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Bisbenzylisoquinolines as Modulators of Chloroquine Resistance in *Plasmodium falciparum* and Multidrug Resistance in Tumor Cells

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Ten naturally occurring bisbenzylisoquinolines (BBIQ) and two dihydro derivatives belonging to five BBIQ subgroups were evaluated in vitro for their ability to inhibit *Plasmodium falciparum* growth and, in drug combination, to reverse the resistance to chloroquine of strain FcB1. The same alkaloids were also assessed in vitro for their potentiating activity against vinblastine with the multidrug-resistant clone CCRF-CEM/VLB, established from lymphoblastic acute leukemia. Three of the BBIQ tested had 50% inhibitory concentrations of less than 1 μM. The most potent antimalarial agent was cocsoline (50% inhibitory concentration, 0.22 μM). Regarding the chloroquine-potentiating effect, fangchinoline exhibited the highest biological activity whereas the remaining compounds displayed either antagonistic or slight synergistic effects. Against the multidrug-resistant cancer cell line, fangchinoline was also by far the most active compound. Although there were clear differences between the activities of tested alkaloids, no relevant structure-activity relationship could be established. Nevertheless, fangchinoline appears to be a new biochemical tool able to help in the comprehension of the mechanism of both chloroquine resistance in *P. falciparum* and multidrug resistance in tumor cells.

One of the most frustrating lessons learned from the use of drugs in the treatment of parasitic diseases such as malaria is the regular emergence of drug resistance. This phenomenon has stimulated intensive efforts directed towards discovery of new bioactive compounds that would retain activity against resistant phenotypes or reverse drug resistance, as well as fundamental work aimed at understanding the mechanism of drug resistance and its reversal.

Bisbenzylisoquinolines (BBIQ) have attracted increasing attention as potential antimalarial drugs and reversing agents (14–16, 18). Tetrandrine, one of the most extensively studied BBIQ, was reported to display antimalarial effects on both chloroquine-susceptible and -resistant strains of *Plasmodium falciparum* and to act synergistically with chloroquine and artemisinin (23). Because drug resistance in malaria has apparent similarities with the multidrug resistance of mammalian tumor cells, the roles of a number of molecules in both biological phenomena have been investigated. Verapamil, a calcium channel blocker, was demonstrated to reverse chloroquine resistance of *P. falciparum* in vitro (17) and to relieve multidrug resistance of cancer cells (20). In this paper, we report the in vitro antiplasmodial activities of 10 naturally occurring BBIQ and 2 dihydro derivatives as well as the effects of coadministration of BBIQ with chloroquine and vinblastine against chloroquine-resistant parasites and multidrug-resistant (MDR) mammalian tumor cells. Some structural requirements for in vitro antiplasmodial action and drug resistance reversal are also discussed.

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MATERIALS AND METHODS

BBIQ were isolated from various plant sources belonging to the families *Annonaceae* and *Menispermaceae*. In *Annonaceae*, monterine and cordobimine were isolated from *Crematosperma* sp. strain RC 3110 (21); *O*-methyl dauricine, dauricoline, and popisonine were isolated from *Popowia pisocarpa* (11); and lindoldhamine was isolated from *Polyalthia nitidissima* (10). In *Menispermaceae*, cocsoline and daphnoline were obtained from *Albertisia papuana* (13), limacine was obtained from *Spirospermum penduliflorum*, and fangchinoline was obtained from *Strychnopsis thouarsii* (18). Dihydrocordobimine *RR* and *RS* were prepared by reduction of cordobimine (21). Chemical structures were unambiguously established in previous phytochemical works.

In vitro *P. falciparum* culture and drug assays. The experiments were performed with the chloroquine-resistant FcB1/Columbia or chloroquine-susceptible F32/Tanzania strains. The parasites were maintained on human type O^+ erythrocytes in RPMI 1640 culture medium supplemented with 27.5 mM $NaHCO_3$, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.4), 11 mM glucose, and 7.5% (vol/vol) heat-inactivated human O^+ serum in an atmosphere of 3% CO_2, 6% O_2, and 91% N_2 at 37°C (22).

P. falciparum susceptibility to chloroquine and to BBIQ was determined in 96-well plates according to the semiautomated microdilution technique of Desjardins et al. (4). Stock solutions of chloroquine diphosphate and BBIQ were prepared in sterile distilled water and dimethyl sulfoxide, respectively. Different concentrations of drugs were added to asynchronous parasite cultures (0.5% parasitemia and 1% hematocrit) for 24 h, at 37°C, prior to the addition of 0.5 μCi of [^{3}H]hypoxanthine monochloride (1 to 5 Ci/mmol; Amersham, Les Ulis, France) per well. After further incubation for 18 h, the cells were harvested from each well with a cell harvester onto glass fiber filters. The dried disks were counted in a scintillation spectrometer.

