Structural Properties of Dibenzosuberanylpiperazine Derivatives for Efficient Reversal of Chloroquine Resistance in *Plasmodium chabaudi*

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Received September 4, 2002

For the purpose of developing chemosensitizers to reverse chloroquine (CQ) resistance in *Plasmodium chabaudi* in vivo, dibenzosuberanylpiperazine (1-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cyclohepten-5-yl)piperazine) (DSP) and its piperazin-1-yl derivatives were synthesized systematically. DSP hydrochloride (**3**) was obtained from the reaction of dibenzosuberanyl chloride with piperazine in the presence of 1,8-diazabicyclo[5,4,0]-7-undecene (DBU). To understand the relationship between the substituent patterns of DSP derivatives and their biological activities, 13 hydroxyalkyl or hydroxyalkenyl derivatives were synthesized by an attack of the piperazine secondary amine of **3** on commercially available epoxides in the presence of triethylamine or DBU, and three alkyl or alkynyl derivatives were synthesized by the reactions of **3** with the corresponding organic chlorides in the presence of DBU. In both reactions, the yield was a maximum of 90%. The biological activities of the synthesized compounds were evaluated on the basis of two values: antimalarial activity and reversal activity. The values of antimalarial activities by single administration of 17 test compounds were not effective, being in the range 67−152% on day 4 after infection of *Plasmodium chabaudi* to mice except for the administration of 3-(dibenzosuberanylpiperazin-1-yl)-1-butene (**29**, 22%). On the other hand, administration of the seven test compounds (50 mg/kg dose) combined with CQ (3−4 mg/kg) gave high reversal activities, namely, low values (0% on day 4). The effective test compounds were those obtained by introducing the following substituents: 2-hydroxybutyl (**24**), 2-hydroxyhexen-5-yl (**27**), 2-hydroxybuten-3-yl (**28a**), 2-substituted 1-hydroxybuten-3-yl (**28b**), 4-acetoxybutyn-2-yl (**30**), 4-hydroxybutyn-2-yl (**31**), and 3-substituted buten-1-yl (**29**), which correspond to the nonbulky groups of hydroxyalkyl (C4), hydroxyalkenyl (C4−C6), hydroxyalkynyl (C4), or alkenyl (C4). These results may lead to the development of an approach to developing clinically applicable chemosensitizers for drug-resistant malaria.

Introduction

The spread of malaria, especially caused by the most deadly as well as the multidrug-resistant (MDR) *Plasmodium* (*P.*) *falciparum*, is becoming a serious problem in endemic countries in the tropical zones of Southeast Asia, Africa, and South America.[1] Furthermore, the inhabitants of malarial vectors (anopheline species) are expanding throughout the world through changes in global ecosystems and weather patterns due to global warming. However, *P. falciparum* promptly built up resistance to various types of antimalarial drugs (e.g., chloroquine, mefloquine, halofantrine, etc.).[2] To maintain MDR *P. falciparum* under control, the development of new MDR-sensitive drugs is essential for the treatment of MDR malaria. The recovery activity of chloroquine (CQ) resistance in *P. falciparum* by the use of verapamil, a calcium channel blocker,[3] chlorpromazine, and prochlorperazine of antipsychotics[4] and desipramine of tricyclic antidepressant drugs, etc.[5] has been reported. Recently, the design, synthesis, and in vitro bioevaluation of chemosensitizers such as phenothiazine, iminodibenzyl, iminostilbene, and diphenylamine derivatives against CQ-resistant *P. falciparum* have been reported.[6]

One of the present authors, Miyata, also found the recovery activity of drug sensitivity by some types of dibenzosuberanylpiperazine (1-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)piperazine, DSP) derivatives against tumors, pathogenic microorganisms, and CQ-resistant rodent malaria.[7] The first DSP derivative against CQ-resistant malaria that did not exhibit sufficient reversibility was a compound substituted by 3-(7-chloro-4-quinolyl)thiopropan-2-ol. We then considered DSP derivatives bearing suitable substituents for MDR-reversing chemosensitizers in human malaria. As an effective bioavailable application of DSP derivatives, Suzuki et al. reported that a derivative of 4-quinolino-2-hydroxypiperazine (5-[3-{4-(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cycloheptane-5-yl)piperazin-1-yl}-2-hydroxypropoxy]quinoline), MS-073, has high MDR reversal activity to tumors without significant toxicity.[8]

We have synthesized a large number of new DSP derivatives having piperazine N substituents and investigated in vivo the usefulness of these compounds for reversal of MDR *P. falciparum*. At first, we surveyed

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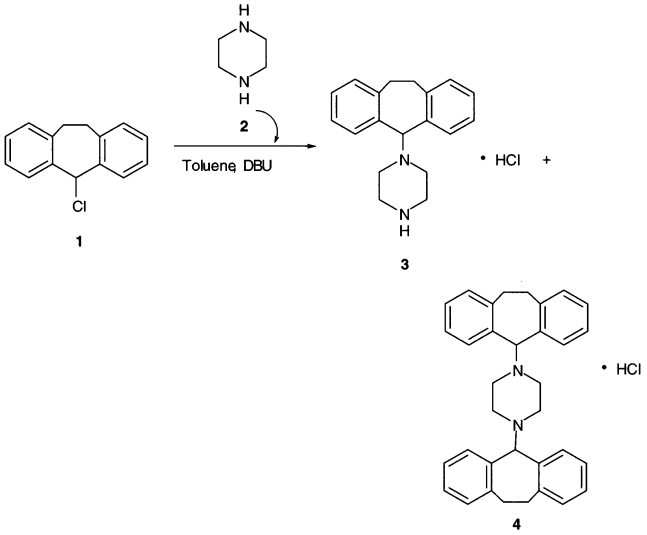
10.1021/jm020379v CCC: $25.00 © 2003 American Chemical Society
Published on Web 04/02/2003

Scheme 1



the reversal effects[9] of CQ resistance in *Plasmodium* (*P.*) *chabaudi*[10,11] of rodent malaria in a search for the most promising substituents of the DSP derivatives as chemosensitizer. Many substituents were introduced to the secondary amine of the DSP piperazine residue by the ring opening of epoxides or by the reaction of organic chlorides. Some structural properties effective to reverse CQ resistance were examined and identified. The mechanism of drug resistance in malaria is not quite clear, even though some persuasive mechanisms suggesting an ATP-dependent efflux pump responsible for MDR observed in tumor cells and *P. falciparum* have been proposed.[12,13] However, if some promising chemosensitizers effective to reverse CQ resistance in *Plasmodium* could be obtained clinically, it would be helpful in achieving low-cost CQ therapy and developing new antimalarial drugs.

Chemical Results

DSP hydrochloride salt (**3**), a basic compound, was obtained from the reaction of dibenzosuberanyl chloride (**1**) with piperazine (**2**) catalyzed by 1,8-diazabicyclo-[5,4,0]-7-undecene (DBU) in toluene in 78% yield (Scheme 1). A byproduct of 1,4-bis(10,11-dihydro-5*H*-dibenzo[*a*,*d*]-cycloheptane-5-yl)piperazine (**4**) was simultaneously obtained as a hydrochloride salt in 22% yield.

