Phosphodiesterase 4 Isoenzyme Inhibitory Activity of 3-Phenylxanthines and 4-Phenyl[*i*]condensed-purines

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Received September 1, 2000; accepted October 31, 2000

On the basis of our study on the structure–activity relationships of 1,3,7-alkylxanthines and condensed-purines on cAMP-phosphodiesterase 4 (PDE 4) isoenzyme inhibitor, we investigated the synthesis and the inhibitory activity of 3-phenylxanthine and 4-phenyl[*i*]condensed-purine derivatives. Xanthines and condensed-purines with the phenyl group exhibited potent and selective PDE 4 inhibitory activity.

Key words phosphodiesterase 4 inhibitor; 3-phenylxanthine; 4-phenyl[*i*]condensed-purine

Inhibitors of cAMP-phosphodiesterase 4 (PDE 4) exhibit relaxation effects of bronchial smooth muscle tissue and anti-inflammatory activities.

On the structure–activity relationships of 1,3,7-alkylxanthines on PDE 4 isoenzyme inhibitors, we reported that the 3-substituents increased both tracheal-relaxant action and positive-chronotropic action, and 1- or 7-substituents were important for bronchoselectivity.[1)] Also, denbufylline (DBF) and XT-44 were effective against osteoporosis in animal models.[2)] Further, we discovered that the heterocycle[*i*]condensed-purines (**3a**—**f**), especially **3c** (R^3=*n*Pr, *n*=2) had potent PDE 4 inhibitory activity and lacked some of the adverse reactions of xanthine derivatives.[3)]

From Vega's report that 3-(*p*-chlorophenyl)-1-propylxanthine (**1**) showed potent bronchodilator activity,[4)] we assumed that PDE 4-inhibitory activity was closely related to the increase of the partition coefficients owning to the replacement of alkyl group to aryl group at 3-position.

The above presumption prompted us to examine PDE 4-inhibitory activities of the corresponding phenyl substituted derivatives (**2**, **4a**—**f**) for comparison with those of DBF and **3a**—**f**. In this paper, we describe the synthesis and inhibitory activity of phenyl substituted derivatives.

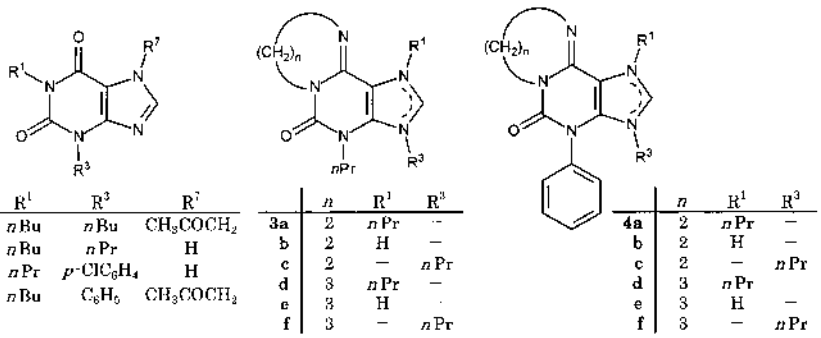
Synthesis 3-Phenyl-7-propylxanthine (**6**) was prepared from 3-phenylxanthine (**5**)[4)] by treatment with *n*-propyl bromide in the presence of potassium carbonate. 7-Acetonyl-1-*n*-butyl-3-phenylxanthine (**2**) was prepared by the following route: 7-benzyl-3-phenylxanthine (**7**), obtained from **5** by benzylation at 7-position, was alkylated with *n*-butyl bromide at 1-position and then treated with 20% palladium hydroxide on carbon to give 1-butyl-3-phenylxanthine (**9**), which afforded **2** by alkylation with bromoacetone in the presence of potassium carbonate (Chart 2).