All the drug concentrations were tested in triplicate for each experiment. The dimethyl sulfoxide concentration never exceeded 0.05%. The growth inhibition for each concentration was determined by comparison of the radioactivity incorporated in the treated cultures with that in the control cultures (without drug) maintained on the same plate. The concentrations causing 50% inhibition (IC_{50}) were obtained from the drug concentration-response curves, and the results were expressed as the means ± the standard deviations determined from several independent experiments.

Drug interactions between chloroquine and BBIQ were estimated by determining the chloroquine IC_{50} for the FcB1 strain, as described above, in the presence of fixed BBIQ concentrations lower than the BBIQ IC_{50}. Isobolograms were constructed by plotting on the horizontal axis the chloroquine fractional IC_{50}, calculated by dividing the IC_{50} of chloroquine combined with the BBIQ by the IC_{50} of chloroquine alone. The corresponding BBIQ fractional IC_{50} was calculated by dividing its fixed concentration by the IC_{50} of BBIQ alone and plotted on the vertical axis. Each value was the mean of the three independent experiments.

Cell line and drug assays. The CCRF-CEM/WT cell line was established from acute lymphoblastic leukemia (7). The MDR clone (CCRF-CEM/VLB line) was selected after treatments in vitro with increasing concentrations of vinblastine (1).

Cells were cultured in R-10 medium (RPMI-1640 [Gibco] supplemented with 10% fetal calf serum and 4 mM glutamine). Vinblastine (500 ng/ml) was added into the culture medium in order to maintain the MDR phenotype of the CCRF-CEM/VLB line.

For drug susceptibility assays, cells were seeded at a density of 4×10^3 cells per 150 μl into 96-well tissue culture plates and incubated at 37°C in an atmosphere of 5% CO_2. After 24 h, 50 μl of six serial dilutions of the tested product was added to evaluate the antiproliferative effect. Each experiment was replicated in triplicate.

For modulator effect assays, the BBIQ solution was added to the six dilutions of vinblastine at a concentration just below that established independently to inhibit growth by 20%.

The cells were allowed to grow for four doubling times. Cell viability was evaluated by the colorimetric assay of endogenous hexosaminidase activity (12). Briefly, after washing the cells with phosphate-buffered saline, 50 μl of a 3.75 mM solution of *p*-nitrophenyl-*N*-acetyl-β-D-glucosamide (Sigma)–Triton X-100 (0.25%) in 50 mM sodium citrate buffer, pH 5, was added. After 60 min of incubation at 37°C, the enzymatic reaction was stopped by adding 100 μl of glycine (50 mM, pH 10.4) and 5 mM EDTA. Absorbances were read at a test wavelength of 405 nm.

Percent survival was defined as percent ratio of optical density measured for the drug-treated cells to that measured for untreated cells. A reversion factor (RF) was defined as the IC_{50} evaluated for vinblastine alone divided by the IC_{50} of vinblastine plus the tested molecule.

RESULTS

BBIQ can be conveniently classified into several types on the basis of the number and the nature (diaryl ether or biphenyl) of the bridges between the two moieties and further subdivided into subgroups according to their relative positions within the dimeric molecules and the absolute configuration of C-1 and C-1′ (2). Thus, the BBIQ investigated were classified into four types and five subgroups as follows (Fig. 1): type 1, BBIQ with one diaryl ether bridge (11 to 12′), subgroup A; type 2, BBIQ with one diaryl ether bridge (8 to 7′) and one biphenyl bridge (11 to 11′), subgroup B; type 3, BBIQ with two diaryl ether bridges (7 to 8′ and 11 to 12′), subgroup C, and (8 to 7′ and 11 to 12′), subgroup D; and type 4, BBIQ with three diaryl ether bridges (6 to 7′, 7 to 8′, and 11 to 12′), subgroup E.

