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Synthesis and Structure–Activity Relationships of 2-Substituted-8-hydroxyadenine Derivatives as Orally Available Interferon Inducers without Emetic Side Effects

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Abstract—Recently we reported the adenine derivatives (**2**–**4**) as new interferon (IFN) inducers. In the present study, we conducted a detailed structure and activity relationship study of **4** and its related derivatives on IFN inducing activity. From this study, we found that compound **4** exhibited the most potent IFN inducing activity in vitro with a minimum effective concentration of 0.01 μM, and **4** also showed strong IFN-inducing activity at doses of more than 0.3 mg/kg by oral administration in mice. This potency was 10-fold stronger than that of Imiquimod. Moreover, **4** did not cause emesis in ferrets even at doses as high as 10 mg/kg, whereas, 80% of animals were emetic when orally administered with the same dose of Imiquimod. These results indicate that compound **4** is superior to Imiquimod with respect to efficacy and safety.

Introduction

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] The current therapy using interferon-α (IFN-α) alone or in combination with Ribavirin is not highly efficacious, especially against genotype 1b viral infections. 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,[3] though, the patients have to suffer regular injections of IFN-α, once or twice a week.

Orally available IFN inducers offer the possible advantages of convenience, prolonged action and avoidance of immunogenicity. According to its expected low price, it could be used for the treatment of other viral infections. Therefore, IFN inducers are expected to be a new therapeutic class drug for viral infections. Although a number of IFN inducers including polyribonucleotide complexes,[4] fluorenones,[5] and pyrimidones[6] have been investigated, no results have been reported for IFN production in humans.[7]

Imiquimod (**1**), developed by 3M Pharmaceuticals, is a low molecular weight IFN inducer (MW 240) and has been reported to induce IFN production in humans.[8] However, the clinical trial for HCV or HBV treatment was discontinued possibly due to its serious side effects such as vomiting and liver dysfunctions. Such side effects have not been previously seen in IFN therapy, therefore, it may be estimated that such side effects are dependent on the action of Imiquimod itself.[9] In fact, Imiquimod is used only for the treatment of papilloma viral infections as external agent to avoid such side effects.

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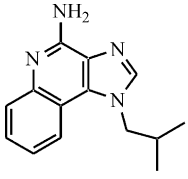
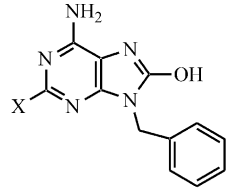
We have recently reported a series of 8-hydroxyadenines as novel IFN inducers (Fig. 1).[10] Compound **2** exhibited the most potent IFN-inducing activity, but the maximum plasma concentration (C_{max}) was low (80 ng/mL) when **2** was orally administered to rats at a dose of 10 mg/kg, and **3** also did not demonstrate a good oral absorption. On the other hand, **4** had a moderate IFN-inducing activity with a high Cmax value (490 ng/mL). To find compounds having potent IFN-inducing activities with good oral absorption, we conducted a detailed structure and activity relationship study on the modification of **4**.

Chemistry

The synthetic route to C(2)-substituted-9-benzyl-8-hydroxyadenine derivatives is shown in Scheme 1. 5-Amino-1-benzylimidazole-4-carboxamide (**5**),[11] prepared from 5-aminoimidazole-4-carboxamide with benzyl chloride in the presence of sodium hydroxide, was heated with an appropriate ethyl ester in the presence of sodium ethoxide to obtain hypoxanthine derivatives (**6e–u**) in good yields. Compounds (**6e–u**) were then chlorinated with phosphorous oxychloride, and aminated with ammonia in an autoclave to give adenine derivatives (**7e–u**). The reaction of **7e–u** with bromine in the presence of sodium acetate gave compounds **8e–u**. The desired compounds (**9e–u**) were obtained by heating **8e–u** in 12N HCl.



1 (Imiquimod)



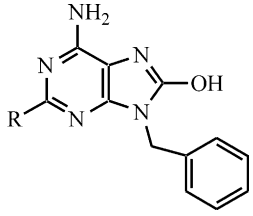
2: X = OBu (MEC = 1 nM)
3: X = NHBu (MEC = 100 nM)
4: X = Bu (MEC = 30 nM)

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

Results and Discussion

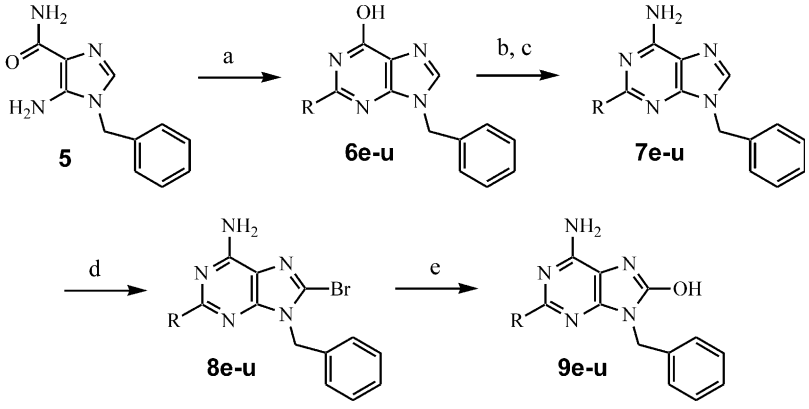
The IFN-inducing activities of the prepared compounds in vitro are shown in Table 1. Mouse splenocytes were cultured with compounds **4** or **9**, and the concentrations of IFN induced by compounds **4** or **9** in the supernatant were measured by bioassay using L929 cells with vesicular stomatitis virus.[12,13]

