2CH_2OH$	H	68	146–148	i-PrOH	$C_{11}H_{16}N_4O_3$
16	$CH_2CH_2OCH_3$	H	55	148–150	AcOEt	$C_{11}H_{16}N_4O_3$
17	$CH_2CH_2OCH_2CH_3$	H	59	153.5–154.5	Benzene	$C_{12}H_{18}N_4O_3$
18	$CH_2CH_2CH_2OCH_3$	H	67	161–162.5	EtOH	$C_{12}H_{18}N_4O_3$
19	CH_2COCH_3	H	60	199–200.5	EtOH	$C_{11}H_{14}N_4O_3$
20	$CH_2CH_2COCH_3$	H	58	198–199	EtOH	$C_{12}H_{16}N_4O_3$
21[20]	$(CH_2)_4COCH_3$	H		146.5–148	MeOH	$C_{14}H_{20}N_4O_3$
22[7]	CH_3	H		220–221	EtOH	$C_9H_{12}N_4O_2$
23[7]	CH_3	Et		114–115	i-Pr_2O	$C_{11}H_{16}N_4O_2$
24[7]	CH_3	*n*-Pr		86–87	i-Pr_2O	$C_{12}H_{18}N_4O_2$
25	CH_3	$CH_2CH{=}CH_2$	67	71–72	i-Pr_2O	$C_{12}H_{16}N_4O_2$
26	CH_3	$CH_2CH_2CH{=}CH_2$	64	61.5–62	i-Pr_2O	$C_{13}H_{18}N_4O_2$
27	CH_3	CH_2CO_2H	58	170–172	AcOEt	$C_{11}H_{14}N_4O_4$
28	CH_3	$CH_2CH_2CO_2H$	81	179–180.5	AcOEt	$C_{12}H_{16}N_4O_4$
29	CH_3	CH_2CONH_2	90	284–285	MeOH	$C_{11}H_{15}N_5O_3$
30	CH_3	$CH_2CH_2CONH_2$	69	214–215.5	MeOH	$C_{12}H_{17}N_5O_3$
31	CH_3	CH_2CN	60	120.5–122	i-Pr_2O	$C_{11}H_{13}N_5O_2$
32	CH_3	CH_2CH_2CN	79	151–152.5	AcOEt	$C_{12}H_{15}N_5O_2$
33	CH_3	$CH_2CH_2N(CH_3)_2$	55	214–216	EtOH	$C_{13}H_{21}N_5O_2{\cdot}HCl$
34	CH_3	$CH_2CH_2CH_2N(CH_3)_2$	75	178–183	i-PrOH	$C_{14}H_{23}N_5O_2{\cdot}HCl$
35	CH_3	CH_2CH_2OH	71	148–151	i-PrOH	$C_{11}H_{16}N_4O_3$
36	CH_3	$CH_2CH_2CH_2OH$	54	87–91	i-PrOH–i-Pr_2O	$C_{12}H_{18}N_4O_3$
37	CH_3	$CH_2CH_2OCH_3$	61	83.5–86	i-Pr_2O	$C_{12}H_{18}N_4O_3$
38	CH_3	$CH_2CH_2OCH_2CH_3$	57	78–79	i-Pr_2O	$C_{13}H_{20}N_4O_3$
39	CH_3	CH_2COCH_3	65	128–129.5	AcOEt	$C_{12}H_{16}N_4O_3$
40	CH_3	$CH_2CH_2COCH_3$	45	117–118.5	i-PrOH–i-Pr_2O	$C_{13}H_{18}N_4O_3$

[a] Yields were of final procedure and purified product. [b] All compounds analyzed for C, H, N; analytical results were within ± 0.4% of theoretical values.

