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2:4-DIAMINOPYRIMIDINES—A NEW SERIES OF ANTIMALARIALS

BY

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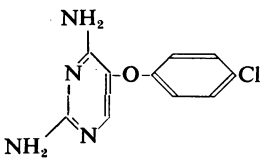
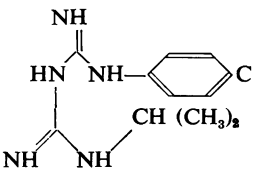
It was found by Hitchings, Elion, VanderWerff, and Falco (1948) that many 2:4-diaminopyrimidines are powerful antagonists of pteroylglutamic acid in cultures of *Lactobacillus casei* (cf. Hitchings, Elion, Falco, Russell, Sherwood, and VanderWerff, 1950). The formal analogy between 2:4-diamino-5-*p*-chlorophenoxypyrimidine (I) and proguanil (" Paludrine ") (II), and the finding that proguanil was also an antagonist of pteroylglutamic acid, suggested that the pyrimidine compound might have antimalarial activity (Falco, Hitchings, Russell, and VanderWerff, 1949).



(I)



(II)

This was shown to be the case, and a short report of the antimalarial activity of another member of this group of compounds was published later by Goodwin (1949) A large number of derivatives of 2:4-diaminopyrimidine substituted in the 5- and 6-positions has now been prepared and tested against laboratory plasmodial infections. The results obtained upon 158 of these substances are recorded in the present paper.

METHODS

Preliminary " screening " tests were carried out against blood-induced infections of *Plasmodium gallinaceum* in chicks and *P. berghei* infections in mice. The more active substances were then assayed against standard antimalarials such as proguanil and quinine. Further tests were carried out with selected compounds against sporozoite-induced infections of *P. gallinaceum* in chicks and against trophozoite and sporozoite infections of *P. cynomolgi* in monkeys.

Acute and chronic toxicity tests and full pharmacological investigations have been made with the more active compounds, and will form the substance of a separate report.

Experimental details

Most of the pyrimidine derivatives were prepared for administration by dissolving the base in water with the addition of lactic acid. One or two compounds were administered as hydrochlorides, and the few which did not form readily soluble salts were suspended in water with the aid of compound powder of tragacanth.

P. gallinaceum *infections*.—The chicks used for the test were Light Sussex X Rhode Island cocks, and were hatched at the Reading University Farm at Shinfield, Berks.

Blood-induced infections.—The method used was similar to that of Davey (1946a). Five-day-old chicks were injected intravenously with 0.2 ml. of a suspension containing approximately 50×10^6 parasitized red cells, the first dose of drug was given orally 2–3 hours later, and further doses twice daily for 3 days. In preparing the inoculum the nomogram devised by Williamson (1948) proved useful and time-saving. Blood films were made at the peak of infection of control birds on the fifth day. Each compound was awarded a "score" according to the following key:

0 = No activity
1 = Slight activity } at 100 mg./kg. in chicks or 50 mg./kg. in mice.
2 = Active at dose levels between 100 and 10 mg./kg.
3 = „ „ „ „ „ 10 and 1 „
4 = „ „ „ „ „ 1 and 0.1 „
5 = „ „ „ „ „ 0.1 and 0.01 „

A dose was considered to be "active" when the mean percentage parasitaemia of the treated group of animals was lowered to 1 per cent or less of the mean percentage parasitaemia of untreated controls.

Active compounds were assayed by the method described by Marshall (1945). The mean percentage parasitaemia in each group of chicks was plotted against the logarithm of the dose, and a curve for each compound was drawn. A curve was determined for a standard antimalarial drug in the same experiment, and the relative activity of the compounds estimated from the ratio of the doses which effected a 50 per cent reduction in parasitaemia compared with untreated controls.

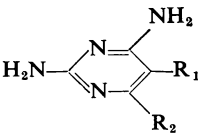
Sporozoite infections were induced by the method of Davey (1946b). Five-day-old chicks were inoculated intravenously with 0.2-ml. quantities of a suspension of sporozoites in a mixture of equal parts of 0.5 per cent glucose-saline and heparinized chick-blood. The suspension was prepared by grinding in a mortar the requisite number of lightly anaesthetized infected *Aedes aegypti* mosquitoes with a little of the saline-blood medium. The suspension was lightly centrifuged and the supernatant sporozoite suspension diluted with medium so that 0.2 ml. contained the equivalent of one mosquito. In untreated controls this inoculum usually caused death from blockage of the brain capillaries with exoerythrocytic schizonts in 9–10 days. Drug treatment was similar to that used for blood infections.

P. berghei *infections*.—The mice used for the test weighed from 20 to 30 grammes. The strain of plasmodium was that described by Vincke and Lips (1948), and was obtained through the kindness of Professor H. E. Shortt. The method used was similar to that described by Goodwin (1949), the mice being infected by intraperitoneal injection of approximately 5×10^6 parasitized red cells suspended in glucose-saline. The first dose of drug was given by mouth 2–3 hours after inoculation and further doses were given twice daily for 3 days. Blood films were made on the seventh day.

Attempts were made to cultivate *Anopheles concolor*, the vector of this parasite (Vincke and Leleup, 1949). Eggs and larvae were kindly sent from the Belgian Congo by Drs.

Vincke and Hanse, but we were unable to obtain the correct conditions for their development. For this reason, no results can as yet be recorded for the effect of the new compounds upon sporozoite-induced infections with *P. berghei*. Tissue-forms of the parasite have been described by Van den Berghe, Vincke, and Chardome (1950) in the histiocytes of the liver and the endothelial cells of the brain capillaries 36 hours after inoculation of infected blood, but we have not yet satisfied ourselves that the exoerythrocytic forms occur in mice after blood inoculation.

