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Synthesis and evaluation of 2-substituted 8-hydroxyadenines as potent interferon inducers with improved oral bioavailabilities

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Abstract—In order to create novel compounds which possess potent interferon (IFN) inducing activities with excellent oral bioavailabilities, a series of 8-hydroxyadenines, which have various alkoxy or alkylthio moieties at the adenine C(2)-position, were synthesized and evaluated. The introduction of hydrophobic groups was not considered to be effective for potentiating the IFN-inducing activity, but several compounds having hydrophilic groups were effective. Among the compounds tested, compound **13f** induced IFN from the dosage of 0.03 mg/kg, which was approximately 100-fold more potent than that of Imiquimod, and showed an excellent oral bioavailability (F = 40%) which was 10-fold improved over **5**, a lead compound (F = 4%).

1. Introduction

Infection by the hepatitis C virus (HCV), which is a member of the *Flaviviridae* family of viruses, has been recognized as one of the leading causes of liver impairment such as cirrhosis and hepatocellular carcinoma in humans. It is estimated that more than 170 million people worldwide are infected, and of those infected, approximately 20% are likely to develop liver cirrhosis and 4% hepatocellular carcinoma, in the next decade.[1] The currently approved treatments for chronic hepatitis C are based on interferon-alpha (IFN-α), alone or in combination with ribavirin. Although sustained response rates are markedly improved using combination therapies, at least 50% of patients fail to show a sustained response.[2] Furthermore, IFN therapy is associated with significant problems of patient compliance, a loss of therapeutic efficacy as a result of the formation of neutralizing antibodies against exogenous IFN.[3] The development of effective therapies to treat HCV infection is therefore highly important. Induction of endogenous IFN synthesis with small molecular-weight compounds is one possible approach and orally bioavailable IFN inducing agents are envisaged as a new therapeutic class of drugs for virus infections.

In recent years, several natural and synthetic molecules have been shown to induce IFN.[4] For example, low molecular-weight compounds including the fluorenones,[5] pyrimidinones,[6] and anthraquinones[7] induced IFN in various animal species. However, none of these agents is capable of inducing high levels of IFN in humans.[8] Imiquimod (**1**), discovered and developed by 3M Pharmaceuticals, is a low molecular weight agent (MW 240) with a potent IFN inducing property in humans. It was launched in the US in 1997 for the treatment of external genital and perianal warts/condyloma acuminata.[9] However, in the clinical trial of Imiquimod for HCV, serious side effects such as vomiting and liver dysfunction were observed in some patients.[10] Therefore, its further development was discontinued probably owing to these side effects.

Recently, we reported the discovery of a series of 8-hydroxyadenines as novel IFN inducing agents.[11] The IFN inducing activities were remarkably improved by the introduction of substituents into the adenine C(2)-position (Table 1) and these analogues could be classified to following four types (**2**–**5**; Fig. 1).

Keywords: 8-hydroxyadenines; Orally active; Interferon inducer; HCV; Imiquimod.
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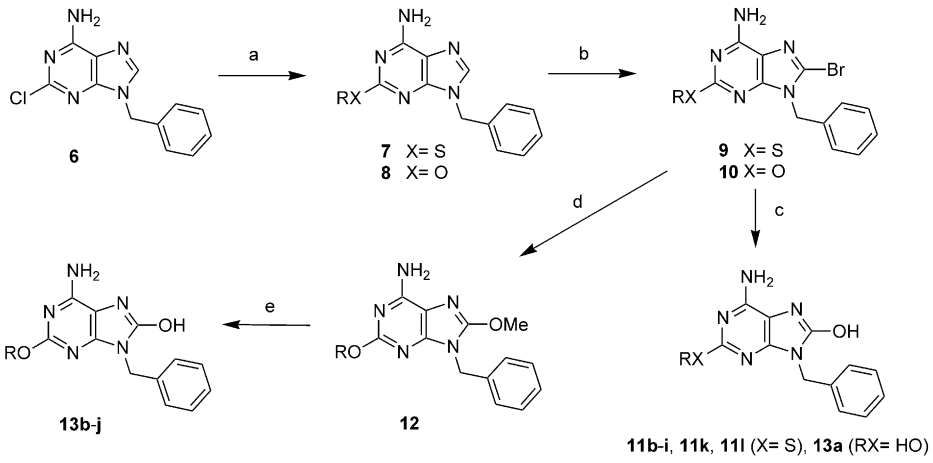
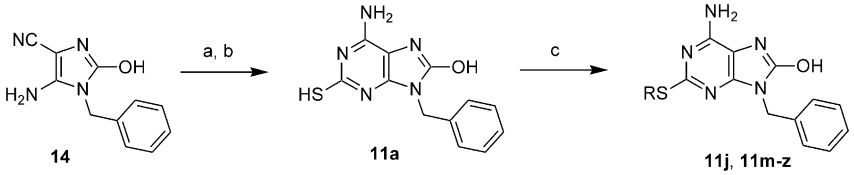


Scheme 1. Reagents and conditions: (a) NaSR, DMF, 110 °C or NaOR, ROH, reflux; (b) Br_2, CH_2Cl_2, rt; (c) 6N HCl, reflux; (d) NaOMe, MeOH, reflux; (e) 12N HCl, rt



Scheme 2. Reagents and conditions (a) BzNCS, THF, rt; (b) 2N NaOH, THF, reflux; (c) RBr or RCl, K_2CO_3, DMF, rt

We have also conducted and disclosed the results of the structure and activity relationship studies (SAR) based on **2** and **3**, but great improvement of the activities both in vitro and in vivo could not be achieved.[12,13] Compared to compounds **2** and **3**, compounds **4** and **5** demonstrated potent in vitro activities. However, these compounds did not show good oral absorption, their oral bioavailabilities in rats were 3% and 4%, respectively. In order to create compounds having potent IFN-inducing activities with improved oral bioavailabilities, we conducted a detailed SAR study based on **4** and **5** by the introduction of various hydrophobic or hydrophilic groups into the 8-hydroxyadenine C(2)-position.

Table 1. Activities of IFN induction

Compound	MEC[a] (μM)	MED[b] (mg/kg)
2	0.03	0.3
3	0.1	0.3
4	0.01	0.3
5	0.001	0.03
1	1	3

[a] MEC: Minimum Effective Concentration (mouse spleen cells).
[b] MED: Minimum Effective Dose (mouse, po).

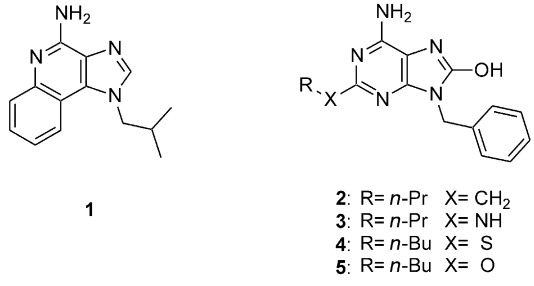


Figure 1. Structures of **1** (Imiquimod) and **2**–**5**.

