Madagascar

TRADITIONAL MEDICINE AND RESISTANCE MODULATORS

RASOANAIVO P., RAKOTONANDRASANA O.L., RAMANITRAHASIMBOLA D. AND RATSIMAMANGA S.

Résumé

Promising resistance modulators were isolated from two endemic medicinal plants of Madagascar, *Strychnos myrtoides* Gilg & Buss and *Erythroxylum pervillei* Baillon as a scientific follow-up of their empirical uses which were communicated by two traditional healers respectively and largely described in this paper. Careful inspection of their structures led to a tentative hypothesis of a basic chemosensitizing pharmacophore identified as S-$(CH2)_n$-S' in which S and S' could be nitrogen or phenyl group. Curiously, this pharmacophore is reminiscent of the polyamine structures, and a possible link between the two entities is discussed in terms of biochemical tools to probe the mechanisms of drug resistance and its reversal as well as chemosensitizers with improved pharmacological profiles.

Key words

Madagascar, Medicinal plants, chemosensitizers, Malagashanine, Pervilleines, Polyamines

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INTRODUCTION

All the living matter evolves from birth to death, and the perpetuity of a given species is continuously sustained by the phenomenon of reproduction. Any factors that interrupt this natural process inevitably lead to the development of survival mechanism in the part of living matter. Thus, in the infectious diseases such as malaria, AIDS, tuberculosis as well as in cancer diseases in which pathogenic micro-organisms or invading cells must be killed by chemotherapy before they kill the host, the micro-organisms deploy all sort of clever and unexpected mechanism known under the general term resistance to escape their predators. Drug resistance in infectious diseases is an adverse factor for the efficacy of several chemotherapeutic treatments. We are therefore in an ambiguous situation or a vicious circle in which more and more powerful drugs are needed to overcome the inevitable phenomenon of resistance while the pathogenic micro-organisms develop various resistance mechanisms to counteract the action of the drugs. But in the absence of relevant alternatives, chemotherapy remains the only valid strategy to kill the pathogenic micro-organisms before the situation becomes fatal for the host.

One relevant strategy to overcome drug resistance is drug combination. At this point, since the pioneering work in cancer (Tsuruo *et al.,* 1981) and malaria chemotherapy (Martin *et al.*, 1987), the use of reversing agents to overcome drug resistance is a potential new treatment strategy both in malaria and cancer. One advantage of this approach in malaria is the possibility of prolonging the useful life of chloroquine which remains the drug of choice because of its rapid onset of action, good tolerability, limited host toxicity, low cost and versatility for both prophylactic and curative uses, but which is becoming less effective because of resistance. Most of active reversing compounds have been of synthetic origin, but naturally-occurring chemosensitizers compounds have been also discovered. We wish to report the story behind the discovery of two medicinal plants of Madagascar, *Strychnos myrtoides* Gilg & Buss (Loganiaceae) and *Erythroxylum pervillei* Baillon (Erythroxylaceae), from which promising chemosensitizing alkaloids were isolated, and we will discuss how these biologically active compounds may provide a useful template for designing novel molecules which can be used to probe the mechanisms of drug resistance and its reversal, or to develop drugs with improved pharmacological profiles.

PLANT STORY

Strychnos myrtoides

The Highlands of Madagascar, characterized by an unstable malaria transmission, have been the subject of sudden and unpredicted malaria epidemics (Blanchy *et al.*, 1993). One of these occurred in 1980s as one of the most devastating of the country's tropical diseases. The severity of the infection is such that local populations now recognise the hitherto unknown condition to which they have given the name *bemangovitra* (disease of great shivering). Shortage of appropriate drugs

