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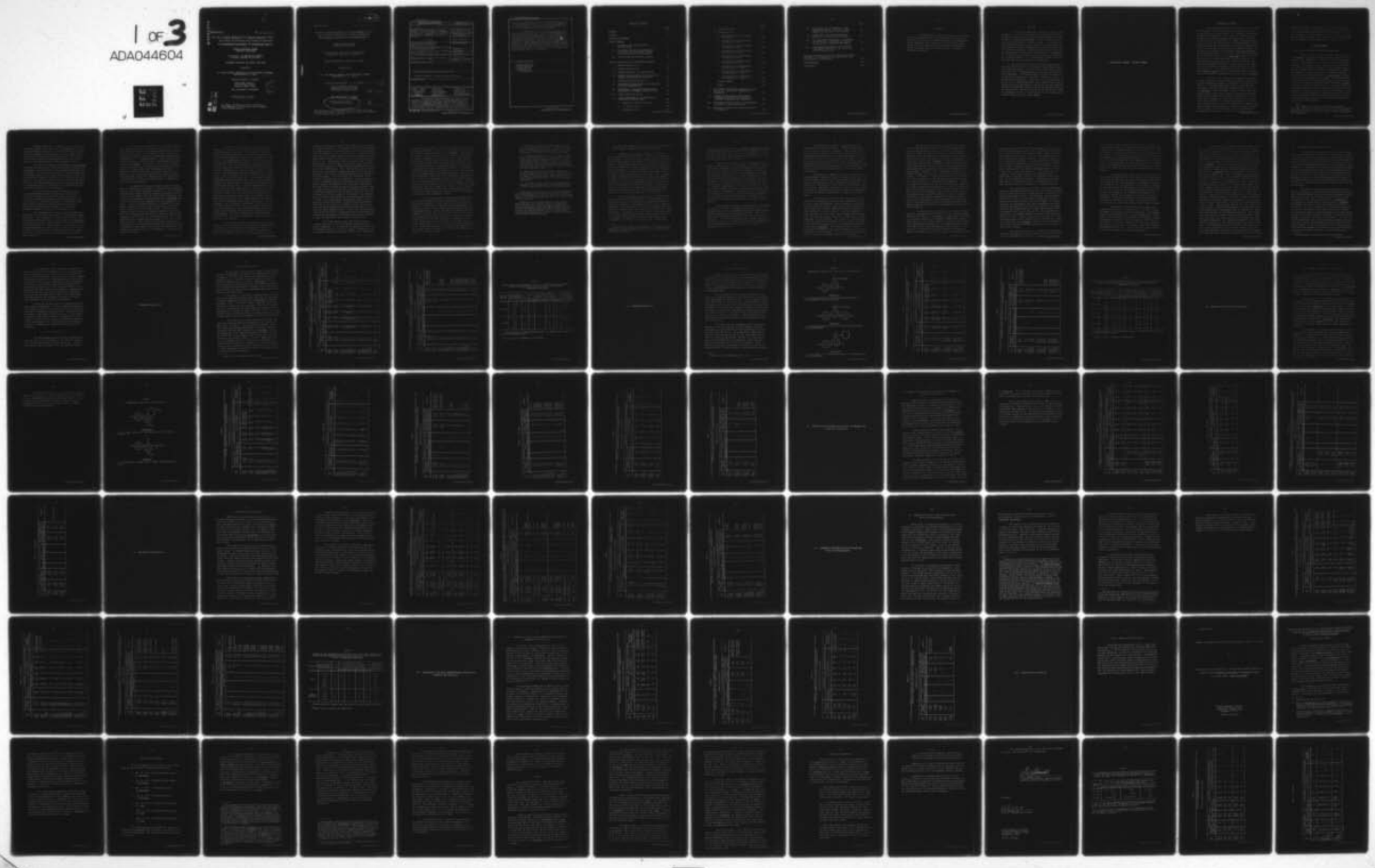

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ANNUAL PROGRESS REPORT
(PROJECT 2284-XXVIII)

For the Period 1 May 1974 to 30 April 1975
(Preparation Completed 30 June 1976)

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

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
WASHINGTON, D. C., 20315

Principal Investigator - L. H. Schmidt

Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205

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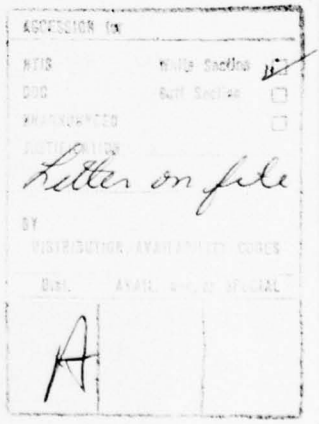

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malaria, simian	owl monkey	suppressive
malaria, human	rhesus monkey	radical curative
P. falciparum	drug-susceptible	potentiation
P. vivax	drug-resistant	quinolinemethanols
P. cynomolgi	prophylactic	pyridinemethanols

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

Investigations undertaken during this Report period included: I. Searches for blood schizonticidal drugs effective against multidrug-resistant (especially chloroquine-resistant) strains of Plasmodium falciparum - pursued in owl monkeys infected with trophozoites of these strains. II. Searches for tissue schizonticidal drugs more effective and better tolerated than primaquine, and for companion drugs that would enhance the

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activities of primaquine without increasing toxicity - pursued in rhesus monkeys infected with sporozoites of P. cynomolgi. III. Studies on IND preparations of new agents, essential to use in human volunteers. IV. Miscellaneous studies on absorption and toxicities of promising new compounds.

The search for new blood schizonticides uncovered two Mannich bases highly active against infections with multi-drug-resistant strains. The search for tissue schizonticides uncovered twelve 8-aminoquinolines as active or more active than primaquine. A 7-chlorolincomycin derivative capable of enhancing the prophylactic and radical curative activities of primaquine was identified. Studies on the IND preparation of WR-184,806, a 4-quinolinemethanol, were completed and reported; studies on the IND preparation of WR-181,023 are well underway. Absorption of the blocd schizonticides WR-30,090 and WR-158,122 was investigated. WR-211,536 and WR-211,537 (the D and L forms of primaquine), WR-211,532, and WR-211,533 were evaluated for subacute toxicity in the rhesus monkey.

19. Key Words Continued

phenanthrenemethanols
diaminoquinazolines
8-aminoquinolines
6-aminoquinolines
1,5-naphthyridines

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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

Investigations undertaken during this Report period included: I. Searches for blood schizonticidal drugs effective against multidrug-resistant (especially chloroquine-resistant) strains of Plasmodium falciparum - pursued in owl monkeys infected with trophozoites of these strains. II. Searches for tissue schizonticidal drugs more effective and better tolerated than primaquine, and for companion drugs that would enhance the activities of primaquine without increasing toxicity - pursued in rhesus monkeys infected with sporozoites of P. cynomolgi. III. Studies on IND preparations of new agents, essential to use in human volunteers. IV. Miscellaneous studies on absorption and toxicities of promising new compounds.

The search for new blood schizonticides uncovered two Mannich bases highly active against infections with multidrug-resistant strains. The search for tissue schizonticides uncovered twelve 8-aminoquinolines as active or more active than primaquine. A 7-chlorolincomycin derivative capable of enhancing the prophylactic and radical curative activities of primaquine was identified. Studies on the IND preparation of WR-184,806, a 4-quinolinemethanol, were completed and reported; studies on the IND preparation of WR-181,023 are well underway. Absorption of the blood schizonticides WR-30,090 and WR-158,122 was investigated. WR-211,536 and WR-211,537 (the D and L forms of primaquine), WR-211,532, and WR-211,533 were evaluated for subacute toxicity in the rhesus monkey.

INTRODUCTORY COMMENT - GENERAL SUMMARY

INTRODUCTORY COMMENT

From September 1967 when this Project was established to the present, its activities have been tightly focused on the initial and evolving missions of the Malaria Chemotherapy Program of the Department of the Army. This focus has been implemented through development, standardization, and application of two new non-human primate - human plasmodium models and reactivation and application of a non-human primate - simian plasmodium model employed intensively in the post-World War II search for generally useful curative antimalarial drugs. The two new models employed the owl monkey (Aotus trivirgatus) as the experimental animal. One involved infections with trophozoites of various strains of P. falciparum (seven in all) of diverse levels of drug susceptibility and resistance. The second dealt with infections with trophozoites of the much used drug-susceptible New Guinea Chesson strain of P. vivax and the pyrimethamine-resistant Vietnam Palo Alto strain of this plasmodium. The "reactivated" model utilized the rhesus monkey (Macaca mulatta) as the experimental animal and dealt with infections with sporozoites of the B strain of P. cynomolgi.

The first of the above models supported and continues to support pursuit of the original and primary mission of the Malaria Chemotherapy Program, development of new drugs fully effective at well-tolerated doses against infections with chloroquine-resistant and other drug-resistant strains of P. falciparum. The second model served to insure that such agents as were active against infections with the malignant tertian parasite would also control the blood phases of infections with P. vivax. The third model supported the second major goal of the Malaria Program, development of new drugs more effective, better tolerated, and more easily administered than primaquine for prophylaxis and radical cure of infections with P. vivax. The problems that had to be surmounted in developing these models to positions of reliability and

utility, the major operational features of the models, and the mission-related accomplishments that can be credited to their application up through the 1973-1974 contract year have been detailed in previous Annual Progress Reports, or in published reports, and will not be dealt with here. This Report will be limited to studies pursued since April 30, 1974.

GENERAL SUMMARY

I. THE SEARCH FOR IMPROVED BLOOD SCHIZONTICIDES

There has been a marked decrease in the use of the *P. falciparum* / *P. vivax* - owl monkey models during the past contract year, continuing a trend which had its beginnings in 1972. This change rested in part on the positive accomplishments of preceding years, during which at least eight new agents were uncovered, any one of which promised to satisfy the original goal of the Malaria Chemotherapy Program, development of a drug fully effective against infections with multidrug-resistant strains of *P. falciparum* and equally active against infections with *P. vivax**. The downward trend in use of these models also reflected the decreased productivity of the primary rodent screen (infections with *P. berghei* in the mouse) which in recent years has uncovered relatively few active agents with novel structures or congeners of older compounds endowed with greater activity and tolerability than derivatives already evaluated. Finally, the trend to reduced use of owl monkey - human plasmodium models was an enforced conservation measure imposed by restrictions on exports of these primates from Colombia.

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*These agents included: the phenanthrenemethanols, WR-33,063, WR-122,455, and WR-171,669; the quinolinemethanols, WR-30,090, WR-142,490, and WR-184,806; the pyridinemethanol, WR-180,409; and the 2,4-diamino-6-sulfur substituted quinazoline, WR-158,122.

Although only four new compounds were presented for evaluation against infections with P. falciparum and P. vivax in the owl monkey, there was a sizeable use of these models. Both were employed systematically to provide supporting data for Investigative New Drug (IND) applications, and to help solve dosage regimen and formulation problems pertinent to most effective administration of new compounds to man.

Of the four new agents evaluated against P. falciparum infections, one, WR-177,602, was the threo-epimer of WR-142,490 (mefloquine), the most active of the 4-quinolinemethanols studied to date. This evaluation, carried out in monkeys infected with the multidrug-resistant Smith strain, showed that WR-177,602 was essentially identical to WR-142,490 in activity and tolerability. In view of the advanced status of the clinical application of the latter compound, WR-177,602 could be considered as a competitor only if it promised to be significantly less expensive to prepare or better tolerated.

The second of the compounds examined was the 4-pyridinemethanol, WR-182,231, an analog of WR-180,409. WR-182,231 differed from the latter agent with respect to side chain configuration, bearing a di-N-butylaminoethyl substituent on the 4-carbinol in place of a 2-piperidyl group. This change in structure affected activity adversely, WR-182,231 exhibiting only one-fourth the activity of WR-180,409 against infections with the Smith strain.

The remaining two new agents were the benzoxazine, WR-204,165, and its open-ring relative (a substituted phenol), WR-194,965. These compounds had essentially identical activity against infections with the Smith strain of P. falciparum. Compared dose-for-dose, their capacities to cure such infections were essentially identical with that of WR-142,490, the 4-quinolinemethanol referred to above. Unless there are toxicologic contraindications, not exhibited in the owl monkey, WR-194,965 or WR-204,165 merits consideration for study in human volunteers.

Four special investigations, each fairly extensive, were pursued with compounds examined in earlier years. One of these studies was aimed at determining whether the therapeutic activity of WR-158,122, a 2,4-diamino-6-sulfur substituted quinazoline, could be enhanced by concomitant administration with WR-180,872, a dihydroquinazoline. Marked synergism had been observed in mice infected with a pyrimethamine-resistant strain of P. berghei. The results of the studies pursued in monkeys infected with the pyrimethamine-resistant Palo Alto strain of P. vivax showed that concomitant delivery of WR-180,872 added nothing to the activity of WR-158,122. This negative result contrasted sharply with the eight to sixteen-fold enhancement achieved by administering very small doses of sulfadiazine (5.0 mg per kg body weight) in combination with the above quinazoline.

The second of the special studies dealt with procedures for enhancing the therapeutic efficiency of the lincomycin derivative, WR-203,661 (formerly designated U-24,729A). Earlier investigations showed that this compound was highly effective in curing experimental infections with either chloroquine-susceptible or chloroquine-resistant strains of P. falciparum and that its activity was not compromised by pyrimethamine resistance. It was also markedly superior in activity to clindamycin. However, like the various tetracyclines, WR-203,661 effects clearance of parasitemia slowly. In an effort to eliminate this deficiency, a study was undertaken of the impacts of loading doses on the rate of clearance of parasitemia in monkeys infected with the Vietnam Smith strain. The results showed that this approach was not beneficial; in fact, at the same total dose, seven fractional daily doses of WR-203,661 effected parasite clearance more rapidly and consistently than a loading dose equivalent to half of the seven-day total on Day 1 followed by equal fractions of the remainder of the total dose on Days 2 through 7.

The third special study was concerned with the comparative activities of two dosage formulations of the quinolinemethanol, WR-30,090. This compound, originally prepared as the hydrochloride salt, exhibited sufficient activity in human volunteers infected with chloroquine-resistant strains of P. falciparum to warrant a small scale trial in Vietnam in military personnel infected with multidrug-resistant strains. Overall, WR-30,090 was remarkably effective in this evaluation. However, there were some patient-to-patient irregularities in rate of response which were attributed to poor absorption resulting from limited water solubility. These irregularities were relieved in part, but not completely, by increasing the dose. In an effort to obtain a more uniform relation between dose and response, systematic attempts were made to improve solubility and absorption from the gastrointestinal tract. A major accomplishment in this direction was made by investigators at $INTER_X$ Research Corporation, who found that WR-30,090 base was soluble up to 20 per cent in oleic acid, and that when such solutions were administered orally to the beagle dog, the concentrations of WR-30,090 in the plasma were eightfold greater than those attained when comparable doses of the hydrochloride salt were delivered. As an essential preliminary to clinical use of WR-30,090 dissolved in oleic acid, therapeutic efficiencies of this preparation and that of WR-30,090 hydrochloride were compared in owl monkeys infected with the Smith strain of P. falciparum. The results showed that the hydrochloride salt was at least twice and perhaps four times as active as the oleic acid solution.

This unexpected result, the opposite of that anticipated, led to a collaborative study with the $INTER_X$ Research Corporation with the objective of measuring the levels of WR-30,090 achieved in plasma when oleic acid solutions of the base and aqueous slurries of the hydrochloride salt were

administered to normal uninfected owl monkeys and rhesus monkeys. As planned originally, ten owl monkeys and eight rhesus monkeys were committed to a crossover study in which each preparation was to be administered in a single dose of 20.0 mg base per kg body weight, via stomach tube, to half of the members of each monkey group on Day 1, with reversal of the delivery order on Day 14 or 21. Blood samples were to be drawn prior to drug delivery and 2, 4, and 6 hours thereafter, packed in wet ice, and shipped promptly to INTER$_X$ Research Corporation for analysis of WR-30,090 content. The treatment phase of the first arm of this experiment was completed without incident, but no more than a trace of the quinolinemethanol could be found in any blood sample. For this reason, the second arm of the crossover experiment was not undertaken. No easy explanation for this remarkable negative finding has been forthcoming. Studies pursued preliminary to this experiment showed that WR-30,090 added to normal owl monkey and rhesus monkey blood or plasma could be recovered with reasonable precision. The preparations employed in this absorption study were the same as those used in the therapeutic evaluations. The monkeys bled in the above experiment were the monkeys who had received the drug. It is conceivable, although not likely, that the drug deteriorated in transit, or that following absorption WR-30,090 was converted to a metabolite which could not be extracted and/or measured by the analytical procedure, or that there was gross analytical error. In any case, the absorption study failed to provide an explanation for the difference in therapeutic performance of WR-30,090 hydrochloride suspended in water and WR-30,090 base dissolved in oleic acid.

The fourth special study dealt with the blood schizonticidal activities of primaquine and WR-181,023 (4-methyl primaquine) as exhibited in owl monkeys infected with the Vietnam Palo Alto strain of P. vivax. Primaquine has an established position as a radical curative drug. WR-181,023

has not yet been evaluated for curative properties in man, but on a dosage comparison, is superior to primaquine in rhesus monkeys infected with sporozoites of P. cynomolgi. When employed for radical cure, both agents are delivered with chloroquine in order to eliminate both erythrocytic and exoerythrocytic forms of the relevant parasites. Since many 8-aminoquinolines have activity against both blood and tissue schizonts, there is a possibility that some compounds might be so well-endowed in both areas as to make concomitant delivery of chloroquine (or other blood schizonticide) unnecessary. The current study, aimed at determining whether WR-181,023 would be likely to meet this requirement, showed that this compound was approximately twice as active as primaquine against infections with trophozoites of the Vietnam Palo Alto strain of P. vivax. Since the dose of WR-181,023 required to cure such infections was approximately ten times that required for radical cure of sporozoite-induced infections, and since such a dose might evoke toxic reactions, it would be unwise to consider this 8-aminoquinoline as a candidate for a mono-drug regimen.

One of the major uses of the human plasmodia - owl monkey models during the current contract year has been to provide data required for FDA clearance of the clinical preparations of WR-184,806, a promising 4-quinolinemethanol and competitor of WR-142,490, and WR-180,409, an equally promising 4-pyridinemethanol. Studies incidental to clearance of these compounds have dealt not only with proof that the activities of the preparation of drug intended for clinical use and the original lot were the same, but with the influences of the dosage regimen and route of delivery on activity. The studies on WR-180,409 are still in progress and will be summarized later. Studies on WR-184,806 were completed and summarized in the Interim Report of December 9, 1974. The conclusions set forth in this Report follow:

"1. When administered orally, WR-184,806-AH regularly cured infections with the various strains at the following total doses (base equivalent): Oak Knoll - 35.0 mg/kg body weight; Smith - 70.0 mg/kg body weight; Palo Alto - 17.5 mg/kg body weight.

"2. At uniformly curative levels (ca CD_{90}), single dose, three consecutive daily dose, and seven consecutive daily dose regimens of WR-184,806 were essentially equally effective. There was a suggestion, however, that the time required for parasite clearance was shorter with single doses than when the same total dose was delivered in equal fractions on seven consecutive days.

"3. When administered intravenously, WR-184,806 regularly cured infections with the various strains at the following total doses (base equivalent): Oak Knoll - 20.0 mg/kg body weight; Smith - ca 30.0 mg/kg body weight; Palo Alto - $>10.0 < 20.0$ mg/kg body weight.

"4. On a dose-for-dose comparison, WR-184,806-AH appeared to be slightly more effective when administered intravenously than when delivered orally.

"Comparison of the above results with those obtained in earlier pilot studies indicates that the phosphate salt of WR-184,806 is at least as effective as the hydrochloride salt (WR-184,806-AA, BN: BB-52,137) and possibly slightly superior.

"Comparison of the data acquired in the current study with those of even more extensive studies on WR-142,490 [α-(2-piperidyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol, hydrochloride] indicates that the activities of WR-184,806-AH are only slightly inferior to those attained with WR-142,490, the most effective of the quinolinemethanols evaluated to date and currently under study in human volunteers."

II. THE SEARCH FOR IMPROVED PROPHYLACTIC AND RADICAL CURATIVE DRUGS (TISSUE SCHIZONTICIDES) AND DRUG REGIMENS

Primaquine is the only prophylactic and radical curative drug generally available*. Although the capacity of this compound to prevent and cure mosquito-induced infections with P. vivax cannot be questioned, it does have certain limitations. To prevent active infections, it must be administered (preferably with a blood schizonticide) both during the period of exposure to infected mosquitoes and for two or more months thereafter. To cure established infections, it must be administered for fourteen consecutive days. These extended treatment requirements are difficult to enforce or monitor. In addition, the doses of primaquine required for prevention and cure are close to the maximum tolerated level, particularly for individuals with G6PD deficiencies. Lastly, there appear to be strains of P. vivax which produce infections which cannot be cured regularly by the conventional dosage regimen, 15.0 mg daily for fourteen consecutive days.

These limitations, brought into clear focus during military operations in Southeast Asia, led to expansion of the mission of the U. S. Army Malaria Chemotherapy Program to include development of new prophylactic and radical curative drugs more effective and better tolerated than primaquine and capable of inducing cures when delivered in single, or at most three doses. Initially, it was hoped that this mission could be served by studies in owl monkeys infected with sporozoites of P. vivax. Unfortunately, such infections could not be developed to the status of a reliable model. Accordingly,

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*Quinocide, an isomer of primaquine, is available and employed in the USSR and satellite countries. Critical experimental and clinical studies indicate that it is inferior to primaquine in activity.

attention was redirected to the P. cynomolgi - Anopheles freeborni - rhesus monkey model, which although theoretically less desirable than one based on P. vivax, has a well-established record for selecting drugs that cure human infections with this parasite and predicting their relative merits.

There were five major facets to the searches for improved curative drugs pursued during the 1974-1975 contract year. These included: (1) pilot assessments of the curative activities of recently synthesized compounds; (2) side-by-side evaluation of the activities of primaquine (a racemate) and its D and L components; (3) expanded comparisons of the curative properties of primaquine and WR-181,023 (4-methyl primaquine); (4) delineation of the effectiveness of single and three-dose regimens of primaquine and WR-181,023; and (5) attempted enhancement of the curative activity of primaquine by concomitant delivery of a companion drug other than chloroquine. In addition, a systematic study was undertaken of the influence of the size of the sporozoite inoculum on the "curative" activity of chloroquine. Combined, these activities entailed work with slightly more than 400 rhesus monkeys.

Seventy-five agents were accorded pilot assessments for curative activity. Included in this total were six 7-aminoquinolines, five 6-aminoquinolines, three naphthyridines, and ten compounds of miscellaneous structure. None of the latter group exhibited curative activity at the largest dose delivered. One of the 7-aminoquinolines had questionable activity at a dose of 10.0 mg per kg. None of the naphthyridines and 6-aminoquinolines exhibited curative activities at the maximum test dose levels.

The remaining 51 compounds submitted for pilot evaluations were 8-aminoquinolines. Twelve of these agents were at least as active as primaquine. Nine of this group were as active as WR-181,023, the most effective of the 8-aminoquinolines evaluated prior to the beginning of this contract year. Four of the nine appeared to be twice as active as WR-181,023; like the latter derivative, they had methyl substitutents in position 4. Three of the four differed from WR-181,023 in side chain configuration, with branching at the distal end of the alkyl bridge rather than at the proximal (1) position.

Considering the numbers of years of work on the 8-aminoquinolines required to develop primaquine, identification in one calendar year of twelve structurally related agents of equal or greater activity is a considerable achievement. The toxicity or tolerability of these agents will determine their real utility. Hopefully, one or more will be at least as well tolerated as primaquine and thereby a significant improvement over this currently available drug in terms of therapeutic index.

The second facet of the search for curative drugs dealt with the activities of WR-211,536 and WR-211,537, the D and L isomers of primaquine. Special interest was attached to this evaluation because studies on the acute toxicity of these agents for the mouse indicated that the L isomer was only one-fourth as toxic as the D. If these isomers had equal curative activity, and if the difference in mouse toxicity carried over to man, the L isomer would have a distinct advantage over the racemate. The results of a side-by-side evaluation showed that primaquine and its isomers had essentially identical capacities to cure sporozoite-induced infections with P. cynomolgi. This favorable result led to a preliminary evaluation of the subacute toxicities of the three agents for the rhesus monkey. The results of this comparison are set forth in a subsequent section of this Summary.

The third major facet of the search for improved curative drugs was concerned with a detailed comparison of the prophylactic and radical curative activities of primaquine and its 4-methyl derivative, WR-181,023. Studies pursued during the previous contract year indicated that the latter compound was two to four times as effective as primaquine in curing infections with P. cynomolgi. Studies pursued in the early 1950's shortly after the initial synthesis of WR-181,023 (then designated CN-1101) indicated that this compound had approximately half the subacute toxicity of primaquine for the rhesus monkey. Together, these assessments suggested that WR-181,023 would have at least a fourfold advantage over primaquine. This promise of superiority called for a detailed and precise comparison of the prophylactic and radical curative properties of these closely related compounds. Side-by-side appraisals showed that the doses of WR-181,023 required to prevent infections with sporozoites, or to cure established sporozoite-induced infections, fell between one-half and one-third the doses of primaquine required for the same result. This demonstration led to the decision to prepare a batch lot of WR-181,023 for ultimate evaluation in human volunteers. This lot has been produced and is currently being subjected to the lower animal toxicity and therapeutic activity examinations required by FDA prior to investigating a new drug in human volunteers.

The fourth facet of the search for improved curative drugs was concerned with the efficacy of short term treatment regimens of WR-181,023. Development of such regimens has been of increasing interest in the Malaria Chemotherapy Program. A single dose or even a three daily dose curative regimen would have considerable advantage over the standard fourteen-consecutive-day or once-a-week-for-fourteen-weeks schedules. The merits of a seven-day regimen were defined in experimental studies carried out earlier in this Project and clinical studies

pursued by Clyde at the University of Maryland. These investigations demonstrated that the curative activity of primaquine was a function of the total dose delivered, specifically that within this dose stricture, seven-day and fourteen-day treatment schedules were equally effective. Because of concerns with acute toxic reactions, it was deemed unwise to evaluate the efficacies of single dose and three-dose regimens of primaquine in man. WR-181,023 appeared to have sufficient therapeutic advantage over the latter 8-aminoquinoline to warrant a test of the "total dose principle". The results of this evaluation showed clearly that at the same total dose, WR-181,023 was equally effective in single dose, three daily dose, and seven daily dose schedules. Extension of the total dose concept to agents more active than WR-181,023, which have emerged from pilot assessments, is clearly indicated.

The fifth facet of the curative drug investigations dealt with attempts to enhance the curative activity of primaquine by administration of a companion drug in addition to, or even more desirable, in place of chloroquine. Such enhancement could lead to use of a lower dose of primaquine than that usually prescribed, thereby reducing the numbers of untoward reactions; or enhancement could make conventional doses more effective against infections with those strains of P. vivax which are now curable only when larger than ordinary doses are employed. The initial focus in this investigation was on WR-203,661 (a 7-chlorolincomycin derivative, formerly designated U-24,729A). Earlier studies had shown that this agent had significant activity against both the primary and secondary tissue forms of P. cynomolgi, resulting in both prophylaxis and radical cure when maximum tolerated doses (40.0 mg per kg daily) were employed.

The initial studies were carried out on established sporozoite-induced infections previously treated with a variety of 8-aminoquinoline derivatives without achieving cure.

Such infections were cured when one-fourth to one-half of the usual curative dose of primaquine was administered with one-sixteenth of the maximum tolerated dose of WR-203,661. This encouraging result led to experiments with previously untreated infections. In these studies, the benefits of treatment with a combination of primaquine and WR-203,661 were evident, but were much less striking than those encountered in retreatment cases. Consideration of the causes of these diverse responses led to the suggestion that the superior activity in retreatment cases could very well be due to a relatively small tissue burden.

The suggestion set forth above was put to critical test in an experiment in which the curative activities of each of two normally non-curative doses of primaquine, administered in combination with one-sixteenth of the maximum tolerated dose of WR-203,661, were evaluated in two groups of monkeys, one inoculated with 10^4 sporozoites, the other with 10^6 sporozoites. The results of this experiment showed clearly that the parasite burden was an important determinant of the capacity of WR-203,661 to enhance the curative activity of primaquine. Cures were obtained in all eight infections induced with the smaller inoculum, as contrasted with one of eight cures in infections induced by the larger inoculum.

The favorable results obtained with WR-203,661 encouraged studies of the capacities of a variety of agents to enhance the curative activity of primaquine. The compounds examined included WR-203,659 (7-chlorolincomycin or clindamycin), oxytetracycline, pyrimethamine, erythromycin, rifampin, valinomycin, cordycepin, and cycloheximide. WR-203,659 (a close chemical relative of WR-203,661) effected a slight enhancement of the activity of primaquine when administered at the maximum tolerated dose. None of the other agents modified the curative potential of this 8-aminoquinoline.

The chemotherapeutic studies summarized to this point were all concerned with development of new curative drugs or improved use of primaquine. One other study of more general import, an outgrowth of the experiments with WR-203,661, was undertaken. This investigation dealt specifically with the influence of the tissue stage burden on the capacity of chloroquine to cure sporozoite-induced infections with the B strain of P. cynomolgi. This issue had been dealt with in an expansive form with a variety of chemotherapeutic agents in the 1946-1948 period when sporozoite-induced infections with the M strain of P. cynomolgi were first employed in the search for curative drugs. The current investigation involved work with four groups of three to five monkeys, inoculated with 5, 5×10^2, 5×10^4, or 5×10^6 sporozoites. Two of five recipients of 5 sporozoites did not become infected. Chloroquine was administered at a dose of 5.0 mg per kg body weight, daily for five days, to each of the other subjects when patency was first established and upon each relapse. Infections in the recipients of 5 sporozoites were cured with a single drug course. Infections in all other subjects are still relapsing ten months after inoculation. Intervals between treatment and relapse are distinctly longer in recipients of 5×10^2 sporozoites (25 to 40 days) than in recipients of 5×10^6 sporozoites (7 to 12 days). These results indicate that the tissue stage burden can be an important determinant of the apparent curative capabilities of chloroquine and the frequency of relapse following delivery of this blood schizonticide. They suggest that evaluations of curative activity are precarious in subjects who have a low tissue burden either because of a small sporozoite inoculum or few remaining secondary tissue forms because of exposure to near curative doses of the same or other drug. These findings reinforce the long standing conviction that where large inocula are routinely employed, as in the pilot and secondary assessments, the sole role of chloroquine is containment of the erythrocytic phase of the malaria infection.

III. MISCELLANEOUS PHARMACOLOGIC STUDIES

Although there has never been a contractual commitment for pharmacologic investigations in this Project, studies in this area have been pursued when it appeared that local expertise and resources were uniquely suited to exploration of a specific problem. During the period covered by this Report, three isolated pharmacologic studies have been pursued. These included: (1) comparison of the absorption of WR-30,090 hydrochloride and WR-30,090 base from the gastrointestinal tracts of the owl monkey and rhesus monkey (already summarized in Section I above); (2) investigation of the absorption of the quinazoline WR-158,122 from the gastrointestinal tracts of the above monkeys; and (3) pilot evaluations of the toxicities of WR-211,532, WR-211,533, WR-211,536, and WR-211,537 for the rhesus monkey.

The study of the absorption of WR-158,122 by owl and rhesus monkeys was part of an investigation which included work on human volunteers,* aimed at acquiring information which might help explain the differences in the activity of this quinazoline in owl monkeys infected with P. falciparum or P. vivax and in rhesus monkeys infected with P. cynomolgi, and suggest what might be anticipated when this agent is used in man. The investigation was a collaborative endeavor in which the animal phase was carried out in this Project, the analytical phase in another Department of the Army Project located at The Christ Hospital Institute for Medical Research, College of Medicine, University of Cincinnati. In brief, the results showed that each of four broadly spaced doses gave rise to plasma levels of microbiologically active drug which were tenfold greater in the owl monkey than in the rhesus monkey. These differences roughly paralleled the activities of WR-158,122 exhibited in malarial infections in these primate hosts.

*Performed by Dr. John Arnold under DADA Contract.

The evaluations of the toxicities of WR-211,532, WR-211,533, WR-211,536, and WR-211,537 for the rhesus monkey were preliminary in scope, but satisfied the purposes for which they were undertaken. The first two of these compounds were among the more active 8-aminoquinolines accorded pilot study, with curative doses comparable to that of primaquine. The results of the toxicity study showed that WR-211,532 and WR-211,533 evoked toxic reactions very similar, if not identical with those produced by primaquine. Quantitatively, WR-211,532 had the same order of toxicity as primaquine; WR-211,533 was less than half as toxic. The latter agent would obviously be the preferable candidate for study in human volunteers were either to be considered.

WR-211,536 and WR-211,537 are respectively the D and L isomers of primaquine. Previous studies had shown that their radical curative activities were equal and identical with that of primaquine (the DL mixture). The results of the toxicologic study showed that the toxicity of the D isomer was equal to that of the racemate or slightly less; the L isomer was twice as toxic. If these relationships carry-over to the human subject, and if the D isomer can be obtained readily, its clinical use should be favored over that of primaquine.

The above Summary brings together in abbreviated form the scope of the activities of the 1974-1975 contract year and the major results of these activities. Detailed descriptions are to be found in the Sections that follow.

I. STUDIES ON WR-177,602

I. STUDIES ON WR-177,602

WR-177,602 [α-(2-piperidyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol] is the isomer (threo-epimer) of WR-142,490 (mefloquine), the most promising of the 4-quinolinemethanols examined to date. Interest in evaluating the activity of WR-177,602 against infections with P. falciparum in the owl monkey rested on the demonstration that its acute oral toxicity for the mouse was approximately one-fourth that of WR-142,490 (LD_{10} - WR-177,602 = 3600 mg per kg body weight; LD_{10} - WR-142,490 = 810 mg per kg body weight).

The results of the pilot assessment of the activity of WR-177,602* against established infections with the multidrug-resistant Vietnam Smith strain of P. falciparum are detailed in Tables 1 and 2. The data in Table 2 show that cures were achieved in five of the ten recipients of WR-177,602 in daily doses of 2.5 mg base per kg body weight and in nine of nine recipients of 5.0 mg per kg doses. WR-177,602 has a steep dose response curve, as indicated by the failure of doses less than 2.5 mg per kg to effect even temporary clearance of parasitemia.

The results of the above evaluation have been compared with those of earlier assessments of the activity of WR-142,490 (cf Table 3). This comparison indicates that the two isomers have essentially identical activities against infections with the Smith strain of P. falciparum. A careful comparison of the subacute toxicities of WR-177,602 and WR-142,490 for various animal species would seem to be an investigation of high priority. If such a comparison shows that the subacute toxicity of WR-177,602 is regularly and significantly less than that of WR-142,490, this threo-epimer clearly merits evaluation for both tolerability and activity in human volunteers, the advanced status of clinical studies on mefloquine notwithstanding.

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*Prepared as the monohydrochloride salt.

TABLE 1

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-177,602 AGAINST ESTABLISHED INFECTIONS 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
7918	0.625	108	136	490	352	Dose increased						
7951	0.625	50	54	200	96	Dose increased						
7952	0.625	110	72	123	50	Dose increased						
7953	1.25	88	98	84	24	7	3	<1	<1	-	-	-
7954	1.25	22	30	40	40	20	16	4	<1	<1	<1	<1
7958	1.25	120	42	50	270	425	290	133	48	Dose increased		
7823	2.5	4	14	2	<1	<1	-	-	-	-		-
7824	2.5	10	87	36	6	1	<1	-	-	-		-
7825	2.5	4	54	6	1	<1	-	-	-	-		-
7961	2.5	106	120	18	8	1	<1	<1	<1	<1	-	-
7975	2.5	171	134	110	51	4	3	<1	<1	-	-	-
7918r	2.5	352	920	290	100	20	7	2	<1	<1	<1	-
7951r	2.5	96	300	129	91	34	14	3	<1	<1	-	-
7952r	2.5	50	300	70	28	2	<1	<1	-	-	-	-
7953r	2.5	30	11	3	<1	<1	<1	-	-	-	-	
7954r	2.5	140	235	138	44	3	<1	<1	<1	<1	<1	-
7826	5.0	8	30	8	<1	<1	-	-	-	-		-
7858	5.0	6	51	5	<1	<1	-	-	-	-		-
7859	5.0	6	82	22	3	<1	<1	<1	-	-		-
7823r	5.0	10	2	<1	<1	-	-	-	-	-	-	-
7958r	5.0	48	34	6	<1	<1	<1	-	-	-	-	
7975r	5.0	22	40	9	<1	<1	<1	-	-	-	-	
7918rr	5.0	2	<1	<1	<1	-	-	-	-	-	-	-
7951rr	5.0	<1	<1	<1	<1	<1	<1	-	-	-	-	
7952rr	5.0	30	68	18	12	4	<1	<1	-	-	-	
7860	10.0	4	14	3	2	<1	<1	-	-	-		-
7861	10.0	12	57	15	2	<1	<1	-	-	-	-	
7862	10.0	8	60	10	6	<1	<1	-	-	-		-

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TABLE 2

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-177,602 AGAINST ESTABLISHED INFECTIONS 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			
7918	0.625		±		n.a.	n.a.	Dose increased
7951	0.625		±		n.a.	n.a.	Dose increased
7952	0.625		+		n.a.	n.a.	Dose increased
7953	1.25			+	8	35	
7954	1.25		+		n.a.	n.a.	
7958	1.25		±		n.a.	n.a.	
7823	2.5			+	5	13	
7824	2.5			+	6	n.a.	Cured
7825	2.5			+	5	n.a.	Cured
7961	2.5			+	9	n.a.	Cured
7975	2.5			+	8	17	
7918r	2.5			+	10	46	
7951r	2.5			+	9	34	
7952r	2.5			+	7	41	
7953r	2.5			+	6	n.a.	Cured
7954r	2.5			+	10	n.a.	Cured
7826	5.0			+	5	n.a.	Cured
7858	5.0			+	5	n.a.	Cured
7859	5.0			+	7	n.a.	Cured
7823r	5.0			+	4	n.a.	Cured
7958r	5.0			+	6	n.a.	Cured
7975r	5.0			+	6	n.a.	Cured
7918rr	5.0			+	4	n.a.	Cured
7951rr	5.0			+	6	n.a.	Cured
7952rr	5.0			+	7	n.a.	Cured
7860	10.0			+	6	n.a.	Cured
7861	10.0			+	6	n.a.	Cured
7862	10.0			+	6	n.a.	Cured

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TABLE 3

COMPARISON OF THE ACTIVITIES OF WR-177,602 AND WR-142,490 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Compound WR- No.	Daily Dose* Mg Base/Kg Body Weight	No. of Infections Treated					Days from Initial R_x to Parasite Clearance**
		Total	Response to Treatment				
			None	Suppressed	Cleared	Cured	
177,602	0.625	3	-	3	-	0	n. a.
	1.25	3	-	2	1	0	n. a.
	2.5	10	-	-	10	5	7
	5.0	9	-	-	9	9	6
	10.0	3	-	-	3	3	6
142,490	1.25	2	-	2	-	0	n. a.
	2.5	6	-	-	6	2	8
	5.0	14	-	-	14	14	7
	10.0	2	-	-	2	2	7

*Administered as the hydrochloride salt, via stomach tube, once daily for seven consecutive days.

**Median day to clearance of parasitemia.

II. STUDIES ON WR-182,231

II. STUDIES ON WR-182,231

WR-182,231 [α-(di-N-butylaminoethyl)-2-(4-trifluoromethylphenyl)-6-trifluoromethyl-4-pyridinemethanol] was deemed worthy of pilot evaluation because of its structural similarities to WR-172,435 and WR-180,409 (cf Figure 1). The latter agents are essentially equally active and the most effective of the 4-pyridinemethanols examined to date in the owl monkey - P. falciparum model.

The assessment of the activity of WR-182,231* was limited to experiments on monkeys infected with the Smith strain of P. falciparum (cf Tables 4 and 5). As the summary observations show (Table 5), this agent was routinely curative when administered in daily doses of 20.0 mg base per kg body weight. Clearance of parasitemia was attained regularly by delivery of 10.0 mg per kg doses, but only two of six infections so treated were cured. Doses of 2.5 mg per kg were essentially without effect on the course of infection. Limited suppression of parasitemia was achieved in three of six recipients of doses of 5.0 mg per kg.

The results of this evaluation of the activity of WR-182,231 have been compared with the results of earlier assessments of the activities of the hydrochloride salts of WR-172,435 and WR-180,409. This comparison, summarized in Table 6, shows clearly that WR-182,231 is no more than one-fourth as active as WR-180,409. WR-182,231 is definitely less active than WR-172,435, although the data on the latter agent are too limited for a precise assessment. Unless WR-182,231 has major toxicologic advantages over WR-172,435 and WR-180,409, it would not appear to merit further attention.

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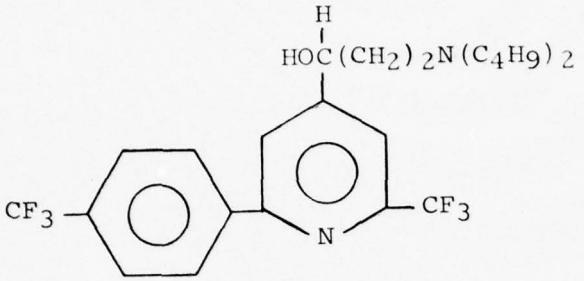
*Prepared as the monohydrochloride salt.

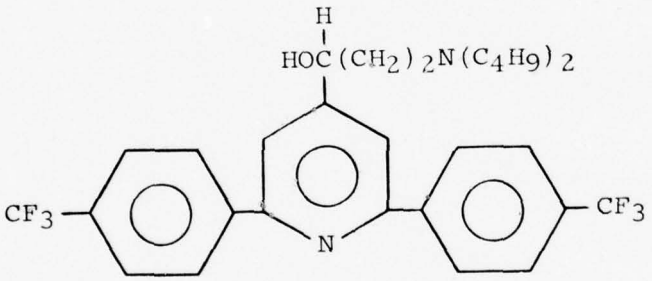
FIGURE 1

STRUCTURES OF WR-182,231, WR-172,435, AND WR-180,409



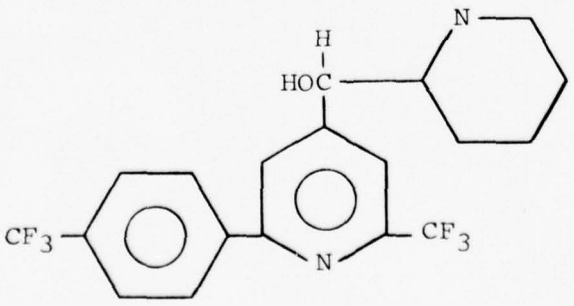
WR-182,231

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



WR-172,435

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



WR-180,409

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

TABLE 4

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-182,231 AGAINST ESTABLISHED INFECTIONS 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
7691	2.5	118	230	204	700	200	Dose increased					
7692	2.5	81	202	156	430	220	Dose increased					
7693	2.5	186	458	488	700	500	Dose increased					
7694	5.0	27	38	27	23	32	15	22	12	2	<1	2
7707	5.0	118	200	276	250	210	55	30	3	<1	<1	<1
7710	5.0	112	58	78	12	24	10	6	1	<1	<1	<1
7691r	5.0	200	185	63	30	4	<1	<1	-	-	-	-
7692r	5.0	220	102	74	48	21	4	1	<1	-	-	-
7693r	5.0	500	318	64	108	15	2	<1	-	-	-	-
7711	10.0	117	78	116	27	22	4	<1	<1	<1	<1	-
7712	10.0	206	138	220	100	27	4	<1	<1	<1	<1	-
7719	10.0	142	32	72	19	14	4	<1	<1	<1	<1	-
7694r	10.0	54	38	<1	<1	<1	-	-	-	-	-	-
7707r	10.0	<1	<1	-	-	-	-	-	-	-	-	-
7710r	10.0	<1	<1	-	-	-	-	-	-	-	-	-
7720	20.0	37	19	5	2	<1	<1	<1	<1	-	-	-
7721	20.0	51	24	9	1	<1	<1	-	-	-		
7723	20.0	88	39	11	1	<1	<1	-	-	-		
7711r	20.0	2	2	1	<1	<1	-	-	-	-	-	-
7712r	20.0	<1	6	<1	<1	<1	-	-	-	-	-	-
7719r	20.0	<1	<1	<1	-	-	-	-	-	-	-	-

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TABLE 5

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-182,231 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Summary Observations

Atr No.	Daily Dose Mg/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			
7691	2.5	+			n.a.	n.a.	Dose increased
7692	2.5	+			n.a.	n.a.	Dose increased
7693	2.5	+			n.a.	n.a.	Dose increased
7694	5.0		+		n.a.	n.a.	
7707	5.0		+		n.a.	n.a.	
7710	5.0		+		n.a.	n.a.	
7691r	5.0			+	7	23	
7692r	5.0			+	8	19	
7693r	5.0			+	7	16	
7711	10.0			+	10	13	
7712	10.0			+	10	13	
7719	10.0			+	10	9	
7694r	10.0			+	5	25	
7707r	10.0			+	3	n.a.	Cured
7710r	10.0			+	3	n.a.	Cured
7720	20.0			+	8	n.a.	Cured
7721	20.0			+	6	n.a.	Cured
7723	20.0			+	6	n.a.	Cured
7711r	20.0			+	5	n.a.	Cured
7712r	20.0			+	5	n.a.	Cured
7719r	20.0			+	3	n.a.	Cured

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TABLE 6

A COMPARISON OF THE ACTIVITIES OF WR-182,231, WR-172,435, AND WR-180,409 AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Compound WR- No.	Daily Dose* Mg Base/Kg Body Weight	No. of Infections Treated					Days from Initial R_x to Parasite Clearance**
		Total	Response to Treatment				
			None	Suppressed	Cleared	Cured	
182,231	2.5	3	3	-	-	0	n.a.
	5.0	6	-	3	3	0	n.a.
	10.0	6	-	-	6	2	n.a.
	20.0	6	-	-	6	6	5
172,435	1.25	3	3	-	-	0	n.a.
	5.0	6	-	-	6	5	9
	10.0	1	-	-	1	1	6
180,409	1.25	6	2	3	1	1	n.a.
	2.5	13	1	1	11	0	n.a.
	5.0	21	-	1	20	19	6
	10.0	6	-	-	6	6	5

*Administered as the hydrochloride salt, via stomach tube, once daily for seven consecutive days.

