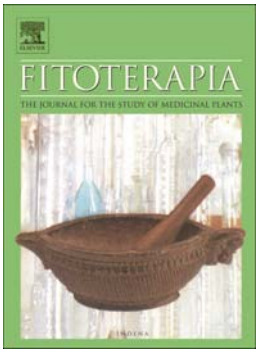

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Nitric oxide inhibitory constituents from the barks of *Cinnamomum cassia*

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ABSTRACT

Six new compounds including one γ-butyrolactone, cinncassin A (**1**), two tetrahydrofuran derivatives, cinncassins B and C (**2**, **3**), two lignans, cinncassins D and E (**4**, **5**), and one phenylpropanol glucoside, cinnacassoside D (**6**), together with 14 known lignans (**7**–**20**) were isolated from the barks of *Cinnamomum cassia*. The structures of **1**–**6** were elucidated by extensive 1D and 2D NMR spectroscopic data analysis as well as chemical methods, and the absolute configurations were established by experimental and calculated ECD data. The anti-inflammatory activities of the isolates were evaluated on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced BV-2 microglial cells. Compounds **5**, **7**, **8**, and **15** showed potent inhibition activities with IC_{50} values of 17.6, 17.7, 18.7, and 17.5 μM, respectively.

Keywords:

Cinnamomum cassia

Lignans

γ-Butyrolactone

Tetrahydrofuran derivatives

Nitric oxide

1. Introduction

The plants *Cinnamomum cassia* Presl (Lauraceae) have been widely cultivated in the tropical or subtropical areas, such as Yunnan, Guangdong, Guangxi, Hainan, Guizhou, and Taiwan in China, as well as India, Vietnam, Indonesia, Laos, Thailand, and Malaysia. The dried barks of *C. cassia*, well known as Cinnamomi Cortex, are not only used as a spice and flavoring agent, but also used as a traditional medicine in the world for the treatment of amenorrhea, rheumatoid arthritis, cardiac palpitation, diarrhea, and gastrointestinal neurosis[1]. Previous phytochemical and pharmacological studies on this plant have resulted in the isolation of several kinds of bioactive compounds such as *trans*-cinnamaldehyde and 2-methoxycinnamaldehyde from the essential oil of *C. cassia* which have been reported to inhibit NF-κB[2], and cinnamaldehyde has been shown to possess potential *in vitro* and *in vivo* anti-inflammatory activities[5]. There are some other kinds of bioactive compounds[3-8] such as flavonoids, diterpenoids, comarins and polyphenols which exhibited antimicrobial, antifungal, immunosuppressive, cytotoxic , and antioxidant

activities.

In order to search for more new bioactive components from *C. cassia*, a systematic phytochemical investigation was carried out. Six new compounds including one γ-butyrolactone, cinncassin A (**1**), two tetrahydrofuran derivatives, cinncassins B and C (**2**, **3**), two lignans, cinncassins D and E (**4**, **5**), and one phenylpropanol glucoside, cinnacassoside D (**6**), together with 14 known lignans (**7**–**20**) were isolated from the barks of *C. cassia*. Herein, the isolation and structural elucidation of the six new compounds and the inhibitory effects of the isolates on LPS-stimulated NO production in BV-2 microglial cells are reported.

2. Experimental

2.1. General experimental procedures

Optical rotation was measured on an Autopol III automatic polarimeter. UV spectra were obtained on a Shimadzu UV-2450 spectrophotometer. IR spectra were achieved on a Nicolet Nexus 470 FT-IR spectrometer by KBr pellets. NMR spectra were recorded on a Varian INOVA-500 FT NMR spectrometer. HR mass spectra were obtained from a Bruker APEX IV FT-MS (7.0 T) mass spectrometer fitted with an ESI source. CD spectra were acquired on a JASCO J-810 spectropolarimeter. Analytical HPLC was performed on an Agilent 1100 series equipped with an Extend-C_{18} column (5 μm, 4.6 × 250 mm) and a DAD detector. Semi-preparative HPLC was performed on an Agilent 1260 system equipped with an Agilent ZORBAX SB-C_{18} column (5 μm, 9.4 mm × 250 mm; flow rate 3.0 mL/min). Column chromatography (CC) was

performed over silica gel (200–300 mesh, Qingdao Marine Chemistry Co., Ltd., China), Sephadex LH-20 (Amersham Biosciences, Sweden), and ODS C_{18} (40–63 μm; Merck, Germany). TLC was carried out on glass precoated silica gel (GF_{254}) plates (Qingdao Marine Chemistry Co., Ltd., China). Spots were visualized under UV light or by spraying with 2% vanillin-sulfuric acid solution.

2.2. Plant material

Cinnamomum cassia was purchased from Guangdong Province, China, and authenticated by one of the authors, Prof. P.-F. Tu. The voucher specimen (no. 20130326) has been deposited at the herbarium of Modern Research Center for Traditional Chinese Medicine, Peking University.

2.3. Extraction and isolation

The dried barks of *C. cassia* (100 kg) was powered and extracted with 85% aqueous EtOH for 3 times, each for 3 h. After removal of EtOH under reduced pressure, the aqueous brownish syrup was suspended in water and partitioned with petroleum, EtOAc, and *n*-BuOH, successively.

The EtOAc extract was concentrated in vacuo to yield a black residue (3.8 kg) which was chromatographed on a silica gel CC eluting with gradient of $CHCl_3$-MeOH (from 30:1 to 1:1) to yield eight fractions (Frs.1→8). Fr.8 (159 g) was separated on a silica gel CC ($CHCl_3$-MeOH, 20:1 to 1:1) to give six major subfractions (Frs. A→F). Fr. A (20 g) was purified by Sephadex LH-20, eluting with $CHCl_3$-MeOH (1:1), to give Frs. A1, A2, and A3. Fr. A1 (200 mg) was crystallized from cold MeOH to afford **18** (20 mg). Fr. A2 (50.1 mg) was subjected to