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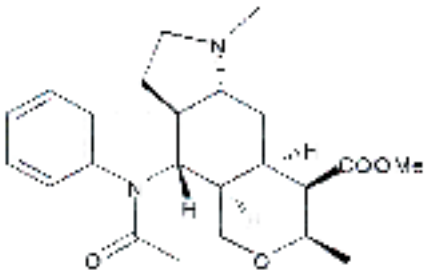
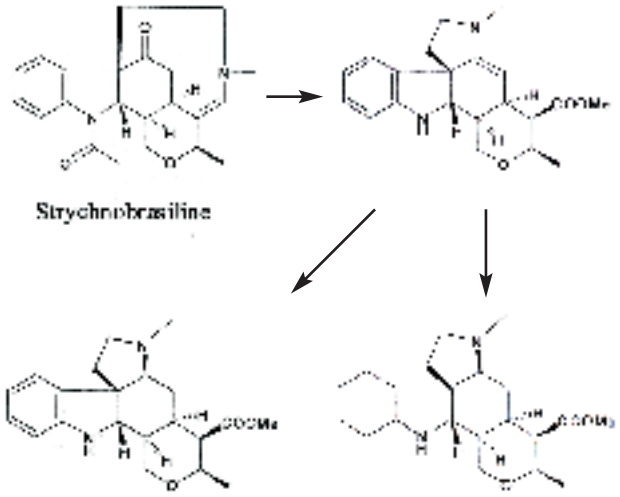
at that time and also attachment to traditional treatments led the population back to the massive use of herbal remedies. During a series of ethnobotanical field works conducted in 1990-1992 with our friend ethnobotanist J. P. Abrahama in the Eastern rain forests, one of us (PR) observed that he used discretely an infusion of one scrapped stem of a plant when we went to the forests. Our Colleague tactfully asks him about the purpose of this practice. He told our Colleague that the infusion of this plant, known under the vernacular name *retendrika* identified later on as *Strychnos myrtoides*, protects him against malaria. This plant grows in Ankarafantsika, West part of Madagascar. To the best of our knowledge, this is the first information about the prophylactic use of an herbal remedy against malaria in Madagascar. Assuming first that, like chloroquine, the infusion of this plant may have antimalarial activity with long-lasting effect, we evaluated in our institute the *in vitro & in vivo* antiplasmodial activity of various extracts, but the results were rather disappointing since they all had a low activity. Although we felt that we required quickly more details on the use of *retendrika*, we did not press immediately to obtain them in order to avoid any resentment or reticence. Many months later, our Colleague tactfully made more inquiries into Abrahama's recipe. He maintained the use of *retendrika* as efficient prophylactic remedy against malaria. But our Colleague also learned that he has a personal recipe : he and his family use the infusion of the scrapped stem of *Strychnos myrtoides* in combination with 1-2 tablets of chloroquine as a curative treatment against chronic malaria. This subsequently guided our work into drug combination assessment, which resulted in the isolation of the two bioactive constituents, the known alkaloid strychnobrasiline and a new compound named malagashanine (Rasoanaivo *et al.*, 1994).



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* (Martin *et al.*, 1999). 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. This confirmed the validity of the traditional recipe in the experimental models. After basic toxicity studies, an infusion of stem barks of *Strychnos myrtoides* was shown to be effective when combined with chloroquine or quinine in a clinical observational study (Ramialiharisoa *et al.*, 1994). However, the claimed prophylactic effect has not been hitherto confirmed in the existing experimental models (Mazier *et al.*, unpublished results).

Strychnobrasiline was by far the major constituent of *S. myrtoides*. As it was devoid of *in vivo* activity (Rafatro *et al.*, 2000), it was used as a starting material for the hemisynthesis of malagashanine derivatives which were patented (Trigalo *et al.*, 2002), and briefly summarized in the scheme below:



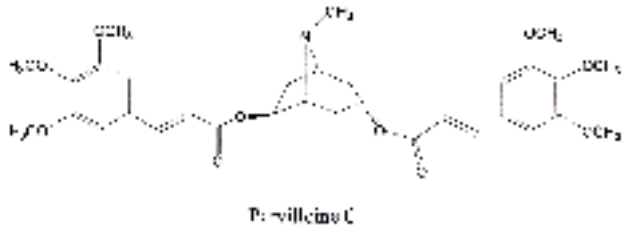
Malagashanine derivatives were found to enhance *in vitro* both chloroquine action against resistant stains of malaria parasites and doxorubicine activity against doxorubicine-resistant P388 cell lines (Trigalo *et al.*, 2002).

