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3, 4-*seco*-Labdane diterpenoids from the leaves of *Callicarpa nudiflora* with anti-inflammatory effects

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[ABSTRACT] Four new 3, 4-*seco*-labdane diterpenoids, nudiflopenes J−M, were isolated from the leaves of *Callicarpa nudiflora* along with six known compounds. The structures of these diterpenoids were determined by comprehensive spectroscopic analysis. All the isolated compounds were evaluated for their inhibitory effects on NO production in LPS-stimulated RPMs and RAW264.7 cells. The results suggest that nudiflopenes J−M and other four known compounds showed significant inhibitory effects against NO production comparable to the positive control dexamethasone.

[KEY WORDS] *Callicarpa nudiflora*; Verbenaceae; 3, 4-*seco*-Labdane diterpenoid; Anti-inflammatory; RPMs; RAW264.7

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Introduction

Callicarpa nudiflora Hook. et Arn., belonging the genus *Callicarpas* of the family Verbenaceae, is a shrub or small tree distributed mainly along tropical and subtropical Asia and Oceanica [1]. The leaves of *Callicarpa nudiflora,* which are included in the Chinese Pharmacopoeia, are commonly used as a traditional Chinese folk medicine for the treatment of respiratory tract infections, hepatitis and bleeding [2]. *C. nudiflora* exhibits anti-inflammatory, hemostatic, antibacterial, cytotoxic and immuno-enhancing activities [3]. The chemical constituents of *C. nudiflora* mainly include diterpenoids, triterpenoids, flavonoids and phenolic acids [4−10]. In a continuing search for anti-inflammatory agents, four undescribed 3, 4-*seco*-Labdane diterpenoids and six other known compounds were isolated. Their anti-inflammatory effects were evaluated by measuring NO release in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage and resident peritoneal macrophages (RPMs).

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These authors have no conflict of interest to declare.

Results and Discussion

Structural elucidation

The air-dried leaves of *C. nudiflora* were extracted with methanol. The crude extract was suspended in H_2O and then partitioned excessively with petroleum ether and chloroform. The chloroform fraction was repeatedly subjected to silica gel, reversed phase (RP) C_{18} silica gel, and Sephadex LH-20 column chromatography as well as HPLC with an Cosmosil Packed Column 5PYE to yield four undescribed 3, 4-*seco*-labdane diterpenoids and six known compounds. The structures of compounds **1**−**10** are presented (Fig. 1).

Compound **1**, a colorless oil, had a molecular formula of $C_{19}H_{28}O_3$ by HR-ESI-MS at *m/z* 303.1955 $[M - H]^-$ (Calcd. for 303.1966 $[M - H]^-$). The ^1H NMR (Table 1) and ^{13}C NMR spectrum (Table 2) indicated the existence of an iso-

propenyl group [δ_H 1.78 (3H, br s), 4.77(1H, br s), 4.91 (1H, br s), δc 24.1, 114.8, 147.9], olefinic methylene signals [δ_H 4.44 (1H, br s), 4.83 (1H, br s), δc 109.8, 149.8], a methyl bound to a quaternary carbon [δ_H 0.96 (3H, s), δc 17.6], a carboxylic group [δc 175.9] and methoxy signals[δ_H 3.61(3H, s), δc 51.7]. The remaining signals were at higher field and were deduced to be four methylenes, two methines, and one quaternary carbon from the DEPT spectra data. These spectroscopic features suggested that compound **1** might be a diterpenoid. The HMBC and ^{1}H-^{1}H COSY correlations (Fig. 2) further indicated that compound **1** had a 3, 4-*seco*-labdane skeleton similar to that of ent-3, 4-*seco*-14-carbonyl-15, 16-epoxy-4(18), 8(17), 13(14)-labdatrien-3-oic acid [8]. However, some differences in the chemical shifts for the side

