Cipadessains A–K, eleven limonoids from the fruits of Cipadessa cinerascens†

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Eleven new mexicanolide-type limonoids, cipadessains A–K (1–11), were isolated from the fruits of Cipadessa cinerascens (Pellegr) Hand.-Mazz. Their planar structures were determined based on IR, UV, 1D and 2D NMR spectra and HRESIMS data. The absolute configuration of 1 was elucidated by single-crystal X-ray diffraction using mirror Cu Kα radiation, and that of compounds 2–8 were determined by ECD analysis. Two mexicanolides bearing methoxybutenolide moiety originated from the furan ring 3 and 6, showed significant cytotoxicity against HepG2 cell line with IC50 values of 5.23 ± 0.12, 8.67 ± 1.02 μM, respectively; and NO inhibitory activities in LPS-activated RAW 264.7 macrophages at nontoxic concentration (IC50 5.79 ± 0.18, 6.93 ± 0.89 μM, respectively).

Introduction

Cipadessa cinerascens (Pellegr) Hand.-Mazz. is a species of Cipadessa genus and known as ‘Ya Luo Qing’ or ‘Lao Ya Fan’ by the Dai.1 Its roots and leaves have been used in Chinese folk medicine for the treatment of rheumatism, stomachache, dysentery, malaria, scald, and skin itch.1–3 Previous reports demonstrated that terpenoids, flavonoids, steroids, and limonoids are the main bioactive constituents of Cipadessa genus.2,3 Among those compounds, limonoids have become a point of interest in the field of natural products, because of its diverse skeletons (mexicanolides, methyl angolensates, trijugins, and cipadesins) and various biological e-ects such as cytotoxic, insecticidal, antioxidant, and trypanocidal activities.2–6 Previous studies on Cipadessa genus focused on their leaves, roots, barks and stems.4–15 To our knowledge, there have been few phytochemical investigations on the fruits of Cipadessa species. In continuing search for new bioactive limonoids, 11 new mexicanolide limonoids with various modified furan rings, cipadessains A–K (1–11), were isolated from the fruits of C. cinerascens. All the isolates were evaluated for NO inhibitory activities in LPS activated RAW 264.7 macrophages and cytotoxicity against HepG2 cell line. In this paper, we describe the isolation, identification of 1–11, as well as their bioactivity screening.

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† Electronic supplementary information (ESI) available: HRESIMS, 1D and 2D NMR spectra of compounds 1–11 and ECD spectrum of 1–8 are available as supporting information. CCDC 1815118. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c8ra00728d

Results and discussion

Cipadessain A (1), was isolated as white powder, and assigned to a molecular formula of C32H40O9 on the basis of HRESIMS at m/z 591.2560 [M + Na]+ (calcd C32H40O9Na, 591.2565), corresponding with 13 degrees of unsaturation. Its IR absorption bands at 3460 and 1728 cm−1 showed the presence of hydroxyl and carbonyl functionalities. The NMR data (Table 1) revealed the characteristic resonances for β-subsutituted furan ring [δH 7.93 (H-21), 7.45 (H-23), and 6.50 (H-22), δC 143.4 (C-23), 142.4 (C-21), 120.5 (C-20), 109.7 (C-22)] four tertiary methyls [δC 1.42, 1.11, 0.85, 0.83]; a characteristic downfield H-17 signal (δH 5.43); a ketone carbonyl (δC 217.3), three ester carbonyls (δC 175.1, 168.6, 167.2) and two unsaturated bonds (δH 6.90, 5.39; δC 139.6, 137.5, 127.6, 125.6). In the HMBC spectrum (Fig. 2), both angular methyls (C-28, C-29) were attached to C-4 (δC 38.7), were elucidated by the cross peaks from H2-28 (δH 0.83 to C-29 (δC 20.3), C-5 (δC 41.8) and C-3 (δC 77.6), and from H2-29 (δH 0.85) to C-28 (δC 22.9), C-5 (δC 41.8) and C-3 (δC 77.6); a C-6/7 esterified appendage (δH 3.74; δC 52.3, 175.1) was easily verified by the HMBC cross peaks from H2-6 (δH 2.98, 2.40) to C-5 (δC 41.8), C-7 (δC 175.1) and from OCH3 (δH 3.74) to C-7 (δC 175.1). From cross peaks of H-3 (δH 4.83) to C-5 (δC 41.8), C-30 (δC 125.6), of H-30 (δH 5.39) to C-9 (δC 63.9), C-1 (δC 217.3), and of H2-19 (δH 1.42) to C-1 (δC 217.3), C-5 (δC 41.8), and C-9 (δC 63.9), the characteristic [3, 3, 3] A/B ring system could be elucidated.16 Furthermore, the HMBC correlations from H-17 (δH 5.43) to C-12 (δC 45.4), C-18 (δC 21.4), C-14 (δC 45.4), of H-15b (δH 2.73) to C-13 (δC 37.4), C-16 (δC 168.6), from H-15a (δH 2.85) to C-8 (δC 137.5), from H2-18 (δH 1.11) to C-17 (δC 76.7), C-14 (δC 45.4) indicated the ring C/D in 1.16 These aforementioned data strongly suggested 1 was a B,D-seco mexicanolide-type limonoid,7,18 typical constituent of Cipadessa genus.
Table 1  

| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 1        | 217.3     |           | 216.9     |           | 217.2     |           | 216.6     |           |
| 2        | 3.56, dd (8.8, 7.7) | 48.9 | 3.50, t (9.7) | 48.9 | 3.50$^b$ | 49.2 | 3.56, t (10.0) | 48.8 |
| 3        | 4.83, d (9.4) | 77.6 | 4.81, d (9.5) | 77.2 | 4.83, d (9.2) | 77.3 | 4.83, d (9.3) | 77.3 |
| 4        | 38.7     |           | 38.7     |           | 38.9     |           | 38.7     |           |
| 5        | 3.49, d (10.4) | 41.8 | 3.39, dd (7.9, 3.8) | 41.4 | 3.31$^b$ | 40.4 | 3.37, d [9.5] | 41.7 |
| 6        | 2.40, dd (16.8, 10.8) | 32.2 | 2.38, m | 33.0 | 2.36, dd (17.3, 10.4) | 33.1 | 2.39, dd (17.0, 9.5) | 32.8 |
| 7        | 2.98, d (16.8) | 2.38, m |           |           | 2.48, d (17.3) |           | 2.23, d (17.0) |           |
| 8        | 175.1    |           | 174.1    |           | 173.3    |           | 174.5    |           |
| 9        | 137.5    |           | 141.2    |           | 138.0    |           | 137.5    |           |
| 10       | 2.19, d (9.8) | 63.9 | 2.72, dd (12.2, 5.5) | 53.0 | 2.20$^b$ | 56.5 | 2.25$^b$ | 56.4 |
| 11       | 4.60, td (10.4, 4.6) | 65.8 | 2.07, dd (13.0, 4.1) | 20.2 | 1.73$^b$ | 21.1 | 2.07, m | 20.5 |
| 12       | 1.39, d (11.8) | 45.4 | 1.33, d (13.5) | 28.6 | 1.43, td (14.0, 3.5) | 34.8 | 1.59, m | 34.2 |
| 13       | 1.82, d (11.8) | 45.4 | 1.98, dd (13.5, 4.1) | 1.91, d (14.0) |           |           | 1.83, m |           |
| 14       | 2.24, d (5.8) | 45.4 | 73.4 | 2.22$^b$ | 45.4 | 2.24$^b$ | 45.4 |
| 15       | 2.85, dd (18.8, 5.8) | 30.0 | 2.99, d (18.0) | 39.2 | 2.79, m | 29.2 | 2.80, m | 29.5 |
| 16       | 2.73, d (18.8) | 2.92, d (18.0) | 2.78, m |           |           |           | 2.79, m |           |
| 17       | 168.6    |           | 168.6    |           | 167.8    |           | 167.0    |           |
| 18       | 76.7     | 5.66, s | 77.4     | 5.54, s | 76.6     | 5.73, s | 78.1     |           |
| 19       | 1.11, 3H, s | 21.4 | 1.08, 3H, s | 15.9 | 1.00, 3H, s | 22.8 | 1.07, 3H, s | 21.9 |
| 20       | 1.42, 3H, s | 18.7 | 1.16, 3H, s | 15.9 | 1.16, 3H, s | 15.7 | 1.16, 3H, s | 15.8 |
| 21       | 120.5    |           | 120.1    |           | 136.1    |           | 164.1    |           |
| 22       | 7.93, s | 142.4 | 7.84, s | 142.4 |           |           | 168.2    | 4.98, dd (18.0, 2.8) |
| 23       | 6.50, s | 109.7 | 6.49, s | 110.0 | 7.23, s | 147.8 | 6.21, d (1.08) | 118.7 |
| 24       | 7.45, s | 143.4 | 7.42, s | 143.0 | 5.80, s | 102.4 |           | 172.9 |
| 25       | 0.83, 3H, s | 22.9 | 0.78, 3H, s | 22.5 | 0.79, 3H, s | 22.6 | 0.78, 3H, s | 22.5 |
| 26       | 0.85, 3H, s | 20.3 | 0.82, 3H, s | 20.6 | 0.81, 3H, s | 20.9 | 0.82, 3H, s | 20.4 |
| 27       | 5.39, td (7.5, 11.7) | 125.6 | 5.63, d (6.8) | 124.5 | 5.34, d (6.6) | 123.4 | 5.35, d (6.9) | 124.3 |
| 28       | 3.74, 3H, s | 52.3 | 3.71, 3H, s | 52.3 | 3.64, 3H, s | 52.1 | 3.69, 3H, s | 52.5 |
| 29       | 7-OCH$_3$ | 3.74, 3H, s | 52.3 | 3.64, 3H, s | 52.1 | 3.69, 3H, s | 52.5 |
| 30       | 7- OCH$_3$ |           |           |           |           |           |           |           |
| 31       | 1'       | 167.2    |           | 176.7    |           | 167.3    |           | 167.1    |
| 32       | 2'       | 127.6    | 2.62, m | 33.9 | 127.8 | 127.7 |
| 33       | 3'       | 139.6    | 1.15, 3H$^b$ | 19.2 | 6.91, m | 139.3 | 6.88, m | 139.4 |
| 34       | 4'       | 1.80, 3H, s | 11.9 | 1.17, 3H$^b$ | 18.6 | 1.84, 3H, s | 12.0 | 1.82, 3H, m$^b$ | 12.0 |
| 35       | 5'       | 1.68, 3H, d (7.1) | 14.7 | 1.82, 3H, d (7.3) | 14.8 | 1.82, 3H, m$^b$ | 14.9 |

$^a$ $^1$H (500 MHz) NMR data of compounds. $^b$ Signals were overlapped.

