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Furan type lignans with antimycobacterial activity

[Lignanos del tipo furano con actividad antimicobacterial]

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Abstract: Compounds such as triclosan, diclofenac and trimetropin 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 ug/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 ug/mL; el compuesto metilpluviatilol, aislado de mostró una inhibición del 99% a esa concentración. Esos 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, biensayos, antimicobacterial

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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 dibenzylbutyrolactone 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)

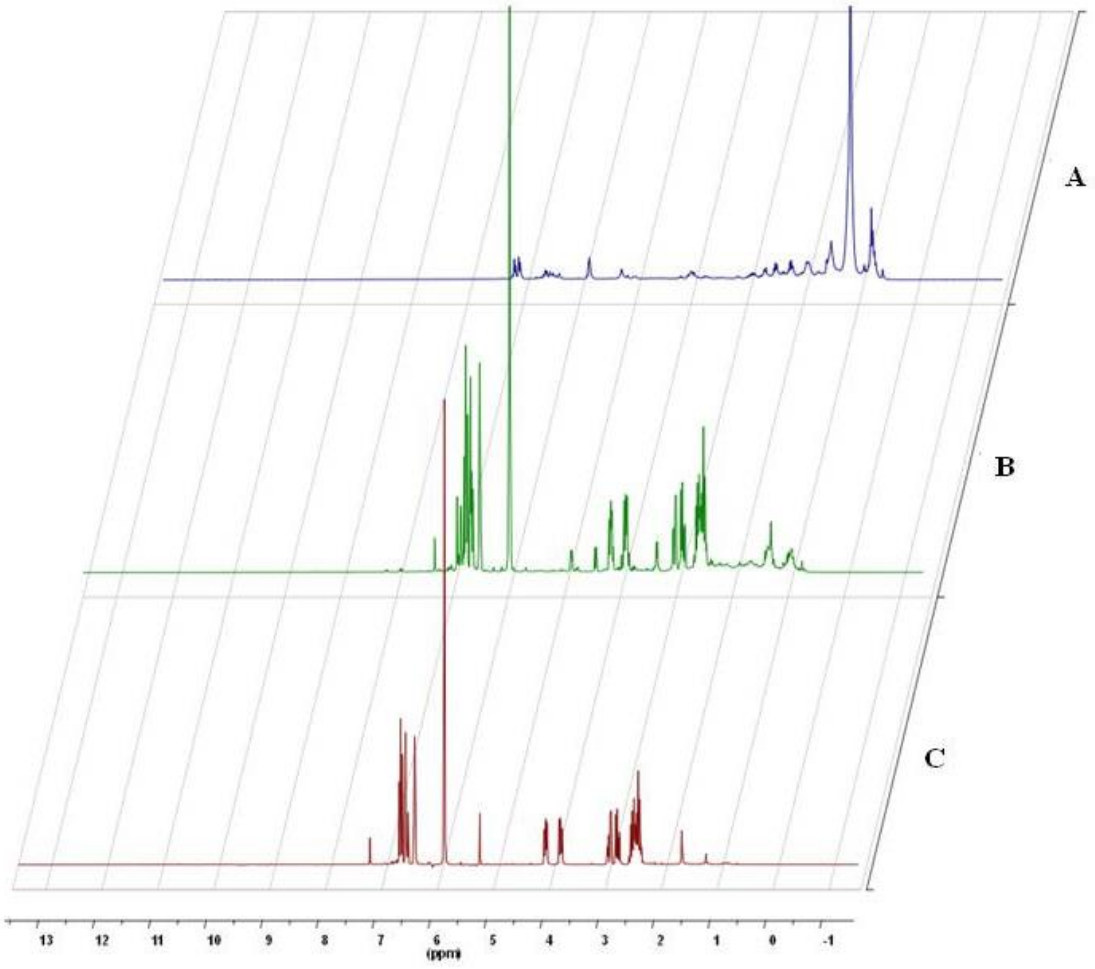
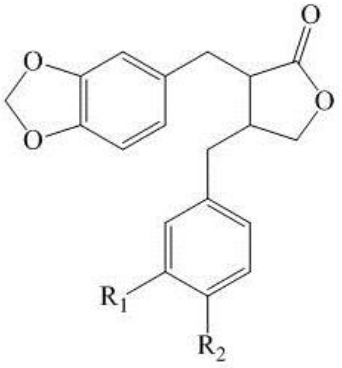
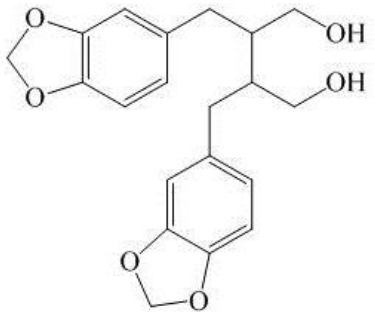