the maximum level. The bronchodilatory effect of theophylline (30 mg/kg) rapidly appeared and continued for 110 min, but at this dosage the heart stimulation also continued at a high level. The appearance of effect of 1,3-dipropylxanthine (**3**) was delayed much longer than theophylline, suggesting low absorbability from the intestine, but the effect of compound **24**, which has also long alkyl groups at the 3- and 7-positions, rapidly appeared and declined. The bronchodilator effect of compound **12** rapidly appeared and continued 50 min after the administration, and thereafter decreased. The effects of compounds substituted with the carbonylalkyl groups at the 1- or 7-position (**21**, **39**, denbutylline) reached the maximum level 5 to 20 min after administration, and then gradually decreased, suggesting metabolic degradation of the carbonyl group, as reported for pentoxifylline.[11,12] Compounds having the alkoxyalkyl group at the 1-position were comparatively long-acting, particularly, compound **17** continuously inhibited the acetylcholine-induced bronchospasm without any effect on the heart. Furthermore, when these xanthine derivatives were orally administered to ddY mice, the alkoxyalkyl compounds caused mild decrease of motor activity over 30 mg/kg as well as pentoxifylline and denbufylline, while theophylline increased motor activity over 3 mg/kg. Consequently, we could obtain favorable bronchodilators from 1-alkoxyalkyl-substituted xanthines, which were completely free from the drawbacks of theophylline.

There are a number of hypotheses for the mechanism of pharmacological activities of xanthines from cyclic AMP-PDE inhibition and adenosine antagonism. Persson *et al.*[2,13] indicated that enprofylline, which is a negligible antagonist of adenosine, has a potent bronchodilator effect like theophylline. Denbufylline is a selective inhibitor of PDE IV,[14] a cyclic AMP-specific PDE,[15] and improvement of peripheral circulation by pentoxifylline and its analog propentofylline was elucidated from PDE inhibition[16] and adenosine-uptake inhibition.[17] Adenosine is a candidate as a mediator of asthma, and xanthines antagonize the A_1-mediated contractile reaction.[18] We have reported that the tracheal relaxant activities of xanthines increased in cyclic AMP-PDE inhibitory activities with prolongation

Table II. *In Vitro* Pharmacological Activities of 3-*n*-Propylxanthine Derivatives

compd no.	substitution R¹	substitution R⁷	tracheal relaxation: EC_{50}, μM	heart stimulation: EC_{15}, μM	broncho-selectivity[a]
1	H	H	7.2 ± 1.3	18.3 ± 5.6	2.5
2	CH_2CH_3	H	1.3 ± 0.3	6.1 ± 0.5	4.7
3	$CH_2CH_2CH_3$	H	0.05 ± 0.04	20.0 ± 2.2	400
4	$CH_2CH{=}CH_2$	H	1.6 ± 0.3	5.4 ± 1.5	3.4
5	$CH_2CH_2CH{=}CH_2$	H	0.20 ± 0.06	4.5 ± 0.3	22.5
6	CH_2CO_2H	H	41.0 ± 5.1	>100	2.4
7	$CH_2CH_2CO_2H$	H	21.2 ± 1.7	>100	4.7
8	CH_2CONH_2	H	>100	>100	1
9	$CH_2CH_2CONH_2$	H	13.1 ± 3.3	31.2 ± 7.9	2.4
10	CH_2CN	H	4.2 ± 0.7	1.7 ± 0.4	0.4
11	CH_2CH_2CN	H	1.7 ± 0.5	4.3 ± 1.3	2.5
12	$CH_2CH_2N(CH_3)_2$	H	2.7 ± 0.6	>100	37.0
13	$CH_2CH_2CH_2N(CH_3)_2$	H	20.5 ± 2.1	>100	4.9
14	CH_2CH_2OH	H	0.51 ± 0.06	2.0 ± 0.3	3.9
15	$CH_2CH_2CH_2OH$	H	5.1 ± 0.7	26.4 ± 5.1	5.2
16	$CH_2CH_2OCH_3$	H	0.70 ± 0.21	6.3 ± 0.9	9.0
17	$CH_2CH_2OCH_2CH_3$	H	0.07 ± 0.02	25.3 ± 1.5	361
18	$CH_2CH_2CH_2OCH_3$	H	2.9 ± 0.7	31.3 ± 7.1	10.8
19	CH_2COCH_3	H	14.9 ± 3.6	45.7 ± 6.3	3.1
20	$CH_2CH_2COCH_3$	H	3.5 ± 0.8	27.0 ± 3.7	7.7
21[b]	$CH_2CH_2CH_2CH_2COCH_3$	H	0.06 ± 0.02	3.6 ± 0.7	62
22	CH_3	H	1.6 ± 0.2	3.3 ± 0.6	2.1
23	CH_3	CH_2CH_3	5.9 ± 2.3	>100	16.9
24	CH_3	$CH_2CH_2CH_3$	5.0 ± 1.2	>100	20.0
25	CH_3	$CH_2CH{=}CH_2$	11.2 ± 2.2	22.4 ± 2.7	2.0
26	CH_3	$CH_2CH_2CH{=}CH_2$	16.8 ± 3.6	30.6 ± 5.3	1.8
27	CH_3	CH_2CO_2H	>100	>100	1
28	CH_3	$CH_2CH_2CO_2H$	>100	>100	1
29	CH_3	CH_2CONH_2	>100	>100	1
30	CH_3	$CH_2CH_2CONH_2$	>100	>100	1
31	CH_3	CH_2CN	35.3 ± 7.5	>100	2.8
32	CH_3	CH_2CH_2CN	27.6 ± 3.6	>100	3.6
33	CH_3	$CH_2CH_2N(CH_3)_2$	>100	>100	1
34	CH_3	$CH_2CH_2CH_2N(CH_3)_2$	>100	>100	1
35	CH_3	CH_2CH_2OH	31.8 ± 3.1	>100	3.1
36	CH_3	$CH_2CH_2CH_2OH$	22.6 ± 3.6	>100	4.4
37	CH_3	$CH_2CH_2OCH_3$	36.1 ± 5.7	>100	2.8
38	CH_3	$CH_2CH_2OCH_2CH_3$	4.6 ± 1.2	>100	21.7
39	CH_3	CH_2COCH_3	1.9 ± 0.5	37.5 ± 3.6	19.7
40	CH_3	$CH_2CH_2COCH_3$	12.2 ± 3.1	>100	8.2
theophylline			28.2 ± 2.1	62.0 ± 4.3	2.2
pentoxifylline[b]			28.3 ± 1.3	52.4 ± 11.6	1.9
denbufylline			0.01 ± 0.003	6.0 ± 3.5	600

