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Withanolides from aerial parts of *Nicandra physalodes*

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ABSTRACT

Twenty withanolides, including previously unknown nicanlodes A–M, were isolated from aerial parts of *Nicandra physalodes*. Their structural elucidations were unambiguously achieved through interpretation of extensive spectroscopic data (NMR and HRMS) and by comparison with literature data. Nicanlodes A and B have an unusual aromatic amine moiety. The isolated compounds were evaluated for their cytotoxicity against five human cancer cell lines.

1. Introduction

Withanolides, a class of naturally occurring C_{28} steroid derivatives, possess a δ- or γ-lactone ring between C-26 and C-22 or C-23 in the side-chain (Chen et al., 2011). This type of compound has been extensively reported from plants of the Solanaceae family in the last 50 years, especially in the genera *Withania*, *Physalis*, *Datura*, *Salpichroa*, *Nicandra*, *Lycium*, *Tubocapsicum*, and *Jaborosa* (Glotter, 1991). Due to their structural diversity, withanolides display a wide spectrum of bioactive properties, such as antimicrobial (Alali et al., 2014), antitumor (Roy et al., 2013; Reyes-Reyes et al., 2013), anti-inflammatory (Yang et al., 2014a,b), immunomodulatory (Yang et al., 2014a,b; Furmanowa et al., 2001), insect-antifeedant (Mareggiani et al., 2000; Vaccarini and Bonetto, 2000), and insecticidal (Nalbandov et al., 1964) activities.

The genus *Nicandra* comprises three species. *N. john-tyleriana* and *N. yacheriana* grow in Peru, while *N. physalodes*, which is a well-known and most widespread species of the genus, occurs in regions from Peru to northern Argentina, as well as being found as a ruderal species in tropical and subtropical areas worldwide (Nicolás et al., 2015). This plant is also distributed widely in Southwest China and its whole plant has been used in folk medicine for the treatment and prevention of sedation, as an expectorant, for fever relief, and for detoxification (Editorial Board of National Herbal Compendium, 1975). Literature reports indicate that *N. physalodes* is an abundant source of withanolides with about twenty withanolides having antifeedant (Andrews-Smith et al., 1991), insecticidal (Nalbandov et al., 1964), and cytotoxic effects (Gunasekera et al., 1981) being identified from this species. Thus, to further discover structurally diverse and biologically significant withanolides from the title plant, a phytochemical investigation on *N. physalodes* was carried out. This resulted in isolation of 13 new withanolides, nicanlodes A–M (**1**–**13**), together with seven known compounds, including nic 1 (**14**) (Begley et al., 1976), salpichrolide A (**15**) (Veleiro et al., 1992), nacaphysalin C (**16**) (Shingu et al., 1994), nic 2 (**17**) (Bates and Morehead, 1974), nicaphysalin A (**18**) (Shingu et al., 1994), withahisolide I (**19**) (Cao et al., 2014), and nic 11 (**20**) (Begley et al., 1973) (Fig. 1). Herein, the isolation, structural

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elucidation, as well as cytotoxicities of these compounds, are reported.

2. Results and discussion

Thirteen new withanolides, nicanlodes A–M (**1**–**13**), together with seven known compounds, were isolated from the ethyl acetate soluble fraction of the aerial parts of *N. physalodes*. Compounds **1**, **3**, **4**, **6**, **8**, **10**, **11**, and **13** were isolated as white amorphous powders, while compounds **2**, **5**, **7**, **9**, and **12** were isolated as light yellow amorphous powders.

2.1. Structural elucidation of new compounds

The molecular formula of nicanlode A (**1**), $C_{35}H_{39}NO_7$, was deduced from its HRESIMS at *m/z* 608.2623 $[M + Na]^+$ (calcd. for $C_{35}H_{39}NO_7Na$, 608.2619) and ^{13}C NMR data. Its 1D (^1H and ^{13}C) NMR spectra (Tables 1 and 3) showed the presence of four methyls [δ_H 1.24 (s, H_3-19), δ_C 14.0; δ_H 1.10 (d, $J = 7.1$ Hz, H_3-21), δ_C 18.2; δ_H 1.44 (s, CH_3-28), δ_C 19.0; and δ_H 1.41 (s, CH_3-27), δ_C 17.4], four methylenes, sixteen methines, nine quaternary carbons and two carbonyl carbons. Among them, the characteristic signals at δ_C 202.7 (C-1), δ_C 128.8 (C-2), and δ_C 140.0 (C-3), along with two methine resonances at δ_C 56.9 (C-6), δ_C 55.7 (C-7), and one oxygenated signal at δ_C 72.8 (C-5) indicated that compound **1** possessed a 1-one-2-ene-5α-hydroxy-6α(7α)-epoxy moiety in rings A and B. Furthermore, resonances for aromatic ring D and a 22,26-epoxy moiety in the side-chain [δ_H 3.81 (m, H-22), 5.11 (d, $J = 7.5$ Hz, H-26), 7.05 (d, $J = 7.9$ Hz, H-15), 6.83 (d, $J = 7.9$ Hz, H-16), 6.77 (s, H-18); δ_C 136.7 (C-13), 134.4 (C-14), 123.7 (C-15), 124.8 (C-16), 142.4 (C-17), 128.0 (C-18), 68.0 (C-22), 79.2 (C-26)] were also observed in its 1D NMR spectrum. In addition, a 24,25-epoxy moiety in the side-chain [63.7 (C-24), 63.3 (C-25)] was indicated by analysis of the NMR spectrum. The above featured fragments were further confirmed by 2D NMR correlations. These data illustrated that compound **1** was a typical withanolide and resembled Nic 1 (**14**), a compound with a 6-membered hemiacetal side-chain.

1D NMR spectra of **1** also showed additional signals for another aromatic moiety [δ_H 6.57 (d, $J = 8.5$ Hz, H-3′), 7.12 (m, H-4′), 6.64 (m, H-5′), and 7.96 (dd, $J = 8.0$, 1.2 Hz, H-6′); δ_C 110.3 (C-1′), 150.0 (C-2′), 113.8 (C-3′), 134.6 (C-4′), 116.2 (C-5′), 132.0 (C-6′), and 171.6 (C-7′)]. The HMBC and ^1H-^1H COSY spectra (Fig. 2), as well as its molecular formula, indicated that the aromatic moiety was 2′-aminobenzeoic acid. The key HMBC correlations of H-26 (δ_H 5.11 d, $J = 7.5$ Hz) with C-2′ established that this substituent was located at C-26, coincident with the downfield shift of C-26 (δ_C 79.2).

