

Anti-inflammatory Benzofurans from the Heartwood of *Dalbergia cochinchinensis* Pierre ex Laness

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Abstract: A new benzofuran, Cochinfuran A (**1**), and four known benzofurans (**2-5**) were isolated from the heartwood of *Dalbergia cochinchinensis* Pierre ex Laness. The chemical structure of the new benzofuran was determined based on broad NMR and mass spectrometry evaluation. Bioactivity assays showed that the compounds **1-3** and **5** were found as anti-inflammatory agents with IC₅₀ values 49.01 ± 1.54, 1368.93 ± 0.98, 67.48 ± 0.92 and 77.91 ± 1.53 μM, respectively. They could reduce the production of NO (*P*<0.001) with 3.52~7.04, 1.96~7.84, 1.85~7.40 and 0.98~3.93 μM and restrained LDH (*P*<0.01) with 3.52~7.04, 3.93~7.84, 3.70~7.40 and 0.98~3.93 μM in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, respectively, the compound **4** (IC₅₀ 218.20 ± 3.39 μM) showed no anti-inflammatory activity.

Keywords: *Dalbergia cochinchinensis* Pierre ; benzofurans; anti-inflammatory activity. © 2022 ACG Publications. All rights reserved.

1. Plant Source

The heartwood of *Dalbergia cochinchinensis* Pierre ex Laness was purchased from the Fang cheng gang, Guangxi and identified by one of the authors (Ronghua-Liu). The plant specimen (DC.201306) was deposited at the Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of Chinese Medicine, Nanchang, China.

2. Previous Studies

Dalbergia cochinchinensis Pierre ex Laness, commonly known as red rosewood, is mainly distributed in the Southeast Asian countries such as Thailand, Cambodia, Laos and Vietnam [1]. *Dalbergia cochinchinensis* Pierre ex Laness is a traditional Thai herbal medicine, which is mainly used for the clinical

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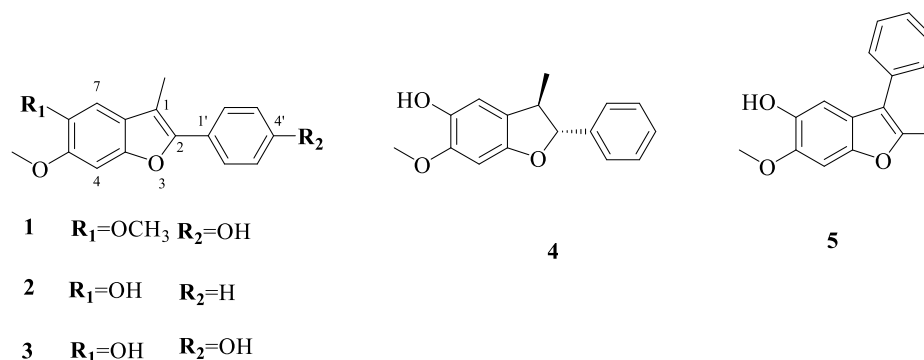
treatment of blood stasis and cancer [2]. Studies in China and abroad showed that this plant mainly contained neoflavonoids, flavonoids, isoflavones, flavanes, isoflavanes, and benzofurans [3-10], among which benzofuran compounds have been reported as anti-inflammatory, antibacterial, anti-tumor, antiviral agents from the species [11-14].

