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During this project period a total of 41 new compounds were studied for antileishmanial activity against Leishmania donovani in hamsters. Only one had measurable suppressive activity and this compound was toxic.

One new compound was studied for efficacy against Leishmania braziliensis panamensis and this compound was not active and was toxic when administered via the intramuscular route.

A computer search of a total of 736 compounds that have been tested against L. b. panamensis since 1980 identified a total of 37 compounds with suppressive activity equal to or greater than the standard reference compound, Glucantime. These compounds were then studied in detail to determine the most promising drug and the most promising routes of administration and dosage regimen against L. b. panamensis. For comparative purposes these compounds were tested simultaneously against L. donovani and L. b. panamensis.

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Of the 37 compounds identified, 32 were 8-aminoquinolines. This class of compounds contains the most potent compounds studied thus far in this laboratory for suppressive activity against visceral and cutaneous leishmaniasis. With two exceptions, these compounds were several fold more active against *L. donovani* than against *L. b. panamensis*. Ten of these 37 compounds were sufficiently active against *L. b. panamensis* to be of interest and the most active of the group was WR049577 (SD_{50} = 3.76 mg/kg). Unfortunately this compound was not active when administered orally and is toxic in hamsters at a dosage level as low as 26 mg/kg, a dosage which suppresses *L. b. panamensis* lesions by only 76%.

Regarding the other two active compounds, a phosphonium compound and Sinefungin were noted to be active but the former was toxic and the activity of neither was remarkable.

A total of 31 oligonucleotides was studied for inhibition of multiplication of promastigotes of *L. donovani in vitro*. Only one of these had any suppressive activity.

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FOREWORD

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PI Signature William L. Hanson Date October 28, 1992

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INTRODUCTION

Protozoan parasites of the genus Leishmania are widespread throughout the world where they cause a complex of visceral or cutaneous diseases in human beings as well as some animals including dogs in numerous tropical and sub-tropical countries (1,2,3). Since the leishmaniases commonly exist as zoonoses, these diseases pose a significant potential threat to military personnel as well as military dogs throughout endemic areas. Recent publicity regarding infection of personnel involved in Operation Desert Storm has reemphasized the military significance of the leishmaniases.

Better drugs are needed for the treatment of the leishmaniases since those currently available are often not satisfactorily effective and are potentially toxic to man and animals.

This laboratory has been involved for several years in studies to identify new compounds for antileishmanial activity against both visceral (Leishmania donovani) and cutaneous (Leishmania braziliensis panamensis) leishmaniasis. Among the most promising active compounds found against visceral leishmaniasis during these studies is the 8-aminoquinoline, WR06026. This compound is now undergoing clinical trials in Kenyan visceral leishmaniasis patients. Screening for compounds active against visceral leishmaniasis has continued during this year in the event that WR06026 does not perform in the field as expected.

Since 1980 a total of 736 compounds has been tested for activity against cutaneous leishmaniasis. Most of these compounds were selected on the basis of previous activity against visceral leishmaniasis although a few were selected specifically for testing against cutaneous leishmaniasis. Generally, compounds active against visceral leishmaniasis proved to be either inactive or much less active against cutaneous leishmaniasis. The 8-aminoquinoline, WR06026, mentioned in the preceding paragraph is the most potent compound found thus far against visceral leishmaniasis but this compound has given highly variable results when studied against cutaneous leishmaniasis and when active, is much less active against cutaneous leishmaniasis in hamsters than against visceral leishmaniasis in the same host. Based on available evidence, it appears possible that WR06026 may be effective in human beings for visceral but not for cutaneous leishmaniasis. In order to ascertain the possibility of there being other promising compounds with possible efficacy against cutaneous infection, a search was made of the entire cutaneous data base to identify compounds that had been equally or more potent against cutaneous leishmaniasis than the reference drug, Glucantime. A total of 37 compounds were identified, of which 32

were 8-aminoquinolines. These compounds were then studied in detail against both visceral and cutaneous leishmaniasis during this year to determine the most promising drug against cutaneous infections. Since 8-aminoquinolines such as WR06026 are more potent against visceral leishmaniasis when administered orally, it was decided to test compounds of this class both orally and intramuscularly. The remaining compounds were tested by either the oral or intramuscular route.

This report summarizes the results of studies conducted for this contract during the period September 28, 1991 - September 27, 1992.

MATERIALS AND METHODS

A. 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 X 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 (4,5,6) as modified by Hanson et al. (7). 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 and nature of the compound.

The vehicle for the test compounds was 0.5% hydroxyethyl-cellulose-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 hamsters received the 0.5% HEC-Tween vehicle only and the reference compound, Glucantime® was given at 3 drug dosage levels (208, 52, and 26 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, et al. (4,5,6).

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 of the animals was also considered indicative of significant drug toxicity.

After determining the ratio of numbers of amastigotes per 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 per organ, and percent parasite suppression. The computer program then performs linear and non-linear regression analysis and calculates a SD_{50} for each active compound from each

of the analyses (drug dosage resulting in 50% suppression of amastigotes). 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.

B. Primary Cutaneous Test System

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

Promastigotes for establishing experimental infections in hamsters were grown in Schneider's Drosophila Medium (Hendricks, et al., 8) and quantitated using procedures described previously (Hanson and Roberson, 9). 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 approximately 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 postinfection, and continued through day 22 postinfection. 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., 10). 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 an 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. The linear regression analysis is performed for comparison with the non-linear analysis.