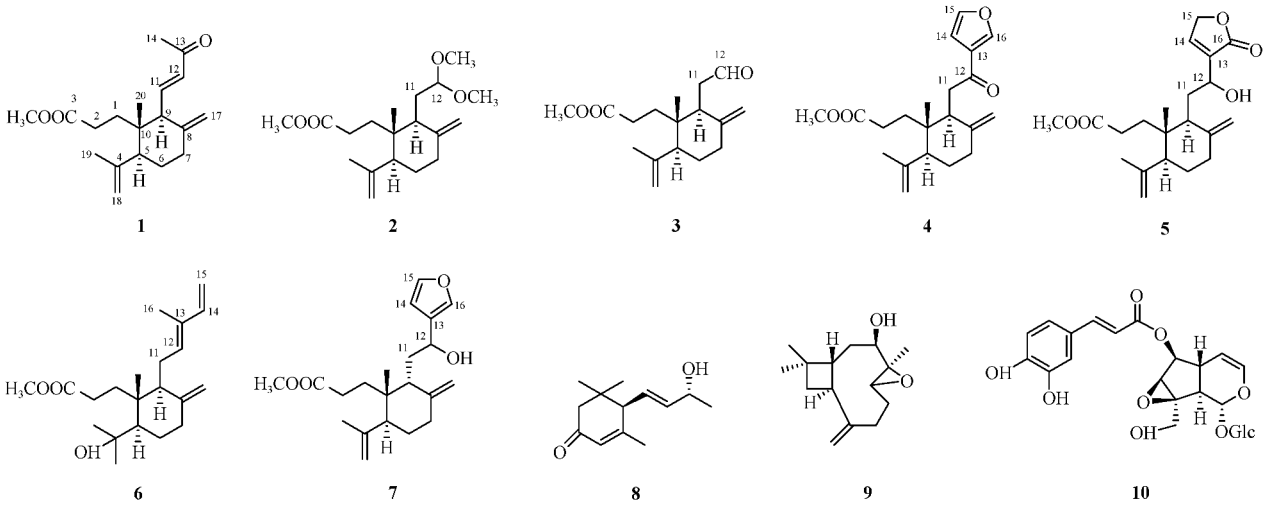

Fig. 1 Structures of compounds 1−10

Table 1 The ^{1}H NMR data for compounds 1 and 3−5 (400 MHz)

No.	1 [a]	3 [b]	4 [b]	5 [a]
1	1.42 (1H, m) 1.67 (1H, m)	1.58 (1H, m) 1.79 (1H, m)	1.61 (1H, m) 1.79 (1H, m)	1.59 (1H, m) 1.66 (1H, m)
2	2.35 (1H, m) 2.47 (1H, m)	2.25 (1H, m) 2.48 (1H, m)	2.32 (1H, m) 2.49 (1H, m)	2.44 (1H, m) 2.71 (1H, m)
5	2.33 (1H, m)	2.31 (1H, m)	2.35 (1H, m)	2.36 (1H, m)
6	1.58 (1H$_\alpha$, m) 1.82 (1H$_\beta$, m)	1.61 (1H$_\alpha$, m) 1.73 (1H$_\beta$, m)	1.61 (1H$_\alpha$, m) 1.73 (1H$_\beta$, m)	1.59 (1H$_\alpha$, m) 1.74 (1H$_\beta$, m)
7	2.16 (1H$_\alpha$, m) 2.41 (1H$_\beta$, m)	2.09 (1H$_\alpha$, td, 13.0, 5.2) 2.38 (1H$_\beta$, m)	2.15 (1H$_\alpha$, td, 13.1, 5.0) 2.35 (1H$_\beta$, m)	2.07 (1H$_\alpha$, td, 13.0, 5.0) 2.38 (1H$_\beta$, m)
9	2.88 (1H, br d, 10.4)	2.52 (1H, br d, 12.1)	2.83 (1H, br d, 11.2)	2.38 (1H, m)
11	7.01 (1H, dd, 16.0, 10.4)	2.35 (1H, m) 2.65 (1H, m)	2.60 (1H, dd, 17.1, 2.1) 3.12 (1H, dd, 17.1, 11.2)	1.40 (1H, br dd, 14.0, 10.8) 1.99 (1H, ddd, 14.0, 12.0, 1.0)
12	6.19 (1H, d, 16.0)	9.67 (1H, br d, 3.1)	–	4.45 (1H, br d, 10.8)
13	–	–	–	–
14	2.29 (3H, s)	–	6.78 (1H, dd, 1.9, 0.8)	7.52 (1H, br s)
15	–	–	7.44 (1H, dd, 1.9, 1.4)	4.86 (2H, br s)
16	–	–	8.11 (1H, dd, 1.4, 0.8)	–
17	4.44 (1H, br s) 4.83 (1H, br s)	4.45 (1H, br s) 4.87 (1H, br s)	4.37 (1H, br s) 4.75 (1H, br s)	4.94 (1H, br s) 4.99 (1H, br s)
18	4.77 (1H, br s) 4.91 (1H, br s)	4.71 (1H, br s) 4.89 (1H, br s)	4.72 (1H, br s) 4.89 (1H, br s)	4.70 (1H, br s) 4.87 (1H, br s)
19	1.78 (3H, br s)	1.74 (3H, br s)	1.76 (3H, br s)	1.74 (3H, br s)
20	0.96 (3H, s)	0.74 (3H, s)	0.80 (3H, s)	0.72 (3H, s)
OCH_3	3.61 (3H, s)	3.65 (3H, s)	3.62 (3H, s)	3.64 (3H, s)