Erythroxylum pervillei

Because of the aridity of the climate, the vegetation of the South part of Madagascar is basically xerophytic, characterised by thorny or succulent species with deciduous or reduced leaves, staggered boughs and bottle-like trunks with tubers. Large species are rare but shrubs and bushes dominate in the area. These adaptations enable the species to grow in the harsh conditions of the South. The endemicity is comparatively high, with more than 90% of species which are reported to be specific to the region (Phillipson, 1996). Since 1982, one of us (PR) has been having useful links with local populations. Particularly, with the help of a Colleague working at the University of Toliara, he had the opportunity to know an Ombiasy (traditional healer) and also Mpisikidy (foreteller, diviner) whose name is Longonanake. The foreteller makes a diagnosis of diseases with a traditional method called *sikidy*, using seeds of *Tamarindus indica* which are arranged in a special way (Dahle, 1886). The *sikidy* is also known to be a mean if one wants to look into the future, to detect enemies or dangers, to find out what is to be his lot of good or evil. Our Colleague did few ethnobotanical field works with Longonanake, and occasionally attended ritual sessions of *sikidy*. One day he noticed in the Longonanake's house roots or stems of plants scattered in the ground. He kindly requested him the traditional uses of theses roots. Longonanake told him that they are mainly used for ritual ceremonies *in sikidy*, but some of them have also medicinal virtues. One of these plants called *Tsivano* is used to treat several illnesses, particularly diseases called '*bay*' in the local

language. ‘Bay’ itself refers to various diseases including inflammation (*bay mivonto*), tumours (*bay tsy janga*), fungal skin diseases (*fandikesa*), furuncles and acne (*bay farasisa*). Roosts are moistened with water and scrapped against a stone to yield a powdered material which is applied topically. *Tsivano* is the vernacular name used to name some *Erythroxylum* species in the South part of Madagascar, and this vernacular name is sometimes misleading when collecting the right species. Our Colleague took sample roots of the plant for anticancer screening carried out at the University of Illinois at Chicago within an official agreement.

Several new tropane alkaloid aromatic esters named pervilleines A-F isolated from *E. pervillei* were found to be excellent modulators of the MDR phenotype, with magnitude comparable to the standard MDR modulators verapamil and cyclosporine A (Silva *et al.*, 2001; Mi *et al.*, 2002). These compounds are being further investigated within the Rapid Access and Intervention and Development (RAID) Program of the National Cancer Institute, under a formal agreement with the Malagasy partners.

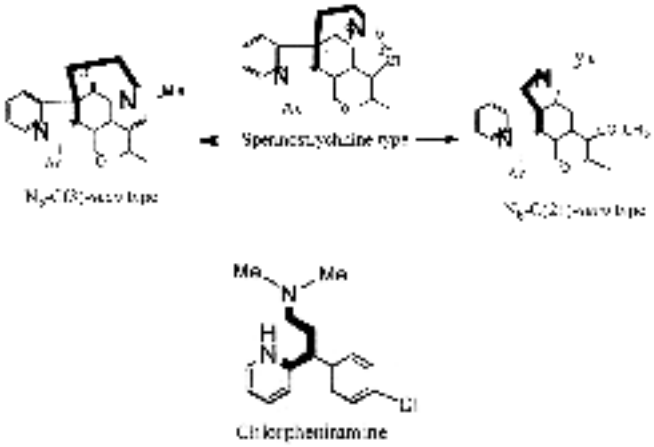


Pervilleine C

DISCUSSION

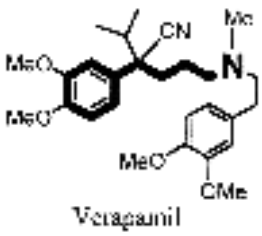
Madagascar has unique floristic biodiversity with unparalleled degree of endemism. Such biodiversity has already given unique chemical structures, to cite the antitumours drugs vinblastin and vincristin isolated from the Malagasy periwinkle *Catharanthus roseus*. Although the *Strychnos* and *Erythroxylum* genera have been the subject of several phytochemical and biological investigations (Bosh *et al.*, 1996; Griffin & Lin, 2000), *Strychnos myrtoides* and *Erythroxylum pervillei* both endemic to Madagascar hold new structures endowed with drug resistance reversal activities. To the best of our knowledge, such activities in *Strychnos* indole alkaloids and in *Erythroxylum* tropane alkaloids were first discovered from Malagasy plants.