The ROESY correlations of H_3-19/H-8/H-7/H-6, of H-20/H-22/H-23b, and H-23a/H_3-27/H_3-28/H-26 established the relative configuration of the 6,7-epoxy group to be β-oriented while H-26 in the α plane (Fig. 2). Therefore, structure **1** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α-hydroxy-26-(2′-amino benzoic acid)-17(13 → 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

A molecular formula of $C_{35}H_{39}NO_8$ was assigned to nicanlode B (**2**) by HRESIMS at *m/z* 624.2566 $[M + Na]^+$ (calcd. for $C_{35}H_{39}NO_8Na$, 624.2568) and ^{13}C NMR data. Its 1D NMR data (Tables 1 and 3) were similar to those of **1** except for the signals of the aromatic substituent. Careful comparison of ^1H NMR spectroscopic data in the downfield region between **2** and **1** showed characteristic signals at H-2′ (δ_H 7.01, d, $J = 1.5$ Hz), H-5′ (δ_H 6.80, d, $J = 8.3$ Hz), and H-6′ (δ_H 7.39, d, $J = 8.1$ Hz) for an ABX system in **2**. The observed HMBC correlations of H-2′ and H-6′ with C-7′ (δ_C 170.0), and those of H-2′ and H-5′ with C-3′ (δ_C 133.3) and C-4′ (δ_C 148.9), further verified the presence of a 3′-hydroxy-4′-aminobenzoic acid substituent. Furthermore, H-26 showed a HMBC cross-peak with C-4′, which indicated that this substituent was connected to C-26. Similar ROESY correlations between **2** and **1** proved they shared the same relative configuration. Consequently, structure **2** was established as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α-hydroxy-26-(3′-hydroxy-4′-aminobenzoic acid)-17(13 → 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

The molecular formula of nicanlode C (**3**) was deduced as $C_{28}H_{32}O_7$ by HRESIMS at *m/z* 503.2041 $[M + Na]^+$ (calcd. for $C_{28}H_{32}O_7Na$, 503.2040) and ^{13}C NMR data, requiring 13 degrees of unsaturation. Comparison of 1D NMR data (Tables 1 and 3) of **3** and **14** showed that they were similar withanolides with a 6-membered hemiacetal side-chain, differing only in the presence of a carbonyl group in **3** instead of a methylene at C-12 observed in **14**. This was further supported by HMBC correlations of H-18 (δ_H 8.23), H-9 (δ_H 2.96), and H-11 (δ_H 2.57, 4.25) with C-12 (δ_C 197.9), together with ^1H-^1H COSY correlations of H-6/H-7/H-8/H-9/H-11. The ROESY spectrum showed that compounds **3** and **14** possessed similar configuration. Therefore, structure **3** was assigned as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,26-dihydroxy-17(13 → 18)-abeo-ergost-2,13,15,17-tetraen-1,12-dione.

Nicanlode D (**4**) was assigned a molecular formula of $C_{28}H_{36}O_6$ based on its HRESIMS (*m/z* 491.2404 $[M + Na]^+$) and NMR data with two more hydrogen atoms than Nic 1 (**14**). Detailed comparison of its 1D NMR data (Tables 1 and 3) with **14** showed an oxymethine rather than an unsaturated carbonyl in ring A of **4**, which was further supported by HMBC correlations of H_3-19 (δ_H 0.84) with C-1 (δ_C 70.6), of H-1 (δ_H 3.71) with C-2 (δ_C 129.5), C-3 (δ_C 124.0), and C-19 (δ_C 14.5), of H-2 (δ_H 5.99) and H-3 (δ_H 5.78) with C-1 and C-4 (δ_C 35.7), respectively. Furthermore, ROESY correlations between H_3-19β and H-1 proved that the configuration of OH-1 was α-oriented, and that correlations of H-22β with H-26 were absent in the ROESY spectrum, which also further confirmed the configuration of the side-chain. Thus, structure **4** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-1α,5α,26-trihydroxy-17(13 → 18)-abeo-ergost-2,13,15,17-tetraene.

The molecular formula of nicanlode E (**5**) was established to be $C_{28}H_{34}O_5$ by analysis of the HRESIMS (*m/z* 473.2298 $[M + Na]^+$) and ^{13}C NMR data. Its 1D NMR data (Tables 1 and 3) was similar to Nic 1 (**14**), except for the replacement of 6α,7α-epoxide moiety in ring B in **14** by a double bond [δ_H 5.80 (1H, dd, $J = 10.0$, 2.7 Hz, H-6), 6.48 (1H, dd, $J = 10.0$, 1.8 Hz, H-7); δ_C 130.1 (C-6), 130.8 (C-7)] in **5**. HMBC correlations from H_3-19 (δ_H 1.27) to C-5 (δ_C 74.4), from H-4β (δ_H 2.99) to C-5/C-6 (δ_C130.1), and from H-6 (δ_H 5.80) to C-4/C-10, confirmed the above deduction. Furthermore, similar ROESY correlations between **5** and **4** implied identical relative configurations. Accordingly, structure **5** was identified as 20*S*,22*R*,24*S*,25*S*,26*R*)22(26),24(25)-diepoxy-5α,26-dihydroxy-17(13 → 18)-abeo-ergost-2,6,13,15,17-pentaen-1-one.

A molecular formula $C_{28}H_{34}O_6$ of nicanlode F (**6**) was determined on the basis of its HRESIMS (*m/z* 489.2254 $[M + Na]^+$) and ^{13}C NMR data. Inspection of the 1D and 2D NMR spectra suggested that it was also a withanolide similar to salpichrolide A (**15**), except for the presence of an extra 7-hydroxy group [4.70 (1H, m, H-7); δ_C 64.5 (C-7)] in **6**. HMBC correlations of CH_3-19 (δ_H 1.36)/C-5, H-4β (δ_H 2.01) with C-5/C-6, and H-6 with C-4/C-10 suggested this inference. Furthermore, the 7-hydroxy group and the 5,6-epoxide in **6** were both determined to be in an α-orientation based on ROESY correlations of H-8/H-7/H-6/H_3-19. Consequently, structure **6** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)5α(6α),22(26),24(25)-triepoxy-7α,26-dihydroxy-17(13 → 18)-abeo-ergost-2,13,15,17-tetraen-1-one.

The HRESIMS (*m/z* 511.2669 $[M + Na]^+$) and NMR data of nicanlode G (**7**) established the molecular formula $C_{28}H_{40}O_7$. Its 1D NMR spectra (Tables 1 and 3) exhibited the presence of five methyls [δ_H 0.75 (s, H_3-18), δ_C 8.0; δ_H 1.16 (s, H_3-19), δ_C 14.6; δ_H 1.06 (d,

