The Synthesis of Some Heterocyclic Derivatives of Biguanide with Antibacterial Activity

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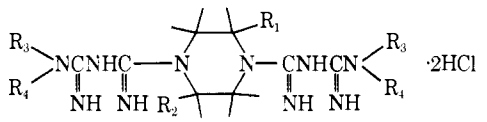
A series of N^5-alkyl or -aryl mono- and bisguanides in which the N^1 atom is part of a heterocyclic nucleus has been prepared by reaction of an appropriate cyanoguanidine with an amine hydrochloride. Compounds have been prepared from piperazine, 2-methyl-, 4-methyl-, and 2,5-dimethylpiperazine, 2-aminoethylpiperazine, 4-methylpiperidine, piperidine, and morpholine. Several highly active antibacterial compounds are reported, especially 1,4-bis[N^1-(N^1-*p*-chlorophenylamidino)amidino]piperazine dihydrochloride (picloxydine),[1] which shows high activity against a wide variety of gram-negative and gram-positive organisms.

Many substituted biguanides have been shown to possess chemotherapeutic activity against, for instance, protozoa, bacteria, and viruses. Thus N^1-*p*-chlorophenyl-N^5-isopropylbiguanide (chlorguanil) was shown by Curd and Rose[2] to be active against the malaria parasite. Later Rose and Swain[3] synthesized a series of bisbiguanides from which 1,6-bis(N^5-*p*-chlorophenyl-N^1-biguanido)hexane dihydrochloride (IV, $n = 6$) (chlorhexidine), a compound of high antibacterial activity, was developed. A comprehensive series of over 200 biguanides has also been reported by Weinberg,[4] many of which have significant antibacterial activity. This last series included a few compounds (*e.g.*, Ib, X = H, R_3 = H, R_4 = 2,4-dichlorobenzyl) in which the N^1 nitrogen atom of the biguanide was contained within a heterocyclic system. Another compound of this type, N^1,N^1-anhydrobis(β-hydroxyethyl)biguanide (Ib, X = O, R_3 = R_4 = H) has been reported as an antiviral agent by Sjoberg[5] and Melander[6] effective against influenza in man and by Schersten[7] against herpes.

It was the object of work now reported to study in some detail a new, closely related series of mono- and bisbiguanides derived from some simple heterocyclic systems particularly with regard to their antibacterial activity in relation to their chemical structure.

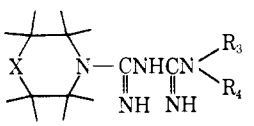
Piperazine and some of its simple alkyl analogs have afforded bisbiguanides (Table I) of structure Ia.



Ia

R_1, R_2 = H, CH_3; R_3, R_4 = H, CH_3, *i*-C_3H_7, C_6H_4Y, $C_6H_3Y_2$; Y = F, Cl, Br, CH_3

Monobiguanides (Table II) of similar type (Ib) have



Ib

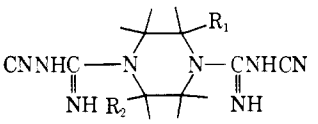
X = CH_2, NCH_3, $CHCH_3$, O, $NCH_2CH_2NHCNHCNR_3R_4$; (NH NH)

R_3, R_4 = H, CH_3, $CH(CH_3)_2$, *p*-C_6H_4Cl

(1) J. W. James and L. F. Wiggins, British Patent 855,017 (1959).
(2) F. H. S. Curd and F. L. Rose, *J. Chem. Soc.*, 729 (1946).
(3) F. L. Rose and G. Swain, *ibid.*, 4422 (19561.

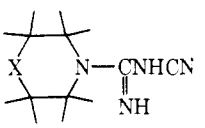
been prepared from 4-methylpiperazine, 4-methylpiperidine, and morpholine. Another bisbiguanide was prepared from the N-2-aminoethylpiperazine (Table II) in which only one of the biguanide groups formed part of a heterocyclic ring.

