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Synthesis and Structure–Activity Relationships of 2-Amino-8-hydroxyadenines as Orally Active Interferon Inducing Agents

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Abstract—Recently, we have reported the 8-hydroxyadenine derivatives (**2**–**4**) as a novel class of interferon (IFN) inducing agents. In the present study, a series of 8-hydroxyadenines, which possess various amino moieties at the adenine C(2)-position, were synthesized and evaluated for their ability to induce endogenous IFN in comparison to the known active agent, Imiquimod. Among the compounds prepared, compound **9o** possessing a 2-methoxyethylamino group at C(2)-position of adenine was found to exhibit potent IFN inducing activity in vivo. Compound **9o** induced IFN from the dosage of 0.1 mg/kg, which was 30-fold potent than that of Imiquimod, and showed a good oral bioavailability ($F = 81\%$).

Introduction

Significant advances in antiviral therapeutics have occurred in recent years. Hepatitis C virus (HCV) is a member of the *Flaviviridae* family of viruses and is a causative agent of chronic non-A non-B hepatitis, which can lead to cirrhosis and hepatocellular carcinoma in humans. It is estimated that more than 170 million people worldwide are infected, and of those infected, approximately 20 and 4% are likely to develop liver cirrhosis and hepatocellular carcinoma, respectively, in the next decade.[1] Interferon (IFN) is one of the naturally occurring cytokines that have diverse biological functions, including antiviral, antiproliferative, and immunomodulatory activities.[2] Currently, the available treatments for hepatitis C are based upon alpha-IFN alone or in combination with ribavirin and are only effective in a limited number of cases.[3] IFN therapy is associated with significant problems of patient compliance, a loss of therapeutic efficacy as a result of the formation of neutralizing antibodies against IFN.[4] Enhancing the release of endogenous IFN by the administration of small molecular-weight compounds is one approach to achieve an equal IFN therapeutic effect. Therefore, orally available IFN inducing agents are envisaged as a new therapeutic class drugs and is currently an intensive area of the research.

Recently, a number of IFN inducing agents have been reported.[5] Among them, low molecular-weight compounds including the fluorenones,[6] pyrimidinones,[7] and anthraquinones[8] induced IFN in various animal species. However, none of these agents is capable of inducing high levels of IFN in humans.[9] Imiquimod (**1**), developed by 3M Pharmaceuticals, is a low molecular weight agent (M_W 240) with a potent IFN inducing property in humans[10] and has shown good efficacies in the treatment of external genital and perianal warts caused by papillomavirus infection.[11] However, in the clinical trial of Imiquimod for HCV, side effects such as vomiting and liver dysfunction were observed in some patients.[12] Therefore, further development of it was discontinued probably due to these side effects.

We have recently described the discovery of a series of 8-hydroxyadenines as novel IFN inducing agents.[13] The IFN inducing activities were significantly improved by the introduction of the substituents into the adenine

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C(2)-position (Table 1) and the analogues could be classified to following three types (**2**–**4**; Fig. 1). In spite of its weak activity in vitro, **2** showed equipotent activity with **3** and **4** in vivo. Compounds **2**–**4** possessed almost the same logP* values, but only **2** exhibited good solubility. These results suggested that **2** had good oral absorption due to its good solubility compared to **3** and **4**. Actually, the oral bioavailabilities of these compounds were 12, 3, and 4%, respectively, when orally administered at a dose of 10 mg/kg to rats. From these results, we examined detailed structure and activity relationship studies on **2**.

Chemistry

The synthetic route to the compound (**9a**–**9i**, **9o**, **9q**, **9r**, **9x** and **9ab**–**ad**) is shown in Scheme 1. Compound **6** was prepared by the reaction of 2,6-dichloropurine (**5**) with ammonia followed by benzylation.[14] Treatment of **6** with appropriate amines gave the corresponding C(2)-substituted intermediates (**7**). Bromination at the C(8)-position was selectively carried out with bromine to give the 8-bromo analogues (**8**). The target compounds (**9**)[15] were obtained by the treatment of 8-bromo analogues with 6N HCl at reflux.

The synthetic route to the compound (**9j**–**9n**, **9p**, **9s**–**9w**, **9y**, **9z**, **9aa** and **9ae**) is shown in Scheme 2. Compound **10** was obtained by the bromination of **6**. Treatment of **10** with 12 N HCl in *n*-BuOH gave **11**. Compound **11** was converted into the corresponding C(2)-substituted derivatives (**9**) by the reaction with appropriate amines.

Table 1. Activities of IFN induction and physicochemical properties

Compd	MEC[a] (μM)	MED[b] (mg/kg)	Solubility[c] (μg/mL)	LogP*
2	0.1	0.3	307	1.50
3	0.01	0.3	0.3	1.86
4	0.001	0.1	1.8	1.97
1	1	3	nt[d]	nt[d]

[a]MEC, Minimum Effective Concentration (mouse spleen cells).
[b]MED, Minimum Effective Dose (mouse, po).
[c]Solubility to pH 2.5 phosphate buffer.
[d]nt, not tested.

