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Alkaloids from plants of Madagascar in the treatment of drug-resistant *Plasmodium* malaria

P. Rasoanaivo and S. Ratsimamanga-Urverg

Institut Malgache de Recherches Appliquées, B.P. 3833, 101-Antananarivo, Madagascar.

Abstract. Various plant species termed chloroquine (CQ) adjuvants, have been used to supplement CQ effectiveness in Madagascar. As guided by bioassay to reverse the CQ-resistant strain FCM29 of *Plasmodium falciparum* , two known bisbenzylisoquinoline alkaloids, three novel isoquinoline dimers and five indole alkaloids, of which three are new, were isolated from four plant species. Some structural requirements for drug resistance reversal were identified.

INTRODUCTION

Despite decades of effort aimed at the control of malaria,the disease remains a serious problem, affecting in particular the populations of developing countries, with an estimated 200-300 million cases and 2 million death reported each year (1). The persistence of the disease in many areas and its increased incidence in others are attributable to the spread of chloroquine (CQ)-resistant strains of *Plasmodium falciparum* and the alarming emergence of multi-drug resistant strains of this organism. To make matters worse, only few antimalarials are available for clinical uses and the development of new ones both costly and time-comsuming. It is therefore our conviction that this disease will be with us for some time to come.

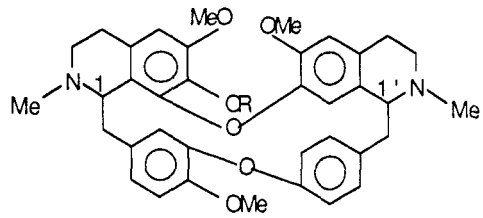
Of the new strategies available for the control of malaria, reversal of drug resistance has dominated the field of malaria chemotherapy in recent years (2). It has been shown, for example, that Ca^{2+} channel blockers such as verapamil, as well as several tricyclic antidepresants and various tricyclic antihistaminics are able to restore CQ sensitivity to resistant clones of *P. falciparum* . However, hopes for the rapid application of combination therapy with these drugs are probably premature since some such combinations increase chloroquine toxicity to host cells (3). On the other hand, very little is known about resistance-reversing agents from plants although relevant data exists in folklore medicines. This has prompted us to evaluate the ability of some Malagasy plants to potentiate CQ action.

WORKING HYPOTHESIS

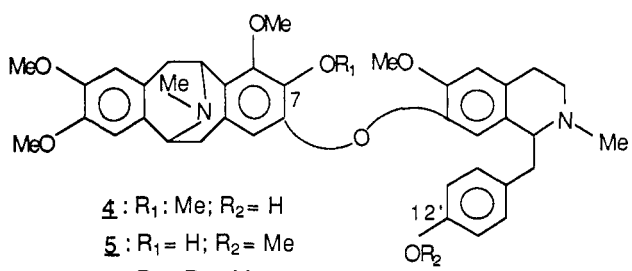
During the 1980's, malaria re-emerged in Madagascar as the most devastating of the country's tropical diseases (4). The severity of infection is such that local populations now recognize the hitherto unknown condition to which they have given the name *bemangovitra* (disease of great shivering). In the course of our ethnobotanical field work, we have learned that most rural people treat malaria by means of self-medication with 1-2 tablets of CQ, (a dose thought to promote CQ resistance), together with a decoction made from various plant species, termed CQ-adjuvants (5). We have assumed that some of these species may enhance CQ efficiency and might therefore reverse CQ resistance on the part of the malaria parasite.

RESULTS AND DISCUSSION

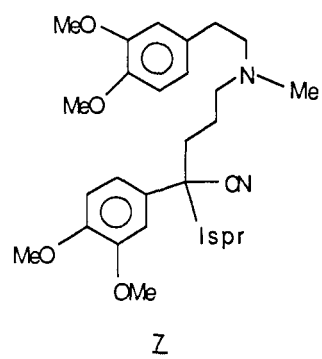
We have been able to demonstrate that the bisbenzylisoquinoline (BBIQ) frangchinoline 1 and its enantiomer limacine 2 respectively isolated from roots of the CQ adjuvant plants *Strychnopsis thouarsii* Baillon and *Spirospermum penduliflorum* Thouars (Menispermaceae) have significant intrinsic *in vitro* antimalarial effect (IC_{50} = 0.7 µg/ml), and also reverse *in vitro* CQ resistance in malaria (6). The configuration at the C-1 and C-1' chiral carbons slightly affects the CQ enhancing activity. Compound 1 having the "S" isomeric configuration is more potent in effecting CQ resistance reversal than its enantiomer 2. Frangchinoline 1 and tetrandrine 3 have been investigated independently by other research group for their multi-drug resistance reversal in malaria and patented together with other derivatives including compound 2 (7).



