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In vitro and *In vivo* Antimalarial Activity of Amphiphilic Naphthothiazolium Salts with Amine-Bearing Side Chains

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Abstract. Because of emerging resistance to existing drugs, new chemical classes of antimalarial drugs are urgently needed. We have rationally designed a library of compounds that were predicted to accumulate in the digestive vacuole and then decrystallize hemozoin by breaking the iron carboxylate bond in hemozoin. We report the synthesis of 16 naphthothiazolium salts with amine-bearing side chains and their activities against the erythrocytic stage of *Plasmodium falciparum in vitro*. KSWI-855, the compound with the highest efficacy against the asexual stages of *P. falciparum in vitro*, also had *in vitro* activity against *P. falciparum* gametocytes and *in vivo* activity against *P. berghei* in a murine malaria model.

INTRODUCTION

Plasmodium falciparum malaria kills up to one million persons annually and accounts for up to 250 million clinical cases.[1] Only drugs can be used to cure these potentially fatal infections.[2] The efficacy of most currently used antimalarial drugs has been compromised by the development of resistance.[3] In many different malaria-endemic areas, low to high-level resistance in the predominant malaria parasites, *Plasmodium falciparum* and *P. vivax*, has been observed for chloroquine, amodiaquine, mefloquine, primaquine, and sulfadoxone-pyrimethamine. As a result, the mainstay of antimalarial treatment has become artemisinin combination therapies. However, the efficacy of even this therapy has been threatened by the emergence of delayed parasite response to artemisinin combination therapies.[4–9] Fortunately, significant progress has been made to identify multiple new preclinical leads and some promising compounds are in the early stages of development.[10] However, novel chemical classes of antimalarial compounds are still urgently needed to refresh the pipeline of new and effective antimalarial drugs and sustain progress in malaria control.[11]

Our goal was to design and test a novel chemical class of antimalarial drugs using a rational structure based drug discovery process. The hemoglobin degradation pathway is an attractive antimalarial target because of its specificity and necessity for parasite metabolism.[12] The breakdown of host hemoglobin, although essential for malaria parasite metabolism, results in near molar levels of toxic-free heme that require neutralization to prevent cellular damage. The heme moiety consists of the porphyrin ring Fe(II)-protoporphyrin IX (FP), which, as free FP, readily undergoes redox chemistry, thus generating toxic free radicals and causing oxidative damage via either the ferric ion co-ordinated in the heme moiety[13] or free hematin.[14,15]

To prevent host cell damage during intraerythrocytic growth, the malaria parasite has evolved a unique survival mechanism for detoxifying the heme groups: it crystallizes free heme molecules into an insoluble crystalline material called hemozoin. We have exploited the fact that *P. falciparum* is dependent on hemozoin formation for survival to design a new class of anti-malarial compounds that target this pathway.

We describe the synthesis and testing of a series of amphiphilic napthothiazolium drugs for antimalarial activity. These compounds were designed to bind heme via a stacking interaction with the porphyrin moiety of the heme to prevent heme dimer formation and then decrystallize hemozoin by reducing the Fe^{+3} in hemozoin to its Fe^{+2} oxidation state, thereby breaking the iron carboxylate bonds holding the crystal structure together. The most effective compound, KSWI-855 was evaluated *in vitro* against the asexual and sexual blood stages of *P. falciparum in vitro* and against *P. berghei* in a murine model of cerebral malaria.

MATERIALS AND METHODS

Chemistry. *Chemical synthesis.* 5-Hydroxynaptho[1,2-*d*] thiazolium salts are conveniently prepared by reaction of a suitably substituted thiourea derivative with a naphthoquinone in ethanolic HCl (Figure 1A). Naphthothiazolium salts were prepared by using the general procedure described.[16] Synthesis involves the reaction of a two-fold excess of a 1,4-quinone, such as 2-methyl-1,4-naphthoquinone (menadione) with an N-substituted or an N,N'-disubstituted thiourea derivative in ethanol in the presence of one equivalent of hydrochloric acid at room temperature in the dark, typically for 24–48 hours. Products were characterized by ^{1}H nuclear magnetic resonance spectroscopy, elemental analysis, and melting point. Syntheses of KSWI compounds 853, 854, 869, 870, 871, 872, 873, 874, and 875 was performed as described.[16] For the remaining seven compounds, the following syntheses are illustrative.

