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Article

chiro-Inositol Derivatives from *Chisocheton paniculatus* Showing Inhibition of Nitric Oxide Production

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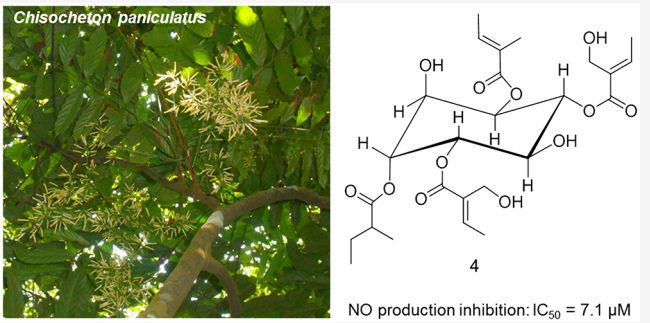
ABSTRACT: Six new *chiro*-inositol derivatives (**1**−**6**) were isolated from the leaves of *Chisocheton paniculatus* collected in Vietnam. Their chemical structures were elucidated by 1D and 2D NMR and HRESIMS analyses. All isolated compounds were evaluated for their inhibitory activity against lipopolysaccharide-induced nitric oxide (NO) production in the RAW 264.7 macrophage cell line. Compound **4** exhibited potent inhibitory activity for NO production with an IC_{50} value of 7.1 μM.



The genus *Chisocheton* belonging to the family Meliaceae is composed of 53 species. Plants of this genus are distributed in the tropical and subtropical regions of Asia, mostly in India and Malaysia.[1] The *Chisocheton* genus has attracted some attention by phytochemical researchers owing to the novel structures and potential bioactivities of its constituents. Biological studies have led to reports that this genus possesses potential anticancer, anti-inflammatory, antiobesity, antifungal, antibacterial, and antimalarial properties. The chemical constituents isolated from 11 plants of the *Chisocheton* genus have shown the presence of limonoids and protolimonoids in addition to triterpenes, steroids, sesquiterpenes, anthraquinones, spermidine alkaloids, coumarins and phenolic compounds.[2] In previous investigations, we reported a new limonoid, 6α,7α-diacetoxy-3-oxo-24,25,26,27-tetranorapotirucalla-1,14,20(22)-trien-21,23-lactam, with nitric oxide (NO) production inhibitory activity against lipopolysaccharide (LPS)-stimulated BV2 cells as well as three new inositol derivatives, *chiro*-inositol-4,5-di-5-hydroxytiglate-1,3-ditiglate, *chiro*-inositol-2-acetate-4,5-di-5-hydroxy-tiglate-3-tiglate, and *chiro*-inositol-2-acetate-4,5-di-5-hydroxytiglate-3-2-methylbutyrate, from *Chisocheton paniculatus* Hiern collected in Vietnam.[3,4]

As a part of ongoing studies on biological compounds from this species, we herein report the structural elucidation and NO production inhibitory activities of compounds **1**−**6** (Figure 1).

Compound **1** was obtained as a pale yellow oil. Its molecular formula was deduced as $C_{26}H_{38}O_{12}$ by HRESIMS in conjunction with NMR spectroscopic data analysis. The IR spectrum revealed the presence of hydroxy (3446 cm^{-1}), ester (1718 cm^{-1}), and olefinic (1649 cm^{-1}) functional groups in the molecule of **1**. The ^{1}H NMR spectrum of **1** (Table 1) showed characteristic signals of three olefinic methine protons [δ_H 7.02, 6.91, 6.86], six methyl groups [δ_H 1.00 (3H, t, J = 7.5 Hz), 1.27 (3H, d, J = 7.1 Hz), 1.78 (6H), 1.88 (3H, d, J = 7.2 Hz), and 1.92 (3H, d, J = 7.2 Hz)], and 10 oxygenated methine and/or methylene protons (δ_H 4.14−5.72). Analysis of the ^{13}C NMR (Table 2) and HSQC spectra of **1** revealed the presence of four carbonyl carbons [δ_C 177.2 (C-1′), 168.7 (C-1″), 167.7 (C-1‴), 168.1 (C-1⁗)], six olefinic carbons (δ_C 129.1−143.8), six oxygenated methine carbons [δ_C 74.7 (C-1), 74.5 (C-5), 73.3 (C-3), 71.4 (C-4), 68.4 (C-6), 68.2 (C-2)], two oxygenated methylene carbons [δ_C 56.2 (C-5⁗), 56.1 (C-5‴)], and eight sp^3 carbons (δ_C 11.9−42.4).

