Twelve Novel and Diverse 16-Norphragmalin-Type Limonoids from *Chukrasia tabularis* var. *velutina*

Jun Luo, Yi Li, Jun-Song Wang, Juan Lu, Xiao-Bing Wang, Jian-Guang Luo, and Ling-Yi Kong

*Department of Natural Medicinal Chemistry, China Pharmaceutical University; 24 TongJiaXiang, Nanjing 210009, China; and *Testing & Analysis Center, Nanjing Normal University; Nanjing 210046, China.

Received September 22, 2011; accepted November 16, 2011; published online November 24, 2011

A series of novel and structurally related C-15-acyl 16-norphragmalin-type limonoids, chuktabrins C—J (1—8) and chuktabarins U—X (9—12), were isolated from the stem bark of *Chukrasia tabularis* var. *velutina*. Their structures were established on the basis of detailed spectroscopic analysis, and the absolute configuration of compound 1 was determined by a single-crystal X-ray study using a mirror Cu Ka radiation. Compounds 7 and 8 were unprecedented C-15-acyl 16-norphragmalins with ketonic alkyl appendage at C-15, and compounds 4 and 8 were first examples of limonoid with a characteristic carbonate moiety esterified at OH-9/OH-8 or OH-1/OH-8 respectively. A biosynthetic pathway of these limonoids was reasonably presumed based on the novel and diverse structures isolated, which provides a new insight into the plausible biosynthesis of C-15-acyl 16-norphragmalin-type limonoids. The anti-inflammatory activity of major isolates were evaluated for inhibitory activity against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line, with IC50 value ranging from 2.40 to 16.90 μM.

**Key words** phragmalin-type limonoid; anti-inflammatory; *Chukrasia tabularis* var. *velutina*

C-15-acyl phragmalin-type limonoids are a series of novel limonoids with a biosynthetically extended acyl side chain at C-15, which distributed mainly in the genus *Chukrasia*, *Entandrophragma*, and *Neobeguea* of the Meliaceae family. The first C-15-acyl phragmalin was isolated in 1972, and thereafter, a series of this type of limonoids have been reported, which possess an characteristic β-ketolactone moiety between C-16 and C-15 appendage. In recent years, C-15-acyl phragmalins have been the research focus limonoids from family Meliaceae, and several C-15-acyl 16-norphragmalins possessing an precedent 16-decarboxylated skeleton were reported. Up to now, twenty three C-15-acyl 16-norphragmalins with ketal appendage at C-15 and only one enolic alkyl derivates have been isolated from plants of genera *Chukrasia*.

*Ch. tabularis* var. *velutina*, a timber tree, grows mainly in tropical areas of Asia such as India and southern mainland China, which stem bark has been used traditionally as an astringent, antidiarrheal, and anti-influenza agents in China. Previous research work indicated phragmalin-type limonoids as its main constituent. In our present research, a series of novel and structurally related 16-norphragmalin-type limonoids with alkyl appendage at C-15 (Fig. 1), chuktabrins C—J (1—8) and chuktabarins U—X (9—12), were isolated from chloroform extract of the stem bark of title plant. These novel compounds were isolated by combining with various methods of column chromatographs, such as silica gel, octadecyl silica (ODS), and Sephadex LH-20, and most of which were purified by preparative HPLC finally. Their structures were established on the basis of detailed spectroscopic analysis, and that of I as well as the absolute configuration were determined by a single-crystal X-ray study using a mirror Cu Ka radiation. Compounds 7 and 8 were unprecedented 16-norphragmalins with ketonic alkyl appendage at C-15. In structures of I—8, a characteristic carbonate moiety esterified at OH-30/OH-8, OH-1/OH-8 or OH-9/OH-8, former two of which have never been reported. The anti-inflammatory activity of major compounds (2—9) was evaluated for inhibitory activity against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line. The results exhibited that these C-15-acyl 16-norphragmalins possess significant activity with IC50 value from 2.40 to 16.90 μM.