semi-preparative reverse-phase HPLC with CH_3CN-H_2O (18:82) to afford **17** (8 mg; t_R 10.3 min) and **1** (5.1 mg; t_R 19.7 min). Fr. B (17 g) was chromatographed on Sephadex LH-20 and its main fraction was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (20:80) to yield **5** (5 mg; t_R 15.1 min), **7** (6.1 mg; t_R 20.6 min), **8** (5.7 mg; t_R 25.9 min), and **14** (5.6 mg; t_R 32.3 min). Fr. C (5 g) was subjected to semi-preparative HPLC eluting with CH_3CN-H_2O (30:70) to give **11** (4.5 mg; t_R 21.5 min), **13** (3.0 mg; t_R 28.9 min), and **16** (5.4 mg; t_R 36.0 min). Fraction D (25 g) was separated by Sephadex LH-20 (MeOH-H_2O, 70:30) to give Frs.D1→D4. Compounds **2** (3.1 mg; t_R 26.8 min), **3** (3.0 mg; t_R 34.1 min), and **15** (5.7 mg; t_R 41.2 min) were obtained from Fr. D1 after purification by semi-preparative HPLC eluting with CH_3CN-H_2O (18:82). Fr. D2 (800 mg) was first subjected to a silica gel CC ($CHCl_3$-MeOH, 15:1 to 1:1) and then purified by semi-preparative HPLC eluting with CH_3CN-H_2O (18:82) to yield **12** (5.5 mg; t_R 17.5 min), **19** (16.3 mg; t_R 23.2 min), and **20** (30 mg; t_R 38.1 min). Compounds **4** (4.5 mg; t_R 18.8 min), **9** (4.2 mg; t_R 23.9 min), and **10** (12 mg; t_R 35.0 min) were obtained from Fr. D3 (220 mg) after purification by semi-preparative HPLC eluting with CH_3CN-H_2O (15:85). Fr. F (2 g) was chromatographed on Sephadex LH-20 (MeOH-H_2O, 50:50) to give Frs. F1→F3. Frs. F3 (50 mg) was purified by semi-preparative HPLC eluting with CH_3CN-H_2O (7:93) to yield **6** (10.2 mg; t_R18.9 min)

*2.3.1. Cinncassin A (**1**)*

Yellow amorphous powder; $[\alpha]^{20}_D$ +42 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.46), 224 (2.06), 281 (1.56) nm; IR (KBr) ν_{max} 3367, 1760, 1596, 1517 cm^{-1};

CD (MeOH, $\Delta\varepsilon$) 204 (–13.95), 230 (–7.53) nm; HRESIMS $[M–H]^-$ *m/z* 285.0770 (calcd for $C_{16}H_{13}O_5$, 285.0763); for ^{1}H NMR and ^{13}C NMR data, see Table 1.

*2.3.2. Cinncassin B (**2**)*

White amorphous powder; $[\alpha]^{20}_D$ +61 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 206 (2.76), 273 (1.71), 304 (1.51) nm; IR (KBr) ν_{max} 3370, 1594, 1519, 1464 cm^{-1}; CD (MeOH, $\Delta\varepsilon$) 206 (+61.42), 226 (–2.39), 242 (+2.22) nm; HRESIMS $[M+Na]^+$ *m/z* 415.1354 (calcd for $C_{20}H_{24}O_8Na$, 415.1369); for ^{1}H NMR and ^{13}C NMR data, see Table 1.

*2.3.3. Cinncassin C (**3**)*

White amorphous powder; $[\alpha]^{20}_D$ –27 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.63), 225 (2.26), 280 (1.75) nm; IR (KBr) ν_{max} 3397, 1765, 1610, 1515 cm^{-1}; CD (MeOH, $\Delta\varepsilon$) 214 (–13.82), 228 (+5.71), 240 (–1.70), 254 (+3.63) nm; HRESIMS $[M+Na]^+$ *m/z* 457.1477 (calcd for $C_{22}H_{26}O_9$ Na, 457.1475); for ^{1}H NMR and ^{13}C NMR data, see Table 1.

*2.3.4. Cinncassin D (**4**)*

White amorphous powder; $[\alpha]^{20}_D$ –36 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 207 (2.65), 235 (2.23), 289 (1.98), 307(1.88) nm; IR (KBr) ν_{max} 3467, 1766, 1593, 1463 cm^{-1}; CD (MeOH, $\Delta\varepsilon$) 212 (+178.85), 226 (–5.27), 240 (+6.40), 256 (–0.23), 294 (+9.36) nm; HRESIMS $[M+H]^+$ *m/z* 509.1801 (calcd for $C_{28}H_{29}O_9$, 509.1812); for ^{1}H NMR and ^{13}C NMR data, see Table 2.

*2.3.5. Cinncassin E (**5**)*

White amorphous powder; $[\alpha]^{20}_D$ –27 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε)

205 (2.65), 303 (2.05), 337 (2.09) nm; IR (KBr) ν_{max} 3466, 1734, 1596, 1511, 1426 cm^{-1}; CD (MeOH, $\Delta\varepsilon$) 230 (–0.58) nm; HRESIMS $[M–H]^-$ *m/z* 403.1391 (calcd for $C_{21}H_{23}O_8$, 403.1393); for ^1H NMR and ^{13}C NMR data, see Table 2.

*3.3.6. Cinnacassoside D (**6**)*

White amorphous powder; $[\alpha]^{20}_D$ +11.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 207 (3.52), 255 (2.78); IR (KBr) ν_{max} 3367, 2927, 1705, 1650, 1601, 1453 cm^{-1}; HRESIMS *m/z* 329.1236 $[M–H]^-$ (calcd for $C_{15}H_{21}O_8$ 329.1236); for ^1H and ^{13}C NMR spectral data, see Table 2.

2.4. Acid hydrolysis and GC analysis of compound **6**

Compound **6** (4.9 mg) was stirred at 100 °C for 12 h with 2 M CF_3COOH (3 mL). After being cooled to room temperature, the solution was extracted with $CHCl_3$ for three times. The $CHCl_3$ extract was concentrated in vacuo to yield a white residue (A), and the water solution was freeze-dried to give a white residue (B).

Residue B was redissolved in 2 mL pyridine with 0.1 M L-cysteine methyl ester hydrochloride and stirred at 60 °C for 1.5 h. After being cooled and concentrated, the mixture was treated with hexamethyl-disilazane (HMDS) and trimethylchlorosilane (TMSCl) (2:1, v/v, 0.6 mL) inpyridine (2 mL), followed by stirring at 60 °C for 0.5 h. Then, the solution was concentrated to dryness, and the residue was separated with H_2O and *n*-hexane (2 × 3 mL) [9,10]. The *n*-hexane fraction was analyzed by GC using a HP-5 column (0.25 mm × 30 m). The temperature of injector was 250 °C, and the temperature of detector was 280 °C. A gradient temperature system was applied to the oven, starting at 160 °C and increasing up to 280 °C at a rate of 5 °C/min, then

keeping 280 °C for 10 min. The peak of the sugar derivative in the hydrolysate were identified by comparison of the retention time with the authentic sample of D-glucose (24.9 min) after similar treatment with TMSCl/HMDS.