To compare the reversal activities of CQ resistance with piperazine-N-substituted DSP derivatives synthesized by structural design, **3** was reacted with commercially available epoxides (**5**−**16**) in the presence of triethylamine (TEA) or DBU in methanol (MeOH) to produce the DSP derivatives (**17**−**28**) as shown in Scheme 2. The yield of products is also shown in Scheme 2. The use of TEA to produce **27** gave a low yield of 33%. However, the use of DBU to produce **27** raised the yield to 71%. This tendency was generally observed in small carbon-chain substituents probably because of the high basicity of DBU.

Reaction of epoxides **5**−**15** gave only **17**−**27**. By the reaction of **3** with epoxide **16**, isomer products **28a** and **28b** were obtained and separated by a silica gel column chromatography with yields of 48% and 27%, respectively.

3-Substituted buten-1-yl derivative (**29**) was synthesized by the reaction of **3** and 3-chloro-1-butene in the presence of DBU in 51% yield. The 4-acetoxybutyn-2-yl derivative (**30**) was prepared in 74% yield by the reaction of **3** and 4-acetoxy-1-chloro-2-butyne that was obtained from the reaction of 2-butyne-1,4-diol with acetic anhydride and then with thionyl chloride. Because the derivative **30** was syrupy just after being prepared and was unstable in standing at room temperature (changing from colorless to brown), it was converted to the crystalline deacetate **31** (70% yield) using K_2CO_3 as a base. However, syrupy **30** solidified when kept in a refrigerator for a while.

Biological Results

In addition to evaluating the antimalarial activity and the CQ-resistant reversal activity of the synthesized DSP derivatives, 4- to 5-week-old female mice (ICR Crj: CD-1, Charles River Japan, Inc.) were injected intravenously (iv) with 5×10^6 parasitized red blood cells (PRBC) of the CQ-resistant (3CQ) line of the AS strain of *P. chabaudi*.[11] These in vivo tests were carried out according to the method of Tanabe et al.[11b] except for injection of a CQ dose of 4 mg/kg. After 2 h of PRBC injection into the mice, the test compounds (5−50 mg/kg) and CQ (3 or 4 mg/kg) were subsequently injected intraperitoneally (ip) into the mice, and the injections were repeated for an additional 3 days (four total injections). The test compounds were dissolved in a physiological sodium chloride solution (saline) containing 10% (w/v) dimethyl sulfoxide (DMSO) for **4**, **17**−**23**, and **25**−**28** or in absolute DMSO for **3**, **24**, and **28**−**31**. CQ diphosphate salt (Sigma, C-6628) was dissolved in sterilized saline or dispersed in absolute DMSO. Four groups of mice were injected with either DMSO alone, CQ alone, test compound alone, or CQ combined with test compounds. Parasitemias (numbers of PRBC per 10 000 RBCs in Giemsa stained thin blood films) were microscopically monitored daily for 5 days.

The data of the antimalarial and the reversal activities were determined using the following two equations:

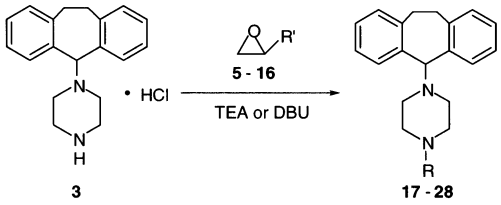
$$\text{value of antimalarial activity (\%)} = \text{(no. of PRBC by administering the compound only)/(no. of PRBC by no administering)} \times 100$$

$$\text{value of reversal activity (\%)} = \text{(no. of PRBC by administering the compound and chloroquine)/(no. of PRBC by administering chloroquine only)} \times 100$$

where the number of PRBC is per 10 000 RBCs in thin blood film. In both equations above, the lower the value (%) of antimalarial and reversal activities, the higher the antimalarial and reversal efficacies are expected.

Table 1 shows both values for the antimalarial and the reversal activities of 18 test compounds including 17 newly synthesized DSP derivatives at day 4 of administration (the values obtained on days 0−3 are not shown). As shown in the table, test compounds are classified into two groups mainly by the values of reversal activity. Group 1 includes 11 compounds, namely, **17**−**23**, **25**, **26**, **3**, and **4** showing generally high values of reversal activity (except for **3**, which shows a low value of reversal activity) obtained by a set of two mice. Group 2 includes seven compounds, namely, **24**

Scheme 2



	R'		R	Base	Yields (%)
5	$-CH_2O-C_6H_5$	**17**	$-CH_2CH(OH)-CH_2O-C_6H_5$	TEA	33
6	$-CH_2O-C_6H_4-OCH_3$	**18**	$-CH_2CH(OH)-CH_2O-C_6H_4-OCH_3$	TEA	38
7	$-(CH_2)_{15}CH_3$	**19**	$-CH_2CH(OH)(CH_2)_{15}CH_3$	TEA	82
8	$-(CH_2)_{13}CH_3$	**20**	$-CH_2CH(OH)(CH_2)_{13}CH_3$	TEA	76
9	$-(CH_2)_{11}CH_3$	**21**	$-CH_2CH(OH)(CH_2)_{11}CH_3$	TEA	81
10	$-(CH_2)_7CH_3$	**22**	$-CH_2CH(OH)(CH_2)_7CH_3$	TEA	42
11	$-(CH_2)_5CH_3$	**23**	$-CH_2CH(OH)(CH_2)_5CH_3$	TEA	57
12	$-CH_2CH_3$	**24**	$-CH_2CH(OH)CH_2H_3$	DBU	90
13	$-(CH_2)_6CH=CH_2$	**25**	$-CH_2CH(OH)(CH_2)_6CH=CH_2$	DBU	72
14	$-(CH_2)_4CH=CH_2$	**26**	$-CH_2CH(OH)(CH_2)_4CH=CH_2$	DBU	73
15	$-(CH_2)_2CH=CH_2$	**27**	$-CH_2CH(OH)(CH_2)_2CH=CH_2$	DBU	71
16	$-CH=CH_2$	**28a**	$-CH_2CH(OH)CH=CH_2$	DBU	75
		28b	$HOCH_2CH(OH)CH=CH_2$		(**28a** **28b**=48%:27%)

and **27**−**31** showing almost zero value of reversal activity (high reversal activity) obtained by more than four mice. Three and five mice were used to obtain the values of antimalarial activity of **28a** and **28b**, respectively. Therefore, the bioactivities of group 1 compounds have not been pursued further and the study of bioactivities was focused on group 2 compounds.

Each dose of the test compounds to the mice was 50 mg/kg except for **28b** (15 mg/kg). For administration of CQ, a dose of 3 or 4 mg/kg was chosen for **4**, **17**−**23**, and **25**−**27**, or for **3**, **24**, and **28**−**31**, respectively. The use of a higher CQ dose (4 mg/kg) would demonstrate the reversal efficacy more clearly. The order of the test compounds (with values less than 100%) effective for antimalarial action was **29**, **19** ∼ **25** ∼ **3**, **28a**, **23**, **24**. Other compounds gave values of antimalarial activity greater than 100%. Compounds **24**−**31** and **3** gave very low values of reversal activity when combined with the CQ dose.

