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THE USE OF AOTUS TRIVIRGATUS AND MACACA MULATTA AS TOOLS FOR STUDIES ON PREVENTION AND THERAPY OF INFECTIONS WITH PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX (U)

FINAL PROGRESS REPORT
(PROJECT 2284-XXIX)

For the Period 1 May 1975 to 30 April 1976
(Preparation Completed 13 August 1976)

COVERING CONTRACT NO. DADA 17-69-C-9104

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C., 20314

Principal Investigator- L. H. Schmidt

Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205

DDC AVAILABILITY STATEMENT

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potentiation
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pyridinemethanols

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
As in previous years, the activities of this Project were focused on development of: (a) agents fully effective against infections with blood schizonts of multidrug-resistant strains of Plasmodium falciparum and P. vivax in the owl monkey; and (b) compounds more active than primaquine against the tissue schizonts of P. cynomolgi in the rhesus monkey, and better tolerated.

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Forty-five newly prepared compounds, including thirty-eight 8-aminoquinolines, were examined for tissue schizonticidal activity in the rhesus monkey - P. cynomolgi model. Evaluations of thirteen previously submitted 8-aminoquinolines were completed. Six of these fifty-one quinolines, each at least four times as active as primaquine, were evaluated in-depth for curative activities and were accorded preliminary toxicologic studies. A lot of 4-methyl primaquine (WR-181,023), prepared for clinical trials, was evaluated in detail. Additional studies included: (a) comparison of the curative activities and toxicities of the D and L forms of WR-181,023; and (b) a reappraisal of the need for large sporozoite inocula in assessments of radical curative (tissue schizonticidal) activities.

19. Key Words Continued

Mannich bases
8-aminoquinolines
1,5-naphthyridones
toxicity

TABLE OF CONTENTS

FOREWORD

In conducting the research described in this Report the investigator adhered to the principles set forth in the Guide For Care And Use Of Laboratory Animals as promulgated by the Committee on Revision of the Guide For Laboratory Animal Facilities And Care of the Institute for Laboratory Animal Resources, National Research Council - National Academy of Sciences.

ABSTRACT

As in previous years, the activities of this Project were focused on development of: (a) agents fully effective against infections with blood schizonts of multidrug-resistant strains of Plasmodium falciparum and P. vivax in the owl monkey; and (b) compounds more active than primaquine against the tissue schizonts of P. cynomolgi in the rhesus monkey, and better tolerated.

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GENERAL SUMMARY

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The period covered by this Report rounds out nine years of investigations aimed at developing new, well-tolerated blood schizonticidal drugs equally effective against infections with both drug-susceptible and multidrug-resistant strains of *Plasmodium falciparum*. This endeavor has been supported by a recently developed animal model - established infections with trophozoites of various drug-susceptible and drug-resistant strains of *P. falciparum* (and companion infections with *P. vivax*) in the owl monkey (*Aotus trivirgatus*). Development of this model, accomplished between September 1967 and May 1969, has provided investigators in malaria chemotherapy with their first opportunity to evaluate new agents in an experimental host infected with the human plasmodia. A sizeable fraction of the important accomplishments of the Malaria Chemotherapy Program of the Department of the Army rests on exploitation of this model.

The Report period also rounds out more than three years of investigations directed toward developing more effective and better tolerated tissue schizonticidal (radical curative) drugs than primaquine. This activity has been supported by a slightly modified version of a model introduced to malaria chemotherapy during the post-World War II search for curative drugs - infections with sporozoites of *P. cynomolgi* in the rhesus monkey (*Macaca mulatta*). The modifications of the old model include substitution of the *B* for the *M* strain of *P. cynomolgi* and *Anopheles freeborni* for *A. quadrimaculatus* as the insect vector. The predictive value of this simian model for infections with *P. vivax* in man was established nearly twenty years prior to initiation of the current Malaria Chemotherapy Program.

As is implied from the above comments, work with the owl monkey - human plasmodium model was the sole concern of this Project for some six years when the efforts of the Malaria Chemotherapy Program were focused almost exclusively on development of new blood schizonticidal drugs effective against infections with chloroquine-resistant strains of P. falciparum. Way was made for work on the rhesus monkey - P. cynomolgi model in 1972 shortly after the mission of the Malaria Chemotherapy Program was broadened to include the search for improved radical curative drugs. Initially, this activity did not affect the tempo of the search for new blood schizonticides; for somewhat more than the past two years, efforts in this latter area have declined, while work on new tissue schizonticides has been intensified.

A number of factors have contributed to the above change in emphasis. First, there has been a marked reduction in the output of compounds from the primary P. berghei screen deemed worthy of evaluation in the owl monkey model. Secondly, pressures for identifying new compounds with promising blood schizonticidal activity have lessened because of the backlog of highly interesting agents already awaiting evaluation in human volunteers. Lastly, the embargo on exportation of owl monkeys from Colombia, instituted in September 1974, has all but eliminated the possibilities of immediate use of this host for malaria or other research in the U. S. A.* These factors

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*Evaluations pursued since September 1974 have been supported by a stock of 150 recently imported monkeys undergoing conditioning at the time of the embargo and approximately an equal number of conditioned, but previously used monkeys, acquired via an exchange arrangement with the Biomedical Research Institute, Boston. The latter animals had been used for research on the eye, but had no previous exposure to malaria. By strictest economies in assignments and serial use in evaluating new agents against infections with both P. falciparum and P. vivax, these subjects provided support for a sizeable group of studies.

suffice to explain why only three new agents have been evaluated in the owl monkey model during the period of this Report and why this model has been utilized primarily for assessing the activities of drug preparations destined for study in human volunteers in the near future.

None of the three new agents examined during the current Report period came directly from the P. berghei screen. Two of the compounds, WR-199,426, a 4-quinolinemethanol, and WR-216,100, an 8-aminoquinoline, were deemed worthy of study because of activity exhibited in rhesus monkeys infected with trophozoites of P. cynomolgi. In preliminary assessments, the first of these compounds, WR-199,426, exhibited limited activity against infections with the Smith strain of P. falciparum; the second, WR-216,100, had no more than marginal activity against infections with the Palo Alto strain of P. vivax. Neither agent would seem to merit more extensive evaluation.

The third compound, WR-226,253, received its primary evaluation in the owl monkey. This 4-quinolinemethanol had been designed by investigators in the Division of Medicinal Chemistry, WRAIR, so as to incorporate what were believed to be the more desirable structural features of WR-30,090 and WR-142,490. The first of these compounds had exhibited significant activity in human volunteers infected with chloroquine-resistant strains of P. falciparum and had shown considerable promise in preliminary field studies. WR-142,490 (mefloquine) was substantially more active than WR-30,090 in human volunteers infected with multidrug-resistant strains of P. falciparum and effectively suppressed infections with such strains when administered at weekly or even less frequent intervals. The results of the preliminary assessments of the activity of WR-226,253 in owl monkeys infected with the Smith strain of P. falciparum and the Palo Alto strain

of P. vivax indicated: (1) that WR-226,253 was at least twice as active as WR-142,490 in treating established infections; and (2) that the duration of protection against trophozoite challenge, accorded by a single dose of WR-226,253, was substantially greater than that provided by mefloquine. There is urgent need for additional critical evaluations in the owl monkey model directed toward determining whether WR-226,253 has sufficient superiority over WR-142,490 to merit consideration for study in human volunteers.

The major use of the P. falciparum - owl monkey model has been in studies aimed primarily at insuring that specific lots of WR-172,435, WR-180,409, and WR-194,965 were suitable for clinical trial. The first two of these agents were 4-pyridinemethanol derivatives; the third was a Mannich reaction product. Each had been selected as a candidate for examination in human volunteers. The batch lots prepared for these evaluations were compared with the original lots for activity in owl monkeys infected with the Smith strain and in addition, were accorded critical dosage regimen evaluations aimed at identifying the most effective treatment schedule. Because of a shortage of monkeys, the majority of the dosage regimen evaluations were carried out in animals infected with the Palo Alto strain of P. vivax.

The search for improved radical curative drugs, carried out in rhesus monkeys infected with sporozoites of P. cynomolgi, can be divided into six discrete compartments. The first of these was concerned with pilot evaluations of new agents. Forty-five compounds were submitted for such evaluations: five were in the heterogeneous structure category; two were 1,5-naphthyridone derivatives; thirty-eight were 8-aminoquinolines. None of the first two groups exhibited reproducible curative activity. Eleven of the 8-aminoquinolines were at least as active as primaquine; three of this number were from two to four times as active. In addition,

six other representatives of this class were at least half as active as primaquine. Overall, the yield of 8-aminoquinolines with noteworthy activity (43 per cent of the total studied during the Report period) was remarkably high, especially so for a chemical class that has been subjected to intensive, although intermittent, investigations for at least fifty years.

The second and third compartments of the search for new curative drugs were extensions of earlier assessments of the activity of WR-181,023 (4-methyl primaquine). The first was concerned with the radical curative and prophylactic activities of the so-called IND lot prepared for possible study in human volunteers. The second involved comparison of the activities of two lots of WR-181,023 prepared in the current Malaria Chemotherapy Program with the activities of two lots synthesized in 1949 by Elderfield and coworkers when 4-methyl primaquine was known as CN-1101. These investigations revealed no biological nor statistically significant differences in the curative activities of the various preparations of WR-181,023. From the activity viewpoint, the IND lot was as acceptable as any lot for delivery to human volunteers.

The fourth compartment dealt with a comparison of the curative activities of WR-181,023 (a racemate) and its D and L components (respectively, WR-221,033 and WR-221,036). The resolution of WR-181,023 and study of the activities of its isomers were stimulated by the results of an earlier investigation of the activities of primaquine and its isomers. This earlier study showed that the curative activities of primaquine and its isomers were identical, but that the L isomer (WR-211,537) was from two to four times as toxic for the rhesus monkey as the D isomer (WR-211,536), thus conferring a significant therapeutic advantage on the latter agent. The current evaluation showed that the radical curative activities of WR-181,023 and its isomeric components (WR-221,033 and WR-221,036) were

essentially identical. This finding led to a preliminary assessment of the toxicities of these agents for the monkey. The results of this appraisal will be detailed in a subsequent section of this Report.

The fifth compartment of the search for more effective and better tolerated curative drugs involved in-depth evaluations of the curative properties of six 8-aminoquinoline derivatives which in pilot studies appeared to be at least twice as active as WR-181,023 and four times as active as primaquine. The group included WR-212,579, WR-215,296, WR-215,761, WR-216,804, WR-221,527, and WR-222,671. Five of this group were 4-methyl substituted congeners of WR-181,023. The exception, WR-222,671, was 2-methyl substituted. WR-216,804, the 5-methoxy analog of WR-181,023, was the only compound bearing the primaquine side chain. The other five agents had chains with novel branching. The results of the in-depth evaluations of the activities of the above group of compounds showed unequivocally that they were endowed with a greater level of curative activity than any other 8-aminoquinolines studied thus far. The results also showed that the curative activities of these agents were dependent upon the total dose of compound delivered and that in keeping with this principle, three-day and seven-day treatment schedules were equally effective.

The sixth and last facet of the investigations with the *P. cynomolgi* - rhesus monkey model involved an evaluation of the influence of the size of the sporozoite inoculum on the curative activities of the 8-aminoquinolines. Both primaquine and WR-181,023 were utilized in this study in which inocula were varied from 4×10^1 to 1.1×10^6 sporozoites. The results of this study showed that with inocula of 4×10^4 sporozoites or fewer, the curative activities of these 8-aminoquinolines were not markedly influenced by inoculum size. However, increasing the inoculum to 4×10^5 sporozoites or greater led

to a marked reduction in curative activity. This influence of inoculum size on curative activity was more marked with primaquine than with 4-methyl primaquine (WR-181,023). As will be pointed out in more detail later in this Report, this relation between inoculum size and "basic" drug efficacy should be considered whenever the same monkey is employed for serial evaluations of agents endowed with marginal levels of curative activity.

Investigations pursued during this Report period have been concerned with two very preliminary, but probably important toxicologic studies, both dealing with the reactions of rhesus monkeys to various 8-aminoquinolines. Although there has been no major contractual commitment to this activity, it has been undertaken in part because of special expertise in the area, but primarily because past experience with this class of compounds has shown that evaluations of toxicity are critically important to determining whether a real gain has been achieved when agents of greater therapeutic activity are discovered.

The first of these studies was concerned with the subacute toxicities of WR-221,033 and WR-221,036, respectively the D and L isomers of WR-181,023. The results of these studies showed that the reactions of the rhesus monkey to the D and L forms and to the D,L mixture (WR-181,023) were quantitatively identical. This finding is in striking contrast to the results of a comparable study on WR-211,536 and WR-211,537, the D and L components of primaquine, which showed that the L form was distinctly more toxic than the D form and significantly more toxic than primaquine. This observation led to the suggestion that consideration be given to substituting WR-211,536 for primaquine in curative drug regimens. Obviously, a similar shift to one of the isomers of WR-181,023 is contraindicated.

The second of the studies was concerned with the toxicities of WR-212,579, WR-215,296, WR-215,761, WR-216,804, WR-221,527, and WR-222,671, the group of agents endowed with unusually high levels of curative activity referred to in an earlier section of this Summary. In brief, the results of these studies have shown that WR-212,579, WR-215,296, and WR-215,761 are at least twice as toxic as primaquine; WR-222,671 is approximately three times as toxic, whereas WR-216,804 and WR-221,527 are more than four times as toxic. These findings leave these "promising" agents with therapeutic indices very similar to the therapeutic index of primaquine. How this position may influence interest in evaluating one or more of the new compounds in human volunteers will be considered in a later section of this Report.

The above is a concise summary of the scope and major results of the activities pursued in the 1975-1976 contract year. Detailed descriptions of these endeavors are presented in the sections that follow.

I. PILOT EVALUATIONS OF NEW BLOOD SCHIZONTICIDES

I. PILOT EVALUATIONS OF NEW BLOOD SCHIZONTICIDES

A. WR-199,426

WR-199,426 was one of the two 4-quinolinemethanol derivatives evaluated during the current contract year. This compound has structural features somewhat different from those of WR-30,090 and WR-142,490, two of the more promising quinolinemethanols (cf Figure 1). These features, coupled with demonstrable activity against infections with trophozoites of the B strain of P. cynomolgi in the rhesus monkey, stimulated a preliminary assessment of the activity of WR-199,426* against infections with the multidrug-resistant Smith strain of P. falciparum in the owl monkey.

As the results of this study show (cf Tables 1 and 2), WR-199,426 failed to control parasitemia when administered in daily doses of 1.25, 2.5, 5.0, or 10.0 mg base per kg body weight. Control was irregular with daily doses of 20.0, 40.0, and 80.0 mg per kg. Clearance of parasitemia and cure were achieved in both recipients of doses of 20.0 mg per kg and in one of each pair of recipients of doses of 40.0 and 80.0 mg per kg. The infection in the second recipient of 40.0 mg doses recrudesced twenty days after the end of treatment. Parasitemia was reduced but not cleared in the second recipient of 80.0 mg doses. These irregularities are puzzling. They may have been related to a defect in absorption from the gastrointestinal tract associated with anorexia (and accompanying weight loss), a reaction exhibited in all recipients of 40.0 and 80.0 mg per kg doses.

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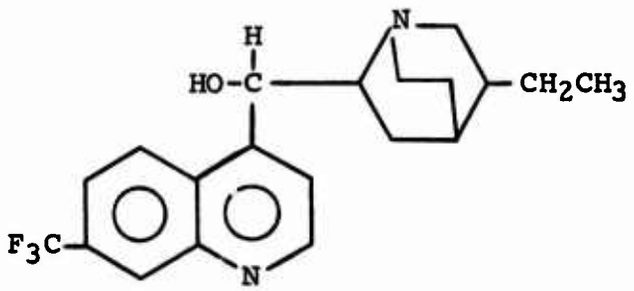
*Used as the dihydrochloride salt.

The shortage of owl monkeys precluded evaluating the activities of WR-199,426, WR-30,090, and WR-142,490 in parallel. The next best appraisal, a comparison of the results of the current evaluation of the activity of WR-199,426 with the results of earlier, far more extensive evaluations of the activities of WR-30,090 and WR-142,490, shows that the former agent is no more than one-half as active as WR-30,090 or one-eighth as active as WR-142,490 against infections with the multidrug-resistant Smith strain of P. falciparum.* According to this comparison, WR-199,426 would be a poor competitor of WR-30,090 and afford even less competition to WR-142,490 or to its close and slightly less active relative, WR-184,806 (cf Annual Report, 1974-1975: SORI-KM-76-319).

*The hydrochloride salts of WR-30,090 and WR-142,490 were employed in these evaluations.

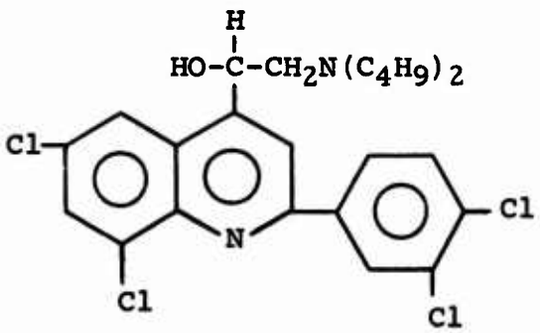
FIGURE 1

STRUCTURES OF WR-199,426, WR-30,090, AND WR-142,490



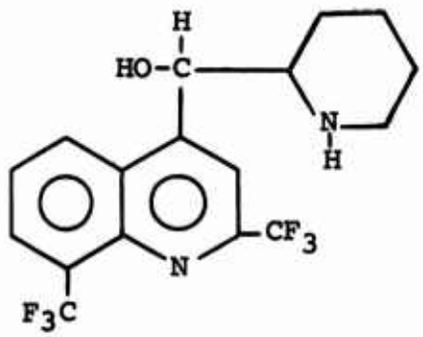
WR-199,426

α-(5-ethyl-2-quinuclidinyl)-7-trifluoromethyl-4-quinoline-methanol



WR-30,090

α-(di-n-butylaminomethyl)-2-(3,4-dichlorophenyl)-6,8-dichloro-4-quinolinemethanol



WR-142,490

α-(2-piperidyl)-2,8-di-trifluoromethyl-4-quinolinemethanol

TABLE 1

THE ACTIVITY OF WR-199,426 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg Base/Kg Body Weight*	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment							Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
8085	1.25	63	100	265	135	Dose increased						
8088	1.25	111	410	1120	1520	Dose increased						
8089	2.5	27	70	375	1100	Dose increased						
8094	2.5	72	240	392	960	Dose increased						
8103	5.0	12	18	112	81	Dose increased						
8105	5.0	44	48	170	78	Dose increased						
8106	10.0	66	136	213	231	320	48	51	20	15	3	10
8107	10.0	216	740	808	345	156	210	21	28	39	21	19
8085r	20.0	135	180	18	4	<1	<1	<1	-	-	-	-
8088r	20.0	1520	820	120	12	2	<1	<1	-	-	-	-
8089r	40.0	1100	960	130	12	2	<1	<1	-	-	-	
8094r	40.0	960	960	210	18	3	<1	<1	-	-	-	-
8103r	80.0	81	135	90	30	12	9	3	<1	<1	<1	<1
8105r	80.0	78	136	10	5	<1	<1	-	-	-	-	-
8108	-	33	60	150	896	1000	1400	1990	2630	2710	3960	1310

*Administered via stomach tube, once daily for seven consecutive days, except when it was necessary to increase the dose to control parasitemia.

TABLE 2

THE ACTIVITY OF WR-199,426 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg Base/Kg Body Weight*	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recrudescence	Remarks
		None	Suppressed	Cleared			
8085	1.25	+			n.a.	n.a.	Dose increased
8088	1.25	+			n.a.	n.a.	Dose increased
8089	2.5	+			n.a.	n.a.	Dose increased
8094	2.5	+			n.a.	n.a.	Dose increased
8103	5.0		+		n.a.	n.a.	Dose increased
8105	5.0		+		n.a.	n.a.	Dose increased
8106	10.0		+		n.a.	n.a.	
8107	10.0		+		n.a.	n.a.	
8085r	20.0			+	7	n.a.	Cured
8088r	20.0			+	7	n.a.	Cured
8089r	40.0			+	7	20	
8094r	40.0			+	7	n.a.	Cured
8103r	80.0		+		n.a.	n.a.	
8105r	80.0			+	6	n.a.	Cured

*Administered via stomach tube, once daily for seven consecutive days, except when it was necessary to increase the dose to control parasitemia.

B. WR-216,100

It has been hoped that the search for more effective radical curative drugs would uncover a compound well endowed with both blood and tissue schizonticidal activities. The availability of such an agent would make it possible to cure naturally acquired infections with a single drug regimen. Primaquine has activity against both blood and tissue schizonts, but its blood schizonticidal potency is not great enough at tolerated doses to be useful. Evaluations pursued in owl monkeys infected with the Palo Alto strain of P. vivax have shown that in terms of activity against blood schizonts, WR-181,023 (4-methyl primaquine) is only a slight improvement over primaquine. The dose of WR-181,023 required to control infections with blood schizonts of P. vivax is at least ten times the dose required for cure of infections with the tissue schizonts of P. cynomolgi.

WR-216,100, a 5,6-substituted 8-aminoquinoline (cf structure Figure 2), has been suggested as a possible candidate for use as both a tissue and blood schizonticide. This compound has exhibited significant activity against the blood parasites of P. berghei and P. cynomolgi. Its tissue schizonticidal activity falls between that of WR-181,023 and primaquine. These qualities, together with some structural novelty, stimulated the evaluation summarized below.

The results of a preliminary assessment of the activity of WR-216,100 against established infections with trophozoites of the Palo Alto strain of P. vivax have been summarized in Tables 3 and 4*. Data on primaquine and WR-181,023 are included for comparative purposes. As indicated in Tables 3 and 4, some of the observations on the latter compounds were derived from the current evaluation, the majority from assessments performed at earlier dates.

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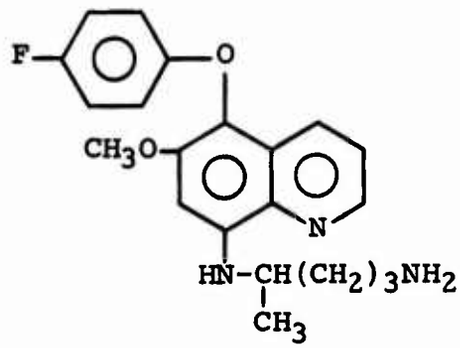
*WR-216,100 was administered as the citrate salt in these studies.

The observations summarized in Table 4 show that WR-216,100 had relatively low activity against the blood schizonts of the Palo Alto strain of P. vivax, failing to clear parasitemias when delivered at daily doses of 2.5 mg per kg and less, and effecting only temporary clearance when administered in doses of 5.0 and 10.0 mg per kg. In comparison, 2.5 mg doses of primaquine effected clearance regularly, as did 1.25 mg doses of WR-181,023. Cures were achieved in approximately 50 per cent of the recipients of this latter 8-aminoquinoline in doses of 1.25 or 2.5 mg per kg. If these comparative data are an indication of what can be expected of WR-216,100 in humans infected with P. vivax, this agent is likely to be of little value in a monodrug curative regimen.

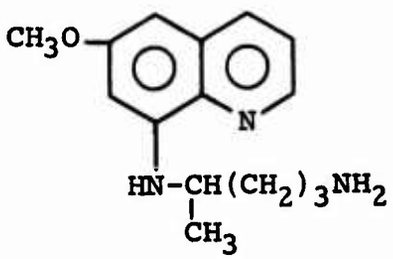
FIGURE 2

STRUCTURES OF WR-216,100, PRIMAQUINE, AND WR-181,023



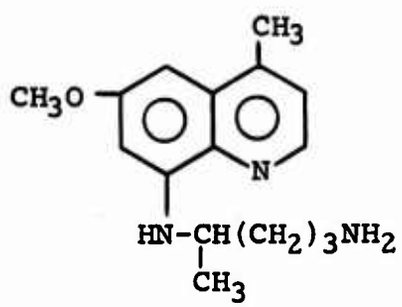
WR-216,100

5-(4-fluorophenoxy)-6-methoxy-8-(4-amino-1-methylbutylamino)-quinoline



Primaquine

6-methoxy-8-(4-amino-1-methylbutylamino)-quinoline



WR-181,023

4-methyl-6-methoxy-8-(4-amino-1-methylbutylamino)-quinoline

TABLE 3

COMPARISON OF THE ACTIVITIES OF WR-216,100, PRIMAQUINE, AND WR-181,023 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight**	Parasitemia - No. Parasites/10^4 Erythrocytes										
		Day Pre-treatment	Day of Treatment							Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
WR-216,100												
7701	0.3125	24	34	62	72	24	24	22	12	20		
7716	0.3125	14	66	72	246	280	192	246	243	110		
7761	0.625	44	81	58	86	156	104	46	17	42		
7763	0.625	24	16	26	16	8	16	2	<1	<1		
7766	1.25	26	42	74	88	56	30	54	14	22		
7833	1.25	10	64	58	134	117	72	36	5	6		
7876	2.5	8	50	86	120	144	66	75	50	69		
7897	2.5	6	20	32	28	27	8	20	12	1		
7701r	5.0	20	10	8	2	<1	<1	<1	<1	<1	<1	2
7761r	5.0	42	75	4	4	<1	<1	-	-	-	-	-
7766r	10.0	22	5	<1	1	<1	<1	-	-	-	-	-
7876r	10.0	69	66	4	1	<1	<1	<1	<1	-	-	-
Primaquine												
7735*	0.3125	12	8	15	12	16	10	18	Regimen changed			
7736*	0.3125	18	32	70	180	120	112	140	84	Regimen changed		
7737*	0.625	12	48	120	160	72	160	108	105	Regimen changed		
7738*	0.625	20	88	100	104	44	48	60	60	Regimen changed		
7755*	1.25	18	14	4	2	10	3	<1	<1	<1	<1	1
7758*	1.25	8	27	3	1	16	12	4	1	14	20	
7763r	1.25	<1	-	-	-	-	-	-	-	-	-	-
7897r	1.25	1	1	<1	<1	<1	<1	<1	<1	<1	<1	<1
7768*	2.5	4	6	2	<1	<1	<1	<1	-	-	-	
7769*	2.5	4	4	1	<1	<1	<1	<1	-	-	-	
7977	2.5	9	15	2	<1	<1	-	-	-	-	-	
7978	2.5	12	9	1	<1	<1	-	-	-	-	-	

TABLE 3 - CONTINUED

Atr No.	Daily Dose Mg/Kg Body Weight**	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment							Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
WR-181,023												
7770*	0.156	36	160	133	140	123	130	180	60	Regimen changed		
7779*	0.156	14	16	34	39	46	69	72	150	Regimen changed		
7780*	0.3125	6	20	15	27	26	6	3	2	6	4	
7872*	0.3125	4	3	1	4	<1	<1	<1	<1	<1	<1	4
7873*	0.625	16	30	24	24	16	2	<1	<1	-	-	
7882*	0.625	26	33	60	35	30	14	2	<1	<1	<1	<1
7900*	1.25	6	12	8	4	<1	<1	<1	-	-	-	
7902*	1.25	12	64	80	30	2	<1	<1	-	-	-	-
7833r	1.25	6	4	1	<1	<1	-	-	-	-	-	-
7716r	1.25	110	54	2	8	1	<1	<1	-	-	-	-
7977r	1.25	22	6	<1	<1	-	-	-	-	-	-	-
7897rr	1.25	12	10	6	<1	<1	<1	-	-	-	-	-
7701rr	1.25	6	12	4	<1	<1	<1	-	-	-	-	-
7975	2.5	14	6	<1	<1	<1	-	-	-	-	-	
7976	2.5	9	30	14	2	<1	<1	-	-	-	-	
7770r*	2.5	60	15	6	4	1	<1	<1	-	-	-	
7779r*	2.5	150	48	4	1	<1	<1	<1	-	-	-	
7897rrr	2.5	8	2	2	<1	<1	-	-	-	-	-	-
Controls												
7706	-	10	76	144	110	144	150	117	170	400	460	550
7982	-	28	58	159	210	230	123	96	140	72	46	100

*Monkeys used in earlier assessments of the activities of primaquine and WR-181,023.

**Administered via stomach tube, once daily for seven consecutive days, except when it was necessary to increase the dose to control parasitemia.

TABLE 4

COMPARISON OF THE ACTIVITIES OF WR-216,100, PRIMAQUINE, AND WR-181,023 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight**	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
		None	Suppressed	Cleared			
WR-216,100							
7701	0.3125		±		n. a.	n. a.	
7716	0.3125	+			n. a.	n. a.	
7761	0.625		+		n. a.	n. a.	
7763	0.625		+		n. a.	n. a.	
7766	1.25		+		n. a.	n. a.	
7833	1.25		+		n. a.	n. a.	
7876	2.5		+		n. a.	n. a.	
7897	2.5		+		n. a.	n. a.	
7701r	5.0		+		n. a.	n. a.	
7761r	5.0			+	6	12	
7766r	10.0			+	6	15	
7876r	10.0			+	8	8	
Primaquine							
7735*	0.3125		+		n. a.	n. a.	Regimen changed
7736*	0.3125	+			n. a.	n. a.	Regimen changed
7737*	0.625	+			n. a.	n. a.	Regimen changed
7738*	0.625		±		n. a.	n. a.	Regimen changed
7755*	1.25		+		n. a.	n. a.	
7758*	1.25		+		n. a.	n. a.	
7763r	1.25			+	2	12	
7897r	1.25		+		n. a.	n. a.	
7768*	2.5			+	7	7	
7769*	2.5			+	7	10	
7977	2.5			+	5	11	
7978	2.5			+	5	21	

TABLE 4 - CONTINUED

Atr No.	Daily Dose Mg/Kg Body Weight**	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recru-descence	Remarks
		None	Suppressed	Cleared			
				WR-181,023			
7770*	0.156	+			n.a.	n.a.	Regimen changed
7779*	0.156		±		n.a.	n.a.	Regimen changed
7780*	0.3125		+		n.a.	n.a.	
7872*	0.3125		+		n.a.	n.a.	
7873*	0.625			+	8	14	
7882*	0.625		+		n.a.	n.a.	
7900*	1.25			+	7	21	
7902*	1.25			+	7	7	
7833r	1.25			+	5	34	
7716r	1.25			+	7	n.a.	Cured
7977r	1.25			+	4	n.a.	Cured
7897rr	1.25			+	6	13	
7701rr	1.25			+	6	n.a.	Cured
7975	2.5			+	5	16	
7976	2.5			+	6	16	
7770r*	2.5			+	7	n.a.	Cured
7779r*	2.5			+	7	n.a.	Cured
7897rrr	2.5			+	5	n.a.	Cured

*Monkeys used in earlier assessments of the activities of primaquine and WR-181,023.

**Administered via stomach tube, once daily for seven consecutive days, except when it was necessary to increase the dose to control parasitemia.

C. WR-226,253

The development of WR-226,253 [α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinolinemethanol] was somewhat unusual. Synthesis was undertaken in an attempt to build into a single compound what were believed to be the most promising features of WR-30,090 and WR-142,490 (mefloquine), two 4-quinolinemethanols which have exhibited highly promising activity in human volunteers. From the most simplistic biological viewpoint, the structure was designed to encompass the high tolerability of WR-30,090 and the high activity of WR-142,490. Synthesis was completed on December 31, 1975. Contrary to usual practice, biological activity was assessed first in owl monkey - human plasmodium models rather than in mice infected with P. berghei (Rane test). These simian studies were initiated on January 16, 1976.

The scope of the evaluation of the activity of WR-226,253 was severely limited, primarily because of the limited availability of owl monkeys (especially previously uninfected subjects), but also because of the short time interval between submission of the compound and termination of the laboratory phases of this project. Despite these strictures, four separate experiments were mounted, three with monkeys infected with the Palo Alto strain of P. vivax, and one with monkeys infected with the Smith strain of P. falciparum. The first experiments with P. vivax and the sole P. falciparum study were conventional studies with special attention to the influence of the dosage regimen on activity. The third and last experiment with P. vivax was designed to measure the duration of protection attained via delivery of a single dose of WR-226,253*.

The results of the assessments of the activity of WR-226,253 against infections with the pyrimethamine-resistant Palo Alto strain of P. vivax have been set forth in Tables 5

*WR-226,253 was administered as the hydrochloride salt throughout these studies.

and 6. The summary data in the latter table indicate that this compound was curative when administered in a total dose of 8.75 mg base per kg body weight. The results obtained with doses of 4.375 mg per kg, although very limited, suggest that this quinolinemethanol may be more effective when delivered in a single dose than in three or seven fractions on as many consecutive days.

Although the evaluation of the activity of WR-226,253 was severely restricted, data were obtained (cf Tables 7 and 8) which suggested that this compound has a very high order of activity against the chloroquine-, quinine-, pyrimethamine-resistant Smith strain of P. falciparum. Cures were achieved with a total dose of 8.75 mg per kg. A single dose at this level was as effective as the same total amount delivered in three or seven equal fractions on as many days.

A very preliminary effort was made to determine the duration of protection accorded by a single dose of WR-226,253 against infection with the Palo Alto strain of P. vivax. A single dose level of WR-226,253, 17.5 mg per kg, was used in this study - at least twice the dose required to cure an established infection. Eleven monkeys were assigned to the experiment, one untreated control and five pairs of animals treated with WR-226,253, two each at four hours or four, seven, fourteen, or twenty-one days prior to inoculation. Each subject was inoculated intravenously with 5×10^6 parasites*.

The results of this experiment have been summarized in Figure 4. The data therein show that a dose of WR-226,253 of 17.5 mg per kg accorded full protection for at least four days. No parasites, normal or abnormal, could be found at any time during the thirty day observation period. In terms of developing infection, full protection was achieved in both

*A critically located monkey, one of two challenged fourteen days after dosage, was lost to the experiment on the day after inoculation. Death was accidental, occurring during the intramuscular injection of procaine penicillin prescribed for treating a newly diagnosed respiratory illness.

subjects treated seven days prior to challenge. One of the pair never displayed parasites on thick films. The second exhibited distinctly abnormal early schizonts for three days after challenge; blood films became negative on the fourth day and remained so through the observation period. The parasitemia of the sole survivor of the pair treated fourteen days prior to inoculation was fully suppressed for seventeen days. On day eighteen, normal parasites reappeared in the blood with parasitemia progressing normally thereafter. Neither of the recipients of WR-226,253 twenty-one days prior to challenge exhibited evidence of protection. Together, these findings suggest that effective blood schizonticidal levels of WR-226,253 persist for at least seven days after delivery of a dose of 17.5 mg per kg and possibly somewhat longer.

The composite results of the studies set forth in the preceding section have led to the conclusion that on the basis of its antimalarial activity, WR-226,253 has slight but probably significant advantages over WR-142,490 and is markedly superior to WR-30,090. On a dose-for-dose comparison, this newly developed 4-quinolinemethanol is conservatively twice as active as WR-142,490 and more than ten times as active as WR-30,090 against infections with the Smith strain of P. falciparum or the Palo Alto strain of P. vivax. The duration of protection accorded by a single dose of WR-226,253 against infections with this strain of P. vivax is substantially greater than that accorded by WR-142,490 against challenge with the Malayan Camp-CH/Q strain of P. falciparum, suggesting that in the owl monkey, at least, the former agent is the more persistent. Together, these superior qualities of WR-226,253 should encourage in-depth studies at the very earliest opportunity*.

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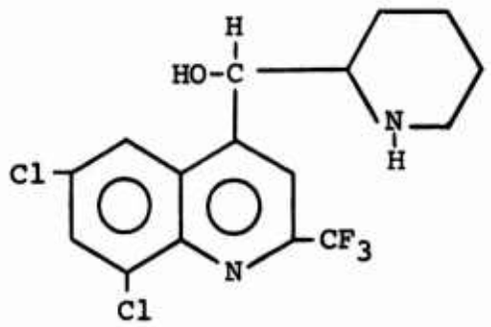
*In order for WR-226,253 to compete fully with WR-142,490, it will be necessary to develop a stable salt with greater water solubility than the currently available hydrochloride. The limited solubility of this latter salt would probably preclude parenteral administration of WR-226,253 to those individuals who could not accept the drug orally.

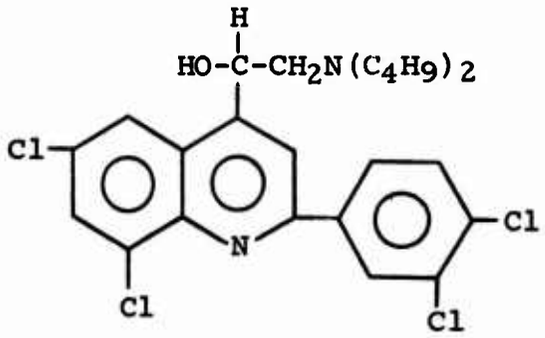
FIGURE 3

STRUCTURES OF WR-226,253, WR-30,090, AND WR-142,490



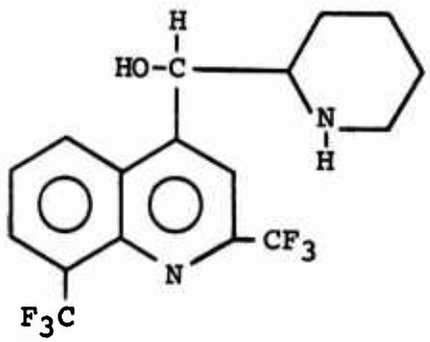
WR-226,253

α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinoline-methanol



WR-30,090

α-(di-n-butylaminomethyl)-2-(3,4-dichlorophenyl)-6,8-dichloro-4-quinolinemethanol



WR-142,490

α-(2-piperidyl)-2,8-di-trifluoromethyl-4-quinolinemethanol

TABLE 5

PRELIMINARY EVALUATION OF THE ACTIVITY OF WR-226,253 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH SPECIAL EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8113	1.1	1	1.1	2	18							21	13	16
8119	0.365	3	1.1	6	40	31	75					168		
8126	0.156	7	1.1	6	7	7	11	18	Dose increased					
8114	2.2	1	2.2	15	11							1	<1	<1
8113r	2.2	1	2.2	16	6							5	<1	
8121	0.73	3	2.2	27	18	5	1					<1	-	-
8119r	0.73	3	2.2	168	138	54	18					9	-	-
8127	0.312	7	2.2	18	26	34	100	114	Dose increased					
8117	4.375	1	4.375	10	16							<1	<1	-
8114r	4.375	1	4.375	3	<1							<1	<1	-
8113rr	4.375	1	4.375	3	1							<1	<1	<1
8122	1.46	3	4.378	12	22	4	<1					<1	-	-
8132	0.625	7	4.375	33	10	4	1	<1	<1	<1	-	-	-	-
8126r	0.625	7	4.375	18	39	10	2	<1	<1	-	-	-	-	
8127r	0.625	7	4.375	114	93	96	57	42	48	10	3	<1	<1	<1

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment						Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8118	8.75	1	8.75	12	4							1	<1	-
8117r	8.75	1	8.75	3	2							<1	<1	-
8072	2.92	3	8.76	33	33	2	-					-	-	-
8073	2.92	3	8.76	33	39	2	-					-	-	-
8122r	2.92	3	8.76	6	1	<1	<1					-	-	-
8101	1.25	7	8.75	18	16	10	<1	-	-	-	-	-	-	-
8103	1.25	7	8.75	NC	117	88	8	<1	<1	-	-	-	-	-
8127rr	1.25	7	8.75	6	3	2	2	<1	<1	-	-	-	-	
8079	5.84	3	17.5	24	9	NC	<1					<1	-	-
8106	2.5	7	17.5	10	12	<1	-	-	-	-	-	-	-	-
8126rr	2.5	7	17.5	3	<1	<1	<1	-	-	-	-	-	-	
8083	11.68	3	35.0	36	9	1	<1					-	-	-
8107	5.0	7	35.0	54	21	6	<1	<1	-	-	-	-	-	-
8089	23.36	3	70.0	12	21	3	<1					-	-	-
8136	10.0	7	70.0	6	2	<1	<1	-	-	-	-	-	-	-
8142	10.0	7	70.0	12	9	4	<1	<1	-	-	-	-	-	-
8071	-	-	-	8	6	n.a.	12	21	30	32	60	63	62	61
8133	-	-	-	14	56	36	74	84	84	123	90	75	116	140

TABLE 6

PRELIMINARY EVALUATION OF THE ACTIVITY OF WR-226,253 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH SPECIAL EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_X			Days from Initial R_X to Parasite Clearance	Days from Final R_X to Recrudescence	Remarks*
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8113	1.1	1	1.1		+		n.a.	n.a.	Dose increased
8119	0.365	3	1.1		+		n.a.	n.a.	Dose increased
8126	0.156	7	1.1		+		n.a.	n.a.	Dose increased
8114	2.2	1	2.2		+		n.a.	n.a.	Dose increased
8113r	2.2	1	2.2		+		n.a.	n.a.	Dose increased
8121	0.73	3	2.2			+	5	n.a.	Cured
8119r	0.73	3	2.2			+	6	n.a.	Died Negative Day 32 Post R_X**
8127	0.312	7	2.2	+			n.a.	n.a.	Dose increased
8117	4.375	1	4.375			+	4	25	
8114r	4.375	1	4.375			+	4	n.a.	Negative Day 68 Post R_X
8113rr	4.375	1	4.375			+	5	n.a.	Negative Day 55 Post R_X
8122	1.46	3	4.378			+	5	23	
8132	0.625	7	4.375			+	7	n.a.	Cured
8126r	0.625	7	4.375			+	6	19	
8127r	0.625	7	4.375			+	14	29	

TABLE 6 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks*
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8118	8.75	1	8.75			+	4	n.a.	Cured
8117r	8.75	1	8.75			+	4	n.a.	Negative Day 50 Post R_x
8072	2.92	3	8.76			+	3	n.a.	Cured
8073	2.92	3	8.76			+	3	n.a.	Cured
8122r	2.92	3	8.76			+	4	n.a.	Negative Day 48 Post R_x
8101	1.25	7	8.75			+	4	n.a.	Cured
8103	1.25	7	8.75			+	6	n.a.	Cured
8127rr	1.25	7	8.75			+	6	n.a.	Negative Day 30 Post R_x
8079	5.84	3	17.5			+	5	n.a.	Cured
8106	2.5	7	17.5			+	3	n.a.	Cured
8126rr	2.5	7	17.5			+	4	n.a.	Negative Day 41 Post R_x
8083	11.68	3	35.0			+	4	n.a.	Cured
8107	5.0	7	35.0			+	5	n.a.	Cured
8089	23.36	3	70.0			+	4	n.a.	Cured
8136	10.0	7	70.0			+	4	n.a.	Cured
8142	10.0	7	70.0			+	5	n.a.	Cured

*The status of each treated subject at termination of this Project is indicated. Infections which had not recrudesced within forty days of the last drug dose were probably cured.

**Death resulted from physical trauma.

TABLE 7

PRELIMINARY EVALUATION OF THE ACTIVITY OF WR-226,253 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM WITH SPECIAL EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8158	4.38	1	4.38	96	183							176		
8160	1.46	3	4.38	96	57	198	Dose increased							
8152	0.625	7	4.375	81	84	302	Dose increased							
8151	8.75	1	8.75	183	147							36	3	<1
8153	8.75	1	8.75	24	33							3	2	<1
8158r	8.75	1	8.75	176	202							60	25	6
8154	2.92	3	8.76	33	18	6	1					<1	2	<1
8162	2.92	3	8.76	144	165	90	22					6	1	<1
8160r	2.92	3	8.76	198	118	20	3					<1	<1	-
8150	1.25	7	8.75	12	14	13	13	1	<1	<1	-	-	-	-
8155	1.25	7	8.75	144	201	202	230	290	85	86	24	16	3	<1
8152r	1.25	7	8.75	302	117	44	42	2	<1	<1	<1	<1	-	-
8159	17.5	1	17.5	72	42							10	1	<1
8156	5.84	3	17.5	18	8	4	1					<1	<1	<1
8162r	5.84	3	17.5	18	1	<1	<1					-	-	-
8161	2.5	7	17.5	27	30	9	2	<1	<1	-	-	-	-	-
8155r	2.5	7	17.5	<1	2	15	6	<1	<1	-	-	-	-	-
8157	-	-	-	117	66	238	152	1070	950	1610	1070	1750	1410	1390

TABLE 8

PRELIMINARY EVALUATION OF THE ACTIVITY OF WR-226,253 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM WITH SPECIAL EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

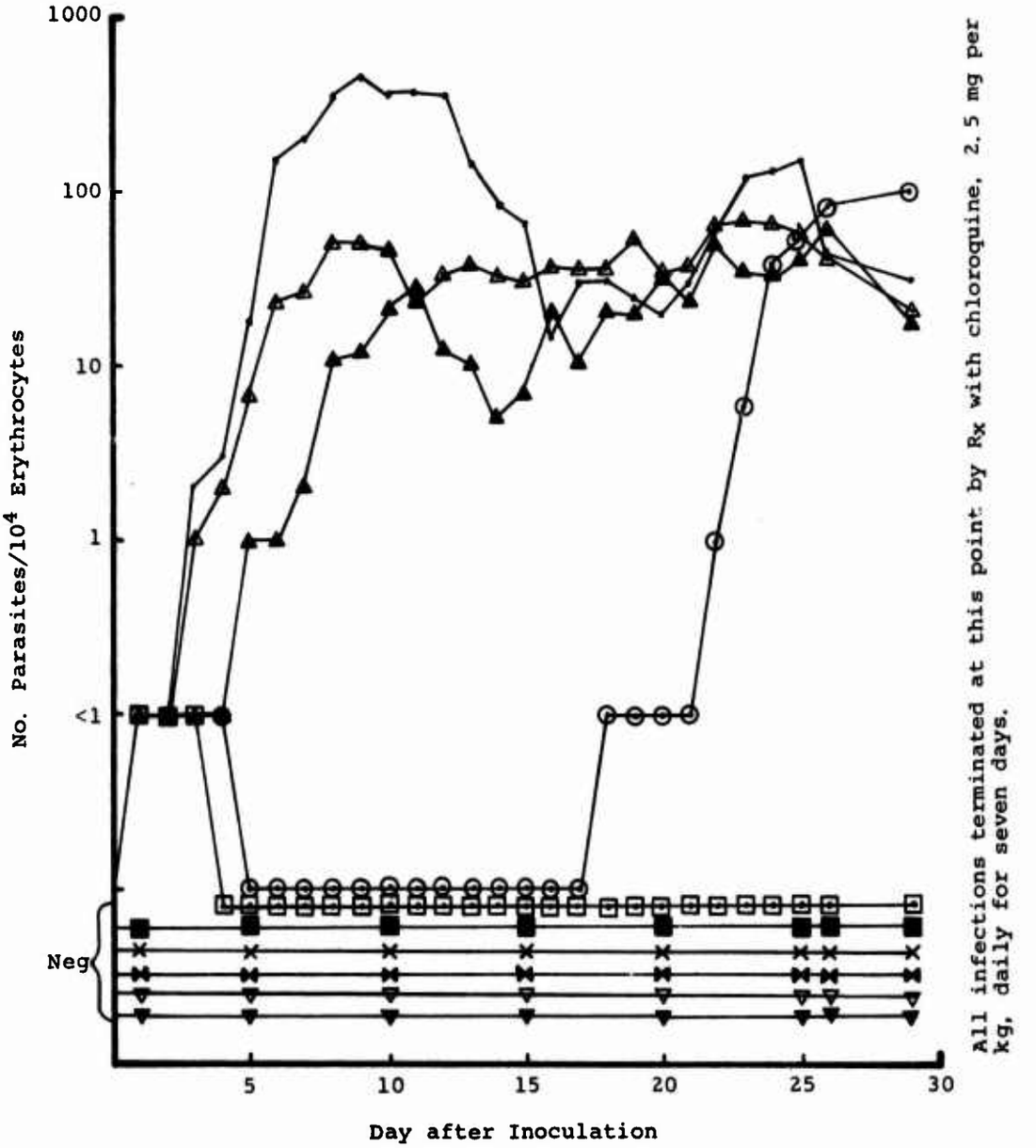
Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_X			Days from Initial R_X to Parasite Clearance	Days from Final R_X to Recrudescence	Remarks*
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8158	4.38	1	4.38	+			n.a.	n.a.	Dose increased
8160	1.46	3	4.38	+			n.a.	n.a.	Dose increased
8152	0.625	7	4.375	+			n.a.	n.a.	Dose increased
8151	8.75	1	8.75			+	8	n.a.	Negative Day 69 Post R_X
8153	8.75	1	8.75			+	6	n.a.	Negative Day 69 Post R_X
8158r	8.75	1	8.75			+	7	n.a.	Negative Day 67 Post R_X
8154	2.92	3	8.76			+	7	n.a.	Negative Day 67 Post R_X
8162	2.92	3	8.76			+	8	31	
8160r	2.92	3	8.76			+	7	n.a.	Negative Day 65 Post R_X
8150	1.25	7	8.75			+	7	n.a.	Negative Day 63 Post R_X
8155	1.25	7	8.75		+		n.a.	n.a.	
8152r	1.25	7	8.75			+	9	n.a.	Negative Day 61 Post R_X
8159	17.5	1	17.5			+	6	n.a.	Negative Day 70 Post R_X
8156	5.84	3	17.5			+	7	n.a.	Negative Day 67 Post R_X
8162r	5.84	3	17.5			+	5	n.a.	Negative Day 33 Post R_X
8161	2.5	7	17.5			+	6	n.a.	Negative Day 64 Post R_X
8155r	2.5	7	17.5			+	6	n.a.	Negative Day 48 Post R_X

*The status of each treated subject at termination of this Project is indicated. Infections which had not recrudesced within forty days of the last drug dose were probably cured.

FIGURE 4

THE DURATION OF PROTECTION ACCORDED BY A SINGLE DOSE OF WR-226,253 AGAINST INFECTION WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX



LEGEND:

Atr 8141 Control

Atr 8120, Atr 8125 } R_x Day 21 pre-inoc.

Atr 8129 R_x Day 14 pre-inoc.

Atr 8131, Atr 8137 } R_x Day 7 pre-inoc.

Atr 8138, Atr 8139 } R_x Day 4 pre-inoc.

Atr 8148, Atr 8149 } R_x 4 hours pre-inoc.

R_x = single dose - 17.5 mg WR-226,253 per kg body weight.

II. SPECIAL STUDIES ON BLOOD SCHIZONTICIDES

II. SPECIAL STUDIES ON BLOOD SCHIZONTICIDES

Detailed studies of three promising blood schizonticides (WR-180,409, WR-172,435, and WR-194,965) have been completed during the period covered by this Report. The first two compounds are 4-pyridinemethanol derivatives, close relatives of the 4-quinolinemethanols. The third compound, WR-194,965, is a Mannich reaction product, the most active of this class of agents uncovered to date. Each of the studies was pursued with the objective of providing information that would assist prospective evaluations in human volunteers. The lots of WR-180,409 and WR-172,435 utilized in these detailed assessments were prepared specifically for IND use. Because of the delay in submitting the IND lot of WR-194,965 and the need for information on the efficacy of various dosage regimens, evaluations were carried out on one of the early test lots of this compound.

Summaries of the results of these detailed studies have already been prepared and submitted. These are reproduced in their entireties as Sections II-A, II-B, and II-C of this Report.

A. WR-180,409-AC (BN: BE-56,685): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX IN THE OWL MONKEY (AOTUS TRIVIRGATUS)

SORI-KM-75-309

SUMMARY OF STUDIES CARRIED OUT UNDER CONTRACT NO. DADA 17-69-C-9104

ON

WR-180,409-AC (BN: BE-56,685): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX IN THE OWL MONKEY (AOTUS TRIVIRGATUS)

Southern Research Institute
2000-Ninth Avenue South
Birmingham, Alabama 35205
July 1, 1975

Project 2284-XXIV

WR-180,409-AC (BN: BE-56,685): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THE OWL MONKEY (*AOTUS TRIVIRGATUS*)

INTRODUCTORY COMMENT

Eight 4-pyridinemethanol derivatives*, selected on the basis of activities exhibited against infections with *P. berghei* in the mouse, have been evaluated for curative capabilities in owl monkeys infected with the chloroquine-quinine-resistant, pyrimethamine-susceptible Vietnam Oak Knoll strain and/or the chloroquine-quinine-pyrimethamine-resistant Vietnam Smith strain of *P. falciparum*. The structures of these pyridinemethanols and the daily doses required to cure infections with the Oak Knoll strain have been summarized in Table 1. Based on curative dosage, WR-180,409 [α-2'-piperidyl-2-(4-trifluoromethylphenyl)-6-trifluoromethyl-4-pyridinemethanol**] was clearly superior to six of the derivatives and slightly superior to the seventh (WR-172,435). The difference between the positions of WR-180,409 and WR-172,435 widens, however, when the therapeutic indices (TI) are considered, the former agent having a TI of at least 16, as opposed to 4 for the latter.

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*Actually, nine compounds were examined. The ninth, WR-180,117, was a stereoisomer of WR-180,409. Since the two isomers exhibited identical activity, only WR-180,409 will be referred to here.

**The initial investigations of the antimalarial activities of WR-180,409 were carried out with the hydrochloride salt, designated WR-180,409-AB (BN: BC-09,640).

The performance of WR-180,409 in the pilot studies referred to above has been summarized in Table 2 (detailed in Tables 1 through 4, Addendum). Since the activity of this 4-pyridinemethanol compared favorably with that of WR-142,490 (mefloquine*), it was decided to pursue the appropriate preclinical therapeutic and toxicologic studies required for clinical evaluations. These studies were preceded by the search for a more water soluble salt of WR-180,409 than the hydrochloride evaluated originally. This effort led to preparation of the phosphate salt which exhibited both greater water solubility and longer shelf stability than the hydrochloride.

The studies summarized in the following sections of this Report were carried out on a sample of the batch lot of WR-180,409 phosphate prepared for preclinical and clinical evaluation, specifically WR-180,409-AC, BN: BE-56,685. The objectives of the various studies were: (1) to ascertain whether the phosphate salt had the same bioavailability as the hydrochloride; (2) to determine the influence of the dosage regimen on activity; and (3) to appraise the acceptability and effectiveness of this pyridinemethanol when administered via the intravenous route. Objectives (1) and (2) were pursued in owl monkeys infected with the multidrug-resistant Vietnam Smith strain of P. falciparum and the pyrimethamine-resistant Vietnam Palo Alto strain of P. vivax. Because of short supply of owl monkeys, pursuit of objective (3) was restricted to monkeys infected with P. vivax.

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*WR-142,490 was the most active of the 4-quinolinemethanols evaluated against infections with drug-susceptible and drug-resistant strains of P. falciparum and P. vivax in the owl monkey. Studies in human volunteers have shown that a single dose of 1000 mg has high activity against infections with the multidrug-resistant Marks strain of P. falciparum.

METHODS AND PROCEDURES

The data presented in this Report were derived from the following four experiments, designed for the purposes indicated.

A. Assessments of the activity of WR-180,409-AC, delivered via the oral route, with emphasis on the impacts of the dosage regimen.

1. February 21, 1975 - Vietnam Smith strain, *P. falciparum*.

2. March 28, 1975 - Vietnam Smith strain, *P. falciparum*.

3. March 7, 1975 - Vietnam Palo Alto strain, *P. vivax*.

B. Assessment of the tolerability and efficacy of WR-180,409-AC administered intravenously.

1. April 10, 1975 - Vietnam Palo Alto strain, *P. vivax*.

The following procedures were common to each of the experiments. Owl monkeys (Aotus trivirgatus grisiemembra), imported directly from areas of Colombia adjacent to Barranquilla, were used exclusively. These subjects were vaccinated against Herpes tamarinus and Herpes simplex and conditioned for a minimum period of sixty days via procedures detailed elsewhere (Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:446-74, 1973). They were then assigned to assessments of the activities of WR-180,409-AC against infections with the Vietnam Smith strain of P. falciparum. Monkeys previously infected with this or other strains of P. falciparum, and cured via application of appropriate drugs other than WR-180,409, were utilized in assessing the activity of this pyridinemethanol against infections with the Vietnam Palo Alto strain of P. vivax*,**.

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*The responses of infections with the two test strains to currently available antimalarial drugs have been detailed elsewhere (Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:446-74, 1973). In brief, infections with the Vietnam Smith strain are fully resistant to treatment with the maximum tolerated doses of chloroquine, quinine, and pyrimethamine. Infections with the Vietnam Palo Alto strain are susceptible to treatment with chloroquine or quinine, but are resistant to treatment with pyrimethamine or proguanil.

**The owl monkey closely resembles man in that previous or current infection with P. falciparum does not alter host susceptibility to infection with P. vivax or vice versa. This makes it possible to utilize an animal for evaluating the activity of a drug against infections with P. falciparum and when cure of such infection is assured, to assign the same monkey to assessment of the activity of a different drug against infection with P. vivax. Such multiple use, a routine in our chemotherapeutic program, reduces operational costs and conserves numbers of monkeys that must be imported for these therapeutic assessments.

Three groups of 25 and one group of 7 monkeys were utilized in the various experiments. Infections were induced by intravenous inoculation of 5 x 10^6 erythrocytic parasites derived from monkeys of the passage lines of the various strains*. Measurements of parasitemias on thick and thin blood films stained with Giemsa were initiated three days after inoculation, at which time thick blood films were invariably positive. Thick and thin blood films were prepared daily thereafter until densities of 10 to 50 parasites per 10^4 erythrocytes** were attained. At this time, treatment with WR-180,409-AC was initiated, for some subjects orally, for others via the intravenous route. In the former case, the requisite dose of the compound (always calculated as base equivalent), dissolved in 10 ml of distilled water, was delivered by stomach tube, followed by a 3 ml water rinse. When administered intravenously, WR-180,409-AC, dissolved in sterile distilled water in a concentration of 5 mg base per ml, was injected into the mid-saphenous vein at the rate of 10 mg per kg per minute. Irrespective of route of delivery, WR-180,409-AC was administered within one hour of solution preparation.

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*The passage line of the Vietnam Smith strain of P. falciparum is maintained by serial transfer of parasitized erythrocytes through normal untreated owl monkeys every seven to ten days. The passage line of the Vietnam Palo Alto strain of P. vivax is maintained by serial transfer every twenty-one to twenty-eight days. Inocula for the chemotherapeutic studies are obtained by appropriate dilution in iced saline of heparinized blood drawn from a passage monkey. Dilutions for this strain of P. falciparum varied from 1:100 to 1:200; dilutions for the strain of P. vivax varied from 1:10 to 1:20.

**Such densities are equivalent to parasite populations of 5,000 to 25,000 per cmm of blood.

The effects of treatment on parasitemias were assessed on thick and thin blood films stained with Giemsa. Such films were prepared just prior to drug delivery during the treatment period and daily thereafter until thick films were parasite negative for at least four consecutive days. Film preparation and study were then reduced to a twice-weekly level (Monday and Thursday or Tuesday and Friday) for two consecutive weeks, and if results were uniformly negative during this interval, to a once-weekly level for ten additional weeks. Infections were considered cured if blood films were negative during the entire post-treatment period.

If parasitemias persisted at the initial or even lower levels, or increased in intensity during delivery of WR-180,409-AC, or if there was a reappearance of parasites after an apparent blood-negative interval, a second drug course was delivered, either at a higher total dose via the same regimen or at the same total dose via a different regimen. Whenever an infection was retreated either early or late, an r was added to the Atr number. Thus the number of r's following a monkey number indicates the number of retreatment courses the animal has received. This procedure has two advantages: (1) it expands the information on drug activity that can be obtained via use of a single monkey; and (2) it can signal emergence of drug-resistant plasmodia, if such occurs, and the rapidity with which this undesirable event appears.

In all of the studies, attention was directed to the impacts of the drug on the normal development of the parasite as indicated by alterations in morphology. This focus provided a gauge of the rapidity with which WR-180,409-AC affected parasite growth and the type of activity which this agent possesses.

Every experiment included at least one untreated control monkey. Inclusion of such subjects made it possible to monitor the virulence of the parasites in each inoculum.

RESULTS

The results of the four experiments referred to above have been detailed in Tables 3 through 8. The activities of various dosage regimens of WR-180,409-AC delivered orally to monkeys infected with the Vietnam Smith strain of P. falciparum are to be found in Tables 3 and 4. Similar data acquired in subjects infected with the Vietnam Palo Alto strain of P. vivax have been set forth in Tables 5 and 6. The effectiveness of WR-180,409-AC, administered intravenously to monkeys infected with the Palo Alto strain, has been detailed in Tables 7 and 8. These data on individual monkeys have been summarized in Tables 9 and 10. The latter tables are the focus of the following comments.

1. Comparison Of The Hydrochloride And Phosphate Salts Of WR-180,409 For Bioavailability

This comparison has been limited to the activity of seven-day treatment regimens in monkeys infected with the Vietnam Smith strain of P. falciparum. The data in Table 9 show that daily oral administration of the phosphate salt (WR-180,409-AC) in doses of 2.5 and 5.0 mg base equivalent per kg body weight cured 3 of 4 and 8 of 8 infections, respectively. The data acquired in pilot studies, summarized in Table 2, show that daily oral administration of the hydrochloride salt (WR-180,409-AB) in doses equivalent to 2.5 mg base failed to cure any of 13 infections; 19 of 21 infections

were cured with doses of 5.0 mg per kg; and 6 of 6 infections were cured with doses of 10.0 mg per kg body weight. This comparison indicates that WR-180,409 phosphate is at least as effective as the hydrochloride salt (probably more effective) in curing infections with the multidrug-resistant Smith strain of P. falciparum.

2. The Influence Of The Dosage Regimen On The Activity Of WR-180,409-AC

The dosage regimen comparison in Table 9 suggests strongly that WR-180,409-AC is more effective against infections with the Smith strain when delivered in three-day or seven-day courses than when administered in a single dose. There are suggestions that the seven-day dosage schedule may be slightly superior to the three-day schedule. However, it would be desirable to expand the data on these regimens substantially before accepting this conclusion. The single dose regimen not only was less effective than divided dose schedules with respect to cures, but its use was associated with erratic results - doses of 70.0 mg per kg being less effective than doses of 35.0 mg per kg. Although blood level data are not available, it seems likely that the larger of these doses had an adverse effect on the gastrointestinal tract which interfered with absorption.

The experience with the pyrimethamine-resistant Palo Alto strain of P. vivax, summarized in the lower section of Table 9, indicates that WR-180,409-AC was at least as effective against infections with this plasmodium as against infections with P. falciparum. The comparative curative activities of total doses of 8.75 and 17.5 mg per kg and the more rapid clearance of parasitemia suggest that WR-180,409-AC may actually be more effective against infections with P. vivax.

There were slight differences in the effectiveness of various dosage regimens against infections with the Palo Alto strain. Overall, however, the three- and seven-day divided dose schedules were superior to the single dose regimen.

3. The Acceptability And Effectiveness Of WR-180,409-AC Administered By The Intravenous Route

WR-180,409-AC was administered intravenously to ten subjects infected with the Palo Alto strain of P. vivax in single doses of 4.4 to 17.5 mg base equivalent per kg body weight and to eight other subjects in three divided daily doses at the same total levels (cf Table 10). There were no untoward systemic reactions in any recipient of these doses and no sign of phlebitis or irritation to tissue about the injection site. This experience indicates that intravenous delivery of WR-180,409 as the phosphate salt is feasible.

