Antimalarial Iron Chelator, FBS0701, Shows Asexual and Gametocyte *Plasmodium falciparum* Activity and Single Oral Dose Cure in a Murine Malaria Model

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**Abstract**

Iron chelators for the treatment of malaria have proven therapeutic activity *in vitro* and *in vivo* in both humans and mice, but their clinical use is limited by the unsuitable absorption and pharmacokinetic properties of the few available iron chelators. FBS0701, (S)3''-(HO)-desazadesferrithiocin-polyether [DADFT-PE], is an oral iron chelator currently in Phase 2 human studies for the treatment of transfusional iron overload. The drug has very favorable absorption and pharmacokinetic properties allowing for once-daily use to deplete circulating free iron with human plasma concentrations in the high µM range. Here we show that FBS0701 has inhibition concentration 50% (IC₅₀) of 6 µM for *Plasmodium falciparum* in contrast to the IC₅₀ for deferoxamine and deferiprone at 15 and 30 µM respectively. In combination, FBS0701 interfered with artemisinin parasite inhibition and was additive with chloroquine or quinine parasite inhibition. FBS0701 killed early stage *P. falciparum* gametocytes. In the *P. berghei* A3 model, the parasites were suboptimum in an uncomplicated malaria trial because of poor iron clearance after oral absorption. Iron regulation is known but evidence points to a iron chelator mechanism of parasiticidal action of artemisinin drugs [9,10].

**Introduction**

Iron metabolism is a proven target for many malaria drugs. The quinolines like chloroquine and quinine interfere with iron protoporphyrin IX crystallization in the digestive vacuole [1]. The artemisinins are activated by iron to generate carbon-centered radicals that rapidly kill parasites [2]. Iron chelators have been explored as alternative malaria drugs for decades [3]. Intravenous deferoxamine does increase clearance of parasites [4] but in an important clinical trial deferoxamine did not effect outcome from severe cerebral malaria [5]. The orally available deferiprone was also suboptimum in an uncomplicated malaria trial because of poor iron clearance after oral absorption [6]. Iron regulation is potent in limiting liver stage multiplication mediated, in part, by hepcidin pathway [7]. Iron supplementation increases hepatic parasites [8] and iron chelators decrease hepatic parasites [7]. For intraerythrocyte *Plasmodium*, the literature describes chelation of both labile iron pools in parasites as well as infected erythrocyte cytosol. Deferoxamine decreases available iron in the nucleus and parasite cytosol. The exact mechanism of parasite killing is not known but evidence points to a iron chelator mechanism of limiting iron for ribonucleotide reductase production of nucleotides, however earlier studies also suggest a toxic mechanism of erythrocytic *Plasmodium* killing [3]. A drawback for *Plasmodium* iron chelation chemotherapy is interference with the parasiticidal action of artemisinin drugs [9,10].

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so known as (S)-3’-(HO)-desazadesferrithiocin-polyether [DADFT-PE] or more simply as FBS0701 has a very high affinity and specificity for Fe (III) with an equilibrium constant of $10^{29}$ Molar [11]. The molecular weight of the formulated salt is 441 while free base is 400. The iron clearing efficiency in rodents is 10.6% [11,12]. In contrast the iron clearing efficiency for defereroxamine is 2.8% and 5.5% and deferiprone is 1.2% and 2.1% in rodents and monkeys, respectively. In normal human volunteers dosed with FBS0701 with 6, 10 or 16 mg/kg, the pharmacokinetics across the dosing range indicates high bioavailability $C_{\text{max}}$ of 22.7, 41.5 and 87.0 $\mu$M respectively, dose-proportionality and a terminal $t_1/2$ permitting once-daily dosing [13].

Here we investigated the actions of this iron chelator on *P. falciparum* blood stage parasites, its action against gametocytes and efficacy in murine malaria models.

**Methods**

**Materials**

FBS0701 was obtained from Aptuit (Kansas City) LLC under good manufacturing practice. All other reagents and chemicals are from Sigma unless otherwise noted. *P. falciparum* isolates 3D7, Indo and KMVII were obtained from ATCC-BEI MR4 repository.

