Bulletin of the World Health Organization, 59 (3): 463-479 (1981)

New tissue schizontocidal antimalarial drugs

DAVID E. DAVIDSON, JR,[1] ARBA L. AGER,[2] JOHN L. BROWN,[3] FRANK E. CHAPPLE,[3] RICHARD E. WHITMIRE,[3] & RICHARD N. ROSSAN[4]

Over 700 causal prophylactic and radical curative antimalarial drugs have been discovered during the screening of approximately 4000 chemical compounds in rodent and simian malaria models. Causal prophylactic activity in the Plasmodium berghei*–rodent model was demonstrated by 10 distinct groups of chemicals: 1) tetrahydrofolate dehydrogenase inhibitors, 2) naphthoquinones, 3) dihydroacridinediones, 4) tetrahydrofurans, 5) guanylhydrazones, 6) analogues of clopidol, 7) quinoline esters, 8) dibenzyltetrahydropyrimidines, 9) 6-aminoquinolines, 10) 8-aminoquinolines.*

Of the causal prophylactic compounds, only the 6- and 8-aminoquinolines were capable of curing persistent exoerythrocytic infections of P. cynomolgi *in rhesus monkeys. The 6-aminoquinolines were substantially less active than primaquine.*

This report describes a series of 4-methyl-5-phenoxy-6-methoxy-8-aminoquinolines, which are potent blood schizontocides and radical curative drugs. The most active member of this series, 4-methyl-5-(3-trifluoromethylphenoxy)-6-methoxy-8-[(4-amino-1-methylbutyl) amino]quinoline succinate (WR 225448), was 5 times more active than primaquine in curing persistent exoerythrocytic infections of P. cynomolgi *in rhesus monkeys.*

As a blood schizontocide, WR 225448 was effective in animal models against P. berghei, P. cynomolgi, P. vivax, *and both drug-sensitive and drug-resistant strains of* P. falciparum. *WR 225448 was also more toxic than primaquine in rats on subacute (28-day) administration.*

The number of antimalarial drugs currently available for clinical use, as causal prophylactic or radical curative drugs, is extremely limited. Only primaquine, the 8-aminoquinoline introduced nearly 30 years ago, is clinically effective against the persistent tissue stages of *Plasmodium vivax* or *P. ovale* in man. Close analogues, such as pentaquine, isopentaquine, pamaquine, quinocide, etc., are either less effective or more toxic than primaquine in clinical use.

The toxicity of primaquine limits its clinical usefulness in both prophylactic and therapeutic applications. The most serious side-effect is haemolysis, which occurs in individuals who are genetically deficient in glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (*1, 2*). Methaemoglobinaemia, abdominal cramping, and epigastric distress are also significant side-effects of many 8-aminoquinolines (*3*). The 8-aminoquinolines as a class are hepatotoxic (*4*), and, while this is seldom a problem when primaquine is administered in acceptable dosages, it is a potential hazard of overdosing.

Drug resistance has developed to almost every blood schizontocidal drug currently in clinical use. Fortunately, although there are geographic differences in the susceptibility of persistent tissue stages of vivax malaria to primaquine (*3, 5*), no true drug resistance has been unequivocally demonstrated. However, the ease with which Arnold et al. (*6*) were able to induce primaquine resistance experimentally in vivax malaria emphasizes the need for alternative tissue schizontocides.

Because primaquine is unique as a radical curative drug and because the associated side-effects severely limit its use, it was decided several years ago to initiate a modest screening effort to search for alternative drugs, as part of the US Army Antimalarial Program. Approximately 4000 compounds of diverse structure have been screened, a high proportion of which were analogues of compounds previously reported to have tissue schizontocidal activity.

In developing the screening strategy, existing animal models for causal prophylactic and radical curative testing were used. Tissue schizontocidal testing is intrinsically more complex and difficult than blood schizontocidal testing, and none of the existing models were capable of supporting economical, large-scale screening. It was necessary, therefore, to develop models to meet the needs of the programme. Several models were developed and operated by collaborating

[1] Chief, Department of Parasitology, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington, DC 20012, USA.

[2] Director, Rane Research Laboratory, University of Miami Medical Center, Miami, FL, USA.

[3] Department of Veterinary Medicine, Armed Forces Research Institute of the Medical Sciences, Bangkok, Thailand.

[4] Gorgas Memorial Laboratory, Panama City, Panama.

laboratories, and some of these will be described.

The data provided are intended to highlight some of the approaches that have yielded tissue schizontocidal compounds with improved activity. The approaches that were unproductive will also be indicated.

METHODS

In addition to the causal prophylactic testing against sporozoite-induced *P. yoelii* malaria in rodents, which was performed by the authors, test data were also provided by Wallace Peters of the Liverpool School of Tropical Medicine, and by Harry Most of New York University. Radical curative testing against sporozoite-induced *P. cynomolgi* malaria in rhesus monkeys was performed by Leon Schmidt of Southern Research Institute, Birmingham, Alabama, and by the authors. Toxicological studies were performed by C.C. Lee of Midwest Research Institute.

The primary mouse prophylactic screening was performed using methods described by Rane & Kinnamon (*7*). In this screen, the test compound is administered subcutaneously to ICR/Ha mice, and followed two hours later by an intraperitoneal inoculation of a lethal dose of *P. yoelii* sporozoites. If the compound has activity against the pre-erythrocytic stages, or if it is a blood schizontocide and persists long enough to suppress the blood forms emerging after day 3, survival time of the treated mice is prolonged. In this screening model, there is a 99% mortality rate among infected, untreated controls, with deaths occurring between days 6 and 17 (mean survival time, 9.8 days). A test compound is considered active if 2 or more treated mice survive to 30 days at any drug dose.

It is to be emphasized that this screening test is not specific, since both causal prophylactic compounds and persistent blood schizontocides give positive results. Nevertheless, it does provide for rapid, inexpensive screening of large numbers of compounds, and identifies the majority of blood schizontocides that are not persistent.

Compounds that were active in the primary prophylactic mouse screen were evaluated in one of the more definitive rodent tests, which are capable of distinguishing causal prophylactic compounds from those possessing only residual blood schizontocidal activity.

The mouse causal prophylactic test described by Gregory & Peters (*8*) uses mice inoculated with sporozoites of *P. yoelii*, with parasitized blood, and with both. By a mathematical analysis of the effects of the test compound on the subsequent parasitaemias, it is possible to identify compounds that are truly causal prophylactics, and to estimate the level of activity.

