Natural Products and Drug Discovery through a Network of Partnerships

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Abstract
We present here relevant result obtained from a network of collaborations. Malaria programme ranging from screening to investigation of mechanism of action has been conducted in collaboration with the *Muséum National d'Histoire Naturelle* in Paris, the *Institut de Chimie des Substances Naturelles* (CNRS), the *Istituto Superiore di Sanità di Roma*, the *Università degli Studi di Roma "La Sapienza"*, the *Université de Montpellier II*, the *Université des Sciences et Technologies de Lille*, the University of Cape Town. The cancer MDR modulator programme has been done at the University of Illinois at Chicago, in collaboration with our Institute. The antiviral and anti-inflammatory programme has been done in collaboration with the *Università del Piemonte Orientale*, and the University of British Columbia in Vancouver.

Key words: Drug biodiscovery; partnerships; malaria; antiviral; anti-inflammatory; integrated approaches.

Introductory remarks

Although powerful new technologies such as high-throughput screening and combinatorial chemistry are revolutionizing drug discovery, natural products still offer structural diversity which makes them a valuable source of novel lead compounds, allowing the design and rational planning of new drugs. In addition, some natural products to cite muscarine, cannabinoids, forskolin, colchichine, are important tools used in pharmacological, physiological and biochemical studies.

Basically, drug development involving natural products is a multi-disciplinary process. The basic sciences involved are botany, ethnobotany, chemistry and pharmacology. Any research into pharmacological active natural compounds depends on the integration of these sciences. The way they are integrated and the extent of integration depend on the objective of the study. For researchers in Africa, successful drug biodiscovery needs multi-centre partnerships with a complementary expertise; otherwise the results may not be robust enough and may lead to breakdown of the process. We report here relevant results through a network of partnerships.

Malaria programme

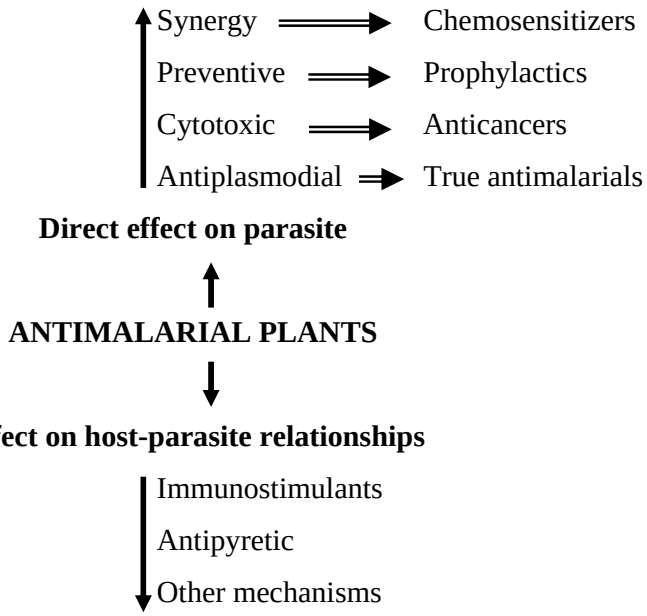
Background

Despite strong efforts to control it, malaria still remains a disease of importance afflicting particularly the populations of developing countries, with an estimated 300 millions cases and 2 millions deaths each year, and the global morbidity and mortality have not significantly changed over the past 50 years. One main reason for continued or, in some areas, rising rate of malaria infection are attributable to the spread of drug-resistant strains of *Plasmodium*

malaria, and the failure to get the existing, effective drugs to be applied in those areas where they can be of most benefit.

During the 1985's, malaria re-emerged in the central Highlands of Madagascar as the most devastating of the country's tropical diseases. The severity of the infection is such that local populations now recognize the hitherto unknown condition to which they have given the name *bemangovitra* (disease of great shivering). Shortage of appropriate antimalarial drugs at that time led the populations, especially those living in the countryside, back to the massive use of traditional herbal remedies. Following this sudden recrudescence of malaria, our Institute started in 1988 a research programme, in collaboration with French and Italian laboratories, with the aim of evaluating the antiplasmodial activity of medicinal plants used to treat malaria.