C. Comparative Antileishmanial Activity of Selected Compounds Against L. donovani and L. braziliensis panamensis.

The most active compounds in the primary cutaneous test system were selected for these studies from the data base. Thirty-seven compounds were tested simultaneously in the primary cutaneous and primary visceral test systems using the procedures described above in sections A and B. Since most of the active compounds were 8-aminoquinolines, two routes of administration (i.e. intramuscular and oral) were used against each parasite. Dosage levels for the cutaneous test system were generally higher than that used for the visceral test system due to the fact that the reference compound Glucantime requires approximately a four fold higher dosage level in the cutaneous system than in the visceral test system for activity.

D. In Vitro Studies of Oligonucleotides Against L. donovani

Promastigotes of L. donovani were cultured from an infected hamster spleen in Schneider's Drosophila Medium (Hendricks, et al., 8) and quantitated using procedures described previously (Hanson and Roberson, 9). Promastigotes from four-day cultures (fourth to twelfth subpassage) were used in this work. (Unpublished data indicates that this age culture is the best for establishing infections in hamsters.)

Cultures were harvested by centrifugation and resulting pellets were resuspended in Schneider's Drosophila Medium to a final concentration of 6.5×10^6 per ml. Using round bottom microtiter plates (Dynatech), 200 μl of the parasite suspension was added to each well and plates incubated at 26°C (Day 0).

Approximately 24 hours later, the oligonucleotides were added to appropriate wells at 30 micromolar concentrations (Day 1). Sets of four cultures were used for each as well as for untreated controls. Cultures were again incubated until Day 4 at which time total numbers of promastigotes/ml for each well were determined using the procedures described by Hanson and Roberson (9).

Mean numbers of parasites per well for each treated well and for untreated wells were calculated. Percent suppression or inhibition of parasite growth was determined using the following formula:

> Percent Suppression = mean number of parasites for the untreated controls minus the mean number of parasites for the test compound divided by the mean number of parasites for the untreated control times 100.

Negative percent suppression indicated enhanced growth of parasites in the treated wells as compared to growth in the untreated wells.

RESULTS

A. Primary Visceral Test System

During this reporting period a total of 41 compounds were studied for efficacy against Leishmania donovani infections in hamsters. One compound was administered via three different routes at three different dosage levels for each route, two compounds were administered via two routes at three dosage levels per route, one compound was administered via a single route at three dosage levels, and all others were administered via one route at two dosage levels (Table I). Only one (ZP10397) of the compounds studied in this system was considered active (greater than 50% parasite suppression) and this compound was toxic at the highest dosage level studied. Three of the inactive compounds were also toxic in hamsters.

B. Primary Cutaneous Test System

One compound (BM10620) was studied for efficacy against L. b. panamensis in the primary cutaneous test system. This compound was administered via the intramuscular, subcutaneous, and oral routes at three dosage levels for each route (Table II). Although this compound was toxic when administered via the intramuscular route, some suppression of parasite induced cutaneous lesions was noted. This compound was neither active nor toxic when administered via the other routes.

C. Comparative Antileishmanial Activity of Selected Compounds Against L. donovani and L. braziliensis panamensis

A group of compounds (Figure 1) which were selected from the cutaneous test system data base because they had been found to have antileishmanial activity equal to or greater than the reference compound, Glucantime, were studied simultaneously via the oral and intramuscular routes for efficacy against both L. donovani and L. b. panamensis for comparative purposes as well as to determine the compound most active against L. b. panamensis as indicated in Table III.

It was noted from the results obtained that, with two exceptions (WR049577 and WR027794), those compounds that were active at all against L. b. panamensis were considerably more active against L. donovani. For example, four 8-aminoquinoline compounds (WR211789, WR211666, WR223658, WR223756) were 99-100% suppressive against L. donovani at the lowest dosage levels tested (either 6.5 or 13 mg/kg) when administered either orally or via the intramuscular route. Additional studies (Table IV) were done on these compounds to determine the SD_{50} for comparative purposes. In contrast, only WR211789 and WR223658

were active against L. b. panamensis and the SD_{50}'s of each of these compounds were in excess of 100 mg/kg against this parasite. All of these compounds except WR211789 showed evidence of toxicity to hamsters when administered at dosage levels of 104 or 208 mg/kg against L. b. panamensis.

Ten of the 37 compounds studied in these experiments were sufficiently active against L. b. panamensis to be of interest. Eight of these were 8-aminoquinoline compounds. Among these, WR006007 with an SD_{50} of 79.8 mg/kg was approximately seven times less efficacious against L. b. panamensis than against L. donovani when administered via the intramuscular route. Similar comparative studies using the oral route of administration could not be done because of the insufficient quantity of this compound available. WR027794 was approximately 3-4 times less potent against L. b. panamensis than L. donovani and this compound appeared to be equally effective when administered via the oral or intramuscular routes. Similarly the efficacy of WR027779 was approximately two fold more active against L. donovani and this compound was approximately equally active when administered orally or intramuscularly. The difference in potency of WR027780 against L. b. panamensis and L. donovani was likewise approximately two fold but this compound was about twice as active when administered via the intramuscular route than via the oral route. The efficacy of both WR006877 and WR006021 was two to three times greater against L. donovani than against L. b. panamensis. Although the activity of these compounds against L. donovani was similar when administered either orally or intramuscularly, these compounds were active against L. b. panamensis only when administered via the intramuscular route. WR006881 (SD_{50} =77.7 mg/kg) was only slightly less potent against L. b. panamensis than L. donovani.

The most active compound against L. b. panamensis was the 8-aminoquinoline, WR049577 (SD_{50} =3.76 mg/kg). Although this compound was the most potent compound studied against L. b. panamensis, it was not active when administered orally and is toxic (causing weight loss in recipient hamsters) at dosage levels as low as 26 mg/kg while suppressing lesion size by only 76% at this same dosage.

