# Discovery of 8-Hydroxyadenines as a Novel Type of Interferon Inducer

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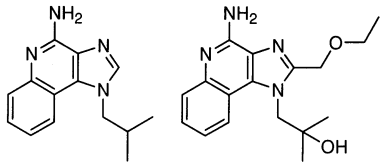
**Abstract:** 9-Benzyl-8-hydroxyadenine (**6**) was found to possess interferon-inducing activity in vitro as a lead compound. Although replacement of the 9-benzyl group of **6** did not improve the activity, the introduction of a substituent such as alkyl, alkylthio, alkylamino, and alkoxy groups into the 2-position of the adenine ring resulted in a remarkable increase in the activity. The 2-alkylthio (**30**−**32**), 2-butylamino (**41**), and 2-butoxy (**47**) analogues indicated the highest activities by oral administration to mice.

**Introduction.** Hepatitis C is induced by infection with the hepatitis C virus (HCV) through contact with blood products, which might occur by accidentally being stuck with a dirty (used) needle, using IV drugs and sharing needles, or getting a blood transfusion. The major problems of HCV infection are the progression of hepatitis C to cirrhosis, liver failure, and liver cancer after a long period of time. All current treatment protocols for hepatitis C are based on the use of various preparations of interferon alpha (IFN-α) alone or in combination with ribavirin.[1] Recombinant (synthetic) IFNs, which currently dominate the IFN market, are genetically engineered and consequently are sometimes recognized as "foreign" by the body's immune system. Treatment with recombinant IFN may cause unfavorable immune responses and the formation of neutralizing antibodies that reduce the effectiveness of a particular therapy.[2] Furthermore, as IFN preparations are administered by intramuscular or subcutaneous injection, the treatment with IFN causes pain and irritation at the site of injection. The cost of treatment is very high because of the inordinately expensive IFN preparations. Therefore, enhancing the release of endogenous IFN by the oral administration of small-molecular-weight compounds is one approach to anti-HCV therapeutics. Development of small-molecule IFN inducers has been ardently desired for a long time to avoid such drawbacks of IFN injections.

Various compounds possessing IFN-inducing activity have been hitherto reported.[3] Among them, small-molecule IFN inducers include tilorone,[4] BL-20803,[5] ataburine,[6] CP-28888,[7] ABMP,[8] DRB,[9] 10-carboxymethyl-9-acridone,[10] bropirimine,[11] and imiquimod.[12,13] Imiquimod, which is clinically used in the United States




Imiquimod Resiquimod

for treatment of exophytic 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 for hepatitis C as a chemotherapy drug. Subsequently, imiquimod analogues such as R-842[14] (a metabolite of imiquimod) and resiquimod[13,15] were found to be more effective IFN inducers than imiquimod. However, no IFN inducer has yet been clinically employed for the treatment of hepatitis C.

To search for novel IFN inducers, we have focused on the screening of pyrimidine and purine derivatives stocked in our compound library. Herein we describe the discovery of a novel type of lead compound possessing IFN-inducing activity and its chemical modification and qualitative structure−activity relationships (SAR).

**Chemistry.** 8-Chloro-9-benzyladenine (**2**) was prepared by chlorination of 9-benzyladenine (**1**) by using a combination of titanium tetrachloride and hydrogen peroxide. Other various 8-substituted 9-benzyladenine derivatives (**3**−**10**) were synthesized from **1** according to usual methods[16] employed for the preparation of 8-substituted purines. 9-Unsubstituted 8-hydroxyadenine (**11**)[17] and a variety of 9-substituted 8-hydroxyadenine derivatives (**12**−**22**)[18] were synthesized according to the method previously reported.

The methods for the synthesis of 9-benzyl-8-hydroxyadenines possessing various substituents at the 2-position are shown in Schemes 1 and 2. 5-Amino-1-benzyl-4-cyano-2-hydroxyimidazole (**23**) is a useful intermediate for the synthesis of 8-hydroxyadenines such as 2-methyl (**24**), 2-phenyl (**28**), 2-amino (**37**), and 2-hydroxy (**43**) derivatives.[18] Condensation of **23** with substituted imidates afforded the corresponding 2-alkyl-8-hydroxyadenines **25**−**27**. Although analogous condensation with thiourea failed to obtain the expected 2-mercapto-8-hydroxyadenine (**29**), the reaction with benzoyl isothiocyanate and successive treatment with sodium hydroxide resulted in the successful formation of it. Subsequent alkylation of **29** with alkyl halides selectively on the sulfur atom proceeded to give 2-alkylthio analogues **30**−**35**. Oxidation of the 2-butylthio derivative (**33**) with *m*-chloroperbenzoic acid afforded the corresponding *S*-oxide (**36**). 2-Alkylamino analogues **39**−**42** were prepared by the reductive alkylation of 2-aminoadenine (**37**) with aliphatic aldehydes and sodium cyanoborohydride. The alkylation was proceeded regioselectively at the 2-amino group. On the other hand, alkylation of 2-hy-

