Synthesis of 6-Substituted 9-Benzyl-8-hydroxypurines with Potential Interferon-Inducing Activity

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Various 6-substituted 9-benzyl-8-hydroxypurines were synthesized in order to investigate the structure–activity relationship at the 6-position of 9-benzyl-8-hydroxyadenine (1), which is a lead compound for the screening of interferon (IFN)-inducing activity. 6-Unsubstituted, mercapto-, methylthio- and hydroxy-9-benzyl-8-hydroxypurines (2—5) were prepared from 5-amino-1-benzyl-4-cyano-2-hydroxyimidazole (9). Synthesis of a 6-methoxy analog (6) was conducted from 5-amino-4-benzylamino-6-chloropyrimidine (13). 6-Alkylamino and acylaminopurines (7 and 8) were also prepared by alkylation and acylation of 1, respectively. Since these compounds (2—8) indicated no activity, it was found that a free amino group of 1 is required for the expression of IFN-inducing activity.

Key word interferon-inducing activity; 8-hydroxypurine; 9-benzyl-8-hydroxyadenine; 5-amino-4-cyano-2-hydroxyimidazole; reductive alkylation

8-Hydroxypurine derivatives have been reported to have a wide range of biological activities, such as corticotropin-releasing hormone receptor antagonism,[1,2] anti-rhinovirus activity,[3] xanthine oxidase inhibiting activity[4] and excellent binding affinity to a benzodiazepine receptor.[5] Recently, we found a novel type of lead compound, 9-benzyl-8-hydroxyadenine (**1**), possessing potent interferon (IFN)-inducing activity and conducted substituent modifications at the 2-, 8- and 9-positions of **1** to clarify the structure–activity relationships.[6] Consequently, we found that the 8-hydroxyl and 9-benzyl groups of **1** are required for the expression of IFN-inducing activity and the introduction of an appropriate alkyl-chain substituent at the 2-position of **1** remarkably increases the activity.[6] The focus of our attention was turned to the effect of the 6-amino group of **1** on the IFN-inducing activity. In this report, the synthesis of various 6-substituted 9-benzyl-8-hydroxypurines (**2**—**8**) shown in Fig. 1 and evaluation of their IFN-inducing activity are described.

Preparation of 6-substituted 9-benzyl-8-hydroxypurines was carried out as shown in Charts 1—3. Reaction of imidazole **9**[7] with thiobenzamide[8] afforded 4-thiocarbamoyl imidazole (**10**) in 69% yield, followed by cyclization with formamidine hydrochloride in refluxing 2-methoxyethanol to give 6-mercapto derivative (**3**) in 87% yield. Subsequently, **3** was selectively *S*-methylated with methyl iodide in the presence of potassium carbonate to give 6-methylthio derivative (**4**). 9-Benzyl-8-hydroxypurine (**2**) was obtained by desulfurization of **3** with Raney Ni. 6-Hydroxy derivative (**5**) was synthesized by hydrolysis of the cyano group of **9** under acidic conditions and subsequent cyclization of the resulting **11** with formamidine hydrochloride. Methylation of **5** did not give the desired 6-methoxypurine (**6**) but 1-methylhypoxanthine (**12**), whose structure was confirmed by nuclear Overhauser effect (NOE) between N^1-methyl and C^2-hydrogens as shown in Chart 1. Therefore, the synthesis of **6** was carried out using 5-amino-4-benzylamino-6-chloropyrimidine (**13**)[9] as the starting material (Chart 2). 6-Chloro-8-hydroxypurine (**14**) was easily prepared by cyclization of **13** with triphosgene in 88% yield. While conversion of **14** into 6-methoxypurine (**6**) in the presence of sodium methoxide was unsuccessful with recovery of **14**, 5-amino-4-benzylamino-6-methoxypyrimidine (**15**), which was prepared by methanolysis of **13** using sodium methoxide, was successfully cyclized

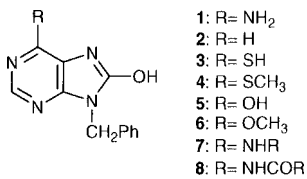
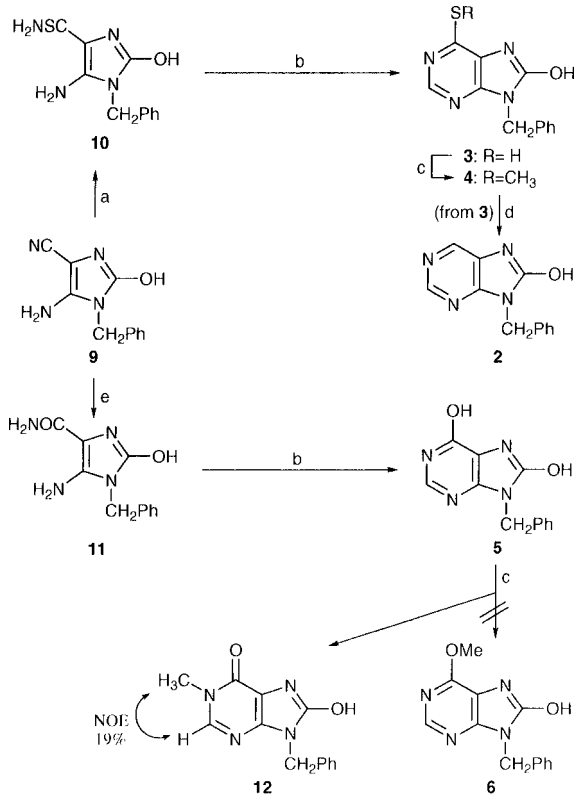


