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Screening of *Leishmania* APRT enzyme inhibitors

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Adenine phosphoribosyltransferase (APRT) enzyme from *Leishmania tarentolae* has been proposed as a target for the rational search of new leishmanicidal drugs. In this paper, we describe the evaluation of the inhibitory activity on *L. tarentolae* APRT enzyme of 46 crude extracts of Meliaceae and Rutaceae plants, besides three furoquinolone alkaloids. The results showed that 21 extracts were able to decrease the APRT enzymatic activity (IA% $\geq$ 50). The methanolic extracts from roots and leaves of *Cedrela fissilis* and from fruits, branches and leaves of *Cipadessa fruticosa* have showed strong activities. Therefore, these species could be a promising source of lead compounds for the rational design of new leishmanicidal drugs. The phytochemical investigation of an active fraction from *Almeidea rubra* afforded the alkaloids isodutaduprine, isoskimmianine and isokokusagine, which showed low to moderate activity on APRT.

1. Introduction

Leishmaniasis comprises a group of tropical diseases caused by different species of haemoflagellate protozoa parasites belonging to the genus *Leishmania* and transmitted zoonotically by the female flying insects of the genus *Phlebotomus* and *Lutzomyia*. The various manifestations of the disease have been used by the World Health Organization as the basis to classify leishmaniasis in: visceral, cutaneous and mucocutaneous. Leishmaniasis, in each of its three clinical forms, remains a major public health problem throughout much of the tropical and subtropical world. According to recent World Health Organization reports, this ailment affects 12 million people in 88 countries, with 350 million of people under risk of infection and about 2–3 million new estimated cases each year. Recently, there has been an increase in visceral leishmaniasis and HIV-infection, especially in southern Europe (WHO, 2004).

In the absence of availability of anti-*Leishmania* vaccines, chemotherapy remains the mainstay for the treatment of this disease (Davis et al. 2004). However, despite all efforts employed in the search of new drugs, the treatment is still based on the use of the potentially toxic pentavalent antimonials, such as meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®), which must be given as daily intramuscular injections, producing unpleasant side effects. Besides, those drugs are expensive and not always effective (Olliaro and Bryceson 1993). Therefore, there is a great and urgent need for the development of new, effective and safe drugs for the treatment of leishmaniasis.

The rational design of a drug is usually based on biochemical and physiological differences between pathogens and host. Perhaps the most remarkable differences between protozoan parasites and their human host are the pathways by which the two synthesize purine. Purine nucleotides, which are essential for the synthesis of nucleic acids, proteins, and other metabolites as well as for energy-requiring reactions, can be synthesized by the *de novo* and/or the so-called "salvage" pathways. Unlike their mammalian host, most parasites lack the pathways for *de novo* purine biosynthesis and rely on the salvage pathways to meet their purine demands. In addition, because of the great phylogenic separation between the host and the parasite, there are sufficient distinctions between corresponding enzymes of the purine salvage from the host and the parasite that can be exploited to design specific inhibitors for the parasitic enzymes. Therefore, enzymes responsible for the salvage of purines in parasites constitute excellent potential targets for chemotherapy against these organisms (el Kouni 2003).

Parasites from the genus *Leishmania* possess three enzymes involved in the recycling of purine nucleotides, adenine phosphoribosyltransferase (APRT), hypoxanthine-guanine phosphoribosyltransferase (HGPRT), and xanthine phosphoribosyltransferase (XPRT) (Ulman and Carter 1997). APRT is responsible for catalyzing the reaction of adenine and α-D-5-phosphoribosyl-1-pyrophosphate (PRPP), arising adenosine-5-monophosphate (AMP) and pyrophosphate (PPi) (Musick 1981). This enzyme from *Leishmania donovani* and *L. tarentolae* has been investigated by Allen et al. (1989, 1995), Bashor et al. (2002), Phillips et al. (1996, 1999), Silva et al. (2004), and Thiemann et al. (1998). Moreover, our group proposed this enzyme as a target for the rational search of new leishmanicidal drugs (Napolitano et al. 2003a, b; Silva et al. 2003).

