Supporting Information

Novel endoperoxide-based transmission-blocking antimalarials with liver- and blood-schizontocidal activities

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Synthesis of hybrid compounds 5, 8, 10, 12, 16 and 18

General experimental details

Solvents were of analytical reagent grade from Merck. Tetrahydrofurane (THF) and diethyl ether were dried from sodium benzophenone. Dichloromethane (DCM), 1,2-dichloroethane (DCE), methanol and ethanol were from $CaCl_2$. All chemicals were reagent-grade from Sigma-Aldrich, Merck or Alfa Aesar. Melting points were determined using a Kofler camera Bock Monoscope M or a Gallemkamp melting point apparatus. Analytical thin layer chromatography (TLC) was performed on pre-coated silica gel (0.25 mm layer of silica gel F254) aluminium sheets. UV light (254 nm) and *p*-anisaldehyde was used for detection and flash column chromatography was performed using Merck 938S Kieselgel 60 Silica gel. The IR spectra were recorded on a Nicolet Impact 400 FTIR spectrophotometer, and only the most significant absorption bands are reported. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker 400 Ultra-Shield spectrometer in $CDCl_3$ solutions unless otherwise stated; chemical shifts, δ, are expressed in ppm in reference to Me_4Si (TMS); coupling constant, *J*, are reported in hertz. High resolution mass spectra (HRMS) were performed on a Bruker MicrOTOF equipped with ESI ion source (University of Santiago de Compostela). Low-resolution mass spectra were recorded using a Micromass Quattro micro API mass spectrometer. Elemental analyses were performed using a CE Instruments EA 1110 automatic analyzer (University of Santiago de Compostela).

Experimental details for compound 5

To a solution of **4** (0.31 mmol, 1.0 eq) in DCM (7 mL), stirred at 0 °C, were added TEA (0.47 mmol, 1.5 eq) and TBTU (0.31 mmol, 1.0 eq). After 1 h a solution of primaquine diphosphate (0.31 mmol, 1.0 eq), and triethylamine (0.33 mmol, 1.0 eq) in DCM (2 mL), was added. The reaction mixture was allowed to warm up to room temperature and monitored by TLC. After completion, it was diluted with ethyl acetate (50mL) and then with saturated $NaHCO_3$ (20 mL). The layers were separated, and the aqueous layer was extracted with AcOEt (2x 50 mL). All the combined organic layers were treated with saturated $NaHCO_3$ (60 mL) and brine solution (60 mL), and dried over anhydrous Na_2SO_4. The solvent was removed, and the crude product purified by column chromatography on silica gel, to give **5** as a yellow solid (44% yield); mp: 78-82°C; ^{1}H NMR [400 MHz, $CDCl_3$] δ 8.53 (1H, dd, *J* = 4.2, 1.6 Hz), 7.93 (1H, dd, *J* = 6.8, 1.6 Hz), 7.31 (1H, dd, *J* = 6.8, 4.2 Hz), 6.34 (1H, d, *J* = 2.4 Hz), 6.28 (1H, d, *J* = 2.4 Hz), 5.98 (1H, d, *J* = 8.4 Hz), 5.58-5.50 (1H, m), 3.89 (3H, s), 3.69-3.56 (1H, m), 3.34-3.20 (2H, m), 3.18-3.02 (2H, m), 2.10–1.46 (24H, m), 1.29 (3H, d, *J* = 6.4 Hz) ppm; ^{13}C NMR [101 MHz, $CDCl_3$] *δ* 174.3, 159.3, 144.8, 144.3, 135.3, 134.8, 129.9, 121.9, 110.5, 107.1, 96.9, 91.7, 55.2, 47.7, 44.1, 39.3, 36.9, 33.8, 33.1, 26.9, 26.1, 20.6 ppm; IR (υ_{max}, KBr): 3383, 3300, 2933, 1641, 1060,

997 cm^{-1}; HRMS [ESI] m/z = 565.3229 (calculated for $C_{32}H_{43}N_3O_6$+H: 565.3152); Elem. Anal. C, 67.57; H, 7.52; N, 7.07 (calculated for $C_{32}H_{43}N_3O_6$: C, 67.94; H, 7.66; N, 7.43).

