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A Unique Ring-Expanded Acyclic Nucleoside Analogue that Inhibits Both Adenosine Deaminase (ADA) and Guanine Deaminase (GDA; Guanase): Synthesis and Enzyme Inhibition Studies of 4,6-Diamino-8*H*-1-hydroxyethoxymethyl-8-iminoimidazo[4,5-*e*][1,3]diazepine

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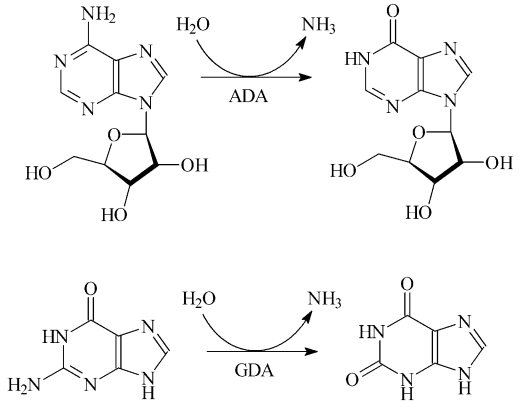
Dedicated to Professor Nelson J. Leonard on the occasion of his 85th birthday.

Abstract—The synthesis and enzyme inhibition studies of a novel ring-expanded acyclic nucleoside analogue **1** are reported. Compound **1** has been found to be a competitive inhibitor of both adenosine deaminase (ADA) and guanine deaminase (GDA; guanase) with K_i's equal to $1.52 \pm 0.34 \times 10^{-4}$ M and $2.97 \pm 0.25 \times 10^{-5}$ M, respectively. Inhibition of two enzymes of purine metabolism may bear beneficial implications in antiviral therapy.

Adenosine deaminase (ADA; EC 3.5.4.4)[1] and guanine deaminase (GDA; guanase; EC 3.3.4.3)[2] are two important enzymes of purine metabolism (Scheme 1), whose inhibition carries beneficial implications in antiviral therapy.[3,4] While the two enzymes are functionally closely related in that they both catalyze the hydrolysis of an amino group of a purine derivative, they are nevertheless farther apart in terms of the geometric and spatial requirements of the substrates which they can accommodate in their active sites. The substrate for ADA is adenosine, a nucleoside, whereas that for GDA is guanine, a heterocyclic base. Furthermore, the two amino groups are present at different locations of the purine ring: position 6 in adenosine and position 2 in guanine. The two enzymes also differ in their metabolic functions and pathways with which they are associated. While both ADA and GDA are catabolic enzymes, and are both part of the purine salvage metabolic pathway,[5] the principal function of ADA nevertheless appears to be related to the development of immune system and cell differentiation in humans.[6] GDA, on the other hand, is predominantly an enzyme of the purine salvage synthesis, especially in microorganisms.[5a]

Although it is neither clear nor proven if the metabolic pathways of the two functionally similar enzymes are also connected in any way, such a connection can, nevertheless, be invoked in principle. For example, there exists more than one metabolic pathway for the production of xanthosine-5′-monophosphate (XMP), a



Scheme 1.

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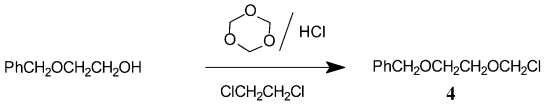
crucial intermediate in the de novo biosynthesis of guanosine-5′-monophosphate (GMP), a building block for nucleic acid synthesis.[7] GMP synthetase converts XMP to GMP via reaction with glutamine accompanied by hydrolysis of a molecule of ATP.[7] One of the pathways for the formation of XMP can be outlined as sequential reactions involving hydrolysis of adenosine to inosine by ADA,[1] phosphorylation of inosine by inosine kinase to produce inosine-5′-monophosphate (IMP),[8] and oxidation of IMP to XMP by IMP dehydrogenase.[9] An alternative pathway for XMP could consist of hydrolysis of guanine to xanthine, catalyzed by GDA, followed by phosphoribosylation of xanthine by hypoxanthine-guanine-xanthine phosphoribosyl transferase (HGXPRT).[10] Therefore, the production of GMP would be retarded by simultaneous inhibition of both ADA and GDA. Furthermore, since IMP is an important de novo biosynthetic intermediate for both adenine- and guanine-based nucleosides and nucleotides,[5] any decrease in IMP levels would have further deleterious consequences on nucleic acid synthesis as a whole.

