

## Effects of Alkyl Substitutions of Xanthine Skeleton on Bronchodilation

Ryosuke Sakai,<sup>†</sup> Kayo Konno,<sup>†</sup> Yasunori Yamamoto,<sup>†</sup> Fujiko Sanae,<sup>†</sup> Kenzo Takagi,<sup>‡</sup> Takaaki Hasegawa,<sup>‡</sup> Nobuhiko Iwasaki,<sup>§</sup> Masato Kakiuchi,<sup>§</sup> Hideo Kato,<sup>§</sup> and Ken-ichi Miyamoto<sup>\*†</sup>

Research Laboratory for Development of Medicine, Hokuriku University, School of Pharmacy, Ho-3 Kanagawa-machi, Kanazawa 920-11, Japan, Nagoya University School of Medicine, Tsurumai, Nagoya 466, Japan, and Hokuriku Seiyaku Co. Ltd., Inokuchi, Katsuyama 911, Japan

Received March 23, 1992

Structure-activity relationships in a series of 1,3,7-trialkyl-xanthine were studied with guinea pigs. Relaxant actions in the tracheal muscle were increased with alkyl chain length at the 1- and 3-positions of the xanthine skeleton, but decreased by alkylation at the 7-position. Positive chronotropic actions in the right atrium were potentiated with 3-alkyl chain length but tended to decrease with 1-alkylation and diminish by 7-substitution. Consequently, while the 1- and 3-substitutions were equally important for the tracheal smooth muscle relaxation, the substitution at the 1-position was more important than the 3-substitution for bronchoselectivity. The 7-alkylation may be significant to cancel heart stimulation. There were good correlations between the smooth muscle relaxant action and the cyclic AMP-PDE inhibitory activity in 3-substituents and the affinity for adenosine (A<sub>1</sub>) receptors in 1-, 3-, and 7-substituents. This suggests that not only the cyclic AMP-PDE inhibitory activity but also the adenosine antagonistic activity is important in the bronchodilatory effects of alkylxanthines. Among these xanthine derivatives, 1-butyl-3-propylxanthine and its 7-methylated derivative showed high bronchoselectivity in the in vitro and in vivo experiments compared to theophylline and enprofylline and may be new candidates for bronchodilator.

### Introduction

Theophylline is one of the most effective and frequently used drugs for the treatment of asthma. However, its adverse reactions on cardiovascular and central nervous systems are well known and impair its usefulness.<sup>1,2</sup> Moreover, the mechanism of bronchodilatory action of theophylline is not completely known.

Studies on structure-activity relationship give strategies to synthesize progressively more potent drugs and evidence for the action mechanism. Persson et al.<sup>2-4</sup> studied the variety of methylxanthines and developed 3-propylxanthine (enprofylline), which is a negligible antagonist of adenosine, having a potent bronchodilatory effect like theophylline. We have studied the structure-activity relationships of 3-alkyl-1-methylxanthines and indicated that the tracheal muscle relaxant activity and cyclic AMP-phosphodiesterase (PDE) inhibitory activity are increased with alkyl chain length at the 3-position of the xanthine nucleus,<sup>5</sup> and methylation at the 1-position induces further potent activities.<sup>6,7</sup> Thus, there is now only fragmentary evidence for structure-activity relationship to obtain selective bronchodilators from xanthine derivatives and

to understand the action mechanisms. In this study, we synthesized a series of alkylxanthines and examined the effects of the substitutions on the broncho muscle, heart, cyclic AMP-PDE, and adenosine receptors.

### 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: <sup>1</sup>H-NMR spectra with JEOL JNM FX-90Q (90 MHz) or JEOL JNM A-500 (500 MHz) spectrometer with tetramethylsilane (TMS) as an internal standard; mass spectra (MS) with JEOL JMS-DX 300 or JMS-D 300 mass spectrometer; IR spectra with 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<sub>254</sub> Merck). Xanthine (1), 1-methylxanthine (2), 3-methylxanthine (6), and theophylline (7) were purchased from Sigma Chemical Co., St. Louis, MO.

(7) Miyamoto, K.; Takagi, K.; Sakai, R.; Wakusawa, S.; Koshiura, R.; Nadai, M.; Apichartpichean, R.; Hasegawa, T. Correlation between hydrophobicity of N-alkylxanthine derivatives and their biological activities on guinea-pig isolated tracheal smooth muscle. *J. Pharm. Pharmacol.* 1989, 41, 844-847.

(8) Persson, C. G. A.; Gustafsson, B.; Kjellin, G. Tracheal relaxation and adenosine antagonism by 1- and/or 3-alkylxanthines. *Curr. Clin. Pract. Ser.* 1985, 19, 447-453.

(9) Dalt, J. W.; Padgett, W. L.; Shamim, M. T. Analogs of caffeine and theophylline: Effect of structural alterations on affinity at adenosine receptors. *J. Med. Chem.* 1986, 29, 1305-1308.

(10) Croce, M. A.; Kramer, G. L.; Garbers, D. L. Inhibition of alkaline phosphatase by substituted xanthines. *Biochem. Pharmacol.* 1979, 28, 1227-1231.

(11) Wooldridge, K. R. H.; Slack, R. The synthesis of some 6-thio-xanthines. *J. Chem. Soc.* 1962, 1863-1868.

(12) Ohtsuka, Y. Oxazolopyrimidines. VI. Formation of 3-substituted xanthines via 7(6H)-iminooxazolopyrimidines. *Bull. Chem. Soc. Jpn.* 1973, 46, 506-509.

(13) G. D. Searle & Co. 2,4-Pyrimidones. U.S. Pat. 2,602,795 (*Chem. Abstr.* 47, 4920g).

(14) Blicke, F. F.; Godt, H. C., Jr. Diuretics. I. 3-Substituted paraxanthines. *J. Am. Chem. Soc.* 1954, 76, 3653-3655.

(15) Beavo, J. A.; Rogers, N. L.; Crofford, O. B.; Hardman, J. G.; Sutherland, E. W.; Newman, E. V. Effects of xanthine derivatives on lipolysis and on adenosine 3',5'-monophosphate phosphodiesterase activity. *Mol. Pharmacol.* 1970, 6, 597-603.

<sup>†</sup> Hokuriku University.

<sup>‡</sup> Nagoya University.

<sup>§</sup> Hokuriku Seiyaku Co. Ltd.

(1) Piasfsky, K. M.; Ogilvie, R. I. Dosage of theophylline in bronchial asthma. *New Engl. J. Med.* 1975, 292, 1218-1222.

