Supporting Information

Structure-Activity Relationship Study of Dibenzocyclooctadiene Lignans Isolated from *Schisandra chinensis* on Lipopolysaccharide-Induced Microglia Activation

Di Hu¹, ², Na Han¹, Xuechun Yao³, Zhihui Liu¹, Yu Wang⁴, Jingyu Yang³, Jun Yin¹

**Affiliations**

¹Development and Utilization Key Laboratory of Northeast Plant Materials of Liaoning Province, Department of Pharmacognosy, Shenyang Pharmaceutical University, Shenyang, China

²College of Pharmacy, Harbin University of Commerce, Harbin, Heilongjiang Province, China

³Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang, China

⁴The Chinese People's Liberation Army 463 Hospital, Shenyang, China

**Correspondence**

*Prof. Jun Yin*

Department of Pharmacognosy, College of Chinese Herbs 48º

Shenyang Pharmaceutical University, 103 Wenhua Road

Shenyang 110016, China

Phone: +86 24 23986491

Fax: +86 24 23986460

yinjun2002@yahoo.com
**Compound isolation**

The dried fruits of *S. chinensis* (9.5 kg) were ground and flash extracted three times with EtOAc, 3 min per time. The filtrate was then concentrated under reduced pressure to yield an extract (1375 g). The EtOAc extract was chromatographed on a silica gel column (10 × 115 cm, 200-300 mesh, 2000 g) with a gradient of petroleum ether-EtOAc (100:0, 50:1, 25:1, 10:1, 5:1, 2:1, and 0:100, v/v) to obtain 8 fractions. Compounds 6 (20 mg) and 7 (3.38 g) were recrystallized from fraction 2 (37 g) and fraction 3 (56 g), respectively. Fraction 4 (324 g) was subjected to silica gel column chromatography (10 × 115 cm, 200-300 mesh, 2000 g) eluted with petroleum ether-EtOAc (20:1 to 2:1, v/v) to afford compound 6 (50 mg) and other 7 subfractions, 4.1-4.7. Fraction 4.1 (4.2 g) was then separated by preparative HPLC with MeOH-H$_2$O (70:30, v/v) to obtain compounds 6 (8.2 mg), 15 (5.8 mg), and 22 (8.1 mg). Fraction 4.2 (25.6 g) was further subjected to flash silica gel column chromatography (5 × 50 cm, 200-300 mesh, 650 g) with mixtures of petroleum ether-Me$_2$CO (25:1, v/v) to afford fraction 4.2.1 (2.4 g). Compounds 10 (77.4 mg) and 11 (44.2 mg) were obtained from fraction 4.2.1 by preparative HPLC with MeOH-H$_2$O (70:30, v/v). Fraction 4.3 (4.4 g) was purified by an ODS column (3.3 × 13 cm, 40 g) using solvent system MeOH-H$_2$O (55:45) to give compounds 6 (36.7 mg), 7 (6.1 mg), and 17 (2.9 mg). Fraction 4.4 (3.8 g) was purified by repeated preparative HPLC with the mobile phase MeOH-H$_2$O (70:30, v/v) to give compounds 10 (25.1 mg), 11 (4.5 mg), and 23 (4.6 mg). Fraction 4.5 (2.1 g) was subjected to preparative HPLC with MeOH-H$_2$O (70:30, v/v) to produce compounds 5 (2.1 mg)
and 12 (7.4 mg). Fraction 4.6 (1.4 g) was separated by preparative HPLC chromatography with MeOH-H₂O (70:30, v/v) to produce compound 13 (5.4 mg). Fraction 4.7 (62.7 g) was chromatographed on a silica gel column (200-300 mesh, 5 × 50 cm, 650 g) using petroleum ether-EtOAc (5:1) and then purified by preparative HPLC chromatography with solvent system MeOH-H₂O (70:30, v/v) to provide compounds 1 (46.9 mg), 2 (7.3 mg), 3 (20 mg), 13 (18.1 mg), 14 (40.7 mg), and 18 (5.6 mg). Fraction 5 (8.9 g) was purified by silica gel column chromatography (200-300 mesh, 3 × 32 cm, 310 g) using petroleum ether-EtOAc (10:1 to 2:1, v/v), preparative TLC with petroleum ether-Me₂CO (7:1, v/v) and preparative HPLC with MeOH-H₂O (70:30, v/v) to afford compounds 5 (10 mg), 8 (9.7 mg), 12 (1.8 mg), 13 (2.5 mg), 14 (0.7 mg), and 25 (4.2 mg). Fraction 6 (38.7 g) was further chromatographed on a flash silica gel column (5 × 50 cm, 200-300 mesh, 600 g), eluted with mixtures of petroleum ether-Me₂CO (20:1 to 5:1, v/v) to afford three subfractions. Compounds 20 (2.6 mg) and 21 (46.5 mg) were obtained from fraction 6.1 (7.2 g) by Sephadex LH-20 gel column chromatography with CHCl₃-MeOH (1:1, v/v) and preparative HPLC with MeOH-H₂O (70:30, v/v). Fraction 6.2 (6.8 g) was subjected to Sephadex LH-20 gel column chromatography with CHCl₃-MeOH (1:1, v/v) as the mobile phase, then purified by preparative HPLC with MeOH-H₂O (70:30, v/v) to yield compounds 13 (19.6 mg), 19 (8.5 mg), and 25 (12.8 mg). Fraction 6.3 (15.8 g) was purified by Sephadex LH-20 gel column chromatography with the elution of CHCl₃-MeOH (1:1, v/v) as the mobile phase, then purified by preparative HPLC with MeOH-H₂O (70:30, v/v) to afford compounds 4 (46.5 mg), 8 (50.5 mg),
and 18 (188.9 mg). Fraction 7 (21.2 g) was subjected to a silica gel column (5 × 50 cm, 200-300 mesh, 600 g) by using petroleum ether-Me₂CO (7:1, v/v) to afford compounds 8 (14.6 mg), 9 (2.7 g), and fraction 7.1 (3.1 g). Fraction 7.1 was further purified by preparative TLC with petroleum ether-Me₂CO (7:1, v/v) and Sephadex LH-20 gel column chromatography with CHCl₃-MeOH (1:1, v/v) to give compound 24 (26.8 mg).