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# Platelet-activating factor (PAF) receptor binding antagonists from *Alpinia officinarum*

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**Abstract**—The bioassay-guided purification of ether extracts of *Alpinia officinarum* led to the isolation of two new compounds 6-hydroxy-1,7-diphenyl-4-en-3-heptanone (**1**) and 6-(2-hydroxy-phenyl)-4-methoxy-2-pyrone (**4**) as well as three known compounds 1,7-diphenyl-4-en-3-heptanone (**2**), 1,7-diphenyl-5-methoxy-3-heptanone (**3**), and apigenin (**5**). Their structures were established on the basis of spectral methods. All three diarylheptanoids **1**, **2**, and **3** exhibited potent PAF receptor binding inhibitory activities with an $IC_{50}$ of 1.3, 5.0, and 1.6 μM, respectively. These studies have identified diarylheptanoids as a novel class of potent PAF antagonists.


Platelet-activating factor (PAF, 1-*O*-alkyl-2(*R*)-acetyl-glyceryl-3-phosphorylcholine) is an endogenous phospholipid inflammatory mediator. Since the discovery of platelet-activating factor (PAF) in 1972 and its first synthesis in 1979, it has been recognized that PAF plays a wide range of physiological and pathological roles. Inflammatory cells such as alveolar macrophage, eosinophils, platelets, and neutrophils generate PAF in response to inflammatory and immune stimuli. Then PAF acting on specific G-protein-coupled receptors results in a series of biological responses including increased vascular permeability, hemoconcentration, hypotension, ulcerogenesis, bronchoconstriction, triggering of airway hyperresponsiveness, and platelet degranulation. These proinflammatory activities indicate that PAF could be an important mediator in a wide range of pathological conditions that have an inflammatory component. These would include septic shock, asthma, ischemia/reperfusion injury, pancreatitis, inflammatory bowel disease, and rhinitis.[1]

In our previous work, a series of medicinal plants which have been used to treat PAF related diseases were collected and screened for PAF receptor binding inhibitory effects. Among the screened plants, *Alpinia officinarum* showed significant inhibitory effects on the platelet-activating factor (PAF) receptor binding.[2]

*Alpinia officinarum*, as a folk medicine with local drug names: Gao-liang-jiang, Go-ryang-gang, is widely used to treat epigastric pains, nausea, indigestion, gastritis, gastric and duodenal ulcer, gastroenteritis, and tinea versicolor infection.[3] Diarylheptanoids were one of the main chemical constituents found in the rhizome of *A. officinarum*, including 7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3,5-heptadione, 5-hydroxy-7-(4″-hydroxyphenyl)-1-phenyl-3-heptenone, 5-methoxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptenone;[4] 7-(4″-hydroxyphenyl)-1-phenyl-4-en-3-heptanone, 5-methoxy-1,7-diphenyl-3-heptanone, 5-methoxy-7-(4″-hydroxyphenyl)-1-phenyl-3-heptenone;[5] octahydrocurcumin, (3*R*,5*R*)-1-(4-hydroxyphenyl)-7-phenylheptane-3,5-diol;[6] *trans*,-*trans*-1-(3′-methoxy-4′-hydroxyphenyl)-7-phenyl-5-ol-4,6-dien-3-heptanone.[7] Other chemical constituents such as phenylpropanoids,[8] neolignans[9] have also been reported.

In an attempt to identify potent and novel PAF antagonist from medicinal plants, the bioassay-guided isolation and purification of the extracts of *A. officinarum* led to two new 6-hydroxy-1,7-diphenyl-4-en-3-heptanone (**1**) and 6-(2-hydroxy-phenyl)-4-methoxy-2-pyrone (**4**) together with three known diarylheptanoids **2**, **3** and flavonoid **5** (Fig. 1).

*Keywords*: *Alpinia officinarum*; Platelet-activating factor (PAF); Antagonist; Diarylheptanoid.
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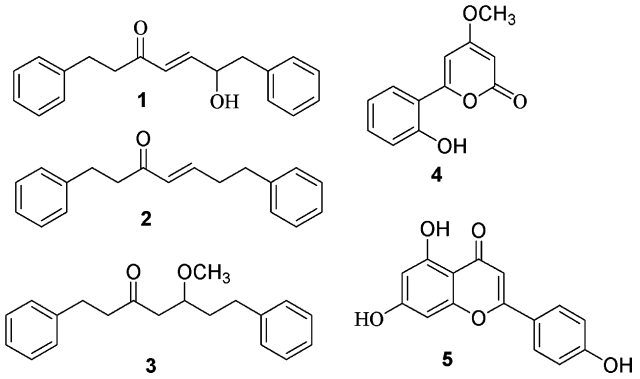


**Figure 1.** Compounds isolated from *Alpinia officinarum*.

Diethyl ether extracts of *A. officinarum* were subjected to silica gel column chromatography eluted with hexane/EtOAc (50:1 in volume) to give eight fractions. All fractions were evaluated for their PAF receptor binding inhibitory activities according to the method of Valone with some modification.[10] The fraction which showed the most significant inhibitory activities with 69% inhibitory effect at a concentration of 200 μg/mL was applied to column chromatography (hexane/EtOAc 10:1 in volume) to afford eight sub-fractions. The sub-fractions showing 66% and 82% inhibitory effect at a concentration of 200 μg/mL, respectively, were subjected to repeated column chromatography. Three diarylheptanoids **1**, **2**, and**3** were isolated from the most significantly inhibitory (82%) sub-fraction, and compounds **4** and **5** were obtained from the 66% inhibitory sub-fraction.