**Median day to clearance of parasitemia.

III. STUDIES ON WR-204,165 AND WR-194,965

III. STUDIES ON WR-204,165 AND WR-194,965

The structures of WR-204,165 and WR-194,965 are indicated in Figure 2. Both of these agents can be considered as Mannich bases. There was considerable interest in Mannich reaction products during the World War II Malaria Chemotherapy Program. A significant group of such agents exhibited activity against infections with erythrocytic parasites of *P. gallinaceum* and *P. lophurae* in the chicken and *P. knowlesi* in the rhesus monkey. Four were evaluated against infections with *P. vivax* in human volunteers with disappointing results.

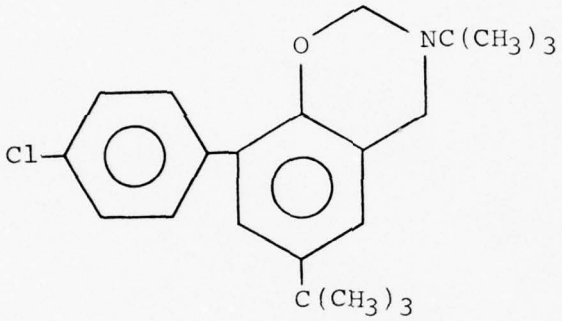
WR-204,165, one of the Mannich bases prepared in the current Malaria Chemotherapy Program, exhibited significant activity against trophozoite-induced infections with *P. berghei* in the mouse and *P. cynomolgi* in the rhesus monkey. Since the level of activity encountered appeared much superior to that of any derivative examined in the World War II Malaria Program, it was decided to undertake a pilot assessment of the activity of WR-204,165 against established infections with the multidrug-resistant Smith strain of *P. falciparum*. The demonstrated effectiveness of this compound led to similar studies on WR-194,965, which was looked upon as a likely metabolite of WR-204,165.

The results of the pilot evaluations of the activities of WR-204,165 and WR-194,965 have been summarized in Tables 7-10. Although data on WR-194,965 are less extensive than those on WR-204,165, it seems likely that these agents are essentially equally active, curing infections with a high degree of regularity when administered in doses of 5.0 mg base per kg body weight, daily for seven consecutive days. Both agents have steep dose response curves, as indicated by failure to achieve clearance of parasitemia at doses of 1.25 mg per kg, irregular clearance in some recipients of 2.5 mg per kg, cure of infections in others, and full curative activity at doses of 5.0 mg per kg.

The activities of WR-204,165 and WR-194,965 exhibited above are very similar to those displayed by the most active of the 4-quinolinemethanols and 4-pyridinemethanols. If the tolerability of these Mannich reaction products compares favorably with that of the latter agents, trials in human volunteers would seem indicated.

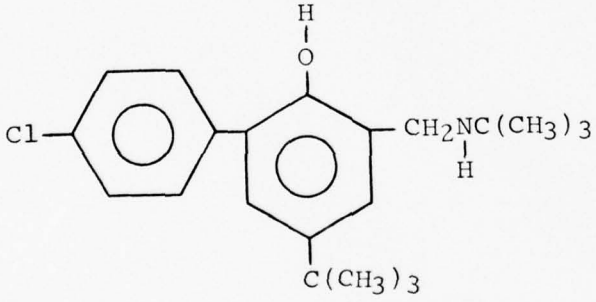
FIGURE 2

STRUCTURES OF WR-204,165 AND WR-194,965



WR-204,165

3,6-bis-(tert. butyl)-8-(4-chlorophenyl)-2H,4H-1,3-benzo-oxazine.



WR-194,965

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

TABLE 7

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-204,165 AGAINST ESTABLISHED INFECTIONS 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
7976	0.625	72	36	460	110	Dose increased						
7977	0.625	15	68	104	63	Dose increased						
7978	0.625	75	132	500	340	Dose increased						
7982	1.25	84	78	322	285	575	340	304	195	Dose increased		
7983	1.25	50	84	235	430	280	650	266	210	Dose increased		
7984	1.25	72	68	111	54	96	65	30	9	8	2	6
7863	2.5	8	69	22	40	12	15	2	<1	-	-	-
7864	2.5	3	50	14	16	2	8	1	<1	-	-	-
7865	2.5	4	42	17	16	10	<1	<1	<1	-	-	-
7967	2.5	9	22	8	4	<1	<1	<1	-	-	-	
7970	2.5	10	66	18	16	3	<1	<1	-	-	-	
7985	2.5	28	28	18	14	5	9	3	<1	<1	<1	<1
7986	2.5	40	34	36	18	3	<1	<1	<1	<1	<1	<1
7976r	2.5	110	690	420	200	36	4	<1	<1	<1	<1	<1
7977r	2.5	63	108	45	15	1	<1	<1	-	-	-	-
7978r	2.5	340	940	510	260	34	6	1	<1	<1	<1	<1
7982r	2.5	195	76	26	19	5	2	<1	<1	<1	-	-
7983r	2.5	210	36	6	<1	<1	<1	-	-	-	-	
7984r	2.5	<1	<1	<1	<1	-	-	-	-	-	-	-

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TABLE 7 - 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
7866	5.0	6	129	9	4	<1	<1	-	-	-		-
7934	5.0	11	93	15	78	30	31	4	<1	<1	-	-
7935	5.0	8	42	33	2	<1	<1	-	-	-		-
7973	5.0	8	30	8	5	<1	<1	<1	-	-	-	
7974	5.0	28	27	3	<1	<1	<1	-	-	-	-	
7863r	5.0	9	2	<1	<1	<1	<1	-	-	-	-	
7864r	5.0	10	3	<1	<1	<1	-	-	-	-	-	-
7970r	5.0	4	12	6	8	6	2	<1	-	-	-	
7985r	5.0	8	<1	<1	<1	-	-	-	-	-	-	-
7986r	5.0	6	6	12	10	1	<1	<1	-	-	-	-
7976rr	5.0	<1	<1	<1	-	-	-	-	-	-	-	-
7977rr	5.0	<1	<1	<1	-	-	-	-	-	-	-	
7982rr	5.0	1	1	<1	<1	<1	-	-	-	-	-	-
7936	10.0	13	60	24	4	<1	<1	<1	<1	-	-	-
7937	10.0	7	22	4	<1	<1	<1	-	-	-		-
7938	10.0	9	26	12	10	<1	<1	<1	-	-		-
7934r	10.0	16	68	11	8	2	<1	<1	-	-	-	
7970rr	10.0	30	12	4	<1	<1	-	-	-	-	-	

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TABLE 8

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-204,165 AGAINST ESTABLISHED INFECTIONS 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			
7976	0.625	+			n.a.	n.a.	Dose increased
7977	0.625	+			n.a.	n.a.	Dose increased
7978	0.625	+			n.a.	n.a.	Dose increased
7982	1.25		+		n.a.	n.a.	Dose increased
7983	1.25		+		n.a.	n.a.	Dose increased
7984	1.25		+		n.a.	n.a.	
7863	2.5			+	8	34	
7864	2.5			+	8	41	
7865	2.5			+	8	n.a.	Cured
7967	2.5			+	7	n.a.	Cured
7970	2.5			+	7	15	
7985	2.5		+		n.a.	n.a.	
7986	2.5			+	13	23	
7976r	2.5			+	11	21	
7977r	2.5			+	7	27	
7978r	2.5			+	12	n.a.	Cured
7982r	2.5			+	9	15	
7983r	2.5			+	6	n.a.	Cured
7984r	2.5			+	4	n.a.	Cured

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TABLE 8 - 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			
7866	5.0			+	6	n.a.	Cured
7934	5.0			+	9	34	
7935	5.0			+	6	n.a.	Cured
7973	5.0			+	7	n.a.	Cured
7974	5.0			+	6	n.a.	Cured
7863r	5.0			+	6	n.a.	Cured
7864r	5.0			+	5	n.a.	Cured
7970r	5.0			+	7	27	
7985r	5.0			+	4	n.a.	Cured
7986r	5.0			+	7	n.a.	Cured
7976rr	5.0			+	3	n.a.	Cured
7977rr	5.0			+	4	n.a.	Cured
7982rr	5.0			+	5	n.a.	Cured
7936	10.0			+	8	n.a.	Cured
7937	10.0			+	6	n.a.	Cured
7938	10.0			+	7	n.a.	Cured
7934r	10.0			+	7	n.a.	Cured
7970rr	10.0			+	5	n.a.	Cured

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TABLE 9

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-194,965 AGAINST ESTABLISHED INFECTIONS 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
7929	1.25	15	38	190	140	302	272	152	88	66	48	
7930	1.25	11	16	10	1	<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	-	-	-	-	-	
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	-	-	-	-	-

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TABLE 10

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

Summary Observations

Atr No.	Daily Dose Mg/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			
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

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IV. STUDIES ON THE CAPACITY OF WR-180,872 TO ENHANCE THE ACTIVITY OF WR-158,122

IV. STUDIES ON THE CAPACITY OF WR-180,872 TO ENHANCE THE ACTIVITY OF WR-158,122

Studies pursued in preceding years showed that WR-158,122 [2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline] has remarkable activity in owl monkeys infected with the drug-susceptible Chesson strain of *P. vivax* or the chloroquine-, quinine-resistant Oak Knoll strain of *P. falciparum*. The activity of this quinazoline derivative was reduced markedly in monkeys infected with pyrimethamine-resistant strains of either of these plasmodia. To a very great extent, the liabilities of pyrimethamine resistance could be eliminated by administering small doses of sulfadiazine in combination with WR-158,122 (cf Report SORI-KM-73-246, July 23, 1973).

Studies pursued by Thompson and coworkers in mice infected with a pyrimethamine-resistant strain of *P. berghei* seemed to offer an alternate approach to use of a quinazoline-sulfonamide mixture. These investigations showed that the liabilities of pyrimethamine resistance could be abolished by administering WR-180,872 [2,4-diamino-6-(2-naphthylsulfonyl)-5,6,7,8-tetrahydroquinazoline] in combination with WR-158,122. Experiments were carried out to determine whether similar benefits could be achieved in monkeys infected with the pyrimethamine-resistant Vietnam Palo Alto strain of *P. vivax*. The results have been summarized in Tables 11 and 12.

As shown in Table 12, the largest dose of WR-158,122 employed alone in this evaluation, 1.56 mg per kg body weight daily, effected temporary clearance of parasitemia. No cures were achieved in this study; in a much larger experiment, five of ten infections were cured. The largest dose of WR-180,872 utilized alone was 25.0 mg per kg daily. Previous studies had shown that such an amount would cure infections with the chloroquine-resistant, pyrimethamine-susceptible Oak Knoll strain of

P. falciparum. In the current investigation (Table 12), this dose level failed to control the parasitemia in monkeys infected with the pyrimethamine-resistant Palo Alto strain.

As the data summarized in Table 12 show, little, if anything, was gained by administering WR-158,122 and WR-180,872 in combination. Doses of 0.39, 1.56, or 6.25 mg of the latter quinazoline added nothing to the activity of 0.39 mg per kg or lesser doses of WR-158,122. This negative contribution stands in striking contrast to the benefits derived from administering WR-158,122 in combination with sulfadiazine, where concomitant delivery of 0.098 mg per kg doses of this quinazoline and 5.0 mg per kg doses of the sulfonamide was a uniformly curative regimen.

TABLE 11

ASSESSMENT OF THE CAPACITY OF WR-180,872 TO ENHANCE THE ACTIVITY OF WR-158,122 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		
	WR-158,122	WR-180,872		1	2	3	4	5	6	7	1	2	3
6076	0.39	-	36	60	82	54	40	28	38	45	24	30	14
6989	0.39	-	10	24	34	50	24	52	11	2	1	2	4
7170	0.39	-	16	14	40	24	24	8	8	14	10	12	16
7196	1.56	-	15	18	2	2	<1	<1	<1	-	-	-	-
7251	1.56	-	36	32	38	42	10	4	<1	-	-	-	-
7307	1.56	-	12	14	20	2	<1	<1	<1	-	-	-	-
7311	-	1.56	5	46	74	38	52	62	76	29	60	40	54
7316	-	1.56	3	9	15	16	22	30	10	32	8	30	36
7340	-	1.56	8	22	27	36	14	14	18	6	15	6	9
7358	-	6.25	22	54	64	70	38	12	10	6	9	6	4
7365	-	6.25	15	34	44	22	14	10	12	6	16	10	12
7367	-	6.25	17	34	16	8	10	8	6	7	6	14	22
7372	-	25.0	32	70	102	104	88	126	154	156	180	170	218
7374	-	25.0	1	2	15	8	12	6	9	14	4	12	16
7375	-	25.0	28	26	22	24	32	36	20	12	15	4	16
7376	0.098	0.39	3	5	20	8	11	32	38	48	16	60	28
7377	0.098	0.39	20	18	4	14	20	26	20	20	18	13	13
7380	0.098	0.39	18	32	30	66	26	2	<1	2	4	2	1
7383	0.098	1.56	39	52	122	102	56	30	16	18	14	2	18
7393	0.098	1.56	16	30	42	43	52	3	40	27	56	58	57
7394	0.098	1.56	4	12	11	19	12	2	<1	15	16	13	10
7395	0.098	6.25	3	4	2	1	<1	<1	<1	<1	<1	<1	<1
7398	0.098	6.25	8	10	8	12	3	6	7	4	4	10	24
7407	0.098	6.25	1	2	6	6	3	3	<1	6	3	4	6

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TABLE 11 - CONTINUED

Atr No.	Daily Dose Mg/Kg Body Weight		Parasitemia - No. Parasites/10^4 Erythrocytes									
			Day Pre-treatment	Day of Treatment						Day Post-treatment		
	WR-158,122	WR-180,872		1	2	3	4	5	6	7	1	2	3
7431	0.39	0.39	14	22	60	110	114	52	12	6	14	24	19
7432	0.39	0.39	12	8	6	2	<1	<1	<1	2	2	10	30
7433	0.39	0.39	22	42	69	58	60	44	30	50	150	122	172
7454	0.39	1.56	32	94	340	400	820	380	Regimen changed				
7455	0.39	1.56	20	26	52	72	70	27	24	18	27	36	33
7498	0.39	1.56	5	1	2	2	5	2	1	<1	<1	3	9
7502	0.39	6.25	10	2	2	3	<1	<1	<1	<1	<1	12	14
7507	0.39	6.25	1	4	2	<1	<1	<1	<1	<1	<1	<1	<1
7509	0.39	6.25	4	2	1	1	<1	<1	<1	<1	<1	2	8

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TABLE 12.

ASSESSMENT OF THE CAPACITY OF WR-180,872 TO ENHANCE THE ACTIVITY OF WR-158,122 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 Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recrudescence	Remarks
	WR-158,122	WR-180,872	None	Suppressed	Cleared			
6076	0.39	-		+		n.a.	n.a.	
6989	0.39	-		+		n.a.	n.a.	
7170	0.39	-		+		n.a.	n.a.	
7196	1.56	-			+	7	12	
7251	1.56	-			+	7	9	
7307	1.56	-			+	7	12	
7311	-	1.56	+			n.a.	n.a.	
7316	-	1.56		+		n.a.	n.a.	
7340	-	1.56		+		n.a.	n.a.	
7358	-	6.25		+		n.a.	n.a.	
7365	-	6.25		+		n.a.	n.a.	
7367	-	6.25		+		n.a.	n.a.	
7372	-	25.0	+			n.a.	n.a.	
7374	-	25.0		+		n.a.	n.a.	
7375	-	25.0		+		n.a.	n.a.	
7376	0.098	0.39		+		n.a.	n.a.	
7377	0.098	0.39		+		n.a.	n.a.	
7380	0.098	0.39		+		n.a.	n.a.	
7383	0.098	1.56		+		n.a.	n.a.	
7393	0.098	1.56		+		n.a.	n.a.	
7394	0.098	1.56		+		n.a.	n.a.	
7395	0.098	6.25		+		n.a.	n.a.	
7398	0.098	6.25		+		n.a.	n.a.	
7407	0.098	6.25		+		n.a.	n.a.	

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TABLE 12 - CONTINUED

Atr No.	Daily Dose Mg/Kg Body Weight		Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx to Recrudescence	Remarks
	WR-158,122	WR-180,872	None	Suppressed	Cleared			
7431	0.39	0.39		+		n.a.	n.a.	
7432	0.39	0.39		+		n.a.	n.a.	
7433	0.39	0.39		+		n.a.	n.a.	
7454	0.39	1.56	+			n.a.	n.a.	Regimen changed
7455	0.39	1.56		+		n.a.	n.a.	
7498	0.39	1.56		+		n.a.	n.a.	
7502	0.39	6.25		+		n.a.	n.a.	
7507	0.39	6.25		+		n.a.	n.a.	
7509	0.39	6.25		+		n.a.	n.a.	

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V. THE ACTIVITY OF WR-203,661

V. THE ACTIVITY OF WR-203,661

There has been intermittent interest in the antimalarial properties of WR-203,661 and WR-203,659 (clindamycin) since 1967, when Lewis first reported on the activities of these lincomycin derivatives in mice infected with P. berghei. The most recent interest stemmed from a preliminary report by Powers on the activity of WR-203,661 (originally coded U-24,729A) in owl monkeys infected with the chloroquine-resistant Oak Knoll strain of P. falciparum. This observation was confirmed and extended [cf Annual Report (this Project) 1973-1974] to investigations with the multidrug-resistant Smith strain.

The results of these previously reported studies showed that WR-203,661 was fully active in the face of chloroquine, quinine, or pyrimethamine resistance and that it was approximately eight times as active as the closely related WR-203,659. However, parasitemias were controlled slowly when either agent was administered in a seven consecutive daily dose regimen. Attempts have been made to determine whether this liability could be reduced by modifying the dosage regimen. This modification has taken two forms: (1) delivery of the total dosage of WR-203,661 in a single dose or three doses instead of seven; and (2) delivery of an initial loading dose of this agent, followed by a series of small fractional doses.

The results of a preliminary study, summarized in Tables 13 and 14, show that the curative activity of WR-203,661 was less when the same total amount was administered in a single dose or three divided doses than when seven consecutive daily fractions were delivered. The rate of parasite clearance was not improved in either the short term or loading dose regimens. Although the data supporting these conclusions are limited, their consistency suggests that further pursuit of dosage regimen studies would not be rewarding.

Somewhat unrelated studies, described in Section XIII of this Report, indicated that the simultaneous delivery of WR-203,661 would enhance the capacity of primaquine to cure infections with sporozoites of P. cynomolgi. Since this finding might be applied to human infections with P. vivax, it became important to know whether the trophozoite phase of such infections could be controlled by WR-203,661 or whether it would still be necessary to employ chloroquine or other agent as a blood schizonticide. This led to a limited assessment of the capacity of this lincomycin derivative to cure established infections with the Vietnam Palo Alto strain of P. vivax.

The results of the above evaluation, summarized in Tables 15 and 16, show that the curative activity of WR-203,661 was characterized by a flat dose response curve. A significant proportion of infections was cured by the least daily dose used in this study (1.25 mg per kg body weight); a daily dose of 10.0 mg per kg was required for a uniformly curative response. At any of the doses employed, parasitemias were cleared slowly. These findings suggest that in order to make effective use of the capacity of WR-203,661 to enhance the radical curative activity of primaquine, it would be desirable to include a rapidly acting blood schizonticide in the treatment regimen.

TABLE 13

PRELIMINARY APPRAISAL OF THE INFLUENCE OF THE DOSAGE REGIMEN AND LOADING DOSES ON THE CAPACITY OF WR-203,661 TO CURE ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

Detailed Effects On Parasitemia

Atr No.	Dosage Regimen Mg/Kg Body Weight		Parasitemia - No. Parasites/10^4 Erythrocytes									
	Individual Dose	Total Dose	Day Pre-treatment	Day of Treatment						Day Post-treatment		
				1	2	3	4	5	6	7	1	2	3
7683	35.0 x 1	35.0	72	155	303	240	60	40	4	<1	<1	-	-
7684	35.0 x 1	35.0	90	340	390	220	22	1	<1	<1	<1	-	-
7686	12.0 x 3	36.0	72	260	80	15	1	<1	<1	-	-	-	
7687	12.0 x 3	36.0	26	80	100	18	6	<1	<1	-	-	-	
7683r	12.0 x 3	36.0	8	32	30	26	6	7	4	Regimen changed			
7684r	12.0 x 3	36.0	6	1	<1	-	-	-					-
7690	20.0(D-1) + 2.5(D-2 to 6)	35.0	66	390	520	320	60	4	<1	-	-	-	
7695	20.0(D-1) + 2.5(D-2 to 6)	35.0	63	155	220	58	8	<1	<1	-	-	-	
7688	5.0 x 7	35.0	54	145	65	18	6	<1	<1	-	-	-	
7689	5.0 x 7	35.0	96	225	130	98	4	<1	<1	-	-	-	
7683rr	5.0 x 7	35.0	4	3	<1	<1	<1	-	-	-	-	-	
7696	70.0 x 1	70.0	42	122	100	21	4	<1	<1	-	-	-	
7697	70.0 x 1	70.0	78	75	56	33	4	<1	<1	-	-	-	
7698	24.0 x 3	72.0	70	184	250	250	75	4	<1	<1	<1	-	-
7699	24.0 x 3	72.0	98	129	145	42	5	<1	<1	-	-	-	
7696r	24.0 x 3	72.0	20	10	12	10	9	2	<1	-	-	-	-
7697r	24.0 x 3	72.0	30	16	28	2	<1	<1	<1	-	-	-	
7702	40.0(D-1) + 5.0(D-2 to 6)	70.0	80	180	220	102	15	3	<1	-	-	-	
7713	40.0(D-1) + 5.0(D-2 to 6)	70.0	30	21	40	20	1	<1	<1	-	-	-	
7700	10.0 x 7	70.0	40	125	50	36	2	<1	<1	-	-	-	
7701	10.0 x 7	70.0	82	200	260	28	18	1	<1	-	-	-	

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TABLE 14

PRELIMINARY APPRAISAL OF THE INFLUENCE OF THE DOSAGE REGIMEN AND LOADING DOSES ON THE CAPACITY OF WR-203,661 TO CURE ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF *PLASMODIUM FALCIPARUM*

Summary Observations

Atr No.	Dosage Regimen 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
	Individual Dose	Total Dose	None	Suppressed	Cleared			
7683	35.0 x 1	35.0			+	9	30	
7684	35.0 x 1	35.0			+	9	39	
7686	12.0 x 3	36.0			+	7	n.a.	Cured
7687	12.0 x 3	36.0			+	7	n.a.	Cured
7683r	12.0 x 3	36.0		+		n.a.	n.a.	Dose increased
7684r	12.0 x 3	36.0			+	3	n.a.	Cured
7690	20.0(D-1) + 2.5(D-2 to 6)	35.0			+	7	n.a.	Cured
7695	20.0(D-1) + 2.5(D-2 to 6)	35.0			+	7	n.a.	Cured
7688	5.0 x 7	35.0			+	7	n.a.	Cured
7689	5.0 x 7	35.0			+	7	n.a.	Cured
7683rr	5.0 x 7	35.0			+	5	n.a.	Cured
7696	70.0 x 1	70.0			+	7	17	
7697	70.0 x 1	70.0			+	7	26	
7698	24.0 x 3	72.0			+	9	n.a.	Cured
7699	24.0 x 3	72.0			+	7	n.a.	Cured
7696r	24.0 x 3	72.0			+	7	n.a.	Cured
7697r	24.0 x 3	72.0			+	7	n.a.	Cured
7702	40.0(D-1) + 5.0(D-2 to 6)	70.0			+	7	n.a.	Cured
7713	40.0(D-1) + 5.0(D-2 to 6)	70.0			+	7	n.a.	Cured
7700	10.0 x 7	70.0			+	7	n.a.	Cured
7701	10.0 x 7	70.0			+	7	n.a.	Cured

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TABLE 15

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-203,661 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
7735r	1.25	18	8	24	30	10	8	9	3	<1	<1	<1
7758r	1.25	20	20	40	6	2	<1	<1	<1	<1	<1	<1
7768r	1.25	11	10	8	20	4	<1	<1	<1	-	-	-
7873r	1.25	<1	<1	<1	<1	<1	<1	-	-	-	-	-
7736r	2.5	84	189	188	220	150	64	12	2	1	<1	<1
7780r	2.5	4	6	10	2	<1	<1	<1	-	-	-	-
7900r	2.5	10	5	4	4	<1	<1	-	-	-	-	-
7902r	2.5	6	3	8	8	4	<1	<1	<1	-	-	-
7737r	5.0	105	84	85	70	80	14	2	<1	<1	-	-
7755r	5.0	10	2	2	<1	<1	<1	-	-	-	-	
7882r	5.0	<1	<1	<1	<1	-	-	-	-	-	-	
7736rr	5.0	<1	<1	<1	<1	<1	<1	<1	-	-	-	-
7873rr	5.0	4	20	10	12	6	1	<1	<1	<1	<1	<1
7902rr	5.0	20	10	2	<1	<1	-	-	-	-	-	-
7738r	10.0	60	51	12	10	4	2	<1	<1	<1	-	-
7769r	10.0	1	2	4	<1	<1	<1	-	-	-		
7872r	10.0	20	40	12	6	<1	<1	<1	-	-	-	
7768rr	10.0	3	2	4	<1	<1	-	-	-	-	-	-
7736rrr	10.0	<1	<1	<1	-	-	-	-	-	-	-	-

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TABLE 16

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF WR-203,661 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 Recru-descence	Remarks
		None	Suppressed	Cleared			
7735r	1.25			+	11	n.a.	Cured
7758r	1.25			+	12	n.a.	Cured
7768r	1.25			+	8	19	
7873r	1.25			+	6	26	
7736r	2.5			+	11	23	
7780r	2.5			+	7	n.a.	Cured
7900r	2.5			+	6	n.a.	Cured
7902r	2.5			+	8	25	
7737r	5.0			+	9	n.a.	Cured
7755r	5.0			+	6	n.a.	Cured
7882r	5.0			+	4	n.a.	Cured
7736rr	5.0			+	7	26	
7873rr	5.0			+	11	n.a.	Cured
7902rr	5.0			+	7	36	
7738r	10.0			+	9	n.a.	Cured
7769r	10.0			+	6	n.a.	Cured
7872r	10.0			+	7	n.a.	Cured
7768rr	10.0			+	5	n.a.	Cured
7736rrr	10.0			+	3	n.a.	Cured

SOUTHERN RESEARCH INSTITUTE

VI. COMPARATIVE STUDIES ON WR-30,090 BASE AND WR-30,090 HYDROCHLORIDE

VI. COMPARATIVE STUDIES ON WR-30,090 BASE AND WR-30,090 HYDROCHLORIDE

WR-30,090 [α-(di-N-butylaminomethyl)-2-(3,4-dichlorophenyl)-6,8-dichloro-4-quinolinemethanol], one of the first promising compounds to emerge from the experimental component of the Malaria Chemotherapy Program, was evaluated in human volunteers for tolerability and for capacity to cure infections with chloroquine-resistant, pyrimethamine-resistant strains of P. falciparum. The favorable results of these studies led to closely supervised field trials in military service personnel in South Vietnam. Overall, the results of these studies were promising. However, there were some irregularities in response which required special monitoring of patient status and adjustment of the dosage of WR-30,090. These irregularities were attributed to erratic absorption of WR-30,090 which was administered as the relatively insoluble monohydrochloride salt.

In an effort to circumvent such response irregularities, attention was directed to development of a more soluble form of WR-30,090. This endeavor, pursued by investigators at the INTER$_X$ Research Corporation, led to the discovery that WR-30,090 base was highly soluble in oleic acid, up to 20 per cent by weight, and readily absorbed from such solution. A limited study pursued in the dog showed that the peak plasma or whole blood levels of "WR-30,090" were eightfold greater following oral administration of the base in oleic acid than after delivery of the equivalent dose of the hydrochloride salt. On the basis of this observation, plans were made for evaluating the therapeutic activity of oleic acid solutions of WR-30,090 base in human volunteers. As a prelude to these evaluations, the bioavailability of

WR-30,090 base and WR-30,090 hydrochloride was compared in owl monkeys infected with the Vietnam Smith strain of Plasmodium falciparum*.

The results of this comparison have been detailed in Tables 17 and 18 and summarized in Table 19. As the data in the latter table show, there was a striking difference between the therapeutic efficacy of WR-30,090 base and WR-30,090 hydrochloride salt. Contrary to what would have been expected from the absorption data, the base had very limited activity. In recipients of the base in doses of 5.0 to 40.0 mg per kg, clearance of parasitemia was achieved in but six of fifteen subjects, and cure of the infection in but one individual. Within the same dose range, delivery of the hydrochloride salt led to clearance of parasitemia in 26 of 27 recipients, cures in 21 of 27.

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*Since the oral administration of oil solutions to experimental animals is not a conventional procedure, a brief description of the method of delivery of both WR-30,090 base and the hydrochloride salt seems indicated. (1) WR-30,090 base: A stock solution of base in oleic acid was prepared and diluted serially with oleic acid to give a range of concentrations such that the dose per kg of monkey was contained in a 0.2 ml volume. The volume actually required per monkey was measured via use of a 0.25 ml tuberculin syringe into an opened No. 5 gelatin capsule. After capping, the latter was placed in the non-constricted end of a No. 14 French urethral catheter which was passed into the stomach by conventional technique. The capsule was promptly ejected from the catheter by slowly compressing the air in a 5 ml hypodermic syringe attached to the constricted end of the catheter. (2) WR-30,090 hydrochloride: A stock solution was prepared by an initial grinding of the requisite quantity of this salt with 0.1 ml Tween 80, followed by grinding to a homogeneous suspension with the required quantity of distilled water. The appropriate volume of this suspension was placed in a 50 ml Erlenmeyer flask and diluted to 10.0 ml with water. This volume was introduced into the stomach through a No. 14 French urethral catheter, fitted with a 10.0 ml glass hypodermic syringe, and followed by rinsing of flask and syringe with 3 to 5 ml distilled water.

These perplexing and unexpected results suggested that there might be major differences in the absorption of WR-30,090 base and hydrochloride salt in different animal species; either that or the blood levels of "WR-30,090" were unrelated to antimalarial activity. Certainly, if plasma and blood levels were similar in owl monkey and dog, it would have to be concluded that what was being measured as "WR-30,090" was probably a mixture of derivatives and unchanged drug, a large component of which was devoid of antimalarial activity. In order to assist in resolving these issues, a comparison was undertaken of the levels of "WR-30,090" in blood following oral administration of equivalent doses of the base and hydrochloride salt to non-infected owl monkeys. A companion rhesus monkey component was added to this experiment to assist in appraising species variability.

Ten owl monkeys and eight rhesus monkeys were assigned to what was to have been a two-armed crossover experiment. In one arm, five owl monkeys and four rhesus monkeys were to receive a single 20.0 mg per kg dose of WR-30,090 base in oleic acid; the remaining five owl monkeys and four rhesus monkeys were to receive a single 20.0 mg per kg dose of WR-30,090 hydrochloride in water*. Blood samples were to be obtained just prior to dosage and 2, 4, and 6 hours thereafter. These samples were to be refrigerated on collection and shipped in wet ice the next morning to INTER$_X$ Research Corporation. Two weeks later the procedure was to be repeated with reversal of the assignment of the monkeys to the two preparations.

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*The procedure for administering the oleic acid solution of WR-30,090 base and suspension of WR-30,090 hydrochloride to the owl monkey was identical with that already described. Except for size of capsule (No. 0) and urethral catheter (No. 20 Fr), and volume of suspension, the same method was used for administering these agents to the rhesus monkey.

Technically, the delivery of drug, collection of blood, and shipment to $INTER_X$ went off without incident. To the bewilderment of all, no more than a trace of WR-30,090 could be found in any blood sample. Because of this, it was decided to postpone the second portion of the planned study, pending resolution of what appeared to be a serious analytical problem. To this time, the problem has not been resolved.

TABLE 17

THE COMPARATIVE ACTIVITIES OF WR-30,090 BASE (IN OLEIC ACID) AND WR-30,090 HYDROCHLORIDE (IN WATER) AGAINST ESTABLISHED INFECTIONS 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
WR-30,090 Base - In Oleic Acid											
7884	1.25	44	105	170	80	236	90	505	330	Dose increased		
7885	1.25	40	132	70	64	36	16	35	28	Dose increased		
7896	1.25	30	52	204	142	312	180	250	114	Dose increased		
7897	2.5	30	68	135	196	344	565	970	690	Dose increased		
7898	2.5	15	34	98	72	102	45	56	20	Dose increased		
7899	2.5	30	64	430	412	1960	2960	5640	4200	Died		
7907	5.0	28	60	174	184	424	160	300	390	Dose increased		
7909	5.0	6	40	16	7	4	20	24	24	Dose increased		
7910	5.0	24	18	21	1	<1	<1	<1	<1	<1	-	-
7915	10.0	28	44	56	76	41	28	34	24	Dose increased		
7916	10.0	6	23	4	<1	<1	<1	<1	<1	-	-	-
7922	10.0	18	54	144	78	28	20	10	12	48	46	266
7884r	20.0	330	180	60	5	1	<1	<1	-	-	-	
7896r	20.0	114	62	22	32	7	6	6	6	2	<1	<1
7898r	20.0	20	18	2	1	<1	<1	-	-	-	-	
7909r	20.0	24	28	4	1	<1	<1	<1	<1	<1	3	2
7885r	40.0	28	94	36	110	9	3	<1	<1	<1	<1	<1
7897r	40.0	690	480	400	132	20	2	<1	<1	<1	-	-
7907r	40.0	390	840	930	1550	1240	Regimen changed					
7915r	40.0	24	40	40	16	9	10	16	14	14	33	
7922r	40.0	266	220	623	324	1090	770	Regimen changed				

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TABLE 17 - 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-30,090 HCl - In Water												
7900	5.0	10	17	1	<1	<1	<1	<1	-	-	-	
7901	5.0	51	146	551	605	206	40	57	42	Dose increased		
7902	5.0	13	57	75	132	144	63	125	87	Dose increased		
7904	10.0	8	34	36	78	100	35	50	6	Dose increased		
7917	10.0	34	59	22	3	<1	<1	<1	<1	-	-	-
7923	10.0	10	64	129	31	10	5	3	<1	<1	-	-
7909rr	10.0	2	<1	<1	-	-	-	-	-	-	-	
7915rr	10.0	33	10	3	<1	<1	-	-	-	-	-	-
7755	20.0	8	20	2	<1	<1	<1	<1	-	-	-	
7756	20.0	4	4	1	<1	<1	<1	-	-	-	-	
7758	20.0	4	2	1	<1	-	-	-	-	-	-	
7900r	20.0	4	5	1	<1	<1	<1	-	-	-	-	
7901r	20.0	42	57	36	21	2	<1	<1	-	-	-	
7902r	20.0	87	30	20	4	<1	<1	-	-	-	-	
7904r	20.0	6	2	1	<1	<1	<1	-	-	-	-	
7910r	20.0	16	18	3	<1	<1	<1	-	-	-	-	
7916r	20.0	2	5	3	<1	<1	<1	-	-	-	-	
7923r	20.0	20	94	24	3	<1	<1	-	-	-	-	
7884rr	20.0	4	<1	<1	<1	-	-	-	-	-	-	
7885rr	20.0	<1	<1	<1	-	-	-	-	-	-	-	
7897rr	20.0	<1	<1	<1	<1	<1	-	-	-	-	-	-
7898rr	20.0	1	<1	<1	<1	-	-	-	-	-	-	
7922rr	20.0	770	430	90	20	10	3	<1	<1	<1	<1	<1
7759	40.0	8	11	2	<1	-	-	-	-	-	-	
7886	40.0	10	16	2	<1	-	-	-	-	-	-	
7887	40.0	22	10	6	<1	-	-	-	-	-	-	
7901rr	40.0	9	10	3	<1	<1	<1	<1	-	-	-	-
7907rr	40.0	1240	596	210	19	<1	<1	<1	<1	<1	-	-
7916rr	40.0	20	8	2	<1	<1	-	-	-	-	-	
7884rrr	40.0	90	142	12	1	<1	<1	<1	-	-	-	
7901rrr	80.0	22	23	4	<1	<1	<1	-	-	-	-	-

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TABLE 18

THE COMPARATIVE ACTIVITIES OF WR-30,090 BASE (IN OLEIC ACID) AND WR-30,090 HYDROCHLORIDE (IN WATER) AGAINST ESTABLISHED INFECTIONS 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 Recru-descence	Remarks
		None	Suppressed	Cleared			
WR-30,090 Base - In Oleic Acid							
7884	1.25		+		n.a.	n.a.	Dose increased
7885	1.25		+		n.a.	n.a.	Dose increased
7896	1.25		+		n.a.	n.a.	Dose increased
7897	2.5		±		n.a.	n.a.	Dose increased
7898	2.5		+		n.a.	n.a.	Dose increased
7899	2.5	+			n.a.	n.a.	Died - Malaria
7907	5.0		+		n.a.	n.a.	Dose increased
7909	5.0		+		n.a.	n.a.	Dose increased
7910	5.0			+	9	10	
7915	10.0		+		n.a.	n.a.	Dose increased
7916	10.0			+	8	12	
7922	10.0		+		n.a.	n.a.	
7884r	20.0			+	7	36	
7896r	20.0			+	12	n.a.	Cured
7898r	20.0			+	6	7	
7909r	20.0		+		n.a.	n.a.	
7885r	40.0		+		n.a.	n.a.	
7897r	40.0			+	9	30	
7907r	40.0		±		n.a.	n.a.	Regimen changed
7915r	40.0		+		n.a.	n.a.	
7922r	40.0	+			n.a.	n.a.	Regimen changed

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TABLE 18 - 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-30,090 HCl - In Water							
7900	5.0			+	7	10	
7901	5.0		+		n.a.	n.a.	Dose increased
7902	5.0		+		n.a.	n.a.	Dose increased
7904	10.0		+		n.a.	n.a.	Dose increased
7917	10.0			+	8	n.a.	Cured
7923	10.0			+	9	10	
7909rr	10.0			+	3	n.a.	Cured
7915rr	10.0			+	5	n.a.	Cured
7755	20.0			+	7	n.a.	Cured
7756	20.0			+	6	n.a.	Cured
7758	20.0			+	4	n.a.	Cured
7900r	20.0			+	6	n.a.	Cured
7901r	20.0			+	7	14	
7902r	20.0			+	6	n.a.	Cured
7904r	20.0			+	6	n.a.	Cured
7910r	20.0			+	6	n.a.	Cured
7916r	20.0			+	6	10	
7923r	20.0			+	6	n.a.	Cured
7884rr	20.0			+	4	14	
7885rr	20.0			+	4	n.a.	Cured
7897rr	20.0			+	5	n.a.	Cured
7898rr	20.0			+	4	n.a.	Cured
7922rr	20.0			+	12	n.a.	Cured
7759	40.0			+	4	n.a.	Cured
7886	40.0			+	4	n.a.	Cured
7887	40.0			+	4	n.a.	Cured
7901rr	40.0			+	6	15	
7907rr	40.0			+	9	n.a.	Cured
7916rr	40.0			+	4	n.a.	Cured
7884rrr	40.0			+	7	n.a.	Cured
7901rrr	80.0			+	6	n.a.	Cured

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TABLE 19

SUMMARY OF THE COMPARATIVE ACTIVITIES OF WR-30,090 BASE AND WR-30,090 HYDROCHLORIDE AGAINST ESTABLISHED INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

WR-30,090	Daily Dose* Mg Base/Kg Body Weight	No. of Infections Treated					Days from Initial R_x to Parasite Clearance**
		Total	Response to Treatment				
			None	Suppressed	Cleared	Cured	
Base	1.25	3	-	3	-	0	n.a.
	2.5	3	1	2	-	0	n.a.
	5.0	3	-	2	1	0	n.a.
	10.0	3	-	2	1	0	n.a.
	20.0	4	-	1	3	1	n.a.
	40.0	5	1	3	1	0	n.a.
Hydro-chloride	5.0	3	-	2	1	0	n.a.
	10.0	5	-	1	4	3	>5
	20.0	15	-	-	15	12	6
	40.0	7	-	-	7	6	4

*Administered via stomach tube once daily for seven consecutive days.

**Median day to clearance of parasitemia.

VII. COMPARISON OF THE BLOOD SCHIZONTICIDAL ACTIVITIES OF PRIMAQUINE AND WR-181,023

VII. COMPARISON OF THE BLOOD SCHIZONTICIDAL ACTIVITIES OF PRIMAQUINE AND WR-181,023

As a class, the 8-aminoquinolines have activity against all forms of P. vivax, including the blood schizonts. Unfortunately, the latter parasite stages are less susceptible than the tissue schizonts to the action of agents such as primaquine, isopentaquine, and pentaquine. In fact, the dose of any one of these compounds required to eradicate the erythrocytic parasites of P. vivax is at least four times the dose required for radical cure. Since such large doses approach the maximum tolerated level, it is not possible to exploit the broad spectrum of activity of the 8-aminoquinolines. Interest in such a possibility, renewed with the development of WR-181,023, led to a direct comparison of the capacities of this agent and primaquine to cure established infections with trophozoites of the Vietnam Palo Alto strain of P. vivax, with the results set forth in Tables 20 to 23.

The observations summarized in Tables 21 and 23 show that the curative activity of WR-181,023 is clearly superior to that of primaquine. Daily doses of 2.5 mg WR-181,023 per kg appear to be curative; doses of 1.25 mg per kg effected temporary clearance of parasitemia. Doses of 2.5 mg primaquine were not curative. Overall, its activity fell between one-half and one-fourth that of its 4-methyl congener. Although the demonstrated superiority of WR-181,023 is encouraging, its blood schizonticidal activity is not great enough to have therapeutic applicability. Even with its improved activity, the dose of WR-181,023 required for control of the erythrocytic phase of P. vivax infections exceeds by almost a log the dose required for elimination of tissue schizonts and approaches a level which could provoke hepatotoxicity.

TABLE 20

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF PRIMAQUINE 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
7735	0.3125	12	8	8	15	12	16	10	18	Regimen changed		
7736	0.3125	18	32	70	180	122	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	
7768	2.5	4	6	2	<1	<1	<1	<1	-	-	-	
7769	2.5	4	4	1	<1	<1	<1	<1	-	-	-	

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TABLE 21

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF PRIMAQUINE 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 Recru-descence	Remarks
		None	Suppressed	Cleared			
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.	
7768	2.5			+	7	7	
7769	2.5			+	7	10	

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TABLE 22

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF 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
7770	0.156	36	160	133	140	123	130	180	60	Dose increased		
7779	0.156	14	16	34	39	46	69	72	150	Dose increased		
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	-	-	-	-
7770r	2.5	60	15	6	4	1	<1	<1	-	-	-	
7779r	2.5	150	48	4	1	<1	<1	<1	-	-	-	

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TABLE 23

A PRELIMINARY ASSESSMENT OF THE ACTIVITY OF 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 Recru-descence	Remarks
		None	Suppressed	Cleared			
7770	0.156	+			n.a.	n.a.	Dose increased
7779	0.156	+			n.a.	n.a.	Dose increased
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	20	
7902	1.25			+	7	7	
7770r	2.5			+	7	n.a.	Cured
7779r	2.5			+	7	n.a.	Cured

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VIII. STUDIES ON WR-184,806-AH

VIII. STUDIES ON WR-184,806-AH

WR-184,806-AH, the phosphate salt of α-(tert. butylaminoethyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol, was prepared in a batch quantity sufficient for evaluations in human volunteers, and for the preclinical investigations required for FDA approval of a new drug trial. One component of the latter investigations required subjecting this preparation to a series of studies aimed at quantifying its antimalarial activity and determining to what extent such activity was influenced by the dosage regimen and route of delivery. The results of these explorations were summarized on December 9, 1974 for inclusion in an IND application. This summary is reproduced in its entirety as part of this Annual Report.

SORI-KM-74-400

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

ON

WR-184,806-AH (BN: BD-99,078): 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
December 9, 1974

Project 2284-XXII

WR-184,806-AH (BN: BD-99,078): ITS ACTIVITIES AGAINST ESTABLISHED INFECTIONS WITH PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX IN THE OWL MONKEY (AOTUS TRIVIRGATUS)

INTRODUCTORY COMMENT

WR-184,806-AA* [α-(tert-butylaminoethyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol, hydrochloride] ranked high among the quinolinemethanols with respect to activity against infections with Plasmodium berghei in the mouse (Rane Model). The above compound also exhibited a high order of activity against infections with P. falciparum in the owl monkey (Aotus trivirgatus). In pilot evaluations in this latter model, the activities of WR-184,806-AA against infections with the pyrimethamine-resistant Malayan Camp-CH/Q strain, the chloroquine-resistant Vietnam Oak Knoll strain, and the multi-drug resistant (pyrimethamine, chloroquine, quinine) Vietnam Smith strain, far exceeded the activities of its earlier relative, WR-30,090**, and fell only slightly short of the activities of WR-142,490†, the most effective of the quinolinemethanols studied to date.

The comparative activities of these three quinolinemethanols, as exhibited in pilot evaluations in owl monkeys infected with the above strains, are set forth in Table 1. The results of these assessments, together with

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* WR-184,806-AA (BN: BB-52,137).

** WR-30,090 [α-[(di-N-butyl)-aminoethyl]-2-(3,4-dichlorophenyl)-6,8-dichloro-4-quinolinemethanol, hydrochloride] has exhibited significant activity against P. falciparum infections in human volunteers and in the field.

† WR-142,490 [α-(2-piperidyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol, hydrochloride] has exhibited striking activity against infections with P. falciparum in human volunteers.

the results of special toxicity studies pursued elsewhere, led to the decision to evaluate the tolerability and antimalarial properties of WR-184,806 in human volunteers. Prior to embarking on these investigations, attempts were made to acquire a salt of WR-184,806 with greater solubility and better shelf life than the hydrochloride. These efforts led to preparation of the phosphate salt which exhibited physical characteristics superior to those of the hydrochloride. This finding led to manufacture of a batch lot of WR-184,806 phosphate for ultimate use in human volunteers and in pilot field studies. New drug development regulations made it necessary to examine this lot for antimalarial activity and toxicity. This Report is concerned with the examination for antimalarial activity.