As plants have provided many lead molecules for the design of new antileishmanial drugs (Akendengue et al. 1999; Chan-Bacab and Pena-Rodriguez 2001; de Carvalho and Ferreira 2001; Iwu et al. 1994; Phillipson and Wright 1991a, b), and especially because of antiprotozoal activity described for Meliaceae and Rutaceae plants (Ambrozin et al. 2004; Fournet et al. 1994a; Mafezoli et al. 2000; Vieira et al. 2001; Weniger et al. 2001), in combination

with the urgent necessity of new drugs, we propose the evaluation of Meliaceae and Rutaceae plant extracts on a spectrophotometric APRT inhibition assay, as the way to search new anti-*Leishmania* drugs. Thus, this paper reports the APRT inhibition activity of 46 plant extracts from Meliaceae and Rutaceae plants, and three furoquinolone alkaloids isolated from an active fraction.

2. Investigations and results

In the present paper, the inhibitory activity on *L. tarentolae* APRT enzyme of 46 crude extracts of Meliaceae and Rutaceae plants was evaluated. Table 1 summarizes the results obtained from these extracts, showing that 21 of them were able decrease the enzymatic activity (IA% $\geq$ 50). All of the active extracts belong to the Meliaceae family. The methanolic extracts from roots and leaves of *C. fissilis* (CRM, CFM) and from fruits, branches and leaves of *C. fruticosa* (CFFRM, CFGM, CFFM) were the most active ones.

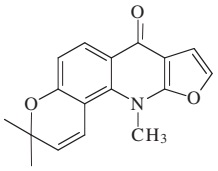
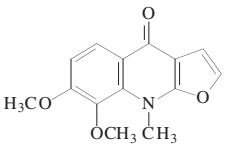
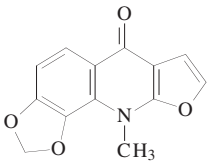
Although none of Rutaceae extracts showed activity, the phytochemical investigation of the methanolic extract from leaves of *Almeidea rubra* (ALFM) afforded an ethyl acetate fraction (ALFMA), which decrease the specific activity of APRT in 90.7%. From this fraction were obtained three furoquinolone alkaloids, isodutaduprine (**1**), isoskimmianine (**2**) and isokokusagine (**3**), which were assayed on APRT enzyme. The results (Table 2) reveal that they were active and could be associated with the observed activity of the ALFMA fraction.

Table 1: APRT inhibitory activity of Meliaceae and Rutaceae extracts

Family/species	Plant part	Extraction solvent	Crude extract	IA[a] (%)
Meliaceae:				
Cedrela fissilis	Fruits	Hexane	CFRH	34.2
		Dichloromethane	CFRD	60.3
		Methanol	CFRM	56.7
	Branches	Hexane	CGH	62.8
		Dichloromethane	CGD	62.4
		Methanol	CGM	63.2
	Stem	Hexane	CCH	52.7
		Dichloromethane	CCD	24.5
		Methanol	CCM	64.8
	Root	Hexane	CRH	63.4
		Dichloromethane	CRD	46.3
		Methanol	CRM	84.5
	Leaves	Hexane	CFH	72.2
		Dichloromethane	CFD	52.7
		Methanol	CFM	80.1
Cipadessa fruticosa	Fruits	Hexane	CFFRH	18.8
		Dichloromethane	CFFRD	63.6
		Methanol	CFFRM	78.7
	Branches	Hexane	CFGH	70.1
		Dichloromethane	CFGD	61.0
		Methanol	CFGM	90.8
	Leaves	Hexane	CFFH	65.1
		Dichloromethane	CFFD	56.4
		Methanol	CFFM	90.3
Trichilia ramalhoi	Branches	Hexane	TRGH	0
		Methanol	TRGM	68.3
	Leaves	Hexane	TRFH	0
		Methanol	TRFM	0
Rutaceae:				
Almeidea coerulea	Branches	Hexane	AGH	0
		Methanol	AGM	23.2
Almeidea rubra	Stem	Hexane	ALCH	9.2
		Methanol	ALCM	15.5
	Leaves	Hexane	ALFH	1.1
		Methanol	ALFM	26.9
Conchocarpus heterophyllus	Stem	Hexane	AHCH	0
		Methanol	AHCM	0
	Leaves	Hexane	AHFH	37.0
		Methanol	AHFM	0
Galipea carinata[b]	Stem	Hexane	GCH	33.0
		Methanol	GCM	32.1
	Leaves	Hexane	GFH	0
		Methanol	GFM	20.8
Galipea carinata[c]	Stem	Hexane	GCCH	0
		Methanol	GCCM	0
	Leaves	Hexane	GCFH	36.8
		Methanol	GCFM	25.5

