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2-Alkynyl-8-aryladenines Possessing an Amide Moiety: Their Synthesis and Structure–Activity Relationships of Effects on Hepatic Glucose Production Induced Via Agonism of the A_{2B} Adenosine Receptor

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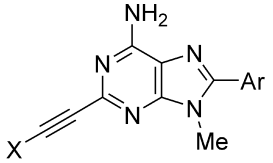
Abstract—A series of 2-alkynyl-8-aryladenine derivatives bearing an amide moiety at the 9-position of adenine was synthesized. These analogues were evaluated for inhibitory activity on *N*-ethylcarboxamidoadenosine (NECA)-induced glucose production in primary cultured rat hepatocytes. The *m*-primary benzamide derivative **15f** was the most potent compound ($IC_{50} = 0.017$ μM), being 15-fold more active than the corresponding 9-methyl derivative (**1**). Compound **15f** showed 72- and 5.2-fold selectivity for human A_{2B} receptor versus human A_1 and A_{2A} receptors, respectively. Structure–activity relationship (SAR) studies of the synthesized compounds indicated that a three-carbon linker, fixed in the form of a benzene ring, between the adenine core and the amide moiety is important for both A_{2B} antagonistic activity and selectivity. The IC_{50} values in rat hepatocyte glucose assay correlated well with the IC_{50} values in cAMP assay using Chinese hamster ovary cells stably transfected with human A_{2B} receptors ($r^2 = 0.94$). The A_1 and A_{2A} affinities showed no correlation with the potency to inhibit NECA-induced glucose production. These results strongly support our previous conclusion that adenosine agonist-induced hepatic glucose production in rat hepatocytes is mediated through the A_{2B} receptor.

Introduction

The purine nucleoside, adenosine, is produced in many organs and tissues, and has a variety of biological actions via adenosine receptors.[1] So far, four subtypes of adenosine receptors (i.e., A_1, A_{2A}, A_{2B}, and A_3) have been defined on the basis of pharmacological and molecular cloning characterization.[2–5] Activation of A_1 and A_3 receptors can lead to inhibition of adenylate cyclase activity, while this activity is stimulated by the activation of A_{2A} and A_{2B} receptors.[1]

Recently, we have discovered novel adenosine antagonists, 2-alkynyl-8-aryl-9-methyladenine derivatives (**1** and its analogues, Chart 1), as candidate antidiabetic agents by means of structure–activity relationship (SAR) studies on inhibitory activity towards NECA-induced glucose production in primary cultured rat hepatocytes, and we suggested that these hepatic effects are mediated through the A_{2B} receptor.[6] We have also shown that one of these compounds has hypoglycemic activity in genetically diabetic KK-A^y mice.[6]

In our previously reported SAR studies, we had fixed the 9-substituent of the adenine ring as a methyl group,[6] so we could not examine the role of the substituent at this position in A_{2B} antagonistic potency and selectivity. The nonselective adenosine agonist, NECA,[7] is a potent



1; Ar = 3-fluorophenyl

X = c-C_6H_{10}(OH)

Chart 1. Structure of 2-alkynyl-8-aryl-9-methyladenine derivatives.

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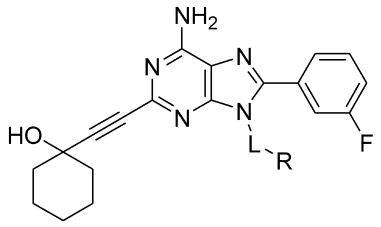


L = alkylene, phenyl ring

R = amide, carbamate, sulfonamide

Chart 2. Structure of 2-alkynyl-8-aryladenines possessing an amide moiety.

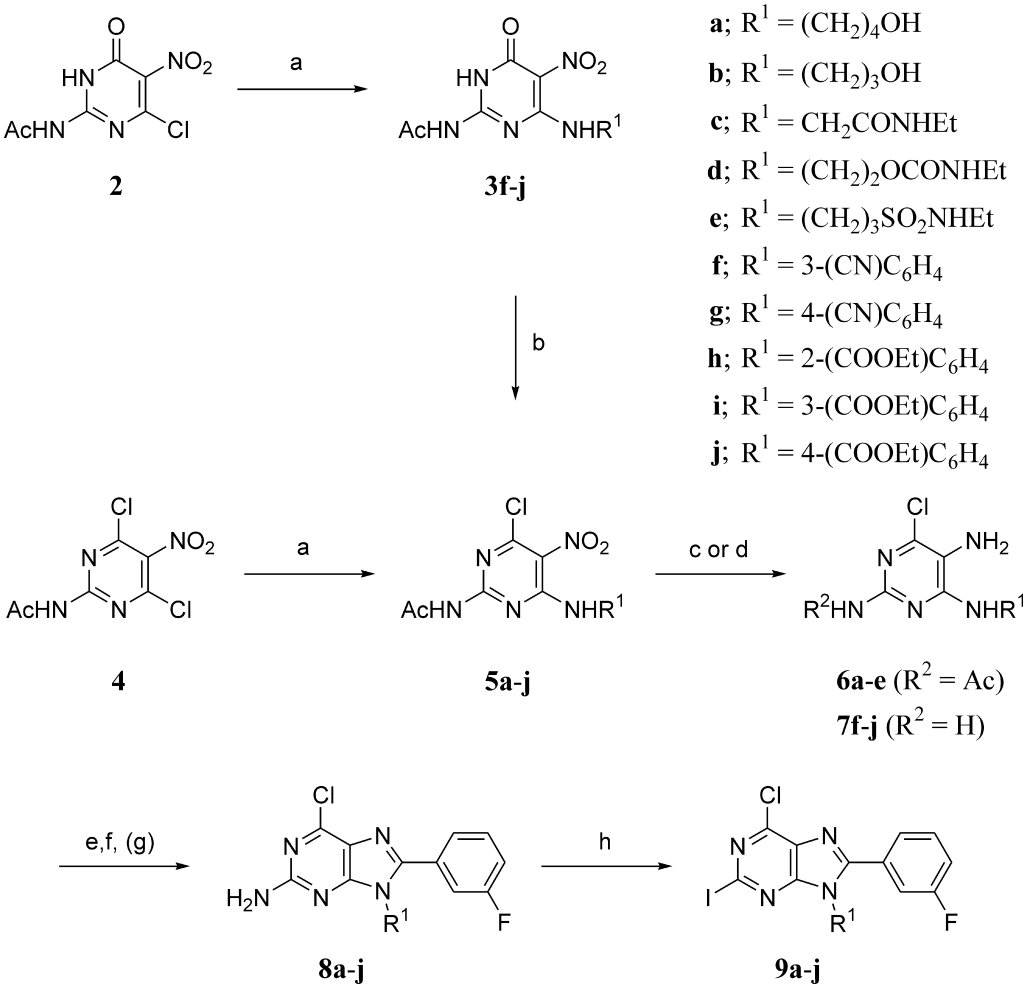
A_{2B} receptor ligand,[8,9] and its 5′-amide group has been found to be favorable for high-affinity binding to the A_{2B} receptor.[9] Therefore, it is of interest to introduce various substituents bearing an amide moiety at the 9-position of 2-alkynyl-8-aryladenines (Chart 2).

In the present study, as a continuation of our previous work,[6] we synthesized a series of 2-alkynyl-8-aryladenine derivatives bearing an amide moiety at the 9-position of the adenine core, and evaluated their inhibitory activities toward NECA-induced glucose production in rat hepatocytes. The A_{2B} antagonistic activities of these compounds were also measured in terms of the inhibitory activities on NECA-induced cyclic AMP accumulation in CHO.K1 cells stably transfected with human adenosine A_{2B} receptor cDNA.[4b] Moreover, to examine the selectivity of our compounds, we tested their affinities for human A_1 and A_{2A} receptors using a radioligand binding technique.

Results and Discussion

Chemistry

The 2-alkynyl-8-aryl-9-amidoalkyladenine derivatives (**15a** and **15b**) were synthesized by using 6-chloro-2-iodo-9-hydroxyalkylpurine derivatives (**9a** and **9b**, respectively) as key intermediates, as previously reported in the synthesis of 2-alkynyl derivatives of NECA.[10,11] These intermediates were prepared starting from *N*1-(4,6-dichloro-5-nitropyrimidin-2-yl)acetamide (**4**),[12] and 4-amino-1-butanol or 3-amino-1-propanol according to our established method for the synthesis of 2-alkynyl-8-aryl-9-methyladenine derivatives (Scheme 1).[6]

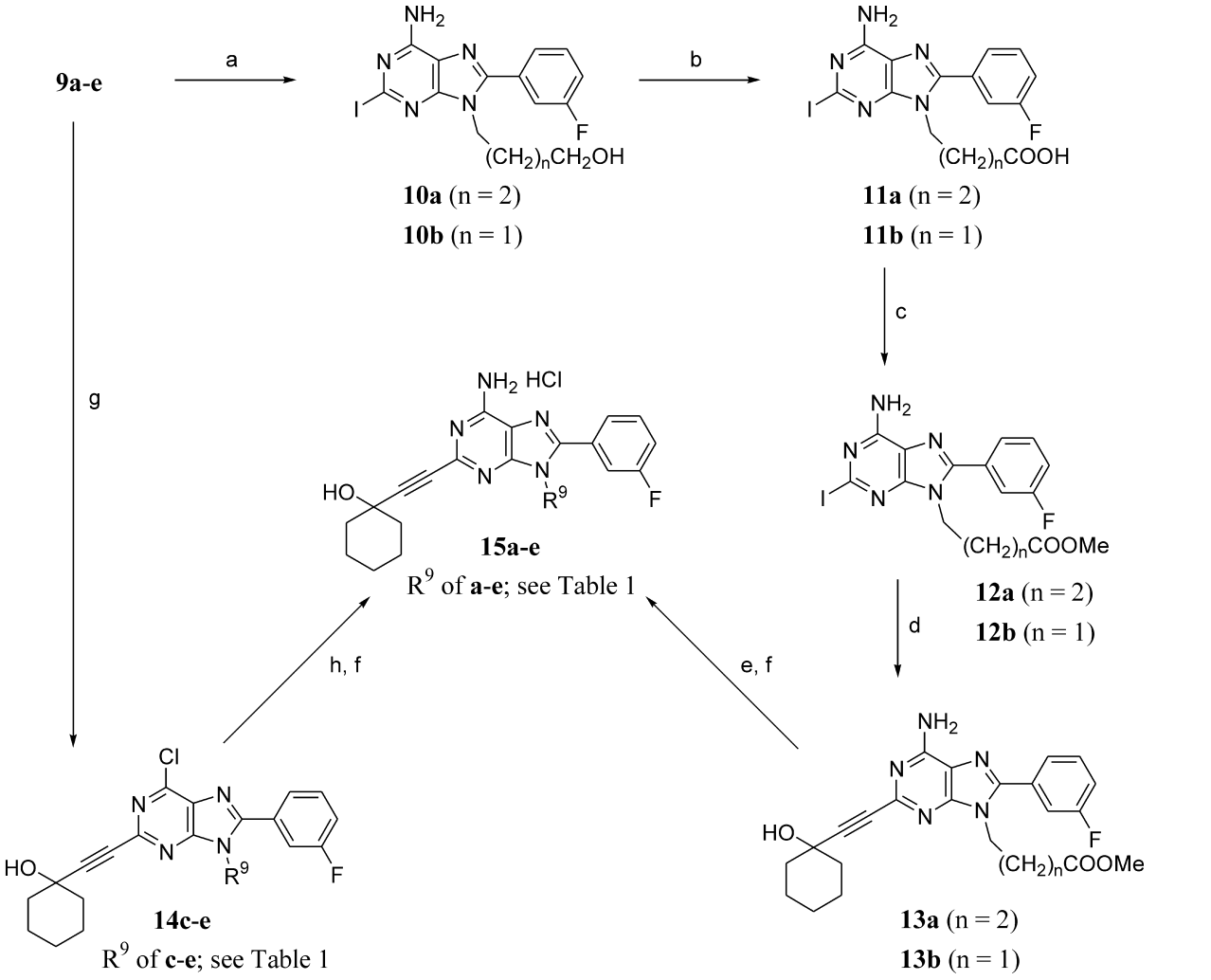


Scheme 1. Preparation of 8-aryl-6-chloro-2-iodopurine intermediates. Reagents: (a) R^1NH_2, AcOH/THF(–MeOH); (b) $POCl_3$, *N*,*N*-dimethylaniline, Et_4NCl/CH_3CN; (c) H_2, Raney Ni/MeOH; (d) $NaBH_4$, $SnCl_2{\cdot}2H_2O$/EtOH; (e) 3-fluorobenzaldehyde, AcOH/MeOH; (f) $FeCl_3$/EtOH; (g) HCl/H_2O–THF; (h) CH_2I_2, isoamyl nitrite, CuI/THF.