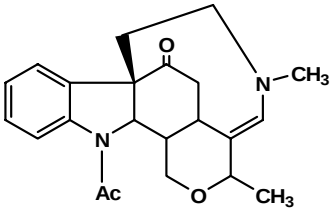
After seven years experience in ethnobotanical field work related to malaria, we came up with an integrated approach to antimalarial plants. We therefore expanded our screening programme to the evaluation of the cytotoxicity and the immunomodulating activities of plant extracts. And recently, we set up and implemented *in vivo* antiplasmodial screening in the liver stage of malaria parasites, while the *in vitro* tests are yet done at *Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, Centre Hospitalier-Universitaire Pitié-Salpêtrière, Université Pierre et Marie Curie* in Paris. This integrated approach can be summarized in the diagram below.



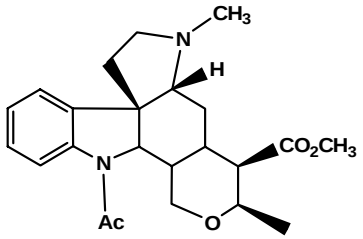
Chemosensitizers in malaria

At the inception of our programme on malaria chemotherapy, we focused our studies on medicinal plants that could reverse chloroquine resistance in malaria. To this end, we learned from ethnobotanical field works conducted in the period 1987-1992 that rural populations in Madagascar treat *bemangovitra* by means of self-medication with 1-2 tablets of chloroquine (a dose thought to promote chloroquine resistance), together with a decoction made from various plant species termed chloroquine-adjuvants [1]. Among several plants investigated,

Madagascan *Strychnos* species yielded two major bioactive constituents, malagashanine and strychnobrasiline [2].



Strychnobrasiline

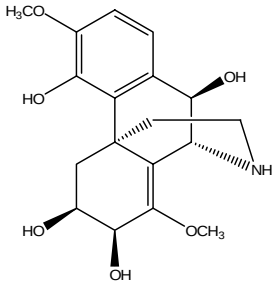


Malagashanine

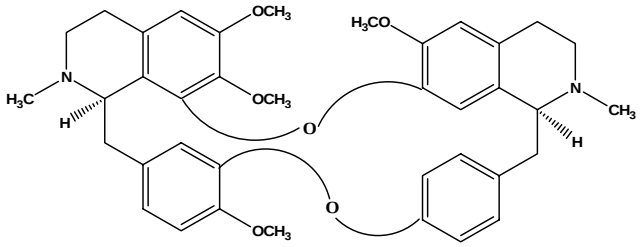
Malagashanine turned out to be the parent compound of a new subtype of *Strychnos* alkaloids, the C-21,N_b-secocuran indole alkaloids, isolated so far from Malagasy *Strychnos.* It had a weak antiplasmodial action, but when combined to chloroquine at concentrations much lower than required for antimalarial effect, it enhanced *in vitro* and *in vivo* chloroquine action against chloroquine-resistant strains of *Plasmodium* malaria. Minor alkaloids structurally related to the parent compound malagashanine were also isolated [3. 4]. This part of the work was done in collaboration with the *Muséum National d'Histoire Naturelle* in Paris, the *Institut de Chimie des Substances Naturelles*, CNRS, the *Istituto Superiore di Sanità* in Roma, and the *Unversità degli Studi di Roma* "La Sapienza".

