any dose was noted as a crude index of drug efficacy and indirect measure of intrinsic activity.

Cardiovascular Evaluation in the Cat. Six mongrel cats (2.5–4.0 kg) were anesthetized with an intravenous injection of chloralose (80 mg/kg) and placed in a stereotaxic apparatus. The femoral artery and vein were cannulated in order to monitor arterial blood pressure and for intravenous drug injections, respectively. Heart rate (HR) was triggered from the EKG. A glass trachea tube was inserted and rectal temperature was maintained using a heating pad and/or lamp. Animals were allowed to breath spontaneously. Sympathetic nerve discharge (SND) was recorded under mineral oil from the isolated left inferior cardiac nerve using a bipolar platinum electrode with capacity coupled preamplification at low and high frequency half-amplitude responses at 1 and 500 Hz, respectively. SND was quantitated using cumulative integration (i.e. summation of voltage contained in sympathetic slow waves).

Mean arterial blood pressure (MAP), HR, and SND were allowed to equilibrate for 1 h after surgery. Pretreatment values for MAP, HR, and SND were averaged over the last 10 min of the equilibration period. A cumulative dose–response curve was constructed to determine the effects of U-86192A on MAP, HR, and SND. The starting dose of U-86192A was 0.01 mg/kg iv, and the dose of the drug was increased every 20 min to achieve total cumulative dose of 0.03, 0.1, 0.3 and 1.0 mg/kg iv. The effects of U-86192A on MAP, HR, and SND were determined 15–20 min after each dose. Following the last dose of U-86192A, the 5-HT_{1A} antagonist spiperone was administered intravenously at a dose of 1 mg/kg.

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Adenosine A_1 Antagonists. 2.† Structure–Activity Relationships on Diuretic Activities and Protective Effects against Acute Renal Failure

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Diuretic activities of xanthine or nonxanthine adenosine antagonists and their ameliorative effects against glycerol-induced acute renal failure in rats were investigated in order to clarify the physiological and pathological function of adenosine receptors in the kidney. Diuretic and natriuretic activities of a variety of adenosine antagonists clarified systematically for the first time that the blockade of A_1 receptors is more important than that of A_2 receptors in sodium and water excretion and support the hypothesis that endogenous intrarenal levels of adenosine directly enhance tubular sodium readsorption. Studies of structure–activity relationships of 8-substituted xanthines in the acute renal failure demonstrated that the activation of adenosine A_1 receptor was an important factor in developing such a renal failure. A series of 8-(3-noradamantyl)xanthines exhibited the extremely potent diuretic and natriuretic activities (**24**; 2.5 μg/kg, po, the ratio of urinary excretion value in treated rats to urinary excretion value in control rats = 1.69, the ratio of Na^+/K^+ in treated rats to Na^+/K^+ in control rats = 1.76) and potent ameliorative effects against glycerol-induced acute renal failure (**24**; 10 μg/kg, ip, 55% inhibition). From our detailed studies of structure–activity relationships, we can speculate that some tissue differences of the adenosine A_1 receptor might exist between kidney and brain and sites of action for adenosine antagonists could be different between two renal pharmacological assays. 1,3-Dipropyl-8-(3-noradamantyl)xanthine, KW-3902 (**24**), was chosen for further studies and is under development as a drug for treating the acute renal failure.

Introduction

The relatively weak diuretic effects of xanthines such as caffeine (**1**) or theophylline (**2**) has been described for more than century.[1] Recently, the pharmacological basis for these diuretic actions has been proposed to be adenosine receptor antagonism.[2] In fact, exogenous adenosine produces intense antidiuretic and antinatriuretic effects in many species,[3] and these effects are competitively antagonized by theophylline[4] and mimicked by several adenosine analogues.[5]

Adenosine receptors are subdivided into subtypes, designated as A_1 and A_2.[6,7] A_1 receptors exhibit relatively high affinity to adenosine in binding studies (nM) and some are coupled to and inhibit adenylate cyclase. In contrast, A_2 receptors exhibit at lower affinity to adenosine (μM) and some are coupled to, but stimulate, adenylate cyclase.[8] Adenosine receptors, when activated, can elicit either vasoconstriction (A_1) or vasodilation (A_2). This biphasic action of exogenous adenosine has created uncertainty over the specific role of endogenous adenosine in the control of renal function. In the isolated kidney, perfused at constant pressure, stimulation of A_1 receptors

† Part 1 in the series of Adenosine A_1 Antagonist is ref 33c.

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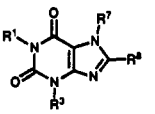
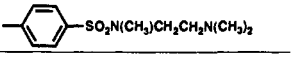
produces a primary vasoconstriction of the afferent arteriole and a decrease in glomerular filtration rate, and could be antidiuretic.[9] Conversely, in the same preparation, stimulation of adenosine A_2 receptors produces vasodilation of both the afferent and efferent arterioles and an increase in glomerular filtration rate. Theophylline, an adenosine receptor antagonist with low A_1/A_2 selectivity, has been noted to produce diuresis and natriuresis even in the absence of detectable increases in glomerular filtration rate and renal blood flow.[10] Therefore, it seems reasonable to assume that adenosine-induced antidiuresis and antinatriuresis (and by inference, xanthine-induced diuresis and natriuresis) can be mediated by both renal hemodynamic and direct tubular mechanisms. But which receptor subclass (A_1, A_2) mediates the diuretic effect of xanthines remains to be fully elucidated.

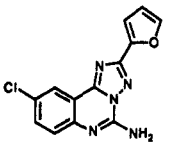
It was proposed that in the kidney, adenosine mediates the hemodynamic changes observed in acute renal failure, and that the changes are pathogenic in reducing glomerular filtration rate.[11] This hypothesis is supported by the following observation. First, in the initial stage of glycerol-induced acute renal failure, renal blood flow is reduced[12] and the resultant ischemia is likely to evoke production and release of adenosine.[13] Second, acute renal failure is associated with a fall in glomerular filtration rate, which is also produced by an infusion of adenosine.[14] Theophylline (1)[15] or 8-phenyltheophylline (2)[16] can reduce

Xanthines



no.	R^1	R^3	R^7	R^8
1	methyl	methyl	H	H
2	methyl	methyl	methyl	H
3	methyl	methyl	H	phenyl
4	propyl	propyl	H	dicyclopropylmethyl
5	propyl	propyl	H	cyclopentyl
6	propyl	propyl	H	
7	propyl	propyl	H	

Nonxanthines



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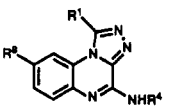

no.	R^1	R^4	R^8
9	CF_3	cyclopentyl	Cl
10	CF_3	cyclopentyl	H
11	phenyl	H	Cl

Figure 1. Chemical structure of reference compounds.

the severity of glycerol-induced acute renal failure, whereas the adenosine uptake inhibitor dipyridamole[17] potentiates the severity of acute renal failure. Glycerol-induced acute renal failure produces a myohemoglobinuric state in rats which is similar to clinical rhabdomyolysis.[18] Thus it is an interesting model for the evaluation of new drugs for treating acute renal failure.

In recent years, considerable efforts have been dedicated to the development of potent and selective adenosine antagonists.[19-33] One of the most potent classes of A_1 an-

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tagonists identified was 1,3-dialkyl-8-polycycloalkylxanthine derivatives.[33] Besides xanthines, a wide range of other heterocycles have been found to be antagonists at adenosine receptors. The representatives of xanthine

Table I. A_1 and A_2 Adenosine Receptor Binding of Reference Compounds[a]

no.	K_i,[a] nM A_1	K_i,[a] nM A_2	K_i ratio A_2/A_1
1 (theophylline)	23000 ± 330 (9700 ± 900)[b] (8470)[c]	16000 ± 2200 (25300)[d]	0.70
2 (caffeine)	100000 ± 2000	27000 ± 1700	0.27
3 (8-phenyltheophylline)	1600 (86.0)[c]	2300 (848)[d]	1.4
4 (8-(dicyclopropylmethyl)-1,3-dipropylxanthine)	3.0 ± 0.21 (0.99 ± 0.04)[b]	430 ± 5.8	140
5 (8-cyclopentyl-1,3-dipropylxanthine)	6.4 ± 0.35 (0.49 ± 0.06)[b] (0.46)[c] (0.9)[e]	590 ± 48 (410)[d]	92
6 (XAC)	11 (1.2)[e]	21 (63)[d]	1.9
7 (PD-115199)	140 (13.9)[f]	26 (15.5)[d]	0.19
8 (CGS-15943)	10 (20.5)[g]	0.73 (3.3)[h]	0.073
9	36 (5.5)[i]	710 (2100)[h]	20
10 (CPQ)	76 (7.3)[c]	1300 (1010)[d]	17
11 (CP-66713)	6900 ± 1600 (270)[i]	5.1 ± 1.1 (21)[h]	0.00074
12 (furosemide)	>100000	>100000	