Table 1. IFN inducing activity in mouse splenocytes



Compd	R	IFN (U/mL)					
		Concentration of test compound (μM)					
		0.01	0.03	0.1	0.3	1	10
9a[15]	H					—	1.8
9b[10]	Me				—	4.2	14.6
9c[10]	Et			—	6	11.8	
9d[10]	Pr		—	1.6	12.2	8.2	
4	Bu	1.4	13.6	12.4			
9e	Pen	—	4.4	15.8	9.8		
9f	Hep				—	2.4	8.8
9g	*i*-Pr		—	11.4	11.8		
9h	*c*-Pr		—	4.6	13.7	4.5	
9i	*i*-Bu				—	15.8	
9j	*c*-Hex			—	14.4	15.4	
9k	CH_2-*c*-Pen		—	9.4	12.4	12	
9l	CH_2-*c*-Hex		—	5.2	10.2		
9m	Phenyl					—	6.4
9n	3-Pyridyl					—	4.8
9o	Benzyl		—	1.8	12.2	12	
9p	4-F-Benzyl		—	11.6	15	14.4	
9q	CF_3		—	13	13		
9r	CHF_2		—	1.9	6.7	8.8	
9s	CF_2CF_3			—	5.4	14	
9t	$(CF_2)_2CF_3$			—	1.1	8.3	
9u	$(CH_2)_2CF_3$		—	4.2	16.4		
Imiquimod	—			—	3.4	5.8	5.8

IFN concentration was measured by the method as described in the Experimental. Each value represents the mean of duplicate assay. —Not detected (detection limit was 0.4 IU/mL).



Scheme 1. Synthetic route to compounds **9e–u**. Reagents and conditions. (a) R-COOEt, Na, EtOH, reflux; (b) $POCl_3$, reflux; (c) NH_3 (28% in water), BuOH, 130 °C in autoclave; (d) Br_2, AcONa, AcOH, 110 °C; (e) 12 N HCl, 100 °C.

The IFN-inducing activity was enhanced by elongation of the methylene chain length at the C(2)-position, and the most suitable methylene length was found to be 4 or 5 (**4**, **9e**). The minimum effective concentration (MEC) of **4** was 0.01 μM, which was 30-fold stronger than that of Imiquimod. Branched or cyclic alkyl groups (**9g–l**) exhibited moderate activities, but were inferior to **4**. Compared to alkyl substituents, aromatic substituents (**9m**,[14] **9n**) showed decreased activities (MEC = 10 μM). However, the activities of the benzyl compounds (**9o**, **9p**) were moderate (MEC = 0.1 μM), and were the same as that of the cyclohexylmethyl compound (**9l**). These results show that a directly bound aromatic moiety did not result in potent activity. As the activities of **9o** and **9p** were 10-fold weaker than that of **4**, we did not conduct further studies on other arylalkyl substituents, such as 2-phenylethyl. The MEC of the trifluoromethyl compound (**9q**) was 0.1 μM, which was 10-fold potent than that of the methyl compound (**9b**: MEC = 1 μM), and considering the results of straight alkyl compounds (**9b–d**), we expected that the elongated fluorinated alkyl groups would improve the activity. But the elevation of the potency could not be achieved, for example, the pentafluoroethyl compound (**9s**: 0.3 μM), and the heptafluoropropyl compound (**9t**: 0.3 μM).

Before the in vivo studies were started, we confirmed that the subtype of IFN induced by the prepared compounds was identified to be mainly alpha IFN by using anti-human neutralizing antibodies to various types of IFN. Briefly, the anti-viral activity of IFN was neutralized by more than 90% by addition of the antibody against alpha IFN into the supernatant of mouse splenocytes. Whereas, the addition of the beta IFN antibody had no effect on the anti-viral activity (data not shown).

The results of the in vivo studies are shown in Table 2. The test compounds were orally administered 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. As shown in Table 2, compound **4** showed the most potent IFN-inducing activity and the order of potency was found to be closely correlated with that found in the in vitro studies. The potency of compound **4** was 10-fold higher than that of Imiquimod. The in vivo activities of compounds **9e**, **9q**, and **9u** seemed to be weaker compared with their in vitro activities. The discrepancy of these results may be due to differing oral bioavailabilities.

Table 2. IFN concentrations in mouse plasma 2 h after oral administration

Compd	R	IFN (U/mL)		
		Dose (mg/kg)		
		0.3	3	30
9a	H		<7	510±172
9b	Me	<7	76±23	3302±310
9c	Et		859±133	3208±1027
9d	Pr		230±117	2204±661
4	Bu	173±41	1476±146	1146±132
9e	Pen	21±6	942±265	6054±1792
9g	*i*-Pr		259±64	1015±204
9k	CH_2-*c*-Pen	<7	205±61	1157±278
9m	Phenyl		<7	10±5
9o	Benzyl		250±113	2545±863
9p	4-F-benzyl		458±160	
9q	CF_3	<7	174±59	1747±67
9u	$CH_2CH_2CF_3$		62±2	426±23
Imiquimod	—		67±21	1672±614

IFN concentration was measured by the method as described in the Experimental. Each value shows the mean±SE of three mice.

In general, it is reported that the IFN concentration in plasma is required to be controlled between the level of 50–100 U/mL in treatment of the HCV infection. In fact, the clinical trials of Imiquimod for treatment of HCV or HBV infections, were conducted at a dosage ranging between 100 and 200 mg per day (2–4 mg/kg), and the reduction of virus titer and the improvement of liver function were observed in patients. The result of this study in mice (67 U/mL @ 3 mg/kg of Imiquimod) was corresponded well with that of the clinical study. According to the present comparative study of Imiquimod and **4** in mice, it is estimated that **4** could induce IFN at a dose of 0.3 mg/kg in humans corresponding to a dose that is 10-fold lower than that of Imiquimod.