The activity data in the upper section of Table 10 show that WR-180,409-AC was highly effective in curing established infections with the Palo Alto strain when administered intravenously in either single doses of 8.75 or 17.5 mg per kg or three daily doses of equivalent totals. At a total dose of 8.75 mg per kg, the divided dose regimen appeared more effective than the single dose schedule. More experience would be needed to firm up this conclusion.

Comparison of the above data with those acquired in the companion arm of the evaluation in which WR-180,409-AC was administered by the oral route, indicates that there was a twofold gain in activity when this pyridinemethanol was administered parenterally. Since blood level data are lacking, one can do little more than suggest that the inferior results achieved with oral delivery reflect either incomplete absorption or restrictions on systemic availability of WR-180,409, possibly resulting from enterohepatic cycling.

SUMMARY AND CONCLUSIONS

The studies designed to evaluate the batch lot of WR-180,409-AC prepared for preclinical pharmacologic studies and subsequent evaluation in human volunteers have shown that:

1. WR-180,409-AC, delivered at a dose equivalent to 2.5 mg base per kg body weight daily for seven days, regularly cures established infections with either the multidrug-resistant Vietnam Smith strain of P. falciparum or the pyrimethamine-resistant Vietnam Palo Alto strain of P. vivax.

2. In a seven-day treatment regimen, WR-180,409-AC (phosphate salt) is at least as effective as WR-180,409-AB (hydrochloride salt) in curing established infections with the Vietnam Smith strain of P. falciparum. There are indications that the more water soluble phosphate is superior to the less water soluble hydrochloride.

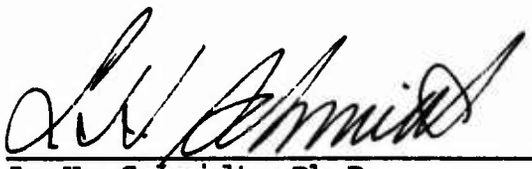
3. The total dose of WR-180,409-AC required for cure of infections with either the Smith strain of P. falciparum or the Palo Alto strain of P. vivax is the same for both three-day and seven-day dosage regimens. A single dose regimen is slightly less effective than either multiple dose regimen.

4. A single dose of WR-180,409-AC, equivalent to 17.5 mg base per kg body weight, produces no untoward symptoms when delivered via the intravenous route.

5. WR-180,409-AC is slightly but significantly more effective in curing infections with the Palo Alto strain of P. vivax when administered intravenously than when delivered via the oral route.

6. Although the studies summarized above were not designed to cover assessments of tolerability, observations on food consumption, maintenance of body weight, physical activity, immediate post-treatment behavior, and irritability were made routinely during all therapeutic assessments. No monkey committed to the diverse experiments exhibited untoward reactions in any of these areas. Thus it may be concluded that therapeutic benefits of WR-180,409-AC were achieved without any evidence of host toxicity.

The studies summarized in this Report were designed, supervised, and evaluated by the undersigned.



L. H. Schmidt, Ph.D.
Principal Investigator, DADA 17-69-C-9104

Kettering-Meyer Laboratory
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July 1, 1975

Project 2284-XXIV

TABLE 1

THE STRUCTURES OF A GROUP OF SELECTED PYRIDINEMETHANOLS AND THEIR CURATIVE ACTIVITIES AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

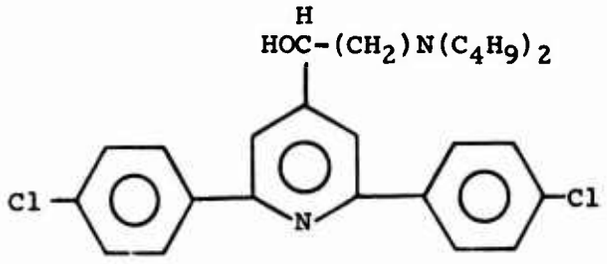
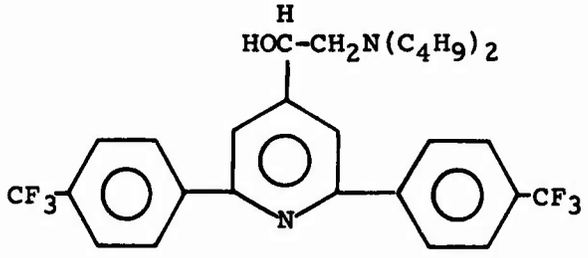
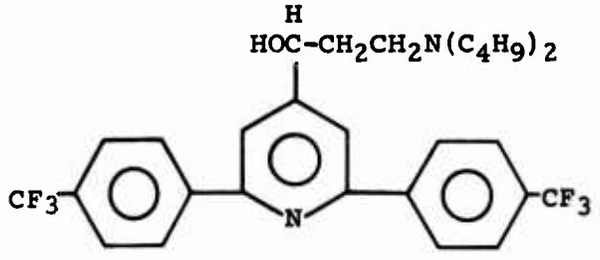
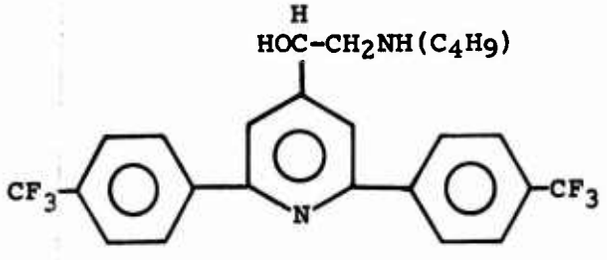
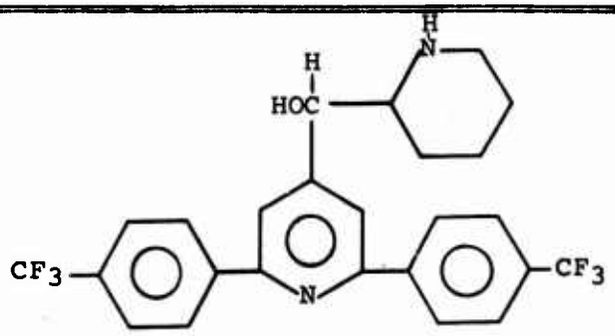
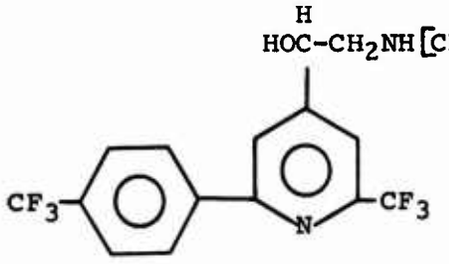
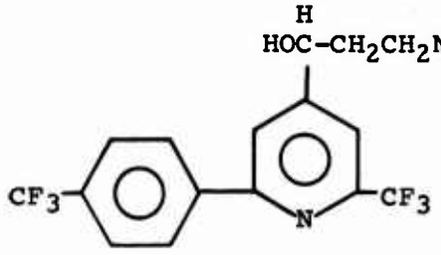
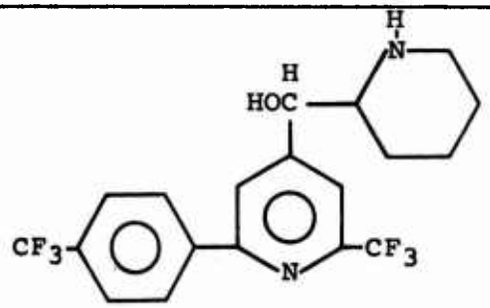
Compound WR- No.	Structure	Approximate Curative Dose Mg/Kg x 7
98,057		>200.0*
148,496		> 12.5
172,435		5.0
151,312		12.5

TABLE 1 - CONTINUED

Compound WR- No.	Structure	Approximate Curative Dose Mg/Kg x 7
154,904		12.5
178,919		> 40.0
175,039		40.0
180,409		2.5

* The activity of WR-98,057 was assessed against infections with the Vietnam Monterey strain. This strain has a spectrum of responses to chloroquine, quinine, and pyrimethamine identical with the responses of the Oak Knoll strain.

TABLE 2

THE CAPACITY OF WR-180,409 TO CURE ESTABLISHED INFECTIONS WITH THE VIETNAM OAK KNOLL AND VIETNAM SMITH STRAINS OF PLASMODIUM FALCIPARUM

Daily Dose Mg WR-180,409 Base*/Kg Body Weight x 7	No. Infections Cured/No. Infections Treated	
	Vietnam Oak Knoll Strain	Vietnam Smith Strain
0.6	0/3	-
1.25	1/5	1/6
2.5	8/10	0/13
5.0	2/2	19/21
10.0	2/2	6/6
40.0	2/2	2/2

* WR-180,409-AB (BN: BC-09,640), hydrochloride salt, was used in the evaluations summarized in this table.

TABLE 3

THE ACTIVITY OF WR-180,409-AC ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM - WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment									
					1	2	3	4	5	6	7	8	9	10
6994	8.75	1	8.75	8	350	16	<1	<1	<1	<1	<1	<1	<1	<1
6998	8.75	1	8.75	8	48	16	1	<1	5	24	101			
8017	3.0	3	9.0	40	184	69	10	1	<1	<1	<1	<1	<1	-
8018	3.0	3	9.0	57	87	10	<1	<1	<1	<1	<1	<1	<1	-
8028	1.25	7	8.75	56	150	125	25	40	24	12	2	3	1	8
8029	1.25	7	8.75	32	300	150	50	3	1	<1	<1	<1	<1	<1
7847	17.5	1	17.5	18	20	4	<1	<1	<1	<1	-	-	-	
7849	17.5	1	17.5	28	280	36	10	1	<1	<1	-	-	-	
8049	17.5	1	17.5	15	84	16	2	<1	-	-	-		<1	<1
8051	17.5	1	17.5	15	108	56	5	4	<1	<1	<1	<1	<1	<1
6994r	17.5	1	17.5	6	3	1	<1	<1	<1	<1	-	-	-	
6998r	17.5	1	17.5	101	36	6	2	-	-	-			-	
8019	6.0	3	18.0	14	140	27	3	<1	<1	<1	-	-	-	
8020	6.0	3	18.0	15	140	55	6	<1	6	6	2	15	58	
8017r	6.0	3	18.0	24	3	<1	<1	<1	-	-	-			
8018r	6.0	3	18.0	1	6	3	<1	-	-	-		-		
8042	2.5	7	17.5	51	232	32	18	1	<1	<1	-	-	-	
8043	2.5	7	17.5	18	276	320	330	170	220	28	14	2	<1	<1
8028r	2.5	7	17.5	2	4	2	<1	<1	<1	<1	-	-	-	
8029r	2.5	7	17.5	4	1	<1	<1	<1	<1	-	-	-	-	

TABLE 3 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment									
					1	2	3	4	5	6	7	8	9	10
7894	35.0	1	35.0	32	190	16	2	<1	6	16	220			
7895	35.0	1	35.0	44	110	14	1	<1	<1	<1	-	-	-	
8052	35.0	1	35.0	30	76	40	<1	<1	-	-	-		-	
8053	35.0	1	35.0	15	160	184	300	462	1210					
7847r	35.0	1	35.0	<1	<1	<1	<1	<1	-	-	-			
7849r	35.0	1	35.0	8	2	<1	<1	-	-	-	-			
8049r	35.0	1	35.0	2	2	<1	<1	-	-	-			-	
8051r	35.0	1	35.0	1	3	<1	<1	-	-	-			-	
6994rr	35.0	1	35.0	2	1	<1	<1	<1	-	-	-	-	-	
6998rr	35.0	1	35.0	<1	<1	-	-	-	-	-		-		
8021	12.0	3	36.0	20	260	171	40	1	<1	<1	-	-	-	
8023	12.0	3	36.0	168	92	56	9	1	<1	<1	-	-	-	
8019r	12.0	3	36.0	3	1	<1	<1	<1	<1	<1	-	-	-	
8020r	12.0	3	36.0	58	10	5	2	<1	<1	-	-	-	-	
8018rr	12.0	3	36.0	<1	2	2	<1	-	-	-				
8045	5.0	7	35.0	24	200	40	24	4	1	<1	<1	-	-	-
8046	5.0	7	35.0	58	414	99	33	5	2	<1	<1	-	-	-
8043r	5.0	7	35.0	2	3	2	<1	<1	<1	-	-	-	-	
8056r	5.0	7	35.0	184	210	250	90	14	3	<1	<1	-	-	-
7894rr	5.0	7	35.0	1	<1	-	-	-	-	-	-	-	-	-
8016rr	5.0	7	35.0	2	<1	<1	<1	<1	-	-	-	-	-	
8053rr	5.0	7	35.0	120	39	12	2	<1	<1	-	-	-	-	-
7849rrr	5.0	7	35.0	4	8	2	<1	-	-	-	-	-	-	

TABLE 3 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment									
					1	2	3	4	5	6	7	8	9	10
8015	70.0	1	70.0	105	190	90	40	6	<1	<1	-	-	-	
8016	70.0	1	70.0	51	272	260	400	440	Dose repeated					
8056	70.0	1	70.0	30	186	160	180	95	184					
8057	70.0	1	70.0	27	98	32	<1	<1	-	-	-		-	
7894r	70.0	1	70.0	220	60	6	<1	-	-	-		-		
8016r	70.0	1	70.0	440	480	110	64	64	12	6	<1	3	2	
8053r	70.0	1	70.0	1210	2420	1040	240	90	80	96	62	120		
7849rr	70.0	1	70.0	2	<1	<1	<1	<1	2	4				
8024	24.0	3	72.0	60	105	105	40	4	<1	<1	-	-	-	
8026	24.0	3	72.0	56	63	14	2	<1	<1	<1	-	-	-	
8048	10.0	7	70.0	17	140	30	3	<1	<1	<1	-	-	-	
8054	10.0	7	70.0	36	81	34	4	<1	<1	<1	-	-	-	
8055	-	-	-	20	350	590	1270	4040	1856	3620	2100	Dead		
8058	-	-	-	15	168	500	670	480	920	880	1360	1090	1500	1320

TABLE 4

THE ACTIVITY OF WR-180,409-AC ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM - WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recrudescence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
6994	8.75	1	8.75		+		n.a.	n.a.	
6998	8.75	1	8.75		+		n.a.	n.a.	
8017	3.0	3	9.0		+		n.a.	n.a.	
8018	3.0	3	9.0			+	10	38	
8028	1.25	7	8.75		+		n.a.	n.a.	
8029	1.25	7	8.75		+		n.a.	n.a.	
7847	17.5	1	17.5			+	7	28	
7849	17.5	1	17.5			+	7	28	
8049	17.5	1	17.5		+		n.a.	n.a.	
8051	17.5	1	17.5		+		n.a.	n.a.	
6994r	17.5	1	17.5			+	7	24	
6998r	17.5	1	17.5			+	5	13	
8019	6.0	3	18.0			+	5	10	
8020	6.0	3	18.0		+		n.a.	n.a.	
8017r	6.0	3	18.0			+	5	n.a.	Cured
8018r	6.0	3	18.0			+	4	19	
8042	2.5	7	17.5			+	7	n.a.	Cured
8043	2.5	7	17.5		+		n.a.	n.a.	
8028r	2.5	7	17.5			+	7	n.a.	Cured
8029r	2.5	7	17.5			+	6	n.a.	Cured

TABLE 4 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recru-descence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7894	35.0	1	35.0		+		n. a.	n. a.	
7895	35.0	1	35.0			+	7	n. a.	Cured
8052	35.0	1	35.0			+	5	n. a.	Cured
8053	35.0	1	35.0	+			n. a.	n. a.	
7847r	35.0	1	35.0			+	5	n. a.	Cured
7849r	35.0	1	35.0			+	4	18	
8049r	35.0	1	35.0			+	4	n. a.	Cured
8051r	35.0	1	35.0			+	4	n. a.	Cured
6994rr	35.0	1	35.0			+	5	n. a.	Cured
6998rr	35.0	1	35.0			+	2	n. a.	Cured
8021	12.0	3	36.0			+	5	n. a.	Cured
8023	12.0	3	36.0			+	5	n. a.	Cured
8019r	12.0	3	36.0			+	7	n. a.	Cured
8020r	12.0	3	36.0			+	4	n. a.	Cured
8018rr	12.0	3	36.0			+	4	n. a.	Cured
8045	5.0	7	35.0			+	8	n. a.	Cured
8046	5.0	7	35.0			+	8	n. a.	Cured
8043r	5.0	7	35.0			+	6	n. a.	Cured
8056r	5.0	7	35.0			+	8	n. a.	Cured
7894rr	5.0	7	35.0			+	2	n. a.	Cured
8016rr	5.0	7	35.0			+	5	n. a.	Cured
8053rr	5.0	7	35.0			+	6	n. a.	Cured
7849rrr	5.0	7	35.0			+	4	n. a.	Cured

TABLE 4 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_X			Days from Initial R_X to Parasite Clearance	Days from Final R_X to Recrudescence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
8015	70.0	1	70.0			+	7	n.a.	Cured
8016	70.0	1	70.0		+		n.a.	n.a.	Dose repeated
8056	70.0	1	70.0		+		n.a.	n.a.	
8057	70.0	1	70.0			+	5	n.a.	Cured
7894r	70.0	1	70.0			+	5	25	
8016r	70.0	1	70.0		+		n.a.	n.a.	
8053r	70.0	1	70.0		+		n.a.	n.a.	
7849rr	70.0	1	70.0		+		n.a.	n.a.	
8024	24.0	3	72.0			+	5	n.a.	Cured
8026	24.0	3	72.0			+	5	n.a.	Cured
8048	10.0	7	70.0			+	7	n.a.	Cured
8054	10.0	7	70.0			+	7	n.a.	Cured

TABLE 5

THE ACTIVITY OF WR-180,409-AC ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX - WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment								
					1	2	3	4	5	6	7	8	9	10
7745	8.75	1	8.75	87	14	4	<1	-	-	-		-		
7750	8.75	1	8.75	4	2	<1	-	-	-			-		
7807	3.0	3	9.0	42	57	147	180	12	6	12	18			
7823	3.0	3	9.0	66	81	30	<1	<1	-	-	-			
7898	1.25	7	8.75	10	18	14	19	2	<1	<1	-	-	-	
7910	1.25	7	8.75	36	100	42	22	15	2	<1	-	-	-	
7760	17.5	1	17.5	12	28	1	<1	<1	-	-	-	-		
7762	17.5	1	17.5	10	4	<1	<1	-	-	-		-		
7745r	17.5	1	17.5	19	16	<1	<1	-	-	-		-		
7824	6.0	3	18.0	24	6	1	-	-	-			-		
7826	6.0	3	18.0	26	12	3	-	-	-			-		
7807r	6.0	3	18.0	18	12	4	<1	<1	<1	<1	-	-	-	-
7915	2.5	7	17.5	8	8	6	2	<1	<1	-	-	-	-	
7916	2.5	7	17.5	22	40	44	24	6	<1	<1	-	-	-	
7898r	2.5	7	17.5	6	10	24	2	<1	-	-	-	-	-	
7910r	2.5	7	17.5	1	1	<1	<1	-	-	-	-	-	-	

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment									
					1	2	3	4	5	6	7	8	9	10
7764	35.0	1	35.0	4	4	<1	<1	-	-	-				
7791	35.0	1	35.0	36	27	32	6	24	12	Dose repeated				
7791r	35.0	1	35.0	12	6	4	1	<1	<1	-	-	-	-	
7859	12.0	3	36.0	20	21	29	<1	-	-			-		
7862	12.0	3	36.0	3	2	<1	-	-	-			-		
7807rr	12.0	3	36.0	26	27	2	<1	<1	-	-	-	-		
7917	5.0	7	35.0	16	24	4	<1	<1	-	-	-	-	-	
7922	5.0	7	35.0	20	24	4	<1	<1	-	-	-	-	-	
7806r	5.0	7	35.0	2	4	<1	<1	<1	-	-	-	-	-	-
7792	70.0	1	70.0	15	2	<1	-	-	-			-		
7806	70.0	1	70.0	8	10	<1	-	-	-			-		
7870	24.0	3	72.0	24	9	<1	<1	-	-	-		-		
7896	24.0	3	72.0	8	2	<1	<1	-	-	-		-		
7986	10.0	7	70.0	16	24	2	<1	<1	-	-	-	-	-	
7987	10.0	7	70.0	12	8	2	<1	<1	-	-	-	-	-	
7988	-	-	-	9	10	32	56	28	35	18	56	28	15	13

TABLE 6

THE ACTIVITY OF WR-180,409-AC ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX - WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7745	8.75	1	8.75			+	5	18	
7750	8.75	1	8.75			+	3	n. a.	Cured
7807	3.0	3	9.0		+		n. a.	n. a.	
7823	3.0	3	9.0			+	5	n. a.	Cured
7898	1.25	7	8.75			+	7	9	
7910	1.25	7	8.75			+	7	16	
7760	17.5	1	17.5			+	5	n. a.	Cured
7762	17.5	1	17.5			+	4	n. a.	Cured
7745r	17.5	1	17.5			+	4	n. a.	Cured
7824	6.0	3	18.0			+	3	n. a.	Cured
7826	6.0	3	18.0			+	3	n. a.	Cured
7807r	6.0	3	18.0			+	8	19	
7915	2.5	7	17.5			+	6	n. a.	Cured
7916	2.5	7	17.5			+	7	n. a.	Cured
7898r	2.5	7	17.5			+	5	n. a.	Cured
7910r	2.5	7	17.5			+	4	n. a.	Cured

TABLE 6 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recru-descence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7764	35.0	1	35.0			+	5	n.a.	Cured
7791	35.0	1	35.0		+		n.a.	n.a.	Dose repeated
7791r	35.0	1	35.0			+	6	n.a.	Cured
7859	12.0	3	36.0			+	4	n.a.	Cured
7862	12.0	3	36.0			+	3	n.a.	Cured
7807rr	12.0	3	36.0			+	5	n.a.	Cured
7917	5.0	7	35.0			+	5	n.a.	Cured
7922	5.0	7	35.0			+	5	n.a.	Cured
7806r	5.0	7	35.0			+	5	n.a.	Cured
7792	70.0	1	70.0			+	3	n.a.	Cured
7806	70.0	1	70.0			+	3	12	
7870	24.0	3	72.0			+	4	n.a.	Cured
7896	24.0	3	72.0			+	4	n.a.	Cured
7986	10.0	7	70.0			+	5	n.a.	Cured
7987	10.0	7	70.0			+	5	n.a.	Cured

TABLE 7

THE INFLUENCE OF THE ROUTE OF ADMINISTRATION ON THE ACTIVITY OF WR-180,409-AC IN OWL MONKEYS INFECTED WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment									
					1	2	3	4	5	6	7	8	9	10
Intravenous Route Of Administration														
7612	4.4	1	4.4	11	2	<1	<1	2	3	15				
7692	4.4	1	4.4	1	3	<1	1	4	2	12				
7777	1.5	3	4.5	10	18	2	<1	<1	-	-	-		-	
7863	1.5	3	4.5	16	84	76	21	3	<1	<1	<1	<1	<1	<1
7707	8.75	1	8.75	3	2	<1	<1	-	-	-			-	
7710	8.75	1	8.75	6	4	<1	<1	<1	-	-	-		-	
7612r	8.75	1	8.75	15	4	1	<1	-	-	-				-
7692r	8.75	1	8.75	12	2	1	<1	-	-	-				
7864	3.0	3	9.0	3	8	2	<1	<1	-	-	-		-	
7865	3.0	3	9.0	8	2	3	<1	<1	-	-	-		-	
7777r	3.0	3	9.0	1		<1	-	-	-					-
7863r	3.0	3	9.0	8	1	<1	<1	<1	<1	-	-	-	-	
7720	17.5	1	17.5	13	8	1	<1	-	-	-			-	
7776	17.5	1	17.5	16	8	1	<1	-	-	-			-	
7710r	17.5	1	17.5	3		<1	-	-	-					-
7612rr	17.5	1	17.5	<1	4	<1	-	-	-			-		
7866	6.0	3	18.0	12	10	1	<1	-	-	-			-	
7918	6.0	3	18.0	18	10	1	<1	-	-	-			-	

TABLE 7 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Day Pre-treatment	Day from Beginning of Treatment									
					1	2	3	4	5	6	7	8	9	10
Oral Route Of Administration														
7934	4.4	1	4.4	36	56	184	132	152	90	80				
7935	4.4	1	4.4	6	32	18	13	8	10	22				
7946	1.5	3	4.5	10	32	84	60	46	22	24				
7948	1.5	3	4.5	8	14	48	22	24	4	15	22	8		
7936	8.75	1	8.75	8	4	<1	<1	<1	<1	-	-	-		
7937	8.75	1	8.75	20	8	1	<1	<1	-	-	-		-	
7934r	8.75	1	8.75	80	30	5	1	-	-	-				-
7935r	8.75	1	8.75	22	1	<1	<1	-	-	-				-
7951	3.0	3	9.0	2	8	2	2	<1	-	-	-		-	
7952	3.0	3	9.0	9	40	12	6	<1	-	-	-		-	
7946r	3.0	3	9.0	24	18	4	<1	-	-	-				-
7948r	3.0	3	9.0	8	6	<1	-	-	-			-		
7938	17.5	1	17.5	22	12	1	<1	-	-	-			-	
7944	17.5	1	17.5	12	6	1	<1	-	-	-			-	
7937r	17.5	1	17.5	2	1	<1	<1	-	-	-	-			
7934rr	17.5	1	17.5	15	3	1	<1	-	-	-			-	
7935rr	17.5	1	17.5	<1	2	<1	-	-	-			-		
7953	6.0	3	18.0	10	4	1	<1	<1	-	-	-		-	
7958	6.0	3	18.0	12	20	6	<1	<1	-	-	-		-	
7952r	6.0	3	18.0	1	3	1	<1	<1	-	-		-		
7946rr	6.0	3	18.0	18	21	7	2	<1	<1	-	-	-		
7961	-	-	-	24	58	33	91	128	93	114	34	40	48	56

TABLE 8

THE INFLUENCE OF THE ROUTE OF ADMINISTRATION ON THE ACTIVITY OF WR-180,409-AC IN OWL MONKEYS INFECTED WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
Intravenous Route Of Administration									
7612	4.4	1	4.4		+		n. a.	n. a.	
7692	4.4	1	4.4		+		n. a.	n. a.	
7777	1.5	3	4.5			+	5	18	
7863	1.5	3	4.5		+		n. a.	n. a.	
7707	8.75	1	8.75			+	4	n. a.	Cured
7710	8.75	1	8.75			+	5	20	
7612r	8.75	1	8.75			+	4	21	
7692r	8.75	1	8.75			+	4	n. a.	Cured
7864	3.0	3	9.0			+	5	n. a.	Cured
7865	3.0	3	9.0			+	5	n. a.	Cured
7777r	3.0	3	9.0			+	3	n. a.	Cured
7863r	3.0	3	9.0			+	6	n. a.	Cured
7720	17.5	1	17.5			+	4	n. a.	Cured
7776	17.5	1	17.5			+	4	n. a.	Cured
7710r	17.5	1	17.5			+	3	n. a.	Cured
7612r	17.5	1	17.5			+	3	n. a.	Cured
7866	6.0	3	18.0			+	5	n. a.	Cured
7918	6.0	3	18.0			+	5	n. a.	Cured

TABLE 8 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recru-descence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
Oral Route Of Administration									
7934	4.4	1	4.4	+			n.a.	n.a.	
7935	4.4	1	4.4		+		n.a.	n.a.	
7946	1.5	3	4.5	+			n.a.	n.a.	
7948	1.5	3	4.5	+			n.a.	n.a.	
7936	8.75	1	8.75			+	6	n.a.	Cured
7937	8.75	1	8.75			+	4	13	
7934r	8.75	1	8.75			+	4	18	
7935r	8.75	1	8.75			+	4	21	
7951	3.0	3	9.0			+	5	n.a.	Cured
7952	3.0	3	9.0			+	5	14	
7946r	3.0	3	9.0			+	4	18	
7948r	3.0	3	9.0			+	3	n.a.	Cured
7938	17.5	1	17.5			+	4	n.a.	Cured
7944	17.5	1	17.5			+	4	n.a.	Cured
7937r	17.5	1	17.5			+	4	n.a.	Cured
7934rr	17.5	1	17.5			+	4	n.a.	Cured
7935rr	17.5	1	17.5			+	3	n.a.	Cured
7953	6.0	3	18.0			+	5	n.a.	Cured
7958	6.0	3	18.0			+	5	n.a.	Cured
7952r	6.0	3	18.0			+	5	n.a.	Cured
7946rr	6.0	3	18.0			+	6	n.a.	Cured

TABLE 9

SUMMARY: EVALUATION OF THE INFLUENCE OF THE DOSAGE REGIMEN ON THE ACTIVITIES OF WR-180,409-AC ADMINISTERED ORALLY TO MONKEYS INFECTED WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM OR VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Smith Strain - P. falciparum								
8.75	1	8.75	2	0	2	0	0	n. a.
3.0	3	9.0	2	0	1	1	0	10
1.25	7	8.75	2	0	2	0	0	n. a.
17.5	1	17.5	6	0	2	4	0	ca 6
6.0	3	18.0	4	0	1	3	1	ca 5
2.5	7	17.5	4	0	1	3	3	ca 7
35.0	1	35.0	10	1	1	8	7	ca 5
12.0	3	36.0	5	0	0	5	5	5
5.0	7	35.0	8	0	0	8	8	6
70.0	1	70.0	8	0	5	3	2	ca 6
24.0	3	72.0	2	0	0	2	2	5
10.0	7	70.0	2	0	0	2	2	7
Vietnam Palo Alto Strain - P. vivax								
8.75	1	8.75	2	0	0	2	1	4
3.0	3	9.0	2	0	1	1	1	5
1.25	7	8.75	2	0	1	2	0	7
17.5	1	17.5	3	0	0	3	3	ca 4
6.0	3	18.0	3	0	0	3	2	ca 5
2.5	7	17.5	4	0	0	4	4	ca 6
35.0	1	35.0	3	0	1	2	2	ca 6
12.0	3	36.0	3	0	0	3	3	4
5.0	7	35.0	3	0	0	3	3	5
70.0	1	70.0	2	0	0	2	1	3
24.0	3	72.0	2	0	0	2	2	4
10.0	7	70.0	2	0	0	2	2	5

* Median day to parasite clearance.

TABLE 10

SUMMARY: THE INFLUENCE OF THE ROUTE OF ADMINISTRATION ON THE ACTIVITY OF WR-180,409-AC AGAINST INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Intravenous Route Of Administration								
4.4	1	4.4	2	0	2	0	0	n.a.
1.5	3	4.5	2	0	1	1	0	5
8.75	1	8.75	4	0	0	4	2	ca 4
3.0	3	9.0	4	0	0	4	4	ca 5
17.5	1	17.5	4	0	0	4	4	ca 3.5
6.0	3	18.0	2	0	0	2	2	5
Oral Route Of Administration								
4.4	1	4.4	2	1	1	0	0	n.a.
1.5	3	4.5	2	2	0	0	0	n.a.
8.75	1	8.75	4	0	0	4	1	ca 4.5
3.0	3	9.0	4	0	0	4	2	ca 4.5
17.5	1	17.5	5	0	0	5	5	4
6.0	3	18.0	4	0	0	4	4	5

* Median day to parasite clearance.

ADDENDUM

TABLE 1

THE ACTIVITY OF WR-180,409-AB ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment							Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
7340	0.6	14	72	132	88	66	28	16	8	6	1	<1
7342	0.6	50	318	890	1200	Dose increased						
7344	0.6	78	202	500	730	Dose increased						
7350	1.25	46	178	420	260	40	8	1	<1	-	-	-
7352	1.25	98	258	580	520	272	8	4	6	2	2	<1
7354	1.25	38	110	38	26	5	2	<1	-	-	-	
7342r	1.25	1200	1780	1470	720	69	6	4	1	<1	<1	<1
7344r	1.25	730	340	44	3	<1	<1	<1	<1	<1	-	
7214	2.5	50	84	56	12	<1	<1	-	-	-	-	
7215	2.5	48	88	132	15	<1	<1	-	-	-	-	
7356	2.5	36	66	22	3	<1	-	<1	-	-	-	
7357	2.5	96	134	54	4	1	<1	-	-	-	-	
7382	2.5	134	84	106	48	8	1	<1	<1	-	-	-
7340r	2.5	<1	<1	<1	<1	-	-	-	-	-	-	
7350r	2.5	12	30	24	46	12	3	<1	<1	<1	-	-
7352r	2.5	104	28	42	8	4	<1	<1	<1	<1	-	-
7354r	2.5	18	230	136	38	12	<1	<1	<1	-	-	-
7342rr	2.5	44	17	1	<1	<1	-	-	-	-	-	
7382r	5.0	244	100	35	4	<1	<1	<1	-	-	-	-
7340rr	5.0	1	<1	1	<1	-	-	-	-	-	-	
7216	10.0	42	51	22	8	<1	-	-	-	-	-	
7217	10.0	56	48	16	1	<1	-	-	-	-	-	
7218	40.0	46	19	12	1	<1	-	-	-	-	-	
7219	40.0	76	55	5	1	<1	-	-	-	-	-	
7227	-	46	141	1090	894	2290	1270	2120	1140	1060	1270	570
7409	-	96	302	348	1130	1800	2420	1230	1490	1000	1760	2600

TABLE 2

THE ACTIVITY OF WR-180,409-AB ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
		None	Suppressed	Cleared			
7340	0.6		+		n.a.	n.a.	
7342	0.6	+			n.a.	n.a.	Dose increased
7344	0.6		±		n.a.	n.a.	Dose increased
7350	1.25			+	8	10	
7352	1.25		+		n.a.	n.a.	
7354	1.25			+	7	17	
7342r	1.25		+		n.a.	n.a.	
7344r	1.25			+	9	n.a.	Cured
7214	2.5			+	6	n.a.	Cured
7215	2.5			+	6	n.a.	Cured
7356	2.5			+	7	n.a.	Cured
7357	2.5			+	6	n.a.	Cured
7382	2.5			+	8	12	
7340r	2.5			+	4	30	
7350r	2.5			+	9	n.a.	Cured
7352r	2.5			+	9	n.a.	Cured
7354r	2.5			+	8	n.a.	Cured
7342rr	2.5			+	5	n.a.	Cured
7382r	5.0			+	7	n.a.	Cured
7340rr	5.0			+	4	n.a.	Cured
7216	10.0			+	5	n.a.	Cured
7217	10.0			+	5	n.a.	Cured
7218	40.0			+	5	n.a.	Cured
7219	40.0			+	5	n.a.	Cured

TABLE 3

THE ACTIVITY OF WR-180,409-AB ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment						Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
7302	1.25	19	104	132	150	284	118	96	34	150		
7303	1.25	24	58	30	10	8	30	8	12	8	7	11
7304	1.25	58	80	80	100	60	16	10	2	1	<1	<1
7339	1.25	24	180	66	310	94	330	210	Dose increased			
7558	1.25	35	300	328	1350	Dose increased						
7559	1.25	51	420	780	1760	Dose increased						
7228	2.5	108	490	830	Dose increased							
7229	2.5	40	40	50	34	22	4	1	<1	-	-	-
7305	2.5	19	154	74	40	25	36	15	12	3	12	11
7306	2.5	29	66	24	18	14	4	<1	<1	-	-	-
7307	2.5	22	40	58	72	52	28	8	2	<1	<1	<1
7365	2.5	31	86	36	102	9	4	<1	<1	-	-	-
7367	2.5	28	30	22	6	1	<1	-	-	-	-	-
7560	2.5	18	96	38	68	25	36	10	2	<1	-	-
7561	2.5	37	100	96	110	48	22	8	4	1	<1	-
7302r	2.5	150	20	15	6	<1	<1	-	-	-	-	-
7339r	2.5	210	76	24	18	2	<1	<1	-	-	-	-
7558r	2.5	1350	410	332	230	36	8	<1	-	-	-	
7559r	2.5	1760	1780	1250	1050	440	110	27	16	5	4	<1

TABLE 3 - CONTINUED

Atr No.	Daily Dose Mg/Kg Body Weight	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment						Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
7308	5.0	22	58	15	8	4	<1	<1	-	-	-	
7309	5.0	12	172	64	22	4	3	<1	-	-	-	
7310	5.0	44	164	34	16	8	<1	<1	-	-	-	
7374	5.0	4	2	<1	<1	<1	-	-	-	-	-	
7377	5.0	23	88	20	5	2	<1	-	-	-	-	-
7567	5.0	28	41	36	36	25	22	4	2	<1	<1	<1
7568	5.0	44	30	29	4	<1	<1	<1	<1	<1	-	-
7228r	5.0	830	540	134	52	8	<1	-	-	-	-	
7229r	5.0	1	1	<1	<1	-	-	-	-	-	-	
7303r	5.0	53	24	12	<1	<1	<1	<1	-	-	-	-
7305r	5.0	74	50	9	16	<1	<1	<1	-	-	-	-
7306r	5.0	60	100	60	20	5	<1	<1	<1	-	<1	-
7307r	5.0	<1	<1	<1	<1	<1	-	-	-	-	-	
7365r	5.0	<1	<1	-	-	-	-	-	-	-		
7367r	5.0	9	6	3	1	<1	-	-	-	-		
7560r	5.0	5	6	2	<1	<1	<1	-	-	-		
7561r	5.0	15	6	2	<1	<1	<1	-	-	-		
7302rr	5.0	6	7	6	6	<1	<1	-	-	-	-	
7339rr	5.0	5	3	<1	<1	<1	-	-	-	-	-	
7558rr	5.0	28	111	112	60	90	40	12	2	<1	<1	-
7559rr	5.0	22	60	80	60	8	2	<1	<1	-	-	
7232	10.0	90	40	6	<1	-	-	-	-	-	-	
7238	10.0	85	52	32	12	<1	<1	-	-	-	-	
7393	10.0	30	56	10	6	<1	<1	-	-	-	-	-
7394	10.0	21	12	3	<1	<1	-	-	-	-	-	-
7567r	10.0	2	1	<1	<1	<1	-	-	-	-	-	-
7560rr	10.0	60	203	85	84	42	10	2			-	
7239	40.0	72	22	8	4	<1	-	-	-	-	-	
7240	40.0	104	18	1	<1	-	-	-	-	-	-	
7252	-	120	300	770	1260	1020	1630	1060	1580	3350	5400	Dead
7381	-	11	74	150	470	1090	1590	3740	2370	4440	Dead	
7581	-	27	306	360	1230	852	1700	1230	1470	1500	1700	3380

TABLE 4

THE ACTIVITY OF WR-180,409-AB ADMINISTERED ORALLY TO OWL MONKEYS INFECTED WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
		None	Suppressed	Cleared			
7302	1.25		+		n.a.	n.a.	
7303	1.25		+		n.a.	n.a.	
7304	1.25			+	20	n.a.	Cured
7339	1.25		+		n.a.	n.a.	Dose increased
7558	1.25	+			n.a.	n.a.	Dose increased
7559	1.25	+			n.a.	n.a.	Dose increased
7228	2.5	+			n.a.	n.a.	Dose increased
7229	2.5			+	8	56	
7305	2.5		+		n.a.	n.a.	
7306	2.5			+	8	22	
7307	2.5			+	16	43	
7365	2.5			+	7	14	
7367	2.5			+	6	17	
7560	2.5			+	9	10	
7561	2.5			+	10	10	
7302r	2.5			+	6	26	
7339r	2.5			+	7	50	
7558r	2.5			+	6	21	
7559r	2.5			+	11	34	

TABLE 4 - CONTINUED

Atr No.	Daily Dose Mg/Kg Body Weight	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
		None	Suppressed	Cleared			
7308	5.0			+	7	n. a.	Cured
7309	5.0			+	7	n. a.	Cured
7310	5.0			+	7	n. a.	Cured
7374	5.0			+	5	n. a.	Cured
7377	5.0			+	6	n. a.	Cured
7567	5.0		+		n. a.	n. a.	
7568	5.0			+	9	n. a.	Cured
7228r	5.0			+	6	n. a.	Cured
7229r	5.0			+	5	n. a.	Cured
7303r	5.0			+	7	n. a.	Cured
7305r	5.0			+	7	n. a.	Cured
7306r	5.0			+	10	n. a.	Cured
7307r	5.0			+	5	n. a.	Cured
7365r	5.0			+	3	n. a.	Cured
7367r	5.0			+	5	n. a.	Cured
7560r	5.0			+	6	25	
7561r	5.0			+	6	n. a.	Cured
7302rr	5.0			+	6	n. a.	Cured
7339rr	5.0			+	5	n. a.	Cured
7558rr	5.0			+	10	n. a.	Cured
7559rr	5.0			+	8	n. a.	Cured
7232	10.0			+	4	n. a.	Cured
7238	10.0			+	6	n. a.	Cured
7393	10.0			+	6	n. a.	Cured
7394	10.0			+	5	n. a.	Cured
7567r	10.0			+	5	n. a.	Cured
7560rr	10.0			+	10	n. a.	Cured
7239	40.0			+	5	n. a.	Cured
7240	40.0			+	4	n. a.	Cured

B. WR-172,435-AH (BN: BG-00,530): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THE OWL MONKEY (*AOTUS TRIVIRGATUS*)

SORI-KM-76-253

SUMMARY OF STUDIES CARRIED OUT UNDER
CONTRACT NO. DADA 17-69-C-9104

ON

WR-172,435-AH (BN: BG-00,530): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THE OWL MONKEY (*AOTUS TRIVIRGATUS*)

Southern Research Institute
2000-Ninth Avenue South
Birmingham, Alabama 35205
May 28, 1976

Project 2284-XXVI

WR-172,435-AH (BN: BG-00,530): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX IN THE OWL MONKEY (AOTUS TRIVIRGATUS)

INTRODUCTORY COMMENT

The 4-pyridinemethanols are one of the more promising classes of compounds developed in the Malaria Chemotherapy Program of the Department of the Army. Numerous representatives of this class exhibited curative activity at seemingly well-tolerated doses in mice infected with Plasmodium berghei. Nine derivatives, selected for high activity and novelty of structure, have undergone preliminary assessments of activity in owl monkeys infected with Plasmodium falciparum. Two of these agents, designated WR-172,435 and WR-180,409, were clearly more active than any of the other seven against established infections with the chloroquine-resistant Vietnam Oak Knoll strain and the pyrimethamine-resistant Malayan Camp-CH/Q strain. In complementary studies, both derivatives were highly active against infections with the pyrimethamine-resistant, chloroquine-resistant Vietnam Smith strain. The distinguishing structural features of WR-172,435 and WR-180,409 are in the substituents at position 6 of the pyridine ring and at the 4-methanol group of the side chain (cf Figure 1).

In preliminary assessments, WR-180,409 appeared to be slightly more active than WR-172,435 - by a factor of not more than two. WR-180,409 also appeared to be better tolerated than WR-172,435 and thus had a better therapeutic index. These attributes led to selection of WR-180,409 as a candidate for study in human volunteers and to the preclinical evaluation required for such study. The results of the experimental components of these preparatory studies were set forth in a special report dated July 1, 1975.

Because of its activity and "desirable" structural features, interest in WR-172,435 was sustained. This interest received an important stimulus when critical examination of the toxicity components of the original studies on this pyridine-methanol led to the conclusion that the deaths encountered in these appraisals were probably due to incompletely defined animal health problems rather than being drug induced. This conclusion, supported by the results of a very preliminary toxicity study carried out on non-infected owl monkeys, led to plans for evaluating the activity of WR-172,435 in human volunteers.

Prior to implementing these plans, attention was directed to procurement of a salt of WR-172,435 possessing greater water solubility and longer shelf life than the dihydrochloride monohydrate employed in the preliminary assessments*. This effort resulted in synthesis of the methanesulfonic acid salt and preparation of a batch lot of this derivative (designated WR-172,435-AH; BN: BG-00,530) for use in the projected clinical trials as well as in the preclinical studies required to clear a new agent for use in human volunteers.

Two major studies were carried out in this laboratory as part of the requisite preclinical evaluation. The first, pursued in owl monkeys infected with the Palo Alto strain of P. vivax, was concerned with the comparative activities of WR-172,435-AH (methanesulfonate) and WR-172,435-AC (dihydrochloride). The second, pursued in infections with the above strain of P. vivax and the Smith strain of P. falciparum, was concerned with the influence of the dosage regimen on the therapeutic activity of WR-172,435-AH. These studies were preceded by a very abbreviated comparison of the tolerability of WR-172,435-AC and WR-172,435-AH in the owl monkey. The technical features of these experiments and their results are summarized in the following sections of this Report.

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*The preparation of this salt used in the initial studies in owl monkeys infected with P. falciparum was designated WR-172,435-AC; BN: AY-98,670.

METHODS AND PROCEDURES

The data presented in this Report were derived from the following experiments, initiated on the dates indicated:

1. A preliminary comparison of the tolerance of WR-172,435-AC and WR-172,435-AH.

   Experiment of June 24, 1975, involving 14 owl monkeys.

2. A comparison of the activities of WR-172,435-AC and WR-172,435-AH against established infections with the Vietnam Palo Alto strain of P. vivax.

   Experiment of July 11, 1975, involving 38 owl monkeys.

3. Extended assessments of the activity of WR-172,435-AH against established infections with the Vietnam Palo Alto strain of P. vivax, with emphasis on the effectiveness of various dosage regimens.

   a. Experiment of July 22, 1975, involving 13 owl monkeys.

   b. Experiment of September 12, 1975, involving 13 owl monkeys.

4. Assessment of the activity of WR-172,435-AH against established infections with the Vietnam Smith strain of P. falciparum, with emphasis on the effectiveness of various dosage regimens.

   a. Experiment of August 1, 1975, involving 19 owl monkeys.

   b. Experiment of July 22, 1975, involving four owl monkeys whose infections were not controlled by treatment with WR-199,426.

The comparison of the toxicities of the original (AC) and IND (AH) lots of WR-172,435 was limited in scope to effects on body weight and survival. The owl monkeys committed to this evaluation had exhibited weight stability and satisfied all other requirements of superior health for 90 to 120 days after reaching "discard" status (cured after serial commitment to therapy of infections with P. falciparum and P. vivax). Pairs of these animals (one male and one female) were assigned to dosage with 20.0, 40.0, or 80.0 mg WR-172,435-AC or WR-172,435-AH per kg body weight, administered once daily (8:00 a.m.) for seven consecutive days. A stock suspension containing 10.0 mg drug base per ml was prepared fresh daily. The requisite volume of this suspension, diluted to 10.0 ml with distilled water, was administered via stomach tube, followed by a 5.0 ml aqueous rinse. Body weights were obtained daily (8:00 a.m.) during the period of drug dosage and thereafter on alternate days for nine weighings (18 days).

The following procedures were common to each of the therapeutic experiments. Infections were induced by intravenous inoculation of 5×10^6 erythrocytic parasites derived from monkeys of the passage lines of either the Smith strain of P. falciparum or the Palo Alto strain of P. vivax*. Measurements of parasitemias on thick and thin blood films stained with Giemsa were initiated three days after inoculation, at which time thick blood films were invariably positive. Thick and thin blood films were prepared daily thereafter until densities

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*The passage line of the Vietnam Smith strain of P. falciparum is maintained by serial transfer of parasitized erythrocytes through normal untreated owl monkeys every seven to ten days. The passage line of the Vietnam Palo Alto strain of P. vivax is maintained by serial transfer every twenty-one to twenty-eight days. Inocula for the chemotherapeutic studies are obtained by appropriate dilution in iced saline of heparinized blood drawn from a passage monkey. Dilutions for this strain of P. falciparum varied from 1:100 to 1:200; dilutions for the strain of P. vivax varied from 1:10 to 1:20.

of 10 to 50 parasites per 10^4 erythrocytes* were attained. At this time, treatment with WR-172,435 was initiated via the oral route. The requisite dose of the appropriate lot (always calculated as base equivalent**), dissolved in 10 ml of distilled water, was delivered by stomach tube, followed by a 5 ml rinse. WR-172,435 was always administered within one hour of solution preparation.

The effects of treatment on parasitemia were assessed on thick and thin blood films stained with Giemsa. Such films were prepared just prior to drug delivery during the treatment period and daily thereafter until thick films were parasite negative for at least four consecutive days. Film preparation and study were then reduced to a twice-weekly level (Monday and Thursday or Tuesday and Friday) for two consecutive weeks, and if examinations were uniformly negative during this interval, to a once-weekly level for ten additional weeks. Infections were considered cured if blood films were negative during the entire post-treatment period.

If parasitemias persisted at the initial or even lower levels, or increased in intensity during delivery of WR-172,435, or if there was a reappearance of parasites after an apparent blood negative interval, a second drug course was delivered, either at a higher total dose via the same regimen or at the same total dose via a different regimen. Whenever an infection was retreated, either early or late, an r was added to the Atr number. Thus the number of r's following a monkey number indicates the number of retreatment courses the animal has received. This serial treatment procedure makes it possible to expand the information on drug activity that can be obtained via use of a single monkey and will often signal emergence of drug-resistant plasmodia and the rapidity with which this undesirable event appears.

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*Such densities are equivalent to parasite populations of 5,000 to 25,000 per cmm of blood.

**The conversion factors, salt to base, are 1.16 and 1.2, respectively, for WR-172,435-AC and WR-172,435-AH.

In all of the studies, attention was directed to the impacts of the drug on the morphology of the parasite and the time when morphologic changes occurred. This focus provided a gauge of the rapidity with which WR-172,435 affected parasite growth and the type of activity which this agent possesses.

Every experiment included at least one untreated control monkey, serving among other purposes to check on the virulence of the parasites in the inoculum.

The owl monkeys used in these experiments (Aotus trivirgatus grisiemembra) had been imported directly from areas of Colombia adjacent to Barranquilla. These subjects had been vaccinated against Herpes tamarinus and Herpes simplex and conditioned for a minimum period of 60 days via procedures detailed elsewhere (Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:446-74, 1973). Only previously uninfected monkeys were used in assessing the activity of WR-172,435-AH against infections with the Vietnam Smith strain of P. falciparum. Monkeys previously infected with this or other strains of P. falciparum and cured via application of appropriate drugs other than WR-172,435, were utilized in assessing the activities of the two preparations of this pyridinemethanol against infections with the Vietnam Palo Alto strain of P. vivax*,**.

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*The responses of infections with the two test strains to currently available antimalarial drugs have been detailed elsewhere (Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:446-74, 1973). In brief, infections with the Vietnam Smith strain are fully resistant to treatment with the maximum tolerated doses of chloroquine, quinine, and pyrimethamine. Infections with the Vietnam Palo Alto strain are susceptible to treatment with chloroquine or quinine, but are resistant to treatment with pyrimethamine or proguanil.

**The owl monkey closely resembles man in that previous or current infection with P. falciparum does not alter host susceptibility to infection with P. vivax or vice versa. This makes it possible to utilize an animal for evaluating the activity of a drug against infections with P. falciparum and when cure of such infection is assured, to assign the same monkey to assessment of the activity of a different drug against infection with P. vivax. Such multiple use, a routine in our chemotherapeutic program, reduces operational costs and conserves numbers of monkeys that must be imported for these therapeutic assessments.

RESULTS

1. The Comparative Toxicities Of WR-172,435-AC And WR-172,435-AH

The summary in Table 1 shows that as measured by alterations in body weight there was very little toxicity associated with administration of either the original (AC) or IND (AH) preparations of WR-172,435 at daily doses equivalent to 20.0, 40.0, or 80.0 mg base per kg body weight. A single recipient of WR-172,435-AC at a dose of 80.0 mg per kg, Atr 7873, exhibited a small but significant weight loss (ca nine per cent of base weight), but recovered to pre-treatment weight status within six days after receiving the last dose of drug. This transitory weight change was the sole untoward reaction associated with delivery of seven daily doses of WR-172,435-AC or WR-172,435-AH in amounts up to 80.0 mg per kg body weight.

Although this study failed to provide data on the comparative toxicities of the AC and AH lots of WR-172,435*, its essentially negative features were important. The acceptability of WR-172,435-AC in the current investigation contrasted sharply with the progressive cachexia encountered when lesser doses of the same lot were employed in the pilot assessments of antimalarial activity. This acceptability provides important support for the earlier reassessment which led to the tentative conclusion that the fatalities that occurred in the 1972-1973 pilot experiments (which delayed continued study of WR-172,435-AC) were due to colony health problems rather than to drug toxicity.

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*Limitations in supply of WR-172,435-AC and stocks of discarded owl monkeys precluded repetition or expansion of the toxicity assessments utilizing larger drug doses.

2. A Comparison Of The Activities Of WR-172,435-AC And WR-172,435-AH Against Established Infections With The Palo Alto Strain Of *Plasmodium vivax*

Before summarizing the results of this study, it might be useful to draw attention to the general pattern of data presentation followed in this and subsequent sections of the Report. In each case the responses of infections in individual monkeys have been detailed in two tables. The first table in each pair (cf Tables 2, 4, and 6) sets forth the immediate effects of drug delivery on parasite densities; the second (cf Tables 3, 5, and 7) focuses on end results. The data in the odd numbered tables are of primary concern in the analysis that follows.

As shown in Table 3, cure of infections with the Palo Alto strain of *P. vivax* was achieved with delivery of either WR-172,435-AC or WR-172,435-AH at a total dose of 18.75 mg base equivalent per kg body weight. At this dosage level, a single dose was as effective as three or seven fractional doses administered on as many consecutive days.

Since 18.75 mg per kg was the least dose used in the study, and since this dose was uniformly curative, this experiment, like the preceding toxicologic evaluation, failed in its primary mission. A supplemental experiment with a downward titration of the doses of WR-172,435-AC and WR-172,435-AH was planned, but because of the shortage of owl monkeys, only the arm concerned with the activity of the IND preparation could be implemented. The results of this exploration are dealt with in the section that follows.

3. Extended Evaluation Of The Activity Of WR-172,435-AH (IND Preparation) Against Established Infections With The Palo Alto Strain Of Plasmodium vivax

As indicated in Table 5, this assessment was concerned with the activities of total doses of ca 1.1, 2.2, 4.4, and 8.75 mg base equivalent per kg body weight. The lowest of these doses had little, if any, effect on parasitemia. Parasite clearance did occur in some of the recipients of doses of 2.2 mg per kg, but in no subject was the infection cured. There was a high proportion of cures among monkeys receiving a total dose of 4.38 mg per kg. A dose of 8.75 mg per kg was uniformly curative.

One of the missions of this study, as well as the experiment that preceded it, was to determine the influence of the dosage regimen on the activity of the IND preparation of WR-172,435. The data acquired in the current study are quite relevant to this issue. Taken together, they show that at total dose levels of 2.2, 4.38, or 8.75 mg per kg, WR-172,435-AH was more effective when administered in a single dose than when delivered in seven fractions on consecutive days. The yardsticks used to identify the efficiencies of the dosage regimens varied with the dosage level. At 2.2 mg per kg, the differences were related to capacity to clear parasitemia. Here, parasite clearance occurred in three of four recipients of single doses, contrasted with no clearances in four recipients of seven fractional doses. At the 4.38 mg per kg dosage, the differences were related to curative activity. Cures were achieved in five of six recipients of a single dose as compared with two of six recipients of the fractional dose regimen. At the 8.75 mg per kg level, uniformly curative in all regimens, the differences were in speed of control of parasitemia. Such was achieved regularly in four days in recipients of single doses, as contrasted with five and six days in four of the six recipients of divided doses. The effectiveness of the three dose regimen fell between that of the single and the seven dose schedules.

4. The Activity Of WR-172,435-AH (IND Preparation) Against Established Infections With The Smith Strain Of *Plasmodium falciparum*

As shown in Table 7, a high proportion of infections with the Smith strain were cured by a total dose of 17.5 mg WR-172,435-AH (base equivalent) per kg body weight. A total dose of 35.0 mg per kg was uniformly curative. Cure of previously untreated infections could not be obtained with doses less than 17.5 mg per kg. Comparison of these results with those set forth in the preceding section lead to the conclusion that WR-172,435-AH is considerably more effective against infections with the Palo Alto strain of *P. vivax* than against infections with the Smith strain of *P. falciparum*.

Evaluation of the influence of the dosage regimen was one of the missions of this experiment. The data in Table 7 indicate that at a total dosage of 17.5 or 35.0 mg per kg, the curative activities of the three and seven divided dose schedules were the same. As measured by the response to the 17.5 mg per kg dosage, the single dose regimen seems to be slightly less effective than the divided schedules. However, if there is any regimen difference, it is relatively small.

COMMENTS

The essential features of the evaluations of the activities of WR-172,435-AH against established infections with the Smith strain of *P. falciparum* and Palo Alto strain of *P. vivax* (derived from the detailed data in Tables 3, 5, and 7) have been summarized in Table 8. This summary simplifies identification of the various aspects of the activity of this IND preparation, especially those concerned with the influence of the dosage regimen. It also provides readily accessible evidence relative to the intramural consistency of various therapeutic evaluations.

For reasons at present not remediable, it was not possible to make the side-by-side evaluations required to determine whether the antimalarial activities and toxicity of the IND preparation were identical with those of the original (AC) preparation. All that can be stated here with respect to toxicity is that both preparations appeared to be well-tolerated by the owl monkey at daily doses of 80.0 mg base per kg body weight up to a total dose of 560.0 mg per kg. Studies pursued elsewhere on other experimental animals either have quantified or will quantify the limit of tolerability of WR-172,435-AH and the quality of untoward reactions.

The most that can be done to fill the deficit in comparing the antimalarial properties of the two preparations is to refer to the results of pilot assessments of the activities of the original preparation, WR-172,435-AC, carried out in 1972-73. These results (detailed in Appendix Tables 1-6) have been summarized in Table 9. Included in this table are the summary data acquired in the current study on the activity of the AC preparation against infections with the Palo Alto strain of P. vivax. These data place the dose of WR-172,435-AC required to cure infections with either the Smith, Oak Knoll, or Malayan Camp-CH/Q strain of P. falciparum at 5.0 mg base per kg body weight daily or a total of 35.0 mg per kg. This dose is essentially identical (perhaps slightly greater) with the dose of the IND preparation required to cure infections with the Smith strain of P. falciparum (cf data on seven day regimens, Table 8). Considering the time interval between pursuit of the studies on the original and IND preparations, these small differences should probably be discounted. It therefore seems reasonable to conclude that the antimalarial activities of the two lots are essentially the same.

As with all studies, careful attention was given to signs of emergence of resistance to WR-172,435-AH. The most reliable signal of this event is failure to achieve cure of retreatment cases with doses of drug that invariably cure in the original treatment setting. Such a signal was uniformly absent in both the pilot and current assessments, suggesting that evolution of resistance to WR-172,435 will not be a serious problem.

In concluding this Report, brief comment should be made of the relative merits of WR-172,435 and WR-180,409, the first pyridinemethanol derivative selected as a candidate for preclinical and subsequent clinical study. Comparison of the data on WR-172,435-AH summarized in this Report with the data on WR-180,409-AC summarized in the July 1, 1975 Report indicates that the two agents are essentially equally active against infections with the Smith strain of P. falciparum, but that WR-172,435 is approximately twice as active as WR-180,409 against infections with the Palo Alto strain of P. vivax. This would suggest that but for one attribute, choice between use of these agents would have to rest on such issues as tolerability, cost, and shelf life rather than antimalarial potency. WR-180,409 does have the advantage of greater water solubility which makes it useful for parenteral administration at times when it is necessary to employ this route of delivery.

SUMMARY AND CONCLUSIONS

Studies designed to evaluate the batch preparation of WR-172,435-AH (BN: BG-00,530) synthesized for preclinical pharmacologic studies and subsequent evaluation in human volunteers have shown that:

1. WR-172,435-AH evokes no symptoms of toxicity when administered to the owl monkey in daily doses of 80.0 mg base per kg body weight to a total dose of 560.0 mg per kg.

2. WR-172,435-AH regularly cures established infections with the pyrimethamine-, chloroquine-, quinine-resistant Smith strain of P. falciparum at a total dose between 17.5 and 35.0 mg base per kg body weight. A single dose at the upper of these levels is as effective as the same amount delivered in three or seven fractional daily doses.

3. WR-172,435-AH regularly cures infections with the pyrimethamine-resistant Palo Alto strain of P. vivax at a total dose of 8.75 mg base per kg body weight. Against infections with this plasmodium this pyridine-methanol derivative appears to be regularly more active when delivered in a single dose than in seven fractional daily doses.

4. Restrictions on supplies of owl monkeys and the original preparation of WR-172,435 (Lot AC) precluded side-by-side comparison of the activity and toxicity of this lot and the IND preparation. However, comparison of the results obtained in original pilot assessments pursued with WR-172,435-AC with those obtained in the current investigation with WR-172,435-AH indicates that the antimalarial activities of these preparations are very similar, if not identical.

The studies summarized in this Report were designed, supervised, and evaluated by the undersigned.

L. H. Schmidt, Ph. D.
Principal Investigator, DADA-17-69-C-9104

ACKNOWLEDGEMENT: We are indebted to Ruth Crosby and Howard Washington for execution of the above described experiments and to Lee Vogel for secretarial assistance in preparing this Report.

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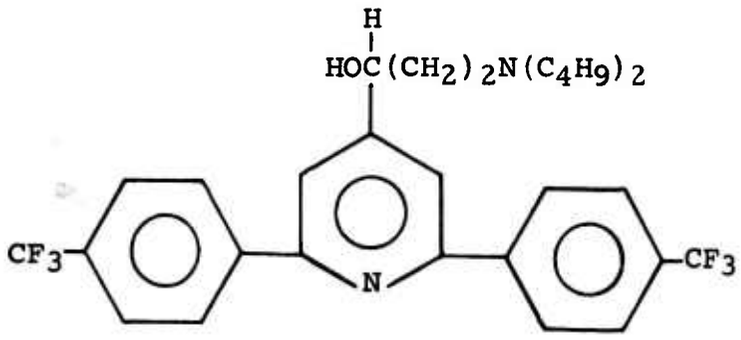
Project 2284-XXVI

FIGURE 1

STRUCTURES OF WR-172,435 AND WR-180,409



WR-172,435

α-(di-N-butylaminoethyl)-2,6-di-(4-trifluoromethylphenyl)-4-pyridinemethanol

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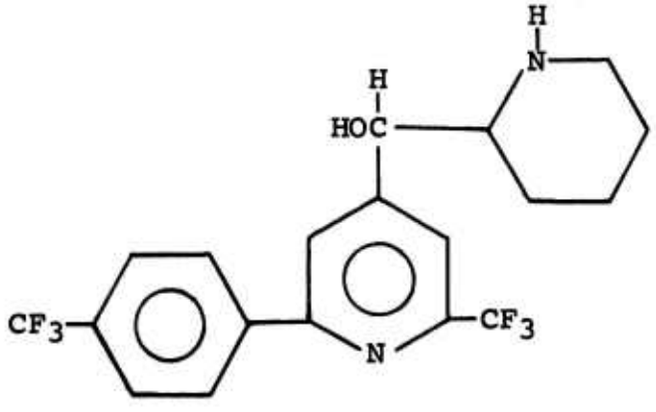

WR-180,409

α-2'-piperidyl-2-(4-trifluoromethylphenyl)-6-trifluoromethyl-4-pyridinemethanol

TABLE 1

A COMPARISON OF THE CAPACITIES OF WR-172,435-AC AND WR-172,435-AH TO MODIFY THE WEIGHT OF THE NON-INFECTED OWL MONKEY

(Pilot Evaluation)

WR- No. of Preparation	Daily Dose* Mg Base/Kg Body Weight	Atr No.	Body Weight - Kg				
			Day 1 of R_x	Day after End of R_x			
				1	6	12	18
172,435-AC	20.0	7768	0.94	0.93	0.94	0.94	0.93
	20.0	7778	0.84	0.83	0.82	0.81	0.83
	40.0	7801	1.05	1.04	1.05	1.05	1.05
	40.0	7806	0.92	0.90	0.94	0.94	0.96
	80.0	7817	0.96	0.93	0.95	0.93	0.93
	80.0	7873	1.03	0.94	1.01	0.99	1.00
172,435-AH	20.0	7870	0.95	0.95	0.93	0.93	0.93
	20.0	7896	1.09	1.05	1.07	1.07	1.06
	40.0	7898	0.98	0.96	0.97	0.96	0.95
	40.0	7922	1.09	1.10	1.14	1.14	1.14
	80.0	7986	1.10	1.06	1.10	1.11	1.10
	80.0	7987	1.03	1.02	1.04	1.02	1.02
None	-	7791	0.93	0.95	0.96	0.95	0.96
	-	7792	0.90	0.90	0.90	0.89	0.89

*Administered once daily for seven consecutive days.