The BBIQ IC_{50} (in vitro growth) for the chloroquine-resistant FcB1 are summarized in Table 1. All the BBIQ tested exhibited IC_{50} lower than 5 μM, and four BBIQ IC_{50} were less than 1 μM. The most potent antimalarial agent was cocsoline, belonging to the structural subgroup E, followed by two BBIQ representative of subgroup B: cordobimine and dihydro-cordobimine *RR*, with IC_{50} of 0.22, 0.58, and 0.37 μM, respectively. The IC_{50} of the remaining BBIQ were in the range 0.93 to 4.2 μM. Under test conditions, chloroquine presented an IC_{50} of 0.1 μM. The IC_{50} of chloroquine for susceptible strain Fc32 was 0.01 μM.

BBIQ were tested in vitro on the resistant strain FcB1 for their ability to interact with the chloroquine inhibitory effect. Limacine and fangchinoline, two other BBIQ, which have been described as chloroquine-potentiating drugs on chloroquine-resistant strains of *P. falciparum* (18), were evaluated under the same conditions. Isobolograms of interactions constructed from the experimental data are reproduced in Fig. 2. A curve above the diagonal, a curve falling below the diagonal, and a curve superimposed on the diagonal indicate, respectively, antagonism, synergism, and a simple additive effect of BBIQ on chloroquine inhibition (19). In order to facilitate comparison between the interaction of each BBIQ and chloroquine, an interaction factor (IF) was deduced from each isobologram curve. This factor was defined as the reciprocal of the chloroquine fractional IC_{50} obtained from the experimental curve for half of the IC_{50} of each BBIQ tested. The IF equals 2, <2, or >2 for additive, antagonistic, or synergistic effects of BBIQ on chloroquine inhibition, respectively: the higher the BBIQ IF value, the higher its potentiating activity on chloroquine.

Among the BBIQ presenting a synergistic effect on chloroquine activity, fangchinoline had the most efficient potentiating activity on the FcB1 strain of *P. falciparum*, with an IF of 12.5. The other BBIQ potentiated the chloroquine activity with IF values ranging from 3.33 to 5 (Table 1).

BBIQ were then tested for their properties to modulate the multidrug resistance of the CCRF-CEM/VLB cell line. Preliminary experiments showed that only *O*-methyl dauricine, cocsoline, cordobimine, limacine, and fangchinoline had a synergistic effect on vinblastine activity on this MDR cell line (data not shown). Hence, they were selected for further studies and compared with verapamil and tetrandrine, the 7-*O*-methyl derivative of fangchinoline, known as a modulator of chloroquine resistance in *P. falciparum* (23) (Fig. 1).

Cytotoxicity curves for the CCRF-CEM/VLB MDR cell line were constructed for the selected drugs, and the IC_{50} were determined (Table 2). BBIQ presented IC_{50} ranging from about 0.9 μM for cocsoline to more than 10 μM for fangchinoline. It should be noted that vinblastine presented an IC_{50} of 0.7 μM for this MDR cell line; hence, about a 700% increase in IC_{50} was obtained compared with the initial vinblastine-sensible CCRF-CEM/WT cell line (vinblastine IC_{50} = 1 nM).

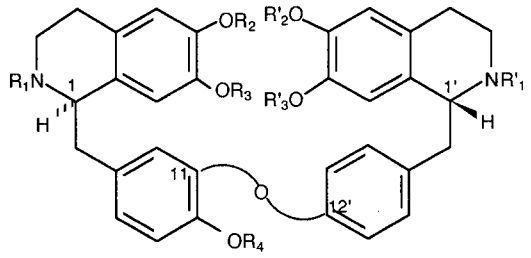
The modulator effects of BBIQ on vinblastine activity were determined for BBIQ concentrations causing less than 20% growth inhibition of the MDR cell line. An RF was defined as the IC_{50} evaluated for vinblastine alone divided by the IC_{50} obtained for vinblastine in the presence of BBIQ or verapamil: the higher the BBIQ RF value, the stronger its potentiating activity. Vinblastine IC_{50} in the presence or in the absence of BBIQ or verapamil and their corresponding RF values are listed in Table 3. As a control, the effects of the BBIQ and verapamil concentrations used on the MDR cell line were evaluated independently. In each case, the concentrations used presented a cell survival value higher than 80% (data not shown).

Among the BBIQ alkaloids tested, only fangchinoline and, to a lesser extent, its two analogs tetrandrine and limacine presented a clear potentiating activity on vinblastine, with RF values of 87.5, 17.5, and 4.4, respectively. At the same concentrations (2 μM), fangchinoline appeared about fourfold more potent than tetrandrine and 20-fold more potent than limacine. Compared with fangchinoline, tetrandrine, and limacine, the other BBIQ, as well as verapamil, had low potentiating activities (RF values of 1.8 for BBIQ and 2.3 for verapamil).

