2-Alkynyl-8-aryl-9-methyladenines as Novel Adenosine Receptor Antagonists: Their Synthesis and Structure−Activity Relationships toward Hepatic Glucose Production Induced via Agonism of the A_{2B} Receptor

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Novel adenosine antagonists, 2-alkynyl-8-aryl-9-methyladenine derivatives, were synthesized as candidate hypoglycemic agents. These analogues were evaluated for inhibitory activity on *N*-ethylcarboxamidoadenosine (NECA)-induced glucose production in primary cultured rat hepatocytes. In general, aromatic moieties at the 8-position and alkynyl groups at the 2-position had significantly increased activity compared to unsubstituted compounds. The preferred substituents at the 8-position of adenine were the 2-furyl and 3-fluorophenyl groups. In modifying the alkynyl side chain, change of the ring size, cleavage of the ring, and removal of the hydroxyl group were well tolerated. The order of the stimulatory effects of adenosine agonists on rat hepatocytes was NECA > CPA > CGS21680, which is consistent with involvement of the A_{2B} receptor. In Chinese hamster ovary cells stably transfected with human A_{2B} receptor cDNA, one of the compounds potent in hepatocytes, **15o** ($IC_{50} = 0.42\ \mu M$), antagonized NECA-induced stimulation of cyclic AMP production ($IC_{50} = 0.063\ \mu M$). This inhibitory effect was much more potent than those of FK453, KF17837, and L249313 which have been reported to be respectively A_1, A_{2A}, and A_3 selective antagonists. These findings agree very well with the result that, compared to **15o**, these selective antagonists for each receptor subtype showed only marginal effects in rat hepatocytes. These results suggest that adenosine agonist-induced glucose production in rat hepatocytes is mediated through the A_{2B} receptor. Furthermore, **15o** showed hypoglycemic activity in an animal model of noninsulin-dependent diabetes mellitus, the KK-A^y mice. It is possible that inhibition of hepatic glucose production via the A_{2B} receptor could be at least one of the mechanisms by which **15o** exerts its *in vivo* effects. Further elaboration of this group of compounds may afford novel antidiabetic agents.

Introduction

Adenosine is an autocoid produced in many organs and tissues and has a variety of actions mediated by adenosine receptors.[1] So far, four receptor subtypes, termed A_1, A_{2A}, A_{2B}, and A_3, have been identified and cloned.[2−5] Activation of A_1 and A_3 receptors inhibits adenylate cyclase through G_i coupling, while activation of A_{2A} and A_{2B} receptors stimulates adenylate cyclase through G_s coupling.[1]

Noninsulin-dependent diabetes mellitus (NIDDM) is a complex disorder of heterogeneous etiology and is characterized by abnormal insulin secretion, decrease in the response of peripheral tissues to insulin (insulin resistance), and increased hepatic glucose production. These metabolic abnormalities cause hyperglycemia in NIDDM patients, and this hyperglycemia is regarded as the most important cause of diabetic complications. Therefore, a major therapeutic goal in NIDDM patients is to optimize blood glucose control to prevent the risk of complications resulting from vascular disease.[6]

Adenosine is involved in glucose homeostasis.[7−10] It has been reported that A_1 adenosine receptor antagonism improves glucose tolerance by increasing glucose uptake in skeletal muscle in Zucker rats.[10] Studies with specific agonists and antagonists suggested that adenosine's hepatic effects are mediated by the A_2 receptor,[7] but it remains unclear which A_2 (A_{2A} or A_{2B}) receptor is involved. It has also been reported that abnormal hepatic glucose production rather than decreased muscle glucose uptake is the major factor responsible for both fasting and postprandial hyperglycemia in NIDDM.[11] In our preliminary in vitro studies, adenosine agonists stimulated hepatic glucose production, and a nonselective adenosine antagonist, 8-phenyltheophylline (8-PT, **1**; Chart 1),[12,13] inhibited a nonselective adenosine agonist, *N*-ethylcarboxamidoadenosine (NECA, **5**; Chart 2),[12,14,15]-induced glucose production in primary cultured rat hepatocytes, both in a dose-dependent manner. This accumulation of facts prompted us to investigate the possibility of developing a novel type of antidiabetic agent, active via adenosine A_2 antagonism.

9-Methyladenine derivatives[12,16] lacking ribose have been reported to be antagonists of adenosine, but not agonists. During the previous search for adenosine A_2 receptor agonists, alkynyl, alkylamino, and alkoxy groups at the 2-position of adenine have been found to

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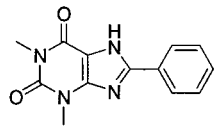
Chart 1. Structures of Adenosine Antagonists[a]



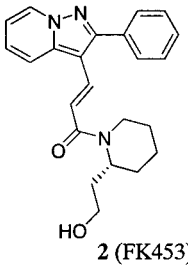
1 (8PT)

A_1: 1.34 (human)

A_{2A}: 0.454 (human)

A_{2B}: 0.415 (human)

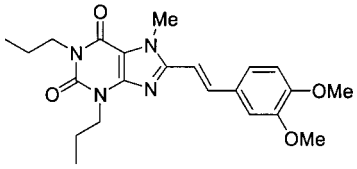
A_3: 1.25 (human)



2 (FK453)

A_1: 0.0172 (rat)

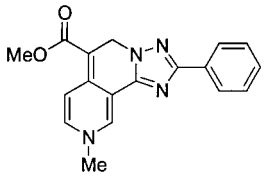
A_{2A}: 11.3 (rat)



3 (KF17837)

A_1: 0.062 (rat)

A_{2A}: 0.0010 (rat)



4 (L249313)

A_1: 5.3 (human)

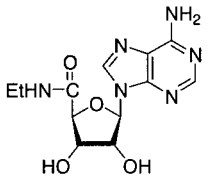
A_{2A}: 27.5 (human)

A_{2B}: >10 (human, cAMP)

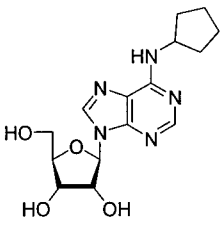
A_3: 0.013 (human)

[a] Binding Affinity, K_i or Inhibitory Activity of Cyclic AMP Production, and IC_{50} values are listed in μM.[23,24b,25b]

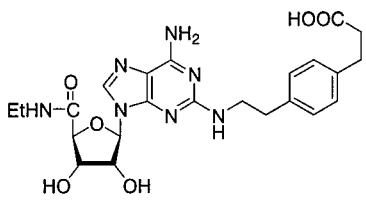
Chart 2. Structures of Adenosine Agonists



5 (NECA)



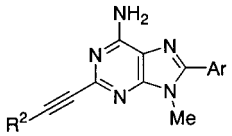
6 (CPA)



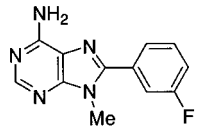
7 (CGS21680)

be favorable for high-affinity binding to adenosine receptors and also to impart an A_2 selectivity.[17−19] Recently Cristalli and co-workers reported 2-substituted 9-alkyladenine derivatives as adenosine antagonists,[20] while preliminary research suggested 8-aryl substitution leads to an increase in activity (Table 1, **15a** and **22**, and other unpublished results). 2-Alkynyl-8-aryladenine derivatives are structurally new. As an approach to a novel class of antidiabetic agent acting through adenosine A_2 antagonism, we synthesized various kinds of 8-aryl-9-alkylpurine derivatives (Chart 3)[21] and evaluated them for their effect on NECA-induced glucose production in primary cultured rat hepatocytes. Of these compounds, our present report focuses on a series of

Table 1. Inhibitory Activities of 2-Alkynyl-8-aryl-9-methyladenine Derivatives on NECA-Induced Glucose Production in Primary Cultured Rat Hepatocytes and on NECA-Induced Cyclic AMP Accumulation in CHO.K1 Cells