TABLE 2

SIDE-BY-SIDE COMPARISON OF THE ACTIVITIES OF WR-172,435-AC AND WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
WR-172,435-AC														
6461	17.5	1	17.5	10	4							<1	<1	-
7756	17.5	1	17.5	6	4							<1	<1	-
7932	6.0	3	18.0	21	2	<1	<1					-	-	-
7959	6.0	3	18.0	7	8	<1	<1					-	-	-
8016	2.5	7	17.5	11	8	1	<1	-	-	-	-	-	-	
8021	2.5	7	17.5	12	4	<1	<1	-	-	-	-	-	-	
7860	35.0	1	35.0	8	2							<1	-	-
7884	35.0	1	35.0	11	5							<1	<1	-
7967	12.0	3	36.0	3	1	<1	<1					-	-	-
7973	12.0	3	36.0	10	2	<1	<1					-	-	-
8030	5.0	7	35.0	16	2	<1	<1	-	-	-	-	-	-	
8037	5.0	7	35.0	3	6	<1	<1	-	-	-	-	-	-	
7887	70.0	1	70.0	5	3							<1	-	-
7930	70.0	1	70.0	10	6							<1	-	-
7995	24.0	3	72.0	4	5	<1	<1					-	-	-
7999	24.0	3	72.0	6	6	<1	<1					-	-	-
8046	10.0	7	70.0	16	8	<1	<1	-	-	-	-	-	-	
8049	10.0	7	70.0	11	4	<1	-	-	-	-	-	-	-	

TABLE 2 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
					WR-172,435-AH									
7359	17.5	1	17.5	8	1							<1	<1	-
7759	17.5	1	17.5	10	4							<1	<1	-
7933	6.0	3	18.0	9	14	<1	-					-	-	-
7964	6.0	3	18.0	1	3	<1	-					-	-	-
8017	2.5	7	17.5	6	18	1	<1	-	-	-	-	-	-	
8028	2.5	7	17.5	6	8	<1	<1	-	-	-	-	-	-	
7861	35.0	1	35.0	16	4							<1	<1	-
7886	35.0	1	35.0	2	2							<1	<1	-
7970	12.0	3	36.0	1	<1	<1	-					-	-	-
7974	12.0	3	36.0	2	1	<1	-					-	-	-
8033	5.0	7	35.0	18	9	<1	<1	-	-	-	-	-	-	
8045	5.0	7	35.0	16	6	<1	-	-	-	-	-	-	-	
7929	70.0	1	70.0	10	6							<1	-	-
7931	70.0	1	70.0	10	2							<1	-	-
7998	24.0	3	72.0	14	8	<1	-					-	-	-
8000	24.0	3	72.0	16	6	<1	-					-	-	-
8048	10.0	7	70.0	6	6	<1	-	-	-	-	-	-	-	
8051	10.0	7	70.0	8	5	<1	-	-	-	-	-	-	-	
8052	-	-	-	18	48	80	144	301	240	310	330	130	230	170
8057	-	-	-	14	18	16	39	32	10	14	16	8	10	16

TABLE 3

SIDE-BY-SIDE COMPARISON OF THE ACTIVITIES OF WR-172,435-AC AND WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
WR-172,435-AC									
6461	17.5	1	17.5			+	4	n.a.	Cured
7756	17.5	1	17.5			+	4	n.a.	Cured
7932	6.0	3	18.0			+	4	n.a.	Cured
7959	6.0	3	18.0			+	4	n.a.	Cured
8016	2.5	7	17.5			+	4	n.a.	Cured
8021	2.5	7	17.5			+	4	n.a.	Cured
7860	35.0	1	35.0			+	3	n.a.	Cured
7884	35.0	1	35.0			+	4	n.a.	Cured
7967	12.0	3	36.0			+	4	n.a.	Cured
7973	12.0	3	36.0			+	4	n.a.	Cured
8030	5.0	7	35.0			+	4	n.a.	Cured
8037	5.0	7	35.0			+	4	n.a.	Cured
7887	70.0	1	70.0			+	3	n.a.	Cured
7930	70.0	1	70.0			+	3	n.a.	Cured
7995	24.0	3	72.0			+	4	n.a.	Cured
7999	24.0	3	72.0			+	4	n.a.	Cured
8046	10.0	7	70.0			+	4	n.a.	Cured
8049	10.0	7	70.0			+	3	n.a.	Cured

TABLE 3 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
					WR-172,435-AH				
7359	17.5	1	17.5			+	4	n.a.	Cured
7759	17.5	1	17.5			+	4	n.a.	Cured
7933	6.0	3	18.0			+	3	n.a.	Cured
7964	6.0	3	18.0			+	3	n.a.	Cured
8017	2.5	7	17.5			+	4	n.a.	Cured
8028	2.5	7	17.5			+	4	n.a.	Cured
7861	35.0	1	35.0			+	4	n.a.	Cured
7886	35.0	1	35.0			+	4	n.a.	Cured
7970	12.0	3	36.0			+	3	n.a.	Cured
7974	12.0	3	36.0			+	3	n.a.	Cured
8033	5.0	7	35.0			+	4	n.a.	Cured
8045	5.0	7	35.0			+	3	n.a.	Cured
7929	70.0	1	70.0			+	3	n.a.	Cured
7931	70.0	1	70.0			+	3	n.a.	Cured
7998	24.0	3	72.0			+	3	n.a.	Cured
8000	24.0	3	72.0			+	3	n.a.	Cured
8048	10.0	7	70.0			+	3	n.a.	Cured
8051	10.0	7	70.0			+	3	n.a.	Cured

TABLE 4

EXTENDED EVALUATION OF THE ACTIVITY OF WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment						Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
6750	1.1	1	1.1	3	<1							1	4	12
7746	1.1	1	1.1	17	34							18	57	27
7906	0.375	3	1.13	16	12	7	2					9	3	7
7991	0.375	3	1.13	32	28	12	2					<1	<1	<1
8036	0.156	7	1.09	20	9	32	81	90	100	18	2	4	18	15
8039	0.156	7	1.09	18	26	62	129	57	20	21	100	78	44	51
7838	2.2	1	2.2	22	4							<1	<1	-
7875	2.2	1	2.2	4	3							<1	<1	-
6750r	2.2	1	2.2	16	18							10	25	30
7746r	2.2	1	2.2	21	7							1	<1	<1
8018	0.75	3	2.25	4	5	2	2					<1	-	-
8034	0.75	3	2.25	10	16	9	18					21	24	21
7906r	0.75	3	2.25	3	9	2	<1					<1	-	-
8047	0.313	7	2.19	6	3	<1	2	<1	<1	<1	<1	<1	<1	<1
8053	0.313	7	2.19	2	3	24	42	24	14	2	<1	<1	<1	<1
8036r	0.313	7	2.19	33	21	27	15	21	30	18	18	18		
8039r	0.313	7	2.19	51	24	72	21	24	18	18	12	14	20	14

TABLE 4 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment						Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
7744	4.38	1	4.38	10	1							<1	<1	-
7925	4.38	1	4.38	12	4							<1	<1	-
7838r	4.38	1	4.38	6	2							<1	<1	-
7875r	4.38	1	4.38	2	2							<1	<1	<1
6750rr	4.38	1	4.38	30	20							4	<1	-
7746rr	4.38	1	4.38	14	12							1	<1	<1
7971	1.5	3	4.5	32	33	8	<1					-	-	-
7990	1.5	3	4.5	6	18	2	<1					-	-	-
7991r	1.5	3	4.5	2	12	12	6					2	<1	-
8018r	1.5	3	4.5	1	3	<1	-					-	-	
8034r	1.5	3	4.5	2	3	<1	-					-	-	
7906rr	1.5	3	4.5	6	1	<1	<1					-	-	-
8040	0.625	7	4.38	18	30	28	14	2	<1	<1	<1	-	-	-
8042	0.625	7	4.38	27	8	20	3	<1	<1	-	-	-	-	-
8047r	0.625	7	4.38	1	2	<1	2	<1	<1	<1	<1	<1	<1	<1
8053r	0.625	7	4.38	10	9	3	<1	<1	-	-	-	-	-	-
8036rr	0.625	7	4.38	18	20	11	14	8	6	2	<1	<1	<1	<1
8039rr	0.625	7	4.38	7	8	12	2	<1	<1	<1	<1	-	-	-

TABLE 4 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose Mg/Kg	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.			1	2	3	4	5	6	7	1	2	3
7927	8.75	1	8.75	6	1							<1	<1	-
7956	8.75	1	8.75	2	2							<1	<1	-
7925r	8.75	1	8.75	6	12							<1	<1	-
8015	3.0	3	9.0	30	8	3	<1					-	-	-
8029	3.0	3	9.0	12	2	<1	<1					-	-	-
7991rr	3.0	3	9.0	5	<1	<1	-					-	-	-
7906rrr	3.0	3	9.0	1	1	<1	<1					<1	-	-
8043	1.25	7	8.75	21	6	1	<1	<1	-	-	-	-	-	-
8054	1.25	7	8.75	36	39	15	4	<1	<1	-	-	-	-	-
8042r	1.25	7	8.75	8	6	<1	-	-	-	-	-			
8047rr	1.25	7	8.75	5	1	<1	<1	<1	<1	-	-	-	-	-
8036rrr	1.25	7	8.75	2	<1	<1	<1	<1	-	-	-	-	-	-
8039rrr	1.25	7	8.75	4	3	<1	<1	-	-	-	-	-	-	-
8056	-	-	-	39	96	64	72	26	12	16	18	28	6	6

TABLE 5

EXTENDED EVALUATION OF THE ACTIVITY OF WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_X			Days from Initial R_X to Parasite Clearance	Days from Final R_X to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
6750	1.1	1	1.1		+		n. a.	n. a.	
7746	1.1	1	1.1	+			n. a.	n. a.	
7906	0.375	3	1.13		+		n. a.	n. a.	
7991	0.375	3	1.13		+		n. a.	n. a.	
8036	0.156	7	1.09	+			n. a.	n. a.	
8039	0.156	7	1.09	+			n. a.	n. a.	
7838	2.2	1	2.2			+	4	11	
7875	2.2	1	2.2			+	4	24	
6750r	2.2	1	2.2	+			n. a.	n. a.	
7746r	2.2	1	2.2			+	5	18	
8018	0.75	3	2.25			+	5	11	
8034	0.75	3	2.25		+		n. a.	n. a.	
7906r	0.75	3	2.25			+	5	19	
8047	0.313	7	2.19		+		n. a.	n. a.	
8053	0.313	7	2.19		+		n. a.	n. a.	
8036r	0.313	7	2.19	+			n. a.	n. a.	
8039r	0.313	7	2.19		±		n. a.	n. a.	

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_X			Days from Initial R_X to Parasite Clearance	Days from Final R_X to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
7744	4.38	1	4.38			+	4	n. a.	Died*
7925	4.38	1	4.38			+	4	20	
7838r	4.38	1	4.38			+	4	n. a.	Cured
7875r	4.38	1	4.38			+	4	n. a.	Cured
6750rr	4.38	1	4.38			+	4	n. a.	Cured
7746rr	4.38	1	4.38			+	5	n. a.	Cured
7971	1.5	3	4.5			+	4	n. a.	Cured
7990	1.5	3	4.5			+	4	n. a.	Cured
7991r	1.5	3	4.5			+	6	11	
8018r	1.5	3	4.5			+	3	n. a.	Cured
8034r	1.5	3	4.5			+	3	n. a.	Cured
7906rr	1.5	3	4.5			+	4	23	
8040	0.625	7	4.38			+	8	n. a.	Cured
8042	0.625	7	4.38			+	6	23	
8047r	0.625	7	4.38		+		n. a.	n. a.	
8053r	0.625	7	4.38			+	5	n. a.	Cured
8036rr	0.625	7	4.38		+		n. a.	n. a.	
8039rr	0.625	7	4.38			+	8	25	

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
7927	8.75	1	8.75			+	4	n. a.	Cured
7956	8.75	1	8.75			+	4	n. a.	Cured
7925r	8.75	1	8.75			+	4	n. a.	Cured
8015	3.0	3	9.0			+	4	n. a.	Cured
8029	3.0	3	9.0			+	3	n. a.	Cured
7991rr	3.0	3	9.0			+	5	n. a.	Cured
7906rrr	3.0	3	9.0			+	5	n. a.	Cured
8043	1.25	7	8.75			+	6	n. a.	Cured
8054	1.25	7	8.75			+	3	n. a.	Cured
8042r	1.25	7	8.75			+	6	n. a.	Cured
8047rr	1.25	7	8.75			+	5	n. a.	Cured
8036rrr	1.25	7	8.75			+	4	n. a.	Cured
8039rrr	1.25	7	8.75						

*Died parasite negative on Day 57 Post-R_x.

TABLE 6

THE ACTIVITY OF WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8106r	4.38	1	4.38	35	9							4	<1	<1
8107r	4.38	1	4.38	20	12							2	<1	<1
8103rr	1.5	3	4.5	23	27	12	2					<1	<1	-
8106rr	1.5	3	4.5	2	<1	<1	-					-	-	-
8071	8.75	1	8.75	22	18							2	<1	-
8072	8.75	1	8.75	21	54							24	<1	-
8089rr	8.75	1	8.75	55	160							18	1	<1
8107rr	8.75	1	8.75	1	<1							<1	-	-
8083	3.0	3	9.0	4	18	<1	-					-	-	-
8086	3.0	3	9.0	24	177	40	4					<1	<1	-
8106rrr	3.0	3	9.0	12	4	<1	-					-	-	-
8107rrr	3.0	3	9.0	<1	<1	<1	-					-	-	-
8096	1.25	7	8.75	39	64	20	10	15	15	3	1	<1	<1	<1
8097	1.25	7	8.75	32	148	40	9	4	1	<1	<1	-	-	-

TABLE 6 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment						Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8073	17.5	1	17.5	32	15							6	1	<1
8079	17.5	1	17.5	6	26							<1	-	-
8071r	17.5	1	17.5	10	2							<1	<1	-
8072r	17.5	1	17.5	18	9							2	<1	<1
8089rrr	17.5	1	17.5	3	1							<1	<1	-
8087	6.0	3	18.0	10	60	12	<1					-	-	-
8092	6.0	3	18.0	12	27	8	<1					-	-	-
8083r	6.0	3	18.0	22	33	8	<1					-	-	
8086r	6.0	3	18.0	6	1	<1	-					-	-	-
8098	2.5	7	17.5	36	64	15	2	<1	-	-	-	-	-	-
8099	2.5	7	17.5	39	52	7	<1	<1	-	-	-	-	-	-
8096r	2.5	7	17.5	11	4	8	2	<1	<1	<1	-	-	-	-
8097r	2.5	7	17.5	14	3	<1	<1	-	-	-	-	-	-	-
8081	35.0	1	35.0	26	33							28	<1	<1
8082	35.0	1	35.0	15	87							24	<1	-
8073r	35.0	1	35.0	4	6							1	-	-
8079r	35.0	1	35.0	2	<1							<1	-	-
8093	12.0	3	36.0	8	6	<1	-					-	-	-
8095	12.0	3	36.0	24	126	21	3					<1	-	-
8100	5.0	7	35.0	21	87	20	1	<1	-	-	-	-	-	-
8101	5.0	7	35.0	24	90	12	<1	<1	-	-	-	-	-	-
8102	-	-	-	24	36	135	65	235	310	240	700	420	Shipped - WRAIR	
8108	-	-	-	33	60	150	896	1000	1400	1990	2630	2710	3960	Died

TABLE 7

THE ACTIVITY OF WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8106r	4.38	1	4.38			+	5	19	
8107r	4.38	1	4.38			+	5	12	
8103rr	1.5	3	4.5			+	6	n.a.	Cured
8106rr	1.5	3	4.5			+	3	13	
8071	8.75	1	8.75			+	4	23	
8072	8.75	1	8.75			+	4	16	
8089rr	8.75	1	8.75			+	5	64	
8107rr	8.75	1	8.75			+	3	17	
8083	3.0	3	9.0			+	3	14	
8086	3.0	3	9.0			+	6	15	
8106rrr	3.0	3	9.0			+	4	n.a.	Cured
8107rrr	3.0	3	9.0			+	3	n.a.	Cured
8096	1.25	7	8.75		+		n.a.	n.a.	
8097	1.25	7	8.75			+	8	18	

TABLE 7 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recrudescence	Remarks
	Daily Dose		Total Dose Mg/Kg	None	Suppressed	Cleared			
	Mg/Kg	No.							
8073	17.5	1	17.5			+	5	24	
8079	17.5	1	17.5			+	3	30	
8071r	17.5	1	17.5			+	4	n. a.	Cured
8072r	17.5	1	17.5			+	5	n. a.	Cured
8089rrr	17.5	1	17.5			+	4	n. a.	Cured
8087	6.0	3	18.0			+	4	n. a.	Cured
8092	6.0	3	18.0			+	4	n. a.	Cured
8083r	6.0	3	18.0			+	4	n. a.	Cured
8086r	6.0	3	18.0			+	3	n. a.	Cured
8098	2.5	7	17.5			+	5	n. a.	Cured
8099	2.5	7	17.5			+	5	n. a.	Cured
8096r	2.5	7	17.5			+	7	n. a.	Cured
8097r	2.5	7	17.5			+	4	n. a.	Cured
8081	35.0	1	35.0			+	6	n. a.	Cured
8082	35.0	1	35.0			+	4	n. a.	Cured
8073r	35.0	1	35.0			+	10	n. a.	Cured
8079r	35.0	1	35.0			+	3	n. a.	Cured
8093	12.0	3	36.0			+	3	n. a.	Cured
8095	12.0	3	36.0			+	5	n. a.	Cured
8100	5.0	7	35.0			+	5	n. a.	Cured
8101	5.0	7	35.0			+	5	n. a.	Cured

TABLE 8

SUMMARY: THE ACTIVITIES OF WR-172,435-AH AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM AND THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Dosage Regimen			No. of Infections Treated					Days from Initial Rx to Parasite Clearance*
Total Dose Mg/Kg	No. of Doses	Daily Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Smith Strain - P. falciparum								
4.38	1	4.38	2	0	0	2	0	5
4.5	3	1.5	2	0	0	2	1	5
8.75	1	8.75	4	0	0	4	0	4
9.0	3	3.0	4	0	0	4	2	4
8.75	[illegible]	1.25	2	0	1	1	0	n.a.
17.5	1	17.5	5	0	0	5	3	4
18.0	3	6.0	4	0	0	4	4	4
17.5	7	2.5	4	0	0	4	4	5
35.0	1	35.0	4	0	0	4	4	4
36.0	3	12.0	2	0	0	2	2	4
35.0	7	5.0	2	0	0	2	2	5

TABLE 8 - CONTINUED

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Total Dose Mg/Kg	No. of Doses	Daily Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Palo Alto Strain - P. vivax								
1.1	1	1.1	2	1	1	0	0	n. a.
1.13	3	0.375	2	0	2	0	0	n. a.
1.09	7	0.156	2	2	0	0	0	n. a.
2.2	1	2.2	4	1	0	3	0	4
2.25	3	0.75	3	0	1	2	0	5
2.19	7	0.313	4	1	3	0	0	n. a.
4.38	1	4.38	6	0	0	6	5	4
4.5	3	1.5	6	0	0	6	4	4
4.38	7	0.625	6	0	2	4	2	n. a.
8.75	1	8.75	3	0	0	3	3	4
9.0	3	3.0	4	0	0	4	4	4
8.75	7	1.25	6	0	0	6	6	5
17.5	1	17.5	2	0	0	2	2	4
18.0	3	6.0	2	0	0	2	2	3
17.5	7	2.5	2	0	0	2	2	4
35.0	1	35.0	2	0	0	2	2	4
36.0	3	12.0	2	0	0	2	2	3
35.0	7	5.0	2	0	0	2	2	4
70.0	1	70.0	2	0	0	2	2	3
72.0	3	24.0	2	0	0	2	2	3
70.0	7	10.0	2	0	0	2	2	3

*Median day to parasite clearance.

TABLE 9

SUMMARY: THE ACTIVITIES OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH, VIETNAM OAK KNOLL, AND MALAYAN CAMP-CH/Q STRAINS OF PLASMODIUM FALCIPARUM AND THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Total Dose Mg/Kg	No. of Doses	Daily Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Smith Strain - P. falciparum								
8.75	7	1.25	3	3	0	0	0	n.a.
35.0	7	5.0	6	0	0	6	5	9
70.0	7	10.0	1	0	0	1	1	6
140.0	7	20.0	1	0	0	1	1	9
Vietnam Oak Knoll Strain - P. falciparum								
8.75	7	1.25	2	2	0	0	0	n.a.
17.5	7	2.5	2	1	0	1	1	n.a.
35.0	7	5.0	5	0	0	5	5	7
70.0	7	10.0	3	0	0	3	3	8
140.0	7	20.0	3	0	0	3	3	5
Malayan Camp-CH/Q Strain - P. falciparum								
17.5	7	2.5	2	1	0	1	0	n.a.
35.0	7	5.0	2	0	0	2	2	11

TABLE 9 - CONTINUED

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Total Dose Mg/Kg	No. of Doses	Daily Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Palo Alto Strain - P. vivax								
17.5	1	17.5	2	0	0	2	2	4
18.0	3	6.0	2	0	0	2	2	4
17.5	7	2.5	2	0	0	2	2	4
35.0	1	35.0	2	0	0	2	2	4
36.0	3	12.0	2	0	0	2	2	4
35.0	7	5.0	2	0	0	2	2	4
70.0	1	70.0	2	0	0	2	2	3
72.0	3	24.0	2	0	0	2	2	4
70.0	7	10.0	2	0	0	2	2	4

*Median day to parasite clearance.

APPENDIX

TABLE 1-A

PILOT ASSESSMENT OF THE ACTIVITY OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF *PLASMODIUM FALCIPARUM*

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment						Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
6719	1.25	192	56	1550	1050	Dose increased						
6720	1.25	130	30	960	580	Dose increased						
6722	1.25	184	30	1240	470	Dose increased						
6728	5.0	142	44	312	70	47	8	2	<1	<1	-	-
6729	5.0	158	52	96	3	<1	1	<1	<1	-	-	-
6730	5.0	162	72	427	272	36	15	2	<1	<1	-	-
6719r	5.0	1050	3560	2540	660	66	14	4	2	<1	<1	-
6720r	5.0	580	2010	1330	840	310	58	4	3	<1	<1	-
6722r	5.0	470	2080	900	84	2	<1	-	-	-	-	
6730r	10.0	15	8	6	1	<1	<1	-	-	-	-	-
6731	20.0	176	104	45	28	24	9	3	1	<1	Died	
6742	20.0	112	98	70	24	12	6	3	Died			
6743	20.0	166	82	190	30	32	15	2	<1	<1	-	-

TABLE 2-A

PILOT ASSESSMENT OF THE ACTIVITY OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recru-descence	Remarks
		None	Suppressed	Cleared			
6719	1.25	+			n.a.	n.a.	Dose increased Day 4
6720	1.25	+			n.a.	n.a.	Dose increased Day 4
6722	1.25	+			n.a.	n.a.	Dose increased Day 4
6728	5.0			+	9	n.a.	Cured
6729	5.0			+	8	n.a.	Cured
6730	5.0			+	9	15	
6719r	5.0			+	10	n.a.	Cured
6720r	5.0			+	10	n.a.	Cured
6722r	5.0			+	6	n.a.	Cured
6730r	10.0			+	6	n.a.	Cured
6731	20.0		+		n.a.	n.a.	Died Day 3 Post R_x
6742	20.0		+		n.a.	n.a.	Died Day 1 Post R_x
6743	20.0			+	9	n.a.	Cured

TABLE 3-A

PILOT ASSESSMENT OF THE ACTIVITY OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment						Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
6675	1.25	31	52	540	Dose increased							
6676	1.25	40	50	420	Dose increased							
6293	2.5	260	230	50	2	4	<1	<1	<1	<1	<1	-
6294	2.5	134	166	1490	1750	Dose increased						
6677	5.0	26	82	146	28	6	3	<1	<1	-	-	-
6678	5.0	30	13	32	3	<1	<1	-	-	-	-	
6679	5.0	40	32	42	4	<1	<1	-	-	-	-	
6675r	5.0	540	170	110	24	2	<1	<1	-	-	-	
6676r	5.0	420	290	46	22	4	<1	<1	<1	-	-	-
6295	10.0	222	138	28	1	<1	<1	<1	-	-	-	-
6296	10.0	176	70	13	2	<1	<1	<1	<1	-	-	-
6294r	10.0	1750	670	362	36	6	3	1	<1	<1	-	-
6680	20.0	21	2	10	1	<1	<1	-	-	-	-	
6681	20.0	20	14	4	<1	<1	-	-	-	-	-	
6682	20.0	68	10	28	1	<1	-	-	-	-	-	
6297	40.0	270	104	23	3	<1	<1	<1	<1	-	-	-
6298	40.0	160	192	26	17	5	<1	<1	Died			

TABLE 4-A

PILOT ASSESSMENT OF THE ACTIVITY OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
		None	Suppressed	Cleared			
6675	1.25	+			n.a.	n.a.	Dose increased Day 3
6676	1.25	+			n.a.	n.a.	Dose increased Day 3
6293	2.5			+	10	n.a.	Cured
6294	2.5	+			n.a.	n.a.	Dose increased Day 4
6677	5.0			+	8	n.a.	Cured
6678	5.0			+	6	n.a.	Cured
6679	5.0			+	6	n.a.	Cured
6675r	5.0			+	7	n.a.	Cured
6676r	5.0			+	8	n.a.	Cured
6295	10.0			+	7	n.a.	Cured
6296	10.0			+	8	n.a.	Cured
6294r	10.0			+	9	n.a.	Cured
6680	20.0			+	6	n.a.	Cured
6681	20.0			+	5	n.a.	Cured
6682	20.0			+	5	n.a.	Cured
6297	40.0			+	8	n.a.	Died Day 8 Post R_x
6298	40.0			+?	n.a.	n.a.	Died Day 1 Post R_x

TABLE 5-A

PILOT ASSESSMENT OF THE ACTIVITY OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE MALAYAN CAMP-CH/Q STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment						Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
6210	2.5	66	51	828	344	78	14	4	3	<1	<1	<1
6211	2.5	30	40	396	684	1500	2160	Dose increased				
6210r	5.0	44	57	60	34	6	<1	<1	<1	<1	<1	-
6211r	5.0	2160	1060	590	116	54	10	6	4	<1	<1	<1
6212	10.0	22	52	62	10	8	1	<1	<1	-	-	Died
6213	10.0	46	32	3	<1	<1	<1	-	-	-	Died	
6214	40.0	72	3	17	<1	<1	<1	-	-	-	-	
6215	40.0	11	3	2	<1	<1	<1	<1	<1	-	Died	

TABLE 6-A

PILOT ASSESSMENT OF THE ACTIVITY OF WR-172,435-AC AGAINST ESTABLISHED INFECTIONS WITH THE MALAYAN CAMP-CH/Q STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recru-descence	Remarks
		None	Suppressed	Cleared			
6210	2.5			+	12	12	
6211	2.5	+			n. a.	n. a.	Dose increased Day 5
6210r	5.0			+	10	n. a.	Cured
6211r	5.0			+	12	n. a.	Cured
6212	10.0			+	8	n. a.	Died Day 4 Post R_x
6213	10.0			+	6	n. a.	Died Day 3 Post R_x
6214	40.0			+	6	n. a.	Died Day 5 Post R_x
6215	40.0			+	8	n. a.	Died Day 3 Post R_x

C. WR-194,965-AB (BN: BE-13,813): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THE OWL MONKEY (*AOTUS TRIVIRGATUS*)

SORI-KM-76-261

SUMMARY OF STUDIES CARRIED OUT UNDER
CONTRACT NO. DADA 17-69-C-9104

ON

WR-194,965-AB (BN: BE-13,813): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THE OWL MONKEY (*AOTUS TRIVIRGATUS*)

Southern Research Institute
2000-Ninth Avenue South
Birmingham, Alabama 35205
June 1, 1976

Project 2284-XXVII

WR-194,965-AB (BN: BE-13,813): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH *PLASMODIUM FALCIPARUM* AND *PLASMODIUM VIVAX* IN THE OWL MONKEY (*AOTUS TRIVIRGATUS*)

INTRODUCTORY COMMENT

The primary and secondary testing phases of the Malaria Chemotherapy Program of the Department of the Army have identified a substantial number of highly active agents. Many of these compounds are as active as chloroquine, or even more active, against infections with drug susceptible strains of *P. falciparum* or *P. vivax*, and are fully effective in the face of chloroquine, quinine, and pyrimethamine resistance. Almost without exception, these agents are analogs of classes of compounds long-known to be active against the blood schizonts of various plasmodia, including the 4-quinolinemethanols, 9-phenanthrenemethanols, and diverse types of dihydrofolic acid reductase inhibitors such as the 2,4-diamino-aryl-substituted pyrimidines, 2,4-diaminotriazines, 2,4-diamino-6-substituted quinazolines, sulfonamides, and sulfones. The 4-pyridinemethanols, whose activity has been uncovered recently, can be considered as close relatives of the 4-quinolinemethanols.

In the face of such limitation of antimalarial activity to a relatively few chemical classes, the emergence of several highly active polysubstituted phenols in the primary *P. berghei* screen commanded special interest. One of the most active of these agents against infections with *P. berghei* in the mouse, WR-194,965 (cf structure in Figure 1), was submitted to a limited pilot assessment in owl monkeys infected with the multidrug-resistant Smith strain of *P. falciparum*. The exhibition in this demanding model of curative activity at daily doses of 2.5 to 5.0 mg base per kg body weight gave WR-194,965 a potentially competitive position with WR-142,490 (mefloquine), the most effective of the 4-quinolinemethanols.

The results of the experiment with the Smith strain of P. falciparum, initiated in January, 1975, led to plans for expanded studies on WR-194,965, with ultimate evaluation in human volunteers. These plans included preparation of a batch lot of this agent, to be used in the initial clinical studies, the expanded experimental therapeutic evaluations, and the toxicologic studies in lower animals required for approval of trials of a new drug in man. A segment of these plans had to be modified because of delays in preparing this batch lot and because of the limited time availability of the owl monkey-human malaria models. In order to make use of these models, it was deemed necessary to utilize the preparation of WR-194,965 employed in the pilot evaluation* for expanded assessment of activity, focusing on the importance of the dosage regimen as a determinant of efficacy. The results of these studies are summarized in this Report.

METHODS AND PROCEDURES

The data presented in this Report were derived from the following six experiments, designed for the purposes indicated.

A. Pilot assessment of the activity of WR-194,965-AB against infections with the Smith strain of P. falciparum.

Experiment of January 3, 1975, involving 9 owl monkeys.

B. Expanded assessment of the activity of WR-194,965-AB against infections with the Smith strain of P. falciparum, with emphasis on the influence of the dosage regimen.

Experiment of November 7, 1975, involving 19 owl monkeys.

Experiment of December 1, 1975, involving 7 owl monkeys.

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*The lot utilized in these studies was a hydrochloride salt, designated WR-194,965-AB (BN: BE-13,813), with a base content of 87.7 per cent (CF = 1.14).

C. Assessment of the activity of WR-194,965-AB against infections with the Palo Alto strain of P. vivax, with emphasis on the influence of the dosage regimen.

Experiment of May 2, 1975, involving 8 owl monkeys.

Experiment of June 16, 1975, involving 21 owl monkeys.

Experiment of October 27, 1975, involving 11 owl monkeys.

The owl monkeys (Aotus trivirgatus grisiemembra) utilized in the above experiments were all of Colombian origin. Those with numbers of 8056 and lower were imported directly from areas of Colombia adjacent to Barranquilla, vaccinated against Herpes tamarinus and Herpes simplex, and conditioned for a minimum period of 60 days via procedures detailed elsewhere (Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:446-74, 1973). Such subjects were used exclusively in the January 3, 1975 pilot assessment of the activity of WR-194,965-AB against infections with the Smith strain of P. falciparum. They were also used in the May 2 and June 16 segments of the evaluations of the activity of WR-194,965-AB against infections with the Palo Alto strain of P. vivax. This latter assignment was a sequel to earlier participation in evaluations of the activities of drugs other than WR-194,965-AB against infections with either the Smith or Oak Knoll strains of P. falciparum*.

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*The owl monkey closely resembles man in that previous or current infection with P. falciparum does not alter host susceptibility to infection with P. vivax or vice versa. This makes it possible to utilize an animal for evaluating the activity of a drug against infections with P. falciparum and when cure of such infection is assured, to assign the same monkey to assessment of the activity of a different drug against infection with P. vivax. Such multiple use, a routine in our chemotherapeutic program, reduces operational costs and conserves numbers of monkeys that must be imported for these therapeutic assessments.

Owl monkeys with numbers of 8057 and greater were obtained from the Boston Biomedical Research Institute (BBRI) in exchange for monkeys discarded from this project following serial use in experiments with P. falciparum and P. vivax. These monkeys had been imported from Colombia by the Tarpon Zoo, Tarpon Springs, Florida, conditioned in the importer's facilities for 30 days or longer, and then transferred to BBRI where they were utilized for a variety of studies on the eye, usually involving surgical manipulation. These investigations invariably resulted in unilateral loss of vision, but had no impact on general health status. Such monkeys were adapted to the facilities and handling procedures of this Institute for 30 days prior to assignment to an experiment. They were used exclusively in the November 7 and December 1, 1975 evaluations of the activity of WR-194,965-AB against infections with the Smith strain of P. falciparum and in the October 27, 1975 evaluation against infections with the Palo Alto strain of P. vivax. Although because of nervousness (probably stemming from unilateral vision) they were more difficult to handle than directly imported owl monkeys, they supported growth of the Smith and Palo Alto strains in a normal reproducible manner.

The following procedures were common to each of the experiments. Infections were induced by intravenous inoculation of 5×10^6 erythrocytic parasites derived from monkeys of the passage lines of the two test strains*. Measurements

*The passage line of the Vietnam Smith strain of P. falciparum is maintained by serial transfer of parasitized erythrocytes through normal untreated owl monkeys every seven to ten days. The passage line of the Vietnam Palo Alto strain of P. vivax is maintained by serial transfer every twenty-one to twenty-eight days. Inocula for the chemotherapeutic studies are obtained by appropriate dilution in iced saline of heparinized blood drawn from a passage monkey. Dilutions for this strain of P. falciparum varied from 1:100 to 1:200; dilutions for the strain of P. vivax varied from 1:10 to 1:20.

of parasitemias on thick and thin blood films stained with Giemsa were initiated three days after inoculation, at which time thick blood films were invariably positive. Thick and thin blood films were prepared daily thereafter until densities of 10 to 50 parasites per 10^4 erythrocytes* were attained. At this time, treatment with WR-194,965-AB was initiated via the oral route. The requisite amount of this agent, calculated as base, dissolved or suspended in 10.0 ml of distilled water, was administered by stomach tube, followed by a 5.0 ml water rinse. Treatments were always carried out between 8:00 and 9:00 a.m., and completed within one hour of preparation of the working solution or suspension.

The effects of treatment on parasitemia were assessed on thick and thin blood films stained with Giemsa. Such films were prepared just prior to drug delivery during the treatment period and daily thereafter until thick films were parasite negative for at least four consecutive days. Film preparation and study were then reduced to a twice-weekly level (Monday and Thursday or Tuesday and Friday) for two consecutive weeks, and if results were uniformly negative during this interval, to a once-weekly level for ten additional weeks. Infections were considered cured if blood films were negative during the entire post-treatment period.

If parasitemias persisted at the initial or even lower levels, or increased in intensity during delivery of WR-194,965-AB, or if there was a reappearance of parasites after an apparent blood-negative interval, a second drug course was administered, either at a higher total dose via the same regimen or at the same total dose via a different

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*Such densities are equivalent to parasite populations of 5,000 to 25,000 per cmm of blood

regimen. Whenever an infection was retreated, either early or late, an r was added to the Atr number. Thus the number of r's following a monkey number indicates the number of retreatment courses the animal has received. The procedure has two advantages: (1) it expands the information on drug activity that can be obtained via use of a single monkey; and (2) it can signal emergence of drug-resistant plasmodia, if such occurs, and the rapidity with which this undesirable event appears*.

In all of the studies, attention was directed to the impacts of the drug on the morphology of the parasite. This focus provided a gauge of the rapidity with which WR-194,965-AB affected parasite growth and the type of activity which this agent possesses.

Each experiment included one untreated control monkey, thus making it possible to check the virulence of the parasites in the inoculum.

RESULTS

The results of the various experiments have been detailed in Tables 1 through 6. The odd numbered tables are concerned with immediate effects of drug delivery on parasite densities. The even numbered tables focus on end results. Major attention will be given the latter in the comments that follow.

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*The responses of infections with the two test strains to currently available antimalarial drugs have been detailed elsewhere (Transactions of the Royal Society of Tropical Medicine and Hygiene, 67:446-74, 1973). In brief, infections with the Vietnam Smith strain are fully resistant to treatment with the maximum tolerated doses of chloroquine, quinine, and pyrimethamine. Infections with the Vietnam Palo Alto strain are susceptible to treatment with chloroquine or quinine, but are resistant to treatment with pyrimethamine or proguanil.

1. The Activity Of WR-194,965-AB Against Established Infections With The Smith Strain Of Plasmodium falciparum

Tables 1 and 2 are concerned with the results of the pilot evaluation of the activity of WR-194,965-AB against infections with the multidrug-resistant Smith strain. As the data in Table 2 show, daily doses of 5.0 and 10.0 mg base per kg body weight were uniformly curative. Cures were achieved in two of four recipients of daily doses of 2.5 mg per kg. Parasitemias cleared rather slowly in all recipients of curative doses, usually on the sixth or seventh treatment day.

The results of the two succeeding experiments, brought together in Tables 3 and 4, provide full support for the results of the pilot evaluation. The observations summarized in Table 4 show that daily doses of 5.0 mg per kg to a total dose of 35.0 mg per kg, were uniformly curative (five of five recipients) while daily doses of 2.5 mg per kg provided 50 per cent cures (three of six recipients). Doses of 1.25 mg per kg did not effect parasite clearance, indicating that WR-194,965-AB has a fairly steep dose-response curve.

The data in Table 4 also show that at the same total dose, the curative activities of WR-194,965-AB were essentially identical for single dose, three daily dose, and seven daily dose regimens. It is noteworthy that the time required for parasite clearance was not influenced by the dosage regimen.

2. The Activity Of WR-194,965-AB Against Established Infections With The Palo Alto Strain Of Plasmodium vivax

The results of the three experiments with the Palo Alto strain have been brought together in Tables 5 and 6. As the summary observations in the latter table show, a total dose of 17.5 mg base per kg body weight, or greater, was uniformly curative, irrespective of the dosage schedule. At a

total dose of 8.75 mg per kg body weight a single dose regimen appeared to be more effective than a seven divided daily dose schedule; thus cures were achieved in six of six recipients of a single dose as compared with six of ten recipients of divided doses. The three divided daily dose regimen occupied an intermediary position with respect to curative activity. Although a total dose of 4.38 mg WR-194,965-AB per kg body weight was not regularly curative in any regimen, a single dose at this level had greater capacity to clear the parasitemia than seven divided doses. The rate of parasite clearance was essentially the same with all curative doses, irrespective of the dosage schedule. The time required for clearance was shorter in infections with P. vivax than in infections with P. falciparum.

COMMENTS

The most important features of the various evaluations of the activity of WR-194,965-AB against established infections with the Smith strain of P. falciparum and Palo Alto strain of P. vivax have been summarized in Table 7. This summary serves to reinforce the conclusions detailed in the Results section with respect to: (a) consistency of the various assessments; (b) differences in the activity of WR-194,965-AB against infections with P. falciparum and P. vivax; and (c) similarities in the effectiveness of various dosage regimens.

It is clear from the summary in Table 7 that the activity of WR-194,965-AB is not prejudiced by the presence of multidrug resistance. The relatively small difference in activity of this compound against infections with the pyrimethamine-resistant Vietnam Palo Alto strain of P. vivax and chloroquine-, quinine-, pyrimethamine-resistant Smith strain of P. falciparum has its counterpart in assessments of diverse classes of compounds (4-quinolinemethanols, 9-phenanthrenemethanols, 4-pyridinemethanols). The difference in efficacy

is probably related to the inaccessibility to the action of any drug of the larger developmental stages of P. falciparum that are sequestered in tissue capillaries.

The relatively slow clearance of parasitemia in recipients of WR-194,965-AB is an interesting but perplexing phenomenon. Careful examination of parasites on thin films indicates that all forms suffer severe injury within twenty-four hours of exposure to this compound. Parasite numbers decrease promptly. However, unlike experiences with all therapeutic agents except the "anti-fols", final clearance of the last injured parasites occurs slowly. The meaning of this event is by no means clear.

In all investigations pursued in this program, attention is given to developments which signal emergence of resistance to the agent under study. The most sensitive indicator of this event is failure to achieve cure of re-treatment cases with doses of a compound which invariably cure previously untreated infections. Such signals were uniformly absent in the various assessments of the activity of WR-194,965-AB.

On the basis of activity only, WR-194,965-AB would appear to have considerable promise, and merits consideration for evaluation in human volunteers. Unfortunately, there are no toxicologic data available which would help identify the safety margin of this compound or the quality of untoward reactions which it evokes. Acquisition of such information is a matter of high priority which will doubtless be developed promptly when the "IND" preparation of WR-194,965 becomes available.

SUMMARY AND CONCLUSIONS

Evaluation of the activity of WR-194,965-AB in owl monkey-human plasmodium models has shown that:

1. Infections with the chloroquine-, quinine-, pyrimethamine-resistant Smith strain of P. falciparum are regularly cured by a total dose of 35.0 mg base per kg body weight, and in a high proportion of cases, by a total dose of 17.5 mg per kg. These dosage levels are equally effective whether delivered as single doses or in three or seven fractions on as many successive days.

2. Infections with the pyrimethamine-resistant Palo Alto strain of P. vivax are regularly cured by a total dose of 17.5 mg base per kg body weight, and in a large fraction of cases, by a total dose of 8.75 mg per kg. A single dose at the above, and especially lower levels, appears to be slightly, but probably significantly, more effective than the same amounts split into seven consecutive daily doses.

These assessments place the activity of WR-194,965-AB very close to that of the most effective of the 4-quinolinemethanols, 9-phenanthrenemethanols, and 4-pyridinemethanols. Unless there are significant toxicologic contraindications, WR-194,965 deserves consideration for evaluation in human volunteers.

The studies summarized in this Report were designed, supervised, and evaluated by the undersigned.

L. H. Schmidt, Ph.D.
Principal Investigator, DADA 17-69-C-9104

ACKNOWLEDGEMENT: We are indebted to Ruth Crosby and Howard Washington for execution of the above described experiments and to Lee Vogel for secretarial assistance in preparing this Report.

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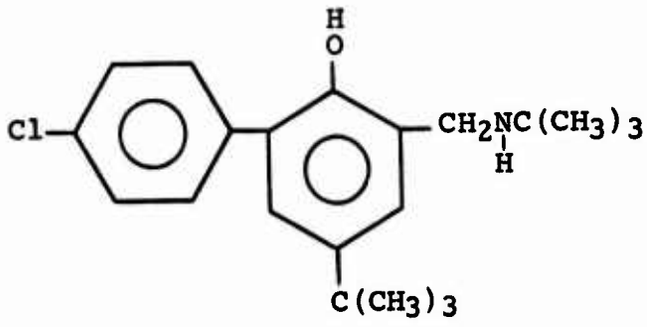
Project 2284-XXVII

FIGURE 1

STRUCTURE OF WR-194,965



2-(4-chlorophenyl)-4-tert. butyl-6-(tert. butylaminomethyl)-phenol

TABLE 1

PRELIMINARY EVALUATION OF THE ACTIVITY OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Parasitemia - No. Parasites/10^4 Erythrocytes									
		Day Pre-treatment	Day of Treatment							Day Post-treatment		
			1	2	3	4	5	6	7	1	2	3
7929	1.25	15	38	190	140	302	272	152	88	66	48	
7930	1.25	11	16	10	1	<1	<1	-	-	-	-	-
7931	2.5	12	21	24	11	3	<1	<1	<1	<1	<1	<1
7932	2.5	14	24	13	29	4	4	<1	<1	<1	<1	1
7929r	2.5	48	61	12	2	<1	<1	<1	<1	<1	<1	-
7930r	2.5	17	10	3	<1	<1	<1	-	-	-	-	-
7933	5.0	34	123	27	4	<1	<1	<1	-	-	-	-
7959	5.0	5	12	4	1	<1	<1	<1	-	-	-	-
7931r	5.0	3	3	2	<1	<1	-	-	-	-	-	-
7932r	5.0	8	2	<1	<1	<1	<1	<1	-	-	-	-
7964	10.0	15	22	6	1	<1	<1	<1	-	-	-	-
7965	10.0	2	24	3	<1	<1	<1	-	-	-	-	-
7926	-	20	86	66	740	940	1530	790	1730	1290	890	2740

TABLE 2

PRELIMINARY EVALUATION OF THE ACTIVITY OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/Kg Body Weight x 7	Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
		None	Suppressed	Cleared			
7929	1.25		+		n.a.	n.a.	
7930	1.25			+	7	13	
7931	2.5			+	11	13	
7932	2.5		+		n.a.	n.a.	
7929r	2.5			+	10	n.a.	Cured
7930r	2.5			+	5	n.a.	Cured
7933	5.0			+	7	n.a.	Cured
7959	5.0			+	7	n.a.	Cured
7931r	5.0			+	5	n.a.	Cured
7932r	5.0			+	7	n.a.	Cured
7964	10.0			+	7	n.a.	Cured
7965	10.0			+	6	n.a.	Cured

TABLE 3

THE ACTIVITY OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose Mg/Kg	Day Pre-treatment	Day of Treatment						Day Post-treatment		
	Mg/Kg	No.			1	2	3	4	5	6	7	1	2	3
8113	8.75	1	8.75	54	30							22	10	23
8114	8.75	1	8.75	48	114							30	29	43
8121	2.92	3	8.76	60	78	14	7					1	<1	<1
8122	2.92	3	8.76	39	60	22	14					8	4	3
8129	1.25	7	8.75	30	108	50	112	22	12	6	1	<1	<1	<1
8134	1.25	7	8.75	24	102	232	244	142	108	116	46	21		
8117	17.5	1	17.5	42	105							26	10	12
8118	17.5	1	17.5	36	36							6	4	<1
8138	17.5	1	17.5	27	18							3	<1	<1
8139	17.5	1	17.5	9	6							1	<1	<1
8113r	17.5	1	17.5	120	33							2	<1	<1
8114r	17.5	1	17.5	216	72							18	4	<1
8124	5.84	3	17.5	21	24	2	<1					<1	<1	-
8125	5.84	3	17.5	48	135	16	2					<1	<1	-
8140	5.84	3	17.5	21	15	36	4					<1	<1	<1
8141	5.84	3	17.5	27	27	12	1					<1	<1	-
8121r	5.84	3	17.5	3	2	<1	<1					-	-	-
8122r	5.84	3	17.5	8	8	1	<1					<1	<1	-
8130	2.5	7	17.5	9	45	12	12	2	<1	<1	-	-	-	-
8131	2.5	7	17.5	18	54	28	11	1	<1	<1	<1	-	-	-
8144	2.5	7	17.5	39	10	21	33	12	6	2	<1	-	-	-
8145	2.5	7	17.5	15	32	72	45	9	<1	3	1	1	<1	<1
8129r	2.5	7	17.5	64	7	<1	<1	<1	-	-	-	-	-	-
8134r	2.5	7	17.5	51	9	12	<1	-	-	-	-	-	-	-

TABLE 3 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8119	35.0	1	35.0	33	80							18	1	<1
8120	35.0	1	35.0	15	30							4	<1	<1
8117r	35.0	1	35.0	117	81							18	3	<1
8118r	35.0	1	35.0	6	6							1	<1	<1
8126	11.68	3	35.0	20	33	2	<1					<1	-	-
8127	11.68	3	35.0	9	33	8	1					<1	<1	-
8120r	11.68	3	35.0	24	30	7	1					<1	<1	-
8125r	11.68	3	35.0	24	27	22	<1					-	-	-
8140r	11.68	3	35.0	56	90	28	3					<1	-	-
8132	5.0	7	35.0	7	6	8	9	4	<1	<1	<1	-	-	-
8133	5.0	7	35.0	42	21	9	1	<1	<1	-	-	-	-	-
8131r	5.0	7	35.0	11	14	6	1	<1	<1	-	-	-	-	-
8144r	5.0	7	35.0	6	12	1	<1	<1	<1	<1	-	-	-	-
8145r	5.0	7	35.0	12	4	2	<1	<1	<1	-	-	-	-	-
8135	-	-	-	6	15	264	450	1460	1210	Transferred to WRAIR				
8146	-	-	-	21	56	234	516	860	1310	1920	1920	5080	7600	Dead

TABLE 4

THE ACTIVITY OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8113	8.75	1	8.75		+		n. a.	n. a.	
8114	8.75	1	8.75		+		n. a.	n. a.	
8121	2.92	3	8.76		+		n. a.	n. a.	
8122	2.92	3	8.76		+		n. a.	n. a.	
8129	1.25	7	8.75		+		n. a.	n. a.	
8134	1.25	7	8.75		+		n. a.	n. a.	
8117	17.5	1	17.5		+		n. a.	n. a.	
8118	17.5	1	17.5		+		n. a.	n. a.	
8138	17.5	1	17.5			+	7	n. a.	Cured
8139	17.5	1	17.5			+	5	n. a.	Cured
8113r	17.5	1	17.5			+	7	n. a.	Cured
8114r	17.5	1	17.5			+	7	n. a.	Cured
8124	5.84	3	17.5			+	6	n. a.	Cured
8125	5.84	3	17.5			+	6	22	
8140	5.84	3	17.5			+	7	20	
8141	5.84	3	17.5			+	6	n. a.	Cured
8121r	5.84	3	17.5			+	4	n. a.	Cured
8122r	5.84	3	17.5			+	6	n. a.	Cured
8130	2.5	7	17.5			+	7	n. a.	Cured
8131	2.5	7	17.5			+	8	11	
8144	2.5	7	17.5			+	8	11	
8145	2.5	7	17.5		+		n. a.	n. a.	
8129r	2.5	7	17.5			+	5	n. a.	Cured
8134r	2.5	7	17.5			+	5	n. a.	Cured

TABLE 4 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recru-descence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8119	35.0	1	35.0			+	7	n. a.	Cured
8120	35.0	1	35.0			+	5	17	
8117r	35.0	1	35.0			+	6	n. a.	Cured
8118r	35.0	1	35.0			+	6	n. a.	Cured
8126	11.68	3	35.0			+	5	n. a.	Cured
8127	11.68	3	35.0			+	6	n. a.	Cured
8120r	11.68	3	35.0			+	6	n. a.	Cured
8125r	11.68	3	35.0			+	4	n. a.	Cured
8140r	11.68	3	35.0			+	5	n. a.	Cured
8132	5.0	7	35.0			+	7	n. a.	Cured
8133	5.0	7	35.0			+	6	n. a.	Cured
8131r	5.0	7	35.0			+	6	n. a.	Cured
8144r	5.0	7	35.0			+	7	n. a.	Cured
8145r	5.0	7	35.0			+	6	n. a.	Cured

TABLE 5

THE ACTIVITY OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes									
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment						Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
8081	4.38	1	4.38	9	7							1	<1	<1
8082	4.38	1	4.38	9	9							<1	<1	<1
8095r	4.38	1	4.38	2	1							<1	<1	-
8088rr	4.38	1	4.38	<1	<1							<1	-	-
8088	1.46	3	4.38	13	9	9	2					<1	<1	-
8081r	1.46	3	4.38	10	12	2	1					<1	-	-
8095rr	1.46	3	4.38	1	<1	<1	-					-	-	
8095	0.625	7	4.38	6	10	6	2	<1	<1	-	-	-	-	<1
8098	0.625	7	4.38	8	10	12	2	<1	<1	-	-	-	-	-
7976r	0.625	7	4.38	5	24	4	<1	<1	<1	3	1	1	2	1
8088r	0.625	7	4.38	2	6	1	<1	<1	<1	-	-	-	-	-
7763rr	0.625	7	4.38	1	2	1	2	<1	<1	<1	<1	<1	<1	<1
8081rr	0.625	7	4.38	19	15	6	1	<1	<1	<1	<1	-	-	-

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
7790	8.75	1	8.75	18	3							<1	-	-
7800	8.75	1	8.75	6	3							<1	-	-
8085	8.75	1	8.75	4	4							1	<1	-
8087	8.75	1	8.75	12	7							1	<1	-
7994r	8.75	1	8.75	1	<1							<1	-	-
8100r	8.75	1	8.75	16	12							1	<1	<1
7883	2.92	3	8.76	20	16	<1	<1					-	-	-
7894	2.92	3	8.76	60	34	4	<1					-	-	-
7895	2.92	3	8.76	16	4	<1	-					-	-	-
8019	2.92	3	8.76	28	4	<1	-					-	-	-
8094	2.92	3	8.76	13	22	18	1					<1	-	-
7761rrr	2.92	3	8.76	8	6	<1	-					-	-	-
7994	1.25	7	8.75	2	4	18	3	<1	<1	<1	-	-	-	-
7997	1.25	7	8.75	6	4	3	<1	-	-	-	-	-	-	-
8099	1.25	7	8.75	9	1	<1	<1	<1	-	-	-	-	-	-
8100	1.25	7	8.75	9	10	21	4	<1	<1	-	-	-	-	
7894r	1.25	7	8.75	4	8	24	16	3	<1	<1	<1	-	-	-
7975r	1.25	7	8.75	<1	<1	<1	<1	1	<1	<1	-	-	-	-
7978r	1.25	7	8.75	6	1	<1	<1	<1	-	-	-	-	-	-
7761rr	1.25	7	8.75	9	7	2	2	1	<1	-	-	-	-	-
7833rr	1.25	7	8.75	<1	<1	<1	-	-	-	-	-	-	-	-
8095rrr	1.25	7	8.75	<1	<1	<1	-	-	-	-	-	-	-	-

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Parasitemia - No. Parasites/10^4 Erythrocytes										
	Daily Dose		Total Dose	Day Pre-treatment	Day of Treatment							Day Post-treatment		
	Mg/Kg	No.	Mg/Kg		1	2	3	4	5	6	7	1	2	3
7805	17.5	1	17.5	27	9							<1	<1	-
7815	17.5	1	17.5	12	15							<1	-	-
7766rr	17.5	1	17.5	26	2							<1	<1	<1
7976rr	17.5	1	17.5	8	3							<1	<1	-
7954	5.84	3	17.5	39	5	<1	-					-	-	-
7981	5.84	3	17.5	8	12	<1	<1					<1	-	-
8003	2.5	7	17.5	18	9	9	1	-	-	-	-	-	-	-
8020	2.5	7	17.5	8	6	2	<1	-	-	-	-	-	-	-
7841	35.0	1	35.0	6	2							<1	-	-
7858	35.0	1	35.0	20	2							<1	<1	-
7984	11.68	3	35.0	6	3	<1	-					-	-	-
7985	11.68	3	35.0	4	1	<1	-					-	-	-
8023	5.0	7	35.0	6	2	<1	<1	<1	-	-	-	-	-	-
8024	5.0	7	35.0	10	2	<1	-	-	-	-	-	-	-	-
7982	-	-	-	28	58	159	210	230	123	96	140	72	46	100
8026	-	-	-	6	21	14	57	16	26	27	40	25	52	34
8105	-	-	-	6	14	18	22	28	34	30	105	16	27	32

TABLE 6

THE ACTIVITY OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Summary Observations

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
8081	4.38	1	4.38			+	6	16	
8082	4.38	1	4.38			+	5	n.a.	Cured
8095r	4.38	1	4.38			+	4	13	
8088rr	4.38	1	4.38			+	3	n.a.	Cured
8088	1.46	3	4.38			+	6	14	
8081r	1.46	3	4.38			+	5	23	
8095rr	1.46	3	4.38			+	3	31	
8095	0.625	7	4.38		+		n.a.	n.a.	
8098	0.625	7	4.38			+	6	n.a.	Cured
7976r	0.625	7	4.38		+		n.a.	n.a.	
8088r	0.625	7	4.38			+	6	35	
7763rr	0.625	7	4.38		+		n.a.	n.a.	
8081rr	0.625	7	4.38			+	8	n.a.	Cured

TABLE 6 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			Days from Initial R_x to Parasite Clearance	Days from Final R_x to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
7790	8.75	1	8.75			+	3	n. a.	Cured
7800	8.75	1	8.75			+	3	n. a.	Cured
8085	8.75	1	8.75			+	4	n. a.	Cured
8087	8.75	1	8.75			+	4	n. a.	Cured
7994r	8.75	1	8.75			+	3	n. a.	Cured
8100r	8.75	1	8.75			+	5	n. a.	Cured
7883	2.92	3	8.76			+	4	n. a.	Cured
7894	2.92	3	8.76			+	4	25	
7895	2.92	3	8.76			+	3	n. a.	Cured
8019	2.92	3	8.76			+	3	n. a.	Cured
8094	2.92	3	8.76			+	5	n. a.	Cured
7761rrr	2.92	3	8.76			+	3	n. a.	Cured
7994	1.25	7	8.75			+	7	19	
7997	1.25	7	8.75			+	4	n. a.	Cured
8099	1.25	7	8.75			+	5	n. a.	Cured
8100	1.25	7	8.75			+	6	23	
7894r	1.25	7	8.75			+	7	n. a.	Cured
7975r	1.25	7	8.75			+	7	13	
7978r	1.25	7	8.75			+	5	n. a.	Cured
7761rr	1.25	7	8.75			+	6	16	
7833rr	1.25	7	8.75			+	3	n. a.	Cured
8095rrr	1.25	7	8.75			+	3	n. a.	Cured

TABLE 6 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recrudescence	Remarks
	Daily Dose		Total Dose						
	Mg/Kg	No.	Mg/Kg	None	Suppressed	Cleared			
7805	17.5	1	17.5			+	4	n.a.	Cured
7815	17.5	1	17.5			+	3	n.a.	Cured
7766rr	17.5	1	17.5			+	6	n.a.	Cured
7976rr	17.5	1	17.5			+	4	n.a.	Cured
7954	5.84	3	17.5			+	3	n.a.	Cured
7981	5.84	3	17.5			+	5	n.a.	Cured
8003	2.5	7	17.5			+	4	n.a.	Cured
8020	2.5	7	17.5			+	4	n.a.	Cured
7841	35.0	1	35.0			+	3	n.a.	Cured
7858	35.0	1	35.0			+	4	n.a.	Cured
7984	11.68	3	35.0			+	3	n.a.	Cured
7985	11.68	3	35.0			+	3	n.a.	Cured
8023	5.0	7	35.0			+	5	n.a.	Cured
8024	5.0	7	35.0			+	3	n.a.	Cured

TABLE 7

SUMMARY: THE ACTIVITIES OF WR-194,965-AB AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM AND THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Total Dose Mg/Kg	No. of Doses	Daily Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Smith Strain - P. falciparum								
8.75	1	8.75	2	0	2	0	0	n. a.
8.76	3	2.92	2	0	2	0	0	n. a.
8.75	7	1.25	4	0	3	1	0	n. a.
17.5	1	17.5	6	0	2	4	4	7
17.52	3	5.84	6	0	0	6	4	6
17.5	7	2.5	10	0	1	9	6	7
35.0	1	35.0	4	0	0	4	3	6
35.04	3	11.68	5	0	0	5	5	5
35.0	7	5.0	9	0	0	9	9	7
70.0	7	10.0	2	0	0	2	2	6

TABLE 7 - CONTINUED

Dosage Regimen			No. of Infections Treated					Days from Initial R_x to Parasite Clearance*
Total Dose Mg/Kg	No. of Doses	Daily Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Palo Alto Strain - P. vivax								
4.38	1	4.38	4	0	0	4	2	5
4.38	3	1.46	3	0	0	3	0	5
4.38	7	0.625	6	0	3	3	2	6
8.75	1	8.75	6	0	0	6	6	4
8.76	3	2.92	6	0	0	6	5	4
8.75	7	1.25	10	0	0	10	6	5
17.5	1	17.5	4	0	0	2	4	4
17.52	3	5.84	2	0	0	2	2	4
17.5	7	2.5	2	0	0	2	2	4
35.0	1	35.0	2	0	0	2	2	4
35.04	3	11.68	2	0	0	2	2	3
35.0	7	5.0	2	0	0	2	2	4

*Median day to parasite clearance.

III. PILOT ASSESSMENTS OF THE ACTIVITIES OF POTENTIALLY CURATIVE DRUGS

III. PILOT ASSESSMENTS OF THE ACTIVITIES OF POTENTIALLY CURATIVE DRUGS

During the period covered by this Report, forty-five compounds were submitted for pilot assessments of capacity to cure established infections in rhesus monkeys inoculated with large numbers (5×10^5 to 2×10^6) of sporozoites of the B strain of P. cynomolgi. The procedures utilized in these assessments, identical in all respects with those used during the past three years, have been presented in reasonable detail in previous Annual Reports, and will not be described here.

Five of the forty-five compounds submitted for study were unrelated in structure and were added to the "Diverse Structure" compartment of this section. Two were 1,5-naphthyridones and were placed in the 1,5-naphthyridine compartment. The remaining thirty-eight were 8-aminoquinolines; these compounds were placed in subcompartments of this general class according to the location of substituent on the quinoline nucleus*.

In the tabulation that follows, data are presented on all compounds of diverse structure, all 1,5-naphthyridines, and all 8-aminoquinolines evaluated in this laboratory in the past three years, during the search for a curative drug or drugs more effective and better tolerated than primaquine. Compounds tested prior to the current Report period have been identified by a dagger suffix to the WR- code number, located in the first column of each table. This procedure makes it possible to assess the accomplishments of the entire search as well as what has been accomplished in the current year.

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*The specific lot and salt of each compound tested can be found on pages 333-337.

A. Compounds Of Diverse Structure

The five new agents in this category included WR-81,817 (a dihydropyridazone), WR-219,384 (an isoquinoline with two methoxy substituents), WR-223,660 (a diphenyl ether), and WR-225,717 (a 3-aminoquinoline with methyl and methoxy substituents in the 4 and 6 positions). The first of these agents carried a dialkylaminoalkylamino side chain; the latter three carried the 4-amino-1-methylbutylamino chain of primaquine. These structural features made it possible to examine the question of whether the 8-substituted quinoline nucleus is a unique requirement for curative activity. In keeping with the results of a number of earlier studies, this test provided no evidence to the contrary. As shown in Table 9, none of the three agents with the primaquine side chain exhibited traces of curative activity when administered in daily doses up to 3.33 or 10.0 mg per kg body weight. Similarly, negative results were obtained with the dihydropyridazone, WR-81,817, carrying the di-*n*-butylamino-*n*-propylamino side chain.

