Inhibition of Adenosine Deaminase by Novel 5:7 Fused Heterocycles Containing the Imidazo[4,5-*e*][1,2,4]triazepine Ring System: A Structure−Activity Relationship Study

Ayub Reayi and Ramachandra S. Hosmane*

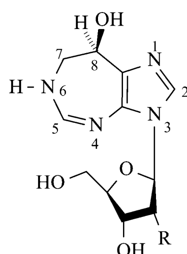
Laboratory for Drug Design and Synthesis, Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

Received September 2, 2003

As part of a program to explore structure−activity relationships for the extremely tight binding inhibition characteristics of coformycins to adenosine deaminase, a series of analogues (**1a**−**1h**) containing the imidazo[4,5-*e*][1,2,4]triazepine ring system has been synthesized and screened in vitro against a mammalian adenosine deaminase for inhibitory activity. While compounds **1a** and **1b** were synthesized in five steps starting from 4-nitroimidazole, others were derived from **1a** through simple exchange reactions with the appropriate alcohols. The observed kinetics profiles and K_i values suggest that the target compounds are competitive inhibitors that bind 6−9 orders of magnitude less tightly to the enzyme. Compounds **1c** and **1d** were the most active in the series with K_i's ranging from 12 to 15 μM.

Introduction

Adenosine deaminase (ADA, EC 3.5.4.4) catalyzes the hydrolytic deamination of adenosine and 2′-deoxyadenosine to the corresponding inosines.[1] ADA is a pivotal enzyme of purine metabolism, whose genetic deficiency is associated with a phenotype of severe combined immunodeficiency disorder (SCID).[2] As SCID is caused by high elevations in the concentration of 2′-deoxyadenosine-5′-triphosphate (dATP), which is lymphotoxic, inhibitors of ADA became the target of many investigations for the selective treatment of lymphoproliferative malignancies.[3,4] Indeed, the potent antitumor properties of two natural products, coformycin (**1**)[5−8] and 2′-



R=OH (Coformycin)
R=H (2'-Deoxycoformycin or Pentostatin)

deoxycoformycin (pentostatin) (**2**),[9−14] the strongest inhibitors of ADA known to-date, with K_i ranging from 10^{-11} to 10^{-12} M,[15−20] were found to bear a relationship with their ability to mimic ADA deficiency and act as immunosuppressants to control cancers associated with the hyperimmune system, such as leukemia and lymphoma.[21−23] Furthermore, the coformycins were discovered to cause synergistic effects upon coadministration with other antitumor compounds that are analogues of adenosine, such as Ara-A,[24] formycin,[25] or cordycepin,[26] which would otherwise be hydrolyzed by ADA into their inactive inosine counterparts.

The high promise of coformycins in cancer treatment, however, met severe setbacks in the clinic due to high incidences of unacceptable toxicities involving liver, kidney, and the central nervous system.[27−29] The long and nearly irreversible, extremely tight binding inhibition of intracellular ADA has been given as the logical explanation for the observed toxicities with the coformycin therapy.[30] The synthesis of a new enzyme molecule was believed necessary for recovering each time from the toxic effects of coformycin inhibition.[30] Therefore, an analogue of coformycin that is somewhat less tight binding to ADA, is readily reversible, and has a shorter duration of action to allow faster enzyme recovery will be needed for overcoming the undesirable toxic effects. While a few synthetic analogues of coformycin have been reported,[31−33] an ideal clinical candidate with the desired characteristics is yet to be found. To this end, our lab has been involved in performing systematic structure−activity relationship (SAR) studies in this area and is currently focusing on probing the molecular basis of tight-binding interactions of coformycin with ADA.[34,35] The outcome of such studies is believed to provide the much needed guidance for eventually rational structural modifications of coformycin that would afford to somewhat loosen its extremely tight protein binding without significantly compromising its efficacy.

The single-crystal X-ray structure of a complex of ADA with 2′-deoxycoformycin (dCF) bound at the active site,[36,37] schematically shown in Figure 1, surprisingly revealed the absence of any H-bonding interactions of the heterocyclic ring nitrogen atoms of the ligand with the protein. As a matter of fact, the only conspicuous interaction of the heterocycle is a coordination bond between the 8-OH group of dCF and the active site zinc of ADA.[36,37] However, the crystal structure of the complex did show the existence of several hydrogen

* To whom the correspondence should be addressed. Tel: (410) 455-2520. Fax: (410) 455-1148. E-mail: hosmane@umbc.edu.

10.1021/jm0304257 CCC: $27.50 © 2004 American Chemical Society
Published on Web 01/13/2004

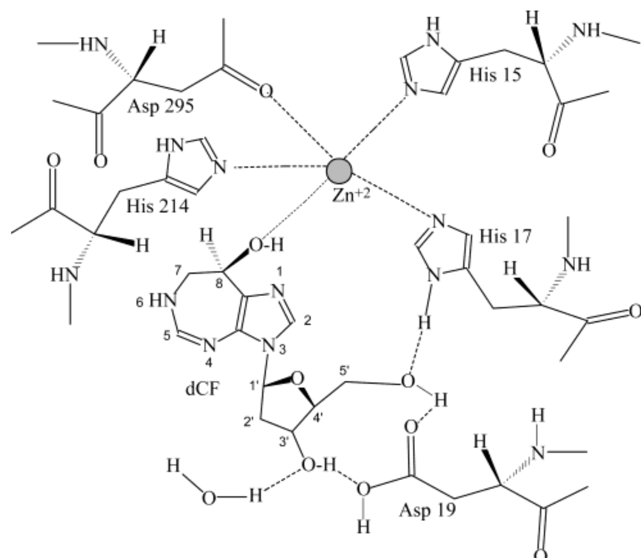
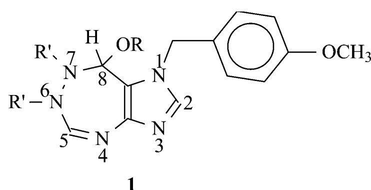


Figure 1. Schematic representation of important protein−ligand interactions in the reported[36,37] crystal structure of ADA−dCF complex.

bonds between the sugar hydroxyls of dCF and the active site amino acid residues of ADA, in particular His 117 and Asp 19, in addition to H-bonding with an adventitious molecule of water.

A close inspection of the X-ray structure suggested that the elimination of H-bonding interactions of the sugar hydroxyls, coupled with the weakening of the coordination bond between the 8-OH and zinc, would considerably loosen the tight-binding interactions of coformycins with ADA. In this regard, compounds bearing the general structure **1** were envisioned to fulfill both of these demands, and are thus the targets of the present investigation.

