Synthesis and Antimalarial Effects of Phenothiazine Inhibitors of a *Plasmodium falciparum* Cysteine Protease

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Acridinediones have previously been shown to have potent antimalarial activity. A series of sulfur isosteres of acridinediones have been synthesized and evaluated for their inhibition of the *Plasmodium falciparum* cysteine protease falcipain and for their antimalarial activity. A number of these phenothiazines inhibited falcipain and demonstrated activity against cultured *P. falciparum* parasites at low micromolar concentrations. We propose that the compounds exerted their antimalarial effects by two mechanisms, one of which involves the inhibition of falcipain and a consequent block in parasite degradation of hemoglobin. These compounds and related phenothiazines are worthy of further study as potential antimalarial agents.

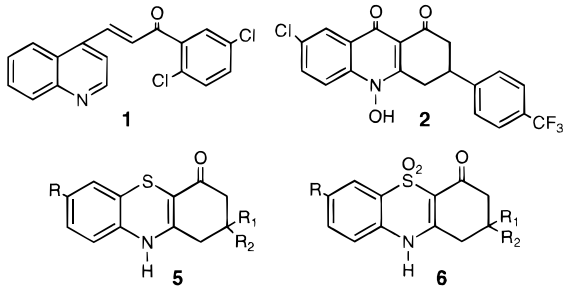
Introduction

Malaria remains one of the most important infectious disease problems in the world. Hundreds of millions of cases of malaria occur annually, and *Plasmodium falciparum*, the most serious human malaria parasite, causes over one million deaths per year.[1] A significant and increasing problem in malaria control is the resistance of malaria parasites to available chemotherapeutic agents.[2] There is, therefore, a pressing need to identify new antimalarial drugs. Ideally, new drugs will act against targets not previously exploited in antimalarial drug development.

Potential targets for chemotherapy include falcipain, a *P. falciparum* papain family cysteine protease that is a hemoglobinase required for normal parasite development.[3,4] Malaria parasites degrade hemoglobin in an acidic food vacuole to provide free amino acids for parasite protein synthesis.[5] When cultured parasites are treated with cysteine protease inhibitors directed against falcipain, hemoglobin degradation is blocked, parasite food vacuoles fill with undegraded hemoglobin, and parasite development is halted.[6]

Inhibitors of falcipain have been studied as potential antimalarial agents. Selected peptidyl fluoromethyl ketones inhibited falcipain and blocked hemoglobin degradation and development by cultured parasites at nanomolar concentrations.[6] One of these compounds was also effective in vivo against murine malaria.[7] Peptidyl vinyl sulfone inhibitors of falcipain have also demonstrated potent antimalarial effects.[8]

Nonpeptide inhibitors of falcipain have been developed through a molecular modeling approach.[9] A number of heterocyclic compounds, including a series of chalcones (**1**), have been synthesized and shown to inhibit falcipain and block the development of cultured parasites.[10] In independent studies, floxacrine (**2**) and several series of related acridinediones have been evaluated as potential antimalarials. Many of these compounds had strong activity against murine malaria parasites in vivo[11−17] and a smaller number demonstrated activity against the human parasite *P. falciparum* in culture[16] and in primate models.[16−18] In an effort to develop improved heterocyclic antimalarials potentially acting against falcipain, we have synthesized sulfur isosteres of acridinediones[19] including phenothiazin-4-one (**5**) and phenothiazin-4-one 5,5-dioxide (**6**) derivatives. In this report we describe the synthesis of **5** and **6** and their derivatives and the effectiveness of these compounds in inhibiting falcipain activity and in blocking the metabolism and development of cultured malaria parasites.



Chemistry

Derivative **5** was easily synthesized by a one-pot reaction involving condensation and oxidative cyclization, with quite good yields.[20] The preparation of 2-amino-5-halobenzenethiol derivatives[21] as key intermediates allowed us to access fluorinated compounds at position C-7 for structures **5** and **6** (Scheme 1). The intermediate 1,3-cyclohexanedione derivatives (**4**) could readily be prepared in large quantities.[22] These structures allowed the incorporation of phenyl groups substituted at different positions. Treatment of 2-amino-5-halobenzenethiol derivatives (**3**) with 1,3-cyclohexanedione derivatives (**4**) in refluxing dimethyl sulfoxide led to formation of **5**, which was monitored by TLC. The cyclization reached completion in 45 min. The products were easier to characterize after the solids were recrystallized and their structures confirmed by spectral and analytical data, as the phenothiazin-4-ones (**5a**−**p**) could then be stored under nitrogen atmosphere indefinitely

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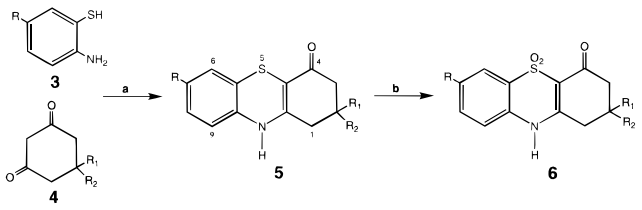
Scheme 1[a]



[a] (a) DMSO, reflux, 45 min; (b) 30% H_2O_2, AcOH, 2.5 h; R, R_1, and R_2 are as in Table 1.

or used directly for the final stage of the synthetic sequence shown in the scheme.

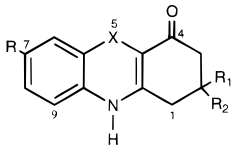
The analogs **6a**−**k** were easily obtained from compound **5** and its derivatives by treatment with 30% hydrogen peroxide in glacial acetic acid under reflux for 2.5 h. Aqueous workup resulted in material with, based on all available evidence, sulfone derivatives (**6**) as the sole product. Physicochemical data for these compounds are displayed in Table 1.

Biological Results

Ten of the phenothiazines were fairly potent inhibitors of falcipain. Each of these compounds inhibited the hydrolysis of the substrate benzyloxycarbonyl-Phe-Arg-7-amino-4-methylcoumarin (Z-Phe-Arg-AMC) with an IC_{50} of 4−5 μM (Table 1). The ability of these compounds to inhibit the development of cultured *P. falciparum* parasites was then assessed. Development in the presence of the phenothiazines was compared with that in control cultures by measuring the uptake of [^{3}H]-hypoxanthine and by counting parasites after the completion of a cycle of develoment. By each assay, most of the phenothiazines studied inhibited parasite development at low micromolar concentrations (Table 2). Two compounds, **6a** and **6k**, which lacked a phenyl ring at C-2, were much less effective. Cellular toxicity of selected compounds was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[23] and cultured Madin Darby canine kidney (MDCK) cells. One compound, **5h**, showed cellular toxicity, with an IC_{50} for toxicity of 10 μM. None of the other compounds tested showed measurable toxicity at concentrations up to 100 μM (Table 2).