[a] A_1 binding was carried out with N^6-[^{3}H]cyclohexyladenosine in guinea pig forebrain membranes as described,[34,33c] and A_2 binding was carried out with N-[^{3}H]ethyladenosin-5'-uronamide in the presence of 50 nM cyclopentyladenosine in rat striatal membranes.[22,33c] [b] A_1 binding measured as inhibition of N^6-[^{3}H]cyclohexyladenosine to rat forebrain membranes in our laboratory.[33c] [c] A_1 binding measured as inhibition of N^6-[^{3}H]cyclohexyladenosine to rat whole brain membranes.[22,27a,31] [d] A_2 binding measured as inhibition of N-[^{3}H]ethyladenosin-5'-uronamide to rat striatal membranes.[22,28c,29,31] [e] A_1 binding measured as inhibition of (*R*)-N^6-(2-[^{3}H]phenyl-1-methylethyl)adenosine to rat cortical membranes.[28c] [f] A_1 binding measured as inhibition of N^6-[^{3}H]cyclohexyladenosine to rat cortical membranes.[29] [g] IC_{50} values for inhibition of N^6-[^{3}H]cyclohexyladenosine binding in rat whole brain membranes.[32a] [h] IC_{50} values for inhibition of N-[^{3}H]ethyladenosin-5'-uronamide binding in rat striatal membranes.[30,32a] [i] IC_{50} values for inhibition of N^6-[^{3}H]cyclohexyladenosine binding in rat cortical membranes.[30]

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Table II. Oral Diuretic Activities of Reference Compounds[a]

no.	dose (mg/kg, po)	UV[b] (mL/100 g per 6 h) control	UV[b] (mL/100 g per 6 h) treated	T/C[c]	Na^+ (mequiv/100 g per 6 h) control	Na^+ (mequiv/100 g per 6 h) treated	T/C[c]	Na^+/K^+[d]
1	25	0.91 ± 0.08	1.22 ± 0.03	1.34	0.193 ± 0.016	0.417 ± 0.007	2.16	1.08
	6.25	0.83 ± 0.08	1.18 ± 0.08	1.42	0.132 ± 0.010	0.201 ± 0.010	1.52	1.31
	1.6	0.69 ± 0.03	0.71 ± 0.07	1.03	0.079 ± 0.007	0.090 ± 0.009	1.14	1.06
2	25	1.21 ± 0.08	1.84 ± 0.12	1.68	0.180 ± 0.014	0.280 ± 0.014	1.55	1.28
	6.25	0.87 ± 0.04	0.80 ± 0.03	0.92	0.148 ± 0.011	0.162 ± 0.008	1.10	1.10
3	25	0.80 ± 0.04	1.10 ± 0.06	1.38	0.134 ± 0.004	0.201 ± 0.009	1.50	1.39
	6.25	0.80 ± 0.02	1.43 ± 0.09	1.79	0.146 ± 0.005	0.250 ± 0.026	1.71	1.54
	1.6	1.40 ± 0.05	0.88 ± 0.09	0.63	0.261 ± 0.008	0.175 ± 0.021	0.67	0.87
4	25	0.85 ± 0.06	2.07 ± 0.18	2.44	0.162 ± 0.018	0.350 ± 0.024	2.16	1.71
	6.25	1.00 ± 0.12	2.39 ± 0.03	2.39	0.138 ± 0.011	0.372 ± 0.010	2.69	2.11
	1.6	0.83 ± 0.07	1.83 ± 0.22	2.20	0.122 ± 0.008	0.284 ± 0.021	2.33	1.56
	0.4	0.94 ± 0.03	1.25 ± 0.06	1.33	0.175 ± 0.014	0.255 ± 0.016	1.45	1.27
5	25	1.00 ± 0.13	1.93 ± 0.18	1.93	0.129 ± 0.026	0.316 ± 0.029	2.46	1.88
	6.25	1.26 ± 0.21	2.35 ± 0.15	1.87	0.191 ± 0.029	0.378 ± 0.012	1.98	1.57
	1.6	0.91 ± 0.08	2.54 ± 0.08	2.79	0.193 ± 0.016	0.449 ± 0.023	2.33	1.76
	0.4	0.83 ± 0.08	2.05 ± 0.10	2.47	0.132 ± 0.010	0.302 ± 0.006	2.28	1.54
	0.1	1.10 ± 0.09	1.36 ± 0.14	1.24	0.169 ± 0.018	0.215 ± 0.023	1.27	1.03
6	25	1.04 ± 0.11	1.05 ± 0.04	1.05	0.095 ± 0.009	0.113 ± 0.026	1.19	1.24
7	12.5	0.94 ± 0.03	1.54 ± 0.13	1.64	0.175 ± 0.014	0.210 ± 0.011	1.78	1.56
	6.25	0.91 ± 0.08	1.06 ± 0.13	1.17	0.193 ± 0.016	0.205 ± 0.021	1.06	1.00
8	25	0.90 ± 0.09	0.92 ± 0.07	1.02	0.113 ± 0.009	0.164 ± 0.011	1.46	1.07
9	25	0.91 ± 0.04	1.74 ± 0.19	1.91	0.186 ± 0.020	0.323 ± 0.025	1.74	1.31
	6.25	0.75 ± 0.12	1.63 ± 0.03	2.17	0.179 ± 0.038	0.326 ± 0.023	1.82	1.46
	1.6	0.93 ± 0.03	1.07 ± 0.13	1.15	0.173 ± 0.015	0.212 ± 0.014	1.22	0.98
10	25	0.87 ± 0.04	1.86 ± 0.15	2.13	0.134 ± 0.017	0.314 ± 0.010	2.33	1.71
	6.25	0.99 ± 0.09	1.53 ± 0.08	1.55	0.349 ± 0.040	0.268 ± 0.003	0.77	1.35
11	25	1.02 ± 0.02	0.87 ± 0.05	0.85	0.209 ± 0.007	0.186 ± 0.007	0.89	1.05
12	25	1.21 ± 0.08	2.12 ± 0.58	1.75	0.180 ± 0.015	0.296 ± 0.069	1.64	1.05
	6.25	0.69 ± 0.03	1.10 ± 0.05	1.59	0.079 ± 0.007	0.128 ± 0.009	1.62	1.22
	1.6	0.85 ± 0.10	0.85 ± 0.07	1.00	0.161 ± 0.013	0.152 ± 0.001	0.94	0.87

[a] Compounds were administered orally to three groups of three male rats and urine was collected for 6 h; see the Experimental Section for details. Values are expressed as means ± SEM of control and drug treatment values, respectively. [b] Urine volume. [c] T/C means the ratios of urinary excretion value in treated rats to urinary excretion value in control rats. [d] Values are ratios of Na^+/K^+ in treated rats to Na^+/K^+ in control rats.

(1–6) or nonxanthine antagonists (7–10) reported to date are shown in Figure 1. The availability of a variety of adenosine antagonists with several A_1/A_2 selectivity prompted us to study the physiological and pathological roles of endogenous adenosine in the kidney. In the present study, we investigated diuretic and renal protective activities of 8-substituted xanthines to elucidate the function of renal adenosine A_1 receptors.

Chemistry

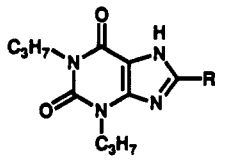
A series of 1,3-dipropylxanthine derivatives containing substituted methyl or polycycloalkyl group at 8-position was synthesized from corresponding 5,6-diaminouracils as reported previously.[33]

Results and Discussion

1. Diuretic Activity. All compounds were evaluated for the diuretic activity after oral administration to conscious and saline-loaded rats. It should be noted, therefore, that the reported diuretic activity is dependent not only on differences in adenosine antagonism but also on possible differences in the pharmacokinetics. At first, the diuretic activities of these representatives (Table I) were examined and shown in Table II. Theophylline (1) showed diuretic and natriuretic activities at the dose of 6.25 mg/kg. Furosemide (12), the representative as a loop diuretic, showed a diuretic activity at the same dose, but it increased both sodium and potassium excretion. Caffeine (2) showed a weaker diuretic activity than theophylline or furosemide. 8-Phenyltheophylline (3) with 10-fold more potent affinities both at A_1 and A_2 receptors than theophylline, showed a nearly equipotent diuretic activity to theophylline. The potent and selective adenosine A_1 antagonist 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (4)[33] or 8-cyclopentyl-1,3-dipropylxanthine (5)[24,27] showed significant diuretic and natriuretic activities at doses of 0.4–6.25 mg/kg. Xanthine adenosine antagonists with low selectivity, such as 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (6)[26] or 8-[4-[[*N*-[2-(dimethylamino)ethyl]-*N*-methylamino]sulfonyl]phenyl]-1,3-dipropylxanthine (7),[29] showed only weak diuretic activity. On the other hand, nonxanthine adenosine antagonists, [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives (9[30] or 10[31]), with A_1 selectivity showed comparable diuretic and natriuretic activities to 8-phenyltheophylline. The compounds with A_2 selectivity, (9-chloro-2-(2-furyl)-[1,2,4]triazolo[1,5-*c*]quinazolin-4-amine (8)[32] and 8-chloro-1-phenyl[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (11),[30] did not show any diuretic nor natriuretic activities. Thus selective and potent A_1 antagonism seems to be important for diuretic and natriuretic activities of adenosine antagonists. Previous studies have shown that adenosine and its analogues can produce significant reduction in sodium and water excretion.[3c,4,5,9b] Our data clarified systematically for the first time that the blockade of A_1 receptors is more important than that of A_2 receptors in sodium and water excretion and support the hypothesis that endogenous intrarenal levels of adenosine directly enhance tubular sodium reabsorption.

