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STUDIES IN ANTIMALARIALS

Part VI. Sulphabiguanide Derivatives*

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Sulphamerazine (2-sulphanilamido-6-methylpyrimidine) and sulphamethazine (2-sulphanilamido-4: 6-dimethyl-pyrimidine) are two alkyl substituted analogues of sulpha-diazine, both of which while equally effective against bacterial infections have the further advantage that the first is not readily eliminated from the system and the second is 6 to 15 times more soluble than sulphadiazine (Northey, E. H., *Ind. and Eng. Chem.*, 1943, **35**, 829). Antimalarial activity of these two drugs have also been evaluated. Testing the causal prophylactic property of these two drugs against primary exoerythrocytic forms of *P. gallinaceum* it was found that they possessed definite prophylactic property although inferior to that of paludrine and M. 4430 (Curd, F. H. S., *et al.*, *Ann. Trop. Med. and Parasitol.*, 1945, **39**, 208; Davey, D. G., *ibid.*, 1946, **40**, 453). When tested against malignant tertian malaria in human beings, these two drugs showed marked suppressive effect though less than that of quinine or atebrin (Findlay, G. M., *et al.*, *Ann. Trop. Med. and Parasitol.*, 1946, **40**, 358).

There is increasing evidence that the sulphanilamides might be acting on malaria parasites by replacing *p*-aminobenzoic acid which is one of the essential metabolites for the growth of malaria organisms (Findlay, *loc. cit.*; Seeler, A. O., Graessle, O., and Dunsenbery, E. D., *J. Bact.*, 1943, **45**, 205). In fact, the action of sulphamerazine has been inhibited by *p*-aminobenzoic acid in the case of *P. lophuræ* in duckling (Seeler, *et al.*, *loc. cit.*) while in the case of *in vitro* culture of *P. knowlesi*, *p*-aminobenzoic acid has been found to be the chief nutrient under the conditions (Ball, E. G., Anfinsen, C. B., *et al.*, *Science*, 1945, **101**, 542; Gieman, Q. M., *et al.*, *J. Exptl. Med.*, 1946, **84**, 583; Anfinsen, C. B., *et al.*, *ibid.*, 607), which adequately explains the exceptional potency of sulphas against monkey malaria (*P. knowlesi*). Such a mode of action of sulphas is different from that of quinine or atebrin and it is possible that more active sulphas might be of value in cases where quinine or atebrin fail to control malaria fever or to remove parasite from blood or where there is idiosyncrasy to these drugs (Findlay, G. M., *et al.*, *loc. cit.*).

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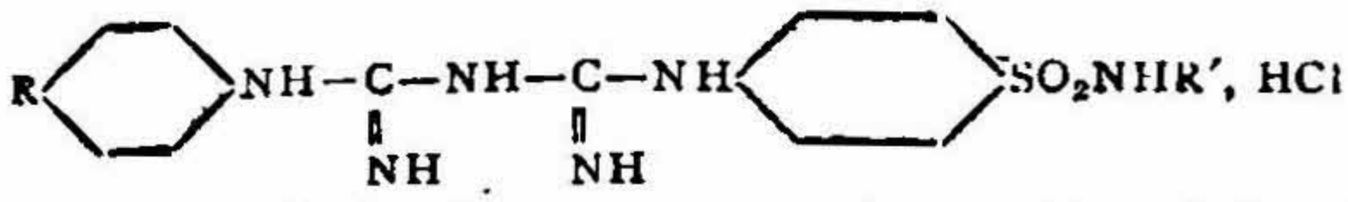
In a previous communication (Bami, Iyer and Guha, *J. Indian Inst. Sci.*, 1947, **29 A**, 15) some sulphabiguanide derivatives of type 'A' have been reported which possess a tautomeric biguanide structure combined with some well-known sulpha-drugs.

