

END COPY

2

AD _____

TESTING OF EXPERIMENTAL COMPOUNDS FOR EFFICACY AGAINST LEISHMANIA

Annual Report

William L. Hanson, Virginia B. Waits,
and Willie L. Chapman, Jr.

February 28, 1990

(For the period 1 January 1989 - 31 December 1989)

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-85-C-5012

University of Georgia Research Foundation
Athens, Georgia 30602

DTIC
SELECTE
APR 05 1990
E D

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of The Army Position unless so designated by other authorized documents.

00 04 02 122

AD-A219 986

AD _____

TESTING OF EXPERIMENTAL COMPOUNDS FOR EFFICACY AGAINST LEISHMANIA

Annual Report

William L. Hanson, Virginia B. Waits,
and Willie L. Chapman, Jr.

February 28, 1990

(For the period 1 January 1989 - 31 December 1989)

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-85-C-5012

University of Georgia Research Foundation
Athens, Georgia 30602

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of The Army Position unless so designated by other authorized documents.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188
Exp. Date: Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION Unclassified			1b. RESTRICTIVE MARKINGS		
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE			4. PERFORMING ORGANIZATION REPORT NUMBER(S)		
5. MONITORING ORGANIZATION REPORT NUMBER(S)			6a. NAME OF PERFORMING ORGANIZATION University of Georgia Research Foundation		
6b. OFFICE SYMBOL (If applicable)			7a. NAME OF MONITORING ORGANIZATION		
6c. ADDRESS (City, State, and ZIP Code) Athens, Georgia 30602			7b. ADDRESS (City, State, and ZIP Code)		
8a. NAME OF FUNDING / SPONSORING ORGANIZATION US Army Medical Research & Development Command			8b. OFFICE SYMBOL (If applicable)		
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-85-C-5012			10. SOURCE OF FUNDING NUMBERS		
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012			PROGRAM ELEMENT NO. 62770A	PROJECT NO. 3M1- 6277A870	TASK NO. AM
			WORK UNIT ACCESSION NO. 037		
11. TITLE (Include Security Classification) Testing of Experimental Compounds for Efficacy Against <u>Leishmania</u> (U)					
12. PERSONAL AUTHOR(S) W. L. Hanson, V. B. Waits, and W. L. Chapman, Jr.					
13a. TYPE OF REPORT Annual		13b. TIME COVERED FROM 1/1/89 TO 12/31/89		14. DATE OF REPORT (Year, Month, Day) 90/2/28	
15. PAGE COUNT 36					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
06	13		Leishmania donovani; WR06026 RA 1		
06	20		chemotherapy; berberine pyrimidine nucleotides		
			golden hamster; Sinefungin antileishmanials; (KT)		
19. ABSTRACT (Continue on reverse if necessary and identify by block number) Six of a total of 312 compounds of diverse nature which were tested in the primary visceral screening system were noted to have suppressive activity against <u>Leishmania donovani</u> (suppressed parasite numbers in the liver by at least 50% at one of the dosages used). The 8-aminoquinolines were the most active compounds studied with approximately 100% suppression of hepatic parasites at 52 mg/kg dosage. Pyrimidine nucleotides had relatively low efficacy as has been noted with this group of compounds in the past. One of 250 pyrazine or quinazoline inhibitors of dihydrofolate reductase which were tested was active.					
In special studies, administration of WR06026 and derivatives to hamsters as a single dose 3 days prior to infection with <u>Leishmania donovani</u> resulted in lower efficacy in suppressing parasite numbers in the liver than when administered 3 days subsequent to infection. Activity in the spleen was less than that in the liver when these compounds were administered after infection and approximately equal when the compounds were administered					
20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED / UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Mary Frances Bostian			22b. TELEPHONE (Include Area Code) 301-663-7325		22c. OFFICE SYMBOL SGRD-RMI-S

prior to infection. Increased dosages of WR06026 resulted in enhanced suppressive activity in the liver when administered prior to infection and enhanced activity in the spleen when administered subsequent to infection. The efficacy of selected 8-aminoquinolines was approximately equivalent when administered either orally or via the intramuscular route.

In other special studies the quaternary alkaloid, berberine, and three of its derivatives (8-cyanodihydroberberine, tetrahydroberberine, and N-methyl-tetrahydroberberinium iodide) were suppressive against L. donovani in hamsters and berberine and one derivative (8-cyanodihydroberberine) produced 56% and 46% suppression of cutaneous lesion areas respectively in hamsters infected with Leishmania braziliensis panamensis. Structure activity relationship studies suggested that a quaternary nitrogen in these compounds was associated with antiparasitic activity against both visceral and cutaneous leishmaniasis but appeared also to be associated with toxicity to the host.

Results of special studies with combinations of Sinefungin and selected purine analogs indicated that when Sinefungin was administered to hamsters infected with L. donovani alone or in combination with any one of six selected purine analogs, no antiparasitic activity significantly greater than that attributable to Sinefungin alone was observed.

ACKNOWLEDGEMENT

The contributions of David Barnard, Richard Bloodworth, LaVonda Cheek, Sriyani Ekanayake, and Sung Shik Shin to this work are gratefully acknowledged.

Accession For	
NTIS GRA&I	☒
DTIC TAB	☒
Unannounced	☐
Justification	
By	
Distribution/	
Availability Codes	
Serial and/or	
Dist	Special
A-1	



FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

 Where copyrighted material is quoted, permission has been obtained to use such material.

 Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

 Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

 ✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

 For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

 In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

William L. Hansen Feb. 28, 1990
PI Signature Date

TABLE OF CONTENTS

Acknowledgement	1
Foreword	2
Introduction	5
Materials and Methods	
I. Primary Visceral Test System.	7
II. Primary Cutaneous Test System	8
III. Special Experiments	9
Results	
I. Studies Involving <u>Leishmania donovani</u>	
A. Primary Screening Data.	10
B. Glucantime Reference Data	10
II. Studies Involving <u>Leishmania braziliensis panamensis</u>	11
III. Special Studies	
A. Combination Drug Studies	11
B. Studies with Derivatives of Berberine	11
C. 8-Aminoquinolines	12
Discussion	14
Conclusions.	16
Equipment and Personnel.	17
Literature Cited	33
Distribution List.	36

TABLES

Table	Page
I. Compounds with > 50% suppressive activity in the visceral test system	18
II. Toxicity of compounds active in the visceral primary screening test system	19
III. Summary of the activity of the reference compound, Glucantime (BL09186) against <u>Leishmania donovani</u> in the primary visceral test system during the period 1 January through 31 December 1989	20
IV. Suppressive activity of combined Sinefungin and purine analogs against <u>Leishmania donovani</u> in the golden hamster.	21
V. Effect of berberine analogs and glucantime on numbers of amastigotes in livers of hamsters infected with <u>L. donovani</u>	22
VI. Effect of berberine analogs and glucantime on lesion size of hamsters infected with <u>L. braziliensis panamensis</u>	23
VII. Effect of route of administration on efficacy of selected 8-aminoquinolines against <u>Leishmania donovani</u> in the golden hamster.	24
VIII. Suppressive activity in liver and spleen of WR06026 administered 3 days prior to or 3 days post infection	25
IX. Suppressive activity in liver and spleen of WR06026 administered either 3 days prior to or 3 days post infection.	26

FIGURES

1. Chemical structures of compounds found active in the primary visceral test system.	27
2. Structures of berberine alkaloids	29
3. Structures of 8-aminoquinolines used in the study of the effect of route of administration on efficacy against <u>Leishmania donovani</u>	30
4. Chemical structures of WR06026 analogs.	32

INTRODUCTION

(Statement of the Problem and Background)

The leishmaniasis, the group of diseases caused by protozoan parasites of the family Trypanosomatidae, genus Leishmania, are widely distributed throughout the world and are found on every inhabited continent except Australia (Kinnamon et al., 1). These diseases occur in such important countries as Russia, China, India, Pakistan, Egypt, Sudan, Israel, Syria, Iran, Saudi Arabia, Brazil, Venezuela, Panama, Mexico, Argentina, and many others. These parasites are transmitted by several species of phlebotomine flies and in most areas the leishmaniasis are zoonoses with canines, rodents, or other mammals serving as reservoir hosts.