Experimental details for intermediates 6 and 7

Compound 6. A suspension of *O,N*-dimethylhydroxylamine hydrochloride (0.93 mmol, 1.1 eq) in DCM (1mL) and triethylamine (0.93 mmol, 1.1 eq) was added to a solution of the acid **4** (0.84 mmol, 1.0 eq) in DCM (3 ml), TBTU (0.84 mmol, 1.0 eq) and triethylamine (0.84 mmol, 1.0 eq). The reaction mixture was monitored by TLC. After stirring for 7h, the reaction mixture was diluted with DCM (50 mL), the organic phase washed with saturated $NaHCO_3$ (20 mL), treated with brine solution (20 mL) and dried over anhydrous Na_2SO_4. Purification by column chromatography on silica gel gave the Weinreb amide **6** as a white solid (92% yield); mp: 115-118 °C; ^{1}H NMR [400 MHz, $CDCl_3$] δ 3.70 (3H, s), 3.18 (3H, s), 2.80-2.69 (1H, m), 2.04-1.50 (22H, m) ppm; IR (υ_{max}, KBr): 2935, 1660, 1060, 997 cm^{-1}.

Compound 7. To a solution of compound **6** (0.71 mmol) in dry THF (8 mL) at 0ºC was added lithium aluminum hydride (0.89 mmol, 1.2 eq). The mixture was stirred for 1 h at 0ºC under nitrogen atmosphere, after which it was quenched with an aqueous solution of $KHSO_4$ (1.4 mmol in 2 mL of water), diluted with water (40 mL), and extracted with ethyl ether (3x 100 mL). The combined organic extracts were washed with saturated $NaHCO_3$ (40 mL), and brine (40 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the aldehyde **7** as colorless oil in quantitative yield. ^{1}H NMR [400 MHz, $CDCl_3$] δ 9.65 (1H, s), 3.17 (1H, brs), 2.80 (1H, brs), 2.39-2.29 (1H, m), 2.04-1.50 (20H, m) ppm; IR (υ_{max}, KBr): 2918, 1714, 1062, 997 cm^{-1}.

Experimental details for compounds 8 and 12

General procedure

To a solution of compounds **7** or **11** (1.7 mmol) in dry DCM (15 mL) and under nitrogen atmosphere, was added primaquine **2** free base (1.9 mmol, 1.1 eq). The mixture was allowed to stir at room temperature for 30 minutes. Then, glacial acetic acid (2.6 mmol, 1.5 eq) and $NaBH(AcO)_3$ (2.6 mmol, 1.5 eq) were added and the stirring continued at room temperature for 16 h. For the synthesis of compound **8**, an additional amount of glacial acetic acid (1.7 mmol, 1 eq) and $NaBH(AcO)_3$ (1.7 mmol, 1 eq) was added to allow the reaction to go to completion. The mixture was diluted and treated with saturated aqueous $NaHCO_3$ until pH 10, and the aqueous phase extracted with DCM (2 x 80 mL) and ethyl acetate (80 mL). The combined organic extracts were washed with brine (30 mL), dried

over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography on silica gel afforded **8** and **12** as pure compounds.

Compound 8. Yellow syrup (65% yield); ^{1}H NMR [400 MHz, $CDCl_3$] δ 8.52 (1H, dd, *J* = 4.2, 1.6 Hz), 7.91 (1H, dd, *J* = 8.2, 1.6 Hz), 7.30 (1H, dd, *J* = 8.2, 4.2 Hz), 6.33 (1H, d, *J* = 2.4 Hz), 6.28 (1H, d, *J* = 2.4 Hz), 6.03 (1H, d, *J* = 7.6 Hz), 3.88 (3H, s), 3.68-3.56 (1H, m), 3.10 (2H, sl), 2.63 (2H, t, *J* = 6.8 Hz), 2.47 (2H, d, *J* = 6.0 Hz), 2.03-1.41 (26H, m), 1.30 (3H, d, *J* = 6.4 Hz) ppm; ^{13}C NMR [101 MHz, $CDCl_3$] *δ* 159.6, 145.2, 144.5, 135.6, 134.9, 130.1, 122.0, 110.5, 108.3, 96.9, 91.8, 55.4, 55.3, 50.1, 48.2, 37.2, 37.1, 34.5, 33.3, 27.3, 26.6, 20.8 ppm; IR (υ_{max}, KBr): 3294, 3203, 1660, 1093, 971 cm^{-1}. HRMS [ESI] m/z = 551.3425 (calculated for $C_{32}H_{45}N_3O_5$+H: 551.3359); Elem. Anal. C, 69.52; H, 8.51; N, 7.31 (calculated for $C_{32}H_{45}N_3O_5$: C, 69.66; H, 8.22; N, 7.62).