To establish the precise relationship of adenosine A_1 antagonism with diuretic and natriuretic activities, 8-cycloalkyl-substituted xanthines (Table III) were investigated as shown in Table IV. Incorporation of a quaternary carbon to cycloalkyl group (13, 14) or introduction of a conformationally restricted cyclopentyl moiety[33] (15–20, 22, 24) enhanced the A_1 antagonism, but the potency of the A_1 binding did not always parallel that of the diuretic activity. 8-(1-Adamantyl)-1,3-dipropylxanthine (22) was a potent and selective (390-fold) A_1 antagonist, but it showed a weak diuretic activity at the dose of 1.6

Table III. A_1 and A_2 Adenosine Receptor Binding of 8-Substituted 1,3-Dipropylxanthines[a]



no.	R	K_i,[a] nM A_1	K_i,[a] nM A_2	K_i ratio A_2/A_1
13	1-methylcyclohexyl	11	1200	110
14	2,2,5,5-tetramethylcyclopentyl	22	>100000	>4500
15	(1*R**,2*R**,5*R**)-bicyclo[3.3.0]octan-2-yl	3.5 ± 0.20	330 ± 4.7	94
16	(1*R**,2*S**,5*R**)-bicyclo[3.3.0]octan-2-yl	5.6 ± 0.19	560 ± 26	100
17	2-*endo*-norbornen-5-yl	4.3 ± 0.62	480 ± 18	110
18	2-*exo*-norbornen-5-yl	3.4 ± 0.41	210 ± 12	62
19	2-*endo*-norbornyl	3.8 ± 0.32	440 ± 42	120
20	2-*exo*-norbornyl	4.4 ± 0.13	290 ± 54	66
21	(2-*exo*-norbornyl)methyl	80 ± 9.5	1000	13
22	1-adamantyl	13 ± 2.8	5100 ± 1100	390
23	(1-adamantyl)methyl	880	>100000	>110
24	3-noradamantyl	1.3 ± 0.12 (0.19 ± 0.04)[b]	380 ± 30	290

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

mg/kg. On the other hand, 8-(3-noradamantyl)-1,3-dipropylxanthine (**24**, KW-3902) was a 10-fold more potent A_1 antagonist than **22**, while the diuretic and natriuretic activities were over 1000-fold more potent than **22**. The diuretic and natriuretic activities of **24** were extremely more potent than those of other xanthine adenosine A_1 antagonists such as **4** and **5** which show similar affinity to the A_1 receptor. It neither affected five different types of phosphodiesterase at μM nor receptor binding by a variety of ligands including histamine, muscarine, and catecholamine at 10 μM.

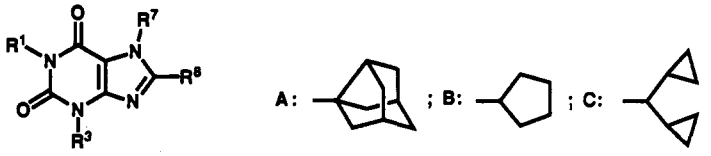
3-Noradamantyl (**24**; type A), cyclopentyl (**5**; type B), and dicyclopropylmethyl (**4**; type C) substitution appeared to be beneficial to both A_1 binding and diuretic activities among the series of 8-substituted xanthine derivatives. These findings prompted us to further investigate the effects of 1-, 3-, or 7-substitution of xanthines (type A, B, and C; Table V) on diuretic activity (Table VI). Propyl substitution at both the 1- and 3-position (**24**, **5**, **4**) was optimum to the A_1 antagonism in three sets of 8-substituted xanthines. The order of the affinity with regard to the substituents was similar in all three sets of xanthines; type A and C, 1,3-dipropyl (**24**, **4**) > 1,3-diethyl (**25**, **36**) ≥ 1,3-dibutyl (**26**, **37**) > 3-isobutyl-1-methyl (**28**, **39**) > 1,3-dimethyl (**28**, **39**) > 3-propyl (**29**, **40**); type B, 1,3-dibutyl (**31**) ≥ 1,3-dipropyl (**5**) > 1,3-diethyl (**30**) > 3-isobutyl-1-methyl (**32**) > 1,3-dimethyl (**33**) > 3-propyl (**34**). On the other hand, the order of the diuretic activities in a series of 8-(3-noradamantyl)xanthines (type A) was as follows: 1,3-dipropyl (**24**) > 1,3-dibutyl (**26**) > 1,3-dimethyl (**28**) > 1,3-diethyl (**25**), 3-isobutyl-1-methyl (**27**) > 3-propyl (**29**). The order in a series of 8-cyclopentylxanthines (type B) and 8-(dicyclopropylmethyl)xanthines (type C) was similar to each other as follows: 3-isobutyl-1-methyl (**32**, **38**) > 1,3-dipropyl (**4**, **5**), 1,3-diethyl (**30**, **36**) > 1,3-dibutyl (**31**, **37**) > 1,3-dimethyl (**33**, **39**), 3-propyl (**34**, **40**). Substitution at the 7-position reduced the affinity both at A_1 and A_2 receptors and the diuretic activity (**35**, **41–44**). The results of these studies confirmed that renal adenosine A_1 blockade potently induce diuresis. However, the structure–activity relationship with respect to the diuretic and

Table IV. Oral Diuretic Activities of 8-Substituted 1,3-Dipropylxanthines[a]

no.	dose (mg/kg, po)	UV[a] (mL/100 g per 6 h) control	UV[a] (mL/100 g per 6 h) treated	T/C[a]	Na^+ (mequiv/100 g per 6 h) control	Na^+ (mequiv/100 g per 6 h) treated	T/C[a]	Na^+/K^+[a]
13	25	0.92 ± 0.13	1.88 ± 0.16	2.04	0.158 ± 0.020	0.377 ± 0.007	2.39	1.71
	1.6	0.86 ± 0.09	2.60 ± 0.11	3.02	0.174 ± 0.013	0.466 ± 0.018	2.68	1.77
	0.4	1.06 ± 0.02	2.51 ± 0.06	2.37	0.173 ± 0.016	0.378 ± 0.019	2.18	1.93
14	25	0.96 ± 0.20	1.34 ± 0.14	1.40	0.146 ± 0.017	0.172 ± 0.026	1.18	1.21
	1.6	1.06 ± 0.02	1.50 ± 0.04	1.42	0.173 ± 0.016	0.213 ± 0.008	1.23	1.12
15	25	0.96 ± 0.06	1.80 ± 0.12	1.88	0.143 ± 0.019	0.298 ± 0.020	2.09	1.58
	6.25	1.10 ± 0.09	2.64 ± 0.12	2.40	0.195 ± 0.015	0.429 ± 0.014	2.20	1.55
	1.6	1.20 ± 0.14	2.52 ± 0.04	2.10	0.252 ± 0.022	0.448 ± 0.009	1.78	1.16
	0.4	0.80 ± 0.04	2.25 ± 0.20	2.81	0.134 ± 0.004	0.358 ± 0.022	2.67	2.10
	0.1	0.95 ± 0.03	0.86 ± 0.25	0.91	0.189 ± 0.005	0.176 ± 0.023	0.94	0.92
16	6.25	0.66 ± 0.04	2.84 ± 0.20	4.30	0.138 ± 0.008	0.487 ± 0.024	3.52	1.88
	1.6	0.79 ± 0.12	2.22 ± 0.22	2.81	0.105 ± 0.002	0.307 ± 0.023	2.93	2.16
17	25	1.20 ± 0.14	2.47 ± 0.06	2.06	0.252 ± 0.022	0.434 ± 0.019	1.72	1.27
	6.25	0.80 ± 0.02	2.05 ± 0.05	2.56	0.146 ± 0.005	0.365 ± 0.009	2.51	2.03
	0.4	1.40 ± 0.05	1.30 ± 0.06	0.93	0.261 ± 0.008	0.233 ± 0.012	0.89	0.98
18	25	0.94 ± 0.04	1.75 ± 0.17	1.87	0.130 ± 0.009	0.271 ± 0.016	2.09	1.25
19	25	1.00 ± 0.09	2.54 ± 0.13	2.54	0.180 ± 0.024	0.427 ± 0.021	2.37	1.84
	1.6	0.79 ± 0.12	2.48 ± 0.21	3.14	0.105 ± 0.002	0.337 ± 0.021	3.22	2.04
20	25	1.00 ± 0.09	2.13 ± 0.03	2.13	0.180 ± 0.024	0.372 ± 0.010	2.06	1.63
	6.25	1.64 ± 0.70	1.84 ± 0.16	1.12	0.265 ± 0.099	0.336 ± 0.017	1.27	1.18
21	25	0.69 ± 0.03	1.66 ± 0.08	2.41	0.079 ± 0.007	0.230 ± 0.002	2.91	2.12
	1.6	1.12 ± 0.33	0.86 ± 0.07	0.77	0.158 ± 0.034	0.131 ± 0.019	0.83	0.85
22	25	0.69 ± 0.03	1.58 ± 0.08	2.29	0.079 ± 0.007	0.226 ± 0.021	2.86	2.09
	3.1	1.18 ± 0.11	1.67 ± 0.16	1.42	0.227 ± 0.027	0.299 ± 0.035	1.32	1.23
	1.6	1.12 ± 0.33	1.39 ± 0.14	1.24	0.158 ± 0.034	0.227 ± 0.011	1.44	1.22
23	25	0.80 ± 0.13	0.51 ± 0.13	0.64	0.160 ± 0.017	0.108 ± 0.025	0.67	0.84
24	25	0.66 ± 0.04	2.74 ± 0.07	4.15	0.138 ± 0.008	0.475 ± 0.015	3.44	2.05
	6.25	0.80 ± 0.02	2.32 ± 0.20	2.90	0.146 ± 0.005	0.384 ± 0.027	2.64	2.07
	1.6	1.06 ± 0.02	2.77 ± 0.15	2.61	0.173 ± 0.016	0.399 ± 0.024	2.30	1.93
	0.4	1.40 ± 0.05	2.64 ± 0.16	1.86	0.261 ± 0.008	0.447 ± 0.03	1.70	1.42
	0.1	0.92 ± 0.05	2.44 ± 0.16	2.65	0.143 ± 0.013	0.334 ± 0.027	2.34	1.90
	0.025	0.82 ± 0.05	1.75 ± 0.39	2.13	0.183 ± 0.012	0.331 ± 0.067	1.81	1.45
	0.005	0.76 ± 0.01	1.39 ± 0.04	1.83	0.160 ± 0.006	0.256 ± 0.010	1.60	1.11
	0.0025	0.95 ± 0.12	1.61 ± 0.25	1.69	0.146 ± 0.006	0.258 ± 0.021	1.76	1.59