These parasites are a significant health hazard to humans in these areas. Visceral leishmaniasis, the most severe type, is endemic in many areas where epidemics occur (TDR Publ, 7th Program Rpt., 2) with mortality reported to reach as high as 98 percent in untreated cases (Biagi, 3; Steck, 4). While it is difficult to obtain an accurate estimate of the number of human beings infected with the leishmaniasis throughout the world, (TDR Publ, 7th Program Rpt., 2) some estimates indicate that at least 12 million persons have one of the different forms of the disease caused by infection with these parasites (Mahmoud and Warren, 5; Croft, 6) and outbreaks often involving additional thousands of persons occur periodically (Peters, 7; TDR Publ, 7th Program Rpt., 2; Perea, 8). Some believe as many as 2 to 3 million new cases per year occur worldwide (Croft, 6).

Infection with these parasites represents a significant health hazard to military personnel operating in many areas of the world. For example in World War II where troops were operating in an endemic area of the Persian Gulf 630 cases were reported in a three-month period (Most, 9). Subsequently during troop movements in another endemic area, 50 percent of certain Israeli forces experienced infections (Naggan et al., 10). In addition, although relatively few troops were involved, 10 to 45 cases per year have been reported among U.S. troops in the Canal Zone (Walton et al, 11) and a subsequent report indicated an overall infection rate of 1.6% in one U.S. Army battalion that was deployed to Fort Sherman in the Canal Zone for jungle warfare training (Takafuji et al., 12). Although mortality may occur, the primary problem is the considerable loss in duty time in infected individuals. For example it has been estimated that each individual having visceral leishmaniasis lost at least one year duty time (Most, 9) and in one instance in which 20 cases of cutaneous leishmaniasis occurred in troops in the Canal Zone, two man-years of duty time were lost (Walton et al, 11).

While chemotherapy is currently the only practical means of control for these parasites, it has not been consistently successful (Neal, 13; Croft, 6).

The first line drugs currently available to treat the leishmaniasis are the pentavalent antimony compounds (Neal, 13; Bryceson, 14). While these drugs are less toxic than previously believed (Bryceson, 14), they have significant limitations in that they must be administered via the parenteral route and often repeated injections are required. In addition, these compounds are often not curative and evidence of antimony resistance among the Leishmania is increasing. For example a strain of L. donovani from Kenya has been shown to be considerably more insensitive to antimony than a strain which has been in the laboratory for many years (Hanson et al., 15) and reports of leishmaniasis in humans unresponsive to pentavalent antimony therapy are reasonably common (Mebratu et al, 16.) Furthermore, antimony resistant strains of L. donovani and L. braziliensis panamensis have been developed experimentally in this laboratory (Waits, et al., unpublished). Other evidence for the presence of drug resistance among the Leishmania has been reviewed by Croft (6). The backup drugs for treatment of the leishmaniases, Amphotericin B or Pentamidine have even more problems in that they cause adverse effects in humans as a result of toxicity (see Jackson, et al for references, 17).

The current prospects for new drugs for the treatment of visceral leishmaniasis are quite limited (WHO Publication, 18; Neal, 13; Croft, 6). Drugs with proved efficacy in laboratory animals and which are currently undergoing pre-clinical or clinical studies are WR06026 and allopurinol riboside. Drugs or delivery systems in various stages of development and showing some promise are Sinefungin, formycin B, miconazole, and liposomes. Formycin B has been observed in this laboratory to be extremely toxic in dogs and only marginally active and thus additional study of this compound is probably not warranted. In our experience Sinefungin is also toxic and this toxicity probably will preclude further consideration of this compound for future practical use. Considerable work remains to be done before any of the others will be useful on a practical basis. Furthermore, the possibility that Leishmania already exists which are resistant to WR06026 (an 8-aminoquinoline) must be considered since these infections occur in areas of the world where 8-aminoquinolines have been used against malaria in humans.

Because of the potential importance of the leishmaniases to the health and performance of military personnel in many parts of the world and the need for improved and more satisfactory chemical compounds for consistent successful treatment of these diseases, this project was initiated to test experimental compounds for efficacy against Leishmania donovani and L. braziliensis panamensis infections in the golden hamster as the primary test systems and in non-human primates as a secondary test system. This is the fifth annual progress report for this project and covers the period 1 January 1989 through December 31, 1989. It describes the test procedures used and summarizes the results obtained. The test results obtained have been sent to appropriate officials at The Walter Reed Army Institute of Research as they became available during the contract year.

MATERIALS AND METHODS (Approach to the Problem)

I. Primary Visceral Test System

A Khartoum strain of L. donovani (WR378) was used and the golden hamster (Mesocricetus auratus), 50-70 gm, served as the host animal. Suspensions of amastigotes for infection of experimental hamsters were prepared by grinding heavily infected hamster spleens in sterile saline in a Ten Broeck tissue grinder and diluting the suspensions so that 0.2 ml contained approximately 10×10^6 amastigotes. Each experimental hamster was infected via the intracardiac injection of 0.2 ml of the amastigote suspension.

The testing procedure used was that described by Stauber and his associates (19, 20, 21) as modified by Hanson et al. (22). On day 3 following infection, hamsters were divided randomly into experimental groups consisting of a minimum of 6 animals per group, initial group weights were obtained, and administration of test compounds was initiated. Each compound was tested at 2 or 3 drug dosage levels dependent on the priority rating of the compound. Generally the test compounds with high priority ratings were studied initially via the intramuscular route (I.M.) at total dosages of 416, 208 and 52 mg/kg (milligrams/kilogram) while those compounds received with a routine or low priority rating were studied at 208 and 52 total mg/kg only. Other drug dosage levels determined by the quantity of compound available or previous toxicity data were used also.

The vehicle for the test compounds was 0.5% hydroxethylcellulose-0.1% Tween 80 (HEC-Tween). Each test group contained 6 hamsters and received one of the desired drug dosage levels. A control group of 6 to 8 hamsters received the 0.5% HEC-Tween vehicle only and the reference compound, Glucantime[®] was given at 2 or 3 drug dosage levels (208, 52, and 26 total mg/kg or 208 and 52 total mg/kg) based on antimony content. All test compounds were administered routinely twice daily via the intramuscular route on days 3 through 6. Final group weights were obtained on all experimental hamsters on day 7 and all animals were killed, livers removed, weighed and liver impressions made for enumeration of amastigotes. Subsequently, the total number of parasites per liver was determined as described by Stauber (20).

In addition to recording body weight changes as a general indicator of toxicity of the test compounds experimental hamsters were observed for such clinical signs of toxicity as nervous disorders, roughened hair coat, and sluggish activity. Deaths also were recorded. Weight loss of 15% or greater and/or death of the animals was considered indicative of significant drug toxicity.

After determining the ratio of numbers of amastigotes/host cell nucleus the weight of the organ, and initial and final weights of the hamsters, the raw data was evaluated with an IBM PC XT microcomputer using a program which calculates percent weight change, total numbers of parasites, mean numbers of parasites/organ, and percent parasite suppression. The computer program then performs linear and non-linear regression analysis and calculates a SD_{50} (drug dosage resulting in 50% suppression of amastigotes) for each active compound from each of the analyses. The SD_{50} from the non-linear analysis is used for a comparison of the relative efficacy of the test compounds and the efficacy of test compounds relative to that of the reference compound, Glucantime. The linear regression analysis is included only for comparison with the non-linear analysis.

Additional information on the antileishmanial activity of each active compound was obtained by comparing the percent suppression of numbers of amastigotes it exhibits with the percent suppression observed with Glucantime, the reference compound. This comparative measure (referred to as the Glucantime Index or "G") was determined by the following formula:

$$\text{Glucantime Index} = \frac{SD_{50} \text{ for Glucantime}}{SD_{50} \text{ for new test compound}}$$

II. Primary Cutaneous Test System

Leishmania braziliensis panamensis (strain WR539) was used in these studies. Male golden hamsters, 50-70 grams, served as experimental hosts.

