Furan type lignans with antimycobacterial activity

[Lignanos del tipo furano con actividad antimicobacterial]

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Abstract: Compounds such as triclosan, diclofenac and trimetoprim posses antibacterial activity, including mycobacterial; their structures are based on two aromatic rings joined by a methylene or a heteroatom. Since a similar structural system is found in natural diarylfuran-based lignans, we studied plants known with this type of lignans, as potential active against Mycobacterium tuberculosis. Fractions of the active extracts were tested for anti-TB activity and their chemical constituents analyzed by NMR spectroscopy. Several extracts and chromatographical fractions exhibited > 90% inhibition of M. tuberculosis at 128 μg/mL. Methylpluviatilol, a pure compound isolated from Virola sebifera, was active at this concentration. These findings suggest that plant species of the families here studied may yield novel lead compounds for the development of antimycobacterial agents.

Keywords: Arylfuran, lignans, tuberculosis, bioassay, antimycobacterial

Resumen: Compuestos tales como triclosan, diclofenac y trimetoprim poseen actividad antibacterial, incluyendo la antimicobacterial; sus estructuras están basadas en dos anillos aromáticos unidos por un metileno o un heteroátomo. Debido a que en la naturaleza se encuentra un sistema estructural similar del tipo diarilfurano en los lignanos, así como otros subtipos, nosotros estudiamos plantas contra Mycobacterium tuberculosis, de las que se sabe contienen lignanos. Las fracciones cromatográficas de los extractos activos fueron ensayadas para actividad anti-Tb y sus constituyentes químicos se analizaron por espectroscopía de RMN. Varios extractos y fracciones cromatográficas exhibieron una inhibición superior al 90% a 128 μg/mL; el compuesto metilpluviatilol, aislado de Virola sebifera, fue activo a esta concentración. Estos hechos sugieren que las especies de plantas de las familias aquí estudiadas podrían suministrar nuevos compuestos líderes para el desarrollo de agentes antimicobacteriales.

Palabras clave: Arilfurano, lignanos, tuberculosis, bioensayo, antimycobacterial

Received: June 2, 2014
Accepted: September 1, 2014
Declaramiento de intereses: This work was supported by COLCIENCIAS (COLOMBIA) and Universidad de Antioquia.

INTRODUCTION
More than 36 million people were infected with tuberculosis between 1995 and 2008, causing 6 million deaths (Lonnroth et al., 2010). These facts make TB a serious and an ever increasing global health problem, partly due to HIV co-infection and the emergence of drug resistant strains of *M. tuberculosis*. About 10% of HIV-infected patients die of TB and every year nearly a half million multidrug resistant cases appear (WHO, 2002). Consequently new treatments are needed for TB control; one of them being the search for new antimycobacterial molecules.

In 2001 several 2,5-diphenylfuran synthetic-type compounds were reported to have MIC ≤ 16 μg/ml against *M. tuberculosis* (Stephens et al., 2001). These molecules are structural analogues of lignans, natural substances found in more than 70 plant families (Saleem et al., 2005). Additionally, natural lignans are chemically highly diverse, specifically on their furan-type rings. Therefore, these compounds may represent new potential antimycobacterial compounds with novel chemical structures and mechanism of action. Based on the above, we carried out bioassay of thirteen Colombian plant species belonging to the Magnoliaceae, Piperaceae, Myristicaceae and Lauraceae.

RESULTS AND DISCUSSION
Structure
Partitions of extracts and chromatographic fractions afforded several subfractions or pure compounds with NMR lignan signals, as it can be seen in Figure 1. However, in some cases additional fractionation was not conducted due to the very low availability of plant material.

Fractionation of *Virola flexuosa* and *Virola sebifera* allowed isolation of furan, furofuran, and dibenzylbutyro lactone lignans as well as flavonoids. Their structures were assigned on the basis of 1H and 13C NMR and compared with spectral properties reported in literature. (Biavatti et al., 2001; Souza Filho et al., 2006; Gomes et al., 2006) Figure 2 shows the structure of these compounds.