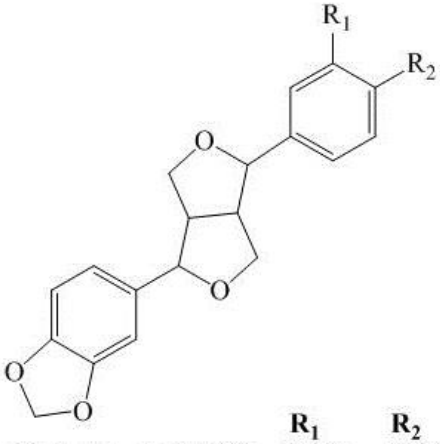
Figure 2
Structures and activity of compounds from V. flexuosa (2, 3, 6, 7 and 8) and V. sebifera (1, 4 and 5). GI%: % growth inhibition at 128 µg/mL



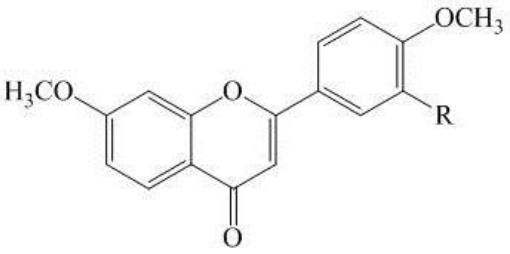
	R_1	R_2	GI%
Kusunokinin **(1)**	OCH_3	OCH_3	67
Hinokinin **(2)**	OCH_2O		80.8



	GI%
Dihydrocubebin **(3)**	65



	R_1	R_2	GI%
Methylpluviatilol **(4)**	OCH_3	OCH_3	98.6
Episesamin **(5)**	OCH_2O		60



	R	GI%
7,4'-dimethoxiflavone **(6)**	H	N.D
Thithonine **(7)**	OH	13.9
7,3',4'-trimethoxiflavone **(8)**	OCH_3	N.D

Table 1
Grow Inhibition (GI%) of CE and fractions at 128 µg/mL against *M. tuberculosis* H_{37}Rv. 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.

	Botanical name / Part of plant / Origin / Voucher specimen	CE	CE GI %	FA	FA GI%
Myristicaceae	*Virola* sp. / Leaves	EtOH	38	2	64
				3	62
				5	19
	V. peruviana / Leaves	EtOH	31	7	29
				8	20

	V. calophylla / Bark / Sonaña-Vaupes-Colombia / COL231575	EtOH	29	6	58
				7	48
				5	97
	V. calophylla / Leaves	EtOH	89	2	92
				2B	61
				3	45
				4	36
				4B	75
				4C	41
	V. flexuosa / Leaves / Puerto Berrio-Antioquia-Colombia / HUA8967	Hexane	94	4	92
				5	32
				6	94
		EtOH	95	2	58
				3	90
				4	74
				5	54
	V. flexuosa / Bark	Hexane	74	-	-
		ETOH	10	-	-
Piperaceae	*Piper* sp / Leaves	EtOH	92	-	-
	P. peltatum / Leaves	EtOH	35	-	-
	P. hispidum / Leaves / Nocaima-Cundinamarca-Colombia COL510518	EtOH	27	-	-
	P. hispidum / Stem	EtOH	95	-	-
	P. auriculatum / Leaves	EtOH	27	-	-
Magnoliaceae	*Talauma arcabucoana* / Leaves	EtOH	81	3	99
				4	52
				8	98
				13	78
	T. arcabucoana / Bark	EtOH	77	4	50
				8	91
				6	73
	Magnolia yarumalense / Leaves	$CHCl_3$	98	-	-
Lauraceae	*Pleurothyrium cinereum* / Leaves	EtOH	57	1	68
				2	69
				3	74
				4	36

				5	65
	O. puberula / Leaves / Carepa-Antioquia-Colombia / HUA8876	Hexane	75	3	79
		EtOH	70	9	97
	Persea Americana / Leaves /	EtOH		2	18
				3	29
				4	24
Control	Rifampin	95			
	Isoniazid	96			

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 sesquiterpeno 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 (^{1}H and ^{13}C) were recorded on a Bruker AMX 300 spectrometer in $CDCl_3$. 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

(Sigma) column using hexane-CH_2Cl_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.