Promastigotes for establishing experimental infections in hamsters were grown in Schneider's Drosophila Medium (Hendricks et al., 23) and quantitated using procedures described previously (Hanson and Roberson, 24). In preparation for infection and weekly during the experiment, the hair was clipped on the dorsal tail head and a commercial depilatory agent applied to the area to remove the remaining hair. Each hamster was inoculated via the intradermal route with 1.5×10^7 promastigotes of L. braziliensis panamensis near the base of the tail using a 0.25 ml glass syringe equipped with a 30 gauge x 1/2" needle. Each experimental group consisted of six hamsters. Initial body weights were obtained and administration of therapy, generally via the intramuscular route, was initiated on day 19 post infection, and continued through day 22 post infection. Glucantime was included at two dosage levels (832 and 208 total mg Sb/kg) as the reference compound and a group of six hamsters received vehicle only (HEC-Tween). Test compounds were administered generally at 416 and 208 total mg/kg.

Lesion area of each experimental hamster was determined with the aid of a template made at WRAIR and calibrated according to the formula $r_1 r_2 \pi$ where r_1 is the major radius of the lesion and r_2 is the minor radius, (Wilson et al., 25). The mean lesion area of each experimental group was obtained and the percent suppression of lesion size calculated by comparing the mean lesion area of each treated group with that of the group receiving vehicle only with the aid of a computer program and an IBM PC XT microcomputer. The computer program performs linear and non-linear regression analysis and calculates a SD_{50} for each active compound using both analyses.

The SD_{50} obtained from the non-linear analyses is used for a rough comparison of the relative efficacies of the test compounds and the relative efficacy of test compounds with that of the reference compound, Glucantime. This may be expressed as the Glucantime Index as described in section I. The linear regression analysis is performed for comparison with the non-linear analysis.

III. Special Experiments

Procedure variations of the two test systems were used in certain experiments. These variations are noted in the appropriate results sections.

RESULTS

I. Studies Involving Leishmania donovani

A. Primary Screening Data

A total of 312 compounds were tested in the primary visceral screening system. Among those tested, 6 compounds (2%) were active, i.e. suppressed parasite numbers by at least 50% at one of the doses administered. The results for active compounds are shown in Table I, and their structures are illustrated in Figure 1.

As has been the case in the past, 8-aminoquinolines (BH56265, BL52749, BL53308, and ZP30451) were the most active compounds. As can be seen from weight loss data (Table II), all of these compounds also showed evidence of toxicity at one or both of the test doses. The fact, however, that the compounds were 100% suppressive at the lowest dose level tested (52 mg/kg) raises the possibility that these compounds will be highly effective at lower less toxic doses.

The pyrimidine nucleotides (like BL55928) were no more active or less toxic than similar compounds tested in the past.

AJ07615, a diaminopyrazine, was the only active compound among approximately 250 pyrazine or quinazoline inhibitors of dihydrofolate reductase tested. Compounds of these classes and biologic activity are an expressed interest of the World Health Organization Steering Committee on the Chemotherapy of Leishmaniasis. In our system the compounds show little activity and are frequently toxic.

Detailed results for all active and inactive compounds are on file in computer data bases at the Division of Experimental Therapeutics, WRAIR.

B. Glucantime Reference Data

The reference compound, Glucantime, is included in each experiment routinely at three dosage levels. These dosage levels are based on an assay of antimony content. The SD_{50} is calculated for the reference compound in each experiment and is used for comparison of the efficacy of any active compounds in that experiment. To provide some indication of the variation of the reference data, the mean and standard deviation for the SD_{50} values for Glucantime from 30 experiments conducted during this report period were calculated. The data from individual experiments is summarized in Table III. The mean SD_{50} for Glucantime was calculated to be 60.8 mg/kg ($SD \pm 36.4$).

Dosage levels of 26, 52, and 208 mg/kg were used for all experiments in order to accurately estimate the SD_{50} and avoid the artifactual variations in the dose response curve and inflated SD_{50} that have been noted when higher doses have been used. Natural variation in response to the drug, particularly at the lowest dose, still results in some degree of variation of the SD_{50} . This points to the importance of including the reference compound in each experiment rather than using a historical mean value for the reference compound to compare the efficacy of test compounds.

II. Studies Involving Leishmania braziliensis panamensis

Primary Cutaneous Test System

See. III B. below

III. Special Studies

A. Combination Drug Studies

A preliminary study was performed to determine the effects of combined treatment with Sinefungin (WR254847) and each of 6 purine analogs that had previously been active against L. donovani infection in our model system. Animals were infected, treated, and necropsied according to the schedule routinely used for the primary visceral screening model. Each treatment consisted of indicated doses of Sinefungin administered intramuscularly and one of the purine analogs administered per os by gavage, these routes having proven to be optimal for these types of compounds in previous experiments. Dose levels were selected based upon previous results to be in the range of the SD₅₀ for Sinefungin and below the SD₅₀ or below the toxic dose for the purine analogs.

Inspection of the results in Table IV reveals that no antiparasitic activity significantly greater than that attributable to Sinefungin alone was observed when animals were treated with both Sinefungin and any one of the purine analogs.

B. Studies with Derivatives of Berberine

The quaternary alkaloid berberine and several of its derivatives (Figure 2) were tested for efficacy against both Leishmania donovani and Leishmania braziliensis panamensis.

The activities of these compounds in the primary visceral test system are shown in Table V. Only 8-cyanodihydroberberine at a total dose of 208 mg/kg and tetrahydroberberine and N-methyltetrahydroberberinium iodide, both at 416 mg/kg suppressed parasite numbers by 50% or more. Among these compounds, 8-cyanodihydroberberine appeared to be the most toxic as evidenced by a loss of 18% total group body weight; however, the 11% weight loss in the group treated with N-methyltetrahydroberberinium iodide suggests that this compound may be more toxic than the equally antiparasitic tetrahydroberberine. None of the compounds showed 50% suppressive activity at 52 mg/kg. In contrast, the reference compound Glucantime suppressed parasite numbers by 72% at this dose level. Therefore, tetrahydroberberine, the most potent and least toxic of the compounds was found to be less effective than Glucantime against L. donovani.

Data in Table VI show the percent suppression of lesion area in hamsters infected with L. brasiliensis panamensis. At the indicated doses all of the compounds tested were relatively nontoxic, and with the exception of berberine itself, appeared to be less effective (structures 2,4,7,8,9) or equipotent (structures 3,5,6) in the cutaneous test system as in the visceral test

system. The two most active compounds, berberine and 8-cyanodihydroberberine, produced 56% and 46% suppression of lesion area respectively when administered at a total dose of 208 mg/kg. In comparison Glucantime suppressed lesion development by 66% at this dose. These data suggest that berberine and 8-cyanodihydroberberine are approximately as effective as Glucantime against L. braziliensis panamensis in our model.

In regard to structure-activity relationships, in the visceral test system the best activity at 416 mg/kg was seen with tetrahydroberberine and N-methyletetrahydroberberinium iodide, both of which have a tetrahydroberberine skeleton. The weight loss associated with the quaternary N-methyltetrahydroberberinium iodide suggests that this compound is more toxic than the almost equally active tetrahydroberberine. It would thus appear that the further investigation of tetrahydroberberine derivatives without a quaternary nitrogen for treatment of visceral leishmaniasis is warranted. In the cutaneous test system, berberine and 8-cyanodihydroberberine were the only compounds showing suppressive activity. The activity of 8-cyanodihydroberberine may have resulted in part from its oxidation to the corresponding quaternary structure; an analogous oxidation has been reported (Devi, 26) for dihydroberberine (structure 2). Another quaternary ammonium compound, methylbenzethonium chloride, has demonstrated activity against Old World (L. major) cutaneous leishmaniasis (El-on and Messeri, 27) suggesting that a quaternary nitrogen atom is a necessary pharmacophore in alkaloids useful against cutaneous leishmaniasis. Surprisingly, however, palmatine chloride (Structure 2), a quaternary derivative which differs from berberine only in the substitution of a methylenedioxy for a bis-methoxy at the 2,3 position, was inactive.