[a] See footnote *a–d* in Table II.

Table V. Effects of Substituents in the 1-, 3-, and 7-Positions on the Binding Affinity at A_1 and A_2 Adenosine Receptors[a]



no.	R^1	R^3	R^7	R^8	K_i,[a] nM A_1	K_i,[a] nM A_2	K_i ratio A_2/A_1
25	ethyl	ethyl	H	A	7.1 ± 0.88	1600 ± 430	230
26	butyl	butyl	H	A	10 ± 0.83	1100 ± 77	110
27	methyl	isobutyl	H	A	15 ± 0.88	850 ± 130	57
28	methyl	methyl	H	A	41 ± 3.1	1200 ± 33	29
29	H	propyl	H	A	370	>100000	>270
30	ethyl	ethyl	H	B	19	440	23
31	butyl	butyl	H	B	4.2	170	41
32	methyl	isobutyl	H	B	33	880	27
33	methyl	methyl	H	B	95	1200	13
34	H	propyl	H	B	1400	>100000	
35	propyl	propyl	methyl	B	3900	>10000	
36	ethyl	ethyl	H	C	13 ± 1.5	690 ± 66	53
37	butyl	butyl	H	C	5.5 ± 0.52	440 ± 69	80
38	methyl	isobutyl	H	C	12 ± 4.6	410 ± 140	34
39	methyl	methyl	H	C	81 ± 3.7	2700 ± 270	33
40	H	propyl	H	C	1300 ± 58	>100000	>77
41	propyl	propyl	methyl	C	7400 ± 1000	29000 ± 3500	3.9
42	propyl	propyl	ethyl	C	1600 ± 150	14000 ± 670	8.8
43	propyl	propyl	propyl	C	13000 ± 1600	6300 ± 660	2.1
44	propyl	propyl	carboxymethyl	C	6900 ± 1400	>100000	>14

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

natriuretic activities apparently show the profile slightly different from that of the A_1 binding. Since the structures of 8-substituted xanthines are similar, the differences of the potency of diuretic activities might be resulted from tissue differences of adenosine A_1 receptors between brain and kidney.

2. Renal Protective Activity. Following a subcutaneous injection of glycerol, serum urea nitrogen and creatinine concentrations increase 7–10-fold within 24 h in rats.[35] Serum urea nitrogen and creatinine concentrations in glycerol-injected rats treated intraperitoneally with a variety of adenosine antagonists or vehicle were shown in Table VII. In our model, aminophylline, ethylenediamine salt of theophylline (**1**), did not show any protective action at the dose of 10 mg/kg. 8-Phenyltheophylline (**3**), which is a more potent adenosine receptor antagonist than aminophylline, showed the protective effect at the same dose. The A_1 selective and potent antagonist 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (**4**) or 8-cyclopentyl-1,3-dipropylxanthine (**5**) showed potent protective effects against the acute renal failure, whereas the nonselective but potent antagonist 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (**6**) or 8-[4-[[*N*-[2-(dimethylamino)ethyl]-*N*-methylamino]sulfonyl]phenyl]-1,3-dipropylxanthine (**7**) showed only moderate protection at the dose of 10 mg/kg. Thus selective adenosine A_1 antagonism seems to be more important for the protective activity than the potency of affinity at the A_1 receptor itself. In a series of nonxanthine adenosine antagonists, the same situation was observed, since the A_1-selective adenosine antagonist such as [1,2,4]triazolo[4,3-*a*]quinoxaline derivative (**9** or **10**) also showed the amelioration at the doses of 1 and 10 mg/kg, respectively, and the A_2-selective ligand 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-4-amine (**8**) or 8-chloro-1-phenyl[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (**11**) increased the severity of the acute renal failure. From these results, it is suggested that the glycerol-induced acute renal failure was developed via the activation of adenosine A_1 receptor and was suppressed via the activation of A_2 receptor. The representative diuretic, furosemide (**12**), increased the severity. Thus, diuretic action could not explain the ameliorative effects of adenosine A_1 antagonists.

The activity of the polycycloalkyl-substituted xanthines against the acute renal failure is shown in Table VIII. The compounds with selective and potent A_1 antagonism (**13**, **15**, **17–20**, **24**) showed more potent ameliorative effects than 8-phenyltheophylline (**3**). The separation of the polycycloalkyl substituent from the xanthine by a methylene group (**21**, **23**) caused reduction of both the binding affinity and ameliorative effect. Compounds **14** and **16** are potent and selective A_1 antagonists, but these compounds showed moderate effects against the acute renal failure. The weak activity of **14**, the most A_1 selective compound, might be explained by its poor bioavailability, since **14** also showed weak diuretic activity. On the other hand, **16** showed potent diuretic activity (Table IV). We need more studies in order to speculate the presence of different subtypes in the renal A_1 receptor.

To further optimize ameliorative compounds in the renal failure, the effects of the 1-, 3-, and 7-substituent of three sets of 8-substituted xanthines (type A, 3-noradamantyl; type B, cyclopentyl; type C, dicyclopropylmethyl) were investigated (Table IX). The structure–activity relationships in the protective activity against the acute renal failure were generally in accord with those in binding assay with an exception (R^1 = Me, R^3 = isobutyl, **27**, **32**, **38**). Compounds with 1,3-dibutyl substitution (**26**, **31**, **37**) and 1,3-dimethyl substitution (**28**, **33**, **39**) showed relatively weak protective effects, whereas compounds with 1-methyl-3-isobutyl substitution (**27**, **32**, **38**) showed potent effects in spite of their relatively low affinity at the A_1 receptor. For example, compounds **37** and **39** did not show any protective effects at the dose of 1 mg/kg, while compound **38** showed the protective effects at the dose of 0.1

(35) Bowmer, C. J.; Yates, M. S.; Emmerson, J. The Effect of Acute Renal Failure on the Pharmacokinetics of Indocyanine Green in the Rat. *Biochem. Pharmacol.* **1982**, *31*, 2531–2538.