*1-(3-Dimethylaminopropyl)-2-cyclohexylamino-5-hydroxy-4-methylnaphtho[1,2-*d*]thiazolium chloride hydrochloride (KSWI-855).* The synthesis of KSWI-855 (Figure 1B) is representative of the synthesis of the 1,N^2-disubstituted napthothiazolium derivatives KSWI-887, KSWI-888, and KSWI 889. A solution of cyclohexyl isothiocyanate (5.65 g, 40 mmole) (Aldrich Chemicals, Milwaukee, WI) in disethyl ether (15 mL) was added to a stirred solution of 3-dimethylaminopropylamine (4.2 g, 41 mmole) (Aldrich Chemicals) in isopropanol (15 mL) with cooling in an ice bath. The mixture was allowed to reach room temperature and was stirred for 16 hours. The crystalline N-cyclohexyl-N'-(3-dimethylaminopropyl)thiourea that separated was filtered and washed with ether, yielding 7.3 g (75%) of white solid, melting point = 62–67°C. A portion of this thiourea derivative

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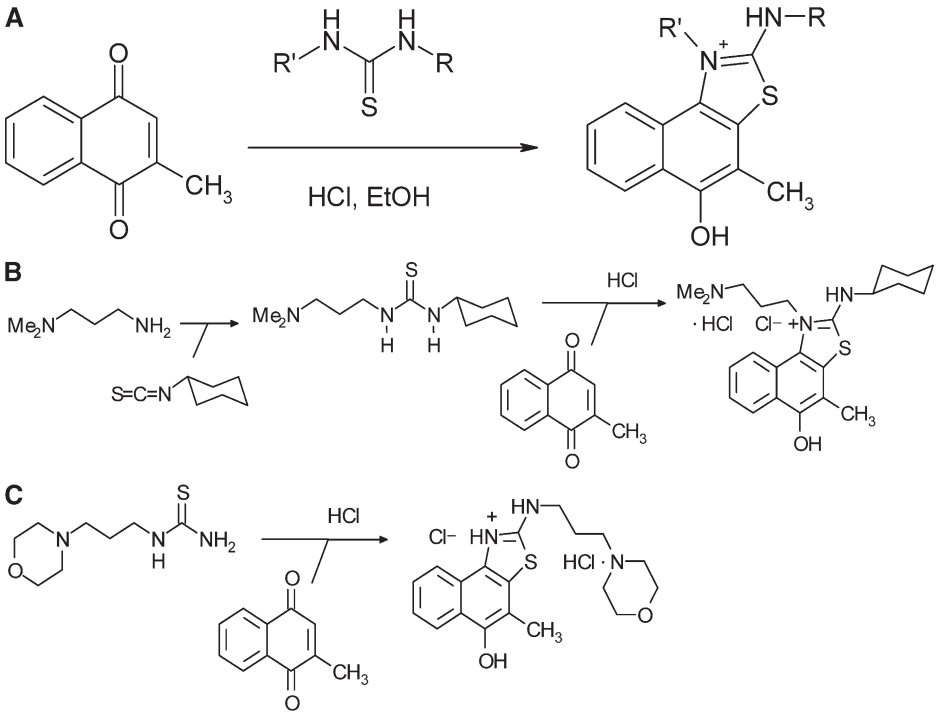


FIGURE 1. **A**, Synthesis of napthothiazolium. Naphthothiazolium salts, were prepared by mixing a two-fold excess of a 1,4-quinone, such as 2-methyl-1,4-naphthoquinone (menadione), with an N-substituted or an N,N′-disubstituted thiourea derivative in ethanol in the presence of one equivalent of hydrochloric acid at room temperature in the dark, typically for 24–48 hours. **B**, Synthesis of KSWI-855. Cyclohexyl isothiocyanate in diethyl ether was added to dimethylaminopropylamine in isopropanol. The resultant crystalline N-cyclohexyl-N′-(3-dimethylaminopropyl)thiourea was subsequently mixed with 2-methyl-1,4-naphthoquinone to form 1-(3-dimethylaminopropyl)-2-cyclohexylamino-5-hydroxy-4-methylnaphtho [1,2-*d*]thiazolium chloride hydrochloride (KSWI-855). **C**, Synthesis of KSWI-878. N-(3-(4-morpholino)propylamino)thiourea in ethanol was added to 2-methyl-1,4-naphthoquinone in ethanol to form 2-(3-(4-morpholino)propylamino)-4-methyl-5-hydroxynaphtho[1,2-*d*]thiazolium chloride hydrochloride (KSWI-878).

(2.43 g, 10 mmole) was dissolved in ethanol (12.5 mL) containing aqueous concentration HCl (1.75 mL, 20 mmole). To this stirred solution, a hot solution of 2-methyl-1,4-naphthoquinone (3.44 g, 20 mmole) in ethanol (25 mL) was added. The hot mixture was allowed to cool and was stored at room temperature in the dark for two days. The crude product that separated was filtered and washed with ethyl acetate to yield 1.86 g (40%) of crude product. Of this product, 1.5 g was recrystallized by dissolving in 20 mL of hot ethanol and adding 120 mL of warm acetone; the white powder that separated on cooling was filtered and washed with ethyl acetate to give 1.14 g (30%) of KSWI-855, melting point = 250–255°C (with decomposition).

