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<sub>1A</sub> antagonist spiperone was administered intravenously at a dose of 1 mg/kg.

Acknowledgment. We thank Gurnam S. Gill and Phil G. Zaworski (Molecular Biology Research, Upjohn Laboratories) for providing membranes of 5-HT<sub>2</sub> receptor expressing clones for the binding studies.

# Adenosine A<sub>1</sub> Antagonists. 2.<sup>†</sup> Structure–Activity Relationships on Diuretic Activities and Protective Effects against Acute Renal Failure

Fumio Suzuki,\* Junichi Shimada, Hideaki Mizumoto, Akira Karasawa, Kazuhiro Kubo, Hiromi Nonaka, Akio Ishii, and Takashi Kawakita

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken 411, Japan. Received January 3, 1992

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  $\mu$ g/kg, po, the ratio of urinary excretion value in treated rats to urinary excretion value in control rats = 1.69, the ratio of Na<sup>+</sup>/K<sup>+</sup> in treated rats to Na<sup>+</sup>/K<sup>+</sup> in control rats = 1.76) and potent ameliorative effects against glycerol-induced acute renal failure (24; 10  $\mu$ g/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 sizes 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.<sup>1</sup> Recently, the pharmacological basis for these diuretic actions has been proposed to be adenosine receptor antagonism.<sup>2</sup> In fact, exogenous adenosine produces intense antidiuretic and antinatriuretic effects in many species,<sup>3</sup> and these effects are competitively antagonized by theophylline<sup>4</sup> and mimicked by several adenosine analogues.<sup>5</sup>

Adenosine receptors are subdivided into subtypes, designated as  $A_1$  and  $A_2$ .<sup>6.7</sup>  $A_1$  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 ( $\mu$ M) and some are coupled to, but stimulate, adenylate cyclase.<sup>8</sup> 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

- Fredholm, B. B. Cardiovascular and Renal Actions of Methyl-xanthines. Prog. Clin. Biol. Res. 1984, 158, 303-330.
- (2) Persson, C. G. A.; Erjefält, I.; Edholm, L. E.; Karlsson, J. A.; Lamm, C. J. Tracheal Relaxant and Cardiostimulant Actions of Xanthines can be Differentiated from Diuretic and CNS-Stimulant Effects. Role of Adenosine Antagonism? *Life Sci.* 1982, 31, 2673-2681.
- (3) (a) Osswald, H.; Schmitz, H.-J.; Heindenreich, O. Adenosine Response of the Rat Kidney after Saline Loading, Sodium Restriction and Hemorrhagia. *Pfluegers Arch.* 1975, 357, 323-333. (b) Osswald, H.; Schmitz, H.-J.; Kemper, R. Renal Action of Adenosine: Effect on Renin Secretion in the Rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1978, 303, 95-99. (c) Osswald, H.; Spielman, W. S.; Knox, F. G. Mechanism of Adenosine-Mediated Decreases in Glomerular Filtration Rate in Dogs. *Cir. Res.* 1978, 43, 465-469.
- (4) Osswald, H. Renal Effects of Adenosine and Their Inhibition by Theophylline in Dogs. Naunyn-Schmiedeberg's Arch. Pharmacol. 1975, 288, 79–86.
- (5) Churchill, P. C.; Bidani, A. Renal Effects of Selective Adenosine Receptor Agonists in Anesthetized Rats. Am. J. Physiol. 1987, 252, F299-F303.

<sup>&</sup>lt;sup>†</sup>Part 1 in the series of Adenosine  $A_1$  Antagonist is ref 33c.

#### Adenosine A1 Antagonists

produces a primary vasoconstriction of the afferent arteriole and a decrease in glomerular filtration rate, and could be antidiuretic.<sup>9</sup> Conversely, in the same preparation, stimulation of adenosine A<sub>2</sub> 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.<sup>10</sup> 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.<sup>11</sup> This hypothesis is supported by the following observation. First, in the initial stage of glycerol-induced acute renal failure, renal blood flow is reduced<sup>12</sup> and the resultant ischemia is likely to evoke production and release of adenosine.<sup>13</sup> Second, acute renal failure is associated with a fall in glomerular filtration rate, which is also produced by an infusion of adenosine.<sup>14</sup> Theophylline (1)<sup>15</sup> or 8-phenyltheophylline (2)<sup>16</sup> can reduce

- (6) Londos, C.; Wolff, J. Two Distinct Adenosine-Sensitive Sites on Adenylate Cyclase. Proc. Natl. Acad. Sci. U.S.A. 1977, 74, 5482-5486.
- (7) Van Calker, D.; Müller, M.; Hamprecht, B. Adenosine Regulates via Two Different Types of Receptors, the Accumulation of Cyclic AMP in Cultured Brain Cells. J. Neurochem. 1979, 33, 999-1005.
- (8) Linden, J. Structure and Function of A<sub>1</sub> Adenosine Receptors. FASEB J. 1991, 5, 2668-2676.
- (9) (a) Murray, R. D.; Churchill, P. C. The Effects of Adenosine Receptor Agonists in the Isolated, Perfused Rat Kidney. Am. J. Physiol. 1984, 247, H343-H348. (b) Rossi, N. F.; Churchill, P. C.; Jacobson, K. A.; Leahy, A. E. Further Characterization of the Renovascular Effects of N<sup>6</sup>-Cyclohexyladenosine in the Isolated Perfused Rat Kidney. J. Pharmacol. Exp. Ther. 1987, 240, 911-915.
- (10) (a) Spielman, W. S. Antagonistic Effects of Theophylline on the Adenosine-Induced Decreases in Renin Release. Am. J. Physiol. 1984, 247, F246-F251. (b) Arend, L. J.; Thompson, C. I.; Spielman, W. S. Dipyridamole Decreases Glomerular Filtration in the Sodium-Depleted Dog. Evidence for Mediation by Intrarenal Adenosine. Cir. Res. 1985, 56, 242-251.
- (11) Churchill, P. C.; Bidani, A. K. Hypothesis: Adenosine Mediates Hemodynamic Changes in Renal Failure. Med. Hypotheses 1982, 8, 275-285.
- (12) Stein, J. H.; Lifeschitz, M. D.; Barnes, L. D. Current Concepts on the Pathophysiology of Acute Renal Failure. Am. J. Physiol. 1978, 234, F171-F181.
- (13) Miller, W. L.; Thomas, R. A.; Berne, R. M.; Rubio, R. Adenosine Production in the Ischemic Kidney. Cir. Res. 1978, 43, 390-397.
- (14) Churchill, P. C. Renal Effects of 2-Chloroadenosine and Their Antagonism by Aminophylline in Anesthetized Rats. J. Pharm. Exp. Ther. 1982, 222, 319–323.
- (15) Bidani, A. K.; Churchill, P. C.; Packer, W. Theophylline-Induced Protection in Myoglobinuric Acute Renal Failure: Further Characterization. Can. J. Physiol. Pharmacol. 1987, 65, 42-45.
- (16) (a) Bowmer, C. J.; Collis, M. G.; Yates, M. S. Effects of the Adenosine Antagonist 8-Phenyltheophylline on Glycerol-Induced Acute Renal Failure in the Rat. Br. J. Pharmacol. 1986, 88, 205-212. (b) Collis, M. G.; Baxter, G. S.; Keddie, J. R. The Adenosine Receptor Antagonist, 8-Phenyltheophylline, Causes Diuresis and Saliuresis in the Rat. J. Pharm. Pharmacol. 1986, 38, 850-852.