Data are the mean ± SEM of three to five experiments. [a] Defined as the ratio of stimulatory activity in the right atrium (EC_{15}, μM) to relaxant activity in the tracheal muscle (EC_{50}, μM). The value over 100 μM was calculated as 100 μM. [b] Data from previous literature.[6]

Table III. *In Vivo* Pharmacological Actions

compd	bronchodilator action: ED_{50} (mg/kg, id)	positive chronotropic action: ED_{15} (mg/kg, id)	broncho-selectivity[a]
theophylline	23.4 (15.1–33.2)	20.2[c]	0.9
1	25.7 (18.2–35.0)	95.1	3.7
3	4.69 (3.40–5.50)	25.5	5.4
5	1.84 (1.40–2.42)	11.0	6.0
12	5.65 (4.29–7.51)	>100	17.7
17	1.70 (1.17–2.33)	20.0	11.8
18	0.72 (0.53–0.96)	10.7	14.9
21	2.55 (1.75–3.96)	13.5	5.3
pentoxifylline	43.0 (35.3–57.6)	39.4	0.9
22	4.44 (3.40–5.50)	5.70	1.3
24	27.5 (21.5–42.3)	>100	3.6
38	12.1 (10.6–13.7)	25.2	2.1
39	1.49 (1.04–1.94)	30.0	20.1
denbufylline	5.90 (4.43–7.45)	90.0	15.3

[a] Defined as the ratio of positive chronotropic action (ED_{15}) to bronchodilator action (ED_{50}). [b] The mean (95% confidence interval) from three to five animals. [c] The mean from three to five animals.