*2-(3-(4-Morpholino)propylamino)-4-methyl-5-hydroxynaphtho [1,2-*d*]thiazolium chloride hydrochloride (KSWI-878).* A solution of N-(3-(4-Morpholino)propylamino)thiourea (1.016 g, 5 mmole) (Trans World Chemicals Inc., Rockville, MD) in ethanol (5 mL) was added to a suspension of 2-methyl-1,4-naphthoquinone (1.72 g, 10 mmole) in ethanol (Figure 1C). The mixture was treated with concentrated aqueous HCl (0.83 mL, 10 mmole) and heated until dissolved. The mixture was allowed to cool and was stored for three days at room temperature in the dark. The solvent was removed *in vacuo* and the residue was triturated with 1:1 ethanol-ethyl acetate. The light pink-tan powdery solid was filtered and dried (weight = 1.479 g). Recrystallization of 1.0 g by dissolving in 50 mL of hot methanol and diluting with 100 mL warm ethyl acetate gave on filtration and drying the title compound as 0.883 g (63%) of off-white powder, melting point = 266–270°C (with decomposition).

2-[[3-[1-[2- (aminoiminomethyl) hydrazono]ethyl]phenyl]-amino]-4-methylnaphthol[1,2-d]thiazol-5-ol dihydrochloride (KSWI-856). 3-acetylaniline (6.76 g, 50 mmole) in ethanol (50 mL) was treated with methyl isothiocyanate (3.42 mL, 50 mmole). After stirring for 18 hours at room temperature, the crystalline N-(3-acetylphenyl)-N′-methylthiourea product was filtered (7.75 g, 75% yield, melting point = 119–120°C). This thiourea (3.5 g, 16.8 mmole) was combined with 2-methylnaphthoquinone (5.8 g, 33.6 mmole) in 35 mL of ethanol in the presence of 12N aqueous HCl (1.4 mL, 16.8 mmole). After 24 hours, filtration and washing with ethanol, ethyl acetate, and ether and air drying gave 2-(3-acetylphenyl) amino-1,4-dimethyl-5-hydroxynaphtho[1,2-*d*]thiazolium chloride (1.62 g, 24%). This keto naphthothiazolium salt (0.80 g, 2.0 mmole) was heated at reflux in 80% methanol (12 mL) containing aminoguanidine hydrochloride (0.24 g, 2.2 mmole) for 40 hours and allowed to cool. Filtration yielded 0.80 g of an off-white powder. Recrystallization of 0.75 g from methanol gave 0.42 g the title compound as a pale yellow powder, melting point 252–257°C (with decomposition).

8-[1-[2-(aminoiminomethyl) hydrazono]ethyl]- 2-(butylimino)-1,2-dihydro-1,4- dimethylnaphtho[1,2-d]thiazol-5-ol monohydrochloride (KSWI-886). 6′-Methyl-2′-acetonaphthone (5.52 g, 30 mmole) (Aldrich Chemicals) (5.52 g, 30 mmole) in acetic acid (22 mL) was treated dropwise with a solution of chromium trioxide (15 g, 150 mmole) in aqueous 45% (v/v) acetic acid (20 mL) with stirring and cooling in a 25°C water bath. After stirring for 16 hours at room temperature, the mixture was diluted with 200 mL of water. The yellow solid that

separated was filtered and recrystallized from isopropanol (40 mL) to give 6-acetyl-2-methyl-1,4-naphthoquinone (2.82 g, 44%, melting point = 126°C). This quinone (1.284 g, 6.0 mmole) was combined with N-butyl-N'-methylthiourea (52) (0.584 g, 4 mmole) in ethanol (20 mL), treated with 12N HCl (0.34 mL, 4 mmole), heated to reflux, and allowed to cool. After 42 hours at room temperature, filtration gave 8-acetyl-2-butylamino-1,4-dimethyl-5-hydroxynaphtho[1,2-*d*]thiazolium chloride (0.515 g, 34%). This keto thiazolium salt (0.284 g, 0.75 mmole) was heated at reflux with aminoguanidine HCl (0.110 g, 1 mmole) in aqueous 85% ethanol (7 mL) for 44 hours. Cooling and filtration gave KSWI-886 (0.170 g, 48%, melting point = 260–264°C (with decomposition).

***In vitro* testing using *P. falciparum* asexual stages.** *Plasmodium falciparum* chloroquine-sensitive strain D10 and chloroquine-resistant strain Dd2 were grown in A+ human erythrocyte suspensions using RPMI 1640 (GIBCO, Gaithersburg, MD) medium supplemented with 25 mM HEPES (pH 7.35), 0.2% NaHCO3 (23 mM), 0.2% (D)-glucose, and 10% human A+ plasma and maintained at 37°C in candle jars. Cultures were synchronized to within 4–6 hours of each other by treating cultures with 5% D-sorbitol to select for ring stages. Parasite growth was determined by measuring incorporation of ^{3}H-hypoxanthine into the nucleic acids of the parasite as described.[17] Compound IC_{50} values (molar concentration that decreases 3H-hypoxanthine incorporation by 50% compared with compound-free controls) were calculated by extrapolation of the log dose-response curves by using curve fitting software (Origin; Microcal Software, Northampton, MA).