2. Chemistry

The 2-alkylthio derivatives **11b**–**i**, **k**, **l** and the 2-alkoxy derivatives **13a**–**j** were prepared by the method outlined in Scheme 1. Treatment of **6**[14] with appropriate sodium thioalkoxides or alkoxides afforded the corresponding 2-alkylthioadenines **7** or 2-alkoxyadenines **8**. Bromination at the C(8)-position was selectively carried out with bromine to provide the 8-bromoadenines **9** or **10**. The 2-alkylthio derivatives **11b**–**i**, **k**, and **l** were obtained by the treatment of **9** with 6N HCl at reflux. In contrast, the same treatment of 2-alkoxy-8-bromoadenines **10** gave only **13a**. In order to obtain the 2-alkoxy derivatives **13b**–**j**, we employed another synthetic route, that is, the reaction of **10** with sodium methoxide which afforded the corresponding 8-methoxydenines **12** which were then treated with 12N HCl at room temperature. This route allowed the synthesis of the desired 2-alkoxy derivatives **13b**–**j** without any accompanying dealkylation product (**13a**).

The 2-thiol **11a** and the 2-alkylthio derivatives **11j**, **m**–**z** were prepared as outlined in Scheme 2. The reaction of **14** with benzoyl isothiocyanate followed by treatment with sodium hydroxide afforded 2-mercaptoadenine **11a**.[15] Subsequent *S*-alkylation with alkyl halides proceeded selectively to give the 2-alkylthio derivatives **11j**, **m**–**z**.

3. Results and discussion

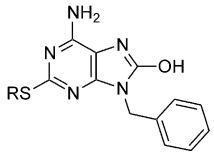
For in vitro studies, mouse spleen cells were cultured with the prepared compounds, and the amounts of IFN in supernatant were measured by a bioassay system using L929 cells infected with vesicular stomatitis virus.[16] The results of the ability of the prepared compounds to induce IFN are summarized in Tables 2 and 3.

In the case of the 2-alkylthio derivatives (Table 2), IFN-inducing activity was improved by the elongation of the alkyl chain, and the most suitable alkyl chain length was 3 or 4, (**4**, **11d**). The minimum effective concentrations (MECs) of these compounds were 0.01 μM, which was approximately 100-fold stronger than that of Imiquimod (**1**). The alkyl groups with the linear configuration (**4**, **11d**) were more potent than those of the branched (**11f–h**) and cycloalkyl ones (**11i**, **j**). From the results of compound **11k–m**, directly binding an aromatic group to the adenine was not preferable (**11k**), and the optimal alkyl chain length between the adenine and the phenyl group was found to be one (**11l**). While the electronic effects of the phenyl ring substituents were investigated using **11l**. The analogues possessing electron-withdrawing groups, like chloro (**11n**, **o**), were less active than the parent compound (**11l**), the analogues possessing electron-donating groups, like methoxy (**11p**, **q**) showed equipotent activities with the parent compound (**11l**). Thus, lead compound (**4**) exhibited the most potent activity and the improvement of the IFN-inducing activity was not achieved by the introduction of hydrophobic groups into the C(2)-position. Next, in order to evaluate the influence of hydrophilic groups at the C(2)-position on the IFN-inducing activities, we synthesized compounds containing ether (**11r–u**), dimethylamino (**11v**, **w**) and hydroxy (**11x–z**) groups at the C(2)-alkyl side chain.

Among the ether analogues, **11r** showed a potent activity and the MEC was equivalent with the propyl analogue (**11d**). Further elongation of the side chain resulted in decreased activities (**11s–u**). In the dimethylamino analogues, **11v** showed a weak activity, but the one methylene elongated compound (**11w**) exhibited a moderate activity. Considering the results of the straight alkyl analogues (**4**, **11b–e**) and the ether analogues (**11r–u**), further elongation of the methylene chain seemed not to be effective in obtaining potent activities. From the results of these types of analogues, it seemed that the substituent at the C(2)-position required a suitable chain length. Different from the above mentioned results, all the compounds having hydroxy groups (**11x–z**) showed the same MECs (0.1 μM). The alkyl chain length had no influence on the activity in this case.

Next, we investigated the activities of the 2-alkoxy derivatives (Table 3). Among the straight chain alkyl derivatives (**5**, **13b–e**), the most potent activity was observed in the butyl derivative (**5**), this is a similar

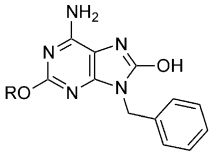
Table 2. IFN inducing activities of 2-alkylthioderivatives in mouse spleen cells



Compd	R	IFN (IU/mL)[a]				
		Drug concentration (μM)				
		0.001	0.01	0.1	1	10
11a	H				<1.8	3.4
11b	Me		<1.8	92.6	46.3	30.5
11c	Et		<1.8	114.0	15.9	17.8
11d	*n*-Pr	<1.8	4.1	66.0	33.7	17.8
4	*n*-Bu	<1.8	13.6	68.3	29.5	40.9
11e	*n*-Pent		<1.8	19.4	23.0	22.9
11f	*iso*-Pr		<1.8	42.4	38.3	28.4
11g	*iso*-Bu		<1.8	87.7	26.0	9.2
11h	*s*-Bu		<1.8	73.9	22.0	16.8
11i	Cyclohexyl		<1.8	7.8	53.9	57.7
11j	Cyclohexylmethyl		<1.8	11.8	28.1	20.0
11k	Ph			<1.8	79.5	48.3
11l	Bn		<1.8	68.4	59.1	35.4
11m	$(CH_2)_2Ph$		<1.8	2.0	27.3	43.4
11n	3-Chlorobenzyl			<1.8	47.2	45.2
11o	4-Chlorobenzyl			<1.8	15.8	nt[b]
11p	3-Methoxybenzyl		<1.8	12.4	28.3	17.0
11q	4-Methoxybenzyl		<1.8	13.9	35.8	40.0
11r	CH_2OMe	<1.8	2.2	21.3	13.1	10.5
11s	$(CH_2)_2OMe$		<1.8	28.4	13.6	11.8
11t	$(CH_2)_2OEt$		<1.8	17.8	16.9	9.5
11u	$(CH_2)_3OEt$		<1.8	58.1	38.4	19.7
11v	$(CH_2)_2NMe_2$				<1.8	14.6
11w	$(CH_2)_3NMe_2$		<1.8	1.8	30.2	30.3
11x	$(CH_2)_2OH$		<1.8	32.8	14.0	13.3
11y	$(CH_2)_3OH$		<1.8	30.5	12.0	12.7
11z	$(CH_2)_4OH$		<1.8	21.5	18.5	20.0
1				<1.8	26.2	19.6

[a] Each value represents the mean of duplicate assay (IU/mL).
[b] nt: not tested.

