

## Article

# Diterpenoid compounds isolated from *Chloranthus oldhamii* Solms exert anti-inflammatory effects by inhibiting the IKK/NF- $\kappa$ B pathway

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## Supplementary information: Methods

### Isolation of compounds from *Chloranthus oldhamii* Solms

The *n*-hexane and EtOAc fractions showed a highly repetitive profile in the TLC analysis; thus, we combined them together (as fr. HE) for the following chromatography separation experiments. Fr. HE (105 g) was then subjected to column chromatography on silica gel (8 × 65 cm) and eluted with a solvent mixture of EtOAc/*n*-hexane (in ratios of 10, 20, 30, 40, 65, 80, and 100%, each 3.5 liter) and then with 5%, 10%, and 20% MeOH/EtOAc to supply 12 fractions (Frs. 1–12). Fr. 4 (18 g) was chromatographed on a silica gel (eluted with 20%–80% EtOAc/*n*-hex) to supply frs. 4-1–4-8. Fr. 4-3 was chromatographed on a semipreparative HPLC (LiChrospher® RP-select B, 10 × 250 mm, 5  $\mu$ m, eluent: 80% ACN/H<sub>2</sub>O, flow rate: 4.0 ml/min) to give CO-8 (3.9 mg) and CO-9 (21.2 mg). Fr. 4-5 and Fr. 4-6 were individually chromatographed on a semipreparative HPLC (LiChrospher® RP-select B, 10 × 250 mm, 5  $\mu$ m, eluent: 45% ACN/H<sub>2</sub>O, flow rate: 3.7 ml/min). Afterwards, CO-11 (2.8 mg) was isolated from Fr. 4-5 and CO-10 (61.0 mg) and CO-12 (4.7 mg) were isolated from Fr. 4-6. 4-9 was chromatographed on a Sephadex-LH-20 (eluted with acetone) and preparative TLC (developed with 60% EtOAc/*n*-hex) to give CO-15 (46.0 mg). Fr. 9 was chromatographed on a silica gel (eluted with 2%–10% MeOH/CHCl<sub>3</sub>) to give Frs. 9-1–9-6. Fr. 9-4 was chromatographed on a Sephadex-LH-20 (eluted with acetone) and on a semipreparative HPLC (Cosmosil 5C18-AR-II, 10 × 250 mm, 5  $\mu$ m, solvent: 35–40% ACN/0.1% formic acid for 20 min, flow rate: 3.7 ml/min) to supply CO-13 (3.6 mg), CO-14 (2.3 mg), and CO-17 (9.6 mg). Fr. 9-5 was chromatographed on a Sephadex-LH-20 (eluted with 50% EtOAc/MeOH) and on a semipreparative HPLC (Cosmosil 5C18-AR-II, 10 × 250 mm, 5  $\mu$ m, eluent: 50% ACN/0.1% formic acid, flow rate: 3.7 ml/min) to supply CO-18 (2.8 mg) and CO-20 (32.5 mg). Using a similar chromatographic technique, CO-1 (7.5 mg), CO-2 (74.3 mg), CO-6 (6.8 mg), CO-7 (7.2 mg), and CO-16 (1.5 mg) were isolated from Fr. 3; CO-19 (3.5 mg) was isolated from Fr. 10; and CO-3 (29.8 mg), CO-4 (13.8 mg), and CO-5 (48.5 mg) were isolated from Fr. 12.

**Citation:** Chiu, L.-C.; Wong, J.-Y.; Lin, C.-H.; Hsu, C.-H.; Lin, L.-C.; Fu, S.-L. Diterpenoid compounds isolated from *Chloranthus oldhamii* exert anti-inflammatory effects by inhibiting the IKK/NF- $\kappa$ B pathway. *Molecules* **2021**, *26*, 6540. <https://doi.org/10.3390/molecules26216540>