[a] Measured in CD_3OD; [b] in $CDCl_3$

chain were observed between them. The side chain connectivity was deduced from HMBC correlations shown by H-11 (δ_H 7.01) with C-13 (δ_C 199.4), C-8 (δ_C 149.8), C-9 (δ_C 54.6) and C-10 (δ_C 42.0); H-12 (δ_H 6.19) with C-13 (δ_C 200.8), C-11 (δ_C 147.9), C-9 (δ_C 54.6) and C-14 (δ_C 27.2); and H_3-14 (δ_H 2.29) with C-13 (δ_C 200.8), C-12 (δ_C 134.5) and C-11 (δ_C 147.9), indicating that the side-chain moiety was attached to C-9. Thus, the planar structure of compound **1** was finally confirmed.

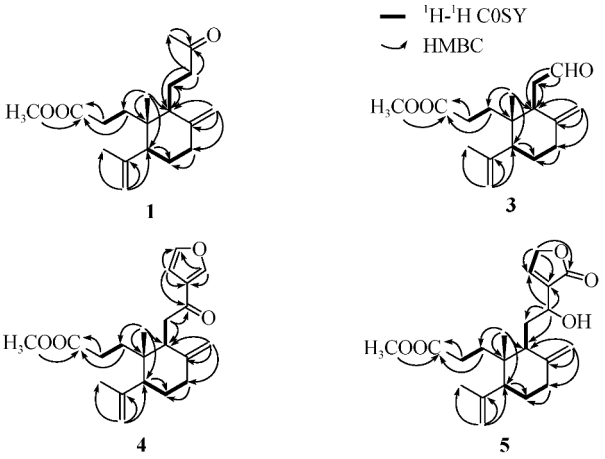

Fig. 2 ^{1}H-^{1}H COSY and key HMBC correlations of compounds 1 and 3–5

The relative configuration of compound **1** was determined by a NOESY experiment (Fig. 3). The correlations of H-9/H-7α, H-9/H-5, H-5/H-7α, H-11/H-17, H_3-20/H-11, H_3-20/H-18 and H_3-20/H-7β suggested that the six-membered ring exists in a twist-chair conformation with H-5 and H-9 in α-axial positions and Me-20 in a β-axial position.

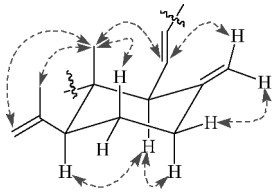

Fig. 3 Key NOESY correlations of compound 1

The absolute configuration of compound **1** was deduced by comparing the experimental and calculated CD spectra (Fig. 4). Its geometrie was optimized with the software package Gaussian 09 (Revision A.01, 2009, Gaussian, Inc., Wallingford CT) at the B3LYP/6-31G (2d, p) level. Then the 60 lowest electronic transitions were calculated using time-dependent density functional theory (TD-DFT) methods [11] at the B3LYP/6-31G (2d, p) level. The overall theoretical CD spectra were obtained according to the Boltzmann weighting of each conformers. In the CD spectra, the band at ca. 221.5 nm of **1a** (5*S*, 9*S*, 10*S*-isomer) and **1b** (5*R*, 9*R*, 10*R*-isomer) were positive cotton effect and negative cotton effect respectively. The theoretically calculated CD spectra of **1a** was in good agreement with the experimental CD spectra of compound 1, which suggested the absolute configurations of chiral carbons in six-membered ring to be 5*S*, 9*S*, and 10*S*. Based on the above results, the structure of compound **1** was defined and given the name nudiflopene J according methods of predecessors [4].