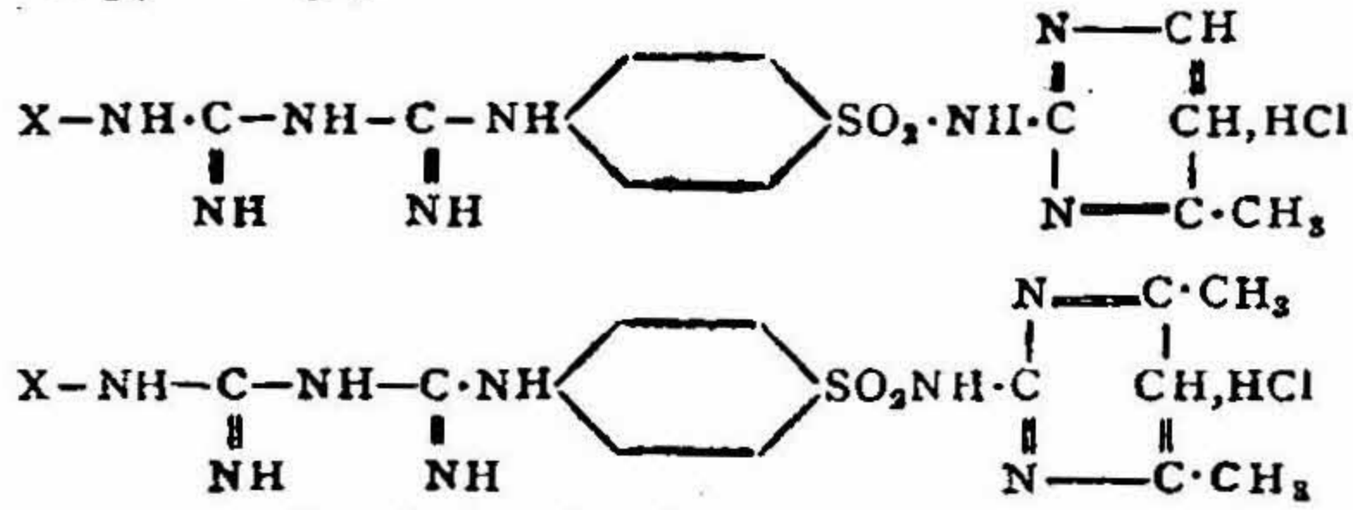


Type A.

R = H, Cl, Br, Me, etc. R' = H, 2-thiazolyl, 2-pyrimidyl.

Some of these compounds were tested against *P. gallanaceum* infection in young chicks and it was found that some of the compounds of type A (where R' = 2-pyrimidyl) are the most active of this series (Bami, *et al.*, *Current Science*, 1947, **16**, 386). This fact seems to be in accordance with the well-known fact that sulphadiazine has so far been the most active of all the sulphas.

Encouraged by these results and supported by the general considerations discussed already, it was thought worthwhile to extend this series of compounds to include sulphamerazine and sulpha-methazine as well. Accordingly compounds of type B and C were synthesised to study the effect of alkyl substituents in the pryimidine molecule of the compounds of type A (R' = 2-pyrimidyl).



Type B

Type C

X = substituted aryls.

The compounds of type B and C have been prepared by general methods previously employed (Bami, *et al.*, *loc. cit.*). Sulphamerazine and sulphamethazine were refluxed for a few hours with appropriate arylcyanoguanidines in 90 per cent. ethanol or dilute dioxane containing enough hydrochloric acid to form the hydrochloride of the sulphanilamide to give the compounds of type B and C, respectively. Out of the nine arylcyanoguanidines employed in the present study, two of them, *viz.*, *p*-iodophenylcyanoguanidine and 2:4-dichlorophenylcyanoguanidine have been prepared and reported for the first time.

While compounds of type B were purified by crystallising them from suitable solvents like ethanol, methanol, dioxane and acetone, compounds of type C were usually purified by suitably removing the starting materials

and subsequently triturating the product with dry acetone. All these sulpha-biguanide derivatives have been isolated as hydrochloride salts and are white amorphous powders (except nitro-derivatives which were light yellow) with high melting points. These compounds are amphotaric in character because they also form sodium salts due to the free amido-hydrogen available in the sulpha-group.

Curd and Rose (*Nature*, 1946, **158**, 707) have put forward an explanation for a possible mode of action of paludrine, wherein the activity is attributed to the formation of metallic complexes (chelates) akin to porphyrin, with trace elements *in vivo*, which might be interfering with the porphyrin system highly specific to malaria parasite. It has been shown that in the case of diaryl substituted biguanides, the metallic complexes are formed with considerable difficulty (Ray, P., *J. Indian Chem. Soc.*, 1937, **14**, 670; Curd and Rose, *J.C.S.*, 1946, 729) and in fact N^1-N^5-diarylbiguanides have been shown to be inactive as antimalarials (Curd and Rose, *loc. cit.*).

The present attempts to form the copper chelates of compounds of type A, B and C by Andreasch's method (*Monatsh*, 1927, **48**, 147) revealed that while sulphabiguanides derived from simple sulphanilamide (type A, R′=H) showed a feeble tendency towards chelation, the rest did not chelate at all.

Considering the antimalarial activity of compounds of type A (R′ = 2-thiazolyl and R′ = 2-pyrimidyl), it appears that antimalarial activity of these compounds largely depends upon the nature of substituents at the either end of the biguanide structure. Formation of metallic complexes need not be the only explanation because even in the absence of a metal, the hydrogen bonding between N_2 and N_4 will allow the molecule to retain some characteristics of chelates.