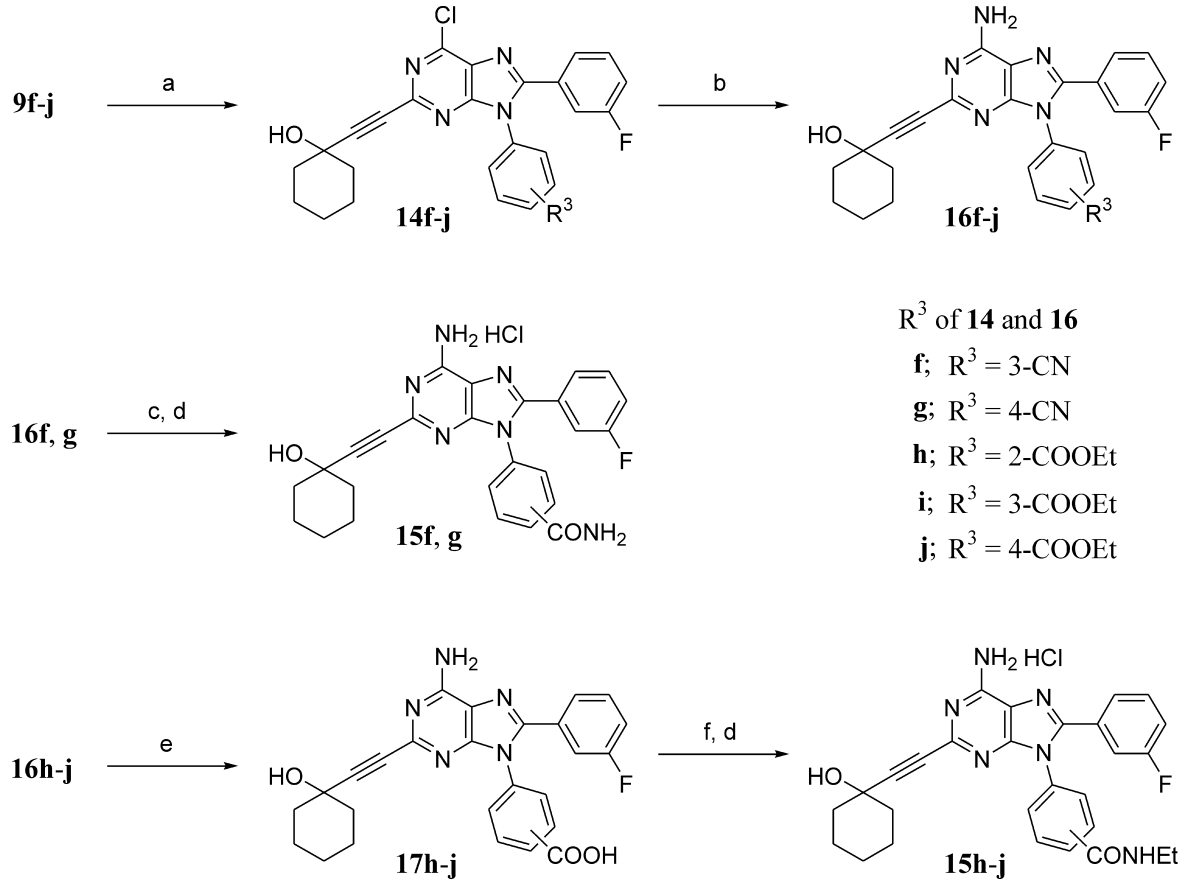
Regioselective amination[13] of the 6-position of **9** with saturated ammonia in MeOH in a sealed tube at 60 °C yielded the 6-amino compound (**10**). Oxidation of the hydroxyl group in **10** with RuO_4 (prepared from $RuO_2{\cdot}H_2O$ with $NaIO_4$ in a mixture of CH_3CN, $CHCl_3$, and H_2O),[11,14] followed by esterification[11] with thionyl chloride in methanol afforded the methyl ester (**12**). Palladium-catalyzed cross-coupling reaction[11,15] of **12** with terminal alkyne yielded the 2-alkynyl compound (**13**). Reaction[11] of **9** with ethylamine in EtOH–H_2O in a sealed tube at 80 °C followed by treatment with 20% HCl/EtOH gave the desired compounds (**15a** and **15b**) as the HCl salts (Scheme 2).

In the synthesis of **15c**, since oxidation of the primary alcohol was unsuccessful, we examined an alternative route in which the ethylamide moiety was introduced beforehand. That is, glycine ethylamide (**20**), readily prepared from *Z*-glycine (**18**) in two steps (Scheme 4), was reacted with **4** to give **5c** in the first step (Scheme 1). With the exception of the palladium-catalyzed cross-coupling reaction prior to ammonolysis, the following steps were basically the same as described above (Scheme 2).[6] The carbamate and sulfonamide derivatives (**15d** and **15e**) were similarly synthesized starting from readily available 2-aminoethyl *N*-ethylcarbamate (**24**) and *N*-ethyl-3-aminopropanesulfonamide (**29**), respectively (Schemes 2 and 4).

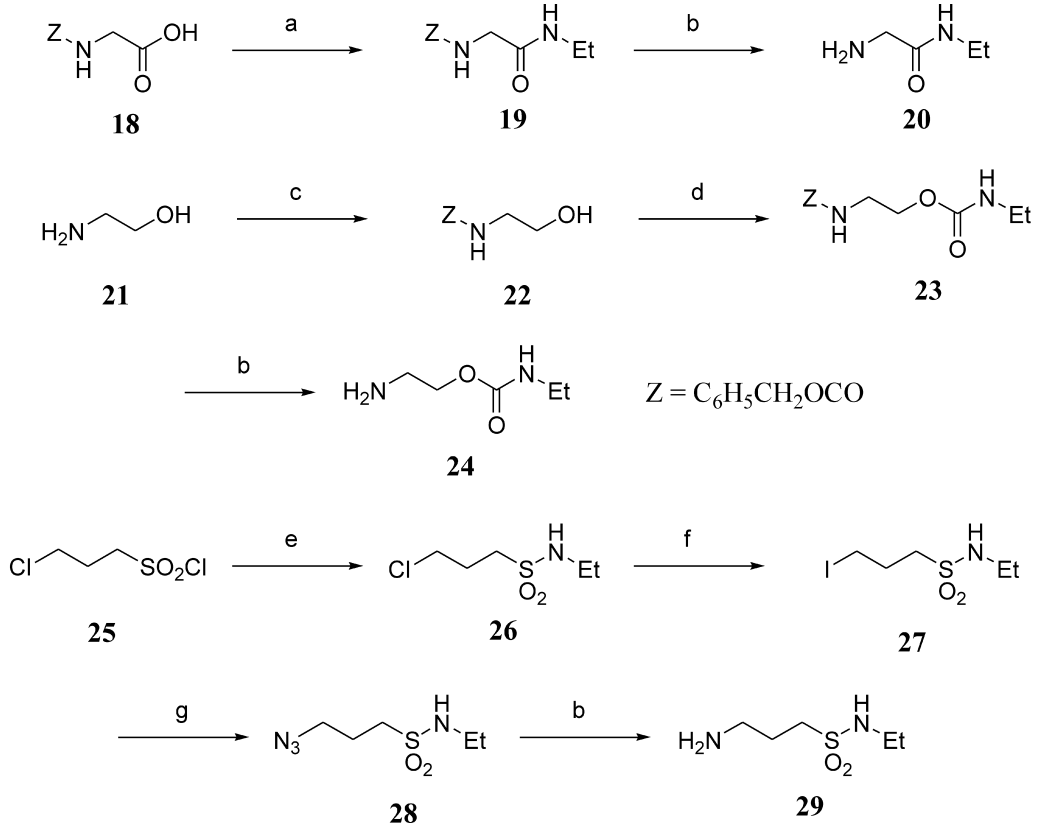
Primary benzamide derivatives (**15f** and **15g**) were obtained by oxidative hydrolysis[16] of the cyano group in **16f** and **16g**, respectively (Scheme 3). In the synthesis of these compounds, however, the reaction of **4** with aminobenzonitrile provided the diarylamino compound, as well as the desired monoarylamino compound (**5f** and **5g**). Monoarylamino pyrimidines (**5f** and **5g**) were obtained by replacement of the chlorine atom of *N*1-(6-chloro-3,4-dihydro-5-nitro-4-oxopyrimidine-2-yl)acetamide (**2**)[12] by aminobenzonitrile, followed by chlorination of the 4-oxo group. Additionally, because of the sensitivity of the cyano group to Raney nickel hydrogenation, reduction of the nitro groups of **5f** and **5g** was achieved by using the sodium borohydride-stannous chloride system,[17] along with deacetylation. Protection of the 2-amino group of pyrimidine with an acetyl group was not indispensable for ring closure to purine (Scheme 1).



Scheme 2. Synthesis of 9-amidoalkylademine derivatives. Reagents: (a) NH_3/MeOH; (b) $RuO_2{\cdot}H_2O$, $NaIO_4$/CH_3CN–$CHCl_3$–H_2O; (c) $SOCl_2$/MeOH; (d) 1-ethynyl-1-cyclohexanol, $(PPh_3)_2PdCl_2$, CuI, Et_3N/DMF; (e) $EtNH_2$/EtOH–H_2O; (f) HCl/MeOH; (g) 1-ethynyl-1-cyclohexanol, $(PPh_3)_2PdCl_2$, CuI, Et_3N/THF; (h) NH_3/EtOH.



Scheme 3. Synthesis of 9-(*N*-ethyl)benzamidoadenine derivatives. Reagents: (a) 1-ethynyl-1-cyclohexanol, $(PPh_3)_2PdCl_2$, CuI, Et_3N/THF; (b) NH_3/EtOH; (c) NaOH, H_2O_2/$CHCl_3$–MeOH–H_2O; (d) HCl/MeOH or EtOH; (e) NaOH/$CHCl_3$–MeOH–H_2O; (f) $EtNH_2$·HCl, Et_3N, WSC, HOBt/DMF.



Scheme 4. Preparation of alkylamines possessing an amide moiety. Reagents: (a) $EtNH_2$·HCl, Et_3N, WSC·HCl, HOBt/THF; (b) H_2, 10% Pd/C/MeOH; (c) *Z*-Cl, K_2CO_3/H_2O; (d) EtNCO, pyridine/CH_2Cl_2; (e) $EtNH_2$·HCl, Et_3N/CH_2Cl_2; (f) NaI/2-butanone; (g) NaN_3/MeOH–H_2O.

The synthesis of *N*-ethylbenzamide derivatives (**15h–j**) was achieved by condensation[18] of the carboxyl group in **17h–j** with ethylamine (Scheme 3). Compounds **17h–j** were obtained by alkaline hydrolysis of carboxylic ester derivatives (**16h–j**) prepared in a similar manner to that described for **16f** and **16g**.

Our synthetic method allowed the introduction of a variety of substituents (substituted alkyl and aromatic groups) at the 9-position of adenine.

Pharmacology

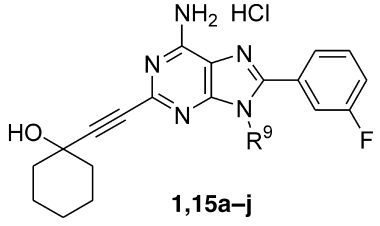
All the synthesized compounds were tested in rat hepatocyte glucose assay. The methods for the preparation of hepatocytes and for the inhibition assay of glucose production are described in the Experimental. The 9-methyladenine compound (**1**)[6] was used as an active control in each screening assay. The inhibitory activities of the title compounds toward NECA-induced glucose production in rat hepatocytes are summarized in Table 1.

The 9-(*N*-ethylamidopropyl)adenine compound (**15a**) showed about 3.5-fold more potent inhibitory activity than the 9-methyl compound (**1**)[6] (**15a**, $IC_{50} = 0.071$ μM; **1**, $IC_{50} = 0.25$ μM). The potency was significantly attenuated when the number of methylene groups between the adenine backbone and the amide group was decreased (**15b**, $IC_{50} = 1.6$ μM; **15c**, $IC_{50} = 23$ μM). The optimum length of the alkyl linker for activity appeared to be three methylene groups. The carbamate derivative (**15d**) and sulfonamide derivative (**15e**) showed significant decreases in activity ($IC_{50} = 14$ and 8.2 μM, respectively).

N-Ethylbenzamide derivatives (**15h–j**) were also synthesized and evaluated in the rat hepatocyte glucose assay. Among the three regioisomers, the *meta*-derivative (**15i**), with three carbons between the adenine backbone and the amide group as in **15a**, was the most potent ($IC_{50} = 0.044$ μM). Moving the amide group of **15i** from the *meta*- to the *para*- or the *ortho*-position markedly decreased the activity (**15j**, $IC_{50} = 0.52$ μM; **15h**, $IC_{50} > 30$ μM). The primary benzamide analogue of **15i** (**15f**) displayed more potent inhibitory activity than its parent compound (**15f**, $IC_{50} = 0.017$ μM; **15i**, $IC_{50} = 0.044$ μM). Movement of the amide group from the *meta*- to the *para*-position diminished the potency (**15g**, $IC_{50} = 0.098$ μM; **15f**, $IC_{50} = 0.017$ μM), but this tendency was not as marked as that observed in the *N*-ethylbenzamide derivatives. This may be attributed to the existence of steric limitation at the *para*-position of the 9-phenyl ring.

The A_{2B} antagonistic activities of the newly synthesized compounds were also measured in terms of inhibitory

Table 1. Inhibitory activities on NECA-induced glucose production in primary cultured rat hepatocytes and on NECA-induced cyclic AMP accumulation in CHO.K1 cells, and adenosine A_1 and A_{2a} receptor binding affinities



1,15a–j

Compd	R^9	IC_{50} (μM)[a]		K_i (μM)[b]	
		Hepatocyte	A_{2B}-CHO.K1 cell	A_1	A_{2A}
15a	$(CH_2)_3CONHEt$	0.071 ± 0.020	0.0080	0.0074	0.0080
15b	$(CH_2)_2CONHEt$	1.6	0.23	0.078	0.014
15c	$CH_2CONHEt$	23	1.8	1.4	0.025
15d	$(CH_2)_2OCONHEt$	14	1.8	0.90	0.18
15e	$(CH_2)_3SO_2NHEt$	8.2	0.96	0.081	0.028
15f	3-$(CONH_2)C_6H_4$	0.017 ± 0.002	0.0025	0.18	0.013
15g	4-$(CONH_2)C_6H_4$	0.098 ± 0.027	0.0040	0.086	0.0098
15h	2-(CONHEt)C_6H_4	> 30[c]	> 10[c]	> 10[c]	> 10[c]
15i	3-(CONHEt)C_6H_4	0.044 ± 0.014	0.024	0.36	0.019
15j	4-(CONHEt)C_6H_4	0.52	0.063	0.064	0.045
1[d]	Me	0.25 ± 0.04	0.023 ± 0.003	0.014 ± 0.003	0.016 ± 0.001
FK453[d]		8.1 ± 1.6	0.98 (0.92–1.0)	0.018 ± 0.002	1.3 ± 0.4
KF17837[d]		> 10 (> 10, > 10)[c]	1.5 (1.4–1.7)	> 10[c]	0.071 ± 0.014

[a]IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least three points). In cases where the number of independent experiments (n) > 2, data are shown as mean IC_{50} values (μM) ± SEM. In cases where $n = 2$, IC_{50} values are the means of two independent experiments (values of individual measurements in parentheses). In other cases where $n = 1$, the values are the results of one experiment performed at least in duplicate and in which individual determinations varied by less than 10%.

[b]K_i values were determined in radioligand binding assays for recombinant human A_1 and A_{2A} receptors expressed in CHO.K1 cells versus [^{3}H]CCPA and HEK-293 cells versus [^{3}H]CGS21680, respectively. Concentration-inhibition curves were obtained using 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.[21] In cases where $n > 2$, data are shown as mean K_i values (μM) ± SEM. In cases where $n = 1$, the values are the results of one experiment performed at least in duplicate and in which individual determinations varied by less than 10%.

[c]> 10 and > 30 mean that the IC_{50} values were greater than 10 and 30 μM, respectively.

[d]Data from our previous report.[6]

activities on NECA-induced cyclic AMP accumulation in CHO.K1 cells stably transfected with human adenosine A_{2B} receptor cDNA. The compounds antagonized NECA-induced stimulation of cyclic AMP production in a dose-dependent manner, and the IC_{50} values are listed in Table 1.