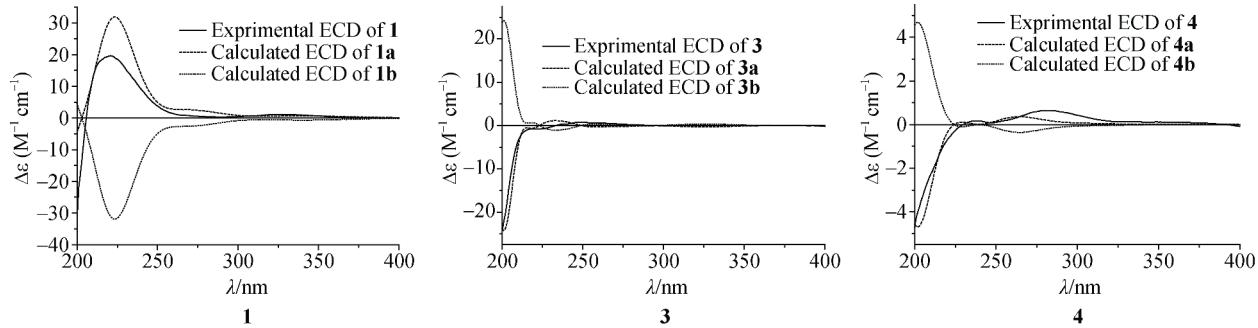

Fig. 4 The CD spectrum of compounds 1, 3 and 4

Compound **3** was obtained as a colorless oil and possessed the molecular formula $C_{17}H_{26}O_3$ as determined by HR-ESI-MS at *m/z* 277.1798 $[M - H]^-$ (Calcd. for 277.1809 $[M - H]^-$). The ^{1}H NMR (Table 1) and ^{13}C NMR (Table 2) spectroscopic data of compound **3** revealed the presence of a 3, 4-*seco*-labdane skeleton, which indicated that its structure was similar to that of compound **1** with the exception of the side chain. The ^{1}H and ^{13}C NMR spectrum revealed the appearance of one methylene [δ_H 2.65 (1H, m, H-11a), 2.35(1H, m, H-11b) and δ_C 39.6 (C-11)] and one aldehyde group [δ_H 9.67 (1H, br d, *J* = 3.1 Hz, H-12) and δ_C 202.2 (C-12)], and HMBC correlations (Fig. 2) from H-12 to C-11 and C-9 implied the residual moiety formed a acetaldehyde placed at C-11. The NOESY spectrum disclosed that the six-membered ring in compound **3** had the same relative configurations as **1**. The absolute configurations of chiral carbons C-5, C-9, and C-10 in the six-membered ring were inferred to be 5*S*, 9*S*, and 10*S* *via* comparison of experimental and calculated CD spectra (Fig. 4). Thus, the structure of compound **3** was defined and given the name nudiflopene K.

Compound **4** was obtained as white amorphous powder and possessed the molecular formula $C_{21}H_{28}O_4$ as determined

by HR-ESI-MS at *m/z* 343.1907 $[M - H]^-$ (Calcd. for 343.1915 $[M - H]^-$). The ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectroscopic data of compound **4** also revealed the presence of a 3, 4-*seco*-labdane skeleton, which indicated that the structure was similar to that of compounds **1**–**3** with the exception of the side chain. The residual moiety comprising C-12–C-16 was extrapolated to form a partially *β*-substituted furan ring olefin attached at C-13, which was confirmed by the ^1H-^1H COSY coupling of H-14/H-15 and the HMBC couplings of H-14 to C-12, C-13, C-15 and C-16. Similarly, HMBC correlations (Fig. 2) from H_2-11 to C-12, C-10, C-9 and C-8 implied the furan moiety placed at C-12 and the side chain placed at C-11. The NOESY spectrum disclosed that the six-membered ring in compound **4** had the same relative configurations as **1**. The absolute configurations of chiral carbons C-5, C-9, and C-10 in the six-membered ring were inferred to be 5*S*, 9*S*, and 10*S* *via* comparison of experimental and calculated CD spectra (Fig. 4). Thus, the structure of compound **4** was defined and given the name nudiflopene L.

