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# 4-(1*H*)-Quinolones and 1,2,3,4-Tetrahydroacridin-9(10*H*)-Ones Prevent the Transmission of *Plasmodium falciparum* to *Anopheles freeborni*

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**Malaria kills approximately 1 million people a year, mainly in sub-Saharan Africa. Essential steps in the life cycle of the parasite are the development of gametocytes, as well as the formation of oocysts and sporozoites, in the *Anopheles* mosquito vector. Preventing transmission of malaria through the mosquito is necessary for the control of the disease; nevertheless, the vast majority of drugs in use act primarily against the blood stages. The study described herein focuses on the assessment of the transmission-blocking activities of potent antierythrocytic stage agents derived from the 4(1*H*)-quinolone scaffold. In particular, three 3-alkyl- or 3-phenyl-4(1*H*)-quinolones (P4Qs), one 7-(2-phenoxyethoxy)-4(1*H*)-quinolone (PEQ), and one 1,2,3,4-tetrahydroacridin-9(10*H*)-one (THA) were assessed for their transmission-blocking activity against the mosquito stages of the human malaria parasite (*Plasmodium falciparum*) and the rodent parasite (*P. berghei*). Results showed that all of the experimental compounds reduced or prevented the exflagellation of male gametocytes and, more importantly, prevented parasite transmission to the mosquito vector. Additionally, treatment with ICI 56,780 reduced the number of sporozoites that reached the *Anopheles* salivary glands. These findings suggest that 4(1*H*)-quinolones, which have activity against the blood stages, can also prevent the transmission of *Plasmodium* to the mosquito and, hence, are potentially important drug candidates to eradicate malaria.**

There were an estimated 154 million to 289 million cases and 610,000 to 971,000 deaths from malaria in 2010 (1). *Plasmodium falciparum*, the deadliest of the five malaria species that infect humans, mainly affects children under the age of 5 years in Africa (2–6). In areas where malaria is endemic, such as Africa, Southeast Asia, and South America, *P. falciparum* has developed resistance to many commercially available antimalarials, such as chloroquine (CQ), mefloquine (MFQ), and sulfadoxine-pyrimethamine (SP) (7). Currently, artemisinin derivatives, which have potent activity against blood stages and early-stage gametocytes, are the only drugs that are effective for treating drug-resistant *P. falciparum* (8, 9). However, recent evidence suggests that parasite resistance to artemisinin and its derivatives is emerging in Southeast Asia (4, 10), thus indicating the need for new drugs to combat this disease.

Development of new antimalarials has traditionally been focused on the asexual blood stages, which are responsible for the proliferation of the parasite in the human host and for the clinical symptoms of the disease (11). However, gametocytes (i.e., the sexual stages), as well as the mosquito stages (i.e., ookinetes, oocysts, and salivary gland sporozoites), are important drug targets, because they are necessary for disease transmission (12). Currently, there are a limited number of antimalarials that are effective against the sexual and the vector stages of malaria parasites. Therefore, we investigated the transmission-blocking activity of 4(1*H*)-quinolones that have activity against the blood and liver stages of parasites in the avian, rodent, and rhesus monkey malaria models (13–15). Recent studies have demonstrated that 4(1*H*)-quinolones are active against *P. falciparum* blood and liver stages *in vitro* (16–25), as well as against *P. berghei* rodent malaria liver stages *in vitro* and *in vivo* (26).

In this work, we tested three 3-alkyl- or 3-phenyl-4(1*H*)-quinolones (P4Qs; P4Q-95, P4Q-105, P4Q-146), one 7-(2-phenoxyethoxy)-4(1*H*)-quinolone (PEQ; ICI 56,780), and one 1,2,3,4-tetrahydroacridin-9(10*H*)-one (THA-93) (16, 17, 23) for their gametocytocidal, gametocidal, and sporozontocidal activity against *P. falciparum* as well as their transmission activities *in vivo* against *P. berghei*. These compounds were chosen because of their strong efficacy against blood stages *in vitro* (16, 17, 23). We found that most of the compounds were not effective in killing early- and late-stage gametocytes, although they reduced or prevented male gametocyte exflagellation and subsequent vector infection *in vitro* and *in vivo*. Additionally, when administered to infected mosquitoes, ICI 56,780 inhibited sporozoite infection of mosquito salivary glands. Our results show that members of the class of 4(1*H*)-quinolones not only have blood-stage activity but also have potent transmission-blocking activity.

## MATERIALS AND METHODS

**Chemicals.** The experimental compounds used in this study were P4Q-95 (16), P4Q-105 (16), P4Q-146 (26), ICI 56,780 (23), and THA-93 (17), whose structures are shown in Fig. 1. The compounds were synthesized and purified by the laboratory of R. Manetsch, Department of Chemistry, University of South Florida. Dihydroartemisinin (DHA; AVA Scientific

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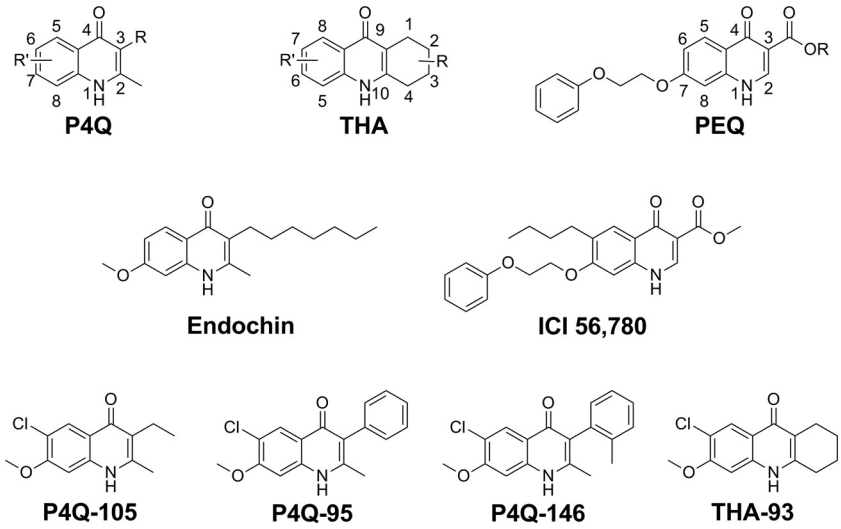


**FIG 1** Structures of the compounds tested in this study.

Chemicals, Mumbai, India) and the 8-aminoquinoline primaquine (PMQ; Sigma, St. Louis, MO) were used as controls. For the *in vitro* studies, compounds were reconstituted in dimethyl sulfoxide (DMSO; Sigma) and then diluted to appropriate concentrations in RPMI 1640 (Life Technologies, Grand Island, NY) before use. For the *in vivo* studies, compounds were reconstituted in polyethylene glycol 400 (Sigma).