Journal of Medicinal Chemistry, 1992, Vol. 35, No. 16 3067



Nonxanthines



Figure 1. Chemical structure of reference compounds.

the severity of glycerol-induced acute renal failure, whereas the adenosine uptake inhibitor dipyridamole<sup>17</sup> potentiates the severity of acute renal failure. Glycerol-induced acute renal failure produces a myohemogloblinuric state in rats which is similar to clinical rhabdomyolysis.<sup>18</sup> 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.<sup>19-33</sup> One of the most potent classes of  $A_1$  an-

- (17) (a) Lin, J.-J.; Churchill, P. C.; Bidani, A. K. The Effect of Dipyridamole on the Initiation Phase of Post-Ischemic Acute Renal Failure in Rats. Can. J. Physiol. Pharmacol. 1987, 65, 1491-1495. (b) Spielman, W. S.; Arend, L. J. Adenosine Receptors and Signaling in the Kidney. Hypertension 1991, 17, 117-130.
- (18) Thiel, G.; Wilson, D. R.; Arce, M. L.; Oken, D. E. Glycerol Induced Hemoglobinuric Acute Renal Failure in the Rat. Nephron 1967, 4, 276-297.
- (19) Williams, M. Adenosine Antagonists. Med. Res. Rev. 1989, 9, 219-243.
- (20) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine Receptors: Pharmacology, Structure-Activity Relationships, and Therapeutic Potential. J. Med. Chem. 1992, 35, 407-422.
- (21) 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.
- (22) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A<sub>2</sub> Adenosine Receptor Labeled by [<sup>3</sup>H]NECA in Rat Striatal Membranes. *Mol. Pharmacol.* 1986, 29, 331-346.
- (23) Schwabe, U.; Ukena, D.; Lohse, M. J. Xanthine Derivatives as Antagonists at A<sub>1</sub> and A<sub>2</sub> Adenosine Receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 1985, 330, 212-221.

tagonists identified was 1,3-dialkyl-8-polycycloalkylxanthine derivatives.<sup>33</sup> Besides xanthines, a wide range

- (24) Martinson, E. A.; Johnson, R. A.; Wells, J. N. Potent Adenosine Receptor Antagonists that are Selective for the A<sub>1</sub> Receptor Subtype. *Mol. Pharmacol.* 1987, 31, 247-252.
- (25) (a) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Haleen, S. J.; Steffen, R. P. Synthesis of Xanthines as Adenosine Antagonists, a Practical Quantitative Structure-Activity Relationship Application. J. Med. Chem. 1985, 28, 1071-1079. (b) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. 1,3-Dialkyl-8-(p-sulfophenyl)xanthines: Potent Water-Soluble Antagonists for A<sub>1</sub>- and A<sub>2</sub>-Adenosine Receptors. J. Med. Chem. 1985, 28, 487-492. (c) Daly, J. W.; Padgett, W. L.; Shamim, M. T. Analogues of 1,3-Dipropyl-8-phenylxanthines: Enhancement of Selectivity at A<sub>1</sub>-Adenosine Receptors by Aryl Substituents. J. Med. Chem. 1986, 29, 1520-1524.
- (26) (a) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. Functionalized Congeners of 1,3-Dialkylxanthines: Preparation of Analogues with High Affinity for Adenosine Receptors. J. Med. Chem. 1985, 28, 1334-1340. (b) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. A Functionalized Congener Approach to Adenosine Receptor Antagonists: Amino Acid Conjugates of 1,3-Dipropylxanthine. Mol. Pharmacol. 1986, 29, 126-133.
- (27) (a) 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<sub>1</sub>-Selective Adenosine Antagonist 8-Cyclopentyl-1,3-dipropylxanthine to Rat Brain Membranes. Naunyn-Schmiedeberg's Arch. Pharmacol. 1987, 335, 59-63. (b) Lohse, M. J.; Klotz, K.-N.; Lindenborn-Fotinos, J.; Reddington, M.; Schwabe, U.; Olsson, R. A. 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX)-A Selective High Affinity Antagonist Radioligand for A1 Adenosine Receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 1987, 336, 204-210. (c) Collis, M. G.; Shaw, G.; Keddie, J. R. Diuretic and Saliuretic Effects of 1,3-Dipropyl-8-cyclopentylxanthine, a Selective A1-Adenosine Receptor Antagonist. J. Pharm. Pharmacol. 1991, 43, 138-139. (d) Kellet, R.; Bowmer, C. J.; Collis, M. G.; Yates, M. S. Amelioration of Glycerol-Induced Acute Renal Failure in the Rat with 8-Cyclopentyl-1,3-dipropylxanthines. Br. J. Pharmacol. 1989, 98, 1066-1074.
- (28) (a) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W. 8-Aryl- and 8-Cycloalkyl-1,3-dipropylxanthines: Further Potent and Selective Antagonists for A1-Adenosine Receptors. J. Med. Chem. 1988, 31, 613-617. (b) Katsushima, T.; Nieves, L.; Wells, J. N. Structure-Activity Relationships of 8-Cycloalkyl-1,3-dipropylxanthines as Antagonists of Adenosine Receptors. J. Med. Chem. 1990, 33, 1906-1910. (c) Jacobson, K. A.; Kiriasis, L.; Barone, S.; Bradbury, B. J.; Kammula, U.; Campagne, J. M.; Secunda, S.; Daly, J. W.; Neumeyer, J. L.; Pfleiderer, W. Sulfur-Containing 1,3-Dialkylxanthine Derivatives as Selective Antagonists at A1-Adenosine Receptors. J. Med. Chem. 1989, 32, 1873-1879. (d) Erickson, R. H.; Hiner, R. N.; Feeney, S. W.; Blake, P. R.; Rzeszotarski, W. J.; Hicks, R. P.; Costello, D. G.; Abreu, M. E. 1,3,8-Trisubstituted Xanthines. Effects of Substitution Pattern upon Adenosine Receptor A<sub>1</sub>/A<sub>2</sub> Affinity. J. Med. Chem. 1991, 34, 1431-1435. (e) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. Effects of 8-Phenyl and 8-Cycloalkyl Substituents on the Activity of Mono-, Di-, and Trisubstituted Alkylxanthines with Substitution at the 1-, 3-, and 7-Positions. J. Med. Chem. 1989, 32, 1231-1237.
- (29) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hays, S. J. PD 115, 199: An Antagonist Ligand for Adenosine A<sub>2</sub> Receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 1987, 335, 64-69.
- (30) Sarges, R.; Howard, H. R.; Browne, R. G.; Lebel, L. A.; Seymour, P. A.; Kenneth Koe, B. 4-Amino[1,2,4]triazolo[4,3-a]-quinoxalines. A Novel Class of Potent Adenosine Receptor Antagonists and Potential Rapid-Onset Antidepressants. J. Med. Chem. 1990, 33, 2240-2254.
- (31) Trivedi, B. K.; Bruns, R. F. [1,2,4]Triazolo[4,3-a]quinoxalin-4-amines: A New Class of A<sub>1</sub> Receptor Selective Adenosine Antagonists. J. Med. Chem. 1988, 31, 1011-1014.