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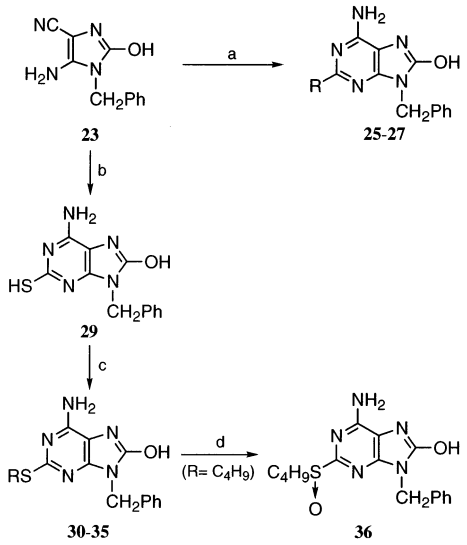
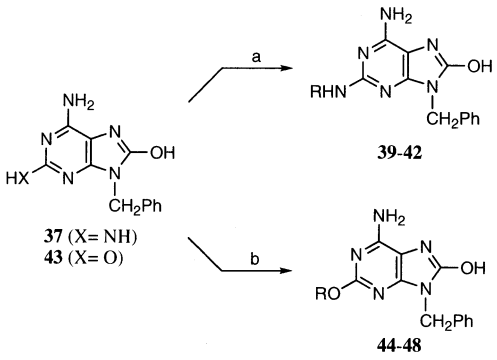
**Scheme 1**[a]



[a] Reagents and conditions: (a) $RCH(NH)OC_2H_5 \cdot HCl$, Na, $C_2H_5OH$, reflux, 2 d (32−40%); (b) (i) BzNCS, THF, rt, 1 d; (ii) 2 N NaOH−THF (1:10), reflux, 2 d; (c) RX, $K_2CO_3$, DMF, rt, 6 h (34−43% from **23**); (d) *m*CPBA, THF, rt, 2 h (58%).

**Scheme 2**[a]



[a] Reagents and conditions: (a) aldehyde, $NaBH_3CN$, $CH_3OH$, rt, 2 d (63−87%); (b) RX, LiH, DMF, rt, 1 d (6−13%).

droxyadenine (**43**) with alkyl halides in the presence of lithium hydride gave a mixture of 2-*O*-mono- and 2-*O*,7-*N*-dialkylated products, from which the desired 2-alkoxy analogues **44**−**48** were purified in low yields.

**Results and Discussion.** First, a lead compound for the potential IFN-inducing activity was found among several 8-substituted 9-benzyladenine derivatives (**1**−**10**), whose IFN-inducing activities were evaluated by using mice spleen cells[19] and are shown in Table 1. 9-Benzyl-8-hydroxyadenine (**6**) and 9-benzyl-8-mercaptoadenine (**8**) showed the activities with a minimum effective concentration (MEC) of 10 $\mu$M, although their activities were weaker than that of the reference drug, imiquimod. 8-Hydroxyadenine **6** was selected as a lead compound because the 8-mercapto analogue (**8**) did not indicate in vivo activity,[19] having a minimum effective dose (MED) >30 mg/kg, which was measured in mice. Interestingly, other analogues, **1**−**5**, **7**, **9**, and **10**, possessing no acidic proton on the 8-substituents, were inactive.

Variation of substituents at the 9-position of lead compound **6** was investigated (Table 2). Although the α-naphthylmethyl derivative (**16**) indicated an activity equivalent to that of **6**, unsubstituted (**11**), butyl (**12**), cyclopentyl (**13**), phenyl (**14**), and phenethyl (**15**) analogues were inactive. Introduction of some substituents onto the benzene ring of the 9-benzyl group had no significant effect on the activity (compounds **17**−**22**).

To explore the effects of the substituents at the 2-position on the IFN-inducing activity, we tested a series of 2-substituted derivatives **24**−**48** and show the results in Table 3. A dramatic improvement of the activity was realized by introduction of chain substituents into the 2-position. In the alkyl-substituted series, we found that the introduction of a longer alkyl chain led to stronger activity. For example, the 2-butyl analogue (**27**) had more than 100-fold greater activity (MEC = 0.03 $\mu$M) than lead compound **6**. On the other hand, the 2-phenyl analogue (**28**) was ineffective in improving the activity. In the 2-alkylthio series, a similar tendency was indicated. 2-Propylthio (**32**) and