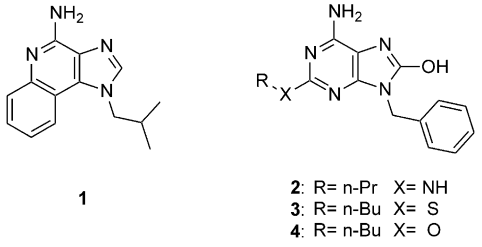
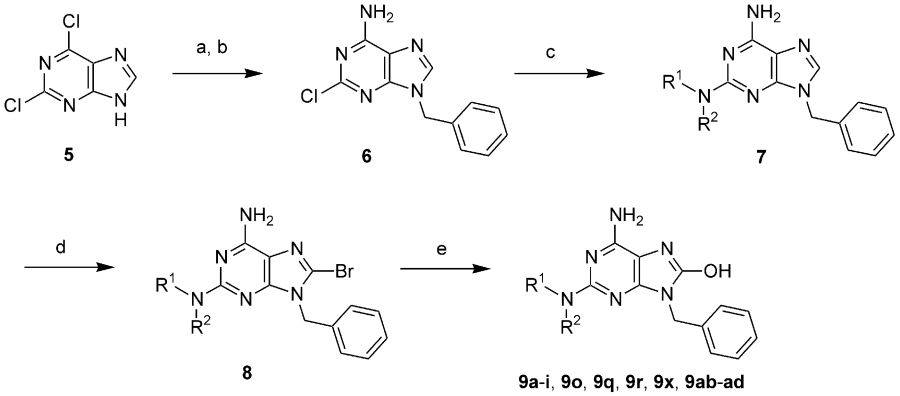


Figure 1. Structures of **1** (Imiquimod) and **2**–**4**.

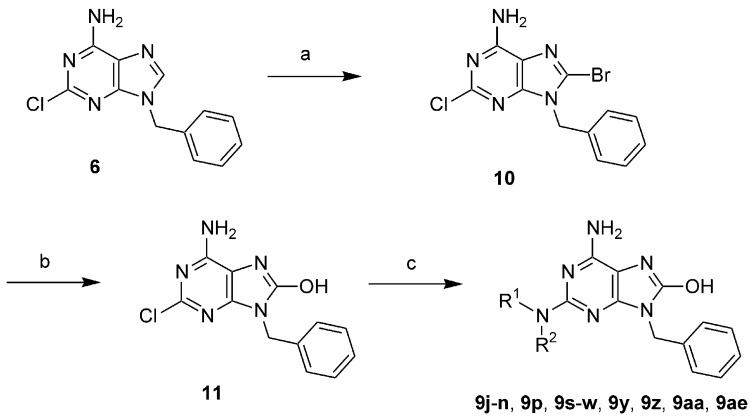
Results and Discussion

For in vitro studies, mouse spleen cells were cultured with the prepared compounds, and the amounts of IFN in supernatant were measured by bioassay system using L929 cells infected with vesicular stomatitis virus.[16] The results of their ability toward IFN induction is summarized in Table 2.

The IFN-inducing activity was improved with the elongation of the alkyl chain. Indeed, propyl (**2**), butyl (**9d**) and pentyl (**9e**) derivatives showed increased activities compared to methyl (**9b**) and ethyl (**9c**) derivatives. The branched alkyl (**9g**, **9h**) and cyclohexyl (**9i**) compounds showed equipotent activities with those of the straight alkyl chain compounds (**2**, **9d**, **9e**). Minimum effective concentrations (MECs) of these compounds were 0.1 μM. Introduction of polar groups into the alkyl side chain was investigated with respect to the activity. The activities were greatly decreased or diminished in the compounds having hydroxy groups (**9j**–**l**) or dimethylamino groups (**9m**, **9n**), suggesting that the presence of hydrophilic groups in the side chain were not compatible with potent activity. However, alkoxyalkyl derivatives (**9o**, **9p**) exhibited equipotent activities with those of the straight alkyl chain derivatives (**2**, **9d**, **9e**). The most potent activity was observed when the benzyl group (**9q**) was introduced into the C(2)-position of adenine. MEC of **9q** was 0.01 μM, which was 10-fold more potent than that of lead compound **2**. To determine the optimal methylene chain length between the adenine and the phenyl group, we prepared compounds **9r** and **9s**. The benzyl derivative (**9q**) exhibited more potent activity than those of phenyl

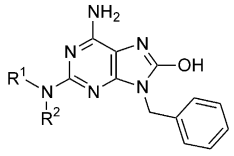


Scheme 1. Reagents and conditions: (a) 30% NH_3–MeOH, 100 °C in autoclave; (b) BnBr, K_2CO_3/DMF, rt; (c) R^1R^2NH, 120 °C in autoclave; (d) Br_2/CH_2Cl_2, rt; (e) 6 N HCl, reflux.



Scheme 2. Reagents and conditions: (a) Br_2, AcONa/AcOH, 70 °C; (b) 12 N HCl, *n*-BuOH, 100 °C; (c) R^1R^2NH, 120 °C in autoclave.

Table 2. IFN inducing activity in mouse spleen cells



Compd	R^1	R^2	IFN (IU/mL)[a]				
			Drug concentration (μM)				
			0.001	0.01	0.1	1	10
9a	H	H				<1.8	65.3
9b	Me	H			<1.8	2.8	24.8
9c	Et	H			<1.8	37.5	18.2
2	*n*-Pr	H		<1.8	95.4	18.3	15.1
9d	*n*-Bu	H		<1.8	3.4	33.7	17.0
9e	*n*-Pent	H		<1.8	92.0	49.6	32.7
9f	*iso*-Pr	H			<1.8	49.1	22.7
9g	*iso*-Bu	H		<1.8	5.3	46.8	38.2
9h	*s*-Bu	H		<1.8	33.3	49.4	23.5
9i	Cyclohexyl	H		<1.8	41.3	21.1	12.3
9j	$(CH_2)_2OH$	H			<1.8	5.1	5.5
9k	$(CH_2)_3OH$	H			<1.8	10.2	3.7
9l	$(CH_2)_4OH$	H			<1.8	14.4	2.5
9m	$(CH_2)_2NMe_2$	H					<1.8
9n	$(CH_2)_3NMe_2$	H				<1.8	5.8
9o	$(CH_2)_2OMe$	H		<1.8	7.1	18.1	11.6
9p	$(CH_2)_3OMe$	H		<1.8	10.2	3.4	3.4
9q	Bn	H	<1.8	44.4	80.4	18.6	17.3
9r	Ph	H			<1.8	2.1	<1.8
9s	$(CH_2)_2Ph$	H			<1.8	8.3	2.2
9t	3-MeO-Bn	H			<1.8	6.8	2.4
9u	4-MeO-Bn	H			<1.8	2.4	8.9
9v	4-Me-Bn	H		<1.8	14.7	7.4	7.4
9w	4-NMe_2-Bn	H			<1.8	5.5	2.9
9x	4-F-Bn	H			<1.8	8.9	4.4
9y	2-Pyridylmethyl	H			<1.8	23.9	5.6
9z	3-Pyridylmethyl	H			<1.8	7.7	2.2
9aa	4-Pyridylmethyl	H		<1.8	5.5	1.8	2.2
9ab	Bn	Me				<1.8	4.3
9ac	$-(CH_2)_2NMe(CH_2)_2-$				<1.8	6.3	3.4
9ad	$-(CH_2)_2O(CH_2)_2-$					<1.8	5.1
9ae	$-(CH_2)_2CHMe(CH_2)_2-$				<1.8	11.1	6.0
1					<1.8	26.2	19.6