A second causal prophylactic model developed by Most & Montouri (*9*) also distinguishes between causal prophylactic and suppressive prophylactic activity. In this model, rats are given the test drug on two consecutive days, and, on the second day, are also inoculated intravenously with either 10 000 or 250 000 *P. berghei* sporozoites. Those receiving the higher sporozoite inoculum are sacrificed 43–45 h after inoculation and their livers are removed, sectioned, stained, and examined microscopically for exoerythrocytic forms. Significant reduction or absence of exoerythrocytic forms is indicative of causal prophylaxis. The course of the parasitaemia following drug administration is monitored in the rats that received the lower sporozoite inoculum. Absence of parasitaemia on examination of stained blood films, confirmed by subinoculation of blood into mice, indicates a parasitological cure, which, in the absence of a cure of exoerythrocytic forms in the liver at 43–45 h, is indicative of suppressive prophylaxis.

Radical curative testing against persistent tissue stages of *P. cynomolgi bastianelli* malaria in rhesus monkeys was performed using the methods described by Schmidt et al. (*10*). In this model, the test compound is administered orally for 7 consecutive days, beginning 10–12 days after intravenous inoculation of $0.5–1.5 \times 10^6$ sporozoites (2–4 days after the appearance of parasitaemia). Chloroquine phosphate (5 mg/kg of body weight per day) is administered concomitantly with the test compound to eliminate blood forms and to permit assessment of the drug's activity against tissue stages, which are unaffected by chloroquine. Relapse of parasitaemia after completion of the drug regimen is indicative of the failure of the test compound to eliminate all exoerythrocytic parasites. If parasitaemia does not reappear within 100 days (or within 30 days in the experiments in which splenectomy was carried out), the test compound is considered to have cured the exoerythrocytic infection.

In the prophylactic rodent models, blood schizontocidal activity has an important impact on the interpretation of results because the tissue stages of rodent malaria are present for such a short time. For this reason, data on the blood schizontocidal activity of compounds were also obtained using the Rane model (*11*), which is a primary blood schizontocidal screen in mice. In this test, infected mice surviving for 60 days after administration of a single subcutaneous dose of the test compound are considered cured. Deaths occurring before day 6 are considered to be a result of drug toxicity.

The blood schizontocidal activities of 7-day regimens of WR 225448 and primaquine were also assessed against trophozoite-induced infections of *P. cynomolgi* in rhesus monkeys (*12*) and against *P. vivax* in *Aotus trivirgatus* monkeys of Panamanian origin, using methods described by Schmidt for Colombian owl monkeys (*13*).

RESULTS

The results of tissue schizontocidal tests for non-aminoquinolines are presented in Table 1. Only compounds that have been sufficiently well tested to provide assurance of true tissue schizontocidal activity are included. Furthermore, for each class of compound, only the most active representatives are mentioned.

The compounds in Table 1 are grouped according to chemical class. Each compound is identified by its Walter Reed (WR) accession number. The corresponding structural formulae are given in Annex 1. Primary prophylactic mouse screening results are expressed as a 50% effective dose (ED_{50}), which is defined as the lowest test dose (administered orally or subcutaneously) that permitted at least 50% of the animals to survive the otherwise lethal sporozoite inoculum. In each case in which true causal prophylactic activity was confirmed in one or both of the secondary rodent models (*8, 9*), this is indicated. A primaquine index, indicating the activity of the test compound relative to primaquine, is provided for the cases where the data obtained in the Peters model were adequate.

Results of radical curative testing in the rhesus monkey–*P. cynomolgi* model are also provided, and again expressed as a primaquine index. The test compound is considered inactive if no monkey was cured at the maximum dose tested (generally 10 mg/kg of body weight per day for 7 days).

Results of blood schizontocidal testing in the Rane mouse model are presented as an approximate 50% curative dose (CD_{50}), which is defined as the lowest dose at which 50% or more of the mice were cured. Compounds producing no increase in survival time at the highest dose tested (640 mg/kg of body weight) are scored as inactive. For compounds that significantly increased survival time, but that were not sufficiently active to effect cures, the highest dose tested is indicated.

Tetrahydrofolate dehydrogenase inhibitors

Because of the many reports in the scientific literature indicating causal prophylactic activity of this class of compound both in animal models and in man, many diaminopyrimidines, triazines, quinazolines, pteridines, and related inhibitors of tetrahydrofolate dehydrogenase (dihydrofolate reductase) (EC 1.5.1.3) have been screened for tissue schizontocidal activity. A few examples are presented in Table 1. In general, these compounds, which are blood schizontocides, are also potent causal prophylactic agents in the rodent models. None of the compounds, however, have ever exhibited activity against persistent tissue forms in the rhesus monkey model.

Naphthoquinones

A number of analogues of the well-known causal prophylactic compound menoctone (*14*) have been tested. Many, including WR 6012 and WR 25175, have exhibited causal prophylactic activity in the rodent models, but none has been more active than menoctone. Furthermore, neither menoctone nor its analogues have exhibited radical curative activity in the rhesus model. Because naphthoquinones are not readily absorbed in rhesus monkeys when administered orally, the intramuscular route was used for the radical curative tests.

Dihydroacridinediones

A number of analogues of the Hoechst compound Floxacrine (WR 233602)[a] have been screened for tissue schizontocidal activity. Like Floxacrine (*15*), the analogues listed in Table 1 exhibited potent causal prophylactic activity in the rodent models, but none had radical curative activity against *P. cynomolgi* in rhesus monkeys. As yet, only Floxacrine has been tested for causal prophylactic activity in the rhesus model (*15*).

Tetrahydrofurans

The causal prophylactic activity of this class of compounds has been described by Peters (*16*). On this basis, a number of analogues have been screened, and have exhibited causal prophylactic activity. These compounds reversibly inhibit the growth of folate-dependent bacteria (C.C. Smith, personal communication, 1971), and may be tetrahydrofolate dehydrogenase inhibitors. Like other compounds with this mechanism of action, they have causal prophylactic activity in the rodent models, but have no radical curative activity against *P. cynomolgi.* Curiously, the compounds with a complete furan ring system are more potent blood schizontocides, while those that may be viewed as an opened ring (e.g., WR 179305 and WR 199334), appear to be more potent causal prophylactics.

Guanylhydrazones

A number of guanylhydrazones have been found to have modest blood schizontocidal and causal prophylactic activity in the rodent models. One of these, WR 9792, has been tested against *P. cynomolgi*, but had no radical curative activity.

Clopidol analogues

Clopidol (WR 61112) and one of two analogues tested had weak causal prophylactic activity in the

[a] 7-chloro-3,4-dihydro-10-hydroxy-3-[4-(trifluoromethyl)phenyl] -1,9 (2*H*, 10*H*)-acridinedione.

