Design, Synthesis, and Evaluation of New Chemosensitizers in Multi-Drug-Resistant *Plasmodium falciparum*

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A series of new chemosensitizers (modulators) against chloroquine-resistant *Plasmodium falciparum* were designed and synthesized in an attempt to fabricate modulators with enhancing drug-resistant reversing efficacy and minimal side effects. Four aromatic amine ring systems—phenothiazine, iminodibenzyl, iminostilbene, and diphenylamine—were examined. Various tertiary amino groups including either noncyclic or cyclic aliphatic amines were introduced to explore the steric tolerance at the end of the side chain. The new compounds showed better drug-resistant reversing activity in chloroquine-resistant than in mefloquine-resistant cell lines and were generally more effective against chloroquine-resistant *P. falciparum* isolates from Southeast Asian (W2 and TM91C235) than those from South America (PC49 and RCS). Structure−activity relationship studies revealed that elongation of the alkyl side chain of the molecule retained the chemosensitizing activity, and analogues with four-carbon side chains showed superior activity. Furthermore, new modulators with phenothiazine ring exhibited the best chemosensitizing activity among the four different ring systems examined. Terminal amino function has limited steric tolerance as evidenced by the dramatic lose of the modulating activity, when the size of substituent at the amino group increases. The best new modulator synthesized in this study possesses all three optimized structural features, which consist of a phenothiazine ring and a pyrrolidinyl group joined by a four-carbon alkyl bridge. The fractional inhibitory concentration (FIC) index of the best compound is 0.21, which is superior to that of verapamil (0.51), one of the best-known multi-drug-resistant reversing agents. Some of the analogues displayed moderate intrinsic in vitro antimalarial activity against a W-2 clone of *P. falciparum*.

Introduction

The increasing prevalence of multiple-drug-resistant (MDR) strains of *Plasmodium falciparum* in most malaria-endemic areas has significantly reduced the efficacy of current antimalarial drugs for treating or preventing malaria. For instance, resistance to the inexpensive antimalarial mainstays, such as chloroquine, is worldwide. Similarly, resistance to mefloquine, which was proposed as the drug of choice for chloroquine-resistant malaria, has been reported from Africa and Southeast Asia.[1,2] Although drug resistance is a common problem in the treatment of most microbial infections, malaria, and many neoplasms, the impact is more acute for malaria chemotherapy because of the limited number of clinically useful antimalarial drugs. Recently, considerable public attention has been directed to address this increasingly serious problem.

A wide variety of drugs representing different drug classes and diverse chemical structures have been shown to reverse chloroquine resistance in *P. falciparum*.[3−5] These include calcium channel blockers (verapamil) and calmodulin antagonists (trifluoperazine and phenothiazines), which could be coadministered with chloroquine to effectively potentiate its efficacy against chloroquine-resistant cell lines.[6,7] However, the clinical value of these modulators as MDR reversing agents was impaired by their profound antipsychotic, antihistaminic, or cardiovascular effects. The effective dose of these compounds as chemosensitizers is generally close to or higher than the therapeutic dose for other clinical applications. Therefore, the principal goal of this study is to fabricate novel chemosensitizing agents with improved anti-MDR efficacy and reduced side effects, and eventually to restore the clinical efficacy of the first-line antimalarial drugs.

Extensive structure−activity relationship studies of neoplastic MDR modulators with diverse chemical structures have established two essential features of the molecule for drug resistance reversal activity: a hydrophobic tricyclic aromatic ring and an alkyl side chain with two amino groups separated by two or three carbons. Tertiary substituted terminal amines were more potent than secondary or primary amines. However, good antipsychotic and antihistaminic agents also shared similar structural features with anti-MDR agents, except for the length of side chain.[8] Phenothiazines with ring nitrogen and side-chain nitrogen separated by two carbons showed the best antihistaminic activity, and those separated by three carbons exhibited the strongest antipsychotic activity. Thus, the length of side chain plays a crucial role in determining the pharmacological properties of the molecules. Because of the availability, the reported phenothiazines or related anti-MDR agents were compounds with side-chain length limited to 2−3 carbons.

While the optimal side-chain length of phenothiazines and related compounds for antihistaminic and antipsy-

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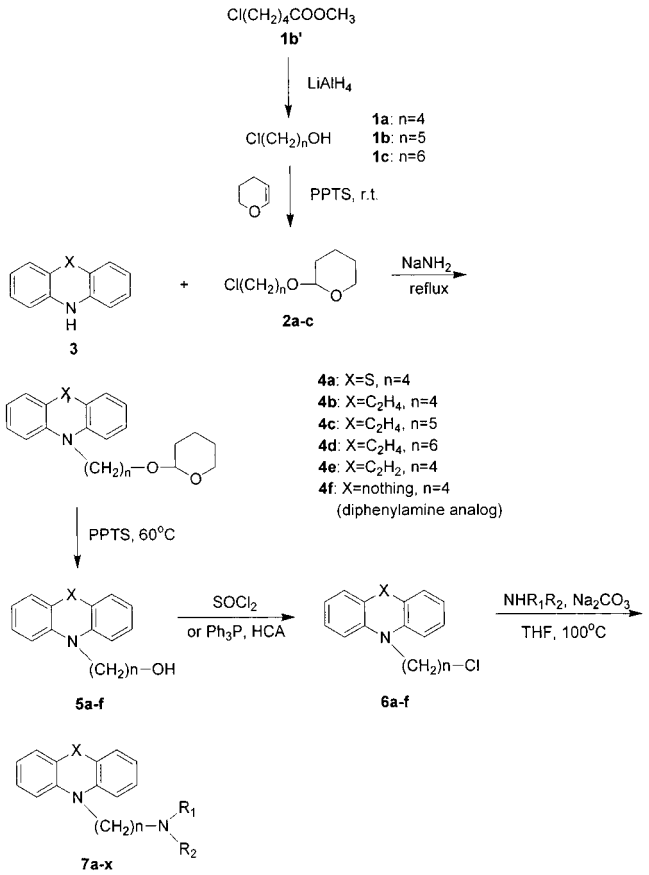
Scheme 1



chotic activities were reported to be limited to 2−3 carbons, the optimal side-chain length for anti-MDR activity has yet to be discovered. To explore the optimal length of side chain and the aromatic ring system for optimal anti-MDR activity and minimal side effects, we synthesized a series of new chemosensitizers, which included derivatives of four different heterocyclic aromatic ring systems—phenothiazine, iminodibenzyl, diphenylamine, and iminostilbene—with side-chain length from four to six carbons. Various tertiary amino groups including both cyclic and noncyclic aliphatic amines were introduced to explore steric tolerance at the terminal of the side chain. The anti-MDR activity of the target compounds were evaluated in several chloroquine- and/or mefloquine-resistant *P. falciparum* clones in vitro.

