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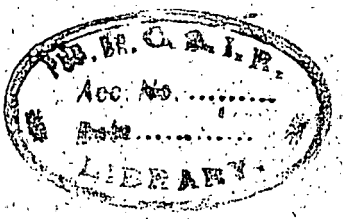

STUDIES IN ANTIMALARIALS

PART V. SOME SULPHABIGUANIDE DERIVATIVES

BY H. L. BAMI, B. H. IYER AND P. C. GUHA

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STUDIES IN ANTIMALARIALS

Part V. Some Sulphabiguanide Derivatives*

BY H. L. BAMI, B. H. IYER AND P. C. GUHA

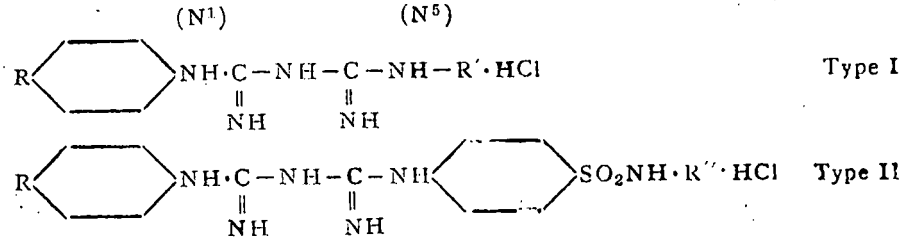
Considering the wide range of activity of sulphonamide group of drugs, these drugs were also tried for their usefulness in experimental and human malaria. Diaz de Leon (*Public Health Repts., Wash*, 1937, **52**, 1460) first discovered the antimalarial action of sulphonamides and this finding was followed by large amount of work with varying results (Singh and Singh, *J. Mal. Inst. India*, 1939, **2**, 181; Coggeshall, *Am. J. Trop. Med.*, 1938, **18**, 715; *J. Exptl. Med.*, 1940, **71**, 13; *J. Amer. Med. Assoc.*, 1941, **117**, 1077; Dikshit and Ganapathi, *J. Mal. Inst. India*, 1940, **3**, 525; Marshall and co-workers, *J. Pharmacol.*, 1942, **75**, 89). At present sulphadiazine, sulphathiazole, sulphaguanidine and sulphanilamide are in descending order of activity against malarial infections (Coatney and Cooper, *U. S. Pub. Health Repts., Wash.*, 1944, **59**, 1455; Van Dyke, *Proc. Soc. Exptl. Biol. and Med.*, 1941, **48**, 368; Curd, *Ann. Trop. Med. and Parasitol.*, 1943, **37**, 115; *Ibid.*, 1945, **39**, 147, 157; *cf.*, Bami, Iyer and Guha, *Science and Culture*, 1947, **13**, 18) and out of these sulphadiazine is the most outstanding due to the fact that it has slight but definite causal prophylactic action which is not demonstrated by other antimalarials. In general, the activity of sulphanilamide group of drugs against human malaria is of lower order than that of other antimalarials like quinine, atebrin, plasmoquine and paludrine, and is inhibited by para-aminobenzoic acid which goes to prove that anti-malarial property of these drugs is due to their bacteriostatic action (Marshall *et al.*, *loc. cit.*).

Recently, another pyrimidine sulphonamide derivative, *viz.*, meta-chloridine, being 2-metanilamido-5-chloro-pyridine, has been added as a suppressive antimalarial (English *et al.*, *J. Amer. Chem. Soc.*, 1946, **68**, 1039) and it has been found to be 16 times as active as quinine and six times as active as sulphadiazine.

Considering the activity of sulpha-derivatives of pyrimidines, Curd and Rose (*J.C.S.*, 1946, 343) first prepared sulphur-free analogues of phenyl substituted pyrimidine type and their later work culminated in the discovery of paludrine, which is a substituted biguanide derivative (Curd and Rose, *J.C.S.*, 1946, 720; Bami, Iyer and Guha, *J. Indian Inst. Sci.*, 1946, **29**A, 1; *cf.*, *Science and Culture*, 1946, **12**, 448).