To assess the selectivity of our compounds for the A_{2B} receptor, their affinities for human A_1 and A_{2A} receptors were evaluated using a radioligand binding technique. An A_1 selective antagonist, (*E*)-(2*R*)-1-[3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl)acryloyl]-2-piperidineethanol (FK453)[8,19] and an A_{2A} selective antagonist, (*E*)-1,3-dipropyl-8-(3,4-dimethoxystyryl)-7-methylxanthine (KF17837),[8,20] were used as positive controls for A_1 and A_{2A} binding assay, respectively. The results are summarized in Table 1.

Our previously reported 9-methyl derivative (**1**) was a nonselective adenosine antagonist.[6] Among the 9-aliphatic amide derivatives (**15a**–**e**), there was no A_{2B}-selective compound. Fixing the amide moiety of **15a** with a benzene ring increased the selectivity for A_{2B} receptor versus A_1, but not A_{2A} (**15i**, 15-fold vs A_1). However, the *para*-isomer of **15i** (**15j**) lost this selectivity. The primary benzamide derivatives tended to be more A_{2B}-selective than the corresponding *N*-ethylbenzamide compounds (**15f** vs **15i**, **15g** vs **15j**). The *m*-primary benzamide compound (**15f**) showed 72- and 5.2-fold selectivity for human A_{2B} receptor versus human A_1 and A_{2A} receptors, respectively. Movement of the amide moiety of **15f** from the *meta*- to the *para*-position of the 9-phenyl ring decreased the selectivity (**15g**, 22- and 2.5-fold vs A_1 and A_{2A} receptors, respectively). These results indicate that fixing the amide moiety at the *meta*-position

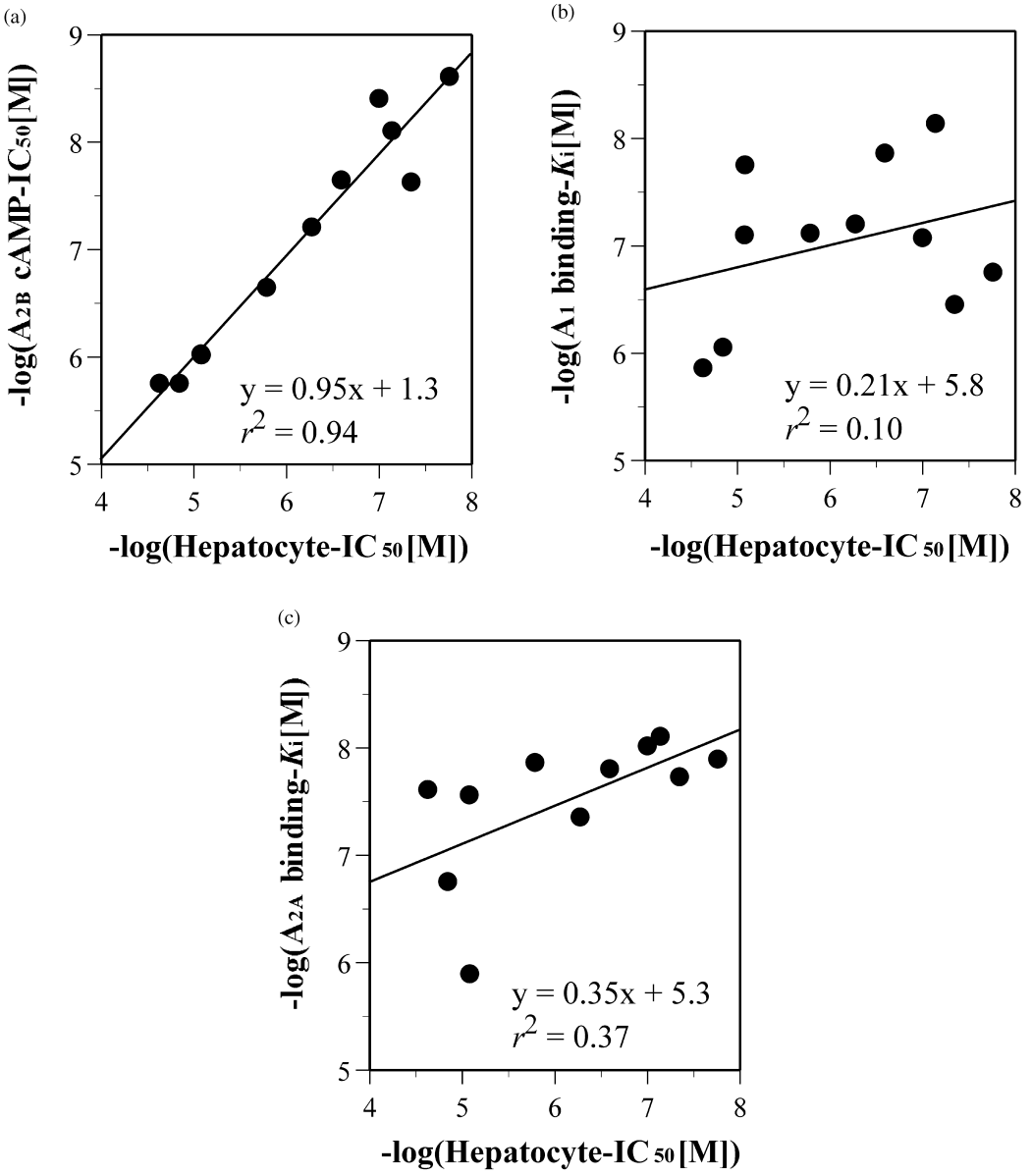


Figure 1. Correlation between inhibitory activities on NECA-induced glucose production in primary cultured rat hepatocytes and (a) inhibitory activities on NECA-induced cyclic AMP accumulation in CHO.K1 cells, (b) A_1 binding affinities, and (c) A_{2A} binding affinities (least-squares regression analysis).

of a benzene ring is favorable for not only A_{2B}-antagonistic activity, but also A_{2B} selectivity. Furthermore, it is worthy of note that the *N*-ethylacetamide derivative (**15c**) was highly selective for the A_{2A} receptor (56- and 72-fold vs A_1 and A_{2B} receptors, respectively).

The IC_{50} values in rat hepatocyte glucose assay were found to be well correlated with IC_{50} values in the A_{2B} cAMP assay [$r^2 = 0.94$, Fig. 1(a)]. The A_1 and A_{2A} affinities showed no correlation with the potency to inhibit NECA-induced glucose production [$r^2 = 0.10$ for A_1 receptor K_i and hepatocyte IC_{50}, Fig. 1(b); $r^2 = 0.37$ for A_{2A} receptor K_i and hepatocyte IC_{50}, Fig. 1(c)]. These results are consistent with our previous conclusion that adenosine agonist-induced hepatic glucose production in rat hepatocytes results from the activation of the A_{2B} receptor.

Conclusion

A series of 2-alkynyl-8-aryladenine derivatives bearing an amide moiety at the 9-position of adenine was synthesized. Using the screening system of adenosine agonist-induced hepatic glucose production, compounds **15a**, **15f**, and **15i** were found to be about 3.5–15-fold more potent than our previously reported compound (**1**). The most potent compound in hepatocyte glucose assay, **15f** ($IC_{50} = 0.017$ μM), showed 72- and 5.2-fold selectivity for human A_{2B} receptor versus human A_1 and A_{2A} receptors, respectively. SAR studies of our compounds indicated that a three-carbon linker between the adenine core and the amide moiety was important for A_{2B} antagonistic activity, and fixing the conformation of the amide group at the *meta*-position of a benzene ring imparted A_{2B} selectivity. Functional assay using recombinant human A_{2B} receptors confirmed that our compounds show A_{2B} antagonistic activity. The correlation between the IC_{50} values in rat hepatocyte glucose assay and in A_{2B} cAMP assay strongly support our previous conclusion that adenosine agonist-induced hepatic glucose production in rat hepatocytes is mediated through the A_{2B} receptor.

Experimental

Chemistry

Column chromatography was performed on silica gel (Merck, particle size 0.063–0.200 mm). TLC analyses were done 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) were obtained on a JEOL SX102 mass spectrometer. Mass spectra and elemental analyses were performed at the Analytical Chemistry Section of Eisai Research Laboratories.

***N*1-[4-Chloro-6-[(4-hydroxybutyl)amino]-5-nitro-2-pyrimidinyl]acetamide (5a). General procedure.** Glacial acetic acid (2.6 mL, 45.4 mmol) was carefully added with cooling to 4-amino-1-butanol (4.1 g, 46.0 mmol) in MeOH (10 mL). This solution was added dropwise to a stirred solution of *N*1-(4,6-dichloro-5-nitro-2-pyrimidinyl)acetamide (**4**) (5.00 g, 19.9 mmol) in THF (50 mL), previously cooled to 0 °C over 1 h. The mixture was stirred at this temperature for an additional 1.5 h, acidified with 1 N HCl to pH 2, and then diluted with EtOAc. The insoluble material was removed by filtration. The organic layer of the filtrate was washed with 1 N HCl, saturated aqueous $NaHCO_3$, and saturated aqueous NH_4Cl, dried over Na_2SO_4, and then concentrated. The residue was suspended in MeOH. The precipitated material was collected by filtration and washed with MeOH to give **5a** (2.98 g, 49%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.39–1.47 (2H, m, CH_2), 1.56–1.64 (2H, m, CH_2), 2.25 (3H, s, Ac), 3.40 (2H, t, $J = 6.4$ Hz, CH_2O), 3.48 (2H, dt, $J = 6.0$ and 7.2 Hz, NCH_2), 4.41 (1H, br, OH), 8.55 (1H, t, $J = 6.0$ Hz, N*H*CH_2), 10.78 (1H, s, N*H*Ac).

***N*1-[5-Amino-4-chloro-6-[(4-hydroxybutyl)amino]-2-pyrimidinyl]acetamide (6a). General procedure.** A suspension of **5a** (2.8 g, 9.22 mmol) in MeOH (60 mL) was hydrogenated in the presence of Raney nickel (2.8 g wet, washed with H_2O and MeOH) at room temperature and atmospheric pressure. The theoretical amount of H_2 was absorbed within 17 h. The reaction mixture was filtered through a Celite pad, which was then washed with MeOH, and the filtrate was evaporated. The residue was suspended in EtOH, and the resulting precipitate was collected by filtration and washed with EtOH to afford **6a** (1.43 g, 57%) as a pale yellow solid. ^{1}H NMR (DMSO-d_6) δ 1.40–1.50 (2H, m, CH_2), 1.52–1.62 (2H, m, CH_2), 2.09 (3H, br s, Ac), 3.30–3.43 (4H, m, CH_2O and NCH_2), 4.38 (1H, t, $J = 5.2$ Hz, OH), 4.67 (2H, br s, NH_2), 6.85 (1H, br, N*H*CH_2), 9.70 (1H, br s, N*H*Ac).

4-[2-Amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]-1-butanol (8a). General procedure. A mixture of **6a** (5.0 g, 18.3 mmol), 3-fluorobenzaldehyde (2.7 g, 21.8 mmol), and acetic acid (1.2 mL) in MeOH (100 mL) was stirred at room temperature for 28.5 h. The reaction mixture was concentrated to dryness under reduced pressure. The residue was azeotroped with toluene (50 mL×2), and used directly in the next step. To a suspension of the crude imine in EtOH (100 mL) was added a solution of anhydrous $FeCl_3$ (3.0 g, 18.5 mmol) in EtOH (20 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 **8a** (3.60 g) as a pale yellow solid. Examination of the ^{1}H NMR spectrum indicated that a small amount of the *N*-acetyl purine derivative remained in the crude material. A solution of crude **8a** (3.60 g) in 1 N HCl (25 mL)–THF (100 mL) was heated under reflux for 1 h 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 **8a** (2.86 g, 47%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.21–1.28 (2H, m, CH_2), 1.60–1.69 (2H, m, CH_2), 3.26 (2H, t, J = 6.4 Hz, CH_2O), 4.19 (2H, t, J = 7.2 Hz, NCH_2), 7.02 (2H, br s, NH_2), 7.40–7.47 (1H, m, phenyl), 7.60–7.66 (3H, m, phenyl).

4-[6-Chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]-1-butanol (9a). General procedure. Isoamyl nitrite (3.0 mL, 22.3 mmol) was added to a mixture of **8a** (2.50 g, 7.45 mmol), CH_2I_2 (3.0 mL, 37.2 mmol), and CuI (1.42 mg, 7.46 mmol) in THF (50 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 the aqueous ammonia layer was extracted with EtOAc. The combined organic layer was washed with saturated aqueous NH_4Cl, 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) to give a crude material. This crude product was suspended in Et_2O, and the resulting precipitate was collected by filtration to afford **9a** (1.78 g, 54%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.23–1.31 (2H, m, CH_2), 1.65–1.74 (2H, m, CH_2), 3.28 (2H, dt, J = 5.6 and 6.0 Hz, CH_2O), 4.36 (2H, t, J = 7.2 Hz, NCH_2) 4.38 (1H, t, J = 5.6 Hz, OH), 7.50–7.56 (1H, m, phenyl), 7.66–7.74 (3H, m, phenyl).

4-[6-Amino-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]-1-butanol (10a). General procedure. A solution of **9a** (2.51 g, 5.60 mmol) in methanolic ammonia (saturated at 0 °C) (250 mL) in a sealed steel tube was heated at 60 °C for 48 h and then allowed to cool to room temperature. The solvent was removed in vacuo, and EtOH and H_2O were added. The resulting precipitate was filtered off and washed with EtOH and Et_2O to give **10a** (1.50 g, 63%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.18–1.26 (2H, m, CH_2), 1.58–1.67 (2H, m, CH_2), 3.26 (2H, dt, J = 5.2 and 5.6 Hz, CH_2O), 4.22 (2H, t, J = 7.2 Hz, NCH_2), 4.36 (1H, t, J = 5.2 Hz, OH), 7.39–7.46 (1H, m, phenyl), 7.59–7.66 (3H, m, phenyl), 7.75 (2H, br s, NH_2).