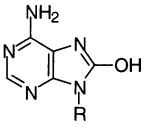
**Table 1.** IFN-inducing Activities of 8-Substituted 9-Benzyladenines

**1-10**

| compd | R | MEC[a] ($\mu$M) | MED[b] (mg/kg) |
|---|---|---|---|
| **1** | H | >10 | |
| **2** | Cl | >10 | |
| **3** | Br | >10 | |
| **4** | I | >10 | |
| **5** | $CH_3$ | >10 | |
| **6** | OH | 10 | 10 |
| **7** | $OCH_3$ | >10 | |
| **8** | SH | 10 | >30 |
| **9** | $SCH_3$ | >10 | |
| **10** | $NHNH_2$ | >10 | |
| imiquimod | | 1 | 3 |

[a] Minimum effective concentration (mice spleen cells): a concentration of compounds required for more than 1 IU/mL induction of IFN. [b] Minimum effective dose (mice po): a dose of compounds required for more than 100 IU/mL induction of IFN.

**Table 2.** IFN-inducing Activities of 9-Substituted 8-Hydroxyadenines

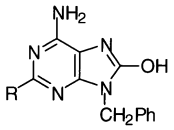


**6, 11-22**

| compd | R | MEC[a] ($\mu$M) |
|---|---|---|
| **6** | $CH_2Ph$ | 10 (10)[b] |
| **11** | H | >10 |
| **12** | $C_4H_9$ | >10 |
| **13** | cyclopentyl | >10 |
| **14** | Ph | >10 |
| **15** | $CH_2CH_2Ph$ | >10 |
| **16** | α-naphthylmethyl | 10 |
| **17** | $CH_2C_6H_4$ (4-F) | 10 (30)[b] |
| **18** | $CH_2C_6H_4$ (4-Cl) | 10 (30)[b] |
| **19** | $CH_2C_6H_4$ (4-$CH_3$) | 10 |
| **20** | $CH_2C_6H_4$ (2-$OCH_3$) | 10 |
| **21** | $CH_2C_6H_4$ (3-$OCH_3$) | 10 |
| **22** | $CH_2C_6H_4$ (4-$OCH_3$) | 10 |
| imiquimod | | 1 (3)[b] |

[a] Minimum effective concentration (mice spleen cells): a concentration of compounds required for more than 1 IU/mL induction of IFN. [b] Value in parentheses is MED (mg/kg) (mice po): a dose of compounds required for more than 100 IU mL induction of IFN.

**Table 3.** IFN-Inducing Activities of 2-Substituted 9-Benzyl-8-hydroxyadenines



**6**, **24**-**48**

| compd | R | MEC[a] ($\mu$M) | MED[b] (mg/kg) |
|---|---|---|---|
| **6** | H | 10 | 30 |
| **24** | $CH_3$ | 1 | 3 |
| **25** | $C_2H_5$ | 1 | 3 |
| **26** | $C_3H_7$ | 1 | 3 |
| **27** | $C_4H_9$ | 0.03 | 0.3 |
| **28** | Ph | 10 | >30 |
| **29** | SH | 10 | nt[c] |
| **30** | $SCH_3$ | 0.1 | 0.1 |
| **31** | $SC_2H_5$ | 0.1 | 0.1 |
| **32** | $SC_3H_7$ | 0.01 | 0.1 |
| **33** | $SC_4H_9$ | 0.01 | 0.3 |
| **34** | $SC_5H_{11}$ | 10 | 1 |
| **35** | $SCH_2Ph$ | 0.1 | >1 |
| **36** | $S(O)C_4H_9$ | 0.01 | 1 |
| **37** | $NH_2$ | 10 | nt[c] |
| **38** | $NHCH_3$ | 1 | nt[c] |
| **39** | $NHC_2H_5$ | 1 | nt[c] |
| **40** | $NHC_3H_7$ | 0.1 | 1 |
| **41** | $NHC_4H_9$ | 0.1 | 0.1 |
| **42** | $NHC_5H_{11}$ | 0.1 | 0.3 |
| **43** | OH | 0.1 | 1 |
| **44** | $OCH_3$ | 0.1 | >1 |
| **45** | $OC_2H_5$ | 0.1 | 0.3 |
| **46** | $OC_3H_7$ | 0.01 | 0.3 |
| **47** | $OC_4H_9$ | 0.001 | 0.1 |
| **48** | $OC_5H_{11}$ | 0.01 | 0.3 |
| imiquimod | | 1 | 3 |

[a] Minimum effective concentration (mice spleen cells): a concentration of compounds required for more than 1 IU/mL induction of IFN. [b] Minimum effective dose (mice po): a dose of compounds required for more than 100 IU/mL induction of IFN. [c] Not tested.