DISCUSSION

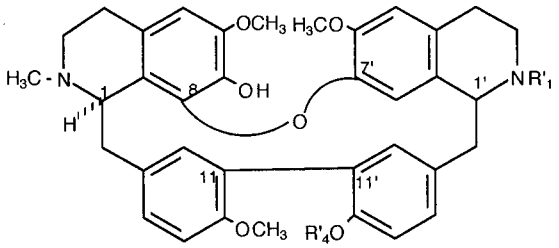
From the data shown in Table 1, it appears that three of the BBIQ possessed interesting intrinsic antimalarial activity, with in vitro IC_{50} of less than 0.6 μM, against the chloroquine-resistant FcB1 strain of *P. falciparum*. The most potent antimalarial agents were cocsoline, belonging to structural subgroup E, and two BBIQ representative of subgroup B: cordobimine and dihydrocordobimine *RR*. These results revealed differences between the activities of tested alkaloids,

Subgroup A



	Configuration C_1	Configuration C_1'	R_1	R_2	R_3	R_4	R'_1	R'_2	R'_3
O-Methyl dauricine	R	R	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3
Dauricoline	R	R	CH_3	H	CH_3	H	CH_3	H	CH_3
Popisonine	R	R	CH_3	H	CH_3	CH_3	CH_3	H	CH_3
Lindoldhamine	R	R	H	CH_3	H	H	H	CH_3	H

Subgroup B



	Configuration C_1	Configuration C'_1	R'_1	R'_4
Cordobimine	R	(C'_1 = N)		H
Dihydrocordobimine RR	R	R	H	H
Dihydrocordobimine RS	R	S	H	H
Monterine	R	S	CH_3	CH_3

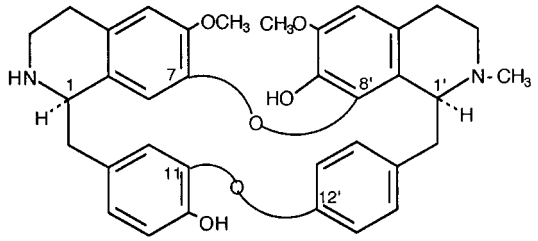
FIG. 1. Structures of BBIQ used. Subgroups are defined in Results.

but the structure-activity relationship could not be easily established. BBIQ are of several structural types. The two monomeric units are linked by one diaryl ether bridge, and the other bridges may be one or two diaryl ether linkages or a carbon-carbon linkage. Antimalarial activity was observed for BBIQ possessing *R* or *S* configuration at C-1 and C-1′. In subgroup B, a direct comparison could be made for dihydrocordobimine *RR* and *RS*, which had IC_{50} of 0.37 and 1.42 μM, respectively. Finally, from results obtained with subgroups A and B, it appears that substituents present on benzylisoquinoline moieties (phenolic or corresponding methyl ether) do not influence antiplasmodial activity significantly.

In addition to the antimalarial activity in vitro, some BBIQ possess potentiating activity against chloroquine. Here again, these molecules exhibit a range of different structures, and no clear structure-activity relationship could be established. In subgroup B, the status of C-1′ configuration is essential, as evidenced by dihydrocordobimine *RR* being antagonistic, whereas its isomer dihydrocordobimine *RS* presents a synergistic effect. A phenolic or corresponding methyl ether present in each benzylisoquinoline moiety may influence interaction significantly. This is illustrated by the synergistic effect observed for *O*-methyl dauricine, a polymethylated molecule, compared with the additive effect of the other BBIQ analogs of subgroup A.

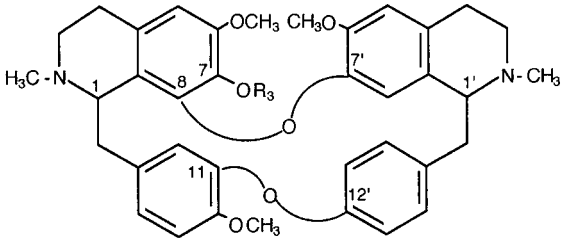
However, limacine and fangchinoline, two isomeric BBIQ of subgroup D, with two diaryl ether bridges (8 to 7′ and 11 to 12′), were the most efficient potentiating drugs, with fangchinoline (*SS* configuration) being significantly more active than limacine (*RR* configuration). These results obtained with the resistant strain FcB1 confirm the higher potentiating activity of fangchinoline compared with that of limacine observed with the chloroquine-resistant strain FcM29 (18). The O-methylated derivative of fangchinoline, tetrandrine, has been shown to act synergisti-