Fig. 1. 6-Substituted 9-Benzyl-8-hydroxypurines



Reagents and conditions: (a) $PhCSNH_2$, neat; (b) $HC(NH)NH_2 \cdot HCl$, 2-methoxyethanol, reflux; (c) CH_3I, K_2CO_3, DMF, rt; (d) Raney Ni, NH_4OH, CH_3OH, reflux (from **3**); (e) *c*. H_2SO_4, 0 °C→ rt.

Chart 1

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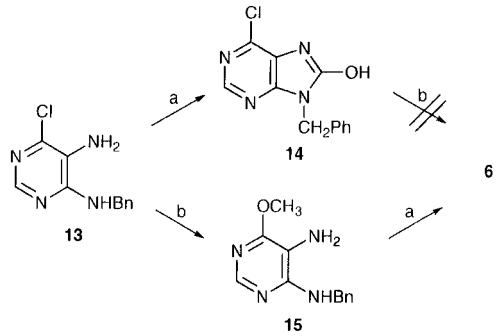
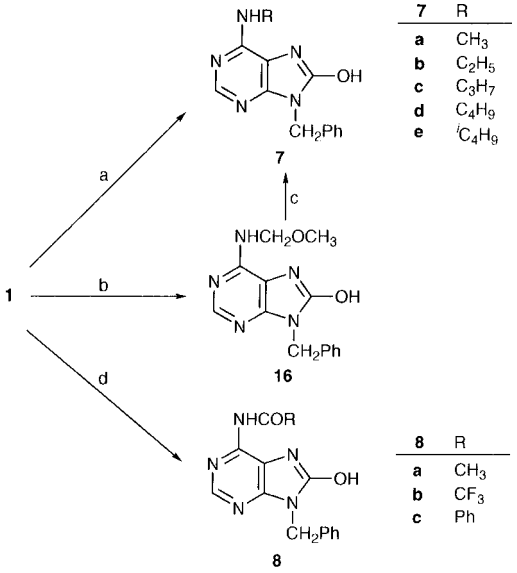


Reagents and conditions: (a) $(Cl_3CO)_2CO$, $(C_2H_5)_3N$, THF, rt; (b) $NaOCH_3$, CH_3OH, reflux.

Chart 2



7	R
a	CH_3
b	C_2H_5
c	C_3H_7
d	C_4H_9
e	$^iC_4H_9$

8	R
a	CH_3
b	CF_3
c	Ph

Reagents and Conditions: (a) aldehyde, $NaBH_3CN$, CH_3OH, 50 °C; (b) $(HCHO)_n$, $NaBH_4$, CH_3OH, 50 °C; (c) $NaBH_4$, CF_3CO_2H, THF, rt; (d) $(RCO)_2O$ (see experimental section).

Chart 3

with triphosgene to give **6**.

N^6-Alkyl-9-benzyl-8-hydroxyadenine derivatives (**7a**—**e**) were synthesized from 9-benzyl-8-hydroxyadenine (**1**)[7] by reductive alkylation using an appropriate aldehyde and sodium cyanoborohydride (Chart 3).[10] The alkylation of **1** proceeded in methanol at 50 °C to give **7b**—**d** in 51—78% yields. In the case of isobutyraldehyde, the reaction was slow and the corresponding product **7e** was obtained in 35% yield together with recovery of **1** in 52% yield. Although the treatment of **1** with paraformaldehyde in the presence of $NaBH_4$ gave only N^6-methoxymethyladenine derivative (**16**) in 69% yield instead of the desired product **7a**, **16** could be easily converted into **7a** by simple reduction using $NaBH_4$ as a reductant under acidic conditions.[11] Synthesis of N^6-acylated derivatives (**8a**—**c**) was achieved by chemoselective acylation of the 6-amino group of **1** using acid anhydride.

The *in vitro* assay for IFN-inducing activities of 9-benzyl-8-hydroxypurine derivatives (**2**—**8**, **14**) synthesized above was examined by a typical method for determining IFN titer check.[12,13] The activity is indicated by MEC (minimum effective concentration), which is the concentration of the test compounds required for more than 1 IU/ml induction of IFN. The 6-unsubstituted and 6-substituted purines (**2**—**8**, **14**) were inactive at the maximum concentration tested (10 μM), while lead compound **1** showed MEC of 10 μM.[6]

In conclusion, we have synthesized various 6-substituted 9-benzyl-8-hydroxypurine derivatives and evaluated their IFN-inducing activities. We revealed that the 6-amino group of the lead compound **1** is essential for the expression of the IFN-inducing activity. These results may provide useful information for mechanistic pharmaceutical studies of the 8-hydroxyadenine series.

