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1 Antimalarial Activity of the 8-Aminoquinolines

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INTRODUCTION

Malaria is the world's most ravaging infectious disease. Rampant throughout much of the tropics and some of the temperate areas, its numbers beggar the imagination. It threatens a third of the world's population, presently afflicts hundreds of millions of people, causes several million deaths annually and may generate as many as 92 million new clinical cases each year [1, 2]. Its socio-economic drain is enormous.

The resurgence of this pestilence during the past two decades has stimulated the search for a vaccine but, despite a prodigious research effort and some cautious optimism, an effective vaccine is still far from fruition [3–10]. In the interim, there must be continued reliance on drugs for prophylaxis and therapy. Research leading to the currently available antimalarial agents has been detailed in a number of comprehensive reviews [11–29]. Unfortunately, most of these agents are obsolescent because of the facility with which the malarial parasite produces drug-resistant mutants [30]. The need for more effective antimalarials is therefore critical. In a search for such drugs, a small group of investigators has returned to an old, very heavily worked and seemingly exhausted mine, the 8-aminoquinolines. The evolution of an extremely promising series of new, broad-spectrum, antimalarial 8-aminoquinolines is described in this chapter. The new drugs are unique in their dual efficacy against the blood and tissue forms of the disease.

THE PARASITE

Malaria is caused by protozoan parasites of the genus *Plasmodium*. There are four species of *Plasmodia* pathogenic to man: *P. falciparum* (malignant tertian malaria), *P. vivax* (benign tertian malaria), *P. malariae* (quartan malaria) and

P. ovale, *P. falciparum* and *P. vivax*, together, cause 95% of human malaria. *P. ovale*, the rarest of the four species, occurs mainly in West Africa and is much like *P. vivax*. *P. malariae* causes a generally mild but extraordinarily tenacious form of the disease which can persist in the blood, with or without symptoms, for a lifetime.

The life cycle of the parasite has three phases: exoerythrocytic (tissue) and erythrocytic (blood) in man; sporogony in the mosquito.

EXOERYTHROCYTIC PHASE

Each species begins its cycle with the injection of thread-like, motile organisms (sporozoites) into the host's bloodstream during the bite of an infected female anopheles mosquito. Within an hour, the sporozoites leave the bloodstream, enter the liver and invade hepatocytes. Most of the sporozoites develop into primary tissue schizonts (schizogony) which undergo repeated asexual division to form thousands of merozoites. At maturity, the merozoites burst from the hepatocytes into the bloodstream, attack red blood cells and begin the erythrocytic phase of the cycle. Some of the sporozoites of *P. vivax* and *P. ovale* remain in the liver as latent forms called hypnozoites [31]. The hypnozoites become active intermittently, for years, releasing new merozoites into cleared blood and causing relapses. *P. falciparum* and *P. malariae* have no hypnozoite stage and, therefore, no potential for relapse.

ERYTHROCYTIC PHASE

On invading an erythrocyte, the merozoite assumes a ring shape called a trophozoite and begins to feed on haemoglobin. The globin fraction is metabolized and the haem fraction is deposited in the tissues as granules called haemozoin or malaria pigment. When the trophozoite matures and begins to divide, it becomes a blood schizont. Division continues causing cell lysis and the discharge of new merozoites which invade fresh red cells for another asexual cycle. It is the periodic rupture of erythrocytes, with the expulsion of merozoites, their waste products and cellular debris, which is responsible for the paroxysmal nature of malaria. Some of the merozoites enter red cells and develop into trophozoites but form no schizonts. Instead, their nuclei remain intact and they differentiate into sexual forms, male and female, known as gametocytes. The gametocytes undergo no further development in man but circulate in the bloodstream until ingested during an anopheline blood meal.

SPOROLOGY

In the mosquito midgut, all stages of the parasite except the gametocytes are destroyed. On maturation, the sperm-like male (microgamete) fertilizes the egg-like female (macrogamete) forming a zygote. The zygote becomes a motile, saclike structure called an ookinete which penetrates the stomach wall and forms an oocyst beneath the wall's outer membrane. Development of the oocyst leads to the formation of large numbers of infectious sporozoites which escape and migrate to the mosquito's salivary glands. With the insect's next bite, the parasite's life cycle is renewed. Sporogony takes from 2 to 3 weeks, depending on the species.

THE DISEASE

Malaria becomes manifest with a general malaise followed by a series of recurrent, three-stage paroxysms of shaking chills, a temperature which can rise as high as 106°F and a drenching sweat. The paroxysms, which signal the completion of each asexual cycle, can recur at regular intervals – about 48 h for *P. vivax*, *P. ovale* and *P. falciparum* and about 72 h for *P. malariae*. With the non-falciparum malarias, some degree of immunity eventually develops, parasitemia is diminished and, despite relapses and considerable debilitation, death is uncommon, even without treatment. However, an untreated falciparum infection frequently leads to a fulminating, pathophysiologic cascade (non-responsive hypotension; anaemia, hyperpyrexia; hypoglycaemia; lactic acidosis; fluid and electrolyte disturbances; septicemia; hemoglobinuria; pulmonary oedema; failure of hepatic, cerebral and renal function; shock; coma) and death may occur soon after onset of symptoms [32, 33]. The mortality rate from the cerebral form of severe falciparum malaria may be as high as 50% [34]. It is mainly the rapid geographic spread of extremely dangerous, multi-drug-resistant *P. falciparum* which makes the need for new antimalarial drugs so urgent.

CLASSIFICATION OF ANTIMALARIAL DRUGS

The aim of antimalarial chemotherapy is a cheap, safe, stable drug which could be administered in a single, well-tolerated, long-lasting, oral dose and which would interrupt all of the stages in the parasite's life cycle without the selection of resistant strains. No such drug exists. Because of their relatively narrow activity spectra, the presently available antimalarials are classified according to

their mode of attack. Thus, drugs which destroy the intrahepatic parasites, before their emergence into the circulation, are termed pre-erythrocytic or primary tissue schizontocides or causal prophylactics. True prophylactics, which would act on the sporozoites during the brief interval between anopheline injection and hepatic sequestration, are unknown. Destruction of the asexual blood forms of the relapsing malaras, with the resulting elimination of clinical symptoms, is effected by agents called suppressive drugs or blood schizontocides. However, complete elimination of the relapsing malaras (radical cure) requires a second drug which can eradicate the persistent liver forms. Such drugs are known variously as radically curative drugs, antirelapse agents, secondary tissue schizontocides or hypnozoitocides. Radical cure of malariae and falciparum malaria, which have no persistent liver forms, can be achieved with blood schizontocides. The parasites may also be attacked during their sexual development in the host (gametocytocide) or mosquito (sporontocide), thereby preventing transmission.

The most pressing present needs in malaria chemotherapy are a safe anti-relapse drug and a blood schizontocide which can eliminate resistant *P. falciparum* without selecting new troublesome mutants. Thus far, there is no single clinical drug which can carry out both functions. Among the drugs and drug combinations in current use as blood schizontocides are chloroquine, amodiaquine, amopyroquine, quinine, proguanil, chlorproguanil, cycloguanil, pyrimethamine, trimethoprim, sulfadoxine, sulfalene, dapsone, mefloquine, halofantrine, qinghaosu and its derivatives, doxycycline, tetracycline, pyrimethamine-sulfadoxin (Fansidar), pyrimethamine-sulfadoxine-mefloquine (Fansimef), pyrimethamine-sulfadoxine-amodiaquine, pyrimethamine-dapsone (Maloprim), quinine-quinidine-cinchonine (Falcimax) and quinine-tetracycline. In stark contrast, there are presently only two drugs in clinical use as tissue schizontocides (radical curative or antirelapse drugs); those drugs are the 8-aminoquinolines, primaquine and, to a lesser extent, quinocide.

HISTORY

The germinal work in the evolution of primaquine occurred a century ago when Guttman and Ehrlich treated malaria with methylene blue (1) after noting the dye's selective staining of intraerythrocytic plasmodia *in vitro* [35]. Schulemann and colleagues subsequently found that the activity of methylene blue could be improved by substituting the basic dialkylaminoalkyl chain, $-(CH_2)_2NEt_2$, for one of the dye's methyl groups to give compound (2) [36]. After attachment to a number of diverse structures, the chain found its way to an 8-aminoquinoline producing the avian antimalarial (3) [37]. The activity of (3), and

Germany's need to find a substitute for the quinine interdicted by World War I, triggered a massive investigation of the 8-aminoquinolines which led through thousands of variants to the first practical synthetic antimalarial, pamaquine (4) [38–45]. Most of the original German research was never published and, despite some interesting detective work by Steck [14], the exact sequence, from methylene blue in 1891 to pamaquine in 1926, remains somewhat nebulous.