**Table I.**  $A_1$  and  $A_2$  Adenosine Receptor Binding of Reference Compounds<sup>6</sup>

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

<sup>a</sup> A<sub>1</sub> binding was carried out with N<sup>6</sup>-[<sup>3</sup>H]cyclohexyladenosine in guinea pig forebrain membranes as described, <sup>34,33c</sup> and A<sub>2</sub> binding was carried out with N-[<sup>3</sup>H]ethyladenosin-5'-uronamide in the presence of 50 nM cyclopentyladenosine in rat striatal membranes.<sup>22,33c</sup> <sup>b</sup> A<sub>1</sub> binding measured as inhibition of N<sup>6</sup>-[<sup>3</sup>H]cyclohexyladenosine to rat forebrain membranes in our laboratory.<sup>33c</sup> <sup>c</sup> A<sub>1</sub> binding measured as inhibition of N<sup>6</sup>-[<sup>3</sup>H]cyclohexyladenosine to rat striatal membranes.<sup>22,23c,29,31</sup> <sup>e</sup> A<sub>1</sub> binding measured as inhibition of N<sup>6</sup>-[<sup>3</sup>H]cyclohexyladenosine to rat whole brain membranes.<sup>22,27a,31</sup> <sup>d</sup> A<sub>2</sub> binding measured as inhibition of N-[<sup>3</sup>H]ethyladenosin-5'-uronamide to rat striatal membranes.<sup>22,28c,29,31</sup> <sup>e</sup> A<sub>1</sub> binding measured as inhibition of (R)-N<sup>6</sup>-(2-[<sup>3</sup>H]phenyl-1-methylethyl)adenosine to rat cortical membranes.<sup>22</sup> <sup>f</sup> A<sub>1</sub> binding measured as inhibition of N<sup>6</sup>-[<sup>3</sup>H]cyclohexyladenosine to rat cortical membranes.<sup>29</sup> <sup>f</sup> C<sub>50</sub> values for inhibition of N<sup>6</sup>-[<sup>3</sup>H]cyclohexyladenosine binding in rat whole brain membranes.<sup>32a</sup> <sup>h</sup> IC<sub>50</sub> values for inhibition of N-[<sup>3</sup>H]ethyladenosine binding in rat cortical membranes.<sup>30</sup>

of other heterocycles have been found to be antagonists at adenosine receptors. The representatives of xanthine

- (32) (a) Williams, M.; Francis, J.; Ghai, G.; Braunwalder, A.; Psychoyos, S.; Stone, G. A.; Cash, W. D. Biochemical Characterization of the Triazoloquinazoline, CGS 15943, a Novel, Non-Xanthine Adenosine Antagonist. J. Pharm. Exp. Ther. 1987, 241, 415-420. (b) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; Stone, G. A.; Desai, M.; Williams, M. Structure-Activity Profile of a Series of Novel Triazolo-quinazoline Adenosine Antagonists. J. Med. Chem. 1988, 31, 1014-1020.
- (33) (a) Suzuki, F.; Shimada, J.; Kubo, K.; Ohno, T.; Karasawa, A.; Ishii, A.; Nonaka, H. Xanthine Derivatives. Eur. Patent 386 675, 1990 (Priority Data: March 6, 1989, Japan Patent Application 53 376). (b) Shimada, J.; Suzuki, F.; Nonaka, H.; Karasawa, A.; Mizumoto, H.; Ohno, T.; Kubo, K.; Ishii, A. 8-(Dicyclopropylmethyl)-1,3-dipropylxanthine: A Potent and Selective Adenosine A<sub>1</sub> Antagonist with Renal Protective and Diuretic Activities. J. Med. Chem. 1991, 34, 466-469. (c) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A. 8-Polycycloalkyl-1,3-dipropylxanthines as Potent and Selective Antangonists for A<sub>1</sub>-Adenosine Receptors. J. Med. Chem. 1992, 35, 924-930.
- (34) Bruns, R. F.; Daly, J. W.; Snyder, S. H. Adenosine Receptors in Brain Membranes: Binding of N<sup>6</sup>-Cyclohexyl[<sup>8</sup>H]adenosine and 1,3-Diethyl-8-[<sup>3</sup>H]phenylxanthine. Proc. Natl. Acad. Sci. U.S.A. 1980, 77, 5547-5551.