In present research, the novel and diverse structures of isolated compounds, especially the precedent ketonic alkyl appendage derivates and C-8/C-9, C-8/C-30 type carbonate moiety, interested and allowed us to presume the biogenetic pathway of them, which provides new insight into the biosynthesis of C-15-acyl 16-norphragmalin-type limonoids. The C-15-acyl 16-norphragmalins isolated in this investigation and reported previously could be correlated well by the plausible biosynthetic pathway, and we think that the origin of carbonate moiety in 16-norphragmalin limonoids (1—8) maybe comes from the 16-carbonyl of phragmalin skeleton which degradated through decarboxylation. Herein, the isolation, structural elucidation, the anti-inflammatory activity, as well as the proposed biosynthetic pathway of these novel compounds were reported.

**Results and Discussion**

**Structural Elucidation. 16-Norphragmalins with Enolic Alkyl Appendage at C-15 (1—6)** Chuktabrin C (1) was isolated as white amorphous powder, and had the molecular formula of C38H46O18Na as determined by high resolution-electron ionization-mass spectra (HR-ESI-MS), which showed a quasi-molecular ion at m/z 813.2543 (Calcd: C38H44O11Na, 813.2576). In 1D- and 2D-NMR spectra of 1, a proton signal at δH 6.49, 7.38, 7.94; δC 119.5, 109.2, 143.4, 143.4), a pair of geminal doublets of characteristic carbonate moiety, interested and allowed us to presume the biogenetic pathway of these novel compounds were reported.
(C-1’), instead of the correlation between H-15 and COOH-16 in common phragmalins. Furthermore, the carbon at δC 152.3 (C-1’) showed obvious correlations with ethyl proton signals [δH 2.22, m, 2H; δH 1.13, t (7.5), 3H] and proton signal of H-14 (δH 2.79). The above analyses indicated that compound 1 was a phragmalin-type limonoid deriver through demolishment the COOH-16 and a propionyl was extended to C-15 and a double bond was formed between C-15 and the carbonyl (C-1’) of propionyl as chuktabrin A.14) Degrees of unsaturation and a characteristic signal at δC 152.3 indicated that a carbonate moiety and an additional ring between carbon signal at δC C-1’ and 16-norphragmalin skeleton existed in structure of 1,14) however, the site of lactonization and the additional ring could not be determined directly by the HMBC spectrum for no valuable correlations were observed. Fortunately, after many attempts with different solvents, a single crystal was successfully obtained and the structure of 1 was finally accomplished as shown in Fig. 1 by single-crystal X-ray diffraction analysis using a mirror CuKα radiation, which possess an ether linkage between C-30 and C-1’ and the carbonate moiety esterified at OH-9/OH-8 as chuktabrin A.14) Thus, the absolute configuration of 1 was unambiguously determined to be 1R, 2S, 3S, 4R, 5S, 6R, 8R, 9R, 10R, 11R, 12R, 13R, 14R, 17S, and 30R.

Chuktabrin D (2), white amorphous powder, had the molecular formula of C32H40O14 as determined by HR-ESI-MS, which showed a quasi-molecular ion at m/z 671.2332 (Calcd: C32H40O14Na, 671.2310). The whole feature of the 1H- and 13C-NMR spectral data indicated that 2 possessed the same 16-norphragmalin skeleton with an 3,4-dihydro-2H-pyran was formed via an ether bond between C-30 and C-1’ in the bio-

---

Fig. 1. Structures of Compounds 1—12

---

Fig. 2. Important HMBC Correlations and X-Ray Structure of 1
synthetically extended propionyl at C-15.\(^\text{14}\) The simialrity of chemical shift of C-8, C-9, C-30 indicated that the carbonate moiety (δC 153.0) esterified at OH-8 and OH-9 as 1. HMBC correlation from proton signal of H-17 (δH 5.84) and ester carbonyl carbon at δC 168.0 indicated that the only acetyl was esterified at OH-17. Thus, the structure of 2 was established as 6-deacteoxyl-19-O-acetyl derivatives of 1.