[a] Enzyme inhibitory activity
[b] *Galipea carinata* specimen collected in 01/18/93
[c] *Galipea carinata* specimen collected in 05/18/00

Table 2: APRT inhibitory activity of isodutaduprine (1), isoskimmianine (2) and isokokusagine (3) isolated from *Almeidea rubra*

Compound	Concentration (μmol/L)	% IA
 **1**	35.6	21.6
 **2**	38.6	39.1
 **3**	41.2	44.6

3. Discussion

These results indicated that, among the assayed species, *C. fissilis* and *C. fruticosa* (Meliaceae) are promising sources of lead compounds for the rational design of new leishmanicidal drugs.

The family Meliaceae is distinguished by the frequent occurrence of limonoids (da Silva et al. 1984), for which several biological activities were described, mainly insecticidal action (Champagne et al. 1992). Moreover, an antiplasmodial activity of several gedunin limonoids was reported (MacKinnon et al. 1997).

Previously, from *C. fissilis* limonoids were isolated (Taylor 1984; Zelnik 1966, 1970) and *C. fruticosa* has been reported to contain *ent*-clerodanes and labdanes diterpenoids (Rojatkar and Nagasampagi 1994; Rojatkar et al. 1994), limonoids, steroids, sesquiterpenoids, heneicosene derivatives and one coumarin (Luo et al. 2000, 2001). Since these genera are characterized by the presence of limonoids, phytochemical studies of the active crude extracts are underway in order to isolate the compounds which could be associated with the observed activities. We hope to isolate limonoids, and, for the first time, evaluate their capacity of inhibition the specific activity of APRT enzyme.

The inhibitory activities of the alkaloids **1**–**3** against the APRT enzyme have been reported for the first time. The inhibitory activities of these compounds are in agreement with previous results, which indicate that alkaloids are the most important natural compounds with antileishmanial activity (Akendengue et al. 1999). In fact, aryl-2 and alkyl-2 quinoline alkaloids isolated from *Galipea longiflora* (Rutaceae) (Fournet et al. 1989, 1993a, b, 1994b, c, 1996) and 4-quinolone alkaloids obtained from *Dictyoloma peruviana* (Rutaceae) (Lavaud et al. 1995) showed high leishmanicidal activities. Besides, sitamaquine, an oral leishmanicidal drug, which is under clinical evaluation (Croft and Coombs 2003), is also an 8-aminoquinoline alkaloid.

Rational design of new leishmanicidal drugs through screening of *L. tarentolae* APRT inhibitors could be successful because of the essential role of this enzyme for survival of the parasite (el Kouni 2003). Moreover, despite this enzyme can be isolated from *L. tarentolae*, which is unable to infect man (Croan et al. 1997; Noyes et al. 1997), it shares 87% amino acid sequence identity with the homologous protein from *L. donovani* (Phillipis et al. 1999; Thiemann et al. 1998). Therefore, their three-dimensional structures should be similar, and the *L. tarentolae* APRT enzyme could be used as a target in the search of new drugs against leishmaniasis. Also, as development of new antileishmanial drugs has been impeded by the lack of a simple and rapid screening system (Christensen et al. 1994), the APRT assay could contribute to a solution this problem.

