Synthesis and Biological Evaluation of Novel 9-Substituted-8-Hydroxyadenine Derivatives as Potent Interferon Inducers

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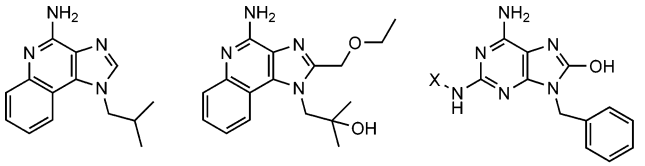
Recently we reported the adenine derivatives **3a**−**d** as novel interferon (IFN) inducers. In the present study, we conducted a detailed structure−activity relationship study of analogues of **3a**−**d** with respect to their IFN-inducing activity, mainly focusing on the N(9)-position of the adenine. From this study, we found that introduction of the 3-pyridylmethyl moiety was effective to increase in vitro activity, and compound **9ae** was identified as being the most potent IFN inducer. This compound gave a minimum effective concentration (MEC) of 3 nM, which is comparable with that of R-848, a second generation IFN inducer. Compound **9ae** also demonstrated potent IFN-inducing activity at a dose of 0.1 mg/kg by oral administration in mice. Furthermore, compound **9ae** induced IFN in monkeys in a dose dependent manner, with a potency superior to that of R-848. In addition, **9ae** did not cause emesis in ferrets even at a dose of 30 mg/kg. In this study the maximum plasma concentration of **9ae** was 1019 ng/mL (ca. 3.1 μM), which was approximately 1000-fold higher than the MEC value. Therefore, with respect to both the efficacy and the safety margin, compound **9ae** (SM-276001) is considered to be a promising compound as an orally active IFN inducer.

Hepatitis C virus (HCV) is a small enveloped RNA virus that belongs to the *Flavviridae* family. It causes chronic liver disease, including chronic active hepatitis in up to 80% of infected patients, as well as cirrhosis and hepatocellular carcinoma.[1] HCV infection is a significant clinical problem affecting an estimated 150 million patients worldwide. Chronic infection can lead to liver cirrhosis and associated development of hepatocellular carcinoma within 10−15 years.[2] At present the recommended treatment for patients with HCV is a combination of pegylated interferon-α (IFN-α) and ribavirin. The sustained virological response rate of this combination therapy is 42−48% for patients with genotype 1 after a course of 48 weeks and 80% for patients with genotype 2 or 3 after a course of 24 weeks.[3,4] IFN-α therapy is very expensive, and the formation of antibodies against IFN-α is observed in some patients. Treatment with IFN-α over a 6 month period enhances the efficacy and reduces the occurrence of hepatocellular carcinoma,[5] though the patients have to suffer regular injections of IFN-α once a week.

Orally available IFN inducers offer the possible advantages of convenience, prolonged action, and avoidance of immunogenicity. Therefore, IFN inducers are expected to be a new therapeutic class drug for treating viral infections. Although a number of IFN inducers including polyribonucleotide complexes,[6] fluorenones,[7] and pyrimidones[8] have been investigated, no results have so far been reported for IFN production in humans.[9]

Imiquimod (**1**), developed by 3M Pharmaceuticals, is a low molecular weight IFN inducer and has been reported to induce IFN production in humans.[10] However, the potential systemic usefulness of **1** was limited because it was emetic in clinical study.[11] The emetic activity of it was also reported in the experimental animal model.[12] However, imiquimod is used as a topical agent in the treatment of papilloma viral infections possibly to avoid such side effects.

Chart 1. Structures of **1** (Imiquimod), **2** (R-848), and **3a**−**d**



In our previous papers we reported on a series of 8-hydroxyadenine derivatives as novel structural IFN inducers,[13−16] especially focusing on the substituent at the C(2)-position of the adenine. In the course of the structure−activity relationship (SAR) study we found that compounds **3a**−**d** exhibited moderate IFN-inducing activities which were superior to that of imiquimod (**1**) but not to that of R-848 (**2**) (Chart 1). Our previous work suggested that the amino group at C(6)-position and the hydroxy at C(8)-position were essential to demonstrate IFN-inducing activity.[13] To find compounds having potent IFN-inducing activities both in vitro and in vivo, we now report a detailed SAR study on the modification of the N(9)-substituent based on **3a**.

Chemistry

The synthetic routes to 9-substituted-8-hydroxyadenine derivatives are shown in Schemes 1−4. The reaction of **4** with an appropriate alkyl or benzyl chloride in the presence of potassium carbonate gave corresponding compounds **5** in good yield. Compounds **6** were obtained by heating **5** with butylamine in an autoclave at 120 °C (method A). Compounds **6** could be also prepared using method B. The replacement of the chlorine atom at the C(2)-position in **4** with butylamine was carried out

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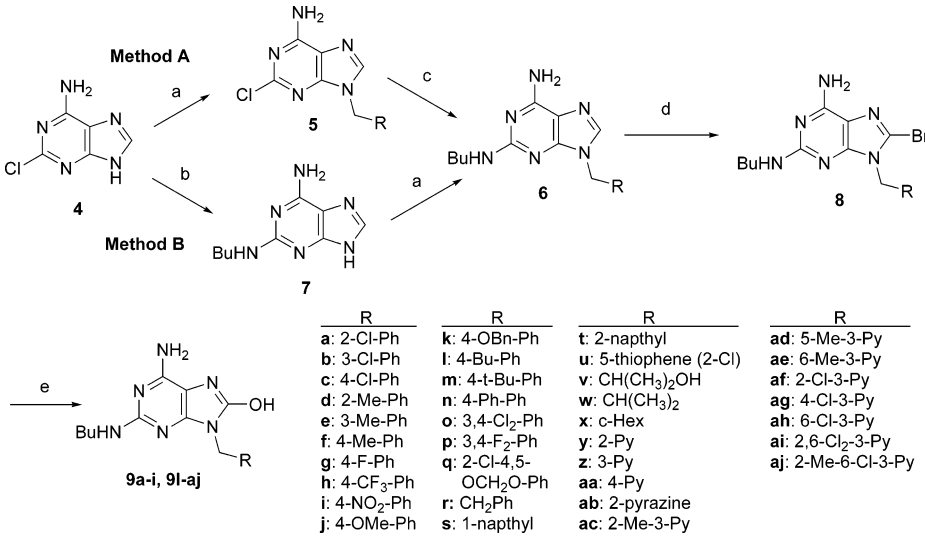
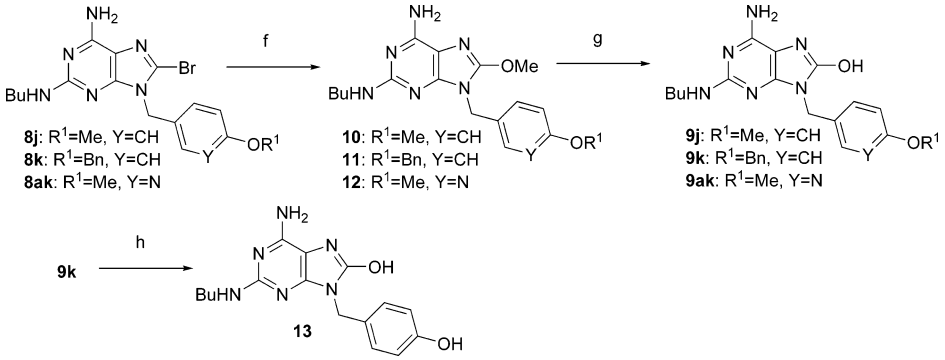
Scheme 1. Synthetic Route to Compounds **9a**−**i**,**l**−**aj**[a]



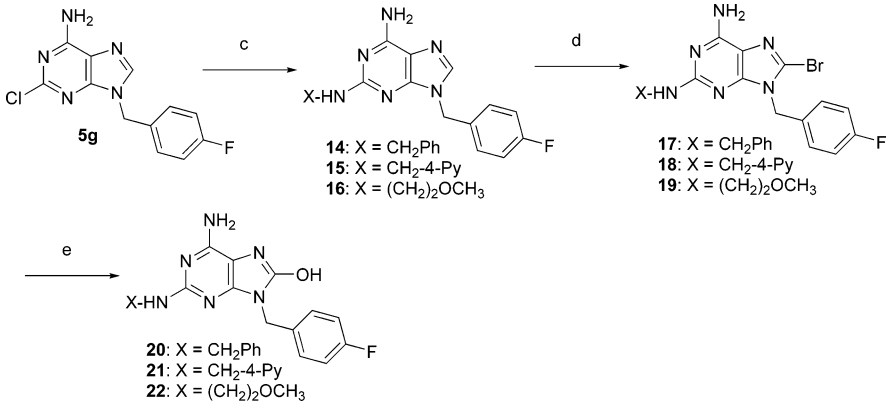
[a] Reagents and conditions: (a) R−CH_2Cl, K_2CO_3, DMF; (b) $BuNH_2$, H_2O; (c) $BuNH_2$, PrOH, 120 °C; (d) Br_2, $CHCl_3$; (e) 12 N HCl, 1,4-dioxane, 110 °C.