Chuktabrins E (3) and F (4) were isolated as a pair of mixture, which was inseparable using various techniques including silica gel, ODS, Sephadex LH-20, and preparative HPLC. The HR-ESI-MS spectra of this mixture exhibited a single ion peak at m/z 813.2588 (Calcd: C₃₆H₄₀O₁₈Na, 813.2576), which indicated that 3 and 4 were a pair of isomers with a same molecular formula of C₃₆H₄₀O₁₈. The structural elucidation and resolution of the 1D-NMR data about these were proposed after extensive 2D-NMR analyses in mixture.

The \(^1\)H- and \(^{13}\)C-NMR (Tables 1, 3) data and the information from the subsequent 2D-NMR studies (HMBC, HSQC and NOESY) suggested that 3 was an isomer of 1 with acetoxyl group in different location. Two oxygenated methylene signals at δH 4.58 and 5.25 (d, J=11.0Hz) were attributable to C-19 by the observed HMBC correlations with carbons at δC 52.5 (C-10), 85.8 (C-9), 37.6 (C-5) and 170.2 (C-19–OCO), which suggested that the 19-methyl had been acetylated. Thus, the structure of 3 was established as 6-deacetoxy-19-O-acetyl derivatives of 1.

The key HMBC correlations between proton signals of skeleton and ester carbonyl carbons indicated that the locations of the ester function of 4 were the same as those of 3. The only variable factor was the location of the carbonate. Comparison the \(^1\)H- and \(^{13}\)C-NMR between 3 and 4, the most obvious change was the chemical shift of C-9 from δC 85.8 in 3 to δC 79.4 in 4, accompanying one proton of H-29 downfield shift from δH 2.05 to δH 2.66 and one proton of H-19 upfield shift from δH 5.25 to δH 4.71 due to the changes of shielding effect generated from the carbonate to these two protons. Meanwhile, the chemical shift of C-2 (δC 73.3) indicated that hydroxyl group at C-2 in 4 was also free.\(^\text{13,14}\) Above changes indicated that the location of the carbonate transferred from OH-9 in 3 to OH-1 in 4. Thus, the structure of 4 was established as depicted, which was the first example of limonoid with C-1/C-8 carbonate.

Chuktabrin G (5), white amorphous powder, showed a quasi-molecular ion at m/z 739.2226 (Calcd: C₃₆H₄₀O₁₈Na, 739.2209), which indicated the molecular formula of C₃₆H₄₀O₁₈. The \(^1\)H- and \(^{13}\)C-NMR (Tables 1, 3) data and the information from the subsequent 2D-NMR studies (HMBC, HSQC and NOESY) suggested the similar 16-norphythalimides skeleton of 5 as 1 but with a C-7/C-19 δ-lactone ring.\(^\text{13}\) Specifcation of the sites of three acetoxyl groups to C-11, C-12, and C-17 were obtained by HMBC correlations.
e.g., H-11 (δ_H 5.34, brs) to δ_C 169.0, H-12 (δ_H 4.61, brs) to δ_C 169.7, and H-17 (δ_H 5.93, s) to δ_C 168.5. The key NOESY correlations, from H-11 to H-5, H-12, and H-17, H-17 to H-5 and H-30, H-14 with Me-18, H-29a with H-3, and H-29b with H-19b, indicated that the relative stereochemistry of the key asymmetric carbons of 5 was well matched with that of 1 obtained by X-ray crystallographic study. Thus, the structure of 5 was determined as depicted.

The molecular formula of Chuktabrin H (6), C_{34}H_{42}O_{15}, was determined by the HR-ESI-MS ion at m/z 799.2425 (Caled: C_{34}H_{42}O_{15}Na, 799.2420). The similarity of the 1H- and 13C-NMR spectral data of 7 (Table 1, 3) to those of 1 indicated that 7 possessed the same 16-norphragmal skeleton.