Table II. Oral Diuretic Activities of Reference Compounds<sup>a</sup>

	dose	UV <sup>b</sup> (mL/1)	00 g per 6 h)		Na <sup>+</sup> (mequiv/	'100 g per 6 h)		
no.	(mg/kg, po)	control	treated	T/C <sup>c</sup>	control	treated	$T/C^{c}$	Na <sup>+</sup> /K <sup>+ d</sup>
1	25	$0.91 \pm 0.08$	$1.22 \pm 0.03$	1.34	$0.193 \pm 0.016$	$0.417 \pm 0.007$	2.16	1.08
	6.25	$0.83 \pm 0.08$	$1.18 \pm 0.08$	1.42	$0.132 \pm 0.010$	$0.201 \pm 0.010$	1.52	1.31
	1.6	$0.69 \pm 0.03$	$0.71 \pm 0.07$	1.03	0.079 🏚 0.007	$0.090 \pm 0.009$	1.14	1.06
2	25	$1.21 \pm 0.08$	$1.84 \pm 0.12$	1.68	$0.180 \pm 0.014$	$0.280 \pm 0.014$	1.55	1.28
	6.25	$0.87 \pm 0.04$	$0.80 \pm 0.03$	0.92	$0.148 \pm 0.011$	$0.162 \pm 0.008$	1.10	1.10
3	25	$0.80 \pm 0.04$	$1.10 \pm 0.06$	1.38	$0.134 \pm 0.004$	$0.201 \pm 0.009$	1.50	1.39
	6.25	$0.80 \pm 0.02$	$1.43 \pm 0.09$	1.7 <del>9</del>	$0.146 \pm 0.005$	0.250 单 0.026	1.71	1.54
	1.6	$1.40 \pm 0.05$	$0.88 \pm 0.09$	0.63	$0.261 \pm 0.008$	$0.175 \pm 0.021$	0.67	0.87
4	25	$0.85 \pm 0.06$	$2.07 \pm 0.18$	2.44	$0.162 \pm 0.018$	$0.350 \pm 0.024$	2.16	1.71
	6.25	$1.00 \pm 0.12$	$2.39 \pm 0.03$	2.39	$0.138 \pm 0.011$	$0.372 \pm 0.010$	2.69	2.11
	1.6	$0.83 \pm 0.07$	$1.83 \pm 0.22$	2.20	$0.122 \pm 0.008$	$0.284 \pm 0.021$	2.33	1.56
	0.4	$0.94 \pm 0.03$	$1.25 \pm 0.06$	1.33	$0.175 \pm 0.014$	$0.255 \pm 0.016$	1.45	1.27
5	25	$1.00 \pm 0.13$	$1.93 \pm 0.18$	1.93	$0.129 \pm 0.026$	$0.316 \pm 0.029$	2.46	1.88
	6.25	$1.26 \pm 0.21$	$2.35 \pm 0.15$	1.87	$0.191 \pm 0.029$	$0.378 \pm 0.012$	1.98	1.57
	1.6	$0.91 \pm 0.08$	$2.54 \pm 0.08$	2.79	$0.193 \pm 0.016$	$0.449 \pm 0.023$	2.33	1.76
	0.4	$0.83 \pm 0.08$	$2.05 \pm 0.10$	2.47	$0.132 \pm 0.010$	$0.302 \pm 0.006$	2.28	1.54
	0.1	$1.10 \pm 0.09$	$1.36 \pm 0.14$	1.24	$0.169 \pm 0.018$	$0.215 \pm 0.023$	1.27	1.03
6	25	$1.04 \pm 0.11$	$1.05 \pm 0.04$	1.05	$0.095 \pm 0.009$	$0.113 \pm 0.026$	1.19	1.24
7	12.5	0.94 ± 0.03	$1.54 \pm 0.13$	1.64	$0.175 \pm 0.014$	$0.210 \pm 0.011$	1.78	1.56
	6.25	$0.91 \pm 0.08$	$1.06 \pm 0.13$	1.17	$0.193 \pm 0.016$	$0.205 \pm 0.021$	1.06	1.00
8	25	$0.90 \pm 0.09$	$0.92 \pm 0.07$	1.02	$0.113 \pm 0.009$	$0.164 \pm 0.011$	1.46	1.07
9	25	$0.91 \pm 0.04$	$1.74 \pm 0.19$	1.91	$0.186 \pm 0.020$	$0.323 \pm 0.025$	1.74	1.31
	6.25	$0.75 \pm 0.12$	$1.63 \pm 0.03$	2.17	$0.179 \pm 0.038$	$0.326 \pm 0.023$	1.82	1.46
	1.6	$0.93 \pm 0.03$	$1.07 \pm 0.13$	1.15	$0.173 \pm 0.015$	$0.212 \pm 0.014$	1.22	0.98
10	25	$0.87 \pm 0.04$	1.86 ± 0.15	2.13	$0.134 \pm 0.017$	$0.314 \pm 0.010$	2.33	1.71
	6.25	$0.99 \pm 0.09$	$1.53 \pm 0.08$	1.55	$0.349 \pm 0.040$	$0.268 \pm 0.003$	0.77	1.35
11	25	$1.02 \pm 0.02$	$0.87 \pm 0.05$	0.85	$0.209 \pm 0.007$	$0.186 \pm 0.007$	0.89	1.05
12	25	$1.21 \pm 0.08$	$2.12 \pm 0.58$	1.75	$0.180 \pm 0.015$	0.296 ± 0.069	1.64	1.05
	6.25	$0.69 \pm 0.03$	$1.10 \pm 0.05$	1.59	$0.079 \pm 0.007$	$0.128 \pm 0.009$	1.62	1.22
	1.6	$0.85 \pm 0.10$	$0.85 \pm 0.07$	1.00	$0.161 \pm 0.013$	$0.152 \pm 0.001$	0.94	0.87

<sup>a</sup>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  $\pm$  SEM of control and drug treatment values, respectively. <sup>b</sup>Urine volume. <sup>c</sup>T/C means the ratios of urinary excretion value in treated rats to urinary excretion value in control rats. <sup>d</sup>Values are ratios of Na<sup>+</sup>/K<sup>+</sup> in treated rats to Na<sup>+</sup>/K<sup>+</sup> 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.<sup>33</sup>

### **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 the ophylline, showed a nearly equipotent diuretic activity to theophylline. The potent and selective adenosine A1 antagonist 8-(dicyclopropylmethyl)-1,3-dipropylxanthine (4)<sup>33</sup> 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)<sup>26</sup> 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  $(9^{30} \text{ or } 10^{31})$ , with A<sub>1</sub> selectivity showed comparable diuretic and natriuretic activities to 8-phenyltheophylline. The compounds with A2 selectivity, (9-chloro-2-(2-furyl)-[1,2,4]triazolo[1,5-c]quinazolin-4-amine  $(8)^{32}$  and 8chloro-1-phenyl[1,2,4]triazolo[4,3-a]quinoxalin-4-amine (11),<sup>30</sup> 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.<sup>3c,4,5,9b</sup> 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, 8cycloalkyl-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<sup>33</sup> (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-Substituted1,3-Dipropylxanthines<sup>a</sup>