Academic Editor: Maria da Graça Costa G. Miguel

Received: 18 October 2021

Accepted: 28 October 2021

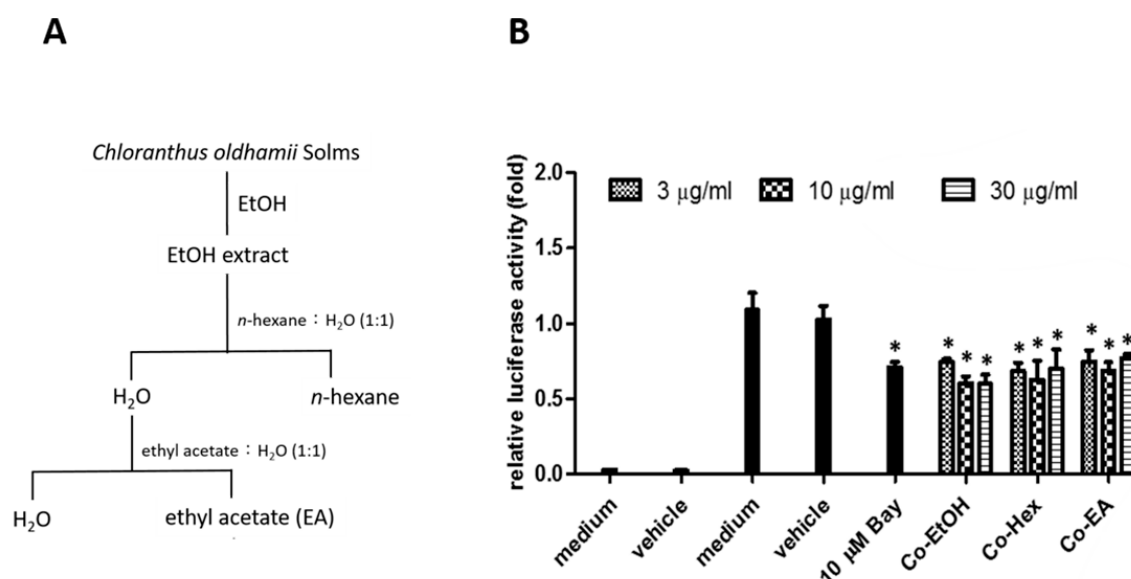
Published: 29 October 2021

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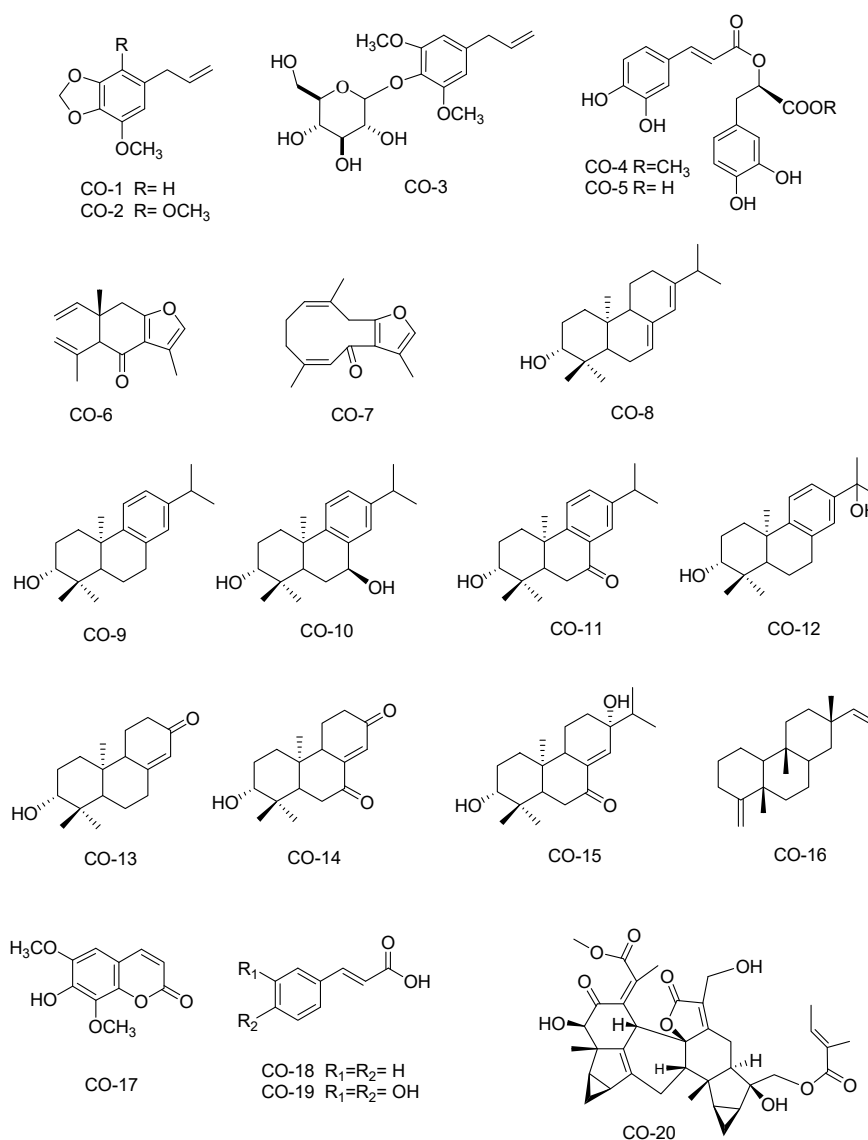