Experimental

p-*Iodophenylcyanoguanidine.*—*p*-Iodoaniline (44 g.) was dissolved in hydrochloric acid (50 c.c.) and diazotised with sodium nitrite solution (15 g. in 50 c.c. water) at 0° C. The diazonium chloride solution was added to dicyandiamide (20 g.) dissolved in water (1 l.) and the mixture made strongly alkaline with sodium hydroxide solution. After 3 hours, the solution was acidified and the precipitated *p*-iodophenylazocyanoguanidine (referred to as triazine) filtered, washed with water and dried well on the filter-paper.

The triazine was added gradually to a mixture of alcohol (200 c.c.) and sulphuric acid (25 c.c.) in such a way that temperature did not rise above 40°. After half-an-hour when the evolution of nitrogen ceased, the clear

solution was diluted with water and chilled. The crude product was filtered off and purified by dissolving in dilute alkali, treating it with norite and reprecipitating the product with mineral acid. Finally the product was crystallised from alcohol; m.p. 217°; yield 25 g. (Found: N, 19·61; $C_8H_7N_4I$ requires N, 19·58 per cent.)

2:4-*Dichlorophenylcyanoguanidine*.—2:4-Dichloroaniline hydrochloride (20 g.) was taken in hydrochloric acid (20 c.c.) and diazotised with sodium nitrite solution (7 g. in 15 c.c. of water) at 0° C. The clear diazonium solution (may be filtered if there is a little diazoamino compound) was added to a solution of dicyandiamide (10 g. in 300 c.c. of water) and the total solution made alkaline. The triazine was precipitated by acid and the filtrate on re-alkalization and re-acidification yielded a little more of the product. The total triazine was denitrogenated at 30–35° in a mixture of acetone (100 c.c.) and hydrochloric acid (20 c.c.) in about half-an-hour. The product was isolated by diluting the acetone solution and purified as detailed above. White silky needles, m.p. 217° C. Yield 5 g. (Found: N, 24·77; $C_8H_6N_4Cl_2$ requires N, 24·45 per cent.).

N^1-Phenyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsulphonamide-biguanide-hydrochloride (type B; X = Phenyl) (I).—Phenylcyanoguanidine (3·2 g.) and sulphamerazine (5·3 g.) were refluxed in a mixture of alcohol (15 c.c.) and hydrochloric acid (2 c.c.) for 10 hours. The resulting solid was filtered off, washed with little alcohol, and crystallised twice from dilute alcohol. White crystalline solid, m.p. 194°. Yield 3 g. (Found: N, 23·97; $C_{19}H_{21}O_2N_8ClS$ requires N, 24·32 per cent.).

N^1-p-Chlorophenyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride (type B; X = p-chlorophenyl) (II).—*p*-Chloro-phenylcyanoguanidine (4 g.) and sulphamerazine (5 g.) were refluxed in a mixture of alcohol (15 c.c.) and hydrochloric acid (2 c.c.) for 5 hours. The separated product was filtered off after chilling and crystallised from dilute alcohol or dioxane. White crystalline product, m.p. 238°. Yield 3 g. (Found: N, 22·32; $C_{19}H_{20}O_2N_8Cl_2S$ requires N, 22·64 per cent.).

N^1-2:4-Dichlorophenyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride (type B; X = 2:4-dichlorophenyl) (III).—2:4-Dichlorophenylcyanoguanidine (1·9 g.) and sulphamerazine (2·6 g.) were refluxed in a mixture of alcohol (10 c.c.) and hydrochloric acid (1 c.c.) for six hours. The separated solid was filtered and crystallised twice from dilute acetone. White crystalline powder, m.p. 220°, yield 2 g. Found: N, 21·41; $C_{19}H_{19}O_2N_5Cl_3S$ requires N, 21·15 per cent.).

N^1-p-Bromophenyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride (type B. X = *p*-bromophenyl) (IV).—*p*-Bromo-phenylcyanoguanidine (2·4 g.) and sulphamerazine (2·6 g.) were refluxed together in a mixture of alcohol (10 c.c.) and hydrochloric acid (1 c.c.) for 5 hours. The separated solid was filtered and crystallised from dilute alcohol. White crystalline solid, m.p. 231°. Yield 2·5 g. (Found: N, 20·71; $C_{19}H_{20}O_2N_8ClBrS$ requires N, 20·75%).