It would therefore appear that a quaternary nitrogen was associated with antiparasitic activity in both test systems, but was associated with toxicity in the visceral test system. The latter observation may be an artifact of the systems. The compounds were administered to visceraally infected animals earlier than to cutaneously infected animals. The treatment of L. donovani infected hamsters at a time when the animals were undergoing a period of rapid growth possibly made them more sensitive to treatment associated weight loss or inhibition of growth than were the larger, somewhat more mature L. braziliensis panamensis infected animals. Therefore, conclusions regarding the toxicity of the test compounds based upon weight loss alone can not be considered absolute, but this parameter is a useful indicator of relative toxicity of compounds within the same test system.

C. 8-Aminoquinolines

1. The effect of route of administration on efficacy against Leishmania donovani was determined for six 8-aminoquinolines (Figure 3). Infection, treatment, and necropsy were performed according to the schedule for routine primary screening against this organism: however, 3 dose levels were used for each compound, and compounds were administered either intramuscularly or per os.

Table VII compares the suppressive activities of the compounds administered by the two routes. All compounds were very active and virtually abolished infection when administered either intramuscularly or orally at 3.25 mg/kg total dose. With the possible exception of AP45845, no significant

differences in suppressive activity was observed between the two routes of administration at any dose level.

2. In order to ascertain the effects of administration of WR06026 prior to infection on parasite burdens in extrahepatic infection sites, animals were treated with a single oral dose of WR06026 or one of its putative metabolites (Figure 4) either 3 days before or 3 days after infection, necropsied 7 days after infection, and the numbers of parasites in their spleens and livers estimated.

In this experiment, the parent compound, WR06026, was less active in suppressing numbers of parasites in the liver when given 3 days prior to infection than when administered 3 days after infection (Table VII). In contrast, the data for suppression of parasite numbers in the spleen are somewhat more variable, and little difference is evident between the two regimens. Activity in the spleen was less than activity in the liver when the drug was administered post infection. When the drug was administered pre-infection, liver and spleen parasite suppression can be interpreted to be approximately equal.

In all cases, the analogs were quite active in suppressing numbers of hepatic parasites at the single dose level tested when administered post infection. This activity dropped significantly when the analogs were administered pre-infection. In the spleen the analogs were, like the parent compound, much less active than in the liver when administered post infection, but little difference between pre- and post infection treatment was seen in suppression of numbers of parasites in the spleen by any of the analogs, with the possible exception of BL34296. In three instances (BL52196, BL52749, BL53308) suppressive activity was apparently greater in the spleen than in the liver when the compounds were administered prior to infection.

A followup experiment was performed to determine if suppression of parasite numbers in the spleen could be enhanced if a larger dose of WR06026 was administered. The experiment was performed exactly as the previous one, but only WR06026 was used and the highest dose level was increased to 6.5 mg/kg administered as a single oral dose.

The results of this experiment are shown in Table IX. In comparison to equivalent groups in the previous experiment, increased drug doses resulted in greater suppressive activity in the liver when animals were treated preinfection and in the spleen when animals were treated post infection; however, even a four-fold increase in the amount of drug administered failed to significantly increase its suppressive activity in spleens of animals treated prior to infection.

DISCUSSION

During the course of this and previous contract periods, 8-aminoquinolines have proven to be the most consistently active compounds against Leishmania donovani of any class of compounds tested thus far. The antileishmanial activity of one 8-aminoquinoline, the lepidine WR06026, was discovered early in the course of an initial USAMRDC contract held by this laboratory (Kinnamon et al, 28) and is now in the early stages of clinical testing. Primary screening of other 8-aminoquinolines has continued principally because of the possibility of discovery of even more potent and less toxic compounds of this class that might serve as alternatives to WR06026 should the latter compound demonstrate less than desirable clinical activity during the course of its development. This potentiality is especially important in regard to the lack of consistent evidence in animal models for efficacy of WR06026 against New World cutaneous leishmaniasis. In view of the equivocal results that have been obtained in previous years with WR06026 in our cutaneous test system, it is important to discover compounds of this class that have activity against visceral leishmaniasis approximating that of WR06026, while demonstrating more consistent efficacy against cutaneous infection. Continued emphasis will be placed upon discovery of compounds of this class that are equally or more effective administered orally as parenterally as was the case with the 8-aminoquinolines of this type investigated during this reporting period. Compounds of this type will be tested for efficacy against cutaneous leishmaniasis in future screening trials.

Continued experimental interest in WR06026 centers upon this drug's metabolism, in particular the duration of its metabolites' antiparasitic activity and their distribution to infected tissues other than the liver. In the experiments performed during this reporting period, it was more clearly demonstrated that the suppressive activity of WR06026 in extrahepatic infection sites such as the spleen is dose dependent. It would appear that significantly higher drug doses are required to clear parasites from the spleen and by extension the bone marrow than from the liver. This observation could be significant for the eventual clinical use of WR06026. Additional studies resulting from our initial observations that the active metabolite(s) of WR06026 have a more extended life than that of previously identified and characterized metabolites and that no single known metabolite is alone as potent as the parent compound were confirmed by our most recent studies. (Hanson, et al, 29) Furthermore, in animals treated 3 days prior to infection, it was observed that the active metabolite(s) is not only relatively long-lived, but that it is able to be delivered to extrahepatic sites for at least 3 days after administration. In contrast to animals treated post infection, no dose dependent suppression in the spleen was observed in pretreated animals, even at higher drug doses.

Despite the amount of pharmacologic and parasitologic work that has been devoted to the study of metabolism of WR06026, many questions remain. The performance of this drug in clinical trials will help to answer some of these questions. It is anticipated; however, that clinical trials will generate further questions regarding the bioavailability and metabolism of this compound, and that these questions will continue to be addressed initially in the hamster model.

Because Leishmania cannot synthesize purines de novo, but must rely on salvage pathways, purine analogs have received a good deal of attention as possible antileishmanial agents, including work done in this laboratory (Berman, et al, 30; Hanson, et al, 29). Synergism between some of these compounds and Sinefungin against L. donovani promastigotes in vitro has been reported (Nolan, 31). In our visceral test system, neither synergy nor an additive effect was observed between Sinefungin and any one of the purine analogs tested. It is possible that the absence of effect may have been due to administration of suboptimal doses of purine analogs. The experiment is, therefore, being repeated with selected purine analogs administered at higher dose levels in order to examine this possibility.

There have been several reports regarding the experimental and clinical efficacy of berberine against both visceral (DasGupta and Dikshit, 32; Ghosh, et al, 33; Ghosh, et al, 34) and cutaneous leishmaniasis (DasGupta and Dikshit, 32; Devi, 26; Karamchandani, 35; Varma, 36); however, information regarding antileishmanial activities of berberine derivatives has been anecdotal (Putzer, 37). The synthesis of several berberine derivatives by the Department of Medicinal Chemistry, WRAIR, provided an opportunity to test these alkaloids in controlled experiments for antileishmanial efficacy in visceral and cutaneous leishmaniasis. Although several of these derivatives showed activity against either Leishmania donovani or Leishmania braziliensis panamensis or both, their activities and toxicities relative to the reference compound, Glucantime are not presently encouraging as to their potential widespread clinical utility. Several interesting problems do, however, remain, e.g. clarification of the contribution of a quaternary nitrogen to the activity and/or toxicity of the compounds and the potential for local administration of the compounds in cases of cutaneous leishmaniasis due to nondisseminating Old World species.

CONCLUSIONS

1. The 8-aminoquinolines remain the most active antileishmanial compounds studied thus far for efficacy against Leishmania donovani. The 8-aminoquinoline WR06026 and its analogs are highly efficacious in suppressing numbers of hepatic amastigotes and are less active against splenic parasites when administered as a single dose 3 days after infection. When administered prior to infection the activity in the liver and spleen is approximately equal. Increase in dosage levels of WR06026 results in greater suppressive activity in the liver when treatment precedes infection and in the spleen when treatment is subsequent to infection.