**P. falciparum culture**

Direct effect of FBS0701 on *P. falciparum* was determined by SYBR green I (Invitrogen-Molecular Probes, Eugene OR) based assay as described earlier [14]. Briefly, the inhibition assay was initiated by adding serially diluted FBS0701 in 96 well microplates followed by sorbitol synchronized 1% ring stage parasites at 1% hematocrit in RPMI 1640 media with glutamine and HEPES supplemented with 10% human serum and 50 $\mu$g/ml hypoxanthine. Culture plates were then transferred to 37°C incubator and maintained in the environment of 5% O$_2$, 5% CO$_2$ and 90% N$_2$ for 72 hrs. 2x SYBR Green I solution in lysis buffer was added after one cycle of freeze and thaw. Plates were then placed at room temperature in dark for 1–2 hours and fluorescence values were quantitated in a plate reader (HTS 7000, Perkin Elmer) at emission and excitation wavelengths of 535 and 485, respectively. For fractional inhibition analysis quinine, quinidine, chloroquine and artesiminin were used at concentrations ranging from 1 to 100 nM and FBS0701 from 1–15 $\mu$M. The fractional inhibition was analyzed according to the original Elion and Hitchings paper [15] with modifications by Bell [16]. The fraction of the chloroquine, quinine or artesiminin drug in combination with FBS0701 that produced an IC$_{50}$ was divided by the IC$_{50}$ of the chloroquine, quinine or artesiminin alone for the ratio on the y-axis. The x-axis was fraction of FBS0701 that produced an IC$_{50}$.

To test the effect of FBS0701 on gametocyte stages, *P. falciparum* NF54 cultures were initiated in 24 well plates at 0.5% asexual parasitemia and 4% hematocrit [17]. Medium was changed daily up to day 18, without addition of fresh RBCs. Continuous cultivation without dilution leads to concomitant crash of asexual parasitemia and induction of gametocytogenesis by day 5. The gametocytemia was approximately 7% at start of treatment. FBS0701 was dosed in wells at Days 9–10, when majority of gametocytes were in early stage of development (Stage I, II) or Days 14–13 when majority of gametocytes have matured to stage III to V. Levels of gametocytemia were determined on day 18 and the mean number of gametocytes was calculated by counting 10 high-powered (1000×) fields from triplicate wells per condition. More than 1000 erythrocytes were enumerated by random scanning across Giemsa blood film.

**Animal Ethics**

All animal experiments were performed on protocol “Malaria Drug Testing in Mice” (Approval ID-MO09H401) approved by The Johns Hopkins Animal Care and Use Committee in accordance with institutional standards.

**Malaria Murine Drug Testing**

Male 5–6 week old at approximately 25±2 g C57/BL6 mice were purchased from Jackson Laboratories (Maine, USA). The *P. berghei* ANKA strain was obtained from ATCC; the *P. yoelii* 17× lethal strain was the gift of James Burns (Drexel University). Freshly made solutions of FBS0701 in water were used for all the experiments and drug was administered by oral cannulation at indicated times. Murine infection was initiated by intraperitoneal inoculation of a million parasites for *P. berghei* and 10 million for *P. yoelii*. Blood was taken from the tail vein for blood smears. Parasitemias were determined in a blinded fashion by counting four fields of approximately 200 erythrocytes per field. Mice that survived for 30 days post infection with complete disappearance of parasitemia and no recrudescence within the next 30 days were considered cured.

**Pharmacokinetics**

30 Balb/c mice were dosed with 100 mg/kg of FBS0701 by oral cannulation and blood was drawn by cardiac puncture during anesthesia. Whole blood was separated into plasma and erythrocytes and plasma sent for analysis by Covance Laboratories (Madison, WI) using a validated assay [13].

