In Vitro Inhibitory Effect of Rubraxanthone Isolated from *Garcinia parvifolia* on Platelet-Activating Factor Receptor Binding

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Abstract

Rubraxanthone and isocowanol isolated from *Garcinia parvifolia* Miq. were investigated for their inhibitory effects on platelet-activating factor (PAF) binding to rabbit platelets using ^3H-PAF as a ligand. Rubraxanthone showed a strong inhibition with IC_{50} value of 18.2 μM. The IC_{50} values of macluraxanthone, 6-deoxyjacareubin, 2-(3-methylbut-2-enyl)-1,3,5-trihydroxyxanthone, 2-(3-methylbut-2-enyl)-1,3,5,6-tetrahydroxyxanthone and 1,3,5-trihydroxy-6,6′-dimethylpyrano(2′,3′:6,7)-4-(1,1-dimethylprop-2-enyl)-xanthone were also determined for comparison. In the course of our study on structure-activity relationship of xanthones, the results revealed that a geranyl group substituted at C-8 was beneficial to the binding while a hydroxylated prenyl group at C-4 resulted in a significant loss in binding to the PAF receptor.

Platelet-activating factor (PAF) has been reported to be involved in many pathological conditions such as inflammation [1], allergy [2], asthma [3] and thrombosis [4]. Compounds which inhibit the specific binding between PAF and receptors found in a variety of cell membranes including those from platelets, have been extensively sought to be used as leads in the development of therapeutic agents in a variety of inflammation, respiratory, immunological, and cardiovascular disorders.

The methanol extract of the bark of *Garcinia parvifolia* Miq. (Guttiferae) showed a strong inhibitory effect on platelet-activating factor receptor binding *in vitro*. In this paper, we report on the effects of rubraxanthone and isocowanol isolated from the plant, on the binding of ^3H-PAF to washed rabbit platelets.

Inhibitory effects of xanthones isolated from *Garcinia parvifolia* on PAF receptor binding to platelets at various concentrations and their IC_{50} values are compared with those of xanthones which were isolated from other Guttiferae species as described previously [5], [6] (Table **1**). The IC_{50} values of the latter xan-

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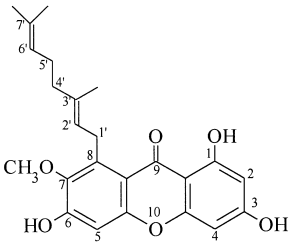
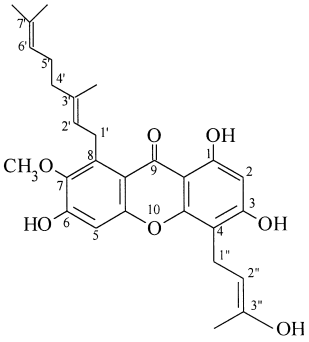
Received: April 5, 2002 · **Accepted:** July 13, 2002

Bibliography: Planta Med 2002; 68: 1133–1134 · © Georg Thieme Verlag Stuttgart · New York · ISSN 0032-0943



Rubraxanthone



Isocowanol

thones have also been determined by Jantan et al. previously [7]. The compounds showed dose-dependent responses, i.e., as the concentration of the compound increased the % inhibition increased. Among them, rubraxanthone showed strong inhibition of PAF receptor binding, with inhibitory effect of 76% at 18.2 μg/ml and with an IC_{50} value of 18.2 μM. The IC_{50} value of rubraxanthone was comparable to those of most of the xanthones studied but slightly higher than that of the positive control, cedrol [7].

The results in Table **1** are in agreement with the previous structure-activity relationship study which revealed that a prenyl group at C-2, a dimethylprop-2-enyl group at C-4 and a hydroxy group at C-5 were all beneficial to the binding of xanthones to the PAF receptor, while a hydroxy group at C-6 and a hydroxylated prenyl group at C-3 resulted in significant loss in binding [7]. This study also showed that the presence of a geranyl group substituted at C-8 (rubraxanthone) was preferable in binding to the receptor, indicating a possible strong hydrophobic interaction. However, the presence of a hydroxylated prenyl group at C-4 in addition to the geranyl group at C-8 (isocowanol) significantly increased the IC_{50} values by 12.5 fold, indicating a significant loss in binding to the PAF receptor. The results further revealed that xanthones can represent a new class of natural products that can bind strongly to PAF receptor.