of alkyl chain length only at the 3-position and closely correlated with the affinity for A_1 receptors in alkyl substitutions at the 1-, 3-, and 7-positions.[3-5,7] Then, to see the biochemical mechanism for tracheal muscle relaxant activities of xanthine derivatives, we measured the activity on cyclic AMP-PDE in guinea pig tracheal muscle and affinities for adenosine A_1 and A_2 receptors in the brain membranes (Table IV). The inhibitory activity on cyclic AMP-PDE and affinities for adenosine receptors were increased by substitutions with low-polarity groups at the 1-position and decreased by 7-substitutions, as observed in the tracheal relaxant activity. The affinity for A_2 receptors of these xanthines was generally less than that for A_1 receptors and was negligible in polar compounds. Then, the relationships between the tracheal muscle relaxant activities and cyclic AMP-PDE inhibitory activities and affinities for A_1 receptors were examined. The PDE inhibitory activities of 1- and 7-position substituents were related to their tracheal relaxant activities with $r = 0.69$ and 0.65, respectively. The affinity for the A_1 receptor of these xanthine derivatives had certain correlations with the tracheal relaxant action ($r = 0.77$ in 1-substituents and $r = 0.70$ in 7-substituents). These results and our previous evidence[7] suggest that not only the cyclic AMP-PDE inhibitory activity but also the adenosine (A_1) antagonistic action is involved in the bronchodilatory effect of the xanthine derivatives. On the other hand, xanthines substituted with low polar groups generally induced tracheal relaxation at lower

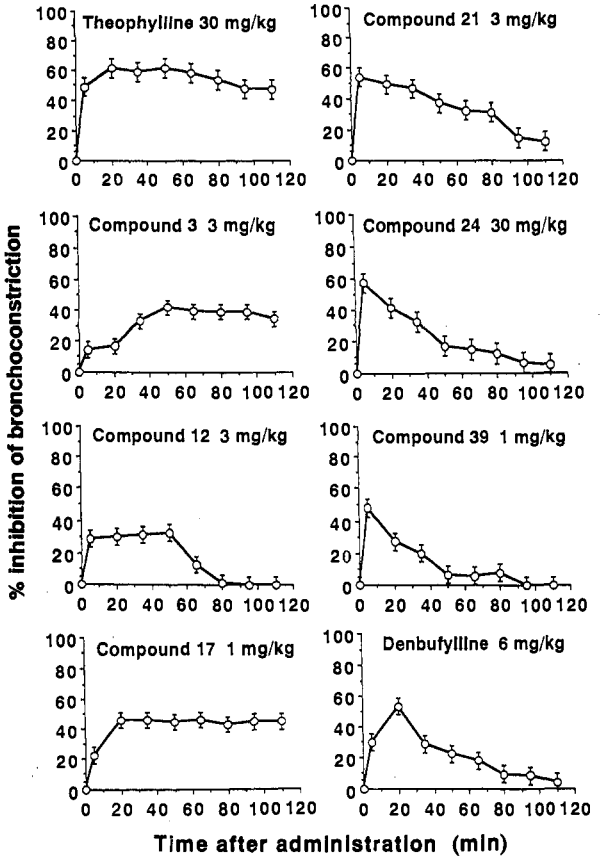


Figure 1. Courses of inhibitory effects of xanthine derivatives on acetylcholine-induced bronchospasm in guinea pigs. Compounds were intraduodenally administered to anesthetized guinea pigs, and then acetylcholine was intravenously injected at a dose of 40 μg/kg at the indicated intervals. Data are expressed as the mean ± SEM of three to five experiments.

concentrations than the K_i values for cyclic AMP-PDE inhibition and adenosine A_1 affinity. This also suggests that these modifications of the xanthine skeleton may contribute to increase of the affinity and/or permeability for the trachea more than the heart and provide strong tracheal relaxation and bronchoselectivity.

In conclusion, this study indicates that the substitutions with none or low-polarity functional groups at the 1-position increase tracheal muscle relaxant activity and improve the bronchoselectivity, and among them 1-(2′-ethoxyethyl)-3-propylxanthine (17) is a good bronchodilator, without drawbacks of theophylline.

Experimental Section

Chemistry. All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: ^{1}H-NMR spectra with a JEOL JNM FX-90Q(90 MHz) or a JEOL JNM A-500 (500 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard; mass spectra (MS) with a JEOL JMS-DX 300 or a JMS-D 300 mass spectrometer; IR spectra with a Hitachi 270-30 spectrometer. Column chromatography was done with Kieselgel 60 (Merck). Elemental analyses were done with a Yanagimoto MT-3 or MT-5 elemental analysis apparatus. TLC was done on a 0.25-mm precoated silica gel plate ($60F_{254}$ Merck). Theophylline was purchased from Sigma Chemical Co., St. Louis, MO. Pentoxifylline,[19] 1-(5′-oxohexyl)-3-propylxanthine (21),[20] and denbufylline[21] were prepared by previously reported methods.