4-[6-Amino-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]butanoic Acid (11a). General procedure. Ruthenium(IV) oxide hydrate (61 mg, 0.458 mmol) was added to a suspension of **10a** (1.30 g, 3.04 mmol) and $NaIO_4$ (3.30 g, 15.4 mmol) in a mixture of CH_3CN (26 mL), $CHCl_3$ (26 mL), and H_2O (39 mL). The mixture was vigorously stirred at room temperature for 4 h. The reaction was quenched by dropwise addition of 2-propanol (2.0 mL), and the mixture was adjusted with 1 N NaOH to pH 12–13. The insoluble material was removed by filtration and washed with MeOH. The organic solvent of the filtrate was evaporated and acidified with 1 N HCl to pH 2–3. The resulting precipitate was collected by filtration and washed with H_2O to give **11a** (1.21 g, 90%) as a grayish solid. ^{1}H NMR (DMSO-d_6) δ 1.86 (2H, quint, J = 7.2 Hz, NCH_2CH_2), 2.16 (2H, t, J = 7.2 Hz, CH_2CO), 4.23 (2H, t, J = 7.2 Hz, NCH_2), 7.39–7.46 (1H, m, phenyl), 7.57–7.66 (3H, m, phenyl), 7.76 (2H, br s, NH_2).

Methyl 4-[6-amino-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]butanoate (12a). General procedure. $SOCl_2$ (0.66 mL, 9.05 mmol) was added dropwise to a suspension of **11a** (800 mg, 1.81 mmol) in MeOH (20 mL) over 1 h at 2–5 °C. The mixture was stirred at room temperature for 1 h and concentrated to dryness under reduced pressure. The residue was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The separated organic phase was washed with saturated aqueous $NaHCO_3$ and saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was suspended in MeOH, and the precipitated product was collected by filtration and washed with MeOH to give **12a** (720 mg, 87%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.87 (2H, quint, J = 7.2 Hz, NCH_2CH_2), 2.22 (2H, t, J = 7.2 Hz, CH_2CO), 3.48 (3H, s, CH_3), 4.24 (2H, t, J = 7.2 Hz, NCH_2), 7.39–7.46 (1H, m, phenyl), 7.59–7.66 (3H, m, phenyl), 7.76 (2H, br s, NH_2).

Methyl 4-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]butanoate (13a). General procedure. Triethylamine (0.27 mL, 1.93 mmol) was added dropwise to a mixture of **12a** (450 mg, 0.989 mmol), CuI (19 mg, 0.0997 mmol), dichlorobis(triphenylphosphine)palladium(II) (69 mg, 0.0983 mmol), and 1-ethynyl-1-cyclohexanol (246 mg, 1.98 mmol) in *N*,*N*-dimethylformamide (5 mL). The mixture was stirred under an atmosphere of N_2 at room temperature for 13.5 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 MeOH. The precipitated product was filtered off and washed with MeOH to give **13a** (286 mg, 64%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.21–1.34 (1H, m, c-C_6H_{10}), 1.42–1.70 (7H, m, c-C_6H_{10}), 1.80–1.88 (2H, m, c-C_6H_{10}), 1.89 (2H, quint, J = 7.2 Hz, NCH_2CH_2), 2.22 (2H, t, J = 7.2 Hz, CH_2CO), 3.48 (3H, s, CH_3), 4.29 (2H, t, J = 7.2 Hz, NCH_2), 5.55 (1H, s, OH), 7.39–7.46 (1H, m, phenyl), 7.50 (2H, br s, NH_2), 7.59–7.66 (3H, m, phenyl).

***N*1-Ethyl-4-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]butanamide hydrochloride (15a). General procedure.** A solution of **13a** (663 mg, 1.47 mmol) in a 70% aqueous solution of ethylamine (20 mL) and MeOH (40 mL) in a sealed steel tube was heated at 80 °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 CH_2Cl_2, CH_2Cl_2/MeOH = 100:1, 50:1, 40:1, 30:1, 20:1) to give a crude material. The crude product was suspended in EtOAc, and the resulting precipitate was collected by filtration to afford the title compound (396 mg) as the free form. This was suspended in MeOH, 20% HCl/EtOH, 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 **15a** (400 mg, 54%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 0.91 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.20–1.32 (1H, m, c-C_6H_{10}), 1.40–1.67 (7H, m, c-C_6H_{10}), 1.78–1.87 (4H, m, c-C_6H_{10} and

NCH_2CH_2), 1.93 (2H, t, $J=7.1$ Hz, CH_2CO), 2.94 (2H, dq, $J=5.5$ and 7.2 Hz, CH_2CH_3), 4.25 (2H, t, $J=7.1$ Hz, NCH_2CH_2), 7.40–7.45 (1H, m, phenyl), 7.58–7.65 (3H, m, phenyl), 7.72 (1H, t, $J=5.5$ Hz, NHEt); MS *m/e* (FAB) 465 (MH^+). Anal. calcd for $C_{25}H_{29}FN_6O_2\cdot HCl\cdot{}^1/_2H_2O$: C, 58.88, H, 6.13, N, 16.48; found: C, 58.80, H, 6.18, N, 16.85.

***N*1-[4-Chloro-6-[(3-hydroxypropyl)amino]-5-nitro-2-pyrimidinyl]acetamide (5b).** This compound (pale yellow solid, 44% yield) was prepared in a manner similar to that described for **5a**, except that 3-amino-1-propanol was used instead of 4-amino-1-butanol. ^{1}H NMR (DMSO-d_6) δ 1.72 (2H, quint, $J=6.4$ Hz, $CH_2CH_2CH_2$), 2.25 (3H, s, Ac), 3.47 (2H, t, $J=6.4$ Hz, CH_2O), 3.55 (2H, dt, $J=5.6$ and 6.4 Hz, NCH_2), 4.56 (1H, br, OH), 8.63 (1H, t, $J=5.6$ Hz, $NHCH_2$), 10.82 (1H, s, NHAc).

***N*1-[5-Amino-4-chloro-6-[(3-hydroxypropyl)amino]-2-pyrimidinyl]acetamide (6b).** This compound (pale brown solid, 59% yield) was prepared in a manner similar to that described for **6a**, except that **5b** was used instead of **5a**. ^{1}H NMR (DMSO-d_6) δ 1.69 (2H, quint, $J=6.4$ Hz, $CH_2CH_2CH_2$), 2.08 (3H, br s, Ac), 3.37–3.47 (4H, m, CH_2O and NCH_2), 4.47 (1H, t, $J=5.4$ Hz, OH), 4.67 (2H, s, NH_2), 6.86 (1H, t, $J=5.2$ Hz, $NHCH_2$), 9.76 (1H, br s, NHAc).

3-[2-Amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]-1-propanol (8b). This compound (white solid, 46% yield) was prepared in a manner similar to that described for **8a**, except that **6b** was used instead of **6a**. ^{1}H NMR (DMSO-d_6) δ 1.78–1.87 (2H, m, $CH_2CH_2CH_2$), 3.34 (2H, t, $J=5.8$ Hz, CH_2O), 4.24 (2H, t, $J=7.6$ Hz, NCH_2), 7.02 (2H, br, NH_2), 7.40–7.46 (1H, m, phenyl), 7.59–7.69 (3H, m, phenyl).

3-[6-Chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]-1-propanol (9b). This compound (white solid, 62% yield) was prepared in a manner similar to that described for **9a**, except that **8b** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 1.82–1.92 (2H, m, $CH_2CH_2CH_2$), 3.36 (2H, dt, $J=4.8$ and 5.6 Hz, CH_2O), 4.40 (2H, t, $J=7.6$ Hz, NCH_2) 4.56 (1H, t, $J=4.8$ Hz, OH), 7.49–7.55 (1H, m, phenyl), 7.65–7.76 (3H, m, phenyl).

3-[6-Amino-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]-1-propanol (10b). This compound (white solid, 72% yield) was prepared in a manner similar to that described for **10a**, except that **9b** was used instead of **9a**. ^{1}H NMR (DMSO-d_6) δ 1.77–1.86 (2H, m, $CH_2CH_2CH_2$), 3.30–3.37 (2H, m, CH_2O), 4.25 (2H, t, $J=7.4$ Hz, NCH_2), 4.58 (1H, t, $J=4.8$ Hz, OH), 7.39–7.45 (1H, m, phenyl), 7.58–7.66 (3H, m, phenyl), 7.75 (2H, br s, NH_2).

3-[6-Amino-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]propanoic acid (11b). This compound (grayish solid, 85% yield) was prepared in a manner similar to that described for **11a**, except that **10b** was used instead of **10a**. ^{1}H NMR (DMSO-d_6) δ 2.75 (2H, t, $J=7.2$ Hz, CH_2CO), 4.38 (2H, t, $J=7.2$ Hz, NCH_2), 7.39–7.46 (1H, m, phenyl), 7.58–7.66 (3H, m, phenyl), 7.76 (2H, br s, NH_2).

Methyl 3-[6-amino-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]propanoate (12b). This compound (white solid, 77% yield) was prepared in a manner similar to that described for **12a**, except that **11b** was used instead of **11a**. ^{1}H NMR (DMSO-d_6) δ 2.82 (2H, t, $J=7.0$ Hz, CH_2CO), 3.49 (3H, s, CH_3), 4.43 (2H, t, $J=7.0$ Hz, NCH_2), 7.40–7.46 (1H, m, phenyl), 7.58–7.66 (3H, m, phenyl), 7.77 (2H, br s, NH_2).

Methyl 3-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]propanoate (13b). This compound (white solid, 76% yield) was prepared in a manner similar to that described for **13a**, except that **12b** was used instead of **12a**. ^{1}H NMR (DMSO-d_6) δ 1.21–1.33 (1H, m, c-C_6H_{10}), 1.42–1.70 (7H, m, c-C_6H_{10}), 1.80–1.90 (2H, m, c-C_6H_{10}), 2.85 (2H, t, $J=7.2$ Hz, CH_2CO), 3.49 (3H, s, CH_3), 4.47 (2H, t, $J=7.2$ Hz, NCH_2), 5.55 (1H, s, OH), 7.40–7.46 (1H, m, phenyl), 7.51 (2H, br s, NH_2), 7.61–7.67 (3H, m, phenyl).

***N*1-Ethyl-3-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]propanamide hydrochloride (15b).** This compound (pale yellow solid, 40% yield) was prepared in a manner similar to that described for **15a**, except that **13b** was used instead of **13a**. ^{1}H NMR (DMSO-d_6) δ 0.90 (3H, t, $J=7.2$ Hz, CH_2CH_3), 1.18–1.33 (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}), 2.55 (2H, t, $J=7.5$ Hz, CH_2CO), 2.93 (2H, dq, $J=5.5$ and 7.2 Hz, CH_2CH_3), 4.43 (2H, t, $J=7.5$ Hz, NCH_2CH_2), 7.39–7.45 (1H, m, phenyl), 7.58–7.66 (3H, m, phenyl), 7.90 (1H, t, $J=5.5$ Hz, NHEt); MS *m/e* (FAB) 451 (MH^+). Anal. calcd for $C_{24}H_{27}FN_6O_2\cdot HCl\cdot H_2O$: C, 57.08, H, 5.99, N, 16.64; found: C, 57.26, H, 5.72, N, 16.80.

Benzyl *N*-[2-(Ethylamino)-2-oxoethyl]carbamate (19). To a mixture of 2-[(benzyloxycarbonyl)amino]acetic acid (**18**) (15.0 g, 71.7 mmol), ethylamine hydrochloride (17.5 g, 215 mmol), 1-hydroxybenzotriazole (13.1 g, 85.6 mmol), and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (16.5 g, 86.1 mmol) in THF (300 mL) was added dropwise triethylamine (30.0 mL, 215 mmol) over 10 min at 5–10 °C. The mixture was stirred at room temperature for 13 h. The reaction mixture was partitioned between EtOAc and saturated aqueous NH_4Cl. The separated organic phase was washed with 1 N NaOH and saturated aqueous NH_4Cl, dried over Na_2SO_4, and concentrated. The residue was suspended in Et_2O, and the precipitated product was collected by filtration and washed with Et_2O to give **19** (10.4 g, 61%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 1.13 (3H, t, $J=7.2$ Hz, CH_2CH_3), 3.31 (2H, dq, $J=6.6$ and 7.2 Hz, CH_2CH_3), 3.84 (2H, d, $J=6.0$ Hz, CH_2CO), 5.13 (2H, s, CH_2Ph), 5.42 (1H, br, NH), 5.96 (1H, br, NH), 7.31–7.38 (5H, m, Ph).

***N*1-Ethyl-2-aminoacetamide (20).** A solution of **19** (9.0 g, 38.1 mmol) in MeOH (400 mL) was hydrogenated in the presence of 10% Pd/C (1.0 g) at room temperature and atmospheric pressure. The theoretical amount of H_2 was absorbed within 12.5 h. The reaction mixture was filtered through a Celite pad, which was then washed with MeOH, and the filtrate was evaporated to give **20**

(3.86 g, 99%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.02 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.04 (2H, s, CH_2CO), 3.07 (2H, br, NH_2), 3.10 (2H, dq, J = 5.8 and 7.2 Hz, CH_2CH_3), 7.79 (1H, br, NHEt).

***N*1-Ethyl-2-[2-(acetylamino)-6-chloro-5-nitro-4-pyrimidinyl]amino]acetamide (5c).** This compound (pale yellow solid, 44% yield) was prepared in a manner similar to that described for **5a**, except that **20** was used instead of 4-amino-1-butanol. ^{1}H NMR (DMSO-d_6) δ 1.00 (3H, t, J = 7.4 Hz, CH_2CH_3), 2.24 (3H, s, Ac), 3.08 (2H, dq, J = 5.6 and 7.4 Hz, CH_2CH_3), 4.06 (2H, d, J = 5.6 Hz, CH_2CO), 8.04 (1H, t, J = 5.6 Hz, NH), 8.79 (1H, t, J = 5.6 Hz, NH), 10.82 (1H, s, NHAc).

***N*1-Ethyl-2-[2-(acetylamino)-5-amino-6-chloro-4-pyrimidinyl]amino]acetamide (6c).** This compound (brown solid, 75% yield) was prepared in a manner similar to that described for **6a**, except that **5c** was used instead of **5a**. ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.06 (3H, s, Ac), 3.05 (2H, dq, J = 5.2 and 7.2 Hz, CH_2CH_3), 3.89 (2H, d, J = 5.2 Hz, CH_2CO), 4.73 (1H, br, NH), 7.34 (1H, t, J = 5.2 Hz, NH), 8.09 (2H, br s, NH_2), 9.87 (1H, br s, NHAc).

***N*1-Ethyl-2-[2-amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]acetamide (8c).** This compound (white solid, 27% yield) was prepared in a manner similar to that described for **8a**, except that **6c** was used instead of **6a**. ^{1}H NMR (DMSO-d_6) δ 0.97 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.07 (2H, dq, J = 5.2 and 7.2 Hz, CH_2CH_3), 4.73 (2H, s, CH_2CO), 7.03 (1H, br s, NH_2), 7.38–7.45 (1H, m, phenyl), 7.50–7.63 (3H, m, phenyl), 8.30 (1H, t, J = 5.2 Hz, NHEt).

