Journal of Medicinal and Pharmaceutical Chemistry

VOL. 4, No. 1 (1961)

Antibacterial Agents. Some New Guanylhydrazone Derivatives

G. CAVALLINI, E. MASSARANI, D. NARDI, L. MAURI and P. MANTEGAZZA, *Medicinal Chemistry Research Laboratories, Vister-Casatenovo Brianza (Como), Italy*

In the continuation of our studies on the effect on 'supporting moieties' in the structures of pharmacodynamically active drugs,[1] we have turned our attention to the question of whether this hypothesis could also prove useful in the field of chemotherapy, especially for compounds with potential *in vivo* antiviral activity.[2,3] Since certain guanylhydrazones are active as antibacterial agents, [4-7] we considered it of interest to join the guanylhydrazone radical, —CH=NNHC(=NH)NH_2, to benzene and in the 4- and 4,4′-positions, to biphenyl, diphenyl ether, diphenyl sulphide, diphenyl sulphone, diphenylmethane, diphenylethane and stilbene, in the hope that antibacterial activity would increase in some of the molecules containing these 'supporting moieties'.*

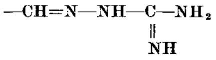
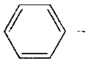
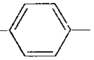
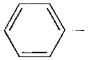
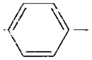
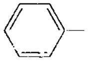
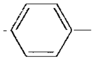
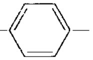
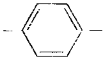
The aldehydes needed in our experiments, with the exception of diphenylethane-4-aldehyde and 4,4′-diphenyl sulphide dialdehyde, were already known. These compounds were prepared from the corresponding chloromethyl derivatives by the Sommelet reaction. The guanylhydrazones were obtained from the corresponding aldehydes with 4-aminoguanidine in acid solution; their hydrochlorides were sufficiently soluble in water for biological testing. The guanylhydrazones prepared are listed in Table I.

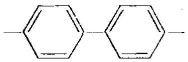
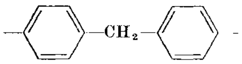
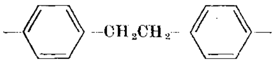
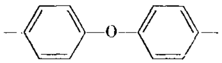
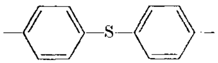
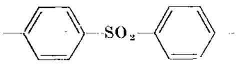
The purity of the guanylhydrazones was confirmed by monodimensional ascending paper chromatography; their R_f values are listed in Table II.

---

* While our work was in progress, the guanylhydrazones of 4,4′-biphenyldialdehyde and 4,4′-diphenyl ether dialdehyde were also synthesized and tested for antibacterial activity in another laboratory.[8]

Table I. Guanylhydrazones: chemical and physical data

	 $—CH=N—NH—\underset{\underset{NH}{\|}}{C}—NH_2$	Yield, %	m.p., °C	Solvent of cryst.[a]	Empirical formula	Analysis									
						Calcd.					Found				
						C	H	Cl	N	S	C	H	Cl	N	S
I	C_6H_5 	84	225	M	$C_{14}H_{14}N_4$	70·56	5·92		23·51		70·33	5·71		23·10	
			201–202	A–E	$C_{14}H_{14}N_4.HCl$			12·90	20·39				12·75	20·05	
II	$C_6H_5CH_2$ 	83	177–178	A–W	$C_{15}H_{16}N_4$				22·21					21·97	
			198–200	A–E	$C_{15}H_{16}N_4.HCl$			12·28	19·40				12·50	19·10	
III	$C_6H_5CH_2CH_2$ 	82	180–181	A–W	$C_{16}H_{18}N_4$	72·15	6·81		21·04		72·37	6·85		20·92	
			228–229	A–E	$C_{16}H_{18}N_4.HCl$			11·71	18·50				11·75	18·20	
IV	$C_6H_5CH=CH$ 	82	286–287	M–E	$C_{16}H_{16}N_4.HCl$			11·79	18·62				12·10	18·10	
V	C_6H_5O 	79	179–179	A–W	$C_{14}H_{14}N_4O$				22·04					22·50	
			173–175	A–E	$C_{14}H_{14}N_4O.HCl$			12·19	19·27				12·35	18·94	
VI	C_6H_5S 	35	180–181	Ac–B	$C_{14}H_{14}N_4S$[b]				20·73	11·84				20·50	11·60
VII	$C_6H_5SO_2$ 	87	264–265	M–E	$C_{14}H_{14}N_4O_2S.HCl$			10·46	16·54	9·46			10·55	16·26	9·42
VIII					$C_{10}H_{14}N_8$[c]				45·50					45·61	
		90	318[d]	M–E	$C_{10}H_{14}N_8.2HCl$			22·23	35·14				22·20	35·08	