Chemistry

The chemical syntheses of the new chemosensitizing agents are shown in the synthetic Scheme 1. The hydroxyl groups of the starting materials, 4-chloro-1-butanol (**1a**) and 6-chloro-1-hexanol (**1c**), were protected by forming tetrahydropyran acetal (THP, **2**), with pyridium *p*-toluenesulfonate (PPTS) as catalyst (Scheme 1). Five-carbon side chain 5-chloro-1-pentanol (**1b**) was not commercially available and was prepared from its corresponding ester, methyl 5-chlorovalerate (**1b′**), by $LiAlH_4$ reduction of the ester group to give the starting alcohol **1b**.

The key step to the synthesis of the target compounds was the conjugation of aliphatic amine side chain with heterocyclic rings. Phenothiazine and related heterocyclic ring nitrogen failed to react with alkyl halides due to poor basicity of the heterocyclic ring nitrogen. However, N-alkylation of the ring nitrogen can be accomplished with preformed heterocyclic amine salt instead of free amine. The general procedure for the coupling of the heterocyclic rings and the alkyl side chains involved salt formation of the heterocyclic ring nitrogen with $NaNH_2$, followed by condensation of the sodium salt **3** with requisite alkyl halides (**2a**−**c**) in anhydrous xylene under reflux to afford the conjugates **4a**−**f** in 20−80% yields. The THP (tetrahydropyran) protecting group was readily removed under the catalysis of pyridinium *p*-toluenesulfonate (PPTS) in MeOH/THF (1:1) solution to give the desired alcohols **5a**−**f**. The hydroxyl groups of **5a**−**f** were converted to the corresponding chlorides **6a,b** by thionyl chloride. In some instances, thionyl chloride failed to provide satisfactory yields of the desired alkyl chlorides. In such cases, an alternative chlorinating agent, hexachloroacetone (HCA)/triphenylphosphine (Ph_3P), was employed to prepare halides **6c**−**f** in yields ranging from 60% to 90%.[9] Treatment of **6a**−**f** with appropriate amines in the presence of sodium carbonate gave the final products **7a**−**x** in 25−93% yields. The identities of the final products and the intermediates were confirmed by NMR and mass spectra and elemental analyses.

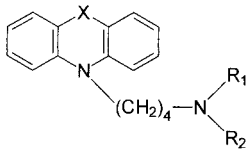
Results and Discussion

The in vitro anti-MDR effects of the new chemosensitizers were evaluated in chloroquine- and mefloquine-resistant TM91C235 cell lines, and the results are shown in Table 1. Each drug was tested at three different concentrations: 5000, 500, and 50 ng/mL in the presence of 10 ng/mL chloroquine or 5 ng/mL mefloquine. Chloroquine and mefloquine at above dose level alone showed no cell growth inhibition to TM91C235. Most of the new compounds exhibited moderate to good anti-MDR activity. For example, compounds **7e**, **7i**, **7j**, and **7m** at a concentration of 50 ng/mL completely restored the sensitivity of TM91C235 to chloroquine as observed by 99% cell suppression. Unlike chloroquine, coadministration of the test compounds **7e**, **7i**, **7j**, and **7m** with mefloquine (5 mg/mL) did not improve the cell growth inhibitory activity against TM91C235. However, compounds **7e**, **7i**, **7j**, **7k**, and **7m** exhibited moderate antimalarial activity (30%−62% cell growth suppression) in the absence of chloroquine or mefloquine, indicating that these modulators possess intrinsic antimalarial activity at <50 ng/mL concentration when used alone. Furthermore, the drug-resistant reversing efficacy of the new compounds decreased as the size of the substituents on the terminal amino group increased from dimethyl and diethyl (**7e**, **7i**, and **7j**) to methylbenzyl or dibenzyl (**7c**, **7d**, **7k**, and **7l)**.

To extensively study the magnitude of chemosensitizing potentiation of chloroquine by the new chemosensitizers in chloroquine-resistant cell lines, the combined effects of chloroquine and modulators were studied by isobologram analysis in Asian isolate W2 clone. Verapamil was used as a reference drug in this study. The *x*-axis in the isobologram graphs (Figures

1−3) represents the fractional inhibitory concentration (FIC) of chloroquine, and the *y*-axis is the FIC of

Table 1. In Vitro Reversal Activity of New Modulators in TM91C235 Cells[a]

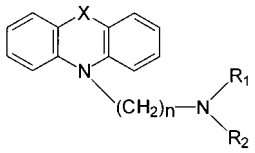


X = S Phenothiazines
X = C_2H_4 Imipramines

			control[b] (% sup)			chloroquine[c] (% sup)			mefloquine[d] (% sup)		
compd	X	R_1, R_2	5000 ng/mL	500 ng/mL	50 ng/mL	5000 ng/mL	500 ng/mL	50 ng/mL	5000 ng/mL	500 ng/mL	50 ng/mL
7a	S	CH_3, CH_3	92	27	17	100	97	30	92	31	2
7b	S	C_2H_5, C_2H_5	100	38	26	100	99	31	100	30	17
7c	S	CH_3, benzyl	35	18	13	62	27	14	13	0	0
7d	S	benzyl, benzyl	22	10	7	25	16	12	9	3	0
7e	S	pyrrolidinyl	96	70	38	100	99	99	95	67	33
7i	C_2H_4	CH_3, CH_3	96	73	59	100	99	99	95	70	43
7j	C_2H_4	C_2H_5, C_2H_5	100	95	62	100	100	99	100	95	64
7k	C_2H_4	CH_3, benzyl	60	32	30	33	20	10	41	22	20
7l	C_2H_4	benzyl, benzyl	13	17	11	13	8	6	19	22	14
7m	C_2H_4	pyrrolidinyl	100	98	58	100	100	99	100	96	64

[a] Resistant to both chloroquine and mefloquine. [b] Test compounds only. [c] Combination of 10 ng/mL of chloroquine (no effect on cell growth inhibition at this concentration alone) and test compounds. [d] Combination of 5 ng/mL of mefloquine (no effect on cell growth inhibition at this concentration alone) and test compounds. [#] % suppression of cell growth.