The complete structure of **1** was assigned based on COSY, HSQC, and HMBC data (Figure 2). The presence of six oxygenated methine carbons (δ_C 68.2−74.7) as well as the lack of the anomeric signal in the ^{13}C NMR spectrum indicated the presence of an inositol moiety in **1**. This finding was supported by the closed spin system [C(1)H−C(2)H−C(3)H−C(4)H−C(5)H−C(6)H−C(1)H] in the COSY spectrum. The coupling patterns of six oxygenated methine protons in **1** included three axial/equatorial and/or equatorial/equatorial couplings with small J values of 3.7, 3.9, and 4.0 Hz as well as three *trans*-diaxial couplings with large J values of 10.0, 10.2,

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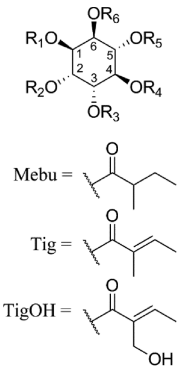

	R_1	R_2	R_3	R_4	R_5	R_6
1	Mebu	H	Tig	TigOH	TigOH	H
2	Tig	H	Mebu	TigOH	TigOH	H
3	Mebu	H	Mebu	TigOH	TigOH	H
4	H	Mebu	TigOH	H	TigOH	Tig
5	H	Mebu	TigOH	H	TigOH	Mebu
6	H	H	Tig	TigOH	TigOH	Tig

Figure 1. Structures of Compounds **1**–**6** from *Chisocheton paniculatus*.

Table 1. ^{1}H (500 MHz) NMR Data for 1–6 in CD_3OD (δ in ppm and J Values in (Hz) in Parentheses)