The investigations summarized below had multiple objectives: first and primarily, to assess the bioavailability of the preparation of the phosphate salt destined for clinical trials; secondly, to determine the influence of the dosage regimen on the activity of this salt; and thirdly, to examine the tolerability and efficacy of WR-184,806 phosphate delivered via the intravenous route. These evaluations were carried out in owl monkeys bearing infections with the Vietnam Oak Knoll and Vietnam Smith strains of P. falciparum and the Vietnam Palo Alto strain of P. vivax.

METHODS AND PROCEDURES

The data summarized in this Report were derived from the seven separate experiments listed below.

1. May 28, 1974 - Vietnam Oak Knoll strain, *P. falciparum*.

2. June 12, 1974 - Vietnam Oak Knoll strain, *P. falciparum*.

3. May 31, 1974 - Vietnam Smith strain, *P. falciparum*.

4. June 28, 1974 - Vietnam Smith strain, *P. falciparum*.

5. May 10, 1974 - Vietnam Palo Alto strain, *P. vivax*.

6. June 14, 1974 - Vietnam Palo Alto strain, *P. vivax*.

7. July 26, 1974 - Vietnam Palo Alto strain, *P. vivax*.

The same preparation of WR-184,806, designated WR-184,806-AH phosphate, BN: BD-99,078, was used in each of the above experiments. The base content of this salt was 80 per cent.

The following experimental procedures were common to all of the studies. Owl monkeys, imported directly from the Barranquilla area of Colombia, were used exclusively. These subjects were vaccinated against Herpes tamarinus and Herpes simplex and conditioned for a minimum sixty-day period, 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-184,806 against infections with either the Vietnam Oak Knoll or Vietnam Smith strains of P. falciparum. Monkeys previously infected with these or other strains of P. falciparum, and cured via application of appropriate drugs other than WR-184,806, were assigned to assessments of the activity of this quinolinemethanol against infections with the Vietnam Palo Alto strain of P. vivax*,**.

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* The responses of infections with the three 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 Oak Knoll strain are fully resistant to treatment with maximum tolerated doses of chloroquine and quinine, but are susceptible to treatment with pyrimethamine or proguanil. Infections with the Vietnam Smith strain are fully resistant to treatment with each of the above drugs. Infections with the Vietnam Palo Alto strain are susceptible to treatment with chloroquine or quinine, but are resistant to pyrimethamine or proguanil.

** The owl monkey closely resembles man in that previous or current infection with P. falciparum does not alter 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 is assured, to assign the same monkey to assessment of the activity of a different drug against infection with P. vivax. Such multiple use, which is a routine in our chemotherapeutic program, reduces operational costs and conserves numbers of monkeys that must be imported for these therapeutic assessments.

Groups of 26 to 42 monkeys were utilized in the various experiments. Infections were induced by the intravenous inoculation of 5×10^6 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-184,806 was started, 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-184,806, dissolved in sterile distilled water in a concentration of 10 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-184,806 was administered within one hour of solution preparation.

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* The passage lines of the Vietnam Oak Knoll and Vietnam Smith strains of P. falciparum are 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 the strains 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 parasitic response to treatment was 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 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 period.

If parasitemias persisted at the initial or even lower levels, or increased in intensity during delivery of WR-184,806, or if there was a reappearance of parasites after an apparent blood-negative interval, a second drug course was delivered, either at a higher dose via the same regimen or at the same 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 the emergence of drug resistant plasmodia, if such appear, and the rapidity with which this undesirable event occurs.

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-184,806 affected parasite growth and the type of activity which this agent possesses.

Every experiment included at least one untreated control monkey and one monkey treated with either chloroquine or pyrimethamine. Inclusion of these subjects made it possible to monitor the virulence and chemotherapeutic stability of the parasites in each inoculum and thereby guard against loss of virulence or change in response to "standard drugs".

RESULTS

The results of the seven experiments which form the basis of this Report have been summarized, monkey-by-monkey, in Tables 2 through 13. The activities of WR-184,806 administered via the oral route are set forth in Tables 2 through 7; activities achieved via intravenous delivery in Tables 8 through 13. In each set, the even numbered tables detail the effects of drug treatment on parasitemia; the odd numbered tables assess the ultimate response to drug delivery. No discussion of these data will be undertaken here. They are included for reviewer analysis and independent validation of conclusions drawn from overall summaries contained in Tables 14 and 15.

Table 14 sums up the activities of various dosage schedules of WR-184,806, administered orally, against infections with diverse strains of P. falciparum and P. vivax. The data show that the curative activity of this compound was a function of the total dose. For example, a single dose of 70.0 mg per kg achieved the same end result in monkeys infected with the Smith strain as three daily doses of 24.0 mg per kg or seven daily doses of 10.0 mg per kg. Measured by rapidity of control of parasitemia, single doses were more effective than divided doses in subjects infected with the Smith strain of P. falciparum and Palo Alto strain of P. vivax.

The data in Table 14 also show that there were strain and/or species variations in the therapeutic effectiveness of WR-184,806 phosphate, just as there were in the effectiveness of the hydrochloride salt (cf Table 1). Thus, the total doses that were uniformly curative were 17.5, 35.0, and 70.0 mg per kg, respectively, for infections with the Palo Alto strain of P. vivax and the Oak Knoll and Smith strains of P. falciparum. As related elsewhere in this Report, each of these strains had a different spectrum of response to chloroquine, quinine, and pyrimethamine. However, no particular spectrum seemed to be a determinant of the effectiveness of WR-184,806. The responsiveness of infections with the pyrimethamine-resistant Palo Alto strain and the chloroquine-resistant Oak Knoll strain indicates that the activity of WR-184,806, like that of its older relatives WR-30,090 and WR-142,490, was not compromised by either pyrimethamine or chloroquine resistance.

Table 15 sums up the results achieved when WR-184,806 was administered intravenously. Two regimens were compared; a single dose and three equal daily doses. The results show that a high proportion of cures, approaching 100 per cent, were achieved when total doses of 20.0 mg per kg were delivered to monkeys infected with either the Oak Knoll strain of P. falciparum or the Palo Alto strain of P. vivax. Although the data on these strains are limited, there is a suggestion that WR-184,806 delivered in a single dose of 20.0 mg per kg was more effective than three doses of 6.67 mg per kg delivered on consecutive days.

As shown in Table 15, WR-184,806 was substantially less effective against infections with the Smith strain of P. falciparum than it was against infections with the Oak Knoll strain. Thus, doses of 20.0 mg per kg effected cure of 11 of 15 infections with the Oak Knoll strain and but 3 of 12 infections with the Smith strain. Even doses of 30.0 mg per kg cured but 9 of 16 infections with the latter strain.

The therapeutic effectiveness of larger doses was not assessed because preliminary studies in discarded owl monkeys showed that rapid delivery of single doses of 40.0 mg per kg and greater produced severe convulsions which in some subjects terminated fatally. This acute toxic reaction to WR-184,806 did not occur, however, when doses as great as 80.0 mg per kg were infused intravenously over a 20 minute period. This latter observation could be significant in view of its relevance to the procedures usually employed in delivering drugs parenterally to man.

There is at present no documented explanation for the finding that WR-184,806 is less effective against infections with the Smith strain of P. falciparum than against infections with the Oak Knoll strain of this plasmodium or the Palo Alto strain of P. vivax. It is possible that the various parasitic stages of the Smith strain are inherently less susceptible to this quinolinemethanol than corresponding stages of the other strains. This possibility might be evaluated by assessing strain susceptibility in vitro. An alternate explanation for the lesser activity of WR-184,806 against infections with the Smith strain may be related to degree of sequestration of more mature parasitic stages in blood vessels outside of the peripheral circulation. This sequestration, also found in human infections with many strains of P. falciparum, is extremely marked in the early phases of infections with the Smith strain, but is not a prominent feature of infections with the Oak Knoll strain at the corresponding stage. Such sequestration may provide a form of pharmacologic sanctuary.

Although evaluation of the toxicity of WR-184,806 was not a part of this study, it is noteworthy that no immediate or delayed untoward effects, attributable to this quinolinemethanol, were encountered in any treated subject. The absence of toxic symptoms suggest that therapeutic benefits of WR-184,806 were attainable without significant cost in host toxicity. This compound probably has a very acceptable therapeutic index.

SUMMARY AND CONCLUSIONS

WR-184,806-AH (BN: BD-99,078), the phosphate salt of α-(tert-butylaminoethyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol, has been evaluated for activity in owl monkeys (Aotus trivirgatus) bearing established infections with the Vietnam Oak Knoll and Vietnam Smith strains of P. falciparum and the Vietnam Palo Alto strain of P. vivax. This evaluation has included comparisons of the effectiveness of various dosage regimens of WR-184,806-AH administered either orally or intravenously. Following are the more significant results of this investigation:

1. When administered orally, WR-184,806-AH regularly cured infections with the various strains at the following total doses (base equivalent): Oak Knoll - 35.0 mg/kg body weight; Smith - 70.0 mg/kg body weight; Palo Alto - 17.5 mg/kg body weight.

2. At uniformly curative levels (ca CD_{90}), single dose, three consecutive daily dose, and seven consecutive daily dose regimens of WR-184,806 were essentially equally effective. There was a suggestion, however, that the time required for parasite clearance was shorter with single doses than when the same total dose was delivered in equal fractions on seven consecutive days.

3. When administered intravenously, WR-184,806 regularly cured infections with the various strains at the following total doses (base equivalent): Oak Knoll - 20.0 mg/kg body weight; Smith - ca 30.0 mg/kg body weight; Palo Alto - >10.0 <20.0 mg/kg body weight.

4. On a dose-for-dose comparison, WR-184,806-AH appeared to be slightly more effective when administered intravenously than when delivered orally.

Comparison of the above results with those obtained in earlier pilot studies indicates that the phosphate salt of WR-184,806 is at least as effective as the hydrochloride salt (WR-184,806-AA,BN: BB-52,137) and possibly slightly superior.

Comparison of the data acquired in the current study with those of even more extensive studies on WR-142,490 [α-(2-piperidyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol, hydrochloride] indicates that the activities of WR-184,806-AH are only slightly inferior to those attained with WR-142,490, the most effective of the quinolinemethanols evaluated to date and currently under study 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
Associate Director, Chemotherapy Research

APPROVED:

F. M. Schabel, Jr.
Director, Chemotherapy Research

Kettering-Meyer Laboratory
Southern Research Institute
Birmingham, Alabama 35205
December 13, 1974

Project 2284-XXII

TABLE 1

PILOT ASSESSMENTS OF THE ACTIVITIES OF WR-30,090, WR-142,490, AND WR-184,806 AGAINST INFECTIONS WITH THE MALAYAN CAMP-CH/Q, VIETNAM OAK KNOLL, AND VIETNAM SMITH STRAINS OF P. FALCIPARUM

Compound WR- No.	Daily Dose - Mg Base*/Kg Body Weight Required for Cure of Established Infections		
	Malayan Camp-CH/Q	Vietnam Oak Knoll	Vietnam Smith
30,090	ca 40.0	10.0	ca 40.0
142,490	3.125	3.125	5.0
184,806	5.0 → 10.0†	5.0 → 10.0†	10.0

* Each of the test compounds was administered as the hydrochloride salt - WR-30,090 as a suspension in 0.5% aqueous Tween 80, WR-142,490 and WR-184,806 as aqueous solutions.

† The accuracy of these assessments was compromised by early post-treatment deaths - probably due to an intercurrent infection of unknown etiology.

TABLE 2

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED ORALLY AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

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
7739	17.5	1	17.5	108	72	12	<1	<1	-	-	-	-		
7740	17.5	1	17.5	86	48	1	<1	<1	-	-	-	-		
7754rr	17.5	1	17.5	40	168	78	8	2	<1	<1	<1	<1	<1	<1
7741	35.0	1	35.0	152	69	2	<1	<1	-	-	-	-		
7742	35.0	1	35.0	114	38	14	<1	<1	-	-	-	-		
7747	70.0	1	70.0	56	9	1	<1	<1	-	-	-	-		
7748	70.0	1	70.0	110	42	6	<1	<1	-	-	-	-		
7749	6.0	3	18.0	192	56	12	<1	-	-	-	-	-		
7751	6.0	3	18.0	121	56	3	<1	-	-	-	-	-		
7754r	6.0	3	18.0	29	57	44	18	2	<1	<1	<1	<1	<1	-
7752	12.0	3	36.0	124	21	2	<1	-	-	-	-	-		
7753	12.0	3	36.0	144	58	4	<1	-	-	-	-	-		

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TABLE 2 - 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 of Treatment							Day Post-treatment		
					1	2	3	4	5	6	7	1	2	3
7843	1.25	7	8.75	21	132	62	7	<1	<1	-	-	-	-	
7844	1.25	7	8.75	4	17	13	6	<1	-	-	-	-	-	
7754	2.5	7	17.5	68	70	18	6	2	<1	-	-	-		
7760	2.5	7	17.5	54	98	44	18	2	<1	-	-	-		
7845	2.5	7	17.5	35	80	12	2	<1	<1	-	-	-	-	
7846	2.5	7	17.5	36	82	6	4	<1	<1	-	-	-	-	
7843r	2.5	7	17.5	54	76	32	10	1	<1	<1	<1	-	-	-
7857rrr	2.5	7	17.5	16	7	3	<1	<1	<1	<1	-	-	-	-
7876rrr	2.5	7	17.5	<1	<1	<1	<1	<1	<1	-	-	-	-	-
7877rrr	2.5	7	17.5	1	1	<1	<1	-	-	-	-	-	-	-
7761	5.0	7	35.0	142	180	9	<1	<1	-	-	-	-		
7762	5.0	7	35.0	164	108	33	1	<1	-	-	-	-		
7843rr	5.0	7	35.0	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
7754rrr	5.0	7	35.0	<1	-	-	-	-	-	-	-	-		
7877rrrr	5.0	7	35.0	3	5	<1	<1	-	-	-	-	-	-	-

SOUTHERN RESEARCH INSTITUTE

AD-A044 604 SOUTHERN RESEARCH INST BIRMINGHAM ALA KETTERING-MEY--ETC F/G 6/15
THE USE OF AOTUS TRIVIRGATUS AND MACACA MULATTA AS TOOLS FOR ST--ETC(U)
JUN 76 L H SCHMIDT DADA17-69-C-9104
UNCLASSIFIED SORI-KM-76-319 NL

2 OF 3
ADA044604

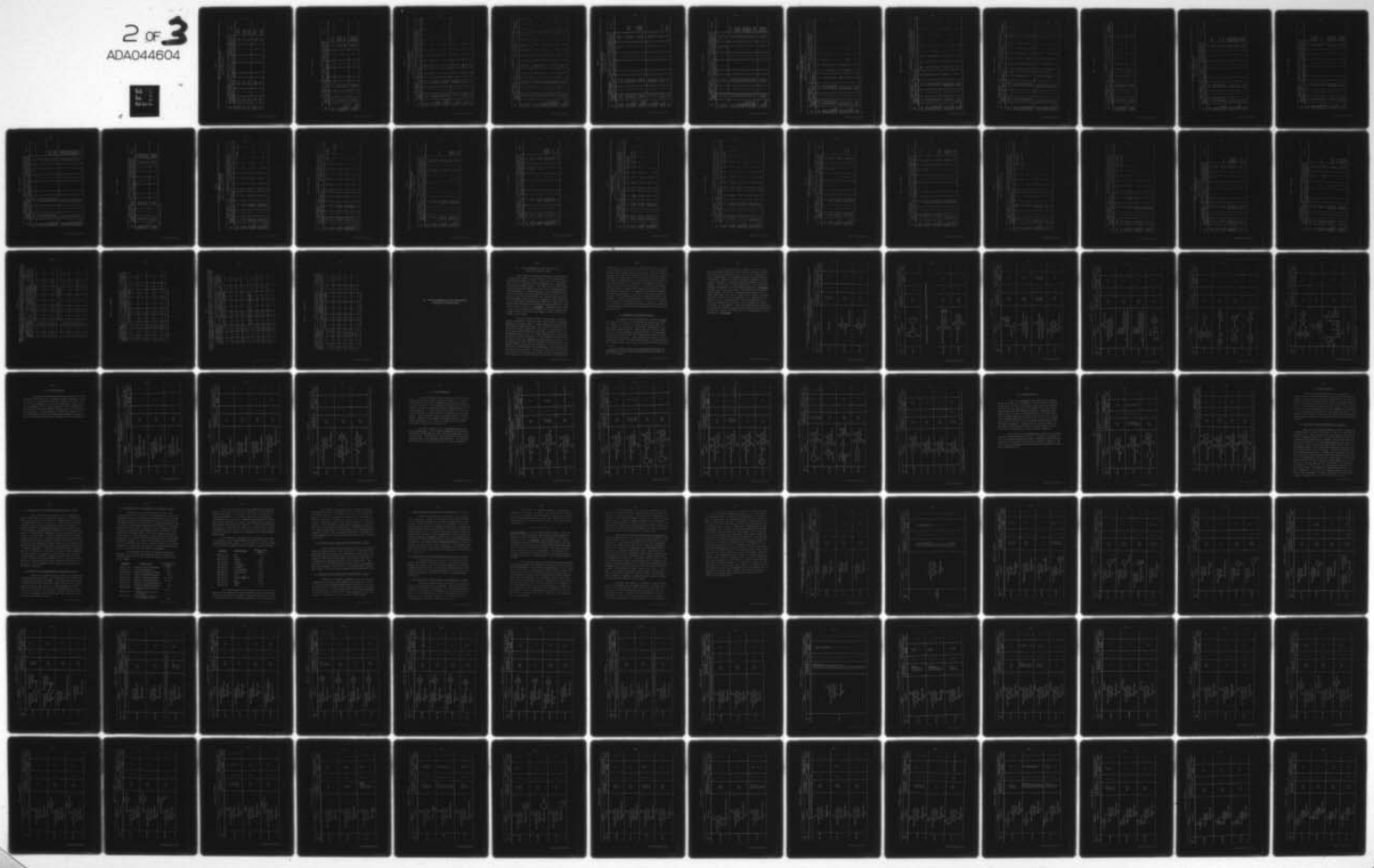

TABLE 3

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED ORALLY AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

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			
7739	17.5	1	17.5			+	5	n.a.	Cured
7740	17.5	1	17.5			+	5	n.a.	Cured
7754rr	17.5	1	17.5			+	13	19	
7741	35.0	1	35.0			+	5	n.a.	Cured
7742	35.0	1	35.0			+	5	n.a.	Cured
7747	70.0	1	70.0			+	5	n.a.	Cured
7748	70.0	1	70.0			+	5	n.a.	Cured
7749	6.0	3	18.0			+	4	n.a.	Cured
7751	6.0	3	18.0			+	4	n.a.	Cured
7754r	6.0	3	18.0			+	10	18	
7752	12.0	3	36.0			+	4	n.a.	Cured
7753	12.0	3	36.0			+	4	n.a.	Cured

SOUTHERN RESEARCH INSTITUTE

TABLE 3 - 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 Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7843	1.25	7	8.75			+	6	12	
7844	1.25	7	8.75			+	5	n.a.	Cured
7754	2.5	7	17.5			+	6	10	
7760	2.5	7	17.5			+	6	n.a.	Cured
7845	2.5	7	17.5			+	6	n.a.	Cured
7846	2.5	7	17.5			+	6	n.a.	Cured
7843r	2.5	7	17.5			+	8	22	
7857rrr	2.5	7	17.5			+	7	n.a.	Cured
7876rrr	2.5	7	17.5			+	6	n.a.	Cured
7877rrr	2.5	7	17.5			+	5	10	
7761	5.0	7	35.0			+	5	n.a.	Cured
7762	5.0	7	35.0			+	5	n.a.	Cured
7843rr	5.0	7	35.0			+	12	n.a.	Cured
7754rrr	5.0	7	35.0			+	3	n.a.	Cured
7877rrrr	5.0	7	35.0			+	4	n.a.	Cured

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TABLE 4

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED ORALLY AGAINST INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

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
7735	17.5	1	17.5	35	35	3	1	<1	<1	<1	<1	<1	<1	<1
7736	17.5	1	17.5	26	39	13	1	<1	<1	<1	<1	<1	7	
7745r	17.5	1	17.5	42	30	<1	<1	-	-	-				-
7764r	17.5	1	17.5	12	10	6	<1	<1	-	-	-	-		-
7737	35.0	1	35.0	51	100	19	8	2	<1	-	-	-		
7738	35.0	1	35.0	99	90	20	4	<1	<1	-	-	-		
7765r	35.0	1	35.0	2	1	1	<1	-	-	-	-	-		
7766r	35.0	1	35.0	8	1	<1	-	-	-	-	-			
7745rr	35.0	1	35.0	3	<1	<1	<1	-	-	-				-
7769rr	35.0	1	35.0	2	<1	<1	<1	-	-	-				-
7770rr	35.0	1	35.0	3	6	1	<1	-	-	-	-			-
7743	70.0	1	70.0	66	48	36	6	<1	<1	-	-	-		
7744	70.0	1	70.0	16	52	6	1	<1	<1	-	-	-		
7735rrr	70.0	1	70.0	<1	<1	-	-	-	-	-	-	-	-	-
7736rrr	70.0	1	70.0	12	8	3	<1	<1	<1	-	-	-	-	-
7738rrr	70.0	1	70.0	<1	<1	1			-	-				-
7745	6.0	3	18.0	84	176	69	26	5	1	<1	<1	3	10	42
7764	6.0	3	18.0	36	224	24	10	1	<1	-	-	-	-	
7769r	6.0	3	18.0	4	6	2	<1	-	-	-			-	
7770r	6.0	3	18.0	11	4	2	<1	<1	-	-	-	-		-
7735rr	6.0	3	18.0	14	4	5	<1	<1	<1	-	-	-		
7736rr	6.0	3	18.0	1	<1	<1	-	-	-				-	
7765	12.0	3	36.0	54	124	30	8	5	<1	-	-	-		
7766	12.0	3	36.0	36	130	24	6	1	<1	-	-	-		
7775r	12.0	3	36.0	20	9	2	<1	<1	<1	-	-	-		
7738rr	12.0	3	36.0	116	273	230	4	1	<1	<1	-	-	-	-
7767	24.0	3	72.0	48	74	9	1	<1	<1	-	-	-		
7768	24.0	3	72.0	100	254	19	6	<1	-	-	-		-	

SOUTHERN RESEARCH INSTITUTE

TABLE 4 - 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 of Treatment							Day Post-treatment		
					1	2	3	4	5	6	7	1	2	3
7716	2.5	7	17.5	3	50	4	38	14	13	1	<1	<1	1	2
7717	2.5	7	17.5	14	102	36	234	63	134	29	25	5	7	15
7769	2.5	7	17.5	66	194	96	42	34	10	3	<1	<1	<1	<1
7770	2.5	7	17.5	69	444	162	72	20	10	1	<1	-	-	-
7735r	2.5	7	17.5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
7736r	2.5	7	17.5	18	39	16	26	10	2	<1	<1	<1	<1	<1
7718	5.0	7	35.0	4	70	28	24	1	<1	<1	-	-	-	
7746	5.0	7	35.0	14	130	17	4	<1	<1	-	-	-	-	
7775	5.0	7	35.0	30	238	36	21	4	<1	<1	-	-	-	
7776	5.0	7	35.0	51	428	46	18	6	<1	<1	-	-	-	
7716r	5.0	7	35.0	2	8	<1	<1	-	-	-	-	-	-	
7717r	5.0	7	35.0	15	10	2	<1	-	-	-	-	-	-	
7737r	5.0	7	35.0	1	<1	<1	<1	<1	-	-	-	-	-	
7738r	5.0	7	35.0	40	94	48	16	4	4	<1	<1	<1	-	-
7809r	5.0	7	35.0	4	30	4	2	<1	<1	-	-	-		
7764rr	5.0	7	35.0	7	4	10	<1	-	-	-	-	-	-	-
7745rrr	5.0	7	35.0	7	1	<1	-	-	-	-	-	-		
7750rrr	5.0	7	35.0	3	1	<1	<1	<1	<1	<1	-	-	-	-
7769rrr	5.0	7	35.0	6	<1	<1	<1	-	-	-	-	-	-	
7770rrr	5.0	7	35.0	<1	<1	<1	<1	-	-	-	-	-	-	-
7790rrr	5.0	7	35.0	1	<1	<1	<1	-	-	-	-	-	-	-
7800rrr	5.0	7	35.0	72	243	185	56	6	<1	<1	<1	-	-	-
7715rrrr	5.0	7	35.0	4	1	<1	<1	<1	-	-	-	-	-	-
7777	10.0	7	70.0	38	166	7	3	<1	<1	-	-	-	-	
7779	10.0	7	70.0	64	238	20	12	2	<1	-	-	-	-	
7746r	10.0	7	70.0	10	28	2	<1	<1	<1	<1	<1	<1	<1	-
7738rrrr	10.0	7	70.0	1	<1	<1	<1	<1	<1	-	-	-	-	-
7790rrrr	10.0	7	70.0	8	1	<1	<1	<1	-	-	-	-	-	-

SOUTHERN RESEARCH INSTITUTE

TABLE 5

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED ORALLY AGAINST INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

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			
7735	17.5	1	17.5		+		n. a.	n. a.	
7736	17.5	1	17.5		+		n. a.	n. a.	
7745r	17.5	1	17.5			+	4	17	
7764r	17.5	1	17.5			+	5	17	
7737	35.0	1	35.0			+	6	16	
7738	35.0	1	35.0			+	6	10	
7765r	35.0	1	35.0			+	4	n. a.	Cured
7766r	35.0	1	35.0			+	3	n. a.	Cured
7745rr	35.0	1	35.0			+	4	17	
7769rr	35.0	1	35.0			+	4	31	
7770rr	35.0	1	35.0			+	4	36	
7743	70.0	1	70.0			+	6	n. a.	Cured
7744	70.0	1	70.0			+	6	n. a.	Cured
7735rrr	70.0	1	70.0			+	2	n. a.	Cured
7736rrr	70.0	1	70.0			+	6	n. a.	Cured
7738rrr	70.0	1	70.0			+	2	32	
7745	6.0	3	18.0		+		n. a.	n. a.	
7764	6.0	3	18.0			+	6	11	
7769r	6.0	3	18.0			+	4	13	
7770r	6.0	3	18.0			+	5	11	
7735rr	6.0	3	18.0			+	6	32	
7736rr	6.0	3	18.0			+	4	24	
7765	12.0	3	36.0			+	6	20	
7766	12.0	3	36.0			+	6	20	
7775r	12.0	3	36.0			+	6	n. a.	Cured
7738rr	12.0	3	36.0			+	7	16	
7767	24.0	3	72.0			+	6	n. a.	Cured
7768	24.0	3	72.0			+	5	n. a.	Cured

SOUTHERN RESEARCH INSTITUTE

TABLE 5 - CONTINUED

Atr No.	Dosage Regimen			Response of Parasitemia to R_x			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			
7716	2.5	7	17.5		+		n.a.	n.a.	
7717	2.5	7	17.5		+		n.a.	n.a.	
7769	2.5	7	17.5		+		n.a.	n.a.	
7770	2.5	7	17.5			+	8	7	
7735r	2.5	7	17.5		+		n.a.	n.a.	
7736r	2.5	7	17.5		+		n.a.	n.a.	
7718	5.0	7	35.0			+	7	n.a.	Cured
7746	5.0	7	35.0			+	6	15	
7775	5.0	7	35.0			+	7	15	
7776	5.0	7	35.0			+	7	n.a.	Cured
7716r	5.0	7	35.0			+	5	n.a.	Cured
7717r	5.0	7	35.0			+	5	n.a.	Cured
7737r	5.0	7	35.0			+	5	n.a.	Cured
7738r	5.0	7	35.0			+	9	11	
7809r	5.0	7	35.0			+	6	n.a.	Cured
7764rr	5.0	7	35.0			+	5	n.a.	Cured
7745rrr	5.0	7	35.0			+	4	n.a.	Cured
7750rrr	5.0	7	35.0			+	7	n.a.	Cured
7769rrr	5.0	7	35.0			+	5	n.a.	Cured
7770rrr	5.0	7	35.0			+	4	n.a.	Cured
7790rrr	5.0	7	35.0			+	4	23	
7800rrr	5.0	7	35.0			+	8	n.a.	Cured
7715rrrr	5.0	7	35.0			+	5	n.a.	Cured
7777	10.0	7	70.0			+	6	n.a.	Cured
7779	10.0	7	70.0			+	6	n.a.	Cured
7746r	10.0	7	70.0			+	10	n.a.	Cured
7738rrrr	10.0	7	70.0			+	6	n.a.	Cured
7790rrrr	10.0	7	70.0			+	6	n.a.	Cured

SOUTHERN RESEARCH INSTITUTE

TABLE 6

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED ORALLY AGAINST INFECTIONS 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
7511	4.37	1	4.37	6	3	<1	-	-	-			-		
7514	4.37	1	4.37	4	6	<1	<1	<1	-	-	-			
7339	8.75	1	8.75	4	2	<1	<1	-	-			-		
7396	8.75	1	8.75	17	2	<1	<1	-	-			-		
7499	8.75	1	8.75	16	1	<1	<1	-	-			-		
7614	8.75	1	8.75	5	6	<1	-	-	-	-		-		
7616	8.75	1	8.75	21	23	1	<1	<1	-	-	-			
7511r	8.75	1	8.75	14	8	<1	-	-	-				-	
7514r	8.75	1	8.75	6	<1	<1	-	-			-			
7558r	8.75	1	8.75	9	<1	<1	-	-	-		-			
7579rr	8.75	1	8.75	<1	<1		-	-	-				-	
7601rr	8.75	1	8.75	3	<1	<1	-	-		-				-
7528	17.5	1	17.5	12	4	<1	-	-	-			-		
7540	17.5	1	17.5	19	2	<1	-	-	-			-		
7547	17.5	1	17.5	4	2	<1	-	-	-			-		
7617	17.5	1	17.5	3	4	<1	-	-	-	-		-		
7625	17.5	1	17.5	32	38	<1	-	-	-	-		-		
7614r	17.5	1	17.5	2	<1	<1	<1	-	-				-	
7616r	17.5	1	17.5	8	8	1	<1	<1	<1	-	-			
7511rr	17.5	1	17.5	1	<1	<1	-	-	-					-
7499rrr	17.5	1	17.5	<1	-	-	-	-				-		
7550	35.0	1	35.0	4	1	<1	<1	<1	-	-	-	-		
7553	35.0	1	35.0	3	<1	<1	-	-	-			-		
7554	35.0	1	35.0	10	<1	-	-	-				-		

SOUTHERN RESEARCH INSTITUTE

TABLE 6 - 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
7627	1.5	3	4.5	64	142	124	13	3	<1	<1	<1	<1	8	24
7629	1.5	3	4.5	26	80	40	<1	-	-	-		-		
7555	3.0	3	9.0	12	5	<1	<1	-	-			-		
7558	3.0	3	9.0	24	24	<1	-	-	-			-		
7559	3.0	3	9.0	16	16	<1	-	-	-			-		
7630	3.0	3	9.0	21	22	6	<1	-	-	-		-		
7641	3.0	3	9.0	19	8	1	<1	<1	<1	-	-			
7579r	3.0	3	9.0	2	7	<1	<1	-	-	-	-			
7601r	3.0	3	9.0	26	33	5	<1	<1	<1	-	-			-
7602r	3.0	3	9.0	3	6	<1	<1	<1	-	-	-	-		
7627r	3.0	3	9.0	44	15	3	<1	<1	-	-	-			
7629r	3.0	3	9.0	<1	<1	-	-	-	-		<1	2	2	
7499rr	3.0	3	9.0	1	3	<1	-	-	-			-		
7561	6.0	3	18.0	28	8	<1	-	-	-			-		
7571	6.0	3	18.0	8	1	<1	<1	-	-			-		
7572	6.0	3	18.0	20	18	<1	-	-	-			-		
7642	6.0	3	18.0	7	5	1	<1	-	-	-		<1	-	-
7654	6.0	3	18.0	5	5	<1	<1	-	-	-		-		
7627rr	6.0	3	18.0	2	1	<1	<1	-	-	-	-	-	-	
7629rr	6.0	3	18.0	2	13	2	<1	<1	<1	-	-	-	-	
7573	12.0	3	36.0	8	3	<1	-	-	-			-		
7574	12.0	3	36.0	32	8	<1	-	-	-			-		
7578	12.0	3	36.0	6	<1	-	-	-	-			-		
7629rrr	12.0	3	36.0	2	1	<1	<1	<1	<1	-	-	-		

SOUTHERN RESEARCH INSTITUTE

TABLE 6 - 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 of Treatment							Day Post-treatment		
					1	2	3	4	5	6	7	1	2	3
7655	0.625	7	4.375	14	9	17	12	11	6	2	2	<1	2	7
7656	0.625	7	4.375	5	3	5	2	2	4	3	4	2	2	1
7579	1.25	7	8.75	6	7	4	<1	<1	-	-	-	-	-	-
7601	1.25	7	8.75	26	27	21	6	<1	-	-	-	-	-	-
7602	1.25	7	8.75	22	16	7	<1	<1	-	-	-	-	-	-
7673	1.25	7	8.75	14	9	23	12	2	<1	<1	-	-	-	-
7687	1.25	7	8.75	16	10	11	<1	1	<1	<1	-	-	-	-
6076r	1.25	7	8.75	16	10	20	30	10	22	7	5	1	<1	<1
7196r	1.25	7	8.75	<1	5	7	<1	<1	-	-	-	-	-	-
7367r	1.25	7	8.75	60	65	36		<1	-	-	-	-		
7383r	1.25	7	8.75	7	4	12	2	7	<1	<1	-	-		
7432r	1.25	7	8.75	70	36	7	<1	-	-	-	-	-		
7499r	1.25	7	8.75	2	10	20	10	4	3	<1	<1	<1	-	-
7655r	1.25	7	8.75	4	1	1	<1	-	-	-	-	-		
7656r	1.25	7	8.75	4	2	<1	<1	<1	<1	-	-	-		
7558rr	1.25	7	8.75	2	4	10	1	<1	<1	-	-	-	-	-
7603	2.5	7	17.5	3	8	6	<1	<1	-	-	-	-	-	-
7608	2.5	7	17.5	24	8	2	<1	<1	-	-	-	-	-	-
7609	2.5	7	17.5	4	7	1	<1	<1	-	-	-	-	-	-
7688	2.5	7	17.5	9	11	1	<1	<1	<1	-	-	-	-	-
7689	2.5	7	17.5	12	3	<1	<1	-	-	-	-	-		
6989r	2.5	7	17.5	6	4	<1	-	-	-	-	-	-		
7251r	2.5	7	17.5	5	1	<1	<1		-	-	-	-	-	-
7372r	2.5	7	17.5	22	4	<1	<1	-	-	-	-	-		Died
7393r	2.5	7	17.5	118	126	36	6	<1	-	-	-	-		
7433r	2.5	7	17.5	204	210	70	16	<1	<1	-	-	-		
7673r	2.5	7	17.5	1	2	7	2	<1	<1	-	-	-	-	
7687r	2.5	7	17.5	3	4	4	<1	<1	<1	<1	-	-	-	
6076rr	2.5	7	17.5	4	9	5	1	<1	-	-	-	-	-	
7367rr	2.5	7	17.5	4	2	<1	<1	<1	<1	-	-	-	-	
7601rrr	2.5	7	17.5	<1	-	-	-	<1	<1	<1	<1	<1	<1	<1

SOUTHERN RESEARCH INSTITUTE

TABLE 6 - 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 of Treatment							Day Post-treatment		
					1	2	3	4	5	6	7	1	2	3
7690	5.0	7	35.0	5	5	<1	<1	-	-	-	-	-		
7699	5.0	7	35.0	2	2	<1	<1	<1	-	-	-	-		
7170r	5.0	7	35.0	56	22	4	<1	-	-	-	-	-		
7307r	5.0	7	35.0	<1	<1	<1	-	-	-	-	-	-	-	-
7374r	5.0	7	35.0	4	2	<1	-	-	-	-	-	-		
7394r	5.0	7	35.0	34	14	<1	<1	-	-	-	-	-		
7455r	5.0	7	35.0	24	6	<1	<1	<1	<1	-	-	-		
7367rrr	5.0	7	35.0	<1	<1	-	-	-	-	-	-	-	-	
7311r	10.0	7	70.0	40	12	1	<1	-	-	-	-	-		
7375r	10.0	7	70.0	6	<1	-	-	-	-	-	-	-		
7395r	10.0	7	70.0	7	3	<1	<1	<1	-	-	-	-		
7498r	10.0	7	70.0	60	4	<1	-	-	-	-	-	-		

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TABLE 7

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED ORALLY AGAINST INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

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			
7511	4.37	1	4.37			+	3	9	
7514	4.37	1	4.37			+	6	12	
7339	8.75	1	8.75			+	4	n. a.	Cured
7396	8.75	1	8.75			+	4	n. a.	Cured
7499	8.75	1	8.75			+	4	18	
7614	8.75	1	8.75			+	3	16	
7616	8.75	1	8.75			+	5	13	
7511r	8.75	1	8.75			+	3	21	
7514r	8.75	1	8.75			+	4	n. a.	Cured
7558r	8.75	1	8.75			+	3	18	
7579rr	8.75	1	8.75			+	3	n. a.	Cured
7601rr	8.75	1	8.75			+	3	16	
7528	17.5	1	17.5			+	3	n. a.	Cured
7540	17.5	1	17.5			+	3	n. a.	Cured
7547	17.5	1	17.5			+	3	n. a.	Cured
7617	17.5	1	17.5			+	3	n. a.	Cured
7625	17.5	1	17.5			+	3	n. a.	Cured
7614r	17.5	1	17.5			+	4	n. a.	Cured
7616r	17.5	1	17.5			+	6	n. a.	Cured
7511rr	17.5	1	17.5			+	4	n. a.	Cured
7499rrr	17.5	1	17.5			+	2	n. a.	Cured
7550	35.0	1	35.0			+	5	n. a.	Cured
7553	35.0	1	35.0			+	3	n. a.	Cured
7554	35.0	1	35.0			+	2	n. a.	Cured

SOUTHERN RESEARCH INSTITUTE

TABLE 7 - 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			
7627	1.5	3	4.5		±		n.a.	n.a.	
7629	1.5	3	4.5			+	4	10	
7555	3.0	3	9.0			+	4	n.a.	Cured
7558	3.0	3	9.0			+	3	18	
7559	3.0	3	9.0			+	3	n.a.	Cured
7630	3.0	3	9.0			+	4	n.a.	Cured
7641	3.0	3	9.0			+	6	n.a.	Cured
7579r	3.0	3	9.0			+	4	32	
7601r	3.0	3	9.0			+	6	14	
7602r	3.0	3	9.0			+	5	n.a.	Cured
7627r	3.0	3	9.0			+	5	21	
7629r	3.0	3	9.0			+	3	5	
7499rr	3.0	3	9.0			+	3	19	
7561	6.0	3	18.0			+	3	n.a.	Cured
7571	6.0	3	18.0			+	4	n.a.	Cured
7572	6.0	3	18.0			+	3	n.a.	Cured
7642	6.0	3	18.0			+	4	n.a.	Cured
7654	6.0	3	18.0			+	4	n.a.	Cured
7627rr	6.0	3	18.0			+	4	n.a.	Cured
7629rr	6.0	3	18.0			+	6	10	
7573	12.0	3	36.0			+	3	n.a.	Cured
7574	12.0	3	36.0			+	3	n.a.	Cured
7578	12.0	3	36.0			+	2	n.a.	Cured
7629rrr	12.0	3	36.0			+	6	n.a.	Cured

SOUTHERN RESEARCH INSTITUTE

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 Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7655	0.625	7	4.375		+		n.a.	n.a.	
7656	0.625	7	4.375		+		n.a.	n.a.	
7579	1.25	7	8.75			+	5	19	
7601	1.25	7	8.75			+	5	9	
7602	1.25	7	8.75			+	5	10	
7673	1.25	7	8.75			+	7	12	
7687	1.25	7	8.75			+	7	11	
6076r	1.25	7	8.75		+		n.a.	n.a.	
7196r	1.25	7	8.75			+	5	n.a.	Died Day 9 Post Rx*
7367r	1.25	7	8.75			+	5	22	
7383r	1.25	7	8.75			+	7	n.a.	Cured
7432r	1.25	7	8.75			+	4	n.a.	Cured
7499r	1.25	7	8.75			+	9	12	
7655r	1.25	7	8.75			+	5	n.a.	Cured
7656r	1.25	7	8.75			+	6	n.a.	Cured
7558rr	1.25	7	8.75			+	6	n.a.	Cured
7603	2.5	7	17.5			+	5	n.a.	Cured
7608	2.5	7	17.5			+	5	n.a.	Cured
7609	2.5	7	17.5			+	5	n.a.	Cured
7688	2.5	7	17.5			+	6	n.a.	Cured
7689	2.5	7	17.5			+	4	n.a.	Cured
6989r	2.5	7	17.5			+	3	n.a.	Cured
7251r	2.5	7	17.5			+	5	n.a.	Cured
7372r	2.5	7	17.5			+	4	n.a.	Cured
7393r	2.5	7	17.5			+	5	n.a.	Cured
7433r	2.5	7	17.5			+	6	n.a.	Cured
7673r	2.5	7	17.5			+	6	n.a.	Cured
7687r	2.5	7	17.5			+	7	n.a.	Cured
6076rr	2.5	7	17.5			+	5	n.a.	Cured
7367rr	2.5	7	17.5			+	6	26	
7601rrr	2.5	7	17.5			+	11	n.a.	Cured

SOUTHERN RESEARCH INSTITUTE

TABLE 7 - 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			
7690	5.0	7	35.0			+	4	n.a.	Cured
7699	5.0	7	35.0			+	5	n.a.	Cured
7170r	5.0	7	35.0			+	4	n.a.	Cured
7307r	5.0	7	35.0			+	4	n.a.	Cured
7374r	5.0	7	35.0			+	3	n.a.	Cured
7394r	5.0	7	35.0			+	4	n.a.	Cured
7455r	5.0	7	35.0			+	6	n.a.	Cured
7367rrr	5.0	7	35.0			+	3	n.a.	Cured
7311r	10.0	7	70.0			+	4	n.a.	Cured
7375r	10.0	7	70.0			+	2	n.a.	Cured
7395r	10.0	7	70.0			+	5	n.a.	Cured
7498r	10.0	7	70.0			+	3	n.a.	Cured

* Death due to physical trauma.

