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SYNTHESIS AND GUANASE INHIBITION STUDIES OF A NOVEL RING-EXPANDED PURINE ANALOGUE CONTAINING A 5:7-FUSED, PLANAR, AROMATIC HETEROCYCLIC RING SYSTEM

Vasanthakumar Rajappan and Ramachandra, S. Hosmane*

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

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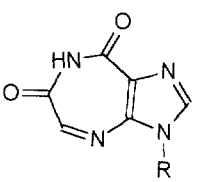
Abstract: The synthesis of a novel planar, potentially aromatic, ring-expanded xanthine analogue (**1**), containing the 5:7-fused imidazo[4,5-e][1,4]diazepine ring system, along with guanase inhibition studies are reported. The compound was synthesized in six steps, starting from 1-benzyl-5-nitroimidazole-4-carboxylic acid (**2**), and was biochemically screened against rabbit liver guanase. Compound **1** is a moderate competitive inhibitor of the enzyme with a K_i of $2.27 \pm 0.66 \times 10^{-4}$ M.

Introduction

Ring-expanded nucleic acid bases, nucleosides, and nucleotides are of chemical, biochemical, biophysical, as well as medicinal interest.[1] From a chemical standpoint, studies relating to their synthesis, structure, acid-base properties, and tautomerism would be appealing, while from a biochemical perspective, they are potentially a rich source of substrates or inhibitors of enzymes of purine metabolism as well as of those requiring energy cofactors such as ATP or GTP. In addition, their unique structural features, steric constraints, and conformational characteristics allow them to be potential probes of nucleic acid metabolism, structure, and function.



1

(R = CH_2Ph)

While a number of ring-expanded heterocycles, nucleosides, and nucleotides have been reported by us[1] and others[2] in recent years, almost all of them, however, are nonplanar as they contain a puckered 7-membered ring in their structural skeletons. We report here the synthesis and guanase inhibition studies of a novel 5:7-fused heterocycle **1** that is anticipated to be planar and potentially aromatic. Compound **1** can be viewed as a ring-expanded xanthine analogue, and since xanthine is a common structural precursor to other planar purine analogues[3] including guanine, adenine, and 2,6-diaminopurine, the successful synthesis of **1** opens up new avenues for a broad area of research related to planar ring-expanded nucleosides and nucleotides. The syntheses of planar, aromatic 5:7-fused heterocycles have often been proven difficult and challenging due to facile, opportunistic rearrangements.[4]

Guanase is an important enzyme in the salvage pathway of purine metabolism, catalyzing the hydrolysis of guanine to xanthine. The inhibition of guanase has beneficial implications in both cancer[5,6] and viral chemotherapy.[7,8] To date, there are only a few known inhibitors of guanase that have moderate activities at best.[1d,9,10] As such, a potent guanase inhibitor would further assist in the in vitro investigations of biochemical processes involving a number of metabolic disorders in which the enzyme shows up either as an indicative or a causative factor. For example, the serum guanase activity is elevated in patients with liver diseases such as hepatitis,[11] failed liver transplants,[12] and multiple sclerosis.[13] Although a number of other enzymes of purine metabolism can be conceived as potential targets for **1** or the appropriate nucleoside/nucleotide analogues derived from **1**, our choice of guanase as the initial target for biochemical screening is largely based upon our renewed interest in the chemotherapy of hepatitis viral infections[14,15]