Table 3. IFN inducing activity of 2-alkoxy derivatives in mouse spleen cells



Compd	R	IFN (IU/mL)[a]				
		Drug concentration (μM)				
		0.001	0.01	0.1	1	10
13a	H		<1.8	22.6	23.8	21.7
13b	Me		<1.8	110.0	26.3	16.8
13c	Et		<1.8	123.0	23.8	15.3
13d	*n*-Pr	<1.8	43.0	71.1	23.1	16.1
5	*n*-Bu	2.4	33.0	56.5	24.0	20.4
13e	*n*-Pent	<1.8	36.9	29.8	24.6	19.8
13f	$(CH_2)_2OMe$	<1.8	1.9	46.6	25.9	24.9
13g	$(CH_2)_2OEt$		<1.8	25.4	18.0	17.2
13h	$(CH_2)_3OEt$			<1.8	23.3	25.0
13i	$(CH_2)_2OH$			<1.8	26.5	41.3
13j	$(CH_2)_3OH$			<1.8	49.4	18.3
1				<1.8	26.2	19.6

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

result with that of the 2-alkylthio derivatives. The MEC of **5** was 0.001 μM, which was approximately 1000-fold more potent than that of Imiquimod (**1**). In addition, we investigated the activities of the compounds having ether (**13f–h**) and hydroxy (**13i**, **j**) groups, and obtained similar results with those of the 2-alkylthio derivatives.

The amounts of induced IFN by all these derivatives including Imiquimod (**1**) exhibited bell-shaped dose–response curves. Cytotoxicity could be considered as one of the possibilities for the bell-shape phenomenon, however, cytotoxicity was not observed in our assay system. It is reported that Imiquimod exhibited a bell-shape type IFN-induction,[17] but the reason for this bell-shape is still not clear.

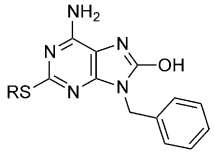
In addition to the in vitro study, the compounds with potent activities were further evaluated in an in vivo study to select compounds for further investigations. The results of the in vivo studies are shown in Table 4 and Table 5. The test compounds were orally administered to male Balb/c mice and then the concentration of IFN in plasma 2 h after dosing was measured by the bioassay as mentioned above. In the preliminary experiment, IFN concentration in mouse plasma reached a maximum at 2 h after oral administration of the compounds (data not shown). In the straight alkylthio derivatives (**4**, **11c–e**), IFN-inducing activities in vivo correlated well with the results of the in vitro study, among them **11d** showed the most potent activity. Compounds **11r** and **11x** exhibited potent activities as expected from their in vitro potentials. However, in other alkylthio derivatives the expected in vivo activities were not observed. We considered that poor oral absorptions of them caused those results. The straight alkyloxy derivatives (**5**, **13b–e**) also showed similar results with those of the alkylthio derivatives. In addition to **5**, the ether type compound (**13f**) exhibited a most potent IFN-inducing activity in vivo. Among these tested compounds, **5**, **11d** and **13f** had induced IFN from the dosage of 0.03 mg/kg, which was approximately 100-fold more potent than that of Imiquimod. The in vivo activity of compound **13f** seemed to be stronger compared with its in vitro potential. The discrepancy of this result may be due to the excellent oral absorption.

Finally, several compounds described above were tested in a rat pharmacokinetic model and we found that **13f**, in particular, showed a good oral absorption. The oral bioavailability of **13f** was calculated as 40% (Table 6). Thus, we were able to find a compound that exhibited potent IFN-inducing activity both in vitro and in vivo and possessing excellent oral bioavailability.

Akira et al. has recently reported that Imiqimod (**1**) induces IFN via Toll-like receptor 7 (TLR7) signaling pathway.[18] Therefore, the action mechanisms of our compounds toward TLR7 is under investigation.

In conclusion, a series of 8-hydroxyadenines, having various alkoxy or alkylthio moieties at the adenine C(2)-position, were synthesized and evaluated for their ability to induce IFN both in vitro and in vivo. We found that

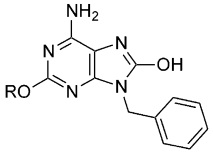
Table 4. IFN inducing activity of 2-alkylthioderivatives in mouse (in vivo)



Compd	R	IFN (IU/mL)[a]				
		Dose (mg/kg)				
		0.03	0.01	0.3	1	3
11c	Et		143	786	2387	
11d	*n*-Pr	99	308	452	225	
4	*n*-Bu		<	918	935	
11e	*n*-Pent				107	
11f	*iso*-Pr				<	
11g	*iso*-Bu			51	516	
11i	Cyclohexyl				<	
11j	Cyclohexylmethyl				<	
11l	Bn				71	
11r	CH_2OMe	<	322	542	1827	
11s	$(CH_2)_2OMe$			152	456	
11w	$(CH_2)_3NMe_2$				<	
11x	$(CH_2)_2OH$		50	226	393	
11y	$(CH_2)_3OH$			169	389	
11z	$(CH_2)_4OH$				44	
1						64

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

Table 5. IFN inducing activity 2-alkoxy derivatives in mouse (in vivo)



Compd	R	IFN (IU/mL)[a]				
		Dose (mg/kg)				
		0.03	0.01	0.3	1	3
13a	H				26	142
13b	Me			59	95	55
13c	Et		28	293	703	836
13d	*n*-Pr		20	318	569	330
5	*n*-Bu	60	186	317	318	877
13e	*n*-Pent	<	610	356	908	894
13f	$(CH_2)_2OMe$	75	314	496	2171	2872
13g	$(CH_2)_2OEt$		<	285	1085	
1						64

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

Table 6. Pharmacokinetic parameters after oral administration of Bf to rats at 3 mg/kg[a]

Compd	F (%)	C_{max} (ng/mL)	T_{max} (hr)	MRT (min)	CL (l/hr/kg)	V_d (l/kg)
13f	40	41.0	0.5	46.4	2.74	2.12

[a] The results are expressed as the mean of three rats.

compound **13f** (SM-360320) induced IFN from the dosage of 0.03 mg/kg, which was approximately 100-fold more potent than that of Imiquimod, and showed good oral bioavailability (F = 40%). Additional studies are underway to further evaluate the therapeutic potential of these derivatives.

