Communications to the Editor

Potential Antitumor Agents. 21.[1] Structure Determination and Antitumor Activity of Imidazo[2,1-*b*]thiazole Guanylhydrazones

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In the course of the past 25 years, guanylhydrazones have been reported in the literature as antiprotozoal,[2] antibacterial,[3] antidepressive,[4] antiinflammatory,[5] platelet aggregation inhibiting,[6,7] antihypertensive,[8-10] antiviral,[11,12] antiarrhythmic,[13] cardiotonic,[14-16] and antimitotic or antitumor[17-25] agents. This last aspect is the most interesting for us since we have devoted a large part of our research efforts to the synthesis of potential antitumor agents; in particular, some of our previous papers were related to hydrazone derivatives of indoles[26-31] and imidazo[2,1-*b*]thiazoles.[32,33]

The history of hydrazone derivatives as antitumor agents begins in 1956 when R. W. Brockman et al. reported the antileukemic activity of pyridine-2-carboxaldehyde thiosemicarbazone.[34] Two years later the antileukemic activity of glyoxal bisguanylhydrazone was reported.[35] These two

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(1) Potential antitumor agents. 20. 6-Anilinoimidazo[2,1-b]thiazoles. For part XX, see: Andreani, A.; Rambaldi, M.; Locatelli, A.; Bossa, R.; Fraccari, A.; Galatulas, I. unpublished results.

(2) Sundberg, R. J.; Dahlhausen, D. J.; Manikumar, G.; Mavunkel, B.; Biswas, A.; Srinivasan, V.; Musallam, H. A.; Reid, W. A., Jr.; Ager, A. L. Cationic Antiprotozoal Drugs. Trypanocydal Activity of 2-(4'-Formylphenyl)imidazo[1,2-a]pyridinium Guanylhydrazones and Related Derivatives of Quaternary Heteroaromatic Compounds. *J. Med. Chem.* **1987**, *33*, 298-307.

(3) Noel-Artis, A.; Berge, G.; Fulcrand, P.; Castel, J. Q. S. A. R. et Activite Antimicrobienne in vitro de Phenoxyacetaldehyde-guanylhydrazones. *Eur. J. Med. Chem.* **1985**, *20*, 25-32.

(4) Mutschler, E.; Springer, J.; Wassermann, O. Guanylhydrazones, Another Class of Monoamine Oxidase Inhibitors. *Biochem. Pharmacol.* **1970**, *19*, 9-15.

(5) Konieczny, M.; Teodorczyc, J.; Bierowska, D.; Gieldanowski, J. Synthesis and Pharmacological Properties of Guanylhydrazones of Barbituric Acid Derivatives. *Arch. Immunol. Ther. Exp.* **1972**, *20*, 385-398.

(6) Berge, G.; Darmanaden, R.; Artis-Noel, A.; Fulcrand, P.; Castel, J.; Orzalesi, H. Substituted guanylhydrazones: Q.S.A.R. and in vitro inhibition of platelet aggregation. *Eur. J. Med. Chem.* **1983**, *18*, 45-50.

(7) Desideri, N.; Sestili, I.; Manarini, S.; Cerletti, C.; Stein, M. L. Guanylhydrazones of 3-Substitutes 2-Pyridinecarboxaldehyde and of (2-Substituted 3-pyridinyloxy) Acetaldehyde as Prostanoid Biosynthesis and Platelet Aggregation Inhibitors. *Eur. J. Med. Chem.* **1991**, *26*, 455-460.

(8) Foye, W. O.; Almassian, B.; Eisenberg, M. S.; Maher, T. J. Synthesis and Biological Activity of Guanylhydrazones of 2- and 4-Pyridine and 4-Quinoline Carboxaldehydes. *J. Pharm. Sci.* **1990**, *79*, 527-530.

(9) Guanabenz. Drugs Future **1976**, *1*, 523-525.

(10) Hagen, A.; Froemmel, B.; Kuehmstedt, H.; Wunderlich, I.; Kottke, K.; Goeres, E. Pharmacological Action Spectrum of Guanylhydrazones. *Pharmazie* **1979**, *34*, 330-332.