1 : R= H : (1S, 1'S)
2 : R= H : (1R, 1'R)
3 : R= CH_3 : (1S, 1'S)



4 : R_1 : Me; R_2= H
5 : R_1 = H; R_2 = Me
6 : R_1 = R_2 = Me



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Continued exploration of this class of compounds for their potential to afford new and more potent agents which might be subjected to *in vivo* testing, led to our isolating from *Hazomalania voyronii* R. Capuron (Hernandiaceae), by bioassay-guided fractionation based on reversal of CQ resistance (8), three isoquinoline alkaloid dimers named herveline A 4 herveline B 5 and herveline C 6. These compounds possess novel structures comprising pavine and benzylisoquinoline (BIQ) moities. Chemical structure was established by spectral methods, and the use of various 2D-NMR techniques (COSY, COSY LR, BIRD, HMBC) permitted complete assignement of their ^{13}C and ^{1}H NMR data (9). As regards biological activity, compounds 4, 5 and 6 exhibited moderate inherent antiplasmodial activity (IC_{50}= 1.5 -3 µg/ml). However drug combination methods using isobolograms (Figure 1) demonstrated that herveline B & C reversed *in vitro* CQ resistance in a dose dependent manner, whereas herveline A did not show any reversal activity (10).

Some structural requirements for CQ potentiating action in the series of isoquinoline dimers have been identified. First, it appears that the OMe group at C-12' is of paramount importance for biological activity. Moreover, there is some similarity between the hemicyclic structure of verapamil 7 and the BIQ part of isoquinoline dimers. Thus, BIQ

can be considered as verapamil derivatives with less flexibility, which would result in more selectivity in biological properties. Verapamil is a calcium channel blocker whereas tetrandrine and its derivatives do not show an affinity for calcium channel blocking. We therefore believe that if any future work on the CQ resistance reserval of isoquinoline alkaloids is to have any meaning, it will be essential to associate it with the synthesis of BIQ analogues. Other compounds structurally unrelated to isoquinoline alkaloids are also needed.

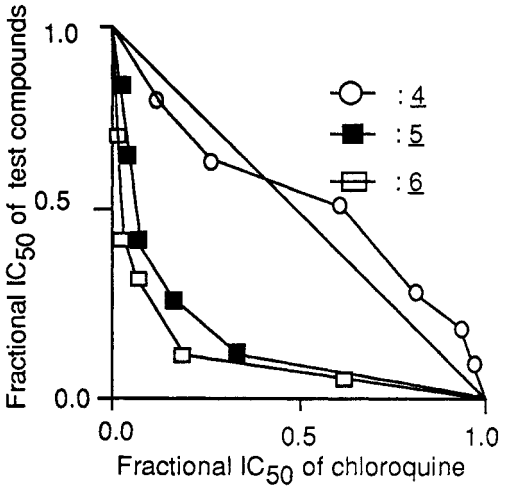


Fig1. Isobolograms of drug interaction *in vitro*

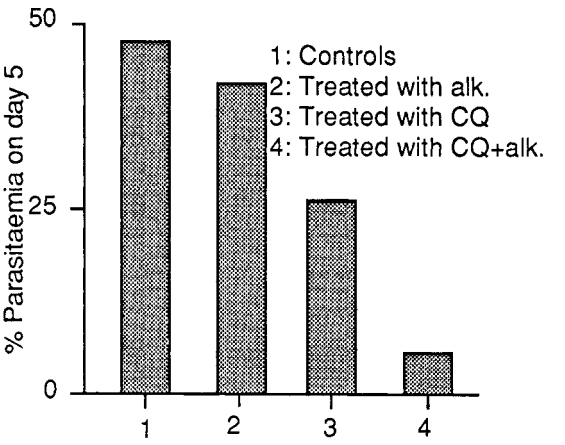
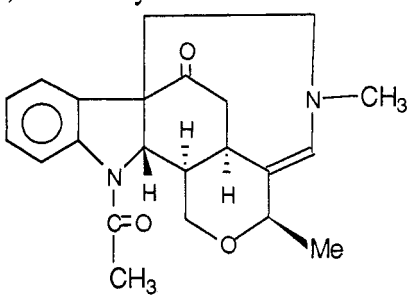


Fig 2: *In vivo* CQ potentiating action of crude alkaloid extract of *S. myrtoides*

Indole alkaloids possess various biological activities. By combining data on chemical systematics and ethnobotany, we have identified *Strychnos myrtoides* Gilg & Buss (Loganiaceae) as a plant worthy of further studies. Crude alkaloids significantly enhanced *in vitro & in vivo* CQ action (Figure 2). Bioassay-directed fractionation led to the isolation of the two major bioactive constituents namely strychnobrasiline 8 and malagashanine 9. These two compounds were devoid of any intrinsic *in vitro* antiplasmodial action (IC_{50} = 60 µg/ml).

