Synthesis and Biological Activity of Guanylhydrazones of 2- and 4-Pyridine and 4-Quinoline Carboxaldehydes

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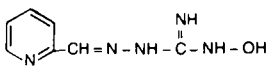
Abstract □ A series of guanylhydrazones derived from 2- and 4-pyridine and 4-quinoline carboxaldehydes was synthesized from *S*-methylisothiosemicarbazide hydroiodide using known procedures. The compounds are analogous to anticancer and antiviral thiosemicarbazones, but several of the guanylhydrazones derived from 4-quinoline carboxaldehyde showed no activity against P388 lymphocytic leukemia in mice. Guanylhydrazones derived from all three heterocyclic aldehydes revealed significant blood pressure lowering effects in the rat, however.

The strongly basic guanidine function appears in a number of biologically active compounds, most notably in those with anticancer and antihypertensive activities. Bis(guanylhydrazones) have been shown to be active against leukemias and lymphomas in mice[1] and in some types of human cancer.[2] Those derived from anthracene-9,10-dicarboxaldehyde are effective against both leukemias and solid tumors.[3] The most effective compound of this type, bisantrene, is believed to act by intercalation of DNA,[3,4] but there is also evidence that the antileukemic action of the bis(guanylhydrazones) may be associated with an immunostimulatory effect.[5] An antitumor antibiotic, spergualin, and its derivatives, contain a guanidine function, and a series of *N*-hydroxyguanidine derivatives (1) has shown antileukemic activity against L1210 cell cultures.[6] A number of thiosemicarbazones having heterocyclic rings with a hetero nitrogen adjacent to the thiosemicarbazone function (2) have also been effective against experimental tumors.[7]



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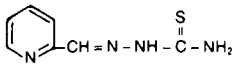

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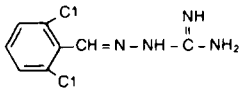

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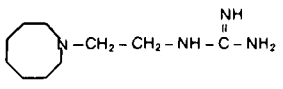

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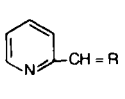

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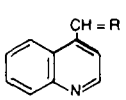

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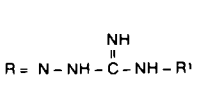

A number of guanidine derivatives having antihypertensive activity are known. Perhaps the best known are guanabenz (3), guanethidine (4) clonidine, prazosin, and minoxidil. No explanation relating the common occurrence of this function to activity in either the anticancer or antihypertensive series has been advanced. Several postulations regarding the anticancer activity of the related thiosemicarbazones have been made, including inhibition of ribonucleotide reductase,[8] possibly by complexation of the iron atom present.[9] The antitumor hydrazones of *N*-hydroxy-N^1-aminoguanidines[6] were also found to be inhibitors of ribonucleotide reductase.[10] In order to investigate a wider range of structures with the guanidine function, a series of guanylhydrazones (5–7) related to the more active of the anticancer thiosemicarbazones has been synthesized, and the anticancer and antihypertensive activities of some of the compounds have been measured. Activity found for either type could then lead to mechanism studies. Inclusion of the imino in place of the thio function might also overcome the aqueous solubility and toxicity problems associated with the thiosemicarbazones.

The guanylhydrazones were prepared by the condensation of *S*-methylisothiosemicarbazide hydroiodide with the pyridine or quinoline aldehyde, followed by reaction with the appropriate amine (method A). In some cases, it was preferable to carry out the condensation of the *S*-methylisothiosemicarbazide hydroiodide with the amine to form the aminoguanidine and then condense this product with the aldehyde (method B). Spectral and analytical data for all intermediates and final products were consistent with the proposed structures.

The substituents on the N^1-nitrogen (R^1) were selected to provide a range of lipophilic and/or hydrophilic properties. In general, the products were isolated as hydroiodides, but in some cases, better stability was obtained with the hydrochlorides or hydrobromides. Physical properties of the compounds synthesized are listed in Table I.

Experimental Section

Melting points were determined on a Mel-Temp capillary melting point block and are uncorrected. Infrared absorption spectra were determined on a Perkin-Elmer 457-A grating spectrophotomer, and proton nuclear magnetic resonance spectra were determined on a Varian T60 spectrometer. Elemental analyses were done by Multi Chem Laboratories, Lowell, MA. Thin-layer chromatography was performed using Eastman silica gel plates with fluorescent indicator.

Organic chemicals were purchased from Aldrich Chemical Company. Solvents were obtained from Fisher Scientific Company, and dried with 3 or 4 Å molecular sieves.