Compound 12. Brown powder (54% yield); mp: 60-62°C; ^{1}H NMR [400 MHz, $CDCl_3$] δ 8.53 (1H, dd, *J* = 4.2, 1.6 Hz), 7.92 (1H, dd, *J* = 8.3, 1.6 Hz), 7.31 (1H, dd, *J* = 8.2, 4.2 Hz), 6.34 (1H, d, *J* = 2.5 Hz), 6.29 (1H, d, *J* = 2.4 Hz), 6.01 (1H, d, *J* = 8.3 Hz), 3.89 (3H, s), 3.66-3.57 (1H, m), 3.74-3.62 (3H, m), 2.12-1.26 (27H, m) 1.30 (3H, d, *J* = 6.4 Hz) ppm; ^{13}C NMR [101 MHz, $CDCl_3$] *δ* 159.8, 145.3, 144.8, 135.8, 135.2, 130.3, 122.3, 110.8, 107.7, 97.3, 92.1, 55.7, 53.9, 48.4, 47.0, 37.3, 34.6, 33.5, 27.4, 20.9 ppm. HRMS [ESI] m/z = 538.3262 (calculated for $C_{31}H_{44}N_3O_5$+H: 538.3281); Elem. Anal. C, 69.24; H, 8.05; N, 7.79 (calculated for $C_{31}H_{45}N_3O_5$: C, 69.25; H, 8.06; N, 7.81).

Experimental details for compound 10

To a solution of compound **9** (0.5 mmol, 1.0 eq.) in dry DCM (30 mL) were added triethylamine (0.75 mmol, 1.5 eq) and ethylchloroformate (0.5 mmol, 1.0 eq). The reaction mixture was stirred for 60 minutes at 0^{o}C. Primaquine diphosphate salt (0.5 mmol, 1.0 eq) was added and after 30 minutes of stirring, the reaction mixture was warmed to room temperature and stirred for further 90 minutes. The reaction mixture was then diluted with water (5 mL) and extracted with DCM (3x 20mL). The organic extracts were washed with brine and dried over anhydrous Na_2SO_4. Purification by flash column chromatography on silica gel gave **10** as a brown powder (78% yield); mp: 64-66°C; ^{1}H NMR [400 MHz, $CDCl_3$] δ 8.24 (1H, dd, *J* = 4.2, 1.5 Hz), 7.81 (1H, dd, *J* = 8.3, 1.5 Hz), 7.19 (1H, dd, *J* = 8.3, 4.3 Hz), 6.17 (1H, d, *J* = 2.5 Hz), 5.87 (1H, d, *J* = 8.4 Hz), 5.54 (1H, t, *J* = 5.5 Hz), 3.78 (3H, s), 3.58-3.45 (1H, m), 3.23-3.08 (2H, m), 1.18 (3H, d), 1.19-1.00 (30H, m) ppm; ^{13}C NMR [101 MHz, $CDCl_3$] *δ* 172.2, 159.8, 145.2, 144.8, 153.7, 135.3, 130.3, 122.2, 110.8, 108.1, 97.3, 92.2, 55.6, 48.2, 43.8, 39.9, 37.4, 34.4, 33.9, 33.6, 32.0, 27.5, 26.6, 23.1, 21.1 ppm. HRMS [ESI] m/z = 580.3404 (calculated for $C_{33}H_{46}N_3O_6$+H: 580.3387); Elem. Anal. C, 68.36; H, 7.81; N, 7.24 (calculated for $C_{33}H_{46}N_3O_6$: C, 68.37; H, 8.82; N, 7.25).

Experimental details for intermediates 14 and 15

Compound 14. Prepared according to the procedure for intermediate **6**. Yellow oil (56% yield); ^{1}H NMR [400 MHz, $CDCl_3$] δ 5.38 (1H, s), 4.91 - 4.83 (1H, m), 3.74 (3H, s), 3.23 (3H, s), 2.97-2.88 (1H, m), 2.86-2.79 (1H, m), 2.53-2.44 (1H, m), 2.41-2.30 (1H, m), 2.08-2.0 (1H, m), 1.97-1.59 (3H, m), 1.44 (3H, s), 1.53-1.24 (5H, m), 0.99 (3H, d, J = 6.0 Hz), 0.97-0.93 (1H, m), 0.91 (3H, d, J = 7.2 Hz) ppm; IR (υ_{max}, KBr): 2940, 2874, 1709, 1013, 941, 878 cm^{-1}.

