Supporting Information

Lignan Derivatives from *Krameria lappacea* Roots Inhibit Acute Inflammation in Vivo and Pro-inflammatory Mediators in Vitro

*Lisa Baumgartner,[†] Silvio Sosa,[‡] Atanas G. Atanasov,[§] Antje Bodensieck,[⊥] Nanang Fakhrudin,[§] Julia Bauer,[∥] Giorgia Del Favero,[‡] Cristina Ponti,[‡] Elke H. Heiss,[§] Stefan Schwaiger,[†] Angela Ladurner,[c] Ute Widowitz,[⊥] Roberto Della Loggia,[‡] Judith M. Rollinger,[†] Oliver Werz,[∥,∆] Rudolf Bauer,[⊥] Verena M. Dirsch,[§] Aurelia Tubaro,[‡] Hermann Stuppner[†,]**

[†]Institute of Pharmacy/Pharmacognosy and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Josef-Moeller-Haus, Innrain 52c, 6020 Innsbruck, Austria.

[‡] Department of Life Sciences, University of Trieste, Via A. Valerio 6, 34127 Trieste, Italy

[§]Department of Pharmacognosy, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

[⊥]Institute of Pharmaceutical Sciences, Pharmacognosy, Karl-Franzens University Graz, Universitätsplatz 4, 8010 Graz, Austria

[∥]Department of Pharmaceutical Analytics, Pharmaceutical Institute, Eberhard-Karls-University Tuebingen, Auf der Morgenstelle 8, 72076 Tuebingen, Germany

[∆]Department of Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Friedrich-Schiller-University Jena, 07743 Jena, Germany

*Corresponding author: Tel.: 0043-(0)512-507-5300. Fax: 0043-(0)512-507-2939. E-mail: Hermann.Stuppner@uibk.ac.at

1. IKK2 activity:

To measure the IKK2 activity we utilized the ELISA-based (K-LISA™) IKK2 enzymatic activity assay (Calbiochem, VWR, Austria) as previously described [1]. GST-IκBα 50-amino acid peptide that includes Ser32 and Ser36 IKK2 phosphorylation sites, is used as a substrate and incubated for 30 min at 30 °C with purified human IKK2 in a glutathione-coated 96-well plate, allowing substrate phosphorylation and capture in a single step. The phosphorylated GST-IκBα substrate was subsequently detected using anti-phospho IκBα (Ser32/Ser36) as first antibody, followed by the HRP-conjugated secondary antibody. The color development of the HRP substrate was monitored at 450 nm on a GeniosPro plate reader (Tecan, Austria) and the absorbance intensity was used as a measure for the IKK2 activity.

2. PPARα-, PPARγ- and GR-activation:

To estimate PPAR activation potential, PPAR-luciferase reporter gene assay was utilized as previously described [2]. HEK293 cells were transiently transfected with PPARα, PPARγ, or GR receptor expression plasmid, reporter plasmid (tk-PPREx3-luc, or 2xGRE-tk-luc, respectively), and green fluorescent protein plasmid (pEGFP-N1) as an internal control. The cells were harvested 6 h after the transfection and reseeded in 96-well plates (5×10^4 cells/well). Transfected cells were treated with the investigated compounds, dissolved in DMSO. To account for potential effects of the solvent vehicle 0.1% DMSO served as vehicle control. As positive controls 50 nM GW7647, 5 µM troglitazone, or 2.5 µM dexamethasone were used to activate PPARα, PPARγ, or GR respectively. Treated cells were incubated for 18 h. After cell lysis, the luminescence of the firefly luciferase and the fluorescence of EGFP were quantified on a GeniosPro plate reader (Tecan, Salzburg, Austria). The luminescence

signals were normalized to the EGFP-derived fluorescence to account for differences in cell number or transfection efficiency.

3. Free radical scavenging activity:

To determine free radical scavenging activity in a cell free system a 96-well format DPPH assay was performed. DPPH was dissolved in 80% ethanol and used in a final concentration of 250 μM per well. The test samples were first dissolved in 100 % DMSO and diluted to 0.335% DMSO in 200 μl overall assay volume. Absorbance was measured at 550 nm after 30 min in a Tecan/SUNRISE™ photometer. The known radical scavenger ascorbic acid was used as a positive control.

