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Antimycobacterial activity of lichen substances

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ABSTRACT

We describe here the extraction and identification of several classes of phenolic compounds from the lichens *Parmotrema dilatatum* (Vain.) Hale, *Parmotrema tinctorum* (Nyl.) Hale, *Pseudoparmelia sphaerospora* (Nyl.) Hale and *Usnea subcavata* (Motyka) and determined their anti-tubercular activity. The depsides (atranorin, diffractaic and lecanoric acids), depsidones (protocetraric, salazinic, hypostictic and norstictic acids), xanthones (lichexanthone and secalonic acid), and usnic acid, as well seven orsellinic acid esters, five salazinic acid 8',9'-*O*-alkyl derivatives and four lichexanthone derivatives, were evaluated for their activity against *Mycobacterium tuberculosis*. Diffractaic acid was the most active compound (MIC value 15.6 μg/ml, 41.6 μM), followed by norstictic acid (MIC value 62.5 μg/ml, 168 μM) and usnic acid (MIC value 62.5 μg/ml, 182 μM). Hypostictic acid (MIC value 94.0 μg/ml, 251 μM) and protocetraric acid (MIC value 125 μg/ml, 334 μM) showed moderate inhibitory activity. The other compounds showed lower inhibitory activity on the growth of *M. tuberculosis*, varying from MIC values of 250 to 1370 μM.

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Introduction

Tuberculosis is an infectious bacterial disease caused by *Mycobacterium tuberculosis*, which most commonly affects the lungs, and is responsible for approximately 2 million deaths annually. In addition, Tuberculosis is a leading cause of HIV-related deaths worldwide (http://www.who.int/topics/tuberculosis/en – accessed January 21, 2009).

Despite the fact that tuberculosis control programs have been in place for decades, an elevated number of persons are latently infected with *M. tuberculosis* and there is an emergence of multidrug-resistant tuberculosis, adding to the ability of tuberculosis bacillus to persist in macrophages for a long time. The search for new drug leads that are effective against multidrug-resistant (MR) strains of *M. tuberculosis* and that are able to kill the "dormant bacteria" in macrophages is therefore an urgent need (Quenelle et al. 2001; Suksamrarn et al. 2003; Caballeira 2008; Koul et al. 2008; Wagner and Ulrich-Merzenich 2009). Active tuberculosis is generally treated using more than one drug and efforts have been developed to provide novel anti-tuberculosis agents, natural or synthethic, that are more potent and less toxic. The combination of first-line drugs, such as rifampicin and isoniazid with 7-methyljuglone (7-MJ), a natural product, reduced the MIC values of both compounds four-fold and six-fold, respectively, and showed both extracellular and intracellular synergistic activity against the tested strains of *M. tuberculosis* (Bapela et al. 2006).

Synergistic effects can be produced if the constituents of an extract or a drug combination affect different targets or interact with one another in order to improve the solubility and thereby enhance the bioavailability of one or more substances in the mixture (Wagner and Ulrich-Merzenich 2009). A great number of researchers have dedicated efforts to discover new structural classes of compounds in order to develop agents to replace or supplement the established drugs (Lenaerts et al. 2007). Compounds of several classes, isolated from plants, including phenols, quinones, xanthones, alkaloids, terpenes, steroids and others, exhibit wide-ranging *in vitro* potency against *M. tuberculosis* (Copp and Pearce 2007).

Phenols are a group with a large structural diversity and several biological and/or pharmacological activities. Among the phenolic compounds obtained from nature are those produced by lichens.

Lichens, symbiotic associations of a fungus and one or more algae, produce several classes of phenolic compounds, including: depsides, depsidones, usnic acids, dibenzofuranes, xanthones, anthraquinones, in addition to pulvinic acid derivatives and aliphatic acids. Many of these compounds are exclusive to lichens, but some may also be found in fungi not lichenized and in superior plants (Hale 1983).