Compound 15. Prepared according to the procedure for intermediate **7**. Colorless oil (85% yield); ^{1}H NMR [400 MHz, $CDCl_3$] δ 9.83-9.79 (1H, m), 5.34 (1H, s), 5.01-4.93 (1H, m), 2.83-2.65 (2H, m), 2.51-2.43 (2H, m), 2.41-2.30 (1H, m), 2.10-2.02 (1H, m), 1.99-1.66 (3H, m), 1.43 (3H, s), 1.50-1.22 (5H, m), 0.99 (3H, d, J = 6.0 Hz), 0.97-0.92 (1H, m), 0.89 (3H, d, J = 7.6 Hz) ppm; IR (υ_{max}, KBr): 2926, 2872, 1722, 1043, 880 cm^{-1}.

Experimental details for compound 16

Prepared according to general procedure for compounds **8** and **12**. Yellow solid (63% yield); mp: 53-56°C; ^{1}H NMR [400 MHz, $CDCl_3$] δ 8.54 (1H, dd, J = 4.2, 1.6 Hz), 7.94 (1H, brd, J = 8.2 Hz), 7.32 (1H, dd, J = 8.2, 4.2 Hz), 6.35 (1H, d, J = 2.4 Hz), 6.29 (1H, d, J = 2.4 Hz), 6.03 (1H, brd), 5.36 (1H, s), 4.35-4.24 (1H, m), 3.91 (3H, s), 3.72-3.55 (1H, m), 3.06-2.82 (3H, m), 2.80-2.57 (2H, m), 2.37-2.20 (1H, m), 2.09-1.56 (12H, m), 1.44-1.17 (4H, m), 1.36 (3H, s), 1.32 (3H, d, J = 6.4 Hz), 0.99-0.82 (7H, m) ppm; ^{13}C NMR [101 MHz, $CDCl_3$] δ 159.9, 145.1, 144.7, 135.5, 132.8, 130.5, 122.3, 103.5, 97.9, 92.6, 90.3, 81.3, 75.4, 55.7, 52.2, 48.8, 48.0, 47.8, 47.6, 43.8, 37.6, 36.8, 34.6, 33.7, 32.3, 30.8, 26.3, 25.0, 23.6, 20.8, 20.4, 12.7 ppm; IR (υ_{max}, KBr): 3387, 2926, 2874, 1051, 878 cm^{-1}. HRMS [ESI] m/z = 553.3549 (calculated for $C_{32}H_{47}N_3O_5$+H: 553.3516); Elemental analysis for $C_{32}H_{47}N_3O_5$ C: 69.71, H: 8.90, N: 7.48 (required values, C: 69.41, H: 8.56, N: 7.59).

Experimental details for compound 18

Prepared according to the procedure described for compound **5** using artelinic acid as starting material.[1] Yellow solid (72% yield); mp: 84-88°C; ^{1}H NMR [400 MHz, $CDCl_3$] δ 8.55 (1H, dd, J = 4.0, 1.4 Hz), 7.95 (1H, dd, J = 8.2, 1.4 Hz), 7.70 (2H, d, J = 8.2 Hz), 7.37-7.31 (1H, m), 7.35 (2H, d, J = 8.2 Hz), 6.37 (1H, d, J = 2.4 Hz), 6.32 (1H, d, J = 2.4 Hz), 6.22 (1H, t, J = 5.6 Hz), 6.04 (1H, d, J = 8.4 Hz), 5.47 (1H, s), 4.94 (1H, d, J = 12.8 Hz), 4.92 (1H, d, J = 2.8 Hz), 4.57 (1H, d, J = 12.8 Hz), 4.92 (1H, d, J = 2.8 Hz), 3.90 (3H, s), 3-75-3.64 (1H, m), 3.60-3.43 (2H, m), 2.76-2.64 (1H, m), 2.46-2.34 (1H, m), 2.11-2.02 (1H, m), 1.97-1.76 (7H, m), 1.68-1.60 (1H, m), 1.58-1.46 (2H, m), 1.48 (3H, s), 1.34 (3H, d, J = 6.4 Hz), 1.37-1.25 (2H, m), 0.97 (3H, d, J = 7.2 Hz), 0.96 (3H, d, J = 6.0 Hz), 0.94-0.88 (1H, m) ppm; ^{13}C NMR [101 MHz, $CDCl_3$] δ 167.3, 159.4, 144.9, 144.4, 141.8, 135.3, 134.8, 133.7, 129.9, 127.1, 126.9, 121.9, 104.2, 101.5, 96.9, 91.8, 88.0, 81.1, 69.2, 55.2,