***N*1-Ethyl-2-[6-chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]acetamide (9c).** This compound (pale brown solid, 68% yield) was prepared in a manner similar to that described for **9a**, except that **8c** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 0.97 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.07 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 4.95 (2H, s, CH_2CO), 7.48–7.54 (1H, m, phenyl), 7.61–7.70 (3H, m, phenyl), 6.15 (1H, t, J = 5.6 Hz, NHEt).

***N*1-Ethyl-2-[6-chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]acetamide (14c).** This compound (white solid, 88% yield) was prepared from **9c** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.22–1.37 (1H, m, c-C_6H_{10}), 1.44–1.74 (7H, m, c-C_6H_{10}), 1.84–1.96 (2H, m, c-C_6H_{10}), 3.08 (2H, dq, J = 6.0 and 7.2 Hz, CH_2CH_3), 4.98 (2H, s, CH_2CO), 5.72 (1H, s, OH), 7.48–7.56 (1H, m, phenyl), 7.61–7.72 (3H, m, phenyl), 8.41 (1H, t, J = 6.0 Hz, NHEt).

***N*1-Ethyl-2-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]acetamide Hydrochloride (15c).** A solution of **14c** (157 mg, 0.344 mmol) in ethanolic ammonia (saturated at 0 °C) (40 mL) in a sealed steel tube was heated at 100 °C for 12 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 CH_2Cl_2, CH_2Cl_2/MeOH = 100:1, 40:1, 30:1, 20:1) to give the title compound (90 mg) 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 **15c** (86 mg, 53%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.18–1.31 (1H, m, c-C_6H_{10}), 1.41–1.67 (7H, m, c-C_6H_{10}), 1.78–1.90 (2H, m, c-C_6H_{10}), 3.06 (2H, dq, J = 5.5 and 7.2 Hz, CH_2CH_3), 4.84 (2H, s, CH_2CO), 7.38–7.44 (1H, m, phenyl), 7.52–7.63 (3H, m, phenyl), 8.40 (1H, t, J = 5.5 Hz, NHEt); MS *m*/*e* (FAB) 437 (MH^+). Anal. calcd for $C_{23}H_{25}FN_6O_2 \cdot HCl \cdot H_2O$: C, 56.27, H, 5.75, N, 17.12; found: C, 55.97, H, 5.72, N, 16.93.

Benzyl *N*-(2-hydroxyethyl)carbamate (22). To a solution of ethanolamine (**21**) (15.0 g, 245 mmol) in H_2O (150 mL) were simultaneously added dropwise benzyl chloroformate (35.0 mL, 245 mmol) and aqueous 2 N Na_2CO_3 (125 mL, 250 mmol) over 2 h at 0–8 °C. After the addition was complete, the mixture was stirred at 0 °C for an additional 1 h and extracted with EtOAc. The organic layer was washed with H_2O, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated. The residue was diluted with hexane, and the resulting precipitate was collected by filtration and washed with hexane to afford **22** (40.0 g, 83%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 2.76 (1H, br s, OH), 3.32 (2H, q, J = 5.2 Hz, NCH_2), 3.67 (2H, t, J = 5.2 Hz, CH_2CH_2O), 5.09 (2H, s, CH_2Ph), 5.36 (1H, br, NH), 7.26–7.41 (5H, m, phenyl).

2-[(Benzyloxy)carbonyl)]amino]ethyl *N*-ethylcarbamate (23). To a solution of **22** (10.0 g, 51.2 mmol) and pyridine (1 drop) in CH_2Cl_2 (100 mL) was added dropwise ethyl isocyanate (10.6 mL, 134 mmol) at 0 °C, and the mixture was stirred at this temperature for 62 h. The solvent was removed under reduced pressure. The residue was diluted with Et_2O, and the resulting precipitate was collected by filtration and washed with Et_2O to afford **23** (9.0 g, 66%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.97 (2H, dq, J = 6.0 and 7.2 Hz, CH_2CH_3), 3.13–3.23 (2H, m, NCH_2CH_2), 3.93 (2H, t, J = 6.0 Hz, CH_2CH_2O), 5.01 (2H, s, CH_2Ph), 7.08 (1H, br, NH), 7.25–7.45 (6H, m, NH and phenyl).

2-Aminoethyl *N*-ethylcarbamate (24). A solution of **23** (8.50 g, 31.9 mmol) in MeOH (400 mL) was hydrogenated in the presence of 10% Pd/C (1.0 g) at room temperature and atmospheric pressure. The theoretical amount of H_2 was absorbed within 13 h. The reaction mixture was filtered through a Celite pad, which was then washed with MeOH, and the filtrate was evaporated to give **24** (4.38 g, quant) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.00 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.69 (2H, t, J = 6.0 Hz, NCH_2CH_2), 2.99 (2H, dq, J = 5.8 and 7.2 Hz, CH_2CH_3), 3.10 (2H, br, NH_2), 3.87 (2H, t, J = 6.0 Hz, CH_2O), 7.06 (1H, br, NH).

2-[2-(Acetylamino)-6-chloro-5-nitro-4-pyrimidinyl]amino]-ethyl *N*-ethylcarbamate (5d). This compound (pale

yellow solid, 57% yield) was prepared in a manner similar to that described for **5a**, except that **24** was used instead of 4-amino-1-butanol. ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.25 (3H, s, Ac), 2.97 (2H, dq, J = 5.2 and 7.2 Hz, CH_2CH_3), 3.69 (2H, dt, J = 5.2 and 5.8 Hz, NCH_2CH_2), 4.15 (2H, t, J = 5.8 Hz, CH_2O), 7.11 (1H, t, J = 5.2 Hz, NH), 8.56 (1H, t, J = 5.2 Hz, NH), 10.83 (1H, s, NHAc).

2-[2-(Acetylamino)-5-amino-6-chloro-4-pyrimidinyl]amino]-ethyl *N*-ethylcarbamate (6d). This compound (pale yellow solid, 73% yield) was prepared in a manner similar to that described for **6a**, except that **5d** was used instead of **5a**. ^{1}H NMR (DMSO-d_6) δ 1.00 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.09 (3H, br s, Ac), 3.00 (2H, dq, J = 5.2 and 7.2 Hz, CH_2CH_3), 3.53–3.63 (2H, m, NCH_2CH_2), 4.13 (2H, t, J = 5.8 Hz, CH_2O), 4.72 (2H, br s, NH_2), 7.04 (1H, t, J = 5.2 Hz, NH), 7.12 (1H, t, J = 5.2 Hz, NH), 9.80 (1H, s, NHAc).

2-[2-Amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]-ethyl *N*-ethylcarbamate (8d). This compound (white solid, 51% yield) was prepared in a manner similar to that described for **8a**, except that **6d** was used instead of **6a**. ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.84 (2H, dq, J = 5.2 and 7.2 Hz, CH_2CH_3), 4.23 (2H, t, J = 5.0 Hz, CH_2O), 4.36 (2H, t, J = 5.0 Hz, NCH_2CH_2), 6.96 (1H, t, J = 5.2 Hz, NHEt), 7.03 (2H, br s, NH_2), 7.39–7.46 (1H, m, phenyl), 7.56–7.64 (3H, m, phenyl).

2-[6-Chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]ethyl *N*-ethylcarbamate (9d). This compound (white solid, 60% yield) was prepared in a manner similar to that described for **9a**, except that **8d** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.81 (2H, dq, J = 5.0 and 7.2 Hz, CH_2CH_3), 4.22 (2H, t, J = 4.0 Hz, CH_2O), 4.56 (2H, t, J = 4.0 Hz, NCH_2CH_2), 6.95 (1H, t, J = 5.0 Hz, NHEt), 7.48–7.56 (1H, m, phenyl), 7.63–7.76 (3H, m, phenyl).

2-[6-Chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]ethyl *N*-ethylcarbamate (14d). This compound (colorless foam, 92% yield) was prepared from **9d** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.22–1.36 (1H, m, c-C_6H_{10}), 1.44–1.74 (7H, m, c-C_6H_{10}), 1.86–1.95 (2H, m, c-C_6H_{10}), 2.80 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 4.24 (2H, t, J = 4.8 Hz, CH_2O), 4.60 (2H, t, J = 4.8 Hz, NCH_2CH_2), 6.94 (1H, t, J = 5.6 Hz, NHEt), 7.48–7.58 (1H, m, phenyl), 7.59–7.78 (3H, m, phenyl).

2-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]ethyl *N*-ethylcarbamate hydrochloride (15d). This compound (white solid, 67% yield) was prepared in a manner similar to that described for **15c**, except that **14d** was used instead of **14c**. ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.19–1.32 (1H, m, c-C_6H_{10}), 1.41–1.70 (7H, m, c-C_6H_{10}), 1.80–1.92 (2H, m, c-C_6H_{10}), 2.80 (2H, dq, J = 5.5 and 7.2 Hz, CH_2CH_3), 4.19 (2H, t, J = 5.7 Hz, CH_2O), 4.44–4.52 (2H, m, NCH_2CH_2), 6.96 (1H, t, J = 5.5 Hz, NHEt), 7.40–7.48 (1H, m, phenyl), 7.58–7.66 (3H, m, phenyl); MS *m*/*e* (FAB) 467 (MH^+). Anal. calcd for $C_{24}H_{27}FN_6O_3$·HCl: C, 57.31, H, 5.61, N, 16.71; found: C, 57.12, H, 5.76, N, 16.61.

***N*1-Ethyl-3-chloro-1-propanesulfonamide (26).** Triethylamine (31.2 mL, 224 mmol) was added dropwise to a suspension of ethylamine hydrochloride (18.2 g, 223 mmol) in CH_2Cl_2 (150 mL) over 20 min with stirring at 2–5 °C, and 3-chloropropanesulfonyl chloride (**25**) (13.2 g, 74.6 mmol) was added dropwise at 3–10 °C for 1 h. The mixture was stirred at room temperature for a further 19 h. The reaction mixture was poured into H_2O, and the organic phase was washed with 1 N HCl and saturated aqueous $NaHCO_3$, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give **26** (10.4 g, 75%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.25–2.32 (2H, m, $CH_2CH_2CH_2$), 3.19 (2H, dq, J = 6.4 and 7.2 Hz, CH_2CH_3), 3.19 (2H, dd, J = 6.0 and 7.2 Hz, CH_2S), 3.69 (2H, t, J = 6.0 Hz, CH_2Cl), 4.26 (1H, br, NH).

***N*1-Ethyl-3-iodo-1-propanesulfonamide (27).** A mixture of **26** (10.0 g, 53.9 mmol) and NaI (24.2 g, 161 mmol) in 2-butanone (200 mL) was stirred under reflux for 6.5 h. The mixture was cooled, the solvent was evaporated, and the residue was partitioned between EtOAc and H_2O. The separated organic phase was washed with brine, dried over Na_2SO_4, and concentrated to give **27** (14.0 g, 84%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.29–2.36 (2H, m, $CH_2CH_2CH_2$), 3.07–3.26 (4H, m, CH_2CH_3 and CH_2S), 3.31 (2H, t, J = 6.4 Hz, CH_2I), 4.23 (1H, br, NH).

1-[3-(Ethylamino)sulfonyl]propyl]-1,2-triazadien-2-ium (28). A mixture of **27** (14.0 g, 45.3 mmol) and NaN_3 (7.6 g, 115 mmol) in H_2O (43 mL)–MeOH (15 mL) was stirred under reflux for 3 h. After having been cooled, the reaction mixture was diluted with EtOAc, washed with H_2O (×2) and brine, then dried over Na_2SO_4, and concentrated to give **28** (6.4 g, 74%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 1.23 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.04–2.11 (2H, m, $CH_2CH_2CH_2$), 3.10 (2H, dd, J = 6.0 and 7.2 Hz, CH_2S), 3.19 (2H, dq, J = 6.0 and 7.2 Hz, CH_2CH_3), 3.50 (2H, t, J = 6.4 Hz, CH_2N_3), 4.21 (1H, br, NH).

***N*1-Ethyl-3-amino-1-propanesulfonamide (29).** A solution of **28** (6.0 g, 31.2 mmol) in MeOH (250 mL) was hydrogenated in the presence of 10% Pd/C (3.0 g) at room temperature and atmospheric pressure. The theoretical amount of H_2 was absorbed within 10 h. The reaction mixture was filtered through a Celite pad, which was then washed with MeOH, and the filtrate was evaporated to give **29** (4.92 g, 95%) as pale yellow needles. ^{1}H NMR (DMSO-d_6) δ 1.07 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.65–1.72 (2H, m, $CH_2CH_2CH_2$), 2.60 (2H, t, J = 6.4 Hz, CH_2NH_2), 2.94 (2H, q, J = 7.2 Hz, CH_2CH_3), 2.98–3.03 (2H, m, CH_2S), 3.12 (2H, br, NH_2).

***N*1-[4-Chloro-6-[3-[(ethylamino)sulfonyl]propyl]amino]-5-nitro-2-pyrimidinyl]acetamide (5e).** This compound

(yellow powder, 54% yield) was prepared in a manner similar to that described for **5a**, except that **29** was used instead of 4-amino-1-butanol. ^{1}H NMR (DMSO-d_6) δ 1.05 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.90–2.00 (2H, m, $CH_2CH_2CH_2$), 2.23 (3H, s, Ac), 2.93 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 3.00–3.08 (2H, m, CH_2S), 3.58 (2H, dt, J = 5.6 and 6.4 Hz, NCH_2CH_2), 6.98 (1H, t, J = 5.6 Hz, N*H*), 8.63 (1H, t, J = 5.6 Hz, N*H*), 10.85 (1H, s, N*H*Ac).

***N*1-[5-Amino-4-chloro-6-[3-[(ethylamino)sulfonyl]propyl]-amino]-2-pyrimidinyl]acetamide (6e).** This compound (pale yellow solid, 57% yield) was prepared in a manner similar to that described for **6a**, except that **5e** was used instead of **5a**. ^{1}H NMR (DMSO-d_6) δ 1.05 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.90–2.00 (2H, m, $CH_2CH_2CH_2$), 2.10 (3H, br s, Ac), 2.95 (2H, dq, J = 6.0 and 7.2 Hz, CH_2CH_3), 3.02–3.10 (2H, m, CH_2S), 3.48 (2H, q, J = 6.0 Hz, NCH_2CH_2), 4.70 (2H, br s, NH_2), 7.00 (2H, two t, J = 6.0 Hz, two NH), 9.79 (1H, br s, N*H*Ac).