Surprisingly, the two series of unrelated structures have similar drug resistance reversal activity, and this is also true for the synthetic chemosensitizers in malaria to cite the calcium entry blockers, some tricyclic antidepressants and some tricyclic antihistaminics. This raises the problem of functional *versus* chemical diversity. We assumed that basic chemosensitizing pharmacophores might be present in most malaria reversers. At this point, one important result that came up from our investigation of four *Strychnos* species was the finding that the spermostrychnine type and two *seco* derivatives, the N_b-C(3) and the N_b-C(21) *seco* curane types, all reverse *in vitro* chloroquine resistance against resistant strains of *Plasmodium* malaria. The reduction of the benzene ring of the indoline group did not affect the activity, indicating that this functional group was not directly involved in the chemosensitizing activity of the concerned indole alkaloid (Trigalo *et al.*, 2002).

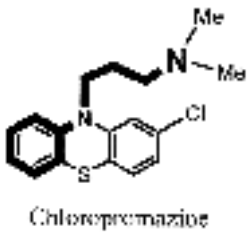


Although the number of existing naturally-occurring chemosensitizing structures were low to draw a valid structure-activity relationship without computational processing in a hotly debated and controversial topic such as chloroquine resistance and its reversal, we tentatively proposed a hypothesis in which we assumed that the 1,4-diamino unit in *Strychnos* alkaloids might be a basic structure requirement for chemosensitizing activity in malaria. In line with this hypothesis, some synthetic chemosensitizers such as chlorpheniramine have a 1,4-diamino fragment.

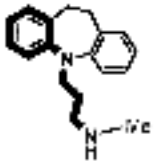
How this hypothesis could be applied to the synthetic malaria reversers? At this point, based on the assumption that phenyl group is claimed to have an attractive effect with another phenyl group if they are perpendicular each other (Dive, personal communication), we replaced one nitrogen atom by a phenyl group in the above basic pharmacophore. Thus, 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 below, the 1-amino-4-phenyl 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. In some structures, a nitrogen atom replaces one carbon between the two functional groups.



