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Triterpenoids from the stems of *Kadsura ananosma*

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ABSTRACT

An extensive study of the triterpenoids produced by the stems of *Kadsura ananosma*, has led to the isolation of eleven new ones, kadnanolactones A-I (**1**–**4**, **7**–**11**) and kadnanosic acids A (**5**) and B (**6**), and six known analogues. Their structures were elucidated mainly by comprehensive spectroscopic analysis, and DFT computational methods were applied to validate the stereochemistry of an epoxide in compound **7**. All triterpenoids were evaluated for their cytotoxicity against HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3 human cancer cells.

1. Introduction

Recently, we have initiated a program to discover structurally unique and bioactive natural products from different *Kadsura* species, and a series of triterpenoids exhibiting different carbon frameworks and oxygenated pattern, such as kadlongilactone,[1] longipedlactone,[2] and nortriterpenoid[3] skeletons, have been so far reported from different species of geographically distinct regions. In this study, chemical constituents of *Kadsura ananosma* have been extensively investigated. Previous phytochemical study on this plant resulted in the discovery of longipedlactone and lanostane triterpenoids, sesquiterpenoids, and lignans.[4–9] Further studies led to the isolation of eleven new triterpenoids, kadnanolactones A-I (**1**–**4**, **7**–**11**) and kadnanosic acids A (**5**) and B (**6**), along with six known analogues, kadcoccilactone R (**12**),[10] schisanlactone F (**13**),[11] manwuweizic acid (**14**),[12] micrandilactones B (**16**) and C (**15**),[13] and wuweizidilactone H (**17**).[14] To further validate the relative configuration of an epoxide in **7**, DFT computational methods were applied. The cytotoxicity activity of all compounds were tested against human tumor cell lines of HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3 by the MTT method,[15] but none was active.

2. Results and discussion

Kadnanolactone A (**1**) was isolated as white amorphous powder, and gave an $[M+Na]^+$ ion peak at *m*/*z* 491.3129 in the HRESIMS, consistent with a molecular formula of $C_{30}H_{44}O_4$ (calcd 491.3137), requiring 9° of unsaturation. The ^1H NMR spectrum showed signals for an olefinic proton (δ_H 6.61), an isopropenyl group (δ_H 4.93, 4.82, 1H each, s, and δ_H 1.77, 3H, s), an oxygenated methine (δ_H 4.47), an olefinic methyl (1.92, 3H, s), a secondary methyl (δ_H 0.97, 3H, d, J=8.3 Hz), and two tertiary methyl groups (δ_H 0.84, 0.85, 3H, each s) (Table 1). The ^{13}C NMR and DEPT spectra revealed 30 carbon signals, consisting of five methyls, eleven methylenes, seven methines (including an oxidized carbon), and seven quaternary carbons (including an oxidized, two olefinic and two ester groups) (Table 3). These data suggested that **1** was likely to be a 3,4:9,10-seco-cycloartane-type triterpenoid, which was similar to schinalactone B,[16] but a pair of double bonds between C-4 and C-30 was exhibited in **1** instead showed a tertiary alcohol in schinalactone B. The oxygen atom of the spiro-ring on C-10 was in the α-orientation as the same as schinalactone B, which was determined by ROESY correlations (between H-1α and H-8β, 11β) (Fig. 2) and by comparison of its spectroscopic data to those of schinalactone B. Therefore, the structure of **1** was assigned as shown in Fig. 1, named kadnanolactone A.

Kadnanolactone B (**2**) was isolated as white amorphous powder. On the basis of HRESIMS (found 507.3100 $[M+Na]^+$), the molecular formula of **2** was defined as $C_{30}H_{44}O_5$, containing 9° of unsaturation.

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Table 1
^{1}H NMR data of compounds **1**–**6** (δ in ppm, J in Hz)

H	**1**[a]	**2**[b]	**3**[c]	**4**[d]	**5**[b]	**6**[b]
1α	1.75 (overlap)	6.13 (t, 9.5)	1.75 (overlap)	2.73 (m)	3.89 (m)	1.82 (overlap)
1β	2.33 (m)		1.69 (overlap)	1.69 (overlap)	2.13 (overlap)	1.45 (overlap)
2α	1.76 (overlap)	3.56 (m)	2.63 (m)	2.37 (m)	2.83 (overlap)	2.58 (overlap)
2β	2.47 (m)	3.56 (m)	2.54 (m)	1.99 (overlap)	2.40 (m)	2.44 (m)
5	2.56 (br d, 12.0)	2.86 (m)	1.97 (overlap)	1.51 (overlap)	1.78 (d, 12.0)	1.63 (overlap)
6α	1.68 (br s)	1.82 (m)	2.06 (overlap)	1.62 (m)	1.65 (overlap)	1.45 (overlap)
6β	1.45 (overlap)	2.15 (overlap)	1.55 (m)	1.45 (overlap)	1.48 (overlap)	1.42 (overlap)
7α	1.67 (br s)	1.35 (overlap)	1.75 (overlap)	1.99 (overlap)	1.98 (overlap)	2.02 (overlap)
7β	1.53 (overlap)	1.55 (overlap)	1.48 (overlap)	1.99 (overlap)	1.39 (overlap)	2.02 (overlap)
8	1.92 (overlap)	2.03 (overlap)				
9	1.46 (overlap)					
11α	1.68 (br s)	5.29 (m)	1.72 (overlap)	1.70 (overlap)	1.71 (overlap)	2.02 (overlap)
11β	1.51 (overlap)		1.65 (overlap)	1.25 (overlap)	1.63 (overlap)	2.02 (overlap)
12α	1.53 (overlap)	2.03 (overlap)	2.11 (m)	1.83 (overlap)	1.98 (overlap)	1.76 (overlap)
12β	1.53 (overlap)	1.82 (m)	2.02 (overlap)	1.48 (overlap)	1.98 (overlap)	1.62 (overlap)
15α	1.30 (m)	1.33 (overlap)	1.25 (overlap)	1.82 (overlap)	1.63 (overlap)	1.62 (overlap)
15β	1.25 (m)	1.33 (overlap)	1.25 (overlap)	1.75 (overlap)	1.63 (overlap)	1.21 (overlap)
16α	1.79 (overlap)	1.57 (overlap)	1.43 (overlap)	1.98 (overlap)	1.39 (overlap)	1.74 (overlap)
16β	1.34 (overlap)	1.24 (m)	2.01 (overlap)	2.12 (m)	1.39 (overlap)	1.98 (overlap)
17	1.53 (overlap)	1.52 (overlap)	1.51 (overlap)	1.62 (overlap)	1.54 (m)	1.74 (overlap)
18	0.85 (s)	0.67 (s)	0.77 (s)	0.82 (s)	0.78 (s)	0.77 (s)
19α	1.85 (overlap)	3.41 (d, 16.7)	1.23 (s)	1.20 (s)	1.39 (s)	1.03 (s)
19β	1.71 (overlap)	2.91 (d, 16.7)				
20	2.04 (m)	1.92 (overlap)	2.02 (overlap)	1.92 (m)	1.50 (overlap)	2.08 (overlap)
21	0.97 (d, 8.3)	0.91 (d, 8.1)	1.00 (d, 6.6)	1.00 (d, 6.7)	1.01 (d, 7.4)	1.12 (d, 7.5)
22α	4.47 (br d, 16.5)	4.40 (br d, 16.2)	4.47 (br d, 13.1)	4.45 (td, 3.7, 13.8)	1.65 (overlap)	5.36 (m)
22β					1.26 (m)	
23α	2.11 (m)	1.91 (overlap)	2.38 (m)	2.31 (overlap)	2.89 (overlap)	2.53 (overlap)
23β	2.50 (m)	2.13 (overlap)	2.38 (m)	2.26 (m)	2.73 (overlap)	2.53 (overlap)
24	6.61 (d, 6.4)	6.48 (d, 6.3)	6.60 (d, 6.0)	6.71 (d, 6.3)	6.04 (t, 8.6)	7.26 (t, 6.9)
27	1.92 (s)	1.92 (s)	1.91 (s)	2.04 (s)	2.13 (s)	2.15 (s)
28	0.84 (s)	0.82 (s)	0.88 (s)	0.96 (s)	0.97 (s)	0.95 (s)
29	1.77 (s)	1.45 (s)	1.51 (s)	1.28 (s)	1.50 (s)	1.13 (s)
30a	4.93 (s)	1.40 (s)	1.43 (s)	1.25 (s)	1.47 (s)	1.05 (s)
30b	4.82 (s)					
OCH_3				3.58 (s)		
OAc						2.02 (s)