TABLE I

2: 4-Diamino-5-phenylpyrimidines 

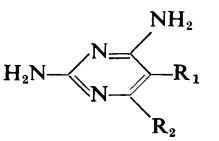
Reference No.	R_1	R_2	Score of activity; proguanil equivalents in parentheses	
			P. gallinaceum	*P. berghei*
48–187	phenyl	H	2	2
50–101	4′-methylphenyl	H	2	2
50–100	4′-methoxyphenyl	H	3	2
50–103	4′-nitrophenyl	H	2	2
50–82	2′-chlorophenyl	H	1	2
50–26	3′-chlorophenyl	H	2 (0.3 P)	2 (0.5 P)
49–248	4′-chlorophenyl	H	3 (0.4 P)	4 (30 P)
49–370	4′-bromophenyl	H	3 (0.6 P)	4 (10 P)
50–308	4′-fluorophenyl	H	3 (0.6 P)	4 (7 P)
50–59	phenyl	Me	3	2
50–200	phenyl	Et	3	2
50–201	4′-methylphenyl	Me	2	0
50–143	2′-chlorophenyl	Me	2	2
50–338	3′-fluorophenyl	Me	3	3
50–142	3′-chlorophenyl	Me	3	3
50–329	3′-bromophenyl	Me	3	3
50–283	4′-fluorophenyl	Me	4	3
50–58	4′-chlorophenyl	Me	4 (15 P)	4 (40 P)
50–322	4′-bromophenyl	Me	4	4
50–307	4′-fluorophenyl	Et	4 (5 P)	4 (7 P)
50–63	4′-chlorophenyl	Et	5 (60 P)	5 (200 P)
50–238	4′-bromophenyl	Et	4 (30 P)	4 (80 P)
50–148	4′-chlorophenyl	*n*-Pr	4 (20 P)	3 (5 P)
50–172	4′-chlorophenyl	*n*-Bu	4 (7 P)	3 (1 P)
50–146	4′-chlorophenyl	*iso*-Bu	4 (10 P)	3 (3 P)
50–109	4′-chlorophenyl	*n*-Am	4 (40 P)	3 (8 P)
50–256	4′-chlorophenyl	*n*-hexyl	4 (5 P)	3 (2 P)
50–251	4′-chlorophenyl	*n*-heptyl	4 (5 P)	3 (6 P)
50–141	4′-chlorophenyl	*n*-undecyl	3 (4 P)	3 (9 P)
50–115	4′-chlorophenyl	phenyl	3	2
50–230	4′-chlorophenyl	3-pyridyl	3 (0.5 P)	1
50–339	4′-chlorophenyl	4-pyridyl	2	0
50–198	4′-chlorophenyl	methoxymethyl	3	0
50–126	4′-nitrophenyl	Me	3 (2 P)	3 (2 P)
50–199	4′-nitrophenyl	Et	4 (4 P)	3
50–99	2′: 4′-dichlorophenyl	H	2	3 (1 P)
50–11	3′: 4′-dichlorophenyl	H	3 (0.7 P)	3
50–197	3′: 4′-dichlorophenyl	Me	4 (14 P)	5 (130 P)
50–276	3′: 4′-dichlorophenyl	Et	4 (20 P)	5 (190 P)
50–280	3′: 4′-dichlorophenyl	*n*-Pr	4	4
50–292	3′: 4′-dichlorophenyl	*n*-Bu	4	3

P. cynomolgi *infections*.—We are also indebted to Professor H. E. Shortt for trophozoite- and sporozoite-inoculated monkeys. Trophozoite infections were induced by the intraperitoneal injection of infected citrated blood. For infection with sporozoites, monkeys under pentobarbitone anaesthesia were placed in a cage containing a large number of infected mosquitoes which were allowed to feed. In neither trophozoite nor sporozoite infections was there any evaluation of the number of parasites inoculated. The monkeys were dosed orally with drugs, and stained thick and thin blood films prepared and examined at frequent intervals. Four animals with established, long-standing sporozoite infections were selected for investigation of the effect of prolonged treatment with large doses of three of the more active compounds. This experiment also served as a chronic toxicity test, and determinations of haemoglobin and red and white cell counts were made at intervals.

Toxicity tests.—Toxicity determinations were made in mice with some of the more active compounds and with a series of well-known antimalarial drugs. Two dose-levels were used for each compound, with a group of 10 mice at each level. Doses of drug were given by mouth, in solution or suspension in a volume of 0.5 ml. per 20 g. body weight

TABLE II

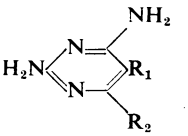
2 : 4-Diamino-5-benzylpyrimidines



Reference No.	R_1	R_2	Score of activity; proguanil equivalents in parentheses	
			P. gallinaceum	*P. berghei*
48–224	benzyl	H	2	2
49–294	4′-methylbenzyl	H	2	0
49–169	2′-chlorobenzyl	H	1	2
48–257	4′-chlorobenzyl	H	2	2 (0.6 P)
50–62	4′-nitrobenzyl	H	3 (0.6 P)	2
49–161	4′-methoxybenzyl	H	2 (0.2 P)	2
49–210	3′ : 4′-dimethoxybenzyl	H	3	1
49–267	4′-dimethylaminobenzyl	H	2 (0.15 P)	0
48–228	benzyl	Me	2	3
49–172	2′-chlorobenzyl	Me	2	2
49–224	4′-chlorobenzyl	Me	3 (0.4 P)	3 (2 P)
49–315	4′-bromobenzyl	Me	3 (0.3 P)	3 (1 P)
50–203	4′-chlorobenzyl	Et	3	2
49–336	4′-chlorobenzyl	*n*-Pr	2	1
49–302	3′-methylbenzyl	Me	2	0
49–371	4′-nitrobenzyl	Me	3 (1 P)	3 (1.5 P)
50–88	4′-aminobenzyl	Me	2	0
49–291	4′-methoxybenzyl	Me	2	1
49–292	2′ : 4′-dichlorobenzyl	Me	2	3
50–61	3′ : 4′-dichlorobenzyl	Me	2	2
50–2	3′ : 4′-dimethoxybenzyl	Me	2 (0.2 P)	0
49–299	3′ : 4′-methylenedioxybenzyl	Me	2	0
50–110	4′-acetamidobenzyl	Me	3	1
48–251	benzyl	phenyl	0	1
49–223	benzyl	Cl	0	0
48–243	benzyl	NH_2	2 (0.05 P)	1 (0.1 P)
49–295	4′-chlorobenzyl	NH_2	2	2

