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# Novel phomactin analogues as PAF receptor ligands

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**Abstract**—A range of natural and unnatural phomactins, recently synthesised in our laboratory, were found to exhibit PAF antagonism with $pIC_{50}$ values in the range 5.6–6.2. The variation in structural and stereochemical features between the phomactins was found to have only a modest effect on the inhibition of binding of PAF to its human platelet receptors.

Platelet activating factor (PAF) is a naturally occurring ether phospholipid (1-*O*-alkyl-2*R*-acetylglycerol-3-phosphorylcholine), which regulates important biological functions such as platelet aggregation, vascular permeability, smooth muscle contraction and hypotension. PAF is also implicated in a number of inflammatory and respiratory diseases (e.g., asthma), and in organ transplant rejection.[1] Inhibition of PAF binding to its receptor, or inhibition of its production therefore offers a potential treatment for some of these debilitating conditions.

The ‘phomactins’ are a family of oxygenated diterpene natural products based on a core bicyclo[9.3.1]pentadecane ring system isolated from the marine fungus *Phoma* sp.[2] This novel group of diterpenes has been found to be active as PAF antagonists, inhibiting PAF-induced platelet aggregation and binding of PAF to its receptors. Within this family of natural products phomactin D (**4**) has been shown to exhibit the highest level of PAF antagonism.[2d] Other diterpenes such as ginkgolides A–C, isolated from the Chinese tree *Ginkgo biloba* L.,[3] and forskolin, isolated from the roots of *Coleus forskholii*,[4] as well as several lignans, for example, kadsurenone isolated from the plant *Piper futokadsurae*,[5] also exhibit PAF antagonist activity.

We have had a long-standing interest in these structurally unique and biologically important natural products which, recently, culminated in the first total syntheses of phomactins A (**1**) and G (**3**),[6] as well as a number of related unnatural phomactins.[7] It is likely the biosynthesis of phomactin A involves the enzymatic oxidation of phomactin G to the epoxy alcohol **2**, which has not yet been isolated (Fig. 1).[6c] Furthermore, phomactins D and G share an interesting structural homology and are related by tautomerisation, isomerisation and cyclisation steps. With a wide range of both natural and unnatural phomactins available, we have now studied

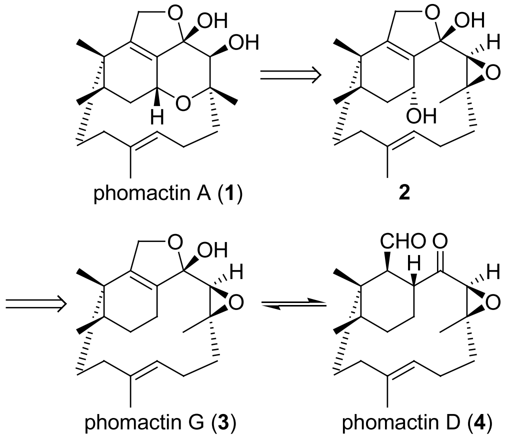

**Figure 1.** Likely biogenetic relationships between natural phomactins A (**1**), G (**3**) and D (**4**).

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*Keywords*: Phomactin; Platelet activating factor; PAF; Antagonist; Diterpene.

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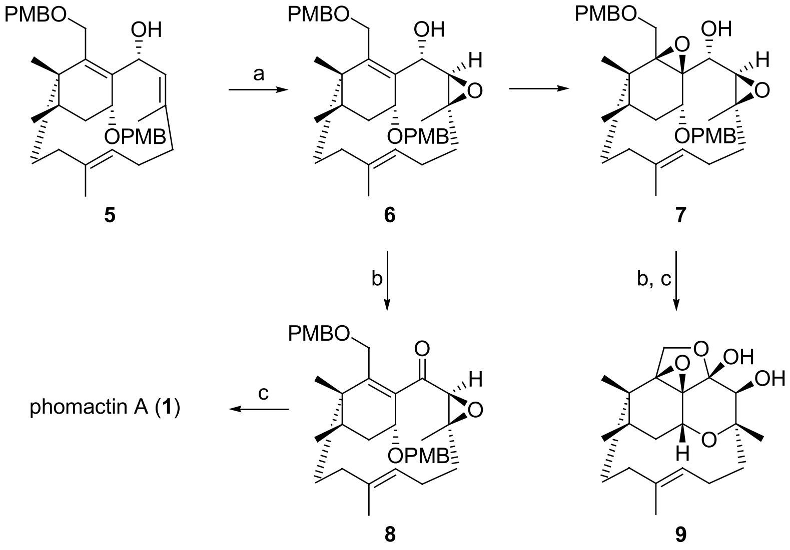