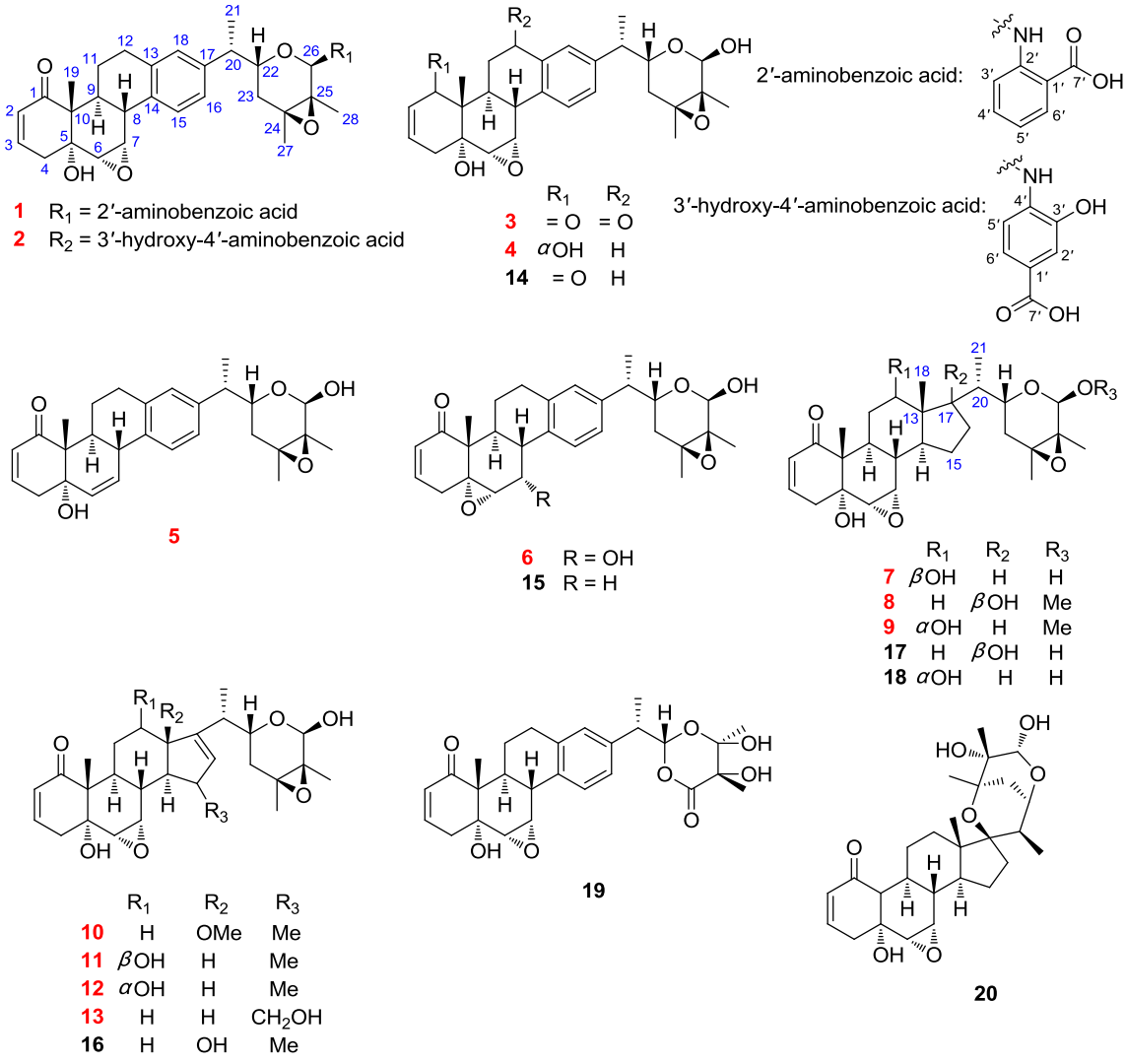

Fig. 1. Chemical structures of **1**–**20** isolated from the aerial parts of *N. physalodes*.

J = 7.0 Hz, H_3-21), δ_C 14.5; δ_H 1.42 (s, H_3-27), δ_C 16.6, and δ_H 1.40 (s, H_3-28), δ_C 19.0], four methylenes, twelve methines, and six quaternary carbons. Among them, some characteristic signals were assigned to an α,β-unsaturated carbonyl in ring A, a 5α-hydroxy-6α,7α-epoxide in B ring, and a 6-membered hemiacetal in the side-chain on the basis of analysis of the HMBC, HSQC and ^{1}H-^{1}H COSY spectra. The above data showed that **7** was similar to nicaphysalin A (**18**). However, the downfield shift of C-12 (δ_C 72.5 → 76.9) in **7** suggested that it could be an OH-12 epimer of **18**. Furthermore, correlations of H-12/H-9α in the ROESY spectrum established OH-12 to be β-oriented. Accordingly, structure **7** was established as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,12β,26-trihydroxy-5α-ergost-2-en-1-one.

The molecular formula of nicanlode H (**8**) was established as $C_{29}H_{42}O_7$, according to the HRESIMS (m/z 525.2822 [M + Na]$^+$) and ^{13}C NMR data. Analysis of the 1D NMR spectroscopic data of (Tables 2 and 3) **8** and **17** showed that an additional methoxy group (δ_H 3.44) was present in **8** instead of a hydroxy group in **17**. Moreover, the HMBC correlation of δ_H 3.44 with C-26 (δ_C 99.4) indicated that the methoxy group was located at C-26. ROESY cross-peaks between H-26 and H_3-28 also suggested the methoxy group as being β-oriented. Accordingly, structure **8** was assigned as 20*R*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,17β-dihydroxy-26-methoxy-5α-ergost-2-en-1-one.

Nicanlode I (**9**) was assigned the same molecular formula as that of compound **8**, while its 1D NMR spectra (Tables 2 and 3) showed some similarities with compound **18**. The obvious difference was the presence of a methoxy group at C-26 in **9**, instead of a hydroxy in **18**, which was confirmed by HMBC correlations of OCH_3 (δ_H 3.42) with C-26 (δ_C 99.4). Furthermore, ROESY correlations of H-12/H_3-18β suggested that 12-OH was α–oriented. Consequently, structure **9** was elucidated as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,12α-dihydroxy-26-methoxy-5α-ergost-2-en-1-one.

Nicanlode J (**10**) was assigned the molecular formula $C_{29}H_{40}O_7$ on the basis of HRESIMS (m/z 523.2665 [M + Na]$^+$) and ^{13}C NMR data. Its 1D NMR data (Tables 2 and 3) resembled those of compound **16**. The main difference between them was the presence of a methoxy at C-15 in **10** rather than a hydroxy in **16**. The above difference was supported by obvious HMBC correlations of OCH_3 (δ_H 3.27) to C-15 (δ_C 81.2). The remaining HMBC cross peaks from H_3-18 (δ_H 1.11) to C-17 (δ_C 163.3), and from H-15 (δ_H 4.20) to C-13 (δ_C 48.2) and C-17 suggested a 15-methoxy-16(17)-ene moiety in D ring. Furthermore, the 6α,7α-epoxy group and 15β-OMe were explicated by ROESY correlations of H-8/H-7, H-7/H-6, and H-14/H-15. Consequently, structure **10** was elucidated to be 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,26-dihydroxy-15β-

Table 1
^{1}H NMR spectroscopic data for compounds **1**–**7** (δ in ppm, J in Hz).