Table 1. Antimalarial activity of non-aminoquinolines

WR compound no.	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
	Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
Tetrahydrofolate dehydrogenase inhibitors					
2978	1.25[a,b]	2.5	inactive CP active[c]	80	160
5473	5[a,b]	2.5	inactive CP active[c]	> 640	> 640[d]
38839	0.63[a]	0.63	inactive[e]	80	> 640
159412	1.25[a]	1.25	inactive[e]	10	> 640
206891	2.5[a,b]	2.5	inactive	10	> 640
Naphthoquinones					
49808	10[a]	10[a]	inactive (intramuscular) [inactive orally]	320	> 640
6012	40[a,b]	40[a]	inactive (intramuscular)	320	> 640
25175	40[a,b]	40[a]	inactive (intramuscular)	> 640	> 640
Dihydroacridinediones					
233602	2.5	2.5	inactive (intramuscular) CP active[c]	20	640
226626	10[a,b] (P = 20.6)[f]	10[a] (P = 0.6)[f]	inactive	160	> 640
226970	2.5	10	inactive	160	> 640
234062	160	40	inactive	> 640	> 640
Tetrahydrofurans					
93133	40[a]	40[a]	inactive[e]	80	640
190729	160[b]	160	inactive	80	640
179305	10[a,b]	10	inactive[e]	> 640	320
199334	40	10		> 640	640
Guanylhydrazones					
9792	40[b]	10	inactive	640	160
99682	160	40		80	> 640
91808	40[a]	40		80	> 640
Clopidol analogues					
61112	160[b]	160	inactive	> 640	> 640
156949	160	160		inactive	> 640
167655	inactive	inactive	inactive[e]	> 640	> 640
Quinoline esters					
7295	160	160		> 640	> 640
194905	0.63[a]	0.63[a]	inactive	> 640	> 640
Dibenzyl pyrimidines					
158124	40	40	inactive	inactive	> 640
214235	160	160			
214705	160	160			

[a] Causal prophylactic activity confirmed in secondary rodent model by Peters et al. (8).

[b] Causal prophylactic activity confirmed in secondary rodent model by Most & Montouri (9).

[c] CP = Causal prophylactic test: *P. cynomolgi* in rhesus monkey. Data provided by Leon H. Schmidt.

[d] No toxic deaths at highest dose tested, i.e., 640mg/kg of body weight.

[e] Data provided by Leon H. Schmidt, Southern Research Institute, Birmingham, Alabama.

[f] P = Primaquine index in secondary rodent model of Peters et al. (8).

rodent models. Radical curative activity in the rhesus model was not detected.

Quinolones

Two analogues of the quinolones and acetoxy derivatives described by Ryley & Peters (*17*) were tested. One of these, WR 194905, was among the most active causal prophylactics in the rodent model, although its blood schizontocidal activity was weak. In the rhesus model, there was no evidence of radical curative activity.

Dibenzylpyrimidines

Three compounds of this class have exhibited activity in the causal prophylactic mouse screen. While this has not as yet been confirmed in secondary rodent testing, results in mice suggest that the activity is not due to residual blood schizontocidal activity. Only one of these compounds, WR 158124, has been tested for radical curative activity and it was ineffective.

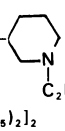
6-Aminoquinolines

The results of testing of a group of 5, 8-dimethoxy-6-aminoquinolines are presented in Table 2. All of the compounds listed were tested in the rhesus monkey radical curative test, but rodent causal prophylactic and blood schizontocidal data are not available for several compounds. Two compounds in this group, WR 188438 and Ni-147/36, exhibited modest radical curative activity in the rhesus model, although both were considerably less active than primaquine. WR 188438, WR 203766, and Ni-147/36 also exhibited causal prophylactic activity in the rodent systems.

7-Aminoquinolines

The results of testing a group of 5, 8-dimethoxy-7-aminoquinolines are presented in Table 3. None of these compounds had any activity in either the prophylactic or blood schizontocidal rodent models. One compound, WR 213640, had weak radical curative activity in the rhesus model.

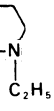
Table 2. Antimalarial activity of 6-aminoquinolines[a]

Compound no.	R_2	R_4	R	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
				Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
181614	$-CH_3$	H	$-CH(CH_3)(CH_2)_3NH-$ 			Inactive[b]		
182144	$-CH_3$	$-CH_3$	$-CH(CH_3)(CH_2)_3NH-$ 			Inactive[b]		
182146	$-CH_3$	$-CH_3$	$-CH(CH_3)(CH_2)_3NH_2$			Inactive[b]		
188438	$-CH_3$	$-CH_3$	$-CH(CH_3)(CH_2)_3NH-$ 	160	40	0.16[b]	Inactive	640
199065	$-CH_3$	$-CH_3$	$-[(CH_2)_2N(C_2H_5)_2]_2$	Inactive		Inactive[b]	Inactive	> 640
203766	-H	$-CH_3$	$-CH(CH_3)-(CH_2)_3NH_2$	40	40	Inactive[b]		
Ni-147/36	$-CH_3$	$-CH_3$	$-CH(CH_3)-(CH_2)_3N(C_2H_5)_2$			< 0.5[b]		

[a] Basic chemical structure given in Annex 1.

[b] Data provided by L. H. Schmidt, Southern Research Institute, Birmingham, Alabama.

Table 3. Antimalarial activity of 7-aminoquinolines[a]

Compound no.	R_2	R_4	R	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
				Sub-cutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
207766	$-CH_3$	$-CH_3$	$-CH(CH_3)-(CH_2)_3N(C_2H_5)_2$	Inactive		Inactive[b]	Inactive	> 640
213640	-H	-H	$-CH(CH_3)-(CH_2)_3N(C_2H_5)_2$	Inactive	Inactive	< 0.1[b]		
217270	$-CH_3$	$-CH_3$	$-CH(CH_3)-(CH_2)_3NH_2$			Inactive[b]		
218336	-H	-H	$-CH(CH_3)-(CH_2)_3NH_2$	Inactive		Inactive[b]		
218677	-H	$-CH_3$	$-CH(CH_3)-(CH_2)_3N(C_2H_5)_2$			Inactive[b]		
218948	$-CH_3$	$-CH_3$	$-CH(CH_3)-(CH_2)_3NH-$ 	Inactive		Inactive[b]		
219008	-H	-H	$-(CH_2)_4-N$ 	Inactive		Inactive[b]		

[a] Basic chemical structure given in Annex 1.

[b] Data provided by L. H. Schmidt, Southern Research Institute, Birmingham, Alabama.

1-Aminonaphthalenes

The results of testing a group of 1-aminonaphthalenes analogous to the 6-methoxy-8-aminoquinolines are presented in Table 4. None of these compounds exhibited blood or tissue schizontocidal activity.

8-Aminoquinolines

The results of testing of an interesting class of 5-aryloxy-8-aminoquinolines are presented in Tables 5–10. Although side-chain variants of this class have been synthesized and tested, only compounds with the primaquine side-chain are included in the tables.