**Scheme 1.** Reagents and conditions: (a) VO(acac)$_2$, *t*-BuOOH, PhH, rt, 86%, ca. 1:1 (**6** and **7**); (b) Dess–Martin periodinane, NaHCO$_3$, CH$_2$Cl$_2$, 0 °C to rt, 80–99%; (c) DDQ, CH$_2$Cl$_2$–H$_2$O (18:1), 0 °C to rt, 62–83%.

their relative PAF antagonistic activities in order to secure more detail on the structural and stereochemical features required of these compounds for maximum inhibition.[8]

During our synthetic investigation towards phomactin A (**1**) we also prepared its epoxide **9**. Treatment of the known bicyclic alcohol **5**[6b] with VO(acac)$_2$ and *t*-BuOOH gave the β-orientated epoxide **6** accompanied by the corresponding bis-epoxide **7**, which were produced in equal amounts and in 86% overall yield (Scheme 1). To complete our synthesis of phomactin A (**1**) we oxidised the secondary alcohol in **6**, using Dess–Martin periodinane, and then treated the resulting epoxyketone **8** with DDQ in CH$_2$Cl$_2$–H$_2$O at 0 °C, which resulted in simultaneous deprotection of both the primary and secondary PMB ether groups followed by spontaneous cyclic hemiacetal and pyran ring formation leading directly to (±)-phomactin A (**1**) in 83% yield. Using a sequence of steps identical to the conversion of **6** into **1** via **8**, the bis-epoxide **7** was converted into the epoxide **9** of phomactin A in 62% overall yield.

In a similar manner, and from related intermediates, cf. **5**, the unnatural phomactins **10**–**13** were synthesised (Fig. 2). For example, we prepared the epoxy alcohols **10** and **11**,[7] which are hydroxy and epoxy diastereoisomers, respectively, of the possible biogenetic intermediate **2**. Furthermore, the epimeric epoxide **12**, and regioisomeric epoxide **13**, of phomactin G (**3**) were also synthesised.[6c,9] We have taken these novel phomactins and evaluated their capacity to compete for [$^3$H]-PAF binding to human platelets in comparison with phomactins A (**1**) and G (**3**).

To achieve this objective human platelets were used to examine affinity at the human PAF receptor (Table 1), following the method of Tahraoui et al.[10] Thus, blood was obtained from informed consent volunteers and platelet-rich plasma was prepared. After washing and lysis cycles with homogenisation and centrifugation,

