Supporting Information

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SI Materials and Methods

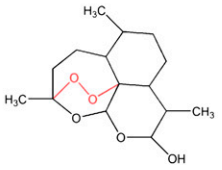
To generate our summary of the gametocyte inhibition data (Fig. P1*A*), we used the luciferase values from those drug assays (Figs. 3 and 4) at 5× the IC_{50} (Table 1). These values indicate the portion of inhibition attributable to drug action. On examination of the data, we observed that the luciferase counts typically fell by a constant proportion every treatment day, with that proportion being unique to each drug (Fig. S1) at each stage. In our fitting, $y(t)$ is the luciferase value for a given drug before ($t = 0$) and after ($t > 0$) treatment. We thus curve fit the equation $y(t) = y(0) \cdot \alpha^t$ for days $t \in (0.\ t_{SSE})$. We call α the remaining fraction and t_{SSE} the stage-specific end time (defined below). The curve fitting was done by maximizing the R^2 goodness of fit measure.

The luciferase data curve fitting of $y(t)$ was applied for the days in which gametocytes would be expected to remain within that developmental stage. For example, when curve fitting the stages I and II data, we fit our equation to days 1–4 posttreatment, because gametocytes are in stages I and II for ∼4 d. For stage III data, we fit to days 1–3 posttreatment, because gametocytes are in stage III for ∼3 d; for stage IV, we fit to days 1–3 posttreatment, and for stage V, we fit to days 1–4 posttreatment.

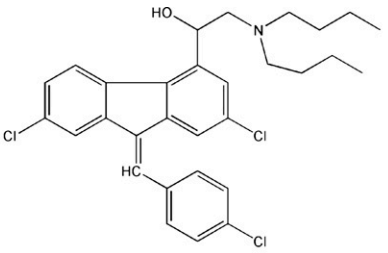
After the remaining fraction constants were obtained for each stage, we applied them to generate a loss curve over all of the gametocyte developmental stages. This curve stipulated an upper bound of $\alpha = 1$ (i.e., we assumed that gametocyte populations could not increase during drug treatment). Starting with a hypothetical population of gametocytes, we multiplied the appropriate remaining fraction to the population as it aged to simulate the effects of constant drug exposure. Curves generated for days 0–12 are displayed in Fig. P1*A*.

After analyzing these curve fits, we noticed that the action of certain drugs on later gametocyte stages did not follow a smooth pattern. Rather than exhibiting a monotonic decline after drug exposure or no robust trend at all, certain drugs showed a small rise in luciferase counts before these values consistently fell. For these drugs, including atovaquone, tafenoquine, and lumefantrine, it seemed that the drug action against later stage gametocytes was delayed by a few days. Thus, for these drugs, we repeated the curve fitting exercise by delaying the curve fitting until the initial peak in luciferase counts had fallen and a downward trend was observed.

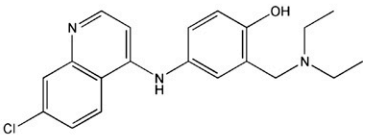
The stage-specific remainder fractions for drug assays at 5× the IC_{50} for all drugs and atovaquone, tafenoquine, and lumefantrine adjusted for delayed action are shown in Table S5.



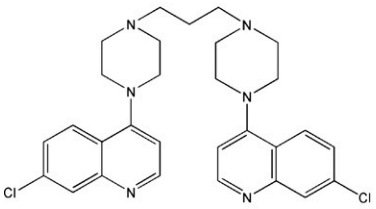
dihydroartemisinin (DHA)



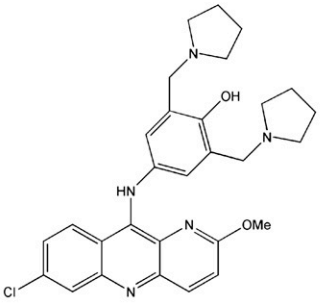
lumefantrine (LMF)



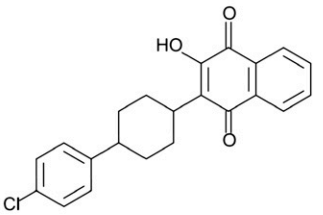
amodiaquine (ADQ)



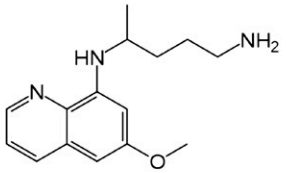
piperaquine (PPQ)



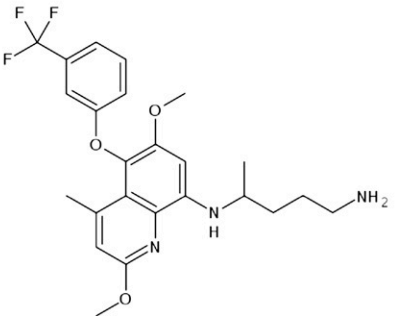
pyronaridine (PND)



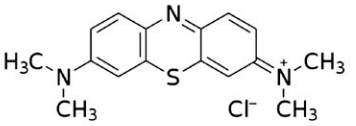
atovaquone (ATQ)



primaquine (PMQ)



tafenoquine (TFQ)



methylene blue (MB)

Fig. S1. Structures of antimalarials tested for gametocytocidal activity.