The results for 5-phenoxy compounds without methyl substitution on the quinoline ring are listed in Table 5. It is particularly notable that none of the compounds in this group had causal prophylactic activity in the rodent models, yet all of them had radical curative activity in the rhesus model. With the exception of WR 215295 (primaquine index 3.2), the radical curative activity was modest, with primaquine indices ranging from 0.2 to 1.4. Blood schizontocidal activity, like that of primaquine, was low in the mouse model. In general, these compounds extended survival time, but did not cure even at the highest dose tested. WR 216100, WR 235720, and WR 215295 were slightly more active than primaquine as blood schizontocides, curing at 320–640 mg/kg of body weight. It is also notable that all of these 5-phenoxy compounds were substantially less toxic than primaquine, and only WR 235724 produced toxic deaths at 640 mg/kg of body weight, whereas primaquine is toxic at 160 mg/kg of body weight.

The results for 5-phenoxy compounds with 2-methyl substitution on the quinoline ring are listed in Table 6. These were also inactive in the causal prophylactic mouse screen, but had radical curative activity in the rhesus model. Again, all the 2-methyl compounds were less toxic than primaquine in mice, and had only weak blood schizontocidal activity. The 2-methyl-4-chlorophenoxy and 4-fluorophenoxy compounds had substantially stronger radical curative activity than their non-methyl substituted analogues (3.1 and 3.0 versus 1.0 and 1.4, respectively). The 3-trifluoro-

Table 4. Antimalarial activity of aminonaphthalenes[a]

Compound no.	R_1	R_2	R_3	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
				Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
180128	$-OCH_3$	-H	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2$			Inactive		
218575	$-OCH_3$	$-OCH_3$	$-CH(CH_3)(CH_2)_3N(C_2H_5)_2$			Inactive[b]		
232036	$-OCH_3$	$-OCH_3$	$-CH(CH_3)(CH_2)_3NH_2$	Inactive		Inactive	Inactive	640
232143	$-OCH_3$	$-OCH_3$	$-(CH_2)_3CH(CH_3)-NH_2$	Inactive		Inactive	Inactive	160
232439	$-OCH_3$	$-OCH_3$	$-CH(C_2H_5)-(CH_2)_3-NH_2$	Inactive		Inactive	Inactive	320

[a] Basic chemical structure given in Annex 1.

[b] Data provided by L. H. Schmidt, Southern Research Institute, Birmingham, Alabama.

Table 5. Antimalarial activity of 5-phenoxy-8-aminoquinolines[a]

Compound no.	Radical	Salt	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
			Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
225374	-H	H_3PO_4	inactive	inactive	1.3	> 640	> 640
182232	4-Cl	H_2O	inactive	inactive	1.0	> 640	> 640
216100	4-F	Succinate	inactive	inactive	1.4	320	> 640
235720	$4-OCH_3$	Citrate	inactive		0.2	640	> 640
235724	$4-OCF_3$	Citrate	inactive		0.2	> 640	640
215295	$3-CF_3$	Succinate	inactive	inactive	3.2[b]	640	> 640
233878	2,4-Cl	H_3PO_4	inactive	inactive	0.4	> 640	> 640
233881	3,4-Cl	Fumarate	inactive		0.4	> 640	> 640
234738	$3,5-CF_3$	$2H_3PO_4$	inactive		0.6	> 640	> 640
Primaquine		$2H_3PO_4$	50[c, d]	50[c]	1.0	> 640	160

[a] Basic chemical structure given in Annex 1.

[b] Data provided by L. H. Schmidt, Southern Research Institute, Birmingham, Alabama.

[c] Causal prophylactic activity confirmed in secondary rodent model by Peters et al. (*8*).

[d] Causal prophylactic activity confirmed in secondary rodent model by Most & Montouri (*9*).

Table 6. Antimalarial activity of 2-methyl-5-phenoxy-8-aminoquinolines[a]

Compound no.	Radical	Salt	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
			Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
211532	4-Cl	Fumarate	inactive	inactive	3.1[b]	320	> 320
224097	4-F	Fumarate	inactive		3.0	320	> 640
224486	3-CF_3	Fumarate	inactive	inactive	1.7	> 640	320
2-Methyl-primaquine		2HCl	40[c,d] (P = 3.8)[e]	40	1.0	> 640	320

[a] Basic chemical structure given in Annex 1.
[b] Data provided by L. H. Schmidt, Southern Research Institute, Birmingham, Alabama.
[c] Causal prophylactic activity confirmed in secondary rodent model by Peters et al. (*8*).
[d] Causal prophylactic activity confirmed in secondary rodent model by Most & Montouri (*9*).
[e] Primaquine index in secondary rodent model of Peters et al. (*8*).

methylphenoxy analogue was less active as the 2-methyl substituted compound.

Only one 3-methyl compound has been tested so far (Table 7). In the radical curative test, its primaquine index was only 1.5, but it had a high level of blood schizontocidal activity and was less toxic than primaquine in the mouse. It was also effective in the mouse prophylactic screen, although residual blood schizontocidal activity has not yet been ruled out. It is notable that 3-methylprimaquine itself is not a potent blood schizontocide, although its radical curative potency is comparable with that of WR 235485, and it is a powerful causal prophylactic in the mouse model.

4-Methyl substitution on 5-phenoxy-8-aminoquinolines appeared to enhance substantially both blood and tissue schizontocidal activity, without increasing toxicity (Table 8). All the 4-methyl compounds listed had potent radical curative activity, with primaquine indices ranging from 4.2 to 4.8, and all were potent blood schizontocides, curing mice at doses of 5–40 mg/kg of body weight. All the compounds were highly active in the primary prophylactic mouse screen, but secondary test in the Peters model showed that this was attributable to residual blood schizontocidal activity. However, the Most technique showed that WR 225448 was a true causal prophylactic in the rat. By comparison, 4-methylprimaquine itself was a true causal prophylactic in the

Table 7. Antimalarial activity of 3-methyl-5-phenoxy-8-aminoquinolines[a]

Compound no.	Radical	Salt	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
			Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
235485	3-CF_3	Succinate	20	10	1.5	20	> 640
3-Methyl-primaquine		2HCl	40[b] (P = 37.0)[c]	40[b] (P = 3.8)[c]	1.3[d]	> 160	80

[a] Basic chemical structure given in Annex 1.
[b] Causal prophylactic activity confirmed in secondary rodent model by Peters et al. (*8*).
[c] Primaquine index in secondary rodent model.
[d] By both oral and subcutaneous administration.