	·····	K <sub>i</sub> ,ª	nM	K. ratio
no.	R	A1	A <sub>2</sub>	$A_2/A_1$
13	1-methylcyclohexyl	11	1200	110
14	2,2,5,5-tetramethyl- cyclopentyl	22	>100000	>4500
15	(1R*,2R*,5R*)-bicyclo- [3.3.0]octan-2-yl	$3.5 \pm 0.20$	330 ± 4.7	94
16	(1R*,2S*,5R*)-bicyclo- [3.3.0]octan-2-yl	5.6 ± 0.19	$560 \pm 26$	100
17	2-endo-norbornen-5-yl	$4.3 \pm 0.62$	$480 \pm 18$	110
18	2-exo-norbornen-5-yl	$3.4 \pm 0.41$	$210 \pm 12$	62
19	2-endo-norbornyl	3.8 ± 0.32	$440 \pm 42$	120
20	2-exo-norbornyl	$4.4 \pm 0.13$	290 ± 54	66
21	(2- <i>exo</i> -norbornyl)- methyl	$80 \pm 9.5$	1000	13
22	1-adamantyl	$13 \pm 2.8$	$5100 \pm 1100$	390
23	(1-adamantyl)methyl	880	>100000	>110
24	3-noradamantyl	$1.3 \pm 0.12$ (0.19 ± 0.04) <sup>b</sup>	$380 \pm 30$	290

<sup>a</sup>See footnote a in Table I. <sup>b</sup>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  $\mu M$  nor receptor binding by a variety of ligands including histamine, muscarine, and catecholamine at 10  $\mu M$ .

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) $\geq$  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  $(31) \ge 1,3$ -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<sub>1</sub> 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<sup>a</sup>

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

<sup>a</sup>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<sub>1</sub> and A<sub>2</sub> Adenosine Receptors<sup>a</sup>



<sup>a</sup>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.<sup>35</sup> 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]-Nmethylamino]sulfonyl]phenyl]-1,3-dipropylxanthine (7) showed only moderate protection at the dose of 10 mg/kg. Thus selective adenosine A1 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<sub>2</sub>-selective ligand 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-4-amine (8) or 8chloro-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  $(\mathbb{R}^1 = \mathbb{M}e, \mathbb{R}^3 = \text{isobutyl}, 27, 32, 38)$ . 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 1methyl-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

<sup>(35)</sup> 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<sup>a</sup>