Table VI. Effects of Substituents in the 1-, 3-, and 7-Positions on the Binding Affinity at A_1 and A_2 Adenosine Receptors and the Diuretic Activity of 8-Substituted Xanthine Derivatives[a]

no.	dose (mg/kg, po)	UV[a] (mL/100 g per 6 h) control	UV[a] (mL/100 g per 6 h) treated	T/C[a]	Na^+ (mequiv/100 g per 6 h) control	Na^+ (mequiv/100 g per 6 h) treated	T/C[a]	Na^+/K^+[a]
25	1.6	0.97 ± 0.05	2.90 ± 0.02	2.99	0.169 ± 0.012	0.492 ± 0.019	2.92	1.97
	0.4	1.72 ± 0.31	2.29 ± 0.16	1.33	0.267 ± 0.044	0.356 ± 0.008	1.33	1.29
26	1.6	1.12 ± 0.05	2.52 ± 0.18	2.25	0.189 ± 0.005	0.426 ± 0.035	2.26	1.61
	0.4	0.97 ± 0.05	2.50 ± 0.14	2.58	0.169 ± 0.012	0.412 ± 0.013	2.45	1.89
	0.1	0.96 ± 0.09	1.76 ± 0.05	1.83	0.147 ± 0.015	0.279 ± 0.005	1.89	1.58
	0.01	1.05 ± 0.11	0.78 ± 0.09	0.74	0.180 ± 0.017	0.176 ± 0.023	0.98	1.05
27	1.6	0.97 ± 0.05	2.43 ± 0.15	2.51	0.169 ± 0.012	0.409 ± 0.014	2.43	1.73
	0.4	1.72 ± 0.31	2.41 ± 0.04	1.40	0.267 ± 0.044	0.367 ± 0.016	1.38	1.37
28	1.6	0.97 ± 0.05	2.27 ± 0.11	2.34	0.169 ± 0.012	0.398 ± 0.006	2.36	1.67
	0.4	0.96 ± 0.09	1.59 ± 0.05	1.66	0.147 ± 0.015	0.257 ± 0.003	1.74	1.54
	0.1	1.05 ± 0.11	0.86 ± 0.15	0.82	0.180 ± 0.017	0.163 ± 0.026	0.91	0.97
29	25	1.64 ± 0.70	2.34 ± 0.29	1.43	0.265 ± 0.099	0.400 ± 0.040	1.51	1.29
	6.25	1.13 ± 0.11	1.42 ± 0.23	1.26	0.161 ± 0.027	0.240 ± 0.031	1.49	1.43
30	1.6	0.86 ± 0.09	2.09 ± 0.06	2.43	0.174 ± 0.013	0.363 ± 0.012	2.09	1.56
	0.4	1.15 ± 0.17	1.89 ± 0.09	1.64	0.191 ± 0.020	0.342 ± 0.012	1.79	1.44
	0.1	1.05 ± 0.11	1.09 ± 0.04	1.03	0.180 ± 0.017	0.206 ± 0.015	1.15	1.00
31	1.6	0.86 ± 0.09	1.60 ± 0.23	1.86	0.174 ± 0.013	0.299 ± 0.021	1.72	1.32
	0.4	1.15 ± 0.17	1.45 ± 0.04	1.26	0.180 ± 0.017	0.309 ± 0.009	1.61	1.23
32	1.6	0.93 ± 0.03	1.97 ± 0.19	2.12	0.173 ± 0.015	0.379 ± 0.014	2.19	1.57
	0.1	0.75 ± 0.12	1.59 ± 0.17	2.12	0.179 ± 0.038	0.308 ± 0.040	1.72	1.55
	0.01	1.15 ± 0.17	1.31 ± 0.07	1.14	0.191 ± 0.020	0.257 ± 0.009	1.35	1.22
33	6.25	1.15 ± 0.17	1.88 ± 0.21	1.64	0.191 ± 0.020	0.349 ± 0.017	1.82	1.46
	1.6	0.86 ± 0.09	1.06 ± 0.14	1.23	0.174 ± 0.013	0.217 ± 0.040	1.25	1.12
34	6.25	0.92 ± 0.06	1.62 ± 0.13	1.76	0.128 ± 0.007	0.264 ± 0.014	2.07	1.82
35	25	0.74 ± 0.07	1.07 ± 0.17	1.45	0.172 ± 0.015	0.184 ± 0.026	1.07	1.00
36	1.6	0.96 ± 0.09	1.72 ± 0.14	1.79	0.147 ± 0.015	0.312 ± 0.024	2.12	1.71
	0.4	1.15 ± 0.17	2.11 ± 0.11	1.84	0.191 ± 0.020	0.433 ± 0.003	2.27	1.63
	0.1	1.15 ± 0.12	0.83 ± 0.07	0.72	0.133 ± 0.022	0.113 ± 0.011	0.85	1.37
37	1.6	0.96 ± 0.09	1.59 ± 0.07	1.66	0.147 ± 0.015	0.282 ± 0.009	1.91	1.66
	0.4	1.15 ± 0.17	1.24 ± 0.18	1.08	0.191 ± 0.020	0.276 ± 0.036	1.44	1.45
38	1.6	0.93 ± 0.03	1.93 ± 0.08	2.08	0.173 ± 0.015	0.345 ± 0.014	1.99	1.37
	0.1	0.75 ± 0.12	1.26 ± 0.25	1.68	0.179 ± 0.038	0.250 ± 0.022	1.40	2.13
	0.01	1.15 ± 0.17	1.15 ± 0.14	1.00	0.191 ± 0.020	0.212 ± 0.012	1.11	1.09
39	1.6	0.96 ± 0.09	0.87 ± 0.15	0.91	0.147 ± 0.015	0.183 ± 0.020	1.24	1.18
40	25	0.74 ± 0.05	1.85 ± 0.11	2.50	0.152 ± 0.013	0.328 ± 0.027	2.16	1.40
	6.25	0.95 ± 0.12	1.35 ± 0.09	1.42	0.146 ± 0.006	0.246 ± 0.012	1.69	1.51
	1.5	1.01 ± 0.08	1.13 ± 0.11	1.12	0.165 ± 0.008	0.202 ± 0.033	1.22	1.13
41	6.25	0.92 ± 0.05	1.64 ± 0.13	1.78	0.143 ± 0.013	0.306 ± 0.013	2.14	1.78
	1.6	0.82 ± 0.05	1.48 ± 0.13	1.80	0.183 ± 0.012	0.271 ± 0.018	1.48	1.21
	0.4	0.99 ± 0.07	0.96 ± 0.19	0.97	0.191 ± 0.021	0.174 ± 0.014	0.91	0.94
42	25	0.89 ± 0.06	2.15 ± 0.06	2.42	0.162 ± 0.006	0.348 ± 0.012	2.14	1.51
	6.25	1.14 ± 0.02	1.36 ± 0.06	1.19	0.173 ± 0.005	0.214 ± 0.016	1.24	1.12
43	25	0.76 ± 0.01	1.12 ± 0.05	1.47	0.160 ± 0.006	0.188 ± 0.007	1.18	0.92
44	25	0.89 ± 0.06	1.02 ± 0.06	1.15	0.162 ± 0.006	0.185 ± 0.006	1.14	0.95

[a] See footnote *a–d* in Table II.

Table VII. Renal Protective Activities of Reference Compounds[a]

no.	dose (mg/kg, ip)	Cr (mg/dL) vehicle	Cr (mg/dL) treated	% inhibn[b]	UN (mg/dL) vehicle	UN (mg/dL) treated	% inhibn[b]
1	10	5.43 ± 0.11	5.32 ± 0.19	2	182.5 ± 3.4	174.0 ± 10.4	5
2	10	4.41 ± 0.16	2.85 ± 0.36**	35	145.1 ± 7.4	109.0 ± 15.4**	25
	1	4.70 ± 0.17	3.68 ± 0.35*	22	154.2 ± 5.0	125.7 ± 12.4*	18
3	10	3.94 ± 0.24	1.74 ± 0.19***	56	141.3 ± 6.6	62.4 ± 8.8***	56
	1	3.86 ± 0.27	3.14 ± 0.38	19	178.0 ± 11.3	148.3 ± 13.8	17
4	1	5.43 ± 0.11	2.99 ± 0.28***	45	182.5 ± 3.4	107.6 ± 10.2***	41
5	1	5.43 ± 0.11	3.75 ± 0.38**	31	182.5 ± 3.4	131.7 ± 9.3***	27
6	10	2.74 ± 0.27	2.07 ± 0.30	24	97.7 ± 8.7	70.1 ± 8.9*	28
7	10	2.89 ± 0.30	2.31 ± 0.20	20	93.6 ± 8.6	62.6 ± 6.7*	33
8	10	2.72 ± 0.22	3.00 ± 0.70	−10	ND[c]		
9	1	4.70 ± 0.17	2.58 ± 0.30***	45	154.2 ± 5.0	88.7 ± 10.2***	42
10	10	2.89 ± 0.18	1.52 ± 0.10***	48	138.4 ± 9.8	58.9 ± 5.6***	60
	1	2.89 ± 0.42	2.60 ± 0.44	10	107.7 ± 15.2	92.0 ± 13.5	15
11	10	3.38 ± 0.29	4.58 ± 0.34*	−36	114.0 ± 8.7	137.8 ± 9.3	−21
12	10	3.22 ± 0.35	4.17 ± 0.41	−30	110.7 ± 9.4	150.3 ± 13.7*	−36