The hepatic stage of malaria parasite

The inaugural meeting of RITAM (**R**esearch **I**nitiative on **T**raditional **A**ntimalaria **M**ethods) held in Moshi (Tanzania) in December 1999 opened a new way to the Malaria Laboratory of IMRA for the screening of extracts that may display antiplasmodial activities in the hepatic stage of malaria parasites. To this end, few selected antimalarial plants that were previously screened for antiplasmodial/chemosensitizing activities in the erythrocytic stage of malaria parasites were screened against the *Plasmodium* liver stage. As a result, aqueous extract of *Strychnopsis thouarsii* Baillon (Menispermaceae) exhibited significant *in vitro* and *in vivo* activities against the hepatic *Plasmodium* schizonts. A new morphinan alkaloid named tazopsine was found to be the active constituent [5, 6]. To he best of our knowledge, this is the first report on natural products that are active in the hepatic stage of malaria parasites. All the biological work was done at the INSERM U 511, *Centre Hospitalier-Universitaire Pitié-Salpêtrière, Université Pierre & Marie Curie*, Paris. This species was previously investigated for intrinsic antiplasmodial effects and drug reversing activities, and a known *bis*-benzylisoquinoline, fangchinolin, were shown to be the bioactive constituent [7].



Tazopsine



Fangchinolin

Semisynthesis

Going back to malaria chemosensitizers, it was useful to transform strychnobrasiline which was inactive *in vivo* in drug combination into malagashanine derivatives. Thus, the oxidation of strychnobrasline with *meta*-chloroperbenzoic acid led to an unexpected Bayer-Villiger rearrangement [8]. Then the hemisynthesis of malagashanine derivatives using deacetyl-strychnobrasiline as starting material was achieved in nine steps [9].

Based on the relevant results in which the introduction of a ferrocene into the side chain of chloroquine has led to a very active derivative, a ferrocene derivative of strychnobrasiline, was also prepared [10]. This was done in collaboration with the *Université des Sciences et Technologies de Lille*,

Chemosensitizing pharmacophore: hypothesis and synthesis

All naturally-occurring and hemisynthetic *Strychnos* alkaloids we isolated or prepared reversed *in vitro* chloroquine resistance. Careful examination of these structures suggested that the 1,4-diamino unit or N-phenyl-1,3-diamino propane of the tryptamine moieties might be a basic structure requirement for chemosensitizing activity in malaria. Careful inspection of the structures of synthetic chemosensitizers surprisingly showed that, although they appear to possess unrelated structures, most of them have in common a basic fragment. As shown in Figure 1, this fragment can be found embedded in the calcium entry blockers such as verapamil, in some tricyclic antidepressants to cite desipramine, amitriptyline, and in some tricyclic antihistaminics to name cyproheptadine, chloropromazine and penfluridol.

