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ORIGINAL ARTICLE

Lanostane triterpenoids from fruiting bodies of basidiomycete *Stereum* sp., structures and biological activities

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Twelve new lanostane triterpenoids, sterenoids A–L (1–12) have been isolated from fruiting bodies of the basidiomycete *Stereum* sp. Compounds 1–12 are rare 14(13 → 12)*abeo*-lanostane triterpenoids featuring remarkable 13*R* configurations that discriminate from the previously covered counterparts. Their structures and absolute configurations are assigned on the basis of in-depth one- and two-dimensional NMR spectroscopic analysis, as well as unbiased quantum chemical NMR and electronic CD calculations. All isolates are evaluated for their *in vitro* cytotoxicity against five human tumor cell lines. Compound 5 exhibits potent cytotoxic activities against tumor cell lines HL-60 and SMMC-7721 with IC_{50} values of 4.7 and 7.6 μM, respectively.
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INTRODUCTION

Ubiquitous fungi, hailed as high-performance creators of natural occurrences, produce overwhelming second metabolites with diverse original scaffolds and versatile biological activities.[1] These attractive characteristics render fungi as an integral part of mining groundbreaking drug candidates and novel small-molecule probes.[2] Higher fungi, which are typically spore-bearing fruiting bodies of fungi, are a paradigm of fabricating useful natural products for the upstream of drug development.[3] The genus *Stereum* is noted for producing a variety of biologically active second metabolites, including sesquiterpenes,[4–8] dimeric sesquiterpenes,[9–11] isoindolinone alkaloids,[12,13] and vibralactone derivatives.[14–18] Moreover, the isolates of this genus have gained organic chemists extraordinary interests. Elegant total synthesis of several isolates has been achieved.[19–22] In addition, the biosynthetic pathway for vibralactone, a pancreatic lipase inhibitor from *S. vibrans* has been deciphered and a monooxygenase from *S. vibrans* is also identified.[23,24]

Previous chemical investigations of the genus *Stereum* mainly centered on culture broth, which were capable of producing diverse second metabolites by scale-up fermentation or using different culture media. Our follow-up search for bioactive natural products from higher fungi, twelve new chemical entities, sterenoids A–L (**1–12**, Figure 1), were isolated from fruiting bodies of *Stereum* sp., which is a wood decaying fungus dwelling at Xishuangbanna Tropical Botanical Garden. Compounds **1–12** are rare 14(13 → 12)*abeo*-lanostane-type triterpenoids featuring distinctive 13*R* configurations that are incompatible with previously covered counterparts.[25,26] To the best of our knowledge, the triterpenoid, possessing this 14(13 → 12)*abeo*-lanostane-type-6/6/5/6 ring core skeleton, was first synthesized occasionally and then was isolated from the medical plant *Kadsura heteroclite*.[26,27] Intriguingly, triterpenoids with this tetracyclic rearranged scaffold also originated from mushrooms *Tyromyces fissilis* and *Ganoderma lucidum*.[28–30] All isolated compounds are evaluated for their cytotoxicity *in vitro* against five human tumor cell lines. Herein, the isolation, structure elucidation and biological evaluation of new compounds **1–12** are discussed.

RESULTS AND DISCUSSION

Structure elucidation

Compound **1**, amorphous powder, had a molecular formula $C_{30}H_{46}O_3$ with eight double bond equivalents as unraveled by the sodium adduct (+)-HR–ESI–MS ion at *m/z* 477.3350 $[M+Na]^+$ (calcd for 477.3339) and the ^{13}C NMR data. The ^1H NMR spectrum (Table 1) of **1** revealed typical resonances for one secondary methyl at δ_H 0.99 (d, $J = 6.8$ Hz, H_3-21), seven tertiary methyls at δ_H 1.05 (H_3-29), 1.11 (H_3-28), 1.14 (H_3-19), 1.21 (H_3-18), 1.23 (H_3-30), 1.60 (H_3-27) and 1.65 (H_3-26), one olefinic proton at δ_H 5.12 (t, $J = 7.2$ Hz, H-24). A thorough analysis of the ^{13}C NMR data (Table 2), with the aid of distortionless enhancement by polarization transfer (DEPT) and HSQC spectra, unlocked 30 carbon signals, including two carbonyls (δ_C 208.4 and δ_C 216.0), 2 double bonds, 8 methyls, 8 sp^3 methylenes, 4 sp^3 methines and 4 sp^3 quaternary carbons (1 oxygenated). One proton resonance at δ_H 3.56 showed no correlations with any carbons in the HSQC spectrum and thus was designated to the hydroxy group. The aforementioned functionalities carbonyls and double bonds accounted for four of the eight degrees of unsaturation and the

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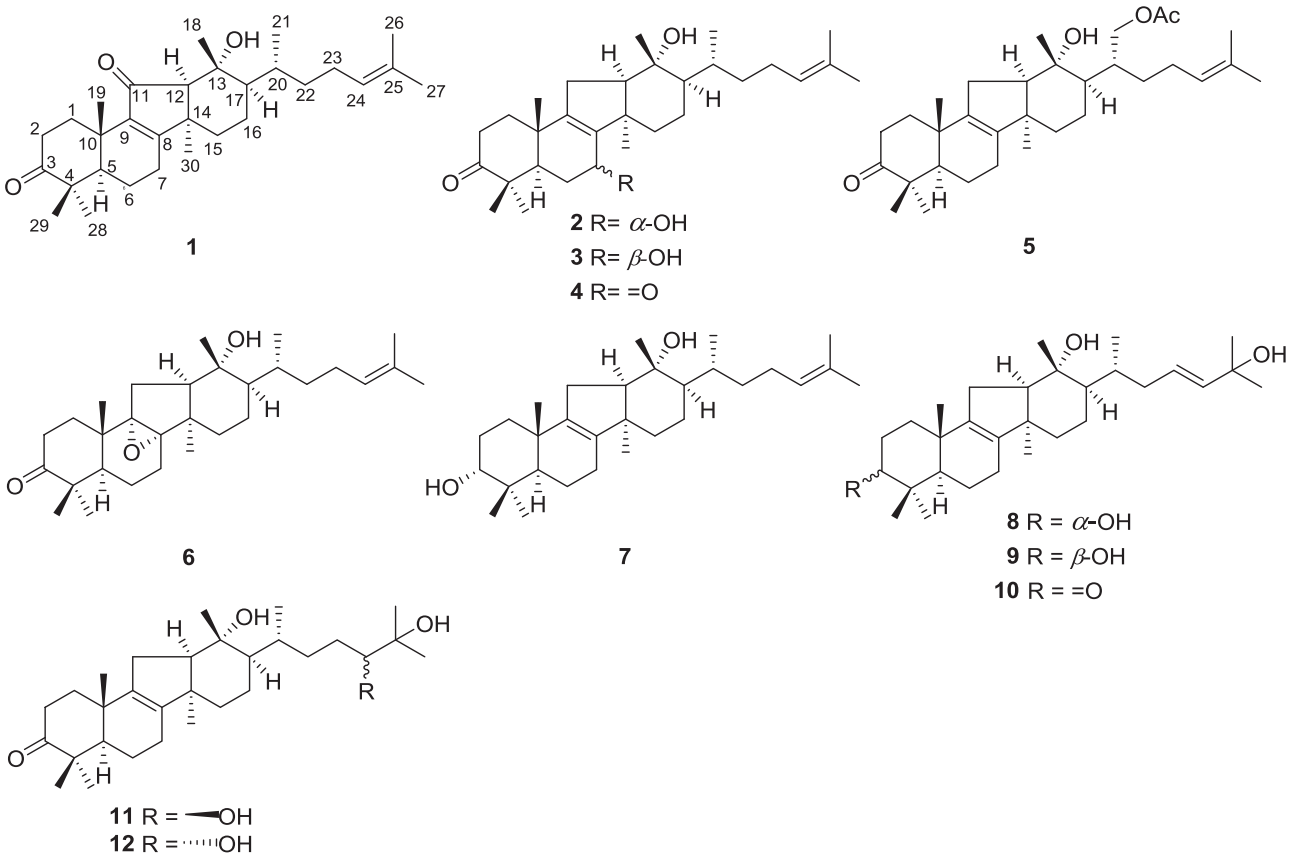

