Short communication

Synthetic butanolide and tetrahydrofuran lignans with platelet activating factor antagonist activity

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Summary — Several butanolide and tetrahydrofuran lignans were synthesized to be comparatively tested as platelet activating factor (PAF) antagonists. In particular, the influence of the tetrahydrofurans ether oxygen as compared with the γ-lactone system of butanolides was evaluated, showing that the two classes of compounds were practically bioisosters. Molecular modelling and semi-empirical calculation were used to rationalize the collected data.

Résumé — Lignanes butanolidiques et tétrahydrofuraniques de synthèse à activité antagoniste vis-à-vis du facteur d'activation des plaquettes (PAF). Quelques lignanes butanolidiques et tétrahydrofuraniques ont été synthétisés pour être testés comparativement comme antagonistes du facteur d'activation des plaquettes. En particulier, l'influence de l'oxygène de la fonction éther des tétrahydrofurannes a été évaluée en comparaison avec le système gamma-lactonique des butanolides, montrant ainsi que les deux groupes de composés étaient pratiquement bioisostères. L'étude des modèles moléculaires et des calculs semi-empiriques a été utilisée pour rationaliser les données recueillies.

Introduction

Various biological activities have been ascribed to molecules belonging to the lignan class [1], including platelet activating factor antagonist activity which has been repeatedly reported in the last few years [2-4]. In particular for some butanolide lignans and especially for 3,4-disubstituted and 2,3,4,5-tetra-substituted furanoid lignans, anti PAF activities ranging from IC50 800 to 0.6 μM have been observed [4]. More recently synthetic studies based on the natural tetrahydrofuran framework led to trans-2,5-bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran (Merck L-652,731) and trans-2-(3-methoxy-5-methylsulfonyl-4-propoxyphenyl)-5-(3,4,5-trimethoxyphenyl)tetrahydrofuran (Merck L-659,989), which were several times more potent than the natural products [5, 6].

One of the inferences that could be drawn from these reports is that the presence of a tetrahydrofuran instead of a γ-butyrolactone ring is one of the structural requirements for enhancing activity.

With the aim of verifying this particular aspect of the problem, we decided to comparatively test a series
of butanolide lignans and the corresponding tetrahydrofurans in the same biological test set to obtain optimal cross comparison of the activity of these 2 kinds of compound. For this purpose, compounds trans-2,3-bis-(3-hydroxybenzyl)-4-butanolide (1), trans-2,3-bis-(3-methoxybenzyl)-4-butanolide (2), trans-2,3-bis(3,4-dimethoxybenzyl)-4-butanolide (3), trans-2,3-bis(3,4-methoxybenzyl)-4-butanolide (4), trans-2,3-bis(3,4,5-trimethoxybenzyl)-4-butanolide (5), trans-2,3-bis(3,4-methylenedioxybenzyl)-4-butanolide (6), trans-3,4-bis(3-mercaptoxybenzyl)tetrahydrofuran (7), trans-3,4-bis(3,4-dimethoxybenzyl)tetrahydrofuran (8) and trans-3,4-bis(3,4,5-trimethoxybenzyl)tetrahydrofuran (9), were synthesized and tested for their ability to displace 3H-PAF specific binding from isolated rabbit platelets.

**Chemistry**

The synthesis of the butanolide lignan series 1, 2, 3, 4, 5, and 6 was carried out with a ruthenium complex catalyzed hydrogenation of the corresponding Stobbe's fulgenic acids, as recently reported by us [7].

In order to obtain the corresponding tetrahydrofurans, we exploited the ability of γ-Al₂O₃ Ketjen Akzo in dehydrating 1,4 diols to give ring closure to tetrahydrofurans in very high yield.

This procedure was first tried out on a diol obtained as usual by reduction with LiAlH₄ of the corresponding butanolide lignan [8]. To this end 2,3-bis-(3,4,5-trimethoxybenzyl)butane-1,4-diol (10) was prepared from 5. This diol was readily converted to 3,4-bis(3,4,5-trimethoxybenzyl)tetrahydrofuran (9), almost completely in trans form, by heating in toluene in the presence of γ-Al₂O₃. In GC analysis with a programmed temperature vaporizer (PTV), the diol was also prone to dehydration, depending on injection conditions; if PTV was used in cold injection mode, the diol survived almost completely giving a single peak; if the injection was carried out with the PTV hold at 300°C, 2 more peaks appeared together with the diol, totalling 10–15% of the peak area of the diol itself. GC-MS experiments revealed that the 2 additional compounds were the trans and cis isomers of 3,4-bis(3,4,5-trimethoxybenzyl)tetrahydrofuran, since they had the same mass spectra (molecular ion and fragmentation consistent with the proposed structure) [9].

