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Effect of Natural 2,5-Diaryl-3,4-dimethyltetrahydrofuran Lignans on Complement Activation, Lymphocyte Proliferation, and Growth of Tumor Cell Lines

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Abstract

(+)-Machillin F, isolated from the bark of *Persea membranacea*, Kosterm and four other 2,5-diaryl-3,4-dimethyltetrahydrofuran–type lignans from *Talauma hodgsonii* Hook, F. & Thoms., (−)-galbacin, (−)-talaumidin, (−)-talaumidin methyl ether, and acetyl talaumidin, were evaluated for their influence on the classical and alternative pathways of activation of human complement system, mitogen-induced proliferation of human lymphocytes, and growth of human tumor cell lines. All these lignans exhibited moderate inhibitory activities. (+)-Machillin F showed the highest anticomplementary activity, and (−)-talaumidin methyl ether exhibited the strongest suppressive effect on lymphocyte proliferation. (−)-Talaumidin, acetyl talaumidin, and (+)-machillin F inhibited the growth of MCF-7, TK-10, and UACC-62 cell lines, whereas (−)-galbacin was inactive against UACC-62 and (−)-talaumidin methyl was inactive against both TK-10 and UACC-62. A characterization of the structural features of these lignans that is important to their biological activities is discussed.

Keywords: Anticomplementary, antiproliferative, antitumor activity, lignans, *Persea membranacea*, *Talauma hodgsonii.*

Introduction

Lignans are a large group of phenylpropanoid metabolites found throughout the plant kingdom and known by their diverse and broad spectrum of biological activities, including bactericidal, fungicidal, antiviral, antioxidant, anti-inflammatory, immunomodulatory, and antitumor effects (Ayres & Loike, 1990; Ward, 1997; Cho et al., 1999, 2001; Su et al., 1999). Although 2,5-diaryl-3,4-dimethyltetrahydrofuran derivatives are a prolific group of lignans, the studies of their biological activities are sparse. It has been reported that some tetrahydrofuran lignans were potent platelet activating factor antagonists (Biftu & Stevensen, 1987). So far, nectandrin B is the only 2,5-diaryl-3,4-dimethyltetrahydrofuran lignan reported to suppress the mitogen-induced proliferation of human lymphocytes (Hirano et al., 1991). To our knowledge, the anticomplementary activity of this type of lignan has never been described. In this study, we have investigated the effect of (+)-machillin F (**3**; Fig. 1), isolated from *Persea membranacea* Kosterm (Lauraceae), together with four other structurally related 2,5-diaryl-3,4-dimethyltetrahydrofuran lignans, namely (−)-galbacin (**1**), (−)-talaumidin (**2a**), (−)-talaumidin methyl ether (**2b**), and acetyl talaumidin (**2c**) (Fig. 1), previously isolated from *Talauma hodgsonii* Hook, F. & Thoms. (Magnoliaceae) (Vieira et al., 1998), on the classical (CP) and alternative (AP) pathways of activation of the human complement system as well as their effect on the mitogenic response of human lymphocytes to phytohemagglutinin (PHA). For the first time, the capacity of 2,5-diaryl-3,4-dimethyltetrachydrofuran lignans to inhibit the *in vitro* growth of the human tumor

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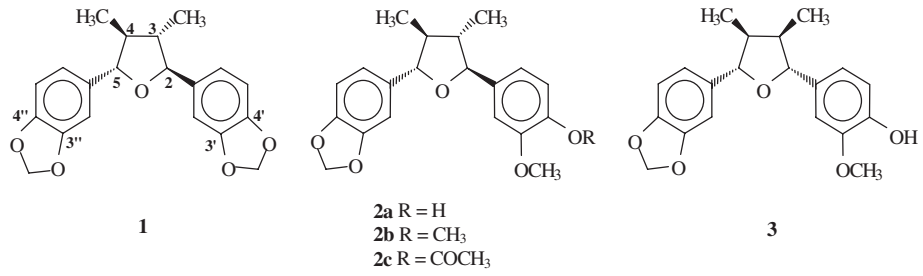

1 **2a** R = H, **2b** R = CH_3, **2c** R = $COCH_3$ **3**

Figure 1. Chemical structures of the 2,5-diaryl-3,4-dimethyltetrahydrofuran lignans under study.

cell lines MCF-7 (breast cancer), TK-10 (renal cancer), and UACC-62 (melanoma), was assessed.