position	1	2	3	4	5	6
1	5.27 dd (3.9, 4.0)	5.31 dd (3.7, 3.7)	5.25 dd (3.8, 3.9)	4.11[a]	4.06 dd (3.0, 3.4)	4.19 brs
2	4.14 dd (3.7, 3.9)	4.11 dd (2.9, 3.7)	4.07 dd (3.5, 3.5)	5.41[a]	5.39 dd (3.4, 2.9)	4.19 brs
3	5.17 dd (3.7, 10.5)	5.18 dd (2.9, 10.5)	5.17 dd (2.9, 10.5)	5.41[a]	5.40 dd (2.9, 9.6)	5.41 dt (7.5, 2.5)
4	5.72 dd (10.5, 10.2)	5.69 dd (10.5, 10.2)	5.68 dd (10.5, 10.0)	4.11[a]	4.08 dd (9.6, 9.9)	5.76 m
5	5.46 dd (10.2, 10.0)	5.47 dd (10.2, 10.0)	5.44 dd (9.8, 10.0)	5.64 dd (10.0, 10.4)	5.60 dd (9.9, 10.1)	5.76 m
6	4.30[a]	4.32[a]	4.32[a]	5.15 dd (2.9, 10.4)	5.14 dd (3.0, 10.1)	5.41 dt (7.5, 2.5)
	Mebu-1	Mebu-3	Mebu-1	Mebu-2	Mebu-2	Tig-3
2′	2.55 m	2.40 m	2.55 m	2.51 m	2.50 m	
3′	1.78 m	1.60 m	1.77 m	1.74 m	1.72 m	6.88 q (7.3)
	1.57 m	1.41 m	1.58 m	1.56 m	1.56 m	
4′	1.00 t (7.5)	0.82 t (7.4)	0.99 t (7.4)	0.98 t (7.5)	0.97 t (7.5)	1.78 d (7.3)
5′	1.27 d (7.1)	1.09 d (6.9)	1.27 d (7.1)	1.22 d (7.0)	1.22 d (7.0)	1.79 s
	Tig-3	Tig-1	Mebu-3	Tig-6	Mebu-6	Tig-6
2″			2.40 m		2.40 m	
3″	6.86 m	7.03[a]	1.58 m	6.88 m	1.60 m	6.88 q (7.3)
			1.42 m		1.43 m	
4″	1.78[a]	1.90 d (7.2)	0.82 t (7.4)	1.78[a]	0.83 t (7.4)	1.78 d (7.3)
5″	1.78[a]	1.93[a]	1.09 d (7.0)	1.78[a]	1.10 d (7.0)	1.79 s
	TigOH-4	TigOH-4	TigOH-4	TigOH-3	TigOH-3	TigOH-4
3‴	6.91 q (7.2)	7.03[a]	6.94 q (7.2)	7.06 q (7.2)	7.06[a]	6.91 q (7.2)
4‴	1.88 d (7.2)	1.91 d (7.2)	1.92 d (7.3)	1.95 d (7.2)	1.95 d (7.3)	1.87 d (7.2)
5‴	4.24 s	4.25 s	4.25 s	4.37[a], 4.33[a]	4.37[a],4.33[a]	4.24 s
	TigOH-5	TigOH-5	TigOH-5	TigOH-5	TigOH-5	TigOH-5
3⁗	7.02 q (7.2)	6.95 q (7.2)	7.01 q (7.3)	7.02 q (7.2)	7.06[a]	6.95 q (7.2)
4⁗	1.92 d (7.2)	1.93[a]	1.89 d (7.3)	1.93 d (7.2)	1.93 d (7.3)	1.87 d (7.2)
5⁗	4.33 d (12.0)	4.33 d (11.9)	4.33 d (12.0)	4.37[a]	4.37[a]	4.24 s
	4.30 d (12.0)	4.30 d (11.9)	4.29 d (12.0)	4.33[a]	4.33[a]	

[a]Overlapping signals.

and 10.5 Hz. Furthermore, the key correlations of H-3 (δ_H 5.17) to H-5 (δ_H 5.46) and H-4 (δ_H 5.72) to H-6 (δ_H 4.30) were detected in the NOESY spectrum (Figure 3). Thus, a *chiro*-form was assigned for the inositol moiety of **1**.[5,6]

The cross-peaks of H_3-4′ (δ_H 1.00)/H_3-5′ (δ_H 1.27) to C-2′ (δ_C 42.4)/C-3′ (δ_C 27.6) and H_3-5′ to C-1′ (δ_C 177.2) in the HMBC spectrum and the linear spin system [(C-4′)H_3−(C-3′)H_2−(C-2′)H−(C-5′)H_3] in the COSY spectrum (Figure 1) supported the presence of a 2-methylbutyroyloxy moiety in **1**. In the same manner, the presence of a (*E*)-2-methylbut-2-enoyloxy (trivial name: tigloyloxy) and two (*E*)-2-(hydroxymethyl)but-2-enoyloxy (trivial name: 5-hydroxytigloyloxy) moieties in **1** was deduced from analysis of the HMBC, COSY, and NOESY spectra (Figures 1 and 2). The HMBC correlations from the oxygenated methine protons of the inositol moiety to the carbonyl carbons of the ester groups [H-1/C-1′, H-3/C-1″, H-4/C-1‴, and H-5/C-1⁗] allowed the 2-methylbutyroyloxy, tigloyloxy, and two 5-hydroxytigloyloxy moieties to be located at C-1, C-3, C-4, and C-5, respectively. Consequently, compound **1** was elucidated as 4,5-di-*O*-5-hydroxytigloyl-1-*O*-2-methylbutyroyl-3-*O*-tigloyl-*chiro*-inositol.