Table 2. FIC[a] Indices of New Modulators in *Plasmodium falciparum* W2 Clone[b]



compd	X	n	R_1, R_2	FIC
7a	S	4	CH_3, CH_3	0.23
7b	S	4	C_2H_5, C_2H_5	0.23
7e	S	4	pyrrolidinyl	0.21
7f	S	4	piperidinyl	0.49
7g	S	4	morpholinyl	0.39
7h	S	4	4-methylpiperazinyl	0.4
7i	C_2H_4	4	CH_3, CH_3	0.23
7j	C_2H_4	4	C_2H_5, C_2H_5	0.39
7m	C_2H_4	4	pyrrolidinyl	0.32
7n	C_2H_4	4	piperidinyl	0.45
7o	C_2H_4	4	morpholinyl	0.31
7p	C_2H_4	4	4-methylpiperazinyl	0.52
7q	C_2H_2	4	C_2H_5, C_2H_5	0.53
7r	C_2H_2	4	pyrrolidinyl	0.33
7s	N/A	4	C_2H_5, C_2H_5	0.48
7t	N/A	4	pyrrolidinyl	0.45
7u	C_2H_4	5	C_2H_5, C_2H_5	0.44
7v	C_2H_4	5	pyrrolidinyl	0.48
7w	C_2H_4	6	C_2H_5, C_2H_5	0.48
7x	C_2H_4	6	pyrrolidinyl	0.57
verapamil				0.51

[a] FIC = fractional inhibitory concentration (1:1 combination of drug and chloroquine). [b] Resistant to chloroquine.

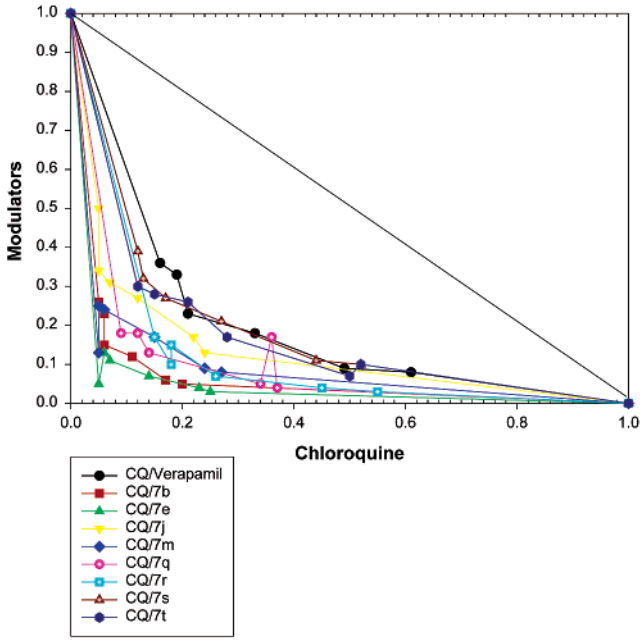


Figure 1. Isobologram of the interaction of chloroquine with modulators againts W2 clone.

the modulator. The FIC is the actual IC_{50} of one drug in the presence of the second drug but is expressed as a fraction of its IC_{50} when used alone. If the isobologram graph is a straight line, it represents an additive effect of the two drugs. If the graph forms a concave curve below the line, it indicates synergy or potentiation of the combination. If the curve is above the line, it indicates an antagonism between the two drugs. Thus, the more concave the curve exhibited, the more effective the modulator. For easy comparison, the FIC index of each tested compound is listed in Table 2. This index is a mathematical representation of an isobologram. FIC index is a combination of the FIC of both drugs. An FIC index of 1 represents the additive effect of the combination, less than 1 represents synergism, and greater than 1 means antagonism.

To study the effects of polycyclic aromatic moiety on the anti-MDR activity, modulators with four different aromatic rings—phenothiazine, iminodibenzyl, iminostilbene, and the flexible diphenylamine—were prepared. The isobolograms of the interaction of chloroquine with modulators against a W-2 clone are presented in Figures 1−3. Phenothiazine analogues **7b** and **7e**, which are represented by the most concave curves in Figure 1, showed the best MDR-reversing activity. FIC indices of these two compounds are 0.23 and 0.21 respectively (Table 2). Compounds containing saturated (**7j** and **7m**) and unsaturated seven-membered central rings (**7q** and **7r**) possessed similar activity, with FIC indices in the range of 0.32−0.53, but were less active than phenothiazine analogues. Diphenylamine analogues **7s** and **7t** were not as potent as their tricyclic

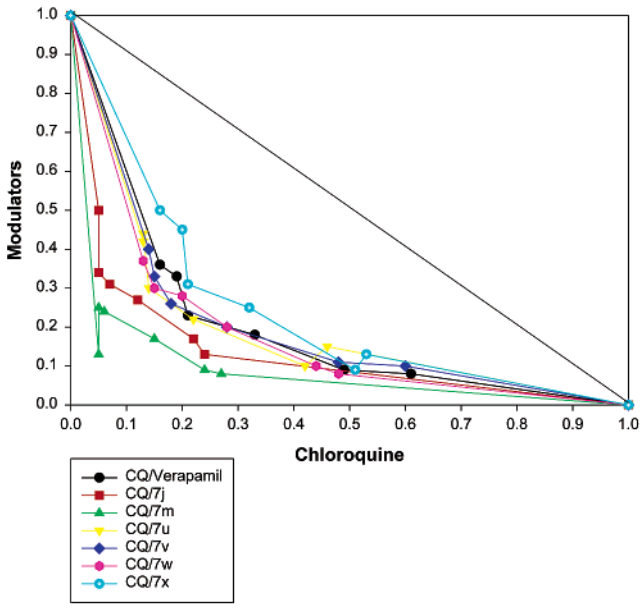


Figure 2. Isobologram of the interaction of chloroquine with imipramine derivatives against W2 clone.

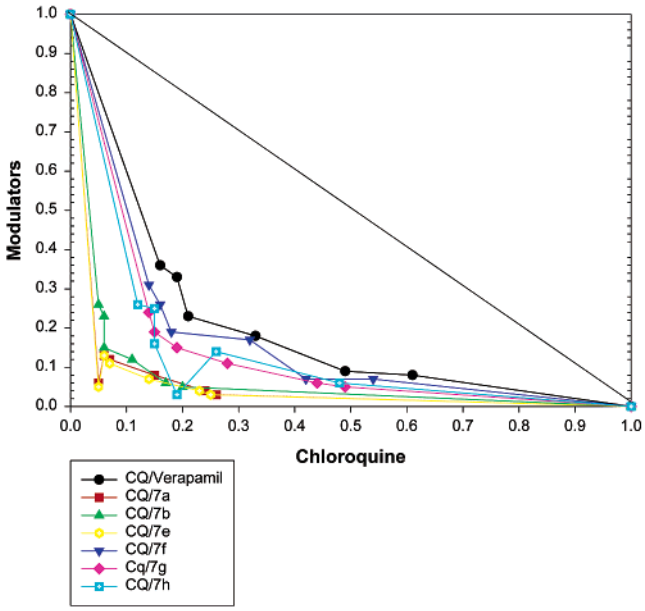


Figure 3. Isobologram of the interaction of chloroquine with phenothiazine derivatives against W2 clone.

ring counterparts and were 2-fold less active than phenothiazines **7b** and **7e** as compared by their FIC indices (Table 2). Generally, all of these compounds displayed better modulating activity than verapamil in chloroquine-resistant cell lines.