The fifth compound, WR-225,508, classified as commercially discreet, was administered intravenously in a solution containing glucose and ascorbic acid, together with chloroquine administered via the oral route. Daily doses of 0.5, 1.0, 3.33, or 10.0 mg per kg body weight were devoid of curative activity and did nothing to prolong the relapse interval (cf Table 9).

The addition of these five agents brings to twenty-eight the number of compounds of diverse chemical structure examined for curative activity in the past three years (cf Table 9). Some were selected for study because of exhibition of curative activity in chickens infected with sporozoites of *P. gallinaceum* or mice infected with sporozoites of *P. berghei*.

Others were chosen because of their activity as coccidiostats and the hope that such activity might carry over to other protozoa. Two pyrocatechol derivatives (WR-198,559 and WR-198,560) were examined as structural variants of RC-12, an agent which has highly reproducible curative and prophylactic activities in rhesus monkeys infected with sporozoites of P. cynomolgi. The majority of compounds were examined to test structural principles, hoping to develop leads for synthesis programs.

The net result of these efforts has been at least superficially negative. Only one of sixty-six treated infections was cured. This cure was achieved in a recipient of the maximum tolerated dose of WR-3,396, an organic tin derivative. With the exception of the recipients of WR-12,921 and WR-102,796, relapse intervals after treatment were extremely short, comparable to those obtained when the companion drug, chloroquine, was administered alone. Thus there was no indication of even borderline injury to tissue schizonts. The intervals between relapses were prolonged markedly in the recipients of WR-12,921 and WR-102,796. In the case of the latter compound (and probably WR-12,921 as well), this prolongation reflected the persistence of the agent at levels effective against blood schizonts. Although not useful as tissue schizonticides, one or both of these compounds might have some utility as repository type blood schizonticides.

The negative results with the two pyrocatechol derivatives (WR-198,559 and WR-198,560) should not discourage further explorations of this chemical series. Several representatives of this chemical class have exhibited relatively high orders of prophylactic and curative activities against infections with P. cynomolgi. RC-12 [1,2-dimethoxy-4-(bis-diethylaminoethyl)-amino-5-bromobenzene], the most active of the group against this infection, failed to exhibit either prophylactic or curative activity in human volunteers inoculated with sporozoites of the Chesson strain of P. vivax.

Unfortunately, efforts were not made to determine (a) whether there was a pharmacologic basis for this failure and if such existed whether it could be circumvented, or (b) whether for the first time, the P. cynomolgi - rhesus monkey model had provided a false lead with respect to radical curative activity. In view of the paucity of classes of compounds with curative activity, it would seem well to clear up these issues before turning away from the pyrocatechols completely. By so doing, it would also be possible to either enhance or qualify the confidence now placed in the P. cynomolgi - rhesus monkey model as a predictive system.

TABLE 9

PILOT ASSESSMENTS OF THE RADICAL CURATIVE ACTIVITIES OF A MISCELLANEOUS GROUP OF COMPOUNDS AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

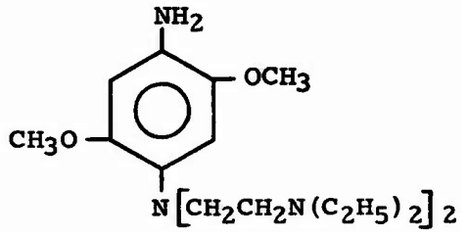
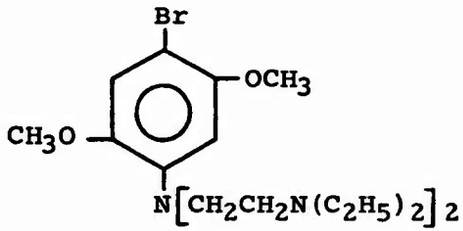
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
3,396†	$(C_4H_9)_3SnCl$	0.25	+	4	-
		0.5	+	4	-
		0.5	+	9	-
		1.0	+	9	-
		1.0	-	-	+
198,559†		2.0	+	5	-
		20.0	+	9	-
198,560†		2.0	+	2	-
		20.0	+	15	-

TABLE 9 - CONTINUED

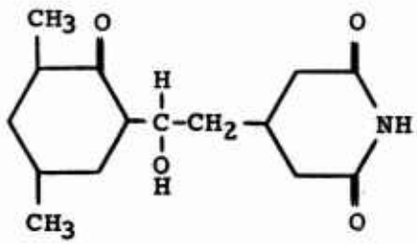
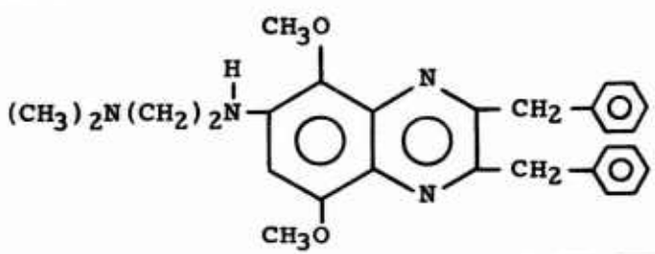
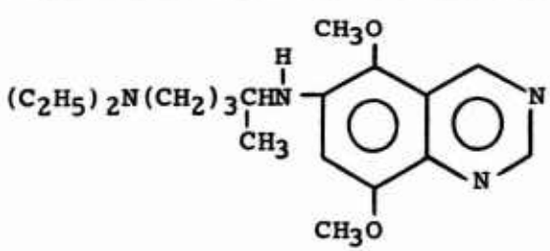
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
13,255†		1.0 10.0	+ +	7 7	- -
219,124†	"Commercially Discreet"	1.0 10.0	+ +	5 8	- -
193,713†		1.0 10.0	+ +	7 8	- -
198,782†		1.0 10.0	+ +	4 7	- -

TABLE 9 - CONTINUED

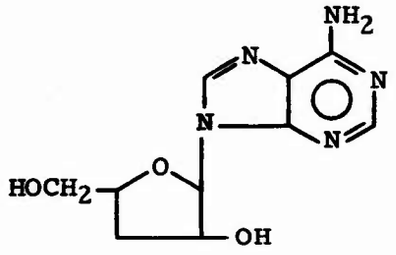
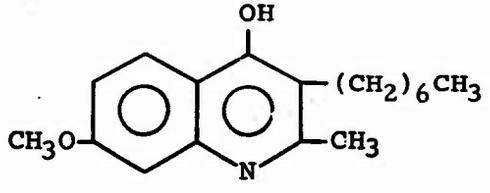
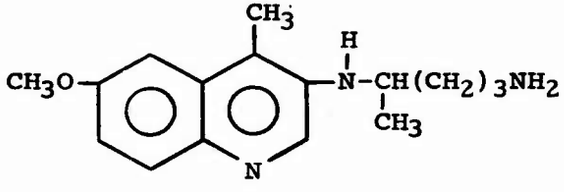
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
191,994†		1.0 5.0 10.0	+ + +	8 8 9	- - -
7,295†		2.0 10.0 20.0	+ + +	12 13 13	- - -
225,717		1.0 3.33	+ +	7 6	- -

TABLE 9 - CONTINUED

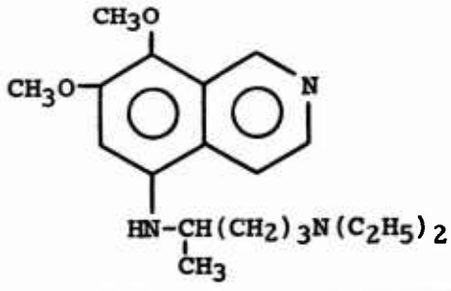
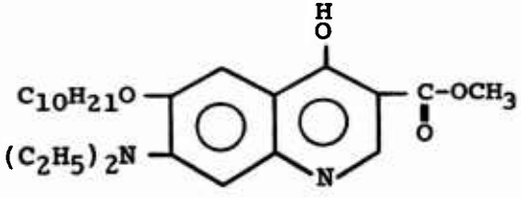
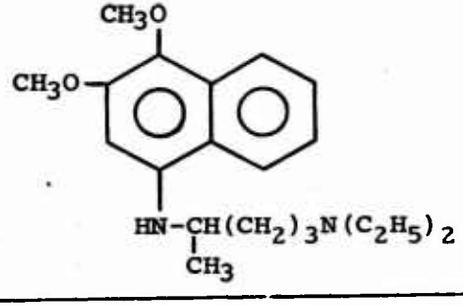
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_X and Relapse	Infection Cured
219,384		1.0 10.0	+ +	9 7	- -
102,796†		10.0 10.0 10.0 10.0 10.0	+ + + + +	48 48 56 121 133	- - - - -
218,575†		1.0 3.33 10.0	+ + +	7 9 78	- - -

TABLE 9 - CONTINUED

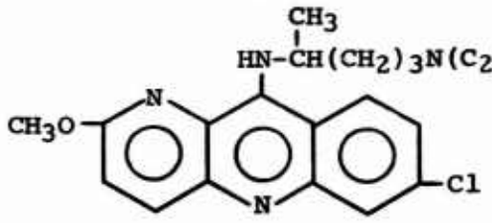
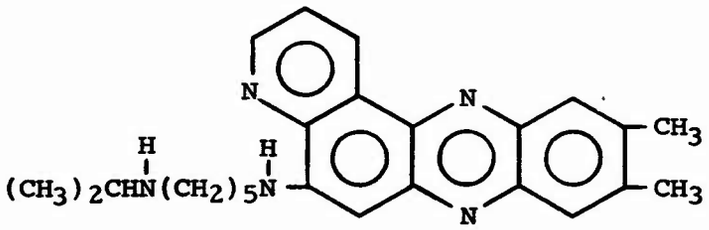
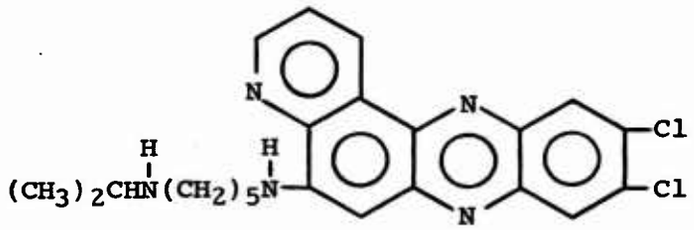
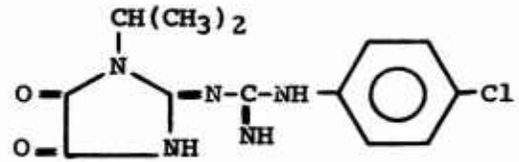
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
12,921†		1.0 10.0	+ +	44 33	- -
205,446†		1.0 10.0	+ +	7 6	- -
202,833†		1.0 10.0	+ +	2 4	- -
182,058†		1.0 10.0	+ +	6 8	- -

TABLE 9 - CONTINUED

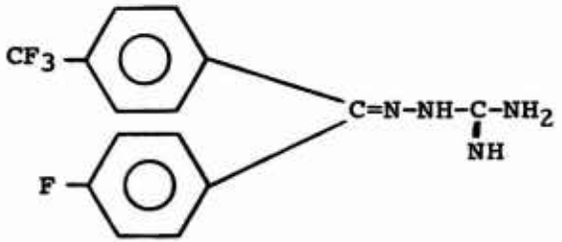
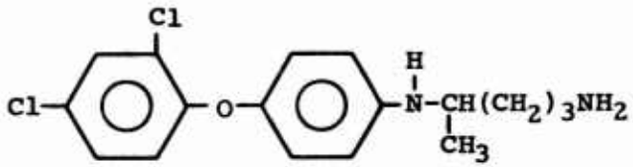
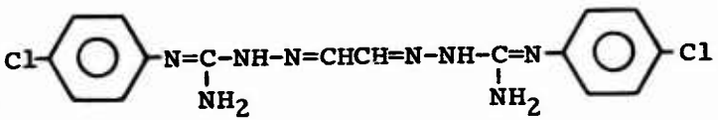
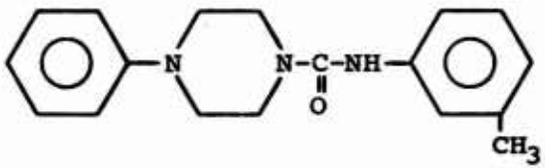
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
9,792†		0.5 5.0	+ +	8 24	- -
223,660		1.0 3.33 10.0	+ + +	8 8 6	- - -
25,981†		1.0 10.0	+ +	6 7	- -
31,877†		1.0 10.0	+ +	3 2	- -

TABLE 9 - CONTINUED

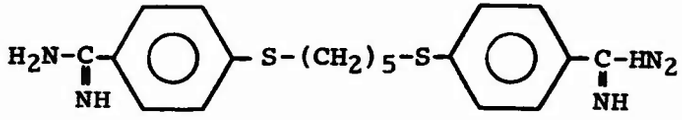
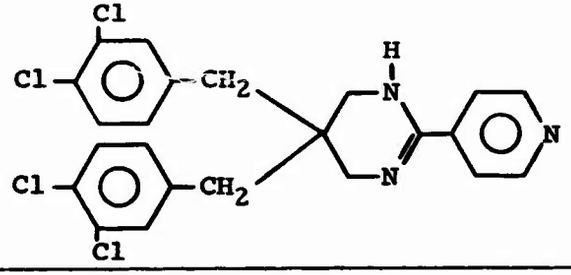
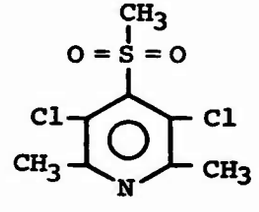
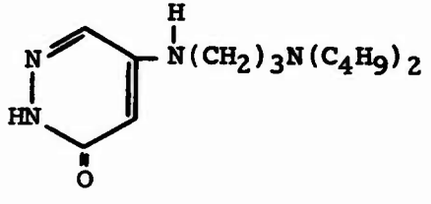
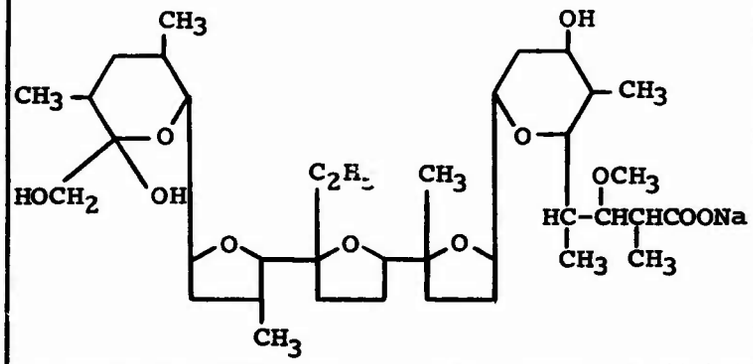
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
190,830†		10.0	+	10	-
158,124†		10.0	+	14	-
167,655†		10.0	+	7	-
81,817		0.5 1.0 3.33	+ + +	7 6 6	- - -

TABLE 9 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
124,905†		1.0 10.0	+ +	8 10	- -
124,892†	Valinomycin	1.0 5.0	+ +	7 7	- -
225,508	"Commercially Discreet"	0.5 1.0 3.33 10.0	+ + + +	7 6 6 9	- - - -

*Dose administered via stomach tube, once daily for seven days with chloroquine at a dose of 2.5 mg base per kg body weight.

†Compounds examined for curative activity in previous Project years.

B. 1,5-Naphthyridines

Two naphthyridone derivatives, WR-222,119 and WR-222,121, have been added to this chemical series. These agents differed from each other with respect to substitution at the 2 position. WR-222,119 was unsubstituted. WR-222,121 had a trifluoroethoxy substituent. As indicated in Table 10, one of twelve infections treated with WR-222,119 at doses ranging from 0.25 to 3.33 mg per kg was cured. This cure was obtained in the first recipient of this naphthyridone at a dose of 1.0 mg per kg. One of six infections treated with WR-222,121 was cured. This cure was achieved at a daily dose of 1.0 mg per kg, again in the first recipient of the test compound. A second recipient of the same dose had passed the forty-seventh negative post-treatment day at the time follow-up study had to be terminated. Thus, the possibility exists of a second cure at 1.0 mg per kg, interspersed between two treatment failures at a dose of 3.33 mg per kg and one at a dose of 1.0 mg per kg.

The random cures encountered with WR-222,119 and WR-222,121 are perplexing and as is evident from the following analysis, cannot be explained entirely on the basis of the therapeutic histories of the subjects concerned. The cure obtained with the first of these agents occurred in a monkey whose primary attack and first relapse had been treated successively with doses of 0.5 and 1.0 mg per kg WR-219,635 (an 8-aminoquinoline), with reappearance of parasitemia in six and thirteen days, respectively. The second relapse, to which WR-222,119 was applied, should have provided a significant challenge to any potentially curative compound. The infection cured by WR-222,121 may have been compromised by previous drug exposure. As a result of delivery of primaquine in a prophylactic regimen two hours prior to sporozoite challenge, the onset of a patent parasitemia was delayed for seven days,

suggesting destruction of a significant fraction of the sporozoite inoculum. Treatment of the primary attack with WR-214,420 (the n-propyl analog of pentaquine) was not curative, but relapse was delayed for thirty-three days. This background of events associated with previous drug exposure could have reduced the tissue schizont burden to a level where even a weak agent administered with chloroquine could lead to cure. Such a situation did not exist in the subject whose infection remained negative (probably cured) for forty-seven days after delivery of WR-222,121. This infection had been exposed previously to WR-226,253 (a 4-quinolinemethanol), with reappearance of parasitemia fifteen days after the administration of the last drug dose. Since WR-226,253 is strictly a blood schizonticide, there is no reason to believe that there had been a reduction in tissue schizonts. That such a burden was heavy is indicated by the results obtained when WR-222,121 was administered to other monkeys in the same experiment undergoing their fourth relapse. In these subjects, there was a reappearance of parasitemia six and seven days after delivery of the seventh dose of 3.33 mg per kg.

The only explanation that can be advanced for the random cures obtained with WR-222,119 and WR-222,121 is that these compounds do possess weak tissue schizonticidal activity and that such activity surfaces when there are peculiarities in physiologic disposition or metabolism, leading to unusually high blood and tissue concentrations of parent drug or metabolites. It might be worthwhile testing this hypothesis by delivering either larger single doses of the above agents or the lower doses at frequent intervals throughout the day. Such studies probably should be carried out before further syntheses in the 1,5-naphthyridine or 1,5-naphthyridone series are undertaken. Conceivably, the results might convert a series with little apparent promise to one worthy of extended study.

TABLE 10

PILOT ASSESSMENTS OF THE RADICAL CURATIVE ACTIVITIES OF A GROUP OF 1,5-NAPHTHYRIDINE DERIVATIVES AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

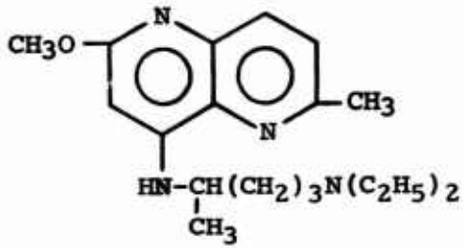
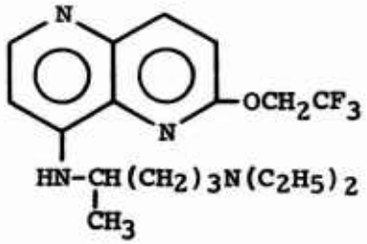
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
202,927†		1.0 10.0	+ +	6 6	- -
217,125†		1.0 10.0	+ +	8 8	- -
202,928†		2.0 20.0	+ +	5 9	- -

TABLE 10 - CONTINUED

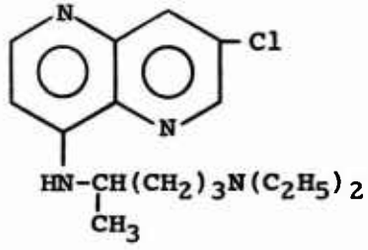
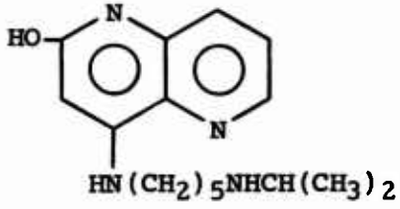
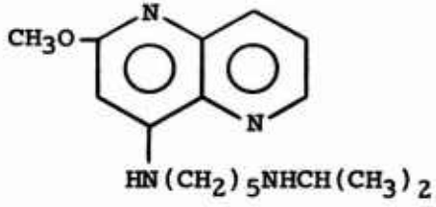
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
180,411†		10.0	+	7	-
206,287†		1.0 10.0	+ -	7 -	- +
206,283†		1.0 10.0	+ +	7 7	- -

TABLE 10 - CONTINUED

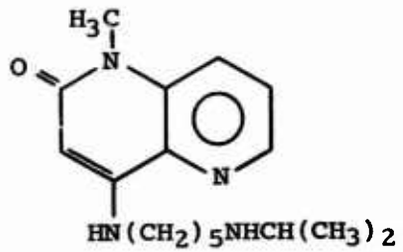
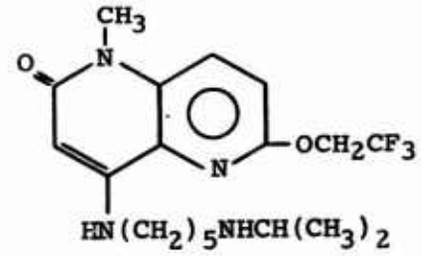
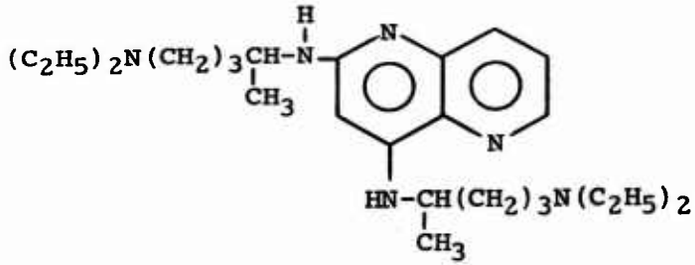
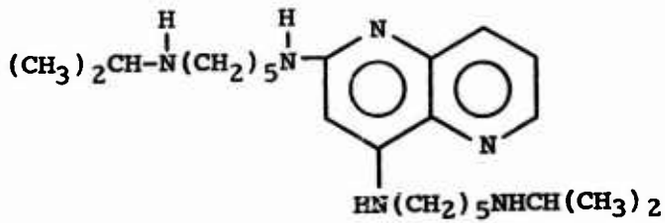
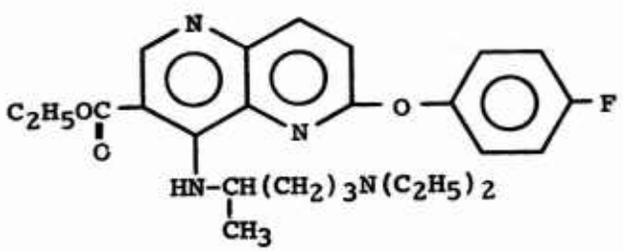
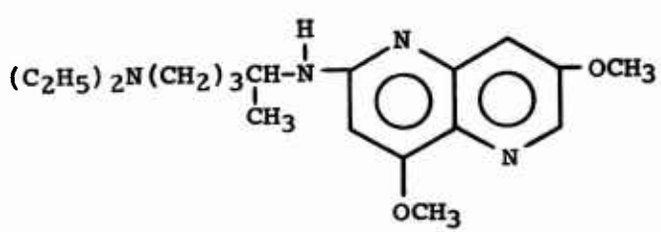
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
222,119		0.25	+	5	-
		0.25	+	5	-
		0.5	+	6	-
		0.5	+	6	-
		0.5	+	13	-
		1.0	+	5	-
		1.0	+	6	-
		1.0	+	13	-
		1.0	+	14	-
		1.0	-	-	+
		3.33	+	7	-
		3.33	+	7	-
222,121		0.5	+	9	-
		1.0	+	9	-
		1.0	-	-	?(>47)
		1.0	-	-	+
		3.33	+	9	-
		3.33	+	9	-
210,304†		1.0	+	12	-
		10.0	+	7	-

TABLE 10 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
210,442†		1.0 10.0	+ +	12 7	- -
145,023†		1.0 10.0	+ +	6 7	- -
216,010†		1.0 10.0	+ +	7 7	- -

*Dose administered via stomach tube, once daily for seven days with chloroquine at a dose of 2.5 mg base per kg body weight.

†Compounds examined for curative activity in previous Project years.

C. 8-Aminoquinolines

During the period covered by this Report, thirty-eight newly synthesized 8-aminoquinoline derivatives were submitted for pilot appraisals of radical curative activity. Evaluations pursued on these agents were extensive enough to either identify the curative dose or to ascertain that the compound was not curative when administered in daily doses of 1.0 mg per kg. Assessments were completed on thirty-one derivatives. The remaining seven were submitted too late in the Project period to obtain a full 105 day post-treatment follow-up on every subject. Follow-up intervals on recipients of six of these compounds were sufficiently long, however, to make prospects of relapse unlikely. Therefore, these subjects have been accorded tentative curative status, so indicated by +(> days) in the last column of Table 11. The follow-up periods on recipients of the seventh compound, WR-223,745, were too brief for even a tentative assessment of curative dose.

Evaluations of the curative activities of thirteen 8-aminoquinolines submitted for study during previous project periods were expanded. Most of these agents had been administered in daily doses of 0.5 mg per kg in the initial appraisals. Where this dose had not effected cure, the current evaluation was directed to determining the efficacy of 1.0 mg per kg doses. When a cure was obtained in the initial trials, the effectiveness of doses less than 0.5 mg per kg was examined.

Although not strictly a pilot investigation, special attention has been given to strengthening earlier observations on the curative activities of WR-212,579, WR-215,296, WR-215,761, WR-216,100, and WR-216,804, compounds which in initial pilot investigations appeared to be as active as WR-181,023 or significantly more active. The results of these studies will be considered in the General Summary at the end of this section.

The results of all three groups of investigations have been summarized in Table 11, along with the results of earlier evaluations on related derivatives. As in previous Reports, the compounds have been separated into thirteen compartments according to the location of substituents on the quinoline nucleus. Although the analysis that follows will be limited to those compartments which contain agents investigated during the current Report period, an effort will be made in the General Summary to appraise the overall contribution of all pilot studies and to suggest areas where further research might be profitable or needed.

1. Derivatives with substituents at position 6.

There were five newly synthesized compounds in this category. Included were WR-221,661, WR-222,122, WR-224,485, WR-224,586, and WR-225,635. All but the first of these agents had a 6-methoxy quinoline nucleus. WR-221,661 carried a 4-chlorophenoxy substituent at position 6 and the primaquine side chain. WR-222,122 was the N^4-oxide of pamaquine. WR-225,635 was a hydrazino derivative with the first carbon of the methylbutyl group of primaquine replaced by nitrogen. WR-224,485 and WR-224,586 were substituted piperazines with symmetrical linkage of two 6-methoxy 8-aminoquinoline nuclei through N-butyl or carbamoylpropyl groups. WR-221,661, WR-222,122, and WR-224,586 were not curative at doses of 1.0 mg per kg; WR-224,485 and WR-225,635 were not curative at doses of 0.5 mg per kg*, the largest doses of these agents to be tested.

*It will be noted that a single infection with each of these compounds was cured. This aberrant activity should probably be discounted. In both cases, cure was obtained in a subject previously assigned to evaluation of the prophylactic activity of WR-181,023. Although this exposure did not provide complete protection, it did lead to lengthening of the incubation period. In addition, these infections were used to evaluate the radical curative activities of WR-127,854 and WR-223,442. Although these agents were not curative at the doses delivered, the parasite negative periods following completion of treatment were extended to 25 and 28 days. It is quite likely that this series of drug exposures reduced tissue schizont burdens to such low levels that these subjects presented a marginal challenge to the curative potential of WR-224,485 and WR-225,635.

The explorations also included an expanded evaluation of the activity of WR-214,420, the n-propyl analog of pentaquine. This derivative was not curative when administered in doses of 1.0 mg per kg.

2. Derivatives with substituents at positions 2 and 6.

Five new derivatives in this compartment were examined along with three compounds studied previously. The new derivatives included WR-219,634, WR-219,635, WR-222,671, WR-228,849, and WR-224,398. Each had a methoxy substituent at position 6; with the exception of WR-222,671, each carried the primaquine side chain. WR-219,634, WR-219,635, and WR-222,849, with chloro, 3-trifluoromethylbenzyloxy, and ethyl substituents at position 2, failed to exhibit curative activity at daily doses of 1.0 mg per kg. WR-224,398, 2-amino primaquine, was curative at daily doses of 0.5 mg per kg. WR-222,671, with a 2-methyl substituent and an amino-4-methylbutylamino side chain at position 8, exhibited curative activity at daily doses of 0.25 mg per kg. WR-222,671 appeared to be twice as active as WR-182,234, a structural isomer with an amino-1-methylbutylamino side chain.

The three previously evaluated compounds, examined in greater detail, included WR-217,124, WR-217,154, and WR-218,669. Each carried a primaquine side chain. WR-217,124, with a 4-trifluoromethylbenzyloxy substituent at position 2, and WR-217,154 with a 4-fluorobenzyloxy substituent, were curative at a dose of 1.0 mg per kg. WR-218,669, with a 2-methoxy substituent, was not curative at this dose level.

3. Derivatives with substituents at positions 3 and 6.

There were no new additions to this series. However, the expanded study of WR-211,814 (3-methyl primaquine) indicated that this agent was curative at a daily dose of 3.33 mg per kg. This was doubtless close to the maximum tolerated level since doses of 10.0 mg per kg provoked a lethal reaction in one of two recipients. Gross examination indicated that the death of this subject was due to hepatotoxicity.

4. Derivatives with substituents at positions 4 and 6.

Fifteen newly synthesized members of this class were submitted for study. This large addition reflected continued interest in the series generated by the rediscovery of WR-181,023 (4-methyl primaquine) and the demonstration that this compound was clearly superior to primaquine with respect to radical curative activity. These interests have been considerable, resulting in synthesis and at least preliminary study of sixty 4-substituted derivatives, approximately 35 per cent of the entire group of 8-aminoquinolines prepared in the search for an agent with greater curative activity and better tolerability than primaquine.

Five of this group of fifteen compounds were congeners of primaquine with diverse substituents at position 4. Included in this subcategory were WR-225,503 with a methylvinyl substituent; WR-223,138 with a 2-methylbutyl substituent; WR-223,137 with an ethylmercapto-4-fluorophenyl substituent; WR-219,894 with a methylcyclohexyl substituent; and WR-220,226 with a n-propylcyclohexyl substituent. None of these compounds exhibited curative activity at a daily dose of 1.0 mg per kg. Thus, they were less than one-fourth as active as WR-181,023 and less than one-half as active as primaquine.

The remaining ten compounds were side chain variants of WR-181,023. In two, the chain terminated with a primary amino group. WR-225,742 had an amino-n-hexylamino side chain; WR-219,373 had an amino-1-ethylpentylamino chain. Neither compound exhibited curative activity at a daily dose of 1.0 mg per kg. Five compounds were secondary amine derivatives: WR-127,854 had an isopropylamino-n-pentylamino chain; WR-211,666 had an isopropylamino-n-hexylamino chain; WR-223,442 had a secondary butylamino-n-hexylamino chain; WR-224,382 had an isopropylamino-n-heptylamino chain; WR-225,741 had a N^1-ethyl-3-piperidylamino-n-hexylamino side chain. The first two members of this subgroup exhibited curative activity at doses of 1.0 mg per kg. The

last three listed did not display curative activity when administered in such doses. The last three compounds in this group of ten had side chains terminating in tertiary amino groups. WR-6,026, with a diethylamino-n-hexylamino chain, and WR-223,658, with a diethylamino-n-heptylamino chain, were curative at daily doses of 0.5 mg per kg and 1.0 mg per kg, respectively. WR-225,845, the N-oxide of WR-6,026, was not curative at a dose of 0.5 mg per kg.

Overall, the activities of the above series of 4,6-substituted 8-aminoquinolines were disappointing. Eleven failed to exhibit curative activity when administered in daily doses of 1.0 mg per kg - indicating that they were less than half as active as primaquine, if indeed they were active at all. Three compounds, curative at the 1.0 mg per kg dose level, had half the curative activity of primaquine. WR-6,026, curative at daily doses of 0.5 mg per kg, was probably the equal of primaquine in radical curative activity, but less than half as active as WR-181,023.

In addition to the studies on newly synthesized agents summarized above, seven older representatives of this 4,6-substituted chemical series were accorded additional evaluations aimed primarily at determining whether they were curative when administered at a daily dose of 1.0 mg per kg or slightly greater. Six of these seven agents had methoxy substituents at position 6. Three members of this group carried the primaquine side chain at position 8 and diverse alkyl substituents at position 4. Included in this category were: WR-218,806 with a n-propyl substituent; WR-218,805 with a n-butyl substituent; and WR-218,636 with a β-methyl-vinyl substituent. None of these derivatives exhibited curative activity at a dose of 1.0 mg per kg.

Three other members of this older group had methyl substituents at position 4, but differed from each other with respect to substituent at the 8 position; in other words, these were side chain variants of WR-181,023. This category

included WR-147,657, with a diethylamino-n-propylamino substituent at position 8, and WR-218,335, with an amino-4-ethylbutylamino substituent. Each of these derivatives was curative at a daily dose of 0.5 mg per kg. It also included WR-212,624, with an amino-1-methylpentylamino substituent, curative at a dose of 1.0 mg per kg.

The seventh compound, WR-217,159, the 6-fluoro analog of WR-181,023, did not exhibit curative activity when administered in daily doses up to 3.33 mg per kg. In examining the detailed data on this compound (cf Table 11, page 192), it will be noted that cure was achieved in one of eight treated subjects, a recipient of doses of 0.5 mg per kg. However, there was no evidence of tissue schizonticidal activity in two other recipients of such doses or two recipients of 1.0 mg per kg. In these subjects, the time between completion of treatment and reappearance of parasitemia ranged from seven to ten days, relapse intervals comparable to those obtained in recipients of chloroquine alone. No reasonable explanation can be advanced for the single cure. The monkey concerned had been inoculated with 8×10^5 sporozoites, had developed a patent parasitemia eight days after inoculation, and received the first dose of WR-217,159 on the sixth day of the primary attack*.

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*It will doubtless be noted that aberrant cures were obtained with four other drugs in this chemical compartment; specifically, with WR-223,137, WR-223,138, WR-223,442, and WR-224,382. The four monkeys in which curative results were obtained were derived from a study of the prophylactic activity of WR-181,023. In each case, the incubation period was prolonged markedly. In each case, the primary attack had been treated with WR-181,023 (0.125 mg per kg) and chloroquine (2.5 mg per kg) for seven days. Although this treatment was not curative, it did extend the relapse interval significantly. Although not perceived at the time, this previous exposure to WR-181,023 doubtless reduced the burden of tissue schizonts to a level incompatible with the continued invasion of hepatic cells. In that setting, cure could be achieved by delivery of an effective blood schizonticide such as chloroquine. For what it may be worth, new agents are no longer evaluated against infections which exhibit extended incubation periods following exposure to prophylactic regimens.

5. Derivatives with substituents at positions 5 and 6.

Six new representatives of this class were submitted for study during this Report period. Each had a methoxy substituent at position 6. Four compounds had the primaquine side chain at position 8. These included: WR-221,041 with a methylmercapto substituent at position 5; WR-224,640 with a dimethylamino substituent; WR-223,745 with a trifluoroethanone substituent; and WR-219,785 with an acetamidophenoxy substituent. WR-223,745 and WR-221,041 were curative at doses of 0.5 and 1.0 mg per kg, respectively. WR-224,640 and WR-219,785 were not curative at the larger of these doses.

The fifth and sixth agents in this compartment were WR-222,890 (with a 3-trifluoromethylphenoxy substituent at position 5 and an amino-4-methylbutylamino side chain at position 8) and WR-224,639 (with a dimethylamino substituent at position 5 and an amino-1-methylbutyl-4-phthalimido side chain). This latter compound was not curative at a dose of 1.0 mg per kg; WR-222,890 was curative at this dose and eradicated one of two infections treated with doses of 0.5 mg per kg.

WR-218,676 was the only carry-over from previous Project periods submitted to additional study. This compound, 5-ethoxy primaquine, received in February 1975, was evaluated against but a single infection in the 1974-1975 period at a dose of 0.5 mg per kg with a curative result. A follow-up series of evaluations led into the current Project period. In all, eight infections were treated with various doses of the compound. Including the result on the first subject, cures were achieved in two of two recipients of 0.5 mg per kg doses, three of three recipients of 0.25 mg per kg, and one of four recipients of doses of 0.125 mg per kg. This result places the curative activity of WR-218,676 at twice that of primaquine and equal to that of 4-methyl primaquine (WR-181,023).

6. Derivatives with substituents at positions 2, 5, and 6.

There were three new agents in this compartment, each with a methyl substituent at position 2 and a methoxy substituent at position 6. They differed with respect to substituents at position 5 and side chain at position 8. Two of the compounds, WR-224,097 and WR-224,486, had the primaquine side chain; WR-224,097 had a 4-fluorophenoxy substituent at position 5; WR-224,486 had a 3-trifluoromethylphenoxy substituent at this position. Although post-treatment follow-up periods were limited, WR-224,097 promised to be curative at a daily dose of 0.25 mg per kg, WR-224,486 at a dose of 0.5 mg per kg.

The third compound in this group, WR-222,418, carried an amino-4-methylpentylamino side chain at position 8 and a 4-chlorophenoxy substituent at position 5. This agent was curative at a daily dose of 1.0 mg per kg.

7. Derivatives with substituents at positions 4, 5, and 6.

There were four newly synthesized derivatives in this compartment. Each had a methyl substituent at position 4 and a methoxy substituent at position 6. Two of the group carried the amino-1-methylbutylamino side chain of primaquine at position 8. One of this pair, WR-219,874, with a fluoro substituent at position 5, was curative at a daily dose of 0.5 mg per kg. The second, WR-225,448, with a 3-trifluoromethylphenoxy substituent at position 5, was evaluated late in the Project period, thereby limiting the follow-up period severely. There are good prospects, clearly in need of confirmation, that this agent will be curative at a dose of 0.125 mg per kg.

The third compound in this compartment, WR-221,527, with a methoxy substituent at position 5, was a structural isomer of WR-216,804 (the 5-methoxy congener of WR-181,023 and the most active 8-aminoquinoline derivative identified thus far) with a 4-methylbutyl group separating the side chain nitrogens in place of the 1-methylbutyl group of WR-216,804. WR-221,527 was curative at a daily dose of 0.125 mg per kg. Thus it equalled WR-216,804 in activity, was twice as active as WR-181,023, and four times as active as primaquine.

The last of the four compounds, WR-219,423, had methyl and methoxy substituents at positions 4 and 5, respectively, and an isopropylamino-n-pentylamino side chain. This change from the primaquine side chain led to a marked reduction in activity, a daily dose of 1.0 mg per kg being required for cure.

General Summary: Pilot assessments for radical curative activity were initiated on July 2, 1972. From that date to the end of this Project period, one hundred seventy-two 8-aminoquinoline derivatives were evaluated in rhesus monkeys infected with sporozoites of P. cynomolgi. Seven hundred twenty treatment courses were delivered during the initial, confirmatory, and expanded assessments of the activities of these agents. The results of these appraisals, communicated promptly to those responsible for the Malaria Chemotherapy Program, have helped to guide the synthesis of new derivatives. They have also focused attention on agents most worthy of consideration for trial in human volunteers and have led to the acquisition of dosage regimen data pertinent to such trials. In the comments that follow, an attempt will be made to summarize the positive aspects of the pilot studies and to identify some of the more important areas worthy of future investigation.

In order to simplify this summation of accomplishments, the structural compartmentalization of compounds utilized in the detailed analysis of Table 11 has been followed. Compounds within a compartment have been rated according to minimum dose required to cure infections in more than 50 per cent of the recipients; a daily dose of 1.0 mg base per kg body weight has been set as the cutoff point in this grading. Table 12 lists the numbers of compounds within each compartment that were curative at this dose, at a dose of 0.5 mg per kg or less, and at a dose of 0.25 mg per kg or less.

As shown in the bottom line of Table 12, fifty-one compounds exhibited curative activity at a daily dose of 1.0 mg per kg or less. Of this number, thirty-three were curative at a dose of 0.5 mg per kg or less; thirteen were curative at a dose of 0.25 mg per kg or less. Thus, 19 per cent of the one hundred seventy-two compounds examined for curative activity were at least as effective as primaquine and 8 per cent were at least twice as active as this well-established drug. Considering the attention accorded the 8-aminoquinolines in the 1926-1939 period by German, French, and Russian investigators, and the intense domestic focus on this class during the 1943-1950 interval, the development of such a substantial fraction of agents equally active or more active than primaquine is a very substantial accomplishment. It is the more remarkable when it is recognized that despite all of the previous attention to structure - activity relationships, relatively uncultivated classes of 8-aminoquinoline derivatives with remarkable curative activity have been uncovered.

As indicated in Table 12, the 6-substituted 2,6-disubstituted, 4,6-disubstituted, and 5,6-disubstituted 8-aminoquinolines received the greatest attention in the current search. Together, these compound classes accounted for one hundred forty-two (82 per cent) of all derivatives

examined for curative activity. The 6-substituted derivatives contributed twenty-nine members to the above total; however, only one agent in this class equalled primaquine in curative activity. This low yield of "actives" is not surprising since the 6-substituted 8-aminoquinolines have received more intensive study than any other group and were almost the sole focus of interest in the intensive search for a useable curative drug pursued during the World War II Malaria Chemotherapy Program*. Twenty-two 2,6-disubstituted compounds were evaluated; this class contributed five agents with activity equal to that of primaquine and one compound that was at least twice as active. The group of sixty agents in the 4,6-substituted class contained eleven members with activity equal to that of primaquine and four with greater activity. Among the group of thirty-one 5,6-substituted derivatives, there were six compounds with activity equal to that of primaquine and two that were at least twice as active. Although the contributions of these classes to the total number of agents with interesting activity were impressive, they were exceeded percentagewise by the contribution of the 2,5,6- and 4,5,6-trisubstituted categories. The seven representatives of the 2,5,6-substituted series yielded four compounds that were as active as primaquine; three that were at least twice as active. Among six 4,5,6-substituted derivatives, there were four compounds with activity equal to that of primaquine, three with greater activity. Thus these two groups, constituting but 7.5 per cent of all agents evaluated, contributed 46 per cent of the compounds with activity clearly superior to that of primaquine. If further structural modifications of these multi-substituted classes are chemically feasible, they should receive high priority attention in the synthesis program.

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*The 4-substituted derivatives (lepidines) received considerable attention in the post-War extension of the World War II Malaria Chemotherapy Program.

The chemical structures of the twelve most active compounds, referred to in the above analysis, and the dimensions of the expanded pilot studies pursued on these agents, have been presented in Table 13. In connection with the latter issue, it should be noted that the evaluation of WR-225,448 is incomplete. Data on primaquine and WR-182,234 (2-methyl primaquine) have been included for reference purposes.

Although the results of the expanded assessments of curative activity will not be detailed here, a very substantial body of data is available on each agent. In all, two hundred thirty-three treatment courses were delivered; excepting WR-225,448, the numbers of courses per compound ranged from eight to thirty-four, and exceeded fourteen for seven of the twelve agents. This series of evaluations does not include targetted assessments pursued on groups of monkeys inoculated for more specific purposes.

The structural segment of Table 13 shows that like primaquine, each of the twelve "more active" agents had a methoxy substituent at position 6. Ten of the twelve were methyl substituted: three (WR-222,671, WR-215,733, and WR-211,532) at position 2; seven (WR-181,023, WR-215,296, WR-215,761, WR-212,579, WR-216,804, WR-221,527, and WR-225,448) at position 4. Seven of the twelve agents had substituents at position 5. Two of these (WR-218,676 with an ethoxy substituent and WR-215,295 with a 3-trifluoromethylphenoxy substituent) were not substituted at positions 2 or 4. Two of the 5-substituted compounds (WR-215,733 with a fluoro substituent and WR-211,532 with a 4-chlorophenoxy substituent) were 2-methyl substituted. Three of the 5-substituted compounds (WR-216,804 and WR-221,527, both with a methoxy substituent, and WR-225,448, with a 3-trifluoromethylphenoxy substituent) were 4-methyl substituted.

Reference to Table 11 shows that a fairly wide variety of substituents was introduced into the 2 and 4 positions of these derivatives. The methyl group was the only substituent that enhanced activity. Although it is not clear what methyl substitution accomplishes, it is obviously a unique modification, a contribution which mechanistically deserves special investigation. Explorations might include study of: (1) the impacts of 2- and 4-methylation on the stability or lability of the relevant compound in various metabolic systems; (2) the effects of such substitution on localization in tissue schizonts; (3) localization of such substituted compounds in hepatocytes; or (4) whether they affect hepatocyte metabolism in a manner which interferes with maturation of tissue schizonts. The unusual hepatotoxicity of the 2- and 4-methylated derivatives makes the latter two possibilities especially attractive.

Because of the variety of 5-substituents in the "more active" series, it is difficult to generalize about the contributions of 5-substitution. The superiority of derivatives with a methoxy substituent at position 5 might be ascribed to their facilitating formation of active quinoline-quinones. It may be questioned, however, whether fluoro, trifluoromethylphenoxy, and chlorophenoxy substitution leads to a similar end. It is clear that the enhanced activity of 5-substituted 8-aminoquinolines is not limited to the presence of any one substituent as it is with the 2- and 4-substituted derivatives. This variety of 5-substituents found among compounds of high activity opens the way to preparation and examination of numerous similarly substituted congeners.

As shown in Table 13, all twelve of the "more active" compounds carried branched side chains with four or five carbons separating the 8-amino group and terminal unsubstituted amino group. In seven of the twelve compounds (WR-181,023, WR-218,676, WR-215,295, WR-215,733, WR-211,532, WR-216,804, and WR-225,448) a 1-methylbutyl group separated the side chain nitrogens, as it does in primaquine and 2-methyl primaquine (WR-182,234). In three of the remaining five compounds (WR-222,671, WR-215,296, and WR-221,527), a 4-methyl group separated the side chain nitrogens. The fourth agent (WR-212,579) carried a 5-methylpentyl group; the fifth compound (WR-215,761) had a 1-ethylbutyl branched chain.

On first examination, it appeared that curative activity was enhanced when branching occurred at the distal end of the alkyl chain. This observation was surprising because WR-152,149, with a 4-methylbutylamino chain, has distinctly less curative activity than its 1-methylbutyl-amino isomer, primaquine, against infections with P. cynomolgi in the monkey and P. vivax in man. As more compounds were examined, it became clear that distally branched derivatives were not uniformly more active than proximally branched agents. Among the small numbers of paired agents available for comparison, primaquine and WR-215,295, both proximally branched, exhibited greater curative activity than their distally branched isomers, WR-152,149 and WR-222,890. On the other hand, the curative activities of the proximally branched WR-182,234, WR-181,023, and WR-212,624 were distinctly inferior to those of their distally branched relatives, WR-222,671, WR-215,296, and WR-212,579. Complicating matters further is the observation that another pair of agents, WR-216,804 and WR-221,527, had essentially identical activity. Obviously, further study of additional pairs of compounds with diversely branched chains will have to be undertaken in order to clarify the impacts of these structural alterations.

To this point, attention has been focused on those compounds which appear to have greater curative activity than primaquine. This focus is compatible with one facet of the search for new curative drugs, development of an agent more active than primaquine. However, for a new agent to be an improvement, it must also have a therapeutic index superior to that of primaquine. In turn, this means that if the new compound is more active, it must be no more toxic and hopefully less toxic than this established drug. Since previous experience indicates that as a class 8-aminoquinolines have relatively small margins of safety, toxicologic studies assume unusual importance and should be made an integral portion of the evaluation of any 8-aminoquinoline which in pilot studies exhibits activity greater than that of primaquine. However, compounds with demonstrable activity but no more active than primaquine should not be neglected. It could well turn out that one or more of the nineteen compounds that appears to have no more curative activity than primaquine (or for that matter, one or more of the additional nineteen with half such activity) could have so much less toxicity that it would have a markedly greater therapeutic index and fill the need for a better tolerated radical curative drug.

TABLE 11

PILOT ASSESSMENTS OF THE RADICAL CURATIVE ACTIVITIES OF VARIOUS 8-AMINOQUINOLINE DERIVATIVES AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

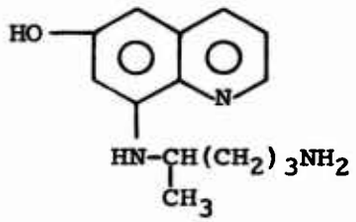
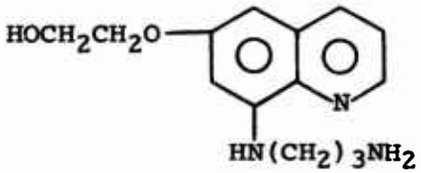
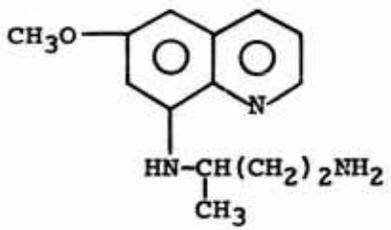
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Position 6					
199,508†		1.0 10.0	+ +	6 6	- -
29,633†		1.0 10.0	+ +	4 10	- -
211,664†		1.0 10.0	+ -	10 -	- +

TABLE 11 - CONTINUED

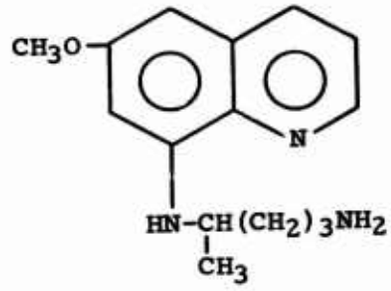
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
2,975† (Primaquine)		0.25	+	7	-
		0.25	+	7	-
		0.25	+	11	-
		0.25	+	11	-
		0.25	+	11	-
		0.25	+	11	-
		0.25	+	18	-
		0.25	+	32	-
		0.375	+	11	-
		0.375	+	14	-
		0.5	+	11	-
		0.5	+	22	-
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.75	-	-	+
		0.75	-	-	+
		0.75	-	-	+
		0.75	-	-	+

TABLE 11 - CONTINUED

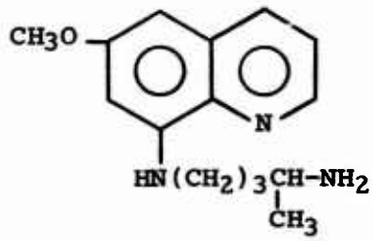
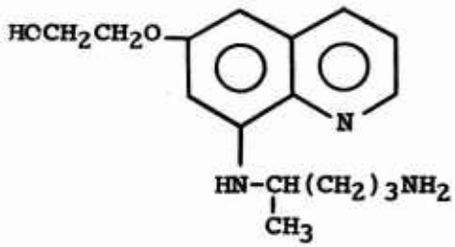
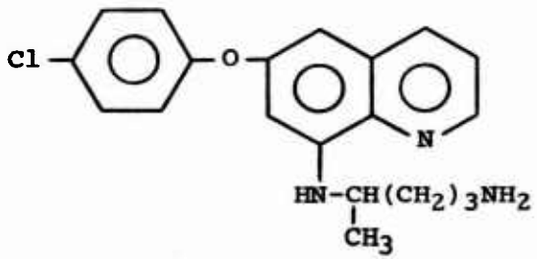
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
152,149†		0.5 0.5 0.5 1.0 1.0	+ + - + -	8 8 - 24 -	- - + - +
186,370†		1.0 10.0	+ +	7 3	- -
221,661		0.5 1.0	+ +	11 12	- -

TABLE 11 - CONTINUED

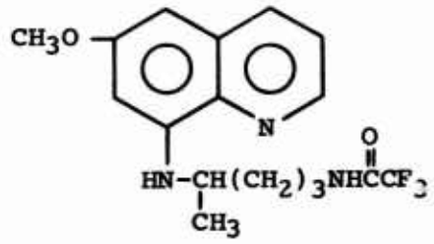
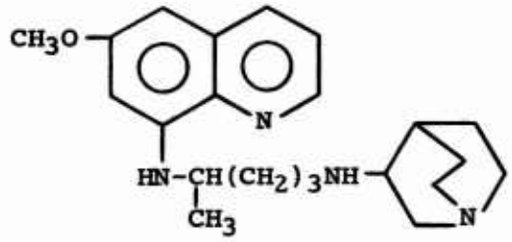
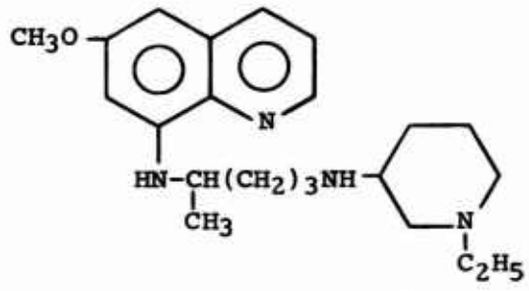
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
215,730†		1.0	+	7	-
161,085†		1.25 2.5 2.5 5.0 5.0 20.0	+ + - - - -	11 27 - - - -	- - + + + +
180,125†		1.0 10.0	+ -	9 -	- +

TABLE 11 - CONTINUED

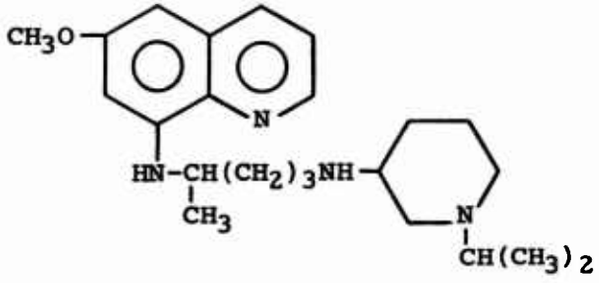
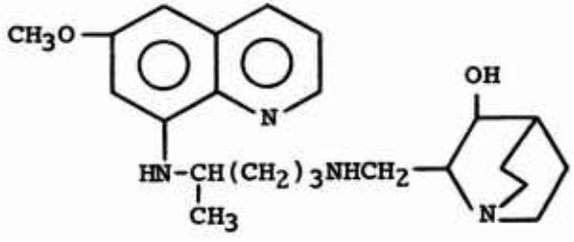
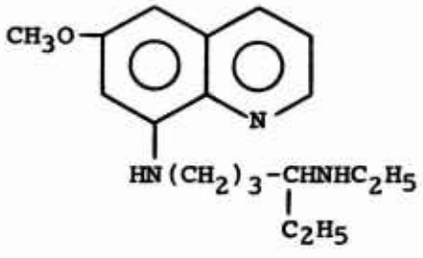
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
197,624†		1.0 3.33 10.0	+ - -	6 - -	- + +
199,981†		1.0 10.0	+ -	6 -	- +
29,594†		0.25 0.5 0.5 1.0 1.0	+ + + - -	12 8 15 - -	- - - + +

TABLE 11 - CONTINUED

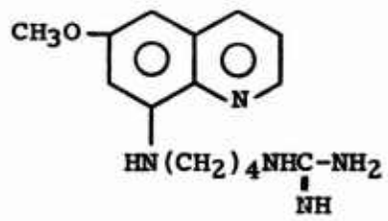
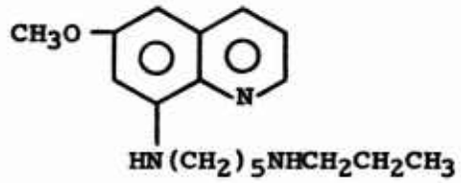
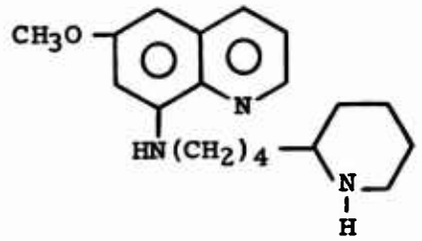
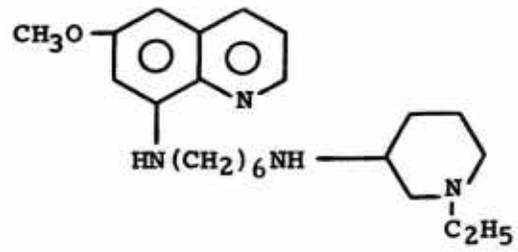
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
27,757†		1.0 10.0	+ +	7 8	- -
214,420†		0.5 1.0	+ +	5 33	- -
29,606†		1.0 10.0	+ -	42 -	- +
190,285†		1.0 3.33 10.0	+ - -	10 - -	- + +

TABLE 11 - CONTINUED

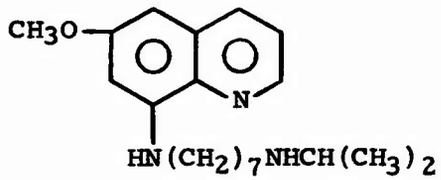
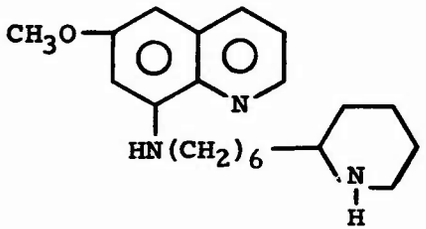
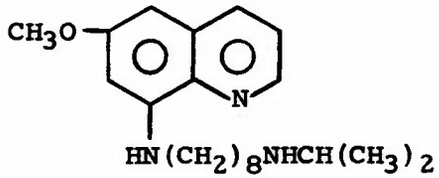
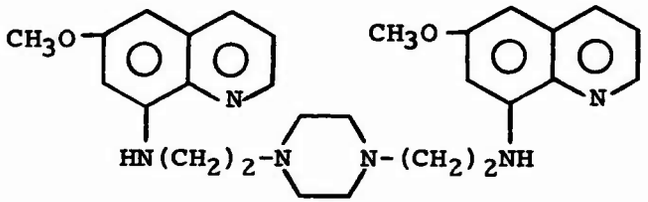
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
193,127†		1.0 10.0	+ -	7 -	- +
29,616†		0.5 1.0 3.33	+ + +	38 13 32	- - -
181,441†		1.0 10.0	+ +	5 5	- -
187,427†		0.5 1.0 1.0 10.0	+ + + Died Day 6 of R_x-Drug Toxicity	7 25 40	- - -

TABLE 11 - CONTINUED

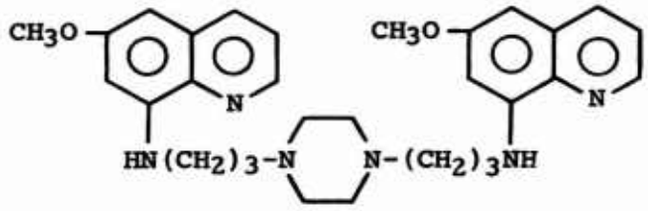
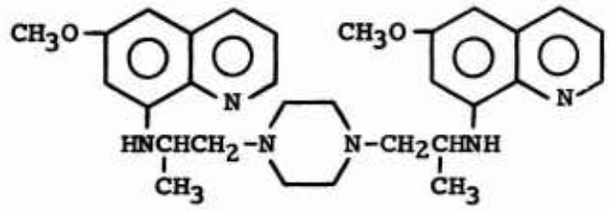
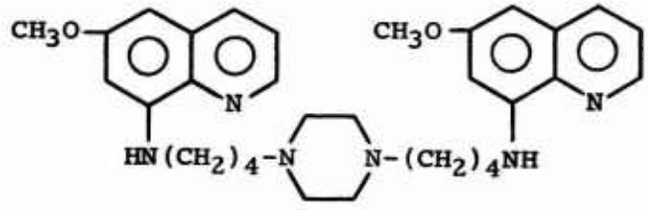
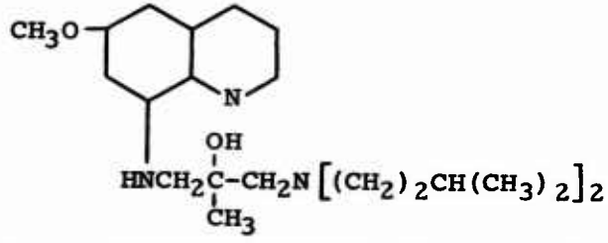
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
187,428†		1.0 2.0 5.0 5.0 10.0	+ + - - -	12 8 - - -	- - + + +
185,306†		1.0 10.0	+ +	18 13	- -
224,485		0.25 0.5 0.5	+ + -	5 7 -	- - +
7,312†		1.0 10.0	+ +	9 10	- -

TABLE 11 - CONTINUED

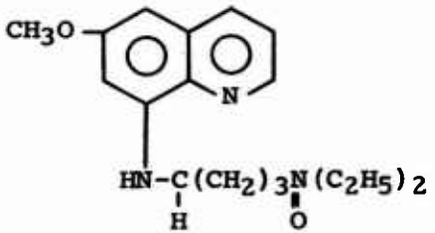
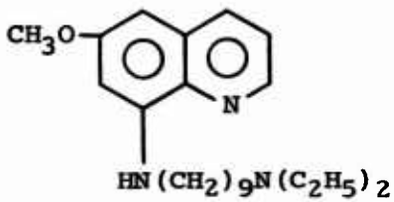
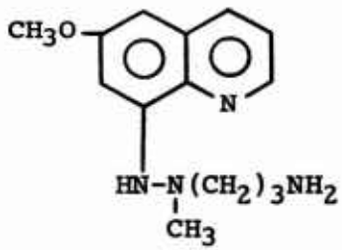
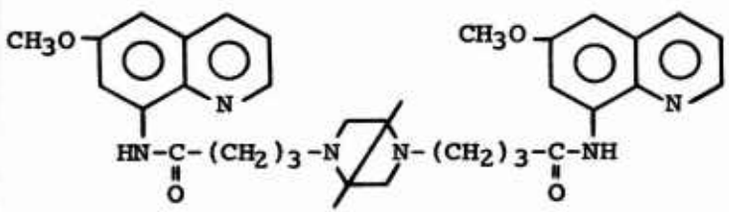
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
222,122		0.5 1.0	+ +	11 19	- -
29,634†		1.0 10.0	+ +	7 16	- -
225,635		0.125 0.25 0.25 0.5	+ + - +	6 7 - 6	- - + -
224,586		0.5 1.0	+ +	17 4	- -

TABLE 11 - CONTINUED

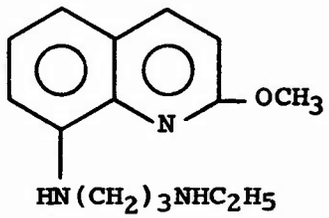
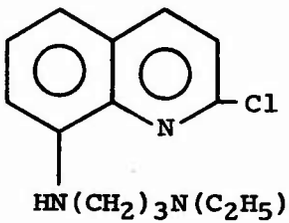
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Position 2					
184, 544†		1.0 10.0	+ +	7 7	- -
212, 231†		1.0 10.0	+ +	6 9	- -