Fig. 1  
Chemical structures of cipadessains A–K (1–11).
Furthermore, the existence of $\Delta^{8,30}$ double bond was determined by the HMBC correlations from H-30 ($\delta_H$ 5.39) to C-9 ($\delta_C$ 63.9) and C-14 ($\delta_C$ 45.4), and from H-2 ($\delta_H$ 3.56) to C-1 ($\delta_C$ 217.3), C-30 ($\delta_C$ 125.6). The correlation from H-3 ($\delta_H$ 4.83) to C-1’ ($\delta_C$ 167.2) of the tigloxyl moiety [$\delta_H$ 6.90 (H-3’), 1.80 (H-4’), 1.68 (H-5’); $\delta_C$ 167.2 (C-1’), 139.6 (C-3’), 127.6 (C-2’), 14.7 (C-5’), 11.9 (C-4’)] showed the presence of a tigloxyl group at C-3. The NMR data of compound 1 were similar to those of 6-desoxyswietenine. The ROESY correlations between H-17 ($\delta_H$ 4.60) with C-11 ($\delta_C$ 65.8) and HMBC correlations from H-9 ($\delta_H$ 2.19) and H-12 ($\delta_H$ 1.82, 1.39) to C-11 ($\delta_C$ 65.8). Thus, the planar structure of 1 was determined as shown in Fig. 1.

The ROESY correlations between H-17 ($\delta_H$ 5.43)/H-11 ($\delta_H$ 4.60), H-5 ($\delta_H$ 3.49)/H-28 ($\delta_H$ 0.83) suggested these protons were the same configuration (Fig. 2). In turn, the ROESY correlations between H-2 ($\delta_H$ 3.56)/H-3 ($\delta_H$ 4.83), H-3 ($\delta_H$ 4.83)/H-19 ($\delta_H$ 0.85), H-14 ($\delta_H$ 2.24)/H-19 ($\delta_H$ 1.42) and H-9 ($\delta_H$ 2.19)/H-19 ($\delta_H$ 1.42) revealed that they were co-facially oriented. Accordingly, the structure of 1 was proposed as shown. Fortunately, compound 1 was recrystallized in the CH$_2$Cl$_2$/MeOH (1:1) mixture to yield prisms. On the basis of single-crystal X-ray diffraction data (CCDC 1815118), the absolute stereochemistry of 1 was elucidated to be 2S, 3R, 5S, 9S, 10S, 11R, 13S, 14R, 17S (Fig. 3). Finally, the structure of 1, with a $\Delta^{8,30}$ double bond and a 11-hydroxyl group, was established as depicted in Fig. 1.

Compound 2, was obtained as a colorless needles, which had a sodium adduct ion peak at $m/z$ 579.2568 [M + Na]$^+$ in the HRESIMS spectrum corresponding to a molecular formula of C$_{31}$H$_{40}$O$_9$. The NMR spectroscopic data of 2 (Table 1) exhibited close similarity to those of 1. In comparison to 1, the correlations from H$_3$-3’ (H$_3$-4’) to C-1’ ($\delta_C$ 176.7) and from C-2’ ($\delta_C$ 33.9), H-3 ($\delta_H$ 4.81) to C-1’ ($\delta_C$ 176.7) in the HMBC spectrum indicated the presence of an isobutyryloxyl group at C-3 in 2 instead of a tigloxyl group in 1. An upfield shift at $\delta_C$ 20.2 ($\Delta = -45.6$ ppm) of C-11 and a downfield shift at $\delta_C$ 73.4 ($\Delta = +28.0$ ppm) of C-14 in the $^{13}$C NMR spectrum of 2 as compared with the corresponding signals of 1 implied that the hydroxyl group was located at C-14. The deduction was supported by the HMBC correlations of C-14 ($\delta_C$ 73.4) and H$_2$-15 ($\delta_H$ 2.99, 2.92), H-17 ($\delta_H$ 5.66), H-30 ($\delta_H$ 5.63). The z-oriented of OH-14 was determined by comparison of NMR data of 2 with those of two known compounds Khasenegasin D and Khasenegasin E. Therefore, the structure of 2 was established as shown (Fig. 1).

Compound 3 was isolated as a white amorphous powder and showed the HRESIMS ion peak at $m/z$ 621.2669 [M + Na]$^+$ (calcd for C$_{31}$H$_{40}$O$_9$Na, 621.2670). The spectroscopic data of 3 were similar to those of 6-desoxyswietenine, except for the presence of 23-methoxybutenolide ring moiety in 3 instead of the $\beta$-substituted furan moiety as found in 6-desoxyswietenine. This was confirmed by the HMBC correlations of OCH$_3$-23 ($\delta_C$ 3.58)/C-23 ($\delta_C$ 102.4)/H-23 ($\delta_H$ 5.80)/C-21 ($\delta_C$ 168.2), C-20 ($\delta_C$ 136.1); of H-22 ($\delta_H$ 7.23)/C-23 ($\delta_C$ 102.4), C-20 ($\delta_C$ 136.1), C-21 ($\delta_C$ 168.2), of H-17 ($\delta_C$ 5.54)/C-21 ($\delta_C$ 168.2), C-20 ($\delta_C$ 136.1). Thus, the planar structure of 3 was identified as shown. The relative configuration of 3 was mainly elucidated by its ROESY data and comparison of its NMR spectroscopic data with those of similar reported compounds. The correlations between H-17/H-11b ($\delta_H$ 2.29), H-17/H-15b ($\delta_H$ 2.78), H-17/H-5 ($\delta_H$ 3.51), indicated these groups were on the same side of the structure, and they were assigned as $\beta$-configuration. Correlations of H$_2$-19 ($\delta_H$ 1.16)/H-9 ($\delta_H$ 2.20)/H-2 ($\delta_H$ 3.50), H-9/H-3 ($\delta_H$ 4.83)/H-29 ($\delta_H$ 0.81), H-3/H-14 ($\delta_H$ 2.22) and cross peaks of OCH$_3$-23/CH$_3$-18 revealed that these protons were z-oriented. Hence, the structure of 3 was elucidated as shown in Fig. 4 and named cipadessain C.