Figure 1 Chemical structures of isolated compounds **1**–**12**.

remaining four degrees of unsaturation exactly constructed four rings in the molecule.

The planar structure of **1** was established by detailed deciphering of 2D NMR spectra. ^{1}H-^{1}H COSY spectrum indicated eighteen proton-bearing fragments as delineated in bold bonds (Figure 2). Quaternary carbons were attached to these fragments to form the scaffold of **1** by the HMBC correlations. The multiple HMBC correlations of H_3-28/C-3, C-4 and C-5; H_3-29/C-3, C-4 and C-5; H_3-19/C-1, C-5, C-9 and C-10, H_a-1/C-3 and H-7/C-8, C-9 along with COSY correlations of H_a-1/H_a-2, H-5/H_b-6 and H_b-6/H-7 constructed the two six-membered carbon rings A and B. The chemical shifts of 179.7 (C-8), 145.0 (C-9) and 208.4 (C-11) suggested the presence of an α,β-unsaturated ketone, which was appended by one methine (C-12) and one quaternary carbon (C-14) to form the five-membered ring C via HMBC correlations of H_3-30/C-8, C-14, C-15 and H-12/C-8, C-11. Furthermore, the ^{1}H-^{1}H COSY revealed the connection of C-15, C-16, C-17, C-20, C-22, C-23 and C-24. The key HMBC correlations of H_3-18/C-12, C-13 and C-17 were responsible for the linkage of the six-membered ring D. The hydroxy group at δ_H 3.56 was fixed at C-13 via the HMBC correlations of the hydroxy proton signal to C-12, C-13 and C-17. Therefore, the tetracyclic triterpenoid scaffold was established. The COSY spin systems between H-17 (δ_H 1.63) and H-20 (δ_H 1.81), as well as HMBC correlations of the methyl protons H_3-21 (δ_H 0.99) to C-17 (δ_C 50.2), C-20 (δ_C 33.3) and C-22 (δ_C 34.3) offered solid evidence that the side chain containing eight carbons was fixed to C-17. Overall, the analysis strongly hinted that **1** had a rare 14(13→12)*abeo*-lanostane-type triterpenoid, which was similar to neokadsuranic acid A.[26] The ROESY correlations of H-5 with H_3-28 and H-12 with H-17, H_3-30, HO-13, and H-17 with H_3-21 revealed that H_3-28, H-5, H-12, HO-13, H_3-30, H-17 and H_3-21 were cofacial and were assigned to be β-oriented (Figure 2). The ROESY cross-peaks of H_3-19/H_3-29 and H_3-18/H-20 showed that they had α-orientations.[31] Intriguingly, the absolute configuration of C-13 was *R*, which discriminated from the previously covered 14(13→12)*abeo*-lanostane-type triterpenoids.[25,32] This arbitrary assumption was confirmed by ROESY and comparison of the experimental electronic CD with quantum chemical calculated electronic CD spectra as shown in Figure 3. Hence, the absolute configuration of **1** was determined as 5*R*, 10s, 12*R*, 13*R*, 14*R*, 17*R*, 21*R*. Taken together, the structure of **1**, sterenoid A, was unambiguously characterized as 24(*E*)-3,11-dioxo-13α-hydroxy-14(13→12)*abeo*-lanosta-8,24-dien.

Compound **2** and **3** were isolated as optically active, white amorphous solid, which had identical molecular formulas $C_{30}H_{48}O_3$ determined by HR–ESI–MS measurements of the sodium adduct ion at *m/z* 479.3488 $[M+Na]^+$ and 479.3490 $[M+Na]^+$ (calcd for 479.3496). Analysis of the NMR data of **2** (Tables 1 and 2) suggested that it was a derivative of **1**, except for the absence of the carbonyl group for C-11 and an additional hydroxy group at C-7. The ^{1}H-^{1}H COSY correlation of H-7 (δ_H 4.24) with H_b-6 (δ_H 1.70) and the HMBC cross-peaks of H-7 with C-8 and C-9 supported the above deduction. The α-oriented hydroxy group at C-7 was defined by the ROESY spectrum via the correlations between H-7/H_3-29 and H_3-28/H-5. The remaining ROESY correlations suggested that **2** shared the same configuration with that of **1**, except for the configuration of hydroxy group for C-7. In parallel, compound **3** was defined as the C-7 epimer of **2** and the configuration of hydroxy group for C-7 was