Emesis, one of the serious side effects, was observed in the clinical study of Imiquimod. Therefore, we chose a ferret emesis model to evaluate the emetic side effect. The effect was evaluated by the absence or presence of vomiting behavior during 6 h after administration of the compounds. When **4** was orally administered at a dose of 10 mg/kg, no vomiting was observed in any of three ferrets, while the administration of Imiquimod at the same dose caused vomiting in four out of five ferrets. The plasma concentrations of these compounds at 6 h postdosing were 2.5 μM for **4** and 4 μM for Imiquimod. Thus, although Imiquimod induced IFN production, it was strongly emetic. These results indicate that compound **4** exerts a highly potent IFN-inducing activity and is superior to Imiquimod in terms of the margin between the IFN-inducing and emetic doses. As for the possible reason for this result, we hypothesize that **4** is not able to transmigrate into the brain effectively due to 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 **4** is expected to be a useful IFN inducer for treatment of viral infections such as HCV and HBV. At present, further optimization studies of the substituents present in **4** are still underway.

Experimental

Chemistry

General. All reagents and solvents were obtained from commercial suppliers and were used without further purification. Melting points were measured with a Büchi

535 melting point apparatus and were uncorrected. Proton NMR spectra were recorded on a JEOL GSX270 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. TOF MS (time-of-flight mass spectrometry) was recorded on a Kompact MALDI 3 V4.0.0 spectrometer. Elemental analyses were performed at the Toray Research Center. Wakogel C-200 (Wako; 70–150 mm) was used for column chromatography. 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).

9-Benzyl-2-butylhypoxanthine. Ethyl valerate (6.0 g, 46 mmol) and **5** (1.3 g, 5.6 mmol) was added to a solution of sodium (1.1 g, 47 mmol) in EtOH (30 mL), and the reaction mixture was refluxed for 10 h. The reaction mixture was concentrated, water (30 mL) was added to the residue and neutralized by 12 N HCl. The precipitate was collected to give the title compound as a white solid (1.4 g, 89%); ^{1}H NMR (DMSO-d_6) δ 12.13 (1H, s), 8.11 (1H, s), 7.28–7.37 (5H, m), 5.32 (2H, s), 2.62 (2H, t, J = 7.3 Hz), 1.61–1.72 (2H, m), 1.23–1.36 (2H, m), 0.88 (3H, t, J = 7.3 Hz); MS (TOF) m/z 283 $(M+H)^+$.

Compounds **6e–u** were also prepared from appropriate commercially available esters using the same procedure described above.

6e: ^{1}H NMR (DMSO-d_6) δ 12.13 (1H, s), 8.11 (1H, s), 7.28–7.35 (5H, m), 5.33 (2H, s), 2.61 (2H, t, J = 7.3 Hz), 1.69 (2H, tt, J = 7.3, 7.3 Hz), 1.23–1.33 (4H, m), 0.85 (3H, t, J = 7.3 Hz); MS (TOF) m/z 297 $(M+H)^+$. **6f**: ^{1}H NMR (DMSO-d_6) δ 12.14 (1H, s), 8.12 (1H, s), 7.26–7.37 (5H, m), 5.33 (2H, s), 2.59 (2H, t, J = 7.3 Hz), 1.68 (2H, tt, J = 7.3, 7.3 Hz), 1.23–1.25 (8H, m), 0.84 (3H, t, J = 7.3 Hz); MS (TOF) m/z 325 $(M+H)^+$. **6g**: ^{1}H NMR (DMSO-d_6) δ 12.10 (1H, s), 8.12 (1H, s), 7.27–7.36 (5H, m), 5.32 (2H, s), 2.94 (1H, m), 1.24 (6H, d, J = 6.8 Hz); MS (TOF) m/z 269 $(M+H)^+$. **6h**: ^{1}H NMR (DMSO-d_6) δ 12.39 (1H, s), 8.07 (1H, s), 7.26–7.38 (5H, m), 5.24 (2H, s), 2.02 (1H, tt, J = 5.9, 5.9 Hz), 1.04 (4H, m); MS (TOF) m/z 267 $(M+H)^+$. **6i**: ^{1}H NMR (DMSO-d_6) δ 12.14 (1H, s), 8.12 (1H, s), 7.32 (5H, m), 5.33 (2H, s), 2.48 (2H, m), 2.05–2.20 (1H, m), 0.89 (6H, d, J = 6.5 Hz); MS (TOF) m/z 283 $(M+H)^+$. **6j**: ^{1}H NMR (DMSO-d_6) δ 12.06 (1H, s), 8.11 (1H, s), 7.29–7.36 (5H, m), 5.32 (2H, s), 2.58–2.69 (1H, m), 1.49–1.89 (7H, m), 1.14–1.37 (3H, m); MS (TOF) m/z 309 $(M+H)^+$. **6k**: ^{1}H NMR (DMSO-d_6) δ 12.13 (1H, s), 8.11 (1H, s), 7.28–7.32 (5H, m), 5.32 (2H, s), 2.60 (2H, d, J = 7.8 Hz), 2.26–2.36 (1H, m), 1.46–1.72 (6H, m), 1.12–1.24 (2H, m); MS (TOF) m/z 309 $(M+H)^+$. **6l**: ^{1}H NMR (DMSO-d_6) δ 12.12 (1H, s), 8.13 (1H, s), 7.28–7.34 (5H, m), 5.32 (2H, s), 1.77–1.87 (1H, m), 1.58–1.66 (6H, m), 1.46–1.72 (6H, m), 0.88–1.26 (6H, m); MS (TOF) m/z 323 $(M+H)^+$. **6n**: ^{1}H NMR (DMSO-d_6) δ 12.70 (1H, s), 9.27 (1H, s), 8.72–8.74 (1H, m), 8.44–8.49 (1H, m), 8.26 (1H, s), 7.55–7.60 (1H, m), 7.30–7.39 (5H, m), 5.44 (2H, s); MS (TOF) m/z 304 $(M+H)^+$. **6o**: ^{1}H NMR (DMSO-d_6) δ 12.41 (1H, s), 8.14 (1H, s), 7.23–7.34 (10H, m), 5.30 (2H, s), 3.95 (2H, s); MS (TOF) m/z 317 $(M+H)^+$. **6p**: ^{1}H NMR (DMSO-d_6) δ 12.41 (1H, s), 8.12 (1H, s), 7.28–7.39 (7H, m), 7.11–7.18 (2H, m), 5.28 (2H, s), 3.94 (2H, s); MS (TOF) m/z 336 $(M+H)^+$. **6q**: ^{1}H NMR (DMSO-d_6) δ 7.88 (1H, s), 7.25–7.36 (5H, m), 5.26 (2H, s); MS (TOF) m/z 295 $(M+H)^+$. **6r**: ^{1}H NMR (DMSO-d_6) δ 13.15 (1H, s), 8.32 (1H, s), 7.28–7.39 (5H, m), 6.84 (1H, t, J = 52.4 Hz), 5.41 (2H, s); MS (TOF) m/z 277 $(M+H)^+$. **6s**: ^{1}H NMR (DMSO-d_6) δ 7.87 (1H, s), 7.32 (5H, m), 5.23 (2H, s); MS (TOF) m/z 345 $(M+H)^+$. **6t**: ^{1}H NMR (DMSO-d_6) δ 13.74 (1H, s), 8.57 (1H, s), 7.28–7.36 (5H, m), 5.45 (2H, s); MS (TOF) m/z 395 $(M+H)^+$. **6u**: ^{1}H NMR (DMSO-d_6) δ 7.87 (1H, s), 7.23–7.36 (5H, m), 5.26 (2H, s), 2.63–2.82 (4H, s); MS (TOF) m/z 323 $(M+H)^+$.