To compare the reversal activity for CQ resistance with the carbon-chain length of piperazine N-substitutents of DSP derivatives, the values (%) of reversal activities for the derivatives with hydroxy-saturated carbon chains and with hydroxy-unsaturated carbon chains C 4−10 are shown in Figure 1. The values of reversal activities from day 0 to day 4 of the potent compounds **24**, **27**, **28a**, **29**, **30**, and **31** having high reversal effects are plotted in Figure 2.

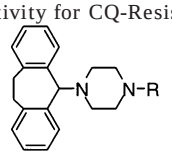
Compound **28** was isolated in two isomeric forms (**28a**, **28b**). The values of the reversal activity of **28a** at the different doses of 5, 10, 15, 30, 40, and 50 mg/kg are shown in Table 2 and Figure 3. Only the lower level doses (5, 10, and 15 mg/kg) of **28b** were administered to mice as shown in Table 2, since the higher level doses (30, 40, and 50 mg/kg) were expected to give 0% reversal activity.

Discussion

Compound **3** was isolated as the HCl salt and therefore was easy to handle. From this reaction, the sole byproduct formed was 1,4-bis(10,11-dihydro-*5H*-dibenzo[*a*,*d*]cycloheptane-5-yl)piperazine hydrochloride (**4**). The structure of **3** was confirmed by X-ray crystallographic analysis[14] as well as spectroscopic analysis. A free form of **3**, DSP, was easily liberated from **3** through Dowex 8 in 95% yield, and its spectral data were identical to those reported in previous papers.[8a,15] Even though the conventionally stable conformation of cycloheptane is a chair form,[16] X-ray analysis showed that the cycloheptane ring of **3** takes a boat form and the plane of the piperazine ring is almost perpendicular to that of the 1,2:4,5-dibenzocycloheptane skeleton. The structure of the piperazine ring was confirmed to be a chair form.

Thirteen hydroxy DSP derivatives were newly synthesized in satisfactory yields by an attack of the

Table 1. Values (%) of Antimalarial Activity and Reversal Activity for CQ-Resistant Malaria of Test Compounds



	Compd.	R	antimalarial activity (%) [a, b] 4 d	reversal activity (%) [a] CQ (mg/kg)	4 d
Group 1	**17**	-CH_2CH(CH_2O-Ph)OH	136 ± 17	3	113 ± 13
	18	-CH_2CH(CH_2O-Ph-OCH_3)OH	147 ± 4	3	90 ± 16
	19	-CH_2CH(OH)$(CH_2)_{15}CH_3$	67 ± 10	3	68 ± 6
	20	-CH_2CH(OH)$(CH_2)_{13}CH_3$	122 ± 18	3	110 ± 19
	21	-CH_2CH(OH)$(CH_2)_{11}CH_3$	138 ± 21	3	64 ± 21
	22	-CH_2CH(OH)$(CH_2)_7CH_3$	152 ± 21	3	84 ± 17
	23	-CH_2CH(OH)$(CH_2)_5CH_3$	92 ± 4	3	102 ± 6
	25	-CH_2CH(OH)$(CH_2)_6CH{=}CH_2$	68 ± 6	3	6.8 ± 2.8
	26	-CH_2CH(OH)$(CH_2)_4CH{=}CH_2$	108 ± 17	3	8.9 ± 1.5
	3 [c]	H	68 ± 16	4	2.0 ± 2.8
	4		108 ± 2	3	81 ±24
Group 2 [d]	**24**	-CH_2CH(OH)CH_2CH_3	99 ± 1	4	0
	27	-CH_2CH(OH)$(CH_2)_2CH{=}CH_2$	101 ± 1	3	0
	28a [e]	-CH_2CH(OH)$CH{=}CH_2$	82 ± 5	4	0
	28b [e]	$HOCH_2$CH(OH)$CH{=}CH_2$	103 ± 14	4	2.6 ± 0.8
	29	CH_3CH(OH)$CH{=}CH_2$	22 ± 15	4	0
	30	-CH_2-C≡C-CH_2OAc	116 ± 3	4	0
	31	-CH_2-C≡C-CH_2OH	121 ± 11	4	0

[a] Dose of 50 mg/kg except for **28b** (15 mg/kg). [b] Two mice were used. [c] Compound **3** was only dissolved in saline. [d] Reliability on reversal activity of group 2 obtained using more than four mice is for $P < 0.05$. [e] Three mice for **28a** and five for **28b** were used for antimalarial activity.

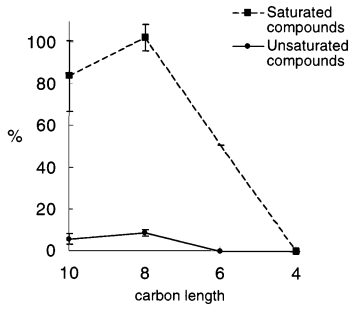


Figure 1. Reversal activities (%) of DSP derivatives with hydroxy-saturated substituents (**22**−**24**), and with hydroxy-unsaturated substituents (**25**−**28**) at 50 mg/kg dose of the test compounds combined with 3 mg/kg dose (**24** with 4 mg/kg) of CQ against carbon chain length.

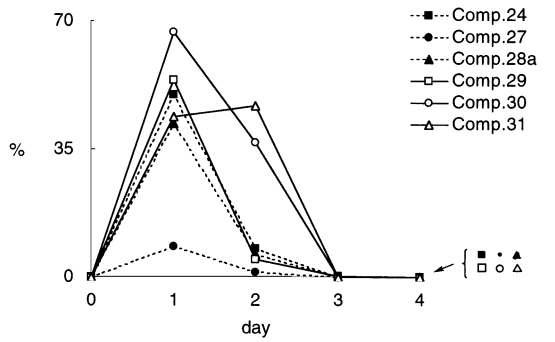


Figure 2. Preliminary results of reversal activities from day 0 to day 4 of potent compounds **24**, **27**, **28a**, **29**, **30**, and **31** at 50 mg/kg dose combined with 4 mg/kg dose of CQ (except **27**, with 50 mg/kg dose combined with 3 mg/kg dose of CQ).

secondary amine of **3** on the corresponding epoxides in the presence of TEA or DBU. Since the use of DBU tended to raise the yield of the DSP derivatives, DBU was usually used to synthesize the derivatives possessing a short hydroxy carbon chain. The synthesized

Table 2. Reversal Activities (%) of **28a** and **28b** at Different Doses from Days 0−4 Combined with 4 mg/kg Dose of CQ[a]

	daily reversal activity (%) of **28a**					daily reversal activity (%) of **28b**				
dose (mg/kg)	day 0	day 1	day 2	day 3	day 4	day 0	day 1	day 2	day 3	day 4
5	0	76	32	12	11	0	69	35	33	17
10	0	62	21	7.9	6	0	59	37	23	8.6
15	0	49	16	9.5	5.4	0	37	13	4	2.6
30	0	55	38	2.9	0					
40	0	33	10	0	0					
50	0	42	6.2	0	0					

[a] Reliability of all the data at different doses is for $P < 0.05$ except for the reversal activity at 5 mg/kg dose of **28b** on day 3 ($P < 0.1$).