3,7-Dihydro-7-(4-methoxybenzyl)-3-propyl-1*H*-purine-2,6-dione (I). A slurry of 3-propylxanthine (1) (6.68 g, 34 mmol), *p*-methoxybenzyl chloride (4.90 mL, 36 mmol), and anhydrous K_2CO_3 (4.75 g, 34 mmol) in DMF (70 mL) was heated at 60 °C for 2 h. The reaction mixture was poured into ice–water and neutralized with 2 N hydrochloric acid. The resultant precipitate was filtered, washed with water, and dried to yield 8.00 g (74%) of pale yellow solid, which was recrystallized from MeOH, giving analytically pure I as colorless needles: mp 192–193 °C; MS (M^+) 314; ^{1}H NMR (90 MHz, CD_3OD) δ 0.94 (t, 3H, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.74 (sextet, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 3.76 (s, 3H, OCH_3), 3.97 (t, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 5.41 (s, 2H, CH_2-Ar), 6.88 (d, 2H, J = 9 Hz, 3,5-ArH), 7.35 (d, 2H, J = 9 Hz, 2,6-ArH), 7.97 (s, 1H, 8-H). Anal. ($C_{16}H_{18}N_4O_3$) C, H, N.

3,7-Dihydro-7-(4-methoxybenzyl)-1-(2-propenyl)-3-propyl-1*H*-purine-2,6-dione (II, X = CH=CH$_2$, *n* = 1). A slurry of I (4.0 g, 13 mmol), allyl bromide (1.7 mL, 19 mmol), and anhydrous K_2CO_3 (1.8 g, 13 mmol) in DMF (25 mL) was heated at 60 °C for 4 h. The reaction mixture was poured into ice–water. The resultant precipitate was filtered, washed with water and *n*-hexane, and dried to yield 4.3 g (96%) of II (X = CH=CH_2, n = 1), which was recrystallized from i-Pr_2O, giving an analytically pure sample as colorless needles: mp 98–100 °C; MS (M^+) 354; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.96 (t, 3H, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.78 (sextet, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 3.80 (s, 3H, OCH_3), 4.05 (t, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 4.63 (dt, 2H, J = 5.5, 1.5 Hz, CH_2CH=CH_2), 5.18 (dq, 1H, J = 10, 1.5 Hz, CH_2CH=CHH(cis)), 5.24 (dq, 1H, J = 17.5, 1.5 Hz, CH_2CH=CHH(trans)), 5.42 (s, 2H, CH_2Ar), 5.95 (ddt, 1H, J = 17.5, 10, 5.5 Hz,