52.5, 47.9, 44.3, 39.9, 37.4, 36.4, 34.6, 33.9, 30.9, 26.4, 26.2, 24.7, 24.5, 20.7, 20.4, 13.1 ppm; IR (υ_{max}, KBr): 3389, 2920, 1636, 1614, 1520, 1385, 1099, 874 cm^{-1}. HRMS [ESI] m/z = 660.3631 (calculated for $C_{38}H_{49}N_3O_7$+H: 659.36); Elemental analysis for $C_{38}H_{49}N_3O_7$ C: 69.17, H: 7.40, N: 6.37 (required values, C: 68.71, H: 7.32, N: 6.00).

***In vitro* metabolism studies**

Metabolic stability was evaluated by incubating compounds **5** and **8** at 37 °C at 0.1 μM in rat pooled liver microsomes (0.2 mg of protein/mL, BD Gentest, Woburn, MA), suspended in 10 mM pH 7.4 phosphate buffer solution. The activity of the rat microsomes was determined by their CYP2E1-catalyzed p-nitrophenol hydroxylation capacity, applying a methodology described elsewhere.[2] Reactions were initiated by the addition of a NADPH regenerating system A (31 mM $NADP^+$, 66 mM glucose 6-phosphate, and 0.67 mM $MgCl_2$) and NADPH regenerating system B (BD Gentest, containing 40 U/mL glucose 6-phosphate dehydrogenase in 5 mM sodium citrate). At appropriate intervals, aliquots were removed and diluted with acetonitrile. The samples were centrifuged at 4000g for 10 min, and the supernatant was analysed using a LichroCART RP-18 (5 μm × 250 mm) analytical column on a LabChrom L7400 Merck Hitachi instrument, with acetonitrile-water (25:75 ratio) as eluent at a flow rate of 1 mL/min. In the assay without NADPH generating system, solutions A and B were replaced by purified water. The reactions followed strict first-order kinetics for at least three half-lives, with $R^2 > 0.95$. The corresponding half-life values, $t_{1/2}$, were then used to calculate the *in vitro* intrinsic clearance ($CL_{int,in\ vitro}$), predicted *in vivo* intrinsic clearance value (CL_{int}), and predicted *in vivo* hepatic extraction ratio (E_H), assuming predominantly hepatic cytochrome P450-mediated clearance *in vivo* and the following scaling parameters for rat: hepatic blood flow of 55.2 mL/min/kg body weight, liver weight of 40 g liver/kg body weight, and microsomal content of 45 mg microsomal protein/g liver.[3-5]

***In vitro* activity assay against blood stage of infection**

Synchronized ring-stage *P. falciparum* strain W2 parasites were cultured with multiple concentrations of test compounds (added from 1,000 × stocks in dimethyl sulfoxide [DMSO]) in RPMI 1640 medium with 10% human serum. After 48 h of incubation, when control cultures contained new rings, parasites were fixed with 1% formaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 48 h at room temperature; then they were labeled with YOYO-1 (1 nM; Molecular Probes) in 0.1% Triton X-100 in PBS. Parasitemia was determined from dot plots (forward scatter versus fluorescence) acquired on a FACSort flow cytometer using CellQuest software (Becton Dickinson). Fifty percent inhibitory concentrations (IC_{50} values) for growth inhibition were determined with GraphPad Prism software from plots of the percentage of parasitemia of the control relative to the inhibitor

concentration. In each case, the goodness of the curve fit was documented by R^2 values of 0.95.