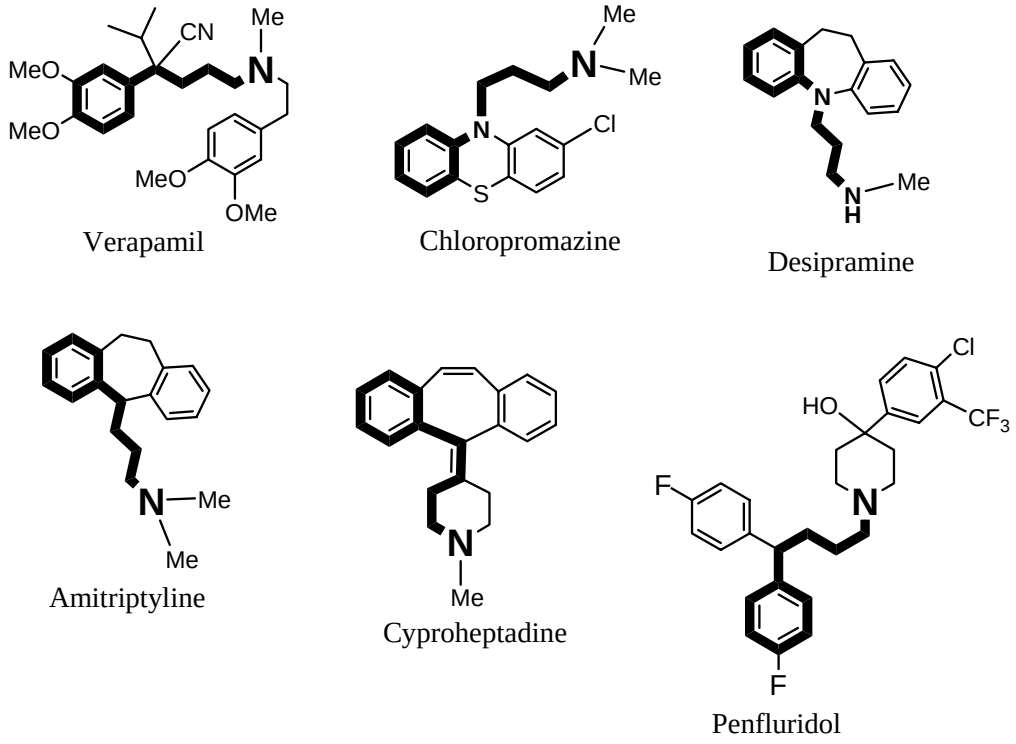


Fig. 1: Postulated reversing pharmacophore (in bold)
in synthetic malaria chemosensitizers

This led us to postulate the unifying structure requirement for reversal activity -S-$(C)_n$-S', in which S and S' = aromatic group or nitrogen and n = 3, 4, 5, 6. Synthesis of derivatives was achieved, and pharmacological testings confirmed our assumption [11]. This part of the work was done in collaboration with the Department of Chemistry of the University of Cape Town.

The 1,4-diamino structure is reminiscent of that of polyamines, namely putrescine, and by extrapolation spermidine and spermine which play a central role in cellular growth, differentiation and neoplastic transformation. Accordingly, polyamine-based structures may offer a wide range of structural possibilities, basically by substituting some of the nitrogen atoms by phenyl groups, in designing new antimalarial drugs or chemosensitizers with useful clinical relevance in the treatment of malaria and other parasitic diseases. In this regard, our on-going synthesis work done in collaboration with the *Université des Sciences et technologies de Lille* within the frame of PhD training has generated bioactive compounds and may yield promising leads [12].

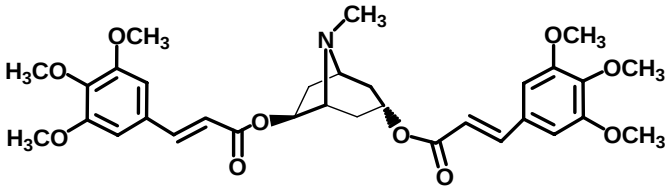
Malagashanine as biochemical tool

Surprisingly, chloroquine has both a 1,4-diamino fragment in its structure. Is it coincidence with our proposed pharmacophore or whether the data are relevant in understanding chloroquine resistance? We have used malagashanine as a biochemical tool to probe the mechanism of chloroquine resistance and its reversal. At this point, we came up with a hypothesis in which we postulated for the first time the existence of a bi-directional, reversible chloroquine transporter [13]. Reversing chloroquine resistance would consist in restoring the initial chloroquine importer function of this transporter. This work was done in collaboration with the *Université of Montpellier*.

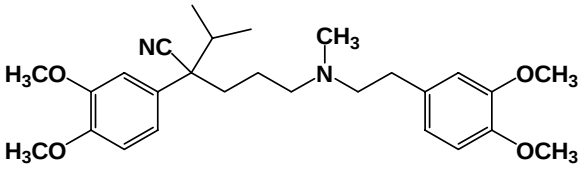
MDR in cancer and chemosensitizers

Within the frame of the official collaboration with the *Institut Malgache de Recherches Appliquées* and the College of Pharmacy of the University of Illinois at Chicago, several plant extracts were screened in 1992-1995 for anticancer/chemosentizing activities. One prominent result arose from this collaborative work. In effect, several new tropane alkaloid aromatic esters named pervilleines A-F were isolated from *Erythroxylum pervillei* Baillon (Erythroxylaceae). They were found to be excellent modulators of the MDR phenotype, with magnitude comparable to the standard MDR modulators verapamil and cyclosporine [14]. These compounds are being further investigated within the Rapid Access to Invention Development (RAID) Programme of the US National Cancer Institute.