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Investigations into the activity of extracts and of pure compounds isolated from lichens have been conducted for many years, mainly against microorganisms including the *Mycobacterium* genus (Vartia 1973). Atranorin and usnic, lobaric, salazinic and (+) protolichesterinic acids were tested against *M. aurum*, a rapidly growing non-pathogenic organism with a similar drug sensitivity profile to *M. tuberculosis*. The results indicated that usnic acid is the most active compound among those studied (Ingólfsdóttir et al. 1998). More recently, Gupta et al. reported tests of ethanol extracts of nine lichen species against *M. tuberculosis* $H_{37}Rv$ and $H_{37}Ra$ strains. All extracts showed weak activity (Gupta et al. 2007).

Continuing our investigation of bioactive phenolic substances isolated from lichens, we relate here the results of the activity against *M. tuberculosis* of twenty-six compounds: depsides, depsidones and xanthones, usnic acid, derivatives from salazinic and lecanoric acids and lichexanthone.

Material and methods

Lichens

The lichens *Parmotrema dilatatum* (Vain.) Hale, *Parmotrema tinctorum* (Nyl.) Hale, *Pseudoparmelia sphaerospora* (Nyl.) Hale and *Usnea subcavata* (Motyka) were collected in Mato Grosso do Sul State, Brazil; *Ramalina sp.* and *Parmotrema lichexanthonicum* Eliasaro & Adler were obtained from decoration shops. The identification was conduced by Prof. Dr. Mariana Fleig of the Universidade Federal do Rio Grande do Sul and Prof. Dr. Marcelo P. Marcelli of the Instituto de Botânica de São Paulo. A voucher specimen of each species has been retained in our laboratory for future reference.

Extraction and isolation of compounds

The talli of the lichens were powdered and extracted with chloroform and acetone exhaustively, at room temperature. The extracts were concentrated *in vacuo*.

The extract obtained from each lichen was fractionated by silica gel column chromatography and eluted with hexane-chloroform or hexane-acetone mixtures, in crescent polarity, to yield atranorin [**1**] (*P. dilatatum*, and *P. tinctorum),* usnic acid [**2**] (*U. subcavata* and *Ramalina* sp.), diffractaic acid [**3**] (*U. subcavata*) lichexanthone [**4**] (*P. lichexanthonicum),* secalonic acid [**5**] (*P. sphaerospora*). From the acetone extract of the lichen *P. tinctorum*, lecanoric acid [**6**] was isolated and purified according to the method reported by Ahmann and Mathey (1967). From the species *P. dilatatum, P. lichexanthonicum*, *P. sphaerospora* and *Ramalina* sp. were isolated, respectively, protocetraric acid [**7**], salazinic acid [**8**], hypostatic acid [**9**], and norstitic acid [**10**]. The purification of these compounds was conducted by treatment with a small volume of acetone in an ice bath and centrifugation.

The degree of purity for all lichen compounds was >95% as determined by thin layer chromatography (TLC) and nuclear magnetic resonance (NMR) analyses.

Derivatives

Procedure for the preparation of derivatives

Preparation of orsellinates (Lopes et al. 2008). The preparation of the orsellinates [**11**–**17**] was carried out through the reaction of lecanoric acid (200 mg) with 50 ml of alcohol at 40 °C in a steam bath. After completion of the reaction, the mixture was concentrated and the compounds were separated by chromatography on a silica gel column with chloroform and chloroform/acetone gradient. In all reactions, orsellinic acid and the corresponding esters 2,4-dihydroxy-6-methylbenzoates (orsellinates), [**11–17**], were obtained.

Salazinic acid derivatives (Micheletti et al. 2009). A mixture of salazinic acid (150.0 mg, 0.4 mmol) and 25.0 ml of an alcohol (*n*-propanol, *n*-butanol, *n*-pentanol, *n*-hexanol and *iso*-propanol) was heated in a reactional flask at a temperature of 90 °C, except for *iso*-propanol, which was heated under reflux. Reaction mixtures were monitored by TLC. After solvent evaporation, the products were subjected to column chromatography and eluted with a gradient system (hexane/chloroform), to yield compounds [**18–22**].