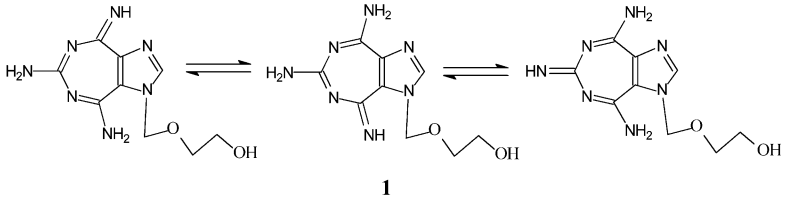
We report here the synthesis and enzyme inhibition studies of a novel ring-expanded purine analogue **1**,[11] (Scheme 2) which incorporates the structural features of both adenosine and guanine, and is found to inhibit both ADA and GDA. To the best of our knowledge, this is the first example of a purine analogue that simultaneously inhibits both of these enzymes of purine metabolism. In designing such a dual inhibition, we took into consideration that **1** would mimic both a nucleoside, a requirement for ADA, and a heterocyclic base, a necessity for GDA. An acyclic sugar moiety as in **1** was perceived to meet such a criterion. Secondly, **1** should also manifest the molecular traits of both adenine and guanine in its heterocyclic moiety. In that regard, because of its multiple amino/imino functionalities, **1** is theoretically capable of existing in several tautomeric forms in solution, and depending upon which particular tautomeric form it assumes, **1** can mimic adenine, guanine, 2,6-diaminopurine or isoguanine as shown in Scheme 2. Furthermore, in view of its structural similarity to these various natural purine derivatives, compound **1** and its nucleoside/nucleotide analogues are potentially a rich source of substrates or inhibitors of a number of enzymes of purine metabolism besides ADA and GDA, as well as of those enzymes that utilize energy co-factors such as ATP or GTP.

The target acyclic nucleoside **1** was synthesized (Scheme 3) from the corresponding triamino heterocyclic base **2** whose synthesis we have reported a few years ago.[12] Compound **2** was condensed with 1-benzyloxy-2-chloromethoxyethane (**4**).[13] The latter was prepared by saturation of a mixture of 2-benzyloxyethanol and 1,3,5-trioxane with dry HCl gas as shown in eq 1.[13] The Vorbrüggen glycosylation[14] was the method of choice for the condensation of **2** and **4**, which involved initial silylation of **2** with *N,O*-bis(trimethylsilyl)trifluoroacetamide, followed by reaction with **4** catalyzed by trimethylsilyl triflate. The target **1** was obtained in 86% yield as a crystalline triflate salt. The ^{1}H and ^{13}C NMR, elemental microanalyses, as well as mass spectral data are consistent with the assigned structure.[11]

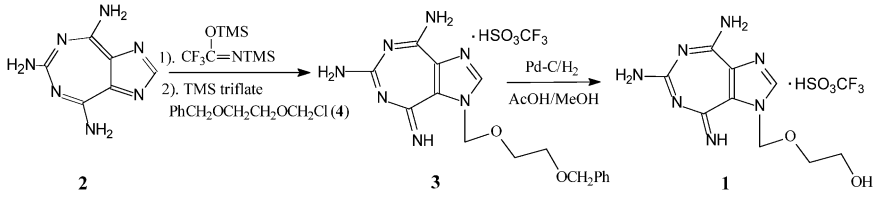


(1)

Inhibition of adenosine deaminase (ADA) by **1** was investigated in vitro against adenosine deaminase from calf intestinal mucosa in a 50 mM phosphate buffer (pH 7.4) at 25 °C, by spectrophotometrically monitoring the rate of hydrolysis of substrate adenosine at 265 nm. Stock solutions of the substrate, enzyme, and inhibitors were prepared using a 50 mM phosphate buffer (pH 7.4). The enzyme kinetics was followed by measuring the change in optical density (decrease in absorbance) per minute of the substrate adenosine. By keeping the concentration of the inhibitor constant, and by varying the substrate concentration, a set of kinetic data was obtained. Additional sets of data were generated using different concentrations of the inhibitor. The substrate concentration in each assay ranged between 20 and 40



Scheme 2.



Scheme 3.

μM; a total of six different concentrations were employed. The inhibitor concentration used in each assay was 46 or 92 μM. The amount of enzyme used in each assay was 0.0216 unit. The K_i for inhibition assays was computed from Lineweaver–Burk plots. The Lineweaver–Burk plot (1/V vs 1/[S]) as well as the linear regression analysis of kinetic data were performed using the program Quattro Pro (version 6.01) for Windows™. Compound **1** was found to be a competitive inhibitor of adenosine deaminase with a $K_i = 1.52 \pm 0.34 \times 10^{-4}$ M, as computed from the Lineweaver–Burk plots.