9-Benzyl-2-butyladenine. A suspension of 9-benzyl-2-butylhypoxanthine (1.4 g, 5 mmol) in $POCl_3$ (15 mL) was refluxed for 3 h, and the reaction mixture was concentrated. Next, it was poured into crashed ice and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography using CH_2Cl_2 as an eluent to give 9-benzyl-2-butyl-6-chloropurine as a pale yellow oil (1.39 g, 93%). A suspension of 9-benzyl-2-butyl-6-chloropurine (1.38 g, 5 mmol) and 28% ammonium solution (12 mL) in EtOH (30 mL) was heated at 130 °C for 12 h in an autoclave. The reaction mixture was concentrated to give the title compound as a pale yellow solid (1.08 g, 84%); ^{1}H NMR (DMSO-d_6) δ 8.18 (1H, s), 7.26–7.33 (7H, m), 5.34 (2H, s), 2.66 (2H, t, J = 7.6 Hz), 1.67–1.73 (2H, m), 1.27–1.35 (2H, m), 0.89 (2H, t, J = 7.6 Hz); MS (TOF) m/z 282 $(M+H)^+$.

Compounds **7e–u** were also prepared using the same procedure described above.

7e: ^{1}H NMR (DMSO-d_6) δ 8.14 (1H, s), 7.24–7.33 (5H, m), 7.07 (2H, s), 5.33 (2H, s), 2.63 (2H, t, J = 7.6 Hz), 1.68–1.74 (2H, m), 1.26–1.30 (4H, m), 0.85 (3H, t, J = 7.6 Hz); MS (TOF) m/z 296 $(M+H)^+$. **7f**: ^{1}H NMR (DMSO-d_6) δ 8.17 (1H, s), 7.19–7.33 (7H, m), 5.33 (2H, s), 2.64 (2H, t, J = 7.6 Hz), 1.70–1.73 (2H, m), 1.24–1.27 (8H, m), 0.84 (3H, t, J = 7.6 Hz); MS (TOF) m/z 324 $(M+H)^+$. **7g**: ^{1}H NMR (DMSO-d_6) δ 8.14 (1H, s), 7.26–7.38 (5H, m), 7.05 (2H, s), 5.32 (2H, s), 2.92 (1H, m), 1.24 (6H, d, J = 6.8 Hz); MS (TOF) m/z 268 $(M+H)^+$. **7h**: ^{1}H NMR (DMSO-d_6) δ 8.10 (1H, s), 7.25–7.37 (5H, m), 7.03 (2H, s), 5.24 (2H, s), 2.02 (1H, m), 1.04 (4H, t, J = 2.7 Hz); MS (TOF) m/z 266 $(M+H)^+$. **7i**: ^{1}H NMR (DMSO-d_6) δ 8.14 (1H, s), 7.20–7.33 (5H, m), 7.08 (2H, s), 5.33 (2H, s), 2.18 (2H, m), 0.88 (6H, d, J = 6.5 Hz); MS (TOF) m/z 282 $(M+H)^+$. **7j**: ^{1}H NMR (DMSO-d_6) δ 8.14 (1H, s), 7.27–7.35 (5H, m), 7.00 (2H, s), 5.32 (2H, s), 2.50–2.63 (1H, m), 1.51–1.91 (7H, m), 1.15–1.40 (3H, m); MS (TOF) m/z 308 $(M+H)^+$. **7k**: ^{1}H NMR (DMSO-d_6) δ 8.14 (1H, s), 7.24–7.33 (5H, m), 7.07 (2H, s), 5.32 (2H, s), 2.63 (2H, d, J = 7.8 Hz), 2.30–2.50 (1H, m), 1.44–1.69 (6H, m), 1.15–1.27 (2H, m); MS (TOF) m/z 308 $(M+H)^+$. **7l**: ^{1}H NMR (DMSO-d_6) δ 8.15 (1H, s), 7.25–7.35 (5H, m), 7.11 (2H, s), 5.32 (2H, s), 2.54 (1H,