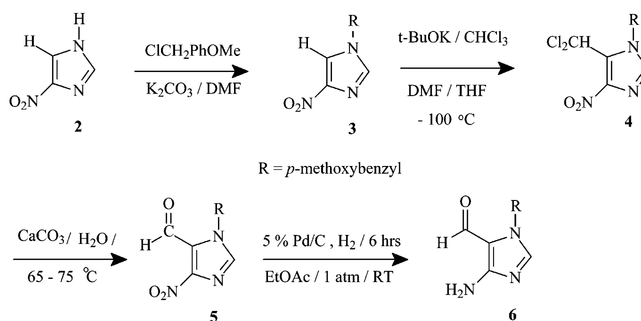


1

a; R = R' = CH_3
b; R = CH_2CH_3, R' = CH_3
c; R = $(CH_2)_2CH_3$; R' = CH_3
d; R = $(CH_2)_3CH_3$; R' = CH_3
e; R = $CH(CH_3)_2$; R' = CH_3
f; R = $(CH_2)_2OCH_3$; R' = CH_3
g; R = CH_2Ph-(*p*-OCH_3); R' = CH_3
h; R = CH_2Ph-(*m*-CH_3); R' = CH_3

The important structural features of compounds **1a**−**1h** include (a) the total absence of the sugar moiety, and hence, the inability to form the crucial H-bonds associated with the sugar hydroxyls of coformycins. The sugar moiety is not absolutely essential for activity, as we had already discovered from our earlier SAR studies.[34,35] While ADA inhibitory activity has also been reported with many other different classes of compounds besides nucleosides, including flavanoids,[38,39] steroids,[40] terpenoids,[40] and other natural products,[41,42] it is not clear if these compounds bind to the active or allosteric site of the enzyme. (b) The presence of an aralkyl substituent at position-1 is significant. This is based on our earlier

Scheme 1

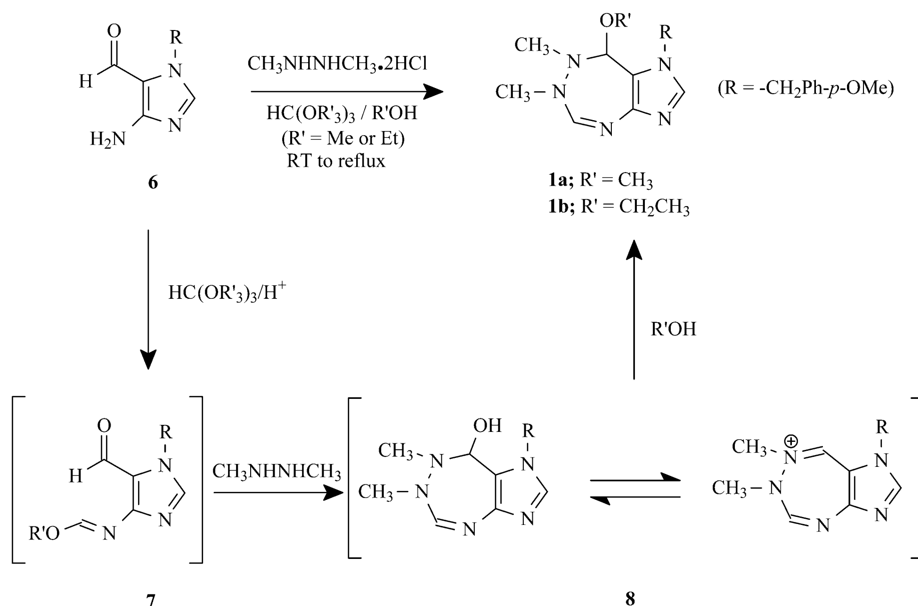


structure−activity relationship (SAR) studies,[34,35] which indicated that the complete removal of the sugar moiety of coformycins leads to total inactivity against ADA, while the substitution of a benzyl group in place of the sugar retains a good part of the original activity. We also speculated that an aralkyl group at position-1 may be preferred to position-3, as it would avoid the unfavorable hydrophobic interactions of the aromatic ring in a presumably hydrophilic sink, where the original sugar hydroxyls of coformycins lay in the active site of ADA. Furthermore, there exist several hydrophobic residues in the active site of ADA where the departing molecule of ammonia exits,[36] so the hydrophobic interactions of those residues with an aralkyl group of **1** should be favorable. (c) The somewhat weakened nucleophilicity of the respective 8-OH group by the electron-withdrawing inductive effect of an additional ring nitrogen atom inserted at position-7 is important. (d) The attachment of a methyl group at each of the positions 6 and 7 of the heterocyclic ring is consequential. This is because the 5:7-fused imidazotriazepine ring system of **1** is prone to facile opportunistic rearrangements to form a 5:6-fused system,[43−46] unless substituted at one or both of 6- and 7-positions of the triazepine ring. For the same reason, an analogue containing the parent 8-OH function (**1**, R = H), as in coformycins, was excluded from the target list, as such a compound would constitute the characteristics of a carbinolamine, which is prone to facile ring-opening to form an aldehyde derivative of an imidazole. We present here our SAR studies with eight analogues of coformycins, **a**−**h**, bearing the general structural features of **1**.

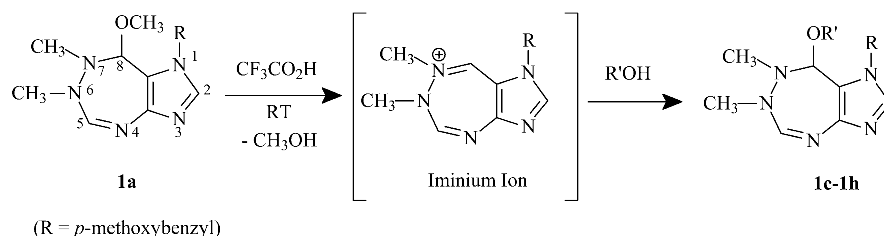
Results and Discussion

Chemistry. The common starting material for the target compounds is 4-amino-1-*p*-methoxybenzylimidazole-5-carbaldehyde (**6**) (Scheme 1), which was synthesized in four steps starting from the commercially available 4-nitro-1*H*-imidazole (**2**). The latter was condensed with *p*-methoxybenzyl chloride, catalyzed by potassium carbonate in dimethylformamide (DMF), to obtain 4-nitro-1-*p*-methoxybenzylimidazole (**3**) in >90% yield. The position of attachment of the *p*-methoxybenzyl group was confirmed by two-dimensional nuclear Overhauser and exchange spectroscopy (NOESY), which showed correlation between the methylene protons of the *p*-methoxybenzyl substituent and the proton at position-5. A dichloromethyl group was then introduced at position-5, employing the vicarious nucleophilic substitution procedure of Ostrowski.[47] Thus, the reaction of **3** with chloroform in the presence of potassium

Scheme 2



Scheme 3



tert-butoxide in DMF at < −60 °C afforded **4** in 53% yield. Compound **4** was hydrolyzed to the aldehyde **5** by reaction with aqueous calcium carbonate at 65−75 °C in 61% yield. The reduction of **5** to the desired **6** was accomplished by catalytic hydrogenation over 5% Pd on carbon in methanol in 34% yield. Although the use of ethyl acetate as the solvent medium for this reaction gave even lower yield than the one with methanol, the product was cleaner and did not require any chromatographic purification.