Experimental

Melting points were determined on a Yanagimoto melting-point apparatus and are uncorrected. UV absorption spectra were recorded on a Shimadzu 260 spectrophotometer. IR spectra were measured using Perkin Elmer 1640 FT-IR spectrometer. ^{1}H-NMR spectra were recorded on a JEOL GX-270 (270 MHz) or JEOL JNM EX-400 (400 MHz) spectrometer using DMSO-d_6 as a solvent. Chemical shifts are given in ppm (δ) relative to internal solvent signals. Coupling constants (J) are given in Hz and splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; *, deuterium exchangeable. ^{13}C-NMR spectra were recorded on a JEOL JNM EX-400 (100 MHz) spectrometer using DMSO-d_6 as a solvent. Chemical shifts are given in ppm (δ) relative to internal solvent signals. Mass spectra were recorded on a JMS-SX 102A spectrometer. Elemental analyses were performed on Yanagimoto MT-3, and the results (C, H, N) were within ±0.3% of the theoretical values. Thin-layer chromatographic (TLC) analyses were carried out on 0.25 mm Silica Gel 60 F_{254} plates (Art 5715, Merck). The silica gel used for column chromatography was Silica Gel 60 (230—400 mesh, Merck).

5-Amino-4-cyano-2-hydroxy-4-thiocarbamoylimidazole (10) A mixture of 5-amino-1-benzyl-4-cyano-2-hydroxyimidazole (**9**, 200 mg, 0.93 mmol)[7] and thiobenzamide (641 mg, 4.67 mmol) was heated under Ar atmosphere at 140 °C for 15 min. The residue was triturated with MeOH (3 ml) and the resulting precipitate was filtered to give **10** (159 mg, 69%): mp 237—239 °C (washed with AcOEt); ^{1}H-NMR: 4.82 (2H, s, 1-CH_2), 7.24—7.36 (5H, m, 1-Ph), 7.39 (2H, br s*), 8.14 (2H, s*), 9.76 (1H, s*); ^{13}C-NMR: 42.2, 96.8, 127.0, 127.4, 128.5, 136.4, 146.2, 148.6, 174.2; IR (KBr) ν cm^{-1}: 3344, 1715, 1624, 1539, 1296, 736; UV (EtOH) λ_{max} 269.0 nm (ε 8400), 352.4 nm (ε 21600); MS (EI) *m/z* 248 (M^+), 214, 189, 91; HR-MS (EI) Calcd for $C_{11}H_{12}N_4OS$ (M^+): 248.0732. Found: 248.0734; *Anal*. Calcd for $C_{11}H_{12}N_4OS \cdot 1/10H_2O$: C, 52.83; H, 4.92; N, 22.40. Found: C, 53.00; H, 4.91; N, 22.24.

9-Benzyl-8-hydroxy-6-mercaptopurine (3) A mixture of **10** (400 mg, 1.61 mmol) and formamidine hydrochloride (519 mg, 6.45 mmol) in 2-methoxyethanol (10 ml) was refluxed under Ar atmosphere for 60 h. The solvent was removed under reduced pressure and the residue was triturated with H_2O (10 ml). The resulting precipitate was filtered to give **3** (363 mg, 87%): mp 277—279 °C (recrystallized from EtOH); ^{1}H-NMR: 4.94 (2H, s, 9-CH_2), 7.24—7.33 (5H, m, 9-Ph), 8.16 (1H, s, 2-H), 11.59 (1H, s*), 13.83 (1H, br s*); ^{13}C-NMR: 42.7, 121.9, 127.3, 127.5, 128.6, 136.4, 141.9, 145.5, 152.4, 161.3; IR (KBr) ν cm^{-1}: 3098, 1730, 1622, 1500, 1379, 1174; UV (EtOH) λ_{max} 240.0 nm (ε 12100), 315.6 nm (ε 12400), 336.6 nm (ε 11700); MS (EI) *m/z* 258 (M^+), 91; HR-MS (EI) Calcd for $C_{12}H_{10}N_4OS$ (M^+): 258.0575. Found: 258.0570; *Anal*. Calcd for $C_{12}H_{10}N_4OS$: C, 55.80; H, 3.90; N, 21.69. Found: C, 55.61; H, 4.00; N, 21.50.

9-Benzyl-8-hydroxy-6-methylthiopurine (4) A mixture of **3** (100 mg, 0.39 mmol) and K_2CO_3 (64 mg, 0.47 mmol) in dry *N,N*-dimethylformamide (DMF) (8 ml) was stirred under Ar atmosphere at room temperature for 1 h. To the mixture was added methyl iodide (29 μl, 0.47 mmol) and the mixture was stirred at room temperature for 12 h. After evaporation, the residue was triturated with H_2O (3 ml). The resulting suspension was neutralized with 10% $NaHSO_4$ solution and extracted with AcOEt (10 ml). The organic layer was washed with brine (10 ml) and dried over $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica gel (ether/hexane=2/1) to give **4** (21 mg, 20%): mp 250—253 °C; ^{1}H-NMR: 2.60 (3H, s, 6-CH_3), 4.97 (2H, s, 9-CH_2), 7.24—7.33 (5H, m, 9-Ph), 8.47 (1H, s, 2-H), 11.73 (1H, br s*); ^{13}C-NMR: 11.5, 42.6, 118.3, 127.4, 127.5, 128.6, 136.6, 144.3, 147.0, 150.5, 152.8; IR (KBr) ν cm^{-1}: 3446, 1709, 1612, 1482; UV (EtOH) λ_{max} 222.2 nm (ε 15700), 295.4 nm (ε 16000); MS (EI) *m/z* 272 (M^+), 91; HR-MS (EI) Calcd for $C_{13}H_{12}N_4OS$ (M^+): 272.0732. Found:

272.0725.