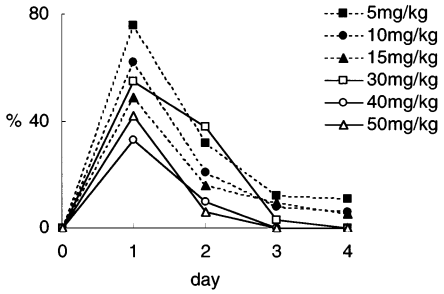


Figure 3. Reversal activities (%) of **28a** at 5, 10, 15, 30, 40, and 50 mg/kg doses from day 0 to day 4 combined with 4 mg/kg dose of CQ.

compounds shown in Table 1 were mainly chosen from the viewpoint of bulkiness, unsaturation, or a lipophilic/hydrophilic balance of substituents for biological efficacies.

Derivatives **17**−**28** were obtained as racemates because no optical rotation was indicated. Regioselective nucleophilic attack of the piperazine secondary amine of **3** on epoxides **5**−**15** was estimated by a one-side attack because of their bulky residues. The reaction of **3** with epoxide **16** having an ethylene side chain gave two nucleophilic products of **28a** (having a 2-hydroxybuten-3-yl) and **28b** (having a 1-hydroxybuten-3-yl). On the other hand, the reaction of **3** with epoxide **12** having an ethyl side chain and the same carbon number as epoxide **16** produced only one product. Although the reason for the difference between epoxides **12** and **16** is not certain, attack on the epoxide ring of compound **16** might have occurred on both sides because of a mesomeric effect of the ethylene side chain. In addition, the derivatives **29** of 3-substituted buten-1-yl, **30** of 4-acetoxybutyn-2-yl, and **31** of 4-hydroxybutyn-2-yl were synthesized by the reactions of **3** with the corresponding organic chlorides. Compound **29** is also a racemate, same as **17**−**28**, but **30** and **31** are not racemates.

All products were analyzed with NMR spectrometry and mass spectrometry as well as by elemental analysis. In addition, the structures of the isomeric crystalline products **28a** and **28b** were analyzed by X-ray crystallography, and the result is shown in Figure 4.[17] The structures of the cycloheptane ring and the piperazine moiety of **28a** and **28b** were confirmed to be a boat and a chair form, respectively, and were characterized in that the plane of the piperazine ring is almost perpendicular to that of dibenzocycloheptane, similar to the conformational structure of **3**. It was proved that the CQ-resistant (3CQ) line of the AS strain of *P. chabaudi* has a resistance for CQ by monitoring PRBC after administering only CQ (3 or 4 mg/kg).

After careful screening of solvents that can dissolve the test compounds, we decided to use DMSO. The

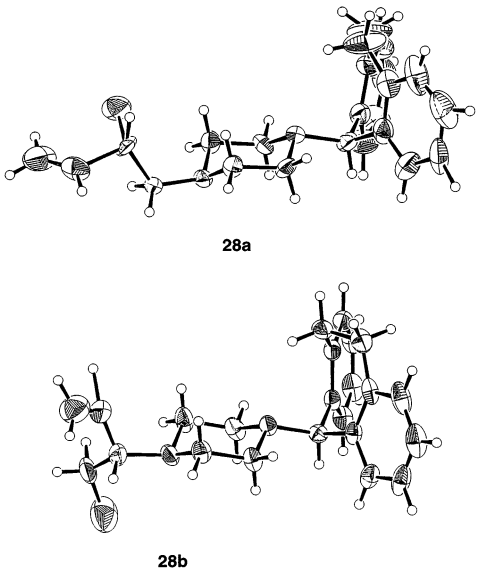


Figure 4. ORTEP plots of **28a** and **28b**.

solubility of the test compounds in water was generally low, and the use of olive oil, for instance, would form an emulsifying solution. It was observed that ip injection of an emulsifying solution into mice would cause skin swelling near the injected site of the mice abdomen. The test compounds dissolved in DMSO easily. Prior to the biological experiments, it was determined that no toxic symptoms of the mice occurred with a 0.2 mL dose of DMSO per mouse (weight of mouse, ca. 25 g). Low toxicity of DMSO to mammals has been reported,[18a] and its acute ip LD_{50} to mice is 12.6 mL/kg.[18b] A 50 mg/kg dose level (ca. 1.25 mg/mouse) of the test compounds was dissolved in DMSO, and then the solution was diluted with saline to make a 10% (w/v) DMSO solution. Because the DMSO solution would become cloudy by dilution of saline, however, absolute DMSO containing the test compounds (**24**, **28**−**31**) was used in more recent biological experiments. It was confirmed that the reversal activity of **28a** and **28b** were almost the same in a 10% DMSO solution and in absolute DMSO. The highest dose level of the test compounds (50 mg/kg dissolved in DMSO) in mice did not reveal any side effects. These results may suggest that the DSP derivatives tested are sufficiently low in toxicity.

Even if DMSO was nontoxic in the present study, its use in human application should be avoided because DMSO is a foreign matter to the human body. Some methods to dissolve the DSP derivatives in water such as formation of an inclusion complex with cyclodextrine are being examined.

As shown in Table 1, compounds **24**, **27**, **28a**, **28b**, **30**, and **31** did not have intrinsic antimalarial activity

(antimalarial activity: **24** , 99%; **27**, 101%; **28a**, 82%; **28b**, 103%; **30**, 116%; **31**, 121%) but acted as chemosensitizing agents. On the other hand, **29** had considerably high antimalarial activity as follows: 129% (day 1), 19% (day 2), 31% (day 3), and 22% (day 4) (only the value at day 4 is shown in Table 1). This compound also has a high reversal activity (0%). Therefore, it will be of interest to pursue studies on **29** for its antimalarial and reversal activities.

The curve of reversal activity for the dose of the test compounds combined with CQ dose to mice usually shows the maximum value on day 1 after administration and then decreases (sometimes to 0%) on subsequent days. The values of reversal activities of compounds **17**−**23** were high on day 4, but those of compounds **25**−**31** were less than 9% on day 4. The common characteristics of the latter compounds are that they have short carbon-chain substituents (≤10) as well as an unsaturated bond. For instance, compounds **26** (8 carbons with its terminal double bond) and **25** (10 carbons with its terminal double bond) gave lower values of reversal activities than compounds **23** (8 carbons without a double bond) and **22** (10 carbons without a double bond) (Table 1 and Figure 1). Both compounds **30** and **31** having four carbon skeleton chains with a triple bond showed 0% reversal activities on day 3 with a slow but sharp change (37% and 47% on day 2, respectively) as shown in Figure 2. As shown in Table 2 and Figure 3, the values of reversal activity of the two positional isomers **28a** and **28b** at the doses of 5, 10, and 15 mg/kg together with a 4 mg/kg dose of CQ were found to be almost the same. Though the higher doses of 30, 40, and 50 mg/kg of **28b** were not examined, similar results of **28a** and **28b** will be expected. The above results also suggest that the position of the hydroxy group on the side chain could be indifferent to the reversal activity. As already mentioned, Table 1 shows that the DSP derivatives having bulky substituents are not effective for CQ-resistant malaria.