4. Experimental

4.1. Chemistry

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

4.2. 9-Benzyl-2-methylthioadenine (7b)[19]

A mixture of **6** (100 mg, 0.385 mmol) and sodium thiomethoxide (270 mg, 3.852 mmol) in DMF (10 mL) was heated at 110 °C for 3.5 h. The mixture was evaporated in vacuo and the residue was partitioned with water (50 mL) and ethyl acetate (150 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (1% MeOH–$CHCl_3$) to give **7b** (64 mg, 61%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 7.63 (1H, s), 7.34 (5H, m), 5.45 (2H, s), 5.31 (2H, s), 2.58 (3H, s).

4.2.1. 9-Benzyl-2-methoxyadenine (8b).[14a] A solution of **6** (200 mg, 0.770 mmol) and sodium methoxide (208 mg, 3.850 mmol) in MeOH (20 mL) was heated to reflux for 30 h. The mixture was evaporated in vacuo and the residue was partitioned with water (50 mL) and $CHCl_3$ (150 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (2% MeOH–$CHCl_3$) to give **8b** (151 mg, 77%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 8.05 (1H, s), 7.37–7.25 (7H, m), 5.26 (2H, s), 3.81 (3H, s).

4.2.2. 9-Benzyl-8-bromo-2-methylthioadenine (9b). To a solution of **7b** (100 mg, 0.369 mmol) in CH_2Cl_2 (100 mL) was added bromine (0.5 mL, 9.76 mmol) dropwise and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into 10% aq $Na_2S_2O_3$. The organic layer was separated, washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (0.5% MeOH–$CHCl_3$) to give **9b** (10 mg, 8%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 7.34 (5H, m), 5.64 (2H, s), 5.33 (2H, s), 2.57 (3H, s); MS (EI) *m*/*z* 350 (MH^+).

4.2.3. 9-Benzyl-8-bromo-2-methoxyadenine (10b). To a solution of **8b** (118 mg, 0.462 mmol) in CH_2Cl_2 (50 mL) was added bromine (0.5 mL, 9.76 mmol) dropwise and the mixture was stirred at room temperature for 5 h. The reaction mixture was poured into 10% aq $Na_2S_2O_3$. The organic layer was separated, washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (1% MeOH–$CHCl_3$) to give **10b** (90 mg, 58%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 7.48 (2H, br s), 7.39–7.24 (5H, m), 5.26 (2H, s), 3.82 (3H, s); MS (EI) *m*/*z* 334 (MH^+).

4.2.4. 9-Benzyl-8-hydroxy-2-methylthioadenine (11b). A solution of **9b** (10 mg, 0.026 mmol) in 6N HCl (10 mL) was heated to reflux for 4 h. After evaporation, water (10 mL) was added to the residue and the solution was basified with 28% aq NH_3. The resulting precipitate was collected by filtration to give **11b** (8 mg, 96%) as a colorless solid: mp 298–300 °C; ^{1}H NMR (DMSO-d_6) δ 9.60 (1H, s), 7.31 (5H, m), 6.53 (2H, s), 4.88 (2H, s), 2.42 (3H, s); MS (FAB) *m*/*z* 288 (MH^+); HRMS calcd for $C_{13}H_{14}N_5OS$ 288.0919, found 288.0915. Anal. calcd for $C_{13}H_{13}N_5OS{\cdot}1/10H_2O$: C, 54.00; H, 4.60; N, 24.22. Found: C, 53.75; H, 4.61; N, 24.13.

Compounds **11c–i**, **11k** and **11l** were prepared using similar procedures as for **11b**.

4.2.5. 9-Benzyl-2-ethylthio-8-hydroxyadenine (11c). A colorless solid (4% for 3 steps from **6**), mp 292–293 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.31 (5H, m), 6.51 (2H, s), 4.88 (2H, s), 2.97 (2H, q, *J* = 7.3 Hz), 1.25 (3H, t, *J* = 7.3 Hz); MS (FAB) *m*/*z* 302 (MH^+); HRMS calcd for $C_{14}H_{16}N_5OS$ 302.1076, found 302.1086. Anal. calcd for $C_{14}H_{15}N_5OS{\cdot}1/10H_2O$: C, 55.46; H, 5.05; N, 23.10. Found: C, 55.39; H, 4.99; N, 23.16.

4.2.6. 9-Benzyl-8-hydroxy-2-propylthioadenine (11d). A colorless solid (12% for 3 steps from **6**), mp 288–290 °C; ^{1}H NMR (DMSO-d_6) δ 10.19 (1H, s), 7.31 (5H, m), 6.55 (2H, s), 4.87 (2H, s), 2.98 (2H, t, *J* = 6.9 Hz), 1.61 (2H, m), 0.94 (3H, t, *J* = 7.2 Hz); MS (FAB) *m*/*z* 316 (MH^+); HRMS calcd for $C_{15}H_{18}N_5OS$ 316.1232, found 316.1241. Anal. calcd for $C_{15}H_{17}N_5OS{\cdot}1/10H_2O$: C, 56.80; H, 5.47; N, 22.08. Found: C, 56.75; H, 5.43; N, 22.11.

4.2.7. 9-Benzyl-8-hydroxy-2-pentylthioadenine (11e). A colorless solid (11% for 3 steps from **6**), mp 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 10.05 (1H, s), 7.30 (5H, m), 6.50 (2H, s), 4.88 (2H, s), 2.99 (2H, t, *J* = 7.3 Hz), 1.59 (2H, m), 1.30 (4H, m), 0.84 (3H, t, *J* = 7.3 Hz); MS (FAB) *m*/*z*334 (MH^+); HRMS calcd for $C_{17}H_{22}N_5OS$ 334.1545, found 334.1523. Anal. calcd for $C_{17}H_{21}N_5OS{\cdot}1/4H_2O$: C, 58.68; H, 6.23; N, 20.13. Found: C, 58.52; H, 6.17; N, 20.31.

4.2.8. 9-Benzyl-8-hydroxy-2-isopropylthioadenine (11f). A colorless solid (13% for 3 steps from **6**), mp 310–313 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.32 (5H, m), 6.50 (2H, s), 4.87 (2H, s), 3.78 (1H, m), 1.30 (6H, d, J = 6.9 Hz); MS (FAB) m/z 316 (MH^+); HRMS calcd for $C_{15}H_{18}N_5OS$ 316.1232, found 316.1240. Anal. calcd for $C_{15}H_{17}N_5OS{\cdot}2/5H_2O$: C, 55.85; H, 5.56; N, 21.71. Found: C, 55.76; H, 5.31; N, 21.91.