Table 1 ^{1}H NMR spectroscopic data of compounds 1–6

No.	***1***[a,b]	***2***[b,c]	***3***[b,c]	***4***[b,c]	***5***[b,c]	***6***[a,b]
1	2.51m; 2.78m	1.67m; 1.88m	1.60m; 1.89m	1.81m; 2.06m	2.40m; 2.49m	1.88m; 1.89m
2	1.47m; 1.65m	1.88m; 2.55m	2.47m; 2.56m	2.54m; 2.69m	1.61m; 1.87m	2.41 ddd (4.6, 6.3, 15.7) 2.56 ddd (8.7, 9.5, 15.7)
5	1.78m	1.98m	1.57m	2.17 dd (3.1, 14.0)	1.56m	1.96m
6	1.59m; 1.85	1.70m; 1.80m	1.57m; 2.03m	2.30 dd (3.1, 16.2) 2.49 dd (14.0, 16.2)	1.51m; 1.69m	1.60m; 2.01m
7	2.32m; 2.50m	4.24m	4.35 br s		1.82m; 1.96m	1.41m; 1.54m
11		2.30 d (17.0); 2.41 dd (6.4, 17.0)	1.06m; 2.31m	1.77 d (5.0) 2.59 d (4.8)	1.31m; 2.30m	1.76 dd (8.2, 13.5) 1.82 dd(11.2, 13.5)
12	2.01s	1.75 d (6.4)	1.68m	1.77 dd (4.8, 5.0)	1.73m	1.52m
15	1.59m; 1.72m	1.38m; 1.98m	1.38m; 2.18m	1.60m; 2.26m	1.27m;1.78m	1.19m; 1.34m
16	1.25m; 1.77m	1.04m; 1.62m	1.27m; 1.61m	1.67m; 2.06m	1.56m;1.12m	1.35m; 2.05m
17	1.63m	1.20m	1.21s	1.30m	1.39m	1.40m
18	1.21s	0.94s	1.07s	1.02s	0.97s	1.28s
19	1.14s	1.02s	1.17s	1.31s	1.07s	1.19s
20	1.81m	1.80m	1.8m	1.77m	2.03m	1.73m
21	0.99 d (6.8)	0.98 d (6.8)	0.98 d (6.8)	0.99 d (6.8)	3.97 dd (4.3,10.9) 4.08 dd (7.3 10.9)	1.04 d (6.5)
22	0.99m; 2.50m	0.98m; 1.40m	1.00m; 1.46m	1.06m; 1.43m	1.16m; 1.38m	1.14 1.62
23	1.89m; 2.02m	1.87m; 2.05m	1.88m; 2.05m	1.88m; 2.06m	1.94m;2.07m	1.93 2.09
24	5.12 t (7.2)	5.09s	5.09 t (6.9)	5.09 t (6.9)	5.08 t (7.2)	5.14 t (7.1)
26	1.65s	1.67s	1.67s	1.67s	1.67s	1.67s
27	1.60s	1.59s	1.59s	1.59s	1.58s	1.18s
28	1.11s	1.13s	1.09s	1.11s	1.10s	1.02s
29	1.05s	1.07s	1.08s	1.12s	1.06s	1.01s
30	1.23s	1.16s	1.06s	1.15s	0.95s	1.61s
-OH	3.56s					
-OAc					1.58s	

[a]Measured in acetone-d_6.
[b]Data were measured at 500, 600 and 800 MHz, respectively.
[c]Measured in $CDCl_3$.

thus assigned to be β-oriented. This assignment was supported by 2D NMR spectra analysis, especially the ROESY correlations of H-7 with H-5 and H_3-30. Reinspection of the ^{13}C NMR data of **2** and **3** uncovered that the chemical shift of C-5 (δ_C 46.4) in **2** was major difference ($\Delta\delta_C$ 4.7 p.p.m.) relative to that of **3** (δ_C 50.2), suggesting the very existence of *γ-gauche* effects on the ^{13}C NMR chemical shifts. The HO-7 and H-5 of **2** were 1,3-diaxially bonded and the HO-7 was mainly responsible for steric interactions with the H-5. On the contrary, the HO-7 of **3** was equatorial orientation and thus gave less steric hinderance. Consequently, the chemical shift of C-5 (δ_C 46.4) in **2** was relatively upfield in comparison with that of C-5 (δ_C 50.2) in **3**. The above-discussed key differences in chemical shifts allowed a clear assignment of α or β steric position of the 7-substitutent on the basis of *γ-gauche* effects.[33,34] The structures of **2** and **3**, namely sterenoids B and C, were thus established as 24(*E*)-3-oxo-7*α*,13*α*-dihydroxy-14(13→12)*abeo*-lanosta-8,24-dien and 24(*E*)-3-oxo-7*β*,13*α*-dihydroxy-14(13→12)*abeo*-lanosta-8,24-dien, respectively.

Compound **4** gave a molecular formula of $C_{30}H_{46}O_3$, as established on the basis of ^{13}C NMR and HR–ESI–MS spectra, indicating compound **4** was two less hydrogen atoms than that of **2**. Their ^{1}H and ^{13}C NMR data (Tables 1 and 2) were similar. The major differences were that the resonances assigned to the hydroxy group in **2** replaced by a carbonyl group, together with the shift of the signals corresponding to C-7 from δ_H 4.24, δ_C 63.4 in **2** to δ_C 196.8 in **4**. This change was verified by the HBMC correlations of H-5 to C-7 (δ_C 196.8), H_a-6 (δ_H 2.30) to C-7 and H_b-6 (δ_C 2.49) to C-7. The ROESY correlation of H_3-18 with H-20 revealed that the OH-13 was *α*-oriented. 2D NMR data analysis substantiated the one-dimensional NMR data, relative configuration and regiochemical assignments. Accordingly, the structure of **4**, sterenoid D, was deduced as 24(*E*)-3,7-dioxo-13*α*-hydroxy-14(13→12)*abeo*-lanosta-8,24-dien, a congener of **2**.

Compound **5** was assigned as the molecular formula $C_{32}H_{50}O_4$ by the HRESIMS ion at *m/z* 521.3606 $[M+Na]^+$ (calcd for 521.3601). Its ^{1}H and ^{13}C NMR data (Tables 1 and 2) were closely related to those of **1**, with the main difference occurring for the signals of the 11-substituent and 21-substituent. The 11-substituent in **5** was shifted as a methylene (δ_H 1.31, 2.30; δ_C 29.5) from the carbonyl (δ_C 208.4) in **1** and the 21-substituent in **5** comprised the resonances of an ester carbonyl (δ_C 171.6) and a tertiary methyl (δ_H 1.58; δ_C 21.2). Further analysis of the HMBC spectrum confirmed an acetoxy group for the 21-substituent. Based on the ROESY spectrum and similar NMR patterns, the relative configuration of all the stereogenic centers was assigned to be identical with those of **1**. The structure of **5**, sterenoid E, was thus established as 24(*E*)-3-oxo-13*α*-hydroxy-21-acetoxy-14(13→12)*abeo*-lanosta-8,24-dien.