15a–s, 22

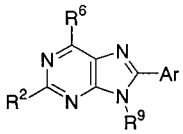


17

			IC_{50} ± SEM (μM)[b]	
cmpd[a]	Ar	R^2	hepatocyte	CHO.K1 cell
15a	C_6H_5	c-C_6H_{10}(OH)	0.76[c]	
15b	2-furyl	c-C_6H_{10}(OH)	0.22[c]	0.014 ± 0.002
15c	2-thienyl	c-C_6H_{10}(OH)	0.75[c]	
15d	2-pyridyl	c-C_6H_{10}(OH)	1.3[c]	
15e	2-FC_6H_4	c-C_6H_{10}(OH)	0.44 ± 0.10	0.077 ± 0.012
15f	3-FC_6H_4	c-C_6H_{10}(OH)	0.25 ± 0.04	0.023 ± 0.003
15g	4-FC_6H_4	c-C_6H_{10}(OH)	1.2 ± 0.4	0.16 ± 0.04
15h	3-ClC_6H_4	c-C_6H_{10}(OH)	0.49[c]	
15i	4-ClC_6H_4	c-C_6H_{10}(OH)	6.3[c]	0.50 (0.40−0.60)
15j	3-(CN)C_6H_4	c-C_6H_{10}(OH)	0.41[c]	0.072 ± 0.029
15k	3-(CF_3)C_6H_4	c-C_6H_{10}(OH)	>10 (>10, >10)[d]	5.6 ± 0.8
15l	3-MeC_6H_4	c-C_6H_{10}(OH)	0.97 (0.72−1.2)	
15m	3-MeOC_6H_4	c-C_6H_{10}(OH)	1.6 (1.4−1.9)	
15n	3-F-4-MeOC_6H_3	c-C_6H_{10}(OH)	>10[c,d]	
15o	3-FC_6H_4	c-C_5H_8(OH)	0.42 ± 0.05	0.063 ± 0.010
15p	3-FC_6H_4	c-C_7H_{12}(OH)	0.44 ± 0.12	0.049 ± 0.009
15q	3-FC_6H_4	$(CH_3)_2$(OH)C	0.51[c]	0.15 ± 0.02
15r	3-FC_6H_4	$(C_2H_5)_2$(OH)C	0.54[c]	0.14 ± 0.01
15s	3-FC_6H_4	c-C_5H_9	0.72[c]	
17			5.3[c]	
22	H	c-C_6H_{10}(OH)	4.4[c]	
1			1.1 ± 0.1	0.21 ± 0.02
2			8.1 ± 1.6	0.98 (0.92−1.0)
3			>10 (>10, >10)[d]	1.5 (1.4−1.7)
4			>10[c,d]	3.0 ± 0.4

[a] Compounds **15**, **17**, and **22** were prepared as their HCl salts except **15s**. [b] IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least three points). Repeated experiments were made unless otherwise noted. In cases where the number of independent experiments (n) > 2, data are shown as mean IC_{50} values (μM) ± SEM. In other cases where n = 2, IC_{50} values are the means of two independent experiments (values of individual measurements between parentheses). [c] The values are the results of one experiment performed in triplicate and individual determinations varied by less than 10%. [d] >10 means that the IC_{50} was greater than 10 μM.

Chart 3. Structures of 8-Aryl-9-alkylpurine Derivatives



Ar: aromatic ring

R^2: alkynyl, alkylamino, alkoxy

R^6:(alkyl)amino, alkoxy

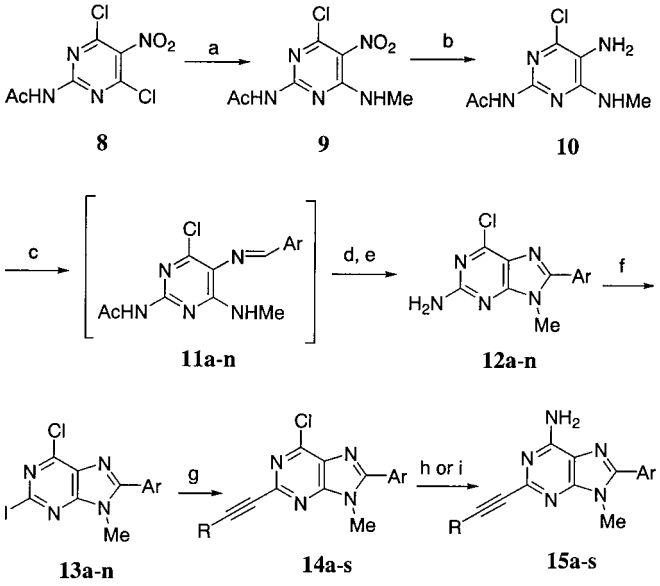
R^9: alkyl

8-Aryl-9-alkylpurine derivatives

2-alkynyl-8-aryl-9-methyladenine derivatives which possess potent activities, not only *in vitro*, but also *in vivo* (hypoglycemic activities in a murine diabetic model).

To characterize which adenosine receptor subtype stimulates hepatic glucose production, we examined the order of potency of the stimulatory effects of various adenosine agonists (Chart 2)[12,14,15,18a,22] on rat hepatocytes, and the inhibitory effects both of our and of known antagonists (Chart 1)[12,23−25] on NECA-induced cyclic AMP production in Chinese hamster ovary (CHO.K1) cells expressing the human adenosine A_{2B} receptor.[4b] Moreover, the hypoglycemic activity of one of our

Scheme 1. Synthesis of 2-Alkynyl-8-aryl-9-methyladenine Derivatives[a]



[a] Reagents: (a) 40% $MeNH_2$, AcOH/THF; (b) H_2, Raney Ni/MeOH; (c) ArCHO, AcOH/MeOH; (d) $FeCl_3$/EtOH; (e) HCl/H_2O−THF; (f) CH_2I_2, isoamyl nitrite, CuI/THF; (g) alkyne, $(PPh_3)_2PdCl_2$, CuI, Et_3N/THF; (h) NH_3/EtOH; (i) NH_3/H_2O−1,2-dimethoxyethane.

compounds was also examined in genetically diabetic KK-A^y mice.[26]

Chemistry

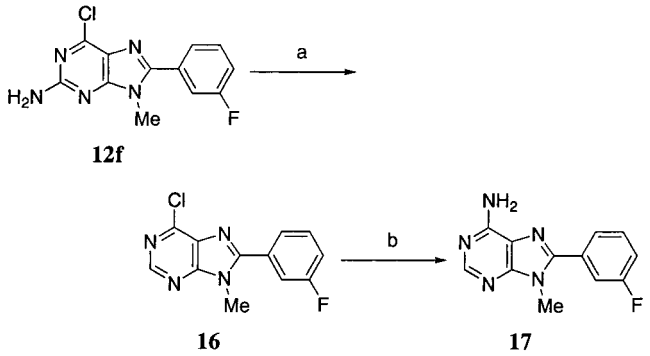
The 2-alkynyl-8-aryl-9-methyladenine derivatives were prepared by the procedures outlined in Scheme 1. Treatment of the previously reported *N*1-(4,6-dichloro-5-nitropyrimidin-2-yl)acetamide (**8**)[27] with an aqueous solution of methylamine neutralized with acetic acid gave the monomethylamino derivative (**9**) in good yield.[28] Hydrogenation[27] of **9** in the presence of Raney nickel provided the 5-amino compound (**10**). This was condensed with an aldehyde to form the Schiff base (**11**) which was directly converted to 2-amino-8-aryl-6-chloropurine (**12**) by successive treatments with anhydrous $FeCl_3$ and aqueous HCl. This procedure of oxidative ring closure via $FeCl_3$, generally used in the preparation of 8-phenylxanthine derivatives,[29] was demonstrated for the first time to be convenient for the synthesis of adenine. Transformation to the 2-iodo compound (**13**) was accomplished by diazotization-substitution reaction[17b] of **12** with the combination of isoamyl nitrite, CH_2I_2, and CuI in THF. Palladium-catalyzed cross-coupling reaction[17c] of **13** with a terminal alkyne at room temperature afforded the 2-alkynyl compound (**14**) regioselectively. Amination of **14** with ammonia in a sealed tube gave our target (**15**).

Reductive deamination of 2-amino-8-(3-fluorophenyl)-6-chloropurine (**12f**), followed by ammonolysis of **16**, provided 2-*H*-derivative (**17**) (Scheme 2).