We next sought to determine whether the antimalarial effects of the phenothiazines were due to the inhibition of falcipain. In our experience with multiple other classes of inhibitors, the cellular inhibition of falcipain is accompanied by the appearance of an abnormal parasite food vacuole that fills with undegraded hemoglobin.[3,6,8] Inhibited parasites subsequently halt their development, and daughter ring-stage parasites are not formed. Parasites were incubated with the phenothiazines for 24 h beginning at the ring stage and then evaluated microscopically. Typical swollen, hemoglobin-containing food vacuoles were identified after inhibition of parasites with 100 μM concentrations of most of the phenothiazines studied, indicating that these compounds had blocked hemoglobin degradation (Table 3, Figure 1). A block in hemoglobin degradation was confirmed by SDS−PAGE; proteins isolated from parasites incubated with **6a** and **6k** included much more undegraded globin than did proteins from control parasites (Figure 2). In addition to the accumulation of

Table 1. Physicochemical and Biochemical Data for Phenothiazine Derivatives



compd	R	R_1	R_2	X	mp (°C)	solvent[a]	IC_{50}[b]
5a	Cl	H	H	S	217−220	A	40
5b	Cl	CH_3	CH_3	S	272	A	30
5c	Cl	H	C_6H_5	S	260−263	B	10
5d	Cl	H	3-$CH_3OC_6H_4$	S	265−268	B	20
5e	Cl	H	4-$CH_3OC_6H_4$	S	235	B	10
5f	Cl	H	2,3-$(CH_3O)_2C_6H_3$	S	270−273	B	10
5g	Cl	H	3,4-$(CH_3O)_2C_6H_3$	S	230	B	30
5h	Cl	H	4-ClC_6H_4	S	228−230	A	4
5i	Cl	H	2,4-$Cl_2C_6H_3$	S	261−264	B	10
5j	F	CH_3	CH_3	S	282−283	A	60
5k	F	H	C_6H_5	S	250	B	20
5l	F	H	3-$CH_3OC_6H_4$	S	263	B	20
5m	F	H	2,3-$(CH_3O)_2C_6H_3$	S	280−281	B	20
5n	F	H	2,4-$Cl_2C_6H_3$	S	240−242	B	10
5o	F	H	4-ClC_6H_4	S	178−180	A	5
5p	F	H	3,4-$(CH_3O)_2C_6H_3$	S	250−253	C	20
6a	Cl	CH_3	CH_3	SO_2	340−342	C	5
6b	Cl	H	C_6H_5	SO_2	202	C	4
6c	Cl	H	4-$CH_3OC_6H_4$	SO_2	200	C	30
6d	Cl	H	2,3-$(CH_3O)_2C_6H_3$	SO_2	144−146	C	4
6e	Cl	H	3,4-$(CH_3O)_2C_6H_3$	SO_2	330−331	B	50
6f	Cl	H	4-ClC_6H_4	SO_2	238	B	4
6g	Cl	H	2,4-$Cl_2C_6H_3$	SO_2	180	C	30
6h	F	H	3-$CH_3OC_6H_4$	SO_2	296−297	B	5
6i	F	H	3,4-$(CH_3O)_2C_6H_3$	SO_2	344	C	5
6j	F	H	4-ClC_6H_4	SO_2	198	B	5
6k	F	CH_3	CH_3	SO_2	220	C	5

[a] Recrystallization solvents: A, EtOH; B, DMF; C, DMF/H_2O. [b] IC_{50} for inhibition of falcipain activity, measured as the hydrolysis of Z-Phe-Arg-AMC.

Table 2. Inhibition of Biological Activities by Phenothiazine Derivatives

	IC_{50} (μM)[a]		
compd	parasite metabolism[b]	parasite development[c]	MDCK cell metabolism[d]
5h	6	3	10
5o	4	3	>100
6a	60	20	>100
6b	5	5	ND[e]
6d	2	2	>100
6f	8	4	ND
6h	3	3	>100
6i	3	1	ND
6j	4	6	ND
6k	100	30	>100

[a] Extrapolated from curves of percent activity vs concentration for each compound. [b] Uptake of [^{3}H]hypoxanthine by cultured parasites. [c] Ring-form parasitemia, indicating completion of a developmental cycle, counted from Giemsa-stained smears after a 48 h incubation. [d] Uptake of MTT by cultured MDCK cells. [e] Not done.

Table 3. Abnormal Parasite Morphology Caused by Phenothiazine Derivatives

	parasite morphology[a]	
compd	food vacuole hemoglobin accumulation[b]	cytoplasmic vacuolization[c]
5h	0	+
5o	2+	+
6a	3+	−
6b	2+	+
6d	3+	+
6f	1+	+
6h	2+	+
6i	1+	+
6j	1+	+
6k	3+	−

[a] The morphology of trophozoites on Giemsa-stained smears was assessed after a 24h incubation with the compound. [b] After incubation with 100 μM compound, parasites were scored based on the percentage of organisms containing dark, swollen food vacuoles indicative of a block in hemoglobin degradation as follows: 0 (<10%); 1+ (10−50%); 2+ (50−90%); 3+ (>90%). [c] After incubation with 10 μM compound, parasite cytoplasmic morphology was compared to that of controls and scored as abnormal (+) or normal (−).

hemoglobin in food vacuoles, most of the phenothiazines studied also caused other morphologic abnormalities in the cultured parasites. After incubation with 10 μM **5h**, **5o**, **6b**, **6d**, **6f**, **6h**, **6i,** and **6k**, the parasites were smaller than untreated controls, with apparent vacuolization of parasite cytoplasm and limited development beyond the trophozoite stage (Table 3). These abnormalities were most pronounced with **5h**, the compound that also was toxic to MDCK cells (Table 2). Food vacuole accumulation of hemoglobin was particularly pronounced with **6a** and **6k**, the two compounds that were significantly less effective in inhibiting parasite development, but these compounds did not cause identifiable morphologic abnormalities independent of the changes in the food vacuole noted. The effects of compounds **6a** and **6k** therefore appeared to be similar to those of leupeptin, E-64, and other peptide cysteine protease inhibitors, which appear to exert antimalarial effects through a specific inhibition of falcipain.

Discussion

We have shown that phenothiazine isosteres of previously evaluated acridinediones are effective antimalarial agents, although the phenothiazines studied to date do not appear to be as potent as the most effective acridinediones. A direct comparison of results with the two classes of compounds is difficult, however, as the acridinediones have mostly been studied against murine malaria in vivo, and our studies of phenothiazines were against the human malaria parasite *P. falciparum* in vitro. In any event, the antimalarial effects of the small group of phenothiazines studied to date suggest that additional evaluation of this class is warranted.