2-butylthio (**33**) derivatives indicated high activities, with an MEC of 0.01 $\mu$M. However, the 2-pentylthio analogue (**34**) exhibited not so impressive activity (MEC = 10 $\mu$M). Additionally, the sulfoxide analogue (**36**) also showed an excellent activity (MEC = 0.01 $\mu$M). Similar tendency was also found in the case of the 2-alkylamino and 2-alkoxy series. Among them, the 2-butoxy analogue (**47**) indicated the most potent activity, with an MEC of 0.001 $\mu$M under the in vitro assay conditions used, and it had a potency more than 10000-fold greater than that of lead compound **6**. Compound **47** also showed a high activity with an MEC of 0.01 $\mu$M in the in vitro assay using human PBMC (MEC is a concentration of **47** required to induce more than 1 pg/mL IFN). Replacement of the 6-amino group of **6** with other substituents such as hydrogen, hydroxy, and mercapto groups resulted in the disappearance of the activity, although no data are shown here.

The remarkable features of the qualitative SAR may be summarized as follows: (1) 9-Benzyl-8-hydroxyadenine is required as the simplest structure for the expression of IFN-inducing activity. (2) At the 9-position, a benzyl-type substituent is essential for the activity. (3) The introduction of several substituents (4-F, 4-Cl, 4-Me, 2-OMe, 3-OMe, or 4-OMe) on the benzene ring of the 9-benzyl group did not improve the activity. (4) The introduction of some alkyl-chain substituents (i.e., alkyl, alkylthio, alkylamino, alkoxy) at the 2-position of the adenine ring strongly enhances the activity.

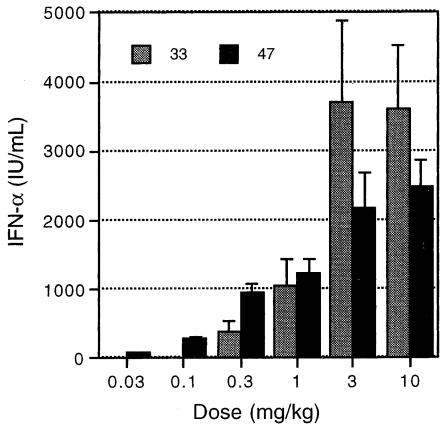



**Figure 1.** Dose-dependent effects of compounds **33** and **47** on IFN-inducing activity in mice by oral administration.

Optimal IFN-inducing activity is seen when the chain length of the 2-substituent is 4−6 atoms.

The in vivo activity[19] of the IFN induction was examined by oral administration to mice to select candidates possessing an excellent bioavailability. As shown in Table 3, several compounds such as 2-propylthio (**32**), 2-butylamino (**41**), and 2-butoxy (**47**) analogues, which indicated excellent activities (MED = 0.1 mg/kg), showed greater than 100-fold improvement of the in vivo activity over lead compound **6**.

The production of IFN in mice by 8-hydroxyadenine derivatives was dose-dependent as shown in Figure 1. The 2-butoxy analogue (**47**) induced IFN at a dose as low as 0.1 mg/kg. The IFN production by **33** and **47** plateaued between 3 and 10 mg/kg. In general, IFN concentration in plasma is required to be 50−100 IU/mL for the treatment of patients infected by HCV. Compounds **33** and **47** induced IFN more than 250 IU/mL at doses of 0.3 and 0.1 mg/kg in mice, respectively. These results indicated that both compounds exhibit enough activities to treat patients at lower concentrations than that of imiquimod (67±21 IU/mL at a dose of 3 mg/kg).

The cytotoxic activity of compound **47** was also determined by using the MTS assay. Little cytotoxicity was identified by **47** at the highest concentration tested (10 $\mu$M) in this assay (detailed data not shown).

Akira et al. has recently reported that imiquimod induces IFN via stimulation of the toll-like receptor 7.[20] Therefore, the action mode of 8-hydroxyadenines toward the receptor is under investigation.

**Conclusion.** In summary, we discovered a novel type of IFN inducer, 9-benzyl-8-hydroxyadenine (**6**) as a lead compound. The substituent modification at the 2-position of the adenine ring brought remarkable improvement of IFN induction. In the in vitro assay, the 2-butoxy analogue (**47**) emerged as having the most potent activity. The in vivo activity was examined by oral administration into mice, and 2-alkylthio (**30**−**32**), 2-butylamino (**41**), and 2-butoxy (**47**) analogues have been selected for further pharmacological investigation. Compounds based on this novel template are the subjects of continuous investigations toward the development of therapeutically useful IFN inducers that avoid side effects such as vomiting.

**Supporting Information Available:** Synthetic procedures and characterization data for compounds **2**, **25**−**27**, **30**−**36**, **39**−**42**, and **44**−**48** and procedures for IFN induction assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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