8-*H*-Derivative (**22**) was obtained in a manner similar to that described for **15**, except that triethyl orthoformate was used to cyclize 5-aminopyrimidine (**10**) to 2-amino-6-chloropurine (**19**) (Scheme 3).[28]

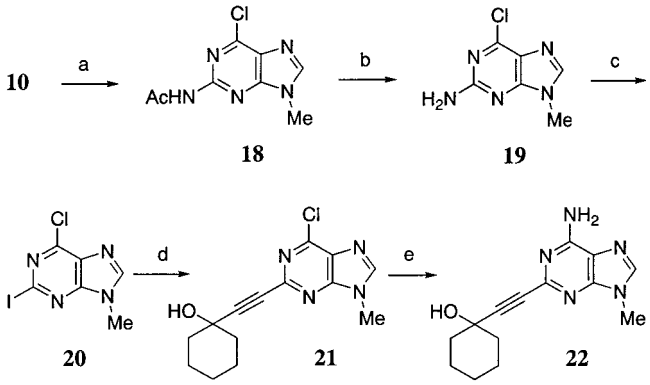
In the course of our study, we prepared various key intermediates (9-alkyl congeners of **13**) by using other alkylamines in place of methylamine. On the basis of the differing reactivity[17c,20,30] of the 6-chloro and 2-iodo substituents to nucleophiles, these intermediates were led to various 8-aryl-9-alkylpurine derivatives (Chart 3) (detailed procedures not shown). This synthetic method therefore allowed us to introduce a variety of substituents at the 2-, 6-, and 9-position of 8-arylpurines, and, moreover, should be applicable to large-scale synthesis.[21]

Scheme 2. Synthesis of 2-*H*-Derivative[a]



[a] Reagents: (a) isoamyl nitrite, CuI/THF; (b) NH_3/H_2O−1,2-dimethoxyethane.

Scheme 3. Synthesis of 8-*H*-Derivative[a]



[a] Reagents: (a) triethyl orthoformate, cHCl/MeOH; (b) HCl/H_2O−THF; (c) CH_2I_2, isoamyl nitrite, CuI/THF; (d) 1-ethynyl-1-cyclohexanol, $(PPh_3)_2PdCl_2$, CuI, Et_3N/THF; (e) NH_3/H_2O−1,2-dimethoxyethane.

Results and Discussion

The preparation of rat hepatocytes and the assay of inhibition of glucose production are briefly described in the Experimental Section. Compound **1** was used as an active control in each screening assay for the inhibition of hepatic glucose production. The inhibitory activities of 2-alkynyl-8-aryl-9-methyladenine derivatives (**15**) toward NECA-stimulated glucose production in rat hepatocytes are summarized in Table 1.

Our first new compound (**15a**) inhibited NECA-stimulated glucose production in a dose-dependent manner (IC_{50} = 0.76 μM). This inhibitory effect was more potent than that of the standard compound, **1** (IC_{50} = 1.1 μM). To explore the structure−activity relationships of this series of compounds, we introduced various heteroaromatic rings and (substituted) phenyl rings into the 8-position of 9-methyladenine, and other alkynyl groups into the 2-position.

Aromatic groups in the 8-position significantly increased the potency in the following order: 2-furyl (**15b**,

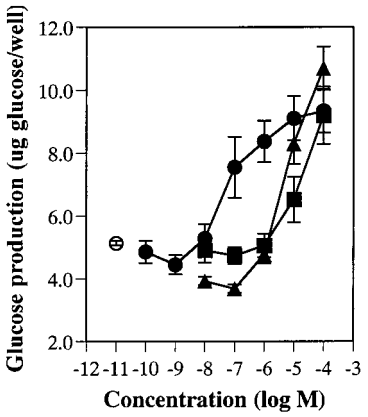


Figure 1. Dose−response curves of the effects of adenosine agonists on glucose production in primary cultured rat hepatocytes. Each point represents the mean ± SEM of three separate dose−response studies. Open circle, control; closed circles, **5**; closed triangles, **6**; closed squares, **7**.

IC_{50} = 0.22 μM) > phenyl (**15a**, IC_{50} = 0.76 μM), 2-thienyl (**15c**, IC_{50} = 0.75 μM) > 2-pyridyl (**15d**, IC_{50} = 1.3 μM) ≫ H (**22**, IC_{50} = 4.4 μM).

The introduction of electron-withdrawing substituents such as F (**15f**, IC_{50} = 0.25 μM), Cl (**15h**, IC_{50} = 0.49 μM), and CN (**15j**, IC_{50} = 0.41 μM) at the 3-position of the phenyl ring resulted in enhancement of potency, except for trifluoromethyl substitution (**15k**, IC_{50} > 10 μM). This effect was not observed with electron-donating substituents such as methyl (**15l**: IC_{50} = 0.97 μM) or methoxyl (**15m**, IC_{50} = 1.6 μM). Additional substitution at the 4-position reduced potency (**15g**, IC_{50} = 1.2 μM; **15i**, IC_{50} = 6.3 μM; and **15n**, IC_{50} > 10 μM), presumably for steric reasons. The 2-fluorine compound (**15e**, IC_{50} = 0.44 μM) was a little less potent than the 3-fluorine compound (**15f**, IC_{50} = 0.25 μM).

Alkynyl groups in the 2-position of the adenine core enhanced potency by an order of magnitude (**15f**, **15o**−**s**, IC_{50} = 0.25−0.72 μM; **17**, IC_{50} = 5.3 μM). Modifying the alkynyl side chain of **15f**, by altering the ring size, opening the ring, or removing the hydroxyl led to a decrease in, but not loss of, inhibitory activity (**15f**, IC_{50} = 0.25 μM; **15o**, IC_{50} = 0.42 μM; **15p**, IC_{50} = 0.44 μM; **15q**, IC_{50} = 0.51 μM; and **15r**, IC_{50} = 0.54 μM; **15s**, IC_{50} = 0.72 μM).

Moreover, in the absence of NECA-stimulation, our antagonists had no effect on basal hepatic glucose production (**15f**, **15o**: 0.01−10 μM; **15a**−**d**: 10 μM) (data not shown), showing that they lack partial agonist activity.

Next, we performed further studies on the mechanism of action of our antagonists versus agonist-induced hepatic glucose production. To identify the adenosine receptor subtype involved in agonist-induced glucose production in rat hepatocytes, we examined the stimulatory potency of different kinds of adenosine agonist (**5**−**7**, Chart 2) on hepatic glucose production. A nonselective agonist (**5**),[12,14,15] an A_1 selective agonist, N^6-cyclopentyladenosine (CPA, **6**; Chart 2),[12,14,15] and an A_{2A} selective agonist, 2-[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS21680, **7**; Chart 2)[12,18a,22] stimulated glucose production in a dose-dependent manner (Figure 1). Their order of potency was **5** > **6** > **7**, consistent with the previously reported order of response toward the A_{2B} receptor.[12,22,31] Furthermore, to demonstrate our compounds do indeed block the A_{2B} receptor, we tested the inhibitory effects of some of our compounds along with those of known antagonists (Chart 1) on NECA-induced cyclic AMP accumulation in CHO.K1 cells stably transfected with human adenosine A_{2B} receptor cDNA. They antagonized NECA-induced stimulation of cyclic AMP production in a dose-dependent manner. The IC_{50} values of these compounds are listed in Table 1. The order of potency was similar to that described in rat hepatocytes glucose assay. These findings using agonists and antagonists are in good accord with the result that an A_1 selective antagonist, (*E*)-(2*R*)-1-[3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)acryloyl]-2-piperidineethanol (FK453, **2**; Chart 1),[12,23] an A_{2A} selective antagonist, (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF17837, **3**; Chart 1),[12,24] and an A_3 selective antagonist, 6-carboxymethyl-5,9-dihydro-9-methyl-2-phenyl-[1,2,4]-triazolo[5,1-*a*][2,7]naphthyridine (L249313, **4**; Chart 1),[12,25] showed only marginal effects on NECA-induced glucose production in rat hepatocytes (**2**, IC_{50} = 8.1 μM; **3**, IC_{50} > 10 μM; **4**, IC_{50} > 10 μM). From the results of binding studies, our antagonists had high affinity for both A_1 and A_{2A} receptors as well as A_{2B} (Table 2). However we may reasonably conclude that adenosine agonist-induced glucose production in primary cultured rat hepatocytes may result from the activation of the A_{2B} receptor.