TABLE 11 - CONTINUED

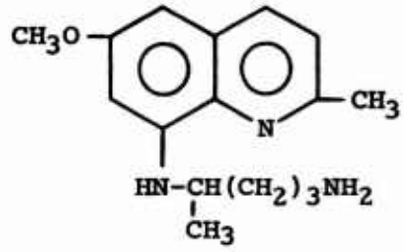
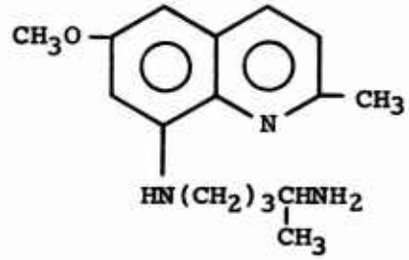
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 2 and 6					
182,234†		0.125	+	10	-
		0.125	+	10	-
		0.125	+	16	-
		0.25	+	11	-
		0.25	+	17	-
		0.25	+	25	-
		0.25	+	28	-
		0.25	+	32	-
		0.25	+	38	-
		0.25	-	-	+
		0.25	-	-	+
		0.5	+	26	-
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+
		1.0	-	-	+
		10.0	-	-	+
222,671		0.125	+	8	-
		0.125	+	13	-
		0.125	+	16	-
		0.125	-	-	+
		0.25	+	14	-
		0.25	+	30	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+ (>86)

TABLE 11 - CONTINUED

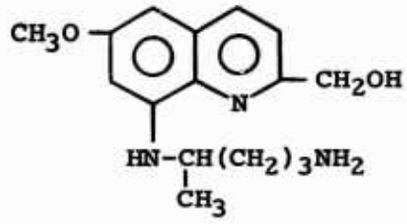
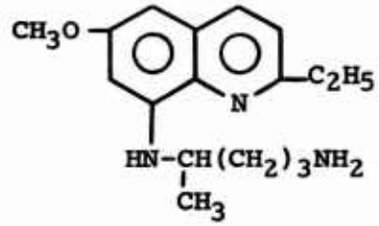
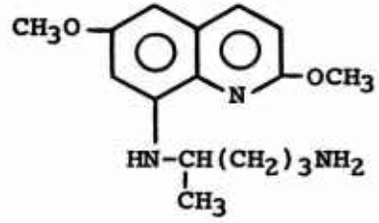
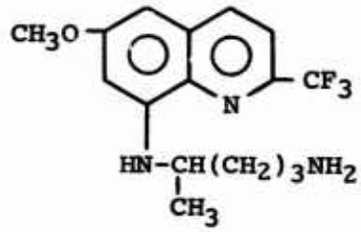
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
213,472†		1.0 10.0	+ +	9 28	- -
222,849		0.25 0.5 1.0	+ + +	17 12 20	- - -
218,669†		0.5 1.0	+ +	12 11	- -
211,077†		1.0 10.0	+ +	13 8	- -

TABLE 11 - CONTINUED

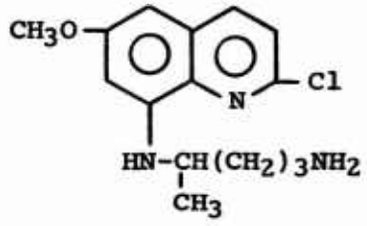
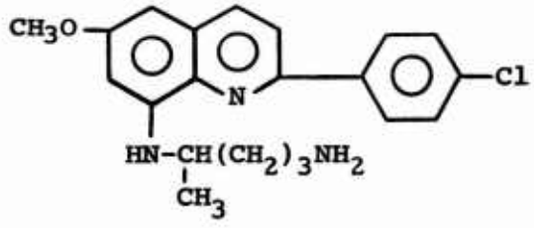
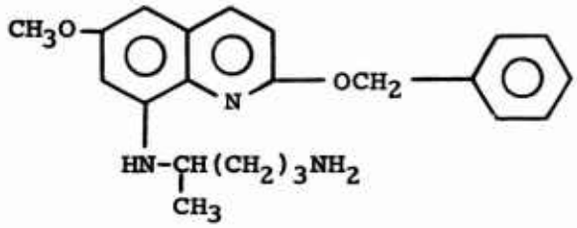
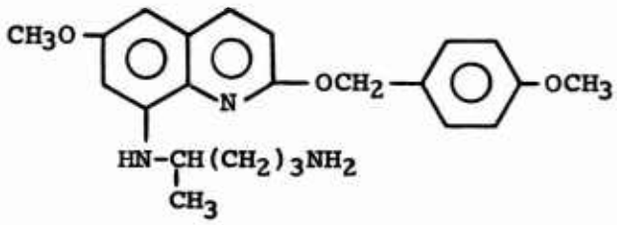
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
219,634		0.5 1.0	+ +	8 10	- -
121,508†		1.0 10.0	+ +	12 22	- -
106,147†		0.375 1.5 1.5 1.5 1.5 3.0 3.0	+ - - - - - -	11 - - - - - -	- + + + + + +
205,438†		1.0 10.0	+ +	7 8	- -

TABLE 11 - CONTINUED

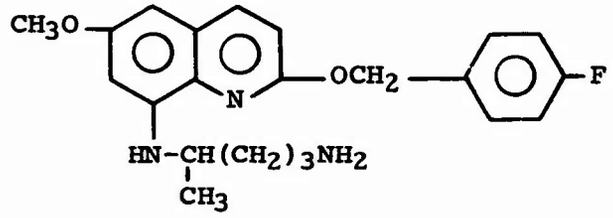
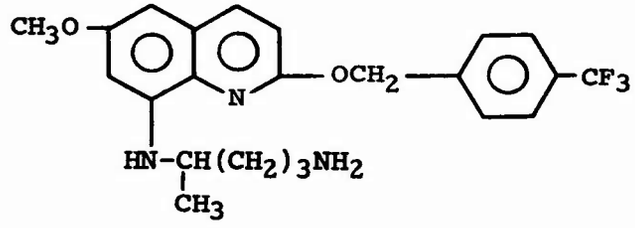
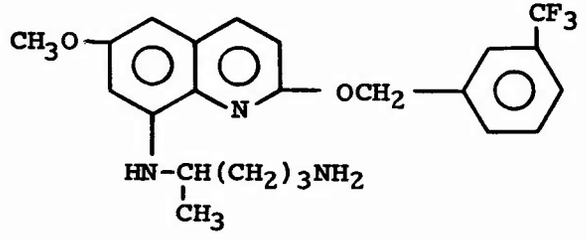
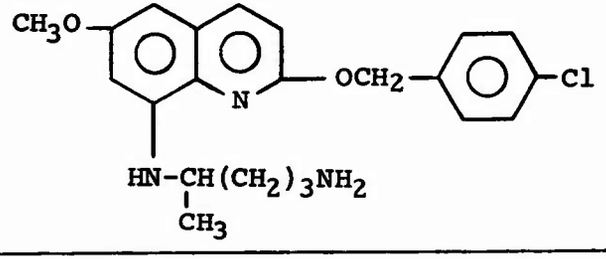
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
217,154†		0.25 0.25 0.25 0.5 0.5 1.0	+ + - + - -	5 16 - 91 - -	- - + - + +
217,124†		0.25 0.5 0.5 1.0 1.0	+ + - - -	9 5 - - -	- - + + +
219,635		0.5 1.0	+ +	6 13	- -
202,790†		1.0 10.0	+ Died Day 7 Post R_x - Hepatotoxicity	9	-

TABLE 11 - CONTINUED

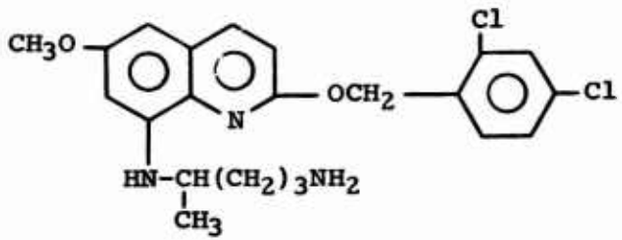
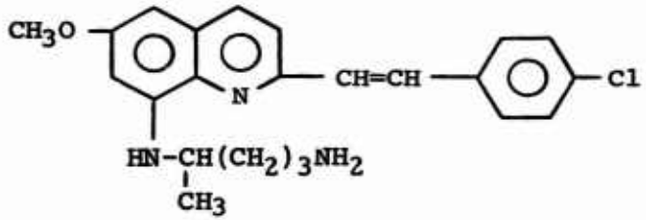
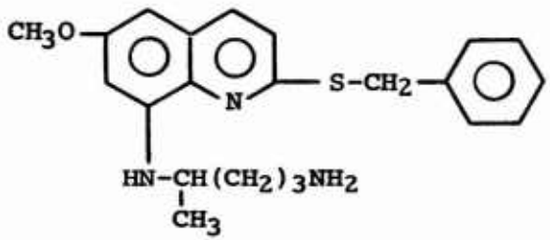
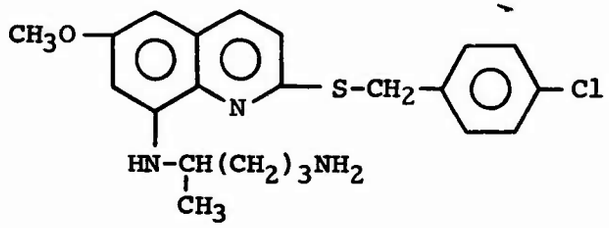
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
205,439†		0.5 1.0	+ -	8 -	- +
183,538†		1.0 10.0	+ -	15 -	- +
212,216†		0.5 1.0 1.0	+ + -	9 15 -	- - +
216,893†		0.5 10.0	+ +	7 14	- -

TABLE 11 - CONTINUED

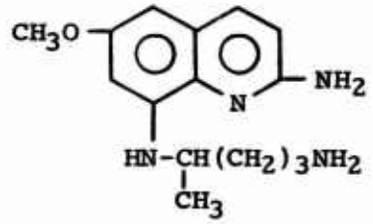
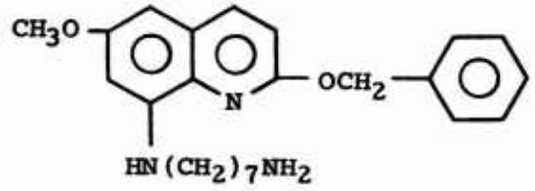
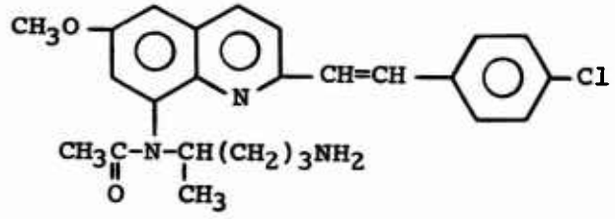
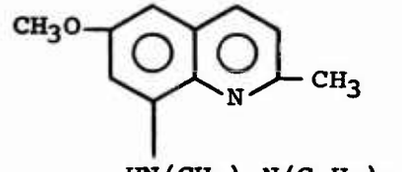
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
224,398		0.125 0.25 0.25 0.5	+ + - -	6 34 - -	- - ?(>42) +(>57)
199,368†		1.0	+	9	-
183,064†		1.0 10.0	+ +	5 9	- -
217,038†		0.5 1.0 1.0	+ + -	7 11 -	- - +

TABLE 11 - CONTINUED

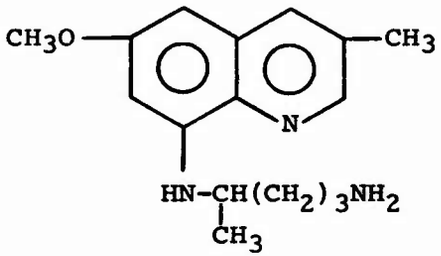
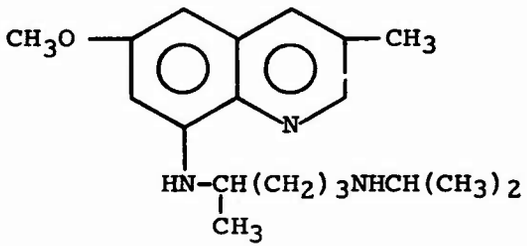
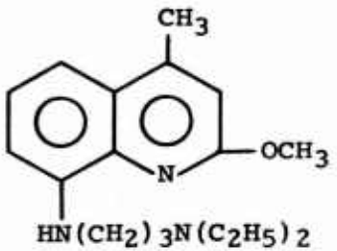
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 3 And 6					
211,814†		1.0 3.33 10.0 10.0	+ - - Died 6 hours after Dose 3 - Hepatotoxicity	17 - -	- + +
211,815†		1.0 10.0	+ -	10 -	- +
Derivative With Substituent At Positions 2 And 4					
211,820†		1.0 10.0	+ -	6 -	- +

TABLE 11 - CONTINUED

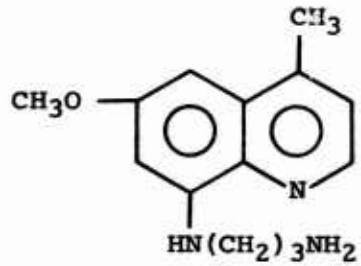
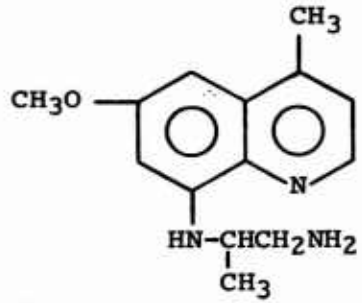
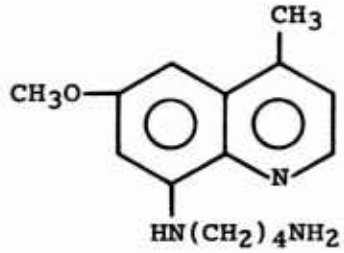
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 4 And 6					
206,027†		1.0 10.0	+ +	6 9	- -
147,778†		1.0 10.0	+ -	7 -	- +
136,479†		1.0 10.0	+ +	6 39	- -

TABLE 11 - CONTINUED

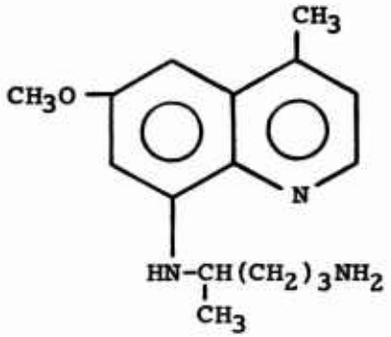
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
181,023†		0.125	+	7	-
		0.125	+	7	-
		0.125	+	10	-
		0.125	+	10	-
		0.125	+	11	-
		0.125	-	-	+
		0.25	+	14	-
		0.25	+	15	-
		0.25	+	16	-
		0.25	+	22	-
		0.25	+	27	-
		0.25	+	34	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	+	23	-
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+

TABLE 11 - CONTINUED

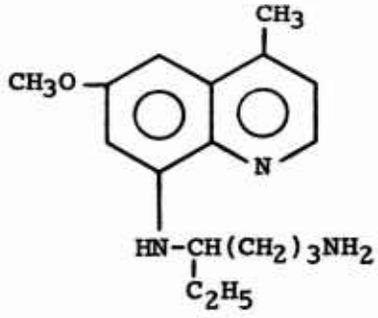
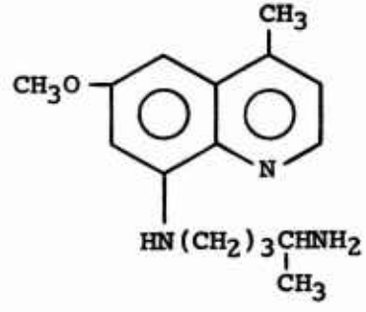
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
215,761†		0.0625	+	9	-
		0.0625	+	10	-
		0.125	+	18	-
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		1.0	-	-	+
215,296†		0.0625	+	19	-
		0.0625	+	23	-
		0.0625	+	54	-
		0.125	+	35	-
		0.125	+	35	-
		0.125	+	85	-
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	--	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+

TABLE 11 - CONTINUED

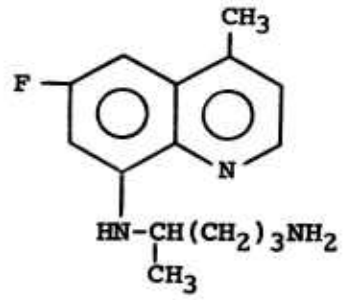
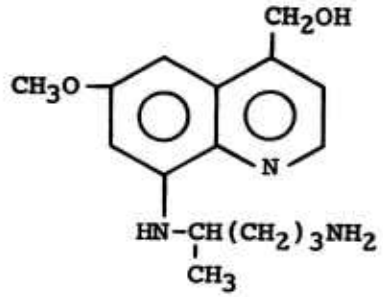
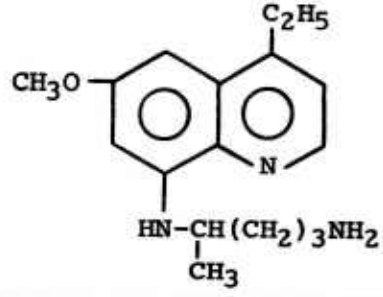
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
217,159†		0.125	+	8	-
		0.25	+	8	-
		0.5	+	7	-
		0.5	+	10	-
		0.5	-	-	+
		1.0	+	8	-
		1.0	+	10	-
		3.33	+	23	-
215,300†		1.0	+	13	-
		10.0	-	-	+
208,442†		0.125	+	9	-
		0.25	+	7	-
		0.25	+	80	-
		0.5	+	28	-
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	+	37	-
		1.0	-	-	+

TABLE 11 - CONTINUED

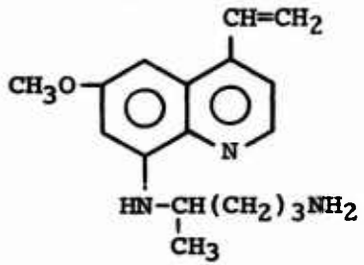
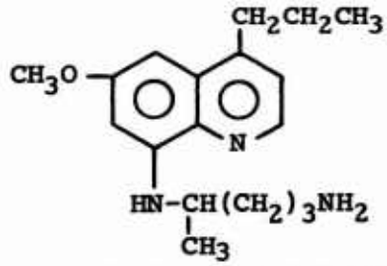
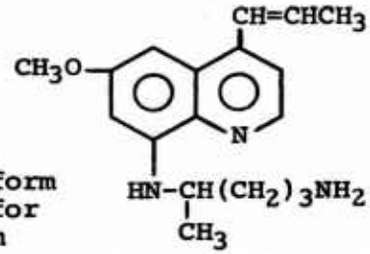
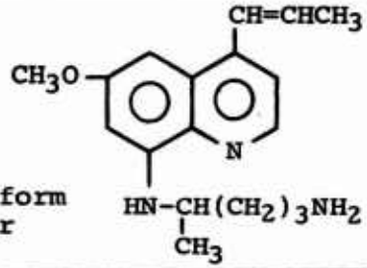
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
211,663†		0.25 0.5 0.5 1.0 1.0	+ + - + -	7 10 - 24 -	- - + - +
218,806†		0.5 1.0	+ +	7 23	- -
225,503	 N.B. "cis" form cf 218,636 for "trans" form	0.25 0.5 1.0	+ + +	7 6 6	- - -
218,636†	 N.B. "trans" form cf 225,503 for "cis" form	0.5 1.0	+ +	8 18	- -

TABLE 11 - CONTINUED

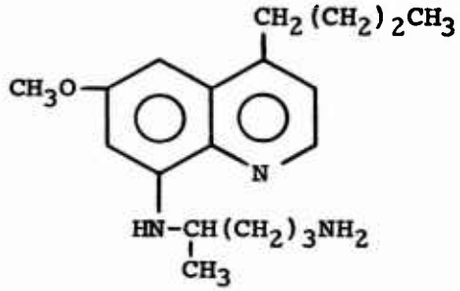
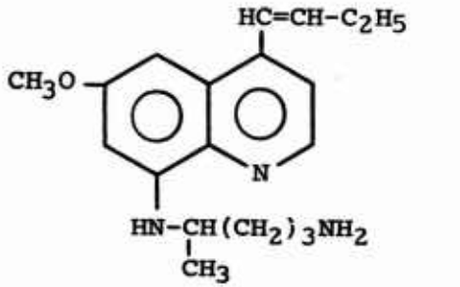
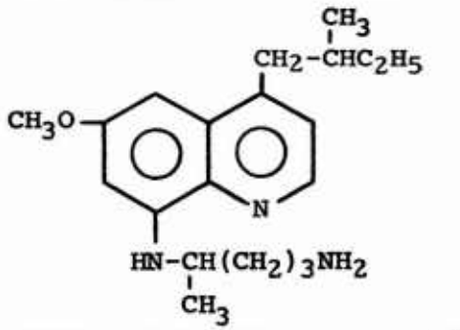
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
218,805†		0.5 1.0	+ +	5 22	- -
218,574†		0.5 1.0	+ +	6 10	- -
223,138		0.125 0.25 0.25 0.25 0.5 0.5 1.0 1.0	+ + + - + + + +	6 6 9 - 7 8 9 14	- - - + - - - -

TABLE 11 - CONTINUED

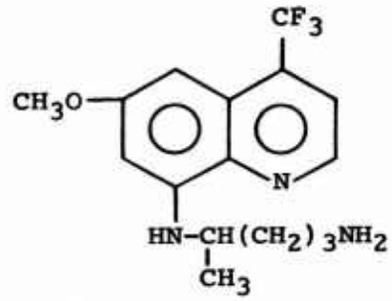
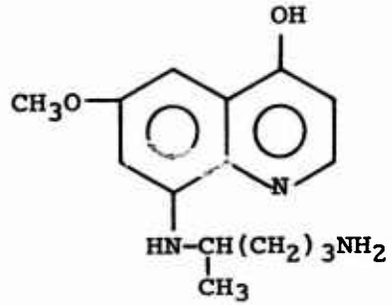
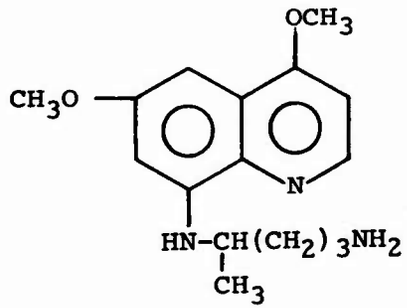
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
216,837†		0.5 10.0	+ +	7 7	- -
214,198†		1.0	+	9	-
217,271†		0.5 1.0	+ +	5 14	- -

TABLE 11 - CONTINUED

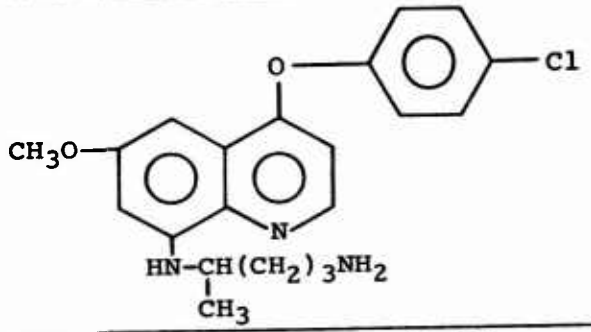
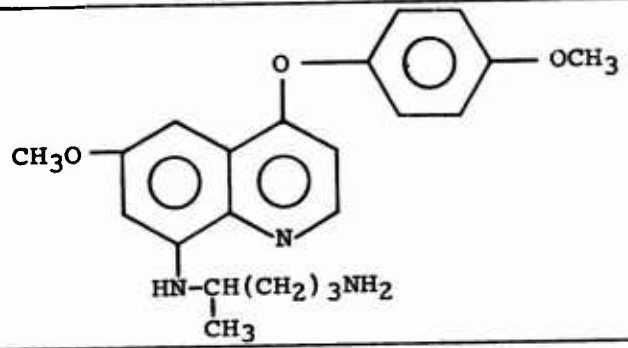
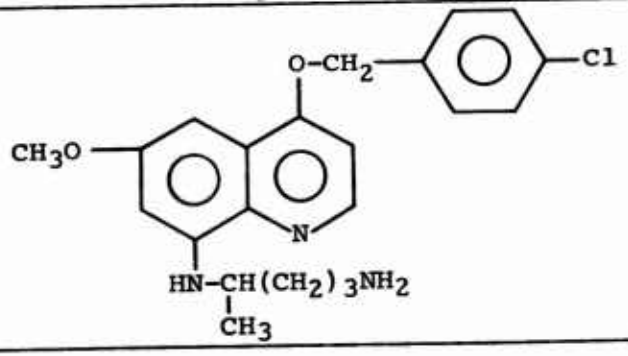
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
208,557†		0.5 1.0 10.0	+ + +	8 56 12	- - -
209,785†		1.0 10.0	+ +	13 7	- -
208,814†		1.0 10.0	+ +	11 13	- -

TABLE 11 - CONTINUED

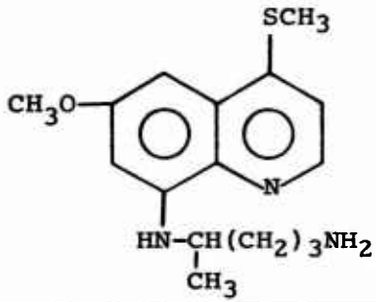
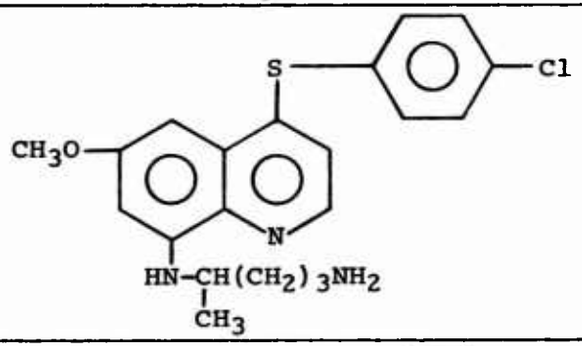
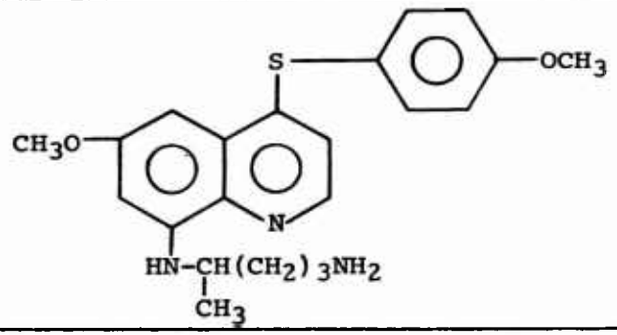
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
214,703†		1.0	+	9	-
209,522†		1.0 10.0	+ +	12 7	- -
209,521†		1.0 10.0	+ +	16 9	- -

TABLE 11 - CONTINUED

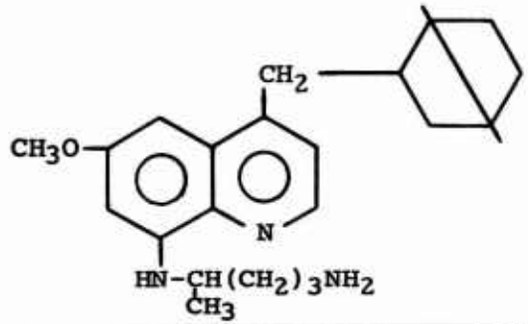
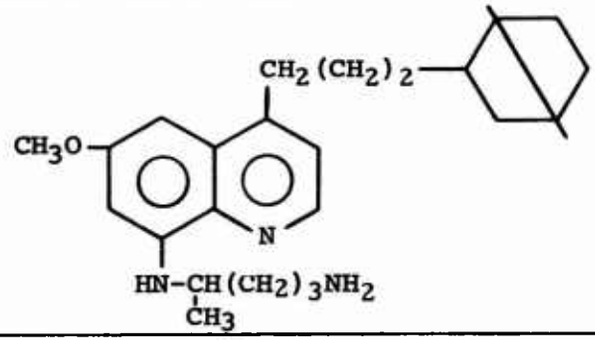
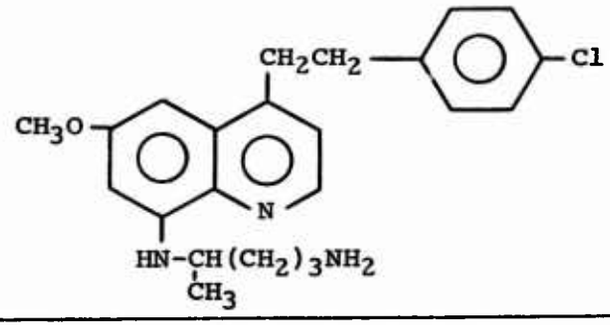
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
219,894		0.5 1.0	+ +	6 7	- -
220,226		0.5 1.0	+ +	6 8	- -
211,975†		1.0 10.0	+ +	7 16	- -

TABLE 11 - CONTINUED

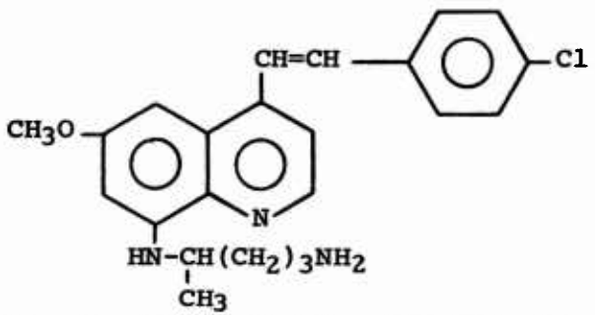
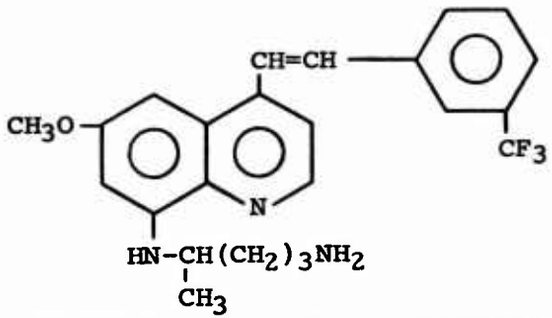
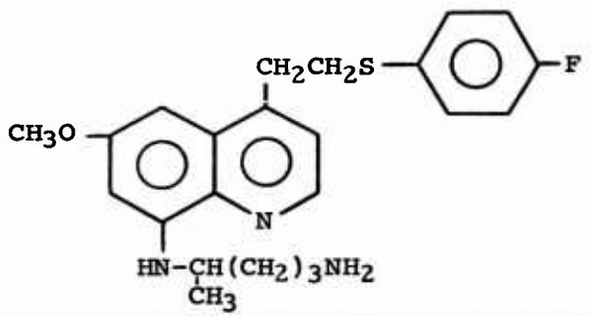
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
189,279†		1.0 10.0	+ +	7 21	- -
199,793†		1.0 10.0	+ -	7 -	- +
223,137		0.25 0.5 0.5 1.0 1.0	+ + - + +	13 7 - 6 7	- - + - -

TABLE 11 - CONTINUED

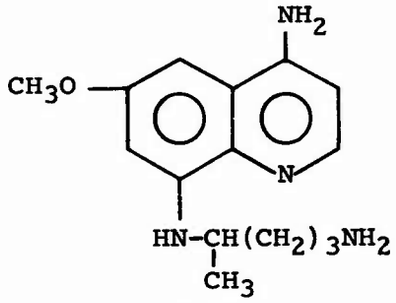
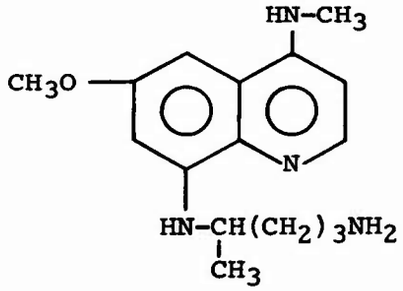
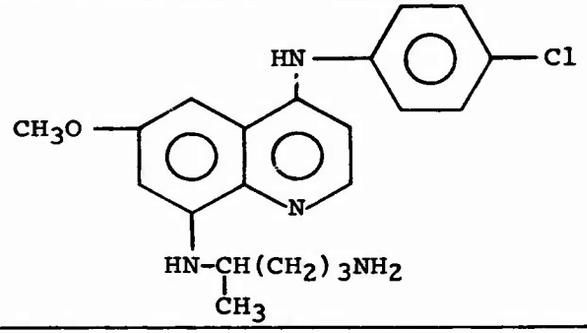
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
212,293†		0.5 1.0 1.0 1.0 1.0 3.33 10.0	+ + + + - + +	5 5 7 49 - 7 9	- - - - + - -
218,573†		0.5 1.0	+ +	6 10	- -
212,302†		1.0 10.0	+ +	11 13	- -

TABLE 11 - CONTINUED

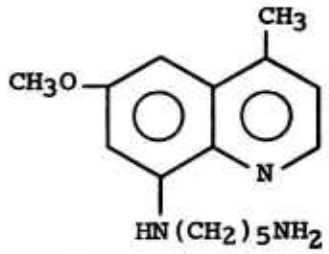
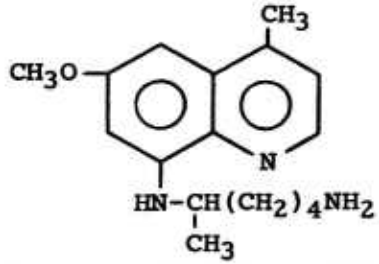
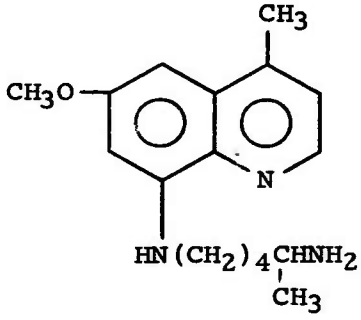
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
201,678†		1.0 10.0	+ +	10 13	- -
212,624†		0.5 0.5 0.5 0.5 1.0 1.0	+ + + - - -	13 20 21 - - -	- - - + + +
212,579†		0.0625 0.0625 0.0625 0.125 0.125 0.125 0.125 0.125 0.125 0.25 0.25 0.25 0.25 0.25 0.25 0.5 0.5 1.0	+ + + + + - - - - - - - - - - - - -	10 10 16 73 81 - - - - - - - - - - - - -	- - - - - + + + + + + + + + + + + +

TABLE 11 - CONTINUED

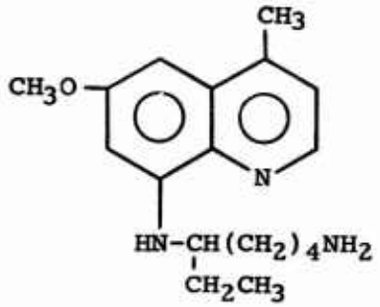
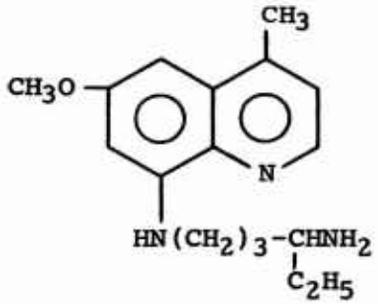
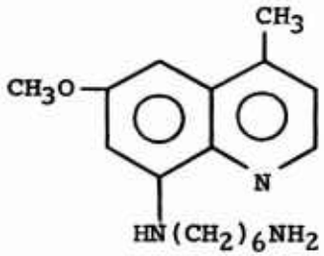
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
219,373		0.125 0.25 0.5 1.0	+ + + +	5 8 7 53	- - - -
218,335†		0.125 0.25 0.25 0.25 0.5 0.5 0.5 1.0	+ + + + + - - -	11 10 12 23 23 - - -	- - - - - + ++ +
225,742		0.25 0.5 1.0	+ + +	7 6 6	- - -

TABLE 11 - CONTINUED

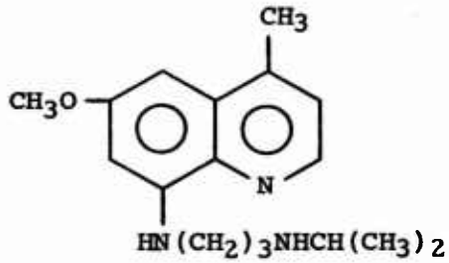
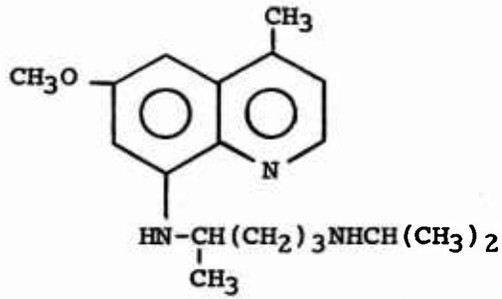
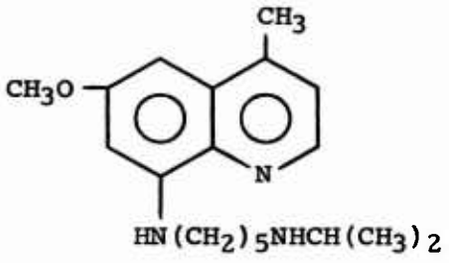
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
6,028†		0.125	+	9	-
		0.25	+	9	-
		0.5	+	12	-
		0.5	+	12	-
		0.5	+	14	-
		0.5	-	-	+
		1.0	+	13	-
		1.0	+	17	-
		1.0	-	-	+
		1.0	-	-	+
		2.0	-	-	+
6,027†		0.125	+	10	-
		0.25	+	11	-
		0.5	+	21	-
		1.0	+	9	-
		1.0	+	28	-
127,854		0.25	+	25	-
		0.5	+	22	-
		0.5	-	-	+
		1.0	-	-	+

TABLE 11 - CONTINUED

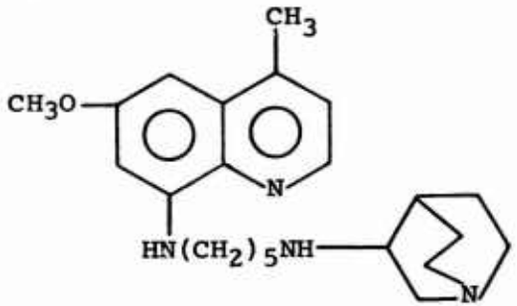
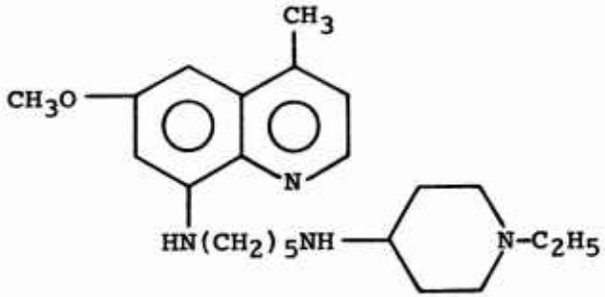
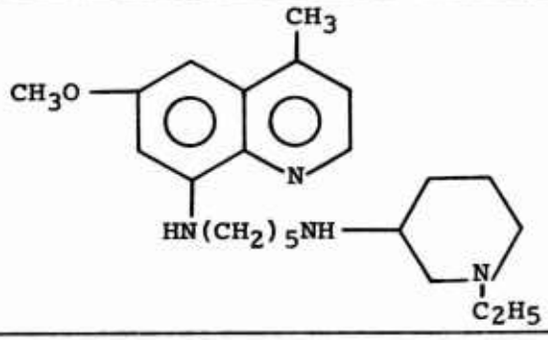
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
202,437†		1.0 1.0 3.33 10.0	+ + - -	16 19 - -	- - + +
203,607†		1.0 10.0	+ -	11 -	- +
203,608†		1.0 10.0	+ -	7 -	- +

TABLE 11 - CONTINUED

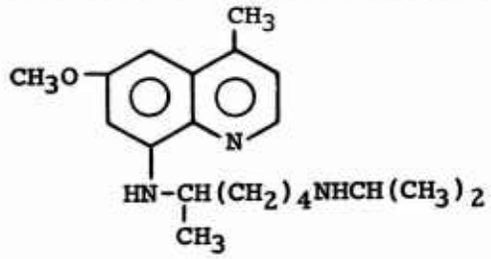
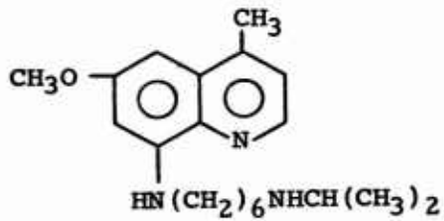
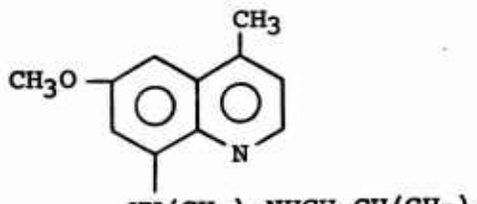
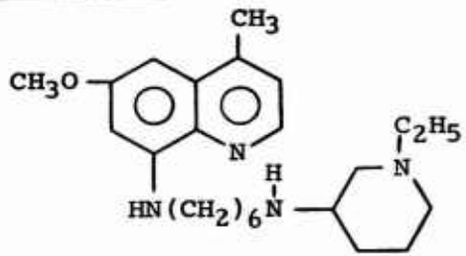
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
211,208†		0.25 0.5 0.5 1.0	+ - - -	15 - - -	- + + +
211,666		0.5 0.5 1.0 1.0	+ + - -	14 16 - -	- - + +
223,442		0.25 0.25 0.5 0.5 1.0	+ - + - +	7 - 8 - 28	- + - ?(>43) -
225,741		0.25 0.5 1.0	+ + +	7 6 16	- - -

TABLE 11 - CONTINUED

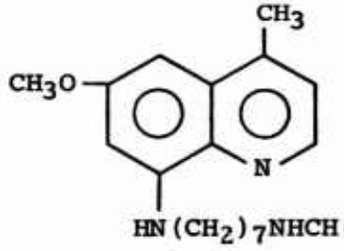
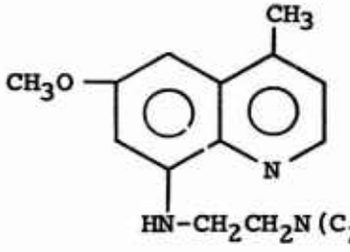
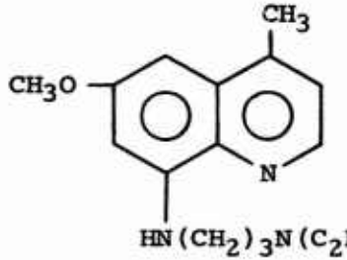
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
224,382		0.25 0.25 0.5 1.0	+ - + +	28 - 42 41	- + - -
211,665†		1.0 10.0	+ +	4 14	- -
147,657†		0.25 0.25 0.25 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 1.0 1.0	+ + + + + + + - - - - - - -	10 10 14 27 30 46 46 - - - - - - -	- - - - - - - + + + + + + +

TABLE 11 - CONTINUED

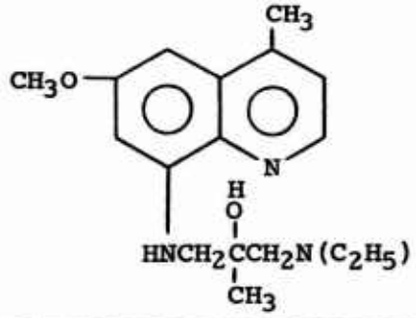
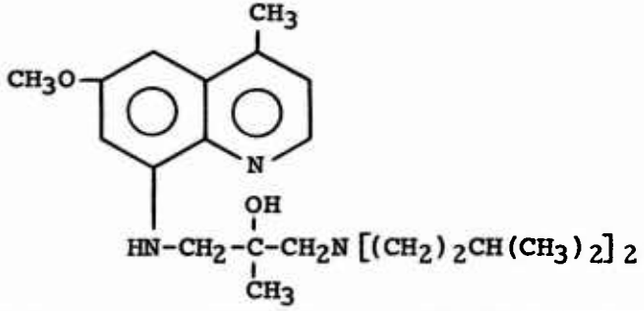
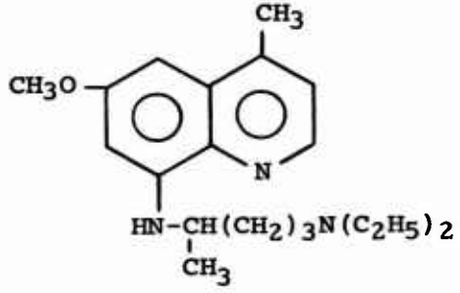
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
214,787†		1.0 10.0	+ -	15 -	- +
202,438†		1.0 3.33 10.0	+ - -	9 - -	- + +
211,816†		1.0 10.0	+ -	15 -	- +

TABLE 11 - CONTINUED

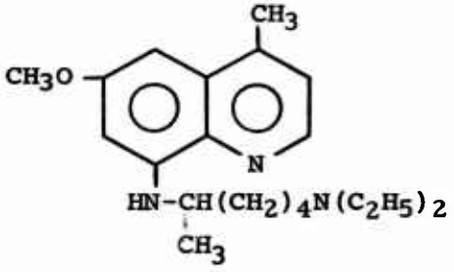
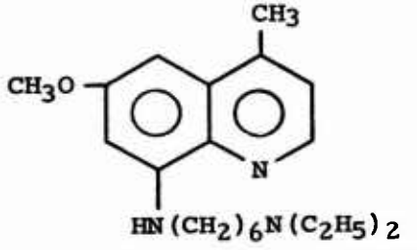
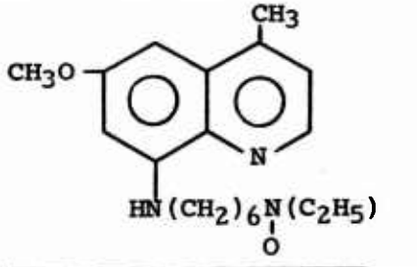
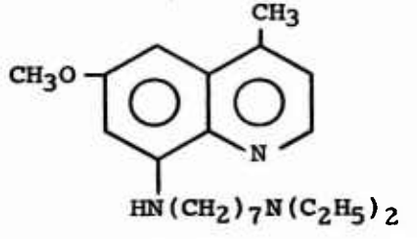
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
212, 223†		0.25 0.25 0.25 0.25 0.25 0.5 0.5 0.5 0.5 1.0	+ + + - - + - - - -	7 16 24 - - 19 - - - -	- - - + + - + + + +
6,026		0.25 0.5 1.0	+ - -	13 - -	- + +
225,845		0.25 0.5 1.0	+ + -	7 26 -	- - ?(>30)
223,658		0.125 0.25 0.5 1.0 1.0	+ + + - -	27 9 25 - -	- - - + +

TABLE 11 - CONTINUED

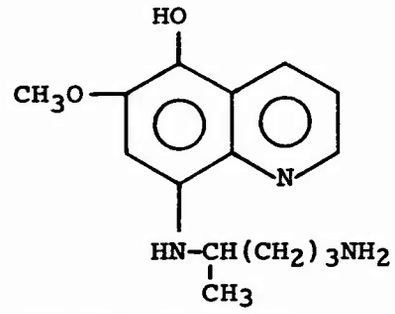
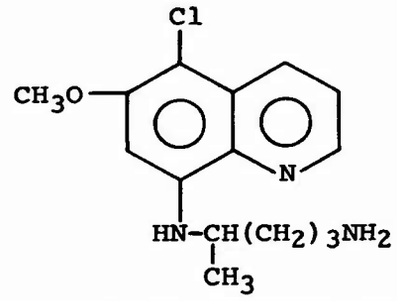
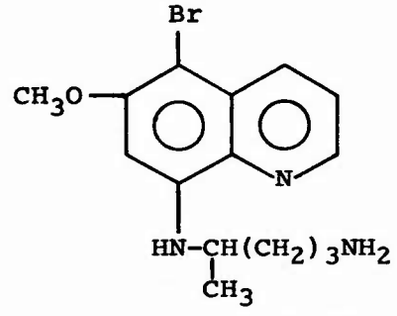
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 5 And 6					
199,507†		1.0 10.0	+ +	3 5	– –
194,333†		1.0 2.0 10.0	+ + –	8 22 –	– – +
200,073†		1.0 10.0	+ –	6 –	– +

TABLE 11 - CONTINUED

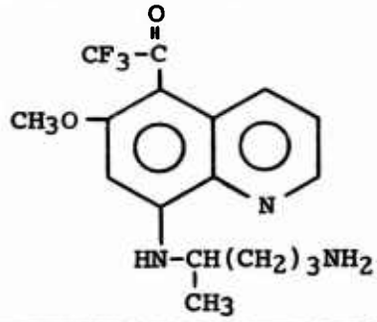
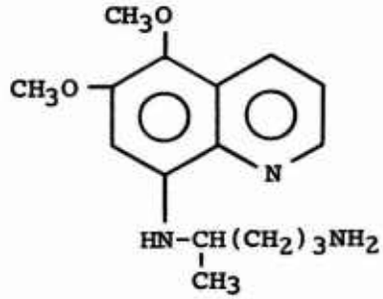
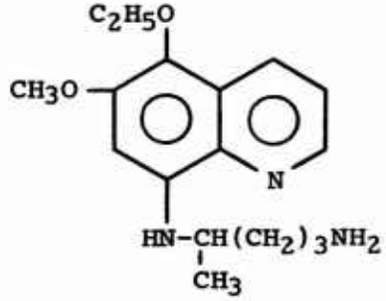
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
223,745		0.25	+	7	-
		0.25	+	13	-
		0.25	+	86	-
		0.5	+	13	-
		0.5	-	-	?(>43)
		0.5	-	-	+(>63)
		0.5	-	-	+(>82)
		1.0	-	-	+
5,990†		0.25	+	7	-
		0.5	+	17	-
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+
		1.0	-	-	+
218,676†		0.125	+	8	-
		0.125	+	8	-
		0.125	+	10	-
		0.125	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+

TABLE 11 - CONTINUED

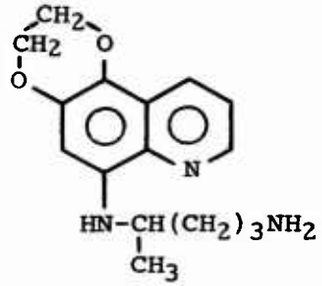
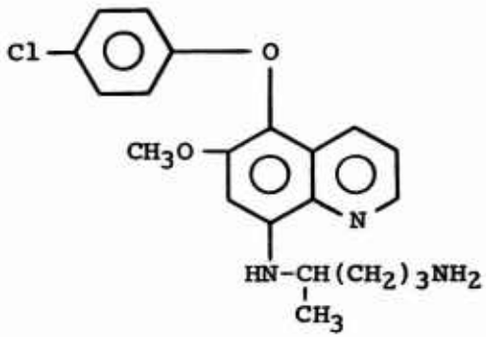
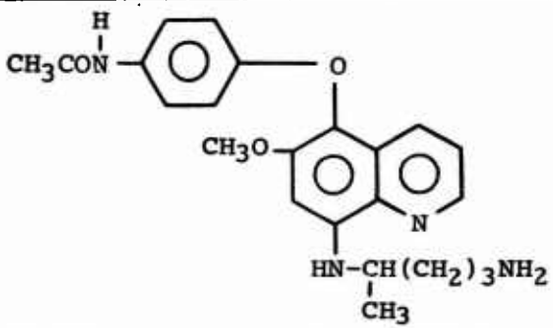
Compound		Daily Dose Mg Base/Kg Body Weight	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
184,118†		1.0 10.0	+ Died Day 1 Post R_x - Hepatotoxicity	5	-
182,232†		0.25 0.5 0.5 0.75 0.75 0.75 0.75 0.75 1.0 1.0 1.0 10.0	+ + + + - - - - - - - -	5 10 22 22 - - - - - - - -	- - - - + + + + + + + +
219,785		0.5 1.0	+ +	9 10	- -

TABLE 11 - CONTINUED

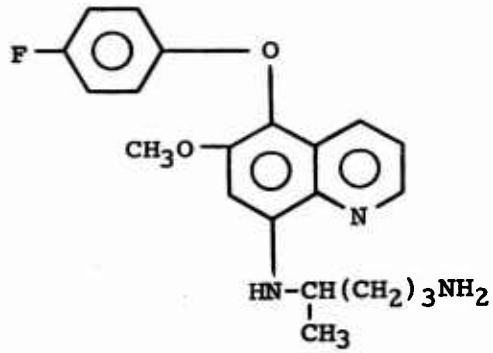
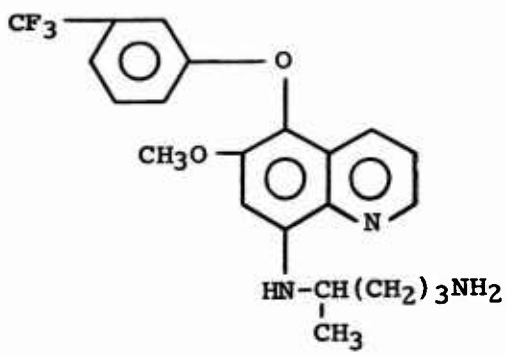
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between Rx and Relapse	Infection Cured
216,100†		0.125	+	8	-
		0.125	+	8	-
		0.125	+	9	-
		0.125	+	12	-
		0.125	+	19	-
		0.25	+	12	-
		0.25	+	18	-
		0.25	+	20	-
		0.25	+	55	-
		0.25	+	59	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		0.5	-	-	+
215,295†		0.125	+	12	-
		0.25	+	12	-
		0.25	+	20	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+

TABLE 11 - CONTINUED

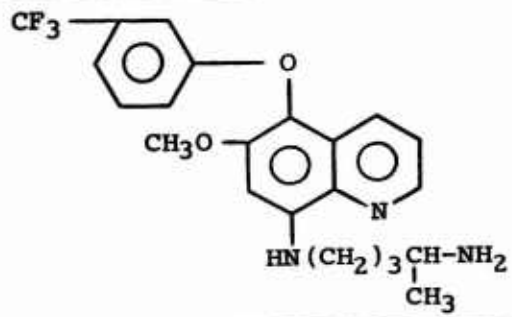
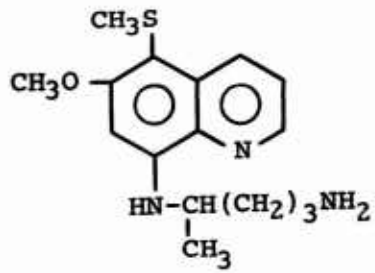
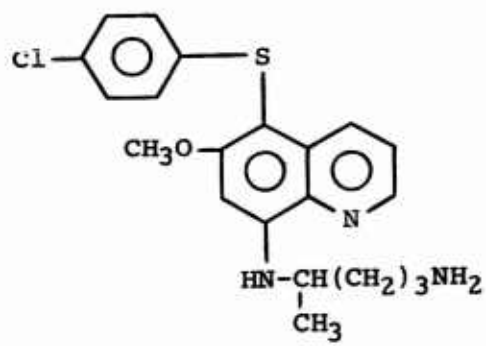
Compound		Daily Dose Mg Base/Kg* Body Weight	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
222,890		0.25 0.25 0.5 0.5 1.0	+ + + - -	6 56 14 - -	- - - + +
221,041		0.5 1.0	+ -	14 -	- +
183,489†		0.125 0.25 0.5 0.5 0.5 1.0 1.0 1.0 1.0 1.0 2.0 10.0 10.0	+ + + + + + + + + - - - -	8 9 11 14 24 16 24 57 59 - - - -	- - - - - - - - - + + + +

TABLE 11 - CONTINUED

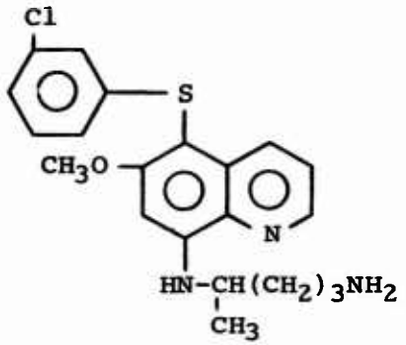
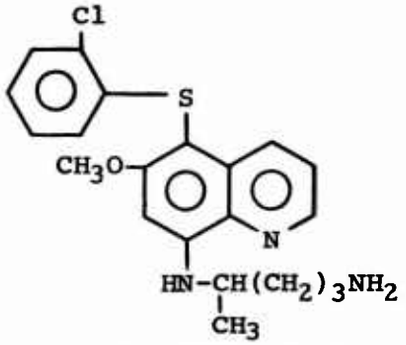
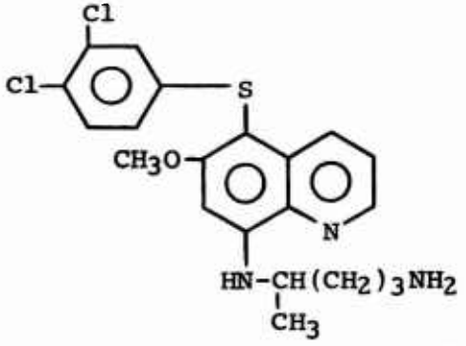
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
206, 428†		1.0 10.0	+ -	12 -	- +
209, 154†		1.0 10.0	+ -	15 -	- +
208, 189†		1.0 10.0	+ -	20 -	- +

TABLE 11 - CONTINUED

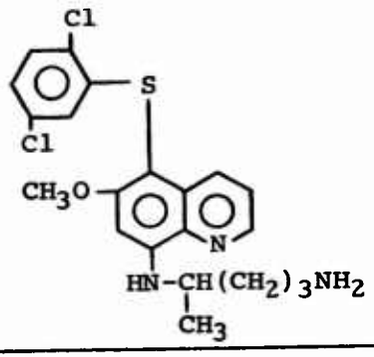
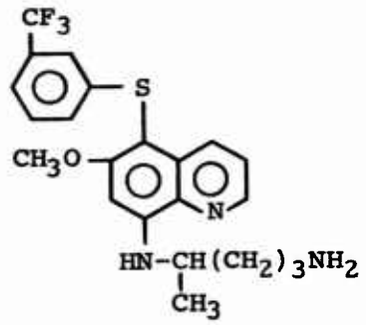
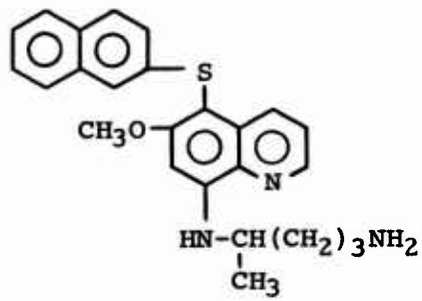
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
209,845†		1.0 10.0	+ -	12 -	- +
208,441†		0.5 1.0	+ -	30 -	- +
211,078†		1.0 10.0	+ -	27 -	- +

TABLE 11 - CONTINUED

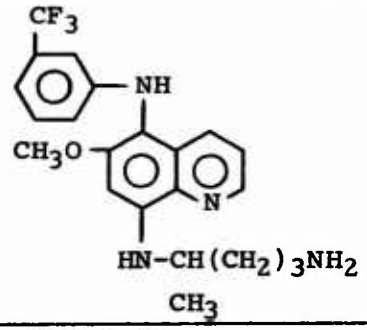
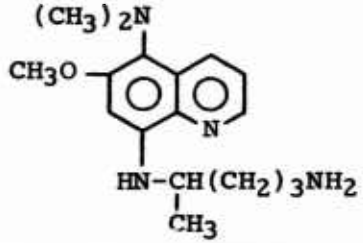
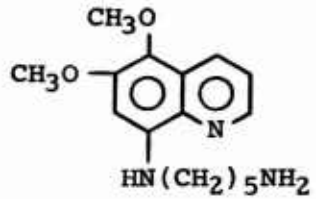
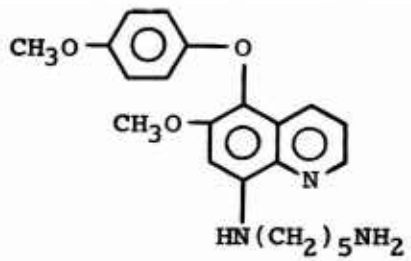
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
188,304†		1.0 10.0	+ +	7 8	- -
224,640		0.25 0.5 1.0	+ + +	7 6 13	- - -
207,610†		1.0 10.0	+ +	15 11	- -
194,341†		1.0 10.0	+ +	15 9	- -

TABLE 11 - CONTINUED

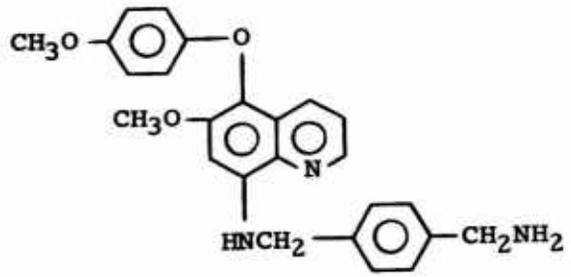
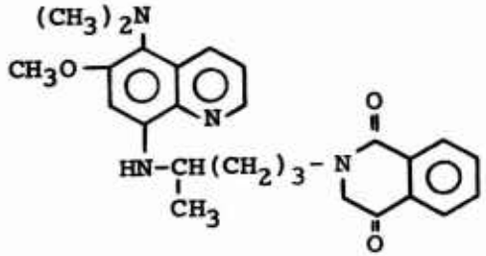
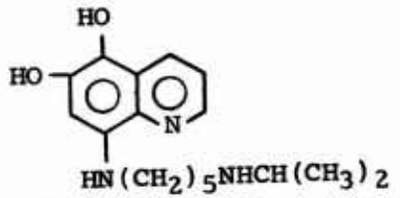
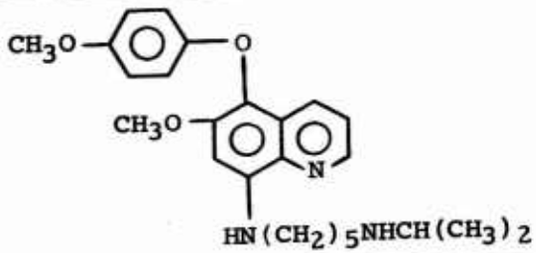
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between Rx and Relapse	Infection Cured
202,789†		1.0 10.0	+ +	6 7	- -
224,639		0.5 1.0	+ +	7 6	- -
49,577†		1.0 10.0	+ +	6 11	- -
194,343†		1.0 10.0	+ -	8 -	- +

TABLE 11 - CONTINUED

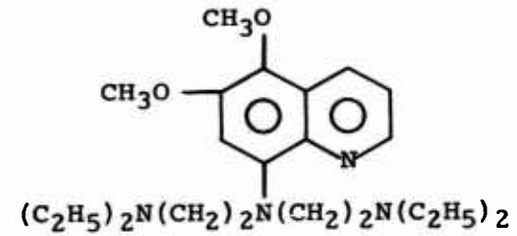
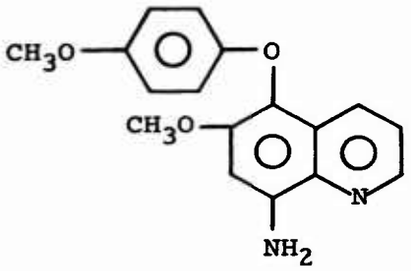
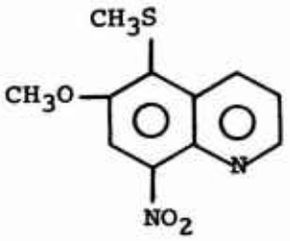
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
181,205†		1.0 10.0	+ +	2 5	- -
189,283†		1.0 10.0	+ +	6 1	- -
184,852†		1.0 10.0	+ +	6 7	- -