***In vitro* testing using *P. falciparum* gametocytes.** To test the effect of drugs on gametocyte stages, *P. falciparum* NF54 cultures were initiated in 24-well plates at 0.5% asexual parasitemia and 4% hematocrit.[18] Medium was changed daily up to day 18, without addition of fresh erythrocytes. Continuous cultivation without dilution leads to concomitant crash of asexual parasitemia and induction of gamteocytogenesis by day 5. The gametocytemia was approximately 6% at start of treatment. Drugs were dosed in wells at days 14 and 15 when most gametocytes have matured to stages III–V. Levels of gametocytemia were determined on day 18 and the mean number of gametocytes was calculated by counting 10 high-powered (1,000×) fields from triplicate wells per condition. More than 500 erythrocytes were enumerated by random scanning across a Giemsa-stained blood film.

***In vivo* testing using *P. berghei*.** C57Bl/6 mice were injected on day 0 with 10^{6} *P. berghei* (MRA-865).[19] Mice (n = 6 mice/group) were dosed intraperitoneally with each of the compounds (KSWI-854, 855, 887, 888, and 889) by using 25 mg/kg, 10 mg/kg, or 1 mg/kg at each of the following time points after infection: 2, 24, 48, and 72 hours. Blood smears were prepared on day 4 (96 hours post-infection) and stained with Giemsa. Parasitemia was assessed by counting the number of infected erythrocytes per 1,000 total erythrocytes.

RESULTS

Activity against chloroquine sensitive and multi-drug resistant *P. falciparum* strains *in vitro*. A panel of sixteen 5-hydroxynaptho[1,2-d]thiazolium salts napthothiazolium salts were synthesized. Compounds were first tested *in vitro* against chloroquine-sensitive *P. falciparum* (strain D10, IC_{50} for chloroquine = 15–30 nM[20,21]). As shown in Table 1, ten of the compounds, KSWI-854, 855, 856,869, 872, 878, 886, 887, 888, and 889, displayed potent activity *in vitro* and showed IC_{50} values ≤ 1 μM. The most effective compound, KSWI-855, had an IC_{50} of 75 nM. All of the active compounds have their amine-bearing side chain attached to the sterically crowded ring nitrogen. Lower activity was associated with absence of a side chain bearing a distal amine.

Five of the active compounds were further evaluated for activity against a multi-drug resistant *P. falciparum* strain (strain Dd2, IC_{50} for chloroquine = 60–160 nM[20,22,23]). IC_{50} values ranging from 500 nM to 75 nM for KSWI-854 and KSWI-855, respectively, were obtained. KSWI-855, [2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3-(dimethylamino) propyl]naphtho-[1,2-d]thiazol-5-ol dihydrochloride], was the most active compound against the chloroquine-sensitive *P. falciparum* strain (D10) and the multi-drug resistant strain (Dd2). KSWI-855 was further evaluated for activity against *P. falciparum* gametocytes stages III–V *in vitro* and showed modest efficacy. As shown in Figure 2, 47% of the late stage gametocytes were killed at a concentration of 4 uM. Although stage III–IV gametocytes (strain NF54) are generally less sensitive to antimalarial agents, they are sensitive to chloroquine at micromolar concentrations.

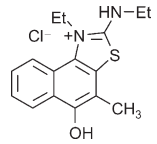
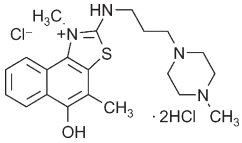
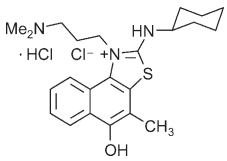
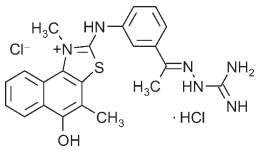
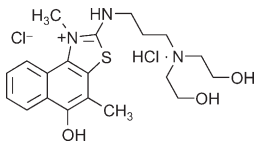
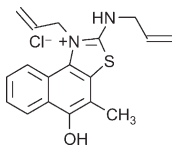
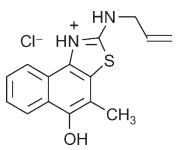
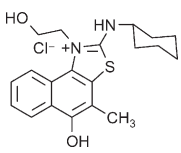
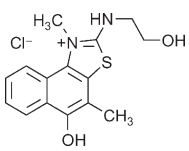
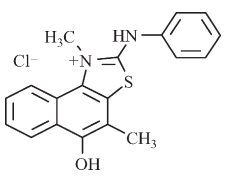
Activity against *P. berghei in vivo*. The five compounds with the lowest IC_{50} values *in vitro* against *P. falciparum,* were subsequently tested *in vivo* against the mouse malaria parasite *P. berghei* in a murine model of cerebral malaria.[24] Each compound was injected intraperitoneally at 2, 24, 48, and 72 post-infection at a concentration of 25 mg/kg. Peripheral blood smears were prepared on day 4 (96 hours post-infection) and parasitemia was assessed (Table 2). All compounds were active, although none cleared the parasitemia by day 4. KSWI-855 continued to be the most active compound and inhibited parasitemia by 84% on day 4. Survival was assessed in the mice through day 15. At day 15, survival in the untreated mice was 0%, 66% in the mice treated with KSWI-889 and 100% in the mice treated with KSWI-854, 855 and 888.