Table 2. Adenosine A_1, A_{2A}, and A_3 Receptor Binding Affinity

	K_i ± SEM (μM)[a]		
cmpd	A_1	A_{2A}	A_3
15b	0.025 ± 0.009	0.011 ± 0.000	
15e	0.019 ± 0.001	0.015 ± 0.001	
15f	0.014 ± 0.003	0.016 ± 0.001	0.54[b]
15j	0.13 ± 0.01	0.013 ± 0.002	
15o	0.020 ± 0.000	0.0098 ± 0.0039	1.1 ± 0.2
15p	0.027 ± 0.003	0.019 ± 0.004	
15q	0.030 ± 0.007	0.0091 ± 0.0016	2.1 ± 0.2
15r	0.029 ± 0.006	0.013 ± 0.003	0.33[b]
2	0.018 ± 0.002	1.3 ± 0.4	2.8 ± 0.7
3	>10[c]	0.071 ± 0.014	2.5 (2.2−2.8)
4	>10[c]	>10[c]	0.0013 ± 0.0007

[a] K_i values were determined in radioligand binding assays for recombinant human A_1, A_{2A}, and A_3 receptors expressed in CHO.K1 cells vs [^{3}H]CCPA, HEK-293 cells vs [^{3}H]CGS21680, and HEK-293 cells vs [^{125}I]AB-MECA, respectively. Concentration−inhibition curves were carried out with three or more concentrations of each test agent, and IC_{50} values were determined from the logarithmic concentration−inhibition curve (at least three points). IC_{50} values were converted to K_i values using the Cheng−Prusoff equation.[34] Repeated experiments were made unless otherwise noted. In cases where the number of independent experiments (n) > 2, data are shown as mean K_i values (μM) ± SEM. In other cases where n = 2, K_i values are the means of two independent experiments (values of individual measurements between parentheses). [b] The values are the results of one experiment performed in duplicate and individual determinations varied by less than 10%. [c] >10 means that K_i was greater than 10 μM.

We examined the in vivo efficacy of **15o**, a potent derivative having a favorable pharmacokinetic profile, in KK-A^y mice (Figure 2).[26] A single oral administration of **15o**, at a dose of 10 or 30 mg/kg, significantly reduced the blood glucose level in KK-A^y mice in a dose-dependent manner. The potency of **15o** at 10 mg/kg was greater than those of a sulfonyl urea drug, *N*-*p*-[2-(5-chloro-2-methoxybenzamido)ethyl]benzenesulfonyl-*N*′-cyclohexylurea (glybenclamide, **23**; Chart 4),[32] at 30 mg/kg and a biguanide drug, 1,1-dimethylbiguanide hydro-

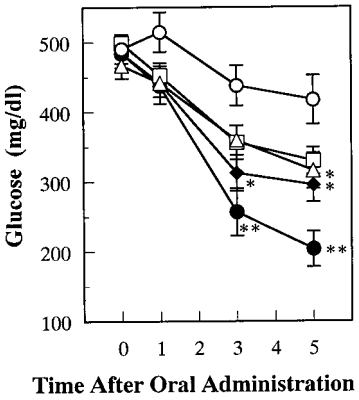
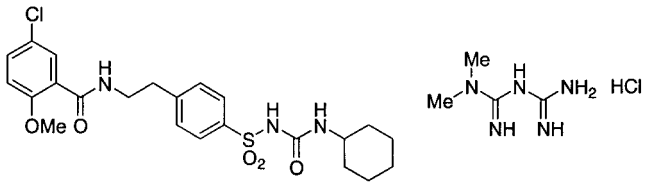


Figure 2. Time course of the hypoglycemic effect of **15o** in KK-A^y mice. Each point represents the mean blood glucose level ± SEM ($n = 5$). Statistically significant differences from the vehicle control levels: $*p < 0.05$, $**p < 0.01$. Open circles, control; closed diamonds, **15o** (10 mg/kg); closed circles, **15o** (30 mg/kg); open triangles, **23** (30 mg/kg); open squares, **24** (100 mg/kg).

Chart 4. Structures of Antidiabetic Drugs



23 (glybenclamide) **24** (metformin)

chloride (metformin, **24**; Chart 4),[33] at 100 mg/kg. Although it has been reported that A_1 adenosine receptor antagonism improves glucose tolerance by increasing glucose uptake in skeletal muscle,[10] the A_1 selective antagonist (**2**),[12,23] at a dose of 100 mg/kg, had no effect on the blood glucose level in this model (data not shown). We postulate that inhibition of hepatic glucose production via the A_{2B} receptor is one reason for the in vivo efficacy of **15o**.

In summary, we have synthesized a series of novel adenosine antagonists, 2-alkynyl-8-aryl-9-methyladenine derivatives.[21] On the basis of screening of these compounds for inhibitory activity in an adenosine agonist-induced hepatic glucose production assay, we selected **15o** for further examination in a murine diabetic model, and found that it is an orally active hypoglycemic agent. From the results of a functional assay using recombinant human A_{2B} receptors, **15o** was found to have A_{2B} antagonistic activity. The poor efficacy of selective antagonists for the A_1, A_{2A}, and A_3 receptors and the order of potency of the stimulatory effects of various adenosine agonists in rat hepatocytes indicate that the A_{2B} receptor participates in adenosine agonist-induced glucose production in primary cultured rat hepatocytes. Further development of this group of compounds may yield novel antidiabetic agents. Compound **15o** is a nonspecific adenosine antagonist, but we hypothesize that its inhibition of hepatic glucose production via the A_{2B} receptor could be at least one of the mechanisms of the in vivo activity of **15o**. To prove this hypothesis, research to find an A_{2B} selective antagonist is currently continuing.

Experimental Section

Chemistry. Column chromatography was performed on silica gel (Merck, particle size 0.063−0.200 mm). TLC analyses were performed on silica gel plates (Merck, Art 5715). All ^{1}H NMR spectra were measured on a Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in δ units from tetramethylsilane (TMS) as an internal standard. Coupling constants (J) are reported in hertz. Abbreviations are as follows; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; br broad peak; Hz, hertz. Mass spectra (FAB-MS or ESI-MS) were obtained on a JEOL SX102 or Thermo Quest SSQ7000 mass spectrometer, respectively. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. Mass spectra and elemental analyses were performed at the Analytical Chemistry Section of Eisai Research Laboratories.

***N*1-[4-Chloro-6-(methylamino)-5-nitro-2-pyrimidinyl]-acetamide (9).** Glacial acetic acid (1.5 L, 26 mol) was carefully added with cooling to a 40% aqueous solution of methylamine (2.0 L, 23 mol). This solution (1.8 L) was added dropwise to a stirred solution of *N*1-(4,6-dichloro-5-nitro-2-pyrimidinyl)acetamide (**8**) (1.5 kg, 6.0 mol) in THF (15 L) previously cooled to 0 °C. The internal temperature was maintained at 4 °C, and the addition of the solution of methylamine took 1 h. Thirty minutes later, a further solution of methylamine (450 mL) was added dropwise, and this was repeated after another 30 min interval. The mixture was stirred at this temperature for an additional 40 min, and crushed ice (4.5 kg) and cooled H_2O (10 L) were added to afford a precipitate. This was collected by filtration, washed with H_2O and Et_2O, and dried at 50 °C for 6 h to give **9** (1253 g, 85%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.27 (3H, s, Ac), 2.97 (3H, d, $J = 4.4$ Hz, NMe), 8.55 (1H, q, $J = 4.4$ Hz, N*H*Me), 10.80 (1H, s, N*H*Ac).