As shown in Figure 2, the potent compounds of **24**, **27**, **28a**, **29**, **30**, and **31** for CQ-resistant malaria revealed a significantly low value of reversal activity before day 3 after administration of these compounds combined with CQ. The values of reversal activity became 0% on days 3 and 4 except for compounds **27** and **29** (0.1% and 0.2%, respectively, on day 3 and both 0% on day 4). Concerning reversal efficiency alone, derivatives **24**, **28a**, **29**, **30**, and **31** having the substituents of the C4 side chain were the most effective. In addition, derivatives **28a**, **29**, **30**, and **31** having an unsaturated bond seemed to exhibit no clear toxic symptoms as judged from an exterior view of the injected mice. Compound **28b** may be regarded as a potent candidate because its pharmacological results as well as its toxicity at low dose are almost the same as those of **28a**. The reversal activity of the basic compound **3** (DSP hydrochloride salt), which corresponds to a hydrogen substituent (carbon 0), also was low (2%). These results suggest that the efficacy of the test compounds is suppressed by steric unfitness of bulky substituents introduced at the moment of uptake of the compounds into the target cells (erythrocytes and/or *Plasmodium* cells).

On the basis of the above viewpoint regarding the reversal activity and low toxicity to mice, the DSP derivatives having an unsaturated C4 side chain with or without a hydroxy group gave the best potent results for CQ-resistant malaria. The only exception is **24**, which has a saturated C4 side chain with a hydroxy group. Detailed experiments will also afford similar potent results for the derivatives with a C6 side chain. Some studies on the DSP derivatives having shorter side chains than C4 are now in progress.

Even though the pfmdr 1 gene in MDR *P. falciparum*[19] has been identified, the mechanism of MDR in *P. falciparum* is not well understood, in contrast to MDR tumor cells, which have a demonstrable efflux mechanism through P-glycoprotein involved in the ABC transport system.[10, 11]

DSP has a structure similar to that of diphenylpiperazine, which is known to have the basic structure of a Ca antagonist and an inhibitor of the ABC transporter. In general, the DSP derivatives not only have a weak efficacy as Ca antagonists,[8a,20] compared with their strong ABC transporter inhibition effect, but also have a weak effect on the central nervous system (CNS), despite its tricyclic structure. In the application of a 50 mg/kg dose of most of the test compounds to mice, no extraordinary behavior that might be derived from the CNS effects was observed. This might be due to the absence of a heteroatom on the tricyclic group. From this viewpoint, the DSP structure could be suitable as an MDR reversal agent.

Conclusion

Thirteen dibenzosuberanylpiperazine derivatives, **17**−**28**, possessing hydroxyalkyl or hydroxyalkenyl substituents were newly synthesized by the reaction of **3** with the corresponding 2-alkyl or 2-alkenyl epoxides in satisfactory yield. Three other DSP derivatives, **29**−**31**, were synthesized by the reaction of **3** with the corresponding organic chlorides**.** These products were confirmed by MS and NMR spectral results as well as elemental analysis results. The structures of the isomeric products **28a** and **28b** were confirmed by X-ray analysis.

The reversal activities of the synthesized compounds for CQ resistance in *P. chabaudi* were examined, and seven compounds, **24**, **27**, **28a**, **28b**, **29**, **30**, and **31**, were found to be potent chemosensitizers for CQ resistance. The values of antimalarial activity of these compounds on day 4 after *P. chabauli* infection were in the range 82−121% except for 3-(dibenzosuberanylpiperazin-1-yl)-1- butene (**29**) (22%). In addition, **29** revealed high reversal activities with low values of 0% on days 3 and 4. The common structural properties of substituents in these potent compounds are characterized as follows. (i) DSP-N-substituted derivatives, (ii) the introduced side chains of a nonbulky group consisting of a hydroxyalkyl (C4), hydroxyalkenyl (C4−C6), hydroxyalkynyl (C4), or alkenyl (C4) group, and (iii) unsaturated double and triple bonds probably reduce side effects and enhance the efficacies of the chemosensitizer.

Experimental Section

Chemical Methods. Melting points were measured on a YAZAWA BY-10 and are uncorrected. Spectral measurements

were recorded on a JMS AX-505HA or a JMS-700MAStation mass spectrometer (for MS) and on a Varian VXR-300, a Unity-400 or a Unity-Inova-600 spectrometer (for ^{1}H and ^{13}C NMR). Piperazine and dibenzo[*a,d*]cycloheptane used in the NMR measurements are abbreviated as P and D, respectively. TLC was carried out on a Merck silica gel 60 F_{254} using the same solvent systems as that used in column chromatography. The spots were visualized under UV light (254 nm) or by charring with molybdophosphoric acid (4% H_2SO_4, 0.85% phosphoric acid, and 0.25% molybdic acid in water). Column chromatography was performed on silica gel 60 (63-210 mesh, neutral, Merck). Elemental analyses were performed on an MT-5 elemental analyzer. The products were crystallized from MeOH/H_2O or ethanol/H_2O mixed solution.

1-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazine Hydrochloride (3) and 1,4-Bis(10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)piperazine Hydrochloride (4). To a solution of dibenzosuberanyl chloride (**1**) (100 mg, 0.437 mmol) and MS-4A in dry toluene (0.5 mL), a solution of piperazine (**2**) (37.6 mg, 0.437 mmol) in dry toluene (1.2 mL) was added dropwise under argon at 0 °C. Then DBU (16.5 μL, 0.11 mmol) was added and the reaction mixture was stirred overnight. The mixture was filtered through a pad of Celite. Then the solvent was evaporated in vacuo and the residue was dissolved in MeOH. On the other hand, byproduct **4** was not found to be soluble in MeOH. After removal of the byproduct (**4**, 24 mg, 22%) by filtration, the filtrate was evaporated to dryness to yield **3** as a yellow solid (107 mg, 78%): mp 246−246.5 °C (crystallized from $CHCl_3$/AcOEt, 1:1); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.58−2.65 (4H, broad, piperazine (abbreviated as P)), 2.80, 2.95 (1H, m, dibenzo[*a,d*]cycloheptane (abbreviated as D) H-10a, 11a), 3.06−3.14 (4H, broad, P), 3.54, 3.89 (2H, m, D H-10b, 11b), 4.10 (1H, s, D H-5), 7.05−7.24 (8H, benzene), 9.62 (1H, broad, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 31.71 (D C-10, 11), 43.69, 48.19 (P), 78.12 (D C-5), 125.78, 125.83, 128.07, 130.26, 137.51, 138.36, 139.61 (benzene); MS (FAB) *m/z* 279 $(M + H)^+$. Anal. ($C_{19}H_{22}N_2 \cdot HCl \cdot 0.5H_2O$) C, H, N.

4: mp 249−250 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.10−2.35 (8H, broad, P), 2.76, 2.79 (4H, m, D 2 × H-10a, 11a), 3.91 (2H, s, D 2 × H-5), 4.00, 4.03 (4H, m, D 2 × H-10b, 11b), 6.95−7.15 (16H, benzene); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.79 (D C-10, 11), 52.31 (P), 79.14 (D C-5), 125.26, 127.50, 130.58, 130.63, 139.40, 139.68 (benzene); MS (FAB) *m/z* 471 $(M + H)^+$, 493 $(M + Na)^+$. HRMS (FAB) calcd for $C_{34}H_{35}N_2$ $(M + H^+)$, 471.2800; found, 471.2779.