***N*1-Ethyl-3-[2-amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]-1-propanesulfonamide (8e).** This compound (pale yellow solid, 32% yield) was prepared in a manner similar to that described for **8a**, except that **6e** was used instead of **6a**. ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.03 (2H, quint, J = 7.2 Hz, $CH_2CH_2CH_2$), 2.85 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 2.92 (2H, t, J = 7.2 Hz, CH_2S), 4.28 (2H, t, J = 7.2 Hz, NCH_2CH_2), 6.96 (1H, t, J = 5.6 Hz, N*H*Et), 7.03 (2H, br s, NH_2), 7.40–7.48 (1H, m, phenyl), 7.58–7.78 (3H, m, phenyl).

***N*1-Ethyl-3-[6-chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]-1-propanesulfonamide (9e).** This compound (white solid, 29% yield) was prepared in a manner similar to that described for **9a**, except that **8e** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 1.01 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.08 (2H, quint, J = 7.2 Hz, $CH_2CH_2CH_2$), 2.88 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 3.02 (2H, t, J = 7.2 Hz, CH_2S), 4.46 (2H, t, J = 7.2 Hz, NCH_2CH_2), 6.98 (1H, t, J = 5.6 Hz, N*H*Et), 7.50–7.57 (1H, m, phenyl), 7.65–7.73 (3H, m, phenyl).

***N*1-Ethyl-3-[6-chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]-1-propanesulfonamide (14e).** This compound (colorless foam, 81% yield) was prepared from **9e** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 1.00 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.25–1.37 (1H, m, c-C_6H_{10}), 1.45–1.74 (7H, m, c-C_6H_{10}), 1.86–1.95 (2H, m, c-C_6H_{10}), 2.09 (2H, quint, J = 7.6 Hz, $CH_2CH_2CH_2$), 2.86 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 3.02 (2H, t, J = 7.6 Hz, CH_2S), 4.50 (2H, t, J = 7.6 Hz, NCH_2CH_2), 5.71 (1H, s, OH), 6.97 (1H, t, J = 5.6 Hz, N*H*Et), 7.50–7.58 (1H, m, phenyl), 7.59–7.75 (3H, m, phenyl).

***N*1-Ethyl-3-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]-1-propanesulfonamide hydrochloride (15e).** This compound (pale yellow solid, 53% yield) was prepared in a manner similar to that described for 15c, except that 14e was used instead of 14c. ^{1}H NMR (DMSO-d_6) δ 0.93–1.00 (3H, m, CH_2CH_3), 1.19–1.32 (1H, m, c-C_6H_{10}), 1.40–1.68 (7H, m, c-C_6H_{10}), 1.77–1.90 (2H, m, c-C_6H_{10}), 1.95–2.14 (2H, m, $CH_2CH_2CH_2$), 2.75–2.87 (2H, m, CH_2CH_3), 2.90–2.98 (2H, m, CH_2S), 4.35–4.42 (2H, m, NCH_2CH_2), 6.94–7.02 (1H, m, N*H*Et), 7.40–7.48 (1H, m, phenyl), 7.58–7.78 (3H, m, phenyl); MS *m*/*e* (FAB) 501 (MH^+). Anal. calcd for $C_{24}H_{29}FN_6O_3S{\cdot}HCl{\cdot}^1/_2H_2O$: C, 52.79, H, 5.72, N, 15.39; found: C, 52.83, H, 5.62, N, 15.40.

***N*1-[4-(3-Cyanoanilino)-5-nitro-6-oxo-1,6-dihydro-2-pyrimidinyl]acetamide (3f). General procedure.** *N*1-(6-Chloro-3,4-dihydro-5-nitro-4-oxopyrimidine-2-yl)acetamide (**2**) (15.0 g, 64.5 mmol) was added to a mixture of 3-aminobenzonitrile (19.0 g, 161 mmol) and glacial acetic acid (9.2 mL, 161 mmol) in THF (150 mL) at 5 °C, and the mixture was stirred at room temperature for 4 h. The precipitated solid was collected by filtration, washed with THF, H_2O, MeOH, and diethyl ether, and dried to give **3f** (19.0 g, 94%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 2.20 (3H, s, Ac), 7.60 (1H, t, J = 8.0 Hz, phenyl), 7.68–6.72 (1H, m, phenyl), 7.85–7.90 (1H, m, phenyl), 8.09 (1H, t, J = 2.0 Hz, phenyl), 11.07 (1H, s, N*H*Ph), 11.70 (2H, br, *N*1-H and N*H*Ac).

***N*1-[4-Chloro-6-(3-cyanoanilino)-5-nitro-2-pyrimidinyl]-acetamide (5f). General procedure.** A mixture of **3f** (5.0 g, 15.9 mmol), Et_4NCl (5.3 g, 32.0 mmol), *N*,*N*-dimethylaniline (4.0 mL, 31.6 mmol), and $POCl_3$ (15.7 mL, 191 mmol) in CH_3CN (100 mL) was heated under reflux for 10 h. After having been cooled, the mixture was poured into crushed ice (200 g) and vigorously stirred for 2 h. The resulting precipitate was collected by filtration, washed with H_2O, MeOH, and Et_2O, and dried to give **5f** (4.27 g, 81%) as a yellow solid. ^{1}H NMR (DMSO-d_6) δ 2.14 (3H, s, Ac), 7.52–7.52 (2H, m, phenyl), 8.03–8.09 (1H, m, phenyl), 8.64 (1H, s, phenyl), 10.10 (1H, s, N*H*Ph), 11.14 (1H, s, N*H*Ac).

3-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]benzonitrile (7f). General procedure. A stirred mixture of **5f** (3.80 g, 11.4 mmol) and $SnCl_2{\cdot}2H_2O$ (12.9 g, 57.2 mmol) in EtOH (380 mL) was heated to 60 °C, and $NaBH_4$ (216 mg, 5.71 mmol) in EtOH (38 mL) was added dropwise. The mixture was stirred at this temperature for 5.5 h. After having been cooled, the reaction mixture was concentrated to dryness, and the residue was diluted with H_2O. The mixture was neutralized with 1 N NaOH. The insoluble material was removed by filtration and washed thoroughly with THF and EtOAc. The filtrate was diluted with saturated aqueous NH_4Cl. The separated organic phase was washed with saturated aqueous NH_4Cl, dried over Na_2SO_4, and evaporated to give **7f** (2.10 g, 71%) as a pinkish solid. ^{1}H NMR (DMSO-d_6) δ 4.25 (2H, br s, NH_2), 6.09 (2H, br s, NH_2), 7.39–7.44 (1H, m, phenyl), 7.49 (1H, t, J = 8.0 Hz, phenyl), 7.99–8.04 (1H, m, phenyl), 8.35 (1H, t, J = 2.0 Hz, phenyl), 8.63 (1H, s, N*H*Ph).

3-[2-Amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]benzonitrile (8f). General procedure. A mixture of crude 2,5-diaminopyrimidine (**7f**) (8.0 g, 30.7 mmol), 3-

fluorobenzaldehyde (5.0 mL, 47.5 mmol), and acetic acid (14.0 mL) in MeOH (450 mL) was stirred at room temperature for 4.5 h. The reaction mixture was concentrated to dryness under reduced pressure. The residue was azeotropically distilled with toluene (×2), and used directly in the next step. To a suspension of the crude imine in EtOH (450 mL) was added a solution of anhydrous $FeCl_3$ (6.5 g, 40.1 mmol) in EtOH (50 mL) at room temperature, and the mixture was stirred for 1 h. The solvent was removed under reduced pressure, and the residue was suspended in a small amount of EtOH. The resulting precipitate was filtered off, washed with EtOH, and dried to give **8f** (7.1 g, 63%) as a slightly brown solid. The filtrate was evaporated to dryness. The residue was dissolved in EtOAc, washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was suspended in a small amount of EtOH, and the resulting precipitate was filtered off, washed with EtOH, and dried to give additional **8f** (2.1 g, 19%) as a slightly brown solid. ^{1}H NMR (DMSO-d_6) δ 7.12 (2H, s, NH_2), 7.21–7.34 (3H, m, 8-phenyl), 7.40–7.46 (1H, m, 8-phenyl), 7.72–7.81 (2H, m, 9-phenyl), 7.98–8.03 (1H, m, 9-phenyl), 8.09–8.12 (1H, m, 9-phenyl).

3-[6-Chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]benzonitrile (9f). This compound (white solid, 50% yield) was prepared in a manner similar to that described for **9a**, except that **8f** was used instead of **8a**. ^{1}H NMR ($CDCl_3$) δ 7.19–7.27 (2H, m, 8-phenyl), 7.31–7.40 (2H, m, 8-phenyl), 7.57–7.60 (1H, m, 9-phenyl), 7.64–7.66 (1H, m, 9-phenyl), 7.68–7.72 (1H, m, 9-phenyl), 7.84–7.87 (1H, m, 9-phenyl).

3-[6-Chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzonitrile (14f). This compound (pale yellow solid, 70% yield) was prepared from **9f** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR ($CDCl_3$) δ 1.27–1.40 (1H, m, c-C_6H_{10}), 1.57–1.78 (7H, m, c-C_6H_{10}), 2.01–2.08 (2H, m, c-C_6H_{10}), 2.13 (1H, s, OH), 7.18–7.24 (1H, m, 8-phenyl), 7.25–7.28 (1H, m, 8-phenyl), 7.32–7.40 (2H, m, 8-phenyl), 7.55–7.58 (1H, m, 9-phenyl), 7.66–7.72 (2H, m, 9-phenyl), 7.83–7.86 (1H, m, 9-phenyl).

3-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzonitrile (16f). This compound (pale yellow solid, 73% yield) was prepared in a manner similar to that described for **15c**, except that **14f** was used instead of **14c**. ^{1}H NMR (DMSO-d_6) δ 1.19–1.31 (1H, m, c-C_6H_{10}), 1.38–1.66 (7H, m, c-C_6H_{10}), 1.75–1.83 (2H, m, c-C_6H_{10}), 5.50 (1H, s, OH), 7.20–7.24 (1H, m, 8-phenyl), 7.27–7.34 (2H, m, 8-phenyl), 7.40–7.47 (1H, m, 8-phenyl), 7.69 (2H, br s, NH_2), 7.73–7.81 (2H, m, 9-phenyl), 8.02 (1H, dt, J = 2.0 and 7.2 Hz, 9-phenyl), 8.04–8.06 (1H, m, 9-phenyl).

3-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzamide hydrochloride (15f). General procedure. A mixture of **16f** (1.40 g, 3.10 mmol), 1 N NaOH (1.55 mL, 1.55 mmol), and 30% H_2O_2 (1.55 mL, 13.66 mmol) in MeOH (70 mL) was stirred at room temperature for 4 h. The precipitated solid was collected by filtration, washed with H_2O, and dried to give the desired compound (1.14 g) as the free form. This was suspended in EtOH, 6 N HCl was added, and the solvent was evaporated to give **15f** (1.14 g, 67%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.15–1.28 (1H, m, c-C_6H_{10}), 1.25–1.63 (7H, m, c-C_6H_{10}), 1.72–1.80 (2H, m, c-C_6H_{10}), 7.22–7.30 (3H, m, 8-phenyl), 7.37–7.43 (1H, m, 8-phenyl), 7.54–7.58 (1H, m, 9-phenyl), 7.60–7.64 (1H, m, 9-phenyl), 7.77 (1H, br, $CONH_2$), 7.89–7.91 (1H, m, 9-phenyl), 8.01–8.04 (1H, m, 9-phenyl), 8.08 (1H, br s, $CONH_2$); MS *m*/*e* (FAB) 471 (MH^+). Anal. calcd for $C_{26}H_{23}FN_6O_2 \cdot HCl \cdot {}^3/_4H_2O$: C, 60.00, H, 4.94, N, 16.15; found: C, 60.19, H, 4.72, N, 16.38.

***N*1-[4-(4-Cyanoanilino)-5-nitro-6-oxo-1,6-dihydro-2-pyrimidinyl]acetamide (3g).** This compound (pale yellow solid, quantitative yield) was prepared in a manner similar to that described for **3f**, except that 4-aminobenzonitrile was used instead of 3-aminobenzonitrile. ^{1}H NMR (DMSO-d_6) δ 2.19 (3H, s, Ac), 7.83 (4H, s, phenyl), 11.09 (1H, br s, N*H*Ph), 11.70 (2H, br, *N*1-H and N*H*Ac).

***N*1-[4-Chloro-6-(4-cyanoanilino)-5-nitro-2-pyrimidinyl]acetamide (5g).** This compound (yellow powder, 86% yield) was prepared in a manner similar to that described for **5f**, except that **3g** was used instead of **3f**. ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s, Ac), 7.77–7.81 (2H, m, phenyl), 8.16–8.21 (2H, m, phenyl), 10.17 (1H, br, N*H*Ph), 11.16 (1H, br s, N*H*Ac).

4-[(2,5-Diamino-6-chloro-4-pyrimidinyl)amino]benzonitrile (7g). This compound (brown powder, 88% yield) was prepared in a manner similar to that described for **7f**, except that **5g** was used instead of **5f**. ^{1}H NMR (DMSO-d_6) δ 4.32 (2H, br s, NH_2), 6.09 (2H, br s, NH_2), 7.71 (2H, d, J = 8.8 Hz, phenyl), 8.03 (2H, d, J = 8.8 Hz, phenyl), 8.76 (1H, br, N*H*Ph).

4-[2-Amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]benzonitrile (8g). This compound (ocher powder, 76% yield) was prepared in a manner similar to that described for **8f**, except that **7g** was used instead of **7f**. ^{1}H NMR (DMSO-d_6) δ 7.11 (2H, br s, NH_2), 7.18–7.23 (1H, m, 8-phenyl), 7.24–7.34 (2H, m, 8-phenyl), 7.39–7.46 (1H, m, 8-phenyl), 7.68 (2H, d, J = 8.4 Hz, 9-phenyl), 8.05 (2H, d, J = 8.4 Hz, 9-phenyl).

4-[6-Chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]benzonitrile (9g). This compound (white solid, 70% yield) was prepared in a manner similar to that described for **9a**, except that **8g** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 7.26–7.30 (1H, m, 8-phenyl), 7.33–7.44 (2H, m, 8-phenyl), 7.46–7.53 (1H, m, 8-phenyl), 7.72–7.76 (2H, m, 9-phenyl), 8.09–8.14 (2H, m, 9-phenyl).