Subgroup C



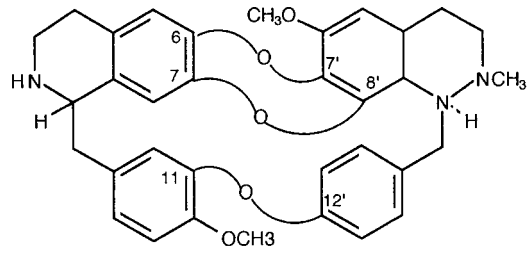
9 Daphnoline (1R, 1'S configuration)

Subgroup D



	Configuration C1	Configuration C'1	R_3
Limacine	R	R	H
Fangchinoline	S	S	H
Phaeanthine	R	R	CH_3
Tetrandrine	S	S	CH_3

Subgroup E



14 Cocsoline (1S, 1'S)

FIG. 1—*Continued.*

TABLE 1. In vitro antimalarial activity (IC_{50}) and in vitro chloroquine-potentiating action of BBIQ alkaloids (IF) against the chloroquine-resistant *P. falciparum* strain FcB1[a]

Subgroup	IC_{50} (μM)	IF
A		
O-methyl dauricine	1.96 ± 1.06[b]	3.56
Dauricoline	2.09 ± 0.70[b]	1.85
Popisonine	1.11 ± 0.31[b]	1.88
Lindoldhamine	4.29 ± 0.54[c]	1.56
B		
Cordobimine	0.58[d]	1.38
Dihydrocordobimine *RR*	0.37 ± 0.02[b]	1.0
Dihydrocordobimine *RS*	1.42 ± 0.22[b]	4.76
Monterine	1.59 ± 0.35[b]	4.16
C		
Daphnoline	1.75 ± 0.67[e]	1.17
D		
Limacine	1.27 ± 0.26[b]	5
Fangchinoline	0.93[d]	12.5
E		
Cocsoline	0.22 ± 0.10[b]	3.33

[a] IC_{50} were obtained from the drug concentration-response curves and are expressed as the means ± the standard deviations from several independent experiments. Drug interactions between chloroquine and BBIQ were evaluated by determining chloroquine IC_{50} in the presence of fixed BBIQ concentrations. Isobolograms of interactions were constructed from the experimental data (Fig. 2). An IF was defined as the reciprocal of the chloroquine fractional IC_{50} obtained for half of the IC_{50} of each BBIQ tested. The IF equals 2, <2, or >2 for additive, antagonistic, and synergistic effects of BBIQ on chloroquine inhibition, respectively. The IC_{50} of chloroquine diphosphate is 0.10.

[b] $n = 3$.

[c] $n = 5$.

[d] $n = 2$.

[e] $n = 4$.

cally with chloroquine against the chloroquine-resistant strain *P. falciparum* W-2 (23), whereas its isomer phaeanthine (*RR* configuration) showed an additive effect upon the chloroquine-resistant strain K (5). Our results and data in the literature underline the role of the configuration of C-1 and C-1′: fangchinoline and tetrandrine possessing configuration *SS* appear to be more potent potentiating drugs than their isomers of *RR* configuration.

The number of BBIQ able to enhance or potentiate the activity of vinblastine against the MDR CCRF-CEM human cell line was limited. Among the different alkaloids tested, only the BBIQ in subgroup D potentiate vinblastine. At the same concentration (2 μM), fangchinoline appears to be fourfold more potent than tetrandrine and 20-fold more potent than limacine. These results underline again the role of the configuration of the carbons C-1 and C-1′. Fangchinoline, which possesses an *SS* configuration, is much more active than limacine, with an *RR* configuration.

The substituents present in the isoquinoline moiety influence multidrug resistance reversing activity. A comparison of tetrandrine (RF = 17.5) and fangchinoline (RF = 87.5) indicates that methylation of the C-7 hydroxyl decreases activity. The other structural-type BBIQ, dehydrocordobimine, cocsoline, and *O*-methyl dauricine, displayed a comparatively very low potentiating activity.

Bearing in mind the different experimental conditions used and the genetic heterogeneity, it could be concluded from this work and data in the literature that fangchinoline and tetrandrine have reversal activities like that of verapamil against the chloroquine-resistant strains of *P. falciparum* and the MDR cell line.