**Measurement of Bioavailable Labile Iron in Erythrocytes**

Blood was collected from healthy subjects washed 2× with PBS. Erythrocytes were then incubated with 0.125 $\mu$M calcein (Invitrogen) in PBS for 15 minutes at 37°C, washed 2× with PBS and then allowed to rest for 10 minutes at 37°C in the dark [18,19,20]. Cells were incubated with PBS, 100 $\mu$M FBS0701 or 100 $\mu$M deferiprone for 1 hour at 37°C. Cells were analyzed by flow cytometry using a modified FACS-Calibur with 2 lasers 30 mW 488 Diode Pumped Solid State laser and a 25 mW 637 red diode laser (FACS-Calibur; Becton Dickinson, Mount View CA, modified by Cytek Development). Data was collected using FlowJo CE and analyzed using Summit v4.3.01. The emission of 400,000 cells was analyzed using logarithmic amplification for fluorescence signal height (FL-1, 530/30) and linear amplification for forward scatter (FSC) and side scatter (SSC). The threshold was set at FSC to exclude cell debris and microparticles. The mean fluorescence intensity (MFI) was calculated using Summit v4.3.01. Individual experiments were performed in triplicate and also biologic duplicate with different human erythrocyte donors. The statistical significance was calculated using the Student’s t-test.

**Results**

**P. falciparum Blood Stage Inhibition**

In the chloroquine and quinine sensitive isolate 3D7, the IC$_{50}$ with continuous drug exposure was 6 $\mu$M (IC$_{50}$ = 3 $\mu$M and IC$_{90}$ = 10 $\mu$M). Testing in the chloroquine and quinine resistant parasites Indo and KMVII showed a similar inhibition to 3D7. Morphologically the FBS0701 exposed parasites were shrunken. Stage and concentration dependence of FBS0701 iron chelation showed that for continuous drug exposure starting at ring and trophozoite stage the IC$_{50}$ was similar at 6 $\mu$M, but when drug
exposure was initiated at schizont stage after DNA replication, the IC_{50} increased by almost three fold to 15–17 μM. Investigation of dose-dependent killing of gametocytes shows that 10 μM inhibited more than 90% of early stage I and II gametocytes but only about 40% of late stage III or IV gametocytes, 10 μM FBS0701 had minimal 37% decrease in the number of gametocytes (Figure 1). Because of the known interactions of iron chelators with the artemisinins [9,16,21], we investigated interaction in vitro with P. falciparum. Fractional inhibition indicates interference with artemisinin and an additive action with the quinolines like chloroquine or quinine (Figure 2).

Murine Malaria Inhibition

In the lethal P. berghei/ANKA mouse model, FBS0701 showed a 50% reduction in day 5 parasitemia and delayed death by more than ten days at the 100 mg/kg dose, which was independent of single day dosing for one day, three days or seven days (not shown). The lethal P. yoelii model was used next because while lethal infection results from invasion of both normocytes and reticulocytes a recent vaccine study demonstrated that by largely restricting invasion to reticulocytes the infection was no longer lethal. [22]. We dosed mice with the FBS0701 by single oral dose either seven days or one day prior to parasite inoculation and also dosed a day after inoculation. All infected untreated control mice

Figure 1. FBS0701 inhibits early stage not late stage gametocytes. Gametocyte cultures of P. falciparum NF54 strain were initiated at 0.5% asexual parasitemia and 4% hematocrit in 24 well culture plates. Gametocytes were visualized and counted on Day 18 with no drug (A) or 100 μM FBS0701 dosed in wells at Days 9–10 (B) or Days 14–15 (C) post-culture initiation. Dose dependent inhibition is more pronounced with early gametocyte stage I and II (day 9–10 dosing) than gametocyte late stages III-IV (day 14–15 dosing) (D). Gametocytes were inoculated at 0.5% parasitemia, resulting in gametocyte induction, followed by dosing of FBS0701 on indicated days. Gametocytes per 10 high power fields (excess of 1000 erythrocytes) in each of three replicate wells for each condition were counted on Day 18. Control wells had 7.4% mean gametocytes. Data expressed as percent inhibition of control. Standard deviation of triplicate observations for each of the three wells is shown.