*Norlichexanthone [**23**] (Micheletti et al. 2009).* Lichexanthone [**4**] (53.7 mg, 0.19 mmol) was dissolved in anhydrous dichloromethane (10 ml), by shaking under N_2 atmosphere, and the solution was maintained in ice bath for 30 min. 0.4 ml (4.17 mmol) of BBr_3 was then added and the temperature was gradually increased until it reached 30 °C. The reaction was monitored by TLC and stopped after 18 h by the addition of water. The aqueous phase was filtered, the residue dissolved in acetone, and the solution dried with anhydrous sodium sulfate (Na_2SO_4) and evaporated under reduced pressure. The product was then purified using silica gel chromatography (hexane/ethyl acetate gradient).

3,6-di-O-prenyl-norlichexanthone **[24]** *(Micheletti et al. 2009).* Norlichexanthone [**23**] (50.0 mg, 0.19 mmol) and K_2CO_3 (43.0 mg, 0.31 mmol) were dissolved in acetone (10.0 ml). To the solution were added 40 μl (0.35 mmol) of 3,3-dimethylallil bromide and the mixture was stirred at room temperature (approximately 30 °C). The reaction was monitored by TLC, stopped after 23 h and the solvent evaporated. The reaction mixture was fractionated by preparative TLC using hexane/ethylacetate 30%.

3,6-bis[(3,3-dimethyloxyran-2-il)methoxy]-1-hydroxy-8-methyl-9H-xanten-9-one **[25]** *(Micheletti et al. 2009).* To a solution of 31.2 mg (0.085 mmol) of 3,6-di-*O*-prenyl-norlichexanthone [**24**] in chloroform (15.0 ml) were added 47.6 mg (0.31 mmol) of 3-chlorobenzoic acid. The mixture was stirred through 170 h and the reaction was stopped by addition of water. A saturated solution of Na_2CO_3 was added to the reaction mixture, which was extracted with chloroform. The organic phase was dried with Na_2SO_4 and the solvent evaporated. The residue was fractionated by column chromatography on silica gel, eluted with a hexane/ethyl acetate gradient.

3-O-methyl-6-O-benzyl-norlichexanthone **[26]** (Micheletti 2007). To a solution of 13.5 mg (0.05 mmol) of 3-*O*-methylnorlichexanthone in acetone (15.0 ml) were added 10.0 μl (0.075 mmol) of benzyl chloride and 14.0 mg (0.1 mmol) of K_2CO_3. The mixture was stirred over a period of 7 days. After this time the reaction was stopped, the solvent evaporated and the residue dissolved in ethyl acetate and extracted with water. The organic phase was dried with Na_2SO_4 and the solvent removed under reduced pressure.

The structures were confirmed by NMR spectra (1D and 2D) obtained in a Brucker DPX300 instrument and by electron impact mass spectrometry (EI-MS) spectra.

Anti-M. tuberculosis activity

The anti-*M. tuberculosis* activity of the tested compounds and the standard drug isoniazid (Difco laboratiories, Detroit, MI, USA) were determined in triplicate through the Microplate Alamar Blue Assay (MABA), according to the method reported by Franzblau et al. (1998). In this technique, the Minimal Inhibitory

Concentration (MIC) value of a compound necessary to inhibit the growth of *M. tuberculosis* H37Rv in 90% is determined using Alamar Blue as a fluorescent vital dye. The bacterial suspension used was prepared by adjusting its turbidity in the McFarland scale to number 1, and further dilution (1:25 v/v) in the culture medium 7H9. Concentrations of the tested compounds and isoniazid ranged from 3.9 to 125.0 μg/ml and 0.015 to 0.50 μg/ml, respectively. A negative control was run without bacterial *inoculum* to confirm the absence of reaction of the compounds themselves with Alamar Blue. The fluorescence of the dye (indicative of bacterial growth) was measured in a SPECTRAfluor Plus microplate reader fluorimeter (Tecan®) in bottom reading mode, with excitation at 530 nm and emission at 590 nm (Collins and Franzblau 1997).