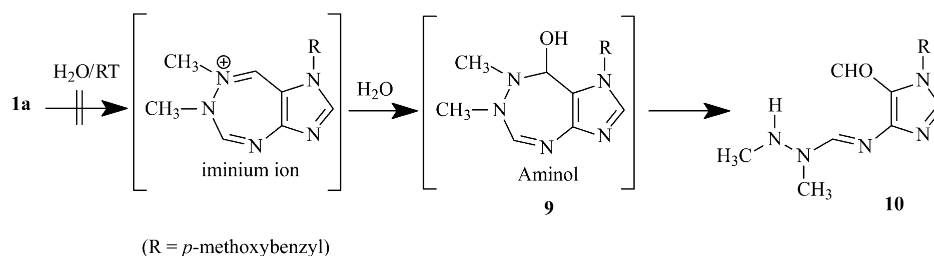
The target compounds **1a** and **1b** were synthesized in 62% and 30% yields, respectively, in a single-pot reaction (Scheme 2) of **6** with 1,2-dimethylhydrazine dihydrochloride and trimethyl or triethyl orthoformate in the corresponding alcohol used as a solvent. The reaction pathway to the products is believed to involve the initial formation of the imidate **7**, which upon ring-closure would form an equilibrium mixture containing the aminol−iminium species (**8**). The latter upon reaction with the alcohol will form the target imidazotriazepinols **1a** and **1b**. In corroboration of this mechanism is the isolation of **7** in a separate reaction of **6** with triethyl orthoformate at reflux, whether in the presence or absence of an acid catalyst.

The speculated intermediacy of aminol−iminium species **8** in the formation of the target **1a** and **1b** further suggested that the remaining targets **1c**−**1h** could simply be prepared through exchange reactions of **1a** or **1b** with the appropriate alcohols. This was indeed proven to be correct as compounds **1c**−**1h** were all obtained (Scheme 3) by reactions of **1a** with *n*-propanol, *n*-butanol, 2-propanol, 2-methoxyethanol, 4-methoxybenzyl alcohol, and 3-methylbenzyl alcohol, respectively, under catalysis by trifluoroacetic acid at room temperature, in yields of 32%−77%.

The above exchange reactions could also be carried out without the acid catalysis by simply heating the mixture of **1a** in the appropriate alcohol at reflux temperature. This observation, however, raised some concern about the stability of the final products in an aqueous medium that is to be employed for the intended ADA inhibition studies. The iminium ion intermediate, if formed at room temperature even without the catalysis, could produce an aminol (**9**) (Scheme 4) upon reaction with water and would subsequently ring-open to produce an aldehyde (**10**). We were relieved to find out that such a transformation does not occur without the acid catalysis, as shown in Scheme 3, or without heating at high temperature. The ^{1}H NMR spectrum of **1a** in a 4:1 mixture of D_2O−DMSO-d_6 exhibited no change, even after standing at room temperature for more than 20 h.

The target compounds **1a**−**1h** are somewhat unstable, especially when the trace solvent molecules that tend to adhere to the compounds are completely stripped off by elaborate drying. However, the compounds are reasonably stable as a solution in dimethyl sulfoxide and/or ethanol or when allowed to retain residual amounts of these solvents during the purification process. Therefore, satisfactory microanalytical data for these compounds could only be obtained by accounting for the adventitious solvent molecules. This observation is also consistent with the reported instability and elemental microanalytical data of the aglycon of

Scheme 4



(R = *p*-methoxybenzyl)

Table 1. Inhibitory Activities of Target Compounds **1a**–**1h** against Calf Spleen Adenosine Deaminase[a]

compd	K_i (μM)	compd	K_i (μM)
1a	24 ± 0.5	**1e**	30 ± 5.5
1b	20 ± 0.6	**1f**	23 ± 5.4
1c	12 ± 5.8	**1g**	77 ± 24.3
1d	15 ± 1.0	**1h**	93 ± 30.6

[a] K_i values were computed from Lineweaver–Burk plots.

pentostatin, which carried an adventitious molecule of DMSO. In any case, the ^{1}H NMR spectra, coupled with the high-resolution mass spectral data, leave little doubt about the accuracy of the molecular structures of **1a**–**1h**. An interesting feature of the ^{1}H NMR spectra of the target compounds is the resolution of the two methylene protons of the *p*-methoxybenzyl groups into two distinct doublets, separated by as much as 0.14 ppm, with a large (~15 Hz) coupling constant. This appears to due to the restricted rotation of the methylene protons, imposed by steric interactions with the *peri*-alkoxy group at position-8. Our molecular modeling studies (Insight/Discover)[48] revealed that the alkoxy oxygen atom of **1** lies within 2.5 Å of the methylene protons of the aralkyl group. The restricted rotation could also be discerned in the two methylene protons of the 8-alkoxy group of compounds **1b**, **1c**, and **1f**–**1h**, as they appeared as two distinct, broad multiplets.

Biochemistry. Compounds **1a**–**1h** were screened for inhibition of ADA from calf spleen (Sigma) in a 50 mM phosphate buffer (pH 7.0) at 25 °C by spectrophotometrically monitoring the rate of hydrolysis of the substrate adenosine into product inosine at 265 nm. The K_i's were computed from Lineweaver–Burk plots (see Figure 2 of the Supporting Information) and are listed in Table 1. All of the target compounds were determined to be competitive inhibitors of ADA with K_i's in the range ~10–100 μM. The *n*-propyl (**1c**) and *n*-butyl (**1d**) analogues were found to be the most potent compounds among the group.

Conclusions

The observed K_i's of **1a**–**1h** suggest that they bind about 6– 9 orders of magnitude less tightly to ADA than do coformycin ($K_i = 10^{-11}$)[15] and deoxycoformycin ($K_i = 10^{-12}$).[15] The considerably weaker inhibition of **1a**–**1h** compared to coformycins is consistent with the loss of the sugar hydroxyl–protein hydrogen bonds, which are partly responsible for the extremely tight enzyme binding of compounds of general formula **1**. In the homologous series **1a**–**1d**, the inhibition seems to correlate with the hydrophobicity of the alkyl group (note: although compound **1c** appears to deviate slightly from the trend, the larger error bar associated with its K_i as compared to **1d** will make this correlation still consistent). The increasing inhibitory activity in going from **1a** to **1d** may well be due to the parallel increase in electron-donating inductive effect of the alkyl group, which can render the 8-OR moiety increasingly electron-rich and, in turn, strengthen the 8-OR-mediated coordination bond between the heterocycle and the active site zinc of the protein. However, it is also possible that the increased steric bulk associated with the increasing inductive effect of the alkyl homologues will offset some of the gain made in bond strength by electronic factors. This is evident in the increased K_i of compounds **1e**, **1g**, and **1h**, where steric interference appears to suppress the electronic effect. In the case of **1g** and **1h**, the bad steric interactions with the *p*-methoxybenzyl group at the peri position may further distort and weaken the zinc coordination. Finally, the replacement of a terminal methylene group of **1d** with an electron-withdrawing oxygen atom, as in **1f**, results in a slight increase of K_i, as anticipated.

Compounds **1a**–**1h** contain a chiral center at position 8, so each can exist in two stereoisomeric forms, *R* and *S*. In view of the fact that the *R* isomer of coformycin is considerably more potent than the *S*,[15] it is similarly likely that one of the two isomers in each of the racemic final products reported herein would be more potent than the other. Nevertheless, no further attempts were made to separate the two enantiomers from the racemic mixture, as the observed inhibition was only modest, coupled with the fact that the thrust of the present study was rather to explore the molecular basis of tight-binding interactions of coformycin with ADA than to discover an ideal inhibitor of the enzyme, per se.