TABLE 11 - CONTINUED

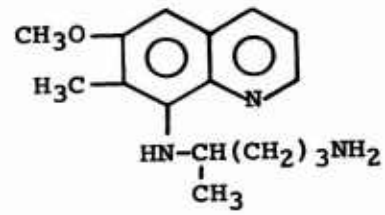
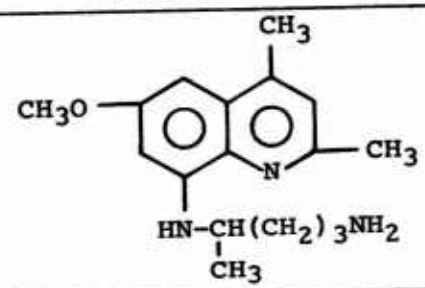
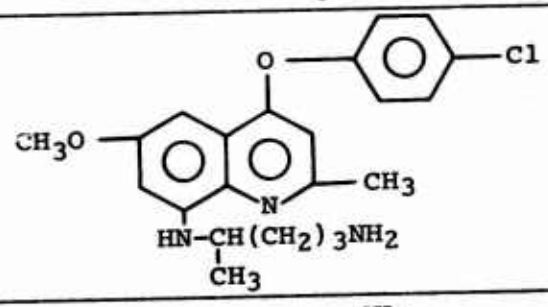
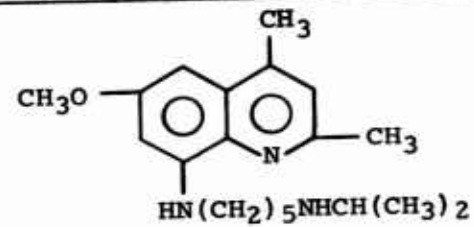
Compound WR-No.	Compound Structure	Daily Dose Mg Base/Kg Body Weight*	Response to Treatment: Relapse	Response to Treatment: Days Between R_x and Relapse	Response to Treatment: Infection Cured
Derivative With Substituent At Positions 6 And 7					
215,732†		1.0 10.0	+ +	4 8	- -
Derivatives With Substituents At Positions 2, 4, And 6					
192,515†		0.125 0.25 0.25 0.5 0.5 1.0 10.0	+ + + - - - Died Day 7 of R_x-Hepatotoxicity	5 9 11 - - -	- - - + + +
218,334†		0.5 1.0	+ +	8 14	- -
211,990†		1.0 10.0	+ Died Day 7 of R_x-Drug Toxicity	7	-

TABLE 11 - CONTINUED

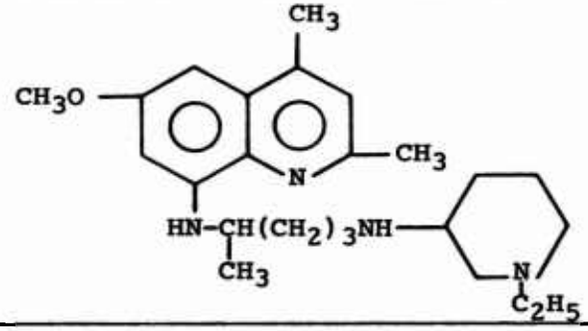
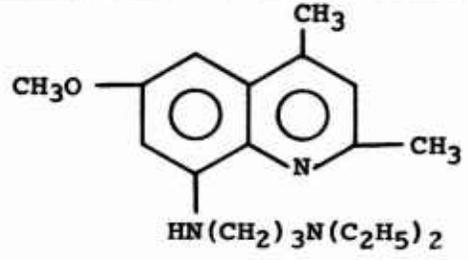
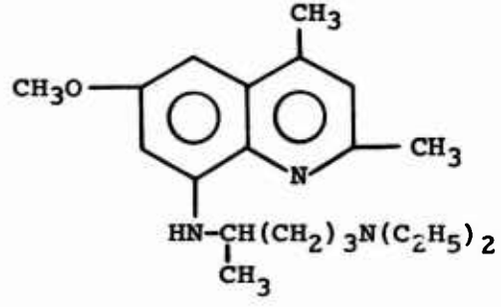
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
193,130†		1.0 3.33 10.0	+ - -	9 - -	- + +
211,533†		0.25 0.25 0.5 0.5 1.0	+ + - - -	7 12 - - -	- - + + +
197,063†		1.0 10.0	+ -	9 -	- +

TABLE 11 - CONTINUED

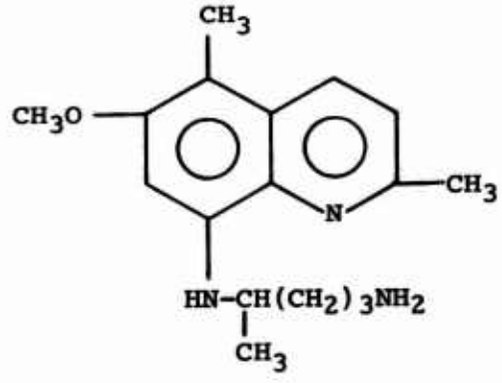
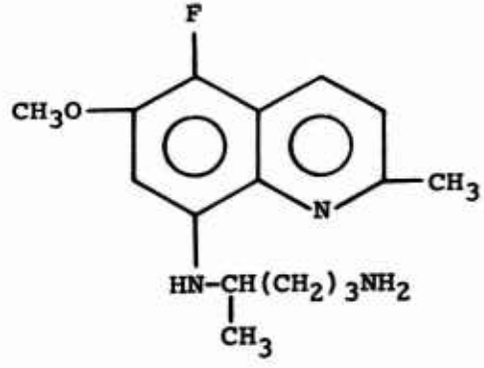
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 2, 5, And 6					
210,810†		1.0 10.0	+ -	15 -	- +
215,733†		0.125 0.125 0.25 0.25 0.25 0.5 0.5 1.0	+ + + - - - - -	22 23 27 - - - - -	- - - + + + + +

TABLE 11 - CONTINUED

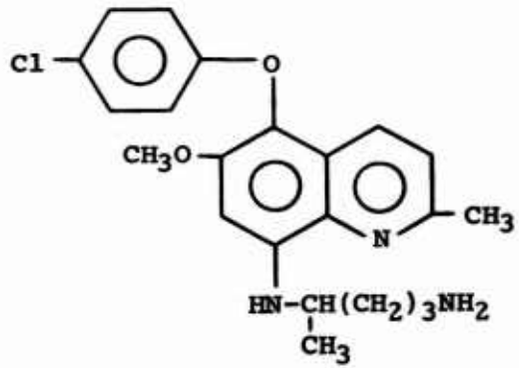
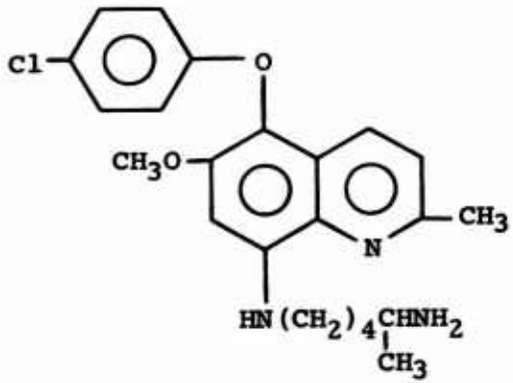
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
211,532†		0.125	+	12	-
		0.125	+	15	-
		0.125	+	33	-
		0.125	+	33	-
		0.125	-	-	+
		0.25	+	56	-
		0.25	+	74	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+
222,418		0.125	+	6	-
		0.125	+	53	-
		0.25	+	8	-
		0.5	+	26	-
		1.0	-	-	+
		1.0	-	-	+

TABLE 11 - CONTINUED

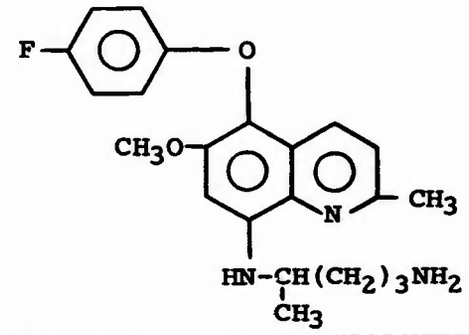
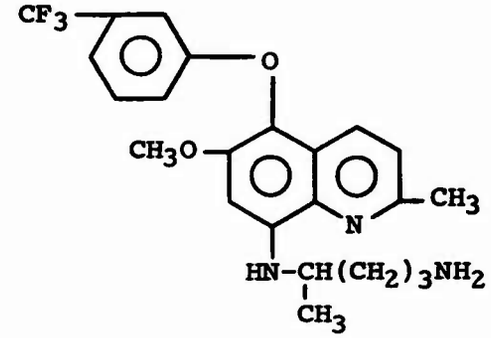
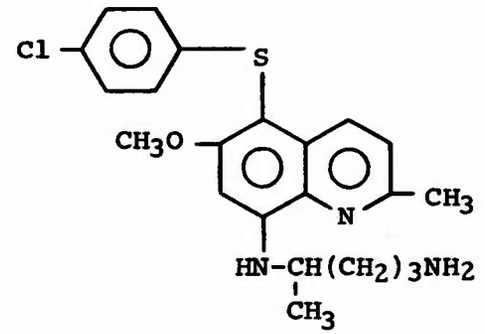
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
224,097		0.125 0.25 0.25 0.25 0.5 0.5	+ + - - - -	10 12 - - - -	- - ?(>39) + ?(>34) +
224,486		0.25 0.5 0.5	+ - -	7 - -	- ?(>43) +
210,805†		0.5 1.0 10.0	+ + -	8 41 -	- - +

TABLE 11 - CONTINUED

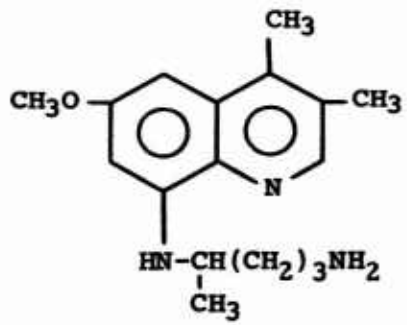
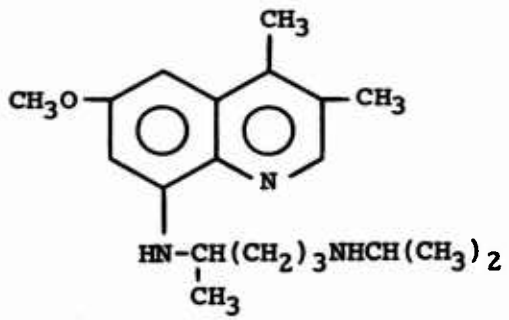
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 3, 4, And 6					
210,550†		0.5 1.0	+ -	8 -	- +
210,551†		1.0 10.0	+ -	13 -	- +

TABLE 11 - CONTINUED

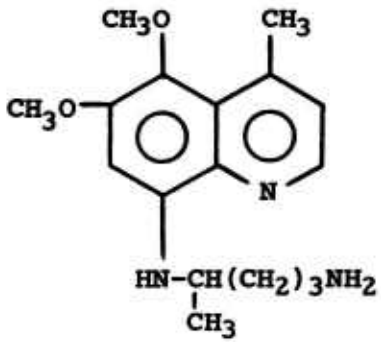
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 4, 5, And 6					
216,804†		0.0625	+	11	-
		0.0625	+	11	-
		0.0625	+	11	-
		0.0625	+	21	-
		0.0625	+	40	-
		0.125	+	13	-
		0.125	+	16	-
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.25	+	22	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+

TABLE 11 - CONTINUED

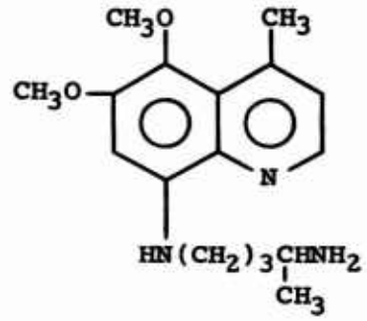
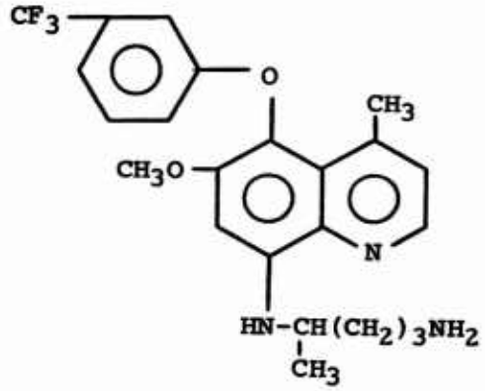
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
221,527		0.0625	+	9	-
		0.0625	+	12	-
		0.125	+	13	-
		0.125	+	21	-
		0.125	+	24	-
		0.125	+	26	-
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.125	-	-	+
		0.25	+	49	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
225,448		0.125	+	25	-
		0.125	-	-	?(>29)
		0.125	-	-	?(>29)
		0.25	-	-	?(>23)
		0.25	-	-	?(>26)
		0.25	-	-	+

TABLE 11 - CONTINUED

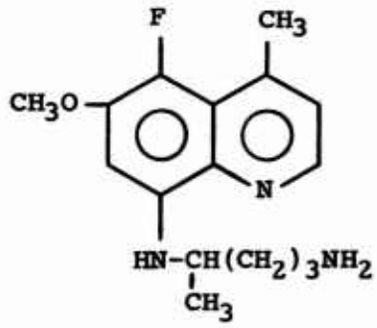
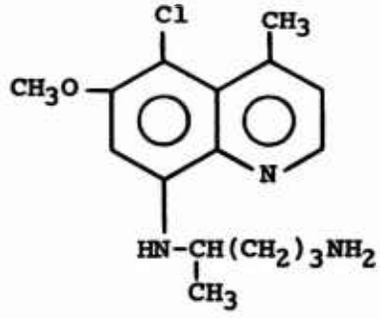
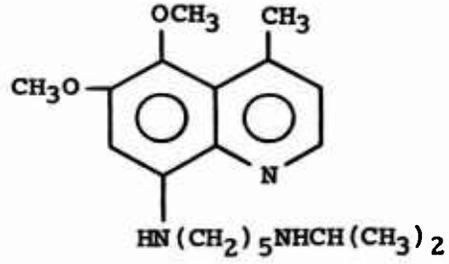
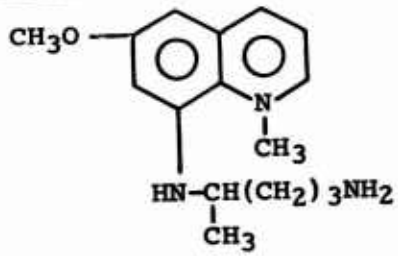
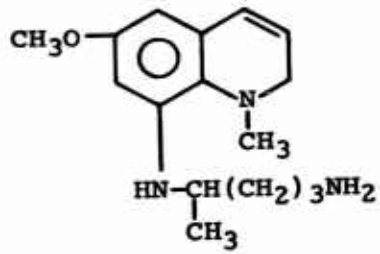
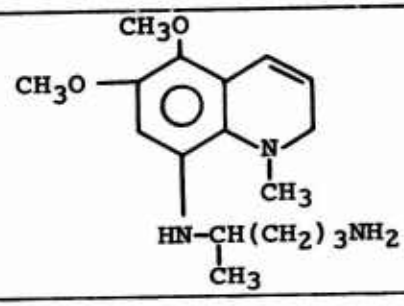
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_X and Relapse	Infection Cured
219,874		0.125 0.25 0.5 0.5 0.5	+ + - - -	5 5 - - -	- - + + +
218,681†		0.5 1.0	+ +	30 13	- -
219,423		0.5 1.0	+ -	29 -	- +

TABLE 11 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With N-Methyl Substituents					
204,510†		1.0 10.0	+ +	10 13	- -
208,071†		1.0 10.0	+ -	11 -	- +
210,448†		1.0 10.0	+ -	12 -	- +

*Dose administered via stomach tube, once daily for seven days with chloroquine at a dose of 2.5 mg base per kg body weight.

†Compounds examined for curative activity in previous Project years.

TABLE 12

THE CONTRIBUTIONS OF VARIOUS CLASSES OF NUCLEAR SUBSTITUTED 8-AMINOQUINOLINES TO THE ROSTER OF DRUGS WITH CURATIVE ACTIVITY AT DAILY DOSES OF 1.0 MG PER KG OR LESS

Position of Substituent on 8-Aminoquinoline Nucleus	No. of Derivatives				
	Total in Category	No. Exhibiting Curative Activity At Daily Dose - Mg/Kg Body Weight			
		Total 1.0 or <	1.0	0.5 or <	0.25 or <
2	2	0	0	0	0
6	29	3	2	1	0
2 and 4	1	0	0	0	0
2 and 6	22	9	4	5	1
3 and 6	2	0	0	0	0
4 and 6	60	17	6	11	4
5 and 6	31	9	3	6	2
6 and 7	1	0	0	0	0
2, 4, and 6	6	2	0	2	0
2, 5, and 6	7	5	1	4	3
3, 4, and 6	2	1	1	0	0
4, 5, and 6	6	5	1	4	3
N-Methyl	3	0	0	0	0
All categories	172	51	18	33	13

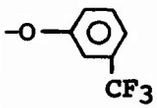
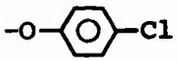
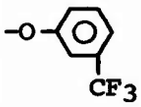
TABLE 13

STRUCTURAL FEATURES OF THE TWELVE MOST ACTIVE AGENTS AND SUMMARY OF RESULTS OF EXPANDED PILOT ASSESSMENTS OF THEIR CURATIVE ACTIVITIES

Data On Primaquine And 2-Methyl Primaquine (WR-182,234) Included For Reference Purposes

Compound WR- No. or Name	Substituent at Position					No. of Infections Cured/No. of Infections Treated					
						Daily Dose - Mg Base/Kg Body Weight*					
	8	6	5	4	2	0.0625	0.125	0.25	0.375	0.5	0.75
Primaquine (2,975)	$-NH-\underset{CH_3}{\underset{\vert}{CH}}(CH_2)_3NH_2$	$-OCH_3$	-	-	-	-	-	0/8	0/2	10/12	4/4
182,234	$-NH-\underset{CH_3}{\underset{\vert}{CH}}(CH_2)_3NH_2$	$-OCH_3$	-	-	$-CH_3$	-	0/3	2/8	-	9/10	-
222,671	$-NH(CH_2)_3\underset{CH_3}{\underset{\vert}{CH}}-NH_2$	$-OCH_3$	-	-	$-CH_3$	-	1/4	6/8	-	1/1	-
181,023	$-NH-\underset{CH_3}{\underset{\vert}{CH}}(CH_2)_3NH_2$	$-OCH_3$	-	$-CH_3$	-	-	1/6	8/14	-	13/14	-
215,296	$-NH(CH_2)_3\underset{CH_3}{\underset{\vert}{CH}}-NH_2$	$-OCH_3$	-	$-CH_3$	-	0/3	5/8	8/8	-	2/2	-
215,761	$-NH-\underset{C_2H_5}{\underset{\vert}{CH}}(CH_2)_3NH_2$	$-OCH_3$	-	$-CH_3$	-	0/2	6/7	4/4	-	1/1	-
212,579	$-NH(CH_2)_4\underset{CH_3}{\underset{\vert}{CH}}-NH_2$	$-OCH_3$	-	$-CH_3$	-	0/3	5/7	6/6	-	2/2	-

TABLE 13 - CONTINUED

Compound WR- No. or Name	Substituent at Position					No. of Infections Cured/No. of Infections Treated					
						Daily Dose - Mg Base/Kg Body Weight*					
	8	6	5	4	2	0.0625	0.125	0.25	0.375	0.5	0.75
218,676	$-NH-CH(CH_3)(CH_2)_3NH_2$	$-OCH_3$	$-OC_2H_5$	-	-	-	1/4	3/3	-	2/2	-
215,295	$-NH-CH(CH_3)(CH_2)_3NH_2$	$-OCH_3$		-	-	-	0/1	3/5	-	2/2	-
215,733	$-NH-CH(CH_3)(CH_2)_3NH_2$	$-OCH_3$	$-F$	-	$-CH_3$	-	0/2	3/4	-	2/2	-
211,532	$-NH-CH(CH_3)(CH_2)_3NH_2$	$-OCH_3$		-	$-CH_3$	-	1/5	5/7	-	2/2	-
216,804	$-NH-CH(CH_3)(CH_2)_3NH_2$	$-OCH_3$	$-OCH_3$	$-CH_3$	-	0/5	11/13	3/4	-	1/1	-
221,527	$-NH(CH_2)_3CH(CH_3)-NH_2$	$-OCH_3$	$-OCH_3$	$-CH_3$	-	0/2	6/10	4/5	-	1/1	-
225,448	$-NH-CH(CH_3)(CH_2)_3NH_2$	$-OCH_3$		$-CH_3$	-	-	2(?)/3	3(?)/3	-	-	-

*Dose administered via stomach tube, once daily for seven days, with chloroquine at a dose of 2.5 mg base per kg body weight.

IV. STUDIES ON THE IND PREPARATION OF WR-181,023 (LOT AG: BE-50,003)

IV. STUDIES ON THE IND PREPARATION OF WR-181,023 (LOT AG: BE-50,003)

The results of pilot appraisals of the curative activity of WR-181,023 (4-methyl primaquine), together with the results of supplemental evaluations of both curative and prophylactic activities, led to serious consideration of this 8-aminoquinoline for IND study in human volunteers. In order to facilitate this consideration, a batch lot of compound was synthesized. This preparation, designated WR-181,023-AG (BN: BE-50,003) and tentatively termed the IND lot, was subjected to a series of studies aimed at determining: (a) whether its curative activity equalled the activities of lots prepared previously; (b) the influence of the dosage regimen on activity; and (c) whether curative responses could be obtained regularly with a dosage schedule of no more than three days. The results of these investigations, together with results of earlier evaluations, were summarized in a special report (SORI-KM-76-058, January 31, 1976). This summary is set forth in its entirety in the following pages of this Annual Report.

SORI-KM-76-058

SUMMARY OF STUDIES CARRIED OUT UNDER
CONTRACT NO. DADA 17-69-C-9104

ON

WR-181,023 (4-METHYL-PRIMAQUINE)

RADICAL CURATIVE AND PROPHYLACTIC ACTIVITIES AS EXHIBITED AGAINST INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI WITH SPECIAL EMPHASIS ON THE PROPERTIES OF WR-181,023-AG (BE-50,003)

Southern Research Institute
2000-Ninth Avenue South
Birmingham, Alabama 35205
January 31, 1976

Project 2284-XXV

WR-181,023 (4-METHYL-PRIMAQUINE)

RADICAL CURATIVE AND PROPHYLACTIC ACTIVITIES AS EXHIBITED AGAINST INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI WITH SPECIAL EMPHASIS ON THE PROPERTIES OF WR-181,023-AG (BE-50,003)

INTRODUCTORY COMMENT

Development of a drug or drugs which would prevent and cure naturally acquired infections with Plasmodium vivax, and be more generally acceptable and more easily administered than primaquine, has been one of the two major missions of the Malaria Chemotherapy Program of the Department of the Army. In pursuing this mission, sizeable numbers of compounds have been procured or synthesized and tested for radical curative activity in rhesus monkeys infected with sporozoites of P. cynomolgi.* Pilot assessments in this animal model, covering representatives of many chemical classes, indicated that curative activity was restricted to 6-aminoquinoline and 8-aminoquinoline derivatives. All active representatives of the former class suffered from low therapeutic indices. The 8-aminoquinolines appeared to offer greater promise,

*As originally projected, this search was to have been carried out in owl monkeys infected with sporozoites of P. vivax. This approach was abandoned after a two-year effort because of the variability in the evolution of such infections, and because many were cured with chloroquine alone. Attention was then turned to the well-documented P. cynomolgi-Anopheles quadrimaculatus or Anopheles freeborni-Macaca mulatta model which has been shown to be a reasonably precise biologic and chemotherapeutic replica of human infections with P. vivax. This model was used extensively during the post-World War II search for a radical curative drug and identified primaquine as the most effective of the then available 8-aminoquinolines.

there being a modest number of derivatives which exhibited sufficient activity to merit expanded evaluation. By the end of 1974, at least four agents had been identified with curative activity equal to or greater than that of primaquine. WR-181,023,* the 4-methyl analog of primaquine, appeared to be the most promising of the group. In a dose-for-dose comparison, this 8-aminoquinoline was approximately twice as active as primaquine in curing established sporozoite-induced infections and in preventing development of infections subsequent to sporozoite inoculation.

This exhibition of superior radical curative and prophylactic properties led to the decision to prepare WR-181,023 for evaluation in human volunteers infected with P. vivax. A batch lot of the compound, designated WR-181,023-AG (BE-50,003), was synthesized for this purpose and for preclinical toxicologic clearance studies. In keeping with the practices of the Malaria Chemotherapy Program, this Lot,

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*WR-181,023 [4-methyl-6-methoxy-8-(4-amino-1-methylbutylamino)-quinoline] although newly synthesized in the current Malaria Chemotherapy Program is not a new compound. It had been prepared in late 1949 under the code number CN 1101 by Elderfield and coworkers, Columbia University, during systematic study of the radical curative properties of various 4-methyl-substituted 8-aminoquinolines. On December 7, 1949, it was submitted to Schmidt and coworkers, The Christ Hospital Institute for Medical Research, for assessments of radical curative and prophylactic activities in rhesus monkeys infected with sporozoites of the M strain of P. cynomolgi. The results of these evaluations, reported in late 1950 (cf Appendix Table 1), led to the conclusion that CN 1101 was clearly superior to primaquine (and all other 8-aminoquinoline derivatives tested to that time) with respect to either radical curative or prophylactic activities. Despite general interest in this conclusion and the supporting data, CN 1101 was not evaluated in human volunteers. Such studies were actually discouraged because of the recent Korean experience which suggested that primaquine met existing needs for a curative drug and because research on malaria chemotherapy was being de-emphasized and funds for evaluating a new agent in man were not available.

identified as the IND preparation, was also assessed for therapeutic and prophylactic activities to insure that its antimalarial potencies were identical with those of earlier preparations. In addition, this preparation was evaluated in a series of dosage regimen experiments aimed at determining the feasibility of reducing the duration of treatment required for cure, an important goal of the Malaria Chemotherapy Program.

This Report, to be incorporated with other items in an IND application, summarizes the scope and results of the diverse therapeutic and prophylactic studies carried out on various preparations of WR-181,023. Special emphasis is given to the data acquired on Lot AG (BE-50,003).

METHODS AND PROCEDURES

The assessments of the radical curative and prophylactic properties of WR-181,023 described in this Report were carried out according to well-established, fully documented procedures which will be referred to only briefly here. Directly imported, locally conditioned, tuberculin negative, subadult rhesus monkeys, weighing from 3.5 to 5.5 kg at the beginning of study, were used in all experiments.*

*Prior to assignment of monkeys to any experiment, thick blood films stained with Giemsa were prepared and searched thoroughly in order to rule out the possibility of introducing subjects with naturally acquired infections. This might be considered an unnecessary exercise since only 5 of more than 2400 rhesus monkeys received between 1969 and 1976 have had such infections. These 5 infected monkeys were derived from two shipments of 50 each, probably reflecting the locale(s) in which the members of these shipments were trapped. Although the benefits of pre-inoculation examinations appear to be few, the security which they provide against waste, because of use of naturally infected monkeys, justifies the small effort.

The B strain of P. cynomolgi was utilized for all appraisals of antimalarial activity. This strain, acquired on May 29, 1959, has been maintained since that date by serial monkey-to-mosquito-to-monkey passages. A strain of Anopheles freeborni, provided in 1957 by Dr. Burgess, Columbia, South Carolina, served as the insect vector.

Assessments of both radical curative and prophylactic activities were performed in groups of from 8 to 42 rhesus monkeys. These subjects were inoculated intravenously (via the mid-saphena) with a suspension of 5×10^5 to 2×10^6 sporozoites derived from lots of heavily infected A. freeborni (20 to 80 oocysts per gut) which had fed 13 to 15 days previously on non-treated infected monkeys bearing gametocytes. The sporozoite suspension was prepared by grinding the thoraces of the requisite numbers of mosquitoes in 4.0 to 8.0 ml of 1:1 normal monkey serum-saline. Gross particulates were sedimented by centrifugation at 1000 rpm for one minute. The supernatant containing the sporozoites was removed by aspiration and diluted with iced monkey serum-saline to give a final volume such that each ml contained the sporozoites derived from ten mosquitoes. The numbers of sporozoites in this volume were measured by conventional counting procedures. Inoculation of the monkeys was always completed within 20 to 50 minutes of the time of preparation of the suspension.

In both types of assessments, parasitologic examinations (utilizing Giemsa stained thick and thin blood films) were initiated on Day 7 after sporozoite challenge (counting the day of inoculation as Day 0). In assessments of curative activity, these examinations were repeated daily until densities of 10 to 40 parasites per 10^4 erythrocytes were attained. Drug treatment was started on that day and repeated daily thereafter for as long as was required by the chosen dosage regimen. Parasitologic examinations were carried out on

alternate days throughout the treatment period and/or until at least three successive negative thick films were obtained. At that point, examinations were reduced to a twice weekly schedule (Monday-Thursday or Tuesday-Friday) and maintained there for the next four weeks unless relapse occurred. Weekly examinations were performed during the ensuing ten weeks, at which time (ca 100 to 110 days after the last positive film) the infections were considered cured* and the monkeys placed in a discard pool for use either locally or elsewhere in studies not prejudiced by previous malarial infection.

In assessments of prophylactic activity, parasitologic examinations were made daily until parasitemia appeared, or in the absence of parasitemia, for 28 consecutive days after the infection in the untreated control became patent. Thereafter, films were examined twice weekly for an additional four to seven weeks. At that time, monkeys with consistently negative blood films were rechallenged by the intravenous inoculation of 5×10^5 sporozoites. Without exception, such rechallenges led to patency eight to nine days after reinoculation, thus demonstrating full susceptibility to infection.

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*Prior to 1964, all treated monkeys were splenectomized 90 days after the last positive blood film and were subjected to continuing parasitologic examinations for 30 days post-splenectomy. Absence of parasites in the peripheral blood throughout the pre- and post-splenectomy intervals was considered proof that infection had been cured. Systematic review of the data on approximately 8000 treated monkeys, infected with either the M or B strains of P. cynomolgi or their drug-resistant variants, showed that splenectomy added nothing to information developed prior to spleen removal. Therefore, failure to relapse after splenectomy has been abandoned as the ultimate yardstick of cure. It is still used to ascertain whether untreated infections have undergone self-cure or are merely quiescent since splenectomy will evoke relapses in approximately 20 per cent of untreated monkeys who have had consistently negative blood films for 80 or more days.

WR-181,023 and primaquine were administered via the oral route. The appropriate base equivalents of these compounds, dissolved in 30 ml of distilled water, were delivered via stomach tube, followed by a 20 ml distilled water rinse. In all assessments of radical curative activity, chloroquine was administered concomitantly in daily doses of 2.5 mg per kg body weight for seven-day curative drug regimens and in the early appraisals of the benefits of one to four-day regimens. In later appraisals of the benefits of single-day and three-day regimens, chloroquine was administered in doses of 5.84 mg per kg body weight on three consecutive days*.

Three different preparations of WR-181,023, diphosphate salt, were used in the studies summarized in this Report: WR-181,023-AC (BC-57,244); WR-181,023-AE (BD-57,427); and the IND lot, WR-181,023-AG (BE-50,003). Each preparation had a calculated base content of 58.2 per cent. A single preparation of primaquine, diphosphate salt, was employed. This lot, a gift of Sterling Winthrop Research Institute, carried the designation N-698BB, with base content of 56.9 per cent. The preparation of chloroquine, as the diphosphate salt with a base content of 62 per cent, was also a gift of the Sterling Winthrop Research Institute and bore the designation N-122ZL.

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*It should be stressed that the sole function of chloroquine is to eliminate the blood schizonts. It is entirely without activity against the exoerythrocytic parasites responsible for relapse of infections with P. cynomolgi and P. vivax. A total dose of 17.5 mg per kg, administered in either three or seven daily fractions, regularly cures trophozoite-induced infections with the B strain of P. cynomolgi.

RESULTS

The results of various assessments of the curative and prophylactic activities of WR-181,023, the comparative efficacies of this 8-aminoquinoline and primaquine, and the influences of the dosage regimen on activity have been set forth in detail in Appendix Tables 2 to 9. These tables provide precise information on the date of each evaluation, the drug preparation employed, the monkey treated, and the status of the animal in terms of previous therapy*, as well as dose, therapeutic regimen, and end result. These detailed data have been condensed into Summary Tables 1 to 8, the contents of which form the basis of the discussion that follows. For obvious reasons, this discussion has been divided into two compartments; the first relating to the primary evaluations of the antimalarial properties of WR-181,023 based on use of preparations BC-57,244 and BD-57,427, the second relating to similar and complementary studies on the activities of the IND preparation, BE-50,003.

A. Primary Evaluation Of The Radical Curative And Prophylactic Activities Of WR-181,023

1. Initial assessments of curative activity.** The results of the pilot and supplemental appraisals of the radical curative activity of WR-181,023, summarized in Table 1 (cf

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*The attack treated has been identified in the detailed tables in the Appendix by a suffix to the Mmu number. P refers to primary attack and first treatment. R_1 refers to first relapse and second treatment, R_2 to second relapse and third treatment, etc. Extensive experience has shown that evaluations of curative activity are not compromised by use of previously treated infections as long as the interval between prior treatment and relapse is 20 days or less, a period similar to that found in infections treated with chloroquine alone.

**WR-181,023 (BC-57,244) was used throughout these evaluations.

TABLE 1

SUMMARY: PILOT AND SUPPLEMENTAL EVALUATIONS OF THE CAPACITY OF WR-181,023 (BC-57,244) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF OF PLASMODIUM CYNOMOLGI

Daily Dose Mg/Kg Body Weight*	No. of Monkeys/Dose	Response to Treatment		
		No. of Relapses	Days from R_x to Relapse	No. of Cures
0.125	4	3	10, 10, 12	1
0.25	9	5	11, 14, 16, 22, 27	4
0.5	11	1	23	10
1.0	1	0	-	1
10.0	1	0	-	1

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

Appendix Table 2 for details), show that this compound cured approximately 90 per cent of established infections when administered in daily doses of 0.5 mg base per kg body weight for seven consecutive days, concomitantly with 2.5 mg per kg doses of chloroquine. When delivered in the same regimen, doses of 0.25 mg per kg cured approximately 50 per cent of the infections.

Primaquine, against which any new curative agent must compete, was not evaluated simultaneously in the experiments which provided the above data. However, numerous appraisals of the activity of primaquine had been carried out in other studies pursued shortly before or during the time when WR-181,023 was being evaluated. The data from these experiments have been summarized in Table 2 (cf Appendix Table 3 for details). This summary shows that daily doses of 0.75 mg primaquine per kg body weight for seven days, together with 2.5 mg per kg doses of chloroquine, cured 39 of 40 infections. Doses of 0.375 mg per kg cured but 3 of 20 infections. This comparison indicated that the curative activity of primaquine was inferior to that of WR-181,023 and encouraged a side-by-side comparison of the two compounds utilizing the same doses of drugs or dosage increments, and the same sporozoite preparations and inocula.

2. Side-by-side comparisons of the curative activities of WR-181,023 and primaquine. The data summarized in Table 3 are a composite of two studies; one performed with the original preparation of WR-181,023 (BC-57,244), the other performed with the second sample, (BD-57,427). Since the results of these evaluations were in essential agreement (cf Appendix Table 4), they have been combined in Table 3. The data therein show that 7 of 10 infections were cured when WR-181,023 was delivered in a seven daily dose regimen of 0.25 mg per kg; 7 of 7 infections were cured at a daily dose of 0.5 mg per kg (with daily chloroquine administered as described above). Primaquine, on the other

TABLE 2

SUMMARY: MISCELLANEOUS EVALUATIONS OF THE CAPACITY OF PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Daily Dose Mg/Kg Body Weight*	No. of Monkeys/Dose	Response to Treatment		
		No. of Relapses	Days from R_x to Relapse	No. of Cures
0.188	2	2	4, 8	0
0.375	20	17	6, 11, 11, 11, 12, 12, 13, 13, 14, 15, 16, 16, 36, 38, 41, 61, 73	3
0.5	3	0	-	3
0.75	40	1	11	39
1.0	1	0	-	1
1.5	13	0	-	13

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

TABLE 3

SUMMARY: COMPARISON OF THE CAPACITIES OF WR-181,023 AND PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound WR- No.	Daily Dose Mg/Kg Body Weight*	No. of Monkeys/Dose	Response to Treatment		
			No. of Relapses	Days from R_x to Relapse	No. of Cures
181,023†	0.125	2	2	7, 7	0
	0.25	10	3	34, 37, 62	7
	0.5	7	0	-	7
2,975	0.25	8	8	7, 7, 10, 11, 11, 12, 18, 66	0
	0.375	2	2	11, 14	0
	0.5	12	2	9, 26	10
	0.75	6	0	-	6

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

†Data derived from administration of Lots BC-57,244 and BD-57,247 combined.

hand, failed to cure any infection at a daily dose of 0.25 mg per kg; 10 of 12 infections were cured at a dose of 0.5 mg per kg. The results of this comparison not only support the suggested superiority of WR-181,023 provided by the separate appraisals, but place the activity of this compound at about twice that of primaquine.

3. A preliminary comparison of the influences of the dosage regimen on the curative activities of WR-181,023 and primaquine.* Development of a drug which would effect radical cure of infections with P. vivax when delivered in a single dose, or for no more than three doses, was one of the targets of the Malaria Chemotherapy Program. Primaquine is conventionally administered in a fourteen-day regimen, but is probably equally effective in a seven-day regimen if the fourteen-day total dose is compressed into the shorter period. Further reduction of the treatment period has not been attempted in man because of dose-limiting toxicity. Since there are indications from studies in monkeys infected with P. cynomolgi that cure is a function of the total dose delivered rather than duration of treatment, a drug significantly more active than primaquine might be effective in short term regimens. This concept led to an evaluation of the effectiveness of single-dose, two-dose, four-dose, and seven-dose treatment regimens of WR-181,023 and primaquine.

This evaluation, summarized in Table 4 (cf Appendix Table 5 for details), was disappointing because it failed to provide solid appraisals of the influence of the dosage regimen on the activities of either WR-181,023 or primaquine.

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*WR-181,023 (BD-57,427) was used in this investigation.

TABLE 4

SUMMARY: COMPARATIVE EVALUATIONS OF THE INFLUENCE OF THE DOSAGE REGIMEN ON THE CAPACITIES OF WR-181,023 (BD-57,427) AND PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound WR- No.	Dosage Regimen*			No. of Monkeys/Dose	Response to Treatment		
	Daily Dose		Total Dose Mg/Kg		No. of Relapses	Days from R_x to Relapse	No. of Cures
	Mg/Kg	No.					
181,023	1.75	1	1.75	2	0	-	2
	0.875	2	1.75	3	1	40	2
	0.44	4	1.76	3	1	8	2
	0.25	7	1.75	4	1	43	3
	3.5	1	3.5	2	0	-	2
	1.75	2	3.5	3	1	42	2
	0.875	4	3.5	2	0	-	2
	0.5	7	3.5	3	0	-	3
2,975	3.5	1	3.5	2	0	-	2
	1.75	2	3.5	2	0	-	2
	0.875	4	3.5	2	0	-	2
	0.5	7	3.5	4	1	19	3
	7.0	1	7.0	1	0	-	1
	3.5	2	7.0	2	0	-	2
	1.75	4	7.0	2	0	-	2
	1.0	7	7.0	2	0	-	2
	0.5	14	7.0	1	0	-	1

*Chloroquine, 2.5 mg per kg body weight, administered daily for seven days beginning on Day 1 of delivery of curative drug.

This failure resulted from the delivery of doses of these compounds that were highly effective in all regimens.* All that can be concluded from these results is that WR-181,023 produced a high fraction of cures at a total dose of 1.75 mg per kg body weight irrespective of the dosage regimen, and that the same end was attained with primaquine at a total dose of 3.5 mg per kg.

4. A comparison of the prophylactic activities of WR-181,023 and primaquine.** In this comparison, both compounds were administered the day prior to inoculation, the day of inoculation, and for seven days thereafter. This regimen provides daily contact between drug and parasite throughout the incubation period. The results of this comparison, summarized in Table 5 (cf Appendix Table 6 for details), show that WR-181,023 is at least twice as active as primaquine as a prophylactic agent, and possibly even more active. Seven of the ten monkeys who received WR-181,023 in doses of 0.25 to 0.75 mg per kg were protected, as compared with one of six recipients of these same doses of primaquine.

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*When the inadequacy of this assessment was recognized, plans were made to repeat the evaluations at lower doses of both WR-181,023 and primaquine. The IND preparation of WR-181,023 became available before these plans could be implemented. They were abandoned in favor of pursuing dosage regimen effect evaluations with the lot of drug destined for use in humans.

**WR-181,023 (BD-57,427) was used in this evaluation.

TABLE 5

SUMMARY: THE COMPARATIVE PROPHYLACTIC ACTIVITIES OF WR-181,023 (BD-57,427) AND PRIMAQUINE

Compound WR- No.	Daily Dose Mg/Kg Body Weight*	No. of Monkeys Challenged	Monkeys Infected		No. of Monkeys Protected
			No.	Days Delay in Patency	
181,023	0.125	2	2	4,4	0
	0.25	4	2	5,15	2
	0.375	2	1	5	1
	0.5	2	0	-	2
	0.75	2	0	-	2
2,975	0.25	1	1	2	0
	0.375	2	2	6,8	0
	0.5	1	1	8	0
	0.75	2	1	18	1
	1.0	1	0	-	1

*Dose delivered the day prior to sporozoite challenge, two hours before challenge, and daily for seven days thereafter.

B. Evaluation Of The Radical Curative And Prophylactic Activities Of WR-181,023 (BE-50,003, IND Preparation)

1. The radical curative activity of WR-181,023 (BE-50,003) compared with that of primaquine in various dosage schedules. These explorations covered two groups of experiments. The first was concerned with the effectiveness of single-dose, three-dose, and seven-dose regimens of WR-181,023 (cf Table 6 and Appendix Table 7). The second compared the activities of WR-181,023 and primaquine in similar dosage regimens (cf Table 7 and Appendix Table 8).

The data provided by the first of these evaluations indicate that the curative activity of the IND preparation in a seven-day dose regimen closely approximates the activities of earlier preparations as recorded in Tables 1, 3, and 4. Daily doses of 0.125 mg per kg of preparations BC-57,244 and BD-57,427 cured 1 of 6 infections (17 per cent); this dose of the IND preparation cured 2 of 24 infections (8 per cent). Daily doses of 0.25 mg per kg of the earlier preparations cured 14 of 23 infections (61 per cent); this dose of preparation BE-50,003 cured 21 of 28 infections (75 per cent). At this latter dose, the effectiveness of the IND preparation appeared to be slightly greater than that of earlier lots. This apparent superiority is probably an experimental artefact.*

*The seeming differences in the curative activities of various preparations of WR-181,023 led to a side-by-side comparison of the activities of the IND preparation (BE-50,003), the earlier lot (BD-57,427), the original preparation (CN 1101 - JW-1 - Elderfield, 1949) and the original large batch preparation (CN 1101 - Elderfield, 1950, subsequently coded BD-27,992). Although this comparison is not complete, it has gone far enough to warrant the conclusion that there is no significant difference in the curative activities of these lots.

TABLE 6

SUMMARY: THE CAPACITY OF WR-181,023 (BE-50,003, IND PREPARATION) TO CURE ESTABLISHED INFECTIONS WITH SPORZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Dosage Regimen*			No. of Monkeys/Dose	Response to Treatment		
Daily Dose		Total Dose Mg/Kg		No. of Relapses	Days from R_X to Relapse	No. of Cures
Mg/Kg	No.					
0.44	1	0.44	2	2	8, 8	0
0.875	1	0.875	5	5	11, 11, 12, 15, 25	0
0.292	3	0.876	2	2	12, 13	0
0.125	7	0.875	18	17	7, 7, 7, 8, 8, 8, 9, 9, 10, 12, 12, 14, 16, 31, 43, 79, 91	1
1.75	1	1.75	7	4	12; 21, 28, 40	3
0.584	3	1.75	9	4	33, 35, 37, 97	5
0.25	7	1.75	12	4	23, 27, 28, 40	8
3.5	1	3.5	2	1	18	1
1.17	3	3.5	2	0	-	2
0.5	7	3.5	3	1	23	2

*Chloroquine administered: (a) in a dose of 5.84 mg per kg body weight, daily for three days, to recipients of single and three doses of WR-181,023 or primaquine; or (b) in a dose of 2.5 mg per kg, daily for seven days, to recipients of seven-day courses of the curative drugs.

TABLE 7

SUMMARY: A COMPARISON OF THE CAPACITIES OF WR-181,023 (BE-50,003, IND PREPARATION) AND PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Compound WR- No.	Dosage Regimen*			No. of Monkeys/Dose	Response to Treatment		
	Daily Dose		Total Dose Mg/Kg		No. of Relapses	Days from R_x to Relapse	No. of Cures
	Mg/Kg	No.					
181,023	0.292	3	0.876	4	4	18, 21, 21, 22	0
	0.125	7	0.875	6	5	7, 7, 20, 22, 46	1
	1.75	1	1.75	5	4	15, 15, 22, 77	1
	0.584	3	1.75	16	5	14, 20, 24, 29, 36	11
	0.25	7	1.75	17	3	12, 38, 51	14
	0.875	3	2.625	2	0	-	2
2,975	0.125	7	0.875	3	3	6, 6, 7	0
	1.75	1	1.75	6	4	9, 9, 17, 32	2
	0.584	3	1.75	5	5	13, 13, 16, 19, 96	0
	0.25	7	1.75	12	10	8, 8, 8, 10, 10, 10, 12, 14, 23, 31	2
	3.5	1	3.5	1	0	-	1
	1.17	3	3.5	1	0	-	1
	0.5	7	3.5	9	0	-	9

*Chloroquine administered: (a) in a dose of 5.84 mg per kg body weight, daily for three days, to recipients of single and three doses of WR-181,023 or primaquine; or (b) in a dose of 2.5 mg per kg, daily for seven days, to recipients of seven-day courses of the curative drugs.

The data summarized in Tables 6 and 7 suggest strongly that the curative activity of WR-181,023 (BE-50,003) is less when administered in a single dose of 1.75 mg per kg than when this total dose is delivered in three or seven equal fractions on as many days. The single dose regimen led to cure of 4 of 12 infections (33 per cent); the three-dose regimen to cure of 16 of 25 infections (64 per cent); the seven-dose regimen to cure of 21 of 28 infections (75 per cent). The seven-dose schedule may be slightly superior to the three-dose schedule; a substantially larger body of data than are available would be required to establish this position.

The results of three direct comparisons of the curative activities of WR-181,023 (BE-50,003) and primaquine (cf Table 7 and Appendix Table 8) are in reasonable agreement with the findings in earlier assessments in which older lots of WR-181,023 and the same lot of primaquine were employed. At a total dose of 0.875 mg per kg body weight, administered in three or seven daily fractions, the BE-50,003 preparation of WR-181,023 effected cure of but one of ten infections. Essentially the same fraction of cures was attained when primaquine was administered in a total dose of 1.75 mg per kg. Cure of 25 of 33 infections was achieved with the latter dose of WR-181,023.

2. <u>The prophylactic activity of single doses of WR-181,023 (BE-50,003) compared with the activity of primaquine</u>. In the assessments of prophylactic activity described in Section A-4 of this Report, both WR-181,023 and primaquine were administered continuously during the incubation period. The protection accorded by such a regimen is of theoretical interest, but of little practical importance. Its application in the field would require continuous administration of the prophylactic agent in order to cope with randomly timed exposures to mosquitoes bearing sporozoites of <u>P. vivax</u>. The current evaluation was designed to appraise the activity of the IND preparation in a more realistic manner. Single doses

TABLE 8

SUMMARY: A COMPARISON OF THE CAPACITIES OF SINGLE DOSES OF WR-181,023 (BE-50,003, IND PREPARATION) AND PRIMAQUINE (WR-2,975) TO PREVENT INFECTIONS FOLLOWING INOCULATION WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound WR- No.	Dosage Regimen		No. of Monkeys Challenged	Monkeys Infected		No. of Monkeys Protected
	Mg/Kg Body Weight	Day of Rx re Challenge		No.	Days Delay in Patency	
181,023	0.44	0	2	2	3,4	0
	0.44	3	2	2	2,4	0
	0.44	7	2	2	0,0	0
	0.88	0	2	2	1,14	0
	0.88	3	2	2	5,6	0
	0.88	7	2	2	0,0	0
	1.75	0	2	0	-	2
	1.75	3	2	2	6,6	0
	1.75	7	2	2	11,14	0
	3.5	0	2	0	-	2
	3.5	3	2	2	10,20	0
	3.5	7	2	1	15	1
2,975	1.75	0	2	2	2,7	0
	1.75	3	2	2	2,2	0
	1.75	7	2	2	5,6	0
	3.5	0	2	2	7,7	0
	3.5	3	2	2	3,3	0
	3.5	7	2	2	7,7	0

of WR-181,023 or primaquine were administered at the time of sporozoite challenge or three or seven days later. This design made it possible to assess activity against the sporozoite or the recently established tissue form, the well-established tissue stage, and the mature tissue stage just prior to liberation of merozoites.

The results of this evaluation, summarized in Table 8 (cf Appendix Table 9 for details), show that single doses of 0.44 and 0.875 mg WR-181,023 (BE-50,003) per kg body weight, delivered on either Day 0, 3, or 7, did little more than prolong the incubation period. Single doses of 1.75 or 3.5 mg per kg, delivered on Day 0, provided complete protection. The effectiveness of WR-181,023 was reduced greatly when such doses were administered on Day 3 or 7. A significant delay in the onset of patency in all recipients and complete protection in one were achieved with these dosage schedules, suggesting either destruction of a very large fraction of the tissue stages (probably four logs) or substantial injury to such stages with slow recovery.

The summary shows that under the same experimental conditions, the prophylactic activity of primaquine was distinctly less than that of WR-181,023, with no subject protected completely. The delays in onset of patent infections in recipients of 3.5 mg per kg doses of primaquine and 0.875 mg per kg doses of WR-181,023 were similar, suggesting that the prophylactic activities of the two compounds differed by a factor of four.

COMMENTS AND CONCLUSIONS

Before commenting on the overall import of the observations summarized in the preceding sections, it might be well to focus attention briefly on the major features and results of evaluations of the radical curative and prophylactic properties of 4-methyl-primaquine performed in 1949-1950. These evaluations were the last to be pursued in the voluntarily coordinated post-World War II effort to achieve one of the major goals of the World War II Malaria Program - specifically, development of a drug or drugs which would effect radical cure of infections with P. vivax at well tolerated doses.

As pointed out earlier in this Report, 4-methyl-primaquine was synthesized by Elderfield and coworkers in 1949 who promptly submitted a sample of this agent, codified CN 1101, to a collaborating group at The Christ Hospital Institute for Medical Research for evaluation of curative activity. This group pursued pilot studies with the original sample, and more extensive therapeutic and prophylactic evaluations and preliminary toxicologic investigations with a portion of an 80 gram lot which exists at present as WR-181,023-AD (BD-27,992). The results of evaluations of the therapeutic and prophylactic activities of CN 1101 were summarized and distributed to members of the collaborative group in late 1950 along with the results of comparable evaluations of the activity of primaquine which then, as now, was the reference agent against which newly discovered drugs with curative activity had to compete. The summary table for this 1950 report has been reproduced as Appendix Table 1.

The experimental procedures employed in the 1949-1950 evaluation of the therapeutic and prophylactic properties of CN 1101, and these and earlier evaluations of primaquine, were for the most part identical with those described in this Report and used in assessing the activities of various preparations of WR-181,023. Note should be taken of four significant differences in procedural detail: (1) The M strain of P. cynomolgi was used as the test plasmodium, rather than the B strain. (2) Anopheles quadrimaculatus served as the insect vector in place of A. freeborni. (3) Quinine, delivered at a dose of 80.0 mg per kg body weight, once daily for seven consecutive days, served as the companion drug in therapeutic assessments, rather than chloroquine. (4) In evaluating prophylactic activity, CN 1101 and primaquine were administered on two occasions; first, four hours after inoculation, and second, four days later, rather than in single doses or daily throughout the incubation period.

The summary table (cf Appendix Table 1) brings together curative data on 59 infections treated with CN 1101 and 144 treated with primaquine. The results show that the dose of CN 1101 required for cure of established infections is from one-half to one-fourth the dose of primaquine required to attain the same endpoint. The dimensions of the prophylactic evaluation were limited as contrasted to the size of the curative evaluation. Nonetheless, the data suffice to show that CN 1101 was two to four times as active as primaquine in preventing infections. These demonstrations of the superiority of CN 1101 over primaquine, in both radical curative and prophylatic areas, developed more than 20 years ago with somewhat different procedures, add weight to the newer observations on the superiority of WR-181,023 over primaquine, and for this reason deserve note.

Whatever the attitude toward the results of the earlier evaluations may be, the results of the present studies by themselves support the following conclusions: (1) WR-181,023 has a significantly greater capacity than primaquine to cure established infections with sporozoites of P. cynomolgi in a variety of dosage schedules. (2) The superiority of WR-181,023 over primaquine with respect to curative activity may be great enough to support use of short term (three-day) treatment schedules. (3) The prophylactic activity of WR-181,023, like its therapeutic activity, is significantly greater than the prophylactic activity of primaquine. (4) Finally, and most relevant to the objective of this Report, the IND preparation of WR-181,023 (BE-50,003) has therapeutic and prophylactic activities essentially identical with those of earlier preparations, and may be regarded as the bioequivalent of the latter.

An issue not dealt with in this Report, most relevant to possible evaluation of WR-181,023 in man, is the relation between the toxicities of this compound and primaquine from both quantitative and qualitative viewpoints. Others have had responsibility for assessing toxicity and will doubtless contribute their findings to the IND application. In principle, it would seem that study of WR-181,023 in human volunteers would not be contraindicated even if this compound had no apparent toxicologic advantage over primaquine. It should be recalled that the toxicities of primaquine and its predecessor, pamaquine, are essentially equal. Yet, because of its greater therapeutic activity, primaquine is relatively useful in curing infections with P. vivax, whereas pamaquine is not.

The studies described in this Report were designed, supervised, and evaluated by the undersigned.

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APPENDIX

TABLE 1*

COMPARATIVE ANTIMALARIAL ACTIVITIES OF PRIMAQUINE AND ITS LEPIDINE, CN 1101

A. Curative Activity Against Sporozoite-Induced Infections. (In each instance, the 8-aminoquinoline was administered with quinine.)

Drug	Daily Dose Mg Base/Kg Body Weight	No. Cures/ No. Infections Treated	Per Cent Cures
Primaquine	0.375	2/14	14
	0.75	40/62	65
	1.5	63/68	93
CN 1101	0.094	0/5	0
	0.188	3/10	30
	0.375	20/22	91
	0.75	16/16	100
	1.5	6/6	100

B. Prophylactic Activity. (In each case the drug was administered on the day of inoculation and four days later.)

Drug	Daily Dose Mg Base/Kg Body Weight	No. Monkeys Protected/ No. Monkeys Inoculated	Per Cent Protection
Primaquine	0.094	0/8	0
	0.375	2/8	25
	1.5	7/8	88
CN 1101	0.024	0/4	0
	0.094	2/8	25
	0.375	5/8	63
	1.5	8/8	100

*Excerpted from Summary Report To Investigators In Malaria Chemotherapy, 1950.

TABLE 2

PILOT AND SUPPLEMENTAL EVALUATIONS OF THE CAPACITY OF WR-181,023 (BC-57,244) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Date of Experiment	Mmu No.	Daily Dose Mg/Kg Body Weight*	Response to Treatment		
			Relapsed	Days from R_x to Relapse	Cured
4/12/73	7793P	1.0			+
	7795P	10.0			+
6/05/73	$7889R_3$	0.125			+
	$7877R_1$	0.25			+
	$7880R_1$	0.25			+
	$7792R_1$	0.5			+
6/06/73	7909P	0.5			+
8/02/73	$7979R_2$	0.125	+	12	
	$7979R_3$	0.25	+	27	
	$7979R_4$	0.5			+
8/09/73	$7950R_2$	0.125	+	10	
	$7950R_3$	0.25	+	14	
	$7936R_4$	0.5			+
	$7950R_4$	0.5			+
9/04/73	7897P	0.125	+	10	
	$7897R_1$	0.25	+	11	
	7948P	0.25			+
	7953P	0.5			+
	$7897R_2$	0.5			+
5/17/74	8265P	0.25	+	16	
	8266P	0.25	+	22	
	8247P	0.25			+
	8269P	0.5	+	23	
	8248P	0.5			+
	$8265R_1$	0.5			+
	$8269R_1$	0.5			+

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

TABLE 3

MISCELLANEOUS EVALUATIONS OF THE CAPACITY OF PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Date of Experiment	Mmu No.	Daily Dose Mg/Kg Body Weight*	Response to Treatment		
			Relapsed	Days from Rx to Relapse	Cured
3/30/72	$7436R_3$	0.375	+	11	
	$7437R_3$	0.375	+	16	
	$7433R_2$	0.375	+	38	
	$7437R_4$	0.75			+
	$7436R_5$	1.5			+
	$7434R_5$	1.5			+
8/11/72	$7549R_1$	0.75			+
	$7492R_2$	0.75			+
	$7579R_2$	0.75			+
	$7587R_2$	0.75			+
9/15/72	$7586R_1$	0.375	+	13	
	$7583R_1$	0.375	+	73	
	$7512R_1$	0.75			+
	$7541R_1$	0.75			+
	$7543R_1$	0.75			+
	$7551R_1$	0.75			+
	$7583R_2$	0.75			+
	$7586R_2$	0.75			+
9/25/72	$7306R_6$	0.375	+	36	
	7385P	0.375	+	41	
	7389P	0.75	+	11	
	12P	0.75			+
	1817P	0.75			+
	6583P	0.75			+
	7119P	0.75			+
	7323P	0.75			+
	7392P	0.75			+
	7406P	0.75			+
	9910P	0.75			+
	$7385R_1$	0.75			+
	$7306R_7$	0.75			+
	21P	1.5			+
	6158P	1.5			+
	6596P	1.5			+
	7286P	1.5			+
	7387P	1.5			+
	7421P	1.5			+
	$7389R_1$	1.5			+

TABLE 3 - CONTINUED

Date of Experiment	Mmu No.	Daily Dose Mg/Kg Body Weight*	Response to Treatment		
			Relapsed	Days from R_x to Relapse	Cured
1/08/73	7744P	0.375	+	6	
	7745P	0.375	+	16	
	7742P	0.375	+	61	
	7747P	0.375			+
	7750P	0.75			+
	7751P	0.75			+
	7755P	0.75			+
	7756P	0.75			+
	7758P	0.75			+
	7763P	0.75			+
	7766P	0.75			+
	7769P	0.75			+
	$7744R_1$	0.75			+
	7770P	1.5			+
	7777P	1.5			+
	7779P	1.5			+
	7782P	1.5			+
4/05/73	7830P	0.375	+	12	
	7832P	0.375	+	12	
	7831P	0.375	+	13	
	7838P	0.75			+
	7839P	0.75			+
	$7830R_1$	0.75			+
	$7831R_1$	0.75			+
	$7832R_1$	0.75			+
8/02/73	$7985R_2$	0.375	+	11	
	$7975R_1$	0.75			+
	$7985R_3$	0.75			+
	$7980R_3$	0.75			+
	8002P	1.0			+
5/15/74	8264P	0.375	+	14	
	$8264R_1$	0.5			+
	8275P	0.75			+
12/23/74	8387P	0.188	+	4	
	8440P	0.188	+	8	
	8441P	0.375	+	11	
	8446P	0.375	+	15	
	$8387R_1$	0.375			+
	$8440R_1$	0.375			+
	$8441R_1$	0.5			+
	$8446R_1$	0.5			+

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

TABLE 4

COMPARISON OF THE CAPACITIES OF WR-181,023 AND PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Date of Experiment	Compound WR- No.	Daily Dose Mg/Kg Body Weight*	Mmu No.	Response to Treatment		
				Relapsed	Days from Rx to Relapse	Cured
10/09/73	181,023†	0.125	7809P	+	7	
		0.125	7810P	+	7	
		0.25	7808P	+	34	
		0.25	7760P			+
		0.25	7820P			+
		0.25	7809R1			+
		0.25	7810R1			+
		0.5	7883P			+
		0.5	7884P			+
		0.5	7808R1			+
	2,975	0.375	7835P	+	11	
		0.375	7659P	+	14	
		0.75	7869P			+
		0.75	7870P			+
		0.75	7835R1			+
		0.75	7859R1			+

†WR-181,023-AC (BC-57,244) administered in this evaluation.

TABLE 4 - CONTINUED

Date of Experiment	Compound WR- No.	Daily Dose Mg/Kg Body Weight*	Mmu No.	Response to Treatment		
				Relapsed	Days from R_x to Relapse	Cured
		0.25	8145P	+	37	
		0.25	8142P	+	62	
		0.25	8143P			+
		0.25	8144P			+
	181,023†	0.25	8146P			+
		0.5	8147P			+
		0.5	8148P			+
		0.5	$8142R_1$			+
		0.5	$8145R_1$			+
		0.25	8131P	+	7	
		0.25	8135P	+	7	
		0.25	8133P	+	10	
		0.25	8134P	+	11	
		0.25	8126P	+	11	
1/28/74		0.25	8132P	+	12	
		0.25	8130P	+	18	
		0.25	8127P	+	66	
		0.5	8139P	+	9	
		0.5	8137P	+	26	
		0.5	8136P	Died Negative Day 66		+(?)
	2,975	0.5	8138P			+
		0.5	$8126R_1$			+
		0.5	$8127R_1$			+
		0.5	$8130R_1$			+
		0.5	$8131R_1$			+
		0.5	$8132R_1$			+
		0.5	$8133R_1$			+
		0.5	$8134R_1$			+
		0.5	$8135R_1$			+
		0.75	$8137R_1$			+
		0.75	$8139R_1$			+

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

†WR-181,023-AE (BD-57,427) administered in this evaluation.

TABLE 5

COMPARATIVE EVALUATIONS OF THE INFLUENCE OF THE DOSAGE REGIMEN ON THE CAPACITIES OF WR-181,023 (BD-57,427) AND PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Date of Experiment	Compound WR- No.	Dosage Regimen*			Mmu No.	Response to Treatment		
		Daily Dose		Total Dose Mg/Kg		Relapsed	Days from R_x to Relapse	Cured
		Mg/Kg	No.					
9/06/74	181,023	0.25	7	1.75	8380P	+	43	
		3.5	1	3.5	8345P			+
		3.5	1	3.5	8377R_1			+
		1.75	2	3.5	8368P	+	42	
		1.75	2	3.5	8363R_1			+
		0.875	4	3.5	8371P			+
		0.5	7	3.5	8374P			+
		0.5	7	3.5	8380R_1			+
		0.25	14	3.5	8377P	+	42	
	2,975	0.5	7	3.5	8376P	+	19	
		0.75	7	5.25	8376R_1			+
		7.0	1	7.0	8337P			+
		3.5	2	7.0	8338P			+
		1.75	4	7.0	8341P			+
		1.0	7	7.0	8348P			+
		0.5	14	7.0	8375P			+
9/16/74	181,023	0.25	7	1.75	8372P			+
		1.75	2	3.5	8289P			+
		0.875	4	3.5	8300P			+
		0.5	7	3.5	8301P			+
		0.25	14	3.5	8350P			+
	2,975	0.5	7	3.5	8356P			+
		3.5	2	7.0	8298P			+
		1.75	4	7.0	8299P			+
		1.0	7	7.0	8307P			+

TABLE 5 - CONTINUED

Date of Experiment	Compound WR- No.	Dosage Regimen*			Mmu No.	Response to Treatment		
		Daily Dose		Total Dose Mg/Kg		Relapsed	Days from R_x to Relapse	Cured
		Mg/Kg	No.					
11/16/74	181,023	1.75	1	1.75	8397P			+
		1.75	1	1.75	8400P			+
		0.875	2	1.75	8401P	+	40	
		0.875	2	1.75	8409P			+
		0.875	2	1.75	$8401R_1$			+
		0.44	4	1.76	8422P	+	8	
		0.44	4	1.76	8410P			+
		0.44	4	1.76	$8422R_1$			+
		0.25	7	1.75	8428P			+
		0.25	7	1.75	8429P			+
	2,975	3.5	1	3.5	8386P			+
		3.5	1	3.5	8389P			+
		1.75	2	3.5	8390P			+
		1.75	2	3.5	8392P			+
		0.875	4	3.5	8393P			+
		0.875	4	3.5	8394P			+
		0.5	7	3.5	8395P			+
		0.5	7	3.5	8396P			+

*Chloroquine, 2.5 mg per kg body weight, administered daily for seven consecutive days beginning on Day 1 of delivery of curative drug.