Regarding the two active compounds that were not 8-aminoquinolines, one (WR122536) is a phosphonium compound which had an SD_{50} of 58 mg/kg when administered via the intramuscular route. Unfortunately, this compound was toxic (caused weight loss in recipient hamsters) at 104 mg/kg.

The other active compound that was not an 8-aminoquinoline was Sinefungin (WR254847). This compound has been tested previously in this laboratory and found to be active against both L. b. panamensis and L. donovani in hamsters (see Final Report, Contract No. DAMD17-85-C-5012, October 31, 1990). The difference

in the activity of this compound against L. b. panamensis and L. donovani was greater in the current experiments than in initial studies.

D. In Vitro Studies of Oligonucleotides Against L. donovani

Table V summarizes the results of the in vitro testing of 31 selected oligonucleotides for inhibition of growth of promastigotes of L. donovani. One oligonucleotide (LE001.01J 910806) appeared to suppress the multiplication of L. donovani in two separate studies (91.4% and 53.0% inhibition). Due to the differences in percent suppression obtained in the two experiments, a confirmatory experiment would be desirable before drawing a final conclusion on the activity of this compound.

DISCUSSION

The main area of emphasis during this contract period was to determine the compound with the best efficacy against cutaneous leishmaniasis caused by L. b. panamensis. To this end computer analysis of the results from the testing of approximately 736 compounds screened since 1980 was done and as a result 37 compounds were selected from the data base as having efficacy against cutaneous leishmaniasis in the hamster which was equal to or greater than that of the standard reference compound, Glucantime.

These compounds were then studied simultaneously in detail for activity against L. b. panamensis as well as L. donovani. The latter parasite was included for comparative purposes. It is interesting that of the ten compounds identified by these studies as the most active against L. b. panamensis, all but two were 8-aminoquinolines.

Studies in this laboratory have historically shown that the 8-aminoquinolines are the most active compounds against both L. donovani and L. b. panamensis in hamsters. In addition, as verified in these current studies, the 8-aminoquinolines have almost always been more active against L. donovani than against L. b. panamensis. The same is true for the reference compound, Glucantime.

The reasons for the higher efficacy of the 8-aminoquinolines against L. donovani are unknown but it may be due to the fact that liver parasites are more accessible to the parent compounds and their metabolites since this class of compounds are metabolized in the liver. Apparently, less compound and/or metabolites is distributed to sites distant to the liver, a hypothesis supported by observations in this laboratory of less activity of these compounds against spleenic parasites than liver parasites in L. donovani infections in hamsters (see Final Report, Contract No. DAMD17-85-C-5012, October 31, 1990).

This suggested problem of bioavailability appears to be an especially important one in cutaneous leishmaniasis. It is possible that this question could be addressed by regimen variation or possibly application of the drug directly onto the lesion.

Several metabolites of the 8-aminoquinoline, WR06026, the most active drug against L. donovani in hamsters, have been found to be active against L. donovani. It is suggested that these should be tested against L. b. panamensis. In addition more definitive studies of Sinefungin and some of its analogs, when available, should be tested against L. b. panamensis.

Antisense RNA's have been exploited with varying success to block the activity of specific genes to inhibit the replication of viruses as well as various human cancer cells (11, 12). Dr. R. Meyer, Microprobe, Inc., under a separate contract (DAMD17-88-C-8201) developed the idea to apply this technology against Leishmania and has synthesized a number of antisense as well as sense oligonucleotides for possible inhibition of the growth of Leishmania. A number of these preparations were studied during the past contract period (see Annual Report, Contract No. DAMD17-90-C-0131, October 27, 1991) and these studies have continued during this contract period. These oligonucleotides were supplied to our laboratory for testing. Thus far this approach has not appeared to be especially promising although some suggestion of inhibition of growth of Leishmania donovani in vitro was observed. One possible explanation for the lack of inhibition observed in these experiments is the fact that it is sometimes difficult to get the oligonucleotides into cells at the right time to block messenger RNA activities (11). Work in this area is continuing. (See report by Dr. Meyer for additional details.)

CONCLUSIONS

1. Since 8-aminoquinolines remain the most active compounds screened to date against both visceral and cutaneous leishmaniasis, it would be advisable to continue to study this class and especially the metabolites for activity against both visceral and cutaneous leishmaniasis.

2. A more definitive testing of Sinefungin and some of its analogs when available should be conducted.

3. Promising novel compounds as indicated by published *in vitro* antileishmanial studies or by reports of activity against other organisms should be screened for antileishmanial activity *in vivo*.

4. Any deoxyoligonucleotides that show *in vitro* activity should be tested for *in vivo* antileishmanial activity.

5. Continued *in vitro* testing of selected oligonucleotides would be useful.

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APPENDIX 1

Table I. Summary of compounds studied for suppressive activity against *Leishmania donovani* in the primary visceral test system.