Antimycobacterial activity
Table 1 displayed the inhibitory activity of crude extracts (CE) and fractions (FA) against *M. tuberculosis* H37Rv. Methylpluviatilol (4) and hinokinin (2), isolated from *V. sebifera* and *V. flexuosa* respectively, were more active than similar compounds such as episesamin (5), kusunokinin (1) and dihidrocubebin (3). Since all of these compounds possess methylenedioxy substituents, other structure activity relationship studies should be carried out to clarify this effect. Additionally, fraction 3 from the ethanol extract of *V. flexuosa* afforded 7,4’-dimethoxy (6) and 7,3’,4’ trimethoxyflavone (8). Although neither of these compounds was evaluated; the flavone thithonin (7) was not significantly active.
Figure 1
Monitoring of fractionation process by NMR. $^1$HNMR spectrum of V. flexuosa hexane extract (A), fraction 5 of V. flexuosa hexane extract (B), and lignan hinokinin obtained from fraction 5 (C)
Figure 2
Structures and activity of compounds from *V. flexuosa* (2, 3, 6, 7 and 8) and *V. sebifera* (1, 4 and 5). GI%: % growth inhibition at 128 μg/mL

Table 1
Grow Inhibition (GI%) of CE and fractions at 128 μg/mL against *M. tuberculosis* H₃₇Rv. Although extracts of *V. calophylla*, *T. arcabucoana* and *O. puberula* were not significantly active, chromatographic separation yielded fractions with improved activity. These active fractions showed NMR signals attributable to the presence of lignans, e.g. methylenedioxy, methoxy, methyl groups and aromatic protons.

<table>
<thead>
<tr>
<th>Myristicaceae</th>
<th>Botanical name / Part of plant / Origin / Voucher specimen</th>
<th>CE</th>
<th>CE GI %</th>
<th>FA</th>
<th>FA GI %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Virola</em> sp. / Leaves</td>
<td>EtOH 38</td>
<td>2</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
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<td>3</td>
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<td></td>
<td>5</td>
<td>19</td>
<td></td>
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<tr>
<td><em>V. peruviana</em> / Leaves</td>
<td>EtOH 31</td>
<td>7</td>
<td>29</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>20</td>
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<td></td>
</tr>
<tr>
<td>Plant Species</td>
<td>Sample Type</td>
<td>Solvent</td>
<td>MIC Value (μg/mL)</td>
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<td></td>
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<td><em>V. calophylla</em> / Bark / Sonaña-Vaupes-Colombia / COL231575</td>
<td>EtOH</td>
<td>29</td>
<td>6 58</td>
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<td><em>V. calophylla</em> / Leaves</td>
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<td>89</td>
<td>2 92</td>
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<td></td>
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<td><em>V. flexuosa</em> / Leaves / Puerto Berrio-Antioquia-Colombia / HUA8967</td>
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<td>94</td>
<td>4 92</td>
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<td></td>
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<tr>
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<td><em>P. peltatum</em> / Leaves</td>
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<tr>
<td><em>P. hispidum</em> / Leaves / Nocaima-Cundinamarca-Colombia COL510518</td>
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<td>27</td>
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<tr>
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<td>EtOH</td>
<td>95</td>
<td>-</td>
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<td><em>P. auriculatum</em> / Leaves</td>
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<td><em>Talauma arcabucoana</em> / Leaves</td>
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<td>81</td>
<td>3 99</td>
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<td><em>T. arcabucoana</em> / Bark</td>
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<td>4 50</td>
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<td>EtOH</td>
<td>57</td>
<td>1 68</td>
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</tbody>
</table>

**Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas/ 175**
All compounds isolated in this work have been reported previously in phytochemical studies of Myristicaceae and related families (Biavatti et al., 2001; Carvalho et al., 1999; Seo et al., 2008). Studies of Virola species reported antifungal (Lopez et al., 1999; Zacchino et al., 1998) activity against gram positive and gram negative bacteria (Barbosa et al., 2006), and hinokinin and other cubebin derivatives have shown antimicrobial activity against oral pathogens (Silva et al., 2007). To date, there have not been any reports of antimycobacterial activity of lignans belonging to this genus.