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Protection in animals pre-treated day +1 on day 0. A single oral dose of FBS0701 at 100 mg/kg given parasitemia was near 50% but confined to reticulocytes rather surviving mice, which had been dosed with FBS0701, the day 16 model we again saw a small reduction in day 3 parasitemia. In all lived suggesting a lingering effect after the drug was cleared. In this Figure 3. FBS0701 cures lethal 27 and day 1 dosing and significant levels at one hour that by 8 hours was close to 1 mM (Figure 5). These concentrations are below those easily achieved in humans [13].

Removal of Labile Erythrocyte Iron
Calcine has been used as an intracellular iron probe [23,24]. Labile iron bound to low molecular weight, low affinity chelators binds and quenches calcine to generate the baseline equilibrium fluorescent intensity. The labile iron pools in normal erythrocytes has been determined to be approximately 1 mM [18,19]. Similar to deferiprone, the iron chelator FBS0701 at 100 µM was able to enter erythrocytes as evidenced by the increase in fluorescent intensity consistent with the release of iron from calcine (Figure 6A and B). Incubation of the FBS0701 and deferiprone for either one or two hours showed no difference in endpoint fluorescence intensity. To measure the removal of intracellular iron by chelation, erythrocytes at 4 x 10^7 cells/ml (0.125% hematocrit) were incubated for an hour with 100 µM of either deferiprone or FBS0701, washed and then placed in PBS overnight. Figure 6C shows that fluorescence in erythrocytes pre-treated with FBS0701 increased slightly from a mean fluorescence intensity (MFI) of 17 to 20. Pre-treatment with deferiprone showed a slight though statistically insignificant decrease in MFI. Addition of either iron chelators to cells previously incubated with FBS0701 showed a small rise of approximately 5 MFI compared to an increase of approximately 15 seen with either PBS or deferiprone-incubated cells demonstrating that in erythrocytes, FBS0701 depletes the labile pool of intracellular iron.

Discussion
A bioavailable oral iron chelator is an attractive and proven objective as an antimalarial agent. Much of the lack of efficacy of previous iron chelators for malaria therapy can be attributed to pharmacokinetic limitations including bioavailability, poor iron clearance efficiency and short-half-life. FBS0701 has high solubility and a log p of −1.22 permitting good oral bioavailability and much better iron clearance than defereroxamine and deferiprone. Here we demonstrate activity against blood stage P. falciparum and also single dose cure in a lethal mouse malaria model. A potential limitation is the known interference to the artemisinin class of drugs. However, the evidence from this work indicates that FBS0701 may remove an irreplaceable source of iron in normocytes in mice. Previous studies on where iron is removed with deferoxamine in the calcine measurements showed a decrease in labile iron in both infected and uninfected erythrocytes. We have not demonstrated a decrease in nonheme iron in these studies but suggest that the effect we see in vivo is not from a new recompartmentalization of iron but from a decrease in erythrocyte levels. Consistent with other reports [9,16,21], we have demonstrated in vitro interference when both iron chelators and artemisinin are present simultaneously, but have not tested whether pre-treatment with FBS0701 before infection interferes with artemisinin activity.

Interestingly, the dose of iron chelator largely restricted lethal P. yoelii to young erythrocytes even almost 28 days after the drug was dosed and three weeks into infection. Mouse erythrocytes have a life span of about 60 days [25,26]. Additional studies of the timing...
of iron chelator days to weeks before lethal \textit{P. yoelii} infection will provide evidence to replenishment of this erythrocyte bioavailable source of iron for \textit{Plasmodium}.