Table 2 The ^{13}C NMR data for compounds 1 and 3–5 (100 MHz)

No.	1 [a]	3 [b]	4 [b]	5 [a]
1	35.2 (t)	32.4 (t)	32.7 (t)	33.5 (t)
2	28.7 (t)	27.8 (t)	27.8 (t)	28.2 (t)
3	175.9 (s)	174.0 (s)	174.3 (s)	176.5 (s)
4	147.9 (s)	146.6 (s)	146.9 (s)	148.7 (s)
5	51.3 (d)	50.5 (d)	50.5 (d)	51.8 (d)
6	30.4 (t)	29.6 (t)	29.7 (t)	31.5 (t)
7	37.1 (t)	36.9 (t)	36.9 (t)	38.9 (t)
8	149.8 (s)	147.3 (s)	148.2 (s)	148.7 (s)
9	54.6 (d)	43.3 (d)	43.5 (d)	45.6 (d)
10	42.0 (s)	40.6 (s)	40.5 (s)	41.9 (s)
11	147.9 (d)	39.6 (t)	36.0 (t)	32.2 (t)
12	134.5 (d)	202.2 (d)	193.5 (s)	65.9 (d)
13	200.8 (s)	–	128.1 (s)	139.3 (s)
14	27.2 (q)	–	108.7 (d)	147.6 (d)
15	–	–	144.2 (d)	72.3 (t)
16	–	–	146.8 (d)	175.3 (s)
17	109.8 (t)	108.9 (t)	107.2 (t)	108.9 (t)
18	114.8 (t)	114.1 (t)	113.9 (t)	114.1 (t)
19	24.1 (q)	23.5 (q)	23.6 (q)	24.0 (q)
20	17.6 (q)	17.6 (q)	18.0 (q)	18.2 (q)
OCH_3	52.1 (q)	51.7 (q)	51.6 (q)	52.1 (q)

[a] Measured in CD_3OD; [b] in $CDCl_3$

The molecular formula of compound **5** was assigned as $C_{21}H_{30}O_5$ according to the HR-ESI-MS ion at *m/z* 361.2011 $[M - H]^-$ (Calcd. for 361.2021 $[M - H]^-$). The 1D (Tables 1 and 2) and 2D NMR data of compound **5** showed high similarity to those of compound **4**, implying a structurally similar 3, 4-*seco*-labdane skeleton. The residual moiety comprising C-12–C-16 was extrapolated to form a partially *α*, *β*-unsaturated-*α*-lactone moiety placed at C-13, which was confirmed by the ^1H-^1H COSY coupling of H-14/H-15 and the HMBC couplings of H-15 to C-16 and C-14 as well as H-14 to C-12. The ^1H and ^{13}C NMR spectrum also revealed the appearance of a methine group bearing a hydroxyl group [δ_H 4.45 (1H, br d, J = 10.8) and δ_C 65.9(C-12)], and HMBC correlations (Fig. 2) from H-12 to C-13, C-14, C-11 and C-9 implied the *α*, *β*-unsaturated-*α*-lactone moiety placed at C-12 and the side chain placed at C-11. The NOESY spectrum disclosed that the six-membered ring in compound **5** had the same relative configuration as compound **1**. The absolute configurations of chiral carbons C-5, C-9 and C-10 in the six-membered ring were not confirmed *via* comparison of experimental CD spectra because of its own reason of **5**. The relative configuration of compound **5** was defined and given the name nudiflopene M.

The structure of the known compounds **2** and **6**–**10** were elucidated as nudiflopene E (**2**) [4]; nudiflopene I (**6**) [4]; *syn*-3, 4-*seco*-12*R*-hydroxy-15, 16-epoxy-4(18), 8(17), 13(16), 14(15)-labdatetraen-3-oic acid (**7**) [8]; (7*E*, 6*R*, 9*S*)-9-hydroxy-4, 7-megastigmadien-3-one (**8**) [12]; suberosols B (**9**) [13]; and nudifloside (**10**) [14] by comparison of their spectroscopic data with those of published papers.