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TABLE 8

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

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
7854	2.5	1	2.5	18	76	24	8	9	42	Dose increased				
7855	2.5	1	2.5	16	25	5	1	<1	<1	2	2	Dose increased		
7856	5.0	1	5.0	15	18	8	1	<1	-	-	-		-	
7857	5.0	1	5.0	25	74	5	<1	<1	<1	-	-	-	-	
7854r	5.0	1	5.0	42	42	4	<1	<1	<1	<1	<1	5		
7855r	5.0	1	5.0	2	6	<1	<1	-	-	-	-		<1	1
7872	10.0	1	10.0	40	70	8	<1	<1	-	-	-		-	
7873	10.0	1	10.0	36	64	4	<1	<1	-	-	-		-	
7856r	10.0	1	10.0	25	19	4	<1	<1	<1	<1	-	-	-	
7857r	10.0	1	10.0	15	7	1	<1	-	-	-	-			-
7854rr	10.0	1	10.0	5	1	<1	<1	<1	<1	<1	<1	4	14	
7855rr	10.0	1	10.0	30	14	4	<1	<1	<1	<1	<1	2		
7874	20.0	1	20.0	11	11	5	<1	<1	-	-	-		-	
7875	20.0	1	20.0	27	52	3	<1	<1	-	-	-		-	
7873r	20.0	1	20.0	51	84	20	<1	<1	-	-				-
7856rr	20.0	1	20.0	26	63	9	<1	<1	<1	<1	<1	<1	<1	<1
7857rr	20.0	1	20.0	2	5	6	4	13	12	7	14	16		
7854rrr	20.0	1	20.0	14	4	<1		-	<1			-	-	-
7855rrr	20.0	1	20.0	2	<1	<1	-	-	-	-	-	-	-	-

SOUTHERN RESEARCH INSTITUTE

TABLE 8 - 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
7876	0.83	3	2.5	78	144	78	41	12	11	76	Dose increased			
7877	0.83	3	2.5	53	114	268	104	22	12	66	Dose increased			
7878	1.67	3	5.0	23	62	50	10	2	<1	<1	<1	<1	<1	<1
7879	1.67	3	5.0	54	340	143	22	3	<1	<1	<1	<1	3	
7876r	1.67	3	5.0	76	74	26	4	1	<1	<1	6	Dose increased		
7877r	1.67	3	5.0	66	54	9	3	1	<1	<1	2	Dose increased		
7880	3.33	3	10.0	58	22	8	<1	<1	<1	<1	-	-	-	
7881	3.33	3	10.0	80	66	12	<1	<1	<1	<1	-	-	-	
7878r	3.33	3	10.0	19	24	4	<1	<1	<1	<1	<1	<1	<1	<1
7879r	3.33	3	10.0	3	2	<1	<1	<1	<1	<1	<1	<1	<1	<1
7882	6.67	3	20.0	34	34	4	<1	<1	<1	-	-	-	-	
7883	6.67	3	20.0	33	48	7	<1	<1	<1	-	-	-	-	
7880r	6.67	3	20.0	2	1	<1	<1	<1	<1	<1	<1	-		
7881r	6.67	3	20.0	8	24	3	<1	<1	-	-	-	-	-	
7876rr	6.67	3	20.0	6	2	<1	-	-	-	-	-			-
7877rr	6.67	3	20.0	2	1	<1	-	-	-	-	-			-
7878rr	6.67	3	20.0	12	101	32	<1	<1	<1	<1	-	-	-	-
7879rr	6.67	3	20.0	9	26	8	<1	<1	<1	-	-			
7879rrr	10.0	3	30.0	28	38	5	1	<1	<1	-	-	-	-	-

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TABLE 9

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL STRAIN OF PLASMODIUM FALCIPARUM

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			
7854	2.5	1	2.5		+		n.a.	n.a.	
7855	2.5	1	2.5		+		n.a.	n.a.	
7856	5.0	1	5.0		+		n.a.	n.a.	
7857	5.0	1	5.0		+		n.a.	n.a.	
7854r	5.0	1	5.0		+		n.a.	n.a.	
7855r	5.0	1	5.0		+		n.a.	n.a.	
7872	10.0	1	10.0			+	5	n.a.	Cured
7873	10.0	1	10.0			+	5	16	
7856r	10.0	1	10.0		+		n.a.	n.a.	
7857r	10.0	1	10.0			+	4	22	
7854rr	10.0	1	10.0		+		n.a.	n.a.	
7855rr	10.0	1	10.0		+		n.a.	n.a.	
7874	20.0	1	20.0			+	5	n.a.	Cured
7875	20.0	1	20.0			+	5	n.a.	Cured
7873r	20.0	1	20.0			+	5	n.a.	Cured
7856rr	20.0	1	20.0			+	12	n.a.	Cured
7857rr	20.0	1	20.0		+		n.a.	n.a.	
7854rrr	20.0	1	20.0			+	6	n.a.	Cured
7855rrr	20.0	1	20.0			+	3	n.a.	Cured

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TABLE 9 - 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			
7876	0.83	3	2.5		+		n.a.	n.a.	
7877	0.83	3	2.5		+		n.a.	n.a.	
7878	1.67	3	5.0		+		n.a.	n.a.	
7879	1.67	3	5.0		+		n.a.	n.a.	
7876r	1.67	3	5.0		+		n.a.	n.a.	
7877r	1.67	3	5.0		+		n.a.	n.a.	
7880	3.33	3	10.0			+	7	22	
7881	3.33	3	10.0			+	7	11	
7878r	3.33	3	10.0		+		n.a.	n.a.	
7879r	3.33	3	10.0		+		n.a.	n.a.	
7882	6.67	3	20.0			+	6	n.a.	Cured
7883	6.67	3	20.0			+	6	n.a.	Cured
7880r	6.67	3	20.0			+	8	n.a.	Cured
7881r	6.67	3	20.0			+	5	n.a.	Cured
7876rr	6.67	3	20.0			+	3	36	
7877rr	6.67	3	20.0			+	3	22	
7878rr	6.67	3	20.0			+	7	n.a.	Cured
7879rr	6.67	3	20.0			+	6	19	
7879rrr	10.0	3	30.0			+	6	n.a.	Cured

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TABLE 10

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

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
7715	5.0	1	5.0	5	44	21	62	309	Dose increased					
7750	5.0	1	5.0	23	126	42	111	192	Dose increased					
7789	10.0	1	10.0	23	60	13	16	3	16	Dose increased				
7790	10.0	1	10.0	13	172	23	7	<1	<1	<1	<1	<1	10	
7715r	10.0	1	10.0	309	48	36	20	14	10	Dose increased				
7750r	10.0	1	10.0	192	66	10	4	2	<1	<1	1	<1	<1	-
7791	20.0	1	20.0	12	48	6	1	<1	<1	<1	-	-	-	
7800	20.0	1	20.0	34	108	12	<1	<1	<1	<1	<1	<1	5	
7789r	20.0	1	20.0	16	9	1	<1	<1	<1	-	-	-		
7790r	20.0	1	20.0	10	14	6	<1	<1	-	-	-			
7715rr	20.0	1	20.0	10	11	<1	<1	-	-	-	-			
7801	30.0	1	30.0	26	50	6	<1	-	-	-	-		-	
7802	30.0	1	30.0	11	64	10	<1	-	-	-	-		-	
7791r	30.0	1	30.0	9	22	<1	<1	-	-	-		-		
7800r	30.0	1	30.0	5	7	<1	<1	<1	-	-	-			
7750rr	30.0	1	30.0	3	2	<1	<1	-	-	-	-		-	
7790rr	30.0	1	30.0	4	4	1	<1	<1	-	-	-	-		
7715rrr	30.0	1	30.0	68	100	60		<1	-	-	-			

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TABLE 10 - 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
7803	1.67	3	5.0	17	136	48	171	64	236	Dose increased				
7804	1.67	3	5.0	10	124	44	178	64	158	Dose increased				
7694rr	1.67	3	5.0	28	14	12	7	4	2	<1	<1	-	-	-
7805	3.33	3	10.0	14	54	6	3	<1	<1	<1	<1	<1	5	
7806	3.33	3	10.0	16	74	6	2	<1	<1	<1	8	5		
7803r	3.33	3	10.0	236	90	110	26	2	<1	<1	<1	<1	<1	4
7804r	3.33	3	10.0	158	94	56	16	3	1	1	<1	<1	6	14
7691rr	3.33	3	10.0	4	1	<1	<1	-	-	-	-			-
7807	6.67	3	20.0	7	30	16	2	<1	<1	<1	-	-	-	
7808	6.67	3	20.0	20	82	22	2	<1	<1	<1	-	-	-	
7805r	6.67	3	20.0	5	<1	<1	-	-	-		-			
7806r	6.67	3	20.0	5	5	<1	<1	-	-	-		-		
7692rr	6.67	3	20.0	3	1	<1	-	-	-	-			-	-
7803rr	6.67	3	20.0	5	2	<1	<1	<1	<1	<1	<1	-	-	-
7804rr	6.67	3	20.0	14	4	<1	<1	-	-	-				
7809	10.0	3	30.0	16	38	2	<1	-	-	-	-	-	-	
7817	10.0	3	30.0	37	101	32	3	<1	<1	-	-	-	-	
7802r	10.0	3	30.0	3	3	2	<1	<1	-	-	-	-	-	
7807r	10.0	3	30.0	4	<1	<1	-	-	-	-				-
7808r	10.0	3	30.0	28	16	6	<1	<1	<1	-	-	-	-	
7693rr	10.0	3	30.0	18	4	<1	<1	-	-	-				-
7800rr	10.0	3	30.0	1	<1	<1	-	-	-	-			<1	
7805rr	10.0	3	30.0	3	20	<1	<1	<1	-	-	-	-	-	
7806rr	10.0	3	30.0	48	20	21	2	<1	-	-	-			

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TABLE 11

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS WITH THE VIETNAM SMITH STRAIN OF PLASMODIUM FALCIPARUM

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			
7715	5.0	1	5.0		+		n.a.	n.a.	
7750	5.0	1	5.0		+		n.a.	n.a.	
7789	10.0	1	10.0		+		n.a.	n.a.	
7790	10.0	1	10.0		+		n.a.	n.a.	
7715r	10.0	1	10.0		+		n.a.	n.a.	
7750r	10.0	1	10.0		+		n.a.	n.a.	
7791	20.0	1	20.0			+	7	12	
7800	20.0	1	20.0		+		n.a.	n.a.	
7789r	20.0	1	20.0			+	6	12	
7790r	20.0	1	20.0			+	5	12	
7715rr	20.0	1	20.0			+	4	21	
7801	30.0	1	30.0			+	4	n.a.	Cured
7802	30.0	1	30.0			+	4	16	
7791r	30.0	1	30.0			+	4	n.a.	Cured
7800r	30.0	1	30.0			+	5	18	
7750rr	30.0	1	30.0			+	4	27	
7790rr	30.0	1	30.0			+	5	22	
7715rrr	30.0	1	30.0			+	5	30	

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TABLE 11 - 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 Recru-descence	Remarks
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7803	1.67	3	5.0		+		n.a.	n.a.	
7804	1.67	3	5.0		+		n.a.	n.a.	
7694rr	1.67	3	5.0			+	8	n.a.	Cured
7805	3.33	3	10.0		+		n.a.	n.a.	
7806	3.33	3	10.0		+		n.a.	n.a.	
7803r	3.33	3	10.0		+		n.a.	n.a.	
7804r	3.33	3	10.0		+		n.a.	n.a.	
7691rr	3.33	3	10.0			+	4	n.a	Cured
7807	6.67	3	20.0			+	7	12	
7808	6.67	3	20.0			+	7	11	
7805r	6.67	3	20.0			+	4	17	
7806r	6.67	3	20.0			+	4	20	
7692rr	6.67	3	20.0			+	3	n.a.	Cured
7803rr	6.67	3	20.0			+	8	n.a.	Cured
7804rr	6.67	3	20.0			+	4	n.a.	Cured
7809	10.0	3	30.0			+	4	19	
7817	10.0	3	30.0			+	7	n.a.	Cured
7802r	10.0	3	30.0			+	5	n.a.	Cured
7807r	10.0	3	30.0			+	4	n.a.	Cured
7808r	10.0	3	30.0			+	8	n.a.	Cured
7693rr	10.0	3	30.0			+	4	n.a.	Cured
7800rr	10.0	3	30.0			+	3	7	
7805rr	10.0	3	30.0			+	5	n.a.	Cured
7806rr	10.0	3	30.0			+	5	n.a.	Cured

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TABLE 12

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS 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
7427	1.25	1	1.25	12	18	36	47	44	12	Dose increased				
7456	1.25	1	1.25	2	4	4	2	2	1	Dose increased				
7515	2.5	1	2.5	2	<1	<1	<1	<1	<1	<1	<1	<1	1	3
7517	2.5	1	2.5	4	3	8	14	16	21	Dose increased				
7456r	2.5	1	2.5	1	<1	-	-	-	-	-				<1
7520	5.0	1	5.0	3	<1	<1	-	-	-			<1	1	2
7521	5.0	1	5.0	7	6	<1	<1	<1	-	-	-			
7427r	5.0	1	5.0	12	4	<1	-	-	-	-				-
7517r	5.0	1	5.0	21	4	<1	<1	-	-	-	-			-
7530	10.0	1	10.0	19	28	<1	-	-	-	-		-		
7541	10.0	1	10.0	8	5	7	<1	-	-	-		-		
7515r	10.0	1	10.0	10	3	<1	-	-				-		
7520r	10.0	1	10.0	2	1	<1	<1	-	-				-	
7521r	10.0	1	10.0	4	2	<1	-	-	-				-	
7456rr	10.0	1	10.0	<1	<1	<1	-	-	-	-	-			
7517rr	10.0	1	10.0	2	1	<1	<1	-	-	-	-		-	
7542	20.0	1	20.0	9	8	<1	<1	-	-	-		-		
7546	20.0	1	20.0	3	3	<1	<1	-	-	-		-		
7530r	20.0	1	20.0	>1	7	1	<1	<1	-	-	-	-		
7542r	30.0	1	30.0	1	<1	<1	-	-	-	-	-			

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TABLE 12 - 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
7551	0.415	3	1.25	5	10	54	38	40	15	Dose increased				
7560	0.415	3	1.25	25	82	172	60	175	195	Dose increased				
7562	0.83	3	2.5	6	14	53	66	112	144	Dose increased				
7568	0.83	3	2.5	9	14	35	20	48	52	Dose increased				
7569	1.67	3	5.0	8	16	50	5	<1	-	-	-	-		
7580	1.67	3	5.0	7	6	9	5	<1	-	-	-	-	-	-
7551r	1.67	3	5.0	15	12	2	<1	-	-	-	-			-
7560r	1.67	3	5.0	195	46	10	3	<1	<1	-	-			<1
7562r	1.67	3	5.0	144	96	18	2	<1	<1	-	-			<1
7568r	1.67	3	5.0	52	36	14	10	7	2	<1	<1	<1	2	
7583	3.33	3	10.0	16	16		<1	-	-	-		-		
7604	3.33	3	10.0	12	16	5	<1	-	-	-		-		
7580r	3.33	3	10.0	18	11	<1	<1	-	-	-	-	-		
7560rr	3.33	3	10.0	3	2	<1	<1	<1	-	-	-	-		
7562rr	3.33	3	10.0	2	4	<1	<1	<1	-	-	-	-		
7568rr	3.33	3	10.0	2	2	<1	<1	-	-			-		
7611	6.67	3	20.0	18	10	<1	<1	<1	-	-	-	-	-	-
7626	6.67	3	20.0	4	10	<1	-	-	-	-		-		
7569r	6.67	3	20.0	16	10	<1	<1	<1	-	-				-
7583r	6.67	3	20.0	7	4	<1	<1	-	-	-				-
7604r	6.67	3	20.0	2	<1	<1	<1	-	-				-	
7562rrr	6.67	3	20.0	40	4	<1	-	-	-	-				

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TABLE 13

THE ACTIVITY OF WR-184,806-AH, ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS WITH THE VIETNAM PALO ALTO STRAIN OF PLASMODIUM VIVAX

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			
7427	1.25	1	1.25	+			n.a.	n.a.	
7456	1.25	1	1.25		±		n.a.	n.a.	
7515	2.5	1	2.5		+		n.a.	n.a.	
7517	2.5	1	2.5		±		n.a.	n.a.	
7456r	2.5	1	2.5			+	3	10	
7520	5.0	1	5.0			+	3	8	
7521	5.0	1	5.0			+	5	15	
7427r	5.0	1	5.0			+	3	n.a.	Cured
7517r	5.0	1	5.0			+	4	19	
7530	10.0	1	10.0			+	3	25	
7541	10.0	1	10.0			+	4	n.a.	Cured
7515r	10.0	1	10.0			+	3	n.a.	Cured
7520r	10.0	1	10.0			+	4	n.a.	Cured
7521r	10.0	1	10.0			+	3	n.a.	Cured
7456rr	10.0	1	10.0			+	3	n.a.	Cured
7517rr	10.0	1	10.0			+	4	n.a.	Cured
7542	20.0	1	20.0			+	4	42	
7546	20.0	1	20.0			+	4	n.a.	Cured
7530r	20.0	1	20.0			+	5	n.a.	Cured
7542r	30.0	1	30.0			+	3	n.a.	Cured

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TABLE 13 - 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 Mg/Kg	No. of Doses	Total Dose Mg/Kg	None	Suppressed	Cleared			
7551	0.415	3	1.25	+			n.a.	n.a.	
7560	0.415	3	1.25	+			n.a.	n.a.	
7562	0.83	3	2.5	+			n.a.	n.a.	
7568	0.83	3	2.5	+			n.a.	n.a.	
7569	1.67	3	5.0			+	5	13	
7580	1.67	3	5.0			+	5	10	
7551r	1.67	3	5.0			+	4	n.a.	Cured
7560r	1.67	3	5.0		+		n.a.	n.a.	
7562r	1.67	3	5.0		+		n.a.	n.a.	
7568r	1.67	3	5.0		+		n.a.	n.a.	
7583	3.33	3	10.0			+	4	13	
7604	3.33	3	10.0			+	4	14	
7580r	3.33	3	10.0			+	4	n.a.	Cured
7560rr	3.33	3	10.0			+	5	n.a.	Cured
7562rr	3.33	3	10.0			+	5	18	
7568rr	3.33	3	10.0			+	4	n.a.	Cured
7611	6.67	3	20.0			+	5	n.a.	Cured
7626	6.67	3	20.0			+	3	n.a.	Cured
7569r	6.67	3	20.0			+	5	n.a.	Cured
7583r	6.67	3	20.0			+	4	n.a.	Cured
7604r	6.67	3	20.0			+	4	n.a.	Cured
7562rrr	6.67	3	20.0			+	3	n.a.	Cured

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TABLE 14

SUMMARY OF EVALUATIONS OF INFLUENCE OF DOSAGE REGIMEN ON THE ACTIVITIES OF WR-184,806-AH ADMINISTERED ORALLY AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL AND VIETNAM SMITH 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*
Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Oak Knoll Strain - P. falciparum								
17.5	1	17.5	3	0	0	3	2	8
6.0	3	18.0	3	0	0	3	2	6
2.5	7	17.5	8	0	0	8	5	6
35.0	1	35.0	2	0	0	2	2	5
12.0	3	36.0	2	0	0	2	2	4
5.0	7	35.0	5	0	0	5	5	6
70.0	1	70.0	2	0	0	2	2	5
Vietnam Smith Strain - P. falciparum								
17.5	1	17.5	4	0	2	2	0	n.a.
6.0	3	18.0	6	0	1	5	0	>6
2.5	7	17.5	6	0	5	1	0	n.a.
35.0	1	35.0	7	0	0	7	2	4
12.0	3	36.0	4	0	0	4	1	6
5.0	7	35.0	17	0	0	17	13	6
70.0	1	70.0	5	0	0	5	4	4
24.0	3	72.0	2	0	0	2	2	6
10.0	7	70.0	5	0	0	5	5	7

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TABLE 14 - CONTINUED

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 Palo Alto Strain - P. vivax								
4.37	1	4.37	2	0	0	2	0	5
1.5	3	4.5	2	0	1	1	0	n.a.
0.625	7	4.37	2	0	2	0	0	n.a.
8.75	1	8.75	10	0	0	10	4	4
3.0	3	9.0	11	0	0	11	5	4
1.25	7	8.75	14	0	1	13	5	6
17.5	1	17.5	9	0	0	9	9	3
6.0	3	18.0	7	0	0	7	6	4
2.5	7	17.5	15	0	0	15	14	6
35.0	1	35.0	3	0	0	3	3	3
12.0	3	36.0	4	0	0	4	4	4
5.0	7	35.0	8	0	0	8	8	4
10.0	7	70.0	4	0	0	4	4	4

* Reference here is the median day to parasite clearance.

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TABLE 15

SUMMARY OF EVALUATIONS OF INFLUENCE OF DOSAGE REGIMEN ON THE ACTIVITIES OF WR-184,806-AH ADMINISTERED INTRAVENOUSLY AGAINST INFECTIONS WITH THE VIETNAM OAK KNOLL AND VIETNAM SMITH 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*
Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg	Total	Response to Treatment				
				None	Suppressed	Cleared	Cured	
Vietnam Oak Knoll Strain - P. falciparum								
2.5	1	2.5	2	0	2	0	0	n.a.
0.83	3	2.5	2	0	2	0	0	n.a.
5.0	1	5.0	4	0	4	0	0	n.a.
1.67	3	5.0	4	0	4	0	0	n.a.
10.0	1	10.0	6	0	3	3	1	n.a.
3.33	3	10.0	4	0	2	2	0	n.a.
20.0	1	20.0	7	0	1	6	6	6
6.67	3	20.0	8	0	0	8	5	5
Vietnam Smith Strain - P. falciparum								
5.0	1	5.0	2	0	2	0	0	n.a.
1.67	3	5.0	3	0	2	1	1	n.a.
10.0	1	10.0	4	0	4	0	0	n.a.
3.33	3	10.0	5	0	4	1	1	n.a.
20.0	1	20.0	5	0	1	4	0	5
6.67	3	20.0	7	0	0	7	3	5
30.0	1	30.0	7	0	0	7	2	4
10.0	3	30.0	9	0	0	9	7	5

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TABLE 15 - CONTINUED

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 Palo Alto Strain - P. vivax								
1.25	1	1.25	2	1	1	0	0	n.a.
0.415	3	1.25	2	2	0	0	0	n.a.
2.5	1	2.5	3	0	2	1	0	n.a.
0.83	3	2.5	2	2	0	0	0	n.a.
5.0	1	5.0	4	0	0	4	1	4
1.67	3	5.0	6	0	3	3	1	n.a.
10.0	1	10.0	7	0	0	7	6	4
3.33	3	10.0	6	0	0	6	3	4
20.0	1	20.0	3	0	0	3	2	4
6.67	3	20.0	6	0	0	6	6	4

* Reference here is the median day to parasite clearance.

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IX. PILOT ASSESSMENTS OF THE ACTIVITIES OF POTENTIALLY CURATIVE DRUGS

IX. PILOT ASSESSMENTS OF THE ACTIVITIES OF POTENTIALLY CURATIVE DRUGS

This section has been divided into five compartments to facilitate analysis and discussion of data presented in corresponding groups of tables. Except for the first compartment, which is concerned with observations on a chemically heterogeneous group of agents, the other four are built about studies on specific chemical classes; the 1,5-naphthyridines, 6-aminoquinolines, 7-aminoquinolines, and 8-aminoquinolines. In order to place the investigations of the past year in perspective with the overall search for curative drugs, data have been presented on all derivatives examined in the Department of the Army Malaria Chemotherapy Program for capacity to cure established infections in rhesus monkeys inoculated with sporozoites of the B strain of *P. cynomolgi*. Compounds tested prior to the period covered by this Report have been identified by a dagger suffix to the WR- code number, placed in the first column of each table.

The pilot studies followed the general procedural pattern developed several years ago. Except when an 8-aminoquinoline was under study, or where there were already known toxicologic contraindications, the evaluation began with delivery of the test compound in a dose of 1.0 mg base (or acid) per kg body weight, daily for seven days (together with 2.5 mg chloroquine base per kg) to a single monkey with a developed sporozoite-induced infection. If this regimen failed to cure, the dose of the test compound was increased to 10.0 mg per kg and administered to either the animal treated originally or to another monkey with an active infection. If this larger dose proved to be non-curative, the evaluation of the new agent was terminated at this point. If cure was achieved with the original dose of 1.0 mg per kg, an attempt was made simultaneously to (a) confirm this result and (b) to ascertain the activity of

a daily dose of 0.5 mg per kg. Because of limited activity, the appraisals of new agents, other than certain 8-aminoquinolines, almost always ended at this point. When an 8-aminoquinoline was under investigation, the evaluation was usually initiated at a test dose of 0.5 mg base per kg body weight. This lower starting point was selected because each agent was first looked upon as a competitor of primaquine which has a CD_{90} at the 0.5 mg per kg level. If cure was not attained with this dose, a second infection was treated with 1.0 mg per kg doses whenever time, compound, and infected monkey availability permitted. If a dose of 1.0 mg per kg was not curative, the evaluation of the test agent was usually terminated. If the 0.5 mg per kg dose was curative (or appeared to be curative), the activities of 0.25, 0.125, or 0.0625 mg per kg doses were examined in stepwise order with attempted confirmation of the activity of what appeared to be the least curative dose*.

A. Compounds Of Diverse Structure

Ten agents of varied structure were examined de novo for radical curative activity during the period covered by this Report (cf Table 24). Three of the agents, WR-3,396 (an organic tin derivative), WR-124,892 (valinomycin), and WR-191,994 (cordycepin) were studied because of their capacities to inhibit oxidative phosphorylation. Two compounds, WR-102,796 and WR-124,905, were evaluated because of their anticoccidial activities. The remaining five agents, including WR-12,921, WR-13,255, WR-25,981, WR-218,575, and WR-219,124, were submitted for study primarily because of chemical novelty.

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*In all studies, cure of an established infection was synonymous with the absence of parasitemia for fifteen or more consecutive weeks after delivery of the last dose of the test compound.

As the data summarized in Table 24 show, none of these compounds exhibited radical curative activity at daily doses up to and including 10.0 mg base or acid equivalent per kg body weight. Two compounds, WR-102,796 and WR-12,921, although not curative, produced profound extensions of the relapse intervals, far beyond anything attainable with chloroquine. WR-102,796 is a hydroxyquinoline; its capacity to suppress multiplication of the blood schizonts of P. cynomolgi for long periods was described in some detail in an earlier Annual Report. WR-12,921, although defined chemically as a diaza-anthracene, can also be looked on as either a 4-aminoquinoline derivative with chloroquine as a component of the molecule or as a aza-quinacrine. The data bearing on the long term suppressive activity of this compound are limited to studies on two monkeys. These observations merit extension. If confirmed, it would be important to determine whether WR-12,921 is effective against infections with a chloroquine-resistant strain of P. falciparum.

TABLE 24

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

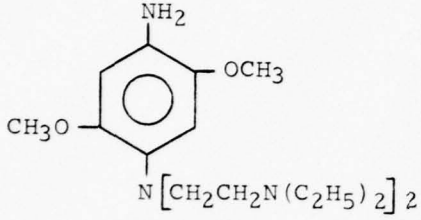
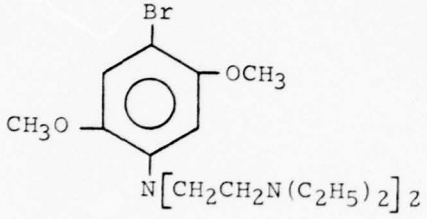
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
3,396	$(C_4H_9)_3SnCl$	0.25	+	4	-
		0.5	+	4	-
		0.5	+	9	-
		1.0	+	9	-
		1.0	-	-	+
198,559†		2.0	+	5	-
		20.0	+	9	-
198,560†		2.0	+	2	-
		20.0	+	15	-

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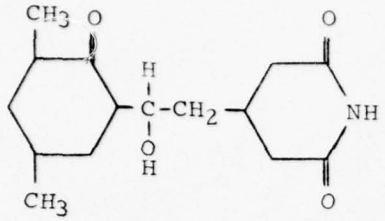
TABLE 24 - CONTINUED

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	- -

Proprietary Information: Available through Burroughs-Wellcome Company

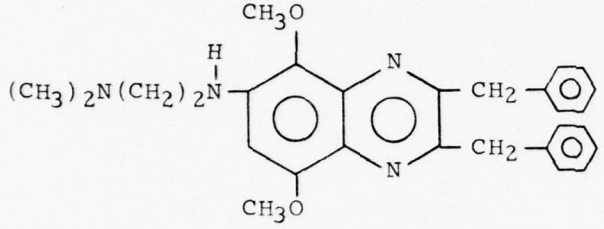
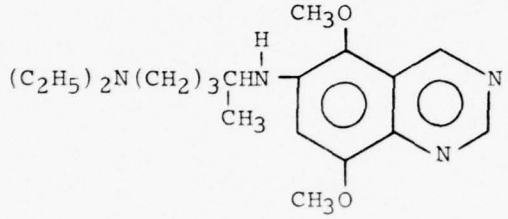
193,713†		1.0 10.0	+ +	7 8	- -
198,782†		1.0 10.0	+ +	4 7	- -

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TABLE 24 - CONTINUED

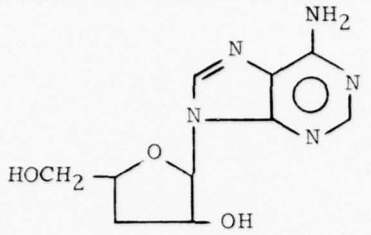
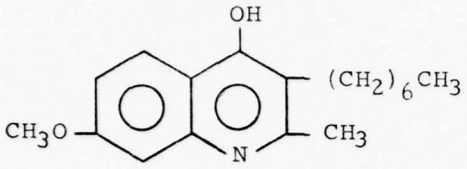
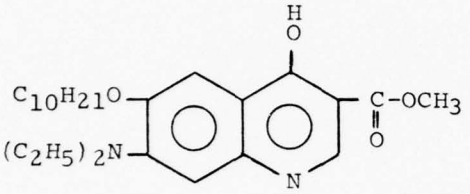
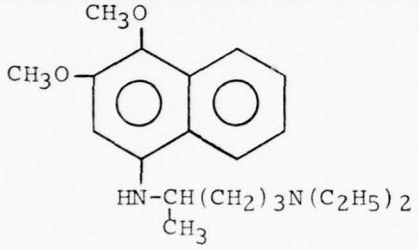
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
191,994		1.0 5.0 10.0	+ + +	8 8 9	- - -
7,295†		2.0 10.0 20.0	+ + +	12 13 13	- - -
102,796		10.0 10.0 10.0 10.0 10.0	+ + + + +	48 48 56 121 133	- - - - -
218,575		1.0 10.0	+ +	7 78	- -

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TABLE 24 - CONTINUED

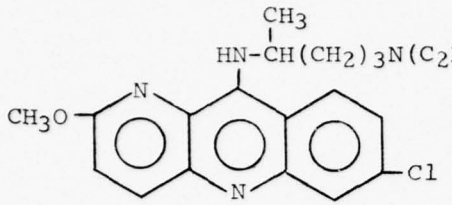
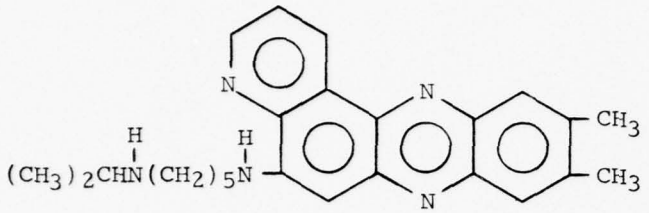
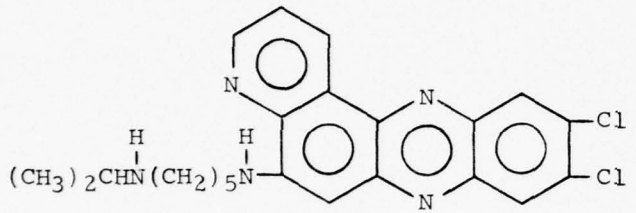
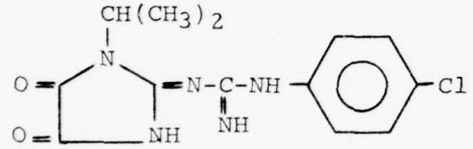
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
12,921		1.0 10.0	+ +	44 33	- -
205,446†		1.0 10.0	+ +	7 6	- -
202,833†		1.0 10.0	+ +	2 4	- -
182,058†		1.0 10.0	+ +	6 8	- -

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TABLE 24 - CONTINUED

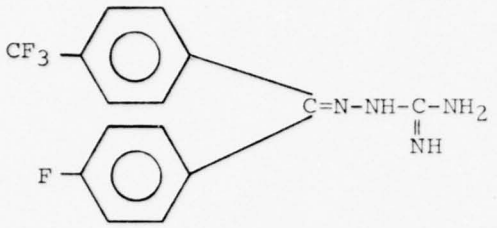
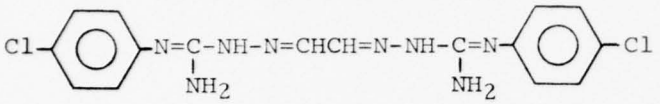
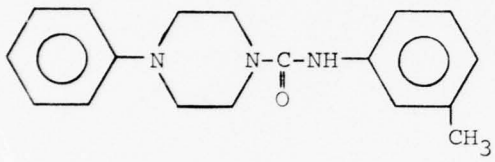
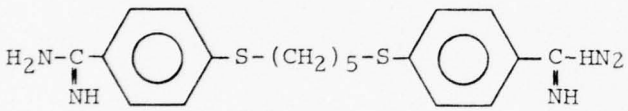
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
9,792†		0.5 5.0	+ +	8 24	- -
25,981		1.0 10.0	+ +	6 7	- -
31,877†		1.0 10.0	+ +	3 2	- -
190,830†		10.0	+	10	-

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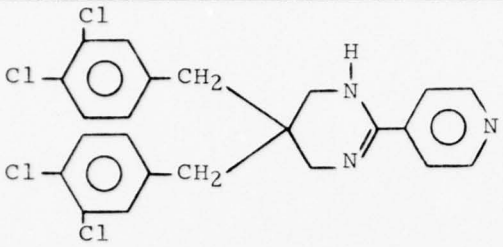
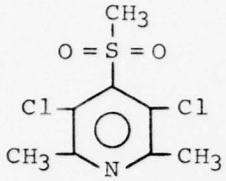
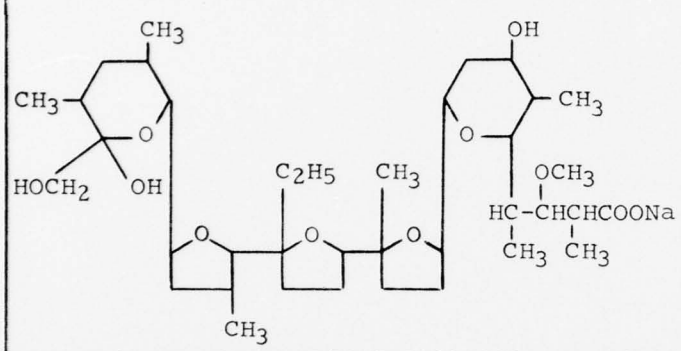
TABLE 24 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
158,124†		10.0	+	14	-
167,655†		10.0	+	7	-
124,905		1.0 10.0	+ +	8 10	- -
124,892	Valinomycin	1.0 5.0	+ +	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.

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B. 1,5-Naphthyridines

None of the three naphthyridine derivatives (WR-217,125, WR-210,442, and WR-216,010), evaluated during the Report period (cf Table 25), exhibited evidence of curative activity. As a class, these compounds have not been promising. Only one of the ten members of this series examined to date (WR-206,287) has exhibited curative activity at daily doses of 10.0 mg per kg body weight. Unfortunately, the limited availability of this compound (the only 6-OH substituted compound examined to date) precluded confirmation or extension of this observation.

TABLE 25

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

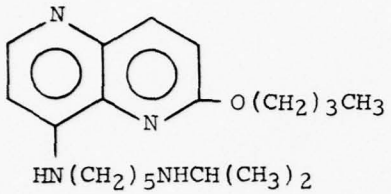
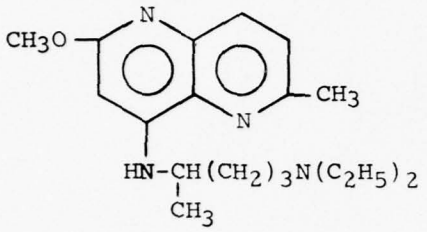
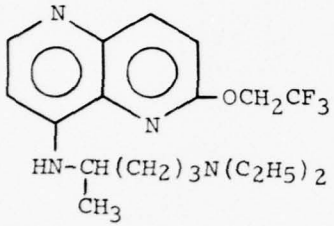
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
202, 927†		1.0 10.0	+ +	6 6	- -
217, 125		1.0 10.0	+ +	8 8	- -
202, 928†		2.0 20.0	+ +	5 9	- -

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TABLE 25 - CONTINUED

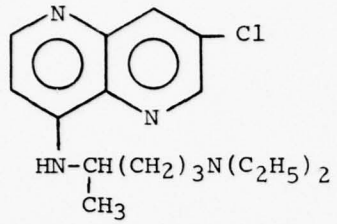
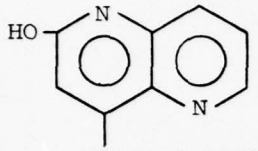
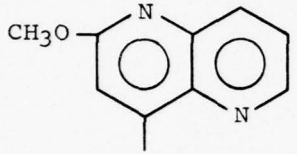
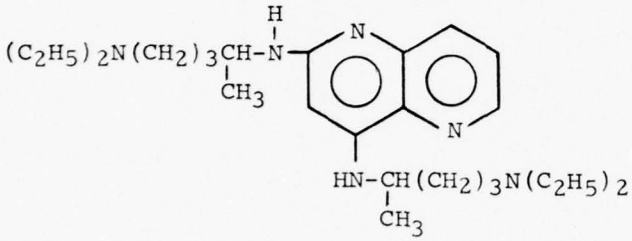
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
180,411†		10.0	+	7	-
206,287†		1.0 10.0	+ -	7 -	- +
206,283†		1.0 10.0	+ +	7 7	- -
210,304†		1.0 10.0	+ +	12 7	- -

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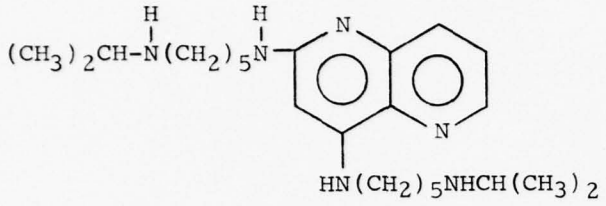
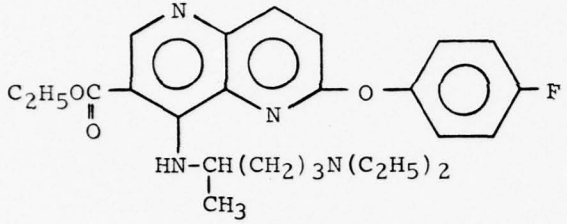
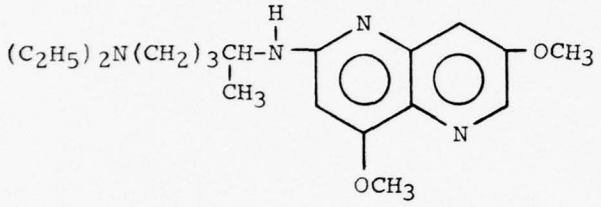
TABLE 25 - CONTINUED

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.

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C. 6-Aminoquinolines

Five newly prepared representatives of this series were evaluated for curative activity (Table 26). Results obtained on four of these compounds were entirely negative at daily doses up to and including 10.0 mg base per kg body weight. The results obtained on the fifth compound (WR-217,162) are perplexing. A single cure was obtained in one of the three recipients of daily doses of 1.0 mg base per kg body weight. This favorable result, obtained against a primary attack, contrasts with complete treatment failures in two other recipients of 1.0 mg per kg doses and in a single recipient of doses of 3.33 mg per kg.

To date, studies on the 6-aminoquinolines have been disappointing. Of the sixteen compounds evaluated, two (Ni-147/36 and WR-188,438) have exhibited unequivocal curative activity. Unfortunately, this favorable action was obtainable only at the maximum tolerated doses. At one-half this dose level, there was no sign of curative potential.

TABLE 26

PILOT ASSESSMENTS OF THE RADICAL CURATIVE ACTIVITIES OF VARIOUS 6-AMINOQUINOLINE DERIVATIVES AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

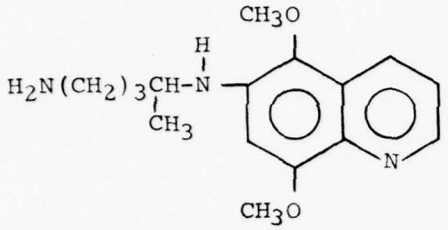
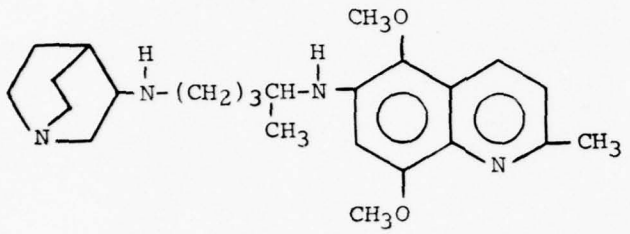
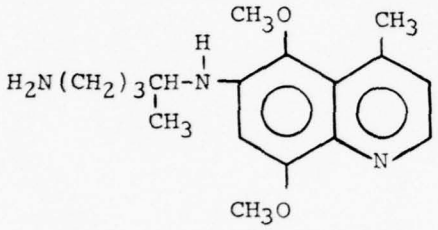
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
219,120		1.0 10.0	+ +	5 8	- -
181,614†		1.25 2.5 5.0 10.0 20.0	+ + + + +	7 14 7 29 15	- - - - -
203,766†		1.0 10.0	+ Died Day 3 of R_x - Hepatotoxicity	6	-

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TABLE 26 - CONTINUED

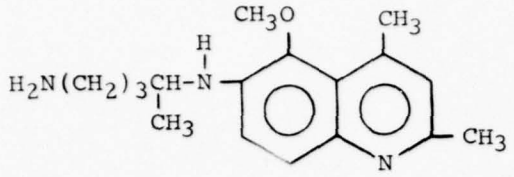
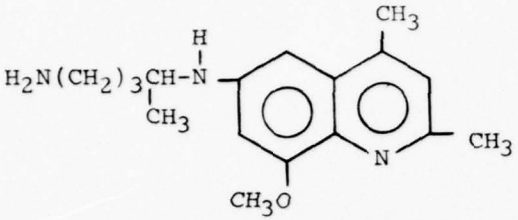
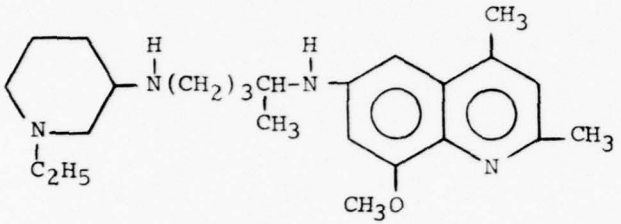
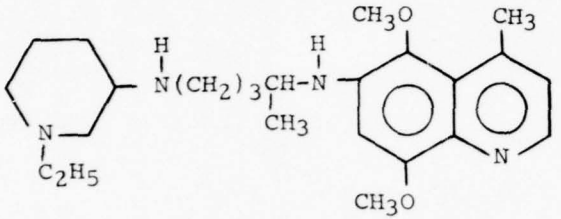
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
218,632		1.0 10.0	+ +	8 10	- -
217,162		0.25 0.5 1.0 1.0 1.0 3.33	+ + + + - +	8 9 7 7 - 10	- - - - + -
216,686		1.0 5.0	+ +	7 7	- -
204,659†		1.0 10.0	+ +	7 19	- -

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TABLE 26 - CONTINUED

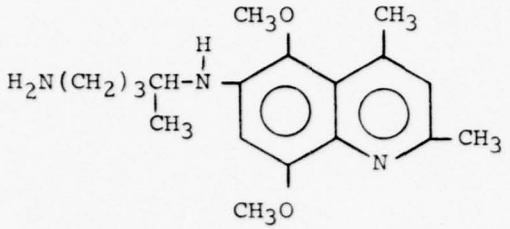
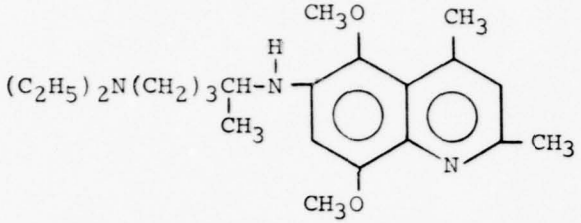
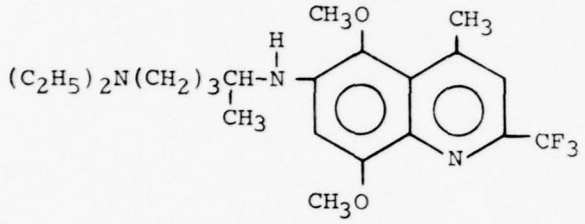
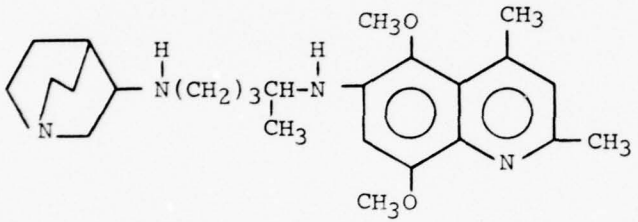
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
182,146†		1.0 10.0	+ +	5 9	- -
Ni-147/36†		1.25 1.25 1.25 1.25 2.5	+ - - - Died Day 7 of R_x - Hepatotoxicity	16 - - -	- + + +
215,627		1.0	+	7	-
182,144†		10.0	+	10	-

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TABLE 26 - CONTINUED

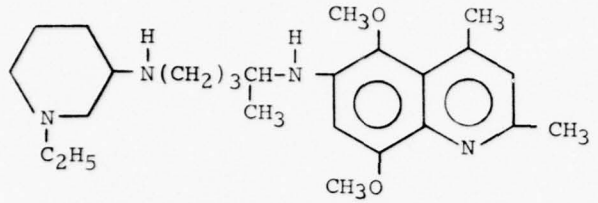
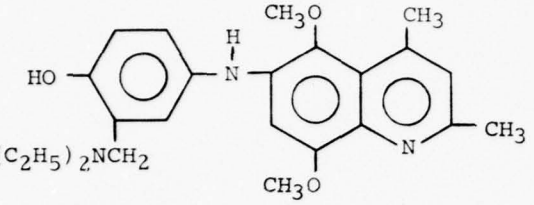
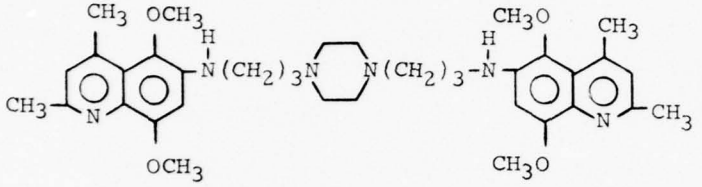
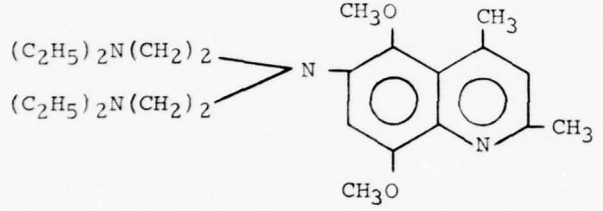
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_X and Relapse	Infection Cured
188,438†		1.0	+	5	-
		2.5	+	9	-
		2.5	+	9	-
		5.0	+	35	-
		5.0	-	-	+
		5.0	-	-	+
		5.0	-	-	+
		10.0	-	-	+
		10.0	Died Day 2 Post R_X - Hepatotoxicity		
199,066†		1.0	+	4	-
		10.0	+	7	-
208,060†		1.0	+	10	-
		10.0	+	19	-
199,065†		1.0	+	9	-
		10.0	+	11	-

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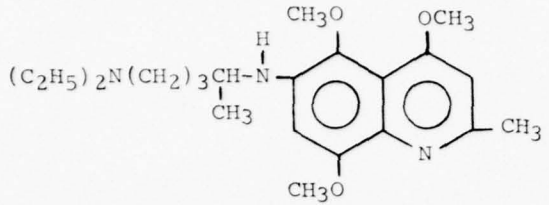
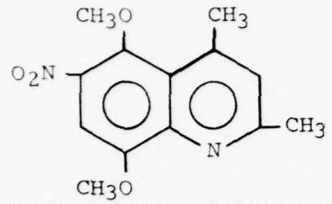
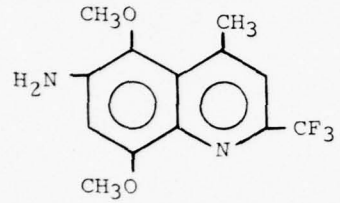
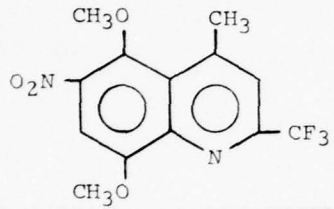
TABLE 26 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Ba-138/111†		1.25 2.5 5.0	+ + +	11 12 13	- - -
182,148†		1.0 10.0	+ +	8 10	- -
190,733†		1.0 10.0	+ +	7 9	- -
199,063†		1.0 2.0 5.0 10.0	+ + + -	11 54 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.