Typical Procedure To Produce 1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)piperazin-1-yl](alkan, alken)-2-ol (17−28). A mixture of **3** (139 mg, 0.44 mmol) and the appropriate 1,2-epoxy compounds (**5**−**16**, 1.01 mmol) was stirred in the presence of TEA (36 μL, 0.26 mmol) for **17**−**23** or in the presence of DBU (38 μL, 0.26 mmol) for **24**−**28** in MeOH (3 mL) at room temperature overnight. The reaction mixture was evaporated to dryness, and the residue was purified on a silica gel column with $CHCl_3$/MeOH, 10:1, to obtain **17**−**28**.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]-3-*O*-phenylpropan-2-ol (17): yield 33%; mp 132−133 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.30−2.40, 2.60−2.70 (8H, broad, P), 2.80 (2H, m, D H-10a, 11a), 3.97 (1H, s, D H-5), 3.98 (2H, m, D H-10b, 11b), 4.04 (1H, m, H-2), 6.88−6.97, 7.04−7.30 (13H, benzene); MS (EI) *m/z* 428 $(M)^+$. Anal. ($C_{28}H_{32}N_2O_2$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]-3-*O*-(4-methoxyphenyl)propan-2-ol (18): yield 38%; mp 105.0−105.5 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.30−2.40, 2.60−2.70 (8H, broad, P), 2.40−2.60 (4H, m, H-1, 3), 2.80 (2H, m, D H-10a, 11a), 3.76 (3H, s, $-OCH_3$), 3.96 (2H, m, D H-10b, 11b), 3.97 (1H, s, D H-5), 4.02 (1H, m, H-2), 6.80−6.89, 7.04−7.20 (12H, benzene); MS (EI) *m/z* 458 $(M)^+$, 459 $(M + 1)^+$. Anal. ($C_{29}H_{34}N_2O_3 \cdot 0.5H_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]octadecan-2-ol (19): yield 82%; mp 78−79 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (3H, t, H-18), 1.20−1.42 (28H, broad, H-4−17), 2.21−2.29 (4H, broad, H-1, 3), 2.37−2.70 (8H, broad, P), 2.71−2.90, (2H, m, D H-10a, 11a), 3.61 (1H, H-2), 3.99 (1H, s, D H-5), 4.00−4.11 (2H, m, D H-10b, 11b), 7.03−7.21 (8H, benzene); MS (EI) *m/z* 546 $(M)^+$. Anal. ($C_{37}H_{58}N_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]hexadecan-2-ol (20): yield 76%; mp 79−80 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (3H, t, H-16), 1.20−1.50 (24H, broad, H-4−15), 2.21−2.42 (4H, m, H-1,3), 2.36−2.70 (8H, broad, P), 2.73−2.89 (2H, m, D H-10a,11a), 3.61 (1H, H-2), 3.99 (1H, s, D H-5), 4.00−4.11 (2H, m, D H-10b, 11b), 7.05−7.19 (8H, benzene); MS (EI) *m/z* 518 $(M)^+$, 519 $(M + H)^+$. Anal. ($C_{35}H_{54}N_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]tetradecan-2-ol (21): yield 81%; mp 74−75 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (3H, t, H-14), 1.20−1.45 (20H, broad, H-4−13), 2.20−2.40 (4H, m, H-1, 3), 2.41−2.72 (8H, broad, P), 2.69−2.91 (2H, m, D H-10a,11a), 3.60 (1H, H-2), 3.99 (1H, s, D H-5), 4.01−4.10 (2H, m, D H-10b,11b), 7.05−7.20 (8H, benzene); MS (FAB) *m/z* 491 $(M + H)^+$, 513 $(M + Na)^+$. Anal. ($C_{33}H_{50}N_2O \cdot 0.25H_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]decan-2-ol (22): yield 42%; mp 56.5−57.0 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (3H, t, H-10), 1.20−1.51 (12H, broad, H-4−9), 2.21−2.32 (4H, m, H-1, 3), 2.30−2.72 (8H, broad, P), 2.80 (2H, m, D H-10a, 11a), 3.62 (1H, H-2), 3.99 (1H, s, D H-5), 4.00 (2H, m, D H-10b, 11b), 7.04−7.20 (8H, benzene); MS (FAB) *m/z* 433 $(M - H)^+$, 457 $(M + Na)^+$. Anal. ($C_{29}H_{42}N_2O \cdot 0.25H_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]octan-2-ol (23): yield 57%; oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (3H, t, H-18), 1.21−1.49 (8H, broad, H-4−7), 2.30−2.42 (4H, m, H-1, 3), 2.40−2.62, 2.91−3.22 (8H, broad, P), 2.85 (2H, m, D H-10a, 11a), 3.71 (1H, m, H-2), 3.95 (2H, m, D H-10b, 11b), 4.14 (1H, s, D H-5), 7.05−7.20 (8H, benzene); MS (EI) *m/z* 406 $(M)^+$. Anal. ($C_{27}H_{38}N_2O \cdot 0.25H_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]butan-2-ol (24): yield 90%; mp 82−83 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (3H, dd, H-4), 1.37, 1.46 (2H, ddd, H-3a, 3b), 2.20−2.45 (10H, m, H-1, P), 2.60 (1H, broad, OH), 2.77, 2.81 (2H, m, D H-10a, 11a), 3.56 (1H, m, H-2), 3.96 (1H, s, D H-5), 3.97, 4.00 (2H, m, D H-10b,11b), 7.00−7.20 (8H, benzene); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 9.80 (C-4), 27.75 (C-3), 31.75 (D C-10, 11), 51.94, 53.58 (P), 63.74 (C-1), 67.42 (C-2), 79.07 (D C-5); MS (EI) *m/z* 350 $(M)^+$. Anal. ($C_{23}H_{30}N_2O \cdot 0.5H_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]-9-decen-2-ol (25): yield 72%; mp 72−73 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25−1.50 (10H, broad, H-3−7), 2.04 (2H, m, H-8), 2.22 (1H, dd, H-1a), 2.26 (1H, dd, H-1b), 2.29−2.70 (8H, broad, P), 2.80 (2H, m, D H-10a, 11a), 3.61 (1H, H-2), 3.97 (1H, s, D H-5), 4.00 (2H, m, D H-10b, 11b), 4.93 (1H, H-10a), 4.99 (1H, H-10b), 5.81 (1H, qd, H-9), 7.04−7.20 (8H, benzene); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 25.53 (C-3), 28.82 (C-7), 29.02 (C-5), 29.61 (C-6), 31.69 (D C-10, 11), 33.75 (C-8), 34.89 (C-4), 51.95, 53.50 (P), 64.05 (C-1), 66.06 (C-2), 79.02 (D C-5), 114.11 (C-10), 139.59 (C-9), 125.42, 127.63, 130.67, 139.12, 139.21, 139.24 (benzene); MS (FAB) *m/z* 432 $(M)^+$. Anal. ($C_{29}H_{40}N_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]-7-octen-2-ol (26): yield 73%; mp 93.0−94.0 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.30−1.50 (6H, broad, H-3−5), 2.04 (2H, broad, H-6), 2.44 (2H, broad, H-1), 2.46−2.70 (8H, broad, P), 2.80 (2H, m, D H-10a, 11a), 3.80 (1H, broad, H-2), 3.95 (2H, m, D H-10b, 11b), 4.03 (1H, s, D H-5), 4.93 (1H, dddd, H-8a), 4.98 (1H, dddd, H-8b), 5.79 (1H, qd, H-7), 7.04−7.20 (8H, benzene); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.93 (C-4), 28.87 (C-5), 31.75 (D C-10, 11), 33.61 (C-6), 34.76 (C-3), 50.43, 53.88 (P), 64.15 (C-1), 65.68 (C-2), 78.49 (D C-5), 114.39 (C-8), 139.55 (C-7), 125.63, 127.89, 130.73, 130.89, 138.44, 138.75 (benzene); MS (FAB) *m/z* 404 $(M)^+$. Anal. ($C_{27}H_{36}N_2O \cdot 0.25H_2O$) C, H, N.