Representative Synthetic Procedures—*1-(2-Pyridylmethinyl)amino-S-methylisothiourea Hydroiodide*—*S*-Methylisothiosemicarbazide hydroiodide[11] (23.3 g, 0.1 mol) was added to a solution of 2-pyridinecarboxaldehyde (12.85 g, 0.12 mol) in 150 mL of ethanol. The mixture was refluxed overnight with continuous stirring. It was cooled in an ice bath and filtered, and the product was recrystallized from ethanol. After being dried at 60 °C under reduced pressure,

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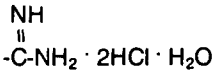
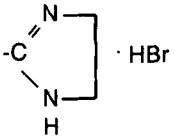
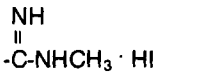
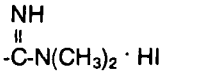
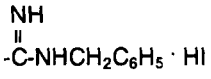
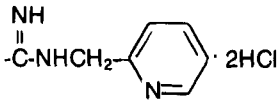
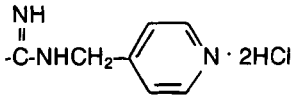
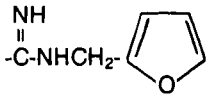
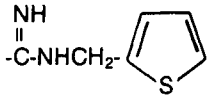
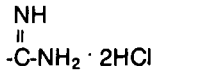
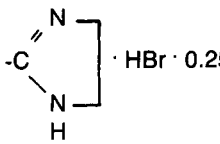
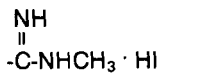
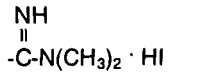
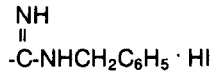
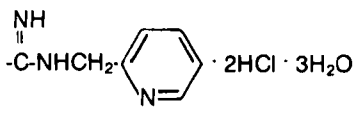
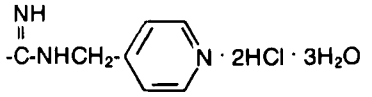
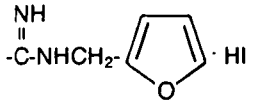
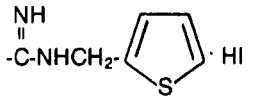
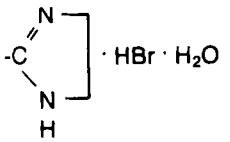
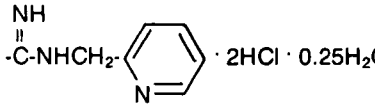
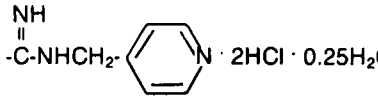
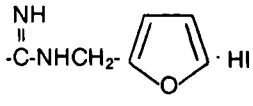
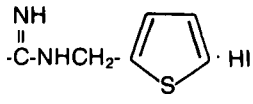
Table I—2-Pyridine-, 4-Pyridine-, and 4-Quinolinecarboxaldehyde Guanylhydrazones (Ar—CH=N—NH—R)

No.	Ar	R	Method	Mp, °C	Recryst'n Solvent	Yield, %[a]
1	2-Pyridine		A	232-238	C_2H_5OH	51
2	2-Pyridine		A	228-229	C_2H_5OH	41
3	2-Pyridine		A	190-191	$C_6H_5CH_3$	23
4	2-Pyridine		B	214-216	C_2H_5OH	30
5	2-Pyridine		A	156-157	CH_2Cl_2	16
6	2-Pyridine		B	218-226	C_2H_5OH	17
7	2-Pyridine		B	232-235	C_2H_5OH/H_2O	16
8	2-Pyridine		A	179-180	C_2H_5OH	51
9	2-Pyridine		A	156-158	CH_2Cl_2	58
10	4-Pyridine		A	267-270	C_2H_5OH	77
11	4-Pyridine		A	274-278	C_2H_5OH	70
12	4-Pyridine		B	252-254	C_2H_5OH	35
13	4-Pyridine		B	224-225	C_2H_5OH	95
14	4-Pyridine		B	218-220	C_2H_5OH	21

Continued

Table I—Continued

No.	Ar	R	Method	Mp, °C	Recryst'n Solvent	Yield, %[a]
15	4-Pyridine		B	261-266	C_2H_5OH/H_2O	30
16	4-Pyridine		B	253-254	C_2H_5OH/H_2O	71
17	4-Pyridine		A	232-234	C_2H_5OH	29
18	4-Pyridine		A	234-235	C_2H_5OH	30
19	4-Quinoline		A	263-264	C_2H_5OH	66
20	4-Quinoline		A	243-244	C_2H_5OH	30
21	4-Quinoline		B	226-227	C_2H_5OH	37
22	4-Quinoline		B	250-253	C_2H_5OH	35
23	4-Quinoline		B	203-205	C_2H_5OH	36
24	4-Quinoline		B	235-236	C_2H_5OH/H_2O	32
25	4-Quinoline		B	234-236	C_2H_5OH/H_2O	24
26	4-Quinoline		A	176-177	C_2H_5OH	53
27	4-Quinoline		A	179.5-180.5	C_2H_5OH	17

[a] Satisfactory analytical data (±0.4% for C, H, N) were obtained for all new compounds listed in the table, with the following exceptions. **1**: calc. N, 27.55%, found N, 28.00; **20**: calc. C, 46.17%, found C, 46.74%; and **21**: calc. C, 40.07, found C, 40.52.

