SUPPLEMENTAL MATERIAL

Exploring ubiquinone biosynthesis inhibition as a strategy for improving atovaquone efficacy in malaria

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Running title: Drug-targeting ubiquinone for malaria

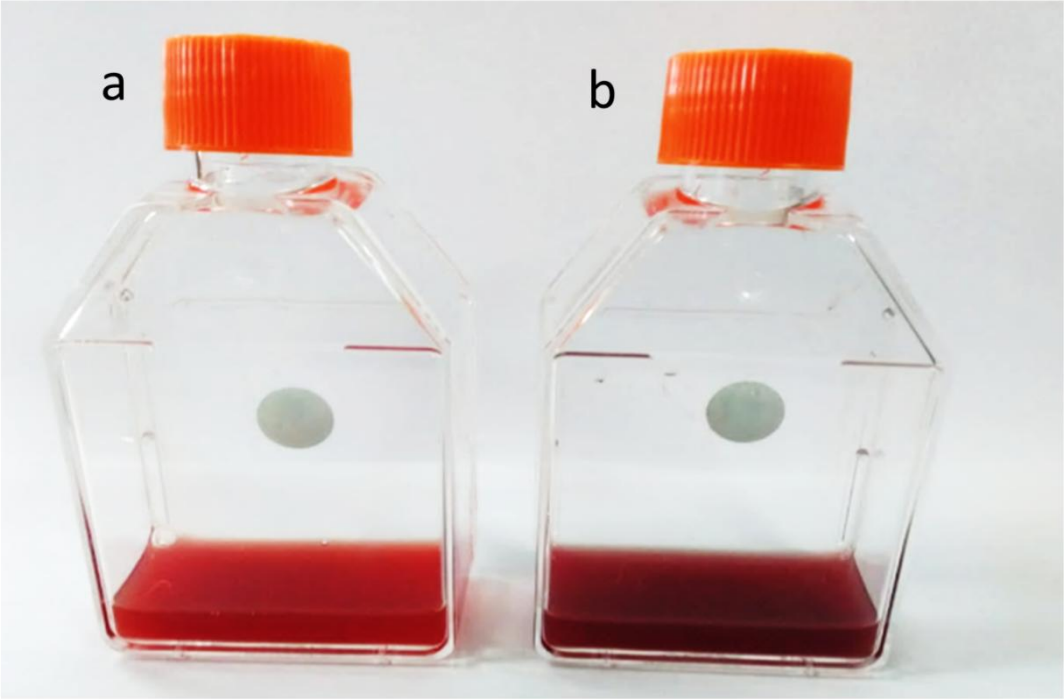

Fig S1. Malaria parasite culture under normal or microaerophilic conditions. *P. falciparum* cultured under normal (a) or microaerophilic conditions (b). This was adopted to observe the changes in blood color. *P. falciparum* 3D7 isolate was cultured *in vitro* as described in the materials and methods and a gaseous mixture of 5% CO_2, 5% O_2, and 90% N_2 was employed as a reference. However, oxygen-free mixtures (5% CO_2 and 95% N_2) were used in certain experiments until the culture attained a chocolate-brown color.