It was found that the standard methods[2,8] for preparing N^1,N^5-substituted biguanides from primary aliphatic amines were applicable to secondary heterocyclic amines. Thus the heterocyclic amine hydrochlorides were heated in butanol, or in some instances water, with sodium dicyanimide to give the cyanoguanidines of the type IIa and b (Table III). Suspensions



IIa

R_1, R_2 = H or CH_3



IIb

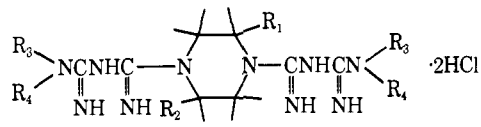
X = NCH_3, O, $NCH_2CH_2NHCNHCN$, CH_2, $CHCH_3$ (NH)

of the cyanoguanidines so prepared were heated in boiling 2-ethoxyethanol with various aromatic and aliphatic amine hydrochlorides to give the 1,5-substituted biguanides of structures Ia and b. Some of the biguanides, particularly those substituted with alkyl groups at N^5, were obtained by treating the heterocyclic amine with the cyanoguanidine derived from the aromatic or aliphatic amine. The biguanides (Ia, Ib where R_3 and R_4 = H) were prepared by fusing cyanoguanidine with the heterocyclic amine hydrochloride.[9]

Biological Results.—All of the compounds listed in Tables I–III were screened initially for antibacterial activity by the conventional *in vitro* "zone of inhibition" method against a variety of bacteria. None of the cyanoguanidines or N^5-alkyl-substituted biguanides showed any activity at all. The N^5-aryl-substituted

(4) E. D. Weinberg, *Antibiot. Chemotherapy*, **11**, 572 (1961).
(5) B. Sjoberg, *Antibiot. Med. Clin. Therapy*, **7**, 97 (1960).
(6) B. Melander, *Antibiot. Chemotherapy*, **10**, 34 (1960).
(7) B. Schersten, *Svenska Lakartidn.*, **56**, 3563 (1956).
(8) F. H. S. Curd, J. A. Hendry, T. S. Kenny, A. G. Murray, and F. L. Rose, *J. Chem. Soc.*, 1630 (1948).
(9) E. Bamberger and L. Seeberger, *Ber.*, **24**, 900 (18911.

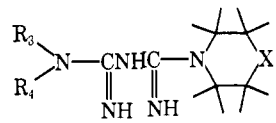
TABLE I



R_1	R_2	R_3	R_4	Mp, °C	Procedure	Reaction time, hr	Yield, %	Recrystn solvent[a]	Formula[e]
H	H	CH_3	p-ClC_6H_4	281–282	2	16	60	A	$C_{20}H_{28}Cl_2N_{10}\cdot 2H_2O$
H	H	H	p-ClC_6H_4	279–282	2	14	79	A	$C_{20}H_{26}Cl_4N_{10}$
H	H	CH_3	p-ClC_6H_4	281–282	2	16	60	A	$C_{22}H_{28}Cl_4N_{10}$
CH_3	H	H	p-ClC_6H_4	251–253	2	6	44	A–B	$C_{21}H_{28}Cl_4N_{10}\cdot 0.5H_2O$
CH_3	CH_3	H	p-ClC_6H_4	257–260	2	8	60	A–B	$C_{22}H_{30}Cl_4N_{10}$
H	H	H	m-ClC_6H_4	234–235	2	12	11	C	$C_{20}H_{26}Cl_4N_{10}$[f]
H	H	H	3,4-$Cl_2C_6H_3$	253–255	2	18[b]	10	A	$C_{20}H_{24}Cl_6N_{10}$[f]
H	H	H	p-BrC_6H_4	275–280	2	8[b]	23	A–C	$C_{20}H_{26}Br_2Cl_2N_{10}$[f]
H	H	H	p-FC_6H_4	273	2	24	23	A–C	$C_{20}H_{26}Cl_2F_2N_{10}\cdot H_2O$[f]
H	H	H	p-$CH_3OC_6H_4$	261	2	12	19	A–D	$C_{22}H_{32}Cl_2N_{10}O_2$
H	H	H	p-$CH_3C_6H_4$	251	2	12	15	D–E	$C_{22}H_{32}Cl_2N_{10}$
H	H	H	H	257[c]	4	4	25	A	$C_8H_{20}Cl_2N_{10}$
CH_3	H	H	H	264–265[d]	4	4	26	C	$C_8H_{22}Cl_2N_{10}$
CH_3	CH_3	H	H	280–284	4	6	35	C	$C_{10}H_{24}Cl_2N_{10}$
H	H	H	$CH(CH_3)_2$	259–261	3a	24	70	A	$C_{14}H_{32}Cl_2N_{10}$
CH_3	CH_3	H	$CH(CH_3)_2$	255–257	3a	4	41	A	$C_{16}H_{36}Cl_2N_{10}\cdot 2H_2O$
H	H	CH_3	CH_3	273–275	3b	14	84	A	$C_{12}H_{28}Cl_2N_{10}\cdot 0.5H_2O$
CH_3	H	CH_3	CH_3	248–250	3b	4	89	A–F	$C_{13}H_{30}Cl_2N_{10}\cdot 2H_2O$
CH_3	CH_3	CH_3	CH_3	283–284	3b	14	71	A	$C_{14}H_{32}Cl_2N_{10}\cdot 2H_2O$