N^1-p-Iodophenyl-N^5-p-(6-methyl-2-pyrimidyl)phenylsulphonamide-biguanide hydrochloride (type B; X = *p*-iodophenyl) (V).—*p*-Iodo-phenylcyanoguanidine (2·8 g.) and sulphamerazine (2·6 g.) were reacted together in alcoholic hydrochloric acid and the product isolated and crystallised as under (IV). White powder, m.p. 227° (slight decomp.). Yield 2 g. (Found: N, 19·16; $C_{19}H_{20}O_2N_8ClIS$ requires N, 10·11 per cent.).

N^1-p-Methylphenyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsuphonamido-biguanide hydrochloride (type B; X = *p*-methylphenyl) (VI).—Methylphenylcyanoguanidine (3·5 g.) and sulphamerazine (5·3 g.) were refluxed together in a mixture of alcohol (12 c.c.) and hydrochloric acid (2 c.c.) for 7 hours. The separated solid was filtered, washed with little alcohol and crystallised from dilute acetone, m.p. 231°. Yield 5 g. (Found: N, 23·20; $C_{20}H_{23}O_2N_8ClS$ requires N, 23·61 per cent.).

N^1-2:3-Dimethyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsulphonamido-biguanide-hydrochloride (type B; X = 2:3-dimethylphenyl) (VII).—2:3-Dimethylcyanoguanidine (1·9 g.) and sulphamerazine (2·6 g.) were reacted together in alcoholic hydrochloric acid and the product isolated and crystallised as under (IV). White crystalline powders m.p. 220°. Yield 2 g. (Found: N, 23·27; $C_{21}H_{25}O_2N_8ClS$ requires N, 22·94 per cent.).

N^1-p-Methoxyphenyl-N^5-p-(6-methyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride (type B; X = *p*-methoxyphenyl) (VIII).—*p*-Methoxyphenylcyanoguanidine (1·9 g.) and sulphamerazine (2·6 g.) refluxed in alcoholic hydrochloric acid (10:1) and the product obtained as under (IV). White powder, m.p. 200°. Yield 2·5 g. (Found: N, 22·92; $C_{20}H_{23}O_3N_8ClS$ requires N, 22·83 per cent.).

N^1-p-Nitrophenyl-N^5-[p-(6-methyl-2-pyrimidyl)-phenylsulphonamide]-biguanide hydrochloride (type B; X = *o*-Nitrophenyl) (IX).—*p*-Nitrophenyl-cyanoguanidine (2 g.) and sulphamerazine (2·6 g.) were reacted in alcoholic hydrochloric acid and the product obtained as under (IV). Light yellow powder, m.p. 230° (slight decomp.). Yield 1·5 g. (Found: N, 24·94%. $C_{19}H_{20}O_4N_9ClS$ requires N, 24·92 per cent.).

N^1-Phenyl-N^5 p-(4 : 6-*dimethyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride* (type C; X = phenyl) (X).—Phenylcyanoguanidine (1·6 g.) and sulphamethazine (2·8 g.) were refluxed in a mixture of alcohol (7 c.c.) and hydrochloric acid (1 c.c.) for six hours. The product was diluted with water (50 c.c.) and the resulting semisolid washed thrice with cold water. The semisolid product was dissolved in alcohol (25 c.c.), treated with norite and filtered. The alcoholic solution was evaporated to dryness at 110° and the resulting solid refluxed with acetone (30 c.c.) for 2 hours when it turned into a white amorphous powder. The product was filtered, washed repeatedly with acetone and dried. White amorphous powder, m.p. 222°. Yield 3 g. (Found: N, 23·95; $C_{20}H_{23}O_2N_8ClS$ requires N, 23·60 per cent.).

N^1-p-Chlorophenyl-N^5-p-(4 : 6-dimethyl-2-pyrimidyl)-phenylsulphonamide biguanide hydrochloride (type C; X = Chloro-phenyl) (XI).—*p*-Chlorophenylcyanoguanidine (3·8 g.) and sulphamethazine (5·5 g.) were refluxed together in alcohol (10 c.c.), hydrochloric acid (2 c.c.) mixture for 12 hours. The solution was diluted with water (50 c.c.) and the resulting semisolid mass washed repeatedly with cold water. The product was crystallised from dilute dioxane. White crystalline powder, insoluble in dry acetone and dioxane, m.p. 225°. Yield 4 g. (Found: N, 22·12; $C_{20}H_{22}O_2N_9ClS$ requires N, 22·00 per cent.).