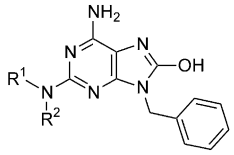
[a]Each value represents the mean of duplicate assays (IU/mL).

(**9r**) and phenethyl (**9s**) derivatives. Next, to explore the electronic effects of the benzyl moiety, we synthesized the analogues containing electron-donating moieties (**9t–w**) and an electron-withdrawing one (**9x**). These analogues showed decreased activities compared to the parent compound (**9q**), and clear electronic effects were not observed. When we replaced the benzyl group with pyridylmethyl groups (**9y–aa**), 4-pyridylmethyl analogue (**9aa**) showed the most potent activity of the pyridylmethyl derivatives, but its MEC was 10-fold weaker than that of **9q**. We examined the importance of NH at the C(2)-position. The hydrogen in **9q** was replaced with a methyl group (**9ab**), and piperazino (**9ac**), morpholino (**9ad**) and piperidino moieties (**9ae**) were introduced into the C(2)-position. All of these compounds showed decreased activities. The amount of induced IFN by all these derivatives including Imiquimod exhibited bell-shaped dose–response curves. Cytotoxicity could be considered as one of the possibilities for the bell-shape syndrome, however, cytotoxicity was not observed in our assay system. It is reported that Imiquimod exhibited a bell-shape type IFN-induction,[17] but the reason for this bell-shape is still not clear.

In addition to in vitro study, the compounds with moderate activities (MECs were under 0.1 μM) were further evaluated against in vivo study. The compounds were orally administered to male Balb/c mice and then the concentration of IFN in plasma 2 h after dosing was measured by the bioassay as mentioned above. In the preliminary experiment, IFN concentration in mouse plasma reached the maximum at 2 h after the oral administration of the compounds (data not shown). The results of the in vivo studies are shown in Table 3. The in vivo potencies of many of the compounds correlated with the results of the in vitro study. The in vivo activities of **9g**, **9i**, **9p**, and **9v** seemed to be weaker compared with their in vitro potentials. The discrepancy of these results may be due to the difference in oral bioavailability. Among these compounds, **9d**, **9o** and **9q** induced IFN from the dosage of 0.1 mg/kg, which were 30-fold more potent than that of Imiquimod.

Finally, we examined the oral bioavalabilities of these compounds by using rats, and found that **9o** showed a good oral absorption. The bioavailability of **9o** was calculated as 81%, whereas the those of **9d** and **9q** were very poor (<5%). Akira et al. have recently reported that imiqimod induces IFN via Toll-like receptor 7 (TLR7) signaling pathway.[18] Therefore, the action

Table 3. IFN inducing activity in mouse (in vivo)



Compd	R^1	R^2	IFN (IU/mL)[a]				
			Dose (mg/kg)				
			0.03	0.1	0.3	1	3
2	*n*-Pr	H			46	104	440
9d	*n*-Bu	H	<12	113	465	1180	1531
9e	*n*-Pent	H		<15	186	877	568
9g	*iso*-Bu	H				36	114
9h	*s*-Bu	H			30	261	757
9i	Cyclohexyl	H			<22	134	532
9o	$(CH_2)_2OMe$	H		44	639	974	
9p	$(CH_2)_3OMe$	H				<23	168
9q	Bn	H	<15	203	398	876	1110
9v	4-Me-Bn	H			<23		816
9aa	4-Pyridylmethyl	H			73		2295
1							64

[a]Each value shows the mean of three mice (IU/mL).

mechanisms of 8-hydroxyadenines toward TLR7 is under investigation.

In conclusion, a series of 8-hydroxyadenines, having various amino moieties at the C(2)-position, were synthesized and evaluated for their ability to induce IFN. We found that compound **9o** induced IFN from the dosage of 0.1 mg/kg, which was 30-fold potent than that of Imiquimod, and showed a good bioavailability. Additional studies are underway to further evaluate the therapeutic potential of these derivatives.

Experimental

Chemistry

Melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded at ambient temperature on a JEOL JNM LA-300 or a Bruker Avance 400 FT NMR spectrometer. Chemical shifts are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard, and signals are expressed s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Mass spectra (MS) were measured on a JEOL JMS-AX505W or JEOL JMS-SX102A mass spectrometer. Elemental analyses were performed by Sumika Chemical Analysis Service, Osaka, Japan. Commercial reagents and solvents were of reagent grade and used without further purification. Thin-layer chromatography (TLC) was performed on Merk Kieselgel 60 F_{254} precoated plates and components were visualized using UV light. Flash chromatography was conducted using Merk Kieselgel 60 F_{254} or Cica-Reagent Silica Gel 60.