(2) Persson, C. G. A.; Andersson, K. E.; Kjellin, G. Effects of enprofylline and theophylline may show the role of adenosine. *Life Sci.* 1986, 38, 1057-1072.

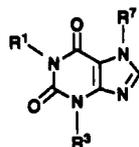
(3) Persson, C. G. A.; Kjellin, G. Enprofylline, a principally new antiasthmatic xanthine. *Acta Pharmacol. Toxicol.* 1981, 49, 313-316.

(4) Karlsson, J.-A.; Kjellin, G.; Persson, C. G. A. Effects on tracheal smooth muscle of adenosine and methylxanthines, and their interaction. *J. Pharm. Pharmacol.* 1982, 34, 788-793.

(5) Takagi, K.; Hasegawa, T.; Kuzuya, T.; Ogawa, K.; Watanabe, T.; Satake, T.; Miyamoto, K.; Wakusawa, S.; Koshiura, R. Structure-activity relationship in N3-alkyl-xanthine derivatives. *Jpn. J. Pharmacol.* 1988, 46, 373-378.

(6) Ogawa, K.; Takagi, K.; Satake, T. Mechanism of xanthine-induced relaxation of guinea-pig isolated trachealis muscle. *Br. J. Pharmacol.* 1989, 97, 542-546.

Table I. Physicochemical Data for Mono-, Di-, and Trialkyl-Substituted Xanthines



compd no.	R <sup>1</sup>	R <sup>3</sup>	R <sup>7</sup>	yield <sup>b</sup> (%)	mp (°C)	recryst solvent	formula <sup>c</sup>
1 <sup>a</sup> (xanthine)	H	H	H				
2 <sup>a</sup> (1-methylxanthine)	Me	H	H				
3 <sup>a</sup>	Et	H	H	41	>300	MeOH	C <sub>7</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub>
4 <sup>b</sup>	<i>n</i> -Pro	H	H	54	272–278	MeOH	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>
5 <sup>b</sup>	<i>n</i> -Bu	H	H	74	272.5–274	MeOH	C <sub>9</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>
6 <sup>a</sup> (3-methylxanthine)	H	Me	H				
7 <sup>a</sup> (theophylline)	Me	Me	H				
8 <sup>9</sup>	Et	Me	H	55	226–228	MeOH	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>
9 <sup>10</sup>	<i>n</i> -Pro	Me	H	63	205–207	AcOEt	C <sub>9</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>
10 <sup>11</sup>	<i>n</i> -Bu	Me	H	85	205–207	AcOEt	C <sub>10</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>
11 <sup>12</sup>	H	Et	H	31	>300	DMF–Et <sub>2</sub> O	C <sub>7</sub> H <sub>8</sub> N <sub>4</sub> O <sub>2</sub>
12	Me	Et	H	88	281–282	DMF	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>
13 <sup>12</sup>	H	<i>n</i> -Pro	H	85	291–292	DMF–Et <sub>2</sub> O	C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>
14	Me	<i>n</i> -Pro	H	39	220–221	EtOH	C <sub>9</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>
15 <sup>13</sup>	Et	<i>n</i> -Pro	H	70	180–181	EtOH	C <sub>10</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>
16 <sup>13</sup>	<i>n</i> -Pro	<i>n</i> -Pro	H	84	205–207	MeOH	C <sub>11</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub>
17	<i>n</i> -Bu	<i>n</i> -Pro	H	82	210–212	MeOH	C <sub>12</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>
18 <sup>12</sup>	H	<i>n</i> -Bu	H	59	281–283	DMF–Et <sub>2</sub> O	C <sub>9</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>
19 <sup>13</sup>	Me	<i>n</i> -Bu	H	96	210–212	MeOH	C <sub>10</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>
20	H	<i>n</i> -Pro	Me	74	242–243	EtOH	C <sub>9</sub> H <sub>12</sub> N <sub>4</sub> O <sub>2</sub>
21 <sup>14</sup>	Me	<i>n</i> -Pro	Me	39	99–100	AcOEt– <i>n</i> -hex	C <sub>10</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>
22	Et	<i>n</i> -Pro	Me	50	108.5–110	<i>i</i> -Pr <sub>2</sub> O	C <sub>11</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub>
23	<i>n</i> -Pro	<i>n</i> -Pro	Me	67	116–117	<i>i</i> -Pr <sub>2</sub> O	C <sub>12</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>
24	<i>n</i> -Bu	<i>n</i> -Pro	Me	76	123.5–125	<i>i</i> -Pr <sub>2</sub> O	C <sub>13</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub>
25	H	<i>n</i> -Pro	Et	66	151–152	<i>i</i> -PrOH	C <sub>10</sub> H <sub>14</sub> N <sub>4</sub> O <sub>2</sub>
26	Me	<i>n</i> -Pro	Et	65	114–115	<i>i</i> -Pr <sub>2</sub> O	C <sub>11</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub>
27	H	<i>n</i> -Pro	<i>n</i> -Pro	56	156–157	<i>i</i> -PrOH	C <sub>11</sub> H <sub>16</sub> N <sub>4</sub> O <sub>2</sub>
28	Me	<i>n</i> -Pro	<i>n</i> -Pro	96	86–87	<i>i</i> -Pr <sub>2</sub> O	C <sub>12</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>
29 <sup>15</sup>	H	<i>n</i> -Pro	<i>n</i> -Bu	83	148–149	<i>i</i> -PrOH	C <sub>12</sub> H <sub>18</sub> N <sub>4</sub> O <sub>2</sub>
30	Me	<i>n</i> -Pro	<i>n</i> -Bu	89	62–63	<i>n</i> -hexane	C <sub>13</sub> H <sub>20</sub> N <sub>4</sub> O <sub>2</sub>

<sup>a</sup> Commercial compound (see text). <sup>b</sup> Yields were of final procedure and purified product. <sup>c</sup> All compounds analyzed for C, H, N; analytical results were within  $\pm 0.4\%$  of theoretical values.