Unfortunately, the early enthusiasm for pamaquine's ability to cure vivax infections [46–48] was soon tempered by the frequency with which it caused methaemoglobinemia and acute haemolytic anaemia [14]. When World War I ended, research on the 8-aminoquinolines became less intense [49–58] and at the beginning of World War II, pamaquine was still the only antirelapse drug known. When malaria's terrible toll on military personnel created an urgent need for an antirelapse drug, a number of investigators re-evaluated pamaquine. They confirmed its activity but found a level of toxicity which precluded its use [59–62].

In an effort to improve the therapeutic index of pamaquine, the U.S. Office of Scientific Research and Development (OSRD) supported the synthesis of hundreds of new 8-aminoquinolines [63–80]. By the end of World War II, this programme had produced pentaquine (5) [81–84]. Following the war, the U.S. Public Health Service sponsored a programme from which emerged isopentaquine (6), SN-3883 (7) and primaquine (8). Although compounds (5)–(8) were all better than pamaquine, it was the pressure of another war, the Korean conflict (1950–1953), which ultimately established primaquine (8) as the drug of choice for the radical cure of the relapsing malaras [85–93].

(4) $\text{R} = \text{CHMe}(\text{CH}_2)_3\text{NEt}_2$	pamaquine (Plasmochin)
(5) $\text{R} = (\text{CH}_2)_5\text{NHCHMe}_2$	pentaquine
(6) $\text{R} = \text{CHMe}(\text{CH}_2)_3\text{NHCHMe}_2$	isopentaquine
(7) $\text{R} = (\text{CH}_2)_4\text{NH}_2$	SN - 3883
(8) $\text{R} = \text{CHMe}(\text{CH}_2)_3\text{NH}_2$	primaquine
(9) $\text{R} = (\text{CH}_2)_3\text{CHMeNH}_2$	quinocide

Quinocide (9), an isomer of primaquine, is used in Eastern Europe and the USSR but it is more toxic than primaquine [94–101]. Primaquine is presently the preferred radical curative drug in most parts of the world. The work leading from pamaquine to primaquine has been detailed in a number of reviews [11, 12, 62]. Primaquine has a broad range of antimalarial activity. In addition to its radical curative activity, primaquine is a causal prophylactic, a gametocytocide and a sporontocide. It can also be produced at low cost and has been relatively slow to select resistant strains [102–104]. Despite these attributes, primaquine is far from the ideal antimalarial. It is a poor blood schizontocide and, although it is the best of the 8-aminoquinolines, its clinical use as a tissue schizontocide is still limited by serious side-effects such as haemolysis, methaemoglobinemia and gastrointestinal distress. Particularly prone to haemolytic reactions are patients with a genetic deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) [105]. Also troublesome is the need to administer primaquine in divided doses over an extended period (15 mg daily for 14 days) since, in addition to its toxicity, it is rapidly absorbed, metabolized and eliminated. Clinicians have therefore been using primaquine with some trepidation and with the desire for a safer, more effective congener.

The most recent surge in antimalarial chemotherapeutic research started with the disturbing discovery in 1960 that *P. falciparum* was becoming resistant to the major blood schizontocide, chloroquine [106–108]. In 1963, the U.S. Army Medical Research and Development Command (USAMRDC) initiated the Antimalarial Drug Development Program because resistant *P. falciparum* was endangering U.S. forces in Vietnam. This monumental program, coordinated through the Walter Reed Army Institute of Research (WRAIR), Washington, DC, has resulted in the primary screening of over 300,000 compounds. From these compounds emerged a number of amino alcohol blood schizontocides, most notably mefloquine, which are relatively safe and presently effective against resistant *P. falciparum*. Amazingly, even though mefloquine is not yet in general use, resistant cases have been observed in Thailand [109] and in Tanzania [110]. Thus, after 20 years of development, and while still in field trials, this cream of the blood schizontocidal crop is already under a cloud.

The Vietnam experience also stressed the need for a better radical curative drug than primaquine [111–114]. Accordingly, in 1968, the Army shifted its emphasis to the discovery of more effective, less toxic 8-aminoquinolines. In 1975, the Army's effort was bolstered by the initiation of the research program of the Scientific Working Group (SWG) on the Chemotherapy of Malaria (CHEMAL), organized by the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) [115]. A third

source of funds, the U.S. Agency for International Development, has concentrated its support on vaccine development. In the interval since 1968, the USAMRDC has supported the synthesis of a great number of new aminoquinolines [116–145]. Another intensive effort to improve primaquine is under way in the People's Republic of China [146–159]. The USAMRDC program has produced new primaquine derivatives which, in animal models, combine low toxicity and a remarkable dual efficacy as blood and tissue schizontocides. This research is continuing.

METHODS FOR ANTIMALARIAL DRUG EVALUATION

The methods devised to screen candidate compounds for antimalarial activity have been described in a number of detailed reviews: avian [11, 12, 160–164], rodent [165–169], simian [170–173], human [174–176] and *in vitro* [177–180].

An early avian model, *P. relictum* in canaries, led to the development of pamaquine [38]. Other avian plasmodia, *P. gallinaceum* in chicks and *P. lophurae* in ducklings, were used for the mass screening of potential antimalarials in the World War II programme and helped in the development of pentaquine [11, 12]. Although the avian plasmodia had provided some valuable information, they are quite different from mammalian species and they ultimately fell into disrepute as uncertain predictors of drug effects in humans.

A major advance occurred with the isolation, in 1948, of a rodent plasmodium, *P. berghei*, in a wild African rat [181]. This parasite readily infected laboratory rats and mice and was soon the basis for a number of more reliable, standardized tests [182–185]. One of the tests, the Rane screen, [185] has been used by the USAMRDC as a primary screen for over 300 000 candidate antimalarials and has generated a number of promising new leads [29]. Another important stride was the demonstration, by Schmidt in 1947, that infection with sporozoites of *P. cynomolgi* in rhesus monkeys is the biological and chemotherapeutic counterpart of similarly induced infections with *P. vivax* in humans [24, 62, 186]. It was the *P. cynomolgi*-rhesus screen which pointed to primaquine as a superior weapon against *P. vivax* [187]. The subsequent discovery that the South American owl monkey (*Aotus trivirgatus*) and the squirrel monkey (*Samiri sciureus*) were susceptible to *P. falciparum* and *P. vivax* made it possible to carry out *in vivo* preclinical studies on human malaria [188–190]. However, the price and scarcity of monkeys have stimulated the search for *in vitro* screens which may eventually eliminate the need for preclinical testing in animals [177–180].

With antimalarials, as with all drugs designed for human use, the ultimate

screening animal is man. From 1917 to 1939, antimalarials were screened in volunteers and in patients with neurosyphilis who received therapeutic malaria for the beneficial effect of the induced fever [191]. The advent of penicillin caused a drastic drop in the number of neurosyphilitics and minimized the utility of malariotherapy as a screening device. Since 1939, clinical trials have been carried out in volunteers; particularly helpful during World War II and the conflicts in Korea and Vietnam were prison inmates and military personnel [11, 19, 62, 192–196]. Public concern caused the discontinuation of the prison programme in 1975 and clinical trials are now conducted with paid civilian volunteers.

The screens most relevant to the work discussed in this chapter are the Rane blood schizontocidal test (*P. berghei*-mouse) [185] and the Schmidt radical curative test (*P. cynomolgi*-rhesus) [23, 197, 198]; these are the preclinical screens most commonly used by the U.S. Army Antimalarial Drug Development Program. None of the promising new 8-aminoquinolines has reached the clinical stage.