In addition, the efficacy of lower dosages of KSWI-855 and 854 was tested by administering each compound intraperitoneally at 2, 24, 48, and 72 hours post-infection at 10 mg/kg or 1 mg/kg. For both compounds, 10 mg/kg was more active than 1 mg/kg and there was an observed reduction in the mortality rate, but none of the treatments cured the mice. Treatment with KSWI-854 at a concentration of 10 mg/kg resulted in survival of 100% of the mice. Treatment with either KSWI-855 at a concentration of 10 mg/kg or KSWI-854 at a concentration of 1 mg/kg resulted in 80% survival, and treatment with KSWI- 855 at a concentration of 1 mg/kg resulted in 60% survival (Figure 3). KSWI-855 had no antimalarial activity when administered orally at a concentration of 25 mg/kg at 2, 24, 48, and 72 hours post-infection.

DISCUSSION

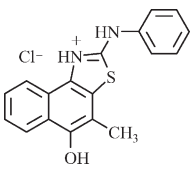
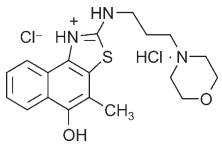
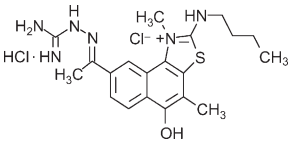
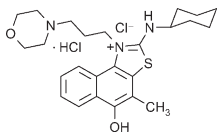
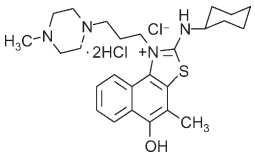
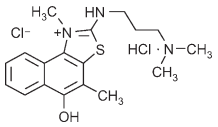
We describe the antimalarial activity of amphiphilic naphthothizolium salts with amine-bearing side chains. The most active compound, KSWI-855, has an IC_{50} of 75 nM against the chloroquine-sensitive *P. falciparum* (strain D10) and the multi-drug resistant *P. falciparum* (strain Dd2) and 4 μM against late stage (stages III–V) *P. falciparum* gametocytes. In addition, KSWI-855 demonstrates antimalarial activity

TABLE 1
Activity of napthothiazolium salts against *Plasmodium falciparum in vitro**

Structure	Compound number and chemical name	IC_{50} vs. *P. falciparum*
	KSWI-853 1-ethyl-2-(ethyl-imino)-1,2-dihydro-4-methyl-naphtho[1,2-d]thiazol-5-ol monohydrochloride	2.5 μM CQS
	KSWI-854 1,2-dihydro-1,4-dimethyl-2-[[3-(4-methyl-1-piperazinyl) propyl]imino]naphtho[1,2-d]- thiazol-5-ol trihydrochloride	0.075 μM CQS 0.5 μM CQR
	KSWI-855 2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3- (dimethylamino) propyl]naphtho- [1,2-d]thiazol-5-ol dihydrochloride	0.075 μM CQS 0.075 μM CQR
	KSWI-856 2-[[3-[1-[2- (aminoiminomethyl) hydrazono]ethyl]phenyl]-amino]-4-methylnaphthol[1,2-d]thiazol-5-ol dihydrochloride	0.83 μM CQS
	KSWI-869 2-[[3-[bis(2-hydroxyethyl)amino] propyl]imino]-1,2-dihydro-1,4-dimethylnaphtho[1,2-d]- thiazol-5-ol dihydrochloride	0.2 μM CQS
	KSWI-870 1,2-dihydro-4-methyl-1-(2-propenyl)-2-(2- propenylimino)naphtho[1,2-d]thiazol-5-ol monohydrochloride	6.0 μM CQS
	KSWI-871 4-methyl-2-(2-propenylamino) naphtho[1,2-d]-thiazol-5-ol monohydrochloride	6.3 μM CQS
	KSWI-872 2-(cyclohexylimino)-5-hydroxy-4-methyl-naphtho[1,2-d]thiazole-1(2H)-ethanol monohydrochloride	1.0 μM CQS
	KSWI-873 1,2-dihydro-2-[(2-hydroxyethyl)imino]-1,4-di-methylnaphtho[1,2-d]thiazol-5-ol monohydrochloride	1.4 μM CQS
	KSWI-874 1,2-dihydro-1,4-dimethyl-2-(phenylimino)-naphtho[1,2-d]thiazol-5-ol monohydrochloride	10 μM CQS

(continued)