9-Benzyl-2-chloroadenine (6). A solution of 2,6-dichloropurine (500 mg, 2.646 mmol) in 30% NH_3–MeOH (50 mL) was heated at 100 °C in autoclave for 12 h. The reaction mixture was evaporated in vacuo and water was added to the residue. The resulting precipitate was collected by filtration to give 2-chloroadenine (448 mg, quant). To a suspension of 2-chloroadenine (295 mg, 1.740 mmol) and K_2CO_3 (481 mg, 3.480 mmol) in DMF (10 mL) was added benzyl bromide (357 mg, 2.087 mmol) and the reaction mixture was stirred at room temperature for 4 h. The mixture was evaporated in vacuo and the residue was partitioned with water (100 mL) and $CHCl_3$ (200 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (2% MeOH–$CHCl_3$) and recystalized from EtOH to give **6** (200 mg, 44%) as a colorless solid: mp 216–218 °C; ^{1}H NMR (DMSO-d_6) δ 8.26 (1H, s), 7.81 (2H, s), 7.31 (5H, m), 5.34 (2H, s).

9-Benzyl-2-(methylamino)adenine (7b). A solution of **6** (200 mg, 0.770 mmol) in 40% $MeNH_2$–MeOH (50 mL) was heated at 120 °C in autoclave for 20 h. The mixture was evaporated in vacuo and the residue was partitioned with water (50 mL) and $CHCl_3$ (150 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel columnchromatography (2% MeOH–$CHCl_3$) to give **7b** (163 mg, 83%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 7.78 (1H, s), 7.36–7.26 (5H, m), 6.68 (2H, s), 6.20 (1H, q, J=4.8 Hz), 5.19 (2H, s), 2.76 (3H, d, J=4.8 Hz).

9-Benzyl-8-bromo-2-(methylamino)adenine (8b). To a solution of **7b** (75 mg, 0.295 mmol) in CH_2Cl_2 (50 mL) was added bromine (0.5 mL, 9.76 mmol) dropwise and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into 10% $Na_2S_2O_3$ aq The organic layer was separated, washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (1% MeOH–$CHCl_3$) to give **8b** (73 mg, 74%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 7.38–7.22 (5H, m), 6.90 (2H, s), 6.39 (1H, q, J=4.8 Hz), 5.18 (2H, s), 2.75 (3H, d, J=4.8 Hz).

9-Benzyl-8-hydroxy-2-(methylamino)adenine (9b). A solution of **8b** (55 mg, 0.165 mmol) in 6 N HCl (30 mL) was heated to reflux for 5 h. After evaporation, water (10 mL) was added to the residue and the solution was basified with 28% NH_3 aq. The resulting precipitate was collected by filtration to give **9b** (42 mg, 94%) as a colorless solid: mp 271–272 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 7.31–7.24 (5H, m), 6.19 (1H, q, J=4.8 Hz), 6.06 (2H, s), 4.81 (2H, s), 2.69 (3H, d, J=4.8 Hz); MS (EI) m/z 270 (M^+); HRMS calcd for $C_{13}H_{14}N_6O$ 270.1229, found 270.1221. Anal. calcd for $C_{13}H_{14}N_6O\cdot1/3HCl$: C, 55.28; H, 5.12; N, 29.75. Found: C, 55.25; H, 5.07; N, 29.60.

Compounds **9a**, **9c–i**, **9o**, **9q**, **9r**, **9x** and **9ab–ad** were prepared by using the similar procedures as for **9b**.

2-Amino-9-benzyl-8-hydroxyadenine (9a). A colorless solid (11% for three steps from **6**), mp 267–268 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (1H, s), 7.34–7.22 (5H, m), 6.02 (2H, s), 5.74 (2H, s), 4.81 (2H, s); MS (EI) *m*/*z* 256 (M^+); HRMS calcd for $C_{12}H_{12}N_6O$ 256.1072, found 256.1089. Anal. calcd for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.72; N, 32.79. Found: C, 56.07; H, 4.62; N, 32.78.

9-Benzyl-2-(ethylamino)-8-hydroxyadenine (9c). A colorless solid (46% for three steps from **6**), mp 269–272 °C; ^{1}H NMR (DMSO-d_6) δ 9.65 (1H, s), 7.34–7.24 (5H, m), 6.18 (1H, t, *J* = 5.5 Hz), 6.01 (2H, s), 4.81 (2H, s), 3.19 (2H, m), 1.06 (3H, t, *J* = 7.1 Hz); MS (EI) *m*/*z* 284 (M^+); HRMS calcd for $C_{14}H_{16}N_6O$ 284.1385, found 284.1363. Anal. calcd for $C_{14}H_{16}N_6O{\cdot}1/4H_2O$: C, 58.22; H, 5.76; N, 29.10. Found: C, 58.45; H, 5.64; N, 29.23.

9-Benzyl-2-(butyllamino)-8-hydroxyadenine (9d). A colorless solid (75% for three steps from **6**), mp 248–250 °C; ^{1}H NMR (DMSO-d_6) δ 9.64 (1H, s), 7.29–7.24 (5H, m), 6.19 (1H, t, *J* = 6.2 Hz), 6.00 (2H, s), 4.80 (2H, s), 3.15 (2H, m), 1.43 (2H, m), 1.28 (2H, m), 0.87 (3H, t, *J* = 7.3 Hz); MS (EI) *m*/*z* 312 (M^+); HRMS calcd for $C_{16}H_{20}N_6O$ 312.1698, found 312.1671. Anal. calcd for $C_{16}H_{20}N_6O{\cdot}1/2H_2O$: C, 59.80; H, 6.59; N, 26.15. Found: C, 59.71; H, 6.55; N, 25.88.