In order to study the enzyme–inhibitor binding mechanism as well to assess the ADA inhibition, the following experiments were conducted using **1**. Two experiments were designed according to Agarwal et al.:[15] (a) pre-incubation of the enzyme with the inhibitor; here the enzyme (ADA 10 μL) and inhibitor were preincubated for 10 min in a calculated amount of phosphate buffer (50 mM, pH 7.5) at 25 °C and the reaction was initiated by the addition of substrate, adenosine, and (b) without preincubation; here the reaction was started by addition of ADA (10 μL) to a mixture of adenosine and inhibitor in the phosphate buffer at 25 °C. When the inhibitor **1** was examined with and without preincubation with ADA, identical reaction velocities were observed over a 3 min incubation period. This indicated that compound **1** is a reversible inhibitor with a rapid establishment of equilibrium between E, ES, and EI. Therefore, it was possible to employ the initial velocities to determine both the inhibition mechanism and the K_i value, using the Lineweaver–Burk analyses.

Compound **1** was also assayed in vitro for inhibition of guanine deaminase (GDA; guanase) from rabbit liver in a 50 mM Tris–HCl buffer (pH 7.4) at 27 °C, by spectrophotometrically monitoring the rate of hydrolysis of substrate guanine at 243 nm. Stock solutions of the substrate, enzyme, and inhibitors were prepared using a 50 mM Tris–HCl buffer (pH 7.4). The enzyme kinetics was followed by measuring the change in optical density (decrease in absorbance) per minute of the substrate guanine. By keeping the concentration of the inhibitor constant, and by varying the substrate concentration, a set of kinetic data was obtained. Additional sets of data were generated using different concentrations of the inhibitor. The substrate concentration in each assay ranged between 6 and 20 μM; a total of six different concentrations were employed. The inhibitor concentration used in each assay was 30 or 40 μM. The amount of enzyme used in each assay was 0.0076 unit. The K_i for inhibition assays was computed from Lineweaver–Burk plots. The Lineweaver–Burk plot (1/V vs 1/[S]), as well as the linear regression analysis of kinetic data, were performed using the program Quattro Pro (version 6.01) for Windows.™ Compound **1** was found to be a competitive inhibitor of guanine deaminase (GDA; guanase) with a $K_i = 2.97 \pm 0.25 \times 10^{-5}$ M, as computed from the Lineweaver–Burk plots.

Analogous preincubation experiments as described for ADA above were performed with GDA in order to explore the mode of binding of the inhibitor to the enzyme. Once again, identical reaction velocities were obtained with or without preincubation, suggesting that **1** is a readily reversible inhibitor of GDA as well.

Conclusion

The target acyclic nucleoside **1** is a competitive inhibitor of both adenosine deaminase and guanase with K_i's equal to $1.52 \pm 0.34 \times 10^{-4}$ M and $2.97 \pm 0.25 \times 10^{-5}$ M, respectively. The heterocyclic ring of **1** can be regarded as a ring-expanded purine. The observed ADA inhibition is only modest with respect to some of the best known inhibitors of ADA, for example, 2′-deoxycoformycin (pentostatin), an irreversible, tight-binding inhibitor of ADA, with a K_i equal to $\sim 10^{-12}$ M,[16] or EHNA, a reversible inhibitor with a K_i of $\sim 10^{-9}$ M.[17] On the other hand, the observed inhibition against GDA is somewhat comparable in terms of the known inhibitors of GDA, most of which have K_i's in the 10^{-5} to 10^{-6} M range.[18] In any case, the fact that **1** is able to inhibit two different enzymes of purine metabolism that have different specificities for substrates, bears important implications in further development of antiviral agents based on dual enzyme inhibition strategy. Furthermore, since many different tautomeric structures can be drawn for its heterocyclic moiety, **1** is potentially capable of mimicking a wide variety of natural purines including adenine, guanine, isoguanine and 2,6-diaminopurine. This property of **1** may have further ramifications for inhibition of other enzymes of purine metabolism besides ADA and GDA. The readily reversible inhibition of both ADA and GDA is an added benefit to **1** since it is well known that the extremely tight-binding, totally irreversible inhibition of ADA is what leads to the clinical toxicity and failure of pentostatin as an antitumor drug.[19]