Compound **6** was obtained as a white, amorphous solid. Its molecular formula $C_{30}H_{48}O_3$, with seven indices of hydrogen deficiency, was established from the HR–ESI–MS sodium adduct ion at *m/z* 479.3492 $[M+Na]^+$ (calcd for 479.3496). The ^{1}H and ^{13}C NMR data (Tables 1 and 2) highly resembled those of **1**, suggesting that these two compounds should be homologous carbocyclic skeletons and substitution patterns, except for the existence of diagnostic resonances of one sp^3 methylene (δ_C 28.4), two oxygenated quaternary carbons C-8 (δ_C

Table 2 ^{13}C NMR spectroscopic data of compounds 1–6

No.	**1**[a,b]	**2**[b,c]	**3**[b,c]	**4**[b,c]	**5**[b,c]	**6**[a,b]
1	34.0 CH_2	34.4 CH_2	35.1 CH_2	34.4 CH_2	34.2 CH_2	34.1 CH_2
2	34.1 CH_2	34.0 CH_2	34.3 CH_2	34.1 CH_2	34.8 CH_2	34.6 CH_2
3	216.0 C	217.6 C	216.6 C	214.8 C	218.0 C	215.4 C
4	47.5 C	46.4 C	46.8 C	47.0 C	47.0 C	47.4 C
5	51.8 CH	45.5 CH	50.2 CH	51.4 CH	51.7 CH	45.2 CH
6	20.0 CH_2	30.9 CH_2	31.7 CH_2	37.3 CH_2	20.1 CH_2	21.0 CH_2
7	25.0 CH_2	63.4 CH	68.0 CH	196.8 C	22.9 CH_2	19.6 CH_2
8	179.7 C	140.7 C	142.0 C	141.3 C	140.9 C	75.3 C
9	145.0 C	147.2 C	146.1 C	170.7 C	140.2 C	73.9 C
10	35.5 C	36.3 C	36.3 C	37.5 C	35.7 C	35.9 C
11	208.4 C	29.5 CH_2	29.9 CH_2	31.5 CH_2	29.5 CH_2	28.4 CH_2
12	67.0 CH	59.3 CH	57.6 CH	57.5 CH	58.4 CH	51.1 CH
13	76.6 C	76.4 C	76.5 C	76.3 C	76.0 C	74.6 C
14	44.2 C	48.4 C	49.7 C	48.5 C	48.7 C	42.1 C
15	30.1 CH_2	32.1 CH_2	34.6 CH_2	30.8 CH_2	32.6 CH_2	28.7 CH_2
16	20.9 CH_2	21.9 CH_2	22.2 CH_2	21.1 CH_2	22.4 CH_2	19.8 CH_2
17	50.2 CH	53.5 CH	53.6 CH	52.5 CH	48.7 CH	50.9 CH
18	23.7 CH_3	19.4 CH_3	20.1 CH_3	20.3 CH_3	19.0 CH_3	29.8 CH_3
19	19.5 CH_3	17.9 CH_3	19.1 CH_3	17.6 CH_3	19.2 CH_3	17.6 CH_3
20	33.3 CH	30.7 CH	30.8 CH	31.6 CH	35.4 CH	32.3 CH
21	20.7 CH_3	21.6 CH_3	21.7 CH_3	21.4 CH_3	68.0 CH_2	22.8 CH_3
22	34.3 CH_2	33.5 CH_2	33.6 CH_2	33.4 CH_2	28.3 CH_2	35.4 CH_2
23	26.9 CH_2	26.6 CH_2	26.6 CH_2	26.4 CH_2	26.6 CH_2	26.4 CH_2
24	125.7 CH	124.7 CH	124.8 CH	124.7 CH	124.2 C	125.4 CH
25	131.4 C	131.4 C	131.4 C	131.5 C	132.0 C	131.7 C
26	25.8 CH_3	25.7 CH_3	25.7 CH_3	25.7 CH_3	25.7 CH_3	25.9 CH_3
27	17.6 CH_3	17.6 CH_3	17.6 CH_3	17.6 CH_3	17.7 CH_3	21.0 CH_3
28	27.4 CH_3	27.3 CH_3	26.7 CH_3	26.2 CH_3	27.3 CH_3	27.6 CH_3
29	21.0 CH_3	20.9 CH_3	21.1 CH_3	21.2 CH_3	21.0 CH_3	21.3 CH_3
30	27.5 CH_3	31.6 CH_3	29.6 CH_3	29.0 CH_3	29.0 CH_3	21.0 CH_3
OAc					171.6 C	
					21.2 CH_3	

[a]Measured in acetone-d_6.
[b]Data were measured at 125, 150 and 200 MHz, respectively.
[c]Measured in $CDCl_3$.

75.3) and C-9 (δ_C 73.9) replacing those of the α,β-conjugated carbonyl group (δ_C 208.4, 179.7 and 145.0) of **1** (Tables 1 and 2) in the B/C-ring, respectively. This deduction was further illustrated by complete examination of 2D NMR spectra. The relative configuration of **6** was assigned via ROESY data in comparison with the counterpart of stereogenic centers in **1**, with the exception of 8,9-oxirane moiety. The ^{13}C NMR calculations with quantum-based methods pinpointed the relative configuration of epoxide ring motif in **6** as previously reviewed.[35] The density functional theory (DFT) calculations of ^{13}C NMR data of the two possible stereoisomers of **6a** and **6b** were performed (Supplementary Figure S100; for details, see the NMR calculations for compound **6** in the Supplementary Information). The calculated NMR data of the isomer **6b** were much closer to the experimental data of **6** as weighed by the linear correlation coefficients (R^2) and root-mean-square deviations, suggesting that the epoxide ring motif was α-oriented. The compound of **6**, sterenoid F, was elucidated as 24(*E*)-3-oxo-8(9)-epoxy-13α-hydroxy-14(13→12)*abeo*-lanosta-24-en.

Compound **7** was isolated as a white amorphous solid. It gave a molecular formula $C_{30}H_{50}O_2$ based on the HR–ESI–MS ion at *m/z* 465.3705 $[M+Na]^+$ (calcd for 465.3703), corresponding to six double bond equivalents. A comprehensive analysis of ^{1}H NMR and ^{13}C NMR data (Tables 3 and 4) revealed that **7** shared a common A–D ring system with that of **1**, occurred an oxygenated methine (δ_C 76.2)

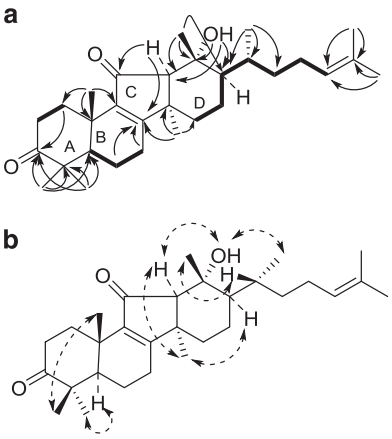

Figure 2 ^{1}H – ^{1}H COSY and selected HMBC (**a**) and key ROESY (**b**) correlations of **1**.

and an additional sp^3 methylene (δ_C 29.7), and disappeared two carbonyls. One carbonyl at C-3 was shifted to a hydroxy carbon (δ_C 76.2) and the other at C-11 was interchanged by the sp^3 methylene, respectively. This plausible hypothesis was verified by the HMBC correlations from H_3-28/H_3-29 to C-3 (δ_C 76.2), C-4 (δ_C 37.4), C-5 (δ_C 45.9) and from H_a-11 (δ_H 2.24) to C-8 (δ_C 139.0), C-9 (δ_C 142.6), C-12 (δ_C 58.1) and C-14 (δ_C 48.7). The ROESY correlations of H_3-19/H_a-2, H_a-2/H-3 and H-3/H_3-29 showed that they were cofacial, indicating the hydroxy group at C-3 was α-oriented. Taken together, compound **7**, sterenoid G, was thus characterized as 24(*E*)-3α,13α-dihydroxy-14(13→12)*abeo*-lanosta-8,24-dien.