Effect of compounds 1-10 on NO expression level

Macrophages are versatile cells that play many roles. Some stimuli, such as lipopolysaccharide (LPS), could active macrophages to produce pro-inflammatory mediators to induce inflammation. Nitric oxide (NO) is one of the proinflammatory factors that could induce inflammation in bodies. NO could activate the nuclear factor kappa B (NF-*κ*B) pathway to induce the expression proinflammatory cytokines, such as interleukin 1*β* (IL-1*β*) and tumor necrosis factor α (TNF-*α*). During inflammation, NO overexpression enhances the inflammatory effect and cause harm to normal cells. Therefore, we evaluated the anti-inflammatory activity of compounds **1**–**10** using the LPS-stimulated RAW264.7 cell model and LPS-stimulated RPM cell model (Fig. 5). As shown in Fig. 5A, compounds **5**, **6**, and **9** exhibited a significant effect on reducing the NO expression level in the culture medium in LPS-stimulated RPMs. Fig. 5B demonstrates that compounds **1**–**3**, **5**–**7**, and **9** exhibited significant effects on reducing the NO expression level in the culture medium in LPS-stimulated RAW 264.7 cells. Given that the RPMs cell model is more similar to the *in vivo* conditions than the RAW264.7 cell model, Compounds **5**, **6**, and **9**, which reduced the NO expression level in the RPM cell model and RAW 264.7 cell model, may have better potential value for future developments and studies.

Interestingly, in the RPMs, dexamethasone (DEX) exhibited a better NO reducing ability than compound **5**; however, compound **5** inhibited NO expression more in RAW 264.7 cells compared with RPMs. The RAW 264.7 cell line is a murine macrophage cell line established from an ascites of a tumor induced in a male mouse by intraperitoneal injection of

Abselon Leukemia Virus (A-MuLV). The RPMs were isolated from SD Rats. The species difference of these two types of cells may cause the different results in the study. In addition, RAW 264.7 cells are immortalized cells, whereas RPMs are primary cells. Since the phenotype and function of the cells may change with continuous culture [15], these two types of cells may not react similar to the positive control drug DEX.

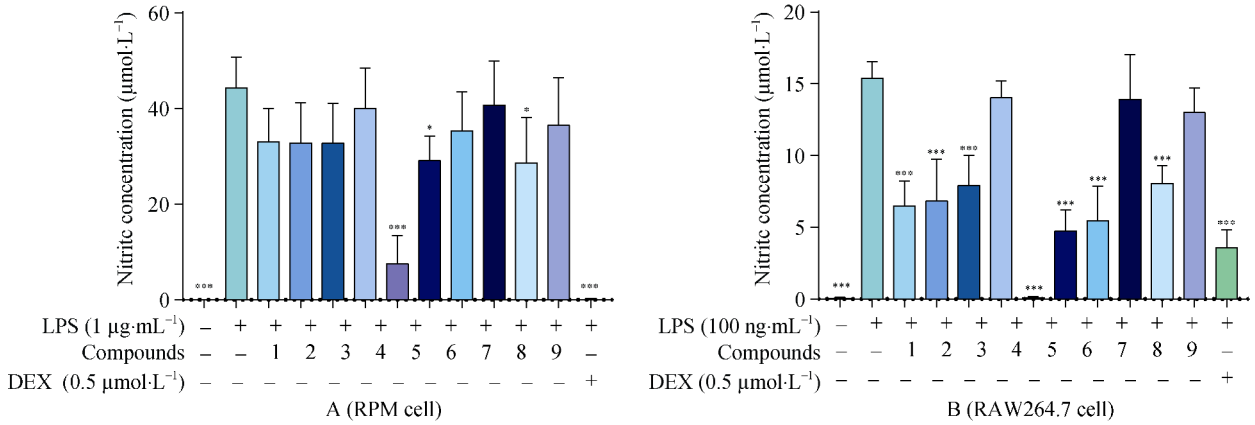

Fig. 5 Effect of compounds 1−10 on NO expression in LPS-stimulated RPMs and RAW264.7 cells (mean ± SD, $n = 3$, $^{*}P < 0.05$, $^{***}P < 0.001$ vs LPS group)