[1] Noha, S.; Atanasov, A. G.; Schuster, D.; Markt, P.; Fakhrudin, N.; Heiss, E. H.; Schrammel, O.; Rollinger, J. M.; Stuppner, H.; Dirsch, V. M.; Wolber, G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 577-583.

[2] Fakhrudin, N.; Ladurner, A.; Atanasov, A. G.; Heiss, E. H.; Baumgartner, L.; Markt, P.; Schuster, D.; Ellmerer E.-P.; Wolber, G.; Rollinger, J..M.; Stuppnner, H.; Dirsch, V. M. *Mol. Pharmacol.* **2010**, *77*, 559-566.

Table S1. Effect of Compounds **5** and **7**, Indomethacin and Hydrocortisone on the Global Edematous Response and Leukocyte infiltrate up to 48 h.

substance	dose (μmol/cm^2)	edema AUC (mg x h) Mean ± S.E.	% reduction	cell infiltrate AUC (EU x h) Mean ± S.E.	% reduction
control	--	136.6 ± 4.0	--	1659.7 ± 87.6	--
5	0.40	72.6 ± 6.3*	47	1129.3 ± 56.5*	32
7	0.40	75.6 ± 2.9*	45	1041.8 ± 53.0*	37
indomethacin	0.40	103.3 ± 3.8*	24	967.7 ± 48.1*	42
hydrocortisone	0.04	42.8 ± 2.4*	69	815.4 ± 44.9*	51

AUC = area under the curve; EU = enzyme units; * $p<0.05$ at the analysis of variance, as compared to controls.