The mouse pharmacokinetic parameters are close to those obtained in the rat model [12]. Most of the iron-FBS0701 complex was excreted in the bile while free FBS0701 has renal excretion [12]. The relative level of FBS0701 in the rodent shows liver > kidney > plasma > pancreas > heart. Interestingly, humans show approximately ten times higher plasma concentrations compared to mouse for a given mg/kg dose. However, in studies comparing dose proportionality for cancer drugs where toxicity is a larger issue 100 mg/kg dose in mice represents a dose of 300 mg/m² while a 10 mg/kg in humans is equivalent to 370 mg/m² [27]. The implications are that in humans effective drug concentrations above minimal \textit{P. falciparum} inhibition concentrations last less than ten hours. In the \textit{P. falciparum} in vitro drug testing the drug is at continuous concentration for three days. In the mouse \textit{in vivo} studies here we have demonstrated a persistent effect weeks after the drug was dosed. This data indicate that iron chelation is able to remove a bioavailable source which persists and plays a role in parasite inhibition. Unlike many of the long half-life blood stage active quinolines which rely on time above inhibition concentration to kill parasites, FBS0701 has an effect possibly by perturbing an iron compartment in the circulating erythrocytes.

The determination of relative labile iron concentrations using calcine indicates FBS0701 is able enter erythrocytes and bind iron bound to calcine. More importantly, in contrast to deferiprone, FBS0701 was able to remove labile iron from erythrocytes, an effect that persisted for at least 16 hours. This is the first study to demonstrate the egress of labile iron complexed with a chelator complex from erythrocytes. This has important clinical implica-

**Figure 4. Protection is associated with exclusion of infection from normocytes.** On day 3 blood films from control (A) and FBS0701 (B) treated mice both show infection in normocytes. On day 16 \textit{P. yoelii} infection is largely restricted to young reticulocytes with only a few in normocytes in mice treated on day – 7 (C) or day – 1 (D). Quantification of blood films (E) indicated that on day 3 infected normocytes (black bars) out numbered by more than 20 fold infected reticulocytes (hatched bar). On day 16 most of the erythrocytes are either infected (hatched bars) or uninfected reticulocytes (clear bars). By day 23 more than 80 to 90% of the erythrocytes are uninfected normocytes. Parasitemia on day 3 is reduced compared to control animals which are absent in subsequent days due to control animal death. Quantification was performed on counting at least 500 erythrocytes per mouse and averaged in the groups of 4 or number of remaining mice. Counts are represented as number of infected or uninfected normocytes or reticulocytes out of 100. Error is standard deviation of means between up to 4 mice.

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**Figure 5. Pharmacokinetics of FBS0701 in mice show high peak values.** 21 fed mice were given single oral dose at 100 mg/kg (filled triangle) and were sacrificed at indicated time with whole blood separated into erythrocytes and plasma. At least 200 microliters of plasma was analyzed for drug levels in the three mice at each time point. Error is standard deviation of the mean.

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Figure 6. FBS0701 treatment chelates intracellular erythrocytic iron to the same extent as deferiprone but unlike deferiprone, removes iron from erythrocytes. Aliquots 1–5×10⁶ erythrocytes from two separate healthy donors were stained in the dark with 0.125 μM calcein-AM for 15 minutes at 37°C. Cells were washed twice with PBS and allowed to rest for 10 minutes at 37°C in the dark. Flow cytometry on FACS-Calibur records fluorescent signal height of calcein on 40,000 cells. A. Calcein-AM fluorescence histograms of unstained (no calcein) erythrocytes (red) and calcein-stained erythrocytes (green) followed by incubation without (green) or with iron chelator L1 (blue) or FBS0701(yellow) show an increase in fluorescent signal intensity with iron chelator indicating displacement of labile iron from calcein. The L1 and FBS0701 peaks overlap. B. Washed erythrocytes after calcein-AM staining were incubated with PBS (green hatched bars), 100 μM L1 (deferiprone) (red dotted bars) or 100 μM FBS0701 (blue solid bars) chelator for 1 hr or 2 hr. Both chelators were able to enter erythrocyte and chelate iron once bound to calcein to increase MFI. C. Erythrocytes were incubated with PBS, 100 μM L1 (deferiprone) or 100 μM FBS0701 chelator for 1 hr and washed twice with PBS and incubated overnight in PBS. Erythrocytes were then washed, stained with calcein-AM and incubated with either L1 (red dotted bars) or FBS0701 (blue solid bars). The baseline calcein fluorescence in FBS0701 pre-treated cells was higher indicating a lower concentration of labile iron in these erythrocytes, while baseline MFI in cells incubated with deferiprone have is essentially unchanged. D. Change in Mean Fluorescent Intensity is shown for each of the conditions described in C. In the FBS0701 pre-treated cells, the addition of 100 μM of either chelator resulted in a change in MFI of 5 compared to a change of 15 in cells treated with either PBS or deferiprone. The data in B was performed in three replicates on RBCs from a single blood donor while C and D was performed using RBCs from two different donors each in triplicate and mean and standard deviation (SD) of the 6 data points is shown. Student’s t-test shows a statistical significant difference in the change in MFI for both iron chelators and PBS in B and C. In C, baseline FBS0701 MFI is higher than both PBS and deferiprone (p<0.05) and in D the change in MFI with FBS0701 after the overnight pre-treatment is less than with PBS and deferiprone (student’s t-test, p<0.003).