**Parasites.** In these studies, we used *P. falciparum* and *P. berghei* parasites. For *in vitro* studies, we used *P. falciparum* NF54 (27), which was cultured in type A-positive red blood cells (RBCs) at 5% hematocrit in RPMI 1640 medium containing 0.5% AlbuMAX at 37°C in the presence of 90% $N_2$, 5% $O_2$, and 5% $CO_2$, as previously described (28). For *in vivo* studies, we used a transgenic *P. berghei* ANKA line (GFPcon 259cl2) obtained through the Malaria Research and Reference Reagent Resource Center (MR4; Manassas, VA), supported by the National Institute of Allergy and Infectious Diseases: *Plasmodium berghei* (ANKA) GFPcon 259cl2 (MRA-865; deposited by C. J. Janse and A. P. Waters). This line was designed to constitutively express a green fluorescent protein (GFP) fusion protein under the control of the *eef1*α promoter (29).

***In vitro P. falciparum* gametocyte production.** Asexual and mature gametocytes of NF54 parasites were cultivated as previously described (30, 31) in six-well plates. We used the CQ-sensitive strain NF54 because of its ability to form viable male and female gametocytes and to infect mosquitoes. In brief, gametocyte production was induced by use of a low parasite inoculum (0.1%) and a high hematocrit (7.5%) in 2.5 ml of culture (day 0), and gametocytes were grown for 3 days. On day 4 postinoculation (p.i.) the volume of medium was doubled. The medium was changed every day until day 15, when the gametocytes were mature (stage V). The plates were maintained in a gassing incubator in 90% $N_2$, 5% $O_2$, 5% $CO_2$ at 37°C.

**Assessment of gametocytocidal activity.** All drugs were dissolved in DMSO at a concentration of 5 mg/ml and then diluted to the desired concentrations in culture medium before use. To determine the effect of the compounds against gametocytes, cultures were treated with 0.1, 1.0, or 10 μM experimental or control compound at the early stage (I, II, III) on days 7, 8, and 9 p.i. or at the late stage (III, IV, V) on days 11, 12, and 13 p.i. Blood smears were prepared daily starting on day 7 p.i. to document the progression of asexual-stage as well as sexual-stage parasitemia, and a minimum of 1,000 cells was counted per treatment group. Finally, on day 15 p.i., gametocytes were checked for exflagellation using previously described methods (30). All experiments were conducted in duplicate (Fig. 2A).

**Assessment of gametocidal activity of tested compounds *in vitro*.** Female *Anopheles freeborni* mosquitoes (4 to 5 days old), obtained from MR4 (MRA-130; Manassas, VA), were used for *P. falciparum* infection. Red blood cells infected with mature male and female gametocytes at 37°C were fed to mosquitoes by membrane feeds by the method previously described (31, 32). The blood meal was provided to mosquitoes with artificial feeders (Hemotek membrane feeding system; Accrington, Lancashire, United Kingdom). Briefly, infected RBCs with mature gametocytes (day 15 p.i.) previously treated in early or late stages were mixed with fresh RBCs and type AB human serum. The mixture was placed on the Hemotek feeders, and 50 *A. freeborni* females were allowed to feed for 20 min. Following the blood meal, the mosquitoes were kept at 26°C and 80% humidity with sucrose *ad libitum*. Midguts ($n = 10$) were dissected on day 25 p.i. and stained with 0.5% mercurochrome for 5 min to enhance the identification of oocysts. The oocyst number for each mosquito was determined by light microscopy (Fig. 2A). This study was conducted in duplicate.

**Assessment of sporozontocidal activity of tested compounds.** To determine the sporozontocidal effect of the compounds against *P. falciparum in vitro*, blood containing untreated mature gametocytes was fed to *A. freeborni* females as described above, and on day 8 postexposure (PE) the presence of oocysts in the midguts was confirmed. Later, noninfected blood containing the test compounds (1.0 μM) was fed to the mosquitoes. On day 18 postfeeding (day 35 p.i.), 10 mosquitoes (i.e., 20 salivary glands) were dissected and the presence of sporozoites was recorded. The number of infected glands was determined by light microscopy as previously described (32) (Fig. 2B). This study was conducted in duplicate.

**Evaluation of effect of drug treatment on vertebrate host transmission to mosquitoes.** To determine if treating infected mice would prevent parasite transmission to *A. stephensi* mosquitoes, experimental mice were infected with $1 \times 10^6$ *P. berghei* GFP-ANKA parasites (MRA-865; MR4, Manassas, VA). On day 4 PE, when levels of parasitemia were ≥3.0%, Giemsa-stained blood smears were prepared and the presence of gametocytes was verified prior to treatment. Six groups consisting of two mice each ($n = 2$) were treated with ICI 56,780, THA-93, or P4Q-95 (0.1, 0.3, 1.0, 3.0, and 10.0 mg/kg of body weight) or 10 mg/kg of the control drugs, PMQ and DHA (Fig. 3). Untreated mice were included as an infection control. At 1 h posttreatment (PT), the mice were anesthetized and exposed to 100 naive *A. stephensi* mosquitoes for 20 min, as described above. Following the blood feed, unfed mosquitoes were removed. On day 10 PE, midguts ($n = 40$) from mosquitoes in each treatment group were dissected to assess oocyst prevalence and numbers using light microscopy, as previously described (31, 33, 34).

