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STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS: PART III. ANTIMALARIAL COMPOUNDS

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When we first established our method for examining the antituberculous activity of compounds in mice (Martin, 1946), work in these laboratories which subsequently led to the development of the drug paludrine made available to us a number of new types of compounds showing marked antimalarial activity. We examined these by the new procedure, with the result that promising activity was obtained with certain compounds. The principal stages in the chemical investigations which led ultimately to the discovery of antimalarial activity in N^1 - *p* - chlorophenyl-N^5 - *iso*propylbiguanide ("paludrine") have been described by Curd and Rose (1946), and are indicated in Table I below, which also shows the compounds in which antituberculous activity has been found.

RESULTS

Therapeutic tests against *Mycobacterium tuberculosis* in mice have been carried out on each of the main classes of compounds and the results are listed below (Tables II, III, IV). We also thought it proper to examine other well-known antimalarial drugs; no activity was found with quinine or mepacrine at doses of 0.5, 1.0, or 2.0 mg. per 20 g. mouse, nor with pamaquin at doses of 0.05, 0.1, or 0.2 mg. per 20 g. mouse. The test method was that previously described (Martin, 1946). Briefly, this consisted in the infection of mice by the intravenous route and their treatment by drugs administered twice daily by syringe and catheter at doses ranging downwards from the maximum tolerated. The results are presented in the form used in

TABLE I

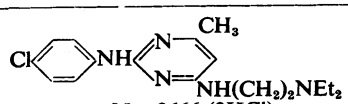
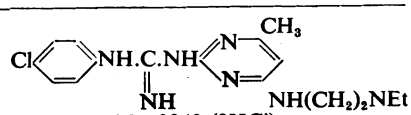
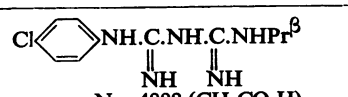
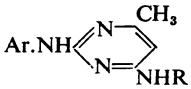
Type of compound	Activity: Antimalarial	Activity: Antituberculous	Representative compound
2-arylamino-4-dialkylaminoalkylamino-6-methylpyrimidines ..	active	active	 No. 2666 (2HCl)
2-arylguanidino-4-dialkylaminoalkylamino-6-methylpyrimidines ..	more active	inactive	 No. 3349 (2HCl)
N^1-aryl-N^5-alkylbiguanides ..	very active	inactive	 No. 4888 (CH_3CO_2H) ("paludrine")

TABLE II

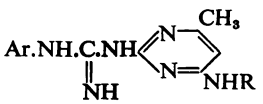
Therapeutic tests on 2-arylamino-4-dialkylaminoalkylamino-6-methylpyrimidines given orally to mice infected with *Mycobacterium tuberculosis*. Doses given twice daily, as dihydrochlorides



No.	Ar	R	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
2666	*p*-chlorophenyl	β-diethylaminoethyl ..	1.0	+2.3	1.6
3711	*p*-chlorophenyl	γ-dimethylaminopropyl	0.5 1.0 1.5 2.0	0 +2.2 +2.0 +1.7	1.7
3299	*p*-chlorophenyl	γ-diethylaminopropyl ..	0.5 1.0	+2.6 +3.2	1.9
			1.0 1.5	+3.4 +2.2	1.7
3300	*p*-chlorophenyl	δ-diethylamino-α-methylbutyl	1.0	+5.5	1.6
			1.5 2.0	+4.2 +4.2	1.7
3502	6-bromo-2-naphthyl	β-diethylaminoethyl ..	1.0 1.5 2.0	+5.5 +4.0 +2.4	1.4
			1.0 2.0 4.0	+3.9 +3.4 +0.4	1.7

TABLE III

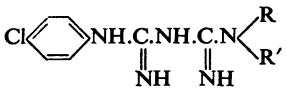
Therapeutic tests on 2-arylguanidino-4-dialkylaminoalkylamino-6-methylpyrimidines given orally to mice infected with *Mycobacterium tuberculosis*. Doses given twice daily, as dihydrochlorides



No.	Ar	R	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
3349	*p*-chlorophenyl	β-diethylaminoethyl ..	1.0 2.0	−2.1 −0.6	1.8
3672	*p*-chlorophenyl	γ-diethylaminopropyl ..	0.1 0.5 1.0	−0.4 0 −0.9	1.7
4926	6-bromo-2-naphthyl	β-diethylaminoethyl ..	0.25 0.5	−0.7 −1.2	1.7

TABLE IV

Therapeutic tests on N^1-aryl-N^5-alkylbiguanides (and related compounds) given orally to mice infected with *Mycobacterium tuberculosis*. Doses given twice daily, No. 4967 as base, No. 4095 as sulphate, No. 5114 as carbonate, and the rest as acetates