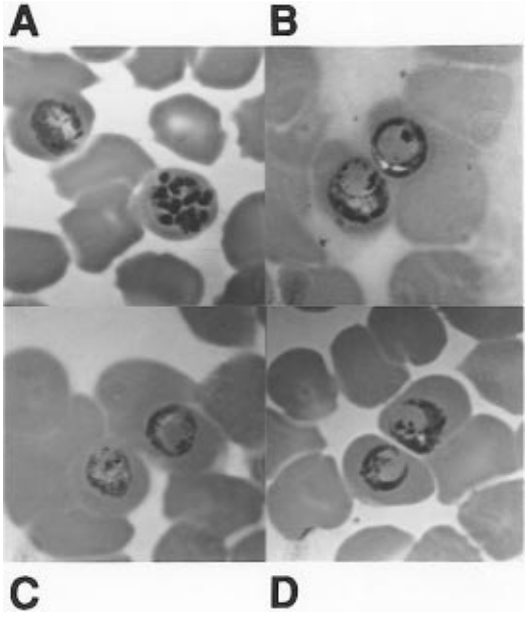


Figure 1. Morphological effects of phenothiazines. Cultured *P. falciparum* parasites were incubated with 1% DMSO (A) or the phenothiazines **6a** (100 μM; B), **6d** (100 μM; C), or **6k** (100 μM; D) for 24 h, beginning at the late ring stage. The parasites were then evaluated on Giemsa-stained smears. Control cultures (A) contained healthy late trophozoite and schizont-stage parasites. Cultures incubated with the three phenothiazines each contained abnormal forms with swollen, dark-staining food vacuoles indicative of a block in hemoglobin degradation.

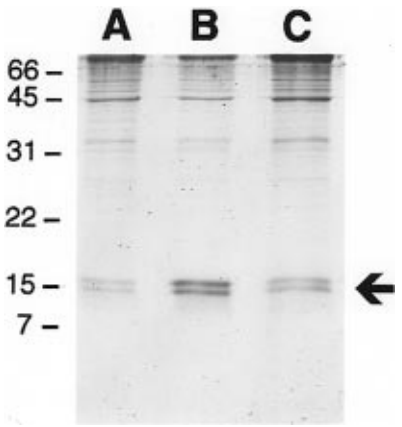


Figure 2. Inhibition of hemoglobin degradation by phenothiazines. Cultured *P. falciparum* parasites were incubated with 1% DMSO (lane A) as a control or with 100 μM **6a** (B) or **6k** (C) for 24 h beginning at the ring stage. Trophozoite-infected erythrocytes were then collected, lysed with saponin, and washed extensively, and parasite proteins were electrophoresed in 15% SDS−polyacrylamide gels and stained with Coomassie blue. The contents of about 10^7 parasites were included in each lane. Parasites incubated with the phenothiazines accumulated much more undegraded globin (arrow) than did controls incubated with the DMSO solvent. The locations of molecular weight standards are indicated in kilodaltons.

Of greatest biological interest, the phenothiazines studied inhibited the malarial cysteine protease falcipain and blocked the hydrolysis of hemoglobin, the natural substrate of falcipain. This inhibition may have been due to the binding of the phenothiazines to the falcipain active site in a manner analogous to that predicted for chalcones and other heterocyclic compounds that inhibit falcipain at similar concentrations.[10] Of particular interest, compounds **6a** and **6k** were considerably less potent antimalarials than compounds containing a C-2 phenyl group, but they inhibited falcipain activity and parasite hemoglobin degradation at concentrations similar to those at which the other phenothiazines inhibited these processes.

We propose that the phenothiazines we evaluated elicit antimalarial effects through two independent mechanisms. The first mechanism is probably the same as that of the acridinediones that have been previously identified as promising antimalarial drugs. For both acridinediones[12,15,17] and phenothiazines (compounds **6a** and **6k**), compounds lacking a phenyl ring at C-2 had decreased antimalarial efficacy. The mechanism of action of the acridinediones is unknown, but a number of the compounds have demonstrated efficacy in murine models of malaria and selected compounds cured *P. falciparum*-infected primates at doses of 20 mg/kg or less.[16,17] However, limited solubility and significant toxicity of floxacrine and perhaps other acridinediones are concerns. Phenothiazines should offer improved solubility, and the extensive use of other phenothiazines in clinical medicine suggests that toxicity may not be a problem with these compounds. The optimal phenothiazine in our studies, **6d**, may thus be considered a lead compound for the development of phenothiazine antimalarials.

The second mechanism of action of the phenothiazines against *P. falciparum* appears to involve the inhibition of the hemoglobinase falcipain. The phenothiazines effectively inhibited falcipain in an assay utilizing a standard peptide substrate. As seen with peptidyl inhibitors of falcipain, considerably higher concentrations of the phenothiazines were required for biological effects, presumably due to limitations on transport into the parasite food vacuole, the organelle in which falcipain resides and hemoglobin degradation takes place. At these higher concentrations, the phenothiazines clearly blocked hemoglobin degradation and parasite development. Antimalarial effects due to falcipain inhibition appeared to require about 10−20 times the concentration of phenothiazine required for effects due to the acridinedione-related mechanism. For compounds that elicited antimalarial effects by both of the mechanisms, the inhibition of hemoglobin degradation was overshadowed by the acridinedione-related mechanism; most inhibitors caused food vacuole hemoglobin accumulation, but parasites were clearly altered due to other effects. For compounds **6a** and **6k**, however, which lacked a phenyl ring at C-2, the only apparent antimalarial effect was that due to the inhibition of falcipain and subsequent blocks in hemoglobin degradation and parasite development. Compounds **6a** and **6k** may thus be considered lead compounds for the development of nonpeptide antimalarial agents that act specifically via the inhibition of falcipain.

In summary, our studies suggest that phenothiazines resembling previously evaluated acridinediones may be of interest as antimalarial drugs for two reasons. First, the phenothiazines share the antimalarial efficacy of the acridinediones, and this class of compounds may provide agents with improved solubility and toxicity profiles. Second, the phenothiazines inhibited the hemoglobinase falcipain and blocked parasite development through the inhibition of hemoglobin degradation. While this latter effect required quite high concentrations of the phenothiazines, modifications of these compounds, particularly with consideration of the predicted structure of falcipain,[9] should provide improved efficacy. Thus, further evaluation of phenothiazines related to the acridinedione antimalarials is likely to identify compounds with improved antimalarial efficacy.