[a] Recorded in $CDCl_3$, 500 MHz.
[b] Recorded in C_5D_5N, 400 MHz.
[c] Recorded in $CDCl_3$, 400 MHz.
[d] Recorded in $(CD_3)_2CO$, 400 MHz.

Extensive analysis of the NMR spectroscopic data of **2** showed a close resemblance with lancilactone C.[17] The most prominent differences of **2** and lancilactone C were the location of the double bonds. Two double bonds in **2** formed between C-1 and C-10, and between C-9 and C-11, determined by the HMBC correlations from H-1 (δ_H 6.13) to C-3, C-5, and C-19, from H-2, H-5, and H-19 to C-10 (δ_C 142.1, s), from H-11 (δ_H 5.29) to C-8, C-13, and C-19, and from H-12, H-19 to C-9 (δ_C 141.1, s). This deducation was further confirmed by the ^{1}H NMR signals of one AB spin system doublet (δ_H 3.41, d, J=16.7 Hz, and δ_H 2.91, d, J=16.7 Hz, H_2-19) appeared in the lower field region. The relative stereochemistry of **2** was determined to be same as that of lancilactone C by the analysis of ROESY spectrum and its spectroscopic data. Therefore, compound **2** was determined as shown in Fig. 1, named kadnanolactone B.

Kadnanolactone C (**3**) was obtained as white amorphous powder. HRESIMS analysis of **3** showed that it has the molecular formula $C_{30}H_{44}O_4$ (m/z 469.3322 $[M+H]^+$ calcd 469.3317), indicating 9° of unsaturation. The ^{1}H and ^{13}C NMR data (Tables 1 and 3) of **3** showed similarities with the analogous values for compound **12**, with the only difference was in ring A. The molecular formula of **3** revealed the occurrence of seven-membered lactone in ring A, which was in accord with the observation of remarkable downfield shift of the signal of C-4 from δ_C 74.5 in **12** to δ_C 86.1 in **3**. The relative configurations of all stereocenters of **3** were identical to those of **12** determined by the observed ROESY correlations and by comparison of NMR data of both compounds. The CH_3-18 and CH_3-19 are inferred to be β, and CH_3-28 and H-17 to be α, judging from ROESY correlations from CH_3-18 to H-11β and H-20β, from CH_3-19 to H-1β and H-11β, and from CH_3-28 to H-7α and H-17α. Accordingly, the structure of **3** was assigned to be kadnanolactone C.

Kadnanolactone D (**4**), white amorphous powder, whose molecular formula was determined as $C_{31}H_{48}O_5$ by the HRESIMS (m/z 523.3391 $[M+Na]^+$ calcd 523.3399). Detailed comparison of NMR data of **4** with those of **12** indicated that these two compounds were almost identical, except for the evidence for an evident methoxy group (δ_C 51.5, q) in **4**. The HMBC correlation of δ_H 3.58 (3H, s, OCH_3) with δ_C 175.5 (s, C-3) revealed that the methoxy group is located at C-3. ROESY correlations suggested the same relative stereochemistry as that of **12**. Thus, the structure of **4**, named as kadnanolactone D, was unambiguously determined.