(11) Andreani, A.; Cavrini, V.; Giovanninetti, G.; Mannini-Palenzona, A.; Franchi, L. Potential antiviral agents. 2. Derivatives of N-substituted 2-chloro-3-formylindoles. *Farmaco, Ed. Sci.* **1975**, *30*, 440-448.

(12) Garuti, L.; Giovanninetti, G.; Baserga, M.; Mannini-Palenzona, A. Potential antiviral agents. 19. Amidinohydrazones of formyl esters and formyl thioesters of N-heterocyclic aromatic compounds. *Farmaco, Ed. Sci.* **1981**, *36*, 779-784.

(13) Richter, P.; Besch, A.; Wunderlich, I.; Hagen, A. Preparation of 2-Substituted Benzophenone Amidinohydrazones. (1991) Ger (East) DD 290184; *Chem. Abstr.* **1991**, *115*, 255827*k*.

(14) Gelbart, A.; Thomas, R. Cardenolide Analogues. 7. Synthesis and Biological Activity of Some New Steroidal Guanylhydrazones. *J. Med. Chem.* **1978**, *21*, 284-287.

(15) Schuetz, S.; Meyer, K. H.; Kraetzer, H. Guanylhydrazones with Positive Inotropic Effect on the Heart. *Arzneim-Forsch.* **1969**, *19*, 69-75.

(16) Mutschler, E.; Scherf, H.; Voss, P.; Wassermann, O. Relations between Electron Distribution in Monoguanyl Hydrazones and their Pharmacological Action. *Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol.* **1967**, *256*, 367-382; *Chem. Abstr.* **1967**, *67*, 63978*d*.

(17) Muntoni, S. Antimitotic Activity of a Steroid Guanylhydrazone. *Nature* **1967**, *215*, 969.

(18) Korytnyk, W.; Ghosh, A. C.; Angelino, N.; Dave, C. Guanylhydrazones with Potential Antileukemic Activity. 1. Aza Analogs of 4,4'-Diacetyldiphenylurea Bis(guanylhydrazone). *J. Med. Chem.* **1973**, *16*, 959-961.

(19) Carlsson, F. H. H.; Charlson, A. J.; Watton, E. C. Biological Activity of Some Guanylhydrazones and Thiosemicarbazones of Alifatic Carbonyl Compounds. *Carbohydr. Res.* **1974**, *36*, 359-368.

(20) Orzalesi, H.; Castel, J. Pharmaceutical and Agrochemical Guanylhydrazones. (1978) Ger Offen 2745596; *Chem. Abstr.* **1978**, *89*, 163248*p*.

(21) Korytnyk, W.; Angelino, N.; Dave, C.; Caballes, L. Guanylhydrazones with Potential Antileukemic Activity. 2. Synthesis and S.A.R. of Analogues of 4,4'-Diacetyl-N,N'-diphenylurea Bis(guanylhydrazone). *J. Med. Chem.* **1978**, *21*, 507-513.

(22) Denny, W. A.; Cain, B. F. Potential Antitumor Agents. 31. Q.S.A.R. for the Antileukemic Bis(guanylhydrazones). *J. Med. Chem.* **1979**, *22*, 1234-1238.

(23) Noel, A.; Ryznerski, Z.; Berge, G.; Fulcrand, P.; Chevallet, P.; Castel, J.; Orzalesi, H. Phenoxyacetaldehyde-guanylhydrazones as growth inhibitors of Lepidium sativum L. roots. *Eur. J. Med. Chem.* **1979**, *14*, 135-142.

(24) Alves, A.; Fulcrand, P.; Berge, G.; Noel-Artis, A.; Castel, J.; Orzalesi, H. QSAR of new substituted guanylhydrazones which inhibit plant growth. *Eur. J. Med. Chem.* **1986**, *21*, 297-304.

(25) Antineoplastic activity and toxicity of dihydroambazone in comparison to ambazone (1,4-benzoquinone guanylhydrazone thiosemicarbazone). Gutsche, W.; Hartl, A.; Baumgart, J.; Schulze, W. *Pharmazie* **1990**, *45*, 55-57.

(26) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Filippeschi, S.; Pintus, C. Potential antitumor agents. 1. Nitrophenylhydrazones of 2-chloro-3-formyl indoles. *Boll. Chim. Farm.* **1977**, *116*, 370-373.

