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2-*tert*-butyl-primaquine exhibit potent blood schizontocidal antimalarial activity via inhibition of heme crystallization

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Background

Primaquine (PQ) is the only 8-quinolinamine available to treat the malarial parasites in the infections caused by *Plasmodium vivax* and *P. ovale.* PQ has broad range of antimalarial activities, including efficacy as a causal prophylactic, gametocytocide, and sporontocide. These encouraging pharmacological properties make PQ an ideal drug to emulate while designing new antimalarials with improved activities ([1]). The placement of a metabolically stable *tert*-butyl group at the C-2 position of a quinoline ring in PQ (2-*tert*-Butyl-Primaquine - BPQ) results in a tremendous improvement in blood schizontocidal antimalarial activity ([1,2]). Because free heme released from hemoglobin catabolism in a malarial parasite is highly toxic, the parasite protects itself mainly by crystallization of heme into insoluble nontoxic hemozoin ([3]). In this study, we investigate the mechanism of blood schizontocidal activity of BPQ.

Results

The ability of 2-*tert*-butylprimaquine to inhibit in vitro beta-hematin formation (see Table 1), to form a complex with heme with a stoichiometry of 1:1 (see Figure 1 and Figure 2), and to enhance heme-induced hemolysis (see Figure 3) were demonstrated.

Table 1 IC50 values for inhibition of *P. falciparum* growth and heme crystallization

Drug	IC50 (μM) for inhibition of	
	*P. falciparum*D6 clone growth	BH formation
CQ	0.3	15.4
PQ	ND*	ND*
BPQ	0.1	2.9

*ND, not detected.

Conclusion

The results described herein indicate that a major improvement in the blood-schizontocidal antimalarial activity of 2-*tert*-butylprimaquine might be due to a disturbance of heme catabolism pathway in the malarial parasite.

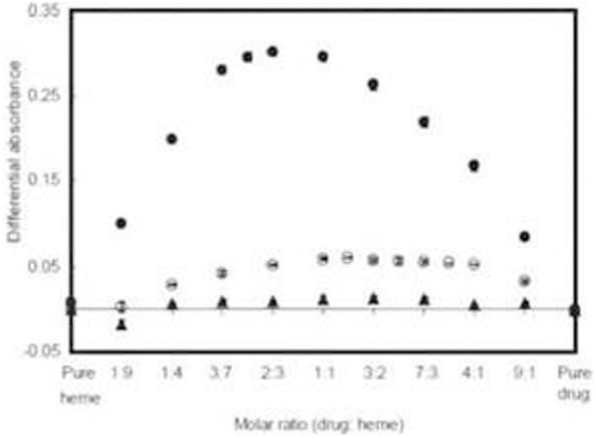

Figure 1 (abstract P69). Job plots of heme-CQ (closed circles), heme-PQ (closed triangles), and heme-BPQ (open circles) interaction. The total final combined concentration of heme and drug in the mixtures was constant at 10 μM in 40% aqueous DMSO. The pH and the temperature were constant at pH 7.4 and 25°C. The differential absorbance at 400 nm was recorded after incubation for 30 min. Values are the means ± standard errors of the means of three independent experiments. The results are reproducible.

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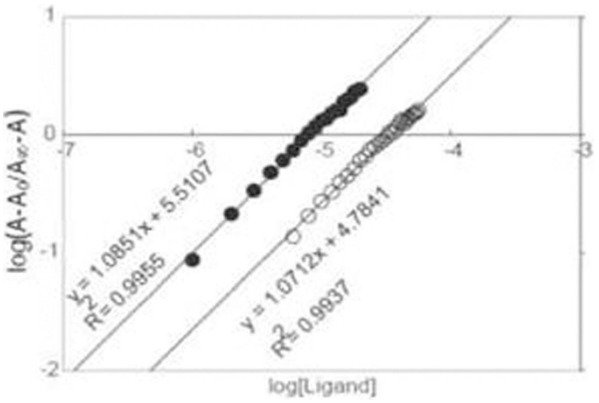

Figure 2 (abstract P69). Hill plots of heme-CQ (closed circles) and heme-BPQ (open circles) association. The *n* values correspond to individual slopes. The *n* and *Ka* values for heme-CQ association were 1.09 and 3.24×10^5 M^{-1}, respectively. The *n* and *Ka* values for heme-BPQ association were 1.07 and 0.61×10^5 M^{-1}, respectively.

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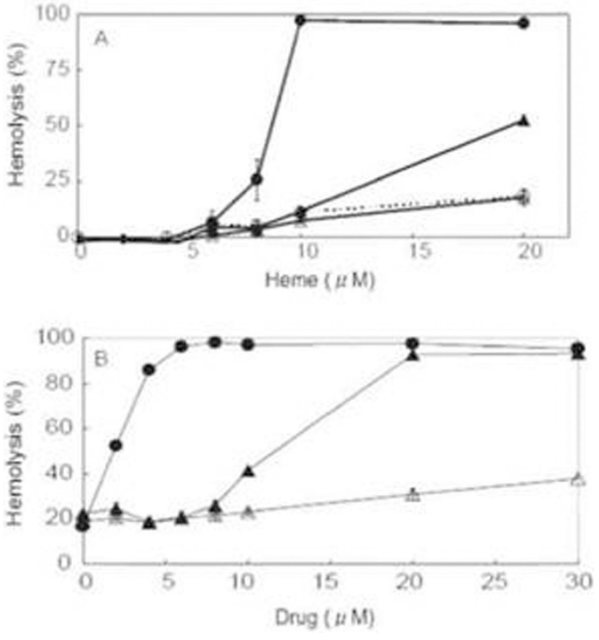

Figure 3 (abstract P69). Effects of heme-induced hemolysis by drugs. **(A)** Suspensions of erythrocytes were incubated in the presence of heme (final concentrations ranged from 0 to 20 μM) without (open circles) or with 10 μM CQ (closed circles), 10 μM PQ (open triangles), or 10μM BPQ (closed triangles). **(B)** Suspensions of erythrocytes were incubated in the presence of 10 μM of heme with various concentrations of the drug CQ (closed circles), PQ (open triangles), or BPQ (closed triangles). Error bars indicate standard deviations.

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