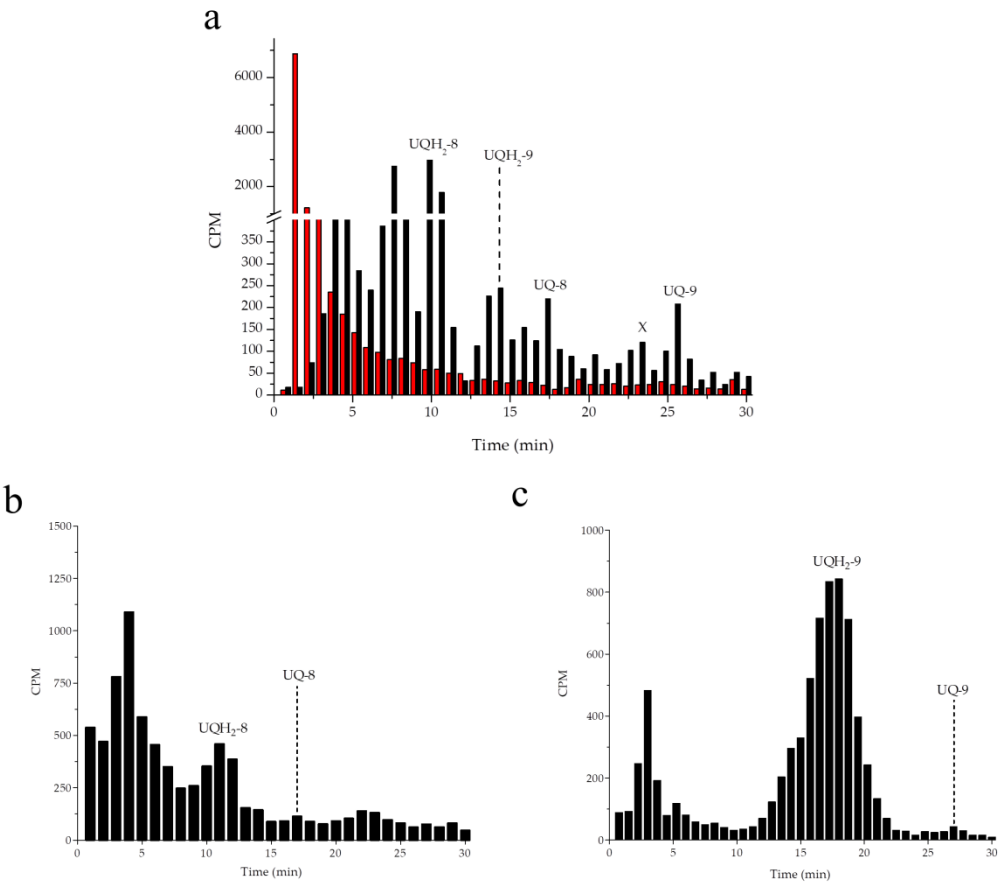

Fig S2. [^{3}H] GGPP incorporation into ubiquinones. a: RP-HPLC metabolic profiles of erythrocytes uninfected or infected with *P. falciparum* schizonts. Uninfected or infected erythrocytes were incubated with [^{3}H] GGPP. **b** and **c**: UQ-8 (b) or UQ-9 (c) from [^{3}H] GGPP-radiolabeled parasites were purified by RP-HPLC. Both UQ homologs were chemically reduced and analyzed by the same chromatography method. In all experiments, samples were co-injected with UQ standards. The fractions were collected at 1 min. CPM: counts per minute. This experiment was performed twice with similar results.