4.2.9. 9-Benzyl-8-hydroxy-2-isobutylthioadenine (11g). A colorless solid (20% for 3 steps from **6**), mp 276–281 °C; ^{1}H NMR (DMSO-d_6) δ 10.10 (1H, s), 7.35–7.26 (5H, m), 6.51 (2H, s), 4.87 (2H, s), 2.93 (2H, d, J = 6.6 Hz), 1.83 (1H, m), 0.93 (6H, d, J = 6.6 Hz); MS (FAB) m/z 330 (MH^+); HRMS calcd for $C_{16}H_{20}N_5OS$ 330.1389, found 330.1382.

4.2.10. 9-Benzyl-2-(*s*-butylthio)-8-hydroxyadenine (11h). A colorless solid (2% for 3 steps from **6**), mp 299–301 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.35–7.24 (5H, m), 6.50 (2H, s), 4.87 (2H, s), 3.65 (1H, m), 1.61 (2H, m), 1.28 (3H, d, J = 7.0 Hz), 0.93 (3H, t, J = 7.3 Hz); MS (FAB) m/z 330 (MH^+); HRMS calcd for $C_{16}H_{20}N_5OS$ 330.1389, found 330.1366. Anal. calcd for $C_{16}H_{19}N_5OS{\cdot}1/10H_2O$: C, 58.02; H, 5.84; N, 21.14. Found: C, 57.97; H, 5.83; N, 21.32.

4.2.11. 9-Benzyl-2-cyclohexylthio-8-hydroxyadenine (11i). A colorless solid (24% for 3 steps from **6**), mp 304–307 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.31 (5H, m), 6.49 (2H, s), 4.87 (2H, s), 3.62 (1H, m), 2.00 (2H, m), 1.68 (2H, m), 1.62–1.56 (1H, m), 1.35 (5H, m); MS (FAB) m/z 356 (MH^+); HRMS calcd for $C_{18}H_{22}N_5OS$ 356.1545, found 356.1560. Anal. calcd for $C_{18}H_{21}N_5OS{\cdot}2/5H_2O$: C, 59.61; H, 6.06; N, 19.31. Found: C, 59.38; H, 5.90; N, 19.58.

4.2.12. 9-Benzyl-8-hydroxy-2-phenylthioadenine (11k). A colorless solid (8% for 3 steps from **6**), mp 281–283 °C; ^{1}H NMR (DMSO-d_6) δ 9.50 (1H, s), 7.55 (2H, m), 7.46 (3H, m), 7.28 (3H, m), 7.13 (2H, m), 6.55 (2H, s), 4.67 (2H, s); MS (FAB) m/z 350 (MH^+); HRMS calcd for $C_{18}H_{16}N_5OS$ 350.1076, found 350.1069.

4.2.13. 9-Benzyl-2-benzylthio-8-hydroxyadenine (11l). A colorless solid (3% for 3 steps from **6**), mp 284–287 °C; ^{1}H NMR (DMSO-d_6) δ 10.12 (1H, s), 7.34–7.19 (10H, m), 6.58 (2H, s), 4.91 (2H, s), 4.29 (2H, s); MS (FAB) m/z 364 (MH^+); HRMS calcd for $C_{19}H_{18}N_5OS$ 364.1232, found 364.1225.

4.2.14. 9-Benzyl-8-hydroxy-2-mercaptoadenine (11a). To a solution of **14** (31.3 g, 146 mmol) in THF (1500 mL) was added benzoyl isothioyanate (41 mL, 305 mmol) dropwise and the mixture was stirred at room temperature for 12 h. After evaporation, the residue was triturated with diethyl ether (150 mL) and the resulting precipitate was collected by filtration. To a solution of the obtained solid in THF (1500 mL) was added 2N NaOH (150 mL) and the mixture was heated to reflux for 50 h. The reaction mixture was acidified to pH 3 with 10% aq $NaHSO_4$ and the resulting precipitate was collected by filtration to give a crude product (27.8 g). This crude product was used next step without further purification. The crude product (30 mg) was recrystalized from ethyl acetate to give **11a** (10 mg) as a yellow solid: mp 276–278 °C; ^{1}H NMR (DMSO-d_6) δ 12.10 (1H, br s), 10.06 (1H, s), 7.36–7.24 (5H, m), 6.74 (2H, s), 4.85 (2H, s); MS (FAB) m/z 274 (MH^+); HRMS calcd for $C_{12}H_{12}N_5OS$ 274.0763, found 274.0777.

4.2.15. 9-Benzyl-2-cyclohexylmethylthio-8-hydroxyadenine (11j). To a suspension of crude **11a** (200 mg) and K_2CO_3 (102 mg, 0.738 mmol) in DMF (60 mL) was added cyclohexylmethyl bromide (130 mg, 0.734 mmol) and the mixture was stirred at room temperature for 9 h. The mixture was evaporated in vacuo and the residue was purified by silica gel column chromatography (3% MeOH–$CHCl_3$) to give **11j** (93mg, 24% for 2 steps from **14**) as a colorless solid: mp 285-288 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.30 (5H, m), 6.50 (2H, s), 4.87 (2H, s), 2.93 (2H, d, J = 6.6 Hz), 1.78–0.88 (11H, m); MS (FAB) m/z 370 (MH^+); HRMS calcd for $C_{19}H_{24}N_5OS$ 370.1702, found 370.1702. Anal. calcd for $C_{19}H_{23}N_5OS{\cdot}1/5H_2O$: C, 61.17; H, 6.32; N, 18.77. Found: C, 61.12; H, 6.24; N, 19.05.

Compounds **11m–z** were prepared using similar procedures as for **11j**.

4.2.16. 9-Benzyl-8-hydroxy-2-(2-phenylethylthio)adenine (11m). A colorless solid (44% for 2 steps from **14**), mp 271–272 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 7.28 (10H, m), 6.56 (2H, s), 4.92 (2H, s), 3.22 (2H, t, J = 6.6 Hz), 2.89 (2H, t, J = 6.6 Hz); MS (FAB) m/z 378 (MH^+); HRMS calcd for $C_{20}H_{20}N_5OS$ 378.1389, found 378.1394. Anal. calcd for $C_{20}H_{19}N_5OS{\cdot}1/10H_2O$: C, 63.34; H, 5.10; N, 18.47. Found: C, 63.27; H, 5.10; N, 18.58.

4.2.17. 9-Benzyl-2-(3-chlorobenzylthio)-8-hydroxyadenine (11n). A colorless solid (33% for 2 steps from **14**), mp 276–277 °C; ^{1}H NMR (DMSO-d_6) δ 10.14 (1H, s), 7.45 (1H, s), 7.27 (8H, m), 6.61 (2H, s), 4.90 (2H, s), 4.30 (2H, s); MS (FAB) m/z 398 (MH^+); HRMS calcd for $C_{19}H_{17}ClN_5OS$ 398.0842, found 398.0837. Anal. calcd for $C_{19}H_{16}ClN_5OS$: C, 57.35; H, 4.05; N, 17.60. Found: C, 57.12; H, 4.05; N, 17.68.