Experimental Section

Evaluation of Biological Effects. Assays of the hydrolysis of the fluorogenic substrate Z-Phe-Arg-AMC by soluble parasite extracts containing falcipain were performed as previously described.[8] For all assays, 30 nM falcipain was incubated with 10 mM dithiothreitol and phenothiazines added from 100× stocks (in DMSO) in 0.1 M sodium acetate, pH 5.5, for 30 min at room temperature before the Z-Phe-Arg-AMC substrate (final concentration 50 μM) was added. Fluorescence caused by the cleavage of the substrate (excitation 380 nm, absorbance 460 nm) was then monitored continuously over 30 min. The rate of hydrolysis of substrate (increase in fluorescence over time) in the presence of phenothiazine inhibitors was compared with the rate of hydrolysis in controls incubated with an equivalent volume of DMSO. In each experiment, multiple concentrations of phenothiazines were evaluated in duplicate or triplicate, and IC_{50} values were extrapolated from curves of % control activity over concentration.

Parasite metabolic activity and development were evaluated by standard assays as previously described.[8] Parasites were of the Itg2 chloroquine-resistant *P. falciparum* strain. For the [^{3}H]hypoxanthine uptake assay,[24] 300 μL cultures of ring stage parasites in 96-well plates were incubated with phenothiazines (from 100× stocks in DMSO) for 24 h, [^{3}H]hypoxanthine was added (0.0144 μg per well; 1.2 μCi per well), and the cultures were maintained for an additional 18 h. The cells were then harvested and deposited on glass-fiber filters that were washed with water and dried with ethanol. [^{3}H]Hypoxanthine uptake by the parasites was quantitated by scintillation counting of the filters, the uptake by treated cultures was compared with that by control cultures containing 1% DMSO, and IC_{50} values were extrapolated from curves of percent control activity over concentration. To assess effects on parasite development, 2 mL cultures containing ring-stage parasites were incubated with phenothiazines from 100× stocks in DMSO. Medium was changed after 24 h, with maintenance of the appropriate inhibitor concentration. Parasites were collected after 24 h, when control cultures contained trophozoites, and 48 h, when controls contained nearly all new ring-stage parasites. Giemsa-stained smears were made at each time point. Parasites were evaluated microscopically for abnormal trophozoite morphology at 24 h. For evaluation of development, new ring forms per 1000 erythrocytes were counted in the 48 h smears, and the counts were compared with those of controls cultured in 1% DMSO. To quantitate the accumulation of hemoglobin in treated parasites, trophozoite-infected erythrocytes incubated with DMSO or phenothiazines as described above were collected after 24 h, treated with saponin as described previously[25] to lyse erythrocyte membranes, washed extensively, and centrifuged, and supernatants were electrophoresed in 15% SDS−polyacrylamide gels.

The cellular toxicity of phenothiazines was evaluated with a standard assay that evaluates the reduction of MTT, which requires the mitochondrial enzyme succinate dehydrogenase.[23] MDCK cells were cultured overnight in phenyl red-free RPMI with 1−100 μM concentrations of selected phenothiazines (from 100× stocks in DMSO). The culture medium was then

changed to phenyl red-free RPMI containing MTT (1 mg/mL), and after 2 h culture supernatants were removed, 50 μL of ethanol was added to each well, and MTT reduction, as indicated by absorbance at 560 nm, was compared with that of control cells that had been incubated overnight with 1% DMSO.

Chemistry. Reagent grade solvents were used without further purification. All starting materials were purchased from Aldrich. All organic extracts were dried over $MgSO_4$. TLC plates coated with silica gel 60 F_{254} (Merck) were used; detection by UV light (254 nm) or I_2 vapor was applied. Melting points were determined with a Thomas micro hot stage apparatus and are uncorrected. Proton NMR spectra (NMR) were recorded on a JEOL GSX (270 MHz) spectrometer; δ values in ppm relative to tetramethylsilane are given. IR spectra (IR) were recorded as KBr pellets using a Shimadzu model 470 spectrometer. Elemental analyses were performed by Dr. H. Daniel Lee, Purdue University; results were within 0.4% of predicted values for all compounds. Liquid secondary ion mass spectrometry (LSIMS) spectra were obtained at the Instituto Venezolano de Petroleo mass spectrometry facility.

General Method for the Synthesis of Phenothiazin-4-one Derivatives (5a−p). A mixture of 2-amino-5-halobenzenethiol (**3**; 3.6 mmol) and the appropriate 1,3-dicyclohexanedione (**4**; 3.6 mmol) was dissolved in DMSO (10 mL) and heated under reflux for 45 min. The mixtures were left to cool at ambient temperature and poured into water. The solids thus obtained were collected and recrystallized (see Experimental Section **5a−p**). In the experimental section, when R_2 in **5** or **6** includes a phenyl ring, the positions are numbered 1′−6′.

General Procedure for the Synthesis of Phenothiazin-4-one 5,5-Dioxide Derivatives (6a−k). Hydrogen peroxide (30%; 0.7 mL) was added to a solution of phenothiazin-4-one (**5**; 0.7 mmol) in glacial acetic acid (7 mL) and refluxed for 15 min. Additional 30% hydrogen peroxide (0.7 mL) was added. The reaction mixture was again refluxed for 2.5 h. The excess of solvent was removed under reduced pressure, and the mixture was poured into ice−water. The residue obtained was filtered off, washed with water repeatedly, and recrystallized from the appropriate solvent (see Experimental Section **6a−k**).

7-Chloro-1,2-dihydro-4(3*H*,10*H*)-phenothiazinone (5a) (0.46 g, 52% yield): IR 1578 (C═O), 3488 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.76−1.85 (m, 1H, CH_2, 2-H), 2.25 (t, 2H, CH_2, 1-H), 2.31 (t, 2H, CH_2, 3-H), 6.51 (d, 1H, 9-H, J = 8.7 Hz), 6.84 (d, 1H, 6-H, J = 1.48 Hz), 6.9 (dd, 1H, 8-H, J = 9.4, 1.0 Hz), 9.0 (s, 1H, NH); LSIMS m/z (M + H) 252.02. Anal. ($C_{12}H_{10}ClNSO$) C, H, N.

7-Chloro-1,2-dihydro-2,2-dimethyl-4(3*H*,10*H*)-phenothiazinone (5b) (0.80 g, 85% yield): IR 1581 (C═O), 3536 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.98 (s, 6H, CH_3), 2.14 (s, 2H, CH_2), 2.15 (s, 2H, CH, 1-H), 6.84 (s, 1H, 6-H), 6.89 (d, 1H, 8-H, J = 8.4 Hz), 6.5 (d, 1H, 9-H, J = 8.4 Hz), 8.96 (s, 1H, NH); LSIMS m/z (M + H) 280.04. Anal. ($C_{14}H_{14}ClNSO$) C, H, N.