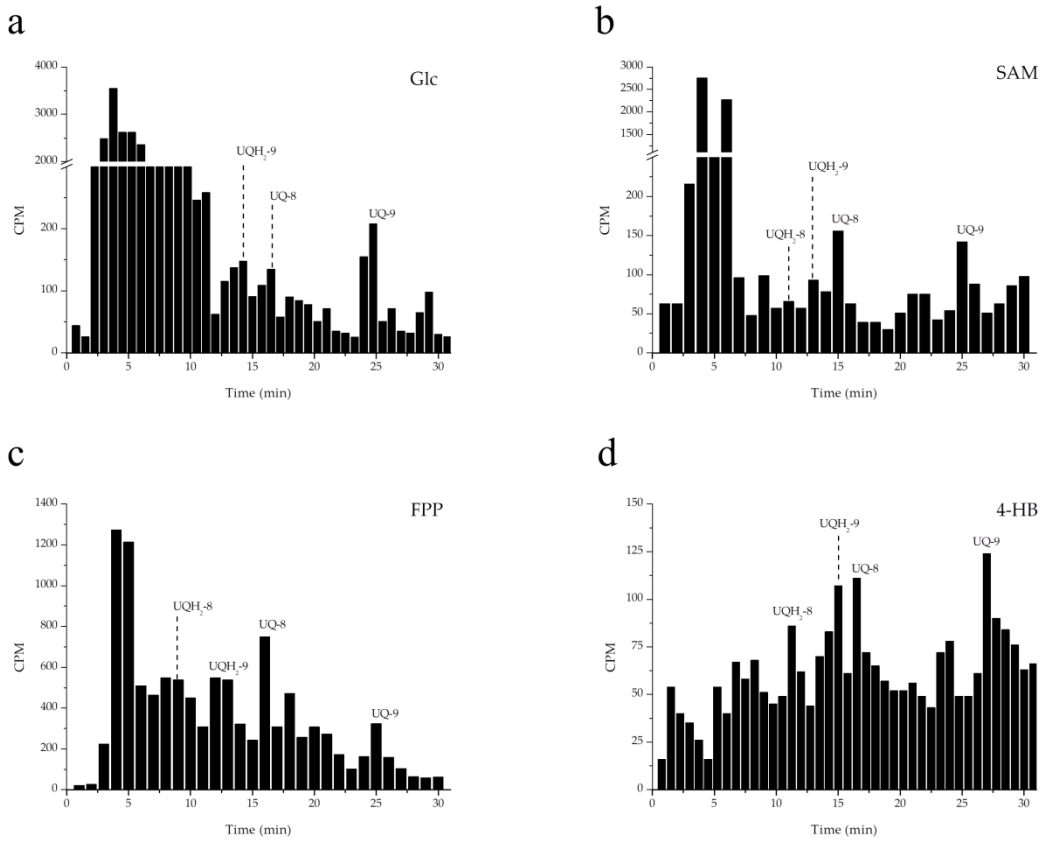

Fig S3. Incorporation of radiolabeled precursors into ubiquinones. RP-HPLC metabolic profiles of erythrocytes infected with *P. falciparum* schizonts incubated with different radiolabeled metabolites, as indicated. In all experiments, the samples were co-injected with UQ standards. The fractions were collected at 0.75 min. CPM: counts per minute. Glc: [^{14}C-U] Glc, SAM: [methyl-^{3}H] SAM, FPP: [^{3}H] FPP, 4-HB: [ring-^{14}C]-4-HB. These experiments were performed twice for each radiolabeled precursor and all experiments yielded similar results.