Compound **1** was isolated as colorless oil. The EI-mass spectra of compound **1** showed a molecular ion peak at *m/z* 280 ($M^+$) shifting 16 mass units relative to **2** and the molecular formula was determined to be $C_{19}H_{20}O_2$. The IR spectra showed the presence of hydroxyl group (3550 $cm^{-1}$) as well as a α,β-unsaturated carbonyl group (1690 and 1626 $cm^{-1}$). The NMR spectra of **1** corresponded closely with those of compound **2**. Besides showing the two phenyl groups {$\delta$ 7.18–7.40 (m, 10H)}, the $^1H$ NMR spectra also showed the conjugated double bond {$\delta$ 6.32 (dd, $J$ = 1.5, 15.9 Hz, 1H), 6.84 (dd, $J$ = 4.8, 15.9 Hz, 1H)}, however, both peaks of the alkene protons showed double doublets instead of double triplets as those of compound **2**. That means the hydroxyl group located at C-6 position. The whole structure was further confirmed by the fragment ions *m/z* 189 $[M-PhCH_2]^+$ and 159 $[M-PhCH_2CHOH]^+$ in the mass spectrum. The structure of **1** was therefore assigned as 6-hydroxy-1,7-diphenyl-4-en-3-heptanone.[11]

Compound **4** was isolated as light yellow powder. The IR spectrum of compound **4** showed the presence of hydroxyl group (3580 $cm^{-1}$) and conjugated lactone carbonyl group (1645 $cm^{-1}$). The NMR showed a disubstituted phenyl group {$\delta$ 8.26 (ddd, $J$ = 0.6, 1.5, 8.4 Hz, 1H), 8.01 (ddd, $J$ = 0.6, 1.2, 8.4 Hz, 1H), 7.67 (ddd, $J$ = 1.5, 6.6, 8.1 Hz, 1H), 7.43 (ddd, $J$ = 1.2, 6.6, 8.4 Hz, 1H)} and methoxyl group {$\delta$ 4.42 (s, 3H)}. The position of methoxy group at C-4 was confirmed by the correlation between methoxy group and H-3 [$\delta$ 7.05 (d, $J$ = 2.7 Hz, 1H)] and H-5 [$\delta$ 7.61 (d, $J$ = 2.7 Hz, 1H)] in NOESY spectra. The structure of **4** was therefore assigned as 6-(2-hydroxy-phenyl)-4-methoxy-2-pyrone.[12]

Compounds **2**, **3**, and **5** were identified as 1,7-diphenyl-4-en-3-heptanone,[13] 1,7-diphenyl-5-methoxy-3-heptanone,[5] and apigenin, respectively, by spectral analysis and comparison of their physical and spectral data with those of reference data. Of the five isolated substances, all three diarylheptanoids **1**, **2**, and **3** showed potent PAF receptor binding antagonistic activities with an $IC_{50}$ value of 1.3, 5.0, and 1.6 μM, respectively. The inhibitory activities of compounds **4** and **5** have not been evaluated.

In recent year, a series of potent PAF antagonists with a wide variety of structural types including synthetic PAF analogues,[14] biphenylcarboxamide,[15] *N*-(acyloxyalkyl) pyridinium salts,[16] 3-acylindole imidazopyridine,[17] and naturally occurring Ginkgolide B[18] have been reported. Several potent PAF antagonists have also been isolated by our group, such as Pinusolide[19] from *Biota orientalis* and diacylglycolipid[20] from *Kalimeris indica*. The diarylheptanoids that we have identified represent a novel structural class of potent PAF receptor binding inhibitory compounds which can be used as leading compounds for further structure–activity studies.

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bound $^{3}H$-PAF in the absence and presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. In a set of experiments, $^{3}H$-PAF was incubated with different concentrations of sample, and the inhibitory effect of the sample on the specific binding is expressed as percent inhibition of the control. The $IC_{50}$ value of a sample was defined as the final concentration of the sample required to block 50% of the specific $^{3}H$-PAF binding to rabbit platelet receptors.

11. Compound **1**: IR: 3550, 1690, 1626, 1480, 1168 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 7.18–7.40 (m, 10H), 6.84 (dd, $J$ = 4.8, 15.9 Hz, 1H), 6.32 (dd, $J$ = 1.5, 15.9 Hz, 1H), 4.55 (br m, 1H), 2.90 (m, 6H); $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 199.3, 146.7, 141.0, 136.7, 129.4 (2C), 128.7 (2C), 128.4 (2C), 128.3 (2C), 128.2, 127.0, 126.1, 71.8, 43.3, 42.4, 29.9; EI-MS *m/z* 280 (M+), 189, 159, 103, 91; HR-MS Calcd for $C_{19}H_{20}O_2$ 280.1463, found: 280.1460.
12. Compound **4**: mp 135–137 °C; IR: 3580, 1645, 1558, 1493, 1250, 1050 $cm^{-1}$; $^{1}H$ NMR ($CDCl_3$, 300 MHz) $\delta$ 8.26 (ddd, $J$ = 0.6, 1.5, 8.4 Hz, 1H), 8.01 (ddd, $J$ = 0.6, 1.2, 8.4 Hz, 1H), 7.67 (ddd, $J$ = 1.5, 6.6, 8.1 Hz, 1H), 7.61 (d, $J$ = 2.7 Hz, 1H), 7.43 (ddd, $J$ = 1.2, 6.6, 8.4 Hz, 1H), 7.05 (d, $J$ = 2.7 Hz, 1H), 4.42 (s, 3H). $^{13}C$ NMR ($CDCl_3$, 75 MHz) $\delta$ 163.7, 156.5, 145.1, 144.6, 129.8, 127.6, 123.9, 122.3, 118.2, 105.7, 103.6, 59.7; HR-MS Calcd for $C_{12}H_{10}O_4$ 218.0579, found: 218.0573.
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