9-Benzyl-8-hydroxy-2-(pentylamino)adenine (9e). A colorless solid (37% for three steps from **6**), mp 250–252 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (1H, s), 7.30–7.24 (5H, m), 6.19 (1H, t, *J* = 5.3 Hz), 5.99 (2H, s), 4.80 (2H, s), 3.19–3.11 (2H, m), 1.48–1.43 (2H, m), 1.27-1.24 (4H, m), 0.85 (3H, t, *J* = 7.0 Hz); MS (EI) *m*/*z* 326 (M^+); HRMS calcd for $C_{17}H_{22}N_6O$ 326.1855, found 326.1872. Anal. calcd for $C_{17}H_{22}N_6O{\cdot}1/4H_2O$: C, 61.71; H, 6.85; N, 25.40. Found: C, 61.54; H, 6.73; N, 25.39.

9-Benzyl-8-hydroxy-2-(isopropylamino)adenine (9f). A colorless solid (59% for three steps from **6**), mp 243–246 °C; ^{1}H NMR (DMSO-d_6) δ 9.64 (1H, s), 7.34–7.21 (5H, m), 5.99 (2H, s), 5.98 (1H, d, *J* = 8.2 Hz), 4.80 (2H, s), 4.00–3.90 (1H, m), 1.08 (6H, d, *J* = 6.4 Hz); MS (EI) *m*/*z* 298 (M^+); HRMS calcd for $C_{15}H_{18}N_6O$ 298.1542, found 298.1530. Anal. calcd for $C_{15}H_{18}N_6O{\cdot}1/4H_2O$: C, 59.49; H, 6.16; N, 27.75. Found: C, 59.45; H, 5.97; N, 27.79.

9-Benzyl-8-hydroxy-2-(isobutyllamino)adenine (9g). A colorless solid (26% for three steps from **6**), mp 251–253 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (1H, s), 7.30–7.22 (5H, m), 6.24 (1H, t, *J* = 6.0 Hz), 5.99 (2H, s), 4.80 (2H, s), 2.99 (2H, t, *J* = 6.0 Hz), 1.84–1.75 (1H, m), 0.84 (6H, d, *J* = 6.8 Hz); MS (EI) *m*/*z* 312 (M^+); HRMS calcd for $C_{16}H_{20}N_6O$ 312.1698, found 312.1692. Anal. calcd for $C_{16}H_{20}N_6O{\cdot}11/10H_2O$: C, 57.85; H, 6.74; N, 25.30. Found: C, 57.63; H, 6.60; N, 25.50.

9-Benzyl-2-(*s*-butylamino)-8-hydroxyadenine (9h). A colorless solid (27% for three steps from **6**), mp 234–235 °C; ^{1}H NMR (DMSO-d_6) δ 9.63 (1H, s), 7.31–7.24 (5H, m), 5.97 (2H, s), 5.95 (1H, d, *J* = 8.6 Hz), 4.80 (2H, s), 3.82–3.74 (1H, m), 1.51–1.34 (2H, m), 1.04 (3H, d, *J* = 6.4 Hz), 0.83 (3H, t, *J* = 7.3 Hz); MS (EI) *m*/*z* 312 (M^+); HRMS calcd for $C_{16}H_{20}N_6O$ 312.1698, found 312.1723. Anal. calcd for $C_{16}H_{20}N_6O{\cdot}1/4H_2O$: C, 60.65; H, 6.52; N, 26.52. Found: C, 60.70; H, 6.33; N, 26.53.

9-Benzyl-2-(cyclohexylamino)-8-hydroxyadenine (9i). A colorless solid (9% for three steps from **6**), mp 245–248 °C; ^{1}H NMR (DMSO-d_6) δ 9.62 (1H, s), 7.28 (5H, m), 5.96 (3H, s), 4.78 (2H, s), 3.58 (1H, m), 1.80 (2H, m), 1.65 (2H, m), 1.56 (1H, m), 1.27–1.06 (5H, m); MS (EI) *m*/*z* 338 (M^+); HRMS calcd for $C_{18}H_{22}N_6O$ 338.1855, found 338.1842. Anal. calcd for $C_{18}H_{22}N_6O{\cdot}3/4H_2O$: C, 61.43; H, 6.73; N, 23.88. Found: C, 61.72; H, 6.74; N, 23.95.

9-Benzyl-8-hydroxy-2-[(2-methoxyethyl)amino]adenine (9o). A colorless solid (19% for three steps from **6**), mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 9.66 (1H, s), 7.34–7.26 (5H. m), 6.14 (1H, t, *J* = 7.8 Hz), 6.05 (2H, s), 3.37 (4H, m), 3.22 (3H, s); MS (EI) *m*/*z* 314 (M^+); HRMS calcd for $C_{15}H_{18}N_6O_2$ 314.1491, found 314.1476. Anal. calcd for $C_{15}H_{18}N_6O_2{\cdot}1/10H_2O$: C, 56.99; H, 5.80; N, 26.58. Found: C, 56.96; H, 5.70; N, 26.37.

9-Benzyl-2-(benzylamino)-8-hydroxyadenine (9q). A colorless solid (8% for three steps from **6**), mp 241–242 °C; ^{1}H NMR (DMSO-d_6) δ 9.75 (1H, s), 7.31–7.15 (10H, m), 6.83 (1H, t, *J* = 6.4 Hz), 6.10 (2H, s), 4.78 (2H, s), 4.40 (2H, d, *J* = 6.4 Hz); MS (FAB) *m*/*z* 347 (MH^+); HRMS calcd for $C_{19}H_{19}N_6O$ 347.1620, found 347.1625.