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D. 7-Aminoquinolines

Of the six 7-aminoquinoline derivatives examined during the Report period (Table 27), five showed no evidence of curative activity. The results with the sixth compound (WR-213,640) were equivocal, but suggested that this agent might have borderline curative activity. Infections were cured in one recipient of daily doses of 1.0 mg per kg and in the second recipient of doses of 10.0 mg per kg. In two other infected subjects (one receiving 1.0 mg per kg doses, the other 10.0 mg per kg), the relapse intervals were significantly prolonged. Opposed to this, was the absence of any sign of curative activity in five other subjects. There is no clear explanation for these variable responses.

The variety of 7-aminoquinoline derivatives examined to date has been extremely limited. It would be desirable to have a much broader experience before concluding that this class either has or does not have curative potential. Agents in this group are clearly better tolerated than their counterparts among the 6-aminoquinolines.

TABLE 27

PILOT ASSESSMENTS OF THE RADICAL CURATIVE ACTIVITIES OF VARIOUS 7-AMINOQUINOLINE DERIVATIVES AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

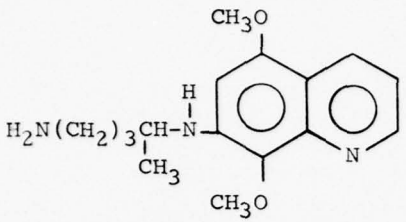
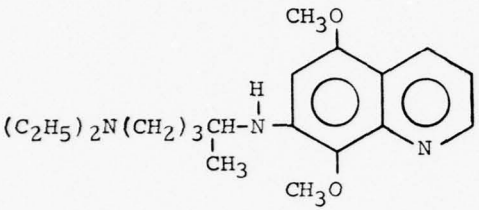
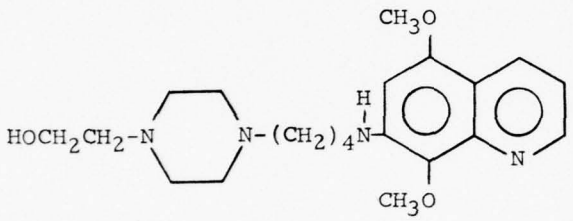
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
218,336		1.0 10.0	+ +	6 10	- -
213,640		0.5 1.0 1.0 1.0 3.33 3.33 10.0 10.0 10.0	+ + + - + + + + -	13 13 26 - 16 16 15 44 -	- - - + - - - - +
219,008		1.0 10.0	+ +	5 5	- -

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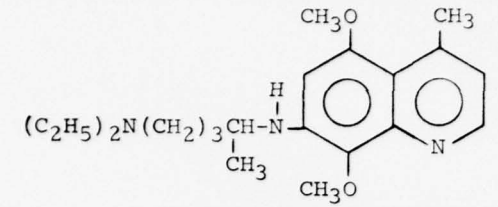
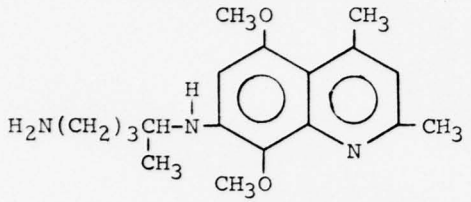
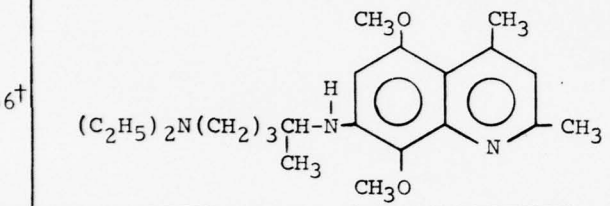
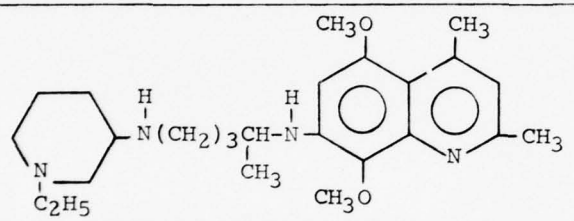
TABLE 27 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
218,677		1.0 10.0	+ +	8 9	- -
217,270		1.0 10.0	+ +	8 10	- -
207,766†		1.0 10.0	+ +	26 26	- -
218,948		1.0 10.0	+ +	5 5	- -

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

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E. 8-Aminoquinolines

Fifty-one 8-aminoquinolines were evaluated for radical curative properties during the period covered by this Report. The results of these pilot assessments have been summarized in Table 28, along with the results of similar appraisals of 83 related derivatives carried out in preceding contract years. To facilitate discussion of the results, these 134 derivatives have been arranged in thirteen compartments according to the location of substituents on the quinoline nucleus. The analysis that follows is restricted to those nine compartments which contained agents first evaluated during the current Report period.

1. Derivatives with substituents at position 6

The six newly studied compounds in this category included WR-29,633, WR-186,370, WR-215,730, WR-27,757, WR-214,420, and WR-152,149. None exhibited activity equal or superior to that of primaquine (WR-2,975). WR-29,633 and WR-186,370 had a 6-hydroxyethoxy substituent in common, but differed from each other with respect to the alkyl group separating the side chain nitrogens; WR-29,633 had an n-propyl group, WR-186,370, a 1-methylbutyl group. Neither derivative displayed curative activity at daily doses of 10.0 mg per kg. WR-215,730 and WR-27,757 had 6-methoxy substituents in common, but differed with respect to (a) the alkyl group separating the side chain nitrogens; and (b) the substituent on the terminal amino group. Neither compound exhibited curative activity at the largest dose tested. WR-214,420, the n-propyl analog of pentaquine, was inactive at a dose of 0.5 mg per kg. The evaluation of WR-152,149, a structural isomer of primaquine (designated Quinocide), is quite incomplete. Even so, this compound appears to be less active than primaquine, a finding consistent with appraisals in human volunteers carried out twenty years earlier.

2. Derivatives with substituents at positions 2 and 6

Eight derivatives were added to this compartment. They included WR-213,472, WR-218,669, WR-217,154, WR-217,124, WR-212,216, WR-216,893, WR-199,368, and WR-217,038; each had a methoxy substituent at position 6. The first six of these compounds carried the primaquine side chain at position 8. WR-199,368 had a 7-amino-n-heptylamino side chain, WR-217,038 a 3-diethylamino-n-propylamino side chain. The distinguishing moiety in this group of compounds was the substituent at position 2: a methyl group in the case of WR-217,038; a hydroxymethyl in WR-213,472; a methoxy in WR-218,669; substituted or unsubstituted benxyloxy groups in WR-217,154, WR-217,124, and WR-199,368; and benzylthio groups in WR-212,216 and WR-216,893. None of these derivatives was as active as primaquine or WR-182,234 (2-methyl primaquine). The most effective of these compounds, WR-217,154, WR-217,124, WR-212,216, and WR-217,038, exhibited curative activity at a dose of 1.0 mg per kg, twice the dose of primaquine or 2-methyl primaquine required for the same result. WR-199,368 was not curative at a 1.0 mg per kg dose; WR-213,472 and WR-216,893 were not curative at a dose of 10.0 mg per kg. The assessment of the activity of WR-218,669 is currently incomplete.

3. Derivatives with substituents at positions 3 and 6

WR-211,814 and WR-211,815, the first 3,6-substituted 8-aminoquinolines evaluated in this Project, had 3-methyl and 6-methoxy substituents in common. They differed with respect to substituent at the 8-position. WR-211,814 carried a primaquine side chain, WR-211,815, an isopentaquine side chain. Both derivatives were less active than primaquine, but were curative at doses of 10.0 mg per kg. Whether they could compete with primaquine would depend upon their toxicity, which has not been examined to date.

4. Derivatives with substituents at positions 4 and 6

Twenty-three new representatives of this class (approximately two-fifths of all 8-aminoquinolines studied in this Report period) were accorded pilot evaluations. This large addition reflected interests generated by the rediscovery of the superior activity of WR-181,023 (4-methyl primaquine) and the abilities of collaborating chemists to synthesize targetted compounds of this class. Twenty-two of the twenty-three new derivatives carried methoxy substituents at position 6; the twenty-third compound (WR-217,159) had a fluoro substituent at this position. The dimensions of this series of twenty-two agents make it possible to deal effectively with two important questions, namely: (1) What are the impacts of alterations in the 8-amino side chain on the curative activity of 4-methyl primaquine? (2) What are the impacts of various substitutions in the 4-position on the activity of primaquine?

The compounds that aided in answering the first question, their distinguishing substituents at position 8, and the doses at which they exhibited curative activity are listed below:

Compound	8-Substituent	Curative Dose Mg/Kg
WR-147,778	2-amino-1-methylethylamino	10.0
WR-136,479	4-amino-n-butylamino	>10.0
WR-181,023	4-amino-1-methylbutylamino	0.25
WR-215,296	4-amino-4-methylbutylamino	0.25
WR-215,761	4-amino-1-ethylbutylamino	ca 0.125
WR-218,335	4-amino-4-ethylbutylamino	ca 0.5
WR-212,624	5-amino-1-methylpentylamino	1.0
WR-212,579	5-amino-5-methylpentylamino	0.25
WR-006,028	3-isopropylamino-n-propyl-amino	ca 1.0
WR-006,027	2-isopropylamino-1-methyl ethylamino	> 1.0
WR-214,787	3-diethylamino-2-methyl-2-hydroxypropylamino	10.0

This listing identifies five compounds with activity equal to or greater than that of primaquine, and four agents with activity equal to or greater than that of WR-181,023 (4-methyl primaquine). All of these agents have butylamino or pentylamino side chains with unconventional branching. Within this series, however, the impacts of branching were irregular, making broad generalizations impossible. Apart from identifying the impact of branching, the data in the listing point to the association of low curative activity with side chains containing fewer than five carbons or secondary or tertiary terminal amino groups.

The compounds concerned with identifying the impacts of variation in the 4-substituent on curative activity, their distinguishing substituents, and the doses at which they exhibited or failed to exhibit curative activity are listed below:

Compound	4-Substituent	Curative Dose Mg/Kg
WR-214,198	-OH	> 1.0
WR-181,023	$-CH_3$	0.25
WR-215,300	$-CH_2OH$	10.0
WR-211,663	$-CH=CH_2$	ca 0.5
WR-218,806	$-CH_2CH_2CH_3$	> 0.5
WR-218,636	$-CH=CHCH_3$	> 0.5
WR-218,805	$-CH_2CH_2CH_2CH_3$	> 0.5
WR-218,574	$-CH=CHCH_2CH_3$	> 1.0
WR-216,837	$-CF_3$	>10.0
WR-211,975	$-CH_2CH_2-C_6H_4-Cl$	>10.0
WR-212,293	$-NH_2$	>10.0
WR-218,573	$-NHCH_3$	> 1.0
WR-217,271	$-OCH_3$	> 1.0

This summary shows that the curative activity of primaquine was not enhanced by any of the above listed substitutions. Only one agent (WR-211,663) with a vinyl substituent at position 4 exhibited curative activity approximating that of primaquine.

The status of the twenty-third 4,6-substituted compound (WR-217,159), the 6-fluoro analog of 4-methyl primaquine, is somewhat uncertain. No curative activity was exhibited at doses of 0.125 or 0.25 mg per kg; one of three infections was cured at a dose of 0.5 mg per kg. If this cure was valid, it is quite likely that doses of 1.0 mg per kg would be regularly curative. This possibility seems worth exploring; first, because of its impact on the dogma that a 6-methoxy substituent is a requisite for curative activity among the 8-aminoquinolines; secondly, because of past experience showing that 6-halogen substituted compounds are better tolerated than those with 6-methoxy substituents.

5. Derivatives with substituents at positions 5 and 6

Four new representatives of this class were evaluated during the Report period. Each differed from 5-methoxy primaquine (WR-5990) with respect to substituent at position 5. Two 5-phenoxy substituted derivatives, WR-216,100 and WR-215,295, exhibited curative activity equal to or greater than that of primaquine and approaching the activity of WR-181,023. The activity of the 5-ethoxy derivative, WR-218,676, approximated that of primaquine. WR-184,118, a 5,6-ethylenedioxy congener, was clearly less active than primaquine, and probably more toxic.

6. Derivatives with substituents at positions 6 and 7

WR-215,732 (7-methyl primaquine), the first and only representative of this class submitted for study, was devoid of curative activity when administered in daily doses of 1.0 and 10.0 mg per kg. Based on this single experience, one might suggest that there is little future in 6,7-substituted compounds. It would be well to defer such a conclusion until a broad experience is obtained with a variety of 7-substituents.

7. Derivatives with substituents at positions 2, 4, and 6

Three representatives of this class were accorded pilot evaluation. These included WR-211,533 and WR-211,990, the 2,4-dimethyl congeners of Plasmocid and pentaquine, respectively. WR-211,533 was the equal of primaquine in activity, with a curative dose of 0.5 mg per kg body weight, and appeared to be well tolerated at a daily dose of 1.0 mg per kg. WR-211,990 was not curative at a dose of 1.0 mg per kg, but was lethal at a dose of 10.0 mg per kg. This compound was clearly less active than 2,4-dimethyl primaquine (WR-192,515), but appeared to be no less toxic. The third representative of this class, WR-218,334 [2-methyl-4-(4-chlorophenoxy) primaquine] exhibited no curative activity at doses of 0.5 or 1.0 mg per kg. This compound was clearly less active than either primaquine or 2-methyl primaquine (WR-182,234), both of which are curative at a daily dose of 0.5 mg per kg.

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

Two representatives of this class, WR-215,733 and WR-211,532, curative at daily doses of 0.25 mg per kg body weight, were twice as active as either primaquine or 2-methyl primaquine (WR-182,234). These agents can be viewed as congeners of WR-182,234 (2-methyl primaquine): WR-215,733 as the 5-fluoro derivative, WR-211,532 as the 5-(4-chlorophenoxy) derivative.

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

WR-216,804 and WR-218,681, the first representatives of this class submitted for evaluation, exhibited markedly different activities. WR-216,804, the 5-methoxy congener of WR-181,023, was curative at daily doses of 0.125 mg per kg body weight. It was clearly more active than WR-181,023 and at this stage of assessment, appeared to be the most active 8-aminoquinoline derivative examined to date.

WR-218,681, the 5-chloro congener of WR-181,023, exhibited no evidence of curative activity at daily doses of 0.5 and 1.0 mg per kg. The differences in effects of 5-chloro and 5-methoxy substitution are thus very striking, but have their antecedents in the results of earlier structure-activity comparisons.

General Summary: In the interval between July 7, 1972 and April 30, 1975, one hundred thirty-four 8-aminoquinoline derivatives were accorded pilot evaluations for curative activity in the P. cynomolgi - rhesus monkey model. These evaluations have provided the underpinning for both the chemical synthesis and prospective clinical evaluation components of the search for a more active and/or more generally useful radical curative drug than primaquine. The comments that follow will address attention to what are believed to be the accomplishments and the shortcomings of the studies pursued to date.

The first concern of the Malaria Chemotherapy Program has been development of a compound with greater curative activity than primaquine. To facilitate consideration of this issue, the results of the assessments detailed in Table 28 have been digested and the digest presented in Table 29 according to: (1) the numbers of each class of nuclear substituted compounds that have been examined; (2) the numbers within each class which have exhibited activity at a daily dose of 1.0 mg base per kg body weight (twice the curative dose of primaquine); (3) the numbers which have exhibited activity at a daily dose of 0.5 mg per kg (the curative dose of primaquine); and (4) the numbers which have displayed activity at a dose of 0.25 mg per kg or less (one-half or less than one-half the curative dose of primaquine).

As shown in Table 29, thirty-two compounds, 24 per cent of the total evaluated, exhibited curative activity at a dose of 1.0 mg per kg or less. Eighteen of the thirty-two (13.4 per cent of the total) displayed activity at a dose of 0.5 mg per kg or less. Eight of these eighteen (6 per cent of the total) were curative at a dose of 0.25 mg per kg or less. In other terms, eighteen of the one hundred thirty-four derivatives had activity equal to that of primaquine; eight were at least twice as active as this established curative drug.

Four classes of nuclear substituted 8-aminoquinolines made up approximately 83 per cent of the agents studied. One of these four classes, the 6-substituted derivatives, did not contribute a single derivative more active than primaquine. This is not surprising in view of the extensive examination of this class during the World War II and post-World War II search for a curative antimalarial drug. One of the other classes accorded major attention in the current Program, the 2,6-substituted group, contributed but a single agent equal to primaquine in activity. The contributions of the 4,6- and 5,6-disubstituted groups were more impressive. Together, these classes contributed twelve of the eighteen compounds equal to primaquine in activity and five of the eight with greater activity. Little studied classes, such as the 2,4,6-, 2,5,6-, and 4,5,6-trisubstituted groups contributed a surprisingly large fraction of agents with activity equal to or greater than primaquine - probably a signal that more attention should be given these categories in the future.

By almost any measure, the output of significantly active 8-aminoquinolines has been impressive - the more so in view of the attention this class received in the 1944-1951 search for a curative drug superior to pamaquine. The relatively high output of agents with significant activity is a creditable reflection of the attention given to structure - activity relationships in directing synthesis of new congeners.

The second concern of the search for curative drugs in the current Malaria Chemotherapy Program is the development of a compound better tolerated than primaquine. This component of the mission has probably not been given the attention that it should have received. Obviously, if enhanced activity is associated with added toxicity, there may have been no real gain in therapeutic utility. Conversely, a new agent with equal or slightly less curative activity than primaquine and substantially less toxicity would be a real advance in the approach to a more generally useful compound. These simple principles deserve more attention than they have received. We would suggest that evaluation of subacute toxicity for the rhesus monkey be made an immediate follow-up to the demonstration of activity superior to that of primaquine, and that if at all possible, such evaluations should also be accorded every agent with activity equal to that of primaquine; for in that larger pool may reside the compound of greater therapeutic index and utility. The information derived from such routine toxicity studies would provide a valuable guide to chemical synthesis and focus attention early on agents that merit consideration for study in human volunteers. If limited in scope, as they should be at an early stage, these toxicity evaluations would not require a significant expenditure of investigator time or draw on the limited animal supply; they could be pursued on monkeys previously used in therapeutic evaluations whose infections had been cured.

TABLE 28

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

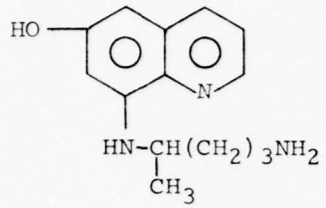
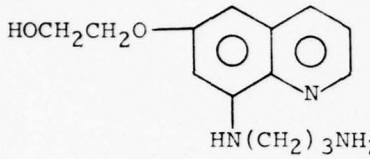
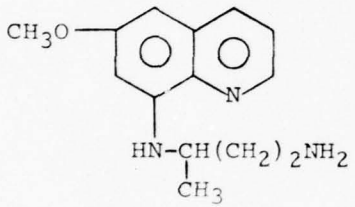
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Position 6					
199,508†		1.0 10.0	+ +	6 6	- -
29,633		1.0 10.0	+ +	4 10	- -
211,664†		1.0 10.0	+ -	10 -	- +

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TABLE 28 - CONTINUED

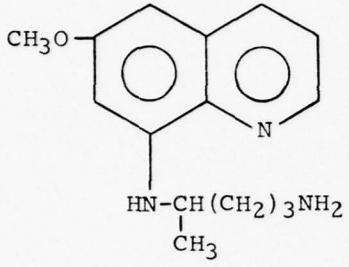
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
2,975† (Primaquine)		0.25	+	7	-
		0.25	+	7	-
		0.25	+	11	-
		0.25	+	11	-
		0.25	+	11	-
		0.25	+	11	-
		0.25	+	18	-
		0.25	+	32	-
		0.375	+	11	-
		0.375	+	14	-
	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

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TABLE 28 - CONTINUED

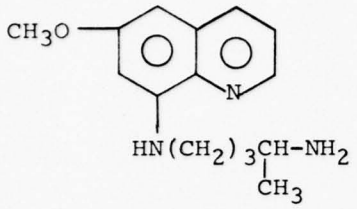
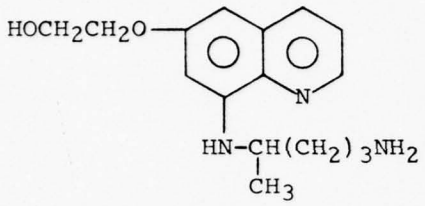
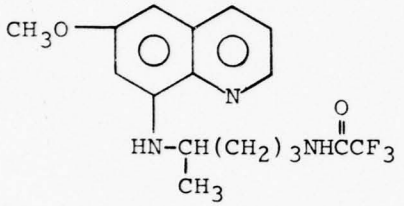
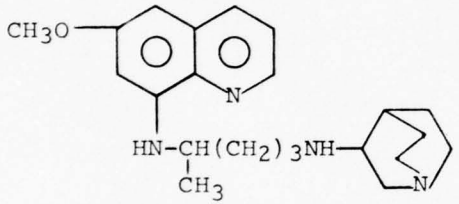
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
152,149		0.5 0.5 0.5 1.0 1.0	+ + - + -	8 8 - 24 -	- - + - +
186,370		1.0 10.0	+ +	7 3	- -
215,730		1.0	+	7	-
161,085†		1.25 2.5 2.5 5.0 5.0 20.0	+ + - - - -	11 27 - - - -	- - + + + +

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TABLE 28 - CONTINUED

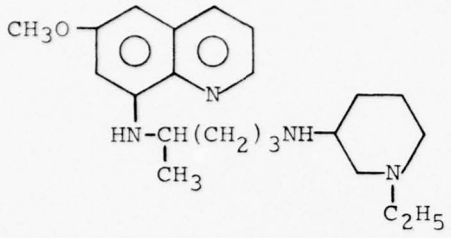
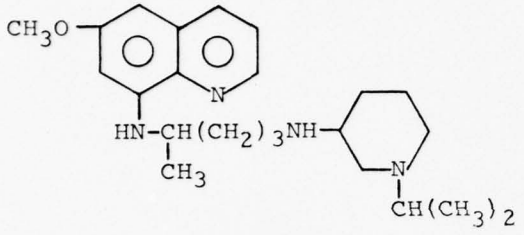
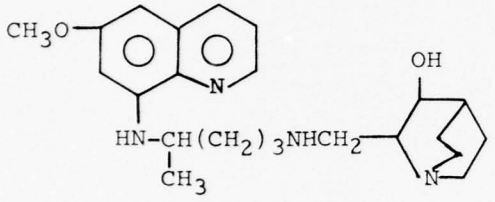
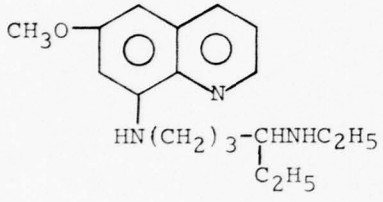
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
180,125†		1.0 10.0	+ -	9 -	- +
197,624†		1.0 3.33 10.0	+ - -	6 - -	- + +
199,981†		1.0 10.0	+ -	6 -	- +
29,594†		0.25 0.5 0.5 1.0 1.0	+ + + - -	12 8 15 - -	- - - + +

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TABLE 28 - CONTINUED

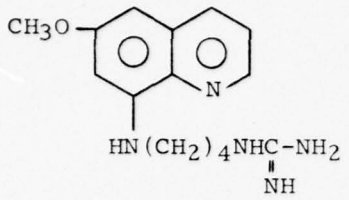
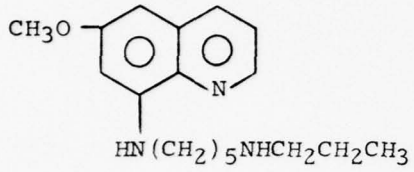
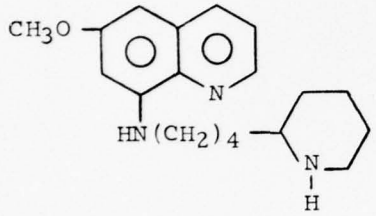
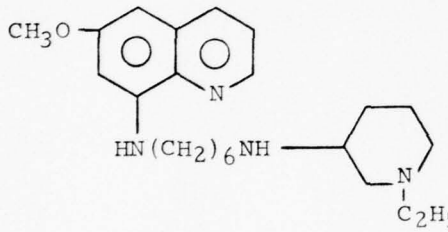
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
27,757		1.0 10.0	+ +	7 8	- -
214,420		0.5	+	5	-
29,606†		1.0 10.0	+ -	42 -	- +
190,285†		1.0 3.33 10.0	+ - -	10 - -	- + +

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TABLE 28 - CONTINUED

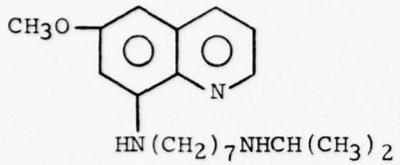
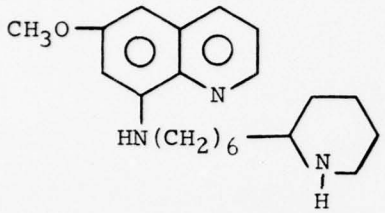
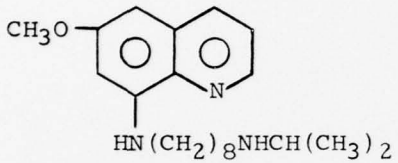
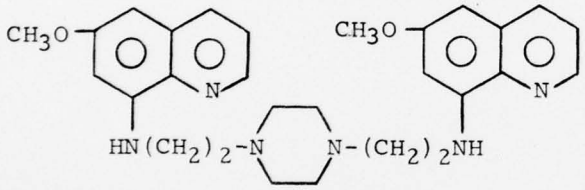
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
193,127†		1.0 10.0	+ -	7 -	- +
29,616†		0.5 1.0 3.33	+ + +	38 13 32	- - -
181,441†		1.0 10.0	+ +	5 5	- -
187,427†		0.5 1.0 1.0 10.0	+ + + Died Day 6 of R_x-Drug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

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TABLE 28 - CONTINUED

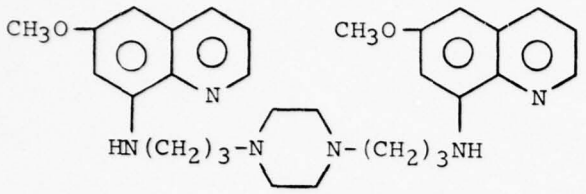
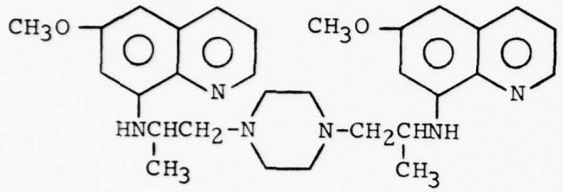
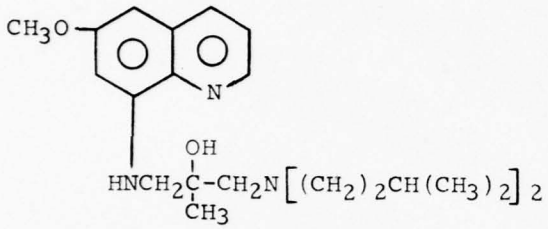
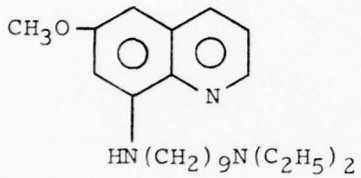
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
187,428†		1.0 2.0 5.0 5.0 10.0	+ + - - -	12 8 - - -	- - + + +
185,306†		1.0 10.0	+ +	18 13	- -
7,312†		1.0 10.0	+ +	9 10	- -
29,634†		1.0 10.0	+ +	7 16	- -

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TABLE 28 - CONTINUED

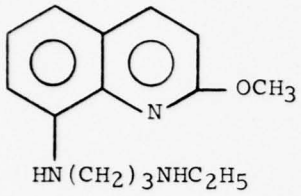
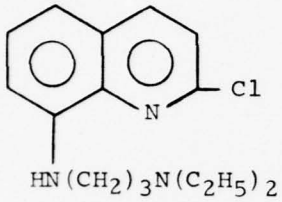
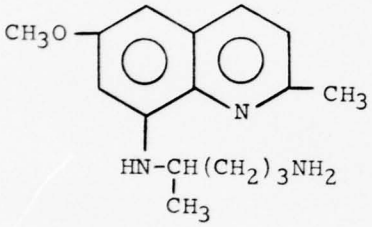
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Position 2					
184, 544†		1.0 10.0	+ +	7 7	- -
212, 231†		1.0 10.0	+ +	6 9	- -
Derivatives With Substituents At Positions 2 And 6					
182, 234†		0.125 0.25 0.5 1.0 10.0	+ + - - -	10 28 - - -	- - + + +

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TABLE 28 - CONTINUED

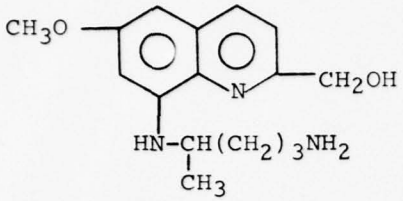
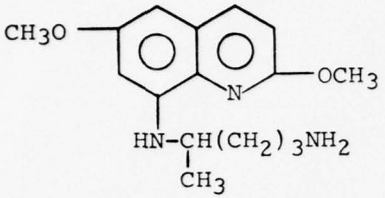
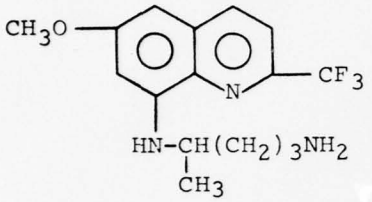
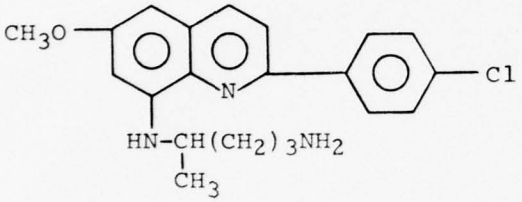
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
213,472		1.0 10.0	+ +	9 28	- -
218,669		0.5	+	12	-
211,077†		1.0 10.0	+ +	13 8	- -
121,508†		1.0 10.0	+ +	12 22	- -

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TABLE 28 - CONTINUED

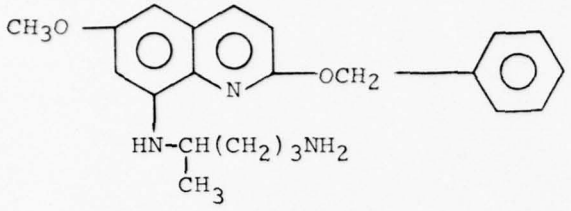
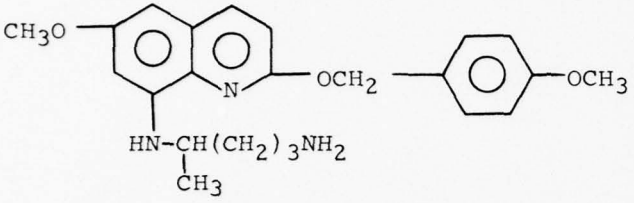
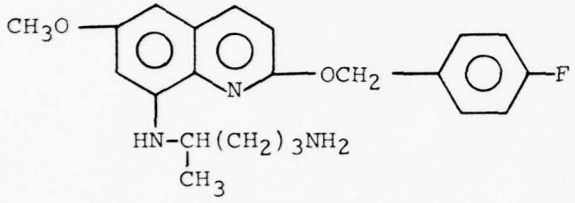
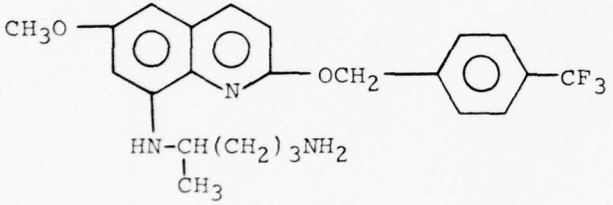
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
106,147†		0.375 1.5 1.5 1.5 1.5 3.0 3.0	+ - - - - - -	11 - - - - - -	- + + + + + +
205,438†		1.0 10.0	+ +	7 8	- -
217,154		1.0	-	-	+
217,124		0.25 0.5 1.0	+ + -	9 5 -	- - +

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TABLE 28 - CONTINUED

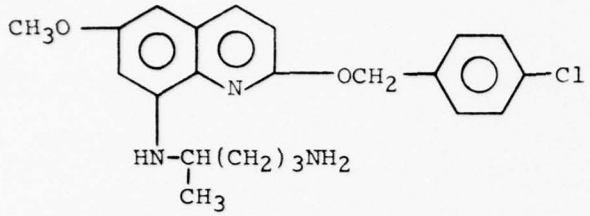
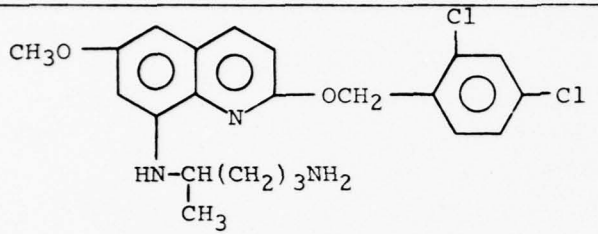
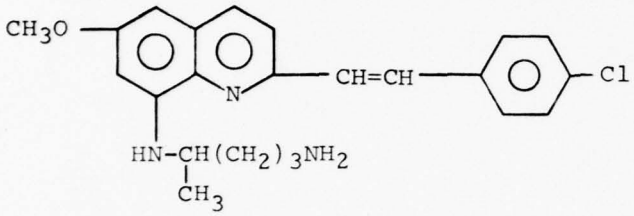
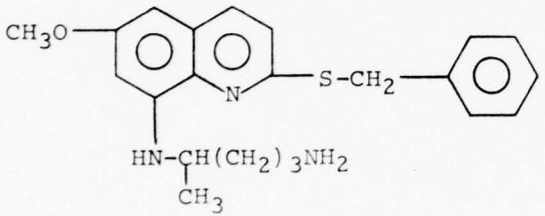
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
202,790†		1.0 10.0	+ Died Day 7 Post R_x-Hepatotoxicity	9	-
205,439†		0.5 1.0	+ -	8 -	- +
183,538†		1.0 10.0	+ -	15 -	- +
212,216		0.5 1.0 1.0	+ + -	9 15 -	- - +

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TABLE 28 - CONTINUED

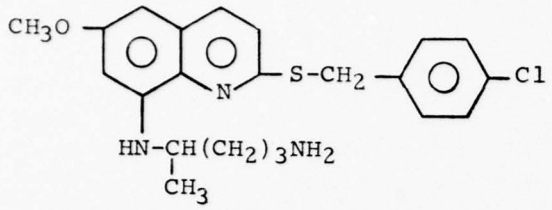
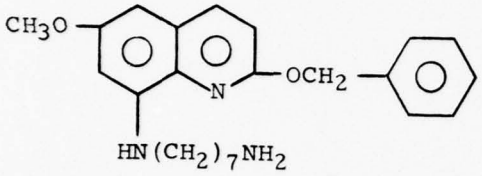
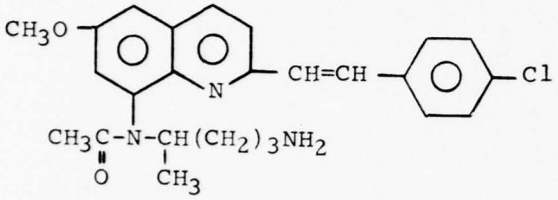
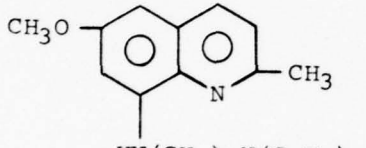
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between Rx and Relapse	Infection Cured
216,893		0.5 10.0	+ +	7 14	- -
199,368		1.0	+	9	-
183,064†		1.0 10.0	+ +	5 9	- -
217,038		0.5 1.0 1.0	+ + -	7 11 -	- - +

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TABLE 28 - CONTINUED

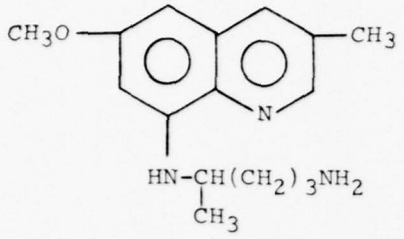
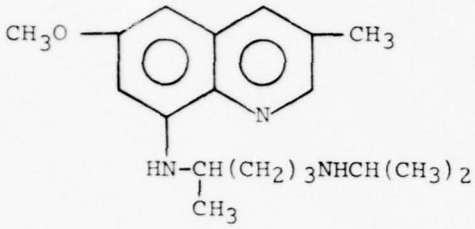
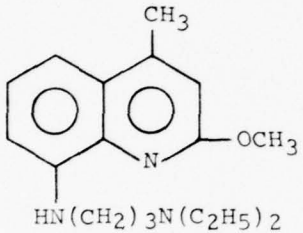
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 3 And 6					
211,814		1.0 10.0	+ -	17 -	- +
211,815		1.0 10.0	+ -	10 -	- +
Derivative With Substituent At Positions 2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

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TABLE 28 - CONTINUED

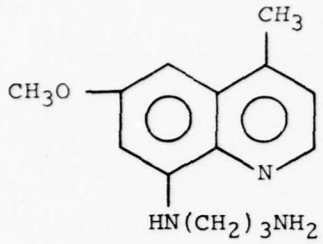
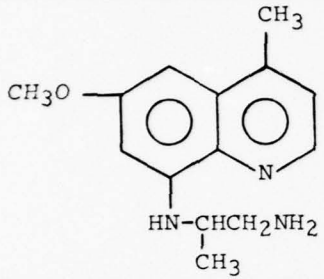
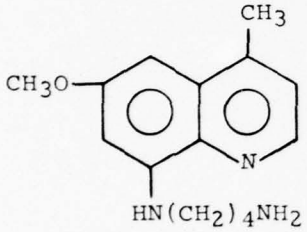
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 4 And 6					
206,027†		1.0 10.0	+ +	6 9	- -
147,778		1.0 10.0	+ -	7 -	- +
136,479		1.0 10.0	+ +	6 39	- -

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TABLE 28 - CONTINUED

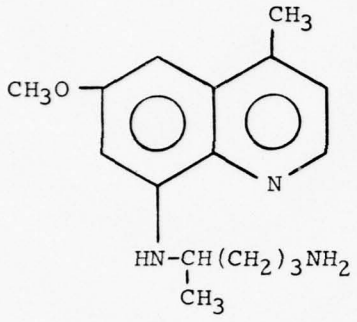
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
181,023†		0.125	+	7	-
		0.125	+	7	-
		0.125	+	10	-
		0.125	+	10	-
		0.125	+	11	-
		0.125	-	-	+
		0.25	+	14	-
		0.25	+	15	-
		0.25	+	16	-
		0.25	+	22	-
		0.25	+	27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

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TABLE 28 - CONTINUED

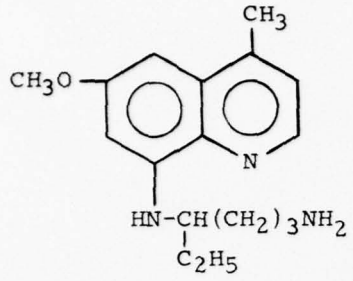
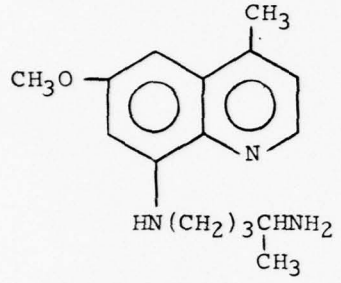
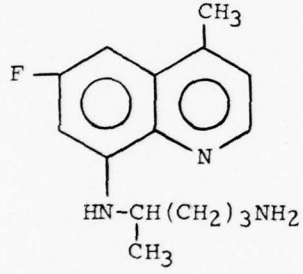
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between Rx and Relapse	Infection Cured
215,761		0.0625	+	9	-
		0.0625	+	10	-
		0.125	+	18	-
		0.125	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		1.0	-	-	+
215,296		0.125	+	35	-
		0.125	+	35	-
		0.125	+	85	-
		0.125	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+
217,159		0.125	+	8	-
		0.25	+	8	-
		0.5	+	7	-
		0.5	+	10	-
		0.5	-	-	+

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TABLE 28 - CONTINUED

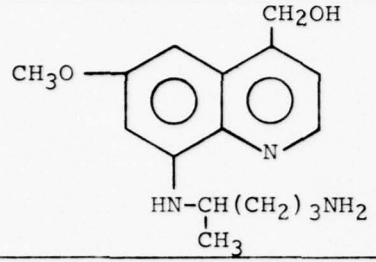
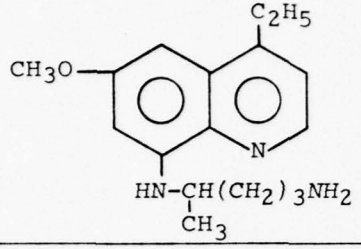
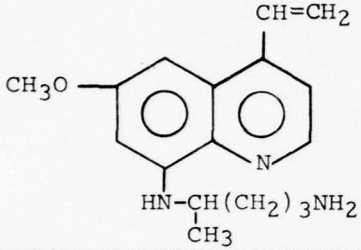
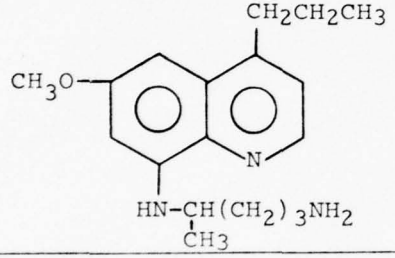
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
215,300		1.0 10.0	+ -	13 -	- +
208,442†		0.125 0.25 0.25 0.5 0.5 0.5 0.5 1.0 1.0	+ + + + - - - + -	9 7 80 28 - - - 37 -	- - - - + + + - +
211,663		0.25 0.5 0.5 1.0 1.0	+ + - + -	7 10 - 24 -	- - + - +
218,806		0.5	+	7	-

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TABLE 28 - CONTINUED

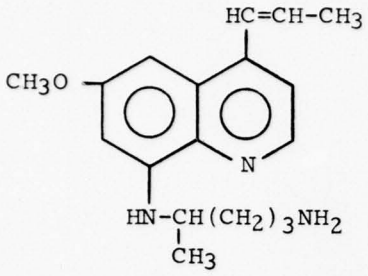
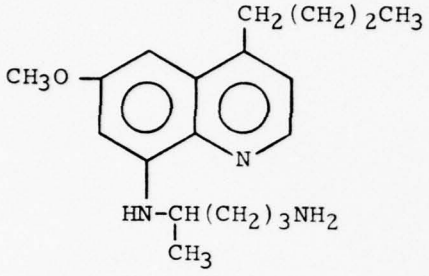
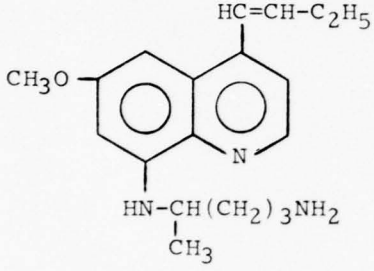
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
218,636		0.5	+	8	-
218,805		0.5	+	5	-
218,574		0.5 1.0	+ +	6 10	- -

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TABLE 28 - CONTINUED

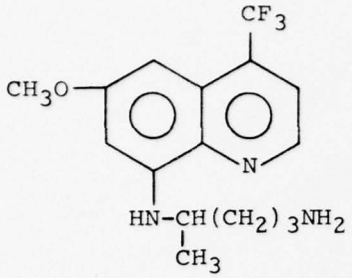
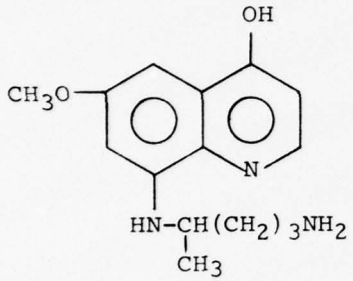
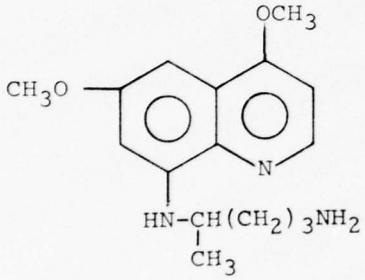
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
216,837		0.5 10.0	+ +	7 7	- -
214,198		1.0	+	9	-
217,271		0.5 1.0	+ +	5 14	- -

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TABLE 28 - CONTINUED

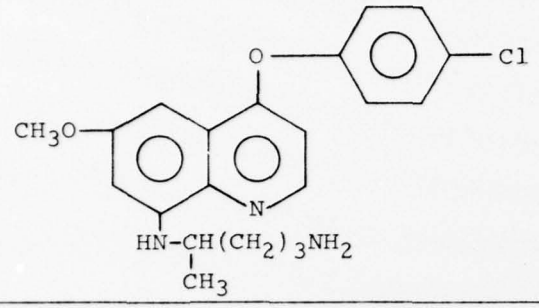
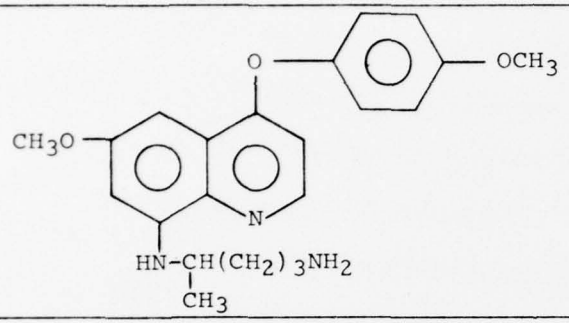
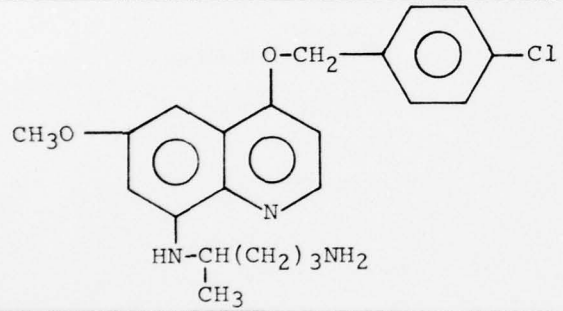
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
208,557†		0.5 1.0 10.0	+ + +	8 56 12	- - -
209,785†		1.0 10.0	+ +	13 7	- -
208,814†		1.0 10.0	+ +	11 13	- -