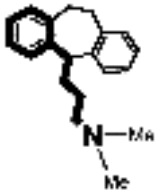
Verapamil



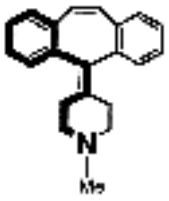
Chloropromazine



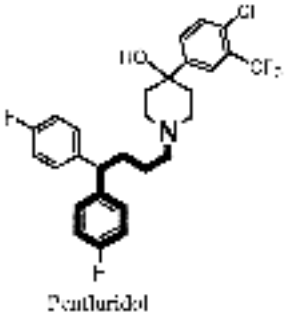
Desipramine



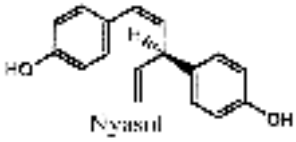
Amitriptyline



Cyproheptadine



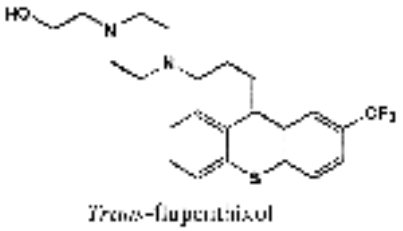
Penfluridol



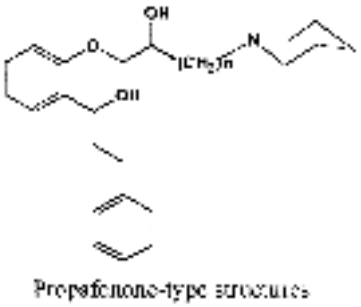
Nyasol

This led us to postulate a unifying hypothesis in which **-N-(C)$_n$-S** (S = aromatic group or nitrogen and n = 3, 4, 5, 6) might be a basic structure requirement for reversal activity. Structures with S = phenyl and n > 5 may be considered as an extended verapamil-based structures. To the best of our knowledge, the approach leading to this unifying hypothesis was first presented as plenary lecture in the *5ème Colloque Produits Naturels d'Origine Végétale*, Québec, 7-9 August 2001 (Rasoanaivo *et al.*, 2001). Clearly, it is easy to extract an extended verapamil pharmacophore from the pervilleine structures. It can be deduced from the unifying hypothesis that 1,n-diphenyl structures (n = 3, 4, 5, 6) might possess chemosensitizing activities. Indeed, the norlignan nyasol was reported to enhance the antifungal activities of azole agents against *Candida albicans* (Iida *et al.*, 2002). In line with this, we assumed the chloroquine reversal activity of *bis*-benzylisoquinolines might be due to the presence of phenyl groups which are located at the required distance for biological activity (Ratsimamanga-Urverg *et al.*, 1992).

In connection with our hypothesis, useful structure-activity relationships have been identified in the field of MDR in cancer. Thus the investigation of the rational design of chemosensitizing phenothiazines revealed three important components including the hydrophobicity of the tricyclic ring, the length of the alkyl bridge and the charge of the terminal amino group, and trans-flupenthixol was found to be in agreement with these structure requirements (Hait & Aftab, 1992). In line with the hydrophobicity requirement of the aromatic ring for good reversing activity, the introduction of a hydrophobic group at position 4 in chalcones led to much more active compounds than the parent chalcone (Bois *et al.*, 1999). Regarding the length between the two functional groups, it was reported in propafenone-type modulators that the activity increased with increasing number of methylene groups, whereby it reaches a plateau for compounds with more than five methylene groups between the ether oxygen and the nitrogen atom (Chiba *et al.*, 1998). It can be deduced that lipid solubility at physiological pH, cationic charge and distance between the two functional groups are important physical properties for drug reversal. Using a photoactivable analogues of vinblastine as probe, it was also reported that two planar aromatic domains and a basic nitrogen atom were established as important structural features for MDR modulating activities (Pearce *et al.*, 1990).



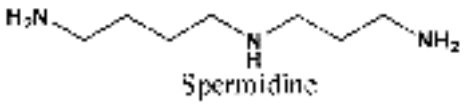
Trans-flupenthixol

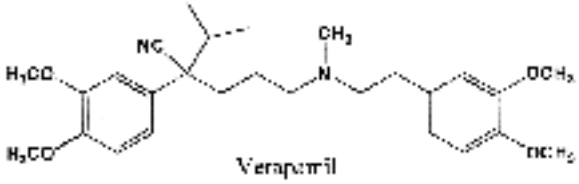


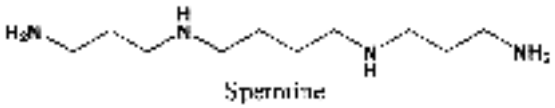
Propafenone-type structures

The 1,4-diamino structure is reminiscent of that of polyamines, namely putrescine, spermidine and spermine which play a central role in cellular growth, differentiation and neoplastic transformation. It is evident that we have tried to link our hypothesis to the pharmacological activities of these cationic molecules. At this point, spermine was found to modulate the accumulation and cytotoxicity of cis-diaminedichloroplatinium in sensitive and resistant human ovarian carcinoma cells (Marverti *et al.*, 1997). Study of the interaction between polyamine transporters and P-glycoproteins (P-gp) reported that a functioning polyamine transport system may be a requirement for MDR transporter activity, possibly by activating the three-dimensional organisation of P-gp in the cell membrane, while the expression of functioning P-gp was found to down-regulate the polyamine transporter activity (Aziz *et al.*, 1998). Based on these findings, resistance modulators having polyamine-like structures as defined in our hypothesis might have several alternative mechanisms as working hypothesis, namely by inhibiting the polyamine transport system or by competitively binding to the polyamine transporter. Polyamine derivatives were found to have antimalarial activity (Bitonti *et al.*, 1989; Bergeron *et al.*, 1994), and in recent years, polyamine biosynthetic enzymes have attracted attention as drug targets because they might reveal novel antiparasitic therapies (Müller *et al.*, 2001). According to our hypothesis, polyamine-based structures may also offer a wide range of structural possibilities, basically by replacing some of the nitrogen atoms by a phenyl group, in designing new chemosensitizers with useful clinical relevance in the treatment of malaria and cancer, rescuing previously highly successful treatment regimens for future use.