	dose	$UV^a (mL/10)$	00 g per 6 h)		$Na^+$ (mequiv/100 g per 6 h)			
no.	(mg/kg, po)	control	treated	$T/C^{\alpha}$	control	treated	$T/C^{\alpha}$	Na <sup>+</sup> /K <sup>+</sup> <sup>a</sup>
25	1.6	$0.97 \pm 0.05$	$2.90 \pm 0.02$	2.99	$0.169 \pm 0.012$	$0.492 \pm 0.019$	2.92	1.97
	0.4	$1.72 \pm 0.31$	2.29 ± 0.16	1.33	$0.267 \pm 0.044$	$0.356 \pm 0.008$	1.33	1.29
26	1.6	$1.12 \pm 0.05$	$2.52 \pm 0.18$	2.25	$0.189 \pm 0.005$	$0.426 \pm 0.035$	2.26	1.61
	0.4	$0.97 \pm 0.05$	$2.50 \pm 0.14$	2.58	$0.169 \pm 0.012$	$0.412 \pm 0.013$	2.45	1.89
	0.1	$0.96 \pm 0.09$	$1.76 \pm 0.05$	1.83	$0.147 \pm 0.015$	$0.279 \pm 0.005$	1.89	1.58
	0.01	$1.05 \pm 0.11$	$0.78 \pm 0.09$	0.74	$0.180 \pm 0.017$	$0.176 \pm 0.023$	0.98	1.05
27	1.6	$0.97 \pm 0.05$	$2.43 \pm 0.15$	2.51	$0.169 \pm 0.012$	$0.409 \pm 0.014$	2.43	1.73
	0.4	$1.72 \pm 0.31$	$2.41 \pm 0.04$	1.40	$0.267 \pm 0.044$	$0.367 \pm 0.016$	1.38	1.37
28	1.6	$0.97 \pm 0.05$	$2.27 \pm 0.11$	2.34	$0.169 \pm 0.012$	$0.398 \pm 0.006$	2.36	1.67
	0.4	$0.96 \pm 0.09$	$1.59 \pm 0.05$	1.66	$0.147 \pm 0.015$	$0.257 \pm 0.003$	1.74	1.54
	0.1	$1.05 \pm 0.11$	$0.86 \pm 0.15$	0.82	$0.180 \pm 0.017$	$0.163 \pm 0.026$	0.91	0.97
29	25	$1.64 \pm 0.70$	$2.34 \pm 0.29$	1.43	$0.265 \pm 0.099$	$0.400 \pm 0.040$	1.51	1.29
	6.25	$1.13 \pm 0.11$	$1.42 \pm 0.23$	1.26	$0.161 \pm 0.027$	$0.240 \pm 0.031$	1.49	1.43
30	1.6	$0.86 \pm 0.09$	$2.09 \pm 0.06$	2.43	$0.174 \pm 0.013$	$0.363 \pm 0.012$	2.09	1.56
	0.4	$1.15 \pm 0.17$	$1.89 \pm 0.09$	1.64	$0.191 \pm 0.020$	$0.342 \pm 0.012$	1.79	1.44
• •	0.1	$1.05 \pm 0.11$	$1.09 \pm 0.04$	1.03	$0.180 \pm 0.017$	$0.206 \pm 0.015$	1.15	1.00
31	1.6	$0.86 \pm 0.09$	$1.60 \pm 0.23$	1.86	$0.174 \pm 0.013$	$0.299 \pm 0.021$	1.72	1.32
	0.4	$1.15 \pm 0.17$	$1.45 \pm 0.04$	1.26	$0.180 \pm 0.017$	$0.309 \pm 0.009$	1.61	1.23
32	1.6	$0.93 \pm 0.03$	$1.97 \pm 0.19$	2.12	$0.173 \pm 0.015$	$0.379 \pm 0.014$	2.19	1.57
	0.1	$0.75 \pm 0.12$	$1.59 \pm 0.17$	2.12	$0.179 \pm 0.038$	$0.308 \pm 0.040$	1.72	1.55
	0.01	$1.15 \pm 0.17$	$1.31 \pm 0.07$	1.14	$0.191 \pm 0.020$	$0.257 \pm 0.009$	1.35	1.22
33	6.25	$1.15 \pm 0.17$	$1.88 \pm 0.21$	1.64	$0.191 \pm 0.020$	$0.349 \pm 0.017$	1.82	1.46
	1.6	$0.86 \pm 0.09$	$1.06 \pm 0.14$	1.23	$0.174 \pm 0.013$	$0.217 \pm 0.040$	1.25	1.12
34	6.25	$0.92 \pm 0.06$	$1.62 \pm 0.13$	1.76	$0.128 \pm 0.007$	$0.264 \pm 0.014$	2.07	1.82
35	25	$0.74 \pm 0.07$	$1.07 \pm 0.17$	1.45	$0.172 \pm 0.015$	$0.184 \pm 0.026$	1.07	1.00
36	1.6	$0.96 \pm 0.09$	$1.72 \pm 0.14$	1.79	$0.147 \pm 0.015$	$0.312 \pm 0.024$	2.12	1.71
	0.4	$1.15 \pm 0.17$	$2.11 \pm 0.11$	1.84	$0.191 \pm 0.020$	$0.433 \pm 0.003$	2.27	1.63
07	0.1	$1.15 \pm 0.12$	$0.83 \pm 0.07$	0.72	$0.133 \pm 0.022$	$0.113 \pm 0.011$	0.85	1.37
37	1.6	$0.96 \pm 0.09$	$1.59 \pm 0.07$	1.66	$0.147 \pm 0.015$	$0.282 \pm 0.009$	1.91	1.66
	0.4	$1.15 \pm 0.17$	$1.24 \pm 0.18$	1.08	$0.191 \pm 0.020$	$0.276 \pm 0.036$	1.44	1.45
38	1.0	$0.93 \pm 0.03$	$1.93 \pm 0.08$	2.08	$0.173 \pm 0.015$	$0.345 \pm 0.014$	1.99	1.37
	0.1	$0.75 \pm 0.12$	$1.26 \pm 0.25$	1.68	$0.179 \pm 0.038$	$0.250 \pm 0.022$	1.40	2.13
90	0.01	$1.10 \pm 0.17$	$1.10 \pm 0.14$	1.00	$0.191 \pm 0.020$	$0.212 \pm 0.012$	1.11	1.09
39	1.0	$0.90 \pm 0.09$	$0.67 \pm 0.10$	0.91	$0.147 \pm 0.015$	$0.183 \pm 0.020$	1.24	1.18
40	20	$0.74 \pm 0.00$	$1.60 \pm 0.11$	2.50	$0.152 \pm 0.013$	$0.328 \pm 0.027$	2.16	1.40
	0.20	$0.90 \pm 0.12$	$1.30 \pm 0.09$	1.42	$0.146 \pm 0.006$	$0.246 \pm 0.012$	1.69	1.51
41	1.0	$1.01 \pm 0.08$	$1.13 \pm 0.11$	1.12	$0.160 \pm 0.008$	$0.202 \pm 0.033$	1.22	1.13
41	0.20	$0.92 \pm 0.00$	$1.04 \pm 0.13$ 1 49 $\pm 0.19$	1.70	$0.143 \pm 0.013$ 0.199 ± 0.019	$0.306 \pm 0.013$	2.14	1.78
	1.0	$0.62 \pm 0.05$	$1.40 \pm 0.13$	1.80	$0.183 \pm 0.012$ 0.101 $\pm 0.001$	$0.271 \pm 0.018$	1.48	1.21
49	0.4	$0.99 \pm 0.07$	0.90 = 0.19	0.97	$0.191 \pm 0.021$	$0.174 \pm 0.014$	0.91	0.94
42	20	$0.09 \pm 0.00$	$2.10 \pm 0.00$	2.42	0.102 = 0.006 0.172 $\pm$ 0.007	$0.348 \pm 0.012$	2.14	1.51
49	0.20	1.14 = 0.02 0.76 $\pm$ 0.01	1 19 1 0.00	1.19	$0.173 \pm 0.005$	$0.214 \pm 0.016$	1.24	1.12
40	20	$0.70 \pm 0.01$	$1.12 \pm 0.00$ $1.09 \pm 0.00$	1.4/	$0.100 \pm 0.006$	$0.185 \pm 0.007$	1.18	0.92
44	20	$0.59 \pm 0.06$	$1.02 \pm 0.06$	1.19	$0.162 \pm 0.006$	$0.185 \pm 0.006$	1.14	0.95

<sup>a</sup>See footnote a-d in Table II.

Table VII. Renal Protective Activities of Reference Compounds<sup>a</sup>

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

<sup>a</sup> Bowmer's method<sup>35</sup> 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  $\pm$  SEM; significant difference from vehicle-treated group (\*, p < 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001). <sup>b</sup>Percent inhibition denotes ratios of Cr or UN values in treated rats to Cr or UN values in vehicle-treated rats. <sup>c</sup>Not determined.

mg/kg. In addition, caffeine (2) showed more potent protective activity than theophylline (1). These unex-

pected results might be explained by some other mechanism of action.<sup>36</sup> Introduction of the 7-substituent to the

Table VIII. Renal Protective Activities of 8-Substituted 1,3-Dipropylxanthines<sup>a</sup>