TABLE 1
Continued

Structure	Compound number and chemical name	IC_{50} vs. *P. falciparum*
	KSWI-875 1,2-dihydro-4-methyl-2-(phenylimino)naphtho-[1,2-d]thiazol-5-ol monohydrochloride	4.2 μM CQS
	KSWI-878 4-methyl-2-[[3-(4-morpholino)propylamine]-naphtho[1,2-d]thiazol-5-ol dihydrochloride	0.6 μM CQS
	KSWI-886 8-[1-[2-(aminoiminomethyl) hydrazono]ethyl]-2-(butylimino)-1,2-dihydro-1,4-dimethylnaphtho[1,2-d]thiazol-5-ol monohydrochloride	0.6 μM CQS
	KSWI-887 2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3-(4-morpholino)propyl]naphtho[1,2-d]thiazol-5-ol dihydrochloride	0.33 μM CQS 0.19 μM CQR
	KSWI-888 2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3-(4-methyl-1-piperazinyl)propyl]naphtho[1,2-d]thiazol-5-ol trihydrochloride	0.16 μM CQS 0.17 μM CQR
	KSWI-889 2-[[3-(dimethylamino)propyl]imino]-1,2-dihydro-1,4-dimethylnaphtho[1,2-d]thiazol-5-ol dihydrochloride	0.21 μM CQS 0.25 μM CQR

* Compounds were screened *in vitro* for activity against *P. falciparum* chloroquine-sensitive strain D10 (CQS) and *P. falciparum* multidrug-resistant strain Dd2 (CQR) in a 96-hour growth assay. Parasite growth was determined by measuring incorporation of ^{3}H-hypoxanthine into the nucleic acids of the parasite. IC_{50} values are the molar drug concentrations that decrease ^{3}H-hypoxanthine incorporation by 50% compared with drug-free samples.

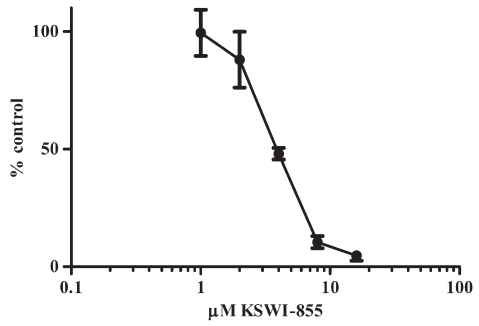
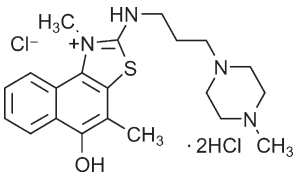
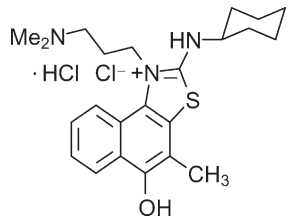
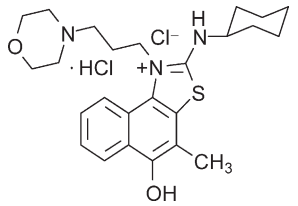
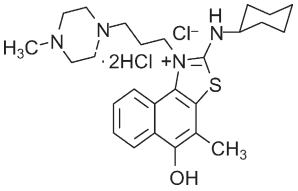
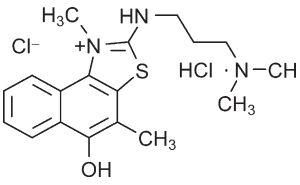


FIGURE 2. Activity of KSWI 855 against late stage *Plasmodium falciparum* gametocytes. On days 14–15 after initiation of gametocytes, increasing concentrations of KSWI-855 were effective at killing stage III–V gametocytes. On day 18, no drug control wells numbered 6.1% gametocytes per total cells of more than 500 cells counted in replicate wells. Data are expressed as percent inhibition of control wells. Error bars indicate 95% confidence intervals.

in vivo in a murine model of malaria when administered intraperitoneally but not if dosed orally.

This series of compounds was designed to hypothetically accumulate in the digestive vacuole of the malaria parasite and catalyze the decrystallization of hemozoin. Hemozoin is found in all species of *Plasmodium*, including all the currently known human pathogens (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and *P. knowlesi*.[25] Hemozoin can be found in all of the erythrocytic stages of the parasite, including the late-stage metabolically inactive stage V gametocytes. The hemoglobin degradation pathway is an attractive antimalarial target because of its specificity and necessity for parasite metabolism.[26] As the *P. falciparum* parasite grows inside the host erythrocyte, it digests and metabolizes up to 80% of the erythrocyte hemoglobin in the parasitic food vacuole.[27] Hemoglobin is hydrolyzed by a multiprotein complex containing aminopeptidases, aspartic proteases (plasmepsins), and cysteine proteases (falcipains).[28,29] This degradation of large amounts of hemoglobin is believed to be critical to provide a source of amino acids and to help maintain intracellular osmolarity during rapid parasite growth.[30] However, the breakdown of