9-Benzyl-8-hydroxypurine (2) To a solution of **3** (300 mg, 1.16 mmol) in MeOH (25 ml) and 28% NH_4OH solution (5 ml) was added Raney Ni (1 ml of suspension in H_2O) and the mixture was refluxed for 4 h. After removal of the catalyst by filtration using celite cake, the filtrate was concentrated *in vacuo*. The residue was chromatographed on silica gel ($CHCl_3$/MeOH=66/1) to give **2** (95 mg, 36%): mp 197—198 °C; ^{1}H-NMR: 5.00 (2H, s, 9-CH2), 7.24—7.32 (5H, m, 9-Ph), 8.26 (1H, s, 2-H), 8.57 (1H, s, 6-H), 11.50 (1H, br s*); ^{13}C-NMR: 42.4, 122.0, 127.47, 127.54, 128.6, 132.8, 136.4, 149.5, 150.5, 153.0; IR (KBr) ν cm^{-1}: 3422, 3125, 1719, 1493, 1379, 702; UV (EtOH) λ_{max} 239.4 nm (ε 3600), 279.0 nm (ε 10900); MS (EI) *m/z* 226 (M^+), 197, 121, 91; HR-MS (EI) Calcd for $C_{12}H_{10}N_4O$ (M^+): 226.0855. Found: 226.0844; *Anal.* Calcd for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.77. Found: C, 63.58; H, 4.49; N, 24.65.

5-Amino-1-benzyl-4-carbamoyl-2-hydroxyimidazole (11) To concentrated H_2SO_4 (3 ml) in an ice bath was added **9** (300 mg, 1.40 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was diluted with ice water (15 ml) and neutralized with 28% NH_4OH solution. The mixture was kept standing overnight in a refrigerator (at 5 °C). The resulting precipitate was filtered to give **11** (294 mg, 91%): mp 206—208 °C (washed with AcOEt); ^{1}H-NMR: 4.76 (2H, s, 1-CH_2), 6.10 (2H, s*), 6.45 (2H, s*), 7.23—7.34 (5H, m, 1-Ph), 9.42 (1H, s*); ^{13}C-NMR: 41.8, 90.5, 127.0, 127.2, 128.4, 137.2, 140.2, 149.6, 162.3; IR (KBr) ν cm^{-1}: 3372, 3204, 1685, 1551, 1403, 739, 697; UV (EtOH) λ_{max} 289.2 nm (ε 14300); MS (EI) *m/z* 232 (M^+), 189, 91; HR-MS (EI) Calcd for $C_{11}H_{12}N_4O_2$ (M^+): 232.0960. Found: 232.0966; *Anal*. Calcd for $C_{11}H_{12}N_4O_2 \cdot 1/7H_2O$: C, 56.27; H, 5.27; N, 23.86. Found: C, 56.37; H, 5.24; N, 23.67.

9-Benzyl-6,8-dihydroxypurine (5) A mixture of **11** (300 mg, 1.29 mmol) and formamidine hydrochloride (416 mg, 5.17 mmol) in 2-methoxyethanol (8 ml) was refluxed under Ar atmosphere for 60 h. The solvent was removed under reduced pressure and the residue was triturated with H_2O (10 ml). The resulting precipitate was filtered to give **5** (124 mg, 40%): mp >300 °C (recrystallized from EtOH); ^{1}H-NMR: 4.91 (2H, s, 9-CH_2), 7.23—7.33 (5H, m, 9-Ph), 7.95 (1H, s, 2-H), 11.36 (1H, s*), 12.53 (1H, br s*); ^{13}C-NMR: 42.6, 107.8, 127.3, 127.4, 128.5, 137.0, 144.8, 145.1, 150.7, 152.2; IR (KBr) ν cm^{-1}: 3446, 3037, 1721, 1551, 1452, 1370; UV (EtOH) λ_{max} 257.8 nm (ε 10400); MS (EI) *m/z* 242 (M^+), 91; HR-MS (EI) Calcd for $C_{12}H_{10}N_4O_2$ (M^+): 242.0804. Found: 242.0812; *Anal*. Calcd for $C_{12}H_{10}N_4O_2 \cdot 1/3H_2O$: C, 58.06; H, 4.33; N, 22.57. Found: C, 58.17; H, 4.27; N, 22.62.