No.	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[a]
1				3.71 d (5.0, 11.5)			
2	5.92 dd (2.3, 10.1)	5.90 dd (2.3, 10.1)	6.00 d (10.1)	5.99 m	5.94 dd (2.7, 10.1)	6.04 dd (2.0, 10.1)	5.86 dd (2.3, 10.2)
3	6.65 ddd (2.2, 5.1, 10.1)	6.64 ddd (2.0, 4.9, 10.0)	6.61 m	5.78 m	6.63 ddd (2.0, 5.1, 10.0)	6.80 ddd (2.2, 5.0, 10.0)	6.60 ddd (2.1, 5.1, 10.0)
4	2.65 m	2.64 d (5.3)	2.66 dd (4.7, 18.8)	2.34 d (17.9)	2.46 dd (5.2, 19.0)	2.01 dd (4.9, 8.5)	2.54 dd (5.2, 18.7)
	2.75 m	2.73 d (18.6)	2.76 d (18.9)	2.41 dd (4.9, 17.8)	2.69 d (19.0)	3.15 dt (2.5, 19.4)	2.68 d (18.6)
6	3.25 d (3.9)	3.21 d (3.8)	3.36 s	3.16 d (11.7)	5.80 dd (2.7, 10.0)	3.55 d (5.3)	3.04 d (3.9)
7	3.96 brs	3.91 brs	4.06 s	4.07 brs	6.48 dd (1.8, 10.0)	4.70 m	3.32 m
8	2.94 brd (11.3)	2.93 brd (11.5)	3.26 brd (11.2)	3.11br d (11.7)	3.22 brd (11.0)	2.82 dd (4.1, 12.3)	1.64 overlapped
9	1.82 td (3.4, 11.1)	1.72 m	2.96 t (12.3)	2.01 td (3.0, 11.5)	2.22 td (3.7, 10.8)	2.21 td (2.1, 12.1)	1.64 overlapped
11	1.46 m	1.42 m	2.57 m	1.56 m	1.66 m	1.30 dd (3.7, 12.5)	1.29 m
	2.75 m	2.59 overlapped	4.25 d (17.2)	2.14 m	2.89 m	2.61 d (10.6)	2.88 m
12	2.56 m	2.42 m		2.89 m	2.86 m	2.72 m	3.53 m
	2.63 m	2.59 overlapped		2.94 m	3.04 m	2.88 m	
14							1.37 m
15	7.05 d (7.9)	7.00 d (8.3)	7.72 d (7.9)	7.40 d (8.0)	7.21 d (7.8)	7.25 d (8.2)	1.37 m
							1.84 m
16	6.83 d (7.9)	6.78 t (8.1)	7.56 brs	7.05 dd (1.4, 8.0)	7.01 d (9.3)	7.05 d (8.2)	1.65 overlapped
							1.74 m
17							1.58 m
18	6.77 s	6.71 s	8.23 s	6.97 s	7.00 s	6.93 s	0.75 s
19	1.24 s	1.20 s	1.24 s	0.84 s	1.27 s	1.36 s	1.16 s
20	2.58 m	2.61 m	2.90 m	2.75 m	2.75 m	2.74 m	1.72 m
21	1.10 d (7.1)	1.09 d (7.0)	1.25 d (7.4)	1.25 d (7.2)	1.25 d (7.3)	1.24 d (7.2)	1.06 d (7.0)
22	3.81 m	3.82 t (9.2)	4.45 m	3.86 ddd (2.3, 5.7, 11.2)	3.85 ddd (2.4, 5.8, 11.3)	3.85 ddd (2.3, 5.6, 11.2)	3.84 ddd (2.5, 5.6, 11.2)
23	1.65 m	1.70 m	1.62 m	1.58 m	1.57 m	1.58 m	1.65 overlapped
	2.12 m	2.20 d (14.3)	1.94 d (14.0)	1.85 dd (2.3, 14.4)	1.84 dd (2.3, 14.5)	1.85 dd (2.3, 14.4)	1.80 m
26	5.11 d (7.5)	5.05 d (5.7)	5.40 s	4.99 d (10.1)	4.99 d (10.0)	4.98 d (9.8)	5.03 d (10.1)
27	1.41 s	1.44 s	1.42 s	1.39 s	1.39 s	1.38 s	1.42 s
28	1.44 s	1.46 s	1.28 s	1.36 s	1.36 s	1.35 s	1.40 s
2′		7.01 d (1.5)					
3′	6.57 d (8.5)						
4′	7.12 ddd (1.7, 7.0, 8.6)						
5′	6.62 ddd (1.1, 6.8, 8.2)	6.80 d (8.3)					
6′	7.96 dd (1.7, 8.1)	7.39 d (8.1)					
H-NH	8.51 d (7.5)	5.08 d (5.8)					
1-OH				3.39 d (11.6)		3.47 overlapped	
7-OH							
26-OH				3.42 d (10.0)	3.41 d (10.1)	3.47 overlapped	

[a] Measured in $CDCl_3$.
[b] Measured in C_5D_5N.

methoxy-5α-ergost-2,16-dien-1-one.

Nicanlode K (**11**) and nicanlode L (**12**) possessed the same molecular formula of $C_{28}H_{38}O_7$ as that of **16**, on the basis of their HRESIMS and ^{13}C NMR data. Moreover, further analyses of their 1D NMR spectra (Tables 2 and 3) showed that they shared the same overall skeleton. However, analysis of the 1D NMR data of **11** and **12** showed two main differences between them and known compound **16**. Firstly, there was the presence of an oxymethine at C-12 in both **11** and **12**, rather than a methylene in **16**. ^{1}H-^{1}H COSY correlations of H-6/H-7/H-8/H-9/H-11/H-12, along with the HMBC

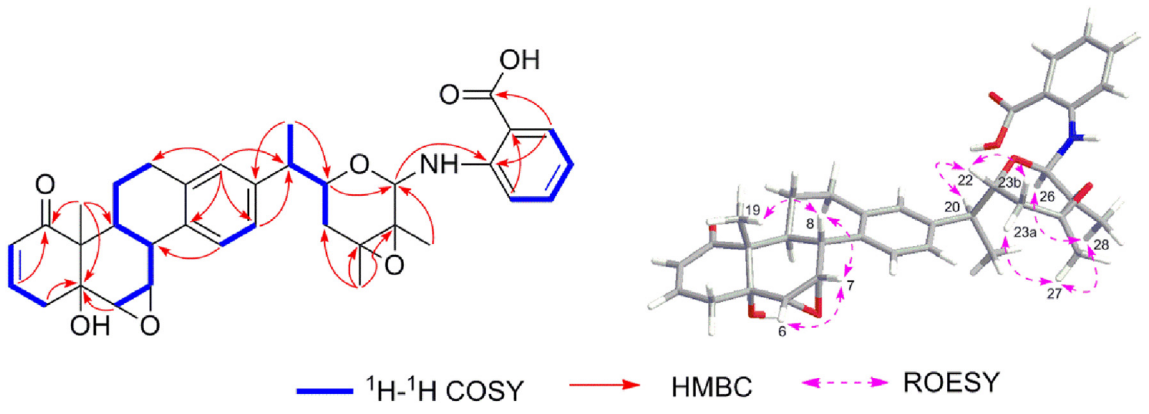

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC, and ROESY correlations of **1**.