**3-Ethyl-3,7-dihydro-1H-purine-2,6-dione (11).** A suspension of 6-amino-5-(formylamino)-1-ethyl-2,4-(1*H*,3*H*)-pyrimidinedione (I, R<sup>1</sup> = Et, R<sup>3</sup> = H) (6.0 g, 30 mmol) in 2 N NaOH (30 mL, 60 mmol) was refluxed for 1.5 h. The mixture was neutralized with 10% hydrochloric acid, and the resultant precipitate was filtered, washed with water, and dried. Recrystallization from DMF–Et<sub>2</sub>O gave 1.7 g (31%) of 11 as colorless crystals: mp >300 °C; MS (M<sup>+</sup>) 180; <sup>1</sup>H-NMR (90 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.22 (t, 3 H, *J* = 7 Hz, CH<sub>3</sub>), 3.98 (q, 2 H, *J* = 7 Hz, CH<sub>2</sub>), 7.95 (s, 1 H, 8-H), 10.87 (br s, 1 H, 1-H), 13.32 (br s, 1 H, 7-H). Anal. (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

Compounds 12–14, 18, and 19 were prepared from corresponding I by a similar procedure as above, and physicochemical data are shown in Table I.

**3,7-Dihydro-3-(4-methoxybenzyl)-1H-purine-2,6-dione (II).** With a procedure analogous to that reported in the patent literature,<sup>16</sup> II was obtained as yellowish orange prisms after recrystallization from DMF–EtOH: mp 288–293 °C dec; MS (M<sup>+</sup>) 272; <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.71 (s, 3 H, OCH<sub>3</sub>), 5.05 (s, 2 H, CH<sub>2</sub>), 6.85 (d, 2 H, *J* = 8.5 Hz, 3,5-ArH), 7.30 (d, 2 H, *J* = 8.5 Hz, 2,6-ArH), 7.98 (s, 1 H, 8-H), 11.01 (s, 1 H, 1-H), 13.42 (s, 1 H, 7-H). Anal. (C<sub>13</sub>H<sub>12</sub>N<sub>4</sub>O<sub>3</sub>) C, H, N.

**3,7-Bis(4-methoxybenzyl)-3,7-dihydro-1H-purine-2,6-dione (III).** A slurry of II (17.0 g, 63 mmol), *p*-methoxybenzyl chloride (10.8 g, 69 mmol), and anhydrous K<sub>2</sub>CO<sub>3</sub> (8.6 g, 63 mmol) in DMF (85 mL) was heated at 60 °C for 1 h. The reaction mixture was poured into ice-water and neutralized with diluted hydrochloric acid. The resultant precipitate was filtered, washed with water, and dried. Recrystallization from acetone gave 20.9 g (72%) of III as dark yellow crystals: mp 174–176.5 °C; MS (M<sup>+</sup>) 392; <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  3.70 (s, 3 H, OCH<sub>3</sub>), 3.72 (s, 3 H, OCH<sub>3</sub>), 5.01 (s, 2 H, CH<sub>2</sub>), 5.35 (s, 2 H, CH<sub>2</sub>Ar), 6.84

(d, 2 H, *J* = 8.5 Hz, 3,5-ArH), 6.89 (d, 2 H, *J* = 8.5 Hz, 3,5-ArH), 7.28 (d, 2 H, *J* = 8.5 Hz, 2,6-ArH), 7.33 (d, 2 H, *J* = 8.5 Hz, 2,6-ArH), 8.14 (s, 1 H, 8-H), 11.07 (s, 1 H, 1-H). Anal. (C<sub>21</sub>H<sub>20</sub>N<sub>4</sub>O<sub>4</sub>) C, H, N.

**1-Ethyl-3,7-dihydro-3,7-bis(4-methoxybenzyl)-1H-purine-2,6-dione (IV; R<sup>1</sup> = Et).** A slurry of III (3.0 g, 7.7 mmol), ethyl iodide (0.9 mL, 11 mmol), and anhydrous K<sub>2</sub>CO<sub>3</sub> (1.5 g, 11 mmol) in DMF (18 mL) was heated at 70 °C for 13 h, and DMF was removed in vacuo. Water was added to the residue, and the aqueous solution was extracted with Et<sub>2</sub>O (50 mL  $\times$  2). The combined organic layer was washed with water and brine, dried with MgSO<sub>4</sub>, and concentrated. The product was purified by flash column chromatography (*n*-hexane–AcOEt, 3:2) to give 2.79 g (87%) of IV (R<sup>1</sup> = Et) as a yellow viscous oil: MS (M<sup>+</sup>) 420; <sup>1</sup>H-NMR (90 MHz, CDCl<sub>3</sub>)  $\delta$  1.24 (t, 3 H, *J* = 7 Hz, CH<sub>3</sub>), 3.75 (s, 3 H, OCH<sub>3</sub>), 3.79 (s, 3 H, OCH<sub>3</sub>), 4.07 (q, 2 H, *J* = 7 Hz, CH<sub>2</sub>), 5.19 (s, 2 H, CH<sub>2</sub>Ar), 5.40 (s, 2 H, CH<sub>2</sub>Ar), 6.81 (d, 2 H, *J* = 9 Hz, 3,5-ArH), 6.87 (d, 2 H, *J* = 9 Hz, 3,5-ArH), 7.30 (d, 2 H, *J* = 9 Hz, 2,6-ArH), 7.47 (d, 2 H, *J* = 9 Hz, 2,6-ArH), 7.50 (s, 1 H, 8-H).

**1-Ethyl-3,7-dihydro-1H-purine-2,6-dione (3).** A solution of IV (R<sup>1</sup> = Et) (2.7 g, 6.4 mmol), concentrated sulfuric acid (0.6 g, 6.4 mmol), and anisole (2.1 mL, 19 mmol) in TFA (27 mL) was refluxed for 19 h. Concentrated sulfuric acid (5 drops) was added to the reaction mixture, which was then refluxed for 7 h and concentrated. The oily residue was diluted with water and isopropyl ether, and adjusted with 20% NaOH to pH 5 with stirring. The resultant precipitate was filtered, washed with water and isopropyl ether, and dried. Recrystallization from MeOH gave 0.5 g (41%) of 3 as colorless crystals: >300 °C; MS (M<sup>+</sup>) 180; <sup>1</sup>H-NMR (90 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  1.12 (t, 3 H, *J* = 7 Hz, CH<sub>3</sub>), 3.89 (q, 2 H, *J* = 7 Hz, CH<sub>2</sub>), 7.88 (s, 1 H, 8-H), 11.20–13.76 (br s, 2 H, 3-H and 7-H). Anal. (C<sub>7</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

Compound 4 and 5 were prepared via corresponding IV by a similar procedure as above and physicochemical data are shown in Table I.

(16) Draco, A. B. Antiasthma and cardiotonic agents [alkylpurine-diones]. Jpn. Kokai Tokkyo Koho JP 80 57,517 (CA 94,47360y).