IX		70	328–329	M–E	$C_{16}H_{18}N_8.2HCl$	48·62	5·10	17·94	28·32		48·11	5·29	17·48		
X		70	268–270[d]	M–W	$C_{17}H_{20}N_8$	60·69	5·99		33·31		60·32	6·35		32·70	
XI		80	271[d]	A–W	$C_{18}H_{22}N_8$	61·69	6·33		31·98		61·54	6·58		31·93	
XII			282–284		$C_{16}H_{18}N_8O$[c]				33·90					33·40	
		75	245–247	A–E	$C_{16}H_{18}N_8O.HCl$			17·24	27·24				17·18	26·98	
XIII		75	265	A–W	$C_{16}H_{18}N_8S.2H_2O$	49·21	5·68		28·70		49·58	6·18		28·28	
XIV		80	175	A	$C_{16}H_{18}N_8O_2S$				29·00	8·28				28·48	8·05
			310	A–E	$C_{16}H_{18}N_8O_2S.2HCl$			15·43	24·41	6·98			15·60	23·98	6·85

---

[a] M = Methanol; A = Ethanol; E = Ether; W = Water; Ac = Ethyl acetate; B = Petroleum ether (30–50°).

[b] The base was extracted with ethyl acetate.

[c] The base was obtained with aqueous NaOH from the aqueous solution of the hydrochloride.

[d] Decomposition.

Table II.[a] R_f values of guanylhydrazones

Compound	Solvents[b]			
	A	B	C	D
I	0·85	0·76	0·75	0·46
II	0·89	0·99	0·76	0·61
III	0·89	0·99	0·72	0·52
IV	0·80	0·73	0·45[c]	0·15[c]
V	0·82	0·99	0·74	0·65
VI	0·90	0·99	0·78	0·55
VII	0·89	0·96	0·89	0·76
VIII	0·36	0·13	0·78	0·55
IX	0·49	0·16	0·59	0·28[c]
X	0·75	0·67	0·80	0·56
XI				
XII	0·75	0·44	0·76	0·59
XIII				
XIV	0·69	0·43	0·88	0·73

[a] The table shows the R_f values of the ascending paper chromatography carried out on Whatman paper No. 1. Temperature of chromatography room 20°±1°. The length of the run was 25 cm. The spots were detected by spraying the strips with Dragendorff's $KBiI_4$ reagent.

[b] A = *n*-Butanol saturated with water and acetic acid; B = Ethanol 80, water 10, hydrochloric acid 10; C = 30% aqueous acetic acid; D = 15% aqueous acetic acid.

[c] These compounds gave elongated spots.

Experimental†

A. General method for the preparation of the guanylhydrazones. A suspension of 4-aminoguanidine hydrochloride (2·72 g, 0·02 mole) in water (30 ml) was brought into solution by addition of 35 per cent hydrochloric acid (2 ml), a solution of the aldehyde (0·02 mole) in ethanol (50 ml) was added, and the mixture was heated with stirring for 2 h. After cooling, water was added, the base was precipitated with sodium hydroxide solution, the precipitate was centrifuged, washed with water, and crystallized from an appropriate solvent. The substances appeared as colourless crystals. Their properties and analytical data are listed in Table I.