Scheme 2. Synthetic Route to Compounds **9j**,**k**,**ak** and **13**[a]



[a] Reagents and conditions: (f) NaOH/MeOH, reflux; (g) 12 N HCl, room temp; (h) 10% $Pd(OH)_2$/C, HCO_2H.

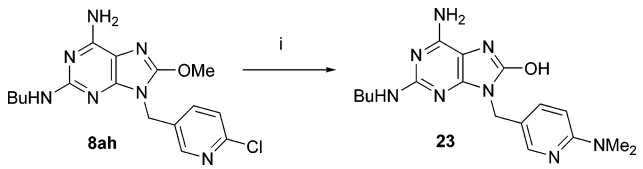
Scheme 3. Synthetic Route to Compounds **20**−**22**[a]



[a] Reagents and conditions: (c) X−NH_2, PrOH, 120 °C; (d) Br_2, $CHCl_3$; (e) 12 N HCl, 1,4-dioxane, 110 °C.

by heating in an autoclave at 180 °C to give **7**. This was followed by a reaction with an appropriate alkyl or benzyl chloride in the presence of potassium carbonate to give corresponding compounds **6**. As shown, compound **7** was considered as a common intermediate, so method B was more convenient than method A to use to make various derivatives. The bromination of **6** was easily achieved by the addition of bromine to give **8**. The desired compounds (**9a**−**i**,**l**−**aj**) were obtained by heating the corresponding **8** in 12 N HCl. In the case of compounds **9j**,**k**,**ak**, heating of the corresponding **8** in 12 N HCl brought about decomposition of the products.

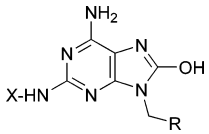
Scheme 4. Synthetic Route to Compound **23**[a]



[a] Reagent and condition: (i) Me_2NH, 140 °C.

Therefore, compounds **10**−**12** were prepared as shown in Scheme 2 by replacement of the bromine atom with a methoxy

Table 1. IFN-Inducing Activities of the Test Compounds Both in Vitro and in Vivo (IU/mL)



			in vitro (nM)				in vivo (mg/kg)	
compd	X	R	3	10	30	100	0.1	0.3
3a	Bu	Ph			<0.5	3.4	113	465
3b	$MeO(CH_2)_2$	Ph			<0.5	7.1	44	639
3c	$PhCH_2$	Ph	<0.5	44.4		80.4	203	398
3d	4-Py−CH_2	Ph	<0.5	2.2	3.9	9.1	<5	73
9a	Bu	2-Cl−Ph		<0.5	2	4.5		nd[a]
9b	Bu	3-Cl−Ph		<0.5	2.8	4.4		nd[a]
9c	Bu	4-Cl−Ph	<0.5	8.5	8.2	7.4	7	219
9d	Bu	2-Me−Ph		<0.5	3	3.6		nd[a]
9e	Bu	3-Me−Ph		<0.5	3	5.5		nd[a]
9f	Bu	4-Me−Ph	<0.5	9.2	6.2	2	28	356
9g	Bu	4-F−Ph	<0.5	12.8		4.2	139	313
9h	Bu	4-CF_3−Ph	<0.5	2.9	10.7	15.2	8	276
9i	Bu	4-NO_2−Ph	<0.5	11.6	4.5	2	61	444
9j	Bu	4-OMe−Ph	<0.5	6.5	4.7	2	73	933
9k	Bu	4-OBnn−Ph			<0.5	10.6		nd[a]
9l	Bu	4-*n*-Bu−Ph			<0.5	11		<5
9m	Bu	4-*t*-Bu−Ph		<0.5	4.4	13		<5
9n	Bu	4-Ph−Ph			<0.5	3.4		nd[a]
13	Bu	4-OH−Ph	<0.5	12.4		3.1		20
9o	Bu	3,4-Cl_2−Ph	<0.5	0.8		10	<5	136
9p	Bu	3,4-F_2−Ph	<0.5	6.4	14.7	7.1	35	338
9q	Bu	2-Cl−4,5-OCH_2O−Ph			<0.5	2.1		<5
9r	Bu	CH_2Ph				<0.5		nd[a]
9s	Bu	1-naphthyl	<0.5	2.2	34.0	15.4	<5	39
9t	Bu	2-naphthyl		<0.5	7.1	17.4		<5
9u	Bu	5-thiophen(2-Cl)	<0.5	8.9	11.3	7.2	34	484
9v	Bu	CMe_2OH			<0.5	0.6		<5
9w	Bu	*i*-propyl			<0.5	4.1		nd[a]
9x	Bu	*c*-hexyl		<0.5	1.7	3.7		nd[a]
9y	Bu	2-Py		<0.5	7.4	9.1		<5
9z	Bu	3-Py	1.6	32.5	16.6	8.1	76	327
9aa	Bu	4-Py	<0.5	4.2	11.9	4.3	21	332
9ab	Bu	2-pyrazine		<0.5	2.9	4.2		nd[a]
20	$MeO(CH_2)_2$	4-F−Ph		<0.5	4.8	8.3	54	521
21	$PhCH_2$	4-F−Ph			<0.5	4.5		<5
22	4-Py−CH_2	4-F−Ph	<0.5	11.1		5.2	19	492
2			2.8	11	7	4	1176	1714

[a] nd: not determined.

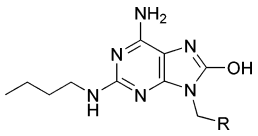
group with the use of sodium methoxide. The cleavage of the methoxy group in **10**−**12** by stirring in 12 N HCl at room temperature gave the desired compounds (**9j**,**k**,**ak**). The catalytic hydrogenation of **9k** gave **13**. As shown in Scheme 3, the replacement of the chlorine atom at the C(2)-position in **5g** with appropriate amines led to compounds **14**−**16** which were converted to the desired products **20**−**22** in the same way. The reaction of **8ah** with dimethylamine in an autoclave at 140 °C afforded **23** (Scheme 4).

Results and Discussions

The in vitro IFN-inducing activities of the prepared compounds were conducted by the following method. Mouse splenocytes were cultured with the compounds, and the concentrations of IFN induced by the compounds in the supernatant were measured by bioassay using L929 cells with vesicular stomatitis virus.[17,18] With regard to the in vivo test, we orally administered the test compounds to male Balb/c mice and then the concentrations of IFN in the plasma were measured by the bioassay mentioned above. In the preliminary experiment, the IFN concentration in mouse plasma reached a maximum 2 h after oral administration of the test compounds (data not shown), so the IFN concentration was measured at this point.