**3,7-Dihydro-7-methyl-3-propyl-1H-purine-2,6-dione (20).** To a mixture of V ( $R^3 = n\text{-Pro}$ ) (1.9 g, 10 mmol) and anhydrous  $K_2CO_3$  (1.4 g, 10 mmol) in DMF (20 mL) was added methyl iodide (0.8 mL, 12 mmol) at 5 °C, and the mixture was stirred at room temperature for 1 h. After neutralization with 10% hydrochloric acid, the mixture was concentrated. The product was washed with water and dried. Recrystallization from EtOH gave 1.5 g (74%) of 20 as colorless needles: mp 242–243 °C; MS ( $M^+$ ) 208;  $^1H\text{-NMR}$  (90 MHz, DMSO- $d_6$ )  $\delta$  0.87 (t, 3 H,  $J = 7.5$  Hz,  $CH_3$ ), 1.67 (sext, 2 H,  $J = 7.5$  Hz,  $CH_2$ ), 3.86 (s, 3 H, 7- $CH_3$ ), 3.87 (t, 2 H,  $J = 7.5$  Hz,  $CH_2$ ), 7.92 (s, 1 H, 8-H), 10.89 (br s, 1 H, 1-H). Anal. ( $C_9H_{12}N_4O_2$ ) C, H, N.

Compound 25, 27, and 29 were prepared from corresponding V by a similar procedure as above and physicochemical data are shown in Table I.

**1-Ethyl-3,7-dihydro-7-methyl-3-propyl-1H-purine-2,6-dione (22).** A mixture of 20 (2.0 g, 9.6 mmol), ethyl iodide (1.5 mL, 9 mmol), and anhydrous  $K_2CO_3$  (2.1 g, 15 mmol) in DMF (12 mL) was heated at 60 °C for 9 h. The reaction mixture was poured into ice-water, and the resultant precipitate was filtered, washed with water, and dried. Recrystallization from isopropyl ether gave 1.1 g (50%) of 22 as colorless needles: mp 108.5–110 °C; MS ( $M^+$ ) 236;  $^1H\text{-NMR}$  (90 MHz,  $CDCl_3$ )  $\delta$  0.98 (t, 3 H,  $J = 7.5$  Hz,  $CH_3$  (3- $n\text{-Pro}$ )), 1.25 (t, 3 H,  $J = 7$  Hz,  $CH_3$  (1-Et)), 1.80 (sext, 2 H,  $J = 7.5$  Hz,  $CH_2$  (3- $n\text{-Pro}$ )), 3.99 (s, 3 H, 7- $CH_3$ ), 4.04 (t, 2 H,  $J = 7$  Hz,  $CH_2$  (1-Et)), 4.10 (q, 2 H,  $J = 7.5$  Hz,  $CH_2$  (3- $n\text{-Pro}$ )), 7.50 (s, 1 H, 8-H). Anal. ( $C_{11}H_{16}N_4O_2$ ) C, H, N.

Compound 21, 23, 24, 26, 28, and 30 were prepared from corresponding compounds (20, 25, 27, 29) by a similar procedure as above and physicochemical data are shown in Table I.

**3,7-Dihydro-7-(4-methoxybenzyl)-3-methyl-1H-purine-2,6-dione (VI,  $R^3 = Me$ ).** A slurry of 3-methylxanthine (V,  $R^3 = Me$ ) (18.4 g, 0.11 mol), *p*-methoxybenzyl chloride (15.8 mL, 0.12 mol), and anhydrous  $K_2CO_3$  (15.3 g, 0.11 mol) in DMF (20 mL) was heated at 60 °C for 2 h. The reaction mixture was poured into ice-water and neutralized with diluted hydrochloric acid (10%). The resultant precipitate was filtered, washed with water, and dried. Recrystallization from DMF gave 28.4 g (90%) of VI ( $R^3 = Me$ ) as pale yellow crystals: mp 271–273 °C; MS ( $M^+$ ) 286;  $^1H\text{-NMR}$  (90 MHz, DMSO- $d_6$ )  $\delta$  3.33 (s, 3 H, 3- $CH_3$ ), 3.72 (s, 3 H,  $OCH_3$ ), 5.36 (s, 2 H,  $CH_2Ar$ ), 6.87 (d, 2 H,  $J = 9$  Hz, 3,5- $ArH$ ), 7.31 (d, 2 H,  $J = 9$  Hz, 2,6- $ArH$ ), 8.10 (s, 1 H, 8-H), 10.92 (s, 1 H, 1-H). Anal. ( $C_{14}H_{14}N_4O_3$ ) C, H, N.

**1-Ethyl-3,7-dihydro-7-(4-methoxybenzyl)-3-methyl-1H-purine-2,6-dione (VII,  $R^1 = Et$ ,  $R^3 = Me$ ).** A slurry of VI ( $R^3 = Me$ ) (5.0 g, 18 mmol), ethyl iodide (2.1 mL, 26 mmol), and anhydrous  $K_2CO_3$  (2.42 g, 18 mmol) in DMF (35 mL) was heated at 50 °C for 3 h. The reaction mixture was poured into ice-water. The resultant precipitate was filtered, washed with water, and dried. Recrystallization from ethyl acetate gave 5.0 g (90%) of VII ( $R^1 = Et$ ,  $R^3 = Me$ ) as pale yellow needles: mp 108–109 °C; MS ( $M^+$ ) 314;  $^1H\text{-NMR}$  (90 MHz,  $CDCl_3$ )  $\delta$  1.26 (t, 3 H,  $J = 7$  Hz,  $CH_3$  (1-Et)), 3.57 (s, 3 H, 3- $CH_3$ ), 3.80 (s, 3 H,  $OCH_3$ ), 4.10 (q, 2 H,  $J = 7$  Hz,  $CH_2$  (1-Et)), 5.43 (s, 2 H,  $CH_2Ar$ ), 6.88 (d, 2 H,  $J = 9$  Hz, 3,5- $ArH$ ), 7.31 (d, 2 H,  $J = 9$  Hz, 2,6- $ArH$ ), 7.51 (s, 1 H, 8-H). Anal. ( $C_{16}H_{18}N_4O_3$ ) C, H, N.