(27) Andreani, A.; Bonazzi, D.; Rambaldi, M.; Mungiovino, G. Potential antitumor agents. 2. Hydrazone derivatives of 2-chloroindoles. *Boll. Chim. Farm.* **1979**, *118*, 18-22.

(28) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Greci, L.; Andreani, F. Study on Compounds with Potential Antitumor Activity. III. Hydrazone Derivatives of 5-Substituted 2-Chloro-3-formyl-6-methylindole. *Farmaco, Ed. Sci.* **1979**, *34*, 132-138.

(29) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Greci, L.; Andreani, F. Potential antitumor agents. 4. Thiosemicarbazones of 2-chloro-3-formyl-6-methylindoles and -indole quinones. *Farmaco, Ed. Sci.* **1979**, *34*, 1062-1070.

(30) Andreani, A.; Rambaldi, M.; Bonazzi, D. Potential antitumor agents. 5. Hydrazone derivatives of 6-substituted 5-formylimidazo[2,1-b]thiazoles and 2,3-dihydro-5-formylimidazo[2,1-b]thiazoles. *Farmaco, Ed. Sci.* **1980**, *35*, 573-580.

(31) Andreani, A.; Rambaldi, M.; Locatelli, A.; Andreani, F.; Aresca, P.; Bossa, R.; Galatulas, I. Potential Antitumor Agents. XVII. Cytotoxic Agents from Indole Derivatives and their Intermediates. *Acta Pharm. Nord.* **1990**, *2*, 407-414.

(32) Andreani, A.; Bonazzi, D.; Rambaldi, M. Potential Antitumor Agents. VII. 5-Substituted 6-Phenylimidazo[2,1-b]thiazoles. *Arch. Pharm.* **1982**, *315*, 451-456.

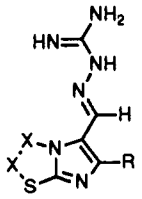
(33) Andreani, A.; Rambaldi, M.; Andreani, F.; Bossa, R.; Galatulas, I. Potential Antitumor Agents. XVI. Imidazo[2,1-b]thiazoles. *Eur. J. Med. Chem.* **1988**, *23*, 385-389.

(34) Brockman, R. W.; Thomson, J. R.; Bell, M. J.; Skipper, H. E. Observations on the Antileukemic Activity of Pyridine-2-carboxaldehyde Thiosemicarbazone and Thiocarbohydrazone. *Cancer Res.* **1956**, *16*, 167-170.

(35) Freedlander, B. L.; French, F. A. Carcinostatic Action of Polycarbonyl Compounds and Their Derivatives. II. Glyoxal Bis(guanylhydrazone) and Derivatives. *Cancer Res.* **1958**, *18*, 360-363.

0022-2623/92/1835-4634$03.00/0

Table I. Guanylhydrazones 1–10



compd	X	R	formula (mw)	mp, °C	ν_{max},[a] cm^{-1}
1	CH	Cl	$C_7H_7ClN_6S\cdot HCl\cdot H_2O$ (297.2)	283–285	1670, 1630, 1330, 1245
2	CH	CH_3	$C_8H_{10}N_6S\cdot 2HCl\cdot H_2O$ (313.2)	255–257	1675, 1610, 1210, 1155
3	CH	C_6H_5	$C_{13}H_{12}N_6S\cdot 2HCl\cdot H_2O$ (375.3)	235–238	1680, 1630, 1605, 1160
4	CH	C_6H_4Cl-*p*	$C_{13}H_{11}ClN_6S\cdot 2HCl\cdot H_2O$ (409.8)	300–303	1675, 1630, 1560, 1235
5	CH	$C_6H_4CH_3$-*p*	$C_{14}H_{14}N_6S\cdot 2HCl\cdot H_2O$ (389.3)	255–258	1670, 1600, 1490, 1160
6	CH_2	Cl	$C_7H_9ClN_6S\cdot HCl$ (281.2)	290–292	1665, 1620, 1235, 1155
7	CH_2	CH_3	$C_8H_{12}N_6S\cdot 2HCl\cdot H_2O$ (315.2)	288–291	1670, 1620, 1495, 1160
8	CH_2	C_6H_5	$C_{13}H_{14}N_6S\cdot 2HCl\cdot H_2O$ (377.3)	250–253	1675, 1610, 1495, 1160
9	CH_2	C_6H_4Cl-*p*	$C_{13}H_{13}ClN_6S\cdot 2HCl\cdot H_2O$ (411.8)	234–237	1675, 1620, 1600, 1170
10	CH_2	$C_6H_4CH_3$-*p*	$C_{14}H_{16}N_6S\cdot 2HCl\cdot H_2O$ (391.3)	253–256	1675, 1610, 1500, 1165