7-Chloro-1,2-dihydro-2-phenyl-4(3*H*,10*H*)-phenothiazinone (5c) (1.03 g, 88% yield): IR 1594 (C═O), 3424 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.38−2.45 (m, 2H, CH_2, 1-H), 2.53−2.68 (m, 2H, CH_2, 3-H), 3.4−3.6 (m, 1H, 2-H), 6.52 (d, 1H, 9-H, J = 8.4 Hz), 6.87 (s, 1H, 6-H), 6.91 (dd, 1H, 8-H, J = 8.15, 1.2 Hz), 7.32 (m, 4H, Ar), 7.2−7.26 (m, 1H, 4′-H), 9.1 (s, 1H, NH); LSIMS m/z (M + H) 328.82. Anal. ($C_{18}H_{14}ClNSO$) C, H, N.

7-Chloro-1,2-dihydro-2-(3-methoxyphenyl)-4(3*H*,10*H*)-phenothiazinone (5d) (0.98 g, 77% yield): IR 1600 (C═O), 3440 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.37−2.72 (m, 4H, CH_2, 1,3-H), 3.73 (s, 3H, OCH_3), 6.52 (d, 1H, 9-H, J = 8.2 Hz), 6.80 (dd, 1H, 4′-H, J = 8.6, 1.2 Hz), 6.86−6.92 (m, 4H, Ar), 7.23 (dd, 1H, 5′-H, J = 7.2, 7.2 Hz), 9.1 (s, 1H, NH); LSIMS m/z (M + H) 358.05. Anal. ($C_{19}H_{16}ClNSO_2$) C, H, N.

7-Chloro-1,2-dihydro-2-(4-methoxyphenyl)-4(3*H*,10*H*)-phenothiazinone (5e) (1.10 g, 85% yield): IR 1600 (C═O), 3472 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.37−2.44 (m, 2H, CH_2, 1-H), 2.57−2.67 (m, 2H, CH_2, 2-H), 3.73 (s, 3H, OCH_3), 6.52 (d, 1H, 9-H, J = 8.4 Hz), 6.84 (d, 1H, 6-H, J = 2.2 Hz), 6.88 (d, 1H, 8-H, J = 3.4 Hz), 6.91 (d, 2H, 3′,5′-H, J = 5.32 Hz), 7.23 (d, 2H, 2′,6′-H, J = 5.32 Hz), 9.0 (s, 1H, NH); LSIMS m/z (M + H) 358.05. Anal. ($C_{19}H_{16}ClNSO_2$) C, H, N.

7-Chloro-1,2-dihydro-2-(2,3-dimethoxyphenyl)-4(3*H*,10*H*)-phenothiazinone (5f) (1.20 g, 87% yield): IR 1603 (C═O), 3456 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.32−2.46 (m, 2H, CH_2, 1-H), 2.54−2.69 (m, 2H, CH_2, 3-H), 3.52−3.61 (m, 1H, 2-H), 3.74 (s, 3H, OCH_3), 3.8 (s, 3H, OCH_3), 6.52 (d, 1H, 9-H, J = 8.4 Hz), 6.85−6.94 (m, 3H, Ar), 6.97 (d, 1H, 6-H, J = 1.5 Hz), 7.05 (dd, 1H, 5′-H, J = 7.9, 7.9 Hz), 9.0 (s, 1H, NH); LSIMS m/z (M + H) 388.07. Anal. ($C_{20}H_{18}ClNSO_3$) C, H, N.

7-Chloro-1,2-dihydro-2-(3,4-dimethoxyphenyl)-4(3*H*,10*H*)-phenothiazinone (5g) (1.16 g, 84% yield): IR 1600 (C═O), 3453 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.36−2.71 (m, 4H, CH_2, 1,3-H), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 6.52 (d, 1H, 9-H, J = 8.4 Hz), 6.87 (m, 1H, 6′-H), 6.9 (s, 1H, 2′-H), 6.93−6.96 (m, 2H, 6,8-H), 7.05 (d, 1H, 5′-H, J = 8.4 Hz), 9.0 (s, 1H, NH); LSIMS m/z (M + H) 388.07. Anal. ($C_{20}H_{18}ClNSO_3$) C, H, N.

7-Chloro-1,2-dihydro-2-(4-chlorophenyl)-4(3*H*,10*H*)-phenothiazinone (5h) (1.02 g, 79% yield): IR 1594 (C═O), 3440 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.38−2.71 (m, 4H, CH_2, 1,3-H), 3.38 (m, 1H, 2-H), 6.52 (d, 1H, 9-H, J = 8.7 Hz), 6.86 (s, 1H, 6-H), 6.91 (d, 1H, 8-H, J = 8.2, 1.6 Hz), 7.35 (d, 2H, 2′,6′-H, J = 9.1 Hz), 7.38 (d, 2H, 3′,5′-H, J = 9.3 Hz), 9.1 (s, 1H, NH); LSIMS m/z (M + H) 362.03. Anal. ($C_{18}H_{13}Cl_2NSO$) C, H, N.

7-Chloro-1,2-dihydro-2-(2,4-dichlorophenyl)-4(3*H*,10*H*)-phenothiazinone (5i) (0.99 g, 70% yield): IR 1578 (C═O), 3440 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.39−2.53 (m, 2H, CH_2, 1-H), 2.6−2.74 (m, 2H, CH_2, 3-H), 3.57−3.66 (m, 1H, 2-H), 6.50 (d, 1H, 9-H, J = 8.4 Hz), 6.52 (d, 1H, 6-H, J = 2.2 Hz), 6.89 (dd, 1H, 8-H, J = 6.2, 2.5 Hz), 7.48 (m, 2H, 5′,6′-H), 7.63 (s, 1H, 3′-H), 9.2 (s, 1H, NH); LSIMS m/z (M + H) 395.97. Anal. ($C_{18}H_{12}Cl_3NSO$) C, H, N.

7-Fluoro-1,2-dihydro-2,2-dimethyl-4(3*H*,10*H*)-phenothiazinone (5j) (0.71 g, 76% yield): IR 1613 (C═O), 3472 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.96 (s, 6H, CH_3), 2.12 (s, 2H, CH_2, 1-H), 2.16 (s, 2H, CH_2, 3-H), 6.51 (dd, 1H, 9-H, J = 9.3 Hz, J = 5.2 Hz), 6.63−6.7 (m, 2H, 6,8-H), 8.9 (s, 1H, NH); LSIMS m/z (M + H) 264.08. Anal. ($C_{14}H_{14}FNSO$) C, H, N.