TABLE III

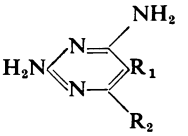
2 : 4-Diamino-5-alkoxy- and -aryloxy-pyrimidines



Reference No.	R_1	R_2	Score of activity; proguanil equivalents in parentheses	
			P. gallinaceum	*P. berghei*
49–243	methoxy	H	0	0
48–211	ethoxy	H	2 (0.1 P)	1
49–251	*n*-butoxy	H	2 (0 2 P)	1 (0.3 P)
50–174	*n*-octyloxy	H	2	0
49–249	benzyloxy	H	2	2 (0.8 P)
49–200	phenoxy	H	2	1
49–173	4′-methylphenoxy	H	0	1
49–220	4′-tertbutylphenoxy	H	1	1
49–155	4′-*iso*-cctylphenoxy	H	0	0
49–269	3′ : 4′-dimethylphenoxy	H	1	0
49–272	2′-*iso*propyl-5′-methylphenoxy	H	0	0
49–69	α-naphthoxy	H	1	1
48–256	β-naphthoxy	H	2	2
49–170	β-naphthoxy	phenyl	1	0
49–74	6′-bromo-β-naphthoxy	H	1	1
49–70	2′ : 6′-dichloro-α-naphthoxy	H	0	2
48–284	4′-phenylphenoxy	H	2	3 (1 P)
48–285	4′-benzylphenoxy	H	2	2 (1 P)
49–231	4′-hydrindylphenoxy	H	3	2
49–72	4′-hydroxyphenoxy	H	1	1
49–209	3′-methoxyphenoxy	H	1	1
49–229	4′-methoxyphenoxy	H	2	0
49–369	2′ : 6′-dimethoxyphenoxy	H	0	0
49–42	4′-benzyloxyphenoxy	H	1	0
48–221	2′-chlorophenoxy	H	1	1
48–287	3′-chlorophenoxy	H	2 (0.05 P)	1
48–122	4′-chlorophenoxy	H	2	2
49–277	4′-bromophenoxy	H	0	1
48–152	2′ : 4′-dichlorophenoxy	H	0	1
49–19	2′ : 4′-dibromophenoxy	H	0	3
49–225	2′ : 4′ : 5′-trichlorophenoxy	H	0	0
49–160	4′-chloro-3′-methylphenoxy	H	1	0
49–226	4′-tertbutyl-2′-chlorophenoxy	H	1	0
49–159	4′-chloro-2′-phenylphenoxy	H	0	0
49–171	4′-chloro-5′-methyl-2′-*iso*propyl phenoxy	H	1	2
49–284	phenoxy	Me	2	1
50–104	4′-propionylphenoxy	Me	3	1
49–318	3′ : 4′-dimethylphenoxy	Me	0	0
49–273	4′-methoxyphenoxy	Me	2	0
50–1	4′-phenylphenoxy	Me	2	2 (0.4 P)
49–142	β-naphthoxy	Me	0	1
49–175	β-naphthoxy	*n*-Pr	1	0
49–265	3′-chlorophenoxy	Me	3 (0.5 P)	1
48–210	4′-chlorophenoxy	Me	3 (0.4 P)	3 (0.7 P)
50–193	4′-chlorophenoxy	Et	3	1
50–78	4′-chlorophenoxy	*n*-Pr	2	1
49–233	4′-chlorophenoxy	phenyl	0	1
50–57	4′-nitrophenoxy	Me	3 (2 P)	3
50–73	4′-aminophenoxy	Me	2	0
50–87	4′-acetamidophenoxy	Me	1	1
50–167	4′-(*p*-chlorobenzyloxy) phenoxy	Me	2	1
50–183	4′-benzenesulphonylphenoxy	Me	1	0

TABLE IV

Miscellaneous derivatives of 2 : 4-diaminopyrimidine



Reference No.	R_1	R_2	Score of activity	
			P. gallinaceum	*P. berghei*
50–117	α-naphthyl	H	0	0
50–330	β-phenoxyethyl	H	0	0
50–346	β-phenoxyethyl	Me	1	0
49–250	nitro	H	0	0
49–376	aminomethyl	H	1	1
49–68	formylamino	H	0	0
49–92	chloroacetylamino	H	0	0
48–124	H	phenyl	0	0
49–61	H	β-naphthyl	0	0
48–29	Me	Me	2	0
48–174	*n*-propyl	Me	0	1
50–202	2-furyl	Me	1	1
50–249	2-thienyl	Me	1	0
50–305	dibromothienyl-2-	Me	3	4
50–173	2′-phenylthiazolyl-4-	Me	2	0
50–171	α-4′-chlorophenethyl	Me	2	0
48–98	bromo	Me	0	0
48–244	*n*-butyl	amino	2	1
49–8	Me	4′-chlorophenyl	0	0
Methiodides				
50–186	4′-chlorophenyl	H	0	1
50–284	4′-chlorophenoxy	Me	2	2

Results and Discussion

The results are presented in Tables I–IX. The activity of each compound is recorded as a " score " from 0 to 5 according to the key on p. 186, and the proguanil equivalents of the more potent substances are also recorded in parentheses.