The target compounds of this study contained a hydrophobic substituent at the N-1 position of **1**. It would be interesting to explore how the hydrophilic groups attached to either the N-1 or the N-3 atom of **1** would affect the overall enzyme binding and inhibition. The synthesis and screening of a variety of additional analogues of **1** are anticipated to shed further light on the SAR of coformycin, and such an endeavor is currently in progress in our laboratory.

Experimental Section

(A) Organic Synthesis. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR were obtained on a 300-MHz instrument equipped with the NMR software Aquerius by Techmag. Chemical shifts are reported downfield from DMSO-d_6. Spectral data are reported as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, dt = doublet of triplets, q = quartet, m = multiplet, and b = broad. Thin-layer chromatography (TLC) was carried out on Kieselgel silica gel 60 F_{254} plates. Column chromatography was performed using 32–63 mesh silica gel. Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia. Mass

spectra were recorded at the Mass Spectral Facility, Department of Biochemistry, Michigan State University or the University of Maryland, College Park. Anhydrous solvents DMF and acetonitrile were purchased and used as is. All alcohols and THF were dried over sodium metal.

1-*p*-Methoxybenzyl-4-nitroimidazole (3). A suspension of 4-nitroimidazole (14.9 g, 131.8 mmol) and potassium carbonate (21.9 g, 158.4 mmol) in 80 mL of anhydrous DMF was stirred for 5 min, and it became creamy yellow. To the reaction mixture was added *p*-methoxybenzyl chloride (21.6 mL, 159.3 mmol), followed by 20 mL of anhydrous DMF. The mixture was stirred at room temperature overnight. The creamy yellow reaction mixture was rotary evaporated under high vacuum at 50 °C to remove DMF. Ice water was then added to the reaction mixture and left at room temperature for 1 h to dissolve the potassium carbonate. The crude product was suction filtered and washed successively with water and methanol to obtain **3** as a white crystalline solid (28.9 g, 94%): mp 132−135 °C; ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1H, H-5), 7.98 (s, 1H, H-2), 7.37 (d, 2H, J = 9.0 Hz, Ph-H), 6.92 (d, 2H, J = 8.4 Hz, Ph-H), 5.22 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 159.2 (C-4), 147.0 (C-5), 136.8 (C-2), 129.4 (Ph), 127.8 (Ph), 120.8 (Ph), 114.2 (Ph), 54.9 (CH_2 of benzyl), 50.1 (OCH_3); MS (FAB) m/z 234 (MH^+). Anal. C, H, N.

5-Dichloromethyl-1-*p*-methoxybenzyl-4-nitroimidazole (4). To a 250-mL round-bottom flask was added *t*-BuOK (6 g, 53.5 mmol) and a mixture of dry THF−DMF (2:1, 45 mL). The reaction mixture was stirred vigorously under N_2 at −100 °C (dry ice/ether). In a separate flask, 1-*p*-methoxybenzyl-4-nitroimidazole (**3**) (3 g, 12.9 mmol) was dissolved in dry chloroform (1.2 mL, 14.9 mmol), and the solution was added to 10 mL of anhydrous DMF. This solution was then added to the 250-mL round-bottom flask during a period of 10 min. The reaction mixture was stirred for 10 min and then an additional 0.5 mL (6.3 mmol) of dry chloroform was added. The reaction mixture continued to stir for an additional 10 min at −100 °C and was quenched with a solution of glacial acetic acid (5 mL) in 5 mL of methanol, when it turned reddish brown. To the acidified mixture, 100 mL of ice water was added. The mixture was extracted with dichloromethane (25 mL × 3), the organic layers were combined, washed twice with water, and dried over anhydrous magnesium sulfate. After filtration, the dichloromethane was removed in low vacuum at 40 °C and the DMF was removed under high vacuum at 75 °C. The crude product was purified using silica gel column chromatography [eluent: *n*-heptane to a mixture of *n*-heptane-chloroform (1:1) to chloroform]. The desired fractions were pooled and the solvent was removed. Addition of a minimal amount of ethyl acetate followed by the addition of hexane precipatated **4** as white crystals (2.15 g, 53%): mp 97−100 °C; ^{1}H NMR (DMSO-d_6) δ 8.01 (s, 1H, $CHCl_2$), δ 7.96 (s, 1H, H-2), 7.27 (d, 2H, J = 8.4 Hz, Ph-H), 6.95 (d, 2H, J = 8.4 Hz, Ph-H), 5.56 (s, 2H, CH_2), 3.73 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 159.1 (C-4), 142.0 (C-5), 138.3 (C-2), 129.0 (Ph-C), 126.7 (Ph-C), 126.4 (Ph-C), 114.0 (Ph-C), 58.5 ($CHCl_2$), 54.9 (CH_2 of benzyl), 49.3 (OCH_3); MS (EI) m/z 315, 317 (M^+). Anal. C, H, N, Cl.

1-*p*-Methoxybenzyl-4-nitroimidazole-5-carbaldehyde (5). In a three-neck round-bottom flask, crushed calcium carbonate (6 g, 60 mmol) was stirred in 80−100 mL of water for 30 min at 65−75 °C. To the flask was added **4** (6 g, 18.9 mmol), and the heterogeneous reaction mixture was stirred for 40−48 h at 65−75 °C until no further starting material was present as shown by TLC [chloroform/methanol (50:1)]. After cooling, the calcium carbonate was filtered and washed with a minimum amount of acetone, the filtrate was extracted with dichloromethane (3 × 25 mL), and the combined organic extracts were washed with 25 mL of water. After silica gel chromatography (eluent 1:1 → 2:1 chloroform/heptane), the desired fractions were pooled and rotary evaporated. Addition of ethyl acetate to the residue, followed by hexane, yielded **5** as yellow crystals (3 g, 61%). Compound **5** is light sensitive and discolors in daylight; therefore, it needs to be stored in the dark: mp 87−90 °C; ^{1}H NMR (DMSO-d_6) δ 10.22 (s, 1H, CHO), 8.34 (s, 1H, H-2), 7.23 (d, 2H, J = 8.4 Hz, Ph-H), 6.89 (d, 2H, J = 8.7 Hz, Ph-H), 5.50 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 181.4 (CHO), 159.1 (C-4), 150.4 (C-5), 140.1 (C-2), 129.0 (Ph-C), 127.5 (Ph-C), 124.6 (Ph-C), 114.0 (Ph-C), 55.1 (CH_2 of benzyl), 49.9 (OCH_3); MS (EI) m/z 261 (M^+). Anal. C, H, N.