Materials and Methods

Plant material

Bark of *Persea membranacea* Kosterm was collected in Peat Swamp Forest, Narathiwat Province, Thailand, in March 1992. A voucher specimen was deposited in the herbarium of the Royal Forest Department, Bangkok, Thailand.

Extraction and isolation

Dry powdered bark (4.5 kg) was percolated by MeOH (10 l) to exhaustion. The MeOH extract was evaporated at reduced pressure to furnish 228 g of crude extract that was dissolved in $CHCl_3$ at room temperature with the aid of ultrasound and filtered. The process was repeated until the solution became pale yellow. Evaporation of the $CHCl_3$ solution at reduced pressure afforded 35 g of crude extract, which was chromatographed on a Si gel column (200 g) using 80,500-ml fractions ($CHCl_3$-petrol) of increasing polarity. Extensive rechromatography at preparative thin-layer chromatography (PTLC) of the various subfractions resulted in the isolation of (+)-machillin F (**3**, 190 mg), *meso*-dihydroguaiaretic acid (210 mg) and (+)-guaiacin (270 mg). Melting points (m.p.), $[\alpha]_D$, mass spectra (MS), ^1H and ^{13}C NMR spectra of these lignans corresponded to those in the literature (Ito et al., 1984; Shimomura et al., 1988).

(−)-Galbacin (**1**), (−)-talaumidin (**2a**), and (−)-talaumidin methyl ether (**2b**) were isolated from the bark of *Talauma hodgsonii* Hook. F. & Thoms. (Magnoliaceae) as previously described by us (Vieira et al., 1998). Acetyl talaumidin (**2c**) was obtained by acetylation of **2a** with Ac_2O/Py.

Reagents and samples for biological assays

Fetal bovine serum (FBS) and RPMI-1640 medium were obtained from Gibco BRL (Scotland, UK). Cyclosporin A, dextran sulfate, dimethylformamide (DMF), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), gentamicin, L-glutamine, Histopaque-1077, phytohemagglutinin (PHA), podophyllotoxin, sodium lauryl sulfate (SDS), and sulforhodamine B (SRB) were purchased from Sigma Chemical Co (St. Louis, MO, USA).

Stock solutions of lignans **1**, **2a**, **2b**, **2c**, and **3** were prepared in DMSO and then diluted to the desired final concentrations with the appropriate diluents just prior to the different biological assays. Final concentrations of DMSO did not interfere with any of the biological activities tested.

Complement assays

The effect of lignans on the classical (CP) and alternative pathway (AP) of human complement system was determined by hemolytic assays as previously described by us (Gonzalez et al., 1999). Dextran sulfate was used as positive control.

Lymphocyte assays

The effect of lignans on the mitogenic response of human lymphocytes to PHA (10 µg/ml) was evaluated using a modified version of the colorimetric MTT-assay (Mosmann, 1983), which was previously described by our group (Gonzalez et al., 1999). Mononuclear cells were isolated from heparinized peripheral blood of healthy volunteers by Histopaque-1077. Cyclosporin A was used as a positive control. To evaluate a possible toxicity of lignans against human lymphocytes, a MTT-assay was also used, which measured the ability of viable cells to reduce the colorless tetrazolium salt MTT to the colored formazan product. Briefly, in flat-bottom 96-well plates, nonstimulated human mononuclear cells were treated with serial concentrations of each lignan for 24 h at 37 °C. Following this incubation period, the MTT solution (1 mg/ml) was added. After 4 h of incubation, the MTT formazan products were solubilized with the SDS/DMF solution (20% SDS in a 50% solution of DMF, pH 4.7) overnight at 37 °C. Absorbance of the colored solution was measured at 550 nm. Lymphocyte toxicity, determined in terms of the percentage of viable cells, was present when the viability of the treated cells, compared with that of the nontreated control cells, was less than 70%.