In *P. falciparum*, the mechanism of activity of chloroquine as well as the mechanism of resistance to this drug is poorly understood. Several observations suggested similarities between the mechanism of chloroquine resistance and the mechanism of multidrug resistance in mammalian cells (3). Drug resistance in cancer cells is associated with an increase of drug efflux mediated by an amplification of *mdr* genes (9) and/or an overexpression of its product, the ATP-dependent p.glycopro-

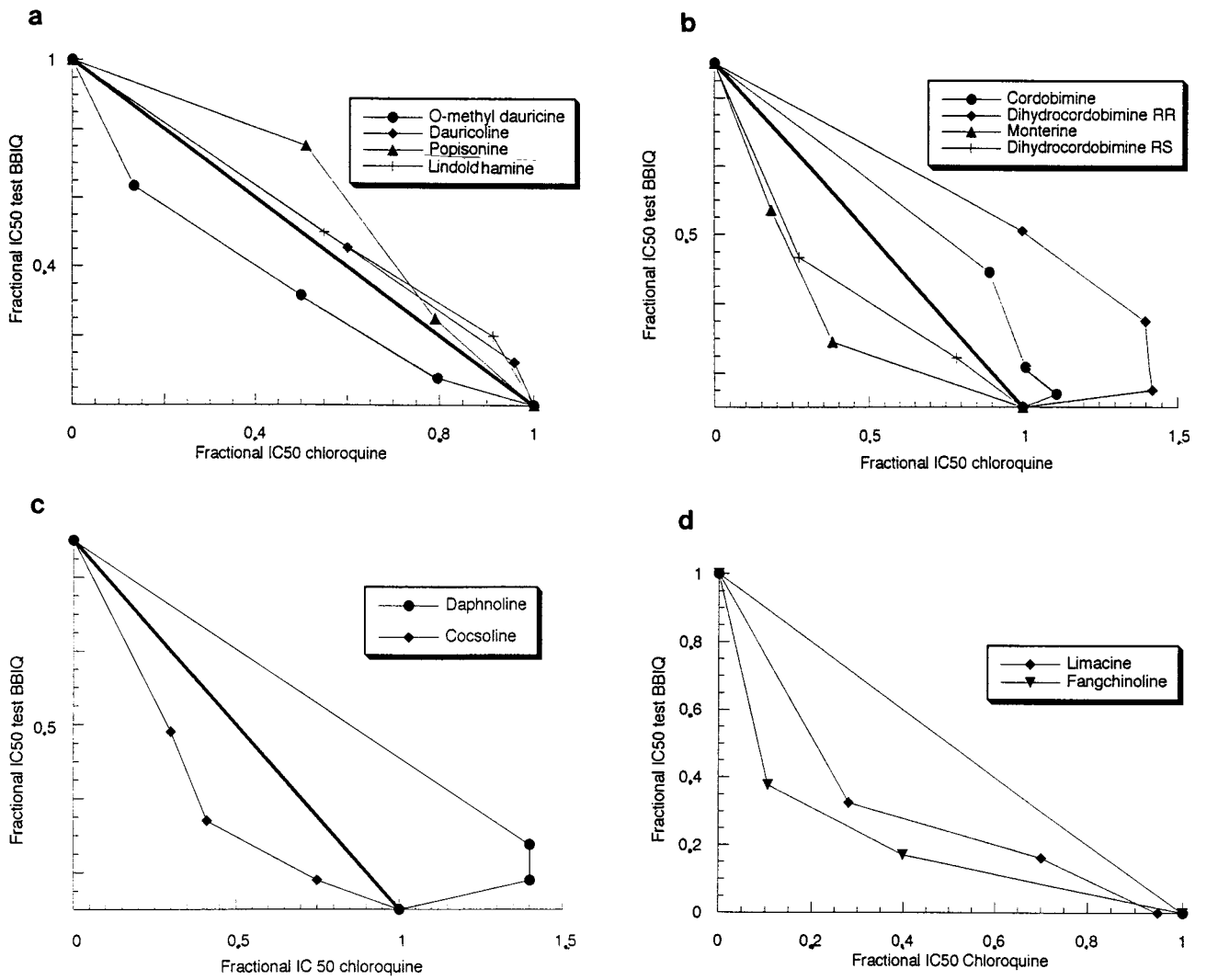


FIG. 2. Isobolograms of in vitro drug interaction between test BBIQ (subgroup A [a], subgroup B [b], subgroup E [c], and subgroup D [d]) and chloroquine against the chloroquine-resistant strain FcB1. Each point in the isobolograms was obtained by dividing the IC_{50} of chloroquine plus test alkaloids by the IC_{50} of chloroquine alone (abscissa) and by dividing the fixed test BBIQ concentration by the IC_{50} of its intrinsic activity (ordinate). Results are means of three independent experiments.