A characteristic ketonic carbonyl signal at δ_C 207.9 was observed in 13C-NMR spectra of 7, showing obvious HMBC correlation (Fig. 3) with H-14 [δ_H 4.09, brd (5.5)]; H-15 [δ_H 3.04, dd (19.5, 7.0); 2.84, dd (19.5, 2.0)] and a set of ethyl group [δ_H 2.50, m, 2H; δ_H 1.09, t (7.5), 3H]. Above information indicated that a propionyl biosynthetically extended to C-15 existed in form of ketone alkyl appendage instead of the enol form in 1—6. A characteristic carbonate signal at δ_C 152.1 showed HMBC correlations with H-30 (δ_H 5.08), indicating one of the esterification position was at OH-30. The similarity of chemical shift of C-8 indicated that another branch of carbonate moiety (δ_C 152.1) esterified at OH-8 as 1.24. The relative configuration of key asymmetric carbons in 7 was established to be as same as those of 1 by its NOESY experi-
Chuktabarin U (9) gave the molecular formula of C_{33}H_{40}O_{15} as deduced from the HR-ESI-MS ion at m/z 699.2237 (Calcd: C_{33}H_{39}O_{15}Na, 699.2235). The whole feature
of the \textsuperscript{1}H- and \textsuperscript{13}C-NMR data indicated that 9 was also a 16-norphragmalin-type limonoid.\textsuperscript{13}

In HMBC spectra, a ketal resonance at $\delta_C$ 110.6 (C-1') showed cross-peaks to H-15a ($\delta_H$ 2.46), H-30 ($\delta_H$ 4.64, s), and a methyl ($\delta_H$ 1.62, s, 3H) suggested that an acetyl biosynthetically extended to C-15 existed in form of ketal alkyl appendage instead of the enol in 1—6 or ketone in 7 and 8. Degrees of unsaturation and the similarity of chemical shift of C-8 and C-9 with chuktabularins E—T indicated that C-30 and C-8 linked with C-1' through an ether linkage forming the characteristic ketal moiety.\textsuperscript{13} Chemical shift of C-2 and HMBC correlations between proton signals of skeleton and ester carbonyl carbons indicated that three acetyl groups were esterified at OH-2, OH-3, OH-17 respectively. Thus, the structure of 9 was established as demonstrated.

Chuktabularins V (10) and W (11) were also isolated as a pair of mixture. In spite of the best of efforts as those 3 and 4, we were unable to separate out these two compounds. The HR-ESI-MS spectra of this mixture exhibited a two ion peak at m/z 811.2822 (Calced: C$_{39}$H$_{48}$O$_{17}$Na, 811.2784) and 825.2975 (Calced: C$_{40}$H$_{50}$O$_{17}$Na, 825.2940), corresponding the molecular formula of C$_{39}$H$_{48}$O$_{17}$ (10) and C$_{40}$H$_{50}$O$_{17}$ (11). The characteristic ketal carbon signals at $\delta_C$ 111.0 and 113.2 indicated that the carbon skeleton of these two compounds was 16-norphragmalin with a ketal moiety as 9. The structural elucidation and resolution of the 1D-NMR data about these were proposed after extensive 2D-NMR analyses in mixture.

The key HMBC correlations about the ketal carbons revealed that the main difference of these two compounds was the acyl appendage at C-15, i.e. an acetyl in 10 and a propionyl in 11. The locations of the substituted esterifications were determined by the HMBC correlations from proton signals of skeleton to ester carbonyl carbons and the chemical shift of relative carbons. Thus, the structures of 10 and 11 were determined as depicted in Fig. 3.

