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ENGLISH ONLY

(avec résumé en français)

SEARCHING FOR NEW ANTIMALARIAL COMPOUNDS¹

by

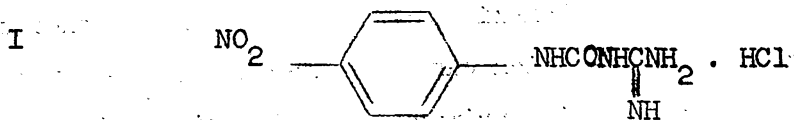
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INTRODUCTION

The finding that some substituted amidineureas were active against Plasmodium gallinaceum in vivo was reported in 1960 (Chin et al., 1960 a & b, WHO 1961).

One of these compounds, 1-(p-nitrophenyl)-2-amidineurea hydrochloride (I), known as T 72 or Nitroguanil, was assessed for its toxicity. This compound:



was subsequently used in a field trial on more than 500 subjects infected with P. falciparum, P. malariae, P. vivax and P. ovale and gave fairly satisfactory results though it showed no advantage in comparison with proguanil (Urbanski et al., 1964). A detailed investigation of this compound, the method of production, analysis, form of the drug and pharmacology was also carried out (Urbanski et al., 1962; Jakimowski et al., 1964).

The antimalarial activity of some derivatives of amidineurea was the starting point of our present work on the synthesis and antimalarial properties of a series of monosubstituted amidineureas (II) and biguanides (III) and the products of their cyclization resulting in formation of the corresponding pyrimidine derivatives (IV).

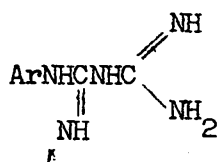
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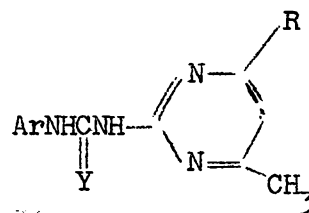
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II



III



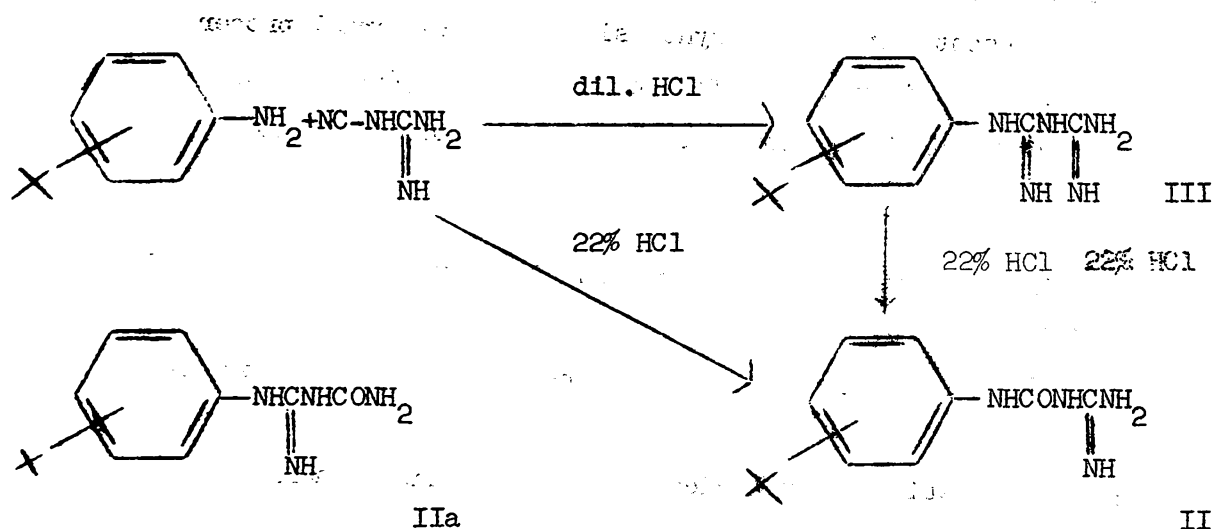
IV

Y = O, NH
R = OH, CH₃

CHEMISTRY

The biguanides (III) were obtained in the usual way from the salts of primary amines and cyanoguanidine in aqueous solution.