The MDR-reversing activity is also affected by the length of the alkyl bridge, which connects the hydrophobic aromatic ring and the terminal amino group. Elongation of the alkyl side chain of the modulators from three carbons to 4−6 carbons retained the chemosensitizing activity. However, the anti-MDR efficacy decreased as the length of alkyl bridge increased from four to six carbons. This trend was clearly demonstrated in Figure 2. For example, the curve of compound **7m**, which has a four-carbon alkyl linker, was more concave toward the left of the central line as compared to its five-carbon (**7v**) and six-carbon (**7x**) analogues. The results indicated that **7m** was a more potent anti-MDR agent than **7v** and **7x** (FIC = 0.32 vs 0.48 and 0.57, respectively). A similar relationship was observed with compounds **7j**, **7u**, and **7w**.

Figure 3 showed the effects of a series of phenothiazine derivatives in which the size of terminal amino functions was varied. Phenothiazine derivatives having noncyclic amines **7a** (dimethylamino) and **7b** (diethylamino) and five-membered cyclic amine **7e** (FIC values 0.23, 0.23, and 0.21, respectively) were more active chemosensitizers than were those with six-membered cyclic amines, **7f**, **7g**, and **7h** (FIC values 0.49, 0.39, and 0.4, respectively).

The anti-MDR activity and structural requirements of compounds synthesized in this study are reminiscent of those found to be important for interactions between phenothiazines and CaM (calmodulin).[10a] The relationship between structure and hydrophobicity for anti-MDR activities suggests that, similar to CaM, chemosensitizers interact in both a hydrophobic and an electrostatic manner with a protein target. Reid et al., who studied molecular modeling of phenothiazines and their interaction with the target protein, calmodulin, have proposed that there are two binding sites at a distance of half a helical turn.[10b] A hydrophobic pocket containing two aromatic phenylalanine residues interacts with the tricyclic nucleus, and a hydrophilic region, which is composed of three glutamic acid residues, interacts with the positively charged nitrogen atom of the side chain in an electrostatic manner. Similar to the proposal of Reid on the interaction between phenothiazines and calmodulin, our results indicated that the optimal length of alkyl linker probably dictated by the distance of the two binding sites in target protein. Elongation of the alkyl bridge from four carbons to 5−6 carbons may affect the binding of the ring nucleus and the amino group of the modulators to their corresponding binding sites, resulting in less active compounds. In addition, the compromised activity of **7s** and **7t** as MDR modulators suggested that the structure of diphenylamine is too flexible to fit snugly in the hydrophobic binding pocket. Thus, compounds with a restricted tricyclic ring system were better modulators than those with flexible diphenylamines. Compounds with bulky substituent at the side-chain amino group interacted poorly with acidic residues aligned in the hydrophilic pocket as indicated by the loss of MDR modulating activity in compounds containing *N*-methyl-*N*-benzylamino (**7k**) or *N*,*N*-dibenzylamino (**7l**) groups.

Drug-resistant parasites from different regions of the world responded differently to the test compounds as shown in Table 3, which listed the FIC indices of the new modulators against TM91C235 (from Thailand), RCS (from Brazil), and Peruvian PC49 (from South America). In general, the new modulators exhibited better anti-MDR activity in isolates from Southeast Asia (W2 and TM91C235) than in isolates from South America (RCS and PC49). In addition, the new test compounds also demonstrated clear differences in chemosensitizing activity observed in the two South American chloroquine-resistant isolates, RCS and PC49. The test compounds exhibited better reversing effects in the Brazil isolate (RCS) than in the Peru isolate (PC 49). While a number of compounds—**7a**, **7b**, **7e**, **7g**, **7h**, **7m**, **7q**, and **7u**—displayed significant reversing activity

Table 3. FIC Indices of New Modulators in Parasites from Different Regions

compd	TM91C235[a]	RCS[b]	Peruvian PC49[c]
verapamil	0.46	0.63	0.61
7a	0.28	0.51	NT[d]
7b	0.29	0.41	0.41
7e	0.25	0.51	0.5
7f	0.52	0.68	0.83
7g	0.32	0.37	0.81
7h	0.48	0.49	0.98
7i	0.26	0.52	0.62
7j	0.41	0.57	0.57
7m	0.42	0.51	0.66
7n	0.54	0.36	0.73
7o	0.54	0.89	0.78
7p	0.54	0.66	NT
7q	0.33	0.39	0.6
7r	0.49	0.59	0.74
7s	0.3	0.33	NT
7t	0.32	0.37	NT
7u	0.48	0.36	0.82
7v	0.55	0.37	0.59
7w	0.59	0.72	NT
7x	0.54	0.60	0.92

[a] Isolate from Southeast Asia. [b] Isolate form Brazil. [c] Isolate form South America. [d] NT, not tested.

in W2, TM91C235, and RCS (FIC = 0.5), the same test compounds exhibited weak to marginal anti-MDR activity in PC49 (FIC > 0.6).

The assessment of anti-MDR activity of the most active compound **7e** in Aotus monkey against chloroquine-resistant *P. falciparum* is currently in progress.

Conclusions

Structure−activity relationship studies of the new chemosensitizers synthesized in this study indicated that elongation of the alkyl side chain from three to six carbons retained the modulating activity, with four-carbon bridge analogues being the most active. The phenothiazine ring exhibited the best anti-MDR activity among the four different ring systems studied. Steric tolerance at the terminal amino function is limited, as evidenced by the dramatic loss of the modulating activity when the size of the substituent on the amino group increased. The most active compound **7e** combines all three optimized structural features, which consist of a phenothiazine ring and a pyrrolidinyl group joined by a four-carbon alkyl bridge. Compound **7e** (FIC = 0.21) is more than twice as active as verapamil (FIC = 0.51), one of the best-known chemosensitizers.

Experimental Section

Chemistry. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker AC-300 or Avance-600 spectrometer with Me_4Si(TMS) as the internal reference. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA, where analyses are indicated by symbols of the elements; the analytical results obtained were within ±0.4% of the theoretical values. Mass spectra were recorded on a Finnigan LCQ mass spectrometer. Silica gel (70−230 mesh), from EM, was used for column chromatography.