2.5. Cell viability

MTT assay was used for Cell viability detection as previously reported [10–12]. Briefly, BV-2 cells (mouse microglial cells) were purchased from Peking Union Medical College, Cell Bank (Beijing, China) and maintained in DMEM medium supplemented with 10% Fetal Bovine Serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere with 5% CO_2. Then, BV-2 cells were seeded in 48-well plates (5×10^4 cells/well) for 24 h incubation and treated with compounds isolated from *C. cassia* for 24 h with LPS at the concentration of 1μg/mL (*Escherichia coli* 0111:B4, Sigma, MO, USA). After that, BV-2 cells were incubated with MTT solution (500 μL, 0.5 mg/mL in culture medium) at 37 °C for 4 h. Then the culture supernatant was removed and the residue was dissolved in DMSO (500 μL). The absorbance was detected under 540 nm by using a microplate reader (Tecan Trading AG).

2.5. Inhibition of NO production

The production of NO was determined by detecting cell culture supernatants for nitrite, a major stable product of NO, by Griess reagent[10]. 5×10^4 cells/well were seeded in 48-well culture plates for 24 h. Then the cells were treated with different concentrations of compounds and stimulated with LPS at the concentration of 1μg/mL for 24 h. After that, the cell culture supernatant (300 μL) was collected to react with

Griess reagent (100 μL, 1% sulfanilamide/0.1% naphthylethylene diamine dihydrochloride/2% phosphoricacid) for 10 min in the darkness at room temperature. The absorbance was measured at 540 nm, using a microplate reader (Tecan Trading AG). The experiments were performed in parallel for three times. The IC_{50} was generated by GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, California) and quercetin was used as a positive control (IC_{50} = 15.6 μM).

2.6. ECD calculations

The 3D structures were subjected to random conformation analysis by using Sybyl-X 1.1 with the MMFF94s molecular mechanics force field. Then, the minimum conformers were optimized using DFT at the B3LYP/6-31G(d) level in the gas phase in the Gaussian 09 program. The B3LYP/6-31G(d) harmonic vibrational frequencies were further calculated to confirm their stability. ECD calculations were performed using the time-dependent density functional theory (TDDFT) method at the B3LYP/6-311++G (2d, 2p) level through the CPCM model (MeOH). ECD spectra of different conformers were simulated using SpecDis with a half-band width of 0.3 ev, and the final ECD spectra were obtained according to the Boltzmann-calculated contribution of each conformer.

3. Results and discussion

Cinncassin A (**1**) was obtained as a yellow amorphous powder. Its molecular formula was assigned as $C_{16}H_{14}O_5$ on the basis of a quasimolecular ion at *m/z* 285.0770 $[M–H]^-$ (calcd for $C_{16}H_{13}O_5$, 285.0763) in its HRESIMS. The IR spectrum

indicated that **1** possesses hydroxy (3367 cm^{-1}), carbonyl (1760 cm^{-1}), and phenyl (1596 and 1517 cm^{-1}) functionalities. The ^{1}HNMR data revealed the presence of a set of ABX coupled phenyl protons at δ_H 6.71 (1H, d, J = 7.5 Hz), 6.69 (1H, d, J = 1.0 Hz) 6.60 (1H, dd, J = 7.5, 1.0 Hz), a group of A_2B_2 coupled phenyl protons at δ_H 7.00 (2H, d, J = 8.5 Hz), 6.73 (2H, d, J = 8.5 Hz), and four aliphatic proton signals at δ_H 4.61 (1H, br t, J = 8.5 Hz), 4.26 (1H, br t, J = 9.5 Hz), 3.91 (1H, d, J = 12.0 Hz), 3.68 (1H, td, J = 12.0, 8.5 Hz). The ^{13}C NMR data (Table 1) showed 16 carbon resonances comprising 12 phenyl carbons, one carbonyl carbon, and three aliphatic carbons. The HMBC correlations of H-3 and H-5 with C-2 as well as ^{1}H-^{1}H correlations of H-3/H-4/H-5 revealed the presence of a furanone moiety. The linkage positions of the two phenyl groups were deduced to be at C-3 and C-4 of the furanone moiety from the HMBC correlations of H-3 (δ_H 3.91) with C-1' (δ_C 128.1), C-2' (δ_C 130.8), and C-6' (δ_C 130.8), and H-4 (δ_H 3.68) with C-1" (δ_C 130.1), C-2" (δ_C 115.6), and C-6" (δ_C 119.9). Three hydroxy groups were deduced to be attached to the two phenyl groups to match the molecular composition and the chemical shifts of those aromatic carbons. The relative configuration of **1** was deduced based on the NOESY spectrum, in which H-3 was correlated with H-5β (δ_H 4.26), and H-4 with H-5α (δ_H 4.61). The absolute configuration of **1** was determined by comparing the experimental and calculated ECD spectra. The experimental ECD spectrum of **1** showed negative Cotton effects at 204 and 230 nm, which are in agreement with the calculated ECD data of (3*R*,4*R*)-**1** (Fig. 3). Based on the above evidences, the structure of cinncassin A (**1**) was elucidated as

(+)-(3*R*,4*R*)-4-(3",4"-dihydroxyphenyl)-3-(4'-hydroxyphenyl)-dihydrofuran-2-one.