Bottle #	Route	Dose1	Suppres1	Dose2	Suppres2	Dose3	Suppres3
BM10620	IM	104*	38	52	18	13	29
	PO	104	-6	52	22	13	17
	SQ	104	19	52	27	13	18
BM10371	IM	208	25	52	37	ND	ND
BL21100	IM	208	17	52	14	ND	ND
BL59588	IM	208	-44	52	-42	ND	ND
BL56390	IM	208	-18	52	-34	13	-12
BL34170	IM	208	-5	52	2	ND	ND
BL29759	IM	208	25	52	24	ND	ND
AX26839	IM	208*	-3	52	19	ND	ND
AY97173	IM	208	36	52	33	ND	ND
AY97315	IM	208	10	52	35	ND	ND
AH90393	IM	208	16	52	33	ND	ND
AG66089	IM	208	12	52	12	ND	ND
AG50330	IM	208	-1	52	0	ND	ND
AD60466	IM	208	3	52	8	ND	ND
BM12991	IM	208	-14	52	-7	ND	ND
AR81714	IM	208	40	52	23	ND	ND
AP64866	IM	208	20	52	12	ND	ND
AN35100	IM	208	6	52	36	ND	ND
AN15359	IM	208	9	52	19	ND	ND
BM12508	IM	52	11	ND	ND	ND	ND
	PO	208	0	52	-6	13	-3
BM12491	IM	52	-13	ND	ND	ND	ND
	PO	208	-10	52	7	13	1
AE95204	IM	208	22	52	-14	ND	ND
ZP10397	IM	208	D	52	51	ND	ND
AR94417	IM	208	28	52	36	ND	ND
ZC07760	IM	208	-4	52	3	ND	ND
ZC07751	IM	208	0	52	-15	ND	ND
ZA01419	IM	208	15	52	-20	ND	ND
BL86558	IM	208	-9	52	-19	ND	ND
AS64898	IM	208	-17	52	-21	ND	ND
AQ07393	IM	208	28	52	7	ND	ND
AN39528	IM	208*	35	52	13	ND	ND
AM04315	IM	208	-11	52	-12	ND	ND
AJ91813	IM	208	4	52	-19	ND	ND
AR02802	IM	208	-3	52	-10	ND	ND
AP86979	IM	208	-4	52	-7	ND	ND
ZG81239	IM	208	16	52	1	ND	ND
AL02996	IM	208	13	52	22	ND	ND
AH69718	IM	208	-23	52	8	ND	ND
AG53859	IM	208	14	52	-12	ND	ND
AG53840	IM	208	25	52	16	ND	ND
AG53831	IM	208	22	52	27	ND	ND

See following page for footnotes.

Table I. (continued)

* Toxic as indicated by death of hamsters and/or 15% or greater loss of body weight
D: All hamsters receiving this drug dosage level died
IM: Intramuscular route of drug administration
PO: Oral route of drug administration
SQ: Subcutaneous route of drug administration
ND: Not done

Table II. Summary of compounds studied for suppressive activity against Leishmania braziliensis panamensis in the primary cutaneous test system.

Bottle #	Route	Dose1	Suppres1	Dose2	Suppres2	Dose3	Suppres3
BM10620	IM	104*	73	52*	51	13	7
	PO	104	11	52	-7	13	-7
	SQ	104	15	52	-11	13	-59

* Toxic as indicated by death of hamsters and/or 15% or greater loss of body weight
IM: Intramuscular route of drug administration
PO: Oral route of drug administration
SQ: Subcutaneous route of drug administration

Table III. Summary of results obtained from studies on the comparative activity of selected compounds against both *Leishmania donovani* and *Leishmania braziliensis panamensis*.

Table III. (continued)

WRNO	BN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY?
WR061250	AX26820	L. don.	im	26	-18	52	-28	104	21		
			po		-24		-32		-20		
		L. bras.	im	52	23	104	15	208	49		
			po		15		8		19		
WR211789	BK50713	L. don.	im	6.5	99	13	100	52	100	<6.5	
			po		99		100		100	<6.5	
		L. bras.	im	13	0	52	35	104	50	104.00	
			po		15		28		19		
WR211666	BG11417	L. don.	im	6.5	100	13	100	52	100	<6.5	
			po		100		100		100	<6.5	
		L. bras	im	13	12	52	25	104	ND		104
			po		13		45		ND		104
WR122536	AG78374	L. don.	im	6.5	-2	13	2	52	61	<52	
			po	ND							
		L. bras.	im	26	15	52	48	104	70	58.00	104
			po	ND							
WR006023	AG98545	L. don.	im	13	-18	52	67	104	ND	41.70	104
			po		18		51		86	45.70	
		L. bras.	im	6.5	-8	26	-4	52	0		
			po		-24		-44		-48		
WR099029	AH16404	L. don.	im	6.5	4	13	9	26	9		
			po	ND							
		L. bras.	im	6.5	0	13	-4	26	33		
			po	ND							
WR249668	BJ92403	L. don.	im	13	17	52	91	104	100	24.30	
			po		-20		62		98	47.00	
		L. bras.	im	13	0	52	16	104	29		
			po		-36		-28		12		
WR223658	BG21744	L. don.	im	13	100	52	100	104	100	<13	
			po		100		100		100	<13	
		L. bras.	im	26	16	104	46	208	ND		208
			po		-28		23		62	175.00	

Table III. (continued)