7-Fluoro-1,2-dihydro-2-phenyl-4(3*H*,10*H*)-phenothiazinone (5k) (0.92 g, 83% yield): IR 1600 (C═O), 3472 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.16 (m, 2H, CH_2, 3-H), 2.38−2.45 (m, 2H, CH_2, 1-H), 6.68−6.76 (m, 2H, 6,8-H), 6.54 (dd, 1H, 9-H, J = 8.3 Hz, 4.9 Hz), 7.22−7.34 (m, 5H, Ar), 9.1 (s, 1H, NH); LSIMS m/z (M + H) 312.08. Anal. ($C_{18}H_{14}FNSO$) C, H, N.

7-Fluoro-1,2-dihydro-2-(3-methoxyphenyl)-4(3*H*,10*H*)-phenothiazinone (5l) (0.95 g, 78% yield): IR 1597 (C═O), 3456 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.37−2.73 (m, 4H, CH_2, 1,3-H), 3.29 (m, 1H, 2-H), 3.74 (s, 3H, OCH_3), 6.54 (dd, 1H, 9-H, J = 8.2, 5.2 Hz), 6.71−6.9 (m, 5H, Ar), 7.24 (dd, 1H, 5′-H, J = 8.2, 8.2 Hz), 9.1 (s, 1H, NH); LSIMS m/z (M + H) 342.09. Anal. ($C_{19}H_{16}FNSO_2$) C, H, N.

7-Fluoro-1,2-dihydro-2-(2,3-dimethoxyphenyl)-4(3*H*,10*H*)-phenothiazinone (5m) (1.06 g, 80% yield): IR 1613 (C═O), 3504 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.3−2.69 (m, 4H, CH_2, 1,3-H), 3.47−3.59 (m, 1H, 2-H), 3.73 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 6.53 (dd, 1H, 9-H, J = 8.2, 5.2 Hz), 6.68−6.75 (m, 2H, 6,8-H), 6.9 (d, 1H, 4′-H, J = 7.7 Hz), 6.95 (d, 1H, 6′-H, J = 7.7 Hz), 7.05 (m, 1H, 5′-H), 9.1 (s, 1H, NH); LSIMS m/z (M + H) 372.10. Anal. ($C_{20}H_{18}FNSO_3$) C, H, N.

7-Fluoro-1,2-dihydro-2-(2,4-dichlorophenyl)-4(3*H*,10*H*)-phenothiazinone (5n) (0.60 g, 44% yield): IR 1590 (C═O), 3456 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35−2.7 (m, 4H, CH_2, 1,3-H), 3.6 (m, 1H, 2-H), 6.67−6.74 (m, 2H, 6,8-H), 6.51−6.54 (m, 1H, 9-H), 7.46 (m, 2H, 5′,6′-H), 7.61 (s, 1H, 13-H), 9.12 (s, 1H, NH); LSIMS m/z (M + H) 380.05. Anal. ($C_{18}H_{12}FCl_2NSO$) C, H, N.

7-Fluoro-1,2-dihydro-2-(4-chlorophenyl)-4-(3*H*,10*H*)-phenothiazinone (5o) (0.94 g, 76% yield): IR 1581 (C═O), 3472 (NH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35−2.7 (m, 4H, CH_2, 1,3-H), 6.5−6.56 (m, 1H, 9-H), 6.66−6.73 (m, 2H, 6,8-H), 7.38 (d, 2H, 3′,5′-H, J = 8.9 Hz), 7.35 (d, 2H, 2′,6′-H, J = 8.9 Hz), 9.04 (s, 1H, NH); LSIMS m/z (M + H) 346.04. Anal. ($C_{18}H_{13}FClNSO$) C, H, N.

7-Fluoro-1,2-dihydro-2-(3,4-dimethoxyphenyl)-4(3*H*,-10*H*)-phenothiazinone (5p) (0.99 g, 75% yield): IR 1594 (C═O), 3520 (NH) cm^{-1}; ^1H NMR (DMSO-*d*) δ 2.37−2.71 (m, 4H, CH_2, 1,3-H), 3.21 (m, 1H, 2-H), 3.71 (s, 3H, OCH_3), 3.73 (s, 3H OCH_3), 6.67−6.73 (m, 2H, 7,8-H), 6.51−6.56 (m, 1H, 9-H), 6.80 (d, 1H, 6′-H, J = 8.15 Hz), 6.88 (d, 1H, 5′-H, J = 8.15 Hz), 6.95 (s, 1H, 2′-H), 9.05 (s, 1H, NH); LSIMS *m/z* (M + H) 372.10. Anal. ($C_{20}H_{18}FNSO_3 \cdot H_2O$) C, H, N.

7-Chloro-1,2-dihydro-2,2-dimethyl-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6a) (0.19 g, 66% yield): IR 1635 (C═O), 3536 (NH) 1312, 1270 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.04 (s, 6H, CH_3), 2.32 (s, 2H, CH_2, 1-H), 2.72 (s, 2H, CH_2, 3-H), 7.38 (d, 1H, 9-H, J = 8.91 Hz), 7.70 (d, 1H, 8-H, J = 1.49, 8.78 Hz), 7.85 (d, 1H, 6-H, J = 2.24 Hz), 11.48 (s, 1H, NH); LSIMS *m/z* (M + H) 312.04. Anal. ($C_{14}H_{14}ClNSO_3$) C, H, N.

7-Chloro-1,2-dihydro-2-phenyl-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6b) (0.18 g, 71% yield): IR 1648 (C═O), 3488 (NH) 1319, 1296 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.49−2.59 (m, 2H, CH_2, 1-H), 2.78−2.98 (m, 2H, CH_2, 3-H), 3.27 (m, 1H, 2-H), 7.37 (m, 5H, Ar), 7.62−7.68 (m, 1H, 4′-H), 7.87 (s, 1H, 6-H), 11.54 (s, 1H, NH); LSIMS *m/z* (M + H) 360.04. Anal. ($C_{18}H_{14}ClNSO_3$) C, H, N.

7-Chloro-1,2-dihydro-2-phenyl-2-(4-methoxyphenyl)-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6c) (0.20 g, 78% yield): IR 1699 (C═O), 3488 (NH) 1299, 1180 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.74−2.94 (m, 2H, CH_2, 1-H), 3.73 (s, 3H, OCH_3), 6.91 (d, 2H, 3′,5′-H, J = 8.64 Hz), 7.28 (d, 2H, 2′,6′-H, J = 8.64 Hz), 7.37 (d, 1H, 9-H, J = 8.9 Hz), 7.69 (dd, 1H, 8-H, J = 8.8, 2.21 Hz), 7.87 (d, 1H, 6-H, J = 2.21 Hz), 11.54 (s, 1H, NH); LSIMS *m/z* (M + H) 390.05. Anal. ($C_{19}H_{16}ClNSO_4$) C, H, N.