Methylation of 9-Benzyl-6,8-dihydropurine 5 (Preparation of 12) A mixture of **5** (274 mg, 1.13 mmol) and K_2CO_3 (188 mg, 1.36 mmol) in dry DMF (20 ml) was stirred under Ar atmosphere at room temperature for 1 h. To the mixture was added methyl iodide (85 μl, 1.36 mmol) and the mixture was stirred at room temperature for 24 h. After evaporation, the residue was triturated with H_2O (5 ml). The resulting suspension was neutralized with 10% $NaHSO_4$ solution and extracted with AcOEt (20 ml). The organic layer was washed with brine (10 ml) and dried over $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica gel ($CHCl_3$/MeOH=100/1) to give **12** (119 mg, 41%): mp 269—270 °C; ^{1}H-NMR: 3.47 (3H, s, 1-CH_3), 4.91 (2H, s, 9-CH_2), 7.23—7.33 (5H, m, 9-Ph), 8.27 (1H, s, 2-H), 11.39 (1H, s*); ^{13}C-NMR: 33.8, 42.5, 107.1, 127.3, 127.4, 128.5, 137.0, 144.3, 147.7, 150.7, 152.3; IR (KBr) ν cm^{-1}: 3448, 3140, 1702, 1539, 1457, 1346, 726; UV (EtOH) λ_{max} 259.0 nm (ε 8700), 286.6 nm (ε 4200); MS (EI) *m/z* 256 (M^+), 91; HR-MS (EI) Calcd for $C_{13}H_{12}N_4O_2$ (M^+): 256.0960. Found: 256.0958; *Anal*. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.87; H, 4.82; N, 21.63.

9-Benzyl-6-chloro-8-hydroxypurine (14) To a solution of 5-amino-4-benzylamino-6-chloropyrimidine (**13**, 416 mg, 1.77 mmol)[9] and triethylamine (0.49 ml, 3.55 mmol) in dry tetrahydrofuran (THF) (10 ml) was added triphosgene (526 mg, 1.77 mmol) in dry THF (5 ml) dropwise and the mixture was stirred under Ar atmosphere at room temperature for 1 h. After evaporation, the resulting residue was chromatographed on silica gel ($CHCl_3$/MeOH=200/1) to give **14**[14] (408 mg, 88%): mp 256—257 °C (washed with ether); ^{1}H-NMR: 5.00 (2H, s, 9-CH_2), 7.24—7.32 (5H, m, 9-Ph), 8.43 (1H, s, 2-H), 12.19 (1H, s*); ^{13}C-NMR: 43.0, 119.7, 127.5, 127.6, 128.6, 134.6, 136.1, 150.2, 150.6, 152.8; IR (KBr) ν cm^{-1}: 3424, 3007, 1715, 1493, 1375, 1158, 735; UV (EtOH) λ_{max} 246.4 nm (ε 3700), 281.2 nm (ε 12400); MS (EI) *m/z* 262 (M^++2), 260 (M^+), 231, 155, 91; HR-MS (EI) Calcd for $C_{12}H_9ClN_4O$ (M^+): 260.0465. Found: 260.0458; *Anal*. Calcd for $C_{12}H_9ClN_4O$: C, 55.29; H, 3.48; N, 21.49. Found: C, 55.23; H, 3.38; N, 21.63.

5-Amino-4-benzylamino-6-methoxypyrimidine (15) To a solution of Na (300 mg, 13.05 mmol) in dry MeOH (20 ml) was added **13** (300 mg, 1.28 mmol) and the mixture was refluxed under Ar atmosphere for 3 d. The mixture was diluted with H_2O (20 ml) and the solution was neutralized with 1 N HCl solution. The resulting solution was extracted with AcOEt (50 ml) and the organic layer was washed with brine (30 ml) and dried over $MgSO_4$. The solvent was evaporated off and the residue was chromatographed on silica gel ($CHCl_3$) to give **15** as a crude product, which was used for the next reaction without further purification: ^{1}H-NMR: 3.83 (3H, s, 6-CH_3), 4.18 (2H, s*, 5-NH_2), 4.59 (2H, d, *J*=5.9 Hz, 4-CH_2), 6.66 (1H, t*, *J*=5.9 Hz, 4-NH), 7.20—7.30 (5H, m, 4-Ph), 7.70 (1H, s, 2-H).

9-Benzyl-8-hydroxy-6-methoxypurine (6) To a solution of crude product **15** and triethylamine (92 μl, 0.66 mmol) in dry THF (5 ml) was added triphosgene (98 mg, 0.33 mmol) in dry THF (2 ml) and the mixture was stirred under Ar atmosphere at room temperature for 1.5 h. The solvent was removed under reduced pressure and the solidified product was chromatographed on silica gel to give **6** (36 mg, 11% in 2 steps): mp 266—267 °C; ^{1}H-NMR: 3.99 (3H, s, 6-CH_3), 4.98 (2H, s, 9-CH_2), 7.23—7.33 (5H, m, 9-Ph), 8.30 (1H, s, 2-H), 11.58 (1H, s*); ^{13}C-NMR: 42.7, 53.7, 106.3, 127.4, 127.5, 128.5, 136.7, 149.8, 149.9, 151.3, 152.8; IR (KBr) ν cm^{-1}: 3027, 1706, 1647, 1376, 1096; UV (EtOH) λ_{max} 266.2 nm (ε 13600); MS (EI) *m/z* 256 (M^+), 227, 151, 91; HR-MS (EI) Calcd for $C_{13}H_{12}N_4O_2$ (M^+): 256.0960. Found: 256.0966; *Anal*. Calcd for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.70; H, 4.78; N, 21.62.