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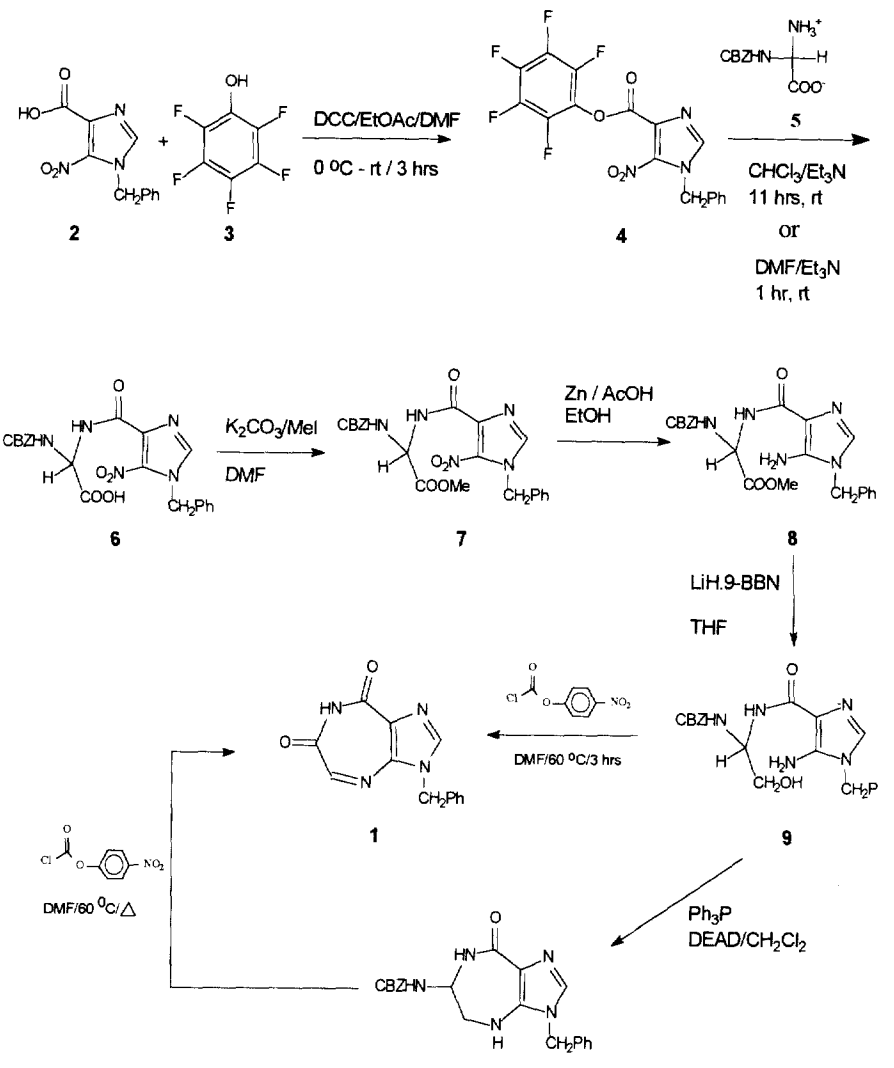
wherein guanase most often reveals itself as a metabolic signature of the disease.[11]

Results And Discussion

Chemistry

Compound **1** was synthesized in six steps, commencing with 1-benzyl-5-nitroimidazole-4-carboxylic acid (**2**)[16] (Scheme 1). The DCC-mediated condensation of **2** with pentafluorophenol gave the ester **4** (mp 115–117 °C) in nearly quantitative yield. The latter was reacted with α-(*N*-benzyloxycarbonyl)aminoglycine (**5**)[17] to provide the amide-acid **6** (mp 92–95 °C, 85%), which was further converted into the corresponding amide-ester (**7**) (mp 75–76 °C, 85%) by base-catalyzed alkylation with methyl iodide. Sequential reductions of the nitro and the ester group of **7** with zinc/acetic acid and lithium hydrido[9-borabicyclo[3.3.1]nonane], respectively, provided **8** (176–178 °C, 65%) and **9** (125–126 °C, 66%).[18]