Other new tropane ester alkaloids were isolated from a large re-collection of the species as minor constituents [15].



Pervilleine C



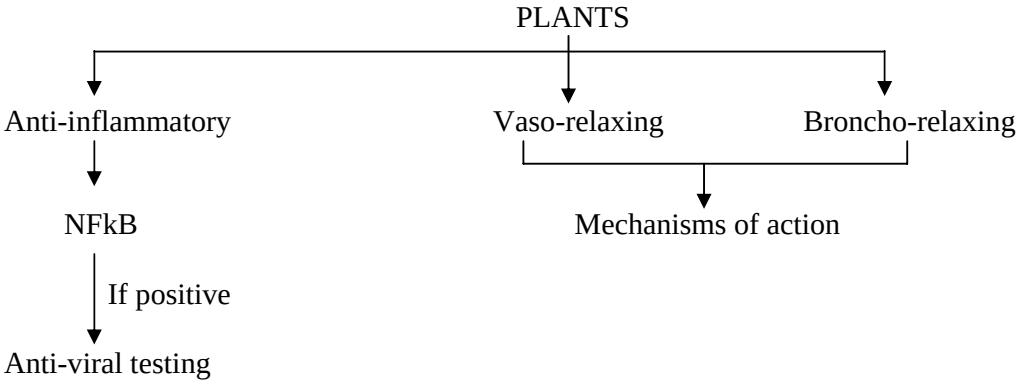
Verapamil

Fig. 2: Similarities between verapamil structure and a chemosensitizing pharmacophore (in bold) identified from a pervilleine structure

In carefully dissecting the structure of the reference compound verapamil and those of pervilleine series (Fig. 2), it is easy to extract a verapamil-like pharmacophore, which further supports our unifying chemosensitizing pharmacophore.

Anti-inflammatory - antiviral pathway

We learned from a Colleague at the *Università del Piemonte Orientale*, Milano, that Italian *Helichrysum* species hold phloroglucinol derivatives with good antiparasitic and anti-infectious activities. Based on this information, we selected relevant Madagascan *Helichrysum* for biological screening within a collaborative research. *H. cordifolium* was found to have strong and selective antiviral activities, and also inhibited NFκB [16]. Independently, following a long-standing contact with the University of British Columbia in Vancouver, we learned that Baobab had strong antiviral activities [17]. We then tested leaves of *Adansonia madagascariensis* within this programme, and found that it displayed very potent antiviral activities and also anti-inflammatory potency by measuring several different cytokines. We therefore came up with an integrated approach outlined below to screen medicinal plants used to treat vascular and pulmonary disorders, using isolated-based techniques available in our Institute.



Within the frame of vaso-/bronxcho-relaxing plant programme, we investigated *Phymatodes scolopendria* used to treat asthma in the east region of Madagascar, and found coumarin to be the active constituent [18].

Conclusion

The combinatorial chemistry is now making very large numbers of compounds of pre-selected structural types available for screening. It is hard to compete with such a potential for discovery. Despite this, natural products remain an untapped source of leads whose structures may challenge the imagination of medicinal chemists. In our opinion, one area of importance for Africa is the discovery of new drugs for the treatment of parasitic diseases. They are the biggest and most paralyzing health problem of African countries. We believe that modern natural products research has a chance to discover such drugs. Referring to malaria, quinine was discovered from the South-American biodiversity, and artemisinin from the Asian biodiversity. It is hoped that the next powerful naturally-occurring antimalarial will come from African biodiversity.

Acknowledgments

Financial support from *Agence Universitaire de la Francophonie*, Programme PAL$^+$ of the French Ministry of Education and Research, the International Development Research Centre, and the International Foundation for Science, is gratefully acknowledged.

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