7.35 g (10.5% yield) of yellow crystals were obtained; mp 169–170 °C; IR (KBr): ν 3400–3100 (N—H), 1610–1550 (C=N,=NH_2), 1300 (S—CH_3), and 1100 (C—N) cm^{-1}; ^1H NMR (Me_2SO—d_6): δ 2.75 (s, 3H, SCH_3), 4.20 (bs, 1H, NH), 7.40–8.75 (m, 4H, pyridine), and 8.45 ppm (s, 1H, CH=).

Anal.—Calc. for $C_8H_{11}N_4SI$: C, 29.83; H, 3.44; N, 17.39. Found: C, 29.82; H, 3.47; N, 17.42.

1-(2-Pyridylmethinyl)amino-3-methylguanidine Hydroiodide—1-(2-Pyridylmethinyl)amino-*S*-methylisothiourea hydroiodide (1.0 g, 3.1 mmol) was suspended in 15 mL of toluene and methylamine (0.48 g, 6.2 mmol). The mixture was refluxed overnight with stirring, the heating was discontinued, and the mixture was stirred 4 h more. After concentration of the mixture under reduced pressure and allowing the residue to stand overnight at room temperature, a precipitate appeared. It was filtered, recrystallized from ethanol, and dried at 60 °C under reduced pressure, giving 0.2 g (21% yield) of yellow crystals; mp 190–191 °C; IR (KBr): ν 3400 (N—H), 3100 (=C—H), 1660–1590 (C=N, arom C—C, =N—H), and 1290 (C—N) cm^{-1}; ^1H NMR (Me_2SO—d_6): δ 2.95 (s, 3H, N—CH_3), 3.40 (s, HDO), 7.50–8.95 (m, 4H, pyridine), 7.8 (s, 1H, CH=), and 8.4 ppm (bs, 1H, NH).

Anal.—Calc. for $C_8H_{12}N_5I$: C, 31.49; H, 3.96; N, 22.95. Found: C, 31.43; H, 3.94; N, 22.85.

Antileukemia Screening—Antileukemia testing of several of the 4-quinolyl guanylhydrazones was done at the National Cancer Institute in mice with P388 lymphocytic leukemia using the NCI protocol.[12] Compounds were administered ip in CD_2F_1 mice.

Cardiovascular Activity—Effects of the guanylhydrazones on mean arterial blood pressure (MAP) were measured. The compounds were administered at a dose of 2 mg/kg intravenously in a volume of 1 mL/kg to chloralose:urethane (50:500 mg/kg) anesthetized adult male Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 263 to 319 g. Systolic and diastolic pressures were recorded from a cannula placed in the carotid artery at 0.5, 1, 2, 3, 4, 5, and 10 min after drug administration and corrected for the effect of an equal volume of saline. Saline-induced changes in MAP ranged from −4.87 to 2.92 mmHg during the 10-min test period. Peak changes in MAP were calculated for the test period for all compounds tested and compared with peak saline-induced MAP changes by ANOVA and Dunnett's Test. Significance was set at the $p < 0.05$ and $p < 0.01$ levels. Results are shown graphically in Figure 1.

Results and Discussion

Four of the 4-quinolyl guanylhydrazones (**19**, **20**, **23**, and **25**) were tested for antileukemic activity in mice at dosage levels of 7.5–240 mg/kg. None of the compounds showed activity in this test. A thiosemicarbazone with a related structure, having a 2-pyridylmethinyl and a simulated ribose function,[13] had positive activity in the P388 leukemia screen, however.

Eight of the compounds, including examples of the 2-pyridyl, 4-pyridyl, and 4-quinolyl derivatives, were tested for their effect on MAP. All but one (**10**) produced significant changes in MAP compared with saline controls (Figure 1). The most effective compound (**5**) produced a rapid decrease in MAP (33 mmHg) which readjusted slightly above baseline (4.5 mmHg) within 3 min. Compounds **3** and **24** produced a rapid decrease in MAP (21–27 mmHg) which plateaued within 3 min at a level of 10–15 mmHg below baseline for the remainder of the test period. Compounds **2** and **16** produced a gradual decrease in MAP (11–20 mmHg) 1–2 min after administration which was sustained for the 10-min period. Compounds **5** and **21**, after an initial slight increase in MAP (0.3–5 mmHg), produced a decrease in MAP (3–9 mmHg) for 5 min and rose toward baseline between 5 and 10 min.