Kadnanosic acid A (**5**) was isolated as colorless solid, and yielded a pseudo-molecular ion peak in the positive HRESIMS spectrum at m/z 511.3393 $[M+Na]^+$, indicative of the molecular formula $C_{30}H_{48}O_5$ (calcd 511.3399). The ^{13}C NMR and DEPT spectra exhibited 30 carbon signals, including nine quaternary carbons (an oxygenated, three olefinic and two carboxylic ones), four methines (an olefinic), ten methylenes, and seven methyls (one secondary and six tertiary). The chemical shifts at δ_C 139.3 (s), 131.6 (s) indicated that a double bond is at C-8 and C-9.[10] The HMBC correlations observed from CH_3-19 to C-1, C-5, C-9, and C-10, indicated **5** is a lanostane triterpenoid.[7] Careful comparison of the NMR data of **5** with those of **12** showed similarities except that the six-membered lactone ring in the side chain of **12** was replaced by an angelic acid in **5**. The ROESY correlation of CH_3-27 with H-24 supported the Z-type olefinic bond between C-24 and C-25. The rest of the relative stereochemistry of **5** was assigned as being the same as those of **12**

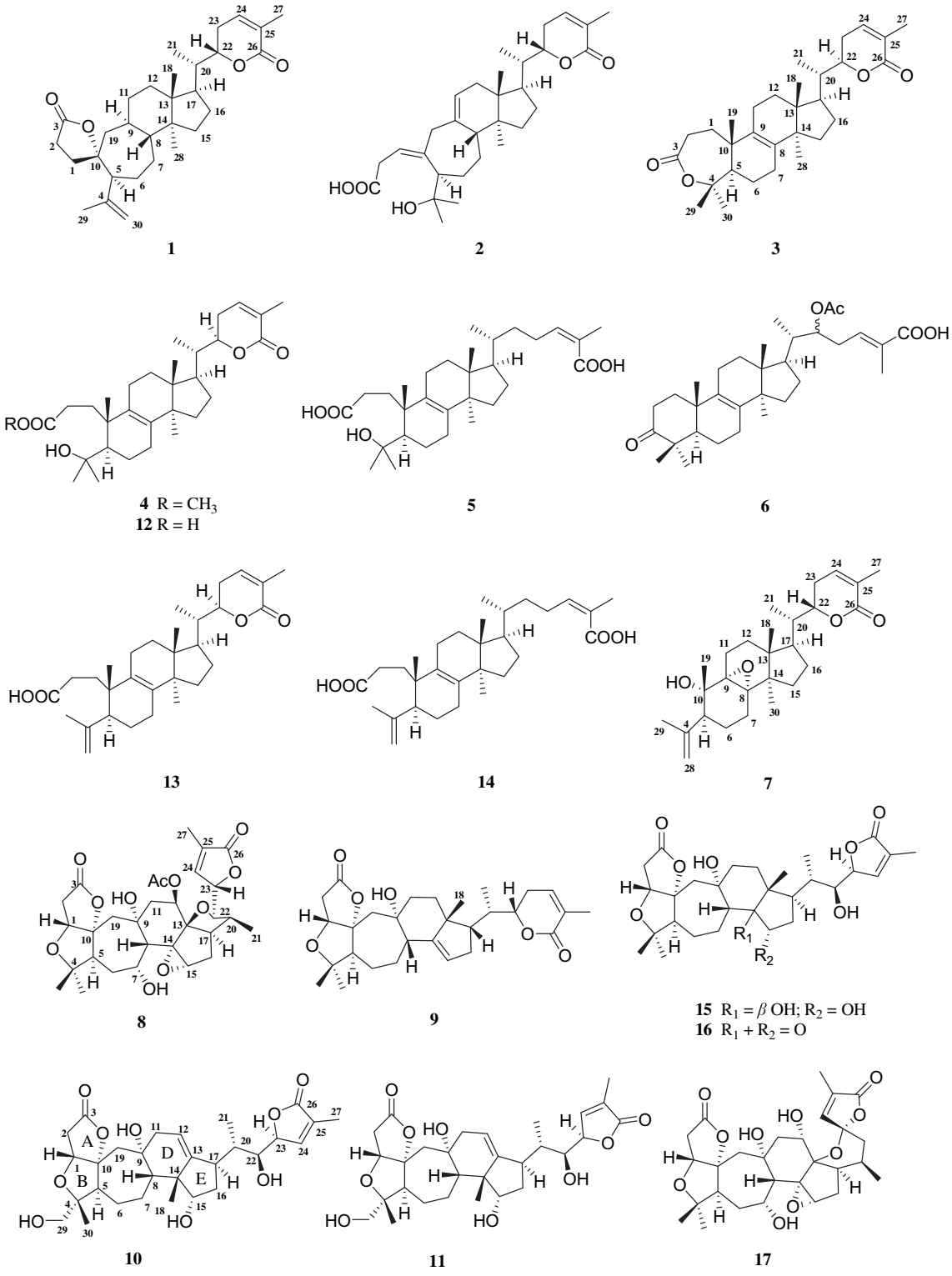

Fig. 1. The structures of compounds **1**–**17**.

from the further analysis of the ROESY spectrum. Consequently, the structure of **5**, kadnanosic acid A, was elucidated as shown.

Kadnanosic acid B (**6**) obtained as white amorphous powder had the molecular formula $C_{32}H_{48}O_5$ as revealed by its HRESIMS data (*m/z* 535.3392 $[M+Na]^+$ calcd 535.3399). Extensive analysis of the 2D NMR spectral data led to the establishment of a structure of lanostane triterpenoid was the same as (3α,24*E*)-3-hydroxy-lanosta-7,9(11),24-trien-26-oic acid.[18] C-3 in **6** was substituted by a ketone group, and a double bond was assigned to C-8 and C-9 on the basis of the HMBC correlations from H_3-29 and H_3-30 to C-3 (δ_C 216.3), from H_3-28 to C-8 (δ_C 135.2, s), and from H_3-19 to C-9 (δ_C 133.8, s). The correlation observed from H-22 (δ_H 5.36, m) to an acetyl carbonyl (δ_C 170.3, s) indicated that the acetyl group is located at C-22. Moreover, the relative configuration of **6** was determined to be the same as that of (3α,24*E*)-3-hydroxy-lanosta-7,9(11),24-trien-26-oic acid by the ROESY spectrum except for C-22, due to free rotation of the side chain. Thus, the structure of **6** was determined to be as shown in Fig. 1.

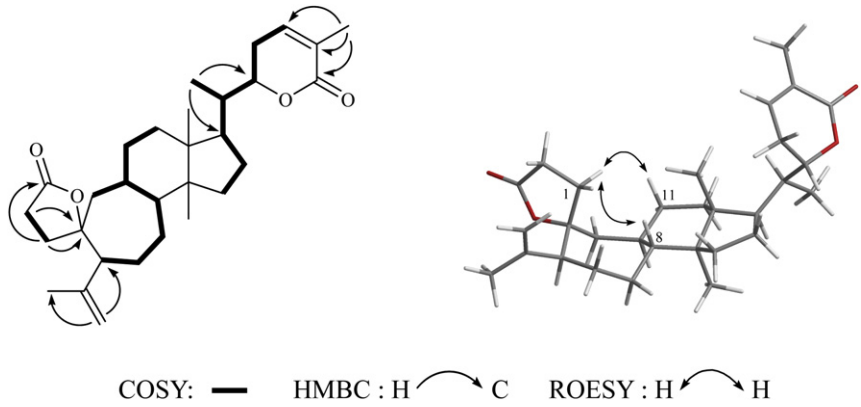

Fig. 2. Key COSY, HMBC, and ROESY correlations of **1**.