Finally, most of the assayed active extracts belongs to the family Meliaceae. In particular, those of *C. fissilis* and *C. fruticosa* seems to be promising sources of lead compounds for the rational design of new drugs against leishmaniasis. Moreover, the furoquinolone alkaloids bring about the enzymatic inhibition, showing that these compounds may serve as lead structures in the search of leishmanicidal drugs.

4. Experimental

4.1. Plant material

Cedrela fissilis Vell. was collected in São Carlos (SP, Brazil) and identified by Dr. Maria Inês Salgueiro Lima from the Department of Botany, Federal University of São Carlos, Brazil. It was deposited with voucher number – 6701 – in the Herbarium HUFSCar. *Cipadessa fruticosa* Bl. was collected in Viçosa, (MG, Brazil) and identified by Dr. José R. Pirani from the Department of Botany, University of São Paulo, Brazil, where a voucher specimen (110.664) was deposited. The other species *Almeidea coerulea* A. St.-Hil., *Almeidea rubra* A. St.-Hil., *Conchocarpus heterophyllus* (A. St.-Hil.) Kallunki & Pirani, *Galipea carinata* Pirani (sp. nov.) and *Trichilia ramalhoi* Rizzini were collected in Southeastern Brazil, and identified by Dr. José R. Pirani. The voucher herbarium specimens were deposited at the Herbarium of Department of Botany, University of São Paulo, Brazil and are described by Ambrozin et al. (2004).

4.2. Preparation of crude extracts

Selected parts of the plants (branches, fruits, leaves, roots, and/or stems) were dried carefully by forced air at 40 °C and reduced to powder. The powdered air-dried plant material was extracted by maceration three times with hexane at room temperature for 72 h. After the evaporation of the solvent under reduced pressure, crude hexane extracts were obtained. This process was repeated with dichloromethane and methanol for *C. fissilis* and *C. fruticosa*, and only with methanol for the other species. Thus, the hexane, dichloromethane and/or methanol extracts obtained were assayed on *L. tarentolae* APRT enzyme.

4.3. Isolation of alkaloids

The powdered air dried leaves (313.5 g) from *A. rubra* were extracted first with hexane and then with methanol. Part of the methanol extract (15.9 g) was submitted to vacuum liquid chromatography over silica gel 60 (70–230 mesh) using a gradient hexane, dichloromethane, ethyl acetate and methanol, to yield the corresponding fractions. The active ethyl acetate fraction (ALFMA; 3.32 g) was subjected to silica gel (230–400 mesh) CC ($\phi \times h = 4.3 \times 41.0$ cm) using hexane, hexane-ethyl acetate and methanol as mobile phase, to give eleven fractions. The alkaloids isodutaduprine (**1**) (10.3 mg), and isoskimmianine (**2**) (43.3 mg) were crystallized in MeOH from fractions seven (317.3 mg) and nine (104.5 mg), respectively. The other alkaloid isokokusagine (**3**) (14.3 mg) was purified from the fraction eight (90.3 mg) on sephadex LH-20, using an isocratic elution (methanol). Compounds **1**–**3** were identified by MS and NMR spectroscopy. The experimental data were compared with the literature (Santos et al. 1998; Wu et al. 1999).