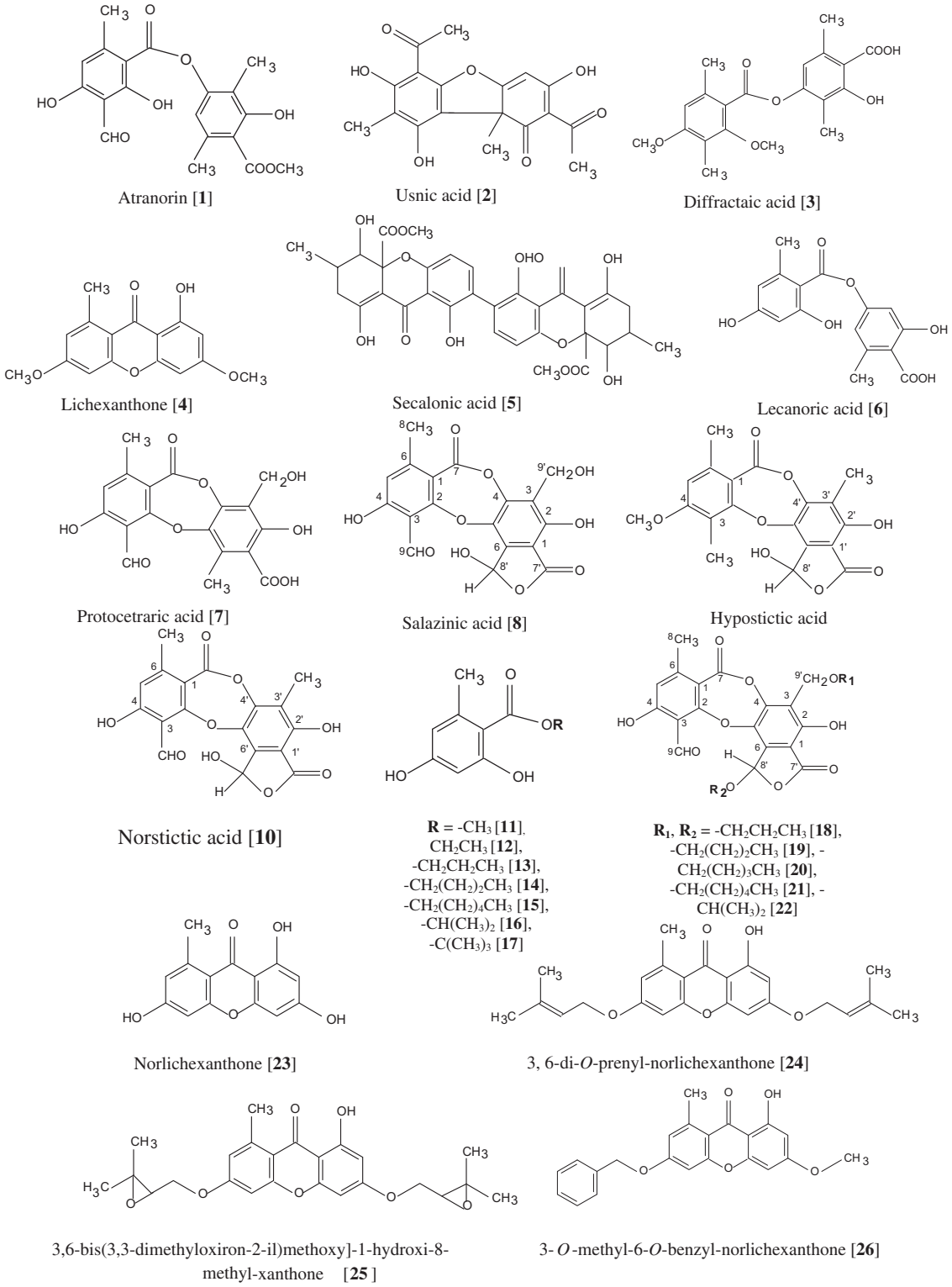

Fig. 1. Structures of the compounds evaluated against *Mycobacterium tuberculosis*.

