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NOVEL INHIBITORS OF GUANASE

Anila Bhan and Ramachandra S. Hosmane*

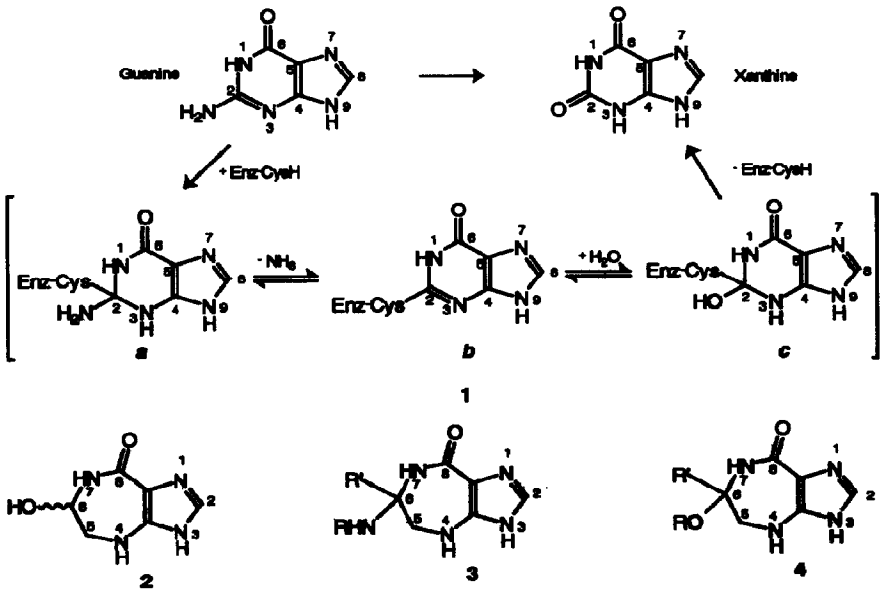
Laboratory for Drug Design & Synthesis
Department of Chemistry and Biochemistry
University of Maryland Baltimore County
Baltimore, Maryland 21228

Abstract. Synthesis and guanase inhibitory activity of two novel 5:7-fused heterocyles, **15** and **16**, containing the imidazo[4,5-*e*][1,4]diazepine ring system, have been reported.

Several reports have recently appeared regarding the detection of abnormally high levels of serum guanase activity in patients with liver diseases like hepatitis.[1] High serum guanase activity has also been reported to be a biochemical indicator of rejection in liver transplant recipients.[2] Further, it has been shown that patients with multiple sclerosis (MS) exhibit significantly elevated levels of guanase activity in their cerebral spinal fluids (CSF), and that there is a close correlation between the extent of disability and the level of CSF guanase activity.[3] In this respect, it is timely that a suitable guanase inhibitor may assist in exploring the biochemical mechanisms of these metabolic disorders as well as in understanding the specific physiological role played by guanase.

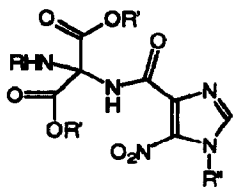
Guanase (guanine deaminase or guanine aminohydrolase, EC 3.5.4.3) catalyzes the hydrolysis of guanine to xanthine (**Scheme 1**) via the putative sequence of intermediates,

Scheme 1

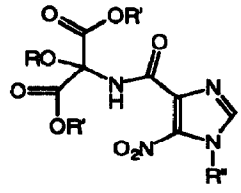


collectively depicted as **1**.[4] Azepinomycin (**2**) is a naturally occurring antitumor antibiotic, and is considered to be the transition state analogue inhibitor of this enzyme.[5] However, an examination of **1** reveals that both **1a** and **1c** contain a quaternary carbon at the 2-position, the site of hydrolysis, with geminal thio/amino or thio/hydroxy functionalities. Therefore, a more appropriate inhibitor to mimic the transition state of the deaminase reaction might be a compound such as **3** or **4** having a quaternary carbon attached to two substituents at the 6-position of the imidazo[4,5-*e*][1,4]diazepine ring, as contrasted with azepinomycin that lacks such a quaternary carbon. We report here the synthesis and biochemical screening of two compounds, **15** and **16**, which incorporate this important structural characteristics of **3** and **4** that is missing in azepinomycin.

A retrosynthetic analysis of **3** and **4** called for a synthon such as **5** and **6** which contained a unique diamino- or alkoxyaminomalonate side chain, and for which there is little literature precedent. A logical precursor to both **5** and **6** is the aminomalonate derivative **7**[6]



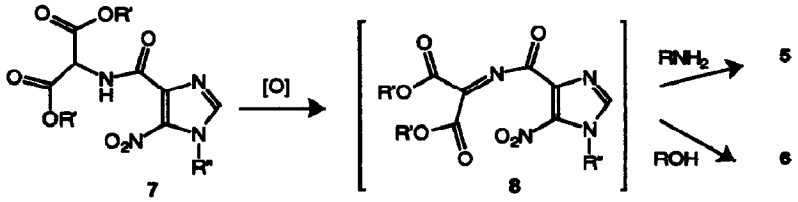
5



6

(**Scheme 2**). Oxidation of **7** to produce the iminomalonate intermediate **8**, followed by conjugate addition by an amine or alkoxy nucleophile would yield the desired diamino- and alkoxyaminomalonate synthons **5** and **6**, respectively. Indeed, bromination of **7** in the presence of sodium hydride, followed by quenching with benzylamine or methanol, afforded the desired