Typical Procedure for the Synthesis of 7b—e To a suspension of **1** (0.42 mmol) and $NaBH_3CN$ (2.45 mmol) in dry MeOH (20 ml) was added an aldehyde [3.32 mmol except for the case of the preparation of **7b** (acetaldehyde was used 14.09 mmol)] and the mixture was stirred under Ar atmosphere at 50 °C for 8 days. After evaporation, the residue was chromatographed on silica gel.

9-Benzyl-N^6-ethyl-8-hydroxyadenine (**7b**): Yield 78%; mp 253—255 °C (recrystallized from 1,4-dioxane); ^{1}H-NMR: 1.16 (3H, t, *J*=7.1 Hz, 6-CH_3), 3.40—3.46 (2H, m, 6-CH_2), 4.91 (2H, s, 9-CH2), 6.46 (1H, t*, *J*=5.4 Hz, 6-NH), 7.22—7.32 (5H, m, 9-Ph), 8.08 (1H, s, 2-H), 10.18 (1H, s*); ^{13}C NMR: 15.0, 34.9, 42.4, 103.4, 127.4, 128.5, 137.1, 145.9, 146.5, 151.1, 151.9; IR (KBr) ν cm^{-1}: 3242, 1710, 1638, 1348, 890, 780, 697; UV (EtOH) λ_{max} 273.6 nm (ε 16700); MS (EI) *m/z* 269 (M^+), 254, 178, 91; HR-MS (EI) Calcd for $C_{14}H_{15}N_5O$ (M^+): 269.1277. Found: 269.1270; *Anal*. Calcd for $C_{14}H_{15}N_5O$: C, 62.44; H, 5.61; N, 26.01. Found: C, 62.37; H, 5.70; N, 25.98.

9-Benzyl-8-hydroxy-N^6-propyladenine (**7c**): Yield 51%; mp 226—228 °C (recrystallized from toluene); ^{1}H-NMR: 0.91 (3H, t, *J*=7.3 Hz, 6-CH_3), 1.51—1.60 (2H, m, 6-CH_2), 3.37 (2H, q, *J*=6.7 Hz, 6-CH2), 4.91 (2H, s, 9-CH_2), 6.48 (1H, t*, *J*=6.7 Hz, 6-NH), 7.24—7.32 (5H, m, 9-Ph), 8.08 (1H, s, 2-H), 10.20 (1H, s*); ^{13}C-NMR: 11.3, 22.5, 41.7, 42.4, 103.3, 127.3, 127.4, 128.4, 137.1, 146.0, 146.5, 151.0, 151.9; IR (KBr) ν cm^{-1}: 3358, 1703, 1641, 1458, 1371, 699, 632; UV (EtOH) λ_{max} 274.4 nm (ε 16700); MS (EI) *m/z* 283 (M^+), 268, 254, 241, 192, 91; HR-MS (EI) Calcd for $C_{15}H_{17}N_5O$ (M^+): 283.1433. Found: 283.1427; *Anal.* Calcd for $C_{15}H_{17}N_5O$: C, 63.59; H, 6.05; N, 24.72. Found: C, 63.32; H, 5.90; N, 24.69.

9-Benzyl-N^6-butyl-8-hydroxyadenine (**7d**): Yield 58%; mp 201—203 °C (recrystallized from toluene); ^{1}H-NMR: 0.90 (3H, t, *J*=7.3 Hz, 6-CH_3), 1.31—1.40 (2H, m, 6-CH_2), 1.49—1.56 (2H, m, 6-CH_2), 3.41 (2H, q, *J*=6.5 Hz, 6-CH_2), 4.91 (2H, s, 9-CH_2), 6.44 (1H, t*, *J*=6.5 Hz, 6-NH), 7.24—7.32 (5H, m, 9-Ph), 8.08 (1H, s, 2-H), 10.18 (1H, s*); ^{13}C-NMR: 13.6, 19.5, 31.3, 42.4, 103.3, 127.3, 127.4, 128.4, 137.1, 146.0, 146.5, 151.0, 151.8; IR (KBr) ν cm^{-1}: 3353, 2957, 1702, 1642, 1456, 698, 632; UV (EtOH) λ_{max} 274.2 nm (ε 16800); MS (EI) *m/z* 297 (M^+), 281, 268, 254, 241, 91; HR-MS (EI) Calcd for $C_{16}H_{19}N_5O$ (M^+): 297.1590. Found: 297.1597; *Anal*. Calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55. Found: C, 64.79; H, 6.42; N, 23.51.