TABLE 2
Activity of naphthothiazolium salts against *Plasmodium berghei in vivo**

Compound number and chemical name	Structure	% Inhibition of parasitemia on day 4	% Survival on day 15
	KSWI-854 1,2-dihydro-1,4-dimethyl-2-[[3-(4-methyl-1-piperazinyl) propyl]imino]naphtho[1,2-d]- thiazol-5-ol trihydrochloride	79	100
	KSWI-855 2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3- (dimethylamino) propyl]naphtho- [1,2-d]thiazol-5-ol dihydrochloride	84	100
	KSWI-887 2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3-(4-morpholino)propyl]naphtho[1,2-d]thiazol-5-ol dihydrochloride	49	0
	KSWI-888 2-(cyclohexylimino)-1,2-dihydro-4-methyl-1-[3-(4-methyl-1-piperazinyl)propyl]naphtho[1,2-d]thiazol-5-ol trihydrochloride	82	100
	KSWI-889 2-[[3-(dimethylamino)propyl]imino]-1,2-dihydro-1,4-dimethylnaphtho[1,2-d]thiazol-5-ol dihydrochloride	82	66

* Compounds were screened for *in vivo* activity by using five *berghei*. C57Bl/6 mice were injected on day 0 with 10^6 parasitized erythrocytes and then dosed intraperitoneally with 25 mg/kg of the indicated drug at each of the following time points: 2, 24, 48, and 72 hours (n = 6 mice/group). Peripheral blood smears were prepared on day 4 and parasitemia was determined. Mice were monitored daily for 15 days. Inhibition of parasitemia on day 4 = 100 – ([mean parasitemia treated/mean parasitemia control] × 100).[16] Percent survival is the percentage of mice that were alive at day 15 post-infection. The five most active compounds are shown.

host hemoglobin, results in near molar levels of toxic-free heme. The heme moiety, consisting of the porphyrin ring Fe(II)-protoporphyrin IX (FP), can readily undergo redox chemistry, generate toxic free radicals, and cause oxidative damage.[13,31] To prevent host cell damage during intraerythrocytic growth, the malaria parasite has evolved a unique survival mechanism for detoxifying heme: it crystallizes free heme molecules into hemozoin, an insoluble crystalline material.[32]

It has proven difficult to obtain an x-ray diffraction pattern of native hemozoin, but the structure of its synthetic analogue, β-hematin, has been solved definitively by x-ray powder diffraction pattern.[33] β-Hematin is not a polymer of single heme units as had been believed,[34] but rather it is composed of cyclic dimers of Fe^{+3}-protoporphyrin IX in which one propionate group of each Fe^{+3}-PPIX molecule coordinates to the Fe^{+3} center of its partner, and the dimers are linked through hydrogen bonding of the other propionic acid groups.[33] The iron-carboxylate bonds in hemozoin, which are critical to the stability of the polymer, are stable only when the iron is in the Fe+3 state. This information encouraged us to attempt the design of compounds that could hypothetically decrystallize hemozoin. The chemical challenge of this problem is formidable because the only known solubilizing agent for hemozoin is strong alkali (0.1 N NaOH).

We designed compounds that are predicted to change from complete neutrality from their initial multi-protonated state: The 2-amino group on the thiazolium ring has a relatively low pKa and can be reversibly deprotonated to a neutral thiazolone imine with concurrent deprotonation of the other side chain amine(s). We postulated that the planar naphthothiazolium ring structure in these compounds could align geometrically with the heme group in hemozoin through the formation of pi-pi complexes while the amine-bearing side chain interacted with a heme carboxylate side chain

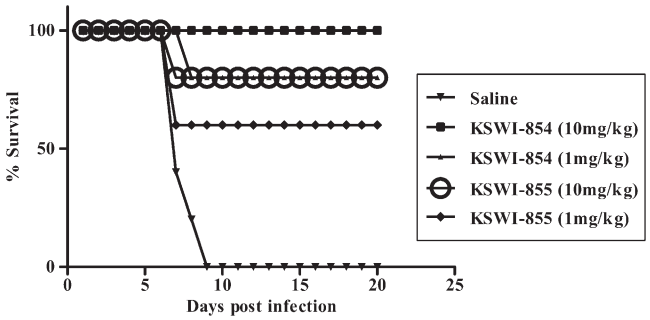


FIGURE 3. Dose effect of KSWI-855 and KSWI-854 *in vivo* in a *Plasmodium berghei* malaria model. C57Bl/6 mice were infected intraperitoneally with 2×10^7 *P. berghei* parasitized erythrocytes. KSWI-854 and KSWI-855 were injected intraperitoneally with 10 mg/kg or 1 mg/kg at 2, 24, 48, and 72 hours post-infection (n = 6 mice/group). Data for treatment groups was compared with that of the saline control group and analyzed by sing the log-rank (Mantel-Cox) test. **KSWI-854 (10 mg/kg): $P = 0.0015$. **KSWI-54 (1 mg/kg): $P = 0.008$. * KSWI-855 (10 mg/kg): $P = 0.0374$. KSWI-55 (1 mg/kg): $P = 0.0807$.