Table 2
^{1}H NMR spectroscopic data for compounds **8**–**13** in $CDCl_3$ (δ in ppm, J in Hz).

No.	8	9	10	11	12	13
1						
2	5.85 d (10.1)	5.85 dd (2.3, 10.1)	5.84 dd (2.3, 10.1)	5.85 dd (2.4, 10.1)	5.83 dd (2.3, 10.1)	5.84 dd (2.4, 10.1)
3	6.59 dd (5.0, 10.0)	6.61 m	6.59 ddd (2.2, 5.2, 10.1)	6.59 ddd (1.9, 5.0, 10.0)	6.58 ddd (2.1, 5.2, 10.1)	6.59 ddd (2.1, 5.1, 10.1)
4a	2.53 dd (4.9, 18.7)	2.54 dd (5.1, 18.7)	2.53 dd (5.2,18.7)	2.53 dd (5.1, 18.7)	2.53 dd (4.9, 14.1)	2.53 dd (5.1, 18.7)
4b	2.68 d (18.7)	2.68 d (18.8)	2.69 d (18.0)	2.68 d (19.8)	2.68 m	2.69 d (18.7)
6	3.04 d (2.7)	3.03 d (3.8)	3.04 d (3.9)	3.05 d (3.8)	3.03 d (3.8)	3.04 d (3.8)
7	3.31 s	3.31 brs	3.67 dd (1.8, 3.7)	3.39 brs	3.38 m	3.42 brs
8	1.75 overlapped	1.75 overlapped	2.17 m	1.86 m	1.98 dt (1.7, 11.2)	2.08 m
9	1.55 d (10.3)	2.05 t (10.1)	1.66 overlapped	1.68 m	2.11 m	1.70 m
11	1.29 m	1.29 overlapped	1.44 overlapped	1.36 m	2.04 m,	1.40 m
	2.80 d (13.4)	1.69 m	2.85 m	2.96 dt (4.0, 12.9)	2.34 ddd (3.0, 6.5, 14.3)	2.87 m
12	1.66 m, 1.66 m	4.02 brs	1.44 overlapped	3.81 dd (4.5, 10.6)	4.05 brs	1.41 m
			1.66 overlapped			1.90 overlapped
14	2.05 m	1.93 m	1.63 m	1.63 overlapped	2.42 td (6.6, 11.5)	1.90 overlapped
15	1.31 m	1.29 overlapped	4.20 dd (2.5, 5.2)	2.11 m	1.60 m	2.23 ddd (3.0, 7.0, 14.4)
	1.90 m	1.83 overlapped		2.26 ddd (3.0, 6.3, 14.3)	2.91 dt (3.3, 14.5)	2.39 m
16	1.80 m	1.29 overlapped	5.79 d (2.1)	5.48 s	5.48 s	5.75 s
	1.98 m	1.83 overlapped				
17		1.83 overlapped				
18	0.83 s	0.76 s	1.11 s	0.90 s	0.90 s	3.45 dd (6.0, 11.8)
						3.79 dd (6.0, 11.8)
19	1.18 s	1.17 s	1.22 s	1.19 s	1.17 s	1.20 s
20	1.96 m	1.67 m	2.24 m	2.65 m	2.49 m	2.14 m
21	0.98 d (6.8)	1.01 d (6.7)	0.99 d (7.1)	1.10 d (7.2)	1.15 d (7.2)	0.95 d (7.0)
22	4.05 br d (11.3)	3.82 dt (5.2, 11.2)	3.79 ddd (2.5, 7.7, 13.0)	3.87 ddd (2.1, 6.0, 11.0)	3.74 ddd (2.5, 5.2, 11.2)	3.77 ddd (2.3, 6.7, 12.5)
23	1.75 overlapped	1.25 m	1.66 overlapped	1.63 overlapped	1.74 dd (11.3, 14.5)	1.57 m
	2.11 d (14.5)	1.63 m	1.99 dd (2.5, 14.4)	1.83 m	1.84 dd (2.5, 14.6)	2.16 m
26	4.62 s	4.64 s	5.02 d (10.2)	5.03 d (8.6)	5.03 s	4.96 d (10.8)
27	1.37 s	1.37 s	1.43 s	1.42 s	1.39 s	1.42 s
28	1.36 s	1.37 s	1.42 s	1.40 s	1.39 s	1.42 s
15-OMe			3.27 s			
26-OMe	3.44 s	3.42 s				
5-OH	3.16 s	3.19 s	3.19 s	3.15 s	3.24 s	3.11 overlapped
13-CH_2OH			3.42 d (10.2)			3.11 overlapped
26-OH						3.58 d (10.9)

correlations of H-18 with C-12/C-13/C-14/C-17, also indicated that the hydroxy group was located at C-12 in **11** and **12**. In addition, the methylene moiety was assigned at C-15 in **11** and **12**, rather than as an oxymethine in **16**, which was further supported by ^{1}H-^{1}H COSY correlations of H-8/H-14/H-15/H-16 as well as the HMBC correlations of H-16 with C-13/C-14/C-15/C-17/C-20. Hence, **11** and **12** were deduced to be the positional isomers of **16**. Furthermore, comparison of ROESY spectra of **11** and **12** displayed key correlations of H-12/H-9 in **11**, while there were cross-peaks of H-12/H_3-18 in **12**. Therefore, **11** and **12** were epimers at C-12 due to different orientations of the OH group. Based on the above evidence, structures **11** and **12** were elucidated as being 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,12β,26-trihydroxy-5α-ergost-2,16-dien-1-one and 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,12α,26-trihydroxy-5α-ergost-2,16-dien-1-one, respectively.

Similar to **11**, **12**, and **16**, the HRESIMS (*m/z* 509.2516 $[M + Na]^+$) and ^{13}C NMR data suggested the molecular formula of nicanlode M (**13**) as $C_{28}H_{38}O_7$. The 1D NMR and HSQC spectra of **13** indicated that it had the same structural skeleton as those of **10**–**12** and **16**. According to the NMR data, a characteristic signal at δ_C 63.4 (t, C-18) indicated the presence of a CH_2OH group in **13**. The connectivity of CH_2OH with C-13 was confirmed by obvious HMBC correlations of δ_H 3.45, and 3.79 with C-14, C-16, C17, and C-13. These observations suggested that the methyl (C-18) was oxidized to CH_2OH. Furthermore, the CH_2OH-13 was assigned a β-configuration by the ROESY correlations of H_2-18/H-8. Accordingly, structure **13** was established as 20*S*,22*R*,24*S*,25*S*,26*R*)6α(7α),22(26),24(25)-triepoxy-5α,18,26-trihydroxy-5α-ergost-2,16-dien-1-one.

In addition to nicanlodes A–M (**1**–**13**), seven known withanolides (**14**–**20**) were isolated from *N. physalodes* and identified by comparing their physical and spectroscopic data with reported values. Compounds **1** and **2** are the first withanolides with an unusual aromatic amine moiety at C-26. In addition, compounds **15** and **19** were reported for the first time from the genus *Nicandra*. Further LC-MS analysis of the crude extract and **8**–**10** from title species indicated that **8**–**10** were all new compounds rather than artificial ones during extraction and isolation procedures.