RANE BLOOD SCHIZONTOCIDAL SCREEN (*P. BERGHEI* INFECTION IN MICE)

This simple model has served as the principal primary screening test for blood schizontocidal activity since 1964. It benefits from the genetic homogeneity of laboratory mice, their resistance to interfering bacterial and viral pathogens, their small body size, ready availability, ease of handling and low cost [199]. The Rane screen is highly reproducible and responds to a broad spectrum of chemical classes. It is based on a comparison of responses by groups of treated and control mice, five in each group, after infection with *P. berghei* KBG 173. Utilizing young ICR/HA Swiss mice and a standard inoculum of *P. berghei*, it is possible to produce a uniform disease fatal to 100% of untreated animals, within 6 to 8 days, with a mean survival time of 6.2 days. Test animals of approximately the same age and weight are housed in metal-topped plastic cages, given a standard laboratory diet and water *ad libitum*. The animals receive an intraperitoneal injection of 0.5 ml of 1 : 100 dilution of heparinized heart's blood with a minimum of 90% parasitized cells (4×10^7 cells), drawn from donor mice infected 1 week earlier with *P. berghei*. The donor strain is maintained by weekly passages in separate groups of mice inoculated with 0.5 ml of 1 : 500 dilution of heparinized heart's blood. Test compounds are administered after dissolution or suspension in peanut oil. A single dose is given subcutaneously 72 h after the mice are infected with *P. berghei*. At this time a 10–15% parasitemia has developed; the disease is well established, but has not produced sufficient debility to alter the response of the host to any toxic effects

of the test compound. Since treatment is withheld for 3 days and death occurs in untreated controls within 6–8 days, this system presents a candidate drug with the maximum challenge. In order to check factors such as changes in the infectivity of *P. berghei* or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with pyrimethamine at dose levels producing definite increases in survival time is included as a positive control in every experiment. In each experiment, test compounds are administered in graded doses; 640, 160 and 40 mg/kg in the first series and, if activity warrants, 640, 320, 160, 80, 40 and 20 mg/kg in the second series. If activity is still evident, the dose is halved, stepwise, until activity disappears. With highly active compounds, increases in dose levels are usually followed by increases in the survival time of the treated mice. However, if an active drug is toxic for the host, its toxicity may become a limiting factor; a continued increase in dose levels also increases the toxic effects and may result in the diminution of survival times. Deaths prior to the 6th day, when untreated controls begin to die, are regarded as non-parasitic and become the basis for toxicity evaluation. A mean survival time twice that of the controls is evidence of activity and after 60 days survivors are considered cured. Toxic deaths and 60-day survivors are not included in calculating mean survival time.

SCHMIDT RADICAL CURATIVE SCREEN (*P. CYNOMOLGI* INFECTION IN RHESUS MONKEYS)

As noted earlier, sporozoite-induced cynomolgi malaria in rhesus monkeys (*Macaca mulatta*) is much like a vivax infection in man. *P. cynomolgi*'s persistent tissue forms, resembling human hypnozoites, make it an excellent model for the evaluation of candidate radical curative drugs. Well-conditioned Indian rhesus monkeys of either sex, weighing 2–4 kg, are utilized. *P. cynomolgi* (Bastianelli strain) sporozoites are prepared by grinding heavily infected anopheles balabacensis salivary glands in 1 : 1 monkey serum-saline vehicle. Monkeys are infected by i.v. injection of 10^6 freshly isolated *P. cynomolgi* sporozoites on day 0. A rapidly rising parasitemia develops after a 7–9-day prepatent period, and administration of the test drug is initiated when the rising parasite count exceeds 5000 per mm^3 (typically day 10–12). Test drugs are administered orally, by nasogastric intubation, once daily for 7 consecutive days in aqueous solution or, if insoluble, in suspension in 0.3% methylcellulose solution. Chloroquine diphosphate (3.1 mg/kg base orally per day) is always administered concurrently with the test drug for 7 days to eliminate blood schizonts. Thus, any tissue schizontocidal activity of the test drug will always be apparent even if it lacks blood schizontocidal activity. A vehicle control monkey and a positive

drug control (primaquine) monkey are included in each group of inoculated monkeys. The effect of the test drug is determined by counting blood parasites. Parasite counts are made daily through day 20 and every 2 days thereafter. Initially, a clearance of blood parasites is observed due to the blood schizontocidal action of chloroquine. If exoerythrocytic parasites (tissue schizonts) survive the action of the test drug, because it is inactive or marginally active, there will be a relapse of blood parasites. If there is no relapse within 20 days of the initial clearance of parasitemia, parasitemia is followed for an additional 80 days. If there is no relapse within this period, the experiment is terminated and the monkey is considered cured. Primaquine diphosphate cures 90% of monkeys in this test system when administered at a dose of 1.3 mg/kg per day for 7 days (1.0 mg/kg free base) in combination with chloroquine.

In an early version of this screen, at the Southern Research Institute in Birmingham, Alabama, Schmidt used set drug doses of 10, 1, 0.75, 0.5, 0.25, 0.125 and 0.0625 mg/kg. A subsequent version directed by John Brown and Frank Chapple of the SEATO Medical Research Group, Bangkok, Thailand, used doses of 10, 3.16, 1.0, 0.316 and 0.1 mg/kg. The same doses are at present being used by M.M. Dhar in the screen's most recent home in the Central Drug Research Institute, Lucknow, India.

STRUCTURE-ACTIVITY RELATIONSHIPS

Primaquine was itself the culmination of more than a half century of intensive research in the U.S.A., the United Kingdom, Germany, France and Russia. The determination of the U.S. Army Medical Research and Development Command to improve on primaquine therefore presented a daunting challenge. Fortunately, the earlier avian work [11, 12] had provided a useful pad from which to launch the new programme. Despite the multiplicity of avian models and the differences between avian and mammalian malarias, it was cautiously assumed that among the 8-(aminoalkylamino)quinolines (10):

chloroquine

mefloquine

(10)

- (a) A methoxy group at position 6 would elevate both activity and toxicity; 2-, 4- and 5-methoxy groups would be less beneficial than the 6-methoxy group.

- (b) The hydrogen, hydroxy, ethoxy, *n*-butoxy and hydroxyethoxy groups would be less effective at position 6 than the methoxy group.
- (c) The 2,6- and 5,6-dimethoxyquinolines and 5,6-methylenedioxyquinoline would be less active and less toxic than the corresponding 6-methoxy derivative, with toxicity decreasing more than activity for an increase in therapeutic index.
- (d) A 5-phenoxy group would reduce toxicity much more than activity, thus producing a high therapeutic index; a 5-phenyl group would eliminate activity.
- (e) One or more halogens at positions 3, 4 or 6 would diminish activity and toxicity.
- (f) The 5-, 6- and 7-methyl groups would contribute little of value; the 4-methyl group would diminish toxicity without affecting activity; a pair of methyls at positions 2 and 4 would have about the same effect as a single methyl at position 4.
- (g) An amino or anilino group at position 5 of a 6-methoxyquinoline would reduce toxicity more than activity.
- (h) A 4-methylthio group or a 4-benzyl group would reduce activity and toxicity.
- (i) Saturation of the quinoline ring would reduce efficacy.
- (j) A primary terminal amino group in the side-chain would be less toxic than a secondary or tertiary amino group; the aromatic 8-amino group should be secondary.
- (k) The number of methylene groups separating the 8-amino nitrogen atom and the terminal nitrogen atom of the side-chain should be greater than three to avoid the severe irreversible neurotoxicity of antimalarials like rhodoquine (plasmocid); the optimal number of methylenes between nitrogen atoms would probably be 4–6.

On this tentative basis and with the support of the new mammalian screens, major work on the design and synthesis of improved 8-aminoquinoline antimalarials was undertaken in 1968, by the American teams of Blanton (University of Georgia, Athens, GA), Carroll (Research Triangle Institute, Research Triangle Park, NC), LaMontagne (Ash Stevens, Inc., Detroit, MI) and Nodiff (Franklin Research Center, Philadelphia, PA). Additional synthetic contributions were provided by Archer (Rensselaer Polytechnic Institute, Troy, NY), Cheng (Midwest Research Institute, Kansas City, MO), Klayman (Walter Reed Army Institute of Research, Washington, DC) and Werbel (Warner-Lambert/ Parke-Davis, Ann Arbor, MI). During the past decade a number of Chinese investigators have also entered the field (Institute of Parasitology and the Second Military Medical College, Shanghai; Military Academy of Medical

Sciences, Beijing). The new programme placed primary emphasis on 8-aminoquinolines in which the 6-methoxy group and the primaquine side-chain were held constant while the nature, number and position of ring substituents were varied. Extensive synthesis of side-chain variants of primaquine, in earlier programs, had produced none with a better therapeutic index than primaquine itself.

2-SUBSTITUTED ANALOGUES OF PRIMAQUINE

Blanton and co-workers assumed that the activity of the 8-aminoquinolines stemmed from their metabolic conversion to the labile 5,6-quinones and that these quinones could be stabilized by appropriate substitution at position 2 of the quinoline nucleus [122]. This group therefore embarked on a program which produced compounds (11)–(25) in *Table 1.1*. Also included in *Table 1.1* are derivatives prepared in the laboratories of the Franklin Research Center (26) [140] and the Second Military Medical College, Shanghai (27–37) [151].