7-Chloro-1,2-dihydro-2-(2,3-dimethoxyphenyl)-4(1*H*,-10*H*)-phenothiazinone 5,5-dioxide (6d) (0.18 g, 62% yield): IR 1712 (C═O), 3504 (NH), 1290, 1126 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.74−2.90 (m, 2H, CH_2, 1-H), 3.76 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.95−7.1 (m, 2H, 6,4′-H), 7.72 (d, 1H, 8-H, J = 7.4 Hz), 6.92 (d, 1H, 9-H, J = 7.43 Hz), 7.4 (m, 1H, 6′-H), 7.56 (m, 1H, 5′-H), 11.49 (s, 1H, NH); LSIMS *m/z* (M + H) 420.10. Anal. ($C_{20}H_{18}ClNSO_5$) C, H, N.

7-Chloro-1,2-dihydro-2-(3,4-dimethoxyphenyl)-4-(3*H*,-10*H*)-phenothiazinone 5,5-dioxide (6e) (0.17 g, 58% yield): IR 1645 (C═O), 3472 (NH), 1306, 1146 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.72−2.95 (m, 2H, CH_2, 1-H), 3.73 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 6.84 (d, 1H, 5′-H, J = 8.15 Hz), 6.91 (d, 1H, 6′-H, J = 8.15 Hz), 7.01 (s, 1H, 2′-H), 7.38 (d, 1H, 9-H, J = 8.9 Hz), 7.71 (d, 1H, 8-H, J = 8.4 Hz), 7.89 (s, 1H, 6-H), 11.57 (s, 1H, NH); LSIMS *m/z* (M + H) 420.06. Anal. ($C_{20}H_{18}ClNSO_5$) C, H, N.

7-Chloro-1,2-dihydro-2-(4-chlorophenyl)-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6f) (0.18 g, 66% yield): IR 1645 (C═O), 3504 (NH) 1302, 1149 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.77−2.95 (m, 2H, CH_2, 1-H), 3.17−3−31 (m, 2H, CH_2, 3-H), 3.48−3.58 (m, 1H, 2-H), 7.38 (d, 1H, 9-H, J = 8.9 Hz), 7.41 (d, 2H, 2′,6′-H, J = 8.4 Hz), 7.42 (d, 2H, 3′,5′-H, J = 8.4 Hz), 7.8 (d, 1H, 6-H, J = 2.5 Hz), 11.57 (s, 1H, NH); LSIMS *m/z* (M + H) 394.27. Anal. ($C_{18}H_{13}Cl_2NSO_3$) C, H, N.

7-Chloro-1,2-dihydro-2-(2,4-dichlorophenyl)-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6g) (0.23 g, 78% yield): IR 1702 (C═O), 3472 (NH) 1393, 1152 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.79−2.99 (m, 2H, CH_2, 1-H), 3.76−3−87 (m, 1H, 2-H), 7.38 (d, 1H, 9-H, J = 8.67 Hz), 7.50 (m, 2H, 5′,6′-H), 7.65−7.72 (m, 2H, 8,3′-H), 7.88 (s, 1H, 6-H), 11.64 (s, 1H, NH); LSIMS *m/z* (M + H) 427.96. Anal. ($C_{18}H_{12}Cl_3NSO_3$) C, H, N.

7-Fluoro-1,2-dihydro-2-(3-methoxyphenyl)-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6h) (0.17 g, 66% yield): IR 1645 (C═O), 3520 (NH) 1312, 1146 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.77−2.97 (m, 2H, CH_2, 1-H), 3.18−3.28 (m, 2H, CH_2, 3-H), 3.74 (s, 3H, OCH_3), 6.81−6.85 (m, 1H, 6′-H), 6.92−6.95 (m, 2H, 2′,4′-H), 7.41 (dd, 1H, 9-H, J = 9.03, 4.5 Hz), 7.56 (m, 1H, 8-H), 7.73 (dd, 1H, 6-H, J = 2.73, J = 7.9 Hz), 11.51 (s, 1H, NH); LSIMS *m/z* (M + H) 373.08. Anal. ($C_{19}H_{16}FNSO_4$) C, H, N.

7-Fluoro-1,2-dihydro-2-(3,4-dimethoxyphenyl)-4(3*H*,-10*H*)-phenothiazinone 5,5-dioxide (6i) (0.13 g, 46% yield): IR 1642 (C═O), 3472 (NH), 1306, 1146 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.75−2.94 (m, 2H, CH_2, 1-H), 3.72 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 6.84 (d, 1H, 5′-H, J = 8.15 Hz), 6.90 (d, 1H, 6′-H, J = 7.67 Hz), 7.40 (dd, 1H, 9-H, J = 3.8, 8.53 Hz), 7.56 (m, 1H, 8-H), 7.72 (d, 1H, 6-H, J = 7.4 Hz), 11.51 (s, 1H, NH); LSIMS *m/z* (M + H) 404.07. Anal. ($C_{20}H_{18}FNSO_5$) C, H.

7-Fluoro-1,2-dihydro-2-(4-chlorophenyl)-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6j) (0.21 g, 79% yield): IR 1706 (C═O), 3472 (NH) 1306, 1123 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.76−2.95 (m, 2H, CH_2, 1-H), 7.30 (m, 1H, 9-H), 7.41 (d, 2H, 2′,6′-H, J = 7.7 Hz), 7.44 (d, 2H, 3′,5′-H, J = 7.7 Hz), 7.56 (m, 1H, 8-H), 7.73 (dd, 1H, 6-H, J = 2.9, 8.02 Hz), 11.53 (s, 1H, NH); LSIMS *m/z* (M + H) 378.81. Anal. ($C_{18}H_{13}FClNSO_3$) C, H, N.

7-Fluoro-1,2-dihydro-2,2-dimethyl-4(3*H*,10*H*)-phenothiazinone 5,5-dioxide (6k) (0.1 g, 50% yield): IR 1699 (C═O), 3504 (NH), 1312, 1155 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.04 (s, 6H, CH_3), 2.31 (s, 1H, 1-H), 2.72 (s, 1H, 3-H), 7.41 (dd, 1H, 9-H, J = 9, 4.5 Hz), 7.55 (m, 1H, 8-H), 7.7 (dd, 1H, 6-H, J = 7.70, 2.73 Hz), 11.43 (s, 1H, NH); LSIMS *m/z* (M + H) 296.07. Anal. ($C_{14}H_{14}FNSO_3$) C, H, N.