The molecular formula $C_{30}H_{50}O_3$ was assigned to **8** with six indices of hydrogen deficiency by the ^{13}C NMR data and the HR–ESI–MS ion at *m/z* 481.3653 $[M+Na]^+$ (calcd for 481.3625), which was more 16 mass units attributable to oxygenated motif than that of **7**. The NMR data (Tables 3 and 4) of **8** were highly consistent with those of **7**, except for the side-chain moiety. The emerging chemical shifts of ^{1}H and ^{13}C NMR spectra in **8** were assignable to three methyls (δ_H 0.95, d, J = 6.9 Hz; δ_H 1.30, s and δ_H 1.30, s), one methylene, one methine, one persubstituted double bond (δ_H 5.59, m; δ_H 5.90, d, J = 18.1 Hz) and one oxygenated quaternary carbon (δ_C 70.7). The ^{1}H-^{1}H COSY correlations of H-17/H-20/H_a-22/H-23 and HMBC cross-peaks from H_3-21 to C-17, C-20 and C-22, from H_3-26 and H_3-27 to C-24 and C-25 established the side chain as depicted. The geometry of the $\Delta^{23,24}$ double bond was assigned as *E* based on coupling constant (18.1 Hz). A thorough analysis of the ROESY spectrum and NMR patterns revealed that the hydroxy group at C-3 was α-oriented and the other stereogenic centers were assigned to be identical with those of **1**. Compound **8**, sterenoid H, was thereby elucidated as 24(*E*)-3α,13α,25-trihydroxy-14(13→12)*abeo*-lanosta-8,23-dien.

Analysis of HR–ESI–MS and ^{13}C NMR data indicated that compound **9** had the same molecular formula with that of **8**. The ^{1}H and ^{13}C NMR data (Tables 3 and 4) of **9** were highly analogous to those of **8**, except for minor variations at the A ring, suggesting that **9** should be the C-3 epimer of **8** and HO-3 was determined to be β-oriented.[25] This deduction was further confirmed by the ROESY correlation of H-3 with H-5. Therefore, compound **9**, 24(*E*)-3β,13α,25-trihydroxy-14(13→12)*abeo*-lanosta-8,23-dien, was given trivial name sterenoid I.

The HR–ESI–MS ion at *m/z* 479.3493 $[M+Na]^+$ (calcd for 479.3496) and ^{13}C NMR data of compound **10** revealed the molecular

formula $C_{30}H_{48}O_3$ with seven indices of hydrogen deficiency. NMR data (Tables 3 and 4) showed that **10** was structurally related to **8** with the discrepancy of replacing HO-3 via the carbonyl group. The HMBC correlations from H_3-28 (δ_H 1.10) and H_3-29 (δ_H 1.08) to C-3 (δ_C 218.1), C-4 (δ_C 47.0) and C-5 (δ_C 51.7) supported this hypothesis. In addition, the ROESY spectrum uncovered that all stereogenic centers were agreement with those of **1**. Compound **10**, sterenoid J, was thus deducted as 23(*E*)-3-oxo-13α,25-dihydroxy-14(13→12)*abeo*-lanosta-8,23-dien.

Compounds **11** and **12** exhibited the identical molecular formula $C_{30}H_{50}O_4$ as deduced from the HRESIMS ion at *m/z* 497.3603 and 497.3600 $[M+Na]^+$ (calcd for 497.3601), respectively. Comparison of the one- and two-dimensional NMR data of **11** and **12** showed similarities, except for the subtle variations of the chemical shifts of C-22 ($\Delta\delta_C$ 2.7), C-23 ($\Delta\delta_C$ 0.7) and C-24 ($\Delta\delta_C$ 1.2) in the side-chain motif, indicating that they were a pair of C-24 epimers. The general features of the ^{1}H and ^{13}C NMR spectra of **11** closely resembled those of **1**, except that the $\Delta^{23,24}$ double bond was subject to the direct hydration. This change was supported by ^{1}H-^{1}H COSY correlations between H_b-23 (δ_H 1.15) and H-24 (δ_H 3.28), as well as the HMBC correlations from H_3-26 (δ_H 1.20) and H_3-27 (δ_H 1.15) to C-24 (δ_C 79.5) and C-25 (δ_C 73.2). Thus, the structure of **11** was thus deducted as shown (Figure 1). It was not reliable to distinguish the stereogenic centers at C-24 between **11** and **12** on the basis of available NMR data. Thus, the absolute configuration of C-24 was defined by utilizing the $Mo_2(OAc)_4$-induced CD experiment for vicinal diols.[36] As a result, compound **11** displayed a positive Cotton effect at 313 nm, indicating the 24*S* configuration for **11**. Accordingly, the 24*R* configuration for **12** was postulated. Therefore, **11** and **12** were named sterenoids K and L, and were assigned as 3-oxo-13α,24*S*,25-trihydroxy-14(13→12)*abeo*-lanosta-8-en and 3-oxo-13α,24*R*,25-trihydroxy-14(13→12)*abeo*-lanosta-8-en, respectively.

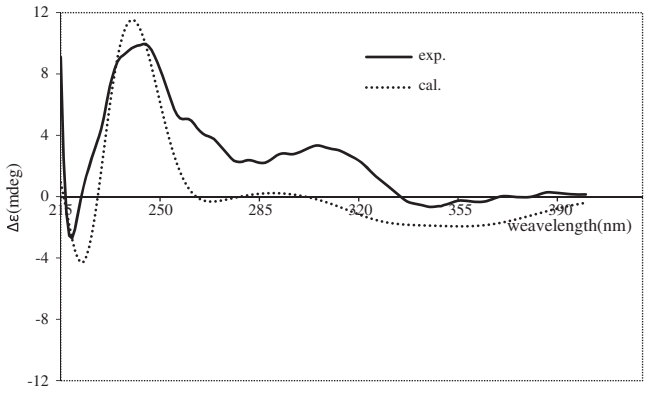

Figure 3 Experimental and calculated electronic CD (ECD) spectra of **1** (full line, experimentally recorded in methanol; dashed line, calculated for 5*R*, 10*S*, 12*R*, 13*R*, 14*R*, 17*R* and 21*R* configuration in methanol).