The efficacy of the 8-aminoquinolines is approximately equal against Leishmania donovani when administered orally or via the intramuscular route.

2. As noted in the past with similar compounds, pyrimidine nucleotides have relatively low efficacy against Leishmania and often the large quantity of compound required to eliminate the parasites is toxic to the host.
3. As a group, the pyrazine or quinazoline inhibitors of dihydrofolate reductase do not appear to be promising candidates for antileishmanial drugs since only one of 250 studied had significant activity. These compounds generally have little antileishmanial activity and are often toxic to the host.
4. Treatment of hamsters infected with Leishmania donovani with both Sinefungin and any one of six selected purine analogs does not result in antiparasitic efficacy significantly greater than that attributable to Sinefungin alone.
5. The quaternary alkaloid, berberine, and three derivatives (8-cyano-dihydroberberine, tetrahydroberberine, and N-methyltetrahydroberberinium iodide) have activity against Leishmania donovani and berberine and one derivative (8-cyanodihydroberberine) have activity against Leishmania braziliensis panamensis. Both antileishmanial activity and toxicity to the host appear to be associated with the presence of a quaternary nitrogen.

EQUIPMENT AND PERSONNEL

A. Equipment Purchased During This Report Period:

None

B. Personnel

Funded Positions	Percent Effort
2 Graduate Assistants	50%
1 Research Coordinator	66%
1 Laboratory Technician	100%
1 Student Assistant	50%
Non-funded Positions	
1 Principal Investigator	40%
1 Co-Investigator	25%
1 Research Coordinator	34%
1 Administrative Secretary	15%

Positions were occupied 100% of the period covered by this report with the exception of the full time technician and one of the graduate assistants. These positions were vacated 1 October 1989 and were not filled due to the short period of time remaining in this contract.

Table I. Compounds with > 50% suppressive activity in the primary visceral test system.

Bottle Number	Dose*	%Suppress	Dose*	%Suppress	SD50*
BG56265	52	100	208	100	25.1
BL52749	52	100	208	100	25.1
AJ07615	52	27	208	55	179.0
BL53308	52	100	208	99	25.1
BL55928	52	67	208	ND#	38.4
ZP30451	52	100	208	ND#	25.0

*mg/kg

#not determined due to animal mortality at this dose.

Table II. Toxicity of compounds active in the visceral primary screening test system.

Bottle Number	Dose*	Wt Change#	Dose*	Wt Change#
BG56265	52	- 17	208	- 30
BL52749	52	- 01	208	- 20
AJ07615	52	04	208	- 07
BL53308	52	03	208	- 22
BL55928	52	- 24	208	ND
ZP30451	52	02	208	ND

*mg/kg

% change in treatment group weight

ND = not determined due to animal mortality at this dose.

Table III. Summary of the activity of the reference compound, Glucantime (BL09186) against Leishmania donovani in the primary visceral test system during the period 1 January through 31 December 1989.

Exp No.	Dose 1	Suppress 1	Dose 2	Suppress 2	Dose 3	Suppress 3
457	26	17	52	63	208	80
458	26	54	52	68	208	95
459	26	50	52	58	208	94
460	26	39	52	43	208	82
461	26	23	52	53	208	79
462	26	17	52	35	208	74
463	26	31	52	56	208	76
465	26	36	52	62	208	83
467	26	17	52	57	208	83
468	26	49	52	77	208	88
469	26	33	52	65	208	90
470	26	27	52	54	208	85
471	26	16	52	55	208	81
472	26	32	52	52	208	80
474	26	27	52	50	208	75
475	26	3	52	88	208	93
476	26	11	52	31	208	82
477	26	20	52	63	208	90
478	26	14	52	44	208	84
479	26	43	52	72	208	89
480	26	18	52	55	208	77
483	26	27	52	36	208	63
484	26	21	52	39	208	74
485	26	39	52	59	208	71
486	26	43	52	54	208	73
488	26	17	52	50	208	56
489	26	34	52	62	208	82
490	26	37	52	45	208	87
491	26	8	52	68	208	85
492	26	3	52	49	208	80

Table IV . Suppressive activity of combined Sinefungin and purine analogs against Leishmania donovani in the golden hamster.*

Compound	Dose#	Alone	+Sinefungin	
			+6.5#	3.25#
Sinefungin	-		59	48
Allopurinol riboside	104	- 11	61	43
Oxyformycin B	104	18	73	34
Allopurinol	104	19	60	35
3-deazoguanosine	13	17	63	41
Thiopurinol riboside	13	18	41	44
Formycin B	3.25	22	57	51

*Results are expressed as % suppression of parasite numbers as compared to untreated controls.

#total mg/kg

Table V. Effect of berberine analogs and glucantime on numbers of amastigotes in livers of hamsters infected with L. donovani.

<u>Compound</u>	<u>Dose(mg/kg)^a</u>	<u>Suppression (%)^b</u>	<u>% Wt. Change^b</u>
Berberine chloride (1)	52	20	+5
	208 ^c	36	-10
Palmatine chloride (2)	52	14	0
	416	28	-11
Oxyberberine (3)	52	0	+9
	416	27	+6
Dihydroberberine (4)	52	23	+7
	416	34	+9
8-Cyano dihydroberberine (5)	52	22	+8
	208 ^c	54	-18
Tetrahydroberberine N-oxide (6)	52	2	+8
	416	13	+11
Tetrahydroberberine (7)	52	17	+9
	416	50	+7
N-Methyl tetrahydro berberinium iodide (8)	52	10	+9
	416	56	-11
Berberine betaine (9)	52	15	+8
	416	23	-11
Glucantime ^d	52	72	+10
	208	84	+11

a. Total dose administered over a four day period.

b. As compared to animals receiving HEC-Tween vehicle only; each treatment group included 6 hamsters and 7 hamsters were included in the control groups.

c. Compounds administered at a maximum total dose of 208 mg/kg due to deaths among groups treated at 416 mg/kg in preliminary experiments.

d. Meglumine antimonate

Table VI. Effect of berberine analogs and glucantime on lesion size of hamsters infected with L.braziliensis panamensis.

<u>Compound</u>	<u>Dose (mg/kg)^a</u>	<u>Suppression (%)^b</u>	<u>% Wt. Change^b</u>
Berberine chloride (1)	52	22	-2
	208	56	-1
Palmatine chloride (2)	52	0	0
	208	0	-6
Oxyberberine (3)	52	0	+15
	208	21	0
Dihydroberberine (4)	52	0	+1
	208	3	-1
8-Cyano dihydroberberine(5)	52	39	-1
	208	46	-6
Tetrahydroberberine N-oxide (6)	52	8	+1
	208	11	-1
Tetrahydro berberine (7)	52	0	0
	208	26	-1
N-Methyl tetrahydro berberinium iodine (8)	52	0	0
	208	8	-5
Berberine betaine (9)	52	11	+2
	208	5	-5
Glucantime ^c	52	22	+2
	208	66	-3

a. Total dose administered over a four day period.

b. As compared to animals receiving HEC-Tween vehicle only; each treatment group consisted of 6 hamsters, and 7 hamsters were included in the control group.

c. Meglumine antimonate

Table VII. Effect of route of administration on efficacy of selected 8-aminoquinolines against Leishmania donovani in the golden hamster.

Compound	Dose (mg/kg)	Route	%Suppression	
			I.M.	P.O.
BK84200	0.20		0	2
	0.81		27	20
	3.25		98	95
ZP45845	0.20		- 6	26
	0.81		66	90
	3.25		99	90
BK99121	0.20		17	21
	0.81		80	76
	3.25		100	98
BL03308	0.20		25	18
	0.81		71	86
	3.25		98	100
BL50021	0.21		33	20
	0.81		70	73
	3.25		99	99
BL51297	0.21		37	13
	0.81		76	40
	3.25		98	99

Table VIII. Suppressive activity in liver and spleen of WR06026 administered either 3 days prior to or 3 days post infection.