Conversely, magnolol, honokiol, 3,5’-diallyl-2’-hydroxy-4-methoxy-biphenyl (MIC 2.5-7.5 μg/mL) (Clark et al., 1981) and the sesquiterpene lactone parthenolide (MIC 16 μg/mL) (Fischer et al., 1998) have been isolated from Magnolia sp., all of which have positive for antimycobacterial activity. In this study, NMR signals for sesquiterpene lactones were detected in FA2 from M. yarumalense. Moreover, Piper sanctus yielded several antimycobacterial constituents especially pyrone-type compounds. A double bond and methylenedioxy group may be involved in this activity. Horsfieldin, (+)-sesamin and furofuran lignans from P. sarmentosum was considered inactive with MIC > 200 μg/mL (Tuntiwachwuttikul et al., 2006), but some neolignans displayed antimycobacterial activity (Leon-Diaz et al., 2010).

### Experimental

#### General experimental procedures

NMR spectra (¹H and ¹³C) were recorded on a Bruker AMX 300 spectrometer in CDCl₃. The presence of lignans in extracts and fractions was correlated with the presence of several signals attributable to methoxy, methylenedioxy and alkoxy groups in addition to aromatic rings and lactones in NMR spectroscopy. NMR data of pure isolated compounds was compared with published data.

#### Plant Material

Leaves and bark of 13 plants were collected from several locations in Colombia from February to June 2007. Table 1 summarize the studied plant specimens; vouchers were deposited at the herbariums of Universidad de Antioquia (HUA), Nacional Colombiano, (COL) and Amazonico Colombiano (COAH). Plant extracts of Virola sp., V. peruviana, Piper sp., P. peltatum, P. auriculatum, Talauma arcabucoana and Pleurothyrium cinereum were kindly provided by professor Luis Enrique Cuca of Universidad Nacional de Colombia-Bogota and the Magnolia yarumalense extract by professor Benjamin Rojano of Universidad Nacional de Colombia-Medellin.

#### Extract Preparation and Fractionation

Dried and ground plant material (1.0 Kg) was extracted by percolation using hexane and ethanol (EtOH). Extracts were concentrated under vacuum, yielding crude extracts (CE), which were fractionated on a Sephadex LH-20.
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(Sigma) column using hexane-CH$_2$Cl$_2$-MeOH (2:1:1, v/v) to obtain several fractions (FA). CE and FA were analyzed by $^1$H and $^{13}$C NMR spectroscopy to detect lignans and then were submitted for antimycobacterial activity. Finally, lignans 2, 3, 6, 7, and 8 (40, 20, 45, 50, 30 mg, respectively) from V. flexuosa and 1, 4 and 5 (70, 25, 25 , respectively) from V. sebifera, were obtained.

**Antimycobacterial Assay**

Activity against *M. tuberculosis* H37Rv (ATCC 27294; American Type Culture Collection, Rockville, Md.) was assessed using Microplate Alamar Blue Assay (Collins & FRanzblau, 1997), but 7H12 medium was used instead (Falzari et al., 2005). Activity of crude extracts, fractions and isolated compounds was evaluated at 128µg/mL in triplicate and results were expressed as the average percentage of growth inhibition. Extracts with >90% inhibition were considered to be candidates for further separation.

**CONCLUSIONS**

The search for new bioactive molecules from nature has been based on ethnobotanical reports, bioassay-guided isolation and chemotaxonomic relationships. Additionally, active secondary metabolites are often transformed into other derivatives to obtain better pharmacological or toxicological compounds. In spite of this, a new approach has been taken in this study: synthetic compounds with reported bioactivity, aside from yielding closely related compounds, thus facilitating early structure-activity relationships, have served as templates to guide research on natural sources of related compounds. In this study, we selected several plant species whose families are recognized by their production of lignans, especially arylfuran-type lignan. The detection of several active extracts and one pure compound appear to validate this approach.

**ACKNOWLEDGMENTS**

This work was supported by COLCIENCIAS (COLOMBIA) and Universidad de Antioquia. Thanks to professors Ramiro Fonfegra, Luis E. Cuca and Benjamin Rojano for material collection or extracts supplied.

**REFERENCES**