4.4. APRT enzymatic assay

The *L. tarentolae* APRT enzyme was purified from *Escherichia coli* in a recombinant system, as described by Silva et al. (2004). The purified protein was stocked at −80 °C in 100 mM Tris-HCl pH 7.4, 5 mM $MgCl_2$, glycerin 10% (v/v) at 1 μg/mL. The enzymatic assay was developed by modification from spectrophotometric protocol previously reported by Tutle and Krenitsky (1980). The principle of the assay permits to monitor the rate of change in absorbance resulting from the conversion of natural substrates to adenosine monophosphate (AMP), at 259 nm. The final concentration of components in the reaction mixture (500 μL) was 100 mM Tris-

HCl, pH 7.4, 200 μM 5′-phospho-α-D-ribosyl-1′-pyrophosphate (PRPP), 100 μM adenine, 5 mM $MgCl_2$. All assays were started by addiction of enzyme 2 μg/mL (final concentration) and carried out at 25 °C by 60 s. For enzyme inhibitors screening, the stock solution of both, pure compounds and extracts fractions were made in dimethyl sulfoxide (DMSO) at 1 mg/mL. Further dilutions were made in the enzymatic reaction mixture immediately before use. The final concentration of DMSO in the enzymatic reaction did not exceed 5% (v/v), a concentration at which the solvent did not affect the enzymes activities. The inhibitory activities of each compound or extract were measured in triplicate. The rate of increase in AMP formation was used to calculate the APRT specific activity (SA). So, the enzyme inhibitory activity percentage (IA%) from screened molecules was obtained by analyses of APRT specific activities with or without inhibitor. Thus, extracts were tested at a final concentration of 50 μg/mL and the pure compounds at 10 μg/mL.

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References

Akendengue B, Ngou-Milama E, Laurens A, Hocquemiller R. (1999) Recent advances in the fight against leishmaniasis with natural products. Parasite 6: 3–8.

Allen T, Henschel EV, Coons T, Cross L, Conley J, Ullman B. (1989) Purification and characterization of the adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase activities from *Leishmania donovani*. Mol Biochem Parasitol 33: 273–282.

Allen T, Hwang HY, Wilson K, Hanson S, Jardim A, Ullman B (1995) Cloning and expression of the adenine phosphoribosyltransferase gene from *Leishmania donovani*. Mol Biochem Parasitol 74: 99–103.

Ambrozin ARP, Vieira PC, Fernandes JB, da Silva MFGF, de Albuquerque S (2004) Trypanocidal activity of Meliaceae and Rutaceae plant extracts. Mem Inst Oswaldo Cruz 99: 227–231.

Bashor C, Denu JM, Brennan RG, Ullman, B (2002) Kinetic mechanism of adenine phosphoribosyltransferase from *Leishmania donovani*. Biochemistry-US 41: 4020–4031.

Champagne DE, Koul O, Isman MB, Scudder GGE, Towers GHN (1992) Biological activity of limonoids from the Rutales. Phytochemistry 31, 377–394.

Chan-Bacab MJ, Pena-Rodriguez M (2001) Plant natural products with leishmanicidal activity. Nat Prod Rep 18: 674–688.

Christensen SB, Ming C, Andersen L, Hjorne U, Olsen CE, Cornett C, Theander TG, Kharazmi A (1994) An antileishmanial chalcone from Chinese licorice roots. Planta Med 60: 121–123.

Croan DG, Morrison DA, Ellis JT (1997) Evolution of the genus *Leishmania* revealed by comparison of DNA and RNA polymerase gene sequences. Mol Biochem Parasitol 89: 149–159.

Croft SL, Coombs GH (2003) Leishmaniasis – current chemotherapy and recent advances in the search for novel drugs. Trends Parasitol 19: 502–508.

da Silva MFGF, Gottlieb OR, Dreyer DL (1984) Evolution of limonoids in the Meliaceae. Biochem Syst Ecol 12: 299–310.

Davis AJ, Murray HW, Handman E (2004) Drugs against leishmaniasis: a synergy of technology and partnerships. Trends Parasitol 20: 73–76.

de Carvalho PB, Ferreira EI (2001) Leishmaniasis phytotherapy. Nature's leadership against an ancient disease. Fitoterapia 72: 599–618.

el Kouni MH (2003) Potential chemotherapeutic targets in the purine metabolism of parasites. Pharmacol Therapeut 99: 283–309.