Table IV. Biochemical Activities of 3-*n*-Propylxanthine Derivatives

compd no.	cyclic AMP-PDE inhibition: K_i, μM	affinity: A_1 receptor: K_i, μM	affinity: A_2 receptor: K_i, μM
1	43.1 ± 0.4	60.7 ± 4.6	90.6 ± 9.4
2	24.1 ± 8.7	1.4 ± 0.6	3.9 ± 0.7
3	26.1 ± 10.1	0.21 ± 0.07	2.4 ± 0.5
4	27.3 ± 1.4	1.7 ± 1.0	11.8 ± 3.2
5	14.6 ± 3.7	0.06 ± 0.02	3.2 ± 1.3
6	>200	>200	>200
7	60.0 ± 9.0	>200	>200
8	>200	39.7 ± 11.5	>200
9	56.3 ± 10.3	31.5 ± 8.7	>200
10	23.8 ± 1.9	2.1 ± 0.5	81.5 ± 23.6
11	40.8 ± 3.3	15.9 ± 3.3	162 ± 34
12	9.0 ± 1.9	4.3 ± 0.3	>200
13	92.0 ± 5.7	43.2 ± 15.4	>200
14	14.1 ± 3.2	50.0 ± 16.3	>200
15	12.6 ± 2.0	12.0 ± 3.5	68.7 ± 21.6
16	8.2 ± 1.7	2.5 ± 1.1	21.6 ± 5.2
17	22.3 ± 3.7	1.9 ± 1.0	12.0 ± 3.3
18	48.7 ± 7.2	12.4 ± 2.8	15.7 ± 1.9
19	46.4 ± 3.9	11.4 ± 1.7	>200
20	13.0 ± 0.5	11.2 ± 2.1	144 ± 16.4
21[a]	16.4 ± 2.4	23.1 ± 4.7	–[b]
22	10.6 ± 3.5	1.9 ± 0.2	8.0 ± 3.2
23	32.3 ± 6.0	14.2 ± 1.8	21.5 ± 1.6
24	30.8 ± 1.4	9.4 ± 5.1	43.8 ± 1.3
25	35.8 ± 3.5	15.4 ± 2.2	12.1 ± 1.6
26	29.9 ± 1.7	6.0 ± 2.3	5.6 ± 1.2
27	>200	>200	>200
28	>200	>200	>200
29	119 ± 24	>200	>200
30	92.4 ± 45.3	>200	>200
31	16.5 ± 2.5	6.6 ± 0.3	12.8 ± 3.4
32	46.0 ± 5.0	88.6 ± 21.2	>200
33	53.0 ± 12.2	>200	>200
34	>200	>200	>200
35	28.7 ± 11.3	41.4 ± 17.3	>200
36	26.2 ± 6.3	112 ± 36	>200
37	72.7 ± 21.8	12.4 ± 2.3	58.7 ± 26.9
38	136 ± 33	24.3 ± 5.7	22.9 ± 3.2
39	20.7 ± 4.0	95.7 ± 5.9	>200
40	32.8 ± 1.3	30.0 ± 11.7	>200
theophylline	56.8 ± 8.9	9.8 ± 0.4	16.6 ± 2.5
pentoxifylline[a]	37.7 ± 3.5	180 ± 2	–
denbufylline	44.9 ± 3.9	16.5 ± 4.0	31.5 ± 11.6

Data are the mean ± SEM of three to five measurements. [a] Data from previous literature.[6] [b] Not determined.

$CH_2CH{=}CH_2$), 6.89 (d, 2H, J = 9 Hz, 3,5-ArH), 7.32 (d, 2H, J = 9 Hz 2,6-ArH), 7.51 (s, 1H, 8-H). Anal. ($C_{19}H_{22}N_4O_3$) C, H, N.

3,7-Dihydro-1-(2-propenyl)-3-propyl-1*H*-purine-2,6-dione (4). A solution of II (X = $CH{=}CH_2$, n = 1) (3.6 g, 10 mmol), concentrated sulfuric acid (8 drops), and anisole (1.5 g, 13 mmol) in TFA (21 mL) was refluxed for 10 h and then evaporated. The oily residue was diluted with water and i-Pr_2O and neutralized with 20% NaOH to pH 6 with stirring. The resultant precipitate was filtered, washed with water and i-Pr_2O, and dried to yield 1.8 g (75%) of 4, which was recrystallized from AcOEt, giving analytically pure 4 as colorless plates: mp 161–163 °C; MS (M^+) 234; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.99 (t, 3H, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.83 (sextet, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 4.14 (t, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 4.72 (d, 2H, J = 5.5 Hz, $CH_2CH{=}CH_2$), 5.19 (dd, 1H, J = 10, 1.5 Hz, $CH_2CH{=}CHH$(cis)), 5.25 (dd, 1H, J = 17, 1.5 Hz, $CH_2CH{=}CHH$(trans)), 5.97 (ddt, 1H, J = 17, 10, 5.5 Hz, $CH_2CH{=}CH_2$), 7.81 (s, 1H, 8-H), 13.0 (br s, 1H, N-H). Anal. ($C_{11}H_{14}N_4O_2$) C, H, N.

Compounds **5**, **8–20**, and III were prepared via corresponding II by a similar procedure to the above and physicochemical data are shown in Table I.