Table 1.1. 2-SUBSTITUTED ANALOGUES OF PRIMAQUINE

(11) – (37)

Compound No.	R	Compound No.	R
11	PhCH ₂ O	25	Me ₂ N
12	4-FC ₆ H ₄ CH ₂ O	26	Me
13	4-ClC ₆ H ₄ CH ₂ O	27	3-ClC ₆ H ₄ CH ₂ O
14	2,4-Cl ₂ C ₆ H ₃ CH ₂ O	28	3,4-Cl ₂ C ₆ H ₃ CH ₂ O
15	3-CF ₃ C ₆ H ₄ CH ₂ O	29	4-BrC ₆ H ₄ CH ₂ O
16	4-CF ₃ C ₆ H ₄ CH ₂ O	30	PhO
17	4-MeOC ₆ H ₄ CH ₂ O	31	4-FC ₆ H ₄ O
18	PhCH ₂ S	32	4-ClC ₆ H ₄ O
19	4-ClC ₆ H ₄ CH ₂ S	33	2,4,5-Cl ₃ C ₆ H ₂ O
20	MeO	34	4-BrC ₆ H ₄ O
21	Cl	35	4-MeC ₆ H ₄ O
22	Et	36	piperidino
23	CH ₂ = CH	37	pyrrolidino
24	NH ₂		

In the Rane blood schizontocidal screen (*P. berghei*, mouse), the Blanton group's lead compound (11) was slightly active and non-toxic at doses up to 640 mg/kg [122]; primaquine was toxic at 160 mg/kg in the same screen. On learning that (11) was also active in the radical curative screen (*P. cynomolgi*; rhesus), Blanton's group extended its work to include the substituted benzyloxy derivatives (12)–(17) and their sulphur isosteres (18, 19) [119]. All of the new compounds had toxic doses greater than 640 mg/kg in the Rane screen, but only (12), (14) and (19) had even marginal activity in the same model. In the radical curative screen, the best of the new analogues, namely, the 4-fluoro (12) and 2,4-dichloro (14) derivatives were slightly less potent than primaquine. A final attempt to optimize this series provided Blanton with compounds (20)–(25) [118]. Unfortunately, the methoxy (20), chloro (21), vinyl (23) and dimethylamino (25) derivatives had lost all of primaquine's radical curative activity while retaining its toxicity; no data were available for the amine (24). The ethyl derivative (22), a weak, non-toxic blood schizontocide, was also inactive in the radical model. Addition of a 2-Me group to primaquine to give (26) resulted in a small increase in radical curative activity and an attenuation of acute toxicity (1/5 toxic deaths (*T*) at 320 mg/kg vs. 2/5*T* at 160 mg/kg for primaquine). Stimulated by this work, Xu's group prepared compounds (27)–(37) and resynthesized (11) and (13) [151]. The most effective member of the Chinese series, the phenyl ether (30) was only as effective as primaquine for the radical cure of cynomolgi malaria in the monkey. Thus, introduction of a lone substituent at position 2 of primaquine offers, at best, a small improvement in therapeutic index for the radical cure of malaria.

3-SUBSTITUTED ANALOGUES OF PRIMAQUINE

There is a dearth of information on primaquine with a single substituent at position 3. 3-Methylprimaquine [140] is a little more toxic than primaquine (1/5*T* at 80 mg/kg vs. 2/5*T* at 160 mg/kg) and considerably more toxic than 2-methylprimaquine (1/5*T* at 320 mg/kg) [140]; it has insignificant activity in the blood schizontocidal screen and is slightly better than primaquine in the tissue schizontocidal model (2/2 cures (*C*) at 1.0 mg/kg vs. 1/2*C* for primaquine at the same dose). 3-Methylprimaquine is a powerful causal prophylactic in the mouse model [23].

4-SUBSTITUTED ANALOGUES OF PRIMAQUINE

4-Methylprimaquine ((38); Table 1.2), a promising antimalarial originally prepared by Elderfield, *et al.* in the World War II programme [80] was resynthe-

sized by Nodiff's group for evaluation in the new mammalian screens. This analogue was somewhat superior to primaquine as a radical curative and blood schizontocidal agent and less acutely toxic in mice [140]. However, the sub-acute oral toxicity of 4-methylprimaquine in dogs and monkeys was prohibitively greater than that of primaquine [200] and work on (38) was discontinued. In an effort to improve the therapeutic index of (38), additional 4-mono-substituted primaquines (Table 1.2) were prepared by Carroll *et al.* [128]

Table 1.2. 4-SUBSTITUTED ANALOGUES OF PRIMAQUINE

Compound No.	R	Compound No.	R
38	Me	53	MeO
39	Et	54	MeS
40	CH ₂ = CH	55	NH ₂
41	Pr	56	CH ₃ CONH
42	Bu	57	MeNH
43	EtCH(Me)CH ₂	58	OH
44	C ₆ H ₁₁ CH ₂	59	4-ClC ₆ H ₄ O
45	C ₆ H ₁₁ (CH ₂) ₃	60	4-MeOC ₆ H ₄ O
46	MeCH = CH	61	3,4-Cl ₂ C ₆ H ₃ O
47	EtCH = CH	62	3-CF ₃ C ₆ H ₄ O
48	C ₆ H ₁₁ CH = CH	63	4-ClC ₆ H ₄ NH
49	cis-MeCH = CH	64	4-ClC ₆ H ₄ S
50	4-FC ₆ H ₄ SCH ₂	65	4-MeOC ₆ H ₄ S
51	4-ClC ₆ H ₄ S(CH ₂) ₂	66	4-ClC ₆ H ₄ CH ₂ O
52	4-MeOC ₆ H ₄ S(CH ₂) ₂		

(39–52), and LaMontagne *et al.* [139] (53–66). Of moderate interest among these compounds were (39), (40) and (59). In the blood schizontocidal screen, the ethyl (39) and vinyl (40) derivatives were slightly less active than primaquine at doses up to 80 mg/kg and were less toxic than primaquine at the higher doses. The ethyl derivative, the better of the two, was non-toxic over the range 20–640 mg/kg and it was 40% and 100% curative at 320 mg/kg and 640 mg/kg, respectively. In the radical curative test, the ethyl derivative was as active as primaquine and the vinyl compound was slightly less active. The

4-ClC₆H₄O derivative (59) was 60% curative in the Rane screen at 160 mg/kg and 100% curative at 320–640 mg/kg. However, in contrast to the ethyl (39) and vinyl (40) analogues, the phenoxy derivative (59) was devoid of radical curative activity at the highest dose tested (10 mg/kg).

It now seemed clear that monosubstitution on the pyridine ring of primaquine would, at best, attenuate acute toxicity without the desired concomitant enhancement of radical curative activity. Additional work at positions 2, 3 and 4 was therefore held in abeyance.

5-SUBSTITUTED ANALOGUES OF PRIMAQUINE

The most effective 8-aminoquinoline to emerge from the earlier avian compilation [12] was the 5-aryloxy derivative (67). This compound had a thera-

peutic index of 177 compared with 57 for pentaquine (5) and 30 for primaquine (8). With (67) as a lead, Nodiff's group prepared the various 5-phenoxy-, 5-phenylthio- and 5-anilinoprimaquinines (68–88) [142, 144, 145] shown in *Table 1.3*. Prompted by this work, Zheng *et al.* resynthesized (69), (70), (71) and (74) and added the new analogues, (89)–(92) [159]; Xu *et al.* resynthesized (68), (69), (70), (73), (89) and (90) and introduced (93)–(98) [149, 156].

All of the compounds in *Table 1.3* were less toxic than primaquine in the murine blood schizontocidal screen. With the exception of (72), none produced acute lethality at the highest dose tested (640 mg/kg); (72) caused a single death at this dose. The most active blood schizontocides in *Table 1.3* were the fluorine-containing derivatives, 4-fluoro (70) and 3-trifluoromethyl (71), which were completely curative at 320 and 640 mg/kg, respectively. The compound containing both 4-F and 3-CF₃ (77) was less active (2/5C at 640 mg/kg) than either of the single substituted derivatives. The 4-MeO derivative (73), closely related to the lead compound (67), was almost equipotent (4/5C at 640 mg/kg) with (71). The best of these compounds in the radical curative test (70), (71) and (77) were slightly more active than primaquine. The most interesting compound among the Chinese contributions was the resynthesized analogue (70). Zheng *et al.* [159] reported that, against *P. yoelii* in the mouse, (70) was 20-times less toxic and 4–5-times less effective than primaquine. According to

Table 1.3. 5-PHENOXY, 5-PHENYLTHIO AND 5-ANILINO ANALOGUES OF PRIMAQUINE

(68) - (98)

Compound No.	X	R	Compound No.	X	R
68	O	H	84	S	3,4-Cl ₂
69	O	4-Cl	85	S	2,5-Cl ₂
70	O	4-F	86	S	4-MeO
71	O	3-CF ₃	87	S	3-CF ₃
72	O	4-CF ₃ O	88	S	3,4-benzo
73	O	4-MeO	89	O	4-Br
74	O	2,4-Cl ₂	90	O	4-Me
75	O	3,4-Cl ₂	91	O	4-I
76	O	3,5-(CF ₃) ₂	92	O	2-Cl
77	O	4-F-3-CF ₃	93	O	3-F
78	O	4-MeCONH	94	O	3-Cl
79	NH	4-Cl	95	O	3-Br
80	NH	3-CF ₃	96	O	3-Me
81	S	2-Cl	97	O	3,4-Me ₂
82	S	3-Cl	98	O	3,5-Me ₂
83	S	4-Cl			

Zheng *et al.* the fluorophenoxy (70) was also less effective than primaquine against *P. cynomolgi* in the monkey.