Compound **2** was isolated as a pale yellow oil. The HRESIMS and NMR data indicated this isolate to have the same molecular formula and substituent groups present as those of **1**. The downfield chemical shifts of H-1 (δ_H 5.31), H-3 (δ_H 5.18), H-4 (δ_H 5.69), and H-5 (δ_H 5.47) also suggested that **2** possesses a 1,3,4,5-tetra-substituted inositol ring as in **1**. The positions of the two 5-hydroxytigloyloxy moieties were determined at C-4 and C-5 based on the HMBC correlations from H-4 to C-1‴ (δ_C 167.6) and H-5 to C-1⁗ (δ_C 168.1), respectively. Moreover, the HMBC correlations of H-1 to C-1″ (δ_C 168.3) and H-3 to C-1′ (δ_C 177.7) demonstrated that

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Table 2. ^{13}C (125 MHz) NMR Data for 1−6 in CD_3OD

position	1	2	3	4	5	6
1	74.7	74.9	74.7	68.6	68.6	71.3
2	68.2	68.4	68.2	71.9	71.9	71.3
3	73.3	72.7	72.6	73.3	73.0	73.2
4	71.4	71.3	71.3	70.9	71.1	71.8
5	74.5	74.7	74.7	73.6	73.5	71.8
6	68.4	68.5	68.3	73.1	72.7	73.2
	Mebu-1	Mebu-3	Mebu-1	Mebu-2	Mebu-2	Tig-3
1′	177.2	177.7	177.2	176.6	176.6	168.8
2′	42.4	42.1	42.4	42.5	42.5	129.2
3′	27.6	27.7	27.6	27.8	27.8	139.8
4′	11.9	11.8	11.9	12.0	12.0	14.4
5′	17.2	16.5	17.2	17.2	17.2	12.0
	Tig-3	Tig-1	Mebu-3	Tig-6	Mebu-6	Tig-6
1″	168.7	168.3	177.7	168.8	177.7	168.8
2″	129.1	129.3	42.1	129.1	42.2	129.2
3″	139.9	139.8	27.7	139.9	27.7	139.8
4″	14.4	14.5	11.8	14.4	11.8	14.4
5″	12.0	12.3	16.5	12.1	16.6	12.0
	TigOH-4	TigOH-4	TigOH-4	TigOH-3	TigOH-3	TigOH-4
1‴	167.7	167.6	167.6	167.6	167.6	167.7
2‴	133.2	133.1	133.2	133.4	133.4	133.2
3‴	143.6	143.9	143.9	143.7	143.7	143.6
4‴	14.4	14.4	14.4	14.4	14.3	14.4
5‴	56.1	56.1	56.1	56.2	56.2	56.1
	TigOH-5	TigOH-5	TigOH-5	TigOH-5	TigOH-5	TigOH-5
1⁗	168.1	168.1	168.1	167.9	167.8	167.7
2⁗	133.2	133.1	133.2	133.5	133.5	133.2
3⁗	143.8	143.9	143.9	143.2	143.5	143.6
4⁗	14.4	14.4	14.4	14.4	14.3	14.4
5⁗	56.2	56.2	56.2	56.2	56.2	56.1

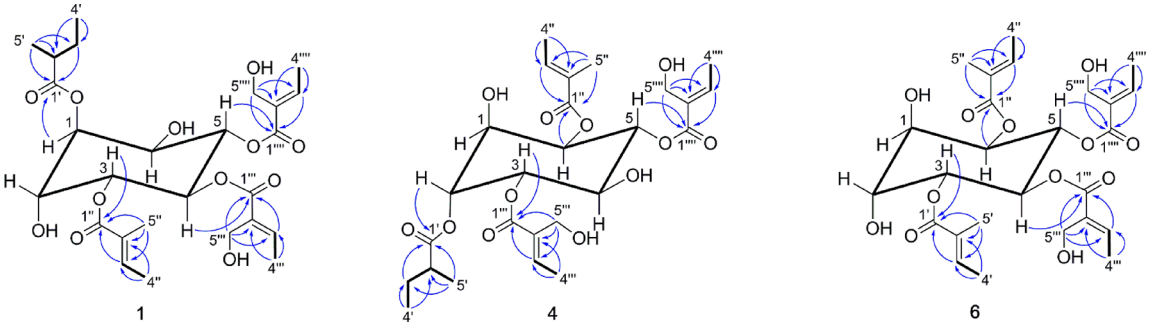

Figure 2. Key HMBC ($^1H \rightarrow {}^{13}C$, arrows) and COSY (bold lines) correlations of compounds **1**, **4**, and **6**.