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tions for the use of an iron chelator in the treatment of malaria as well as the ability of FBS0701 to remove iron from other cell types.

In the malaria iron chelator literature there is some debate whether iron chelators inhibit by limiting DNA replication which in many bacteria and mammalian cells is cytostatic or whether iron chelators are toxic and therefore parasiticidal to *Plasmodium*. Early stage gametocytes do not replicate DNA, so the data which show early stage inhibition with FBS0701 may indicate an additional toxic mechanism of inhibition at the high dose used in the gametocyte assay at the stages digesting the iron and hemoglobin rich erythrocyte cytoplasm.

In summary, FBS0701 demonstrates more potent inhibition for *P. falciparum* than previous iron chelators in clinical use. The activity persists in mice after the drug is below inhibition concentrations of approximately 5 μM. Our gametocyte data suggest a combined toxic mechanism in presence of erythrocyte cytosol ingestion in addition to the proposed mechanism of limiting DNA synthesis. In the lethal *P. yoelii* murine malaria model we show single oral dose cure. The removal of erythrocytic iron from intracellular bioavailable pools likely contributes to both the antimalarial activity and duration of effect of FBS0701 accounting for these effects in the relative absence of drug in plasma. FBS0701 may find clinically utility as monotherapy, a malarial prophylactic or, more likely, in combination with other antimalarials after further preclinical testing to investigate liver stage activity, duration of blood stage interference with the artemisinins or possibly inhibition of transmission in mosquito stages ingesting FBS0701 exposed gametocytes.

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**Author Contributions**

Conceived and designed the experiments: PF AKT MAC CCH DJS. Performed the experiments: PF AKT MAC CCH DJS. Analyzed the data: PF AKT MAC CCH DJS. Contributed reagents/materials/analysis tools: PF AKT MAC CCH DJS. Wrote the paper: PF AKT MAC CCH HYR DJS.

**References**

1. Sullivan DJ Jr., Gluzman IY, Russell DG, Goldberg DE (1996) On the molecular mechanism of chloroquine’s antimalarial action. Proc Natl Acad Sci U S A 93: 11865–11870.

2. O’Neill PM, Posner GH (2004) A medicinal chemistry perspective on artemisinin and related endoperoxides. J Med Chem 47: 2965–2964.

3. Maheza GF, Loyevsky M, Gordeuk VR, Weiss G (1999) Iron chelation therapy for malaria: a review. Pharmacol Ther 81: 53–75.

4. Gordeuk VR, Thuma PE, Brittenham GM, Zuhu S, Simwanza G, et al. (1992) Iron chelation with deferoxamine B in adults with asymptomatic *Plasmodium falciparum* parasitemia. Blood 79: 305–312.

5. Gordeuk V, Thuma P, Brittenham G, McLaren C, Parry D, et al. (1992) Effect of iron chelation therapy on recovery from deep coma in children with cerebral malaria. N Engl J Med 327: 1477–1477.

6. Thuma PE, Olivo AI, Maheza GF, Bemba G, Parry D, et al. (1998) Assessment of the effect of the oral iron chelator desferiprone on asymptomatic *Plasmodium falciparum* parasitemia in humans. Am J Trop Med Hyg 58: 538–564.