Chuktabularin X (12), white amorphous powder, gave the molecular formula of C$_{33}$H$_{40}$O$_{15}$ as deduced from the HR-ESI-MS ion at m/z 699.2237 (Calced: C$_{33}$H$_{40}$O$_{15}$Na, 699.2259). The whole feature of the \textsuperscript{1}H- and \textsuperscript{13}C-NMR data indicated that 12 was also a 16-norphragmalin-type limonoid with ketal moiety. HMBC cross-peaks from the ketal resonance at $\delta_C$ 111.3 (C-1') to H-15a ($\delta_H$ 2.13, dd, J=11.7, 8.8Hz), H-30 ($\delta_H$ 4.49, s), and ethyl proton signals ($\delta_H$ 1.91, q (7.8), 2H; $\delta_H$ 0.96, t (7.8), 3H) suggested that a propionyl biosynthetically extended to C-15 existed in form of ketal alkyl appendage as that in 11. HMBC correlation between H-17 ($\delta_H$ 6.28, s) and ester carbonyl carbon at $\delta_C$ 168.5 indicated that only one acetyl group was located at OH-17. Thus, the structure of 12 was established as demonstrated.

**Proposition of Biosynthetic Pathway** In our previous papers, we have reported the plausible biosynthetic pathway of phragmalin-type limonoids with enolic alkyl at C-15\textsuperscript{12} and 16-norphragmalins with ketal alkyl at C-15,\textsuperscript{12,13} In present research, the novel and diverse 16-norphragmalins, especially with unprecedented ketonic alkyl appendage and C-8/C-9, C-8/C-30 type carbonate moiety, interested and allowed us to presume the biogenetic pathway for these compounds, which provide new insight into the biosynthesis of 16-norphragmalin type limonoids with an alkyl appendage at C-15.

The biosynthetic origin of 1—12 (Chart 1) was proposed to be the phragmalin-type limonoid with esterfunction at C-30.\textsuperscript{11} Shift of the acyl unit from C-30 through a claisen reaction and concurrent the cleavage of C-16/C-17 $\delta$-lactone, would produce a key intermediate b. The electronegativity of C-15, electropositivity of carbonyl of esterfunction at C-30, and the adjacent spatial position of these two carbons were the beneficial condition for the claisen reaction. The intermediate b plays a key role in the biosynthesis of all the phragmalins with an alkyl appendage at C-15.

There are two plausible pathways for phragmalins with ketal alkyl appendage at C-15. First, the instability of $\beta$-dicarbonyl moiety of intermediate b made it prone to lose the COOH-16 and yield another intermediate c. After a series of ketal formation and esterification, compounds 9—12 with ketal alkyl at C-15 were obtained.\textsuperscript{12} Another pathway was also a plausible procedure for forming intermediate c. In intermediate b, esterification between adjacent COOH-16 and OH-8 would give intermediate d, which contains more unstable $\alpha$-acylated $\gamma$-lactone ring. So, the carbon bond between C-15 and COOH-16 was broken easily and afford intermediate e whose OH-8 esterified by the carboxic acid (C-16). Degradation of the carbon dioxide (CO$_2$) would give intermediate e.

In my opinion, the second procedure was the origin for phragmalins with ketonic or enolic alkyl at C-15 (compounds 1—8). In intermediate e, OH-1, OH-9, and OH-30 were spatially adjacent to carboxic acid at OH-8, so, if the esterified reaction replaced the degradation of the carbon dioxide, the characteristic carbonate moiety was biosynthetically extended to the mother nucleus of compounds 1—8. Intermediate f with C-1/C-8 carbonate moiety could be finally converted into 4 by enolization of the carbonyl in acyl appendage, the followed dehydration and esterifications; The key intermediate of compounds 1—3, 5, 6, 8 with C-9/C-8 carbonate moiety (g) was formed by the esterified reaction with OH-9, which converted to 8 with ketonic acyl at C-15 by esterification directly and through enolization, dehydration, and esterification to give 1—3, 5, 6 with enolc acyl at C-15. Intermediate h with C-30/C-8 carbonate moiety could be esterified to afford 7.