TABLE 6

EVALUATION OF THE PROPHYLACTIC ACTIVITY OF WR-181,023 (BD-57,427) WITH SIDE-BY-SIDE COMPARISON WITH THE ACTIVITY OF PRIMAQUINE (WR-2,975)

Compound WR- No.	Daily Dose Mg/Kg Body Weight*	Mmu No.	Day of Patency after Challenge	Days Delay in Onset of Patency
181,023	0.125	8247	12	4
	0.125	8248	12	4
	0.25	8265	13	5
	0.25	8266	23	15
	0.25	7969†	Protected against infection[a]	
	0.25	8225†	Protected against infection[a]	
	0.375	8269	13	5
	0.375	8270	Protected against infection[a]	
	0.5	8231†	Protected against infection[a]	
	0.5	8233†	Protected against infection[a]	
	0.75	8234†	Protected against infection[a]	
	0.75	8235†	Protected against infection[a]	
2,975	0.25	8236†	10	2
	0.375	8158	14	6
	0.375	8159	16	8
	0.5	8241†	16	8
	0.75	8159	Protected against infection[a]	
	0.75	8242†	18	10
	1.0	8243†	Protected against infection[a]	
-	-	7956	8	-
-	-	8244†	8	-
-	-	8318	8	-

*Dose delivered the day prior to sporozoite challenge, two hours before challenge, and daily for seven days thereafter.

†Monkeys involved in side-by-side comparison of the activity of WR-181,023 and primaquine.

[a]Protection synonymous with absence of parasitemia for 70-76 days after onset of infection in untreated control monkeys and proof of susceptibility by appearance of parasitemia eight days after rechallenge with 5×10^5 sporozoites.

TABLE 7

THE CAPACITY OF WR-181,023 (BE-50,003, IND PREPARATION) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Date of Experiment	Mmu No.	Dosage Regimen*			Response to Treatment		
		Daily Dose		Total Dose	Relapsed	Days from R_x to Relapse	Cured
		Mg/Kg	No.	Mg/Kg			
3/13/75	8482P	0.44	1	0.44	+	8	
	8483P	0.44	1	0.44	+	8	
	8489P	0.875	1	0.875	+	11	
	8499P	0.875	1	0.875	+	11	
	$8482R_1$	0.875	1	0.875	+	12	
	8486P	0.875	1	0.875	+	15	
	$8483R_1$	0.875	1	0.875	+	25	
	8551P	0.125	7	0.875	+	7	
	8550P	0.125	7	0.875	+	7	
	8532P	1.75	1	1.75	+	12	
	$8486R_1$	1.75	1	1.75	+	21	
	8511P	1.75	1	1.75	+	28	
	$8482R_2$	1.75	1	1.75	+	40	
	$8499R_1$	1.75	1	1.75			+
	$8489R_1$	1.75	1	1.75			+
	$8483R_2$	1.75	1	1.75			+
	$8551R_3$	0.584	3	1.75	+	35	
	$8482R_3$	0.584	3	1.75	+	37	
	$8486R_2$	0.584	3	1.75			+
	$8511R_2$	0.584	3	1.75			+
	$8550R_2$	0.584	3	1.75			+
	$8550R_1$	0.25	7	1.75	+	28	
	$8551R_1$	0.25	7	1.75	+	20	
	$8482R_4$	0.25	7	1.75			+
	$8551R_4$	0.25	7	1.75			+
	$8511R_1$	3.5	1	3.5	+	18	
	$8532R_1$	3.5	1	3.5	Died Negative Day 88		+(?)
	$8551R_2$	0.5	7	3.5	+	23	
5/29/75	8639P	0.292	3	0.88	+	12	
	8640P	0.292	3	0.88	+	13	
	8652P	0.584	3	1.75	+	33	
	8651P	0.584	3	1.75	+	97	
	$8640R_1$	0.584	3	1.75			+
	$8639R_1$	0.584	3	1.75			+
	$8651R_1$	1.17	3	3.5			+
	$8652R_1$	1.17	3	3.5			+

TABLE 7 - CONTINUED

Date of Experiment	Mmu No.	Dosage Regimen*			Response to Treatment		
		Daily Dose		Total Dose Mg/Kg	Relapsed	Days from R_x to Relapse	Cured
		Mg/Kg	No.				
6/27/75	8641P	0.125	7	0.875	+	14	
	8642P	0.125	7	0.875	+	79	
	8649P	0.25	7	1.75			+
	8650P	0.25	7	1.75			+
	$8641R_1$	0.25	7	1.75			+
	$8642R_1$	0.25	7	1.75			+
8/08/75	8693P	0.125	7	0.875	+	8	
	8694P	0.125	7	0.875	+	43	
	$8694R_1$	0.25	7	1.75	+	23	
	$8693R_1$	0.25	7	1.75	+	27	
	8699P	0.25	7	1.75			+
	8700P	0.25	7	1.75			+
	$8694R_2$	0.5	7	3.5			+
	$8693R_2$	0.5	7	3.5			+
9/15/75	8736P	0.125	7	0.875	+	7	
	8735P	0.125	7	0.875	+	8	
	8780P	0.125	7	0.875	+	8	
	8732P	0.125	7	0.875	+	9	
	8779P	0.125	7	0.875	+	9	
	8737P	0.125	7	0.875	+	10	
	8741P	0.125	7	0.875	+	12	
	8770P	0.125	7	0.875	+	12	
	8733P	0.125	7	0.875	+	16	
	8740P	0.125	7	0.875	+	31	
	8765P	0.125	7	0.875	+	91	
	8734P	0.125	7	0.875			+

*Chloroquine administered: (a) in a dose of 5.84 mg per kg body weight, daily for three days, to recipients of single and three doses of WR-181,023 or primaquine; or (b) in a dose of 2.5 mg per kg, daily for seven days, to recipients of seven-day courses of the curative drugs.

TABLE 8

A COMPARISON OF THE CAPACITIES OF WR-181,023 (BE-50,003, IND PREPARATION) AND PRIMAQUINE (WR-2,975) TO CURE ESTABLISHED INFECTIONS WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI WITH EMPHASIS ON THE INFLUENCE OF THE DOSAGE REGIMEN

Date of Experiment	Compound WR- No.	Dosage Regimen*			Mmu No.	Response to Treatment		
		Daily Dose		Total Dose		Relapsed	Days from Rx to Relapse	Cured
		Mg/Kg	No.	Mg/Kg				
2/25/75	181,023	0.292	3	0.876	8571P	+	18	
		0.292	3	0.876	8569P	+	21	
		0.292	3	0.876	8568P	+	14	
		0.292	3	0.876	8570P	+	22	
		0.125	7	0.875	8549P	+	20	
		0.125	7	0.875	8548P	+	22	
		0.125	7	0.875	8552P	+	46	
		0.584	3	1.75	8571R$_1$	+	20	
		0.584	3	1.75	8574P	+	24	
		0.584	3	1.75	8579P			+
		0.584	3	1.75	8580P			+
		0.584	3	1.75	8581P			+
		0.584	3	1.75	8568R$_1$			+
		0.584	3	1.75	8569R$_1$			+
		0.584	3	1.75	8570R$_1$			+
		0.25	7	1.75	8553P			+
		0.25	7	1.75	8564P			+
		0.25	7	1.75	8565P			+
		0.25	7	1.75	8548R$_1$			+
		0.25	7	1.75	8549R$_1$			+
		0.25	7	1.75	8552R$_1$			+
		0.875	3	2.625	8574R$_1$			+
		0.875	3	2.625	8571R$_2$			+
	2,975	0.25	7	1.75	8534P	+	10	
		0.25	7	1.75	8539P	+	14	
		0.25	7	1.75	8538P			+
		0.5	7	3.5	8542P			+
		0.5	7	3.5	8543P			+
		0.5	7	3.5	8547P			+
		0.5	7	3.5	8534R$_1$			+
		0.5	7	3.5	8539R$_1$			+

TABLE 8 - CONTINUED

Date of Experiment	Compound WR- No.	Dosage Regimen*			Mmu No.	Response to Treatment		
		Daily Dose		Total Dose Mg/Kg		Relapsed	Days from R_x to Relapse	Cured
		Mg/Kg	No.					
		1.75	1	1.75	8567P	+	15	
		1.75	1	1.75	8588P	+	15	
		1.75	1	1.75	8566P	+	22	
		1.75	1	1.75	8556P	+	77	
		1.75	1	1.75	8597R_2			+
		0.584	3	1.75	8599P	+	14	
		0.584	3	1.75	8598P	+	29	
		0.584	3	1.75	8597P	+	36	
		0.584	3	1.75	8605P			+
	181,023	0.584	3	1.75	8567R_1			+
		0.584	3	1.75	8588R_1			+
		0.584	3	1.75	8556R_1			+
		0.584	3	1.75	8666R_1			+
		0.25	7	1.75	8597R_1	+	51	
		0.25	7	1.75	8606P			+
		0.25	7	1.75	8607P			+
		0.25	7	1.75	8608P			+
		0.25	7	1.75	8609P			+
		0.25	7	1.75	8598R_1			+
		0.25	7	1.75	8599R_1			+
5/1/75								
		1.75	1	1.75	8610P	+	9	
		1.75	1	1.75	8615P	+	9	
		1.75	1	1.75	8623R_1	+	17	
		1.75	1	1.75	8620R_2	+	29	
		1.75	1	1.75	8626R_1			+
		1.75	1	1.75	8621R_2			+
		0.584	3	1.75	8621P	+	13	
		0.584	3	1.75	8623R_2	+	13	
		0.584	3	1.75	8610R_1	+	16	
		0.584	3	1.75	8620P	+	19	
	2,975	0.584	3	1.75	8615R_1	+	96	
		0.25	7	1.75	8623P	+	8	
		0.25	7	1.75	8626P	+	8	
		0.25	7	1.75	8610R_2	+	12	
		0.25	7	1.75	8621R_1	+	14	
		0.25	7	1.75	8620R_1	+	31	
		0.25	7	1.75	8615R_2			+
		3.5	1	3.5	8610R_3			+
		1.17	3	3.5	8620R_3			+
		0.5	7	3.5	8623R_3			+

TABLE 8 - CONTINUED

Date of Experiment	Compound WR- No.	Dosage Regimen*			Mmu No.	Response to Treatment		
		Daily Dose		Total Dose Mg/Kg		Relapsed	Days from R_x to Relapse	Cured
		Mg/Kg	No.					
	181,023	0.125	7	0.875	8743P	+	7	
		0.125	7	0.875	8747P	+	7	
		0.125	7	0.875	8746P			+
		0.25	7	1.75	$8747R_2$	+	12	
		0.25	7	1.75	$8747R_1$	+	38	
		0.25	7	1.75	$8747R_3$			+
		0.25	7	1.75	$8743R_1$			+
9/22/75	2,975	0.125	7	0.875	8730P	+	6	
		0.125	7	0.875	8731P	+	6	
		0.125	7	0.875	8742P	+	7	
		0.25	7	1.75	$8742R_1$	+	8	
		0.25	7	1.75	$8730R_1$	+	10	
		0.25	7	1.75	$8731R_1$	+	10	
		0.5	7	3.5	$8730R_2$			+
		0.5	7	3.5	$8742R_2$			+
		0.5	7	3.5	$8731R_2$			+

*Chloroquine administered: (a) in a dose of 5.84 mg per kg body weight, daily for three days, to recipients of single and three doses of WR-181,023 or primaquine; or (b) in a dose of 2.5 mg per kg, daily for seven days, to recipients of seven-day courses of the curative drugs.

TABLE 9

A COMPARISON OF THE CAPACITIES OF SINGLE DOSES OF WR-181,023 (BE-50,003, IND PREPARATION) AND PRIMAQUINE (WR-2,975) TO PREVENT INFECTIONS FOLLOWING INOCULATION WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Date of Experiment	Compound WR- No.	Dose Mg/Kg Body Weight	Day of R_x after Challenge	Mmu No.	Day of Patency after Challenge	Days Delay in Onset of Patency
9/15/75	181,023	0.44	0	8732	11	3
		0.44	0	8733	12	4
		0.44	3	8736	10	2
		0.44	3	8737	12	4
		0.44	7	8765	8	0
		0.44	7	8770	8	0
		0.88	0	8734	22	14
		0.88	0	8735	9	1
		0.88	3	8740	13	5
		0.88	3	8741	14	6
		0.88	7	8779	8	0
		0.88	7	8780	8	0
10/24/75	181,023	1.75	0	8789	Protected against infection[a]	
		1.75	0	8808	Protected against infection[a]	
		1.75	3	8796	14	6
		1.75	3	8810	14	6
		1.75	7	8800	19	11
		1.75	7	8818	22	14
		3.5	0	8739	Protected against infection[a]	
		3.5	0	8816	Protected against infection[a]	
		3.5	3	8755	28	20
		3.5	3	8824	18	10
		3.5	7	8801	23	15
		3.5	7	8830	Protected against infection[a]	
	2,975	1.75	0	8756	10	2
		1.75	0	8707	15	7
		1.75	3	8790	10	2
		1.75	3	8809	10	2
		1.75	7	8799	13	5
		1.75	7	8817	14	6
		3.5	0	8738	15	7
		3.5	0	8815	15	7
		3.5	3	8754	11	3
		3.5	3	8823	11	3
		3.5	7	8775	15	7
		3.5	7	8829	15	7

[a]Protection synonymous with absence of parasitemia for 70-76 days after onset of infection in untreated control monkeys and proof of susceptibility by appearance of parasitemia eight days after rechallenge with 5×10^5 sporozoites.

ADDENDUM

PROCEDURE FOR PREPARING THE SPOROZOITE INOCULUM

Review of the report on the IND preparation of WR-181,023 drew attention to the need for a brief description of procedures employed in obtaining lots of infected mosquitoes and sporozoites for inoculation purposes. The abbreviated description that follows should meet this need as it relates to all evaluations (pilot and special) of curative drug activities.

Groups of Anopheles freeborni (1,000 to 1,200 in number and six to seven days post-emergence in age) are allowed to feed for four to six minutes on non-anaesthetized, non-sedated infected control (untreated) rhesus monkeys during the ascending phases of their initial parasitemias or of their first or second recrudescences. Engorged mosquitoes are separated from unfed mosquitoes within an hour of biting and transferred in groups of 250 to 300 to the required number of 12 x 12 x 12 inch wooden frame cages. These cages have a flex-board floor, plastic screening on three sides and the top, and a nylon bobbinet sleeve, eighteen inches long, on the fourth side (the latter for access purposes). This procedure yields 800 to 1,000 well fed mosquitoes. Service of these cages, daily throughout the holding period, includes counting and removal of dead mosquitoes, discarding old food, and provision of fresh food. The latter, placed on a clean 100 mm glass petri dish top or bottom, consists of an absorbent cotton pad saturated with 5 per cent Karo syrup, raisins soaked in diluted Karo, and three to four 4 mm thick cross sections of a ripe banana. For the first six days after feeding, cages are maintained in the insectary at a temperature of 26.5 ± 1.0°C. If retained after the sixth day, they are placed in a cold box at 21.0 ± 0.2°C.

On Day 6 after feeding, gut dissections are carried out on a group of five fed mosquitoes, randomly selected from one of the above cages. Only those lots that are 100 per cent infected with oocyst averages of 20 to 80, and include no mosquito with less than 10 oocysts nor more than 100, are held for future inoculation purposes. Lots exhibiting lesser or greater gut infections are destroyed. This practice is based on a very large experience showing: (1) that lots with oocyst averages of 20 to 80 invariably yield heavy sporozoite infections; (2) that the majority of the lots with average gut infections of 10 or less do not yield large numbers of sporozoites; and (3) that lots with oocyst averages in excess of 100 invariably have a poor survival record and mosquitoes that do survive do not develop sporozoite infections.

On Day 12 to 14 after feeding, lots of mosquitoes with appropriate gut infections are examined for sporozoite numbers. In this preliminary examination, dissected thoraces from ten mosquitoes are ground lightly in a glass mortar with 1.0 ml of iced 1:1 normal monkey serum-saline. The homogenate is transferred to a 15 ml conical centrifuge tube held in an ice bath. After five minutes standing, 5 cmm aliquots of the supernatant are spread over each 10 mm diameter circle of a fluorescent antibody slide. After air drying, the spread is fixed in absolute methanol, stained with Giemsa, and examined microscopically at 1000x magnification. If one or more sporozoites are found per every two oil immersion fields, the lot is considered suitable for use in curative or prophylactic experiments. With rare exceptions, lots with the appropriate oocyst averages have met this yardstick.

Lots of mosquitoes which have passed the preliminary check are processed for inoculation purposes, usually on Days 13 to 15 after feeding. The number of monkeys to be inoculated determines the number of mosquitoes processed. For example, 150 mosquitoes are prepared for a group of twelve monkeys,

300 for a group of twenty-four, 450 for a group of thirty-six. The actual preparation is as follows. Groups of five mosquitoes are sucked from the holding cage into a 12 mm bore glass tube, stunned, and placed on a sterile 3 x 1 inch microscope slide for dissection. The thoraces, freed of all appendages and contiguous parts, are collected to a total of 150 in a sterile 2 ounce smooth walled (unetched) glass mortar maintained in an ice bath. Dissection is continued until the total numbers of thoraces required for inoculation purposes have been obtained. At this time, grinding is initiated with successive attention to the thoraces in each mortar. The contents of each of these vessels are very lightly ground with a smooth surface glass pestle with three successive 2 ml volumes of sterile iced 1:1 normal monkey serum-saline, with decantation of each of these volumes into a sterile, screw-capped 15 ml conical centrifuge tube. The total volume is mixed thoroughly by manual rotation of the tube. The same procedure is followed with each group of 150 mosquitoes, each homogenate being placed in a separate tube. When grindings are completed, sporozoites (and most bacteria, etc.) are separated from mosquito tissue or debris by centrifuging for one minute at 1000 rpm (approximately 400 G). This sedimentation procedure is always carried out in an International Equipment Company Model K, Size 2 Centrifuge equipped with No. 250 horizontal head, No. 320 cup, and No. 325 trunion ring; with this apparatus, the radius from center of axle to tip of its 15 ml conical tube is 27.1 cm.

Upon completion of centrifuging, the supernatants are removed from each of the centrifuge tubes with a sterile capillary pipette, pooled in a sterile, screw-capped Erlenmeyer flask, and diluted with sterile iced 1:1 monkey serum-saline so that each ml of the final volume contains the sporozoites derived from ten mosquito thoraces. One ml aliquots are injected immediately into the mid-saphena of monkeys assigned to the study. The time period between homogenization of thoraces and injection of the processed homogenate never exceeds 50 minutes.

The numbers of sporozoites in the inoculum are determined as follows. Five cmm aliquots of the dilution, prepared for inoculation purposes, are spread as evenly as possible over each of six 10 mm diameter circles on fluorescent antibody slides. The spread is air dried, fixed in absolute methanol, and stained for 45 minutes in diluted Giemsa stain (16 ml of concentrate in 400 ml of pH-7.2 buffer, the latter prepared by dissolving 1.0 gram of buffer salt in 1000 ml of distilled water). When staining is completed, the slides are rinsed in freshly prepared buffer solution and air dried. The inoculum size is determined by counting the numbers of sporozoites in 100 oil immersion fields in each of four different circles, employing two horizontal and two vertical passes across each circle. Actual measurements with a microscope fitted with a 100x objective and 10x oculars (1000x magnification) have shown that there are 5000 ± 100 oil immersion fields in the 10 mm circles. This gives a factor of $5000 \times \frac{1000}{5}$ or 10^6 for conversion of average numbers of sporozoites per oil immersion field to numbers of sporozoites per ml of inoculum.

The above enumerative procedure does not include any assessment of the qualities of the sporozoites on the slide. Morphologic variations are always noted, however, since some sporozoites are less mature than others, and a great preponderance of immature forms can reduce infectivity.

V. THE COMPARATIVE CURATIVE ACTIVITIES OF VARIOUS PREPARATIONS OF WR-181,023

V. THE COMPARATIVE CURATIVE ACTIVITIES OF VARIOUS PREPARATIONS OF WR-181,023

Since 1949, when it was first recognized (cf Figure 5) that the curative activity of 4-methyl primaquine (later designated WR-181,023) was superior to that of primaquine*, a number of different preparations of the phosphate salt have been synthesized and evaluated in monkeys infected with sporozoites of P. cynomolgi. There were some differences in the biological features of these evaluations. In the 1949-1951 studies, monkeys were infected with sporozoites of the M strain of P. cynomolgi derived from A. quadrimaculatus. Quinine was employed as the blood schizonticide. In studies carried out in 1972 and later, monkeys were infected with sporozoites of the B strain of P. cynomolgi derived from A. freeborni. Chloroquine was utilized as the blood schizonticide. Although all preparations of CN-1101 or WR-181,023 satisfied the most stringent requirements for chemical purity available at the times of synthesis, they did exhibit color and solubility differences. Whether these diverse qualities affected the biological activities of the preparations was not known. Interest in this issue led to a limited comparison of the curative activities of the original 1949 preparation of CN-1101 (JW-1), the lot prepared in 1950 for anticipated evaluation in human volunteers** (now coded BD-27,992), lot BD-57,427, prepared in late 1973, and lot BE-50,003 prepared in early 1975 for prospective IND explorations.

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*At this time, these compounds were coded as CN-1101 and SN-13,272, respectively.

**The human volunteer studies were to have been conducted by the late Dr. Alf Alving, University of Chicago at the Illinois State Penitentiary, Joliet.

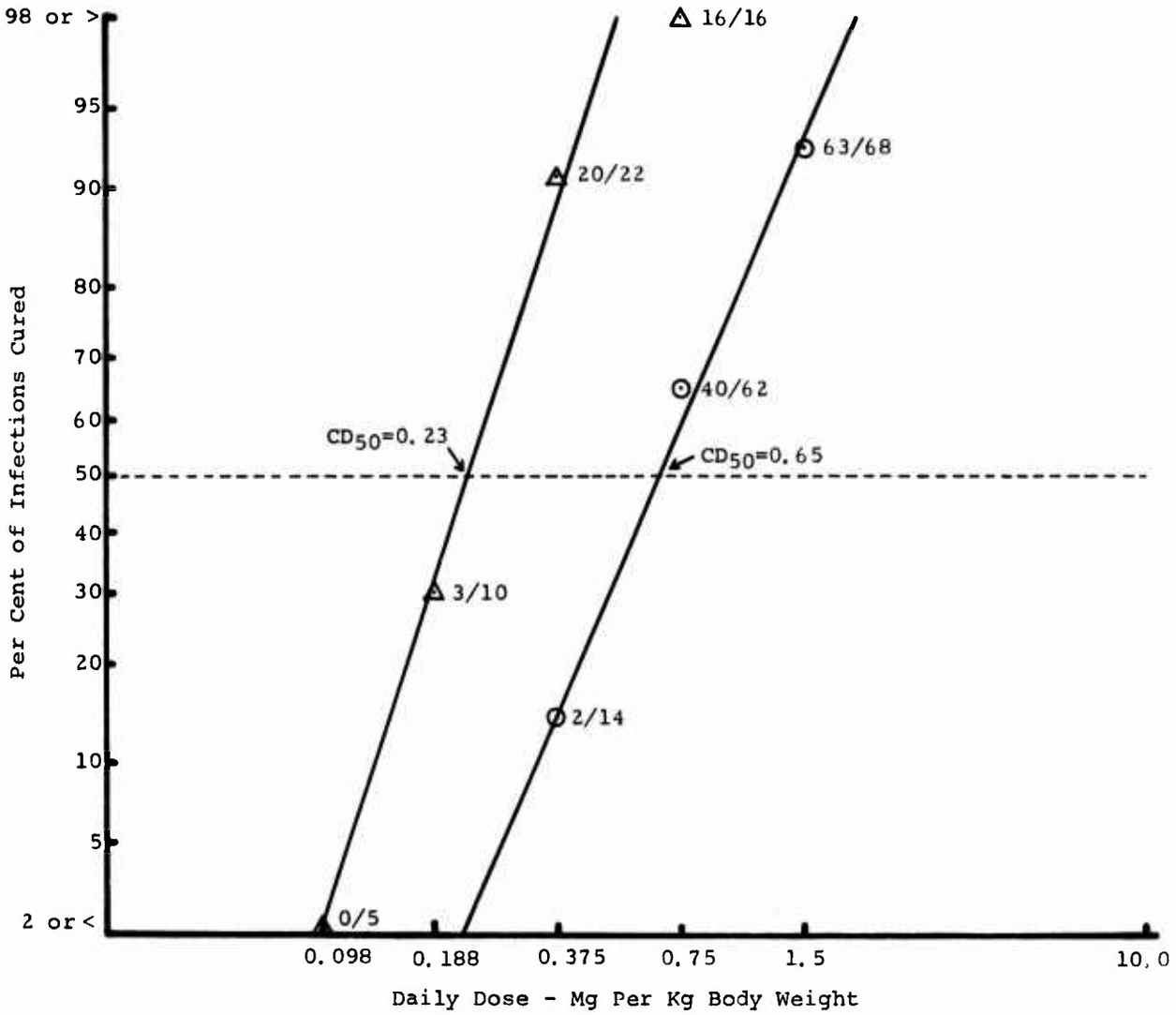
Twenty rhesus monkeys were committed to this comparative study; each was inoculated with 1.6×10^6 sporozoites. Subgroups of five monkeys were assigned to each of the four preparations. In dealing with primary attacks, three monkeys were treated with daily doses of 0.125 mg per kg, two with doses of 0.25 mg per kg. Management of treatment failures was accomplished by stepwise increases in dosage.

The results of this comparison, summarized in Table 14, suggest that there might be differences in the curative activities of the various preparations. Thus, at a daily dose of 0.25 mg per kg body weight, two of six infections treated with JW-1, one of six treated with BD-27,992, four of five treated with BD-57,427, and three of seven treated with BE-50,003 were cured. There are reasons, however, for believing that these seeming differences in activity may be no more than chance variation. Figure 6 records the results of a much larger previous experience with lots BD-57,427 and BE-50,003, plus experience with lot BC-57,244, the first preparation of WR-181,023 to be examined in the current Malaria Chemotherapy Program. Although these evaluations were pursued at different times in this Program, the results were remarkably similar. The CD_{50} of each preparation was calculated to be 0.22 mg per kg. Furthermore, overplot of the 1949-1950 data on lot JW-1, extracted from Figure 5, shows that despite the aforementioned variations in experimental procedures, the CD_{50} of this preparation was 0.23 mg per kg. The slopes of the dose response curves for all four preparations were remarkably similar. This comparison should eliminate concerns with differences in the biologic potency of the diverse preparations which might be generated by the results of the current, more restricted study.

FIGURE 5

THE COMPARATIVE CURATIVE ACTIVITIES OF 4-METHYL PRIMAQUINE (CN-1101) AND PRIMAQUINE AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE M STRAIN OF PLASMODIUM CYNOMOLGI*

(Summary Of 1949-1950 Evaluations)**



*Duration of treatment - seven days. Quinine administered as the companion drug at a daily dose of 80.0 mg base per kg body weight. △——△ = recipients of CN-1101; ⊙——⊙ = recipients of primaquine. Figures adjacent to symbols = number of cures per number of infections treated.

**These evaluations were carried out by the Principal Investigator at The Christ Hospital Institute For Medical Research with the partial support of RG-47, National Institute of Health.

TABLE 14

COMPARATIVE CURATIVE ACTIVITIES OF VARIOUS PREPARATIONS OF WR-181,023 AGAINST ESTABLISHED INFECTIONS INDUCED BY SPOROZOITES OF THE B STRAIN OF *PLASMODIUM CYNOMOLGI*

Preparation of WR-181,023	Daily Dose Mg Base/Kg Body Weight*	Mmu No.	Response to Treatment		
			Relapsed	Days from R_x to Relapse	Cured
CN-1101 JW-1	0.125	8783P	+	7	-
	0.125	8784P	+	8	-
	0.125	8782P	+	20	-
	0.25	8785P	+	12	-
	0.25	8786P	+	35	-
	0.25	$8782R_1$	+	43	-
	0.25	$8784R_1$	+	47	-
	0.25	$8783R_1$	-	-	+
	0.25	$8786R_1$	-	-	+
	0.5	$8784R_2$	+	47	-
	0.5	$8785R_1$	-	-	+
	0.5	$8782R_2$	-	-	+(>82)
CN-1101 BD-27,992	0.125	8791P	+	7	-
	0.125	8787P	+	10	-
	0.125	8788P	+	13	-
	0.25	8792P	+	12	-
	0.25	$8791R_1$	+	15	-
	0.25	$8788R_1$	+	24	-
	0.25	$8787R_1$	+	27	-
	0.25	$8791R_2$	+	55	-
	0.25	8793P	-	-	+
	0.5	$8791R_3$	+	16	-
	0.5	$8792R_1$	-	-	+
	0.5	$8787R_2$	-	-	+
	0.5	$8788R_2$	-	-	+
BD-57,427	0.125	8797P	+	10	-
	0.125	8794P	+	12	-
	0.125	8798P	-	-	+
	0.25	$8797R_1$	+	24	-
	0.25	8804P	-	-	+
	0.25	8806P	-	-	+
	0.25	$8794R_1$	-	-	+
	0.25	$8797R_2$	-	-	+

TABLE 14 - CONTINUED

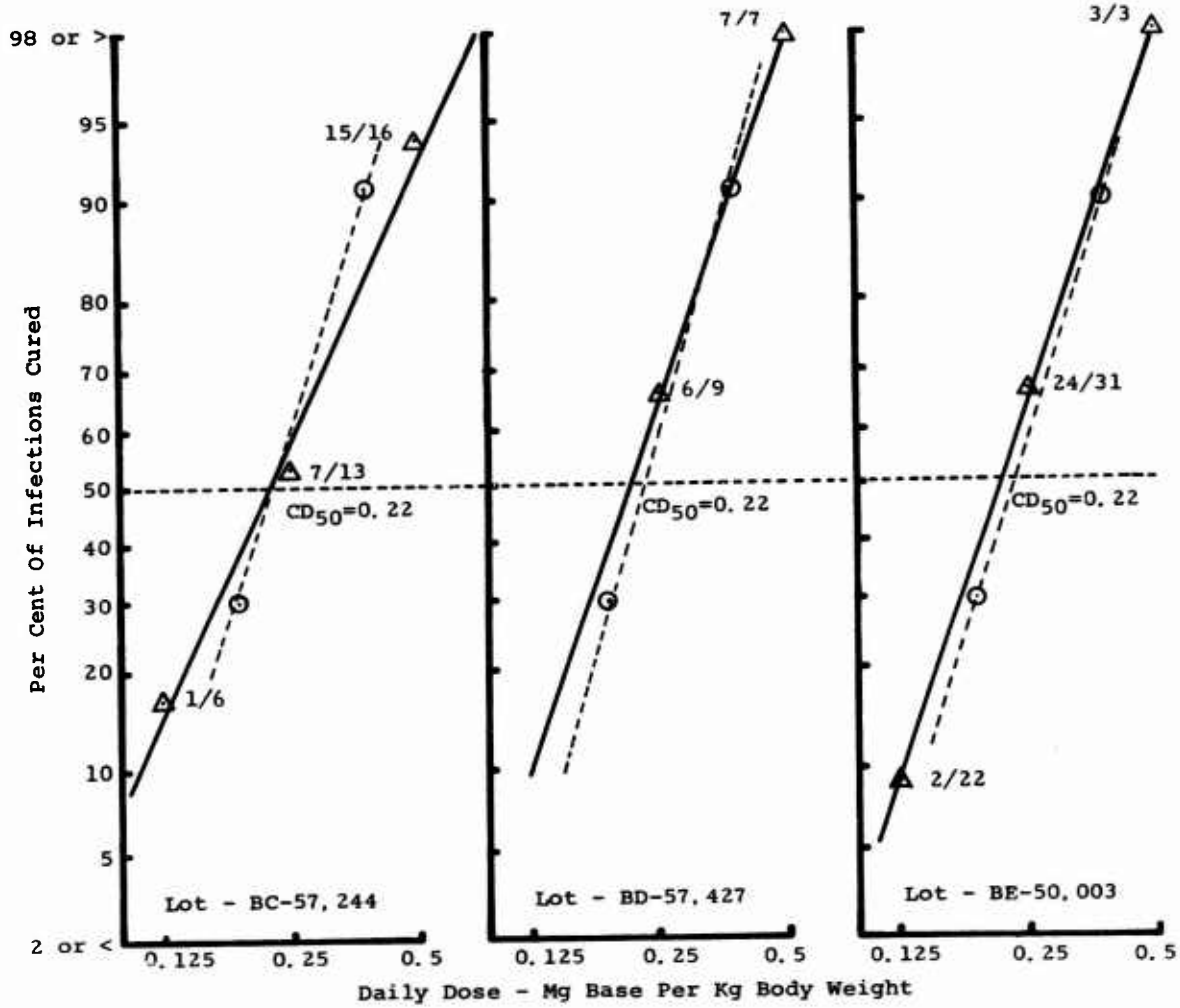
Preparation of WR-181,023	Daily Dose Mg Base/Kg Body Weight*	Mmu No.	Response to Treatment		
			Relapsed	Days from R_x to Relapse	Cured
BE-50,003 (IND lot)	0.125	8814P	+	8	-
	0.125	8820P	+	16	-
	0.125	8813P	+	23	-
	0.25	8825P	+	8	-
	0.25	$8820R_1$	+	13	-
	0.25	$8827R_1$	+	21	-
	0.25	8827P	+	44	-
	0.25	$8813R_1$	-	-	+
	0.25	$8814R_1$	-	-	+
	0.25	$8820R_2$	-	-	+
	0.5	$8825R_1$	-	-	+
	0.5	$8827R_2$	-	-	+(>88)

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

FIGURE 6

THE COMPARATIVE CURATIVE ACTIVITIES OF VARIOUS PREPARATIONS OF 4-METHYL PRIMAQUINE (WR-181,023) AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI*

(Summary Of 1973-1976 Evaluations)



*Duration of treatment - seven days. Chloroquine administered as the companion drug at a daily dose of 2.5 mg per kg body weight. Figures adjacent to Δ = number of cures per number of infections treated; O——O = reference plot of 1949-1951 evaluations (cf Figure 5).

VI. THE CURATIVE ACTIVITIES OF WR-221,033 AND WR-221,036, THE D AND L COMPONENTS OF WR-181,023

VI. THE CURATIVE ACTIVITIES OF WR-221,033 AND WR-221,036, THE D AND L COMPONENTS OF WR-181,023

As reported previously (cf SORI-KM-76-319), the D and L isomers of primaquine (respectively, WR-211,536 and WR-211,537) were endowed with essentially identical radical curative activities as measured by capacity to cure established infections with sporozoites of P. cynomolgi. However, the subacute toxicity of the D isomer for the rhesus monkey was approximately one-fourth that of the L isomer and one-half that of primaquine, the racemate. These findings led to the suggestion that consideration be given to comparing the activity and tolerability of WR-211,536 and primaquine in human volunteers, and if the relationships exhibited in the monkey carried over to man, and the costs of preparing the better tolerated agent were not prohibitive, substitution of the D isomer for the racemate.

Although the requisite studies in human volunteers have not yet been undertaken, there has been an expanding interest at the experimental level in the activities and toxicities of isomers of both blood schizonticidal and tissue schizonticidal compounds. Resolution of the D and L components of 4-methyl primaquine (respectively, WR-221,033 and WR-221,036) has made it possible to pursue studies comparable to those carried out with the isomers of primaquine. The results of the curative activity component of this investigation will be dealt with here.

The curative activities of WR-221,033, WR-221,036, and WR-181,023 were compared in parallel in a group of twenty-four monkeys inoculated with 6×10^5 sporozoites of the B strain of P. cynomolgi. Subgroups of eight were assigned to the evaluation of each compound, four to be treated initially with daily doses of 0.125 mg per kg body weight, four with doses of 0.25 mg per kg.

The results of this comparative study (cf Table 15) indicate that the radical curative activities of WR-181,023 and its D and L isomers are essentially the same. At a daily dose of 0.25 mg per kg, infections were cured in six of eight recipients of WR-181,023, five of nine recipients of WR-221,033, and four of eight recipients of WR-221,036. At a dose of 0.125 mg per kg, there were no cures among four infections treated with the racemate, as compared with one cure among each four infections treated with the D and L isomers. Thus, as with the primaquine isomers, the potential utility of the isomers of WR-181,023 will be determined by their relative toxicities. The results of a preliminary study of the subacute toxicities of WR-221,033 and WR-221,036 will be summarized in Section X of this Report.

TABLE 15

COMPARISON OF THE RADICAL CURATIVE ACTIVITIES OF WR-181,023 AND ITS D AND L ISOMERS (WR-221,033 AND WR-221,036) AGAINST INFECTIONS INDUCED BY SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound WR- No.	Daily Dose Mg Base/Kg Body Weight*	Mmu No.	Response to Treatment		
			Relapsed	Days from Rx to Relapse	Cured
181,023	0.125	8693P	+	9	-
	0.125	8641P	+	14	-
	0.125	8694P	+	45	-
	0.125	8642P	+	78	-
	0.25	8694R_1	+	23	-
	0.25	8693R_1	+	27	-
	0.25	8649P	-	-	+
	0.25	8650P	-	-	+
	0.25	8699P	-	-	+
	0.25	8700P	-	-	+
	0.25	8641R_1	-	-	+
	0.25	8642R_1	-	-	+
	0.5	8693R_2	-	-	+
	0.5	8694R_2	-	-	+
221,033	0.125	8707P	+	9	-
	0.125	8658P	+	15	-
	0.125	8708P	+	41	-
	0.125	8657P	-	-	+
	0.25	8667P	+	13	-
	0.25	8668P1	+	16	-
	0.25	8708R_1	+	16	-
	0.25	8711P	+	65	-
	0.25	8713P	-	-	+
	0.25	8658R_1	-	-	+
	0.25	8667R_1	-	-	+
	0.25	8707R_1	-	-	+
	0.25	8708R_2	-	-	+
	0.5	8668R_1	-	-	+
	0.5	8711R_1	-	-	+

TABLE 15 - CONTINUED

Compound WR- No.	Daily Dose Mg Base/Kg Body Weight*	Mmu No.	Response to Treatment		
			Relapsed	Days from R_x to Relapse	Cured
221,036	0.125	8714P	+	13	-
	0.125	8716P	+	13	-
	0.125	8674P	+	18	-
	0.125	8673P	-	-	+
	0.25	$8714R_1$	+	13	-
	0.25	$8716R_1$	+	23	-
	0.25	8681P	+	29	-
	0.25	8676P	+	41	-
	0.25	8722P	-	-	+
	0.25	8723P	-	-	+
	0.25	$8674R_1$	-	-	+
	0.25	$8714R_2$	-	-	+
	0.5	$8716R_2$	+	58	-
	0.5	$8676R_1$	-	-	+
	0.5	$8681R_1$	-	-	+
	0.5	$8716R_3$	-	-	+

*Dose indicated administered once daily for seven days with chloroquine, 2.5 mg base per kg body weight.

VII. SIDE-BY-SIDE COMPARISONS OF THE CURATIVE ACTIVITIES OF WR-212,579, WR-215,296, WR-215,761, WR-216,804, AND WR-221,527 WITH THE ACTIVITIES OF WR-181,023 AND PRIMAQUINE

VII. SIDE-BY-SIDE COMPARISONS OF THE CURATIVE ACTIVITIES OF WR-212,579, WR-215,296, WR-215,761, WR-216,804, AND WR-221,527 WITH THE ACTIVITIES OF WR-181,023 AND PRIMAQUINE

Late in the previous Project period, a group of four compounds, each approximately twice as active as WR-181,023 and four times as active as primaquine, emerged from the pilot evaluations. All of these agents were 4-methyl substituted, closely related structurally to WR-181,023. Three of the compounds, WR-212,579, WR-215,296, and WR-215,761, differed from 4-methyl primaquine with respect to branching in the side chain. The fourth compound, WR-216,804, was the 5-methoxy congener of WR-181,023. The structures of the four derivatives are shown in Figure 7, with the structure of WR-181,023 for comparison.

As indicated in Table 11, the initial pilot appraisals of the curative activities of WR-212,579, WR-215,296, WR-215,761, and WR-216,804 were expanded substantially. For the most part, this expansion was implemented by use of infections treated repetitively, without benefit, with diverse agents submitted for pilot appraisals of curative activity. The results of these assessments against previously treated infections agreed well with the results of the original pilot evaluations pursued in previously untreated infections.

Despite the sizeable volume of supporting data, concerns have been expressed that the curative activities of WR-212,579, WR-215,296, WR-215,761, and WR-216,804 might have been favored by use of previously treated infections. To allay these concerns, a series of evaluations on previously untreated infections were undertaken. Two of these appraisals involved side-by-side comparisons of the curative activities of these four agents with those of WR-181,023 or

primaquine*, limited attention being given to the impacts of the dosage regimen on such activity. Three separate experiments were carried out, involving fifty-eight rhesus monkeys subjected to a total of one hundred twelve treatment courses. The first experiment dealt with the comparative activities of all seven agents delivered in seven-day dosage regimens; the second compared the effectiveness of single dose, three daily dose and seven daily dose regimens of WR-212,579; the third dealt with the comparative efficacies of three dose regimens of WR-212,579, WR-215,296, WR-215,761, WR-216,804, and WR-181,023.

The detailed results of these three experiments have been combined in Table 16 and summarized in Table 17. The seven-dose treatment regimen data in this Table show that the curative activities of WR-212,579, WR-215,296, WR-215,761, WR-216,804, and WR-221,527 are clearly superior (by a factor of two or greater) to the activity of WR-181,023, which as usual was more active than primaquine. The first four of the above agents exhibited similar superiority over 4-methyl primaquine when administered in a three-dose regimen.

As indicated in Table 17, WR-215,296, WR-215,761, and WR-216,804 appeared to have somewhat greater curative activity when administered in a seven-dose regimen than when administered in a three-dose regimen. On the other hand (cf Tables 16 and 17), the curative activity of WR-212,579 appeared to be independent of the dosage regimen, a single dose being as effective as seven divided doses.

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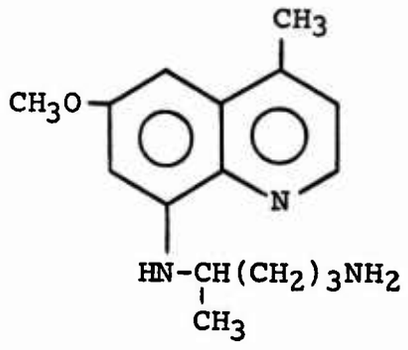
*A fifth highly active derivative, WR-221,527, was included in the most recent of the evaluations. This compound emerged from pilot studies early in the current Project period. Structurally, it was an isomer of WR-216,804 with a 4-methylbutyl group separating the side chain nitrogens rather than the more conventional 1-methylbutyl group (cf Figure 7).

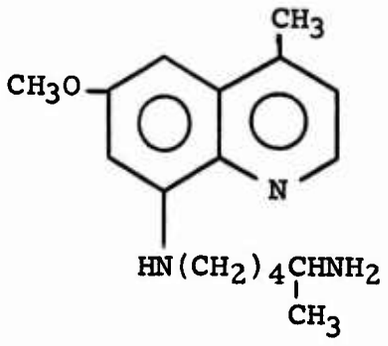
Comparison of the data recorded in Tables 11 and 17 shows that there is remarkably good agreement between the results of the older pilot assessments of the activities of WR-212,579, WR-215,296, WR-215,761, WR-216,804, and WR-221,527 carried out in subjects with random therapeutic experiences and the results of the current specially targetted evaluations pursued against previously untreated infections. In both situations, each of the five agents exhibited a high order of curative activity at a daily dose of 0.125 mg base per kg body weight for seven days, or a total dose of 0.875. This demonstration clearly places these compounds in the front rank of all 8-aminoquinolines examined to date.

FIGURE 7

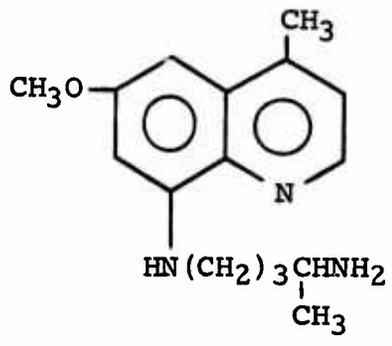
STRUCTURES OF WR-181,023, WR-212,579, WR-215,296, WR-215,761
WR-216,804, AND WR-221,527



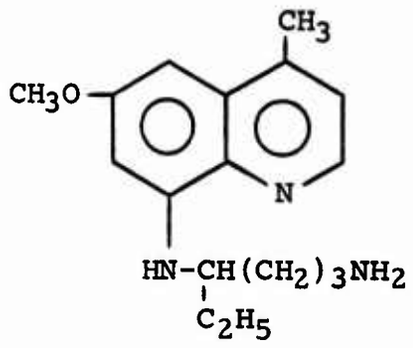
WR-181,023



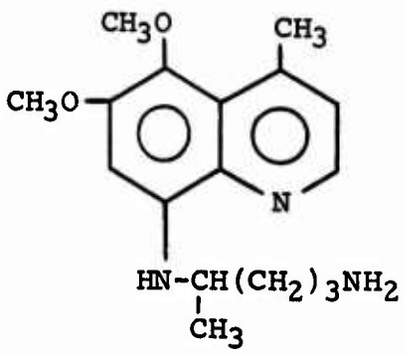
WR-212,579



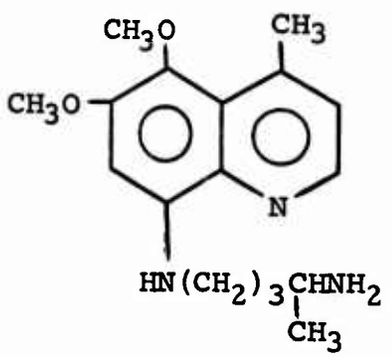
WR-215,296



WR-215,761



WR-216,804



WR-221,527

TABLE 16

COMPARATIVE EVALUATION OF THE CURATIVE ACTIVITIES OF PRIMAQUINE, WR-181,023, WR-212,579, WR-215,296, WR-215,761, WR-216,804 AND WR-221,527 IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Detailed Observations

Compound WR- No. or Name	Dosage Regimen*			Mmu No.	Response to Treatment		
	Daily Dose		Total Dose		Relapsed	Days from R_x to Relapse	Cured
	Mg/Kg	No.	Mg/Kg				
Primaquine	0.125	7	0.875	8731P	+	6	-
	0.125	7	0.875	8730P	+	7	-
	0.125	7	0.875	8742P	+	9	-
	0.25	7	1.75	$8742R_1$	+	9	-
	0.25	7	1.75	$8730R_1$	+	10	-
	0.25	7	1.75	$8731R_1$	+	10	-
	0.5	7	3.5	$8730R_2$	-	-	+
	0.5	7	3.5	$8731R_2$	-	-	+
	0.5	7	3.5	$8742R_2$	-	-	+
181,023	0.292	3	0.876	8639P	+	10	-
	0.292	3	0.876	8640P	+	15	-
	0.125	7	0.875	8743P	+	8	-
	0.125	7	0.875	8747P	+	8	-
	0.125	7	0.875	8746P	-	-	+
	0.584	3	1.75	8652P	+	38	-
	0.584	3	1.75	8651P	+	102	-
	0.584	3	1.75	$8639R_1$	-	-	+
	0.584	3	1.75	$8639R_2$	-	-	+
	0.25	7	1.75	$8747R_1$	+	41	-
	0.25	7	1.75	$8747R_2$	+	12	-
	0.25	7	1.75	$8743R_1$	-	-	+
	1.168	3	3.5	$8651R_1$	-	-	+
	1.168	3	3.5	$8652R_1$	-	-	+
	0.5	7	3.5	$8743R_2$	-	-	+

TABLE 16 - CONTINUED

Compound WR- No. or Name	Dosage Regimen*			Mmu No.	Response to Treatment		
	Daily Dose		Total Dose		Relapsed	Days from R_x to Relapse	Cured
	Mg/Kg	No.	Mg/Kg				
212,579	0.438	1	0.438	8698P	+	10	-
	0.438	1	0.438	8689P	+	11	-
	0.146	3	0.438	8710P	+	6	-
	0.146	3	0.438	8654P	+	8	-
	0.146	3	0.438	8653P	+	15	-
	0.146	3	0.438	8709P	+	18	-
	0.0625	7	0.438	8726P	+	9	-
	0.0625	7	0.438	8727P	+	9	-
	0.0625	7	0.438	8748P	+	10	-
	0.0625	7	0.438	8749P	+	15	-
	0.875	1	0.875	8706P	+	13	-
	0.875	1	0.875	8705P	+	17	-
	0.875	1	0.875	$8689R_1$	-	-	+
	0.875	1	0.875	$8698R_1$	-	-	+
	0.292	3	0.876	8719P	+	7	-
	0.292	3	0.876	8656P	+	19	-
	0.292	3	0.876	$8654R_1$	+	24	-
	0.292	3	0.876	8655P	-	-	+
	0.292	3	0.876	8720P	-	-	+
	0.292	3	0.876	$8653R_1$	-	-	+
	0.292	3	0.876	$8709R_1$	-	-	+
	0.292	3	0.876	$8719R_1$	-	-	+
	0.292	3	0.876	$8710R_1$	-	-	+(?)†
	0.125	7	0.875	8750P	+	31	-
	0.125	7	0.875	$8748R_1$	+	12	-
	0.125	7	0.875	$8727R_1$	+	16	-
	0.125	7	0.875	$8749R_1$	+	17	-
	0.125	7	0.875	$8726R_1$	+	24	-
	0.125	7	0.875	8728P	-	-	+
	0.125	7	0.875	8729P	-	-	+
	0.125	7	0.875	8751P	-	-	+
	1.75	1	1.75	$8705R_1$	-	-	+
	1.75	1	1.75	$8706R_1$	-	-	+
	0.584	3	1.75	$8656R_1$	-	-	+
	0.584	3	1.75	$8654R_2$	-	-	+
	0.25	7	1.75	$8750R_1$	-	-	+
	0.25	7	1.75	$8726R_2$	-	-	+
	0.25	7	1.75	$8727R_2$	-	-	+
	0.25	7	1.75	$8748R_2$	-	-	+
	0.25	7	1.75	$8749R_2$	-	-	+

TABLE 16 - CONTINUED

Compound WR- No. or Name	Dosage Regimen*			Mmu No.	Response to Treatment		
	Daily Dose		Total Dose		Relapsed	Days from R_x to Relapse	Cured
	Mg/Kg	No.	Mg/Kg				
215, 296	0.0625	7	0.438	8753P	+	10	-
	0.0625	7	0.438	8752P	+	16	-
	0.292	3	0.88	8661P	+	15	-
	0.292	3	0.88	8662P	+	15	-
	0.125	7	0.875	8758P	+	18	-
	0.125	7	0.875	8753R_1	+	29	-
	0.125	7	0.875	8759P	-	-	+
	0.125	7	0.875	8752R_1	-	-	+
	0.584	3	1.75	8663P	-	-	+
	0.584	3	1.75	8664P	-	-	+
	0.584	3	1.75	8661R_1	-	-	+
	0.584	3	1.75	8662R_1	-	-	+
	0.25	7	1.75	8758R_1	-	-	+
	0.25	7	1.75	8753R_2	-	-	+
215, 761	0.0625	7	0.438	8760P	+	17	-
	0.0625	7	0.438	8761P	+	22	-
	0.292	3	0.88	8665P	+	16	-
	0.292	3	0.88	8666P	+	19	-
	0.125	7	0.875	8763P	+	13	-
	0.125	7	0.875	8762P	-	-	+
	0.125	7	0.875	8761R_1	-	-	+
	0.125	7	0.875	8763R_1	-	-	+
	0.584	3	1.75	8665R_1	+	19	-
	0.584	3	1.75	8669P	-	-	+
	0.584	3	1.75	8670P	-	-	+
	0.584	3	1.75	8666R_1	-	-	+
	1.168	3	3.5	8665R_2	-	-	+

TABLE 16 - CONTINUED

Compound WR- No. or Name	Dosage Regimen*			Mmu No.	Response to Treatment		
	Daily Dose		Total Dose		Relapsed	Days from R_x to Relapse	Cured
	Mg/Kg	No.	Mg/Kg				
	0.146	3	0.438	8678P	+	18	-
	0.146	3	0.438	8677P	+	32	-
	0.0625	7	0.438	8767P	+	8	-
	0.0625	7	0.438	8768P	+	22	-
	0.292	3	0.875	8683P	+	20	-
	0.292	3	0.875	8684P	+	32	-
	0.292	3	0.875	$8678R_1$	+	15	-
216,804	0.292	3	0.875	$8677R_1$	-	-	+
	0.125	7	0.875	8771P	-	-	+
	0.125	7	0.875	8772P	-	-	+
	0.125	7	0.875	$8767R_1$	-	-	+
	0.125	7	0.875	$8768R_1$	-	-	+
	0.584	3	1.75	$8634R_1$	-	-	+
	0.584	3	1.75	$8684R_1$	-	-	+
	0.584	3	1.75	$8678R_2$	-	-	+
	0.0625	7	0.438	8773P	+	24	-
	0.0625	7	0.483	8774P	+	24	-
221,527	0.125	7	0.875	8777P	-	-	+
	0.125	7	0.875	8778P	-	-	+
	0.125	7	0.875	$8773R_1$	-	-	+
	0.125	7	0.875	$8774R_1$	-	-	+

*Chloroquine administered concomitantly in a dose of 5.84 mg base per kg body weight for three consecutive days to recipients of single dose or three dose regimens, or in a dose of 2.5 mg base per kg body weight for seven consecutive days to recipients of seven dose regimens.

†Negative at death Day 51 post-R_x.

TABLE 17

COMPARATIVE EVALUATIONS OF THE CURATIVE ACTIVITIES OF PRIMAQUINE, WR-181,023, WR-212,579, WR-215,296, WR-215,761, WR-216,804, AND WR-221,527 IN MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Summary Observations

Dosage Regimen*			No. of Infections Cured/No. Treated						
Daily Dose		Total Dose	Compound - WR- No. or Name						
Mg/Kg	No.	Mg/Kg	Primaquine	181,023	212,579	215,296	215,761	216,804	221,527
0.146	3	0.438	-	-	0/4	-	-	0/2	-
0.0625	7	0.438	-	-	0/4	0/2	0/2	0/2	0/2
0.292	3	0.876	-	0/2	6/9	0/2	0/2	1/4	-
0.125	7	0.875	0/3	1/3	3/8	2/4	3/4	4/4	4/4
0.584	3	1.75	-	2/4	2/2	4/4	3/4	3/3	-
0.25	7	1.75	0/3	1/3	5/5	2/2	-	-	-
1.168	3	3.5	-	2/2	-	-	1/1	-	-
0.5	7	3.5	3/3	1/1	-	-	-	-	-

*Chloroquine administered concomitantly in a dose of 5.84 mg base per kg body weight for three consecutive days to recipients of three dose regimens, or in a dose of 2.5 mg base per kg body weight for seven consecutive days to recipients of seven dose regimens.

VIII. A PRELIMINARY COMPARISON OF THE CURATIVE ACTIVITIES OF WR-182,234 AND WR-222,671

VIII. A PRELIMINARY COMPARISON OF THE CURATIVE ACTIVITIES OF WR-182,234 AND WR-222,671

WR-182,234 (2-methyl primaquine) attracted considerable interest three years ago as one of the few novelly substituted compounds with activity equal to that of primaquine. This interest diminished when compounds more active than primaquine were uncovered and following the demonstration that the subacute toxicity of WR-182,234 for the rhesus monkey was essentially identical, qualitatively and quantitatively, to the toxicity of primaquine.

WR-222,671, a structural isomer of WR-182,234, with a 4-methylbutyl group separating the side chain nitrogens in place of the 1-methylbutyl group, was submitted for pilot evaluation in the mid-portion of the current Project period. The results of the initial appraisal and expanded studies indicated that this isomeric 2-methyl primaquine was at least twice as active as WR-182,234 or primaquine and was the equal of WR-181,023. This preliminary finding placed WR-222,671 among the more active and interesting 8-aminoquinolines. To solidify these preliminary observations, the activity of WR-222,671 was evaluated in parallel with the activity of WR-182,234. This comparative study was initiated on January 23, 1976 with full recognition that completion prior to the end of the Project period might not be possible, but with hope that sufficient information could be generated in the time available to determine whether interest in WR-222,671 should be sustained.

Sixteen rhesus monkeys with established but previously untreated infections were committed to this study, with groups of eight assigned to evaluation of each compound, subgroups of four to each of two dose levels. WR-182,234 was administered initially in daily doses of 0.25 or 0.5 mg per kg body weight; WR-222,671 was administered in doses of 0.125 or 0.25 mg per kg; treatment failures were treated with progressively doubled doses.

As indicated in Table 18, it was not possible to reach the fifteen week post-treatment follow-up period customarily required for determining curative status. However, a seven-plus week follow-up was achieved in all but two subjects (Mmu 8888 and Mmu 8902). Since relapses rarely occur later than fifty days after completion of treatment with rapidly metabolized agents, it is possible to provide a tentative assessment of curative potential with less than a 105 day follow-up with reasonable assurance that the conclusion is valid. Within this boundary, the results of the direct comparison, summarized in Table 18, indicate that WR-222,671 is twice as active as WR-182,234. Four of four infections treated with 0.125 mg per kg doses of WR-222,671 relapsed, 14, 18, 21, and 21 days after the seventh dose. Four of four infections treated with 0.25 mg per kg doses of WR-182,234 relapsed 7, 7, 8, and 24 days after the seventh dose. Four of eight infections treated with 0.25 mg per kg doses of WR-222,671 reached tentative "cure" status; three of seven infections treated with 0.5 mg per kg doses of WR-182,234 reached this endpoint. The results of this parallel assessment are in essential agreement with those of the more or less random pilot evaluations recorded in Table 11. From the curative activity viewpoint, WR-222,671 may be considered the equal of WR-181,023. Information on the toxicity of this 2-methyl primaquine isomer is essential to determining whether it has any advantage over 4-methyl primaquine. This issue will be dealt with in Section X-C of this Report.

TABLE 18

SIDE-BY-SIDE COMPARISON OF THE RADICAL CURATIVE ACTIVITIES OF WR-182,234 AND WR-222,671 AS EXHIBITED IN INFECTIONS INDUCED BY SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

(Preliminary Evaluation)

Compound WR- No.	Daily Dose Mg Base/Kg Body Weight*	Mmu No.	Response to Treatment		
			Relapsed	Days from R_x to Relapse	Cured**
182,234	0.25	8882P	+	7	-
	0.25	8888P	+	7	-
	0.25	8898P	+	8	-
	0.25	8902P	+	24	-
	0.5	8910P	+	14	-
	0.5	$8888R_1$	+	20	-
	0.5	$8882R_1$	+	36	-
	0.5	$8902R_1$	-	-	?(>45)
	0.5	$8898R_1$	-	-	+(>64)
	0.5	8905P	-	-	+(>80)
	0.5	8909P	-	-	+(>80)
	0.5	8912P	-	-	+(>80)
	1.0	$8888R_2$	-	-	?(>37)
	1.0	$8910R_1$	-	-	+(>58)
222,671	0.125	8918P	+	14	-
	0.125	8919P	+	18	-
	0.125	8914P	+	21	-
	0.125	8916P	+	21	-
	0.25	$8914R_1$	+	6	-
	0.25	8921P	+	14	-
	0.25	8923P	+	21	-
	0.25	$8918R_1$	+	27	-
	0.25	$8916R_1$	-	-	+(>51)
	0.25	$8919R_1$	-	-	+(>52)
	0.25	8920P	-	-	+(>80)
	0.25	8928P	-	-	+(>80)
	0.5	$8914R_2$	+	13	-
	0.5	$8923R_1$	-	-	+(>51)
	0.5	$8921R_1$	-	-	+(>58)

*Dose indicated administered once daily for seven days along with 2.5 mg chloroquine per kg body weight.

**All cures indicated in this column are tentative. The figures in parentheses refer to the days of consistently negative thick blood films after the seventh dose of drug.

IX. THE INFLUENCE OF THE SIZE OF THE SPOROZOITE INOCULUM ON THE CURATIVE ACTIVITIES OF WR-181,023 AND PRIMAQUINE

IX. THE INFLUENCE OF THE SIZE OF THE SPOROZOITE INOCULUM ON THE CURATIVE ACTIVITIES OF WR-181,023 AND PRIMAQUINE

The influence of the size of the sporozoite inoculum on the curative activities of pamaquine, pentaquine, and isopentaquine was examined in some depth during the 1946-1947 period shortly after the introduction of mosquito-induced infections with P. cynomolgi in the rhesus monkey as a tool for predicting the capacities of new compounds to cure infections with P. vivax in man. This examination showed that the capacities of the above 8-aminoquinolines to cure either the primary infection or the first two to three relapses were influenced by the size of the sporozoite inoculum. In brief, doses of the above agents required to cure primary infections induced by inocula of 10^2 to 10^4 sporozoites were one-half to one-fourth those required to cure infections induced by inocula of 10^5 to 10^6 sporozoites. More importantly, when infections were induced by the smaller of these inocula, first or second relapses were frequently cured by administration of quinine (then used routinely as a companion drug), chloroquine, or proguanil. In infections induced by the larger of the inocula, even the last of a series of relapses, up to nine in number, was not cured by delivery of these blood schizonticidal drugs. These observations resulted in routine use of infections induced by inocula of 10^5 to 10^6 sporozoites throughout the 1946-1951 search for a curative drug more effective and better tolerated than pamaquine.

Essentially the same routines have been followed in searches for curative drugs pursued since 1951. However, there have been a number of changes in experimental procedures since that date. The M strain of P. cynomolgi, maintained by serial trophozoite passage since isolation in 1936, was replaced in 1959 by the more recently isolated B strain which has since been maintained by serial sporozoite transfers. A. quadrimaculatus, a relatively poor vector for P. cynomolgi, was replaced in 1957

by A. freeborni, a more efficient vector. The diet of the rhesus monkey colony was changed in 1952 from a fruit - vegetable - low protein (< 10 per cent) quality to a mixed grain - soy bean cake - fully vitamin supplemented - high protein (25 per cent) quality with profound improvement in general health of the monkey colony.

Together, these modifications have made for a more easily manageable, more productive test system. It has been assumed, however, that they did not alter the need to employ large (10^5 or >) sporozoite inocula in the systematic search for evaluation of curative drugs. This assumption deserves examination and possible affirmation for two reasons. In the first place, evaluation of the activities of potentially curative drugs is being turned over to a new group of investigators with limited experience with the biology of the P. cynomolgi - A. freeborni - rhesus monkey model. Secondly, in the interests of conserving the limited supplies of rhesus monkeys and reducing project costs, the repetitive use of the same infection for successive evaluation of new agents (when such lack activity at the dose delivered) is now established practice. The studies described below represent an abbreviated examination of the influence of inoculum size on the curative activities of WR-181,023, primaquine, and chloroquine in the model system in current use.

