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Synthesis and Biological Evaluation of 2,8-Disubstituted 9-Benzyladenines: Discovery of 8-Mercaptoadenines as Potent Interferon-Inducers

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Abstract—Recently, we have identified 9-benzyl-8-hydroxyadenines bearing an appropriate substituent (a butoxy, propylthio or butylamino group) at the 2-position as potent interferon (IFN)-inducers. Herein we report the design, synthesis, and IFN-inducing activity of 8-substituted 9-benzyladenines possessing such an appropriate substituent at the 2-position. Introduction of the appropriate substituent into the 2-position of the adenine nucleus gave rise to expression of the activity even in 9-benzyladenines bearing no hydroxyl group at the 8-position. An amino group at the 6-position and a hydroxyl or thiol group carrying an acidic proton at the 8-position are required to express excellent IFN-inducing activity. 9-Benzyl-2-butoxy-8-mercaptoadenine (**9**) indicated the most potent activity with MEC of 0.001 μM.
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Introduction

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3–4 million persons are newly infected each year.[1] It has been a serious problem of HCV infection that the proportion of progression of hepatitis C to cirrhosis, liver failure, and liver cancer over a long period is very high.

The major therapeutic option of HCV carriers is interferon (IFN). Various clinical trials have been conducted to optimize IFN treatment of HCV-infected patients.[2] However, administration of recombinant IFN frequently induces neutralizing antibodies, which diminish the antiviral activity of IFN.[3] Furthermore, the administration of IFN by intramuscular or subcutaneous injection causes severe pain and irritation at the site of injection. The cost of IFN therapy is also very high because of the inordinately expensive IFN preparations. To avoid such drawbacks, the development of an IFN inducer, which enhances the release of endogenous IFN by oral administration, has been ardently desired for a long time.

Various compounds possessing IFN-inducing activity have been hitherto reported.[4] Since tilorone was reported as an IFN inducer,[5] a variety of low-molecular-weight compounds including BL-20803,[6] atabrine,[7] CP-28888,[8] ABMP,[9] DRB,[10] 10-carboxymethyl-9-acridone,[11] bropirimine[12] and imiquimod[13,14] have been reported as IFN inducers. Among them, imiquimod, which is clinically used in the USA for treatment of exophylic warts caused by the human papillomavirus, is especially a potent IFN inducer. However, its serious side-effects such as vomiting and hepatopathy found during the clinical trial stage forced abandonment of its further development as a chemotherapy drug for hepatitis C. Subsequently, imiquimod analogues such as R-842 (a hydroxylated metabolite of imiquimod)[15] and resiquimod[14,16] were found to be more effective IFN inducers than imiquimod (Fig. 1). However, no IFN inducer has yet been clinically employed for the treatment of hepatitis C.

Recently, we found 9-benzyl-8-hydroxyadenine (**1**) possessing an IFN-inducing activity as a lead compound in our first screening[17] and its substituent modifications at the 2-, 6- and 9-positions were conducted to investigate structure–activity relationships for IFN-inducing activity.[18,19] Consequently, it was found that the introduction of an appropriate chain substituent at the 2-position remarkably increases the activity. Among various 2-substituted 9-benzyl-8-hydroxyadenines,

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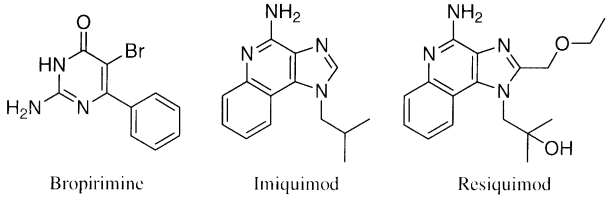
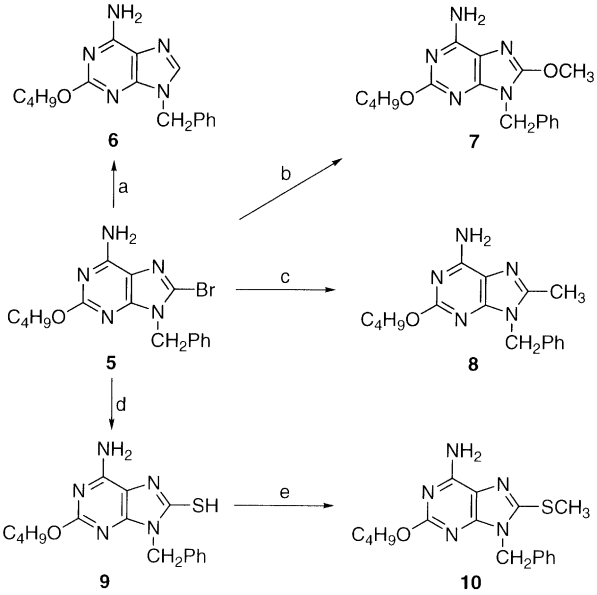


Figure 1. Some advanced low-molecular-weight interferon inducers.

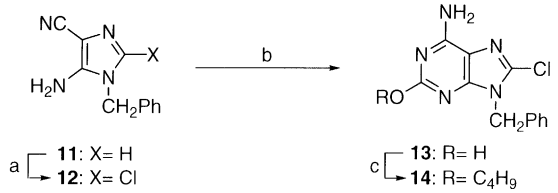
2-butoxy (**2**), 2-propylthio (**3**) and 2-butylamino (**4**) analogues indicated potent IFN-inducing activity.[17] On the basis of the results mentioned above, our attention was directed to the chemical modification at the 8-position of 9-benzyladenines possessing a substituent having considerable influence on the activity such as a butoxy, propylthio or butylamino group at the 2-position. In this report, we describe the synthesis and evaluation of 8-substituted 9-benzyladenines (**A**) carrying an appropriate substituent at the 2-position as shown in Figure 2. In addition, in order to examine the importance of the 6-amino group, some 6-substituted 9-benzyl-8-hydroxypurines possessing such a chain substituent at the 2-position were synthesized and the IFN-inducing activity was evaluated.