4.2.18. 9-Benzyl-2-(4-chlorobenzylthio)-8-hydroxyadenine (11o). A colorless solid (27% for 2 steps from **14**), mp 305-306 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 7.29 (9H. m), 6.59 (2H, s), 4.91 (2H, s), 4.26 (2H, s); MS (FAB) m/z 398 (MH^+); HRMS calcd for $C_{19}H_{17}ClN_5OS$ 398.0842, found 398.0824. Anal. calcd for $C_{19}H_{16}ClN_5OS{\cdot}1/5H_2O$: C, 56.84; H, 4.12; N, 17.44. Found: C, 56.78; H, 4.08; N, 17.61.

4.2.19. 9-Benzyl-8-hydroxy-2-(3-methoxybenzylthio)adenine (11p). A colorless solid (34% for 2 steps from **14**), mp 247–248 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 7.28 (5H, m), 7.15 (1H, m), 6.96 (2H, m), 6.79 (1H, m), 6.59 (2H, s), 4.89 (2H, s), 4.27 (2H, s), 3.68 (3H, s); MS (FAB) m/z 394 (MH^+); HRMS calcd for $C_{20}H_{20}N_5O_2S$ 394.1338, found 394.1334. Anal. calcd for $C_{20}H_{19}N_5O_2S{\cdot}1/10H_2O$: C, 60.77; H, 4.90; N, 17.72. Found: C, 60.70; H, 4.86; N, 17.93.

4.2.20. 9-Benzyl-8-hydroxy-2-(4-methoxybenzylthio)adenine (11q). A colorless solid (29% for 2 steps from **14**), mp 2760–278 °C; ^{1}H NMR (DMSO-d_6) δ 10.13 (1H, s), 7.28 (7H, m), 6.73 (2H, d, J = 8.9 Hz), 6.57 (2H, s), 4.92 (2H, s), 4.22 (2H, s), 3.69 (3H, s); MS (FAB) m/z 394 (MH^+); HRMS calcd for $C_{20}H_{20}N_5O_2S$ 394.1338, found 394.1337. Anal. calcd for $C_{20}H_{19}N_5O_2S{\cdot}1/10H_2O$: C, 60.77; H, 4.90; N, 17.72. Found: C, 60.63; H, 4.84; N, 17.86.

4.2.21. 9-Benzyl-8-hydroxy-2-(methoxymethylthio)adenine (11r). A colorless solid (48% for 2 steps from **14**), mp 272–273 °C; ^{1}H NMR (DMSO-d_6) δ 10.15 (1H, s), 7.31 (5H, m), 6.59 (2H, s), 5.29 (2H, s), 4.89 (2H, s), 3.21 (3H, s); MS (FAB) m/z 318 (MH^+); HRMS calcd for $C_{14}H_{16}N_5O_2S$ 318.1025, found 318.1034. Anal. calcd for $C_{14}H_{15}N_5O_2S$: C, 52.98; H, 4.76; N, 22.07. Found: C, 52.80; H, 4.68; N, 22.04.

4.2.22. 9-Benzyl-8-hydroxy-2-(2-methoxyethylthio)adenine (11s). A colorless solid (8% for 2 steps from **14**), mp 273–275 °C; ^{1}H NMR (DMSO-d_6) δ 10.11 (1H, s), 7.30 (5H, m), 6.55 (2H, s), 4.87 (2H, s), 3.50 (2H, t, J = 6.6 Hz), 3.22 (3H, s), 3.21 (2H, t, J = 6.6 Hz); MS (FAB) m/z 332 (MH^+); HRMS calcd for $C_{15}H_{18}N_5O_2S$ 332.1181, found 332.1194. Anal. calcd for $C_{15}H_{17}N_5O_2S$: C, 54.36; H, 5.17; N, 21.13. Found: C, 54.19; H, 5.09; N, 21.24.

4.2.23. 9-Benzyl-2-(2-ethoxyethylthio)-8-hydroxyadenine (11t). A colorless solid (8% for 2 steps from **14**), mp 260–261 °C; ^{1}H NMR (DMSO-d_6) δ 10.11 (1H, s), 7.30 (5H, m), 6.54 (2H, s), 4.88 (2H, s), 3.54 (2H, t, J = 6.9 Hz), 3.43 (2H, q, J = 7.0 Hz), 3.18 (2H, t, J = 6.6 Hz), 1.08 (3H, t, J = 6.9 Hz); MS (FAB) m/z 346 (MH^+); HRMS calcd for $C_{16}H_{20}N_5O_2S$ 346.1338, found 346.1323.

4.2.24. 9-Benzyl-2-(3-ethoxypropylthio)-8-hydroxyadenine (11u). A colorless solid (21% for 2 steps from **14**), mp 246–249 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.31 (5H, m), 6.51 (2H, s), 4.87 (2H, s), 3.44-3.34 (4H, m), 3.03 (2H, t, J = 8.9 Hz).1.83 (m, 2H), 1.08 (3H, t, J = 6.9 Hz).; MS (FAB) m/z 360 (MH^+); HRMS calcd for $C_{17}H_{22}N_5O_2S$ 360.1494, found 360.1485. Anal. calcd for $C_{17}H_{21}N_5O_2S$: C, 56.80; H, 5.89; N, 19.48. Found: C, 56.83; H, 5.78; N, 19.48.

4.2.25. 9-Benzyl-2-(2-dimethyaminoethylthio)-8-hydroxyadenine (11v). A colorless solid (3% for 2 steps from **14**), mp 245–247 °C; ^{1}H NMR (DMSO-d_6) δ 10.11 (1H, s), 7.29 (5H, m), 6.53 (2H, s), 4.88 (2H, s), 3.11 (2H, t, J = 7.6 Hz), 2.49 (2H, t, J = 7.6 Hz), 2.14 (6H, s); MS (FAB) m/z 345 (MH^+); HRMS calcd for $C_{16}H_{21}N_6OS$ 345.1498, found 345.1494.

4.2.26. 9-Benzyl-2-(3-dimethyaminopropylthio)-8-hydroxyadenine (11w). A colorless solid (5% for 2 steps from **14**), mp 252–255 °C; ^{1}H NMR (DMSO-d_6) δ 10.10 (1H, s), 7.30 (5H, m), 6.50 (2H, s), 4.87 (2H, s), 3.00 (2H, t, J = 7.6 Hz), 2.26 (2H, t, J = 7.3 Hz), 2.08 (6H, s), 1.72 (2H, m); MS (FAB) m/z 359 (MH^+); HRMS calcd for $C_{17}H_{23}N_6OS$ 359.1654, found 359.1657. Anal. calcd for $C_{17}H_{22}N_6OS{\cdot}1/3H_2O$: C, 56.03; H, 6.27; N, 23.06. Found: C, 55.95; H, 6.12; N, 23.07.