4-Phenyltetrahydroimidazo[*i*]purine (**4a**, **b**) and 4-phenyltetrahydropyrimido[*i*]purine (**4d**, **e**) were prepared by the following route: treatment of 3-phenylxanthine (**5**; R=H and **6**; R=*n*Pr) with Lawesson's reagent or phosphorus pentasulfide, respectively, gave 3-phenyl-6-thioxoxanthine (**10a**, **b**), which were treated with 2-aminoethanol or 3-aminopropanol to give the corresponding 6-hydroxyalkyl compounds (**11a**, **b**, **d**, **e**). **11a**, **b**, **d**, and **e** were treated with methanesulfonyl chloride in the presence of triethylamine to afford the corresponding 4-phenyl[*i*]condensed-purines (**4a**, **b**, **d**, **e**) (Chart 3).

Attempts to synthesize **4c** and **f** (R=*n*Pr) from **4b** and **e** (R=H) by treatment with *n*-propyl bromide in the presence of potassium carbonate were unsuccessful. These products were unexpectedly **4a** and **4d**, although the synthesis of **3c** and **3f** having 3-*n*-propyl group had been achieved by similar treatment of **3b** and **3e**.[3)] These results indicate that the steric hindrance by the replacement of 3-*n*-propyl group to 3-phenyl group might have occurred.

Biological Results and Discussion

The inhibitory activities of 3-phenylxanthines (**2**, **5**, **6**, **9**) and 4-phenyl[*i*]condensed-purines (**4a**, **b**, **d**, **e**) against PDE 1



	R^1	R^3	R^7
DBF	*n*Bu	*n*Bu	CH_3COCH_2
XT-44	*n*Bu	*n*Pr	H
1	*n*Pr	*p*-ClC_6H_4	H
2	*n*Bu	C_6H_5	CH_3COCH_2

	n	R^1	R^3
3a	2	*n*Pr	–
b	2	H	–
c	2	–	*n*Pr
d	3	*n*Pr	–
e	3	H	–
f	3	–	*n*Pr

	n	R^1	R^3
4a	2	*n*Pr	–
b	2	H	–
c	2	–	*n*Pr
d	3	*n*Pr	
e	3	H	–
f	3	–	*n*Pr

Chart 1

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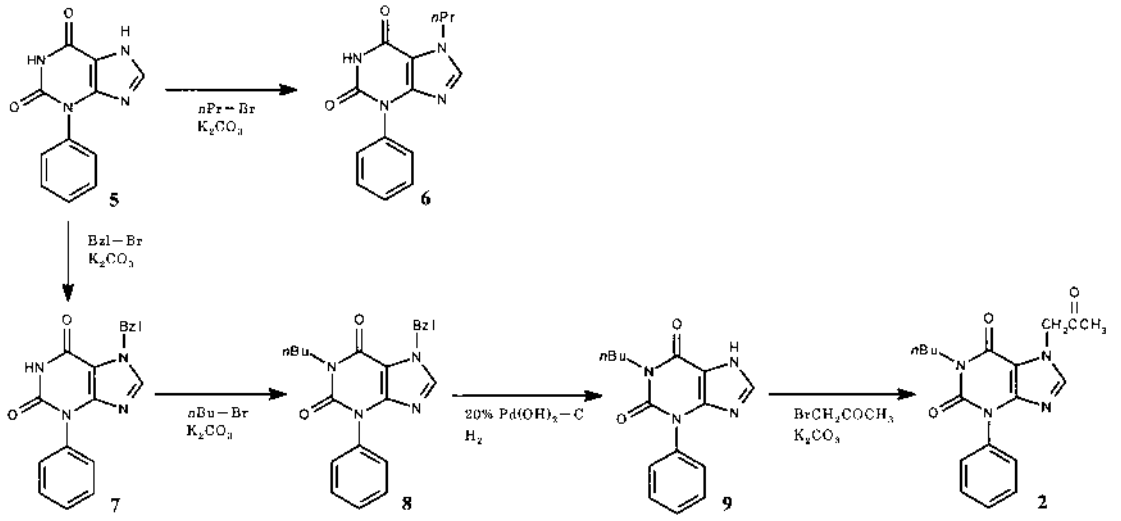