Compound 4, a white, amorphous powder, displayed a molecular formula C$_{32}$H$_{40}$O$_{10}$ on the basis of its HRESIMS ion at $m/z$ 591.2565 ([M + Na]$^+$, C$_{32}$H$_{40}$O$_{10}$Na, calcd 591.2565). The $^1$H and $^{13}$C NMR data (Table 1) of 3 and 4 were almost the same except for signals of the E ring. The appearance of $\alpha,\beta$-unsaturated-$\gamma$-lactone ring portion linked to C-17 in 4 was determined by the HMBC correlations between H-17 ($\delta_H$ 5.73)/C-20 ($\delta_C$ 164.1), C-21 ($\delta_C$ 72.2), C-22 ($\delta_C$ 118.7) and H$_2$-21 ($\delta_H$ 4.98)/C-20 ($\delta_C$ 164.1), C-22 ($\delta_C$ 118.7), C-23 ($\delta_C$ 172.9). The ROESY correlations were observed between H-17 ($\delta_H$ 5.73)/H-5 ($\delta_H$ 3.37), H-5/ H-11b ($\delta_H$ 2.07), H-5/H$_3$-28 ($\delta_H$ 0.78), H$_2$-29 ($\delta_H$ 0.82)/H-3 ($\delta_H$ 4.83), H-3/H-2 ($\delta_H$ 3.56) suggesting the relative configuration of
the cross peaks of H3-4 6.22 and by carbon signals at C33H44O11Na, 639.2776). The 1D and 2D NMR data (Table 2) of 17 in additional presence of 21-hydroxybutenolide unit attached to C-29 (ðH 10440 | D 625.2619). The 1H and 13C-NMR data of of the ROESY correlations based on the general rule, con spectrum, the cross peak of H-23 (ðC 103.1); H-23 (ðH 5.03) to C-20 (ðC 127.6/127.2), of H-3 (ðH 4.97 was correlated with the appearance of pairs of proton and carbon resonances in the NMR spectra of 5 indicated the presence of epimers at C-21. Thus, based on these data, the planar structure of 5 was established.

Compound 6 gave a molecular formula of C33H44O11 from the positive ion peak at m/z 639.2771 [M + Na]+ (calcd for C32H42O11Na, 639.2776). The 1D and 2D NMR data (Table 2) of 6 were similar to those of 2′R-cipadesin A,† a mexicanolide-type limonoid with a Δ6,30 epoxide ring from Cipadessa fruticosa. The structures 6, featuring 23-methoxybutenolide ring in the C-17 side-chain, was verified by the key HMBC cross peaks of OCH3-C17 (ðC 103.1); H-23 (ðH 5.82/5.78)/C-21 (ðH 5.03) to C-20 (ðC 127.6/127.2), of H-3 (ðH 4.97) correlated with and concomitantly a γ-hydroxybutenolide group. The detailed analysis of the ROESY correlations between H3-4 (ðH 1.90) and H-15 (ðC 167.0), C-3′ (ðC 140.6/140.2), of H-2′ (ðH 2.55) and H-15x (ðH 3.50), between H-1′ (ðH 1.24) and H2-29 (ðH 0.78) confirmed its relative configurations were as the same as that of cipadesin A.

The molecular formula of 8 was determined to be C34H44O11 from the HRESIMS peak at m/z 623.2464 (calcd for C34H40O11Na, 623.2463), 2 mass units less than 7. The NMR data of 8 (Table 2) exhibited overall similarity to those of 7, but revealed the presence of a tigloxyl moiety instead of a 2-methylbutenyl moiety attached to C-3. This was proved by the HMBC correlations of H-3 (ðH 1.91) to C-1′ (ðC 167.0), C-3′ (ðC 140.6/140.2), of H-2′ (ðH 1.90) to C-2′ (ðC 127.6/127.2), of H-3 (ðH 5.03) to C-1′ (ðC 167.0). Therefore, the planar structure of 8 was assigned as shown. The α-configuration of epoxide ring at C8/30 was determined by the coupling constant value of H-30 (J = 2.3 Hz). The relative configuration of the tigloxyl moiety at C-3 was β-oriented according to the observed ROESY correlations between H-3 and H-2, and the coupling constant value of H-3 (J = 9.4 Hz). The appearance of pairs of most proton and carbon resonances in the NMR spectra (Table 2) of 7 identified the presence of C-21 epimers. Thus, compound 7 was elucidated as a mexicanolide-type limonoid bearing γ-hydroxybutenolide group. According to the general ruler, the ROESY correlations between H3-5 (ðH 0.97) and H-30 (ðH 3.30), between H-2′ (ðH 2.55) and H-15x (ðH 3.50), between H3′-5′ (ðH 1.24) and H2-29 (ðH 0.78) confirmed its relative configurations were as the same as that of cipadesin A.

The similar electronic circular dichroism spectra of 1-8 (see S1 in ESI†) indicated that the basic skeletons of these compounds possessed absolute configurations as Fig. 1. Compound 9 was isolated as a white powder. Its molecular formula, C33H42O10 was deduced from the HRESIMS data (m/z 567.2597 [M – H]−, calcd for C33H40O10, 567.2600). Analysis of its NMR spectra indicated that 9 was an analogue of proceranolide. A proton signal at ðH 4.97 was correlated with a carbon signal at ðC 65.4 in HSQC spectrum and the HMBC correlation from the hydroxyl group (ðH 3.18 br s) to C-15 (ðC 65.4) indicated that the hydroxyl group was located at C-15. Furthermore, a tigloxyl moiety was located at C-3′, which was confirmed by the HMBC correlation from H-3 (ðH 4.89) to C-
### Table 2
$^1$H NMR and $^{13}$C NMR spectral data of compounds 5–8 in CDCl$_3$ ($\delta$ in ppm, J in Hz)