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TABLE 28 - CONTINUED

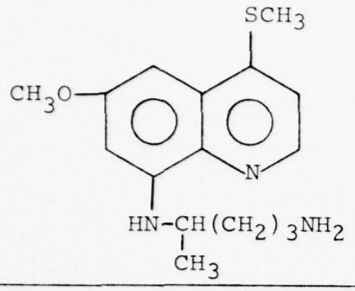
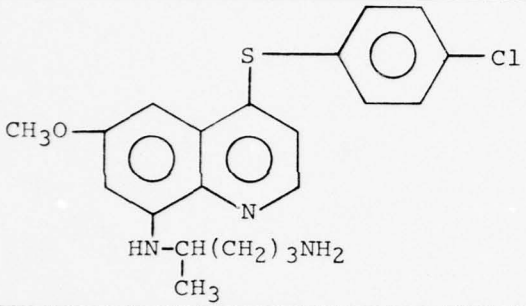
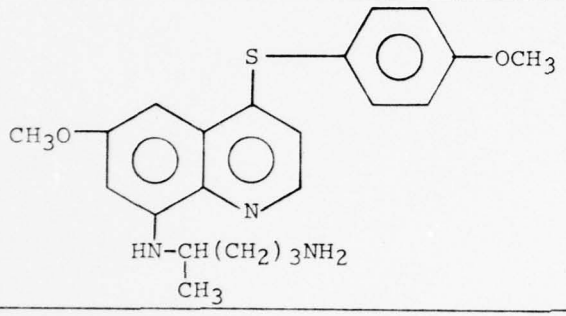
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
214,703		1.0	+	9	-
209,522†		1.0 10.0	+ +	12 7	- -
209,521†		1.0 10.0	+ +	16 9	- -

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TABLE 28 - CONTINUED

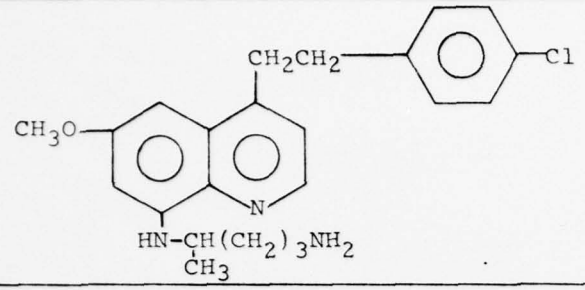
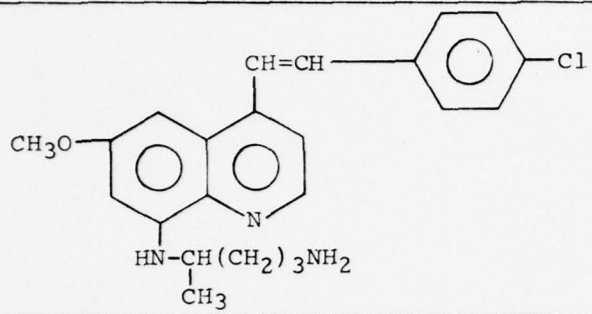
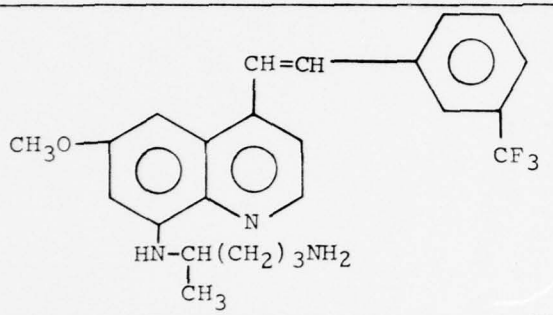
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
211,975		1.0 10.0	+ +	7 16	- -
189,279†		1.0 10.0	+ +	7 21	- -
199,793†		1.0 10.0	+ -	7 -	- +

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TABLE 28 - CONTINUED

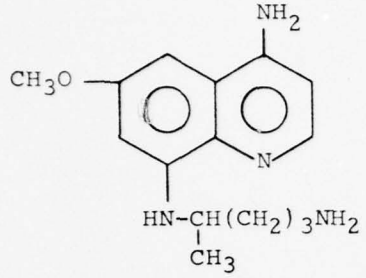
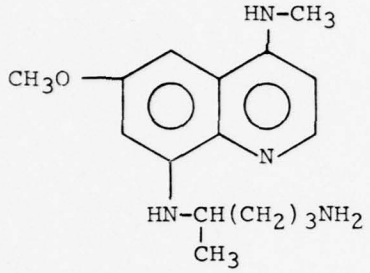
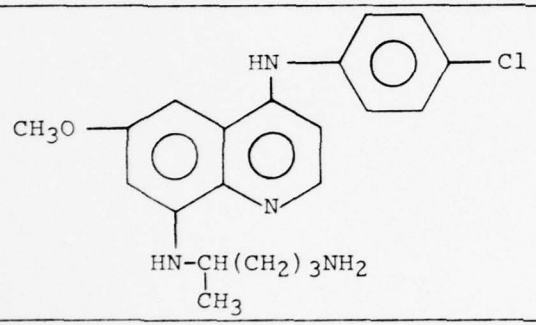
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
212,293		0.5 1.0 1.0 1.0 1.0 3.33 10.0	+ + + + - + +	5 5 7 49 - 7 9	- - - - + - -
218,573		0.5 1.0	+ +	6 10	- -
212,302†		1.0 10.0	+ +	11 13	- -

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TABLE 28 - CONTINUED

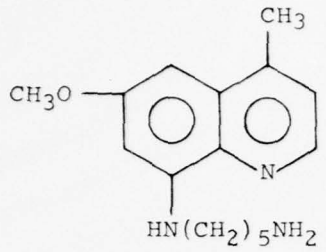
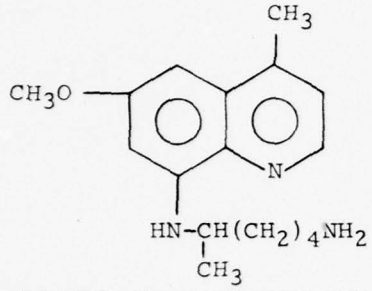
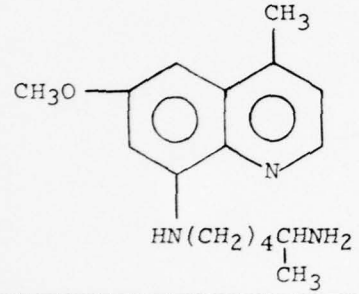
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
201,678†		1.0 10.0	+ +	10 13	- -
212,624		0.5 0.5 1.0	+ + -	13 20 -	- - +
212,579		0.0625 0.0625 0.0625 0.25 0.25 0.5 0.5 1.0	+ + + - - - - -	10 10 16 - - - - -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

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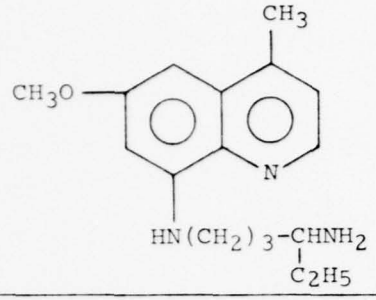
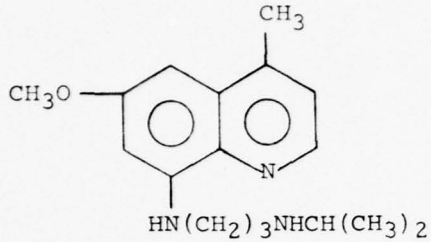
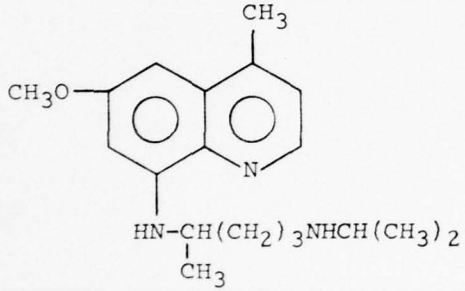
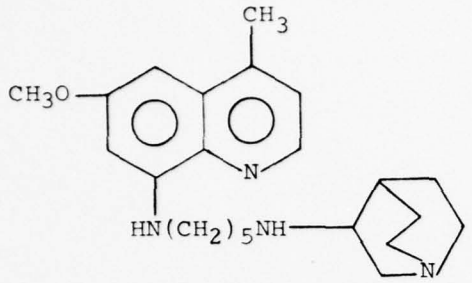
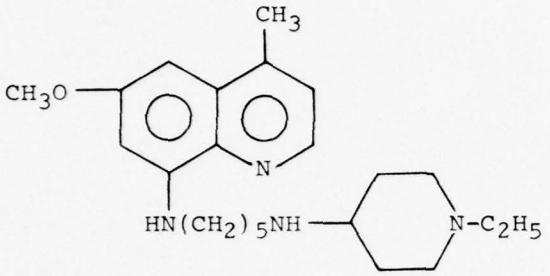
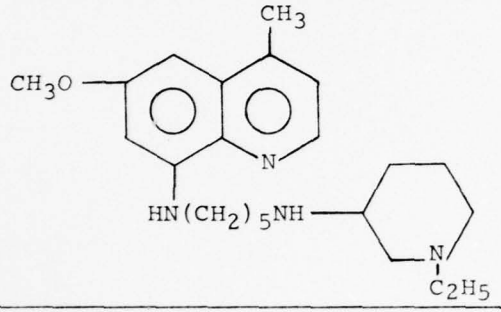
TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
218,335		0.125	+	11	-
		0.25	+	10	-
		0.25	+	12	-
		0.25	+	23	-
		0.5	+	23	-
		0.5	-	-	+
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	-

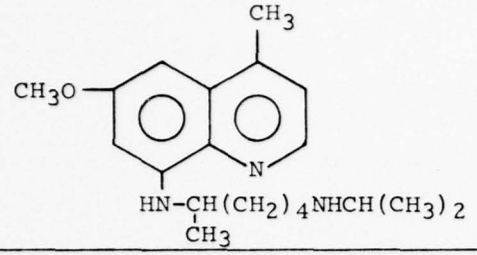
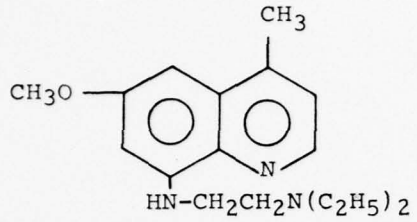
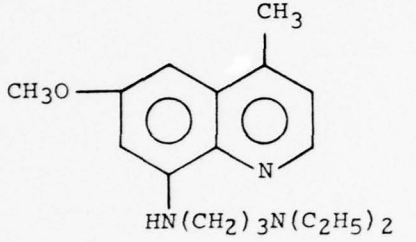
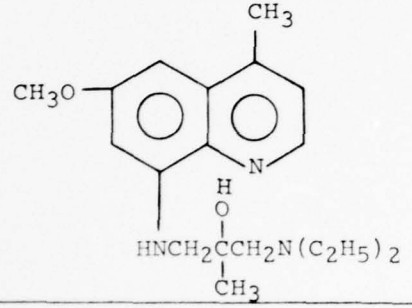
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - 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 -	- +

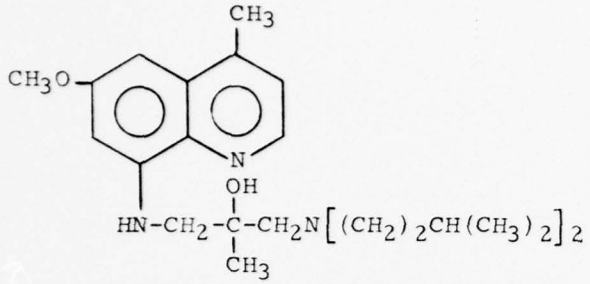
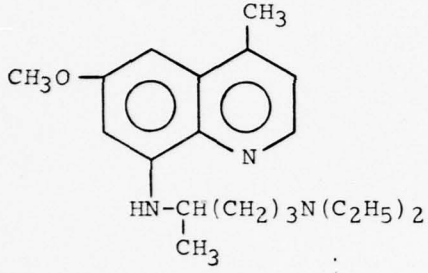
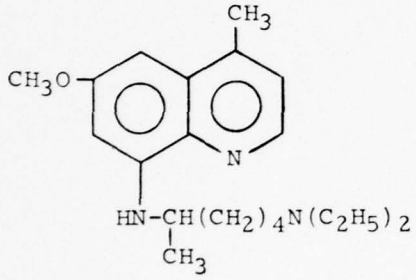
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - 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 - - -	- + + +
211,665		1.0 10.0	+ +	4 14	- -
147,657†		0.25 0.5 0.5 1.0 1.0	+ + - - -	10 30 - - -	- - + + +
214,787		1.0 10.0	+ -	15 -	- +

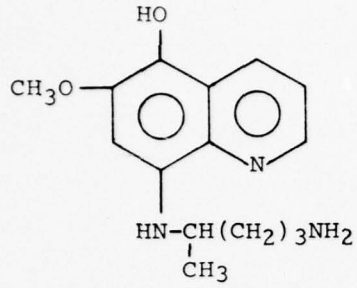
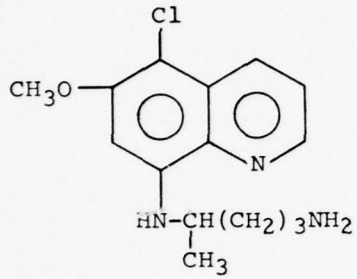
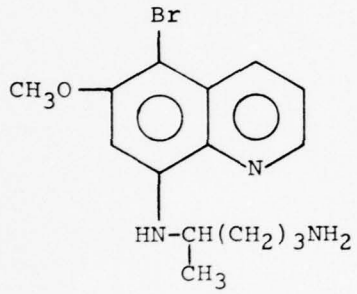
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - 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, 438†		1.0 3.33 10.0	+ - -	9 - -	- + +
211, 816†		1.0 10.0	+ -	15 -	- +
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 - - - -	- - - + + - + + + +

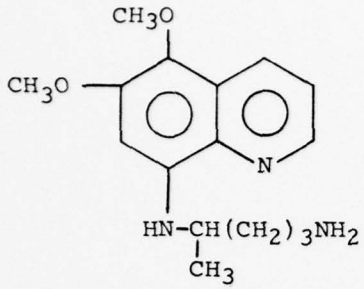
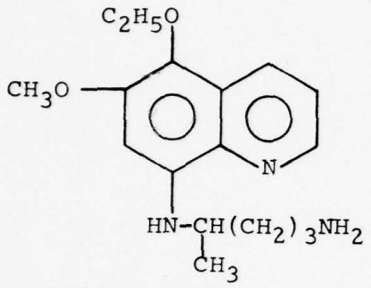
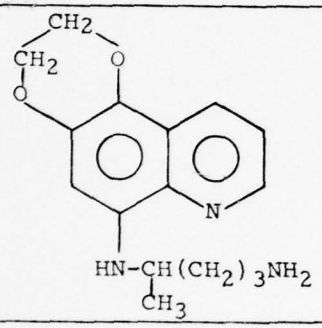
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - 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 -	- +

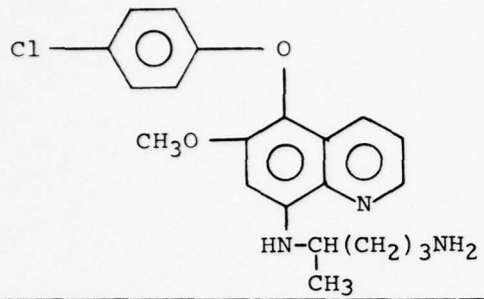
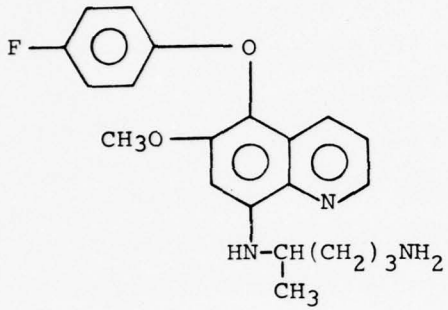
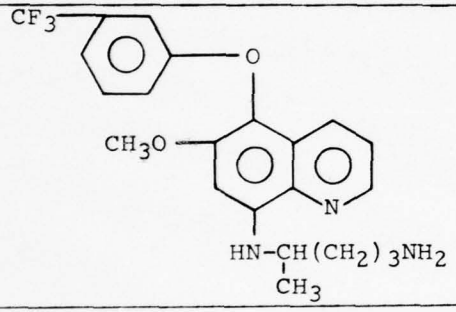
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
5,990†		0.25 0.5 0.5 0.5 0.5 1.0 1.0	+ + - - - - -	7 17 - - - - -	- - + + + + +
218,676		0.5	-	-	+
184,118		1.0 10.0	+ Died Day 1 Post R_x-Hepatotoxicity	5	-

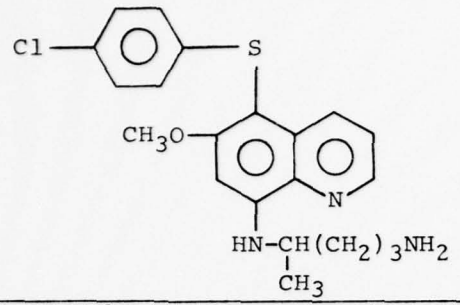
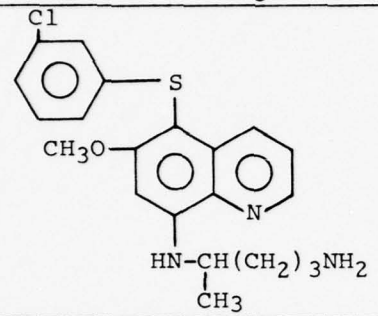
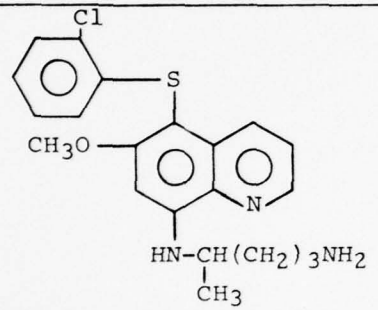
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
182,232†		0.25	+	5	-
		0.5	+	10	-
		0.75	-	-	+
		1.0	-	-	+
		10.0	-	-	+
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	-	-	+
215,295		0.125	+	12	-
		0.25	+	12	-
		0.25	+	20	-
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+

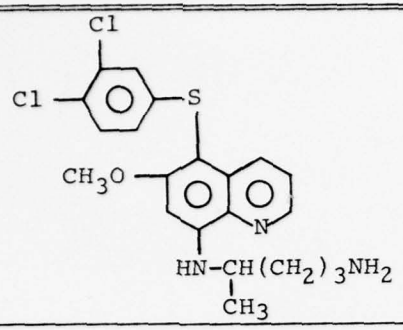
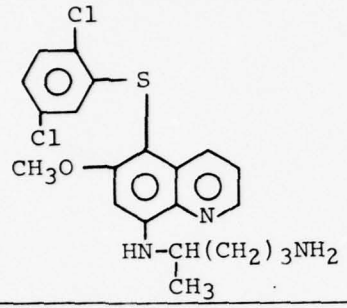
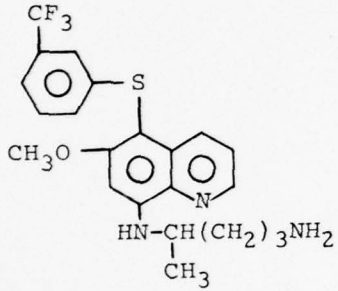
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg* Body Weight	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
183,489†		0.125 0.25 0.5 1.0 1.0 10.0	+ + + + - -	8 9 14 57 - -	- - - - + +
206,428†		1.0 10.0	+ -	12 -	- +
209,154†		1.0 10.0	+ -	15 -	- +

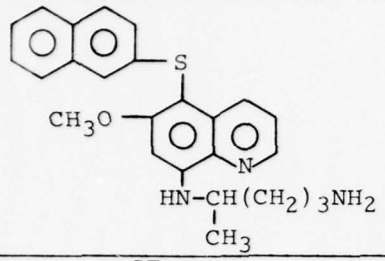
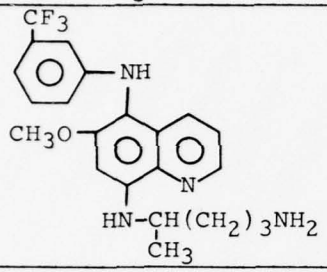
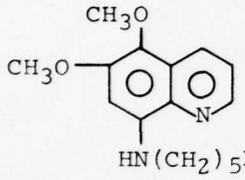
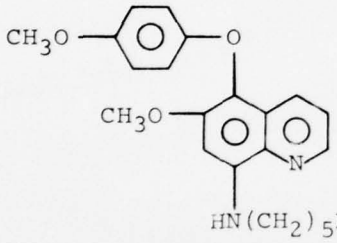
SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
208,189†		1.0 10.0	+ -	20 -	- +
209,845†		1.0 10.0	+ -	12 -	- +
208,441†		0.5 1.0	+ -	30 -	- +

SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
211,078†		1.0 10.0	+ -	27 -	- +
188,304†		1.0 10.0	+ +	7 8	- -
207,610†		1.0 10.0	+ +	15 11	- -
194,341†		1.0 10.0	+ +	15 9	- -

SOUTHERN RESEARCH INSTITUTE

AD-A044 604 SOUTHERN RESEARCH INST BIRMINGHAM ALA KETTERING-MEY--ETC F/G 6/15
THE USE OF AOTUS TRIVIRGATUS AND MACACA MULATTA AS TOOLS FOR ST--ETC(U)
JUN 76 L H SCHMIDT DADA17-69-C-9104
UNCLASSIFIED SORI-KM-76-319 NL

3 OF 3
ADA044604

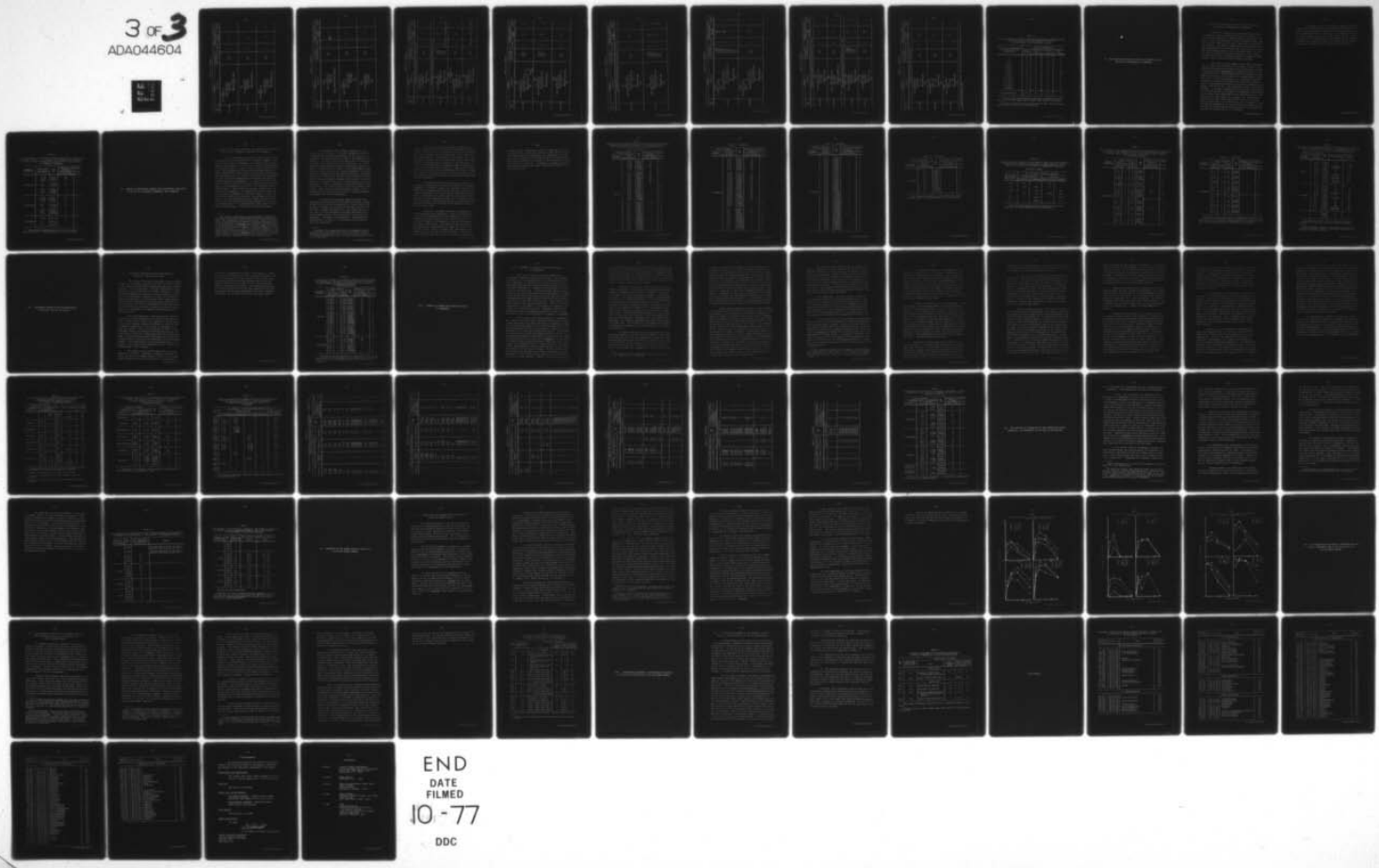


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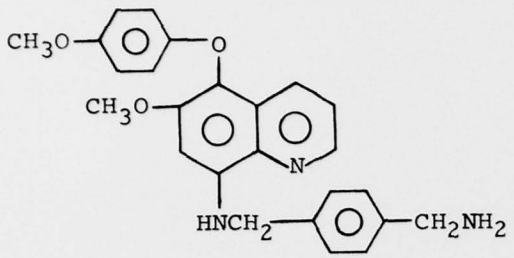
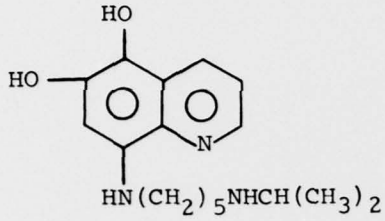
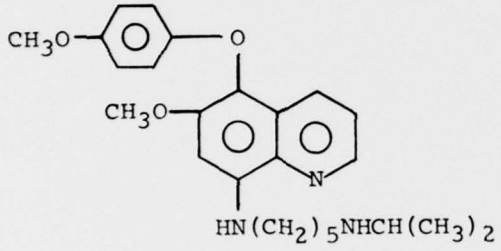
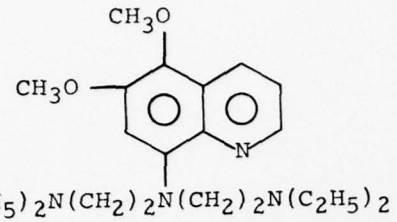
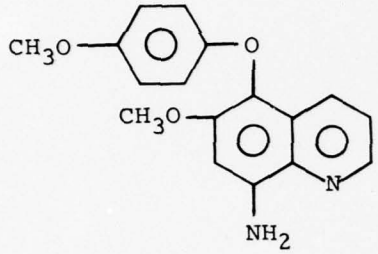
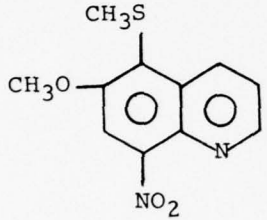
TABLE 28 - CONTINUED

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

SOUTHERN RESEARCH INSTITUTE

TABLE 28 - 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	- -

SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

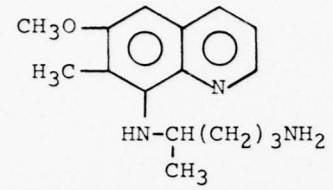
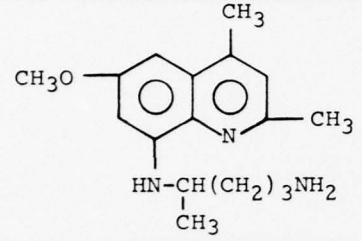
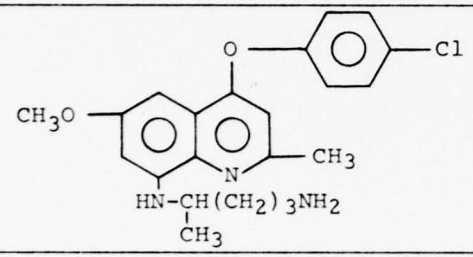
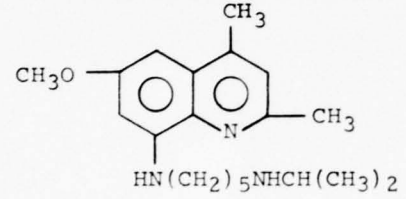
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivative With Substituent At Positions 6 And 7					
215,732		1.0 10.0	+ +	4 8	- -
Derivatives With Substituents At Positions 2, 4, And 6					
192,515†		0.125 0.25 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	-

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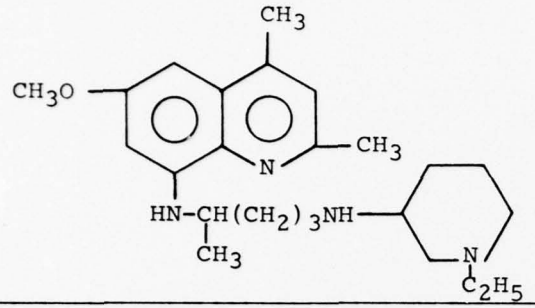
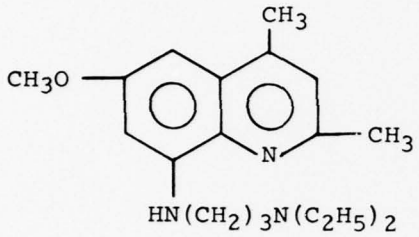
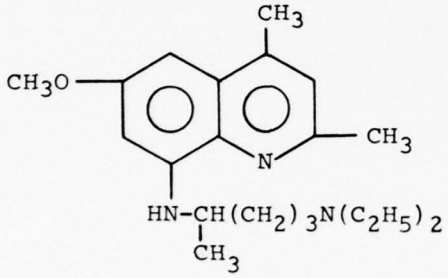
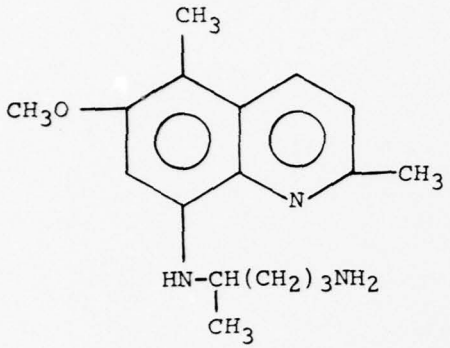
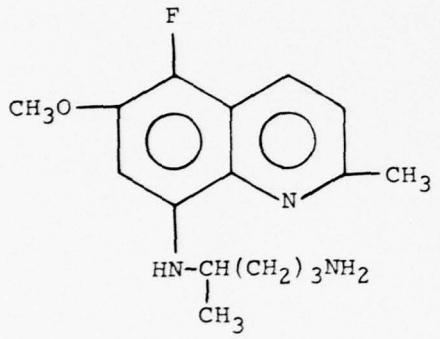
TABLE 28 - CONTINUED

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 -	- +

SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between Rx 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 0.25 0.5 0.5 1.0	+ + + - - - - - -	22 23 27 - - - - - -	- - - + + + + + +

SOUTHERN RESEARCH INSTITUTE

TABLE 28 - CONTINUED

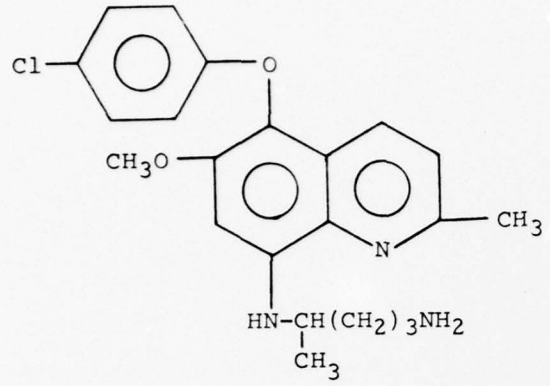
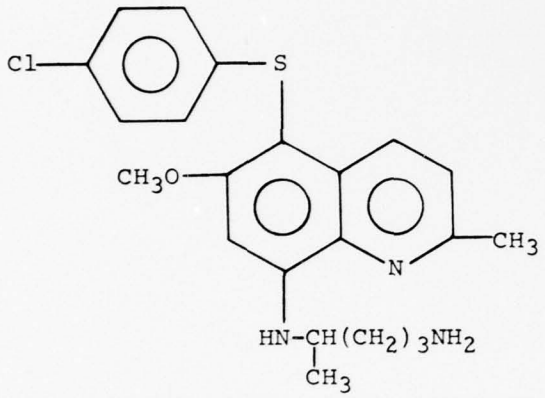
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
211,532		0.125	+	12	-
		0.125	+	15	-
		0.125	+	33	-
		0.125	+	33	-
		0.125	-	-	+
		0.25	+	56	-
		0.25	+	74	-
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.25	-	-	+
		0.5	-	-	+
		0.5	-	-	+
		1.0	-	-	+
210,805†		0.5	+	8	-
		1.0	+	41	-
		10.0	-	-	+

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TABLE 28 - CONTINUED

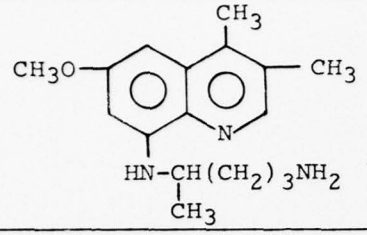
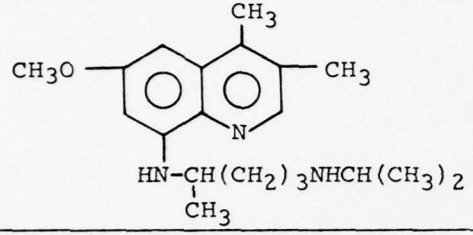
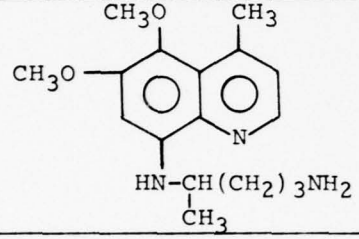
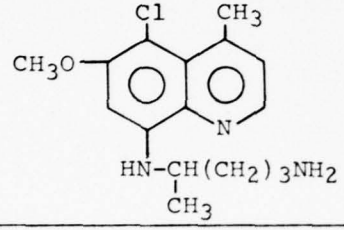
Compound		Daily Dose Mg Base/Kg Body Weight*	Response to Treatment		
WR-No.	Structure		Relapse	Days Between R_x and Relapse	Infection Cured
Derivatives With Substituents At Positions 3, 4, And 6					
210,550†		0.5 1.0	+ -	8 -	- +
210,551†		1.0 10.0	+ -	13 -	- +
Derivatives With Substituents At Positions 4, 5, And 6					
216,804		0.0625 0.0625 0.125 0.125 0.25 0.25 0.5	+ + - - - - -	11 11 - - - - -	- - + + + + +
218,681		0.5 1.0	+ +	30 13	- -

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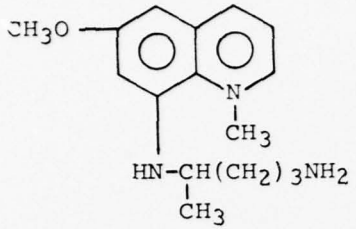
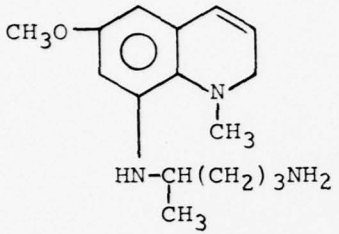
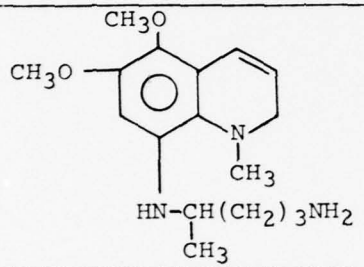
TABLE 28 - CONTINUED

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.

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TABLE 29

THE CONTRIBUTIONS OF VARIOUS 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 in 8-Aminoquinoline Compartment	No. of Derivatives			
	Total Tested	Curative @ 1.0 mg/kg	Curative @ 0.5 mg/kg*	Curative @ <0.5 mg/kg
6	24	1	0	0
2	2	0	0	0
2 and 4	1	0	0	0
2 and 6	17	7	1	0
3 and 6	2	0	0	0
4 and 6	45	11	8	4
5 and 6	25	7	4	1**
6 and 7	1	0	0	0
2, 4 and 6	6	2	2	0
2, 5 and 6	4	2	2	2
3, 4 and 6	2	1	0	0
4, 5 and 6	2	1	1	1
N-methyl	3	0	0	0
All derivatives	134	32	18	8

*Curative dose of primaquine; therefore, compounds tabulated here have activity equal to or greater than that of primaquine.

**Refers to WR-215,295 which cured two of four infections when administered in doses of 0.25 mg per kg.

X. THE CURATIVE ACTIVITIES OF WR-211,536 AND WR-211,537, THE D AND L COMPONENTS OF PRIMAQUINE

X. THE CURATIVE ACTIVITIES OF WR-211,536 AND WR-211,537, THE D AND L COMPONENTS OF PRIMAQUINE

WR-211,536 and WR-211,537, the D and L components of primaquine, have recently been prepared in quantities sufficient for evaluation of their biological properties. The first of these investigations, pursued elsewhere, dealt with the acute oral toxicities of these isomers and the racemate for the mouse. Its results showed that the LD_{50} of WR-211,537 was approximately four times the LD_{50} of WR-211,536 and twice that of primaquine, leading to the conclusion that the L isomer has but one-fourth the acute toxicity of the D isomer. The dimension of this difference prompted a comparison of the radical curative activities of the three compounds.

The results of this comparison (cf Table 30) showed that the capacities of WR-211,536 (D isomer), WR-211,537 (L isomer) and primaquine (D,L mixture) to cure infections induced by sporozoites of *P. cynomolgi* were essentially identical. This finding could be of considerable practical importance if the subacute toxicities of the isomers in the human subject follow the pattern of the acute toxicities in the mouse (cf above). Given such a toxicity differential and identical curative activity, consideration would have to be given to replacing primaquine with WR-211,537. The advantages of such a shift would include: (1) greater acceptability of doses used routinely in the fourteen-day curative regimens; (2) reasonable acceptability of the larger than conventional doses required for cure of infections with some strains of *P. vivax*; (3) possibility of delivering the total dose required for cure in a seven-day period rather than over fourteen days; and (4) opening the way to cautious explorations of the efficacy and acceptability of a three-day treatment regimen coinciding with the conventional interval for administration of chloroquine.

As indicated above, the relative subacute toxicities of WR-211,537, WR-211,536, and primaquine would determine whether these seeming advantages of WR-211,537 could be realized. The critical importance of this issue led to a preliminary comparison of the subacute toxicities of WR-211,536, WR-211,537, and primaquine for the rhesus monkey. The results of this comparison and their significance for use of WR-211,537 in place of primaquine are dealt with in Section XVI of this Report.

TABLE 30

DIRECT COMPARISON OF THE RADICAL CURATIVE ACTIVITIES OF WR-211,536 (D ISOMER), WR-211,537 (L ISOMER), AND PRIMAQUINE (D,L MIXTURE) IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound Name/WR-No.	Daily Dose Mg Base/Kg Body Weight*	Mmu No.	Response to Treatment	
			Relapsed: Days Between R_X and Relapse	Cured
WR-211,536	0.375	8227P	10	-
	0.375	8228P	10	-
	0.375	8240P	-	+
	0.5	$8228R_1$	28	-
	0.5	$8227R_1$	-	+
	0.75	8249P	-	+
	0.75	8250P	-	+
	0.75	$8228R_2$	-	+
WR-211,537	0.375	8254P	11	-
	0.375	8257P	21	-
	0.375	8253P	-	+
	0.5	$8257R_1$	16	-
	0.5	$8254R_1$	-	+
	0.75	8258P	-	+
	0.75	8263P	-	+
	0.75	$8257R_2$	-	+
Primaquine	0.375	8264P	11	-
	0.5	$8264R_1$	-	+
	0.75	8275P	-	+

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

XI. STUDIES ON THE RADICAL CURATIVE AND PROPHYLACTIC ACTIVITIES OF WR-181,023 (4-METHYL PRIMAQUINE) AND PRIMAQUINE

XI. STUDIES ON THE RADICAL CURATIVE AND PROPHYLACTIC ACTIVITIES OF WR-181,023 (4-METHYL PRIMAQUINE) AND PRIMAQUINE

Pilot evaluations carried out in 1973 indicated that the curative activity of WR-181,023 (4-methyl primaquine) was at least twice that of primaquine. This observation was important for two reasons: (1) it was made early in the Department of the Army search for a more active and better tolerated compound than primaquine and thereby provided encouragement to those concerned with various aspects of synthesis and curative drug evaluation; and (2) it focused attention on the potentials of 4-substituted 8-aminoquinolines in general and especially 4-methyl substituted compounds*. As a result of this preferential position, WR-181,023 has been studied for both radical curative and prophylactic activities in an unusually comprehensive manner. Some evaluations involved side-by-side comparison with primaquine, the standard drug against which any new curative or prophylactic agent must compete. Events may prove that WR-181,023 did not merit such in-depth attention; even so, the investigations served a useful function in providing base line data and delineating patterns of evaluation which should facilitate investigations of more effective and better tolerated agents than WR-181,023 when such appear.

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*The 4-methyl substituted 8-aminoquinolines (designated lepidines) were studied intensively during the post-World War II search for a generally useful curative drug. Four of the most active of these derivatives against infections with sporozoites of the M strain of P. cynomolgi were examined in human volunteers infected with the Chesson strain of P. vivax. None proved to be more useful than primaquine. Although WR-181,023 (designated CN-1101 by Dr. R. C. Elderfield when he submitted it for study in 1949) exhibited greater activity than primaquine or any other lepidine in the P. cynomolgi model, it was not studied in human volunteers because primaquine was already well-tested and believed to meet foreseeable needs for a curative drug.

The results of the extended assessments of the radical curative activity of 4-methyl primaquine and those of parallel or side-by-side appraisals of the activity of primaquine have been detailed in Table 31 and summarized in Table 32. The data in the latter table have been separated according to the status of the test infection; i.e., previously untreated (primary attack) or previously treated (relapse). Examination of the data in these compartments shows that the curative activity of 4-methyl primaquine is superior to that of primaquine in either type of infection. The dimensions of this superiority are difficult to measure precisely. However, minimal curative responses were achieved with daily doses of 0.125 mg WR-181,023 per kg body weight or 0.375 mg primaquine. Curative responses were obtained regularly with daily doses of 0.25 and 0.5 mg per kg of the respective compounds. These results provide reasonable support for the conclusion that the curative activity of 4-methyl primaquine is roughly twice that of primaquine.

The detailed assessments summarized above were followed by evaluations of the influence of the dosage regimen on the curative activity of WR-181,023. These studies were designed to compare the efficacy of selected total doses of the above compound delivered either as a single dose or in two, four, or seven fractions on as many consecutive days. They included side-by-side evaluations of the efficacy of primaquine. Throughout these appraisals, chloroquine was administered at a dose of 2.5 mg base per kg body weight, daily for seven days*.

---

*Although it was recognized that a chloroquine regimen identical with the schedule of delivery of WR-181,023 and primaquine would be ideal, it was felt unwise in this study to alter dosage schedules for both tissue and blood schizonticides at the same time.

The results of these experiments, summarized in Table 33, show that irrespective of the frequency of drug delivery, total doses of 1.75 mg WR-181,023 per kg body weight or 3.5 mg primaquine per unit body weight were essentially uniformly curative. Since these were the lowest total doses employed, the studies did little more than suggest that there would be no striking loss of activity if the total dose of either 4-methyl primaquine or primaquine could be administered in two or four equal daily fractions. Plans for obtaining a more precise appraisal of the influence of the dosage regimen were developed. These were tabled in favor of evaluating the lot of WR-181,023 being prepared for the proposed examination in human volunteers.

Although the above appraisal of the influence of the dosage regimen on the activity of WR-181,023 is obviously incomplete, it provides support for continued search for an agent in the 8-aminoquinoline series that will be curative in a short term regimen. If as the current evaluation indicates, cure is determined by the total dose delivered rather than exposure of tissue schizonts to drug for a set number of days, the real problem is uncovering an agent with sufficiently low toxicity to permit safe delivery of the required total dose in two to three days.

Because of continuing interest in development of a well-tolerated causal prophylactic agent, and because as a class the 8-aminoquinolines have prophylactic activity, attention has been directed to the relative capacities of 4-methyl primaquine and primaquine to prevent development of primary tissue stages. The results of two small scale experiments have been combined and summarized in Table 34. In these studies, groups of monkeys were inoculated intravenously with 5×10^5 or 1×10^6 sporozoites. Drugs were delivered twenty-four hours prior to sporozoite inoculation.

two hours before inoculation, and daily thereafter for seven consecutive days. As the data in Table 34 show, WR-181,023 was strikingly superior to primaquine in a prophylactic setting. Two of four recipients of 0.25 mg per kg doses of WR-181,023 were fully protected, as were four of four recipients of doses of 0.5 and 0.75 mg per kg. In contrast, primaquine conferred protection to but four of eleven recipients of doses of 0.75 and 1.0 mg per kg.