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References

(1) Walsh, J. A. Disease problems in the Third World. *Ann. N.Y. Acad. Sci.* **1989**, *569*, 1−16.
(2) Oaks, S. C., Mitchell, V. S., Pearson, G. W., Carpenter, C. C. J., Eds. *Malaria: Obstacles and Opportunities*; National Academy Press: Washington, D.C., 1991.
(3) Rosenthal, P. J.; McKerrow, J. H.; Aikawa, M.; Nagasawa, H.; Leech, J. H. A malarial cysteine proteinase is necessary for hemoglobin degradation by *Plasmodium falciparum*. *J. Clin. Invest.* **1988**, *82*, 1560−1566.
(4) Salas, F.; Fichmann, J.; Lee, G. K.; Scott, M. D.; Rosenthal, P. J. Functional expression of falcipain, a *Plasmodium falciparum* cysteine proteinase, supports its role as a malarial hemoglobinase. *Infect. Immun.* **1995**, *63*, 2120−2125.
(5) Rosenthal, P. J.; Meshnick, S. R. Hemoglobin catabolism and iron utilization by malaria parasites. *Mol. Biochem. Parasitol.* **1996**, *83*, 131−139.
(6) Rosenthal, P. J.; Wollish, W. S.; Palmer, J. T.; Rasnick, D. Antimalarial effects of peptide inhibitors of a *Plasmodium falciparum* cysteine proteinase. *J. Clin. Invest.* **1991**, *88*, 1467−1472.
(7) Rosenthal, P. J.; Lee, G. K.; Smith, R. E. Inhibition of a *Plasmodium vinckei* cysteine proteinase cures murine malaria. *J. Clin. Invest.* **1993**, *91*, 1052−1056.
(8) Rosenthal, P. J.; Olson, J. E.; Lee, G. K.; Palmer, J. T.; Klaus, J. L.; Rasnick, D. Antimalarial effects of vinyl sulfone cysteine proteinase inhibitors. *Antimicrob. Agents Chemother.* **1996**, *40*, 1600−1603.
(9) Ring, C. S.; Sun, E.; McKerrow, J. H.; Lee, G. K.; Rosenthal, P. J.; Kuntz, I. D.; Cohen, F. E. Structure-based inhibitor design by using protein models for the development of antiparasitic agents. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 3583−3587.
(10) Li, R.; Kenyon, G. L.; Cohen, F. E.; Chen, X.; Gong, B.; Dominguez, J. N.; Davidson, E.; Kurzban, G.; Miller, R. E.; Nuzum, E. O.; Rosenthal, P. J.; McKerrow, J. H. *In vitro* antimalarial activity of chalcones and their derivatives. *J. Med. Chem.* **1995**, *38*, 5031−5037.
(11) Raether, W.; Fink, E. Antimalarial activity of floxacrine (HOE 991) I: studies on blood schizontocidal action of floxacrine against *Plasmodium berghei*, *P. vinckei*, and *P. cynomolgi*. *Ann. Trop. Med. Parasitol.* **1979**, *73*, 505−526.
(12) Dürckheimer, W.; Raether, W.; Seliger, H.; Seidnath, H. 10-Hydroxy-3,4-dihydroacridine-1,9(2H,10H)-diones, a new group of malricidal and coccidiostatic compounds. *Arzneim.-Forsch./Drug Res.* **1980**, *30*, 1041−1046.
(13) Raether, W.; Fink, E. Antimalarial activity of floxacrine (HOE 991) II: studies on causal prophylactic and blood schizontocidal action of floxacrine and related dihydroacridinediones against *Plasmodium yoelii* and *P. berghei*. *Ann. Trop. Med. Parasitol.* **1982**, *76*, 507−516.

(14) Werbel, L. M.; Hung, J.; McNamara, D.; Ortwine, D. F. 3-aryl-7-chloro-3,4-dihydro-1,9(2H, 19H) acridinedione, 1-hydrazones as potent antimalarial agents (1,2). *Eur. J. Med. Chem.* **1985**, *20*, 363−370.
(15) Winkelmann, E.; Raether, W. Antimalarial and anticoccidial activity of 3-aryl-7-chloro-3,4-dihydroacridine-1,9-(2H,10H)-diones: 1-imines, 1-amines, 1-oximes, 1-hydrazones and related compounds. *Arzneim.-Forsch./Drug Res.* **1987**, *37*, 647−661.
(16) Raether, W.; Enders, B.; Hofmann, J.; Schwannecke, U.; Seidenath, H.; Hänel, H.; Uphoff, M. Antimalarial activity of new floxacrine-related acridinedione derivatives: studies on blood schizontocidal action of potential candidates against *P. berghei* in mice and *P. falciparum* in vivo and in vitro. *Parasitol. Res.* **1989**, *75*, 619−626.
(17) Kesten, S. J.; Degnan, M. J.; Hung, J.; McNamara, D. J.; Ortwine, D. F.; Uhlendorf, S. E.; Werbel, L. M. Synthesis and antimalarial properties of 1-imino derivatives of 7-chloro-3-substituted-3,4-dihydro-1,9(2H,10H)-acridinediones and related structures. *J. Med. Chem.* **1992**, *35*, 3429−3447.
(18) Schmidt, L. H. Antimalarial properties of floxacrine, a dihydroacridinedione derivative. *Antimicrob. Agents Chemother.* **1979**, *16*, 475−485.
(19) Hung, J.; McNamara, D. J.; Werbel, L. M. Synthesis of 3,4-dihydrothioxanthene-1,9-dione analogs as potential antimalarial agents [1]. *J. Heterocycl. Chem.* **1983**, *20*, 1575−1580.
(20) Gupta, A.; Saraswat, V.; Gupta, S. K.; Gupta, R.; Mukherj, S. K.; Gupta, R. R. Synthesis of 5,8-dihydro-3-methyl-4H-1,4-benzothiazines and their conversion into sulfones. *Phosphorous Sulfur Silica* **1983**, *85*, 101−106.
(21) Mital, R. L.; Kumar-Jain, S. Synthesis of some 5-substituted 2-aminobenzenethiols and their conversion into phenothiazines via Smiles rearrangement. *J. Chem. Soc. C* **1969**, 2148−2150.
(22) Yoshimoto, Y.; Tamura, Y.; Kimimoto, K.; Toda, S.; Tomita, T.; Wada, T.; Seto, E.; Murayama, M.; Shibata, Y.; Nomura, A.; Chata, K. Nonsteroidal antiinflammatory agents: I. 5-alcoxy-3-biphenylacetic acids and related compounds as new potential antiinflammatory agents. *J. Med. Chem.* **1977**, *20*, 709−714.
(23) Denizot, F.; Lang, R. Rapid colorimetric assay for cell growth and survival. *J. Immunol. Methods* **1986**, *89*, 271−277.
(24) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.
(25) Rosenthal, P. J. *Plasmodium falciparum*: Effects of proteinase inhibitors on globin hydrolysis by cultured malaria parasites. *Exp. Parasitol.* **1995**, *80*, 272−281.

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