Kadnanolactone E (**7**) was isolated as white amorphous powder, and its molecular formula $C_{27}H_{40}O_4$ was established by HRESIMS. Its NMR spectral data suggested **7** to be a 3,4-seco-lanostane-type trinortriterpenoids. A careful analysis of the 2D NMR spectral data and comparison with **13**, led to the conclusions that C-1, C-2, and C-3 were disappeared, and C-8, C-9, and C-10 were oxygenated quaternary carbons on the basis of the HMBC correlations of H-6 with C-8 (δ_C 70.2) and C-10 (δ_C 75.1), and of H-5 with C-9 (δ_C 71.3) and C-10 (δ_C 75.1). Considering the molecular formula and NMR data, an epoxide and a hydroxyl must be located at C-8, C-9, and C-10, respectively. In the ROESY experiment, CH_3-18 showed strong correlation with CH_3-19 indicated CH_3-19 is β-oriented. The α-orientation of the epoxy ring between C-8 and C-9 is also deduced from the key correlation between CH_3-18 and CH_3-19 as shown in computer-generated 3D drawing (Fig. 3), which was minimized using the MM2 force field. To support the position and stereochemical assignment of the epoxide group, DFT caculations[19–21] were employed in ^{13}C NMR data and relative energies (RE) computations of four possible structural formulas of **7** (Fig. 4). The computed results (see Supplementary data) are agreed with our assignment. Therefore, compound **7** was unambiguously determined as shown in Fig. 1, and named kadnanolactone E.

Kadnanolactone F (**8**) was obtained as white amorphous powder, and the molecular formula $C_{30}H_{38}O_{11}$ was assigned through its HRESIMS (*m*/*z* 597.2294, $[M+Na]^+$), requiring 12° of unsaturation. Comparison of the spectroscopic data of **8** with those of wuweizidilactone B[21] indicate that **8** was a 18-nor-schiartane skeleton dinortriterpenoid substituted by a hydroxyl group at C-7, instead of an angeloyl in wuweizidilactone B. This assumption was subsequently confirmed by the strong HMBC correlations of H-7 (δ_H 4.37) with C-5, C-9, and C-14, and $^1H-^1H$ COSY correlations of H-5/H-6/H-7/H-8. Furthermore, in the ROESY spectrum, the correlations of H-7 with H-8β and H-15β suggested the α-orientation of HO-7. Thus, the complete structure of **8** was established as shown in Fig. 1.

Kadnanolactone G (**9**) was assigned the molecular formula $C_{29}H_{40}O_6$, which was deduced by means of HRESIMS analysis (*m*/*z* 507.2741 $[M+Na]^+$). The NMR spectroscopic data were analogous to those of kadcoccilactone D[3] belonging to schiartane skeleton nortriterpenoid. The only difference observed in the ^{13}C NMR spectrum was that the formyloxy group at C-9 in kadcoccilactone D was

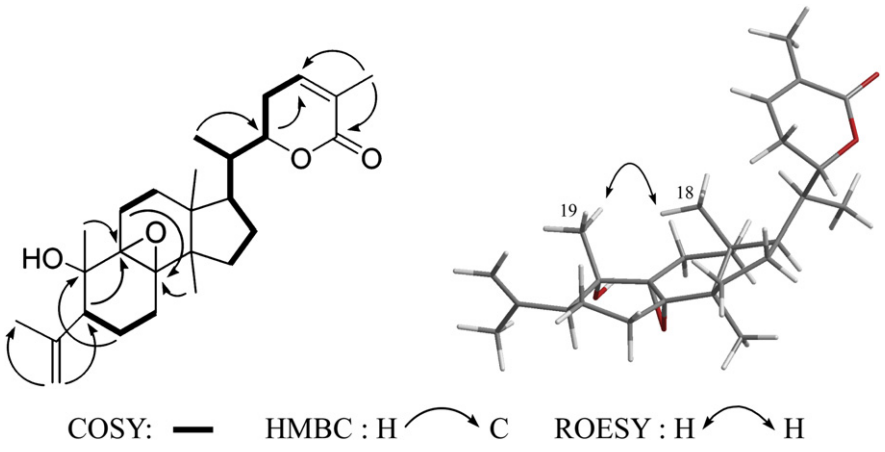

Fig. 3. Key COSY, HMBC, and ROESY correlations of **7**.

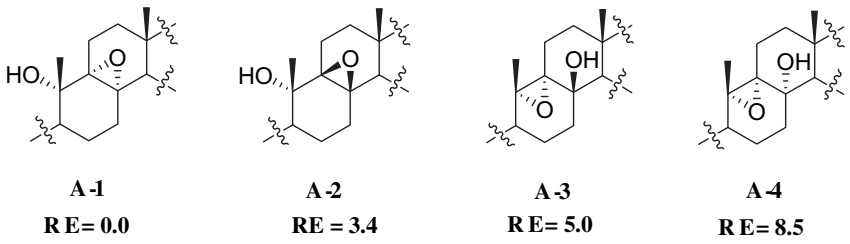

Fig. 4. B3LYP/6-31G* predicted relative energies (RE, in kcal/mol) of four possible isomers of **7**.

absent, but showed a hydroxyl group in **9**. The key HMBC correlations from H-7, H-12, and H-19 to C-9 (δ_C 73.2) were observed. In addition, the ^{13}C NMR signal for C-9 shifted upfield (δ_C 73.2) in **9** compared with kadcoccilactone D (δ_C 83.6), which further corroborated the structural feature that a hydroxyl group located at C-9. The ROESY correlations of H-8β with H-19β, and H-11β with H-19β were deduced to be sharing the same α-hydroxyl group at C-9. Therefore, the gross structure of **9** was determined.