(2,3,6,7-Tetrahydro-2,6-dioxo-3-propyl-1*H*-purin-1-yl)acetic Acid (6). A solution of ethyl (2,3,6,7-tetrahydro-2,6-dioxo-3-propyl-1*H*-purin-1-yl)acetate (III; X = CO_2Et, n = 1) (2.1 g, 7.5 mmol) and 2 N NaOH (11 mL) in EtOH (21 mL) was stirred at room temperature for 1.5 h and then evaporated. The residue was diluted with water. An insoluble material was filtered off, and the filtrate was adjusted with 10% HCl to pH 3. The resultant precipitate was filtered and washed with water to yield 1.9 g (98%) of 6, which was recrystallized from a mixture of DMF and MeOH, giving analytically pure 6 as colorless needles: mp > 300 °C, MS (M^+) 252; ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.88 (t, 3H, $CH_2CH_2CH_3$), 1.71 (sextet, 2H, $CH_2CH_2CH_3$), 3.98 (t, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 4.54 (s, 2H, CH_2CO_2H), 8.00 (s, 1H, 8-H), 13.44 (br s, 1H, N-H). Anal. ($C_{10}H_{12}N_4O_4$) C, H, N.

Compound **7** was prepared via corresponding III by a similar procedure to the above, and physicochemical data are shown in Table I.

3,7-Dihydro-1-methyl-7-(2-propenyl)-3-propyl-1*H*-purine-2,6-dione (25). To a mixture of 3,7-dihydro-1-methyl-3-propyl-1*H*-purine-2,6-dione (**22**) (2.0 g, 9.6 mmol) and anhydrous K_2CO_3 (1.5 g, 11 mmol) in DMF (20 mL) at room temperature was added allyl bromide (0.9 mL, 11 mmol), and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated. The oily residue was diluted with water and extracted with Et_2O. The ether layer was washed with water three times, dried, and concentrated to give a pale yellow solid, which was recrystallized from i-Pr_2O, yielding 1.6 g (67%) of pure **25** as colorless plates: mp 71–72 °C; MS (M^+) 248; ^{1}H NMR (90 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.88 (sextet, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 3.41 (s, 3H, OCH_3), 4.08 (t, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 4.95 (dt, 2H, J = 5.5, 1.5 Hz, $CH_2CH{=}CH_2$), 5.25 (dq, 1H, J = 16.5, 1.5 Hz, $CH_2CH{=}CHH$(trans)), 5.32 (dq, 1H, J = 10.5, 1.5 Hz, $CH_2CH{=}CHH$(cis)), 6.08 (ddt, 1H, J = 16.5, 10.5, 5.5 Hz, $CH_2CH{=}CH_2$), 7.56 (s, 1H, 8-H). Anal. ($C_{12}H_{16}N_4O_2$) C, H, N.

Compounds **26**, **29–40**, and IV were prepared from corresponding **22** by a similar procedure to the above, and physicochemical data are shown in Table I.

(2,3,6,7-Tetrahydro-1-methyl-2,6-dioxo-3-propyl-1*H*-purin-7-yl)acetic Acid (27). A solution of ethyl (2,3,6,7-tetrahydro-1-methyl-2,6-dioxo-3-propyl-1*H*-purin-7-yl)acetate (IV: X = CO_2Et, n = 1) (1.9 g, 6.5 mmol) and 2 N NaOH (9.7 mL) in EtOH (18 mL) was stirred at room temperature for 1.5 h and then evaporated. The residue was diluted with water. An insoluble material was filtered off, and the filtrate was adjusted with 10% HCl to pH 3. The resultant precipitate was filtered and washed with water to yield 0.99 g (58%) of **27**, which was recrystallized from AcOEt, giving analytically pure **27** as colorless needles: mp 170–172 °C; MS (M^+) 266; ^{1}H NMR (90 MHz, DMSO-d_6) δ 0.89 (t, 3H, J = 7.5 Hz, $CH_2CH_2CH_3$), 1.71 (sextet, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 3.21 (s, 3H, NCH_3), 3.97 (t, 2H, J = 7.5 Hz, $CH_2CH_2CH_3$), 5.07 (s, 2H, CH_2CO_2H), 8.00 (s, 1H, 8-H).