In another study, infected mice were treated with 1.0 mg/kg of ICI 56,780 (the minimal transmission-blocking concentration) and then ex-

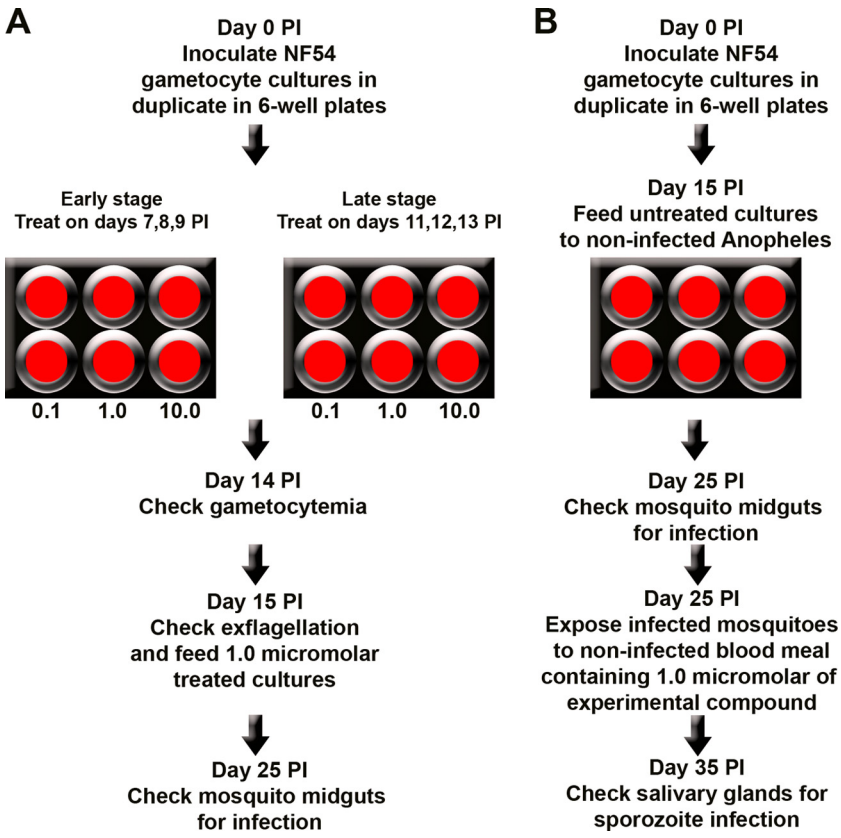


**FIG 2** Summary of the methods used in this study. (A) Assessment of gametocytocidal and gametocidal activity of experimental compounds. To test the gametocytocidal activity of the compounds, early-stage (stages I, II, and III) and late-stage (stages III, IV, and V) gametocytes were treated with the compounds in duplicate for three consecutive days at 0.1, 1.0, and 10.0 μM concentrations. The gametocytemia on day 14 p.i. was used as a measure of the effect of the experimental compounds on gametocyte development. Gametocidal activity was determined by assessing the effect of the test compounds (1.0 μM) on male gamete exflagellation on day 15 p.i. and subsequent oocyst development in the mosquito midgut. The number of oocysts per midgut on day 25 p.i. was used as a measure of gametocidal activity. (B) Assessment of sporozontocidal activity of the test compounds. On day 15 p.i., untreated gametocyte cultures were fed to noninfected *A. freeborni* females. On day 25 p.i., mosquitoes were exposed to a noninfected blood meal containing 1.0 μM the test compound, and on day 35 p.i., salivary glands were checked for infections.

posed to noninfected mosquitoes at 1, 6, 12, and 24 h PT. Untreated noninfected and infected mice were included as controls.

**Ethics statement.** All mice used in these experiments were female BALB/c mice (average weight, approximately 18 g) obtained from Harlan (Fredrick, MD). This study was conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* of the National Research Council of the National Academies (35). The protocol was approved by the University of South Florida Institutional Animal Care and Use Committee. The numbers of animals used were the minimum required for obtaining scientifically valid data. Experimental procedures were designed to minimize harm and included predefined parasitological endpoints to avoid unnecessary suffering.

**Statistical analysis.** All statistical analyses were performed using GraphPad Prism, version 5, software (GraphPad Software Inc., La Jolla, CA). A $P$ value of $<0.05$ was considered statistically significant. Data were analyzed using one-way analysis of variance with Dunnett's *post hoc* test, comparing treated groups to untreated controls.

## RESULTS

**Assessment of gametocytocidal activity.** To assess the gametocytocidal activity of the 4(*1H*)-quinolones, we tested the compounds against early- and late-stage gametocytes and determined the total number of gametocytes as well as the number of stage V gametocytes on day 14 p.i. We observed 88%, 58%, and 89% reductions in stage V gametocytemia when 0.1 μM P4Q-95, ICI 56,780, and P4Q-146, respectively, were added to early-stage gametocytes. We found 78%, 67%, 83%, and 73% reductions when 1.0 μM THA-93, P4Q-105, ICI 56,780, and P4Q-146, respectively, were added. Furthermore, we saw 83%, 89%, 79%, 80%, and 91% reductions when 10.0 μM THA-93, P4Q-95, P4Q-105, ICI 56,780, and P4Q-146, respectively, were added. However, we found that, compared to the untreated control, there was no significant reduction in total or in stage V gametocytemia (Dunnett's test). In contrast, DHA completely suppressed the formation of gametocytes at all concentrations (Fig. 4). When late-stage gametocytes were treated with these compounds, no compound produced a significant reduction in stage V gametocytemia compared to that for the untreated control. PMQ did not affect gametocytemia when added to late-stage gametocytes (Fig. 4).