WRNO	BN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY?
WR223756	BG22125	L. don.	im	13	100	52	100	104	100	<13	
			po		100		100		100	<13	
		L. bras.	im	52	4	104	4	208	4		
			po		-16		24		ND		208
WR049577	AH07870	L. don.	im	6.5	14	13	26	26	34		
			po		-27		-16		-30		
		L. bras.	im	6.5	61	13	61	26	76	3.76	26
			po		0		-7		-3		
WR006007	AJ36812	L. don.	im	13	54	52	90	104	98	11.90	
			po	ND							
		L. bras.	im	13	7	52	37	104	61	79.80	
			po	ND							
WR006917	AH32668	L. don.	im	13	-13	52	2	104	-15		
			po	ND							
		L. bras.	im	52	0	104	3	208	-3		
			po	ND							
WR006014	AJ09575	L. don.	im	13	-4	52	1	104	84	82.30	
			po		10		25		48		
		L. bras.	im	26	24	104	11	208	19		
			po		-30		15		15		
WR007561	AJ09851	L. don.	im	13	16	52	40	104	44		
			po	ND							
		L. bras.	im	26	-19	104	-4	208	53		
			po	ND							
WR027795	BE20532	L. don.	im	6.5	3	13	-1	52	0		
			po		-4		14		10		
		L. bras.	im	13	7	52	11	104	11		
			po		-14		-7		14		
WR057023	BB18813	L. don.	im	13	11	52	56	104	99	49.40	
			po		-36		60		98	47.60	
		L. bras.	im	52	-7	104	19	208	-7		
			po		-21		-7		7		

Table III. (continued)

WRNO	PIN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY
WR053215	BB19758	L. don.	im	13	-14	26	-24	104	-25		
			po		-22		-27		-17		
		L.bras.	im	26	-4	104	0	208	11		
			po		-25		-2		-7		
WR006027	BE20112	L. don.	im	13	99	26	99	104	100	4.93	
			po		99		100		100	4.65	
		L. bras.	im	26	21	104	48	208	ND		208
			po		28		17		ND		208
WR027788	BE20318	L. don.	im	13	-17	26	-5	104	11		
			po		-35		-11		5		
		L. bras.	im	26	14	104	5	208	ND		208
			po		7		7		-3		
WR027792	BE20345	L. don.	im	13	-11	26	2	104	4		
			po		16		50		55	92.00	
		L. bras.	im	26	21	104	37	208	ND		208
			po		28		-3		14		
WR027793	BE20354	L. don.	im	6.5	15	13	50	52	77	26.90	
			po		26		28		77	29.90	
		L. bras.	im	13	30	52	47	104	67	59.30	
			po		7		44		72	61.00	
WR027794	BE20498	L. don.	im	13	22	26	30	104	84	53.20	
			po		20		40		68	52.80	
		L. bras.	im	26	37	104	46	208	60	133.00	208
			po		-15		15		53	199.00	
WR027796	BE20603	L. don.	im	ND							
			po	6.5	29	13	35	52	78	24.60	
		L. bras.	im	ND							
			po	13	9	52	4	104	26		
WR006881	BE20792	L. don.	im	6.5	25	26	13	52	46		
			po		21		28		20		
		L. bras.	im	6.5	2	26	42	104	54	77.70	
			po		-19		-26		-30		

Table III. (continued)

WRNO	BN	PARASITE	ROUTE	DOSE1	SUPPRESS1	DOSE 2	SUPPRESS2	DOSE 3	SUPPRESS3	SD50	TOXICITY
WR027742	BE20925	L. don.	im	6.5	10	26	44	52	82	28.70	
			po		16		33		60	41.60	
		L. bras.	im	6.5	10	26	53	104	78	25.90	
			po		17		13		38		
WR027785	BE20943	L. don.	im	ND							
			po	6.5	26	13	27	52	52	48.50	
		L. bras.	im	ND							
			po	13	-35	52	4	104	58	96.10	
WR006877	ZN29695	L. don.	im	6.5	-37	13	17	52	72	35.60	
			po		-25		-13		76	38.20	
		L. bras.	im	13	13	52	41	104	71	65.00	
			po		-17		17		43		
WR027779	BE21039	L. don.	im	6.5	17	26	61	52	75	20.70	
			po		9		58		72	23.60	
		L. bras.	im	13	25	52	71	208	81	33.70	208
			po		20		57		75	44.30	
WR006020	BE20166	L. don.	im	6.5	7	26	15	52	57	23.60	
			po		1		14		47		
		L. bras.	im	13	16	52	42	104	32		
			po		-10		10		19		
WR027780	BE21084	L. don.	im	6.5	-1	26	65	52	83	18.80	
			po		-5		26		58	44.10	
		L. bras.	im	26	23	52	52	104	79	50.10	104
			po		-23		19		55	96.30	
WR007296	BE21511	L. don.	im	6.5	-41	13	-24	52	14		
			po		-7		1		26		
		L. bras.	im	13	-13	26	0	104	6		
			po		6		6		3		
WR006021	BE21799	L. don.	im	6.5	9	13	3	52	78	34.80	
			po		3		5		61	34.80	
		L.bras.	im	13	-12	26	-8	104	55	104.00	
			po		14		8		42		

Table III. (continued)

WRNO	BN	PARASITE	ROUTE	DOSE 1	SUPPRESS 1	DOSE 2	SUPPRESS 2	DOSE 3	SUPPRESS 3	SD50	TOXICITY
WR052252	AT63681	L. don.	im	13	3	52	22	104	-9		
			po	ND							
		L. bras.	im	52	-15	104	-13	208	-13		
			po	ND							
WR254419	BL05848	L. don.	im	6.5	74	13	93	52	99	4.16	
			po		64		83		98	4.47	
		L. bras.	im	13	15	52	17	104	ND		104
			po		-19		-4		ND		104
WR254847	BL58705	L. don.	im	6.5	79	26	89	52	85	1.71	
			po	ND							
		L. bras.	im	13	35	52	43	104	56	79.60	
			po	ND							
WR007511	AJ15304	L. don.	im	13	7	26	22	52	34		
			po	ND							
		L. bras.	im	3.25	-9	13	-30	52	9		
			po	ND							
WR006561	AT56097	L. don.	im	13	13	52	51	104	38	51.40	
			po	ND							
		L. bras.	im	52	9	104	-4	208	13		
			po	ND							

Table IV. Summary of results of additional testing of compounds found highly suppressive against *Leishmania donovani*.