[a] A = H_2O, B = 2-ethoxyethanol, C = EtOH, D = HOAc, E = EtOAc, F = Me_2CO. [b] Solvent partially removed from reaction mixture before filtration. [c] J. Bourdais [*Bull. Soc. Chim. France*, 1174 (1962)] reports 288–290°. [d] Lit.[c] mp 294–296°. [e] Analyzed for C, H, Cl, N unless otherwise noted. [f] Analyzed for C, H, N.

TABLE II

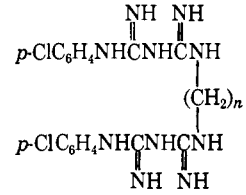


R_3	R_4	X	Salt	Mp, °C	Procedure	Reaction time, hr	Yield, %	Recrystn solvent[a]	Formula[f]
H	p-ClC_6H_4	–N(Y)–[e]	2HCl	256–258	2	4	31	A	$C_{22}H_{31}Cl_4N_{11}\cdot H_2O$
H	p-ClC_6H_4	$–CH_2–$	HCl	252	2	1	20	A–F	$C_{13}H_{19}Cl_2N_5$[g]
H	p-ClC_6H_5	–CH(CH_3)–	HCl	251	2	4	48	A–F	$C_{14}H_{21}Cl_2N_5$
H	p-ClC_6H_4	–O–	HCl	242–244	2	4[b]	35	C	$C_{12}H_{11}Cl_2ON_5$
H	p-ClC_6H_4	–N(CH_3)–	2HCl	235	2	4[b]	48	A–F	$C_{13}H_{21}Cl_2N_6$
H	H	$–CH_2–$	2HCl	215[c]	4	3	44	A–C	$C_7H_{17}Cl_2N_2$
H	H	–CH(CH_3)–	2HCl	225–226	4	3	35	A–C	$C_8H_{17}Cl_2N_5$
H	H	–O–	HCl	205–208[d]	4	2	31	C	$C_6H_{14}ClON_5$
H	H	–N(CH_3)–	2HCl	212	4	3.5	62	C–E	$C_7H_{20}Cl_2N_6\cdot 0.5H_2O$[g]
H	$CH(CH_3)_2$	–O–	HCl	223–225	3a	4	62	C	$C_9H_{20}ClON_5$
CH_3	CH_3	–O–	HCl	233–235	3b	4	80	C	$C_8H_{18}ClON_5$

[a] A = H_2O, C = EtOH, E = EtOAc, F = Me_2CO. [b] Solvent partially removed from reaction mixture before filtration. [c] Bamburger[9] reports 217°. [d] B. Melander and T. Nelson [U. S. Patent 3,073,744 (1963)] report 210–212°. [e] Y = $CH_2CH_2NHC(=NH)$-$NHC(=NH)NH(p$-$ClC_6H_4)$. [f] Analyzed for C, H, Cl, N unless otherwise noted. [g] Analyzed for C, H, N.

compounds showed considerable activity and were studied further to determine their minimum inhibitory concentrations against various bacteria. These results are tabulated (Tables IV and V) and show several of the compounds to possess high antibacterial activity and allow the following conclusions to be drawn.

(1) The bisbiguanides show significant antibacterial activity. This activity is of a lower order in the monobiguanides similarly substituted suggesting that a difunctional molecule is required for optimum activity. In comparing the activity of **2** (Table IV) and **13** (Table V), it is interesting that the introduction of the $–CH_2–CH_2–$ group between a nitrogen atom of the piperazine molecule and biguanide entity does not affect activity. This is in agreement with the findings of Rose and

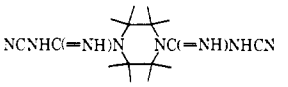
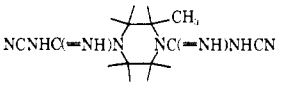
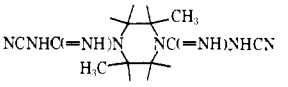
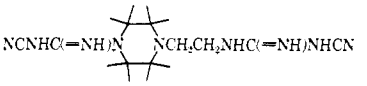
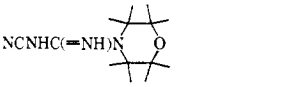
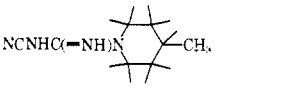
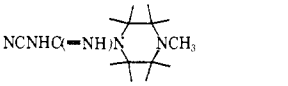


IV

Swain[3] in their polymethylene compounds (IV) where highest antibacterial activity occurred when the substituted biguanide residues are separated by a polymethylene chain of 5–7 units.