	dose	Cr (	mg/dL)		UN	(mg/dL)	
no.	(mg/kg, ip)	vehicle	treated	% inhibnª	vehicle	treated	% inhibnª
13	1	4.74 ± 0.31	$2.04 \pm 0.23^{***}$	57	$138.8 \pm 7.8$	55.2 ± 8.1***	60
14	10	$2.13 \pm 0.27$	$1.43 \pm 0.10*$	33	NDª		
	1	$3.06 \pm 0.68$	$3.03 \pm 0.82$	1	$120.9 \pm 23.3$	113.4 单 26.1	6
15	1	$5.43 \pm 0.11$	$3.32 \pm 0.28 * * *$	31	$182.5 \pm 3.4$	$115.4 \pm 6.2^{***}$	37
16	10	$4.64 \pm 0.24$	$3.79 \pm 0.42$	18	$147.0 \pm 5.8$	$119.1 \pm 9.7*$	19
17	1	$4.41 \pm 0.17$	$2.08 \pm 0.23^{***}$	53	$147.9 \pm 6.1$	$80.4 \pm 10.1^{***}$	56
18	1	5.07 ± 0.33	$2.23 \pm 0.45^{***}$	56	195.8 ± 14.4	$92.7 \pm 18.5^{***}$	53
19	1	$5.07 \pm 0.33$	$2.59 \pm 0.54^{***}$	49	195.8 ± 14.4	$101.0 \pm 19.3^{***}$	48
20	1	$5.07 \pm 0.33$	$2.54 \pm 0.54^{***}$	50	195.8 ± 14.4	$98.8 \pm 14.1^{***}$	49
21	10	$4.26 \pm 0.39$	$2.53 \pm 0.42^{**}$	41	146.7 ± 10.1	90.9 ± 12.0**	38
	1	$3.06 \pm 0.68$	$2.75 \pm 0.79$	10	$120.9 \pm 23.3$	$104.7 \pm 30.0$	13
22	10	$4.26 \pm 0.39$	$1.91 \pm 0.02^{***}$	55	146.7 ± 10.1	77.5 ± 9.6***	47
	1	$4.91 \pm 0.10$	3.45 ± 0.46*	30	<b>154.5 47.0</b>	115.9 ± 15.7*	25
23	10	$3.63 \pm 0.29$	$3.21 \pm 0.52$	12	137.9 ± 8.5	$117.8 \pm 14.9$	15
24	1	$2.75 \pm 0.43$	$2.00 \pm 0.14$ **	47	$123.2 \pm 14.2$	$66.4 \pm 6.3^{**}$	46

<sup>a</sup>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<sup>a</sup>

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

<sup>a</sup>See footnotes a and b in Table VII.

Table X. Analytical Data for 8-Substituted Xanthines

	synthetic	%	mp, °C	
no.	method	yield	(recryst solvent)	formula <sup>a</sup>
4	A	44	129-130 (EtOH/H <sub>2</sub> O)	C <sub>18</sub> H <sub>30</sub> N <sub>4</sub> O <sub>2</sub>
30	В	57	181-182 (cyclohexane)	$C_{14}H_{20}N_4O_2$
31	В	59	172-173 (dioxane/H <sub>2</sub> O)	$C_{18}H_{28}N_4O_2$
32	В	52	$202-204 (2-PrOH/H_2O)$	$C_{15}H_{22}N_4O_2$
33	В	51	244-246 <sup>b</sup> (2-PrOH)	$C_{12}H_{16}N_4O_2$
34	С	66	311-313 (DMF)	$C_{13}H_{18}N_4O_2$
35	С	94	$114-115^d$ (acetone/H <sub>2</sub> O)	$C_{17}H_{26}N_4O_2$
36	D	35	166-169 (dioxane/H <sub>2</sub> O)	$C_{16}H_{22}N_4O_2$
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)	C15H20N4O2
41	С	82	70 (heptane)	$C_{19}H_{28}N_4O_2$
42	С	71	80-83 (MeCN)	C <sub>20</sub> H <sub>30</sub> N <sub>4</sub> O <sub>2</sub>
				0.5MeCN <sup>/</sup>
43	С	82	93-100 (EtOH/H <sub>2</sub> O)	$C_{21}H_{32}N_4O_2$
44	с	99	141-149 (MeCN)	C <sub>20</sub> H <sub>27</sub> N₄O₄Na 0.8H <sub>2</sub> O

<sup>a</sup> All compounds were analyzed for C, H, N. <sup>b</sup> Lit.<sup>36a</sup> mp 247-249 °C. <sup>c</sup>See the Experimental Section. <sup>d</sup> Lit.<sup>28a</sup> mp 114 °C. <sup>c</sup>H: calcd, 6.99; found, 7.42. <sup>f</sup>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<sup>37</sup> 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 (<sup>1</sup>H 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  $\pm 0.4\%$  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),<sup>26</sup> 8-[4-[[N-[2-(dimethylamino)ethyl]-N-methylamino]sulfonyl]phenyl]-1,3-dipropylxanthine (7),<sup>29</sup> 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-4-amine (8),<sup>32</sup> 8-chloro-4-(cyclopentyl] amino)-1-(trifluoromethyl)[1,2,4]triazolo[4,3-a]quinoxaline (9),<sup>30</sup> 4-(cyclopentylamino)-1-(trifluoromethyl)[1,2,4]triazolo[4,3-a]quinoxaline (10),<sup>31</sup> and 8-chloro-1-phenyl[1,2,4]triazolo[4,3-a]quinoxalin-4-amine (11),<sup>30</sup> The synthesis of 8-polycycloalkylsubstituted xanthines (13-29) has been described elsewhere.<sup>33</sup> 1,3-Dialkyl-5,6-diaminouracils<sup>38</sup> were synthesized by published procedure.

Method A. 8-(Dicyclopropylmethyl)-1,3-dipropylxanthine (4). To a solution of dicyclopropylacetic acid<sup>39</sup> (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<sup>25b</sup> (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<sub>3</sub> solution was added, and the mixture was extracted with CHCl<sub>3</sub> three times. The combined organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent was removed under vacuo. Purification on silica gel column chromatography (eluent:1% MeOH/CHCl<sub>3</sub>) afforded 6-amino-5-[[(dicyclopropylmethyl)carbonyl]amino]-1,3-dipropyluracil (1.16 g, 66%): <sup>1</sup>H NMR (CDCl<sub>3</sub>, 90 MHz)  $\delta$ 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<sub>3</sub> was refluxed for 1.5 h. The excess

<sup>(36) (</sup>a) Svedmyr, N. Xanthines. In Asthma: Basic Mechanisms and Clinical Management. Barnes, P. J., Rodger, I. W., Thomson, N. C., Eds.; Academic Press: London, 1988; pp 607-625. (b) Goodsell, E. B.; Stein, H. H.; Wenzke, K. J. & Substituted Theophyllines. In Vitro Inhibition of 3',5'-Cyclic Adenosine Monophosphate Phosphodiesterase and Pharmacological Spectrum in Mice. J. Med. Chem. 1971, 14, 1202-1205. (c) Wells, J. N.; Nieves, L.; Katsushima, T. Structure Activity Relationships of Xanthines as Inhibitors of Adenosine Receptors. In Purines in Cellular Signaling: Targets for New Drugs; Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springler: New York, 1990; pp 365-369.
(37) (a) Shah, S. V.; Walker, P. D. Evidence Suggesting a Role for

 <sup>(37) (</sup>a) Shah, S. V.; Walker, P. D. Evidence Suggesting a Role for Hydroxy Radical in Glycerol-Induced Acute Renal Failure. Am. J. Physiol. 1988, 255, F438-F443. (b) Abul-Ezz, S. R.; Walker, P. D.; Shah, S. V. Role of Glutathione in Animal Model of Myoglobinuric Acute Renal Failure. Proc. Natl. Acad. Sci. U.S.A. 1991, 88, 9833-9837.