* A preliminary communication of this work has been published in *Current Science*, 1947, **16**, 252.

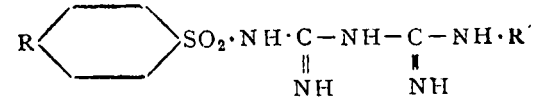
Considering the new field of research in synthetic antimalarials which has been opened by substituted biguanide derivatives, it was thought of interest to seek further possibilities to prepare potential antimalarials in the same field. It has been observed that the compounds of type I where isopropyl radical of paludrine has been replaced by an isocyclic ring are not very active (Curd and Rose, *loc. cit.*). This decrease in activity may be attributed to lack of any potential substituent in the isocyclic ring. In view of the well-established chemotherapeutic property of sulphonamido-group or heterocyclic substituted sulphonamido-group, it has now been successfully tried to substitute sulphamyl radical (substituted or otherwise) at para-position in the N^5-phenyl radical of the biguanide to give the compounds of type II.



R = Cl, Me, MeO, Br, H, Etc.; R′ = phenyl or naphthyl;
R″ = H, 2-thiazolyl and 2-pyrimidyl.

Potential heterocyclic rings like pyrimidine and thiazole have been selected as substituents in the sulphamyl radical due to the considerations already discussed. Due to similar reasons only para-substituted phenyl rings were introduced at N^1-position of the biguanide molecule for the present study.

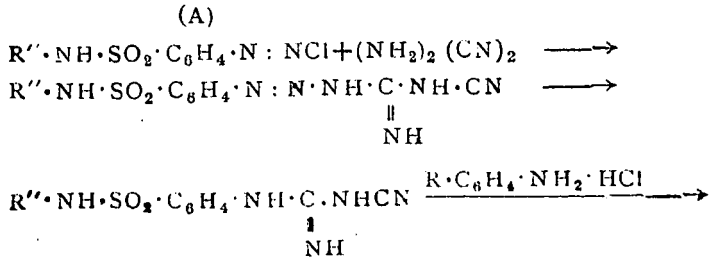
It will be of interest to mention that compounds of type III have been patented as chemotherapeutic agents (Rose, B. P., 1943, 550538; Winnek, U. S. P., 1943, 2295884).



R = NO_2, AcNH, NH_2 R′ = H, alkyl or aryl, etc.

For the synthesis of compounds of type II, one of the following two schemes could be adopted:

(A)



(B)

$$R\cdot C_6H_4\cdot N{:}\,N\cdot Cl + (NH_2)_2\,(CN_2) \longrightarrow R\cdot C_6H_4\cdot N:N\cdot NH\cdot C(=NH)\cdot NHCN \longrightarrow$$

$$R\cdot C_6H_4\cdot NH\cdot C(=NH)\cdot NHCN \xrightarrow{R''\cdot NH\cdot SO_2\cdot C_6H_4\cdot NH_2\cdot HCl} \text{Type II}$$

According to Scheme A, sulphanilamide was diazotised and reacted with cyanoguanidine in alkaline medium. The *p*-sulphamyl-phenyl-azocyano-guanidine (referred to as triazine) thus obtained could not be denitrogenated by any of the methods already used (Bami, *et. al.*, *loc. cit.*). The reason for this failure may be traced to the fact that the sulphamyl group hinders the formation of a labile hydrochloride of the triazine which is essential for denitrogenation [*cf.*, Walther and Greishmer, *J. fur. Prakt. Chem.*, 1915, **19** (ii), 218]. This scheme was therefore abandoned for the present work, but further work on the denitrogenation mechanism of various triazines of this type is in progress and shall be detailed later.