TABLE 31

COMPARATIVE RADICAL CURATIVE ACTIVITIES OF WR-181,023 AND PRIMAQUINE AS EXHIBITED IN PARALLEL EVALUATIONS IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF *PLASMODIUM CYNOMOLGI*

Detailed Observations

Curative Regimen		Mmu No.	Response to Treatment	
Compound Name/WR-No.	Daily Dose Mg/Kg Body Weight*		Relapsed: Days Between Rx and Relapse	Cured
	0.125	7809P	7	-
	0.125	7810P	7	-
	0.125	7897P	10	-
	0.125	$7950R_2$	10	-
	0.125	$7979R_2$	12	-
	0.125	$7889R_3$	-	+
	0.25	$7897R_1$	11	-
	0.25	$7950R_3$	14	-
	0.25	8265P	16	-
	0.25	8266P	22	-
	0.25	$7979R_3$	27	-
	0.25	7808P	34	-
	0.25	8145P	37	-
	0.25	8142P	62	-
	0.25	7760P	-	+
	0.25	7820P	-	+
	0.25	7948P	-	+
	0.25	8143P	-	+
	0.25	8144P	-	+
	0.25	8146P	-	+
181,023	0.25	8247P	-	+
	0.25	$7809R_1$	-	+
	0.25	$7810R_1$	-	+
	0.25	$7877R_1$	-	+
	0.25	$7880R_1$	-	+
	0.5	8269P	23	-
	0.5	7883P	-	+
	0.5	7884P	-	+
	0.5	7909P	-	+
	0.5	7953P	-	+
	0.5	8147P	-	+
	0.5	8148P	-	+
	0.5	8248P	-	+
	0.5	$7792R_1$	-	+
	0.5	$7808R_1$	-	+
	0.5	$8142R_1$	-	+
	0.5	$8145R_1$	-	+
	0.5	$8265R_1$	-	+
	0.5	$8269R_1$	-	+
	0.5	$7897R_2$	-	+
	0.5	$7936R_4$	-	+
	0.5	$7950R_4$	-	+
	0.5	$7979R_4$	-	+

TABLE 31 - CONTINUED

Curative Regimen		Mmu No.	Response to Treatment	
Compound Name/WR-No.	Daily Dose Mg/Kg Body Weight*		Relapsed: Days Between R_x and Relapse	Cured
Primaquine	0.25	8131P	7	-
	0.25	8135P	7	-
	0.25	8133P	10	-
	0.25	8126P	11	-
	0.25	8134P	11	-
	0.25	8132P	12	-
	0.25	8130P	18	-
	0.25	8127P	66	-
	0.375	7744P	6	-
	0.375	7835P	11	-
	0.375	8441P	11	-
	0.375	7985R_2	11	-
	0.375	7436R_3	11	-
	0.375	7830P	12	-
	0.375	7832P	12	-
	0.375	7831P	13	-
	0.375	7659P	14	-
	0.375	8264P	14	-
	0.375	8446P	15	-
	0.375	7745P	16	-
	0.375	7437R_3	16	-
	0.375	8158P	32	-
	0.375	7306R_6	36	-
	0.375	7433R_2	38	-
	0.375	7742P	61	-
	0.375	7747P	-	+
	0.375	7983R_1	-	+
	0.375	7299R_2	-	+
	0.375	7307R_3	-	+
	0.5	8139P	9	-
	0.5	8137P	26	-
	0.5	8136P	-	+
	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	-	+
	0.5	8264R_1	-	+

TABLE 31 - CONTINUED

Curative Regimen		Mmu No.	Response to Treatment	
Compound Name/WR-No.	Daily Dose Mg/Kg Body Weight*		Relapsed: Days Between R_x and Relapse	Cured
	0.75	12P	-	+
	0.75	1817P	-	+
	0.75	6583P	-	+
	0.75	7119P	-	+
	0.75	7323P	-	+
	0.75	7392P	-	+
	0.75	7406P	-	+
	0.75	7750P	-	+
	0.75	7751P	-	+
	0.75	7755P	-	+
	0.75	7756P	-	+
	0.75	7758P	-	+
	0.75	7763P	-	+
	0.75	7766P	-	+
	0.75	7769P	-	+
	0.75	7838P	-	+
	0.75	7839P	-	+
	0.75	7869P	-	+
	0.75	7870P	-	+
	0.75	8275P	-	+
	0.75	9910P	-	+
Primaquine	0.75	7385R_1	-	+
	0.75	7512R_1	-	+
	0.75	7541R_1	-	+
	0.75	7543R_1	-	+
	0.75	7549R_1	-	+
	0.75	7551R_1	-	+
	0.75	7579R_1	-	+
	0.75	7587R_1	-	+
	0.75	7659R_1	-	+
	0.75	7744R_1	-	+
	0.75	7830R_1	-	+
	0.75	7831R_1	-	+
	0.75	7832R_1	-	+
	0.75	7835R_1	-	+
	0.75	7859R_1	-	+
	0.75	7975R_1	-	+
	0.75	8137R_1	-	+
	0.75	8139R_1	-	+
	0.75	7492R_2	-	+
	0.75	7583R_2	-	+
	0.75	7980R_3	-	+
	0.75	7985R_3	-	+
	0.75	7437R_4	-	+
	0.75	7306R_7	-	+

TABLE 31 - CONTINUED

Curative Regimen		Mmu No.	Response to Treatment	
Compound Name/WR-No.	Daily Dose Mg/Kg Body Weight*		Relapsed: Days Between R_x and Relapse	Cured
Primaquine	1.5	21P	-	+
	1.5	6158P	-	+
	1.5	6596P	-	+
	1.5	7286P	-	+
	1.5	7387P	-	+
	1.5	7421P	-	+
	1.5	7770P	-	+
	1.5	7777P	-	+
	1.5	7779P	-	+
	1.5	7782P	-	+
	1.5	$7389R_1$	-	+
	1.5	$7434R_5$	-	+
	1.5	$7436R_5$	-	+

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

TABLE 32

COMPARATIVE RADICAL CURATIVE ACTIVITIES OF WR-181,023 AND PRIMAQUINE AS EXHIBITED IN PARALLEL EVALUATIONS IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Condensed Summary

Daily Dose Mg Base/Kg Body Weight*	No. Cures/No. Infections Treated			
	Primary Attacks		Relapses	
	WR-181,023	Primaquine	WR-181,023	Primaquine
0.125	0/3	-	1/3	-
0.25	7/12	0/8	4/7	-
0.375	-	1/13	-	3/8
0.5	7/8	2/4	10/10	9/9
0.75	-	21/21	-	24/24
1.5	-	10/10	-	3/3

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

TABLE 33

THE INFLUENCE OF THE DOSAGE REGIMEN ON THE RADICAL CURATIVE ACTIVITIES OF WR-181,023 AND PRIMAQUINE AS EXHIBITED IN PARALLEL EVALUATIONS IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound Name/WR-No.	Dosage Regimen*			Mmu No.	Response to Treatment	
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg		Relapsed: Days Between R_x and Relapse	Cured
WR-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	$8401R_1$	-	+
	0.438	4	1.75	8422P	8	-
	0.438	4	1.75	8410P	-	+
	0.438	4	1.75	$8422R_1$	-	+
	0.25	7	1.75	8377P	42	-
	0.25	7	1.75	8380P	43	-
	0.25	7	1.75	8372P	-	+
	0.25	7	1.75	8428P	-	+
	0.25	7	1.75	8429P	-	+
	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	8289P	-	+
	1.75	2	3.5	$8368R_1$	-	+
	0.875	4	3.5	8300P	-	+
	0.875	4	3.5	8371P	-	+
	0.5	7	3.5	8301P	-	+
	0.5	7	3.5	8374P	-	+
	0.5	7	3.5	$8380R_1$	-	+

TABLE 33 - CONTINUED

Compound Name/WR-No.	Dosage Regimen*			Mmu No.	Response to Treatment	
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg		Relapsed: Days Between R_x and Relapse	Cured
Primaquine	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	8376P	19	-
	0.5	7	3.5	8356P	-	+
	0.5	7	3.5	8395P	-	+
	0.5	7	3.5	8396P	-	+
	7.0	1	7.0	8337P	-	+
	3.5	2	7.0	8298P	-	+
	3.5	2	7.0	8338P	-	+
	1.75	4	7.0	8299P	-	+
	1.75	4	7.0	8341P	-	+
	1.0	7	7.0	8307P	-	+
	1.0	7	7.0	8348P	-	+

*Throughout these evaluations, chloroquine was administered in a dose of 2.5 mg base per kg body weight once daily for seven consecutive days. Delivery of chloroquine was initiated on the first day of administration of WR-181,023 or primaquine.

TABLE 34

A COMPARISON OF THE PROPHYLACTIC ACTIVITIES OF WR-181,023 AND PRIMAQUINE AS EXHIBITED IN PARALLEL EVALUATIONS IN RHESUS MONKEYS CHALLENGED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Prophylactic Regimen*		Mmu No.	Day of Patency after Challenge	Days Delay in Onset of Patency
Compound Name/WR-No.	Daily Dose Mg/Kg Body Weight			
181,023	0.125	8247	12	4
	0.125	8248	12	4
	0.25	7969	Protected	> 74**
	0.25	8225	Protected	> 74
	0.25	8265	13	5
	0.25	8266	23	15
	0.375	8269	13	5
	0.375	8270	Protected	> 94
	0.5	8231	Protected	> 74
	0.5	8233	Protected	> 74
	0.75	8234	Protected	> 74
	0.75	8235	Protected	> 74
Primaquine	0.25	8236	10	2
	0.375	8158	18	10
	0.375	8189	16	8
	0.75	7501	Protected	>113
	0.75	7549	17	9
	0.75	7586	19	11
	0.75	8159	Protected	> 79
	0.75	8242	18	10
	1.0	7705	26	18
	1.0	7708	17	9
	1.0	7709	18	10
	1.0	7710	18	10
	1.0	7955	Protected	> 89
	1.0	8243	Protected	> 74
-	-	7956	8	-
	-	8244	8	-
	-	8318	8	-

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

**Where the symbol > appears, observations were terminated on the designated day; absence of parasitemia to such date is synonymous with full protection.

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

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

The results of the studies described in the preceding section of this Report, together with those of studies pursued in the 1949-1951 period (when 4-methyl primaquine was designated CN-1101), led to the decision to initiate evaluations of the curative activity of WR-181,023 in human volunteers. A batch lot of this compound was prepared for these appraisals as well as for the preclinical evaluations of toxicity and efficacy required by FDA prior to testing a new drug in human subjects. The preclinical evaluations of efficacy were designed to determine whether the curative and prophylactic activities of the IND preparation were the same as those of pre-IND lots and to explore the influence of the dosage regimen on both of the above activities.

The first experiment, concerned with the curative activity of the IND preparation, was initiated on February 5, 1975. It dealt with a study of the comparative effectiveness of three and seven-day regimens of WR-181,023, controlled by a companion evaluation of the activity of primaquine in a seven-day regimen. Unlike the evaluations described in the preceding section, the duration of chloroquine delivery coincided with the duration of administration of the 8-aminoquinoline. In the three-day regimen, chloroquine was administered in daily doses of 5.84 mg per kg body weight; in the seven-day regimen, it was administered in the conventional doses of 2.5 mg per kg. The total dose of chloroquine was 17.5 mg base per kg body weight in both regimens.

The results of this initial experiment have been summarized in Table 35. Although the numbers of monkeys involved are small, the summary shows: (1) that in a seven-day dosage regimen the activity of WR-181,023 is approximately

twice that of primaquine, the curative doses being 0.25 mg per kg and 0.5 mg per kg daily for the respective compounds; and (2) at total doses of 1.75 mg WR-181,023 per kg body weight, three-day and seven-day dosage schedules were essentially equally effective, six of eight vs six of six infections cured on the respective regimens. More extensive evaluations of the curative activities of the IND preparation and older preparations and studies of prophylactic activity, incomplete at present, will be summarized in the next Annual Report.

TABLE 35

A PRELIMINARY APPRAISAL OF THE RADICAL CURATIVE ACTIVITY OF THE IND PREPARATION OF WR-181,023 (LOT AG:BE-50,003) AS EXHIBITED IN RHESUS MONKEYS INFECTED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Compound Name/WR-No.	Dosage Regimen*			Mmu No.	Response to Treatment	
	Daily Dose Mg/Kg	No. of Doses	Total Dose Mg/Kg		Relapse: Days Between R_x and Relapse	Cured
181,023	0.292	3	0.876	8568P	14	-
	0.292	3	0.876	8571P	18	-
	0.292	3	0.876	8569P	21	-
	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	$8552R_1$	-	+
Primaquine	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	$8539R_1$	-	+

*Chloroquine was administered in a total dose of 17.5 mg per kg body weight in both regimens; daily doses were 5.84 mg per kg and 2.5 mg per kg, respectively for the three and seven dose schedules.

XIII. ATTEMPTS TO ENHANCE THE CURATIVE ACTIVITY OF PRIMAQUINE

XIII. ATTEMPTS TO ENHANCE THE CURATIVE ACTIVITY OF PRIMAQUINE

There are at least two distinct approaches to developing an improved curative drug regimen. One embodies the search for a single agent more active and better tolerated than primaquine. As indicated in Section IX of this Report, the Malaria Chemotherapy Program has been pursuing this line of attack vigorously at a combined chemical synthesis and biological evaluation level. A second approach embodies efforts to enhance the well-established curative capabilities of primaquine (without decreasing tolerability) by administering this 8-aminoquinoline in combination with a second tissue schizonticidal drug of a different chemical class. This approach has been largely if not completely neglected. One of the factors responsible for the neglect is the paucity of candidates for a place in a combination regimen - compounds other than the 8-aminoquinolines with demonstrated activity against either the early or late tissue schizonts of P. vivax or P. cynomolgi.

In reflecting on possible approaches to development of a combination drug regimen, attention was focused on observations made some seven years ago during a comprehensive study of the antimalarial properties of 7-chlorolincomycin (U-21,251F, now designated WR-203,659) and N-demethyl-4'-pentyl-7-chlorolincomycin (U-24,729A, now designated WR-203,661). Specifically, these observations showed that administration of WR-203,661 at the maximum tolerated dose (MTD) would prevent or cure infections with sporozoites of the B strain of P. cynomolgi. At one-fourth of the MTD, WR-203,661 effected marked delays in onset of parasitemia and prolongation of the relapse intervals. The same studies showed that these effects resulted from a direct action of WR-203,661 on the tissue schizonts. WR-203,659 exhibited similar activity, but much less regularly than WR-203,661 and then only at a dose thirty-twofold greater than the effective dose of the latter compound. Together, these observations led

to the conclusion that the 7-chlorolincomycins, and especially WR-203,661, were endowed with activity against both early and late tissue schizonts. This rare endowment, in compounds structurally different from the 8-aminoquinolines, stimulated a group of preliminary studies aimed at determining whether concomitant administration of WR-203,661 would enhance the prophylactic and radical curative properties of primaquine.

Two pilot studies of the capacity of WR-203,661 to enhance the prophylactic activity of primaquine were undertaken*. A number of compounds other than this lincomycin derivative were included in the initial pilot experiment - essentially as controls. The group consisted of: proguanil, pyrimethamine, and WR-158,122 (a 6-sulfur substituted 2,4-diaminoquinazoline) - all dihydrofolic acid reductase inhibitors; WR-203,659 - the lincomycin derivative referred to above; and oxytetracycline. Each of these agents was administered at approximately one-fourth of the MTD in a nine-day, once daily, dosage regimen, either alone or concomitantly with primaquine at a daily dose of 0.375 mg per kg, half the dose of this 8-aminoquinoline required for prophylaxis. Oxytetracycline was injected intramuscularly; the remaining compounds were administered orally via stomach tube. The second pilot experiment was limited to a preliminary assessment of the dose of WR-203,661 required for prophylaxis.

The combined results of the two experiments have been presented in Table 36. The data therein suggest strongly that the prophylactic activity of primaquine was enhanced by concomitant administration of WR-203,661 and that this goal could be attained with a daily dose of 2.5 mg per kg, equivalent to one-sixteenth of the MTD. WR-203,659, a second lincomycin analog, was the only other compound that conferred protection.

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*The inocula for these experiments were 1.2×10^6 and 1.6×10^6 sporozoites, respectively.

It should be noted that incubation (prepatent) periods were prolonged for eight and ten days in Mmu 8158 and Mmu 8189, the recipients of primaquine in doses of 0.375 mg per kg without companion drug. The incubation period was significantly longer than this in Mmu 8232, Mmu 8224, and Mmu 8201, respectively the recipients of primaquine in combination with proguanil, pyrimethamine, and oxytetracycline. In the case of the latter two drugs, extension of the prepatent period could be attributed to persistence of blood schizonticidal concentrations of drug; however, this may not be the entire explanation even for these agents. It would not account for the extension of the incubation period encountered with proguanil, delivered either alone or in combination with primaquine. It is desirable to recognize the existence of these delays even though explaining them is not possible within the boundaries of the present study.

Monkeys which developed infections in the prophylactic experiments just described were promptly assigned to pilot assessments of the capacities of the various "prophylactic" agents to enhance the radical curative activity of primaquine. With one exception, relating to the length of the post-treatment follow-up period, conventional procedures were utilized in evaluating curative activity. The length of this follow-up period, usually 105 consistently negative days after delivery of the last drug dose, was extended to 210 days because of three very late relapses. Proguanil, pyrimethamine, WR-158,122, oxytetracycline, and WR-203,659 were administered at one-fourth of the MTD, as was WR-203,661, to one subject. Four other monkeys were treated with the latter 7-chlorolincomycin derivative at doses of 2.5 mg per kg. One monkey received chloroquine at the usual companion drug dose of 2.5 mg per kg body weight. Primaquine was administered in daily doses of 0.375 mg per kg body weight to all monkeys except those receiving 2.5 mg WR-203,661 per kg; these received this 8-aminoquinoline at a daily dose of 0.188 mg per kg body weight.

The results of this pilot evaluation, summarized in Table 37, show that cures were not achieved when chloroquine, proguanil, WR-158,122, oxytetracycline, and WR-203,659 were used as companion drugs. Relapse intervals in both recipients of WR-158,122 and in one of the two recipients of WR-203,659 were prolonged markedly. This result was not surprising in the case of the latter compound, which has significant tissue schizonticidal activity. It was very surprising in the case of WR-158,122 and contrasts with the short relapses associated with administration of this quinazoline, alone, to monkeys with developed sporozoite-induced infections.

More importantly, the data in Table 37 show that the combination regimen of 0.188 mg primaquine with 2.5 mg WR-203,661 per kg body weight was uniformly curative. Since in a very large experience this dose of primaquine has never exhibited curative activity* when administered in combination with chloroquine, it seems reasonable to conclude that the capacity of this 8-aminoquinoline to effect radical cure has been enhanced by the concomitant administration of WR-203,661.

Cures achieved with the combination of pyrimethamine and 0.375 mg per kg doses of primaquine (cf Mmu 8223 and Mmu 8224) were not anticipated. However, review of previous studies on this drug combination showed that attention had been given primarily to the dose of this pyrimidine required to cure infections with P. cynomolgi when administered with daily doses of 0.75 mg primaquine per kg body weight. In actuality, there had never been a critical study of the capacity of pyrimethamine to enhance the curative activity of primaquine utilizing a constant dose of the substituted pyrimidine and decreasing doses of the 8-aminoquinoline.

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*These studies have shown that primaquine is rarely curative when administered in daily doses of 0.375 mg per kg, together with chloroquine, 2.5 mg per kg. A daily dose of 0.5 mg per kg is close to a CD_{50}, a dose of 0.75 mg per kg approximates a CD_{99}.

The apparent enhancement of the prophylactic and radical curative activities of primaquine, attained by the concomitant administration of WR-203,661, led to plans for more complete assessment of the radical curative component of the phenomenon. Recognition that pyrimethamine, a well-studied agent, might have similar enhancing activities, resulted in alterations in these plans. These modifications, pursued without increasing the numbers of monkeys committed to the experiment, reduced the strength of the experiment to little more than another pilot investigation. Since the design of the experiment was complicated, a brief description of its major features may be useful.

Each of 24 rhesus monkeys was inoculated with 1.6×10^6 sporozoites. Infections were patent eight days thereafter. When parasitemias approximating 10 per 10^4 erythrocytes were attained, 23 of the monkeys were assigned to various treatment regimens; the 24th subject served as an untreated control. The specific assignments are best indicated by reference to Table 38. It should be noted that chloroquine was a component of all treatment regimens and was administered routinely (starting 48 hours after the last dose of primaquine, WR-203,661, pyrimethamine, or their combinations) to make certain that blood schizonts would be eliminated. Such a procedure was deemed essential since the amounts of WR-203,661 and pyrimethamine included in some combinations were below the doses required for cure of trophozoite-induced infections.

Treatment of relapses was dealt with on a drug rotational basis. For example, monkeys assigned to regimens containing WR-203,661 for treatment of primary attacks were assigned to regimens containing pyrimethamine for treatment of first relapses, then to regimens containing WR-203,661 for second relapses, then to regimens containing pyrimethamine for third relapses, etc. In the majority of cases, follow-up

treatment with chloroquine was not carried out when the doses of WR-203,661 and pyrimethamine were increased to levels known to be capable of curing blood-induced infections.

The results of these evaluations have been summarized in Table 39. The benefits of concomitant administration of WR-203,661 on the radical curative activity of primaquine were apparent in this study, but they were much less impressive than in the pilot experiments described above. The combination of 2.5 mg WR-203,661 and 0.188 mg primaquine per kg body weight evoked marked extension of the time between relapses. Combination of the above dose of WR-203,661 with 0.375 mg primaquine per kg cured four of six infections. Systematic curative activity was not encountered in any pyrimethamine/primaquine regimen; some combinations did lead to extension of the intervals between relapse.

Despite attempts to do so, there was no easy way of reconciling the limited enhancement of the curative activity of primaquine just described with the striking enhancement noted in the original pilot evaluation. In searching for an explanation for these divergent findings, it occurred to us that they could be related to differences in tissue schizont burdens, even though the initial sporozoite inocula were essentially the same. Each of the monkeys employed in the pilot evaluation had received primaquine previously in the prophylactic component of the study. This exposure was associated with significant prolongation of the incubation period. In most cases, this lengthening could not be attributed to persistence of the companion drug in effective blood schizonticidal concentrations. It seemed more reasonable to ascribe the delay in onset of patency to injury and partial destruction of sporozoite or tissue stage progeny. This would have left a residual tissue stage burden much smaller than that which prevails in previously untreated infections. This lesser burden may have

been responsible for the excellent performance of the combinations of WR-203,661 and primaquine in the initial evaluation of curative activity. If so, a similar result should be attainable in previously untreated infections by lowering the sporozoite inoculum. This possibility led to design of an experiment aimed at determining whether the capacity of WR-203,661 to enhance the radical curative activity of primaquine is significantly influenced by the size of the sporozoite inoculum.

Twenty-eight monkeys were assigned to this radical curative study. All were inoculated intravenously with sporozoites derived from the same lot of infected mosquitoes; fourteen received an inoculum of 8.5×10^3 sporozoites; fourteen received an inoculum of 8.5×10^5 sporozoites. All monkeys developed patent infections eight days after inoculation. Drug treatments were initiated as soon as parasitemias approximated 10 per 10^4 erythrocytes. Results have been summarized in Table 40.

Comments will be limited to the results achieved in treating primary attacks since responses of relapses can be compromised by previous drug experience, particularly when such an experience covers an agent like primaquine of proven tissue schizonticidal activity. The data in Table 40 show that WR-203,661 was remarkably effective in enhancing the curative activity of primaquine when delivered to monkeys challenged with approximately 10^4 sporozoites. Thus administration of WR-203,661 in daily doses of 2.5 mg per kg, together with primaquine at doses of either 0.188 or 0.375 mg per kg resulted in cure of eight of eight infections. This response contrasted sharply with that obtained in monkeys inoculated with approximately 10^6 sporozoites. None of four previously untreated infections were cured by administration of primaquine in doses of 0.188 mg per kg and only one of four infections was cured by doses of 0.375 mg per kg.

It should be noted that there were three cures at this dose level among the monkeys previously treated with 0.188 mg per kg doses. These observations emphasize the importance of using relatively modest inocula in preliminary assessments of the capacities of second agents to enhance the curative activity of primaquine. They also provide a reasonable explanation for the remarkable benefits derived from combining WR-203,661 and primaquine in the initial pilot study of curative activity.

Interest generated by the capacity of WR-203,661 to enhance the radical curative activity of primaquine led to pilot explorations of the capacities of other agents to produce similar benefits and specifically to a study of the efficacies of cordycepin, erythromycin, rifampin, cycloheximide, and valinomycin. The reasons for evaluating these agents have been mentioned earlier and will not be repeated here. The experiment followed the pattern of conventional curative drug studies, except that an inoculum of 4.5×10^4 sporozoites was employed. Also, chloroquine was included in each regimen since none of the compounds under study exhibited effective blood schizonticidal activity at the doses employed.

The results of the assessments performed on the above miscellaneous agents (cf Table 41) show that none enhanced the curative activity of primaquine at the doses administered. The few cures obtained in recipients of cordycepin, erythromycin, and rifampin were probably due to the primaquine/chloroquine component of the combination which by itself eliminated previously untreated infections. A striking feature of this study was the failure to extend the relapse interval when any of the compounds was administered in combination with 0.188 mg per kg doses of primaquine.

At present, WR-203,661 stands alone with respect to capacity to enhance the radical curative activity of primaquine. Whether this finding is of practical significance remains to be determined. More information is required on the impacts of variations in sporozoite dosage on this enhancement, particularly as variations in inoculum size occur in humans infected with P. vivax under natural conditions. More information is required on the importance of the dosage regimen of WR-203,661. The need for companion administration of chloroquine is a matter for further study. Evaluations of the toxicity of combinations of WR-203,661 and primaquine (and possibly chloroquine) are essential. Difficulties in this area would not be anticipated because of the dose of WR-203,661 required for enhancement - the human equivalent of 0.8 mg per kg - but hard information on the safety of the combination is required. Finally, if the results of all evaluations favor application of WR-203,661 as a companion drug to primaquine, careful consideration must be given to the impacts of such use on the future of lincomycin and its congeners as antimicrobial drugs.

In concluding this section, it should be stressed that whether combination therapy with primaquine and WR-203,661 proves to be practical or not is probably less important than the demonstration that it is possible to improve the activity of this 8-aminoquinoline. This finding should encourage more extensive investigations of the enhancement approach to development of more effective curative drug regimens in which primaquine (or other 8-aminoquinoline) is a component of the combination.

TABLE 36

THE CAPACITIES OF WR-203,661 AND MISCELLANEOUS BLOOD SCHIZONTICIDES TO ENHANCE THE PROPHYLACTIC ACTIVITY OF PRIMAQUINE *IN RHESUS MONKEYS CHALLENGED WITH SPOROZOITES* OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

Prophylactic Regimen*		Mmu No.	Day of Patency after Challenge	Days Delay in Onset of Patency†
Companion Drug	Primaquine			
Name/WR- No.	Daily Dose Mg/Kg Body Weight			
-	0.375	8158	18	10
-	0.375	8189**	16	8
-	0.75	8159	Protected	>79††
Proguanil 20.0	-	8230	27	19
Proguanil 20.0	0.375	8232	31	23
Pyrimethamine 0.6	-	8223	25	17
Pyrimethamine 0.6	0.375	8224	48	40
WR-158,122 10.0	-	8182	18	10
WR-158,122 10.0	0.375	8183	21	13
Oxytetracycline 80.0	-	8201	12	4
Oxytetracycline 80.0	0.375	8222	28	20
WR-203,659 80.0	-	8192	21	13
WR-203,659 80.0	0.375	8193	Protected	>79
WR-203,661 10.0	-	8159**	30	22
WR-203,661 10.0	-	8188	35	27
WR-203,661 0.156	0.375	8281**	21	13
WR-203,661 0.625	0.375	8193**	50	42
WR-203,661 2.5	0.375	8282**	Protected	>70
WR-203,661 10.0	0.375	8189	Protected	>79

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

**Monkeys so designated were in second pilot experiment.

†Infections in untreated controls were patent on Day 8 after inoculation.

††Absence of parasitemia on these days is synonymous with complete protection.

TABLE 37

THE CAPACITIES OF WR-203,661 AND MISCELLANEOUS BLOOD SCHIZONTICIDES TO ENHANCE THE RADICAL CURATIVE ACTIVITY OF PRIMAQUINE IN RHESUS MONKEYS INFECTED WITH THE B STRAIN OF PLASMODIUM CYNOMOLGI

Curative Regimen*			Mmu No.	Response to Treatment	
Companion Drug		Primaquine		Relapsed	Cured
Name/WR- No.	Daily Dose Mg/Kg Body Weight			Days Between R_x and Relapse	
Chloroquine	2.5	0.375	8158P	38	-
Proguanil	20.0	0.375	8230P	19	-
	20.0	0.375	8232P	43	-
Pyrimethamine	0.6	0.375	8223P	-	+
	0.6	0.375	8224P	-	+
WR-158,122	10.0	0.375	8182P	88	-
	10.0	0.375	8183P	134	-
Oxytetracycline	80.0	0.375	8201P	6	-
	80.0	0.375	8202P	13	-
WR-203,659	80.0	0.375	8192P	21	-
	80.0	0.375	$8201R_1$	101	-
WR-203,661	2.5	0.188	8159P	-	+
	2.5	0.188	8189P	-	+
	2.5	0.188	8193P	-	+
	2.5	0.188	8281P	-	+
	10.0	0.375	8188P	-	+

*Primaquine and companion drug administered simultaneously, once daily for seven consecutive days.

TABLE 38

DOSAGE REGIMENS AND ASSIGNMENTS EMPLOYED IN THE COMPARISON OF THE CAPACITIES OF WR-203,661 AND PYRIMETHAMINE TO ENHANCE THE RADICAL CURATIVE ACTIVITY OF PRIMAQUINE

Mmu No.	Drug Regimen - Mg Base/Kg Body Weight Daily x 7				
	Primary Attack				Post-Primary*
	Primaquine	Pyrimethamine	WR-203,661	Chloroquine	Chloroquine
8332	0.5	0.15	-	-	5.0
8333	0.25	0.15	-	-	5.0
8334	0.125	0.15	-	-	5.0
8335	0.0625	0.15	-	-	5.0
8336	0.25	0.075	-	-	5.0
8340	0.25	0.0375	-	-	5.0
8342	0.25	0.0188	-	-	5.0
8343	0.25	0.0094	-	-	5.0
8346	0.5	-	2.5	-	5.0
8347	0.25	-	2.5	-	5.0
8352	0.125	-	2.5	-	5.0
8353	0.0625	-	2.5	-	5.0
8358	0.25	-	1.25	-	5.0
8359	0.25	-	0.625	-	5.0
8360	0.25	-	0.312	-	5.0
8361	0.25	-	0.156	-	5.0
8362	-	0.15	-	-	5.0
8363	-	0.6	-	-	5.0
8364	-	-	2.5	-	5.0
8365	-	-	10.0	-	5.0
8366	0.25	-	-	-	5.0
8367	0.5	-	-	-	5.0
8378	-	-	-	2.5	5.0
8381	-	-	-	-	-

*Post-primary treatment initiated 48 hours after end of treatment of the primary attack.

TABLE 39

COMPARISON OF THE CAPACITIES OF WR-203,661 AND PYRIMETHAMINE TO ENHANCE THE RADICAL CURATIVE ACTIVITY OF PRIMAQUINE

Daily Dose - Mg/Kg Body Weight x 7				Mmu No.	Response to Treatment	
					Relapsed	
WR-203,661	Pyrimeth-amine	Primaquine	Chloroquine		Days Between R_x and Relapse	Cured
0.156	-	0.25	5.0	8361P	10	-
0.156	-	0.25	5.0	$8343R_1$	97	-
0.312	-	0.25	5.0	8360P	10	-
0.312	-	0.25	5.0	$8342R_1$	23	-
0.625	-	0.25	5.0	8359P	15	-
0.625	-	0.25	5.0	$8340R_1$	19	-
1.25	-	0.25	5.0	8358P	38	-
1.25	-	0.25	5.0	$8336R_1$	27	-
2.5	-	0.0625	5.0	8353P	10	-
2.5	-	0.125	5.0	8352P	17	-
2.5	-	0.125	5.0	$8335R_1$	19	-
2.5	-	0.188	5.0	$8332R_1$	48	-
2.5	-	0.188	-	$8353R_2$	40	-
2.5	-	0.188	5.0	$8359R_2$	32	-
2.5	-	0.188	5.0	$8360R_2$	35	-
2.5	-	0.188	-	$8361R_2$	24	-
2.5	-	0.188	-	$8364R_2$	24	-
2.5	-	0.188	-	$8365R_2$	20	-
2.5	-	0.25	5.0	8347P	13	-
2.5	-	0.25	5.0	$8334R_1$	72	-
2.5	-	0.375	5.0	$8362R_3$	28	-
2.5	-	0.375	5.0	$8363R_3$	22	-
2.5	-	0.375	-	$8358R_2$	-	+
2.5	-	0.375	-	$8335R_3$	-	+
2.5	-	0.375	-	$8336R_3$	-	+
2.5	-	0.375	-	$8340R_3$	-	+
2.5	-	0.5	5.0	8346P	63	-
2.5	-	0.5	5.0	$8333R_1$	-	+

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TABLE 39 - CONTINUED

Daily Dose - Mg/Kg Body Weight x 7				Mmu No.	Response to Treatment	
					Relapsed	
WR-203,661	Pyrimeth-amine	Primaquine	Chloroquine		Days Between R_x and Relapse	Cured
-	0.0094	0.25	5.0	8343P	17	-
-	0.0094	0.25	5.0	8361R_1	12	-
-	0.0188	0.25	5.0	8342P	10	-
-	0.0188	0.25	5.0	8360R_1	33	-
-	0.0375	0.25	5.0	8340P	10	-
-	0.0375	0.25	5.0	8359R_1	31	-
-	0.075	0.25	5.0	8336P	13	-
-	0.15	0.0625	5.0	8335P	6	-
-	0.15	0.125	5.0	8334P	13	-
-	0.15	0.125	5.0	8353R_1	12	-
-	0.15	0.25	5.0	8333P	17	-
-	0.15	0.25	5.0	8352R_1	76	-
-	0.15	0.5	5.0	8332P	73	-
-	0.15	0.5	-	8347R_1	-	+
-	0.6	0.188	-	8358R_1	40	-
-	0.6	0.188	-	8335R_2	31	-
-	0.6	0.188	-	8336R_2	22	-
-	0.6	0.188	-	8340R_2	36	-
-	0.6	0.188	5.0	8342R_2	41	-
-	0.6	0.188	-	8362R_2	20	-
-	0.6	0.188	-	8363R_2	24	-
-	0.6	0.188	-	8346R_1	-	+
-	0.6	0.375	-	8353R_3	84	-
-	0.6	0.375	5.0	8361R_3	47	-
-	0.6	0.375	5.0	8365R_3	43	-
-	0.6	0.375	-	8364R_3	-	+

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TABLE 39 - CONTINUED

Daily Dose - Mg/Kg Body Weight x 7				Mmu No.	Response to Treatment	
					Relapsed	
WR-203,661	Pyrimeth-amine	Primaquine	Chloroquine		Days Between R_x and Relapse	Cured
2.5	-	-	5.0	8364P	10	-
2.5	-	-	5.0	$8362R_1$	12	-
10.0	-	-	5.0	8365P	10	-
10.0	-	-	5.0	$8363R_1$	15	-
-	0.15	-	5.0	8362P	7	-
-	0.15	-	5.0	$8364R_1$	11	-
-	0.6	-	5.0	8363P	15	-
-	0.6	-	5.0	$8365R_1$	11	-
-	-	0.25	5.0	8366P	11	-
-	-	0.5	5.0	8367P	-	+
-	-	0.5	5.0	$8366R_1$	-	+
-	-	-	2.5	8378P	10	-
-	-	-	2.5	$8378R_1$	8	-
-	-	-	2.5	$8378R_2$	9	-
-	-	-	2.5	$8378R_3$	6	-
-	-	-	2.5	$8378R_4$	6	-
-	-	-	2.5	$8378R_5$	7	-
-	-	-	2.5	$8378R_6$	7	-
-	-	-	2.5	$8378R_7$	7	-
-	-	-	2.5	$8378R_8$	6	-
-	-	-	2.5	$8378R_9$	7	-
-	-	-	2.5	$8378R_{10}$	7	-
-	-	-	2.5	$8378R_{11}$	8	-
-	-	-	2.5	$8378R_{12}$	14	-
-	-	-	2.5	$8378R_{13}$	14	-
-	-	-	2.5	$8378R_{14}$	10	-

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TABLE 40

THE INFLUENCE OF THE SIZE OF THE SPOROZOITE INOCULUM ON THE CAPACITY OF WR-203,661 TO ENHANCE THE RADICAL CURATIVE ACTIVITY OF PRIMAQUINE

Daily Dose - Mg/Kg Body Weight x 7			Mmu No.	Response to Treatment	
				Relapsed	Cured
WR-203,661	Primaquine	Chloroquine		Days Between R_x and Relapse	
Inoculum - 10^4 Sporozoites					
2.5	0.094	-	8481R_2	55	-
2.5	0.188	-	8424P	-	+
2.5	0.188	-	8432P	-	+
2.5	0.188	-	8442P	-	+
2.5	0.188	-	8443P	-	+
2.5	0.375	-	8465P	-	+
2.5	0.375	-	8466P	-	+
2.5	0.375	-	8467P	-	+
2.5	0.375	-	8479P	-	+
-	0.188	2.5	8384P	50	-
-	0.188	2.5	8385P	50	-
-	0.375	2.5	8416P	13	-
-	0.375	2.5	8417P	75	-
-	0.375	2.5	8384R_1	-	+
-	0.375	2.5	8385R_1	-	+
-	0.5	2.5	8416R_1	-	+
-	0.5	2.5	8417R_1	-	+
2.5	-	-	8481P	20	-
10.0	-	-	8481R_1	37	-
-	-	2.5	8480P	15	-
-	-	2.5	8480R_1	10	-
-	-	2.5	8480R_2	32	-
-	-	2.5	8480R_3	25	-
-	-	2.5	8480R_4	10	-
-	-	2.5	8480R_5	19	-

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TABLE 40 - CONTINUED

Daily Dose - Mg/Kg Body Weight x 7			Mmu No.	Response to Treatment	
				Relapsed	
WR-203,661	Primaquine	Chloroquine		Days Between R_x and Relapse	Cured
Inoculum - 10^6 Sporozoites					
2.5	0.188	-	8450P	24	-
2.5	0.188	-	8451P	28	-
2.5	0.188	-	8452P	16	-
2.5	0.188	-	8454P	18	-
2.5	0.375	-	8455P	58	-
2.5	0.375	-	8457P	56	-
2.5	0.375	-	8459P	66	-
2.5	0.375	-	$8454R_1$	53	-
2.5	0.375	-	8456P	-	+
2.5	0.375	-	$8450R_1$	-	+
2.5	0.375	-	$8451R_1$	-	+
2.5	0.375	-	$8452R_1$	-	+
10.0	0.188	-	$8455R_1$	34	-
10.0	0.188	-	$8459R_1$	100	-
10.0	0.188	-	$8454R_2$	27	-
10.0	0.188	-	$8474R_3$	44	-
10.0	0.188	-	$8457R_1$	-	+
-	0.188	2.5	8387P	4	-
-	0.188	2.5	8440P	9	-
-	0.375	2.5	8441P	11	-
-	0.375	2.5	8446P	16	-
-	0.375	2.5	$8387R_1$	-	+
-	0.375	2.5	$8440R_1$	-	+
-	0.5	2.5	$8441R_1$	-	+
-	0.5	2.5	$8446R_1$	-	+

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TABLE 40 - CONTINUED

Daily Dose - Mg/Kg Body Weight x 7			Mmu No.	Response to Treatment	
WR-203,661	Primaquine	Chloroquine		Relapsed: Days Between R_x and Relapse	Cured
Inoculum - 10^6 Sporozoites					
2.5	-	-	8474P	10	-
2.5	-	-	8474R_1	24	-
10.0	-	-	8474R_2	40	-
-	-	2.5	8462P	4	-
-	-	2.5	8462R_1	8	-
-	-	2.5	8462R_2	7	-
-	-	2.5	8462R_3	8	-
-	-	2.5	8462R_4	9	-
-	-	2.5	8462R_5	12	-
-	-	2.5	8462R_6	11	-
-	-	2.5	8462R_7	10	-
-	-	2.5	8462R_8	5	-
-	-	2.5	8462R_9	10	-
-	-	2.5	8462R_{10}	10	-
-	-	2.5	8462R_{11}	13	-

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TABLE 41

ASSESSMENTS OF THE CAPACITIES OF CORDYCEPIN, ERYTHROMYCIN, RIFAMPIN, CYCLOHEXIMIDE, AND VALINOMYCIN TO ENHANCE THE RADICAL CURATIVE ACTIVITY OF PRIMAQUINE

Curative Regimen			Mmu No.	Response to Treatment	
Companion Drug		Primaquine		Relapsed	
Name/WR- No.	Daily Dose Mg/Kg Body Weight*			Days Between R_x and Relapse	Cured
-	-	0.188	8447P	39	-
-	-	0.375	8448P	-	+
Cordycepin	1.0	-	8449P	7	-
	1.0	0.188	8463P	8	-
	1.0	0.188	8449R_1	14	-
	1.0	0.375	8463R_1	-	+
	5.0	-	8453P	5	-
	5.0	0.188	8464P	11	-
	5.0	0.188	8453R_1	7	-
	5.0	0.375	8464R_1	-	+
Erythromycin	10.0	-	8470P	4	-
	10.0	0.188	8497P	8	-
	10.0	0.188	8470R_1	9	-
	10.0	0.375	8497R_1	-	+
	50.0	-	8471P	4	-
	50.0	0.188	8498P	8	-
	50.0	0.188	8471R_1	7	-
	50.0	0.375	8498R_1	69	-
Rifampin	5.0	-	8500P	4	-
	5.0	0.188	8518P	8	-
	5.0	0.188	8500R_1	13	-
	5.0	0.375	8518R_1	-	+
	20.0	-	8508P	4	-
	20.0	0.188	8531P	4	-
	20.0	0.188	8508R_1	8	-
	20.0	0.375	8531R_1	10	-
Cycloheximide WR-13,255	10.0	-	8382R_1	7	-
	10.0	0.188	8508R_2	13	-
Valinomycin WR-124,892	5.0	-	8391R_1	7	-
	5.0	0.188	8471R_2	9	-

*Each subject received chloroquine, 2.5 mg/kg daily, concomitantly with primaquine and companion drug.

XIV. THE CAPACITY OF CHLOROQUINE TO CURE SPOROZOITE-INDUCED INFECTIONS - WITH EMPHASIS ON THE SIZE OF THE INOCULUM

XIV. THE CAPACITY OF CHLOROQUINE TO CURE SPOROZOITE-INDUCED INFECTIONS - WITH EMPHASIS ON THE SIZE OF THE INOCULUM

In 1946, when infections with sporozoites of the M strain of P. cynomolgi were first used in the search for a curative 8-aminoquinoline better tolerated and more active than pamaquine, quinine was employed as a companion drug to insure elimination of blood schizonts. Control experiments showed that repetitive delivery of quinine alone (in doses of 80.0 mg per kg daily for seven days or 40.0 mg per kg daily for fourteen days) during the primary attack and successive relapses, spread over a twelve month post-inoculation period, was without curative effect. Similar control experiments were carried out with chloroquine in 1950-1951, when the role of companion blood schizonticide was accorded this 4-aminoquinoline. These showed that repetitive delivery of chloroquine at doses of 2.5 mg per kg daily for seven days or 5.0 mg per kg daily for four days would not cure infections with sporozoites of the M strain*. Comparable results were obtained in early 1960 when the M strain of P. cynomolgi was replaced by the B strain. These studies led to the conclusion that cures resulting from administration of a prospective curative compound with either companion drug would have to be ascribed to the new agent.

In the current Malaria Chemotherapy Program, the same infected monkey has been maintained for evaluating the activities of new agents as long as relapses occur; in some cases, reuses have covered more than a year following sporozoite inoculation**. Throughout this Program, responses to

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*These evaluations were carried out in monkeys inoculated with 10^2 to 10^4 sporozoites.

**In pre-1960 studies, the period of animal use was much shorter than this, because when treatment of a primary attack or first relapse with a new compound did not lead to cure, the second relapse was invariably treated with a known active agent (e.g., pamaquine, pentaquine, or isopentaquine) to determine more effective ways of delivering the compound.

the chloroquine component of the curative dose regimen have been checked repeatedly. No infection has been cured by repeated administration of chloroquine alone in course doses of 2.5 or 5.0 mg base daily for seven days. These checks have provided additional support for the conclusion that chloroquine will not cure infections induced by moderate to large doses of sporozoites when administered repetitively with another drug for periods in excess of a year.

However, the situation when chloroquine is administered by itself may not be quite the same as when chloroquine is administered with barely sub-curative doses of a known curative drug or a new agent with marginal curative activity. In such situations, often identified by association with protracted intervals between relapses, the pool of tissue schizonts may be reduced to a level just large enough to initiate the next relapse, but too small to reseed the hepatocytes. Under this circumstance, elimination of the blood schizonts (by chloroquine) would result in cure. If this possibility is real, and goes unrecognized, time could be lost and false hopes generated by following "leads" on false positives.

The possibility that chloroquine by itself can be curative was explored in relapses occurring after an unduly long parasite negative period (30+ days). The results of this effort were equivocal, probably because of the heterogeneity of the material employed in the test. They led to the decision to measure directly the influence of the size of the sporozoite inoculum (ergo, tissue schizont burden) on the curative capabilities of chloroquine.

Seventeen monkeys were committed to this study. As shown in Table 42, five received inocula of 5 sporozoites, five 5×10^2 sporozoites, three 5×10^4 sporozoites, and four

5×10^6 sporozoites. Two of the recipients of 5 sporozoites did not develop parasitemias*. The other three recipients of 5 sporozoites developed patent infections with incubation periods of 13, 19, and 42 days. All monkeys inoculated with 5×10^2 or larger numbers of sporozoites developed patent infections. The incubation periods were eight days in recipients of 5×10^4 or 5×10^6 sporozoites, nine to eleven days in recipients of 5×10^2 sporozoites.

As soon as it was certain that infections were patent (equivalent to exhibition of two positive thick films on consecutive days), chloroquine treatment was initiated at a dose of 5.0 mg per kg body weight, daily for five consecutive days. Thick film assessments were carried out on an every other day or daily schedule throughout the study. As soon as relapses were identified and confirmed, chloroquine was administered as described above. This procedure precluded development of countable parasitemias and thereby acquisition of a significant level of immunity.

The results of chloroquine treatment, summarized in Table 43, show that infections induced by 5 sporozoites were cured by the first course of chloroquine. No other infections were cured by 9 to 24 chloroquine courses delivered over a period slightly in excess of ten months. The numbers of relapses and the intervals between relapses varied roughly with the size of inoculum. Thus, recipients of 5×10^2 sporozoites exhibited 9 to 12 relapses and median intervals between relapse of 14 to 18.5 days; recipients of 5×10^6 sporozoites exhibited 17 to 24 relapses with median interrelapse intervals of 4.5 to 11 days.