On the basis of compound **3a**, we examined the electronic effect of substituents by use of a chlorine atom, as an electron-withdrawing group, or a methyl group, as an electron-donating group, at various positions on the benzene ring (**9a**−**f**). The results are summarized in Table 1. It can be seen that para-substituted compounds (**9c**,**f**) were 10-fold more potent than unsubstituted **3a**, whereas meta- or ortho-substituted compounds (**9a**,**b**,**d**,**e**) proved less favorable. These results indicated that the electronic component of the substituent was negligible, whereas the correct regiochemistry of the substituent was important. Next, we examined compounds containing various substituents at the para position (**9g**−**n**, **13**). The activities of compounds **9k**,**l**−**n** (MEC = 30 or 100 nM) suggested that bulky substituents and long linear alkyl groups were detrimental to the activity, whereas compounds containing relatively compact groups (**9g**−**j**, **13**) were preferred (MEC = 10 nM). Dihalogenated compounds (**9o**,**p**) showed equipotent activities with the monohalogenated compounds (**9c**,**g**) (MEC = 10 nM) showing that a meta halogen group was tolerated. However, it can be seen that the amount of IFN induced by compounds **9o**,**p** was inferior to that of **9c**,**g**. Interestingly, in this group of potent compounds the 4-fluoro analogue (**9g**) exhibited the most potent

Table 2. IFN-Inducing Activities of the Test Compounds Both in Vitro and in Vivo (IU/mL)



		in vitro (nM)					in vivo (mg/kg)	
compd	R	1	3	10	30	100	0.1	0.3
9z	3-Py	<0.5	1.6	32.5	16.6	8.1	76	327
9ac	2-Me−3-Py		<0.5	12.8	8.3	3.0	186	1462
9ad	5-Me−3-Py	<0.5	1.9	2.4	2.1	4.6		nd[a]
9ae	6-Me−3-Py	<0.5	4.4	7.8	3.7	1.4	393	1891
9af	2-Cl−3-Py		<0.5	3.7	16.3	5.0	<5	94
9ag	4-Cl−3-Py				<0.5	4.2		nd[a]
9ah	6-Cl−3-Py	<0.5	5.6	6.8	2.6	1.5	811	1826
9ak	6-OMe−3-Py	<0.5	8.2	13.4	3.0	1.0	375	1920
23	6-NMe_2−3-Py	<0.5	4.9	9.4	5.1	2.7	<5	403
9ai	2-Cl−6-Me−3-Py		<0.5	8.4		3.4	<5	619
9aj	2,6-Cl−3-Py			<0.5	14.6	5.7	42	414
2		<0.5	2.8	11	7	4	1176	1714

[a] nd: not determined.

Table 3. IFN-Inducing Activities of Compounds **2** and **9ae**,**ah**,**ak** in Cynomolgus Monkeys (IU/mL)

	dose (mg/kg)		
compd	0.1	0.3	1
9ae	121 ± 89	591 ± 411	34810 ± 15915
9ah	0	19 ± 16	3602 ± 1619
9ak	3 ± 3	244 ± 244	17422 ± 14108
2	7 ± 4	13 ± 10	44037 ± 34055

Table 4. Emetic Activities of Compounds **2**, and **9ae**,**ah**,**ak**) in Ferrets

compd	dose (mg/kg)	vomiting	C_{max} (ng/mL)
9ae	0.3	0/5	nd[a]
	3	0/5	20.8
	30	0/5	1019
9ah	0.3	0/5	nd[a]
	3	0/5	20.1
	30	0/5	275.2
9ak	0.3	0/5	nd[a]
	3	0/5	108.9
	30	0/5	1199
2	0.3	2/5	nd[a]
	3	5/5	1181

[a] Nd: not determined.

IFN-inducing activity in vivo which we speculated may be due to its good oral bioavailability.

In our previous paper[15] we disclosed compounds **3b**−**d**; therefore, here we substituted the *N*(9)-benzyl moiety present in **3b**−**d** with a 4-fluorobenzyl moiety with the expectation of an improvement in the activity. Unfortunately, as shown in Table 1, compound **20** demonstrated a 3-fold improvement in the in vitro activity compared with **3b**, whereas **21** and **22** did not improve the activity in comparison with **3c** and **3d**, respectively. However, the in vivo activity of **20** was not significantly improved; therefore, the 4-fluorobenzyl group could not be considered to be a generally useful N(9)-substituent to increase the activity.

As shown in Table 1, although compound **9g** was 10-fold more potent than **3a** in vitro, this did not translate into an increase in the in vivo activity as measured by the quantity of IFN in plasma (139 IU/mL versus 113 IU/mL). To find compounds having activities more potent than **9g**, both in vitro and in vivo, we continued further examination by the replacement of the phenyl ring in **3a** with other moieties. Alkyl compounds (**9r**,**v**−**x**) gave weak or diminished activities, while lipophilic aromatic compounds (**9s**,**u**) exhibited potencies comparable with **9g** (MEC = 10 nM). Unfortunately, again these compounds did not show potent IFN-inducing activity in vivo, as determined by the amount of IFN induced at a dose of 0.1 mg/kg in comparison to **9g**. The in vivo results of para-substituted compounds **9c**,**f**−**j** or **9s**,**u** suggested that lipophilic substituents at N(9)-positions were unfavorable for potent in vivo activity which we suggest may be due to a problem in oral bioavailability. Next we investigated the pyridyl derivatives as examples of hydrophilic aromatic substituents (**9y**−**aa**). As shown in Table 1, the order of the potency was 3-pyridyl (**9z**, 3 nM) > 4-pyridyl (**9aa**, 10 nM) > 2-pyridyl (**9y**, 30 nM). It is noteworthy that **9z** was 30-fold more potent than the benzyl compound **3a**; however, the 2-pyrazine (**9ab**) was equipotent with the 2-pyridyl (**9y**), suggesting that the nitrogen atom at the ortho position might not be advantageous in increasing the potency further. Although compound **9z** induced IFN when orally administered (76 IU/mL at 0.1 mg/kg), this result was unexpectedly inferior to that of **3a** or **9g**. The SAR of **9z** was further investigated by the introduction of substituents onto the pyridine ring, and the results are summarized in Table 2. Unfortunately, against our expectations the in vitro activities of compounds **9ae**,**ah** were evaluated as 3 nM, which were equivalent with that of **9z**. These results were not consistent with those of para-substituted benzyl compounds (**9c**,**f**) where an increase in potency was observed. In addition, the results of compounds **9ac**,**af**,**ag** indicated that substitution at the C2- or C4-positions of the pyridine ring did not lead to an increase in activity. These results are similar to those of ortho-substituted benzyl compounds (**9a**,**d**). Among the compounds tested here, compounds **9ae**,**ah**,**ak** showed potent IFN-inducing activities at a dose of 0.1 mg/kg by oral administration: The induced amounts of IFN were 393 IU/mL for **9ae**, 811 IU/mL for **9ah**, and 375 IU/mL for **9ak**, respectively. Their potencies were severalfold greater than **3a**. The compound R-848 (**2**),[19] developed by 3M Pharmaceuticals, also exhibited potent in vitro IFN-inducing activity, and it induced 1176 IU/mL of IFN at an oral dose of 0.1 mg/kg. This in vivo potency was greater than our compounds, and we speculate that is the case because R-848 might possess superior oral bioavailability than our compounds.

Further biological evaluations of compounds **9ae**,**ah**,**ak** were carried out with comparison to compound **2**. Test compounds were orally administered to cynomolgus monkeys, and the amount of IFN in plasma was measured during 0−24 h after dosing. The results of maximum concentrations of IFN in plasma are summarized in Table 3. It can be seen that compound **9ae** induced IFN in a dose dependent manner, and it is more potent than **9ah** and **9ak**. The compoound R-848 (**2**) also exhibited potent IFN-inducing activity at a dose of 1 mg/kg, but it did not show the clear dose-dependency. Therefore, the activity of compound **9ae** was considered to be superior to that of R-848 at low doses.