B. Microbiology. The antimicrobial and antifungal spectra of all the guanylhydrazones *in vitro* was carried out by the usual

---

† All melting points are uncorrected.

procedures, by the tube dilution method. In Table III, the minimal inhibiting concentrations are reported for *Streptococcus hemolyticus A*, *Micrococcus pyogenes* var. aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Neurospora sitophila*.

Table III. Minimal inhibiting concentration μg/ml of fifteen guanylhydrazones

Compound	Test Organism				
	Micrococcus pyogenes var. *aureus*[a]	*Streptococcus hemolyticus A*[a]	*Escherichia coli*[b]	*Pseudomonas aeruginosa*[b]	*Neurospora sitophila*[c]
XV[d]	0[e]	50	0	0	0
I	3	3	12·5	12·5	12·5
II	3	3	12·5	12·5	25
III	3	1·5	6	12·5	12·5
IV	1·5	1·5	3	12·5	3
V	6	3	25	12·5	50
VI	3	1·5	6	12·5	12·5
VII	100	50	100	100	0
VIII	50	3	50	25	50
IX	12·5	0·7	6	6	6
X	3	3	6	3	6
XI	3	0·4	6	3	3
XII	6	3	25	3	6
XIII	1·5	0·4	3	6	1·5
XIV	0	0	25	50	12·5

[a] Medium: Difco tryptose phosphate broth. [b] Medium: nutrient broth. [c] Medium: Sauburaup liquid medium. [d] Benzalaldehyde guanylhydrazone. [e] The number zero indicates no activity under 100 μg/ml.

These screening data indicate that only certain 'supporting moieties' increase the antibacterial activity of the guanylhydrazone radical to a specific and significant degree. The phenyl and diphenyl sulphone groups do not appear as proper increments, while the biphenyl, diphenylmethane and diphenylethane, stilbene, diphenyl ether and diphenyl sulphide moieties support our explanation of the increased and broadened activity of the respective guanylhydrazones. As previously observed in other series,[9, 10] activity increases from phenyl to biphenyl to stilbene and to diphenylethane groups. 4,4′-Disubstituted derivatives were more active than monosubstituted guanylhydrazones. The two most active derivatives were the bis-guanylhydrazones of 4,4′-diphenylethanedialdehyde (XI) and diphenyl sulphide 4,4′-dialdehyde (XIII).

Summary. A series of guanylhydrazones derivatives of benzene, biphenyl, diphenylmethane, diphenylethane, diphenyl ether, diphenyl sulphide and diphenyl sulphone has been prepared.

All the compounds show a considerable antibacterial activity especially the bis-guanylhydrazones of 4,4′-diphenylethanedialdehyde and diphenyl sulphide 4,4′dialdehyde.

(*Received 8 August, 1960*)

References

[1] Cavallini, G. *Farmaco*, **10**, 644 (1955)
[2] Cavallini, G. and Massarani, E. This Journal, **1**, 365 (1959)
[3] Cavallini, G., Massarani, E., Nardi, D., Magrassy, F., Altucci, P., Lorenzutti, G. and Sapio, U. This Journal, **1**, 601 (1959)
[4] Fujikawa, F., Tokuoka, A., Takimura, M. and Miura, K. *J. pharm. Soc. Japan*, **72**, 518 (1952)
[5] Fujikawa, F., Tokuoka, A., Miura, K., Kometani, E., Nakazawa, S., Omatsu, T. and Toyoda, T. *J. pharm. Soc. Japan*, **73**, 20 (1953)
[6] Hayashi, S. *Kumamoto Pharm. Bull.*, **1**, 93 (1954)
[7] Tetsumoto, S., Okitsu, T. and Fukuda, M. *Bull. Jap. Soc. sci. Fish.*, **20**, 1099 (1955)
[8] Farbenfabriken Bayer A.G., Brit. 768,089, Feb. 13 (1957). *Chem. Abstr.*, **51**, 16539 (1957)
[9] Milla, E. and Grumelli, E. *Farmaco*, **6**, 150 (1951)
[10] Cavallini, G., Mantegazza, P., Massarani, E. and Tommasini, R. *Farmaco*, **8**, 2 (1953)