2-Anilino-9-benzyl-8-hydroxyadenine (9r). A colorless solid (13% for three steps from **6**), mp 220–223 °C; ^{1}H NMR (DMSO-d_6) δ 9.88 (1H, s), 8.79 (1H, s), 7.67 (2H, d, *J* = 8.0 Hz), 7.30–7.17 (5H, m), 7.11 (2H, t, *J* = 8.0 Hz), 6.75 (1H, t, *J* = 8.0 Hz), 6.20 (2H, s), 4.83 (2H, s); MS (FAB) *m*/*z* 333 (MH^+); HRMS calcd for $C_{18}H_{16}N_6O$ 333.1464, found 333.1453.

9-Benzyl-2-[(4-fluorobenzyl)amino]-8-hydroxyadenine (9x). A colorless solid (16% for three steps from **6**), mp 283–285 °C; ^{1}H NMR (DMSO-d_6) δ 9.69 (1H, s), 7.34–7.20 (7H, m), 7.08–7.02 (2H, m), 6.85 (1H, t, *J* = 6.3 Hz), 6.06 (2H, s), 4.79 (2H, s), 4.36 (2H, d, *J* = 6.3 Hz); MS (FAB) *m*/*z* 365 (MH^+); HRMS calcd for $C_{19}H_{18}FN_6O$ 365.1526, found 365.1536. Anal. calcd for $C_{19}H_{17}FN_6O{\cdot}H_2O$: C, 59.68; H, 5.01; N, 21.98. Found: C, 59.46; H, 4.90; N, 22.04.

9-Benzyl-2-(*N*-benzyl-*N*-methylamino)-8-hydroxyadenine (9ab). A colorless solid (54% for three steps from **6**), mp 256–257 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (1H, s), 7.29–7.19 (10H, m), 6.12 (2H, s), 4.81 (2H, s), 4.77 (2H, s), 2.99 (3H, s); MS (EI) *m*/*z* 360 (M^+); HRMS calcd for $C_{20}H_{20}N_6O$ 360.1698, found 360.1676. Anal. calcd for $C_{20}H_{20}N_6O{\cdot}1/5H_2O$: C, 65.99; H, 5.65; N, 23.09. Found: C, 66.08; H, 5.52; N, 23.15.

9-Benzyl-8-hydroxy-2-(4-methylpiperazin-1-yl)adenine (9ac). A colorless solid (17% for three steps from **6**), mp 248–250 °C; ^{1}H NMR (DMSO-d_6) δ 9.77 (1H, s), 7.24–7.32 (5H, m), 6.15 (2H, s), 4.82 (2H, s), 3.58 (4H, s), 2.32–2.27 (4H, m), 2.19 (3H, s); MS (FAB) *m*/*z* 340 (MH^+); HRMS calcd for $C_{17}H_{22}N_7O$ 340.1886, found

340.1887. Anal. calcd for $C_{17}H_{21}N_7O \cdot 1/3HCl$: C, 58.08; H, 6.12; N, 27.89. Found: C, 57.99; H, 6.03; N, 27.94.

9-Benzyl-8-hydroxy-2-(4-morpholinyl)adenine (9ad). A colorless solid (20% for three steps from **6**), mp 291–292 °C; ^{1}H NMR (DMSO-d_6) δ 9.75 (1H, s), 7.22–7.31 (5H, m), 6.15 (2H, s), 4.82 (2H, s), 3.59 (4H, d, J = 4.7 Hz), 3.54 (4H, d, J = 5.7 Hz); MS (FAB) m/z 327 (MH^+); HRMS calcd for $C_{16}H_{19}N_6O_2$ 327.1569, found 327.1579. Anal. calcd for $C_{16}H_{18}N_6O_2 \cdot 1/10H_2O$: C, 58.56; H, 5.59; N, 25.61. Found: C, 58.55; H, 5.44; N, 25.72.

9-Benzyl-8-bromo-2-chloroadenine (10). To a suspension of **6** (1.36 g, 5.2 mmol) and sodium acetate (6.4 g, 78 mmol) in AcOH (58 mL) was added bromine (3.48 mL, 67.6 mmol) dropwise at 0 °C and the mixture was stirred at 70 °C for 5 h. After cooling to ambient temperature, the reaction mixture was poured into 10% $Na_2S_2O_3$ aq and neutralized with 1 N NaOH. The resulting mixture was extracted with $CHCl_3$ and then the organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was triturated with diethy ether and the precipitate was collected by filtration to give **10** (1.16 g, 66%) as a light yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.99 (2H, br s), 7.38–7.29 (3H, m), 7.21–7.19 (2H, m), 5.32 (2H, s).

9-Benzyl-2-chloro-8-hydroxyadenine (11). A suspension of **10** (1.36 g, 5.2 mmol) in 12 N HCl (30 mL) and *n*-BuOH (30 mL) was heated at 100 °C for 7 h. After cooling to ambient temperature, the reaction mixture was evaporated in vacuo. The residue was poured into water and the precipitate was collected by filtration. The obtained solid was dissolved in 3 N NaOH and the solution was extracted with $CHCl_3$. The aqueous layer was neutralized with 12 N HCl and the resulting precipitate was collected by filtration to give **11** (582 mg, 65%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 10.36 (1H, br s), 7.35–7.25 (5H, m), 6.90 (2H, br s), 4.89 (2H, s).

9-Benzyl-2-[(2-dimethylaminoethyl)amino]-8-hydroxyadenine (9m). To a suspension of **11** (100 mg, 0.36 mmol) and *N,N*-dimethylethylenediamine (392 μL, 3.60 mmol) in *n*-BuOH (10 mL) was heated at 120 °C in autoclave for 18 h. The reaction mixture was evaporated in vacuo. The residue was partitioned with $CHCl_3$ (100 mL) and 3 N NaOH (80 mL). The aqueous layer was neutralized with 12 N HCl and extracted with $CHCl_3$ (100 mL). The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was triturated with MeOH/AcOEt (1:1) and the resulting precipitate was collected by filtration to give **9m** (31 mg, 25%) as a colorless solid: mp 197–200 °C; ^{1}H NMR (DMSO-d_6) δ 9.93 (1H, s), 7.29–7.24 (5H, m), 6.17 (2H, s), 5.94 (1H, br s), 4.80 (2H, s), 3.25 (2H, t, J = 6.2 Hz), 2.36–2.31 (2H, m), 2.13 (6H, s); MS (FAB) m/z 328 (MH^+); HRMS calcd for $C_{16}H_{22}N_6O$ 328.1886, found 328.1868.