Table S1. Effect of antimalarials on transmission of drug-treated *Plasmodium falciparum* gametocytes to *Anopheles* mosquitoes: Dihydroartemisinin and lumefantrine (18 d postinduction)

	Experiment 1						Experiment 2					
Treatment	CTRL	DMSO	DHA 1× (24 nM)	DHA 5× (120 nM)	LMF 1× (66 nM)	LMF 5× (330 nM)	CTRL	DMSO	DHA 1× (24 nM)	DHA 5× (120 nM)	LMF 1× (66 nM)	LMF 5× (330 nM)
Mosquitoes	15	15	15	15	17	14	19	16	15	18	19	15
Block in transmission (%)*	0	0	7	13	0	14	0	0	0	0	0	0
Oocyst mean	48	34	26	8	21	4	47	48	36	14	11	8
Oocyst range	5–73	5–70	0–48	0–22	1–17	0–9	2–143	12–97	7–71	2–47	3–33	1–35
Reduction in oocysts (%)†	N/A	N/A	24	76	38	88	N/A	N/A	25	71	77	83
P value‡	N/A	N/A	0.28	<0.0001	0.06	<0.0001	N/A	N/A	0.15	<0.0001	<0.0001	<0.0001

CTRL, untreated control; DHA, dihydroartemisinin; LMF, lumefantrine; N/A, not applicable.
*Percent block in transmission is defined as the percent of mosquitoes that were uninfected after drug treatment of gametocytes. All mosquitoes fed untreated or mock-treated gametocytes were positive (corresponding to an infection prevalence of 100%).
†Compared with DMSO values.
‡Statistical significance was determined using a Student *t* test.

Table S2. Effect of antimalarials on transmission of drug-treated *Plasmodium falciparum* gametocytes to *Anopheles* mosquitoes: Monodesethyl-amodiaquine and piperaquine (17 d postinduction)

	Experiment 1						Experiment 2					
Treatment	CTRL	DMSO	mdAQ 0.5× (30 nM)	mdAQ 2.5× (150 nM)	PPQ 1× (35 nM)	PPQ 5× (175 nM)	CTRL	DMSO	mdAQ 0.5× (30 nM)	mdAQ 2.5× (150 nM)	PPQ 1× (35 nM)	PPQ 5× (175 nM)
Mosquitoes	24	24	24	24	25	23	22	20	22	25	20	25
Block in transmission (%)*	0	0	0	0	0	0	0	0	0	0	0	0
Oocyst mean	67	77	54	100	87	96	44	31	49	38	90	89
Oocyst range	18–134	6–203	9–129	4–212	8–160	3–207	3–105	1–114	12–100	2–90	41–155	3–197
Reduction in oocysts (%)†	N/A	N/A	30	−30	−13	−25	N/A	N/A	−58	−23	−190	−187
P value‡	N/A	N/A	0.06	0.13	0.40	0.24	N/A	N/A	0.02	0.43	<0.0001	<0.0001

CTRL, untreated control; mdAQ, monodesethyl-amodiaquine; N/A, not applicable; PPQ, piperaquine.
*Percent block in transmission is defined as the percent of mosquitoes that were uninfected after drug treatment of gametocytes. All mosquitoes fed untreated or mock-treated gametocytes were positive (corresponding to an infection prevalence of 100%).
†Compared with DMSO values.
‡Statistical significance was determined using a Student *t* test.

Table S3. Effect of antimalarials on transmission of drug-treated *Plasmodium falciparum* gametocytes to *Anopheles* mosquitoes: Pyronaridine and methylene blue (18 d postinduction)

	Experiment 1						Experiment 2					
Treatment	CTRL	DMSO	PND 1× (17 nM)	PND 5× (85 nM)	MB 0.25× (7.5 nM)	MB 1.25× (38 nM)	CTRL	DMSO	PND 1× (17 nM)	PND 5× (85 nM)	MB 0.25× (7.5 nM)	MB 1.25× (38 nM)
Mosquitoes	24	23	20	19	24	18	14	21	17	15	22	23
Block in transmission (%)*	8	0	5	21	12	100	0	0	6	27	5	78
Oocyst mean	16	12	13	4	7	0	13	17	9	2	13	0
Oocyst range	0–53	2–28	0–37	0–8	0–34	0	3–27	2–48	0–20	0–6	0–44	0–1
Reduction in oocysts (%)†	N/A	N/A	−8	67	56	100	N/A	N/A	47	88	0	98
P value‡	N/A	N/A	0.73	0.0001	0.03	—	N/A	N/A	0.02	<0.0001	0.29	<0.0001

CTRL, untreated control; MB, methylene blue; PND, pyronaridine.
*Percent block in transmission is defined as the percent of mosquitoes that were uninfected after drug treatment of gametocytes.
†Compared with DMSO values, except for MB (compared with untreated control).
‡Statistical significance was determined using a Student *t* test.