However, when they were combined with CQ at a dose level much lower than required for



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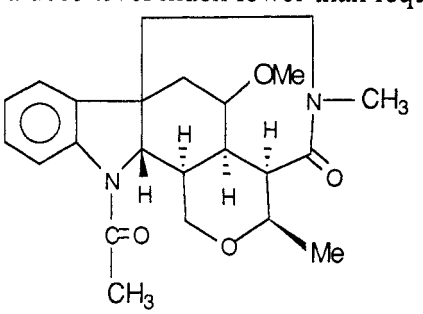

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antimalarial effect, they markedly reversed CQ resistance. Basicity of nitrogen atoms would not be required for reversal activity in this series (11). These findings would suggest a mechanism of action other than that of the isoquinoline alkaloids. Three new minor indole alkaloids were also isolated from *S. myrtoides*. They all reversed CQ resistance. Following toxicity studies, particularly evaluation of relaxant/convulsant effects on isolated organs, preliminary clinical trials were performed with infusions of *S. myrtoides* . Results were significant (12).

CONCLUDING REMARKS

Our results would account for the ethnopharmacological uses of CQ adjuvant plants investigated so far. The evolution of drug resistance is inevitable under drug pressure. We are therefore prospecting other species from the rich and diversified flora of Madagascar for drug resistance reversal, since in all probability the use of enhancers in the chemotherapy of malaria will be seriously considered in the near future.

The present work stems from a complementary North-South collaboration between *Institut Malgache de Recherches Appliquées* (Antananarivo), *Istituto Superiore di Sanità* , Roma, and *Museum National d'Histoire Naturelle* , Paris. All bioassay-guided fractionations have been conducted at the Phytochemistry & Pharmacology Department of IMRA.

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REFERENCES

1. WHO *Weekly Epid. Rec.* **64** , 241 (1989).

2. H. Ginsburg *Exp. Parasitol.* **73** , 227 (1991).

3. G. Watt, G. W. Long, M. Gorgl and S. K. Martin *WHO Report WHO/MAL/90.1056* (1990).

4. J. P. Lepers, P. Deloron, D. Fontenille, P. Coulanges *Lancet* , March 12, 586 (1988).

5. P. Rasoanaivo, A. Petitjean, S. Ratsimamanga-Urverg, A. Rakoto-Ratsimamanga *J. Ethnopharmacol.* **37** , 117 (1992).

6. S. Ratsimamanga-Urverg, P. Rasoanaivo, L. Ramiaramanana, R. Milijaona, H. Rafatro, A. Rakoto-Ratsimamanga, J. Le Bras *Planta Med.* **58** , 540 (1992).

7. K. Van Dyke *US Patent N° 5,025,020* dated June 18 (1991).

8. S. Ratsimamanga-Urgerg, P. Rasoanaivo, R. Milijaona, H. Rafatro, B. Robijaona, A. Rakoto-Ratsimamanga, J. Le Bras *Phyt. Res.* (in press).

9. P. Rasoanaivo, C. Galeffi, M. Nicoletti, S. Ratsimamanga-Urverg, F. Frappier, M.-T. Martin *Tetrahedron* (submitted).

10. S. Ratsimamanga-Urverg, P. Rasoanaivo, H. Rafatro, B. Robijaona, A. Rakoto-Ratsimamanga *Ann. Trop. Med. Parasitol.* (submitted).

11. P. Rasoanaivo, S. Ratsimamanga-Urverg, R. Milijaona, H. Rafatro, A. Rakoto-Ratsimamanga, C. Galeffi, M. Nicoletti *Planta Med.* (in press).

12. A. Ramialiarisoa, J. Ranaivoravo, S. Ratsimamanga-Urverg, P. Rasoanaivo, A. Rakoto-Ratsimamanga *Rev. Med. Pharm. Afr.* (in press).