4-[6-Chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzonitrile (14g). This compound (pale yellow solid, 67% yield) was prepared from **9g** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide

as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 1.22–1.34 (1H, m, c-C_6H_{10}), 1.39–1.52 (3H, m, c-C_6H_{10}), 1.54–1.70 (4H, m, c-C_6H_{10}), 1.80–1.89 (2H, m, c-C_6H_{10}), 5.67 (2H, s, OH), 7.28–7.32 (1H, m, 8-phenyl), 7.35–7.44 (2H, m, 8-phenyl), 7.47–7.54 (1H, m, 8-phenyl), 7.73–7.78 (2H, m, 9-phenyl), 8.10–8.14 (2H, m, 9-phenyl).

4-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzonitrile (16g). This compound (pale yellow solid, 79% yield) was prepared in a manner similar to that described for **15c**, except that **14g** was used instead of **14c**. ^{1}H NMR (DMSO-d_6) δ 1.22–1.34 (1H, m, c-C_6H_{10}), 1.38–1.68 (7H, m, c-C_6H_{10}), 1.74–1.84 (2H, m, c-C_6H_{10}), 5.50 (1H, s, OH), 7.19–7.24 (1H, m, 8-phenyl), 7.27–7.34 (2H, m, 8-phenyl), 7.40–7.48 (1H, m, 8-phenyl), 7.66 (2H, d, J = 8.4 Hz, phenyl), 7.70 (2H, br s, NH_2), 8.05 (2H, d, J = 8.4 Hz, phenyl).

4-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzamide hydrochloride (15g). This compound (white solid, 85% yield) was prepared in a manner similar to that described for **15f**, except that **16g** was used instead of **16f**. ^{1}H NMR (DMSO-d_6) δ 1.16–1.28 (1H, m, c-C_6H_{10}), 1.35–1.64 (7H, m, c-C_6H_{10}), 1.72–1.81 (2H, m, c-C_6H_{10}), 7.22–7.29 (3H, m, 8-phenyl), 7.39–7.44 (1H, m, 8-phenyl), 7.50 (2H, d, J = 8.2 Hz, 9-phenyl), 7.55 (1H, s, $CONH_2$), 7.99 (2H, d, J = 8.2 Hz, 9-phenyl), 8.13 (1H, s, $CONH_2$); MS *m*/*e* (FAB) 471 (MH^+). Anal. calcd for $C_{26}H_{23}FN_6O_2 \cdot HCl \cdot {}^3/_4H_2O$: C, 60.00, H, 4.94, N, 16.15; found: C, 60.00, H, 4.74, N, 16.10.

Ethyl 3-[2-(acetylamino)-5-nitro-6-oxo-1,6-dihydro-4-pyrimidinyl]amino]benzoate (3i). This compound (pale yellow solid, 94% yield) was prepared in a manner similar to that described for **3f**, except that ethyl 3-aminobenzoate was used instead of 3-aminobenzonitrile. ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.17 (3H, s, Ac), 4.34 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.56 (1H, t, J = 8.0 Hz, phenyl), 7.82–7.86 (1H, m, phenyl), 7.88–7.91 (1H, m, phenyl), 7.96 (1H, t, J = 2.0 Hz, phenyl), 11.06 (1H, s, N*H*Ph), 11.63 (2H, br, *N*1-H and N*H*Ac).

Ethyl 3-[(2-amino-6-chloro-5-nitro-4-pyrimidinyl)amino]-benzoate (5i). This compound (yellow solid, 78% yield) was prepared in a manner similar to that described for **5f**, except that **3i** was used instead of **3f**. ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.07 (3H, s, Ac), 4.30 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.49 (1H, t, J = 8.0 Hz, phenyl), 7.70–7.75 (1H, m, phenyl), 8.07 (1H, t, J = 2.0 Hz, phenyl), 8.39–8.44 (1H, m, phenyl), 10.10 (1H, s, N*H*Ph), 10.95 (1H, s, N*H*Ac).

Ethyl 3-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-benzoate (7i). This compound (brown solid, 82% yield) was prepared in a manner similar to that described for **7f**, except that **5i** was used instead of **5f**. ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.2 Hz, CH_2CH_3), 4.28 (2H, br, NH_2), 4.32 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.92 (2H, br s, NH_2), 7.43 (1H, t, J = 8.0 Hz, phenyl), 7.54–7.60 (1H, m, phenyl), 8.10 (1H, t, J = 2.0 Hz, phenyl), 8.04–8.40 (1H, m, phenyl), 8.59 (1H, br, N*H*Ph).

Ethyl 3-[2-amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]benzoate (8i). This compound (pale brown solid, 79% yield) was prepared in a manner similar to that described for **8f**, except that **7i** was used instead of **7f**. ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, J = 7.2 Hz, CH_2CH_3), 4.33 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.09 (2H, br s, NH_2), 7.22–7.32 (3H, m, 8-phenyl), 7.36–7.45 (1H, m, 8-phenyl), 7.65–7.74 (2H, m, 9-phenyl), 8.06–8.12 (2H, m, 9-phenyl).

Ethyl 3-[6-chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]benzoate (9i). This compound (white solid, 66% yield) was prepared in a manner similar to that described for **9a**, except that **8i** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, J = 7.2 Hz, CH_2CH_3), 4.34 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.30–7.42 (3H, m, 8-phenyl), 7.44–7.51 (1H, m, 8-phenyl), 7.70–7.82 (2H, m, 9-phenyl), 8.14–8.19 (2H, m, 9-phenyl).

Ethyl 3-[6-chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoate (14i). This compound (white solid, 74% yield) was prepared from **9i** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 1.31 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.24–1.35 (1H, m, c-C_6H_{10}), 1.40–1.52 (3H, m, c-C_6H_{10}), 1.52–1.70 (4H, m, c-C_6H_{10}), 1.80–1.88 (2H, m, c-C_6H_{10}), 4.33 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.65 (1H, s, OH), 7.32–7.42 (3H, m, 8-phenyl), 7.44–7.52 (1H, m, 8-phenyl), 7.73–7.82 (2H, m, 9-phenyl), 8.14–8.18 (2H, m, 9-phenyl).

Ethyl 3-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoate (16i). This compound (white solid, 90% yield) was prepared in a manner similar to that described for **15c**, except that **14i** was used instead of **14c**. ^{1}H NMR (DMSO-d_6) δ 1.18–1.28 (1H, m, c-C_6H_{10}), 1.31 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.38–1.66 (7H, m, c-C_6H_{10}), 1.74–1.83 (2H, m, c-C_6H_{10}), 4.33 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.49 (1H, s, OH), 7.22–7.33 (3H, m, 8-phenyl), 7.38–7.45 (1H, m, 8-phenyl), 7.66 (2H, br s, NH_2), 7.68–7.72 (2H, m, 9-phenyl), 8.00–8.03 (1H, m, 9-phenyl), 8.07–8.13 (1H, m, 9-phenyl).

3-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoic acid (17i). General procedure. A mixture of **16i** (798 mg, 1.60 mmol) and 1 N NaOH (30 mL) in $CHCl_3$–MeOH (2:1) (90 mL) was stirred at room temperature for 5 h. The mixture was acidified with 1 N HCl, and the precipitated solid was collected by filtration, washed with H_2O, and dried to give **17i** (644 mg, 86%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.16–1.27 (1H, m, c-C_6H_{10}), 1.37–1.65 (7H, m, c-C_6H_{10}), 1.72–1.81 (2H, m, c-C_6H_{10}), 5.49 (1H, br s, OH), 7.20–7.30 (3H, m, 8-phenyl), 7.36–7.43 (1H, m, 8-phenyl), 7.59–7.71 (4H, m, 9-phenyl and NH_2), 7.93–7.96 (1H, m, 9-phenyl), 8.03–8.09 (1H, m, 9-phenyl).

***N*1-Ethyl-3-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzamide hydrochloride (15i). General procedure.** A mixture of **17i** (200

mg, 0.424 mmol), 1-hydroxybenzotriazole (200 mg, 1.31 mmol), and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide (200 mg, 1.29 mmol) in DMF (5.0 mL) was stirred at room temperature for 4 h. Ethylamine hydrochloride (110 mg, 1.35 mmol) and triethylamine (0.18 mL, 1.29 mmol) were added, and the mixture was stirred for an additional 13 h. The solvent was removed in vacuo, and the residue was purified by silica gel column chromatography (eluent CH_2Cl_2, CH_2Cl_2/MeOH = 40:1, 20:1) to give the title compound (136 mg) 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 **15i** (126 mg, 56%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.10 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.14–1.28 (1H, m, c-C_6H_{10}), 1.34–1.64 (7H, m, c-C_6H_{10}), 1.71–1.81 (2H, m, c-C_6H_{10}), 3.26 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 7.22–7.30 (3H, m, 8-phenyl), 7.37–7.44 (1H, m, 8-phenyl), 7.53–7.57 (1H, m, 9-phenyl), 7.62 (1H, t, J = 8.0 Hz, 9-phenyl), 7.89 (1H, m, 9-phenyl), 8.00 (1H, m, 9-phenyl), 8.61 (1H, t, J = 5.6 Hz, NHEt); MS *m*/*e* (FAB) 499 (MH^+). Anal. calcd for $C_{28}H_{27}FN_6O_2 \cdot HCl \cdot {}^5/_4H_2O$: C, 60.32, H, 5.51, N, 15.07; found: C, 60.03, H, 5.68, N, 15.33.

Ethyl 2-[2-(acetylamino)-5-nitro-6-oxo-1,6-dihydro-4-pyrimidinyl]amino]benzoate (3h). This compound (pale yellow solid, 80% yield) was prepared in a manner similar to that described for **3f**, except that ethyl 2-aminobenzoate was used instead of 3-aminobenzonitrile. ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.18 (3H, s, Ac), 4.32 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.36 (1H, t, J = 8.0 Hz, phenyl), 7.66 (1H, dt, J = 1.6 and 8.0 Hz, phenyl), 7.97 (1H, dd, J = 1.6 and 8.0 Hz, phenyl), 8.02 (1H, d, J = 8.0 Hz, phenyl), 11.67 (2H, br, two NH), 12.08 (1H, br s, NH).

Ethyl 2-[(2-amino-6-chloro-5-nitro-4-pyrimidinyl)amino]-benzoate (5h). This compound (ocher powder, 91% yield) was prepared in a manner similar to that described for **5f**, except that **3h** was used instead of **3f**. ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.12 (3H, s, Ac), 4.35 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.29 (1H, t, J = 8.0 Hz, phenyl), 7.65 (1H, dt, J = 1.6 and 8.0 Hz, phenyl), 8.01 (1H, dd, J = 1.6 and 8.0 Hz, phenyl), 8.93 (1H, d, J = 8.0 Hz, phenyl), 11.15 (1H, br s, NHPh), 11.67 (1H, br s, NHAc).

Ethyl 2-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-benzoate (7h). This compound (yellow solid, 93% yield) was prepared in a manner similar to that described for **7f**, except that **5h** was used instead of **5f**. ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.88 (2H, br s, NH_2), 4.34 (2H, q, J = 7.2 Hz, CH_2CH_3), 6.22 (2H, br s, NH_2), 7.07 (1H, t, J = 8.0 Hz, phenyl), 7.57 (1H, dt, J = 1.6 and 8.0 Hz, phenyl), 7.98 (1H, dd, J = 1.6 and 8.0 Hz, phenyl), 8.92 (1H, d, J = 8.0 Hz, phenyl), 10.72 (1H, br s, NHPh).

Ethyl 2-[2-amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]benzoate (8h). This compound (ocher solid, 81% yield) was prepared in a manner similar to that described for **8f**, except that **7h** was used instead of **7f**. ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.88–4.01 (2H, m, CH_2CH_3), 7.03 (2H, br s, NH_2), 7.16–7.30 (3H, m, 8-phenyl), 7.36–7.42 (1H, m, 8-phenyl), 7.68–7.74 (2H, m, 9-phenyl), 7.83 (1H, dt, J = 1.6 and 8.0 Hz, 9-phenyl), 8.01 (1H, dd, J = 1.6 and 8.0 Hz, 9-phenyl).

Ethyl 2-[6-chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]benzoate (9h). This compound (white powder, 65% yield) was prepared in a manner similar to that described for **9a**, except that **8h** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, J = 7.2 Hz, CH_2CH_3), 3.88–4.00 (2H, m, CH_2CH_3), 7.26–7.32 (2H, m, 8-phenyl), 7.35–7.41 (1H, m, 8-phenyl), 7.43–7.50 (1H, m, 8-phenyl), 7.78–7.83 (2H, m, 9-phenyl), 7.91 (1H, dt, J = 1.6 and 8.0 Hz, 9-phenyl), 8.08 (1H, dd, J = 1.6 and 8.0 Hz, 9-phenyl).

Ethyl 2-[6-chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoate (14h). This compound (white solid, 48% yield) was prepared from **9h** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 0.83 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.20–1.32 (1H, m, c-C_6H_{10}), 1.37–1.51 (3H, m, c-C_6H_{10}), 1.51–1.69 (4H, m, c-C_6H_{10}), 1.78–1.88 (2H, m, c-C_6H_{10}), 3.92 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.65 (1H, s, OH), 7.26–7.34 (2H, m, 8-phenyl), 7.34–7.41 (1H, m, 8-phenyl), 7.44–7.50 (1H, m, 8-phenyl), 7.78–7.86 (2H, m, 9-phenyl), 7.93 (1H, dt, J = 1.6 and 8.0 Hz, 9-phenyl), 8.08 (1H, dd, J = 1.6 and 8.0 Hz, 9-phenyl).

Ethyl 2-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoate (16h). This compound (white powder, 95% yield) was prepared in a manner similar to that described for **15c**, except that **14h** was used instead of **14c**. ^{1}H NMR (DMSO-d_6) δ 0.86 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.20–1.30 (1H, m, c-C_6H_{10}), 1.38–1.66 (7H, m, c-C_6H_{10}), 1.73–1.82 (2H, m, c-C_6H_{10}), 3.87–3.96 (2H, m, CH_2CH_3), 5.47 (1H, s, OH), 7.16–7.29 (3H, m, 8-phenyl), 7.36–7.43 (1H, m, 8-phenyl), 7.61 (2H, br s, NH_2), 7.66 (1H, dd, J = 1.2 and 8.0 Hz, 9-phenyl), 7.72 (1H, dt, J = 1.2 and 8.0 Hz, 9-phenyl), 7.83 (1H, dt, J = 1.6 and 8.0 Hz, 9-phenyl), 8.00 (1H, dd, J = 1.6 and 8.0 Hz, 9-phenyl).