Preparation of 5-Chloro-1-pentanol (1b). To a solution of methyl 5-chlorovalerate (180 mg, 1.2 mmol) in 4 mL of anhydrous diethyl ether was added dropwise $LiAlH_4$ (1.2 mL of 1 M LAH solution in THF, 1.3 mmol). After 1 h, the excess of $LiAlH_4$ was decomposed by addition of ice-cold diluted sulfuric acid and the white solid was removed by filtration. The filtrate was extracted with ethyl ether three times. The ether extracts were combined, dried over anhydrous sodium sulfate, concentrated, and chromatrographed on a silica gel column. Elution of the column with 10% ethyl acetate/hexanes afforded 146 mg (100%) of colorless liquid **1b**. ^{1}H NMR ($CDCl_3$, 600 Hz) δ 3.68 (2H, t), 3.57 (2H, t), 1.84 (2H, m), 1.61 (2H, m), 1.54 (2H, m); MS (*m*/*z*) 121, 105. Anal. ($C_5H_{11}OCl$) C, H.

General Procedure for the Preparation of Compounds 2a−c: 2-[(4-Chlorobutyl)oxy]tetrahydropyran (2a). To a mixture of **1a** (0.54 g, 5 mmol) and dihydropyran (0.76 g, 6 mmol) in 15 mL of CH_2Cl_2 was added pyridinium *p*-toluenesulfonate (PPTS, 110 mg, 0.4 mmol). After being stirred at room temperature overnight, the mixture was washed with water, dried over Na_2SO_4, concentrated, and chromatrographed on a silica gel column. Elution of the column with 10% ethyl acetate/hexanes afforded 0.96 g (99%) of colorless liquid **2a**: ^{1}H NMR ($CDCl_3$, 600 Hz) δ 4.57 (1H, s), 3.86−3.75 (2H, m), 3.59−3.41 (4H, m), 1.91−1.51 (10H, m); MS (*m*/*z*) 193, 157, 103. Anal. ($C_9H_{17}O_2Cl$) C, H.

2-[(5-Chloropentyl)oxy]tetrahydropyran (2b): yield 98%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 4.57 (1H, s), 3.89−3.71 (2H, m), 3.56−3.35 (4H, m), 1.86−1.47 (12H, m); MS (*m*/*z*) 207, 105. Anal. ($C_{10}H_{19}O_2Cl\cdot0.25H_2O$) C, H.

2-[(6-Chlorohexyl)oxy]tetrahydropyran (2c): yield 97%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 4.60 (1H, s), 3.89−3.75 (2H, m), 3.57−3.40 (4H, m), 1.84−1.43 (14H, m); MS (*m*/*z*) 221, 119, 101. Anal. ($C_{11}H_{21}O_2Cl$) C, H.

General Procedure for the Preparation of Compounds 4a−f: 10-[4-[(Tetrahydropyran-2-yl)oxy]butyl]-10*H*-phenothiazine (4a). A reaction mixture containing phenothiazine (2.1 g, 10.5 mmol) and sodium amide (0.46 g, 12 mmol) in anhydrous xylenes was refluxed for 2 h. After the mixture was cooled to room temperature, compound **2a** (2.26 g, 12 mmol) was added and the mixture was refluxed for an additional 6 h. To the reaction mixture was added ice water, dropwise, to quench excess sodium amide. The mixture was filtered, washed with water, dried over Na_2SO_4 and concentrated. The residue was purified with a silica gel column and was eluted with 5% ethyl acetate/hexanes to afford 1.46 g (46%) of **4a**. ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.17 (4H, m), 6.92 (4H, m), 4.56 (1H, s), 3.94 (2H, t), 3.88−3.76 (2H, m), 3.53−3.42 (2H, m), 1.94−1.52 (10H, m); MS (*m*/*z*) 355 (M^+), 272, 200. Anal. ($C_{21}H_{25}O_2NS$) C, H, N.

***N*-[4-[(Tetrahydropyran-2-yl)oxy]butyl]iminodibenzyl (4b):** yield 31%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.15 (6H, m), 6.95 (2H, t), 4.55 (1H, s), 3.84−3.71 (4H, m), 3.50−3.39 (2H, m), 3.20 (4H, s), 1.71−1.51 (10H, m); MS (*m*/*z*) 351 (M^+), 268, 250, 196. Anal. ($C_{23}H_{29}O_2N$) C, H, N.

***N*-[5-[(Tetrahydropyran-2-yl)oxy]pentyl]iminodibenzyl (4c):** yield 64%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.14 (6H, m), 6.94 (2H, t), 4.57 (1H, s), 3.86−3.71 (4H, m), 3.87−3.70 (2H, m), 3.20 (4H, s), 1.71−1.44 (12H, m); MS (*m*/*z*) 366 (MH^+), 282, 264, 208. Anal. ($C_{24}H_{31}O_2N$) C, H, N.

***N*-[6-[(Tetrahydropyran-2-yl)oxy]hexyl]iminodibenzyl (4d):** yield 81%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.14 (6H, m), 6.94 (2H, t), 4.57 (1H, s), 3.89−3.70 (4H, m), 3.52−3.35 (2H, m), 3.19 (4H, s), 1.85−1.34 (14H, m); MS (*m*/*z*) 380 (MH^+), 296, 208. Anal. ($C_{25}H_{33}O_2N\cdot0.5H_2O$) C, H, N.

***N*-[4-[(Tetrahydropyran-2-yl)oxy]butyl]iminostilbene (4e):** yield 60%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.24 (2H, m), 7.06−6.94 (6H, m), 6.71 (2H, s), 4.49 (1H, s), 3.82−3.63 (4H, m), 3.47−3.32 (2H, m), 1.77−1.46 (10H, m); MS (*m*/*z*) 349 (M^+), 266, 248, 194. Anal. ($C_{23}H_{27}O_2N$) C, H, N.

Diphenyl[4-[(tetrahydropyran-2-yl)oxy]butyl]amine (4f): yield 55%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.29 (4H, t), 7.0 (4H, d), 6.97 (2H, t), 4.59 (1H, s), 3.87−3.75 (4H, m), 3.52−3.42 (2H, m), 1.80−1.54 (10H, m); MS (*m*/*z*) 325 (M^+), 270, 242, 224. Anal. ($C_{21}H_{27}O_2N$) C, H, N.

General Procedure for the Preparation of Compounds 5a−f: 4-(Phenothiazin-10-yl)butan-1-ol (5a): PPTS (14 mg, 0.056 mmol) was added to the solution of **4a** (214 mg, 0.56 mmol) in 10 mL of a mixture of methanol/THF (1:1). The reaction mixture was heated to 60 °C for 4 h. The solvent was evaporated to dryness under reduced pressure. The residue was dissolved in 10 mL of CH_2Cl_2, washed with water, dried over Na_2SO_4, and concentrated. The crude oil was purified with

a silica gel column and eluted with 2% CH_3OH/CH_2Cl_2 to give 153 mg (100%) of **5a**. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.16 (4H, m), 6.89 (4H, m), 3.90 (2H, t), 3.63 (2H, t), 1.86 (2H, m), 1.68 (2H, m); MS (*m*/*z*) 271 (M^+), 254, 200. Anal. ($C_{16}H_{17}ONS\cdot 0.25H_2O$) C, H, N.