Chemistry

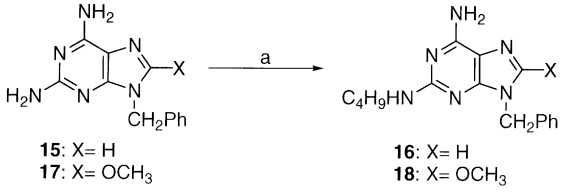
First, synthesis of several 8-substituted 9-benzyl-2-butoxyadenines, whose 2-butoxy substituent induces the highest IFN-inducing activity in the 9-benzyl-8-hydroxyadenine series,[17] was attempted. Most of the 8-substituted 9-benzyl-2-butoxyadenines were prepared from the corresponding 8-bromoadenine (**5**)[18] as shown in Scheme 1. 8-Unsubstituted 9-benzyl-2-butoxyadenine (**6**) was prepared in 94% yield by hydrogenolysis of the 8-bromo group using a Pd/C catalyst in the presence of triethylamine. 8-Methoxy analogue (**7**) was prepared by nucleophilic substitution of **5** with sodium methoxide as previously reported.[18] The synthesis of 8-methyl (**8**) and 8-mercapto (**9**) derivatives was achieved according to the usual methods.[20,21] Cross-coupling of **5** with trimethylaluminum using a palladium catalyst[20] gave 8-methyladenine (**8**) in 96% yield, and refluxing of **5** and thiourea in ethanol[21] afforded 8-mercaptoadenine (**9**) in 86% yield. Subsequently, **9** was selectively *S*-methylated with methyl iodide in the presence of potassium carbonate to give 8-methylthio analogue (**10**) in 88% yield. 8-Chloro analogue (**14**) was prepared from 5-amino-1-benzyl-4-cyanoimidazole (**11**)[22] by the following three steps: chlorination of **11**, cyclization of **12** with urea and selective *O*-butylation of **13** (Scheme 2).



Scheme 1. Reagents and conditions: (a) 10% Pd/C, $(C_2H_5)_3N$, H_2, CH_3OH, rt (94%); (b) ref 18; (c) $Al(CH_3)_3$, Ph_3P, $PdCl_2$, THF, reflux (96%); (d) H_2NCSNH_2, C_2H_5OH, reflux (86%); (e) CH_3I, K_2CO_3, DMF, rt (88%).



Scheme 2. Reagents and conditions: (a) NCS, THF, rt (87%); (b) H_2NCONH_2, neat (78%); (c) C_4H_9Br, K_2CO_3, DMF, rt (66%).



Scheme 3. Reagents and conditions: (a) C_3H_7CHO, $NaBH_3CN$, CH_3OH, rt (**16**: 84%, **18**: 85%).

Some 2-butylaminoadenine derivatives were also prepared, in which the synthetic methods of 8-unsubstituted (**16**) and 8-methoxy (**18**) analogues are illustrated in Scheme 3. 2-Amino-9-benzyladenine (**15**), which was easily prepared by the cyclization of imidazole **11**[22] with guanidine, was treated with butyraldehyde in the presence of sodium cyanoborohydride

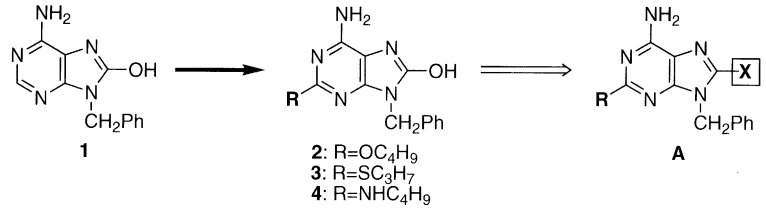
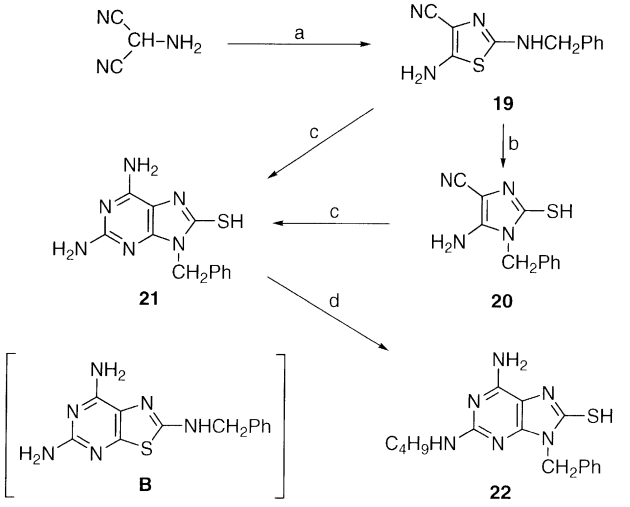


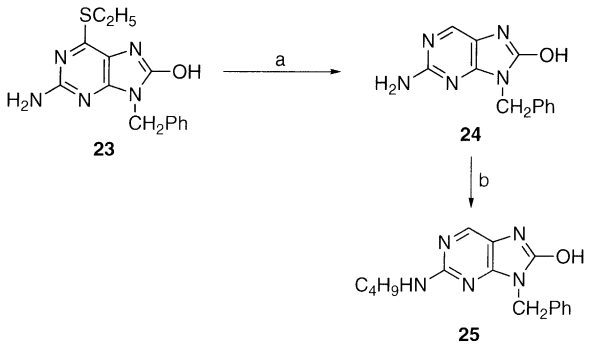
Figure 2. Strategy for structure-optimization of 9-benzyladenines.

to afford the desired 9-benzyl-2-butylaminoadenine (**16**) via regioselective reductive alkylation.[18] Similarly, the synthesis of 9-benzyl-2-butylamino-8-methoxyadenine (**18**) was carried out by reductive alkylation of 2-amino-9-benzyl-8-methoxyadenine (**17**), which was prepared by cyclization of 5-amino-1-benzyl-2-bromo-4-cyanoimidazole[18] with guanidine and subsequent methanolysis of the 8-bromo group of the resulting 2-amino-9-benzyl-8-bromoadenine. The synthetic sequence of 8-mercaptoadenine (**22**) is shown in Scheme 4. 2-Mercaptoimidazole (**20**), which is a key intermediate for the preparation of **22**, was synthesized by treatment of aminomalononitrile with benzyl isothiocyanate and subsequent known ring-transformation[23] of the resulting thiazole (**19**) into imidazole (**20**) in alkali medium. Cyclization of **20** with guanidine gave 8-mercaptoadenine (**21**), which was selectively alkylated at the 2-amino group to yield the expected 9-benzyl-2-butylamino-8-mercaptoadenine (**22**). On the other hand, cyclization of thiazole **19** with guanidine did not give thiazolo[5,4-*d*]pyrimidine (**B**), but 8-mercaptoadenine **21** in 76% yield. This reaction would involve the ring-transformation caused by the basicity of guanidine.