No.	R	R′	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
4967	ethyl	H	0.025	−0.2	1.6
4887	*n*-propyl	H	0.1 0.05	−0.2 −0.4	1.4
4888	*iso*propyl ..	H	0.1 0.25	+0.9 −0.7	1.3 2.2
4565	*n*-butyl	H	0.5	−0.1	1.5
4430	*n*-propyl	methyl	0.25 1.0	+0.5 +0.2	1.3
4095	*n*-butyl	*n*-butyl	1.0 0.5	+0.6 +0.3	1.8
5114	δ-diethylamino-α-methylbutyl	H	1.0 1.0	+0.3 +0.8	1.8

preceding papers of this series (e.g., Hoggarth and Martin, 1948).

It was of interest to see how far the therapeutic effects of the new drugs shown in Table II were dependent upon the method of examination. The high activities of compounds Nos. 3300 and 3502 made them suitable for this purpose. It was possible to demonstrate a therapeutic effect with both of these compounds when certain modified dose schedules, as shown in Table V, were adopted.

It will be seen that compound No. 3300 exerted a slight but definite therapeutic effect when dosing was begun one week after infection, showing that the drug was having an inhibitory effect even on established tuberculous disease. The absence of therapeutic effect with a single dose of drug given either immediately before, or 24 hours after, infection is important, since it shows that the drug is not merely killing freshly introduced organisms, but is exerting an effect upon them while they multiply in the tissues.

As a further modification of the usual procedure, compound No. 3300 was given mixed with the food. The drug was mixed with the powdered food, weighed quantities of which were offered to the mice in special containers designed to minimize loss. The consumption of the drug was estimated by weighing the residual food at daily intervals. The figure given in the " dose " column of Table VI (referring to experiments in which the drug was given by this method) is the estimated average daily

TABLE V

Further therapeutic tests on compounds Nos. 3300 and 3502

No.	Dose mg. per 20 g.	Schedule of dosing	Increased mean survival time (days)	Increase required for significance (days)
3502	2.0	(i) twice daily for first 5½ days ..	4·1	1.6
		(ii) as usual (for 19 days)	4.0	
3300	1.0	(i) one dose only before infection	+0.8	1.4
		(ii) one dose only 24 hours after infection.. ..	−0.1	
		(iii) twice daily for first 5½ days after infection.. ..	+3.4	
		(iv) twice daily from 8 to 13½ days after infection ..	+1.8	
		(v) as usual (for 16 days)	+3.5	

intake. The drug was given to other mice by syringe and catheter as usual for comparison. During week-ends when dosing by syringe was suspended, the drug-diet was replaced by plain food.

TABLE VI

Further examination of compound No. 3300. Comparison of the effect of oral dosing with the administration of the drug in the diet. Treatment given for the first 16 days

Dose mg. per 20 g.	Method of administration	Increased mean survival time (days)	Increase required for significance (days)
0.5	in food (per day)	+0.7	1.6
1.0		+3.2	
2.0		+5.6	
3.0		+4.8	
0.5	by syringe and catheter (twice daily)	+0.8	
1.0		+1.4	
2.0		+4.4	
3.0		+4.8	

Discussion

Previous investigations of the chemotherapeutic activity of synthetic substances in tuberculosis have been almost all concerned with compounds of the sulphone-sulphonamide types. The only exceptions of which we are aware are *p*-aminosalicylic acid (Feldman, Karlson, and Hinshaw, 1947) and certain naphthoquinones (Alcalay, 1947). Attention was first drawn to the compounds reported here because of their antimalarial activity, but it is clear that the two types of therapeutic action are not co-extensive. Furthermore, even in the group of compounds showing both antituberculous and antimalarial activity, the compounds are placed in a different order of activity by the *in vivo* test against *Mycobacterium tuberculosis* in mice and the antimalarial test with *Plasmodium gallinaceum* in chicks (Curd, Davey, and Rose, 1945; Curd and Rose, 1946a). Thus, whereas No. 3300 is more active than No. 2666 in the antituberculous test, the reverse is the case in the antimalarial test. The influence of chemical structure upon therapeutic activity in compounds related to No. 3300 will form the subject of a future communication.

Summary

Antituberculous activity in mice has been demonstrated with a new group of compounds (2-arylamino-4-dialkylaminoalkylamino - 6-methylpyrimidines), some members of which are active as antimalarial drugs. No activity was found with the other antimalarial drugs tested.

Most of the compounds mentioned in this report were prepared by the team of chemists working under the direction of Drs. F. H. S. Curd and F. L. Rose, to whom we wish to express our thanks.

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