Cinncassin B (**2**) was obtained as a white amorphous powder. HRESIMS analysis revealed a molecular formula of $C_{20}H_{24}O_8$ from its pseudomolecular ion at *m/z* 415.1354 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_8Na$, 415.1369). The IR spectrum revealed the absorption bands for hydroxy (3370 cm^{-1}) and phenyl (1594, 1519 and 1464 cm^{-1}) functionalities. The ^1H NMR data showed four aromatic proton signals at δ_H 6.65 (2H, s) and δ_H 6.53 (2H, s), revealing the presence of two tetrasubstituted phenyl groups, four methoxys at δ_H 3.82 (6H, s) and δ_H 3.73 (6H, s), and five aliphatic protons at δ_H 5.14 (1H, d, *J* = 10.5 Hz, H-2), 4.47 (1H, t, *J* = 4.5 Hz, H-4), 4.43 (1H, dd, *J* = 9.5, 4.5 Hz, H-5), 4.01 (1H, d, *J* = 9.5 Hz, H-5)，3.13 (1H, dd, *J* = 10.5, 4.5Hz, H-3). In the ^{13}C NMR spectrum, except the data of two phenyl groups and four methoxyls, there are also four aliphatic carbons, which were proved to the signals of a tetrahydrofuran moiety from the ^1H-^1H COSY and HMBC correlations. The HMBC correlations from H-2 (δ_H 5.14) to C-1' (δ_C 133.1), C-2' (δ_C 104.6), and C-6' (δ_C 104.6), and from H-3 (δ_H 3.13) to C-1" (δ_C 127.8), C-2" (δ_C 108.6), and C-6" (δ_C 108.6) verified that the two phenyl groups are linked to C-2 and C-3 of tetrahydrofuran, respectively. The large coupling constant of H-2 and H-3 (*J* = 10.5 Hz) and no correlation between them in the NOESY spectrum suggested that H-2 and H-3 are *trans*-relationship. While H-3 and H-4 are *cis*-relationship on the basis of their NOE effect and their small coupling constant of *J* = 4.5 Hz. The CD spectrum displayed a typical coupled Cotton effect, positive at 242 nm (Δε +2.22) and negative at 226 nm (Δε –2.39), indicating an exciton coupling between the π→π* transition of the phenyl chromophores. The

positive chirality revealed the 2*S*,3*S*,4*R* configuration for **2**, which was further confirmed by the calculated ECD data of **2** (Fig. 4). Thus, the structure of cinncassin B (**2**) was determined as (+)-(2*S*,3*S*,4*R*)-2,3-*bis*(4-hydroxy-3,5-dimethoxy)-4-hydroxytetrahydrofuran.

Cinncassin C (**3**) was obtained as a white amorphous powder. HRESIMS exhibited a sodium-adduct $[M+Na]^+$ ion at *m/z* 457.1477 (calcd for $C_{22}H_{26}O_9Na$, 457.1475), suggesting a molecular formula of $C_{22}H_{26}O_9$. The ^1H NMR data showed the signals of two sets of 1,3,4,5-tetrasubstituted phenyl groups like **2** [δ_H 6.65 (2H, s), 6.63 (2H, s), 3.82 (6H, s), 3.81 (6H, s)], a doublet methine at δ_H 4.00 (1H, d, J = 7.0 Hz), along with six aliphatic protons at δ_H 2.23–5.33, five of which are deduced to form a 2,4-disubstituted tetrahydrofuran moiety from the ^1H-^1H COSY correlations of H-2/H-3/H-4/H-5 (Fig. 2). The ^{13}C NMR data (Table 1) showed 22 carbon resonances, comprising 12 olefinic carbons, four methoxy groups, six aliphatic carbons and a carboxylic carbon. In the HMBC spectrum, the correlations from H-5 (δ_H 3.77, 3.65), H-4 (δ_H 2.37), and H-3 (δ_H 2.33, 2.23) to -COOH (δ_C 180.7) suggested that -COOH group was linked at C-4. The HMBC correlations of H-6 (δ_H 4.00) and C-1', 1'' (δ_C 132.8), C-2', 6' (δ_C 107.1), C-2'', 6'' (δ_C 107.6), C-2 (δ_C 82.5), and C-3 (δ_C 30.5) indicated that two tetrasubstituted phenyl units were both connected at C-6. The relative configuration of **3** was deduced from the NOESY correlations of H-4 with H-3α (δ_H 2.23), and H-2 with H-3β (δ_H 2.33). The absolute configuration of **3** was determined by comparing the experimental and calculated ECD spectra. Therefore, the structure of cinncassin C (**3**) was assigned as (–)-(2*S*,

4*R*)-2-[6-*bis*(4-hydroxy-3,5-dimethoxy)]-4-carboxyl-tetrahydrofuran.

Cinncassin D (**4**) was obtained as a white amorphous powder. Its HRESIMS showed a $[M + H]^+$ ion at *m/z* 509.1801 (calcd for $C_{28}H_{29}O_9$, 509.1812), suggesting a molecular formula of $C_{28}H_{28}O_9$. Meanwhile, the IR spectrum indicated that **4** possesseshydroxy (3467 cm^{-1}), aldehyde (1766 cm^{-1}), and phenyl (1593 and 1463 cm^{-1}) functionalities. The NMR data of **4** (Table 2) showed the presence of one aldehyde, one 1,3,4-trisubstituted phenyl, two 1,3,4,5-tetrasubstituted phenyl groups, three methoxyls, two oxygenated methines, two hydroxymethylenes, and two methine groups. The NMR data of **4** were similar to those of vitrifol A[13], except for substitution of the *n*-propanolunit in vitrifol A by an aldehyde group in **4**. The HMBC correlations of -CHO (δ_H 9.79) andC-1" (δ_C 132.8), C-6" (δ_C 122.2), and C-2" (δ_C 113.9) provided support that the aldehyde group is linked at C-1". The *trans*-configurations between H-7 and H-8, and H-7' and H-8' were deduced from their coupling constants of *J* = 6.5 Hz, 6.5 Hz[14,15]. The CD spectrum of **4** showed a positive Cotton effect at 294 nm, indicating a 7*S*,8*R*,7'*S*,8'R configuration[16] and the absolute configuration of **4** was also confirmed by the calculated ECD data. Therefore, the structure of cinncassin D (**4**) was determined to be (–)-(7*S*,8*R*,7'*S*,8'*R*)-2,2',3,3'-tetrahydro-2'-(4-hydroxy-3-methoxyphenyl)-3,3'-*bis*(hydroxymethyl)-7,7'-dimethoxy-5-aldehyde-2,5'-bibenzofuran.