tein transporter (20). This efflux can be modulated by verapamil and other calcium channel blockers. Verapamil was also shown to relieve chloroquine resistance in *P. falciparum* (17). However, in contrast to cancer cells, no correlation between the amplification of the *pfmdr1* gene, a parasite gene homologous to the mammalian *mdr* genes, and the resistance status of *P. falciparum* was observed in vitro (3). Whatever the mo-

TABLE 2. Cytotoxicity for the MDR cell line CCRF-CEM/VLB[a]

BBIQ	IC_{50} (μM)
O-methyl dauricine	8.0
Cocsoline	0.9
Fangchinoline	>10
Limacine	3.5
Tetrandrine	5.0
Verapamil	7.0
Vinblastine sulfate	0.7

[a] IC_{50} were obtained from the drug concentration-response curve and expressed as the means from two independent experiments.

TABLE 3. Modulator effects of BBIQ and verapamil on the vinblastine activity on the MDR cell line CCRF-CEM/VLB[a]

Condition	Concn (μM)	IC_{50} (μM)	RF
Vinblastine alone		0.70	
Vinblastine with:			
O-methyl dauricine	1	0.40	1.80
Cocsoline	0.1	0.40	1.80
Cordobimine	10	0.40	1.80
Fangchinoline	2	0.008	87.50
Limacine	2	0.16	4.38
Tetrandrine	2	0.04	17.50
Verapamil	0.5	0.30	2.30

[a] BBIQ and verapamil concentrations causing less than 20% growth inhibition were used. IC_{50} were obtained from the drug concentration-response curve and expressed as the means from two independent experiments. RF was defined as the IC_{50} of vinblastine alone divided by the IC_{50} of vinblastine in combination with BBIQ or verapamil.

lecular mechanism involved in chloroquine resistance, the lower level of accumulation of drug is an obvious characteristic, and resistance is regarded as being due to a decrease in the concentration of drug in the parasite. Two pathways for modulating the intracellular chloroquine concentration are then possible: the resistant parasite can either take up less drug, or it can increase the chloroquine efflux of the infected erythrocytes (8).

Fangchinoline, tested on the susceptible strain F32 for its ability to interact with the chloroquine inhibitory effect, presented a low synergistic effect (IF = 4.15) and appeared to be threefold less potent than it was against the chloroquine-resistant strain. This result supports the hypothesis that the molecular mechanism involved in the fangchinoline activity should be present in the susceptible strain and more efficient in the resistant strain. The future should provide a much clearer understanding of the parasite resistance mechanism and of similarities of the chloroquine resistance phenotype with the multidrug resistance phenotype of mammalian tumor cells. In this context, fangchinoline, which affects these two different types of resistance mechanism, appears to be a new biochemical tool able to help in the comprehension of the chloroquine resistance mechanism of *P. falciparum.*