**Assessment of gametocidal effect.** In order to assess the gametocidal effects of the P4Q, PEQ, and THA compounds, we tested the exflagellation of treated male gametocytes and the capacity of these gametes to form oocysts in the mosquito midgut. We found that most of the tested compounds had an effect on the exflagellation of male gametocytes when the drug was added to stages I to III. Early stages treated with THA-93 at 0.1 and 1.0 μM had 93%

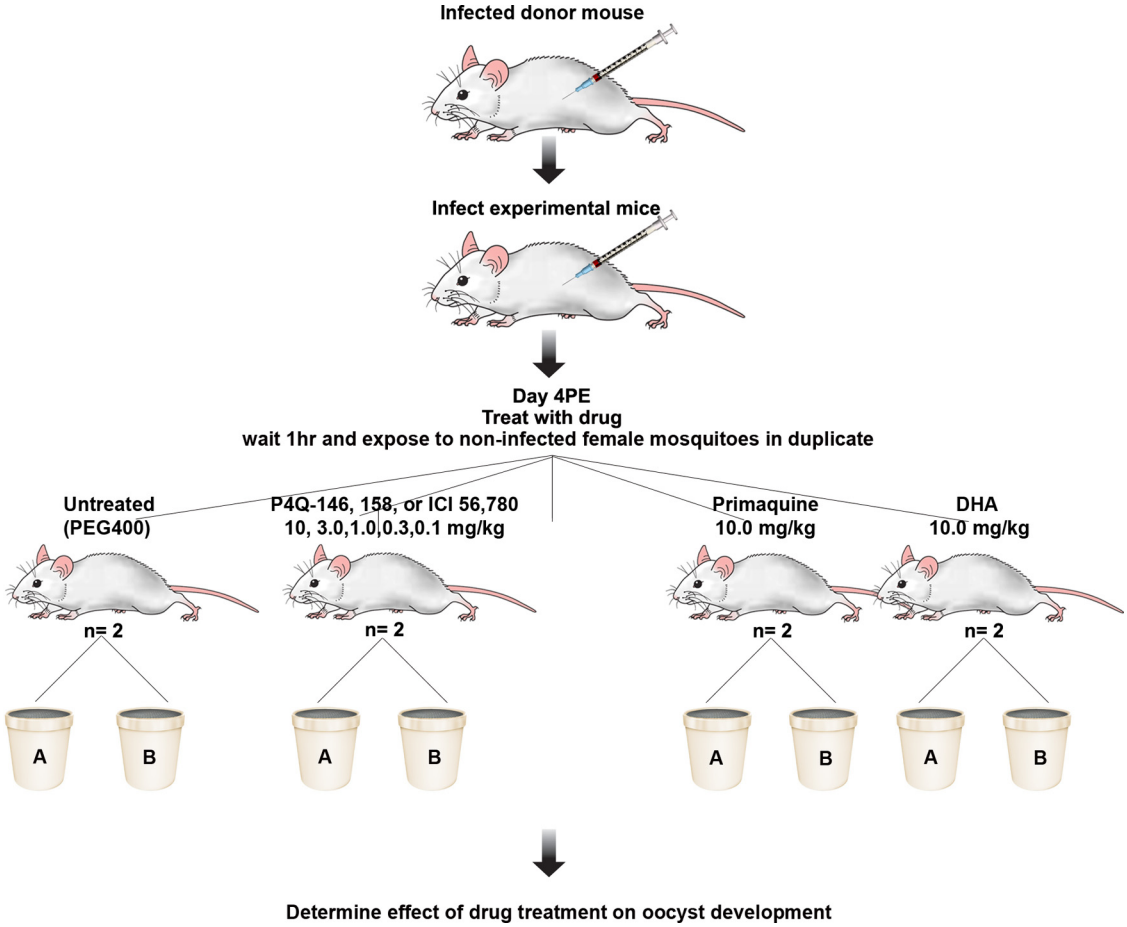


**FIG 3** Transmission-blocking technique. PEG400, polyethylene glycol 400.

and 90% reductions in exflagellation, respectively. P4Q-95 produced no reduction of exflagellation at 0.1 μM but a 96% reduction of exflagellation at 1.0 μM. Early-stage gametocytes treated with P4Q-105 had a 69% reduction of exflagellation at 0.1 μM and a 100% reduction at 1.0 μM. Treatment with ICI 56,780 and P4Q-146 eliminated exflagellation at all tested concentrations. It is important to note that at 10.0 μM treatment concentrations, all of the tested compounds completely prevented exflagellation of male gametocytes (Fig. 5).

The treatment of late-stage gametocytes also showed a reduction in exflagellation. In fact, P4Q-105, P4Q-146, and ICI 56,780 reduced exflagellation at all treatment concentrations. P4Q-105 produced decreases of 93%, 72%, and 100% at 0.1, 1.0, and 10.0 μM, respectively. ICI 56,780 produced reductions of 99%, 83%, and 98% at 0.1, 1.0, and 10.0 μM, respectively. P4Q-146 reduced exflagellation by 99%, 69%, and 100% at 0.1, 1.0, and 10.0 μM, respectively (Fig. 5). In comparison with the untreated control, these reductions were significantly different (Dunnett's multiple-comparison test, $P < 0.001$). It is important to note that PMQ did not prevent the exflagellation of male gametocytes at 0.1 μM or at 1.0 μM (Fig. 5).

To determine the transmission-blocking activity of the P4Q, PEQ, and THA compounds, the treated gametocytes were fed to noninfected mosquitoes and the numbers of infected mosquitoes and oocysts in the midguts were evaluated on day 8 postexposure. The total number of infected mosquitoes in each treatment was significantly affected by the tested compounds (Dunnett's multiple-comparison test, $P < 0.05$). In particular, treatment of early gametocyte stages with 1.0 μM THA-93, ICI 56,780, and P4Q-146 completely prevented the infection in 100% of the mosquitoes, while treatment with P4Q-95 and P4Q-105 reduced the number of infected mosquitoes by 90% (Table 1). Treatment of late-stage gametocytes with 1.0 μM ICI 56,780 and P4Q-146 prevented infection in 100% of mosquitoes, while only 80% of the mosquitoes were infected after treatment with 1.0 μM THA-93. Treatment with 1.0 μM P4Q-95 and P4Q-105 reduced the number of infected mosquitoes by 95% (Table 1).