**1-Ethyl-3,7-dihydro-3-methyl-1H-purine-2,6-dione (8).** A solution of VII ( $R^1 = Et$ ,  $R^3 = Me$ ) (4.3 g, 14 mmol), concentrated sulfuric acid (8 drops), and anisole (2.1 g, 19 mmol) in TFA (25 mL) was refluxed for 7 h and evaporated. The oily residue was diluted with water and isopropyl ether and neutralized with 20% NaOH to pH 5 under stirring. The resultant precipitate was filtered, washed with water and isopropyl ether, and dried. Recrystallization from MeOH gave 1.5 g (55%) of 8 as pale brown needles: mp 226–228 °C; MS ( $M^+$ ) 194;  $^1H\text{-NMR}$  (90 MHz,  $CDCl_3$ )  $\delta$  1.30 (t, 3 H,  $J = 7$  Hz,  $CH_3$  (1-Et)), 3.66 (s, 3 H, 3- $CH_3$ ), 4.17 (q, 2 H,  $J = 7$  Hz,  $CH_2$ ), 7.84 (s, 1 H, 8-H), 13.10 (br s, 1 H, 7-H).

Compounds 9, 10, and 15–17 were prepared via corresponding VI and VII by a similar procedure as 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 all experiments 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  $\mu M$ ) 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 had become constant, the preparation was treated with cumulative concentrations of a compound. Relaxation by 1.0  $\mu M$  isoprenaline was defined as 100%, and the concentration producing 50% relaxation ( $EC_{50}$ ) was calculated.

**Beating Rate of Right Atrium.** The right atrium isolated was placed in an organ bath (25 °C) filled with Krebs–Henseleit solution aerated with a gas of 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 cardiometer triggered by isometric contraction on the atria. Data were expressed as 15% increasing concentration ( $EC_{15}$ ) of the unstimulated beating rate.

**Acetylcholine-Induced Bronchoconstriction.** Under urethane anesthesia (1.5 g/kg, ip), the guinea pigs were respired artificially by means of 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.<sup>17</sup> The heart rate was measured through a cardiometer triggered by the blood pressure pulse of the left jugular artery. Acetylcholine (40  $\mu g/kg$ ) 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 in 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–PDE Assay.** Inhibitory activity of xanthines on cyclic AMP–PDE with low  $K_m$  (0.61  $\mu M$ ) in the 10000g supernatant of trachealis muscle homogenate was measured by the two-step assay system of Thompson and Appleman.<sup>18</sup> The inhibition constant ( $K_i$ ) was calculated by the method of Dixon.<sup>19</sup>

**Adenosine Receptor Binding Assay.** Affinity for the adenosine ( $A_1$ ) receptor of xanthines was measured by a ligand (tritiated 8-cyclopentyl-1,3-dipropylxanthine, [ $^3H$ ]CPX<sup>20</sup>) binding replacement on a membrane preparation of the cerebral cortex.<sup>21</sup> The  $A_2$ -receptor binding assay was done using [ $^3H$ ]GCS-21680<sup>22</sup> on striatal membranes.<sup>23</sup> The dose–inhibition data

(17) Konzett, H.; Rossler, R. Versuchsordnung zu untersuchung an der bronchialmuskulatur. *Naunyn-Schmiedeberg's Arch. Exp. Pathol. Pharmacol.* 1940, 195, 71–74.

(18) Thompson, W. J.; Appleman, M. M. Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochemistry* 1971, 10, 311–316.

(19) Dixon, M. The determination of enzyme inhibitor constants. *Biochem. J.* 1953, 55, 170–171.

(20) 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.

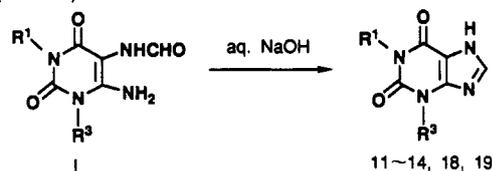
(21) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. [ $^3H$ ]Xanthine amine congener of 1,3-dipropyl-8-phenylxanthine: An antagonist radioligand for adenosine receptors. *Proc. Natl. Acad. Sci. U.S.A.* 1986, 83, 4089–4093.

(22) Hutchison, A. J.; Webb, R. L.; Osei, H. H.; Ghai, G. R.; Zimmerman, M. B.; Williams, M. CGS 21680C, an  $A_2$  selective adenosine receptor agonist with preferential hypotensive activity. *J. Pharmacol. Exp. Ther.* 1989, 251, 47–55.

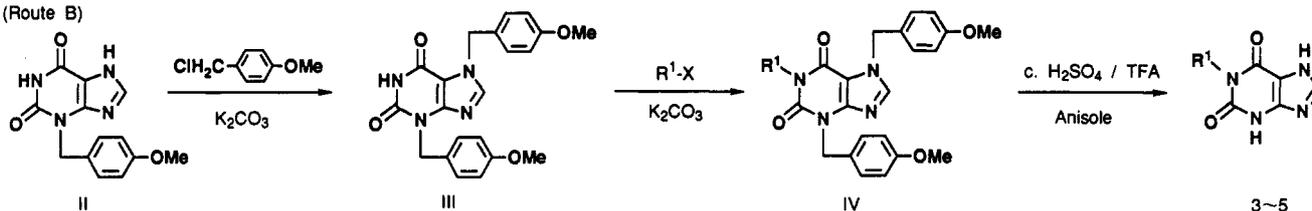
(23) Jarvis, M. F.; Schulz, P.; Hutchison, A. L.; Do, U. H.; Sills, M. A.; Williams, M. [ $^3H$ ]CGS 21680, a selective  $A_2$  adenosine receptor agonist directly label  $A_2$  receptors in rat brain. *J. Pharmacol. Exp. Ther.* 1989, 251, 888–893.

## Scheme I

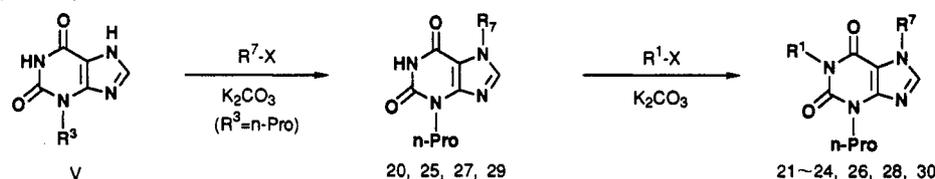
(Route A)



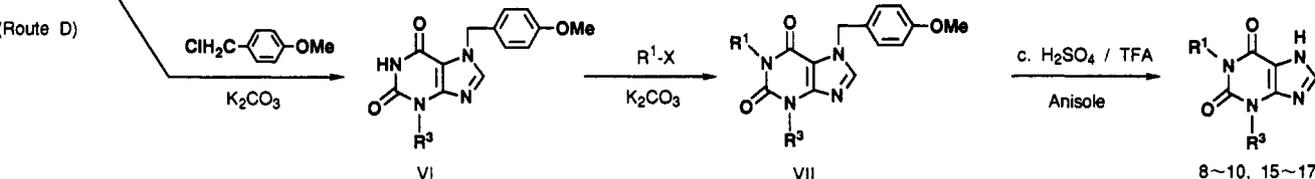
(Route B)



(Route C)



(Route D)



was analyzed using a nonlinear least-squares fit to a competitive inhibition model, and the inhibition constant ( $K_i$ ) was calculated from the Cheng and Prusoff equation.<sup>24</sup>

Protein was measured by the method of Lowry et al.<sup>25</sup>

**Statistical Analysis.** The regression lines were calculated by a nonlinear least-squares method program, MULTI, written by Yamaoka et al.<sup>26</sup> using a personal computer, PC-9801 (Nihon Electric Co., Tokyo).

