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Design of inhibitors against guanase: Synthesis and biochemical evaluation of analogues of azepinomycin

Ravi K. Ujjinamatada, Anila Bhan† and Ramachandra S. Hosmane*

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

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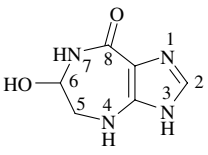
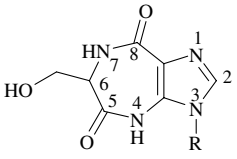
Abstract—As part of a program to design rational, mechanism-based inhibitors of guanase, we report here the synthesis and biochemical screening of two analogues of azepinomycin (**1** and **2**), a naturally occurring inhibitor of guanase, known to mimic the transition-state of the enzyme-catalyzed reaction. Our biochemical results show that compounds **1** and **2** are competitive inhibitors with K_i of $2.01 \pm 0.16 \times 10^{-5}$ and $5.36 \pm 0.14 \times 10^{-5}$ M, respectively.

Guanine deaminase (GDA) or guanase (EC 3.5.4.3) is an enzyme that catalyzes the hydrolytic deamination of guanine to xanthine. This enzyme has been found in human liver, brain, and kidney.[1] There have been reports of abnormally high levels of serum guanase activity in patients with liver diseases,[2–4] and so, the elevated enzyme activity has been suggested as a marker of hepatitis and hepatoma.[3] Furthermore, such a high guanase activity is believed to be a biochemical indicator of rejection in liver transplant recipients.[5] Increased levels of guanase have also been detected in cancerous kidney[6–8] and breast cancer tissues.[7,9] In addition, patients with multiple sclerosis exhibit significantly elevated levels of guanase activity in their cerebral spinal fluids.[10] These observations suggest that a potent guanase inhibitor is necessary for exploring the biochemical mechanisms of the above metabolic disorders as well to understand the specific physiological role played by guanase, and not to mention its potential therapeutic use in treating these disorders. While many studies on guanase inhibition have been reported in the literature,[11–36] a potent guanase inhibitor with a submicromolar or nanomolar K_i has yet to be discovered. We report here the design, synthesis, and guanase inhibitory activity of two analogues (**1** and **2**) of the natural product azepinomycin, a moderate inhibitor of guanase, which is believed to mimic the transition state of the enzyme-catalyzed deamination reaction.[37]



Azepinomycin



1, R = -$CH_2C_6H_5$
2, R = H

Guanase catalyzes the hydrolysis of guanine (**3**) to xanthine (**4**) (Scheme 1) via the tetrahedral intermediate (**5**).[38] The recently solved crystal structure of guanase[39] from *Bacillus subtilis* suggests that the enzyme-catalyzed reaction is assisted by an active site zinc metal ion (Zn^{2+}), which forms a tetrahedral complex with His-53, Cys-83, and Cys-86 of the protein, as well as with an isolated water molecule. Glutamate-55 serves as a proton shuttle, abstracting a proton from the zinc-activated water to form the necessary hydroxide nucleophile, while also enabling protonation at the N-3 site of guanine, thus resulting in the formation of the intermediate **5**, as shown. Glu-55 also assists in protonation of the NH_2 group of **5**, facilitating the elimination of a molecule of ammonia to form the final product

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* Corresponding author. Tel.: +1 410 455 2520; fax: +1 410 455 1148; e-mail: hosmane@umbc.edu
† Present address: G.E Healthcare, BioSciences, Protein Separations, 800 Centennial Ave, Piscataway, NJ 08855, USA.

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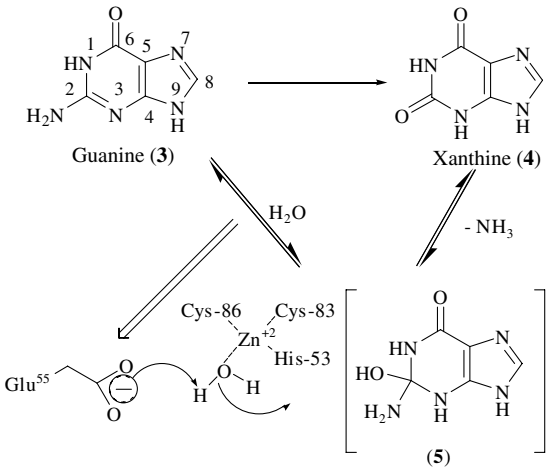


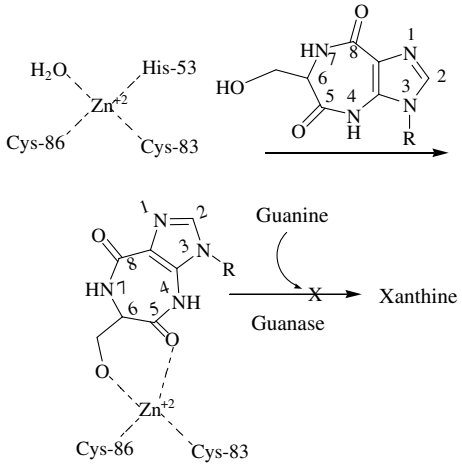
Scheme 1. Zn^{2+}-assisted hydrolysis of guanine to xanthine, catalyzed by guanase.

xanthine.[39] Azepinomycin may interrupt this hydrolytic process via coordination of its own OH group at position –6 with the active site zinc, displacing the crucial active site water molecule involved in hydrolysis.