Fournet A, Vagneur B, Richomme P., Bruneton, J (1989) Aryl-2 et alkyl-2 quinoléines nouvelles isolées d'une Rutacée bolivienne: *Galipea longiflora*. Can J Chem 67: 2116–2118.

Fournet A, Hocquemiller R, Barrios AA, Munoz V, Cavé A, Bruneton J (1993a) 2-Substituted quinolines alkaloids as potential antileishmanial drugs. Antimicrob Agents Chemother 37: 859–863.

Fournet A, Hocquemiller R, Roblot F, Cavé A, Richomme P, Bruneton J (1993b) Les chimanines, nouvelles quinoleines substituees en 2, isolees d'une plante bolivienne antiparasitaire: *Galipea longiflora*. J Nat Prod 56: 1547–1552.

Fournet A, Barrios AA, Munoz V (1994a) Leishmanicidal and trypanocidal activities of Bolivian medicinal plants. J Ethnopharmacol 41: 19–37.

Fournet A, Barrios AA, Munoz V, Hocquemiller R, Roblot F, Cavé A, Richomme P, Bruneton J (1994b) Antiprotozoal activity of quinoline alkaloids isolated from *Galipea longiflora*, a Bolivian plant used as a treatment for cutaneous leishmaniasis. Phytother Res 8: 174–178.

Fournet A, Gantier JC, Gautheret A, Leysalles L, Munos MH, Mayrague J, Moskowitz H, Cavé A., Hocquemiller R (1994c) The activity of 2-substituted quinoline alkaloids in BALB/c mice infected with *Leishmania donovani*. J Antimicrob Chemother 33: 537–544.

Fournet A, Ferreira ME, de Arias AR, Ortiz ST, Fuentes S, Nakayama H, Schinini A, Hocquemiller R (1996) *In vivo* efficacy of oral and intralesional administration of 2-substituted quinolines in experimental treatment of New World cutaneous leishmaniasis caused by *Leishmania amazonensis*. Antimicrob Agents Chemother 40: 2447–2451.

Iwu MM, Jackson JE, Schuster BG (1994) Medicinal plants in the fight against leishmaniasis. Parasitol Today 10: 65–68.

Lavaud C, Massiot G, Vasquez C, Moretti C, Sauvain M, Balderrama L (1995) 4-Quinolinone alkaloids from *Dictyoloma peruviana*. Phytochemistry 40: 317–320.

Luo XD, Wu SH, Ma YB, Wu DG (2000) Components of *Cipadessa baccifera*. Phytochemistry 55: 867–872.

Luo XD, Wu SH, Ma YB, Wu DG (2001) Studies on chemical constituents of *Cipadessa baccifera*. Zhongcaoyao 32: 778–780.

MacKinnon S, Durst T, Arnason JT (1997) Antimalarial activity of tropical Meliaceae extracts and gedunin derivatives. J Nat Prod 60: 336–341.

Mafezoli J, Vieira PC, Fernandes JB, da Silva MFGF, de Albuquerque S (2000) *In vitro* activity of Rutaceae species against the trypomastigote form of *Trypanosoma cruzi*. J Ethnopharmacol 73: 335–340.

Musick WD (1981) Structural features of the phosphoribosyltransferases and their relationship to the human deficiency disorders of purine and pyrimidine metabolism. CRC Crit Rev Biochem 11: 1–34.

Napolitano HB, Silva M, Ellena J, Rocha WC, Vieira PC, Thiemann OH, Oliva G (2003a) Redetermination of skimmianine: a new inhibitor against the *Leishmania* APRT enzyme. Acta Crystallogr E 59, O1503–O1505 part 10.

Napolitano HB, Silva M, Ellena J, Rocha WC, Vieira PC, Thiemann OH, Oliva G (2003b) Redetermination and comparative structural study of isopimpinellin: a new inhibitor against the *Leishmania* APRT enzyme. Acta Crystallogr E 59, O1506–O1508 part 10.