Two separate experiments were undertaken. The first was limited to determining the influence of the size of the sporozoite inoculum on the curative activities of WR-181,023 and primaquine. Thirty-nine rhesus monkeys were assigned to this study. These animals, in groups of thirteen, were inoculated with 4×10^1, 4×10^3, or 4×10^5 sporozoites*. Subgroups of six each were assigned to either WR-181,023 or primaquine dosage regimens, the thirteenth monkey serving as an untreated

*These inocula were derived from the undiluted homogenate of mosquito thoraces and 10^{-2} and 10^{-4} dilutions in normal monkey serum diluted 1:1 with saline. The sequence of inoculation was from the highest to lowest dilution.

control. The second experiment, involving thirty-two rhesus monkeys, was limited to determining the influence of inoculum size on the curative activity of WR-181,023 and chloroquine. Groups of eight monkeys each were inoculated with 1.1×10^6, 1.1×10^4, 1.1×10^2, or 1.1×10^0 (1) sporozoites*. Subgroups of four monkeys were assigned to a WR-181,023 regimen; a subgroup of two received treatment with chloroquine; the eighth monkey served as an untreated control. In both experiments, blood film examinations were initiated on Day 7 after sporozoite inoculation. In order to simplify the presentation, the results of the two experiments have been combined in Tables 19 to 21 and Figure 8.

The data on the relation between size of sporozoite inoculum and incubation or prepatent period have been summarized in Table 19. Data on the eight recipients of the 10^{-6} dilution (ca 1 sporozoite) are not listed because no member of this group developed a patent infection. As the table shows, only five of the fourteen recipients of 4×10^1 sporozoites (first experiment) became infected. This result was somewhat surprising since in previous titrations, doses of 10 or more sporozoites invariably proved to be infective. All recipients of larger inocula developed infections with a consistent (inverse) relationship between the size of the inoculum and the time when parasites were first identified on thick films.** Thus

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*These inocula were derived from the undiluted homogenate of mosquito thoraces and 10^{-2}, 10^{-4}, and 10^{-6} dilutions in normal monkey serum diluted 1:1 with saline. The sequence of inoculation was from the highest to lowest.

**It is quite likely that all infections were patent on Day 8 after inoculation, but that the parasitemias in recipients of the smaller inocula were so low as to be undetectable on thick films. This position is supported by the results of a large scale study carried out in 1946 in which 20 ml samples of blood drawn from monkeys on Day 9 after challenge (the day of tissue schizont maturation of the M strain) with 1×10^1 to 1×10^6 sporozoites were injected into clean recipients. The latter developed patent infections irrespective of the dose of sporozoites delivered to the donor or the time when thick films became positive.

the incubation period increased stepwise from eight days in recipients of 1.1×10^6 sporozoites to twelve to fourteen days in recipients of 4×10^1 sporozoites.

The courses of the parasitemias in the untreated control monkeys for the first ninety days after inoculation have been charted in Figure 8. As would be anticipated, once the infection became patent, the course of the parasitemia during this early period was independent of the size of the sporozoite inoculum.

Table 20 details the observations on the impacts of inoculum size on the curative activities of WR-181,023, primaquine, and chloroquine. The data obtained with this latter drug indicate that the apparent curative activity and relapse pattern are influenced by the size of the sporozoite inoculum. Mmu 8843 and Mmu 8854, inoculated with 1.1×10^6 sporozoites, exhibited a series of six relapses in an observation period of 110 days, with relapse intervals ranging from four to nine days (median six days)*. Mmu 8848 and Mmu 8850, inoculated with 1.1×10^4 sporozoites, each exhibited four relapses in the same time period, with relapse intervals ranging from seven to fifteen days (median ten days). Mmu 8834, one of the subjects inoculated with 1.1×10^2 sporozoites, exhibited four relapses at intervals of ten to thirteen days. Mmu 8844, the second recipient of this inoculum, had but a single relapse. In this subject, there was a thirty-six day parasite free interval between completion of treatment of the primary attack and the first and only relapse. There is a strong suggestion that the treatment of this relapse resulted in cure. Such a response to delivery of chloroquine is not uncommon in infections induced by inocula of 10^3 sporozoites or fewer. It probably

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*It will be noted that clearance of parasitemia was not achieved during treatment of the primary attack. This is not an uncommon occurrence with large sporozoite inoculum. The results of previous studies, which will not be detailed here, suggest that this phenomenon results from steady reseeding of the circulating blood with the progeny of the sporozoites which are developing at different rates in hepatocytes.

signals a negligible burden of tissue schizonts, or perhaps their total elimination. It should be emphasized that the same marginal burden can be achieved in subjects treated with barely sub-effective doses of radical curative drugs.

Table 21 summarizes the observations detailed in Table 20 relating to the influence of the size of the sporozoite inoculum on the curative activities of WR-181,023 and primaquine. In toto they show that the activities of these agents decrease with increase in inoculum size. Thus, among subjects inoculated with 10^4 sporozoites or fewer, seven of fourteen infections were cured by a dosage of 0.125 mg WR-181,023 per kg body weight; eleven of eleven infections were cured by a dosage of 0.25 mg per kg. In monkeys inoculated with 4×10^5 sporozoites and greater, only one of eight infections was cured at the 0.125 mg dose level, five of ten at a dose of 0.25 mg per kg. Although assessment of the influence of inoculum size on the curative activity of primaquine was limited, results were similar to those obtained with WR-181,023.

Although the observations reported here are less extensive than one might like, they are so similar to the results of earlier and far more extensive studies with the M strain of P. cynomolgi and with pamaquine, pentaquine, and isopentaquine, that it seems reasonably safe to conclude that in monkeys infected with the B strain the size of the sporozoite inoculum (ergo the burden of tissue schizonts) is also an important determinant of the real and apparent curative capabilities of test compounds. This principle commands continued attention in any curative drug evaluation program. It argues for inducing test infections with large sporozoite inocula, 10^5 or greater. It supports the repetitive use of the same subject (infection) for drug evaluations as long as relapse intervals are short. It cautions against continued use of such infections whenever relapse intervals are prolonged because such intervals may reflect minimal tissue schizont burden.

TABLE 19

THE DURATION OF THE INCUBATION (PREPATENT) PERIOD OF RHESUS MONKEYS INOCULATED WITH VARYING NUMBERS OF SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

No. of Sporozoites in Inoculum*	Mmu No.	Day of Patency after Sporozoite Inoculation	Remarks
4×10^{1}**	8828	-	Rechallenged with 10^6 sporozoites Day 52; infections patent Day 8 following reinoculation.
	8831	-	
	8832	-	
	8834	-	
	8849	-	
	8850	-	
	8852	-	
	8854	-	
	8856	-	
	8855	12	Experiment 1: Comparison of curative activities of WR-181,023 and primaquine.
	8842	13	
	8871	14	
	8833	14	
	8851	14	
1.1×10^{2}	8834	11	Experiment 2: Comparison of curative activities of WR-181,023 and chloroquine.
	8844	11	
	8849	11	
	8789	12	
	8811	12	
	8821	12	
	7458	13	
	8808	13	
4×10^{3}	8836	10	Experiment 1: Comparison of curative activities of WR-181,023 and primaquine.
	8858	10	
	8860	10	
	8862	10	
	8863	10	
	8864	10	
	8865	10	
	8866	10	
	8867	10	
	8868	10	
	8853	11	
	8857	11	
	8861	11	

TABLE 19 - CONTINUED

No. of Sporozoites in Inoculum*	Mmu No.	Day of Patency after Sporozoite Inoculation	Remarks
1.1×10^4	8739	10	Experiment 2: Comparison of curative activities of WR-181,023 and chloroquine.
	8816	10	
	8822	10	
	8826	10	
	8846	10	
	8848	10	
	8850	10	
	8852	10	
4×10^5	8845	8	Experiment 1: Comparison of curative activities of WR-181,023 and primaquine.
	8847	8	
	8869	8	
	8872	8	
	8874	8	
	8875	8	
	8877	8	
	8879	8	
	8890	8	
	8837	9	
	8838	9	
	8873	9	
	8878	9	
1.1×10^6	8738	8	Experiment 2: Comparison of curative activities of WR-181,023 and chloroquine.
	8744	8	
	8803	8	
	8830	8	
	8835	8	
	8843	8	
	8854	8	
	8856	8	

*Experiment 2 included a group of eight monkeys inoculated with 4 sporozoites; none exhibited a patent infection over an observation period of 48 days. All were rechallenged with 10^6 sporozoites on that day; all exhibited patent infections on Day 8 after rechallenge.

**Inadvertently, a fourteenth monkey was included in this group.

FIGURE 8

THE INFLUENCE OF THE SIZE OF THE SPOROZOITE INOCULUM ON THE COURSE OF THE PARASITEMIA IN UNTREATED CONTROL MONKEYS

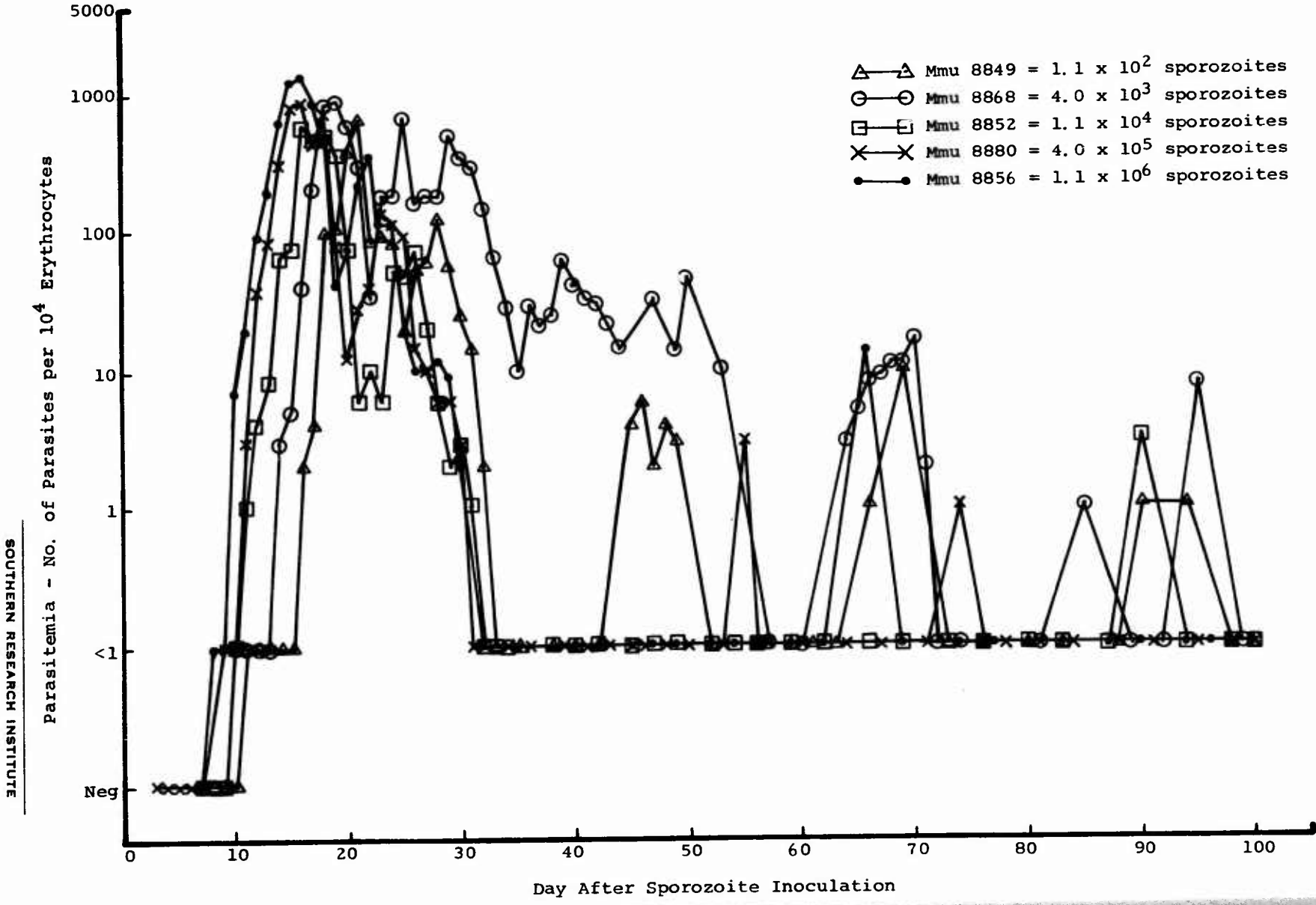

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TABLE 20

THE INFLUENCE OF THE SIZE OF THE SPOROZOITE INOCULUM ON THE RADICAL CURATIVE ACTIVITIES OF WR-181,023, PRIMAQUINE, AND CHLOROQUINE

(Detailed Observations)

No. of Sporozoites in Inoculum	Daily Dose Mg Base/Kg Body Weight			Mmu No.	Response to Treatment		
	WR-181,023	Primaquine	Chloroquine		Relapsed	Days from R_x to Relapse	Cured*
4×10^1	0.125	-	2.5	8851P	-	-	+
	0.25	-	2.5	8855P	-	-	+
	-	0.25	2.5	8871P	-	-	+
	-	0.5	2.5	8833P	-	-	+
	-	0.5	2.5	8842P	-	-	+
1.1×10^2	0.125	-	2.5	8821P	+	11	-
	0.125	-	2.5	8789P	+	14	-
	0.125	-	2.5	8811P	+	15	-
	0.125	-	2.5	8808P	-	-	+(93)
	0.125	-	2.5	7458P	-	-	+(95)
	0.25	-	2.5	$8789R_1$	-	-	+(70)
	0.25	-	2.5	$8811R_1$	-	-	+(70)
	0.25	-	2.5	$8821R_1$	-	-	+(73)
	-	-	2.5	8834P	+	10	-
	-	-	2.5	$8834R_1$	+	10	-
	-	-	2.5	$8834R_2$	+	10	-
	-	-	2.5	$8834R_3$	+	10	-
	-	-	2.5	$8834R_4$	+	13	-
	-	-	2.5	8844P	+	36	-
	-	-	2.5	$8844R_1$	-	-	+(51)
4×10^3	0.125	-	2.5	8863P	+	16	-
	0.125	-	2.5	8862P	-	-	+
	0.125	-	2.5	8864P	-	-	+
	0.25	-	2.5	8865P	-	-	+
	0.25	-	2.5	8866P	-	-	+
	0.25	-	2.5	8867P	-	-	+
	0.25	-	2.5	$8863R_1$	-	-	+(92)
	-	0.25	2.5	8857P	+	9	-
	-	0.25	2.5	8836P	-	-	+
	-	0.25	2.5	8853P	-	-	+
	-	0.5	2.5	8858P	-	-	+
	-	0.5	2.5	8860P	-	-	+
	-	0.5	2.5	8861P	-	-	+
	-	0.5	2.5	$8857R_1$	-	-	+

TABLE 20 - CONTINUED

No. of Sporozoites in Inoculum	Daily Dose Mg Base/Kg Body Weight			Mmu No.	Response to Treatment		
	WR-181,023	Primaquine	Chloroquine		Relapsed	Days from R_x to Relapse	Cured*
	0.125	-	2.5	8739P	+	13	-
	0.125	-	2.5	8826P	+	14	-
	0.125	-	2.5	8816P	+	32	-
	0.125	-	2.5	8822P	-	-	+(98)
	0.125	-	2.5	8846P	-	-	+(98)
	0.25	-	2.5	$8816R_1$	-	-	+(58)
	0.25	-	2.5	$8739R_1$	-	-	+(76)
	0.25	-	2.5	$8846R_1$	-	-	+(76)
1.1×10^4	-	-	2.5	8848P	+	9	-
	-	-	2.5	$8848R_1$	+	14	-
	-	-	2.5	$8848R_2$	+	10	-
	-	-	2.5	$8848R_3$	+	10	-
	-	-	2.5	$8848R_4$	+	9	-
	-	-	2.5	8850P	+	7	-
	-	-	2.5	$8850R_1$	+	15	-
	-	-	2.5	$8850R_2$	+	10	-
	-	-	2.5	$8850R_3$	+	10	-
	-	-	2.5	$8850R_4$	+	13	-
	0.125	-	2.5	8875P	+	7	-
	0.125	-	2.5	8874P	+	9	-
	0.125	-	2.5	8873P	-	-	+
	0.25	-	2.5	8877P	+	28	-
	0.25	-	2.5	8878P	-	-	+
	0.25	-	2.5	8879P	-	-	+
	0.25	-	2.5	$8874R_1$	-	-	+
	0.25	-	2.5	$8875R_1$	-	-	+
	0.5	-	2.5	$8877R_1$	-	-	+(90)
4×10^5	-	0.25	2.5	8838P	+	7	-
	-	0.25	2.5	8837P	+	9	-
	-	0.25	2.5	8845P	+	10	-
	-	0.5	2.5	8847P	+	12	-
	-	0.5	2.5	8872P	+	72	-
	-	0.5	2.5	$8838R_1$	+	91	-
	-	0.5	2.5	8869P	-	-	+
	-	0.5	2.5	$8837R_1$	-	-	+
	-	0.5	2.5	$8845R_1$	-	-	+
	-	1.0	2.5	$8847R_1$	-	-	+
	-	1.0	2.5	$8872R_1$	-	-	+(48)
	-	1.0	2.5	$8838R_2$	-	-	?(14)

TABLE 20 - CONTINUED

No. of Sporozoites in Inoculum	Daily Dose Mg Base/Kg Body Weight			Mmu No.	Response to Treatment		
	WR-181,023	Primaquine	Chloroquine		Relapsed	Days from R_x to Relapse	Cured*
	0.125	-	2.5	8738P	+	6	-
	0.125	-	2.5	8803P	+	7	-
	0.125	-	2.5	8835P	+	7	-
	0.125	-	2.5	8744P	+	11	-
	0.125	-	2.5	8830P	+	24	-
	0.25	-	2.5	$8744R_1$	+	14	-
	0.25	-	2.5	$8835R_1$	+	23	-
	0.25	-	2.5	$8803R_1$	+	30	-
	0.25	-	2.5	$8830R_1$	+	39	-
	0.25	-	2.5	$8738R_1$	-	-	+(86)
	0.5	-	2.5	$8803R_2$	-	-	+(48)
	0.5	-	2.5	$8835R_2$	-	-	+(56)
	0.5	-	2.5	$8744R_2$	-	-	+(58)
1.1×10^6	-	-	2.5	8843P	Parasitemia persisted		
	-	-	2.5	$8843R_1$	+	6	-
	-	-	2.5	$8843R_2$	+	6	-
	-	-	2.5	$8843R_3$	+	6	-
	-	-	2.5	$8843R_4$	+	6	-
	-	-	2.5	$8843R_5$	+	9	-
	-	-	2.5	$8843R_6$	+	9	-
	-	-	2.5	8854P	Parasitemia persisted		
	-	-	2.5	$8854R_1$	+	4	-
	-	-	2.5	$8854R_2$	+	7	-
	-	-	2.5	$8854R_3$	+	6	-
	-	-	2.5	$8854R_4$	+	6	-
	-	-	2.5	$8854R_5$	+	6	-
	-	-	2.5	$8854R_6$	+	6	-

*The figures in parentheses indicate the duration of the thick film negative follow-up period subsequent to delivery of the seventh dose of WR-181,023 or primaquine. It is quite likely that the infection has been cured when the follow-up period exceeds fifty days.

TABLE 21

THE INFLUENCE OF THE SIZE OF THE SPOROZOITE INOCULUM ON THE RADICAL CURATIVE ACTIVITIES OF WR-181,023 AND PRIMAQUINE

Summary Observations

No. of Sporozoites in Inoculum	Compound	No. of Infections Cured/No. Treated			
		Daily Dose - Mg Base/Kg Body Weight*			
		0.125	0.25	0.5	1.0
4×10^1	WR-181,023	1/1	1/1	-	-
1.1×10^2		2/5	3/3	-	-
4×10^3		2/3	4/4	-	-
1.1×10^4		2/5	3/3	-	-
4×10^5		1/3	4/5	1/1	-
1.1×10^6		0/5	1/5	3/3	-
4×10^1	Primaquine	-	1/1	2/2	-
4×10^3		-	1/3	4/4	-
4×10^5		-	0/3	3/6	2/2

*Administered once daily for seven consecutive days together with chloroquine, 2.5 mg base per kg body weight.

X. PRELIMINARY EVALUATIONS OF THE TOXICITIES OF SELECTED 8-AMINOQUINOLINES FOR THE RHESUS MONKEY

X. PRELIMINARY EVALUATIONS OF THE TOXICITIES OF SELECTED 8-AMINOQUINOLINES FOR THE RHESUS MONKEY

Although pursuit of toxicologic investigations has never been a major responsibility of this Project, a number of preliminary studies have been undertaken in this area. These explorations have been quite distinct from the casual identification of symptoms of intolerance during evaluations of blood schizonticidal agents in the owl monkey or tissue schizonticidal compounds in the rhesus monkey. All investigations have been concerned with the reactions of the latter monkey to the 8-aminoquinolines - a small group of derivatives which commanded unusual attention either because of novelty of chemical structure or because of exhibition of unusual levels of curative activity.

A fortuitous set of circumstances made it possible to pursue these preliminary toxicologic investigations locally with more than a little precision, maximal speed, minimal effort and cost, and without disrupting other activities for which there was formal commitment. First, the Principal Investigator had acquired substantial experience in defining the untoward reactions of various laboratory animals to the 8-aminoquinolines during the WW II and post-WW II search for more effective, better tolerated curative drugs. This made it possible to identify the more important features of these agents in small scale studies. Secondly, until June 30, 1975, the Principal Investigator had responsibility for a group of investigators committed to large scale evaluations of the hematologic, biochemical, and tissue reactions of various experimental animals to anticancer drugs. Without significant distraction from their major efforts, these individuals were able to pursue critical assessments of the impacts of the 8-aminoquinolines on formed elements and biochemical constituents of peripheral blood, and gross and microscopic changes in organs and tissues.

Thirdly, a more than adequate supply of healthy, well-conditioned rhesus monkeys, discarded from curative drug evaluations after minimal parasitic experiences, was readily available. This situation held animal costs to daily charges for food, bedding, and essential care. Lastly, hourly surveillance of the monkey colony was an established practice. This insured serial identification of deterioration in well-being, whenever it occurred, and acquisition of tissue samples free of post-mortem changes.

This unique situation was exploited during the preceding Project periods when limited appraisals of the toxicities of primaquine and its isomers (WR-211,536 and WR-211,537), WR-181,023 (4-methyl primaquine), WR-211,532 [2-methyl-5-(4-chlorophenoxy) primaquine], and WR-211,533 (2,4-dimethyl plasmocid) were undertaken. In the current Project period, preliminary studies were pursued on the toxicities of WR-221,033 and WR-221,036 (the D and L components of WR-181,023), WR-212,579, WR-215,296, WR-215,761, WR-216,804, and WR-221,527 (the most active of the 8-aminoquinolines examined to date), and on WR-182,234 and its structural isomer WR-222,671*. The results of these studies are summarized briefly in the subsections that follow.

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*The project concerned with the toxicities of antitumor drugs was terminated on June 30, 1975. This made it necessary to omit all hematologic studies from the current evaluations and to reduce biochemical studies to measurements of bilirubin concentrations and GOT activities in serum. The latter, together with the results of gross and microscopic examinations of the liver, sufficed to identify the levels of hepatotoxicity exhibited by the various agents.

A. The Comparative Toxicities Of WR-181,023 And Its D And L Components, WR-221,033 And WR-221,036

As indicated in Section VI of this Report, the radical curative activities of WR-221,033 and WR-221,036, the D and L components of WR-181,023, were essentially identical with the activity of the racemate. It was generally recognized that continued interest in these isomers would be determined by their toxicities. If they were equal to each other and to WR-181,023 in toxicity, further study would be contraindicated. If, however, one of the isomers was strikingly less toxic than the other, as was the case with the primaquine isomers, WR-211,536 and WR-211,537 (cf SORI-KM-76-319 for details), the less toxic of the pair would merit more extensive investigation since it could well replace WR-181,023, the racemate, as a candidate for evaluation in human volunteers.

The results of a preliminary comparison of the toxicities of WR-221,033, WR-221,036, and WR-181,023 have been summarized in Table 22. As shown in this table, the isomers were examined in single monkeys at daily doses of 3.0, 6.0, 9.0, and 12.0 mg base per kg body weight*. In the current study, WR-181,023 was examined at doses of 3.0 and 6.0 mg per kg. Data on responses to doses of 4.0, 8.0, and 12.0 mg WR-181,023 per kg were drawn from earlier evaluations. The results of this comparison indicate that the toxicities of the D and L isomers and the racemate were very similar. Daily doses of 9.0 mg per kg of either WR-221,033 (D) or WR-221,036 (L) or 8.0 mg per kg of WR-181,023 were uniformly fatal. The total dose of any one of these agents producing a lethal reaction approximated 45.0 mg base per kg body weight. The qualitative aspects of these fatal reactions were similar -

*The limited supplies of the isomers account for abbreviated evaluations referred to here and recorded in Table 22.

anorexia, icterus, loss of skeletal muscle tone, and coma, appearing in rapid succession. These were associated with striking elevations in GOT activity and bilirubin concentrations in serum and with marked enlargement and focal or generalized necrosis of the liver; ascites was a common finding.

The similarity in toxicity of the D and L components of WR-181,023 stands in striking contrast to the difference in the toxicities of the primaquine isomers, where the L isomer was three to four times as toxic as the D. The reasons for this difference in the relative toxicities of the two sets of isomers are not at all clear and deserve careful study. However, from the practical point of view, the current findings offer no immediate stimulus for turning to either isomer of WR-181,023 in the projected studies in human volunteers.

TABLE 22

A PRELIMINARY COMPARISON OF THE SUBACUTE TOXICITIES OF WR-221,033 (D ISOMER), WR-221,036 (L ISOMER) AND WR-181,023 (D,L MIXTURE) FOR THE RHESUS MONKEY

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	No. of Doses	Total Dose Mg Base/Kg Body Weight	Reactions to Treatment			
				Fate	Liver Pathology Gross	Conc. in Serum** GOT Units	Conc. in Serum** Bilirubin Mg per Cent
WR-181,023 (D,L Mixture)							
8462	3.0	7	21.0	Survived: no reaction.	n.a.	26	0.3
8482	3.0	7	21.0	Survived: no reaction.	n.a.	29	0.2
7538	4.0	7	28.0	Survived: no reaction.	n.a.	30	0.4
7963	4.0	7	28.0	Survived: no reaction.	n.a.	40	0.6
8607	6.0	7	42.0	Survived: anorexia; weight loss; icterus; recovery incomplete Day 14.	n.a.	180	1.4
8551	6.0	4	24.0	Died 19 hours after Dose 4.	+	460	2.8
7817	8.0	4	32.0	Died 29 hours after Dose 4.	+	900	5.9
7964	8.0	6	48.0	Died 13 hours after Dose 6.	+	880	3.0
7815	12.0	4	48.0	Died 22 hours after Dose 4.	+	450	4.1
7936	12.0	4	48.0	Died 12 hours after Dose 4.	+	440	3.5
7997	12.0	5	60.0	Died 26 hours after Dose 5.	+	396	7.2

TABLE 22 - CONTINUED

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	No. of Doses	Total Dose Mg Base/Kg Body Weight	Reactions to Treatment			
				Fate	Liver Pathology — Gross	Conc. in Serum** GOT Units	Conc. in Serum** Bilirubin Mg per Cent
WR-221,033 (D Isomer)							
8521	3.0	7	21.0	Survived: no reaction.	n.a.	28	0.5
8649	6.0	7	42.0	Survived: no reaction.	n.a.	32	0.4
8521r	9.0	4	36.0	Died 29 hours after Dose 4.	+	370	2.3
8649r	12.0	5	60.0	Died 23 hours after Dose 5.	+	720	3.5
WR-221,036 (L Isomer)							
8673	3.0	7	21.0	Survived: no reaction.	n.a.	24	0.3
8674	6.0	7	42.0	Survived: no reaction.	n.a.	29	0.4
8673r	9.0	5	45.0	Died 8 hours after Dose 5.	+	480	3.0
8674r	12.0	5	60.0	Died 14 hours after Dose 5.	+	640	4.0

*Dose administered orally, once daily at 8:00 a.m.

**Terminal levels in fatal cases; peak levels during or post-treatment of survivors.

B. The Toxicities Of WR-212,579, WR-215,296, WR-215,761, WR-216,804, And WR-221,527 For The Rhesus Monkey

Studies summarized in Section III-C and confirmed in Section VII indicate that WR-212,579, WR-215,296, WR-215,761, WR-216,804, and WR-221,527 (cf structures in Figure 7) are the most active of all 8-aminoquinolines examined to date. On a comparative dose basis, each of these agents is approximately twice as active as WR-181,023, four times as active as primaquine. These activities are impressive, but may be meaningful for human application only if associated with larger therapeutic indices than are exhibited by primaquine or 4-methyl primaquine. The need for these indices led to preliminary assessments of the subacute toxicity of each of the agents for the rhesus monkey with results summarized in Table 23.

The data set forth in the above table indicate that the total doses of WR-212,579, WR-215,296, and WR-215,761 tolerated by the monkey without evidence of untoward reactions fall between 10.5 and 21.0 mg base per kg body weight. The total tolerated doses of WR-216,804 and WR-221,527 were somewhat less than the above, falling between 5.25 and 10.5 mg per kg. All five compounds produced similar toxic reactions; anorexia, jaundice, loss of muscle tone, coma, associated with hyperbilirubinemia, increases in serum GOT activity, hepatic enlargement, and necrosis. Thus they resembled primaquine and WR-181,023 with respect to the types of reactions evoked; quantitatively, they were significantly more toxic.

Rough calculations based on the routinely curative dose (0.875 mg per kg total) and the probable maximum tolerated dose (10.5 to 21.0 mg per kg total) indicate that WR-212,579, WR-215,296, and WR-215,761 have therapeutic indices between 12 and 24. The corresponding indices for WR-216,804 and WR-221,527 would fall between 6 and 12.

The therapeutic indices of WR-212,579, WR-215,296, and WR-215,761 set forth above are very nearly identical with those of primaquine and 4-methyl primaquine. This similarity in therapeutic index may well imply that these unusually active compounds have no more to offer than primaquine and discourage further examinations. The door should be left open, however, to tempering this implication. The indices referred to are based on lethal toxicity. Lethality is not the reaction which limits the utility of primaquine (nor which limited the utility of its predecessors), but rather the capacity of this agent to induce gastric distress, abdominal cramping, methemoglobin formation, depression of hematopoiesis, and an anemia related to inherited G6PD deficiency. These parameters have not been explored as yet, nor can they be evaluated appropriately in the rhesus monkey. The first three can be assessed in the dog. Therefore, it would be advisable to evaluate these compounds in the latter laboratory animal before setting aside agents that might be far more active than primaquine and potentially useful in developing short term treatment regimens. It should be recalled that there are relatively minor differences between the chemical structures of WR-212,579, WR-215,296, and WR-215,761, and WR-181,023. Yet, these alterations led to substantial improvement in therapeutic activity. It would not be surprising if more comprehensive examinations, particularly in animals other than the rhesus monkey, showed that these structural changes were associated with alterations in qualitative aspects of host toxicity as well.

TABLE 23

PRELIMINARY EVALUATIONS OF THE SUBACUTE TOXICITIES OF WR-212,579, WR-215,296, WR-215,761, WR-216,804 AND WR-221,527 FOR THE RHESUS MONKEY

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	No. of Doses	Total Dose Mg Base/Kg Body Weight	Reactions to Treatment			
				Fate	Liver Pathology Gross	Conc. in Serum** GOT Units	Conc. in Serum** Bilirubin Mg per Cent
WR-212,579							
8648	0.75	7	5.25	Survived: no reaction.	n.a.	19	0.3
8648r	1.5	7	10.5	Survived: no reaction.	n.a.	26	0.4
8691	1.5	7	10.5	Survived: no reaction.	n.a.	23	0.4
8691r	3.0	7	21.0	Survived: elevated bilirubin and GOT Day 1 post R_x; normal Day 5.	n.a.	110	1.1
8687	3.0	7	21.0	Died 57 hours after Dose 7.	+	1020	6.2
8688	6.0	3	18.0	Died 26 hours after Dose 3.	+	340	2.9
WR-215,296							
8693	0.75	7	5.25	Survived: no reaction.	n.a.	24	0.5
8693r	1.5	7	10.5	Survived: no reaction.	n.a.	38	0.4
8708	1.5	7	10.5	Survived: no reaction.	n.a.	27	0.3
8708r	3.0	7	21.0	Survived: elevated bilirubin and GOT Day 1 post R_x; normal Day 5.	n.a.	84	1.0
8714	3.0	5	15.0	Died 30 hours after Dose 5.	+	740	3.4
8690	6.0	5	30.0	Died 9 hours after Dose 5.	+	620	3.7

TABLE 23 - CONTINUED

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	No. of Doses	Total Dose Mg Base/Kg Body Weight	Reactions to Treatment			
				Fate	Liver Pathology Gross	Conc. in Serum** GOT Units	Conc. in Serum** Bilirubin Mg per Cent
WR-215,761							
8717	1.5	7	10.5	Survived: no reaction.	n. a.	28	0.4
8724	3.0	7	21.0	Survived: no reaction.	n. a.	21	0.3
8751	3.0	7	21.0	Survived: elevated bilirubin and GOT Day 1 post R_x; normal Day 11.	n. a.	140	1.4
8717r	3.0	6	18.0	Died 23 hours after Dose 6.	+	940	4.2
8724r	6.0	4	24.0	Died 22 hours after Dose 4.	+	500	3.1
8762	6.0	4	24.0	Died 24 hours after Dose 4.	+	540	4.4
WR-216,804							
8620	0.75	7	5.25	Survived: no reaction.	n. a.	36	0.4
8513	1.5	6	9.0	Died 14 hours after Dose 6.	+	820	4.2
8657	3.0	3	9.0	Died 26 hours after Dose 3.	+	310	2.2
8658	6.0	3	18.0	Died 4 hours after Dose 3.	+	280	2.0
WR-221,527							
8743	0.75	7	5.25	Survived: no reaction.	n. a.	27	0.3
8743r	1.5	5	7.5	Died 37 hours after Dose 5.	+	880	4.0
8778	1.5	3	4.5	Died 9 hours after Dose 3.	+	1240	5.1
8721	3.0	3	9.0	Died 17 hours after Dose 3.	+	340	3.1
8727	6.0	1	6.0	Died 27 hours after Dose 1.	+	160	2.2

*Dose administered orally, once daily at 8:00 a.m.

**Terminal levels in fatal cases; peak levels during or post-treatment of survivors.

C. The Toxicities Of WR-182,234 And WR-222,671 For The Rhesus Monkey

WR-182,234, 2-methyl primaquine, attracted some interest early in the search for improved curative drugs, both because of novel structure and because it was one of the few agents examined which exhibited activity comparable to that of primaquine. This interest was not sustained, in part because of the appearance of WR-181,023 with greater activity, and in part because WR-182,234 exhibited the same order of toxicity as primaquine for the rhesus monkey. Interest in 2-methyl substituted derivatives was revived when it was found that a side chain isomer, WR-222,671, was at least twice as active as WR-182,234. This finding stimulated a preliminary evaluation of the toxicity of WR-222,671. The results of this appraisal are summarized in Table 24, together with the results of the earlier study of the toxicity of WR-182,234, complemented by data on two additional subjects (Mmu 8712 and Mmu 8675) examined currently.

As indicated in Table 24, the lethal toxicity of WR-222,671 is at least twice that of WR-182,234, possibly greater, pointing to a lower therapeutic index. The reactions of the rhesus monkey to lethal doses of the two compounds were quite similar and in no significant way different from the reactions to WR-181,023 or primaquine. This finding provides little encouragement for further studies of WR-222,671 since its activity is quantitatively less than that of the 4-methyl substituted WR-212,579, WR-215,296, and WR-215,761 and since on a dosage basis, it is at least equally toxic. However, because of the novelty of 2-substituted 8-aminoquinolines, it might be well to postpone a totally negative judgment until a pilot study in another animal species can be mounted and evaluated.

TABLE 24

PRELIMINARY EVALUATIONS OF THE SUBACUTE TOXICITIES OF WR-182,234 AND WR-222,671 FOR THE RHESUS MONKEY

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	No. of Doses	Total Dose Mg Base/Kg Body Weight	Reactions to Treatment			
				Fate	Liver Pathology Gross	Conc. in Serum** GOT Units	Conc. in Serum** Bilirubin Mg per Cent
WR-182,234							
8712	3.0	7	21.0	Survived: no reaction.	n.a.	31	0.3
7949	4.0	7	28.0	Survived: no reaction.	n.a.	38	0.6
8675	6.0	7	42.0	Survived: elevated bilirubin and GOT Day 1 post-R_X; normal Day 5.	n.a.	96	0.9
7950	8.0	7	56.0	Survived: elevated bilirubin and GOT Day 1 post-R_X; normal Day 5.	n.a.	110	1.0
7980	12.0	3	36.0	Died 10 hours after Dose 3.	+	430	3.4
7986	18.0	4	72.0	Died 6 hours after Dose 4.	+	340	3.5
WR-222,671							
8763	0.75	7	5.25	Survived: no reaction.	n.a.	26	0.4
8806	1.5	7	10.5	Survived: no reaction.	n.a.	32	0.3
8642	3.0	6	18.0	Died 33 hours after Dose 6.	+	1280	5.2
8777	6.0	4	24.0	Died 9 hours after Dose 4.	+	860	4.1

*Dose administered orally, once daily at 8:00 a.m.

**Terminal levels in fatal cases; peak levels during or post-treatment of survivors.

EPILOGUE

EPILOGUE

The foregoing Report is a milestone, marking the end of the active laboratory phase of DADA 17-69-C-9104 and the completion and termination of thirty-four consecutive years of investigations on the chemotherapy of malaria*. This activity had its origins in 1942 in the Malaria Chemotherapy Program of World War II. Much of the work undertaken in the intervening years has been directed toward meeting the needs of the military, first in the South Pacific (1942-1950), then in Korea (1950-1951), and more recently in Southeast Asia (1967-1976). Investigations carried out in these periods of military need, and those pursued in the 1952-1967 interlude, have contributed to the primary development and/or method of application of every agent that now occupies a place in the therapy of the human malarias and most recently to the establishment of a sizeable pool of candidates awaiting primary examination in human volunteers. For the most part, these contributions have rested on development and exploitation of two experimental animal models, one based on infections with sporozoites of P. cynomolgi in the rhesus monkey, the second on infections induced by drug-susceptible and resistant strains of P. falciparum and P. vivax in the owl monkey**. The first of these models has been utilized primarily, but not exclusively in the search for improved tissue schizonticidal drugs. The second, a more recent development, has been utilized

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*These years covered activities at The Christ Hospital Institute for Medical Research, Cincinnati, 1942-1963, the National Center for Primate Biology, University of California, Davis, 1963-1969, and the Southern Research Institute, 1969-1976.

**During the 1942-1967 period, assessments of antimalarial activities, with studies on physiologic disposition and host toxicity (as encountered in rhesus monkeys and dogs), were pursued in tandem. Since 1967, assessments of antimalarial activities have, with few exceptions, been of sole concern.

exclusively in the search for blood schizonticides, serving primarily to identify agents as effective as chloroquine against infections with chloroquine-susceptible strains and fully active against infections with chloroquine- and multi-drug-resistant strains.

A full review of the efforts and accomplishments of these thirty-four years is clearly beyond the scope of this addendum. The Principal Investigator would like to conclude his formal project reporting with a bit of stock-taking, setting forth briefly his personal opinions as to where the Malaria Chemotherapy Program stands with respect to attainment of its two major missions, what further investigations are clearly indicated, and what priorities the latter command*. In the following comments, needs for primary and expanded clinical evaluations have been stressed quite frequently. This implies neither a "fixation" on such investigations, downgrading of past or current studies in humans, nor depreciation of work in the organic synthesis or preclinical evaluation areas. It reflects a strong personal conviction that further endeavors in the latter laboratory fields cannot be highly productive until it is known what can be accomplished with available agents in the human target and whether these agents have shortcomings that must be eliminated via either chemical synthetic modifications or alterations in drug delivery mechanisms. There is a growing conviction that, however difficult it may be to do so, outlets for controlled evaluations of promising compounds in human subjects must be expanded significantly if the accumulated fruits of laboratory activities are to be harvested in a reasonable time.

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*It will doubtless be noted that there is no mention of the status of pharmacologic studies. This reflects the absence of specific information on activities in this area.

THE SEARCH FOR BLOOD SCHIZONTICIDES

Insofar as the results of experimental laboratory studies* are concerned, substantial progress has been made toward developing a drug or group of drugs fully effective against infections with chloroquine- and other drug-resistant strains of P. falciparum - the original mission of the Malaria Chemotherapy Program. Two compounds, WR-30,090 and WR-33,063, have already exhibited useable activities in both controlled volunteer and limited field studies; however, these are no longer the most promising agents available. There are at least six other compounds which on a dose basis are as active (or more active) against experimental infections with multi-drug-resistant strains of this plasmodium as is chloroquine against infections with susceptible strains. The group includes the 9-phenanthrenemethanol, WR-122,455, two 4-quinolinemethanols, WR-142,490 and WR-184,806, two 4-pyridinemethanols, WR-172,435 and WR-180,409, and the Mannich base, WR-194,965. One additional 9-phenanthrenemethanol, WR-171,669, is only slightly less active than WR-122,455. The results of preliminary laboratory studies, clearly in need of confirmation and expansion, have identified a third 4-quinolinemethanol, WR-226,253, which is somewhat more active on a dose basis than its chemical relatives WR-142,490 and WR-184,806. The combination of WR-158,122 (a 2,4-diamino-6-sulfonyl substituted quinazoline) with a sulfonamide, on a dose basis probably the most active of all single or combination antimalarials, should be added to this group of eight agents. All nine merit controlled evaluation in human subjects infected with multidrug-resistant strains of P. falciparum.

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*Both chemical and biological activities are included in this phrase.

Insofar as we are aware, only one of the above group, WR-142,490, has been evaluated adequately in human subjects. These evaluations have shown that a single well-tolerated dose of this 4-quinolinemethanol effects rapid control of clinical symptoms (chills and fever) and cures infections with blood schizonts of two highly chloroquine-resistant strains of P. falciparum. A single well-tolerated dose confers protracted protection against challenge with blood parasites of these strains. These observations have generated considerable optimism with respect to the future of WR-142,490, an attitude undoubtedly stimulated by the effectiveness of the much less active 4-quinolinemethanol, WR-30,090, in treating infections with chloroquine/pyrimethamine-resistant strains of P. falciparum in Vietnam. This optimism may be justified, but at this point, it should not lead to shelving of the other seven promising derivatives or the WR-158,122 - sulfonamide combination without examination in man. There is clearly room, in fact need, for a variety of effective antimalarial drugs. Recent experience with chloroquine and amodiaquine testifies all too persuasively to the dangers inherent in reliance on a limited drug armamentarium. Evaluation of the utility of WR-142,490 in field situations should be encouraged, but such activity should not lock out attention to other agents which have exhibited as much or greater promise in demanding and relevant laboratory studies.

The uncertainties of the value in human infections of the group of nine compounds referred to above have a chilling effect on new laboratory studies. They make it difficult to develop an orderly or justifiable plan for further synthesis in the relevant compound classes. Until the values and limitations of the currently available agents are known, it would probably be advisable to direct synthesis efforts into those few virgin areas that have not yet been explored in the current malaria program or even to pilot explorations of some wholly theoretical chemical-parasitological concepts.

The above "in limbo" status is shared by those concerned with the biological evaluations of new agents. The activities of representatives of new compound classes and exotic chemical manipulations should probably be the sole concern of the primary screen until the clinical status of the active classes is defined and stimulates new and rational activity. Since the output of new compounds worthy of advanced study is likely to be small, there will be relatively few demands for the more critical evaluations in the owl monkey - human malaria models, leaving this system free for other types of investigations.

The likelihood of emergence of resistance to the promising representatives of the 9-phenanthrenemethanols, 4-quinolinemethanols, 4-pyridinemethanols, and Mannich bases is an important peripheral area which deserves careful study in owl monkeys infected with diverse strains of P. falciparum. This activity could occupy this system until there are new compounds to evaluate. Previous casual studies on development of resistance in this laboratory have yielded totally negative results indicating that evolution of strains resistant to the above agents will be a minor problem, if it occurs at all. Nonetheless, the phenomenon should be investigated more directly and intensively since Peters has reported that lines of P. berghei resistant to WR-122,455 and WR-142,490 develop with relative ease. This finding in a highly labile parasite system is worth noting, but should not cause undue concern unless confirmed in the more stable human plasmodium - owl monkey models. If resistance does develop in any of the simian systems, the search for a companion drug which would delimit the event should be undertaken at once.

THE SEARCH FOR TISSUE SCHIZONTICIDES

Assessment of progress made toward achieving the second and more recently assumed mission of the Malaria Chemotherapy Program - development of tissue schizonticidal drugs more effective, better tolerated, and more easily administered than primaquine - is a more complicated task than appraisal of progress in developing new blood schizonticides. A moderately large group of promising agents has been identified in the highly reproducible and predictive rhesus monkey - P. cynomolgi model. Lacking validation of the activities of any of these tissue schizonticides in human volunteers bearing infections with sporozoites of P. vivax, it is difficult to gauge this accomplishment. Such clinical studies are essential not only for assessing progress, but equally for guiding future chemical synthesis and evaluations in the P. cynomolgi model.

The search for new classes of compounds endowed with tissue schizonticidal activity has not been productive to date. A sizeable group of chemically heterogeneous agents has been studied without indication that any has potential radical curative activity*. Special attention has been accorded the

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*Although this appraisal is correct insofar as evaluations supported by this Project are concerned, it requires qualification to encompass older observations on the pyrocatechol, RC-12. This compound exhibited high orders of activity against the early and late tissue schizonts of the B, RO, and RO/PM strains of P. cynomolgi (the activity against the B strain has been confirmed in two other laboratories). These activities were not replicated in carefully staged evaluations in human volunteers inoculated with sporozoites of P. vivax. A single dosage of RC-12, comparable to that effective against the simian infections, was employed in the human studies. It is unfortunate that the latter investigations were not pursued further. It is most important to know whether apparent failure in man rests on a defect in absorption of RC-12 from the gastrointestinal tract, unusually rapid inactivation or excretion, or whether for the first time the P. cynomolgi model has failed to predict for infections with P. vivax. Until these issues are resolved, it would seem unwise to discount the possibility that RC-12 (and other pyrocatechols) has potential as a tissue schizonticide.

1,5-naphthyridines and 6-aminoquinolines. No member of the former class has exhibited reproducible tissue schizonticidal activity. A number of 6-aminoquinolines have displayed activity, but always at or very close to the maximally tolerated dose. Although moderately sized numbers of both of the above groups have been examined for tissue schizonticidal activity, it appears to a non-chemist that the structural variety of the test compounds has been unduly restricted. It might be well to try to broaden the scope of substitutions on the naphthyridine and quinoline nuclei before concluding that these classes have no future.

The investigations of the 8-aminoquinolines have been highly productive in terms of uncovering agents with tissue schizonticidal activity equal to or exceeding that of primaquine. This productivity is particularly impressive to one familiar with the magnitudes of the post-World War I work of German, French, and Russian investigators and the USA efforts (1944-1951) to develop a more effective and better tolerated curative drug than pamaquine. The current program involving preparation and evaluation of one hundred seventy-two agents has delivered thirty-three compounds (19 per cent of the total examined) with activity equal to or greater than that of primaquine; thirteen of these (8 per cent of the total) are two to four times as active. The remarkable feature of this is that despite all of the chemical modifications resorted to in the post-World War I and World War II searches, the current group of highly active compounds can be looked upon as novel structures*. This is more than a small tribute to the imagination and skills of those who designed and executed synthesis in this chemical series.

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*The 4-methyl-6-methoxy substituted derivatives are an exception. This class was the focus of much activity in the 1946-1951 cooperative program; CN-1101 (prepared independently as WR-181,023) was the outstanding product of this investigation.

A fraction of the thirty-three compounds which might be considered as primaquine competitors or successors has been accorded very preliminary study for subacute toxicity in the rhesus monkey in an effort to obtain a crude therapeutic index. Utilizing maximum non-lethal doses as the toxicologic endpoint, none of the compounds examined to date exhibited a therapeutic index superior to that of primaquine. This result is disappointing, but placed in perspective, it only serves as a stimulus to examine the toxicity of these agents in a manner which is more likely to identify those untoward reactions which restrict the utility of primaquine. These reactions include emesis, abdominal cramping, methemoglobinemia, and an anemia referable to G6PD deficiency. The latter reaction can probably not be identified in any experimental animal. Previous experience has shown that the dog is more likely to exhibit the other manifestations of toxicity than is the rhesus monkey. The latter laboratory animal, and other Old World primates, are excellent for identifying the structure linked - Plasmocid type of neurotoxicity. This type of toxicity was of much concern during the World War II malaria program, but with two exceptions, has not been an issue in these studies.

Following the reasoning set forth above, attempts should be made promptly to quantify and qualify the reactions of the dog to each of the thirty-one 8-aminoquinolines identified as equal to or superior to primaquine in tissue schizonticidal activity. The least toxic (in terms of relevant reactions) and the most active compounds among this group should then be accorded the same type of critical evaluation in human volunteers as was employed so successfully by Alving, Jones, Arnold, and Powell at the Illinois State Penitentiary, Joliet. On the basis of past correlations between activities against infections with P. cynomolgi and activities against infections with P. vivax, it is reasonable to expect that more than one agent will emerge not only more effective than primaquine in the conventional treatment schedule, but effective enough to be utilized in a three-day regimen.

The above plan would place further syntheses in the 8-aminoquinoline area in a qualified position. There is clearly need for preparation and therapeutic evaluation of a larger variety of 2,4,6- and 2,5,6-trisubstituted derivatives, especially compounds with an oxidation retarding group at position 5. Synthesis of such agents should be encouraged. There is also a need to sort out the impacts of methyl substitution at the 1 and 4 positions of the butyl group between the side chain nitrogens. Compounds required to round out the effects of such modifications should be prepared. The merits of synthesis in other directions seems questionable. There are already very substantial bodies of information on the impacts of 6-substitution and 2,6-, 4,6-, and 5,6-disubstitution. Further attention to these areas is not indicated. With the exception of the side chain variants referred to above, there is little need for further activity at the 8 position.

To this point, attention has been directed solely to the search for new tissue schizonticides. There may be another equally rewarding investigative approach - namely, enhancement of the curative activity of primaquine by concomitant administration of a companion drug endowed with tissue schizonticidal activity (not an 8-aminoquinoline). The experience with the lincomycin derivative, WR-203,661, provides encouragement for this approach. This compound might provide a good point of departure for such an endeavor. This might well begin with attempts to determine whether some moiety of the complex WR-203,661 molecule is responsible for the tissue schizonticidal activity of the whole and whether said moiety is amenable to manipulation in the chemical laboratory. Another approach might involve the judicious use of the pyrocatechol, RC-12. The P. cynomolgi - rhesus monkey model could be used effectively in these explorations.

In concluding these comments, the Principal Investigator would like to submit that when viewed from experiences of personal participation in programs related to the chemotherapy of acute bacterial infections, malaria, poliomyelitis, tuberculosis, and cancers, covering a period of forty years, the progress made toward achieving the two major missions of the Malaria Chemotherapy Program has been very substantial and creditable. Were there to be no further activity in either the chemical synthesis or animal model evaluation systems, it is highly likely that the roster of promising blood schizonticides contains a number of agents that could assume the role formerly carried by chloroquine. It is equally likely that the roster of highly active 8-aminoquinolines contains a number of agents superior to primaquine with respect to the tolerability required for wide scale, unsupervised administration and the efficacy required to eradicate tissue schizonts in a relatively brief period of drug delivery. What is needed to realize these potential gains are critically controlled studies in relatively limited numbers of human subjects aimed at unequivocal identification of the most effective agents, followed by validation of the efficacy of these agents in well-designed, thoroughly monitored field studies in a fair spectrum of areas where malarias are still highly prevalent. It could well be that the results of such field studies would be so impressive as to encourage wide scale application of the agents. In combination with vector control procedures, these might well reduce the incidences of the human malarias to levels no longer of concern to the military.

MISCELLANEOUS

COMPOUNDS EVALUATED FOR RADICAL CURATIVE ACTIVITY: PREPARATION (AS IDENTIFIED BY BOTTLE NUMBER) AND SALT EMPLOYED IN PILOT STUDY

Compound WR- No.	Bottle No.	Salt	Reference Page
		Miscellaneous Structures	
3,396	ZC-07,877	-	143
198,559	BC-57,397	di-β-resorcylate	143
198,560	BC-57,404	di-β-resorcylate	143
13,255	AL-27,411	-	144
219,124	BE-69,119	-	144
193,713	BC-41,362	pamoate	144
198,782	BC-58,401	sesqui-β-resorcylate	144
191,994	BE-13,162	-	145
7,295	BB-47,961	-	145
225,717	BG-41,620	diphosphate	145
219,384	BE-71,217	-	146
102,796	BC-78,878	-	146
218,575	BE-66,958	-	146
12,921	AG-16,089	dihydrochloride	147
205,446	BD-54,211	dihydroiodide	147
202,833	BD-26,191	dihydroiodide	147
182,058	AY-98,947	-	147
9,792	AE-07,615	hydrochloride	148
223,660	BG-15,899	dihydrochloride	148
25,981	AG-74,536	-	148
31,877	ZC-03,799	-	148
190,830	BD-29,165	dihydrochloride	149
158,124	BD-22,997	sesquihydrochloride	149
167,655	BC-99,831	-	149
81,817	AJ-76,914	-	149
124,905	BE-52,436	sodium	150
124,892	BE-18,158	-	150
225,508	BG-37,653	-	150
		1,5-Naphthyridines	
202,927	BD-26,422	-	153
217,125	BE-67,286	di-β-resorcylate	153
202,928	BD-26,413	-	153
180,411	BD-95,839	di-β-resorcylate	154
206,287	BD-54,748	-	154
206,283	BD-54,720	β-resorcylate	154
222,119	BG-04,065	β-resorcylate	155
222,121	BG-04,074	β-resorcylate	155
210,304	BE-10,858	tri-β-resorcylate	155
210,442	BE-10,830	di-β-resorcylate	156
145,023	BD-53,812	hydrochloride	156
216,010	BE-17,286	di-β-resorcylate	156

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines	
199,508	BD-24,044	trihydrobromide	172
29,633	BE-20,989	dihydrochloride	172
211,664	BE-21,762	diphosphate	172
2,975	-	diphosphate	173
152,149	BE-66,770	phosphate	174
186,370	BE-19,075	citrate	174
221,661	BG-03,424	phosphate	174
215,730	BE-16,583	-	175
161,085	AX-26,820	bis-1,6-naphthalene-disulfonate	175
180,125	AY-95,455	sesqui-β-resorcylate	175
197,624	BC-09,453	di-β-resorcylate	176
199,981	BD-23,154	di-β-resorcylate	176
29,594	BE-20,014	-	176
27,757	BE-20,863	dinitrate	177
214,420	BE-50,012	phosphate	177
29,606	BE-20,229	dihydrochloride	177
190,285	BB-47,510	di-β-resorcylate	177
193,127	BB-49,416	maleate	178
29,616	BE-21,744	dihydrochloride	178
181,441	AY-97,600	dihydrochloride	178
187,427	BB-44,779	-	178
187,428	BB-44,788	-	179
185,306	BB-42,060	-	179
224,485	BG-32,532	-	179
7,312	BB-47,761	dihydrochloride	179
222,122	BG-03,915	dihydrochloride	180
29,634	BE-12,950	dihydrobromide	180
225,635	BG-41,264	fumarate	180
224,586	BG-32,523	-	180
184,544	BE-20,265	triphosphate	181
212,231	BE-20,201	dihydrobromide	181
182,234	BC-58,572	dihydrochloride	182
222,671	BG-11,891	diphosphate	182
213,472	BE-13,948	fumarate	183
222,849	BG-12,521	maleate	183
218,669	BE-55,795	maleate	183
211,077	BE-11,971	phosphate	183
219,634	BE-59,122	maleate	184
121,508	BE-11,121	dihydrobromide	184
106,147	AY-97,897	dihydrochloride	184
205,438	BD-54,202	maleate	184
217,154	BE-67,204	maleate	185
217,124	BE-43,759	maleate	185
219,635	BE-59,113	maleate	185
202,790	BD-26,217	maleate	185

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines - Continued	
205,439	BD-54,195	maleate	186
183,538	BB-41,885	diphosphate	186
212,216	BD-99,103	maleate	186
216,893	BE-19,477	maleate	186
224,398	BG-32,514	difumarate	187
199,368	BC-59,284	maleate	187
183,064	BB-41,894	hydrochloride	187
217,038	BE-19,584	diphosphate	187
211,814	BE-08,518	diphosphate	188
211,815	BE-08,527	diphosphate	188
211,820	BE-21,593	citrate	188
206,027	BE-20,194	dihydrochloride	189
147,778	BB-18,448	diphosphate	189
136,479	BE-21,575	diphosphate	189
181,023	BC-57,244	diphosphate	190
215,761	BE-16,967	diphosphate	191
215,296	BE-16,378	citrate	191
217,159	BE-67,222	citrate	192
215,300	BE-16,243	fumarate	192
208,442	BD-67,981	dihydrobromide	192
211,663	BE-12,825	fumarate	193
218,806	BE-57,315	fumarate	193
225,503	BG-37,420	fumarate	193
218,636	BE-67,133	fumarate	193
218,805	BE-57,324	fumarate	194
218,574	BE-66,985	fumarate	194
223,138	BG-14,196	fumarate	194
216,837	BE-19,306	fumarate	195
214,198	BE-13,082	dihydrochloride	195
217,271	BE-50,183	phosphate	195
208,557	BD-45,267	diphosphate	196
209,785	BE-10,090	succinate	196
208,814	BD-59,074	phosphate	196
214,703	BE-15,040	sesquiphosphate	197
209,522	BD-59,967	phosphate	197
209,521	BD-59,985	diphosphate	197
219,894	BE-80,163	fumarate	198
220,226	BE-82,809	fumarate	198
211,975	BE-12,996	phosphate	198
189,279	BB-46,540	phosphate	199
199,793	BC-99,939	phosphate	199
223,137	BG-14,187	fumarate	199
212,293	BD-99,907	phosphate	200
218,573	BE-66,994	diphosphate	200
212,302	BD-99,943	succinate	200
201,678	BE-13,304	diphosphate	201
212,624	BE-13,822	diphosphate	201
212,579	BE-13,313	fumarate	201

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines - Continued	
219,373	BE-71,173	diphosphate	202
218,335	BE-66,930	diphosphate	202
225,742	BG-41,095	dihydrochloride	202
6,028	BE-20,032	dihydrochloride	203
6,027	BE-20,112	dihydrochloride	203
127,854	BE-20,087	dihydrochloride	203
202,437	BD-26,164	sesqui-β-resorcylate	204
203,607	BD-27,652	di-β-resorcylate	204
203,608	BD-27,661	di-β-resorcylate	204
211,208	BE-20,005	dihydrochloride	205
211,666	BG-11,417	dihydrochloride	205
223,442	BG-14,472	dihydrochloride	205
225,741	BG-41,086	triphosphate	205
224,382	BG-32,176	diphosphate	206
211,665	BE-20,569	dihydrochloride	206
147,657	BE-20,023	dihydrochloride	206
214,787	BE-15,451	dihydrochloride	207
202,438	BD-26,155	fumarate	207
211,816	BE-20,630	dihydrochloride	207
212,223	BE-20,121	dihydrochloride	208
6,026	BG-14,463	dihydrochloride	208
225,845	BG-43,820	dihydrochloride	208
223,658	BG-21,744	dihydrochloride	208
199,507	BD-24,062	trihydrobromide	209
194,333	BC-06,587	oxalate	209
200,073	BD-23,163	oxalate	209
223,745	BG-21,842	ethanedioate	210
5,990	BC-60.018	diphosphate	210
218,676	BE-55,820	fumarate	210
184,118	BE-20,309	phosphate	211
182,232	BC-99,911	-	211
219,785	BE-75,902	hydrochloride	211
216,100	BE-17,491	citrate	212
215,295	BE-16,378	citrate	212
222,890	BG-13,831	sesquiphosphate	213
221,041	BG-00,496	hemioxalate	213
183,489	BB-42,695	-	213
206,428	BD-55,049	-	214
209,154	BD-59,752	-	214
208,189	BD-45,427	-	214
209,845	BE-10,223	-	215
208,441	BD-57,990	-	215
211,078	BE-12,012	-	215
188,304	BB-45,534	diphosphate	216
224,640	BG-32,550	citrate	216
207,610	BE-11,631	fumarate	216
194,341	BC-06,443	dimaleate	216

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines - Continued	
202,789	BD-26,208	maleate	217
224,639	BG-32,541	-	217
49,577	BC-59,702	trihydrobromide	217
194,343	BC-06,452	maleate	217
181,205	AY-96,907	tricitrate	218
189,283	BB-46,568	-	218
184,852	BB-41,929	-	218
215,732	BE-16,627	diphosphate	219
192,515	BC-58,394	sesqui-β-resorcylate	219
218,334	BE-66,841	phosphate	219
211,990	BD-99.087	dihydrochloride	219
193,130	BC-58,910	di-β-resorcylate	220
211,533	BE-12,601	diphosphate	220
197,063	BC-08,705	di-β-resorcylate	220
210,810	BE-11,677	fumarate	221
215,733	BE-17,026	fumarate	221
211,532	BE-12,567	fumarate	222
222,418	BG-11,449	fumarate	222
224,097	BG-22,198	fumarate	223
224,486	BG-32,738	maleate	223
210,805	BE-11,686	fumarate	223
210,550	BE-11,597	dinitrate	224
210,551	BE-11,588	dinitrate	224
216,804	BE-19,324	diphosphate	225
221,527	BG-03,504	diphosphate	226
225,448	BG-37,402	succinate	226
219,874	BE-79,802	maleate	227
218,681	BE-55,937	hemifumarate	227
219,423	BE-73,480	dihydrochloride	227
204,510	BD-28,971	hemifumarate	228
208,071	BD-45,310	fumarate	228
210,448	BE-11,033	fumarate	228

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INFORMATION

Correction

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Annual ~~FINAL~~ PROGRESS REPORT
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For the Period 1 May 1975 to 30 April 1976
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COVERING CONTRACT NO. DADA 17-69-C-9104

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C., 20314

Principal Investigator- L. H. Schmidt

Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205

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malaria, simian	owl monkey	suppressive
malaria, human	rhesus monkey	radical curative
P. falciparum	drug-susceptible	potentiation
P. vivax	drug-resistant	quinolinemethanols
P. cynomolgi	prophylactic	pyridinemethanols

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

As in previous years, the activities of this Project were focused on development of: (a) agents fully effective against infections with blood schizonts of multidrug-resistant strains of Plasmodium falciparum and P. vivax in the owl monkey; and (b) compounds more active than primaquine against the tissue schizonts of P. cynomolgi in the rhesus monkey, and better tolerated.

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