Tumor cell growth assay

The effect of lignans on the growth of tumor cell lines was evaluated according to the procedure adopted by the National

Cancer Institute (NCI, Bethesda, MD, USA) for the *in vitro* anticancer drug screening that uses the protein-binding dye SRB to assess growth inhibition. The methodology used was the same as originally published by the NCI team (Skehan et al., 1990; Monks et al., 1991). Three human tumor cell lines were used, MCF-7 (breast cancer), TK-10 (renal cancer), and UACC-62 (melanoma). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium supplemented with 5% heat-inactivated FBS, 2 mM glutamine, and 50 µg/ml of gentamicin at 37 °C in a humidified atmosphere containing 5% CO_2. The optimal plating density of each cell line, determined according to their growth profiles that ensure exponential growth throughout all the experimental period, was the same as originally published (Monks et al., 1991) and was, respectively, 1.5×10^5 cells/ml to MCF-7 and TK-10, 1.0×10^5 cells/ml to UACC-62. Cells were exposed for 48 h to 5 concentrations of compounds starting from a maximum concentration of 150 µM. For each test compound and for each cell line, a dose-response curve was generated, and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of compound that inhibits 50% of the net cell growth, was determined as described (Monks et al., 1991). Podophyllotoxin was used as positive control.

Statistical analysis

Results are expressed as mean ± SEM (standard error of the mean). The statistical significance of the difference was assessed by the Student's *t*-test.

Results and Discussion

Effect on the pathways of activation of the human complement system

The effects of lignans **1**, **2a**, **2b**, **2c**, and **3** on the CP and AP of the human complement system are shown in Table 1. All the lignans showed a moderate and dose-dependent inhibitory effect of CP. On a molar basis, compound **3** was the most potent inhibitor of CP, followed by **2b**, whereas **2a** exhibited the lowest activity. Comparing the inhibitory activities of **1**, **2a**, **2b**, and **2c**, all possessing 3″,4″-methylenedioxyphenyl as one of the substituents and whose stereochemistry of the tetrahydrofuran ring is *trans-trans-trans*, it can be concluded that the nature of the substituents on the aromatic portion could influence the effect on the CP of the complement system. Hence, **2b**, with the 3′,4′-dimethoxyphenyl substituent, was significantly more active than **1** ($p < 0.05$), **2a** ($p < 0.01$), and **2c** ($p < 0.001$), which contained a 3′,4′-methylenedioxyphenyl, a 3′-methoxy-4′-hydroxyphenyl, and a 3′-methoxy-4′-acetoxyphenyl substituent, respectively. The stereochemistry of the tetrahydrofuran ring of these lignans seems, however, to play a more important role in their anticomplementary activity. Thus, **3**, whose stereochemistry is *trans-cis-trans*, is about 5-fold more active than **2a**, which possesses the same substituents but different stereochemistry (all *trans*). No detectable effect was observed on the AP with these lignans even when they were tested at concentrations of 1000 µM. Because these compounds inhibited selectively the CP pathway and no effect was detected on AP, it is reasonable to think that this selective inhibition could be due either to a chelation of Ca^{2+}, essential ion for the CP activation, or to interactions with one or more complement factors of the CP pathway.

Table 1. Effect of lignans on the CP and AP of the human complement system (concentration causing 50% inhibition of complement activity).

	IC_{50} (µM)	
Lignans	CP	AP
1 (−)-Galbacin	272.6 ± 15.0	>1000
2a (−)-Talaumidin	479.5 ± 38.8	>1000
2b (−)-Talaumidin methylether	104.7 ± 7.3	>1000
2c Acetyl talaumidin	396.7 ± 2.6	ND
3 (+)-Machillin F	83.8 ± 2.6	ND

Dextran sulfate was used as positive control, IC_{50} (CP) = 1.9 ± 0.7 µg/ml, av. mol. wt. 5000. Results are the mean ± SEM of three to six independent experiments; ND = not determined.

Table 2. Effect of lignans on the mitogenic response of human lymphocytes to PHA (concentration causing 50% inhibition of proliferation) and viability of treated lymphocytes.