Chart 2

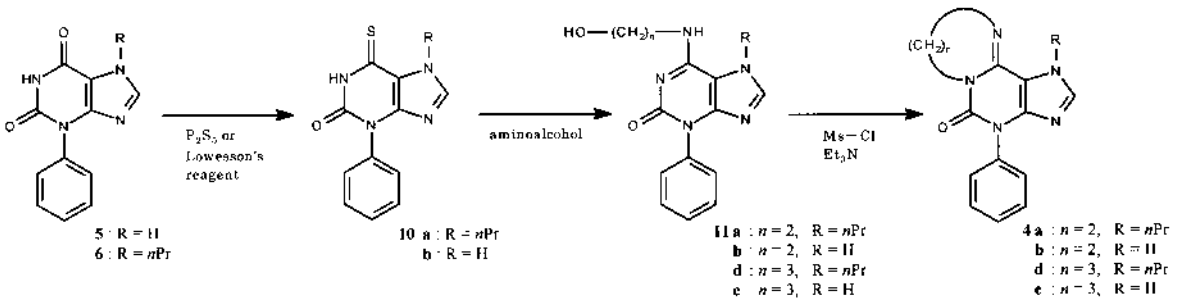

Chart 3

and 4 isoenzymes from a guinea-pig brain and PDE 3 from a guinea-pig heart were measured according to the published methods.[3] The results are shown in Table 1 together with PDE inhibitory activities of known PDE 4 inhibitors DBF, XT-44 and **3c**.

These compounds showed no or very weak inhibitory activity against PDE 1 and 3 isoenzymes. Compound **5**, having no alkyl group at other *N*-positions, was inactive, but the alkyl group at 1- or 7-position (**6**, **9**) induced PDE 4 inhibitory activity. On the other hand, although DBF is a potent and selective PDE 4 inhibitor, its 3-phenyl derivative (**2**) showed much less activity than the parent compound DBF and **9** without 7-acetonyl group.

Heterocycle-condensed purines, **4b** and **4e**, which have no alkyl group at the purine skeleton, were inactive, but compounds **4a** and **4d**, having *n*-propyl group, showed PDE 4 inhibitory activity. Especially, compound **4d** inhibited stronger than **4a**, and with equal or more potency than the reference PDE 4 inhibitors.

Previously, we found that prolongation of the alkyl chain length at the 3-position of the xanthine skeleton increased bronchodilator activity, which is closely related with their PDE 4 inhibitory activity. In addition, we discovered that adverse effects, such as tachycardia, related with their PDE 3 inhibitory activity, and excitation of the central nervous system may be related with their PDE 1 inhibitory activity, and substitution of long alkyl groups at 1-position induced selective PDE 4 inhibitory activity. Consequently, we developed

Table 1. PDE Inhibitory Activities of 3-Phenylxanthines and 4-Phenyl-[*i*]condensed-purines

Compd. No.	IC_{50} (μM)		
	PDE 1	PDE 3	PDE 4
2	>100	>100	44
5	>100	>100	>100
6	>100	>100	76
9	>100	>100	9.4
4a	>100	81	15
4b	>100	>100	>100
4d	>100	>100	5.7
4e	>100	>100	>100
DBF	>100	>100	1.5
XT-44	>100	>100	5.7
3c	>100	53	7.0

Data are the mean of three experiments.

XT-44.[1] Moreover, we indicated that 3-*n*-propyl[*i*]condensed-purines showed selective PDE 4 inhibitory activity and the smaller condensed ring showed stronger inhibitory activity.[3] On the other hand, regarding 3-phenylpurines in this study, the larger heterocycle ring showed stronger inhibitory activity against PDE 4 isoenzyme.

In conclusion, this study indicated that both xanthines and condensed-purines substituted with the phenyl group showed potent and selective PDE 4 inhibitory activity.