Table 8. Antimalarial activity of 4-methyl-5-phenoxy-8-aminoquinolines[a]

Compound no.	Radical	Salt	Mouse causal prophylactic screen ED_{50} (mg/kg of body weight)		Rhesus/ *P. cynomolgi* radical curative test	Mouse blood schizontocidal test (mg/kg of body weight subcutaneous)	
			Subcutaneous	Oral	Primaquine index	CD_{50}	Minimum toxic dose
232956	4-F	H_3PO_4	40[b]	40[b]	4.2	20	320
232584	4-OCH_3	H_3PO_4	40[b]	40[b]	4.3	40	640
225448	3-CF_3	Succinate	20[b]	10[b]	4.8	20	640
233195	2,4-Cl	H_3PO_4	40[b]	40[b]	4.6	20	> 640
233078	3,4-Cl	H_3PO_4	40[b]	40[b]	4.6	5	> 640
4-Methyl-primaquine		$2H_3PO_4$	50[c,d] (P = 0.7)[e]	25[c,d] (P = 2.3)[e]	2.1	640	640

[a] Basic chemical structure given in Annex 1.
[b] Activity in Peters model (*8*) attributed to residual blood schizontocidal properties.
[c] Causal prophylactic activity confirmed in secondary rodent model by Most & Montouri (*9*).
[d] Causal prophylactic activity confirmed in secondary rodent model by Peters et al. (*8*).
[e] Primaquine index in secondary rodent model of Peters et al.

mouse, a weak blood schizontocide, and a radical curative agent in the rhesus monkey with a primaquine index of 2.1.

The results of studies on the blood schizontocidal activity of WR 225448 are presented in Tables 9 & 10. Trophozoite-induced *P. cynomolgi* parasitaemias were consistently cured by a 7-day oral regimen of 1.0 mg/kg of body weight per day or more of the compound, and it was clearly superior to primaquine in this blood schizontocidal model. While transient clearing of parasitaemia was regularly obtained after administration of a total of 2.2 mg of primaquine/kg of body weight or more, blood schizontocidal cure was never attained even at a total dose of 220 mg/kg of body weight.

WR 225448 was also effective as a blood schizontocide against trophozoite-induced Chesson strain *P. vivax* malaria in Panamanian *Aotus trivirgatus* monkeys. It cleared parasitaemia in all monkeys at the

Table 9. Blood schizontocidal activity of a 7-day course of WR 225448 against trophozoite-induced *P. cynomolgi* malaria in rhesus monkeys

Daily oral dose (mg/kg of body weight)	Total dose (mg/kg of body weight)	WR 225448		Primaquine	
		No. cleared	No. cured	No. cleared	No. cured
31.6	220			2/2	0/2
10.0	70	2/2	2/2	2/2	0/2
3.16	22	2/2	2/2	2/2	0/2
1.00	7	2/2	2/2	2/2	0/2
0.316	2.2	2/2	0/2	2/2	0/2
0.100	0.7	2/2	0/2	0/2	0/2
0.0316	0.22	2/2	0/2		

Table 10. Blood schizontocidal activity of a 3-day course of WR 225448 against trophozoite-induced *P. vivax* malaria (Chesson strain) in *Aotus trivirgatus*

Daily oral dose (mg/kg of body weight)	Total dose (mg/kg of body weight)	No. cleared	No. cured
WR 225448			
16	48	3/3	3/3
4	12	4/4	4/4
2	6	5/5	0/5
1	3	3/3	0/3
Primaquine			
53	160	3/3	0/3
27	80	3/3	0/3
13	40	3/3	0/3
3.3	10	0/4	0/4

lowest dose tested (1 mg/kg of body weight per day for 3 days, orally), and was fully curative at a total dose of 12 mg/kg of body weight (4 mg/kg per day for 3 days). Primaquine, tested simultaneously, cleared parasitaemia only in a total 3-day regimen dosage of 40 mg/kg of body weight or more, and was not curative, even at a total dose of 160 mg/kg of body weight.

DISCUSSION

Over a four-year period, approximately 4000 compounds have been screened for causal prophylactic and radical curative activity in one or more animal models. Because compounds have been selected for test on the basis of structural or functional analogy with known causal prophylactic compounds, a large number of them have exhibited such activity. To date, over 700 active compounds, the majority of which are 8-aminoquinolines, have been identified.

There were few active non-aminoquinolines, except for tetrahydrofolate dehydrogenase inhibitors, of which there are many active examples. Compounds identified as having causal prophylactic activity in the rodent models may be grouped into eight general classes:

1. Tetrahydrofolate dehydrogenase inhibitors
2. Naphthoquinones
3. Dihydroacridinediones
4. Tetrahydrofurans
5. Guanylhydrazones
6. Clopidol analogues
7. Quinoline esters
8. Dibenzyltetrahydropyrimidines

For each of these classes, reports of causal prophylactic activity were available prior to testing, and thus no new chemical classes have been identified in this screen. However, specific compounds within these classes have been demonstrated to have activity which has not previously been reported.

It is of interest to note that of the listed non-aminoquinolines exhibiting causal prophylactic activity in rodents, none were active against persistent tissue stages of *P. cynomolgi* in the radical curative test. There are, however, published accounts for a number of the compounds listed in this report indicating activity against pre-erythrocytic tissue forms of *P. cynomolgi* in rhesus monkeys, or pre-erythrocytic forms of *P. falciparum* and *P. vivax* in man. This work has been reviewed by Peters (*18*). In man, tetrahydrofolate dehydrogenase inhibitors, combinations of these with sulfones or sulfonamides, and the 8-aminoquinolines are effective in causal prophylaxis of falciparum or vivax malaria. Limited clinical trials of several non-aminoquinolines have been reported by Canfield et al. (*19*), but none were successful. Menoctone produced no causal prophylactic effect in volunteers receiving 500 mg daily for 3 days after challenge with *P. falciparum*. Clopidol produced unacceptable neurological side-effects, did not control *P. falciparum* parasitaemia, and was excluded from further consideration before causal prophylactic studies could be performed. The pyrocatechol RC-12 (WR 27653), a compound reported to have causal prophylactic activity against *P. cynomolgi* in rhesus monkeys, but ineffective in rodent malaria models (*20*), also failed to exhibit causal prophylactic activity against *P. vivax* challenge. The newer dihydrotriazine, WR 38839 (clociguanil), was effective in causal prophylaxis against *P. falciparum* only when administered in combination with sulfadiazine.

To our knowledge, no representatives of the dihydroacridinedione class, the tetrahydrofurans, the guanylhydrazones, the quinoline esters, or the dibenzylpyrimidines have been evaluated clinically. A quinoline ester (ICI 56780) has been reported to have causal prophylactic activity against *P. cynomolgi* in rhesus monkeys (*18*), and Floxacrine, the dihydroacridinedione, was also active in this simian model (*15*).

Fink (*21*) has suggested that the rodent models may exaggerate the activity of compounds that interfere with nucleic acid synthesis because of the high rate of synthesis in *P. berghei* exoerythrocytic forms. This might also account, in part, for the moderate sensitivity of pre-erythrocytic stages of simian and human malarias to compounds such as the tetrahydrofolate dehydrogenase inhibitors, and the total lack of activity of these compounds against persistent exoerythrocytic forms. Clearly, there is an important need for research into the biochemistry and physiology of exoerythrocytic forms.