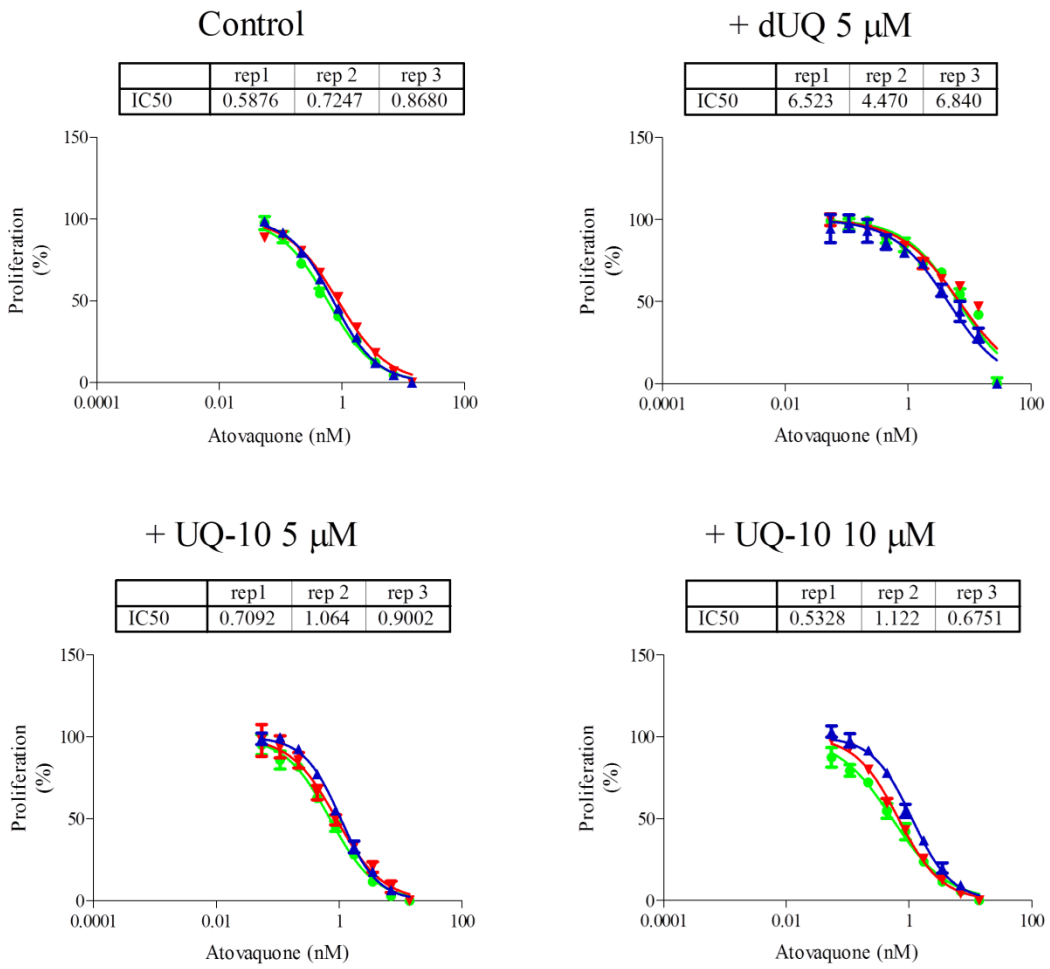

Fig S4. Atovaquone rescue assays. IC_{50} values and sigmoidal dose-response curves of AV at 48 h in the presence or absence of 5 µM dUQ, 5 µM or 10 µM UQ-10 (mean of three identical experiments). This experiment was performed thrice by DNA staining, as described in methodological procedures.

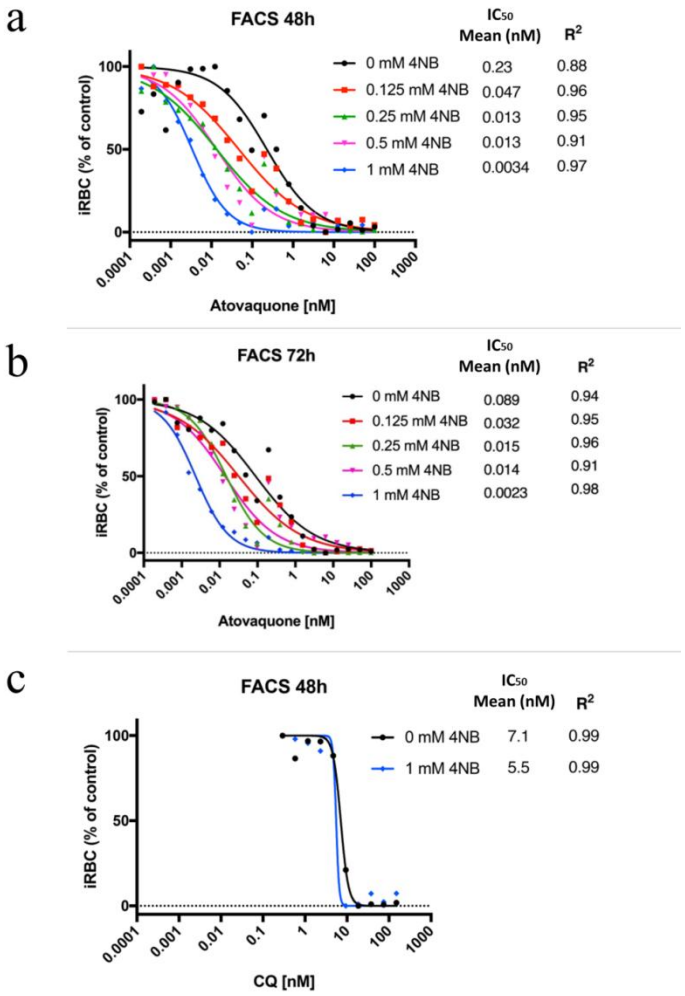

Fig S5. Atovaquone potentiation by 4-nitrobenzoate is not general or nonspecific. The obtained dose-response curves along with the R^2 values (mean of three experiments) corresponding to treatment with AV for 48 h (a) or 72 h (b). The same experiment was performed by treatment with chloroquine (CQ) for 72 h (c). Both assays were performed in the presence or absence of 4-NB at the indicated concentrations, and the effects were observed using flow cytometry (FACS). iRBC: infected red blood cells. Parasites harvested by centrifugation were resuspended in a solution of 10 µg/mL ethidium bromide in PBS and incubated at 37 °C for 20 min. This was followed by washing and resuspension in PBS and analysis using a Guava Easycyte Flow Cytometer (Merck). To confirm that the parasites cultivated in the presence of 4-NB were more susceptible to AV, these were cultured for 48 h

in the presence, or absence of 0.125 to 1 mM 4NB and a series of AV dilutions were prepared. Parasitemia was determined using flow cytometry (FACS) and the results were analyzed as described in materials and methods. The specificity of AV potentiation by 4-NB was investigated by determining chloroquine-sensitivity in the presence and absence of 4-NB. The IC_{50} values of chloroquine were similar, indicating that the effects of 4-NB are not general or nonspecific.