Surprisingly, chloroquine has 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? Importantly, it was reported that chloroquine derivatives with a modified side chain length retained activity against chloroquine-resistant strains of *Plasmodium* malaria, strongly suggesting that the diamino fragment may play an important role in the mechanism of chloroquine resistance (Ridley, 1997). On the other hand, chloroquine derivatives with 1,4- and 1,6-diamino units were reported to reverse MDR in cancer (Chibale *et al*., 2001). In turn, chemosensitizing phenothiazines were transformed into antimalarial drugs by increasing the number of basic groups in the alkylamino side chain (Kalkanidis *et al.*, 2002). All these studies point to the role of the **-N-(C)$_n$-S** hypothesis as defined above in antimalarial activity, drug resistance and reversal. It must be underlined however that there are similarities and differences between resistance in malaria and cancer (Karcz & Cowman, 1991; Bray & Ward, 1998). At this point, a wide variety of chemical structures have been reported to reverse MDR resistance in cancer (Krishma & Mayer, 2000), but not all drugs capable of chemosensitizing MDR tumors are similarly efficacious for chloroquine resistance. In agreement with this, the bis-indole alkaloid voacamine was found to reverse MDR resistance in cancer but was devoid of any similar effect in malaria resistance reversal (Ramanitrahasimbola *et al.*, 2001). The **-N-(C)$_n$-S** hypothesis might be a common point of mechanism in malaria, cancer and probably bacteria resistance. And in connection with the role of the 1,4-diamino side chain of chloroquine in restoring chloroquine sensitivity, a polyamine transporter might be used by resistant parasites to expel the chloroquine from the food vacuoles of the malaria parasite.







Careful analysis of the ethnomedical uses of *E. pervillei* strongly suggests that roots may have antimicrobial/antifungal activities. At this point, it was reported that MDR pump efflux inhibitors can dramatically increase the effectiveness of putative plant antimicrobials (Tegos *et al.*, 2002). Probably, the co-occurrence of pervilleines and the supposed antimicrobial/ antifungal constituents from roots of *E. pervillei* may increase the effectiveness of the antimicrobial/ antifungal constituents, justifying its claimed effectiveness in traditional medicine.

CONCLUDING REMARKS

Drug resistance is one of the main reasons for the failure of malaria eradication, and also one serious problem associated with cancer chemotherapy. A vast amount of work has been done into the characterisation of P-gp in cancerous cell lines. In malaria, it is this resistance which has provided the driving force for research devoted to the understanding of the mechanism of action of chloroquine as well as the process resistance and its reversal. Although decisive advances have been made, the mechanism by which it occurs has raised outcomes of controversy and stimulated much debate. Tropical plants harbour a vast abundance of plants, some of which have yielded natural products with useful chemotherapeutic value, or sources of inspiration for, and messages to be decrypted by medicinal chemists. We hope that the ideas put forward in this paper will raise potentially useful areas of research in drug resistance and its reversal. Work is actively in progress to further explore our working hypotheses.

ACKNOWLEDGMENTS

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REFERENCES

Aziz, S.M., Worthen, D.R., Yatin, M., Ain, K.B. and Crooks, P.A. 1998. A Unique Interaction between Polyamine and Multidrug Resistance (P-glycoprotein) Transporters in Cultured Chinese Hamster Ovary Cells Transfected with Mouse *mdr-1* Gene. Biochemical Pharmacology, 56, 181-187.

Bergeron R.J., McManis J.S., Liu, C.Z., Feng, Y., Weimar, W.R., Luchetta, G.R., Wu, Q., Ortiz-Ocasio, J., Vinson, J.R., and Kramer D. Antiproliferative properties of polyamine analogues: a structure activity study. Journal of Medicinal Chemistry, 37, 3464-3476.

Bitonti A.J., Dumont J.A., Bush, T.L., Edwards, M.L., Stemerick, D. M., McCann P.P. and Sjoerdsma, A. 1989. Bis(benzyl)polyamine analogs inhibit the growth of chloroquine-resistant human malaria parasites (Plasmodium falciparum) in vitro and in combination with alpha-difluoromethylornithine cure murine malaria. Proceedings of the National Academy of Sciences USA, 86, 651-655.