4-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)butan-1-ol (5b): yield 73%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.09 (6H, m), 6.92 (2H, m), 3.75 (2H, t), 3.60 (2H, t), 3.17 (4H, s), 1.70−1.54 (4H, m); MS (*m*/*z*) 268 (MH^+), 250, 196. Anal. ($C_{18}H_{21}ON$) C, H, N.

5-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)pentan-1-ol (5c): yield 91%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.09 (6H, m), 6.89 (2H, m), 3.74 (2H, t), 3.57 (2H, t), 3.17 (4H, s), 1.69−1.32 (6H, m); MS (*m*/*z*) 282 (MH^+), 264, 208. Anal. ($C_{19}H_{23}ON$) C, H, N.

6-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)hexan-1-ol (5d): yield 83%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.08 (6H, m), 6.90 (2H, m), 3.72 (2H, t), 3.58 (2H, t), 3.16 (4H, s), 1.62−1.26 (8H, m); MS (*m*/*z*) 296 (MH^+), 278, 208. Anal. ($C_{20}H_{25}ON\cdot 0.25H_2O$) C, H, N.

4-(Dibenzo[*b*,*f*]azepin-5-yl)butan-1-ol (5e): yield 68%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.26 (2H, m), 7.03 (6H, m), 6.75 (2H, s), 3.76 (2H, t), 3.57 (2H, t), 1.78−1.57 (4H, m); MS (*m*/*z*) 266 (MH^+), 248, 194. Anal. ($C_{18}H_{19}ON\cdot 0.25CH_3CO_2C_2H_5$) C, H, N.

4-(Diphenylamino)butan-1-ol (5f): yield 78%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.26 (4H, m), 6.98 (6H, m), 3.78 (2H, t), 3.67 (2H, t), 1.80−1.55 (4H, m); MS (*m*/*z*) 242 (MH^+), 224, 170. Anal. ($C_{16}H_{19}ON$) C, H, N.

General Procedure for the Preparation of Compounds 6a, 6b, and 6e: 10-(4-Chlorobutyl)phenothiazine (6a). To a solution of **5a** (2.03 g, 7.5 mmol) in 100 mL of dry benzene was added, dropwise, thionyl chloride (2 mL, 10 mmol). The mixture was stirred at room temperature overnight. The solvent was evaporated to dryness under reduced pressure. The residue was applied on a silica gel column and eluted with 10% ethyl acetate/hexanes to yield 2.17 g (62%) of **6a**. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.16 (4H, m), 6.89 (4H, m), 3.89 (2H, t), 3.54 (2H, t), 2.02−1.85 (4H, m); MS (*m*/*z*) 290 (M^+), 254, 212, 199. Anal. ($C_{16}H_{16}NS$) C, H, N.

5-(4-Chlorobutyl)iminodibenzyl (6b): yield 62%;^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.15 (6H, m), 6.98 (2H, m), 3.81 (2H, t), 3.53 (2H, t), 3.21 (4H, s), 1.90−1.67 (4H, m); MS (*m*/*z*) 286 (M^+), 250, 208. Anal. ($C_{18}H_{20}NCl$) C, H, N.

5-(4-Chlorobutyl)-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (6e): yield 80%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.26 (2H, m), 7.02 (6H, m), 6.72 (2H, s), 3.75 (2H, t), 3.48 (2H, t), 1.85 (2H, m), 1.70 (2H, m); MS (*m*/*z*) 284 (M^+), 248, 206. Anal. ($C_{18}H_{18}NCl$) C, H, N.

General Procedure for the Preparation of Compounds 6c, 6d, and 6f:

5-(5-Chloropentyl)-5*H*-dibenzo[*b*,*f*]azepine (6c). To a cooled 25 mL round-bottom flask containing **5c** (50 mg, 0.16 mmol) and Ph_3P (51 mg, 0.2 mmol) was added hexachloroacetone (HCA, 0.05 mL, 0.3 mmol) in 1 mL of CH_2Cl_2. The reaction mixture was allowed to come to room temperature and stirred overnight. The mixture was subjected to purification by flash silica gel column chromatography, with elution first by hexane to remove the HCA and then by 5% ethyl acetate/hexanes to give 49 mg (93%) of **6c**. ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.14 (6H, m), 6.96 (2H, m), 3.78 (2H, t), 3.51 (2H, t), 3.20 (4H, s), 1.76 (2H, m), 1.62 (2H, m), 1.52 (2H, m); MS (*m*/*z*) 300 (M^+), 264, 208. Anal. ($C_{19}H_{22}NCl$) C, H, N.

5-(6-Chlorohexyl)-5*H*-dibenzo[*b*,*f*]azepine (6d): yield 100%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.14 (6H, m), 6.95 (2H, m), 3.77 (2H, t), 3.51 (2H, t), 3.20 (4H, s), 1.73 (2H, m), 1.61 (2H, m), 1.40 (4H, m); MS (*m*/*z*) 314 (M^+), 278, 208. Anal. ($C_{20}H_{24}NCl$) C, H, N.

(4-Chlorobutyl)diphenylamine (6f): yield 65%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.26 (4H, m), 6.97 (6H, m), 3.74 (2H, t), 3.54 (2H, t), 1.82 (4H, m); MS (*m*/*z*) 260 (M^+), 224, 182. Anal. ($C_{16}H_{18}NCl\cdot 0.25H_2O$) C, H, N.

General Procedure for the Preparation of Compounds 7a−x: 10-[4-(Dimethylamino)butyl]phenothiazine (7a). To a solution of **6a** (125 mg, 0.4 mmol) in 1 mL of THF were added 1 mL of dimethylamine (30% in H_2O) and a catalytic amount of Na_2CO_3. The mixture was heated to 100 °C in a sealed tube for 24 h. The reaction mixture was successively filtered, diluted with water, extracted with ethyl acetate, dried over Na_2SO_4, and concentrated. The crude oil was chromatographed on a silica gel column and eluted with 2% CH_3OH/CH_2Cl_2 to yield 56 mg (44%) of **7a**. ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.13 (4H, m), 6.88 (4H, m), 3.87 (2H, t), 2.26 (2H, t), 2.17-(6H, s), 1.83 (2H, m), 1.59 (2H, m); MS (*m*/*z*) 298 (M^+), 254, 200, 100. Anal. ($C_{18}H_{22}N_2S\cdot 0.25CH_3CO_2C_2H_5$) C, H, N.