9-Benzyl-8-hydroxy-N^6-isobutyladenine (**7e**): Yield 35%; mp 206—208 °C (recrystallized from toluene); ^{1}H-NMR: 0.91 (6H, d, *J*=6.4 Hz, 6-CH_3), 1.77—1.87 (1H, m, 6-CH), 3.25 (2H, t, *J*=6.1 Hz, 6-CH_2), 4.91 (2H, s, 9-CH_2), 6.47 (1H, t*, *J*=6.1 Hz, 6-NH), 7.24—7.30 (5H, m, 9-Ph), 8.07 (1H, s, 2-H), 10.22 (1H, s*); ^{13}C-NMR: 20.0, 28.0, 42.4, 47.3, 103.3, 127.3, 127.4, 128.4, 137.1, 146.1, 146.5, 150.9, 151.8; IR (KBr) ν cm^{-1}: 3247, 2960, 1708, 1632, 1354, 891, 782, 734; UV (EtOH) λ_{max} 273.6 nm (ε 17100); MS (EI) *m/z* 297 (M^+), 282, 254, 241, 91; HR-MS (EI) Calcd for $C_{16}H_{19}N_5O$ (M^+): 297.1590. Found: 297.1582; *Anal*. Calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55. Found: C, 64.89; H, 6.37; N, 23.35.

9-Benzyl-8-hydroxy-N^6-methoxymethyladenine (16) A mixture of **1** (300 mg, 1.24 mmol), paraformaldehyde [1.27 g, 42.30 mmol (calculated as formaldehyde)] and $NaBH_4$ (282 mg, 7.46 mmol) in dry MeOH (30 ml) was stirred under Ar atmosphere at 50 °C for 8 d. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel ($CHCl_3$/MeOH=100/1) to give **16** (246 mg, 69%): mp 225—227 °C (recrystallized from MeOH); ^{1}H-NMR: 3.22 (3H, s, 6-CH_3), 4.87 (2H, d, *J*=7.3 Hz,

6-CH_2), 4.94 (2H, s, 9-CH_2), 7.23—7.33 (5H, m, 9-Ph), 7.38 (1H, t*, J=7.3 Hz, 6-NH), 8.14 (1H, s, 2-H), 10.25 (1H, s*); ^{13}C-NMR: 42.4, 54.6, 71.9, 103.9, 127.4, 128.5, 137.0, 145.1, 147.7, 150.7, 152.0; IR (KBr) ν cm^{-1}: 3240, 3043, 1717, 1635, 1368, 1070; UV (EtOH) λ_{max} 273.8 nm (ε 15500); MS (EI) m/z 285 (M^+), 253, 224, 150, 91; HR-MS (EI) Calcd for $C_{14}H_{15}N_5O_2$ (M^+): 285.1226. Found: 285.1222; *Anal.* Calcd for $C_{14}H_{15}N_5O_2$: C, 58.94; H, 5.30; N, 24.55. Found: C, 58.67; H, 5.30; N, 24.39.

9-Benzyl-8-hydroxy-*N*^{6}-methyladenine (7a) To a solution of **16** (100 mg, 0.35 mmol) and $NaBH_4$ (80 mg, 2.10 mmol) in dry THF (15 ml) was added trifluoroacetic acid (0.3 ml, 3.89 mmol) and the mixture was stirred under Ar atmosphere at room temperature for 3 h. After evaporation, the residue was triturated with H_2O (5 ml) and the resulting suspension was neutralized with 1 N NaOH solution. The solution was extracted with AcOEt (20 ml) and the organic layer was washed with brine (10 ml) and dried over $MgSO_4$. The solvent was evaporated off and the residue was chromatographed on silica gel ($CHCl_3$/MeOH=50/1) to give **7a** (24 mg, 27%): mp 242—243 °C (washed with MeOH); ^{1}H-NMR: 2.93 (3H, d, J=4.4 Hz, 6-CH_3), 4.92 (2H, s, 9-CH_2), 6.46 (1H, br q*, J=4.4 Hz, 6-NH), 7.24—7.30 (5H, m, 9-Ph), 8.10 (1H, s, 2-H), 10.21 (1H, s*); ^{13}C-NMR: 27.1, 42.4, 103.6, 127.3, 128.4, 137.1, 146.4, 146.6, 151.0, 152.0; IR (KBr) ν cm^{-1}: 3447, 1696, 1653, 1455, 1375; UV (EtOH) λ_{max} 273.2 nm (ε 15600); MS (EI) m/z 255 (M^+), 226, 164, 150, 91; HR-MS (EI) Calcd for $C_{13}H_{13}N_5O$ (M^+): 255.1120. Found: 255.1123; *Anal*. Calcd for $C_{13}H_{13}N_5O \cdot 1/5$ MeOH: C, 60.59; H, 5.32; N, 26.76. Found: C, 60.73; H, 5.29; N, 26.55.