<sup>(38) (</sup>a) Papesch, V.; Schroeder, E. F. Synthesis of 1-Mono- and 1,3-Disubstituted 6-Aminouracils. Diuretic Activity. J. Org. Chem. 1951, 16, 1879-1890. (b) Speer, J. H.; Raymond, A. L. Some Alkyl Homologs of Theophylline. J. Am. Chem. Soc. 1953, 75, 114-115. (c) Blicke, F. F.; Godt, H. C. Reactions of 1,3-Dimethyl-5,6-diaminouracil. J. Am. Chem. Soc. 1954, 76, 2798-2800. (d) Kramer, G. L.; Garst, J. E.; Mitchel, S. S.; Wells, J. N. Selective Inhibition of Cyclic Nucleotide Phosphodiesterases by Analogues of 1-Methyl-3-isobutylxanthine. Biochemistry 1977, 16, 3316-3321. (e) Draco, A. B. Antiasthma and Cardiotonic Agents. Jpn. Patent 57 517, 1980; Chem. Abstr. 1981, 94, 47360y.

<sup>(39)</sup> Tanimoto, S.; Kita, A.; Okano, M.; Oda, R. Synthesis of Some Derivatives of Dicyclopropyl Ketone. J. Synth. Org. Chem. Jpn. 1969, 27, 444-447.

POCl<sub>3</sub> was removed in vacuo, and the residue was neutralized with 50% NH<sub>4</sub>OH. Usual workup as above and purification on silica gel column chromatography (eluent:10% ethyl acetate/hexane), followed by recrystallization from EtOH/H<sub>2</sub>O, afforded 566 mg (44% overall) of 4 as colorless needles: mp 129–130 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz)  $\delta$  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<sup>-1</sup>. Anal. (C<sub>18</sub>H<sub>30</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

Method B. 8-Cyclopentyl-1,3-diethylxanthine (30). To a solution of 5,6-diamino-1,3-diethyluracil<sup>38b</sup> (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<sub>2</sub>O (1:1) was slowly added portionwise cyclopentanecarboxylic acid (1.2 mL, 11 mmol) with stirring, and the pH was maintained at  $5.0 \pm 0.5$  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; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 90 MHz)  $\delta$  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<sup>-1</sup>. Anal. (C<sub>14</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

8-Cyclopentyl-3-propylxanthine (34). To a suspension of 5,6-diamino-1-propyluracil<sup>38e</sup> (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; <sup>1</sup>H NMR (DMSO- $d_6$ , 270 MHz)  $\delta$  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 $(3 \text{ H}, \text{t}, J = 7.5 \text{ Hz}); {}^{13}\text{C} \text{ NMR} (\text{DMSO-}d_6, 270 \text{ MHz}) \delta 157.7, 154.3,$ 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<sup>-1</sup>. Anal.  $(C_{13}H_{18}N_4O_2)$  C, H, N.

Method C. 8-(Dicyclopropylmethyl)-1,3-dipropyl-7methylxanthine (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<sub>3</sub> 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; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 270 MHz)  $\delta$  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<sup>-1</sup>. Anal. (C<sub>18</sub>H<sub>28</sub>N<sub>4</sub>O<sub>2</sub>) 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; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 90 MHz)  $\delta$  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<sup>-1</sup>. Anal. (C<sub>16</sub>H<sub>22</sub>N<sub>4</sub>O<sub>2</sub>) C, H, N.

[8-(Dicyclopropylmethyl)-1,3-dipropylmethin-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<sub>3</sub> 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<sub>2</sub>Cl<sub>2</sub> (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; <sup>1</sup>H NMR  $(DMSO-d_{6}, 270 \text{ MHz}) \delta 4.58 (1 \text{ H, s}), 3.99 (2 \text{ H, t}, J = 7.0 \text{ Hz}),$ 3.80 (2 H, t, J = 7.4 Hz), 1.86 (1 H, t, J = 8.2 Hz), 1.80-1.50 (4 Hz), 1.80-1.50H, 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<sup>-1</sup>. Anal. (C<sub>20</sub>H<sub>27</sub>N<sub>4</sub>O<sub>4</sub>Na·0.8H<sub>2</sub>O) C, H, N.

**Radioligand Binding Assays.** Inhibition of binding of 1.1 nM of  $N^{6}$ -[<sup>3</sup>H]cyclohexyladenosine to A<sub>1</sub> receptors in guinea pig forebrain membranes was assayed as described,<sup>32c,34</sup> and inhibition of 3.8 nM of N-[<sup>3</sup>H]ethyladenosin-5'-uronamide in the presence of 50 nM  $N^{6}$ -cyclopentyladenosine in rat striatal membranes was assayed as described.<sup>22,33c</sup> Concentration-inhibition curves were carried out in duplicate with five or more concentrations of each test agent, and IC<sub>50</sub> values were calculated from computerization of logit log curve. The inhibition constants ( $K_i$ ) were calculated according to the Cheng and Prusoff equation.<sup>40</sup> When the assays were carried out three or more times, standard errors (SEM) are given in the table.

 $N^{6}$ -[<sup>3</sup>H]Cyclohexyladenosine A<sub>1</sub> 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  $\pm$  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<sup>35</sup> 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  $\pm$  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.

Acknowledgment. We thank Y. Nakasato, M. Takahashi, R. Yanagimoto, M. Ichimura, T. Ohno, and Dr. H. Sato for technical assistance. We are grateful to Dr. T. Hirata and Dr. T. Oka for support and encouragement.

<sup>(40)</sup> Cheng, Y. C.; Prusoff, W. H. Relationship between the Inhibition Constant (K<sub>i</sub>) and the Concentration of Inhibitor which Causes 50% Inhibition (I<sub>50</sub>) of an Enzymatic Reaction. *Biochem. Pharmacol.* 1973, 22, 3099-3108.