All of the anilino and phenylthio derivatives (79)–(88) were nontoxic and inactive or very weakly active at 640 mg/kg in the blood schizontocidal screen. In the radical curative screen, the entire phenylthio series (81)–(88) was curative at 10 mg/kg but only the 4-chloro (83) and 3-trifluoromethyl (87) compounds were curative at 1 mg/kg. The anilino derivative (80) was inactive even at 10 mg/kg. Thus, acute toxicity of primaquine can be diminished by introduction of various phenoxy, phenylthio or anilino groups at position 5. The phenylthio and anilino analogues were consistently less active than their oxygen isosteres, but all three series were unimpressive. There is a notable difference between the 4- and 5-phenoxyprimaquines. While the 5-phenoxy derivatives showed moderate radical curative activity in the monkey, the corresponding 4-phenoxy derivatives were inactive at the highest dose tested. In contrast, the

4-phenoxy derivatives were more potent blood schizontocides than their 5-phenoxy counterparts. Neither group was active in the mouse prophylactic screen.

The lead compound (67) was resynthesized by Kalidas and Blanton [120]. It is ironic that this compound, which had seemed so promising in the avian model and which had inspired so much additional research, was a total failure in the mouse and monkey screens.

5-Methoxyprimaquine (99) had a similar radical curative activity to primaquine and was slightly less toxic; it lacked blood schizontocidal activity. With continuing interest in substitution at position 5, Nodiff's group prepared the higher homologues of (99) shown in *Table 1.4* [140, 141]. The best combination of blood schizontocidal activity and nontoxicity occurred in the *n*-decyl derivative (107). However, this compound was relatively unimpressive with only two cures at 640 mg/kg. On moving from R = decyl down through the homologous series, acute toxicity gradually increased until, when R was butyl or smaller, toxicity was greater than that for primaquine itself. The toxicity increase at the higher doses (80–640 mg/kg) was not accompanied by any elevation in activity at the lower end of the dosage range. With the exception of the *n*-decyloxy derivative (107), which was radically curative only at 10 mg/kg, all of the compounds in *Table 1.4* were completely curative at 1.0 mg/kg. Among these, the propoxy (102), butoxy (103) and pentoxy (104) derivatives were also curative at 0.316 mg/kg, making them somewhat more active than primaquine (1/2C at 1.0 mg/kg).

At this juncture, the 5-phenoxyprimaquines seemed more promising than their excessively toxic 5-alkoxy analogues.

Table 1.4. 5-ALKOXY DERIVATIVES OF PRIMAQUINE

Compound No.	R	Compound No.	R
99	Me	104	<i>n</i> -C ₅ H ₁₁
100	Et	105	<i>n</i> -C ₆ H ₁₃
101	CF ₃ CH ₂	106	<i>n</i> -C ₈ H ₁₇
102	<i>n</i> -Pr	107	<i>n</i> -C ₁₀ H ₂₁
103	<i>n</i> -Bu		

2,4-DISUBSTITUTED ANALOGUES OF PRIMAQUINE

Recognizing the limited ability of a single substituent to improve primaquine's therapeutic index, various investigators introduced more than one. The 2,4-disubstituted analogues (108)–(110), prepared by Carroll *et al.* [127] and shown in Table 1.5, were less toxic and less active than primaquine in the blood schizontocidal screen (no cures or toxic deaths at 640 mg/kg). Primaquine and (108) were equally active as tissue schizontocides.

Table 1.5. 2,4-DISUBSTITUTED ANALOGUES OF PRIMAQUINE

(108) – (110)

Compound No.	R ¹	R ²
108	Me	Et
109	Me	CH ₂ = CH
110	Et	Me

2,5-DISUBSTITUTED ANALOGUES OF PRIMAQUINE

Nodiff and co-workers found that the 2-methyl derivative of 5-(*n*-hexyloxy)-primaquine (111) had slightly greater blood schizontocidal activity than its parent compound (105), at doses from 20 to 160 mg/kg (3C vs. 1C at 160 mg/kg) and was more toxic at 320–640 mg/kg (3T vs. 1T at 640 mg/kg). The radical curative difference between (105) and (111) was slight [140]. It is of interest that 5-(*n*-hexylthio)-2-methylprimaquine (112), the sulphur isostere of (111), was a somewhat better blood schizontocide than (111) with 3C at 160 mg/kg and 5C at 320–640 mg/kg (unpublished data).

(111) X = O
(112) X = S

Xu and Xu [149] and Zheng and Cheng [152] prepared the series of 2,5-disubstituted primaquines (113–119) shown in *Table 1.6*. The fluorine derivative (113) showed some activity against sporozoite-induced infection by *P. yoelii* in mice.

Table 1.6. 2,5-DISUBSTITUTED ANALOGUES OF PRIMAQUINE

Compound No.	R	Compound No.	R
113	4-F	117	4-Me
114	4-Br	118	4-MeO
115	4-Cl	119	H
116	3-CF ₃		

4,5-DISUBSTITUTED AND 2,4,5-TRISUBSTITUTED ANALOGUES OF PRIMAQUINE

After the extensive, above-detailed exploration of peripheral veins by various groups, the teams of Nodiff and LaMontagne seemed to strike the mother lode (or guiding principle). These investigators found that addition of a 4-methyl group to unimpressive 5-aryloxy- or 5-alkoxyprimaquines produced dramatic antimalarial enhancement. As detailed below, some of these 4-methyl compounds are unique in their combination of very low acute toxicity and wide-ranging activity as blood schizontocides, radical curative drugs and causal prophylactics.

4-Alkyl-5-(aryloxy)primaquines

Table 1.7 includes various members of this series prepared by LaMontagne *et al.* (120)–(124) [134, 136], Nodiff *et al.* (125)–(131) [142], (129)–(131) (unpublished data), Carroll *et al.* (132)–(137) [125], Deng *et al.* (138)–(140) [153] and Wang and Xu (141) [150]. Deng resynthesized (120), (121) and (123) and Wang resynthesized (124). Blood schizontocidal and radical curative data for the most effective compounds in this series are compared (in *Table 1.8*) with

Table 1.7. 4-ALKYL-5-(ARYLOXY)PRIMAQUINES^a

No.	R ¹	R ²	No.	R ¹	R ²
120 ^b	3-CF ₃	4-Me	132	3-CF ₃	4-Et
121	2,4-Cl ₂	4-Me	133	3-CF ₃	4-CH ₂ = CH
122	3,4-Cl ₂	4-Me	134	3-CF ₃	4-CH ₂ OH
123	4-MeO	4-Me	135	3-CF ₃	4-MeOCH ₂
124	4-F	4-Me	136	3-CF ₃	4-(MeO) ₂ CH
125	H	4-Me	137	3-CF ₃	4-(3-CF ₃ C ₆ H ₄ OCH ₂)
126	4-F-3CF ₃	4-Me	138	4-Me	4-Me
127	4-F-3CF ₃	3-Me	139	4-Me ₃ C	4-Me
128	3,5-(CF ₃) ₂	4-Me	140	2,4,5-Cl ₃	4-Me
129	4-EtO	4-Me	141	3-F	4-Me
130	4-(<i>n</i> -C ₅ H ₁₁)	4-Me	141a ^c	3-CF ₃	4-Me
131	4-PhO	4-Me			

^a In compounds (120)–(141), R³ = H; in compound (141a), R³ = MeO.

^b WR-225,448.