| Position | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ |
|----------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1        | 217.3/217.2 | 214.3      | 213.9      | 214.2      |
| 2        | 3.49, m    | 48.8       | 48.9       | 49.3/49.1  |
| 3        | 4.75, d (9.2) | 77.4$^b$ | 5.09, d (9.2) | 77.1    | 5.03, d (9.3) | 78.1/77.5 |
| 4        | 38.6       | 39.5       | 39.5/39.3  | 39.4/39.2  |
| 5        | 3.39, d (10.0) | 40.7/40.7 | 3.21, t (5.0) | 42.6    | 3.27, d (9.4) | 43.0/42.6 |
| 6        | 2.43, dd (17.1, 5.8) | 33.1/33.0 | 2.35, d (5.0) | 33.1    | 3.32, d (16.6) | 33.2     |
| 7        | 173.9/173.6 | 174.3      | 174.8      | 174.9      |
| 8        | 137.9/137.8 | 60.6       | 60.1       | 60.1       |
| 9        | 2.23$^b$   | 56.3/56.2  | 1.90$^b$   | 54.9/54.6  |
| 10       | 50.5/50.2  | 48.4       | 48.3/48.2  | 48.3/48.2  |
| 11       | 1.73, br s | 1.76, m    | 1.78, m    | 1.85, m    |
| 12       | 1.92, d (14.0) | 34.5     | 1.13$^b$   | 33.1       |
| 13       | 1.44, m    | 2.25, d (16.3) | 1.39, m    | 33.1       |
| 14       | 36.8/36.7  | 36.8       | 36.5/36.4  | 36.3/26.2  |
| 15       | 45.5       | 1.49$^b$   | 46.4       | 46.5/46.1  |
| 16       | 29.6       | 3.76, m    | 3.50, dd (16.4, 13.7) | 33.6/33.5 |
| 17       | 169.0/169.0 | 2.85, dd (15.5, 3.7) | 2.84, dd (16.4, 4.3) | 2.77, dd (16.5, 4.0) |
| 18       | 77.2       | 5.10, s    | 5.30, br s/5.15, br s | 79.2/78.4 |
| 19       | 1.06/1.04, 3H, s | 23.0     | 1.04, 3H, s | 16.3/16.2 |
| 20       | 15.7/15.7  | 15.8       | 1.08/1.06, 3H, br s | 26.7/26.2 |
| 21       | 135.1/135.0 | 133.7      | 162.7      | 162.7/160.2 |
| 22       | 169.6      | 169.8      | 6.32, br s/6.14, br s | 98.7/97.7 |
| 23       | 7.35/7.34, br s | 149.8/149.6 | 7.28, s    | 122.5/121.8 |
| 24       | 6.18/6.17, br s | 97.1/96.7 | 5.82, br s/5.78, br s | 169.4/169.0 |
| 25       | 22.5       | 21.2       | 0.81, 3H, s | 20.9/20.9 |
| 26       | 210/20.9   | 22.5       | 0.78, 3H, s | 22.7     |
| 27       | 123.3/123.3 | 3.31, br s | 63.7       | 63.6/63.5  |
| 28       | 3.66/3.64, 3H, br s | 52.4/52.3 | 3.72, 3H, s | 52.5     |
| 29       | 3.62, br s/3.56, br s | 57.8     | 3.72, 3H, s | 52.8/52.6  |
| 30       | 52.4/52.3  | 63.6/63.5  | 3.20, d (2.2) | 63.7/63.4 |
| 31       | 7-OCH$_3$  | 3.66/3.64, 3H, br s | 52.4/52.3 | 3.72, 3H, s | 52.8/52.7  |
| 32       | 23-OCH$_3$ | 3.62, br s/3.56, br s | 57.8     | 3.72, 3H, s | 52.8/52.7  |
| 33       | 174.3/174.2 | 175.8      | 176.1/175.9 | 167.0    |
| 34       | 27.2/27.2  | 41.7       | 41.5/41.3  | 127.6/127.2 |
| 35       | 9.0        | 1.54, m    | 26.5       | 140.6/140.3 |
| 36       | 1.25, 3H, d (7.0) | 17.5     | 1.24, 3H, d (7.0) | 17.3/17.3 |
| 37       | 0.97, 3H, t (7.4) | 12.0     | 0.97, 3H, t (7.4) | 11.9/11.8 |

$^a$ $^1$H (500 MHz) NMR data of compounds. $^b$ Signals were overlapped.

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**Fig. 5** Key HMBC and ROESY correlations of compound 6.
' (δC 167.2). In the ROESY spectrum, the H-15 (δH 4.97) was correlated with H-17 (δH 5.54), which suggested the H-15 was β-oriented. The other configurations of 9 were similar to that of proceranolide by ROESY spectroscopic analysis.

Compound 10, was isolated as a white powder and its molecular formula C32H42O10 was determined by the molecular ion peak at m/z 609.2675 (calcd for C32H42O10Na, 609.2670) in the HREIMS. The 1D and 2D NMR data indicated that 10 was also a mexicanolide-type limonoid bearing a γ-hydroxylbutenolide unit as 8. A comparison of their NMR data (Table 3) revealed the main differences being the presence of the Δ8,14 double bond and the absence of the Δ8,20 epoxide ring. The conclusion was confirmed from the HMBC correlations of H-15b (δH 3.46) to C-8 (δC 129.2/128.9), of H-30b (δH 2.12) to C-14 (δC 130.8/130.5), of H-2 (δH 3.19) to C-8 (δC 129.2/128.9). Moreover, the tigoxyl group was replaced by the β-methylbutyryl at C-3 in 10, which can be proved from the H-3 (δH 4.97) to C-1' (δC 176.3). The appearance of pairs of proton and carbon resonances in the NMR spectra of 10 indicated that the presence of epimers at C-21. Therefore, the structure of 10 was shown in Fig. 1. The relative structure of 10 was displayed by the ROESY experiment and general ruler.7