Biogenetically, the conversion of intact lanostane-type triterpenoids to these 14(13→12)*abeo*-lanostane-type-6/6/5/6 ring core triterpenoids is likely to undergo Wagner–Meerwein rearrangement with the carbocation intermediate. The hydroxy group is subsequently embedded in the carbocation to form the 13*R* or 13*S* configurations. From the standpoint of physicochemical stabilization, sterenoids A–L (**1–12**) with 13*R* configurations, which mean that H-12/HO-13 or H-17/HO-13 are syn-coplanar, are more stable than those of 13*S* configuration counterparts. These 13*S* configuration counterparts embody intrinsic reactivity of E2 elimination, which requires that the leaving group and the hydrogen are anti-coplanar. Quantum chemistry calculations for natural products of structure verification are exemplified via compounds **1** and **6**, suggesting that synergistic

Table 3 ^{1}H NMR spectroscopic data of compounds 7–12

No.	***7***[a,b]	***8***[a,b]	***9***[a,b]	***10***[a,b]	***11***[a,b]	***12***[a,b]
1	1.41m; 1.55m	1.41m; 2.32m	1.20m; 1.65m	1.61m; 2.52m	2.50m; 2.54m	2.50m; 2.54m
2	1.62m; 2.00m	1.63m;1.99m	1.60m; 1.70m	1.61m; 1.87m	1.61m; 1.87 ddd (4.6, 7.5, 13.4)	1.60m; 1.86 ddd (4.1, 7.7, 12.2)
3	3.46 br s	3.46 br s	3.24 dd (4.5, 11.3)			
5	1.46m	1.47m	0.95m	1.59m	1.56m	1.58m
6	1.46m; 1.64m	1.47m; 1.64m	1.45m; 1.75m	1.52m; 1.70m	1.10m; 1.56m	1.51m; 1.69m
7	1.81m; 1.90m	1.82m; 1.93m	1.76m; 1.93m	1.82m; 1.99m	1.82m;1.97m	1.11m; 1.57m
11	1.25m; 2.24m	1.25m; 2.24 dd (1.8,16.9)	1.25m; 2.24m	1.25m; 2.28m	2.28 dd (1.8, 16.3); 1.25m	1.25m; 2.28 dd (1.8,16.2)
12	1.69m	1.70m	1.69m	1.73 dd (1.8, 6.5)	1.71 dd (1.8,6.5)	1.73m
15	1.24m; 1.69m	1.25m;1.70m	1.25m; 1.70m	1.28m;1.74m	1.27m; 1.76m	1.28m; 1.77m
16	1.22m; 1.56m	1.26m;1.59m	1.06m; 1.59m	1.59m; 2.06m	1.39m; 1.51m	1.59m; 1.79m
17	1.22m	1.25m	1.25m	1.26m	1.22m	1.23m
18	1.03s	1.06s	1.05s	1.04s	1.02s	1.03s
19	1.03s	1.03s	1.02s	1.07s	1.06s	1.07s
20	1.81s	1.88m	1.88m	1.87m	1.87m	1.82m
21	1.67 br s	0.95 d (6.9)	0.95 d (5.9)	0.95 d (6.8)	0.96 d (6.9)	0.99 d (6.8)
22	0.99m; 1.45m	1.67m; 2.21m	1.65m; 2.20m	1.66m 2.19m	1.69m; 1.50m	1.81m; 1.99m
23	1.88m; 2.05m	5.59m	5.58m	5.59 dd (5.9,16.0)	1.25m; 1.39m	3.28 d (10.2)
24	5.10 t (6.8)	5.90 d (18.1)	5.59 d (16.5)	5.59 d (16.0)	3.35 d (8.9)	1.15m; 1.59m
26	1.68s	1.30s	1.30s	1.30s	1.20s	1.21s
27	1.60s	1.30s	1.30s	1.31s	1.15s	1.15s
28	0.97s	0.98s	1.02s	1.10s	1.10s	1.07s
29	0.89s	0.89s	0.83s	1.08s	1.07s	1.11s
30	0.95s	0.96s	0.95s	0.97s	0.95s	0.96s

[a]Measured in $CDCl_3$.
[b]Data were measured at 600 MHz.

Table 4 ^{13}C NMR spectroscopic data of compounds 7–12

No.	***7***[a,b]	***8***[a,b]	***9***[a,b]	***10***[a,b]	***11***[a,b]	***12***[a,b]
1	30.0 CH_2	30.0 CH_2	35.1 CH_2	34.3 CH_2	34.3 CH_2	34.3 CH_2
2	25.6 CH_2	25.6 CH_2	27.7 CH_2	34.9 CH_2	34.9 CH_2	34.9 CH_2
3	76.2 CH	76.2 CH	79.2 CH	218.1 C	218.1 C	218.1 C
4	37.4 C	37.4 C	38.7 C	47.0 C	47.0 C	47.0 C
5	45.9 CH	45.9 CH	52.0 CH	51.7 CH	51.7 CH	51.7 CH
6	18.4 CH_2	18.4 CH_2	18.6 CH_2	20.2 CH_2	20.1 CH_2	21.8 CH_2
7	23.1 CH_2	23.1 CH_2	23.3 CH_2	22.8 CH_2	21.8 CH_2	22.9 CH_3
8	139.0 C	139.0 C	139.3 C	140.3 C	140.3 C	140.4 C
9	142.6 C	142.6 C	142.3 C	140.6 C	140.7 C	140.7 C
10	35.9 C	35.9 C	36.0 C	35.6 C	35.6 C	35.7 C
11	29.7 CH_2	29.7 CH_2	29.7 CH_2	29.7 CH_2	29.7 CH_2	29.6 CH_2
12	58.1 CH	58.2 CH	58.1 CH	58.2 CH	58.1 CH	58.4 CH
13	76.9 C	77.0 C	76.7 C	76.9 C	77.2 C	76.7 C
14	48.7 C	48.7 C	48.6 C	48.6 C	48.7 C	48.7 C
15	32.5 CH_2	32.4 CH_2	32.5 CH_2	32.5 CH_2	32.6 CH_2	32.7 CH_2
16	21.5 CH_2	21.8 CH_2	21.8 CH_2	21.9 CH_2	30.7 CH_2	30.0 CH_2
17	53.3 CH	52.6 CH	52.7 CH	52.8 CH	53.3 CH	53.5 CH
18	20.2 CH_3	20.3 CH_3	20.2 CH_3	20.0 CH_3	19.8 CH_3	19.8 CH_3
19	19.5 CH_3	19.6 CH_3	19.7 CH_3	20.9 CH_3	20.9 CH_3	21.0 CH_3
20	31.1 CH	31.7 CH	31.7 CH	31.6 CH	31.7 CH	30.8 CH
21	21.6 CH_3	21.3 CH_3	21.3 CH_3	21.3 CH_3	21.7 CH_3	21.4 CH_3
22	33.5 CH_2	36.3 CH_2	36.2 CH_2	36.2 CH_2	22.9 CH_2	20.2 CH_2
23	26.6 CH_2	126.6 CH	126.5 CH	126.5 CH	30.5 CH	29.8 CH_2
24	124.9 CH	138.9 CH	138.9 CH	138.9 CH	79.5 CH_2	78.4 CH
25	131.3 C	70.7 C	70.7 C	70.7 C	73.2 C	73.2 C
26	25.7 CH_3	29.9 CH_3	29.9 CH_3	29.9 CH_3	26.6 CH_3	26.7 CH_3
27	17.6 CH_3	29.8 CH_3	29.8 CH_3	29.8 CH_3	23.1 CH_3	23.1 CH_3
28	28.2 CH_3	28.2 CH_3	28.2 CH_3	27.2 CH_3	27.2 CH_3	27.3 CH_3
29	22.1 CH_3	22.1 CH_3	15.6 CH_3	19.2 CH_3	19.2 CH_3	19.2 CH_3
30	28.9 CH_3	28.8 CH_3	28.9 CH_3	28.8 CH_3	28.9 CH_3	28.9 CH_3