The 1-aryl-3-amidineureas (II) are little known; some of them were prepared by Pellizzari (1923), Passerini (1951), Junod (1952) and Kundu & Ray (1952). In a number of papers (Urbanski et al. 1953 a & b; 1954; 1955; 1956 a & b; 1959 a, b and c; Skowronska-Serafin et al., 1960; Urbanski et al., 1962), a new method of synthesis of these compounds was described, which can be summarized as follows: amidineurea derivatives are formed by refluxing primary aromatic amines with cyanoguanidine in 22 per cent. hydrochloric acid, whereas biguanides are formed from the same starting materials when refluxing in diluted hydrochloric acid (about 12 per cent.). In some instances it is advantageous to use biguanides as starting substances: when refluxed in an excess of hydrochloric acid they furnish the corresponding amidineureas:



Using these two methods, a series of 13 amidineureas and 13 corresponding biguanides were prepared (Table 1). The proof of the correctness of the structure of the amidineureas being (II) and not (IIa) was given through:

1. The aminolysis of (II) yielding disubstituted urea derivatives and guanidine:

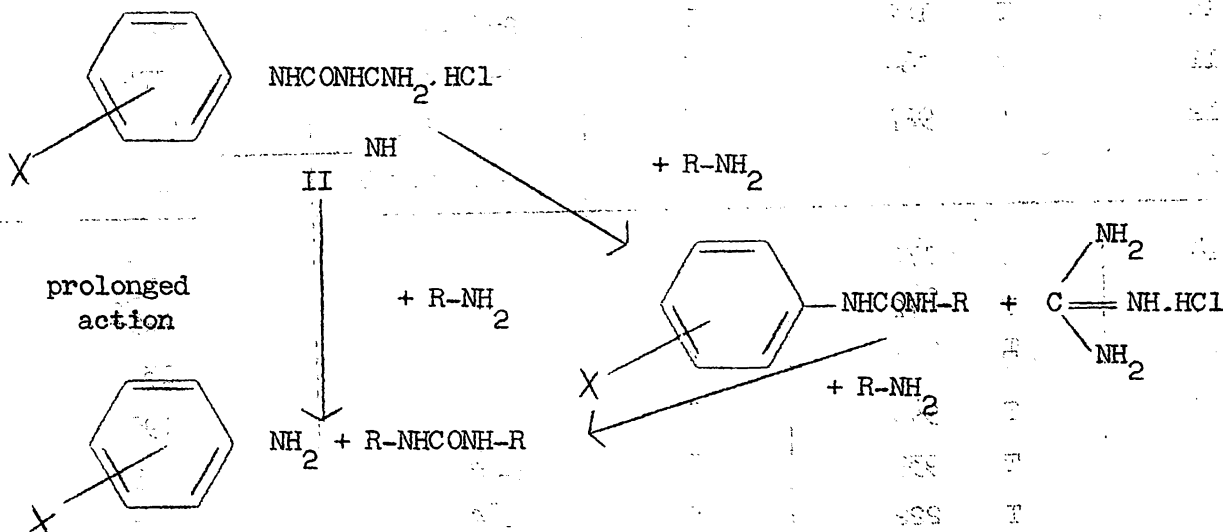
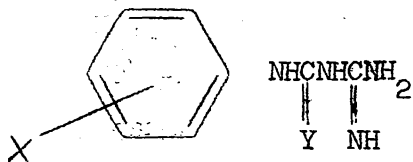


TABLE 1. TOXICITY OF SOME MONOARYL DERIVATIVES OF BIGUANIDE AND OF AMIDINE UREA



Number	Code name	Y	X	DL 50 i.p. mice mg/kg
1	T 71	NH	H	290
2	T 1011	"	m-NO ₂	261
3	T 257	"	p-NO ₂	165
4	T 1014	"	o-Cl	267
5	T 1013	"	m-Cl	187
6	T 260	"	p-Cl	247
7	T 254	"	p-Br	265
8	T 967	"	p-F	262
9	T 74	"	p-COOH	4000

TABLE 1. (continued)