## Results and Discussion

Xanthines substituted with normal alkyl chains at the 1-, 3-, and 7-positions were prepared by the route outlined in Scheme I. We have developed a regioselective introduction of alkyl groups onto xanthine derivatives with the *p*-methoxybenzyl protecting group. 1,3-Disubstituted xanthine derivatives (8-19) were synthesized by the usual method (route A) or by acid deprotection of 7-(*p*-methoxybenzyl) derivatives (VII) (route D). Intermediate VII was prepared from 3-substituted derivatives (V) by treatment with *p*-methoxybenzyl chloride/ $K_2CO_3$ , followed by alkylation with various alkyl halides. Similarly, 1-substituted xanthine derivatives (3-5) were obtained from 3-(*p*-methoxybenzyl)xanthine (II) (route B). On the other hand, 3,7-disubstituted (20, 25, 27, 29) and 1,3,7-substituted xanthines (21-24, 26, 28, 30) were obtained from 3-alkylxanthine derivatives (V) by sequential alkylations

(route C). The generalized chemical structures and compound numbers are shown in Table I.

The pharmacological activities of 30 1,3,7-trialkylxanthines on several tissues from guinea pigs were examined and are shown in Table II. The prolongation of alkyl chain at the 3-position of the xanthine nucleus (1, 2, 6, 7, 11-14, 18, 19) increased not only the relaxant activity on the spontaneous tone of isolated tracheal ring chains, as previously reported,<sup>5-7</sup> but also the positive chronotropic action on the isolated right atrium.

A selectivity for broncho muscle was observed in 1- and 7-substituents. While these pharmacological activities were changed a little or none by 1-alkylation of xanthine without the alkyl group at the 3-position (1-5), the relaxant activity of 3-*n*-propylxanthine derivatives was markedly increased with alkyl chain length at the 1-position, but the positive chronotropic action became weak by changing the 1-position methyl group to a *n*-butyl group (13-17). This trend with the 1-substitutions was also observed in 7-methylated 3-propylxanthine derivatives (20-24). The substitutions at the 7-position appeared to be effective to diminish the heart stimulation; the 15% increasing concentration of beating rate ( $EC_{15}$ ) of almost all 7-substituents was over 100  $\mu M$ , which is the limiting concentration soluble in Krebs-Henseleit solution, whereas the tracheal relaxant activity decreased by methylation was gradually increased with chain prolongation at the 7-position (20, 21, 25-30). From these results, the selectivity defined by the ratio of potency for the heart stimulation ( $EC_{15}$ ) to that for the tracheal relaxation ( $EC_{50}$ ) was very high in compounds having long alkyl chains at both the 1- and 3-positions (16, 17, 23, 24) and at the 7-position

(24) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant ( $K_i$ ) and the concentration of inhibition which causes 50 percent inhibition ( $I_{50}$ ) of enzyme reaction. *Biochem. Pharmacol.* 1973, 22, 3099-3108.

(25) Lowry, O. H.; Rosenbrough, N. T.; Farr, A. L.; Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951, 193, 265-275.

(26) Yamaoka, K.; Tanigawara, Y.; Nakagawa, T.; Uno, T. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobiodyn.* 1981, 4, 879-885.

Table II. In Vitro Pharmacological Activities of 1,3,7-Trialkylxanthines

compound no.	substitution			tracheal relaxation EC <sub>50</sub> , μM	heart stimulation EC <sub>15</sub> , μM	bronchoselectivity <sup>a</sup>	cyclic AMP-PDE inhibition K <sub>i</sub> , μM	affinity for	
	R <sup>1</sup>	R <sup>3</sup>	R <sup>7</sup>					A <sub>1</sub> receptor K <sub>i</sub> , μM	A <sub>2</sub> receptor K <sub>i</sub> , μM
1	H	H	H	>100	>100	1	>200	>200	<i>b</i>
2	Me	H	H	75.9	>100	1.3	163	11.5	67.1
3	Et	H	H	96.2	>100	1.0	26.3	9.8	34.0
4	<i>n</i> -Pro	H	H	62.0	>100	1.6	49.3	3.3	23.4
5	<i>n</i> -Bu	H	H	43.4	>100	2.3	71.1	3.0	15.4
6	H	Me	H	>100	>100	1	156	133	-
7 (theophylline)	Me	Me	H	28.2	62.0	2.2	56.8	9.8	16.6
8	Et	Me	H	15.2	34.0	2.2	25.0	9.8	-
9	<i>n</i> -Pro	Me	H	9.6	36.2	3.7	26.0	3.4	-
10	<i>n</i> -Bu	Me	H	7.6	90.5	11.8	19.1	4.3	-
11	H	Et	H	69.2	>100	1.5	63.0	117	-
12	Me	Et	H	4.7	28.5	6.1	26.0	7.6	25.4
13 (enprofylline)	H	<i>n</i> -Pro	H	7.2	18.3	2.5	43.1	60.7	90.6
14	Me	<i>n</i> -Pro	H	1.6	3.3	2.1	10.6	1.9	8.0
15	Et	<i>n</i> -Pro	H	1.3	6.1	4.7	24.1	1.4	3.9
16	<i>n</i> -Pro	<i>n</i> -Pro	H	0.05	20.0	400	26.1	0.2	3.0
17	<i>n</i> -Bu	<i>n</i> -Pro	H	0.03	29.1	970	14.3	0.02	4.1
18	H	<i>n</i> -Bu	H	8.3	17.0	2.1	32.0	21.4	-
19	Me	<i>n</i> -Bu	H	1.3	2.2	1.7	10.2	1.9	4.8
20	H	<i>n</i> -Pro	Me	27.5	>100	3.6	28.7	90.6	-
21	Me	<i>n</i> -Pro	Me	10.6	45.4	4.3	32.4	14.5	20.1
22	Et	<i>n</i> -Pro	Me	7.6	30.0	4.0	19.4	11.8	-
23	<i>n</i> -Pro	<i>n</i> -Pro	Me	0.6	45.8	76.7	28.6	7.7	-
24	<i>n</i> -Bu	<i>n</i> -Pro	Me	1.4	>100	71.4	30.1	6.0	-
25	H	<i>n</i> -Pro	Et	8.3	>100	12.0	13.2	40.4	-
26	Me	<i>n</i> -Pro	Et	5.9	>100	16.9	32.3	14.2	21.5
27	H	<i>n</i> -Pro	<i>n</i> -Pro	15.5	>100	6.3	35.0	18.2	-
28	Me	<i>n</i> -Pro	<i>n</i> -Pro	5.0	45.2	9.0	30.8	9.4	43.8
29	H	<i>n</i> -Pro	<i>n</i> -Bu	13.8	>100	7.1	36.5	12.1	-
30	Me	<i>n</i> -Pro	<i>n</i> -Bu	3.1	>100	32.3	>200	7.3	15.3