Preparation of 9-benzyl-2-butylamino-8-hydroxypurine (**25**) was performed as shown in Scheme 5. Removal of the 6-ethylthio group in 2-amino-9-benzyl-6-ethylthio-8-hydroxypurine (**23**)[18] using Raney Ni, followed by reductive alkylation using butyraldehyde afforded **25**. N^6-ethyl analogue (**27**) containing a propylthio group at the 2-position was obtained by the reaction of 9-benzyl-8-hydroxy-2-propylthioadenine (**26**)[18] with acetaldehyde in the presence of sodium cyanoborohydride in 69% yield (Scheme 6).



Scheme 4. Reagents and conditions: (a) $PhCH_2NCS$, THF, 40 °C, (86%); (b) 5% Na_2CO_3, reflux (84%); (c) $H_2NC(NH)NH_2{\cdot}HCl$, $NaOC_2H_5$, C_2H_5OH, reflux (from **19**: 76%, from **20**: 69%); (d) C_3H_7CHO, $NaBH_3CN$, CH_3OH, rt (72%).

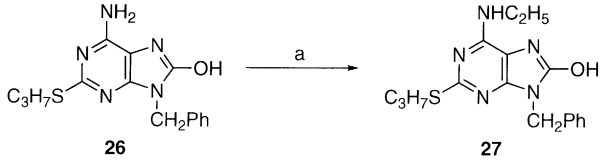


Scheme 5. Reagents and conditions: (a) Raney Ni, NH_4OH, CH_3OH, reflux (61%); (b) C_3H_7CHO, $NaBH_3CN$, CH_3OH, rt (40%).

Results and Discussion

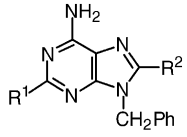
An in vitro assay for IFN-inducing activities of 2,8-disubstituted 9-benzyladenines (**5–10**, **14**, **16**, **18**, **22**) and 2,6-disubstituted 9-benzyl-8-hydroxypurines (**25**, **27**) synthesized above was performed by a typical method for determining IFN titer check.[24,25] The activity is indicated by MEC (minimum effective concentration), which is the concentration of test compounds required for more than 0.9 IU/mL induction of IFN.

The in vitro IFN-inducing activities of 8-substituted 9-benzyl-2-butoxy or 2-butylaminoadenine derivatives (**5–10**, **14**, **16**, **18**, **22**) are summarized in Table 1. In the series of 2-butoxyadenines, 8-unsubstituted (**6**), chloro (**14**), bromo (**5**) and methyl (**8**) analogues, which possess no hydroxyl group at the 8-position, were 10 times more active than the original lead compound **1**. It was recently reported that 9-benzyl-8-mercaptoadenine, an 8-thio analogue of **1**, indicated an equivalent activity of



Scheme 6. Reagents and conditions: (a) CH_3CHO, $NaBH_3CN$, CH_3OH, 50 °C (69%).

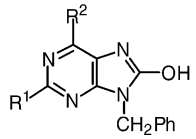
Table 1. IFN-inducing activities of 2,8-disubstituted 9-benzyladenines



Compd	R^1	R^2	MEC[a] (μM)
6	OC_4H_9	H	1
14	OC_4H_9	Cl	1
5	OC_4H_9	Br	1
8	OC_4H_9	CH_3	1
7[18]	OC_4H_9	OCH_3	10
9	OC_4H_9	SH	0.001
10	OC_4H_9	SCH_3	10
16	NHC_4H_9	H	1
18	NHC_4H_9	OCH_3	> 10
22	NHC_4H_9	SH	0.01
1	H	OH	10
2	OC_4H_9	OH	0.001
4	NHC_4H_9	OH	0.1
Imiquimod			1

[a]Minimum effective concentration (mice spleen cells): concentration of compounds required for more than 0.9 IU/mL induction of IFN.

Table 2. IFN-inducing activities of 2,6-disubstituted 9-benzyl-8-hydroxypurines



Compd	R^1	R^2	MEC[a] (μM)
25	NHC_4H_9	H	1
27	SC_3H_7	NHC_2H_5	10
3	SC_3H_7	NH_2	0.01
4	NHC_4H_9	NH_2	0.1
Imiquimod			1

[a]Minimum effective concentration (mice spleen cells): concentration of compounds required for more than 0.9 IU/mL induction of IFN.

1.[17] Similarly, 2-butoxy-8-mercapto analogue (**9**) possessed activity equal to that of the corresponding 8-hydroxyl analogue **2** with MEC of 0.001 μM. However, *S*-methylation of **9** resulted in a drastic decrease of the activity (**10**, MEC = 10 μM). An analogous tendency was observed in the 2-butylamino series. 8-Unsubstituted analogue (**16**) showed the activity with MEC of 1 μM, which was equipotent with the 2-butoxy analogue **6**. 8-Methoxy analogue (**18**) was inactive (MEC = > 10 μM) unlike the case of 2-butoxy analogue **7**. Interestingly, the activity of 2-butylamino-8-mercapto analogue (**22**, MEC = 0.01 μM) was 10-fold more potent than that of the corresponding 8-hydroxyl analogue **4**.

As shown in Table 2, removal of the 6-amino group from 2-butylamino-8-hydroxyadenine **4** resulted in some reduction of IFN-inducing activity (**25**, MEC = 1 μM). On the other hand, the introduction of an ethyl group into the 6-amino group of 8-hydroxy-2-propylthioadenine **3** markedly decreased the activity (**27**, MEC = 10 μM).

Conclusion

In summary, we have synthesized 8-substituted 9-benzyladenines and 6-substituted 9-benzyl-8-hydroxypurines bearing an appropriate chain substituent such as a butoxy, propylthio or butylamino group at the 2-position and evaluated their IFN-inducing activities in order to investigate the structure–activity relationships of 2-substituted 9-benzyl-8-hydroxyadenines (**2**–**4**) and to explore highly active compounds. Among them, 2-butoxy-8-mercaptoadenine derivative (**9**), having an acidic proton at the 8-position like 8-hydroxyadenine **2**, emerged as having the most potent activity. The typical characteristics of the qualitative structure–activity relationship at the 8-position of the adenine nucleus are as follows: OH, SH > > H, halogene, $CH_3 > OCH_3$, SCH_3. It has been found that both of a 6-amino group and an 8-hydroxyl or thiol group are requisite to express excellent IFN-inducing activity and that the presence of the 2-butoxy group brings the most significant increase of the activity.