***N*^{6}-Acetyl-9-benzyl-8-hydroxyadenine (8a)** To a solution of acetic anhydride (5 ml) and concentrated H_2SO_4 (10 μl) was added **1** (200 mg, 0.83 mmol) and the mixture was stirred at room temperature for 30 min. The reaction mixture was dropped into ice water (10 ml) and the solution was neutralized with 28% NH_4OH solution. The precipitated product was filtered off, which was chromatographed on silica gel ($CHCl_3$/MeOH=100/1) to give **8a** (197 mg, 84%): mp 291—292 °C; ^{1}H-NMR: 2.12 (3H, s, 6-CH_3), 5.00 (2H, s, 9-CH_2), 7.25—7.32 (5H, m, 9-Ph), 8.38 (1H, s, 2-H), 10.18 (1H, s*), 10.84 (1H, s*); ^{13}C-NMR: 23.1, 42.6, 110.5, 127.47, 127.52, 128.6, 136.6, 137.5, 150.0, 150.8, 151.7, 169.4; IR (KBr) ν cm^{-1}: 3325, 3022, 1693, 1346; UV (EtOH) λ_{max} 289.0 nm (ε 12900); MS (EI) m/z 283 (M^+), 241, 91; HR-MS (EI) Calcd for $C_{14}H_{13}N_5O_2$ (M^+): 283.1069. Found: 283.1082; *Anal*. Calcd for $C_{14}H_{13}N_5O_2$: C, 59.36; H, 4.63; N, 24.72. Found: C, 59.16; H, 4.60; N, 24.52.

9-Benzyl-8-hydroxy-*N*^{6}-trifluoroacetyladenine (8b) A mixture of **1** (100 mg, 0.41 mmol) and trifluoroacetic anhydride (3 ml) was refluxed under Ar atmosphere for 19 h. The mixture was dropped into ice water (10 ml) and the solution was neutralized with 28% NH_4OH solution and then extracted with AcOEt (10 ml). The organic layer was washed with brine (10 ml) and dried over $MgSO_4$. After evaporation, the solid product was chromatographed on silica gel, ($CHCl_3$/MeOH=100/1) to give **8b** (49 mg, 35%): mp 243—245 °C; ^{1}H-NMR: 5.02 (2H, s, 9-CH_2), 7.25—7.34 (5H, m, 9-Ph), 8.51 (1H, s, 2-H), 11.22 (1H, br s*), 12.33 (1H, br s*); IR (KBr) ν cm^{-1}: 3362, 1709, 1590, 1496, 1222, 1166; UV (EtOH) λ_{max} 293.2 nm (ε 10100); MS (EI) m/z 337 (M^+), 308, 268, 232, 149, 91; HR-MS (EI) Calcd for $C_{14}H_{10}F_3N_5O_2$ (M^+): 337.0787. Found: 337.0793; *Anal*. Calcd for $C_{14}H_{10}F_3N_5O_2$: C, 49.86; H, 2.99; N, 20.77. Found: C, 49.81; H, 3.00; N, 20.73.

***N*^{6}-Benzoyl-9-benzyl-8-hydroxyadenine (8c)** A mixture of **1** (300 mg, 1.24 mmol) and benzoic anhydride (844 mg, 3.73 mmol) was heated under Ar atmosphere at 180 °C for 30 min. The resulting solid was triturated with ether (10 ml) and filtered to give **8c** (316 mg, 74%): mp 234—235 °C (recrystallized from EtOH); ^{1}H-NMR: 5.03 (2H, s, 9-CH_2), 7.25—7.34 (5H, m, 9-Ph), 7.54 (2H, t, J=7.3 Hz, 6-Ph), 7.63 (1H, t, J=7.3 Hz, 6-Ph), 8.04 (2H, d, J=7.3 Hz, 6-Ph), 8.47 (1H, s, 2-H), 10.65 (1H, s*), 11.19 (1H, s*); ^{13}C-NMR: 42.6, 112.4, 127.5, 127.6, 128.3, 128.4, 128.6, 132.3, 132.9, 136.6, 137.9, 149.8, 150.9, 152.1, 165.4; IR (KBr) ε cm^{-1}: 3381, 1722, 1676, 1591, 1495, 1331, 700; UV (EtOH) λ_{max} 232.4 nm (ε 22500), 298.8 nm (ε 14900); MS (EI) m/z 345 (M^+), 317, 105, 91, 77; HR-MS (EI) Calcd for $C_{19}H_{15}N_5O_2$ (M^+): 345.1226. Found: 345.1219; *Anal*. Calcd for $C_{19}H_{15}N_5O_2 \cdot 1/3H_2O$: C, 64.95; H, 4.49; N, 19.93. Found: C, 64.99; H, 4.33; N, 20.02.

IFN Induction in Mouse Splenocyte Cultures Male C3H/HeJ mice (Clea Japan Inc.) aged 8 weeks were sacrificed, spleens were removed from 6 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 DMSO and diluted to 500-fold with supplemented MEM.

Above splenocytes suspension (0.5 ml) and various concentration 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 Analysis Mouse IFN titer in supernatants of splenocytes was quantitated by measuring its antiviral activity in a bioassay using mouse L929 cell monolayers challenged with vesicular stomatitis virus.[12] Results are expressed as IFN IU/ml in terms of the international mouse IFN standard[13] obtained from the National Institute of Health, Bethesda, MD, U.S.A.

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