[a] Bowmer's method[35] was modified to induce acute renal failure. Fifty percent v/v glycerol in sterile saline (0.8 mL/100 g) was injected subcutaneously to rats 30 min after vehicle or compounds treatment. At 24 h after glycerol injection, serum creatinine (Cr) and urea nitrogen (UN) concentrations were determined; see the Experimental Section for details. All values are the means ± SEM; significant difference from vehicle-treated group (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). [b] Percent inhibition denotes ratios of Cr or UN values in treated rats to Cr or UN values in vehicle-treated rats. [c] Not determined.

mg/kg. In addition, caffeine (2) showed more potent protective activity than theophylline (1). These unexpected results might be explained by some other mechanism of action.[36] Introduction of the 7-substituent to the

Table VIII. Renal Protective Activities of 8-Substituted 1,3-Dipropylxanthines[a]

no.	dose (mg/kg, ip)	Cr (mg/dL) vehicle	Cr (mg/dL) treated	% inhibn[a]	UN (mg/dL) vehicle	UN (mg/dL) treated	% inhibn[a]
13	1	4.74 ± 0.31	2.04 ± 0.23***	57	138.8 ± 7.8	55.2 ± 8.1***	60
14	10	2.13 ± 0.27	1.43 ± 0.10*	33	ND[a]		
	1	3.06 ± 0.68	3.03 ± 0.82	1	120.9 ± 23.3	113.4 ± 26.1	6
15	1	5.43 ± 0.11	3.32 ± 0.28***	31	182.5 ± 3.4	115.4 ± 6.2***	37
16	10	4.64 ± 0.24	3.79 ± 0.42	18	147.0 ± 5.8	119.1 ± 9.7*	19
17	1	4.41 ± 0.17	2.08 ± 0.23***	53	147.9 ± 6.1	80.4 ± 10.1***	56
18	1	5.07 ± 0.33	2.23 ± 0.45***	56	195.8 ± 14.4	92.7 ± 18.5***	53
19	1	5.07 ± 0.33	2.59 ± 0.54***	49	195.8 ± 14.4	101.0 ± 19.3***	48
20	1	5.07 ± 0.33	2.54 ± 0.54***	50	195.8 ± 14.4	98.8 ± 14.1***	49
21	10	4.26 ± 0.39	2.53 ± 0.42**	41	146.7 ± 10.1	90.9 ± 12.0**	38
	1	3.06 ± 0.68	2.75 ± 0.79	10	120.9 ± 23.3	104.7 ± 30.0	13
22	10	4.26 ± 0.39	1.91 ± 0.02***	55	146.7 ± 10.1	77.5 ± 9.6***	47
	1	4.91 ± 0.10	3.45 ± 0.46*	30	154.5 ± 7.0	115.9 ± 15.7*	25
23	10	3.63 ± 0.29	3.21 ± 0.52	12	137.9 ± 8.5	117.8 ± 14.9	15
24	1	2.75 ± 0.43	2.00 ± 0.14**	47	123.2 ± 14.2	66.4 ± 6.3**	46

[a] See footnotes *a–c* in Table VII.

Table IX. Effects of Substituents in the 1-, 3-, and 7-Positions on Renal Protective Activity of 8-Substituted Xanthine Derivatives[a]

no.	dose (mg/kg, ip)	Cr (mg/dL) vehicle	Cr (mg/dL) treated	% inhibn[a]	UN (mg/dL) vehicle	UN (mg/dL) treated	% inhibn[a]
24	1	3.75 ± 0.43	2.00 ± 0.14**	47	123.2 ± 14.2	66.4 ± 6.3**	46
	0.1	2.89 ± 0.18	1.40 ± 0.14***	52	138.4 ± 9.8	48.1 ± 7.0***	65
	0.01	4.03 ± 0.23	1.83 ± 0.08***	55	130.5 ± 7.0	45.2 ± 3.9***	65
25	1	4.82 ± 0.17	2.09 ± 0.16***	57	151.5 ± 7.4	65.9 ± 6.4***	56
	0.1	3.92 ± 0.23	1.44 ± 0.18***	63	141.3 ± 8.3	44.5 ± 8.0***	68
	0.01	4.50 ± 0.24	2.82 ± 0.42**	37	130.6 ± 4.1	81.7 ± 13.1**	37
26	1	4.82 ± 0.17	2.63 ± 0.50**	45	151.5 ± 7.4	62.1 ± 10.0***	59
	0.1	3.92 ± 0.23	3.19 ± 0.26	19	141.3 ± 8.3	119.4 ± 11.5	15
27	1	4.82 ± 0.17	2.78 ± 0.64*	42	151.5 ± 7.4	74.0 ± 16.7***	51
	0.1	3.92 ± 0.23	2.04 ± 0.33**	48	141.3 ± 8.3	70.9 ± 14.8**	50
	0.01	4.50 ± 0.24	3.38 ± 0.50*	25	130.6 ± 4.12	115.5 ± 18.0	12
28	1	5.07 ± 0.33	2.88 ± 0.48**	43	195.8 ± 14.4	116.7 ± 15.8**	40
	0.1	4.82 ± 0.17	3.14 ± 0.45**	35	151.5 ± 7.4	97.3 ± 10.8***	36
	0.01	3.92 ± 0.23	3.53 ± 0.41	10	141.3 ± 8.3	133.4 ± 16.2	6
29	10	5.53 ± 0.34	2.83 ± 0.32***	49	177.4 ± 5.3	107.4 ± 14.0***	39
	1	5.07 ± 0.33	4.33 ± 0.45	15	195.8 ± 14.4	164.9 ± 15.1	16
5	1	5.43 ± 0.11	3.75 ± 0.38**	31	182.5 ± 3.4	131.7 ± 9.3***	27
	0.1	4.41 ± 0.17	2.19 ± 0.22***	50	147.9 ± 6.1	82.1 ± 8.9***	44
	0.01	4.03 ± 0.23	3.42 ± 0.39	15	130.5 ± 7.0	101.0 ± 2.9*	23
30	1	4.74 ± 0.31	2.28 ± 0.37***	52	139.8 ± 7.8	65.5 ± 12.7***	53
	0.1	6.90 ± 0.20	5.48 ± 0.46*	21	171.3 ± 6.4	143.9 ± 9.9*	16
31	1	4.74 ± 0.31	2.74 ± 0.25***	42	138.8 ± 7.8	75.0 ± 6.5***	56
	0.1	4.82 ± 0.24	4.03 ± 0.47	16	156.2 ± 6.1	153.6 ± 16.2	2
32	1	4.27 ± 0.24	2.54 ± 0.43**	40	171.5 ± 7.7	109.5 ± 11.8**	36
	0.1	5.08 ± 0.14	2.79 ± 0.31***	45	165.3 ± 4.0	107.3 ± 13.5**	35
	0.01	4.09 ± 0.39	3.08 ± 0.48	25	143.9 ± 12.2	106.4 ± 15.0	26
33	1	4.74 ± 0.31	2.22 ± 0.14***	53	138.8 ± 7.8	63.6 ± 6.4***	54
	0.1	6.90 ± 0.20	7.26 ± 0.10	−5	171.3 ± 6.4	168.7 ± 4.5	1
34	1	4.91 ± 0.10	3.51 ± 0.27**	28	154.5 ± 7.0	117.0 ± 6.1**	24
35	10	4.21 ± 0.21	3.07 ± 0.33**	26	144.1 ± 6.1	107.9 ± 10.8**	25
	1	3.75 ± 0.34	2.62 ± 0.47	30	137.4 ± 16.5	99.9 ± 17.1	27
4	1	5.43 ± 0.11	2.99 ± 0.28***	45	182.5 ± 3.4	107.6 ± 10.2***	41
	0.1	4.91 ± 0.11	3.22 ± 0.37**	33	154.9 ± 4.1	107.0 ± 9.9**	31
	0.01	4.99 ± 0.36	4.29 ± 0.23	14	163.0 ± 10.8	131.3 ± 17.0	19
36	10	3.93 ± 0.45	1.74 ± 0.16***	56	129.1 ± 14.5	50.6 ± 7.5***	61
	1	3.62 ± 0.52	3.24 ± 0.45	10	105.2 ± 14.9	98.5 ± 11.8	6
37	10	3.93 ± 0.45	2.30 ± 0.34*	41	129.1 ± 14.5	81.1 ± 14.7*	37
	1	3.62 ± 0.52	3.63 ± 0.57	0	105.2 ± 14.9	101.1 ± 14.7	4
38	1	6.90 ± 0.20	3.97 ± 0.76**	42	171.3 ± 6.4	110.7 ± 19.9*	35
	0.1	5.08 ± 0.14	3.29 ± 0.33**	35	165.3 ± 4.0	122.0 ± 9.3**	26
	0.01	4.09 ± 0.39	4.28 ± 0.47	−5	143.9 ± 12.2	150.7 ± 12.7	−5
39	10	3.93 ± 0.45	1.78 ± 0.19***	55	129.1 ± 14.5	57.2 ± 8.1***	55
	1	3.62 ± 0.52	4.10 ± 0.51	−13	105.2 ± 14.9	121.4 ± 15.2	−15
40	1	4.19 ± 0.20	3.00 ± 0.27**	28	156.1 ± 8.4	119.1 ± 8.1**	24
	0.1	2.90 ± 0.28	2.96 ± 0.39	−2	93.4 ± 10.0	104.2 ± 16.4	−11
41	1	2.89 ± 0.42	1.63 ± 0.12*	44	107.7 ± 15.2	54.1 ± 6.3**	50
	0.1	3.91 ± 0.37	4.49 ± 0.40	−15	128.9 ± 9.3	136.5 ± 8.1	6
42	10	4.98 ± 0.08	4.50 ± 0.20	10	150.7 ± 3.4	142.7 ± 4.3	5
43	10	4.38 ± 0.17	4.09 ± 0.30	7	164.3 ± 4.9	159.2 ± 10.2	3
44	10	4.98 ± 0.08	4.79 ± 0.13	4	150.7 ± 3.4	145.5 ± 4.0	3

[a] See footnotes *a* and *b* in Table VII.