When early- and late-stage gametocytes treated with 1.0 μM THA-93, P4Q-95, P4Q-105, P4Q-146, and ICI 56,780, were exposed to mosquitoes, a significant reduction in the oocyst number per midgut was observed in comparison to that for the untreated control (Dunnett's multiple-comparison test, $P < 0.001$). In fact, when added to early-stage gametocytes, THA-93, ICI 56,780, and P4Q-146 reduced oocyst numbers by 100%, while both P4Q-95 and P4Q-105 reduced them by 99%. When late-stage gametocytes were treated with P4Q-146 and ICI 56,780 and then fed to noninfected mosquitoes, there was no formation of oocysts in the mosquito midgut, while THA-93, P4Q-95, and P4Q-105 reduced the oocyst numbers by 96.6%, 99.8%, and 99.4%, respectively (Dun-

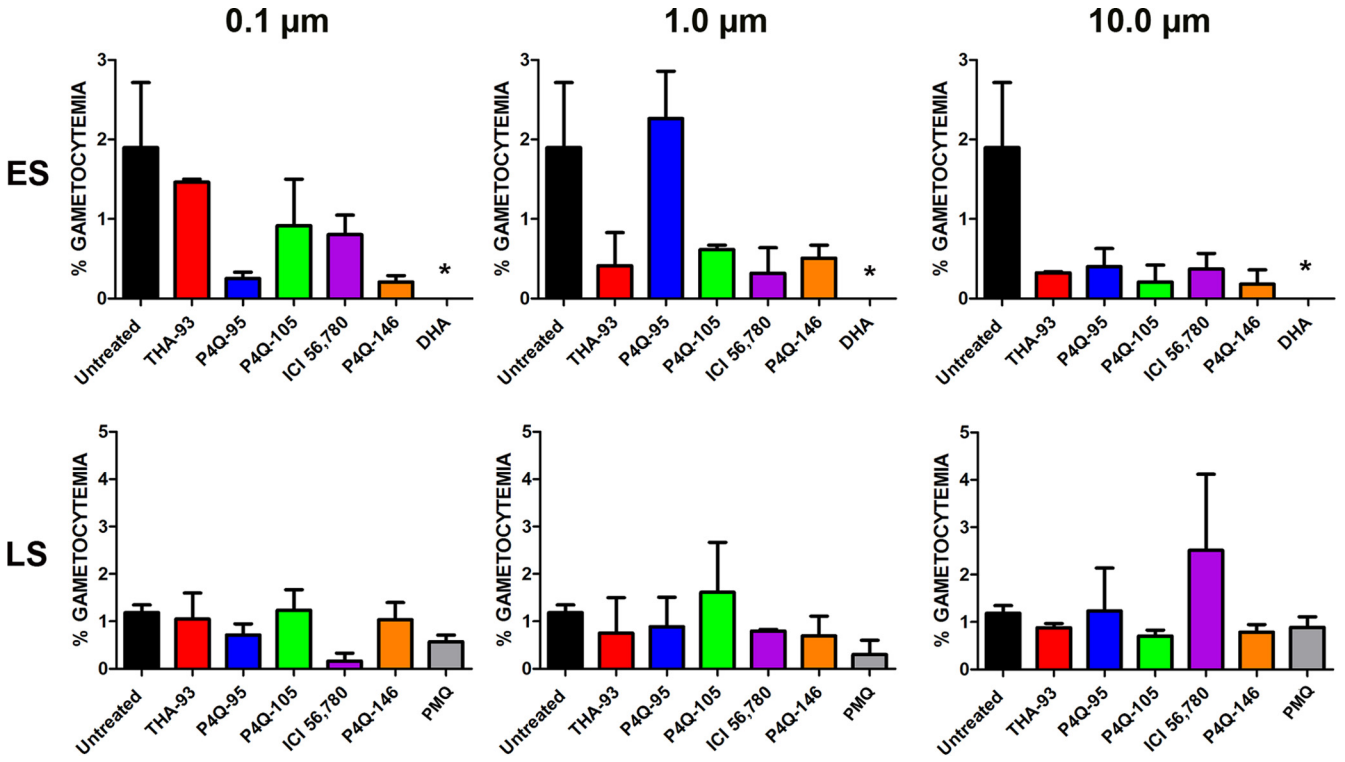


**FIG 4** Stage V gametocytemia on day 14 p.i. following treatment with 0.1, 1.0, and 10.0 μM compound. Treatment of early-stage (ES; stages I to III) and late-stage (LS; stages III to V) gametocytes with 0.1, 1.0, or 10.0 μM compound did not significantly affect gametocyte development compared to that for the untreated controls. Only the control drug DHA significantly diminished the number of stage V gametocytes compared to that for the untreated controls ($P < 0.05$). Interestingly, primaquine did not have a significant effect on the number of stage V gametocytes. *, statistically significant difference.

nett's multiple-comparison test, $P < 0.001$). As expected, PMQ did not decrease the number of oocysts (Table 2).

**Evaluation of effect of drug treatment on vertebrate host transmission to mosquitoes.** Transmission-blocking studies were conducted to determine if THA-93, P4Q-95, P4Q-146, and ICI 56,780 block *P. berghei* transmission from the vertebrate host (e.g., mouse) to the mosquito vector. In this study, a range of concentrations was tested, and the results showed that infected mice treated with 10.0, 3.0, and 1.0 mg/kg of ICI 56,780 and P4Q-146 were unable to transmit infection to mosquitoes (Fig. 6). Mice treated with 0.3 and 0.1 mg/kg of ICI 56,780 remained infective for mosquitoes, which had an average of 128 and 68 oocysts per midgut, respectively. Compared to the untreated group, the number of oocysts in treated animals was significantly lower (Dunnett's multiple-comparison test, $P < 0.01$; Fig. 6). Unlike ICI 56,780 and P4Q-146, when positive-control compounds PMQ and DHA were administered at 10 mg/kg, they did not prevent mosquito infection. However, the average numbers of 12 and 101 oocysts per midgut, respectively, were also significantly lower than those for the untreated group (Dunnett's multiple-comparison test, $P < 0.01$; Fig. 3). Conversely, THA-93 and P4Q-95 were not able to prevent the transmission of *P. berghei* to *Anopheles* mosquitoes.

Subsequently, we evaluated the effectiveness of ICI 56,780 (1 mg/kg) at different exposure times. Mosquitoes were exposed to mice at 1 h, 6 h, 12 h, and 24 h following treatment with a single dose of drug (1 mg/kg). ICI 56,780 blocked transmission up to 12 h posttreatment; however, 24 h later, the mosquitoes developed an infection (Fig. 7).