Kadnanolactones H (**10**) and I (**11**) were obtained as identical molecular formula of $C_{29}H_{40}O_9$ requiring 10° of unsaturation, based on accurate mass measurement and NMR data. Comparison of the spectroscopic data of **10** with those of **9** revealed that they were quite similar. Analysis of the HMBC spectrum of **10**, correlations from H_3-18 to C-8, C-13, C-14, and C-15, and from H-12 (δ_H 5.72) to C-9, C-13, C-14, and C-17 were observed. These data were used to determine that CH_3-18 and a double bond are at C-14, C-12, and C-13, respectively. HMBC correlations from H_3-18 to C-8, C-13, C-14, and C-15 indicated that CH_3-18 is located at C-14. According to the downfield-shifted signal of C-29 (δ_C 69.6) and the molecular formula indicated that a hydroxy group is located at C-29. The strong ROESY correlation of H_3-18 with H-8 was used to place CH_3-18 in a β-orientation (Fig. 5), indicating that CH_3-18 shifted from C-13 to C-14 on the basis of biosynthetic considerations.[22] Therefore, the structure of **10** was assigned as an unusual 3,4-seco-18(13→14)-abeo-artane-type nortriterpenoid, and named kadnanolactone H.

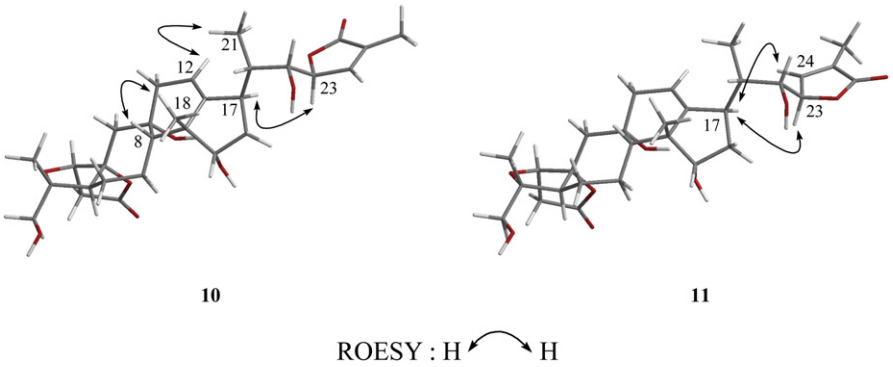

Fig. 5. Key ROESY correlations of **10** and **11**.

Side-by-side comparison of their 1D NMR data and an analysis of 2D NMR data indicate that **10** and **11** possess the same planar structure. The evidences of almost identical 1D NMR data and ROESY correlations from C-1 to C-16 indicated that both had the same rings A-E. The only difference is the strong ROESY correlation of H-24 and H-17 showed in **11**, but disappeared in **10** (Fig. 5), which is the reason responsible for the minor difference of chemical shifts between **10** and **11**, and can be rationalized to the different configuration of C-23. Thus, compound **11** was deduced to be C-23 epimer of **10**, and named kadnanolactone I.

All compounds were tested for their cytotoxicity against the HL-60, SMMC-7721, A-549, PANC-1, and SK-BR-3 human tumor cell lines by the MTT method.[15] All were inactive with IC_{50} values greater than 40 μM.

3. Conclusion

This research led to the isolation of eleven new triterpenoids, together with six known ones, from the stems of *K. ananosma*. The above discovery showed obvious chemical diversity in highly oxygenated triterpenoids, including various seco-triterpenoids, nortriterpenoids, dinortriterpenoids and trinortriterpenoids, which are rare chemical constituents in *Kadsura* species up to now, and also expanded the natural product library of the species of the genus *Kadsura*.

4. Experimental section

4.1. Gerneral

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. A Tenor 27 spectrophotometer was used for scanning IR spectroscopy with KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400 and DRX-500 spectrometers. Chemical shifts (δ) were expressed in parts per million with reference to the solvent signals. Mass spectra were performed on an API QSTAR time-of-flight spectrometer and a VG Autospec-3000 spectrometer, respectively. Column chromatography (CC) was performed with silica gel (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, PR China) and MCI gel (75–150 mm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (9.4 mm×25 cm) column. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

4.2. Plant material

The stems of *K. ananosma* were collected in Simao County of Yunnan Province, People's Republic of China, in August 2006, and identified by Prof. Xi-Wen Li, Kunming Institute of Botany. A voucher specimen has been deposited in the Herbarium of the Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

The air-dried and powdered stems of *K. ananosma* (10 kg) were extracted with 70% aqueous Me_2CO (40 L×3) at room temperature and concentrated in vacuo to yield a residue, which was partitioned between H_2O and EtOAc. The EtOAc extract (300 g) was chromatographed on MCI gel CHP 20P (90% CH_3OH/H_2O). The 90% CH_3OH fraction (245 g) was subjected to silica gel (200–300 mesh, 3.0 kg) column chromatography, eluting with a $CHCl_3/Me_2CO$ gradient system (9:1, 8:2, 2:1, 1:1, 0:1), to give fractions 1–7. Fraction 3 (38 g) was chromatographed on silica gel (eluted with petroleum ether/Me_2CO, 20:1 to 2:1) to give three subfractions 3.1–3.3. Fraction 3.2 (10 g) was further separated by RP-18 column chromatography ($MeOH/H_2O$, 40%–90% gradient system) to afford **13** (2.7 g) and **14** (5.0 g). Fraction 3.3 (250 mg) was purified over Sephadex LH-20 (eluted with $CHCl_3$/MeOH, 1:1) to give **3** (10 mg). Fraction 4 (20 g) was subjected to column chromatography on silica gel with a $CHCl_3/Me_2CO$ gradient system (50:1 to 1:1) to give four subfractions 4.1 to 4.4. Fraction 4.1 (1.5 g) was purification by semi-preparative HPLC (60% CH_3OH/H_2O), to provide **1** (7 mg), **7** (3 mg), and **17** (750 mg). Fraction 4.2 (1.8 g) was separated by repeated silica gel column chromatography (petroleum ether/Me_2CO, 30:1 to

5:1) to yield **9** (3 mg), **5** (13 mg) and **16** (470 mg). Fraction 4.3 (5.7 g) was chromatographed on RP-18 (MeOH/H_2O, 40%–70% gradient system) to afford **2** (4 mg) and **12** (180 mg). Fraction 5 (9 g) was purified by silica gel (eluted with $CHCl_3$–Me_2CO, 30:1 to 2:1), finally separated by semi-preparative HPLC (CH_3CN–H_2O, 30%) to afford **8** (3 mg), **11** (3 mg), **10** (3 mg), **4** (25 mg), **6** (22 mg), and **15** (11 mg).