Scheme 1

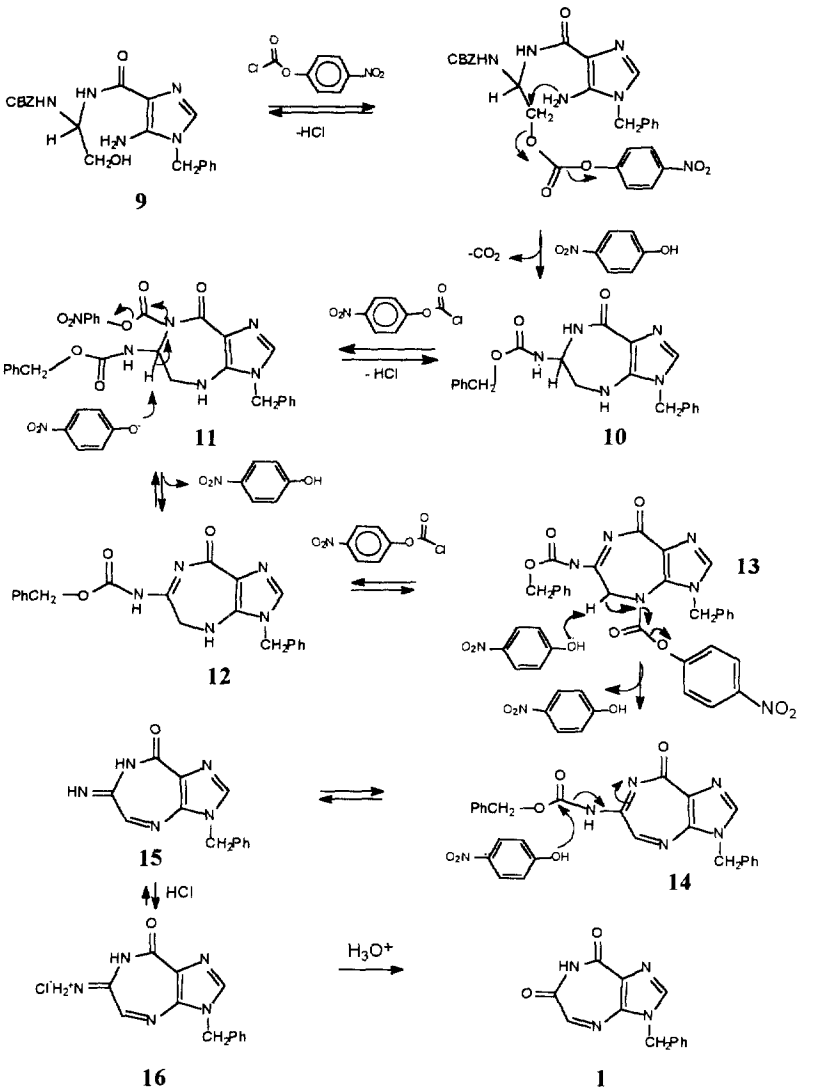


The conversion of the acyclic precursor **9** into the target compound **1** was accomplished in a single step by reaction with excess *p*-nitrophenylchlorofor mate. This conversion essentially involves four synthetic steps combined into one. The 40% yield of the pure, isolated **1** (mp 142–145 °C)[18] from **9** indicates that the average yield for each of the four synthetic steps is approximately 80%. A tentative mechanism for this conversion is outlined in Scheme 2. The support for the proposed mechanism involves the reaction of **10** (mp 164–165 °C),[18] obtained in 52% yield by intramolecular Mitsunobu condensation[19] of **9**, with excess *p*-nitrophenylchloro- formate, which gave **1**. Our computational studies[20] corroborate the anticipation that the heterocyclic ring system of **1** is planar.

Biochemistry

Compound **1** was screened in vitro against guanase from rabbit liver (Sigma) in a Tris buffer (pH 7.4) at 25 °C by spectrophotometric measurements of the rate of hydrolysis of the substrate guanine at λ_{max} 245 nm. Stock solutions of the substrate, enzyme, and inhibitors were prepared using 0.05 M Tris 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 from 5-23 μM; a total of six different concentrations were employed. The inhibitor concentration used in each assay was 80 or 100 μM. The amount of enzyme used in each assay was 0.0077 unit. 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™, and are collected in Figure 1. Our biochemical results suggest that **1** is a moderate competitive inhibitor of the enzyme with a K_i of $2.27 \pm 0.66 \times 10^{-4}$ *M*.