(2) The presence of a free hydrogen atom on the N^1 atom of the biguanide is not essential for antibacterial

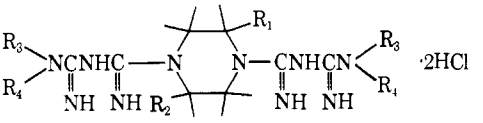
Table III

Compd	Mp, °C	Reaction time, hr	Recrystn solvent[a]	Yield (crude), %
	300	16	...	88
	300	16	...	71
	300	14	...	79
	241–244	12	A	50
	226–227	12	A	67
	159	8	A–C	68
	192	15	A–C	75

[a] A = H_2O, C = EtOH.

Table IV

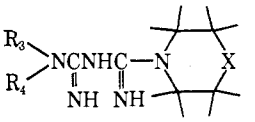
Minimum Inhibitory Concentrations



No.	R_1	R_2	R_3	R_4	MIC, μg/ml *S. aureus*	MIC, μg/ml *Ps. pyocyanea*	MIC, μg/ml *S. typhi*	MIC, μg/ml *E. coli*	LD_{50} (mice), mg/kg ip
1	H	H	H	C_6H_5	31.25	1000	62.5	125	...
2	H	H	H	*p*-ClC_6H_4	1.95	62.5	3.9	7.8	150
3	H	H	CH_3	*p*-ClC_6H_4	15	500	31	250	...
4	CH_3	H	H	*p*-ClC_6H_4	7.8	125	31.35	62.5	250
5	CH_3	CH_3	H	*p*-ClC_6H_4	7.8	125	31.25	62.5	150
6	H	H	H	*m*-ClC_6H_4	3.9	62.5	7.8	15.6	150
7	H	H	H	3,4-$(Cl)_2C_6H_3$	3.9	62.5	31.25	31.25	250
8	H	H	H	*p*-BrC_6H_4	1.95	31.25	3.9	3.9	250
9	H	H	H	*p*-FC_6H_4	1.95	250	31.25	31.25	16
10	H	H	H	*p*-$CH_3OC_6H_4$	60.6	500	62.5	500	50
11	H	H	H	*p*-$CH_3C_6H_4$	7.8	500	15.6	250	50
12	Chlorhexidine			IV, $n = 6$	3	48	1.5	3	

Table V

Minimum Inhibitory Concentrations



No.	R_3	R_4	X	MIC, μg/ml *S. aureus*	MIC, μg/ml *Ps. pyocyanea*	MIC, μg/ml *S. typhi*	MIC, μg/ml *E. coli*	LD_{50} (mouse), mg/kg ip
13	H	*p*-ClC_6H_4	N(Y)[a]	3.9	125	3.9	7.8	150
14	H	*p*-ClC_6H_4	CH_2	48	1500	96		200
15	H	*p*-ClC_6H_4	$CH(CH_3)$	156	156	7.8	78	50
16	H	*p*-ClC_6H_4	-O-	156	625	>1000	625	100
17	H	*p*-ClC_6H_4	$N(CH_3)$	156	625	>1000	625	150

[a] Y = $CH_2CH_2HNC(=NH)NHC(=NH)NHC_6H_4Cl$-*p*.

TABLE VI

BACTERIOSTATIC ACTIVITY OF PICLOXYDINE (**2**)

Organism	MIC, μg/ml
Staphylococcus aureus	3.0
Streptococcus pyogenes Gp.A.	0.76
Streptococcus pneumoniae	3.0
Streptococcus β-haemolyticus	0.76
Bacillus cereus	3.9
Bacillus anthracis	3.0
Clostridium septicum	0.3
Pseudomonas pyocyanea	48.0
Salmonella typhi	6.0
Salmonella typhimurium	19.0
Salmonella pullorum	24.0
Escherichia coli	6.0
Proteus vulgaris	156.0
Shigella sonnei	12.0

activity as shown by the fact that the piperazine residue of **2** replaces the polymethylene chain of **12**, chlorhexidine (IV, $n = 6$), without significantly altering the antibacterial activity.

(3) Activity is reduced in the bisbiguanides when the center portion of the molecule is made more bulky by substitution of methyl groups in the 2 and 5 positions (**4**, **5**) of the piperazine nucleus.