2-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoic acid (17h). This compound (white solid, 85% yield) was prepared in a manner similar to that described for **17i**, except that **16h** was used instead of **16i**. ^{1}H NMR (DMSO-d_6) δ 1.15–1.28 (1H, m, c-C_6H_{10}), 1.33–1.62 (7H, m, c-C_6H_{10}), 1.70–1.80 (2H, m, c-C_6H_{10}), 7.16–7.26 (3H, m, 8-phenyl), 7.33–7.40 (1H, m, 8-phenyl), 7.57 (1H, dd, J = 1.2 and 8.0 Hz, 9-phenyl), 7.60 (2H, br s, NH_2), 7.68 (1H, dt, J = 1.2 and 8.0 Hz, 9-phenyl), 7.88 (1H, dt, J = 1.6 and 8.0 Hz, 9-phenyl), 8.02 (1H, dd, J = 1.6 and 8.0 Hz, 9-phenyl).

***N*1-Ethyl-2-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzamide hydrochloride (15h).** This compound (pale yellow solid, 42% yield) was prepared in a manner similar to that described

for **15i**, except that **17h** was used instead of **17i**. ^{1}H NMR (DMSO-d_6) δ 0.80 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.16–1.27 (1H, m, c-C_6H_{10}), 1.35–1.63 (7H, m, c-C_6H_{10}), 1.70–1.81 (2H, m, c-C_6H_{10}), 2.80–2.91 (1H, m, CH_2CH_3), 2.92–3.03 (1H, m, CH_2CH_3), 7.17–7.25 (2H, m, 8-phenyl), 7.26–7.30 (1H, m, 8-phenyl), 7.32–7.41 (1H, m, 8-phenyl), 7.44–7.49 (1H, m, 9-phenyl), 7.58–7.66 (3H, m, 9-phenyl), 8.28–8.34 (1H, m, NHEt); MS *m*/*e* (FAB) 499 (MH^+). Anal. calcd for $C_{28}H_{27}FN_6O_2 \cdot HCl \cdot {}^1/_2H_2O$: C, 61.82, H, 5.37, N, 15.45; found: C, 61.50, H, 5.53, N, 15.42.

Ethyl 4-[2-(acetylamino)-5-nitro-6-oxo-1,6-dihydro-4-pyrimidinyl]amino]benzoate (3j). This compound (yellow solid, 89% yield) was prepared in a manner similar to that described for **3f**, except that ethyl 4-aminobenzoate was used instead of 3-aminobenzonitrile. ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.21 (3H, s, Ac), 4.32 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.77–7.82 (2H, m, phenyl), 7.94–7.99 (2H, m, phenyl), 11.17 (1H, br s, NHPh), 11.68 (1H, br, NH), 11.73 (1H, br s, NH).

Ethyl 4-[(2-amino-6-chloro-5-nitro-4-pyrimidinyl)amino]-benzoate (5j). This compound (yellow solid, 91% yield) was prepared in a manner similar to that described for **5f**, except that **3j** was used instead of **3f**. ^{1}H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.15 (3H, s, Ac), 4.30 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.93 (2H, d, J = 8.6 Hz, phenyl), 8.12 (2H, d, J = 8.6 Hz, phenyl), 10.15 (1H, br s, NHPh), 11.11 (1H, br s, NHAc).

Ethyl 4-[(2,5-diamino-6-chloro-4-pyrimidinyl)amino]-benzoate (7j). This compound (brown solid, 82% yield) was prepared in a manner similar to that described for **7f**, except that **5j** was used instead of **5f**. ^{1}H NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7.2 Hz, CH_2CH_3), 4.28 (2H, q, J = 7.2 Hz, CH_2CH_3), 4.33 (2H, br s, NH_2), 6.03 (2H, br s, NH_2), 7.86–7.92 (2H, m, phenyl), 7.94–8.00 (2H, m, phenyl), 8.68 (1H, br s, NHPh).

Ethyl 4-[2-amino-6-chloro-8-(3-fluorophenyl)-9*H*-9-purinyl]benzoate (8j). This compound (white powder, 63% yield) was prepared in a manner similar to that described for **8f**, except that **7j** was used instead of **7f**. ^{1}H NMR (DMSO-d_6) δ 1.34 (3H, t, J = 7.2 Hz, CH_2CH_3), 4.35 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.08 (2H, br s, NH_2), 7.21–7.32 (3H, m, 8-phenyl), 7.38–7.45 (1H, m, 8-phenyl), 7.60 (2H, d, J = 8.4 Hz, 9-phenyl), 8.09 (2H, d, J = 8.4 Hz, 9-phenyl).

Ethyl 4-[6-chloro-8-(3-fluorophenyl)-2-iodo-9*H*-9-purinyl]benzoate (9j). This compound (pale yellow powder, 46% yield) was prepared in a manner similar to that described for **9a**, except that **8j** was used instead of **8a**. ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz, CH_2CH_3), 4.37 (2H, q, J = 7.2 Hz, CH_2CH_3), 7.28–7.42 (3H, m, 8-phenyl), 7.44–7.52 (1H, m, 8-phenyl), 7.66 (2H, d, J = 8.4 Hz, 9-phenyl), 8.16 (2H, d, J = 8.4 Hz, 9-phenyl).

Ethyl 4-[6-chloro-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoate (14j). This compound (white powder, 85% yield) was prepared from **9j** in a manner similar to that described for **13a**, except that THF was used instead of *N*,*N*-dimethylformamide as a reaction solvent. ^{1}H NMR (DMSO-d_6) δ 1.35 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.23–1.32 (1H, m, c-C_6H_{10}), 1.40–1.53 (3H, m, c-C_6H_{10}), 1.53–1.70 (4H, m, c-C_6H_{10}), 1.79–1.89 (2H, m, c-C_6H_{10}), 4.37 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.65 (1H, s, OH), 7.31–7.42 (3H, m, 8-phenyl), 7.45–7.53 (1H, m, 8-phenyl), 7.68 (2H, d, J = 8.2 Hz, 9-phenyl), 8.16 (2H, d, J = 8.2 Hz, 9-phenyl).

Ethyl 4-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoate (16j). This compound (white solid, 77% yield) was prepared in a manner similar to that described for **15c**, except that **14j** was used instead of **14c**. ^{1}H NMR (DMSO-d_6) δ 1.20–1.30 (1H, m, c-C_6H_{10}), 1.35 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.38–1.66 (7H, m, c-C_6H_{10}), 1.75–1.83 (2H, m, c-C_6H_{10}), 4.36 (2H, q, J = 7.2 Hz, CH_2CH_3), 5.49 (1H, s, OH), 7.20–7.33 (3H, m, 8-phenyl), 7.39–7.46 (1H, m, 8-phenyl), 7.56–7.60 (2H, m, 9-phenyl), 7.68 (2H, br s, NH_2), 8.08–8.13 (2H, m, 9-phenyl).

4-[6-Amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzoic acid (17j). This compound (white solid, 93% yield) was prepared in a manner similar to that described for **17i**, except that **16j** was used instead of **16i**. ^{1}H NMR (DMSO-d_6) δ 1.18–1.30 (1H, m, c-C_6H_{10}), 1.38–1.66 (7H, m, c-C_6H_{10}), 1.74–1.84 (2H, m, c-C_6H_{10}), 5.53 (1H, br s, OH), 7.22–7.32 (3H, m, 8-phenyl), 7.39–7.46 (1H, m, 8-phenyl), 7.52–7.57 (2H, m, 9-phenyl), 7.66 (2H, br s, NH_2), 8.06–8.12 (2H, m, 9-phenyl).

***N*1-Ethyl-4-[6-amino-8-(3-fluorophenyl)-2-[2-(1-hydroxycyclohexyl)-1-ethynyl]-9*H*-9-purinyl]benzamide hydrochloride (15j).** This compound (white solid, 49% yield) was prepared in a manner similar to that described for **15i**, except that **17j** was used instead of **17i**. ^{1}H NMR (DMSO-d_6) δ 1.12 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.16–1.28 (1H, m, c-C_6H_{10}), 1.36–1.64 (7H, m, c-C_6H_{10}), 1.72–1.82 (2H, m, c-C_6H_{10}), 3.29 (2H, dq, J = 5.6 and 7.2 Hz, CH_2CH_3), 7.21–7.31 (3H, m, 8-phenyl), 7.38–7.45 (1H, m, 8-phenyl), 7.50 (2H, d, J = 8.0 Hz, 9-phenyl), 7.95 (2H, d, J = 8.0 Hz, 9-phenyl), 8.64 (1H, t, J = 5.6 Hz, NHEt); MS *m*/*e* (FAB) 499 (MH^+). Anal. calcd for $C_{28}H_{27}FN_6O_2 \cdot HCl \cdot {}^1/_4H_2O$: C, 62.33, H, 5.32, N, 15.58; found: C, 62.35, H, 5.40, N, 15.52.

Materials. NECA was purchased from Sigma (St. Louis, MO, USA). Compounds **1**,[6] FK453,[19] and KF17837[20c] were synthesized as reported. [^{3}H]-2-Chloro-N^6-cyclopentyladenosine ([^{3}H]CCPA) and [^{3}H]-2-[4-(2-carboxyethyl)phenyl]ethylamino]-5′-*N*-ethylcarboxamidoadenosine ([^{3}H]CGS21680) were obtained from NEN Life Science, Inc. (Boston, MA, USA).

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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-week-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 was added 100 μL of solution containing 0.6 μM NECA and the test antagonist (appropriate concentration). One h thereafter, glucose released from the hepatocytes was assayed using a commercial kit (Glucose CII-test WAKO, Wako Pure Chemical Industries, Ltd., Osaka, Japan).

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 a 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 calf 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 a solution containing 600 μM phosphodiesterase inhibitor Ro20-1724 (Research Biochemicals Inc., Natick, MA, USA), 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 the 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, USA).

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 a 5% CO_2/95% O_2 atmosphere at 37 °C in D-MEM/F-12 (1:1 mixture) medium with 10% fetal calf 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 centrifuged for 15 min at 50,000*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, and filtered, and the collected membranes were 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 bound ligand 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 the radioligand.

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References and Notes

1. Poulsen, S.-A.; Quinn, R. J. *Bioorg. Med. Chem.* **1998**, *6*, 619.
2. (a) Libert, F.; Schiffmann, S. N.; Lefort, A.; Parmentier, M.; Gerard, C.; Dumont, J. E.; Vanderhaeghen, J. J.; Vassart, G. *EMBO J.* **1991**, *10*, 1677. (b) Libert, F.; van Sande, J.; Lefort, A.; Czernilofsky, A.; Dumont, J. E.; Vassart, G.; Ensinger, H. A.; Mendla, K. D. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 919.
3. (a) Maenhaut, C.; Sande, J. V.; Libert, F.; Abramowicz, M.; Parmentier, M.; Vanderhaeghen, J. J.; Dumont, J. E.; Vassart, G.; Schiffmann, S. *Biochem. Biophys. Res. Commun.* **1990**, *173*, 1169. (b) Furlong, T. J.; Pierce, K. D.; Selbie, L. A.; Shine, J. *Mol. Brain Res.* **1992**, *15*, 62.
4. (a) Stehle, J. H.; Rivkees, S. A.; Lee, J. J.; Weaver, D. R.; Deeds, J. D.; Reppert, S. M. *Mol. Endocrinol.* **1992**, *6*, 384.
5. (b) Pierce, K. D.; Furlong, T. J.; Selbie, L. A.; Shine, J. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 86. (a) Zhou, Q. Y.; Li, C. Y.; Olah, M. E.; Johnson, R. A.; Stiles, G. L.; Civelli, O. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7432. (b) Salvatore, C. A.; Jacobson, M. A.; Tailor, H. E.; Linden, J.; Johnson, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10365.
6. Harada, H.; Asano, O.; Hoshino, Y.; Yoshikawa, S.; Matsukura, M.; Kabasawa, Y.; Niijima, J.; Kotake, Y.; Watanabe, N.; Kawata, T.; Inoue, T.; Horizoe, T.; Yasuda, N.; Minami, H.; Nagata, K.; Murakami, M.; Nagaoka, J.; Kobayashi, S.; Tanaka, I.; Abe, S. *J. Med. Chem.* **2001**, *44*, 170.
7. Prasad, R. N.; Bariana, D. S.; Fung, A.; Savic, M.; Tietje, K.; Stein, H. H.; Brondyk, H.; Egan, R. S. *J. Med. Chem.* **1980**, *23*, 313.
8. Müller, C. E.; Stein, B. *Curr. Pharm. Des* **1996**, *2*, 501.
9. de Zwart, M.; Link, R.; von Frijtag Drabbe Künzel, J. K.; Cristalli, G.; Jacobson, K. A.; Townsend-Nicholson, A.; Ijzerman, A. P. *Nucleosides Nucleotides* **1998**, *17*, 969.
10. Cristalli, G.; Eleuteri, A.; Vittori, S.; Volpini, R.; Lohse, M. J.; Klotz, K.-N. *J. Med. Chem.* **1992**, *35*, 2363.
11. Homma, H.; Watanabe, Y.; Abiru, T.; Murayama, T.; Momura, Y.; Matsuda, A. *J. Med. Chem.* **1992**, *35*, 2881.
12. Temple, C., Jr.; Smith, B. H.; Montgomery, J. A. *J. Org. Chem.* **1975**, *40*, 3141.
13. Nair, V.; Young, D. A. *J. Org. Chem.* **1985**, *50*, 406.
14. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936.
15. Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. *J. Med. Chem.* **1992**, *35*, 241.
16. (a) Buck, J. S.; Ide, W. S. *Org. Synth.* **II**, 44. (b) Noller, C. R. *Org. Synth.* **II**, 586.
17. Satoh, T.; Mitsuo, N.; Nishiki, M.; Inoue, Y.; Ooi, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1443.

18. Sheehan, J. C.; Hlavka, J. J. *J. Org. Chem.* **1956**, *21*, 439.
19. Akahane, A.; Katayama, H.; Mitsunaga, T.; Kita, Y.; Kusunoki, T.; Terai, T.; Yoshida, K.; Shinokawa, Y. *Bioorg. Med. Chem. Lett.* **1996**, *7*, 2059.
20. (a) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A.; Ichikawa, S. *J. Med. Chem.* **1992**, *35*, 2342. (b) Nonaka, Y.; Shimada, J.; Nonaka, H.; Koike, N.; Aoki, N.; Kobayashi, H.; Kase, H.; Yamaguchi, K.; Suzuki, F. *J. Med. Chem.* **1993**, *36*, 3731. (c) Suzuki, F.; Shimada, J.; Ishii, A.; Nonaka, H.; Kosaka, N.; Ichikawa, S. PCT Patent WO, 92/06976, 1992.
21. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.