Number	Code name	Y	X	DL 50 i.p. mice mg/kg
10	T 182	NH	p-SO ₃ H	4000
11	T 384	"	p-SO ₂ NH ₂	774
12	T 927	"	m-B/OH/2	4000
13	T 219	"	β-naphthyl ^a	165
14	T 170	O	H	1125
15	T 72	"	p-NO ₂	225 ^b
16	T 941	"	m-NO ₂	130
17	T 940	"	o-Cl	195
18	T 939	"	m-Cl	65
19	T 222	"	p-Cl	82
20	T 259	"	p-Br	71
21	T 944	"	p-F	200
22	T 160	"	p-COOH	1025
23	T 221	"	p-SO ₃ H	2640
24	T 285	"	p-SO ₂ NH ₂	4000
25	T 928	"	m- /OH/2	570
26	T 460	"	β-naphthyl ^c	77
27	T 261	"	p-NH ₂	1000 (p.o.)

^a β-Naphthylbiguanide.

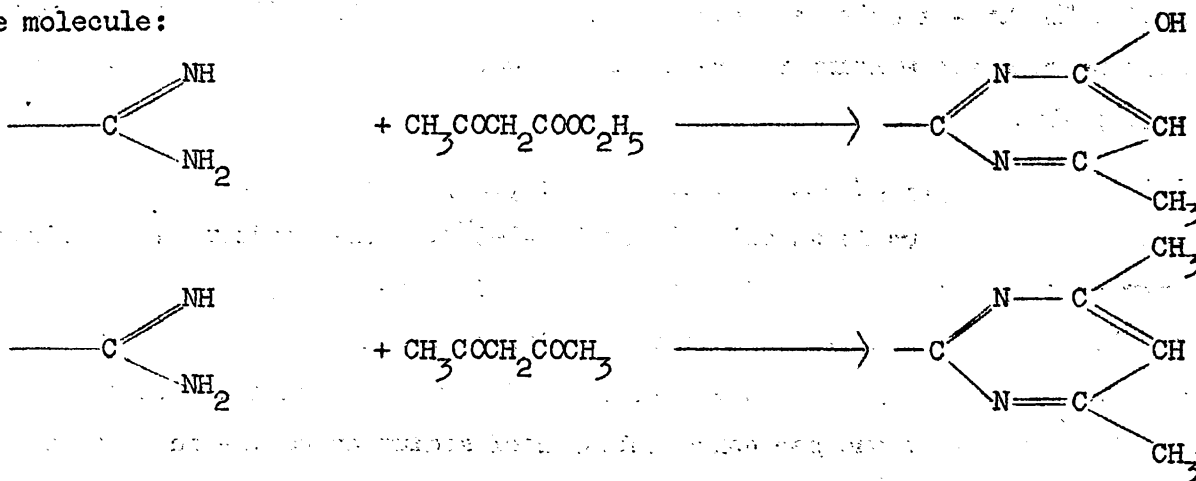
^b see Table 3.

^c 1-β-naphthyl-2-amidineurea.

2. The comparison of infra-red spectra of the two isomers (II) and (IIa), X=H; they were found to differ considerably as shown in the frequencies in cm^{-1} given below:

II	IIa	
3465 3440 3290	3310	NH ₂
1728	1720	C = O, C = NH
1680	1660	C = O in amides
1620 1595 1460 1445	1590 1453	

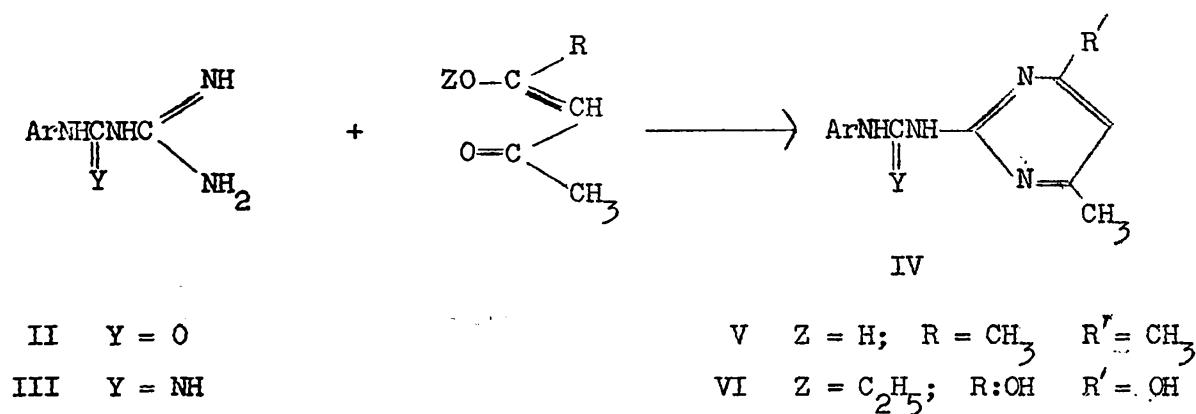
3. The cyclization of amidineureas with acetylacetic ester or acetylacetone resulting in formation of pyrimidine derivatives from the amidine moiety of the side chain of the molecule:



These reactions worked out recently by Zylowski (Serafin 1964) from a new way of synthesis of 1-urea derivatives of pyrimidine.

The question arose whether the compounds (IV) analogous to the amidineureas and biguanides of the general formula (II) and (III), but with a pyrimidine ring instead of the amidine moiety, would be more active against parasites than the straight chain compounds (II) and (III).

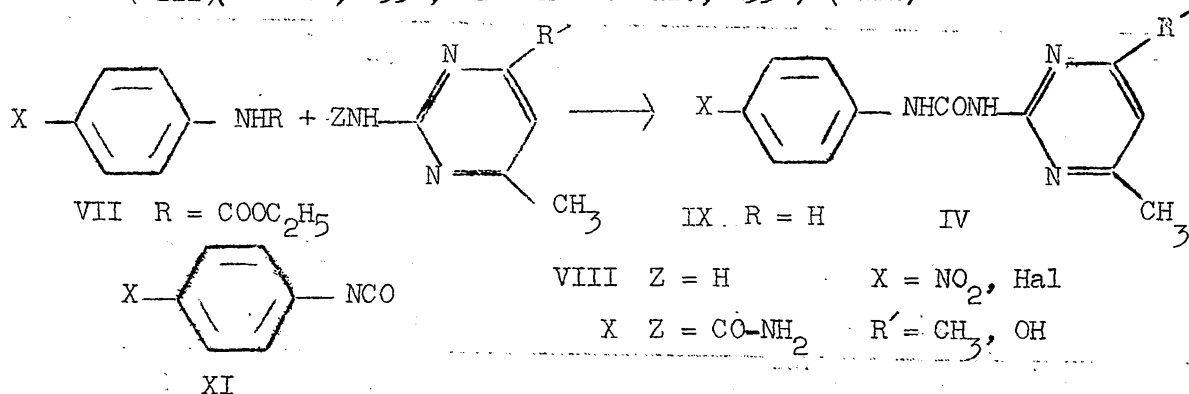
Two methods (A) and (B) were used in the synthesis of these compounds, both involving condensation of arylbiguanides (III) or arylamidineureas (II) with acetyloacetone (V), or ethylacetoacetate (VI):



Method A. One mole of the compound (II) or (III) in a dilute ethanol solution was mixed with 0.5 - 1 mole NaOH and 1 - 2.5 mole of (V) or (VI). After three to five days at room temperature the reaction product (IV) precipitated with 60-80 per cent. yield.

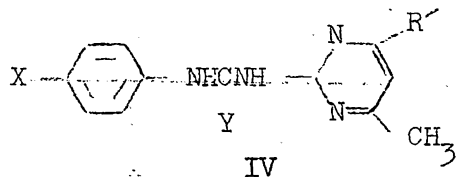
Method B. The mixture of one mole of (II) or (III) with an excess of (V) or (VI) was heated for two hours in an oil bath at 120-140°C. The product was obtained with 30-50 per cent. yield. The crude pyrimidine derivatives (IV), R = CH₃ were purified by crystallization from organic solvents (pyridine, ethanol-acetone mixture, dimethylformamide). The hydroxy derivatives (IV, R = OH) were crystallized in the form of their sodium salts from one per cent. solution of sodium hydroxide in aqueous ethanol.

To verify the structure of the new pyrimidine derivatives of amidineurea (IV, Y = O), we also prepared them condensing aromatic urethanes (VII) with amino-pyrimidines (Ashworth et al., 1948) (VIII), aromatic amines (IX) with pyrimidylureas (X) (Ashworth et al., 1948; Birtwell, 1953) (X), or arylisocyanates (XI) with amino-pyrimidines (O'Neill, 1956; Buu-Hoi et al., 1958) (VIII):



The compounds (IV) and their melting-points are given in Table 2.