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REFERENCES

Barbosa I, Barradas LM, Varella AD, Younes RM. 2006. Antibacterial activity of Brazilian Amazon Plant Extracts. **Braz J Infec Dis** 10: 400 - 402.

Biavatti MW, Vieira PC, Da Silva MF, Fernández JB, Degani AL, Cass QB, Schefer AB, Ferreira AG. 2001. Separation and NMR studies on lignans of *Raulinoa echinata*. **Phytochem Anal** 12: 64 - 68.

Carvalho JCT, Ferreira LP, Da Silva Santos L, Correa MJC, De Oliveira Campos LM, Bastos JK, Sarti SJ. 1999. Anti-inflammatory activity of flavone and some of its derivates from *Virola michelli* Heckel. **J Ethnopharmacol** 64: 173 - 177.

Clark M, El-Feraly F, Li W. 1981. Antimicrobial activity of phenolic constituens of *Magnolia grandiflora* L. **J Pharm Sci** 70: 951 - 952.

Collins LA, Franzblau S. 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.

Falzari K, Zhu Z, Pan D, Liu H, Hongmanee P, Franzblau S. 2005. *In vitro* and *in vivo* activities of macrolide derivatives against *Mycobacterium tuberculosis*. **Antimicrob Agents Chemother** 49: 1447 - 1454.

Fischer NH, Lu T, Cantrell CL, Castañeda Acosta J, Quijano L, Franzblau S. 1998. Antimycobacterial evaluation of germacranolides. **Phytochemistry** 49: 559 - 564.

Gomes V, Da Silva R, Pedersoli S, De Albuquerque S, Bastos J, Andrade e Silva M, Donate P, Da Silva G, Lopes J. 2006. Detailed ^{1}H and ^{13}C NMR Structural assignment of three biologically active lignan lactones. **Spectrochim Acta A Mol Biomol Spectrosc** 63: 234 - 239.

León-Díaz R, Meckes M, Said-Fernández S, Molina-Salinas GM, Vargas-Villarreal J, Torres J, Luna-Herrera J, Jiménez-Arellanes A. 2010. Antimycobacterial neolignans isolated from *Aristolochia taliscan*a. **Mem Inst Oswaldo Cruz** 105: 45 - 51.

Lönnroth K, Castro KG, Chakaya JM, Chauhan LS, Floyd C, Glaziou P, Raviglione MC. 2010. Tuberculosis control and elimination 2010-50: cure, care, and social development. **The Lancet** 375: 1814 - 1829.

Lopez N, Kato M, Yoshida M. 1999. Antifungal constituents from roots of *Virola surinamensis*. **Phytochemistry** 51: 29 - 33.

Saleem M, Kim H, Shaiq Ali M, Lee Y. 2005. An update on bioactive plant lignans. **Nat Prod Rep** 22: 696 - 716.

Seo SM, Lee HJ, Lee OK, Jo HJ, Kang HY, Choi DH, Paik KH, Khan M. 2008. Furofuran lignans from the bark of *Magnolia kobus*. **Chem Org Nat Prod** 44: 419 - 423.

Silva MLA, Coimbra HS, Pereira AC, Almeida VA, Lima TC, Costa ES, Vinhólis AHC, Royo VA, Silva R, Filho AAS, Cunha WR, Furtado NAJC, Martins CHG, Carvalho TC, Bastos JK. 2007. Evaluation of *Piper cubeba* extract, (-)-cubebin and its semi-synthetic derivatives against Oral Pathogens. **Phytother Res** 21: 420 - 422.

Souza Filho APS, Borges FC, Santos LS. 2006. Análise Comparativa dos efeitos alelopáticos das substâncias químicas titonina e titonina acetilada. **Planta Daninha** 24: 205 - 210.

Stephens C, Tanious F, Kim S, Wilson WD, Schell W, Perfect J, Franzblau S, Boykin D. 2001. Diguanidino and reversed diamidino 2,5-diarylfurans as antimicrobial agents. **J Med Chem** 44: 1741 - 1748.

Tuntiwachwuttikul P, Phansa P, Pootaeng-On Y, Taylor W. 2006. Chemical constituents of the roots of *Piper sarmentosum*. **Chem Pharm Bull** 54: 149 - 151.

WHO. 2002. World Health Organization. *Fact sheet N°104*: **Tuberculosis.** WHO: 2002, 1 - 3.

Zacchino S, Rodríguez G, Santecchia C, Pezzenati G, Giannini F, Enriz R. 1998. *In vitro* studies on mode of action of antifungal 8.O.4'-neolignans occurring in certain species of *Virola* and related genera of Myristicaceae. **J Ethnopharmacol** 62: 35 - 41.