Blanchy, S., Rakotonjanabelo, A., Ranaivoson, G. and Rajaonarivelo, E. 1993. Epidémiologie du paludisme sur les Hautes Terres malgaches depuis 1878. Cahiers Santé, 3, 155-161.

Bosch, J.; Bonjoch, J. and Amat, M. 1996. The Strychnos alkaloids, 75-189. In: Alkaloids, Academic Press; Vol. 48.

Bois, F., Boumendiel, A., Mariotte, A.-M., Conseil, G. and Petro A. 1999. Synthesis and biological activity of 4-alkoxy chalcones : potential hydrophobic modulators of P-glycoprotein-mediated multidrug resistance. Bioorganic & Medicinal Chemistry, 7, 2691-2695.

Bray, P.G. and Ward, S.A. 1998. A comparison of the phenomenology and genetics of multidrug resistance in cancer cells and quinoline resistance in Plasmodium falciparum. Pharmacology & Therapeutics, 77(1), 1-28.

Chibale, K., Ojima, I., Haupt, H., Grng X., Pera, P. and Bernacki, R. J. 2001. Modulation of human mammary cell sensitivity to paclitaxel by new quinoline sulfonamides. Bioorganic & Medicinal Chemistry Letters, 11, 2457-2460.

Chiba, P., Annibali, D., Hitzler M., Ritchter E. and Ecker G. 1998. Studies on propafenone-type modulators of multidrug resistance. VI. Synthesis and pharmacological activity of compounds with varied spacer length between the central aromatic ring and the nitrogen atom. Il Farmaco, 53(5), 357-364.

Dahle, L. 1886. Sikidy and vintana: half-hours with Malagasy diviners, Antananarivo Annual X: 219-233; X: 315-324; XI: 457-467.

Griffin, W.J. and Lin, G.D. 2000. Chemotaxonomy and geographical distribution of tropane alkaloids. Phytochemistry, 53, 623-637.

Hait, N.W. and Aftab, D.T. 1992. Rational design and pre-clinical pharmacology of drugs for reversing multidrug resistance. Biochemical Pharmacology, 43, 103-107.

Iida, Y., Oh, K.B., Saito, M., Matsuoka, H. and Kurata H. 2000. In vitro synergism between nyasol, an active compound isolated from Anemarrhena asphodeloides, and azole agents against *Candida albicans*. Planta Medica, 66, 435-438.

Kalkanidis, M., Klonis, N., Tilley, L. and Deady L.W. 2002. Novel phenothiazine antimalarials: synthesis, antimalarial activity, and inhibition of the formation of b-hematin. Biochemical Pharmacology, 63, 833-842.

Karcz, S. and Cowman, A.F. (Similarities and differences between the multidrug resistance phenotype of mamalian tumor cells and chloroquine resistance in Plasmodium falciparum. Experimental Parasitology, 73, 233-240.

Krishma, R. and Mayer, L.D. 2000. Mutidrug resistance (MDR) in cancer: mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. European Journal of Pharmaceutical Sciences, 11, 265-283.

Martin, M.-T., Rasoanaivo, P., Palazzino, G., Galeffi, C., Nicoletti, M., Trigalo, F. and Frappier, F. 1999. Minor Nb-(C21) secocuran alkaloids of *Strychnos myrtoides*, Phytochemistry, 51, 479-486.

Martin, S.K., Oduola, A.M.J. and Milhous, W.K. 1987. Reversal of chloroquine resistance in plasmodium falciparum by verapamil. Science, 235, 899-901.

Marverti, G., Andrews, P.A., Piccinini, G., Ghiaroni, S., Barbieri, D. and Moruzzi, M.S. 1997. Modulation of cis-diaminedichloroplatinium (II) accumulation and cytotoxicity by spermine in sensitive and resistant human ovarian carcinoma cells. European Journal of Cancer, 33(4), 669-675.

Mi, Q., Cui, B., Silva, G. L., Lantvit, D., Lim, E., Chai, H., Hollingshead, M.G., Mayo, J.G., Kinghorn, A.D. and Pezzuto, J.M. 2002. Pervilleines B and C, new tropane alkaloid aromatic esters that reverse the multidrug-resistance in the hollow fiber assay. Cancer Letters, 184, 13-20.