Experimental

Melting points were measured on a Yanagimoto micro melting points hot stage apparatus and are uncorrected. Infrared spectra (IR) were determined with a Horiba FT-720 spectrometer. Nuclear magnetic resonance spectra (^{1}H-NMR) was recorded on a JEOL EX 90A spectrometer. Chemical shifts are quoted in parts per million (ppm) with tetramethyl silane as an internal standard, and coupling constants (*J*) are given in hertz (Hz). Microanalyses were performed in the Micro Analytical Laboratory of this faculty. Yield and physicochemical data of the 6-hydroxyalkylamino-3-phenylxanthines (**11a**, **b**, **d**, **e**) and the 4-phenyl[*i*]condensed-purine (**4a**, **b**, **d**, **e**) are summarized in Table 2 and 3, respectively.

7-Propyl-3-phenylxanthine (6) To a mixture of 3-phenylxanthine (**5**, 500 mg, 2.19 mmol) and anhydrous K_2CO_3 (360 mg, 2.63 mmol) in dimethylformamide (DMF, 10 ml) was added *n*-propyl bromide (900 mg, 5.26 mmol) at 0 °C; the reaction mixture was stirred overnight at room temperature, then concentrated *in vacuo.* The residue was chromatogaraphed on silica gel using $CHCl_3$–MeOH (20 : 1) as an eluent to give **6** (404.5 mg, 68%). mp 224—225 °C (AcOEt–MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, J=7.3 Hz), 1.95 (2H, sext., J=7.3 Hz), 4.26 (2H, t, J=7.3 Hz), 7.37—7.67 (6H, m), 8.39 (1H, br s). IR (KBr) cm^{-1}: 3272, 1712, 1696. *Anal.* Calcd for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.22; N, 20.73. Found: C, 62.04; H, 5.21; N, 20.34.

7-Benzyl-3-phenylxanthine (7) The reaction with **5** (1 g, 4.38 mmol), K_2CO_3 (0.72 g, 5.26 mmol) and benzyl bromide (0.9 g, 5.26 mmol) under the same condition as the preparation of **6** gave **7** (1.03 g, 74%). mp 238—239 °C. ^{1}H-NMR ($CDCl_3$) δ: 5.49 (2H, s), 7.38—7.48 (10H, m), 7.52 (1H, s), 8.09 (1H, br s). IR (KBr) cm^{-1}: 1730, 1716, 1696. *Anal.* Calcd for $C_{18}H_{14}N_4O_2$: C, 67.92; H, 4.43; N, 17.60. Found: C, 68.01; H, 4.62; N, 17.81.

7-Benzyl-1-butyl-3-phenylxanthine (8) The mixture of 3-phenylxanthine (**7**, 200 mg, 0.629 mmol), anhydrous K_2CO_3 (130 mg, 0.944 mmol) and *n*-butyl bromide (129 mg, 0.944 mmol) in DMF (5 ml) was stirred overnight at 60 °C. The reaction mixture was concentrated *in vacuo*, the residue was chromatogaraphed on silica gel using $CHCl_3$–MeOH (6 : 1) as an eluent to give **8** (175.6 mg, 75%). mp 139—140 °C (AcOEt–MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, J=7.5 Hz), 1.21—1.85 (4H, m), 4.06 (2H, t, J=7.5 z), 7.37—7.61 (10H, m), 7.65 (1H, s). IR (KBr) cm^{-1}: 1714, 1672. *Anal.* Calcd for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.50; H, 6.04; N, 14.93.

1-Butyl-3-phenylxanthine (9) The mixture of **8** (620 mg, 0.166 mmol) and 20% palladium hydroxide on carbon (600 mg) in MeOH (20 ml) was shaken under hydrogen (3 atm) for 3 h. The catalyst was removed and the filtrate concentrated *in vacuo*, and the residue crystallized to yield **9** (450 mg, 95%). mp 204—205 °C (AcOEt–MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7.3 Hz), 1.25—1.90 (4H, m), 4.15 (2H, t, J=7.3 Hz), 7.40—7.61 (5H, m), 7.65 (1H, s), 12.96 (1H, br s). IR (KBr) cm^{-1}: 3438, 1748, 171, 1668. *Anal.* Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 61.99; H, 5.97; N, 18.41.