Acknowledgements

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11. **1-Benzyloxyethoxymethyl-4,6-diamino-8*H*-8-iminoimidazo[4,5-*e*][1,3]diazepine (3)**. A 100-mL three-neck round-bottom flask was flame-dried, and was fitted with a serum stopper, a magnetic stirring bar, and a N_2 gas inlet. It was charged with 4,6,8-triaminoimidazo[4,5-*e*][1,3]diazepine (**2**)[12] (0.53 g, 3.0 mmol). Bis(trimethylsilyl) trifluoroacetamide (4 mL, 15 mmol), dry pyridine (0.76 mL, 9.4 mmol), and acetonitrile (10 mL) were added, and the reaction mixture was stirred at room temperature overnight, during which time a nearly clear solution was formed. The solvent and excess reagents were evaporated at 30 °C (10 torr), and the residue was coevaporated with dry acetonitrile (2×10 mL). The residue was kept under vacuum until a syrup was formed. The flask containing the above syrup was fitted with a magnetic stirring bar, and was charged with 20 mL of acetonitrile, and the resulting solution was cooled to −45 °C. To the stirred solution was added 1-benzyloxy-2-chloromethoxyethane (**4**)[13] (1.0 g, 5 mmol) and 0.7 mL of trimethylsilyl trifluoromethane sulfonate (TMS triflate). The temperature of the solution was slowly raised to 0 °C, when a TLC showed that the reaction was complete. The mixture was evaporated to dryness, and 40 mL of methanol was added to decompose the catalyst and dissolve the residue. The mixture was filtered, and a small amount of ethyl acetate was added to the filtrate. A white solid (**3**) that precipitated was filtered and dried. Yield 1.3 g (86%), mp 153–156 °C; ^{1}H NMR (DMSO-d_6) δ 8.77 (br, s, 1H, exchangeable with D_2O, NH), 8.62 (br s, 1H, exchangeable with D_2O, NH), 8.52 (s, 1H, imidazole CH), 8.20–8.13 (d, 2H, exchangeable with D_2O, NH_2), 7.80 (d, 1H, exchangeable with D_2O, NH), 7.30–7.22 (m, 5H, Ph), 5.77 (s, 2H, NCH_2O), 4.40 (s, 2H, $PhCH_2O$), 3.70 (m, 2H, OCH_2), 3.45 (m, 2H, OCH_2); ^{13}C NMR (DMSO-d_6) δ 163.35 (C=N), 157.38 (C=N), 152.33 (C=N), 138.16 (C=C), 137.70 (C=N), 128.17 (C=C), 127.65 (Ph), 127.46 (Ph), 127.43 (Ph), 127.39 (Ph), 127.33 (Ph), 126.61 (Ph), 75.61 (OCH_2N), 71.89 ($PhCH_2O$), 68.01 (OCH_2CH_2O), 67.91 (OCH_2CH_2O); UV λ_{max} (MeOH) 248.6 nm (ε 3.38×10^4); MS (FAB) *m*/*z* 342 (MH^+). Anal. calcd for $C_{16}H_{19}N_7O_2{\cdot}HSO_3CF_3$: C, 41.55; H, 4.10; N, 19.95. Found: C, 41.00; H, 4.20; N, 19.59. **4,6-Diamino-1-hydroxyethoxymethyl-8*H*-8-iminoimidazo[4,5-*e*][1,3]diazepine (1)**. Compound **3** (290 mg, 0.85 mmol) was dissolved in a mixture of glacial acetic acid (100 mL) and MeOH (50 mL) in a hydrogenation bottle. Palladium on carbon (10%, 200 mg) was added to the above solution, and the mixture was hydrogenated in a Parr hydrogenator at 42 psi for 16 h. A TLC [silica gel, $CHCl_3$/MeOH (2:1)] showed a new, more polar (slower moving) product than the starting material. The catalyst was removed by filtration through Celite, and was washed with methanol. The filtrate, along with the washings, was evaporated to dryness under reduced pressure to afford a colorless residue, which was dried in vacuo. The residue was triturated with diethyl ether (2×20 mL), and once again dried in vacuo to obtain **1** as a colorless solid. Yield 210 mg (98%), mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ 8.58 (br, 3H, exchangeable with D_2O, NH_2 + NH), 8.46 (s, 1H, imidazole CH), 7.89 (br d, J = 12 Hz, 2H, exchangeable with D_2O, NH_2), 5.74 (s, 2H, NCH_2O), 4.80 (b, 1H, exchangeable with D_2O, OH), 3.52 (dd, 4H, J = 16.4 Hz, OCH_2CH_2O); ^{13}C NMR (DMSO-d_6) δ 164.05 (C=N), 157.95 (C=N), 152.88 (C=N), 143.68 (C=N), 138.72 (C=C), 127.15 (C=C), 76.25 (OCH_2N), 71.04 (OCH_2CH_2OH), 59.94 (OCH_2CH_2OH); UV λ_{max} (MeOH) 248.0 nm (ε 3.38×10^4); MS (FAB) *m*/*z* 252 (MH^+). Anal. calcd for $C_9H_{13}N_7O_2{\cdot}HSO_3CF_3$: C, 29.93; H, 3.52; N, 24.43. Found: C, 30.00; H, 3.52; N, 24.32.
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