Results and Discussion

The compounds in study, namely: depsides (atranorin [**1**], diffractaic [**3**] and lecanoric [**6**] acids), depsidones (protocetraric [**7**], salazinic [**8**], hypostictic [**9**] and norstictic [**10**] acids), xanthones (lichexanthone [**4**] and secalonic acid [**5**]) and usnic acid [**2**], as well the orsellinic acid esters [**11**–**17**], salazinic acid derivatives [**18**–**22**] and lichexanthone derivatives [**23**–**26**], were evaluated against *M. tuberculosis* $H_{37}Rv$ (structures are shown in Fig. 1).

The diffractaic acid [**3**] was the most active compound (MIC value 15.6 μg/ml, 41.7 μM) followed by norstictic acid [**10**] (MIC value 62.5 μg/ml, 168 μM) and usnic acid [**2**] (MIC value 62.5 μg/ml, 182 μM). The other compounds showed lower inhibitory activity on the growth of *M. tuberculosis*, varying from 250 μM to 1370 μM. Alkyl esters of orsellinic acid [11–17] and the lecanoric acid [6] were only minimally active (Table 1).

The salazinic acid [**8**] a depsidone with lactol ring, was also weakly active against *M. tuberculosis* (MIC value >250 μg/ml, 643 μM). The same result was obtained by Ingólfsdóttir et al. (1998) against *M. aurum*. Lichexanthone [**4**] also was weakly active.

The norstictic [**10**] (MIC value 62.5 μg/ml, 168 μM), hypostictic [**9**] (MIC value 94.0 μg/ml, 251 μM) and protocetraric [**7**] (MIC value 125 μg/ml, 334 μM) acids showed middle inhibitory activity. The difference between the structures of salazinic [**8**] and norstictic [**10**] acids is only with respect to the substituent at C-9', which attributes a more lipophilic feature to the norstictic acid. The hypostictic acid [**9**] and the norstictic acid [**10**] differ at C-3 and C-4. The $-CH_3$ at C-3 and the $-CH_3O$ at C-4 of the hypostictic acid make it more lipophilic than the norstictic acid. A comparison among the Log *P* values (salazinic acid 1.41 ± 0.86; norstictic acid 3.05 ± 0.85 and hypostictic acid 3.29 ± 0.75) shows that the more lipophilic is more active than the less lipophilic compound. However, the protocetraric acid [**7**] and hypostictic acid [**9**] have identical Log *P* values and the first was less active against *M. tuberculosis*. The usnic acid [**2**] (MIC value 62.5 μg/ml, 182 μM) has Log *P* (1.18 ± 0.75) near those of the salazinic acid (Log *P* 1.41 ± 0.86) and it was more active against *M. tuberculosis*. According Ingólfsdóttir et al. (1998), usnic acid is also active against *M. aurum* (MIC value 32 μg/ml). Atranorin [**1**] shows low activity against *M. tuberculosis* and this result is similar to that reported by Ingólfsdóttir et al. (1998) in relation to M. *aurum*.

The diffractaic acid [**3**] (Log *P* 5.48 ± 0.42) was the most active compound and it is more lipophilic than the depsidones and less lipophilic than the depside atranorin. The activity of a compound depends on the transport across the protective lipid membrane bilayer surrounding cells, which is hydrophobic. So, its physico-chemical properties, such as the partition coefficient, expressed by the relative lipophilicity of the molecule (Log *P*), and the ionization coefficient (pKa) are very important. Compounds with a free carboxyl group (-COOH) have pKa around 4.0, while the hydroxyl group at C-4 in most of the compounds evaluated has pKa around 8.0 and is nearly 10% ionized at pH 7.0 (Gomes et al. 2006). The relative contribution of neutral and ionized functional groups is important for the activity of a drug. Drugs with higher Log *P* values cross the hydrophobic biomembranes with greater ease and present higher bioavailability and, consequently, better pharmacological effect (Barreiro and Fraga 2001).