*4.3.1. Kadnanolactone A (**1**).* White amorphous powder; $[\alpha]_D^{21}$ +123.4 (*c* 0.10, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε): 237 (3.00), 224 (2.61), 195 (2.77) nm; IR (KBr) ν_{max} 3436, 2936, 1760, 1713, 1639 cm^{-1}; positive FABMS *m/z* 469 (100) $[M+H]^+$; positive HRESIMS *m/z* 491.3129 $[M+Na]^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3137); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3.

*4.3.2. Kadnanolactone B (**2**).* White amorphous powder; $[\alpha]_D^{21}$ +74.7 (*c* 0.09, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε): 236 (3.09), 211 (2.88), 194 (2.93) nm; IR (KBr) ν_{max} 3425, 2929, 1727 cm^{-1}; positive ESIMS *m/z* 507 (100) $[M+Na]^+$; positive HRESIMS *m/z* 507.3100 $[M+Na]^+$ (calcd for $C_{30}H_{44}O_5Na$, 507.3086); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3.

*4.3.3. Kadnanolactone C (**3**).* White amorphous powder; $[\alpha]_D^{21}$ +113.9 (*c* 0.18, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε): 240 (3.09), 203 (2.82), 193 (2.88) nm; IR (KBr) ν_{max} 3424, 2941, 1720 cm^{-1}; positive ESIMS *m/z* 491 (100) $[M+Na]^+$; positive HRESIMS *m/z* 469.3322 $[M+H]^+$ (calcd for $C_{30}H_{45}O_4$, 469.3317); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3.

*4.3.4. Kadnanolactone D (**4**).* White amorphous powder; $[\alpha]_D^{21}$ +109.8 (*c* 0.08, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε): 236 (3.16), 223 (2.84), 215 (2.90) nm; IR (KBr) ν_{max} 3433, 2947, 1733, 1720, 1634 cm^{-1}; positive ESIMS *m/z* 523 (100) $[M+Na]^+$; positive HRESIMS *m/z* 523.3391 $[M+Na]^+$ (calcd for $C_{31}H_{48}O_5Na$, 523.3399); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3.

*4.3.5. Kadnanosic acid A (**5**).* Colorless solid; $[\alpha]_D^{21}$ +52.9 (*c* 0.09, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε): 236 (3.24), 196 (2.90) nm; IR (KBr) ν_{max} 3426, 2926, 1701, 1639 cm^{-1}; positive ESIMS *m/z* 511 (100) $[M+Na]^+$; positive HRESIMS *m/z* 511.3393 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_5Na$, 511.3399); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3.

*4.3.6. Kadnanosic acid B (**6**).* White amorphous powder; $[\alpha]_D^{21}$ +36.5 (*c* 0.10, $CHCl_3$/MeOH, 1:1); UV (MeOH) λ_{max} (log ε): 236 (3.21), 213 (2.81), 193 (2.91) nm; IR (KBr) ν_{max} 3432, 2949, 1735, 1708, 1690, 1645 cm^{-1}; positive ESIMS *m/z* 535 (100) $[M+Na]^+$; positive HRESIMS *m/z* 535.3392 $[M+Na]^+$ (calcd for $C_{32}H_{48}O_5Na$, 535.3399); for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 3.

*4.3.7. Kadnanolactone E (**7**).* White amorphous powder; $[\alpha]_D^{22}$ +13.1 (*c* 0.15, $CHCl_3$/MeOH, 1:1); UV ($CHCl_3$/MeOH, 1:1) λ_{max} (log ε) 236 (3.06), 212 (2.74), 199 (2.80) nm; IR (KBr) ν_{max} 3411, 2974, 2931, 1712, 1634, 1119 cm^{-1}; positive ESIMS *m/z* 451 (100) $[M+Na]^+$; positive HRESIMS *m/z* 451.2817 $[M+Na]^+$ (calcd for $C_{27}H_{40}O_4Na$, 451.2824); For ^1H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.8. Kadnanolactone F (**8**).* White amorphous powder; $[\alpha]_D^{22}$ +32.5 (*c* 0.08, $CHCl_3$/MeOH, 1:1); UV ($CHCl_3$/MeOH, 1:1) λ_{max} (log ε) 256 (2.53), 235 (2.73), 227 (2.69), 192 (2.93) nm; IR (KBr) ν_{max} 3424, 2974, 2972, 2928, 1761, 1730, 1654, 1119 cm^{-1}; positive ESIMS *m/z* 597 (100) $[M+Na]^+$; positive HRESIMS *m/z* 597.2294 $[M+Na]^+$

Table 2
^1H NMR data of compounds **7**–**11** (δ in ppm, *J* in Hz)