4-Amino-1-*p*-methoxybenzylimidazole-5-carbaldehyde (6). Method A. In a Parr hydrogenation bottle, **5** (1 g, 3.8 mmol) was dissolved in 50−75 mL of methanol. Raney nickel (1 g, wet weight) that was washed several times with methanol was added. The bottle was sealed and hydrogenated at 45 psi at room temperature for 3 h. The catalyst was filtered using Celite and washed with methanol. The filtrate was evaporated, and the residue was purified by silica gel flash chromatography, using a mixture of chloroform−acetone (10:1) as an eluting solvent system. The appropriate fractions were pooled and evaporated to obtain **6** as a white solid (0.3 g, 34%): mp 153−161 °C (dec); ^{1}H NMR (DMSO-d_6) δ 9.42 (s, 1H, CHO), 7.70 (s, 1H, H-2), 7.17 (b, 2H, Ph-H), 6.88 (b, 2H, Ph-H), 6.37 (s, 1H, NH), 5.21 (s, 2H, CH_2), 3.71 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ 174.5 (CHO), 158.6 and 141.5 (C-2 + C-4 + C-5), 128.3 (Ph-C), 113.8 (Ph-C), 54.9 (CH_2 of benzyl), 47.8 (OCH_3). Anal. C, H, N.

Method B. In a 250-mL round-bottom flask, **5** (1 g, 3.8 mmol) was dissolved in 50−75 mL of ethyl acetate, and 5% Pd/C (0.45 g) was added to the flask. The flask was sealed with a rubber septum and evacuated using a needle attached to a hose and connected to the water aspirator. Then a balloon filled with hydrogen gas was attached to the septum using a needle. The reaction was hydrogenated at 1 atm at room temperature while being stirred for 6 h. The reaction mixture was then filtered using Celite and the catalyst was washed with ethyl acetate several times. Rotary evaporation of the filtrate produced a pure light-yellow compound (0.19 g, 22%). The ^{1}H NMR of this compound was identical to that of **6** obtained by method A above.

7,8-Dihydro-8-methoxy-1-*p*-methoxybenzyl-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1a). In a dry round-bottom flask were added consecutively compound **6** (0.08 g, 0.35 mmol), 1,2-dimethylhydrazine dihydrochloride (0.08 g, 0.60 mmol), dry methanol (10−15 mL), and anhydrous trimethyl orthoformate (0.5 mL) (Aldrich). The flask was stirred at room temperature for 1.5 h and then refluxed for 7−8 h until all the starting material disappeared, as observed by TLC [chloroform/methanol (10:1)]. The reaction flask was allowed to cool, then triethylamine (0.3 mL) was added, and the mixture was allowed to stir for 5 min at room temperature. A new spot moving slower than the starting material was observed by TLC [chloroform/methanol (10:1)]. The contents of the flask were rotary evaporated (not to complete dryness) and a minimal amount of chloroform was added. The chloroform mixture was loaded on a silica gel column [eluent: chloroform → chloroform/methanol (10:1)]. The desired fractions were pooled and evaporated to obtain **1a** as a thick brown oil (0.068 g, 62%): ^{1}H NMR (DMSO-d_6) δ 7.53 (s, 1H, H-2), 7.12 (d, 2H, J = 7.8 Hz, Ph-H), 6.98 (s, 1H, H-5), 6.89 (d, 2H, J = 8.1 Hz, Ph-H), 4.98 (m, 2H, CH_2), 4.88 (s, 1H, H-8), 3.71 (s, 6H, OCH_3), 3.30 (H_2O), 3.05 (s, 3H, N^6-CH_3), 2.04 (s, 3H, N^7-CH_3); HRMS (DEI) calcd for $C_{16}H_{21}N_5O_2$ (M^+) m/z 315.1695, found 315.1692. Anal. C, H, N.

7,8-Dihydro-8-ethoxy-1-*p*-methoxybenzyl-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1b). In a dry round-bottom flask were added consecutively compound **6** (0.08 g, 0.35 mmol), 1,2-dimethylhydrazine dihydrochloride (0.08 g, 0.60 mmol), dry ethanol (10−15 mL), and anhydrous triethyl orthoformate (0.5−1.0 mL) (Lancaster). The rest of the procedure is the same as the one described above for **1a**. The desired fractions were pooled and evaporated to obtain **1b** as a thick brown oil (0.034 g, 30%): ^{1}H NMR (DMSO-d_6) δ 7.53 (s, 1H, H-2), 7.10 (d, 2H, J = 7.9 Hz, Ph-H), 6.98 (s, 1H, H-5), 6.88 (d, 2H, J = 8.1 Hz, Ph-H), 5.03 (m, 2H, CH_2), 4.96 (s, 1H, H-8), 3.71 (s, 3H, OCH_3), 3.80 (m, 1H, OCH_2), 3.48 (m, 1H, OCH_2), 3.03 (s, 3H, N^6-CH_3), 2.03 (s, 3H, N^7-CH_3), 1.04 (t, 3H, CH_3, J = 8 Hz, ether CH_3); HRMS (DEI) calcd for $C_{17}H_{23}N_5O_2$ (M^+) m/z 329.1852, found 329.1844. Anal. C, H, N.

7,8-Dihydro-1-*p*-methoxybenzyl-6H-6,7-dimethyl-8-propoxyimidazo[4,5-e][1,2,4]triazepine (1c). Compound **1a** (0.045 g, 0.14 mmol) was dissolved in excess dry *n*-propanol (10−20 mL) in a dry round-bottom flask, and a catalytic amount of dry trifluoroacetic acid (5 μL) was added to the solution. The reaction mixture was stirred at room temperature for 5 days. It was then rotary evaporated (not to complete dryness), and the residue was purified by flash silica gel chromatography [eluent: chloroform → chloroform-methanol (10:1)]. The desired fractions were pooled and evaporated to obtain **1c** as a thick brown oil (0.018 g, 37%): ^{1}H NMR (DMSO-d_6) δ 7.51 (s, 1H, H-2), 7.08 (d, 2H, J = 9 Hz, Ph-H), 6.98 (s, 1H, H-5), 6.89 (d, 2H, J = 9 Hz, Ph-H), 5.03 (m, 2H, CH_2), 4.97 (s, 1H, H-8), 3.76 (m, 1H, OCH_2), 3.62 (m, 1H, OCH_2), 3.71 (s, 3H, OCH_3), 3.04 (s, 3H, N^6-CH_3), 2.04 (s, 3H, N^7-CH_3), 1.44 (q, 2H, J = 9 Hz, CH_2), 0.82 (t, 3H, J = 8 Hz, CH_3); HRMS (FAB) calcd for $C_{18}H_{26}N_5O_2$ (MH^+) m/z 344.2087, found 344.2089. Anal. C, H, N.

8-Butoxy-7,8-dihydro-1-*p*-methoxybenzyl-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1d). Compound **1a** (0.045 g, 0.14 mmol) was dissolved in excess dry butanol (10−20 mL), and a catalytic amount of dry trifluoroacetic acid (5 μL) was added to the solution. The rest of the procedure is the same as the one described above for **1c**. The desired fractions were pooled and evaporated to obtain **1d** as a thick brown oil (0.022 g, 43%): ^{1}H NMR (DMSO-d_6) δ 7.51 (s, 1H, H-2), 7.06 (d, 2H, J = 9 Hz, Ph-H), 6.98 (s, 1H, H-5), 6.88 (d, 2H, J = 9 Hz, Ph-H), 5.02 (m, 2H, CH_2), 4.97 (s, 1H, H-8), 3.78 (m, 1H, OCH_2), 3.64 (m, 1H, OCH_2), 3.71 (s, 3H, OCH_3), 3.37 (H_2O), 3.03 (s, 3H, N^6-CH_3), 2.04 (s, 3H, N^7-CH_3), 1.33 (m, 4H, CH_2), 0.85 (t, 3H, J = 8 Hz, CH_3); HRMS (DEI) calcd for $C_{19}H_{27}N_5O_2$ (M^+) m/z 357.2165, found m/z 357.2174. Anal. C, H, N.