1-[4-(10,11-Dihydro-5*H*-dibenzo[*a,d*]cycloheptan-5-yl)-piperazin-1-yl]-5-hexen-2-ol (27): yield 71%; mp 85−86 °C;

^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.47 (2H, m, H-3), 2.16 (2H, m, H-4), 2.37 (2H, m, H-1), 2.30−2.70 (8H, broad, P), 2.79 (2H, m, D H-10a, 11a), 3.64 (1H, H-2), 3.96 (1H, s, D H-5), 3.99 (2H, m, D H-10b, 11b), 4.94 (1H, dddd, H-6a), 5.02 (1H, dddd, H-6b), 5.82 (1H, m, H-5), 7.03−7.20 (8H, benzene); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 29.84 (C-4), 31.72 (D C-10, 11), 34.07 (C-3), 51.69, 53.45, 53.48 (P), 63.92 (C-1), 65.52 (C-2), 79.03 (D C-5), 114.58 (C-6), 138.48 (C-5), 126.45, 127.66, 130.70 (benzene); MS (EI) *m*/*z* 376 $(M)^+$. Anal. ($C_{25}H_{32}N_2O\cdot0.25H_2O$) C, H, N.

1-[4-(10,11-Dihydro-*5H*-dibenzo[*a*,*d*]cycloheptan-5-yl)-piperazin-1-yl]-3-buten-2-ol (28a): yield 48%; mp 92−93 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.29−2.64 (8H, broad, P), 2.36 (2H, m, H-1a, b), 2.79, 2.80 (2H, m, D H-10a, 11a), 3.97 (1H, s, D H-5), 3.99 (2H, m, D H-10b, 11b), 4.11 (1H, H-2), 5.14 (1H, ddd, H-4a), 5.32 (1H, ddd, H-4b), 5.76 (1H, H-3), 7.05−7.18 (8H, benzene); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.72, 31.74 (D C-10, 11), 51.90, 53.42 (P), 63.53 (C-1), 67.65 (C-2), 79.04 (D C-5), 115.74 (C-4), 138.41 (C-3), 125.46, 127.69, 130.71, 139.19, 139.61 (benzene); MS (EI) *m*/*z* 348 $(M)^+$. Anal. ($C_{23}H_{28}N_2O$) C, H, N.

2-[4-(10,11-Dihydro-*5H*-dibenzo[*a*,*d*]cycloheptan-5-yl)-piperazin-1-yl]-3-buten-1-ol (28b): yield 27%; mp 119−120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.22−2.66 (8H, broad, P), 2.76 (2H, m, D H-10a, 11a), 3.05 (1H, H-2), 3.49, 3.51 (2H, H-1a, H-1b), 3.96 (1H, s, D H-5), 3.98 (2H, m, D H-10b, 11b), 5.15 (1H, ddd, H-4b), 5.25 (1H, ddd, H-4a), 5.72 (1H, H-3), 7.00−7.40 (8H, benzene); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 31.74 (D C-10, 11), 52.22 (P), 60.38 (C-1), 68.17 (C-2), 79.07 (D C-5), 119.63 (C-4), 133.07 (C-3), 125.43, 127.66, 130.68, 139.22, 139.60, 139.64 (benzene); MS (EI) *m*/*z* 348 $(M)^+$. Anal. ($C_{23}H_{28}N_2O$) C, H, N.

X-ray Analysis of 28a:[17] $C_{23}H_{28}ON_2$, M_r = 348.49 g/mol, orthorhombic, space group $Pna2_1$, λ = 1.541 78 Å, a = 33.432-(2) Å, b = 5.684(2) Å, c = 20.310 (2) Å, V = 3859(1) Å^3, Z = 8, ρ_{calcd} = 1.199 g/cm^3, μ = 5.68 cm^{-1}, T = 296 K, $2\theta_{max}$ = 135.2°, scan mode $\omega-2\theta$, 3915 independent reflections, full-matrix least-squares refinement, no. of parameters 471, R indices [$I > -10.00\sigma(I)$], R = 0.088, R_w = 0.200, maximum residual electron density of 0.53 e Å^{-3}.

X-ray Analysis of 28b:[17] $C_{23}H_{28}ON_2$, M_r = 348.49 g/mol, orthorhombic, space group $Pna2_1$, λ = 1.541 78 Å, a = 19.928-(3) Å, b = 15.844(2) Å, c = 6.088(2) Å, V = 1922(1) Å^3, Z = 4, ρ_{calcd} = 1.204 g/cm^3, μ = 5.70 cm^{-1}, T = 296 K, $2\theta_{max}$ = 135.2°, scan mode $\omega-2\theta$, 1998 independent reflections, full-matrix least-squares refinement, no. of parameters 247, R indices [$I > -10.00\sigma(I)$], R = 0.055, R_w = 0.174, maximum residual electron density of 0.13 e Å^{-3}.

3-[4-(10,11-Dihydro-*5H*-dibenzo[*a*,*d*]cycloheptan-5-yl)-piperazin-1-yl]-1-butene (29). To a solution of **3** (278 mg, 0.88 mmol) in dry DMF (1.5 mL), 3-chloro-1-butene (200 μL, 2 mmol) and DBU (300 μL, 2 mmol) were added. The reaction mixture was stirred overnight at room temperature, and then the solvent was evaporated. The residue was purified by silica gel column chromatography, eluting with $CHCl_3$/MeOH (10:1) to provide 172 mg (51%) of yellow syrup, which solidified after long preservation in a refrigerator: mp 79−80 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.13 (3H, d, H-4), 2.10−2.60 (8H, broad, P), 2.76, 2.79 (each 1H, m, D H-10a, 11a), 2.88 (1H, m, H-3), 3.95 (1H, s, D H-5), 3.98, 4.05 (1H, m, D H-10b, 11b), 5.02, 5.07 (each 1H, dd, H-1a, b), 5.76 (1H, m, H-2), 7.00−7.20 (8H, benzene); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 17.47 (C-4), 31.74 (D C-10, 11), 50.15, 52.01 (P), 63.17 (C-3), 79.12 (D C-5), 115.61 (C-1), 125.37, 127. 57, 130.52, 130.72 (benzene), 140.47 (C-2); MS (FAB) *m*/*z* 331 $(M - H)^+$, 355 $(M + Na)^+$. Anal. ($C_{23}H_{28}N_2$) C, H, N.