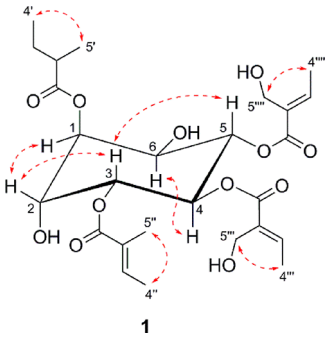

Figure 3. Key NOESY correlations (dashed arrows) of compound **1**.

tigloyloxy and 2-methylbutyroyloxy moieties are attached at C-1 and C-3, respectively. Thus, compound **2** was assigned as 4,5-di-*O*-5-hydroxytigloyl-3-*O*-2-methylbutyroyl-1-*O*-tigloyl-*chiro*-inositol.

Compound **3** was isolated as a pale yellow oil. The HRESIMS exhibited a sodiated molecular ion at *m/z* 567.2418 $[M + Na]^+$, corresponding to the molecular formula $C_{26}H_{40}O_{12}$, requiring seven degrees of unsaturation. The ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) were similar to those of **2**. The main difference was the presence of signals for an additional 2-methylbutyroyloxy group [δ_C/δ_H: 177.2 (C), 42.4/2.55 (CH), 27.6/1.77 and 1.58 (CH_2), 11.9/0.99 (CH_3), 17.2/1.27 (CH_3)] in **3**, instead of a tigloyloxy group in **2**. This group was located at C-1, based on the HMBC correlation from H-1 (δ_H 5.25) to the carbonyl carbon at δ_C 177.2 (C-1′). The remaining 2-methylbutyroyloxy group and the two 5-hydroxytigloyloxy groups in **3** were placed at the same positions as in **2**, based on the downfield chemical shifts

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of H-3 (δ_H 5.17), H-4 (δ_H 5.68), and H-5 (δ_H 5.44) and their HMBC correlations to the carbonyl carbons at δ_C 177.7, 167.6, and 168.1, respectively. Thus, compound **3** was assigned as 4,5-di-*O*-5-hydroxytigloyl-1,3-di-*O*-2-methylbutyroyl-*chiro*-inositol.

Compound **4** was isolated as a pale yellow oil, and its molecular formula was determined as $C_{26}H_{38}O_{12}$ based on the HRESIMS quasimolecular peak at m/z 581.1987 $[M + K]^+$ (calcd for $C_{26}H_{38}O_{12}K$, 581.2000). The NMR and HRESIMS data of **4** revealed this compound to have a close structural resemblance to **1** and **2**, with a *chiro*-inositol skeleton linking to four ester moieties, except for the substituent pattern of the inositol ring. The occurrence of substitution at carbons C-2, C-3, C-5, and C-6 in **4** was verified by the downfield chemical shifts of H-2, H-3 (δ_H 5.41), H-5 (δ_H 5.64), and H-6 (δ_H 5.15) and their HMBC correlations to the corresponding carbonyl carbons in the ester groups. The 2-methylbutyroyloxy and tigloyloxy residues were linked to C-2 and C-6 via the cross-peaks from H-2 to C-1′ (δ_C 176.6) and H-6 to C-1″ (δ_C 168.8). Similarly, the two 5-hydroxytigloyloxy residues at C-3 and C-5 were concluded from the HMBC cross-peaks H-3/C-1‴ (δ_C 167.6) and H-5/C-1⁗ (δ_C 167.9). Hence, the structure of **4** was proposed as 3,5-di-*O*-5-hydroxytigloyl-2-*O*-2-methylbutyroyl-6-*O*-tigloyl-*chiro*-inositol.