Muller, S., Coombs, G H. and Walter R. D. 2001. Targeting polyamines of parasites protozoa in chemotherapy. Trends in Parasitology, 17(5), 242-249.

Pearce, H.-L., Winter, M.A. and Beck, W.T. 1990. Structural characteristics of compounds that modulated P-glycoprotein-associated multidrug resistance. Advances in Enzyme Regulation, 30, 357-373.

Phillipson, P.B. 1996. Endemism and non-endemism in the flora of south-west Madagascar. In: Biogeography of Madagascar, W. R. Lourenço Ed, ORSTOM Editions, Paris, pp 125-136.

Rafatro, H., Ramanitrahasimbola, D., Rasoanaivo, P., Ratsimamanga-Urverg, S., Rakoto-Ratsimamanga, A. and Frappier F. 2000. Reversal activity of the naturally-occurring chemosensitizer malagashanine in *Plasmodium* malaria, *Biochemical Pharmacology*, 59(9), 1053-1061.

Ramanitrahasimbola1, D., Rasoanaivo, P., Ratsimamanga-Urverg, S., Federici, E., Palazzino, G., Galeffi, C. and M. Nicoletti. 2001. Biological Activities of the Plant-derived Bisindole Voacamine with Reference to Malaria. Phytotherapy Research, 15, 30–33.

Ramialiharisoa, A., Ranaivoravo, J., Ratsimamanga-Urverg, S., Rasoanaivo, P., Rakoto-Ratsimamanga, A. 1994. Evaluation en clinique humaine de l'action potentialisatrice d'une infusion de *Strychnos myrtoides* vis-à-vis d'antipaludéens, *Révue de Médecine et de Pharmacopée Africaines* , 8, 123-131.

Rasoanaivo, P., Ratsimamanga-Urverg, S., Milijaona, R., Rafatro, H., Galeffi, C. and Nicoletti M. 1994. *In vitro* and *in vivo* chloroquine potentiating action of *Strychnos myrtoides* alkaloids against chloroquine-resistant strain of *Plasmodium* malaria, Planta Medica, 60, 13-16.
Rasoanaivo, P., Rafatro, H., Ramanitrahasimbola, D., Ranaivoravo, J., Ratsimamanga, S. and Frappier, F. 2001 . Strychnos de Madagascar : de la connaissance traditionnelle à l'essai clinique. 127-140. In : Actes du 5ème Colloque Produits Naturels d'origine Végétale, Guy Collin et François-Xavier Garnier (eds), Université du Québec à Chicoutimi.

Ratsimamanga-Urverg, S., Rasoanaivo, P., Ramiaramanana, L., Milijaona, R., Rafatro, H., Verdier, F., Rakoto-Ratsimamanga, A. and Le Bras, J. 1992. *In vitro* antimalarial activity and chloroquine-potentiating action of BBIQ enantiomers from *Strychnopsis thouarsii* and *Spirospermum penduliflorum*, *Planta Medica*, 58, 540-543.

Silva, G.L., Cui, B., Chavez, D., You, M., Chai, H.B., Rasoanaivo, P., Lynn, S.M., O'Neill, M.J., Lewis, J.A., Besterman, J.M., Monks, A., Farnsworth, N.R., Cordell, G.A., Pezzuto, J.M. and Kinghorn A.D. 2001. Modulation of the multidrug-resistance phenotype by new tropane alkaloid aromatic esters from Erythroxylum pervillei. Journal of Natural Products, 64(12), 1514-1520.

Ridley, R. G. 1997. Haemoglobin degradation and haem polymerization as antimalarial drug targets. Journal of Pharmaceutics and Pharmacology, 49(2) 43-48.

Tegos, G., Stermitz, F.R., Lomovskaya, O. and Lewis, K. 2002. Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrobial Agents and Chemotherapy, 46(10), 3133–3141.

Trigalo, F., Frappier, F., Ratsimamanga-Urverg, S. and Rasoanaivo P. (2002)Tsuruo, T., Iida, H., Tsukagoshi, S. and Sakurai, Y. 1981.