**Assessment of sporozontocidal activity.** We tested the sporozontocidal activity of the P4Q, PEQ, and THA compounds in *P. falciparum*-infected mosquitoes by feeding mosquitoes with 1 μM drug-treated blood. We found that ICI 56,780 was the only compound to significantly reduce salivary gland infections (80%) compared to the results for the untreated control (Dunnett's multiple-comparison test, $P < 0.05$). The sporozontocidal activity of ICI 56,780 was greater than the effect of pyrimethamine (Table 3).

## DISCUSSION

An antimalarial drug effective against more than one stage of the parasite life cycle is advantageous. To date, the vast majority of commercially available drugs are effective against only erythrocytic stages, and only a small number of antimalarials in use have activity against liver and transmission stages. More than 60 years ago, endochin, a 3-heptyl-substituted 4(1*H*)-quinolone, showed promising *in vivo* antimalarial activity, in addition to prophylactic activity, against liver stages of avian malaria and activity against male gametocyte exflagellation of the apicomplexan *Haemoproteus* in finches (14, 15, 36, 37). However, clinical tests with endochin were a failure. Nevertheless, more recently, it was shown that 4(1*H*)-quinolones derived from endochin are potent *in vitro* against atovaquone-resistant strains (16, 21, 22) and warranted further investigation.

Consequently, in this work we tested several compounds derived from endochin for their gametocytocidal, gametocidal, and sporozontocidal activity against *P. falciparum*. Although most of the compounds did not significantly block the gross morphological development of early- or late-stage gametocytes into mature

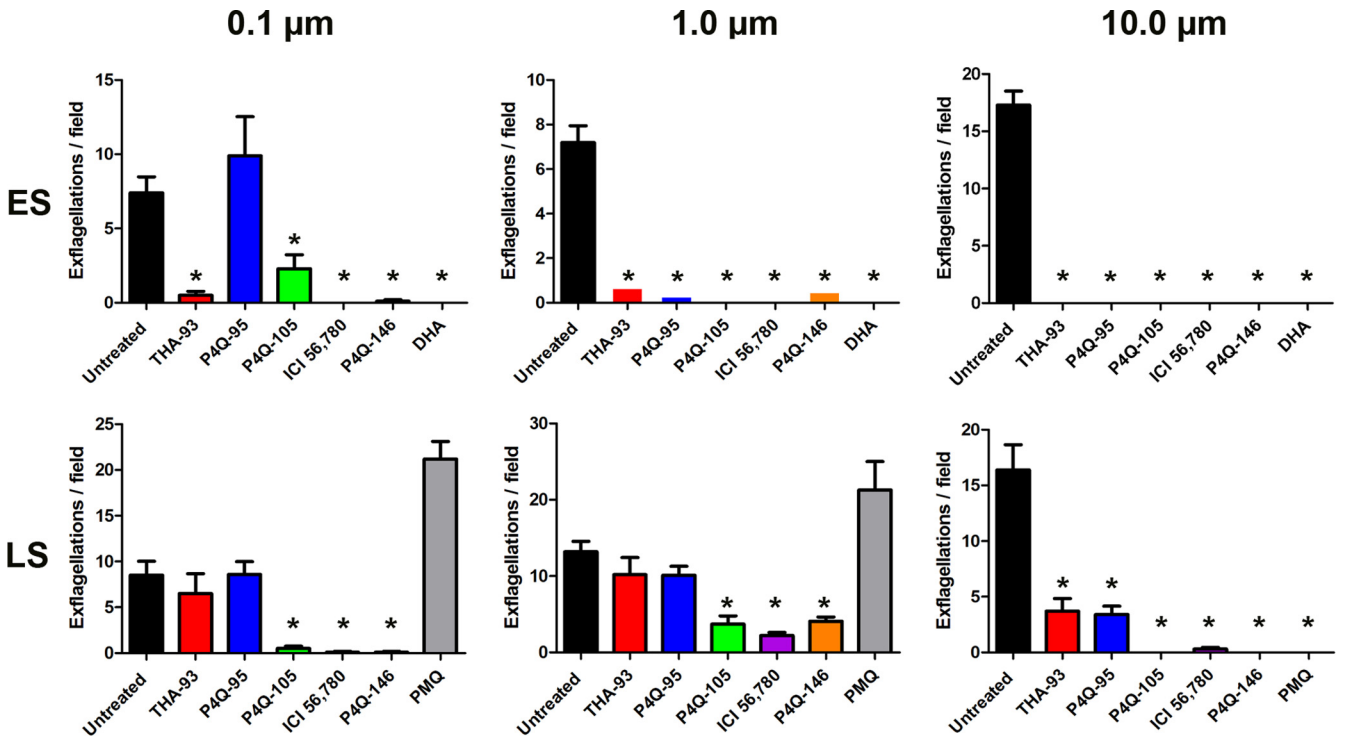


**FIG 5** Effect of test compounds on exflagellation when gametocytes were treated during the early stage (ES; stages I to III) and late stage (LS; stages III to IV). Treatment of early-stage gametocytes with 0.1 μM THA-93, P4Q-105, P4Q-146, and ICI 56,780 significantly reduced male gamete exflagellation compared to that for the untreated control (Dunnett's multiple-comparison test, $P < 0.001$). Treatment of early-stage gametocytes with 1.0 μM and 10.0 μM all test compounds significantly reduced exflagellation compared to that for the untreated control (Dunnett's multiple-comparison test, $P < 0.001$). Late-stage gametocyte treatment with 0.1 μM and 1.0 μM P4Q-105, P4Q-146, and ICI 56,780 significantly reduced male gamete exflagellation compared to that for the untreated control (Dunnett's multiple-comparison test, $P < 0.05$). Treatment of late-stage gametocytes with 10.0 μM all test compounds significantly reduced exflagellation compared to that for the untreated control (Dunnett's multiple-comparison test, $P < 0.001$). *, statistically significant difference.

stage V forms, the three 4(1*H*)-quinolones (P4Q-95, P4Q-105, and P4Q-146), THA-93, and the PEQ ICI 56,780 completely inhibited exflagellation of gametocytes and subsequent mosquito salivary gland infections. In addition, we showed that P4Q-146 and the PEQ ICI 56,780 completely inhibited *P. berghei* transmission to mosquitoes.