Bottle #	Route	Dose1	Suppres1	Dose2	Suppres2	Dose3	Suppres3
BK50713	IM	3.25	97	0.8	-6	0.4	-23
	PO	3.25	94	0.8	6	0.4	-29
BG11417	IM	3.25	99	0.8	59	0.4	29
	PO	3.25	99	0.8	68	0.4	28
BG21744	IM	3.25	100	0.8	12	0.4	-31
	PO	3.25	99	0.8	25	0.4	-5
BG22125	IM	3.25	98	0.8	42	0.4	-16
	PO	3.25	94	0.8	31	0.4	6

IM: Intramuscular route of drug administration
PO: Oral route of drug administration

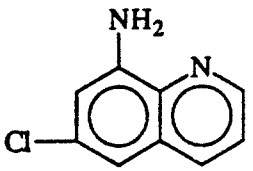
Table V. Summary of results of selected oligonucleotides tested for *in vitro* inhibition of promastigotes of *Leishmania donovani*.

Compound	Percent Suppression
LE502 910410	34.4
LE002.01 J 910415	20.8
LE002.02 J 910415	25.5
LE002.03 J 910416	22.2
LE002.04 J 910416	26.6
LE002.05 J 910716	26.3
LE002.06 J 910716	21.6
LE002.07 J 910716	26.8
LE001.01 J 910806	91.4*
LE001.02 J 910806	14.3
LE001.04 J 910808	47.0
HBV040.01 J 910806	38.8
LE001QX	-76.9**
LE002QX	-222.5
LE001SX	-179.2
LE002SX	-10.7
LE001.01H	17.4
LE002.01H	-14.5
LE001.01Q	-34.9
LE002.01Q	-32.0
LE001.01S	-27.5
LE002.01S	7.3
LE001HX	8.3
LE002HX	-75.2
LE001HY	32.8
LE002HY	27.2
LE001QY	16.0
LE002QY	-71.6
LE001SY	-2.4
LE001.01J	27.2
LE501.01J	53.0

* Based on triplicate cultures

** Negative percent suppression indicates enhancement of parasite numbers

Figure 1. Structures of the most active compounds found in the primary cutaneous test system and tested simultaneously in the primary visceral test system during this contract period.



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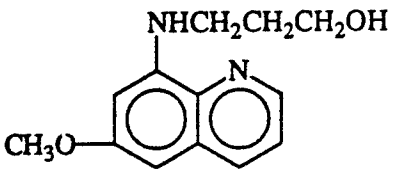

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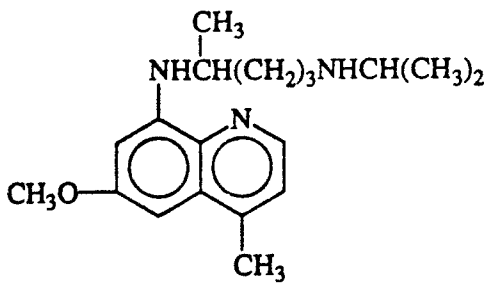

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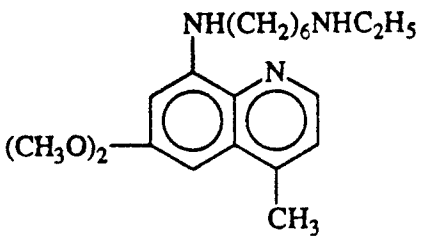

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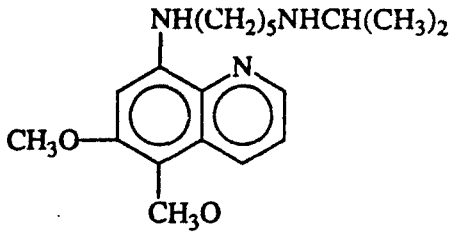

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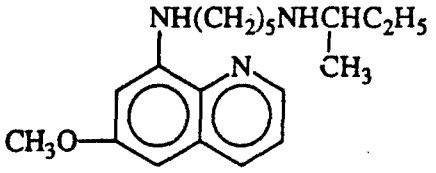

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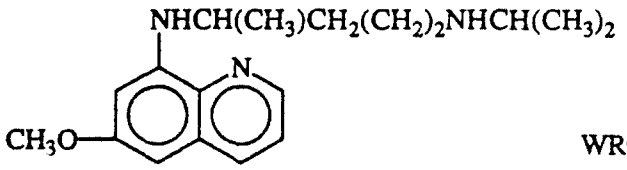

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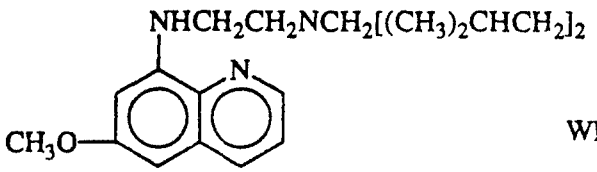

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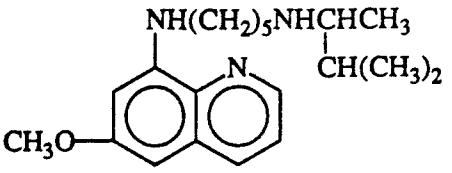

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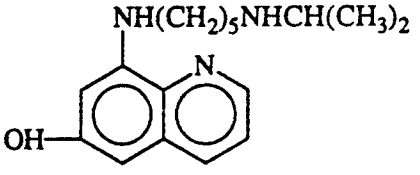