1-[4-(10,11-Dihydro-*5H*-dibenzo[*a*,*d*]cycloheptan-5-yl)-piperazin-1-yl]-4-acetoxy-2-butyne (30). Acetic anhydride (1.88 mL, 20.0 mmol) was added to a solution of 2-butyne-1,4-diol (**32**) (3.44 g, 40.0 mmol) in pyridine (40 mL), and the mixture was stirred at 0 °C for 2 h. Then the solvent was evaporated to dryness. The residue dissolved in $CHCl_3$/EtOAc (1:1) was eluted through a silica gel column to give 4-acetoxy-2-butyn-1-ol (**33**) (1.84 g, 72%). A stirred solution of **33** (1.84 g, 14.4 mmol) in dry benzene (31 mL) was treated with pyridine (124 μL, 1.55 mmol) and thionyl chloride (1.24 mL, 17.1 mmol) in dry benzene (16 mL). The reaction mixture was heated at 60 °C overnight, and then 10 mL of water was added. The reaction mixture was extracted with CH_2Cl_2 and dried with Na_2SO_4. Removal of the solvent in vacuo gave 4-acetoxy-1-chloro-2-butyne (**34**) (1.88 g, 89%). A solution of **34** (100 mg, 0.682 mmol) and DBU (102 μL, 0.682 mmol) in dry DMF (1.0 mL) was added to a solution of **3** (190 mg, 0.682 mmol) in dry DMF (1.0 mL). The reaction mixture was stirred at room temperature overnight, and then the solvent was evaporated in vacuo. The residue was purified by column chromatography on silica gel using toluene/EtOAc (2:1) to give syrupy product **30** (173 mg, 74%), which solidified after long preservation in a refrigerator: mp 74−75 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.08 (3H, s, OAc), 2.30−2.60 (8H, broad, P), 2.79, 2.80 (2H, m, D H-10a, 11a), 3.28 (2H, dd, N−CH_2C≡), 3.97 (1H, s, D H-5), 4.00, 4.01 (2H, m, D H-10b, 11b), 4.68 (2H, dd, C≡C−CH_2-OAc), 7.00−7.20 (8H, benzene); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 20.69 (OAc), 31.73 (D C-10, 11), 46.97 (N−CH_2−C≡), 51.69, 52.40 (P), 52.43 (C−CH_2OAc), 78.88 (C≡C−CH_2OAc), 79.02 (D C-5), 82.15 (N−CH_2C≡), 125.40, 127.65, 130.67, 130.73, 139.18, 139.65 (benzene), 170.18 (C═O); MS (EI) *m*/*z* 388 $(M)^+$. Anal. ($C_{25}H_{28}N_2O_2\cdot0.25H_2O$) C, H, N.

1-[4-(10,11-Dihydro-*5H*-dibenzo[*a*,*d*]cycloheptan-5-yl)-piperazin-1-yl]-2-butyn-4-ol (31). An MeOH (6 mL) solution of **30** (297 mg, 0.764 mmol) was treated with K_2CO_3 (620 mg), and the mixture was stirred at room temperature overnight. The mixture was filtered, and the filtrate was evaporated to dryness. The residue dissolved in $CHCl_3$/MeOH (10:1) was eluted through a silica gel column to give **31** (227 mg, 86%): mp 130−131 °C; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 2.30−2.60 (8H, broad, P), 2.78, 2.80 (2H, m, D H-10a, 11a), 3.26 (2H, dd, N−CH_2C≡), 3.96 (1H, s, D H-5), 3.99, 4.00 (2H, m, D H-10b, 11b), 4.26 (2H, dd, C≡C−CH_2OH), 7.06−7.16 (8H, benzene); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 31.76 (D C-10, 11), 47.04 (N−CH_2−C≡), 51.15, 52.54 (P), 51.69 (C−CH_2OH), 79.05 (C≡C−CH_2OH), 81.08 (D C-5), 83.25 (N−CH_2C≡), 125.43, 127.67, 130.69, 139.19, 139.67 (benzene); MS (FAB) *m*/*z* 347 $(M + H)^+$, 369 $(M + Na)^+$. Anal. ($C_{23}H_{26}N_2O\cdot H_2O$) C, H, N.

Pharmacology. The 4- to 5-week-old female Crj:CD-1 (ICR) mice (Charles River Japan, Inc.) infected with chloroquine-resistant (3CQ) lines of the AS strain of *Plasmodium chabaudi* were used as an in vivo chloroquine-resistant malaria model to evaluate the chloroquine-resistant reversing activity of the synthetic compounds. This test system was also applied to evaluate the antimalarial activity of the synthetic compounds. The 3CQ line of the AS strain of *P. chabaudi*, which was established by Rosario et al.,[10b] was provided by Dr. K. Tanabe of the Osaka Institute of Technology through Dr. D. Walliker of the University of Edinburgh. Prior to experiments, blood was collected from the mice infected with chloroquine-resistant *P. chabaudi*, dispensed into cryotubes, and cryopreserved as described by Tanabe et al.[11b] Just before each experiment, the cryopreserved parasitized red blood cells (PRBC) of *P. chabaudi* were thawed and injected iv into mice. Experiments for screening of the reversing effects of chloroquine resistance by the synthetic compounds on the 3CQ line were carried out by the method of Tanabe et al.[11b] except for the case of the 4 mg/kg chloroqine dose. Mice were injected iv with 5×10^6 PRBC of the 3CQ line and were injected ip with synthetic test compounds (5−50 mg/kg) and subsequently with chloroquine (3−4 mg/kg) at 2 h after the 3CQ line PRBC injection. The compounds and CQ were administered for 4 days (four times). The test compounds were dissolved in 10% DMSO for **4**, **17**−**23**, and **25**−**28** or in absolute DMSO for **3**, **24**, and **28**−**31**. Chloroquine diphosphate salt (Sigma, C-6628) was dissolved in sterilized 0.9% (w/v) saline or dispersed in absolute DMSO. Parasitemias were monitored daily for 5 days for evaluation of the test compounds in contrast with parasitemias of mice, which were injected with only chloroquine or with only test compounds. Two mice were used to obtain the data of antimalarial activity for all the test compounds except for 2**8a** (three mice) and **28b** (five mice). Two mice were used to obtain

the data of reversal activity for the test compounds of **17**−**23**, **25**, **26**, **3**, and **4**, and more than four mice were used to obtain the data of reversal activity for the test compounds of **24** and **27**− **31**. The statistical difference in parasitemias were analyzed by use of the Student's *t* test.

Acknowledgment. This work was supported in part by Pola Chemical Industries, Inc. and Ohyama Health Foundation, Inc.

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JM020379V