H	**7**[a]	**8**[b]	**9**[a]	**10**[b]	**11**[a]
1α		4.22 (d, 4.7)	4.27 (d, 4.8)	4.39 (d, 4.3)	4.39 (d, 4.3)
2α		2.71 (d, 17.8)	2.75 (d, 17.8)	2.69 (d, 17.6)	2.70 (d, 17.6)
2β		2.98 (dd, 17.8, 4.7)	3.01 (dd, 17.8, 4.8)	2.95 (dd, 17.6, 4.3)	2.95 (dd, 17.6, 4.3)
5	2.96 (dd, 13.2, 3.0)	3.21 (dd, 17.4, 4.9)	2.48 (dd, 12.8, 4.6)	3.11 (dd, 14.7, 4.4)	3.11 (dd, 14.7, 4.4)
6α	1.50 (overlap)	2.00 (m)	1.33 (d, 12.8)	1.86 (overlap)	1.86 (overlap)
6β	1.43 (overlap)	1.42 (m)	1.68 (overlap)	1.43 (overlap)	1.43 (overlap)
7α	1.86 (m)	4.37 (d, 7.7)	2.00 (overlap)	2.39 (overlap)	2.39 (overlap)
7β	1.86 (m)		1.93 (overlap)	2.08 (overlap)	2.08 (overlap)
8		2.62 (s)	2.10 (overlap)	1.78 (d, 14.6)	1.78 (d, 14.6)
11α	2.42 (dd, 15.9, 4.8)	2.20 (br d, 17.1)	1.87 (overlap)	2.36 (overlap)	2.36 (overlap)
11β	2.20 (m)	2.29 (d, 17.1)	1.62 (overlap)	2.30 (m)	2.30 (m)
12α	1.42 (overlap)	5.50 (s)	2.15 (overlap)	5.72 (d, 6.8)	5.77 (d, 6.9)
12β	1.42 (overlap)		1.65 (overlap)		
15α	1.60 (m)	3.80 (s)	5.34 (s)	3.97 (s)	3.96 (d, 3.3)
15β	1.28 (t, 12.6)				
16α	1.77 (m)	2.01 (overlap)	2.10 (overlap)	2.37 (overlap)	2.35 (overlap)
16β	1.33 (overlap)	1.68 (m)	2.00 (overlap)	2.19 (overlap)	2.12 (overlap)
17	1.33 (overlap)	2.58 (m)	1.60 (overlap)	3.37 (m)	3.35 (m)
18	0.77 (s)		0.96 (s)	1.11 (s)	1.10 (s)
19α	1.45 (s)	2.12 (overlap)	2.05 (overlap)	2.09 (d, 15.3)	2.10 (d, 15.5)
19β		2.13 (overlap)	2.10 (overlap)	2.18 (d, 15.3)	2.18 (d, 15.5)
20	2.00 (overlap)	2.80 (m)	2.15 (overlap)	2.13 (overlap)	2.13 (overlap)
21	0.95 (d, 6.6)	0.89 (d, 6.7)	0.95 (d, 6.5)	1.32 (d, 7.0)	1.34 (d, 5.7)
22	4.43 (m)	3.93 (dd, 10.1, 2.4)	4.38 (br d, 12.9)	4.06 (m)	4.00 (s)
23α	1.98 (overlap)	5.04 (overlap)	1.70 (overlap)	5.19 (m)	5.27 (s)
23β	2.12 (m)		2.10 (overlap)		
24	6.50 (d, 6.2)	7.19 (overlap)	6.42 (d, 6.8)	7.48 (s)	7.24 (s)
27	1.90 (s)	1.81 (s)	1.92 (s)	1.86 (s)	1.83 (s)
28a	5.10 (s)				
28b	4.83 (s)				
29a	2.07 (s)	1.01 (s)	1.12 (s)	3.84 (d, 15.0)	3.84 (d, 15.0)
29b				3.82 (d, 15.0)	3.82 (d, 15.0)
30	1.04 (s)	1.20 (s)	1.28 (s)	1.40 (s)	1.40 (s)
OAc		2.13 (s)			

[a] Recorded in C_5D_5N, 500 MHz.
[b] Recorded in C_5D_5N, 400 MHz.

Table 3
^{13}C NMR data of compounds **1**–**11** (δ in ppm)

C	**1**[a]	**2**[b]	**3**[a]	**4**[c]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**9**[d]	**10**[b]	**11**[d]
1	31.7 (t)	123.5 (d)	36.3 (t)	33.7 (t)	34.2 (t)	36.2 (t)		82.1 (d)	81.8 (d)	82.6 (d)	82.6 (d)
2	29.5 (t)	35.4 (t)	32.4 (t)	29.8 (t)	30.7 (t)	34.8 (t)		36.0 (t)	36.3 (t)	36.9 (t)	36.9 (t)
3	177.3 (s)	170.8 (s)	174.5 (s)	175.5 (s)	177.8 (s)	216.3 (s)		175.1 (s)	175.2 (s)	175.8 (s)	175.7 (s)
4	146.5 (s)	73.0 (s)	86.1 (s)	75.0 (s)	74.6 (s)	47.4 (s)	149.3 (s)	84.5 (s)	84.8 (s)	88.5 (s)	88.5 (s)
5	54.9 (d)	51.2 (d)	49.9 (d)	49.0 (d)	48.8 (d)	51.3 (d)	46.5 (d)	51.9 (d)	59.8 (d)	54.5 (d)	54.6 (d)
6	31.3 (t)	25.8 (t)	23.4 (t)	23.9 (t)	23.6 (t)	19.7 (t)	25.1 (t)	33.6 (t)	27.3 (t)	28.8 (t)	28.8 (t)
7	32.6 (t)	26.0 (t)	26.9 (t)	27.6 (t)	28.4 (t)	21.3 (t)	24.3 (t)	69.5 (d)	26.6 (t)	25.5 (t)	25.4 (t)
8	31.7 (d)	49.7 (d)	134.2 (s)	139.8 (s)	139.3 (s)	135.2 (s)	70.2 (s)	46.2 (d)	48.8 (d)	58.3 (d)	58.4 (d)
9	48.4 (d)	141.1 (s)	133.7 (s)	132.0 (s)	131.6 (s)	133.8 (s)	71.3 (s)	75.8 (s)	73.2 (s)	70.4 (s)	70.6 (s)
10	91.6 (s)	142.1 (s)	39.4 (s)	43.5 (s)	43.2 (s)	37.1 (s)	75.1 (s)	98.9 (s)	99.8 (s)	99.5 (s)	99.5 (s)
11	30.4 (t)	120.2 (d)	30.9 (t)	30.1 (t)	31.7 (t)	26.6 (t)	22.3 (t)	41.2 (t)	39.1 (t)	42.5 (t)	42.5 (t)
12	29.5 (t)	37.4 (t)	21.4 (t)	27.4 (t)	27.2 (t)	31.3 (t)	27.0 (t)	70.4 (d)	36.7 (t)	120.7 (d)	120.7 (d)
13	45.9 (s)	45.2 (s)	44.5 (s)	45.3 (s)	44.6 (s)	49.9 (s)	44.8 (s)	90.5 (s)	47.3 (s)	147.5 (s)	147.5 (s)
14	48.9 (s)	47.9 (s)	49.7 (s)	51.5 (s)	51.4 (s)	45.2 (s)	49.1 (s)	70.6 (s)	153.0 (s)	52.5 (s)	52.6 (s)
15	33.3 (t)	33.9 (t)	31.0 (t)	30.2 (t)	31.5 (t)	31.3 (t)	32.6 (t)	55.3 (d)	119.0 (d)	76.8 (d)	76.8 (d)
16	26.7 (t)	26.7 (t)	26.3 (t)	21.6 (t)	21.4 (t)	27.3 (t)	27.3 (t)	27.0 (t)	35.0 (t)	40.3 (t)	40.1 (t)
17	46.8 (d)	46.9 (d)	46.2 (d)	47.2 (d)	50.8 (d)	47.8 (d)	44.7 (d)	43.6 (d)	54.6 (d)	42.6 (d)	43.1 (d)
18	14.5 (q)	14.9 (q)	15.7 (q)	16.4 (q)	16.5 (q)	16.0 (q)	16.3 (q)		16.6 (q)	26.2 (q)	26.4 (q)
19	48.7 (t)	43.4 (t)	20.5 (q)	22.4 (q)	22.4 (q)	18.7 (q)	19.5 (q)	46.8 (t)	45.7 (t)	46.0 (t)	45.9 (t)
20	39.1 (d)	39.5 (d)	39.4 (d)	40.4 (d)	36.8 (d)	40.5 (d)	39.6 (d)	36.5 (d)	37.7 (d)	44.3 (d)	44.1 (d)
21	13.4 (q)	13.3 (q)	13.6 (q)	13.8 (q)	18.9 (q)	13.6 (q)	14.0 (q)	11.7 (q)	13.6 (q)	15.9 (q)	15.9 (q)
22	80.5 (d)	80.5 (d)	80.4 (d)	80.8 (d)	36.4 (t)	76.0 (d)	80.3 (d)	84.9 (d)	80.4 (d)	75.0 (d)	74.7 (d)
23	23.5 (t)	23.6 (t)	23.5 (t)	23.9 (t)	27.1 (t)	28.0 (t)	23.7 (t)	81.3 (d)	23.4 (t)	82.6 (d)	82.8 (d)
24	139.5 (d)	140.1 (d)	139.4 (d)	140.6 (d)	142.8 (d)	138.1 (d)	140.2 (d)	146.8 (d)	140.1 (d)	149.1 (d)	148.8 (d)
25	128.2 (s)	128.0 (s)	128.2 (s)	128.4 (s)	128.7 (s)	131.1 (s)	128.0 (s)	130.8 (s)	128.0 (s)	130.2 (s)	130.3 (s)
26	166.7 (s)	166.3 (s)	166.6 (s)	166.3 (s)	170.7 (s)	170.5 (s)	166.4 (s)	174.2 (s)	166.2 (s)	174.6 (s)	174.9 (s)
27	17.0 (q)	17.2 (q)	17.0 (q)	17.1 (q)	21.6 (q)	13.1 (q)	17.3 (q)	10.8 (q)	17.2 (q)	10.8 (q)	10.7 (q)
28	16.8 (q)	18.2 (q)	24.8 (q)	25.1 (q)	24.9 (q)	24.4 (q)	112.5 (t)				
29	22.5 (q)	29.4 (q)	30.6 (q)	32.1 (q)	33.6 (q)	26.4 (q)	27.1 (q)	22.7 (q)	22.7 (q)	69.6 (t)	69.6 (t)
30	115.1 (t)	28.5 (q)	26.2 (q)	30.0 (q)	28.3 (q)	21.2 (q)	20.5 (q)	28.8 (q)	29.2 (q)	19.3 (q)	19.3 (q)
OCH_3				51.5 (q)							
OAc						170.3 (s)		170.9 (s)			
						21.4 (q)		21.4 (q)			