10-[4-(Diethylamino)butyl]phenothiazine (7b): yield 53%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.13 (4H, m), 6.88 (4H, m), 3.87 (2H, t), 2.47 (6H, m), 1.81 (2H, m), 1.59 (2H, m), 0.98 (6H, s); MS (*m*/*z*) 326 (M^+), 311, 254, 128. Anal. ($C_{20}H_{26}N_2S\cdot 0.25H_2O$) C, H, N.

10-[4-(Methylbenzylamino)butyl]phenothiazine (7c): yield 86%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.26 (5H, m), 7.14 (4H, m), 6.88 (4H, m), 3.86 (2H, t), 3.50 (2H, s), 2.43 (2H, t), 2.18 (3H, s), 1.85 (2H, m), 1.68 (2H, m); MS (*m*/*z*) 375 (MH^+), 176. Anal. ($C_{24}H_{26}N_2S\cdot 0.25H_2O$) C, H, N.

10-[4-(Dibenzylamino)butyl]phenothiazine (7d): yield 92%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.26 (10H, m), 7.10 (4H, m), 6.88 (2H, t), 6.76 (2H, t), 3.73 (2H, t), 3.50 (4H, s), 2.41 (2H, t), 1.79 (2H, m), 1.63 (2H, m); MS (*m*/*z*) 451 (MH^+), 252. Anal. ($C_{30}H_{30}N_2S\cdot 0.25CH_3CO_2C_2H_5$) C, H, N.

10-[4-(Pyrrolidin-1-yl)butyl]phenothiazine (7e): yield 34%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.13 (4H, m), 6.88 (4H, m), 3.87 (2H, t), 2.45 (6H, m), 1.85 (2H, m), 1.74 (4H, m), 1.64 (2H, m); MS (*m*/*z*) 324 (M^+), 126. Anal. ($C_{20}H_{24}N_2S$) C, H, N.

10-[4-(Piperidin-1-yl)butyl]phenothiazine (7f): yield 75%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.13 (4H, m), 6.88 (4H, m), 3.85 (2H, t), 2.30 (6H, t), 1.83 (2H, m), 1.64 (2H, m), 1.58 (4H, m), 1.42 (2H, m); MS (*m*/*z*) 338 (M^+), 140. Anal. ($C_{21}H_{26}N_2S\cdot 0.25CH_3-CO_2C_2H_5$) C, H, N.

10-[4-(Morpholin-4-yl)butyl]phenothiazine (7g): yield 73%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.14 (4H, m), 6.89 (4H, m), 3.88 (2H, t), 3.64 (4H, t), 2.33 (6H, t), 1.85 (2H, m), 1.61 (2H, m); MS (*m*/*z*) 340 (M^+), 142. Anal. ($C_{20}H_{24}N_2SO$) C, H, N.

10-[4-(4-Methylpiperazin-1-yl)butyl]phenothiazine (7h): yield 31%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.14 (4H, m), 6.89 (4H, m), 3.87 (2H, t), 2.35 (10H, m), 2.27 (3H, s), 1.84 (2H, m), 1.62 (2H, m); MS (*m*/*z*) 354 (MH^+), 155. Anal. ($C_{21}H_{27}N_3S\cdot 1H_2O$) C, H, N.

[4-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)butyl]dimethylamine (7i): yield 54%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.10 (6H, m), 6.91 (2H, m), 3.74 (2H, t), 3.15 (4H, s), 2.22 (2H, t), 2.18 (6H, s), 1.55 (4H, m); MS (*m*/*z*) 294 (M^+), 100. Anal. ($C_{20}H_{26}N_2\cdot 0.25H_2O$) C, H, N.

[4-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)butyl]diethylamine (7j): yield 42%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.10 (6H, m), 6.90 (2H, m), 3.74 (2H, t), 3.16 (4H, s), 2.45 (4H, q), 2.34 (2H, t), 1.52 (4H, m), 0.95 (6H, t); MS (*m*/*z*) 322 (M^+), 128. Anal. ($C_{22}H_{30}N_2$) C, H, N.

[4-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)butyl]methylbenzylamine (7k): yield 93%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.23 (5H, m), 7.05 (6H, m), 6.89 (2H, t), 3.65 (2H, t), 3.41 (2H, s), 3.20 (4H, s), 2.28 (2H, t), 2.16 (3H, s), 1.52 (4H, m); MS (*m*/*z*) 370 (M^+), 176. Anal. ($C_{26}H_{30}N_2\cdot 0.25CH_3CO_2C_2H_5$) C, H, N.

[4-(10,11-Dihydrodibenzo[*b*,*f*]azepin-5-yl)butyl]dibenzylamine (7l): yield 46%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.23 (10H, m), 7.05 (6H, m), 6.89 (2H, t), 3.62 (2H, t), 3.46 (4H, s), 3.13 (4H, s), 2.33 (2H, t), 1.52 (4H, m); MS (*m*/*z*) 447 (MH^+), 355, 252. Anal. ($C_{32}H_{34}N_2\cdot 0.5H_2O$) C, H, N.

5-[4-(Pyrrolidin-1-yl)butyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*f*]azepine (7m): yield 44%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.09 (6H, m), 6.90 (2H, m), 3.74 (2H, t), 3.16 (4H, s), 2.39 (6H, m), 1.73 (4H, m), 1.57 (4H, m); MS (*m*/*z*) 320 (M^+), 126. Anal. ($C_{22}H_{28}N_2\cdot 0.25H_2O$) C, H, N.

5-[4-(Piperidin-1-yl)butyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*f*]azepine (7n): yield 75%; ^{1}H NMR ($CDCl_3$, 600 Hz) δ 7.13 (6H, m), 6.94 (2H, m), 3.77 (2H, t), 3.20 (4H, s), 2.28 (6H, m), 1.58 (10H, m); MS (*m*/*z*) 334 (M^+), 140. Anal. ($C_{23}H_{30}N_2$) C, H, N.

5-[4-(Morpholin-4-yl)butyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*f*]azepine (7o): yield 88%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.09

(6H, m), 6.90 (2H, m), 3.74 (2H, t), 3.64 (4H, t), 3.15 (4H, s), 2.32 (4H, t), 2.25 (2H, t), 1.55 (4H, m); MS (*m*/*z*) 336 (M^+), 142. Anal. ($C_{22}H_{28}N_2O$) C, H, N.

5-[4-(4-Methylpiperazin-1-yl)butyl]-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (7p): yield 72%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.09 (6H, m), 6.90 (2H, m), 3.73 (2H, t), 3.15 (4H, s), 2.36 (10H, m), 2.26 (3H, s), 1.54 (4H, m); MS (*m*/*z*) 349 (M^+), 155. Anal. ($C_{23}H_{31}N_3 \cdot 0.25H_2O$) C, H, N.