In the clinical study of imiquimod (**1**), one serious side effect, emesis, was observed.[11] Compound R-848, a structurally similar compound to **1**, is being developed as topical agent, and to date no report of any emesis has been published. We chose a ferret emesis model to evaluate this side effect which was evaluated by the absence or presence of vomiting behavior within 6 h after administration of the compounds. As shown in Table 4, when compounds **9ae**,**ah**,**ak** were orally administered at doses of 0.3−30 mg/kg, no vomiting was observed in any of the ferrets, while the administration of **2** at a dose of 3 mg/kg caused vomiting in all animals. The plasma concentrations of these

compounds achieved a sufficient level to induce IFN. The C_{max} value of **9ae** at 30 mg/kg was nearly the same with that of **2** at 3 mg/kg. These results indicated that compound **9ae** exerted a highly potent IFN-inducing activity and was superior to **2** in terms of the margin between the IFN-inducing and emetic doses. As for the possible reason for this, we hypothesize that **9ae** is not able to transmigrate into the brain effectively because of the presence of a polar group (aromatic hydroxy), but the exact details including the action mechanism of emesis are still unclear. From these results, compound **9ae** (SM-276001) is expected to be a useful IFN inducer in treatment of viral infections such as HCV and HBV. It was reported that **1** and **2** induced IFN via the activation of Toll-like receptor-7 (TLR-7).[20] With regard to the mechanism of action of **9ae**, we have identified it as being a TLR-7 agonist and will report further detailed information in appropriate opportunity in the future.

Conclusion

In this study the search for a potent IFN inducer was addressed. Replacement of the benzyl moiety of **3a** at the N(9)-position by para-substituted benzyl groups was shown to increase the in vitro activities by 10-fold (**9c**,**f**−**l**). However, their in vivo activities were not improved in comparison to **3a**, possibly due to poor oral bioavailability. The examination of various lipophilic compounds resulted in no improvement in activities. Among the compounds having a hydrophilic N(9)-substituent, the 3-pyridylmethyl moiety (**9z**) was identified to be a very potent compound with in vitro activity in the nanomolar range. From the SAR study of **9z**, we identified compounds **9ae**,**ah**,**ak** as potent IFN inducers (Table 2). Especially, compound **9ae** demonstrated IFN induction in monkeys in a clear dose-dependent manner by oral administration (Table 3). Furthermore, it did not cause emesis in a ferret model, whereas a literature compound R-848 showed emesis (Table 4). From the viewpoint of the margin between activity and toxicity (emesis) compound **9ae** (SM-276001) is expected to be a promising compound as a novel IFN inducer.

Experimental Section

All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were measured with a Thomas-Hoover capillary melting point apparatus and were uncorrected. Proton NMR spectra were recorded on a Brucker Avance 400 FT NMR spectrometer. Chemical shifts were given in parts per million (ppm) using tetramethylsilane as an internal standard for spectra obtained in DMSO-d_6. Elemental analyses were performed at Sumika Chemical Analysis Service, Osaka, Japan. Wakogel C-200 (Wako; 70−150 mm) was used for column chromatography. The monitoring of reactions was carried out using Merck 60 F_{254} silica gel, glass-supported TLC plates, and visualization with UV light (254 and 365 nm).

Method A. 2-Chloro-9-(2-chlorobenzyl)adenine (5a). To a suspension of **4** (0.68 g, 4 mmol) and potassium carbonate (0.7 g, 5 mmol) in DMF (10 mL) was added 2-chlorobenzyl chloride (1.0 g, 6.2 mmol), and the mixture was heated at 75 °C for 4 h. The reaction mixture was concentrated, and water was added to the residue. The resulting precipitate was collected and washed with diethyl ether to give the title compound as a pale yellow solid (1.03 g, 85%): ^{1}H NMR (DMSO-d_6) δ 8.24 (1H, s), 7.86 (2H, s), 7.53 (1H, d, J = 7.8 Hz), 7.38 (2H, m), 6.95 (1H, dd, J = 7.6, 1.4 Hz), 5.43 (2H, s).

2-Butylamino-9-(2-chlorobenzyl)adenine (6a). A solution of **5a** (0.88 g, 3 mmol) and butylamine (10 mL) in *n*-PrOH (30 mL) was heated at 130 °C for 8 h in an autoclave. The reaction mixture was concentrated, and water was added to the residue. The resulting precipitate was collected and was purified by silica gel chromatography using 1% MeOH in $CHCl_3$ as an eluent to give the title compound as a pale yellow solid (0.62 g, 63%): ^{1}H NMR (DMSO-d_6) δ 7.76 (1H, s), 7.50 (1H, dd, J = 7.6, 1.4 Hz), 7.32 (2H, m), 7.00 (1H, brs), 6.69 (2H, s), 6.26 (1H, t, J = 5.4 Hz), 5.28 (2H, s), 3.19 (2H, q, J = 6.8 Hz), 1.44 (2H, m), 1.28 (2H, m), 0.83 (3H, t, J = 7.6 Hz).

Method B. 2-Butylaminoadenine (7). A suspension of **4** (5.0 g, 30 mmol) and butylamine (30 mL) in H_2O (20 mL) was heated at 180 °C for 11 h in an autoclave. The reaction mixture was concentrated, and water was added to the residue to give the title compound as a dark green solid (5.6 g, 92%): ^{1}H NMR (DMSO-d_6) δ 12.01 (1H, brs), 7.62 (1H, s), 6.53 (2H, s), 6.07 (1H, t, J = 5.6 Hz), 3.18 (2H, q, J = 6.8 Hz), 1.48 (2H, m), 1.31 (2H, m), 0.88 (3H, t, J = 7.2 Hz).

2-Butylamino-9-(4-methylbenzyl)adenine (6f). To a suspension of **7** (118 mg, 0.57 mmol) and potassium carbonate (59 mg, 0.43 mmol) in DMF (5 mL) was added 4-methylbenzyl chloride (120 mg, 0.86 mmol), and the mixture was stirred at room temperature for 18 h. The reaction mixture was concentrated, and the residue was partitioned with water and $CHCl_3$, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel chromatography by use of 2% MeOH in $CHCl_3$ as an eluent to give the title compound as a white solid (125 mg, 71%): ^{1}H NMR (DMSO-d_6) δ 7.75 (1H, s), 7.21 (2H, d, J = 7.6 Hz), 7.12 (2H, d, J = 7.6 Hz), 6.61 (2H, s), 6.21 (1H, t, J = 6.0 Hz), 5.11 (2H, s), 3.22 (2H, q, J = 6.8 Hz), 2.25 (3H, s), 1.48 (2H, m), 1.31 (2H, m), 0.88 (3H, t, J = 7.6 Hz).

8-Bromo-2-butylamino-9-(2-chlorobenzyl)adenine (8a). To a suspension of **6a** (600 mg, 1.8 mmol) in $CHCl_3$ (10 mL) was added bromine (350 mg, 2.2 mmol) in an ice cooling condition, and the reaction mixture was stirred at ambient temperature for 3 h. The resulting precipitate was collected to give the title compound as a pale yellow solid (0.81 g, 91%): ^{1}H NMR (DMSO-d_6) δ 9.42 (1H, brs), 8.54 (1H, brs), 7.55 (2H, m), 7.36 (2H, m), 6.95 (1H, t, J = 6.8 Hz), 5.33 (2H, s), 3.24 (2H, m), 1.45 (2H, m), 1.26 (2H, m), 0.83 (3H, t, J = 7.2 Hz).

2-Butylamino-9-(2-chlorobenzyl)-8-hydroxyadenine (9a). A solution of **8a** (350 mg, 0.7 mmol) and 12 N HCl (10 mL) in 1,4-dioxane (10 mL) was heated at 110 °C for 6 h. The reaction mixture was concentrated, and the residue was neutralized with 1 N NaOH aqueous solution. The precipitate was collected and purified by silica gel chromatography by use of 2% MeOH in $CHCl_3$ as an eluent to give the title compound as a white solid (165 mg, 67%): recrystallized from MeOH, mp 241−244 °C; ^{1}H NMR (DMSO-d_6) δ 10.45 (1H, s), 7.70 (2H, s), 7.47 (1H, d, J = 6.8 Hz), 7.30 (3H, m), 7.15 (1H, s), 5.00 (2H, s), 3.18 (2H, q, J = 6.4 Hz), 1.42 (2H, m), 1.25 (2H, m), 0.83 (3H, t, J = 7.6 Hz). Anal. ($C_{16}H_{19}ClN_6O$) C, H, N.