7. Portugal S, Carret C, Recker M, Armitage AE, Gonzalez LA, et al. (2011) Host-mediated regulation of superinfection in malaria. Nat Med 17: 732–737.

8. Gona J, Renia L, Milten G, Mazier D (1996) Iron overload increases hepatic development of *Plasmodium yoelii* in mice. Parasitology 112 (Pt 2): 163–168.

9. Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, et al. (2003) Artemisinins target the SERCA of *Plasmodium falciparum* malaria. Nature 424: 957–961.

10. Wang J, Huang L, Li J, Fan Q, Long Y, et al. (2010) Artemisinin directly targets malarial mitochondria through its specific mitochondrial activation. PLoS One 5: e9582.

11. Bergeron RJ, Wiegand J, Bharti N, McNamis JS, Singh S (2011) Desferrioxamine analogue iron chelators: iron clearing efficiency, tissue distribution, and renal toxicity. Biomaterials 24: 239–258.

12. Bergeron RJ, Wiegand J, McNamis JS, Bharti N, Singh S (2008) Design, synthesis, and testing of non-nephrotoxic desazadesferrioxamine polyether analogues. J Med Chem 51: 3913–3923.

13. Rienhoff HY Jr., Viprakasit V, Tay I, Harnatt P, Vichinsky E, et al. (2011) A phase 1 dose-escalation study: safety, tolerability, and pharmacokinetics of FBS0701, a novel oral iron chelator for the treatment of transfusional iron overload. Haematologica 96: 521–525.

14. Bennett TN, Pagiuio M, Gligorijevic B, Seidler C, Kusar AD, et al. (2004) Novel, rapid, and inexpensive cell-based quantification of antimalarial drug efficacy. Antimicrob Agents Chemother 48: 1807–1810.

15. Ellen GB, Singer S, Hitchings GH (1934) Antagonists of nucleic acid derivatives. VIII. Synergism in combinations of biochemically related antimalarials. J Biol Chem 208: 477–488.

16. Bell A (2005) Antimalarial drug synergism and antagonism: mechanistic and clinical significance. FEBS Microb Rev 253: 171–194.

17. Buchholz K, Burke TA, Williamson KC, Wiegand RC, Wirth DF, et al. (2011) A high-throughput screen targeting malaria transmission stages opens new avenues for drug development. J Infect Dis 203: 1445–1453.

18. Darbari D, Loyevsky M, Gordeuk V, Kark JA, Castro O, et al. (2003) Fluorescence measurements of the labile iron pool of sickle erythrocytes. Blood 102: 357–364.

19. Loyevsky M, John C, Dickens B, Hu V, Miller JH, et al. (1999) Chelation of iron within the erythrocytic *Plasmodium falciparum* parasite by iron chelators. Mol Biochem Parasitol 101: 43–59.

20. Pons E, Fibach E (2008) The labile iron pool in human erythroid cells. Br J Haematol 142: 301–307.

21. Moshnick SR, Yang YZ, Lima V, Kuypers F, Kamchonwongpaisan S, et al. (1993) Iron-dependent free radical generation from the antimalarial agent chloroquine in pneumocytes. Antimicrob Agents Chemother 37: 1108–1114.

22. Petritus PM, Burns JH Jr. (2006) Suppression of lethal *Plasmodium yoelii* malaria following protective immunization requires antibody-, IL-4-, and IFN-gamma-dependent responses induced by vaccination and/or challenge infection. J Immunol 177: 444–452.

23. Pru S, Fibach E (2008) Flow cytometry measurement of the labile iron pool in human hematopoietic cells. Cytometry A 73: 22–27.

24. Shvartsman M, Fibach E, Cabantchik ZI (2010) Transferrin-iron routing to the cytosol and man. Cancer Chemother Rep 50: 219–244.

25. Walkinger WS, Singer JA, Morrison M, Jackson CW (1984) Preferential phagocytosis of in vivo aged murine red blood cells by a macrophage-like cell line. Br J Haematol 58: 239–266.

26. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50: 219–244.