Compound **5** gave a molecular formula of $C_{26}H_{40}O_{12}$ on the basis of its ^{13}C NMR and HRESIMS data, implying seven degrees of unsaturation. The ^1H and ^{13}C NMR spectroscopic data of **5** were very similar to those of **4**, except for signals showing the replacement of a tigloyloxy group by a 2-methylbutyroyloxy group in **5**. The additional 2-methylbutyroyloxy group at C-6 (δ_C 72.7) was confirmed by an HMBC correlation between H-6 (δ_H 5.14) and C-1″ (δ_C 177.7). Thus, compound **5** was established as 3,5-di-*O*-5-hydroxytigloyl-2,6-di-*O*-2-methylbutyroyl-*chiro*-inositol.

Compound **6** was isolated as a pale yellow oil, and its molecular formula, $C_{26}H_{36}O_{12}$, was determined by the HRESIMS sodiated molecular ion at m/z 563.2088 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_{12}Na$, 563.2104). Surprisingly, the ^{13}C NMR spectrum of **6** exhibited only 13 carbon signals. Therefore, a symmetrical structure was supposed for **6**.[7]

The ^1H, ^{13}C, and HSQC NMR spectra displayed three pairs of signals at δ_C/δ_H 71.3/4.19, 71.8/5.76, and 73.2/5.41, corresponding to six oxygenated methine groups in **6**. Additionally, the signal at δ_H 5.41 (H-3, H-6) showed COSY cross-peaks with signals at δ_H 4.19 (H-1, H-2) and 5.76 (H-4, H-5). Based on this evidence, compound **6** was determined to be an inositol derivative. The large $J_{3,4}$, $J_{5,6}$ values (7.5 Hz) indicated a diaxial relationship between H-3 and H-4 and H-5 and H-6. The observed equatorial orientation was assigned for both H-1 and H-2 due to the small $J_{1,6}$, $J_{2,3}$ values (2.5 Hz). These data suggest that compound **6** possesses a *chiro*-inositol form in its structure. The ^1H and ^{13}C NMR data showed the presence of two tigloyloxy and two 5-hydroxytigloyloxy moieties in **6**, which was confirmed by the HMBC spectrum. In addition, the HMBC correlations of H-3 and H-6 (δ_H 5.41) to carbonyl carbons at δ_C 168.8 (C-1′, C-1″) established the connection from two tigloyloxy units to C-3 and C-6. The positions of two 5-hydroxytigloyloxy units were found to be identical to those of **1**−**3** on the basis of the downfield protons H-4 and H-5 (δ_H 5.76) and their HMBC correlations to two carbonyl carbons at δ_C 167.7 (C-1‴, C-1⁗). Thus, compound **6** was established as 4,5-di-*O*-5-hydroxytigloyl-3,6-di-*O*-tigloyl-*chiro*-inositol.

The anti-inflammatory activities of the isolated compounds were determined by a Griess assay.[8] The isolated compounds **1**−**6** were examined for their inhibition of NO production in LPS-stimulated RAW264.7 cells. In order to screen their cytotoxicity and NO production inhibitory activity, all compounds were evaluated initially at a concentration of 100 μg/mL. None of them showed cytotoxicity (cell viability >85%). Compounds **2** and **5** showed very weak NO production inhibitory activities, with IC_{50} values of 123.7 and 95.6 μM, respectively. Compounds **1**, **3**, and **6** exhibited inhibitory activities, with IC_{50} values of 20.3, 62.9, and 56.7 μM, respectively. Compound **4** displayed the most potent inhibition of NO, with an IC_{50} value of 7.1 μM, among the isolated compounds. Based on the present results, compound **4** in showing a clear anti-inflammatory activity could be selected for further studies.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were recorded with a Shimadzu UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded with an IR Prestige-21 spectrometer (Shimadzu, Kyoto, Japan). NMR spectra were recorded using a Bruker Avance 500 spectrometer (500 MHz for ^1H NMR, 125 MHz for ^{13}C NMR) (Bruker, Billerica, MA, USA) with tetramethylsilane (TMS) as an internal reference. High-resolution electronspray ionization mass spectrometry (HRESIMS) data were acquired with an LCMS-IT-TOF spectrometer (Shimadzu, Kyoto, Japan). Column chromatography was performed with silica gel (60 N, spherical, neutral, 40−50 μm, Kanto Chemical Co., Inc., Tokyo, Japan), reversed-phase C_{18} (RP-18) (Fuji Silysia Chemical Ltd., Kasugai, Aichi, Japan), and Sephadex LH-20 (Dowex 50WX2-100, Sigma−Aldrich, St. Louis, MO, USA). Analytical thin-layer chromatography (TLC) was performed with precoated silica gel $60F_{254}$ and RP-18 F_{254} plates (0.25 or 0.50 mm thickness, Merck KGaA, Darmstadt, Germany). Preparative HPLC was conducted with an Agilent 1260 Infinity II system (Agilent, Santa Clara, CA, USA) using a Zorbax SB−C_{18} column (5 μm particle size, 9.4 × 250 mm) and a DAD detector.