Compounds **9j–l**, **9n**, **9p**, **9s–w**, **9y**, **9z**, **9aa** and **9ae** were prepared by using the similar procedures as for **9m**.

9-Benzyl-8-hydroxy-2-[(2-hydroxyethyl)amino]adenine (9j). A colorless solid (46%), mp 239–241 °C; ^{1}H NMR (DMSO-d_6) δ 9.76 (1H, s), 7.26–7.19 (5H, m), 6.05–6.00 (3H, m), 4.79 (2H, s), 4.58 (1H, t, J = 4.5 Hz), 3.46 (2H, q, J = 4.5 Hz), 3.26 (2H, q, J = 4.5 Hz); MS (FAB) m/z 301 (MH^+); HRMS calcd for $C_{14}H_{17}N_6O_2$ 301.1413, found 301.1422. Anal. calcd for $C_{14}H_{16}N_6O_2 \cdot 1/3HCl$: C, 53.81; H, 5.27; N, 26.90. Found: C, 53.52; H, 5.28; N, 26.62.

9-Benzyl-8-hydroxy-2-[(3-hydroxypropyl)amino]adenine (9k). A colorless solid (47%), mp 228–229 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (1H, s), 7.32–7.26 (5H, m), 6.17 (1H, t, J = 5.4 Hz), 6.04 (2H, s), 4.80 (2H, s), 4.43 (1H, t, J = 5.4 Hz), 3.44 (2H, dt, J = 5.4, 6.5 Hz), 3.22 (2H, dt, J = 5.4, 6.5 Hz), 1.62-1.60 (2H, m); MS (FAB) m/z 315 (MH^+); HRMS calcd for $C_{15}H_{19}N_6O_2$ 315.1569, found 315.1582. Anal. calcd for $C_{15}H_{18}N_6O_2 \cdot 1/10H_2O$: C, 56.99; H, 5.80; N, 26.58. Found: C, 56.89; H, 5.70; N, 26.84.

9-Benzyl-8-hydroxy-2-[(4-hydroxybutyl)amino]adenine (9l). A colorless solid (59%), mp 220–222 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (1H, s), 7.34–7.26 (5H, m), 6.23 (1H, t, J = 4.5 Hz), 5.99 (2H, s), 4.79 (2H, s), 4.33 (1H, t, J = 4.5 Hz), 3.36 (2H, q, J = 4.5 Hz), 3.15 (2H, q, J = 4.5 Hz), 1.44–1.40 (4H, m); MS (FAB) m/z 329 (MH^+); HRMS calcd for $C_{16}H_{21}N_6O_2$ 329.1726, found 329.1707. Anal. calcd for $C_{16}H_{20}N_6O_2 \cdot 3/5H_2O$: C, 56.66; H, 6.30; N, 24.78. Found: C, 56.61; H, 6.25; N, 24.96.

9-Benzyl-2-[(3-dimethylaminopropyl)amino]-8-hydroxyadenine (9n). A colorless solid (14%), mp 225–226 °C; ^{1}H NMR (DMSO-d_6) δ 10.08 (1H, s), 7.31–7.24 (5H, m), 6.23–6.16 (3H, m), 4.80 (2H, s), 3.21–3.13 (2H, m), 2.52–2.50 (2H, m), 2.09 (6H, s), 1.61–1.56 (2H, m); MS (FAB) m/z 342 (MH^+); HRMS calcd for $C_{17}H_{24}N_6O$ 342.2042, found 342.2051.

9-Benzyl-8-hydroxy-2-[3-methoxypropyl]amino]adenine (9p). A colorless solid (46%), mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 9.66 (1H, s), 7.29–7.15 (5H, m), 6.21 (1H, t, J = 5.4 Hz), 6.03 (2H, s), 4.81 (2H, s), 3.51 (3H, s), 3.24–3.20 (2H, m), 1.70–1.68 (4H, m); MS (FAB) m/z 329 (MH^+); HRMS calcd for $C_{16}H_{21}N_6O_2$ 329.1726, found 329.1710. Anal. calcd for $C_{16}H_{20}N_6O_2 \cdot 1/4H_2O$: C, 57.73; H, 6.21; N, 25.25. Found: C, 57.46; H, 6.07; N, 25.34.

9-Benzyl-8-hydroxy-2-[(2-phenylethyl)amino]adenine (9s). A colorless solid (31%), mp 220–223 °C; ^{1}H NMR (DMSO-d_6) δ 10.75 (1H, s), 7.80–7.03 (13H, m), 4.89 (2H, s), 3.52–3.50 (2H, m), 2.82 (2H, t, J = 6.3 Hz); MS (FAB) m/z 361 (MH^+); HRMS calcd for $C_{20}H_{21}N_6O$ 361.1777, found 361.1787. Anal. calcd for $C_{20}H_{20}N_6O \cdot HCl \cdot 5/4H_2O$: C, 57.28; H, 5.65; N, 20.04. Found: C, 57.52; H, 5.47; N, 19.79.

9-Benzyl-8-hydroxy-2-[(3-methoxybenzyl)amino]adenine (9t). A colorless solid (16%), mp 215–217 °C; ^{1}H NMR (DMSO-d_6) δ 9.68 (1H, s), 7.24–6.70 (10H, m), 5.86 (1H, t, J = 6.2 Hz), 5.41 (2H, s), 4.71 (2H, s), 4.35 (2H, d, J = 6.2 Hz), 3.68 (3H, s); MS (FAB) m/z 377 (MH^+); HRMS calcd for $C_{20}H_{21}N_6O_2$ 377.1726, found 377.1737.