Table X. Analytical Data for 8-Substituted Xanthines

no.	synthetic method	% yield	mp, °C (recryst solvent)	formula[a]
4	A	44	129–130 (EtOH/H_2O)	$C_{18}H_{30}N_4O_2$
30	B	57	181–182 (cyclohexane)	$C_{14}H_{20}N_4O_2$
31	B	59	172–173 (dioxane/H_2O)	$C_{18}H_{28}N_4O_2$
32	B	52	202–204 (2-PrOH/H_2O)	$C_{15}H_{22}N_4O_2$
33	B	51	244–246[b] (2-PrOH)	$C_{12}H_{16}N_4O_2$
34	c	66	311–313 (DMF)	$C_{13}H_{18}N_4O_2$
35	C	94	114–115[d] (acetone/H_2O)	$C_{17}H_{26}N_4O_2$
36	D	35	166–169 (dioxane/H_2O)	$C_{16}H_{22}N_4O_2$
37	D	28	116 (heptane)	$C_{20}H_{30}N_4O_2$
38	D	46	170–174 (heptane)	$C_{17}H_{24}N_4O_2$
39	D	64	218–219 (heptane/Tol)	$C_{14}H_{18}N_4O_2$
40	D	64	272–278 (EtOH)	$C_{15}H_{20}N_4O_2$[e]
41	C	82	70 (heptane)	$C_{19}H_{28}N_4O_2$
42	C	71	80–83 (MeCN)	$C_{20}H_{30}N_4O_2 \cdot 0.5MeCN$[f]
43	C	82	93–100 (EtOH/H_2O)	$C_{21}H_{32}N_4O_2$
44	c	99	141–149 (MeCN)	$C_{20}H_{27}N_4O_4Na \cdot 0.8H_2O$

[a] All compounds were analyzed for C, H, N. [b] Lit.[36a] mp 247–249 °C. [c] See the Experimental Section. [d] Lit.[28e] mp 114 °C. [e] H: calcd, 6.99; found, 7.42. [f] C: calcd, 66.55; found, 66.06.

xanthine nuclei caused much weaker affinity and pharmacological activity (35, 41–44). Although the order of renal protective activity in three sets appeared to be similar to that in the affinity at the A_1 receptor, there is a big difference in the potency of the protective activity. 8-(3-Noradamantyl)xanthines (type A) showed the most potent ameliorative effect against the renal failure among the three sets. 8-Cyclopentylxanthines (type B) followed by 8-(dicyclopropylmethyl)xanthines (type C) showed the slightly reduced activity. The minimum effective doses of compounds 25, 30, and 36 were 0.01, 0.1, and 10 mg/kg (ip), respectively. On the other hand, the minimum effective doses of these compounds in the diuresis were 0.4, 0.4, and 0.4 mg/kg (po, Table VI). Thus the differences of the protective effects among these three types of xanthine derivatives was much larger than those of the diuretic activities. This difference might be caused by the differences in pharmacokinetics and by different sites of action between these two renal pharmacological assays. The maximal protection against the renal failure seemed to be approximately 50–60%. These results might be explained by the role of other factors such as oxygen metabolites and glutathione[37] in developing glycerol-induced acute renal failure.

In summary, the diuretic and natriuretic activities of xanthines generally seemed to be correlated to the affinity at the adenosine A_1 receptor. Thus renal adenosine induces antidiuresis and antinatriuresis via the A_1 receptor. The extremely potent diuretic activities of a series of 8-(3-noradamantyl)xanthines would suggest that they had high affinity at adenosine A_1 receptors in the kidney. The activation of adenosine A_1 receptors was an important factor in developing glycerol-induced acute renal failure. From our structure–activity relationships, it is speculated that some tissue differences of adenosine A_1 receptors existed between kidney and brain and that pharmacokinetics and sites of action for adenosine antagonists might be different between two renal pharmacological assays. The further detailed pharmacological profiles of these A_1 antagonists are under active study in our laboratories. Compound 24 (KW-3902) is now under development as a drug for treating the acute renal failure.

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 spectrophotometer. Proton nuclear magnetic resonance (^1H NMR) spectra were measured on a JEOL JNM-PMX60, HITACHI R-90H, or JEOL JNM GX-270 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. Microanalysis was performed on a Perkin-Elmer 2400CHN and agrees within ±0.4% of calculated values unless otherwise noted. For column chromatography, silica gel 60 (E. Merck, 0.063–0.200 mm) was used.

8-Phenyltheophylline (3) and 8-cyclopentyl-1,3-dipropylxanthine (5) were purchased from Research Biochemicals, Inc. (Natick, MA). The following reference compounds were prepared by published procedures: 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine (6),[26] 8-[4-[[*N*-[2-(dimethylamino)ethyl]-*N*-methylamino]sulfonyl]phenyl]-1,3-dipropylxanthine (7),[29] 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-4-amine (8),[32] 8-chloro-4-(cyclopentylamino)-1-(trifluoromethyl)[1,2,4]triazolo[4,3-a]quinoxaline (9),[30] 4-(cyclopentylamino)-1-(trifluoromethyl)[1,2,4]triazolo[4,3-a]quinoxaline (10),[31] and 8-chloro-1-phenyl[1,2,4]triazolo[4,3-a]quinoxalin-4-amine (11).[30] The synthesis of 8-polycycloalkyl-substituted xanthines (13–29) has been described elsewhere.[33] 1,3-Dialkyl-5,6-diaminouracils[38] were synthesized by published procedure.

Method A. 8-(Dicyclopropylmethyl)-1,3-dipropylxanthine (4). To a solution of dicyclopropylacetic acid[39] (700 mg, 5.0 mmol) in pyridine (12.5 mL) was added dropwise thionyl chloride (0.39 mL, 5.5 mmol) at 0 °C with stirring. The reaction mixture was heated at 60 °C for 10 min, and then 5,6-diamino-1,3-dipropyluracil[25b] (1.1 g, 5.0 mmol) in pyridine (10 mL) was slowly added with stirring at 0 °C. After 1 h of stirring at 0 °C, the reaction mixture was concentrated under reduced pressure. A saturated $NaHCO_3$ solution was added, and the mixture was extracted with $CHCl_3$ three times. The combined organic layer was dried over Na_2SO_4, and the solvent was removed under vacuo. Purification on silica gel column chromatography (eluent:1% MeOH/$CHCl_3$) afforded 6-amino-5-[[(dicyclopropylmethyl)carbonyl]amino]-1,3-dipropyluracil (1.16 g, 66%): ^1H NMR ($CDCl_3$, 90 MHz) δ 7.71 (1 H, br s), 5.52 (2 H, br s), 4.00–3.75 (4 H, m), 1.90–1.50 (4 H, m), 1.40–0.20 (17 H, m). A solution of 900 mg (2.6 mmol) of this uracil in 8 mL of $POCl_3$ was refluxed for 1.5 h. The excess

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$POCl_3$ was removed in vacuo, and the residue was neutralized with 50% NH_4OH. Usual workup as above and purification on silica gel column chromatography (eluent:10% ethyl acetate/hexane), followed by recrystallization from $EtOH/H_2O$, afforded 566 mg (44% overall) of **4** as colorless needles: mp 129–130 °C; ^1H NMR ($CDCl_3$, 270 MHz) δ 12.70 (1 H, br s), 4.13 (2 H, t, J = 7.5 Hz), 3.97 (2 H, t, J = 7.5 Hz), 1.90–1.60 (5 H, m), 1.50–1.35 (2 H, m), 1.10–0.90 (6 H, m), 0.75–0.60 (2 H, m), 0.50–0.20 (6 H, m); IR (KBr) 3140, 1700, 1654, 1498 cm^{-1}. Anal. ($C_{18}H_{30}N_4O_2$) C, H, N.