Plant Material. The *C. paniculatus* leaves were collected from Quang Tri Province, Vietnam (geographical coordinates: 17°03′20.4″ N; 107°04′15.4″ E) in August 2018 and identified by Dr. Chinh Tien Vu, Vietnam National Museum of Nature, VAST, Vietnam. A voucher specimen (CP-02) was deposited at the Faculty of Pharmacy, Hue University of Medicine and Pharmacy, Vietnam.

Extraction and Isolation. The dried leaves (4.5 kg) of *C. paniculatus* were extracted three times with MeOH (10.0 L each) at room temperature. The combined MeOH extract was evaporated under reduced pressure to obtain a dark solid extract (357 g). After being suspended in water (2.0 L), the extract was successively partitioned three times with *n*-hexane and ethyl acetate (5.0 L each). The solvents present in the subextracts were then removed in vacuo to yield the *n*-hexane (127 g), ethyl acetate (105 g), and water (W, 98 g)-soluble portions.

The water-soluble extract was applied to a Diaion-HP20 column and eluted with stepwise additions of MeOH in water (0%, 25%, 50%, 75%, 100%) to obtain five major subfractions (W1−W5). Fraction W2 (25.5 g) was chromatographed on a silica gel column, eluted with chloroform−MeOH−water (7:2:0.2, v/v), to give eight fractions (W2.1−W2.8). Fraction W2.3 (2.4 g) was subjected to passage over a RP-18 column, eluted with MeOH−water (1:2, v/v), to obtain five fractions (W2.3.1−W2.3.5). Fraction W2.3.3 (391 mg) was then applied to a Sephadex LH-20 column, eluted with MeOH−water (4:1, v/v), to afford four subfractions (W2.3.3.1−W2.3.3.4). Fraction W2.3.3.2 (90 mg) was further purified by preparative reversed-phase HPLC using MeOH−TFA in water 0.05% (50:50, v/v; flow rate 2.0 mL/min) as the eluent to furnish **1** (5.2 mg), **2** (4.3 mg), **3** (5.7 mg), and **4** (8.1 mg). Fraction W4 (21.0 g) was loaded onto a silica gel

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column, eluted with chloroform−MeOH−water (5:1:0.1, v/v), to give six fractions (W4.1−W4.6). Fraction W4.5 (2.2 g) was next separated by RP-18 column chromatography, eluted with MeOH−water (1:1, v/v), to give five fractions (W4.5.1−W4.5.5). Fraction W4.5.4 (431 mg) was then subjected to purification using a Sephadex LH-20 column, by elution with MeOH−water (4:1, v/v), to afford four fractions (W4.5.4.1−W4.5.4.4). Fraction W4.5.4.2 (161 mg) was successively separated by preparative reversed-phase HPLC using MeOH−TFA in water 0.05% (60:40, v/v; flow rate 2.0 mL/min) as the eluent to afford **5** (4.1 mg) and **6** (3.6 mg).