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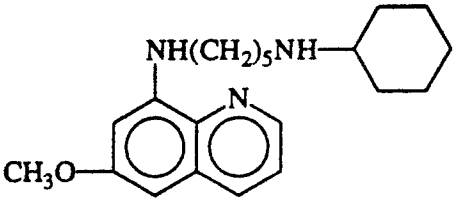

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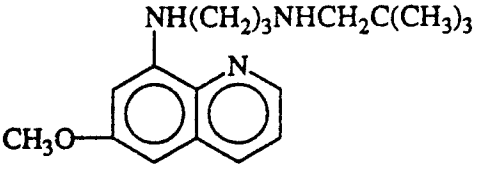

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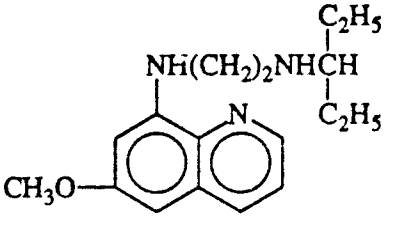

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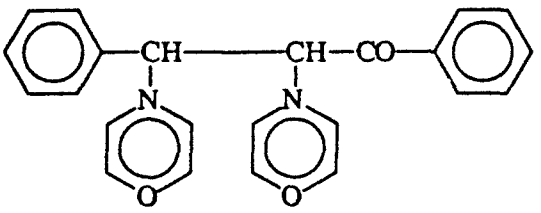

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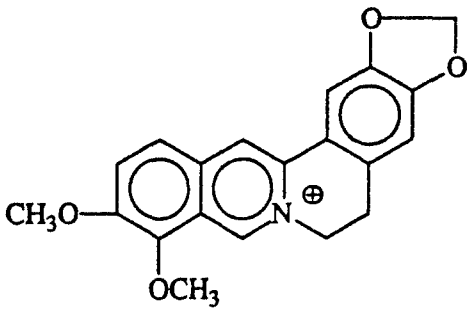

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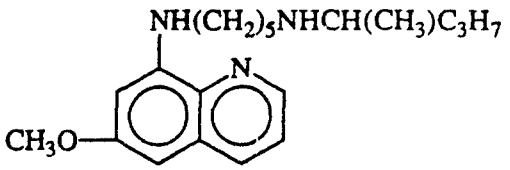

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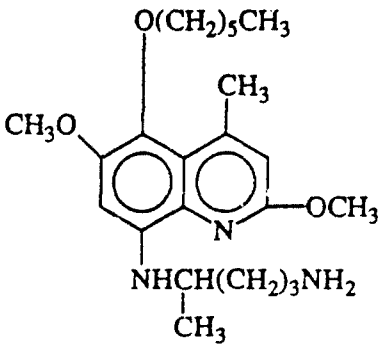

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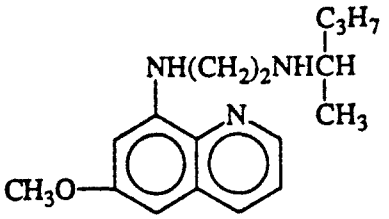

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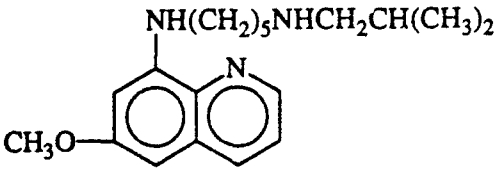

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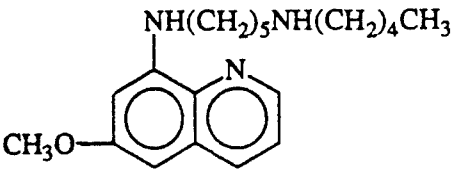

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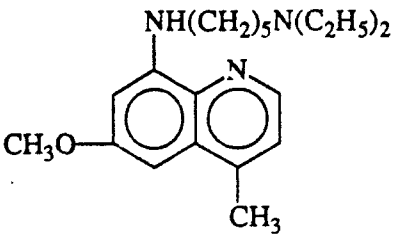

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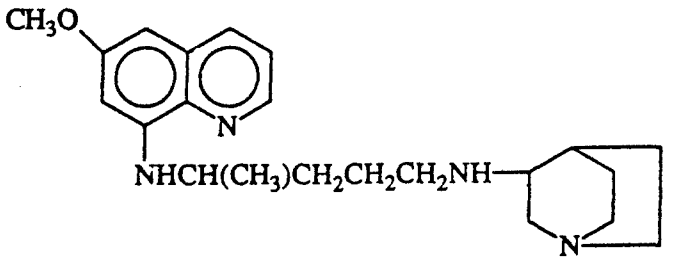

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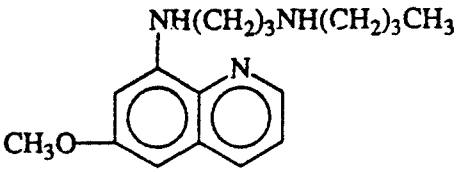

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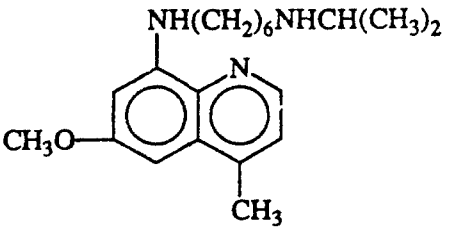