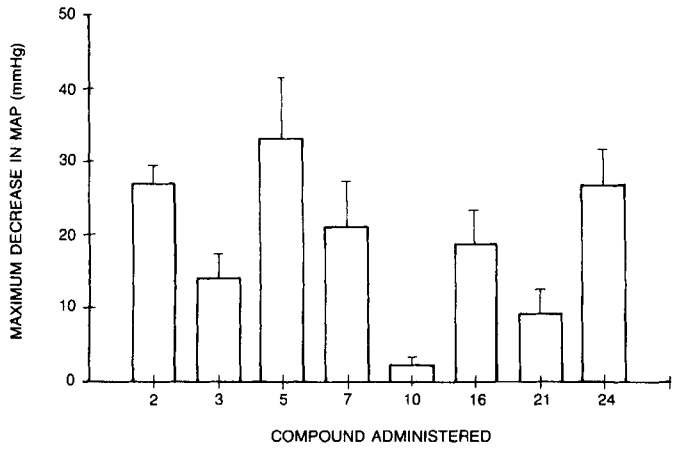


Figure 1—Effect of aminoguanidine derivatives (2 mg/kg) on mean arterial pressure (saline corrected) in the rat.

It is apparent from these results that relatively simple guanidine-containing compounds of the guanylhydrazone type, when attached to a heterocyclic nitrogen function, are capable of reducing MAP. This could be due to the fact that the highly basic guanidine function, having a pK_a value of >12, will exist as a cation under biological conditions. This precludes absorption in the central nervous system, but not at noradrenergic nerve terminals. The rapid and generally short-lived blood pressure effects observed here, however, indicate that the positive charge of the guanidine function most likely does not result in strong or irreversible binding.

The positively charged guanidine function may also mask potential anticancer effects because of poor distribution in the body. Modification by substituents that lower basicity of the guanidine function should be of value. The presence of the sulfur in the thiosemicarbazone group appears to be essential for both anticancer and antiviral[14] activities, however.

Thus, it is unlikely that these compounds produced their vasodepressor responses via a centrally-mediated effect, as is seen with the alpha-2 agonist clonidine. Future studies will be required to elucidate the precise site(s) and mechanism(s) of action of these compounds within the cardiovascular system.

References and Notes

1. Mihich, E. *Arch. Ital. Patol. Clin. Tumori.* **1985**, *8*, 153–206.
2. Mihich, E. In *Handbook of Experimental Pharmacology*, Part 2; Springer Verlag: New York, NY, 1975, pp 766–788.
3. Murdock, K. C.; Child, R. G.; Lin, Y.; et al. *J. Med. Chem.* **1982**, *25*, 505–518.
4. Foye, W. O.; Karnik, P. S.; Sengupta, S. K. *Anti-Cancer Drug Design* **1986**, *1*, 65–71.
5. Mihich, E. *Cancer Res.* **1969**, *29*, 848–854.
6. Tai, A. W.; Lien, E. J.; Lai, M. M. C.; Khwaja, T. A. *J. Med. Chem.* **1984**, *27*, 236–238.
7. French, F. A.; Blanz, E. J., Jr. *J. Med. Chem.* **1966**, *9*, 585–589.
8. Agrawal, K. C.; Booth, B. A.; Michaud, R. L.; Moore, C. E.; Sartorelli, A. C. *Biochem. Pharmacol.* **1974**, *23*, 2421–2429.
9. Brockman, R. W.; Sidwell, R. W.; Arnett, G.; Shaddix, S. *Proc. Soc. Exp. Biol. Med.* **1970**, *133*, 609–614.
10. Weckbecker, G.; Lien, E. J.; Cory, J. G. *Biochem. Pharmacol.* **1988**, *37*, 529–534.
11. Stollé, R. *Chem. Ber.* **1922**, *55*, 1289–1297.
12. Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep. (Part 3)*, **1972**, *3*, 1–103.
13. Foye, W. O.; Banijamali, A. R.; Patarapanich, C. *J. Pharm. Sci.* **1986**, *75*, 1180–1184.
14. Thompson, R. L.; David, J.; Russell, P. B.; Hitchings, G. H. *Proc. Soc. Exp. Biol. Med.* **1953**, *84*, 496–501.

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