9-Benzyl-8-hydroxy-2-[(4-methoxybenzyl)amino]adenine (9u). A colorless solid (15%), mp 233–234 °C; ^{1}H NMR (DMSO-d_6) δ 9.69 (1H, s), 7.23–7.20 (8H, m), 6.77 (2H, d, J = 8.9 Hz), 5.94–5.91 (3H, m), 4.76 (2H, s), 4.29 (2H, d, J = 5.9 Hz), 3.69 (3H, s); MS (FAB) m/z 377 (MH^+); HRMS calcd for $C_{20}H_{21}N_6O_2$ 377.1726, found 377.1744.

9-Benzyl-8-hydroxy-2-[(4-methylbenzyl)amino]adenine (9v). A colorless solid (75%), mp 245–248 °C; ^{1}H NMR (DMSO-d_6) δ 9.89 (1H, s), 7.48–7.00 (9H, m), 5.75 (1H, t, J = 6.3 Hz), 5.20 (2H, s), 4.71 (2H, s), 4.32 (2H, d, J = 6.3 Hz), 2.24 (3H, s); MS (FAB) m/z 361 (MH^+); HRMS calcd for $C_{20}H_{21}N_6O$ 361.1777, found 361.1774.

9-Benzyl-2-[(4-dimethylaminobenzyl)amino]-8-hydroxyadenine (9w). A colorless solid (14%), mp 219–221 °C; ^{1}H NMR (DMSO-d_6) δ 9.81 (1H, s), 7.26–7.09 (7H, m), 6.60–6.57 (3H, m), 6.05 (2H, s), 4.80 (2H, s), 4.25 (2H, d, J = 6.6 Hz), 2.84 (6H, s); MS (FAB) m/z 390 (MH^+); HRMS calcd for $C_{21}H_{24}N_7O$ 390.2042, found 390.2027.

9-Benzyl-8-hydroxy-2-[(2-pyridylmehyl)amino]adenine (9y). A colorless solid (41%), mp 209–210 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 8.47 (1H, d, J = 4.1 Hz), 7.71–7.19 (8H, m), 6.86 (1H, t, J = 5.9 Hz), 6.08 (2H, s), 4.76 (2H, s), 4.50 (2H, d, J = 5.9 Hz); MS (FAB) m/z 348 (MH^+); HRMS calcd for $C_{18}H_{18}N_7O$ 348.1573, found 348.1556. Anal. calcd for $C_{18}H_{17}N_7O{\cdot}HCl{\cdot}3/4H_2O$: C, 54.41; H, 4.95; N, 24.68. Found: C, 54.54; H, 5.00; N, 24.65.

9-Benzyl-8-hydroxy-2-[(3-pyridylmehyl)amino]adenine (9z). A colorless solid (20%), mp 238–242 °C; ^{1}H NMR (DMSO-d_6) δ 9.67 (1H, s), 8.53 (1H, d, J = 1.9 Hz), 8.41–8.39 (1H, m), 7.69–7.66 (1H, m), 7.30–7.24 (6H, m), 6.93 (1H, t, J = 6.2 Hz), 6.08 (2H, s), 4.78 (2H, s), 4.39 (2H, d, J = 6.2 Hz); MS (FAB) m/z 348 (MH^+); HRMS calcd for $C_{18}H_{18}N_7O$ 348.1573, found 348.1567. Anal. calcd for $C_{18}H_{17}N_7O{\cdot}5/4H_2O$: C, 58.45; H, 5.31; N, 26.51. Found: C, 58.44; H, 5.16; N, 26.69.

9-Benzyl-8-hydroxy-2-[(4-pyridylmehyl)amino]adenine (9aa). A colorless solid (30%), mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ 9.69 (1H, s), 8.42 (2H, d, J = 6.0 Hz), 7.25 (2H, d, J = 6.0 Hz), 7.21–7.19 (5H, m), 6.91 (1H, t, J = 6.6 Hz), 6.13 (2H, s), 4.75 (2H, s), 4.40 (2H, d, J = 6.6 Hz); MS (FAB) m/z 348 (MH^+); HRMS calcd for $C_{18}H_{18}N_7O$ 348.1573, found 348.1560. Anal. calcd for $C_{18}H_{17}N_7O{\cdot}2/5H_2O$: C, 60.97; H, 5.06; N, 27.65. Found: C, 61.15; H, 4.91; N, 27.35.

9-Benzyl-8-hydroxy-2-(4-methylpiperidynyl)adenine (9ae). A colorless solid (36%), mp 257–259 °C; ^{1}H NMR (DMSO-d_6) δ 9.70 (1H, s), 7.32–7.24 (5H, m), 6.08 (2H, s), 4.81 (2H, s), 4.53 (2H, br d, J = 13.2 Hz), 2.68 (2H, br t, J = 11.7 Hz), 1.60–0.97 (5H, m), 0.89 (3H, d, J = 6.5 Hz); MS (FAB) m/z 339 (MH^+); HRMS calcd for $C_{18}H_{23}N_6O$ 339.1933, found 339.1941.

Biology

IFN induction in mouse splenocyte cultures. Male C3H/HeJ mice (Clea Japan Inc.) aged 8 weeks were sacrificed, spleens were removed from six mice. 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 U/mL of penicillin, and 100 μg/mL of streptomycin. The test compounds were dissolved in dimethylsulfoxide and diluted to 500-fold with supplemented MEM. 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 mouse plasma. 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 heparinized tube, under ether anaesthesia, 2 h after test compounds administeration. 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 Institute of Health, Bethesda, MD, USA.

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