<sup>a</sup> Defined as the ratio of stimulatory activity in the right atrium (EC<sub>15</sub>, μM) to relaxant activity in the tracheal muscle (EC<sub>50</sub>, μM). The value over 100 μM was calculated as 100 μM. <sup>b</sup> Not determined.

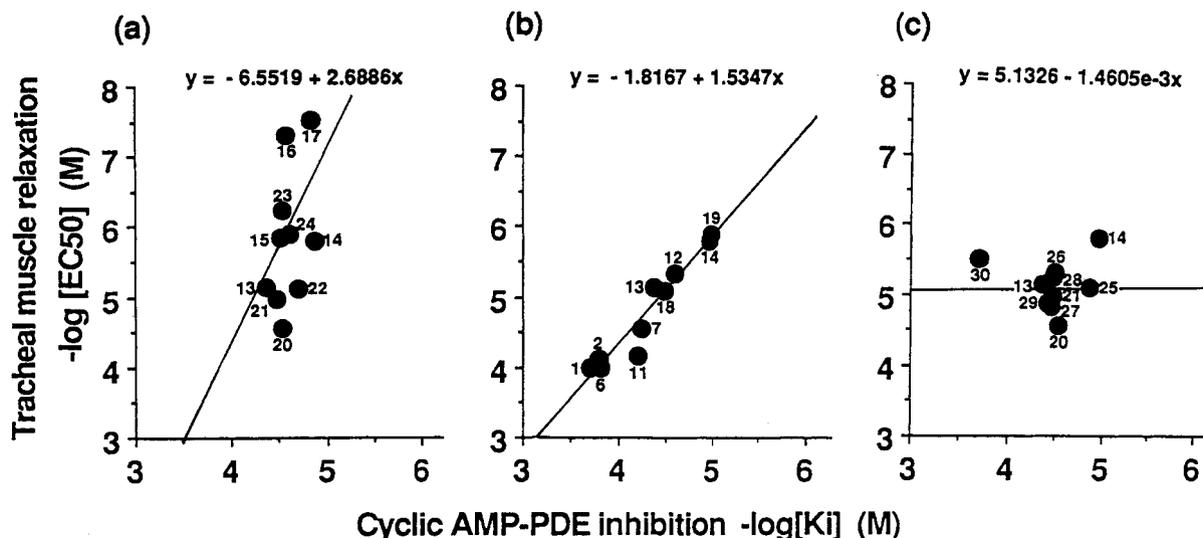


Figure 1. Correlation of the cyclic AMP-PDE inhibitory activities ( $-\log K_i$ ) and the relaxant activities ( $-\log EC_{50}$ ) in the tracheal muscle in (a) 1-substituted 7*H*- and 7-methyl-3-propylxanthines, (b) 3-substituted 1*H*- and 1-methylxanthines, and (c) 7-substituted 1*H*- and 1-methyl-3-propylxanthines. Each point with compound number represents mean of three to five measurements.

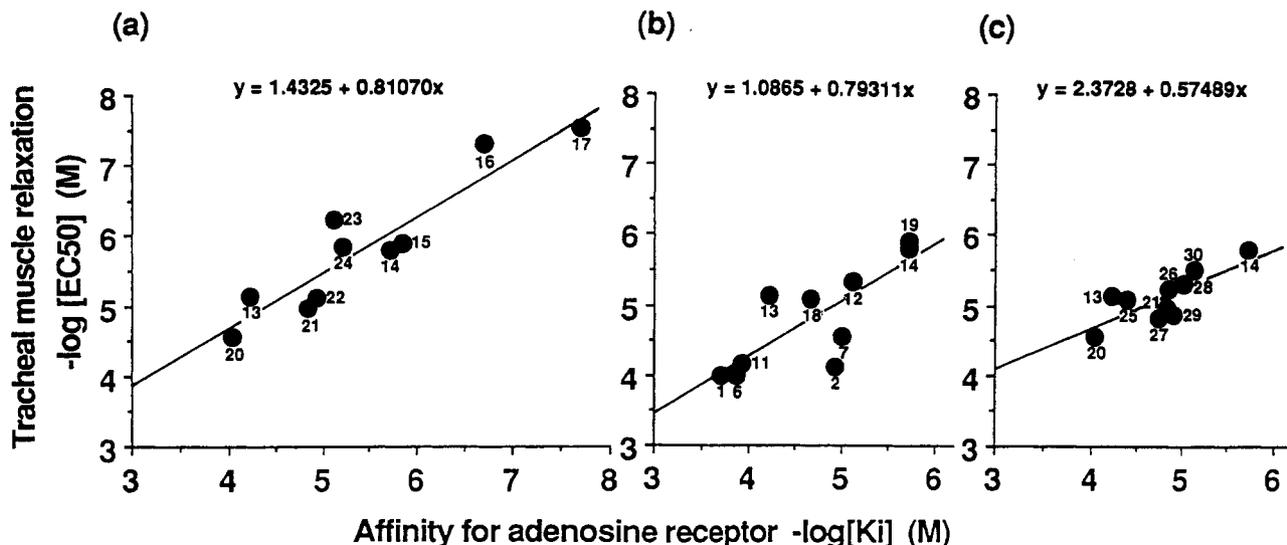
(25–30), while the value being only about two in theophylline (7) and enprofylline (13) (Table II).