*N^1-p-2 : 4-Dichlorophenyl-*N^5-p (4 : 6-*dimethyl-2-pyrimidyl)-phenylsulphonamide-biguaniae hydrochloride* (type C; X = 2 : 4-dichlorophenyl) (XII).—2 : 4- Dichlorophenylcyanoguanidine (2·1 g.) and sulphamethazine (2·8 g.) were refluxed together in a mixture of alcohol (8 c.c.) and hydrochloric acid (1 c.c.) for six hours. The product was isolated and purified as under (X). White amorphous powder, m.p. 223°. Yield 2 g. (Found: N, 20·87; $C_{20}H_{21}O_2N_6Cl_3S$ requires N, 20·60 per cent.).

*N^1-p-Bromophenyl-*N^5-p-(4 : 6-*dimethyl-2-pyrimidyl)-phenylsulphonamido-biguanide hydrochloride* (type C; X *p*-bromophenyl) (XIII).—*p*-Bromophenylcyanoguanidine (2·4 g.) and sulphamethazine (2·8 g.) were refluxed together in a mixture of alcohol (8 c.c.) and hydrochloric acid (1 c.c.) for 6 hours and the product isolated as under (X). White amorphous powder, m.p. 232°. Yield 3 g. (Found: N, 19·88; $C_{20}H_{22}O_2N_8ClBrS$ requires N, 20·23 per cent.).

*N^1-p-Iodophenyl-*N^5-p-(4 : 6-*dimethyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride* (type C; X = *p*-iodophenyl) (XIV).—*p*-Iodophenylcyanoguanidine (2·8 g.) and sulphamethazine (2·8 g.) were refluxed in a mixture of alcohol (8 c.c.) and hydrochloric acid (1 c.c.) for 6 hours. The product was isolated and purified as under (X). White amorphous powder,

m.p. 232° (slight decomp.). Yield 2 g. (Found: N, 18·24; $C_{20}H_{22}O_2N_8$ ClIS requires N, 18·65 per cent.).

N^1-p-Methylphenyl-N^5-p-(4:6-dimethyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride (type C; X = *p*-methylphenyl) (XV).—*p*-Methylphenylcyanoguanidine (1·7 g.) and sulphamethazine (2·8 g.) were reacted together in alcoholic hydrochloric acid and the product obtained as under (X). White amorphous powder, m.p. 238° (slight decomp.). Yield 1·5 g. (Found: N, 23·17; $C_{21}H_{25}O_2N_8ClS$ requires N, 22·92 per cent.).

*N^1-2:3-Dimethylphenyl-*N^5-p-(4:6-*dimethyl-2-pyrimidyl)-phenylsulphonamide biguanide hydrochloride* (type C; X = 2:3-dimethylphenyl) (XVI).—2:3-*D*imethylphenylcyanoguanidine (1·9 g.) and sulphamethazine (2·8 g.) were refluxed together in alcohol (10 c.c.), hydrochloric acid (1 c.c.) mixture for 6 hours. The solution was concentrated to 5 c.c. and the product isolated and purified as under (X). White powder, m.p. 219°. Yield 2 g. (Found: N, 22·36; $C_{22}H_{27}O_2N_8ClS$ requires N, 22·24 per cent.).

N^1-p-Methoxyphenyl-N^5-p-(4:6-dimethyl-2-pyrimidyl)-phenylsulphonamide biguanide hydrochloride (type C; X = *p*-methoxyphenyl) (XVII).—*p*-Methoxycyanoguanidine (3·8 g.) and sulphamethazine (5·5 g.) were refluxed together in alcohol (15 c.c.), hydrochloric acid (2 c.c.) mixture for 10 hours. The solid obtained after keeping overnight was filtered and washed with little alcohol. Crystallised from dilute alcohol. White crystalline powder, m.p. 231°. Yield 4 g. (Found: N, 22·10; $C_{21}H_{25}O_3N_8ClS$ requires N, 22·2 per cent.).

N^1-p-Nitrophenyl-N^5-p-(4:6-dimethyl-2-pyrimidyl)-phenylsulphonamide-biguanide hydrochloride (type C; X = *p*-nitrophenyl) (XVIII).—Sulphamethazine (2·8 g.) and *p*-nitrophenylcyanoguanidine (2·0 g.) were refluxed together in a mixture of alcohol (10 c.c.) and hydrochloric acid (1 c.c.) for 8 hours. After five days, the separated solid, was filtered and dissolved in alcohol, treated with norite and filtered. The filtrate was evaporated to dryness and the product boiled with acetone (25 c.c.) for 1 hour. The light yellow solid was filtered, washed with acetone and dried. Light yellow powder, m.p. 257°. Yield 2·5 g. (Found: N, 24·50; $C_{20}H_{22}O_4N_9ClS$ requires N, 24·25 per cent.).

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