2.2. Cytotoxicities of isolates

Compounds **1**–**4**, **6**–**8**, **11**–**20** were evaluated for their cytotoxicities against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480). Unfortunately, none of the tested compounds exhibited obvious cytotoxic effects at a concentration of 40 μM.

3. Conclusion

In current work, 13 new withanolides, nicanlodes A–M, together with seven known compounds, were obtained from *N. physalodes*. Although, none of the isolates were cytotoxic (against five human cancer cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW480), these findings could enrich the diversity of natural withanolides and their other bioactive properties might be further studied.

4. Experimental

4.1. General experimental procedures

Optical rotations were obtained using a Jasco P-1020

Table 3
^{13}C NMR spectroscopic data for compounds **1**–**13**.

No.	**1**[a]	**2**[a]	**3**[b]	**4**[a]	**5**[a]	**6**[a]	**7**[a]	**8**[a]	**9**[a]	**10**[a]	**11**[a]	**12**[a]	**13**[a]
1	202.7, C	203.6, C	203.3, C	70.59, CH	203.5, C	201.9, C	202.9, C	203.2, C	203.4, C	202.2, C	202.9, C	202.9, C	203.2, C
2	128.8, CH	128.7, CH	128.8, CH	129.5, CH	129.0, CH	128.8, CH	128.9, CH	129.0, CH	128.9, CH	129.1, CH	128.9, CH	129.0, CH	129.0, CH
3	140.0, CH	140.5, CH	141.7, CH	124.0, CH	140.7, CH	142.4, CH	139.8, CH	139.7, CH	140.0, CH	139.6, CH	139.7, CH	139.6, CH	139.6, CH
4	36.9, CH_2	37.0, CH_2	38.2, CH_2	35.7, CH_2	36.4, CH_2	33.3, CH_2	36.7, CH_2	36.7, CH_2	36.7, CH_2	36.7, CH_2	36.6, CH_2	36.6, CH_2	36.7, CH_2
5	72.8, C	73.2, C	73.2, C	70.64, C	74.4, C	67.1, C	73.2, C	73.2, C	73.3, C	73.6, C	73.4, C	73.4, C	73.5, C
6	56.9, CH	57.0, CH	57.3, CH	58.2, CH	130.1, CH	61.5, CH	56.2, CH	56.2, CH	56.1, CH	56.0, CH	56.0, CH	55.9, CH	56.0, CH
7	55.7, CH	55.4, CH	53.6, CH	56.3, CH	130.8, CH	64.5, CH	57.1, CH	57.2, CH	57.1, CH	57.1, CH	57.2, CH	57.3, CH	57.5, CH
8	38.6, CH	38.3, CH	39.6, CH	38.1, CH	39.5, CH	41.1, CH	34.7, CH	36.0, CH	36.0, CH	32.1, CH	33.5, CH	34.5, CH	34.0, CH
9	31.6, CH	31.4, CH	34.2, CH	31.2, CH	36.9, CH	31.1, CH	33.5, CH	35.2, CH	28.6, CH	36.4, CH	34.1, CH	29.9, CH	36.4, CH
10	51.6, C	51.9, C	52.9, C	41.0, C	51.7, C	49.1, C	50.8, C	50.9, C	50.5, C	51.4, C	51.0, C	50.7, C	51.3, C
11	24.4, CH_2	24.3, CH_2	41.9, CH_2	21.8, CH_2	24.3, CH_2	24.9, CH_2	32.0, CH_2	21.7, CH_2	30.0, CH_2	21.3, CH_2	31.1, CH_2	30.0, CH_2	21.5, CH_2
12	29.0, CH_2	29.0, CH_2	197.9, C	29.2, CH_2	29.6, CH_2	30.4, CH_2	76.9, CH	32.4, CH_2	72.6, CH	34.9, CH_2	74.2, CH	70.5, CH	30.7, CH_2
13	136.7, C	137.0, C	132.8, C	136.9, C	137.0, C	138.8, C	48.6, C	48.7, C	46.9, C	48.2, C	52.4, C	53.2, C	52.6, C
14	134.4, C	134.4, C	142.8, C	135.3, C	136.8, C	132.6, C	49.7, CH	45.6, CH	43.6, CH	55.0, CH	50.7, CH	44.2, CH	52.0, CH
15	123.7, CH	123.6, CH	125.8, CH	124.2, CH	123.5, CH	126.9, CH	22.7, CH_2	23.0, CH_2	23.0, CH_2	81.2, CH	29.9, CH_2	29.1, CH_2	31.6, CH_2
16	124.8, CH	125.0, CH	134.0, CH	125.4, CH	125.2, CH	125.8, CH	26.9, CH_2	37.3, CH_2	26.6, CH_2	121.8, CH	125.4, CH	127.4, CH	125.6, CH
17	142.4, C	142.5, C	143.7, C	141.3, C	141.0, C	140.7, C	52.5, CH	84.9, C	43.4, CH	163.3, C	156.2, C	153.8, C	153.2, C
18	128.0, CH	126.9, CH	127.4, CH	129.2, CH	129.0, CH	129.4, CH	8.0, CH_3	15.0, CH_3	12.5, CH_3	22.2, CH_3	11.9, CH_3	18.2, CH_3	63.4, CH_2
19	14.0, CH_3	14.2, CH_3	14.1, CH_3	14.5, CH_3	14.3, CH_3	15.0, CH_3	14.6, CH_3	14.7, CH_3	14.6, CH_3	15.0, CH_3	14.8, CH_3	14.9, CH_3	15.0, CH_3
20	44.0, CH	44.8, CH	44.1, CH	43.1, CH	43.1, CH	43.0, CH	38.3, CH	42.9, CH	39.1, CH	36.6, CH	38.8, CH	38.0, CH	36.6, CH
21	18.2, CH_3	18.8, CH_3	18.3, CH_3	17.3, CH_3	17.4, CH_3	17.1, CH_3	14.5, CH_3	10.4, CH_3	11.7, CH_3	17.2, CH_3	16.7, CH_3	16.1, CH_3	19.4, CH_3
22	68.0, CH	67.6, CH	68.5, CH	67.4, CH	67.4, CH	67.5, CH	66.2, CH	67.6, CH	66.3, CH	66.4, CH	66.8, CH	67.9, CH	68.8, CH
23	35.5, CH_2	36.0, CH_2	35.4, CH_2	33.7, CH_2	33.8, CH_2	33.7, CH_2	32.3, CH_2	33.6, CH_2	29.9, CH_2	34.0, CH_2	34.1, CH_2	33.8, CH_2	35.3, CH_2
24	63.7, C	64.0, C	63.4, C	64.9, C	64.9, C	64.8, C	65.2, C	60.9, C	61.2, C	65.0, C	64.9, C	65.0, C	64.9, C
25	63.3, C	63.7, C	62.3, C	63.7, C	63.7, C	63.6, C	63.8, C	61.5, C	61.4, C	63.9, C	63.7, C	63.7, C	63.8, C
26	79.2, CH	79.8, CH	92.5, CH	91.7, CH	91.6, CH	91.7, CH	91.8, CH	99.4, CH	99.4, CH	91.6, CH	91.6, CH	91.6, CH	91.4, CH
27	17.4, CH_3	17.4, CH_3	17.3, CH_3	16.5, CH_3	16.5, CH_3	16.5, CH_3	16.6, CH_3	17.0, CH_3	17.1, CH_3	16.6, CH_3	16.6, CH_3	16.5, CH_3	16.5, CH_3
28	19.0, CH_3	19.1, CH_3	18.7, CH_3	18.8, CH_3	18.8, CH_3	18.8, CH_3	19.0, CH_3	18.5, CH_3	18.6, CH_3	18.9, CH_3	18.2, CH_3	18.8, CH_3	18.8, CH_3
15-OMe										55.8, CH_3			
26-OMe								55.9, CH_3	55.7, CH_3				
1′	110.3, C	121.1, C											
2′	150.0, C	115.8, CH											
3′	113.8, CH	133.3, C											
4′	134.6, CH	148.9, C											
5′	116.2, CH	113.9, CH											
6′	132.0, CH	122.0, CH											
7′	171.6, C	170.0, C											