Scheme 2



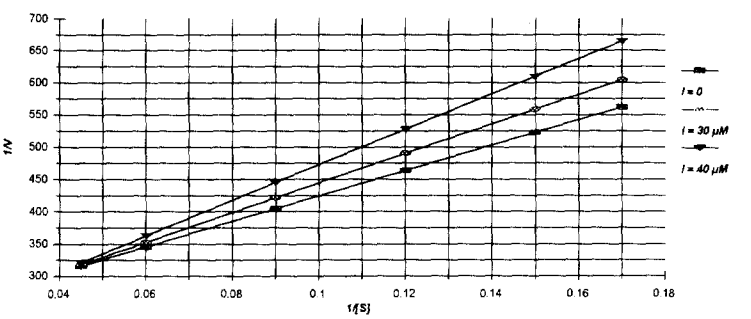


Figure 1: Lineweaver-Burk Plot for Inhibition of Guanase by **1**

Conclusion

The synthesis of the target planar, potentially aromatic, ring-expanded heterocyclic base has been accomplished. The biochemical screening results suggest that **1** is a moderate competitive inhibitor of guanase. As the 5:6-fused, unsubstituted guanine is the natural substrate for guanase, the observed binding of the ring-expanded, benzyl-substituted heterocycle (**1**) to guanase is intriguing, and suggests that the enzyme active site is somewhat more flexible and broad. Furthermore, since azepinomycin,[9] a naturally occurring inhibitor of guanase, has the puckered 5:7-fused ring geometry, the inhibition of guanase by the planar **1** raises questions about the mechanism of action of the enzyme. In any case, the successful synthesis of **1** opens new avenues for the synthesis of ring-expanded nucleoside/nucleotides of not only **1**, but also of other panar, heterocyclic bases derived from **1**, such as the ring-expanded adenine, guanine, and 2,6-diaminopurine.

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18. The data for compounds **1**, **9**, and **10** are as follows: *Compound 1:* ^{1}H NMR (DMSO-d_6) δ 12.30 (s, 1H, NH, ex.w. D_2O), 8.19 (s, 1H, CH), 8.03 (s, 1H, CH), 7.26 (m, 5H, ArH), 5.33 (s, 2H, CH_2), 3.41 (s, 2H, NH_2); MS (FAB) *m/z* 255 (MH^+). *Anal.* C,H, N; *Compound 9:* ^{1}H NMR (DMSO-d_6) δ 7.317 (m, 12 H, ArH & NH), 5.87 (s, 2H, NH_2 ex./w. D_2O), 5.4 (m, 1H, CH), 5.08 (s, 2H, CH_2), 5.00 (s, 2H, CH_2), 5.00-4.96 (t, *J* = 5.4 Hz, 1H, OH, ex./w. D_2O), 3.45 (dd, *J* = 6 and 5.7 Hz), MS (FAB) *m/z* 410 (MH^+); *Anal.* C,H,N; *Compound 10:* ^{1}H NMR (DMSO-d_6) δ 7.85-7.82 (d, *J* = 8.4 Hz, 1H, NH, ex./w. D_2O), 7.29-7.15 (m, 12H, Ar-H, CH, NH), 6.02 (s, 1H, NH, ex./w. D_2O), 5.72 (dt, 1H, CH), 5.10 (s, 2H, OCH_2), 5.02 (s, 2H, NCH_2), 4.37-4.31 (dd, *J* = 9 Hz, 8.7 Hz, 1H, one hydrogen of ring CH_2), 3.94-3.90 (d-d, *J* = 6.3 Hz, 7.5 Hz, 1H, other hydrogen of ring CH_2). ^{13}C NMR (DMSO-d_6) 143.90, 136.87, 131.43, 128.71, 128.07, 127.32, 127.16, 73.03, 69.96, 65.41, 45.98, MS (FAB) *m/z* 392 (MH^+); *Anal.* C,H,N.
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20. Computational studies were performed using QUANTA/ CHARMM™ and INSIGHT II/DISCOVER™ obtained from MSI™.