Efforts to find aminoquinolines, other than 8-aminoquinolines, with tissue schizontocidal activity have not been successful. A few 6-aminoquinolines have exhibited weak causal prophylactic activity in the rodent models, and two analogues (WR 188438 and Ni-147/36) had weak radical curative activity. The 7-aminoquinolines tested were virtually devoid of tissue schizontocidal activity. Several 3-, 4-, and 5-aminoquinolines have also been synthesized and tested, and these exhibited no tissue schizontocidal activity. Aminonaphthalenes were also devoid of antimalarial activity. A variety of isoquinolines and azoquinolines, analogous to the 8-aminoquinolines, have also been tested and were inactive.

The activity of the 4, 5-disubstituted 6-methoxy-8-aminoquinolines is, we believe, an important observation. The marked enhancement of radical curative activity of these disubstituted compounds would not have been expected from the activity of either the

4-methyl or the 5-phenoxy-8-aminoquinolines. The compounds listed in Table 8 have the most potent radical curative activity of all the compounds examined.

The inactivity of the 5-phenoxy-8-aminoquinolines in the rodent causal prophylactic test is notable. We are unaware of any other compound, except the pyrocatechol RC-12 (*20*), with tissue schizontocidal activity against *P. cynomolgi* in rhesus monkeys that is not a causal prophylactic in the rodent models. The reason for this species difference is unknown, although it may be a result of differences in metabolism between mice and monkeys, but this has not yet been tested experimentally. Greenberg (*22*) demonstrated that primaquine and pentaquine were inactive against *P. gallinaceum in vitro* while metabolites of the drugs were active. It has been suggested by Greenberg that 6-quinone and 5, 6-quinone may be the active metabolites, and Smith (*23*) has identified a 5, 6-quinolinequinone metabolite of pentaquine in the rhesus monkey. The rationale for developing these 5-aryloxy analogues was to block metabolism. It will be interesting to determine whether quinone is formed during the metabolism of these new compounds in mice or monkeys. It should also be noted that WR 225448 was a causal prophylactic in the Most rat model (Table 8). If differences in host metabolism are responsible for the variation in activity, then the rat and monkey are different from the mouse.

The potent blood schizontocidal activity of 3- and 4-methyl-8-aminoquinolines was also unexpected, since 3- and 4-methylprimaquine are weak blood schizontocides, as are the 2-methyl and non-methyl substituted 5-phenoxy-8-aminoquinolines. This activity was observed in mice as well as in rhesus and owl monkeys, and was observed against *P. berghei*, *P. cynomolgi*, and *P. vivax*. WR 225448 was also effective as a blood schizontocide against drug-sensitive and drug-resistant strains of *P. falciparum* in owl monkeys (R.N. Rossan, personal communication, 1980), but was inactive against *P. falciparum in vitro* (R. Desjardins, personal communication, 1978).

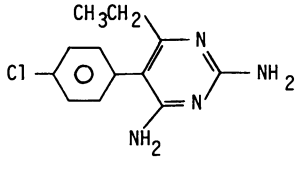
Some preliminary toxicological studies have been performed with WR 225448 (C.C. Lee, personal communication, 1980). On single-dose subcutaneous administration in mice, it was less toxic than primaquine (Table 8). WR 225448 induced methaemoglobinaemia in the dog, but its potency in this respect relative to primaquine is not yet known.

In 28-day oral toxicity studies in rats, WR 225448 was found to be more toxic than primaquine. Hepatotoxicity was more severe, and in addition, WR 225448 produced renal tubular degeneration and lymphoid depletion. Both WR 225448 and primaquine produced degenerative changes in the heart and diaphragmatic muscle. While WR 225448 administered subacutely is qualitatively and quantitatively more toxic than primaquine, it is still not known whether the therapeutic index is better or worse. Further toxicity studies with WR 225448 and other analogues are planned.

Annex 1

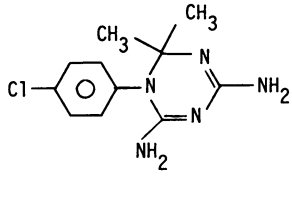
CHEMICAL STRUCTURES OF COMPOUNDS TESTED

NON-AMINOQUINOLINES (TABLE 1)



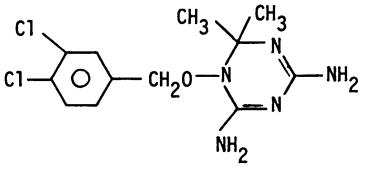
(Pyrimethamine)

2978



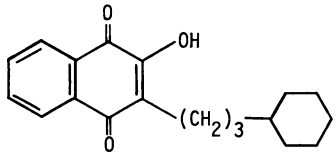
(Cycloguanil pamoate)

5473

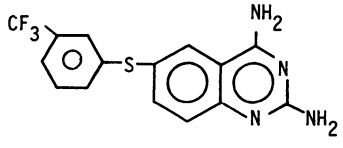


(Clociguanil)

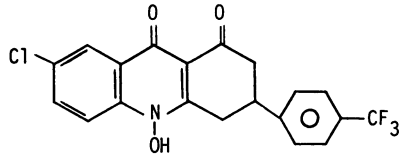
38839



25175

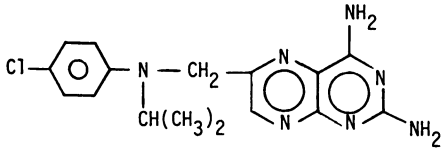


159412

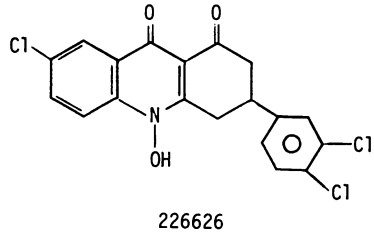


(Floxacrine)

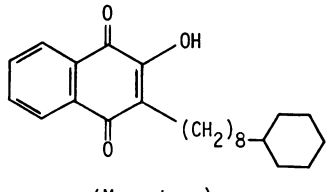
233602



206891

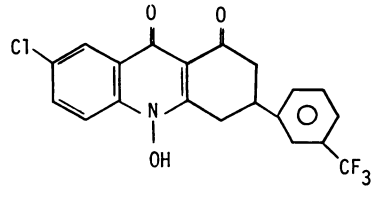


226626

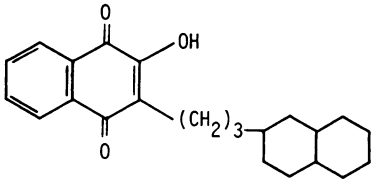


(Menoctone)