***N*1-[5-Amino-4-chloro-6-(methylamino)-2-pyrimidinyl]-acetamide (10).** A suspension of **9** (40.0 g, 0.163 mol) in MeOH (2.0 L) was hydrogenated in the presence of Raney nickel (40.0 g wet, washed with H_2O and MeOH) at room temperature and atmospheric pressure. The theoretical amount of H_2 was absorbed within 3.5 h. The reaction mixture was filtered through a Celite pad, which was then washed with MeOH, and the filtrate was evaporated. The residue was diluted with Et_2O, and the resulting precipitate was collected by filtration to afford **10** (21.5 g, 61%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 2.12 (3H, br s, Ac), 2.88 (3H, d, $J = 4.4$ Hz, NMe), 4.64 (2H, br s, NH_2), 6.97 (1H, q, $J = 4.4$ Hz, N*H*Me), 9.76 (1H, br s, N*H*Ac).

6-Chloro-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinylamine (12f). General Procedure. A mixture of **10** (2.0 g, 9.27 mmol), 3-fluorobenzaldehyde (1.4 g, 11.3 mmol), and acetic acid (1.2 mL) in MeOH (40 mL) was stirred at room temperature for 5.5 h. The reaction mixture was concentrated to dryness under reduced pressure. The residue was azeotropically distilled with toluene (20 mL × 2), and used directly in the next step. To a suspension of crude imine in EtOH (40 mL) was added a solution of anhydrous $FeCl_3$ (1.5 g, 9.25 mmol) in EtOH (10 mL) at room temperature. The mixture was heated under reflux for 1 h and then allowed to cool to room temperature. The solvent was removed under reduced pressure, and the residue was suspended in EtOH and H_2O. The precipitated product was filtered off and washed with H_2O to give crude **12f** (1.26 g) as a pale yellow solid. Examination of the ^{1}H NMR spectrum indicated that a small amount of *N*-acetyl purine derivative remained in the crude material. A solution of crude **12f** (1.26 g) in 1 N HCl (10 mL)-THF (40 mL) was heated under reflux for 40 min and then allowed to cool to room temperature. The mixture was filtered through a Celite pad, which was then washed with MeOH, and the filtrate was evaporated. The residue was suspended in H_2O. The precipitated product was filtered off and washed with H_2O to give **12f** (1.08 g, 42%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.73 (3H, s, Me), 7.01 (2H, s, NH_2), 7.40−7.46 (1H, m, phenyl), 7.60−7.66 (1H, m, phenyl), 7.68−7.74 (2H, m, phenyl).

6-Chloro-8-(3-fluorophenyl)-2-iodo-9-methyl-9*H*-purine (13f). General Procedure. Isoamyl nitrite (1.5 mL, 11.2 mmol) was added to a mixture of **12f** (1.00 g, 3.60 mmol), CH_2I_2 (1.5 mL, 18.5 mmol), and CuI (686 mg, 3.60 mmol) in THF (20 mL). The mixture was heated under reflux for 1 h and then allowed to cool to room temperature. The reaction mixture was partitioned between EtOAc and 1 N HCl. The separated organic phase was washed with concentrated aqueous ammonia and saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was suspended in Et_2O−hexane. The precipitated product was filtered off and washed with hexane to give **13f** (1.02 g, 73%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 3.89 (3H, s, Me), 7.49−7.56 (1H, m, phenyl), 7.66−7.72 (1H, m, phenyl), 7.76−7.82 (2H, m, phenyl).

1-[2-[6-Chloro-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol (14f). General Procedure. Triethylamine (0.27 mL, 1.94 mmol) was added dropwise to a mixture of **13f** (500 mg, 1.28 mmol), CuI (25 mg, 0.131 mmol), dichlorobis(triphenylphosphine)palladium(II) (90 mg, 0.128 mmol), and 1-ethynyl-1-cyclohexanol (192 mg, 1.54 mmol) in THF (10 mL). The mixture was stirred under an atmosphere of N_2 at room temperature for 2.5 h and then partitioned between EtOAc and saturated aqueous NH_4Cl. The separated organic phase was washed with concentrated aqueous ammonia−saturated aqueous NH_4Cl (1:1) and saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was suspended in Et_2O. The precipitated product was filtered off and washed with Et_2O to give **14f** (426 mg, 86%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.23−1.36 (1H, m, c-C_6H_{10}), 1.46−1.74 (7H, m, c-C_6H_{10}), 1.86−1.95 (2H, m, c-C_6H_{10}), 3.92 (3H, s, Me), 5.72 (1H, s, OH), 7.50−7.56 (1H, m, phenyl), 7.67−7.73 (1H, m, phenyl), 7.78−7.84 (2H, m, phenyl).

1-[2-[6-Amino-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15f). General Procedure. A solution of **14f** (12.6 g, 32.7 mmol) in ethanolic ammonia (saturated at 0 °C) (1500 mL) in a sealed steel tube was heated at 100 °C for 20 h and then allowed to cool to room temperature. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (eluent; $CHCl_3$, $CHCl_3$:MeOH = 100:1, 50:1) followed by precipitation with MeOH to give the title compound (9.4 g) as the free form. This was suspended in MeOH, 20% HCl/EtOH was added, and the solvent was evaporated. The residue was diluted with Et_2O, and the precipitated product was filtered off and washed with Et_2O to give **15f** (8.8 g, 67%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.45−1.66 (7H, m, c-C_6H_{10}), 1.83−1.86 (2H, m, c-C_6H_{10}), 3.78 (3H, s, Me), 7.40−7.45 (1H, m, phenyl), 7.60−7.66 (1H, m, phenyl), 7.69−7.72 (2H, m, phenyl); MS *m/e* (FAB) 366 (MH^+). Anal. ($C_{20}H_{20}FN_5O\cdot HCl$) C, H, N.

1-[2-[6-Amino-9-methyl-8-phenyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15a). This compound was prepared in a manner similar to that described for **15f**, except that benzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.32 (1H, m, c-C_6H_{10}), 1.44−1.66 (7H, m, c-C_6H_{10}), 1.81−1.88 (2H, m, c-C_6H_{10}), 3.79 (3H, s, Me), 7.57−7.60 (3H, m, phenyl), 7.85−7.88 (2H, m, phenyl); MS *m/e* (FAB) 348 (MH^+). Anal. ($C_{20}H_{21}N_5O\cdot HCl$) C, H, N.

1-[2-[6-Amino-8-(2-furyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15b). This compound was prepared in a manner similar to that described for **15f**, except that 2-furaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.21−1.34 (1H, m, c-C_6H_{10}), 1.43−1.70 (7H, m, c-C_6H_{10}), 1.82−1.90 (2H, m, c-C_6H_{10}), 3.90 (3H, s, Me), 6.77 (1H, dd, J = 1.6 and 3.6 Hz, furyl), 7.24 (1H, d, J = 3.6 Hz, furyl), 8.00 (1H, d, J = 1.6 Hz, furyl); MS *m/e* (FAB) 338 (MH^+). Anal. ($C_{18}H_{19}N_5O_2\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

1-[2-[6-Amino-9-methyl-8-(2-thienyl)-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15c). This compound was prepared in a manner similar to that described for **15f**, except that 2-thiophenecarboxaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.18−1.34 (1H, m, c-C_6H_{10}), 1.43−1.68 (7H, m, c-C_6H_{10}), 1.79−1.90 (2H, m, c-C_6H_{10}), 3.88 (3H, s, Me), 7.27 (1H, dd, J = 4.0 and 4.8 Hz, thienyl), 7.80 (1H, dd, J = 0.8 and 4.0 Hz, thienyl), 7.82 (1H, dd, J = 0.8 and 4.8 Hz, thienyl); MS *m/e* (ESI) 354 (MH^+). Anal. ($C_{18}H_{19}N_5OS\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

1-[2-[6-Amino-9-methyl-8-(2-pyridyl)-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15d). This compound was prepared in a manner similar to that described for **15f**, except that 2-pyridinecarboxaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.22−1.33 (1H, m, c-C_6H_{10}), 1.46−1.71 (7H, m, c-C_6H_{10}), 1.84−1.94 (2H, m, c-C_6H_{10}), 4.13 (3H, s, Me), 7.58−7.61 (1H, m, pyridyl), 8.05−8.10 (1H, m, pyridyl), 8.26−8.28 (1H, m, pyridyl), 8.77−8.79 (1H, m, pyridyl); MS *m/e* (FAB) 349 (MH^+). Anal. ($C_{19}H_{20}N_6O\cdot HCl\cdot H_2O$) C, H, N.