Activity against blood-induced infections of P. gallinaceum *and* P. berghei

With a large series of substances such as this, which are closely related chemically and many of which have similar solubilities, it is profitable to consider the effect of structure upon therapeutic potency.

The effect of the 5-substituent.—Although some antimalarial activity is shown by substances with simple substituents in the 5-position of the pyrimidine nucleus (Table III, 48–211, 49–251; Table IV, 48–29) the really potent substances are found among those with substituents containing a benzene nucleus. Of these, the phenyl series (Table I) is generally more active than the benzyl series (Table II), which in turn is more active than the phenoxy series (Table III).

An electron-attracting group, such as nitro- or halogen in the *para-* position of the benzene ring enhances activity; *ortho-* and *meta-* derivatives are generally less active than the corresponding *para-* compounds (Table I, 50–82, 50–26, 49–248; Table II, 49–172, 49–224), although in the phenoxy series the *m*-chloro- compounds are about equal in activity to the *p*-chloro compounds against *P. gallinaceum* (Table III, 48–287, 48–122, 49–265, and 48–210). Corresponding chloro-, bromo-, and fluoro- compounds are all of about the same degree of activity when assessed by the

TABLE V

Miscellaneous pyrimidine derivatives and related compounds

Reference No.	Substance	Score of activity	
		P. gallinaceum	*P. berghei*
50–27	4-amino-5-phenylpyrimidine	1	0
50–28	4-amino-5-*p*-chlorophenylpyrimidine	2	0
50–66	4-amino-2-methyl-5-*p*-chlorophenyl pyrimidine	0	0
50–67	4-amino-2: 6-dimethyl-5-*p*-chlorophenyl pyrimidine	1	0
50–125	2-amino-4-hydroxy-5-*p*-chlorophenyl pyrimidine	2	0
48–231	2-amino-4-hydroxy-6-methyl-5-*p*-chlorophenoxy-pyrimidine	1	0
48–194	2-amino-4-mercapto-5-*p*-chlorophenoxy pyrimidine	0	1
50–325	2: 4-*bis* (N-morpholino)-6-phenylpyrimidine	0	0
50–79	4-amino-2-*p*-methylphenyl-5-*p*-chlorophenyl pyrimidine	1	0
49–128	2-amino-4-methylamino-5-*p*-chlorophenoxy pyrimidine	2	1
50–187	2-amino-4-methyl-6-(6′-aminoquinolyl)-pyrimidine	0	0
48–204	2: 4-diamino-6-chloroquinazoline	0	1
49–122	2: 4-diamino-8-*p*-chlorophenylpurine	0	0
49–373	2: 4-diamino-6: 7-di(*p*-chlorophenyl)-pteridine	0	2
49–274	2: 4-diamino-6 or 7-*o*-chlorophenyl-7 or 6-*p*-methoxyphenyl-pteridine	2	1
48–14	2: 6-diaminopurine	1	1
45–70	5-hydroxyuracil	0	1

key on p. 186, but accurate determination of the relative potencies of these derivatives shows that the fluoro- compounds are always slightly less active than the chloro- and bromo- compounds (Fig. 1).

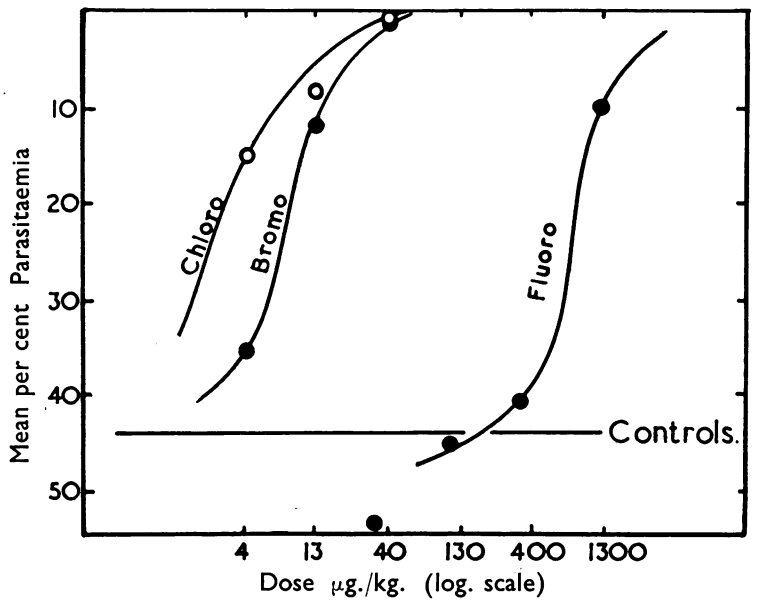


FIG. 1.—A comparison of the activities against *P. berghei* of 2: 4-diamino-5-*p*-chlorophenyl-6-ethyl pyrimidine (50–63) and the corresponding bromo- and fluoro- derivatives. All administered by mouth as solutions of the lactates; a group of 5 mice used at each dose-level.

Dichloro- derivatives in the phenyl series (Table I, 50–99, 50–11, 50–197, 50–276, 50–280, 50–292) are also very potent antimalarials especially against *P. berghei*, and have activities approximately equal to the corresponding *p*-chloro- compounds. The 3′: 4′-dichlorophenyl-6-methyl compound (50–197), although about equal in activity to the corresponding 4′-chlorophenyl compound (50–58) against *P. gallinaceum*, is actually three times as active against *P. berghei*. The 3′: 4′-dichlorophenyl-6-ethyl compound (50–276) has one-third of the activity of the corresponding 4′-chlorophenyl compound (50–63) against *P. gallinaceum*, but the two substances are equally active against *P. berghei*. Phenoxy derivatives with more than one halogen substituent, on the other hand, are almost without action (Table III, 48–152, 49–19, 49–225). Toxicity tests show that the 3′: 4′-dichlorophenyl compounds are rather more toxic than the monochloro-, and delayed deaths are found to occur among the mice for 5 days after oral administration of the drug (Table VI). A similar prolonged toxic action was observed with the *p*-chlorophenyl compound with no 6-substituent (Table I, 49–248).