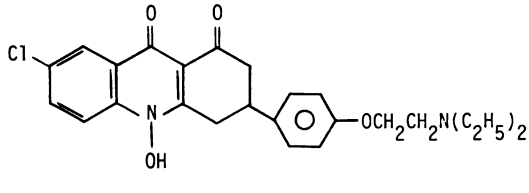
49808



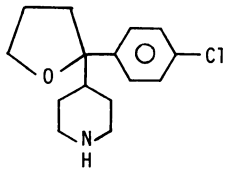
226970



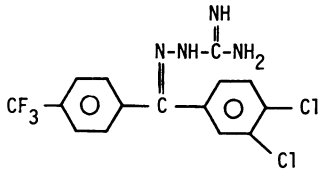
6012



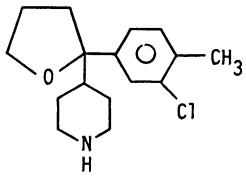
234062



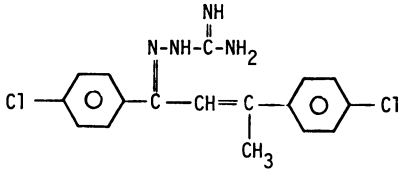
93133



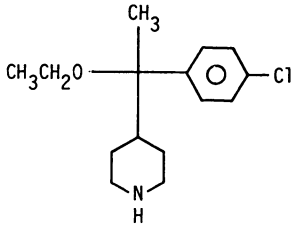
99682



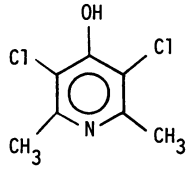
190729



91808

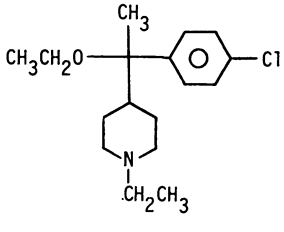


179305

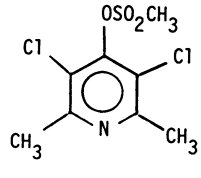


(Clopidol)

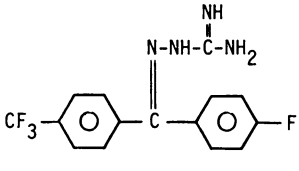
61112



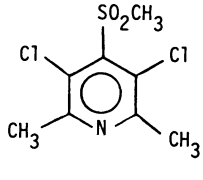
199334



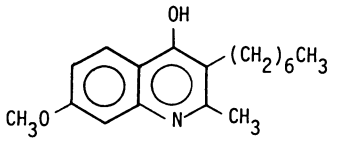
156949



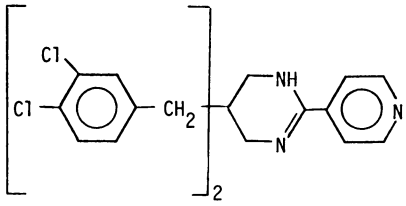
9792



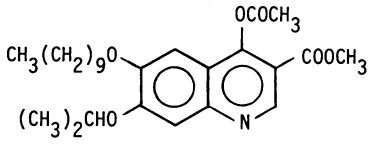
167655



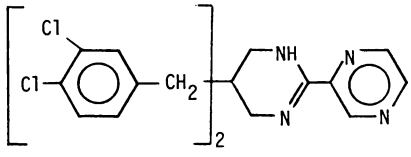
7295



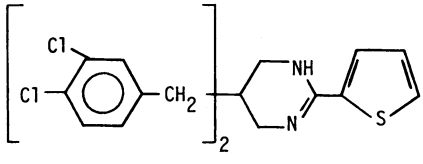
158124



194905

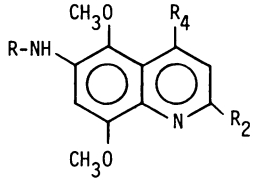


214235

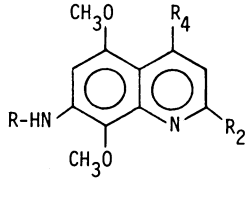


214705

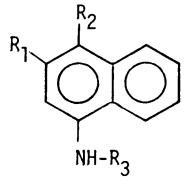
AMINOQUINOLINE DERIVATIVES



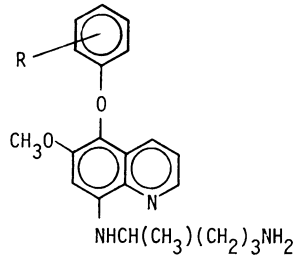
6-Aminoquinoline (Table 2)



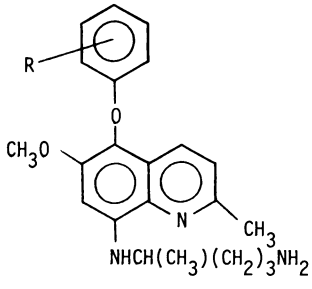
7-Aminoquinoline (Table 3)



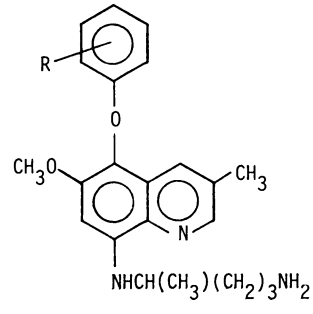
Aminonaphthalene (Table 4)



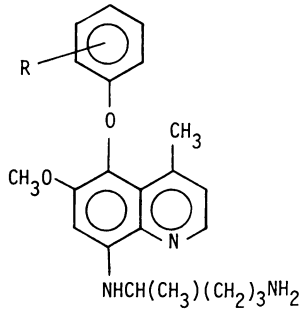
5-Phenoxy-8-aminoquinoline (Table 5)



2-Methyl-5-phenoxy-8-aminoquinoline (Table 6)



3-Methyl-5-phenoxy-8-aminoquinoline (Table 7)



4-Methyl-5-phenoxy-8-aminoquinoline (Table 8)

RÉSUMÉ

NOUVEAUX SCHIZONTOCIDES TISSULAIRES CONTRE LE PALUDISME

Afin de rechercher des schizontocides tissulaires plus sûrs et plus efficaces, on a procédé au criblage d'environ 4000 composés contre *Plasmodium berghei yoelii* dans des modèles murins adaptés aux étioprophylactiques, ou contre *Plasmodium cynomolgi bastianelli* dans un modèle de traitement radical chez le singe rhésus. Les composés sélectionnés pour le criblage étaient en majeure partie des analogues structuraux ou fonctionnels d'étioprophylactiques connus; environ 700 d'entre eux, pour la plupart des amino-8 quinoléines, se sont révélés actifs.

Des composés de 10 familles chimiques différentes étaient doués d'activité étioprophylactique dans les modèles murins: 1) inhibiteurs de la tétrahydrofolate déshydrogénase, 2) naphtoquinones, 3) dihydroacridinediones, 4) tétrahydrofurannes, 5) guanylhydrazones, 6) analogues du clopidol, 7) esters de la quinoléine, 8) dibenzyltétrahydropyrimidines, 9) amino-6 quinoléines, 10) amino-8 quinoléines.

L'activité pour la guérison radicale des infections à *P. cynomolgi* chez le singe rhésus n'a été observée qu'avec les composés appartenant aux familles des amino-6 et amino-8 quinoléines. Toutes les amino-6 quinoléines testées étaient sensiblement moins actives que la primaquine dans l'épreuve de guérison radicale.