m), 1.79–1.93 (6H, m), 1.14–1.25 (4H, m), 0.86–1.01 (2H, m); MS (TOF) m/z 322 $(M+H)^+$. **7n**: ^{1}H NMR (DMSO-d_6) δ 9.50 (1H, d, $J=2.3$ Hz), 8.60–8.64 (1H, m), 8.32 (1H, s), 7.26–7.53 (8H, m), 5.45 (2H, s); MS (TOF) m/z 303 $(M+H)^+$. **7o**: ^{1}H NMR (DMSO-d_6) δ 8.18 (1H, s), 7.18–7.34 (10H, m), 5.33 (2H, s), 3.96 (2H, s); MS (TOF) m/z 316 $(M+H)^+$. **7p**: ^{1}H NMR (DMSO-d_6) δ 8.18 (1H, s), 7.28–7.33 (7H, m), 7.19 (2H, s), 7.04–7.12 (2H, m), 5.32 (2H, s), 3.95 (2H, s); MS (TOF) m/z 335 $(M+H)^+$. **7q**: ^{1}H NMR (DMSO-d_6) δ 8.45 (1H, s), 7.91 (2H, s), 7.26–7.39 (5H, m), 5.42 (2H, s); MS (TOF) m/z 294 $(M+H)^+$. **7r**: ^{1}H NMR (DMSO-d_6) δ 8.37 (1H, s), 7.26–7.39 (5H, m), 7.11 (2H, s), 6.67 (1H, t, $J=52.4$ Hz), 5.41 (2H, s); MS (TOF) m/z 276 $(M+H)^+$. **7s**: ^{1}H NMR (DMSO-d_6) δ 8.47 (1H, s), 7.29–7.36 (5H, m), 7.03 (2H, s), 5.40 (2H, s); MS (TOF) m/z 344 $(M+H)^+$. **7t**: ^{1}H NMR (DMSO-d_6) δ 8.46 (1H, s), 7.28–7.36 (5H, m), 7.05 (2H, s), 5.45 (2H, s); MS (TOF) m/z 394 $(M+H)^+$. **7u**: ^{1}H NMR (DMSO-d_6) δ 8.21 (1H, s), 7.25–7.37 (7H, m), 5.35 (2H, s); MS (TOF) m/z 322 $(M+H)^+$.

9-Benzyl-8-bromo-2-butyladenine (8d). A suspension of 9-benzyl-2-butyladenine (330 mg, 1.2 mmol), bromine (2.8 g, 18 mmol), and NaOAc (1.6 g, 20 mmol) in AcOH (20 mL) was heated at 110 °C for 2 h. The reaction mixture was concentrated, water was added to the residue and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, and concentrated. The residue was purified by silica gel chromatography using 1% MeOH in CH_2Cl_2 as an eluent to give the title compound as a pale yellow solid (215 mg, 51%); ^{1}H NMR (DMSO-d_6) δ 7.22–7.37 (7H, m), 5.32 (2H, s), 2.65 (2H, t, $J=7.6$ Hz), 1.64–1.75 (2H, m), 1.24–1.38 (2H, m), 0.88 (2H, t, $J=7.6$ Hz); MS (TOF) m/z 361 $(M+H)^+$.

Compounds **8e–u** were also prepared using the same procedure described above.

8e: ^{1}H NMR (DMSO-d_6) δ 7.22–7.37 (7H, m), 5.33 (2H, s), 2.61–2.75 (2H, m), 1.66–1.76 (2H, m), 1.28 (4H, m), 0.85 (3H, t, $J=7.3$ Hz); MS (TOF) m/z 375 $(M+H)^+$. **8f**: ^{1}H NMR (DMSO-d_6) δ 7.21–7.36 (7H, m), 5.32 (2H, s), 2.63 (2H, t, $J=7.6$ Hz), 1.65–1.75 (2H, m), 1.23–1.26 (8H, m), 0.84 (3H, t, $J=7.6$ Hz); MS (TOF) m/z 403 $(M+H)^+$. **8g**: ^{1}H NMR (DMSO-d_6) δ 7.25–7.38 (7H, m), 5.32 (2H, s), 2.89 (1H, m), 1.23 (6H, d, $J=6.8$ Hz); MS (TOF) m/z 347 $(M+H)^+$. **8h**: ^{1}H NMR (DMSO-d_6) δ 7.24–7.40 (7H, m), 5.32 (2H, s), 2.11 (1H, m), 1.07 (4H, t, $J=2.7$ Hz); MS (TOF) m/z 345 $(M+H)^+$. **8i**: ^{1}H NMR (DMSO-d_6) δ 7.22–7.36 (7H, m), 5.32 (2H, s), 2.54 (2H, m), 2.13–2.23 (1H, m), 0.88 (6H, d, $J=6.5$ Hz); MS (TOF) m/z 361 $(M+H)^+$. **8j**: ^{1}H NMR (DMSO-d_6) δ 7.24–7.39 (7H, m), 5.32 (2H, s), 2.50–2.59 (1H, m), 1.50–1.91 (7H, m), 1.18–1.40 (3H, m); MS (TOF) m/z 367 $(M+H)^+$. **8k**: ^{1}H NMR (DMSO-d_6) δ 7.23–7.37 (5H, m), 5.32 (2H, s), 2.64 (2H, d, $J=7.3$ Hz), 2.30–2.50 (1H, m), 1.44–1.71 (6H, m), 1.15–1.27 (2H, m); MS (TOF) m/z 367 $(M+H)^+$. **8l**: ^{1}H NMR (DMSO-d_6) δ 7.24–7.37 (7H, m), 5.32 (2H, s), 2.54 (1H, m), 1.80–1.93 (1H, m), 1.59–1.62 (6H, m), 1.13–1.25 (4H, m), 0.89–1.02 (2H, m); MS (TOF) m/z 401 $(M+H)^+$. **8n**: ^{1}H NMR (DMSO-d_6) δ 9.53 (1H, d, $J=1.9$ Hz), 9.12–9.16 (1H, m), 8.91–8.93 (1H, m), 8.01–8.06 (1H, m), 7.83 (2H, brs), 7.28–7.40 (5H, m), 5.49 (2H, s); MS (TOF) m/z 382 $(M+H)^+$. **8o**: ^{1}H NMR (DMSO-d_6) δ 7.18–7.39 (12H, m), 5.32 (2H, s), 3.97 (2H, s); MS (TOF) m/z 395 $(M+H)^+$. **8p**: ^{1}H NMR (DMSO-d_6) δ 7.21–7.41 (9H, m), 7.05–7.12 (2H, m), 5.31 (2H, s), 3.96 (2H, s); MS (TOF) m/z 414 $(M+H)^+$. **8q**: ^{1}H NMR (DMSO-d_6) δ 8.12 (2H, s), 7.31–7.40 (3H, s), 7.21–7.24 (2H, m), 5.40 (2H, s); MS (TOF) m/z 373 $(M+H)^+$. **8r**: ^{1}H NMR (DMSO-d_6) δ 7.89 (2H, s), 7.30–7.38 (3H, m), 7.19–7.22 (2H, m), 6.69 (1H, t, $J=54.8$ Hz), 5.39 (2H, s); MS (TOF) m/z 355 $(M+H)^+$. **8s**: ^{1}H NMR (DMSO-d_6) δ 8.11 (2H, s), 7.25–7.36 (3H, m), 5.38 (2H, s); MS (TOF) m/z 423 $(M+H)^+$. **8t**: ^{1}H NMR (DMSO-d_6) δ 8.12 (2H, s), 7.24–7.36 (5H, m), 5.38 (2H, s); MS (TOF) m/z 473 $(M+H)^+$. **8u**: ^{1}H NMR (DMSO-d_6) δ 7.46 (2H, s), 7.22–7.38 (5H, m), 5.34 (2H, s); MS (TOF) m/z 401 $(M+H)^+$.