Compound 11, was isolated as white and amorphous powder, shown as a positive HRESIMS ion peak at m/z 591.2562 ([M + Na]⁺, calcd for C32H40O10Na 591.2563). The 1H and 13C-NMR data of 11 (Table 3) suggested that its structure was closely related to that of khasengasin N with a Δ8,9 double bond.14 The only difference between 11 and khasengasin N was that a hydroxyl group linked to C-3 as found in khasengasin N was replaced by a tigloxyl group [δH 1.89 (s), δH 1.86 (d, J = 7.1 Hz) and 6.92 (m)] in 11. Further, it was evidenced by HMBC correlation of H-3 (δH 5.17) to C-1' (δC 167.0) of the tigloxyl group. The relative configuration of 11 was assigned by the ROESY experiment and general ruler.8

Table 3 1H NMR and 13C NMR spectral data of compounds 9–11 in CDCl3 (δ in ppm, J in Hz)

| Position | 9α | 10β | 11γ |
|----------|-----|-----|-----|
|          | δH | δC | δH | δC | δH | δC |
| 1        | 217.7 | 217.5/217.3 | 213.4 |
| 2        | 3.26, m | 48.4 | 3.19, m | 48.1 |
| 3        | 4.89, m | 79.3 | 4.97, d (9.8) | 78.0/77.9 |
| 4        | 38.8 | 38.4/38.2 |
| 5        | 3.37, dd (11.0, 1.5) | 40.7 | 3.11, d (10.7) | 41.4/41.2 |
| 6        | 2.42, d (16.5, 11.0) | 33.4 | 2.45, m | 33.9 |
| 7        | 2.37, d (16.5) | 2.36, m |
| 8        | 147.4 | 176.3/175.7 |
| 9        | 2.11, d (6.6) | 51.9 | 2.05ª | 51.3 |
| 10       | 53.6 | 53.1/52.8 |
| 11       | 2.17, m | 18.7 | 1.79, m | 18.7/18.6 |
| 12       | 1.89, m | 1.94, m |
| 13       | 1.00 | 28.6 | 1.99, m | 28.6 |
| 14       | 1.78, m | 1.27, m |
| 15       | 1.02, m | 38.8 | 39.1 |
| 16       | 4.97, s | 65.4 | 3.77, m | 32.9 |
| 17       | 5.54, s | 81.9 | 5.62, br s/5.35, br s | 79.7 |
| 18       | 1.02, 3H, s | 16.9 | 1.08, 3H, s | 17.4 |
| 19       | 1.18, 3H, s | 16.7 | 1.17, 3H, s | 16.9 |
| 20       | 120.6 | 162.8 |
| 21       | 7.62, s | 142.0 | 6.10, s | 98.9/98.0 |
| 22       | 6.51, s | 110.0 | 6.30, s | 122.8/122.1 |
| 23       | 7.42, s | 143.0 |
| 24       | 0.76, 3H, s | 20.4 | 0.68, 3H, s | 23.5 |
| 25       | 0.83, 3H, s | 23.9 | 0.81, 3H, s | 20.6 |
| 26       | 2.99, dd, (15.9, 1.9) | 34.6 | 2.72, d (15.0) | 33.1 |
| 27       | 2.30, dd, (15.9, 6.6) | 21.2, d (6.0) |
| 7-OCH3   | 3.73, 3H, s | 52.6 | 3.71, 3H, s | 52.6/52.2 |
| 15-OH    | 3.18, br s |
| 1'       | 167.2 | 176.3 |
| 2'       | 129.4 | 2.40, m | 41.3 |
| 3'       | 6.90, m | 138.6 | 1.73, dt (20.0, 7.0) | 27.3 |
| 4'       | 1.87, 3H, m | 12.5 | 1.21, 3H, m | 16.2 |
| 5'       | 1.79, 3H, d (7.0) | 14.6 | 0.91, 3H, dd (7.1, 5.5) | 11.3 |

ª 1H (500 MHz) NMR data of compounds. 8 1H (600 MHz) NMR data of compound. 9 Signals were overlapped.
Table 4 Cytotoxicity and NO inhibition activities of compounds 1–11a,b

| Compounds | NO inhibition RAW 264.7 macrophages | Cytotoxicity (HepG2) |
|-----------|-------------------------------------|----------------------|
| 3         | 5.79 ± 0.18                         | 5.23 ± 0.12          |
| 4         | 23.90 ± 2.1                         | >50                  |
| 6         | 6.93 ± 0.89                         | 8.67 ± 1.02          |
| 7         | 20.54 ± 0.63                        | >50                  |
| L-NMMAc   | 41.88 ± 0.91                        | —                    |
| Doxorubicinc | —                                   | 1.04 ± 0.37          |

a Values were expressed as the means ± SD based on three independent experiments. b Compounds 1,2,5,8–11 were inactive. c Positive controls.

experiment, in which key cross-peaks between H-17/H-5, H-5/H-30 indicated H-30 to be β-oriented (Fig. 6). Therefore, the structure of 11 was demonstrated as shown in Fig. 1.

All the isolates were evaluated for their cytotoxicity against HepG2 cancer cell line with doxorubicin as the positive control. As shown in Table 4, compounds 3 and 6 showed significant cytotoxic activities. These isolates were further tested for their inhibitory effects on NO production of LPS-activated RAW 264.7 macrophages with N-nomethyl-L-arginine as the positive control (IC50 41.88 ± 0.91 μM). The results showed that compounds 3 and 6 exhibited significant NO inhibitory activities with IC50 values of 5.79 ± 0.18 μM, 6.93 ± 0.89 μM, respectively. On the contrary, those mexicanolides bearing complete furan moiety or hydroxybutenolide furan moiety were inactive (1, 2, 5, 8–11). According to the continuing study, the modified furan moiety may play important role on the bioactivity of these limonoids.