In this investigation, three kind alkyl appendages at C-15 were isolated, which had several origins from biosynthetic precursors, containing isobutyryl, propionyl, and acetyl at C-30, commonly occurred in phragmalins. This plausible biosynthetic pathway could explain and correlate 16-norphragmalins with ketonic, enolic, or ketal alkyl appendage and the origin of carbonate moiety in some 16-norphragmalin limonoids was the C-16 carboxyl of phragmalin skeleton which...
Chart 1. The Plausible Bisosynthetic Pathway of C-15 Acyl 16-Norphragmalins (Red: C-15 Acyl Appendage; Blue: Conversion of COOH-16 to Carbonate; Colored Structures Shown in the Graphical Abstract)
degraded through decarboxylation. Based on this plausible biosynthetic pathway, a lot of novel but minor 16-norphragmalins with different kinds of alkyl appendage are waiting for natural product researchers to discovery.

Anti-inflammatory Activity of Major Compounds. The anti-inflammatory activities of major compounds (2—9) were evaluated for inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line in this research, representing the first report for C-15-acyl phragmalin-type limonoids with alkyl appendage at C-15, and dexamethasone was used as positive control substance. The results (IC_{50} values) exhibited in Table 4, which indicated these 16-norphragmalins possess significant activity especially 9, 2, 8, 7, and 6 with IC_{50} values at 2.40, 3.81, 7.63, 7.78, and 7.94 μM, respectively. The IC_{50} value data of 5 (16.90) and its 11-O-deacetylated derivative 6 (7.94), and another 11,12-O-bideacetylated derivative 2 (3.81) suggested that major free exocentric hydroxyl groups (OH-3, OH-11, OH-12) may be important factor for anti-inflammatory activity. The data between 8 (7.63) with ketogenic propane revealed that the form of carbonyl of alkyl appendage at C-15 may be another important factor for the anti-inflammatory activity.