In light of these results, in order to improve the overall synthetic course, we set up an autoclave based procedure affording the above-mentioned tetrahydrofurans from the corresponding butanolides in a single step. In fact, as an alternative to LiAlH₄ reduction, the diols can be conveniently obtained by catalytic hydrogenation of the butanolides over barium-promoted copper chromite at 340 bar and 230°C in toluene [10]. Having also observed that adding γ-Al₂O₃ to the reaction medium had no negative effect on the copper chromite catalyst, we carried out the catalytic hydrogenation and the dehydration of the resulting diols simultaneously. In this way the desired compounds 7, 8 and 9 were prepared in almost quantitative yield from the related butanolides 2, 4 and 5 without further manipulation. The reaction was > 96% stereospecific for the trans form, as observed from ¹H NMR data and routinely verified by GC (scheme 1).

¹H NMR measurements of 7, 8 and 9 in non-polar solvent (CDCl₃) show a complex and well-structured multiplet between 2.48 and 2.70 δ for the diastereotopic benzyl protons; however, on passing from CDCl₃ to the more polar CD₂OD, a strong solvent effect is observed, since the system collapses in a much narrower multiplet (2.50–2.60 δ). Further investigation is currently in progress to elucidate this behaviour.

**Pharmacology**

In figure 1 the displacement curves of butanolide lignans 1, 2, 4 and 5 on ³H-PAF specific binding are shown. All these compounds were able to inhibit ³H-PAF binding in a concentration-dependent fashion. On the other hand, compounds 3 and 6, having different substitution patterns on the phenyl rings,
were found to be almost completely inactive. These results seem to indicate that methoxy-substituted derivatives are generally more potent than the corresponding hydroxylated analogs. Moreover, it can be observed that the receptor activity disappears with methylenedioxy substitution. Consequently, only the tetrahydrofuran analogues 7, 8 and 9, corresponding to the most active 2, 4 and 5 compounds, were prepared for comparative testing. The displacement curves obtained for these tetrahydrofuran analogues are illustrated in figure 2. As clearly shown, the same activity pattern as the corresponding butanolides was observed, indicating that butanolides and tetrahydrofurans are substantially bioisosters as far as PAF receptor antagonism is concerned. In table I the displacement potencies ($K_i$, nM) of all the tested molecules are listed with Merck L652,731 as the reference compound.

### Discussion

A putative conformation of PAF platelet membrane binding site has been proposed by Braquet et al [2, 4, 11]. It is suggested that for good receptor affinity the presence of oxygen bearing highly-available electronic doublets is a strict requirement as for the PAF ether function and this applies both to agonist and antagonist activity. Hydrophobic interactions, as well, seem essential for both agonist and antagonist activity.

According to this hypothesis, the lignan derivatives bearing a tetrahydrofuran ring should have been more potent than the corresponding $\gamma$-butyrolactones, since tetrahydrofuran oxygen is more basic and more likely to undergo protonation [4]. On the other hand, our pharmacological data do not agree with this, showing that tetrahydrofuran and butanolide lignans are practically bioisosters. In fact approximately the same activity is observed in tetrahydrofurans and butanolides having the same substitution patterns of aromatic rings. In order to rationalize these results, molecular modelling was applied to tetrahydrofuran lignans. The structures and the heat of formation with dipole moments were calculated by force field MMPMI (an extended program of Allinger’s MM2 to calculate molecules with force field method) and MOPAC [12] software, respectively, and the same results were obtained for 7, 8 and 9 with the exception of a very slight difference in the angular position of their aromatic rings, due to different substitution. Consequently only the molecular structure of 7, taken as representative of all 3 compounds, are reported in figure 3.

### Table I. Inhibitory effect of butanolide and tetrahydrofuran lignan derivatives on $^3$H-PAF specific binding in rabbit platelets.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_i$ (nM) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$7295 ± 1200$</td>
</tr>
<tr>
<td>2</td>
<td>$2650 ± 525$</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 50 000</td>
</tr>
<tr>
<td>4</td>
<td>$1733 ± 245$</td>
</tr>
<tr>
<td>5</td>
<td>$8750 ± 1670$</td>
</tr>
<tr>
<td>6</td>
<td>&gt; 50 000</td>
</tr>
<tr>
<td>7</td>
<td>$3650 ± 480$</td>
</tr>
<tr>
<td>8</td>
<td>$1700 ± 350$</td>
</tr>
<tr>
<td>9</td>
<td>$5175 ± 1250$</td>
</tr>
<tr>
<td>Merck L-652,731</td>
<td>$25 ± 15$</td>
</tr>
</tbody>
</table>
Fig 3. Calculated molecular structures of 7. H = heat of formation (kcal/mol); DM = dipole moment (Debye).
Our empirical calculations show that the structures of figure 3 are energetically equivalent and that their interconversion involves activation energies not exceeding 1.5–2.0 kcal/mol as observed by computing H values for several of the molecular aspects occurring between a, b and c. It must be noted that one of the proposed structures (fig 3b) is substantially identical to that proposed for a typical butanolide lignan by Cooley et al [13].