Compound **28** was prepared via corresponding IV by a similar procedure to the above, and physicochemical data are shown in Table I.

Pharmacological Methods. All compounds were dissolved in Krebs-Henseleit solution or respective assay buffer in the *in vitro* experiments and suspended in 0.5% carboxymethylcellulose in the *in vivo* experiments. Animals used in experiments except for the intoxication test in mice were male Hartley guinea pigs weighing 300–500 g (Nippon SLC, Hamamatsu, Japan).

Tracheal Muscle Relaxation. Relaxation of the spontaneous tone in isolated tracheal muscle ring chains was measured through an isotonic transducer. Briefly, isolated tracheal ring chains were placed in a 10-mL thermostatically controlled organ bath (37 °C) containing Krebs-Henseleit solution (pH 7.4), gassed with 95% O_2–5% CO_2, and isoprenaline (1.0 μM) was added to produce complete relaxation. After isoprenaline was washed out, a tension of 0.5 g was applied to the preparation, and spontaneous tone was allowed to develop. After the tension became constant, the preparation was treated with cumulative concentrations of a compound. Relaxation by 1.0 μM isoprenaline was defined as 100%, and the concentration producing 50% relaxation (EC_{50}) was calculated.

Beating Rate of Right Atrium. The isolated right atrium was placed in an organ bath (25 °C) filled with Krebs-Henseleit solution aerated with 95% O_2–5% CO_2. The preparation was allowed to equilibrate under a tension of 0.5 g and then treated with cumulative concentrations of a compound. The beating rate was recorded through a cardiotachometer triggered by isometric contraction on the atria. Data were expressed as an EC_{15}, the dose causing a 15% increase in heart rate over the unstimulated rate.

Acetylcholine-Induced Bronchoconstriction. Under urethane anesthesia (1.5 g/kg, ip), the guinea pigs were made to respire artifically with a small-animal ventilator. The bronchoconstriction in the animals was recorded using a bronchospasm transducer (Ugo Basile, Comerio-Varese, Italy) by the overflow technique of Konzett and Rossler.[23] The heart rate was measured through a cardiotachometer triggered by the blood pressure pulse of the left carotid artery. Acetylcholine (40 μg/kg) was injected into the jugular vein at 15-min intervals. After three similar responses by acetylcholine, a compound was administered intraduodenally 5 min before the next injection of the spasmogen, and the responses induced by the spasmogen were monitored at 15-min intervals for 110 min. The bronchodilatory effect and the positive chronotropic action of compounds were evaluated by the dose producing 50% inhibition of the spasmogen-induced response (ED_{50}) and the dose producing 15% increase of the heart rate (ED_{15}), respectively, calculated from data from three to five independent experiments. The vehicle (0.5% carboxymethylcellulose) alone did not change the basal responses within the experimental period of 110 min.

Cyclic AMP-Phosphodiesterase Assay. Inhibitory activity of xanthines on cyclic AMP-phosphodiesterase (PDE) with low K_m (0.61 μM) in the 10 000-g supernatant of trachealis muscle homogenate was measured by the 2-step assay system of Thompson & Appleman.[24] The inhibition constant (K_i) was calculated by the method of Dixon.[25]

Adenosine Receptor Binding Assay. Affinity for adenosine (A_1) receptors of xanthines was measured by a ligand (tritiated 8-cyclopentyl-1,3-dipropylxanthine, [^{3}H]CPX[26]) binding replacement on a membrane preparation of the cerebral cortex.[27] The A_2 receptor binding assay was done using [^{3}H]CGS-21680[28] on striatal membranes.[29] The dose-inhibition data was analyzed using a nonlinear least-squares fit to a competitive inhibition model, and the inhibition constant (K_i) was calculated from the Cheng & Prusoff equation.[30]

Protein was measured by the method of Lowry *et al.*[31]

Observation of General Symptoms of Mice. After the oral administration of a compound to male ddY mice (6-weeks old, Nippon SLC), the condition and motor activity of mice were observed.

Statistical Analysis. The regression lines were calculated by a nonlinear least-squares method program, 'MULTI', written by Yamaoka *et al.*[32] using a personal computer, PC-9801 (Nihon Electric Co., Tokyo).

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