Noyes HA, Arana BA, Chance ML, Maingon R (1997) The *Leishmania hertigi* (Kinetoplastida; Trypanosomatidae) complex and the lizard *Leishmania*: their classification and evidence for a neotropical origin of the *Leishmania* endotrypanum clade. J Eukaryot Microbiol 44: 511–517.

Olliaro PL, Bryceson ADM (1993) Pratical progress and new drugs for changing patterns of leishmaniasis. Parasitol Today 9: 323–328

Phillips CL, Ullman B, Brennan RG (1996) Crystallization of the purine salvage enzyme adenine phosphoribosyltransferase. Proteins 25: 510–513.

Phillips CL, Ullman B, Brennan RG, Hill CP (1999) Crystal structures of adenine phosphoribosyltransferase from *Leishmania donovani*. Embo J 18: 3533–3545.

Phillipson JD, Wright CW (1991a) Antiprotozoal agents from plant sources. Planta Med 57: 53–59.

Phillipson JD, Wright CW (1991b) Medicinal plants in tropical medicine: medicinal plants against tropical diseases. T Roy Soc Trop Med H 85: 18–21.

Rojatkar SR, Chiplunkar YG, Nagasampagi BA (1994) A diterpene from *Cipadessa fruticosa* and *Grangea maderaspatana*. Phytochemistry 37: 1213–1214.

Rojatkar SR, Nagasampagi BA (1994) Diterpenes from *Cipadessa fruticosa*. Phytochemistry 37: 505–507.

Santos CS, Januário AH, Vieira PC, Fernandes JB, da Silva MFGF, Pirani JR (1998) Cycloartane triterpenoid and alkaloids from *Almeidea* spp. J Braz Chem Soc 9: 39–42.

Silva M, Napolitano HB, Ellena J, Rocha WC, Vieira PC, Oliva G, Thiemann OH (2003) 3-(5,7-Dimethoxy-2,2-dimethyl-2*H*-benzo[*b*]-pyran-6-yl) propionic acid: a potential inhibitor against *Leishmania*. Acta Crystallogr E 59, O1575–O1577 part 10.

Silva M, Silva CHTP, Iulek J, Oliva G, Thiemann OH (2004) Crystal structure of adenine phosphoribosyltransferase from *Leishmania tarentolae*: potential implications of APRT catalytic mechanism. BBA – Proteins Proteom 1696: 31–39.

Taylor DAH (1984) The chemistry of the limonoids from Meliaceae. Fort Chem Org Naturst 45: 1–102.

Thiemann OH, Alfonzo JD, Simpson L (1998) Cloning and characterization of *Leishmania tarentolae* adenine phosphoribosyltransferase. Mol Biochem Parasitol 95: 141–146.

Tuttle JV, Krenitsky TA (1980) Purine phosphoribosyltransferases from *Leishmania donovani*. J Biol Chem 255: 909–916.

Ullman B, Carter D (1997) Molecular and biochemical studies on the hypoxanthine-guanine phosphoribosyltransferases of the pathogenic haemoflagellates. Int J Parasitol 27: 203–213.

Vieira PC, Mafezoli J, Pupo MT, Fernandes JB, da Silva MFGF, Albuquerque S, Oliva G, Pavão F (2001) Strategies for the isolation and identification of trypanocidal compounds from the Rutales. Pure Appl Chem 73: 617–622.

Zelnik R (1966) Le Fissinolide. Tetrahedron Lett 52: 6441–6444.

Zelnik R. (1970) Tetranortripterpenoids from *Cedrela fissilis*. Phytochemistry 10: 1955–1956.

Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Munoz V, Callapa J, Lobstein A, Anton R (2001) Antiprotozoal activities of Colombian plants. J Ethnopharmacol 78: 193–200.

World Health Organization Division of Control of Tropical Diseases at http://www.who.ch/tdr/diseases/leish, accessed in 05/2004.

Wu TS, Li CY, Leu YL, Hu CQ (1999) Limonoids and alkaloids of the root bark of *Dictamnus angustifolius*. Phytochemistry 50: 509–512.