We can thus assume that both tetrahydrofuran and butanolide lignans, since they have a non-rigid framework, might behave similarly on the receptorial approach to the PAF binding site, thereby corroborating the observed substantial bioisostery of the 2 classes of compounds.

In order to give a further evaluation of the structure–activity relationship of these molecules, the proposed structures were compared with the Merck L-652,731 reference compound (fig 4) and PAF (fig 5) structures calculated in the same manner.

In the Merck L-652,731 trans form molecule, the 2 trimethoxy-substituted phenyl rings are located at a distance (from the center of the rings) of 3.7–3.6 Å from the tetrahydrofuran oxygen. The line linking the 2 lipophilic areas intersects this oxygen, and the 2 phenyl rings and the tetrahydrofuran ring are nearly co-planar.

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**Fig 4.** Calculated molecular structures of the *trans* and *cis* forms of the Merck L-652,731 reference compound.
areas is maintained, but not the alignment and co-planarity. This isomer is seen to be approximately one thousand times less active than the trans isomer [5].

We can presume, from the above-mentioned assertions, that the basic conditions necessary for a good receptorial lignan antagonist affinity may be the following: the presence of 2 hydrophobic areas with a distance of 3.5–5.0 Å from ring oxygen; the exact alignment of the 2 lipophilic areas and the ring oxygen; the co-planarity of 2 lipophilic areas and the ring; finally, lack of hindrance to the attachment of this oxygen to the receptor site.

Consequently, the minor affinity to the PAF receptor of the lignan derivatives as compared with the trans Merck L-652,731 may be attributed to various concurring factors. The distances between the ring oxygen and the 2 lipophilic areas vary, regarding the calculated conformations (ie, all possible ones from an energetic point of view, from 5.2 to 6.0 Å (fig 3)). The line which connects the 2 lipophilic areas does not, in this case, pass closely over the ring oxygen. Furthermore, co-planarity is observed only in some conformations.

However, the affinity to the receptor can be explained by the presence of oxygen which can provide an electronic doublet with no steric hindrances, and by the presence of 2 areas which are able to form hydrophobic bonds, however weak.

**Experimental protocols**

**Chemistry**

Melting points were determined on a Gallenkamp capillary melting point apparatus. IR spectra were measured in nujol mull with a Perkin-Elmer 983 spectrophotometer linked to a Perkin-Elmer 7500 computer (CDS software). 1H NMR spectra were recorded in CDCl₃ and in CD₂OD with Varian Gemini 200 and Brucker MSL 300 instruments, quoting chemical shifts in δ values. Mass spectrometry utilized a VG Analytical 7070 EQ instrument operating in El mode, with 70 eV ionizing potential and 200–300°C source temperature; solid sampling in ion source was performed with the DEI technique [14]. All compounds were found to be amenable to gas chromatography without derivatization, provided that highly inert fused silica capillary columns and a programmed temperature vaporizer (PTV) were employed: a Perkin-Elmer 8320 instrument equipped with PTV was adopted using a SGE 12QC2/BP1 0.25 column.

Industrial grade γ-Al₂O₃ Ketjen Akzo sticks were used after grinding. Barium promoted copper chromite, Aldrich cat No 20932-5, was used as obtained.

**Synthesis of butanolid lignans (1–6)**

These compounds were obtained as described by us in a previous paper [7].

**Synthesis of 2,3-bis(3,4,5-trimethoxybenzyl)butane-1,4-diol (10)**

The procedure proposed by Schrecker et al [8] was adopted with minor modifications. A solution of 1.1 g of (5) in 50 ml
anhydrous THF was added dropwise over 30 min under stirring to an ice-cooled suspension of 450 mg LiAlH4 in 20 ml THF. The mixture was then refluxed for 75 min. After cooling, the LiAlH4 was carefully quenched with water, acidified with diluted H2SO4 and extracted with Et2O. The ether extract was washed with NaCl-saturated H2O to neutrality and dried over anhydrous Na2SO4. Evaporation of the solvent gave 1.07 g of white crude product. Recrystallization from benzene provided 0.9 g of pure 10, mp = 142.5–143.5°C. IR (nujol): 3504, 1238, 1130, 1040, 998 cm⁻¹. 1H NMR (300 MHz, CDCl3): 1.55 2H, -OH, m (W, 27 Hz); 1.86–1.98 H(2), H(3), m; 2.69–2.85 H(5A), H(6A), H(5B), H(6B), m; 3.590 H(1A), H(4A), dd and 3.855 H(1B), H(4B), dd (JAB = 11.4 Hz, JAJB, and JJA = 4.5 Hz, J2B,3 and J5B,4 = 3.0 Hz); 3.815 6H, -OCH₃, para, s; 2.822 12H, -OCH₃, meta, s; 6.361 4H, Ar-H. MS: M⁺ 450(80), 432(18), 182(85), 181(100) m/z.