Compounds **9b**−**i**,**l**−**aj**, and **20**−**22** were also prepared using the similar procedure described above.

2-Butylamino-9-(3-chlorobenzyl)-8-hydroxyadenine (9b). Yield 94%; recrystallized from MeOH, mp 244−246 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 7.38 (3H, m), 7.25 (1H, m), 6.24 (1H, t, J = 6.8 Hz), 6.04 (2H, s), 4.81 (2H, s), 3.16 (2H, q, J = 6.8 Hz), 1.45 (2H, m), 1.28 (2H, m), 0.86 (3H, t, J = 7.6 Hz). Anal. ($C_{16}H_{19}ClN_6O\cdot 0.18H_2O$) C, H, N.

2-Butylamino-9-(4-chlorobenzyl)-8-hydroxyadenine (9c). Yield 85%; recrystallized from EtOH, mp 278−280 °C; ^{1}H NMR (DMSO-d_6) δ 9.56 (1H, s), 7.40 (2H, d, J = 9.2 Hz), 7.29 (2H, d, J = 9.2 Hz), 6.22 (1H, t, J = 5.6 Hz), 6.02 (2H, s), 4.79 (2H, s), 3.15 (2H, q, J = 6.8 Hz), 1.43 (2H, m), 1.27 (2H, m), 0.86 (3H, t, J = 7.6 Hz). Anal. ($C_{16}H_{19}ClN_6O$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-methylbenzyl)adenine (9d). Yield 74%; recrystallized from MeOH−H_2O, mp 195−196 °C; ^{1}H NMR (DMSO-d_6) δ 10.25 (1H, s), 7.16 (7H, m), 4.83 (2H, s), 3.17 (2H, q, J = 6.8 Hz), 2.41 (3H, s), 1.45 (2H, m), 1.27 (2H, m), 0.85 (3H, t, J = 7.2 Hz). Anal. ($C_{17}H_{22}N_6O\cdot HCl\cdot 0.35H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(3-methylbenzyl)adenine (9e). Yield 67%; recrystallized from MeOH, mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 9.62 (1H, s), 7.19 (1H, t, J = 7.2 Hz), 7.08 (3H, m), 6.20 (1H, t, J = 5.6 Hz), 6.00 (2H, s), 4.76 (2H, s), 3.16 (2H, q, J = 6.8 Hz), 2.26 (3H, s), 1.47 (2H, m), 1.27 (2H, m), 0.87 (3H, t, J = 7.2 Hz). Anal. ($C_{17}H_{22}N_6O\cdot 0.1H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(4-methylbenzyl)adenine (9f). Yield 98%; recrystallized from MeOH, mp 282−283 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (1H, s), 7.18 (2H, d, J = 8.0 Hz), 7.10 (2H, d, J = 8.0 Hz), 6.18 (1H, t, J = 5.6 Hz), 5.99 (2H, s), 4.74 (2H, s), 3.15 (2H, q, J = 7.2 Hz), 2.25 (3H, s), 1.44 (2H, m), 1.28 (2H, m), 0.87 (3H, t, J = 7.6 Hz). Anal. ($C_{17}H_{22}N_6O\cdot0.2H_2O$) C, H, N.

2-Butylamino-9-(4-fluorobenzyl)-8-hydroxyadenine (9g). Yield 77%; recrystallized from MeOH, mp 266−267 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 7.33 (2H, dd, J = 8.4, 5.6 Hz), 7.14 (2H, d, J = 8.4, 5.6 Hz), 6.21 (1H, t, J = 5.6 Hz), 6.01 (2H, s), 4.78 (2H, s), 3.16 (2H, q, J = 6.0 Hz), 1.44 (2H, m), 1.29 (2H, m), 0.86 (3H, t, J = 7.7 Hz). Anal. ($C_{16}H_{19}FN_6O\cdot0.1H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(4-trifluoromethylbenzyl)adenine (9h). Yield 85%; recrystallized from MeOH, mp 264−266 °C; ^{1}H NMR (DMSO-d_6) δ 9.73 (1H, s), 7.69 (2H, d, J = 8.0 Hz), 7.46 (2H, d, J = 8.0 Hz), 6.21 (1H, t, J = 5.6 Hz), 6.04 (2H, s), 4.89 (2H, s), 3.14 (2H, q, J = 6.8 Hz), 1.41 (2H, m), 1.26 (2H, m), 0.83 (3H, t, J = 7.6 Hz). Anal. ($C_{17}H_{19}F_3N_6O\cdot0.2H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(4-nitrobenzyl)adenine (9i). Yield 45%; recrystallized from MeOH, mp 216−218 °C; ^{1}H NMR (DMSO-d_6) δ 9.73 (1H, s), 8.19 (2H, d, J = 8.8 Hz), 7.50 (2H, d, J = 8.8 Hz), 6.23 (1H, t, J = 5.6 Hz), 6.05 (2H, s), 4.94 (2H, s), 3.12 (2H, q, J = 6.8 Hz), 1.40 (2H, m), 1.24 (2H, m), 0.83 (3H, t, J = 7.4 Hz). Anal. ($C_{16}H_{19}N_7O_3\cdot1.75H_2O$) C, H, N.

2-Butylamino-9-(4-butylbenzyl)-8-hydroxyadenine (9l). Yield 91%; mp 248−249 °C; ^{1}H NMR (DMSO-d_6) δ 9.61 (1H, s), 7.19 (2H, d, J = 8.2 Hz), 7.11 (2H, d, J = 8.2 Hz), 6.20 (1H, t, J = 5.6 Hz), 5.99 (2H, s), 4.76 (2H, s), 3.14 (2H, m), 2.51 (2H, m), 1.49 (4H, m), 1.28 (4H, m), 0.87 (6H, m). Anal. ($C_{20}H_{28}N_6O\cdot0.12H_2O$) C, H, N.

2-Butylamino-9-(4-*tert*-butylbenzyl)-8-hydroxyadenine (9m). Yield 96%; mp 256−257 °C; ^{1}H NMR (DMSO-d_6) δ 9.66 (1H, s), 7.32 (2H, d, J = 8.4 Hz), 7.22 (2H, d, J = 8.4 Hz), 6.23 (1H, t, J = 5.6 Hz), 6.06 (2H, s), 3.17 (2H, m), 1.45 (2H, m), 1.28 (2H, m), 1.24 (9H, s), 0.87 (3H, t, J = 7.2 Hz). Anal. ($C_{20}H_{28}N_6O$) C, H, N.

9-(4-Biphenylmethyl)-2-butylamino-8-hydroxyadenine (9n). Yield 98%; recrystallized from EtOH, mp 285−287 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 7.62 (4H, m), 7.46 (2H, m), 7.35 (3H, m), 6.24 (1H, t, J = 5.6 Hz), 6.03 (2H, s), 4.85 (2H, s), 3.17 (2H, q, J = 6.8 Hz), 1.46 (2H, m), 1.28 (2H, m), 0.87 (3H, t, J = 7.6 Hz). Anal. ($C_{22}H_{24}N_6O\cdot0.27H_2O$) C, H, N.

2-Butylamino-9-(3,4-dichlorobenzyl)-8-hydroxyadenine (9o). Yield 96%; mp 269−270 °C; ^{1}H NMR (DMSO-d_6) δ 9.73 (1H, s), 7.57 (2H, m), 7.24 (2H, dd, J = 8.8, 1.8 Hz), 6.22 (1H, t, J = 5.6 Hz), 6.05 (2H, s), 4.80 (2H, s), 3.14 (2H, q, J = 6.8 Hz), 1.43 (2H, m), 1.26 (2H, m), 0.85 (3H, t, J = 7.6 Hz). Anal. ($C_{16}H_{18}Cl_2N_6O\cdot0.15H_2O$) C, H, N.