TABLE 2. THE METHOD OF PREPARATION, MELTING-POINT AND TOXICITY OF PYRIMIDINE DERIVATIVES OF GROUPS II AND III



Number	Code name	Y	R'	X	Method of preparation	Melting-point	DL ₅₀ i.p. mice mg/kg
1	T 862*	NH	CH ₃	H	B	207-209°	247
2	T 934*	NH	CH ₃	NO ₂	B	237-239°	>1650
3	T 863*	NH	CH ₃	F	A	223-226°	510
4	T 864*	NH	CH ₃	Cl	A	209-211°	1505
5	T 865*	NH	CH ₃	Br	A	188-191°	900
6	T 1018*	NH	CH ₃	J	A	239-241°	730

TABLE 2 (continued)

Number	Code name	Y	R'	X	Method of preparation	Melting-point	DL ₅₀ i.p. mice mg/kg
7	T 953*	NH	OH	H	A	258-260°	>2000
8	T 935*	NH	OH	NO ₂	B	279-281°	>1000
9	T 880	NH	OH	F	A	263-266°	-
10	T 954	NH	OH	Cl	A	286-288°	>2500
11	T 1020	NH	OH	Br	A	284-285°	2600
12	T 1017	NH	OH	J	A	280-282°	>3000
13	T 881*	O	CH ₃	H	A	198-200°	>2000
14	T 866*	O	CH ₃	NO ₂	B	271-272°	>600
15	T 937	O	CH ₃	F	A	184-186°	-
16	T 869*	O	CH ₃	Cl	A	210-211°	>2000
17	T 868*	O	CH ₃	Br	A	214-216°	-
18	T 951	O	CH ₃	J	A	208-210°	3250
19	T 936*	O	OH	NO ₂	B	300-303°	>1000
20	T 945	O	OH	F	A	297-302°	-
21	T 843	O	OH	Cl	A	292-294°	-
22	T 879	O	OH	Br	A	285-286°	-
23	T 1019	O	OH	J	A	273-275°	-

* Pyrimidine derivatives tested for antimalarial activity.

Toxicity

The toxicity of the biguanide, amidineurea and pyrimidine derivatives was tested using Kärber method (Kärber, 1931): the compounds were administered to white mice, 16-24 g body-weight, parenterally in the form of a suspension in 2.5 per cent. solution of arabic gum. The results are given in Table 1 and Table 2. In general the pyrimidine derivatives were found to be of low toxicity.

The toxicity of T 72 (Nitroguanil) was subject to more detailed examination. The acute toxicity was tested in mice and in rats orally and intraperitoneally; the results are given in Table 3.

TABLE 3. TOXICITY OF NITROGUANIL IN RODENTS

Substance	DL ₅₀ mg/kg	Literature
T-72 (Nitroguanil)	1200 mice, p.o.	Chin et al. 1960
	1790 rats, p.o. ¹	"
	600 rats, p.o. ¹	"
	225 mice, i.p.	Chrusciel, Silesian Medical Academy (unpublished data)
Proguanil	45 mice, p.o.	Chin et al. 1960

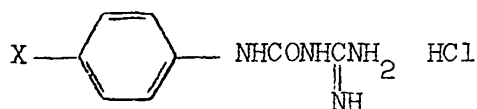
The subacute toxicity was examined in rats, the substance was administered in daily doses of 200 and 100 mg/kg during six weeks. The dose of 200 mg/kg daily was lethal for all the animals after 32 days. The dose of 100 mg/kg daily during six weeks was also toxic.

Antimalarial activity

First experiments on antimalarial activity of amidineurea derivatives were carried out at the Institute "Materia Medica" of the Academia Sinica in Pekin, (Chin et al. 1960 a and b). The in vivo test was carried out on chickens weighing 40-50 g each, inoculating into the jugular vein 25 million parasites of P. gallinaceum. The compounds were administered orally in doses of 0.1 of the respective DL₅₀; the first dose was given in the afternoon following the inoculation, during each of the following three days a similar dose was given twice a day; thus a total of seven doses was administered to each bird; on the fifth day the counts of normal and infected red blood cells were made following Giemsa staining. Proguanil was used as standard. Among the compounds tested, T 72 (Nitroguanil) and T 261 (1-(p-aminophenyl)-3-amidineurea hydrochloride) were found to be active (Table 4).