Cinncassin E (**5**) was obtained as a white amorphous powder. The HRESIMS displayed a pseudomolecular ion $[M–H]^-$at *m/z* 403.1391 (calcd for $C_{21}H_{23}O_8$, 403.1393), consistent with amolecular formula of $C_{21}H_{24}O_8$. The IR absorption bands

indicated the presence of hydroxy (3466 cm^{-1}), aldehyde (1734 cm^{-1}), and phenyl (1596 and 1511cm^{-1}) functionalities. The ^{1}HNMR spectrum revealed the signals for one aldehyde proton at δ_H 9.58 (1H, d, J = 8.0 Hz), a set of ABX coupled phenyl protons at δ_H 7.22 (1H, d, J = 2.0 Hz), 7.16 (1H, dd, J = 8.5, 2.0 Hz), and 7.00 (1H, d, J = 8.5 Hz), a pair of symmetric 1,3,4,5-tetrasubstituted aromatic protons at δ_H 6.72 (2H, s), two *trans*-olefinic protons at δ_H 7.57 (1H, d, J = 16.0 Hz) and 6.66 (1H, d, J = 16.0, 8.0 Hz), four aliphatic protons at δ_H 4.80 (1H, d, J = 6.5 Hz), 4.56 (1H, m), and 3.87 (2H, d, J = 5.0 Hz), and three methoxy groups at δ_H 3.83 (3H, s) and 3.79 (6H, s). The ^{1}H-^{1}H COSY correlations of H-7 (δ_H 4.80)/H-8 (δ_H 4.56)/H_2-9 (δ_H 3.87) and H-7' (δ_H 7.57)/H-8' (δ_H 6.66)/H_2-9' (δ_H 9.58) in combination with HSQC and HMBC correlations revealed the presence of 1,2,3-phenylpropanetriol and cinnamaldehyde groups. The HMBC correlation of H-8 and C-4' verified the 8-*O*-4' linkage; the correlations of methoxyls to C-3', C-3, and C-5 revealed the linkage positions of the methoxyls. The large coupling constant of H-7 and H-8 (J = 6.5 Hz) suggested that H-7 and H-8 are in the *threo*-form[17]. Meanwhile, the CD spectrum showed a negative Cotton effect at 230 nm ($\triangle\varepsilon$ –0.58) , indicating that **5** has a 8*R* absolute configuration[17,18]. Thus, the structure of cinncassin E (**5**) was determined to be (–)-*threo*-(7*R*,8*R*)-7'*E*-4,7,9-trihydroxy-3,3',5-trimethoxy-8-4'-oxyneolignan-7'-en-9'-al.

Cinnacassoside D (**6**) was obtained as a white amorphous powder. The molecular formula of **6** was assigned as $C_{15}H_{22}O_8$ based on the HRESIMS peak at *m/z* 329.1236[M–H]$^-$(calcd for $C_{15}H_{21}O_8$ 329.1236). The IR spectrum of **6** showed the

presence of hydroxy (3367 cm^{-1}) and phenyl (1650, 1601, 1453 cm^{-1}) functionalities. The NMR data of **6** (Table 2) showed the presence of one monosubstituted phenyl [δ_H 7.43 (2H, d, *J* = 7.5 Hz), 7.34 (2H, t, *J* = 7.5 Hz) and 7.26 (1H, t, *J* = 7.0 Hz)], two oxygenated methines [δ_H 4.91 (1H, d, *J* = 4.0 Hz) and 3.95 (1H, m)], one hydroxymethyl [δ_H 3.64 (2H, m)], and a set of *β*-glucopyranosyl moiety [δ_H 4.39 (1H, d, *J* = 7.5 Hz)]. The HMBC correlations from H-7 to C-1, C-2, C-6, C-8 and C-9, as well as ^1H-^1H correlations of H-7/H-8/H_2-9 suggested that **6** is a phenylpropanetriol glucoside. The HMBC correlations from H-8 to C-1 and C-1', and from H-1' to C-8 revealed that the glucopyranosyl moiety is attached to C-8. Based on the above evidences, the structure of **6** was elucidated to be 8-(1-*O*-*β*-glucopyranosyl)-phenylpropanetriol. The small coupling constant of H-7 and H-8 (*J* = 4.0 Hz) and Δ_{C8-C7} = 11.4 ppm (<12.0 ppm) suggested that H-7 and H-8 were in the *erythro*-form[19]. Acid hydrolysis of **6** gave glucose and phenylpropanetriol. The positive [α] value{ $[\alpha]^{20}_D$ +13.7 (*c* 0.1, MeOH)} of phenylpropanetriol indicated that its absolute configuration is 7*S*,8*S*[20,21].The absolute configuration of the glucosyl was determined by analysis of its silane derivative on GC. Therefore, the structure of cinnacassoside D (**6**) was determined to be (+)-(7*S*,8*S*)-8-(1-*O*-*β*-D-glucopyranosyl)-phenylpropanetriol.

The known compounds were identified as (+)-*threo*-(7*S*,8*S*)-guaiacylglycerol-*β*-coniferyl aldehyde ether (**7**)[17], (+)-*erythro*-(7*S*,8*R*)-guaiacylglycerol-*β*-coniferyl aldehyde ether (**8**)[17], (–)-*erythro*-(7*R*,8*S*)-guaiacylglycerol-*β*-*O*-4'-sinapoyl ether (**9**)[22],

(–)-*erythro*-(7*S*,8*R*)-syringylglycerol-8-*O*-4'-(sinapoyl alcohol) ether(**10**)[23], (+)-*erythro*-(7*R*,8*S*)-guaiacylglycerol-8-vanillin ether(**11**)[17,24], (7*S*,8*R*)-lawsonicin (**12**)[14,25], 5'-methoxylariciresinol(**13**)[26], (+)-(7'*R*,8*R*,8'*R*)-5,5'-dimethoxylariciresinol (**14**)[27,28], (+)-(7'*S*,8*R*,8'*R*)-5,5'-dimethoxylariciresinol (**15**) [27,28], picrasmalignan A (**16**)[29], (+)-leptolepisol C (**17**)[19,23], (–)-(7*R*,8*S*,7'*R*,8'*S*)-syringaresinol (**18**)[14,30,31], (+)-isolariciresinol (**19**)[32], and (–)-secroisolariciresinol (**20**)[33,34] on the basis of spectroscopic data analysis and comparison with the physical data reported in the literature (Fig. 1).

One of the clinical usages of *C. cassia* is for the treatment of rheumatoid arthritis and osteoarthritis [1], therefore, LPS-induced NO production in BV-2 microglial cells was used for the evaluation of the anti-inflammatory activities of the isolates of **1**–5 and 7–**20**, and their IC_{50} values are presented in Table 3. Based on the activity assay data, it could be observed that compounds **4**, **5**, **7**, **8**, **10**, **12**, and **15** showed potent inhibitory effects on NO production in LPS-induced BV-2 cells with IC_{50} values of 24.2, 17.6, 17.7, 18.7, 27.0, 20.5, and 17.5 μM respectively. Among the active compounds, the majority are 8-*O*-4'-lignans, so their preliminary structure activity relationship was summarized as follows: 1) compounds with acrylaldehyde group showed potent anti-inflammatory activities, such as compounds **5**, **7**, and **8**. But when the acrylaldehyde was changed to aldehyde, the inhibitory effect became much weaker, such as compound **11**; 2) compounds with acrylaldehyde group showed stronger activity than those having allyl alcohol group (**5**, **7**, **8** > **9**, **10**); 3) the methoxy group in the phenyl did not affect the activities of the compounds with

acrylaldehyde group, for example, the activity of **5** is similar to those of **7** and **8**, whereas it could increase the activity when the acrylaldehyde group was reduced to the allyl alcohol group, such as **10**>**9**.