[a] Measured in $CDCl_3$.
[b] Measured in C_5D_5N.

polarimeter. UV spectra were acquired on a UV 210A spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained using Bruker AV-400 and DRX-600 instruments (Bruker, Zurich, Switzerland), with Me_4Si (TMS) as an internal standard. ESIMS and HRESIMS data were recorded on an API QSTAR Pulsar spectrometer, and infrared spectra were obtained using a Bruker Tensor-27 instrument with KBr pellets. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a ZORBAX SB-C18 (5 μm, 9.4 × 250 mm) column. TLC was performed on pre-coated TLC plates (200–250 μm thickness, F254 Silica gel 60, Qingdao Marine Chemical, Inc.) and compounds were visualized by spraying the dried plates with 10% aqueous H_2SO_4 followed by heating until dryness. Silica gel (200–300 mesh), (Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 μm, Merck) and Sephadex LH-20 (GE Healthcare, Piscataway, NJ, USA) were used for column chromatography (CC).

4.2. Plant material

Aerial parts of *N. Physalodes* were collected in June 2013 from the Baoshan region of Yunnan province, China, and were identified by Prof. Yang Chong-Ren. A voucher specimen (ZDSQ20130601) is deposited in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

Air-dried and powdered aerial parts of *N. physalodes* (50 kg) were extracted for 3h each with MeOH (300 L × 3) under conditions of reflux. The MeOH solubles were combined and concentrated *in vacou* to obtain the crude extract (3 kg), which was then suspended in H_2O and partitioned with petroleum ether (20 L) and EtOAc (20 L), respectively. The EtOAc extract (200 g) was subjected to silica gel CC (10 × 150 cm) with $CHCl_3$/MeOH (150:1–5:1, 15 L) to afford six fractions (Fr.1~Fr.5), whereas Fr.1 (50 g) was applied to silica gel column (10 × 120 cm) that was eluted with petroleum ether/acetone (6:1–2:1, 10 L) to give nic 1 (**14**) (5.0 g). Fr.2 (6.1 g) was subjected to silica gel CC (5 × 50 cm) and eluted with a gradient of petroleum ether/acetone (10:1–1:1, 3 L) to yield seven subfractions (Fr.2-1~Fr.2–7) by TLC analysis. Fr.2-1 (200.0 mg) was isolated by Sephadex LH-20 CC (4 × 150 cm) using MeOH (3 L), silica gel CC (2.5 × 30 cm) with $CHCl_3$/MeOH (250:1–50:1, 800 mL), and by P-TLC (200–250 μm) using $CHCl_3$/*i*-PrOH (25:1) to afford salpichrolide A (**15**) (6.0 mg). Fr.2-2 (279.2 mg) was successively isolated by Sephadex LH-20 CC using $CHCl_3$/MeOH (1:1, 3 L), RP-18 silica gel CC (3 × 20 cm) with a gradient of MeOH/H_2O (40:60 → 100:0), and by P-TLC with $CHCl_3$/MeOH (20:1) to yield **1** (3.0 mg), **7** (4.0 mg), and nacaphysalin C (**16**) (15.0 mg), respectively. Fr.2–3 (184.0 mg) was separated by silica gel CC (3 × 50 cm) with $CHCl_3$/MeOH (100:1, 50:1, 1 L) and P-TLC with $CHCl_3$/*i*-PrOH (20:1) to afford **6** (3.0 mg). Fr.2–6 (215.5 mg) was isolated by silica gel CC (4 × 50 cm) eluting with $CHCl_3$/MeOH (80:1, 50:1, 1 L),

Sephadex LH-20 CC with MeOH (2.5 L), P-TLC with $CHCl_3$/MeOH (20:1) and HPLC eluting with MeOH/H_2O (67:33, 3.0 mL/min, t_R = 28.9 min) to yield nic 11 (**20**) (12.5 mg). Fr.3 (26.1 g) was fractionated by C18 silica gel CC (5 × 50 cm) with a gradient of MeOH/H_2O (50:50 → 100:0) to obtain seven fractions (Fr.3-1~Fr.3–7). Fr.3-1 (2.9 g) was repeatedly subjected to Sephadex LH-20 CC eluting with MeOH (3 L), silica gel CC (3 × 50 cm) using $CHCl_3$/MeOH (80:1–1:1, 2 L), P-TLC with petroleum ether/acetone (8:1), and by semi-prep. HPLC eluting with MeOH/H_2O (70:30, 3.0 mL/min, t_R = 20.7, 23.4, 25.1, 27.0, 30.5 min) to give **2** (10.0 mg), **8** (10.0 mg), **11** (1.5 mg), nic 2 (**17**) (7.5 mg), and nicaphysalin A (**18**) (8.2 mg), respectively. Fr.3-2 (4.0 g) was repeatedly separated by Sephadex LH-20 CC with MeOH (4.5 L), silica gel CC (4 × 50 cm) with $CHCl_3$/MeOH (35:1), and then further purified by P-TLC with $CHCl_3$/*i*-PrOH (15:1) to afford **9** (5.5 mg), **10** (3.0 mg) and withahisolide I (**19**) (10.5 mg). Fr.3–4 (5.4 g) was applied to a Sephadex LH-20 column using MeOH (7 L), then further isolated by silica gel CC (4 × 50 cm) using petroleum ether/acetone (20:1–1:1) and P-TLC using $CHCl_3$/*i*-PrOH (15:1) to give **12** (2.0 mg) and **13** (2.0 mg). Fr.3–7 (1.6 g) was successively isolated by CC on Sephadex LH-20 with MeOH (3 L), silica gel CC (2 × 50 cm) eluting with $CHCl_3$/MeOH (25:1, 1.5 L) and further by P-TLC with $CHCl_3$/*i*-PrOH (15:1) to yield **3** (3.0 mg), **4** (3.5 mg), and **5** (3.0 mg), respectively.