[a]Measured in $CDCl_3$.
[b]Data were measured at 150 MHz.

combination of experimental NMR data and DFT chemical shift predictions is an unbiased way for structure elucidation.

Cytotoxic activities

Compounds **1–12** were screened for cytotoxicity against five cancer cell lines HL-60 (human promyelocytic leukemia), SMMC-7721 (hepatic cancer), A-549 (lung cancer), MCF-7 (breast cancer) and SW-480 (colon cancer) using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) method, with cisplatin and paclitaxel as positive controls. These cytotoxicity results (Table 5) disclosed compound **5** displayed potent cytotoxicity against HL-60 acute leukemia and SMMC-7721 hepatic tumor cell lines with IC_{50} values of 4.7 and 7.6 μM, respectively. Although the remaining compounds (**2–3** and **7–9**) showed weak cytotoxicity against five tumor cell lines and compounds (**1**, **6** and **10–12**) were inactive ($IC_{50} > 40$ μM). Comparison of the cytotoxic compounds **1–5**, **7–9** against HL-60 tumor cell line with **6**, **10–12**, brief structure–activity relationship concludes that $\Delta^{8,9}$ and $\Delta^{24,25}$ double bonds are essential for cytotoxicity.

EXPERIMENTAL PROCEDURES

General experimental procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). UV/Vis spectra were obtained using a Shimadzu UV2401PC spectrometer (Shimadzu, Kyoto, Japan). CD spectra were tested on an Applied Photophysics Chirascan Circular Dichroism Spectrometer (Applied Photophysics Limited, Leatherhead, Surrey, UK). IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Inc., Billerica, MA, USA) with KBr pellets. One- and two-dimensional NMR spectra were measured on a Bruker Avance III 500 MHz, Bruker Avance III 600 MHz and Bruker Accend 800 MHz spectrometers (Bruker Biospin GmbH, Karlsruhe, Germany). HR–ESI–MS were recorded on an Agilent 6200 Q-TOF MS system (Agilent Technologies, Santa Clara, CA, USA). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd, Qingdao City, China) and Sephadex LH-20 (Amersham Biosciences, Upplasa City, Sweden) were used for column chromatography (CC). Medium-pressure LC was performed on a Büchi Sepacore System equipped with pump manager C-615, pump modules C-605 and fraction collector C-660 (Büchi Labortechnik AG, Fällanden, Switzerland), and columns packed with Chromatorex C-18 (40–75 mm, Fuji Silysia Chemical Ltd, Kasugai, Japan). Preparative HPLC was performed on an Agilent 1260 LC system equipped with two types of Zorbax SB-C18 columns (9.4 mm × 150 mm and 21.2 mm × 150 mm, particle size 5 mm).

Fungal material

The fruiting bodies of *Stereum* sp. were collected in October 2013 from Xishuangbanna Tropical Botanical Garden Chinese Academy of Sciences and identified by Professor Yu-Cheng Dai (Beijing Forestry University). A voucher specimen (deposition no.: HPC 20131022) has been deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered fruiting bodies of *Stereum* sp. (848 g) were macerated four times with 95% ethanol to afford crude exact (191 g). The crude extract was suspended in water (800 ml) and partitioned with EtOAc three times to obtain the EtOAc fraction (45 g). The EtOAc fraction was subject to medium-pressure LC with a stepwise gradient elution of MeOH/H_2O (v/v 40:100–100:0) to afford eight fractions (A–H). Fraction F (12 g) was applied to medium-pressure LC with isocratic elution (MeOH/H_2O, 80:20) to obtain seven subfactions (F1–F7) based on TLC analysis. Subfraction F2 was separated by Sephadex LH-20 (methanol) CC to give two fractions (F2a and F2b). Fraction F2a was separated repeatedly by semipreparative HPLC (CH_3CN/H_2O, 40:60 to 70:30, 30 min, 7 ml min^{-1}) to yield **8** (2.5 mg), **10** (9 mg), **11** (15.8 mg) and **12** (3 mg). Subfraction F3 was separated by Sephadex LH-20 (methanol) CC to give four fractions (F3a–F3d) based on TLC analysis. Fraction F3a was purified by Sephadex LH-20 (acetone) CC and then was separated by semipreparative HPLC (CH_3CN/H_2O, 45:55 to 69:31, 30 min, 7 ml min^{-1}) to afford **9** (3.1 mg). Fraction F3b was purified by Sephadex LH-20 (acetone) CC and then was separated by semipreparative HPLC (CH_3CN/H_2O, 50:50 to 71:29, 30 min, 7 ml min^{-1}) to afford **2** (6.2 mg), **3** (4 mg) and **4** (2.3 mg). Subfraction F4 was separated by Sephadex LH-20 (acetone) CC to yield two fractions (F4a and F4b) based on TLC analysis. F4b was subject to a silica gel column with petroleum ether-acetone gradient solvent system (v/v, 10:1 to 2:1) to afford four fractions (F4b1–F4b4). Fraction F4b2 exhibited interesting spots in the TLC, which reacted with anisaldehyde-sulfuric acid and next was separated by preparative HPLC (CH_3CN/H_2O, 62:38 to 86:16, 30 min, 18 ml min^{-1}), further purified by semipreparative HPLC (CH_3CN/H_2O, 62:38 to 86:14, 30 min, 7 ml min^{-1}) to afford **1** (3.6 mg) and **6** (2.1 mg). Subfraction F5 was purified by Sephadex LH-20 (acetone) CC and then was separated by semipreparative HPLC (CH_3CN/H_2O, 50:50 to 71:29, 30 min, 7 ml min^{-1}) to afford **5** (4.5 mg). Subfraction F7 was subject to column chromatography over silica gel, eluting with isocratic petroleum ether-acetone (9:1) to give two fractions (F7a and F7b). Fractions F7b were purified by semipreparative HPLC (CH_3CN/H_2O, 70:30 to 100:0, 30 min, 7 ml min^{-1}) to obtain **7** (3.5 mg).