According to Scheme B, various *p*-substituted anilines were diazotised, reacted with cyano-guanidine and the resulting triazine denitrogenated to the corresponding substituted phenyl-cyanoguanidines. While a simpler method with better yields for the preparation of *p*-bromophenyl-cyano-guanidine and *p*-nitro-phenylcyanoguanidine is being reported, phenyl-cyanoguanidine and its *p*-methyl, methoxy and chloro analogues have been prepared by methods already described (Bami, *et. al.*, *loc. cit.*). Another method for the preparation of such phenylcyanoguanidine is by Wheeler and Jamieson (*J. Amer. Chem. Soc.*, 1903, **25**, 719) which has been further extended by May (*J. Org. Chem.*, 1947, **12**, 437, 443). This method being tedious and lengthy was not considered for the present work.

There are other methods to synthesize compounds of type II but for the present work only Scheme B has been employed because it was the simplest. The substituted phenyl-cyanoguanidines have been reacted with excess of the hydrochloride salt of sulphanilamide, sulphathiazole or suphadiazine in boiling aqueous dioxan or 90% ethanol. The reaction was complete in about 6 hours and the formation of biguanide could be detected by ready formation of a picrate. The product was isolated and crystallised from hot water or dilute alcohol as the case may be. The use of hydrochloride of the amine is due to the reason that a facile reaction with this type of compounds requires the amine to be present in the dissociable form (Curd and Rose, *loc. cit.*), and biguanides are most conveniently isolated as their acid salts.

All these compounds were white crystalline solids with the exception of nitro-derivatives and had fairly high melting points. Pyrimidine derivatives of the type II, however, developed light yellow colour which could not be removed even by treating with norite. The solubility of these compounds was appreciable in the case of sulphanilamide derivatives, but decreased considerably for other derivatives. These compounds were also soluble in dilute alkali due to the presence of sulphamyl group.

Experimental

p-*Bromophenylcyanoguanidine.*—*p*-Bromo-aniline (40 g.) was dissolved in hydrochloric acid (60 c.c.), and water (100 c.c.) and diazotised at 0° C. with sodium nitrite solution (18 g. in 80 c.c. water). The diazonium salt solution was added to dicyandiamide (22 g.) dissolved in water (1500 c.c.) and kept for 2 hours at 10° after having made the solution strongly alkaline with sodium hydroxide solution (30%, 100 c.c.). The coloured solution was acidified and the resulting *p*-bromophenylazocyanoguanidine was filtered off. The semi-solid triazine was added to a mixture of alcohol (200 c.c.) and hydrochloric acid (60 c.c.) in small proportions over a period of 45 minutes so as to keep the temperature below 35°. When the evolution of nitrogen was over the mixture was warmed to 50° in order to complete the reaction. The mixture was diluted with water to one litre and chilled. The crude crystalline product was collected and purified by dissolving it in dilute sodium hydroxide solution and precipitating with acid after treatment with norite; white crystalline product; yield 20 gm. m.p. 198°.

p-*Nitrophenyl cyanoguanidine.*—*p*-Nitro-aniline (27·5 g.) was dissolved by warming in hydrochloric acid (44 c.c.) and water (150 c.c.) and poured on ice pieces to have a uniform paste of the amine hydrochloride. This was diazotized with sodium nitrite solution (14 g. in 60 c.c. water) at 0° C. The diazotized solution was added to dicyandiamide (20 gm.) dissolved in water (1000 c.c.) and the solution made strongly alkaline. The triazine was precipitated from solution with acid after two hours and the filtrate after having removed the triazine was made alkaline again. A second crop of the product was obtained by acidifying the alkaline filtrate. Similarly a third crop was obtained and the total triazine was denitrogenated in acetone (250 c.c.) hydrochloric acid (60 c.c.) mixture at 30° in about two hours. The product was isolated and purified as detailed before. Light yellow powder; yield 14 g., m.p. 243° (Curd and Rose, *loc. cit.*, m.p. 242–243°).

N^1-*phenyl*-N^5-p-*sulphamylphenyl biguanide hydrochloride* (*I*).—Phenylcyanoguanidine (3·7 g.) and sulphanilamide hydrochloride (7 g.) were taken

in dioxan (25 c.c.), and water (10 c.c.) and refluxed for 4 hours. The solvent was removed and the residue solidified on chilling and triturating. The crude product was crystallised twice from hot water with treatment with norite for decolourising. White crystalline powder; yield 4 g., m.p. 228° (Found : N, 22·62. $C_{14}H_{17}O_2N_6ClS$ requires N, 22·79 per cent.).