4.2.27. 9-Benzyl-8-hydroxy-2-(2-hydroxyethylthio)adenine (11x). A colorless solid (32% for 2 steps from **14**), mp 264–265 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.32 (5H, m), 6.52 (2H, s), 4.87 (3H, s), 3.59 (2H, q, J = 5.9 Hz), 3.12 (2H, t, J = 6.6 Hz); MS (FAB) m/z 318 (MH^+); HRMS calcd for $C_{14}H_{16}N_5O_2S$ 318.1025, found 318.1026. Anal. calcd for $C_{14}H_{15}N_5O_2S$: C, 52.98; H, 4.76; N, 22.07. Found: C, 52.70; H, 4.60; N, 22.24.

4.2.28. 9-Benzyl-8-hydroxy-2-(3-hydroxypropylthio)adenine (11y). A colorless solid (43% for 2 steps from **14**), mp 235-238 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.31 (5H, m), 6.50 (2H, s), 4.90 (2H, s), 4.50 (1H, t, J = 5.6 Hz), 3.49 (2H, m), 3.07 (2H, t, J = 6.6 Hz), 1.75 (2H, m); MS (FAB) m/z 332 (MH^+); HRMS calcd for $C_{15}H_{18}N_5O_2S$ 332.1181, found 332.1178.

4.2.29. 9-Benzyl-8-hydroxy-2-(4-hydroxybutylthio)adenine (11z). A colorless solid (4% for 2 steps from **14**), mp 251–253 °C; ^{1}H NMR (DMSO-d_6) δ 10.09 (1H, s), 7.31 (5H, m), 6.50 (2H, s), 4.87 (2H, s), 4.40 (1H, t, J = 5.3 Hz), 3.39 (2H, q, J = 5.3 Hz), 3.02 (2H, t, J = 6.9 Hz), 1.67–1.48 (4H, m); MS (FAB) m/z 346 (MH^+); HRMS calcd for $C_{16}H_{20}N_5O_2S$ 346.1338, found 346.1336. Anal. calcd for $C_{16}H_{19}N_5O_2S{\cdot}1/3H_2O$: C, 54.69; H, 5.64; N, 19.93. Found: C, 54.97; H, 5.60; N, 19.63.

4.2.30. 9-Benzyl-8-hydroxy-2,8-dimethoxyadenine (12b). A solution of **10b** (125 mg, 0.374 mmol) and sodium methoxide (404 mg, 7.479 mmol) in MeOH (10 mL) was heated to reflux for 10 h. The mixture was evaporated in vacuo and the residue was partitioned with water (50 mL) and $CHCl_3$ (150 mL). The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (1% MeOH–$CHCl_3$) to give **12b** (83 mg, 78%) as a colorless solid: ^{1}H NMR (DMSO-d_6) δ 7.73–7.23 (5H, m), 6.90 (2H, s), 5.05 (2H, s), 4.04 (3H, s), 3.78 (3H, s); MS (EI) m/z 286 (MH^+).

4.2.31. 9-Benzyl-2,8-dihydroxyadenine (13a). A solution of **10b** (75 mg, 0.224 mmol) in 6N HCl (15 mL) was heated to reflux for 5 h. After evaporation, water (10 mL) was added to the residue and the solution was basified with 28% aq NH_3. The resulting precipitate was collected by filtration and purified by silica gel column chromatography (0.2% NH_3-5% MeOH–$CHCl_3$) to give **13a** (12 mg, 21%) as a colorless solid: mp 254–256 °C; ^{1}H NMR (DMSO-d_6) δ 9.64 (2H, br s), 7.34–7.22 (5H, m), 6.51 (2H, s), 4.78 (2H, s); MS (FAB) m/z 258 (MH^+); HRMS calcd for $C_{12}H_{12}N_5O_2$ 258.0991, found 258.1008. Anal. calcd for $C_{12}H_{11}N_5O_2{\cdot}H_2O$: C, 52.36; H, 4.76; N, 25.44. Found: C, 52.11; H, 4.58; N, 25.22.

4.2.32. 9-Benzyl-8-hydroxy-2-methoxyadenine (13b). A solution of **12b** (53 mg, 0.186 mmol) in 12N HCl (10 mL) was stirred at room temperature for 5 h. After evaporation, water (10 mL) was added to the residue

and the solution was basified with 28% aq NH_3. The resulting precipitate was collected by filtration to give **13b** (38 mg, 74%) as a colorless solid: mp 308–309 °C; ^{1}H NMR (DMSO-d_6) δ 9.95 (1H, s), 7.35–7.22 (5H, m), 6.50 (2H, s), 4.86 (2H, s), 3.76 (3H, s); MS (FAB) *m*/*z* 272 (MH^+); HRMS calcd for $C_{13}H_{14}N_5O_2$ 272.1148, found 272.1165.

Compounds **13c–j** were prepared using similar procedures as for **13b**.

4.2.33. 9-Benzyl-2-ethoxy-8-hydroxyadenine (13c). A colorless solid (8% for 4 steps from **6**), mp 306–308 °C; ^{1}H NMR (DMSO-d_6) δ 9.96 (1H, s), 7.35–7.23 (5H, m), 6.45 (2H, s), 4.85 (2H, s), 4.19 (2H, q, J = 7.1 Hz), 1.25 (3H, t, J = 7.1 Hz); MS (FAB) *m*/*z* 286 (MH^+); HRMS calcd for $C_{14}H_{16}N_5O_2$ 286.1304, found 286.1294.

4.2.34. 9-Benzyl-8-hydroxy-2-propoxyadenine (13d). A colorless solid (18% for 4 steps from **6**), mp 319–321 °C; ^{1}H NMR (DMSO-d_6) δ 9.96 (1H, s), 7.35–7.22 (5H, m), 6.45 (2H, s), 4.86 (2H, s), 4.10 (2H, t, J = 6.8 Hz), 1.65 (2H, m), 0.93 (3H, t, J = 7.3 Hz); MS (FAB) *m*/*z* 300 (MH^+); HRMS calcd for $C_{15}H_{18}N_5O_2$ 300.1461, found 300.1474. Anal. calcd for $C_{15}H_{17}N_5O_2 \cdot 1/10H_2O$: C, 59.45; H, 5.79; N, 23.12. Found: C, 59.41; H, 5.63; N, 23.16.