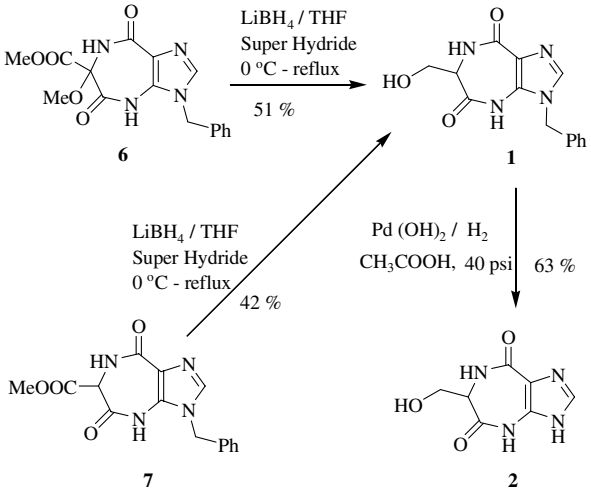
The above zinc-mediated hydrolysis of guanine to xanthine, catalyzed by guanase, led us to the design of the title inhibitors of this paper. In view of only a moderate inhibition of guanase by azepinomycin,[37] which suggested somewhat weak binding of the inhibitor to the enzyme via zinc metal coordination, we hypothesized that the additional metal coordination sites on the inhibitor would strengthen its binding to the protein, and hence enhance its inhibitory potential. The introduction of a carbonyl group at position-5 of the heterocycle, coupled with movement of the 6-hydroxy group away from the ring by an additional carbon atom, would allow excellent coordination of the inhibitor with Zn^{2+} to form a stable, 6-membered ring structure. The latter would use two of the four metal coordination sites, while the other two would be occupied by two of the three original amino acid residues at the enzyme active site, as depicted in Scheme 2 (*Note*: the two amino acids shown to be bonded to the metal are only arbitrary). With this rationale, we set out to synthesize our target inhibitors **1** and **2**. The substitution of a benzyl group at position-3 of **1** was to explore if a hydrophobic moiety in that position would enhance, lower, or unaffect the strength of enzyme binding. In our earlier guanase inhibition studies involving planar, aromatic ring-expanded purine bases, we had noticed that a benzyl group at the N-3 position often potentiated the enzyme inhibition.[30,32]

The target compound **1**[40] was synthesized (Scheme 3) by two different methods, both by reduction mediated by a mixture of lithium borohydride and super hydride in THF at 0 °C. The first method employed 3-benzyl-4,5,7,8-tetrahydro-6-methoxy-6-methoxycarbonyl-6*H*-imidazo[4,5-*e*][1,4]diazepine-5,8-dione (**6**)[20] as the starting material, while the second method used 3-benzyl-4,5,7,8-tetrahydro-6-methoxycarbonyl-6*H*-imidazo[4,5-*e*][1,4]diazepine-5,8-dione (**7**).[41] The detailed multi-step synthesis of both compounds **6** and **7** has been reported by us several years ago,[20,41] employing a common starting material, 1-benzyl-5-nitroimidazole-4-carboxylic acid.[20] The target compound **2**[40] was prepared by debenzylation of **1** using catalytic hydrogenation with palladium hydroxide in acetic acid at 40 psi. The target compounds were characterized by ^{1}H and ^{13}C NMR, and mass spectral data, along with elemental microanalyses.

Guanase from rabbit liver (purchased from Sigma–Aldrich) was employed in the biochemical studies. All studies were carried out at 25 °C and pH 7.4 by spectroscopic measurements of the rate of hydrolysis of the substrate guanine at λ_{max} 245 nm. The change in optical density at λ_{max} 245 nm per unit time is a measure of the guanase activity. A total of seven different concentrations of the substrate, ranging from 5 to 20 μM, was employed for each inhibitor concentration that was either 8 or 25 μM, while the amount of enzyme in each assay was 7.67×10^{-3} unit. The Lineweaver–Burk plots ($1/V$ vs $1/S$) (see Figs. 1A and B) were used to



Scheme 2. The proposed pathway for inhibition of guanase by the title analogues of azepinomycin.



Scheme 3. Synthesis of the target compounds **1** and **2**.