The above results indicate that most of the compounds isolated from *C. cassia* have potent anti-inflammatory effects, which makes a partial contribution for the anti-inflammatory activity of *C. cassia*. The isolates with significant anti-neuroinflammatory activity have potential to be used as candidates for the treatment of various neurodegenerative diseases.

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, inthe online version, athttp://dx.doi.org...

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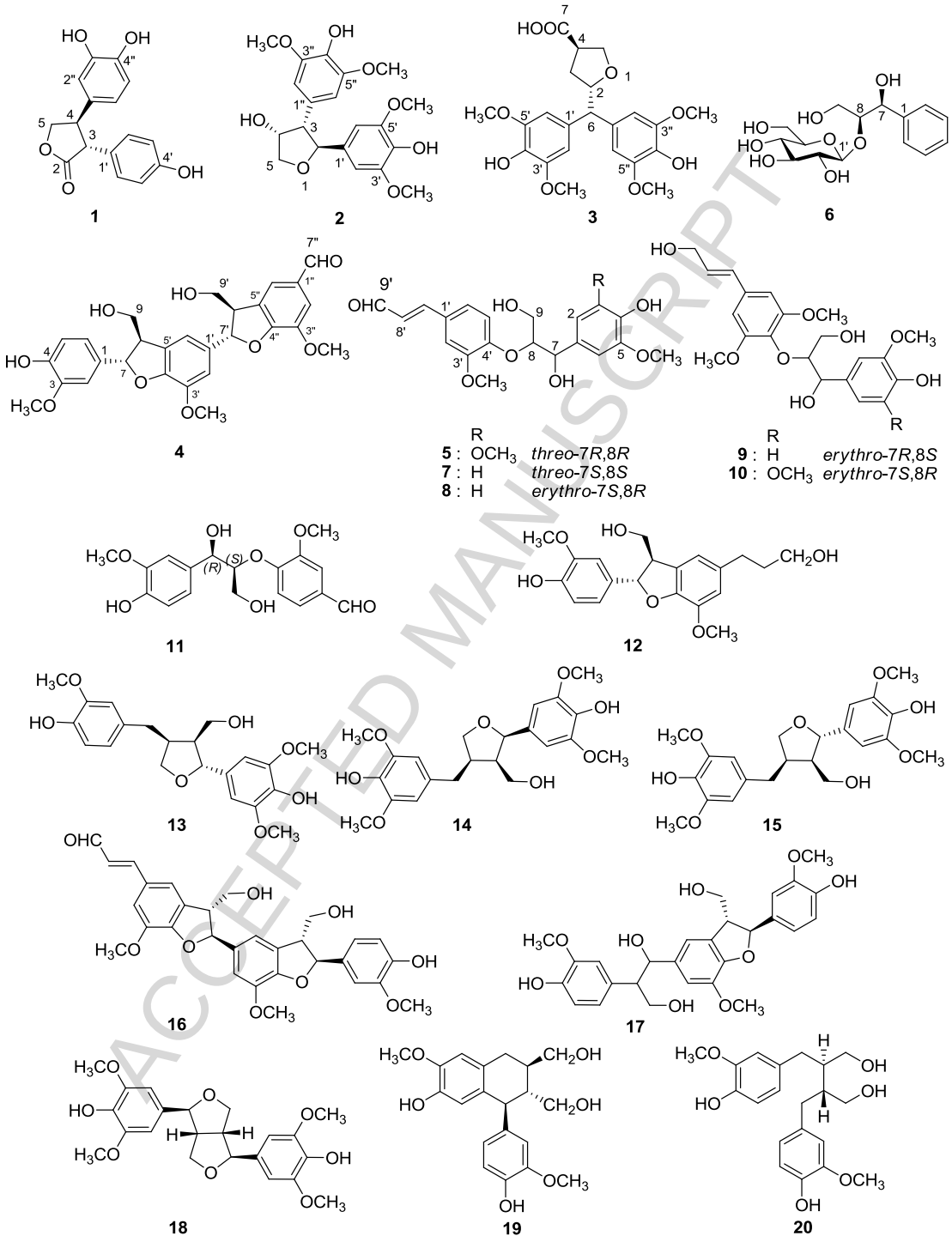


Fig. 1. Structures of compounds **1**–**20** isolated from *C. cassia*.