From the results presented in Table 1, we conclude that the activity against *M. tuberculosis* depends on the physico-chemical parameters (Log *P* and pKa), which are a function of the substituents in each molecule evaluated and of its structural characteristics. Such features may result in intramolecular interactions among these groups, for example, by hydrogen bonds, which affect the lipophilicity and the pKa. For instance, usnic acid [**2**] and salazinic acid [**8**], with similar Log *P* values (1.18 ± 0.75 and 1.41 ± 0.86, respectively), have different structures and inhibitory activities against *M. tuberculosis*. Although diffractaic acid [**3**] was the most active compound among them, it showed lower inhibitory activity (MIC value 41.7 μM) than isoniazid (MIC value 0.109 μM). However, further studies with this compound in

Table 1
MIC values of the compounds evaluated against *Mycobacterium tuberculosis*.

Compounds	MIC (μg/ml)	MIC (μM)	Log *P*[a]
Isoniazid	0.015	0.109	−0.89 ± 0.24
Atranorin **[1]**	250	529	6.14 ± 0.49
Usnic acid **[2]**	62.5	182	1.18 ± 0.75
Diffractaic acid **[3]**	15.6	41.7	5.48 ± 0.42
Lichexanthone **[4]**	>250	>876	4.07 ± 0.51
Secalonic acid **[5]**	>250	>392	3.83 ± 0.92
Lecanoric acid **[6]**	250	785	4.39 ± 0.42
Protocetraric acid **[7]**	125	334	3.29 ± 0.67
Salazinic acid **[8]**	>250	>643	1.41 ± 0.86
Hypostictic acid **[9]**	94	251	3.29 ± 0.75
Norstitic acid **[10]**	62.5	168	3.05 ± 0.85
Methyl orsellinate **[11]**	250	1370	2.38 ± 0.26
Ethyl orsellinate **[12]**	250	1270	2.91 ± 0.26
n-propyl orsellinate **[13]**	125	595	3.44 ± 0.26
n-butyl orsellinate **[14]**	250	1110	3.97 ± 0.26
n-hexyl orsellinate **[15]**	>250	>990	5.04 ± 0.26
Iso-propyl orsellinate **[16]**	125	595	3.26 ± 0.26
Tert-butyl orsellinate **[17]**	125	557	3.61 ± 0.27
8′, 9′-di-*O*-*n*-propyl salazinic acid **[18]**	>250	>529	4.67 ± 0.88
8′, 9′-di-*O*-*n*-butyl salazinic acid **[19]**	>250	>500	5.73 ± 0.88
8′, 9′-di-*O*-*n*-pentyl salazinic acid **[20]**	>250	>473	6.79 ± 0.88
8′, 9′-di-*O*-*n*-hexyl salazinic acid **[21]**	>250	>449	7.85 ± 0.88
8′, 9′-di-*O*-*iso*-propyl salazinic acid **[22]**	>250	>529	4.30 ± 0.88
Norlichexanthone **[23]**	>250	>968	3.02 ± 0.50
3,6-di-*O*-prenyl-norlichexanthone **[24]**	>250	>634	7.83 ± 0.57
3,6-bis(3,3-dimethyloxiron-2-il)methoxy]-1-hydroxi-8-methyl-xanthone **[25]**	>250	>586	3.55 ± 0.58
3-*O*-methyl-6-*O*-benzyl norlichexanthone **[26]**	>250	>690	5.72 ± 0.52

[a] Log *P* was calculated using ACD log *P* version 1994–2002 (www.acdlabs.com – version free).

combination with anti-tuberculosis drugs will be conducted to improve the activity through the synergistic effects.

In conclusion, we confirm the activity of atranorin [**1**], usnic acid [**2**] and salazinic acid [**8**] against *M. tuberculosis*, as already reported by Ingólfsdóttir et al. (1998). The activities of the other compounds are being presented herein for the first time. Diffractaic acid [**3**], isolated from the lichen *Usnea subcavata*, is the most active among them as an inhibitor of *M. tuberculosis* $H_{37}Rv$ growth.