2-Butylamino-9-(3,4-difluorobenzyl)-8-hydroxyadenine (9p). Yield 100%; recrystallized from MeOH−$(CH_3)_2CO$, mp 217−219 °C; ^{1}H NMR (DMSO-d_6) δ 10.54 (1H, s), 7.69 (2H, s), 7.40 (2H, m), 7.16 (1H, t, J = 4.0 Hz), 4.85 (2H, s), 3.16 (2H, m), 1.47 (2H, m), 1.29 (2H, m), 0.84 (3H, t, J = 7.6 Hz). Anal. ($C_{16}H_{18}F_2N_6O\cdot HCl\cdot0.3H_2O$) C, H, N.

2-Butylamino-9-(2-chloro-4,5-methylenedioxylbenzyl)-8-hydroxyadenine (9q). Yield 65%; recrystallized from EtOH, mp 214−215 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (1H, s), 7.10 (1H, s), 6.59 (1H, brs), 6.24 (1H, t, J = 5.6 Hz), 6.05 (2H, s), 6.03 (2H, s), 4.79 (2H, s), 3.13 (2H, q, J = 7.0 Hz), 1.42 (2H, m), 1.27 (2H, m), 0.85 (3H, t, J = 7.4 Hz). Anal. ($C_{17}H_{19}ClN_6O_3$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-phenylethyl)adenine (9r). Yield 93%; recrystallized from MeOH−H_2O, mp 204−205 °C; ^{1}H NMR (DMSO-d_6) δ 9.52 (1H, s), 7.11−7.59 (5H, m), 6.22 (1H, t, J = 5.6 Hz), 5.98 (2H, s), 3.86 (2H, m), 3.17 (2H, m), 2.98 (2H,m), 1.47 (2H, m), 1.31 (2H, m), 0.88 (3H, t, J = 7.6 Hz). Anal. ($C_{17}H_{22}N_6O\cdot1.35H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(1-naphthylmethyl)adenine (9s). Yield 100%; recrystallized from MeOH, mp 249−251 °C; ^{1}H NMR (DMSO-d_6) δ 9.72 (1H, s), 8.39 (1H, d, J = 7.8 Hz), 7.95 (1H, m), 7.86 (1H, m), 7.56 (2H, m), 7.43 (1H, t, J = 7.6 Hz), 7.35 (1H, s), 6.19 (1H, t, J = 5.6 Hz), 6.03 (2H, s), 5.28 (2H, s), 3.12 (2H, m), 1.41 (2H, m), 1.25 (2H, m), 0.83 (3H, t, J = 7.2 Hz). Anal. ($C_{20}H_{22}N_6O$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-naphthylmethyl)adenine (9t). Yield 93%; mp 225−227 °C; ^{1}H NMR (DMSO-d_6) δ 10.08 (1H, s), 7.88 (3H, m), 7.78 (1H, brs), 7.49 (3H, m), 6.85 (2H, s), 5.00 (2H, s), 3.21 (2H, m), 1.46 (2H, m), 1.26 (2H, m), 0.82 (3H, t, J = 7.6 Hz). Anal. ($C_{20}H_{22}N_6O\cdot1.65H_2O$) C, H, N.

2-Butylamino-9-(5-chlorothiophene-2-ylmethyl)-8-hydroxyadenine (9u). Yield 61%; recrystallized from MeOH−$CHCl_3$, mp 268−269 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (1H, s), 6.96 (2H, dd, J = 20.8, 3.6 Hz), 6.28 (1H, t, J = 5.6 Hz), 6.03 (2H, s), 4.87 (2H, s), 3.19 (2H, q, J = 6.8 Hz), 1.48 (2H, m), 1.30 (2H, m), 0.88 (3H, t, J = 7.2 Hz). Anal. ($C_{14}H_{17}ClN_6OS$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-hydroxy-2,2-dimethylethyl)adenine (9v). Yield 92%; mp 214−216 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (1H, s), 6.26 (1H, t, J = 5.6 Hz), 6.12 (2H, s), 5.20 (1H, s), 3.63 (2H, s), 3.15 (2H, m), 1.44 (2H, m), 1.29 (2H, m), 1.09 (6H, s), 0.87 (3H, t, J = 7.2 Hz). Anal. ($C_{13}H_{22}N_6O_2\cdot0.2H_2O$) C, H, N.

2-Butylamino-9-(2,2-dimethylethyl)-8-hydroxyadenine (9w). Yield 62%; mp 213−215 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (1H, s), 6.16 (1H, t, J = 5.6 Hz), 5.95 (2H, s), 3.42 (2H, d, J = 7.2 Hz), 3.17 (2H, q, J = 6.8 Hz), 2.14 (1H, m), 1.46 (2H, m), 1.29 (2H, m), 0.85 (9H, m). Anal. ($C_{13}H_{22}N_6O\cdot0.2H_2O$) C, H, N.

2-Butylamino-9-cyclohexylmethyl-8-hydroxyadenine (9x). Yield 70%; mp 230−231 °C; ^{1}H NMR (DMSO-d_6) δ 9.56 (1H, s), 6.17 (1H, t, J = 5.6 Hz), 5.95 (2H, s), 3.45 (2H, d, J = 7.2 Hz), 3.17 (2H, q, J = 6.8 Hz), 1.82 (1H, m), 1.66 (2H, m), 1.55 (2H, m), 1.46 (2H, m), 1.29 (2H, m), 1.13 (3H, m), 0.95 (3H, m), 0.87 (3H, t, J = 7.2 Hz). Anal. ($C_{16}H_{26}N_6O\cdot0.1H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-pyridylmethyl)adenine (9y). Yield 67%; recrystallized from MeOH, mp 172−174 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 8.46 (1H, m), 7.73 (1H, dt, J = 7.6, 1.8 Hz), 7.25 (1H, 7.12 (1H, d, J = 8.0 Hz), 6.18 (1H, t, J = 5.6 Hz), 5.98 (2H, s), 4.91 (2H, s), 3.09 (2H, q, J = 6.8 Hz), 1.38 (2H, m), 1.20 (2H, m), 0.82 (3H, t, J = 7.2 Hz). Anal. ($C_{15}H_{19}N_7O\cdot H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(3-pyridylmethyl)adenine (9z). Yield 60%; recrystallized from MeOH−H_2O, mp 216−218 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (1H, s), 8.55 (1H, d, J = 1.6 Hz), 8.47 (1H, dd, J = 4.8, 1.6 Hz), 7.68 (1H, d, J = 7.6 Hz), 7.34 (1H, m), 6.23 (1H, t, J = 5.6 Hz), 6.02 (2H, s), 4.83 (2H, s), 3.15 (2H, q, J = 7.0 Hz), 1.44 (2H, m), 1.28 (2H, m), 0.85 (3H, t, J = 7.6 Hz). Anal. ($C_{15}H_{19}N_7O$) C, H, N.