Scheme 2

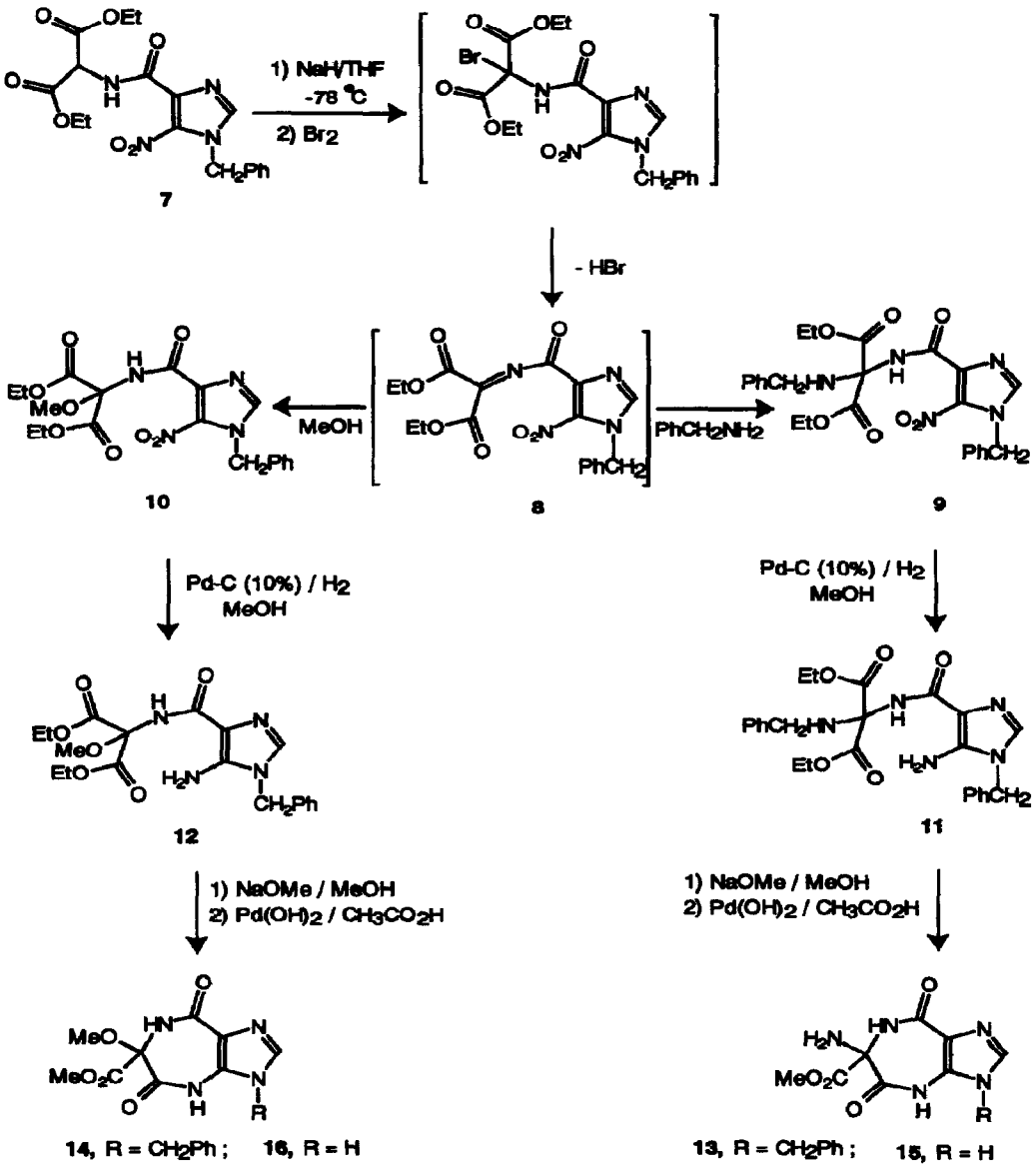


7 **8**

R' = Et, R" = CH_2Ph

diethyl 2-benzylamino-2-[*N*-(1-benzyl-5-nitroimidazolyl-4-carbonyl)amino]malonate (**9**; mp 100-101 °C, ^{1}H NMR, Anal.[7] C,H,N) and diethyl 2-[*N*-(1-benzyl-5-nitroimidazolyl-4-carbonyl)amino]-2-methoxymalonate (**10**; mp 126-127 °C, ^{1}H NMR, Anal.[7] C,H,N), respectively (**Scheme 3**). Reduction of **9** and **10** with Pd-C/H_2 provided the respective 5-aminoimidazole derivatives, **11** (mp 121-123 °C, ^{1}H NMR, Anal.[7] C,H,N) and **12** (mp 162-163 °C, ^{1}H NMR, Anal.[7] C,H,N). Treatment of **11** and **12** with sodium methoxide in refluxing methanol resulted in ring-closure with concomitant exchange of the ester ethoxide group with a methoxide to produce **13** and **14**, respectively. It should be noted that **14** was also formed, along with **13**, during the ring-