Method B. 8-Cyclopentyl-1,3-diethylxanthine (30). To a solution of 5,6-diamino-1,3-diethyluracil[38b] (2.0 g, 10 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (2.1 g, 11 mmol) in 100 mL of dioxane/H_2O (1:1) was slowly added portionwise cyclopentanecarboxylic acid (1.2 mL, 11 mmol) with stirring, and the pH was maintained at 5.0 ± 0.5 by the dropwise addition of 2 N HCl. After 4 h of stirring at room temperature, the reaction mixture was treated with 2 N NaOH (50 mL) and heated under reflux for 10 min. After cooling to 0 °C, the product was precipitated by adjusting the pH to 4.0 with 4 N HCl. Recrystallization from cyclohexane yielded 1.57 g (57%) of **30** as colorless needles: mp 181–182 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ 12.6 (1 H, br s), 4.33 (2 H, t, J = 7 Hz), 4.17 (2 H, t, J = 7 Hz), 3.50–3.20 (1 H, m), 2.25–1.15 (14 H, m); IR (KBr) 3132, 1700, 1646, 1552, 1505, 1400 cm^{-1}. Anal. ($C_{14}H_{20}N_4O_2$) C, H, N.

8-Cyclopentyl-3-propylxanthine (34). To a suspension of 5,6-diamino-1-propyluracil[38a] (30.0 g, 163 mmol) in 60 mL of DMF were added cyclopentanecarboxylic acid (17.7 mL, 163 mmol), 1-hydroxybenzotriazole hydrate (30.0 g, 222 mmol), and dicyclohexylcarbodiimide (50.5 g, 245 mmol). The mixture was stirred at room temperature overnight. After insoluble material was removed by filtration, the filtrate was evaporated under reduced pressure. To the residue was added 600 mL of 4 N NaOH and the solution was refluxed for 10 min. After ice cooling, insoluble material was filtered off again and 50 mL of MeOH was added. The resulting mixture was neutralized with 4 N HCl. The precipitates were collected by filtration to afford 28.3 g (66%) of **34** as a white powder. Analytically pure sample was obtained by recrystallization from DMF: mp 311–313 °C; ^1H NMR (DMSO-d_6, 270 MHz) δ 13.05 (1 H, br s), 10.94 (1 H, br s), 3.86 (2 H, t, J = 7.6 Hz), 3.18–3.04 (1 H, m), 2.05–1.55 (10 H, m), 0.87 (3 H, t, J = 7.5 Hz); ^{13}C NMR (DMSO-d_6, 270 MHz) δ 157.7, 154.3, 150.9, 149.4, 106.5, 43.3, 39.0, 31.9, 25.0, 20.9, 10.9; IR (KBr) 3150, 2880, 1698, 1669 cm^{-1}. Anal. ($C_{13}H_{18}N_4O_2$) C, H, N.

Method C. 8-(Dicyclopropylmethyl)-1,3-dipropyl-7-methylxanthine (41). To a solution of **4** (2.0 g, 6.1 mmol) in 60 mL of DMF were added 2.1 g (15.1 mmol) of potassium carbonate and 0.75 mL (12.1 mmol) of methyl iodide. The mixture was stirred at 50 °C for 1 h and cooled. Water (200 mL) was added, and the mixture was extracted with $CHCl_3$ three times. Usual workup, purification on column chromatography (eluent: 20% ethyl acetate/hexane), followed by recrystallization from heptane, yielded 1.71 g (82%) of **41** as a white powder: mp 70 °C; ^1H NMR ($CDCl_3$, 270 MHz) δ 4.08 (2 H, t, J = 7.3 Hz), 3.96 (2 H, t, J = 7.5 Hz), 3.90 (3 H, s), 1.90–1.60 (5 H, m), 1.40–1.28 (2 H, m), 1.05–0.90 (6 H, m), 0.70–0.60 (2 H, m), 0.50–0.40 (2 H, m), 0.30–0.15 (4 H, m); IR (KBr) 1696, 1660, 1541, 1457 cm^{-1}. Anal. ($C_{19}H_{28}N_4O_2$) C, H, N.

Method D. 8-(Dicyclopropylmethyl)-1,3-diethylxanthine (36). Condensation of dicyclopropylacetic acid (1.56 g, 11.1 mmol) and 5,6-diamino-1,3-diethyluracil (2.0 g, 10.1 mmol) as described in method A afforded 6-amino-1,3-diethyl-5-[[(dicyclopropylmethyl)carbonyl]amino]uracil (1.87 g, 58%). This uracil (1.78 g, 5.56 mmol) was dissolved in 40 mL of 1 N NaOH and 20 mL of dioxane, and heated under reflux for 10 min. Usual workup as described in method B followed by recrystallization from dioxane–water yielded 1.02 g (35% overall) of **36** as colorless needles: mp 166–169 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ 12.0 (1 H, br s), 4.50–4.15 (4 H, m), 2.00–1.15 (9 H, m), 0.80–0.15 (8 H, m); IR (KBr) 3158, 1710, 1657, 1652, 1500 cm^{-1}. Anal. ($C_{16}H_{22}N_4O_2$) C, H, N.

[8-(Dicyclopropylmethyl)-1,3-dipropylxanthin-7-yl]acetic Acid Sodium Salt (44). To a solution of 4.00 g (12.1 mmol) of **4** in 40 mL of DMF was slowly added 60% sodium hydride suspended in mineral oil (582 mg, 14.5 mmol) at 0 °C. After 30 min of stirring at 0 °C, 2.3 mL (2.36 mmol) of *tert*-butyl bromoacetate was dropwise added to the mixture. After 30 min stirring at room temperature, the mixture was poured into water (100 mL), extracted with $CHCl_3$ three times, dried, and concentrated. Purification on silica gel chromatography (eluent: 15% ethyl acetate/hexane) afforded [8-(dicyclopropylmethyl)-1,3-dipropylxanthin-7-yl]acetic acid *tert*-butyl ester (3.35 g, 62%). This ester (3.00 g, 5.51 mmol) was treated with 60 mL of TFA/CH_2Cl_2 (1:1) at 0 °C. After 6 h of stirring at room temperature, the mixture was concentrated under reduced pressure. After usual workup, the residue was dissolved in 30 mL of MeOH and treated with sodium methoxide (25 wt % solution in methanol; 1.13 mL, 5.51 mmol) at 0 °C. The mixture was concentrated and the residue was recrystallized from MeCN two times to afford 2.34 g (61% overall) of **44** as a pale yellow powder: mp 141–149 °C; ^1H NMR (DMSO-d_6, 270 MHz) δ 4.58 (1 H, s), 3.99 (2 H, t, J = 7.0 Hz), 3.80 (2 H, t, J = 7.4 Hz), 1.86 (1 H, t, J = 8.2 Hz), 1.80–1.50 (4 H, m), 1.30–1.15 (2 H, m), 1.00–0.80 (6 H, m), 0.55–0.40 (2 H, m), 0.35–0.20 (4 H, m); IR (KBr) 3200, 1704, 1641, 1614, 1538, 1432, 1399 cm^{-1}. Anal. ($C_{20}H_{27}N_4O_4Na \cdot 0.8H_2O$) C, H, N.

Radioligand Binding Assays. Inhibition of binding of 1.1 nM of N^6-[^{3}H]cyclohexyladenosine to A_1 receptors in guinea pig forebrain membranes was assayed as described,[33c,34] and inhibition of 3.8 nM of *N*-[^{3}H]ethyladenosin-5′-uronamide in the presence of 50 nM N^6-cyclopentyladenosine in rat striatal membranes was assayed as described.[22,33c] Concentration–inhibition curves were carried out in duplicate with five or more concentrations of each test agent, and IC_{50} values were calculated from computerization of logit log curve. The inhibition constants (K_i) were calculated according to the Cheng and Prusoff equation.[40] When the assays were carried out three or more times, standard errors (SEM) are given in the table.

N^6-[^{3}H]Cyclohexyladenosine A_1 binding assay using rat forebrain membranes was performed according to the same protocol as above.

Pharmacology. Diuretic Activity in Saline-Loaded Rats. Fasted male Wistar rats (SLC, ca. 200 g) with free access of water were used. Compounds were suspended with saline and administered orally at a dosage volume of 25 mL/kg. The rats were housed in groups of three in metabolic cages. Urine was collected for the 0–6-h interval in volumetric graduated cylinders and was analyzed for sodium and potassium contents with a flame photometer (Hitachi 775A). Results that are geometric means ± SEM of three cages for each dose level are expressed. Potassium excretion was omitted for clarity in Tables II, IV, and VI.

Renal Protective Activity against Glycerol-Induced Acute Renal Failure in Rats. Bowmer's method[35] was modified to induce acute renal failure. Male Wistar rats (SLC, 250–300 g) were deprived of drinking water for 18 h. Fifty percent v/v glycerol in sterile saline (0.8 mL/100 g) was injected subcutaneously under ether anesthesia 30 min after compounds or vehicle treatment. The drinking water was immediately restored. At 24 h after glycerol injection, the rats were anesthetized with ether and 5 mL of the blood was collected from the descending aorta. Serum creatinine and urea nitrogen were determined by a autoanalyzer (Olympus, AU510). All values are expressed as the means ± SEM (significant difference from vehicle-treated group (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$); unpaired Student's t-test (n = 8–10)) in Tables VII–IX.

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