¹ Different series of experiments.

TABLE 4. ANTI-PARASITIC ACTIVITY OF COMPOUNDS TESTED ON P. GALLINACEUM



Compound	X	Dose mg/kg	Average number of infected erythrocytes per thousand
T 72 (Nitroguanil)	NO ₂	50	-
		100	-
T 259	Br	50	363.0
		100	362.0
T 261	NH ₂	50	11.6
		100	13.0
T 285	SO ₂ NH ₂	50	294.0
		100	365.0
Proguanil	-	4	4.4
		8	-
Control	-	-	377.0

The potency of T 72 (Nitroguanil) was further compared with proguanil, the experimental procedure being the same as that in preliminary experiments. T 72 (Nitroguanil) was found to be active against T. gallinaceum in doses about four times the proguanil dosage. Antimalarial activity of the pyrimidine derivatives (IV) was tested against P. berghei in mice by one of the authors at the National Institute for Medical Research, London. The animals were inoculated intraperitoneally (i.p.) with P. berghei (approximately five million parasites per mouse; the tested compounds were given once daily i.p. during four days, the first dose being given four hours after inoculation. Three mice were used for each dose. On day five, i.e. 24 hours after the last dose, blood films were taken from all the mice and the percentage of red blood corpuscles containing parasites was estimated and compared with the controls. Antimalarial action was indicated by a reduction in the degree of parasitaemia to 10-20 per cent. of that of the controls. Thirteen pyrimidine derivatives (indicated by an asterisk in Table 2) containing different substituents were tested for their antimalarial activity.

At the doses of 0.5, 1.0, 2.0, 2.5, 5.0 and 10.0 mg per 20 g mouse, no action was found. Nitroguanil when given intraperitoneally also showed no antimalarial activity and was highly toxic. In previous experiments, in avian malaria and in clinical trials, this compound was administered orally and proved to be of low toxicity and of a marked activity. The question arose whether Nitroguanil was ineffective against P. berghei or whether the difference depended on the route of administration. Thus, Nitroguanil was tried orally against P. berghei in mice and at a dose 2.0 mg per 20 g mouse on four successive days showed antimalarial activity as great as that of 0.1 mg chloroquine diphosphate given intraperitoneally on four successive days. Nitroguanil was much better tolerated by mouth than intraperitoneally.

In the light of these results it was deemed necessary to re-examine the activity of other compounds by mouth, especially the cyclic Nitroguanil derivatives T 934, T935, T 936 and T 866. But no activity by mouth of these compounds was found. Thus, it is evident that the cyclization of the amidine group into a pyrimidine ring reduces both toxicity and antimalarial activity.

ACKNOWLEDGEMENTS

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RESUME

A la suite des rapports publiés en 1960 sur l'action antipaludique de dérivés de l'amidine-urée contre Plasmodium gallinaceum, un de ces composés connu sous le nom de Nitroguanil a fait l'objet d'un essai concluant sur le terrain. Aussi a-t-on procédé à la synthèse d'une série de dérivés de l'amidine-urée, de biguanides et de dérivés de la pyrimidine. Le document décrit les méthodes de synthèse utilisées.

La toxicité d'un certain nombre de ces composés a été éprouvée sur des souris par la méthode de Kärber, et l'action antipaludique de plusieurs d'entre eux a été essayée sur P. gallinaceum et P. berghei. A l'exception du Nitroguanil, un seul autre composé (chlorhydrate d'amidine-3- (p-aminophényl) 1-urée) a fait preuve d'activité contre P. gallinaceum.

Sur P. berghei, on a trouvé que les dérivés de la pyrimidine et même le Nitroguanil étaient inefficaces par voie intrapéritonéale. Par voie buccale, seul le Nitroguanil a manifesté une action antipaludique, et, par cette voie, il a été bien mieux toléré que par voie intrapéritonéale.

Il est évident que la cyclisation du groupe amidine en un noyau pyrimidine a entraîné une réduction à la fois de la toxicité et de l'effet antipaludique.

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