Experimental

Chemistry

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 JNM EX-400 (400 MHz) spectrometer using DMSO-d_6 as a solvent. Chemical shifts are given in ppm (δ), 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).

9-Benzyl-2-butoxyadenine (6). After two vacuum/H_2 cycles to remove air from the reaction flask, the mixture of **5**[18] (100 mg, 0.27 mmol), 10% Pd/C (10% the weight of **5**) and Et_3N (44 μL, 0.32 mmol) in MeOH (5 mL) was hydrogenated at ambient pressure (balloon) and temperature for 2 h. The reaction mixture was filtered using a Celite cake and the filtrate was evaporated. The residue was chromatographed on silica gel ($CHCl_3$/MeOH = 100/1) to give **6** (75 mg, 94%). Mp 182–183 °C (washed with hexane) (lit.,[26] 174–175 °C); UV (EtOH) λ_{max} 268.8 nm (ε 13,200); IR (KBr) ν cm^{-1}: 3307, 3150, 1660, 1599, 1338; ^{1}H NMR δ 8.02 (1H, s, 8-H), 7.25–7.35 (5H, m, 9-Ph), 7.16 (2H, brs*, 6-NH_2), 5.25 (2H, s, 9-CH_2), 4.20 (2H, t, *J* = 6.6 Hz, 2-CH_2), 1.60–1.67 (2H, m, 2-CH_2), 1.34–1.43 (2H, m, 2-CH_2), 0.91 (3H, t, *J* = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 161.5, 156.7, 151.2, 139.3, 137.2, 128.6, 127.6, 115.0, 65.7, 45.9, 30.6, 18.7, 13.7; MS (EI) *m*/*z* 297 (M^+), 241, 240, 91; HRMS (EI) calcd for $C_{16}H_{19}N_5O$ (M^+): 297.1590. Found: 297.1595; Anal. calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55. Found: C, 64.55; H, 6.38; N, 23.42.

9-Benzyl-2-butoxy-8-methyladenine (8). A mixture of **5**[18] (100 mg, 0.27 mmol), 1 M hexane solution of $AlMe_3$ (0.53 mL, 0.53 mmol), $PdCl_2$ (2.4 mg, 0.01 mmol) and Ph_3P (7.0 mg, 0.03 mmol) in dry THF (5 mL) was refluxed under Ar atmosphere for 10 h. After evaporation, the residue was triturated with $CHCl_3$ (10 mL) and the resulting suspension was filtered using a Celite cake. The filtrate was partitioned between $CHCl_3$ (10 mL) and H_2O (10 mL), whose organic layer was washed with brine (10 mL) and dried over $MgSO_4$. After filtration and evaporation, the residue was chromatographed on silica gel ($CHCl_3$/MeOH = 200/1), which was triturated with hexane (2 mL) to give **8** (80 mg, 96%). Mp 135–136 °C (washed with hexane); UV (EtOH) λ_{max} 269.4 nm (ε 13,600); IR (KBr) ν cm^{-1}: 3313, 3148, 2958,

1654, 1600, 1465, 1342; ^{1}H NMR δ 7.18–7.34 (5H, m, 9-Ph), 7.06 (2H, brs*, 6-NH_2), 5.24 (2H, s, 9-CH_2), 4.19 (2H, t, J = 6.6 Hz, 2-CH_2), 2.34 (3H, s, 8-CH_3), 1.60–1.67 (2H, m, 2-CH_2), 1.34–1.43 (2H, m, 2-CH_2), 0.90 (3H, t, J = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 161.0, 155.8, 152.4, 146.8, 136.9, 128.7, 127.5, 127.0, 113.7, 65.7, 44.7, 30.7, 18.7, 13.7; MS (EI) m/z 311 (M^+), 282, 268, 255, 240, 91; HRMS (EI) calcd for $C_{17}H_{21}N_5O$ (M^+): 311.1746. Found: 311.1754; Anal. calcd for $C_{17}H_{21}N_5O$: C, 65.57; H, 6.80; N, 22.49. Found: C, 65.34; H, 6.71; N, 22.31.

9-Benzyl-2-butoxy-8-thioadenine (9). A mixture of **5**[18] (200 mg, 0.53 mmol) and thiourea (81 mg, 1.06 mmol) in dry EtOH (8 mL) was refluxed under Ar atmosphere for 24 h. After evaporation, the residue was partitioned between AcOEt (20 mL) and H_2O (20 mL). The organic layer was washed with brine (20 mL) and dried over $MgSO_4$. After filtration, the residue was chromatographed on silica gel ($CHCl_3$/MeOH = 50/1) to give **9** (151 mg, 86%). Mp 268–269 °C (recrystallized from EtOH); UV (EtOH) λ_{max} 241.2 nm (ε 15,000), 267.8 nm (ε 7600), 314.8 nm (ε 28,000); IR (KBr) ν cm^{-1}: 3396, 3171, 1678, 1616, 1486, 1354; ^{1}H NMR δ 12.18 (1H, s*, 8-SH), 7.23–7.38 (5H, m, 9-Ph), 6.85 (2H, brs*, 6-NH_2), 5.26 (2H, s, 9-CH_2), 4.17 (2H, t, J = 6.3 Hz, 2-CH_2), 1.58–1.65 (2H, m, 2-CH_2), 1.32–1.41 (2H, m, 2-CH_2), 0.89 (3H, t, J = 7.1 Hz, 2-CH_3); ^{13}C NMR δ 166.0, 161.3, 150.9, 148.6, 136.3, 128.3, 127.8, 127.4, 102.6, 66.1, 45.0, 30.4, 18.6, 13.6; MS (EI) m/z 329 (M^+), 273, 240, 182, 91; HRMS (EI) calcd for $C_{16}H_{19}N_5OS$ (M^+): 329.1310. Found: 329.1304; Anal. calcd for $C_{16}H_{19}N_5OS$: C, 58.34; H, 5.81; N, 21.26. Found: C, 58.28; H, 5.74; N, 21.34.