Conclusions

In summary, eleven new mexicanolide-type limonoids, cipadessains A–K (1–11) were isolated from the fruits of Cipadessa cinerascens. Their structures were elucidated on the NMR data and HRESIMS. The absolute configuration of 1 was determined by X-ray diffraction. In bioactivity screening, two mexicanolides (3 and 6) bearing methoxybutenolide moiety originated from the furan ring showed significant cytotoxicity activities against HepG2 cell line and NO inhibitory activities in LPS-activated RAW 264.7 macrophages, which were more excellent than those limonoids with complete furan moiety or hydroxybutenolide furan moiety. This finding adds the complexity and diversity of mexicanolide-type limonoids in Cipadessa cinerascens. It’s worthy of paying more attention to the cytotoxicity activities against HepG2 cell line of the limonoids with modified furan rings from the Cipadessa genus.

Experimental

General experimental procedures

UV and IR were recorded on a Shimadzu UV-2450 spectrophotometer (Shimadzu, Tokyo, Japan) and Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany), optical rotations were measured on a JASCO P-1020 polarimeter (Jasco, Tokyo, Japan). ECD spectra were obtained on a JASCO J-810 spectropolarimeter (Jasco, Tokyo, Japan) respectively. 1D and 2D NMR spectra were measured by a Bruker AVIII-600 and AVIII-500 NMR instrument at 600 and 500 MHz (1H), 150 MHz (13C) and 125 MHz (13C) in CDCl3. HRESI mass spectra were conducted on an Agilent 6520B UPLC-Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). MCI gel (Mitsubishi Chemical Corp., Tokyo, Japan), MPLC [Beijing H&E Co., Ltd., Beijing, China], and RP-C18 (40–63 μm, Fuji), silica gel (Qingdao Haiyang Chemical Co., Ltd.) were used as column chromatography. Preparative HPLC was performed on a Shimadzu LC-6AD instrument with a SPD-10A detector using a shim-pack reversed-phase C18 column (20 × 200 mm, 10 μm). Analytical HPLC was carried out on an Agilent 1260 Series instrument with a DAD detector using a shim-pack VP-OBS column (250 × 4.6 mm, 5 μm). All solvents used were analytical grade (Jiangsu Hanbon Science and Technology Co., Ltd.). The mouse macrophage cell line RAW264.7 and human hepatocellular carcinoma (HepG2) cell were obtained from the Cell Bank of the Shanghai Institute of Cell Biology and Biochemistry, Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Dulbecco’s modified Eagle medium (DMEM, GIBCO Invitrogen Corp., Carlsbad, CA, USA) with 10% FBS, penicillin (100 U ml−1), and streptomycin (100 μg ml−1) in a humidified atmosphere with 5% CO2 at 37 °C.

Plant material

The air-dried fruits of Cipadessa cinerascens (Pellegr) Hand.-Mazz (Meliaceae) were collected from Xishuangbanna,
Yunnan province, People's Republic of China, in July 2013, and authenticated by Professor Mian Zhang, Research Department of Pharmacognosy, China Pharmaceutical University. A voucher specimen (no. 2015-CCHM) has been deposited in the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and isolation
The dried and powdered fruits of *Cipadessa cinerascens* (5.0 kg) were extracted by refluxing with 95% ethanol (25 L x 4), for 4 h, 4 h, 4 h, 3 h. The ethanol extract was concentrated under reduced pressure to afford the crude extract (637 g), it was suspended in H2O (1.5 L) and partitioned sequentially with petroleum ether (PE), CH2Cl2, EtOAc. The oily PE and CH2Cl2 part were subjected to a silica gel column gradient eluted with EtOH/H2O (30%, v/v) and CH2Cl2/MeOH (100 : 1 to 0:100), further purified by semi-preparative HPLC (MeCN/H2O; 50 : 50, v/v, 10 ml min -1) to yield 14 sub-fractions (C3A-1 to C3A-14).

*Cipadessa A* (1). Colorless crystals (MeOH : CH2Cl2 = 1 : 1); [α]25D −47.6 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 229 (3.58) nm; ECD (MeOH, Δe) 200 (−0.1047), 239 (−1.894), 294 (−4.559) nm; IR (KBr) νmax 4351, 2922, 1640, 1384 cm −1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 591.2560 [M + Na]+ (calcd for C32H40O9Na, 591.2568 [M + Na]+).

*Cipadessa B* (2). Colorless crystals (MeOH : CH2Cl2 = 1 : 1); [α]25D −119.3 (c 0.13, MeOH); UV (MeOH) λmax (log ε) 227 (3.58) nm; ECD (MeOH, Δe) 200 (−11.079), 212 (−4.854), 293 (−2.852) nm; IR (KBr) νmax 3452, 1730, 1640, 1384 cm −1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 579.2568 [M + Na]+ (calcd for C31H39O9Na, 579.2565).

*Cipadessa C* (3). White powder; [α]25D −44.6 (c 0.13, MeOH); UV (MeOH) λmax (log ε) 210 (3.91) nm; ECD (MeOH, Δe) 200 (−43.442), 229 (−5.615), 294 (−5.617) nm; IR (KBr) νmax 3453, 1727, 1647, 1437, 1383, 1263 cm −1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 621.2669 [M + Na]+ (calcd for C31H40O10Na, 621.2670).

*Cipadessa D* (4). White powder; [α]25D −79.1 (c 0.13, MeOH); UV (MeOH) λmax (log ε) 234 (3.47) nm; ECD (MeOH, Δe) 200 (−13.199), 255 (−0.468), 294 (−3.856) nm; IR (KBr) νmax 3455, 1726, 1643, 1455 cm −1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 591.2568 [M + Na]+ (calcd for C32H42O10Na, 591.2565).