4.2.35. 9-Benzyl-8-hydroxy-2-pentoxyadenine (13e). A colorless solid (36% for 4 steps from **6**), mp 307–308 °C; ^{1}H NMR (DMSO-d_6) δ 9.97 (1H, s), 7.35–7.24 (5H, m), 6.44 (2H, s), 4.85 (2H, s), 4.13 (2H, t, J = 6.6 Hz), 1.62 (2H, m), 1.32 (4H, m), 0.88 (3H, t, J = 6.4 Hz); MS (FAB) *m*/*z* 328 (MH^+); HRMS calcd for $C_{17}H_{22}N_5O_2$ 328.1773, found 328.1769. Anal. calcd for $C_{17}H_{21}N_5O_2 \cdot 1/10H_2O$: C, 62.03; H, 6.49; N, 21.27. Found: C, 62.04; H, 6.44; N, 21.22.

4.2.36. 9-Benzyl-8-hydroxy-2-(2-methoxyethoxy)adenine (13f). A colorless solid (16% for 4 steps from **6**), mp 290–292 °C; ^{1}H NMR (DMSO-d_6) δ 9.97 (1H, s), 7.35-7.23 (5H, m), 6.48 (2H, s), 4.86 (2H, s), 4.26 (2H, t, J = 4.6 Hz), 3.58 (2H, t, J = 4.6 Hz), 3.27 (3H, s); MS (FAB) *m*/*z* 316 (MH^+); HRMS calcd for $C_{15}H_{18}N_5O_3$ 316.1410, found 316.1410. Anal. calcd for $C_{15}H_{17}N_5O_3 \cdot 1/10H_2O$: C, 56.81; H, 5.47; N, 22.08. Found: C, 56.75; H, 5.34; N, 22.10.

4.2.37. 9-Benzyl-2-(2-ethoxyethoxy)-8-hydroxyadenine (13g). A colorless solid (26% for 4 steps from **6**), mp 278–279 °C; ^{1}H NMR (DMSO-d_6) δ 9.98 (1H, s), 7.35–7.23(5H, m), 6.48 (2H, s), 4.86 (2H, s), 4.25 (2H, t, J = 4.6 Hz), 3.62 (2H, t, J = 4.6 Hz), 3.45 (2H, q, J = 7.0 Hz), 1.11 (3H, t, J = 7.0 Hz); MS (FAB) *m*/*z* 330 (MH^+); HRMS calcd for $C_{16}H_{20}N_5O_3$ 330.1566, found 330.1549. Anal. calcd for $C_{16}H_{19}N_5O_3 \cdot 1/10H_2O$: C, 58.03; H, 5.84; N, 21.15. Found: C, 58.27; H, 5.71; N, 21.29.

4.2.38. 9-Benzyl-2-(3-ethoxypropoxy)-8-hydroxyadenine (13h). A colorless solid (33% for 4 steps from **6**), mp 297–300 °C; ^{1}H NMR (DMSO-d_6) δ 9.97 (1H, s), 7.31–7.23 (5H, m), 6.45 (2H, s), 4.84 (2H, s), 4.17 (2H, t, J = 6.6 Hz), 3.44 (2H, t, J = 6.6 Hz), 3.38 (2H, q, J = 7.0 Hz), 1.85 (2H, m) 1.08 (3H, t, J = 7.0 Hz); MS (FAB) *m*/*z* 344 (MH^+); HRMS calcd for $C_{17}H_{22}N_5O_3$ 344.1723, found 344.1720. Anal. calcd for $C_{17}H_{21}N_5O_3$: C, 59.46; H, 6.16; N, 20.40. Found: C, 59.29; H, 6.13; N, 20.49.

4.2.39. 9-Benzyl-8-hydroxy-2-(2-hydroxyethoxy)adenine (13i). A colorless solid (12% for 4 steps from **6**), mp 300–302 °C; ^{1}H NMR (DMSO-d_6) δ 9.89 (1H, s), 7.30 (5H, m), 6.46 (2H, s), 4.85 (2H, s), 4.79 (1H, t, J = 5.6 Hz), 4.15 (2H, t, J = 4.9 Hz), 3.65 (2H, m); MS (FAB) *m*/*z* 302 (MH^+); HRMS calcd for $C_{14}H_{16}N_5O_3$ 302.1253, found 302.1242. Anal. calcd for $C_{14}H_{15}N_5O_3 \cdot 1/4H_2O$: C, 54.99; H, 5.11; N, 22.90. Found: C, 54.89; H, 4.92; N, 22.69.

4.2.40. 9-Benzyl-8-hydroxy-2-(3-hydroxypropoxy)adenine (13j). A colorless solid (10% for 4 steps from **6**), mp 294–295 °C; ^{1}H NMR (DMSO-d_6) δ 9.95 (1H, s), 7.30 (5H, m), 6.45 (2H, s), 4.85 (2H, s), 4.50 (1H, t, J = 5.0 Hz), 4.20 (2H, t, J = 6.0 Hz), 3.51 (2H, q, J = 5.0 Hz), 1.79 (2H, m); MS (FAB) *m*/*z* 316 (MH^+); HRMS calcd for $C_{15}H_{18}N_5O_3$ 316.1410, found 316.1407. Anal. calcd for $C_{15}H_{17}N_5O_3 \cdot 1/5H_2O$: C, 56.49; H, 5.50; N, 21.96. Found: C, 56.59; H, 5.42; N, 21.94.

5. Biology

5.1. 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% aq NaCl, 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.

5.2. IFN induction in mouse plasma

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

5.3. 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.

5.4. Parmacokinetic evaluation

Male Sprague–Dawley rats (Charles River Japan Inc.) aged 8–10 weeks were used for pharmacokinetic studies. The test compound **13f** was administered at a dose of 3 mg/kg intravenously (via bolus injection into the tail vein of rats) or 3 mg/kg orally (via gavage to the stomach). For intravenous administration the test compound was formulated as a solution in DMSO-PEG400-1M HCl-saline (1:8:10:81, v/v). For oral administration the test compound was formulated as a suspension in 0.5% sodium carboxymethyl cellulose. At 0.5, 1, 2, 4, 8 and 24 h after administration, rats were anaesthetized with diethyl ether, and heparinized blood samples were collected via caudal vein. Then the plasma samples were prepared by centrifugation (12,000 rpm for 10 min), and stored at-20 °C until analysis.

The plasma samples were mixed with ethyl acetate, and the organic layer obtained was evaporated to dryness at 40 °C, and then reconstituted in 50% methanol. The extract sample was injected into an HPLC system equipped with a Zorbax bonus RP column (4.6×150 mm), and eluted with a linear gradient from 4 to 48% of acetonitrile in 0.1% aq trifluoroacetic acid at a flow rate of 1.0 mL/min and monitored with UV detection at 315 nm. The drug concentration was calculated from the peak area using the calibration curve obtained from standard plasma samples (the lower quantification limit was 10 ng/mL). Pharmacokinetic parameters were determined by non-compartmental method.

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