Compound	Dose*	% Supp. (liver)		% Supp. (spleen)	
		-3d#	+3d	-3d#	+3d
WR06026	1.6	71	100	44	65
	0.4	57	100	70	44
	0.1	14	85	46	56
BL52945	1.6	38	98	45	66
BL52196	1.6	0	91	59	53
BL52749	1.6	34	97	70	58
BL53308	1.6	19	96	46	36
BL34296	1.6	28	82	0	29

*total mg/kg administered in single oral dose.

#-3d Drug given 3 days pre-infection

+3d Drug given 3 days post infection

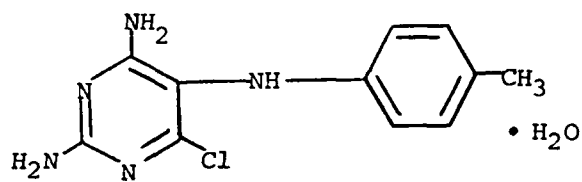
Table IX. Suppressive activity in liver and spleen of WR06026 administered either 3 days prior to or 3 days post infection.

Dose*	% Supp. (liver)		% Supp. (spleen)	
	-3d#	+3d	-3d#	+3d
6.5	86	100	34	100
3.25	74	100	16	97
1.63	60	100	21	66

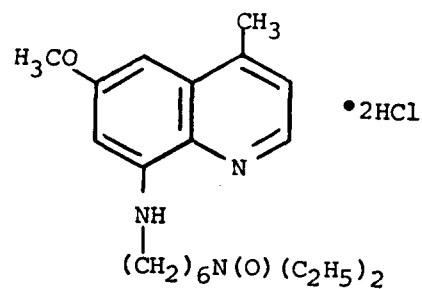
*Total mg/kg administered as a single oral dose.

-3d drug administered 3 days pre-infection
 +3d drug administered 3 days post infection

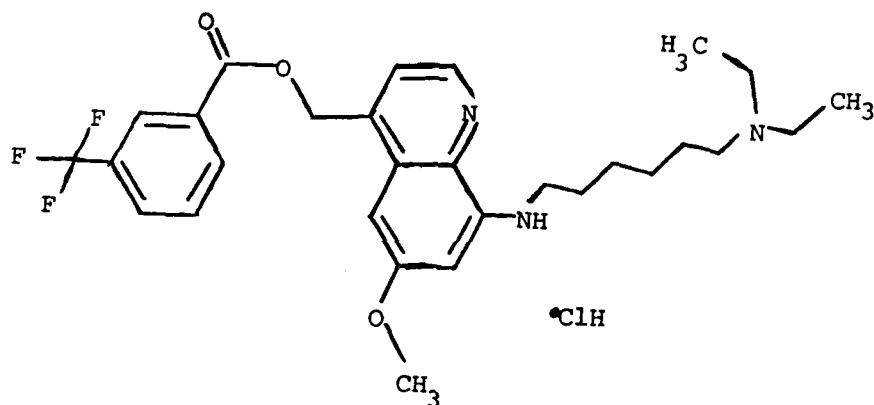
Figure 1. Chemical structures of compounds found active in the primary visceral test system.



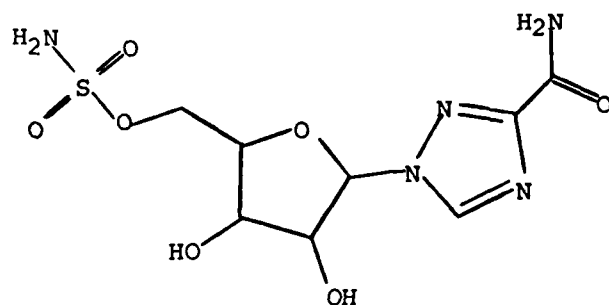
AJ07615



ZP30451



BL53308



BL55928

Figure 1 (Continued)

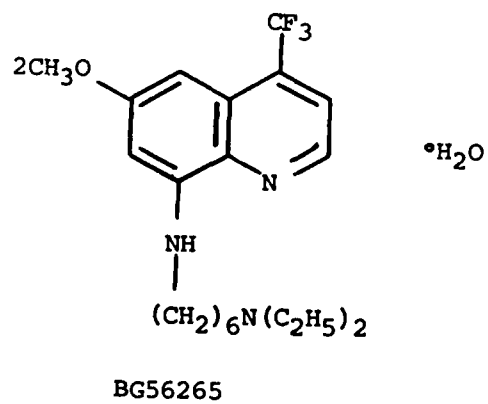
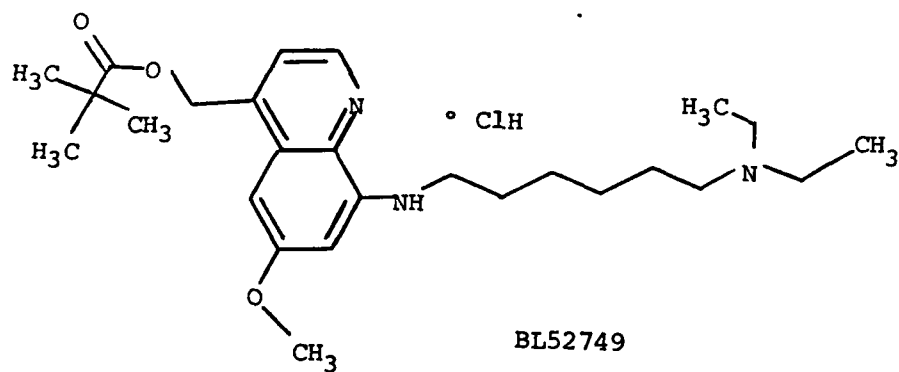


Figure 2. Structures of berberine alkaloids.

(1) berberine chloride, (2) palmatine chloride, (3) oxyberberine, (4) dihydroberberine, (5) 8-cyanodihydroberberine, (6) tetrahydroberberine N-Oxide, (7) tetrahydroberberine, (8) N-methyltetrahydroberberinium iodide, (9) berberine betaine.