[4-(Dibenzo[b,f]azepin-5-yl)butyl]diethylamine (7q): yield 61%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.24 (2H, m), 7.00 (6H, m), 6.71 (2H, s), 3.72 (2H, t), 2.45 (4H, q), 2.35 (2H, t), 1.52 (4H, m), 0.95 (6H, t); MS (*m*/*z*) 320 (M^+), 128. Anal. ($C_{22}H_{28}N_2$) C, H, N.

5-[4-(Pyrrolidin-1-yl)butyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*f*]azepine (7r): yield 48%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.24 (2H, m), 7.00 (6H, m), 6.71 (2H, s), 3.72 (2H, t), 2.40 (6H, m), 1.73 (4H, m), 1.60 (4H, m); MS (*m*/*z*) 319 (MH^+), 126. Anal. ($C_{22}H_{26}N_2 \cdot 0.25H_2O$) C, H, N.

N,N-Diethyl-N′,N′-diphenylbutane-1,4-diamine (7s): Yield 40%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.25 (4H, m), 6.96 (6H, m), 3.71 (2H, t), 2.51 (4H, q), 2.42 (2H, t), 1.65 (2H, m), 1.50 (2H, m), 1.00 (6H, t); MS (*m*/*z*) 297 (MH^+), 224, 128. Anal. ($C_{20}H_{28}N_2 \cdot 0.25H_2O$) C, H, N.

Diphenyl[(4-(pyrrolidin-1-yl)butyl]amine (7t): yield 25%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.25 (4H, m), 6.96 (6H, m), 3.71 (2H, t), 2.51 (4H, m), 2.47 (2H, t), 1.79 (4H, m), 1.70 (2H, m), 1.58 (2H, m); MS (*m*/*z*) 294 (M^+), 126. Anal. ($C_{20}H_{26}N_2 \cdot 0.25CH_3CO_2C_2H_5$) C, H, N.

5-[5-(Diethylamino)pentyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*f*]azepine (7u): yield 29%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.08 (6H, m), 6.90 (2H, m), 3.72 (2H, t), 3.15 (4H, s), 2.47 (4H, q), 2.33 (2H, t), ~1.30−1.62 (6H, m), 0.97 (6H, t); MS (*m*/*z*) 337 (MH^+). Anal. ($C_{23}H_{32}N_2 \cdot 0.25H_2O$) C, H, N.

5-[5-(Pyrrolidin-1-yl)pentyl]-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (7v): yield 50%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.08 (6H, m), 6.89 (2H, m), 3.72 (2H, t), 3.15 (4H, s), 2.44 (4H, m), 2.36 (2H, t), 1.74 (4H, m), ~1.28−1.62 (6H, m); MS (*m*/*z*) 335 (MH^+), 140. Anal. ($C_{23}H_{30}N_2 \cdot 0.25CH_3CO_2C_2H_5$) C, H, N.

5-[6-(Diethylamino)hexyl]-10,11-dihydro-5*H*-dibenzo-[*b*,*f*]azepine (7w): yield 36%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.10 (6H, m), 6.90 (2H, m), 3.71 (2H, t), 3.15 (4H, s), 2.62 (4H, q), 2.47 (2H, t), ~1.20−1.60 (8H, m), 1.05 (6H, t); MS (*m*/*z*) 351 (MH^+). Anal. ($C_{24}H_{34}N_2 \cdot 0.25H_2O$) C, H, N.

5-[6-(Pyrrolidin-1-yl)hexyl]-10,11-dihydro-5H-dibenzo-[b,f]azepine (7x): yield 45%; ^{1}H NMR ($CDCl_3$, 300 Hz) δ 7.09 (6H, m), 6.89 (2H, m), 3.71 (2H, t), 3.15 (4H, s), 2.44 (4H, m), 2.34 (2H, t), 1.75 (4H, m), ~1.20−1.60 (8H, m); MS (*m*/*z*) 349 (MH^+). Anal. ($C_{24}H_{32}N_2 \cdot 0.25H_2O$) C, H, N.

Biology: In Vitro Drug Susceptibility Methods. The in vitro antimalarial drug susceptibility assay used was a modification of the procedures first published by Desjardins et al. with modifications developed by Milhous et al.[11,12] In brief, the assay is based on the incorporation of radiolabeled hypoxanthine by the parasites, and inhibition of isotope incorporation is attributed to activity of known or candidate antimalarial drugs. For each assay, proven antimalarials, such as chloroquine, mefloquine, quinine, artemisinin, pyrimethamine, and sulfadoxine, were used as controls. The incubation period was 66 h and the starting parasitemia was 0.2% with a 1% hematocrit. The medium used was RPMI-1640 culture medium with no folate or *p*-aminobenzoic acid (PABA). Albumax may be used instead of 10% normal heat-inactivated human plasma. The primary difference in Albumax versus human plasma is less protein binding of the drug and, hence, many compounds are slightly more active in this model.

If a candidate compound was tested without any prior knowledge of its activity or solubility, the compound was dissolved directly in dimethyl sulfoxide (DMSO) and diluted 400-fold with complete culture medium. These unknown compounds were normally started at a highest concentration of about 50 000 ng/mL. The compounds were then diluted 2-fold, 11 times, to give a concentration range of about 1048-fold. These dilutions were performed automatically by a Biomek 1000 or 2000 liquid handling system into 96-well microtiter plates. Aliquots (25 μL) of each diluted candidate compound were then transferred to test plates, 200 μL of parasitized erythrocytes (0.2% parasitemia and 1% hematocrit) was added to each aliquot, and the plates were incubated at 37 °C in a controlled environment of 5% CO_2, 5% O_2, and 90% N_2. After 42 h, 25 μL of ^{3}H-hypoxanthine was added and the plates were incubated for an additional 24 h. At the end of the 66 h incubation period, the plates were frozen at −70 °C to lyse the red cells and later thawed and harvested onto glass fiber filter mats by using a 96-well cell harvester. The filter mats were then counted in a scintillation counter and the data were analyzed. For each drug, the concentration−response profile is determined and 50% inhibitory concentrations (IC_{50}) and 90% inhibitory concentrations (IC_{90}) are determined by use of a nonlinear, logistic dose−response analysis program.

Evaluation of Chemosensitizing Activity of the New Modulators. Fifty percent inhibitory concentrations (IC_{50s}) were determined for each candidate compound alone and for candidate compounds in fixed combinations of their respective IC_{50s} (1:1, 1:3, 3:1). These data were used to calculate fractional inhibitory concentration (FICs).[13] The FIC is the actual IC_{50} of one compound in the presence of a second compound but is expressed as a fraction of its IC_{50} when used alone. This index is a mathematical representation of the isobologram such that an FIC index of 1.0 represents the line of additive on the isobologram. An FIC index of less than 1.0 represents synergy or potentiation, and an FIC index of greater than 1.0 represents antagonism.

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