9-Benzyl-2-butoxy-8-methylthioadenine (10). A mixture of **9** (100 mg, 0.30 mmol) and K_2CO_3 (44 mg, 0.32 mmol) in dry DMF (6 mL) was stirred under Ar atmosphere at room temperature for 0.5 h. To the mixture was added methyl iodide (23 μL, 0.36 mmol) and the mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure and 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 (20 mL) and dried over $MgSO_4$. After filtration, the solvent was evaporated and the residue was chromatographed on silica gel ($CHCl_3$/MeOH = 200/1) to give **10** (87 mg, 83%): mp 158–159 °C (recrystallized from EtOH); UV (EtOH) λ_{max} 282.4 nm (ε 16,700); IR (KBr) ν cm^{-1}: 3490, 3101, 1641, 1595, 1354, 1265, 1194, 732; ^{1}H NMR δ 7.22–7.34 (5H, m, 9-Ph), 7.10 (2H, s*, 6-NH_2), 5.16 (2H, s, 9-CH_2), 4.19 (2H, t, J = 6.6 Hz, 2-CH_2), 2.61 (3H, s, 8-CH_3), 1.60–1.67 (2H, m, 2-CH_2), 1.34–1.43 (2H, m, 2-CH_2), 0.90 (3H, t, J = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 160.9, 154.9, 153.3, 146.4, 136.3, 128.6, 127.6, 127.3, 115.1, 65.8, 45.2, 30.6, 18.7, 14.3, 13.7; MS (EI) m/z 343 (M^+), 287, 196, 91; HRMS (EI) calcd for $C_{17}H_{21}N_5OS$ (M^+): 343.1467. Found: 343.1462; Anal. calcd for $C_{17}H_{21}N_5OS$: C, 59.45; H, 6.16; N, 20.39. Found: C, 59.42; H, 6.22; N, 20.31.

5-Amino-1-benzyl-2-chloro-4-cyanoimidazole (12). To a solution of **11**[22] (700 mg, 3.53 mmol) in dry THF (15 mL) was added *N*-chlorosuccinimide (519 mg, 3.89 mmol) in dry THF (15 mL), and the reaction mixture was stirred under Ar atmosphere at room temperature for 10 h. After evaporation, the resulting solid was partitioned between AcOEt (50 mL) and saturated $NaHCO_3$ (50 mL) solution. The organic layer was washed with brine (30 mL) and dried over $MgSO_4$. After filtration, the solvent was evaporated off, and the solidified product was chromatographed on silica gel ($CHCl_3$/MeOH = 50/1) to give **12** (713 mg, 87%): mp 206–208 °C (recrystallized from benzene); UV (EtOH) λ_{max} 249.0 nm (ε 14,000); IR (KBr) ν cm^{-1}: 3334, 3179, 2222, 1650, 1596, 1502, 1195, 725; ^{1}H NMR δ 7.28–7.38 (3H, m, 1-Ph), 7.13 (2H, d, J = 7.8 Hz, 1-Ph), 6.69 (2H, s*, 5-NH_2), 5.12 (2H, s, 1-CH_2); ^{13}C NMR δ 149.2, 135.2, 128.7, 127.7, 126.5, 124.7, 116.2, 88.8, 45.8; MS (EI) m/z 234 (M^+ + 2), 232 (M^+), 91; HRMS (EI) calcd for $C_{11}H_9ClN_4$ (M^+): 232.0516. Found: 232.0512; anal. calcd for $C_{11}H_9ClN_4$: C, 56.78; H, 3.90; N, 24.08. Found: C, 56.91; H, 4.00; N, 24.13.

9-Benzyl-8-chloro-2-hydroxyadenine (13). A mixture of **12** (650 mg, 2.79 mmol) and urea (1.68 g, 27.94 mmol) was heated under Ar atmosphere at 160 °C for 24 h. An additional amount of urea (1.68 g, 27.94 mmol) was added to the reaction mixture and the resulting mixture was heated at 160 °C for 12 h. The residue was triturated with H_2O (20 mL) and the resulting precipitate was filtered to give **13** (604 mg, 78%): mp > 300 °C (washed with hot MeOH and hot H_2O); ^{1}H NMR δ 10.63 (1H, brs*, 2-OH), 7.63 (2H, brs*, 6-NH_2), 7.22–7.36 (5H, m, 9-Ph), 5.13 (2H, s, 9-CH_2); MS (EI) m/z 277 (M^+ + 2), 275 (M^+), 240, 91; HRMS (EI) calcd for $C_{12}H_{10}ClN_5O$ (M^+): 275.0574. Found: 275.0567.

9-Benzyl-2-butoxy-8-chloroadenine (14). A mixture of **13** (400 mg, 1.45 mmol) and K_2CO_3 (602 mg, 4.35 mmol) in dry DMF (8 mL) was stirred under Ar atmosphere at room temperature for 1 h. To the mixture was added butyl bromide (0.47 mL, 4.35 mmol) and the mixture was stirred at room temperature for 2 days. The solvent was removed under reduced pressure and the residue was triturated with H_2O (15 mL). The resulting suspension was neutralized with 10% $NaHSO_4$ solution and extracted with AcOEt (30 mL). The organic layer was washed with brine (30 mL) and dried over $MgSO_4$. After filtration, the solvent was evaporated and the residue was chromatographed on silica gel ($CHCl_3$). The obtained oily product was solidified with ether (3 mL) and filtered to give **14** (319 mg, 66%): mp 144–145 °C (washed with hexane and MeOH); UV (EtOH) λ_{max} 270.6 nm (ε 14,600); IR (KBr) ν cm^{-1}: 3488, 3114, 2958, 1639, 1594, 1348, 731; ^{1}H NMR δ 7.37 (2H, brs*, 6-NH_2), 7.24–7.36 (5H, m, 9-Ph), 5.25 (2H, s, 9-CH_2), 4.21 (2H, t, J = 6.6 Hz, 2-CH_2), 1.61–1.68 (2H, m, 2-CH_2), 1.34–1.43 (2H, m, 2-CH_2), 0.90 (3H, t, J = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 161.6, 155.8, 152.0, 135.9, 134.1, 128.7, 127.8, 127.2, 113.7, 66.0, 45.6, 30.6, 18.7, 13.7; MS (EI) m/z 333 (M^+ + 2), 331 (M^+), 275, 91; HRMS (EI) calcd for $C_{16}H_{18}ClN_5O$ (M^+): 331.1200. Found: 331.1208; Anal. calcd for

$C_{16}H_{18}ClN_5O$: C, 57.92; H, 5.47; N, 21.11. Found: C, 57.90; H, 5.50; N, 21.10.