Experimental

General Experimental Procedures. Optical rotations were measured with a JASCO P-1020 polarimeter. IR (KBr disks) spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on Bruker ACF-500 and 600 NMR instrument, (1H: 500 or 600 MHz, 13C: 125 or 150 MHz), LH-20 (Pharmacia), and RP-C18 (40—63 μm, Fuji) were used for column chromatography. Preparative HPLC was carried out using an Agilent 1100 Series instrument with a Shim-park RP-C18 column (20×200 mm) and a 1100 Series multiple wavelength detector. Mouse macrophage cell line (RAW264.7) was obtained from the Chinese Academy of Science Cell Bank (Shanghai, China). The cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% heat-activated fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (15 μM) at 37°C atmosphere and 5% CO_{2} in CO_{2}-Incubator (Thermo Fisher Scientific, model 311, U.S.A.). The number of cell was observed using inverted microscope (OLYMPUS-CX41, Japan). The OD values were measured on Microplate Absorbance Reader at 540 nm (SUNRISE-BASIC TECAN, Austria). NO kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Plant Material. The air-dried stem bark of C. tabularis var. velutina was collected from Xishuangbanna, Yunnan Province, People’s Republic of China, in March 2007, and was authenticated by Professor Mian Zhang of the Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (No. 2006-MML) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The air-dried stem bark (10kg) was extracted by refluxing with 95% ethanol three times. The EtOH extract was concentrated under reduced pressure (2000 g) and then extracted with CHCl_{3} to give a chloroform extract (300 g). The oily chloroform extract was dissolved in 2L 50% MeOH and H_{2}O and then extracted with petroleum ether. After removal of the fatty components, 210 g of extract were obtained, which was subjected to passage over a silica gel column eluted with CHCl_{3}-MeOH in a gradient (1:0 to 1:2), to afford eight fractions (Fr. A—H), monitored by TLC. Fr. C (22 g) was chromatographed on a column of silica gel, eluted successively with a gradient of petroleum ether–EtOAc (4:1 to 1:2), to give eight sub-fractions (Fr. Cl—C8). Fr. C7 was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H_{2}O (5:7 to 7:3), to give three sub-fractions (Fr. C7a—C7e). Fr. C7c was separated by preparative HPLC using CH$_3$CN–H$_2$O (55:45, 10 mL/min) as the mobile phase to give 10 and 11 in mixture (10 mg). D (30 g) was chromatographed on a column of silica gel eluted successively with a gradient of petroleum ether–EtOAc (5:2 to 1:2) to give seven sub-fractions (Fr. D1—D7). Fr. D3 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H$_2$O (5:7 to 7:3), to give four sub-fractions (Fr. D3a—D3d). Fr. D3d was separated by preparative HPLC using CH$_3$OH–H$_2$O (60:40, 10 mL/min) as the mobile phase, to give 1 (4 mg). Fr. E (20 g) was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H$_2$O (4:6 to 7:3), to give six sub-fractions (Fr. E1—E6). Fr. E2 was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H$_2$O (4:7 to 7:3), to give four sub-fractions (Fr. E3—E5). Fr. E3 was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H$_2$O (4:6 to 7:3), and combined with preparative HPLC using CH$_3$OH–H$_2$O (50:50, 10 mL/min) as the mobile phase to give 6 (3 mg). Fr. E5 was chromatographed on a column of reversed-phase C_{18} silica gel, eluted with MeOH–H$_2$O (2:3 to 7:3), to give four sub-fractions (Fr. E5a—E5d). Fr. E5c was separated by preparative HPLC using CH$_3$CN–H$_2$O (42:58, 10 mL/min) as the mobile phase to give 2 (9 mg). Fr. F (13 g) was chromatographed on a column of silica gel eluted successively with a gradient of petroleum ether–EtOAc (1:1 to 1:4) to give four sub-fractions (Fr. F1—F4). Fr. F2 was chromatographed on a column of reversed-phase C_{18} silica gel eluted with MeOH–H$_2$O (2:3 to 7:3) to give four sub-fractions (Fr. F2a—F2d). Fr. F2d was separated on a column of Sephadex LH-20 eluted with MeOH to give three sub-fractions, and each one was separated by preparative HPLC using CH$_3$OH–H$_2$O (56:44, 10 mL/min) as the mobile fraction.

Table 4. The Anti-inflammatory Activities Data of Tested Compounds (IC_{50} Values)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>No.</th>
<th>IC_{50} values (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of C-15-acyl Enol Ketone Ketal Dexamethasone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>3.81</td>
</tr>
<tr>
<td>3 and 4</td>
<td></td>
<td>15.33</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>16.90</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>7.94</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>7.78</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>7.63</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>2.40</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td>0.06</td>
</tr>
</tbody>
</table>

*The number of cell was observed using inverted microscope (OLYMPUS-CX41, Japan). The OD values were measured on Microplate Absorbance Reader at 540 nm (SUNRISE-BASIC TECAN, Austria). NO kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).*
phase to afford 7 (6 mg), 5 (3 mg), 3 and 4 (8 mg). Fr. F3 was chromatographed on a column of reversed-phase C18 silica gel eluted with MeOH–H2O (2:3 to 7:3) to give four sub-fractions (Fr. F3a—d). Fr. F3c was separated by preparative HPLC using CH3CN–H2O (42:58, 10 mL/min) as the mobile phase to give 8 (8 mg). Fr. F4 was chromatographed on a column of reversed-phase C18 silica gel eluted with MeOH–H2O (2:3 to 7:3) to give four sub-fractions (Fr. F4a—d). Fr. F4c was separated by preparative HPLC using CH3CN–H2O (38:62, 10 mL/min) as the mobile phase to give 12 (3 mg).