3. Present Study

The powdered heartwood of *Dalbergia cochinchinensis* Pierre (18.35 kg) was isolated under reflux by 70% ethyl alcohol thrice. Next, The ethyl alcohol extract was subjected to concentration under decreased pressure to produce a residue (4.97 kg). The extract was dissolved in distilled H₂O and successively partitioned with PE (petroleum ether), CH₂Cl₂ (Dichloromethane), EtOAc (Ethyl acetate) and n-BuOH (n-Butanol). The EtOAc fraction (1.67 kg) was chromatographed on silica gel column chromatography (CC) using PE-EtOAc (from 90:10 to 30:70, v/v) with elutions 8 fractions (Frs.A-Frs.H) are generated. Frs.C (126.3 g) was separated by silica gel CC eluting with petroleum ether-acetone to (50:1 to 1:1, v/v) as eluents to produce seven fractions (Frs.C1- Frs.C7). Fraction C6 (5.6 g) was separated by silica gel CC with petroleum ether-EtOAc (25:1 to 1:1, v/v) as eluents to produce four fractions (Frs.C6a-Frs.C6d). Fraction C6d (1.2 g) was separated by silica gel CC with petroleum ether-acetone to (10:1 to 1:1, v/v) as eluents to produce three fractions (Frs.C6d1-Frs.C6d3). Frs.C6d2 (200 mg) was separated over Sephadex LH-20 with CH₂Cl₂-MeOH (1:1, v/v) to acquire compound **1** (40 mg). Fraction A (10.1 g) was purified by silica gel CC with petroleum ether-EtOAc to (100:1 to 1:1, v/v) elution to produce four fractions (Frs.A1-Frs.A4). Fraction A4 (100 mg) was furtherly chromatographed over Sephadex LH-20 with CH₂Cl₂-MeOH (1:1, v/v) to produce three fractions (Frs.A4a-Frs.A4d). Frs.A4d (50 mg) was on a semi-preparative C₁₈ column (250×10 mM, 10 μM, Phenomenex, USA) with acetonitrile-H₂O (v/v, 60:40, flow rate: 3 mL/min) to acquire compound **2** (3.4 mg, t_R 68.0 minutes). Fraction A4d (50 mg) was on a semi-preparative C₁₈ column (250×10 mM, 10 μM, Phenomenex, USA) with acetonitrile-H₂O (v/v, 60:40, flow rate: 3 mL/min) to acquire compound **4** (6.0 mg, t_R 30.0 minutes). Subfraction D (228.3 g) was separated by CC eluting with petroleum ether-EtOAc to (50:1 to 1:1, v/v) as eluents to produce fifteen fractions (Frs.D1-Frs.D15). Fraction D7 (300 mg) was separated over Sephadex LH-20 eluting with CH₂Cl₂-MeOH (1:1, v/v) to acquire compound **3** (3.4 mg). Fraction A3 (700 mg) was separated over Sephadex LH-20 with CH₂Cl₂-MeOH (1:1, v/v) elution to produce seven fractions (Frs.A3a- Frs.A3g). Frs.A3d (300 mg) was separated by silica gel CC eluting with petroleum ether-acetone to (50:1 to 1:1, v/v) elution to produce three fractions (Frs.A3d1-Frs.A3d3). Finally, Frs.A3d1 (70 mg) was obtained on a semi-preparative C₁₈ column with acetonitrile-H₂O (v/v, 70:30, flow rate: 3 mL/min) to acquire compound **5** (5.4 mg, t_R 45.30 minutes).

Cochinfuran A. (1): Pale yellow powder (Acetone-*d*₆); UV (MeOH) λ_{max}: 250, 320 nm, IR (KBr) ν_{max} cm⁻¹. HR-ESI-MS *m/z* measured 285.11203 ([M+H]⁺), calculated 285.11214 ([M+H]⁺), ¹H-NMR (Acetone-*d*₆, 600 MHz) and ¹³C-NMR (Acetone-*d*₆, 151 MHz): see Table 1.

We studied the chemical constituents from the heartwood of *Dalbergia cochinchinensis* Pierre. A new benzofuran and four known benzofurans were isolated from the heartwood of the species (Figure. 1).

**Figure 1.** Structural features of chemical substances **1-5****Table 1.** The NMR data for **1** in Acetone- d_6 (δ in ppm, J in Hz)

Position	δ_H	δ_C
1	-	109.3
2	-	150.1
3	-	-
4	7.00 (1H, s)	95.4
5	-	148.5
6	-	148.1
7	6.97 (1H, s)	101.8
8	-	123.3
9	-	146.9
1'	-	123.2
2', 6'	7.49 (2H, d, $J = 8.8$ Hz)	127.6
3', 5'	6.34 (2H, d, $J = 8.8$ Hz)	115.5
4'	-	157.0
4'-OH	8.48 (1H, s)	-
1-CH ₃	2.27 (3H, s)	8.6
5-OCH ₃	3.74 (3H, s)	55.7
6-OCH ₃	3.73 (3H, s)	55.9

Compound **1** was isolated as a pale-yellow amorphous powder. Its molecular formula of $C_{17}H_{16}O_4$ was deduced from the HR-ESI-MS peak at m/z 285.1121 ($[M+H]^+$) calcd. for 285.1120 ($[M+H]^+$). The 1H NMR spectrum presented two aromatic peaks (δ_H 7.00 [1H, s, H-4] and 6.97 [1H, s, H-7]), two methoxy groups δ_H 3.74 [3H, s, OCH₃], 3.74 [3H, s, OCH₃], and a hydroxy group δ_H 8.48 (1H, s). The signals at δ_H 2.26 (1H, s, 1-CH₃), C-2 (δ_C 150.1), C-1 (δ_C 109.3), 1-CH₃ (δ_C 8.6) are in agreement with those of pterolinus B derivatives [15]. According to the ^{13}C NMR and HSQC spectra, 12 aromatic carbons [δ_C 157.0, 150.1, 148.4, 148.1, 146.9, 127.6, 123.3, 123.3, 115.5, 109.3, 101.7, 95.5,], two methoxy carbons [δ_C 55.9 OCH₃ and 55.7 OCH₃] and a primary carbon (δ_C 8.7) were observed. The HMBC (see Table S1 in Supporting Information) correlations showed H-7 (δ_H 6.97) to C-9 (δ_C 146.9) and C-5 (δ_C 148.5) along with H-4 (δ_H 7.00) to C-6 (δ_C 148.1) and C-8 (δ_C 123.3). These data were suggested the signals at δ_H 6.97 and 7.00 at ring A and assigned as H-7 and H-4, respectively. In HSQC and HMBC spectra, the correlations between H-4 (δ_H 7.00) and C-5 (δ_C