	T-cell proliferation	
Lignans	IC_{50} (µM)	Viability[a] %
1 (−)-Galbacin	34.1 ± 2.6	96.9
2a (−)-Talaumidin	51.7 ± 5.0	89.9
2b (−)-Talaumidin methylether	23.0 ± 1.1	94.9
2c Acetyl talaumidin	67.4 ± 2.4	ND
3 (+)-Machillin F	101.1 ± 5.0	93.5

Cyclosporin A was used as positive control, IC_{50} = 0.34 ± 0.04 µM. Results are the mean ± SEM of three to six independent experiments. ND = not determined.
[a] Viability of nonstimulated lymphocytes exposed to the concentrations of lignans that cause 50% inhibition of proliferation.

Effect on human lymphocyte proliferation

The effects of lignans **1**, **2a**, **2b**, **2c**, and **3** on the PHA-induced proliferation of human lymphocytes are shown in Table 2. They were found to possess a moderate suppressive effect on the mitogenic response of human lymphocytes to PHA. This antiproliferative effect was shown to be dose-dependent. No toxicity (lymphocytes viability >70%) was observed when the nonstimulated lymphocytes were exposed

Table 3. Effect of lignans on the growth of human tumor cell lines (concentration causing 50% inhibition of cell growth).

Lignans	GI_{50} (μM)		
	MCF-7 (Breast cancer)	TK-10 (Renal cancer)	UACC-62 (Melanona)
1 (−)-Galbacin	44.6 ± 2.9	63.2 ± 8.5	>150
2a (−)-Talaumidin	40.9 ± 9.0	76.8 ± 16.9	76.8 ± 18.4
2b (−)-Talaumidin methylether	101.9 ± 2.5	>150	>150
2c Acetyl talaumidin	32.5 ± 2.2	59.8 ± 2.9	49.4 ± 2.1
3 (+)-Machillin F	83.2 ± 3.8	137.3 ± 5.3	83.8 ± 9.9

Podophyllotoxin was used as positive control, GI_{50} MCF-7 = 8.8×10^{-3} μM; GI_{50} TK-10 = 49.0×10^{-3} μM; GI_{50} UACC-62 = 8.2×10^{-3} μM. Results are the mean ± SEM of three to six independent experiments.

to the IC_{50} concentrations of these lignans (Table 2), which indicates that the suppression of the PHA-induced T-cell proliferation associated with these compounds must be due to a cell growth inhibition rather than to a toxic effect on lymphocytes. The presence of the 3′,4′-dimethoxyphenyl substituent in **2b** was associated with a significantly stronger antiproliferative effect than that of 3′-methoxy-4′-hydroxyphenyl in **2a** ($p < 0.05$) or that of 3′-methoxy-4′-acetoxyphenyl in **2c** ($p < 0.001$). Curiously, (+)-machillin F (**3**), whose stereochemistry is *trans-cis-trans*, presented half the activity of all-*trans* diastereoisomer (−)-talaumidin (**2a**, $p < 0.05$), which emphasizes the importance of the stereochemistry of the ring in these activities.

Effect on the growth of human tumor cell lines

The effect of lignans **1**, **2a**, **2b**, **2c**, and **3** on the growth of the tumor cell lines MCF-7, TK-10, and UACC-62 is summarized in Table 3. Compounds **2a**, **2c**, and **3** showed a mild to moderate inhibitory effect against all the cell lines. However, lignan **1** was found to be moderately active against MCF-7 and TK-10 cell lines but inactive against UACC-62, even when tested at 150 μM. On the other hand, **2b** showed only a poor cytotoxic effect against breast cancer cell line and was completely inactive against renal and melanoma cancer cell lines at the highest concentration tested (150 μM). These growth inhibitory effects were dose-dependent (data not shown) and could not be attributed to a toxic effect, as inferred from the SRB assay (data not shown). This growth inhibitory effect was not clearly influenced by either the nature of the substituents or the stereochemistry of the tetrahydrofuran ring. However, the presence of a methoxy group at 4′ on compound **2b** has caused a significant decrease ($p < 0.05$) of the growth inhibitory effect on the three tumor cell lines, when compared with the hydroxy group in the same position on compound **2a**.

In conclusion, the results of the current study reveal for the 2,5-diaryl-3,4-dimethyltetrahydrofuran lignans moderate but interesting immunomodulatory and antitumor activities, proving that this type of lignan is a promising source of bioactive compounds.

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