The failure of a second chlorine atom in the benzene ring to enhance activity against *P. gallinaceum* in the 2: 4-diaminopyrimidines is interesting in view of the recent publication of Curd, Davey, Hendry, and Rose (1950), in which the dichloro-derivative corresponding to proguanil is shown to be 2.5 to 5 times as active as proguanil. It is also more toxic to some species of animals. We find this compound in preliminary tests to be 1 to 3 times as active as proguanil against *P. berghei*.

The nitro- compounds of the phenyl series are less active than the corresponding chloro- compounds; in the phenoxy and benzyl series the nitro- derivatives are somewhat more active than the chloro-, especially when *P. gallinaceum* is used as the test-organism.

Electron-donating substituents such as alkyl and methoxy groups do not increase antimalarial activity (Table I, 50–101, 50–100).

Alkoxy compounds above methoxy (Table III, 48–211, 49–251, 50–174) have roughly the order of activity of the unsubstituted phenoxy derivatives. However, by appropriate substitutions the activity of the latter may be greatly enhanced, whereas the alkoxy series is not subject to similar modification.

The effect of the 6-substituent.—The antimalarial activity of the *p*-chloro derivatives is enhanced by the introduction of a methyl group in the 6-position of the pyrimidine nucleus. The same generalization probably applies to compounds with other electron-attractive substituents in the benzene nucleus, but does not apply to the dimethylphenoxy or naphthoxy derivatives (Table III, 49–269, 49–318, 48–256, 49–142). When a series of alkyl derivatives was compared the methyl derivative was found to be the most active in both the benzyl (Table II, 49–224, 50–203, 49–336) and phenoxy series (Table III, 48–210, 50–193, 50–78). The effect of the nature and length of the alkyl radical was studied in considerable detail in the phenyl series, in which the peak of activity is reached with the 6-ethyl compound, 50–63 (Table I), which is 60 times as active as proguanil against *P. gallinaceum*, and 200 times as active against *P. berghei*. Higher and lower members of the series are less active, but to a different degree with the two species of plasmodium. *P. berghei* is rather less sensitive than *P. gallinaceum* to the higher members of the series, but these differences may perhaps be explained by differences in the rates of absorption,

metabolism, and excretion of the drugs by the mammalian and avian hosts. In this series it is also noteworthy that the *iso*butyl is more active than the *n*-butyl derivative, and that there is a tendency for compounds with an odd number of C-atoms in the chain to be more active than compounds with an even number of C-atoms. The relative activities of this group of compounds (Table I, 49–248, 50–58, 50–63, 50–148, 50–172, 50–109, 50–256, 50–251, 50–141) are shown diagrammatically in Fig. 2.

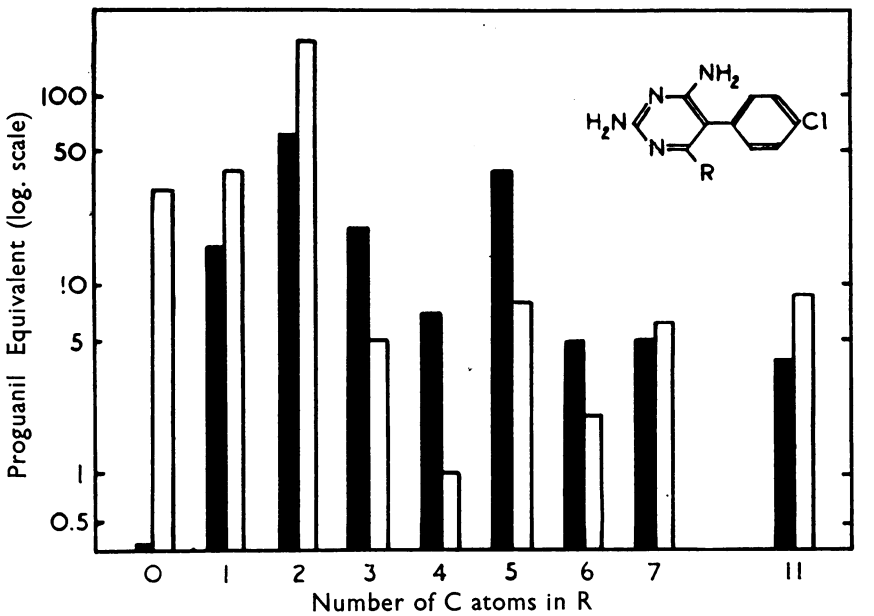


FIG. 2.—The effect upon antimalarial activity of the chain length of the alkyl group in a series of 2: 4-diamino-5-*p*-chlorophenyl-6-alkyl-pyrimidines. Open columns: *P. berghei*; solid columns: *P. gallinaceum*.

The effects of substituents of other types in the 6-position do not lend themselves to ready interpretation. The 6-phenyl derivatives are less active than their alkyl congeners (Table I, 50–115; Table II, 48–251; Table III, 49–233). A 3-pyridyl derivative (Table I, 50–230) has approximately the activity of the phenyl, but the 4-pyridyl is much less active (Table I, 50–339). In the benzyl series the 6-amino derivatives are only slightly less active than the compounds unsubstituted in position 6, but the 6-chloro- compound is essentially inactive (Table II, 48–243, 49–295, 49–223).

Quaternization of a pyrimidine-N atom greatly decreases antimalarial activity (Table IV, 50–186, 50–284).