**Catalytic synthesis of tetrahydrofurans—general procedure**

Heterogeneous catalytic hydrogenation of butanolide lignans to catalytic synthesis of tetrahydrofurans: general procedure

The solution was recovered by filtration. Monitoring by TLC (silica gel, EtOAc as eluent) gave a single spot, showing that under these conditions (toluene) the reaction by-products were retained in the γ-Al₂O₃. The product indeed crystallized as a white microcrystalline powder (mp = 98–100°C) during the reaction, and the stereospecificity.

**trans-3,4-bis(3,4,5-Trimethoxybenzyl) tetrahydrofuran (9)**

0.5 g of the crude diol 10, obtained as above, were dissolved in 50 ml of dry toluene. To this solution 1.5 g of γ-Al₂O₃ were added and the mixture refluxed for 2 h 30 min. After cooling, the solution was recovered by filtration. Monitoring by TLC and GC in order to ascertain the reaction completeness (75 min). After cooling, the solution was monitored by TLC and GC in order to ascertain the reaction completeness (75 min).

**trans-3,4-bis(3-Methoxybenzyl) tetrahydrofuran (7)**

1.3 g of 2, 1.95 g of copper chromite catalyst and 2.60 g of γ-Al₂O₃ gave in 30 h 1.085 g of 7 (GC, 98% trans) as a viscous colorless oil. IR (neat): 1262, 1166, 1154, 1044 cm⁻¹. 1H NMR (300 MHz, CDCl3): 2.09–2.62 H(3), H(4), m; 2.50–2.70 H(6A), H(7A), H(6B), H(7B), m; 3.520 C(2A), H(5A), dd and 3.940 H(2B), H(5B), dd (JAB = 8.7 Hz, J2B,3 and J5B,4 = 6.3 Hz, J2B,3 and J5B,4 = 6.6 Hz); 3.783 6H, -OCH₃, met, s; 6.63–7.21 8H, Ar-H. MS: M⁺ 372(40), 122(100), 121(90) m/z.

**Pharmacology: binding of ³H-PAF to rabbit platelets**

Experiments were performed according to the method described by Nunez et al [15]. Male New Zealand rabbits supplied by Charles River (Calco, Italy) were used. Animals (body weight = 2.0 kg) were kept in our animal care unit for at least 7 days before experiments. Heparinized rabbits (500 IU/animal) were used and blood was collected by intracardiac puncture. ACD buffer (citric acid 0.8%) trisodic citrate 2.2%, glucose 2.45%) was added to collected blood in the ratio 1:1. Blood was centrifuged at 100 g for 15 min, the platelet-rich plasma (PRP) was acidified to pH 6.5 with 0.15 M citric acid and centrifuged at 900 g for 15 min. The platelet was then carefully resuspended in Tyrode buffer, pH 7.5, containing 1.3 mM CaCl₂ and 0.25% bovine serum albumin. Binding studies were performed in 1 ml of Tyrode buffer containing ³H-PAF 0.4 Nm (50 000 dpm), platelet suspension (8 x 10⁷ cells) and 0.1 ml of test drug solutions. Non specific binding was estimated in the presence of 0.5 M of labelled PAF. After 30 min of incubation at 37°C, the filter was washed with Whatman GF/C filters, washed twice with 5 ml ice-cooled Tyrode buffer and the radioactivity on the filters was measured in 20 ml of Filtercount cocktail (Packard) through a Packard TRI-CARB 4530 spectrometer with an efficiency of 60%. In this study, cold PAF and the specific PAF antagonist Merck L 652,731 were included as reference compounds. The displacement capacity of drugs was expressed as ³H-PAF binding inhibition and as Ki values calculated from Cheng and Prusoff’s equation [16]:

\[
K_i = \frac{IC_{50}}{1 + [³H-PAF]} - K_D
\]

where \(K_D\) for ³H-PAF was estimated by Scatchard analysis [17].

Cold PAF was obtained from Sigma, USA. Compound Merck L 652,731 was synthesized by the Department of Chemistry, Zambon Group Research Laboratories (Bresso-Milan, Italy). The other reagents were from commercially available sources.

**Acknowledgment**

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