2-Amino-9-benzyladenine (15). To a solution of Na (0.65 g, 28.25 mmol) in dry EtOH (30 mL) was added guanidine hydrochloride (3.37 g, 35.31 mmol) and the mixture was stirred at room temperature for 5 min. The resulting precipitate was filtered off and the filtrate was refluxed with **11**[22] (0.70 g, 3.35 mmol) under Ar atmosphere for 10 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$/MeOH = 50/1) to give **15** (840 mg, 99%), which was used for the next reaction without further purification: mp 192–193 °C (lit.,[27] 180–181 °C); ^{1}H NMR δ 7.77 (1H, s, 8-H), 7.20–7.34 (5H, m, 9-Ph), 6.69 and 5.80 (each 2H, each s*, 2-NH_2 and 6-NH_2), 5.18 (2H, s, 9-CH_2); ^{13}C NMR δ 160.5, 156.2, 151.8, 137.7, 137.4, 128.6, 127.4, 127.1, 113.1, 45.4.

9-Benzyl-2-butylaminoadenine (16). To a suspension of **15** (100 mg, 0.42 mmol) and $NaBH_3CN$ (157 mg, 2.50 mmol) in MeOH (5 mL) was added butyraldehyde (0.30 mL, 3.33 mmol) and the mixture was stirred under Ar atmosphere at room temperature for 2 days. After evaporation, the residue was chromatographed on silica gel ($CHCl_3$/MeOH = 100/1) to give **16** (104 mg, 84%): mp 147–148 °C (washed with hexane); UV (EtOH) λ_{max} 259.0 nm (ε 9900), 289.4 nm (ε 9500); IR (KBr) ν cm^{-1}: 3322, 2956, 1599, 1539, 1482, 721; ^{1}H NMR δ 7.77 (1H, s, 8-H), 7.23–7.33 (5H, m, 9-Ph), 6.60 (2H, brs*, 6-NH_2), 6.19 (1H, t*, J = 6.7 Hz, 2-NH), 5.16 (2H, s, 9-CH_2), 3.22 (2H, q, J = 6.7 Hz, 2-CH_2), 1.43–1.51 (2H, m, 2-CH_2), 1.25–1.34 (2H, m, 2-CH_2), 0.87 (3H, t, J = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 159.6, 155.9, 151.7, 137.6, 137.1, 128.5, 127.6, 127.5, 113.0, 45.6, 40.7, 31.5, 19.7, 13.9; MS (EI) m/z 296 (M^+), 267, 253, 240, 91; HRMS (EI) calcd for $C_{16}H_{20}N_6$ (M^+): 296.1749. Found: 296.1765; Anal. calcd for $C_{16}H_{20}N_6 \cdot 1/8H_2O$: C, 64.35; H, 6.84; N, 28.14. Found: C, 64.40; H, 6.77; N, 28.08.

2-Amino-9-benzyl-8-methoxyadenine (17). The reaction of 5-amino-1-benzyl-2-bromo-4-cyanoimidazole[18] with guanidine under the same conditions as those for the preparation of **15** gave 2-amino-9-benzyl-8-bromoadenine (for 9 h): Yield 90%; mp 274–275 °C (washed with ether); UV (EtOH) λ_{max} 260.2 nm (ε 9800), 286.0 nm (ε 12700); IR (KBr) ν cm^{-1}: 3466, 3310, 3191, 1595, 1473; ^{1}H NMR δ 7.25–7.35 (3H, m, 9-Ph), 7.16 (2H, d, J = 6.8 Hz, 9-Ph), 6.88 (2H, brs*, 2-NH_2 or 6-NH_2), 5.96 (2H, s*, 2-NH_2 or 6-NH_2), 5.16 (2H, s, 9-CH_2); ^{13}C NMR δ 160.5, 155.1, 153.1, 136.4, 128.7, 127.5, 126.7, 121.2, 113.4, 45.8; MS (EI) m/z 320 ($M^+ + 2$), 318 (M^+), 239, 91; HRMS (EI) calcd for $C_{12}H_{11}BrN_6$ (M^+): 318.0229. Found: 318.0233; Anal. calcd for $C_{12}H_{11}BrN_6$: C, 45.16; H, 3.47; N, 26.33. Found: C, 45.09; H, 3.43; N, 26.18. A mixture of 2-amino-9-benzyl-8-bromoadenine (459 mg, 1.44 mmol) in MeOH (10 mL) and 1 N NaOH solution (10 mL) was refluxed for 2 days. After evaporation, the residue was triturated with H_2O (20 mL) and the mixture was neutralized with 10% $NaHSO_4$ solution. The resulting mixture was extracted with AcOEt (30 mL) and the organic layer was washed with brine (20 mL) and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure, and the product was chromatographed on silica gel ($CHCl_3$/MeOH = 100/1) to give **17** (271 mg, 70%): mp 239–241 °C (washed with ether); UV (EtOH) λ_{max} 251.6 nm (ε 10,600), 283.0 nm (ε 9600); IR (KBr) ν cm^{-1}: 3486, 3322, 3182, 1604, 1401, 1337, 1242; ^{1}H NMR δ 7.23–7.33 (3H, m, 9-Ph), 7.16 (2H, d, J = 7.3 Hz, 9-Ph), 6.34 and 5.64 (each 2H, each s*, 2-NH_2 and 6-NH_2), 4.97 (2H, s, 9-CH_2), 3.97 (3H, s, 8-CH_3); ^{13}C NMR δ 159.2, 154.0, 152.4, 151.5, 137.1, 128.5, 127.3, 126.8, 107.7, 56.5, 43.3; MS (EI) m/z 270 (M^+), 255, 179, 91; HRMS (EI) calcd for $C_{13}H_{14}N_6O$ (M^+): 270.1229. Found: 270.1224; anal. calcd for $C_{13}H_{14}N_6O$: C, 57.77; H, 5.22; N, 31.09. Found: C, 57.89; H, 5.23; N, 30.93.