The relationship of the 2: 4-diaminopyrimidines to similar compounds differing by substitution of the amino groups is worthy of consideration. A large series of pyrimidine derivatives with alkylaminoalkylamino side chains was studied by Davey (1946a) and Curd, Richardson, and Rose (1946). On the basis of their results and those reported in the present paper, at least two types of pyrimidine antimalarials appear to be distinguishable. The first type is exemplified by those pyrimidines carrying phenyl, phenoxy, or benzyl groupings in the 5-position, and in which

substitution of the 2- and/or 4-amino groups appears to diminish the antimalarial activity. For example, 2-*p*-chloroanilino-4-β-diethylaminoethylaminopyrimidine (Curd, Richardson, and Rose, 1946) is less active than 2-amino-4-β-diethylaminoethylaminopyrimidine (Hull, Lovell, Openshaw, and Todd, 1947), which in turn is less active than 2: 4-diamino-5-phenoxypyrimidine (Table III, 49–200). A similar stepwise effect does not appear to be true of the derivatives of 5-benzyl-6-methylpyrimidine, but it is abundantly clear that the unsubstituted 2: 4-diamino-5-benzyl-6-methylpyrimidine (Table II, 48–228) is much more active than the corresponding compounds with substituted amino groups (Hull, Lovell, Openshaw, Payman, and Todd, 1946; Curd, Richardson, and Rose, 1946) and that the 2: 4-diamino-5-

TABLE VI

Acute oral toxicities in mice and antimalarial activities of selected 2: 4-diaminopyrimidines compared with standard antimalarial drugs

Drug	LD50 (mg./kg.)	Limits of error per cent (P=0.99)	Time taken for mice to die	Proguanil equivalent against *P. gallinaceum*	Proguanil equivalent against *P. berghei*
Quinine HCl	1,160	91–111	1 hr.	0.08	0.16
Proguanil acetate	59	83–121	24 hr.	1.0	1.0
Mepacrine HCl	871	82–123	20 min.	0.1	1.7
Chloroquin base	752	88–114	15 min.	1.2	3.3
Pamaquin B.P.	68	85–118	1 hr.	1.2	0.53
2: 4-Diamino-5-(4′-chlorophenoxy)-6-methylpyrimidine (48–210)	ca. 1,000	—	24 hr.	0.4	0.7
2: 4-Diamino-5-(4′-chlorobenzyl)-6-methylpyrimidine (49–224)	79	83–113	½ hr.	0.4	2.0
2: 4-Diamino-5-(4′-chlorophenyl)-pyrimidine (49–248)	250	55–180	Up to 3 weeks	0.4	30
2: 4-Diamino-5-(4′-nitrobenzyl)-6-methylpyrimidine (49–371)	146	93–101	3–4 hr.	1.0	1.5
2: 4-Diamino-5-(4′-chlorophenyl)-6-ethylpyrimidine (50–63)	92	94–108	10 min.	60	200
2: 4-Diamino-5-(3′: 4′-dichlorophenyl)-6-ethylpyrimidine (50–276)	66	82–120	Up to 5 days	20	190

phenylpyrimidine (Table I, 48–187) is much more active than related amino-substituted derivatives (Curd, Richardson, and Rose, 1946). Similarly a 2-amino-4-methylamino-5-*p*-chlorophenoxypyrimidine (Table V, 49–128) is less active than the corresponding 2: 4-diamino derivative (Table III, 48–122).

The second type comprises pyrimidines which are unsubstituted, or carry only alkylsubstituents in the 5-position (those which eventually led to the more active acyclic analogue, proguanil). In this group of compounds, substitution of the amino group is requisite to activity (Basford, Curd, Hoggarth, and Rose, 1947). This difference between the two groups appears to be borne out by the reported absence of cross-resistance between diaminopyrimidines and proguanil which is discussed below.

The essential nature of the 2- and 4-amino group in the pyrimidine nucleus for activity in the present series receives further confirmation from the lower activity

of 2-amino-4-hydroxy (Table V, 50–125, 48–231) and 2-amino-4-mercapto (Table V, 48–194) derivatives as compared with the corresponding 2: 4-diaminopyrimidines (Table III, 48–210, 48–122). Furthermore, 4-amino-5-phenyl- and 4-amino-5-*p*-chlorophenylpyrimidines (Table V, 50–27 and 50–28) are only feebly active as compared with the corresponding diamino-derivatives (Table I, 48–187, 49–248). A number of condensed systems containing the 2: 4-diaminopyrimidine structure show moderate though generally unpromising activity (Table V, 48–204, 48–14). In this category the pteridines (Table V, 49–373, 49–274) possess activities similar to those of the 2: 4-diamino-pteridines studied by Greenberg (1949).

Acute oral toxicities in mice of some of the more active compounds from the three main series are shown in Table VI in comparison with those of standard antimalarial drugs.

TABLE VII

The effect of 2 : 4-diaminopyrimidines upon heavy sporozoite-induced infections of *P. gallinaceum* (1 mosquito per chick)

Reference No.	Substituents	Dose mg./kg.	No. of chicks	No. protected for 28 days	No. living longer than controls	No. showing E.E. forms *post mortem*
Proguanil	—	50	(toxic)			
		40	5	1	2	2
		25	6	0	6	6
		20	10	4	2	3
		12.5	6	0	3	4
		10	5	1	0	2
		5	4	1	0	2
48–210	5-*p*-chlorophenoxy-6-methyl	40	5	1	4	1
		32	6	0	6	6
		20	10	3	8	3
		16	6	0	5	5
		10	5	2	2	3
		8	6	0	5	6
		5	5	0	2	3
49–224	5-*p*-chlorobenzyl-6-methyl	50	(toxic)			
		40	4	2	2	0
		25	6	2	4	3
		20	5	2	3	0
49–248	5-*p*-chlorophenyl	80	(toxic)			
		40	9	2	7	6
		20	9	3	7	5
49–371	5-*p*-nitrobenzyl-6-methyl	40 (toxic to 4/6)	6	2	2	0
		20 (toxic to 3/6)	6	2	3	1
48–211	5-ethoxy	20	5	1	1	3
		10	5	1	1	3
		5	5	0	0	5
49–161	5-*p*-methoxybenzyl	40	5	1	3	4
		20	5	0	1	5