4.3.1. Nicanlode A (**1**)

White amorphous powder; [α]20 D−10.3 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.55), 249 (4.02) nm; IR (KBr) ν_{max} 3442, 2926, 1688, 1580, 1509, 1456, 1380, 1044 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 586 $[M + H]^+$; HRESIMS *m/z* 608.2623 $[M + Na]^+$ (calcd. for $C_{35}H_{39}NO_7Na$, 608.2619).

4.3.2. Nicanlode B (**2**)

Yellow amorphous powder; [α]20 D−97.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 217 (4.37), 263 (3.74) nm; IR (KBr) ν_{max} 3432, 2927, 1687, 1605, 1525, 1453, 1384, 1262, 1043, 921 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 624 $[M + Na]^+$; HRESIMS *m/z* 624.2566 $[M + Na]^+$ (calcd. for $C_{35}H_{39}NO_8Na$, 624.2568).

4.3.3. Nicanlode C (**3**)

White amorphous powder; [α]23 D+68.5 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 252 (4.08), 297 (3.23) nm; IR (KBr) ν_{max} 3443, 2967, 2927, 1679, 1631, 1457, 1424, 1267, 1099, 1033, 916 cm^{-1}; For ^1H (400 MHz, C_5D_5N) and ^{13}C (100 MHz, C_5D_5N) NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 503 $[M + Na]^+$; HRESIMS *m/z* 503.2041 $[M + Na]^+$ (calcd. for $C_{28}H_{32}O_7Na$, 503.2040).

4.3.4. Nicanlode D (**4**)

White amorphous powder; [α]23 D+4.9 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 241 (3.17), 268 (2.99) nm; IR (KBr) ν_{max} 3442, 2963, 2926, 1631, 1460, 1422, 1287, 1087, 1032, 918, 864 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$)NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 491 $[M + Na]^+$; HRESIMS *m/z* 491.2404 $[M + Na]^+$ (calcd. for $C_{28}H_{36}O_6Na$, 491.2404).

4.3.5. Nicanlode E (**5**)

Yellow amorphous powder; [α]21 D+32.0 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.51), 268 (3.41) nm; IR (KBr) ν_{max} 3449, 2927, 1678, 1383, 1262, 1086, 1027, 803 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (600 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 473 $[M + Na]^+$; HRESIMS *m/z* 473.2298 $[M + Na]^+$ (calcd. for $C_{28}H_{34}O_5Na$, 473.2298).

4.3.6. Nicanlode F (**6**)

White amorphous powder; [α]23 D−54.5 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 238 (2.81), 269 (2.80), 275 (2.78) nm; IR (KBr) ν_{max} 3440, 2925, 1684, 1629, 1457, 1382, 1271, 1126, 1079, 1029 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 489 $[M + Na]^+$; HRESIMS *m/z* 489.2254 $[M + Na]^+$ (calcd. for $C_{28}H_{34}O_6Na$, 489.2248).

4.3.7. Nicanlode G (**7**)

Yellow amorphous powder; [α]21 D+11.6 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.13) nm; IR (KBr) ν_{max} 3442, 2929, 1687, 1632, 1461, 1385, 1291, 1092, 1031, 906 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 1 and 3; ESIMS *m/z* 511 $[M + Na]^+$; HRESIMS *m/z* 511.2669 $[M + Na]^+$ (calcd. for $C_{28}H_{40}O_7Na$, 511.2666).

4.3.8. Nicanlode H (**8**)

White amorphous powder; [α]23 D−19.6 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.05) nm; IR (KBr) ν_{max} 3485, 3454, 2926, 1729, 1685, 1630, 1465, 1378, 1292, 1107, 1052, 977 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z* 525 $[M + Na]^+$; HRESIMS *m/z* at 525.2822 $[M + Na]^+$ (calcd. for $C_{29}H_{42}O_7Na$, 525.2823).

4.3.9. Nicanlode I (**9**)

Yellow amorphous powder; [α]23 D−5.3 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.02) nm; IR (KBr) ν_{max} 3556, 3443, 2925, 1729, 1688, 1630, 1465, 1386, 1292, 1105, 1056, 976, 907 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z* 525 $[M + Na]^+$; HRESIMS *m/z* 525.2825 $[M + Na]^+$ (calcd. for $C_{29}H_{42}O_7Na$, 525.2823).

4.3.10. Nicanlode J (**10**)

White amorphous powder; [α]23 D−40.1 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.47) nm; IR (KBr) ν_{max} 3442, 2963, 2926, 1689, 1629, 1460, 1383, 1289, 1084, 1029, 909, 863 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z* 523 $[M + Na]^+$; HRESIMS *m/z* 523.2665 $[M + Na]^+$ (calcd. for $C_{29}H_{40}O_7Na$, 523.2666).

4.3.11. Nicanlode K (**11**)

White amorphous powder; [α]23 D+27.6 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 239 (3.19) nm; IR (KBr) ν_{max} 3464, 2975, 2927, 1675, 1630, 1457, 1383, 1291, 1084, 1048, 1027, 902, 860 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z* 509 $[M + Na]^+$; HRESIMS *m/z* 509.2508 $[M + Na]^+$ (calcd. for $C_{28}H_{38}O_7Na$, 509.2510).

4.3.12. Nicanlode L (**12**)

Yellow amorphous powder; [α]21 D+36.2 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.08) nm; IR (KBr) ν_{max} 3442, 2926, 1686, 1631, 1385, 1257, 1092, 1034, 910 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z* 509 $[M + Na]^+$; HRESIMS *m/z* 509.2513 $[M + Na]^+$ (calcd. for $C_{28}H_{38}O_7Na$, 509.2510).

4.3.13. Nicanlode M (**13**)

White amorphous powder; [α]21 D+22.8 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (2.85) nm; IR (KBr) ν_{max} 3442, 2921, 1685, 1630, 1421, 1265, 1079, 1027, 898 cm^{-1}; For ^1H (600 MHz, $CDCl_3$) and ^{13}C (150 MHz, $CDCl_3$) NMR spectroscopic data, see Tables 2 and 3; ESIMS *m/z* 509 $[M + Na]^+$; HRESIMS *m/z* 509.2516 $[M + Na]^+$ (calcd. for $C_{28}H_{38}O_7Na$, 509.2510).

4.4. Cytotoxicity assay

The cytotoxic activities of isolated compounds except for **5**, **9** and **10** were evaluated against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) using the MTT method described elsewhere (Qin et al., 2016). Briefly, cells in RPMI-1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS, Hyclone) were seeded into a 96-well cell culture plate in the presence of various concentrations of test compounds at 37 °C in a 5% CO_2 humidified incubator for 48 h. Reduced MTS crystals were dissolved in DMSO, and the absorbance (A) was measured at 490 nm in a 96-well microtiter plate reader (Bio-Rad 680) to determine cell growth inhibition. Cisplatin (MW 300) and paclitaxel were included as positive controls.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2017.02.009.

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