7,8-Dihydro-8-isopropoxy-1-*p*-methoxybenzyl-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1e). Compound **1a** (0.045 g, 0.14 mmol) was dissolved in excess dry 2-propanol (10−20 mL), and a catalytic amount of dry trifluoroacetic acid (5 μL) was added to the solution. The rest of the procedure is the same as the one described above for **1c**. The desired fractions were pooled and evaporated to obtain **1e** as a thick brown oil (0.038 g, 77%): ^{1}H NMR (DMSO-d_6) δ 7.46 (s, 1H, H-2), 7.01 (d, 2H, J = 9 Hz, Ph-H), 6.99 (s, 1H, H-5), 6.90 (d, 2H, J = 9 Hz, Ph-H), 5.02 (m, 2H, CH_2), 5.06 (m, 1H from H-8, 1H from CH), 3.71 (s, 3H, OCH_3), 3.3 (H_2O), 3.04 (s, 3H, N^6-CH_3), 2.05 (s, 3H, N^7-CH_3), 1.08 (t, 6H, J = 8 Hz, CH_3); HRMS (FAB) calcd for $C_{18}H_{26}N_5O_2$ (MH^+) m/z 344.2087, found 344.2093. Anal. C, H, N.

7,8-Dihydro-1-*p*-methoxybenzyl-8-(2-methoxyethoxy)-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1f). Compound **1a** (0.045 g, 0.14 mmol) was dissolved in excess dry 2-methoxyethanol (10−20 mL), and a catalytic amount of dry trifluoroacetic acid (5 μL) was added to the solution. The rest of the procedure is the same as the one described above for **1c**. The desired fractions were pooled and evaporated to obtain **1f** as a thick brown oil (0.024 g, 47%): ^{1}H NMR (DMSO-d_6) δ 7.52 (s, 1H, H-2),7.10 (d, 2H, J = 9 Hz, Ph-H), 6.98 (s, 1H, H-5), 6.88 (d, 2H, J = 9 Hz, Ph-H), 5.05 (m, 2H, CH_2), 5.03 (s, 1H, H-8), 3.83 (m, 2H, OCH_2), 3.65 (m, 2H, OCH_2), 3.71 (s, 3H, OCH_3), 3.20 (s, 3H, OCH_3), 3.04 (s, 3H, N^6-CH_3), 2.04 (s, 3H, N^7-CH_3); HRMS (FAB) calcd for $C_{18}H_{26}N_5O_3$ (MH^+) m/z 360.2036, found 360.2039. Anal. C, H, N.

7,8-Dihydro-1-*p*-methoxybenzyl-8-(4-methoxybenzyloxy)-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1g). Compound **1a** (0.08 g, 0.25 mmol) was dissolved in excess 4-methoxybenzyl alcohol (10 mL), and a catalytic amount of dry trifluoroacetic acid (3 μL) was added to the solution. The rest of the procedure is the same as the one described above for **1c**. The purification needed repeated column chromatography. The desired fractions were pooled and evaporated to obtain **1g** as a thick brown oil (0.044 g, 41%): ^{1}H NMR (DMSO-d_6) δ 7.53 (s, 1H, H-2), 7.18 (m, 4H, Ph-H), 7.00 (s, 1H, H-5), 6.83 (m, 4H, Ph-H), 5.04 (m, 2H from CH_2, 1H from H-8), 4.74 (m, 1H, OCH_2), 4.46 (m, 1H, OCH_2), 3.71 (b, 6H, OCH_3), 3.3 (H_2O), 3.09 (s, 3H, N^6-CH_3), 2.05 (s, 3H, N^7-CH_3); HRMS (FAB) calcd for $C_{23}H_{28}N_5O_3$ (MH^+) m/z 422.2192, found 422.2174. Anal. C, H, N.

7,8-Dihydro-1-*p*-methoxybenzyl-8-(3-methylbenzyloxy)-6H-6,7-dimethylimidazo[4,5-e][1,2,4]triazepine (1h). Compound **1a** (0.075 g, 0.24 mmol) was dissolved in excess 3-methylbenzyl alcohol (10 mL), and a catalytic amount of dry trifluoroacetic acid (3 μL) was added to the solution. The reaction mixture was stirred at room temperature for 3−5 days until TLC showed the complete disappearance of the starting material. The rest of the procedure is the same as the one described above for **1c**. The purification needed repeated column chromatography. The desired fractions were pooled and evaporated to obtain **1h** as a thick brown oil (0.031 g, 32%): ^{1}H NMR (DMSO-d_6) δ 7.54 (s, 1H, H-2), 6.78−7.21 (m, H-5 and Ph-H), 5.07 (m, 2H from CH_2, 1H from H-8), 4.49 (m, 1H, OCH_2), 4.79 (m, 1H, OCH_2), 3.68 (s, 3H, OCH_3), 3.3 (H_2O), 3.08 (s, 3H, N^6-CH_3), 2.26 (d, 3H, J = 9 Hz, *m*-CH_3), 2.07 (s, 3H, N^7-CH_3); HRMS (FAB) calcd for $C_{23}H_{28}N_5O_2$ (MH^+) m/z 406.2243, found 406.2250. Anal. C, H, N.

4-(Ethoxymethylene)imino-1-*p*-methoxybenzylimidazole-5-carbaldehyde (7). To a solution of compound **6** (0.1 g, 0.43 mmol) in 10 mL of dry THF was added anhydrous triethyl orthoformate (2 mL), and the reaction mixture was refluxed until all the starting material was consumed (usually overnight). A new spot moving faster than the starting material was formed, as observed by TLC. The reaction mixture was rotary evaporated in high vacuum to remove excess triethyl orthoformate. The solid residue obtained was redissolved in dry THF and rotary evaporated again to obtain **7** as a crystalline solid (0.11 g, 89%): mp 105−106 °C; ^{1}H NMR (DMSO-d_6) δ 9.73 (s, 1H, CHO), 8.53 (s, 1H, H-5), 8.05 (s, 1H, H-2), 7.21 (d, 2H, J = 9 Hz, Ph), 6.87 (d, 2H, J = 9 Hz, Ph), 5.36 (s, 2H, CH_2), 4.28 (q, 2H, OCH_2), 3.69 (s, 3H, OCH_3), 1.27 (t, 3H, J = 9 Hz, ether CH_3); MS (EI) m/z 287 (M^+). Anal. C, H, N.