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*These monkeys were rechallenged 225 and 249 days after the original inoculation and proved to be susceptible.

This experiment is still in progress. It has gone far enough, however, to show that when the burden of tissue stages is barely large enough to initiate an infection, eradication of the blood schizonts by chloroquine can be synonymous with cure of the disease. It would be totally incorrect to ascribe tissue schizonticidal activity to chloroquine in this situation. Nonetheless, the phenomenon is an important one, not just in the readily definable milieu of the current experiment, but in the long standing infection when a substantial interrelapse period may reflect a tissue burden just equal to reinitiating infection of the erythrocytes. From the practical point of view, it may be wise to avoid using long term relapses for evaluation of new agents. The best that might be said for their use is that if another relapse does occur, such shows clearly that the test compound is truly inactive, at least at the dose administered.

TABLE 42

THE INFLUENCE OF INOCULUM SIZE ON THE INCUBATION PERIOD IN RHESUS MONKEYS CHALLENGED WITH SPOROZOITES OF THE B STRAIN OF PLASMODIUM CYNOMOLGI

No. of Sporozoites in Inoculum	Mmu No.	Day of Patency after Sporozoite Inoculation	Remarks
5	7903	-	Rechallenged with 10^6 sporozoites Day 249, infection patent Day 8.
	7908	-	Rechallenged with 10^6 sporozoites Day 225, infection patent Day 8.
	7849	19	
	7901	13	
	7905	42	
5×10^2	7846	11	
	7847	11	
	7848	11	
	7906	9	
	7907	9	
5×10^4	7431	8	
	7841	8	
	7845	8	
5×10^6	7843	8	
	7844	8	
	8150	8	
	8173	8	

TABLE 43

THE INFLUENCE OF THE SPOROZOITE INOCULUM ON THE COURSE OF INFECTIONS WITH THE B STRAIN OF PLASMODIUM CYNOMOLGI AND ON THE CAPACITY OF CHLOROQUINE TO "CURE" SUCH INFECTIONS

No. of Sporozoites in Inoculum	Mmu No.	No. of Relapses Observed*	Days Between Treatment and Relapse	
			Median	Range
5	7849	0	-	-
	7901	0	-	-
	7905	0	-	-
5×10^2	7846	> 9	14.0	11 - 38
	7847	>10	18.5	10 - 29
	7848	>12	16.5	8 - 39
	7906	>12	15.0	7 - 42
	7907	>11	17.0	8 - 36
5×10^4	7431	> 9	12.0	7 - 23
	7841	>16	9.5	3 - 22
	7845	>15†	9.0	5 - 14
5×10^6	7843	>17	11.0	<2 - 32
	7844	>24	4.5	<2 - 18
	8150	>13†	7.0	<2 - 25
	8173	>21	9.0	<2 - 15

*At Day 317 post-inoculation.

†Mmu 7845 died from a pneumococcal lobar pneumonia on Day 255 post-inoculation, six days after Relapse 15; Mmu 8150 died of a peritonitis (Klebsiella pneumoniae) Day 230 post-inoculation, thirteen days after Relapse 13.

XV. COMPARISON OF THE PLASMA LEVELS OF WR-158,122 IN OWL AND RHESUS MONKEYS

XV. A COMPARISON OF THE PLASMA LEVELS OF WR-158,122 IN OWL AND RHESUS MONKEYS

The studies described in this Section represent an effort to find an explanation for some of the unusual and contradictory biological properties of WR-158,122, one of the three most active quinazolines examined in the Malaria Chemotherapy Program, and at one time considered to be a highly promising blood schizonticide. The characteristics of WR-158,122 which have led to this study are summarized briefly below.

WR-158,122 [2,4-diamino-6-(2-naphthyl)-6-sulfonylquinazoline] exhibited remarkable activity in owl monkeys infected with the pyrimethamine-susceptible Oak Knoll strain of P. falciparum, curing established infections at daily doses between 0.05 and 0.1 mg per kg. Activity was reduced substantially in the face of pyrimethamine resistance. However, the liabilities of such resistance could be controlled, if not largely eliminated, by concomitant administration of very small doses of a sulfonamide such as sulfadiazine.

In sharp contrast to its remarkable activity against infections with various human plasmodia in the owl monkey, WR-158,122 exhibited only modest activity against infections with the B strain of P. cynomolgi in the rhesus monkey. Doses of 10.0 mg per kg (one hundred times those that cured infections with the Oak Knoll strain of P. falciparum) were required to cure infections with trophozoites of the above strain of P. cynomolgi. Both strains are pyrimethamine sensitive.

Observations in human volunteers were even more disquieting. Thus, WR-158,122 exhibited a high capacity for inhibiting maturation of parasites of a drug susceptible strain of P. falciparum in Rieckmann's in vitro test when the pure compound was added to human plasma in very small quantities equivalent to a fraction of a microgram per ml. Plasma samples obtained from non-infected volunteers at various periods after ingestion of 250 mg doses of this quinazoline showed no capacity to block maturation of parasites of the same strain. Such doses, the equivalent of 3.5 mg per kg for a volunteer weighing 70 kg, were at least thirty times the dose required to cure an established infection with the Oak Knoll strain in the owl monkey.

It is quite possible that the striking discrepancies in the biological activities of WR-158,122 referred to above could be due to differences in the capacities of rhesus monkeys, owl monkeys, and humans to absorb and metabolize this quinazoline. The studies summarized below represented a multi-institutional effort aimed primarily at investigating the first of these possibilities. They included: (1) collection in this laboratory of plasma samples from owl and rhesus monkeys that had received various doses of WR-158,122; (2) collection in Dr. Arnold's laboratory (Kansas City, Missouri) of plasma samples from human volunteers treated with a similar range of doses; and (3) analysis of the various samples for "WR-158,122" content via use of a microbiological assay procedure in the laboratories of C. Genther and C. Smith (University of Cincinnati).

Twelve owl monkeys and eight rhesus monkeys were committed to the simian component of the study as originally designed. Groups of three owl monkeys were given doses of 0.35, 1.75, 3.5, and 12.5 mg WR-158,122 per kg body weight. Groups of two rhesus monkeys were given doses of 0.35, 1.75, 3.5 and 10.0 mg per kg body weight. Blood samples were withdrawn in heparinized glass syringes one hour prior to drug administration, and one, three, six, and twenty-four hours thereafter;

these samples were obtained from the left ventricular chamber of the owl monkey and from the antecubital vein of the rhesus monkey. Plasma, separated immediately by centrifugation, was transferred to a Vacutainer and stored at 4°C until shipment to Cincinnati, packed in wet ice. All phases of blood collection, plasma separation, and final transfer were carried out aseptically. Because of concerns with the results of the rhesus monkey component of this experiment, a repeat study was undertaken with a new group of eight subjects manipulated precisely as described above. Plasma samples from these monkeys were analyzed with a more sensitive assay procedure.

The results of the owl monkey segment of the above experiment, summarized in chart form in Figure 3*, show that significant concentrations of "WR-158,122" were achieved in the plasma of all subjects and that the average plasma levels at any given period correlated well with dose. There were variations in levels among the recipients of a given dose, but these were not greater than would be anticipated in experimental animals with heterogeneous backgrounds. Figure 3 also shows that with minor exceptions, plasma levels of "WR-158,122" peaked at approximately three hours and declined rather slowly thereafter. In two of each three recipients of doses of 0.35 and 1.75 mg per kg, levels had fallen below measurable concentrations twenty-four hours after dosage. In one of each three recipients of these doses, and in all recipients of doses of 3.5 and 12.5 mg per kg**, readily measurable concentrations of "WR-158,122" were still present at this time. In fact, the twenty-four hour levels in the recipients of the larger of these doses exceeded peak levels in recipients of 0.35 mg per kg doses.

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*The "WR-158,122" measurements recorded in this Figure and Figures 4 and 5 were obtained and provided by Clara Genther, University of Cincinnati.

**This dose and the 10.0 mg per kg dose administered to rhesus monkeys were included in the experiment because on a mg per M^2 basis, they are equivalent to the 250 mg dose which Rieckmann utilized in the human volunteer experiments referred to above.

The results summarized above provide solid support for at least two conclusions: (1) that WR-158,122 is well and regularly absorbed from the gastrointestinal tract of the owl monkey; and (2) that the excretion, tissue localization, and/or metabolism of WR-158,122 in the owl monkey proceed in highly reproducible ways. These qualities are in accord with and provide a reasonable pharmacologic base for the consistent performance of WR-158,122 in therapy of infections with various strains of P. falciparum and P. vivax in the owl monkey.

As indicated above, two studies were pursued in the rhesus monkey. The results of the first of these have been summarized in Figure 4, the results of the second in Figure 5. Note should be taken of the difference in the log scales in these figures, a reflection of the use of a more sensitive bioassay procedure in the second study (Figure 5), which made measurement of the lower concentrations feasible.

In most respects, the results of the first rhesus monkey experiment (Figure 4) are in sharp contrast to the results in the owl monkey arm of the study summarized above. Thus, in the rhesus monkey there were: (1) broad variations in the plasma levels of "WR-158,122" attained in different recipients of the same dose; (2) no apparent relation between height of plasma level and dose; (3) no persistence of "WR-158,122" (all plasma samples obtained twenty-four hours after dosage appeared to be free of "WR-158,122"); and (4) low levels of "WR-158,122" at all sampling periods. The only real similarity in results with the two species was the time at which levels of "WR-158,122" appeared to peak, three hours after dosage in both cases. Overall, the data on the rhesus monkey were difficult to interpret and understand, especially that component which shows no relation between dose and plasma level. This finding is in direct opposition to the dose - therapeutic effect relationship which characterized the activity of WR-158,122 against infections with P. cynomolgi.

In some respects, the results of the second rhesus monkey study were an improvement over the first. The levels of "WR-158,122" in plasma of different recipients of the same dose were more similar. In general, the height of the plasma level increased with dose. "WR-158,122" could be detected in plasma twenty-four hours after administration of WR-158,122, but, except for the recipients of the largest dose (10.0 mg per kg), the concentrations at this period bore little relation to dose. As with the initial rhesus monkey experiment, the levels of "WR-158,122" achieved at any given dose were well below those found in the owl monkey phase of the study.

It is difficult to explain the observations summarized above. It would appear that WR-158,122 is absorbed less well and less regularly in the rhesus monkey than in the owl monkey. It could be that the compound is also excreted or metabolized more rapidly in the former subject. It is highly important for the future of WR-158,122 (and probably for other quinazolines) that efforts be made to determine the cause of the above variability. If the latter results from an absorption defect, measures might be developed which would eliminate that problem. If the variability stems from differences in rate or quality of metabolism, correction could well be an unapproachable task.

The data summarized in the above sections help to understand the striking difference in antimalarial activity noted in the owl and rhesus monkey models. The difference could be strictly related to the dose required to produce a critical plasma level. In this connection, it should be pointed out that in the current study, the lowest dose administered to the owl monkey, 0.35 mg per kg, and the largest dose administered to the rhesus monkey, 10.0 mg per kg, yielded similar concentrations of "WR-158,122" in plasma.

Data on the plasma levels of "WR-158,122" in human volunteers are not available at this time. Correlation of such data, with those of the owl and rhesus monkey experiments, is obviously of critical importance to trials of this quinazoline in human volunteers.

FIGURE 3

PLASMA LEVELS OF "WR-158,122" FOLLOWING ORAL ADMINISTRATION
TO THE OWL MONKEY - MARCH 12, 1974

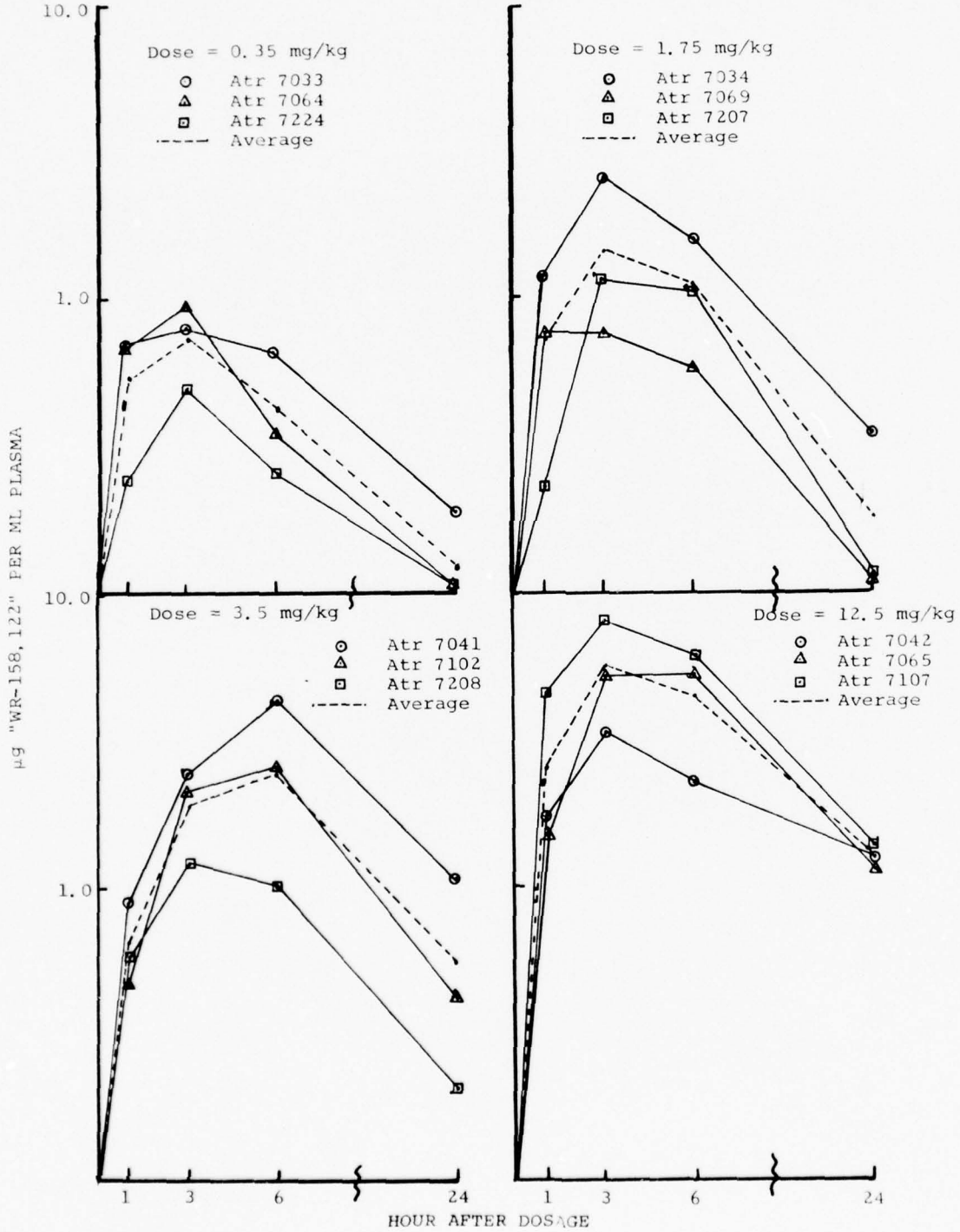

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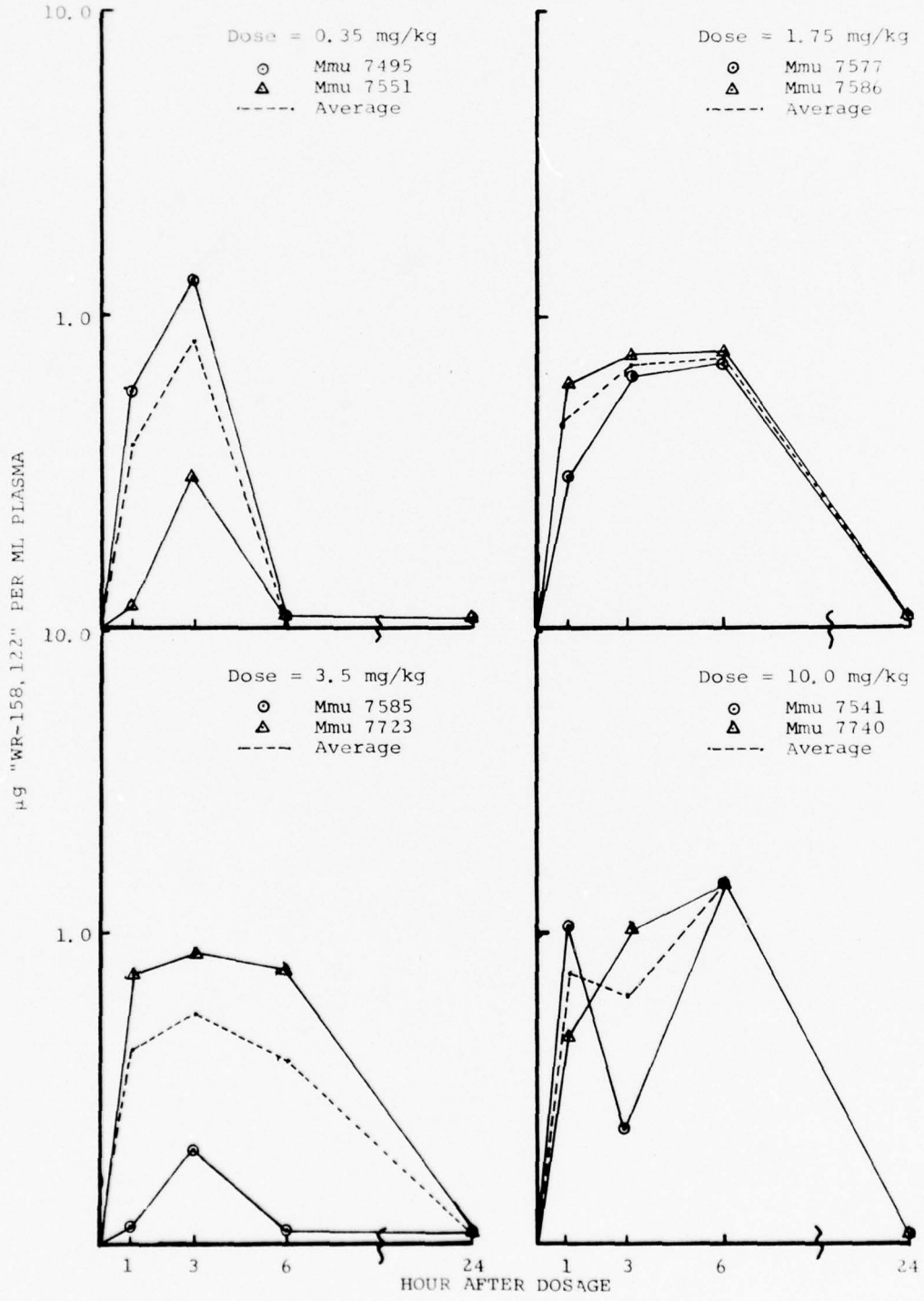
FIGURE 4

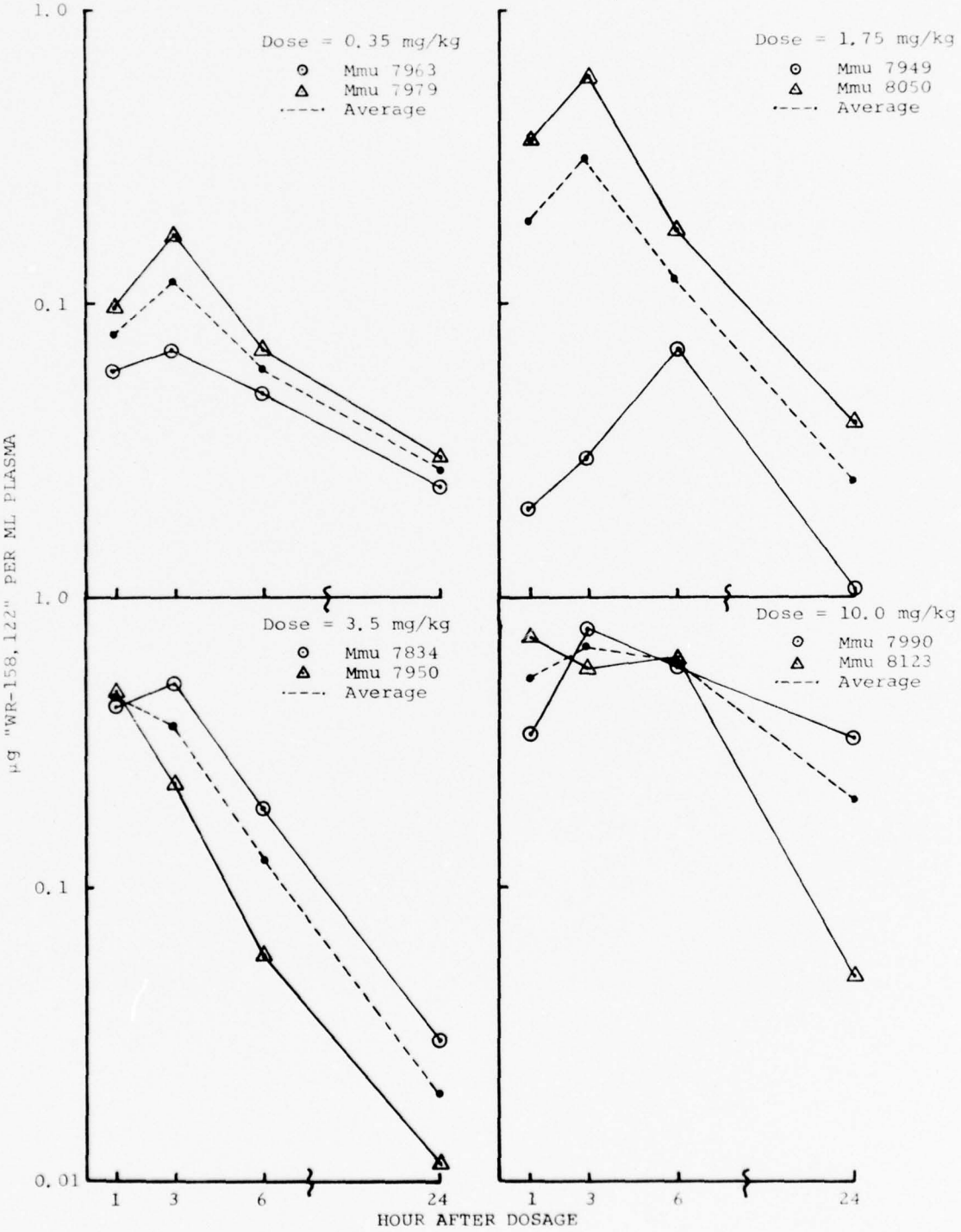
PLASMA LEVELS OF "WR-158,122" FOLLOWING ORAL ADMINISTRATION TO THE RHESUS MONKEY - MARCH 12, 1974



SOUTHERN RESEARCH INSTITUTE

FIGURE 5

PLASMA LEVELS OF "WR-158,122" FOLLOWING ORAL ADMINISTRATION
TO THE RHESUS MONKEY - MAY 3, 1974



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XVI. THE COMPARATIVE TOXICITIES OF PRIMAQUINE AND ITS D AND L COMPONENTS (WR-211,536 AND WR-211,537) FOR THE RHESUS MONKEY

XVI. THE COMPARATIVE TOXICITIES OF PRIMAQUINE AND ITS D AND L COMPONENTS (WR-211,536 AND WR-211,537) FOR THE RHESUS MONKEY

As related in Section X, interest in the D and L forms of primaquine (respectively, WR-211,536 and WR-211,537), stemmed originally from the observation that the acute toxicity of the D form for the mouse is approximately four times that of the L form*. This interest was enhanced by the finding that the radical curative activities of primaquine and its isomers were essentially the same. If the quantitative differences in the toxicities of the D and L isomers noted in the mouse carried over to other species, WR-211,537 offered distinct advantages over primaquine. Concern with this issue led to a preliminary comparison of the subacute toxicity of primaquine and its D and L components for the rhesus monkey.

Thirteen rhesus monkeys, both males and females, 4.0 to 5.0 kg in weight, were used in a direct compound-for-compound comparison. Two of the above number received primaquine,** four the D isomer (WR-211,536), and seven the L isomer (WR-211,537). All monkeys had been used previously in assessing radical curative activities of various new agents and had been discarded as cured after a minimum follow-up period of eighteen weeks since receiving the treatment which terminated their malarial infections.

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*This finding originated at WRAIR, but has been confirmed in principle in this laboratory. When administered orally to female BDF_1 mice in single doses, the LD_{50}'s for WR-211,536, primaquine, and WR-211,537 were 42, 100, and 150 mg base per kg body weight; the LD_{10}'s were 24, 72, and 110 mg base per kg, for the respective compounds.

**The tabulated data list the reactions of nine monkeys treated with primaquine. Mmu 8279 and Mmu 8193 were utilized in the current experiment. The other seven recipients of primaquine had been employed in earlier comparative toxicity studies involving other drugs, investigations pursued with the same methodology as was used in the present study. Inclusion of the data on these seven monkeys strengthens the observations on Mmu 8279 and Mmu 8193.

The experimental procedures used in the current assessment were briefly as follows. Complete blood counts and measurements of a limited group of chemical constituents in peripheral blood were carried out on at least two occasions prior to drug delivery*. Upon assurance of normality and stability of the formed elements and biochemical constituents, administration of the various agents was initiated. The requisite amount of each compound, freshly dissolved in 30 ml of distilled water was delivered via stomach tube and followed by a 20 ml distilled water rinse. Drug administration was accomplished on each of the planned seven consecutive treatment days between 8:00 and 8:15 a.m. Monkeys were weighed daily; drug doses were adjusted when weight loss occurred. Bleedings for hematologic and biochemical measurements were carried out twenty-four hours after delivery of the third and seventh doses and on Tuesdays and Fridays thereafter for twenty-one additional days. The bleeding schedule was modified appropriately for critically ill animals so as to insure obtaining antemortem specimens. Hourly surveillance was maintained throughout the entire day during the period of drug delivery and for forty-eight hours thereafter. Moribund monkeys were euthanized by overdosage with sodium pentobarbital and necropsied immediately thereafter to insure acquisition of tissues free from autolytic changes. Selected tissue specimens were fixed in Zenker-formalin, embedded in paraffin, sectioned at 6 microns, and stained with hematoxylin-eosin for histopathologic examination.

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*The hematologic moieties measured included erythrocytes, hemoglobin, hematocrit, reticulocytes, platelets, total leucocytes, neutrophils (diverse stages), lymphocytes, monocytes, basophils, and eosinophils. The chemical moieties included urea nitrogen, glucose, total protein, albumin, globulin, sodium, potassium, chlorides, bilirubin, methemoglobin, SGOT, and alkaline phosphatase.

Table 44 lists the most pertinent findings in this study. Before summarizing these, a few general comments may be helpful. The spectrum of toxic reactions noted in recipients of primaquine, WR-211,536, and WR-211,537 was essentially the same. Outward reactions to administration of these agents were few. Recipients of sublethal doses exhibited some loss of appetite and a slight weight loss, not more than 12 per cent. Recipients of lethal doses exhibited anorexia, malaise, loss of muscle tone, emesis, jaundice, accumulation of fluid in the abdominal cavity, and coma. There were no symptoms of CNS irritation. There were no striking alterations in the formed elements of peripheral blood. Significant alterations in biochemical elements were limited to those listed in Table 44. Necropsies revealed extensive hepatic pathology (hepatomegaly, bright yellow discoloration, and focal or widespread areas of necrosis). Ascites (up to 60 ml in volume) was common. Kidneys were frequently enlarged. Lungs were often edematous.

The data in Table 44 show that WR-211,537 (L isomer) was significantly more toxic for the rhesus monkey than WR-211,536 (D isomer). The difference was at least twofold and perhaps greater when based on the total dose productive of a lethal result. Fatal reactions followed delivery of 84 mg WR-211,536 per kg body weight and between 18 and 42 mg WR-211,537 per kg. Primaquine evoked fatal reactions at a dose between 36 and 84 mg per kg and thus, as would be expected, occupied an intermediary position.

The three right columns in Table 44 show that each of these agents produced severe alterations in hepatic function and structure. There were striking and progressive increases in GOT activity and bilirubin levels of serum* in all monkeys

*The pretreatment levels of GOT in the sera of these subjects ranged from 23 to 38 units per liter with a median of 30 units. The pretreatment concentrations of bilirubin in serum ranged from 0.1 to 0.5 mg per cent with a median of 0.3 mg per cent.

who succumbed to the test agents. There were no changes in these elements in the recipients of sublethal doses of WR-211, 536 and WR-211, 537. There were significant (in one case striking) but reversible increases in serum GOT activity and bilirubin concentrations in recipients of primaquine at doses of 6.0 and 8.0 mg per kg.

On the basis of the data set forth above, the conclusion that WR-211, 537 (L isomer) has greater subacute toxicity for the rhesus monkey than WR-211, 536 (D isomer) is inescapable. Thus, the relations between acute toxicity of these isomers for the mouse and subacute toxicity for the rhesus monkey are reversed. There are reasons to be confident that this difference is real - not attributable to a mix-up of compound. In the first place, two different preparations of WR-211, 537 gave identical results in the rhesus monkey. Secondly, assessments in this laboratory of the acute toxicity of one of these lots of WR-211, 537 and the preparation of WR-211, 536 gave results essentially identical with those obtained in the original evaluation of mouse toxicity carried out elsewhere.

There is obvious need for studies aimed at explaining the differences in the toxicities of WR-211, 536 and WR-211, 537 for the mouse and monkey. More important than this, however, is the question of how to exploit these agents which have diverse toxicities and identical radical curative properties. The first step would probably be to attempt to confirm the findings on the relative subacute toxicities of WR-211, 536 and WR-211, 537 for the rhesus monkey in an independent laboratory and pursue similar studies on the subacute toxicities of these isomers for the dog. If both investigations show that WR-211, 536 is clearly less toxic than WR-211, 537, evaluation of the former agent in man for both tolerability and efficacy is clearly indicated. If the observations in the rhesus monkey carry-over to man, a shift from primaquine to the D isomer could be expected to produce at least a gain in tolerability of conventional doses

and at best open the way to safe administration of larger than conventional doses. If the dog and monkey toxicity studies do not provide clear indications of the greater tolerability of WR-211,536, it might be well to defer evaluation of this isomer or the L form in human volunteers.

TABLE 44

A PRELIMINARY COMPARISON OF THE SUBACUTE TOXICITIES OF WR-211,536 (D ISOMER), WR-211,537 (L ISOMER), AND PRIMAQUINE (D,L MIXTURE) FOR THE RHESUS MONKEY

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	Reactions to Treatment			
		Fate	Liver Pathology Gross	Conc. in Serum** SGOT Units	Conc. in Serum** Bilirubin Mg Per Cent
		Primaquine			
7794	4.0	Survived: no reaction	n.a.	30	0.5
8279	6.0	Survived: anorexia; weight loss; recovery Day 10 post-R_x	n.a.	1050	2.7
7827	8.0	Survived: anorexia; weight loss; recovery Day 14 post-R_x	n.a.	84	1.1
7974	8.0	Survived: anorexia; weight loss; recovery Day 11 post-R_x	n.a.	64	0.7
8193	12.0	Died 60 hours after Dose 7	+	1320	11.3
7998	12.0	Died 11 hours after Dose 7	+	980	5.7
7975	12.0	Died 26 hours after Dose 3	+	390	3.9
7943	12.0	Died 24 hours after Dose 3	+	500	2.7
7798	18.0	Died 42 hours after Dose 3	+	380	3.0
		WR-211,536 (D isomer)			
8345	6.0	Survived: no reaction	n.a.	38	0.6
8465	6.0	Survived: no reaction	n.a.	40	0.5
8337	12.0	Died 25 hours after Dose 7	+	1600	12.4
8466	12.0	Died 7 hours after Dose 7	+	2200	3.1
		WR-211,537 (L isomer)			
7901	3.0	Survived: no reaction	n.a.	32	0.3
7905	3.0	Survived: no reaction	n.a.	46	0.5
8465r	3.0	Died 16 hours after Dose 6	+	1600	3.5
8412	6.0	Died 9 hours after Dose 7	+	2700	8.2
8338	6.0	Died 6 hours after Dose 7	+	2900	12.7
8300	12.0	Died 6 hours after Dose 3	+	5500	4.6
8348	12.0	Died 4 hours after Dose 3	+	6000	3.6

*Dose administered once daily at 8:00 a.m.; scheduled number of doses - seven.

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

XVII. PRELIMINARY ASSESSMENT OF THE SUBACUTE TOXICITIES OF WR-211,532 AND WR-211,533 FOR THE RHESUS MONKEY

XVII. PRELIMINARY ASSESSMENT OF THE SUBACUTE TOXICITIES OF WR-211,532 AND WR-211,533 FOR THE RHESUS MONKEY

As shown in Table 28 Section IX, WR-211,532 [2-methyl-5-(4-chlorophenoxy)-6-methoxy-8-(4-amino-1-methylbutylamino)-quinoline] was clearly more active than 2-methyl primaquine (WR-182,234). It was one of the comparatively small group of 8-aminoquinolines with curative activity greater than that of primaquine and comparable to that of WR-181,023. WR-211,532 was also at least three times as active as the closely related WR-182,232, which lacks a methyl substituent at position 2. These interesting structure - radical curative activity relationships led to the decision to make a preliminary assessment of the subacute toxicity of WR-211,532. Unfortunately, the amount of compound available for this study was extremely limited, just sufficient for administration to two monkeys.

WR-211,533 [2,4-dimethyl-6-methoxy-8-(3-diethylaminopropylamino)-quinoline] was of interest for several reasons. As shown in Table 28, this compound had curative activity equal to that of primaquine and WR-192,515, the 2-methyl derivative of WR-181,023. Such high activity was remarkable in an 8-aminoquinoline with a terminal tertiary amino group in the side chain. In addition, WR-211,533 carried the diethylaminopropylamino side chain of Plasmocid, which confers a high level of neurotoxicity on all 6-substituted or 5,6-substituted 8-aminoquinolines. WR-211,533 did not exhibit such toxicity at the doses employed in pilot therapeutic studies. Studies pursued in the post-World War II period showed that neurotoxicity of the Plasmocid type was abolished by substitution of a methyl group at position 4. The impacts of an additional substituent at position 2 had never been investigated. Together, these considerations led to the decision to undertake a preliminary study of the subacute toxicity of WR-211,533 for the rhesus monkey. As with WR-211,532, the supply of WR-211,533 on hand at the time of this decision

was just sufficient for work on two monkeys. Approximately a year later, an additional quantity was made available for extension of the toxicity evaluation.

The procedures employed for assessing the toxicities of WR-211,532 and WR-211,533 were identical with those used in evaluating the toxicities of the D and L isomers of primaquine (cf Section XVI) and will not be repeated here. The results of the evaluations have been summarized in Table 45.

The observations on WR-211,532 suggest that the toxicity of this compound is qualitatively and quantitatively very similar to that of primaquine and 2-methyl primaquine (WR-182,234). The toxicity of WR-211,532 is clearly less than the toxicity of WR-181,023.

The data obtained on WR-211,533 indicate that this compound has but one-half or possibly one-third the toxicity of primaquine. They also show that qualitatively, the toxicity of WR-211,533 and primaquine are similar, primarily referable to hepatic injury. There were no signs of neurotoxicity among the recipients of either lethal or just sublethal doses of WR-211,533.

Obviously, these preliminary observations need to be strengthened substantially before making final judgments as to the relative toxicities of either WR-211,532 or WR-211,533. However, such toxicity and curative activity data as are available suggest that either agent has a more favorable therapeutic index than primaquine or 4-methyl primaquine. WR-211,533 with its terminal tertiary amino group in the side chain would seem to command special interest.

TABLE 45

PRELIMINARY ASSESSMENTS OF THE SUBACUTE TOXICITIES OF WR-211,532 AND WR-211,533 FOR THE RHESUS MONKEY

Mmu No.	Daily Dose Mg Base/Kg Body Weight*	Reactions to Treatment			
		Fate	Liver Pathology Gross	Conc. in Serum** SGOT Units	Conc. in Serum** Bilirubin Mg Per Cent
		WR-211,532			
8149	6.0	Survived: weight loss; recovery Day 10 post-R_x	n.a.	34	0.5
8371	12.0	Died 2 hours after Dose 5	+	1800	2.6
		WR-211,533			
8237	6.0	Survived: no reaction	n.a.	41	0.4
8354	12.0	Survived: no reaction	n.a.	53	0.4
8671	18.0	Survived: anorexia; weight loss; recovery Day 14 post-R_x	n.a.	n.a.	0.7
8644	24.0	Died 8 hours after Dose 5	+	n.a.	4.6

*Dose administered once daily at 8:00 a.m.; scheduled number of doses - seven.

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

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	-	111
198,559	BC-57,397	di-β-resorcylate	111
198,560	BC-57,404	di-β-resorcylate	111
13,255	AL-27,411	-	112
219,124	BE-69,119	-	112
193,713	BC-41,362	pamoate	112
198,782	BC-58,401	sesqui-β-resorcylate	112
191,994	BE-13,162	-	113
7,295	BB-47,961	-	113
102,796	BC-78,878	-	113
218,575	BE-66,958	-	113
12,921	AG-16,089	dihydrochloride	114
205,446	BD-54,211	dihydroiodide	114
202,833	BD-26,191	dihydroiodide	114
182,058	AY-98,947	-	114
9,792	AE-07,615	hydrochloride	115
25,981	AG-74,536	-	115
31,877	ZC-03,799	-	115
190,830	BD-29,165	dihydrochloride	115
158,124	BD-22,997	sesquihydrochloride	116
167,655	BC-99,831	-	116
124,905	BE-52,436	sodium	116
124,892	BE-18,158	-	116
		1,5-Naphthyridines	
202,927	BD-26,422	-	118
217,125	BE-67,286	di-β-resorcylate	118
202,928	BD-26,413	-	118
180,411	BD-95,839	di-β-resorcylate	119
206,287	BD-54,748	-	119
206,283	BD-54,720	β-resorcylate	119
210,304	BE-10,858	tri-β-resorcylate	119
210,442	BE-10,830	di-β-resorcylate	120
145,023	BD-53,812	hydrochloride	120
216,010	BE-17,286	di-β-resorcylate	120

Compound WR- No.	Bottle No.	Salt	Reference Page
		6-Aminoquinolines	
219,120	BE-58,652	β-resorcylate	122
181,614	AY-97,959	di-β-resorcylate	122
203,766	BD-27,983	sesqui-β-resorcylate	122
218,632	BE-67,071	fumarate	123
217,162	BE-45,048	sesquifumarate	123
216,686	BE-19,182	di-β-resorcylate	123
204,659	BD-29,405	di-β-resorcylate	123
182,146	BD-26,379	dihydrochloride	124
Ni-147/36	-	naphthylene disulfonate	124
215,627	BE-16,672	fumarate	124
182,144	BB-41,732	di-β-resorcylate	124
188,438	BB-45,436	di-β-resorcylate	125
199,066	BC-58,830	di-β-resorcylate	125
208,060	BD-45,338	-	125
199,065	BC-58,803	di-β-resorcylate	125
Ba-138/111	-	naphthylene disulfonate	126
182,148	AY-99,319	-	126
190,733	BD-29,254	-	126
199,063	BC-58,876	-	126
		7-Aminoquinolines	
218,336	BE-66,832	diphosphate	128
213,640	BE-09,999	dinitrate	128
219,008	BE-58,296	trihydrochloride	128
218,677	BE-55,811	dinitrate	129
217,270	BE-50,174	triphosphate	129
207,766	BD-57,472	dinitrate	129
218,948	BE-57,646	triphosphate	129
		8-Aminoquinolines	
199,508	BD-24,044	trihydrobromide	139
29,633	BE-20,989	dihydrochloride	139
211,664	BE-21,762	diphosphate	139
2,975	-	diphosphate	140
152,149	BE-66,770	phosphate	141
186,370	BE-19,075	citrate	141
215,730	BE-16,583	-	141
161,085	AX-26,820	bis-1,6-naphthylene disulfonate	141
180,125	AY-95,455	sesqui-β-resorcylate	142
197,624	BC-09,453	di-β-resorcylate	142
199,981	BD-23,154	di-β-resorcylate	142
29,594	BE-20,014	-	142

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines - Continued	
27,757	BE-20,863	dinitrate	143
214,420	BE-50,012	phosphate	143
29,606	BE-20,229	dihydrochloride	143
190,285	BB-47,510	di-β-resorcylate	143
193,127	BB-49,416	maleate	144
29,616	BE-21,744	dihydrochloride	144
181,441	AY-97,600	dihydrochloride	144
187,427	BB-44,779	-	144
187,428	BB-44,788	-	145
185,306	BB-42,060	-	145
7,312	BB-47,761	dihydrochloride	145
29,634	BE-12,950	dihydrobromide	145
184,544	BE-20,265	triphosphate	146
212,231	BE-20,201	dihydrobromide	146
182,234	BC-58,572	dihydrochloride	146
213,472	BE-13,948	fumarate	147
218,669	BE-55,795	maleate	147
211,077	BE-11,971	phosphate	147
121,508	BE-11,121	dihydrobromide	147
106,147	AY-97,897	dihydrochloride	148
205,438	BD-54,202	maleate	148
217,154	BE-67,204	maleate	148
217,124	BE-43,759	maleate	148
202,790	BD-26,217	maleate	149
205,439	BD-54,195	maleate	149
183,538	BB-41,885	diphosphate	149
212,216	BD-99,103	maleate	149
216,893	BE-19,477	maleate	150
199,368	BC-59,284	maleate	150
183,064	BB-41,894	hydrochloride	150
217,038	BE-19,584	diphosphate	150
211,814	BE-08,518	diphosphate	151
211,815	BE-08,527	diphosphate	151
211,820	BE-21,593	citrate	151
206,027	BE-20,194	dihydrochloride	152
147,778	BB-18,448	diphosphate	152
136,479	BE-21,575	diphosphate	152
181,023	BC-57,244	diphosphate	153
215,761	BE-16,967	diphosphate	154
215,296	BE-16,378	citrate	154
217,159	BE-67,222	citrate	154
215,300	BE-16,243	fumarate	155
208,442	BD-67,981	dihydrobromide	155
211,663	BE-12,825	fumarate	155
218,806	BE-57,315	fumarate	155

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines - Continued	
218,636	BE-67,133	fumarate	156
218,805	BE-57,324	fumarate	156
218,574	BE-66,985	fumarate	156
216,837	BE-19,306	fumarate	157
214,198	BE-13,082	dihydrochloride	157
217,271	BE-50,183	phosphate	157
208,557	BD-45,267	diphosphate	158
209,785	BE-10,090	succinate	158
208,814	BD-59,074	phosphate	158
214,703	BE-15,040	sesquiphosphate	159
209,522	BD-59,967	phosphate	159
209,521	BD-59,985	diphosphate	159
211,975	BE-12,996	phosphate	160
189,279	BB-46,540	phosphate	160
199,793	BC-99,939	phosphate	160
212,293	BD-99,907	phosphate	161
218,573	BE-66,994	diphosphate	161
212,302	BD-99,943	succinate	161
201,678	BE-13,304	diphosphate	162
212,624	BE-13,822	diphosphate	162
212,579	BE-13,313	fumarate	162
218,335	BE-66,930	diphosphate	163
6,028	BE-20,032	dihydrochloride	163
6,027	BE-20,112	dihydrochloride	163
202,437	BD-26,164	sesqui-β-resorcylate	164
203,607	BD-27,652	di-β-resorcylate	164
203,608	BD-27,661	di-β-resorcylate	164
211,208	BE-20,005	dihydrochloride	165
211,665	BE-20,569	dihydrochloride	165
147,657	BE-20,023	dihydrochloride	165
214,787	BE-15,451	dihydrochloride	165
202,438	BD-26,155	fumarate	166
211,816	BE-20,630	dihydrochloride	166
212,223	BE-20,121	dihydrochloride	166
199,507	BD-24,062	trihydrobromide	167
194,333	BC-06,587	oxalate	167
200,073	BD-23,163	oxalate	167
5,990	BC-60,018	diphosphate	168
218,676	BE-55,820	fumarate	168
184,118	BE-20,309	phosphate	168
182,232	BC-99,911	-	169
216,100	BE-17,491	citrate	169
215,295	BE-16,378	citrate	169
183,489	BB-42,695	-	170
206,438	BD-55,049	-	170
209,134	BD-59,752	-	170

Compound WR- No.	Bottle No.	Salt	Reference Page
		8-Aminoquinolines - Continued	
208,189	BD-45,427	-	171
209,845	BE-10,223	-	171
208,441	BD-57,990	-	171
211,078	BE-12,012	-	172
188,304	BB-45,534	diphosphate	172
207,610	BE-11,631	fumarate	172
194,341	BC-06,443	dimaleate	172
202,789	BD-26,208	maleate	173
49,577	BC-59,702	trihydrobromide	173
194,343	BC-06,452	maleate	173
181,205	AY-96,907	tricitrate	174
189,283	BB-46,568	-	174
184,852	BB-41,929	-	174
215,732	BE-16,627	diphosphate	175
192,515	BC-58,394	sesqui-β-resorcylate	175
218,334	BE-66,841	phosphate	175
211,990	BD-99,087	dihydrochloride	175
193,130	BC-58,910	di-β-resorcylate	176
211,533	BE-12,601	diphosphate	176
197,063	BC-08,705	di-β-resorcylate	176
210,810	BE-11,677	fumarate	177
215,733	BE-17,026	fumarate	177
211,532	BE-12,567	fumarate	178
210,805	BE-11,686	fumarate	178
210,550	BE-11,597	dinitrate	179
210,551	BE-11,588	dinitrate	179
216,804	BE-19,324	diphosphate	179
218,681	BE-55,937	hemifumarate	179
204,510	BD-28,971	hemifumarate	180
208,071	BD-45,310	fumarate	180
210,448	BE-11,033	fumarate	180

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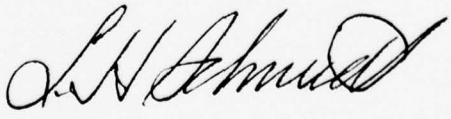

L. H. Schmidt, Principal Investigator

Organic Chemistry Department
Kettering-Meyer Laboratory
Southern Research Institute
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