^c WR-238,605.

corresponding data for primaquine. All of the 4,5-disubstituted derivatives in Table 1.8 were considerably less toxic than primaquine and much more active in both the blood schizontocidal and radical curative screens. The 3-CF₃ and 4-F derivatives (120, 124) were equally active in the blood screen (multiple *C* at 10 mg/kg) and in the radical screen (2/2*C* at 0.316 and 1.0 mg/kg; no *C* at 0.1 mg/kg) and displayed little toxic lethality below 640 mg/kg. The 3,4-Cl₂ analogue (122) was a more effective, less toxic blood schizontocide (80% curative at 5 mg/kg; 100% curative at 10–640 mg/kg) than (120, WR-225,448) or (124) but it was slightly less active than these compounds in the radical model (1/2*C* at 0.316 mg/kg). The phenyl-unsubstituted derivative (125) was more toxic than the other members of this series (3/5*T* at 320–640 mg/kg) but it was the only one to effect any radical cures at the low dose of 0.1 mg/kg. Most of the high blood schizontocidal and radical curative activities and the low toxicity of (120) were retained when its 4-methyl group was replaced by an ethyl (132) or methoxymethyl (135) group. However, replacement of the methyl of (120) with a CH₂ = CH (133), CH₂OH (134), (MeO)₂CH (136) or

Table 1.8. ANTIMALARIAL ACTIVITY AND TOXICITY OF 4-METHYL-5-(ARYLOXY)-PRIMAQUINES

See formula in Table 1.7.

No.	R ¹	Blood schizontocidal activity ^a <i>P. berghei</i> , mouse; dose, mg/kg; cures (C) ^b , toxic deaths (T) ^c or Δ MST ^d								Radical curative activity ^e <i>P. cynomolgi</i> , rhesus; dose, mg/kg; cures/No. of animals		
		5	10	20	40	80	160	320	640	0.1	0.316	1.0
(8) (Primaquine)				4.0	5.0	9.4	2T	5T	5T	0/2	0/2	1/2
120 ^f	3-CF ₃	6.9	2C	5C	5C	5C	5C	5C	1C, 4T	0/2	2/2	2/2
122	3,4-Cl ₂	4C	5C	5C	5C	5C	5C	5C	5C	0/3	1/2	3/3
123	4-MeO	5.5	5.9	1C	5C	5C	5C	5C	2C, 3T	0/2	2/2	3/3
124	4-F	7.0	3C	4C	5C	5C	5C	4C, 1T	3C, 2T	0/3	2/2	3/3
125	H		8.7	4C	3C	4C	4C	3T	3T	1/2	1/1	2/2

^a Activities were determined at the Rane Laboratory, University of Miami, Florida, with five mice per group, via the method of Osdene *et al.* [185].

^b The number of mice surviving at 60 days post-infection.

^c Deaths prior to the 6th day.

^d Increase in mean survival time over controls (MST of control group, 6.1 days).

^e Tests were carried out by the SEATO Medical Research Laboratory, Bangkok and the Central Drug Research Institute, Lucknow, according to the procedure of Schmidt *et al.* [197].

^f R² = 4-Me and R³ = H for compounds (120), (122)–(125).

3-CF₃C₆H₄OCH₂ (137) banished radical curative activity and either eliminated or severely depressed blood schizontocidal activity.

On the basis of blood and tissue data, (120) was judged the most promising member of the series and was selected by the USAMRDC for additional preclinical evaluation [134, 136]. In a repetition of the Rane blood schizontocidal test, which used more than the usual five mice at several concentrations, (120) effected 10/10C at 160 mg/kg and 20 mg/kg and 15/15C at 40 mg/kg but only 2/10C (4 toxic deaths) at 640 mg/kg. Primaquine and (120) were also compared for suppressive activity against *P. cynomolgi* in the rhesus. This test differs from the radical curative test in that parasitemia is induced by intravenous inoculation of parasitized blood rather than by sporozoites and that chloroquine is not co-administered with the test drug [201]. Primaquine was suppressive but did not completely clear blood schizonts at doses from 1.0 to 31.6 mg/kg per day ($\times 7$); (120) was 100% curative at 1.0 mg/kg per day ($\times 7$) and was suppressive but not curative at doses as low as 0.0316 mg/kg.

As mentioned earlier, the toxicity of primaquine precludes administration of a single radically curative dose; in order to achieve radical cure of *P. vivax* in man, multiple doses are required coupled with the blood schizontocide, chloroquine. Accordingly the radical curative screen (*P. cynomolgi* – rhesus) was modified to establish whether a single dose of (120) was effective as both a tissue and blood schizontocide and whether the co-administration of chloroquine was necessary. This test showed that primaquine was effective in two of four monkeys at 3.5 mg/kg ($\times 1$) but permitted 2/2 relapses at 1.75 mg/kg; its fully curative dose was 14 mg/kg ($\times 1$). Primaquine and (120) were about equally active at 3.5 mg/kg without chloroquine, but in the presence of chloroquine the fully curative dose of (120) dropped to 0.875 mg/kg ($\times 1$) compared to 14 mg/kg ($\times 1$) for primaquine; (120) permitted 4/4 relapses at 0.4375 mg/kg. Acute toxicity evaluation in rats and guinea-pigs indicated that (120) was less toxic than primaquine by factors of 1.5–3.2.

Causal prophylaxis, originally attributed to residual blood schizontocidal activity, was confirmed in the Most model [25, 183]. Against a trophozoite-induced vivax infection in the *Aotus trivirgatus* monkey, (120) was 100% curative at a total dose of 12 mg/kg, while primaquine was non-curative even when the total dose was raised to 160 mg/kg [201].

However, the elation elicited by the accumulating positive data for (120) was short-lived. In subacute (60 day) studies with rats, (120) was more toxic than primaquine. Clinical chemistry alterations and tissue lesions were induced by (120) which were not seen when primaquine was administered at equal doses [25]. Of particular concern was the haematotoxicity of (120). A general deficiency of the 8-aminoquinolines is their tendency to produce methaemoglobinemia [202–210]. This condition is characterized by a concentration of methaemoglobin (MHb) in erythrocytes which is greater than the usual 1 to 2%. In methaemoglobin, the normal ferrous iron has been oxidized to the ferric state and is incapable of transporting oxygen. Methaemoglobinemia can thus lead to anoxia and death. According to Anders *et al.* [211], (120) induced in dogs a peak methaemoglobin level which was 4-times higher than that produced by primaquine (25.3% vs. 6.3%; Table 1.9).

Seeking to improve (120), LaMontagne *et al.* returned to the old avian literature and Mislav's report that a 2-methoxy substituent reduced the toxicity of primaquine [64]. This led to the synthesis of (141a, WR-238,605), the 2-methoxy derivative of (120) (Table 1.9) [132]. Compound (141a) had better blood and radical curative activities than its 2-demethoxy parent with no change in acute toxicity but with a third less methaemoglobin induction (16.0% vs. 25.3%; Table 1.9) [132, 133, 211]. It was also significantly less toxic than (120) in the rat either by oral or intraperitoneal administration [132, 133].

Table 1.9. EFFECT OF THE 2-METHOXY GROUP ON THE ACTIVITY, ACUTE TOXICITY AND METHAEMOGLOBIN INDUCTION OF (120)

See formula in Table 1.7.

No.	Blood schizontocidal activity ^a <i>P. berghei</i> , mouse; dose, mg/kg; cures (C) ^b , toxic deaths (T) ^c or Δ MST ^d						Radical curative activity ^e <i>P. cynomolgi</i> , <i>rhesus</i> ; dose, mg/kg; cures/No. of animals			Peak % MHb ^f
	20	40	80	160	320	640	0.1	0.316	1.0	
(8) (Primaquine)	4.0	5.0	9.4	2T	5T	5T	0/2C	0/2C	1/2C	6.3
(120) ^g	1C	4C	5C	5C	5C	4C, 1T	0/2C	2/2C	2/2C	25.3
(141a)	3C	5C	5C	5C	5C	4C, 1T	2/4C	4/4C	2/2C	16.0

^{a-c} See Table 1.8.^f Anders *et al.* [211] performed these evaluations on beagle dogs at the Walter Reed Army Institute of Research, Washington, DC.^g The data reported by LaMontagne for (120), for this comparison [132], were somewhat different from those reported by LaMontagne for the same compound in an earlier study (Cpd (120) in Table 1.8) [136].

Against trophozoite-induced infections of the Chesson strain of *P. vivax* in the *Aotus* monkey, (141a) was slightly better than (120) (2/2C vs. 1/3C at 1 mg/kg $\times$ 3) but it was somewhat less effective than (120) as a causal prophylactic against sporozoite-induced *P. berghei yoelii* in the mouse (0/5C vs. 6/15C at 2.5 mg/kg, subcutaneously) [133]. Replacement of the 2-methoxy of (141a) with Cl, OH, 4-ClC₆H₄O or 4-ClC₆H₄CH₂O gave compounds which were inactive and non-toxic in the Rane screen and were not examined further [132, 133]. Compound (141a) is at present completing advanced preclinical evaluation.