(4) In this series of compounds in order to exhibit maximum antibacterial activity the bisbiguanide entity should be substituted at N^5 by a substituted aryl group.[10] The unsubstituted compound **1** is considerably less active than **2**.

(5) Maximum activity is conferred on the bisbiguanide when the N^5-aryl group is substituted by a halogen, chlorine and bromine (**2**, **8**) being more effective than fluorine (**9**) when these substituents are in the *para* position.

(6) Disubstitution of N^5 nitrogen atoms of the bis molecules by a methyl group (**3**) decreases activity, suggesting that a free hydrogen on N^5 is beneficial.

The activity of **2**, one of the most active compounds of the series, has been determined against a wide variety of bacteria. The results (Table VI) show it to be highly active against both gram-negative and gram-positive organisms. This compound, awarded the British Pharmacopea approved name, picloxydine, has recently been introduced for topical application in both human and veterinary medical areas.

(10) G. E. Davies, J. Francis, A. R. Martin, F. L. Rose, and G. Swain, *Brit. J. Pharmacol.*, **9**, 192 (1954).

Experimental Section

The compounds shown in the tables were prepared by methods closely analogous to those in the specific cases reported below.

Cyanoguanidines (Table III). (1) **1,4-Bis(N^1-cyanoamidino)-piperazine.**—Piperazine hydrochloride (100 g, 0.63 mole) was heated with stirring under reflux for 16 hr with sodium dicyanimide (122.6 g, 1.26 moles) in BuOH (750 ml). The reaction mixture was cooled, filtered, washed thoroughly with H_2O, and dried at 80° to give 121 g (88%) of product, mp 300°.

N^1,N^5-Substituted Phenylbiguanides (Tables I and II). (**2**) **1,4-Bis[N^1-(N^1-*p*-chlorophenylamidino)amidino]piperazine Dihydrochloride.**—1,4-Bis(N^1-cyanoamidino)piperazine (77 g, 0.35 mole) and *p*-chloroaniline hydrochloride (117 g, 0.715 mole) were mixed with 2-ethoxyethanol (1 l.) to which had been added 1 ml of concentrated HCl. The mixture was heated under reflux for 12 hr with efficient stirring, cooled to room temperature, and filtered. The crude material was washed with Me_2CO and dried at 70° *in vacuo* (yield 154 g, 79%). The crude material (10 g) was recrystallized from H_2O (1 l., charcoal), the pH being adjusted to 3 with HCl. The dihydrochloride crystallized as colorless needles, was filtered, washed with H_2O, and dried, mp 279–282° (7 g, 70%).

N^1,N^5-Alkyl-Substituted Biguanides (Tables I and II). (**3a**) **1,4-Bis[N^1-(N^1-*i*-propylamidino)amidino]piperazine Dihydrochloride.**—Isopropyl dicyandiamide[7] (5 g, 0.04 mole) was heated under reflux with piperazine dihydrochloride (3.12 g, 0.02 mole) in 2-ethoxyethanol (50 ml) for 24 hr. During the reaction a solid separated. About half of the 2-ethoxyethanol was distilled at 15 mm after which the reaction mixture was cooled and filtered. Recrystallization from H_2O gave the dihydrochloride, mp 259–261° (5.7 g, 70%).

(**3b**) **1,4-Bis[N^1-(N^1-dimethylamidino)amidino]piperazine Dihydrochloride.**—Dimethyldicyandiamide[7] (5 g, 0.04 mole) and piperazine dihydrochloride (3.12 g, 0.02 mole) were heated under reflux in 2-ethoxyethanol (50 ml) for 14 hr. A solid separated and was filtered after cooling the reaction mixture. The residue was recrystallized from H_2O to give the dihydrochloride, mp 273–275° (7.0 g, 84%).

N^1-Substituted Biguanides (Tables I and II). (**4**) **1,4-Bis[N^1-(N^1-amidino)amidino]piperazine Dihydrochloride.**—An intimate mixture of piperazine hydrochloride (10 g, 0.056 mole) and cyanoguanidine (8.4 g, 0.1 mole) was heated in a flask in an oil bath with stirring until molten (*ca.* 150°). The temperature was maintained for 4 hr. The cold reaction mixture was extracted with boiling H_2O from which the dihydrochloride separated on cooling. The product was recrystallized twice from H_2O; mp 257° (4.7 g, 25%).

Acknowledgments.—The authors which to thank Messrs. D. R. Lewis, J. Weston, and Dr. C. Coutinho for the bacteriological results and Mr. M. Lonsdale for the microanalytical results.