ANTITUBERCULAR ACTIVITY OF GUANIDINE DERIVATIVES

O. A. Malyuga, R. Ya. Galanova,
G. M. Petrenko, N. A. Mukhina,
and G. V. Semukhina

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According to the literature, many guanidine derivatives possess tuberculostatic activity. Thus, some benzthiazolyl derivatives [1], benzalguanyls and their N^4-alkyl-derivatives [2], dichloroalkoxyphenylbiguanides [3], and dipyridylguanides [4] show appreciable antitubercular activity. Streptomycin and dihydrostreptomycin, which contain guanidine groups in their molecules, are highly effective in tuberculosis chemotherapy [5, 6].

The antituberculous activity of a series of diphenyl- and benzylguanidine derivatives [7, 8] was studied by us in a search for active antibacterial compounds.

The tuberculostatic activity of the compounds studied was determined in vitro by the method of serial double dilutions in Sutton's semi-synthetic medium without serum or in the presence of 10% normal horse serum. Mycobacteria of human tuberculosis, Academia and $H_{37}Rv$ strains, and avian tuberculosis, strain Avium P, were used as test cultures. The microbial loading for the $H_{37}Rv$ and Avium P strains for the submerged method of inoculation, checked against a BCG standard, was 0.1 mg of bacilli in 5 ml of the culture. The surface method of cultivation was employed for the Academia strain. The degree of tuberculostatic activity was judged by the magnitude of the minimum bacteriostatic concentration (MBC) which completely arrested the growth of the tuberculosis mycobacteria.

The results of the study of the antituberculous activity of 25 guanidine derivatives are shown in Table 1, the analytical data for the newly prepared compounds is given in Table 2.

Guanidine hydrochloride (I) did not show activity against the strains of tuberculosis microbacteria used. Its N-derivatives (II-IV) were also inactive, with the exception of the sulfate of isonicotinylaminoguanidine (III). The introduction of the isonicotinic acid group led to the production of compounds showing activity at concentration 62.5-125 microgram/ml.

Acylation of guanidine in the N"-position with aliphatic and aromatic acids (compounds V-XVIII) significantly increased the tuberculostatic activity of the compounds. Thus, almost all the benzylguanidine derivatives (V-XVIII) showed moderate antitubercular activity, while the parentbenzylguanidine sulfate (IV) did not possess such activity. Compounds X and XVIII, which contain β-chloroproprionic (X) and p-toluenesulfonic (XVIII) acid groups, were found to be practically inactive. The MBC of these compounds was equal to or greater than 1000 μg/ml. The length of the carbon chain of the aliphatic acyl group did not significantly affect the antitubercular activity of the compounds (V-X).

Introduction of unsubstituted, alkoxy-substituted, and halogenated aromatic acids (compounds XI-XVII) to some degree increased the antituberculous properties of the compounds (MBC 62-125 μg/ml), with the MBC of compound XIII, containing a methoxy group in position 4, equal to 62.5 μg/ml, but introduction of these same groups additionally in positions 3 and 5 (compound XIV) led to a reduction of the activity, MBC of 250-500 μg/ml. The compounds with a halide in the benzene nucleus which were studied (compounds XV-XVII) possessed equal tuberculostatic activity.

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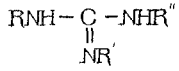
TABLE 1. Antituberculosis Activity of Guanidine Derivatives