9-Benzyl-2-butylamino-8-methoxyadenine (18). Compound **18** was prepared by the reaction of **17** with butyraldehyde in the presence of $NaBH_3CN$ according to the synthetic procedure for the preparation of **16** (for 2 days): Yield 85%; mp 134–135 °C (recrystallized from toluene); UV (EtOH) λ_{max} 253.6 nm (ε 11,700), 288.0 nm (ε 7800); IR (KBr) ν cm^{-1}: 3332, 3197, 2932, 1608, 1528, 1399; ^{1}H NMR δ 7.22–7.33 (5H, m, 9-Ph), 6.27 (2H, s*, 6-NH_2), 6.04 (1H, t*, J = 6.3 Hz, 2-NH), 4.96 (2H, s, 9-CH_2), 3.98 (3H, s, 8-CH_3), 3.19 (2H, q, J = 6.3 Hz, 2-CH_2), 1.42–1.49 (2H, m, 2-CH_2), 1.25–1.34 (2H, m, 2-CH_2), 0.86 (3H, t, J = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 158.5, 153.8, 152.2, 151.4, 137.1, 128.4, 127.3, 127.1, 107.5, 56.4, 43.4, 40.7, 31.5, 19.7, 13.8; MS (EI) m/z 326 (M^+), 297, 283, 270, 91; HRMS (EI) calcd for $C_{17}H_{22}N_6O$ (M^+): 326.1855. Found: 326.1862.

5-Amino-2-benzylamino-4-cyanothiazole (19). To a suspension of aminomalononitrile *p*-toluenesulfonate (2.00 g, 7.90 mmol) in dry THF (20 mL) was added *N*,*N*-diisopropylethylamine (1.10 mL, 6.32 mmol) and the mixture was stirred under Ar atmosphere at room temperature for 5 min. To the solution was added benzyl isothiocyanate (2.09 mL, 15.79 mmol) in dry THF (20 mL) and the mixture was stirred at 40 °C for 72 h. The solvent was removed under reduced pressure, and the residue was partitioned between AcOEt (100 mL) and H_2O (100 mL). The organic layer was washed with brine (50 mL) and dried over $MgSO_4$. The solvent was evaporated and the residue was chromatographed on silica gel ($CHCl_3$) to give **19** (1.57 g, 86%): mp 143–145 °C (washed with hexane); UV (EtOH) λ_{max} 252.2 nm (ε 7600), 305.0 nm (ε 7300); IR (KBr) ν cm^{-1}: 3410, 3300, 3198, 2212, 1612, 1547, 1213, 699; ^{1}H NMR δ 7.54 (1H, t*, J = 5.9 Hz, 2-NH), 7.22–7.34 (5H, m, 2-Ph), 6.45 (2H, s*, 5-NH_2), 4.31 (2H, d, J = 5.9 Hz, 2-CH_2); ^{13}C NMR δ 153.6, 153.4, 139.2, 128.3, 127.4, 126.9, 116.9, 95.8, 46.7; MS (EI) m/z 230 (M^+), 91; HRMS (EI) calcd for $C_{11}H_{10}N_4S$ (M^+): 230.0626. Found: 230.0633; Anal. calcd for $C_{11}H_{10}N_4S$: C, 57.37; H, 4.38; N, 24.33. Found: C, 57.21; H, 4.23; N, 24.10.

5-Amino-1-benzyl-4-cyano-2-mercaptoimidazole (20). The suspension of **19** (500 mg, 2.17 mmol) in 5% Na_2CO_3 solution (15 mL) was refluxed for 2 h. The mixture was neutralized with 1 N HCl solution and the pre-

cipitated solid was collected, which was chromatographed on silica gel ($CHCl_3$/MeOH = 50:1) to give **20** (420 mg, 84%): mp 280–283 °C (recrystallized from MeOH); UV (EtOH) λ_{max} 289.4 nm (ε 17,600); IR (KBr) ν cm^{-1}: 3329, 3183, 2216, 1656, 1468, 1334, 1171, 696; ^1H NMR δ 12.39 (1H, s*, 2-SH), 7.25–7.33 (5H, m, 1-Ph), 6.77 (2H, s*, 5-NH_2), 5.21 (2H, s, 1-CH_2); ^{13}C NMR δ 160.0, 147.1, 135.6, 128.3, 127.3, 127.0, 113.1, 75.1, 45.1; MS (EI) *m/z* 230 (M^+), 91; HRMS (EI) calcd for $C_{11}H_{10}N_4S$ (M^+): 230.0626. Found: 230.0619; Anal. calcd for $C_{11}H_{10}N_4S$: C, 57.37; H, 4.38; N, 24.33. Found: C, 57.39; H, 4.58; N, 24.11.

2-Amino-9-benzyl-8-mercaptoadenine (21). Method A. Compound **21** was prepared by the reaction of **20** with guanidine according to the synthetic procedure for the preparation of **15** in 69% yield (for 2 days).

Method B. To a solution of Na (100 mg, 4.34 mmol) in dry EtOH (3 mL) was added guanidine hydrochloride (415 mg, 4.34 mmol) and the mixture was stirred under Ar atmosphere at room temperature for 5 min. To the resulting mixture was added **19** (100 mg, 0.43 mmol) in dry EtOH (2 mL) and the mixture was refluxed for 17 h. After evaporation, the residue was triturated with H_2O (10 mL). The mixture was neutralized with AcOH and extracted with AcOEt (20 mL). The organic layer was washed with brine (10 mL) and dried over $MgSO_4$. The solvent was evaporated to give **21** (90 mg, 76%): mp 290–292 °C; UV (EtOH) λ_{max} 268.4 nm (ε 9100), 315.8 nm (ε 22,600); IR (KBr) ν cm^{-1}: 3415, 1621, 1476, 1437; ^1H NMR δ 11.93 (1H, s*, 8-SH), 7.23–7.32 (5H, m, 9-Ph), 6.41 and 6.01 (each 2H, each s*, 2-NH_2 and 6-NH_2), 5.21 (2H, s, 9-CH_2); ^{13}C NMR δ 164.4, 160.4, 151.2, 148.3, 136.5, 128.2, 127.3, 127.1, 100.1, 44.7; MS (EI) *m/z* 272 (M^+), 239, 181, 154, 91; HRMS (EI) calcd for $C_{12}H_{12}N_6S$ (M^+): 272.0844. Found: 272.0851.