Sporozoite-induced infections of P. gallinaceum *and* P. cynomolgi

The results of treatment of sporozoite-induced infections of *P. gallinaceum* in chicks with a selection of substances are shown in Tables VII and VIII. It is apparent that the 2: 4-diaminopyrimidines are at least as efficient as proguanil in delaying death from the development of exoerythrocytic schizonts. Greenberg, Trembley, and Coatney (1950) have recently demonstrated that very heavy sporozoite infections such as were used in the experiments recorded in Table VII may give a false impression of the value of antimalarial drugs in the treatment of human malaria. They showed that the results obtained by Alving *et al.* (1948) in natural

TABLE VIII

The effect of 2 : 4-diaminopyrimidines upon heavy and light sporozoite infections of *P. gallinaceum*

Inoculum	Reference No.	Substituents	Dose mg./kg.	No. of chicks	No. protected for 28 days	No. living longer than controls	No. showing E.E. forms *post mortem*
Heavy (One mosquito per chick)	Proguanil		30	6	0	3	3
			15	6	0	3	3
	49–224	5-*p*-chlorobenzyl-6-methyl	25	6	1	2	0
			10	5	1	4	3
	50–58	5-*p*-chlorophenyl-6-methyl	5	(Toxic)			
			2	6	1	1	0
	50–63	5-*p*-chlorophenyl-6-ethyl	5*	Toxic to 5/6 chicks	1	1	0
			2	6	1	1	0
	50–109	5-*p*-chlorophenyl-6-*n*-amyl	5*	6	2	4	0
			2	6	3	4	0
	50–141	5-*p*-chlorophenyl-6-*n*-undecyl	5*	6	0	4	4
			2	4	0	0	2
Light (0.01 mosquito per chick)	Proguanil		30	6	1	2	0
			15	5	2	3	1
	49–224	5-*p*-chlorobenzyl-6-methyl	25	6	0	0	0
			10	6	1	1	0
	50–58	5-*p*-chlorophenyl-6-methyl	5*	6	3	3	0
			2	6	2	2	0
	50–63	5-*p*-chlorophenyl-6-ethyl	5*	5	2	2	0
			2	5	1	2	0
	50–109	5-*p*-chlorophenyl-6-*n*-amyl	5*	6	0	0	0
			2	6	2	2	0
	50–141	5-*p*-chlorophenyl-6-*n*-undecyl	5*	6	0	1	3
			2	6	0	0	4

*Dose given once daily.

TABLE IX

The effect of three 2 : 4-diaminopyrimidines upon *P. cynomolgi* infections in monkeys. Substituents:—49–224: 5-*p*-chlorobenzyl-6-methyl; 49–248: 5-*p*-chlorophenyl; 50–63: 5-*p*-chlorophenyl-6-ethyl

Method of infection	Monkey No.	Drug	Treatment	Result
(1) Blood inoculation	60	49–224	4 mg./kg. twice daily for 6½ days beginning on 10th day after inoculation	Blood became free from parasites on 6th day of treatment. No relapses followed within 8 weeks of splenectomy 20 days later. Conclusion: cured
	63	49–224	15 mg./kg. twice daily for 6½ days beginning on 10th day after inoculation	Blood became free from parasites on 6th day of treatment. No relapse followed within a week of splenectomy 20 days later. Conclusion: probably cured
	62	—	Control	Primary infection reached peak before 10th day and then decreased. Relapsed immediately after splenectomy and again 8 weeks later
(2) Sporozoite infection by mosquito bite	3	49–224	40 mg./kg. given on 4th day after infection	Parasites appeared in the blood 10 days after treatment. Conclusion: no effect upon pre-erythrocytic forms
			4 mg./kg. twice daily for 5 days beginning 14 days after infection	Blood freed from parasites, but a relapse occurred 4 weeks later. Conclusion: blood forms cleared, but no effect upon exoerythrocytic forms
	5	49–224	Two doses of 20 mg./kg. given 1½ hr. before infection	Parasites appeared in the blood 14 days after infection. Conclusion: no effect upon sporozoites
	4	49–248	4 mg./kg. twice daily for 5 days, beginning 14 days after infection	Parasites disappeared from the blood on 3rd day of treatment, but a relapse occurred 4 weeks later. Conclusion: blood forms cleared; no effect on E.E. forms
(3) Sporozoite infection by mosquito bite	11	50–63	5 mg./kg. twice daily for 4½ days beginning 7 days after infection	No parasites appeared until the 30th day. Conclusion: probably a suppressive action due to prolonged presence of the drug in the tissues
	12	—	Control	Parasites appeared in the blood 13 days after infection and persisted for 12 days

After a period of rest, monkeys 11, 5, and 3 were given long courses of substances 50–63, 49–371, and 48–210 respectively. In all, 57 doses each of 5 mg./kg. were given during 6 weeks. No parasites were found in the blood of any monkey during treatment or for 25 days afterwards. The 3 animals, and also monkey 12 to act as a control, were then splenectomized; all four relapsed 4 days later.

human malarial infections with pamaquin treatment were most closely paralleled in *P. gallinaceum* when they used very light infections produced by injecting the equivalent of 0.01 mosquito per chick. We have therefore compared treatment with 2: 4-diaminopyrimidines in heavy and light sporozoite infections, and the results are set forth in Table VIII.

It will be seen that with compounds 49–224, 50–58, 50–63, and 50–109, when the chicks are lightly infected, no exoerythrocytic forms could be found, although some of the birds failed to survive to the end of the test.