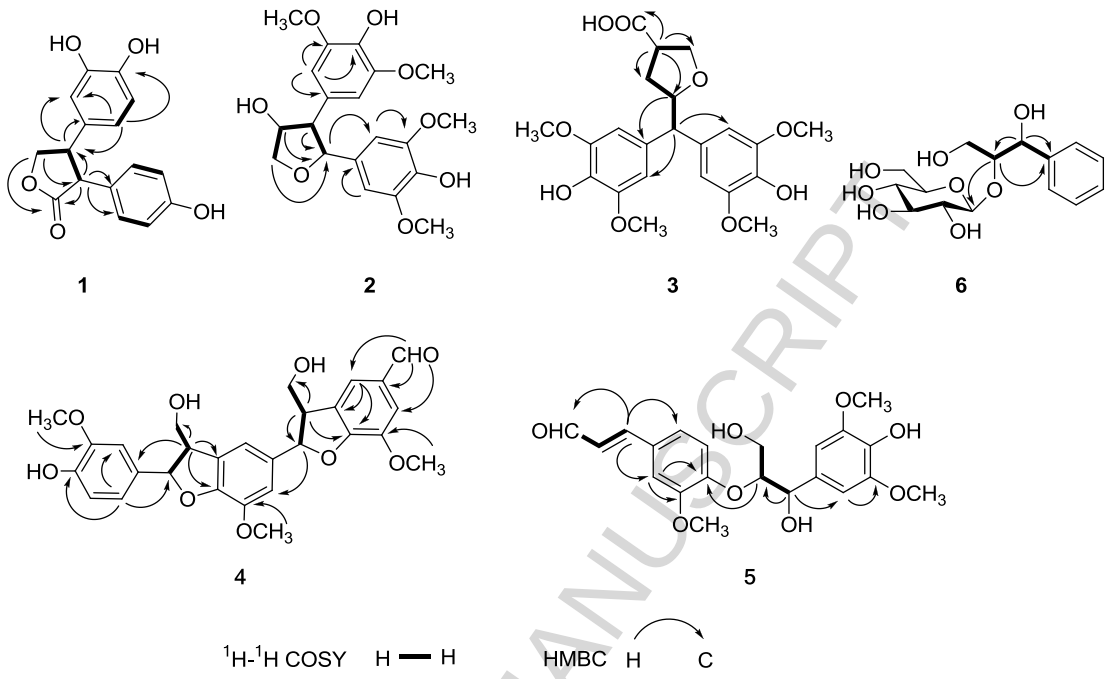


Fig. 2. Key HMBC and ^{1}H-^{1}H COSY correlations of compounds **1**–**6**.

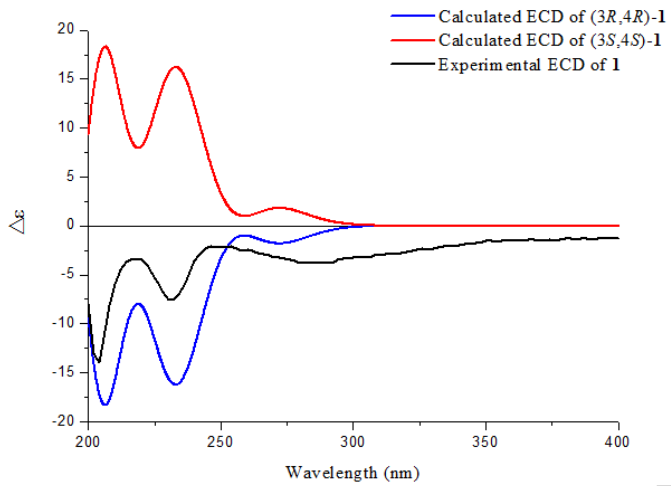


Fig. 3. Comparison of the experimental ECD spectrum of **1** in MeOH with the calculated ECD spectra of (3*R*,4*R*)-**1** and (3*S*,4*S*)-**1**.

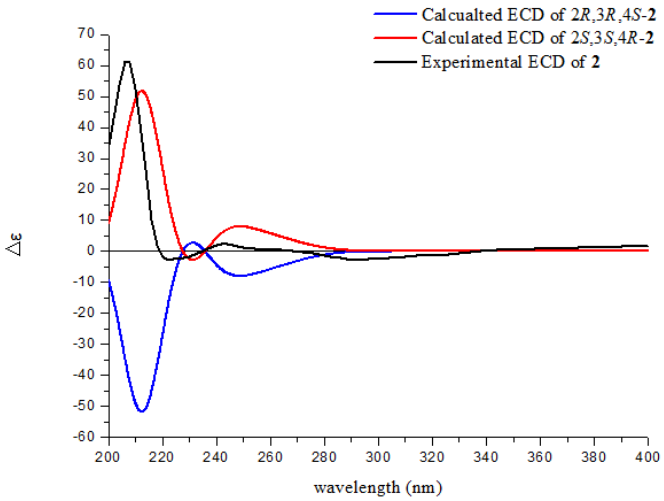
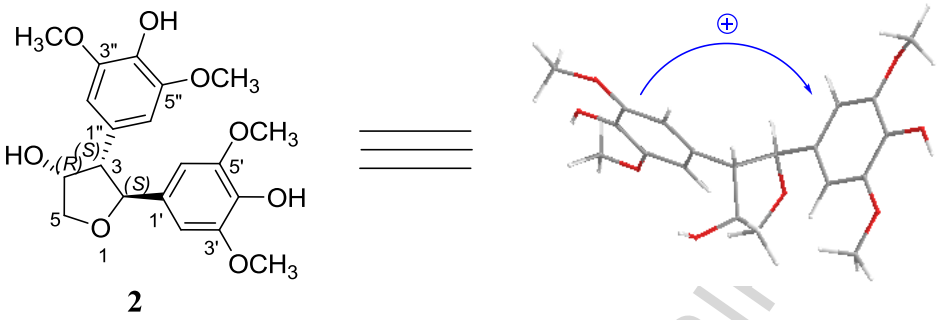


Fig. 4. Comparison of the experimental ECD spectrum of **2** in MeOH with the calculated ECD spectra of (2*R*,3*R*,4*S*)-**2** and (2*S*,3*S*,4*R*)-**2**.



2

Fig. 5. The CD exciton chirality rule applied of **2**.

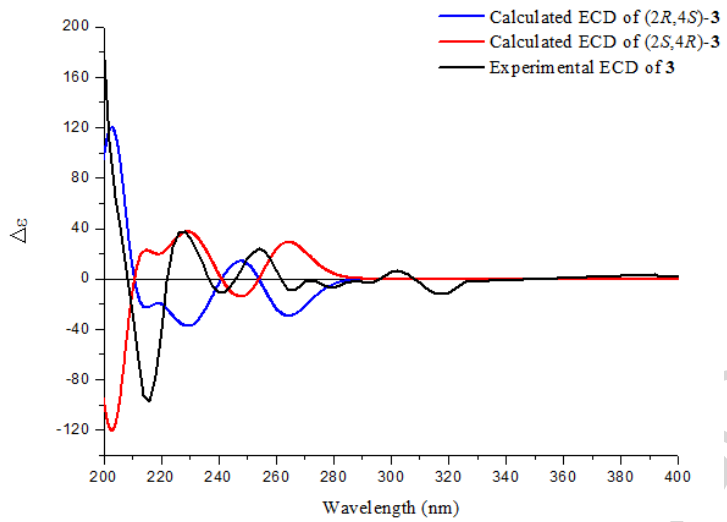


Fig. 6. Comparison of the experimental ECD spectrum of **3** in MeOH with the calculated ECD spectra of (2*S*,4*R*)-**3** and (2*R*,4*S*)-**3**.