9-Benzyl-2-butylamino-8-mercaptoadenine (22). Compound **22** was prepared by the reaction of **21** with butyraldehyde in the presence of $NaBH_3CN$ according to the synthetic procedure for the preparation of **16** (for 2 days): Yield 72%; mp 272–274 °C (recrystallized from EtOH); UV (EtOH) λ_{max} 271.2 nm (ε 12,100), 321.2 nm (ε 23,100); IR (KBr) ν cm^{-1}: 3370, 3129, 1625, 1537, 1460, 1369, 696; ^1H NMR δ 11.89 (1H, s*, 8-SH), 7.23–7.37 (5H, m, 9-Ph), 6.48 (1H, brt*, *J* = 6.5 Hz, 2-NH), 6.39 (2H, brs*, 6-NH_2), 5.20 (2H, s, 9-CH_2), 3.17 (2H, q, *J* = 6.5 Hz, 2-CH_2), 1.40–1.47 (2H, m, 2-CH_2), 1.23–1.32 (2H, m, 2-CH_2), 0.85 (3H, t, *J* = 7.1 Hz, 2-CH_3); ^{13}C NMR δ 164.1, 159.5, 151.1, 148.2, 136.7, 128.2, 127.8, 127.3, 99.9, 44.8, 40.6, 31.3, 19.7, 13.8; MS (EI) *m/z* 328 (M^+), 285, 91; HRMS (EI) calcd for $C_{16}H_{20}N_6S$ (M^+): 328.1470. Found: 328.1462; anal. calcd for $C_{16}H_{20}N_6S$: C, 58.51; H, 6.14; N, 25.59. Found: C, 58.61; H, 6.15; N, 25.64.

2-Amino-9-benzyl-8-hydroxypurine (24). To a suspension of **23**[18] (116 mg, 0.38 mmol) in MeOH (10 mL) and 28% NH_4OH solution (2 mL) was added Raney Ni (1 mL of suspension in H_2O) and the mixture was refluxed for 9 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 = 40:1) to give **24** (56 mg, 61%): mp 268–269 °C; UV (EtOH) λ_{max} 314.0 nm (ε 8300); IR (KBr) ν cm^{-1}: 3462, 1720, 1641, 1453; ^1H NMR δ 10.80 (1H, s*, 8-OH), 7.74 (1H, s, 6-H), 7.24–7.34 (5H, m, 9-Ph), 6.20 (2H, s*, 2-NH_2), 4.87 (2H, s, 9-CH_2); ^{13}C NMR δ 159.2, 153.1, 151.2, 136.8, 134.5, 128.5, 127.4, 127.1, 113.5, 42.0; MS (EI) *m/z* 241 (M^+), 150, 91; HRMS (EI) calcd for $C_{12}H_{11}N_5O$ (M^+): 241.0964. Found: 241.0957.

9-Benzyl-2-butylamino-8-hydroxypurine (25). Compound **25** was prepared by the reaction of **24** with butyraldehyde in the presence of $NaBH_3CN$ according to the synthetic procedure for the preparation of **16** (for 2 days): Yield 40%; mp 224–225 °C (washed with ether); UV (EtOH) λ_{max} 227.4 nm (ε 19,600), 324.2 nm (ε 7900); IR (KBr) ν cm^{-1}: 3354, 2957, 1708, 1639, 1540, 1461, 1132, 698; ^1H NMR δ 10.75 (1H, s*, 8-OH), 7.77 (1H, s, 6-H), 7.23–7.33 (5H, m, 9-Ph), 6.72 (1H, t*, *J* = 6.3 Hz, 2-NH), 4.86 (2H, s, 9-CH_2), 3.18 (2H, t, *J* = 6.3 Hz, 2-CH_2), 1.42–1.49 (2H, m, 2-CH_2), 1.24–1.33 (2H, m, 2-CH_2), 0.86 (3H, t, *J* = 7.3 Hz, 2-CH_3); ^{13}C NMR δ 158.3, 153.1, 151.1, 136.9, 134.4, 128.5, 127.4, 113.2, 42.0, 40.8, 31.2, 19.7, 13.8; MS (EI) *m/z* 297 (M^+), 268, 254, 241, 91; HRMS (EI) calcd for $C_{16}H_{19}N_5O$ (M^+): 297.1590. Found: 297.1587; Anal. calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; N, 23.55. Found: C, 64.36; H, 6.44; N, 23.35.

9-Benzyl-N^6-ethyl-8-hydroxy-2-propylthioadenine (27). To a suspension of **26**[18] (100 mg, 0.32 mmol) and $NaBH_3CN$ (120 mg, 1.90 mmol) in dry MeOH (20 mL) was added acetaldehyde (0.46 mL, 8.24 mmol) and the mixture was stirred under Ar atmosphere at 50 °C for 8 days. After evaporation, the residue was chromatographed on silica gel ($CHCl_3$/MeOH = 100:1) to give **27** (75 mg, 69%): mp 214–215 °C (washed with ether); UV (EtOH) λ_{max} 229.2 nm (ε 20,700), 287.2 nm (ε 16,600); IR (KBr) ν cm^{-1}: 3348, 2965, 1701, 1636, 1452, 1345, 701; ^1H NMR δ 10.01 (1H, s*, 8-OH), 7.23–7.33 (5H, m, 9-Ph), 6.55 (1H, brt*, *J* = 5.4 Hz, 6-NH), 4.86 (2H, s, 9-CH_2), 3.37–3.43 (2H, m, 6-CH_2), 2.97 (2H, t, *J* = 7.3 Hz, 2-CH_2), 1.58–1.67 (2H, m, 2-CH_2), 1.16 (3H, t, *J* = 7.1 Hz, 6-CH_3), 0.93 (3H, t, *J* = 7.1 Hz, 2-CH_3); ^{13}C NMR δ 161.5, 151.7, 147.3, 146.0, 137.1, 128.5, 127.43, 127.37, 100.3, 42.4, 34.9, 32.2, 22.8, 14.9, 13.3; MS (EI) *m/z* 343 (M^+), 328, 310, 91; HRMS (EI) calcd for $C_{17}H_{21}N_5OS$ (M^+): 343.1467. Found: 343.1463; Anal. calcd for $C_{17}H_{21}N_5OS$: C, 59.45; H, 6.16; N, 20.39. Found: C, 59.18; H, 6.15; N, 20.14.

Biology

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.[24] Results are expressed as IFN IU/mL in terms of the international mouse IFN standard[25] obtained from the National Institute of Health, Bethesda, MD, USA.

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