The measure of drug effects on exflagellation has been reported for both commercially available and experimental compounds. For example, pyronaridine, *tert*-butyl isoquine, NPC-1161B, OZ277, and cycloheximide completely inhibited exflagellation at 10 μM (38). Additionally, protease inhibitors such as the cysteine/serine protease inhibitors *N*α-*p*-tosyl-L-lysine chloromethyl ketone and tosylsulfonyl phenylalanyl chloromethyl ketone block microgamete formation at 100 μM (39, 40). More recently, it was reported that 4-quinolones also have activity against different stages of the life cycle of *P. falciparum*; however, the activity against exflagellation was only modest (50% inhibitory concentration, ~10 μM) (19). In contrast, the 4(1*H*)-quinolones tested in our studies, particularly ICI 56,780 and P4Q-146, were able to almost completely inhibit exflagellation at lower concentrations (0.1 μM) compared with the activity of previously tested compounds (38).

**TABLE 1** Average number of infected mosquitoes on day 8 PE following treatment of early- or late-stage gametocytes with 1.0 μM compound

| Compound | No. of infected mosquitoes[a] | |
|---|---|---|
| | Stages I to III | Stages III to V |
| Untreated | 9 ± 1.41 | 8 ± 0.00 |
| THA-93 | 0 ± 0.00* | 4 ± 2.83 |
| P4Q-95 | 1 ± 0.00* | 0.5 ± 0.70* |
| P4Q-105 | 1 ± 1.41* | 0.5 ± 0.70* |
| P4Q-146 | 0 ± 0.00* | 0 ± 0.00* |
| ICI 56,780 | 0 ± 0.00* | 0 ± 0.00* |
| PMQ | Not tested | 9 ± 0.00 |

[a] Data are means ± SDs ($n = 10$). *, means significantly different from those for untreated parasites (Dunnett's multiple-comparison test, $P < 0.05$).

**TABLE 2** Average number of oocysts per mosquito midgut on day 8 PE following treatment of early- or late-stage gametocytes with 1.0 μM compound

| Compound | No. of oocysts per midgut[a] | |
|---|---|---|
| | Stages I to III | Stages III to V |
| Untreated | 15.25 ± 12.74 | 17.7 ± 13.22 |
| THA-93 | 0.00 ± 0.00* | 0.60 ± 0.88* |
| P4Q-95 | 0.10 ± 0.31* | 0.05 ± 0.22* |
| P4Q-105 | 0.15 ± 0.49* | 0.10 ± 0.45* |
| P4Q-146 | 0.00 ± 0.00* | 0.00 ± 0.00* |
| ICI 56,780 | 0.00 ± 0.00* | 0.00 ± 0.00* |
| PMQ | Not tested | 16.80 ± 14.16 |

[a] Data are means ± SDs ($n = 10$). *, means significantly different from those for untreated gametocytes (Dunnett's multiple-comparison test, $P < 0.001$).

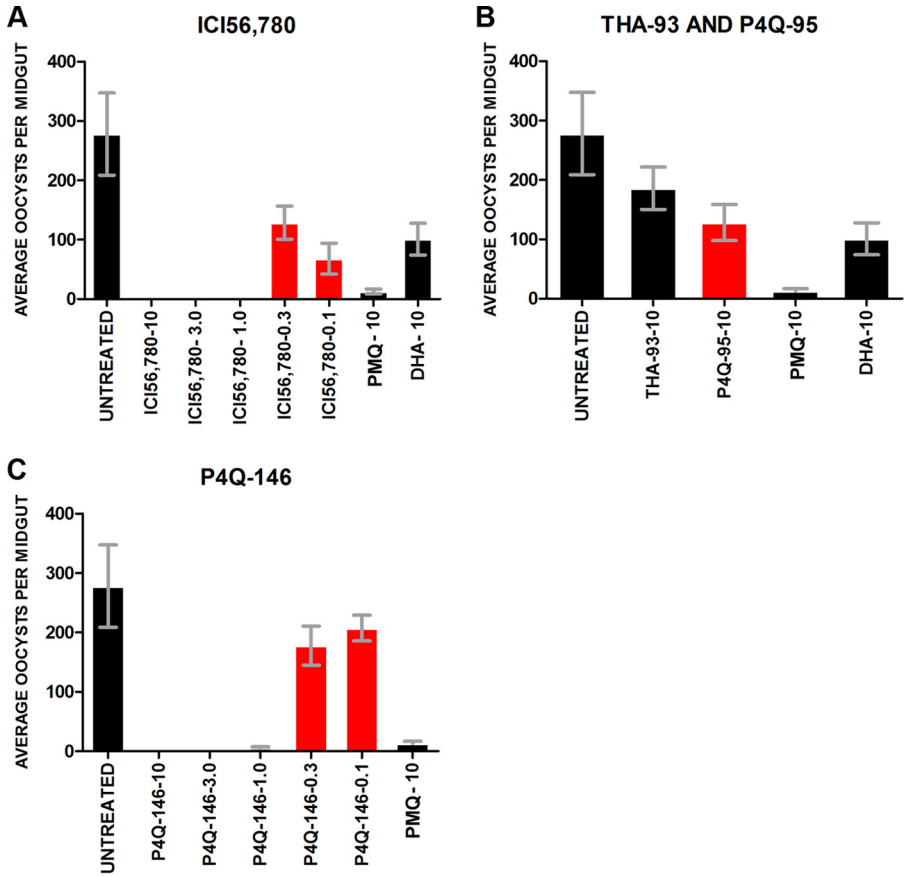


**FIG 6** P4Q-146 and ICI 56,780 block the transmission of *P. berghei* to *A. stephensi in vivo*. (A) ICI 56,780 blocks the transmission at 1, 3, and 10 mg/kg; (B) THA-93 and P4Q-95 do not prevent *P. berghei* transmission to *A. stephensi*; (C) P4Q-146 blocks transmission at 1, 3, and 10 mg/kg.