2-Butylamino-8-hydroxy-9-(4-pyridylmethyl)adenine (9aa). Yield 75%; recrystallized from MeOH−H_2O, mp 205−207 °C; ^{1}H NMR (DMSO-d_6) δ 9.72 (1H, s), 8.49 (1H, dd, J = 4.4, 2.0 Hz), 7.20 (2H, d, J = 7.0 Hz), 6.22 (1H, t, J = 5.6 Hz), 6.05 (2H, s), 4.83 (2H, s), 3.12 (2H, q, J = 7.0 Hz), 1.39 (2H, m), 1.25 (2H, m), 0.83 (3H, t, J = 7.6 Hz). Anal. ($C_{15}H_{19}N_7O\cdot0.5H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-pyrazylmethyl)adenine (9ab). Yield 44%; recrystallized from MeOH−H_2O, mp 188−190 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 8.61 (31H, s), 8.54 (2H, dd, J = 8.8, 2.4 Hz), 6.17 (1H, t, J = 5.6 Hz), 6.02 (2H, s), 4.98 (2H, s), 3.08 (2H, q, J = 6.8 Hz), 1.35 (2H, m), 1.22 (2H, m), 0.81 (3H, t, J = 7.2 Hz). Anal. ($C_{14}H_{18}N_8O\cdot0.65H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(2-methylpyridine-3-ylmethyl)adenine (9ac). Yield 66%; recrystallized from MeOH, mp 215−217 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (1H, m), 8.32 (1H, d, J = 3.1 Hz), 7.37 (1H, d, J = 7.7 Hz), 7.14 (1H, dd, J = 7.7, 3.1 Hz), 6.20 (1H, t, J = 6.4 Hz), 6.00 (2H, s), 4.82 (2H, s), 3.12 (2H, m), 2.60 (3H, s), 1.39 (2H, m), 1.25 (2H, m), 0.84 (3H, t, J = 7.1 Hz). Anal. ($C_{16}H_{21}N_7O\cdot0.25H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(5-methylpyridine-3-ylmethyl)adenine (9ad). Yield 79%; recrystallized from MeOH, mp 221−222 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (1H, m), 8.35 (1H, s), 8.31 (1H, s), 7.50 (1H, s), 6.23 (1H, m), 6.02 (2H, s), 4.80 (2H, s), 3.16 (2H, m), 2.25 (3H, s), 1.44 (2H, m), 1.28 (2H, m), 0.86 (3H, t, J = 7.2 Hz). Anal. ($C_{16}H_{21}N_7O\cdot1.2H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(6-methylpyridine-3-ylmethyl)adenine (9ae). Yield 98%; recrystallized from MeOH, mp 248.5−252 °C; ^{1}H NMR (DMSO-d_6) δ 9.78 (1H, m), 8.42 (1H, d. J = 2.2

Hz), 7.57 (1H, dd, J = 8.0, 2.2 Hz), 7.19 (1H, d, J = 8.0 Hz), 6.22 (1H, t, J = 7.1 Hz), 6.09 (2H, s), 4.78 (2H, s), 3.16 (2H, m), 2.41 (3H, s), 1.44 (2H, m), 1.28 (2H, m), 0.87 (3H, t, J = 7.2 Hz). Anal. ($C_{16}H_{21}N_7O$) C, H, N.

2-Butylamino-9-(2-chloropyridine-3-ylmethyl)-8-hydroxyadenine (9af). Yield 78%; recrystallized from MeOH, mp 235−237 °C; ^{1}H NMR (DMSO-d_6) δ 8.77 (1H, s), 8.33 (1H, dd, J = 4.4, 2.0 Hz), 7.43 (1H, m), 7.39 (1H, m), 6.27 (1H, t, J = 5.6 Hz), 6.08 (2H, s), 3.08 (2H, q, J = 6.8 Hz), 1.38 (2H, m), 1.22 (2H, m), 0.81 (3H, t, J = 7.2 Hz). Anal. ($C_{15}H_{18}ClN_7O\cdot0.9H_2O$) C, H, N.

2-Butylamino-9-(4-chloropyridine-3-ylmethyl)-8-hydroxyadenine (9ag). Yield 47%; recrystallized from MeOH, mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ 9.73 (1H, s), 8.45 (1H, d, J = 5.6 Hz), 8.34 (1H, brs), 7.56 (1H, d, J = 5.6 Hz), 6.22 (1H, t, J = 5.2 Hz), 6.06 (2H, s), 4.94 (2H, s), 3.09 (2H, q, J = 6.8 Hz), 1.36 (2H, m), 1.23 (2H, m), 0.83 (3H, t, J = 7.2 Hz). Anal. ($C_{15}H_{18}ClN_7O\cdot0.2H_2O$) C, H, N.

2-Butylamino-9-(6-chloropyridine-3-ylmethyl)-8-hydroxyadenine (9ah). Yield 86%; recrystallized from MeOH, mp 256−258 °C; ^{1}H NMR (DMSO-d_6) δ 9.71 (1H, s), 8.38 (1H, d, J = 2.4 Hz), 7.73 (1H, dd, J = 8.0, 2.4 Hz), 7.48 (1H, d, J = 8.0 Hz), 6.23 (1H, t, J = 5.6 Hz), 6.04 (2H, s), 4.83 (2H, s), 3.14 (2H, q, J = 6.8 Hz), 1.43 (2H, m), 1.27 (2H, m), 0.86 (3H, t, J = 7.2 Hz). Anal. ($C_{15}H_{18}ClN_7O$) C, H, N.

2-Butylamino-9-(2-chloro-6-methylpyridine-3-ylmethyl)-8-hydroxyadenine (9ai). Yield 76%; recrystallized from MeOH, mp 215−216 °C; ^{1}H NMR (DMSO-d_6) δ 9.74 (1H, s), 7.33 (1H, d, J = 5.8 Hz), 7.22 (1H, d, J = 5.8 Hz), 6.24 (1H, m), 6.06 (2H, s), 4.83 (2H, s), 3.09 (2H, m), 2.42 (3H, s), 1.37 (2H, m), 1.25 (2H, m), 0.82 (3H, t, J = 5.5 Hz). Anal. ($C_{16}H_{20}ClN_7O\cdot0.3H_2O$) C, H, N.

2-Butylamino-9-(2,6-dichloropyridine-3-yl-methyl)-8-hydroxyadenine (9aj). Yield 89%; recrystallized from MeOH, mp 232−234 °C; ^{1}H NMR (DMSO-d_6) δ 9.77 (1H, s), 7.60 (1H, m), 7.53 (1H, d, J = 8.0 Hz), 6.24 (1H, t, J = 5.2 Hz), 6.07 (2H, s), 4.88 (2H, s), 3.09 (2H, q, J = 6.8 Hz), 1.36 (2H, m), 1.23 (2H, m), 0.83 (3H, t, J = 7.2 Hz). Anal. ($C_{15}H_{17}Cl_2N_7O\cdot0.85H_2O$) C, H, N.

9-(4-Fluorobenzyl)-8-hydroxy-2-(2-methoxyethylamino)adenine (20). Yield 68%; recrystallized from EtOH, mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 9.68 (1H, s), 7.34 (1H, dd, J = 8.6, 5.6 Hz), 7.14 (2H, dd, J = 8.6, 5.0 Hz), 6.17 (1H, t, J = 5.6 Hz), 6.07 (2H, s), 4.79 (2H, s), 3.38 (4H, m), 3.24 (3H, s). Anal. ($C_{15}H_{17}FN_6O_2$) C, H, N.

2-Benzylamino-9-(4-fluorobenzyl)-8-hydroxyadenine (21). Yield 95%; recrystallized from EtOH, mp 271−273 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (1H, s), 7.28 (7H, m), 7.07 (2H, m), 6.86 (1H, t, J = 5.6 Hz), 6.06 (2H, s), 4.76 (2H, s), 4.40 (2H, d, J = 6.4 Hz). Anal. ($C_{19}H_{17}FN_6O$) C, H, N.

9-(4-Fluorobenzyl)-8-hydroxy-2-(4-pyridylmethylamino)adenine (22). Yield 90%; recrystallized from EtOH, mp 227−230 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 8.44 (2H, dd, J = 4.4, 1.6 Hz), 7.28 (4H, m), 7.01 (3H, m), 6.08 (2H, s), 4.73 (2H, s), 4.40 (2H, d, J = 6.2 Hz). Anal. ($C_{18}H_{16}FN_7O$) C, H, N.