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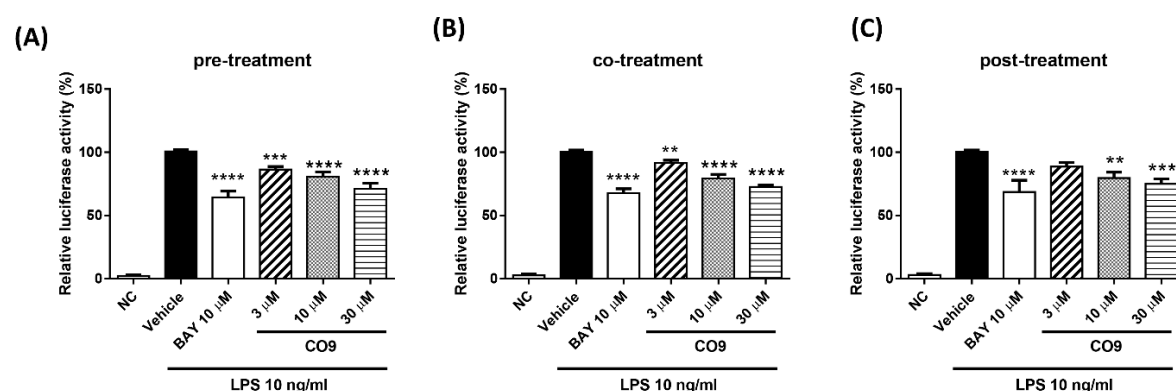
## Supplementary Information: Figures



**Supplementary Figure S1.** The fraction procedure for *Chloranthus oldhamii* and the effects of different fractions on NF- $\kappa$ B activity in LPS-stimulated macrophages. (A) The outline of the fractionation scheme. (B) RAW264.7/Luc-P1 cells ( $3 \times 10^5$  in MP-24 plates) were treated with different herbal fractions or vehicle (0.1% DMSO) for 1 h, followed by LPS treatment (10 ng/ml) for 6 h. Bay117082 (10  $\mu$ M) served as the positive control. Data are expressed as the mean  $\pm$  SD from three independent experiments. \* indicates a significant difference versus the vehicle group ( $p < 0.05$ ).



**Supplementary Figure S2.** The structures of isolated compounds from *C. oldhamii*. CO-1: myristicin, CO-2: apiol, CO-3: 4-allyl-2,6-dimethoxyphenyl glucopyranoside, CO-4: methyl rosmarinate, CO-5: rosmarinic acid, CO-6: curzerenone, CO-7: 1(10)Z,4Z-furanodiene-6-one, CO-8: 3 $\alpha$ -hydroxy-ent-abieta-7,13-diene, CO-9: 3 $\alpha$ -hydroxy-ent-abieta-8,11,13-triene, CO-10: 3 $\alpha$ ,7 $\beta$ -dihydroxy-ent-abieta-8,11,13-triene, CO-11: 3 $\alpha$ -hydroxy-ent-abieta-8,11,13-trien-7-one, CO-12: sessilifol J, CO-13: 3 $\alpha$ -hydroxy-ent-podocarp-8(14)-en-13-one, CO-14: 3 $\alpha$ -hydroxy-ent-podocarp-8(14)-en-7,13-dione, CO-15: decandrin B, CO-16: dolabradiene, CO-17: isofraxidin, CO-18: cinnamic acid, CO-19: caffeic acid, and CO-20: shizukaol C.



**Supplementary Figure S3.** The effect of CO-9 on NF- $\kappa$ B activity in LPS-stimulated macrophages under different treatments. (A) RAW264.7 cells ( $3 \times 10^5$  in MP-24 plates) were treated with various concentrations of drug or vehicle (0.1% DMSO) for 1 h before LPS treatment (10 ng/ml) for 6 hours. (B) Cells were simultaneously treated with 10 ng/ml LPS and various concentrations of drug or vehicle for 6 h. (C) Cells were stimulated with 10 ng/ml LPS for 1 h, followed by treatment with various concentrations of the indicated compounds or vehicle for another 6 h. Data are expressed as the mean  $\pm$  SD from three independent experiments. (\*)  $p < 0.05$ , (\*\*)  $p < 0.01$ , (\*\*\*)  $p < 0.005$ , and (\*\*\*\*)  $p < 0.0001$  indicate significant differences versus the LPS plus vehicle group.