[a] The NH groups give broad bands in the range 3600–2300 cm^{-1}.

works stimulated numerous papers.[36] While the research in this field is still very active, mitoguazone[37,38] and ambazone[39] are under clinical trials, and bisantrene[40] is commercially available in France under the name of Zantrene.[41]

In connection with the aforementioned considerations, we now wish to report the synthesis of a series of guanylhydrazones (see Table I) from imidazo[2,1-*b*]-thiazoles (**1–5**) and thiazolines (**6–10**) bearing a substituent at position 6. The aim of this work is to study the effect of the antitumor activity of this substituent and of the double bond at position 2,3.

Chemistry. Compounds **1–10** were prepared by reaction of aminoguanidine with the appropriate aldehydes previously reported,[32,42] no evidence for the formation of *E–Z* mixtures was noticed. The ^{1}H NMR and MS (Table II) are in agreement with the assigned structures (Table I).

The protons at position 2,3 show the typical aromatic (**1–5**) or aliphatic (**6–10**) pattern. The —CH=N— group at position 5 gives rise to one singlet only in the range 8.1–8.6 ppm. This means that every derivative is a pure geometrical isomer, because in our experience[43] the separation between the resonance of the *E* and *Z* —CH=N— proton is large. This evidence is confirmed by a recent paper on the ^{1}H NMR of bis-guanylhydrazones.[44] Moreover from a literature survey we found three reports only[45–47] dealing with the conversion[45,46] or the separation[47] of the geometrical isomers of guanylhydrazones and when the pharmacological activity was involved,[47] no significant difference was noticed between the activity of the *E* and *Z* isomers.

Nevertheless, as homonuclear NOE (nuclear Overhauser enhancement) difference spectroscopy has been recently defined as a powerful and rapid tool for elucidating the *E–Z* configurations and conformations,[48–52] we decided to apply this method to compound **2** (Scheme I) in DMSO-d_6 solution. First of all we irradiated the methyl group (2.45 ppm) and we found a 5% enhancement of the —CH= group (8.43 ppm); when the NH group (12.15 ppm) was irradiated, a 18% enhancement of the —CH= group was observed; finally the irradiation of the —CH= group gave enhancement of both NH (5%) and CH_3 (3%). This through-space connection between the —CH= proton and the methyl protons is impossible in structures **a** and **c**, and structure **d** may be excluded because it does not agree with the connection between —CH= and NH. As a result of this experiment, only structure **b** fits with the observed values. As far as a possible tautomeric form is concerned (Scheme II), we believe that hydrochlorides **1–10** in DMSO-d_6 solution consist of the tautomer represented by **e** instead of the endiamine **f**. In fact, as shown in Table II, compounds **1–10** give rise to a singlet at ~12 ppm corresponding to one NH group and to a broad signal at ~8 ppm corresponding to one NH and one NH_2 group. As these results are in contrast with those reported in the aforementioned paper,[44] we prepared the free base of compound **6** and we measured its ^{1}H-NMR spectrum in DMSO-d_6. The NH_2 bands were quite different from the hydrochloride and in agreement with the guanylhydrazones previously cited.[44] In fact compound **6** as free base gives two slightly broadened signals (5.60 and 5.83 ppm) with equal intensities (two protons each), thus suggesting

(36) Agrawal, K. C.; Sartorelli, A. C. The Chemistry and Biological Activity of N-Heterocyclic Carboxaldehyde Thiosemicarbazones. *Prog. Med. Chem.* **1978**, *15*, 321–356.

(37) Mitoguazone. *Drugs Future* **1984**, *9*, 199–202.

(38) Mitoguazone. *Drugs Future* **1990**, *15*, 314.

(39) Ambazone. *Drugs Future* **1990**, *15*, 1071–1073.

(40) CL-216,942. *Drugs Future* **1981**, *6*, 762–763.