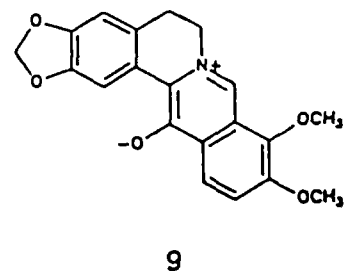
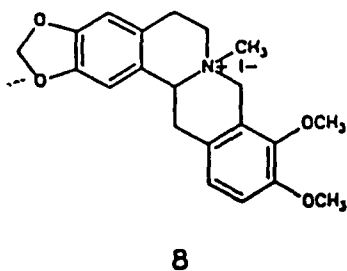
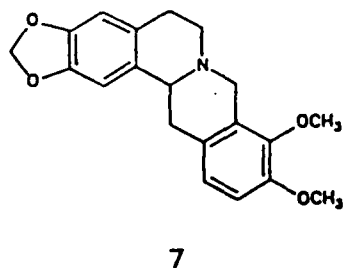
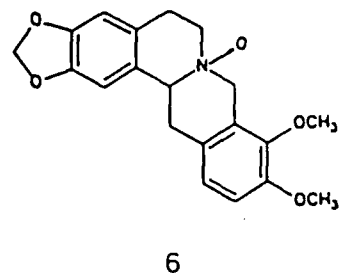
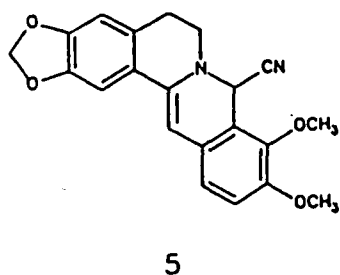
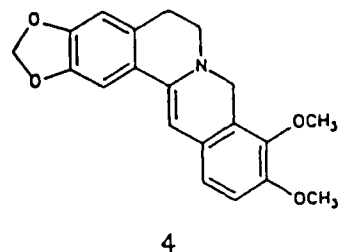
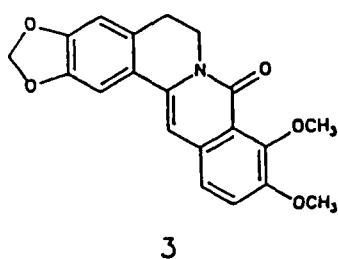
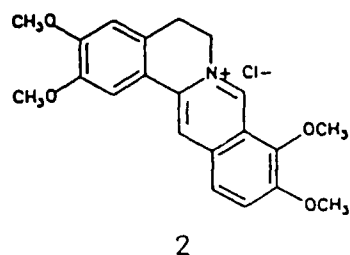
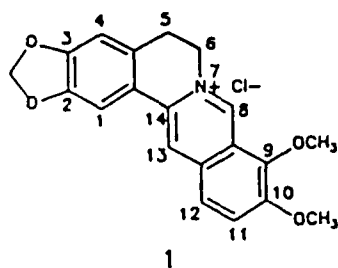
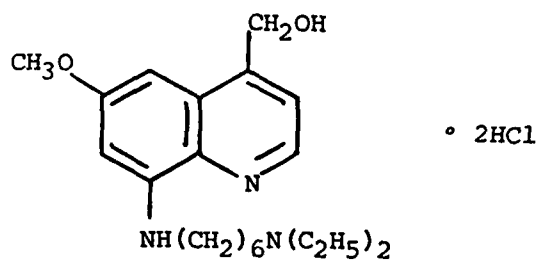
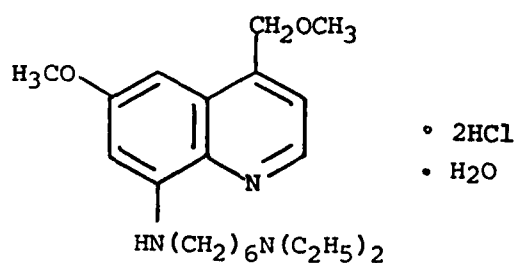


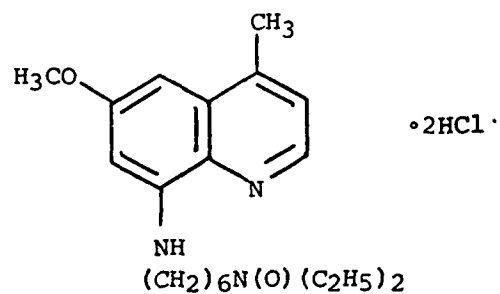
Figure 3. Structures of 8-aminoquinolines used in the study of the effect of route of administration on efficacy against *Leishmania donovani*.



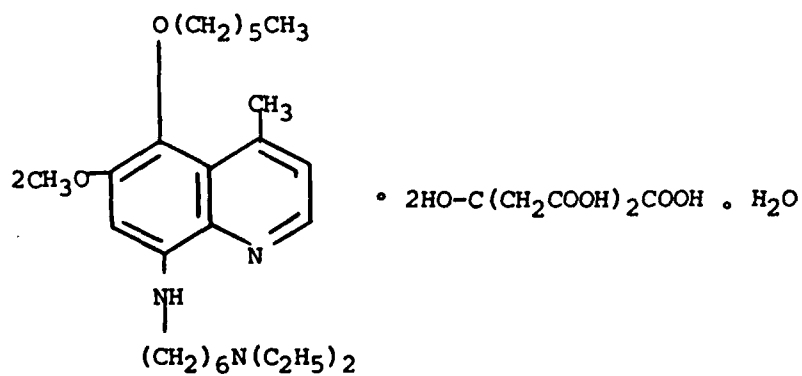
BL51297



BL50021

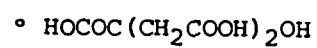
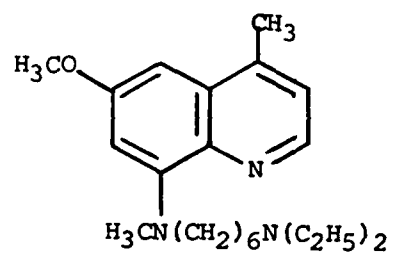


ZP45845



BK84200

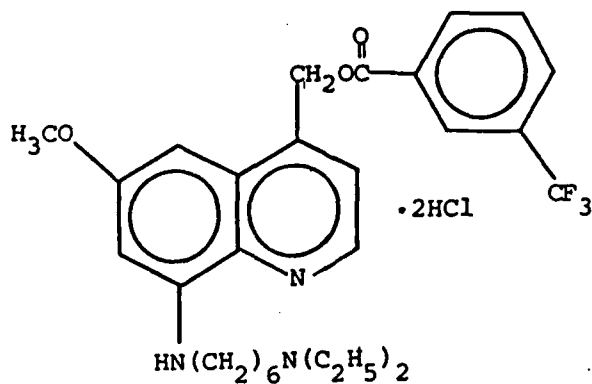
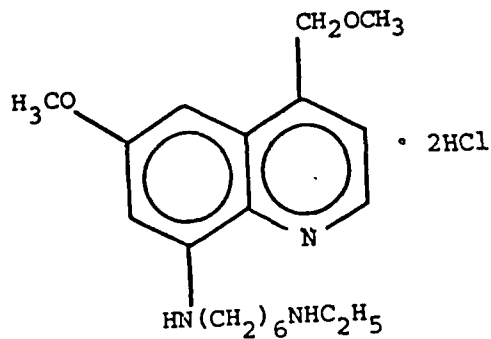
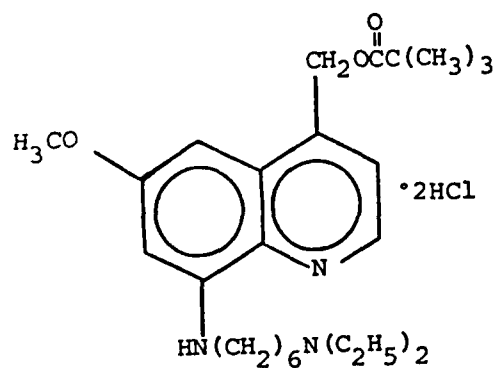
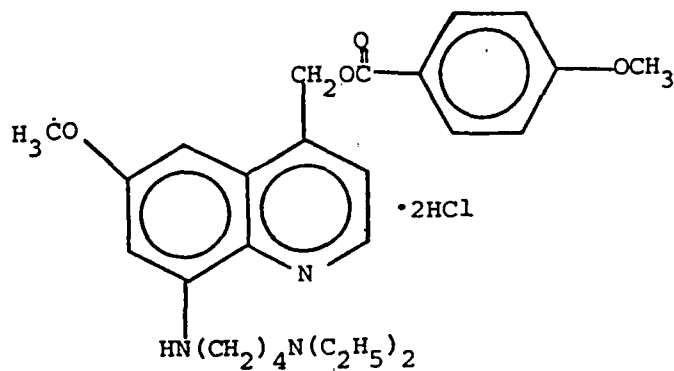
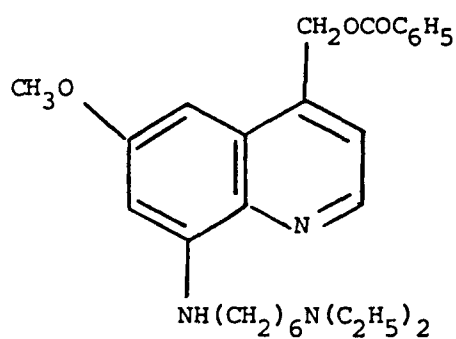
Figure 3 (Continued)



BK99121

BL03308
PROPRIETARY

Figure 4. Chemical structures of WR06026 analogs.