1-[2-[6-Amino-8-(2-fluorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15e). This compound was prepared in a manner similar to that described for **15f**, except that 2-fluorobenzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.32 (1H, m, c-C_6H_{10}), 1.42−1.66 (7H, m, c-C_6H_{10}), 1.80−1.86 (2H, m, c-C_6H_{10}), 3.58 (3H, s, Me), 7.39−7.48 (2H, m, phenyl), 7.63−7.72 (2H, m, phenyl); MS *m/e* (FAB) 366 (MH^+). Anal. ($C_{20}H_{20}FN_5O\cdot HCl\cdot{}^1/_4H_2O$) C, H, N.

1-[2-[6-Amino-8-(4-fluorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15g). This compound was prepared in a manner similar to that described for **15f**, except that 4-fluorobenzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.43−1.68 (7H, m, c-C_6H_{10}), 1.81−1.89 (2H, m, c-C_6H_{10}), 3.79 (3H, s, Me), 7.42−7.47 (2H, m, phenyl), 7.90−7.96 (2H, m, phenyl); MS *m/e* (FAB) 366 (MH^+). Anal. ($C_{20}H_{20}FN_5O\cdot HCl$) C, H, N.

1-[2-[6-Amino-8-(3-chlorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15h). This compound was prepared in a manner similar to that described for **15f**, except that 3-chlorobenzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.42−1.64 (7H, m, c-C_6H_{10}), 1.80−1.89 (2H, m, c-C_6H_{10}), 3.79 (3H, s, Me), 7.54−7.60 (2H, m, phenyl), 7.82−7.85 (1H, m, phenyl), 7.92 (1H, br, phenyl); MS *m/e* (FAB) 382 (MH^+). Anal. ($C_{20}H_{20}ClN_5O\cdot HCl\cdot{}^1/_5H_2O$) C, H, N.

1-[2-[6-Amino-8-(4-chlorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15i). This compound was prepared in a manner similar to that described for **15f**, except that 4-chlorobenzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.19−1.30 (1H, m, c-C_6H_{10}), 1.42−1.65 (7H, m, c-C_6H_{10}), 1.80−1.88 (2H, m, c-C_6H_{10}), 3.77 (3H, s, Me), 7.64 (2H, d, J = 8.0 Hz, phenyl), 7.88 (2H, d, J = 8.0 Hz, phenyl); MS *m/e* (FAB) 382 (MH^+). Anal. ($C_{20}H_{20}ClN_5O\cdot HCl\cdot H_2O$) C, H, N.

3-[6-Amino-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9-methyl-9*H*-8-purinyl]benzonitrile Hydrochloride (15j). This compound was prepared in a manner similar to that described for **15f**, except that 3-cyanobenzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.40−1.70 (7H, m, c-C_6H_{10}), 1.80−1.84 (2H, m, c-C_6H_{10}), 3.80 (3H, s, Me), 7.78 (1H, t, J = 7.8 Hz, phenyl), 8.02(1H, d, J = 7.8 Hz, phenyl), 8.18 (1H, d, J = 7.8 Hz, phenyl), 8.32 (1H, s, phenyl); MS *m/e* (FAB) 373 (MH^+). Anal. ($C_{21}H_{20}N_6O\cdot HCl$) C, H, N.

1-[2-[6-Amino-9-methyl-8-[3-(trifluoromethyl)phenyl]-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15k). This compound was prepared in a manner similar to that described for **15f**, except that 3-(trifluoromethyl)benzaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.42−1.70 (7H, m, c-C_6H_{10}), 1.80−1.89 (2H, m, c-C_6H_{10}), 3.81 (3H, s, Me), 7.82 (1H, t, J = 11.0 Hz, phenyl), 7.93 (1H, d, J = 11.0 Hz, phenyl), 8.19 (1H, d, J = 11.0 Hz, phenyl), 8.21 (1H, s, phenyl); MS *m/e* (FAB) 416 (MH^+). Anal. ($C_{21}H_{20}F_3N_5O\cdot HCl$) C, H, N.

1-[2-[6-Amino-9-methyl-8-(3-methylphenyl)-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15l). This compound was prepared in a manner similar to that described for **15f**, except that *m*-tolualdehyde was used instead of

3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.08−1.31 (1H, m, c-C_6H_{10}), 1.42−1.71 (7H, m, c-C_6H_{10}), 1.82−1.92 (2H, m, c-C_6H_{10}), 2.40 (3H, s, C*H*$_3$phenyl), 3.81 (3H, s, 9-Me), 7.41 (1H, d, J = 7.6 Hz, phenyl), 7.48 (1H, t, J = 7.6 Hz, phenyl), 7.67 (1H, d, J = 7.6 Hz, phenyl), 7.69 (1H, s, phenyl); MS *m/e* (FAB) 362 (MH^+). Anal. ($C_{21}H_{23}N_5O\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

1-[2-[6-Amino-8-(3-methoxyphenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15m). This compound was prepared in a manner similar to that described for **15f**, except that *m*-anisaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.44−1.68 (7H, m, c-C_6H_{10}), 1.83−1.91 (2H, m, c-C_6H_{10}), 3.83 (6H, two s, 9-Me and MeO), 7.18−7.20 (1H, m, phenyl), 7.41−7.42 (1H, m, phenyl), 7.43−7.46 (1H, m, phenyl), 7.51−7.55 (1H, m, phenyl); MS *m/e* (FAB) 378 (MH^+). Anal. ($C_{21}H_{23}N_5O_2\cdot HCl\cdot H_2O$) C, H, N.

1-[2-[6-Amino-8-(3-fluoro-4-methoxyphenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclohexanol Hydrochloride (15n). This compound was prepared in a manner similar to that described for **15f**, except that 3-fluoro-*p*-anisaldehyde was used instead of 3-fluorobenzaldehyde. ^{1}H NMR (DMSO-d_6) δ 1.20−1.30 (1H, m, c-C_6H_{10}), 1.42−1.68 (7H, m, c-C_6H_{10}), 1.80−1.88 (2H, m, c-C_6H_{10}), 3.79 (3H, s, 9-Me), 3.92 (3H, m, MeO), 7.34−7.39 (1H, m, phenyl), 7.66−7.76 (2H, m, phenyl); MS *m/e* (FAB) 396 (MH^+). Anal. ($C_{21}H_{22}FN_5O_2\cdot HCl\cdot{}^1/_4H_2O$) C, H, N.

1-[2-[6-Amino-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cyclopentanol Hydrochloride (15o). This compound was prepared in a manner similar to that described for **15f**, except that 1-ethynyl-1-cyclopentanol was used instead of 1-ethynyl-1-cyclohexanol. ^{1}H NMR (DMSO-d_6) δ 1.66−1.82 (4H, m, c-C_5H_8), 1.87−2.00 (4H, m, c-C_5H_8), 3.86 (3H, s, Me), 7.43−7.50 (1H, m, phenyl), 7.63−7.70 (1H, m, phenyl), 7.71−7.77 (2H, m, phenyl); MS *m/e* (FAB) 352 (MH^+). Anal. ($C_{19}H_{18}FN_5O\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

1-[2-[6-Amino-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinyl]-1-ethynyl]-1-cycloheptanol Hydrochloride (15p). This compound was prepared in a manner similar to that described for **15f**, except that 1-ethynyl-1-cycloheptanol was used instead of 1-ethynyl-1-cyclohexanol. ^{1}H NMR (DMSO-d_6) δ 1.48−1.69 (8H, m, c-C_7H_{12}), 1.78−1.90 (2H, m, c-C_7H_{12}), 1.96−2.06 (2H, m, c-C_7H_{12}), 3.85 (3H, s, Me), 7.44−7.51 (1H, m, phenyl), 7.64−7.71 (1H, m, phenyl), 7.71−7.78 (2H, m, phenyl); MS *m/e* (FAB) 380 (MH^+). Anal. ($C_{21}H_{22}FN_5O\cdot HCl$) C, H, N.