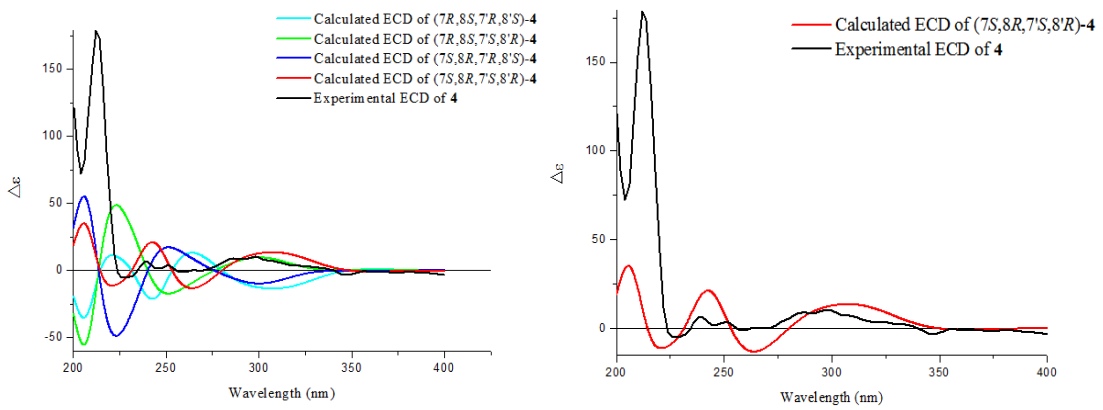


Fig.7. Comparison of the experimental ECD spectrum of **4** in MeOH with the calculated ECD spectra of (7*S*,8*R*,7'*S*,8'*R*)-**4**, (7*S*,8*R*,7'*R*,8'*S*)-**4**,(7*R*,8*S*,7'*S*,8'*R*)-**4** and (7*R*,8*S*,7'*R*,8'*S*)-**4**.

Table 1

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compounds **1**, **2**, and **3** in CD_3OD (δ in ppm).

Position	**1**		**2**		**3**	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
2		179.9	5.14 (d, 10.5)	84.7	5.33 (dt, 6.5, 12.5)	82.5
3	3.91 (d, 12.0)	53.9	3.13 (dd, 10.5, 4.5)	60.5	2.23 (m) 2.33 (m)	30.5
4	3.68 (td, 12.0, 8.5)	51.6	4.47 (t, 4.5)	75.8	2.37 (m)	44.0
5	4.61 (br t, 8.5) 4.26 (br t, 8.5)	73.4	4.43 (dd, 9.5, 4.5) 4.01 (d, 9.5)	77.1	3.77 (m) 3.65 (m)	62.2
6					4.00 (d, 7.0)	57.2
7						180.7
1'		128.1		133.1		132.8
2',6'	7.00 (d, 8.5)	130.8	6.53 (s)	104.6	6.63 (s)	107.1
3',5'	6.73 (d, 8.5)	116.4		149.0		149.2
4'		157.9		135.6		133.6
1"		130.1		127.8		132.8
2"	6.69 (d, 1.0)	115.6	6.65 (s)	108.6	6.65 (s)	107.6
3"		146.6		149.0		149.3
4"		145.9		135.7		133.6
5"	6.71 (d, 7.5)	116.6		149.0		149.3
6"	6.60 (dd, 7.5, 1.0)	119.9	6.65 (s)	108.6	6.65 (s)	107.6
3',5'-OCH_3			3.73 (s)	56.6	3.82 (s)	56.8
3",5"-OCH_3			3.82 (s)	56.8	3.81 (s)	56.9

Table 2

The ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectroscopic data of compounds **4**, **5** and **6** in CD_3OD (δ in ppm).

Position	**4**		**5**		**6**	
	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C
1		134.4		131.5		142.2
2	6.95 (br s)	110.6	6.72 (s)	105.8	7.43 (d, 7.5)	127.9
3		149.1		148.9	7.34 (t, 7.5)	129.2
4		147.6		133.1	7.26 (t, 7.0)	128.5
5	6.76 (d, 8.0)	116.2		148.9	7.34 (t, 7.5)	129.2
6	6.82 (dd, 8.0, 1.0)	119.7	6.72 (s)	105.8	7.43 (d, 7.5)	127.9
7	5.53 (d, 6.5)	89.3	4.80 (d, 6.5)	74.3	4.91 (d, 4.0)	74.5
8	3.50 (m)	55.2	4.56 (m)	85.2	3.95 (m)	85.9
9	3.79 (m)	64.8	3.87 (d, 5.0)	62.6	3.64 (m)	62.3
					3.64 (m)	
1'		135.7		129.1	4.39 (d, 7.5)	104.0
2'	6.92 (br s)	112.0	7.22 (d, 2.0)	112.6	3.24–3.35 (m)	75.2
3'		145.6		151.7	3.24–3.35 (m)	78.0
4'		149.7		152.7	3.24–3.35 (m)	71.5
5'		130.6	7.00 (d, 8.5)	117.0	3.24–3.35 (m)	77.8
6'	6.95 (br s)	115.9	7.16 (dd, 8.5, 2.0)	124.4	3.54 (dd, 12.0, 2.5)	62.6
					3.85 (m)	
7'	5.70 (d, 6.5)	90.7	7.57 (d, 16.0)	155.5		
8'	3.65 (m)	54.4	6.66 (dd, 16.0, 8.0)	127.6		
9'	3.88 (m)	64.5	9.58 (d, 8.0)	196.1		
1"		132.8				
2"	7.46 (br s)	113.9				
3"		146.3				
4"		155.5				
5"		131.1				
6"	7.52 (d, 1.0)	122.2				
7"	9.79 (s)	192.7				

OCH_3-3''	3.93 (s)	56.9		
OCH_3-3'	3.85 (s)	56.7	3.83 (s)	56.6
OCH_3-3	3.81 (s)	56.4	3.79 (s)	56.7
OCH_3-5			3.79 (s)	56.7

Table 3

Inhibitory effects of compounds **1**–5, 7–**20** isolated from *C. cassia* on NO production induced by LPS in BV-2 cells.

Compounds	IC_{50} (μM)	Compounds	IC_{50} (μM)
1	51.3	**12**	20.5
2	48.7	**13**	31.2
3	39.4	**14**	39.4
4	24.2	**15**	17.5
5	17.6	**16**	–
7	17.7	**17**	>100
8	18.7	**18**	50.9
9	37.0	**19**	73.4
10	27.0	**20**	–
11	42.0	Quercetin[a]	15.6

[a]Quercetin was used as a positive control.

Graphical abstract