Table **1** % Inhibition by xanthones on PAF receptor binding to platelets at various concentrations and their IC_{50} values

Compound	*18.2 (µg/ml)*	*9.1*	*4.5*	*1.8*	*IC50 (µM) (mean ± S.D.)*
Rubraxanthone	76*	56	35	13	18.2 ± 2.1
Isocowanol	15	11	4	1	227.8 ± 3.4
Macluraxanthone	84**	58	36	19	15.8 ± 0.6
2-(3-Methylbut-2-enyl)-1,3,5-trihydroxyxanthone	77**	57	47	21	20.9 ± 0.7
2-(3-Methylbut-2-enyl)-1,3,5,6-trihydroxyxanthone	63	42	29	18	35.2 ± 2.3
6-Deoxyjacareubin	75*	53	40	21	22.1 ± 0.8
1,3,5-Trihydroxy-6,6'-dimethyl-pyrano-(2',3':6,7)-4-(1,1-dimethyl-prop-2-enyl)xanthone	68	50	25	17	24.2 ± 1.9
Cedrol	75	66	56	47	10.6 ± 1.2

Data represent mean ± SD of three independent experiments performed in triplicate; *P < 0.05, **P < 0.01 as compared with cedrol (Student's t test).

Materials and Methods

The bark of *Garcinia parvifolia* was collected from West Sumatra, Indonesia and a voucher specimen (no: MT02) was deposited at the Herbarium of Universitas Andalas, Padang, Indonesia. Rubraxanthone (0.006%) and isocowanol (0.0002%) were isolated as pure compounds from *Garcinia parvifolia* by chromatography on silica gel (200 – 300 mesh) using *n*-hexane-EtOAc gradients of increasing polarities at 9 : 1, 7 : 3, 1 : 3, 3 : 7 and 1 : 9. The compounds were identified by spectroscopic techniques and by comparison with published data [8]. Macluraxanthone, 6-deoxyjacareubin, 2-(3-methylbut-2-enyl)-1,3,5-trihydroxyxanthone, 2-(3-methylbut-2-enyl)-1,3,5,6-tetrahydroxyxanthone and 1,3,5-trihydroxy-6,6′-dimethylpyrano(2′,3′:6,7)-4-(1,1-dimethylprop-2-enyl)-xanthone were isolated from *Calophyllum inophyllum* and *G. opaca* as described previously [5], [6]. Radiolabeled PAF (1- *O*-^{3}*H*-octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine, 125 Ci/mmol) was purchased from Amersham (UK). Unlabeled PAF and cedrol were obtained from Sigma Chemical Co. (U.S.A.). The PAF receptor binding inhibitor assay on the xanthones was carried out using washed rabbit platelets according to the modified method of Valone [9]. The reaction mixture consisted of 200 μl of washed rabbit platelet suspension, 25 μl of ^{3}*H*-PAF (2.0 nM) with or without unlabeled PAF (2.0 μM) and 25 μl of sample or control solution. The final concentrations of sample in the reaction mixtures were either 18.2, 9.1, 4.5, 1.8 or 0.9 μg/ml. Cedrol, a known PAF receptor antagonist was used as a positive control in the bioassay [7]. The reaction mixture was incubated at room temperature for 1 h. The free and bound ligands were separated by filtration technique using Whatman GF/C glass fiber filters. The radioactivity was measured by scintillation counting. The difference between total radioactivities of bound ^{3}*H*-PAF in the absence and the presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. The specific binding is expressed as percent inhibition of the control. The IC_{50} values of the samples were obtained from at least duplicate determinations.

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