7-Acetonyl-3-phenylxanthine (2) The reaction with **9** (200 mg, 0.704 mmol), K_2CO_3 (146 mg, 1.06 mmol) and bromo acetone (147 mg, 1.06 mmol) under the same condition as the preparation of **6** gave **2** (163.2 mg, 68%). mp 167—168 °C (*iso*-propylether). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, t, J=7.3 Hz), 1.21—1.75 (4H, m), 2.36 (3H, s), 4.01 (2H, t, J=7.3 Hz), 5.17 (2H, s), 7.32—7.63 (6H, m). IR (KBr) cm^{-1}: 1732, 1706,

Table 2. Physical Properties for Compounds **11** and **4**

Compd. No.	Yield (%)	mp (°C)	Recryst. Solvent	Formula	Analysis (%) Calcd (Found)		
					C	H	N
11a	93	205—206	AcOEt–MeOH	$C_{16}H_{19}N_5O_2$	61.33 (61.29)	6.11 (6.08)	22.35 (22.58)
11b	80	276—277	MeOH	$C_{13}H_{13}N_5O_2$	57.56 (57.40)	4.83 (4.69)	25.82 (25.71)
11d	82	187—188	AcOEt–MeOH	$C_{17}H_{21}N_5O_2$	62.37 (62.14)	6.47 (6.52)	21.39 (21.17)
11e	78	>290	MeOH	$C_{14}H_{15}N_5O_2$	58.94 (58.72)	5.30 (5.14)	24.55 (24.48)
4a	82	204—205	AcOEt–I.P.E[a)]	$C_{16}H_{17}N_5O$	65.07 (65.26)	5.80 (5.71)	23.71 (23.49)
4b	76	>290	AcOEt–MeOH	$C_{13}H_{11}N_5O$	61.65 (61.56)	4.38 (4.60)	27.65 (27.49)
4d	83	233—234	AcOEt–I.P.E[a)]	$C_{17}H_{19}N_5O$	66.00 (65.92)	6.19 (6.02)	22.64 (22.50)
4e	87	>290	AcOEt–MeOH	$C_{14}H_{13}N_5O$	62.91 (62.77)	4.90 (4.88)	26.20 (26.29)

a) *iso*-propylether.

Table 3. Spectral Data for Compounds **11** and **4**

Compd. No.	IR (KBr) cm^{-1}	^{1}H-NMR
11a[a)]	3415, 3282, 1635, 1610	0.95 (3H, t, J=7.3 Hz), 1.85 (2H, sext., J=7.3 Hz), 3.78 (4H, m), 4.35 (2H, t, J=7.3 Hz), 7.29—7.56 (5H, m), 7.83 (1H, s)
11b[a)]	3411, 3317, 3257, 1645, 1603	3.58 (4H, m), 4.09 (1H, br s), 4.91 (1H, br s), 7.27—7.56 (6H, m), 7.78 (1H, s), 12.23 (1H, br s)
11d[a)]	3313, 1631, 1606	0.82 (3H, t, J=7.3 Hz), 1.60—1.91 (4H, m), 3.49—3.56 (5H, m), 4.28 (2H, t, J=7.3 Hz), 7.23—7.54 (5H, m), 7.84 (1H, s)
11e[a)]	3423, 1699, 1606	2.07 (2H, quint., J=5.6 Hz), 3.40 (2H, t, J=5.6 Hz), 3.87 (2H, t, J=5.6 Hz), 7.37—7.55 (6H, m), 11.52 (1H, br s)
4a[b)]	1674	0.96 (3H, t, J=7.3 Hz), 1.94 (2H, sext., J=7.3 Hz), 4.05 (2H, t, J=6.7 Hz), 4.07 (2H, t, J=6.7 Hz), 4.23 (2H, t, J=7.3 Hz), 7.38—7.46 (6H, m)
4b[b)]	3444, 1697, 1685	3.95—3.99 (4H, m), 7.41—7.57 (6H, m)
4d[b)]	1672, 1637	0.93 (3H, t, J=7.3 Hz), 1.75—2.04 (4H, m), 3.57 (2H, t, J=5.8 Hz), 3.93 (2H, t, J=5.8 Hz), 4.28 (2H, t, J=7.3 Hz), 7.37—7.59 (6H, m)
4e[b)]	3429, 1701, 1670	2.28 (2H, quint., J=5.7 Hz), 3.73 (2H, t, J=5.7 Hz), 4.18 (2H, t, J=5.7 Hz), 6.87 (1H, br s), 7.29—7.80 (1H, s)