Experimental

General experimental procedures

UV spectra were measured on a JASCO V-550 UV/VIS spectrophotometer (JASCO International Co., Ltd., Tokyo, Japan). IR spectra were recorded on a JASCO FT/IR-480 Plus Fourier Transform Infrared Spectromter (JASCO International Co., Ltd., Tokyo, Japan). NMR spectra were recorded on a Bruker AV-400 NMR spectrometer (Bruker, Switzerland, 100 MHz for ^{13}C and 400 MHz for ^{1}H), and the chemical shifts were referenced to the solvent residual peak. HR-MS data were acquired on a Thermo Fisher Scientific Q-Exactive LC-MS (Thermo Fisher Scientific Inc., San Jose, CA, USA) fitted with a Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). CD spectra were obtained on a Chirascan CD spectrometer (Applied Photophysics Ltd., England). Silica gel CC was performed on silica gel (100−200 mesh or 200−300 mesh; Qingdao Marine Chemical, Inc.; Qingdao, China) and Sephadex LH-20 (Pharmacia Fine Chemicals Inc., NJ), Semipreparative RP-HPLC was performed using a Shimadzu LC-6AD system with an Cosmosil Packed Column 5PYE (10 mm × 250 mm, NacalaiTesque Inc., Kyoto, Japan). TLC analysis and preparative were performed on precoated silica gel GF254 plates (Qingdao Marine Chemical, Inc.; Qingdao, China). Spots were visualized under UV light (254 and 365 nm) or by heating after spraying with 10% EtOH−H_2SO_4 solution. All the solvents were of analytical grade. The murine macrophage RAW264.7 cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), and resident peritoneal macrophages (RPMs) were isolated from SD rats, which were obtained from the University of Hong Kong.

Plant material

The leaves of *Callicarpa nudiflora* Hook. et Arn. were collected from Po-ya-cun, Yuanmen District, Haikou City, Hainan Province, China in January 2016. Botanical identification was performed by Dr WANG Hong-Gang (school of Traditional Chinese Medicine, Guangdong Pharmaceutical University), and a voucher specimen (No. 20160103) was deposited at the Chinese Herb Medicine Museum, Guangdong Pharmaceutical University.

Extraction and isolation

The air-dried leaves of *C. nudiflora* (5.0 kg) were extracted with MeOH (3 × 30 L) under reflux. The organic solvent was evaporated to afford a crude extract (1205.5 g). The extract was suspended in H_2O (1.2 L) and partitioned with petroleum ether (6 × 1.2 L) and ethyl acetate (5 × 1.2 L) successively. The ethyl acetate soluble-part (124.0 g) was chromatographed over D101 Macroporous Resin eluting with a gradient of MeOH in H_2O (0 : 100−100 : 100, 0.5 L for each gradient elution) to yield nine fractions (F_1−F_9) based on TLC analysis.

Fraction F_4 was subjected to octadecylsilane (ODS) elution with a step gradient 40%−100% MeOH in H_2O to give three subfractions (F_{4-1}−F_{4-3}). Subfraction F_{4-2} was subjected to silica gel CC (CH_2Cl_2−MeOH, 50 : 1−40 : 1) and further purified by semipreparative RP-HPLC using a mobile phase of 55% MeOH in H_2O yielded compound **10** (t_R = 25.32 min, 10.3 mg). Subfraction Fraction F_{4-3} was subjected to silica gel CC (CH_2Cl_2−MeOH, 50 : 1−40 : 1) and Sephadex LH-20 (CH_2Cl_2−MeOH, 1 : 1) and finally subjected to semipreparative RP-HPLC with a gradient of MeOH in H_2O (40%, 0−5 min; 40%−100%, 5−35 min) to obtain compound **8** (t_R = 28.43 min, 15 mg).

Fraction F_5 was subjected to ODS CC elution with a step gradient from 40%−100% MeOH in H_2O to yield eleven subfractions (F_{5-1}−F_{5-11}), successively, and purified by semipreparative RP-HPLC using a gradient of MeOH in H_2O to obtain compound **3** (80%, 0−5 min; 80%−100%, 5−25 min, t_R =

21.19 min, 35 mg) from subfraction F_{5-2}, compound **4** (80%, 0−5 min; 80%−95%, 5−20 min, t_R = 19.12 min, 16.8 mg) from subfraction F_{5-3} and compound **6** (80%, 0−5 min; 80%−96%, 5−21 min, t_R = 20.28 min, 13.0 mg) from subfraction F_{5-6}.