Chuktabrin C (1): White, amorphous powder; [α]D 13 +19 (c = 0.12, CH3OH); IR (KBr) νmax 3473, 2957, 1819, 1755, 1634, 1374, 1233, 1069 cm−1; 1H-NMR, see Table 2, 13C-NMR, see Table 3; negative ESI-MS m/z 753.5 [M+Na]+ (100); positive ESI-MS m/z 794.3 [M+NH4]+ (100); HR-ESI-MS m/z 799.2425 [M+Na]+ (Calcd: C31H26O13Na, 799.2420).

Chuktabrin J (8): White, amorphous powder; [α]D 13 +19 (c = 0.10, CH3OH); IR (KBr) νmax 3457, 2981, 1810, 1746, 1643, 1377, 1236, 1046 cm−1; 1H-NMR, see Table 2, 13C-NMR, see Table 3; negative ESI-MS m/z 769.3 [M+Cl]+ (100); positive ESI-MS m/z 752.3 [M+NH4]+ (100); HR-ESI-MS m/z 757.2308 [M+Na]+ (Calcd: C31H26O13Na, 757.2314).

Chuktabulins V (10) and W(11): White, amorphous powder; 1H-NMR, see Table 2, 13C-NMR, see Table 3; negative ESI-MS m/z 823.4 [M+Cl]+ (100); positive ESI-MS m/z 806.3 [M+NH4]+ (100); HR-ESI-MS m/z 811.2822 [M+Na]+ (Calcd: C35H38O17Na, 811.2781); for 1H-NMR, see Table 2; 13C-NMR, see Table 3; negative ESI-MS m/z 641.4 [M+Cl]+ (100); positive ESI-MS m/z 624.2 [M+NH4]+ (100); HR-ESI-MS m/z 629.2189 [M+Na]+ (Calcd: C29H24O10Na, 629.2205).

Chuktabulins X (12): White, amorphous powder; [α]D 13 +13 (c = 0.15, CH3OH); IR (KBr) νmax 3446, 2989, 1735, 1640, 1370, 1241, 1042 cm−1; 1H-NMR, see Table 2, 13C-NMR, see Table 3; negative ESI-MS m/z 641.4 [M+Cl]+ (100); positive ESI-MS m/z 624.2 [M+NH4]+ (100); HR-ESI-MS m/z 629.2189 [M+Na]+ (Calcd: C29H24O10Na, 629.2205).

**Anti-inflammatory Activity Assay** The anti-inflammatory activities of major compounds (2—9) were evaluated for inhibitory activities against lipopolysaccharide (LPS) induced nitric oxide (NO) production in macrophage (RAW264.7) cell line, which was obtained from the Chinese Academy of Science Cell Bank (Shanghai, China). Cells were cultured at 37°C in 1640 medium supplemented with 10% heated-deactivated FBS, penicillin (100 U/mL), streptomycin (15 mm) in a humidified atmosphere of 5% CO2 and passaged when about 85% confluence were achieved with trypsinase solution [0.25%, dissolved in phosphate buffer saline (PBS)]. RAW 264.7 cells were plated at a density of 1×105 cells in a 24-well cell culture plate with 500 μL culture medium, and incubated for 24 h. The cells were pre-treated with samples for 2 h and stimulated with LPS (2 μg/mL) for 18 h with dexamethasone (DXM) as positive control. The level of NO was determined using the NO kit according to the manufacturer’s protocol.

**Acknowledgment** This research work was supported by the Key Project of National Natural Science Foundation (30830116), the Scaling Project for Innovation Scholars, Natural Science Foundation of Jiangsu Province, China (BK20080309), the Cultivation Fund of the Key Scientific and Technical Innovation Project, Ministry of Education of China (707033), and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions.

**Supplementary Data** The 1H-NMR, 13C-NMR, and HR-ESI-MS spectra of 1—12 can be found in this supplementary data, which will be published along the electronic version.
Reference