All of the P4Q, PEQ, and THA compounds reported on herein showed robust transmission-blocking activity *in vitro* by preventing the development of oocysts in the mosquito at a 1.0 μM treatment concentration. Although when late-stage gametocytes were treated with 1.0 μM the test compounds there was modest or no activity in reducing exflagellation (Fig. 5), most of the compounds did reduce oocyst production by 95% (Table 2). Several compounds have previously been reported to inhibit the development of oocysts in mosquitoes, but at concentrations much higher than what we report here. In particular, several endoperoxides as well as lumefantrine, halofantrine, and mefloquine prevent the development of oocysts at 10.0 μM (38). The transmission-blocking potential of these compounds was confirmed by *in vivo* studies, where we show that treatment of *P. berghei* with P4Q-146 and ICI 56,780 at 1.0 mg/kg inhibited the development of oocysts and that ICI 56,780 activity was retained for up to 12 h posttreatment. The

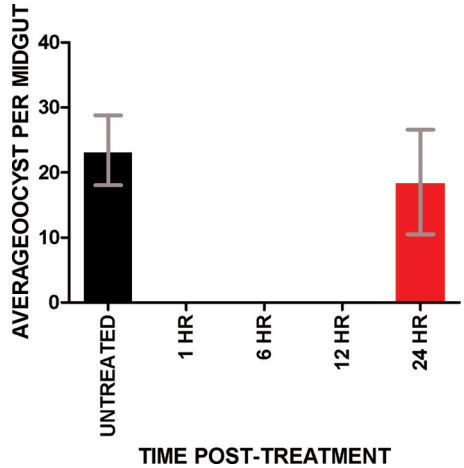


**FIG 7** ICI 56,780 blocks transmission for up to 12 h posttreatment in *P. berghei*-infected mice. Animals with ~3% parasitemia were treated with a single dose of 1 mg/kg ICI 56,780 (*per os*), and then adult female *A. stephensi* mosquitoes were allowed to feed at 1, 6, 12, or 24 h posttreatment. ICI 56,780 completely blocked transmission up to 12 h posttreatment, as assessed by a lack of oocyst development in mosquitoes 8 days after feeding on treated animals.

**TABLE 3** Average number of infected mosquito salivary glands on day 16 PE following treatment of oocyst-positive mosquitoes with 1.0 μM compound

| Compound | No. of infected salivary gland[a] |
|---|---|
| Untreated | 5.0 ± 1.41 |
| THA-93 | 3.0 ± 2.83 |
| PEQ-95 | 3.5 ± 2.12 |
| PEQ-105 | 5.5 ± 0.71 |
| PEQ-146 | 4.0 ± 1.41 |
| ICI 56,780 | 1.0 ± 0.00* |
| Pyrimethamine | 3.5 ± 2.12 |

[a] Data are means ± SDs ($n = 20$ pairs). *, mean significantly different from that for the untreated mosquitoes (Dunnett's multiple-comparison test, $P < 0.05$).

ability of the P4Q and PEQ compounds to prevent mosquito infection and reduce sporozoite viability may give the 4(1*H*)-quinolones an advantage over first-line therapies, such as artemisinin-based combination therapies, which have a minimal impact on mosquito infectivity in zones of intense malaria transmission (41).

The sporozontocidal activity of the P4Q, PEQ, and THA 4(1*H*)-quinolones was also tested, indicating that the PEQ ICI 56,780 significantly inhibited the development of sporozoite infections of mosquito salivary glands at 1.0 μM. Sporozontocidal activity has been previously reported in a small number of compounds. In particular, it was found that pyrimethamine, tafenoquine, the THA floxacrine, and other experimental compounds have an effect on sporogonic development in *P. berghei* and *P. falciparum* (42). Interestingly, in our study, pyrimethamine decreased the number of infected salivary glands by only 30%, whereas ICI 56,780 decreased the number by 80%. The ability of ICI 56,780 to limit the number of sporozoites that reach the salivary glands demonstrates the potential utility of PEQ derivatives as transmission-blocking drugs.

The mechanism of action by P4Qs, PEQs, and THAs in preventing the transmission of parasites to the mosquito and affecting the development of sporozoites is unknown; however, the structural similarities of these compounds to atovaquone and the GlaxoSmithKline pyridones, as well as oxygen consumption tests, indicate that the parasite cytochrome $bc_1$ may be the target for these compounds (16, 21, 22). Early studies noted that the mitochondria of *P. falciparum* are morphologically different between the asexual erythrocytic stages and the sexual blood stages (gametocytes) (43). The asexual stages have tubule-like cristate mitochondria, whereas the gametocyte mitochondria have higher numbers of cristae and contain electron-dense tubular cristae (43). These differences suggest that gametocytes have more metabolically active mitochondria that may be required for survival during transmission. Biochemical evidence supports the hypothesis that mitochondrial function is more active in gametocytes and mosquito stages of the malaria parasite (44–46). In *P. berghei*, complex II is essential for oocyst formation, suggesting that the parasite switches energy metabolism from glycolysis to oxidative phosphorylation in the mosquito (47). Further studies with NDH2 knockouts in *P. berghei* demonstrated arrested development of oocyst maturation, which is consistent with the hypothesis that parasite stages in mosquitoes are dependent upon an active mitochondrial electron transport chain (48). Our observations of the inhibitory effect of the 4(1*H*)-quinolones on transmission stages support the hypothesis that mitochondrial function is crucial for the transmission stages of *Plasmodium*, validating that mitochondria are an important target for transmission-blocking drugs.

In previous studies, it was reported that THA-93, P4Q-95, P4Q-105, P4Q-146, and ICI 56,780 have potent erythrocytic stage activity *in vitro* (16, 17, 23). Moreover, we recently described that P4Q-146 and ICI 56,780 have activity against liver stages *in vivo* and *in vitro* (26). Herein, we show not only that these compounds are potent against blood stages and liver stages but also that they have transmission-blocking activity *in vitro* and *in vivo*. Together, these results indicate that these compounds have multistage activity and show that P4Q, PEQ, and THA 4(1*H*)-quinolones are important drug candidates with the potential to prevent transmission of malaria and have potential for supporting the malaria eradication campaign.

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A.N.L., F.E.S., and D.E.K. conceived and designed the experiments. A.N.L., F.E.S., and K.U. performed the experiments. R.M.C., J.R.M., and R.M. synthesized the compounds. A.N.L., F.E.S., and D.E.K. analyzed the data. A.N.L., F.E.S., and D.E.K. wrote the paper.

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