LITERATURE CITED

1. Kinnamon, K. E., E. A. Steck, P. S. Loizeaux, L. D. Hendricks, V. B. Waits, W. L. Chapman, Jr., and W. L. Hanson. 1979. Leishmaniasis: Military significance and new hope for treatment. Mil. Med. 44(10), 660-664.
2. Tropical Disease Research, Seventh Programme Report, 1 January 1983 - 31 December 1984. UNDP/World Bank/WHO Imprimerie A. Barthelemy, Avignon, France 1985. Pages 7/3-7/18.
3. Biagi, F., 1976. Leishmaniasis. In Tropical Medicine, Hunter, G. H., J. C. Swartzwelder, and D. F. Clyde (Eds.) W. B. Saunders Co., Philadelphia. pp. 411-429.
4. Steck, E. A. 1972. The Chemotherapy of Protozoan Diseases. Walter Reed Army Institute of Research, Vol. II, Sect. 3, pp. 6.1-7.141, published by U.S. Govt. Printing Office.
5. Mahmoud, A. A. F., and K. S. Warren. 1977. Algorithms in the diagnosis and management of exotic diseases. XXIV. Leishmaniasis. J. Infect. Dis. 136, 160-163.
6. Croft, S. L., 1988. Recent developments in the chemotherapy of leishmaniasis. Trends in Pharmacological Sciences. 9(10), 376-378.
7. Peters, W. 1981. The treatment of kala-azar - new approaches to an old problem. Indian J. Med. Res. 73 (Supl.), 1-18.
8. Perea, W. A. 1989. Epidemic visceral leishmaniasis in southern Sudan. The Lancet 2(8673), 1222.
9. Most, H. 1968. Leishmaniasis. In: Internal Medicine in World War II. Volume III. Infectious Diseases and General Medicine. Office of the Surgeon General, Department of the Army, Washington, D.C. pp. 1-48.
10. Naggan, L., A. E. Gunders, R. Dizian et al. 1970. Ecology and attempted control of cutaneous leishmaniasis around Jericho, in the Jordan Valley. J. Infect. Dis. 212, 427-432.
11. Walton, B. C., D. A. Person, and R. Bernstein. 1968. Leishmaniasis in the U.S. Military in the Canal Zone. Am. J. Trop. Med. Hyg. 17 (1), 19-24.
12. Takafuji, E. T., L. D. Hendricks, J. L. Daubek, K. M. McNeil, H. W. Scagliola, and C. L. Diggs, 1980. Cutaneous leishmaniasis associated with jungle training. Am. J. Trop. Med. Hyg. 29 (4), 516-520.

13. Neal, R. A. 1987. Experimental Chemotherapy. In *The Leishmaniasis in Biology and Medicine. Vol II. Clinical Aspects and Control.* Peters, W. and Killick-Kendrick, R. (Eds.). Academic Press, London. pp. 793-845.
14. Bryceson, A. 1987. Therapy in Man. In *The Leishmaniasis in Biology and Medicine. Vol II. Clinical Aspects and Control,* Peters, W. and Killick-Kendrick, R. (Eds.). Academic Press, London pp. 847-907.
15. Hanson, W. L., V. B. Waits, L. D. Hendricks, W. T. Hockmeyer, D. E. Davidson, Jr., and W. L. Chapman, Jr. 1983. Relative insensitivity of a Kenyan strain of Leishmania donovani to pentavalent antimony therapy in hamsters. J. Parasitol. 69, 446.
16. Mebrahtu, Y., P. Lawyer, J. Githure, J. B. Were, R. Muigai, L. Hendricks, J. Leeuwenburg, D. Koech, and C. Roberts, 1989. Visceral leishmaniasis unresponsive to pentostam caused by Leishmania tropica in Kenya. Am. J. Trop. Med. Hyg. 41(3), 289-294.
17. Jackson, J. E., J. D. Tally, and D. B. Tang, 1989. An in vitro micromethod for drug sensitivity testing of Leishmania. Am. J. Trop. Med. Hyg. 41(3), 318-330.
18. WHO Publication TDR/CHEM LEISH/VL. 82.3, 1982. Report of the informal meeting on the chemotherapy of visceral leishmaniasis. Page 4.
19. Stauber, L. A., E. M. Franchino, and J. Grun, 1958. An eight-day method for screening compounds against Leishmania donovani in the golden hamster. J. Protozool. 5, 269-273.
20. Stauber, L. A., 1958. Host resistance to the Khartoum strain of Leishmania donovani. The Rice Institute Pamphlet Vol. XLV (1), 80-96.
21. Stauber, L. A., 1958. Chemotherapy of experimental leishmaniasis. Proc. 6th International Congr. on Trop. Med. & Mal. III, 797-805.
22. Hanson, W. L., W. L. Chapman, Jr., and K. E. Kinnamon. 1977. Testing of drugs for antileishmanial activity in golden hamsters infected with Leishmania donovani. Internat'l. J. Parasitol. 7, 443-447.
23. Hendricks, L. D., D. Wood, and M. Hajduk. 1978. Hemoflagellates: Commercially available liquid media for rapid cultivation. Parasitol. 76, 309-316.
24. Hanson, W. L. and E. L. Roberson. 1974. Density of parasites in various organs and the relation to number of trypomastigotes in the blood during acute infections of Trypanosoma cruzi in mice. J. Protozool. 21, 512-517.
25. Wilson, H. R., B. S. Dieckmann, and G. E. Childs. 1979. Leishmania braziliensis and Leishmania mexicana: Experimental cutaneous infections in golden hamsters. Exptl. Parasitol. 47, 270-283.
26. Devi, A. C. 1929. Berberine sulfate in oriental sore. Indian Med. Gazette 64, 139-140.

27. El-On, J. and G. Messer. 1986. Leishmania major: Antileishmanial activity of methylbenzethonium chloride. Am. J. Trop. Med. Hyg. 35, 1110-1116.
28. Kinnamon, K. E., E. A. Steck, P. S. Loizeaux, W. L. Hanson, W. L. Chapman, Jr., and V. B. Waits. 1978. The antileishmanial activity of Lepidines. Am. J. Trop. Med. Hyg. 27(4), 751-757.
29. Hanson, W. L., V. B. Waits, and W. L. Chapman, Jr., Annual Report. February 28, 1989, Contract No. DAMD17-85-C-5012.
30. Berman, J. D., W. L. Hanson, J. K. Lovelace, V. B. Waits, J. E. Jackson, W. L. Chapman, Jr., and R. S. Klein, 1987. Activity of purine analogs against Leishmania donovani in vivo. Antimicrobial Agents and Chemotherapy, 31(1), 111-113.
31. Nolan, L. Final Report. January 30, 1987. Contract No. DAMD17-81-1198.
32. DasGupta, B. M. and B. B. Dikshit. 1929. Berberine in treatment of oriental sore. Indian Med. Gazette 64, 67-70.
33. Ghosh, A. K., F. K. Bhattacharyya, and D. K. Ghosh. 1985. Leishmania donovani: Amastigote inhibition and mode of action of berberine. Exp. Parasitol. 60, 404-413.
34. Ghosh, A. K., M. M. Rakshit, and D. K. Ghosh. 1983. Effect of berberine chloride on Leishmania donovani. Indian J. Med. Res. 78, 407-416.
35. Karamchandani, P. V. 1927. An analysis of 337 cases of oriental sore treated by various methods. Indian Med. Gazette 62, 558.
36. Varma. R. L. 1927. Berberine sulfate in oriental sore. Indian Med. Gazette 62, 84-85.
37. Putzer, B. 1934. Berberine and its derivatives in the treatment of oriental sore. Med. Chem. Aspects. 2, 274-280.

DISTRIBUTION LIST

5 copies: Director
Walter Reed Army Institute of Research
Walter Reed Army Medical Center
ATTN: SGRD-UWZ-C
Washington, DC 20307-5100

1 copy: Commander
US Army Medical Research and Development Command
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, Maryland 21701-5012

2 copies: Defense Technical Information Center (DTIC)
ATTN: DTIC-FDAC
Cameron Station
Alexandria, VA 22304-6145

1 copy: Dean
School of Medicine
Uniformed Services University of the
Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

1 copy: Commandant
Academy of Health Sciences, US Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234-6100