4-Alkyl-5-(alkoxy)primaquines

Concurrently with their research on the 4-Alkyl-5-(aryloxy)primaquines, Nodiff and co-workers prepared the substituted 5-(alkoxy)primaquines included in Table 1.10 (142–156, 158–170) [140, 141]. Additional analogues were synthesized by LaMontagne *et al.* (157, 171–174) [137] and Carroll *et al.* (175, 176) [124]. R¹ in Table 1.10 encompasses *n*-alkyl, isoalkyl, cycloalkyl, alkenyl, cycloalkylmethyl, phenoxyalkyl, alkoxyalkyl, benzyloxyalkyl and phenylalkyl groups; work on the phenylalkoxy series (167)–(170) has not yet been published.

Table 1.10. 4-METHYL-5-(ALKOXY)PRIMAQUINES

(142) - (176)

No.	R ¹	R ²	No.	R ¹	R ²
142	<i>n</i> -Pr	4-Me	159	PhO(CH ₂) ₆	4-Me
143	<i>n</i> -Bu	4-Me	160	PhO(CH ₂) ₈	4-Me
144	<i>n</i> -C ₅ H ₁₁	4-Me	161	C ₆ H ₁₁ CH ₂	4-Me
145	<i>n</i> -C ₆ H ₁₃	4-Me	162	Cyclopentyl	4-Me
146	<i>n</i> -C ₇ H ₁₅	4-Me	163	CH ₂ = CH(CH ₂) ₂	4-Me
147	<i>n</i> -C ₈ H ₁₇	4-Me	164	Me(CH ₂) ₃ O(CH ₂) ₂	4-Me
148	<i>n</i> -C ₉ H ₁₉	4-Me	165	MeO(CH ₂) ₆	4-Me
149	<i>n</i> -C ₁₀ H ₂₁	4-Me	166	PhCH ₂ O(CH ₂) ₆	4-Me
150	<i>n</i> -C ₁₁ H ₂₃	4-Me	167	Ph(CH ₂) ₄	4-Me
151	<i>n</i> -C ₁₂ H ₂₅	4-Me	168	Ph(CH ₂) ₅	4-Me
152	<i>n</i> -C ₆ H ₁₃	3-Me	169	Ph(CH ₂) ₆	4-Me
153	<i>n</i> -C ₆ H ₁₃	2-Me	170	Ph(CH ₂) ₇	4-Me
154	2-C ₆ H ₁₃	3-Me	171	Me	4-Me
155	3-C ₆ H ₁₃	3-Me	172	Me	2,4-Me ₂
156	<i>n</i> -C ₆ H ₁₃	2,4-Me ₂	173	5,6-OCH ₂ O-	4-Me
157	<i>n</i> -C ₆ H ₁₃	2-MeO,4-Me	174	5,6-O(CH ₂) ₂ O-	4-Me
158	PhO(CH ₂) ₄	4-Me	175	Me	4-CH ₂ = CH
			176	Me	4-(<i>m</i> -CF ₃ PhOCH ₂)

Among these compounds, introduction of a 4-methyl group to the shorter chain (C₃-C₈) 5-alkoxyprimaquines (142-147) generally produced a pronounced blood schizontocidal increase in the lower part of the dosage range and elevated acute toxicity at the higher doses (160-640 mg/kg). There was a progressive diminution in toxicity with increased chain length; the C₃ derivative (142) caused three toxic deaths at 40 mg/kg, while the C₈ derivative (147) caused only two deaths at 640 mg/kg. The long-chain members of the series (C₉-C₁₂; 148-151) were responsible for no toxic deaths even at 640 mg/kg. Among the latter group, activity was inversely related to chain length, with the nonyl derivative (148) producing 2/5C at 10 mg/kg while its dodecyl homologue (151) only provided multiple cures (4/5C) at 160 mg/kg. The most promising members of this series were 4-methyl-5-(*n*-pentoxy)primaquine (144) and 4-methyl-5-(*n*-hexyloxy)primaquine (145). Both of these compounds were curative in the Rane screen at the extremely low dose of 2.5 mg/kg. Their activity in the radical curative screen was also extraordinary, with the pentoxy

compound (144) curing 3/4 monkeys at 0.1 mg/kg and its hexoxy analogue (145, WR-242,511) curing 5/5 monkeys at the same low dose. Compound (145) also displayed potent activity against *P. berghei yoelii* in the causal prophylactic screen, achieving 5/5C at 10 and 40 mg/kg (subcutaneously), 3/5C at 2.5 mg/kg and 1/5C at 0.63 mg/kg.

Unfortunately, both (144) and (145) were about as acutely toxic as primaquine in the upper half of the dosage range of the Rane screen. Furthermore, the hexoxy derivative, (145) induced a worrisome methaemoglobin level in the dog (48.1% vs. 25.3% for (120) and 16.0% for (141a); Table 1.9 [211]. Despite these drawbacks, the USAMRDC still considers (145) a viable antimalarial candidate because of its excellent blood schizontocidal activity (5/5C at 20 mg/kg) and its unrivaled low-dose efficacy in the radical curative screen (5/5C at 0.1 mg/kg). The hexyl ether (145) is at present undergoing intensive preclinical evaluation.

An effort to elevate the therapeutic index of (145), while retaining its dual activity, led to the synthesis of analogues (152)–(176) (Table 1.10) and permitted the following structure–activity correlations among the 5-(alkoxy)-primaquines.

Blood schizontocidal activity

- (a) The 2-Me and 3-Me groups were almost equivalent and had a lesser effect on activity and toxicity than the 4-Me group (153 and 152 vs. 145).
- (b) Paired methyl groups at positions 2 and 4 contributed more to toxicity and less to activity than a single 4-methyl group (156 vs. 145).
- (c) Primaquines bearing a branched-chain alkoxy at position 5 were more toxic than their straight-chain isomer (154 and 155 vs. 152).
- (d) A 4-methylprimaquine with a 5-(unsaturated alkoxy) was less active and toxic than the corresponding saturated compound (163 vs. 143).
- (e) A 5-cycloalkoxy contributed less to activity and toxicity than the corresponding acyclic group (162 vs. 144).
- (f) Replacement of a terminal hydrogen, on a 5-alkoxy group, with a phenoxy moiety had little effect on the activity or toxicity of the resulting primaquine (158–160 vs. 143, 145, 147).
- (g) Replacement of a terminal hydrogen, on a 5-alkoxy group, with an alkoxy diminished activity and increased toxicity (165 vs. 145).
- (h) The 5,6-bridged derivatives were either totally inactive (173) or highly toxic (174).
- (i) Surprisingly toxic, with 2/5T at 20 mg/kg, was the benzyloxyhexyloxy derivative (166). This suggested the possibility that at least part of the

toxicity of the 5-(alkoxy)primaquines stemmed from metabolic oxidation of the terminal methyl to a hydroxymethyl. With this in mind, Nodiff's team protected the terminal methyl with the phenyl group producing the (167)–(170) series (unpublished work). In each case, introduction of a phenyl group caused a striking reduction in toxicity with little or no decrease in activity. Comparative data for the 4-demethyl-5-(alkoxy)primaquines and the corresponding 4-methyl-5-(alkoxy) and 4-methyl-5-(phenylalkoxy) primaquines are grouped in *Table 1.11*. It should be noted that the 5-(phenylheptyloxy) derivative (170), with 5/5C at 10–640 mg/kg and 2C at 5 mg/kg, is considerably more effective than mefloquine, the best of the new wave of blood schizontocides, which is curative at 20 mg/kg [212].

- (j) As described earlier, LaMontagne *et al.* were able to upgrade a 4-methyl-5-(aryloxy)primaquine (120) to (141a); (*Table 1.9*) by inserting an MeO group at position 2 [132]. An attempt to enhance (145) in a similar fashion was unsuccessful (157 vs. 145, *Table 1.11*) [132]. The corresponding 5-(phenylalkoxy) analogue (169, *Table 1.11*) was a better blood schizontocide than either (157) or (145).