Table IX shows the results of treatment of a small number of rhesus monkeys infected with heavy inocula of sporozoites of *P. cynomolgi*. It will be seen that, although small doses of the drugs used were sufficient to suppress the infection, there was little effect upon the tissue forms of the parasite; even 5 mg./kg. given twice daily for six weeks did not prevent or delay the appearance of a heavy relapse of blood infection which occurred a few days after splenectomy.

In spite of the intensive treatment, no ill effects were observed. None of the animals refused food or showed any signs of toxic effects, and the haemoglobin and the red and white cell counts were unaltered. More extensive studies of the toxicity and pharmacology of the 2: 4-diaminopyrimidines will be reported in a further communication.

Action upon other organisms

Many of the compounds, including all the more active antimalarials, were tested against laboratory infections of *Trypanosoma rhodesiense*, *T. congolense*, *T. cruzi*, *Leishmania donovani*, and *Entamoeba histolytica*, by the methods described by Burn, Finney, and Goodwin (1950). No significant activity, apart from slight activity against *T. congolense* by the phenoxy derivatives, was shown by any. However, many of the 2: 4-diaminopyrimidines have powerful antibacterial activity *in vitro*. Individual compounds vary in the degree to which they are affected by the presence of blood and serum, and none has very great antibacterial activity *in vivo*. (Observations of Mr. H. VanderWerff and Mr. S. R. M. Bushby.) It is interesting that the more powerful antimalarials have, in general, the weaker antibacterial action; the best antibacterials are mostly compounds with electron-donating groups in the *para-* position of the 5-benzene nucleus. An important exception to this generalization is found in the phenyl series (Table I), in which the most active antimalarial (50–63) has also the highest antibacterial action. In both the phenoxy and benzyl series the compounds with H in the 6-position are more active than 6-alkyl derivatives against bacteria *in vitro*.

Possible use in human malaria

It may be valuable to speculate upon the possible uses of these compounds in human malaria. In view of the fact that they show very high activity against three species of *Plasmodium* in experimental animals, it is likely that they will also be of use against the parasites of man. The effective dose of the 5-*p*-chlorophenyl-6-ethyl derivative (50–63) is so small that it may well prove an economical compound for suppression, and, because of its relatively low toxicity, a potent drug for the treatment of overt infections. However, as a true causal prophylactic against *P. vivax* it is less likely to be of value, because large doses fail to affect the exoerythrocytic stages of *P. cynomolgi*.

A further consideration is the possibility of the value of these substances in the treatment of the proguanil-resistant strains of human malaria which are now appearing in several parts of the world (Edeson and Field, 1950). Greenberg and Richeson (1950) found that 2: 4-diamino-5-phenoxypyrimidines, like proguanil, acted synergistically with sulphadiazine upon *P. gallinaceum* infections, and therefore were likely to act upon the same metabolic systems of the parasite—probably those mediated by pteroylglutamic acid. However, the modes of action of the pyrimidines and proguanil may not be identical, as antagonism to pteroylglutamic acid and antimalarial activity do not run in parallel, and also a proguanil-resistant strain of *P. gallinaceum* does not show cross-resistance to 2: 4-diamino-5-*p*-chlorophenoxy-6-methylpyrimidine (48–210). Our own experience with drug-resistant strains of *P. gallinaceum* and *P. berghei* has confirmed and amplified these observations, and it is certain that the 2: 4-diaminopyrimidines are valuable in the treatment of proguanil-resistant strains. This work will be reported in detail in a later communication.

Summary

1. A series of derivatives of 2: 4-diaminopyrimidine has been prepared and tested for antimalarial activity.

2. High activity is shown by many members against *P. gallinaceum* in chicks and *P. berghei* in mice.

3. Substances with a 5-phenyl substituent are the most active; 5-benzyl and 5-phenoxy derivatives are somewhat less active.

4. Substitution of halogen or nitro groups in the *para-* position of the benzene nucleus of the 5-substituent enhances activity.

5. Substitution of an alkyl group in the 6-position enhances activity, and, in the 5-phenyl derivatives, a peak of activity is reached with the 6-ethyl compound. 2: 4-Diamino-5-*p*-chlorophenyl-6-ethylpyrimidine is 60 times as active as proguanil against *P. gallinaceum* and 200 times as active against *P. berghei*. Longer chain alkyl derivatives are less active.

6. The drugs are also active against the blood-forms of *P. cynomolgi* in monkeys, but have no pronounced action upon the exoerythrocytic stages.

7. It is hoped that these drugs will prove to be of value in the suppression and treatment of human malaria, and especially in the treatment of proguanil-resistant strains.

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Note added in proof: Two of the compounds, 50–63 (Table I) and 49–224 (Table II), were further tested against blood-induced *P. cathemerium* infections of canaries. The minimum effective dose (MED), here defined as the dose which effected a 50 per cent reduction in parasitaemia compared with untreated controls, was estimated graphically as previously described from percentage parasitaemia counts on the sixth day.

The results are summarized in the Table below. The results with *P. gallinaceum* for 49–224 and 50–63 were estimated from previous assays and are the means of two or more experiments; the results for proguanil in both *P. gallinaceum* and *P. cathemerium* infections were taken from the paper by Davey (1946a).

Drug	*P. gallinaceum* MED	*P. cathemerium* MED	Ratio $\frac{\text{MED } P. \text{ cath.}}{\text{MED } P. \text{ gall.}}$
49–224	3.6 mg./kg. (mean of two)	>15 mg./kg.	>4
50–63	0.026 mg./kg. (mean of four)	0.2 mg./kg.	8
Proguanil ..	0.25 mg./50 g. (5 mg./kg.)	0.5 mg./20 g. (25 mg./kg.)	5

These results show that two members of the new series, previously shown to be highly active against *P. gallinaceum* and *P. berghei*, are considerably less effective against *P. cathemerium*. However, proguanil shows corresponding variations, yet is active against the plasmodia which infect man, and it may be expected that these two compounds act in a similar way.