*4,5-Di-O-5-hydroxytigloyl-1-O-2-methylbutyroyl-3-O-tigloyl-chiro-inositol (**1**):* pale yellow oil; $[\alpha]^{25}_{D}$ +8.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.49) nm; IR (KBr) ν_{max} 3446, 2970, 2936, 1718, 1649, 1450, 1387, 1275, 1221, 1142, 1074, 1032 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 565.2309 $[M + Na]^+$ (calcd for $C_{26}H_{38}O_{12}Na$, 565.2261).

*4,5-Di-O-5-hydroxytigloyl-3-O-2-methylbutyroyl-1-O-tigloyl-chiro-inositol (**2**):* pale yellow oil; $[\alpha]^{25}_{D}$ −3.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.71) nm; IR (KBr) ν_{max} 3447, 2970, 2891, 1715, 1647, 1450, 1389, 1269, 1211, 1140, 1070, 1026 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 565.2216 $[M + Na]^+$ (calcd for $C_{26}H_{38}O_{12}Na$, 565.2261).

*4,5-Di-O-5-hydroxytigloyl-1,3-di-O-2-methylbutyroyl-chiro-inositol (**3**):* pale yellow oil; $[\alpha]^{25}_{D}$ −6.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.23) nm; IR (KBr) ν_{max} 3435, 2970, 2884, 1732, 1649, 1460, 1385, 1279, 1188, 1142, 1072, 1032 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 567.2418 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_{12}Na$, 567.2417).

*3,5-Di-O-5-hydroxytigloyl-2-O-2-methylbutyroyl-6-O-tigloyl-chiro-inositol (**4**):* pale yellow oil; $[\alpha]^{25}_{D}$ +17.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.45) nm; IR (KBr) ν_{max} 3464, 2972, 1713, 1647, 1450, 1387, 1275, 1223, 1142, 1074, 1032 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 581.1987 $[M + K]^+$ (calcd for $C_{26}H_{38}O_{12}K$, 581.2000).

*3,5-Di-O-5-hydroxytigloyl-2,6-di-O-2-methylbutyroyl-chiro-inositol (**5**):* pale yellow oil; $[\alpha]^{25}_{D}$ +9.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.22) nm; IR (KBr) ν_{max} 3402, 2972, 2891, 1711, 1643, 1462, 1389, 1277, 1194, 1144, 1074, 1028 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 567.2363 $[M + Na]^+$ (calcd for $C_{26}H_{40}O_{12}Na$, 567.2417).

*4,5-Di-O-5-hydroxytigloyl-3,6-di-O-tigloyl-chiro-inositol (**6**):* pale yellow oil; $[\alpha]^{25}_{D}$ +32.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 212 (4.46) nm; IR (KBr) ν_{max} 3447, 2965, 2926, 1694, 1433, 1395, 1269, 1205, 1142, 1080, 1028 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz) and ^{13}C NMR (CD_3OD, 125 MHz), see Tables 1 and 2; HRESIMS *m*/*z* 563.2088 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_{12}Na$, 563.2104).

Anti-inflammatory Assay. The nitrite concentration, as an indicator for the presence of NO in the culture medium, was measured by the Griess reaction. Briefly, RAW 264.7 cells were seeded into each well of a 96-well plate at a concentration of 2×10^5 cells/well and maintained in an incubator at 37 °C and 5% CO_2 for 24 h. The cells were then treated with or without test compounds at various concentrations and stimulated with LPS (1 μg/mL) for another 24 h at 37 °C in an incubator. The cell-free supernatant (100 μL) was mixed with an equal volume of the Griess reagent including 50 μL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid and 50 μL of 0.1% (w/v) *N*-1-naphthylethylenediamine dihydrochloride in water to determine nitrite concentrations. Absorbance was measured in a microplate reader at 540 nm with a calibration curve prepared from standard $NaNO_2$ serial dilution. L-NMMA was used as a positive control (IC_{50} value of 30.0 μM). Cell viability of the remaining cells was evaluated using an MTT-based colorimetric assay.[3,9]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01239.

HRESIMS, 1D and 2D NMR, IR, and UV spectra for compounds **1**−**6** and inhibitory activities on NO production against LPS-stimulated RAW264.7 cells (PDF)

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Notes

The authors declare no competing financial interest.

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