(B) Inhibition Studies. The target compounds **(1a−1h)** were screened against calf spleen ADA (Sigma) in vitro in a 50 mM phosphate buffer (pH 7.0) at 25 °C. The rate of hydrolysis of adenosine into inosine was monitored spectrophotometrically by following the absorbance decrease over 2 min at 265 nm using a Varian Cary UV−visible spectrophotometer (50-BIO). All absorbances were maintained below 1 absorbance unit in a 1 cm path length cuvette. The enzyme and substrate stock solutions were prepared using a 50 mM phosphate buffer (pH 7.0). While holding the enzyme and inhibitor concentration constant, the substrate concentration was varied to obtain the kinetic data. The substrate concentration in each assay was measured using up to five different concentrations, ranging from 20 to 60 μM. This was repeated with a different inhibitor concentration. The inhibitor concentration ranged from 8 to 15 μM. The enzyme concentration (0.022 units/mL) was kept constant in all assays. Lineweaver−Burk ($1/V$ vs 1/[S]) plots were constructed for each assay and the K_i was calculated using Graph Pad Prism Software. In each assay, the inhibitors were used without preincubation with enzyme. The substrate and inhibitor were added to the phosphate buffer (50 mM, pH 7.0), followed by the addition of enzyme. The enzyme solution was kept in an ice bath prior to performing each assay. Assays were performed in duplicate and in some cases triplicate.

Acknowledgment. This research was supported by a grant (#9 RO1 AI55452) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health. The authors thank Mrs. Dalia Tadros for assistance in biochemical studies and Dr. Diana S. Hamilton for advice on enzyme kinetics.

Supporting Information Available: Lineweaver−Burk plots used for computation of K_i's of target compounds **1a−1h** (Figure 2).