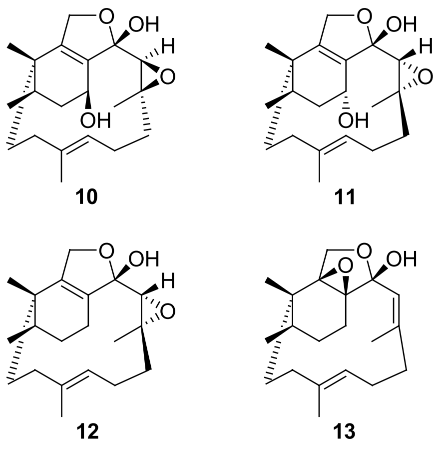

**Figure 2.** Unnatural synthetic phomactins.

**Table 1.** Potency of phomactin analogues at human PAF receptors

| Compound | pIC$_{50}$ (mean ± range)[a] | Hill Slope (mean ± range)[a] |
|---|---|---|
| Phomactin A **1** | 5.90 ± 0.06 | −0.54 ± 0.08 |
| **9** | 5.63 ± 0.09 | −0.62 ± 0.01 |
| **10** | 5.84 ± 0.13 | −3.34 ± 1.68 |
| **11** | 5.95 ± 0.34 | −2.41 ± 1.51 |
| Phomactin G **3** | 6.24 ± 0.39 | −0.55 ± 0.18 |
| **12** | 6.24 ± 0.19 | −2.13 ± 0.88 |
| **13** | 5.94 ± 0.07 | −0.88 ± 0.25 |
| CV6029 | 8.32 ± 0.09 | −1.12 ± 0.36 |
| mc-PAF | 8.44 ± 0.08 | −0.77 ± 0.06 |

[a] Potency and Hill slope determinations of competition analysis at [$^3$H]-PAF receptors. Data are means ± SEM of 2–6 separate experiments, conducted in duplicate.

platelet membranes were incubated at 4 °C for 90 min in the presence of 0.3–1.3 nM [$^3$H]-PAF (6.48 TBq/mmol, Amersham Pharmacia Biotechnology, Herts, UK) 0.5% fatty acid-free bovine serum albumin, 50 mM Tris, 5 mM $MgCl_2$ and 1 mM EDTA (pH 7.4) in a total volume of 0.5 mL. Bound radioligand was harvested by rapid filtration over Whatman GF/C filters and washed with ice-cold buffer (0.1% bovine serum albumin in 50 mM Tris, 1 mM EDTA, pH 7.4). $pIC_{50}$ values (−log of $IC_{50}$ values) were calculated using Prism (GraphPad, California, USA), fitting four parameter logistic equations to model the data. Nonspecific binding was defined as that remaining in the presence of 1 μM CV6029.

The phomactin analogues **9**–**13** were all found to compete for [$^3$H]-PAF binding to human platelet membranes in a concentration-dependent manner (Table 1). The rank order of potency in comparison with phomactin A (**1**) and phomactin G (**3**) was found to be **3** = **12** > **13** = **11** > **1** > **10** > **9**.

The reference compounds displayed potencies consistent with previous reports of CV6029 and mc-PAF affinity.[10] In comparison with the reference compounds, the phomactins were less potent and also showed generally more variable Hill slopes. It is quite likely that this feature may simply reflect either solvent effects (initially dissolved to $10^{-3}$ M in 100% methanol) or the lower solubility of the compounds compared to the reference compounds.

In general, the narrow range of potencies of the phomactin analogues ($pIC_{50}$ values of 5.63–6.24) is indicative of a lack of major impact of structural changes on the affinity for phomactins at the human PAF receptor. However, a comparison between the relative potency of the natural phomactins **1** and **3**, and their unnatural analogues **9**–**13**, might reflect the importance of specific structural and stereochemical features towards the inhibition of binding of PAF to its receptor. For example, modification of phomactin A (**1**), via epoxidation of the tetrasubstituted carbon–carbon double bond, leading to **9** resulted in a decrease in the relative potency of the latter. This decrease in relative potency was also reflected in the epoxide regioisomer **13** of phomactin G (**3**). The comparable potency measured for phomactin G (**3**) and its epoxide diastereoisomer **12** suggests that the stereochemistry of the epoxide played a minor role in the overall potency of these compounds. The higher potency of **12**, relative to its hydroxy analogue **11**, suggests the importance of this hydroxyl in the activity of the phomactins. It then stands to reason that the lower potency of the β-secondary alcohol **10**, in comparison to its α-hydroxy diastereoisomer **11**, reflects the importance of the stereochemistry of this alcohol, over that of the epoxide, cf. **3** and **12**. Furthermore, it is interesting to note that the epoxy alcohol **10**, which possesses the unnatural β-orientated alcohol, is less potent than **11**, and quite possibly phomactin A (**1**).

In conclusion, we have examined a range of natural and unnatural phomactins for their relative binding inhibition of PAF to its receptor on human platelets. These studies highlight the importance of structural and stereochemical features and their relationship to the biological activity of this important family of natural products.

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## Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bmcl.2005.04.048.

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