*Sterenoid A (**1**)*: White solid; $[\alpha]_D^{20}$ +99.8 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 202 (2.89), 240 (3.63) nm; electronic CD (MeOH) λ ($\Delta\varepsilon$) 216 (−3.8), 240 (+11.5) 324 (−0.6) nm; IR (KBr) ν_{max} 3437, 2962, 2877,1696, 1630, 1460, 1383, 1012 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (+)-HRESIMS *m/z* 477.3350 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_3Na$, 477.3339).

*Sterenoid B (**2**)*: White solid; $[\alpha]_D^{20}$ +57.3 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.92), 240 (1.17) nm; IR (KBr) ν_{max} 3445, 2957, 2931, 2867, 1702,

Table 5 Cytotoxicity IC_{50} values (μM) of compounds 1 – 12 against human tumor cell lines

	Cell lines				
Compounds	*HL-60*	*A-549*	*SMMC-7721*	*MCF-7*	*SW480*
1	17.1	>40	>40	>40	>40
2	17.1	16.4	15.3	14.8	13.2
3	16.2	15.7	16.4	17.6	11.3
4	24.8	>40	>40	34.1	>40
5	4.7	16.1	7.6	15.9	15.5
6	>40	>40	>40	>40	>40
7	15.8	21.0	19.7	17.8	13.4
8	16.0	16.1	15.8	15.6	14.7
9	15.8	20.4	17.4	15.3	14.8
10	>40	>40	>40	>40	>40
11	>40	>40	>40	30.95	>40
12	>40	>40	>40	>40	>40
Cisplatin	3.1	18.0	13.7	28.4	14.7
Paclitaxl	<0.008	<0.008	<0.008	<0.008	<0.008

Cell lines: HL-60, acute leukemia; A-549, lung cancer; SMMC-7721, hepatic cancer; MCF-7, breast cancer; SW480, colon cancer. Cisplatin and paclitaxel were used as positive controls.

1458, 1376, 1251, 1111, 1034 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (+)-HR–ESI–MS *m/z* 479.3488 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

Sterenoid C (***3***): White solid; $[\alpha]_D^{20}$ +49.6 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 204 (5.24), 253 (1.24) nm; IR (KBr) ν_{max} 3445, 2958, 2930, 2867, 1701, 1457, 1380, 1251, 1111, 1036 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (+)-HR–ESI–MS *m/z* 479.3488 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

Sterenoid D (***4***): White solid; $[\alpha]_D^{20}$ +11.5 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 202 (2.43), 252 (2.14) nm; IR (KBr) ν_{max} 3435, 2964, 2874, 1707, 1665, 1458, 1381, 1247, 1113, 1034 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (+)-HR–ESI–MS *m/z* 455.3527 $[M+H]^+$ (calcd for $C_{30}H_{47}O_3$, 455.3520).

Sterenoid E (***5***): White solid; $[\alpha]_D^{20}$ +44.4 (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.64) nm; IR (KBr) ν_{max} 3431, 3440, 295, 293, 2868, 1702, 1635, 1460, 1382, 1281, 1125, 1079 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (+)-HR–ESI–MS *m/z* 521.3606 $[M+Na]^+$ (calcd for $C_{32}H_{50}O_4Na$, 521.3601).

Sterenoid F (***6***): White solid; $[\alpha]_D^{20}$ +70.7 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.27) nm; IR (KBr) ν_{max} 3443, 2955, 2933, 2873, 1706, 1632, 1459, 1382, 1118, 1084 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; (+)-HR–ESI–MS *m/z* 479.3492 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

Sterenoid G (***7***): White solid; $[\alpha]_D^{20}$ +14.4 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.23) nm; IR (KBr) ν_{max} 3430, 2955, 2930, 2867, 1707, 1630, 1455, 1383, 1273, 1062 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; (+)-HR–ESI–MS *m/z* 465.3705 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_2Na$, 465.3703).

Sterenoid H (***8***): White solid; $[\alpha]_D^{20}$ +15.5 (*c* 0.42, MeOH); UV (MeOH) λ_{max} (log ε) 203 (1.93) nm; IR (KBr) ν_{max} 3440, 2957, 2936, 1708, 1630, 1457, 1380, 1158, 1062 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; (+)-HR–ESI–MS *m/z* 481.3653 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_3Na$, 481.3652).

Sterenoid I (***9***): White solid; $[\alpha]_D^{20}$ +22.7 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.70), 240 (1.11) nm; IR (KBr) ν_{max} 3428, 2962, 2932, 2868, 1716, 1631, 1456, 1374, 1151, 1030 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; (+)-HR–ESI–MS *m/z* 497.3403 $[M+K]^+$ (calcd for $C_{30}H_{50}O_3K$, 497.3403).

Sterenoid J (***10***): White solid; $[\alpha]_D^{20}$ +6.7 (*c* 0.41, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.92) nm; IR (KBr) ν_{max} 3436, 2962, 2931, 2868, 1703 1631, 1459, 1380,1251, 1149, 1031 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; (+)-HR–ESI–MS *m/z* 479.3493 $[M+Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

Sterenoid K (***11***): White solid; $[\alpha]_D^{20}$ +13.0 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 203 (3.92), 240 (1.17) nm; IR (KBr) ν_{max} 3440, 2956, 2933, 2868, 1701, 1633, 1460, 1381, 1281, 1125, 1078 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; (+)-HR–ESI–MS *m/z* 497.3601 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3603).

Sterenoid L (***12***): White solid; $[\alpha]_D^{20}$ + 34.7 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 203 (1.76), 240 (1.21) nm; IR (KBr) ν_{max} 3443, 2956, 2932, 2866, 1700, 1632, 1461, 1382, 1281, 1124, 1077 cm^{-1}; ^1H and ^{13}C NMR data see Tables 3 and 4; (+)-HR–ESI–MS *m/z* 497.3600 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3601).

Cytotoxicity assays

The human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7 and SW-480 were used in the cytotoxic assay. These cell lines were obtained from ATCC (Manassas, VA, USA). Cells were cultured in RMPI-1640 or DMEM medium (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 10% fetal bovine serum (Biological Industries) at 37 °C in a humidified atmosphere with 5% CO_2. The cytotoxicity assay was evaluated by theMTS, inner salt (Promega, Madison, WI, USA) assay.[37] Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 °C, the test compound (40 μM) was added. After incubated for 48 h, cells were subjected to the MTS assay. Compounds with a growth inhibition rate of 50% were further evaluated at concentrations of 0.064, 0.32, 1.6, 8 and 40 μM in triplicate, with cisplatin and paclitaxel (Sigma, St. Louis, MO, USA) as positive controls. The IC_{50} value of each compound was calculated with the method of Reed and Muench.[38]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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