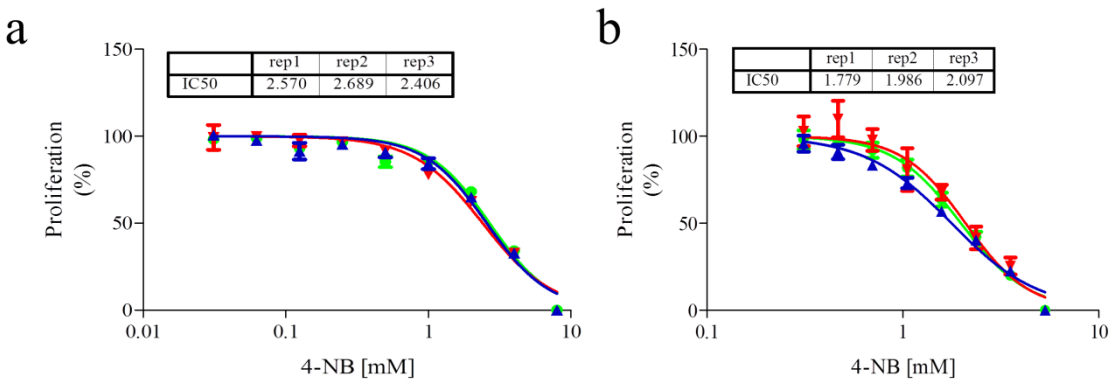

Fig S6. Effects of 4-nitrobenzoate on the growth of malaria parasite. Sigmoidal dose-response curves of the effect of 4-NB at 48 h (a) or 72 h (b). This experiment was performed thrice by DNA staining, as described in materials and methods.

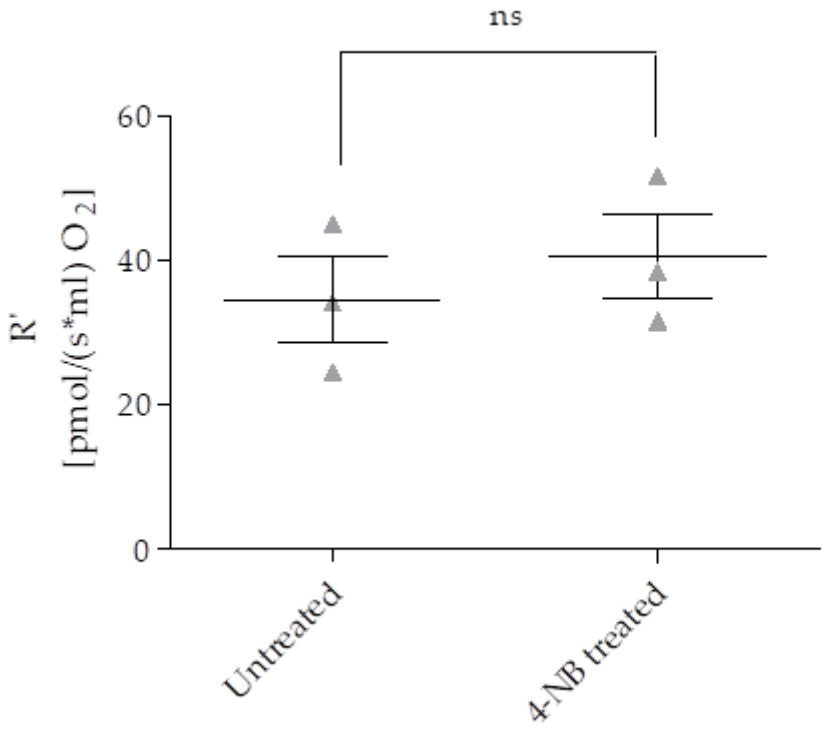

Fig S7. 4-Nitrobenzoate does not affect initial rate of respiration. Comparison of total cellular oxygen consumption (R') between untreated and 4NB-treated *P. falciparum* parasites. This experiment was performed thrice as described in methodological procedures. Unpaired t-test was used for statistical analysis to compare the values corresponding to the R'. ns: difference not statistically significant.