(Figure 4A). This reaction would place a naphtholic hydroxyl group near the heme-Fe3+ (Figure 4B), where it can reduce the iron atom to Fe^{2+}, thereby dissociating the heme monomer from its hemozoin neighbor, releasing free heme (Figure 4C).

We hypothesize that these amphiphilic compounds are able to reversibly enter the hemozoin-containing lipid nanospheres inside the parasite and then decrystallize hemozoin by reducing the Fe^{+3} in hemozoin to its Fe^{+2} oxidation state, thereby breaking the iron carboxylate bonds holding the crystal structure together to release free heme. Work is currently on-going to evaluate this hypothetical mechanism of action and to modify KSWI-855 to increase its hydrophilicity and bioavailability.

In conclusion, we have described the chemical synthesis and the initial characterization of a new chemical class of antimalarial compounds, the napthothiazolium salts, which have efficacy similar to chloroquine. Further studies may lead to the developed of orally active analogues that may be useful for the treatments of acute *P. falciparum* and *P. vivax* infections and to reduce malaria transmission.

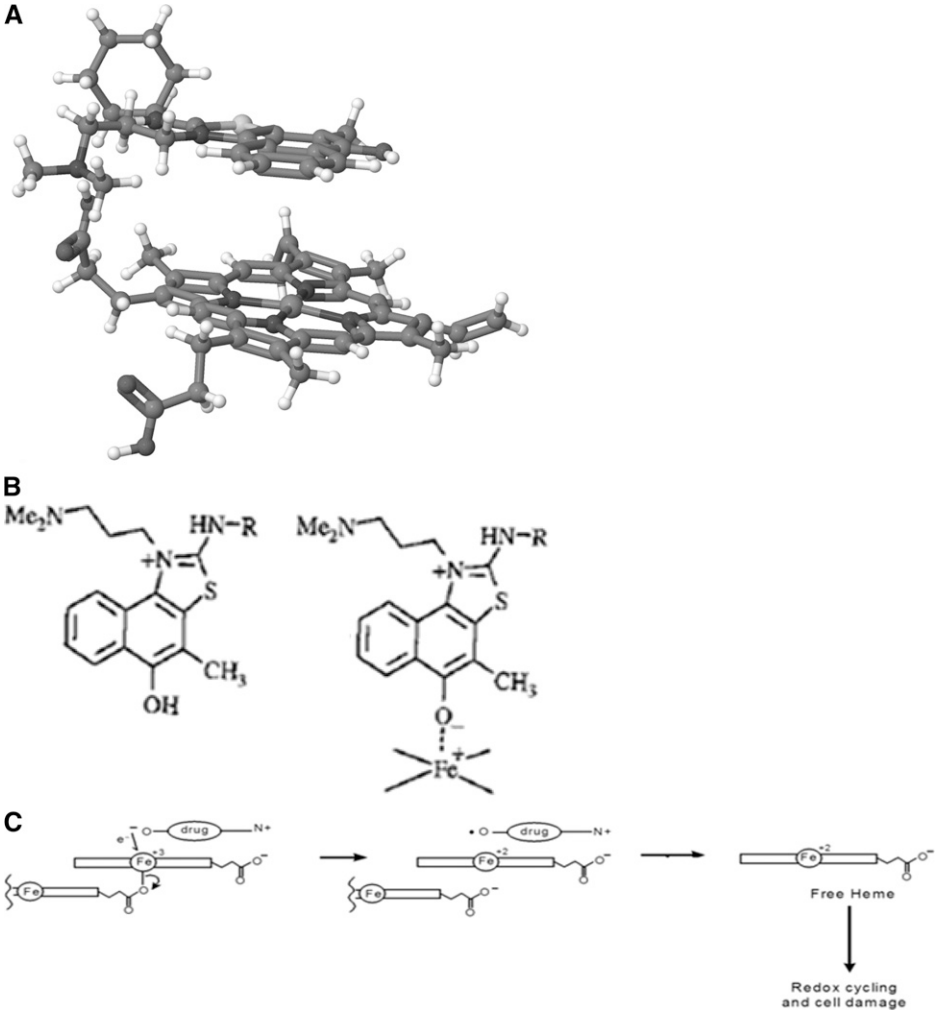


FIGURE 4. Theoretical mechanism of action. **A**, Planar ring of naphthothiazolium salts align with the heme group in hemozoin. **B**, Hydroxyl group nucleophilically attacks the heme-Fe^{3+} in the iron carboxylate bond of hemozoin. **C**, Reduction of heme-Fe^{3+} to form Fe^{2+} then catalyzes the breakdown of hemozoin through nucleophilic attack of the Fe^{3+}-carboxylate bond.

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