***In vitro* activity against liver stage of infection**

Inhibition of liver-stage infection by test compounds was determined by measuring the luminescence intensity in Huh-7 cells infected with a firefly luciferase expressing *P. berghei* line, PbGFP-Luc_{con}, as previously described.[6] Huh-7 cells, from a human hepatoma cell line, were cultured in 1640 RPMI medium supplemented with 10% v/v fetal calf serum, 1% v/v nonessential amino acids, 1% v/v penicillin/streptomycin, 1% v/v glutamine, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7, and maintained at 37 °C with 5% CO_2. For infection assays, Huh-7 cells (1.2 × 104 per well) were seeded in 96-well plates the day before drug treatment and infection. The medium was replaced by medium containing the appropriate concentration of each compound approximately 1 h prior to infection with sporozoites freshly obtained through disruption of salivary glands of infected female *Anopheles stephensi* mosquitoes. Sporozoite addition was followed by centrifugation at 1700g for 5 min. Twenty-four h after infection, the medium was replaced by fresh medium containing the appropriate concentration of each compound. Parasite infection load was measured 48 h after infection. The effect of the compounds on the viability of Huh-7 cells was assessed by the AlamarBlue assay (Invitrogen, U.K.), using the manufacturer's protocol.

***In vivo* activity against blood stage of infection**

C57Bl/6J mice were infected by intraperitoneal inoculations of $2x10^6$ GFP-expressing *P. berghei* ANKA-infected erythrocytes. Parasitemias were monitored daily by microscopy and flow cytometry. Drug treatment was initiated when blood parasitemias were *ca.* 3.9%. At this point, 30 mg/kg b.w. of compound **5** were administered once daily by intraperitoneal injection, for 5 days. An equivalent amount of drug vehicle was injected in control mice. Parasitemias, disease symptoms and survival were monitored daily from the onset of treatment until the end of the experiment. The experiment was terminated following 10 consecutive days of absence of blood parasitemia in treated mice, corresponding to complete clearance of blood stage parasites. Parasitemias of control mice increased steadily until day 6 post-infection, when all mice died with symptoms of experimental cerebral malaria.

Transmission blocking assay

BalbC mice were infected by intraperitoneal inoculations of 10^7 erythrocytes parasitized with *P. berghei* ANKA-GFP. After approximately 4 days, parasitemia (Giemsa stained blood films) typically reached 8% to 12% then, the presence of gametocyte exflagellation (approximately 7/microscope field) was confirmed by observation of fresh preparations of

tail blood. At this point, mice were randomly separated into five different groups of three animals. Each group was treated by intraperitoneal administration (i.p.) with one single dose of each compound and primaquine (3 mice for each dilution, 10 and 25 µmol/kg in inoculation volumes of 0.1-0.2 mL, respectively; controls consisted of mice given a PBS solution). Two hours after administration, mice were anesthetized and placed on top of individual cages containing ca. 50 glucose-starved *Anopheles gambiae* female mosquitoes, which were allowed to feed for 1 h. After the blood meal, unfed females mosquitoes were removed from each cage. Mosquitoes infected with *P. berghei* were kept at 19 ± 1°C for the whole duration of the sporogonic cycle. Ten days after the blood meal, at least 10 mosquitoes of each cage were randomly collected and dissected for microscopic detection of oocysts in midguts.

Chi-square analysis was used to determine if the percentage of treated mosquitoes with oocysts was different from the percentage of control mosquitoes. Mann-Whitney test was used to determine if the number of oocysts per infected mosquito was significantly different from untreated control (Table 1).

Table 1. Effect of Compounds **5**, **8**, **10**, **12** and primaquine on the sporogonic development of *Plasmodium berghei* ANKA in *Anopheles gambiae* mosquitoes.

	Mean nº oocysts per mosquito±SEM[a]				% Infected *mosquitoes*			
Compd	10 µmolkg-1	Mann-Whitney *p*	25 µmol.kg-1	Mann-Whitney *p*	10 µmolkg-1	chit-square *p*	25 µmolkg-1	chit-square *p*
PQ	2.9 ± 1.1	0.0015	1.0 ± 0.0	-	35.5	<0.0001	2.2	<0.0001
5	23.2 ± 5.1	0.0025	0.0 ± 0.0	-	68.6	0.0252	0.0	<0.0001
8	8.0 ± 3.6	0.0008	1.0 ±0.0	-	42.9	<0.0001	5.9	<0.0001
10	26.9[b] ± 6.1	0.9799	1.8 ± 0.8	0.0302	66.7	0.0458	11.4	<0.0001
12	19.3[b] ± 8.0	0.3199	1.0 ± 0.0	-	36.4	0.0003	13.3	0.0087
Control	42.1 ± 4.4			-	83.6			

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