N^1-p-Chlorophenyl-N^5-p-sulphamylphenylbiguanide hydrochloride (II).—Sulphanilamide (13 g.) and *p*-chlorophenylcyanoguanidine (10 g.) was taken in alcohol (50 c.c.) and hydrochloric acid (7·5 c.c.) was then added. The mixture was refluxed for 6 hours and diluted with water to 100 c.c. The crude product was filtered off next morning and crystallised from hot water. White crystalline powder, yield 10 gm., m.p. 233° (Found: N, 20·13 $C_{14}H_{16}O_2N_6Cl_2S$ requires N, 20·84 per cent.).

N^1-p-Bromophenyl-N^5-p-sulphamylphenylbiguanide hydrochloride (III).—*p*-Bromophenylcyanoguanidine (8 g.) and sulphanilamide (10 g.) were added to a mixture of alcohol (30 c.c.) and hydrochloric acid (6 c.c.) and refluxed for 6 hours. The mixture was chilled and the crude product filtered and crystallised from hot water. Yield 10 gm., m.p. 246° (Found: N, 18·45. $C_{14}H_{16}O_2N_6ClBrS$ requires N, 18·77 per cent.).

N^1-p-Nitrophenyl-N^5-p-sulphamylphenylbiguanide hydrochloride (IV).—*p*-Nitrophenylcyanoguanidine (2 g.) and sulphanilamide hydrochloride (4 g.) were refluxed in a mixture of dioxan (7 c.c.) and water (5 c.c.) for 5 hours. The separated solid was filtered off and crystallised from dilute alcohol; light yellow powder, yield 2·5 g., m.p. 217° (with decomp.) (Found: N, 23·24). $C_{14}H_{16}O_4N_7ClS$ requires N, 23·70 per cent.).

N^1-p-Tolyl-N^5-p-sulphamylphenylbiguanide hydrochloride (V).—*p*-Tolylcyanoguanidine (4 g.) and sulphanilamide hydrochloride (7 g.) were refluxed in a mixture of dioxan (25 c.c.) and water (10 c.c.) for 4 hours. The solvent was removed and the residue solidified on triturating and chilling. The product was crystallised twice from water; white amorphous powder, yield 6 g., m.p. 231° (Found: N, 22·22; $C_{15}H_{19}O_2N_6ClS$ requires N, 21·96 per cent.).

N^1-p-Methoxyphenyl-N^5-p-sulphamylphenylbiguanide hydrochloride (VI).–*p*-Methoxyphenylcyanoguanidine (5 g.) and sulphanilamide hydrochloride (7 g.) were refluxed in a mixture of dioxan (25 c.c.) and water (10 c.c.) for 4 hours. The mixture was diluted to 75 c.c. and the separated solid removed next morning and crystallised from water after treating with norite; white crystalline powder, yield 5 g., m.p. 234° (Found: N, 20·80; $C_{15}H_{19}O_3N_6ClS$ requires N, 21·07 per cent.).

N^1-Phenyl-N^5-p-2-*thiazolylsulphamylbiguanide hydrochloride* (*VII*).—Phenylcyanoguanidine (4 g.) and sulphathiazole hydrochloride (7·3 g.) were refluxed in a mixture of dioxan (25 c.c.) and water (10 c.c.) for 3 hours. The solvent was removed and the residue crystallised from dilute alcohol with the treatment of norite; white amorphous powder, yield 4·5 g., m.p. 225° (Found: N, 21·37. $C_{17}H_{17}O_2N_7ClS_2$ requires N, 21·75 per cent.).