*Cipadessa E* (5). White powder; [α]25D −56.8 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 208 (3.61) nm; ECD (MeOH, Δe) 200 (−22.845), 224 (−7.992), 297 (−5.732) nm; IR (KBr) νmax 3455, 1726, 1643, 1455 cm −1; 1H and 13C NMR data, see Table 1; HRESIMS m/z 581.2333 [M + Na]+ (calcd for C32H42O12Na, 581.2357).

*Cipadessa F* (6). White powder; [α]25D −34.0 (c 0.09, MeOH); UV (MeOH) λmax (log ε) 219 (3.64) nm; ECD (MeOH, Δe) 200 (−4.342), 252 (−1.559), 296 (−3.587) nm; IR (KBr) νmax 3451, 1639, 1384, 617 cm −1; 1H and 13C NMR data, see Table 2; HRESIMS m/z 639.2771 [M + Na]+ (calcd for C33H44O11Na, 639.2771).

*Cipadessa G* (7). White powder; [α]25D −40.1 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 210 (3.86) nm; ECD (MeOH, Δe) 209 (−11.148) 246 (+4.267), 294 (−6.172) nm; IR (KBr) νmax 3460, 2969, 1768, 1635, 1460 cm −1; 1H and 13C NMR data, see Table 2; HRESIMS m/z 625.2620 [M + Na]+ (calcd for C32H42O12Na, 625.2619).

*Cipadessa H* (8). White, amorphous powder; [α]25D −54.9 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 230 (3.35) nm; ECD (MeOH, Δe) 200 (−16.272), 224 (−7.992), 297 (−5.732) nm; IR (KBr) νmax 3451, 2969, 1768, 1641, 1457 cm −1; 1H and 13C NMR data, see Table 2; HRESIMS m/z 623.2463 [M + Na]+ (calcd for C32H42O11Na, 623.2464).

*Cipadessa J* (9). White powder; [α]25D −108.41 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 213 (3.80) nm; ECD (MeOH, Δe) 200 (−20.403), 222 (−15.435) nm; IR (KBr) νmax 3400 1725, 1645, 1460 cm −1; 1H and 13C NMR data, see Table 3; HRESIMS m/z 567.2597 [M − H]− (calcd for C32H42O10Na, 567.2600).

*Cipadessa I* (10). White powder; [α]25D −33.1 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 228 (4.14) nm; ECD (MeOH, Δe) 200 (−5.196), 208 (−2.013), 225 (−5.931), 258 (−8.547), 296 (−11.285) nm; IR (KBr) νmax 3445, 1730, 1643, 1142 cm −1; 1H and 13C NMR data, see Table 3; HRESIMS m/z 609.2675 [M + Na]+ (calcd for C32H42O10Na, 609.2670).

*Cipadessa K* (11). Colorless needles (MeOH−H2O); [α]25D −41.5 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 229 (3.48) nm; ECD (MeOH, Δe) 200 (−12.576), 294 (−3.937) nm; IR (KBr) νmax 3460, 2931, 1728, 1648 cm −1; 1H and 13C NMR data, see Table 3; HRESIMS m/z 591.2565 [M + Na]+ (calcd for C32H42O10Na, 579.2565).

X-ray crystallographic analysis of 1. Colorless crystals of 1 were recrystallized from CH2Cl2-MeOH (1 : 1). Crystal data were obtained on a Bruker Smart-1000 CCD with a graphite monochromator with Cu Kα radiation (λ = 1.54184 A) at 290(2) K. The structure was solved by direct methods using the SHELXS-97 (ref. 23) and expanded with difference Fourier techniques, refined with the SHELXL-97.24
Crystal data of 1. C_{32}H_{40}O_{9} (M = 568.64); monoclinic crystal (0.280 × 0.250 × 0.200 mm³); space group P2_1/c; unit cell dimensions a = 1.49100 (10) Å, b = 13.49460 (10) Å, c = 18.7471 (2) Å, V = 2907.05 (5) Å³, θ = 4, T = 290 (2) K, μ (Cu Kα) = 0.776 mm⁻¹, Dcalc = 1.299 g cm⁻³, 26 163 reflections measured (8.072° ≤ 2θ ≤ 142.798°), 5564 unique (Rint = 0.0242, Rsigma = 0.0160) which were used in all calculations. The final R₁ was 0.0420 (I > 2σ (I)) and wR₂ was 0.1182 (all data). Flack parameter = -0.03 (5).

Anti-inflammatory activities

All new compounds were evaluated for their inhibitory effects on NO production in lipopolysaccharide-activated RAW 264.7 macrophages as described in the literature. L-NMMA were used as positive control. All experiments were conducted for three independent replicates.

In vitro cytotoxicity assay

All new compounds were evaluated for their cytotoxicities against HepG2 by MTT assay. Cells were plated in 96-well culture plates (5 × 103 cells per well). After incubation overnight, the cells were treated with different concentrations of each compound for 48 h. DMSO (0.1%) was used as a vehicle. MTT (5 mg mL⁻¹) was dissolved in PBS and filter sterilized, then 20 μL of the prepared solution was added to each well and cells were incubated until a purple precipitate was visible. The formed formazan crystals were dissolved in DMSO (150 μL per well) by constant shaking for 10 min. The absorbance was measured on an ELISA reader (SpectraMax Plus = 384, Molecular Devices, Sunnyvale, CA) at a test wavelength of 570 nm and a reference wavelength of 630 nm. After treatment, cell viability was detected and IC₅₀ values were calculated by the Reed and Muench method. All experiments were performed as three independent replicates.

Conflicts of interest

The authors declare no conflict of interest.

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