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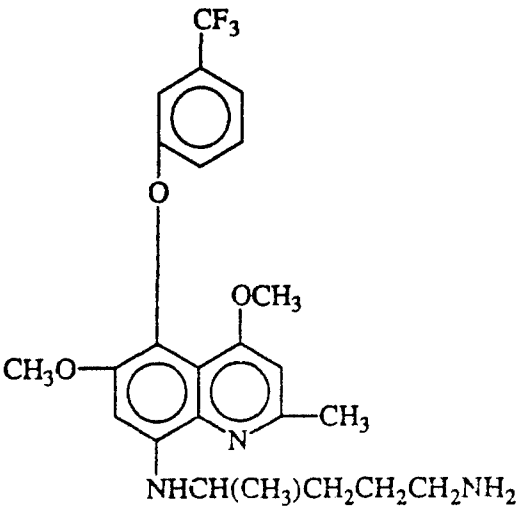

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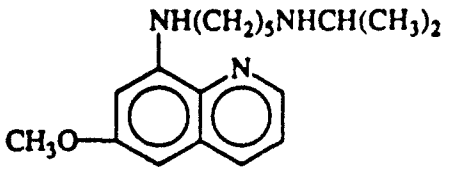

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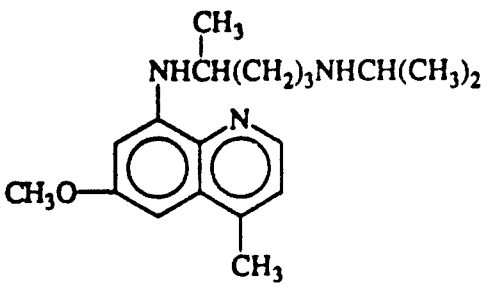

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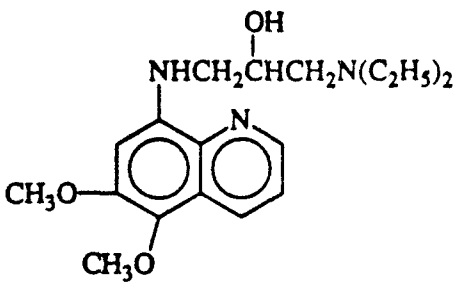

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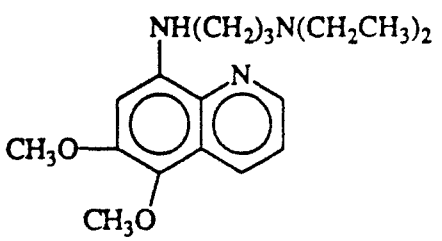

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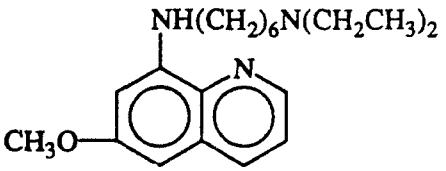

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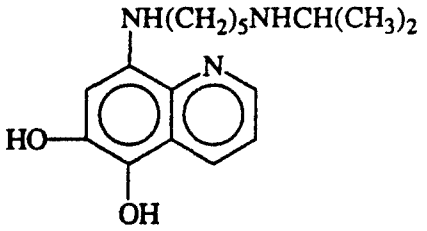

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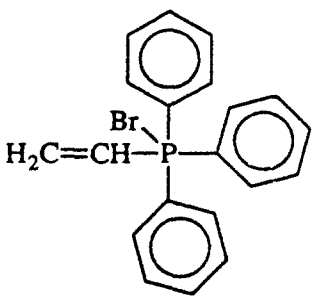

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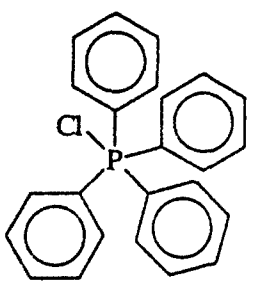

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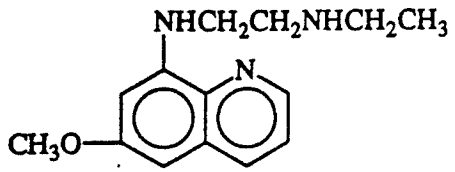

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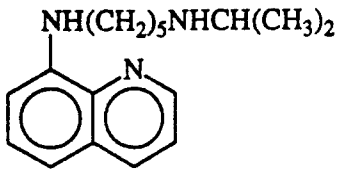

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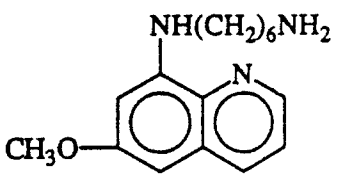

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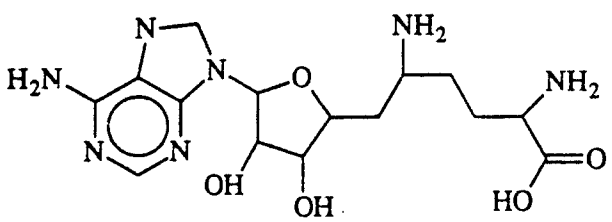

WR254847

APPENDIX 2

PERSONNEL EMPLOYED FROM THIS CONTRACT DURING THIS REPORT PERIOD

Name and Position	Percent Effort	Length of Employment
Barbara Harris Laboratory Technician II	100%	9/23/91 - 12/23/91 1/9/92 - Present
Laura Lamb Graduate Assistant	16%	7/1/92 - 9/30/92
Shannon Waits Student Laboratory Technician	15%	9/28/91 - 6/5/92
Virginia Waits Research Coordinator II	100%	9/28/91 - Present

BIBLIOGRAPHY OF PUBLISHED WORK

None

GRADUATE DEGREES RESULTING FROM THIS CONTRACT

None