^{1}H-NMR spectra were recorded in *a*) DMSO-d_6 and *b*) $CDCl_3$.

1672. *Anal.* Calcd for $C_{18}H_{20}N_4O_3$: C, 63.52; H, 5.92; N, 16.46. Found: C, 63.48; H, 5.97; N, 16.60.

7-Propyl-3-phenyl-6-thioxoxanthine (10a) The mixture of **5** (100 mg, 0.370 mmol) and Lowesson's reagent (150 mg, 0.370 mmol) in dichloroethane (4 ml) was refluxed for 5 h, H_2O was added, and the mixture was extracted with $CHCl_3$. The extracts were washed with brine, dried, and evaporated *in vacuo.* The residue was purified by silica gel column chromatography using $CHCl_3$–AcOEt (4 : 1) as an eluent to give **10** (68.7 mg, 65%). mp 237—238 °C (AcOEt). ^{1}H-NMR (DMSO-d_6) δ: 0.98 (3H, t, J=7.3 Hz), 1.92 (2H, sext., J=7.3 Hz), 4.53 (2H, t, J=7.3 Hz), 7.37—7.58 (5H, m), 9.12 (1H, s). IR (KBr) cm^{-1}: 3415, 1707, 1572. *Anal.* Calcd for $C_{14}H_{14}N_4OS$: C, 58.72; H, 4.93; N, 19.57. Found: C, 58.88; H, 4.91; N, 19.53.

3-Phenyl-6-thioxoxanthine (10b) The mixture of **6** (2 g, 8.77 mmol) and phosphorus pentasulfide (2.42 g, 10.9 mmol) in pyridine (30 ml) was refluxed for 3 h. The reaction mixture was concentrated *in vacuo*, and the residue crystallized to yield **10b** (1.86 g, 87%). mp >290 °C (H_2O). ^{1}H-NMR (DMSO-d_6) δ: 7.24—7.65 (6H, m), 8.77 (1H, br s), 12.50 (1H, br s). IR (KBr) cm^{-1}: 3460, 3437, 1679, 1601. *Anal.* Calcd for $C_{11}H_8N_4OS$: C, 54.09; H, 3.30; N, 22.94. Found: C, 53.88; H, 3.52; N, 22.71.

6-Hydroxyalkylamino-3-phenylxanthines (11a, b, d, e) General Procedure: The mixture of **10** (1 mmol) and 2-aminoethanol or 3-aminopropanol (3 ml) in pyridine (10 ml) was refluxed for 4 h, and concentrated *in vacuo.* The residue was purified by silica gel column chromatography using $CHCl_3$–MeOH (6 : 1) as an eluent to give **11a**, **b**, **d**, and **e.**

Formation of Condensed-purine (4a, b, d, e) General Procedure: To a mixture of **11** (0.2 mmol) and triethylamine (0.24 mmol) in CH_2Cl_2 (5 ml) was added methanesulfonyl chloride (0.24 ml) at 0 °C. The reaction mixture was stirred for 3 h at room temperature and then concentrated *in vacuo.* The residue was chromatographed on silica gel using $CHCl_3$–MeOH (10 : 1) as an eluent to give **4a**, **b**, **d**, and **e**.

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