2-Butylamino-8-methoxy-9-(4-methoxybenzyl)adenine (10). A suspension of **8i** in 5 N NaOH solution (3 mL) and MeOH (3 mL) was refluxed for 4 h. The reaction mixture was concentrated. Then the residue was neutralized with 1 N HCl solution, and the resulting precipitate was collected to give the title compound as a white solid (298 mg, 84%): ^{1}H NMR (DMSO-d_6) δ 7.20 (2H, d, J = 8.6 Hz), 6.88 (2H, m), 6.27 (2H, s), 6.07 (1H, t, J = 5.6 Hz), 4.89 (2H, s), 3.99 (3H, s), 3.71 (3H, s), 3.19 (2H, m), 1.48 (2H, m), 1.31 (2H, m), 0.89 (3H, t, J = 7.2 Hz).

2-Butylamino-8-hydroxy-9-(4-methoxybenzyl)adenine (9j). A solution of **10** (178 mg, 0.5 mmol) in 12 N HCl (5 mL) was stirred at room temperature for 3 h. The reaction mixture was concentrated and neutralized with 1 N NaOH solution. The resulting precipitate was collected and washed with water to give the title compound as a white solid (160 mg, 92%): recrystallized from MeOH, mp 266−268 °C; ^{1}H NMR (DMSO-d_6) δ 9.59 (1H, s), 7.25 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 6.20 (1H, t, J = 5.6 Hz), 5.98 (2H, s), 4.73 (2H, s), 3.71 (3H, s), 3.17 (2H, q, J = 6.8 Hz), 1.45 (2H, m), 1.30 (2H, m), 0.88 (3H, t, J = 7.2 Hz). Anal. ($C_{17}H_{22}N_6O_2\cdot0.2H_2O$) C, H, N.

Compounds **9k**,**ak** were also prepared using the similar procedure used to prepare **9j**.

9-(4-Benzyloxybenzyl)-2-butylamino-8-hydroxyadenine (9k). Yield 92%; mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 9.60 (1H, s), 7.40 (7H, m), 6.94 (2H, dd, J = 6.8, 1.6 Hz), 6.20 (1H, t, J = 5.6 Hz), 5.98 (2H, s), 5.06 (2H, s), 4.73 (2H, s), 3.17 (2H, q, J = 6.8 Hz), 1.46 (2H, m), 1.28 (2H, m), 0.88 (3H, t, J = 7.2 Hz). Anal. ($C_{23}H_{26}N_6O_2$) C, H, N.

2-Butylamino-8-hydroxy-9-(6-methoxypyridine-3-ylmethyl)-adenine (9ak). Yield 49%; mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 9.64 (1H, s), 8.15 (1H, d, J = 2.2 Hz), 7.66 (1H, dd, J = 8.6, 2.2 Hz), 6.77 (1H, d, J = 8.6 Hz), 6.22 (1H, t, J = 5.7 Hz), 6.00 (2H, s), 4.74 (2H, s), 3.80 (3H, s), 3.17 (2H, q, J = 6.8 Hz), 1.44 (2H, m), 1.29 (2H, m), 0.88 (3H, t, J = 7.2 Hz). Anal. ($C_{16}H_{21}N_7O_2\cdot1.5H_2O$) C, H, N.

2-Butylamino-8-hydroxy-9-(4-hydroxybenzyl)adenine (13). To a suspension of **9k** (209 mg, 0.5 mmol) in HCO_2H (3 mL) was added 10% Pd(OH)$_2$/C (30 mg), and the reaction mixture was refluxed for 5 h. The catalyst was removed by filtration, and the resulting solution was concentrated. The residue was neutralized with a 28% ammonium solution, and the resulting solid was collected and washed with water to give the title compound as a white solid (92 mg, 56%): mp 242−245 °C; ^{1}H NMR (DMSO-d_6) δ 9.57 (1H, s), 9.35 (1H, s), 7.14 (2H, d, J = 8.2 Hz), 6.67 (2H, d, J = 8.2 Hz), 6.19 (1H, t, J = 5.6 Hz), 5.97 (2H, s), 4.67 (2H, s), 3.17 (2H, m), 1.46 (2H, m), 1.29 (2H, m), 0.89 (3H, t, J = 7.2 Hz). Anal. ($C_{16}H_{20}N_6O_2\cdot0.5H_2O$) C, H, N.

2-Butylamino-9-(6-dimethylaminopyridine-3-ylmethyl)-8-hydroxyadenine (23). A suspension of **8ah** (250 mg, 3 mmol) and dimethylamine (40% in H_2O, 10 mL) was heated at 140 °C for 6.5 h in an autoclave. The reaction mixture was concentrated, and water was added to the residue. The resulting precipitate was collected and purified by silica gel chromatography using 7% MeOH in $CHCl_3$ as an eluent to give the title compound as a white solid (100 mg, 39%): recrystallized from MeOH, mp 277−278 °C; ^{1}H NMR (DMSO-d_6) δ 9.61 (1H, s), 8.10 (1H, d, J = 2.2 Hz), 7.49 (1H, dd, J = 8.6, 2.2 Hz), 6.56 (1H, d, J = 8.6 Hz), 6.18 (1H, t, J = 5.7 Hz), 5.97 (2H, s), 4.65 (2H, s), 3.18 (2H, q, J = 6.8 Hz), 2.97 (6H, s), 1.48 (2H, m), 1.31 (2H, m), 0.89 (3H, t, J = 7.3 Hz). Anal. ($C_{17}H_{24}N_8O\cdot0.1H_2O$) C, H, N.

IFN Induction by Mouse Splenocyte Cultures. Male C3H/HeJ mice (Clea Japan Inc.) aged 8 weeks were sacrificed, and the spleens were removed from six mice. The spleens were meshed in phosphate buffered saline (PBS) and filtered through nylon mesh. The cell suspension was freed of erythrocytes by hypotonic treatment with 0.2% NaCl solution and washed twice with PBS. Splenocytes were resuspended at a concentration of 2×10^6 cells/mL in MEM supplemented with 5% fetal calf serum, 100 IU/mL of penicillin, and 100 μg/mL of streptomycin. The test compounds were dissolved in dimethyl sulfoxide and diluted to 500-fold with supplemented MEM. The above splenocytes suspension (0.5 mL) and various concentrations of the test compounds solution (0.5 mL) were mixed in 24-well plates and cultured in a humidified 5% CO_2/95% air atmosphere at 37 °C for 18 h. Supernatants were then collected, filter sterilized, and stored at −80 °C until they were analyzed for IFN.

IFN Induction in Mice. The test compounds suspended in 0.5% sodium carboxymethyl cellulose were administered orally to male BALB/c mice (Charles River Japan Inc.) aged 8−10 weeks. Blood was collected by cardiac puncture into a heparinized tube, under ether anesthesia, 2 h after test compounds administration. Plasma samples were obtained by centrifugation and stored at −80 °C until they were analyzed for IFN.

IFN Analysis. Mouse IFN titer in supernatants of splenocytes and plasma sample was quantitated by measuring its antiviral activity in a bioassay using mouse L929 cell monolayers challenged with vesicular stomatitis virus. Results are expressed as IFN IU/mL in terms of the international mouse IFN standard obtained from the National Institutes of Health, Bethesda, MD.

IFN Induction in Monkey. The test compounds suspended in 0.5% sodium carboxymethyl cellulose were administered orally to male cynomolgus monkeys aged 3−5 years. Blood samples (0.5 mL) were taken from a jugular vein, and plasma samples were prepared by centrifugation and stored at −80 °C until they were analyzed for IFN.

Ferret Emesis Analysis. The test compounds suspended in 0.5% sodium carboxymethyl cellulose were administered orally to male ferrets (Charles River Japan Inc.) aged 4 months. Then animals were transferred to individual cages and observed continuously over 6 h. Their behavior was recorded by video cameras, and tapes were subsequently read at the end of the experiment to evaluate emesis. Blood samples (0.5 mL) were taken from a jugular vein, and the serum was obtained by centrifugation at 1630*g* for 10 min at 4 °C and kept at −20 °C until drug analysis. The concentrations of test compounds in the plasma were assayed by an HPLC method.

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Supporting Information Available: ^{1}H NMR data of intermediate compounds and elemental analysis data of final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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