Radical curative activity

- (a) In moving through the homologous 4-methyl-5-(*n*-alkoxy)primaquines, from C₆ (145) to C₁₂ (151), activity gradually decreased, with the C₁₀ (149), C₁₁ (150) and C₁₂ (151) compounds non-curative at 1.0 mg/kg.
- (b) The 2,4-Me₂ analogue (156) of (145) was only half as active as the parent compound (2/4C vs. 5/5C at 0.1 mg/kg) while the 3-Me (152) and 2-Me (153) analogues produced no cures at 0.1 mg/kg.
- (c) The 5-(phenylpentoxy) derivative (168, WR-254,715) and the 5-(phenylhexyloxy) derivative (169) were 100% curative at 0.316 mg/kg (4/4C); (168) also effected 1/2C at 0.1 mg/kg.
- (d) Among the few remaining compounds to achieve cures at 0.1 mg/kg were a 5-(phenoxyoctyloxy)primaquine (160) (1/2C), an analogue of (145) in which the 5-(hexyloxy) chain was interrupted by an oxygen atom (164) (1/3C) and the 2-MeO derivative (157) of (145) (1/4C).

It would thus seem that among the substituted 5-(alkoxy)primaquines, the best combination of low toxicity with high blood and tissue schizontocidal activity resides in 4-methyl-5-(phenylpentoxy)primaquine (168).

Table 1.11. COMPARISON OF ANTIMALARIAL ACTIVITY OF 5-ALKOXY AND 5-(PHENYLALKOXY)PRIMAQUINES

No.	R'	R ²	Blood schizontocidal activity ^a <i>P. berghei</i> , mouse; dose, mg/kg; cures (C) ^b , toxic deaths (T) ^c or ΔMST ^d										Radical curative activity ^c <i>P. cynomolgi</i> , rhesus; dose, mg/kg; cures/No. of animals		
			2.5	5	10	20	40	80	160	320	640	0.1	0.316	1.0	
8	(Primaquine)					4.0	5.0	9.4	2T	5T	5T	0/2	0/2	1/2	
38	(4-Me-Primaquine)					3.1	4.9	5.5	9.0	10.0	3C		0/4	2/2	
103	Me(CH ₂) ₃	H			4.1	6.5	6.7	2T	3T		5T		2/4	2/2	
143	Me(CH ₂) ₃	Me			11.0	5C	3C	4T	5T	5T	5T	0/2	2/2	1/1	
167	Ph(CH ₂) ₄	Me		7.6	2C	4C	4C	5C	3C	0.4	0.0	0/3	1/2	4/4	
104	Me(CH ₂) ₄	H			4.3	4.7	6.1	4.7	4T	5T	2T	0/2	1/2	1/1	
144	Me(CH ₂) ₄	Me	2C	5C	5C	4C	1C	2T	4T	5T	5T	3/4		2/2	
168	Ph(CH ₂) ₅	Me		7.9	3C	4C	5C	5C	5C	3C	1C	1/2	4/4	2/2	
105	Me(CH ₂) ₅	H				3.7	6.5	1C	1C	0.1	1T		0/2	3/3	
145	Me(CH ₂) ₅	Me	1C	1C	1C	5C	5C	4C	3T	5T	5T	5/5	3/3	1/1	
169	Ph(CH ₂) ₆	Me		5.9	1C	3C	4C	4C	3C	3C	4C	0/2	4/4	2/2	
157	Me(CH ₂) ₅	2-MeO-4-Me				10.5	2C	5C	4C	5T	5T	1/4	3/3	2/2	
146	Me(CH ₂) ₆	Me	7.9	3C	3C	5C	4C	2C	1T	5T	5T	0/2	2/2	4/4	
170	Ph(CH ₂) ₇	Me		2C	5C	5C	5C	5C	5C	5C	5C	0/2	0/4	3/3	

^{a-c} See Table 1.8.

MODIFICATION OF THE QUINOLINE HETEROCYCLE

Reduction of the pyridine ring

Encouraged by the high avian antimalarial activity of 8-(5-diethylamino-2-pentylamino)-6-methoxy-1,2,3,4-tetrahydroquinoline (tetrahydropamaquine) [11] Carroll *et al.* prepared the 1,2-dihydroprimaquines (177, 178) and the 1,2,3,4-tetrahydroprimaquine (179) [130]. Mouse and monkey screening data for (177)–(179) suggest that reduction of the pyridine ring in primaquine lessens or eliminates activity.

- (177) R = H; 3,4-double bond
 (178) R = MeO; 3,4-double bond
 (179) R = H

Substitution of naphthalene for quinoline

Archer *et al.* found that several naphthalene analogues (180, 181) of primaquine showed little tissue schizontocidal activity in the *P. cynomolgi* screen [213].

- (180) R = H
 (181) R = Br

Substitution of acridine for quinoline

Klayman and co-workers synthesized acridine derivatives, which may be considered as a benzoprimaquine (182) and a 4-methylbenzoprimaquine (183) [214]. The demethyl derivative (182) was inactive as a blood schizontocide and curative in the radical model at 10 mg/kg. Both derivatives were ineffective at 3.16 mg/kg and were thus less active than primaquine.

(182) R = H

(183) R = Me

Substitution of benzofuran for quinoline

The benzofuranyl isostere of primaquine (184), synthesized by Johnson and Werbel [215], was ineffective as a blood schizontocide and was never evaluated in the *P. cynomolgi*-rhesus model.

(184)

ENANTIOMERS OF PRIMAQUINE

Primaquine is used clinically as a racemic mixture. It was therefore of interest to compare the (+) and (−) enantiomers with the racemate. After Carroll *et al.* resolved the mixture [129], Schmidt *et al.* studied the comparative antimalarial activities and toxicities of the racemate and its enantiomers in mice and rhesus monkeys [216]. According to the latter authors the (+) enantiomer was 4-times more toxic than the (−) enantiomer in mice but 3–5-times less toxic than the (−) form in the rhesus. The radical curative activity of the racemate and its enantiomers against *P. cynomolgi*-rhesus was essentially the same. Assuming that the rhesus data were more predictive for man than the mouse data, Schmidt *et al.* concluded that the (+) form had a 2-fold advantage in therapeutic index over primaquine and suggested clinical evaluation of this enantiomer against *P. vivax*; this has yet to be done.

CHEMISTRY

Typical syntheses are outlined in *Schemes 1.1–1.3*.

Scheme 1.1 Compound (120) (Tables 1.7–1.9) [134, 136]. For Ar, Phth see lower left corner.

CONCLUSION

Primaquine, the best available tissue schizonticide, suffers from a number of serious deficiencies. The concentrate of a 25-year search for an improved tissue schizonticide is the trio of new 8-aminoquinolines compared with primaquine in Table 1.12. All three are at least 10-times more effective than primaquine in the radical curative screen; (145), the best of the three in this screen, is 100% curative at 0.1 mg/kg. Quite unexpectedly, these compounds are also extremely effective blood schizontocides, with (168) providing multiple cures at 10 mg/kg. (The minimum curative dose for mefloquine, the best of the new blood schizontocides, is 20 mg/kg; mefloquine has no radically curative activity.) Despite some uneasiness over the tendency of these drugs to produce

Scheme 1.2 Compound (141a) (Table 1.9) [132, 133]. Abbreviations as in Scheme 1.1.

Scheme 1.3 Compound (145) (Tables 1.10, 1.11) [140, 141].

Table 1.12. ANTIMALARIAL ACTIVITY, ACUTE TOXICITY AND METHAEMOGLOBIN INDUCTION OF COMPOUNDS (141a), (145), (168) AND PRIMAQUINE

No.	<i>R</i> ¹	<i>R</i> ²	Blood schizontocidal activity ^a P. berghei, mouse; dose, mg/kg; cures (C) ^b , toxic deaths (T) ^c , ΔMST ^d							Radical curative activity ^e P. cynomolgi, rhesus; dose, mg/kg; cures/No. of animals			Peak % MHb ^f
			10	20	40	80	160	320	640	0.1	0.316	1.0	
(8) (Primaquine)				4.0	5.0	9.4	2T	5T	5T	0/2	0/2	1/2	6.3
(141a) 3-CF ₃ C ₆ H ₄	MeO			3C	5C	5C	5C	5C	4C, 1T	2/4	4/4	2/2	16.0
(145) <i>n</i> -C ₆ H ₁₃	H		1C	5C	5C	4C	3T	5T	5T	5/5	3/3	1/1	48.1
(168) Ph(CH ₂) ₅	H		3C	4C	4C	5C	5C	3C	1C	1/2	4/4	2/2	

^{a-f} See Table 1.9.

methaemoglobinemia, (141a) is about to enter Phase I clinical evaluation and an application is being prepared for IND approval of (145). Compound (168) with an excellent primary profile, is still awaiting methaemoglobin evaluation. These broad spectrum antimalarials offer the possibility of a single drug that would be effective against all of the relapsing and non-relapsing malarias.

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