References

(1) Cristalli, G.; Costanzi, S.; Lambertucci, C.; Lupidi, G.; Vittori, S.; Volpini, R.; Camaioni, E. Adenosine deaminase: Functional implications and different classes of inhibitors. *Med. Res. Rev.* **2001**, *21*, 105–128.
(2) Aiuti, A. Advances in gene therapy for ADA-deficient SCID. *Curr. Opin. Mol. Therapeut.* **2002**, *4*, 515–522.
(3) Lum, C. T.; Sutherland, D. E.; Najarian, J. R. Inhibition for immunosuppression. *New Eng. J. Med.* **1977**, *296*, 819.
(4) Chassin, M. M.; Chirigos, M. A.; Johns, D. G.; Adamson, R. H. Adenosine deaminase inhibition for immunosuppression. *New Eng. J. Med.* **1977**, *296*, 1232.
(5) Ohno, M.; Yagisawa, N.; Shibahara, S.; Kondo, S.; Maeda, K.; Umezawa, H. Synthesis of coformycin. *J. Am. Chem. Soc.* **1974**, *96*, 4326–4327.
(6) Nakamura, H.; Koyama, G.; Iitaka, Y.; Ohno, M.; Yagisawa, N.; Kondo, S.; Maeda, K.; Umezawa, H. Structure of coformycin, an unusual nucleoside of microbial origin. *J. Am. Chem. Soc.* **1974**, *96*, 4327–4328.
(7) Nakamura, H.; Koyama, G.; Umezawa, H.; Iitaka, Y. The crystal and molecular structure of coformycin. *Acta Crystallogr.* **1976**, *B32*, 1206–1212.
(8) Hawkins, L. D.; Hanvey, J. C.; Boyd, F. L., Jr.; Baker, D. C.; Showalter, H. D. H. Inhibitors of adenosine deaminase. Synthesis of coformycin and 3′-deoxycoformycin. *Nucleosides Nucleotides* **1983**, *2*, 479–494.
(9) Woo, P. W. K.; Dion, H. W.; Lange, S. M.; Dahl, L. F.; Durham, L. J. A novel adenosine and ara-A deaminase Inhibitor, (*R*)-3-(2-deoxy-*β*-D-erythropentofuranosyl)-3,6,7,8-tetrahydroimidazo-[4,5-*d*]diazepin-8-ol. *J. Heterocycl. Chem.* **1974**, *11*, 641–643.
(10) Baker, D. C.; Putt, S. R. A total synthesis of pentostatin, the potent inhibitor of adenosine deaminase. *J. Am. Chem. Soc.* **1979**, *101*, 6127–6128.
(11) Baker, D. C.; Putt, S. R.; Showalter, H. D. H. Studies related to the total synthesis of pentostatin. Approaches to the synthesis of (8*R*)-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol and N-3 alkyl congeners. *J. Heterocycl. Chem.* **1983**, *20*, 629–634.
(12) Chan, E.; Putt, S. R.; Showalter, H. D. H.; Baker, D. C. Total synthesis of (8*R*)-3-(2-deoxy-*β*-D-erythropentofuranosyl-3,6,7,8-tetrahydroimidazo[4,5-*d*][1,3]diazepin-8-ol (pentostatin), the potent inhibitor of adenosine deaminase. *J. Org. Chem.* **1982**, *47*, 3457–3464.
(13) Hanvey, J. C.; Hardman, J. K.; Suhadolnik, R. J.; Baker, D. C. Evidence for the conversion of adenosine to 2′-deoxycoformycin by streptomyces antibiotics. *Biochemistry* **1984**, *23*, 904–907.
(14) Hanvey, J. C.; Hawkins, E. S.; Tunac, J. B.; Dechter, J. J.; Baker, D. C.; Suhadolnik, R. J. Biosynthesis of 2′-deoxycoformycin: Evidence for ring-expansion of the adenine moiety of adenosine to a tetrahydroimidazo[4,5-*d*][1,3]diazepine system. *Biochemistry* **1987**, *26*, 5636–5641.
(15) Agarwal, R. P.; Cha, S.; Crabtree, G. W.; Parks, R. E. Coformycin and deoxycoformycin: Tight-binding inhibitors of adenosine deaminase. *Chemistry and Biology of Nucleosides and Nucleotides*; Academic Press: New York, 1978; pp 159–197.
(16) Wolfenden, R.; Wentworth, D. F.; Mitchell, G. N. Influence of substituent ribose on transition state affinity in reactions catalyzed by adenosine deaminase. *Biochemistry* **1977**, *16*, 5071–5077.
(17) Frick, L.; Wolfenden, R.; Smal, E.; Baker, D. C. Transition-state stabilization by adenosine deaminase: Structural studies of its inhibitory complex with deoxycoformycin. *Biochemistry* **1986**, *25*, 1616–1621.
(18) Kati, W. M.; Wolfenden, R. Contribution of a single hydroxyl group to transition-state discrimination by adenosine deaminase: Evidence for an “entropy trap” mechanism. *Biochemistry* **1989**, *28*, 7919–7927.
(19) Kurz, L. C.; Moix, L.; Riley, M. C.; Frieden, C. The rate of formation of transition-state analogues in the active site of adenosine deaminase is encounter-controlled: Implications for the mechanism. *Biochemistry* **1992**, *31*, 39–48.
(20) Agarwal, R. P.; Spector, T.; Parks, R. E., Jr. Tight-binding inhibitors. IV. Inhibition of adenosine deaminases by various inhibitors. *Biochem. Pharmacol.* **1977**, *26*, 359–367.
(21) Smyth, J. F.; Harrap, K. R. Adenosine deaminase activity in leukemia. *Br. J. Cancer* **1975**, *31*, 544–549.
(22) Smyth, J. F.; Poplack, D. G.; Holiman, B. J.; Leventhal, B. G.; Yarbro, G. Correlation of adenosine deaminase activity with cell surface markers in acute lymphoblastic leukemia. *J. Clin. Invest.* **1978**, *62*, 710–712.
(23) Smyth, J. F.; Prentice, H. G.; Proctor, S.; Hoffbrand, A. V. Deoxycoformycin in the treatment of leukemias and lymphomas. *Ann. N. Y. Acad. Sci.* **1985**, *451*, 123–128.
(24) LePage, G. A.; Worth, L. S.; Kimball, A. P. Enhancement of the antitumor activity of arabinofuranosyladenine by 2′-deoxycoformycin. *Cancer Res.* **1976**, *36*, 1481–1485.
(25) Umezawa, H.; Tsutomu, S.; Yasuo, F.; Ikuyo, H.; Masaaki, I.; Tomio, T. Formycin and formycin B in cells of Ehrlich carcinoma and Escherichia *coli. J. Antibiot. (Tokyo), Ser. A.* **1967**, *20*, 308–316.
(26) Johns, D. G.; Adamson, R. H. Enhancement of the biological activity of cordycepin (3′-deoxyadenosine) by the adenosine deaminase inhibitor 2′-deoxycoformycin. *Biochem. Pharmacol.* **1976**, *25*, 1441–1444.
(27) Major, P. P.; Agarwal, R. P.; Kufe, D. W. Deoxycoformycin: Neurological toxicity. *Cancer Chemother. Pharmacol.* **1981**, *5*, 193–196.
(28) Major, P. P.; Agarwal, R. P.; Kufe, D. W. Clinical pharmacology of deoxycoformycin. *Blood* **1981**, *58*, 91–96.
(29) Grever, M. R.; Siaw, M. F.; Jacob, W. F.; Niedhart, J. A.; Miser, J. S.; Coleman, M. S.; Hutton, J. J.; Balcerzak, S. P. The biochemical and clinical consequences of 2′-deoxycoformycin in refractory lymphoproliferative malignancy. *Blood* **1981**, *57*, 406–417.
(30) Lambe, C.; Bugge, C. J. L.; Lafon, S. W.; Nelson, D. J.; Elion, G. B. *Fed. Proc. Am. Soc. Exp. Biol.* **1979**, *38*, 670.
(31) Acevedo, O. L.; Krawczyk, S. H.; Townsend, L. B. A novel approach towards the synthesis of the 3,4,5-trihydro-1,3-diazepin-5-ol ring structure. *Tetrahederon Lett.* **1983**, *24*, 4789–4792.
(32) Acevedo, O. L.; Krawczyk, S. H.; Townsend, L. B. Synthesis of the new ring system, pyrazolo[3,4-*d*][1,3]diazepines. *J. Heterocycl. Chem.* **1985**, *22*, 349–352.
(33) Acevedo, O. L.; Krawczyk, S. H.; Townsend, L. B. Total synthesis of (4*R*)- and (4*S*)-5,6-dihydro-1-*β*-D-ribofuranosyl-4*H*-pyrazolo-[3,4-*d*][1,3]diazepin-4-ol and (8*R*)- and (8*S*)-7,8-dihydro-3-*β*-D-ribofuranosyl-6*H*-v-triazolo[4,5-*d*][1,3]diazepin-8-ol: Two heterocyclic analogues of the nucleoside antibiotic coformycin. *J. Org. Chem.* **1986**, *51*, 1050–1058.
(34) Hong, M.; Hosmane, R. S. Irreversible, tight-binding inhibition of adenosine deaminase by coformycins: Inhibitor structural features that contribute to the mode of inhibition. *Nucleosides Nucleotides* **1997**, *16*, 1053–1057.
(35) Hosmane, R. S.; Hong, M. How important is the *N*-3 sugar moiety in the tight-binding interaction of coformycin with adenosine deaminase? *Biochem. Biophys. Res. Commun.* **1997**, *236*, 88–93.
(36) Wang, Z.; Quiocho, F. A. Complexes of adenosine deaminase with two potent inhibitors: X-ray structures in four Independent molecules at pH of maximum activity. *Biochemistry* **1998**, *37*, 8314–8324.
(37) Marrone, T. J.; Straatsma, T. P.; Briggs, J. M.; Wilson, D. K.; Quiocho, F. A.; McCammon, J. A. Theoretical study of inhibition of adenosine deaminase by (8R)-coformycin and (8R)-deoxycoformycin. *J. Med. Chem.* **1996**, *39*, 277–284.
(38) Melzig, M. F. Inhibition of adenosine deaminase activity of aortic endothelial cells by selected flavonoids. *Planta Medica* **1996**, *62*, 20–21.
(39) Koch, H. P.; Jager, W.; Groh, U.; Plank, G. In vitro inhibition of adenosine deaminase by flavonoids and related compounds. New insight into the mechanism of action of plant phenolics. *Methods Find. Exp. Clin. Pharmacol.* **1992**, *14*, 413–417.
(40) Koch, H. P.; Aichinger, A.; Bohne, B.; Plank, G. In vitro inhibition of adenosine deaminase by a group of steroid and triterpenoid compounds. *Phytother. Res.* **1994**, *8*, 109–111.
(41) Koch, H. P.; Jager, W.; Hysek, J.; Korpert, B. Garlic and onion extracts. In vitro inhibition of adenosine deaminase. *Phytother. Res.* **1992**, *6*, 50–52.
(42) Sheid, B. Trazodone, a nontricyclic antidepressant, is an inhibitor of adenosine deaminase. *Res. Commun. Chem. Pathol. Pharmacol.* **1985**, *47*, 149–152.
(43) Hosmane, R. S.; Lim, B. B. Chemistry of the 5:7-fused heteroaromatic systems: A novel rearrangement involving the imidazo-[4,5-*e*][1,3,4]triazepine and pyrazolo[3,4-*e*][1,3,4]triazepine systems. *Heterocycles* **1988**, *27*, 31–34.
(44) Hosmane, R. S.; Lim, B. B. A novel method for the synthesis of 6-(*β*-methyl)hydrazinopurine and 4-(*β*-methyl)hydrazinopyrazolo[3,4-*d*]pyrimidine. *Synthesis* **1988**, 242–244.
(45) Hosmane, R. S.; Lim, B. B.; Burnett, F. N. Rearrangements in heterocyclic synthesis: A novel translocation of an (*N*-amino-*N*-methyl)formamidine side-chain to the vinylogous nitrile function. *J. Org. Chem.* **1988**, *53*, 382–386.
(46) Hosmane, R. S.; Lim, B. B.; Summers, M. F.; Siriwardane, U.; Hosmane, N. S.; Chu, S. C. Rearrangements in heterocyclic synthesis. 2. Novel transformations of 2-aminonicotino- and anthranilonitriles. *J. Org. Chem.* **1988**, *53*, 5309–5315.
(47) Ostrowski, S. Synthesis of some new imidazole derivatives. *Polish J. Chem.* **1994**, *68*, 2237–2247.
(48) Molecular modeling studies were performed using Insight/DiscoverTM software package, available from Molecular Simulations, Inc., San Diego, California.

JM0304257