Une famille de méthyl-4 phénoxy-5 méthoxy-6 amino-8 quinoléines était douée d'une activité exceptionnelle pour la guérison radicale des infections à *P. cynomolgi* chez le singe rhésus. De plus, ces composés étaient fortement actifs comme schizontocides sanguins. L'activité optimale comme schizontocides tissulaires a été observée avec le substituant méthyl-4; les analogues non substitués, ou substitués en méthyl-2 et méthyl-3, étaient moins efficaces. L'activité optimale comme schizontocides sanguins s'observait également avec les analogues substitués en méthyl-3 et méthyl-4.

Le représentant le plus efficace de cette nouvelle famille de schizontocides tissulaires était le WR 225448 (succinate de méthyl-4 (trifluorométhyl-3 phénoxy)-5 méthoxy-6 (amino-4 méthyl-1 butylamino)-8 quinoléine). Dans le modèle à singe rhésus, ce composé était cinq fois plus efficace que la primaquine dans la guérison des infections exoérythrocytaires persistantes à *P. cynomolgi*. Dans ce cas, on ne connaît aucun composé plus actif. Comme schizontocide sanguin, le WR 225448 est très actif contre *P. berghei*, *P. cynomolgi* et *P. vivax*, et contre les souches tant résistantes que sensibles de *P. falciparum* dans les modèles animaux.

Des études préliminaires de toxicité sur 28 jours chez le rat montrent que le WR 225448 est plus toxique que la primaquine. Comme cette dernière, il est hépatotoxique, induit une méthémoglobinémie, et provoque une dégénérescence dans les muscles cardiaque et diaphragmatique. En outre, il induit des modifications dégénératives du rein et une hypoplasie lymphocytaire. Des études complémentaires destinées à évaluer et à chiffrer cette toxicité sont en cours. Des études toxicologiques sur les autres analogues sont prévues.

REFERENCES

1. CARSON, P. E. ET AL. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science (New York)*, **124:** 484-485 (1956).
2. ALVING, A. S. ET AL. Malaria, 8-aminoquinolines and haemolysis. In: Goodwin, L. G. & Nimmo-Smith, R. H. ed., *Drugs, parasites and hosts*, London, J. A. Churchill Ltd, 1962, pp. 83-97.
3. ALVING, A. S. ET AL. Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of *Plasmodium vivax* by intermittent regimens of drug administration. A preliminary report. *Bulletin of the World Health Organization*, **22:** 621-631 (1960).
4. SCHMIDT, L. H. ET AL. Comparison of the curative antimalarial activities and toxicities of primaquine and its *d* and *l* isomers. *Antimicrobial agents and chemotherapy*, **12:** 51-60 (1977).
5. BLACK R. H. Results of the clinical use of primaquine for the eradication of relapsing malaria of South-West Pacific origin. *Australasian annals of medicine*, **1:** 259 (1958).
6. ARNOLD, J. ET AL. Induced primaquine resistance in vivax malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **55:** 345-350 (1961).
7. RANE, D. S. & KINNAMON, K.E. The development of a high volume tissue schizontocidal drug screen based upon mortality of mice inoculated with sporozoites of *Plasmodium berghei*. *American journal of tropical medicine and hygiene*, **28:** 937-947 (1979).
8. GREGORY, K. G. & PETERS, W. The chemotherapy of rodent malaria, IX. Causal prophylaxis, Part I: A method for demonstrating drug action on exo-erythrocytic stages. *Annals of tropical medicine and parasitology*, **65:** 15-24 (1970).
9. MOST, H. & MONTOURI, W. A. Rodent systems (*Plasmodium berghei–Anopheles stephensi*) for screening compounds for potential causal prophylaxis. *American journal of tropical medicine and hygiene*, **24:** 179-182 (1975).
10. SCHMIDT, L. H. ET AL. The activity of a repository form of 4, 6-diamino-1-(*p*-chlorophenyl)-1, 2-dihydro-2, 2-dimethyl-*s*-triazine against infections with *Plasmodium cynomolgi*. *American journal of tropical medicine and hygiene*, **12:** 494-503 (1963).
11. OSDENE, T. S. ET AL. 2,4,7-triamino-6-ortho-substituted aryl-pteridines. A new series of potent antimalarial agents. *Journal of medicinal chemistry*, **10:** 431-434 (1967).

12. DAVIDSON, D. E. ET AL. Evaluating new antimalarial drugs against trophozoite-induced *Plasmodium cynomolgi* malaria in rhesus monkeys. *American journal of tropical medicine and hygiene*, **25**: 26-33 (1976).
13. SCHMIDT, L. H. Infections with *Plasmodium falciparum* and *Plasmodium vivax* in the owl monkey—Model systems for basic biological and chemotherapeutic studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **67**: 446-474 (1973).
14. BÊRBERIAN, D. A. ET AL. Causal prophylactic effect of menoctone (a new hydroxynaphthoquinone) against sporozoite-induced *Plasmodium berghei* infection in mice. *Journal of parasitology*, **54**: 1181-1189 (1968).
15. SCHMIDT, L. H. Antimalarial properties of floxacrine, a dihydroacridinedione derivative. *Antimicrobial agents and chemotherapy*, **16**: 475-485 (1979).
16. PETERS, W. Substituted tetrahydrofurans, a new chemical family of antimalarials. The action of 2-(*p*-chlorophenyl)-2-(4-piperidyl) tetrahydrofuran against *Plasmodium berghei* and *Plasmodium chabaudi*. *Annals of tropical medicine and parasitology*, **64**: 189-202 (1970).
17. RYLEY, J. F. & PETERS, W. The antimalarial activity of some quinolone esters. *Annals of tropical medicine and parasitology*, **64**: 209-222 (1970).
18. PETERS, W. *Chemotherapy and drug resistance in malaria*. London, Academic Press, 1970.
19. CANFIELD, C. J. & ROZMAN, R. S. Clinical testing of new antimalarial compounds. *Bulletin of the World Health Organization*, **50**: 203-212 (1974).
20. SCHMIDT, L. H. ET AL. Studies on the antimalarial activity of 1,2-dimethoxy-4-(bis-diethylaminoethyl)-amino-5-bromobenzene. *Bulletin of the World Health Organization*, **34**: 783-788 (1966).
21. FINK, E. Assessment of causal prophylactic activity in *Plasmodium berghei yoelii* and its value for the development of new antimalarial drugs. *Bulletin of the World Health Organization*, **50**: 213-222 (1974).
22. GREENBERG, J. ET AL. Studies on *Plasmodium gallinaceum in vitro*. II. The effects of some 8-aminoquinolines against the erythrocytic parasites. *Journal of infectious diseases*, **88**: 163-167 (1951).
23. SMITH, Metabolism of pentaquine in the rhesus monkey. *Journal of pharmacology and experimental therapeutics*, **116**: 67-76 (1956).