N^1-p-*Chlorophenyl-N^5*-p-2-*thiazolylsulphamylphenylbiguanide hydrochloride* (*VIII*).—*p*-Chlorophenylcyanoguanidine (10 g.) and sulphathiazole (15 g.) were added to a mixture of alcohol (50 c.c.) and hydrochloric acid (6 c.c.), and the mixture refluxed for 7 hours. The product was filtered off and crystallised from dilute alcohol; white crystalline powder, yield 12 g.; m.p. 229° (Found: N, 20·8. $C_{17}H_{16}O_2N_7Cl_2S_2$ requires N, 20·71 per cent.).

N^1-p-*Bromophenyl-N^5*-p-2-*thiazolylsulphamyl-phenyl-biguanide hydrochloride* (*IX*).—*p*-Bromophenylcyanoguanidine (4 g.) and sulphathiazole hydrochloride (5 g.) were refluxed together in dioxan (10 c.c.), and water (5 c.c.) for 4 hours and the mixture diluted to 40 c.c. The product came out on chilling and triturating which was collected and crystallised from dilute alcohol; white crystalline product, yield 3 g.,; m.p. 197° (with decomp.) (Found: N, 18·45; $C_{17}H_{16}O_2N_7ClBrS_2$ requires N, 18·93 per cent.).

N^1-p-*Nitrophenyl-N^5*-p-2-*thiazolylsulphamyl-phenyl-biguanide hydrochloride* (*X*).—*p*-Nitrophenylcyanoguanidine (2 g.) and sulphathiazole hydrochloride (4 g.) were reacted together in aqueous dioxan and the product obtained as detailed under (IV); light yellow powder, yield 2 g., m.p. 267° (Found: N, 23·06. $C_{17}H_{16}O_4N_8ClS_2$ requires N, 23·16 per cent.).

N^1-p-*Tolyl-N^5*-p-2-*thiazolylsulphamylphenylbiguanide hydrochloride* (*XI*). *p*-Tolylcyanoguanidine (3·5 g.) and sulphathiazole hydrochloride (7 g.) were taken in a mixture of dioxan (10 c.c.) and water (5 c.c.) and refluxed for 4 hours. The product was obtained as detailed under (IX); white powder, yield 5 g., m.p. 189–90° (with decomp.) (Found: N, 20·42. $C_{18}H_{19}O_2N_7ClS_2$ requires N, 21·09 per cent.).

N^1-p-*Methoxyphenyl-N^5*-p-2-*thiazolylsulphamylphenylbiguanide hydrochloride* (*XII*)—*p*-Methoxyphenylcyanoguanidine (3·8 g.) and sulphathiazole hydrochloride (6·8 g.) were reacted in a mixture of dioxan (25 c.c.) and water (10 c.c.) by refluxing the mixture for 3 hours. The solvent was removed and the product crystallised from dilute alcohol; white amorphous powder; yield, 3 g.; m.p. 176° (with decomp.) (Found: N, 19·96. $C_{18}H_{19}O_3N_7ClS_2$ requires N, 20·35 per cent.).

N^1-phenyl-N^5-p-2-*pyrimidylsulphamylphenylbiguanide hydrochloride* (*XIII*). —Phenylcyanoguanidine (3·2 g.) and sulphadiazine hydrochloride (5 g.)

were added to a mixture of dioxan (15 c.c.) and water (10 c.c.) and refluxed for 3 hours. After 2 days, the separated solid was filtered and crystallised from dilute alcohol; white amorphous powder; yield 4 g.; m.p. 238° (Found: N, 24·79. $C_{19}H_{18}O_2N_8ClS$ requires N, 24·48 per cent.).

N^1-p-Chlorophenyl-N^5-p-2-pyrimidylsulphamylphenylbiguanide hydrochloride (XIV).—*p*-Chlorophenylcyanoguanidine (10 g.) and sulphadiazine (15 g.) were added to alcohol (40 c.c.), and hydrochloric acid (6 c.c.) and refluxed for 6 hours. The crude product was collected and crystallised from dilute alcohol; light yellow powder; yield 15 g.; m.p. 246° (Found: N, 22·58. $C_{19}H_{17}O_2N_8Cl_2S$ requires N, 22·76 per cent.).