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REFERENCES

1. **Beck, W. T., T. J. Mueller, and L. R. Tanzer.** 1979. Altered surface membrane glycoproteins in vinca-alkaloid-resistant human leukemia lymphoblasts. Cancer Res. **39:**2070–2076.
2. **Cassels, B. K., and M. Shamma.** 1980. A simple relationship between stereostructure and specific rotation for the bis(benzylisoquinoline). Heterocycles **14:**211–230.
3. **Cowman, A. F.** 1991. The P.glycoprotein homologues of *Plasmodium falciparum*: are they involved in chloroquine resistance? Parasitol. Today **7:**70–76.
4. **Desjardins, R. E., C. J. Canfield, J. D. Haynes, and J. D. Chulay.** 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother. **16:**710–718.
5. **Ekong, R., S. J. Partridge, M. M. Anderson, G. C. Kirby, D. C. Warhurst, P. F. Russell, and J. D. Phillipson.** 1991. *Plasmodium falciparum*: effects of phaeanthine, a naturally-occurring bisbenzyl-isoquinoline alkaloid, on chloroquine-resistant and -sensitive parasites *in vitro*, and its influence on chloroquine activity. Ann. Trop. Med. Parasitol. **85:**205–213.
6. **Felix, J. P., V. F. King, J. L. Shevell, M. L. Garcia, G. J. Kaczorowski, I. R. C. Bick, and R. S. Slaughter.** 1992. Bis(benzylisoquinoline) analogs of tetrandrine block L-type calcium channels: evidence for interaction at the diltiazem-binding site. Biochemistry **31:**11793–11800.
7. **Foley, G. E., H. Lazarus, S. Farber, B. G. Human, B. A. Boone, and R. E. McCarthy.** 1965. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer **18:**522.
8. **Foote, S. J., and A. F. Cowman.** 1994. The mode of action and the mechanism of resistance to antimalarial drugs. Acta Trop. **56:**157–171.
9. **Hayes, S. D., and C. R. Wolf.** 1990. Molecular mechanisms of drug resistance. Biochem. J. **272:**281–295.
10. **Jossang, A., M. Leboeuf, P. Cabalion, and A. Cavé.** 1983. Alcaloïdes des Annonacées XLV: alcaloïdes du *Polyalthia nitidissima*. Benth. Planta Med. **49:**20–24.
11. **Jossang, A., M. Leboeuf, A. Cavé, and T. Sevenet.** 1986. Alcaloïdes des Annonacées LXV: alcaloïdes de *Popowia pisocarpa*, lère partie. Nouvelles bibenzyl-isoquinoléines. J. Nat. Prod. **49:**1018–1027.
12. **Landegren, U.** 1984. Measurement of cell numbers by means of the endogenous enzyme hexoaminidase. Applications to detection of lymphokines and cell surface antigens. J. Immunol. Methods **67:**379–388.
13. **Leboeuf, M., M. L. Abouchacra, T. Sevenet, and A. Cavé.** 1982. Alcaloïdes de *Albertisia papuana* Becc., Ménispermacées. Plant. Med. Phytother. **16:**280–291.
14. **Likhitwitayawuid, K., C. K. Angerhofer, G. A. Cordell, J. M. Pezzuto, and N. Ruangrungsi.** 1993. Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Stephania erecta*. J. Nat. Prod. **56:**30–38.
15. **Lin, L. Z., H. L. Shieh, C. K. Angerhofer, J. M. Pezzuto, G. A. Cordell, L. Xue, M. E. Johnson, and N. Ruangrungsi.** 1993. Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Cyclea barbata*. J. Nat. Prod. **56:**22–29.
16. **Marshall, S. J., P. F. Russell, C. W. Wright, M. M. Anderson, J. D. Phillipson, G. C. Kirby, D. C. Warhurst, and P. L. Schiff, Jr.** 1994. In vitro antiplasmodial, antiamoebic, and cytotoxic activities of a series of bisbenzylisoquinoline alkaloids. Antimicrob. Agents Chemother. **38:**96–103.
17. **Martin, S. K., A. M. J. Oduola, and W. Milhous.** 1987. Reversal of chloroquine resistance in *P. falciparum* by verapamil. Science **235:**899–901.
18. **Ratsimamanga-Urverg, R., P. Rasoanaivo, L. Ramiaramanana, R. Milijaona, H. Rafatro, F. Verdier, A. Rakoto-Ratsimamanga, and J. Le Bras.** 1992. *In vitro* antimalarial activity and chloroquine potentiating action of two bisbenzylisoquinoline enantiomer alkaloids isolated from *Strychnopsis thouarsii* and *Spirospermum penduliflorum*. Planta Med. **58:**540–543.
19. **Rideout, D. C., and T. C. Chou (eds.).** 1991. Synergism and antagonism in chemotherapy, p. 3–60. Academic Press, San Diego, Calif.
20. **Rogan, A. M., T. C. Hamilton, R. C. Young, R. W. Klecker, Jr., and R. F. Ozols.** 1984. Reversal of adriamycin resistance by verapamil in human ovarian cancer. Science **224:**994–996.
21. **Saez, J., E. Fernandez, A. Jossang, A. Cavé, and A. Cavé.** 1989. Nouveaux alcaloïdes bisbenzylisoquinoléiques biphényliques de *Crematosperma sp.* Can. J. Chem. **67:**275–278.
22. **Trager, W., and J. B. Jensen.** 1976. Human malaria parasites in continuous culture. Science **163:**673–675.
23. **Ye, Z., K. Van Dyke, and V. Castranova.** 1989. The potentiating action of tetrandrine in combination with chloroquine or qinghaosu against chloroquine sensitive and resistant falciparum malaria. Biochem. Biophys. Res. Commun. **156:**758–765.