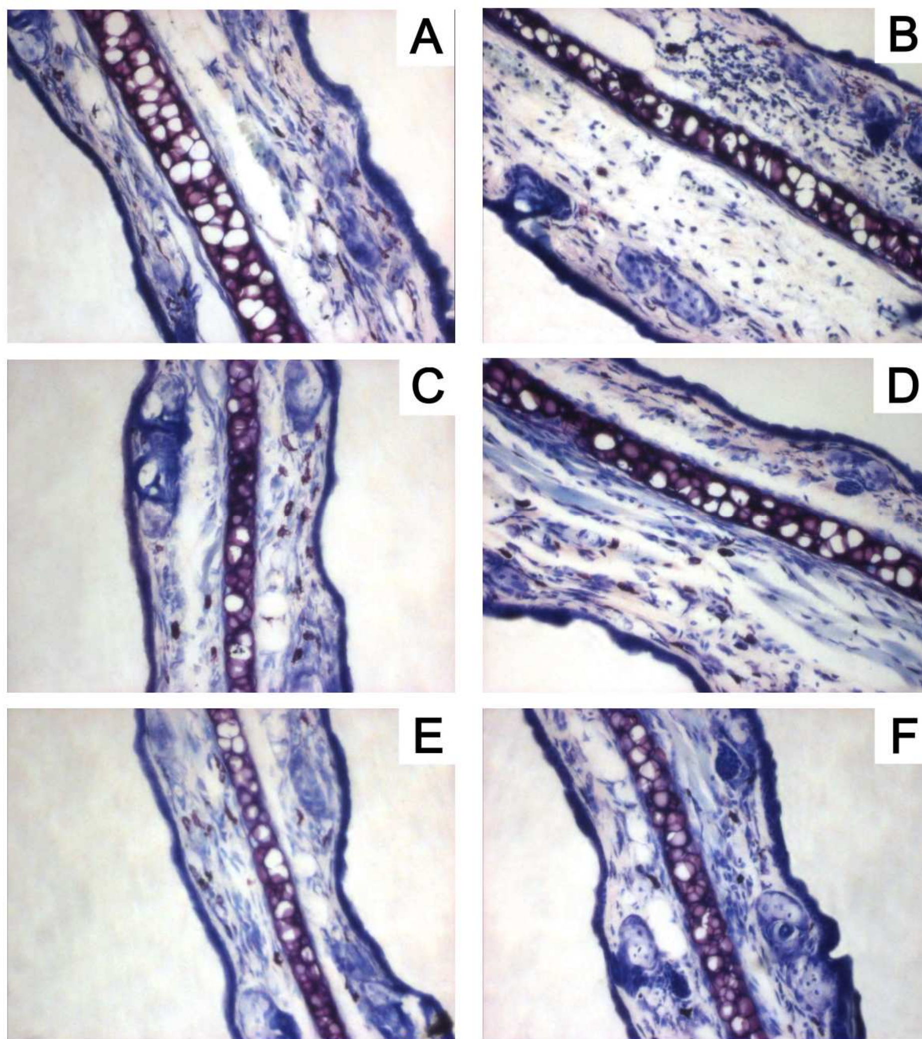

Figure S1. Sections of mouse ears 3 h after the induction of the croton oil dermatitis (A: untreated ear; B: control; C: 0.4 µmol/cm^2 compound **5**; D: 0.4 µmol/cm^2 compound **7**; E: 0.4 µmol/cm^2 indomethacin; F: 0.04 µmol/cm^2 hydrocortisone). Giemsa staining, 25 x magnification.

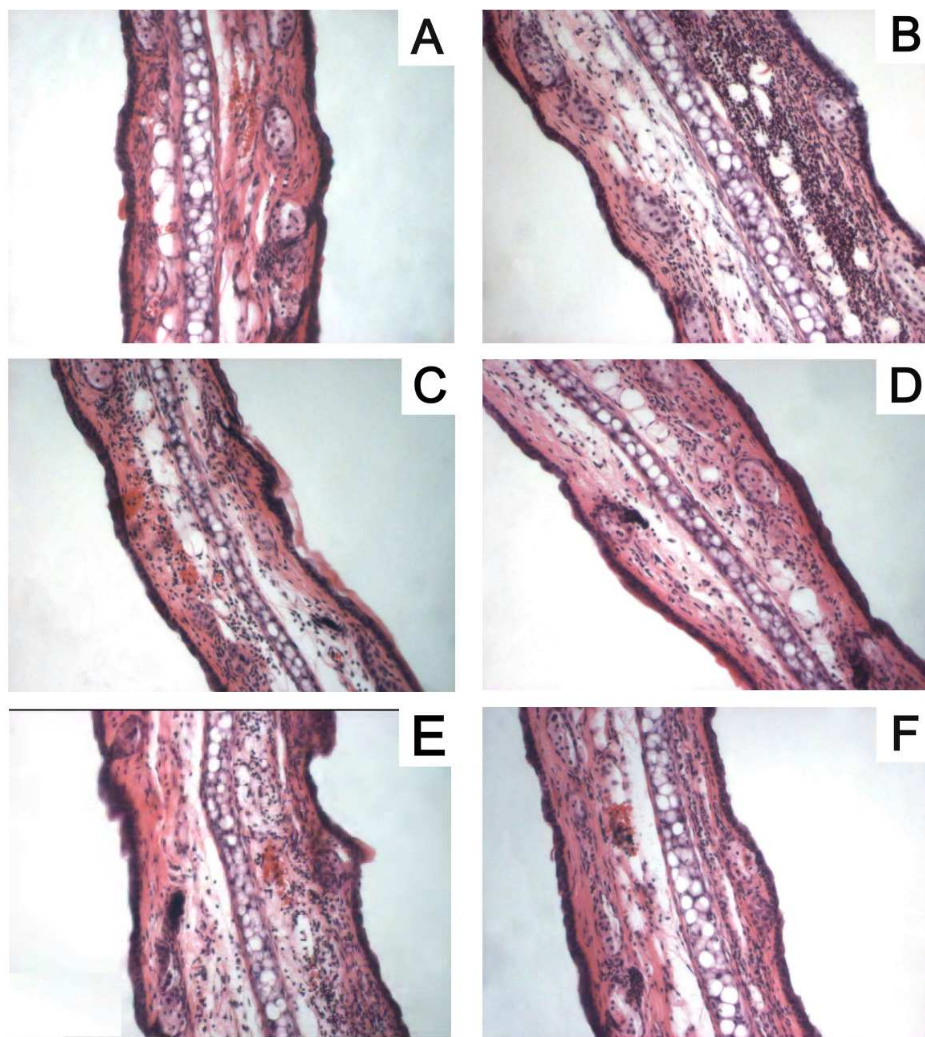

Figure S2. Sections of mouse ears 24 h after the induction of the croton oil dermatitis (A: untreated ear; B, control; C: 0.4 μmol/cm^2 compound **5**; D: 0.4 μmol/cm^2 compound **7**; E: 0.4 μmol/cm^2 indomethacin; F: 0.04 μmol/cm^2 hydrocortisone). Hematoxylin and eosin staining, 25 x magnification.