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References

Ahmann, B., Mathey, A., 1967. Lecanoric acid and some constituents of *Parmotrema tinctorum* and *Pseudevernia intense*. The Bryologist 70, 93–97.

Barreiro, E.J., Fraga, C.A.M., 2001. Química Medicinal. Artmed, Porto Alegre, p. 243.

Bapela, N.B., Lall, N., Fourie, P.B., Franzblau, S.G., Van Rensburg, C.E.J., 2006. Activity of 7-methyljuglone in combination with antituberculous drugs against Mycobacterium tuberculosis. Phytomedicine 13, 630–635.

Caballeira, N.M., 2008. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. Prog. Lipid Res. 47, 50–61.

Copp, B.R., Pearce, N., 2007. Natural product growth inhibitors of *Mycobacterium tuberculosis*. Nat. Prod. Rep. 24, 278–297.

Collins, L.A., Franzblau, S.G., 1997. Microplate Alamar Blue Assay versus BACTEC 460 system for high-throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob. Agents Chemother. 41, 1004–1009.

Franzblau, S.G., Witzig, R.S., McLaughlin, J.C., Torres, P., Madico, G., Hernandez, A., et al., 1998. Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue assay. J. Clin. Microbiol. 36, 362–366.

Gomes, A.T., Honda, N.K., Roese, F.M., Muzzi, R.M., Sauer, L., 2006. Cytotoxic activity of Orsellinates. Z. Naturforsch. C: Biosci. 61, 653–657.

Gupta, V.K., Arokar, M.P., Saikia, D., Pal, A., Fatima, A., et al., 2007. Antimycobacterial activity of lichens. Pharm. Biol. 45, 200–204.

Hale Jr., M.E., 1983. The Biology of Lichens, third ed. Edward Arnold, London.

http://www.who.int/topics/tuberculosis/en – accessed January 21, 2009.

Ingólfsdóttir, K., Chung, G.A.C., Skúlason, V.G., Gissurarson, S.R., Vilhelmdóttir, M., 1998. Antimycobacterial activity of lichen metabolites in vitro. Eur. J. Pharm. Sci. 6, 141–144.

Koul, A., Vranckx, L., Denouga, N., Balemans, W., Van Den Wyngaert, I., et al., 2008. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. J. Biol. Chem. 282, 25273–25280.

Lenaerts, A.J., DeGroote, M.A., Orme, I.M., 2007. Preclinical testing of new drugs for tuberculosis: current challenges. Trends Microbiol. 16, 48–54.

Lopes, T.I.B., Coelho, R.G., Yoshida, N.C., Honda, N.K., 2008. Radical-scavenging activity of orsellinates. Chem. Pharm. Bull. 56, 1551–1554.

Micheletti, A.C., Beatriz, A., de Lima, D.P., Honda, N.K., 2009. Constituintes químicos de *Parmotrema lichexanthonicum* Eliasaro & Adler – Isolamento, Modificações estruturais e avaliação das atividades antibiótica e citotóxica. Quim. Nova 32, 12–20.

Micheletti, A.C., 2007. Preparação de derivados de xantona e depsidona e avaliação da atividade biológica. Dissertation, UFMS, Campo Grande, p. 233.

Quenelle, D.C., Winchester, G.A., Staas, J.K., 2001. Treatment of tuberculosis using a combination of sustained-release rifampin-loaded microspheres and oral dosing with isoniazid. Antimicrob. Agents Chemother. 45, 1637–1644.

Suksamrarn, S., Suwannapoch, N., Phakhoee, W., Thanchranlert, J., Ratananukul, P., et al., 2003. Antimycobacterial activity of prenylated xanthones from the fruits of *Garcinia mangostana*. Chem. Pharm. Bull. 51, 857–859.

Vartia, K.O., 1973. Antibiotics in lichens. In: Ahmadjian, V., Hale, M.E. (Eds.), The Lichens. Academic Press, New York, pp. 547–561.

Wagner, H., Ulrich-Merzenich, G., 2009. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine 16, 97–110.