(41) Bisantrene Hydrochloride. *Drugs Future* **1989**, *14*, 1212.

(42) Andreani, A.; Rambaldi, M.; Bonazzi, D.; Greci, L. 5,6-Disubstituted imidazo[2,1-b]thiazoles with potential antiinflammatory activity. *Boll. Chim. Farm.* **1980**, *119*, 647–652.

(43) Andreani, A.; Rambaldi, M.; Locatelli, A.; Bongini, A.; Bossa, R.; Galatulas, I.; Ninci, M. Synthesis and Cardiotonic Activity of Pyridylmethylene-2-indolinones. *Eur. J. Med. Chem.* **1992**, *27*, 167–170.

(44) Elo, H. Proton Nuclear Magnetic Resonance Spectroscopy of Bis(amidinohydrazones) (Bisguanylhydrazones), and Its Use for Studies on the Isomerism and Tautomerism of the Compounds. *Spectrosc. Lett.* **1989**, *22*, 123–160.

(45) Richter, P.; Besch, A.; Schleuder, M.; Kasbohm, K.; Rintelmann, D.; Heinzig, A.; Albrecht, D.; Hagen, A. Preparation of 2-Amino Substituted Benzophenone Z-Amidinohydrazones. (1991) Ger (East) DD 290185; *Chem. Abstr.* **1991**, *115*, 182855*f*.

(46) Richter, P.; Schleuder, M.; Geissler, G.; Tomaschewski, G.; Hagen, A. Preparation of 2-Substituted Benzophenone Z-Amidinohydrazones. (1991) Ger (East) DD 290183; *Chem. Abstr.* **1991**, *115*, 182856*g*.

(47) Ercoli, A.; Gardi, R.; Gladiali, S.; Tenconi, F.; Vitali, R. Specific Synthesis of Syn and Anti Isomers of Testosterone Amidinohydrazone *Boll. Chim. Farm.* **1977**, *116*, 218–221.

(48) Easmon, J.; Heinisch, G.; Holzer, W. Pyridazines 47. The Configuration of Novel Thiosemicarbazone Derivatives of Pyridazinecarboxaldehydes and Alkyl Pyridazinyl Ketones. *Heterocycles* **1989**, *29*, 1399–1408.

(49) Colombo, A.; Frigola, J.; Pares, J.; Andaluz, B. Synthesis and NMR (^{1}H and ^{13}C) Studies of Azole Analogs of Diclofurime. *J. Heterocycl. Chem.* **1990**, *27*, 615–622.

(50) Heinisch, G.; Holzer, W. Convenient and Rapid Determination of the Configuration of Aldoximes and Ketoximes by Means of NOE Difference Spectroscopy. *Tetrahedron Lett.* **1990**, *31*, 3109–3112.

(51) Heinisch, G., Holzer, W. Configurational Assignement of Aryl Heteroaryl Ketoximes by Means of Homonuclear NOE Difference Spectroscopy. *Collect. Czech. Chem. Commun.* **1991**, *56*, 2251–2257.

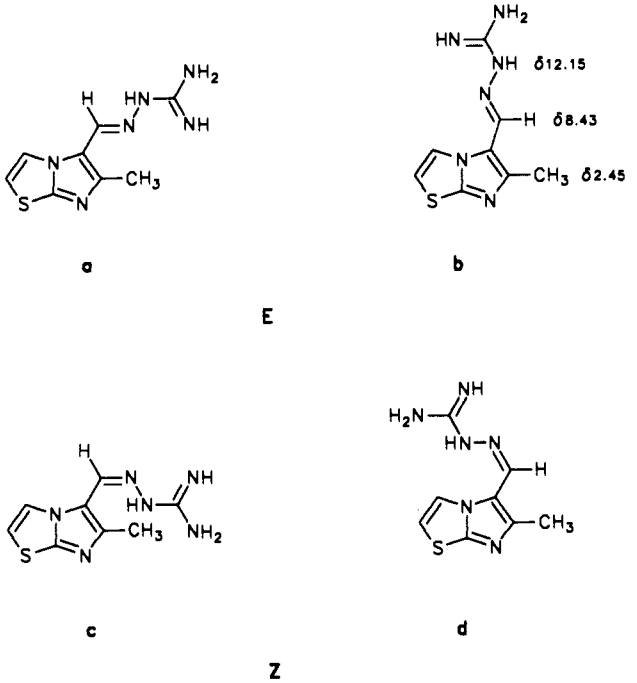
(52) Toth, G.; Levai, A.; Duddeck, H. (1992) Synthesis, Tautomerism and Stereochemistry of Spiropyrazolines. *Magn. Res. Chem.* **1992**, *30*, 235–239.