Table S4. List of oligonucleotides used in this study

Name	Nucleotide sequence	Description
p836	ACGAATTCTTAGCTAATTCGCTTGTAAGA	*PcDT* 5′UTR forward
p838	ACGGTACCGGATATGGCAGCTTAATGTT	*PbDT* 3′UTR reverse
p984	GAATCGATATTGTTACAACACC	Luciferase sequence forward
p1513	gccgGACGTCGAACAAATACATAAGAGCGCCA	*cg6* 5′ forward with AatII site
p1514	CCGCGATGATCCTGACGACGGAGACCGCCGTCGTCGACAAGCCGCATGTTCATGCTCCTCAAC	*cg6* 5′ reverse with attB site
p1515	CGGCTTGTCGACGACGGCGGTCTCCGTCGTCAGGATCATCGCGGGATAATGATAAATGTAT	*cg6* 3′ forward with attB site
p1516	gccgGACGTCCTTTAATTTTATTTTGGTCATGCAATTCTTGCAAC	*cg6* 3′ reverse with AatII site
p2145	GGGCCCGTAGCTATCCAAAAATAAATATCC	*pfs16* forward with ApaI site
p2146	CCTAGGGTTGAAGAAAGTATAAATAGAAAAATGGC	*pfs16* reverse with AvrII site
p2147	GGGCCCCATTTCTATATATTTATGGAGATAAATGTAAGGTAAATATAC	*pfs48/45* forward with ApaI site
p2148	CCTAGGGTATAAAAGAAAAAATTGTAAAAATTTTAAAAAATTTTAAGTGAATATG	*pfs48/45* reverse with AvrII site
p2151	GGGCCCCTCTATATACTATGGAATATGTGC	*mal8p1.16* forward with ApaI site
p2152	CCTAGGTGCGTGGGATTAATATTTTAATG	*mal8p1.16* reverse with AvrII site
p1636	CCTAActcgagAATGGTTGGTTCGCTAAACTGC	h*dhfr* coding sequence forward
p1655	CATTTGAATTATTGCTCAACGCT	*cg6* 5′UTR forward
p1656	CAAACCACAAGCACATAATGGT	*cg6* 3′UTR reverse
p1969	GAAAATATTATTACAAAGGGTGAGG	*cg6* coding sequence forward
p1970	CTCTTCTACTCTTTCGAATTC	*cg6* coding sequence reverse

cg6, glutaredoxin-like gene; h*dhfr*, human *dhfr*; *PbDT*, *Plasmodium berghei* dihydrofolate reductase-thymidylate synthase *(dhfr-ts)*; *PcDT*, *P. chabaudi dhfr-ts*; UTR, untranslated region. *PbDT*, PBANKA_071930 (PlasmoDB); *PcDT*, PCHAS_072830 (PlasmoDB); *cg6*, PF07_0036 (PlasmoDB); h*dhfr*, NG_023304.1 (GenBank); firefly luciferase, M15077.1 (GenBank); *pfs16*, PFD0310w (PlasmoDB); *pfs48*/45, PF13_0247 (PlasmoDB); *mal8p1.16*, MAL8P1.16 (PlasmoDB).

Table S5. Stage-specific remaining fraction constants for *Plasmodium falciparum* gametocyte drug assays

Drug	Stages I and II	Stage III	Stage IV	Stage V
Atovaquone	0.874	1	1	1
Atovaquone	0.874	0.792	0.685	0.704
Tafenoquine	0.823	1	1	1
Tafenoquine	0.823	0.457	0.819	0.713
Lumefantrine	0.776	1	1	1
Lumefantrine	0.776	0.791	0.838	0.889
Piperaquine	0.697	0.895	1	1
Primaquine	0.767	0.821	0.884	0.841
Pyronaridine	0.620	0.858	1	1
md-amodiaquine	0.526	0.775	1	1
Dihydroartemisinin	0.547	0.826	0.812	0.831
Methylene blue	0.333	0.724	0.695	0.605

Values show the stage-specific remainder fractions for drug assays at 5× the IC_{50}. These data were used to generate the loss curves in Fig. P1*A*. Smaller values indicate greater gametocytocidal effect. For atovaquone, tafenoquine, and lumefantrine, the second row provides the remainder fractions adjusted for delayed action (*SI Materials and Methods*). md-amodiaquine, monodesethyl-amodiaquine.