4-[6-Amino-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinyl]-2-methyl-3-butyn-2-ol Hydrochloride (15q). This compound was prepared in a manner similar to that described for **15f**, except that 3-methyl-1-butyn-3-ol was used instead of 1-ethynyl-1-cyclohexanol. ^{1}H NMR (DMSO-d_6) δ 1.47 (6H, s, 2×Me), 3.81 (3H, s, 9-Me), 7.40−7.47 (1H, m, phenyl), 7.60−7.67 (1H, m, phenyl), 7.69−7.74 (2H, m, phenyl); MS *m/e* (FAB) 326 (MH^+). Anal. ($C_{17}H_{16}FN_5O\cdot HCl\cdot{}^1/_{10}H_2O$) C, H, N.

1-[6-Amino-8-(3-fluorophenyl)-9-methyl-9*H*-2-purinyl]-3-ethyl-1-pentyn-3-ol Hydrochloride (15r). This compound was prepared in a manner similar to that described for **15f**, except that 3-ethyl-1-pentyn-3-ol was used instead of 1-ethynyl-1-cyclohexanol. ^{1}H NMR (DMSO-d_6) δ 1.00 (6H, t, J = 7.2 Hz, 2×CH_2CH_3), 1.61−1.71 (4H, m, 2×CH_2CH_3), 3.81 (3H, s, Me), 7.40−7.44 (1H, m, phenyl), 7.61−7.67 (1H, m, phenyl), 7.69−7.74 (2H, m, phenyl); MS *m/e* (FAB) 354 (MH^+). Anal. ($C_{19}H_{20}FN_5O\cdot HCl\cdot{}^1/_2H_2O$) C, H, N.

6-Chloro-2-(2-cyclopentyl-1-ethynyl)-8-(3-fluorophenyl)-9-methyl-9*H*-purine (14s). This compound was prepared in a manner similar to that described for **14f**, except that cyclopentylacetylene was used instead of 1-ethynyl-1-cyclohexanol. ^{1}H NMR (DMSO-d_6) δ 1.54−1.79 (6H, m, c-C_5H_9), 1.99−2.09 (2H, m, c-C_5H_9), 2.97 (1H, quint, J = 7.2 Hz, c-C_5H_9), 3.91 (3H, s, Me), 7.50−7.55 (1H, m, phenyl), 7.66−7.73 (1H, m, phenyl), 7.77−7.83 (2H, m, phenyl).

2-(2-Cyclopentyl-1-ethynyl)-8-(3-fluorophenyl)-9-methyl-9*H*-6-purinylamine (15s). A solution of **15s** (100 mg, 0.282 mmol) in concentrated aqueous ammonia (10 mL)−1,2-dimethoxyethane (20 mL) in a sealed steel tube was heated at 70 °C for 9.5 h and then allowed to cool to room temperature. The reaction mixture was partitioned between EtOAc and saturated aqueous NH_4Cl. The separated organic phase was washed with saturated aqueous NH_4Cl (×2), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane, hexane:EtOAc = 4:1, 2:1, 3:2). This was suspended in Et_2O, and the precipitated product was filtered off and washed with Et_2O to give **15s** (70 mg, 74%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.52−1.78 (6H, m, c-C_5H_9), 1.93−2.04 (2H, m, c-C_5H_9), 2.86 (1H, quint, J = 7.2 Hz, c-C_5H_9), 3.79 (3H, s, Me), 7.37−7.46 (3H, m, NH_2 and phenyl), 7.59−7.66 (1H, m, phenyl), 7.68−7.74 (2H, m, phenyl); MS *m/e* (ESI) 336 (MH^+). Anal. ($C_{19}H_{18}FN_5\cdot{}^1/_4H_2O$) C, H, N.

6-Chloro-8-(3-fluorophenyl)-9-methyl-9*H*-purine (16). Isoamyl nitrite (1.5 mL, 11.2 mmol) was added to a mixture of **12f** (1.00 g, 3.60 mmol) and CuI (686 mg, 3.60 mmol) in THF (20 mL). The mixture was heated under reflux for 2 h and then allowed to cool to room temperature. The reaction mixture was partitioned between EtOAc and 1 N HCl. The separated organic phase was washed with concentrated aqueous ammonia and saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (eluent; hexane, hexane:EtOAc = 10:1, 8:1, 4:1) followed by precipitation with Et_2O to give **16** (446 mg, 47%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 3.95 (3H, s, Me), 7.50−7.56 (1H, m, phenyl), 7.67−7.73 (1H, m, phenyl), 7.79−7.84 (2H, m, phenyl), 8.84 (1H, s, 2-*H*).

8-(3-Fluorophenyl)-9-methyl-9*H*-6-purinylamine Hydrochloride (17). A solution of **16** (250 mg, 0.952 mmol) in concentrated aqueous ammonia (5 mL)-1,2-dimethoxyethane (10 mL) in a sealed steel tube was heated at 70 °C for 8 h and then allowed to cool to room temperature. The reaction mixture was partitioned between EtOAc and saturated aqueous NH_4Cl. The separated organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (eluent; CH_2Cl_2, CH_2Cl_2:MeOH = 40:1, 20:1). This was suspended in MeOH, 5 N HCl was added, and the solvent was evaporated. The residue was diluted with MeOH and Et_2O, and the precipitated product was filtered off and washed with Et_2O to give **17** (224 mg, 84%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 3.91 (3H, s, Me), 7.46−7.53 (1H, m, phenyl), 7.65−7.72 (1H, m, phenyl), 7.72−7.78 (2H, m, phenyl), 8.56 (1H, s, 2-*H*); MS *m/e* (ESI) 244 (MH^+). Anal. ($C_{12}H_{10}FN_5\cdot HCl\cdot{}^1/_{10}H_2O$) C, H, N.

***N*1-(6-Chloro-9-methyl-9*H*-2-purinyl)acetamide (18).** A suspension of **10** (2.30 g, 10.7 mmol) and concentrated HCl (0.7 mL) in triethyl orthoformate (70 mL) was stirred at room temperature for 16 h. The mixture was heated at 80 °C, and MeOH (35 mL) was added and stirred until the insoluble material disappeared (for about 10 min). The reaction mixture was allowed to cool to room temperature, and the solvent was evaporated. The residue was diluted with Et_2O, and the precipitated product was filtered off and washed with Et_2O to give **18** (2.14 g, 89%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 2.19 (3H, s, Ac), 3.77 (3H, s, Me), 8.47 (1H, s, 8-*H*), 10.82 (1H, br s, N*H*Ac).

6-Chloro-9-methyl-9*H*-2-purinylamine (19). A suspension of **18** (2.00 g, 8.86 mmol) in 1 N HCl (10 mL)−THF (40 mL) was heated under reflux for 1 h and then allowed to cool to room temperature. The solvent was evaporated. The residue was dissolved in H_2O and neutralized with saturated aqueous $NaHCO_3$. The resulting precipitate was filtered off and washed with H_2O to give **19** (0.89 g, 55%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 3.63 (3H, s, Me), 6.92 (2H, br s, NH_2), 8.08 (1H, s, 8-*H*).

6-Chloro-2-iodo-9-methyl-9*H*-purine (20). Isoamyl nitrite (1.9 mL, 14.1 mmol) was added to a mixture of **19** (850 mg, 4.63 mmol), CH_2I_2 (1.9 mL, 23.6 mmol), and CuI (882 mg, 4.63 mmol) in THF (20 mL). The mixture was heated under reflux for 1 h and then allowed to cool to room temperature. The reaction mixture was partitioned between EtOAc and 1 N HCl. The separated organic phase was washed with saturated aqueous NH_4Cl. The combined aqueous phase was extracted with EtOAc. The combined organic phase was washed with concentrated aqueous ammonia and saturated

aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was suspended in Et_2O. The precipitated product was filtered off and washed with Et_2O to give **20** (810 mg, 59%) as a brown solid. ^{1}H NMR (DMSO-d_6) δ 3.82 (3H, s, Me), 8.58 (1H, s, 8-*H*).