Table II. ^{1}H NMR and MS of Compounds 1–10

compd	^{1}H NMR in DMSO-d_6:[a] δ, ppm; J, Hz	MS, m/z (%)
1	7.59 (1 H, d, th, J = 4.4), 8.30 (1 H, s, —CH=), 8.66 (1 H, d, th, J = 4.4)	242 (M$^+$, 67), 207 (44), 165 (100), 135 (35)
2	2.45 (3 H, s, CH_3), 7.61 (1 H, d, th, J = 4.4), 8.43 (1 H, s, —CH=), 8.71 (1 H, d, th, J = 4.4)	222 (M$^+$, 100), 180 (18), 163 (50), 150 (58), 137 (15)
3	7.48 (3 H, m, ar), 7.55 (1 H, d, th, J = 4.4), 7.70 (2 H, d, ar), 8.50 (1 H, s, —CH=), 8.72 (1 H, d, th, J = 4.4)	284 (M$^+$, 100), 241 (6), 225 (32), 211 (38), 200 (7)
4	7.52 (1 H, d, th, J = 4.4), 7.55 (2 H, d, ar, J = 7.7), 7.78 (2 H, d, ar, J = 7.7), 8.60 (1 H, s, —CH=), 8.78 (1 H, d, th, J = 4.4)	318 (M$^+$, 100), 275 (4), 260 (23), 245 (10), 234 (6), 211 (23)
5	2.40 (3 H, s, CH_3), 7.37 (2 H, d, ar, J = 7.7), 7.60 (2 H, d, ar, J = 7.7), 7.62 (1 H, d, th, J = 4.4), 8.57 (1 H, s, —CH=), 8.83 (1 H, d, th, J = 4.4)	298 (M$^+$, 100), 255 (12), 239 (57), 225 (33), 214 (24), 211 (33)
6	3.99 (2 H, t, thn, J = 7.5), 4.62 (2 H, t, thn, J = 7.5), 8.14 (1 H, s, —CH=)	244 (M$^+$, 40), 209 (60), 167 (100)
7	2.33 (3 H, s, CH_3), 4.12 (2 H, t, thn, J = 7.5), 4.70 (2 H, t, thn, J = 7.5), 8.25 (1 H, s, —CH=)	224 (M$^+$, 100), 165 (26), 152 (41), 124 (81)
8	4.12 (2 H, t, thn, J = 7.5), 4.80 (2 H, t, thn, J = 7.5), 7.60 (5 H, m, ar), 8.42 (1 H, s, —CH=)	286 (M$^+$, 100), 227 (20), 186 (57)
9	4.14 (2 H, t, thn, J = 7.5), 4.78 (2 H, t, th, J = 7.5), 7.60 (2 H, d, ar, J = 7.7), 7.75 (2 H, d, ar, J = 7.7), 8.44 (1 H, s, —CH=)	320 (M$^+$, 100), 261 (23), 220 (79), 213 (17)
10	2.35 (3 H, s, CH_3), 4.07 (2 H, t, thn, J = 7.5), 4.71 (2 H, t, thn, J = 7.5), 7.30 (2 H, d, ar, J = 7.7), 7.50 (2 H, d, ar, J = 7.7), 8.29 (1 H, s, —CH=)	300 (M$^+$, 100), 241 (31), 200 (73)

[a] The NH groups give a broad signal at δ ~8 (3 H) and a singlet at δ ~12 (1 H). Abbreviations: th = thiazole; thn = thiazoline; ar = aromatic.