Fraction F_6 was chromatographed over a silica gel column and eluted with a step gradient of petroleumether-ethyl acetate (200 : 1−1 : 1) to give fractions (F_{6-1}−F_{6-6}). Subfraction F_{6-2} was subjected to silica gel CC (petroleumether−acetone 100 : 1−100 : 10) and successively purified by semipreparative RP-HPLC with MeOH in H_2O to obtain compounds **1** (92%, t_R = 17.26 min, 7.5 mg), **2** (90%, 0−5 min; 90%−100%, 5−25 min, t_R = 10.65 min, 8.2 mg), **7** (91%, t_R = 49.93 min, 10.5 mg) and **9** (91%, t_R = 51.86 min, 6.0 mg). Subfraction F_{6-6} was further purified by semipreparative RP-HPLC using a gradient of MeOH in H_2O (90%, 0−15 min; 90%−100%, 15−40 min) to obtain compound **5** (t_R = 22.49 min, 11.3 mg).

Nudiflopene J (**1**): colorless oil; UV (MeOH) λ_{max}: 204 nm, 223 nm; IR (MeOH) ν_{max} (cm^{-1}): 3456, 3086, 2934, 2872, 1733, 1644, 1444, 1373, 1177, 1000, 893 cm^{-1}; ^1H NMR (400 MHz, MeOD) and ^{13}C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESI-MS *m/z* 303.1955 $[M - H]^-$, Calcd. for $C_{19}H_{27}O_3$, 303.1966 $[M - H]^-$.

Nudiflopene k (**3**): colorless oil; UV (MeOH) λ_{max}: 203 nm; IR (MeOH) ν_{max} (cm^{-1}): 3451, 3092, 2934, 2857, 1734, 1644, 1446, 1381, 1379, 1179, 664 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HR-ESI-MS *m/z* 277.1798 $[M - H]^-$, Calcd. for $C_{17}H_{25}O_3$, 277.1809 $[M - H]^-$.

Nudiflopene L (**4**): white amorphous powder; UV (MeOH) λ_{max}: 203 nm; IR (MeOH) ν_{max} (cm^{-1}): 3454, 2937, 2864, 1729, 1641, 1444, 1380, 1293, 1174, 899, 677 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Tables 1 and 2; HR-ESI-MS *m/z* 343.1907 $[M - H]^-$, Calcd. for $C_{21}H_{27}O_4$, 343.1915 $[M - H]^-$.

Nudiflopene M (**5**): colorless oil; UV (MeOH) λ_{max}: 203 nm, 243 nm; IR (MeOH) ν_{max} (cm^{-1}): 3442, 3085, 2934, 2867, 1735, 1647, 1587, 1314, 1260, 1201, 1183, 990, 897, 794, 670 cm^{-1}; ^1H NMR (400 MHz, MeOD) and ^{13}C NMR (100 MHz, MeOD) data, see Tables 1 and 2; HR-ESI-MS *m/z* 361.2011 $[M - H]^-$, Calcd. for $C_{21}H_{29}O_5$, 361.2021 $[M - H]^-$.

Bioassay for NO inhibitory activities

RAW 264.7 cells were seeded in 24-wells plate for 18 h. Then, cells were pretreated with compound **1** (50 μmol·L^{-1}), **2** (100 μmol·L^{-1}), **3** (50 μmol·L^{-1}), **4** (25 μmol·L^{-1}), **5** (50 μmol·L^{-1}), **6** (100 μmol·L^{-1}), **7** (50 μmol·L^{-1}), **8** (50 μmol·L^{-1}), **9** (100 μmol·L^{-1}), **10** (100 μmol·L^{-1}), and dexamethasone (0.5 μmol·L^{-1}, DEX) for 1 h. After that, cells were stimulated with LPS (100 ng·mL^{-1}) for 24 h. Nitric oxide concentration was measured using the Griess Reagent System (Promega, Madison, Wisconsin, USA). The data are shown as mean ± SD, n = 3. $^{***}P < 0.001$ *vs* LPS group. Dexamethasone (Sigma Chemical Co., St. Louis, MO, USA) was used as a positive control.

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