9-Benzyl-2-butyl-8-hydroxyadenine (4). A suspension of 9-benzyl-8-bromo-2-butyladenine (1.85 g, 12.0 mmol) in 12 N HCl (27.8 mL) was heated at 100 °C for 8 h. The reaction mixture was concentrated, water (5 mL) was added to the residue and neutralized by 10% NaOH aqueous solution. The precipitate was collected and purified by silica gel chromatography using 3% MeOH in CH_2Cl_2 as an eluent to give the title compound as a white solid (246 mg, 62%), mp 280–283 °C; ^{1}H NMR (DMSO-d_6) δ 10.24 (1H, s), 7.23–7.30 (5H, m), 6.42 (2H, s), 4.89 (2H, s), 2.56 (2H, t, $J=6.6$ Hz), 1.61–1.66 (2H, m), 1.24–1.32 (2H, m), 0.87 (3H, t, $J=6.6$ Hz); MS (TOF) m/z 298 $(M+H)^+$. Anal. calcd for $C_{16}H_{19}N_5O\cdot1.4H_2O$; C, 59.57; H, 6.37; N, 21.71. Found: C, 59.36; H, 6.23; N, 21.48.

Compounds **9e–u** were also prepared by using the same procedure as for **4**.

9-Benzyl-8-hydroxy-2-pentyladenine (9e). White solid (yield 65%), mp 251–253 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.24–7.29 (5H, m), 6.34 (2H, s), 4.88 (2H, s), 2.55 (2H, t, $J=7.2$ Hz), 1.65 (2H, m), 1.26 (4H, m), 0.84 (3H, t, $J=7.2$ Hz); MS (TOF) m/z 312 $(M+H)^+$. Anal. calcd for $C_{17}H_{21}N_5O$: C, 65.57; H, 6.80; N, 22.49. Found: C, 65.54; H, 6.77; N, 22.59.

9-Benzyl-2-heptyl-8-hydroxyadenine (9f). White solid (yield 72%), mp 218–219 °C; ^{1}H NMR (DMSO-d_6) δ 10.08 (1H, s), 7.24–7.29 (5H, m), 6.33 (2H, s), 4.89 (2H, s), 2.56 (2H, t, $J=7.5$ Hz), 1.62–1.67 (2H, m), 1.22–1.24 (8H, m), 0.84 (3H, t, $J=7.5$ Hz); MS (TOF) m/z 340 $(M+H)^+$. Anal. calcd for $C_{19}H_{25}N_5O$: C, 67.23; H, 7.42; N, 20.63. Found: C, 67.11; H, 7.42; N, 20.51.

9-Benzyl-8-hydroxy-2-isopropyladenine (9g). White solid (yield 59%), mp 269–271 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 7.41 (5H, m), 6.39 (2H, s), 4.89 (2H, s), 2.15 (2H, m), 1.51 (6H, d, $J=6.8$ Hz); MS (TOF) m/z 284 $(M+H)^+$. Anal. calcd for $C_{15}H_{17}N_5O$: C, 63.59; H, 6.05; N, 24.72. Found: C, 63.49; H, 6.01; N, 24.51.

9-Benzyl-2-cyclopropyl-8-hydroxyadenine (9h). White solid (yield 69%), mp 263–266 °C; ^{1}H NMR (DMSO-d_6) δ 10.05 (1H, s), 7.25–7.35 (5H, m), 6.30 (2H, s), 4.87 (2H, s), 1.89 (1H, m), 0.82 (4H, m); MS (TOF) m/z 296 $(M+H)^+$. Anal. calcd for $C_{15}H_{15}N_5O\cdot0.1H_2O$: C, 63.68; H, 5.38; N, 24.75. Found: C, 63.59; H, 5.36; N, 25.06.