Scheme I: Compound 2



Scheme II: Compounds 1–10

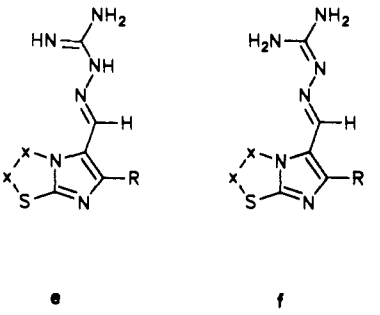


Table III. Antitumor Activity of Compounds 1–10

compd	% T/C	dose, mg/kg ip	compd	% T/C	dose, mg/kg ip
1	200	50	7	100	50
2	100	100	8	100	50
3	120	50	9	180	25
4	190	12.5	10	100	50
5	110	25	5-fluorouracil	180	100
6	180	100			

that, at least under these experimental conditions, it consists of tautomer **f**. The MS of the 2,3-dihydro derivatives (**6–10**) show a simpler fragmentation pattern in comparison to that of the unsaturated analogs (**1–5**). The molecular ion peak is always stable as it is the base peak, except in the 6-chloro derivatives (**1**, **6**), where, however, it is a prominent peak. This result, in contrast with the high melting points, is in agreement with that reported in a recent paper on the MS of bisguanylhydrazones.[53]

From the figures reported in Table II it is easy to recognize that the 6-chloro derivatives (**1**, **6**) give a peak at M – 35 (from loss of chlorine), which then loses 42. All the other compounds directly show a prominent peak at M – 59 from loss of guanidine and/or M – 43 (M – 42) from loss of the terminal amidino group (or its deprotonated form).

Pharmacological Results. The compounds reported in Table I were tested in mice bearing Ehrlich ascites tumor cells (see Experimental Protocols), and the results are reported in Table III.

All four active compounds bear a chlorine at the 6 position (**1**, **6**) or at the phenyl ring of the 6 position (**4**, **9**); the chlorophenyl group seems more suitable than chlorine in terms of therapeutic index.

The effect of the double bond at position 2,3 is much less evident than the effect of the substituent at position 6, but the unsaturated compounds (**1**, **4**) seem slightly more active than the corresponding 2,3-dihydro analogs

(53) Elo, H.; Matikainen, J.; Kaltia, S. Mass Spectroscopy of the Antileukemic Drug MGBG and Related Bis(amidinohydrazones) (Bisguanylhydrazones). *Spectrosc. Lett.* **1990**, *23*, 865–876.

(6, 9). The synthesis of new derivatives is in progress following the preliminary indications provided by these results.

Experimental Protocols. (a) Chemistry. The melting points are uncorrected. Analyses (C, H, N) were within ±0.4% of the theoretical values. TLC of compounds 1–10 as free bases was performed on Bakerflex plates (silica gel IB2-F); the eluent was acetone/petroleum ether/30% NH_4OH in the proportion of 50/49/1. The IR spectra were recorded in Nujol on a Perkin-Elmer 298. The ^1H NMR spectra were recorded on a Varian Gemini 300 using TMS as the internal standard. For the NOE experiments, 512 scans were employed. The EI-MS were recorded at 70 eV on a VG 7070 E.

Guanylhydrazones 1–10. A 10 mmol portion of the appropriate imidazo[2,1-*b*]thiazolecarboxaldehyde[32,42] was dissolved in 50 mL of ethanol and treated with the equivalent of aminoguanidine hydrochloride, prepared from an ethanol suspension of aminoguanidine bicarbonate and 37% hydrochloric acid. The reaction mixture was refluxed for 30 min and the resulting precipitate was collected by filtration with a yield of 85–90% (Tables I and II).

(b) Pharmacology. Eight female Swiss mice (average weight 21 ± 1 g) were implanted with 10^6 Ehrlich ascites tumor cells from donor mice. After 24 h the animals were treated ip with a single dose (100 mg/kg) of the test compound (1–10) dissolved in DMSO; the amount of DMSO, previously used in analogous experiments, did not affect tumor growth. If the dose proved to be active, the test was repeated at lower doses with other groups of eight mice. Deaths were recorded for a period of 60 days. The activity was measured as the ratio of the mean survival time of the test animals to that of the control (10 mice receiving vehicle only) expressed as a percentage (%T/C). Significant activity is achieved as 25% increase in the life span ($T/C \geq 125$).

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Supplementary Material Available: ^1H NMR and NOE spectra of **2** and ^1H NMR spectra of **6** (6 pages). Ordering information is given on any current masthead page.