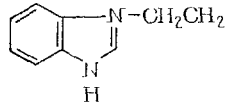
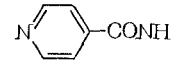
Compound	R	R′	R″	Minimum Bacteriostatic Concentration (in micrograms/ml)			
				strains of Mycobact. tuberculosis			
				$H_{37}Rv$	Academia		Avium P
				with serum	with serum	without serum	with serum
I	H	H	H	>1 000	>1 000	>1 000	>1 000
II		H	H	>1 000	>1 000	>1 000	>1 000
III		H	H	62,5	62,5	31,2	—
IV	$C_6H_5CH_2$	H	H	1 000	1 000	500	1 000
V	$C_6H_5CH_2$	H	C_2H_5CO	250	250	250	250
VI	$C_6H_5CH_2$	H	C_3H_7CO	500	250	250	250
VII	$C_6H_5CH_2$	H	$CH_3(CH_2)_3CO$	250	250	250	—
VIII	$C_6H_5CH_2$	H	$(CH_3)_3CCO$	250	250	250	—
IX	$C_6H_5CH_2$	H	$(CH_3)_2CHCH_2CO$	250	250	250	—
X	$C_6H_5CH_2$	H	$ClCH_2ClCH_2CO$	1 000	≥1 000	1 000	>1 000
XI	$C_6H_5CH_2$	H	$C_6H_5CH{=}CHCO$	62,5	62,5	62,5	250
XII	$C_6H_5CH_2$	H	C_6H_5CO	125	125	125	250
XIII	$C_6H_5CH_2$	H	$4\text{-}CH_3OC_6H_4CO$	62,5	62,5	62,5	250
XIV	$C_6H_5CH_2$	H	$3,4,5\text{-}(CH_3O)_3C_6H_2CO$	250	250	250	250
XV	$C_6H_5CH_2$	H	$4\text{-}BrC_6H_4CO$	62,5	62,5	62,5	250
XVI	$C_6H_5CH_2$	H	$4\text{-}PC_6H_4CO$	125	62,5	62,5	—
XVII	$C_6H_5CH_2$	H	$4\text{-}ClC_6H_4CO$	125	62,5	62,5	—
XVIII	$C_6H_5CH_2$	H	$4\text{-}CH_3C_6H_4SO_2$	>1 000	>1 000	1 000	>1 000
XIX	C_6H_5	H	C_6H_5	125	250	250	1 000
XX	C_6H_5	C_6H_5CO	C_6H_5	>1 000	>1 000	>1 000	>1 000
XXI	C_6H_5	$4\text{-}CH_3OC_6H_4CO$	C_6H_5	≥1 000	>1 000	>1 000	≥1 000
XXII	C_6H_5	$3,4,5\text{-}(CH_3O)\cdot C_6H_2CO$	C_6H_5	≥1 000	≥1 000	>1 000	≥1 000
XXIII	C_6H_5	$4\text{-}BrC_6H_4CO$	C_6H_5	>1 000	≥1 000	1 000	≥1 000
XXIV	C_6H_5	$3\text{-}ClC_6H_4CO$	C_6H_5	≥1 000	≥1 000	>1 000	≥1 000
XXV	C_6H_5	$4\text{-}CH_3C_6H_4SO_2$	C_6H_5	>1 000	>1 000	>1 000	>1 000

TABLE 2. N-Benzyl-N"-acylguanidines

Compound	mp (in °C)	Yield (in %)	Found (in %)	Empirical formula	Calculated (in %)
IV	139—40	47	17,61	$C_{11}H_{15}N_3O \cdot HCl$	17,38
VI	155—6	30,2	16,20	$C_{12}H_{17}N_3O \cdot HCl$	16,43
X	147—9	31	15,08	$C_{11}H_{14}ClN_3O \cdot HCl$	15,22
XI	229—30	25	13,55	$C_{17}H_{17}N_3O \cdot HCl$	13,30
XV	251—3	63	11,42	$C_{15}H_{14}BrN_3O \cdot HCl$	11,43
XVI	245—6	30	13,51	$C_{15}H_{14}FN_3O \cdot HCl$	13,66
XVII	255—6	50	13,42	$C_{15}H_{14}ClN_3O \cdot HCl$	12,95

The sulfate of N,N"-diphenylguanidine (XIX) showed activity of the same order as that of benzylguanidine derivatives.

Acylation of XIX in the N"-position with an aromatic acid anhydride (compounds XX-XXV) led to practically total loss of antituberculous activity. The MBC of all diphenylguanidine derivatives studied was equal to or greater than 1000 μg/ml.

It should be pointed out that the guanidine derivatives studied did not show appreciable inhibiting effect on the growth of Avium P strain microbacteria. Furthermore, the tuberculostatic activity of all the compounds tested did not change in the presence of 10% normal horse serum.

EXPERIMENTAL

N-Benzyl-N"-(acyl)guanidines (V, VI, X, XI, XV-XVII). A mixture of 0.01 mole of the sulfate of IV and 0.04-0.07 mole of the appropriate acid chloride was heated at 90-140°C for 12-24 h. After cooling the reaction mixture, it was worked up with benzene and acetone or ether, the solid residue was recrystallized from alcohol, water, acetone or a mixture of these solvents.

The compounds obtained were white crystalline substances. Yields, mp's, and analytical data are given in Table 2.

Sulfate of Isonicotinylaminoguanidine (III). A mixture of 0.08 mole of isonicotinylhydrazide, 0.04 mole of S-methylisothiourea sulfate and 25 ml of water was heated at 80°C for 9 h. After cooling, the yellow precipitate was filtered off, washed with acetone and ether and then recrystallized from water. This yielded a white powder with a weak greenish tint, mp 252-254°C, yield 90%. Found, %: N 30.80. $C_7H_9N_5O \cdot {}^1/_2H_2SO_4$. Calculated, %: N 30.68.

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