9-Benzyl-8-hydroxy-2-isobutyladenine (9i). White solid (yield 76%), mp 262–265 °C; ^{1}H NMR (DMSO-d_6) δ 7.24–7.31 (5H, m), 6.35 (2H, s), 4.90 (2H, s), 2.45 (2H, d, $J=6.8$ Hz), 2.11 (1H, m), 0.86 (6H, d, $J=6.8$ Hz); MS (TOF) m/z 298 $(M+H)^+$. Anal. calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55. Found: C, 64.56; H, 6.50; N, 23.53.

9-Benzyl-2-cyclohexyl-8-hydroxyadenine (9j). White solid (yield 68%), mp 249–252 °C; ^{1}H NMR (DMSO-d_6) δ 10.14 (1H, s), 7.23–7.33 (5H, m), 6.35 (2H, s), 4.89 (2H, s), 1.16–1.85 (11H, m); MS (TOF) m/z 324 $(M+H)^+$. Anal. calcd for $C_{18}H_{21}N_5O\cdot0.5H_2O$: C, 65.04; H, 6.52; N, 21.07. Found: C, 65.09; H, 6.55; N, 21.02.

9-Benzyl-2-cyclopentylmethyl-8-hydroxyadenine (9k). White solid (yield 60%), mp 230–233 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 7.23–7.32 (5H, m), 6.35 (2H, s), 4.90 (2H, s), 2.56 (2H, d, $J=7.3$ Hz), 2.24–2.35 (1H, m), 1.43–1.68 (6H, m), 1.14–1.24 (2H, m); MS (TOF) m/z 310 $(M+H)^+$. Anal. calcd for $C_{18}H_{21}N_5O\cdot0.2H_2O$: C, 66.11; H, 6.53; N, 21.42. Found: C, 66.02; H, 6.55; N, 21.43.

9-Benzyl-2-cyclohexylmethyl-8-hydroxyadenine (9l). White solid (yield 73%), mp 239–242 °C; ^{1}H NMR (DMSO-d_6) δ 10.12 (1H, s), 7.23–7.31 (5H, m), 6.35 (2H, s), 4.90 (2H, s), 2.45 (2H, d, $J=7.3$ Hz), 1.80–1.83 (1H, m), 1.56–1.61 (5H, m), 1.12–1.23 (3H, m), 0.86–0.98 (2H, m); MS (TOF) m/z 324 $(M+H)^+$. Anal. calcd for $C_{19}H_{23}N_5O$: C, 67.63; H, 6.87; N, 20.76. Found: C, 67.34; H, 6.90; N, 20.41.

9-Benzyl-8-hydroxy-2-phenyladenine (9m). White solid (yield 60%), mp 231–233 °C; ^{1}H NMR (DMSO-d_6) δ 10.31 (1H, s), 8.37 (2H, dd, $J=6.0$, 1.9 Hz), 7.23–7.44 (8H, m), 6.52 (2H, s), 5.01 (2H, s); MS (TOF) m/z 318 $(M+H)^+$. Anal. calcd for $C_{18}H_{15}N_5O\cdot0.3H_2O$: C, 66.98; H, 4.87; N, 21.70. Found: C, 67.07; H, 4.74; N, 21.50.

9-Benzyl-8-hydroxy-2-(3-pyridyl)adenine (9n). Pale Yellow solid (yield 36%), mp 260–263 °C; ^{1}H NMR (DMSO-d_6) δ 10.55 (1H, s), 9.40 (1H, s), 8.60–8.65 (2H, m), 7.55–7.59 (1H, m), 7.23–7.41 (6H, m), 6.73 (2H, s), 5.02 (2H, s); MS (TOF) m/z 319 $(M+H)^+$. Anal. calcd for $C_{17}H_{14}N_6O\cdot1.8H_2O$: C, 58.21; H, 4.54; N, 23.96. Found: C, 58.32; H, 4.78; N, 23.66.

2,9-Dibenzyl-8-hydroxyadenine (9o). White solid (yield 74%), mp 220–222 °C; ^{1}H NMR (DMSO-d_6) δ 10.11 (1H, s), 7.16–7.28 (10H, m), 6.40 (2H, s), 4.89 (2H, s), 3.88 (2H, s); MS (TOF) m/z 332 $(M+H)^+$. Anal. calcd for $C_{19}H_{17}N_5O\cdot0.2H_2O$: C, 68.13; H, 5.24; N, 20.91. Found: C, 68.12; H, 5.06; N, 20.93.

9-Benzyl-2-(4-fluorobenzyl)-8-hydroxyadenine (9p). White solid (yield 47%), mp 209–211 °C; ^{1}H NMR (DMSO-d_6) δ 10.28 (1H, s), 7.25–7.39 (7H, m), 7.04–7.11 (2H, m), 6.45 (2H, s), 4.89 (2H, s), 3.88 (2H, s); MS (TOF) m/z $(M+H)^+$. Anal. calcd for $C_{19}H_{16}FN_5O$: C, 65.32; H, 4.62; N, 20.05. Found: C, 65.31; H, 4.59; N, 20.00.

9-Benzyl-8-hydroxy-2-trifluoromethyladenine (9q). White solid (yield 78%), mp 263–265 °C; ^{1}H NMR (DMSO-d_6) δ 10.69 (1H, s), 7.23–7.35 (5H, m), 7.01 (2H, s), 4.95 (2H, s); MS (TOF) m/z 310 $(M+H)^+$. Anal. calcd for $C_{13}H_{15}F_3N_5O$; C, 50.49; H, 3.26; N, 22.65. Found: C, 50.47; H, 3.37; N, 22.43.