148.5), H-4 (δ_{H} 7.00) and C-6 (δ_{C} 148.1), the OCH_3 (δ_{H} 3.73) and C-6 (δ_{C} 148.1) confirmed that this methoxy group (δ_{C} 3.73) was located at C-6. And the methoxy group OCH_3 (δ_{H} 3.74) was positioned at C-5. The HMBC correlations from CH_3 (δ_{H} 2.26) to C-1 (δ_{C} 109.3), C-2 (δ_{C} 150.1), C-8 (δ_{C} 123.3), and from H-7 (δ_{H} 6.97) to C-1 (δ_{C} 109.3). Based on those data the position of CH_3 at δ_{H} 2.26 was determined as C-1. The HMBC correlations from the 4'-OH (δ_{H} 8.48) and H-2' (δ_{H} 7.49) to C-2 (δ_{C} 150.1) and C-4' (157.0), (δ_{H} 7.50, d, $J = 8.8$ Hz, 2H) and (δ_{H} 6.84, d, $J = 8.8$ Hz, 2H) revealed that the hydroxy group was located at C-4'. Ultimately, compound **1** was identified as Cochinfuran A.

The inhibitory effect of compounds **1-5**, on the release of NO from RAW 264.7 macrophages was induced by lipopolysaccharide, were studied based on the literature method [16,17] and, the results are summarized in Figure 1 for compound **1** and Figure S20 for all compounds (see supporting information). All the tested compounds showed inhibitory effects on the production of nitric oxide in RAW 264.7 macrophages induced by LPS with IC_{50} values ranging from 49.01 ± 1.54 to 1368.93 ± 0.98 μM . Furthermore, compound **4** did not reduce NO production and weaken LDH activity, indicating that the double bond at C-1 and C-2 might enhance the anti-inflammatory activity of RAW 264.7 macrophages, promote NO release and enhance LDH activity. According to those data, we claim that the hydroxy group at C-4' of benzofuran might attenuate the anti-inflammatory activity of the compounds against RAW 264.7 macrophages.

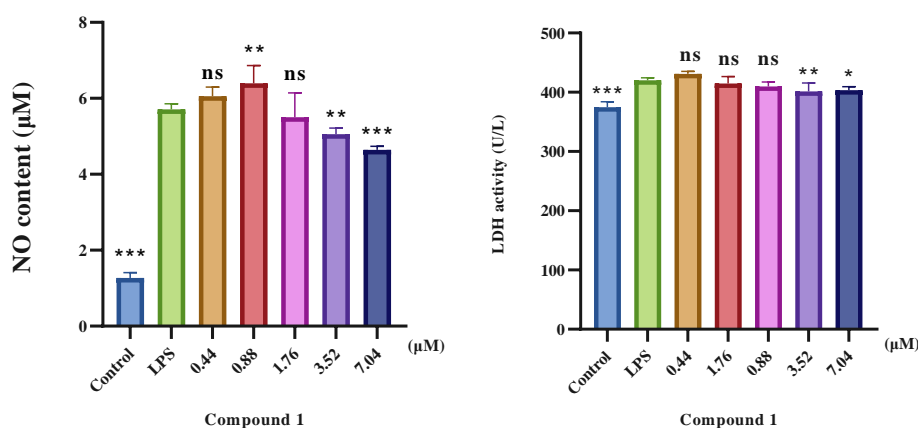


Figure 2. Effects of compound (**1**) on the production of NO and LDH in LPS-induced RAW 264.7 macrophages.

The values shown represent the mean ($n=6$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the LPS group.

The increase of MPO activity will continue the damage of inflammation to tissues. It suggested that inhibiting the release of MPO is a potential target to protect the body tissue injuries [16-20]. The intersecting targets of inflammation and benzofurans (**1-5**) were TERT, PLG, HDAC2, ADA, FGFR1, MPO, and PSEN1, we docked five compounds (**1-5**) into each target using the AUTODOCK 4.2.6 program. The results showed that only the docking score of MPO was basically consistent with the cell experiment results. Therefore, inflammation may be related to MPO. The results confirmed that the compound (**1-3, 5**) showed pretty good binding with MPO protein (Table 2), among them, the compound **3** showed the strongest affinities with MPO protein, with absolute value of 8.63. However, the compound **4** showed the weakest affinities with MPO protein, with absolute value of 4.79. The van der Waals force

between the compound **4** and MPO protein is due to its interaction with ARG-673 and TYR-479 residues in the active cavity of the protein. (Figure S21, see Supporting Information). The result of Molecular docking was found to be like the anti-inflammatory activity of compounds (**1-5**) on the LPS-induced RAW 264.7 macrophages.

Table 2. The scores of compounds (**1-5**) with MPO protein

Compounds	scores	Targeting residues
1	-7.97	ARG-499 MET-253 PHE-252
2	-7.66	ALA-277 GLN-589 LYS-295
3	-8.63	GLU-408 TYR-462
4	-4.79	ARG-673 TYR-479
5	-7.89	ARG-499 HIS-502 ASP-260

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Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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