Scheme 3



closure of **11**. However, since the R_f of **13** was very close to that of **14** in a variety of solvents, it was more convenient to use the mixture of **13** and **14** for the next step than isolate the individual components from the mixture. Thus, debenzylation of **13** and **14** with $Pd(OH)_2/H_2$ in acetic acid provided **15** and **16**, which were separated and/or purified by silica gel flash chromatography, using a gradient of chloroform-methanol as eluent (6:1 → 4:1). The spectral[8]

and analytical data[7] of **15** and **16** were consistent with the assigned structures. The structures of both **15** and **16** were also confirmed by single-crystal X-ray diffraction analysis.[9]

Finally, both **15** and **16** were screened *in vitro* against rabbit liver guanase in a Tris buffer (pH 7.6) at 21 °C, by spectrophotometrically monitoring the rate of hydrolysis of the substrate guanine at 248 nm. Both were found to be inhibitors of this enzyme with K_i's = 1.9 x 10^{-4} *M* and 5.4 x 10^{-4} *M*, respectively. To the best of our knowledge, no K_i has yet been reported for azepinomycin, although its IC_{50} (=0.5 x 10^{-5}) in tissue culture systems is known.[5b] Nevertheless, the more appropriate transition state analogues for the assessment of guanase inhibitory activity might be those with a CH_2 group in place of the C=O functionality at position 5 of **15** and **16**, and an alkyl or a hydroxyalkyl moiety in place of the ester group at position 6. Such an endeavor is currently in progress.

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REFERENCES AND NOTES

[1] (a) Nishikawa, Y.; Fukumoto, K.; Watanabe, F.; Yoshihara, H. *Jpn. J. Clin. Pathol. (Rinsho Byori)* **1989**, *37*, 1392. (b) Shiota, G.; Fukuda, J.; Ito, T.; Tsukizawa, M.; Sato, M. *Jpn. J. Med.* **1989**, *28*, 22. (c) Ito, S.; Tsuji, Y.; Kitagawa, N.; Akihiko, I.; Syundo, J.; Tamura, Y.; Kishi, S.; Mori, H. *Hepatology* **1988**, *8*, 383.

[2] Crary, G.S.; Yasmineh, W.G.; Snover, D.C.; Vine, W. *Transplant Proc.* **1989**, *21*, 2315.

[3] Kaneshige, Y.; Matsumoto, H.; Chiba, S.; Hashimoto, S.; Noro, H. *Clin. Neurol. (Rinsho Shinkeigaku)* **1989**, *29*, 854.

[4] Lewis, A.S.; Glantz, M. D. *J. Biol. Chem.* **1974**, *249*, 3862.

[5] (a) Umezawa, H.; Takeuchi, T.; Iinuma, H.; Hamada, M.; Nishimura, S. **Jpn. Kokai Tokyo Koho JP 58,159,494 [83,159,494]**; *Chem. Abstr.* **1984**, *100*, 137362x. (b) Isshiki, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Umezawa, Y.; Takeuchi, T.; Umezawa, H.; Nishimura, S.; Okada, N.; Tatsuta, K. *J. Antibiot.* **1987**, *40*, 1461. (c) Fujii, T.; Saito, T.; Fujisawa, T. *Heterocycles* **1988**, *27*, 1163.

[6] Bhan, A.; Hosmane, R. S. *J. Heterocycl. Chem.* **1993**, *30*, 1453.

[7] Elemental microanalyses were performed by Atlantic Microlab, Inc., Norcross, Georgia, and the analyses found were within 0.4% of the calculated values.

[8] Compound **15**: mp: sinters at 196 °C and 203 °C (d); ^{1}H NMR (DMSO-d_6) δ 12.93 (br s, 1 H, exchangeable with D_2O, NH), 11.13 (br s, 1 H, exchangeable with D_2O, NH), 7.83 (s, 1 H, exchangeable with D_2O, NH), 7.67 (s, 1 H, imidazole CH), 3.4 (s, 3 H, CO_2Me), 3.0 (s, 2 H, exchangeable with D_2O, NH_2); MS (EI, 70 eV) *m/z* 239 (M^+); Anal. C, H, N.[7]; Compound **16**: mp >280 °C; ^{1}H NMR (DMSO-d_6) δ 12.97 (br s, 1 H, exchangeable with D_2O, NH), 11.36 (br s, 1 H, exchangeable with D_2O, NH), 8.66 (s, 1 H, exchangeable with D_2O, NH), 7.70 (s, 1 H, imidazole CH), 3.73 (s, 3 H, CO_2Me), 3.08 (s, 3 H, OMe); MS (EI, 70 eV) *m/z* 254 (M^+); Anal. C, H, N.[7]

[9] The X-ray diffraction analyses of compounds **15** and **16** were performed at the Department of Chemistry, Southern Methodist University, Dallas, Texas by Dr. Hongming Zhang, and his assistance in this regard is gratefully acknowledged.

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