There are a number of hypotheses for the mechanism of pharmacological activities of xanthines from cyclic AMP-PDE inhibition.<sup>27,28</sup> We measured the activity on cyclic AMP-PDE in guinea pig tracheal muscle (Table

II). The inhibitory activity on the enzyme was increased with the chain length at the 3-position in a manner similar to that observed in the tracheal relaxant activity, and there was a good correlation among these activities ( $r = 0.96$ ,  $P < 0.01$ ) (Figure 1). However, the PDE inhibitory activities of 1- and 7-position substituents did not relate to their actions on the broncho muscle ( $r = 0.38$  and  $0.001$ , respectively). It is also well documented that xanthines exert many pharmacological actions on various tissues through antagonizing endogenous adenosine on its particular receptors,<sup>2,29</sup> and it is suggested that adenosine is a mediator of asthma.<sup>30,31</sup> In the tracheal smooth muscle,

(27) Katsuki, S.; Murad, F. Regulation of cyclic 3',5'-adenosine monophosphate and cyclic 3',5'-guanosine monophosphate levels and contractility in bovine tracheal smooth muscle. *Mol. Pharmacol.* 1977, 13, 330–341.

(28) Polson, J. B.; Krzanowski, J. J.; Szentivanyi, A. Inhibition of high affinity cyclic AMP phosphodiesterase and relaxation of canine tracheal smooth muscle. *Biochem. Pharmacol.* 1982, 31, 3404–3406.



**Figure 2.** Correlation of the affinities for adenosine ( $A_1$ ) receptor ( $-\log K_i$ ) and the relaxant activities ( $-\log EC_{50}$ ) in (a) 1-substituted 7H- and 7-methyl-3-propylxanthines, (b) 3-substituted 1H and 1-methylxanthines, and (c) 7-substituted 1H and 1-methyl-3-propylxanthines. Each point with compound number represents mean of three to five measurements.

adenosine causes contraction through stimulation of the adenosine  $A_1$  receptor and at higher concentrations elicits relaxation via the  $A_2$  receptor, and xanthines antagonize the  $A_1$ -mediated contractile reaction and relax the smooth muscle.<sup>32</sup> The affinity of the xanthine derivatives for adenosine  $A_1$  and  $A_2$  receptors in the brain membranes was measured by the displacement of binding of [ $^3H$ ]CPX and [ $^3H$ ]GCS-21680, respectively (Table II). The affinity for the  $A_2$  receptor of these alkylxanthines was generally less than that for the  $A_1$  receptor. The most significant difference between affinities for the  $A_1$  and  $A_2$  receptors was observed in 1-substituted 3-propylxanthines in which the  $A_1$  affinity was increased 100-fold but the  $A_2$  affinity was changed only 2-fold by changing 1-position methyl group to a *n*-butyl group (14–17). The prolongation of alkyl chain at the 1-position of 3-*n*-propylxanthine (enprofylline, 13), which is a very weak and nonselective adenosine antagonist,<sup>2</sup> increased in  $A_1$  selectivity, as indicated that  $A_1$ -selective antagonists need 1,3-dipropyl moieties rather than small or less substitutions of 8-substituted xanthines.<sup>20,33,34</sup> And the affinity for the  $A_1$  receptor of these alkylxanthines had certain correlations with the tracheal relaxant action ( $r = 0.93$ ,  $P < 0.01$  in 1-alkyl substituents,  $r = 0.79$ ,  $P < 0.01$  in 3-alkyl sub-

**Table III.** In Vivo Pharmacological Actions

compound no.	bronchodilator action $ED_{50}$ (mg/kg, id)	positive chronotropic action $ED_{15}$ (mg/kg, id)
7 (theophylline)	23.4	20.2
13 (enprofylline)	25.7	95.1
17	2.87	>100
24	2.34	>100

stituents, and  $r = 0.78$ ,  $P < 0.01$  in 7-alkyl substituents) (Figure 2). These results suggest that cyclic AMP-PDE inhibitory activity contributes to the tracheal muscle relaxant action in 3-substituted xanthines, and action on the adenosine ( $A_1$ ) receptor involves the pharmacological action of alkylxanthines which include 1-alkyl substituents.

Finally, we evaluated the in vivo pharmacological effects of compounds 17 and 24, which showed markedly high bronchoselectivity in vitro, compared to theophylline (7) and enprofylline (13). When compounds were administered into the duodenum of guinea pigs, both compounds 17 and 24 reduced the acetylcholine-induced bronchospasm with about 10-fold stronger potency than theophylline and enprofylline (Table III). These compounds showed only slight and transient positive-chronotropic action (about 10% increase of the heart rate) and any effect on the blood pressure even at 100 mg/kg, while the existing drugs induced a continuous increase of the heart rate with hypotension. Thus, we confirmed that these new alkylxanthines are potent and selective bronchodilators.

In conclusion, our study found that the substitution at the 1-position is more important than the 3-substitution for bronchoselectivity, while the 1- and 3-substituents were equally important for tracheal smooth muscle relaxation. Moreover, 1,3-dialkylxanthines may also require the alkylation at the 7-position to cancel the action on the heart. We further suggest that not only the cyclic AMP-PDE inhibitory activity but also the adenosine ( $A_1$ ) antagonistic action is important in the bronchodilatory effect of xanthine derivatives.

(29) Daly, J. W. Adenosine receptors. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* 1985, 19, 29–46.

(30) Mentzer, R. M.; Rubio, R.; Berne, R. M. Release of adenosine by hypoxic canine lung tissue and its possible role in pulmonary circulation. *Am. J. Physiol.* 1975, 229, 1625–1631.

(31) Marquardt, D. L.; Parker, C. W.; Sullivan, T. J. Potentiation of mast cell mediator release by adenosine. *J. Immunol.* 1978, 120, 817–878.

(32) Farmer, S. G.; Canning, B. J.; Wilkins, D. E. Adenosine receptor-mediated contraction and relaxation of guinea-pig isolated tracheal smooth muscle: effects of adenosine antagonists. *Br. J. Pharmacol.* 1988, 95, 371–378.

(33) Bruns, R. F.; Daly, J. W.; Snyder, S. H. Adenosine receptor binding: Structure-activity analysis generates extremely potent xanthine antagonists. *Proc. Natl. Acad. Sci. U.S.A.* 1983, 80, 2077–2080.

(34) Erickson, R. H.; Hiner, R. N.; Feeney, S. W.; Blake, P. R.; Rzeszutarski, W. J.; Hicks, R. P.; Costello, D. G.; Abreu, M. E. 1,3,8-Trisubstituted xanthines. Effects of substitution pattern upon adenosine receptor  $A_1/A_2$  affinity. *J. Med. Chem.* 1991, 34, 1431–1435.