9-Benzyl-2-difluoromethyl-8-hydroxyadenine (9r). White solid (yield 40%), mp 253–256 °C; ^{1}H NMR (DMSO-d_6) δ 10.55 (1H, s), 7.24–7.36 (5H, m), 6.84 (2H, s), 6.61 (1H, t, $J=54.5$ Hz), 4.95 (2H, s); MS (TOF) m/z 292 $(M+H)^+$. Anal. calcd for $C_{13}H_{11}F_2N_5O$; C, 53.61; H, 3.81; N, 24.05. Found: C, 53.69; H, 3.91; N, 24.63.

9-Benzyl-8-hydroxy-2-pentafluoroethyladenine (9s). White solid (yield 72%), mp 235–238 °C; ^{1}H NMR (DMSO-d_6) δ 10.62 (1H, s), 7.24–7.32 (5H, m), 7.00 (2H, s), 4.94 (2H, s); MS (TOF) m/z 360 $(M+H)^+$. Anal. calcd for $C_{14}H_{15}F_5N_5O$: C, 46.81; H, 2.81; N, 19.49. Found: C, 46.52; H, 2.91; N, 19.37.

9-Benzyl-2-heptafluoropropyl-8-hydroxyadenine (9t). White solid (yield 96%), mp 234–236 °C; ^{1}H NMR (DMSO-d_6) δ 10.67 (1H, s), 7.24–7.35 (5H, m), 7.03 (2H, s), 4.94 (2H, s); MS (TOF) m/z 410 $(M+H)^+$. Anal. calcd for $C_{15}H_{10}F_7N_5O\cdot0.3H_2O$: C, 43.45; H, 2.58; N, 16.89. Found: C, 43.58; H, 2.66; N, 16.57.

9-Benzyl-8-hydroxy-2-(3,3,3-trifluoropropyl)adenine (9u). White solid (yield 70%), mp 242–245 °C; ^{1}H NMR (DMSO-d_6) δ 10.23 (1H, s), 7.23–7.31 (5H, m), 6.47 (2H, s), 4.91 (2H, s), 2.80–2.86 (2H, m), 2.62–2.77 (2H, m); MS (TOF) m/z 338 $(M+H)^+$. Anal. calcd for $C_{15}H_{14}F_3N_5O\cdot0.8H_2O$: C, 51.22; H, 4.24; N, 19.91. Found: C, 51.12; H, 4.09; N, 19.95.

Biology

IFN induction in mouse splenocyte cultures. Male C3H/HeJ mice (Clea Japan Inc.) aged 8 weeks were sacrificed, and 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 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 heparinized tube, under ether anaesthesia, 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 Institute of Health, Bethesda, MD, USA.

Ferret emesis analysis. The test compounds suspended in 0.5% sodium carboxymethyl cellulose were administered orally to male ferret (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 **1** and **2c** in the serum were assayed by an HPLC method.

References and Notes

1. Alter, H. J. *Blood* **1995**, *85*, 1681.
2. Saito, I.; Miyamura, T.; Ohbayashi, A.; Harada, H.; Katayama, T.; Kikuchi, S.; Watanabe, Y.; Koi, S.; Onji, M.; Ohta, Y.; Choo, Q. L.; Houghton, M.; Kuo, G. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6547.
3. Kasahara, A.; Hayashi, N. *J. Clin. Exp. Med.* **2002**, *200*, 85.
4. Hovanessian, A. G.; Youn, Y. K.; Buffet-Janvresse, C.; Riviere, Y.; Michelson, M.; Lacour, F. *Cancer* **1985**, *55*, 357.
5. Mayer, G. D.; Krueger, R. F. *Science* **1970**, *169*, 1214.
6. Nichol, F. R.; Weed, S. D.; Underwood, G. E. *Antimicrob. Agents Chemother.* **1976**, *9*, 433.
7. Dianzani, F. *J. Interferon Res.* **1992**, *12*, 109.
8. (a) Weeks, C. E.; Gibson, S. J. *J. Interferon Res.* **1994**, *14*, 81. (b) Savage, P.; Horton, V.; Moore, J.; Owens, M.; Witt, P.; Gore, M. E. *Brit. J. Cancer* **1996**, *74*, 1482. (c) Witt, P. L.; Ritch, P. S.; Reding, D.; McAuliffe, T. L.; Westrick, L.; Grossberg, S. E.; Borden, E. C. *Cancer Res.* **1993**, *53*, 5176.
9. (a) Strominger, N. L.; Brady, R.; Gullikson, G.; Carpenter, D. O. *Brain Res. Bull.* **2001**, *55*, 445. (b) Savage, P.; Horton, V.; Moore, J.; Owens, M.; Witt, P.; Gore, M. E. *Brit. J. Cancer* **1996**, *74*, 1482.
10. Hirota, K.; Kazaoka, K.; Niimoto, I.; Kumihara, H.; Sajiki, H.; Isobe, Y.; Takaku, H.; Tobe, M.; Ogita, H.; Ogino, T.; Ichii, S.; Kurimoto, A.; Kawakami, H. *J. Med. Chem.* **2002**, *45*, 5419.
11. Kelly, J. L.; Linn, J. A.; Selway, J. W. T. *J. Med. Chem.* **1989**, *32*, 218.
12. Watanabe, Y.; Kawade, Y. In *Lymphokines and Interferons: A Practical Approach*; Clemens, M. J., Morris, A. G., Gearing, A. J. H., Eds.; IRL: Oxford, 1987; p 6.
13. Pestka, S. In: *Methods in Enzymology*; Pestka, S., Ed.; Academic: New York, 1986; Vol. 119, p 16.
14. Hirota, K.; Kazaoka, K.; Niimoto, I.; Sajiki, H. *Heterocycles* **2001**, *55*, 2279.
15. Altman, J.; Ben-Ishai, D. *J. Heterocycl. Chem.* **1968**, *5*, 679.