1-[2-(6-Chloro-9-methyl-9*H*-2-purinyl)-1-ethynyl]-1-cyclohexanol (21). Triethylamine (0.32 mL, 2.30 mmol) was added dropwise to a mixture of **20** (450 mg, 1.53 mmol), CuI (29 mg, 0.152 mmol), dichlorobis(triphenylphosphine)palladium-(II) (107 mg, 0.152 mmol), and 1-ethynyl-1-cyclohexanol (209 mg, 1.68 mmol) in THF (9 mL). The mixture was stirred under an atmosphere of N_2 at room temperature for 9 h and then partitioned between EtOAc and saturated aqueous NH_4Cl. The separated organic phase was washed with saturated aqueous NH_4Cl, concentrated aqueous ammonia, and saturated aqueous NH_4Cl, dried over Na_2SO_4, and then concentrated. The residue was suspended in Et_2O, and the precipitated product was filtered off and washed with Et_2O to give **21** (345 mg, 78%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.22−1.33 (1H, m, c-C_6H_{10}), 1.43−1.72 (7H, m, c-C_6H_{10}), 1.83−1.93 (2H, m, c-C_6H_{10}), 3.84 (3H, s, Me), 5.70 (1H, s, OH), 8.68 (1H, s, 8-*H*).

1-[2-(6-Amino-9-methyl-9*H*-2-purinyl)-1-ethynyl]-1-cyclohexanol (22). A solution of **21** (200 mg, 0.687 mmol) in concentrated aqueous ammonia (10 mL)-1,2-dimethoxyethane (20 mL) in a sealed steel tube was heated at 70 °C for 5 h and then allowed to cool to room temperature. The reaction mixture was partitioned between EtOAc and saturated aqueous NH_4-Cl. The separated organic phase was washed with saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (eluent; CH_2Cl_2, CH_2Cl_2:MeOH = 40:1, 20:1, 10:1). The product was suspended in MeOH, 5 N HCl was added, and the solvent was evaporated. The residue was diluted with MeOH and Et_2O, and the precipitated product was filtered off and washed with Et_2O to give **22** (97 mg, 46%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.20−1.32 (1H, m, c-C_6H_{10}), 1.43−1.70 (7H, m, c-C_6H_{10}), 1.81−1.90 (2H, m, c-C_6H_{10}), 3.76 (3H, s, Me), 8.01 (2H, br s, NH_2), 8.44 (1H, s, 8-*H*); MS *m*/*e* (ESI) 272 (MH^+). Anal. ($C_{14}H_{17}N_5O\cdot HCl\cdot{}^2/_5H_2O$) C, H, N.

Materials. Compounds **1**, **5**−**7**, **23**, and **24** were purchased from Sigma (St. Louis, MO). Compounds **2**,[23] **3**,[24c] and **4**[25b] were synthesized as reported. [^{3}H]-2-Chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA), [^{3}H]CGS21680, and [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide ([^{125}I]AB-MECA) were obtained from NEN Life Science, Inc. (Boston, MA).

Pharmacology. Inhibitory Activity on NECA-Induced Glucose Production in Primary Cultured Rat Hepatocytes. Hepatocytes were isolated by collagenase type I (Gibco BRL Products, Tokyo, Japan) digestion from livers of 5-weeks-old male Wistar rats (Charles River Japan Inc., Atsugi, Japan). Isolated hepatocytes were cultured in William's E medium. After having been cultured for 24 h, hepatocytes were washed and then incubated in 500 μL of Krebs Ringer bicarbonate buffer containing 0.1% bovine serum albumin for 30 min. To each well were added 100 μL of solution containing 0.6 μM NECA and the test antagonist (appropriate concentration). One hour thereafter, glucose released from the hepatocytes was assayed using a commercial kit (Glucose CII-test WAKO, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Agonist-Induced Glucose Production in Primary Cultured Rat Hepatocytes. Hepatocytes prepared as described above were stimulated with adenosine agonist, **5**, **6**, or **7** for 1 h. Released glucose resulting from glycogenolysis was measured.

Inhibitory Activity on NECA-Induced Cyclic AMP Production in CHO.K1 Cells Expressing Human Adenosine A_{2B} Receptor. A Chinese hamster ovary (CHO.K1) cell stably transfected with human adenosine A_{2B} receptor cDNA[4b] was used in this assay. Cells were cultured under 5% CO_2/95% O_2 atmosphere at 37 °C in D-MEM/F-12 (1:1 mixture) medium (Gibco BRL Products, Tokyo, Japan) with 10% fetal carf serum (Gibco BRL Products, Tokyo, Japan), 100 U/mL penicillin (Gibco BRL Products, Tokyo, Japan), 100 U/mL streptomycin (Gibco BRL Products, Tokyo, Japan), and 1 mg/mL G418 (Gibco BRL Products, Tokyo, Japan). Experimental cultures were grown overnight as a monolayer in 24-well tissue culture plates (1.5×10^5 cells/well). Each well was washed twice with 2 mL of Krebs buffer and then incubated in 0.5 mL of this buffer for 30 min. To each well was added 100 μL of solution containing 600 μM phosphodiesterase inhibitor Ro20-1724 (Research Biochemicals Inc., Natick, MA), 180 nM NECA, 6 U/mL adenosine deaminase, and the test compound (appropriate concentration). After incubation for 15 min, the reaction was terminated by removing medium and adding 0.1 N HCl (300 μL/well). The intracellular cyclic AMP contents were measured using a commercial radioimmunoassay (cyclic AMP EIA Kit, Amersham, Chicago, IL).

Human Adenosine A_1 Receptor Binding Assay. CHO.K1 cells stably transfected with human adenosine A_1 receptor cDNA[2b] were used in this assay. The transfected cells were maintained under 5% CO_2/95% O_2 atmosphere at 37 °C in D-MEM/F-12 (1:1 mixture) medium with 10% fetal carf serum, 100 U/mL penicillin, 100 U/mL streptomycin, and 1 mg/mL G418. Confluent monolayers of the cells were detached by 1 mM EDTA in phosphate buffered saline, then sonicated for about 15 s on ice, and then centrifuged for 15 min at 50000*g* and 4 °C. The membrane pellet was resuspended in HEPES buffer (20 mM HEPES, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.4). The binding assay was performed in a total volume of 500 μL containing membrane suspension, 60 nM [^{3}H]CCPA (25 μL), the appropriate concentration of test antagonist (25 μL), and 1 U/mL adenosine deaminase. The mixture was incubated for 2 h at 25 °C, filtered, and then washed twice with 5 mL of ice-cold buffer containing 50 mM Tris-HCl (pH 7.4) using a Brandel cell harvester. The radioactivity of the ligand bound was measured using a liquid scintillation counter.

Human Adenosine A_{2A} Receptor Binding Assay. Human embryonic kidney (HEK-293) cells stably transfected with human adenosine A_{2A} receptor cDNA[3b] were used in this assay. The A_{2A} receptor binding assay was performed in a manner similar to that described for A_1 binding assay, except that 500 nM [^{3}H]CGS21680 was used as radioligand.

Human Adenosine A_3 Receptor Binding Assay. HEK-293 cells stably transfected with human adenosine A_3 receptor cDNA[5b] was used in this assay. The A_3 receptor binding assay was performed in a manner similar to that described for the A_1 binding assay, except that 2 nM [^{125}I]AB-MECA was used as radioligand and the radioactivity of the ligand bound was measured using a γ-counter.

Hypoglycemic Effect in KK-A^y Mice. Male KK-A^y/Ta Jcl mice (Clea Japan Inc., Tokyo, Japan) were housed in individual cages at a constant temperature of 23 ±1 °C with 55 ± 5% humidity and a 12 h light−dark cycle and were provided with pellet food and water ad libitum. The indicated doses of the test compounds were suspended in 0.5% methylcellulose and orally administered to the mice. Control mice received the vehicle (0.5% methylcellulose) alone. Blood samples were obtained by bleeding from the tail vein at 0, 1, 3, and 5 h after drug administration, and blood glucose levels were monitored. The data were analyzed by one-way analysis of variance followed by Dunnett's multiple range test.

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Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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