N^1-p-Bromophenyl-N^5-p-2-pyrimidylsulphamylphenylbiguanide hydrochloride (XV).—*p*-Bromophenylcyanoguanidine (4 g.) and sulphadiazine hydrochloride (5 g.) were refluxed in dioxan (10 c.c.) and water (5 c.c.) mixture for 4 hours. The solution was diluted to 50 c.c. and the product was filtered off next morning and crystallised from dilute alcohol; white crystalline solid; yield 3 g.; m.p. 202° (Found: N, 21·45. $C_{19}H_{17}O_2N_8ClBrS$ requires N, 20·87 per cent.).

N^1-p-Nitrophenyl-N^5-p-2-pyrimidylsulphamylphenyl-biguanide hydrochloride (XVI).—*p*-Nitrophenylcyanoguanidine (2 g.) and sulphadiazine hydrochloride (3·6 g.) were reacted together and the product obtained as detailed under (XV); yellow powder; yield 2 g.; m.p. 255° (Found: N, 24·81. $C_{19}H_{17}O_4N_9ClS$ requires N, 25·07 per cent.).

N^1-p-Tolyl-N^5-p-2-pyrimidylsulphamylphenyl biguanide hydrochloride (XVII).—*p*-Tolylcyanoguanidine (3·5 g.) and sulphadiazine hydrochloride (6 g.) were reacted together in aqueous dioxan and the product obtained as described under (XIII); light yellow powder; yield 2 g.; m.p. 232° (with decomp.) (Found: N, 23·92. $C_{20}H_{20}O_2N_8ClS$ requires N, 23·75 per cent.).

N^1-p-Methoxyphenyl-N^5-p-2-pyrimidylsulphamylphenylbiguanide hydrochloride (XVIII).—*p*-Methoxyphenylcyanoguanidine (2 g.) and sulphadiazine hydrochloride (4 g.) taken in dioxan (7 c.c.) and water (5 c.c.) were refluxed for 5 hours, diluted to 30 c.c., boiled with norite and filtered. The product was collected and dried the next morning; white crystalline solid; yield 2 g.; m.p. 210–12° (Found: N, 22·51. $C_{20}H_{20}O_3N_8ClS$ requires N, 22·97 per cent.).

Pharmacological Tests

The following typical compounds of the type II and three salts of paludrine reported before (Bami, Iyer and Guha, *loc. cit.*) have been screened for their antimalarial activity against *P. gallinaeceum* in young chicks not

more than six weeks old. The method for screening was as followed by Liverpool workers with suitable modifications to suit our conditions. The drugs were usually water-soluble and hence were fed orally.

Compound	Dosage in milligrams per 100 g. body weight	Activity
1 Paludrine acetate	6	++
2 Paludrine hydrochloride	6	++
3 Paludrine nitrate*	6	−
4 N^1-*p*-chlorophenyl-N^5-*p*-sulphamylphenylbiguanide hydrochloride (II)†	6	−
	12	−
5 N^1-*p*-methoxyphenyl-N^5-*p*-sulphamyl-phenylbiguanide hydrochloride (VI)†	6	−
	12	−
6 N^1-*p*-bromophenyl-N^5-*p*-2-thiazolyl-sulphamylphenyl-biguanide hydrochloride (IX)	20	−
7 N^1-*p*-chlorophenyl-N^5-*p*-2-thiazolyl-sulphamylphenyl-biguanide hydrochloride (VIII)	40	−

* Compound was also found to be non-toxic.

† Compounds were tested for both prophylactic and suppressive action.

As the compounds 4–7 are sulphabiguanide derivatives probably they had no effect against bird malaria. It has been reported that sulphonamides have shown very encouraging results for *P. knowlesi* infection of monkey, and it is not unlikely that these compounds may prove active when tested against this form of malaria. Results of trials of these compounds against simian malaria shall be reported later.

In order to see their bacteriostatic property, compounds 4 and 7, and paludrine hydrochloride were tested against *streptococcal hemolelyticus* and *staphylococcus aureus* both by turbidimetric and Oxford cup method. Paludrine salt was only slightly active while compound 7 showed better activity.

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