[a] Recorded in $CDCl_3$, 100 MHz.
[b] Recorded in C_5D_5N, 100 MHz.
[c] Recorded in $(CD_3)_2CO$, 100 MHz.
[d] Recorded in C_5D_5N, 125 MHz.

(calcd for $C_{30}H_{38}O_{11}Na$, 597.2311); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.9. Kadnanolactone G (**9**).* White amorphous powder; $[\alpha]_D^{21}$ +43.8 (*c* 0.08, $CHCl_3$/MeOH, 1:1); UV ($CHCl_3$/MeOH, 1:1) λ_{max} (log ε) 237 (3.02), 222 (2.81), 205 (2.91), 195 (2.95) nm; IR (KBr) ν_{max} 3441, 2931, 1780, 1713, 1631, 1133 cm^{-1}; positive ESIMS *m*/*z* 507 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 507.2741 [M+Na]$^+$ (calcd for $C_{29}H_{40}O_6Na$, 507.2722); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.10. Kadnanolactone H (**10**).* White amorphous powder; $[\alpha]_D^{21}$ +18.5 (*c* 0.16, $CHCl_3$/MeOH, 1:1); UV ($CHCl_3$/MeOH, 1:1) λ_{max} (log ε) 235 (2.95), 210 (2.91), 198 (2.96) nm; IR (KBr) ν_{max} 3427, 2927, 1780, 1754, 1631, 1066 cm^{-1}; positive ESIMS *m*/*z* 555 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 555.2581 [M+Na]$^+$ (calcd for $C_{29}H_{40}O_9Na$, 555.2570); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

*4.3.11. Kadnanolactone I (**11**).* White amorphous powder; $[\alpha]_D^{22}$ −36.8 (*c* 0.08, $CHCl_3$/MeOH, 1:1); UV ($CHCl_3$/MeOH, 1:1) λ_{max} (log ε) 236 (2.82), 220 (2.63), 200 (2.82), 196 (2.85) nm; IR (KBr) ν_{max} 3422, 2927, 1753, 1068 cm^{-1}; positive ESIMS *m*/*z* 555 (100) [M+Na]$^+$; positive HRESIMS *m*/*z* 555.2563 [M+Na]$^+$ (calcd for $C_{29}H_{40}O_9Na$, 555.2570); for ^{1}H and ^{13}C NMR spectroscopic data, see Tables 2 and 3.

4.4. Cytotoxicity assay

The following human tumor cell lines were used: HL-60, SMMC-7721, A-549, PANC-1 and SK-BR-3. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) at 37°C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO).[15] Briefly, 100 μL adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with initial density of 1×10^5 cells/mL in 100 μL medium. Each tumor cell line was exposed to the test compound at various concentrations in triplicate for 48 h, with 10-hydroxy camptothecin (Sigma) as positive control. After the incubation, MTT (100 μg) was added to each well, and the incubation continued for 4 h at 37°C. The cells were lysed with 100 μL 20% SDS-50% DMF after removal of 100 μL medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method.[15]

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.09.059. These data include

MOL files and InChIKeys of the most important compounds described in this article.

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